NOT MWCH See p18 onwards.

SICKLE CELL ANAEMIA,

SICKLE CELL-HAEMOGLOBIN C DISEASE,

AND

HOMOZYGOUS HAEMOGLOBIN C DISEASE.

Historical Review

and

Clinical Manifestations in Nigerian Children.

T H E S I S

Presented for the Degree of Doctor of Medicine

by

R. G. Hendrickse

M.B., Ch.B., M.R.C.P.

University of Cape Town,

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TO

W. B. DOUGLAS-DRUMMOND and ALAN B. TAYLOR

to both of whom I owe a debt which can

never be repaid.

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INTRODUCTION.

Sickle cell anaemia has long been known to be a congenital haemolytic anaemia, but the nature of the inherited defect in the erythrocyte and the mode of inheritance have only recently been elucidated. It is now known that sickle cell anaemia is caused by homozygous inheritance of an abnormal haemoglobin, and that its heterozygous combination with normal haemoglobin results in a "carrier" state in which sickling of the erythrocytes can be demonstrated, but there is no associated disease. These discoveries led to biochemical and genetic investigations of other haemolytic diseases and the discovery of other abnormal haemoglobins with similar modes of inheritance. Already identification and characterisation of abnormal haemoglobin variants have permitted the analysis of a number of previously obscure syndromes in terms of chemical substances and underlying genetic factors, and "order is beginning to dawn in a field previously consisting of haemolytic curiosities" (Zuelzer, Néel and Robinson 1956).

The historical survey in the first part of this thesis shows the gradual unfolding of knowledge about the presence of an abnormal haemoglobin in this disease, and the fillip this discovery has given to workers in many fields, including medicine, genetics, biochemistry and anthropology, to yet further research. Only the salient features of the early and recent developments have been reviewed, and no attempt has been made to cover completely a subject which has become a focus of interest for many disciplines and the literature on which is now

voluminous. This thesis shows also how the discovery of this abnormal haemoglobin led to the discovery of further abnormal haemoglobins, resulting in the recognition of the nature of the defect in the erythrocyte in other inherited anaemias, and deals specifically with one of these, haemoglobin C, heterozygously in combination with haemoglobin S, and in the homozygous state. There is a survey of the incidence and distribution of the sickle cell and haemoglobin C traits in Africa to indicate the expected incidence of diseases associated with them.

Knowledge of haemoglobin C is so recent and reports on its clinical manifestations, especially in Africa, so few, that the need for information concerning it is obvious. Regarding sickle cell anaemia there have been numerous reports on the clinical manifestations, especially in the American literature, during the past forty years, and most aspects of the disease have been minutely examined. Yet although it has long been recognised that the disease is congenital and that symptoms and signs are usually manifested early in life there have been relatively few papers published which devote particular attention to the disease in children. In Africa so few cases of sickle cell anaemia had been reported prior to 1950 that Raper (1950) expressed doubt as to whether the consequences of homozygous inheritance of the sickling gene were the same in indigenous Africans as in American Negroes. Subsequent reports have shown that when the disease is sought for at the paediatric age level, it is found to occur in a frequency which accords with that expected in terms of the genetic theory advanced by Neel (1947, 1949, 1951) and Best (1949). Most of these reports have, however, contribu-

ted little to knowledge of the clinical manifestations of sickle cell anaemia as it occurs in infancy and childhood in Africa. There is thus a general need for attention to be focussed on sickle cell anaemia as a paediatric problem, and in Africa, a need for specific information about the manifestations of the disease as seen against a background of chronic protein undernutrition, debilitating tropical diseases, and primitive social systems as yet largely uninfluenced by modern thought and practice.

The second part of this thesis deals with the clinical manifestations of these three diseases in Nigerian children. The material which forms the basis of this study was compiled from a study of case records on, and personal observations of, 84 children with these diseases (75 cases of sickle cell anaemia, 8 cases of sickle cell-haemoglobin C disease, and one case of haemoglobin C disease) seen in the Department of Paediatrics of the University College Hospital, Ibadan, Nigeria. With the exception of 7 cases of sickle cell anaemia the diagnosis was in every case confirmed by paper electrophoresis of the haemoglobin. The earliest case record dates back to 1953. My own observations were made between October 1955 and November 1956. Our findings are compared with those reported in children and adults by authors elsewhere in Africa and America. Many differences emerge from the comparison, but the most striking are those relating to the bone changes shown radiologically in sickle cell anaemia. Bone lesions occurred more frequently in our series than in other reported series

and many cases showed lesions which have only rarely been reported. One patient with sickle cell anaemia showed changes not previously described.

It is hoped that this presentation will help to fill in some of the gaps in our knowledge of the natural history of these diseases and their clinical manifestations.

PART ONE

SECTION ONE

AN HISTORICAL SURVEY OF DEVELOPMENTS IN MEDICINE AND ALLIED SCIENCES THAT HAVE RESULTED FROM THE STUDY OF SICKLE CELL ANAEMIA.

Period 1910 to 1920

Sickle cell anaemia was first reported, though not under this name, by Herrick in 1910. In the next decade two more cases were reported, one by Washburn in 1911 and the other by Cook and Meyer in 1915. Emmel (1917), using Cook and Meyer's patient, studied the haematological aspects of the new syndrome and discovered a simple and effective technique for demonstrating erythrocyte sickling. He found that when a drop of the patient's blood was placed on a slide, covered with a coverslip which was then sealed round the edges with vaseline, a marked increase in the number of sickled erythrocytes was evident after a few hours, and sickling progressed to involve 100 per cent. of cells after 24 hours. This technique has been widely used since, and has proved to be of inestimable value because of its simplicity and reliability. Recently it has tended to be replaced by newer techniques but it is still routinely employed in many laboratories, especially those in situations where simplicity and economy are prerequisites for standard techniques. Emmel also deserves mention for first demonstrating that the sickling phenomenon might depend upon an inherited defect in the red blood cell.

Period 1921 to 1930

Mason (1922) describing the fourth reported case of "Herrick's Anaemia" used the term "sickle cell anaemia" for the first time. Huck in 1923 presented 14 "new" cases, but it is apparent from a study of the records that only 2 of these were cases of sickle cell anaemia and the rest were examples of the trait. There was at that time, however, no recognition of the clear distinction between the two conditions. From his family studies, clinical observations and experiments with the erythrocytes of patients with sickle cell anaemia, Huck (1923) concluded that sickle cell anaemia was a not uncommon disease occurring in the Negro race, transmitted in accordance with Mendelian law and having as its cause an inherent defect in the red blood cells. He demonstrated, using Emmel's (1917) technique, that in sealed moist preparations, sickling increases progressively at first, but if such preparations are kept for some time (3 to 42 days), the cells revert to a spherical form. He also showed that sickled preparations when kept in the dark reverted to spherical forms within 2 days, whereas control preparations kept in the light took from 3 to 30 days to undergo the same change. These observations still await explanation. In the same year Taliaferro and Huck (1923), on the basis of a study of one large family, produced support for Huck's view of the inheritance of sickling and concluded that the condition was due to a dominant gene.

Sydenstricker et al (1924) laid stress on the frequent co-

currence of sickle cell anaemia and credit is largely theirs for bringing the disease to the attention of the medical profession at large. They were also the first to recognise that the anaemia is haemolytic in origin; but in common with Huck they were unaware of the clear differences between the trait and the anaemia and considered the former to be the latent and the latter the active form of the same disease.

Cooley and Lee (1926) and Hahn and Gillespie (1927) introduced the terms "sicklaemia" and "sickle cell trait" respectively to denote asymptomatic cases showing the sickling phenomenon. These authors recognised that the sickle cell trait was not a disease in the ordinary sense and was consistent with health and long life.

In 1927 Hahn and Gillespie showed by a series of carefully conducted experiments, using a small gas chamber mounted on a microscope stage, in which a drop of blood was exposed to various gases, that sickling only occurs when haemoglobin is in the reduced state. This observation provided an explanation for Emmel's earlier findings, and provided the basis for many techniques subsequently evolved by other workers to demonstrate sickling. Sciver and Waugh (1930), elaborating on the work of Hahn and Gillespie, did in vivo experiments and showed that blood collected from a digit in which venous stasis had been induced for five minutes, when sealed under a coverslip as in Emmel's technique, showed a greatly accelerated rate of sickling when compared with controls collected without venous stasis.

Cooley and Lee (1926) were the first authors to suggest the anthropological significance of sickling, when they asked "Might men working in Africa, where the negro strains are still well separated, perhaps find a tribe in which sicklaemia is the rule, and from which the condition was imported into this country (America)?". By a curious chance, in the same year, Archibald (1926) reported the first case of sickle cell anaemia found on the African continent. His patient was an Arab youth from the Sudan, however, not a negro. Almost twenty years were to elapse before the "dark continent" made any further contributions to the literature.

This period thus made the following contributions to knowledge of sickle cell anaemias-

In medicine - an awareness of the frequency of the occurrence of the disease; added information about its clinical expression, especially recognition that the anaemia is haemolytic in origin; new techniques for demonstrating sickling; and recognition of the differences in the manifestations of the sickle cell trait and the anaemia.

In biochemistry - the demonstration that sickling occurs when haemoglobin is in the reduced state.

In genetics - proof that sickling is an inherited phenomenon transmitted by a dominant gene. The exact mode of inheritance was ill understood.

In anthropology - a reference to the possibility that the gene for sickling was imported into America from the African continent.

Period 1931 to 1940

During this period there was a rapid growth of the literature on sickle cell anaemia and by 1939 more than 140 publications had appeared (Diggs and Bibb, 1939). Reports covered many aspects of the disease, but in general this was a period of consolidation and elaboration of facts and ideas put forward in the previous decade, and with the exception of work published by Sherman in 1940 (see below) no major advances towards understanding of the basic problems of the disease were recorded.

The following examples have been chosen from the literature to indicate the nature and scope of investigations during the period under review. In 1932 Anderson and Ware reviewed the clinical picture in the 49 cases of sickle cell anaemia that had appeared in the literature and added observations on 6 new cases. Diggs et al (1933) in a survey in Tennessee which included 2,539 Negroes, found that 8.3 per cent of their subjects showed the trait. Their findings were the strongest evidence up to that date of the essentially harmless nature of the trait, and they emphasised that in their experience the trait never gave rise to sickle cell anaemia. Various other authors (cited by Margolies 1951) reported surveys to determine the incidence of sickling in the United States of America. By 1940 the accumulated data showed that amongst 11,990 Negroes tested 7.4 per cent (895 cases) showed the trait, while amongst 2,267 white Americans there were only 4 persons who sickled and of these 3 were Mexicans.

These results seemed to place beyond doubt the observation originally made by Huck (1923) that sickling is a phenomenon peculiar to people of Negro ancestry. Hansen-Pruss (1936) showed that when sealed moist preparations of blood were made using slides previously treated with brilliant cresyl blue, methylene blue, Janus green or sodium cyanide, sickling occurred more rapidly than when the slides were untreated. Aruna (1935 & 1939) and Hughes, Diggs and Gillespie (1940) published information about the cerebrovascular lesions that occur in sickle cell anaemia. The latter authors concluded from a study of 31 cases that involvement of the nervous system is common in sickle cell anaemia. Diggs and Bibb (1939) from personal study of 47 patients and a survey of the literature, published detailed information about the morphology and behaviour of the erythrocyte in sickle cell anaemia. In the same year Bunting (1939) demonstrated that sickled cells from persons with the trait or the anaemia do not form rouleaux and remain unsedimented after one hour, while non-sickled cells from the same subjects form rouleaux and sediment. These and other reports did much to assist clinicians to a clearer understanding of the manifestations of the anaemia and the trait in America.

In 1940 Sherman published the results of experiments done on blood from patients with sickle cell anaemia and persons with the sickle cell trait. He found that sickling increases progressively with lowered oxygen tensions and that cells of the trait require much lower oxygen tensions to sickle than do cells of the anaemia. Stated in another way, red cells in sickle cell anaemia show sickling much more

readily in lowered oxygen tensions than do cells in the trait. The importance of this observation lies in the fact that it was the first demonstration of a physico-chemical difference between the erythrocytes in sickle cell anaemia and the sickle cell trait. Investigators had long been aware of the difference in the clinical manifestations of the trait and the anaemia, but prior to Sherman's work no one had succeeded in demonstrating any constant differences in the behaviour of the erythrocytes in the two conditions.

Period 1941 to 1950

Before this period there had been little interest shown in sickle cell anaemia by workers in Africa. Vandepitte (1955) commenting on this fact states: - "This lack of interest is not very surprising; there were sufficient causes to explain the numerous cases of anaemia in the primitive population, and, in addition, the medical services were not equipped for subtle haematology". However, by 1950 a number of reports on the incidence of sickling in various African communities had appeared. (See section on incidence and distribution of sickle cell and haemoglobin C traits in Africa).

Amongst the earliest reports were those of Smith (1943), Evans (1944),and Findlay et al (1946), from West Africa; English (1945) and Best (1946 and 1947) from East Africa; and Altmann (1945a) from South Africa. These surveys showed that in Africa south of the Sahara desert and north of the Zambesi river sickling occurs in high frequency, and in most groups examined the incidence was higher than that in America. Using data available at the time, Raper (1950)

estimated the number of persons with the sickling trait in Africa to be in the neighbourhood of 40,000,000. Further, he surveyed the literature on the occurrence of sickle cell anaemia in Africa and was able to find less than 100 cases reported, of which one was an Arab (Archibald 1926), three Asiatic Indians (Berk and Bull 1943, and Wright and Pearson 1949), and one a white South African (Altmann 1945b). On the basis of these observations he concluded that sickle cell anaemia depends not only on the extent to which the trait is present in a community, but also on the extent to which admixture with other genetic strains has occurred, and that the disease is more important in the American Negro than in the native African. These findings and conclusions were totally opposed to the hypothesis for the inheritance of sickle cell anaemia put forward by Neel (1947). (See below).

Valentine and Neel (1944) in a haematologic and genetic study of the transmission of thalassaemia, produced striking evidence to support the hypothesis that the mild form of the disease (thalassaemia minor) is due to heterozygosity for a factor which when homozygous results in full-blown thalassaemia (thalassaemia major). Neel (1947) suggested that a similar hypothesis might be applicable to the sickle cell phenomenon, and in 1949, elaborating on this, he presented data which supported the hypothesis that the sickle cell trait is due to heterozygous inheritance of a factor which when homozygous results in sickle cell anaemia. Beet (1949) studied the genetics of sickling in families of the Lala tribe of Bantu in Northern Rhodesia.

In one instance the family tree showed that both parents and six siblings of a fatal case of sickle cell anaemia showed the trait. Beet, employing the analogy of the inheritance of thalassaemia in interpreting his findings, suggested a similar hypothesis to that put forward by Neel, of whose work he was unaware, and his findings and conclusions, in common with those of Neel, deserve credit as original observations of great significance. Beet's work is especially remarkable in that it was carried out in an area remote from all modern facilities, among primitive people, and under conditions which greatly exaggerated the difficulties normally encountered in any investigation of this nature.

Because of the small number of cases of sickle cell anaemia reported from Africa the theory of homozygosity in sickle cell anaemia seemed ill suited to conditions in Africa and gave rise to the opinion expressed by Raper (1950) that the consequences of homozygosity were probably different in Africa and America, and that sickle cell anaemia was probably a more serious problem in America than in Africa. We will return to this question later.

Important advances were meanwhile occurring in the field of biochemistry. Watson et al (1948) in a paper on "The significance of the paucity of sickle cells in the blood of newborn infants" recorded that sickling tests on newborn infants and their mothers showed that the rate of sickling and the percentage of sickled cells was greater in the mothers than the babies. The mothers showed 84 to 100 per cent sickled cells while the babies showed only 0.5 to 29.5 per cent sickling.

In one infant on whom serial tests were done, the percentage of erythrocytes showing sickling gradually increased until at the end of 4½ months 90 per cent of the cells sickled. They related the difference in the behaviour of the infants' erythrocytes to the presence of foetal haemoglobin and hence indicated the role of haemoglobin in the sickling phenomenon. This was the first mention of the possibility that sickling is related to the physico-chemical properties of haemoglobin. A year later Pauling et al (1949) demonstrated that the haemoglobin of persons with the sickle cell trait could be separated into two components by electrophoresis. The one component was shown to be normal adult haemoglobin (haemoglobin A), while the other was a previously unidentified pigment which they called sickle cell haemoglobin (haemoglobin S). Patients with sickle cell anaemia were shown to have only the abnormal haemoglobin in their red blood cells. It was thus established that the phenomenon of sickling depends on the inheritance of an abnormal haemoglobin, and that the difference in the behaviour of cells in the trait and the anaemia observed by Sherman (1940) results from differences in the quantity of sickle cell haemoglobin in the erythrocytes in the two conditions. Pauling and his co-workers (1949) suggested that the erythrocytes of other hereditary haemolytic anaemias be examined for the presence of abnormal haemoglobins.

These biochemical observations lent striking support to the hypothesis for the mode of inheritance of the sickling phenomenon.

The postulated "heterozygotes" were found to possess two components, the one normal and the other abnormal, in their red cells, while the "homozygotes" were shown to have only the abnormal component. The biochemical and genetic observations thus reinforced one another and as Zuelzer, Neel and Robinson (1956) point out "Not only did the electrophoretic analysis provide an objective basis for the distinction, formerly somewhat uncertain, between sickle cell trait and sickle cell anaemia, it also promised to give insight into the mechanisms and quantitative aspects of a human gene with an appreciable frequency, thus lending itself to the exploration of fundamental problems in human biology".

Wells and Itano (1950) found that, contrary to the statement by Pauling et al (1949) that sickle cell haemoglobin is found in the pure form in sickle cell anaemia, an electrophoretically normal haemoglobin, amounting to 5 to 20 per cent of the total pigment, is found in the erythrocytes in certain cases of sickle cell anaemia. This "normal" fraction was subsequently shown to be foetal haemoglobin (Singer 1951).

Further investigation showed that the difference between sickle and normal haemoglobin is located in the globin portion of the haemoglobin molecule. Peruts and Mitchison (1950) showed that whereas the solubility of reduced normal haemoglobin is about half that of oxy-normal haemoglobin, the solubility of reduced sickle cell haemoglobin is only one hundredth that of the oxy-S pigment. They suggested that sickling is caused by "crystalisation" of the sickle haemoglobin within the erythrocytes. Harris (1950) lent support to this view by

demonstrating that in highly concentrated solutions of reduced sickle haemoglobin "tactoid" formation could be seen with a phase microscope. Tactoids are composed of long, thin, rod-like particles which have a parallel and equidistant arrangement (Singer 1950). The tactoids found in the reduced sickle haemoglobin showed a striking resemblance to intact sickled erythrocytes, and Harris concluded that sickled cells represent haemoglobin tactoids, slightly distorted and veiled by the limiting cell membrane.

These observations on the characteristics of sickle cell haemoglobin did much to clarify the physico-chemical aspects of the sickling phenomenon. "The consequences of the deformity of the red corpuscles for the affected individual, and the differences between the carrier of the trait and the patient with anaemia, arthralgia and various other manifestations of sickle cell disease, could now be interpreted in physico-chemical terms. It became clear that in sickle cell trait, where abnormal haemoglobin is in a mixture with normal haemoglobin, the concentration of the former is insufficient to produce actual sickling in vivo, except under very unusual circumstances. Consequently the anomaly is ordinarily without effect for the individual. In sickle cell anaemia on the other hand, where all (or most) of the haemoglobin is of the abnormal variety, lowering of the oxygen tension within the physiologic range can and does produce sickling in vivo. Intravascular sickling is associated with a shortening of the life span of the red cells and with vascular occlusion and the ensuing symptoms typical of the disease". (Zuelzer, Neel and

Robinson 1956).

Itano and Neel (1950) investigated two families in which there occurred one or more children with a haematological picture which resembled sickle cell anaemia, but was of less severity. Further, in each of these families the erythrocytes of only one parent could be induced to sickle, a state of affairs at variance with the hypothesis for the inheritance of sickle cell anaemia (Neel 1949, Beet 1949). Electrophoretic studies on the haemoglobin of the affected children revealed the presence of sickle cell haemoglobin and a new component which migrated as a more positive ion than either normal or sickle cell haemoglobin. The "new" component was found to be present in the red cells of the non-sickling parents. Itano and Neel (1950) concluded from the results of their studies that "a previously unreported protein component, differing in electrophoretic mobility from the haemoglobins of normal and sickle cell anaemic individuals, is present in considerable amounts in the erythrocytes of certain individuals. Other observations indicate this component is indeed another abnormal haemoglobin". (See footnote). Their family studies indicated that it was inherited as if due to a single dominant gene.

Subsequently identified as haemoglobin C.

Period 1951 to the present

The developments recorded in the previous decade opened up new paths of investigation, introduced new concepts in medicine, biochemistry and genetics, and posed new problems. It is not surprising therefore that the last few years have witnessed a remarkable growth of the literature on many aspects of sickle cell anaemia and the other "haemoglobinopathies". As new information appears, shedding light on old observations and resolving old problems, new problems present for further research. The account that follows is an attempt to give a resume of the more important discoveries and trends in thought and investigation in the last seven years. It will be appreciated that a detailed factual review is beyond the scope of the present work. Despite considerable overlap, it will be convenient to consider recent advances under the following headings:- biochemistry, genetics and anthropology. In order to avoid undue repetition, clinical aspects have been omitted as they are covered in the second part of the thesis.

Recent trends in biochemistry.

Kaplan et al (1951) investigated the "abnormal protein" component described by Itano and Neel in 1950 and proved it to be another abnormal haemoglobin, which family studies showed was inherited as a simple Mendelian dominant, and which when combined with normal haemoblogin results in an asymptomatic carrier state in

which the erythrocytes do not sickle but have a high incidence of target cells. Combination of this haemoglobin, which they referred to as haemoglobin III, with sickle cell haemoglobin, results in a distinct haemolytic syndrome, similar to but milder in its manifestations than sickle cell anaemia. (Haemoglobin III was subsequently designated haemoglobin C and the disease resulting from its combination with sickle cell haemoglobin, sickle cell-haemoglobin C disease.)

With the discovery of a third abnormal haemoglobin by Itano (1951), in a white American family, it became clear that a standard system of nomenclature for the abnormal haemoglobins was desirable to avoid confusion based solely on terminological grounds. It was accordingly suggested that haemoglobins be identified by letters of the alphabet, in order of their discovery, the letter "A" being applied to normal adult haemoglobin and the letter "F" to foetal haemoglobin, the only exception being sickle cell haemoglobin, which according to this schema should be designated "B", but in practice is referred to as haemoglobin "S". There is thus no haemoglobin "B". (Conference on Haemoglobin, J.V.Neel (Chairman), 1953 a and b). Neel (1956) commenting on this system of nomenclature states:- "The chief difficulty with this schema is that it did not anticipate the tempo of discovery in the field. Thus it can happen that several groups of investigators will independently describe different new haemoglobins and assign the same letter to each. Thus far a free and healthy exchange of information in the field has minimised this source of confusion". By 1956 eight variants of normal haemoglobin had been des-

cribed, viz., Haemoglobins S, C, D, E, G, H, I and J (Neel 1956, Lehmann 1956, Edington and Lehmann 1956), and several more variants were in the process of investigation (Neel 1956).

In 1953 Spaet, Alway and Ward described homozygous haemoglobin C disease in a four year old Negro boy, and later Ranney et al (1953) and Levin et al (1953) recorded other instances of homozygous haemoglobin C disease in American Negroes. The clinical picture in all these cases was that of a mild haemolytic syndrome with splenomegaly and a preponderance of target cells in the stained films of peripheral blood. Family studies showed that the presence of haemoglobin C is determined by a gene which is transmitted as a Mendelian dominant, and a population survey conducted by Smith and Conley (1953) showed that 2 per cent of 500 Negroes examined showed the gene, while it was not encountered among 500 white Americans. In 1954 Edington and Lehmann reported the occurrence in high frequency of haemoglobin C on the Gold Coast. This was the first record of its occurrence outside the United States of America.

Biochemical and genetical investigation of the abnormal haemoglobins led to the understanding of the factors involved in the aetiology of atypical cases of sickle cell anaemia. The way in which simultaneous inheritance of haemoglobins S and C modifies the clinical expression of the sickling phenomenon has already been referred to. Several authors have reported the occurrence of combination of the thalassaemia and sickle cell genes with resultant severe haemolytic syndromes in some cases (Powell et al 1950, Sturgeon et al 1952, Banks

et al 1952, Neel et al 1953, Edington and Lehmann 1955(b)). It seems desirable therefore to indicate the relationship of thalassaemia to the haemoglobinopathies. In thalassaemia no abnormal haemoglobin has been demonstrated but the "A" gene (i.e. the gene for normal adult haemoglobin) is altered in such a manner as to lower haemoglobin A synthesis. Heterozygous inheritance of the thalassaemia and normal haemoglobin genes results in a more or less symptomless carrier state (analogous to the sickle cell trait), but homozygous inheritance of the altered A gene results in a severe progressive hypochromic anaemia without iron deficiency (Lehmann 1956, Zuelzer et al. 1956). Thalassaemia-sickle cell disease, thalassaemia-haemoglobin C disease and thalassaemia-haemoglobin E disease have been reported. (Reviews by Zuelzer et al. 1956, Lehmann 1956).

Table I, taken from Lehmann (1956), shows the known haemoglobin combinations which have been described since Pauling et al. (1949) initiated the study of the abnormal haemoglobins by demonstrating a haemoglobin abnormality in sickle cell anaemia. The presence of foetal haemoglobin in many of the combinations shown calls for an explanation of the role of this substance in the haemoglobinopathies.

From 50 to 85 per cent of the haemoglobin in newborn infants is of the foetal type. Van Korber (1866) and Van Kruger (1867) (cited by Chernoff & Singer (1952)) demonstrated that haemoglobin from placental blood exhibits a much greater resistance to denaturation by alkaline reagents than haemoglobin from erythrocytes of normal adults. Alkali resistant haemoglobin is always encountered in sickle

TABLE I - KNOWN HAEMOGLOBIN COMBINATIONS (Lehmann 1956).

Genotype			Phenotype
AA			Normal adults
AA		F	Infants
AA	T	F (sometimes)	Thalassaemia minor
AA	TT	F (nearly always present)	Thalassaemia major
SS		F (nearly always present)	Sickle cell anaemia
AS			Sickle cell trait
AS	T	F (presence of A often assumed only)	Microdrepanocytic Disease
CC		F (sometimes present)	Haemoglobin C disease
AC			Haemoglobin C trait
AC	T	F	Haemoglobin C-thalassaemia
SC		F (nearly always)	Sickle cell-haemoglobin C disease
DD			Haemoglobin D disease
AD			Haemoglobin D trait
SD		F	Sickle cell-haemoglobin D disease
EE		F (traces occasionally)	Haemoglobin E disease
AE			Haemoglobin E trait
(A)E	T	F (20 - 40% F, no A found)	Haemoglobin E-thalassaemia
AAE		Traces of an E-like haemoglobin occur sometimes in normal	
AAE	T	F individuals, and in thalassaemia minor (Kunkel-Wallenius phenomenon)	
CC		No anaemia	Homozygous C
AC			Haemoglobin C trait
AI			Haemoglobin I trait
AH		Genetical position not yet clarified	Haemoglobin H disease
AJ			Haemoglobin J trait

Note: No abnormal haemoglobin has been found in thalassaemia. The letter T used in the table indicates the gene for thalassaemia only and has no reference to an abnormal haemoglobin.

Two other abnormal haemoglobins, K and M, have also been discovered, but their characterisation is not yet complete (British Medical Journal 1957).

cell anaemia and thalassaemia major and sometimes in other haemolytic disorders (Singer 1951). Normally most of the foetal haemoglobin present at birth has disappeared by the fourth month of life, but traces may persist into the third year (Chernoff and Singer 1952). When, however, there is in infancy a diminished production of haemoglobin A the faculty for producing haemoglobin F is maintained and may persist into childhood or even adult life. This happens in the haemoglobinopathies and sometimes in other blood diseases. Thus, while haemoglobin F is in itself innocuous, its presence in the erythrocytes after early infancy in any appreciable quantity is usually indicative of some blood disease (Lehmann 1956).

Among the techniques used in the identification of abnormal haemoglobins electrophoresis remains the most important, but the need for employing other techniques is well illustrated by the discovery of haemoglobin D, which on electrophoresis behaves exactly like haemoglobin S, but differs in its solubility properties and is not associated with sickling (Itano 1951). Similarly, haemoglobins A and F show approximately the same electrophoretic mobility but differ markedly in their response to alkaline denaturation.

Neel (1956) lists the following techniques that have been applied to the characterisation of the abnormal haemoglobins-

(1) Crystallography

(2) Solubility studies

(3) Amino acid analysis

(4) Immunological specificity

(5) Stability of haemoglobin in alkali solutions

(6) Electrophoretic mobilities

(7) Spectrophotometric differences

(8) Differences in oxygen dissociation curves

Regarding the identification of "new" haemoglobins, it is apparent that certain "minimum standards" will have to be laid down and Neel (1956) suggests that besides biochemical and haematological criteria, "these 'minimum standards' should include identification of the haemoglobin in at least two related individuals". He also suggests the establishment of a haemoglobin reference laboratory comparable to the Blood Group Reference Laboratory.

Recent Trends in Genetics.

In 1951 Neel published the findings of an investigation of 75 kindreds undertaken to demonstrate the inheritance of the sickling phenomenon, with particular reference to sickle cell anaemia. His results showed that the manner of the association of the trait and the anaemia within families, and the proportions of these two entities, were in good general agreement with the hypothesis for the inheritance of sickle cell anaemia put forward by himself in 1949 and independently by Best in the same year. In tests on 94 parents of children with sickle cell anaemia he found the trait in 93, and 4 children of unions in which one parent had sickle cell anaemia showed the trait. Neel found 2 exceptions to the rule that both parents of a child with sickle cell anaemia, or one parent of a child with the trait, must sickle. He suggested the following possibilities to explain their occurrence:-

(1) That in certain individuals who are heterozygous for the sickling gene, the gene fails to find expression.

(2) That the legal father is not the biological father.

(3) That what appears to be sickle cell disease is actually due to the interaction of a single gene for sickling with as yet unknown environmental or genetic factors.

Or (4) That the apparently normal parent(s) has actually

contributed a sickle cell gene to one or more of his offspring in consequence of a mutation occurring at some stage in the formation of the gametes.

In the United States of America Neel's work was generally regarded as confirmation of the hypothesis of homozygosity in sickle cell anaemia. In Africa, on the other hand, there was considerable controversy. Raper (1950) estimated that whereas in the United States of America the ratio of sickle cell anaemia to sickle cell trait was about 1 to 50, in Africa the available evidence suggested a ratio of less than 1 per 1,000. Jelliffe and Humphries (1952 a and b) in Western Nigeria, and Lehman (1951) in Uganda, supported Raper's observations. An annotation in the British Medical Journal (1952) reviewing the position in Africa, pointed out that the incidence of the trait in some communities implied a frequency of homozygotes of 1 to 6 per cent, and yet the reported incidence of sickle cell anaemia was much less than 1 per cent. It was suggested that a possible explanation might be a high death rate of African homozygotes in infancy, a viewpoint which Neel (1951(b)) had earlier expressed thus- "....that because of the more rigorous conditions obtaining in early life there (Africa), most of the children are eliminated when quite young, either in consequence of a haemolytic crisis or because of intercurrent disease."

In 1951 J. & G. Lambotte-Legrand published their experience of sickle cell anaemia in Leopoldville in the Belgian Congo. They

reported on 88 cases seen amongst infants and young children at the Red Cross Hospital over a period of 3 years. The results of their investigation into the genetics of the inheritance of sickle cell anaemia were in complete agreement with Neel's hypothesis. They also found that the clinical picture presented by their patients differed from that recorded in America, especially in respect of the age incidence. In Leopoldville the vast majority of cases occurred in infancy and very early childhood while cases over the age of 10 years were hardly ever encountered. The progressive disappearance of the syndrome in childhood was shown to be due to a very high mortality in the first two years of life. (In America the disease occurs throughout childhood and adolescence into early adulthood). In later publications Lambette-Legrand (1955 a and b), on the basis of a study of 300 cases, confirmed their earlier observations. Examination of the parents of their cases showed that of 297 mothers tested for sickling 29% were positive, while 259 of 277 of the supposed fathers also showed the trait. Of these 300 cases 150 had died; and 80 per cent of the deaths had occurred before the age of 2 years. Foy and Kondi (1951 and 1952), Foy et al (1951), Vandepitte (1952 and 1955), Hutton (1953) and Welbourne and Raper (1954) lent support to the view expressed by J. & G. Lambette-Legrand that when sickle cell anaemia is sought for at the paediatric level it occurs in the frequency expected. Foy and Kondi (1952) in a survey in Portuguese East Africa found in two tribes, the Makuas and the Makondas, sickling rates of 38 per cent and 40 per cent respectively. Among 210 people examined there were 82 positive

for sickling and 9 of these were found to have sickle cell anaemia. Of these 9 cases, 8 were under 2 years of age. Vandepitte (1955) commenting on 261 cases of sickle cell anaemia discovered over a period of 2 years states: "The infants constituted the majority, as they did in the series of Lambotte & Legrand. Our oldest patient was 23 years; we estimate that in Africa, about one sickle cell anaemia patient in a hundred can reach that age."

