HEMOGLOBIN TYPES IN LEPROSY

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Since 1949, when Pauling and associates [3] described sickle-cell hemoglobin SS, additional abnormal hemoglobins, C,D,E,G and H, have been reported. At pH 8.6 all six abnormal types show less electrophoretic mobility than that of the normal adult Type A hemoglobin. From a practical standpoint, anemias which previously were undiagnosed or considered to be atypical sickle-cell anemia may be recognized now.

Chernoff [1], reviewing the literature on hemoglobin types, states in substance that the adult pattern of hemoglobin is Type A in more than 99 per cent of individuals. It is generally assumed that hemoglobin A is the sole type of hemoglobin produced in the normal adult. However, immunologic and biochemical evidence indicates that small quantities (0.65%) of hemoglobin F (fetal type) are also produced by the mature human being.

Recent observations suggest that the human hemoglobins may form a broad spectrum of compounds, each distinguished from its neighbor by only a slightly different position on paper electrophoresis. As said, several pigments have already been observed which do not correspond with established electrophoresis patterns. It is not known whether they represent new hemoglobins or whether the atypical patterns are the results of other factors.

The objective of the present study was to survey the hemoglobin types found among the leprosy patients at the Public Health Service Hospital at Carville, Louisiana.

MATERIAL AND METHODS

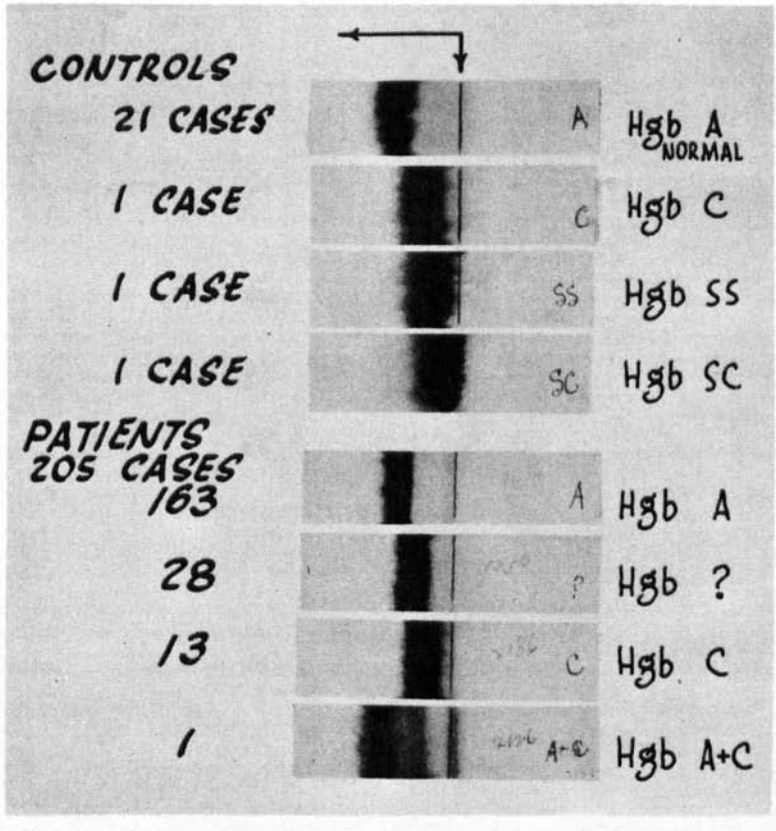
Blood was obtained from 205 patients, 187 of the lepromatous and 18 of the tuberculoid type. These patients represented the following racial backgrounds:

American white	70 patients
American Negro	16 patients
Mexican	81 patients
Chinese	14 patients
Filipino	11 patients
Puerto Rican	5 patients
Greek	3 patients
Italian	2 patients
Samoan	2 patients
Indochinese	1 patient
Total	205

