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A SECOND AND A THIRD ABNORMAL HAEMOGLOBIN IN NORFOLK

HAEMOGLOBIN $G_{Norfolk}$ and HAEMOGLOBIN $D_{Norfolk}$

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In 1958 Ager, Lehmann, and Vella reported that they had discovered a family in Norfolk possessing a new abnormal haemoglobin — haemoglobin Norfolk. To determine the incidence of this haemoglobin in the County of Norfolk 1,000 unrelated inhabitants were examined for the presence of abnormal haemoglobins (Huntsman, 1963). Though no further examples of haemoglobin Norfolk could be traced two Norfolk families with other abnormal haemoglobins were found.

HAEMOGLOBIN $G_{Norfolk}$

Propositus.—The propositus (W. A.), a young married man with two children, was at the time attending the Norfolk and Norwich Hospital for a routine follow-up of tuberculosis of the spine. He was fit and all physical and laboratory examinations yielded normal results. In particular there was no anaemia and all haematological examinations were within normal limits.

Examination of Haemoglobin

No haemoglobin F was found, but on paper electrophoresis at *p*H 8.6 and 8.9 a slow-moving fraction was seen to separate from haemoglobin A; it amounted to about 20% of the total pigment (Fig. 1). The exact position was at all times between haemoglobin A and

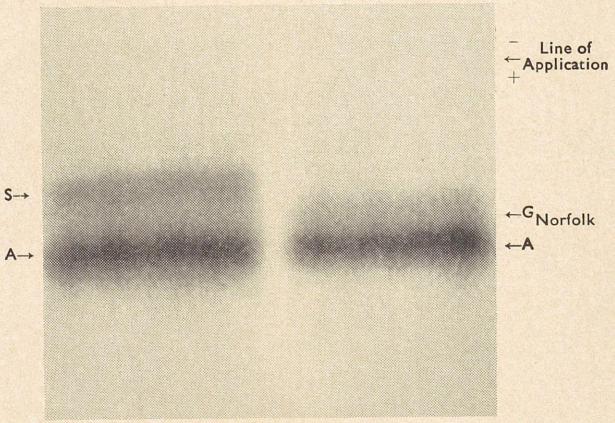

FIG. 1.—Filter-paper electrophoresis, *p*H 8.9, TRIS buffer (Cradock-Watson, Fenton, and Lehmann, 1959). Haemoglobin $G_{Norfolk}$ moves behind haemoglobin A but faster than haemoglobin S.

haemoglobin S controls and corresponded to that of haemoglobin G controls. On column chromatography at *p*H 6 (Huisman and Prins, 1955) with IRC-50 the position was again the same as that of haemoglobin G, between A and S. No separation from haemoglobin A was obtained on paper electrophoresis at *p*H 6.5 or *p*H 7.

When the isolated abnormal fraction was "hybridized" with haemoglobin C (an abnormal β-chain haemoglobin) two additional haemoglobins were formed at alkaline *p*H, on paper and on starch-gel electrophoresis. One of these migrated in the position of haemoglobin A and the other moved behind haemoglobin C, indicating that the new haemoglobin had its mutational change in the α-chain (Fig. 2).

$$\text{Hb G}_{\text{Norfolk}} + \text{Hb C} \rightarrow \text{Hb G}_{\text{Norfolk}} + \text{Hb C} + \text{Hb A} + \text{Hb G}_{\text{Norfolk}}/\text{C}.$$

$$\alpha_2{}^{\text{G}}\beta_2 + \alpha_2\beta_2{}^{\text{C}} \rightarrow \alpha_2{}^{\text{G}}\beta_2 + \alpha_2\beta_2{}^{\text{C}} + \alpha_2\beta_2 + \alpha_2{}^{\text{G}}\beta_2{}^{\text{C}}$$

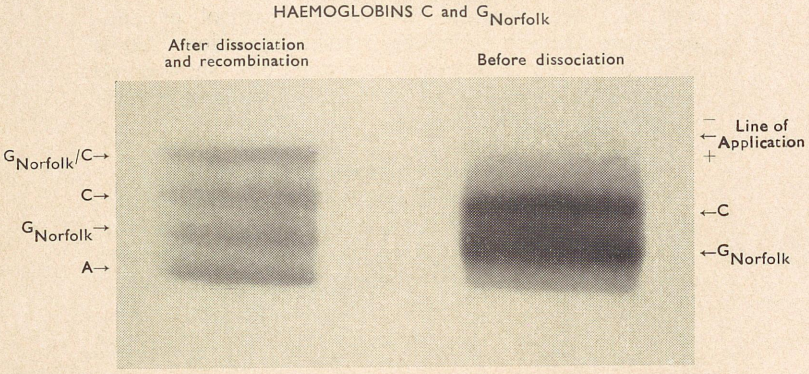

FIG. 2.—Hybridization of haemoglobins C and $G_{Norfolk}$. After dissociation and recombination, electrophoresis on starch gel clearly shows two additional haemoglobins, haemoglobin A and haemoglobin $G_{Norfolk}$ /C. For details of technique see Raper, Gammack, Huehns, and Shooter (1960).

