Hypochromic Anaemias not due to Iron Deficiency: Thalassaemia and Sideroblastic Anaemia

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Most of the hypochromic anaemias encountered in clinical practice are due to simple iron deficiency. Failure of response to iron therapy usually means that the patient is not taking the iron preparation, or that there is a source of chronic blood loss. There are, however, a small group of hypochromic anaemias in which the body iron stores are either normal or increased. In such cases, there is either a defect in haemoglobin synthesis or in transportation of iron from the iron stores to the red cell precursors. These abnormalities may be either congenital or acquired.

CONGENITAL IRON-RESISTANT HYPOCHROMIC ANAEMIAS

The congenital hypochromic anaemias are summarised in Table 1. The commonest are the inherited defects of globin synthesis, the thalassaemia syndromes. Congenital abnormalities of haem production or iron transport are rare.

Thalassaemia Syndromes

The thalassaemias are a group of inherited disorders of globin synthesis (Weatherall, 1965). The condition was first recognised as a distinct clinical

TABLE 1. Congenital Iron-resistant Hypochromic Anaemias

1. Defective globin synthesis	= the thalassaemia syndromes
2. Defective haem synthesis	= sex-linked hypochromic anaemias
3. Abnormalities of iron transport	= transferrin deficiency
	= abnormal delivery of iron

entity in 1925 by a Detroit paediatrician, Thomas Cooley, and was originally thought to be confined to people of Mediterranean origin, but in subsequent years it has been realised that it also occurs very frequently in the Middle and Far East, and sporadically in practically every ethnic group. Since 1949

studies have indicated that the clinical picture of thalassaemia can be caused by several different inherited defects of synthesis of the globin fraction of haemoglobin, and the current classification of the thalassaemias depends on a knowledge of the structure and genetic control of the normal human haemoglobins (Baglioni, 1963; Huehns and Shooter, 1965).

Human adult haemoglobin is a mixture of two proteins, a major fraction, haemoglobin A, and a minor component, haemoglobin A_2. In intra-uterine life, haemoglobin F is the main respiratory pigment; its synthesis ceases at about birth in normal individuals, and it is only found in trace amounts in the blood after the age of six months. Haemoglobins A, F, and A_2 have a very similar structure, consisting of four peptide chains, each associated with one haem unit. All three have one pair of chains in common, the α-chains, but in haemoglobin A these are combined with β-chains ($\alpha_2\beta_2$), in haemoglobin A_2 with δ-chains ($\alpha_2\delta_2$), and in haemoglobin F, with γ-chains ($\alpha_2\gamma_2$). The genetic control of these haemoglobin chains has now been fully worked out. Separate pairs of genes control the synthesis of the α-, β-, δ- and γ-chains. In intra-uterine life, α-chains combine with γ-chains to form haemoglobin F, and in adult life, with α- and β-chains to form haemoglobins A and A_2 respectively.

There are two types of inherited disorders of haemoglobin synthesis, (1) those in which there is an inherited structural abnormality in either the α- or β-chain, sickle-cell disease being the best known example, and (2) those in which there is an inherited defect in the rate of production of either the α- or β-chains, known as the thalassaemia syndromes. They are divided into the α- and β-thalassaemias, depending on which chain is synthesised at a reduced rate.

The β-thalassaemias are characterised by defective haemoglobin synthesis after the third month of life, when normal β-chain synthesis is fully active. In the homozygous state affected infants become anaemic, with failure to thrive and enlargement of the abdomen at about the third month. The subsequent course is one of chronic and severe anaemia, bone deformity, recurrent infection, and the development of gross splenomegaly. Most of these children are kept alive by repeated blood transfusions, and many of them die during the second decade from generalised siderosis, particularly of the myocardium.

The carriers, or heterozygotes, of β-thalassaemia show very varying clinical features, ranging from the almost normal to a condition similar to the homozygous state with severe anaemia and splenomegaly. The blood findings also vary, ranging from an almost normal picture to one characterised by anaemia with gross variation in the size and shape of the red cells.

