

CHAPTER SEVENTEEN

ANEMIA

Learning objectives

At the end of this chapter, the student shall be able to:

- Define anemia
- Describe the classification of anemia
- Explain microcytic anemia
- Describe macrocytic anemia
- Describe normochromic normocytic anemia

Introduction

- ✓ Anemia is a condition in which the amount of hemoglobin in a patient's circulatory system is reduced.
- ✓ A physiologic definition stresses the inability of an anemic individual to maintain normal tissue oxygenation.
- ✓ This may result from decreased hemoglobin content or reduced red cell number.
- ✓ Alterations in total circulating plasma volume as well as of total circulating hemoglobin mass determine the hemoglobin concentration.
- ✓ Reduction in plasma volume (as in dehydration) may mask anemia or even cause polycythemia;
- ✓ Conversely, an increase in plasma volume (as with splenomegaly or pregnancy) may cause anemia even with a normal total circulating red cell and hemoglobin mass.
- ✓ After acute major blood loss, anemia is not immediately apparent since the total blood volume is reduced.
- ✓ It takes up to a day for the plasma volume to be replaced and so for the degree of anemia to become apparent. Regeneration of the hemoglobin mass takes substantially longer.
- ✓ The initial clinical features of major blood loss are, therefore, due to reduction in blood volume rather than to anemia.

Clinical features

- ✓ If the patient does have symptoms, these are usually shortness of breath (particularly on exercise), weakness, lethargy, palpitation and headaches.
- ✓ In older subjects symptoms of cardiac failure, angina pectoris or intermittent claudication or confusion may be present.
- ✓ Visual disturbances due to retinal hemorrhages may complicate very severe anemia, particularly of rapid onset.
- ✓ The signs may be divided into general and specific.
- ✓ General signs include pallor of mucous membrane which occurs if the hemoglobin level is less than 9-10g/ dl.
- ✓ Skin color, on the other hand, is not a reliable sign of anemia; the state of the skin circulation rather than the hemoglobin content of the blood largely determined skin color.
- ✓ Specific signs are associated with particular types of anemia, e.g. koilonychia (spoon nails) with iron deficiency, jaundice with hemolytic or megaloblastic anemias, leg ulcers with sickle cell and other hemolytic anemia, bone deformities with thalassemia major and other severe congenital hemolytic anemias.
- ✓ The association of features of anemia with excess infections or spontaneous bruising suggests that neutropenia or thrombocytopenia may also be present.

Classification of anemias

- Many different classification of anemia have been proposed.
- In Table 17.1 two widely used classifications, morphologic and physiologic, are outlined.

- Used together, these offer a rational pathophysiologic approach to the laboratory diagnosis of anemia.

Table 17.1 Classifications of anemias

I. Morphologic

Normocytic Normochromic normochromic

Blood loss
Hemolytic anemia
Aplastic anemia
Myelophthisic anemia
Chronic disease, renal
Insufficiency

Microcytic hypochromic

Iron efficiency
Sideroblastic anemia
Lead poisoning
Thalassemia

Chronic disease

Macrocytic

Megaloblastic anemias
Liver disease
Post-splenectomy
Hypothyroidism

Stress erythropoiesis

II. Physiologic

Hypoproliferation abnormality

Aplastic anemia
Myelophthisic anemia

Hemolytic anemia
Blood loss

Renal insufficiency
Chronic disease
Endocrine deficiency Stratus

Excessive destruction or loss of red cell Maturation

Megaloblastic anemias
Myelodysplasia ,
including sideroblastic anemia

Thalassemia
Iron deficiency

17.1. Microcytic anemias

- ✓ An important mechanism of anemia is defective hemoglobin synthesis, which results in small, poorly hemoglobinized erythrocytes.
- ✓ After Wright staining, instead of red cells with pink hemoglobin filling the cytoplasm, the cells are pale with only a rim of hemoglobin.
- ✓ Since hemoglobin is made up of two components, either of two pathophysiologic mechanisms can lead to decrease hemoglobin synthesis-defective heme or decreased globin production.
- ✓ Heme is made up of iron and porphyrins; deficiencies in either affect heme production.
- ✓ Deficiency of iron store, failure to utilize iron properly, and defective heme or porphyrin synthesis are characteristic of iron deficiency anemia, anemia of chronic disease, and the sideroblastic anemias, respectively.
- ✓ In thalassemia syndromes, globin production is decreased, thereby hindering hemoglobin synthesis and producing a microcytic anemia.

17.1.1. Iron deficiency anemia

- Iron deficiency is the commonest cause of anemia in every country of the world.
- It is the most important, but not sole, cause of a microcytic, hypochromic anemia, in which all three red cell indices (the MCV, MCH and MCHC) are reduced and the blood film shows microcytic, hypochromic red cells.
- This appearance is due to a defect in hemoglobin synthesis.

Nutritional and metabolic aspects of iron

- Iron is one of the commonest elements in the earth's crust, yet iron deficiencies the commonest cause of anemia.
- This is because the body has a limited ability to absorb iron and excess loss of iron due to hemorrhage is frequent.

I. Body iron distribution

- Hemoglobin contains about two-thirds of body iron.
- Iron is incorporated from plasma transferrin into developing erythroblasts in the bone marrow and into reticulocytes.
- Transferrin obtains iron mainly from reticuloendothelial (RE) cells (macrophages).
- Only a small proportion of plasma iron comes from dietary iron absorbed through the duodenum and jejunum.
- At the end of their life, red cells are broken down in the macrophages of the RE system and their iron is subsequently released into the plasma.
- Some of the iron is also stored in the RE cells as hemosiderin and ferritin, the amount varying widely according to overall body iron status.
- Ferritin is a watersoluble protein-iron complex of molecular weight 465,000. It contains up to 20% of its weight as iron and is not visible by light microscopy.
- Hemosiderin is an insoluble protein-iron complex of varying composition containing about 37% of iron by weight.
- It is probably derived from partial lysosomal digestion of aggregates of ferritin molecules and is visible in macrophages by light microscopy after staining by Perls' (Prussian blue) reaction.
- Iron in ferritin and hemosiderin is in the ferric form. It is mobilized after reduction to the ferrous form, vitamin C being involved.
- Iron is also present in muscle as myoglobin and in most cells of the body in iron containing enzymes, e.g. cytochromes, succinic dehydrogenase, catalase, etc.
- This tissue iron is less likely to become depleted than hemosiderin, ferritin and hemoglobin in states of iron deficiency, but some reduction of heme-containing enzyme may occur in severe chronic iron deficiency.

II. Dietary iron

- Iron is present in food as ferric hydroxides, ferric-protein complexes and heme-protein complexes.
- Both the iron content and the proportion of iron absorbed differ from food to food; in general, meat and, in particular, liver is a better source than vegetables, eggs or dairy foods.
- The average Western diet contains 10-15mg of iron from which only 5-10% is normally absorbed.
- The proportion can be increased to 20-30% in iron deficiency or pregnancy but, even in these situations, most dietary iron remains unabsorbed.

III. Iron absorption

- This occurs through the duodenum and less through the jejunum; it is favored by factors such as acid and reducing agents keeping the iron soluble, particularly maintaining it in the ferrous rather than ferric state.
- Excess iron is combined with apoferritin to form ferritin, which is shed into the gut lumen when the mucosal cell reaches the tip of the intestinal villus.

- In iron deficiency, more iron enters the cell and a greater proportion of this intramucosal iron is transported into portal blood; in iron overload, less iron enters the cell and a greater proportion of this is shed back into the gut lumen.

