A HAEMOGLOBINOPATHY INVOLVING HAEMOGLOBIN H AND A NEW (Q) HAEMOGLOBIN

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The technique of electrophoresis was first used successfully in the study of adult haemoglobin by Pauling, Itano, Singer, and Wells (1949), when they demonstrated that haemolysates obtained from the erythrocytes of patients suffering from sickle-cell anaemia exhibited properties which differed greatly from those of haemolysates of erythrocytes from normal adult individuals. This discovery of the usefulness of electrophoresis in the study of haemoglobin has led to the recognition of other variants of adult human haemoglobin, whose genetic control, somatic effects, and geographical distribution have been under intensive study ever since.

It is the purpose of the present communication to describe in detail the properties of a new haemoglobin which was detected in association with haemoglobin H and to give the clinical and other findings in the patient presenting this new haemoglobinopathy.

Case Report

A Chinese man aged 24 was first admitted to the general Hospital, Singapore, in August, 1953, with a history of shortness of breath, palpitations, giddiness, blurring of vision, and precordial pain on exertion, associated with anorexia and generalized oedema. The onset of these symptoms had been insidious over a period of about three months.

He was found to have a gross hypochromic anaemia, haemoglobin 2.9 g./100 ml., with *Ancylostoma* ova in his stools. Following blood transfusion, treatment with iron, and worming he quickly improved, and when his haemo-

globin level was 10.2 g./100 ml. he was discharged. One year later, in September, 1954, he was readmitted with similar but less severe symptoms, a haemoglobin of 5.1 g./100 ml., and again *Ancylostoma* ova in his stools. He was again wormed and treated with iron. When his haemoglobin reached 9.9 g./100 ml. he was again discharged, and has since been followed continuously as an out-patient. Although there has been no recurrence of his ancylostomiasis, his haemoglobin varies between 3.6 and 11.8 g./100 ml. Treatment with large doses of iron by injection and by mouth, with vitamin B₁₂, folic acid, ascorbic acid, and multiple vitamin preparations, has failed to produce any response.

A recent examination showed him to be a pale, listless, slim young man, with slight sternal tenderness. There were no other abnormal physical signs, and in particular he had no enlarged lymph nodes and his liver and spleen were not palpable. He was not jaundiced.

Radiological Investigations.

Chest radiographs showed no abnormality, but radiographs of the skull showed about 12 small radiotranslucent areas, mostly in the parietal regions (Fig. 1). These areas have a diffuse outline, are roughly circular, and vary from 4 to 11 mm. in diameter. The diploë of the skull were normal. Radiographs of the hands showed small sharply defined radiotranslucent areas in the distal shafts of all the proximal and middle phalanges and also in the bases of both second metacarpals (Fig. 2). Otherwise the bony trabeculae of both hands appeared to be within normal limits. Radiographs of the feet showed similar radiotranslucent areas in the phalanges.

FIG. 2.—Radiograph of hand showing radiotranslucent areas in phalanges and second metacarpal.

FIG. 1.—Radiograph of skull showing radiotranslucent areas in parietal region.

FIG. 3.—Peripheral blood smear (Leishman's stain) showing target cells.

Laboratory Investigations.—Blood Wassermann, Kahn, and V.D.R.L. tests were negative. Serum bilirubin was 0.6 mg./100 ml.; serum iron, 220 μ g./100 ml.; urine urobilinogen, 16 mg. in 24 hours; faecal urobilinogen, 320 mg. in 24 hours. The urine contained no albumin, sugar, or acetone, and the deposit was normal on microscopy. The stools contained no amoebae, cysts, or ova. A histamine test showed a normal gastric hydrochloric acid response. The haematological investigations showed: haemoglobin, 10.1 g./100 ml.; R.B.C., 4,810,000/c.mm.; P.C.V., 43.4%; M.C.V., 90.2 cubic microns; M.C.H., 21.0 μ g.; M.C.H.C., 23.3%; M.C.D., 7.7–7.8 μ (halometry); W.B.C., 8,100/c.mm. (neutrophils 68%, lymphocytes 23%, eosinophils 5%, monocytes 4%); thrombocytes 278,000/c.mm.; reticulocytes 8%.

