abnormal amounts of formiminoglutamic acid (Figlu). A horizontal type, 1,000-volt electrophoresis instrument was used and a clearly recognizable spot of Figlu on the paper strip (a positive test) has been considered indicative of a deficiency in folic-acid metabolism.

The patients studied were 50 cases of megaloblastic anaemia or related conditions. It was found that all of five patients with dietary deficiency of folic acid, four of six patients with cirrhosis, and three of eight patients with a malabsorption syndrome had positive tests, which were easily reverted by the administration of folic acid.

Eleven patients with pernicious anaemia in remission had negative tests, but five out of eight patients with pernicious anaemia in relapse had positive tests. Three of these five patients showed some resistance to folic-acid therapy in reversing the Figlu test to normal. Vitamin B₁₂ alone reverted two of the patients to normal, but in another patient vitamin B₁₂ failed to do this and she required injections of folic acid to achieve it. Other patients tested included cases of erythraemic myelosis and leukaemia with megaloblastic bone-marrow.

It is considered that a positive Figlu test may result from dietary insufficiency of folic acid, malabsorption of folic acid, or from metabolic disturbances in the action of folic acid in the body, one important factor in this regard being vitamin B₁₂ metabolism.

REFERENCES

- Bakerman, H. A., Silverman, M., and Daft, F. S. (1951). *J. biol. Chem.*, **188**, 117.
 Borek, B. A., and Waelsch, H. (1953). *Ibid.*, **205**, 459.
 Carter, F. C., Heller, P., Schaffner, G., and Korn, R. J. (1961). *Arch. intern. Med.*, **108**, 41.
 Herbert, V., Baker, H., Frank, O., Pasher, I., Sobotka, H., and Wasserman, L. R. (1960). *Blood*, **15**, 228.
 Husain, O. A. N., and Ellis, L. (1961). *Lancet*, **1**, 279.
 Knowles, J. P. (1961). *Ibid.*, **1**, 397.
 — Pranker, T. A. J., and Westall, R. G. (1960). *Ibid.*, **2**, 347.
 Kohn, J., Mollin, D. L., and Rosenbach, L. M. (1961). *Ibid.*, **1**, 112.
 Lewis, F. J. W., and Moore, G. R. (1962). *Ibid.*, **1**, 305.
 Lohby, A. L., and Cooperman, J. M. (1961). *Ibid.*, **2**, 490.
 Spray, G. H., and Wits, L. J. (1959). *Ibid.*, **2**, 490.
 Tabor, H., and Wyngarden, L. (1958). *J. clin. Invest.*, **37**, 824.
 Zalusky, R., and Herbert, V. (1962). *Lancet*, **1**, 108.

“ ‘Psychosis arctica’ has been described in the Lapps, and ‘kaamos,’ or depressions, are said to be common in the dark season. It isn’t clear that these are true depressive states, for other observers report that the winter months may be a period of relaxation and enjoyment for northern peoples. The mental problems that result from cultural change are much better known and they are encountered in indigenes and immigrants. The sudden change from modern city life to an apparently unchanging world, devoid of stimuli, can be disturbing for civil servants, settlers, seasonal workers and the personnel of scientific expeditions. They may become desolate and despondent, full of resentment towards families, friends and colleagues whom they believe to be leading an easier life in a more friendly climate and atmosphere. That they volunteered for the work makes no difference. They experience ‘Arctic imprisonment’ which sometimes ends in suicide. Personnel, especially for small expeditions, must be carefully selected, with no history of mental disease, drug addiction, alcoholism or homosexuality. A conference on medicine and public health in the Arctic and Antarctic, which was sponsored recently in Geneva by the World Health Organization, expressed the opinion that especial attention should be given to the study of mental health problems in the high latitudes, particularly of transients and station personnel.” (*World Health*, January 1963.)

A SECOND AND A THIRD ABNORMAL HAEMOGLOBIN IN NORFOLK

HAEMOGLOBIN G_{Norfolk} and HAEMOGLOBIN D_{Norfolk}

BY

R. G. HUNTSMAN, M.B., M.R.C.P.

Chemical Pathologist, Memorial Hospital, Peterborough

MORGAN HALL, M.R.C.S., L.R.C.P.

Director, Department of Pathology, Norfolk and Norwich Hospitals Group

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

Reader in Chemical Pathology, St. Bartholomew's Hospital, London

AND

P. K. SUKUMARAN

Research Assistant, St. Bartholomew's Hospital, London

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 pH 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, pH 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 pH 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 pH 6.5 or pH 7.

When the isolated abnormal fraction was "hybridized" with haemoglobin C (an abnormal β -chain haemoglobin) two additional haemoglobins were formed at alkaline

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 pH 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 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 pH 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 pH. 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

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).

pH, 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).

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

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-

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

had its mutational change in the β -chain (Fig. 5). As one would expect, no additional haemoglobins were formed when the haemoglobin under investigation was "hybridized" with β -chain abnormal variants such as haemoglobin S, C, and D_{Punjab} .

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

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

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 Punjab 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 "T β 4" (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_{Norfolk}. A haemoglobin D was found with its mutational change in the β -chain: haemoglobin D_{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.

REFERENCES

- Ager, J. A. M., Lehmann, H., and Vella, F. (1958). *Brit. med. J.*, 2, 539.
 Baglioni, C. (1962). *Biochim. biophys. Acta (Amst.)*, 59, 437.
 — and Ingram, V. M. (1961). *Ibid.*, 48, 253.
 Bowman, B., and Ingram, V. M. (1961). *Ibid.*, 53, 569.
 Craddock-Watson, J. E., Fenton, J. C., and Lehmann, H. (1959). *J. clin. Path.*, 12, 372.
 Edington, G. M., and Lehmann, H. (1954). *Lancet*, 2, 173.
 Huisman, T. H. J., and Prins, H. K. (1955). *J. Lab. clin. Med.*, 46, 255.
 Huntsman, R. G. (1963). Thesis to be submitted for the M.D. degree, Cambridge University.
 Itano, H. A. (1953). *Arch. Biochem.*, 47, 148.
 Raper, A. B., Gammack, D. B., Huehns, E. R., and Shooter, E. M. (1960). *Brit. med. J.*, 2, 1257.
 Schwartz, H. C., Spaet, T. H., Zuelzer, W. W., Neel, J. V., Robinson, A. R., and Kaufman, S. F. (1957). *Blood*, 12, 238.
 Silvestroni, E., and Bianco, I. (1958). *Polliclinico, Sez. prat.*, 65, 203.
 Swenson, R. T., Hill, R. L., Lehmann, H., and Jim, R. T. S. (1962). *J. biol. Chem.*, 237, 1517.

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).

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.