Accepting that the results published by the authors cited above are representative of the situation in Africa as a whole, a new problem, of particular interest to geneticists, presents itself. If sickle cell anaemia results from the homozygous inheritance of a gene which in the vast majority of instances is lethal before the childbearing period, the steady loss of homozygotes should result in a diminishing incidence of the gene in the population at large, and yet in Africa sickling frequencies of the order of 15 to 30 per cent and more have been observed in many communities. In seeking a genetic explanation for the frequency of the sickle cell trait in Africa there are two obvious alternatives, by no means mutually exclusive. These two alternatives are pithily stated by Neel (1956): "One could, on the one hand, look to what have been termed 'mutable genes'. The alternative explanation is offered by "balanced polymorphism", i.e. a situation wherein the heterozygote for the haemoglobin genes is reproductively superior to either homozygote".

It will be recalled that among the parents of children with

sickle cell anaemia reported by Neel (1951) and Lambotte-Legrand (1951 and 1955) there were certain exceptions to the rule that both parents show the trait, and it was suggested that these exceptional cases might represent the occurrence of gene mutation (Neel 1951). Niermaux (1952) on the basis of a genetic investigation of over 200 families in an ethnic group with high sickling frequency concluded that loss of homozygotes is compensated for by a level of mutation of normal to "S" genes greater than any mutation level known to man. His studies also indicated that the frequency of the mutations is influenced by the line of descent, in other words, that the level of mutation is genetically determined. He compared this finding with the influence of line of descent, established by Morch, on the level of gene mutation in chondrodystrophy. Vandepitte et al (1955) working in Leopoldville in the Belgian Congo, found among a total of 233 mothers of patients with sickle cell anaemia 2 who showed no haemoglobin abnormality. Calculations based on the assumption that both these cases indicated mutations of the sickle gene reveal a very high rate of mutation as judged by usual human mutation rates, but only approximately one tenth of that necessary to offset natural selection in a population with 25 per cent sickling. They concluded that "the available data do not support the hypothesis that in the Belgian Congo mutation alone is responsible for the frequency of the sickling phenomenon, although the possibility that a relatively high mutation rate is a contributory factor in the maintenance of the

gene frequency cannot be denied".

The evidence for "balanced polymorphism" has recently been reviewed by Neel (1956). After careful evaluation of the known facts, he expresses the opinion that "the bulk of the evidence would suggest that the gene frequencies are maintained through balanced polymorphism, though much remains to be done to establish this point".

It is beyond the scope of this work to attempt to cover this wide, complex and controversial subject, but one aspect of it must be mentioned. I refer to Allison's malaria hypothesis (Allison 1954 a, b and c). Prior to the appearance of Allison's publications, Beet (1947) and Brain (1952) had noted that the spleen rate in sicklers tends to be lower than in non-sicklers, and Brain related this finding to a differential susceptibility to malaria, a concept which Allison later employed in his hypothesis. Earlier Beet (1946) had raised the question of malarial susceptibility in relation to the sickling phenomenon, when he found that non-sickling children showed a higher percentage of positive smears for malaria than sicklers, during the time of the year when transmission of malaria was at its lowest. His findings were not, however, statistically significant and he refrained from drawing any conclusions from them.

Allison (1954b) noted the striking correlation between the incidence of the sickle cell trait and malarial severity in population groups. High trait frequencies tend to occur in hyperendemic malarious areas. In experimentally induced malaria he found that sicklers were far less susceptible than non-sicklers. Inoculation

of 15 non-sicklers produced clinical malaria in 14, while in a comparable group of sicklers malaria was only induced in 2 and it was thought that their manifestations of the disease were less severe than they were among the non-sicklers. He also found that among children malarial parasitaemia occurred in higher frequency in non-sicklers than sicklers. Of 43 children with the trait 27.9 per cent had demonstrable parasitaemia, while in a group of 247 non-sicklers 45.7 per cent had malaria. From these and other observations Allison concluded that malaria is an important factor determining the frequency of the sickle cell trait, and suggested that the main effect on the sickle gene frequency is produced by a differential mortality in childhood.

Allison's malaria hypothesis stimulated considerable comment and controversy. Several authors, including Raper (1954 and 1955) and Edington 1954, and Lehmann and Raper (1956), published findings which are in broad general agreement with some of Allison's observations, but several others, including Moore, Brass and Fey (1954), Archibald and Bruce-Chwatt (1955), Fey et al (1955), interpreted their findings in malaria as not supporting the hypothesis. Bentley, Dern and Flanagan (1955) attempted to repeat Allison's observations on the relative susceptibility of sicklers and non-sicklers to experimental malaria. They chose as subjects 16 American Negroes, 8 of whom showed the trait, and all of whom were known not to have been previously exposed to malaria. They found that in the non-sicklers clinical signs and parasite densities were slightly more marked than in sicklers, but concluded that the differences observed were of doubtful significance.

According to Neel (1956), who must be regarded as an authority among geneticists, the malarial hypothesis has attained sufficient prestige to warrant further intensive study to validate or invalidate it. He offers the suggestion, however, that other physiological differences of importance to survival, in addition to malarial susceptibility, will be encountered in elucidating the role of balanced polymorphism in maintaining the high sickling frequencies encountered in Africa.

A third possible mechanism whereby high sickling frequencies might be maintained in Africa has been suggested by Edington and Lehmann (1955a). Their experience in the Gold Coast indicates that sickle cell anaemia does not always carry the gloomy prognosis generally suggested from African sources. Among 50 cases, apparently homozygous for haemoglobin S, which they had observed in persons well past infancy, there were two cases who were completely symptomless. They suggested therefore that the survival of relatively healthy homozygotes may be a potent factor in maintaining high trait frequencies in certain peoples. In a later publication the same authors (Edington and Lehmann (1955b)) reported that further investigation of the two "symptomless haemoglobin S homozygotes" showed that they were in fact heterozygotes for the sickle cell gene and a thalassaemia-like gene. They did not, however, retract their earlier suggestion regarding the maintenance of sickling frequencies in Africa. Reports from elsewhere in Africa indicate that survival of "relatively healthy homozygotes" is not a factor in maintaining sickling frequencies. Lehmann and Raper (1956) found

no homozygotes over 5 years of age amongst 191 sicklers of the Bamba tribe which has a sickling rate of 39 per cent.

Lehmann (1954) pointed out that besides genetic considerations, two other factors must be taken into account when considering the incidence of the sickle cell gene in populations, namely:-

1. The anthropological derivation of the population
2. Sociological factors.

Regarding the latter he points out that the highest sickling rates are found in the socially lowest stratum of a population. "In Africa the Pygmoids of the forest, in India the Veddoids of the Nilgiri, who either are living as serfs or on the lowest possible human level in the jungle, in Arabia again the Akhdam, the lowest social community, were the carriers of the gene. Similarly the Greeks were the slaves of the Turks until recently. These people are forced to live where they are, they are unable to travel, and they do not intermarry with their more fortunate neighbours. Inbreeding, which usually goes hand in hand with a slave-like condition in life, seems to be an important factor in producing a high sickling rate".

Recent Trends in Anthropology.

Early in the history of the discovery of sickle cell anaemia it was recognised that sickling is essentially a Negro characteristic, (Huck 1923) and although reports occasionally appeared of its occurrence in people of European descent, a Negro ancestry could never be

completely excluded. (Lehmann and Cutbush 1952, Lehmann 1954). The demonstration of high sickling frequencies in Africa justified the belief that the sickle cell phenomenon had been introduced into America by slaves originating from Africa. The mode of origin of the sickle cell gene in Africa is, however, not yet clear, and has become a question of great interest to anthropologists.

In 1952 Lehmann and Cutbush investigated the blood of certain aboriginal tribes in Southern India to collect data which might support or refute the idea of a racial linkage between Africa and India, which had been suggested by several leading anthropologists. Their findings were of startling interest. They found sickling in three of the aboriginal Veddian tribes, and in one of these, the Irulas, the incidence was 30 per cent, a figure higher than that encountered in most African populations. Blood group determinations, on the other hand, showed a very low incidence of the Rhesus chromosome Rh_0(cDe) which occurs in high frequency throughout Africa in the indigenous population (Mourant 1954). The authors interpreted their findings as being indicative of an Indian migration to Africa in prehistoric times, a migration in the reverse direction seeming unlikely in view of the very low incidence of the Rh_0(cDe) chromosome in the Indian groups examined. Alternative interpretations of these findings suggested at the time were

1. that the sickle cell gene arose independently in the two continents, and
2. that the peoples in India and Africa inherited the

sickle gene from common, non-African, ancestors (British Medical Journal 1952).

A short while later investigation of a population group in Southern Arabia with ethnological features similar to those of the aboriginal groups investigated in India showed a 23 per cent incidence of sickling, and a frequency of the Rh_o(cDe) chromosome higher than that found in India, but lower than that in Africa (Lehmann 1954). These findings accord well with the hypothesis that the sickle cell gene was introduced into Africa via the former land bridge in the vicinity of Aden and spread from there southward and westward over the continent.

Observations on the distribution in Africa of the shorthorn zebu cattle, believed to have been introduced via Southern Arabia by Indians and Arabs, lends indirect support to the hypothesis for the introduction of the sickle cell gene into Africa (Brain 1953a). It has been shown that the cattle entered Africa in exactly the spot postulated by Lehmann for the introduction of the sickling gene, and that migration then took two routes - westwards to the West coast and southwards to the Zambesi River, which it did not cross (Brain 1953). Brain comments: "Is it possible that the sickle cell gene was distributed in Africa by the custodians of the shorthorn zebu "

Among those who disagreed with the hypothesis for the introduction of the sickle cell gene from India into Africa were Fey and Kendi (1952) and Singer (1954). Singer postulates that the gene could have arisen in central Africa and spread from there over the

continent "wherever the 'Negro' migrated or was carried off in captivity". He suggests that the sickle cell gene was introduced into India by African slaves, and tentatively explains the findings of Lehmann and Cutbush (1952) by invoking the hypothesis of a selective advantage of the sickle cell gene protecting the carriers against malaria, "whereas the Hb_0 gene may have no advantage and subsequently disappear in 'mixed' communities". (Singer 1954). Singer's view accords with the classical concept of sickling as being essentially a negro characteristic which was substantiated by reports from all sources, until Lehmann and Cutbush reported their findings in India. Whatever the final outcome of anthropological investigation of the sickling phenomenon, it is apparent that in the present state of our knowledge it is no longer valid to use sickling as a tracer of African ancestry.

The variability of sickling frequencies among tribal groups in East Africa has been regarded as having ethnological significance by various authors (Lehmann 1954, Lehmann and Raper 1949, Fey et al 1954), but it will readily be seen that, if Allison's malaria hypothesis is correct and sickling incidences can be explained by positive selection in favour of heterozygotes, the relative incidences of the sickle cell gene in populations may be of comparatively small value as indicators of tribal relationships (Mourant 1954). Though balanced polymorphism may account for a relatively high <u>frequency</u> of the gene it cannot, of course, account for the <u>origin</u> of the gene.

Anthropological interest in the other abnormal haemoglobins

centres chiefly on the differential distribution of the haemoglobin variants amongst the human races. Table II sets out the world distribution of haemoglobins S, C, D and E. The other abnormal haemoglobins have only been reported in isolated families and are not shown. "It is very apparent that the intensive study of the distribution of these haemoglobin traits cannot help but provide information of great anthropologic interest - but equally apparent, that at present the same fluidity of thought is required which characterises our thinking about the biochemistry and genetics of the haemoglobins". (Zuelzer, Neel and Robinson, 1956).

TABLE II - WORLD DISTRIBUTION OF HAEMOGLOBINS S, C, D and E.

(Unless otherwise indicated, the information given in the table will be found in the reviews of Mourant (1954), Lehmann (1956) and Zuelzer, Neel and Robinson (1956)).

Haemoglobin	Distribution
S	Tropical Africa North and South America (among people of Negro ancestry) South India Southern Arabia (Lehmann 1954) Certain Mediterranean countries Madagascar (Brain 1956)
C	West Africa Algiers South Africa North America (Negroes)
D	North West India (Isolated reports from North America, Algiers, Britain and Turkey of haemoglobin D in 'white' families)
E	Burma Siam Indonesia Ceylon Malaya India (Bengal) North America (infrequent)

PART ONE

SECTION TWO

THE INCIDENCE AND DISTRIBUTION OF HAEMOGLOBINS S AND C IN AFRICA

A detailed statement of the incidence of the sickle cell trait as recorded up to 1954 is to be found in Dr. A. E. Mourant's book "The Distribution of the Human Blood Groups" (1954). Reports from Africa, cited by the author, give sickling frequencies for over 200 population units from all parts of the continent. These figures will not be reproduced here, but the general pattern of distribution of the sickle cell trait that emerges from a study of these will be discussed. The study of haemoglobin C is very recent, and reports on its incidence in Africa are only beginning to emerge. I have therefore reproduced all the figures which have been published by various authors to date (Table VII). As will be seen, these are comparatively few in number, but already a pattern of distribution is vaguely discernible. We will have to await further investigations to confirm or refute these early impressions.

The Sickle Cell Trait

In Africa North of the Sahara desert sickling is virtually absent. It is found only infrequently in people who have an admixture of Negro blood. A similar situation prevails South of the Zambesi River. In South Africa, Altman (1945a) found only one sickler among 403 Bantu examined, while in Cape Town Rarachowitz et al (1952) found the trait

in only 0.58 per cent of 1,555 Cape Coloured people. Sickling has not been observed in Bushmen (Griffiths 1953 and Budtz-Olsen and Burgers 1955). The sickle cell trait is thus virtually absent from the Union of South Africa. The explanation of this might be that the South African Bantu arrived South of the Zambesi before the haemoglobin S gene was introduced into Africa (Budtz-Olsen and Burgers 1955).

In the rest of Africa, i.e. South of the Sahara and North of the Zambesi, sickling is commonly encountered, but the incidence shows interesting variations, especially when we compare West and East African territories. In West Africa (including the Gold Coast, Nigeria, Gambia, Sierra Leone, the Cameroons, French Togoland and Portuguese Guinea) there appears to be an even distribution of the sickle cell trait and reported frequencies are between 10 and 20 per cent in most populations. To date the lowest and highest frequencies found in West Africa are 3 per cent in 500 natives of Portuguese Guinea (Trincao et al 1950) and 29.1 per cent in 124 natives of the Mende tribe in Sierra Leone (Allison 1956). In no group examined in the whole of West Africa has the sickle cell trait not been found. In East Africa wide variations in sickling frequencies are encountered from tribe to tribe. In many tribes sickling is virtually absent, while in others the incidence exceeds that encountered in West Africa. Analysis of the sickling frequencies given for populations in the East African territories of Uganda, Kenya, Tanganyika, Northern Rhodesia, Nyasaland and Portuguese East Africa (Heurant 1954) shows

that in certain groups, equivalent to approximately 23 per cent of the total sample, sickling was absent or occurred in a frequency not exceeding two per cent. Tables III and IV show population samples from East and West African territories respectively, grouped according to their sickling frequencies, and Table V compares the sickling frequencies in the populations in the two territories.

It will be seen that reports indicate that in West Africa 85.3 per cent of the population show sickling frequencies between 10 and 30 per cent, while in East Africa only 56.3 per cent of the population show frequencies above 10 per cent and as has already been shown 23.4 per cent of the East African samples show virtually no sickling. The impression gained is that not only is sickling more evenly distributed in West Africa but also that its overall frequency is greater than in East Africa.

It was decided to test these impressions by subjecting the data on which they are based to statistical analysis to determine the probability level at which these impressions are acceptable. Sampling errors and overlaps of tribal and geographical distribution, especially in East Africa, must obviously weaken the significance of any conclusions, irrespective of the accuracy of the statistical methods employed. It is probably fair to assume, however, that factors influencing sampling are approximately equal in the two territories and are thus unlikely to be solely responsible for marked differences emerging from a comparison of sickling frequencies in

TABLE III - POPULATION SAMPLES GROUPED ACCORDING TO SICKLING FREQUENCIES

EAST AFRICAN TERRITORIES
(Uganda, Kenya, Nyasaland, Tanganyika and Portuguese East Africa).

Percentage Sickling in Population					
0 to 2	2 to 10	10 to 20	20 to 30	30 and over	Authors
-	1	-	-	-	Trowell & Muwasi (1948)
1	6	6	10	1	Lehmann & Raper (1949)
10	6	3	11	5	Allison (1954) b.
16	7	11	8	2	Foy et al (1954)
-	-	1	2	-	Mackey (1949)
-	1	4	-	-	Beet (1947)
3	6	2	2	-	Brain (1953) b.
30 (23.4%)	26 (20.3%)	31 (24.3%)	23 (25.7%)	8 (6.3%)	Total all authors

Total of all samples - 128. (Figures in brackets indicate percentage of this total).

TABLE V - COMPARISON OF SICKLING INCIDENCES IN EAST AND WEST AFRICAN POPULATION SAMPLES.

Percentage Sickling	Percentage of Population Samples	
	East Africa	West Africa
0 to 2	23.4	0.0
2 to 10	20.3	14.3
10 to 20	24.3	51.0
20 to 30	25.7	34.7
30 to 40	6.3	0.0

TABLE IV - POPULATION SAMPLES GROUPED ACCORDING TO SICKLING FREQUENCIES

WEST AFRICAN TERRITORIES
(Nigeria, Gold Coast, Cameroons, Gambia, Sierra Leone, Portuguese Guinea, French Togoland).

Percentage Sickling in Population					
0 to 2	2 to 10	10 to 20	20 to 30	30 and over	Authors
-	1	-	-	-	Trincao et at (1950)
-	-	-	1	-	Gosden & Reid (1948)
-	-	-	1	-	Colbourne et al (1950)
-	-	1	-	-	Findlay et al (1946)
-	-	1	-	-	Edington (1952)
-	-	3	2	-	Evans (1944)
-	-	1	-	-	Adamson (1951)
-	-	3	-	-	Humphreys (1952)
-	-	-	1	-	Jelliffe & Humphreys (1952)
-	1	-	-	-	Smith (1943)
-	4	13	11	-	Allison (1956)
-	1	2	-	-	Edington & Lehmann (1954 and 1956)
-	-	1	1	-	Walters & Lehmann (1956)
0	7 (14.3%)	25 (51%)	17 (34.7%)	0	Total all authors

Total of all samples = 49. (Figures in brackets indicate percentage of this total).

the two regions.

I am indebted to Dr. E. Dowdle, of the Department of Medicine, University of Cape Town, for assistance with statistical analyses.

Calculations based on the figures presented in Tables III and IV show that:- (See appendix I)

1. Using Fisher's F test, it can be shown with more than 99.9 per cent certainty that the between sample variance for East Africa is greater than the between sample variance for West Africa, i.e., that sickling is more evenly distributed in the latter than the former.
2. Using Student's t test (the value for t with 175 degrees of freedom was 3.1309), the 'impression' that the mean incidence of sickling is greater in West than East Africa was found to be acceptable at a probability level of 1 per cent.

The theory that the sickle cell trait entered Africa from the East and spread westwards and southwards (Lehmann 1952 and 1954), and the theory that the sickling gene originated in East Africa and spread thence (Singer 1954), both imply that the gene was present in East Africa earlier than in West Africa. These theories have been supported, in part at least, by arguments based on the occurrence of very high sickling frequencies in certain East African tribes. We

have seen, however, that these samples are not representative of most populations in East Africa; in fact, they represent only 6.3 per cent of the total (Tables III and V). In our present state of ignorance about the factors that determine gene frequencies, and the role of malaria in determining sickling frequencies, we must resist the temptation to develop strong attachments to theories, attractive though they may be, which utilise sickling frequencies to explain the origin and spread of the gene in Africa. It will readily be seen that the uniform distribution and the higher overall frequency of sickling in West Africa might be interpreted as indicating that the gene has had a longer period in which to become established and gain equilibrium than it has in East Africa, and thus might suggest that spread might have been from West to East, rather than in the reverse direction, as suggested by Lehmann (1952 and 1954), Singer (1954) and Brain (1953). It is interesting to note that the River Niger, which appears to be a no less effective natural barrier to migration than the Zambesi River, has not affected the sickling frequencies in West Africa, despite the fact that it lies directly in the path of the East to West migration proposed by the authors cited above.

Figure I shows the areas in East and West Africa which have been compared, and the migration route proposed by Lehmann for the introduction of the sickling gene into Africa. The inset shows the sickling frequencies in populations (expressed as a percentage of the

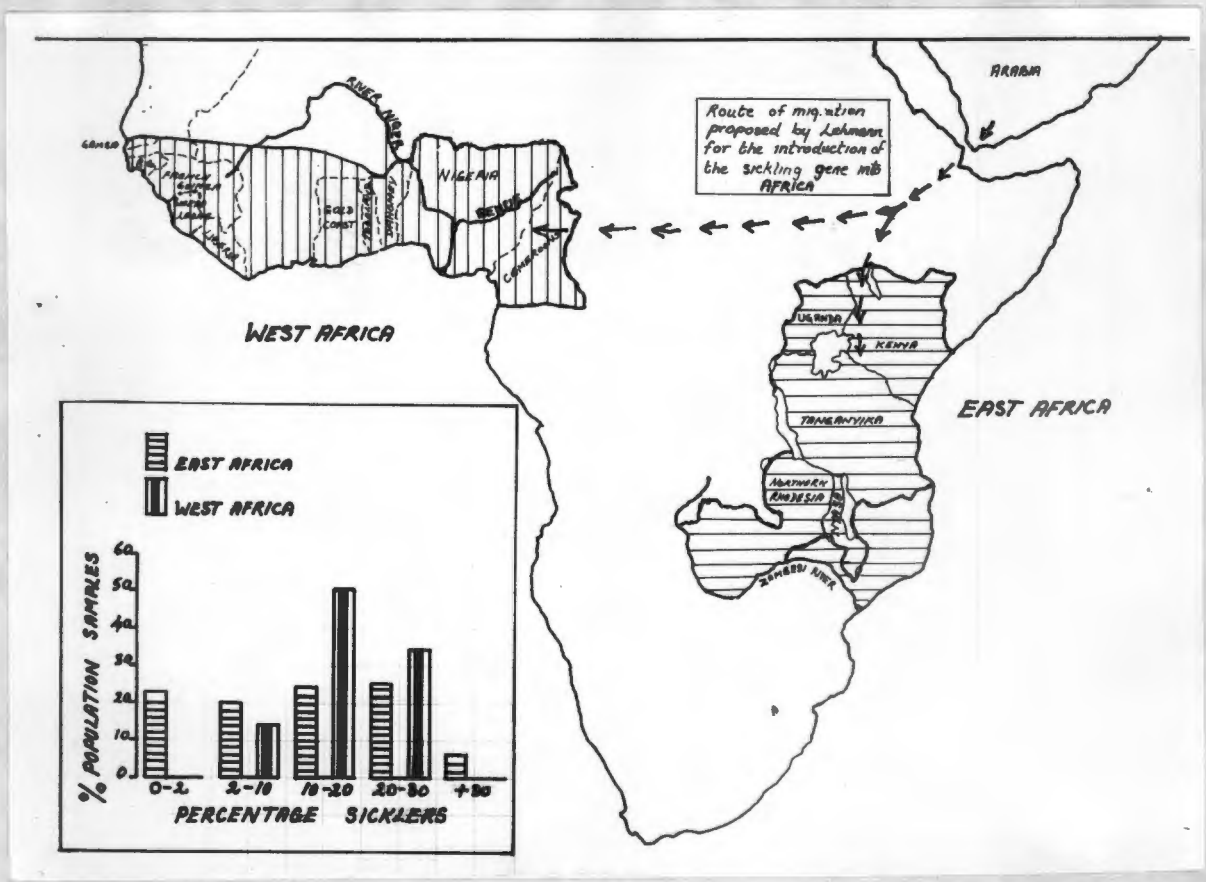


Figure 1: A comparison of sickling frequencies in populations in East and West Africa. The shaded areas on the map indicate the territories compared. The inset shows the percentage of the population samples with various sickling frequencies in each territory. Note that more than 80 per cent of West African samples show frequencies between 10 and 30 per cent, whereas the East African samples show a very much wider frequency range.

total sample) in the two territories.

The material presented in part two of this thesis was collected in Ibadan, which lies in the Western region of Nigeria. Reports show that about 20 per cent or more of the population in this region sickle (Evans 1944, Humphreys 1952, Jelliffe and Humphreys 1952, Walters and Lehmann 1956). Recent work has confirmed the earlier report of Jelliffe and Humphreys (1952) that approximately 25 per cent of the inhabitants of Ibadan show sickling (Garlick 1956).

The Haemoglobin C Trait

Table VI summarises the incidence and distribution of haemoglobin C in Africa. It will be seen that the trait has been found in the West African territories of the Gold Coast, Nigeria, Sierra Leone and the Gambia, but in none of the East African territories on which reports are available. The highest incidence has been recorded in the Northern region of the Gold Coast, where Edington and Lehmann (1956) found 20.1 per cent of 283 persons examined exhibited the trait. In the rest of the Gold Coast approximately 10 per cent of the population show the trait. Reports indicate that there is a decreasing gradient of frequency in West Africa, in all directions, as one moves away from the Gold Coast. In Nigeria Walters and Lehmann (1956) found in 944 Yorubas examined, living West of the River Niger, an incidence of 6.1 per cent, while among the Igala living only 400 miles away, but East of the river, the trait was absent. Figure 2

TABLE VI - INCIDENCE AND DISTRIBUTION OF HAEMOGLOBIN C IN AFRICA

Place	Author	Number Examined	Percentage Hb.-C
West Africa			
Gold Coast	Edington & Lehmann (1954)	200	10.5
Gold Coast (South)	Edington & Lehmann (1956)	183	8.7
Gold Coast (North)	Edington & Lehmann (1956)	283	20.1
Gold Coast	Allison (1956)	1042	10.9
Nigeria	Allison (1956)	247	5.3
Nigeria (Yoruba)	Walters & Lehmann (1956)	944	6.1
Nigeria (Igala)	Walters & Lehmann (1956)	155	0
Sierra Leone	Allison (1956)	218	4.6
Gambia (adults)	Allison (1956)	1442	1.8
Gambia (children)	Allison (1956)	446	0.9
South Africa (Cape Coloured)	Brain (1955)	209	1.9
Sudan	Roberts & Lehmann (1955)	75	0
Tanganyika	Roberts & Lehmann (1955)	100	0
Kenya	Allison (1956)	73	0
Uganda	Jacob (1955)	500	0
Congo	Charles (quoted by Allison 1956)	"hundreds"	0 in indigenous population. One case in a native of Angola.

Haemoglobin C has also been found in Algeria by Portier et al (quoted by Walters & Lehmann 1956).

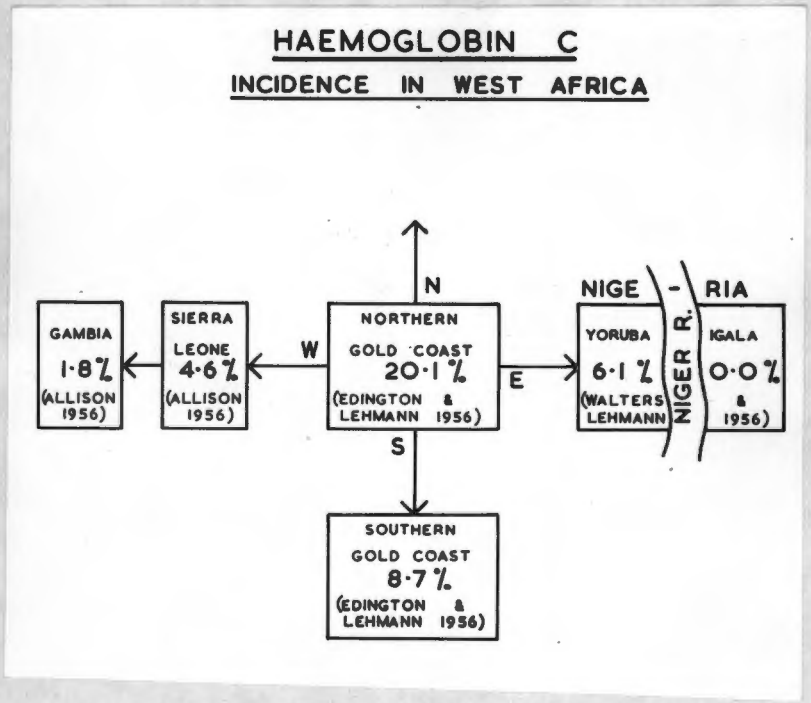


Figure 2: Showing the decline in the incidence of Haemoglobin C as one moves away from the Northern Gold Coast.

illustrates the apparent frequency gradient in West Africa.

The interpretation of these observations must await further investigation, but it seems likely, at least, that the explanation may be that the haemoglobin C gene arose somewhere in the region of the Gold Coast and has disseminated from there. The occurrence of haemoglobin C in South Africa (Brain 1955) and Algeria (Fortier et al quoted by Walters and Lehmann 1956) can easily be accounted for by the importation of slaves from West Africa into these areas. It is known that the first Governor at the Cape, Jan van Riebeeck, imported about 200 slaves from West Africa in the seventeenth century (Allison 1956).

It has been suggested that the haemoglobin C gene might have arisen by a mutation of the sickling gene (Neurant 1954), and Walters and Lehmann (1956) have suggested that the former might confer a better selective advantage (see "balanced polymorphism") and thus be destined ultimately to replace the latter. These suggestions are highly conjectural and their evaluation will have to await much further investigation, but it is interesting to note that Edington and Lehmann (1956) have found an apparent inverse relationship of the genes in two groups investigated in the Gold Coast. Comparing Northern and Southern tribes they found that while the <u>total</u> abnormal haemoglobin incidence (i.e. S plus C) was approximately equal in the two groups, the former showed a higher incidence of haemoglobin C than S, while the

reverse obtained in the latter. Table VII summarises their findings.

TABLE VII

Area	Total Number	Per cent. Abnormal Haemoglobin	S	C
Southern	183	28.4	19.1	9.8
Northern	283	27.6	6.7	21.5

PART TWO

THE CLINICAL MANIFESTATIONS OF SICKLE CELL ANAEMIA, SICKLE CELL-HAEMOGLOBIN C DISEASE AND HAEMOGLOBIN C DISEASE IN NIGERIAN CHILDREN.

Introduction

This section deals with the detailed clinical and haematological study of 75 cases of sickle cell anaemia, 8 cases of sickle cell-haemoglobin C disease, and one case of haemoglobin C disease, seen in the children's department of the University College Hospital in Ibadan, Nigeria. Our observations are compared with similar studies reported from the U.S.A. and certain African territories, in particular the studies of J. & C. Lambotte-Legrand (1951, 1955a and b) from the Belgian Congo, and those of Edington (1953) and Edington and Lehmann (1954) from the Gold Coast.

These observations were made during a period when the clinical departments of the University College, Ibadan, were in temporary premises at the Adeoyo Hospital, Ibadan, and the children's ward had only 37 cots available to cope with all admissions, which in 1956 totalled about 2,000. Admissions were therefore restricted to those cases urgently in need of special care which could not be given at the outpatient or clinic level, and the patients admitted had to be discharged at the earliest possible moment. These conditions, coupled with the heavy outpatient attendance, over 200 daily, limited the scope of investigation and the time available for full documentation of cases.

However, repeated observations on the same patients over a period of time made possible a clearer appreciation of the manifestations of the disease than would have been achieved from a study of admission records only.

Clinical Material

The 75 cases of sickle cell anaemia presented represent the majority of, but not all, the cases of this disease that have been seen in the children's department. Selection has been based on

(1) the certainty with which diagnosis had been established (68 cases having been confirmed by filter paper electrophoresis of the haemoglobin) and

(2) the availability of adequate records.

