Haemoglobin H Disease in an English Family

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Thalassaemia is a hereditary anaemia of widespread geographical distribution with marked variation in clinical severity. Morphological changes in the erythrocytes include microcytosis, anisopoikilocytosis, and the presence of target cells, while an increase in erythrocytic osmotic resistance is characteristic. The haemoglobin content of the erythrocytes is reduced and the present aetiological concept is that of a genetically determined defect in the rate of synthesis of normal Hb A $\alpha_2\beta_2$ (Ingram and Stretton, 1959). In the most frequent form there is a postulated defect of the beta-polypeptide chain synthesis, and this type (beta-thalassaemia) has been reported in English families (Callender, Mallett, and Lehmann, 1961). Less common is alpha-thalassaemia, in which a defect in alpha-chain synthesis is likely, and here, in addition to normal Hb A, there are found varying amounts of Hb H (β_4) and Hb Bart's (γ_4), gamma chains being a normal constituent of foetal haemoglobin $\alpha_2\gamma_2$.

The present report describes an English family which includes two brothers with haemolytic anaemia associated with the presence of haemoglobins H and Bart's, and the effect of splenectomy in one of these brothers.

Case Report

The propositus was a boy aged 14 years. Previous illnesses were chicken-pox, whooping-cough, scarlet fever, and pleurisy. There was no history of jaundice at birth. In May 1961 he had a febrile illness with generalized aches and pains, sore throat, and coryza. He was well after a week, but six weeks later pains developed in the knees, ankles, and wrists. The pain tended to move from joint to joint and there was some morning stiffness but little swelling. In the following month several other joints were involved, some showing considerable swelling. In the first hospital to which he was admitted the objective signs noted were a little swelling of the left wrist and a faint apical systolic murmur. Hb 69%; E.S.R. (Wintrobe) 6 mm.; W.B.C. 7,800/c.mm.; C-reactive protein, negative; urine, normal.

A diagnosis of rheumatic fever was made and he was given a course of calcium aspirin 10 gr. (65 mg.) q.i.d. with much improvement in the joint pain. Over the next few weeks, however, the joint pains returned and an intermittent fever up to 99.2° F. (37.3° C.) was recorded. He looked pale and tended to perspire. The E.S.R. (Wintrobe) was 5 mm., 5 mm., and 3 mm. in one hour on successive occasions, and a test for C-reactive protein was again negative. The A.S.O. titre was 400 u./ml. Chest x-ray picture and E.C.G. were normal. Calcium aspirin was restarted, but the joint pains only gradually disappeared. During this time he complained of some dyspnoea, lassitude, and marked fatigue. In October 1961 he had a sore throat which was treated with oral penicillin.

He was admitted to the Liverpool Royal Infirmary in October 1961 for further investigation. He was a slightly built boy—height 5 ft. 3½ in. (161 cm.), weight 7 st. 2 lb. (45.4 kg.), with a pale sallow complexion. There was minimal pain on passive movement of several joints. The heart was of normal size and a grade 1 apical systolic murmur could be heard. The liver was palpable three fingerbreadths and the spleen two fingerbreadths below the costal margin. X-ray pictures of the chest and skeleton were normal.

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Blood Examination.—Serum protein normal; blood urea 30 mg./100 ml.; E.S.R. 1 mm. in one hour (Wintrobe); serum bilirubin 2.1 mg./100 ml.; serum iron 290 mg./100 ml.; Schumm's test positive; white cells 3,500/c.mm. (differential count normal); red-cell count 4,600,000/c.mm.; haemoglobin 9.9 g./100 ml.; P.C.V. 37%; M.C.V. 81 cubic microns; M.C.H.C. 27%; M.C.H. 22 μ g.; reticulocytes 1-6%. The stained smear showed marked anisopoikilocytosis with target cells, burr forms, and microcytes. The osmotic fragility was reduced (Fig. 1). The direct Coombs test was negative, and no antibodies could be demonstrated in the serum or eluate from the red cells. Red-cell glucose-6-phosphate dehydrogenase activity was increased (Mr. K. A. Fletcher) to 535 units/min./100 ml. (normal 275-425). Sternal marrow showed normoblastic hyperplasia. Incubation of the blood with 1% brilliant cresyl blue for three hours resulted in 32% of the red cells showing inclusion bodies.

FIG. 1.—Osmotic fragility curves of III 1 (O---O) and of II 4 (O—O).

Urinalysis revealed no increase in urobilinogen, coproporphyrin, or porphobilinogen.

Characterization of Haemoglobins.—The haemolysate from the propositus was examined by paper and starch-gel electrophoresis. On paper electrophoresis at pH 8.6 a band which migrated faster than Hb A was seen. Examination by horizontal electrophoresis in starch gel (Tris E.D.T.A. buffer system pH 8.8) again showed a single abnormal band. Using starch-gel electrophoresis at pH 7 (phosphate buffer) (Huehns, Flynn, Butler, and Shooter, 1960), separation of this component into two bands was obtained, both migrating towards the anode (Fig. 2). This strongly suggested that the fast component consisted mainly of Hb H with a smaller amount of Hb Bart's. The abnormal haemoglobins did not separate from each other on starch-block electrophoresis at pH 7, and they were eluted as a single fraction. This fraction was subjected to fingerprint analysis (Ingram, 1958) and the complete absence of alpha-chain peptides was confirmed. The alkali-resistant haemoglobin in the blood of the propositus constituted 3-4% of the total, using the one-minute alkali denaturation test of Singer, Chernoff, and Singer (1951). This slight increase in alkali resistance probably reflects the presence of the small amount of Hb Bart's.

