Anaemia 4.01

Detailed History of Presenting Illness (HPI)

***Presenting Symptoms*:**

- **Fatigue**
- **Dyspnea**
- **Palpitations**
- **headache**
- **tinnitis**
- **anorexia**
- dyspepsia
- bowel disturbance
- angina (if previous coronary artery disease)

***Signs*: (may be absent)**

- **pallor**
- retinal haemorrhages,
- hyperdynamic circulation;
- **tachycardia** (increased cardiac output),
- murmurs and **cardiac enlargement,**
- heart failure may occur,
- **increased respiratory rate and depth,**
- behavioural changes such as slow and **economic movement,**
- koilonychia (spoon-shaped nails),
- angular stomatitis,
- atrophic glossitis,
- **brittle hair,**
- glossitis and dysphagia,
- atrophic gastritis

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The development of the symptoms depends on the
severity,
speed of onset,
adequacy of **compensation mechanisms,**
age (elderly have impaired compensation),
patient expectations,
underlying cause
and associated features and other diseases.

List of Differential Diagnoses (DDx)

- **Chronic fatigue syndrome**
- **Chronic viral infection**
- **Heart failure**
- **Blood loss through GI bleed**
- **Haemolytic anaemia**
- **Under-production of RBCs**
- **Vit B12 deficiency**

Pertinent Findings on History (Hx)

- **history of previous blood examination**
- **history of rejection as a blood donor**
- **family history,** not only for anemia but also for jaundice, cholelithiasis, splenectomy, bleeding disorders,
- **occupations and hobbies,**
- **prior medical treatment**,
- **drugs** (including over-the-counter medications and vitamins),
- and household exposures to potentially noxious agents Eg.
    - **tranquilizers,**
    - **insecticides,**
    - **paints,**
    - **solvents,**
    - **hair dyes .**

- In **searching for blood loss**, carefully document
    - **pregnancies,**
    - **abortions,**
    - **menstrual loss.**

- **tarry stools and general changes in bowel habits** can be useful in uncovering neoplasms of the colon.
- **Hemorrhoidal blood loss**
- **history of gastrointestinal complaints** that may suggest gastritis, peptic ulcers, hiatal hernias, or diverticula.
- **Abnormal urine color** can occur in renal and hepatic disease and in hemolytic anemia.
- A **thorough dietary history** is important in the patient who is anemic and must include both foods the patient eats or avoids and an estimate of their quantity.
    - **A meal-by-meal description** is necessary to obtain appropriate estimates.
    - Question patients specifically regarding consumption of either **clay or laundry starch**. This history will not be provided spontaneously. These substances render iron less absorbable.
    - **Changes in body weight** are important with regard to dietary intake and can suggest the presence of malabsorption or an underlying wasting disease of infectious, metabolic, or neoplastic origin.
- **Nutritional deficiencies may be associated with unusual symptoms** that can be elicited by history.
    - **Patients with iron deficiencies frequently chew or suck ice** (pagophagia).
        - Occasionally, they complain of
        - **dysphasia,**
        - **brittle fingernails,**
        - **relative impotence,**
        - **fatigue,**
        - **cramps in the calves on climbing stairs** that are out of proportion to their anemia.
    - **In vitamin B-12 deficiency,**
        - **early graying of the hair**,
        - **burning sensation of the tongue**,
        - **loss of proprioception** are common.
    - **Suspect loss of proprioception if the patient stumbles in the dark or must look in order to put on pants** in the morning.
    - Patients with folate deficiencies may have a
        - **sore tongue,**
        - **cheilosis,**
        - **symptoms associated with steatorrhea.**
    - **Color, bulk, frequency, and odor of stools and whether the feces float or sink can be helpful in detecting malabsorption**. More sensitive questions to detect steatorrhea include whether the toilet needs to be flushed more than once to rid it of stool and whether an oily substance is floating on the water surface after the first flush.
- **Obtain history or presence of fever**, because infections, neoplasms, and collagen vascular disease can cause anemia. Similarly, the occurrence of purpura, ecchymoses, and petechiae suggest either the occurrence of thrombocytopenia or other bleeding disorders that may be an indication that either more than one bone marrow lineage is involved or that coagulopathy is a cause of the anemia because of bleeding.
- **Cold intolerance** can be an important symptom of hypothyroidism or lupus erythematosus, paroxysmal cold hemoglobinuria, and certain macroglobulinemias.
- **The relation of dark urine to either physical activity or time of day can be important in March hemoglobinuria** and paroxysmal nocturnal hemoglobinuria.

Pertinent findings on Examination (Ex)

- **pallor,**
- abnormal pigmentation,
- icterus,
- spider nevi,
- petechiae,
- purpura,
- angiomas,
- ulcerations,
- palmar erythema,
- coarseness of hair,
- puffiness of the face,
- thinning of the lateral aspects of eyebrows,
- nail defects,
- a usually prominent venous pattern on the abdominal wall.

- **Examine optic fundi**
- **Palpate lymph nodes for evidence of infection or neoplasia**.
- **Bilateral edema**
- **Carefully search for both hepatomegaly and splenomegaly**.
- **Examine rectum + pelvis for neoplasm or haemorrhoids**
- **NEURO: tests of position sense and vibratory sense,** which will be ABNORMAL in pernicious v.B12 anaemia
- **Cardiomegaly**

Tests and Investigations

Full Blood Count + Blood Film Microscopy should always be the first investigation

Expecting a low Hemoglobin

Anaemia may be hidden if the patient is dehydrated so that the haemoglobin concentration appears normal.

Anaemia is defined as below the following values

Adult males	**140g/l**
Adult females	**120g/l**
3 months to puberty	**110g/l**
Birth to 3 months	**150g/l**

Neonates have a high haemoglobin level

HAEMOGLOBIN:

Normo or Hypo Chromic

if this is low, you've got anaemia. IS THE PATIENT HYPERVOLEMIC?

- i.e is the blood diluted? This would make the Hb lower

If this is NORMAL, you might still have anaemia if the patient is dehydrated

i.e blood is concentrated, thus Hb appears normal

MEAN CORPUSCULAR VOLUME :

Micro Normo or Macro Cytic ← DDx

Most commonly:----------

Micro: below 80 fl

- **Iron-deficiency anaemia**,
- thalassaemia,
- sideroblastic anaemia,
- anaemia of chronic disease

Normo: 80-100 fl

- **Acute blood loss,**
- **HAEMOLYSIS**
- chronic disease,
- bone marrow failure,
- renal failure,
- hypothyroidism,
- connective tissue disease,
- pregnancy

Macro: 100+ fl

- **alcohol abuse**
- megaloblastic anaemia

Table 3.7 Laboratory diagnosis of a hypochromic anaemia

	Iron deficiency	Chronic inflammation or malignancy	Thalassaemia trait (α or β)	Sideroblastic anaemia
MCV MCH	Reduced in relation to severity of anaemia	Normal or mild reduction	Reduced; very low for degree of anaemia	Usually low in congenital type but MCV often raised in acquired type
Serum iron	Reduced	Reduced	Normal	Raised
TIBC	Raised	Reduced	Normal	Normal
Serum transferrin receptor	Raised	Normal/low	Variable	Normal
Serum ferritin	Reduced	Normal or raised	Normal	Raised
Bone marrow iron stores	Absent	Present	Present	Present
Erythroblast iron	Absent	Absent	Present	Ring forms
Haemoglobin electrophoresis	Normal	Normal	$Hb A_2$ raised in β form	Normal

MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; TIBC, total iron-binding capacity.

DIAGNOSTIC STRATEGY: ANAEMIA

4.01

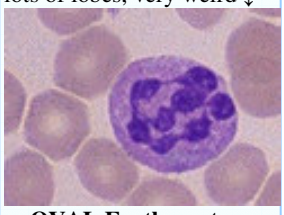
MCV on FBC:

MACROCYTIC → BLOOD FILM:

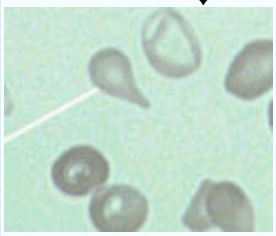
MEGALOBLASTIC:

- **HYPERSEGMENTED NEUTROPHILS**

lots of lobes, very weird ↓



+ OVAL Erythrocytes
+ TEARDROP POIKILOCYTES ↓



→ ALMOST ALWAYS DEFICIENCY OF Vit. B 12 or FOLATE

BONE MARROW BIOPSY

Cannot call it megaloblastic until MEGALOBLASTS are found in the marrow
("erythroid hyperplasia")
+ HYPERCELLULARITY (packed with cells)
+ "GIANT METAMYELOCYTES" are also found; = abnormal leucocyte precursors

WHAT IS MISSING??
Its going to be either **folate or B12; THUS:**
- test→
Serum vitamin B_{12}
and
Red Cell Folate
+ investigate the reason, eg. B12 is down = do **Coombs test for autoantibodies to parietal cells or Intrinsic Factor**
ELSE: (no dietary deficit and no malabsorption)
→ congenital or drug-related (eg. methotrexate)

NON-MEGALOBLASTIC:

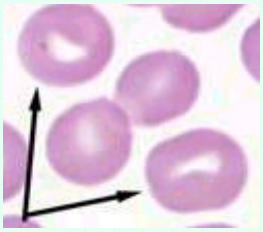
Tends to have **ROUND FLAT MACROCYTES and normal neutrophils = more common than Megaloblastic Anaemia**

RETICULOCYTES:

