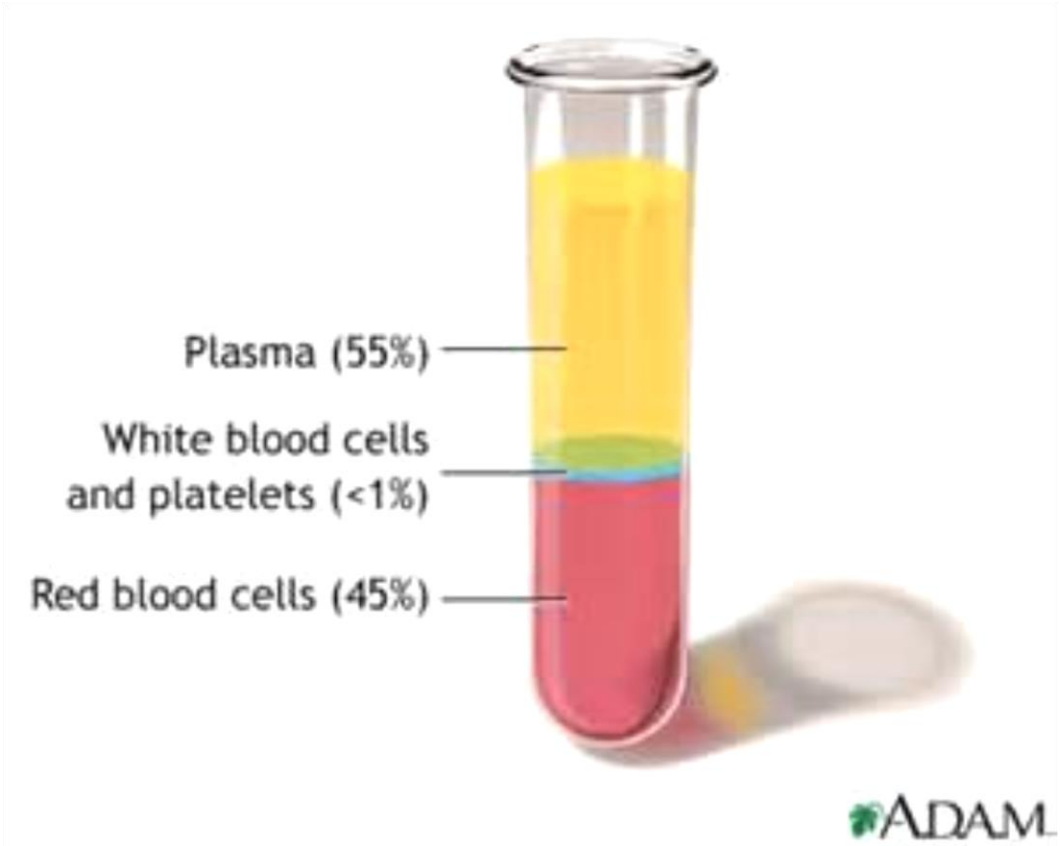
Components of blood

- Blood –collection of fluid and cells
- Fluid component- plasma
- Cells- red cells , white cells and platelets

Components of blood

- Separation of components
- Collect blood sample in to a tube and centrifuge it .
- Cells go to bottom
- Liquid component remains on top of the cell column

- Components of blood



Components of blood

- Red cells- 45%
- Plasma – 55%
- White cells –less than 1%

- Plasma contains clotting factors
- Removal of fibrin and clotting factors from plasma results serum

- Packed cell volume _ haematocrit (PCV-Hct)
  - Height of red cell column as a percentage of total column
  - Done by using Winthrob tube and anticogulted blood
- Rate depends on
  - Number o red cells in plasma
  - Negative charge on red cells
  - Presence of ibrin and **rouleaux** formation

ESR

- Erythrocyte sedimentaion rate
- is the rate at which red blood cells sediment in a period of one hour
- Done using anticoagulated blood and wetergen tube

Anaemia

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Department of Physiology

Erythropoiesis

- Red cell production happens in the bone marrow
- Red cell precursors pass through several stages
- Progressively the precursors mature in to red cells
  - Contain less RNA
  - More haemoglobin

Erythropoiesis

- Erythropoiesis is controlled by hormone erythropoietin
  - Secreted mainly by the kidney-90%
  - liver -10%
- Erythropoietin secretion is mainly regulated by the tissue oxygen tension
- Hypoxia for any reason stimulates erythropoietin secretion

Erythropoiesis

- Factors needed for normal erythropoiesis
  - Iron
  - Vitamin B 12
  - Folate
  - Vitamins- B6 , Thiamine, Riboflavin, Vit C and E
  - Micronutrients – Cobalt
  - Androgens
  - Thyroxin

Red Cell Indices

- Mean corpuscular Volume- MCV (fL)
  - The most useful one to classify anaemia

  = HCT X 10/ Red blood cell count(10^6/microL)
- Mean corpuscular haemoglobin -MCH – (pg)

  =Hb X 10/ Red blood cell count(10^6/microL)
- Mean corpuscular haemoglobin concentration -MCHC (g/dL)

  =Hb X 100/ Hct

Red Cell Indices
Normal Values

Hb	Male- 14.0-17.7g/dl Female- 12.0-16.0g/dl
MCV	80-96fl
MCH	27-33pg
MCHC	32-35 g/dl

- Extramedullary erythropoiesis
  - Erythropoiesis happening in other site except bone marrow
  - Usually the liver and the spleen
  - Results enlargement of the liver and the spleen
  - I.E hepatomegaly and splenomegaly

Anaemia

- Decrease in the level of haemoglobin in the blood below the reference level for the age and the sex of the individual.