40 patients were admitted for treatment two or more times, and collectively they represent 59 admissions. The 35 cases which were not admitted were seen in the outpatient department or at a special clinic to which cases of sickle cell anaemia were referred. The majority were seen repeatedly over a period of several months, and in 11 cases there was continuous follow up over a period of more than one year. Table VIII shows the patients grouped according to where they were examined, the total number of observations on each group, and the total number of observations on all patients.

TABLE VIII - SHOWING THE NUMBER OF OBSERVATIONS RECORDED ON THE CASES OF SICKLE CELL ANAEMIA REPORTED HERE.

Where examined	Number of cases	Number of observations
Outpatient department	14	41
Ward (admissions)	40	59
Clinic only	21	190
Clinic follow up on 30 patients discharged from the ward	(30)	315
Total	75	605

(For the purposes of this table each admission, irrespective of its duration, has been classified as "one observation". The actual number of observations is much larger than indicated, e.g. Case 21, which is represented as one observation was an inpatient for a continuous period of 14 months, during which many examinations were made.)

The 8 cases of sickle cell-haemoglobin C disease and 1 case of homozygous haemoglobin C disease are the only examples of these conditions which we have recognised to date, and in every case diagnosis has been confirmed by filter paper electrophoresis of the haemoglobin. These individual case histories will be presented in full.

Terminology

The term sickle cell disease is currently used to denote all of the disorders in which manifestations of disease seem attributable to the presence of sickle cell haemoglobin, whether the patient is

homozygous for this abnormal pigment as in sickle cell anaemia or heterozygous for the S gene and a gene determining an unrelated red cell abnormality as in sickle cell-haemoglobin C disease.

The sickle cell trait is almost universally regarded as a completely benign abnormality of the red cells unassociated with anaemia or other manifestations of disease. Reinvestigation of some of the cases of sickle cell trait reported with pathologic manifestations has shown that they were heterozygotes for the S gene and a gene determining some other red cell abnormality and thus examples of sickle cell disease and not the trait. (Smith and Conley 1954).

The term "crisis" is used to describe characteristic episodes of fever, pain and other symptoms which occur in association with sicklaemia, irrespective of whether these are associated with increased haemolysis or not.

Laboratory methods used

Haemoglobins were usually estimated using Keeler N.R.C. Grey Wedge Photometers, but some estimations were made with Sahli haemoglobinometers. Thin blood films were stained with Leishman's and thick films (for malarial parasites) with Field's stain. Supravital preparations stained with brilliant cresyl blue (dried alcoholic solutions) were made for reticulocyte determinations (Whitby and Britton 1953). Sickling was tested for by Emmel's technique and

also using a freshly prepared 2 per cent solution of sodium metabisulphite. Filter paper electrophoresis of the haemoglobin was performed by the technique of Smith and Conley (1953). Serum protein estimations were performed by the Biuret method using a photo-electric colorimeter. Liver function tests included thymol turbidity, zinc sulphate turbidity, serum alkaline phosphatase (King-Armstrong), and serum bilirubin, all performed by standard techniques.

CLINICAL FEATURES OF SICKLE CELL ANAEMIA

Race - All our patients were Negroes. The majority belonged to the Yoruba race which predominates in the Western region of Nigeria. There were a few Ibos (Eastern Nigeria) and Hausas (Northern Nigeria).

Sex - There were 33 males and 42 females. It has been suggested that sickle cell anaemia is commoner in females (Diamond 1945), but Table IX, which gives the sex incidence of cases reported by some authors, shows that there are no grounds for this suggestion.

TABLE IX - SEX INCIDENCE OF SICKLE CELL ANAEMIA REPORTED BY VARIOUS AUTHORS.

Authors	Number of Cases	Males	Females
Lambotte-Legrande (1955b)	300	145	155
Scott et al (1951)	37	27	10
Scott et al (1955)	63	39	24
Grover (1947)	48	24	24
Edington (1953)	20	17	3
Our cases	75	33	42
Total	543	285	258

Age at Diagnosis - The age distribution of our cases at diagnosis is shown in figure 3. It will be seen that about 40 per cent were diagnosed during the first 2 years of life and that the percentage in each 2 year age group decreased as the age increased. The average age at diagnosis was 3.8 years. J. & C. Lambotte-Legrand (1955b) record the average age at diagnosis in 300 cases seen in Leopoldville in the Belgian Congo as 1.4 years (1 year 5 months), while Scott et al (1955) found it to be 4.7 years in 63 American Negroes. The reports of the former authors are unique for the exceptionally high incidence of cases diagnosed during the first 18 months of life.

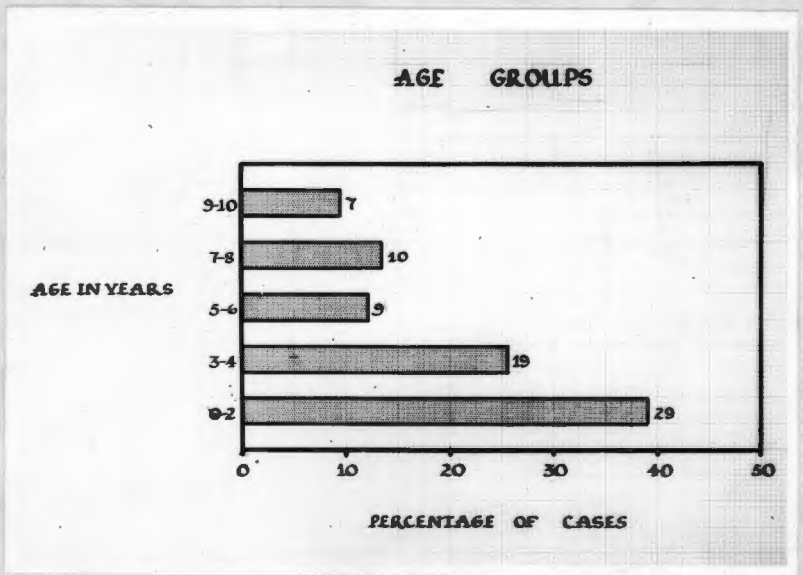


Fig.3 - Showing percentage of patients in 2 year age groups from birth to 10 years. The figure at the end of each column represents the number of patients in each group.

Figure 4 compares the age at diagnosis in the Lambotte-Legrande series with our own cases and shows clearly that in the former the disease was predominantly a problem of infancy, while in the latter, although cases occurred in infancy, the emphasis was on early childhood.

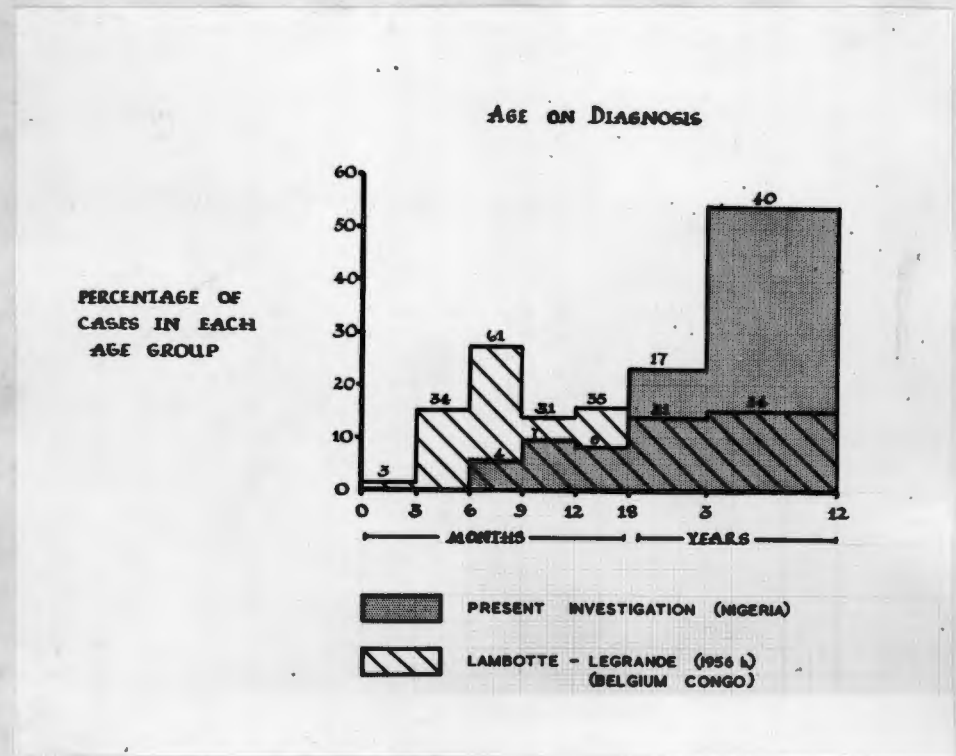


Fig.4 - A comparison of the age on diagnosis in our series with that reported by J. & C. Lambotte-Legrande (1955b) in the Belgian Congo. Note the peak incidence between 6 and 9 months in the latter series.

Age at Onset of Symptoms - It was often difficult to decide from the histories at what age manifestations of sickle cell anaemia first appeared. In 25 cases no decision was possible. The ages at onset of symptoms in the remaining 50 cases, along with the ages on first

attendance at hospital (taken as age at diagnosis) are shown in Table X. Included for comparison are figures given for 37 American Negroes (Scott et al 1951).

TABLE X - A COMPARISON OF THE AGES AT ONSET OF SYMPTOMS WITH THE AGES ON FIRST ATTENDANCE AT HOSPITAL IN 50 OF OUR CASES AND 37 CASES REPORTED FROM THE U.S.A. (SCOTT ET AL 1951).

Age	U.S.A.		NIGERIA	
	On first admission No. of cases	At onset of symptoms No. of cases	On first admission No. of cases	At onset of symptoms No. of cases
Birth to 1 yr.	6	10	11	25
1 to 2 yrs.	2	4	13	7
2 to 3 yrs.	6	3	6	5
3 to 5 yrs.	9	9	9	5
5 to 10 yrs.	9	6	11	8
10 to 12 yrs.	5	5	0	0

It will be seen that in general symptoms started earlier in the Nigerian children. In both series it is obvious that quite a long period must have elapsed between onset of symptoms and first attendance in hospital in many cases, but the "latent" period appears to have been shorter in the Nigerian cases. Reports from the Belgian Congo (Lambotte-Legrande 1951, 1955a and b) indicate that symptoms nearly always start in infancy, and in the majority attendance at hospital follows shortly afterwards.

The age at which patients present for medical treatment must obviously be largely determined by the severity of their symptoms. It is probable that factors such as poor socio-economic conditions, tropical infections, malnutrition and bad sanitation, which are prevalent in tropical Africa, affect patients in such a way as to accelerate the development of the disease and result in treatment being required at an earlier age than in the U.S.A. Differences in the age incidence in the Belgian Congo and Nigeria cannot, however, be readily explained by environmental factors, as conditions are similar in the two territories. It has been suggested that malaria may act as a "trigger" factor in precipitating crises of sickle cell anaemia (Edington 1953). Bruce-Chwatt (1952) showed that in infants in a hyperendemic malarious area the mean parasite rate rises from about 2 per cent at 3 months of age to 80 per cent at one year. Reference to figure 4 will show that in the Belgian Congo more children present with sickle cell anaemia between the ages of 3 months and 1 year than at any other period. In spite of the comment made by the Lambotte-Legrandes (1951) that malaria appeared to be unimportant in their cases, the similarity of the age at which their cases presented and the age of first infection with malaria reported by Bruce-Chwatt suggests that malaria should be considered as a possible factor in determining the age at which sickle cell anaemia presents in the Belgian Congo. Another possible explanation of these age relationships is that some of their cases were examples of primary malarial anaemia in persons with the sickle cell trait. It has been found that the

clinical and haematological findings in infants with malarial anaemia (with or without the sickle cell trait) can closely resemble those encountered in sickle cell anaemia (Hendrickse and King 1957). None of the Lambotte-Legrande series were confirmed by electrophoretic studies of the haemoglobin.

SYMPTOMATOLOGY

Medical histories in primitive societies, especially in paediatric practice, are notoriously unreliable. The average Nigerian mother is not a very good witness; her story is usually influenced by preconceived notions of the relative importance of symptoms. Visible evidence of disease, such as swelling or deformity and gastro-intestinal disturbances, are more seriously regarded than most other symptoms. For example, a child with tuberculous spinal disease will seldom be brought for treatment during the period when pain is the dominant symptom, but the occurrence of deformity or interference with locomotion will usually precipitate a visit to the doctor. The history given will generally only include "swelling of the back" or "not walking" and pain may not be mentioned at all, or may even be denied on direct questioning. When questioned many mothers will admit that the child has had symptoms that they think will please the doctor and deny that it has had others that seem to imply negligence on their part, without regard for the truth. These facts should be borne in mind when considering the symptomatology described in the following section.

Main Symptoms

The complaints most frequently encountered were fever, pain in the limbs, anorexia and vomiting, swelling of the limbs, abdominal swelling, abdominal pain and diarrhoea. The relative frequency of these symptoms is shown in figure 5, and their frequency in relation to age in figure 6. Table XI compares our findings in children with those reported by some authors in Africa and the U.S.A. in children and adults.

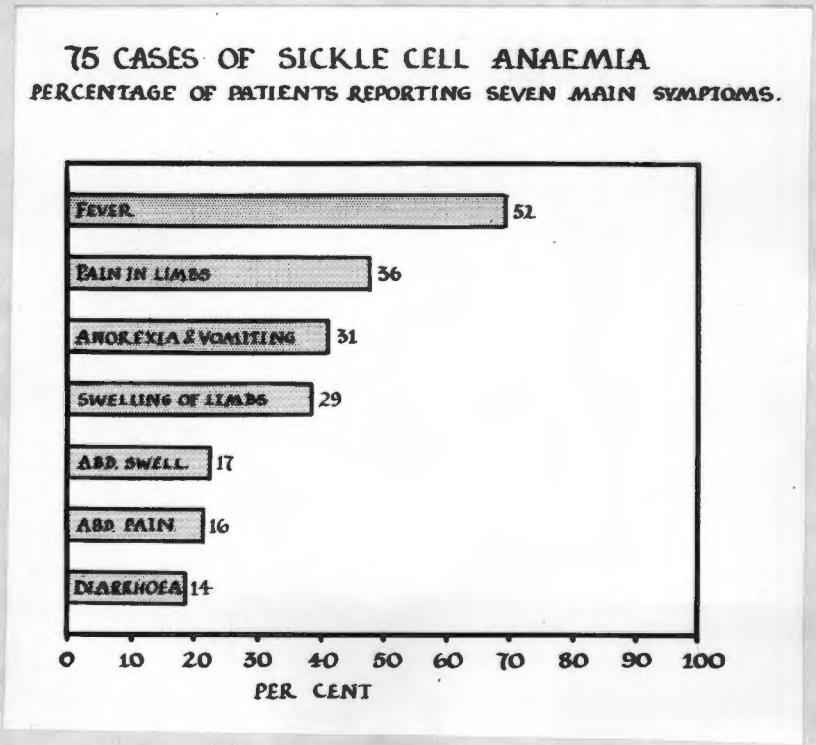


Fig. 5 - The figures at the end of the columns indicate the number of patients reporting these symptoms.

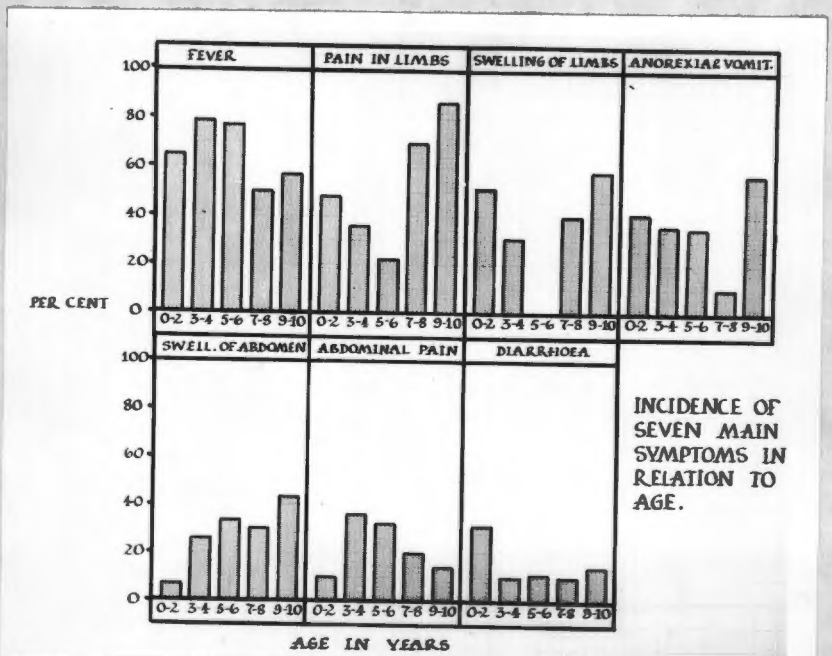

FEVER
PAIN IN LIMBS
SWELLING OF LIMBS
ANOREXIA & VOMIT.
SWELL. OF ABDOMEN
ABDOMINAL PAIN
DIARRHOEA
PER CENT
100
80
60
40
20
0
0-2 3-4 5-6 7-8 9-10
AGE IN YEARS
INCIDENCE OF SEVEN MAIN SYMPTOMS IN RELATION TO AGE.

Fig. 6

TABLE XI - SHOWING MAIN SYMPTOMS, LISTED IN ORDER OF FREQUENCY, REPORTED BY VARIOUS AUTHORS IN AFRICA AND THE U.S.A. IN CHILDREN AND ADULTS.

Place	AFRICA		U.S.A.	
Authors	Present series (75 children)	Edington (1953) (20 older children and adults)	Scott et al (1955) (63 children)	Grover (1947) (48 adults)
Symptoms	Fever	Joint pains	Generalised pain	Limb pains
	Limb pains	Fever	Fever	Abdominal pain
	Anorexia and vomiting	Nausea	Joint pain	Leg ulcers
	Limb swelling	Headache	Abdominal pain	"Colds"
	Abdominal swelling	Abdominal pain	Upper respiratory infection	"Neuro-psychiatric"
	Abdominal pain	Limb pain	Anorexia	Weakness
	Diarrhoea	Chest and lumbar pain		Abdominal swelling

Note.- J. & C. Lambotte-Legrande (1951) did not indicate the relative frequency of the main symptoms in their cases but they laid particular emphasis on limb pain and osteo-articular swellings in the 88 cases they reviewed.

Fever - In our cases fever occurred more frequently in the lower age groups. Scott et al (1955) found that whereas the vast majority of children between the ages of 1 and 9 years had fever, only 33 per cent of patients over 13 years of age showed this symptom. The value of a

history of fever as an aid to diagnosis is negligible in our cases as almost all patients attending the paediatric outpatient department in Ibadan will give a history of fever.

Pain in the limbs - All reports on sickle cell anaemia emphasise limb pain as a common presenting symptom. The intensity of the pain varies considerably from patient to patient and also at different times in the same patient. Sometimes it amounts to little more than vague discomfort or a mild arthralgia, while on other occasions the intensity is so great as to completely incapacitate the patient. Pain may be generalised or localised to particular limbs or specific joints. Not infrequently pain commences in one site, subsides there and then appears elsewhere in a manner reminiscent of the "flitting" pains of acute rheumatic fever. In general, however, unlike in rheumatic fever, pain is seldom confined to joints, but tends rather to be distributed in relation to bones.

In our series limb pain increased in frequency in the latter half of the first decade. This may, however, simply be a reflection of the greater ability of the older children to make their symptoms known.

Swelling of the limbs - This complaint was encountered in 29 of our patients. The swelling was not diffuse, but tended to be localised to the dorsum of the feet, the proximal parts of the fingers, and around joints, and the distribution was often symmetrical. This

finding will be described in more detail later. Apart from the Lambotte-Legrandes (1951), who first observed and stressed the importance of this manifestation of sickle cell anaemia, no other authors have reported this symptom as a prominent feature of the disease.

Anorexia and vomiting - Anorexia with or without vomiting was complained of by 41 per cent of our patients. Usually it occurred in association with the crises of the disease, and often it preceded the onset of other symptoms. Scott et al (1955) states: "We have observed that anorexia, even in the absence of demonstrable infection, is often the forerunner of crisis episodes and may precede by days or weeks the more dramatic onset of pain". Our experience has been that anorexia may persist after all other symptoms of a crisis have subsided, and while it lasts there is always the possibility of a relapse.

Abdominal swelling - Very few children below the age of 2 years presented with this symptom, but over the age of 5 years a third of all patients complained of abdominal distension. Often the parents noted that swelling was associated with splenomegaly. In some of these cases it was obvious that the "swelling" disturbed the parents more than it did the child. As a race the Yorubas are very "belly conscious" and complaints such as "swelling of the abdomen" are frequently met with in children enjoying reasonably good health. However, most reports indicate that abdominal distension, with or without visceromegaly, is a feature of the disease.

Abdominal pain - Abdominal pain occurred most frequently between the ages of 3 and 6 years, after which it declined in frequency. The severity of the pain varied from vague discomfort to severe episodes simulating the surgical "acute abdomen". Pain was sometimes localised to the liver or spleen. Scott et al (1955) found abdominal pain to be a feature of the symptomatology in 75 per cent of their cases which presented between the ages of 2 and 8 years.

Diarrhoea - Diarrhoea occurred in 18 per cent of our patients, the heaviest incidence being in the first 2 years of life. This finding reflects the high incidence of diarrhoeal disorders in the child population generally rather than any special tendency to diarrhoea in sicklaemics.

Symptoms referable to the central nervous system - A number of patients complained of various symptoms referable to the central nervous system. These were:-

1. Disturbances of consciousness (which included convulsions in 4 patients) and other episodes described as "fainting" or "collapse".
2. Disorders of sleep. 8 patients complained of somnolence and 3 of insomnia. It is probable that the latter symptom was caused by restlessness due to pain rather than by a central effect.
3. Headache.

"Weakness" - Grover (1947) lists weakness among the six main symptoms presented by 48 adults with sickle cell anaemia. This complaint was present in 12 of our cases. In some cases the apparent weakness was

simply a disinclination to use limbs that were painful. In others there was a real loss of strength. In young children who had "gone off their legs" it was often difficult to decide whether pain or weakness had caused them to stop walking.

Other symptoms - Other symptoms occasionally encountered were irritability, tiredness, epistaxis, bleeding gums, "dark urine" and various respiratory symptoms.

Family Histories

Satisfactory family histories were recorded in 44 cases representing 41 families, there being 3 families each with 2 children included in the series. Of the 145 children born into these families 29 had died. The causes of death were not known in the majority, but in 4 cases it seemed very likely that the deceased had had sickle cell anaemia. Among the 116 living there were 48 with sickle cell anaemia, 4 of whom are not included in the present review. The remaining 68 children were reported to be in good health. Very few parents were examined for sickling, but all those who were showed the trait. In only 3 cases were both parents examined.

MAIN PHYSICAL SIGNS.

Pallor - As judged by the colour of the mucous membranes on the inside of the lips, the tongue, gums and conjunctivae, all our patients showed pallor of greater or lesser degree. In the younger age groups

paller of the palms and soles was often very striking. Assessment of the degree of anaemia by the appearance of pallor was misleading. It is well known that at all ages, but particularly in childhood, pallor resulting from vasomotor disturbance can simulate anaemia, and conversely that local hyperaemia can effectively mask the milder grades of anaemia.

Our experience with six of the other main physical signs is shown in figure 7 and the age relations of these findings are shown in figure 8. In order of frequency the commonest physical signs encountered were splenomegaly, hepatomegaly, jaundice, limb swellings, cardiac signs and respiratory infection.

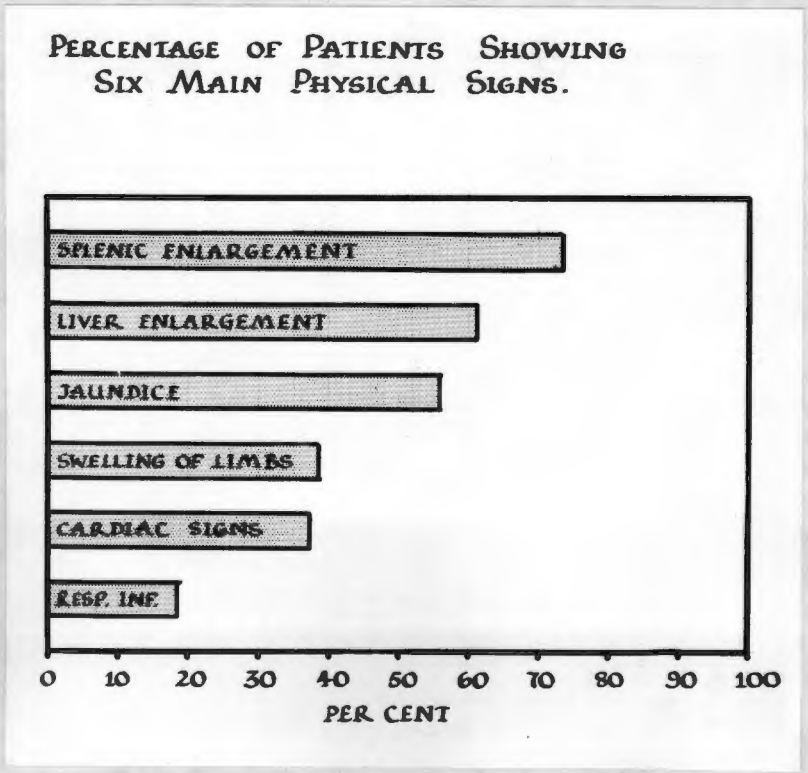


Figure 7.

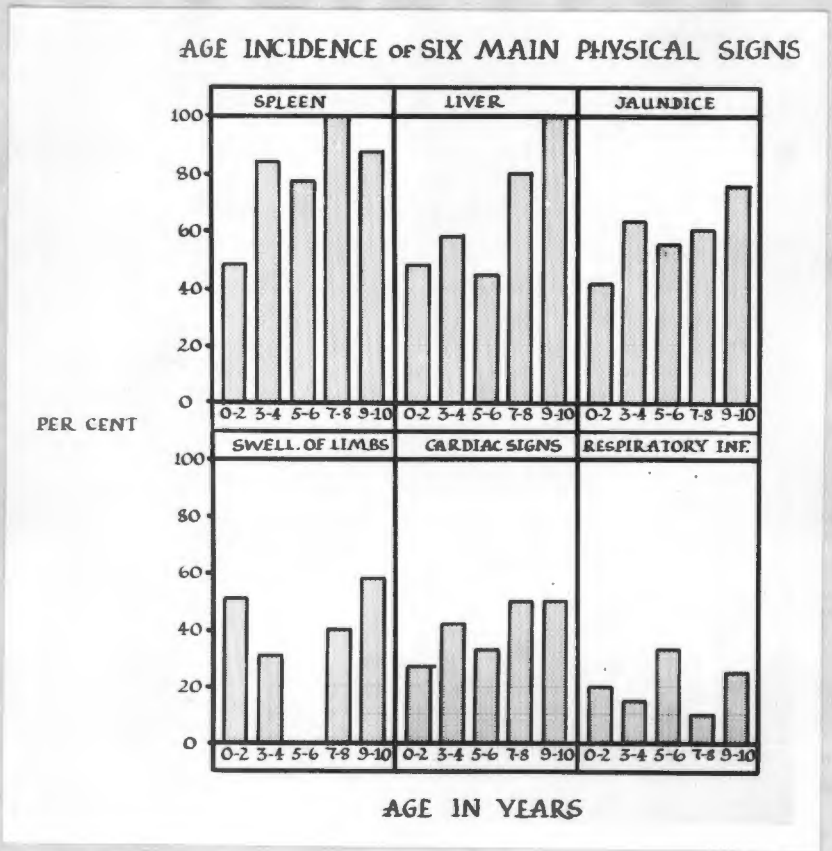


Figure 8

Splenomegaly - 73 per cent of our cases had enlarged spleens. Below the age of 3 the incidence was only 50 per cent but over this age it was more than 80 per cent. Scott et al (1955) found that after 6 years of age the spleen rate declined in their series and Smith and Conley (1954) report: "The spleen in patients with sickle cell anaemia is seldom palpably enlarged past the first few years of life and has never been found enlarged after the age of 10 in any of our group of patients with the disease". Their experience contrasts sharply with our own and that of Edington (1953), who found that 55 per cent of his cases on the Gold Coast, the majority over the age of 10, had splenomegaly. It is

probable that this difference is related to the hyperendemicity of malaria on the Gold Coast and Nigeria, but other factors may be involved.

The spleen size varied from "just palpable" to massive organs that extended to below the umbilicus. This range was encountered in all age groups but most of the very big spleens were found between the ages of 3 and 8 years. Table XII shows the spleen size in relation to age. The spleen size varied in the same patient from time to time. A remarkable feature was the rapidity with which alterations in size occurred in some patients.

TABLE XII - SPLEEN SIZE IN RELATION TO AGE IN SICKLE CELL ANAEMIA IN NIGERIAN CHILDREN.

Spleen size	Age Groups (Years)				
*	Birth to 2 (29)	3 to 4 (19)	5 to 6 (9)	7 to 8 (10)	9 to 10 (7)
Not palpable	15	3	2	0	1
Less than 2 finger-breadths	9	6	4	2	4
2 to 4 finger-breadths	2	3	1	1	0
More than 4 finger-breadths	3	7	2	7	2

* The figures in brackets indicate the total numbers of cases in each age group.

Hepatomegaly - The liver was enlarged in 61 per cent of our patients, but as shown in figure 8 the incidence varied from 48 per cent under 2 years of age to 100 per cent between the ages of 9 and 10. Scott et al (1955) found hepatomegaly in only about 40 per cent of their cases, with a peak incidence between 3 and 9 years. Livers showed marked variations in size from time to time, but there was no constant relationship between this finding and any other feature of the disease. In general, the size increased with age.

Jaundice - Jaundice can easily be overlooked in darkskinned people. A brownish discolouration of the sclera and orange-yellow discolouration of the palms of the hands caused by palm oil, a constituent of the staple diet of Nigerians, is frequently encountered in our practice. These factors combined to make the detection of jaundice very difficult, but in 56 per cent of our cases it was unmistakably present. (See section on liver function tests for bilirubin levels). The incidence of jaundice seemed to increase with age. Scott et al (1955) report a similar finding but the overall incidence of jaundice in their series was lower than in ours.

Limb swellings - Included in this category are all peripheral manifestations of sickle cell anaemia associated with swelling, but excluding oedema from general causes. 38.6 per cent of our cases showed limb swellings of one type or another. Below the age of 2 and above the age of 8 more than 50 per cent of patients were affected, but as shown in figure 9 the anatomical distribution of the swellings varied with age. The characteristic lesion in the younger patients consisted

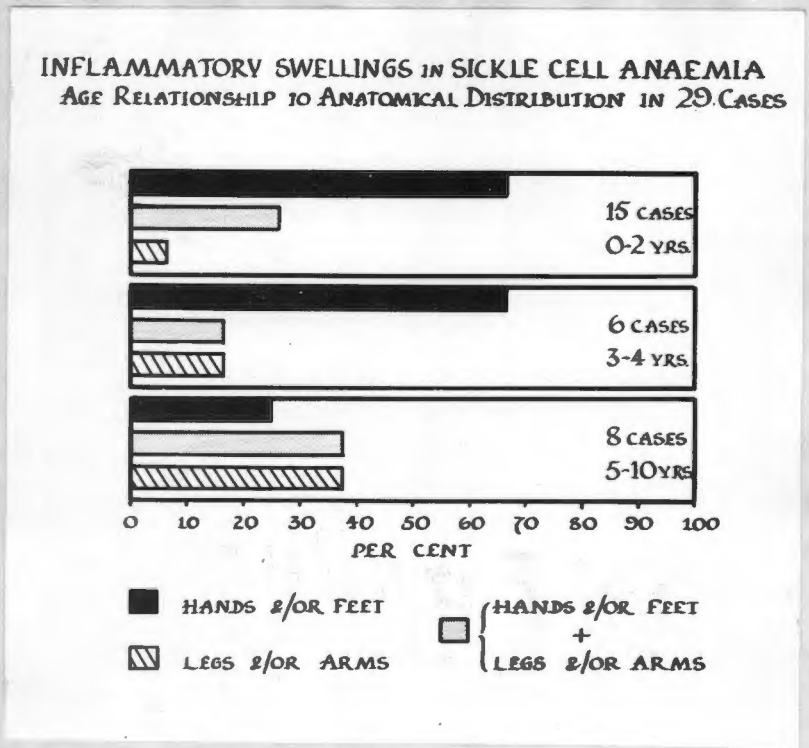


Figure 9

of warm, tender, brawny swelling affecting the proximal parts of the fingers and the dorsum of the feet over the shafts of the metatarsals. They were usually bilateral and often symmetrical. The appearances were often reminiscent of those seen in juvenile rheumatoid arthritis. Our findings agree with those reported by the Lambotte-Legrandes (1951), but whereas the thumbs were always spared in their series, in our series the thumbs were sometimes involved. The swellings usually subsided spontaneously after a variable period of days or weeks. In many swelling was associated with pathological changes in the underlying bones. Usually these were not detectable during the early stages, but showed up some time after the onset of the lesion. In a few

instances the swellings were associated with suppuration. (Further details of the radiological and bacteriological findings in these cases will be given later). Figures 10 and 11 show the typical appearances in the hands and feet respectively. In the older patients findings were more variable. Swellings sometimes involved isolated joints such as a knee or elbow, (see figure 19), or were present over long bones in relation to underlying bone pathology.

