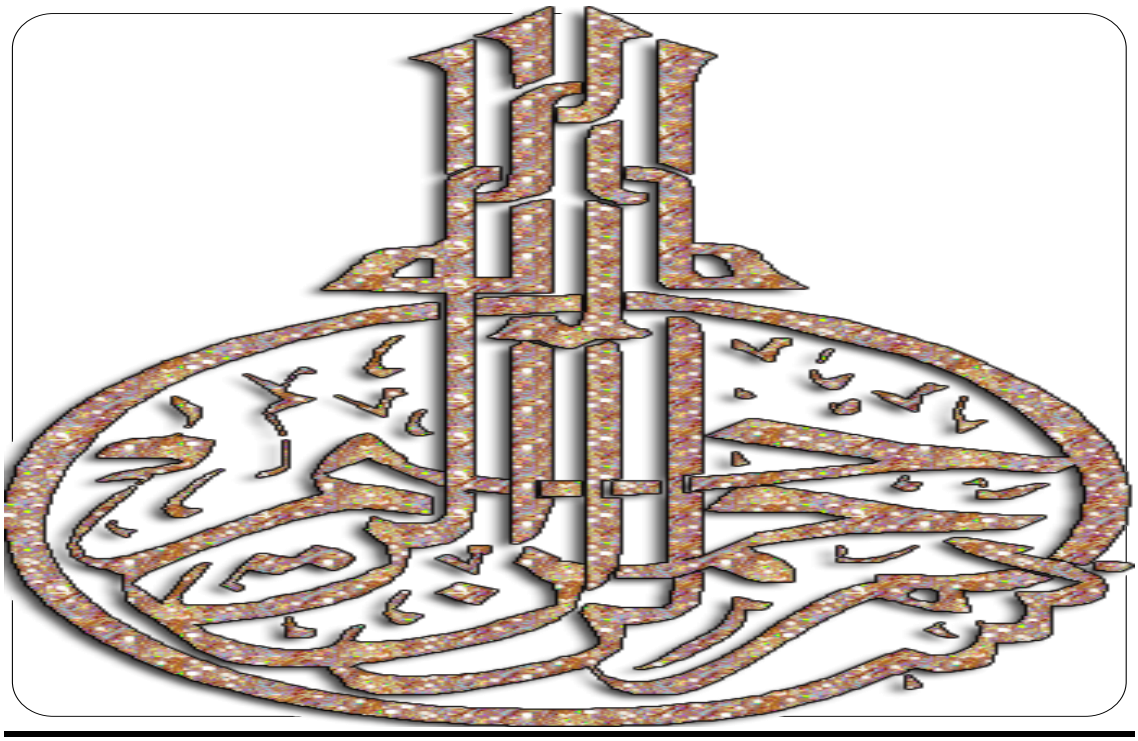
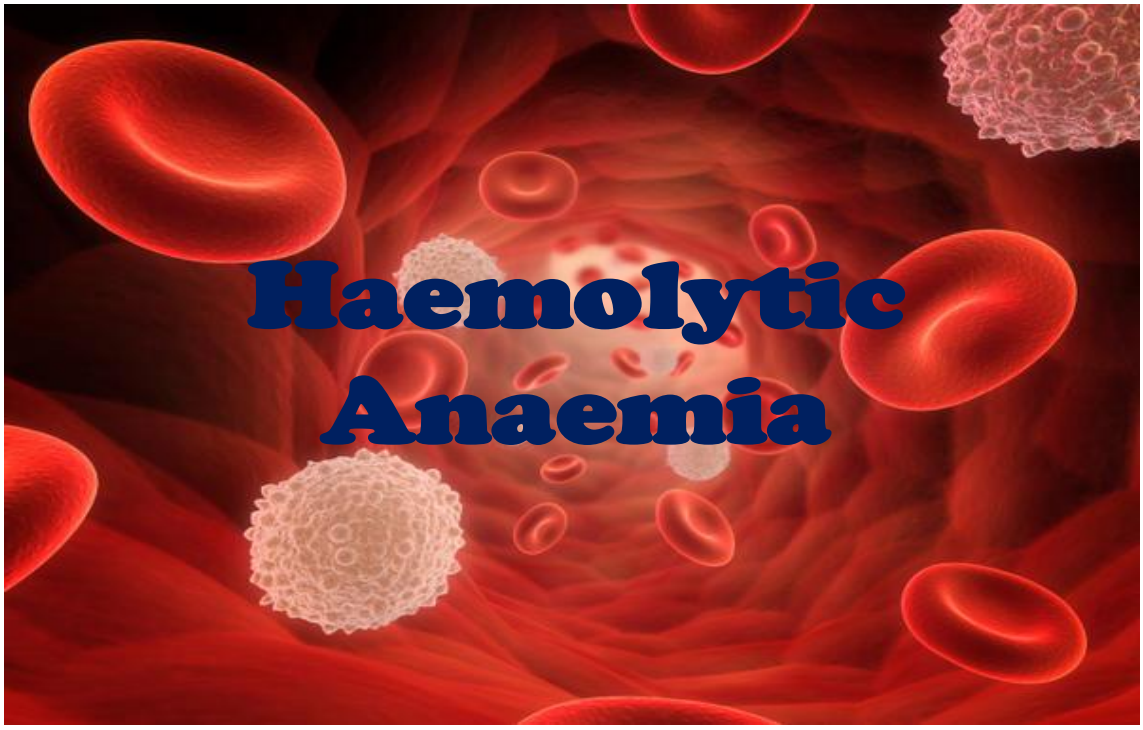