- Common features
  - Malaise, lethargy, tiredness, exertional dyspnoea

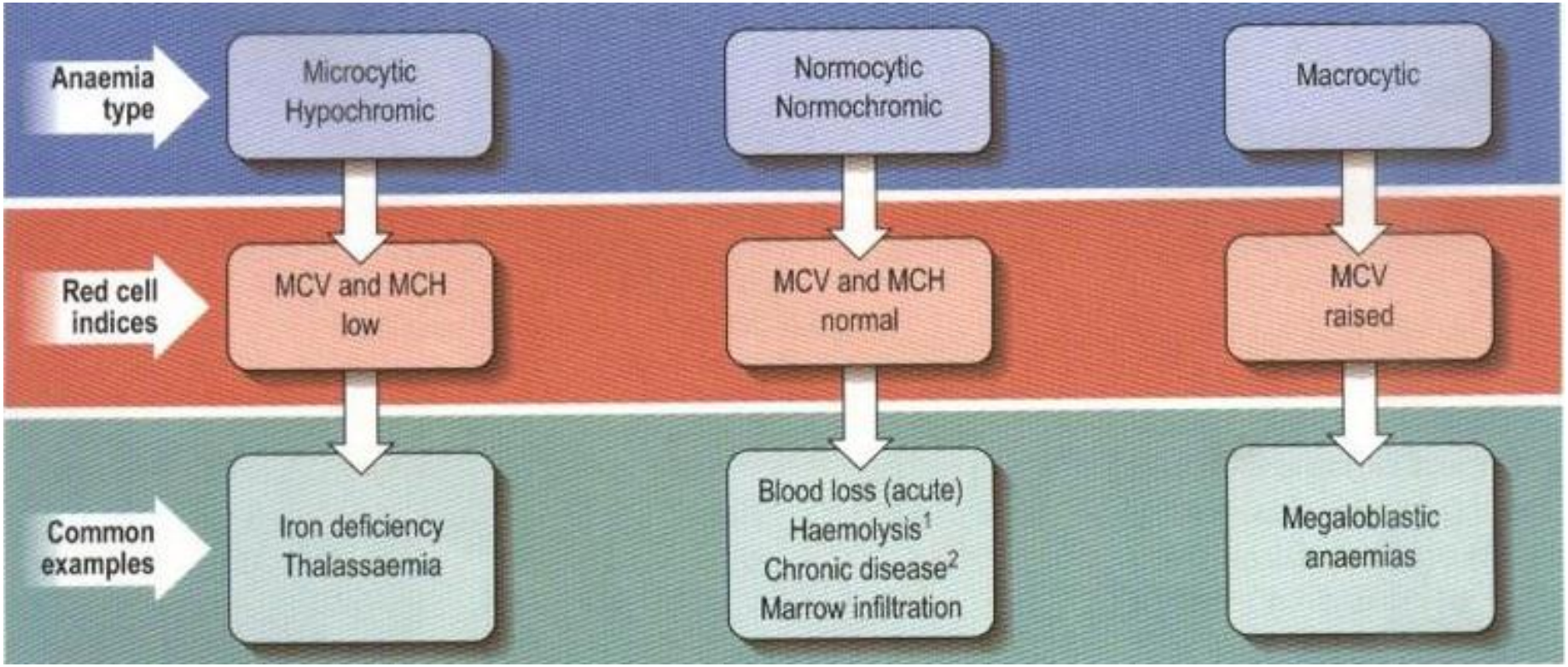
Classification of anaemia

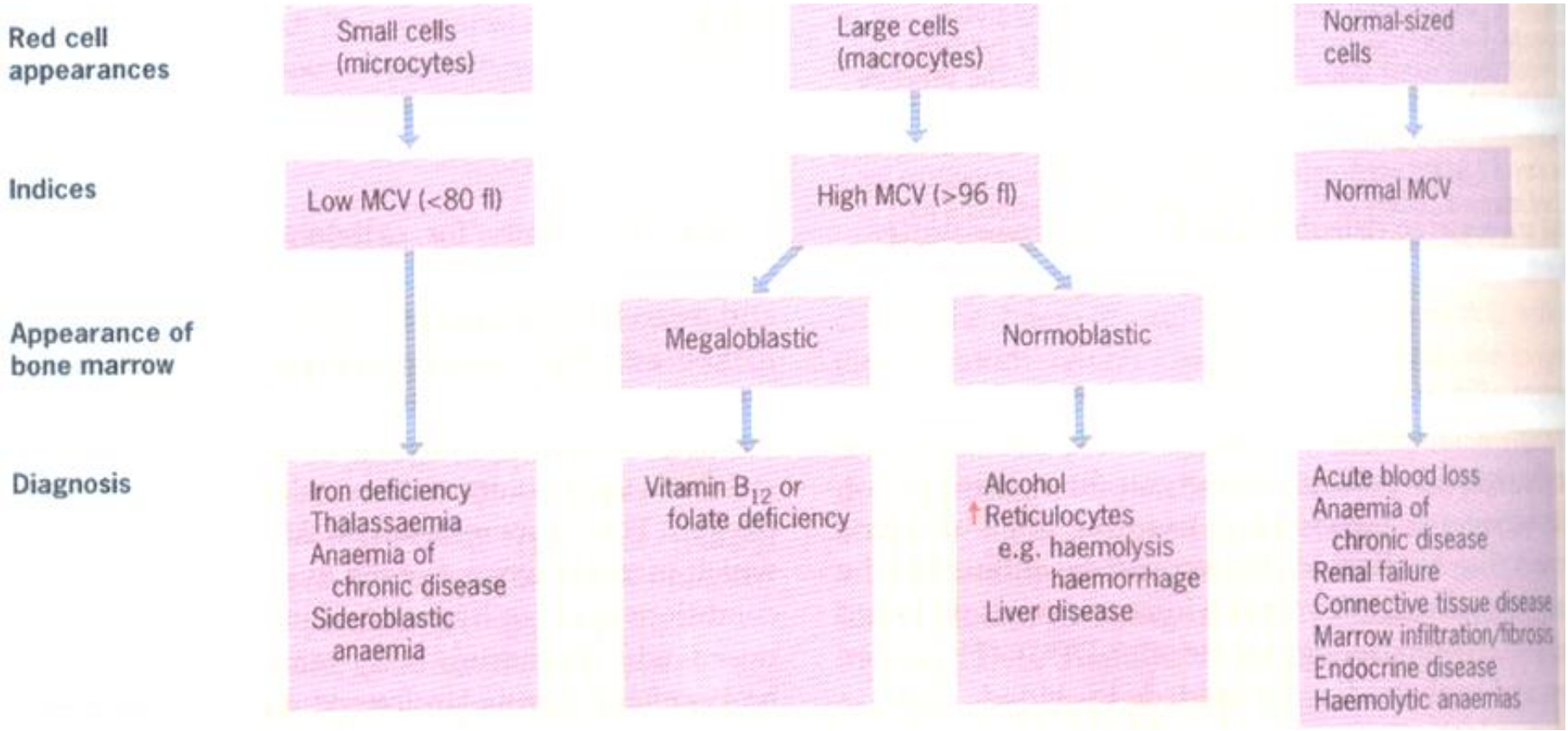
- According to MCV
  1. hypochromic microcytic
  2. normochromic normocytic
  3. macrocytic
- According to basic pathology
  1. Decreased production - marrow failure
  2. Increased loss- chronic bleeding
  3. Increased destruction - haemolysis

	Normal	
	Microcyte	Iron deficiency, Haemoglobinopathies