IV. Iron transport

- Most internal iron exchange is concerned with providing iron to the marrow for erythropoiesis.
- Iron is transported in plasma bound to a β -globulin, transferrin (siderophyllin), of MW 80,000.
- This protein is synthesized in the liver, has a half-life of 8-10 days, and is capable of binding two atoms of iron per molecule.
- It is re-utilized after it has given up its iron.
- Normally it is one-third saturated but there is a diurnal variation in serum iron, the highest values occurring in the morning and the lowest in the evening.
- Transferrin gains iron mainly from the macrophages of the RE system and it is the diurnal variation in their release of iron which explains the diurnal variation in serum iron concentration.
- When plasma iron is raised and transferrin is saturated, the amount of iron transferred to parenchymal cells, e.g. those of the liver, endocrine organs, pancreas and heart, is increased.

V. Iron requirements

- The amount of iron required each day to compensate for losses from the body and growth varies with age and sex; it is highest in pregnancy and in adolescent and menstruating females.
- These groups, therefore, are particularly likely to develop iron deficiency if there is additional iron loss or prolonged reduced intake.
- Normal adult man iron requirement is 0.5–1.0 mg/day.

Iron deficiency

I. Clinical features

- When iron deficiency is developing, the RE stores (hemosiderin and ferritin) become completely depleted for anemia occurs.
- At an early stage, there are usually no clinical abnormalities.
- Later, the patient may develop the general symptoms and signs of anemia.

II. Causes

- Chronic blood loss, especially uterine or from the gastrointestinal tract is the dominant cause.
- Half a liter of whole blood contains approximately 250mg of iron and, despite the increased absorption of food iron at an early stage of iron deficiency; negative iron balance is usually in chronic blood loss.
- Increased demands during infancy, adolescence, pregnancy, lactation and in menstruating women account for the prevalence of latent iron deficiency (absent iron stores without anemia) and a consequent high risk of anemia in these particular clinical groups.
- Newborn infants have a store of iron derived from the breakdown of excess red cells.
- From 3 to 6 months, there is a tendency for negative iron balance to occur due to growth.
- Mixed feeding, particularly with iron fortified foods, prevents iron deficiency.
- It has been estimated to take 8 years for a normal adult male to develop iron deficiency anemia solely due to a poor diet or malabsorption resulting in no iron intake at all.

Laboratory findings

The laboratory findings are summarized and contrasted with those in other hypochromic anemias in Table 17.2

Table 17.2. Laboratory diagnosis of a hypochromic anemia

	<u>Iron Deficiency</u>	<u>Chronic inflammation or malignancy</u>	<u>Thalassemia trait</u> <u>(α or β)</u> <u>Sideroblastic</u> <u>anemia</u>
MCV low in congenital type but MCV often raised in acquired type	All reduced in relation to severity of anemia	Low normal or mild reduction	All reduced; very low for degree of anemia
Serum iron	Reduced	Reduced	Normal
TIBC	Raised	Reduced	Normal
Serum ferritin	Reduced	Normal or raised	Normal
Bone marrow iron stores	Absent	Present	Present
Erythroblast Iron Ring forms	Absent	Absent	Present
Hemoglobin Electrophoresis	Normal	Normal	Hb A ₂ raised
			in β form

Red cell indices and blood films

- o The red cell indices fall and they fall progressively as the anemia becomes more severe.
- o The blood film shows hypochromic, microcytic cells with occasional target cells and pencil-shaped poikilocytes.
- o The reticulocyte count is low in relation to the degree of anemia.
- o When iron deficiency is associated with severe folate or vitamin B₁₂ deficiency a 'dimorphic' film occurs with a dual population of red cells of which one is macrocytic and the other microcytic and hypochromic; the indices may be normal.
- o A dimorphic blood film is also seen in patients with iron deficiency anemia who have received recent iron therapy and produced a population of new wellfilled normal-sized red cells and when the patient has been transfused.
- o The platelet count is often moderately raised in iron deficiency, particularly when hemorrhage is continuing.

Bone marrow iron

- ✓ Bone marrow examination is not essential to assess iron stores except in complicated cases, but iron staining is carried out routinely on all bone marrow aspirations that are performed for any reason.
- ✓ In iron deficiency anemia there is a complete absence of iron from stores (macrophages) and absence of siderotic iron granules from developing erythroblasts.
- ✓ The erythroblasts are small and have a ragged cytoplasm.

Serum iron and total iron-binding capacity (TIBC)

- ✓ The serum iron falls and TIBC rises so that the IBC is less than 10% saturated.
- ✓ This contrasts both with the anemia of chronic disorders when the serum iron and the TIBC are both reduced and with other hypochromic anemias where the serum iron is normal or even raised.

Serum ferritin

- o A small fraction of body ferritin circulates in the serum, the concentration being related to tissue, particularly RE, iron stores.
- o The normal range in men is higher than in women.
- o In iron deficiency anemia, the serum ferritin is very low while a raised serum ferritin indicates iron overload or excess release of ferritin from damaged tissues, e.g. acute hepatitis
- o The serum ferritin is normal or raised in the anemia of chronic disorders.

Free Erythrocyte Protoporphyrin (FEP)

- o This increases early in iron deficiency before anemia develops.
- o Raised FEP levels are, however, also found in lead poisoning, some cases of sideroblastic anemia and in erythropoietic porphyria.
- o The test is not carried out routinely.

17.1.2. Anemia of Chronic disorders

- One of the most common anemias occurs in patients with a variety of chronic inflammatory and malignant diseases.
- Chronic inflammatory diseases include: Infections, e.g. pulmonary abscess, tuberculosis, pneumonia, bacterial endocarditis
- Non-infectious e.g. rheumatoid arthritis, systemic lupus erythematosus (SLE) and other connective tissue diseases Malignant diseases include: Carcinoma, lymphoma, sarcoma.
- The pathogenesis of this anemia appears to be related to decreased release of iron from macrophages to plasma, reduced red cell lifespan and an inadequate erythropoietin response to anemia.
- The anemia is only corrected by successful treatment of the underlying disease and does not respond to iron therapy despite the low serum iron.

The characteristic features are:

- o Normochromic, normocytic or mildly hypochromic indices and red cell morphology.
- o Mild and non-progressive anemia (hemoglobin rarely less than 9.0g/dl)- the severity being related to the severity of the disease.
- o Both the serum iron and TIBC are reduced
- o The serum ferritin is normal or raised
- o Bone marrow storage (RE) iron is normal but erythroblast iron is reduced.

17.1.3. Sideroblastic anemia

- ✓ This is a refractory anemia with hypochromic cells in the peripheral blood and increased marrow iron; it is defined by the presence of many pathological ring sideroblasts in the bone marrow.
- ✓ These are abnormal erythroblasts containing numerous iron granules arranged in a ring or collar around the nucleus instead of the few randomly distributed iron granules seen when normal erythroblasts are stained for iron.
- ✓ The anemia is classified into different types:

Hereditary:

Usually occurs in males, transmitted by females; also occurs rarely in females

Acquired

- Primary: Myelodysplasia FAB Type 2 or
 - Secondary: Other malignant diseases of the marrow, e.g. other types of myelodysplasia, myeloid leukemia, myeloma
 - Drugs, e.g. antituberculous (isoniazid, cycloserine), alcohol, lead
 - Other benign conditions, e.g. hemolytic anemia, megaloblastic anemia, malabsorption
- ✓ There is probably always a defect in heme synthesis.
 - ✓ In the hereditary forms, the anemia is characterized by a markedly hypochromic and microcytic blood picture.
 - ✓ This is due to a congenital enzyme defect, e.g. of δ -aminolevulinic acid synthetase or heme synthetase.
 - ✓ The much more common primary acquired form, is one subtype of myelodysplasia.
 - ✓ It is also termed 'refractory anemia with ring sideroblasts'.
 - ✓ In the hereditary and primary acquired diseases, 15% or more of marrow erythroblasts are ring sideroblasts.
 - ✓ Ring sideroblasts also occur with lesser frequency in the marrow disorders, especially the other types of myelodysplasia, the myeloproliferative diseases, acute myeloid leukemia and myeloma.
 - ✓ They may also occur in the bone marrow of patients taking certain drugs, excess alcohol or with lead poisoning. Vitamin B₆ (pyridoxine) deficiency or vitamin B₆ antagonists (e.g. isoniazid) are rare causes.

