EXPRESSION OF THE SICKLE-CELL GENE IN AFRICA

BY

G. M. EDINGTON, M.D., D.C.P., D.T.M.&H.

Specialist Pathologist, Medical Research Institute, Accra, Gold Coast

AND

H. LEHMANN, M.D., Ph.D., F.R.I.C.

Senior Lecturer in Chemical Pathology, St. Bartholomew's Hospital, London

It has always been difficult to reconcile the accepted theory of the inheritance of sickle-cell anaemia with the facts found in Africa. Neel (1947, 1949) and Beet (1949) concluded that the sickle-cell gene was harmless in the heterozygote but produced sickle-cell anaemia in homozygotes. Although the sickle-cell trait has been found to be present in 20, 30, and even 40% of individuals in some African peoples, the anaemia is considered to be rare in adults. In infants, however, it has been found to occur with a frequency which falls well within the range of the expected incidence of homozygotes (J. and C. Lambotte-Legrand, 1951; Foy, Kondi, and Brass, 1951; Vandepitte and Louis, 1953; Hutton, 1953; Welbourn and Raper, 1954). Nevertheless one of us stated: "I do not believe that in Uganda we could have overlooked sickle-cell anaemia present in all homozygous carriers of the trait. Even if they had died in early infancy, it would have been such a 'slaughter of the innocents' that it could not have escaped our attention" (Lehmann, 1952).

A high incidence of the trait in an adult population with a low incidence of sickle-cell anaemia could be explained most easily by the early death of affected infants. Such a removal of the gene, however, before the age of procreation should lead to a fall in the sickling incidence. Neel (1953) has suggested that the high incidence of the trait found in certain peoples might be explained either (a) by a very high rate of mutation from the gene for normal haemoglobin to that for haemoglobin S, sufficient to replace the loss by death from sickle-cell anaemia, or (b) by a reproductive advantage over the normal or non-sickling homozygote conferred on the heterozygote by the haemoglobin S gene balancing the disadvantage of the anaemia in S homozygotes—the theory of balanced polymorphism. Neel thought that there was some evidence of an increased mutation rate in his family studies in the U.S.A.; but Vandepitte (1954), in the Belgian Congo, could find no evidence of a mutation rate sufficient to replace the loss of S homozygotes in infancy. Balanced polymorphism has been proposed as a possible mechanism for the maintenance of congenital anaemias by Haldane (1949) and for the survival of the sickle-cell trait by Beet (1946, 1947), by Raper (1949), and particularly by Brain (1952a, 1952b). They all suggested that the sickle cell might be more resistant to tropical parasites, particularly the malaria parasite.

Allison (1954) was the first to report very striking differences in the degree of incidence of malaria parasites in sicklers and non-sicklers, though his work has been challenged by Moore, Brass, and Foy (1954). Recently Raper (1954) has reported a statistically significant difference in the degree of parasitaemia of sickling and non-sickling children during the second year of life.

There exists, however, yet a third possible explanation of the high incidence of the S gene which can be added

to those described. If it was found that some healthy African adults were homozygous for the S gene, it would explain in part why sickle-cell anaemia is not found with the expected frequency in adults and why the trait is inherited more often than one would expect if all the homozygotes died in early infancy. One of us (Edington, 1953) was struck by the histories given by patients suffering from sickle-cell anaemia in the Gold Coast. Symptomatically these patients improved with advancing years. One patient aged 44 had been free from crises for nine years and had had six children, three of whom were alive and well; another, aged 39, was suffering from sickle-cell anaemia but had five children who were all alive and well. In view of the discovery, however, of modified sickle-cell anaemia in individuals heterozygous for S and another inherited abnormality of haemoglobin (sickle-cell-thalassaemia, sickle-cell-haemoglobin C disease, sickle-cell-haemoglobin D disease; see Itano, 1953a) there was a possibility that these patients were not the true S homozygotes, as electrophoretic analyses of the haemoglobins had not been performed.