Fig.10: I.A., female, aged 1½ years (series No. 70). Hands, showing bilateral swellings affecting the proximal parts of the fingers. Suppuration occurred in this patient but culture of pus obtained by aspiration on two occasions grew no organisms. The tuberculin skin test and Kahn reaction were negative. X-rays showed destructive changes in the underlying bones.

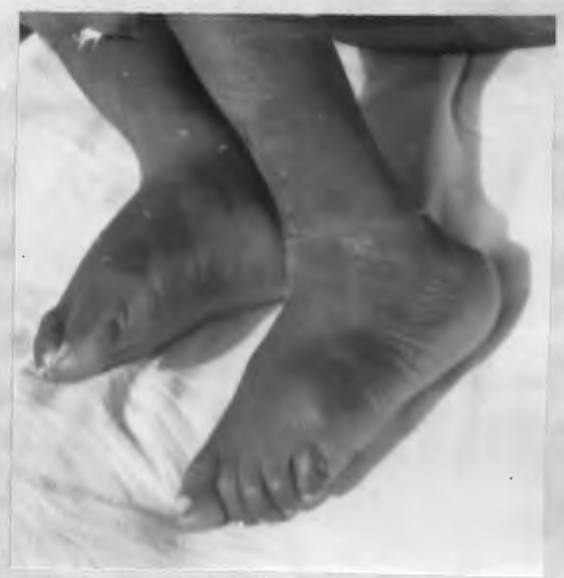


Fig.11: Feet (same case as Fig. 10) showing swellings situated over the metatarsals. No material could be aspirated from these sites but X-rays showed similar bone changes to those found in the hands (See Figs. 43 and 44).

The high incidence of swellings of the hands and feet recorded in the first two years of life in Africa, but not usually reported by American authors, may be related to a custom that is almost universal in tropical Africa. I refer to the manner in which African mothers carry their children. The usual practice is for the child to be placed astride the mother's back with its legs encircling her waist. The position is maintained by a cloth which envelops most of the child, excluding the head and neck, and is tied across the mother's chest and abdomen. In this position the child's limbs are pinioned to the mother and are subjected to steady pressure which probably slightly impairs the peripheral circulation. Such impairment, while of no consequence in normal children, might be sufficient in sickle cell anaemia to provoke intravascular sickling in the extremities, with resultant capillary blockage, tissue damage and reactionary swelling.

Cardiac signs - Abnormal cardiac signs were detected in 37 per cent of our cases and included cardiomegaly, murmurs and gallop rhythms. The incidence of cardiac involvement increased with age, 50 per cent of patients over 6 years being affected. Similar findings in children are reported by Scott et al (1955). Various authors, including Klinefelter (1942) and Smith and Conley (1954) report cardiac abnormality as a feature of almost all adult cases of sickle cell anaemia. In contrast, Edington (1953) found it in only 25 per cent of his cases on the Gold Coast. Lambotte-Legrande found cardiomegaly frequently in their series (mainly infants), but do not quote actual figures.

Respiratory Infection - Respiratory infection was present in 18.6 per cent of our cases. Signs varied from "scattered rhonchi" to evidence of pneumonic consolidation. Most American authors report pulmonary involvement as a frequent occurrence in sickle cell anaemia. Smith and Conley (1954) comment: "Patients with sickle cell anaemia not infrequently develop episodes of pneumonitis not specifically attributable to infection, in some instances probably resulting from pulmonary infarction".

Table XIII compares our main findings with those of Scott et al (1955) in 63 American children, and Edington (1953) in 20 older children and adults on the Gold Coast. The report of the Lambotte-Legrandes (1951) on 88 children in the Belgian Congo does not permit of a comparison of relative frequencies of the main physical signs

they encountered, but it is obvious that pallor, hepato-splenomegaly, osteo-articular swellings and cardiomegaly were their most frequent findings. In common with ours and in contrast with most American reports, lymphadenopathy was not a prominent feature of their cases.

TABLE XIII - PERCENTAGE OF PATIENTS SHOWING CERTAIN MAIN PHYSICAL SIGNS, LISTED IN ORDER OF FREQUENCY, FROM REPORTS BY VARIOUS AUTHORS.

Authors	Scott et al (1955)	Edington (1953)*	Present review
Place	U.S.A.	Gold Coast	Nigeria
Patients	63 children	20 older children and adults	75 children
Signs	Pallor 80%	Splenomegaly 55%	Pallor 100%
	Lymphadenopathy 60%	Jaundice 45%	Splenomegaly 73%
	Cardiac 58%	Hepatomegaly 40%	Hepatomegaly 61%
	Splenomegaly 47%	Leg ulcers 40% (including scars of healed ulcers)	Jaundice 56%
	Jaundice 45%	Lymphadenopathy 40%	Limb swellings 39%
	Hepatomegaly 42%	Cardiomegaly 25%	Cardiac signs 37%
			Respiratory Infection 19%

* Edington does not mention the frequency of pallor in his cases.

NUTRITION AND HABITUS

Nutrition - Reference to figure 12 will show that the majority of our patients were underweight; some grossly so when compared with standard weights laid down for English children (Sheldon 1954). The comparison, however, is not valid because of the vast social, economic, environmental and genetic differences in the two groups. Comparison with the weights of normal Nigerian children of similar ages would be more useful, but unfortunately this information is not at present available. Walters (1956) in a survey in Ilobi, a typical Yoruba village in Western Nigeria, found the average weight of normal children under 5 years old was about 20 per cent below that of English children and that over age 5 the weight deficit increased. If we compare our cases with this standard it becomes apparent that the majority fall within the "normal" weight range. Using weight alone as an index of nutrition, the majority of our patients would thus be considered to be reasonably well nourished. Assessment of nutrition by standards familiar to us in Ibadan agreed with the inference based on weight alone. As a group the older patients appeared to be less well nourished than the younger. A few patients were grossly malnourished and one showed evidence of kwashiorkor.

Habitus - Many authors, including Windsor and Burch (1945), Margolies (1951), Edington (1953), Smith and Conley (1954), have described a characteristic habitus in adults with sickle cell anaemia. The features

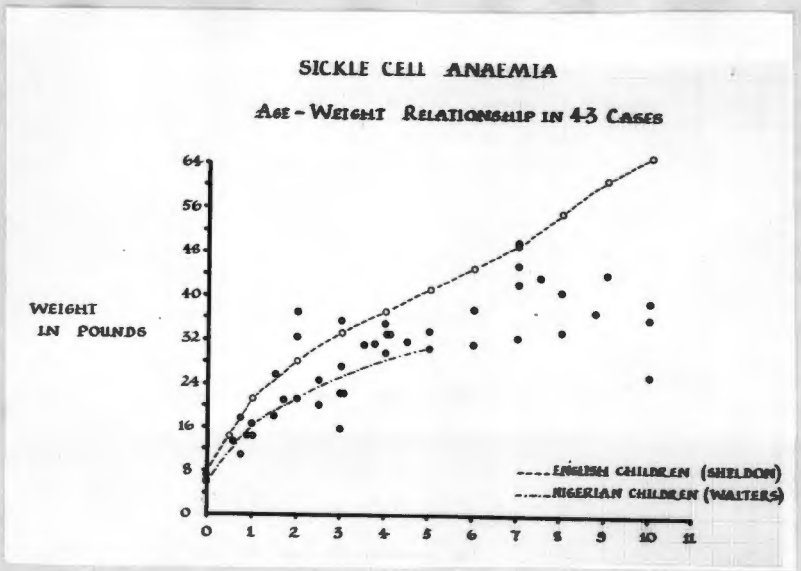


Figure 12.

described include:- narrow shoulders and hips with disproportionately long legs and short trunk; upper dorsal kyphosis and exaggerated lumbar lordosis; abdominal protrusion; relatively short neck; increased antero-posterior diameter of the chest ("hoop" chest); and long tapering extremities. These features, with the exception of abdominal protrusion, were not very noticeable in any of our patients below the age of 7, but some of the older ones showed an abnormal habitus similar to that described in adults. Windsor and Burch (1945) found that during the first decade of life the habitus often shows definite deviations from the normal, the more constant findings being "hoop chest", abdominal distension and thin legs. The features most commonly encountered by us were:-

1. Abdominal distension
2. Increase in the antero-posterior diameter of the chest ("hoop chest")

3. Thin legs, tending to be disproportionately long

4. Exaggeration of the normal spinal curvatures.

Figures 13 to 20 inclusive illustrate nutrition and habitus in some of our patients.

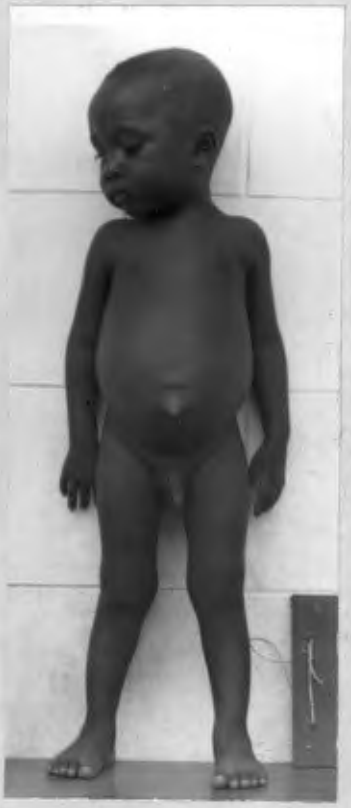


Figure 13

B.O. (series No. 72)
Age 2½ yrs.
Weight 23 lbs.
Showing:
1. Good general nutrition
2. Normal habitus except for abdominal distension.

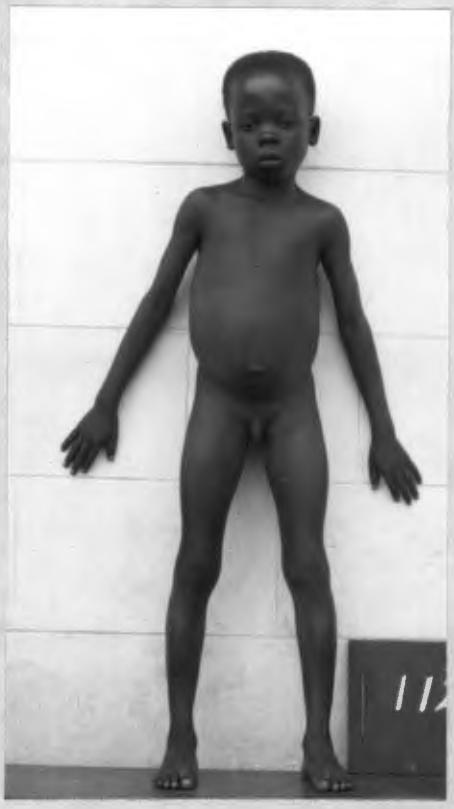
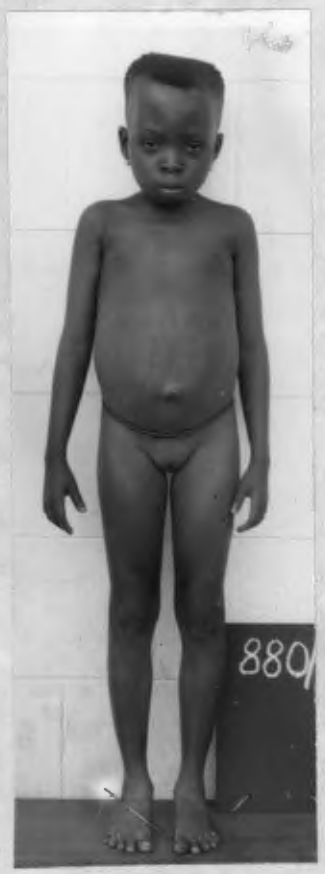
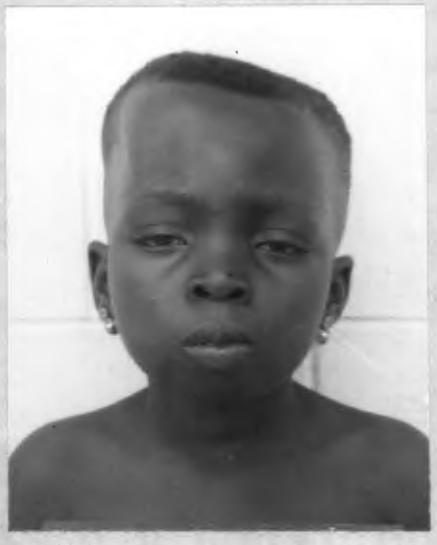


Figure 14

A.P. and Lat. views of L.D. (series No. 28).
Age 10 yrs. Weight 36 lbs. Showing:
1. Submutrition
2. Distended abdomen
3. Increased A.P. diameter of chest
4. Long thin limbs
5. Slightly exaggerated normal dorsal kyphosis and lumbar lordosis.
6. Slight frontal bossing

Figure 15





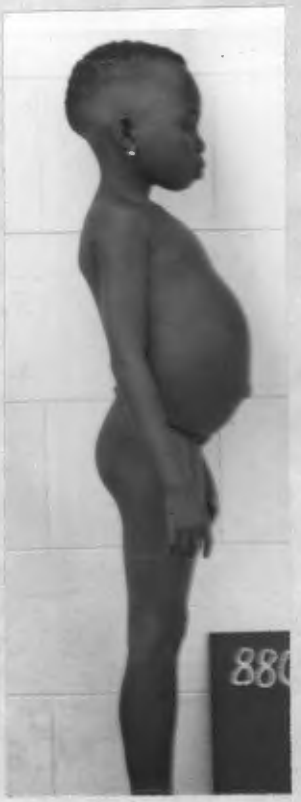


Figure 16 Figure 17 Figure 18

S.O. (series No. 61). Age 7 years. Weight 49 lbs. Showing:

1. Good general nutrition.
2. Distended abdomen
3. Increased antero-posterior diameter of chest
4. Exaggerated normal dorsal kyphosis and lumbar lordosis
5. Bossing of the skull, which presents the unusual feature of being markedly asymmetrical, affecting mainly the right frontal bone.

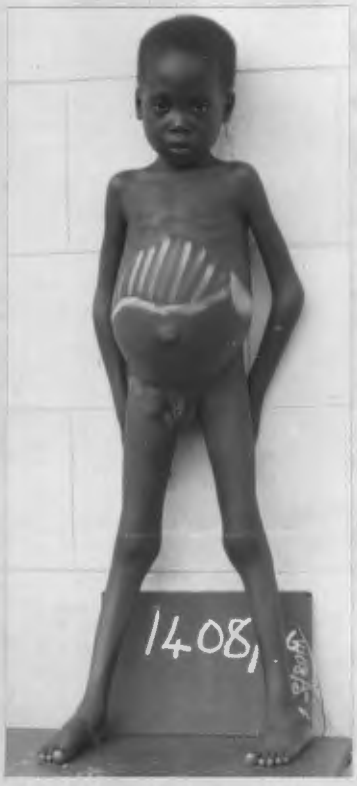


Figure 19

Figure 20

R.A. (series No. 71). Age 10 years. Weight 28 lbs. Showing:

1. Gross malnutrition
2. Distended abdomen associated with hepato-splenomegaly.
3. Increased antero-posterior diameter of chest
4. Disproportionately long limbs especially the legs.
5. Note also lymphadenopathy in right inguinal region and swelling of the left elbow.

The shape of the skull was normal in the majority but some patients showed bossing of the frontal bones. One patient presented the unusual feature of asymmetrical bossing with marked prominence of the right frontal bone (Fig. 17). Another unusual finding was that

of a typical "hot cross Bun" skull caused by symmetrical bossing of frontal and parietal bones.

Other Findings

Clubbing - Finger clubbing was noted in 4 patients, in none of whom did we find evidence of any of the diseases with which clubbing is usually associated. It was presumed that clubbing had occurred as a manifestation of sickle cell anaemia. Fig. 21 shows clubbing in one of our patients. I have not encountered reports of clubbing in the literature consulted, but during the course of a visit to our department, Col. J. Walters, former Director of M.R.C. research in West Africa, commented that he had found instances of clubbing in association with sickle cell anaemia.

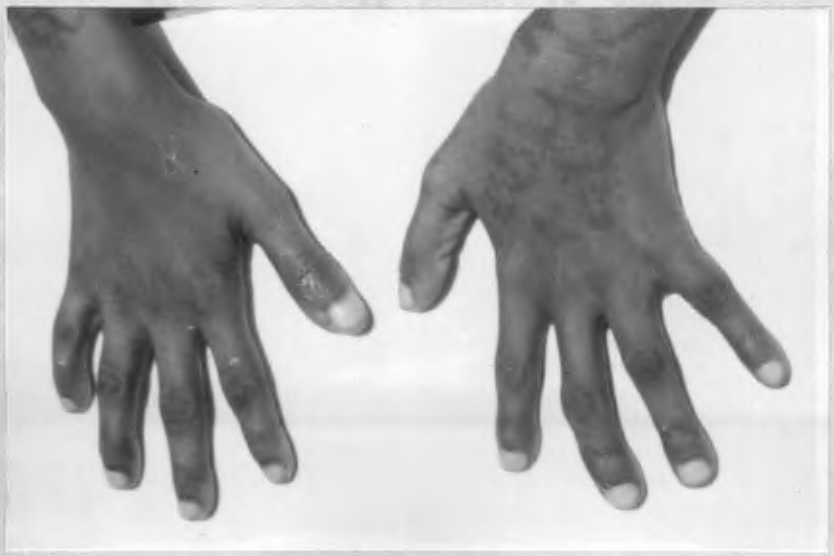


Fig. 21 F.O. Female aged 10 years. (No. 21) Clubbing of the fingers in sickle cell anaemia.

Leg Ulcers - Two patients had ulcers on their legs but in neither could they be regarded as specific manifestations of the disease. In one ulceration had occurred in association with guinea worm infestation and in the other the ulcer was an acute lesion, barely ¼ inch in diameter when first seen, which healed rapidly with no special treatment. Leg ulcers have not been reported as a feature of sickle cell anaemia in children (Scott et al 1951 and 1955, Lambotte-Legrande 1951), though they are commonly found in adults with the disease (Review by Margolies 1951).

Central Nervous System Involvement - Reference has already been made to disturbances of consciousness and sleep in the section on symptomatology. Our own observations confirmed their occurrence. Other signs of central nervous involvement were infrequent. One patient manifested psychotic behaviour during a period of crisis. She became confused, uncooperative and withdrawn, and from time to time indulged in fits of uncontrollable screaming and crying. She eventually recovered full command of her mental faculties. One patient developed purulent meningitis with hemiplegia, and another had pronounced meningism, unassociated with pathological changes in the cerebrospinal fluid. It is interesting to note that the Lambotte-Legrandes report haemophilus meningitis as a not uncommon terminal event in infants in their series. Hughes, Digge and Gillespie (1940), Arena (1939), and several other authors (cited by Margolies 1951) have reported the frequent occurrence of cerebral complications such as coma, hemiplegia, aphasia, convulsions

and meningism in sickle cell anaemia, but others, including Scott (1951 and 1955) and Edington (1953), have found cerebral complications to be infrequent.

Lymphadenopathy - Only one patient showed generalised lymphadenopathy. In 3 the glands were situated in the groins (See Fig. 19). In addition one patient had a mass of tuberculous glands in the right axilla and another had cervical adenopathy associated with a septic scalp lesion. Our findings are in striking contrast with most American reports, which tend to stress lymphadenopathy as a feature of the disease.

HAEMATOLOGICAL FINDINGS

Haemoglobin Level. The haemoglobin level on first examination varied from less than 3 g. per cent to more than 9 g. per cent. Table XIV shows the percentage of patients showing various haemoglobin levels on first examination.

TABLE XIV - HAEMOGLOBIN ON FIRST EXAMINATION IN 67 CASES OF SICKLE CELL ANAEMIA.

Haemoglobin g. per cent (14.8 g. equals 100%)		Percentage of patients
Less than 3	(20%)	3.0
3 to 4.4	(30%)	25.3
4.4 to 5.9	(40%)	22.4
5.9 to 7.4	(50%)	25.3
7.4 to 8.9	(60%)	18.0
8.9 to 9.6	(65%)	6.0

In general anaemia was more severe in the younger patients. Analysis of the figures shown in Table XIV in relation to age shows that whereas 66 per cent of cases under the age of 5 years had haemoglobin levels below 5.9 g. per cent (40%), only 33 per cent of the older children were in the same range. As the first visit was often precipitated by an exacerbation in the severity of the disease, it will be apparent that findings on first examination do not necessarily reflect the usual state of affairs in these patients. Our clinic follow up studies showed that in the majority the haemoglobin was maintained at between 7 and 8 g. per cent (45 to 55%) but some patients tended to maintain slightly lower levels. Sometimes values as low as 6 g. per cent were discovered in patients reporting for routine follow up who complained of no symptoms referable to anaemia per se and who showed no other evidence of exacerbation of the disease.

Patients varied in their ability to maintain their haemoglobin levels. The fact that 35 cases required no admission for transfusion indicates that in many there exists a satisfactory balance between haemolysis and erythrocyte replacement, with maintenance of the haemoglobin at a fairly constant level for long periods. Some patients, however, showed periodic falls in haemoglobin to levels which required to be corrected by transfusion. The rate of fall varied considerably; in some there was a gradual decline over a period of weeks, while in others it occurred within days.

There was no constant relationship between the degree of anaemia and other symptoms such as fever and limb pains. Many patients who were observed during crises of acute pain showed no appreciable increase in the severity of their anaemia. Conversely, exacerbation of anaemia was sometimes observed in patients who were relatively symptome free. The haemoglobin levels in case 72 shown below were recorded during a period when no specific complaints were made. Despite the rapid fall in haemoglobin level he appeared to be quite well, and was ambulant except when administration of blood transfusion necessitated his detention in bed.

The following haemoglobin levels of individual patients illustrate some of the points made above:-

1. A.J. (series No. 6) F. Age 10 years

Date	Hb. g. per cent	Comment
20.12.54	5.6	Amoebic dysentery. Treated with emetine. NOT transfused.
23. 1.55	8.0	
15. 2.55	8.1	
1. 3.55	7.7	
26. 4.55	8.6	
31. 5.55	6.1	Defaulted from clinic for 9 months
20. 2.56	6.8	
14. 3.56	9.6	Last visit

2. D.F. (series No. 44) M. Age 18 months.

Date	Hb. g. per cent	Comment
12.3.56	5.5	Transfused
13.3.56	11.8	Post-transfusion
12.4.56)	Hb. not recorded	
19.4.56)	but comment "no obvious pallor".	
23.6.56	4.14	Transfused
24.6.56	9.6	Post-transfusion
4.8.56	5.2	Transfused
5.8.56	10.35	Post-transfusion (last seen)

3. B.O. (series No. 72) M. Age 2½ years.

Date	Hb. g. per cent	Comment
12.10.56	4.8	Transfused
14.10.56	7.5	
22.10.56	8.1	
29.10.56	6.1	
1.11.56	4.4	Transfused
2.11.56	10.36	
6.11.56	7.4	
13.11.56	4.1	Transfused
15.11.56	11.2	

It will be seen that whereas (1) required no transfusion over a period of 16 months, (2) required transfusions at intervals of months or weeks, and (3) required three transfusions in one month, the second and third being only 12 days apart.

The Red Blood Cells

1. Appearances in stained films of peripheral blood - The red cells were normochromic or slightly hypochromic in the majority, but a few showed quite marked hypochromia. Anisocytosis and poikilocytosis and polychromasia were frequent findings. Target cells and nucleated

erythrocytes were present at some time or another in the majority, and in some the latter predominated in the stained film. Sickled cells were present on direct examination in 22 cases (29 per cent).

2. Appearances in sickled preparations - In specimens prepared by Emmel's (1917) technique or using 2 per cent sodium metabisulphite the erythrocytes showed many bizarre forms, but a constant feature was the appearance of long tapering filaments which projected from the sickled cells. These structures have been reported by most authors who have studied the erythrocytes in sickle cell anaemia, and are clearly depicted in drawings made by Emmel in 1917. They are currently thought to represent haemoglobin tactoids, which form when sickle cell haemoglobin is in the reduced state. (See Part One, page 16.) Figure 22 shows the red cell appearances in sickled preparations.

Fig. 22 - Red cell appearances in "sickled" preparations. Note the variability in shape and the long projecting filaments.

3. Reticulocytes - Figure 23 shows the degree of reticulocytosis in 30 patients on whom estimations were made. It will be seen that reticulocytosis, often of marked degree, was a feature of the blood picture.

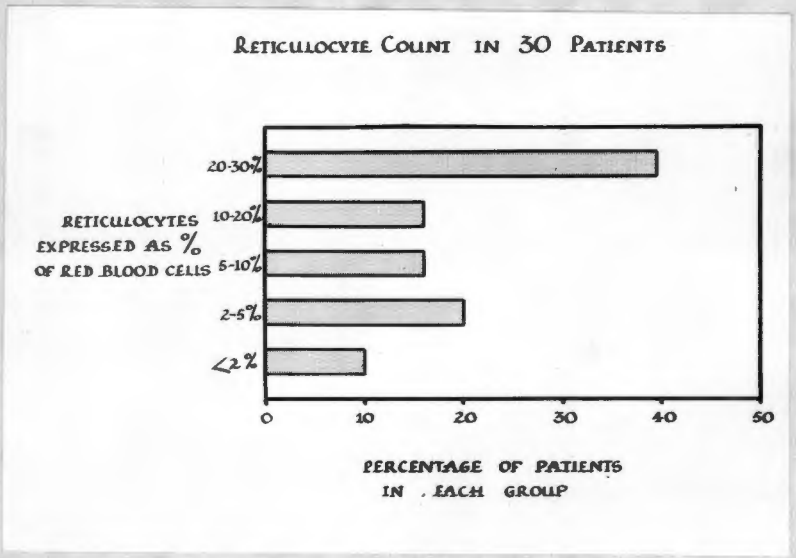


Figure 23.

4. Malarial parasites - Malarial parasites were detected in only 10 cases, in all of whom the parasite was Plasmodium falciparum. In our experience, the incidence of parasitaemia is very much lower among persons with sickle cell anaemia than in the population at large.

* 5. Osmotic fragility of the erythrocytes - The resistance of the erythrocytes to hypotonic saline was greater than normal in all cases tested. In normal blood haemolysis begins in a concentration of about 0.45 per cent sodium chloride and is complete at about 0.32 per cent. In our cases haemolysis often began at about the normal level but was

never complete within the normal range. Table IV shows the results obtained in 3 of our patients and represents fairly typical examples of the usual findings in sickle cell anaemia (Diggs and Bibb 1939).

TABLE XV - OSMOTIC FRAGILITY OF THE ERYTHROCYTES IN SICKLE CELL ANAEMIA.

Sodium chloride % solution	Percentage lysis of erythrocytes Case 13	Case 21	Case 33
.48	0	0	0
.44	0	0	0
.40	5	4	2
.36	12	14	16
.32	25	33	20
.28	45	58	40
.24	65	84	68
.20	80	94	88
.15	92	100	98
.10	100		100

* 6. Foetal (alkaline resistant) haemoglobin content of the erythrocytes -

The percentage of foetal haemoglobin detected in the erythrocytes of 10 cases on whom this investigation was performed is shown in Table XVI.

TABLE XVI - PERCENTAGE FOETAL HAEMOGLOBIN IN THE ERYTHROCYTES IN SICKLE CELL ANAEMIA.

Case No.	Percentage foetal Hb.	Case No.	Percentage foetal Hb.
4	2.9	17	9.7
5	9.3	21	12.0
6	7.7	28	4.0
7	7.1	33	7.7
10	16.8	38	4.5

* 7. Filter paper electrophoresis of the haemoglobin - Diagnosis of sickle cell anaemia was confirmed by this means in 68 cases. The remaining 7 cases were not tested in this way.

8. The bone marrow - The bone marrow was examined in 5 cases only. The picture presented in 4 was that of very active erythropoiesis with marked normoblastic hyperplasia. The white cell precursors and megakaryocytes presented normal appearances. In one case there was no evidence of increased erythropoiesis.

The Leucocytes - Figure 24 shows our findings in respect of the white cell count in our cases. It will be seen that the majority showed a leucocytosis. The high values recorded are in part, however, due to the presence of large numbers of nucleated erythrocytes in many of these patients. In one instance nucleated erythrocytes constituted more than 40 per cent of the total red cell count. It would be more correct thus to regard these findings as representing the "total nucleated cell count" rather than the true leucocyte count. Differential counts showed that even in the youngest patients, the granulocytes predominated. A mild degree of eosinophilia was found in 8 patients. It is interesting to note that Henderson (quoted by Scott et al 1951) found eosinophilia in 45 per cent of 54 cases of sickle cell anaemia.

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* We are indebted to Mr. J. Garlick, who was engaged in independent research on anthropological aspects of the abnormal haemoglobins, for furnishing the results of these investigations, which were not routinely performed by our regular laboratory services.

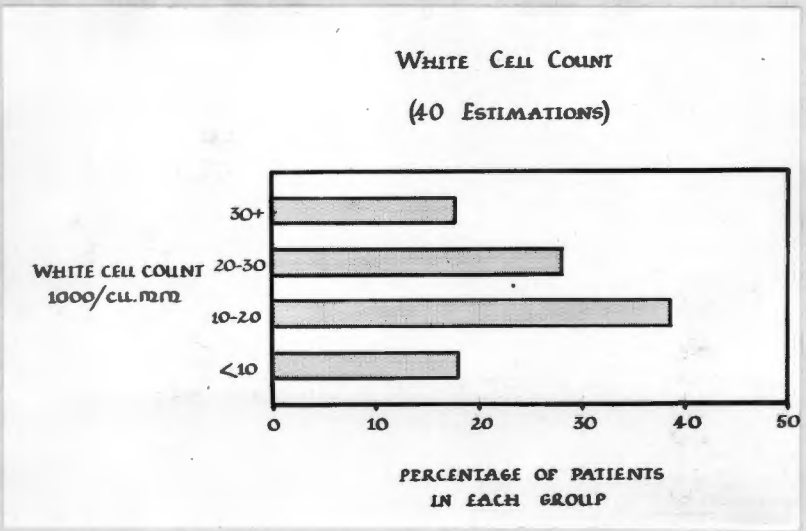


Figure 24

RADIOLOGICAL FINDINGS

The radiological changes in sickle cell anaemia are due to:-

1. Increased erythropoiesis producing hyperplasia of the bone marrow.
2. Chronic anaemia.
3. Changes due to infarctions in the haemopoietic tissues and bones
4. Changes due to greater susceptibility to infections, notably osteomyelitis.

The findings reported here are based on radiographs taken in 46 patients. 14 showed no abnormality. Table XVII shows the number of patients in whom various parts were X-rayed, and the incidence of abnormal findings in these.

TABLE XVII - INCIDENCE OF RADIOLOGICAL ABNORMALITIES IN 46 CASES OF SICKLE CELL ANAEMIA.

Part examined	No. of cases	No. showing abnormality
Chest	24	16
Skull	12	10
Hands and/or feet	29	19
Long bones	17	14
Spine and pelvis	2	1

Chest - The commonest finding was cardiomegaly. The shape of the heart varied. In some there was general enlargement involving the right and left borders equally; in others the cardiac silhouette showed greater prominence of one side or the other. A frequent feature was enlargement of the pulmonary conus giving the cardiac shadow a "mitral" configuration. The pulmonary vessels were normal in the majority, but in a few they appeared to be enlarged.

Parenchymal lung changes were present in 5 cases. In two the appearances were those of bronchopneumonia; two others showed primary tuberculous complexes with hilar adenopathy. One patient showed perihilar calcification, but the tuberculin skin test was negative.

Figures 25 to 28 show chest X-rays in 4 of our cases and demonstrate the variability of the cardiac size and shape.