17.1.4. Lead poisoning

- ✓ Lead inhibits both heme and globin synthesis at a number of points.
- ✓ In addition it interferes with the breakdown of RNA by inhibiting the enzyme pyrimidine 5' nucleotidase, causing accumulation of denatured RNA in red cells, the RNA giving an appearance called basophilic stippling on the ordinary (Romanowsky) stain.
- ✓ The anemia may be hypochromic or predominantly hemolytic, and the bone marrow may show ring sideroblasts.
- ✓ Free erythrocyte protoporphyrin is raised.

17.1.5 Thalassemias

- ✓ These are a heterogeneous group of genetic disorders which result from a reduced rate of synthesis of α or β chains.
- ✓ Clinically they are divided into hydrops fetalis, β -thalassemia major, which is transfusion dependent, thalassemia intermedia characterized by moderate anemia usually with splenomegaly and iron overload, and thalassemia minor, the usually symptomless carrier.

Alpha-thalassemia syndromes

- o These are usually due to gene deletions. As there is duplication of the α -globin gene, deletion of four genes is needed to completely suppress α chain synthesis.
- o Since the α chain is essential in fetal as well as in adult hemoglobin, deletion of both α genes on both chromosomes leads to failure of fetal hemoglobin synthesis with death in utero (hydrops fetalis).
- o The α -thalassemia traits are usually not associated with anemia, but the MCV and MCH are low and the red cell count is over $5.5 \times 10^{12}/l$.
- o Hemoglobin electrophoresis is normal but occasionally Hb H bodies may be observed in reticulocyte preparations.

Beta-thalassemia syndromes

- o Anemia in β -thalassemia is a result of (1) decreased synthesis of the β -globin chains of hemoglobin and (2) precipitation and subsequent removal of excess α -globin chains, which in turn lead to ineffective erythropoiesis and hemolysis.
- o Hypochromia, microcytosis, fragmented forms, and basophilic stippling are found in blood from thalassemia patients. Many target cells are also common.
- o The hypochromia is a result of decreased cellular content of hemoglobin, a major defect in thalassemia.

- o The bone marrow is hyperplastic but the reticulocyte count only moderately increased.
- o The production abnormality is due to ineffective erythropoiesis, that is, destruction of immature erythroid cells in the bone marrow.

17.2 Macrocytic anemia

- Macrocytic anemias are divided into those that are associated with megaloblastic changes in the cells of the marrow and peripheral blood and those that are not (Table 17.3).
- Several forms of macrocytosis are not accompanied by megaloblastic changes and some of these are relatively common.
- The anemia of liver disease is usually associated with mild to moderate macrocytosis, with MCV often in the range of 100 to 110.
- The reason for the macrocytosis of liver disease is unclear.
- It is to be distinguished from the swelling of the red cell membrane that accounts for target cell in some patients with obstructive jaundice.
- Some authors believe that it is the result of the reticulocytosis that accompanies the hemolytic component of the anemia associated with liver dysfunction.
- Similarly, macrocytosis, often in the absence of anemia, is seen in patients who consume large amounts of alcohol, and this is sometimes used as a criterion for the diagnosis of chronic alcoholism.
- Anemia associated with hypothyroidism can have various morphologic characteristics, but is sometimes macrocytic in nature, for reasons that are not entirely clear.
- The postsplenectomy state is often associated with mild macrocytosis, in addition to the formation of some target cells and acanthocytes; these changes are due to the fact that young red cells normally undergo a process of surface remodeling, with loss of some of their redundant red cell membrane, with the spleen, and thus splenectomy may be associated with cells containing excessive plasma membrane material.
- Erythrocytes during the neonatal period are normally macrocytic and are then replaced by cells of normal size.
- The macrocytosis that accompanies “stress” erythropoiesis deserves some attention.
- In the presence of high serum levels of erythropoietin stimulated by anemia and the attendant hypoxemia, there is early release of immature red blood cells from the bone marrow, that is, a “shift” of immature bone marrow reticulocytes into the peripheral blood.
- These immature cells are larger than normal and they are also usually polychromatophilic (gray in color) because they still contain relatively large amounts of RNA (which is blue in stained blood films) and have not yet completed the process of hemoglobin synthesis (they are not yet fully “pinked up”).
- Thus, the MCV is typically moderately elevated with the reticulocytosis and polychromatophilia that accompany the erythroid hyperplasia of the bone marrow in response to hemolysis or bleeding.
- Macrocytosis of mild degree is often seen as well in conditions in which the anemia is due to a decrease in erythropoietic tissue in the bone marrow, for example, aplastic anemia, pure red cell aplasia, or the bone marrow suppression caused by chemotherapy.
- In these situations there is also a high titer of erythropoietin in the plasma, and this causes a rapid rate of ingress of young red blood cells into the peripheral blood.
- Major causes of macrocytic anemia that are megaloblastic in nature are vitamin B12 or folic acid deficiency, both of which have multiple causes.

Table 17.3 Causes of macrocytosis

Non-megaloblastic

- Liver disease, alcoholism
- Hypothyroidism
- Post-splenectomy
- Neonatal macrocytosis

- “Stress” erythropoiesis (with expanded or compromised erythropoiesis in the marrow)

Megaloblastic

- Vitamin B12 deficiency (multiple causes)
- Folic acid deficiency (multiple causes)
- Other causes (antineoplastic drugs, metabolic disorders, neoplastic erythropoiesis)

17.2.1 Megaloblastic anemia

- ✓ The basic underlying defect in megaloblastic anemia is defective DNA synthesis and cell division.
- ✓ This results in ineffective erythropoiesis, that is, death of immature erythroid cells before release from the bone marrow, associated with some early destruction of circulating erythrocytes as well.
- ✓ It is not entirely clear, however, how the deficiency in vitamin B₁₂ or folic acid leads to defective DNA synthesis or how defective DNA synthesis results in premature cell death.
- ✓ It is known that a state of unbalanced growth exists in the marrow cells of patients with megaloblastic anemia.
- ✓ The megaloblasts contain a substantially increased amount of RNA and a normal or slightly increased amount of DNA.
- ✓ This imbalance comes about because there is a delay in cell division due to impaired synthesis of one or more deoxyribonucleotides, the precursors of DNA, while RNA production proceeds normally.
- ✓ It is possible that premature cell death results from this unbalanced cell maturation.
- ✓ Presumably the degree of impairment of DNA synthesis varies from cell to cell and is more prominent among erythroid cells than among granulocyte and platelet precursors.
- ✓ Although most anemias characterized by megaloblastic erythropoiesis are due to either vitamin B12 or folic acid deficiency, there are several other causes of megaloblastic hematopoiesis.
- ✓ Some of these diseases are inherited, others are iatrogenic (drug-induced; e.g. 6-mercaptopurine, cytosine arabinoside, methotrexate), and some may be neoplastic.
- ✓ All fail to respond to replacement therapy with vitamin B12 or folic acid.