Electrophoretic Tests

Until recently the study of the genetics of sickling depended on the testing of whole families for the presence or absence of the sickling character in the red cells and on the clinical condition of those showing sickling; the diagnosis of homozygosity and heterozygosity was subject to considerable doubt. With the introduction of electrophoretic tests on haemoglobin there is now no reasonable doubt that, subject to certain rare exceptions, a determination of the genotype can be made by means of a single test on the blood of an individual, and need not depend on a study of the rest of the family. Normal non-sickling persons show as a rule 100% of haemoglobin A, and in any case no haemoglobin S. Heterozygous sicklers have both types of haemoglobin, while homozygous sicklers have mainly haemoglobin S, together usually with a varying amount of persistent foetal haemoglobin (haemoglobin F) but no haemoglobin A.

There is very strong evidence that genes for haemoglobins A, C, D, E and G, and S belong to a series of multiple alleles, so that all homozygotes and all heterozygous combinations of them can be diagnosed electrophoretically, except that haemoglobins D and S are indistinguishable, as they have the same electrophoretic mobility. Homozygous haemoglobin D (never yet found) could certainly be distinguished by the absence of sickling, but SS and SD can be separated only by solubility measurements. Haemoglobin S is much less soluble in the reduced state than haemoglobin D; thus SS and SD can be separated by their different solubilities. To establish the claim that an individual is a homozygote for haemoglobin S, electrophoretic and solubility studies are therefore necessary. As S homozygotes also possess foetal haemoglobin, a possibility arises that individuals with sickle-cell-thalassaemia disease (SAF) could be mistaken for S homozygotes (SSF).

As Itano (1953a, 1953b) has pointed out, the solubility of the haemoglobin of an SS individual depends on the proportion of F being present; thus the solubility of haemoglobin from an S homozygote with a high proportion of persistent F may be within the same range as the solubility of the haemoglobin of a patient with sickle-cell-thalassaemia disease, in whom the proportion of S is high and that of F moderate and A low. If the amount of A is small—up to 8.2%—it may escape detection by electrophoresis (Singer and Fisher, 1953). Thus a sickle-cell homozygote with a high percentage of foetal haemoglobin and a sickle-cell-thalassaemia individual may not be differentiated by electrophoresis (though this will be exceptional), and the solubility of the two haemoglobin mixtures may be within the same

range, the upper range for that seen in SS homozygotes. If such a finding is made the only recourse is still the family study. However, thalassaemia has never been found in the Gold Coast, in spite of an exhaustive search. This was stimulated particularly by a high incidence of target cells (Edington, 1953).

We have recently conducted a survey of Gold Coast Africans for abnormal haemoglobins (Edington and Lehmann, 1954), and were particularly interested in trying to discover whether in such an investigation symptomless homozygotes for the S haemoglobin could be found. Of the 200 individuals examined, 38 were sicklers, and two of these were homozygous for the S gene (which incidentally would be the expected incidence for homozygotes).

Case 1

J. A. S., a plethoric African man of the Gã tribe aged 40, reported to the out-patient department of the Gold Coast Hospital complaining of epigastric pain occurring about half an hour after meals during the last six weeks. Previous history, family history, physical examination, and laboratory and radiological investigations revealed no abnormality apart from sickling of the erythrocytes and that the patient was symptomatically relieved by alkalis. He had had three children, one of whom, a son aged 22, was alive and well. A boy aged 14 had died of dysentery and a girl aged 7 of "measles." When the analysis of the haemoglobin revealed that J. A. S. was an S homozygote further investigations were made. On direct questioning he admitted that he had suffered from "rheumatism" in childhood. At the age of 8 he had had attacks of pain in the larger joints of the limbs accompanied by fever; these attacks occurred about ten times in that year and gradually lessened in frequency and severity until they ceased at the age of 18.