A very large quantity of foetal haemoglobin is characteristic of homozygotes, and an elevation in the level of the minor component, haemoglobin A_2, of heterozygotes.

It has recently become possible to measure the *in vitro* rate of production of the globin chains and to follow the fate of these chains after synthesis (Weatherall *et al.*, 1965; Bank and Marks, 1966). In the β-thalassaemias there is a defective production of β-chains, resulting in a large excess of α-chains, many of which remain free in the red cell, uncombined with β- or γ-chains. These chains are unstable and rapidly precipitate into the cell stroma, leading to the formation of inclusion bodies (Fessas, 1963), which probably damage the red cell membrane and thus cause the haemolysis which characterises this disorder (Nathan and Gunn, 1966).

It is likely that many cells with large inclusions never leave the bone marrow, and there is evidence of intramedullary destruction of haemoglobin in β-thalassaemia. How these inclusions damage the red cell membrane is uncertain, but it has been shown that the membranes are 'leaky' and abnormally permeable to potassium, an effect that may arise from mechanical damage to the membranes due to the rigidity of the inclusion bodies, or may follow binding of the membrane SH^- groups by the precipitating α-chains. The anaemia of β-thalassaemia is thus a combination of a defective rate of haemoglobin synthesis associated with the deleterious effects of an excess of unstable α-chains.

The α-thalassaemias cause defective haemoglobin synthesis in both intra-uterine and adult life, as both foetal and adult haemoglobin contain α-chains. A severe deficiency of α-chains causes defective foetal haemoglobin synthesis. The production of γ-chains of haemoglobin F is, however, normal, causing an excess of γ-chains, which aggregate to form molecules with the formula γ_4 (Ager and Lehmann, 1959). This haemoglobin, known as haemoglobin Bart's, does not transport oxygen normally. Thus, in addition to having defective haemoglobin production, infants with a severe deficiency of α-chains produce a respiratory pigment which is useless for carrying oxygen. For these reasons homozygous α-thalassaemia causes stillbirth, with the clinical picture of hydrops foetalis, usually around the thirty-second to the thirty-fourth week. This condition is a frequent cause of intra-uterine death in South-East Asia (Lie Injo Luan Eng, 1962).

Individuals heterozygous for milder forms of α-thalassaemia survive into adult life when a deficiency of α-chains causes an excess of β-chains, which aggregate to form a molecule with the formula β_4, or haemoglobin H. This molecule is unstable and tends to precipitate in the older erythrocyte populations and shortens red cell survival in a similar manner to that found in

β-thalassaemia. Thus, in haemoglobin H thalassaemia there is a defect in haemoglobin production and a shortened red cell survival due to the properties of the excess of β-chains.

The thalassaemia syndromes are the commonest of the congenital anaemias, particularly in the Mediterranean region, India, Pakistan, and the Far East. In Thailand alone, there are about 12,000 children with homozygous β-thalassaemia, and 45,000 children possessing the genes for both β-thalassaemia and a structural haemoglobin variant, haemoglobin E. It should be remembered, however, that thalassaemia can be the cause of an iron-resistant hypochromic anaemia in practically any racial group, and recently in Liverpool five British families with a picture of long-standing iron-resistant hypochromic anaemia in more than one member have been observed and the diagnosis of β-thalassaemia clearly established in each case. Two patients were over seventy years old.

The diagnosis of thalassaemia depends on the morphological appearance of the red cells, the presence of increased levels of either haemoglobin F, or haemoglobin A_2, or the presence of haemoglobins Bart's or H. Therefore, the condition can be recognised by the very careful examination of a stained blood film combined with haemoglobin electrophoresis and quantitative determination of the levels of foetal haemoglobin and haemoglobin A_2.

The clinical management of the homozygous state is difficult, but these children should have regular transfusions to maintain the haemoglobin at 10–12 G/100 ml. They should also receive regular folic acid supplements and early treatment of infection with antibiotics. If splenomegaly is marked and if there is evidence of hypersplenism, splenectomy should be considered, but only after the age of five years because of the risk of infection. Attempts are being made to deal with the problem of siderosis by the use of chelating agents such as desferrioxamine and diethylenetriaminepenta-acetate (DTPA). The regime advocated at the moment involves the use of intramuscular desferrioxamine several times a week, combined with a single intravenous dose of DTPA into the transfusion bottle at each blood transfusion (Sephton-Smith, 1964).