	Macrocyte	Megaloblastic anaemia, Liver disease, Hypothyroidism
	Target cell	Iron deficiency, Haemoglobinopathies
	Spherocyte	Hereditary Spherocytosis
	Sickle cell	Sickle cell disease
	Pencil cell	Iron deficiency, Haemoglobinopathies
	Ecchinocyte	Liver disease
	Acanthocyte	Renal failure, liver disease

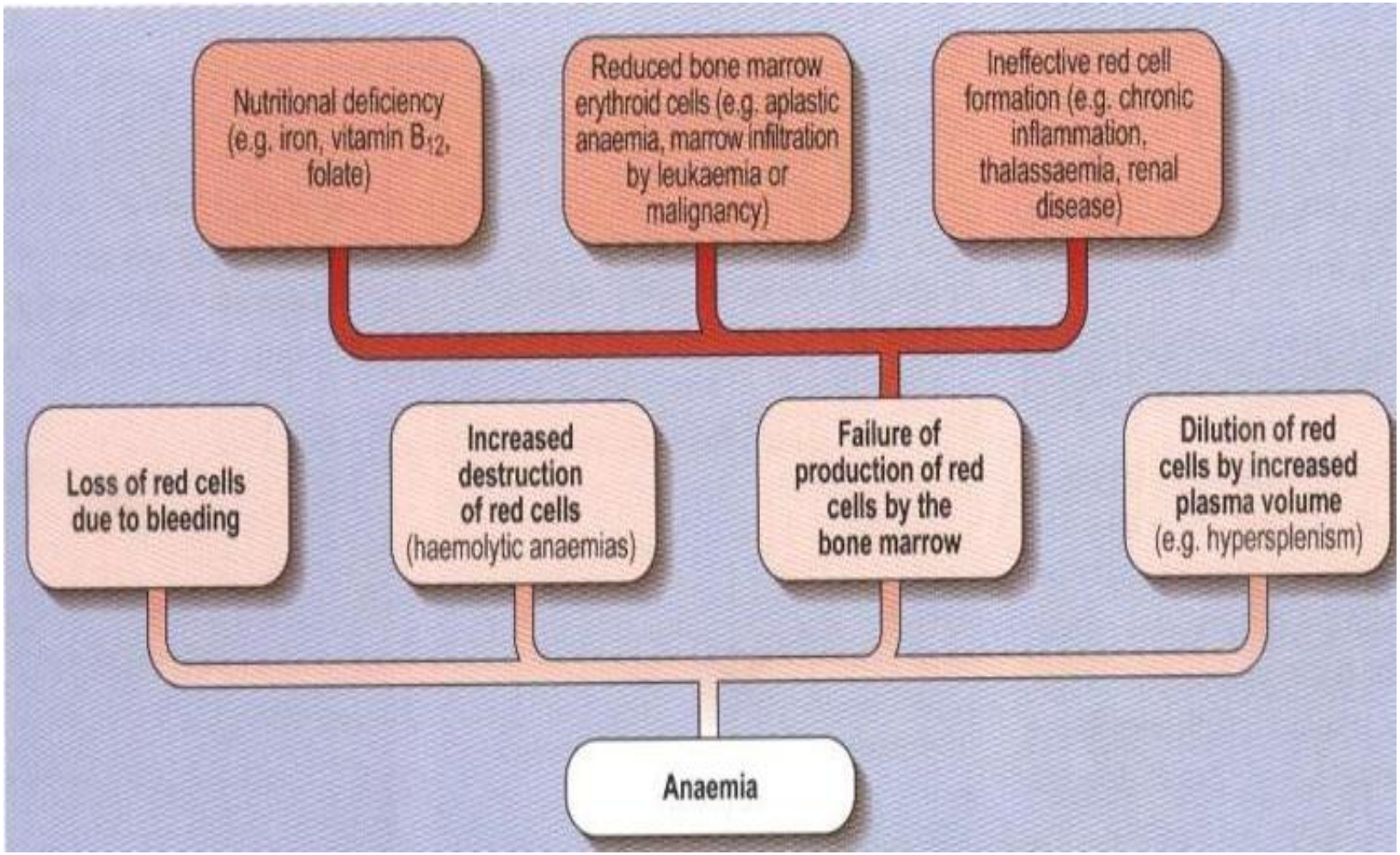
	Stomatocyte	Liver disease, Alcoholism
	Eliptocyte	Hereditary eliptocytosis
	Fragmented cell	Disseminated intravascular coagulation
	Tear drop cell	Extramedullary haemopoiesis, Myelofibrosis