Laboratory Investigations.—Urine: no sugar, bile, albumin, casts, or urobilin. Kahn test negative; van den Bergh negative; serum bilirubin, 0.3 mg. per 100 ml.; haemoglobin (Grey wedge), 13 g. per 100 ml.; red cells, 4,600,000; reticulocytes, 1.2%; white cells, 5,400; P.C.V., 43%; sickling of the erythrocytes—no filamentous forms noted; thin film and differential count revealed no abnormality apart from the presence of scanty target cells (less than 4%).

Case 2

E. B. A. D., a well-built man of the Akwapim tribe aged 30, reported to the out-patient department of the Gold Coast Hospital complaining of a "rash." He was suffering from a mild neurodermatitis which cleared rapidly with treatment. Analysis of the haemoglobin revealed that he was an S homozygote.

On direct questioning he admitted that he had suffered from attacks of pain in the larger joints which had begun at the age of 12 and were worse in the rainy season. During these attacks his eyes were yellowish and his urine was highly coloured. The attacks were never severe, and ceased at the age of 25, when he stopped playing football—to which game he attributed the illness. His parents were dead, cause unknown. He is married with one child alive and well. Physical examination revealed no abnormality. Neither the liver nor the spleen was palpable.

Laboratory Investigations.—Kahn test negative; serum bilirubin, 0.7 mg. per 100 ml.; haemoglobin (Grey wedge) 15.5 g. per 100 ml.; red cells, 4,860,000; reticulocytes, 1%; white cells, 4,600; P.C.V., 48%; no abnormality detected in the thin film. Urine: urobilin present in a dilution of 1:2.

Investigation of the Haemoglobins

The chemical investigation of the two bloods showed all the features usually considered typical of sickle-cell anaemia. Fig. 1 shows the electrophoretic pattern of the haemoglobin of both individuals—a single band in the S position. Both bloods contained foetal haemoglobin determined by alkali denaturation (Singer, Chernoff, and Singer, 1951). The

FIG. 1.—The blood of J. A. S. and E. B. A. D., two Gold Coast Africans, is compared by paper electrophoresis with a blood containing as a "marker" A-C haemoglobins. Both individuals show a single band in the S position. Foetal haemoglobin was found by chemical means, but it does not separate on paper electrophoresis from A or S haemoglobin. The haemoglobins are photographed unstained (for detailed technique see Lehmann and Smith, 1954).

solubility of the haemoglobins determined after Itano (1953b) certainly for the first individual fell well within the range of the solubilities found by that author only in sickle-cell anaemia (<0.44 g./l. of 2.24 M phosphate buffer).

	% Alkali-resistant (Foetal) Haemoglobin	Solubility in g./l. of 2.24 M Phosphate Buffer
J. A. S.	5.2	0.15
E. B. A. D.	24.2	0.50

The second individual shows a high foetal haemoglobin and the solubility is within the range for that seen in both sickle-cell anaemia and sickle-cell-thalassaemia disease (0.44–0.90 g./l. of 2.24 M phosphate buffer). It has already been pointed out that the possibility of finding the sickle-cell-thalassaemia disease in the Gold Coast can be disregarded, and so far as the major argument of this communication is concerned it may be stated that from the accepted point of view both sickle-cell homozygotes (SSF) and sickle-cell-thalassaemia individuals (SAF) should be suffering from anaemia.

It thus seems that, at least in the Gold Coast, the homozygous inheritance of the S gene can be a benign condition which would be missed in ordinary clinical and haematological investigations and which may be detected only by the analysis of the haemoglobin.

The Sickle-cell Trait

While it has been shown that every sickle-cell haemoglobin homozygote need not suffer from a severe disease, it cannot, on the other hand, be taken for granted that the heterozygous condition will always be entirely harmless and express itself solely as a trait rather than as a disease. Haematuria due to pathological lesions in the kidney and ascribed to the presence of the sickle-cell trait has been described in American negroes (Harrison and Harrison, 1952). Vernier (1955) summarizes 35 reports of one-sided renal haemorrhage in sickle cell individuals, most of them sickle cell trait carriers.