Prof. Dr. SHEREEN MOHAMED EL-HOSEINY
Professor of Clinical and Chemical Pathology

Haemolytic Anaemia

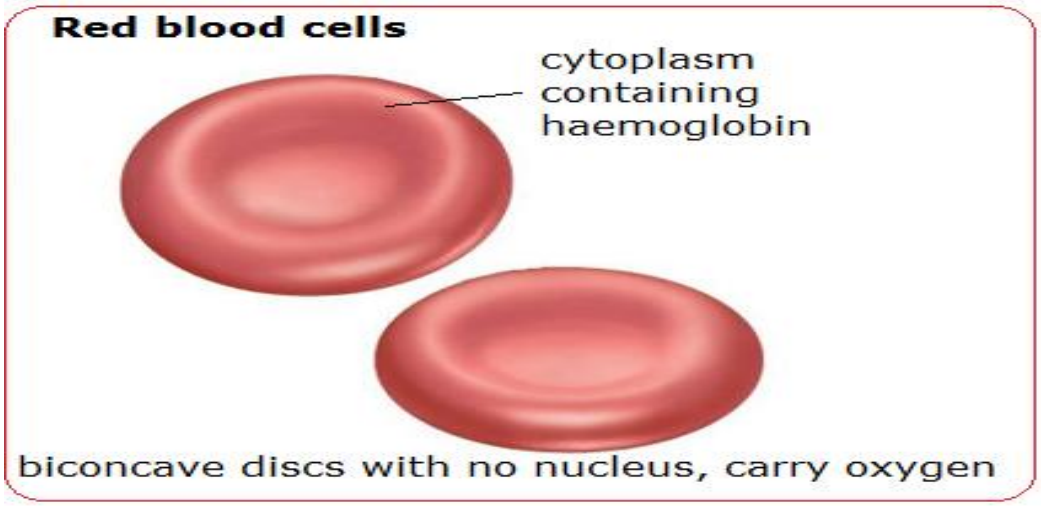
Introduction:

RBCs:

- Non – nucleated cell e' simple constriction.
- Has non-oxidative metabolism (no mitochondria).
- Adapted to its special function.
- Its main function is to carry O2 by Hb from lungs to tissues & CO2 from tissues to lungs.
- In order to perform this function:

-Hb must be in a state suitable for reversible combination e' O2.

- RBCs membrane must be deformable to allow passage through narrow capillaries.



Deformability depends on:

- Flexibility.
- A genetic factor plays a role (normal membrane structure).
- Energy supply to protect membrane from oxidation.
- Normal Hb structure.
- Normally life span of RBCs is 120 days.

