“Pseudo-Abnormal” Hemoglobins

By HOWARD A. PEARSON, JAMES V. McCoo AND SANFORD L. LEIKIN

SINCE the original use of electrophoresis to demonstrate an abnormal human hemoglobin,¹ the number of recognizable variants has rapidly increased. The purpose of this brief recording is not to increase the number of hemoglobin varieties further, but rather to call attention to artifactual “pseudo-abnormal” hemoglobins which have been observed in our laboratories during the past two years, and to caution against the acceptance of similar components as being true hemoglobin variants without repeated electrophoretic confirmation with different blood samples.

METHOD

Blood was collected, using oxalate as the anticoagulant. No sterile precautions were used. Our specimens are received from a widespread area and are often in transit in the mails for many days.

Red cells were washed three times with cool, normal saline, hemolyzed with distilled water and toluene and then clarified by high speed centrifugation. Starch block electrophoresis was performed, using barbital buffer at pH 8.6,² as previously described.

RESULTS

Example 1. This blood specimen was obtained as part of a family study. The blood was not refrigerated and was transported to the laboratory a distance of 300 miles in the trunk of an automobile during the summer months.*

Starch block electrophoresis showed four distinct hemoglobin components (fig. 1-b). There was a component in the position of Hgb. C, accounting for 40 per cent of the total, and a component in the position of Hgb. A, accounting for 19 per cent of the total. An intermediate component, slower than Hgb. S but faster than Hgb. C and A₂, constituted 26 per cent. Finally, a fast minor component in the position of Hgb. Hopkins II was observed, accounting for 15 per cent. It was believed that this individual might simultaneously possess abnormal genes controlling the synthesis of α and β chains such as has recently been described.³ ⁵ The intermediate component could, therefore, be a “hybrid” sharing the electrophoretic abnormalities of both Hgb. C and the fast component. The fast and intermediate components demonstrated the usual spectral absorption curves of cyanmethemoglobins with absorption maxima at 540 and 415 mµ. However, subsequent study of a fresh sample showed only a C-A pattern consistent with Hgb. C thalassemia disease (fig. 1-c). Hgb. C now accounted for 72 per cent of the total hemoglobin.

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Fig. 1.—The starch block pattern of Example 1. Barbital buffer, pH 8.6.

-b. Specimen showing intermediate and fast artefactual components.
-c. Another specimen from same patient —b, showing only C and A components.

Example 2. This specimen was sent by regular mail from a hospital 250 miles from the laboratory, and was in transit four days. Moderate hemolysis of the specimen was observed.

The original electrophoretic pattern is demonstrated in figure 2-a. A fast component accounted for 17 per cent of the total. This component was indistinguishable, spectrophotometrically, from normal cyanmethemoglobin. Electrophoresis of a fresh specimen showed an entirely normal pattern (fig. 2-b).

Example 3. This patient was studied because of hereditary microcytic anemia. Starch block electrophoresis showed a minor hemoglobin component in the S position, accounting for 6.0 per cent of the total hemoglobin. Hgb. A2 was 5.0 per cent (fig. 3-a). Since the patient had the erythrocyte morphology of thalassemia trait, it was felt that this might represent another instance of Lepore trait. However, electrophoresis of a newly drawn specimen showed no intermediate component, and Hgb. A2 was 7.0 per cent (fig. 3-b).

DISCUSSION

The mechanism of the production of these artefactual hemoglobins is uncertain and cannot be related to any single, unique set of conditions. Over 400
Fig. 2.—Starch block pattern of Example 2.

—a. Pattern showing a fast, artefactual component.
—b. New specimen from same patient showing only Hgb. A and A₂.

Fig. 3.—Starch block pattern of Example 3.

—a. Pattern showing elevated Hgb. A₂ and an artefactual intermediate fraction.
—b. In a new specimen, the intermediate fraction is no longer visible.

Blood specimens have been examined by starch block electrophoresis in our laboratories. Many have been transported over large distances by ordinary mail and some of the samples are one or two weeks old when electrophoresed. Oxalate was used as the anticoagulant in the majority of specimens. The spectral absorption patterns of some of these components were identical with that of normal cyanmethemoglobin, suggesting that no great disruption of the hemoglobin molecule had occurred. These transitory components are probably related to Hgb. A₃, a component faster than A₁, which appears to arise from
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this. Significantly, electrophoretic mobilities of the artefactual components were, in general, faster than the hemoglobins from which they presumably were derived. Bacterial action on hemoglobin might also be important.

We have attempted to produce similar "pseudo-abnormal" hemoglobins intentionally by aging and heating other blood samples. However, although distortion and streaking of the starch pattern has been observed, no distinct components such as described here have been seen.

Hill has recently revealed that a hemoglobin "G", which for several years had posed a conundrum for geneticists, was indeed artefactual, similar to our "pseudo-abnormal" hemoglobins. This, combined with the examples described in this report, emphasizes that unusual hemoglobin components should be re-examined with freshly prepared hemolysates before being accepted as real.

SUMMARY AND CONCLUSIONS

Three examples of artefactual "pseudo-abnormal" hemoglobins were observed in the course of starch block electrophoresis analysis of over 400 specimens. In fresh samples from the same patients, these components were no longer demonstrable. It is suggested that repeated examination with different blood specimens should be done before an unusual component is accepted as a true hemoglobin variant.

SUMMARIO IN INTERLINGUA

Tres exemplos de artefactic hemoglobinas "pseudo-anormal" esseva observate in le curso del analyse de 400 specimens per electrophorese a bloco de amylo. In specimens fresce ab le mesme patientes, ille componentes esseva noq plus demonstrabile. Es proponite que repetite examines con differente specimens deberea esser effectuate ante que un componente inusual es acceptate como ver variante hemoglobinic.

REFERENCES


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