REFRACTORY ANAEMIA (FANCONI TYPE)

ITS INCIDENCE IN THREE MEMBERS OF ONE FAMILY, WITH IN ONE CASE A RELATIONSHIP TO CHRONIC HAEMOLYTIC ANAEMIA WITH NOCTURNAL HAEMOGLOBINURIA (MARCHIAFAVA-MICHELÌ DISEASE OR 'NOCTURNAL HAEMOGLOBINURIA')

BY

J. V. DACIE AND A. GILPIN

(From King's College Hospital, London)

Orthochromic or hyperchromic anaemias, resistant to all forms of treatment, comprise a well-recognized, but probably heterogeneous, group of blood disorders for which the general term 'refractory anaemia' has been recently suggested (Bomford and Rhoads, 1941). Their origin is obscure, but the fact that similar syndromes may follow exposure to poisons such as certain aromatic hydrocarbons suggests that in some cases at least the formation of an endogenous haemotoxic metabolite may be a causal factor. That a constitutional element may sometimes be involved is shown by the rare occurrence of severe refractory anaemia in several members of the same family. Fanconi's description in 1927 of fatal anaemia in three brothers, all under seven years of age, appears to be the first recorded example of this association.

The present communication deals with the incidence of anaemia of this type in two brothers observed for a number of years. One patient (C. H.) has died and hypoplasia of the marrow was found at autopsy; the other (A. H.), who has now been ill for ten years, has made a partial recovery. A cousin (M. H.), a girl aged twelve years, has also died recently of 'aplastic anaemia' in another hospital. She was an only child and of similar genetic constitution to that of the other two patients, for her father and mother were brother and sister respectively of the parents of the two affected boys. The boys' three sisters and their parents appear to be healthy, and no other relative, save M. H., has suffered or has died from anaemia as far as could be ascertained. A further unusual feature has been that increased haemolysis has played a part in the development of the anaemias, at least in the case of A. H. In this instance an abnormality affecting the erythrocytes identical with that found in nocturnal haemoglobinuria has been repeatedly demonstrated.

Clinical description

1. C. H. This boy was first under observation in March, 1933 when he was nine and three-quarter years of age. He was said to have been always pale, but definite anaemia dates from a severe epistaxis a month before admission. He did not properly

recover from this and was kept in bed, becoming increasingly pale and listless. He had never been a robust child and had suffered from pneumonia on three occasions, winter bronchitis, measles, mumps and ringworm. He was the third of a family of five children. Physical examination showed a small, extremely pale child, 43½ lb. in weight. The only abnormal physical signs, excluding severe pallor, were haemic cardiac murmurs, scattered purpuric spots on arms and legs and slight oozing from the gums. The edge of the liver was just palpable but no enlargement of the spleen or of the superficial lymph nodes could be detected. There was an ill-defined mass in the abdomen to the left and below the umbilicus which was later found to be an enlarged and displaced left kidney.

Examination of the urine revealed no abnormality. Laboratory investigations showed a severe hyperchromic anaemia with leucopenia and thrombopenia. An average of six blood counts, done before transfusion was resorted to, gave the following figures:—erythrocytes 1,290,000 per c.mm., haemoglobin (Haldane) 30 per cent., colour index 1.15; leucocytes 1,800 per c.mm., neutrophil polymorphonuclears 35 per cent., lymphocytes 59 per cent., monocytes 2 per cent., eosinophils 3 per cent., myelocytes less than 1 per cent. There was slight anisocytosis and poikilocytosis, and occasional normoblasts were seen; reticulocytes averaged 1.8 per cent.; platelets 22,000 per c.mm.; bilirubin 0.1 mgm. per 100 c.c. plasma. Treatment with iron, copper, liver and adrenalin by injection proved ineffective, and he was later twice transfused. Clinically he was much improved by this and the haemoglobin level was raised to 68 per cent. The improvement in his blood was maintained without further transfusion for a period of three months until his discharge from hospital on July 25 (fig. 1). There was some improvement in the total leucocyte count; a maximum of 3,800 per c.mm. of which 43 per cent. were neutrophil polymorphonuclears being reached on June 19. The platelets remained low at 35,000 per c.mm.

He was re-admitted into hospital on November 6. He was transfused twice and discharged once more from hospital on January 21, 1934 with 98 per cent. haemoglobin. He was re-admitted on April 19, having been comparatively well in the meantime. His haemoglobin had by then dropped to 62 per cent. This was raised by transfusion to

FIG. 1.—Erythrocyte counts and haemoglobin and reticulocyte percentages during the first admission of C. H. into hospital, the early part of his second admission and the last two admissions. Erythrocyte counts are linked by continuous lines, haemoglobin percentages by interrupted lines and reticulocyte percentages by dotted lines. Transfusions are denoted by the letter T.