Splenectomy.—The erythrocyte half-life, using ^{51}Cr -labelled cells, was found to be 14 days. Counting was done over the

praecordium, spleen, and liver, and the spleen-liver ratio reached 2.95 after 10 days. It was thus thought that considerable sequestration of red cells must be occurring in the spleen, and because of the patient's persisting disability, with fatigue, over a

FIG. 2.—Starch-gel electrophoresis of haemolysate from *propositus* (pH 7), showing haemoglobins A, H, and Bart's.

period of eight months it was decided to carry out splenectomy. This was performed without incident by Mr. R. Helsby on 10 April 1962. After the immediate post-operative period the patient began to feel stronger and at the time of writing (January 1963) was showing considerable clinical improvement, so that he was able to play games without undue fatigue.

Blood examination after splenectomy showed the presence, both in the unstained film and after staining for one minute with 1% methyl violet or cresyl blue, of numbers of red cells containing single large inclusion bodies. These had been absent before splenectomy. These inclusions have been shown by Rigas and Koler (1961) to be denatured Hb H, and their appearance after splenectomy was noted by them and by other workers.

Haematologically there has been little change. The Hb level has persisted at 9–10 g./100 ml. The reticulocyte count was 2% and the serum bilirubin was within normal limits.

Three months after splenectomy red-cell survival was reassessed. The half-life of the tagged injected erythrocytes was found to be 17 days. A sample of blood from the patient after splenectomy was examined by Dr. H. Lehmann, who found 10% Hb H and 5% Hb Bart's.

Investigation of the Family

The pedigree is shown in Fig. 3. Inquiry showed no evidence of foreign ancestry. The grandmother's parents were both from North Lancashire families. There was no known consanguinity.

FIG. 3.—Pedigree of family. The arrow indicates the *propositus*.

III 2, the brother of the *propositus*, is aged 16 years and in good health. Two years ago he complained of fatigue and his haemoglobin was found to be low. However, no further investigations had been done. On examination in 1962 he was of similar build

and complexion to the patient. The liver and spleen were not palpable. Blood investigation: Schumm's test negative; serum bilirubin 2 mg./100 ml.; Hb 10.6 g./100 ml.; P.C.V. 41%; M.C.V. 82 cubic microns; M.C.H.C. 26%; M.C.H. 21 μ g.; reticulocyte count <1%. Osmotic fragility was markedly decreased. Incubation with brilliant cresyl blue resulted in the appearance of inclusion bodies in 30% of the red cells. A stained smear showed the same appearance as that from the *propositus*. On starch-gel electrophoresis the presence of Hb H and a smaller amount of Hb Bart's was demonstrated.

II 4, the father of the *propositus*, is a healthy, well-built police sergeant. Blood investigation: serum iron 195 μ g./100 ml.; white cells 7,500/c.mm. (normal differential count); red-cell count 5,800,000/c.mm.; Hb 14.9 g./100 ml.; P.C.V. 49%; M.C.V. 84 cubic microns; M.C.H.C. 31%; M.C.H. 26 μ g.; reticulocytes <1%. A stained smear showed slight microcytosis and anisopoikilocytosis. There was moderate hypochromia and moderate numbers of target cells. Osmotic fragility was slightly decreased (see Fig. 1). On incubation with cresyl blue a small number of red cells were seen to contain numerous inclusion bodies (Fig. 4). No abnormal haemoglobins were detected on starch-gel electrophoresis and the alkali-resistant haemoglobin constituted <1% of the total.

I 4, the paternal grandmother, suffers from severe chronic rheumatoid arthritis (she was having no treatment at the time of blood examination). Her red-cell osmotic fragility was considerably reduced. A few inclusion bodies were seen on incubation with cresyl blue. No abnormal haemoglobins were seen on starch-gel electrophoresis.

FIG. 4.—Appearance of inclusions in erythrocyte of II 4 (supravital staining with brilliant cresyl blue).

All other members of the family examined showed no haematological abnormalities. In particular, the mother's blood showed a normal haemoglobin level, peripheral blood smear, and osmotic fragility, while a careful search for inclusion bodies was negative.

Discussion

The mild chronic haemolytic anaemia in the two brothers is characteristic of Hb H disease. The degree of chronic disability in the *propositus* appears, however, to have been disproportionate to the haemoglobin level. The part that the salicylate therapy may have played in aggravating the rate of haemolysis is uncertain, but Rigas and Koler (1961) have described the deleterious effect of sulfisoxazole (sulphafurazole). The place of splenectomy in the treatment of this disease remains uncertain, but there has been marked symptomatic improvement in the case here described. Rigas and Koler (1961) reported clinical benefit in three cases.