Laboratory findings

1. Pancytopenia :
 - ✓ As a result of ineffective erythropoiesis, granulopoiesis, and thrombopoiesis, and premature destruction of defective cells in the peripheral blood, it is unusual to find a patient with megaloblastic anemia who does not have depression of all three cell lines in the peripheral blood.
2. Bone marrow is hyperplastic
 - ✓ Despite this pancytopenia, the bone marrow is hyperplastic-reflecting the fact that ineffective production of blood cells with early death in the marrow is the major pathophysiologic mechanism in megaloblastic anemia.
3. The megaloblastic bone marrow produces macrocytic, oval red cells.
4. Their MCH and MCV are both increased, and the MCHC is normal.
5. Macro-ovalocytosis, as seen in the peripheral blood smear, is a hallmark of megaloblastic anemias.
6. The reticulocyte count is usually less than 1 percent. Occasionally it is 2 to 3 percent, but the reticulocyte production index is low, a reflection of a functionally defective marrow.
7. Poikilocytosis:
 - ✓ Marked abnormalities in the shape of red cells also occur in megaloblastic anemias.
 - ✓ It has been suggested that these abnormalities result from fragmentation of the abnormal large red cells as they pass through small arterioles.

- ✓ As the megaloblastic anemia becomes more severe, bizarre shapes such as triangles and helmets increases proportionately.
8. Hypersegmented polys
 - ✓ The megaloblastic process also leads to abnormalities in white cells.
 - ✓ Cells size and average number of lobes in the mature granulocyte (poly) are increased.
 - ✓ Normally no more than 1 percent of polys have six nuclear lobes, but in megaloblastic anemia many have six or more, even ten, lobes.
 - ✓ The hyperlobulation is presumably due to abnormal nuclear development.
 9. Elevated levels of serum lactic acid dehydrogenase (LDH), bilirubin, and fecal and urinary urobilinogen are due to ineffective erythropoiesis.
 10. Plasma iron turnover and marrow iron uptake are both increased despite decreased incorporation of iron into mature red cells.
 11. Hemolysis or premature death of mature erythrocytes in the peripheral blood. Despite hemolysis the reticulocyte production index is reduced because of the ineffective erythropoiesis in the bone marrow.
 12. Megaloblastic erythropoiesis and giant myeloid forms in the bone marrow.
 - ✓ Morphologically, the megaloblastic erythropoiesis is characterized by the presence of large cells, with asynchronism between nuclear and cytoplasmic development.
 - ✓ This morphologic appearance-an immature nucleus associated with mature cytoplasm-parallels the biochemical abnormality whereby DNA synthesis and maturation of the cell nucleus are impaired while cytoplasmic RNA and hemoglobin synthesis proceed normally.
 13. Giant white cell precursor forms, abnormal megakaryocytes, and hypercellularity of all three cell lines are also features of megaloblastic marrows.

Vitamin B12

- ✓ Since vitamin B12 is common in human diets, almost all deficiencies of vitamin B12 are a result of malabsorption.
- ✓ Vitamin B12 is made up of a porphyrin like structure attached to a nucleotide.
- ✓ This structure is analogous to the porphyrin structure of heme, with position of the heme iron being occupied by a cobalt atom.
- ✓ The ultimate source of vitamin B12 in man is from microbial synthesis.
- ✓ The vitamin B12 synthesized by microbes is deposited in animal tissues, such as liver, eggs, and milk, and is therefore plentiful in fish and meat products.
- ✓ A normal diet contains a large excess of vitamin B12 compared with daily needs (Table 17.4).
- ✓ The average diet contains 5 to 30µg of vitamin B12 daily, 1 to 2µg of which usually is absorbed and retained.
- ✓ In the adult a storage pool of 3000 to 5000µg is present, of which 1000 to 3000µg is stored in the liver.
- ✓ If malabsorption of vitamin B12 occurs, it will take 2 to 5 years before body stores are exhausted and megaloblastic erythropoiesis supervenes.
- ✓ Vitamin B12 binds with a glycoprotein called intrinsic factor (IF), which is secreted by the parietal cell located in the body of the stomach for absorption.
- ✓ The intrinsic factor-B₁₂ complex is absorbed in the distal ileum.
- ✓ The most common disease associated with vitamin B12 malabsorption, pernicious anemia, is caused by failure to secrete adequate amount of IF.
- ✓ Three to four hours after oral ingestion, vitamin B12 is detected in the blood.
- ✓ A peak level is obtained in 8 to 10 hours.
- ✓ The vitamin is attached to three protein binders named transcobalamin (TC) I, TC II, and TC III for transportation.

Table 17.4 Vitamin B12 and folate; nutritional aspects

	<u>Vitamin B12</u>	<u>Folate</u>
Normal daily dietary Intake	5 - 30µg	200-250µg

Main foods greens and yeast	Animal produce only	Most, especially liver,
Cooking	Little effect	Easily destroyed
Minimal adult daily requirement	1 - 2µg	150µg
Body stores 6 months)	3 - 5mg (sufficient for 2-5 years)	5 - 20mg (sufficient for 3-
Absorption site	Ileum	Duodenum and jejunum
Mechanism	Intrinsic factor	Conversion to
methyltetrahydrofolate		
Limit	2-3µg daily	50-80% of dietary content
Enterohepatic Circulation	5-10µg/day	90µg/day
Transport in plasma albumin	Bound to TC	Weakly bound to

Schilling test

- ✓ One of the most useful means of making the diagnosis of vitamin B12 deficiency and determining its etiology is the Schilling test, which measures the absorption of orally administered radiolabeled vitamin B12.
- ✓ The simplest and most commonly employed method is to give the patient a 0.5 or 1µg dose of radiocobalt- labeled cyanocobalamin and either immediately or 2 hours later to administer a 1mg dose of nonradioactive cyanocobalamin intramuscularly.
- ✓ This “flushing” dose is used to saturate vitamin B12 binding sites in the plasma and liver.
- ✓ A 24 hour collection of urine is begun after the radioactive B12 has been ingested.
- ✓ Normal subjects will excrete in their urine 7 percent or more of the radioactivity taken orally, whereas patients with pernicious anemia or other causes of vitamin B12 malabsorption will excrete well less than 7 percent.
- ✓ Renal insufficiency or incomplete collection of urine may result in a spuriously low excretion rate.
- ✓ The second part of the Schilling test is performed only if the first part gives abnormal results.
- ✓ In the second part 60mg of hog IF is given orally along with the radioactive vitamin B12.
- ✓ If there is a defect in the absorption of the vitamin B12-IF complex, then abnormally small amounts of B12 will be excreted.
- ✓ However, if the patient’s gastric secretions lack IF, the addition of hog IF to the vitamin B12 oral dose leads to normal urinary excretion of the vitamin B12.
- ✓ Thus, the part one and part two Schilling tests distinguish IF deficiency from ileal malabsorption of IF-B12 complex.
- ✓ A third part of the Schilling test may be performed to determine if the patient suffers from malabsorption of the IF-B12 complex secondary to small-intestine bacterial overgrowth.
- ✓ In part three of the Schilling test a 2-week course of antibiotic therapy with tetracycline, 250mg four times per day, is prescribed.
- ✓ If bacterial overgrowth was responsible for the abnormal second part of the Schilling test, then tetracycline treatment should normalize vitamin B12 absorption. In practice, the third part of the Schilling test is seldom performed.