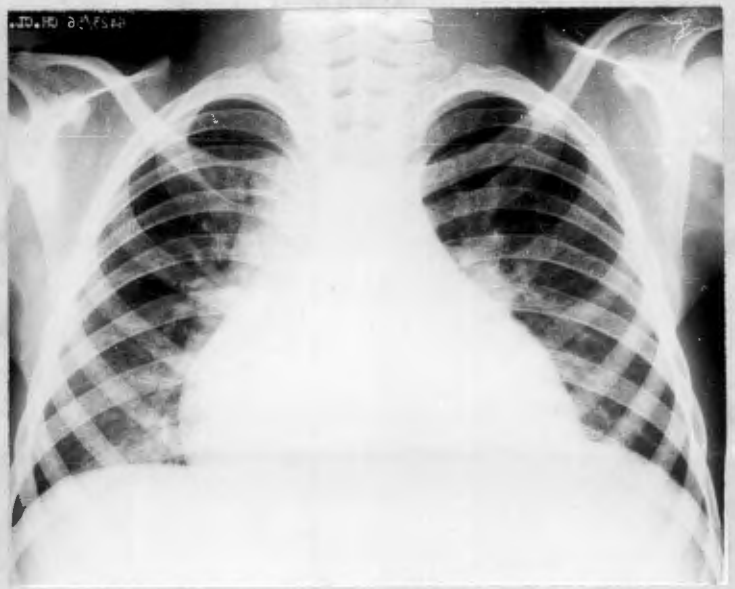


Fig. 25 - F. Age 7. No. 61
General cardiac enlargement; left diaphragm elevated due to splenomegaly; patchy opacity right cardio-phrenic angle.

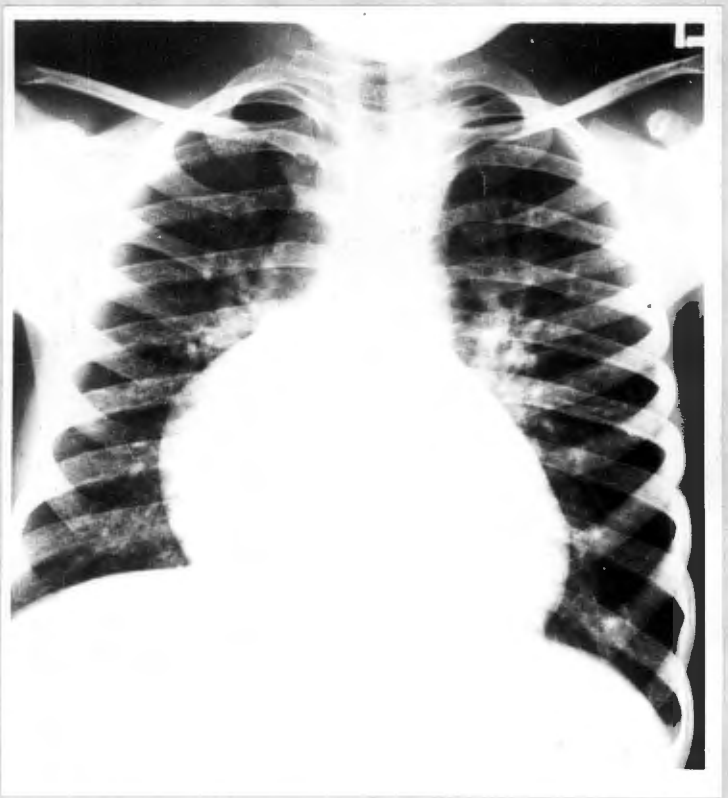


Fig. 26 - M. 9 years. No. 34.
Globular shaped heart with marked prominence of the right border. Lung fields clear but hilar shadows increased. Slight prominence of pulmonary conus.

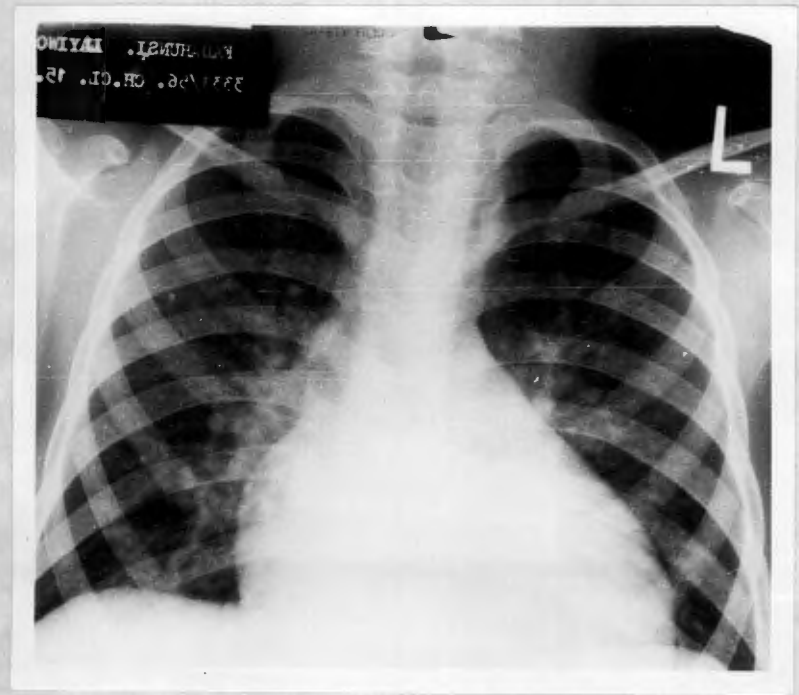


Fig. 27 - M. 10 years. No. 28
Large heart with prominence of the left border. Note pulmonary conus.

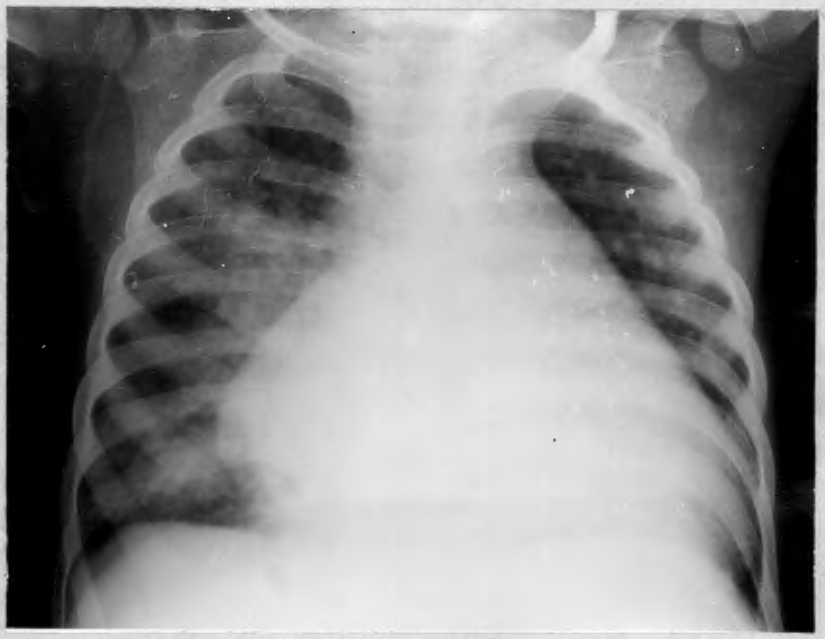


Fig. 28 - F. 15 months. No. 67
Huge heart with a massive pulmonary conus and engorged pulmonary vessels. The clinical and radiological findings suggested an underlying congenital heart lesion probably with a patent ductus arteriosus.

Skull - Our findings were similar to those reported in children by various authors, including Match and Roman (1948), Caffey (1950), and Margolies (1951). The features commonly encountered were:-

1. Widening of the diploë
2. Thinning of the outer table
3. Increase in the thickness of the frontal and parietal bones.

Prominence of the diploeic spaces gave the skull a "ground glass" or "spongy" texture especially noticeable in the frontal region on the lateral view. The "hair on end" appearance produced by radial alignment of the bone trabeculae was encountered in only 2 patients. The relative infrequency of this finding, which also occurs in other haemolytic anaemias, in our cases, is in keeping with the reports of the authors cited above; but Edington (1953) found it the commonest radiological feature in his series. One patient showed small circumscribed areas of rarefaction in the calvarium. A similar finding has been reported by Legant and Ball (1948) who attributed the appearance to bone necrosis following infarction. "Tower skull" produced mainly by thickening of the parietal bones has been reported by Match and Roman (1948). This finding was present, though not marked, in some of our cases. Figures 29 to 32 are examples of skull appearances encountered by us.

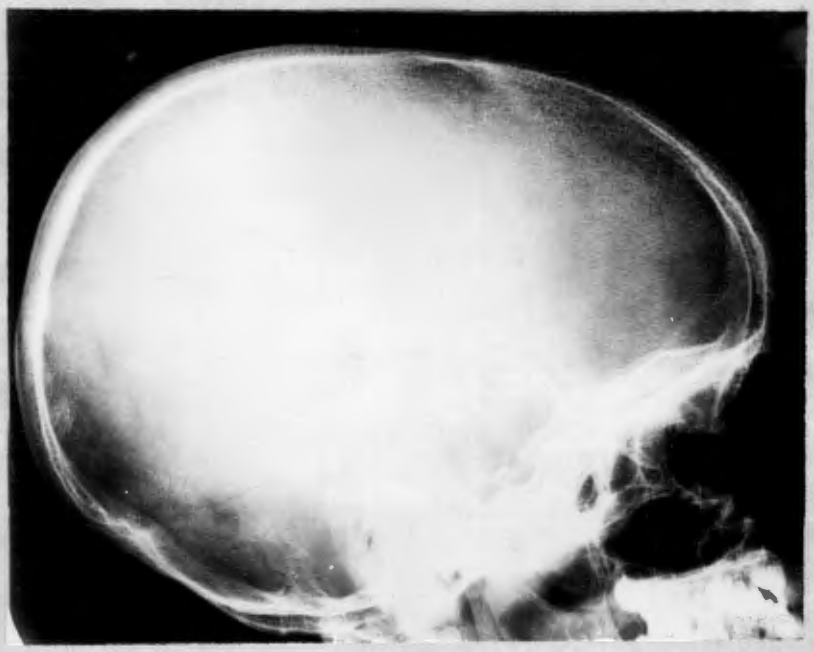


Fig. 29 - M. Age 10 years. No. 28.
Note the "groundglass" or "spongy" texture of the bones and the thin outer table.

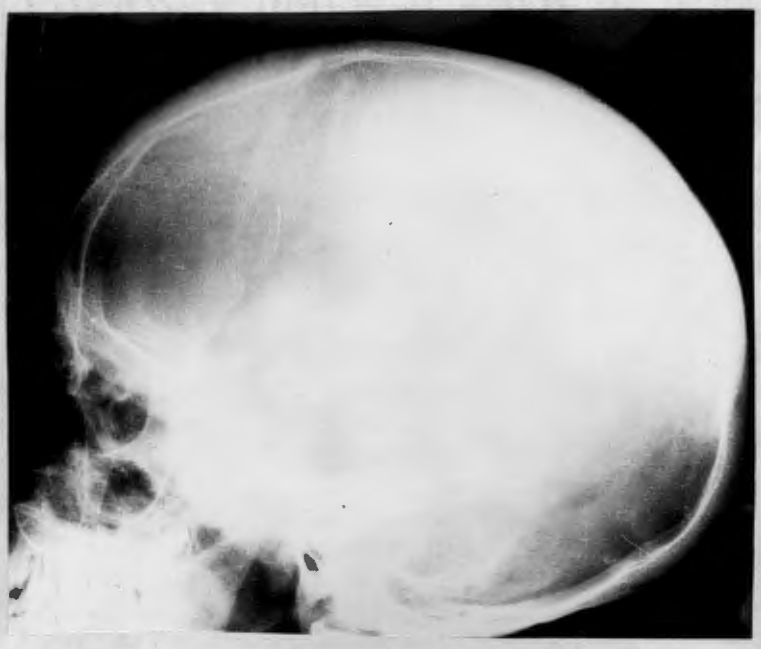


Fig. 30 - F. Age 7 years. No. 61
Note.- 1. Spongy texture in frontal region.
2. "Hair on end" appearance over vertex.
3. Thin outer table and widened diploee.

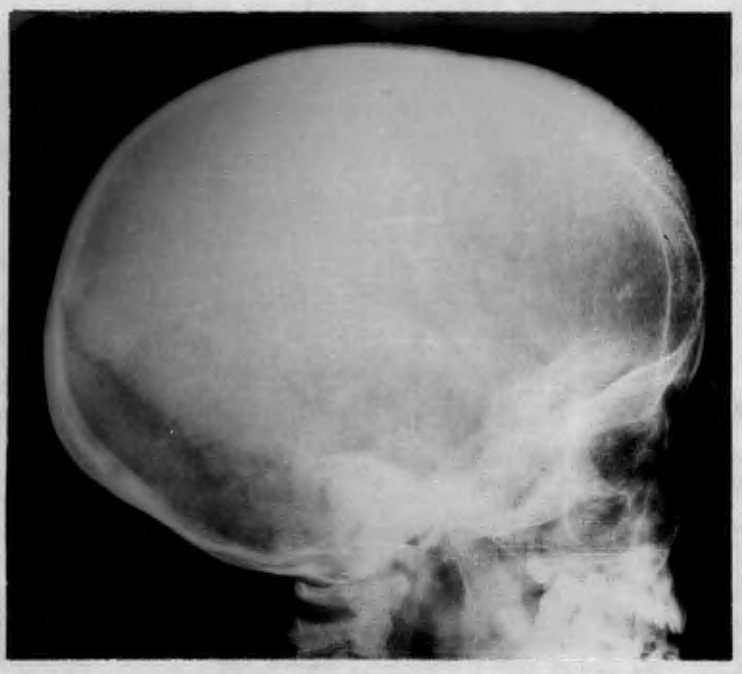


Fig. 31 - F. Age 8 years. No. 7 Changes similar to those in Fig. 30 but more marked. Note the increase in height of the skull caused by thickening of frontal and parietal bones.

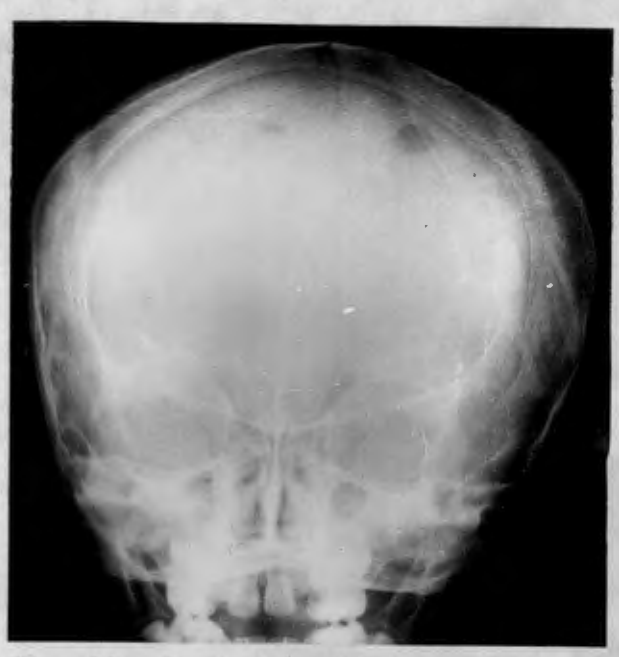


Fig. 32 - M. Age 6 years. No. 64. Showing circumscribed areas of rarefaction probably due to infarcts. Note also the increase in width of the parietal bones.

The long bones - The commonest findings were coarsening of the trabecular pattern, widening of the medullary cavity and thinning of the cortices. These changes are the natural consequence of hyperplasia of the bone marrow (Match and Roman 1948). Other findings were periosteal reaction (Figure 33a) and circumscribed areas of translucency, sometimes surrounded by slightly sclerotic bone, usually situated in the distal half of the shafts of the long bones (Figures 33b, 34, 35a and b). These changes are thought to occur as a result of bone infarction caused by diffuse capillary blockage by clumps of sickled erythrocytes. It is possible, however, that other mechanisms may be involved.

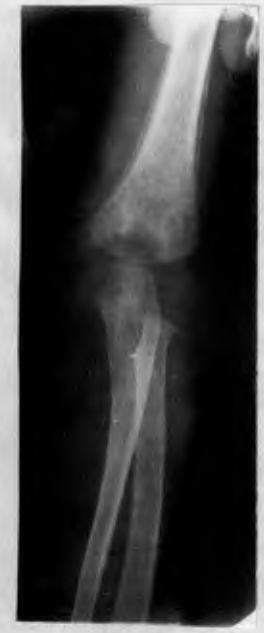


Fig. 33a.
M. 3 years old. No. 46. A.P. and Lat. view same patient. Showing periosteal reaction and an area of rarefaction in the lower end of the right humerus.

Fig. 33b.

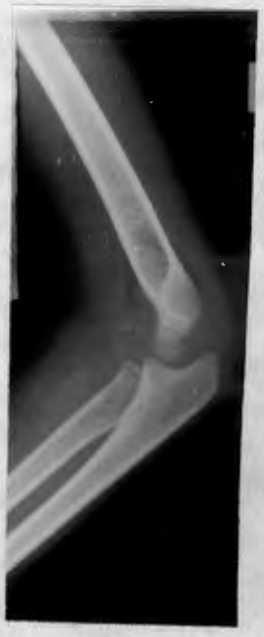


Fig. 34. F. Age 5 years No. 35. Lateral view of left elbow showing circumscribed area of rarefaction in the lower third of the left humerus.

Multiple bone infarcts were observed in several patients and in a few the lesions were symmetrically distributed. Match and Roman (1948) reported two patients with symmetrical infarcts in the lower halves of the femora. Figures 36 to 39 illustrate serial changes observed in a patient in whom both tibiae and fibulae were simultaneously involved. The clinical picture in this case was complicated by suppuration, and a small quantity of pus aspirated on one occasion grew B. coli and streptococci on culture. There seems little doubt, however, that sickle cell anaemia was primarily responsible for the changes observed.

Fig. 35a

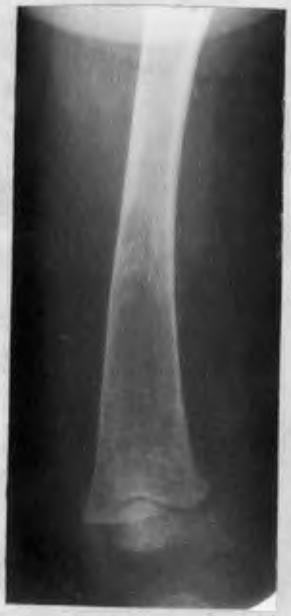


Fig. 35b

A. P. and Lateral views of left femur in the same patient as Fig. 33, showing an area of translucency in the lower half of the bone. Note also the wide medullary cavity, thin cortex and general osteoporosis.

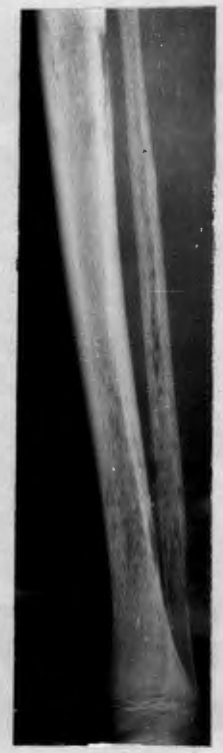


Fig. 36

A.P. (Right) Lat.

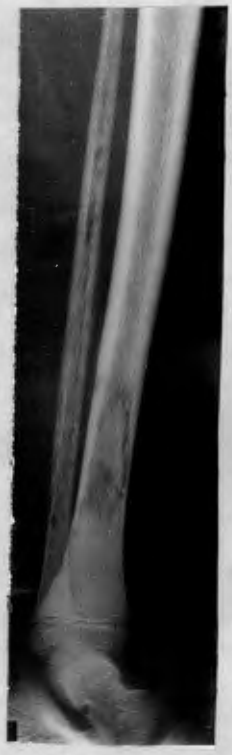


Fig. 37

A.P. (Left) Lat.

7.7.55.

Figures 36 to 39 (See next page for Figs. 38 and 39). F. 8 years. No. 7.
Serial X-rays showing symmetrical involvement of tibiae and fibulae. Note diffuse areas of bone absorption in early plates and localisation in lower third of tibiae in later plates. Suppuration occurred in this case and B. coli and streptococci were isolated on culture of the pus.

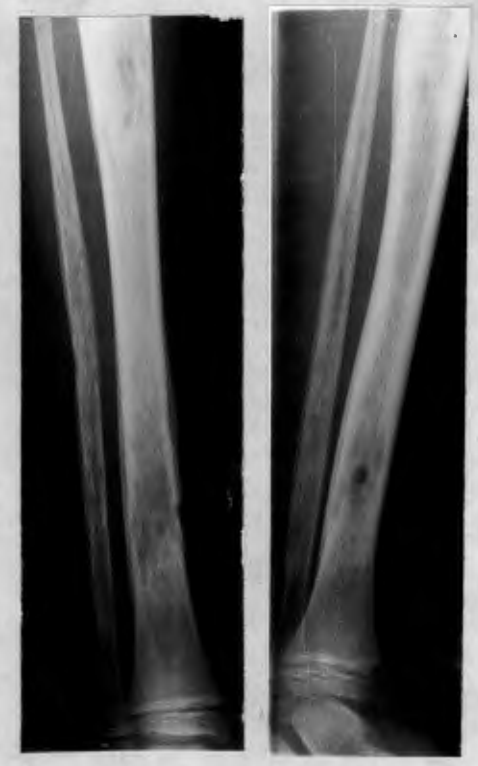


Fig. 38
A.P. (Right) Lat.

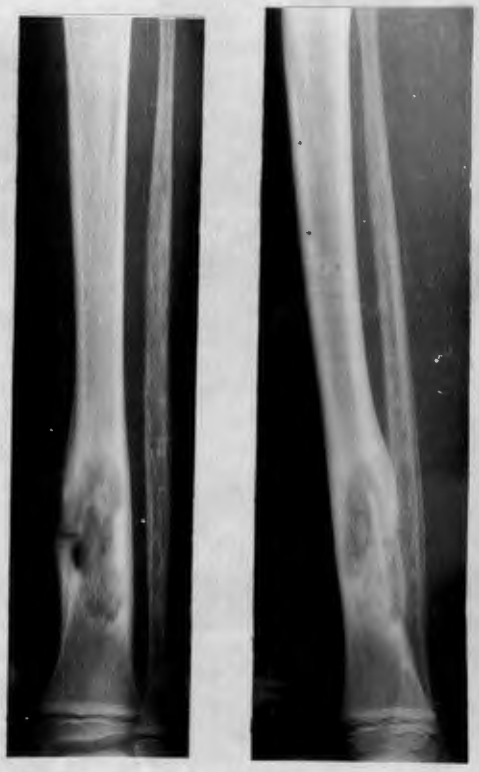


Fig. 39
A.P. (Left) Lat.

10.9.55.

Figures 36 to 39 (See previous page for Figs. 36 and 37). F. 8 years. No. 7. Serial X-rays showing symmetrical involvement of tibiae and fibulae. Note diffuse areas of bone absorption in early plates and localisation in lower third of tibiae in later plates. Suppuration occurred in this case and B. coli and streptococci were isolated on culture of the pus.

Hands and Feet - The common findings here were similar to those described in the long bones, viz. coarsening of the trabeculae and thinning of the cortices. The appearance of "cystic" areas near the distal ends of the shafts of the proximal and middle phalanges was noted in several cases, and the tendency to symmetrical arrangement of these was even more striking than the changes seen in the long bones. (Figure 40).

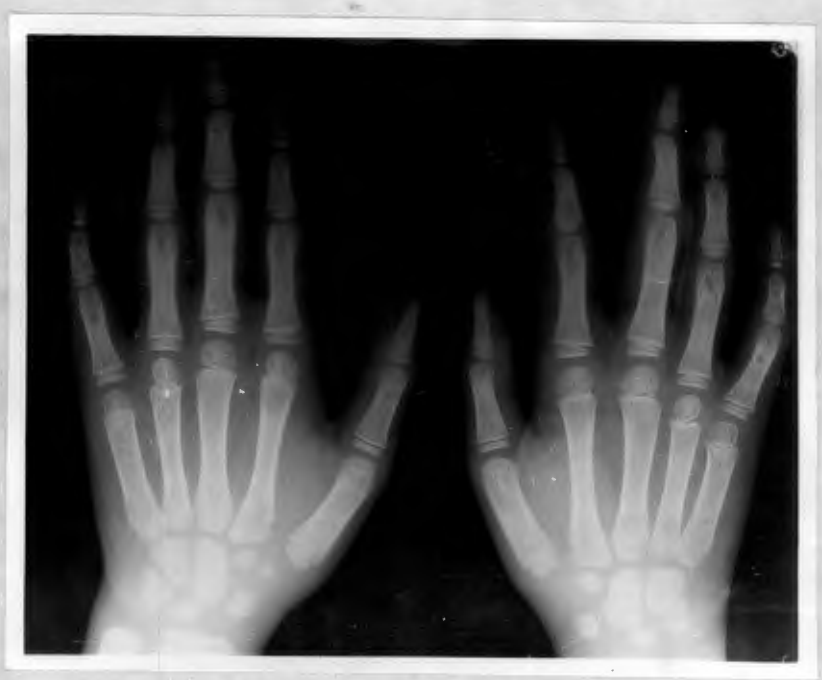
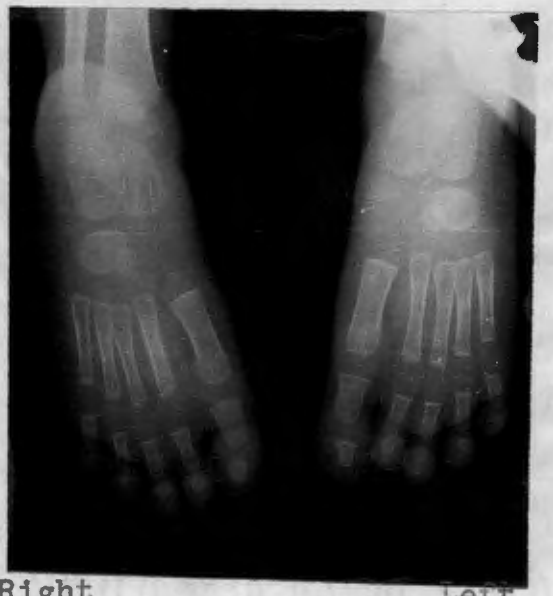


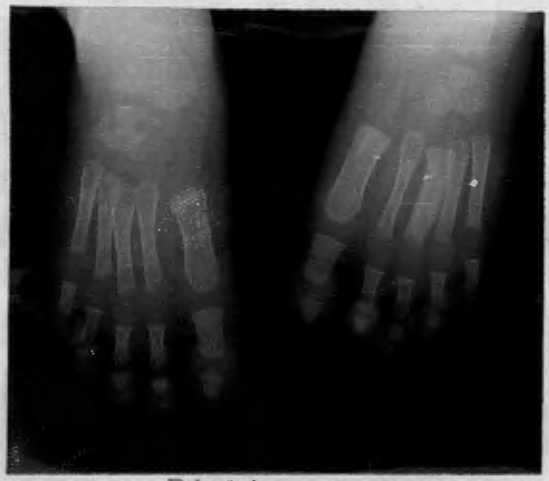
Fig. 40 - F. Age 7 years. No. 74. Hands. "Cystic" areas in the distal ends of the proximal and middle phalanges of both hands.

The swellings of the hands and feet already referred to were usually unassociated with detectable bone involvement in the early stages, but re-examination at a later date showed changes in the bones in many. Figures 41, 43 and 44 show changes which occurred within a few weeks of onset, and are similar to those reported by Carrel and Evans (1949) and Danford et al (1941) in two American Negroes aged 9 months and 8 months respectively. Our experience suggests that this type of bone involvement is not as unusual in sickle cell anaemia as these authors suggest. An unusual finding in 3 of our cases was distortion of shape and increase in density of certain metacarpals or metatarsals as shown on X-rays taken several months after acute symptoms had subsided. The appearances suggest diffuse sclerosis. (Figs. 42,43, and 46).



Right Left

Fig. 41 - Age 1½ years. No. 33. Bone appearances during the acute phase of swelling. Note slight periosteal reaction right second metatarsal.



Right Left

Fig. 42. Same patient 6 months later. Note distortion and sclerosis of left middle metatarsal.

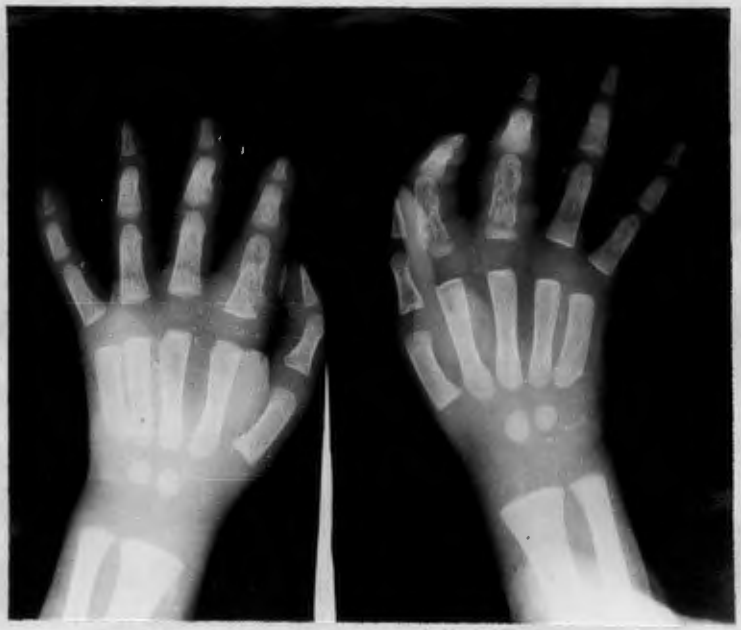


Fig. 43

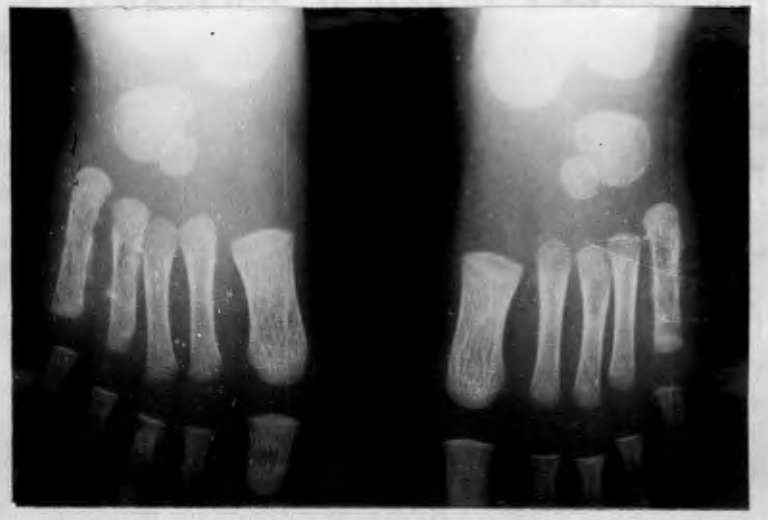


Fig. 44

Figs. 43 and 44. F. 1½ years. No. 70. Hands and feet. Appearances 3 weeks after onset of pain and swelling. Note the soft tissue swelling of fingers, necrosis of proximal phalanges in the hands and metatarsals (both fifth and right fourth), and the coarse trabeculations especially in the first metatarsals. Suppuration occurred in the hands but not the feet. The pus was sterile on culture. The tuberculin skin test and Kahn reaction were negative.

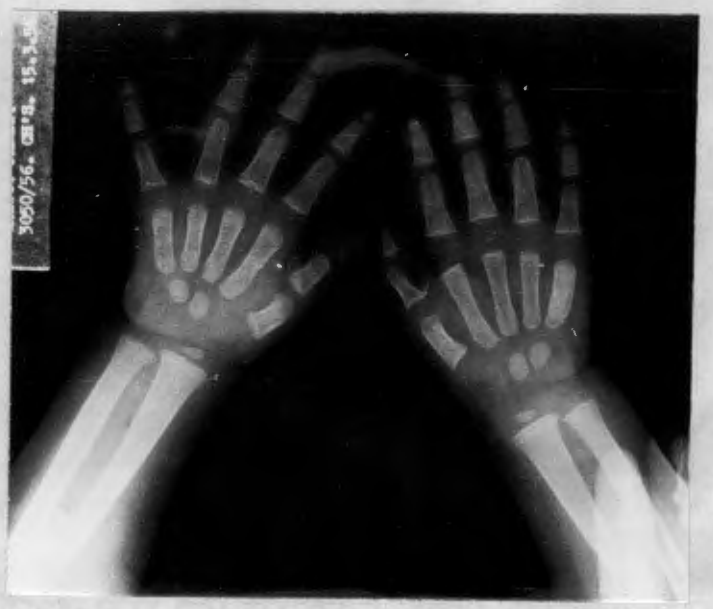


Fig. 45 - F. Age 1 year. No. 43. Hands. Note the distortion and sclerosis of right fifth metacarpal, which occurred some months after swelling of the hands.