Classification of anaemia




Red cell appearances
Small cells (microcytes)
Large cells (macrocytes)
Normal-sized cells
Indices
Low MCV (<80 fl)
High MCV (>96 fl)
Normal MCV
Appearance of bone marrow
Megaloblastic
Normoblastic
Diagnosis
Iron deficiency
Thalassaemia
Anaemia of chronic disease
Sideroblastic anaemia
Vitamin B_{12} or folate deficiency
Alcohol
↑ Reticulocytes
e.g. haemolysis
haemorrhage
Liver disease
Acute blood loss
Anaemia of chronic disease
Renal failure
Connective tissue disease
Marrow infiltration/fibrosis
Endocrine disease
Haemolytic anaemias

Classification of anaemia



Hypochromic microcytic anaemia

- Key features in red cell indices
  - Low MCV
  - Low MCH
- Causes
  - Iron deficiency
  - Haemoglobinopathies
    - Thalassaemia

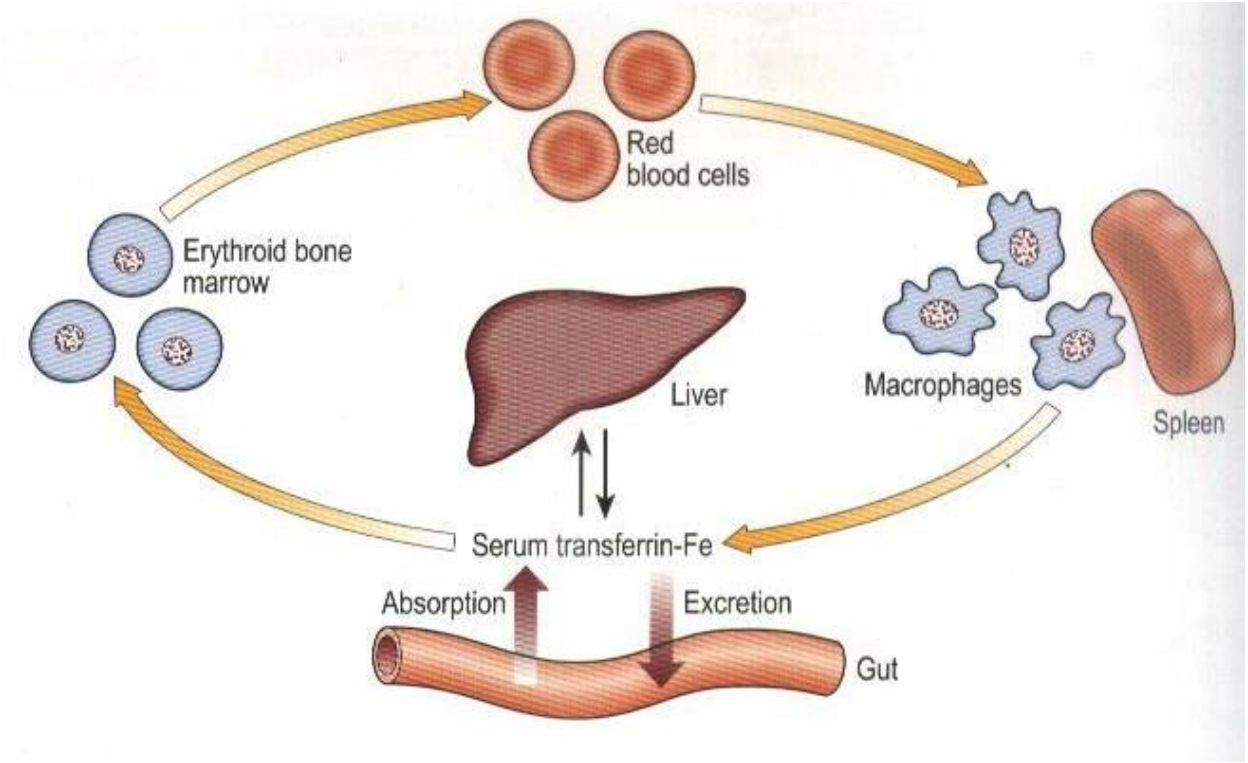
Hypochromic microcytic anaemia

- Iron deficiency anaemia
  - Commonest cause
  - Result of
    - Limited ability of the body to absorb iron
    - Frequent increased loss
- Iron is needed for Hb synthesis