On the basis of its electrophoretic, chromatographic, and hybridization properties the new haemoglobin can be described as haemoglobin $G_{\alpha\ Norfolk}$.

Family Study

Two other relatives of the propositus were found to possess the same abnormal haemoglobin (Fig. 3), but it was not seen in any of the other 999 blood samples in the Norfolk survey, nor was it possible to trace any living parents or cousins on the mother's side of the propositus, from whom the haemoglobin had been inherited. The mother is of East Anglian stock.

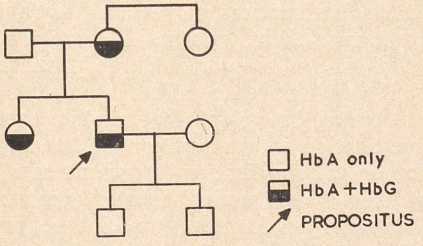

FIG. 3.—Norfolk family with haemoglobin G.

HAEMOGLOBIN $D_{Norfolk}$

Propositus.—The propositus (R. J. H.), an elderly married man with one child, was attending the Norfolk

and Norwich Hospital for routine control of anti-coagulant therapy following cardiac infarction. He is now well and laboratory investigations showed no evidence of anaemia ; all haematological examinations were within normal limits.

Examination of Haemoglobin

No haemoglobin F was found, but on paper electrophoresis at *p*H 8.6 and 8.9 a slow-moving fraction was seen to separate from haemoglobin A, amounting to about 30% of the total pigment (Fig. 4). The exact

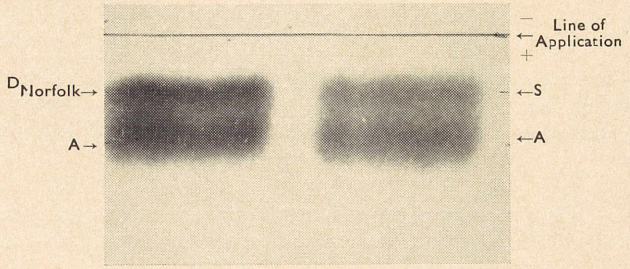

FIG. 4.—Filter-paper electrophoresis *p*H 8.9, TRIS buffer. Haemoglobin $D_{Norfolk}$ moves behind haemoglobin A at the same distance as haemoglobin S.

position was at all times identical to that of haemoglobin S or D. However, repeated attempts failed to produce red-cell sickling and the haemoglobin solubility test (Itano, 1953) was normal. On column chromatography at *p*H 6 with IRC-50 the position was again the same as that of haemoglobin S or D.

When the isolated abnormal fraction was "hybridized" with the original α-chain abnormal haemoglobin Norfolk an additional band of haemoglobin was formed on starch-gel electrophoresis at alkaline *p*H. This fraction migrated between haemoglobin Norfolk and the haemoglobin under investigation in the position of haemoglobin A. As in other " hybridization " experiments between abnormal " fast " and " slow " haemoglobins the doubly abnormal hybrid would also move in the position of haemoglobin A because the opposed negative and positive charges of the two abnormal α_2 and β_2 subunits tend to cancel each other out. This indicated that the new haemoglobin had its mutational change in the β-chain (Fig. 5). As one would expect, no additional haemoglobins were formed when the haemoglobin under

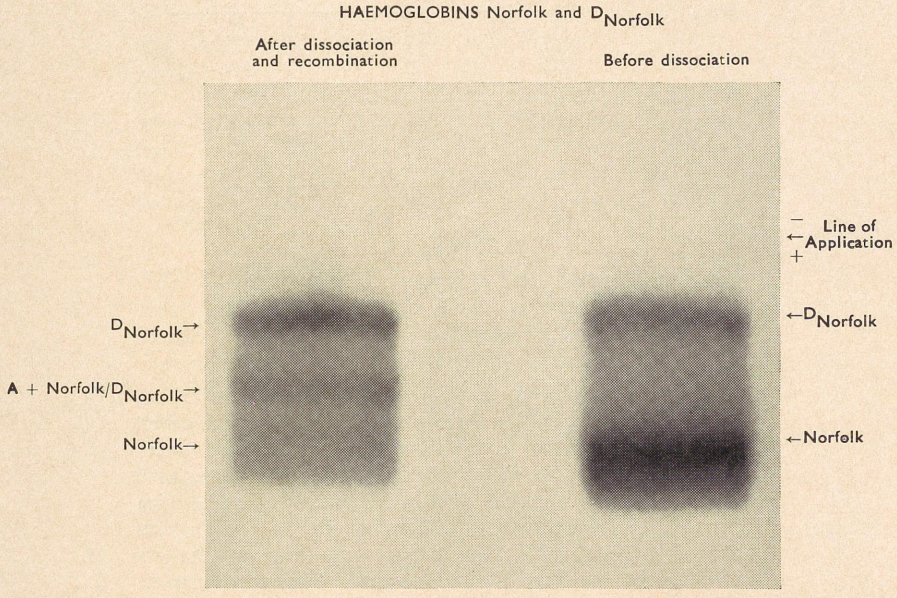

FIG. 5.—Hybridization of haemoglobins Norfolk and D Norfolk. After dissociation and recombination, electrophoresis on starch gel clearly shows an additional line due to the formation of haemoglobin A and haemoglobin Norfolk/$D_{Norfolk}$; these migrate as a single band (see text).