We have recently seen in Accra a girl of the Gã tribe, aged 18, suffering from haematuria, whose haemoglobin on electrophoretic analysis revealed the presence of the trait—that is, of both normal and sickle-cell haemoglobin. No history of previous illness was given and the physical examination revealed no abnormality. The results of the laboratory investigations were as follows. Urine: numerous red blood cells present; a few of them were abnormally shaped, but no definite sickle cells were seen. Culture was sterile and guinea-pig inoculation was negative. Serum bilirubin, 1.2 mg. per 100 ml.; direct van den Bergh test negative. Hb, 12.3 g. per 100 ml.; red cells, 4,320,000; reticulocytes, 2.8%; white cells, 8,100; P.C.V., 33%; sickling positive. A peripheral blood film showed many target cells with an occasional poikilocyte and polychromatic red cell. The alkaline phosphatase and thymol turbidity were within normal limits. The haematuria cleared within a few days, but no follow-up was possible. It cannot be claimed that the presence of the trait was definitely responsible for this girl's symptoms, but in view of the reports in the American literature it must be considered in the differential diagnosis of the condition.

The sickling phenomenon may certainly play a part in the terminal phases of other diseases where the picture of the crises can be seen at necropsy without evidence of previous acute episodes (Thoma, 1953). At the present moment it is not clear whether these crises, which are not uncommon in the Gold Coast (Edington, 1951, 1955), occur most often in the S homozygote, in the heterozygote for S and normal haemoglobin, or in the individual bearing the S gene in combination with some other abnormal haemoglobin. In an attempt to elucidate this problem we have recently begun to examine electrophoretically the blood of all subjects coming to necropsy, and the crisis has been noted in one woman suffering from sickle-cell haemoglobin C disease. In addition, however, the blood of another woman (K.L.), who presented at necropsy the typical features of the crisis, revealed on electrophoretic analysis the presence of the trait only (normal and sickle-cell haemoglobin) (Fig. 2), as is described in detail below.

FIG. 2.—The blood of K.L., who died in sickle-cell crisis, is compared by filter-paper electrophoresis (for details see Lehmann and Smith, 1954) with a "marker" blood from an individual heterozygous for A and C haemoglobins. The haemoglobin of K.L. shows bands in the S and A position. K.L. is heterozygous for A and S haemoglobins.

Crisis in a "Sickle-cell-trait Carrier"

K.L., an African woman of the Gã tribe aged 23, was admitted to the Maternity Hospital, Accra, in a comatose condition. Shortly after admission she died following the expulsion of a hydatidiform mole. She had had one previous child, who had died in infancy. No history of previous illnesses was obtained from the relatives. The patient had been ill for two weeks with fever. Substernal pain and cough had been noted during the last week and she was thought to be three months pregnant.

Necropsy Report.—The body was that of a well-built plethoric African female. The sclerae were slightly jaundiced. There was frothy mucus in the trachea and the lungs were congested and grossly oedematous (795 g.). Heart: the left ventricle was hypertrophied; no lesions of the myocardium, valves, or coronary arteries were noted (320 g.). The liver was congested (1,975 g.). The spleen was enlarged, rubbery in consistency, and dark blue in colour (495 g.). The cut surface was dark red and did not bleed on section. The uterus was enlarged to the size of a three-months pregnancy. No abnormal blood clot was present and the placental site appeared macroscopically normal. The remaining abdominal organs exhibited intense pallor and the kidneys were oedematous. The sternal marrow was dark red and dry. The brain was pale and the vessels contained watery blood. The heart's blood showed sickling on direct examination, and electrophoretic analysis of the haemoglobin revealed the presence of sickle and normal adult haemoglobins (Fig. 2).