The underlying cause of thalassaemia is still uncertain. The disorders are the results of a defect in the rate of synthesis of a protein chain, and recent work on haemoglobin synthesis in β-thalassaemia suggests that the defect is a quantitative reduction of messenger RNA for this particular chain. These studies are of considerable importance because the thalassaemias serve as a model for all disorders characterised by a reduced rate of synthesis of a protein.

Congenital Defects in Haem Synthesis

There are reports of several families in which there is an iron-resistant, hypochromic anaemia, usually affecting males, that appears to be inherited as a sex-linked recessive gene (Losowsky and Hall, 1965). It is characterised by a moderate degree of anaemia with a dimorphic blood picture, the blood film showing a mixture of normal and markedly hypochromic cells; the bone marrow shows erythroid hyperplasia and variable numbers of ringed sideroblasts. Large doses of pyridoxine produce varied responses; there is a rise in haemoglobin and a transient reticulocytosis, but the response is rarely complete.

The precise defect in this disorder is unknown, but it has been suggested that the haem synthetic pathway is involved, possibly at an early stage when glycine and active succinate are converted to δ-aminolaevulinic acid. This hypothesis remains to be explored by further biosynthetic studies.

It must be emphasised that the disorder is quite uncommon and there are only a few adequate studies published.

Congenital Disorders of Iron Transport

These disorders are exceptionally rare. In one, there is a congenital deficiency of the iron-carrying protein, transferrin (Heilmeyer *et al.*, 1961), and affected children have a severe hypochromic anaemia. As these children synthesise some haemoglobin, iron must be transported by other means.

In another type there is failure of iron transport from liver to bone marrow (Shahidi *et al.*, 1964). Affected children have a gross deficiency of iron in the red cell precursors and increased levels of liver iron. Factors other than transferrin are obviously concerned with iron transport.

Acquired Iron-resistant Hypochromic Anaemias

These disorders fall into several clearly defined groups (summarised in Table 2), depending on the distribution of iron in the bone marrow and the

Table 2. Acquired Iron-resistant Hypochromic Anaemias

1. Defective haem synthesis	= sideroblastic anaemias
2. Defective iron movement	= anaemia of chronic disorders
3. Neoplastic conditions	= myelosclerosis
	= chronic di Guglielmo's disease
4. Re-utilisation defects	

body stores. Thus, in the sideroblastic anaemias there is an abnormal accumulation of iron within the red cell precursors, while in the group of hypochromic

anaemias associated with chronic disorders such as carcinoma or rheumatoid arthritis, iron tends to accumulate in the reticuloendothelial part of the bone marrow and is abnormally scarce in the developing red cell precursors. In addition, iron-resistant hypochromic anaemia may characterise the various neoplastic disorders of the bone marrow, such as the myeloproliferative states and the bizarre disorders which go under the general term of the 'di-Guglielmo syndrome'.

Sideroblastic Anaemias

These disorders are characterised by an iron-resistant hypochromic anaemia associated with abnormal sideroblasts in the bone marrow (Mollin, 1965). Most red cell precursors contain small amounts of iron scattered throughout the cytoplasm and are, therefore, correctly designated as 'sideroblasts'. In the sideroblastic anaemia group, however, there is increased iron in the red cell precursors, in both the cytoplasm and the perinuclear region, that is thought to be associated with the mitrochondria. It is this ring of Prussian-blue positive material in the perinuclear region which characterises the red cell precursors in the sideroblastic anaemias.

Several types of sideroblastic anaemia without an obvious cause have been defined (Table 3). One variety that occurs usually late in life and constitutes the primary acquired group of sideroblastic anaemia. The congenital sideroblastic anaemias have already been described. However, a sideroblastic reaction can occur in a variety of acquired disorders, of which the most common are the myeloproliferative states, malignancy, drug reactions, and the various megaloblastic anaemias of folic acid and vitamin B_{12} deficiency.