A peripheral blood film stained with Leishman's stain showed moderate hypochromia, anisocytosis, poikilocytosis, polychromasia, and many target cells (Fig. 3). The leucocytes and platelets appeared normal. Supravital staining with brilliant cresyl blue showed inclusion bodies (Fig. 4) in about 95% of the erythrocytes, as is seen in blood which contains haemoglobin H. Resistance to lysis

FIG. 4.—Peripheral blood. Wet preparation. Supravital staining with brilliant cresyl blue. Note the inclusion bodies.

FIG. 5.—Osmotic fragility.

by hypotonic saline was increased, haemolysis began at 0.45% NaCl and was complete only at 0.10% NaCl (Fig. 5). There was no sickling with 2% sodium metabisulphite and there were no siderocytes. Direct and indirect Coombs tests were negative at 4° C., 20° C., and 37° C.

Investigation of the Haemoglobin

Paper electrophoresis at pH 8.6 showed two components on every occasion that fresh blood samples were examined. The smaller, comprising 20% of the total, had the mobility of haemoglobin H or I, and the remainder moved between haemoglobins A and S. At pH 6.5 the smaller fraction moved towards the anode, which is the unique property of haemoglobin H, and the remainder moved towards the cathode. This is the fifteenth instance of haemoglobin H seen in Singapore, most of the subjects being of Chinese extraction. No alkali-resistant haemoglobin was found, and the haemoglobin A₂ fraction was not increased.

A sample of the haemoglobin was sent to Dr. Lie-Injo Luan Eng, of the University of Indonesia, Djakarta, and she found that the non-haemoglobin H component had a mobility resembling, but slightly differing from, that of haemoglobin S. It differed from her recently observed Buginese "X" component (Eng, 1956, 1957).

The following haemoglobins move on paper electrophoresis at pH 8.6 between A and S in order of mobility: F, G, and L. The non-haemoglobin H component moved more slowly than haemoglobin F; unlike F, it was not

FIG. 6.—Comparison by hanging strip paper electrophoresis at pH 8.6 of haemoglobins A plus D and A plus L with the non-H haemoglobin of the patient. The new haemoglobin moves faster than haemoglobins D and L but more slowly than haemoglobin A. White lines mark centre of density of bands A+D, Q, and A+L.

resistant to alkali-denaturation, and when mixed with haemoglobin A it separated clearly from this haemoglobin, whereas no such separation can be achieved between haemoglobins A and F on paper electrophoresis at alkaline pH. The non-haemoglobin H component moved in advance of haemoglobin L (Fig. 6), and most resembled haemoglobin G in its position. However, there was a slight difference in that the new compound was moving a fraction faster than haemoglobin G (Fig. 7)—not unlike the haemoglobin described by Schwartz and Spaet (1955). The American authors decided that such a slight difference in mobility should not be taken into account for the time being, and called their haemoglobin G (Schwartz, Spaet, Zuelzer, Neel, Robinson, and Kaufman, 1957). In the comparison

FIG. 7.—Comparison by hanging strip paper electrophoresis at pH 8.6 of a mixture of the new haemoglobin with haemoglobin A with haemoglobins A plus G. Note that the new haemoglobin moves slightly faster than haemoglobin G.

of the new haemoglobin by itself with a haemoglobin AG mixture the difference between the new haemoglobin and haemoglobin G was barely noticeable. But if the new haemoglobin by itself was compared with pure haemoglobin G, or if haemoglobin A was added to it and the resulting mixture was compared with a mixture of haemoglobin A plus G,

the fact that it moved faster than haemoglobin G became more obvious. The explanation for this is that pure haemoglobin G moves on paper electrophoresis at alkaline pH a little more slowly than the haemoglobin G of a haemoglobin A plus G mixture. In such a mixture the position of the separated G is just in front of that of pure haemoglobin G—that is, it is found a little nearer to the haemoglobin A fraction. This interaction between haemoglobins which move very similarly on electrophoresis is not unusual. For example, when a haemoglobin A plus J mixture is compared with pure haemoglobin A, the haemoglobin A of the mixture moves a little faster than the pure haemoglobin A.