Vitamin B12 deficiency

- ✓ The deficiency is usually due to pernicious anemia (Table 17.5).
- ✓ Much less commonly the deficiency may be caused by veganism in which the diet lacks B12 (usually in Hindu Indians), gastrectomy or small intestinal lesions.
- ✓ There is no syndrome of B12 deficiency due to increased utilization or loss of the vitamin, so the deficiency inevitably takes at least 2 years to develop, i.e. the time needed for body stores to deplete at the rate of 1-2µg each day when there is no new B12 entering the body from the diet.

Table 17.5 Causes of vitamin B12 deficiency

Decreased intake

Henry Karoki Bsc.| Anaemia

- Strict vegetarianism

Decreased absorption

- Pernicious anemia
- Congenital lack of IF production
- Selective vitamin B12 malabsorption
- Total or partial gastrectomy
- Chronic pancreatitis
- Regional ileitis, particularly with ileal resection
- Ileal resection
- Sprue

Competition

- Fish tapeworm
- Blind loop syndromes
- Small-intestinal diverticulosis

Pernicious anemia (PA)

Characteristically patients with PA are of Northern European extraction and have fair complexions and blue eyes. Long earlobes, prematurely gray hair, and blood group A are other clinical features that have been associated with PA. The patients are usually elderly, but can be of any age. Not all patients fit this description, and many patients who have these clinical findings do not suffer from PA.

Folic acid

- ✓ The terms folic acid and folate refer to a large group of compounds consisting of three moieties, pteridine, paraaminobenzoic acid, and a variable number of glutamic acid units.
- ✓ Folates are widely distributed in a variety of food, including green vegetables, liver, kidney, and dairy products.
- ✓ A daily diet contains fifty to several hundred micrograms of folates.
- ✓ Cooking, particularly boiling, destroys this thermolabile vitamin.
- ✓ During the process of intestinal absorption the folates are converted to 5-methyltetrahydrofolate, which is the main transport and storage form of folate in man.
- ✓ About 50µg of folic acid is required daily from food.

Normally 5 to 20mg of folic acid is stored in the liver and other tissues.

For this reason it takes 3 to 6 months for tissue stores to be completely exhausted in the absence of folate replacement. Folate deficiency is most often due to a poor dietary intake of folate alone or in combination with a condition of increased folate utilization or malabsorption (Table 17.6). Excess cell turnover of any sort, including pregnancy, is the main cause of an increased need for folate.

Decreased intake

- Poor diet or overcooking of folate-containing foods
- Alcoholism (also impairs folate metabolism)

Decreased absorption

- Sprue and other malabsorption syndromes
- Anticonvulsant and oral contraceptive drugs

Increased requirement

- Pregnancy
- Erythroid hyperplasia of the marrow (hemolysis, thalassemia)
- Leukemias and other neoplastic disorders
- Skin disease such as psoriasis, exfoliative dermatitis

Other causes

- Chemotherapy with folic acid antagonists
- Renal dialysis
- Vitamin C deficiency

17.3. Normocytic anemias

17.3.1. Aplastic Anemia

- Aplastic (hypoplastic) anemia is defined as pancytopenia (anemia, leucopenia, and thrombocytopenia) resulting from aplasia of the bone marrow.
- A selective decrease in red cell production is referred to as pure red cell aplasia.
- Patients with aplastic anemia generally have symptoms characteristic of a particular cellular deficiency.
- Those with anemia may be fatigued or short of breath, those with neutropenia may manifest serious infection, and those with thrombocytopenia may demonstrate petechiae or bleeding.
- The diagnosis is suggested by the presence of pancytopenia.
- A low reticulocyte count suggests underproduction rather than increased loss or destruction of red cells.
- The diagnosis is confirmed with a bone marrow biopsy that shows a substantial decrease in the number of red cell, white cell, and platelet precursors, and replacement of the usually cellular bone marrow with fat.
- Aplastic anemia can be mild or severe, and the management of the patient depends on the severity of the illness.
- Failure of the pluripotential stem cells of the bone marrow to maintain bone marrow cellularity and the production of normal numbers of mature red cells, neutrophils, and platelets characterizes aplastic anemia.
- Failure of the pluripotential stem cell can be caused by many different factors (Table 17.7). In most cases of aplastic anemia, the cause is not known.
- These cases are referred to as idiopathic.
- Many agents that cause aplastic anemia, such as benzene and radiation, can on occasion precipitate malignant transformation of the damaged bone marrow stem cells, resulting in the development of acute leukemia.

Table 17.7 Etiologies of aplastic anemia

- Idiopathic
- Radiation
- Drugs
 - Antimicrobials - chloramphenicol
 - Antiepileptics - carbamazepine, diphenylhydantoin
 - Anti-inflammatory - phenylbutazone
 - Antithyroid - methimazole (Tapazole)
 - Gold compounds
 - Organic arsenicals
 - Chemotherapeutic agents - alkylating agents
- Toxins
 - Solvents - benzene
 - Insecticides - DDT
- Viral agents
 - Hepatitis B virus
 - Non-A, non-B hepatitis virus
 - Epstein-Barr virus

Laboratory findings

- Pancytopenia.
- Anemia is normochromic, normocytic or macrocytic.
- Reticulocyte count is low.
- There are no abnormal cells in the peripheral blood.

- Bone marrow shows hypoplasia, with loss of hemopoietic tissue and replacement by fat.
 - Normally, 50 percent of the bone marrow is fat on histologic examination of the bone marrow biopsy, but in aplastic anemia of ten 80 to 95 percent is fat.
- Serum iron, vitamin B12, and folic acid levels are normal or, in the case of iron, often high because of underutilization.

Pure Red cell Aplasia

- Acquired pure red cell aplasia is a rare disorder, usually immunologically mediated, in which there is a specific failure of production of red cells.
- The patient has a normal white blood cell count and normal platelet count.
- There is severe anemia and a low reticulocyte count.
- The bone marrow biopsy shows a selective absence of red blood cell precursors, whereas white cell and platelet precursors are present in normal numbers.

Anemia of Renal Failure

- Patients with significant renal disease almost always have anemia.
- Patients who require dialysis are almost always severely anemic and need repeated transfusions.
- The primary cause of the anemia is a lack of erythropoietin, a hormone necessary for red cell growth and development in the bone marrow.
- Erythropoietin is made in the kidney.

Anemia of Chronic Disease

- In cases of chronic systemic inflammation, infection, or malignancy, the anemia of chronic disease (ACD) will often occur. This is frequently a mild to moderate anemia with hematocrits between 29 and 35%.
- The anemia is usually normocytic and normochromic with a normal reticulocyte percentage.
- About 40 percent of the time, the anemia is microcytic and hypochromic, usually only mildly so, but occasionally sufficient to cause confusion with iron deficiency anemia.
- In ACD the serum iron level is low, but so is the TIBC, in contrast to iron deficiency, in which the serum iron is low but the TIBC is usually elevated.
- The serum ferritin is normal or often elevated in ACD because it is an acute-phase reactant; it is characteristically low in iron deficiency.
- Inspection of the bone marrow usually shows abundant iron in reticuloendothelial cells, but little or no iron in red cell precursors.
- Thus, the patient has adequate iron stores, but is unable to transfer iron from the reticuloendothelial system storage cells to the red cell precursors that need it to form hemoglobin.
- The cause of this block in iron reutilization is uncertain, and there is no effective treatment other than to correct the underlying chronic disease.