DECREASED=

- **Alcoholism**
- **or liver disease**

→ do LFTs
→ look for STOMATOCYTES



and ECCHINOCYTES

- **Hypothyroidism**

- do thyroid function tests

INCREASED=

- **Hemolytic anaemia** (see below and to the right)
- **Recovering from haemorrhage** **(look for evidence of GI bleed, or recent blood loss)**

a Direct Coombs'
Test tells you whether the red blood cells are antibody-coated, and, in the presence of hemolysis, indicates **an immune-mediated process (NOT Very Accurate)**

NORMOCYTIC → BLOOD FILM:

EITHER OVER-DESTRUCTION Or UNDER-CREATION:

Look for **RETICULOCYTES:**
→ HIGH: hemolytic a.
→NONE: aplastic anaemia
(**BM biopsy** will show practically NO erythropoietic activity)

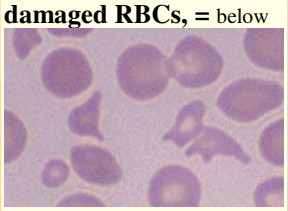
SPLENOMEGALY:
A large hypertensive spleen will sequester any cells it can get its hands on

Evidence of Haemorrhage:
It's the easiest way to lose RBCs

Evidence of **CHRONIC INFECTION**
- GET a BONE MARROW BIOPSY
Macrophages in the marrow accumulate their hemosiderin (considerable increase, use Perle stain or Prussian Blue) and thus the sideroblasts have very little iron.

Evidence of **Kidney Disease**
(decreased erythropoietin)
(→ urea, creatinine, electrolytes)

Evidence of HAEMOLYSIS
Look for LDH
Look for Bilirubin
Look for Haptoglobin
Look for schizocytes (helmet-shaped damaged RBCs, = below

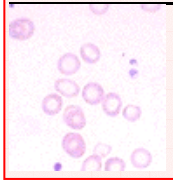


Look for spherocytes (little RBCs with no central pallor)
→ IF THESE ARE INCREASED, it may be autoimmune hemolysis

The direct measurement of red cell survival is made by labelling the patient ' s red cells with chromium-51, the cells being reinjected back into the patient ' s circulation and sampled at intervals of several days to measure the rate of their removal. This investigation is rarely ordered and we rely on other criteria.

MICROCYTIC → BLOOD FILM:

MICROCYTES



← RBCs will be small and hypochromic, with occasional
- **PENCIL CELLS**
- **TARGET CELLS**
- **There will be ANISOCYTOSIS**

SERUM FERRITIN STUDY

HIGH IRON
= TAKE BONE MARROW BIOPSY
=look for increased sideroblast iron = Sideroblastic Anaemia

Total iron Binding Capacity (TIBC)
→ will be very low in IRON OVERLOAD (totally saturatecd)

NORMAL
=Thalassaemia
→ do hemoglobin electrophoresis to check for Hglobinopathy

LOW IRON
= IRON DEFICIENCY: look for GI bleed!
Stool sample,
→ look for blood
Endoscopy,
→look for ulcer

Total iron Binding Capacity (TIBC)
→ will drop in **Iron.def.Anaem.**

Total iron Binding Capacity (TIBC)
→ will remain the same in **Anaemia of chronic disorders**

BONE MARROW FAILURE
Will appear as a
PANCYTOPENIA

Glossary of Useful Blood Count Descriptors

Anisocytosis: red cells of unequal size. Reflected in increased RDW (Red cell Distribution Width.)
Band cell: the stage of neutrophil maturation immediately before full maturity. Named after the shape of its nucleus. Appears in the blood during infections, and other marrow 'stress'
Blast Cell: early committed marrow precursor of mature red and white cells. This cell accumulates in the marrow in acute leukemia, and may appear in the blood in large numbers.
Dimorphic Blood Film: two populations of red cells - one microcytic and the other normocytic. Seen in treated or transfused iron deficiency, and sideroblastic anemia
Erythroblast: any nucleated red cell precursor
Howell-Jolly bodies: round nuclear remnants within the red cells. Indicate splenectomy or hyposplenism
Hypersegmented neutrophils: a neutrophil with six or more lobes. Usually (but not inevitably) means vitamin B12 or folate deficiency
Hypochrom(as)ia: pale red cells. Always accompanied by microcytosis
Left Shift: the presence of slightly immature white cells (eg bands and metamyelocytes), suggesting infection
Leukoerythroblastic: the presence of erythroblasts and myelocytes (which are precursors of mature cells) in the blood. Often indicates marrow infiltration eg by secondary cancer, or fibrosis
Macrocytosis: large red cells
Microangiopathy: indicates mechanical damage to red cells with red cell fragments on the blood film
Microcytosis: small red cells
Metamyelocyte: the stage of neutrophil maturation immediately before the band cell. Appears in the blood during infections, and other marrow 'stress'
Myelocyte: a white cell precursor. A component of the 'leukoerythroblastic' blood film
Pancytopenia: a reduction in all the formed elements of the peripheral blood. May indicate marrow failure
Poikilocytosis: a traditional term for red cells of unequal shape
Polychromasia: grey coloured red cells on film, indicating presence of increased reticulocytes
Reticulocyte: an erythrocyte newly released from the bone marrow, identifiable by a network or 'reticulum' of RNA in its cytoplasm (a special stain is needed to show this). After about 24 h, this RNA disappears. An increased absolute number of reticulocytes indicates increased marrow erythropoiesis.
Rouleaux: red cells in stacks, as coins. Indicates high ESR, eg infection, myeloma, cancer, collagen disease etc.
Schistocyte: a red cell which has undergone mechanical damage - synonymous with red cell fragment
Spherocyte: a spherical red cell due to disproportionate membrane loss. Either inherited, or acquired from (usually) immune causes
Sickle cell: a crescent-shaped red cell characteristic of Sickle Cell Anemia
Target cell: red cell with central area of Hb giving the appearance of a target. Seen in many conditions, including hemoglobinopathy and liver disease

IRON STUDIES → → → → → → → → → → → → → → →

The only cause of low serum ferritin is iron deficiency

Serum Ferritin: Range: 18-300 ng/ml

INCREASED in inflammation, cancer, hemochromatosis, or Hyperthyroidism.

Serum Iron: Measures Transferrin-associated ferric ion, Range: 50 - 175 ug/dl

INCREASED =

- Hemochromatosis
- Hemolysis
- Hemolytic Anemia
- Hemosiderosis
- Hepatic necrosis
- Hepatitis
- Ineffective Erythropoiesis
- Vitamin B12 Deficiency
- Iron Poisoning or Iron Overdose
- Lead Toxicity or Lead Poisoning

DECREASED=

- Chronic Gastrointestinal Blood loss
- Heavy Menstrual Bleeding
- Inadequate iron absorption
- Insufficient Dietary Iron
- Iron Deficiency Anemia
- Malabsorption
- Nephrotic Syndrome
- Third trimester of pregnancy

LOW serum iron and HIGH total iron binding capacity (TIBC) = IRON DEFICIENCY
LOW serum iron and a LOW TIBC = ANAEMIA OF CHRONIC DISORDERS

We have about 3.7 grams of iron in our body, painstakingly gathered from iron in our diet. About 2.5 grams are locked inside the hemoglobin in our blood, where they assist in the transport of oxygen. This is a valuable and essential resource, so special mechanisms for the recycling of this iron have been developed. Another few tenths of a gram are found in myoglobin, which also assists in oxygen management. A remarkably small amount--about 0.02 g--is distributed between the many different proteins that transfer electrons, such as the proteins of the oxidative phosphorylation electron transport chain that create most of our cellular ATP supplies. The rest, about a gram, is stored inside ferritin to fulfill future needs.

Tranferrin 160 to 370 mg/dl (0.16 to 0.37 g/dl); ***Serum half-life: 20 days***

= is the binding protein which carries iron from the liver into the bone marrow.
= It is endocytosed when it connects to a transferrin receptor, THUS → inside cell in tiny vesicle
= **the vesicle is ACIDIFIED and transferrin gives up its iron**
= the empty tranferrin husk is then excreted back into the blood

INCREASED:
- Iron Deficiency Anemia
- Viral Hepatitis
- Medication :Oral Contraceptives

DECREASED:
- Nephrotic Syndrome
- Liver disease
- Chronic inflammatory states
- Chronic illness
- Thalassemia
- Neoplasm
- Protein malnutrition

Total Iron Binding Capacity (TIBC)

Transferrin carries 2 iron atoms per molecule
Transferrin is normally 30% bound to iron
TIBC reflects a measurement of serum Transferrin's available binding sites

Increased TIBC: low iron stores

A. Iron Deficiency Anemia
B. Third trimester Pregnancy
C. Polycythemia Vera

Decreased TIBC over-saturated transferrin

A. Anemia of Chronic Disease
B. Hemolytic Anemia
C. Hemochromatosis
D. Chronic Liver Disease or Cirrhosis
E. Hypoproteinemia
F. Malnutrition
G. Pernicious Anemia
H. Sickle Cell Anemia

IMMUNOLOGICAL TESTING

→ ***Investigating Haemolysis***

A direct Coombs' test detects the two different antigens that might induce hemolysis in the patient's red blood cells.
An indirect Coombs' test looks for antibodies to someone else's red blood cells in the patient's serum (the blood without the cells).
Combining the two tests gives clues to the origin of the hemolysis.

DIRECT COOMBS TEST:

Answers the Question: Is IgG or Complement bound to the RBCs?