Fortunately we were able to differentiate the present haemoglobin more definitely from haemoglobin G by ion-exchange-resin chromatography (Huisman and Prins, 1957). The following is the sequence of the haemoglobins in order of mobility by this technique: H, F, I (A–J–K), G, E (S–D), L, C. The new haemoglobin moved between haemoglobins S or D, and L. It was found close to L, but a mixture of the two divided and the new haemoglobin moved in advance of haemoglobin L (Fig. 8). On acid electrophoresis at

spectrum of the carboxyhaemoglobin showed no difference when compared with haemoglobin A.

The present case shows a haemoglobin which differs from all other known haemoglobins in its electrophoretic behaviour on paper electrophoresis at pH 8.6, although the difference from haemoglobin G is small. It also differs from all other known haemoglobins in its chromatographic behaviour, although it resembles haemoglobin L more than any other. The following haemoglobins are now known and named: A, C, D, E, F, G, H, I, J, K, L, M, and S. The letter N is for the time being reserved for a new haemoglobin from Liberia (Robinson, Zuelzer, Neel, Livingstone, and Miller, 1956). Two other abnormal haemoglobins have been described by Eng (1956, 1957), and by Schneider and Haggard (1957), which are provisionally known as Buginese X and the Galveston type respectively. The new haemoglobin has been compared with Liberia I and II, Buginese X, and the Galveston type haemoglobins, and has been found to differ from them all on paper electrophoresis at pH 8.6 and on resin-chromatography. One abnormal haemoglobin which moved faster than haemoglobin A on paper electrophoresis at alkaline reaction has been seen by Fessas and Papaspyrou (1957) in an infant shortly after birth, but it disappeared later on. It is therefore not yet certain whether this haemoglobin will come under the category of the inherited haemoglobin variants. Leaving the letters N, O, and P for the naming of the haemoglobins from Liberia, Indonesia, and Texas, we are calling the new pigment described here haemoglobin Q.

Family History

Both parents were born in Amoy of Hokien ancestry. They were not blood relatives. The father died as a young man. The mother was an only child and is alive in Singapore. There were five children, of whom two elder sisters are unavailable for study, having been last heard of from China in 1949. Two brothers died, aged 21 and 12, following a haematemesis and an undefined progressive illness respectively.

The patient's mother is about 60 years old and is totally deaf. Haematological investigations on her showed: haemoglobin, 11.7 g./100 ml.; R.B.C., 5,000,000/c.mm.; P.C.V., 36%; M.C.V., 72 cubic microns; M.C.H., 23.2 μ g.; M.C.H.C., 29.5%; M.C.D., 7.2 μ (halometry); W.B.C., 7,800/c.mm. (neutrophils 65%, lymphocytes 28%, eosinophils 4%, monocytes 3%); thrombocytes 275,000/c.mm.; reticulocytes, 1.3%. A peripheral blood film stained with Leishman's stain showed moderately hypochromic normocytic erythrocytes with minimal anisocytosis. Target cells were 1%. The leucocytes and platelets appeared normal. Supravital staining with brilliant cresyl blue showed no inclusion bodies. There was no sickling with 2% sodium metabisulphite. Siderocytes were absent. On paper electrophoresis at pH 8.6 she showed about 60% of haemoglobin A and about 40% of a haemoglobin resembling in mobility the non-haemoglobin H component of her son. Alkali-resistant haemoglobin amounted to 1%.

Haemoglobin Q–H Disease

Haemoglobin H was detected simultaneously in two Chinese siblings in America (Rigas, Koler, and Osgood, 1955, 1956) and in two Greek families (Gouttas, Fessas, Tseverenis, and Xefteri, 1955). It has since been found in Filipinos (Motulsky, 1956), in Thais (Na-Nakorn and Minnich, 1956), in a Malay (Lehmann and Singh, 1956), in a Greek Cypriot (Ager and Lehmann, 1957), in a Trans-Jordanian (White, Beaven, and Ellis, 1956), in Chinese in Indonesia (Eng, Poey, Kho, and Endenburg, 1957), in Singapore (Vella, 1957), and in a Nepalese Gurkha in Singapore (Brain and Vella, 1958).