For normal controls, blood was obtained from 21 nonpatients, 15 white, 6 Negro, and 3 nonpatients exhibiting the following abnormal hemoglobins: C, SC, and SS.[1]

Hemoglobin solutions were prepared from oxalated blood. The blood was centrifuged and the supernatants discarded. The red cells were washed with 0.85 per cent sodium chloride solution until the last supernatant showed no protein to 3 per cent sulfosalicylic acid. The washed cells were then lysed by the addition of an equal volume of 0.05 M barbital buffer and 1/10 volume of toluene. The solutions were then placed in the refrigerator for 18 hours, centrifuged, and the hemolysates filtered. The hemoglobin concentrations were adjusted to approximately 10 gm.

The paper electrophoresis apparatus used was a Swedish make, LKB, illustrated in Fig. 1. The power supply was adjusted so that the current flow through the electrophoresis unit was 6 milliamperes (1 ma. for each strip). The filter paper, Munktell No. 20S, was cut into 25 mm. strips and 6 such strips were used for each run. A barbital buffer, pH 8.6, was used in a concentration of 0.05 ionic strength. The strips were loaded with 10 lambda (0.01 cc.) of hemoglobin in a linear fashion with a Kirk micropipette. Electrophoresis proceeded for a period of 6 hours, with



TEXT-FIG. 1. Paper electrophoresis patterns of hemoglobin observed with specimens from 205 patients and 24 nonpatient controls. Hemoglobin A is the normal migration patterns; the atypical ones are as indicated.

[1]These abnormal hemoglobins were obtained through the courtesy of the Hematological Department of Tulane University School of Medicine, and from the laboratory of the Public Health Service Hospital, New Orleans.

results as illustrated in Fig. 2. The paper strips were removed, dried, and stained with a solution of amidoschwarz. Red cell counts and hemoglobin and hematocrit determinations were made by standard methods. Sickle-cell preparations were made by mixing equal volumes of oxalated blood and 2 per cent sodium metabisulfite. Wright's stain was used for the blood smears.

RESULTS

The hemoglobin patterns observed in the 205 patients with leprosy and the 24 nonpatient controls are shown in Text-fig. 1. Hemoglobin A, which is the normal pattern, was found in 163 (79.5%) of the patients and in all normal controls. Atypical patterns were observed in 28 (13.7%) of the patients. Hemoglobin C patterns occurred in 13 patients, and 1 patient exhibited a hemoglobin C trait pattern, which is a combination of hemoglobins A and C. Of the 13 patients exhibiting the hemoglobin C pattern, 4 were Negroes, 7 were Mexican, and 2 were white. The hemoglobin C trait occurred in a Negro patient.

A clinical diagnosis of amyloid nephrosis was made in 20 patients. That condition was a complicating factor in 13 of the 28 patients showing atypical hemoglobin patterns. Normal patterns were observed in 7 patients with amyloid nephrosis.

Of the patients exhibiting atypical and abnormal hemoglobin patterns, 40 were of the lepromatous and 2 of the tuberculoid type. This group represented the following races:

American white	15 patients
Mexican	15 patients
American Negro	4 patients
Filipino	3 patients
Puerto Rican	1 patient
Chinese	2 patients
Greek	2 patients
Total	42

Sickling of the erythrocytes could not be elicited in any case, and the examination of stained blood films revealed no morphologic abnormalities such as target cells. Fetal hemoglobin F as measured by the alkaline denaturation method of Singer and associates (4) showed values of less than 1 per cent in all of the 42 patients with atypical patterns and hemoglobin C patterns.

Hematologic data on the 42 patients with abnormal hemoglobin migration patterns showed that the red cell counts were below 3.5 million in 5 of them. The hemoglobin levels varied from 6.6 to 14.0 gm., and were below 9.0 gm. in 5 patients.