Iron deficiency anaemia

- Causes
  - Chronic blood loss
  - Increased demand
  - Poor dietary intake
  - Decreased absorption

Iron deficiency anaemia



Iron deficiency anaemia

- Chronic blood loss
  - Mostly form the GI tract
    - Hook worm infestation
    - Carcinoma (cancer large bowel)
  - Form abnormal menstruation in females
- Increased demand
  - Growth and pregnancy
- Poor dietary intake
- Decreased absorption
  - Post gastrectomy

Iron deficiency anaemia

- Features
  - Feature s of anaemia
    - Malaise, lethargy, tiredness, exertional dyspnoea
  - Specific to iron deficiency
    - Spoon shaped nails- koilonychia
    - Brittle nails
    - Angular stomatitis
    - Glossitis – atrophy of tongue papillae

Iron deficiency anaemia

Koilonychia, brittle nails

Glossitis

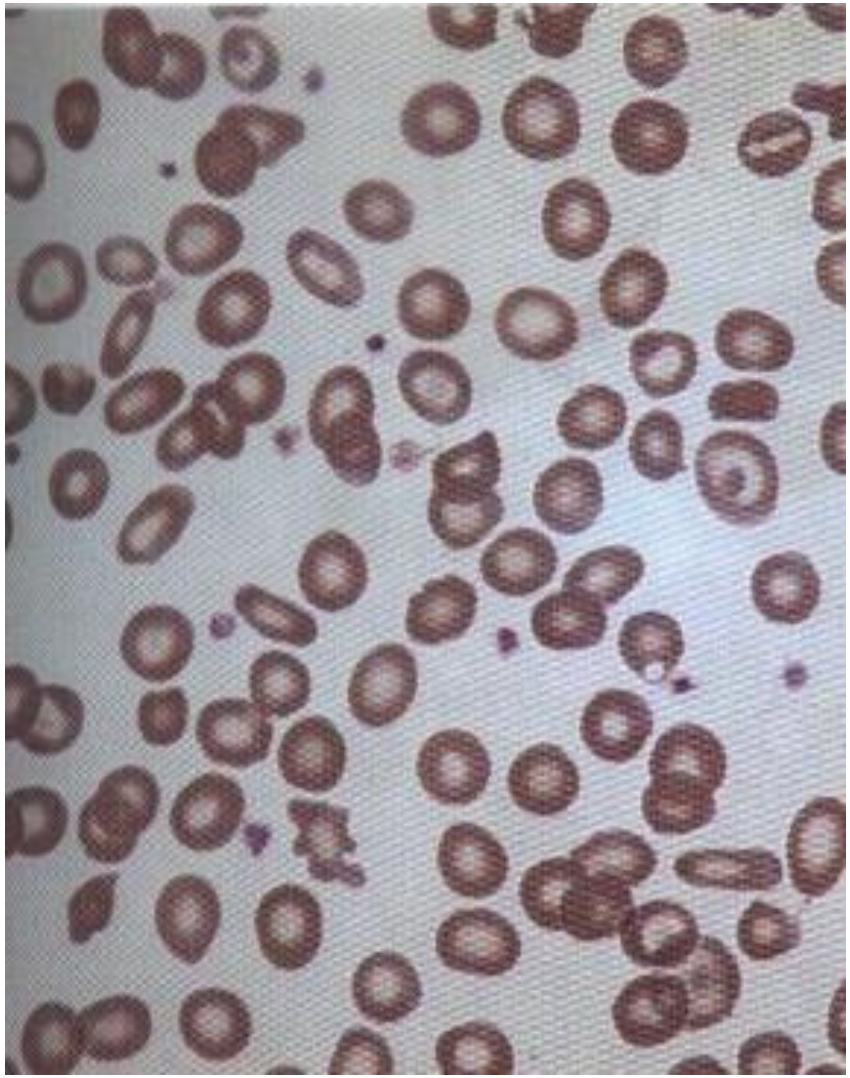
Iron deficiency anaemia

- Investigations
- Hb -low – less than reference value
- MCV – less than 80fl
- MCH- less than 27
- Blood film
- Hypochromic microcytic
- Bone marrow-
  - Erythorid hyperplasis
  - Negative for Perl's reaction- No Prussian-blue granules
- Iron studies

Iron deficiency anaemia

- Iron studies
- Serum iron – low
- Iron binding capacity – TIBC- high
- Serum ferritin- low
  - Reflects the stored iron

Iron deficiency anaemia



Normochromic normocytic anaemia

- Seen in chronic diseases
  - Chronic infections, malignancies
  - Major organ failures
  - Endocrine disodres
    - Hypoadrenalism, hypopituitarism
- Key features in red cell indices
  - Low Hb
  - Normal MCV, MCH