90 per cent. and he was discharged on April 29, then weighing 53 lb.

He was not seen again until May 8, 1936, two years later. He had kept fairly well during the intervening period, but in the month or so prior to re-admission he had become weaker. A remarkable feature was that he had hardly grown at all during the preceding two years; he weighed 51 lb., a little less than his weight in April 1934, and he was but one inch taller in height. A blood count showed a recurrence of severe anaemia; erythrocytes 870,000 per c.mm., haemoglobin 26 per cent., colour index 1.49, cell volume 10.2 per cent., mean corp. vol. 118 cu. μ ; leucocytes 1,600 per c.mm. The percentage of reticulocytes was higher than before; 7.7 per cent. was the average of three observations. Physical examination revealed nothing more except that pallor was now combined with a slight generalized brownish colouration of the skin and this was particularly noticeable around the eyes. Several teeth were carious and his gums were oozing. He was kept under observation in hospital for eight months and was given numerous injections of liver extract (pernaemon forte) and preparations of vitamin A, C and D and anterior pituitary extracts, all without any beneficial effect. He received during this time seven transfusions of between 300 and 550 c.c. of fresh citrated blood. Examination of a gastric residue showed hypochlorhydria, but there was a good secretion of free HCl after the injection of 0.25 mgm. of histamine. An x-ray of the skeleton showed no abnormality except slight coarsening of the trabeculation and decalcification at the ends of the long bones. A barium meal revealed no abnormality in the upper gastrointestinal tract. A sternum puncture showed a moderately active marrow with a leucocyte-nucleated erythrocyte ratio of 3:7 to 1:0.* He was discharged from hospital on January 17, 1937, but was re-admitted on May 19 severely anaemic with 13 per cent. haemoglobin. He was given two

transfusions and was discharged a week later, only to be re-admitted on June 28 again critically ill. It was decided to perform a laparotomy to discover the nature of the puzzling abdominal tumour which had been felt on each admission. At operation the mass was found to be an enlarged kidney low in position and extending across the midline. No other abnormality was found and the spleen which appeared small was not removed. He stood the operation well but subsequently developed an irregular pyrexia ranging between 99° F. to 101° F., which persisted for the six weeks that he was kept under observation. He received three transfusions before the operation and four afterwards, but it was disturbing to notice that the beneficial effects of transfusion were becoming more transitory. A 20 per cent. fall in haemoglobin within four days was noticed on one occasion and a fall of 12 per cent. in the same period after a second transfusion. It seemed probable that erythrocyte formation was becoming increasingly difficult or that blood destruction at an increased rate was taking place. No decisive information was, however, available. He was discharged on August 29.

He was re-admitted for the last time on November 3, 1937, aged fourteen years and weighing 56 lb. He received five transfusions within a fortnight amounting in all to 1,600 c.c. of fresh citrated blood, and the haemoglobin was in this way raised from 15 to 46 per cent. His gums were bleeding on admission and a fortnight later swelling and oedema of the right cheek developed, associated with a sloughing area inside the mouth. The area of necrosis gradually spread until it had involved the palate and tonsillar fossae. His temperature had been gradually rising since admission and for the last fortnight fluctuated between 103° F. and 104° F. He died on December 2. An autopsy was performed ten hours after death.

Post-mortem report. The body was of a small pale boy in a moderate state of nutrition; the subcutaneous fat was limited in amount. There was a generalized dusky pigmentation of the skin particularly marked in the axillae, groins and inner aspect of the thighs. The outer side of the left cheek was discoloured and this corresponded to an area of necrosis within the mouth.

THORAX. The cardiac muscle was soft and pale;

* A differential count gave the following results:—leucocytes: neutrophil polymorphonuclears 4.8 per cent., metamyelocytes (band forms) 4.8 per cent., metamyelocytes (young forms) 42.8 per cent., myelocytes 21.6 per cent., myeloblasts 2.4 per cent., eosinophils 2.0 per cent., monocytes 2.0 per cent., lymphocytes 31.2 per cent., and plasma cells 3.2 per cent.; nucleated erythrocytes; haemocytoblasts 2 per cent., primary erythroblasts 43 per cent., normoblasts 55 per cent. The majority of the primary erythroblasts were late types. No megaloblasts were seen.

there were numerous sub-epicardial haemorrhages and 'tabby-cat' striation beneath the endocardium. The cardiac valves and great vessels were normal. There was an excess of pericardial fluid and haemorrhages beneath the parietal pericardium. The pleurae contained effusions; the bases of the lungs were collapsed and there were many sub-pleural haemorrhages.