Myelophthitic anemia

- Neoplasms, granulomatous infections, or a fibrotic process can directly replace the bone marrow.
- This may lead to a “myelophthitic” blood picture in which early white cell precursors as well as nucleated red cells are found in the peripheral blood, as are giant platelet forms or megakaryocyte fragments.
- Mature red cells often display a teardrop appearance.
- Infectious disease that may invade the bone marrow include tuberculosis and other mycobacterial infections (including atypical mycobacterial infections in patients with AIDS).

Anemias Associated with Endocrine Abnormalities [Hypothyroidism, Hypopituitarism]

- A mild anemia is commonly associated with hypothyroidism.
- This is usually normochromic and normocytic but may be macrocytic.

- The reticulocyte count is low, demonstrating that this is a hypoproliferative anemia, through the actual mechanism is not known.
- A mild normochromic, normocytic anemia is associated with hypopituitarism.
- The mechanism is unclear. Patients with Addison's disease also have a mild normochromic, normocytic anemia.

17.3.2 Hemolytic anemia

- Hemolytic anemias are defined as those anemias which result from an increase in the rate of red cell destruction.
- In hemolytic disorders, red cells are destroyed prematurely, usually in a random fashion.
- If the red blood cell life span is only moderately shortened, the patient will usually have little, if any, anemia because the bone marrow is capable of increasing the rate of new red blood cell production by a factor of 4 to 8.

Normal red cell destruction

- Red cell destruction usually occurs after a mean lifespan of 120 days when the cells are removed extravascularly
- by the macrophages of the reticuloendothelial (RE) system, especially in the marrow but also in the liver and spleen.
- Red cell metabolism gradually deteriorates as enzymes are degraded and not replaced, until the cells become non-viable, but the exact reason why the red cells die is obscure.
- The breakdown of red cells liberates iron for recirculation via plasma transferrin to marrow erythroblasts, and protoporphyrin which is broken down to bilirubin.
- This circulates to the liver where it is conjugated to glucuronides which are excreted into the gut via bile and converted to stercobilinogen and stercobilin (excreted in feces).
- Stercobilinogen and stercobilin are partly reabsorbed and excreted in urine as urobilinogen and urobilin.
- A small fraction of protoporphyrin is converted to carbon monoxide (CO) and excreted via the lungs.
- Globin chains are broken down to amino acids which are reutilized for general protein synthesis in the body.
- Haptoglobins are proteins present in normal plasma capable of binding hemoglobin.
- The hemoglobin-haptoglobin complex is removed from plasma by the RE system.
- Intravascular hemolysis (breakdown of red cells within blood vessels) plays little or no part in normal red cell destruction.

Extravascular Versus intravascular hemolysis

There are two general sites in which hemolysis may take place (Table 17.8). In intravascular hemolysis, which is uncommon, red blood cells are destroyed directly within the circulatory system. Extravascular hemolysis is more common than intravascular hemolysis and involves the destruction of red blood cells within mononuclearphagocytic cells, often in the spleen.

Table 17.8 Hemolytic anemia

Site of hemolysis	
Extravascular	Intravascular
Mechanism of hemolysis	
Extracorporeal	
Intracorporeal	
<ul style="list-style-type: none"> • Extrinsic to RBC • Usually acquired 	<ul style="list-style-type: none"> • Intrinsic to RBC • Usually inherited
• Morphologic stigmata may or may not be present on blood smear	• Morphologic stigmata usually present on smear

Intracorporeal versus Extracorporeal Defects

- o Intracorporeal defects are intrinsic to the red blood cell (Table 17.9).
- o They are usually inherited, and generally (but not always) the abnormality is observable in the peripheral blood smear.

- o Extracorporeal defects refer to problems in the environment of the red blood cell, not in the red blood cell itself
- o Extracorporeal hemolysis is usually acquired and is often but not always discernible in the form of morphologic abnormalities in the peripheral blood smear.

Table 17.9 Intracorporeal hemolytic anemias

Membrane defects

- Congenital spherocytosis
- Congenital elliptocytosis
- Paroxysmal nocturnal hemoglobinuria*

Metabolic defects

- o Pentose shunt defects
- o Embden-Meyerhof pathway defects

Hemoglobin defects

- o Hemoglobinopathies (S,C unstable hemoglobin, etc)
- o *Acquired defect
- o Table 17.10 Extracorporeal hemolytic anemias

Immune disorders

- o ABO incompatibility (transfusion reaction)*
- o Rh incompatibility (erythroblastosis fetalis)
- o Autoimmune or immunohemolytic anemias
- o Warm Cold antibodies
- o IgG Complement Coombs' test
- o Paroxysmal cold hemoglobinuria (Donath- Landsteiner antibody)*

Physical damage

- o Micro-or macroangiopathic hemolytic anemias*
- o Burns*

Chemicals, toxins, drugs

- o H₂O*, *Clostridium welchii* toxin*, spur cell anemia,
- o oxidant drugs*, drugs causing immunohemolytic anemia

Increased activity of reticuloendothelial system

- o Hypersplenism

Infections

- o Malaria*
- o Infectious mononucleosis
- o *Mycoplasma pneumonia*
- o *Cl. Welchii*
- o *Intravascular hemolysis
- o Babesia

Laboratory findings

- o The major criteria for the laboratory diagnosis of hemolytic anemia are reticulocytosis and an increase in serum level of unconjugated bilirubin.
- o Serum level of lactic dehydrogenase (LDH) is elevated.
- o Serum haptoglobin level is decreased.
- o The peripheral blood smear often but not invariably shows morphologic changes in the red blood cells compatible with hemolysis
- o For example, many spherocytes suggest hereditary spherocytosis or immunohemolytic anemia and sickle cells suggest one of the sick cell syndromes.

INHERITED HEMOLYTIC ANEMIA

- ✓ This is a congenital hemolytic anemia, some of which present at birth, and others later in life, while still others may remain silent unless a physiologic stress is superimposed.

Hereditary spherocytosis

- Hereditary spherocytosis (HS) is an autosomal dominant disorder that may become symptomatic shortly after birth, or may not be detected until later life.
- Most patients with HS have spherocytes, splenomegaly, and jaundice.

Laboratory findings

- o Patients with HS have laboratory evidence of hemolysis, including anemia, reticulocytosis, increased serum LDH level, decreased or absent haptoglobin, and often mild hyperbilirubinemia.
- o Except during hemolytic crises they do not have hemoglobinemia or hemoglobinuria.
- o Examination of the peripheral blood smear shows large numbers of spherocytes, often accompanied by polychromatophils.
- o The spherocytes are more susceptible to osmotic stress as measured by the osmotic fragility test.
- o The MCV may be low normal or, with reticulocytosis, moderately elevated
- o The MCHC is characteristically high. HS is one of the few causes of an elevated MCHC.

Hereditary elliptocytosis

- Hereditary elliptocytosis (HE) is an autosomal dominant disorder characterized by excessive numbers of elliptical cells.
- HE cells are biconcave dumbbells with an axial ratio of less than 0.8. In the majority of cases hematocrit levels are normal or near normal with minimal hemolysis; greater than 25 percent (often 75%) of red cells are elliptocytes.
- In 10 to 15 percent of patients with HE, erythrocyte destruction is substantially increased, leading to all the signs and symptoms of a true hemolytic anemia.
- It should be noted that some elliptical cells also occur in thalassemia, iron deficiency, myelophthitic anemias, sickle cell disease, and megaloblastic anemia.
- These disorders, however, are accompanied by other characteristic morphologic changes as well.