The mode of inheritance of Hb H disease is not fully understood (Koler and Rigas, 1961). In the family described here it is evident that the gene for alpha-thalassaemia is possessed by the father and paternal grandmother of the affected brothers. It is likely that more than one gene for alpha-thalassaemia exists, and it is possible that the mother of the *propositus*, although phenotypically normal, has such a gene which interacted with the genotype of her husband to give Hb H disease in two of their three children.

Summary

The occurrence in an English family of two brothers with a mild chronic haemolytic anaemia associated with thalassaemia and the presence of haemoglobins H and Bart's is described. Splenectomy was performed in one of the brothers with symptomatic improvement.

The clinically unaffected father and paternal grandmother showed haematological evidence of thalassaemia, but the mother was haematologically normal.

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examined samples of blood and gave quantitative estimates for the haemoglobin present. Dr. D. Weatherall gave valuable help in the preparation of the paper. We thank Mr. L. C. Cooper for the photomicrograph.

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Medical Memoranda**A Case of Probable Phosgene Poisoning**

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This case is presented to illustrate a potential danger of non-inflammable paint-strippers and to raise the question of safety precautions. Their active components are chlorinated hydrocarbons, which can oxidize to form phosgene.

CASE HISTORY

The patient, an interior decorator aged 67, has had chronic bronchitis and a quiescent duodenal ulcer for several years. After eight hours in a small unventilated room heated by a paraffin stove he experienced breathlessness, giddiness, a tight feeling across the chest, and headache. On admission to hospital next morning the breathlessness was worse, and he had anorexia and retrosternal pain. He was cyanosed, sweating, and tachypnoeic, with negligible chest expansion and extensive coarse rales in both lungs except the right lower lobe; pulse 86, regular; B.P. 95/60. He had extreme right hypochondrial tenderness. Thorough examination revealed no other abnormality.

Investigations showed: Hb 118%; W.B.C. 20,000/c.mm. (P. 38%, L. 11%, M. 1%); E.S.R. 4 mm./hour; blood urea 56 mg./100 ml. Serum electrolytes and liver-function tests revealed nothing abnormal. Chest x-ray examination showed diffuse bronchiolitis in all areas except the R.L.L. Barium-meal examination showed a large hiatus hernia and duodenal bulb deformity.

The alimentary symptoms were thought to be due to oesophagitis and reactivation of the duodenal ulcer. Methylene dichloride itself produces epigastric pain, and the hydrocortisone administered might have been a contributory factor; but as the symptoms began so soon after contact and as the patient had been using paint-strippers previously without symptoms, dissolved phosgene was their most likely cause.

Improvement was slow. Dyspnoea and hypotension persisted for four days, during which time he required intermittent oxygen, intravenous saline, potassium, and glucose. Penicillin and streptomycin were given for 10 days, together with hydrocortisone 100 mg. b.d., which was gradually reduced and after a further week was stopped. Analgesics and antacids were administered symptomatically. Nausea and vomiting continued for one week and anorexia and intractable retrosternal and epigastric pain for four weeks after admission. His chest was clinically and radiologically normal after two weeks. He was discharged five weeks after admission, but lassitude, weakness, and hypochondriasis continued for three months.

COMMENT

Phosgene caused 80% of the gas deaths in the 1914–18 war. Rare cases result from its use industrially as chlorinating agent. Little (1955) demonstrated its production from the chlorinated hydrocarbons in tobacco in negligible quantities. Gerritsen and Buschmann (1960) reported two cases which showed the typical clinical picture of phosgene poisoning in circumstances similar to the present one. They demonstrated the production of phosgene from methylene dichloride and related compounds exposed to a naked flame in quantities sufficient to produce lethal concentrations in a confined space. It is likely that carbon monoxide close to the flame oxidizes the chlorine. The maximum allowable concentration of phosgene in the atmosphere is one p.p.m. of air.

The clinical features of phosgene poisoning are well illustrated by this case. In low concentrations it passes unnoticed, then gross irritation leads to degeneration of mucosal epithelium, haemorrhagic pulmonary oedema, focal pneumonia leading to cardiac decompensation, and peripheral circulatory collapse. Dissolved in saliva it irritates the alimentary mucosae. The slow convalescence and neurosis are also characteristic.

Treatment.—From the experiments of Gerritsen and Buschmann (1960), the size of the room, and the length of contact in this case, it seems likely that this man received a dose exceeding 1 p.p.m. Our successful treatment was based on that administered to their one surviving case, which is therefore commended as the treatment of choice, the notable difference from former therapy being the administration of hydrocortisone.

Preventive Measures.—As someone will always disregard any written instructions when using a substance such as a paint-remover, it is desirable that ultimately it be replaced with safer products. On the basis of so few reported cases it would be an excessive precaution to insist on its withdrawal now. What should be insisted on are appropriate instructions on each tin and a clear warning of the potential danger. Manufacturers should be aware of this hazard and able to explain it on inquiry—which was not the case on this occasion.

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