ABDOMEN. The gastro-intestinal tract was normal except that the organs were pale: the mesenteric and para-aortic glands were hyperplastic. The liver was soft and appeared fatty and was a rich golden brown in colour. The spleen, which weighed 65 gm., appeared normal in colour and structure; within its substance was a cavernous haemangioma, 15 mm. in diameter.

The pancreas, suprarenals, thyroid, thymus, pituitary and testicles were pale but showed no other macroscopic changes.

The kidneys were represented by a single horse-shoe-shaped organ weighing 220 gm.; this was situated on the brim of the true pelvis below the bifurcation of the aorta. The right renal artery was derived from the right common iliac vessel and the left renal artery from the anterior surface of the aorta just above the point of bifurcation. A right and left ureter ran from the anterior surface of the kidney. The kidney substance was pale and soft and macroscopically normal.

BONE MARROW was examined from the sternum, skull, lumbar vertebrae and from the right femur. The marrow from the flat bones was pale red and semi-fluid in consistency; it floated on water. That from the upper third of the femur was deeper red; in the middle third there was an admixture of red marrow with fat, and marrow from the lower third appeared to be entirely fatty.

Histopathology

LIVER. There was a generalized fatty change of moderate degree with congestion and early necrosis of the parenchyma cells at the centre of the lobules. Much iron-containing pigment was distributed in the form of small granules within the liver cells and in Küpfer cells.

KIDNEY. There was no significant deviation from the normal except a moderate degree of granular degeneration and a small amount of fat within the cells of the convoluted tubules. No iron-containing pigment was identified.

SPLEEN. The spleen pulp was cellular and contained a moderate amount of blood. The Malpighian bodies were small. There was a generalized hyperplasia of the reticulum cells of the pulp and of the littoral cells of the venous sinuses, and many lymphocytes and plasma cells and occasional eosinophils and polymorphs were scattered throughout. A great deal of iron-containing pigment was present completely filling the cytoplasm of many reticulum cells. A few erythroblasts and normoblasts could be identified.

The section of the haemangioma showed it to be of the cavernous type with large endothelial lined spaces separated by thin connective tissue septa.

MYOCARDIUM. No significant changes except a slight degree of fragmentation of the muscle fibres.

LUNGS. Generalized oedema and small areas of haemorrhage were present; the latter generally in the vicinity of bronchioles and surrounding large

groups of organisms. There was some accumulation of mononuclear cells around the foci of infection, but few polymorphs.

PANCREAS, THYROID AND PITUITARY. No significant abnormalities.

TESTIS. The spermatic tubules were well developed but spermatogenesis was incomplete.

THYMUS. Sections showed a considerable degree of atrophy. The surviving tissue had been divided into small strips by strands of fat and collagenous tissue. Hassall's corpuscles were inconspicuous.

BONE MARROW. Marrow free from bony spicules was obtained from the centre of the shaft of the femur. Sections showed many fat cells with scattered haemopoietic cells lying between. The cells identified included late primary erythroblasts and normoblasts, and lymphocytes and plasma cells; smaller numbers of myelocytes and eosinophils were present, but haemocytoblasts and adult polymorphs appeared to be absent. Much iron-containing pigment could be seen within phagocytic cells.

2. A. H. This boy aged twelve years, elder brother of C. H., was admitted into King's College Hospital on January 12, 1933. He had become increasingly pale and breathless for the previous three months and was said to have had a similar attack during the preceding summer from which he recovered. He had had no illnesses except chicken-pox, measles, mumps and whooping cough. Physical examination showed an intelligent and extremely pale boy. The lower edge of the liver was just palpable but the spleen could not be felt. There was no detectable enlargement of the superficial lymph nodes and no purpura of skin or mucosae. He weighed 80 lb. Blood counts on admission showed a profound anaemia, leucopenia and thrombopenia. The following figures are averages of fourteen counts made within a period of six weeks before he was transfused. Erythrocytes 860,000 per c.mm., haemoglobin 18 per cent., colour index 1.05: leucocytes 1,600 per c.mm.; polymorphonuclears 35 per cent., lymphocytes 60 per cent., monocytes 2 per cent., and eosinophils 3 per cent.; a very few myelocytes, myeloblasts and normoblasts were seen. There was a small amount of anisocytosis and polychromasia, but little poikilocytosis. There was an average of 4.7 per cent. reticulocytes. Single observations showed that not more than 10,000 platelets per c.mm. were present, and that there was 0.1 mgm. bilirubin per 100 c.c. plasma. Examination of the gastric residue showed slight hypochlorhydria with a good response to histamine. The Wassermann reaction was negative. During this time he was treated intensively with liver by injection and later with ventriculin by mouth, but without benefit (fig. 2).