The haemoglobin H trait usually presents clinically as a moderate or severe hypochromic microcytic anaemia resistant to iron therapy, with or without splenomegaly, radiological changes, and target cells, the finding of intra-

FIG. 8.—Comparison by ion-exchange chromatography between a mixture of haemoglobins A, L, and Q with haemoglobin Q only. It will be noted that haemoglobin Q moves similarly to haemoglobin L, but slightly in front of it. Upper two-thirds of columns shown.

pH 6.5 the compound resembled haemoglobin G in mobility, but on electrophoresis in agar at pH 6.2 (Robinson, Robson, Harrison, and Zuelzer, 1957) it resembled haemoglobin L and moved more slowly than haemoglobin S.

The solubility of the new haemoglobin was established after haemoglobin H had become denatured on storage in the cold and the precipitate had been removed by centrifugation. In Itano's (1953) solubility test the solubility of the new haemoglobin resembled that of haemoglobin G inasmuch as it was slightly lower than, or within the lower range of, the solubility of haemoglobin A. The solubility of the ferrohaemoglobin was 0.65 and 0.64 g. per litre of 2.58 M phosphate buffer. The visible and ultra-violet

erythrocytic inclusion bodies on supravital staining with brilliant cresyl blue, and a decreased erythrocyte osmotic fragility. The present patient's condition would have been diagnosed clinically as thalassaemia, though the marked and unusual radiological findings and the absence of splenomegaly would have aroused suspicion.

Whenever family studies have been possible haemoglobin H has not been found in the phenotype unless one of the parents had thalassaemia. It has been suggested by Rigas *et al.* (1956) and by Motulsky (1956) that the gene for haemoglobin H does not find expression unless the simultaneously present gene for haemoglobin A is modified by the additional inheritance of a thalassaemia gene.

We might speculate that our propositus inherited a gene for haemoglobin Q from his mother, and that the father transmitted a gene for haemoglobin H. Unlike haemoglobin A, haemoglobin Q would then not fully repress haemoglobin H in the non-thalassaemic haemoglobin H heterozygote. However, we have no evidence so far that haemoglobins A and H or haemoglobins Q and H are allelomorphs. Two of various possibilities are that the propositus inherited both genes for haemoglobins A and Q from his mother and a gene for haemoglobin H and one for thalassaemia from his father, or that he inherited one gene for haemoglobin Q from the mother and one for haemoglobin H and one for thalassaemia from the father. In either case the father would have suffered from haemoglobin H disease. If this were so, it would explain why the father died young and why two brothers of the propositus died prematurely. The condition described here may well turn out to be a haemoglobin H-thalassaemia associated with haemoglobin Q rather than with haemoglobin A as in the cases hitherto described. Absence in our patient of haemoglobin F and of a raised haemoglobin A₂ fraction, both of which are often associated with thalassaemia, is not unusual in haemoglobin H-thalassaemia (Motulsky, Fessas, personal communications; and observations of the authors in Singapore and London). Nevertheless on the basis of the available evidence we prefer to call the condition haemoglobin Q-H disease.

Summary

A Chinese male patient in Singapore was suffering from a chronic hypochromic anaemia which did not respond to treatment with iron. There were unusual x-ray findings in the skeleton. The haemoglobin was composed of haemoglobin H and of another new haemoglobin which has been named haemoglobin Q. On paper electrophoresis at alkaline pH it moves more slowly than haemoglobin A and slightly faster than haemoglobin G. The condition has been named haemoglobin Q-H disease. The mother, who is the only member of the family available for study, has been found to be an asymptomatic carrier of haemoglobin Q.