Macrocytic anaemia

- Two forms
- Key features
  - Low Hb
  - High MCV >100
- 1. Megaloblastic
- 2. non Megaloblastic

Megaloblastic anaemia

- Characterised by presence of megaloblasts in the bone marrow
- Megaloblasts
  - Erythroblasts with delayed maturation
  - Due to defective DNA synthesis
  - Large and have immature nuclei

Megaloblastic anaemia

- Occurs in
  - Vitamin B 12 deficiency
  - Folate deficiency
- Investigations
  - Hb- Low
  - MCV – high >100fl
  - Blood film- Macrocytes and hypersegmented neutrophis
  - Bone marrow- megaloblasts

Megaloblastic anaemia

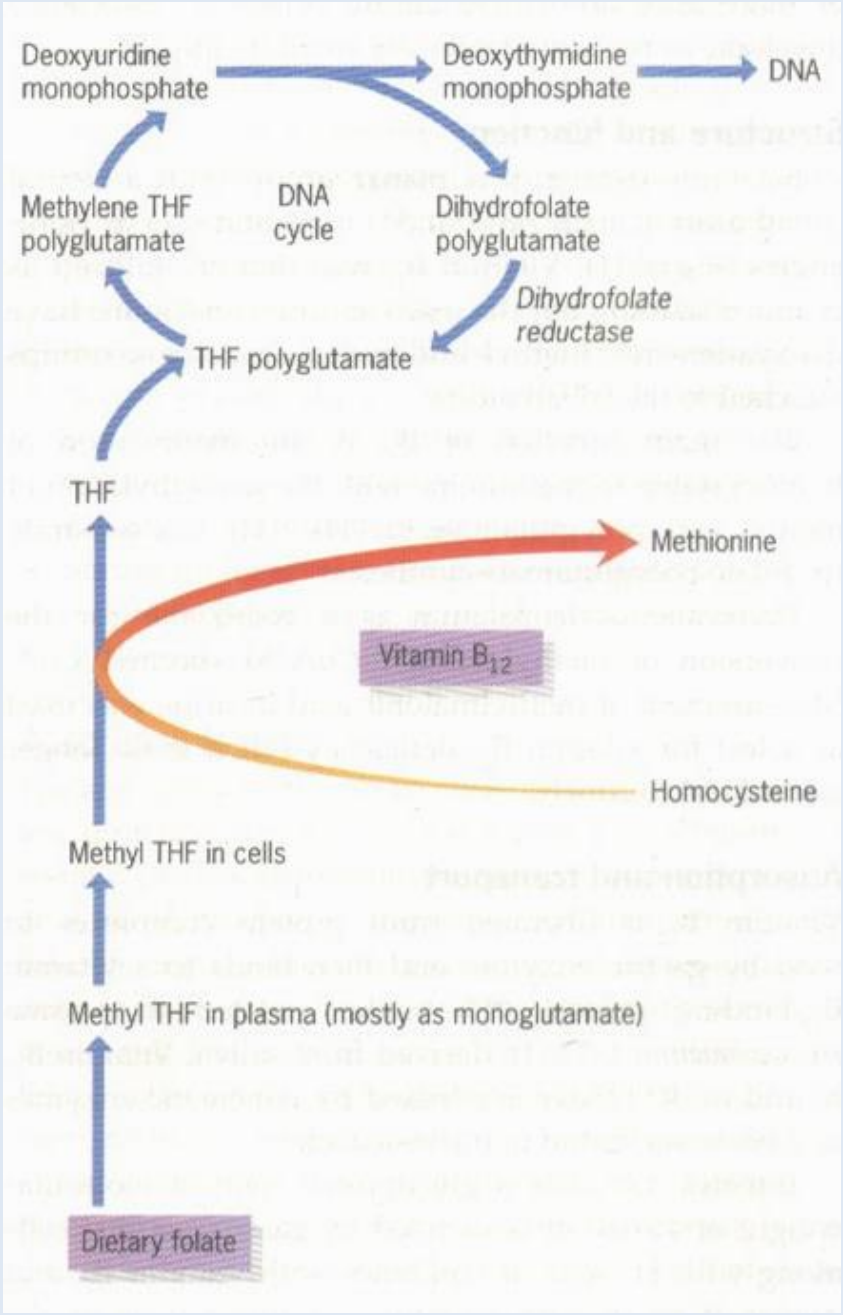
- Biochemical basis
  - Read biochemistry

Block in DNA synthesis

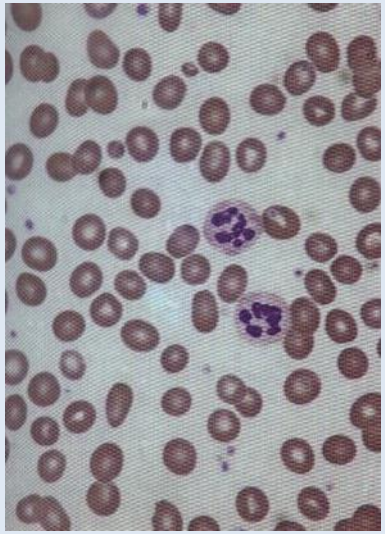
Due to lack of methyl group

Methyl group supplied by tetra hydrofolate

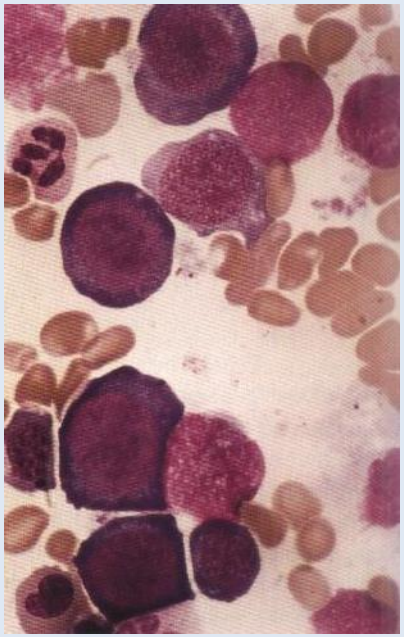
Vitamin B12 deficiency also reduces the formation of tetra hydrofolate