Primary sideroblastic anaemias. Acquired primary sideroblastic anaemia

TABLE 3. The Sideroblastic Anaemias

1. *Primary*	(*a*)	Hereditary
	(*b*)	Acquired
2. *Secondary*	(*a*)	Myeloproliferative disease
	(*b*)	Carcinoma
	(*c*)	Drugs: isoniazid and cycloserine
	(*d*)	Lead poisoning
	(*e*)	Vitamin B_{12} and folic acid deficiency
	(*f*)	Rheumatoid arthritis
	(*g*)	Haemolytic anaemia

occurs in middle and old age and is characterised by the slow onset of an anaemia of moderate severity. Clinical examination usually reveals no abnormality and splenomegaly is quite unusual.

The haematological findings are summarised in Table 4. There is a moderate

degree of anaemia, and the blood picture is typically dimorphic. There are many well-filled erythrocytes interspersed with a line of cells which are quite hypochromic. The platelet and white cell counts are usually normal. The bone marrow shows striking erythroid hyperplasia, and in many cases frank

TABLE 4. Main Features of Sideroblastic Anaemia

1. Moderate anaemia with 'dimorphic' blood picture
2. Erythroid hyperplasia with megaloblastic changes
3. Abnormal 'ringed' sideroblasts present in large numbers
4. Serum iron usually high
5. Variable response to folic acid, pyridoxine, crude liver, corticosteroids, and ascorbic acid

megaloblasts are present. Iron staining shows increased iron in the reticulo-endothelial elements of the marrow and the red cell precursors, of which a high proportion are ringed sideroblasts.

Some patients with this disorder show biochemical evidence of folic acid deficiency combined with a low serum folate level. Additionally, in a considerable number of patients an abnormal xanthurenic excretion test shows evidence of pyridoxine deficiency.

The diagnosis of the primary form of sideroblastic anaemia is made by finding the clinical and haematological features, outlined above, in the absence of any underlying myeloproliferative disease, occult neoplasm, or any of the other conditions listed in Table 4. Treatment with large doses of folic acid is sometimes helpful, particularly in cases with biochemical evidence of folic acid deficiency, and pyridoxine is also helpful in some cases, and large doses up to 100 mg a day may be required to raise the haemoglobin level. The haematological picture rarely reverts completely to normal after treatment with these agents. If there is no response to folic acid or pyridoxine it is worthwhile trying corticosteroids, androgens, or even crude liver extract, as in reported cases all have occasionally helped.

Secondary sideroblastic anaemias. Several disorders have frequently been associated with sideroblastic anaemia (Table 3), the commonest being the myeloproliferative syndromes, occult carcinomas and various drug-induced anaemias, particularly those associated with anti-tuberculous drugs.

All these conditions may be associated with a florid sideroblastic reaction causing severe anaemia and a bone marrow picture identical to the primary sideroblastic anaemia. It is important, therefore, that any patient with a sideroblastic anaemia gives a full drug history and is examined most carefully for a carcinoma, particularly of bronchus, prostate or gastro-intestinal tract. In addition, the peripheral blood and marrow must be examined carefully

for evidence of myeloproliferative disease; it is useful to examine a closed bone marrow biopsy as well as a routine aspirate to estimate the reticulin present, as this may help to detect an underlying myeloproliferative disorder.

Many of the other secondary conditions listed in Table 3 may simply be sideroblastic reactions in primary folic acid or vitamin B_{12} deficiency, or in a haemolytic anaemia, when the marrow rapidly reverts to normal on appropriate treatment. It is probably incorrect to classify them as 'sideroblastic anaemias' and better to designate them as 'sideroblastic reactions'.

The nature of the sideroblastic reaction. Almost nothing is known of the underlying mechanism of the abnormal deposition of iron in the mitochondrial region of the red cell precursors. Recent studies, utilising *in vitro* radioactive iron labelling, revealed no block in haem synthesis nor deficiency of any enzyme along the haem synthetic pathway (Vavra and Poff, 1967). These problems will not be solved until more is known about the mechanisms controlling the entry of iron into the red cell precursor and its movement into haemoglobin.