Since the hemoglobin position on paper electrophoresis did not fit established electrophoresis patterns in a group of 28 patients, advice was sought of Dr. Chernoff. Hemolysates from 12 of the 28 patients and paper strips of 26 of them showing abnormal patterns were sent to him. Included were

36 paper strips of patients showing a normal migration pattern. Although comments could not be made about their normality or abnormality because of the differences in the method used, Chernoff (2) did make an interesting observation. Of the 12 presumably abnormal hemolysates, the following patterns were observed in 5: hemoglobin C trait (hemoglobins A+C), 2; sickle-cell trait, 1; sickle-cell trait with a fast-moving component (unidentified), 1; hemoglobin A plus a fast-moving component (unidentified), 1. The remaining 7 specimens gave a pattern indistinguishable from hemoglobin A by his technique. It was felt that the results obtained would warrant further studies on our patients.

SUMMARY

Paper electrophoresis studies were made to ascertain the frequency of abnormal hemoglobin types among 205 patients (187 lepromatous and 18 tuberculoid) hospitalized at the Public Health Service Hospital, Carville, Louisiana. As controls, blood was obtained from 21 nonpatients with normal hemoglobin patterns and 3 nonpatients having abnormal patterns.

Hemoglobin A, the normal pattern, was found in 163 (79.5%) of the patients. Atypical patterns were observed in 28 of the patients by the method we used. Hemolysates from 12 of the 28 patients were submitted to Chernoff who, using a different method than we had used, identified 5 of the 12 patterns as follows: hemoglobin C trait, 2; sickle-cell trait, 1; sickle-cell trait with a fast-moving component (unidentified), 1; hemoglobin A plus a fast-moving component (unidentified), 1. Hemoglobin C patterns were noted in 13 instances and hemoglobin C trait pattern in 1 instance.

Sickling of the erythrocytes or target cells could not be demonstrated in the group showing atypical patterns, or in those patients exhibiting a hemoglobin C pattern. Fetal hemoglobin F as measured by an alkaline denaturation method showed values within the normal range of less than 1 per cent.

RESUMEN

Se hicieron estudios de la electroforesis con papel para averiguar la frecuencia de tipos anormales de hemoglobina en 205 enfermos recluídos en el Hospital del Servicio de Sanidad Pública, Carville, Louisiana, E. U. A. De estos enfermos, 187 eran lepromatosos y 18 tuberculoideos. Como testigos, se obtuvieron ejemplares de sangre de 21 sujetos que no estaban hospitalizados y tenían patrones normales de hemoglobina y de 3 que tampoco estaban hospitalizados y tenían patrones anómalos de hemoglobina.

En 163 (79.5%) de los enfermos se encontró hemoglobina A, el patrón normal. Con el método usado se observaron patrones atípicos en 28 de los enfermos. Se enviaron hemolisatos de 12 de los 28 enfermos a Chernoff, quien, usando una técnica distinta de la de los AA., identificó así 5 de los 12 patrones: característica de hemoglobina C, 2, característica de célula falciforme, 1; característica de célula falciforme de movimiento rápido (sin identificar), 1; hemoglobina A más componente de movi-

miento rápido (sin identificar), 1. Se observaron patrones de hemoglobina C en 13 casos y patrón de característica de hemoglobina C en 1 caso.

No pudo descubrirse drepanocitosis de los eritrocitos o los glóbulos escudos en el grupo que mostró patrones atípicos ni tampoco en los enfermos que manifestaron un patrón de hemoglobina C. La hemoglobina fetal F, medida por un método de desnaturalización alcalina, reveló valores que quedaban dentro de los límites normales de menos de 1 por ciento.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Rudolph J. Muelling, Jr., Associate Professor of Pathology at the Louisiana State University School of Medicine, who made helpful comments and confirmed the atypical nature of the 28 hemoglobins which have been so described.

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DESCRIPTION OF PLATE

PLATE 14

FIG. 1. The LKB electrophoresis unit used in the study reported. The arrow indicates the hemoglobin loading point.