He was then given within three weeks a series of five transfusions totalling in all 1,850 c.c. of citrated blood and greatly benefited thereby. His haemoglobin was raised to 70 per cent. and only gradually fell to 48 per cent. ten weeks after the last transfusion. He was given three more transfusions and discharged from hospital on July 2 with 81 per cent. haemoglobin. He was seen several times during the next five months and re-admitted into hospital on January 15, 1934 for observation. His haemoglobin, which had fallen to 61 per cent. by September 5, 1933, had risen spontaneously and was now 82

Fig. 2.—Erythrocyte counts and haemoglobin and reticulocyte percentages during the first and last admissions of A. H. into hospital. Erythrocyte counts are linked by continuous lines, haemoglobin percentages by interrupted lines and reticulocyte percentages by dotted lines. Transfusions are denoted by the letter T.

per cent. The leucocyte count had improved also ; there were 5,000 per c.mm., of which 50 per cent. were polymorphonuclears. Reticulocytes were then 7 per cent. and bilirubin 0.6 mgm. per 100 c.c. He was transfused and left hospital on March 4, with a haemoglobin of 88 per cent.

More than two years passed before he was seen again. During this period he had kept well and had not been transfused. Examination of his blood (May 1936) showed a mild degree of anaemia; erythrocytes 3,310,000 per c.mm., haemoglobin 68 per cent.; cell volume 30 per cent., m.c.v. 91 cu. μ , platelets 175,000 per c.mm.; bilirubin 0.3 mgm. per 100 c.c. His blood was again examined in January 1937 and the haemoglobin was found to be 50 per cent. On this occasion he was admitted into hospital with a history of epigastric pain and vomiting of two days' duration. The attack passed off within twenty-four hours and did not recur.

He was not seen again until January 6, 1939, when he was readmitted with a recurrence of severe epigastric pain. The pain was of a continuous nature and was located in the left epigastric region. It was severe enough to prevent sleep and was similar to that experienced three times before in the previous seven weeks. Each attack was accompanied by vomiting and disappeared spontaneously in twenty-four to forty-eight hours. His blood picture had somewhat altered. The colour index was now less than one, and there was considerable anisocytosis, poikilocytosis and polychromasia. Quantitative estimations gave the following figures: erythrocytes 2,760,000 per c.mm., haemoglobin 47 per cent., colour index 0.86; cell volume 22 per cent., m.c.v. 81 cu. μ ; reticulocytes 8.5 per cent. (average of four observations); leucocytes 2,100 per c.mm.; erythrocyte fragility to hypotonic saline, normal; bilirubin 0.3 mgm. per 100 c.c. A sternum puncture showed erythroblastic hyperplasia.*

A transfusion given on January 23 produced a severe febrile reaction lasting for thirty-six hours and

no appreciable rise in haemoglobin, and when a further transfusion of 500 c.c. of fresh citrated blood was given a fortnight later a rise in temperature to 103.8° F. was associated with clinical jaundice the following day. Although there was no haemoglobinuria, a fall in haemoglobin of 2 per cent. indicated that a haemolytic episode had occurred. The patient was group A and had received group A blood; the latter had appeared to be perfectly compatible to the direct matching test, and the fact that he had been subsequently shown to be Rh+* makes it unlikely that the post-transfusion haemolysis was due to iso-immunization conditioned by previous transfusions. Laboratory tests have indeed provided another and quite unexpected explanation, for it has been established that A. H.'s erythrocytes behave in vitro in a similar manner to those of patients with nocturnal haemoglobinuria, a disease in which haemolytic reactions following transfusion are frequently encountered (see Dacie and Firth, 1943). A summary of the laboratory findings on this point is recorded in a later section.