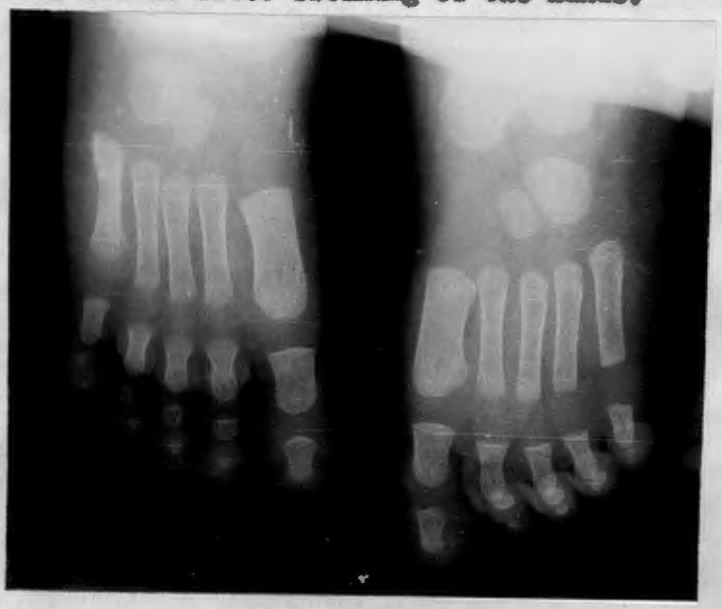


Fig. 46. - M. Age 2 years. No. 44. Feet. Note the distortion and sclerosis of the right fifth metatarsal, which occurred months after swelling of the feet.

Unusual Bone Manifestations.

One patient (Case 21) had very widespread bone involvement and showed most of the radiological features described in the preceding sections. In addition to these she had:-

1. Destructive changes in the body of the 8th dorsal vertebra.
2. Pathological dislocation of the right hip.
3. Pathological fractures of the left femur and left tibia.
4. Suppurative arthritis of the left knee associated with gross destructive changes in the adjacent ends of the femur and tibia.

All investigations in this case indicated that the lesions had resulted primarily from sickle cell anaemia. In the knee and the hip, however, the picture was complicated by infection. Figures 47 to 50 illustrate some of the bone changes seen. A full account of the clinical findings in this patient will be found in Appendix II.

Pathological changes in the spine in sickle cell anaemia were first reported by Leivy and Schnabel (1932). They were subsequently reported by other authors, including Match and Roman (1948), Henkin (1949), Wight and Thompson (1950) and Margolies (1951). The changes reported by these authors were:-

1. Increased translucency with prominence of the trabeculae.
2. Decrease in the height with increase in the width of the vertebral bodies, sometimes associated with "cupping" in relation to the intervertebral discs.

The changes shown in Fig. 48 have only been previously reported in one case of sickle cell anaemia (Carroll and Evans 1949). The lesion resembles spinal tuberculosis, but our investigations did not support this diagnosis.

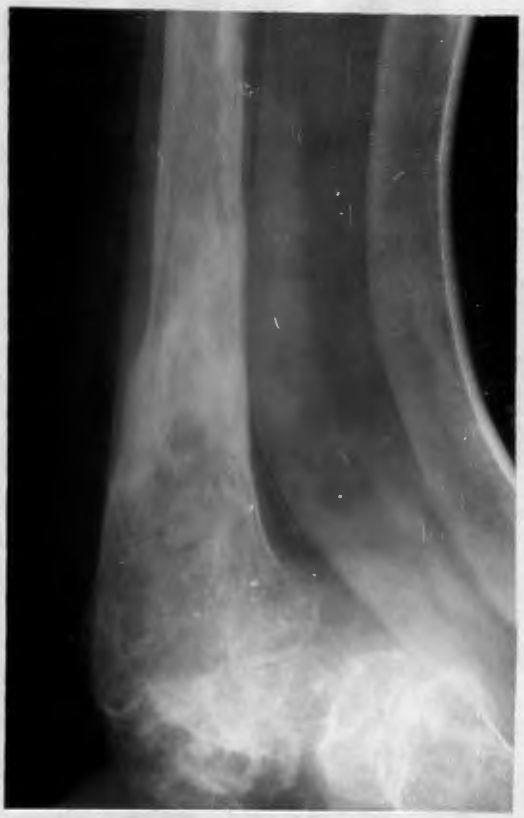


Fig. 47 - Left knee

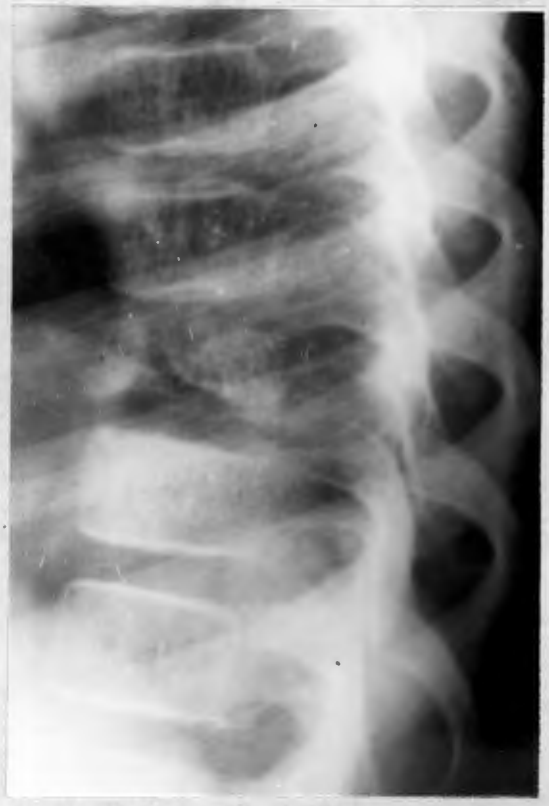


Fig. 48 - Dorsal spine

Figs. 47 and 48. F. 10 years. No. 21. See Appendix II. Note the marked osteoporosis of all bones shown and the destruction in the body of the 8th dorsal vertebra and the bones of the knee joint. A healed pathological fracture is visible in the lower third of the femur. (The spinal lesion differs from that usually encountered in tuberculosis in that the intervertebral discs have been spared, and there is no evidence of "cold abscess" formation. A synovial biopsy of the knee showed no evidence of tuberculosis).

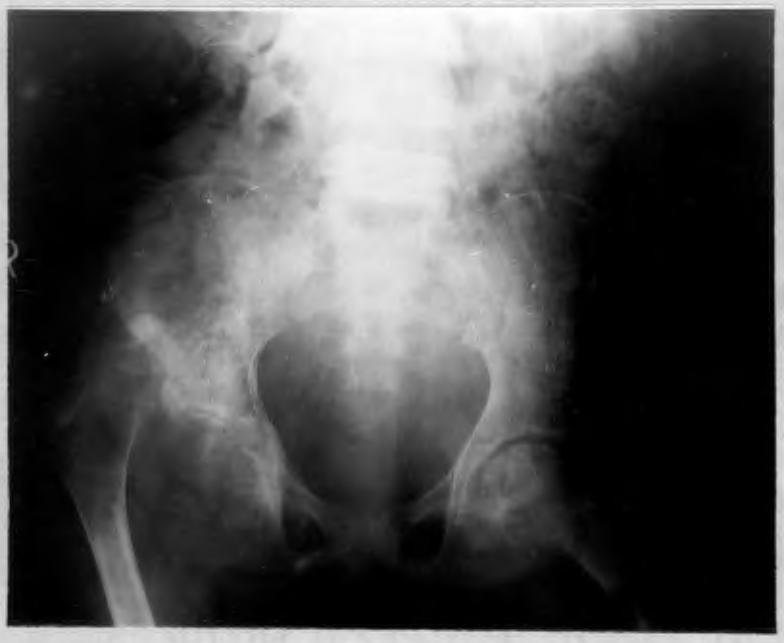


Fig. 49

Pelvis

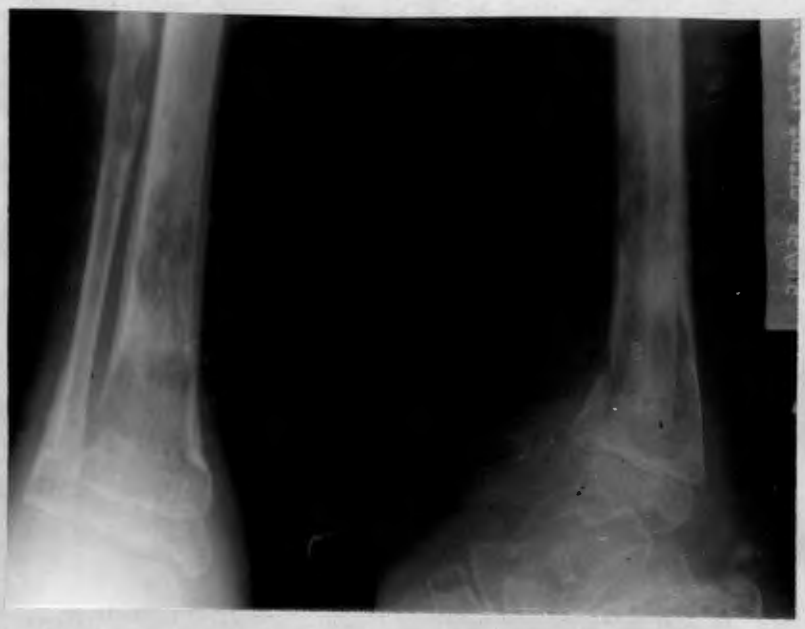


Fig. 50

Left tibia and fibula

Figs. 49 and 50. Same patient as Figs. 47 and 48. Note the gross osteoporosis of all bones. The X-ray of the pelvis shows dislocation of the right hip associated with destructive changes in the acetabulum. The fifth lumbar vertebra is compressed. The tibia and fibula show multiple areas of translucency and periosteal reaction. There is a pathological fracture through one of the translucent areas in the distal end of the tibia. The Kahn test was negative and biopsy of the tibia showed no histological evidence of tuberculosis, syphilis, yaws, or malignant disease.

Osteitis in Sickle Cell Anaemia.

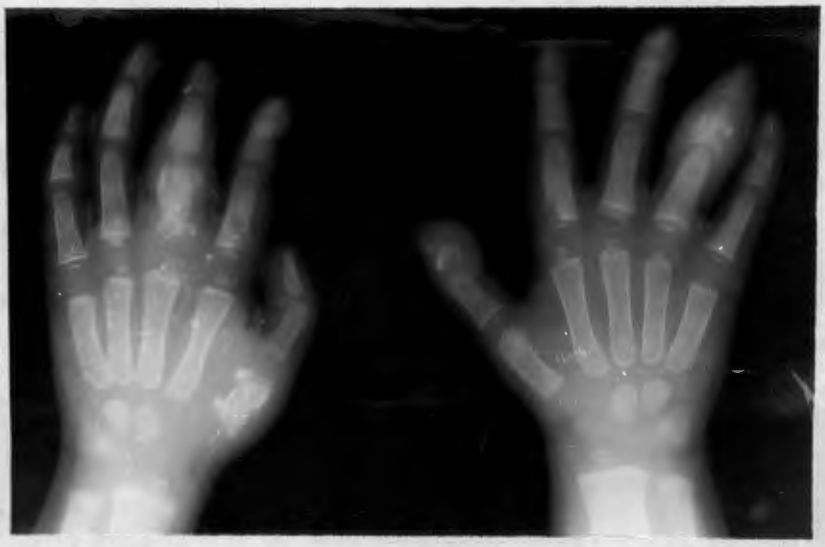
Suppuration occurred in association with bone lesions in 7 of our cases. In 2 no organism could be recovered from the pus. In 2 others mixed organisms were grown on culture - B. coli and Streptococci in one and Staphylococcus pyogenes and B. coli in the other. The remaining 3 patients were infected with strains of Salmonella.

Sickle cell anaemia, even in the absence of detectable infection, can masquerade as osteomyelitis. One of our patients was referred to us as a case of "unusual osteitis with severe anaemia". The X-rays showed a large infarct resembling a Brodie's abscess in the lower end of the femur. In several other patients the combination of fever, local pain and swelling, leucocytosis and radiological changes in the bones, strongly suggested osteitis, but investigation showed that all these findings were attributable directly to sickle cell anaemia.

Salmonella osteitis is a rare clinical entity; by 1950 only 19 cases had been recorded in the world literature (Wigh and Thompson (1950). Patients with sickle cell anaemia, however, seem to be peculiarly susceptible to this complication, instances having been reported by several authors, including Wigh and Thompson (1950), Ellenbogen et al (1955), Cole (1955), and Vandepitte and Oyo (1956). The last mentioned authors cite 5 cases in infants seen over a period of less than 2 years. One of the authors reviewing the epidemiology of Salmonella infections in the Belgian Congo referred to 14 Salmonella cultures

isolated from osteomyelitis in patients with sickle cell anaemia. Figures 51 and 52 show the bone changes in two of our patients. It will be seen that radiologically the lesions resemble those in uncomplicated sickle cell anaemia, both in nature and distribution. The inference seems to be that Salmonella become established in sites previously damaged by pathological processes peculiar to sickle cell anaemia.

Cole (1955) estimates that osteitis is about 75 times more common in sicklaemics than in the general population, and suggests as an explanation that bones damaged by infarction offer ideal sites for organisms to settle in following transient bacteriaemia.



Right Fig. 51 Left

Fig. 51. F. Age 15 months. No. 67. Infected with Salmonella group B. X-rays taken 3 weeks previously had shown only soft tissue swelling. The X-ray presented shows:-
Right hand - destructive changes in the first metacarpal and proximal phalanges of index and middle fingers.
Left hand - similar changes in terminal phalanx of thumb, middle phalanx of ring and proximal phalanx of index fingers. A circumscribed area of translucency was also present in the left radius.

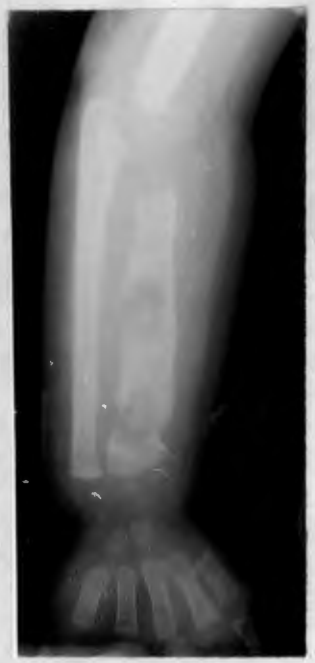


Fig. 52. M. Age 9 months. No. 73. Infected with Salmonella enteritidis. Two large areas of destruction are visible in the left radius. X-rays of the feet showed involvement of both first and the left fifth metatarsals. The hands were swollen and tender but showed no changes in the bones.

Liver Function Tests.

Table XIII shows serum protein estimations, liver function tests and liver sizes recorded in 18 of our cases. Table XIX shows the results of similar investigations in 15 American children with sickle cell anaemia (Green, Conley and Berthrong, 1953). Comparing the tables it will be seen that in all respects our cases showed greater abnormality than the American series. The older patients in our series gave more abnormal results than the younger, but the numbers examined are too small for definite conclusions to be drawn.

Serum Proteins - The total protein estimation fell within the normal range in the majority, but reversal of the normal albumin-globulin ratio was a common finding. Comparison of these results with those

Notes to Table XVIII - see next page.

Alb. = Albumen Glob. = Globulin, both expressed in g. per 100 mls.
Bilirubin expressed in mg. per 100 ml. D = direct reacting bilirubin.
A/phos. = Alkaline phosphatase. K.A. = King Armstrong units.
T.T. = Thymol turbidity.
ZnS. T. = Zinc sulphate turbidity.

* Needle biopsy showed cirrhosis of the liver.

TABLE XVIII - SERUM PROTEINS, LIVER FUNCTION TESTS AND LIVER SIZE IN 18 CASES IN THE PRESENT SERIES.

Series No.	Age Yrs.	Sex	Serum Proteins Alb.	Glob.	A/G	Bilirubin Total		A/Phos. K.A.	T.T. units	ZnS.T. units	Liver cms.
7	8	F	2.6	3.1	0.8	15.8	D	-	9.5	-	10
			2.4	2.6	0.9	17.0	D	-	14.0	-	10
10	3	M	-	-	-	4.8	D	-	6.5	-	8 - 10
17	3	F	-	-	-	15.0	D	9	12.0	12.9	8
						2.3		7.4	3.0	6.8	0
27		M	3.0	4.3	0.7	1.2	D	20.3	6.0	-	3 - 8
21	10	F	1.7	6.0	0.28	8.6	D	33.6	6.5	19.0	0
28	10	M	2.9	3.3	0.87	6.5	D	13.9	4.5	-	8
						1.2		18.0	9.6	13.6	8
			4.4	4.3	1.0	4.0		21.9	16.5	13.5	6
34	9	M	-	-	-	8.8	D	45.7	8.0	10.6	2 above
			-	-	-	25.0	D	33.0	14.2	18.4	umbilicus
			-	-	-	5.0	D	35.2	4.5	-	2 below
			-	-	-	0.45		22.3	5.2	-	umbilicus
38	1½	M	4.4	3.5	1.2	0.2		30.0	3.5	7.5	2
*39	6	M	2.9	3.2	0.9	4.5	D	37.9	5.0	-	"Huge"
41	1	M	-	-	-	1.2		20.9	4.0	-	0
48	3	F	-	-	-	11.0	D	10.5	3.0	7.0	4 - 6
60	5	F	1.9	4.7	0.4	5.6	D	28.0	14.0	18.6	3 above
						5.9	D	54.0	11.0	14	umbilicus
61	7	F	1.8	4.4	0.43	3.0	D	21.1	9.6	19.2	10
			-	-	-	2.2		24.8	6.9	14.0	10
63	3	M	-	-	-	-		30.5	-	-	0
64	6	M	-	-	-	3.3		12.8	2.5	10.0	6 - 8
68	4	F	-	-	-	4.3	D	18.9	4.5	11.0	1 - 4
70	1½	F	-	-	-	0.45		-	7.8	5.6	0
71	9	M	3.0	3.7	0.81	2.56	D	23.9	1.6	4.3	2
			-	-	-	1.5		26.3	1.5	5.8	8

TABLE XIX - LIVER SIZE, SERUM PROTEINS AND LIVER FUNCTION TESTS IN 15 CASES OF SICKLE CELL ANAEMIA IN CHILDREN REPORTED BY GREEN ET AL (1953).

Age Yrs.	Sex	Serum Proteins Alb.	Glob.	A/G	Bilirubin Total (D)	A/Phos. K.A.	T.T. Units	Liver cms.
7	M	4.0	2.6	1.5	1.7 (0.5)	7.6	-	0 to 5
12	M	5.1	2.7	1.9	2.9 (1.2)	6.7	4.5	0 to 4
11	F	4.8	2.5	1.9	2.7 (1.2)	9.12	1.2	0 to 2
11	F	4.7	2.3	2.0	2.0 (1.4)	9.1	4.3	0 to 2
5	M	4.4	2.9	1.5	2.2 (1.5)	8.6	1.5	0
6	F	4.9	2.3	2.1	1.8 (0.8)	1.0	6.4	0 to 1
10	M	4.4	3.5	1.2	2.9	-	-	0
14	M	5.0	2.1	2.4	2.2 (1.5)	7.7	1.3	0 to 3
9	M	5.3	2.4	2.2	2.3 (1.1)	12.8	2.8	0 to 2
11	M	4.7	2.8	1.6	2.7 (1.4)	10.0	1.8	0 to 1
15	M	4.8	2.8	1.7	1.3 (0.8)	8.3	1.4	0 to 1
8	M	5.0	2.4	2.1	2.8 (1.2)	9.4	6.4	6 to 0
12	M	4.3	3.5	1.2	8 (3.4)	15.2	6.4	4
14	M	4.0	4.7	0.85	1.6 (1.0)	8.8	4.8	2 to 3
3	M	4.5	2.7	1.6	4.6 (2.0)	8.8	13.0	1 to 6

Alb.= Albumen Glob.= Globulin, both expressed in g. per 100 ml.
Bilirubin expressed in mg. per 100 ml. D= direct reacting bilirubin.
A/phos. = Alkaline phosphatase. K.A. = King Armstrong units.
T.T. = Thymol turbidity.

recorded for (1) healthy adults, (2) healthy infants, (3) anaemic infants (not due to sickle cell anaemia) in Ibadan shows that our findings in sickle cell anaemia cannot be attributed to racial or environmental factors alone, nor can they be attributed solely to anaemia. (See Table XX).

TABLE XX - SERUM PROTEINS: AVERAGE VALUES RECORDED FOR NORMAL ADULTS, ANAEMIC (ANAEMIA NOT DUE TO SICKLE CELL DISEASE) AND NON-ANAEMIC INFANTS, AND CHILDREN WITH SICKLE CELL ANAEMIA, IN IBADAN, NIGERIA.

Subjects	Number tested	Alb.	Glob.	A/G.	Authors
Healthy adults	200	3.6	3.2	1.12	Edozien (1956)
Healthy infants	9	3.05	2.9	1.05	Hendrickse and King (1957)
Anaemic infants (Hb. less than 5 g. per cent)	12	3.24	3.07	1.05	
Children with sickle cell anaemia.	11	2.8	4.0	0.7	Present investigation

Serum bilirubin - The serum bilirubin was raised in the vast majority and at times the level was very high. In 3 instances the serum bilirubin fell within the normal range, but one of these readings was obtained in a patient who on another occasion gave the highest value recorded in the series (25mg. per cent.). Reference to Table XVIII will show that in the majority of patients a portion of the bilirubin was of the direct reacting type encountered in obstructive jaundice. The urine, however, seldom showed bile pigment. Green et al (1953)

in a review of liver function in 50 cases of sickle cell anaemia reported similar findings in many of their older patients (mainly in the third and fourth decades).

Thymol and Zinc Sulphate Turbidity Tests - our results were highly abnormal when judged by European standards. When judged by standards familiar to us in Ibadan, however, they show a lesser departure from the normal. The average value for the zinc sulphate turbidity test in normal adult Nigerians is 8.5 Maclagan units, with a range of 6 to 14 units (Edozien 1956). Thymol turbidity tends to be raised also. We not infrequently encounter children in whom there is no evidence of hepatic disease who give values up to 8 units. However, even when judged by these relaxed local standards, some of our patients gave abnormal results.

Serum Alkaline Phosphatase - 24 estimations gave abnormally high values in all but 3 instances. Caution must be exercised in interpreting this finding as high levels are commonly encountered in children in Ibadan even in the absence of clinical evidence of hepatic dysfunction or bone disease such as rickets.

Liver Biopsy - One patient who presented with a large tender liver which simulated an amoebic liver abscess was subjected to needle biopsy. The histological report read as follows:- "The specimen consists of a number of pieces of disorganised liver tissue. Liver cells are largely replaced by connective tissue cells and there is heavy deposition of

bile pigment. Some lymphocytic infiltration is present. On the whole the appearances suggest cirrhosis rather than hepatitis".

Comment on liver function tests - While it must be acceptedthat individual tests have little value as indices of liver function, our results taken as a whole in conjunction with liver size indicate that in many patients the disease is associated with hepatic dysfunction. This finding is in keeping with that of Green, Conley and Berthrong (1953) in adults in the U.S.A. These authors concluded that the liver disorder was a specific manifestation of sickle cell anaemia which could not be explained on any other basis. Autopsies on 21 cases showed that hepatic dysfunction probably resulted from severe impairment of blood supply from the combined effects of

1. Anaemia
2. Sickling of erythrocytes in the hepatic sinusoids
3. Obstruction of sinusoids by Kupffer cells engorged with phagocytosed erythrocytes.

Cirrhosis was present in 4 of the cases that came to autopsy and others showed morphological evidence of liver cell injury. These authors, in common with others in the U.S.A., do not describe hepatic dysfunction as a common feature of the disease in the first decade of life, but it is interesting to note that one of their cases with cirrhosis was only 6 years old.

The diet of the general Nigerian population is grossly deficient in animal protein and also lacks adequate non-animal protein

(Woodruff 1951). It is generally accepted that chronic protein deficiency impairs liver function and may lead to diffuse hepatic fibrosis. The likely explanation for the earlier onset of hepatic dysfunction in our cases is that chronic protein deficiency renders the organ more susceptible to damage, and thus accelerates the pathological processes of the disease.

The urine - None of our patients presented any gross urinary abnormalities apart from changes associated with jaundice. The specific gravity of the urine, however, in those cases in which estimations were made, tended to be low. Hyposthenuria has been reported as a constant finding in sickle cell anaemia and other variants of sickle cell disease (Scott et al 1955, Keitel et al 1956). It has also been found in 69 per cent of persons with the sickle cell trait (Keitel et al 1956).

Painless haematuria unassociated with other evidence of renal disease is relatively frequently found in sicklaemics (Smith and Conley 1954, Vernier 1955, Goodwin et al 1950), but the vast majority of cases occur after the first decade of life. Reports also indicate that haematuria occurs more frequently in association with the sickle cell trait and sickle cell-haemoglobin C disease than with sickle cell anaemia. Smith and Conley (1954) also report a peculiar nephritis which may lead to uraemia and death in sickle cell anaemia. This finding has not been reported in children.

Tuberculosis and Sickle Cell Anaemia - 28 patients had tuberculin skin tests performed by the Heaf technique. There were 6 positive reactors and 3 others showed reactions which were classified as "doubtful". Table XXI gives the relevant details in these cases. It will be seen that only 3 cases showed unequivocal evidence of active tuberculosis on investigation, but it is likely that case 5 also had active disease when he first presented for treatment.

Tuberculosis is rife in Ibadan and the incidence among children attending the outpatient department of University College Hospital is high. Accurate statistics, however, are at present not available. * It has been reported that persons with the sickle cell trait and sickle cell anaemia show a higher incidence of the various forms of tuberculosis than do persons who do not show the sickling phenomenon (Weiss and Waife 1952, Weiss and Stecher 1953). Our impression is that tuberculosis is at least as common in patients with sickle cell anaemia as in others attending hospital, but definite conclusions about the relative frequency will have to await further investigation.

* A small study of the incidence of positive tuberculin reactors among children attending our outpatient department was undertaken during 1956. Results obtained in 350 children showed the following:-

Age	under 1 year	4% positive
"	1 to 3 years	14% "
"	3 to 6 "	23% "
"	over 6 years	53% positive

TABLE XXI - SHOWING AGE, SEX, X-RAY FINDINGS AND OTHER RELEVANT DETAILS IN 9 CASES OF SICKLE CELL ANAEMIA WHICH SHOWED A REACTION TO THE TUBERCULIN SKIN TEST.

Case No.	Age Yrs.	Sex	Tuberculin Skin Test	Chest X-ray	Comment
4	5	M	Positive	Enlarged tracheo-bronchial glands	Treated with I.N.H. and P.A.S.
5	¾	M	Positive	Lungfields clear	Treated with I.N.H. and P.A.S. Died in haemolytic crisis at age 1½ years. No post mortem.
10	3	M	Doubtful	Lungfields clear	No extrapulmonary focus of tuberculosis. No treatment.
20	4	F	Positive	Primary complex in right upper lobe	Treated with I.N.H. and P.A.S.
21	10	F	Doubtful	Lungfields clear	Bone and joint lesions resembling tuberculosis but biopsies negative (see Appendix II)
33	1½		Positive	Initial X-ray showed patchy bronchopneumonic consolidation at both bases, but on re-examination a few weeks later the lung fields were clear.	
39	6	M	Doubtful	Lungfields clear	Cirrhosis of the liver
56	7	F	Positive	Lungfields clear	No extrapulmonary tuberculous lesions
60	5	F	Positive	Lungfields clear	Massive tuberculous glands in the right axilla. Treated with I.N.H. and P.A.S.

PROGNOSIS.

Only 3 of our patients are known to have died. In all death occurred in association with acute haemolytic crises, but in one the picture was complicated by severe bronchopneumonia. The ages at death were 18 months, 6 years and 7 years, respectively. The fact that many patients defaulted from the follow up clinic made it impossible to assess prognosis accurately. Since my departure from Ibadan a field worker has been employed to trace cases which have been lost sight of for periods of 6 months or more. So far 10 patients have been traced, all alive and relatively symptom free (Tompkins 1957).

Edington (1953) and Edington and Lehmann (1955a) have indicated that on the Gold Coast prognosis in sickle cell anaemia is similar to that reported by American authors, many patients surviving into the second, third and fourth decades of life. In contrast with these authors, the Lambotte-Legrandes (1955b) report that among 300 cases seen in the Belgian Congo over a period of 6 years, 150 had died, of whom 80 per cent were under 2 years of age. They go on to say, however, that the severity of the disease and the risk of fatal outcome decrease with age.

Prognosis in the Bantu tribe in East Africa appears to be similar to that reported in the Belgian Congo (Lehmann and Raper 1956), but among the Luo (Allison 1956) and the Buganda (Jacob 1957) survival

into adult life of haemoglobin S homozygotes is approximately 20 and 14 per cent respectively.

The reasons for the differences in prognosis (1) in the first 2 years of life in the Belgian Congo and West Africa and (2) between tribal groups in East Africa are not at all clear, but it seems likely that environmental rather than genetically determined factors are responsible.

Our findings in respect of age showed that the numbers of patients presenting for treatment decreased in succeeding age groups. It remains to be seen whether a rising mortality or a decrease in the severity of symptoms accounts for the smaller number of older children seen. Scott et al (1955) found an inverse ratio between the chronological ages of their patients and the incidence of hospital admissions, and attributed this finding to "the attenuating nature of the disease with increasing age". The impression gained from our cases is that the severity of the anaemia decreases with age, but other findings, such as liver dysfunction and crises of pain tend to increase with age. Longer follow up will, however, be required before these impressions can be confirmed.

TREATMENT.

There is no specific or curative treatment for sickle cell anaemia. Among the measures we used to alleviate symptoms, the following were most frequently employed.

Blood transfusions - 35 patients were transfused, 9 of them twice or more often. Transfusions were usually given only when the haemoglobin level fell below 5.7 g. per cent (40%). This policy was dictated by expediency in the first instance, but experience has shown that these patients do not require transfusion when their level of haemoglobin is higher unless there is the added complication of infection or severe pain. It is probable that the hazards of multiple transfusions far outweigh the possible benefit that might accrue from maintaining the haemoglobin at near normal levels. Wherever possible "push-ins", as opposed to "cut-downs", were used when giving blood. Preservation of accessible veins is as important in these cases as in persons with haemophilia.

Antimalarials - It has been suggested that malaria may act as a precipitating factor in the development of crises of sickle cell anaemia (Edington 1953, Colbourne and Edington 1956). Most of our patients presenting with fever and pain were given antimalarial drugs even in the absence of demonstrable parasitaemia. Not infrequently their administration seemed to be followed by relief of symptoms. However, the known tendency to periodic exacerbations and remissions in the disease

and the variability of symptoms during crises makes it impossible to assess the role played by antimalarials.

Antibiotics - 30 patients received penicillin, 9 chloromycetin, 8 aureomycin and 5 streptomycin. In many the use of antibiotics was dictated by recognisable infection such as osteitis, pneumonia and diarrhoea, but in others they were used empirically during crises on the assumption that symptoms might have been precipitated by infection. Many patients in the last group appeared to be improved by their administration.

Analgesics- Acetyl salicylic acid alone or in combination with codeine was frequently prescribed for pain. Results generally were poor, though slight relief was afforded to some. Occasionally pethidine had to be used when other measures failed to relieve severe pain.

Cortisone - 4 patients were given cortisone to control severe pain and swelling in the extremities. 2 showed dramatic improvement, one was not improved and the fourth developed suppuration in the affected parts and purulent meningitis. Suppuration following cortisone may have been purely coincidental but the known susceptibility of these patients to infection demands that caution be exercised in using the drug. Scott et al (1951) report A.C.T.H. and cortisone to be helpful adjuvants to blood transfusion in the control of anaemia, debility and pain.

Diet - Attention to the diet was an obvious necessity in our cases. All patients were advised to increase their protein intake, and some of

the outpatients received a daily ration of milk supplied by the hospital. Many received vitamin supplements, calcium lactate and ferrous sulphate, none of which seemed to have any effect on the clinical course of the disease.