The Anaemias of Chronic Disorders

The haematological findings in patients with disorders such as carcinoma, chronic infection, and rheumatoid arthritis have recently been reviewed by Cartwright (1966). The anaemia is either normochromic or mildly hypochromic, and, even in cases where the blood picture appears to be normochromic, there is some reduction in the mean corpuscular haemoglobin concentration (MCHC).

The characteristic picture is of a mild hypochromic anaemia with an abnormal distribution of iron in the bone marrow. There is a failure of iron transport from the reticuloendothelial stores to the developing red cell precursors. Consequently, iron is extremely scarce in the red cell precursors but is found in normal or increased amounts in the reticuloendothelial parts of the marrow and in the other body iron stores. These findings are associated with a low serum iron and a reduced iron-binding capacity due to defective transferrin production. Therefore, the percentage saturation of the total iron-binding capacity of the serum is not as low as that usually found in classical iron deficiency anaemia.

The true nature of this disorder is not yet apparent, but it seems to be due to a combination of events which include a slightly shortened red cell survival, a failure of iron transport to the developing red cell precursor, and a reduced production of erythropoietin, as many of these defects can be reversed by giving large doses of erythropoietin.

It is very important to recognise this type of anaemia, as patients should not be given iron, and every effort should be made to establish the underlying cause. This disorder underlines the importance of a careful examination of the distribution of iron within the bone marrow.

The Myeloproliferative Disorders

Many patients with myelosclerosis and chronic di Guglielmo's disease show varying degrees of hypochromia on examination of a peripheral blood film, and they are often unsuccessfully treated with iron for a long period before the correct diagnosis is made.

In addition to hypochromia, the red cells in myelosclerosis show gross poikilocytosis with 'tear drop' forms, and there are often both qualitative and quantitative abnormalities of the platelets, together with the presence of young white cells and nucleated red cells. It should be remembered, however, that, early in the course of the disease, the spleen is not always grossly enlarged and the bone marrow may, in fact, be hyperplastic. The diagnosis in these cases can be made by careful examination of the peripheral blood film together with a bone marrow biopsy followed by staining for reticulin.

Di Guglielmo's disease is characterised by gross abnormalities in the size and shape of the red cells, associated with varying numbers of nucleated red cells in the blood and a strikingly abnormal appearance of the bone marrow, which contains many megaloblastic forms and bizarre red cell precursors (Baldini *et al.*, 1959).

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The Manner of a President

Sir William Browne was a delight to gossip writers, his egotism being matched by his garrulity. Educated at Cambridge, he first practiced at Lynn, Norfolk, and was aware of George I's donation of books to the University of Cambridge, having purchased the huge library of the Bishop of Ely. By chance the king had, at the same time, ordered a regiment of cavalry to Oxford. Browne celebrated the two events in verse.

> The king to Oxford sent a troop of horse,
> For Tories own no argument but force;
> With equal skill to Cambridge books he sent,
> For Whigs admit no force but argument.

He was admitted a Fellow of the College in 1726 but only began to practice in London in 1749. He became President of the College in 1765 and was quite the wrong man to deal with the revolt of the Licentiates who, at one time, actually forced their way into Comitia. Sir William, fearing that he would be brought down by them, resigned after his second year of office but managed a long valedictory address.

'The manly age and inclination with conformable studies I diligently applied to the practice of physic in the country, where, as that age adviseth, I sought riches and friendships; but, afterwards, being satiated with friends, whom truth, not flattery, had procured; satiated with riches which Galen, not fortune had presented, I resorted immediately to this College, where, in further obedience to the same adviser, I might totally addict myself to the service of honour. Conducted by your favour instead of my own merit, I have been advanced through various degrees of honour, a most delightful climax indeed, even to the very highest of all which the whole profession of physic hath to confer.' And so on. Sir William certainly managed to hold the stage even if his career left no lasting distinction.