1. Start with patient's Red Blood Cells
2. Add **Anti-human globulin antibodies** (**Coombs reagent**)
   (**Coombs reagent** = Anti-Human Globulin Antibody, binds to human IgG antibody and C3 Complement)

Positive → Agglutination indicates antibody coated RBCs

INDIRECT COOMBS TEST:

Answers the Question: Is Antibody against the patients Red Blood Cells present in serum?

3. Start with patient's serum
4. Add Anti-human globulin antibodies (**Coombs reagent**)
5. Add Indicator Red Blood Cells with known antigens

Indirect Coombs Positive :

1. **Agglutination indicates antibody to RBC in serum**
2. Antibody titer can be obtained by serial dilution

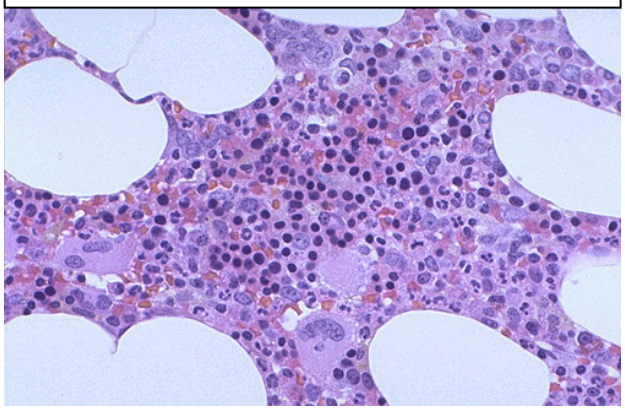
THUS: haemolytic anaemia is autoimmune if:
the RBCs are coated with ANTIBODY, and that ANTIBODY is present in the SERUM

If the Coombs' tests are negative, the anemia is unlikely to be autoimmune, and the hematologist will have to search elsewhere for a cause.

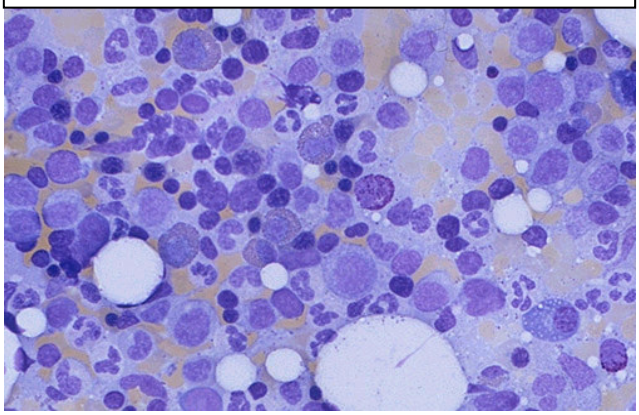
Bone Marrow Biopsy : best objective method of assessing body iron stores

Bone marrow iron is assessed by staining the cells with Perls' stain which stains iron containing material BLUE. Absence of blue colouration indicates iron deficiency.

This is the appearance of normal bone marrow from a middle-aged person. At high magnification. Note the presence of megakaryocytes, erythroid islands, and granulocytic precursors.It is about 50% cellular, with steatocytes mixed in.



This is the appearance of normal bone marrow smear at high magnification. Note the presence of an eosinophilic myelocyte, a basophilic myelocyte, and a plasma cell.



Management

Treating iron deficiency = doses of oral iron, usually in the form of slow release ferrous sulphate tablets

Anaemia of Chronic disease – treat underlying disease. Iron supplementation **will not work**

Sideroblastic anaemia – removal of cause if possible.
Pyridoxine and folate sometimes work.
Often need blood transfusions.
Treat the myelodysplasia.

Treatment of megaloblastic anaemia depends on the type of deficiency.
treat patients with both B12 and folate while awaiting results.
BUT: Folic acid may produce a haematological response in Vit B12 deficiency but may aggravate neuropathy.
Large doses of folate should not be used unless it is known that the Vit B12 levels are normal.
Treatment of B12 deficiency:
Correct dietary lack or Hydroxocobalamin 1000ug intramuscularly to a total of 5-6mg over a course of 3 weeks.
1000ug every 3 months for the rest of the patient's life.
Treatment of folate deficiency: 5mg folic acid daily orally for 3-4 months

Prognosis

Response to Therapy:

As the missing hemotinics are replaced,
- **RETICULOCYTES** should rise with 2-3 days
- Peak at 6-7 days
- **HEMOGLOBIN** should rise by 10g/L per week
- **Bone Marrow** should become **NORMOBLASTIC** in 48hrs

Epidemiology

- UNITED STATES:
  approximately 4% of men and 8% of women have hemoglobin values lower than 125 g/L

- **Internationally:**
  In *underprivileged countries, the prevalence of anemia is 2-5 times greater than in the United States.*
  nutritional factors with iron deficiency and, to a lesser extent, folic acid deficiency play major roles in the increased prevalence of anemia.

Populations with little meat in the diet have a high incidence of iron deficiency anemia because heme iron is better absorbed from food than inorganic iron.

Mortality/Morbidity:

- **The morbidity and mortality of anemias vary greatly depending on etiology.**
- Acute hemorrhage has variable mortality depending upon the site of bleeding
- (80% with aortic rupture, 30-50% with bleeding esophageal varices, approximately 1% with benign peptic ulcers).
- Anemia from gastrointestinal bleeding may be the first evidence of an intestinal malignancy.
- Hereditary spherocytosis may present with either a severe hemolytic anemia or be asymptomatic with compensated hemolysis.
- Similarly, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency may manifest as chronic hemolytic anemia or exist without anemia until the subject receives an oxidant medication.
- The 2-year fatality rate for severe aplastic anemia is 70% without bone marrow transplantation or a response to immunosuppressive therapy.
- Tolerance of anemia is proportional to the anemia's rate of development.
- Symptoms and mortality associated with **rapidly developing anemia are more profound** than in slowly developing anemia.

Race:

- Certain races and ethnic groups have increased prevalence of genetic factors associated with certain anemias.
- Examples are hemoglobinopathies, thalassemia, and G-6-PD deficiency.
- Each of these disorders has different morbidity and mortality in different populations due to differences in the genetic abnormality producing the disorder.
- For example, G-6-PD deficiency and thalassemia have less morbidity in African Americans than in Sicilians because of differences in the genetic fault.
- Conversely, sickle cell anemia has a greater morbidity and mortality in African Americans than among Saudi Arabians.
- Socioeconomic advantages are more prevalent among white individuals than individuals of other races.
- → THUS: decreased prevalence of nutritional anemias and anemia associated with chronic untreated illnesses.

Sex:

- **Overall, anemia is twice as prevalent in females than in males**.
- This difference is significantly greater during the childbearing years due to pregnancies and menses.
- Each healthy pregnancy depletes the mother of approximately 500 mg of iron.
- While a man must absorb about 1 mg of iron to maintain equilibrium, a premenopausal woman must absorb an average of 2 mg daily. Further, since women eat less food than men, they must be more than twice as efficient as men in the absorption of sufficient iron to avoid iron deficiency.
- Women have a markedly lower incidence of anemia from X-linked anemias such as G-6-PD deficiency and sex-linked sideroblastic anemias.

Age:

- During childbearing years, women are more likely to become iron deficient.
- Neoplasia increases in prevalence with each decade of life and can produce anemia from bleeding, replacement of bone marrow with tumor, or by developing anemia associated with chronic disorders.
- Use of aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), and coumadin increases with age and can produce gastrointestinal bleeding.

Pathophysiology

Life history of Erythrocytes: generation loss and destruction

RBCs: form out of **ERYTHROID** STEM CELLS under influence of **ERYTHROPOIETIN** mature through a number of divisions leading from erythroblasts to red cells. (IN **RED MARROW**)

- NORMALLY: production = destruction

WHEN BLOOD LOSS OCCURS production can be upregulated **7 FOLD:**

- This is done by
  - **expanding red marrow volume**
  - **skipping divisions** in maturation

120 day lifespan:

LIMITED BY

- continual loss of membrane components,
- accumulation of products of oxidated damage
- decreased deformability of the aged red cell leaving it unable to squeeze through the minute (1-2 micrometre) fenestrations in the splenic microvasculature.

In children, most bones are filled with **red bone marrow** (capable of erythropoiesis) **which is gradually replaced with fatty yellow bone marrow** except in the sternum, vertebrae, ribs, base of skull and the upper ends of long limb bones. **Yellow bone marrow is capable of reversion in times of need as are the liver and spleen (extramedullary haemopoiesis).** Erythropoiesis occurs in the bone marrow, at the rate of about 2-3 million a second.

DEATH:

phagocytosis by macrophages in reticuloendothelial system
in the **spleen, liver, bone marrow.**

Normal bone marrow function

Haemopoieses: takes place in foetal liver + spleen
After 7 months of gestation, moves to bone marrow

The **haemopoietic stem cell** is capable of self renewal and differentiation to

- erythroid,
- lymphoid
- myeloid

i.e. its PLURIPOTENT
its also morphologically identical to a small to intermediate sized lymphocyte.
WHEN ACTED UPON BY **GROWTH FACTORS**, the pluripotent cell commits to one lineage or the other.

Commitment is **irreversible**. Capacity to self-renew is lost.

Haemopoietic growth factors:
produced by cells in the marrows, all except
!! *Erythropoietin* (main source is the kidney)**!!**
Growth factors are in general stimulatory
although inhibitory factors also exist.