Other therapeutic measures reported: -

1. Infusion of Dextran-fructose solution - Scott et al (1955) and Jenkins, Scott and Ferguson (1956) have reported the use of 6 per cent dextran with 5 per cent fructose as a substitute for blood transfusion in crises of sickle cell anaemia. Of 9 patients treated thus, 6 showed definite improvement of symptoms. An interesting and important observation in these cases was that the administration of the dextran-fructose mixture (given intravenously) was followed by an increase in the total mass of circulating haemoglobin. Jenkins et al (1956) suggest that this change resulted from mobilisation of erythrocytes trapped and stagnating in organ depots and/or small peripheral vessels. They further postulate that infusions of dextran may also be beneficial by exerting a "protein sparing" effect in relationship to liver function. (Observations on plasma volume and plasma proteins in their patients showed that the total plasma protein mass was greater than normal, despite a normal concentration of plasma protein per unit volume). The number of cases reported is small but indications are that the intravenous infusion of dextran-fructose solution will find a useful place in the management of crises of sickle cell anaemia, especially as it avoids many of the hazards of multiple blood transfusions.

2. Priscoline - Smith et al (1953) have reported dramatic relief of symptoms following the administration of Priscoline in crises of sickle cell anaemia. Their results offer indirect support to the theory that much of the pathology of sickle cell anaemia results from vascular spasm (Kimmelstiel 1948).

3. Cobaltous chloride - Cobaltous chloride has been used in an attempt to reduce the anaemia (Gross et al 1955, Scott et al 1955). Results show that following an initial rise the haemoglobin level falls to below the pre-treatment level. Severe toxic reactions to cobalt are reported, an interesting late effect being thyroid enlargement with hypothyroidism (Gross et al 1955).

4. Splenectomy - It is generally held that splenectomy does not benefit patients with sickle cell anaemia, but some authors have reported beneficial effects. Shotton, Crockett and Leavell (1951) and Leavell (1954) report that patients with massive spleens are usually benefited by splenectomy. The former authors also record a case with features of hypersplenism which was dramatically improved by removal of the spleen.

We have not employed splenectomy as a therapeutic measure. It seems extremely unlikely, however, that spleen size could be used as a reliable indication for splenectomy in our cases.

THE CLINICAL MANIFESTATIONS OF SICKLE CELL-HAEMOGLOBIN C DISEASE.

Although the indications are that sickle cell-haemoglobin C disease is by no means rare in Africa and America, relatively few cases have been reported. Schell and McGinley (1956) were able to find only 71 examples of this syndrome in the English language literature. The clinical manifestations of the disease have been very well described in several American reports, notably those of Smith and Conley (1953 and 1954), but reports from African sources, with one exception (Edington and Lehmann 1954) have not included details of clinical findings. Up to the present the syndrome has not been described in African children and American reports contain references to very few patients seen in the first decade of life. It has thus been thought worthwhile to present the individual case histories of our 8 patients, all of whom were under the age of 10 years.

CASE REPORTS.

1. T.A., (O.P. No. 26,027), a 4 year old Yoruba girl, when first seen complained of pain and swelling in both elbows and haematuria, of 3 days' duration. She had had no previous attacks of a similar nature. On examination she was well nourished, and apart from symmetrical, fusiform, tender swelling of both elbow joints and a palpable spleen, all systems were normal. Haemoglobin 11.1 g. per cent. White cell count 20,500 per c.mm., of which 87% were polymorphonuclear leucocytes. The urine contained red cells and a moderate number of pus cells. Culture of the urine showed a growth of coliform bacilli. No sickling of the red cells was seen in a stained film of peripheral blood, but a sealed moist preparation showed 100% sickling after 24 hours. There was reduced osmotic fragility of the red cells, and paper electrophoresis of the haemoglobin showed sickle-haemoglobin C. X-ray examination of both elbows showed no bone abnormality. She was given 600,000 units of

procaine penicillin daily for one week as an outpatient, and symptoms gradually subsided. When seen a fortnight later she was symptom free and no abnormalities were detected. She has not been seen since.

2. I.O., (O.P. No. 5269), an 8 year old Yoruba girl, gave a history of intermittent pain and swelling of the right leg associated with fever for 3 years. There were no other complaints. Her parents were both alive and well and a younger sister was in good health. Two older siblings had died but the causes of death were not known. On examination she was found to be well nourished and the mucosae well coloured. Apart from slight tenderness and nodularity of the subcutaneous surface of the lower half of the right tibia and a palpable spleen, physical examination showed nothing abnormal. Haemoglobin was 11.5 g. per cent; a sealed moist blood preparation showed 100% sickling in 24 hours; red cell osmotic fragility was normal, and paper electrophoresis of the haemoglobin showed sickle-haemoglobin C. X-ray examination of chest, legs and hands:- Chest - lung fields clear, heart normal, calcification present in glands in right hilum. Legs and hands - no bony abnormality detected. Kahn test negative. Despite the absence of malarial parasites from the peripheral blood film she was given chloroquin 0.2 g. twice daily for 2 days. Two weeks later she still complained of fever and pain in the leg, but in the following week symptoms subsided. When next seen 3 weeks later, she complained of pain in both arms, but there were no signs of local inflammation and her general condition was unchanged. She has not attended since.

3. R.A.,(I.P. No. 343), a 6 year old Yoruba girl, presented with a history of severe colicy abdominal pain of 4 days' duration. At the onset of the pain she had vomited copiously, but thereafter vomiting did not occur. The pain was intermittent and so severe that she "doubled up" during bouts. Bowel action had been normal until the day before admission, since when she had been constipated. There were no other symptoms. Prior to the onset of the pain she had been in good health. Both parents and one younger sister were alive and well. On examination she appeared very ill. She was pyrexial and the pulse rate was 120 per minute. There was no pallor of the mucous membranes, but the sclerae were slightly jaundiced. The respiratory and cardiovascular systems were normal. The abdomen was grossly distended, and there was tenderness in the epigastric and periumbilical regions. The spleen was palpable 3 fingerbreadths below the costal margin and was non-tender. Rectal examination elicited tenderness in the pouch of Douglas but no other abnormality. Haemoglobin was 10.85 g. per cent and the white cell count 10,200 per c.mm., with 71% polymorphs, 22% lymphocytes, 6% monocytes and 1% eosinophils. There were no malarial parasites in the peripheral film. A straight X-ray of

the abdomen taken in the erect position showed grossly distended loops of bowel with fluid levels. In spite of the splenomegaly and slight jaundice, the clinical picture simulated a "surgical acute abdomen" and the surgeons were consulted. After lengthy discussion it was decided that the evidence of intestinal obstruction was so striking that laparotomy was obligatory. At laparotomy the large bowel was found to be grossly distended and the mesenteric glands were enlarged, many showing small petechial haemorrhages. There was no other pathological lesion. Postoperatively the haemoglobin level fell sharply, and on the second day it was 4.9 g. per cent. The white cell count rose to 30,200 but a differential cell count showed that 50 per cent of the nucleated cells were of the red cell series. A few sickled erythrocytes were observed in the stained preparation. Using 2% sodium metabisulphite, marked sickling with filaments was demonstrated. Osmotic fragility of the red cells was greatly reduced and paper electrophoresis of the haemoglobin showed sickle-haemoglobin C. A blood transfusion was commenced, but because of a severe pyrexial reaction occurring after about 20 ml. had been given, it was discontinued. Subsequent progress was uneventful. The temperature subsided, the haemoglobin level rose spontaneously to 8 g. per cent on the ninth post-operative day, and the abdominal wound healed well. There was no recurrence of pain or vomiting. When seen 6 weeks after admission she was in good health, symptom free, the mucous membranes well coloured, and there was no jaundice. She has not reported back for further follow up.

4. S.S., (O.P. No. 27816), a 5 year old Yoruba girl, presented with a short history of fever, loss of weight and pain in the left side of the abdomen. There were no other complaints. She had been in good health prior to the onset of these symptoms. Her parents were alive and well. There was one other child alive and two had died. On examination she was found to be well nourished, weight 35 lb 2 ozs., mucous membranes well coloured, and there was no jaundice. The spleen was easily palpable, but there were no other abnormal findings. Haemoglobin was 12.58 g. per cent. A sealed moist blood preparation showed sickling of the red cells with scanty filament formation. The osmotic fragility of the red cells was reduced. Paper electrophoresis of the haemoglobin showed sickle-haemoglobin C. There were no malarial parasites demonstrable in the peripheral blood film. She was given Nivaquin empirically and observed for 4 weeks. Her symptoms gradually subsided. There has been no further follow up.

5. O.A., (O.P. No. 28218), a 9 year old Yoruba girl, complained of pain in both legs, affecting chiefly the ankles and the right knee. Pain had been present for 4 days and was severe enough to prevent her from walking. She also complained of anorexia. 4 months previously she had experienced similar pain in the arms. There were no other symptoms and her past history was of good health. Her parents and two siblings were alive and well. On examination she was slender, but her general nutrition was fair. The mucous membranes were well coloured. The apex beat was in the 4th interspace just external to the mid-clavicular line, and an apical systolic murmur was easily audible. The lung fields were clear. The spleen was just palpable. The liver was not enlarged. The right knee joint was swollen and tender and there appeared to be a small effusion into the joint. The ankles appeared to be normal. An X-ray of the right knee showed no pathological changes. Using 2% sodium metabisulphite sickling of the red cells was demonstrated. The osmotic fragility was reduced (initial 0.40, final 0.15) and paper electrophoresis of the haemoglobin showed sickle-haemoglobin C. The Kahn test was negative. She was given 600,000 units of penicillin daily as an outpatient. 4 days later she was much better and was walking. 9 days later she again complained of pain in the legs, and on examination the right ankle was found to be slightly swollen and very tender. She received 3 more injections of procaine penicillin and improved rapidly. When seen one week later she was symptom free and according to the mother playing normally with other children. She has not been seen since.

6. R.A. (O.P. No. 10154), a 7 year old Yoruba girl, presented with anorexia, fever and jaundice of 7 days' duration. She had had a similar episode one year previously. On examination she was apyrexial and her nutrition good, her weight being 43 lbs. She did not appear ill. The mucous membranes were well coloured, but there was definite scleral jaundice. The heart and lungs were normal. The liver was palpable 6 fingerbreadths below the costal margin. The spleen was not palpable. The haemoglobin was 11.84 g. per cent, and the reticulocyte count was 1.5 per cent. Red cells showed marked anisocytosis and there were numerous target cells. The differential white cell count showed 28% polymorphs, 66% lymphocytes, 3% monocytes and 3% eosinophils. The urine showed an excess of urobilinogen, but no bile pigments were detected. The serum proteins were:- albumen 3.2 g. per 100 ml., globulin 5.5 g. per 100 ml, an albumen/globulin ratio of 0.58. Serum bilirubin level was 5 mg. per 100 ml. and the Vandenbergh test gave a direct reaction. Thymol turbidity was 13 units. Sickling was demonstrated using 2% sodium metabisulphite; the erythrocyte osmotic fragility was markedly reduced; and paper electrophoresis of the haemoglobin showed sickle-haemoglobin C. A diagnosis of

hepatitis occurring in a patient with sickle-haemoglobin C disease was made. Over the next 6 weeks symptoms gradually subsided, jaundice became less marked but never disappeared completely, and the size of the liver remained the same. She defaulted from the clinic and has not been seen again.

7. O.M., a 9 year old Yoruba girl, presented with pain in both legs and arms affecting chiefly the joints, but not associated with any swelling. She had lost weight and was subject to occasional attacks of fever. She also complained of anorexia. These symptoms had been present for 2 months. She had previously been in good health. On examination her weight was 46 lbs. 10 oz. She was slender and her arms and legs appeared disproportionately long. Her fingers were long and tapering. Lumbar lordosis was exaggerated and the abdomen appeared slightly distended. (See Figures 53 a and b). The heart, chest, abdomen and X-rays of the

Fig. 53a Fig. 53b

Abnormal habitus in sickle cell-haemoglobin C disease. Note:-

1. The thin, disproportionately long limbs
2. The long tapering fingers
3. Slight exaggeration of lumbar lordosis
4. Slight abdominal distension.

skull, long bones and hands and feet revealed no abnormalities. The haemoglobin was 10.36 g. per cent, and a sealed moist blood preparation showed marked sickling after 24 hours. The tuberculin skin test was negative. Paper electrophoresis of the haemoglobin showed sickle-haemoglobin C. Symptoms subsided spontaneously during the following week and she was not seen again until 2 months later when she presented with pain in the right leg and arm and fever for one day. Physical findings were as stated above except that the spleen was now palpable. The haemoglobin had fallen to 8.28 g. per cent. The white cell count was 12,700. The red cells were hypochromic and numerous target cells were seen. Symptoms gradually subsided over the next 2 weeks, the spleen was no longer palpable, and the haemoglobin returned to its previous level. She has remained symptom free since.

8. S.A., (I.P. No. 1453/56), a 4 month old Yoruba girl, had been well until 5 days before admission, when she developed fever, diarrhoea, ulceration of the mouth, a slight cough, and swelling of the right elbow and right foot. Her appetite remained good, but despite a good intake she had lost weight. The fever and swelling of the elbow had become progressively worse. Her parents and two older brothers were alive and well. On examination she appeared ill and apathetic; the mucous membranes were very pale, but there was no jaundice; the mouth showed herpetic ulcerative stomatitis; she was pyrexial and there was a marked tachycardia; the heart was normal and the liver and spleen were not palpable; rhonchi were heard over both lung fields and there were scattered crepitations over the left lung. There was a large, tender, warm, fluctuant swelling over the outer side of the right elbow. The right ankle was slightly swollen and tender. X-rays of the elbows and feet showed no evidence of bone pathology. The haemoglobin was 5.32 g. per cent; white cell count 17,500 with 72% polymorphs, 26% lymphocytes, 1% monocytes and 1% eosinophils. The red cells showed poikilocytosis and aniscocytosis and slight polychromasia, and there were numerous target cells. No malarial parasites were seen in the peripheral blood film. Sickling was demonstrated using 2% sodium metabisulphite and electrophoresis of the haemoglobin showed sickle-haemoglobin C. Progress:- She was given penicillin and Nivaquin 0.2 g. twice daily for 2 days. The swelling on the right elbow was incised and thin yellow pus was found, which was sterile on culture. Serum submitted for a Widal test failed to agglutinate any Salmonella suspensions. Her condition improved rapidly; fever subsided and her weight rose from 11 lb. 12 oz. on admission to 12 lb. 4 oz. at the end of the first week. The haemoglobin rose spontaneously to 8.1 g. per cent

in the same period and the limb swellings and ulcerative stomatitis resolved. She was discharged from hospital on the seventh day, but returned 3 days later with fever and pain and swelling of the right ankle. She was put back on penicillin, but 5 days later was still pyrexial and the white cell count was 23,400 per c.mm. Chloromycetin was then prescribed. When next seen a week later, improvement was obvious, and apart from slight thickening of the soft tissues around the right elbow, she appeared to be normal on physical examination. The haemoglobin was 7.4 g. per cent. 2 weeks later the haemoglobin was 11.24 g. per cent, the weight 13 lb. 2 oz., all signs of local inflammation had resolved, and she was in obvious good health. She is still under observation and is progressing satisfactorily.

Table XXII summarises the main findings in our cases. It will be seen that the symptomatology was similar to that in sickle cell anaemia, but physical examination revealed far fewer abnormalities.

Age and Sex - The average age was 6 years as compared with 3.8 years in the sickle cell anaemia group. American reports indicate that the vast majority of patients with sickle cell-haemoglobin C disease are diagnosed in adult life, but Smith and Conley (1954) record that 7 of their patients developed symptoms in the first decade of life.

All our patients were female. No difference in the sex incidence has been recorded by other authors, including Smith and Conley (1953 and 1954), Ranney et al (1953), Neel et al (1953), Kaplan et al (1952). It is probable that our finding has been determined purely by chance.

Symptoms - Pain felt either in the limbs or the abdomen was the commonest presenting symptom. Pain was generally less severe than that encountered in sickle cell anaemia, but in one patient (Case 3)

TABLE XXII - MAIN SYMPTOMS AND SIGNS, HAEMOGLOBIN AND RED CELL FRAGILITY IN 8 CASES OF SICKLE CELL- HAEMOGLOBIN C DISEASE.

	Series No.	Age Yrs.	Sex	Site of Pain	Other Symptoms	Jaundice	Spleen	Liver	Heart	Limb Swellings	Hb. g.%	Osmotic Fragility
	1	4	F	Limbs	Haematuria	-	+	-	N.A.D.	Both elbows	11.1	Slightly reduced
	2	8	F	Limbs	Fever	-	+	-	N.A.D.	Right tibia	11.5	Normal
*	3	6	F	Abdomen	Vomiting Constipation	Slight	2-3 f.	-	N.A.D.	-	4.9 10.8	Greatly reduced
	4	5	F	Abdomen	Fever	-	+	-	N.A.D.	-	12.6	Reduced
	5	9	F	Limbs		-	+	-	Enlarged Systolic Murmur	Right knee and Ankle	Not Pale	Reduced
	6	7	F	-	Anorexia, Fever, Cough,	Marked Bilirubin 5 mg.%	-	6f.	N.A.D.	-	11.8	Reduced
	7	9	F	Limbs	Anorexia,	-	+	-	N.A.D.	-	10.4	Not done
**	8	4/12	F	Limbs	Fever, vomiting, diarrhoea	-	-	-	N.A.D.	Right elbow and ankle	5.3 11.2	Not done

* Abdominal crisis simulating intestinal obstruction. Laparotomy performed.

** Youngest case of sickle cell-haemoglobin C disease reported to date.

abdominal pain was more severe than in any of the cases of sickle cell anaemia. In 4 cases pain was associated with swelling of joints or over bone, but in none of these were the feet or hands involved.

Other symptoms included fever (5), anorexia and vomiting (4), jaundice (1), and haematuria (1).

Nutrition and Habitus - Nutrition was good in all except case 8, who was markedly underweight when first seen. After treatment, however, her weight became normal. Only one patient showed abnormal habitus (See fig. 53) resembling that seen in sickle cell anaemia. Smith and Conley (1954) record a similar finding in one of their cases. These authors report that most adult patients with sickle-cell-haemoglobin C disease have a rather stocky build without leg/trunk disproportion.

Physical signs.

Spleen - 6 patients had palpable spleens but in only one did the spleen extend more than one fingerbreadth below the costal margin. Splenomegaly was very much less marked than in the corresponding age group with sickle cell anaemia. Our findings in this respect are the reverse of those recorded by Smith and Conley (1954) in their comparative study of the genetic variants of sickle cell disease. If, as has been suggested earlier, malaria is causally related to the massive splenomegaly found in many of our cases of sickle cell anaemia, our findings in sickle cell-haemoglobin C disease would seem to imply that these patients are less susceptible to malaria. This is highly

conjectural, but it is interesting to note that none of the sickle cell-haemoglobin C group had malarial parasites demonstrable in their peripheral blood films.

Liver - Only one patient had an enlarged liver. The clinical picture in this case was indistinguishable from acute viral hepatitis. Hepatomegaly is an infrequent finding in sickle cell-haemoglobin C disease and severe hepatic dysfunction has not been recorded (Smith and Conley 1954). It seems unlikely therefore that the liver findings in this patient were a direct consequence of the disease.

Jaundice - 2 patients were jaundiced. In one it was associated with a severe haemolytic crisis and in the other with hepatitis.

Heart - One patient had slight cardiomegaly associated with an apical systolic murmur. The rest showed no cardiac abnormalities. Apical systolic murmurs are occasionally reported in sickle cell-haemoglobin C disease (Scott and Jenkins 1955, Smith and Conley 1954) but in general, reports indicate that abnormal cardiac signs are very much less frequent than in sickle cell anaemia.

Limb swellings - 3 patients had swollen joints (elbows, ankles and knees). One patient had slight nodularity of the subcutaneous surface of the right tibia. In all cases swelling was unassociated with radiological evidence of bone disease. In one patient (case 8) swelling of the elbow was associated with suppuration in the superficial tissues

outside the joint. Pus aspirated was sterile on culture.

Urine - One patient had frank painless haematuria. In spite of the fact that B. coli was isolated on culture it is likely that the haematuria resulted from sicklaemia rather than infection. (See page 122).

Haematological findings.

Haemoglobin - Haemoglobin levels were generally within the range encountered in normal Nigerian children. In 2 cases, however, the level fell markedly during crises, but in both subsequently returned to normal without the aid of blood transfusion.

Erythrocytes - Sickling was demonstrable in all patients. Osmotic fragility of the erythrocytes was reduced in 5 of the 6 cases tested. Our records on the red cell appearances in stained films of peripheral blood are incomplete, but in 3 cases target cells were recorded as being a prominent feature of the blood picture. All available reports indicate that a high percentage of target cells (up to 100%) is an invariable feature of sickle cell-haemoglobin C disease.

Leucocytes - White cell counts in 4 patients ranged from 10,000 to 20,000. In 3 the granulocytes predominated, but in one there were 66% lymphocytes.

Radiological findings.

No pathological changes were detected in any bones X-rayed. The only striking skeletal lesion which has been reported is avascular necrosis of the head of the femur similar to that seen in Legg-Perthes' disease (Ramsey et al 1953, Smith and Conley 1954). This finding was present in 5 of Smith and Conley's patients and in one of them it was bilateral. Avascular necrosis of the femoral head has also been described in 6 patients with sickle cell anaemia (Tanaka et al 1956). With 2 exceptions all cases with this complication have been over 20 years old. Smith and Conley record a case in which symptoms first occurred at 10 years of age and Tanaka et al found it in a boy of 13 years.

PROGNOSIS

There were no deaths. Crises were usually milder and less frequent than in sickle cell anaemia, but in 2 cases (Nos. 3 and 8) crisis was associated with grave illness. Between crises these patients appear to enjoy normal health. Our findings show that the immediate prognosis in sickle cell-haemoglobin C disease is very much better than in sickle cell anaemia. Probably this is true of the ultimate prognosis as well. Edington and Lehmann (1954) have reported a patient in whom symptoms, which started in infancy, gradually decreased in severity, and eventually disappeared at age 30 years. Five years later mild symptomsreappeared in association with pregnancy.

Smith and Conley (1954) have reported 2 patients who remained symptom free into the seventh decade of life. These reports confirm our impression that sickle cell-haemoglobin C disease is compatible with good health and longevity.

DIFFERENTIAL DIAGNOSIS OF SICKLE CELL ANAEMIA AND SICKLE CELL-HAEMOGLOBIN C DISEASE.

Table XXIII compares our findings in sickle cell anaemia and sickle cell-haemoglobin C disease. It will be seen that the groups present many differences but it will also be apparent that these are mainly due to variations in the intensity of the same symptoms and signs. Thus, while "typical" examples of either syndrome can usually be recognised clinically, an intermediate group, comprising mild cases of sickle cell anaemia and cases of sickle cell-haemoglobin C disease with severe manifestations, present diagnostic problems which can only be resolved by electrophoretic study of the haemoglobin. In general, the haemoglobin level and the presence or absence of swellings of the hands and feet and radiological bone changes were our most reliable clinical criteria for differentiation. It seems likely that, in view of the experience of others (Ranney et al 1953, Neel et al 1953, Edington and Lehmann 1954, Smith and Conley 1953 and 1954) a more careful evaluation of the percentage of target cells in the peripheral films in our cases would assist considerably in the clinical differentiation of these syndromes. It has been shown that almost all patients with sickle cell-haemoglobin C disease have more than 50% target cells, whereas patients with sickle cell anaemia rarely, if ever, approximate this figure.

TABLE XXIII - DIFFERENTIAL DIAGNOSIS OF SICKLE CELL ANAEMIA AND SICKLE CELL-HAEMOGLOBIN C DISEASE.

Finding	Sickle cell anaemia	Sickle cell-haemoglobin C disease.
Average age	3.8 years (many under 2 years)	6 years (rare under 2 years.
Pain	Frequent. Severe	Less frequent and less severe, but occasionally marked.
Limb swellings:-		
1. Hands and feet	Frequent	Never
2. Large joints	Sometimes	Sometimes
Habitus	Frequently abnormal	Usually normal
Jaundice	Frequent	Usually absent
Abdominal distension	Often marked	Usually slight or absent
Spleen	Enlarged, often huge	Enlarged, but rarely very big
Liver	Usually enlarged, often massive	Usually not enlarged
Heart	Often abnormal	Rarely abnormal
Radiological bone changes	Frequent	Absent
Osteitis	Frequent	Absent
Anaemia	Moderate to severe	Usually absent or mild, but may be severe in crisis
Stained peripheral blood films:-		
1. Sickling	Often present	Usually absent
2. Target cells	Often present	Always present and numerous
3. Reticulocytosis	Usually present	Absent or minimal
Red cell osmotic fragility	Reduced	Reduced
Sickling (sealed moist preparations or 2% sodium metabisulphite)	Present	Present
Electrophoresis	Haemoglobin S	Haemoglobins S and C
Blood transfusions	Frequently necessary	Only exceptionally

HOMOZYGOUS HAEMOGLOBIN C DISEASE.

There have been few reports of this syndrome. Only one case has been reported previously from Africa (Edington and Lehmann 1954). Table XXIV lists the findings in 11 reported cases. It will be seen that with one exception all reported cases have been adults, the majority over 30 years of age.

Case Report

A.A., (Clinic No. B.101), Yoruba, female, age 6 years. When first seen she complained of cough and fever which had been present for many weeks. There were no other complaints. On examination she was found to be well nourished and normally developed (weight 47½ lbs.), the mucous membranes were well coloured and her general condition was good. Shotty glands were palpable on both sides of the neck and in both groins and axillae. The cardiovascular system and abdomen were normal. There were bilateral rhonchi audible throughout both lungs with persistent sticky crepitations at both bases. The tuberculin skin test was negative. An X-ray of the chest showed only slight basal congestion. No other investigations were done at the time and she was treated for a respiratory infection with sulphamezathine. She was seen several times in the next two months and though the cough persisted all chest signs disappeared and subsequent X-rays were completely normal. She was next seen 6 months later, when she complained of

1. Pain in the right knee, which she attributed to a fall one month previously.
2. Periodic attacks of abdominal pain not associated with bowel disturbance.
3. Slight cough.
4. Passing round worms.

Examination showed no abnormality apart from slight splenomegaly. X-rays of the knees, skull, hands and feet showed no pathological changes. The haemoglobin was 10.5 g. per cent, white cell count 13,400 with 38% polymorphs, 38% lymphocytes, 17% eosinophils and 7% monocytes. There were 0.5% reticulocytes and a stained film of peripheral blood showed many target cells. The red cells could not be induced to sickle. Paper electrophoresis of the haemoglobin showed haemoglobin C only.

Progress:- She was given an anthelminthic and expelled many ascarides. Two weeks later she still complained of pain in the knee and was limping. A few septic superficial skin lesions were present around the affected knee but there was no evidence of bone or joint involvement. Procaine penicillin was prescribed and when next seen a week later she was

TABLE XXIV - MAIN FINDINGS IN 11 REPORTED CASES OF HOMOZYGOUS HAEMOGLOBIN C DISEASE.

Authors	Age Yrs.	Sex	Symptoms	Spleen	Liver	Hb.g.%	Reticulocytes	Target Cells	Osmotic Fragility r.b.c.'s.
(a)	4	M	Haematoma right eye	+		9.8	0.8-1.2	+++	Reduced
(b)	35	F	Joint pains	5 cms.		10.0	3.3	100%	Reduced
(c)	41	F	Fatiguability	++		9.6		++	
(d)	Middle aged	M	Joint pains			12.8		++	
(e)	24	F	Nil	10 cms.				+++	
(f)			Joint and abdominal pain			11.0		60-90%	
(g)	57	M	Cardiac failure, alcoholic	++	++	Not anaemic	1.2	+	
(g)	40	M	Pneumonia	6 cms.	3-4cm.	10.9		+	Reduced
(g)	34	M	"Dizziness" Dyspnoea	8 cm.		13.4	2.2	+	Reduced
(g)	24	F	Dizziness Pain in knees	4 cm.		10.6	4.2-5.8	+	Reduced
(h)	39	F	Anaemia of pregnancy	2 fb.	4 fb.	8-14.7	2.2	++	Reduced

(a) Spaet, Alway and Ward (1953)
(b) Ranney, Larson and McCormack (1953)
(c) Levin et al (1953)
(d) Edington and Lehmann (1954)
(e) Morgan, Bowles and Harris (1955)
(f) Harris, quoted by Morgan et al (1955)
(g) Hartz and Schwartz (1955)
(h) Rice (1957).

completely symptom free. She is still under observation.

(Eosinophilia has not been reported in haemoglobin C disease. It is probable that in this patient it was related to the heavy infestation with Ascaris lumbricoides).

Comment - Haemoglobin C disease is an inherited haemolytic syndrome so far reported only in Negroes. Symptoms are usually mild, but may be absent. The most frequent complaints are pain in the joints and fatiguability. The most constant finding on examination is splenomegaly, which may be very marked. The usual haematological findings are listed below -

1. Anaemia - mild or absent
2. Target cells - invariably present and usually very numerous (up to 100%)
3. Reticulocytosis - mild or absent
4. Erythrocyte osmotic fragility - usually reduced
5. Erythrocyte sickling - never
6. Electrophoresis - haemoglobin C only.

Prognosis - The prognosis appears to be very good.

The haemoglobin C trait is present in high frequency in West Africa and it is likely that there are many C homozygotes in the general population. The low reported incidence of haemoglobin C disease probably only reflects the fact that many cases remain symptom free or present with such mild manifestations that they are overlooked in a population in which splenomegaly and mild anaemia are widely prevalent.

DISCUSSION.

Vandepitte (1955) has estimated that in every generation of Negroes born in Africa more than a million die of sickle cell anaemia. Observed facts in the Belgian Congo accord with this estimation and our experience in Ibadan agrees with this. The 75 cases which have been described are not the only examples of this syndrome which we have seen. Case selection for this presentation was based on absolute certainty of diagnosis and the availability of adequate case records.

Ibadan has a population of at least half a million, of whom approximately one quarter show the sickling trait. In such a population the incidence of sickle cell anaemia can be estimated at 15.6 per thousand births (Vandepitte 1955). It is hardly surprising therefore that the disease is frequently seen, and probably many more cases will be diagnosed as medical services expand to cover a wider section of the population and awareness of the incidence of the disease grows in the ranks of the medical profession.

Our experience is very different from that of Jelliffe and Humphries, who, working in the same locality, reported in 1952: "It has been our experience that in Ibadan examples of sickle cell anaemia are definitely uncommon, especially when considering the large pool of sicklaemics present in the area". (Jelliffe and Humphries, 1952b). The likely explanation for the discrepancy between their findings and ours is that we were more advantageously placed in respect of staff

and laboratory services than they were. It should be noted that sickle cell anaemia accounts for only a relatively small percentage of severely anaemic children seen in Ibadan. During 1956 223 severely anaemic children were admitted to our ward. As Jelliffe and Humphries themselves observed, the average haemoglobin level of children between the ages of 4 months and 2 years in Ibadan is probably between 50 and 60 per cent. Against such a background the diagnosis of sickle cell anaemia, in the absence of adequate clinical and laboratory facilities, may understandably be overlooked.

Our experience in Ibadan strongly reinforces the observations of the Lambotte-Legrands (1951 and 1955 a and b), Vandepitte (1955), Foy and Kondi (1951) and others, that in Africa sickle cell anaemia is a disease mainly of infancy and childhood. This is attested by the fact that during 1955 only 6 cases of the disease were seen in the adult medical wards, whereas about ten times this number were seen in the children's ward. This fact is still not generally appreciated, principally because we have been conditioned to think of the disease in terms of the experience recorded in America, mainly in adults, but also because the clinical picture of sickle cell anaemia in African children as yet lacks definition, and clinicians have little to guide them in their efforts to distinguish the disease from the many other causes of anaemia in the African child.

What are the salient features of the disease in Africa and

how do they differ from those usually recorded by American authors? We have found that sickle cell anaemia in Nigerian children under age 10 years is characterised by frequent episodes of fever, limb pains often associated with inflammatory swellings, and various gastro-intestinal complaints, including anorexia and vomiting, abdominal pain and distension, and diarrhoea. Anaemia is constantly found, but varies in severity. Hepato-splenomegaly is present in about 50 per cent. of patients in the first 2 years of life, and increases in frequency thereafter. Inflammatory swellings of the hands and feet are commonly seen in the early years of life. Many patients have cardiomegaly and cardiac murmurs, and the incidence increases with age. Evidence of hepatic dysfunction is relatively frequent in the latter half of the first decade. Bone changes demonstrable radiologically, which are attributable to marrow hyperplasia and infarction, are commonly seen, and are sometimes complicated by suppuration. Some patients show an abnormal habitus. Leg ulcers do not occur. Lymphadenopathy is rare. The typical haematological findings are normochromic or hypochromic anaemia, reticulocytosis and other evidence of active erythropoiesis, polymorphonuclear leucocytosis, target and sickled cells in stained peripheral films, reduced osmotic fragility of the erythrocytes; and all erythrocytes can be induced to sickle, many showing long tapering filaments.