↓life span =(H.A).

Compensated H.A:

BM can compensate for anaemia by ↑erythropoiesis several times (6-8 times)

If BM fails to compensate → H.A.

Compensated H.A:

Hb e'in N range.

↑ retics.

↓RBCs life span.

Red cell aging:

Changes that occur in aging RBCs to be recognized by MQ in RES :

1-↑ density: ass. e'

- Loss of H20.
- ↑ MCHC.
- Tendency to sphere.
- ↓ O.F.

2-Membrane glycoprotein changes: leads to:

- Changes in antigenicity.
- New Ags reacting e' naturally occurring auto Abs.
- So RBCs r recognized by MQ.

3-↓ATP due to ↓enzymes: leads to:

- ↓ membrane deformability.
- ↓ K, ↑Na+ → membrane defect.

4-↓ **creatine phosphate**

Evidence of H.A:

1- Evidence of ↑RBCs destruction.

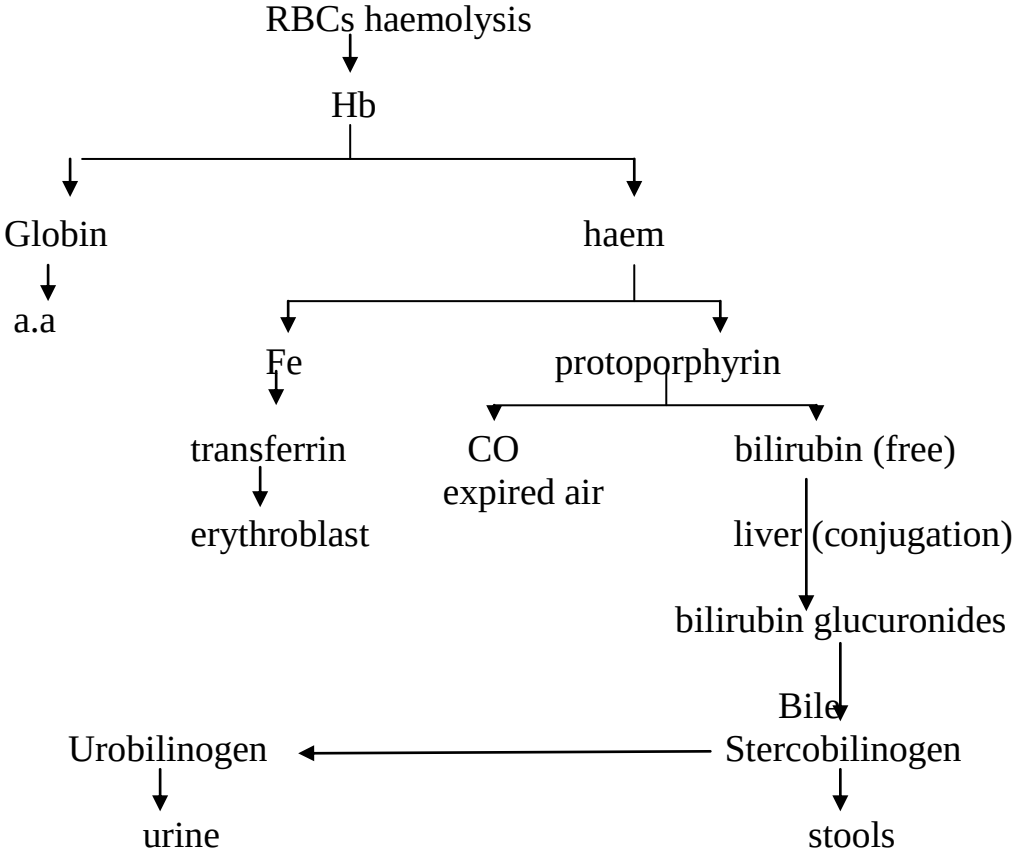
2- ,, ,, ↓,, production.

3- ,, ,, shortened RBCs life span.

4- RBCs morphology.

5- specific tests.