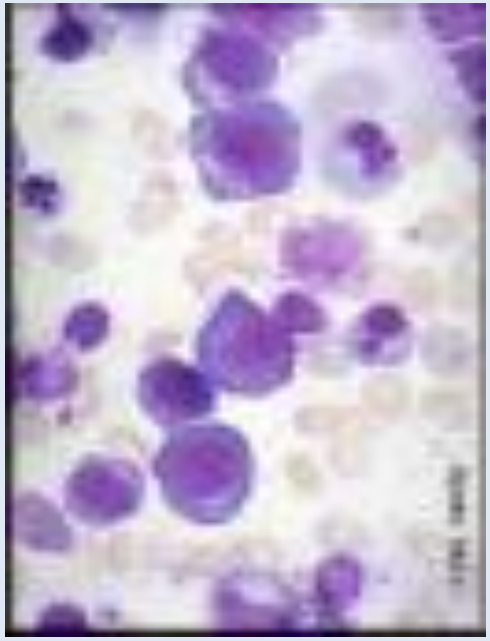

Deoxyuridine monophosphate
Deoxythymidine monophosphate
DNA
Methylene THF polyglutamate
DNA cycle
Dihydrofolate polyglutamate
Dihydrofolate reductase
THF polyglutamate
THF
Methionine
Vitamin B_{12}
Homocysteine
Methyl THF in cells
Methyl THF in plasma (mostly as monoglutamate)
Dietary folate

Megaloblastic anaemia



Macrocytes with hyper-segmented neutrophils





Megaloblasts in the bone marrow

Megaloblastic anaemia

- Vitamin B12 deficiency
- Vitamin B12
  - Synthesised by microbes
  - Humans depend on animal sources
  - Average adult store 2-3mg – mainly in liver
  - Daily loss is small
  - Sufficient for about 2 years –before deficiency sets in

- Vitamin B12
- Structure
  - a cobalamin with a central cobalt atom
- Function
  - Co enzyme for methylation of homocystine to methionine

- Vitamin B 12
- Absorption
  - Liberated from the protein complexes in food by gastric enzymes and HCl
  - Then binds to two B12 binding proteins
    - Intrinsic factor and R binder
  - Vit B12 bound to R factor is released by pancreatic enzymes and get bounds to intrinsic factor
  - IF carries Vit B12 to terminal ileum
  - Terminal ileum mucosa has specific receptors for B12
  - Vit B12 enters in to the cells and IF remains in the lumen

- Vitamin B12
- Transport
- Binding proteins
  - transcobalamin I,II ,III
- Transcobalamin I
  - Main binding protein
  - Unable to deliver B12 to marrow
- Transcobalamin II
  - Transport Vit B12 from the enterocytes to bone marrow
- Transcobalamin III
  - Binding protein unable to deliver B12 to marrow

Megaloblastic anaemia

- Vit B12 deficiency
- Causes
  - Vegans – low intake
  - Impaired absorption
    - Pernicious anaemia
    - Gastrectomy –lack of intrinsic factor
    - Ileal disease or resection
    - Bacterial overgrowth
- Pernicious anaemia
  - Atrophy of gastric mucosa
  - Lack of intrinsic factor

Megaloblastic anaemia

- Vit B 12 deficiency -Features
- Anaemia
- Involvement of the central nervous system
  - Peripheral nerve damage
  - Posterior column damage
  - Pyramidal tract damage
  - Dementia

Megaloblastic anaemia

- Haematological findings
  - Low Hb
  - High MCV
  - Macrocytes in blood film
  - Megaloblasts in bone marrow
  - Low serum B 12 levles
  - Abnormal Schilling test

Megaloblastic anaemia

- Folate deficiency
- Folic acid
  - Tetrahydro folate acts as coenzyme
  - Transfers of single carbon units in DNA synthesis
  - Found in green vegetables
  - Cooking destroys folate

Megaloblastic anaemia

- Folate deficiency
  - Poor dietary intake
  - Excess alcohol
  - Increased utilization
    - Pregnancy, lactation
  - Haematological diseases
    - Haemolysis, malignancies with high cell turn over
  - Anti Folate drugs

Megaloblastic anaemia

- Haematological findings
  - Low Hb
  - High MCV
  - Macrocytes in blood film
  - Megaloblasts in bone marrow
  - Low serum and red cell folate

Megaloblastic anaemia

- Treatment
- Vit B12 or folate therapy
- Folic acid alone must not be used to treat megaloblastic anaemia
  - Coexisting Vit B12 results worsening of the neurological disease

Macrocytic anaemia with out megaloblasts

- Raised MCV
- No megaloblasts in the marrow
- Causes
  - Alcohol excess
  - Liver disease
  - Hypothyroidism