It is obvious that many of the clinical features listed occur in a number of unrelated conditions prevalent in tropical Africa.

Their occurrence in non-sicklers calls for no comment, as routine sickling tests readily eliminate this source of confusion. In children who have the sickling trait and who present with diseases associated with anaemia, splenomegaly, etc., differentiation is less easy, but even without the aid of subtle haematology most cases can still be differentiated. Among the most valuable aids to clinical diagnosis the "rheumatoid" manifestations of sickle cell anaemia (limb pains, joint swellings, swellings of hands and feet, cardiac signs) feature prominently. This is an African paradox. In America, differentiation of sickle cell anaemia from rheumatic fever and other rheumatic disorders constitutes a very real problem. In tropical Africa rheumatic disorders are very rarely seen. During my 14 months in Ibadan no instance of a rheumatic disease was recorded. "Rheumatism" in an anaemic African child, in our experience, is virtually synonymous with sickle cell disease. In particular the swelling of the hands and feet in the very young is an invaluable aid to diagnosis. In cases where clear evidence of sickle cell anaemia is not immediately apparent, diagnosis can usually be made by observing the course of the disease and noting the response to treatment. Malarial anaemia during the first 2 years of life in children with the sickle cell trait can closely simulate sickle cell anaemia, but observation of the response to antimalarial drugs will usually resolve the problem. Adequate treatment of the malaria leads to rapid improvement of the anaemia, the haemoglobin level usually being restored to near normal levels within a matter of weeks (Hendrickse and King 1957).

In addition to the foregoing, we have found radiological bone examination a very useful aid to diagnosis in many cases. Haematological differentiation of anaemic trait carriers and sickle cell anaemia may present difficulties, but usually the appearances in stained peripheral films and in sickled preparations permit reasonably accurate diagnosis. Where doubt persists and facilities for electrophoretic examination are not available, examination of the bone marrow can provide a clear answer. It has recently been demonstrated that in sickle cell anaemia marrow preparations constantly show peculiar accumulations of haemoglobin filaments enclosed between the blood-forming elements. These may be so numerous as to look like mycelia (Vandepitte, 1955).

Differentiation of sickle cell anaemia from its genetic variant, sickle cell haemoglobin C disease, has been dealt with elsewhere and will not be discussed further.

Comparison of our findings and those of others in Africa with the experience recorded by American authors in sickle cell anaemia shows the following differences:- In Africa the age at onset of severe symptoms is earlier, anaemia tends to be more severe, the incidence of splenomegaly is higher and spleens tend to be larger, gross hepatomegaly and evidence of hepatic dysfunction generally occur at an earlier age, limb swellings, especially those involving the hands and feet, in the early years of life, are common whereas they are rarely reported by American authors, radiological evidence of

of bone involvement is more frequent, and ultimate prognosis appears to be infinitely worse.

These differences in general reflect the more rigorous conditions in Africa but certain features of the disease, as seen by us, may be attributable to specific environmental factors. The probable role of protein deficiency in determining the age at onset of liver dysfunction has been mentioned, and reference has also been made to the possible role of a particular African custom in causing the occurrence of swellings of the hands and feet (see page 75). A high incidence of bacterial infections and endemic malaria are two environmental factors which contribute considerably to morbidity and mortality in Africa. The possible effects of these on the course and manifestations of sickle cell anaemia can only be determined in the light of our knowledge of the basic mechanisms underlying the manifestations of the disease. Let us therefore briefly review current knowledge of the pathogenesis of the lesions in sickle cell anaemia before returning to the consideration of the role of infection and malaria in the disease in Africa.

Mechanisms underlying the clinical manifestations of sickle cell anaemia.

The discovery of an abnormal haemoglobin in sickle cell anaemia and analysis of its physico-chemical properties have helped to clarify the mechanism of the sickling phenomenon and permitted

clear differentiation of the sickling trait, the anaemia, and allied syndromes. There are still, however, many features of the disease which, even in the light of the recent remarkable advances in knowledge, have no certain explanation.

It has been clearly demonstrated that the erythrocytes of persons with sickle cell anaemia have a greatly diminished lifespan in vivo. (Singer et al., 1948, Singer, Motulsky and Wile, 1950). The reason for the increased rate of destruction has, however, not yet been satisfactorily explained. An attempt has been made to explain this finding by suggesting that massive intravascular sickling occurs in areas where oxygen tension is low and sickled cells impact in capillaries, with subsequent vascular occlusion and ultimate lysis of the sickled cells. As Singer (1951) and others have pointed out, while vascular occlusion undoubtedly occurs, this mechanism cannot explain the sustained anaemia in patients who remain free of evidence of vascular occlusion. Our own findings show clearly that while anaemia was a constant and unremitting feature of allour cases, many showed no evidence of vascular occlusion. Further, it has been shown that prolonged oxygen administration (80 to 100 per cent. for 8 to 20 days) decreases the degree of intravascular sickling but does not reduce the rate of haemolysis in sickle cell anaemia (Reinhard et al., 1944). It is obvious that some other mechanism must be involved in erythrocyte destruction. It has been postulated that an additional extrinsic or intra-erythrocytic factor which also contributes to premature disinte gration of the erythrocyte may be present in sickle

cell anaemia (Singer, Motulsky and Wile, 1950). Tosteson et al., (cited by Dacie 1954) have shown that deoxygenated sickle cells quickly lose major amounts of potassium and gain substantial amounts of sodium, while sickle trait cells behave normally. Dacie (1954) suggests that these changes may depend on actual damage to the cell membrane and states: "If this is so, this work then provides evidence of an additional and perhaps all important mechanism of cell destruction".

From time to time exacerbations in the severity of the anaemia may occur. Singer et al. (1950) distinguish three mechanisms whereby anaemic crises may be produced in sickle cell anaemia and other haemolytic syndromes, viz.,

1. The "aplastic" type caused by failure of erythropoiesis.
2. The hyperhaemolytic type produced by an abrupt increase in erythrocyte destruction.
3. The "aplastic-hyperhaemolytic" type which results from combination of (1) and (2) above.

These authors described 2 cases of sickle cell anaemia in which severe aplastic crises developed in association with acute respiratory infection. Chernoff and Josephson (1951) have also reported aplastic crises in 4 children with sickle cell anaemia. In 3 crisis was precipitated by upper respiratory infection and in the fourth by infection with Salmonella cholerae-suis. Aplastic crisis in association with infection has also been described in hereditary spherocytosis (various authors cited by Dacie, 1954). The rapidity with which anaemia develops

when erythropoiesis fails is directly proportional to the lifespan of the erythrocyte.

It is important to realise that exacerbation in the severity of the anaemia may occur in the absence of any increase in the severity of other manifestations of the disease, and conversely that "clinical crises" of limb pain, abdominal pain, fever, etc. may occur without increase in severity of the anaemia. Diggs (1956) recently reported findings in 166 patients with sickle cell anaemia seen in 747 "clinical crises". He recorded that there was no evidence of a more severe anaemia during or following crises as compared with the values for erythrocytes and bilirubin previous to crises. Similarly there was no significant change in the reticulocyte count, the nucleated red cells, the percentage of sickled cells or the excretion of urobilinogen. He concluded: "The concept of a haemolytic crisis in sickle cell anaemia is a myth which should not be perpetuated in the light of present knowledge". This is an extreme view which has not taken into account the experience recorded by other workers (see Margolies 1951, Singer et al, 1948), but the data presented to substantiate it are impressive. Our own findings show that "clinical crises" often occur in the absence of exacerbations of the anaemia, but in some patients the two coincide. Available data on our patients do not permit an opinion as to whether increase in anaemia was more often due to temporary bone marrow aplasia or to hyperhaemolysis, but 3 patients who died were thought to have died in "haemolytic" crises. An implication inherent in the lack of correlation between

"clinical" crises and "anaemic" crisis is that different mechanisms are involved in their pathogenesis.

It has been presumed that the clinical manifestations of sickle cell anaemia, other than those referable to anaemia per se and compensatory hyperplasia of erythropoietic tissue, can all be attributed to intravascular sickling and the consequences thereof. Assuming that these are the only factors involved in producing the protean manifestations of the disease, we are still faced with the question, why do periodic exacerbations of intravascular sickling occur. There is reason to doubt the validity of the presumption that intravascular sickling is the sole cause of the clinical manifestations peculiar to sickle cell disease. Prolonged oxygen administration during crises of pain has been shown not to relieve symptoms even though it decreases intravascular sickling (Reinhard et al. 1944). Kimmelstiel (1948) did careful pathological examination of a case of sickle cell anaemia which had succumbed in crisis following cholecystectomy. He found multiple large foci of ischaemic necrosis in the internal organs, including the brain, liver and kidneys. These lesions were not associated with any organic changes in the vascular tree. In particular, thrombi were not observed. He reviewed the literature and found that, in contrast with current opinion, there was a lack of correlation between parenchymatous necrosis and evidence of mechanical vascular occlusion. On the basis of these findings, Kimmelstiel postulated that the crisis of sickle cell

anaemia constitutes a state of shock or pre-shock, initiated by factors which decrease oxygen tension in the peripheral blood). Peripheral vascular spasm occurs as a compensatory mechanism to maintain normal blood pressure. He suggests that vascular spasm may lead directly to ischaemic necrosis or may be followed by capillary dilatation and stasis. During this phase of failure of the peripheral circulation capillaries become packed with sickle cells. He believes this phenomenon, i.e. capillary engorgement with sickled cells, to be the result rather than the cause of stasis.

Certain clinical observations offer indirect support for the hypothesis that vascular spasm may be an important factor in the pathogenesis of the lesions in sickle cell anaemia. Smith et al. (1953) were able to afford dramatic relief of symptoms during crises by administering the vasodilator Priscoline. Our own observation of bilateral symmetrical peripheral lesions are more easily explicable by invoking the concept of vascular spasm followed by capillary blockage by sickled cells than by simply attributing them to diffuse intravascular sickling dependant only on reduction of haemoglobin within the physiological range. The observation recorded by Bark and Bull (1943) that crises seemed to occur in association with cold weather in their patient, may also be attributed to alterations in vascular tone induced by cold. Further, it can be postulated that relief of symptoms during crises afforded by the intravenous infusion

of dextran-fructose solution (Jenkins et al. 1956) may have come about through decrease in vascular spasm following increase in blood volume. The alternative explanation that the solution had a direct inhibitory effect on intravascular sickling seems very much less likely.

There seem to be sufficient grounds to justify serious consideration of the theory of vascular spasm as an important mechanism in the pathogenesis of crises and ischaemic lesions in sickle cell anaemia. Should this prove correct it will provide a rational basis for therapy (especially for the relief of pain) during crises. It may also indicate an additional constitutional defect in sickle cell anaemia.

Whatever the mechanism involved in producing the crises of pain and the ischaemic lesions may finally prove to be, we will still be faced with the problem of determining the factors that precipitate these changes. When discussing exacerbations in the severity of the anaemia, mention was made of the role of infection in producing depression of erythropoiesis. Further consideration of the role of infection in sickle cell anaemia follows.

Role of infection in sickle cell anaemia.

Many facts point to an increased susceptibility to bacterial infection in sickle cell anaemia. American authors, including Scott et al. (1951 and 1955), report upper respiratory infection as a frequent concomitant of crises. The incidence of osteitis in sickle cell anaemia

is very much higher than in the general population. The Lambotte-Legrands (1951) report influenzal meningitis as a frequent terminal event in their cases. A peculiar susceptibility to Salmonella osteitis has also been demonstrated. The likely explanation for these findings appears to be that vascular stasis and foci of ischaemic necrosis, which occur in sickle cell anaemia, offer ideal sites for organisms to lodge in and multiply. Transient bacteriaemia, which in normal persons is usually without sequel, assumes very much graver significance in sickle cell anaemia. Our experience with Salmonella osteitis in sickle cell anaemia reflects this. Many strains of Salmonella abound in the Ibadan area and can be recovered from a number of domestic animals and from lizards, which appear to be more numerous than human inhabitants in the town. Enteral infections with salmonella are encountered at all ages, but osteitis is rarely seen except in sickle cell anaemia.

Are there any reasons for believing that infection may precipitate "clinical" crises? There are definite theoretical grounds for suggesting the possibility, viz.,

1. Infection increases oxygen utilisation in the tissues
   (a) in local inflammatory reaction
   (b) by causing pyrexia which raises the basal metabolic rate.

2. Infection may be associated with widespread alteration in vascular tone, namely arteriolar constriction and venous dilatation (Best and Taylor, 1951).

The combined effects of these changes are to produce exactly the conditions postulated to account for crises in sickle cell anaemia. Clinical observations lend support to theoretical considerations. Case 21 (see Appendix II) had frequent crises during periods when infection was evident. Antibiotics appeared to afford relief during crises in some cases even in the absence of demonstrable infection. Scott et al. (1951 and 1955) have stressed the frequent association of respiratory infection and crises. Infection should thus be regarded as a likely "trigger" factor in precipitating crises and ischaemic lesions in sickle cell anaemia.

Ibadan has a very high incidence of bacterial diseases due mainly to unfavourable environmental factors such as bad sanitation, poor general hygiene and vast natural reservoirs of infection in the animal population. Some of the differences between our cases and those reported by American authors, in particular the high incidence of bone infarction in our series, probably result from differences in the incidence of infection.

Recognition of the several ways in which infection may be deleterious to the patient with sickle cell anaemia, viz.,

1. by causing aplastic crises.
2. by complicating ischaemic lesions.
3. by precipitating "clinical" crises

must influence our management of the disease, especially where en-

virorment presents the unsatisfactory features found in Ibadan. The routine use of antibiotics during crises and long term prophylactic chemotherapy (similar to that employed to prevent relapses in rheumatic fever) are measures which might favourably influence the course of the disease.

Sickle cell anaemia and malaria.

Much attention has been devoted to the role of malaria in determining sickling frequencies in Africa (see Part One), but the effects of malaria on the clinical course of sickle cell anaemia have received little attention.

What is the incidence of malaria in sickle cell anaemia? 10 of our cases (13 per cent) had demonstrable parasitaemia. This is a much lower incidence than is recorded in the general child population in the Western region of Nigeria, where approximately 76 per cent. of children between the ages of 1 and 10 years show parasitaemia (Walters 1956). Our findings in respect of spleen size (compared with American reports) and apparent response to antimalarials might, however, be interpreted as indicating a higher incidence of malaria than was demonstrated by examination of the peripheral blood.

Malaria may cause severe anaemia by haemolysis. It may also cause depression of erythropoiesis (Stitt 1945). Knisely and Block (1942) cited by Edington (1953) have described massive intravascular

agglutination in vivax and falciparum malaria, with local tissue anoxaemia resulting from clumping of erythrocytes. They described a case of sickle cell anaemia in which this occurred. It is very apparent that any of these processes will have more serious consequences in sickle cell anaemia than in normal persons. Their combined effects might be disastrous. Edington (1953) believes that crises of sickle cell anaemia are precipitated by malaria, and has observed that crises occur more frequently during the time of the year when malarial transmission is at its height. It should be noted, however, that in the Gold Coast, where these observations were made, maximal malarial transmission occurs during the cold rainy season. This raises the question whether the crises might have been related to the influence of the weather per se or to intercurrent respiratory infections, which tend to be prevalent during winter months.

There are grounds for contending that persons with sickle cell anaemia are probably less prone to malarial infection and its consequences than normal persons. Allison has demonstrated that the sickling trait confers an advantage against malaria (see Part One). It is reasonable to presume that in sickle cell anaemia, where all or most of the haemoglobin is of the "S" type, resistance to malaria will be even greater. A variable percentage of foetal haemoglobin is present in sickle cell anaemia. It is believed that the presence of this pigment in the erythrocytes of normal newborn babies may account for the fact that malaria is extremely rare in the first weeks of

life, even in children born of infected mothers with heavy placental infection (Bruce-Chwatt 1952). The presence of foetal haemoglobin in sickle cell anaemia may thus also help to prevent malaria. Further, Raper (1956) has reported that he has never encountered cerebral malaria in children with the sickle cell trait although it not infrequently complicates malaria in children without the trait.

In the present state of our knowledge no definite conclusions can be arrived at about the role of malaria in sickle cell anaemia. The relationship between the two conditions offers a fascinating subject for future clinical research. The possibility that malaria might be a factor in the less favourable prognosis of sickle cell anaemia in Africa should be borne in mind and should influence our management of cases.

Finally a few words about haemoglobin C and the diseases associated with its inheritance. Knowledge of haemoglobin C is very recent, but already the pattern of its distribution in Africa has been sketched. The haemoglobin C trait has been found in quite high frequency in several territories West of the River Niger in West Africa. To date the highest incidence has been recorded in the Gold Coast (Part One Section Two). From our knowledge of the distribution of the sickle cell and haemoglobin C genes and their mode of inheritance we can deduce the incidence of diseases caused by homozygous inheritance of either or the heterozygous combination of the two. It can

be assumed that sickle cell-haemoglobin C disease and homozygous haemoglobin C disease must be relatively common in West Africa, yet reports of their clinical recognition are still very few. This is understandable. These diseases in their usual form cause relatively little disability, symptoms as a rule are not dramatic and clinical signs are unimpressive. Seen against the African background with its high incidence of disease with extravagant pathology it is hardly surprising that cases are overlooked. When sickle cell-haemoglobin C disease does occasionally present with dramatic symptoms, the picture is very similar to that of sickle cell anaemia, and as erythrocyte sickling occurs in both conditions, the former may readily be confused with the latter.

The characteristic haematological findings which occur in association with haemoglobin C are-

1. A high incidence of target cells
2. Reduced osmotic fragility of the erythrocytes.

Target cells occur in a number of other conditions, including thalassaemia (target-cell anaemia), sickle cell anaemia, and cirrhosis of the liver. Their occurrence in association with haemoglobin C is thus not specifically related to this pigment in the same way as sickling is to haemoglobin S. However, although not specific for haemoglobin C, a high incidence of target cells discovered in any patient in West Africa should put one in mind of the disease associated with haemoglobin C. As previously mentioned, the percentage

of target cells in sickle cell-haemoglobin C disease is almost invariably above 50 per cent. while in sickle cell anaemia it rarely approaches this figure (Smith and Conley 1954). In this connection it is interesting to note that Edington (1953), reporting "sickle cell anaemia" in the Gold Coast, recorded a very high incidence of target cells in many of his cases. It seems likely, in view of the known high incidence of haemoglobin C in this area, and the fact that many of his patients were relatively symptom-free, with slight or absent anaemia, that some of the cases reported were in fact sickle cell-haemoglobin C disease. Diagnosis was not confirmed by electrophoresis of the haemoglobin.

Differences in the clinical manifestations of sickle cell anaemia and sickle cell-haemoglobin C disease are attributable to differences in the quantity of sickle cell haemoglobin in the erythrocytes in the two conditions. In the former where all or most of the haemoglobin is of the S type, intravascular sickling and the consequences thereof occur very much more readily than in the latter. In homozygous haemoglobin C disease sickling does not occur, but these patients may also have periodic attacks of mild arthralgia and occasionally abdominal pain. Here there is clear indication that these manifestations in sickle cell disease may involve other mechanisms than intravascular sickling.

The origin and significance of haemoglobin C in West Africa have yet to be determined. It has been suggested that the haemo-

globin C gene might have arisen by mutation of the sickling gene (Beet Haemant 1954) and that it might confer a better selective advantage than the S gene in relation to malaria (Walters and Lehmann 1956). In connection with this latter suggestion it is interesting to note that while 13 per cent. of patients with sickle cell anaemia had demonstrable malarial parasitaemia, none of the patients with sickle cell-haemoglobin C disease were found to have parasites. Spleen sizes recorded in the two conditions in our series, compared with results recorded by American authors, also provide some grounds for speculating about differential susceptibility to malaria in favour of haemoglobin C. Such speculation, however, must be tempered by the knowledge that these findings have little or no statistical value.

SUMMARY.

1. The sequence of events, following the first description of sickle cell anaemia, which led to the discovery of the abnormal haemoglobins and their mode of inheritance is described. Recent trends and new problems in medicine, biochemistry, genetics and anthropology which followed these discoveries are briefly reviewed.

2. The incidence of the sickle cell and haemoglobin C traits in Africa is described. Sickling frequencies in East and West Africa are compared and it is shown that the sickle cell trait is more evenly distributed and its overall frequency is higher in West than in East Africa.

3. Clinical findings in Nigerian children with sickle cell anaemia, sickle-cell haemoglobin C disease and homozygous haemoglobin C disease are described.

Data presented on 75 cases of sickle cell anaemia include symptomatology, physical signs, nutrition and habitus, haematological findings, radiological findings, liver function, urinalysis, relationship to tuberculosis, prognosis and treatment.

These findings have been compared in relation to age and have also been compared with those recorded by workers elsewhere in Africa and in America. Many differences emerge from the comparisons, but the most striking have been the high incidence

of "rheumatoid" swellings of hands and feet in African children in the early years of life and of radiological bone changes in our cases generally.

4. 8 case histories of children with sickle cell-haemoglobin C disease are presented. The clinical picture is reviewed and compared with that of sickle cell anaemia. The high incidence of target cells in sickle cell haemoglobin C disease is mentioned and its value in diagnosis is discussed.

5. A case of homozygous haemoglobin C is presented. Findings in other reported cases are summarised. The typical clinical picture and haematological findings are reviewed.

6. The discussion deals with the high incidence of sickle cell anaemia in Africa, the overwhelmingly great incidence of the disease in children in the continent, the clinical picture in African children and its differentiation from other forms of anaemia. The probable mechanisms determining the clinical manifestations of the disease and the onset of crises, and the influence of environment on the course of the disease, with special reference to the role of bacterial infection and malaria, are also discussed. In addition there is a brief discussion of the incidence of diseases associated with haemoglobin C in West Africa, their clinical manifestations, especially the high incidence of target cells, and the relationship of haemoglobin C and malaria.

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APPENDIX I

STATISTICAL METHODS USED

The statistical procedures used to analyse the relative frequencies of the sickling trait in East and West Africa were chosen to test the two hypotheses: -

(a) That sickling is more evenly distributed in West Africa than in East Africa

(b) That the incidence of sickling in West Africa is higher than that in East Africa.

There were, for analysis, figures for 128 samples of the East African population and 49 samples of the West African population. These samples comprised varying numbers of individuals in each sample. The incidence of sickling in each sample was expressed as a percentage of the total number of individuals examined. These percentages were used for calculation without transformation.

The variance between samples for each population was calculated as

$$s^2 = \frac{\sum_k (p)^2 - (\sum_k p)^2 / k}{k - 1}$$

where p_x denotes the percentage for each sample and x runs from 1 to k and where k denotes the total number of samples for each population.

A value for F was obtained as the quotient of the variances for East and West Africa and looked up in a table of F with 127 and 48

degrees of freedom. The variance for East Africa was found to be 136.66 and that for West Africa 39.81, indicating that the mean sickling frequency for West Africa is significantly greater than that for East Africa.

Both of these statistical procedures, it can be seen, assume that the between sample variance is the sole source of variance, or that the within sample variance about the true percentage for each population is nil. The numbers of cases examined in each sample were large enough, it was felt, to assume that their within sample variance, while undoubtedly present, made a negligible contribution to the total variance.

This analysis also presumes that the between sample percentages in each population are distributed normally.

APPENDIX II

Case Report - Sickle cell anaemia with unusual bone changes.

F.O. (Case No. 21), a Yoruba girl aged 10 years, was under observation in the children's ward for a continuous period of 14 months. She was admitted with the following complaints:- Pain in the left knee for 6 months, swelling of the joint for 2 months, and loss of weight over the same period; pain and swelling of the left ankle for 2 weeks and slight cough for 1 week before admission. When first examined she was cheerful and co-operative and displayed normal intelligence. She was very emaciated and showed slight pallor of the mucous membranes, with slight scleral jaundice. The heart was enlarged, the apex beat being in the 5th left interspace outside the midclavicular line. A soft systolic murmur was heard all over the praecordium, but was maximal in the pulmonary area. E.C.G. showed no abnormality. The lung fields were clear. The abdomen was slightly distended, but the liver and spleen were not palpated. (The liver later became palpable 2 fingerbreadths below the costal margin, but at no time was the spleen felt). The central nervous system was normal. Extremities - there was clubbing of the fingers. The left hand showed swelling over the proximal phalanges of the 4th and 5th fingers and the middle phalanx of the middle finger. No swelling was detected in the right hand. The left knee was swollen, warm and tender, and there was a small effusion. There was gross limitation of movement, the joint tending to be helf fixed in 130° of flexion. The left ankle was swollen and tender, the swelling extending to the

dorsum of the foot. A small superficial discharging sinus was present over the medial malleolus and was thought to be the track of a guinea worm, secondarily infected.

Radiological findings:-

Chest - lungfields clear. Large globular heart. The transverse diameter of the heart shadow was greater than half the diameter of the chest.

Skull - marked increase in the diploeic spaces in the frontal and parietal regions. The bone presented a "spongy" appearance.

Hands - left - destruction of the cortex and medulla and proximal phalanges of the 4th and 5th fingers, with pathological fractures through both. Expansion of the middle phalanx of the middle finger with destruction of the central trabeculae. Right - small "cystic area in the middle phalanx of the middle finger".

Left knee - synovial thickening and areas of rarefaction in both femoral condyles. Similar areas of rarefaction in the lower third of the shaft of the femur. (A pathological fracture developed in the lower third of the femur at a later date).

Left ankle - multiple areas of rarefaction and periosteal reaction in the lower ends of the tibia and fibula. A pathological fracture was visible through one of the sites of rarefaction in the tibia.

Laboratory findings:-

(For convenience of presentation results of investigations done over many months will be summarised here).

Haematology - on admission the haemoglobin was 7.4 g. per cent; reticulocytes 4 per cent; sickling test positive using 2% sodium metabisulphite; red cell osmotic fragility was reduced (initial 0.4, final 0.15); the stained peripheral film showed nucleated red cells, target cells and a few sickled cells. The erythrocytes were slightly hypochromic; filter paper electrophoresis of the haemoglobin showed only haemoglobin S; 12 per cent. of the haemoglobin was of the

foetal type; the white cell count was 16,600 with polymorphs 86%, lymphocytes 10% and eosinophils 4%; the haemoglobin level varied considerably during periods of crisis. From time to time the level dropped sharply, sometimes to below 4 g. per cent., and had to be corrected by blood transfusions.

Bone marrow differential cell count -

Blast cells		1%
Neutrophil promyelocytes		2%
" myelocytes		8%
" metamyelocytes		14%
" adults (unsegmented)		23%
" " (segmented)		22%
Eosinophil myelocytes		2%
" adults		2%
Lymphocytes	less than	1%
Pro-erythroblasts		1%
Basophilic normoblasts		3%
Polychromatic "		9%
Pyknotic "		12%
Plasma cells	less than	1%

Serum proteins - initial findings were: - Albumin 3.0 g. per cent, globulin 4.9 g. per cent, albumin globulin ratio 0.6. After almost a year in hospital the values were:- Albumin 1.7 g. per cent, globulin 6.0 g. per cent, albumin globulin ratio 0.28.

Two determinations of the serum calcium, inorganic phosphorus and phosphatases which were done during the period when bone lesions were most marked gave the following results:-

Calcium	8.8 and 8.9 mg. per 100 ml.
Inorganic phosphorus	3.9 and 3.8 "
Alkaline phosphatase	20.8 and 17.5 K.A. units
Acid phosphatase	1.0 "

Urine - A small quantity of albumin was sometimes present, but all other findings, including estimations of the urinary calcium (Sulkowitch) were normal.

Kahn test - negative on two occasions.

Tuberculin skin test - repeated on several occasions, gave equivocal results.

Family studies - Both parents and 2 siblings showed the sickling phenomenon. Electrophoresis of the haemoglobins showed that the parents and one sibling had the sickle cell trait. The other sibling had sickle cell anaemia.

Management and progress

Despite the "doubtful" tuberculin reaction, the bone changes were initially regarded as being tuberculous in origin, and treatment with streptomycin and I.N.H. was started. Penicillin was also given during the first week for the cellulitis around the left ankle. The acute inflammatory reaction around the left ankle subsided rapidly, but apart from this no response to treatment was noted. The diagnosis of tuberculosis complicating sickle cell anaemia was reconsidered and it was decided to seek confirmation by histological examination of biopsy specimens obtained from the knee and certain bones. The histological appearances were as follows:-

Synovium from left knee - "The specimen shows a subacute or chronic inflammatory condition without specific features. Plasma cells predominate. Scanty pus cells were seen but no organisms".

Bone from middle phalanx of left middle finger - "The decalcified tissue consists of bone fragments and granulation tissue. The latter is infiltrated with lymphocytes and plasma cells. There is no evidence of tuberculosis".

Biopsy of an area of rarefaction in the left tibia showed similar features to those recorded above.

Following on the knee biopsy the joint became infected and suppuration ensued. Staphylococcus pyogenes and coliform organisms were isolated from the pus. In spite of the use of broad spectrum antibiotics, blood transfusions and adequate local treatment to the

joint, suppuration continued for about three months. During this period she had frequent crises of severe pain in the limbs and joints, swinging pyrexia, and an increase in the severity of the anaemia. Cessation of suppuration was accompanied by improvement in her general condition. At about this time she complained of pain in her spine. Physical examination showed no evidence of spinal disease, but X-rays showed destructive changes in the body of the 8th dorsal vertebra and general osteoporosis of the spine (see Fig. 48). It should be noted that streptomycin and I.N.H. had been continued, even though the diagnosis of tuberculosis had not been established.

During the 8th month in hospital she again started having fever and frequent episodes of pain in the limbs. During one of these episodes she showed signs of peripheral circulatory collapse, became mentally confused and was very restless. In the weeks that followed she became psychotic. She was withdrawn, unco-operative and confused, and frequently indulged in uncontrollable screaming and crying. Her general condition deteriorated rapidly. No complaints could be elicited, but from her behaviour it was obvious that she was in constant pain.

During the 9th month a large abscess which had been previously overlooked was detected in the right sacro-iliac region. It was immediately incised and a large quantity of pus released. Organisms similar to those previously isolated from the knee were grown on culture of the pus. X-rays of the pelvis showed gross osteoporosis of

all bone and destructive changes in the right acetabulum, associated with pathological dislocation of the hip (See Fig. 49). The same pattern of events that had occurred in association with suppuration of the knee joint was repeated. Pus continued to discharge from the incision for several months, and during this time fever and crises of limb pain constantly recurred. When the suppuration eventually ceased she showed slow but steady improvement.

Between the 12th and 14th months after admission, physical examination of the chest, heart and abdomen showed findings similar to those recorded on admission. Her mental state had returned to normal and no abnormality of the central nervous system was detected. The lymph glands in the axilla, groins and neck were slightly enlarged. In the limbs, finger clubbing was more marked than it had been originally, but the bone lesions in the left hand, left ankle region and left knee had healed. X-rays of the spine showed little change from the previous appearances. It had not been possible to reduce the dislocation of the right hip joint. Her appetite was voracious and she gained weight rapidly. She had occasional episodes of fever and limb pain, much less frequently than before. During the 13th month deep jaundice was noted. Liver function tests done at this time showed:-

> Serum albumin 1.7 g. per 100 ml., globulin 6.0 g. per 100 ml., Albumin globulin ratio 0.28, total serum bilirubin 8.6 mg. per 100 ml., of which 6.1 mg. was of the direct reacting type, thymol turbidity 8.6 units, zinc sulphate turbidity 19 units, alkaline phosphatase 33.6 K.A. units.

Jaundice subsequently cleared. The liver function tests were not repeated.

When last seen she was in reasonably good health and was being detained in hospital chiefly because of the deformity of the right hip. The haemoglobin level had become stabilised at about 8 g. per cent.

Comment - In the absence of evidence to the contrary, the bone lesions have been attributed to sickle cell anaemia complicated by secondary infection following the knee biopsy. During the periods when active infection was present, crises of limb pain were very frequent and the anaemia was worse. The mechanisms underlying the crises of pain in sickle cell anaemia are not understood. Findings in this patient suggest that infection may be an important factor.