I- Evidence of RBCs destruction:



- ↑ Unconjugated hyper bilirubinaemia.
- Stercobilinogen.
- Urobilinogen → red colour e' Erlich.
- CO, ↑ LDH.
- ↓ Haptoglobin (N Hb buffer):

-Normal plasma contains haptoglobin enough to bind free Hb up to 100-200 mg/100 ml . Hb- haptoglobin complex is N removed by liver.

-↑ free plasma Hb → consumption of haptoglobin.

-N plasma Hb is < 4mg/100 ml

-If ↑ 15-20 mg% → amber yellow

-50-100 mg% → red colour

After depletion of haptoglobin → free Hb oxidation met-Hb → brown colour

40-50 % of free Hb → pass in urine.

- ↓ Glycosylated Hb
- ↓ In Hb level at a rate greater than 1gm/dl/week.

II-Evidence of ↑ RBCs production:

1-↑ Retics:

N : 0.2-2%

↑ If = ↑BM power

↑ rate of release of retics from BM:(**premature release):**

-Larger than N retics

-Survive longer in P.B

-Howell-jolly bodies& basophilic stippling

In order to reflect the red BM activity → do correction of retics:

Retics % $\frac{\text{X Hct of patient}}{\text{normal Hct (45\%)}}$

2- Normoblastaemia

3-Macrocytosis

4-Leucocytosis & thrombocytosis

5- ↑BM erythroid hyperplasia:

(N: M/E=2:1-12:1)

In chronic H.A (esp. congenital), cavities of skull & ribs expand ⟶

frontal bossing

hair on end app. In X-ray

bony pains & fractures

6-Ferrokinetics:

↑plasma iron turn over

↑ red cell iron utilization

7- ↑ RBCs creatine:

- It is a better indicator of erythropoiesis rather than retics.
- It is ↑ in young cells (6-9 times than old cells).
- It remains↑ for 20 days, unlike maturation time of retics (1-3 days).
- N RBCs creatine= 1.5-5 0r 9 mg/dl.

III- Evidence of shortening RBCs life span:

1-**Radioactive C51:**

assesses: ↓life span

site of destruction by scanning over liver, spleen or BM.

Calculate time needed for ½ of the dose to disappear N= 25-32 days.

2-Ashby technique:

- Inject red cells gp O M-ve into gp A M+ve.
- Find survival of donor cells by finding non-agglutinable cells to anti-M at intervals till they disappear.
- disadvantages: can't estimate patient's RBCs life span.
  Can produce immunological problems.

3- P32 labelling of RBCs:

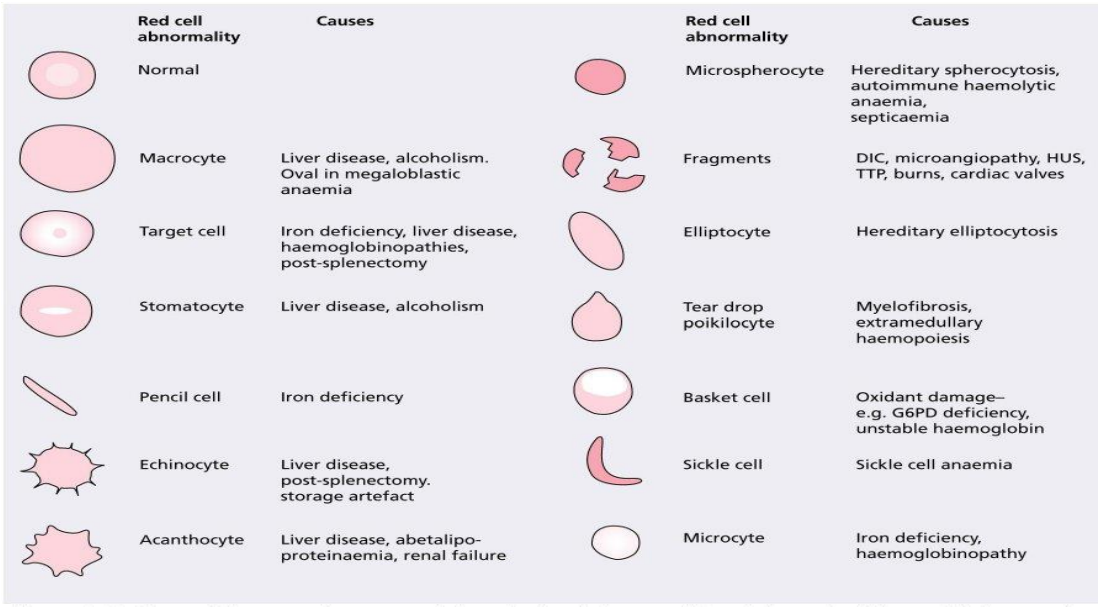
By using di-isopropyl flurophosphate (DFP) labelled e' P32.
Unlike Ashby tech., it can estimate patient' own RBCs life span.