The microenvironment:

- **extra-cellular matrix (ECM)**

= fibronectin, laminin, collagen and proteoglycans.

- microvascular network of **thin walled venous sinusoids**
- **STROMAL CELLS:**

(include macrophages, fibroblasts, reticulum cells, fat cells and endothelial vascular cells)

FUNCTIONS:
Stromal cells are one important source of haemopoietic growth factors and other cytokines.
ADHESION of haemopoietic cells through adhesion molecules
(thus, regulation of where haemopoiesis happens by selective adhesion)

Some haemopoietic growth factors:

EPO (erythropoietin),
G-CSF (Granulocyte - Colony Stimulating factor)
GM-CSF (Granulocyte/Monocyte - Colony Stimulating Factor)

Other factors that are required for normal haemopoiesis (especially erythropoiesis) include:

- Metals - **iron, manganese, cobalt**
- Vitamins –
  vitamin B_{12}
  folic acid,
  Vitamin C, E, B_6 ,
  thiamine,
  riboflavin
  pantothenic acid
- Amino acids
- Hormones

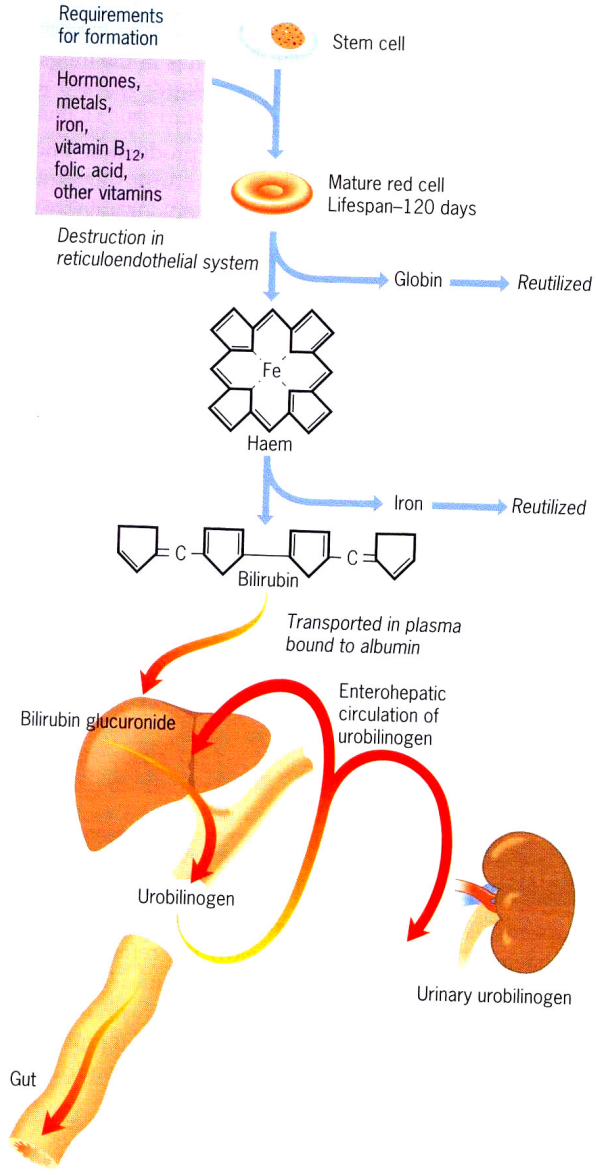
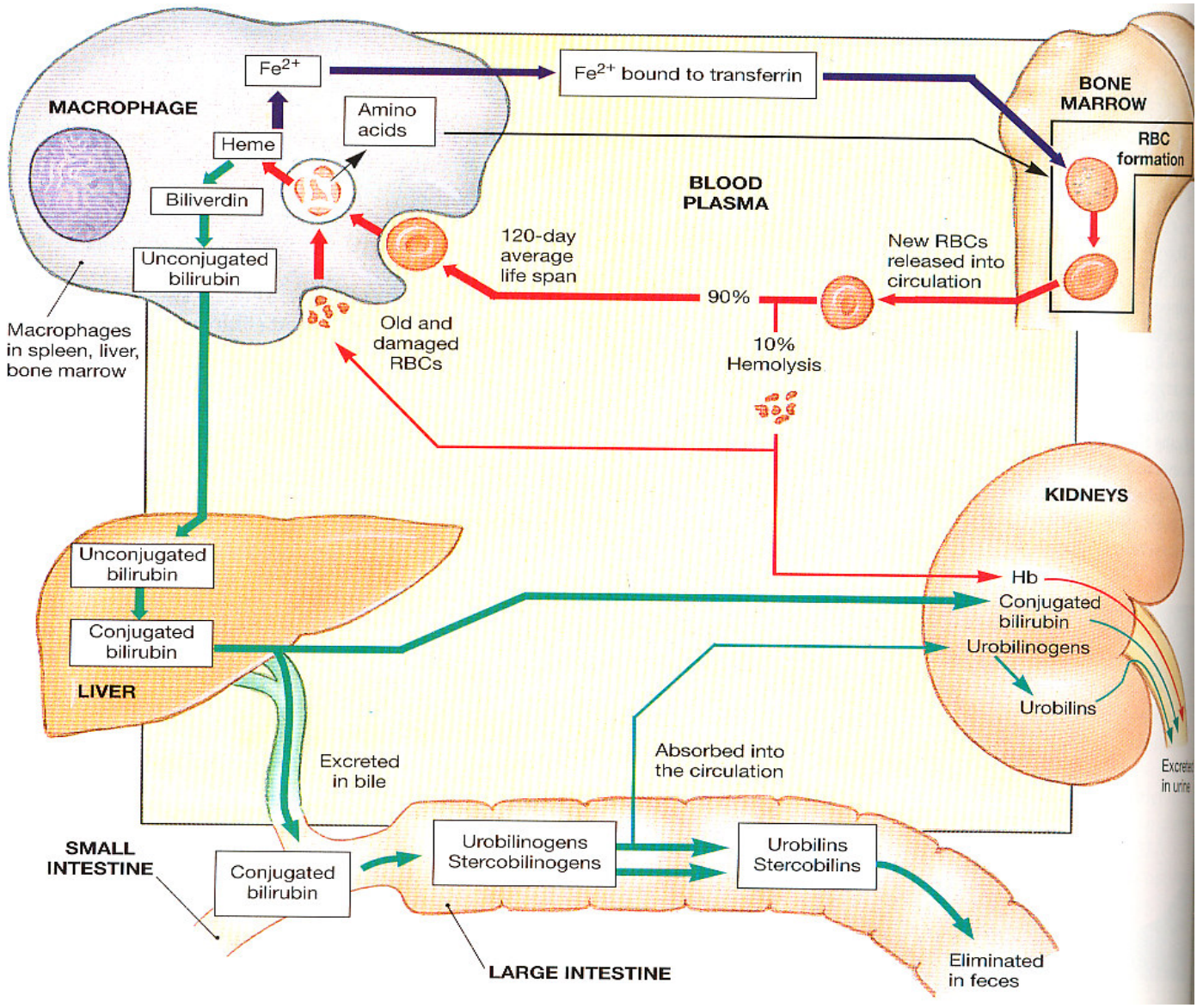


Fig. 8.5 **Red cell production and breakdown.**

The **most common** nutritional deficiency states that lead to alterations in haemopoiesis are **deficiencies of Vitamin B_{12} and folate and the mineral iron.**

The Traffic of Iron *from Martini et.al, "Anatomy and Physiology"*



Consequences of deficiencies of essential haematinics

ESSENTIAL HAEMATINICS:

- **iron,** manganese, cobalt, **vitamin B $_{12}$** , **folic acid**, Vitamin C, E, B $_6$,thiamine, riboflavin, pantothenic acid, amino acids and hormones

Lack of essential elements = ANAEMIA

Iron deficiency is the commonest cause of anaemia
due to:

- gastrointestinal blood loss from hookworm infestation.
- menstrual blood loss in fertile women. (considered almost "normal for age".)
  The diagnosis of iron deficiency which cannot be explained by obvious blood loss
  must be followed by a search for a source of occult bleeding
- is also common in infants and young children due to poor iron intake.

IRON DEFICIENCY ANAEMIA: DDX
The **laboratory hallmark of iron deficiency** is microcytosis:
Normal MCV is 80-100fl but in iron deficiency the MCV falls to under 80fl.
####################
IRON STORAGE STUDIES:
serum iron level,
serum transferrin (the iron carrying protein in the blood)
serum ferritin (the **best measurement** of overall iron stored in the body).

FOLIC ACID / B12 ANAEMIA: DDX
characterised by macrocytosis and specific appearances within the bone marrow (megaloblastosis).

- MCV is over 100fl
- blood film shows large red cells and variations is red cell size and shape.

may also be changes in the neutrophils which can show marked nuclear hypersegmentation.
Not all causes of macrocytosis induce megaloblastosis
Excess alcohol consumption for example is the commonest cause of macrocytosis in our community but is not associated with megaloblastic anaemia.

the lack of folic acid or vitamin B_{12} is **rather uncommon**. However it is important since as well as causing severe anaemia, deficiency of vitamin B_{12} can cause profound neurological damage. **Lack of vitamin B_{12} is most commonly caused by poor absorption from the bowel.**
This in turn is often due to an autoimmune disease known as pernicious anaemia. An antibody produced by the patient's own immune system destroys gastric cells which normally secrete a substance known as **intrinsic factor**. **Intrinsic factor must bind to vitamin B_{12} in the stomach for vitamin B_{12} to be absorbed in the terminal ileum.**

Folic acid absorption occurs in the jejunum and does not require intrinsic factor. Diseases affecting the small bowel (eg coeliac disease) or surgical removal of large segments of small intestine can therefore impair its absorption. Some groups of patients with poor diets eg the elderly, severely depressed individuals and adolescents can also become deficient from inadequate folic acid intake.