Erythrocyte enzyme deficiencies

- Hereditary hemolytic anemia has been associated with at least ten red cell enzyme deficiencies.
- Of these, only three are of clinical significance.

I. Glucose-6-phosphate Dehydrogenase deficiency

- Deficiency of the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) is by far the most common inherited erythrocyte enzyme deficiency, affecting more than 100 million people. The gene for G-6-P is sexlinked.
- Because of the X-linkage, male patients are more severely affected than female patients.
- It is a key enzyme in the hexose monophosphate pathway (HMP).

II. Pyruvate Kinase Deficiency

- Pyruvate kinase (PK) deficiency is much less common than G-6-PD deficiency.
- However, it is the second most common erythrocyte enzyme deficiency.
- The deficiency is not limited to any particular racial or geographically defined population.
- Rather than producing acute hemolysis in association with drug ingestion, it causes a chronic congenital nonspherocytic hemolytic anemia.

III. Pyrimidine-5'-nucleotidase deficiency

- A chronic hemolytic anemia inherited as an autosomal recessive and characterized by large numbers of erythrocytes with basophilic stippling is due to deficiency in an enzyme, Pyrimidine-5'-nucleotidase, which dephosphorylates the ribonucleotides of cytidine and uridine.

Hemoglobinopathies

- Mutations in the DNA sequence controlling the synthesis of globin chains in hemoglobin result in either structurally abnormal hemoglobins or reduced globin chain synthesis or, sometimes, both.
- Generally the term hemoglobinopathy is used to signify a structurally abnormal hemoglobin with at least one amino acid substitution.
- Thalassemia refers to DNA mutations resulting in normally structural globins but with reduced or negligible synthesis rates.
- Structural abnormalities may cause premature red cell destruction; easily denatured hemoglobins; hemoglobins with abnormal oxygen affinity; altered hemoglobin solubility; and, in a few instances, reduced globin synthesis.
- The heme moiety of hemoglobin is synthesized normally and is structurally normal.
- In this topic only the few clinically significant hemoglobinopathies are discussed.

I. Hemoglobin S

- By far the most important hemoglobinopathies are those related to the presence of sickle hemoglobin (HbS).
- This hemoglobin is present in approximately 10 percent of blacks.
- Sickle hemoglobin results from replacement of the sixth amino acid from the N-terminal end of the β - chain, glutamic acid, by valine.
- Invariably sickle cells are typically seen on Wright-stained peripheral blood smears from patients.

II. Hemoglobin C syndromes

- Hemoglobin C (HbC) is probably the second most common hemoglobinopathy (2-3% gene frequency in
- black populations).
- HbC is caused by substitution of lysine for glutamic acid in the sixth position from the Nterminal end of the β -hemoglobin chain (same location as the substitution in HbS).
- The peripheral blood smear shows increased numbers of target cells.

ACQUIRED HEMOLYTIC ANEMIA

- This includes immuno hemolytic Anemias, PNH, fragmentation hemolysis, typersplenism, chemicals and toxins.
- A variety of acquired clinical conditions result in shortened survival of previously normal red cells.
- These include immune-mediated destruction, red cell fragmentation disorders, acquired membrane defects, splenic effects, and the results of infections and environmental toxins.

Immuno hemolytic anemia

- Immuno hemolytic anemias are the result of the binding of antibody, complement, or antibody plus complement to red cells.
- Antibodies formed against erythrocyte antigens may be either warm (active at 37°C) or cold (active at room temperature and below).
- In some cases, these antibodies activate a series of proteins, referred to collectively as complement; in others, the red cells are coated with antibody alone.
- As a result of complement activation by hemolytic antibodies, intravascular red cell lysis and release of hemoglobin may occur.
- Alternatively, and more frequently, immune lysis, due to either antibody or complement, is extravascular and happens slowly within cells of the macrophagic-phagocytic system (RES), particularly in the spleen.
- Immuno hemolytic anemias fall into one of three major categories: autoimmune, in which the patient makes an autoantibody against his or her own red cells; alloimmune, where the patient's antibody is directed against foreign red cells; and drug-induced, where a drug-dependent or related antibody is responsible for hemolysis (Table 17.11).

Table 17.11 Classification of immuno hemolytic anemias

Autoimmune hemolytic anemia

- Warm autoimmune hemolytic anemia
- Cold agglutinin syndrome
- Paroxysmal cold hemoglobinuria

Drug induced hemolytic anemias

Alloimmune hemolytic anemia

- Transfusion reactions
- Hemolytic anemia of the newborn

I. Autoimmune hemolytic anemia (AIHA)

A. Warm AIHA

- ✓ This illness, more common than the two other autoimmune hemolytic anemias, occurs in 1 to 3 individuals per 100000 in the population.
- ✓ The red cells are usually coated with IgG alone, IgG and complement or complement alone, but a minority of cases show IgA or IgM coating alone or combined with IgG antibody.
- ✓ The complement component detectable is C3d, the degraded fragment of C3.
- ✓ The AIHA in SLE is typically of the IgG + complement type. Red cells coated with IgG are taken up the RE macrophages, especially in the spleen, which have receptors for the Fc fragment.
- ✓ Part of the coated membrane is lost so the cell becomes progressively more spherical to maintain the same volume and is ultimately prematurely destroyed, usually predominantly in the spleen.
- ✓ Red cells with complement coating alone or in addition to IgG are destroyed more generally in the RE system, and not particularly in the spleen.
- ✓ The disease may occur at any age in either sex and presents as a hemolytic anemia of varying severity.

Laboratory findings

- ✓ The hematological and biochemical finding are typical of a hemolytic anemia with spherocytosis prominent in the peripheral blood.
- ✓ The hallmark of autoimmune hemolytic anemia is the presence of antibody or complement, or both, on the patient's own red cells. The DAT is positive due to IgG, IgG and complement, IGA or, rarely, IgM on the cells.
- ✓ In some cases, the autoantibody shows specificity within the rhesus system, e.g. anti-c or anti-e.
- ✓ The antibodies both on the cell surface and free in serum are best detected at 37oC.

B. Cold AIHA

- This hemolytic anemia is less common than its warm counterpart.
- In these syndromes the autoantibody, whether monoclonal (as in the idiopathic cold hemeagglutinin syndrome or associated with lymphoproliferative disorders) or polyclonal (as following infection, e.g. infectious mononucleosis or *Mycoplasma pneumonia*) attaches to red cells mainly in the peripheral circulation where the blood temperature is cooled.
- The antibody is usually IgM and binds to red cells best at 4oC.
- Hemolytic syndromes of varying severity may occur depending on the titer of the antibody in the serum, its affinity for red cells, its ability to bind complement, and its thermal amplitude (whether or not it binds to red cells at 37oC).
- Agglutination of red cells by the antibody often causes peripheral circulation abnormalities.
- The antibody may then detach from red cells when they pass to the warmer central circulation but, if complement has been bound, the direct antiglobulin test remains

positive-of complement only type- and the cells are liable to be destroyed in the whole RE system, especially the liver, giving rise to a chronic hemolytic anemia.

- Intravascular hemolysis occurs in some of the syndromes, in which the complement sequence is completed on the red cell surface. Low serum levels of complement in other case may help to protect the patient from a more severe clinical disease.