Whilst in hospital A. H. experienced another severe attack of epigastric pain and this was followed by the passage of a small amount of blood in the faeces. The possibility of a recurring strangulation of the small intestine or of an intussusception was considered and a laparotomy was decided upon. This was performed on February 24, 1939 by Mr. H. C. Edwards, but no abnormality of the intestinal tract was discovered. The spleen was, however, enlarged and was for this reason removed along with two spleniculi, one as large as a grape and the other much smaller.

He made a good recovery from the operation and his haemoglobin rose from 44 per cent. on February 27 to 62 per cent. on March 18 when he left hospital. He was seen one month and two months later and reported that he felt well and was going to start work. His blood count appeared unaltered.

He has been seen four times since 1939; in March, 1940 there were 3,670,000 erythrocytes per c.mm., and the haemoglobin was 60 per cent.; reticulocytes 7 per cent., leucocytes 8,800 per c.mm. and platelets 320,000 per c.mm. In February 1941 a small improvement was evident; the haemoglobin was 70 per cent., but the reticulocyte count was unaltered. In October 1941, when next examined, a blood count showed no change except

* The nucleated erythrocyte-leucocyte ratio was 1.3 to 1.0. A differential count gave the following results:—leucocytes: adult polymorphs 0.5 per cent., metamyelocytes (band forms) 6.5 per cent., metamyelocytes (young forms) 41.5 per cent., myelocytes 17.5 per cent., myeloblasts 2.5 per cent., eosinophils 2.5 per cent., eosinophil myelocytes 4.0 per cent., monocytes 2.5 per cent., lymphocytes 21.5 per cent., and plasma cells 1.5 per cent.; nucleated erythrocytes; primary erythroblasts 74 per cent., normoblasts 26 per cent. The majority of the primary erythroblasts were medium to late forms. No megaloblasts were seen.

* Thanks are due to Dr. P. L. Mollison for performing this test.

that the reticulocyte percentage had fallen to 1·8 per cent. He was last seen in October 1942 having felt well enough in the previous year to work 60 hours a week as a mechanic. There was little change in his blood count; erythrocytes were 3,500,000 per c.mm. and haemoglobin 67 per cent., and reticulocytes 3 per cent. The erythrocyte fragility had been substantially reduced following the removal of the spleen and target cells were present in the peripheral circulation.

Histopathology of spleen. Weight, 350 gm. The cut surface of the spleen was brownish pink in colour with easily visible but small Malpighian bodies. The trabeculae and finer fibrous strands were moderately conspicuous; there was no thickening of the capsule. In consistency, the spleen was firm and on section little blood escaped.

Sections showed that the spleen pulp was relatively abundant and cellular. The Malpighian bodies were normal in size and showed slightly increased activity of their germinal follicles. The pulp contained little blood; its increased cellularity was due to a diffuse hyperplasia of reticulum cells causing partial obliteration of the pulp spaces. The venous sinuses were generally difficult to identify. Small numbers of fine collagen fibres were present within the pulp. There was no erythropoietic activity, but scattered lymphocytes and eosinophils could be seen throughout.

Iron-containing pigment was inconspicuous.

The *in vitro* behaviour of A. H.'s erythrocytes. Laboratory findings demonstrating a relationship to nocturnal haemoglobinuria

It had been observed early in January (1939) that a sterile sample of venous blood allowed to coagulate at 37° C. showed much lysis of the clot after twenty-four hours' incubation, but lack of time had delayed exploration of this phenomenon. A series of investigations undertaken after the haemolytic episode provoked by the transfusion has thrown some light on this mechanism. This appears to be identical with that operating in patients with nocturnal haemoglobinuria. In the course of nine series of observations made between February 1939 and October 1942 it has been demonstrated:—

(1) That haemolysis of venous blood allowed to clot undisturbed in small tubes starts after one hour and is well marked after two hours' incubation; lysis is much slower at room temperature.

(2) That haemolysis of clotted blood could be largely prevented by aeration of the blood by gentle rotation in the tube before the onset of coagulation; tests using washed patient's corpuscles and the patient's serum acidified with lactic acid or hydrochloric acid showed a sensitivity to H-ion concentration. Haemolysis was inhibited at pH 8 and was optimum at pH 7·0 to 7·4.

(3) That haemolysis, using the patient's corpuscles and serum, acidified to pH 7·2 was increased by the addition of fresh guinea-pig serum (complement) from which all anti-human heterolysin had been adsorbed; no haemolysis was caused by guinea-pig serum in the absence of human serum.

(4) That the patient's corpuscles were haemolysed as readily in normal serum as in the patient's own serum.

(5) That normal corpuscles were not haemolysed by the patient's serum.