Histological Examination.—All organs were fixed in 10% formol saline and transferred to formol sublimate saline before embedding. **Liver:** The sinusoids were congested and contained numerous white blood cells; the Kupffer cells were distended and contained numerous sickled erythrocytes; free iron-containing pigment was present in the Kupffer cells and periportal parenchymal cells. Haemofuchsin was not detected; a little malarial pigment was present. **Spleen:** There was gross congestion and haemorrhage and the sinuses were stuffed with sickled cells; the Malpighian corpuscles were widely separated and com-

pressed; the germinal centres were hypertrophied and surrounded by a narrow rim of lymphocytes; traces of free iron-containing pigment were present; a reticulin stain showed interruption of the normal reticulin pattern in the areas of haemorrhage. **Uterus:** Tags of placenta were adherent and a marked polymorphonuclear infiltration with small abscesses was present. **Lung:** The smaller vessels were congested with sickled erythrocytes. The alveoli showed gross oedema and heart failure cells were present. **Kidney:** An occasional hyalinized glomerulus was noted, and there were occasional casts in the collecting tubules. Thrombosed vessels were not noted and no free iron was present. Apart from a normoblastic hyperplasia seen in the sternal marrow no other abnormality was noted in the remaining organs.

Comment.—The gross pathology of the spleen and the histopathology of the liver and spleen were those found in the sickle-cell crisis. Mild haemolysis was also probably present, as shown by the presence of jaundice, iron pigment in the Kupffer cells, and a normoblastic hyperplasia of the sternal marrow. This was probably not of long standing, as there was only a little free iron in the spleen, and none in the kidney. Undoubtedly a septicæmic element was also present, and it is difficult to decide what part this played as an eventual cause of death. It is considered that the terminal crisis was probably initiated by the septicæmic condition and was directly responsible for death.

Discussion

Two Gold Coast Africans, to all intents and purposes healthy, have been found to be homozygous for the sickle-cell gene. Previously it had been thought that most patients suffering from sickle-cell anaemia died in childhood, and it was difficult to explain the high incidence of the trait in certain communities because of this belief. At the present time the mortality occurring in S homozygotes is not known, but it is possible that a considerable number reach adult life, procreate, and lead relatively normal lives. One of us (G.M.E.) has now observed over 50 patients suffering from sickle-cell anaemia, and the prognosis, in the Gold Coast at least, is by no means gloomy. Many of these patients have improved with the passing years and are able to lead useful and normal lives. It is too early yet to venture an opinion on the fertility of these patients, as the majority were children when first seen and have not yet reached the age of marriage, but from the histories obtained in the older patients it is not expected that fertility would be greatly impaired. The survival of the relatively healthy homozygote may be a potent factor in maintaining a high incidence of the trait in certain peoples.

On the other hand, there is no doubt that sickle-cell anaemia is the cause of considerable mortality, the exact extent of which is unknown, and in this communication it has been suggested that the trait itself may be by no means harmless. It is impossible to say to what extent procreation by S homozygotes helps to balance the mortality caused by the sickling phenomenon, but this possibility must be taken into account, in addition to the possible protection afforded by the trait against malaria and a possible high mutation rate of the S gene, when discussing reasons for the persistent high incidence of the trait in certain African and Indian peoples.

Summary

Of 200 individuals examined in the Gold Coast, 19% showed sickling and two (1%) were found to be homozygous for S haemoglobin. They were adults not suffering from sickle-cell anaemia, yet chemically their haemoglobin was indistinguishable from that of such patients.

A sickle-cell crisis is described in a woman heterozygous for sickle-cell and normal haemoglobin.

These findings, and their bearing on the current theories on the expression of the sickle-cell gene and its population dynamics, are discussed.