C. Paroxysmal Cold Hemoglobinuria

- This rare disease, characterized by hemoglobinuria following cold exposure, is caused by an IgG autoantibody to the red cell antigen P.
- This Donath- Landsteiner autoantibody is unique in that it binds to red cells at cold temperatures, in contrast to other IgG antibodies.
- Furthermore, it binds complement well, and brisk hemolysis results when the cells are warmed and the complement sequence proceeds to completion.
- Viral infections and syphilis are predisposing causes.

Laboratory findings

- These are similar to those of warm AIHA, except that spherocytosis is less marked, red cells agglutinate in the cold. E.g. on the blood film made at room temperature, and the DAT reveals complement (C3) only on the red cell surface.
- Serum antibodies often present in high titer are IgM, react best at 4°C. In the rare cold AIHA, paroxysmal cold hemoglobinuria, the antibodies are IgG and have specificity for the P blood group antigens.

II. Drug induced immune hemolytic anemias

- ✓ Four pathophysiologic mechanisms account for most cases of drug-induced red cell sensitization and positive direct Coombs test.
- ✓ The drug-induced problem may result in serious hemolytic anemia on the one hand, or a laboratory abnormality without clinical sequelae on the other.

A. Penicillin: 'Hapten' (drug adsorption) mechanism

- Penicillin binds strongly to the red cell membrane, and can be detected on red cells in many patients who are receiving high doses of this drug.
- Although the drug coating by itself is not harmful, some patients develop high-titer antipenicillin IgG antibodies, which can react with the coated red cells.
- The coombs test is positive due to the presence of IgG; complement is not usually found.

B. Quinidine: 'Innocent bystander' (immune complex) mechanism

- In some patients previously sensitized to quinidine, and taking it again, the drug reacts with a quinidine antibody, often IgM, to form an immune complex.
- This complex is then adsorbed onto the red cell membrane; the red cell is considered an "innocent bystander" because it is not the direct target of the antibody.

- The immune complexes often activate complement, and an abrupt and life-threatening intravascular hemolytic anemia may develop.
- The coombs test is positive for complement; the immune complexes themselves do not bind firmly to the cell.

C. α-methyldopa (Aldomet): Autoimmune hemolysis by unknown mechanism

- In as many as one third of patients taking 2gm α-methyldopa daily, a positive direct Coombs test develops after 3 to 6 months of use.
- Far fewer (between 1 and 5%) develop frank hemolysis.
- The drug itself does not attach to red cells or antibodies, but somehow causes the development of red cell IgG autoantibodies, which closely resemble those found in warm AIHA.
- The direct Coombs test is usually strongly positive with IgG; complement is rarely found.

D. Cephalosporins: Membrane modification

- These drugs cause a positive direct Coombs test through a non-immunologic mechanism.

- Cephalothin and other cephalosporins are capable of altering the red cell membrane so that proteins, including complement and an assortment of γ -globulins, are nonspecifically adsorbed.
- As a result of the presence of these proteins, the direct antiglobulin test is positive.
- However, the red cell eluate does not react with any other cells, because the mixture of γ -globulins does not include any predominantly red cell antigen-specific antibody.
- The antibody screen is negative because no unusually drug-related antibodies are present.
- Hemolytic anemia does not occur in this situation.

III. Alloimmune hemolytic anemias

A. Hemolytic transfusion reactions

- The differential diagnosis of a positive direct antiglobulin test includes not just red cell autoantibodies but also alloantibodies-antibodies in the patient directed against foreign red cell antigens.
- These antibodies are either “naturally occurring,” in that individuals acquire them without specific exposure to the red cell antigen, or “immune,” from red cell transfusions.

Acute hemolytic transfusion reactions

- ABO antibodies are the most important example of naturally occurring red cell antibodies (so-called isoantibodies) that can cause severe, even fatal, hemolysis.
- For example, a patient whose red cells are group O has anti-A and anti-B in his or her serum.
- Like many other IgM antibodies, these isoagglutinins are potent complement fixers.
- If inadvertently transfused with group A red cells, this patient’s anti-A would immediately react with the donor cells. Complement fixation and intravascular hemolysis would result.
- The direct Coombs test is positive due complement fixation, but may become negative within hours to days, depending on how rapidly the group A cells are destroyed.
- The presence of urine hemosiderin beginning 3 to 5 days after the transfusion attests to the recent presence of hemoglobinemia.

Delayed hemolytic transfusion reactions

- In some patients, the titer of non-ABO antibodies after transfusion wanes, and the antibody becomes undetectable.
- If the patient then receives an antigen-positive unit, an anamnestic rise in antibody occurs over the next 3 to 21 days.
- This results in delayed hemolysis.
- Here, red cell destruction is usually leisurely, since the cells are eliminated only after they are coated with sufficient antibody, which depends on the rapidity with which it is produced.
- The direct Coombs test on a Post-transfusion blood specimen is positive due to IgG-coated transfused red cells.
- The test becomes negative as the antibody-coated cells are removed from the circulation.
- The patient’s antibody screen, negative before the transfusion, becomes positive shortly afterward.

B. Hemolytic disease of the newborn

- This hemolytic process actually begins in utero to the baby of a mother with IgG red cell antibodies.
- IgG antibodies readily cross the placenta, as opposed to IgM antibodies, which cannot.
- In the past, many Rh(D)-negative women became sensitized to the red cell antigen D at the time of birth of a first Rh-positive child, because at birth it is common for a small volume of fetal cells to enter the maternal circulation.
- Today, Rh sensitization is much less common and largely preventable.
- Rh-positive fetuses carried by a sensitized Rh-negative mother can be severely affected by the IgG anti-D.
- Some babies develop profound in utero anemia with congestive heart failure (hydrops fetalis), leading to stillbirth.

Paroxysmal nocturnal hemoglobinuria

- Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired membrane disorder than results in red cells becoming unusually sensitive to the hemolytic action of complement.
- The susceptible red cells are clonally derived, meaning that all abnormal cells have descended from a single pluripotential stem cell with the PNH mutation.

Laboratory findings

- Patients with PNH often have severe anemia, but in contrast to other acquired hemolytic anemias, no spherocytes or other morphologic abnormalities are seen on examination of the peripheral blood smear.
- The reticulocyte count is high.
- Over time, some patients develop hypochromic microcytic red cells due to progressive iron deficiency, resulting form hemoglobinuria and hemosiderinuria. Neutropenia and thrombocytopenia are common.
- As in chronic myelogenous leukemia (CML), the leukocyte alkaline phosphatase (LAP) score is reduced in PNH granulocytes.
- Acetylcholinesterase levels in the erythrocytes are also decreased.
- The biochemical hallmark of PNH is the ability to demonstrate hemolysis of the patient's red cells when the pH is dropped to between 6.5 and 7.0.
- The Ham test involves the addition of acidified serum from a normal volunteer to the patient's red cells.
- The normal serum provides complement, which may be depleted in patients with PNH.
- Lysis in this tube as compared to control tubes, in which the normal serum has been heated to destroy complement, is virtually diagnostic of PNH.
- The sucrose hemolysis ("sugar water") test can be used as a simple screening test.
- Here the patient's blood is added to isotonic sucrose, which is a low ionic strength solution.
- Complement is activated by the reduced ionic strength, and hemolysis occurs.
- Since occasional false positives occur, positive results require confirmation with the more complex and rigorous Ham test.

Review Questions

1. Define anemia
2. Describe the different methods of classifying anemia
3. Explain in brief microcytic anemia and the different forms included in this category
4. Describe macrocytic anemia
5. Describe normochromic normocytic anemia