A Variant of Hemoglobin A\textsubscript{2} Found in a Negro Family

By Ralph C. Lee and Titus H. J. Huisman

Three possible variants of Hb-A\textsubscript{2} (a\textsubscript{2}\textdelta\textsubscript{2}) with abnormal \delta polypeptide chains have been discovered, namely, Hb-A\textsubscript{2}' or Hb-B\textsubscript{2} in the Negro race\textsuperscript{1,2,4,11} Hb-K\textsuperscript{12}ln in one family of German descent\textsuperscript{13} and Hb-Flatbush in a family of Puerto Rican ancestry.\textsuperscript{14} In this communication, we report the results of studies of an abnormal minor hemoglobin component, which was observed in four members of a Negro family. The abnormality showed close resemblance to Hb-Flatbush. If further studies prove it to be identical, this report then describes the second family with this hemoglobin abnormality.

Materials and Methods

During an investigation of a large Negro family for the possible presence of certain hemoglobin abnormalities, this unexpected variant was accidently discovered in a 42 year old female, Case II, 2 (fig. 1). Her husband and five children, one brother and his family and the family of one deceased sister were available for further study.

Qualitative analyses of the hemoglobin patterns were carried out by starch gel electrophoresis.\textsuperscript{10} The percentages of the minor hemoglobin fractions were determined with the use of DEAE-cellulose chromatography.\textsuperscript{8} Since the chromatographic mobility of the abnormal variant was only slightly higher than that of Hb-A\textsubscript{0} a slow buffer gradient was applied to a column of 45 X 0.9 cm. The following buffer solutions, designated by their pH values and composed of sodium phosphate and NaCl, prepared in concentrations as described in an earlier publication,\textsuperscript{8} were used in the variable gradient device: 8.6; 8.4; 8.4; 8.1; 8.1; 7.9; 7.7; 7.5; 6.8; 6.5. After elution of the two minor hemoglobin fractions the column was mounted above a volumetric flask (200 ml.) and the remaining hemoglobin eluted with a 0.01 M sodium phosphate buffer pH 6.0 to which 0.3 M NaCl was added. Calculation of the percentages of the hemoglobin fractions was carried out by using the formula presented for routine Hb-A\textsubscript{0} determinations.\textsuperscript{7} Larger quantities of the abnormal components were also prepared by DEAE-cellulose chromatography using the procedure outlined for the purification of Hb-Lepore.\textsuperscript{8} Gross structural abnormalities were studied by hybridization using a modification of the technic described by Gammack et al.\textsuperscript{3} and by starch gel electrophoresis of globin in formate buffer pH 1.9.\textsuperscript{5,12} Both technics have been reviewed recently.\textsuperscript{10} The possible presence of \delta chains, although abnormal, was also studied by immunologic procedures described in a previous paper.\textsuperscript{16} Particularly, the reactions of the abnormal hemoglobin component with anti A and anti A\textsubscript{2} antibodies, before and after adsorption with Hb-F and Hb-A were investigated. Hematological data were obtained using the conventional technics.

Results

Figure 2 (A, sample 1) presents the electrophoretic pattern of the hemoglobin of a heterozygous carrier. The abnormal fraction moved distinctly slower than Hb-F (sample 3) and only slightly faster than Hb-S, as was

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demonstrated by comparing the mobilities of an impure-isolated abnormal fraction (sample 5) with those of the hemoglobins of a sickle cell trait carrier (sample 4) and of cord blood (sample 6). The electrophoretic mobility of the abnormal component is similar to that described for Hb-Flatbush by Ranney et al; for this reason the abnormality found in our family is also tentatively designated as Hb-FlatbushGa.* An example of separation of Hb-A2, Hb-FlatbushGa and Hb-A by DEAE-cellulose chromatography is shown in figure 1C. Since the separation is almost complete, reasonably accurate calculations of the quantities of Hb-A2 and its variant could be carried out.

The hemoglobin variant was demonstrated in three additional cases (fig. 1). The brother of the propositus (II, fig. 1) was heterozygous for Hb-C; no individuals with both Hb-C and Hb-FlatbushGa were found. Data from hematological studies of the four Hb-FlatbushGa carriers were not significantly different from the data for relatives without apparent hemoglobinopathy (table 1). In each of the four carriers of Hb-FlatbushGa, Hb-A2 was notably decreased; values varying between 0.9 and 1.15 per cent were observed. Hb-FlatbushGa accounted for 1.35–1.50 per cent of the total hemoglobin (table 1). In all other family members, whose hemoglobin was found to be normal, Hb-A2 levels were within the normal range.