REFERENCES

- Allison, A. C. (1954). *British Medical Journal*, 1, 290.
 Beet, E. A. (1946). *E. Afr. med. J.*, 23, 75.
 — (1944). *Ibid.*, 21, 212.
 — (1949). *Ann. Eugen. (Camb.)*, 14, 279.
 Brain, P. (1952a). *British Medical Journal*, 2, 880.
 — (1952b). *S. Afr. med. J.*, 26, 925.
 Edington, G. M. (1951). *Trans. roy. Soc. trop. Med. Hyg.*, 44, 559.
 — (1953). *British Medical Journal*, 2, 957.
 — (1955). *Trans. roy. Soc. trop. Med. Hyg.* In press.
 — and Lehmann, H. (1954). *Ibid.*, 48, 332.
 Foy, H., Kondi, A., and Brass, W. (1951). *E. Afr. med. J.*, 28, 1.
 Haldane, J. B. S. (1949). *Ricerca Sci.*, 19, Suppl. p. 68.
 Harrison, F. G., and Harrison, F. G., jun. (1952). *J. Urol.*, 68, 943.
 Hutton, P. W. (1953). *E. Afr. med. J.*, 30, 75.
 Itano, H. A. (1953a). *Science*, 117, 89.
 — (1953b). *Arch. Biochem. Biophys.*, 47, 148.
 Lambotte-Legrand, J., and Lambotte-Legrand, C. (1951). *Ann. Soc. belge Méd. trop.*, 31, 207.
 Lehmann, H. (1952). *Trans. roy. Soc. trop. Med. Hyg.*, 46, 43.
 — and Smith, E. B. (1954). *Ibid.*, 48, 12.
 Moore, R. A., Brass, W., and Foy, H. (1954). *British Medical Journal*, 2, 630.
 Neel, J. V. (1947). *Genetics*, 32, 38.
 — (1949). *Science*, 110, 64.
 — (1953). *Amer. J. hum. Genet.*, 5, 154.
 Raper, A. B. (1949). *E. Afr. med. J.*, 26, 281.
 — (1954). *Rapports et Communications, Ve Congrès International de Transfusion Sanguine, Paris, 1954*, p. 49.
 Singer, K., Chernoff, A. I., and Singer, L. (1951). *Blood*, 6, 413.
 — and Fisher, B. (1953). *J. Lab. clin. Med.*, 42, 193.
 Thoma, G. W. (1953). *Amer. J. med. Sci.*, 226, 412.
 Vandepitte, J. M. (1954). *Rapports et Communications, Ve Congrès International de Transfusion Sanguine, Paris, 1954*, p. 48.
 — and Louis, L. A. (1953). *Lancet*, 2, 806.
 Vernier, R. L. (1955). *Amer. J. Dis. Child.*, 89, 221.
 Welbourn, H., and Raper, A. B. (1954). *British Medical Journal*, 1, 1440.

OSTEOMALACIA IN VON RECKLINGHAUSEN'S NEUROFIBROMATOSIS

METABOLIC STUDY OF A CASE

BY

PAUL D. SAVILLE, M.B., M.R.C.P.

J. R. NASSIM, B.M., F.R.C.P.

F. HARWOOD STEVENSON, M.D., M.R.C.P.

LILY MULLIGAN, B.Sc.

AND

MARGARET CAREY, B.Sc.

From the Royal National Orthopaedic Hospital, London

Von Recklinghausen's neurofibromatosis is a hereditary and familial disease. It is characterized by lesions of skin, nerve, and bone. Skeletal abnormalities are said to occur in 7% of cases (Gould, 1918; Reuben, 1934). The commonest abnormality is scoliosis (Hagelstam, 1946; Fairbank, 1951). There may be overgrowth or undergrowth of individual bones, while neurofibromata may erode the bones from the periosteum or cystic changes may occur in bone.