Haemolytic anaemia

- Increased destruction of the red cells
- Sites of haemolysis
  - Extra vascular
    - In the reticuloendothelial system
    - Mostly in the spleen

Haemolytic anaemia

- Evidence for haemolysis
  - Elevated serum unconjugated bilirubin
    - Liver has about 7 more times capacity than normal for conjugation
    - Therefore mild elevation of unconjugated bilirubin
    - Mild jaundice
  - Increased urinary urobilinogen/stercobilinogen
    - Dark coloured urine on standing
    - Dark coloured stools

Haemolytic anaemia

- Inherited haemolytic anaemias
- Causes
  - Red cell membrane defects
    - Hereditary Spherocytosis
  - Haemoglobinopathies
    - Quantitative abnormalities
    - Qualitative abnormalities
  - Metabolic disorders of the red cells

- Haemoglobinopathies
  - Quantitative abnormalities
    - Normal structure
    - Abnormal levels of either alpha or beta chains
      - Alpha or beta Thalassaemia
  - Qualitative abnormalities
    - Abnormal structure
      - Sickle cell disease

- Metabolic disorders of the red cells
  - G 6PD deficiency
  - Pyruvate kinase deficiency

Haemolytic anaemia

- Red cell membrane defect
- Hereditary Spherocytes
  - Autosomal dominant disease
  - Deficiency of spectrin in the red cell membrane
  - Surface to volume ratio decreases
  - Cells become spherocytes
  - Spherocytes are rigid and less deformable
  - Unable to pass through the spleen

Hereditary Spherocytes

- Features
  - Anaemia
  - Mild unconjugated janundice
  - Splenomegaly
  - Foot ulcers

Hereditary Spherocytes

- Investigations
  - Low Hb
  - Spherocytes in the blood film
  - Increased osmotic fragility

- Osmotic fragility test
  - When red cells placed in increasing hypotinic solutions
  - Red cells take water , swell up
  - Eventually lyse releasing Hb to solution
  - Spherocytes can not tolerate hypotonicity as normal red cells
  - Therefore increased osmotic fragility

Read practical manual

Haemoglobinopathies

- Quantitative Haemoglobinopathies
- Hb molecule structure is normal
- There is reduction of alpha or beta chains
- Reduction of
  - Alpha chains – alpha Thalassaemia
  - Beta chains – beta Thalassaemia

Thalassaemia

	Type of haemoglobin	**Structure**
Normal	HbA_1 92%	$\alpha_2\beta_2$
	HbA_2 2%	$\alpha_2\delta_2$
	HbF <1%	$\alpha_2\gamma_2$
β thalassaemia	Increased HbA_2, HbF	
Alpha thalassaemia Major	Hb Barts	γ_4
Alpha thalassaemia	HbH	β_4

beta Thalassaemia

- Autosomal recessive inheritance
- Normally there is 1:1 production of alpha and beta chains
- In beta thalassaemia beta chain production is reduced
- Excess of alpha and gamma chains precipitates in erythroblasts
- Results haemolysis
- Ineffective erythropoiesis

beta Thalassaemia

- Excess alpha chains combine with whatever
  - Beta, gamma and delta chains available
- Results
  - Increased HbF , HbA_2,
  - Combination of 4 gamma chains- Hb Barts
  - Hb Barts precipitates in the erythroblasts
  - Leading to haemolysis and ineffective erythropoiesis
  -

beta Thalassaemia

- Syndromes
  - Thalassaemia major
  - Thalassaemia trait
- Thalassaemia major
  - Found in Homozygous individuals
  - Severe form of disease
  - Present during first year of life
  - Needs recurrent blood transfusions
  - Leads to iron over load

beta Thalassaemia

- Thalassaemia trait (minor)
  - Found in heterozygous individuals
  - Mild form of disease
  - Usually go unnoticed
  - Iron studies are normal

beta Thalassaemia

- Investigations
  - Low Hb
  - Low MCV – disproportionate to degree of anaemia
  - Low MCH
  - Hypochromic microcytic cells
  - Lots of target cells
  - Increased serum iron , ferritin, low TIBC in major thalassaemia
  - Hb electrophoresis- low HbA1 , high HbF

Alpha Thalassaemia

- Reduction in alpha chain production
- Excess of beta and gamma chains
- Severe form
  - No alpha chains
  - Accumulation of gamma chains- γ_4 – Hb Barts

Haemoglobinopathies

- Qualitative Haemoglobinopathies
  - Normal amounts
  - Abnormal structure
    - HbS –Sickle cell disease
    - HbC, HbD, HbE diseases

Sickle cell disease

- Single base mutation
- Substitution of valine for glutamine
- Deoxygenated HbS
  - Insoluble and polymerize
  - Flexibility of the cells reduced
  - Cells become rigid and assumes sickle shape
- Sickling is produced by
  - Hypoxia, infections, acidosis and dehydration

Sickle cell disease

- Investigations
  - Low Hb
  - Positive sickling test
  - Increased HbS in Hb electrophoresis

Metabolic red cell disorders

- G6PD deficiency
  - Unable to tolerate oxidant stress
  - Results rapid intravascular haemolysis
    - Anaemia
    - Jaundice
    - Haemoglobinuruia

G6PD deficiency

- Causes
  - drug induced – eg. cholroquine
  - Ingestion of fava beans