investigation was " hybridized " with β-chain abnormal variants such as haemoglobin S, C, and D_{Punjab}.

$$\text{Hb Norfolk} + \text{Hb } D_{Norfolk} \rightarrow$$

$$\text{Hb Norfolk} + \text{Hb } D_{Norfolk} + \text{Hb A} + \text{Hb Norfolk}/D_{Norfolk}$$

$$\alpha_2{}^{Norfolk}\beta_2 + \alpha_2\beta_2{}^{D} \rightarrow \alpha_2{}^{Norfolk}\beta_2 + \alpha_2\beta_2{}^{D} + \alpha_2\beta_2 + \alpha_2{}^{Norfolk}\beta_2{}^{D}$$

On the basis of its electrophoretic, chromatographic, hybridization, and solubility properties this new haemoglobin can be described as haemoglobin $D_{\beta\ Norfolk}$.

Family Study

Six other relatives of the propositus were found to possess the same abnormal haemoglobin (Fig. 6), but it was not seen in any of the other 999 blood samples in the Norfolk survey. This haemoglobin was found to be inherited through three generations, originating from the sister of the propositus, who also possessed this variant. Two families (consisting of 11 members examined) descending from a maternal aunt of the propositus did not possess this abnormal pigment. All these families are of East Anglian stock.

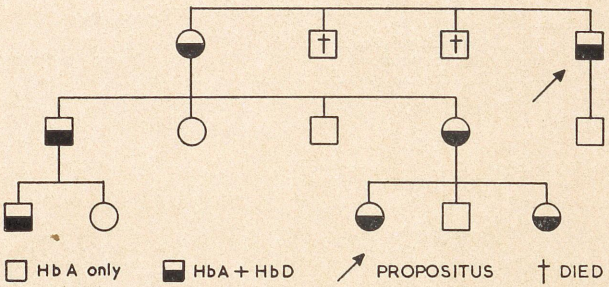

FIG. 6.—Norfolk family with haemoglobin D.

Discussion

Since haemoglobin G was first reported from West Africa by Edington and Lehmann (1954) several other haemoglobins have been described under this letter. The position has recently been summarized by Swenson, Hill, Lehmann, and Jim (1962). Haemoglobins G may carry their abnormality either in the α- or in the β-chains, and with two exceptions—one in Italian immigrants in the United States (Schwartz, Spaet, Zuelzer, Neel, Robinson, and Kaufman, 1957) and the other in Italy (Silvestroni and Bianco, 1958)—the haemoglobin was found either in Africans and their descendants in the New World or in Chinese. Of the African haemoglobins G one, haemoglobin G_{Accra}, is a β-chain mutation, and the others are identical α-chain mutants with lysine as the sixty-eighth residue of the α-chain instead of asparagine (Baglioni and Ingram, 1961). The Chinese haemoglobins G also are an α-chain mutation, but the substitution consists in the replacement of glutamic acid by glutamine in the thirtieth position.

Haemoglobin D has been described sporadically in white families in the U.S.A. and in Europe, but there was usually some possibility of non-European ancestry. It is found at considerable incidence in Punjabis and in Gujeratis, in Pakistan and in India respectively, and so far most of the haemoglobin D samples examined elsewhere have been shown to be identical with the type of haemoglobin found in the Punjab—namely, a substitution has occurred in the 121st position of the β-chain by a glutamine residue for one of glutamic acid (Bowman and Ingram, 1961 ; Baglioni, 1962).

The two haemoglobins will be analysed for their amino-acid substitution. The haemoglobin G differs in

its peptide chromatogram from all previously analysed haemoglobins G, and in the haemoglobin D the abnormality seems to lie in "Tβ4" (personal communications from Professor R. L. Hill, Duke University, North Carolina, and Dr. W. Konigsberg, Rockefeller Institute, New York). But meanwhile it is considered that the finding of two further abnormal haemoglobins in a survey of Norfolk families is an event worthy of record.

Summary

In a survey for abnormal haemoglobins of 1,000 unrelated inhabitants of the County of Norfolk, no further example of haemoglobin Norfolk was discovered; however, two other instances of abnormal haemoglobins in English families were seen. A haemoglobin G was detected and identified as having its mutational change in the α-chain: haemoglobin $G_{\alpha\ \text{Norfolk}}$. A haemoglobin D was found with its mutational change in the β-chain: haemoglobin $D_{\beta\ \text{Norfolk}}$.

We are indebted to Mr. E. Thompson for his help in collecting blood samples in Norwich—in the course of which the present haemoglobins were discovered—and to Mr. V. Price-Jones and Mr. S. Parkin for collecting blood samples from King's Lynn and Wisbech respectively.

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