Evidence for the presence of normal α-polypeptide chains in Hb-FlatbushGa was obtained by recombination experiments utilizing the β-chain abnormality Hb-S and the α-chain abnormality Hb-A2Rus.* No hybrid hemoglobins were produced from mixtures of Hb-S and Hb-FlatbushGa, while two new components were observed when a mixture of Hb-A2Rus and Hb-FlatbushGa was

*It is the opinion of the authors that the abnormality described resembles Hb-Flatbush so closely that it seems not appropriate to give the abnormal hemoglobin a specific designation. The designation Hb-FlatbushGa was selected to indicate that the abnormality was detected in a family living in the state of Georgia. Although this designation is in accordance with the procedure followed for the abnormal hemoglobins named by letters, the novelty of adding one geographic designation as a subscript to another is realized.
studied. One of the new species of hemoglobin possessed an electrophoretic mobility similar to that of Hb-A. The second component moved considerably slower than Hb-Flatbushoa and Hb-ARuss at pH 8.1 and is therefore probably composed of the abnormal α-chains of Hb-ARuss and the abnormal non α-chains of Hb-Flatbushoa. Starch gel electrophoresis of the globin prepared from Hb-Flatbushoa in formate buffer pH 1.9 revealed the presence of two subunits, one with mobility of normal α-chains and a second with a mobility indistinguishable from that of the corresponding subunit of Hb-A2 (fig. 3). This technic seems useful for the demonstration of the types of polypeptide chain present; the separation of the normal α, β, γ and δ chains can easily be obtained. The method is less satisfactory for the demonstration of the possible presence of abnormal α or β or δ chains, since the electrophoretic mobilities of abnormal α-chains as present in Hb-ARuss, Hb-A2Russ, Hb-Do, Hb-A2-Do, of abnormal β-chains as found in Hb-Dpunjab and of abnormal δ-chains, as are probably present in Hb-Flatbushoa, are identical to those of the corresponding normal polypeptide chains (fig.3). Examples of results of immunologic studies with the isolated Hb-Flatbushoa are present in figure 4. It seems evident that the reaction of this abnormal hemoglobin with anti-β-chain antibodies, obtained by absorbing anti Hb-A (α2β2) antibodies with Hb-F (α2γ2), is identical to the reactions given by Hb-A2 (α2δ2) and its variant Hb-A2' and distinctly different from that given by Hb-A. The reaction of Hb-Flatbushoa with anti δ-chain antibodies, obtained by absorbing anti A2 (α2δ2) antibodies with either Hb-F or Hb-A, is again similar to that obtained with Hb-A2. It seems, therefore, that the immunologic reaction of Hb-Flatbushoa differs from that of Hb-A in a way similar to that found for Hb-A2 and Hb-A2'. The difference in reactivity of Hb-Flatbushoa with anti-δ-chain antibodies, obtained after absorbing anti A2 antibodies with Hb-A, may indicate the presence of a structural abnormality in that part of the δ-chain which is responsible for the observed reaction of Hb-A2 and anti-δ-chain antibodies.

**Discussion**

From the data obtained in our studies of the isolated component, it seems evident that the abnormal hemoglobin is composed of normal α-chains and altered δ polypeptide chains. The hemoglobin is distinctly different from two other δ-chain abnormalities, namely Hb-A2' (or Hb-B2) which migrates to the cathode on electrophoresis at pH 8.1,1,2,4,6 and Hb-Köln, which migrates more slowly than Hb-S under the same conditions.10,13 Our electrophoretic data indicate a similarity of the abnormality with Hb-Flatbush;14 the mobilities of the two components in starch gel electrophoresis at alkaline pH are closely similar, if not identical. Although the results of structural studies of the abnormal component found in our family and of that reported by Ranney et al.14 are not yet available, we consider the identity of the two abnormalities most likely.

In the heterozygous state, Hb-Flatbushoa is not associated with any detect
Fig. 2.—Electrophoretic and chromatographic properties of the abnormal minor variant. (A and B) Comparative studies by starch gel electrophoresis; for explanation see text. (C) Separation of Hb-A₂, Hb-Flatbushes, and Hb-A by DEAE cellulose chromatography.
Table 1.—Hematological Data

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VARIANT OF HEMOGLOBIN A2

able hematological abnormality. The Hb-A2 levels are decreased to approximately 1 per cent while the percentages of Hb-Flatbushα are definitely higher, namely 1.35-1.50 per cent. In this respect the Hb-Flatbushα resembles some fast moving β-chain abnormalities as for instance Hb-Jafrica15 and Hb-Jenkins,16 which were also found to be present in the heterozygous state in quantities slightly greater than the corresponding normal hemoglobin type.

Fig. 3.—The separation of normal and abnormal α, β and δ polypeptide chains of human hemoglobin types by starch gel electrophoresis at pH 1.9. 1, Hb-A (α2β2); 2, Hb-A2 (α2δ2); 3, Hb-D-Punjab (α2β2δ); 4, Hb-Russ (α2Russβ2); 5, Hb-A2Russ (α2Russδ2); 6, Hb-Flatbushα; 7, Hb-A2-Dα (α2β2δ); 8, Hb-Dα (α2β2δ).
Fig. 4.—The immunologic behavior of isolated Hb-Flatbush (Hb-A). The results from studies designated to elucidate the structural abnormality of the hemoglobin variant will be the subject of a future report.

SUMMARY

The discovery of an abnormal minor hemoglobin in four heterozygous members of a Negro family is reported. The abnormality seems to be a variant of Hb-A2 and is probably identical with Hb-Flatbush.

SUMMARIO IN INTERLINGUA

Es reportate le discoperta de un anormal minor hemoglobina in quatro membros heterozygotic de un familia negre. Le anormalitate pare esser un variante de Hemoglobina A2 e es probabilemente identic con hemoglobina Flatbush.

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