Apart from neurofibromatous affection of bone, the occurrence of osteomalacia in association with neurofibromatosis has been reported too often to be a chance finding. Gould (1918) reported typical histological findings of osteomalacia in five cases of von Recklinghausen's disease which he examined *post mortem*. He gave no indication of the aetiology. Since then further cases have been reported but without full metabolic investigations. Merklen and Israel (1934) described a case of von Recklinghausen's disease with general rarefaction of bone and Milkman's syndrome. Milkman's syndrome is now generally believed to be invariably due to osteomalacia. Nørgaard (1937) described many radiological abnormalities of the skeleton in two cases.

In Fig. 3, Case 1, of his article it can be seen that the x-ray film of the pelvis shows a pseudo-fracture through the left inferior pubic ramus and another through the superior pubic ramus. The patient was 22 years old, and an x-ray film one year previously showed no pseudo-fracture. This patient then was undoubtedly suffering from osteomalacia. Hernberg and Edgren (1949) reported the case of a woman aged 32 with neurofibromata of skin and multiple pseudo-fractures (Looser-Milkman syndrome). She had a serum inorganic phosphorus of only 2 mg. per 100 ml. with normal calcium, and was given 11 million units of vitamin D in twelve weeks with no apparent effect until eight months later, when healing occurred. They suggested that this might be a "parallel phenomenon" to von Recklinghausen's disease or raised resistance to vitamin D. Since then knowledge of the pathogenesis of osteomalacia and rickets has advanced, and it is now clear that these cases are due to a hereditary abnormality of the renal tubule. Dent (1952) described two cases of osteomalacia in von Recklinghausen's disease which he considered to be due to resistant rickets (Types I and II respectively). Fraser and Nordin (1953) reported a case which they felt was due to resistant rickets. Swann (1954) also reported several cases of this syndrome.

We are recording a further case in which the opportunity was taken to make metabolic studies on varying doses of vitamin D, proving conclusively that this disease falls into the group known as resistant rickets. In addition, biopsies were made from affected bone, and the results of these are also reported.

Case History

The patient was a man aged 55 who for seven months before admission had increasingly severe backache, made worse by coughing and sneezing. More recently he noticed aching pain in his shoulders, left ankle, and left foot. Walking was almost impossible. His general health was satisfactory, but he had always had a "weak bladder" and passed urine every 1½ hours by day, and two or three times at night. He had had no relevant past illnesses, apart from "fits" as a child. His father and paternal grandmother had had neurofibromatosis.

Examination revealed a stunted little man with typical skin lesions, both pigmentation and tumours, covering the trunk, head, neck, and limbs. The right leg was 1½ in. (3.8 cm.) shorter than the left, and all the muscles below the right knee were partially atrophied. There was a tarsal equinovarus deformity of the right foot. He gave the impression of being of rather low intelligence, and was virtually illiterate.

Investigations.—Haemoglobin, 98% (14.5 g. per 100 ml.); W.B.C., 6,750 (normal differential count); E.S.R., 3 mm. (Wintrobe). Urine: normal cytology; sterile on culture, no albumin or sugar. Serum calcium, 9.9 mg. per 100 ml.; phosphorus, 2.2 mg. per 100 ml.; alkaline phosphatase, 14.5 King-Armstrong units; potassium, 5.3 mEq/l.; alkali reserve, 29 mEq/l.; blood urea, 37 mg. per 100 ml.; plasma protein, total 6.9 g. per 100 ml. (albumin 3.2 g., globulin 3.7 g.). Urea clearance, 63% of normal (uncorrected). Glucose-tolerance test, normal, rising from 70 to a maximum of 151 and back to 70 mg. per 100 ml. in two hours. Sugar appeared in the urine at the height of the curve. Paper chromatography of urine: No aminoaciduria; superglycine pattern present, which we have come to associate with osteomalacia in the adult (C. E. Dent). X-ray examination showed multiple fractures of ribs ("There is an opacity in the right lower zone which on lateral view is seen to be below the diaphragm, which is tented up over it.") There was some kyphosis and some demineralization of the