ERYTHROPOIETIN normally produced in the kidneys.
In renal failure the kidneys' ability to produce erythropoietin is impaired.

CANCER ARTHRITIS or INFECTION can mimic this sort of anaemia: some part of the immune response disables the release of iron from body stores.

- Tests in these patients reveal no evidence of iron deficiency and giving extra iron fails to improve the anaemia.
- The only effective therapy is to treat the underlying condition following which the anaemia spontaneously improves.

Premature destruction of red blood cells 4.01

ERYTHROCYTES LIVE 120 days: ANYTHING LESS IS HEMOLYSIS.
Lifespan measured directly by labelling RBCS with chromium-51, releasing them back into the blood stream and then sampling blood at intervals.
← NEVER ORDER THIS TEST.

Rapid falls in haemoglobin concentration can **ONLY** be the result of either **haemolysis or blood loss anaemia**.

SUSPECT HEMOLYSIS?? Look for:

- **scleral jaundice** should be sought (due to bilirubin)
- **SPLENOMEGALY,** when red cell destruction occurs in the spleen

laboratory parameters of hemolysis:

- increased **reticulocytes**
- increased **bilirubin** (due to the breakdown of the haem component of haemoglobin)
- **elevated serum levels of lactic dehydrogenase (LDH).** Contained within erythrocytes, released upon destruction
- **absence of a serum protein known as haptoglobin** which binds haem and prevents its loss through the kidney
- **examination of a well stained blood film for various morphological changes** which help in the diagnosis of haemolytic anaemia

Bone marrow examination may show **erythroid hyperplasis** (increased number of red cell precursors).
SUSPECT IMMUNE MECHANISM? → antiglobulin Coombs test

!! MECHANISM OF HAEMOLYSIS !!

- **Abnormalities of the red cell membrane** and is underlying cystoskeletal proteins which lead to loss of surface lipid and **spherocyte formation** (eg hereditary spherocytosis).
- **Enzyme deficiencies in the red cell**. The commonest enzyme deficiency is glucose 6 phosphate dehydrogenase so that the cell is unable to generate NADPH to counteract oxidant substances which are always present in out circulation. Excessive oxidant stress denatures haemoglobin and leads to red cell destruction.
- **Abnormalities of the haemoglobin molecule structure or synthesis** (usually inherited). Sickle cell anaemia and thalassaemia are typical examples.
- **Immune disorders with antibody-mediated red cell lysis** (thus labelled cells are destroyed during the circulation of the cell through spleen and liver.)

Aetiology: Mechanism of Pathogenesis: PERNICIOUS ANAEMIA

FAILURE of self-tolerance

Production of Autoimmune antibodies
To parietal cells @ stomach
(in 90% of pernicious anaemia)
BUT: also positive in 20% of unaffected relatives)

Or **INTRINSIC FACTOR** itself
(in 70% of affected patients)

Crohn's iliac Disease (destruction of the B12-absorbing cells)

B 12 = THE ONLY LIPID-INSOLUBLE VITAMIN which is stored in the liver

Autoimmune Gastritis:
CD 4+ T-cell mediated attack on parietal cells
- **IFN-gamma**
- **IL-2**
- **TNF**

DECREASED ABSORPTION OF Vitamin B12

Autoantibody neutralisation of intrinsic factor

Without B12 the body CANNOT DEMETHYLATE Methyl-TetrahydroFolate (methyl-THF) into Tetrahydrofolate (THF)
THUS:
DNA syhthesis is impaired

BIOCHEMICAL BACKGROUND (!! Boring !!)
DNA differs from RNA: **THYMINE** is used instead of **URACIL**
THUS: must convert uracil into thymine.
THEY ONLY DIFFER BY 1 METHYL GROUP (which thymine posesses);
FOLATE is required to attach this methyl group.
Vitamin B12 co-factors in this reaction.
FOLATE, through its many transformations, is converted into **H5-methyl-THF**; which is an inactive metabolite.
THIS IS THE "FOLATE TRAP"
The Only Way for H5-methyl-folate to escape is to be converted into **THF** by **Methyl-B12 (**using homocysteine, which incidentally gets turned into **methionine)**
THUS deficiency of B12 causes a build-up of the useless metabolite.
IF THF is NOT PRODUCED, it cannot be converted into the USEFUL N5,N10-methylene-THF which does the adding of the methyl group to uracil.
THUS: NO DNA FOR YOU.

Without B12 homocysteine cannot be reduced into methionine
a reaction in which folate also plays a role)

Accumulation of homocysteine leads to NEUROTOXICITY

Subacute combined degeneration of the DORSAL COLUMN of the spinal cord (major tract of proprioception, ascending into the primary somatosensory cortex)

NEUROPATHY:
- Unsteadiness of Gait
- Altered sensation in distal limbs
- Cannot touch fingers together with eyes closed

PLUS:
Vitamin B12 also co-factors a reaction of the Beta-Oxidation pathway of lypolysis (where fatty acids are broken down for the Krebs Cycle);
THIS PARTICULAR STEP deals with "odd-chain" fatty acids (i.e ones with 3, 5, 7 Carbon atoms);
THUS: if this step is disrupted by B12 deficiency, the odd-chain acids build up inside neurons, and cause untold harm

- **GLOBAL REDUCTION in production of rapidly dividing cells**
- **Eg. HEMOPOIESIS**

CELLS MATURE MORE SLOWLY
…but the normal absorption of haemoglobin which occurs during erythropoiesis is not disturbed; **THUS:** RBCs GORGE ON HEMOGLOBIN and grow large
→MACROCYTOSIS

MYELOCYTES also mature with difficulty
Thus you see **hypersegmented** nuclei in **neutrophils**

These gluttonous macrocytes rarely make it out of the bone marrow, and often get hemolysed within the marrow. This causes a total loss of circulating hemoglobin, and may result in an **elevated LDH**

Relevant anatomy

Bone marrow in adults is confined to the axial skeleton, although erythropoiesis can occur in practically any organ.

Table 1.1 Sites of haemopoiesis

Fetus	0–2 months (yolk sac) 2–7 months (liver, spleen) 5–9 months (bone marrow)
Infants	Bone marrow (practically all bones)
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

Biochemistry + Physiology

Vitamin B12 and the nervous system

vitamin B $_{12}$ appears to be involved in the synthesis of myelin
(a lipoprotein synthesised by glial cells)

When the vitamin is deficient, fatty deposits accumulate patchily in the myelin and coalesce, the largest fibres often being most affected.

THUS the conduction of action potentials is slowed, high frequency information cannot be sustained and ultimately transmission is completely blocked.

Patients describe tingling ("pins and needles" or paraesthesias) in their hands and feet, often symmetrically (a "glove and stocking" distribution).

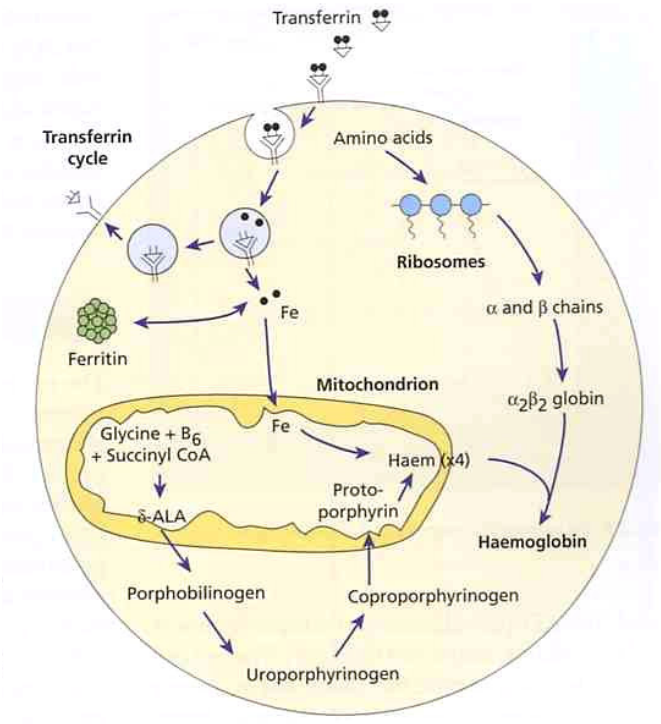
Symptoms due to the slowing and asynchrony of action potentials in sensory neurones.
Numbness or loss of some sensation is associated, demonstrated by neurological testing.
Motor weakness is evident and, in the long term,
wasting of peripheral muscles may occur.
Stretch reflexes are diminished in the affected regions.

A vitamin B $_{12}$ deficiency often affects two major pathways in the white matter of the spinal cord, a "combined degeneration".

- One pathway carries discriminative sensory information from the body surface, joints and muscles to the brain (the dorsal or posterior columns); the cell bodies of these bipolar neurones lie in the dorsal root ganglia.
- The second (**the lateral corticospinal tract**) transmits voluntary signals from the motor cortex to motoneurones projecting directly to muscles from the spinal cord. The affected individual experiences **sensory difficulties with a loss of the sense both of the position of the legs and feet (proprioception) particularly in the dark, and of vibration**.