FIG. 2. Hemoglobin migration observed after six hours of operation of the unit.

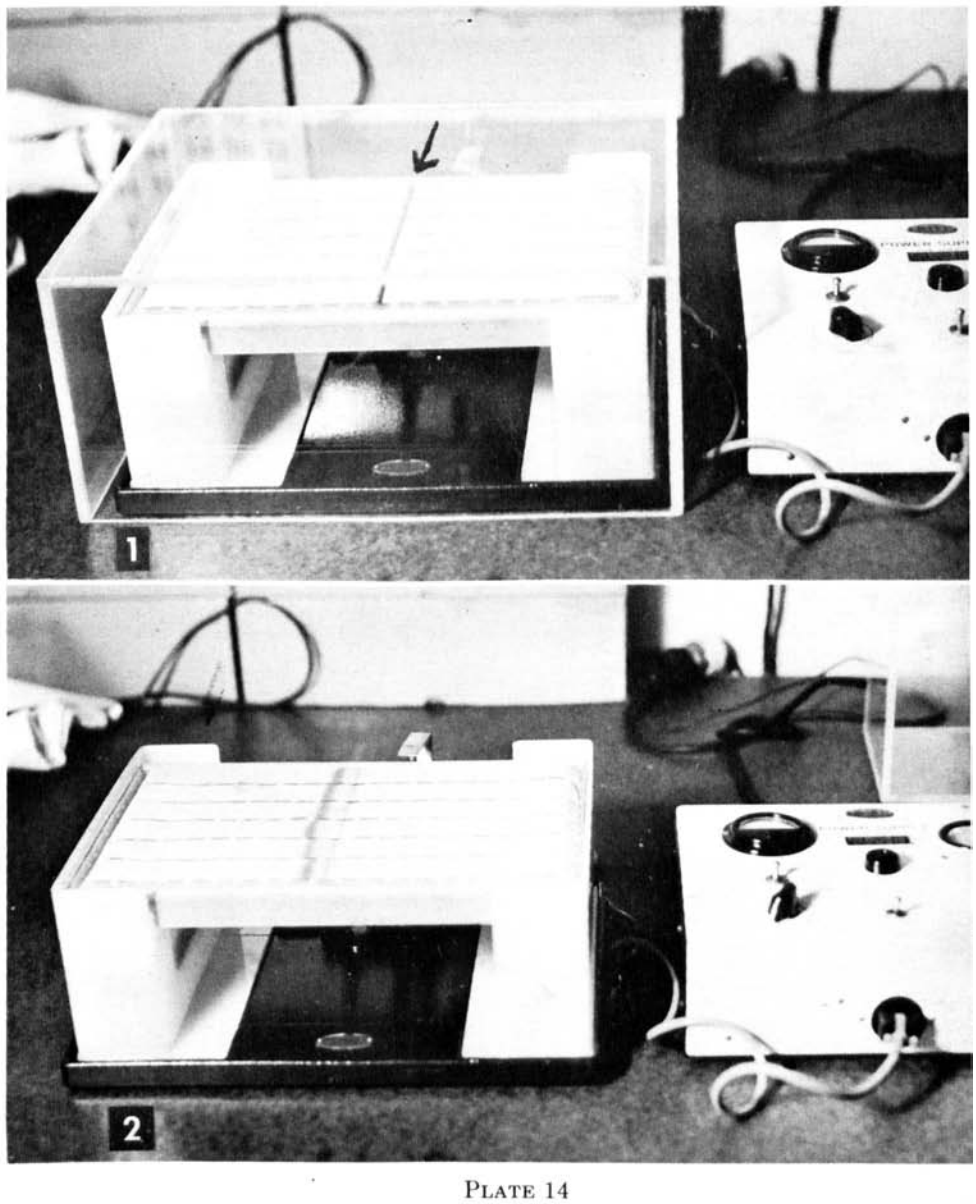

PLATE 14