(6) That the patient's corpuscles were more sensi-

tive than normal cells to the isohaemolysin and to an anti-human haemolytic serum prepared from a rabbit; they were somewhat less sensitive than the corpuscles of a typical case of nocturnal haemoglobinuria investigated at the same time.

The amount of lysis of the patient's corpuscles produced by incubation in his own acidified serum has progressively decreased, and when last tested (October 1942) this was much less easy to demonstrate than in two typical examples of nocturnal haemoglobinuria investigated during the same period. When the abnormality was first discovered in 1939 incubation for one hour caused as much as 25 per cent. haemolysis of a 1 per cent. suspension of patient's cells in acidified serum (1 in 2). When the test was last repeated in October 1942 under the same conditions there was only 5 per cent. lysis. It is interesting to note that the reticulocyte count has fallen from between 9 to 13 per cent. in 1939 to 1·8 to 3 per cent. in 1941–42 suggesting a reduced rate of haemolysis latterly.

Discussion

The original laboratory findings of severe megalocytic hyperchromic anaemia with little anisocytosis poikilocytosis and polychromasia, the leucopenia with a relative lymphocytosis, the thrombopenia, the presence of only a slightly reduced gastric secretion, the absence of hyperbilirubinaemia or of a significant number of abnormal cells in the blood supported the clinical diagnosis of 'aplastic anaemia' in the case of both C. H. and A. H. The lack of response to treatment with liver by injection or to oral administration of hog stomach extract, iron and concentrated vitamin preparations provided additional support for this diagnosis and transfusion was resorted to as the only possible palliative measure.

In C. H. the relatively low reticulocyte count (average 1·8 per cent.) when he was first under observation was quite compatible with an anaemia of aplastic type: the small numbers present being thought to reflect the erythropoietic activity of small surviving foci of active marrow. When re-admitted in June 1936 significantly higher percentages of reticulocytes were observed (5·8 to 11 per cent.) and it seems likely that a measure of increased haemolysis was occurring despite the fact that no increase in plasma bilirubin was demonstrated. It is unfortunate that no *in vitro* tests for abnormal autohaemolysis were undertaken and also that no figures for urobilinogen excretion are available. The marrow puncture made in October 1936 which showed moderate erythroblastic activity does not provide decisive evidence. Therefore, although there is a possibility that increased blood destruction was a factor in the genesis of C. H.'s anaemia, positive evidence for this is lacking. On the other hand, the finding of hypoplastic marrow post-mortem shows that the original conception of C. H.'s anaemia as 'aplastic' was correct, at least in part.

In the case of A. H., the data are more complete; although no figures for pigment excretion are available, the observation of rapid *in vitro* autohaemolysis makes it highly probable that there was

increased blood destruction *in vivo*. It is not known if this was a factor from the first. Although A. H.'s reticulocytes were always increased in number, averaging 4.7 per cent. at his first admission, the profound anaemia, neutropenia and thrombopenia found at that time suggest that an 'aplastic' element was dominant. Again, the favourable effect of transfusions experienced in all his admissions except the last, shows that the haemolytic element was not pronounced at first; four reticulocyte counts as high as 7 per cent. and one of 9 per cent. made in 1933-34 suggest, nevertheless, that some measure of increased haemolysis was occurring. This was never sufficiently rapid to cause jaundice except as a result of the haemolytic crisis provoked by the last transfusion (February 1939). Haemoglobinuria was never present and haemosiderin was not found in the urine deposit.

It is difficult to know what effect splenectomy has had upon the course of the disease; it seems that the 'aplastic' element in his case was never as severe as in his brother even if the haemolytic element was more pronounced. Remissions occurred from the start, and the haemoglobin was not observed to fall below 46 per cent. between February 1934 and January 1939 despite the fact that he received no transfusions during this time. The final, and it is hoped permanent, remission, cannot, therefore, be attributed to removal of the spleen with any surety.

The clinical and pathological observations outlined above are remarkable on two counts: (1) the familial incidence, and (2) the haemolytic phase.

(1) **The familial incidence.** As has been mentioned in the introduction to this article a small number of cases of familial refractory anaemia in children associated with other constitutional abnormalities have been reported.