The motor disturbances are manifested as **unsteadiness when walking**, due both to the damage to the descending motor pathway and the loss of ascending sensory feedback. In the absence of significant peripheral neuropathy, the stretch reflexes are exaggerated, motor tone is increased and the **Babinski sign is present**, all signs of damage to motor pathways from the brain to the motoneurones.
Some **demyelination is sometimes also seen in pathways in the brain, leading to confusion, depression, moodiness, memory losses and even overt psychosis**.
The cerebral manifestations, resembling dementia, usually *yield rapidly to appropriate treatment with vitamin B $_{12}$* .
Recovery from demyelination and associated axonal damage, however, is usually slow, particularly when lesions are longstanding.

The structure of normal haemoglobins



Each molecule of normal adult haemoglobin (Hb) A consists of 4 polypeptide chains $\alpha_2\beta_2$, the **globin** portion, each with its own haem group, four iron-containing, non-protein nitrogenous groups, which is bound to the polypeptide.
Each haemoglobin can combine with 4 O2 molecules.

- Normal adult blood also contains quantities of two other haemoglobins , HbF and HbA_2.
- There is a switch between fetal and adult haemoglobin 3-6 months after birth.
- Haem synthesis largely occurs in the mitochondria.
- As the haemoglobin molecules load and unload O2, the individual globin chains move on each other.
- The $\alpha_1\beta_1$ and $\alpha_2\beta_2$ contacts stabilise the molecule. The β chains slide on the $\alpha_1\beta_1$ and $\alpha_2\beta_2$ contacts during oxygenation and deoxygenation.
- When O2 is unloaded, the β chains are pulled apart, permitting the entry of the metabolite
- 2,3-diphosphoglycerate (2,3-DPG) resulting in a lower affinity of the molecule O2.
- This movement is responsible for the sigmoid form of the haemoglobin O2 dissociation curve.

! Foetal Hb has a higher O_2 affinity than maternal Hb which facilitates the transfer of O_2 from the maternal circulation.

As O2 is poorly soluble in blood,
98.5% is carried on haemoglobin, the other 1.5% making the PO_2.

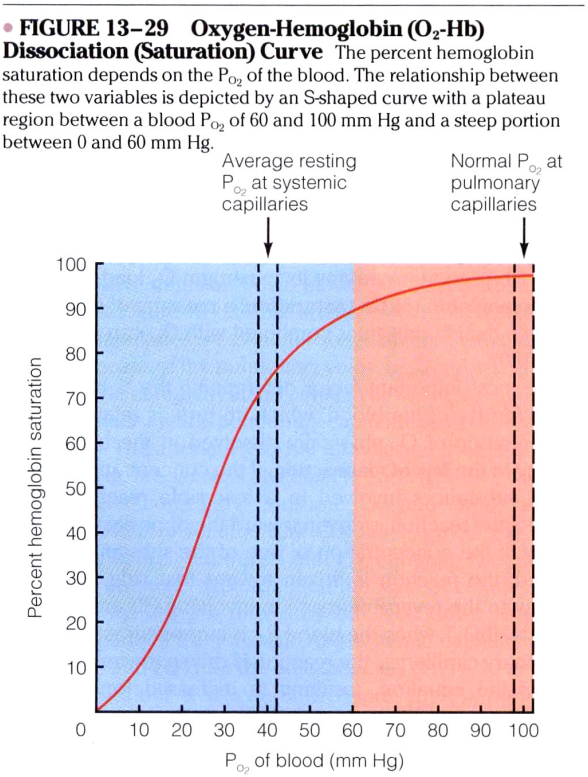


FIGURE 13–29 Oxygen-Hemoglobin (O_2-Hb) Dissociation (Saturation) Curve The percent hemoglobin saturation depends on the P_{O_2} of the blood. The relationship between these two variables is depicted by an S-shaped curve with a plateau region between a blood P_{O_2} of 60 and 100 mm Hg and a steep portion between 0 and 60 mm Hg.

The plateau part of the curve ensures that Hb remains largely saturated until PO_2 has dropped below 60mmHg. **THUS NO OXYGEN IS DISMISSED FROM HEMOGLOBIN IN THE ARTERIES**

This is still well above the partial pressure in the venous system, and therefore allows for imperfections in ventilation.

The steep part of the curve enables the O2 to be unloaded in the capillary system where the PO_2 is approximately 40mmHg.

The Hb in the blood returning to the lungs is still typically 75% saturated.
If the PO_2 is lower, more will be released – there is a reserve.

This transition in affinity for oxygen is achieved through changes in the conformation of haemoglobin induced by allosteric effectors, small molecules which bind at other sites on this protein. The normal position of the disassociation curve depends on the concentration of 2,3-DPG, H^+ ions, temperature and CO2 in the red cell and on the structure of the haemoglobin molecule. High concentrations, high temperature or the presence of sickle haemoglobin shift the curve to the right (O2 given up more easily) whereas foetal haemoglobin (Hb F) (which is unable to bind 2,3-DPG) shift the curve to the left.

The shift to the right of the curve in increased concentrations of CO2 (and hence H^+) assists with the unloading of O2 in areas where CO2 is being produced (metabolism). In areas of increased metabolism, the production of lactic acid and heat also pushes the curve to the right.
The influence of CO2 and H+ on the curve is called the **Bohr effect.**
Both CO2 and H+ are able to bind to the haemoglobin at sites other than the O2 binding sites.
The result is an alteration in the structure of Hb that reduces its affinity for O2.

These effects are reversed at the lungs. The acid forming CO2 is blown off, the blood cooled and the curve shifts to the left, facilitating the loading of O2.

2,3-DPG is produced *within the cell* by RBC metabolism. DPG production gradually increases when Hb in the blood is chronically undersaturated, that is whenever arterial HbO2 is below normal. This condition may occur in individuals living in high altitudes, or suffering from circulatory or respiratory diseases of anaemia. The negative side of DPG production is that it decreases Hb ability to load O2 at the lungs.

10% of CO2 is transported physically dissolved,
30% bound to haemoglobin
60% as HCO_3^-

The enzyme **carbonic anhydrase** helps convert CO2 to bicarbonate ion (HCO_3^-).
Therefore the RBCs both carry CO2 and convert it to HCO_3^-.

$$CO_2 + H_2O = H_2CO_3 = H^+ + HCO_3^-$$

BOHR EFFECT:
CO_2 and acidic pH induces the release of oxygen by hemoglobin

HALDANE EFFECT:
Removal of oxygen from hemoglobin increases its ability to scavenge CO_2

The fact that the removal of O2 from Hb increases the ability of Hb to pick up CO2 and CO2 generated H+ is known as the **Haldane effect.**
The Haldane and Bohr effects work in synchrony to faciliate O2 liberation and CO2 and H+ uptake at tissues.

Cyanocobalamin, Folate and Iron absorption

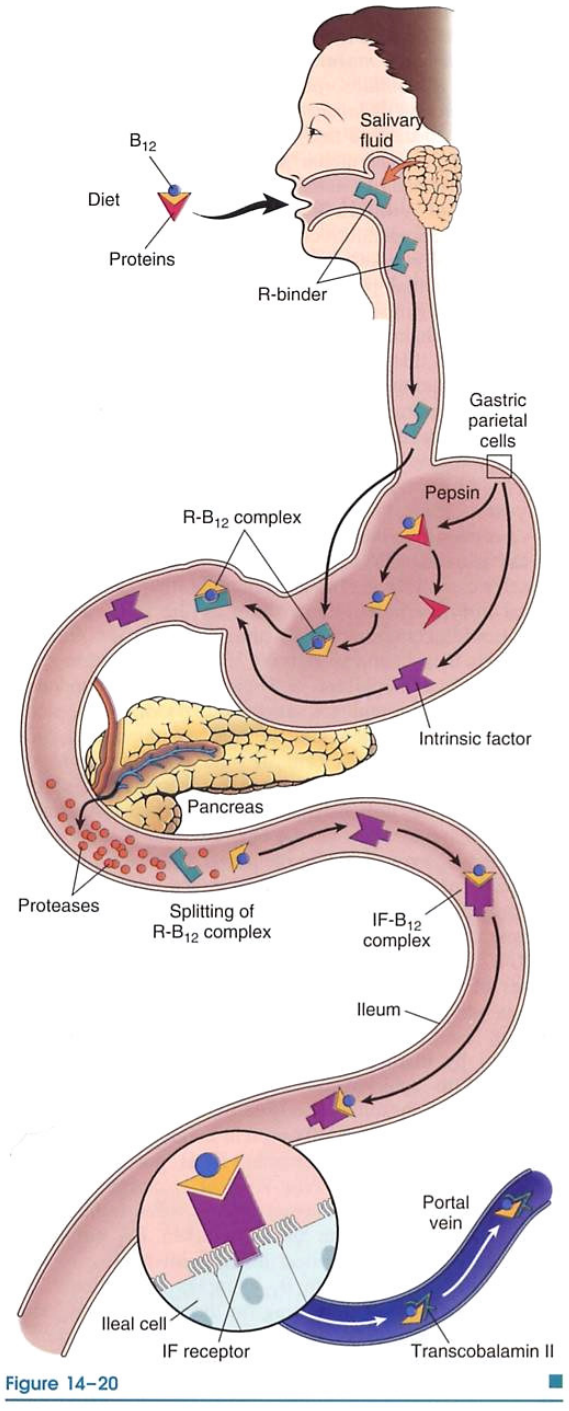


Figure 14–20

Schematic illustration of vitamin B_{12} absorption.