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REFERENCES

- Ager, J. A. M., and Lehmann, H. (1957). *Brit. med. J.*, 1, 1449.
 Brain, M. C., and Vella, F. (1958). *Lancet*, 1, 192.
 Eng, L. I. L. (1956). Thesis, University of Indonesia.
 — (1957). *Lancet*, 2, 1338.
 — Poey, S. H., Kho, L. K., and Edenburg, P. M. (1957). *Acta haemat. (Basel)*, 18, 156.
 Fessas, P., and Papaspyrou, A. (1957). *Science*, 126, 1119.
 Goutas, A., Fessas, P., Tsevenis, H., and Xefteri, E. (1955). *Sang*, 26, 911.
 Itano, H. A. (1953). *Arch. Biochem.*, 47, 148.
 Huisman, T. H. J., and Prins, H. K. (1957). *Clin. chim. Acta (Amst.)*, 2, 307.
 Lehmann, H., and Singh, R. B. (1956). *Nature (Lond.)*, 178, 695.
 Motulsky, A. G. (1956). *Ibid.*, 178, 1055.
 Na-Nakorn, S., and Minnich, V. (1956). *Proc. 6th Congr. int. Soc. Hematol. Boston*, p. 441.
 Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C. (1949). *Science*, 110, 543.
 Rigas, D. A., Koler, R. D., and Osgood, E. E. (1955). *Ibid.*, 121, 372.
 — (1956). *J. Lab. clin. Med.*, 47, 51.
 Robinson, A. R., Robson, M., Harrison, A. P., and Zuelzer, W. W. (1957). *Ibid.*, 50, 745.
 — Zuelzer, W. W., Neel, J. V., Livingstone, F. B., and Miller, M. J. (1956). *Blood*, 11, 902.
 Schneider, R. G., and Haggard, M. E. (1957). *Nature (Lond.)*, 180, 1486.
 Schwartz, H. C., and Spaet, T. H. (1955). *Clin. Res. Proc.*, 1955, 3, 51.
 — Spaet, T. H., Zuelzer, W. W., Neel, J. V., Robinson, A. R., and Kaufman, S. F. (1957). *Blood*, 12, 238.
 Vella, F. (1957). *Proc. Alumni, Ass. Malaya*, 10, 174.
 White, J. C., Beaven, G. H., and Ellis, M. (1956). *Ciba Foundation Symposium on Paper Electrophoresis*, p. 43. Churchill, London.

HAEMOGLOBIN K IN SINGAPORE

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Haemoglobin K is a fast-moving haemoglobin having electrophoretic properties similar to but not identical with those of haemoglobin J. It has so far been reported in three Berber families in North Africa (Cabannes *et al.*, 1956), in a number of Liberian natives (when it was provisionally named "Liberia II") (Robinson *et al.*, 1956), in three members of the Dagomba tribe in Northern Ghana (Edington and Lehmann, 1957), and in two related East Indians resident in London (Ager and Lehmann, 1957).

Report of Two Examples

In the course of a survey of the incidence of abnormal haemoglobin by the technique of paper electrophoresis, haemoglobin K was detected, together with normal haemoglobin, in an Indian patient suffering from a psychosis, in Singapore. With the help of Dr. Gwee Ah Leng a family study was attempted, and it was found that the abnormal haemoglobin was transmitted by the mother to the propositus and to his brother. The father, who is of Indonesian-Malay origin, was carrying normal haemoglobin only. The mother is of north Indian origin and was adopted when young. The exact geographical origin of her family is indefinite. No clinical or haematological abnormality which could be attributed to the presence of the abnormal haemoglobin could be detected in any member of this family.

Another instance of haemoglobin K trait has been found in a Malay woman attending a maternity and child welfare clinic in Singapore. Her parents are pure Malay from Malacca. She is suffering from a hypochromic anaemia attributable to repeated frequent pregnancies and to worm infestation. A family study in this case has not yet proved feasible. This is the only case of haemoglobin K trait so far found in over 1,500 Malays studied.*

Blood samples from the propositus in the Indian family and from the Malay woman were compared by Dr. H. Lehmann against haemoglobins H, J, and K, and both were found to be identical with haemoglobin K.

Though the incidence of this abnormal haemoglobin appears to be very low, it seems clear that it is not restricted to any one ethnic group.

REFERENCES

- Ager, J. A. M., and Lehmann, H. (1957). *Brit. med. J.*, 1, 1449.
 Cabannes, R., Buhr, J. L., and Sandra, L. (1956). *Proc. 6th Congr. int. Soc. Hemat., Boston*, p. 448.
 Edington, G. M., and Lehmann, H. (1957). Quoted by Ager and Lehmann (1957).
 Robinson, A. R., Zuelzer, W. W., Neel, J. V., Livingstone, F. B., and Miller, M. J. (1956). *Blood*, 11, 902.

*Since this note was submitted for publication a further case has been seen in a Malay from Penang; this brings the incidence to two out of 2,400 Malays so far studied.