The three fatal cases described by Fanconi (1927) were boys aged, five, six and seven years. Severe hyperchromic anaemia; leucopenia with thrombopenia and a haemorrhagic diathesis were associated with microcephaly, hypoplasia of the testicles, convergent strabismus, increased tendon reflexes and a generalized brown melanin pigmentation of the skin. In 1938 Weil reported the incidence of refractory hyperchromic anaemia in two out of five children of one family. One boy aged seven years died after a nine months' illness during which haemorrhages were prominent. In addition to anaemia there was a well marked dusky pigmentation of the trunk, an inguinal hernia and an undescended testicle. The second boy was affected when nine years of age. A dusky pigmentation of the skin was associated with obesity the onset of which coincided with the development of anaemia; the right testicle had not descended and that on the left side was small. His anaemia was hyperchromic and there was leucopenia, granulopenia and thrombopenia. He was never as severely ill as his brother and was kept under observation for four years, being treated by transfusions and thymic and testicular extracts. The last appeared to bring about the descent of the right testicle and this was associated with signs of puberty. Samples of marrow from the sternum were examined

on three occasions; at the first puncture few marrow cells were obtained, but the later samples showed erythroblastic hyperplasia. When last seen four years after the onset of anaemia a blood count showed 3,070,000 erythrocytes per c.mm. with 65 per cent. haemoglobin; there were 2,200 leucocytes per c.mm. of which 10 per cent. were polymorphonuclears; reticulocytes were 1.2 per cent. and platelets 75,000 per c.mm. Weil also described a sporadic case which he believed to be of a similar type to the above. This patient was a girl aged six years who suffered from a severe hyperchromic anaemia, leucopenia and thrombopenia and died after an illness of eighteen months' duration. Her anaemia was associated with a brown pigmentation of the skin, particularly of the abdomen; a congenital bilateral anomaly of the thenar muscles preventing opposition was also present. Another example of a familial incidence has been reported by Hjorth (1940); he described a severe hyperchromic anaemia in a brother and sister both aged eight years at the time of onset. The anaemia was associated with leucopenia, thrombopenia, and a haemorrhagic diathesis; in the boy there was hypoplasia of the marrow, thymus and testicles. There were also congenital bony deformities; clubfoot with an absent calcaneus and congenital luxation of the hip respectively. Similar deformities were present in two maternal cousins and a paternal cousin lacked both thumbs. There was a high incidence of abortion in this family affecting ten out of seventeen pregnancies; this was apparently not due to syphilis. Another instance of sporadic anaemia in a boy aged seven years, associated with abnormalities of development has been reported by Uehlinger (1929); in this case, anaemia, leucopenia and thrombopenia were accompanied by pigmentation of the skin, an absence of the left thumb and hypoplasia of the right one, and hypoplasia of the testicles. At autopsy a partially fatty marrow was observed and the right kidney and ureter found to be absent; the left kidney was enlarged with a double pelvis. A similar sporadic case has been recorded by van Leeuwen (1933). The patient was a boy who died after an illness of five years' duration, having then the appearance of an eight- to ten-year-old child, although fourteen years of age. Anaemia was severe and there was thrombopenia and slight leucopenia. Splenectomy was undertaken without benefit. A generalized dusky pigmentation of the skin was present, and his right thumb was deformed. Post-mortem showed, in addition to signs of severe anaemia, marked hypoplasia of the bone marrow, an absence of the right kidney, hypertrophy of the left kidney and ureter, an absence of the first right metacarpal and hypoplasia of testicles and thymus.

The reports of Fanconi, Weil, Hjorth, Uehlinger and van Leeuwen have many features in common; developmental abnormalities, particularly of the hands and kidneys, pigmentation of the skin and hypoplasia of the gonads were in each case associated with a hyperchromic anaemia, leucopenia and thrombopenia. It is likely that the present family belongs to the same group. A. H. is a well-developed young man and presents no unusual stigmata, but in C. H.'s case pigmentation of the skin, and a failure to grow were striking features. His kidneys were fused medially and low in position.

As far as adults are concerned Bomford and Rhoads (1941), who studied sixty-six patients, concluded that there was no evidence of a hereditary predisposition. Nevertheless, they reported that in two instances sisters of their own patients had died of anaemia of unknown nature, and in a further case a brother of the patient had died eighteen years before of benzol anaemia. It is interesting to note that there appeared to be a possible association between endocrine abnormalities and anaemia in certain patients out of the series of Bomford and Rhoads; two patients were eunuchs, in two others hyperthyroidism was also present, and in four the onset was concurrent with the menopause. In several other patients pigmentation of the skin was conspicuous.