Vitamin B12 is received solely in the diet, animal products and dirt bacteria being the sources.

Iron:

The average western diet takes in about 10-15mg of iron a day. Of this 5-10% is typically absorbed which can be raise to 20-30% in pregnancy or anaemia. The best sources are haem sources.

Dietary iron is deconjugated by peptic enzymes and HCl in the stomach and transported to the early part of the duodenum where the soluble iron complexes are absorbed.

Only a small amount comes from the diet, absorbed through the duodenum and jejunum.

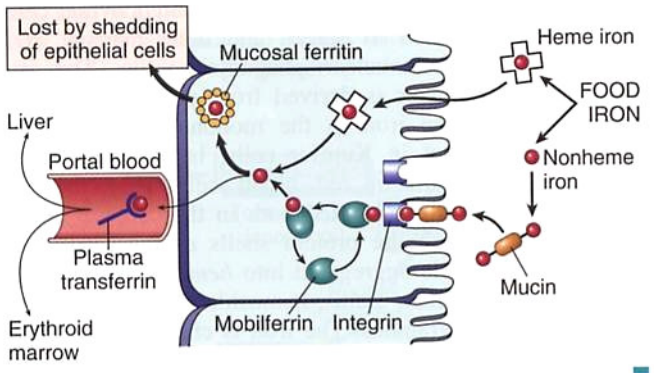


Figure 14–24

Diagrammatic representation of iron absorption. Mucosal uptake of heme and nonheme iron is depicted. Not illustrated is the iron transporter protein Nramp2 that is involved in the passage of iron across the mucosal cell membrane. When the storage sites of the body are replete with iron and erythropoietic activity is normal, most of the absorbed iron is lost into the gut by shedding of the epithelial cells. Conversely, when body iron needs to be increased or when erythropoiesis is stimulated, a greater fraction of the absorbed iron is transferred into plasma transferrin, with a concomitant decrease in iron loss through mucosal ferritin.

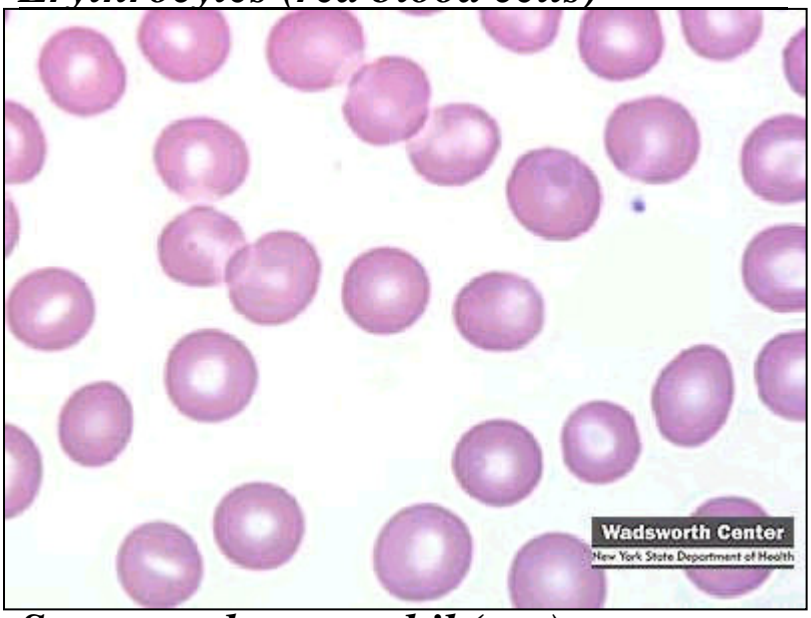
The body regulates absorption according to its iron needs

FACTORS HINDERING OR FAVOURING ABSORPTION

Factors favouring absorption	Factors hindering absorption
Haem iron	Inorganic iron
Ferrous form (Fe^{2+})	Ferric form (Fe^{3+})
Acids	Alkalis – antacids or pancreatic secretions
Solubilizing agents (sugers, AAs)	Precipitating agents
Iron deficiency	Iron excess
Increased erythropoiesis	Decreased erythropoiesis
Pregnancy	Infection
Hereditary haemochromatosis	Tea
Increased expression of DMT-1 and ferroportin in duodenal enterocytes	Decreased expression of DMT-1 and ferroportin in duodenal enterocytes

NORMAL HISTOLOGY OF BLOOD CELLS: a hymn to the peripheral blood

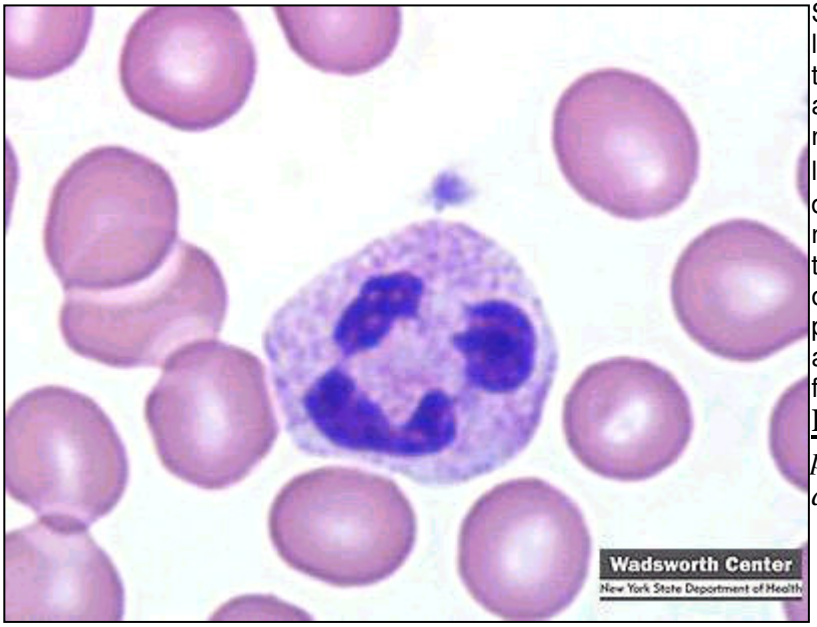
Erythrocytes (red blood cells)



The mature red blood cell (rbc) consists primarily of hemoglobin (about 90%). The membrane is composed of lipids and proteins. In addition, there are numerous enzymes present which are necessary for oxygen transport and cell viability. The main function of the red cell is to carry oxygen to the tissues and return carbon dioxide from the tissues to the lungs. The protein hemoglobin is responsible for most of this exchange. Normal red blood cells are round, have a small area of central pallor, and show only a slight variation in size.

A normal red cell is 6-8 µm in diameter. As the relative amount of hemoglobin in the red cell decreases or increases, the area of central pallor will decrease or increase accordingly.

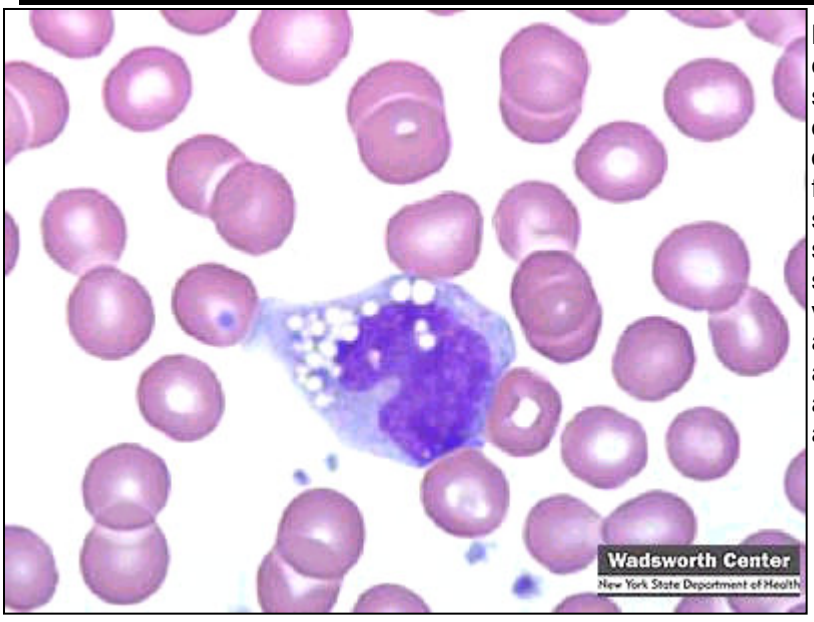
Segmented neutrophil (seg)



Segmented neutrophils (polymorphonuclear leukocytes, or segs) are the mature phagocytes that migrate through tissues to destroy microbes and respond to inflammatory stimuli. Segmented neutrophils comprise 40-75 % of the peripheral leukocytes. They are usually 9 to 16 µm in diameter. The nuclear lobes, normally numbering from 2 to 5, may be spread out so that the connecting filaments are clearly visible, or the lobes may overlap or twist. The chromatin pattern is coarse and clumped. The cytoplasm is abundant with a few nonspecific granules and a full complement of rose-violet specific granules.

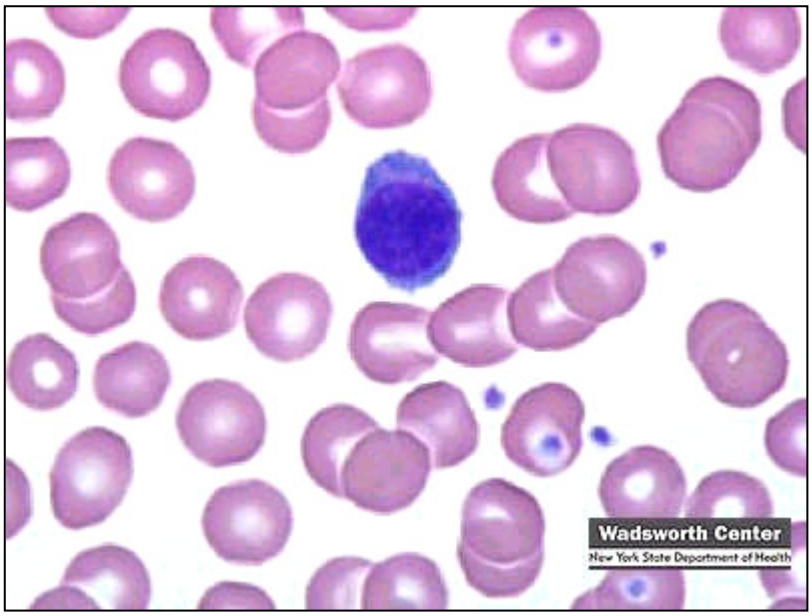
HyperSegmentation of the nucleus *is a pathognomic marker for megaloblastic anaemia*

Monocyte (Macrophage)



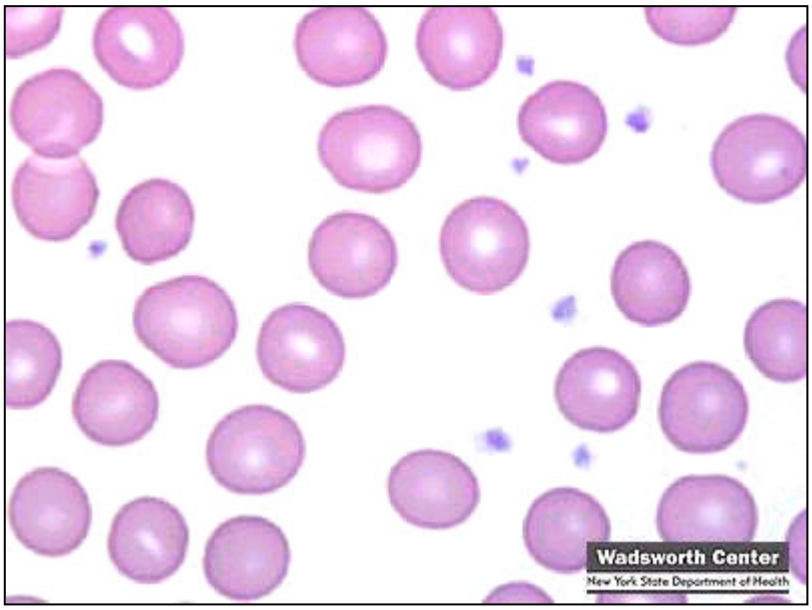
Monocytes are large mononuclear phagocytes of the peripheral blood. They are the immature stage of the macrophage. Monocytes vary considerably, ranging in size from10 to 30 µm in diameter. The nucleus to cytoplasm ratio ranges from 2:1 to 1:1. The nucleus is often band shaped (horseshoe), or reniform (kindey-shaped). It may fold over on top of itself, thus showing brainlike convolutions. No nucleoli are visible. The chromatin pattern is fine, and arranged in skein-like strands. The cytoplasm is abundant and blue gray with many fine azurophilic granules, giving a ground glass appearance. Vacuoles may be present

Lymphocyte



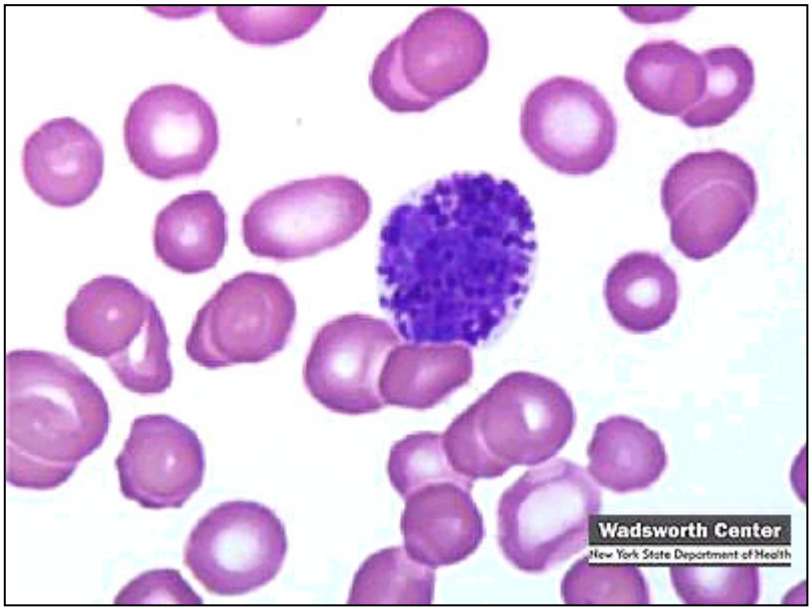
Lymphocytes in the peripheral blood have been described on the basis of size and cytoplasmic granularity. Small lymphocytes are the most common, ranging in size from 6 to 10 µm. The nucleus is usually round or slightly oval, occasionally showing a small indentation due to the adjacent centrosome. Except in the smallest cells, the nucleus is about 7 µm in diameter, a size that has been convenient for estimating the size of the surrounding erythrocytes. Nuclear chromatin stains a dark reddish-purple to blue with large dark patches of condensed chromatin. The nuclear cytoplasm ratio is 5:1 to 3:1, and the cytoplasm is often seen only as a peripheral ring around part of the nucleus.

Platelets



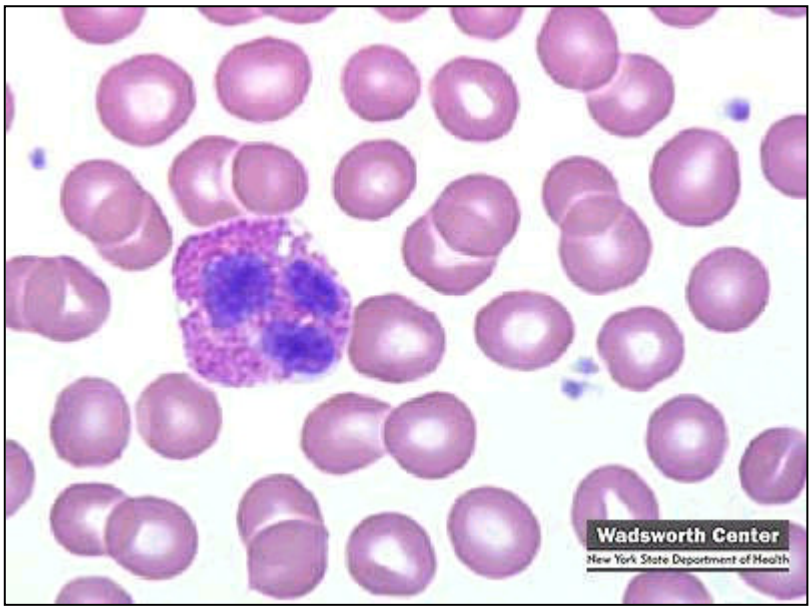
megakaryocytes, circulating as small discs in the peripheral blood. They are responsible for hemostasis (the stoppage of bleeding) and maintaining the endothelial lining of the blood vessels. During hemostasis, platelets clump together and adhere to the injured vessel in this area to form a plug and further inhibit bleeding. Platelets average 1 to 4 µm in diameter. The cytoplasm stains light blue to purple, and is very granular. There is no nucleus present. Normal blood concentrations range from 130,000 to 450,000/µL.

Basophil



Basophils are granulocytes that contain vasoactive compounds. They comprise approximately 0.5% of the total leukocyte count. Basophils participate in immediate hypersensitivity reactions, such as allergic reactions to wasp stings, and are also involved in some delayed hypersensitivity reactions. Basophils are the smallest circulating granulocytes, averaging 10 to 15 µm in diameter. The nucleus to cytoplasm ratio is about 1:1, and the nucleus is often unsegmented or bilobed, rarely with three or four lobes. The chromatin pattern is coarse and patchy, staining a deep blue to reddish-purple. The cytoplasm is a homogenous pale blue, but this is often obscured by the large dark granules.

Eosinophil



Eosinophils are the mature granulocytes that respond to parasitic infections and allergic conditions. Eosinophils comprise about 1 to 4% of the peripheral leukocytes. They are usually 9 to 15 µm in diameter. Granules stain a bright reddish-orange with Wright's or Giemsa stains. The nucleus contains one to three lobes. The chromatin pattern is coarse and clumped. The cytoplasm is abundant with a full complement of bright reddish-orange specific granules.

Behavioural Sciences: Caring for the Extremely Old

Carelink: referral serivice; call regarding any aspect of aged care

Community Care Packages

Specificity eg.

- General
- Dementia-specific
- Multivultural
- Aboriginal

Veterans Home Health care: 20 days or 196 hrs max

Community Nurse: **for personal care, housework and shopping**

A Long Term Carer needs care too:

To relieve the pressure, a Residential Respite service is available for short periods
Carer Information and Support Groups
Housework services eg. lawn-mowing
Long Term Carer Allowance = $85 per 2 weeks