It is doubtful if there is any justification for making any absolute separation between the familial examples of refractory anaemia met with in children and the more frequent sporadic cases of childhood and adult life, at least until observations on pathogenesis demonstrate conclusive differences. It seems likely that neither developmental abnormalities, pigmentation of the skin nor endocrine abnormalities are specifically associated with the familial type, and it may be recalled that Weil, Uehlinger and van Leeuwen have observed cases of refractory anaemia in childhood associated with developmental abnormalities in which no familial incidence could be established. Moreover, it is possible that a hereditary tendency to refractory anaemia is more frequent than the report of Bomford and Rhoads would suggest.

Huber (1939) has recently investigated the blood pictures of members of three families in which 'panmyelophthisis' had occurred. In each case three generations were studied; in his first family twenty-three relatives were investigated, and of these six members had less than 2,500 polymorphonuclears per c.mm., and in three of these there were more lymphocytes than polymorphonuclears. Out of fifteen members of the second family, three had less than 2,500 polymorphonuclears per c.mm. and one member (a male) a slight anaemia (erythrocytes 3,800,000 per c.mm., haemoglobin 75 per cent.). In his third family three out of eighteen members showed a neutropenia, 37 per cent. polymorphonuclears out of a total leucocyte count of 4,600 per c.mm. being the lowest value observed. Huber also investigated eleven relatives of a patient suffering from anaemia due to exposure to trichlorethylene; none showed leucopenia.

These results are interesting and need confirmation. They emphasize the possible rôle of endogenous factors, in part inherited, in the genesis of refractory anaemia.

2. The haemolytic phase and the relationship to nocturnal haemoglobinuria

It has been only recently recognized that increased haemolysis may play a part in the genesis of a substantial proportion of refractory anaemias.

Rhoads (1939) records the results of a quantitative investigation of the excretion of urobilinogen by thirty such patients; half of these showed a daily

excretion greater than the normal of 150 mgm. even when no account was taken of the degree of anaemia (more accurately of the total mass of circulating haemoglobin) at the time. The average daily figure varied from 172 to 570 mgm., the mean of fifteen cases being 308 mgm. This increased breakdown of blood is not confined to those cases of obscure endogenous origin, but may also be observed in poisoning due to benzol and has been produced experimentally in a dog given large doses of oestradiol monobenzoate (Rhoads, 1939).

The part that increased haemolysis probably played in the production of the anaemia affecting the subjects of this report has already been discussed, and attention has been drawn to the ineffectiveness of transfusions given to C. H. during his last two admissions and to the haemolytic reaction ultimately provoked in A. H., which led to the demonstration of *in vitro* autohaemolysis of a type not previously recorded except in patients exhibiting the clinical picture of nocturnal haemoglobinuria. It is possible that this observation may not be as rare as it appears to be, for Rhoads referring to cases of refractory anaemia has recorded that transfusions . . . at certain stages seem to have been positively harmful, leading to acute haemolytic episodes, which suggests that a similar mechanism may have been operable.

The cause of increased haemolysis in refractory anaemia is not known. Except for mentioning that there seemed to be no correlation between the type of bone marrow and the presence or absence of signs of increased haemolysis, and that increased destruction within the marrow was considered possible, its mechanism is not discussed by either Rhoads and Barker (1938), Rhoads (1939) or Bomford and Rhoads (1941), who reported on the patients investigated in the Rockefeller Institute.

It is not suggested that the type of haemolytic mechanism demonstrated in A. H. is necessarily that operable in other examples of refractory anaemia in which there is increased blood destruction. Rather it is possible that refractory anaemia and nocturnal haemoglobinuria are disorders of parallel pathogenesis, perhaps both due to metabolic abnormalities, the one affecting erythropoiesis and the other disturbing factors, at present unknown, which may prevent or control the haemolysis of corpuscles *in vivo*. It may be that in the two patients we described both types of disorder were combined.

Summary

1. The incidence of refractory anaemia in three members of a family is described; of the two boys personally investigated one, C. H., died after an illness of four years' duration and the other A. H., is alive after ten years' observation.

2. C. H. failed to grow during the last two years of his illness, and a dusky pigmentation of the skin developed. At autopsy his bone marrow was found to be hypoplastic: a developmental abnormality of the renal tract was also present, a single

horseshoe-shaped kidney being situated on the brim of the true pelvis.

3. A. H. has made a partial recovery. Investigation of a transfusion reaction led to the discovery of rapid autohaemolysis in vitro. Subsequent investigations showed that the mechanism of haemolysis was the same as in nocturnal haemoglobinuria.

4. The literature is reviewed and the few reports of familial refractory anaemia described. The similarity between the first patients and those investigated by Fanconi and later authors is stressed.

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