IV- RBCs morphology:

Sickle cells, spherocytes, elliptocytes, stomatocytes, fragmented cells.

V- Special tests:

- O.F: in spherocytosis
- G6PD enzyme : in G6PD def.
- Coomb's test: in AIHA.
- Ham's test: in PNH
- Hb electrophoresis: in thalassamia, sickle cell an.



Red cell abnormality	Causes	Red cell abnormality	Causes
Normal		Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease, alcoholism	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
Pencil cell	Iron deficiency	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
Echinocyte	Liver disease, post-splenectomy. storage artefact	Sickle cell	Sickle cell anaemia
Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure	Microcyte	Iron deficiency, haemoglobinopathy

Figure 2.16 Some of the more frequent variations in size (anisocytosis) and shape (poikilocytosis) that may be found in different anaemias. DIC, disseminated intravascular coagulopathy; G6PD, glucose-6-phosphate dehydrogenase; HUS, haemolytic uraemic syndrome; TTP, thrombotic thrombocytopenic purpura.

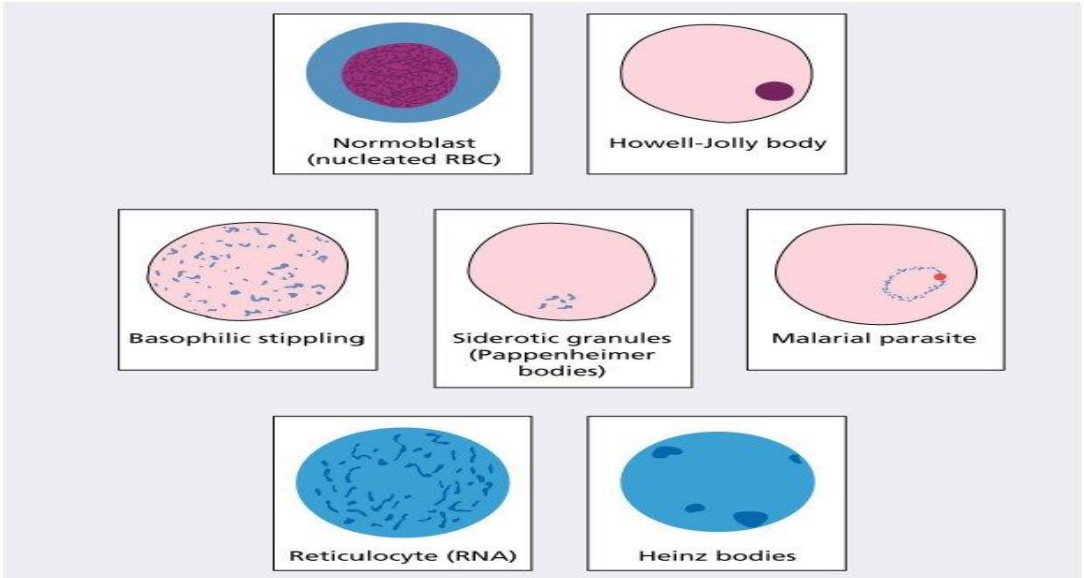


Figure 2.17 Red blood cell (RBC) inclusions which may be seen in the peripheral blood film in various conditions. The reticulocyte RNA and Heinz bodies are only demonstrated by supravital staining (e.g. with new methylene blue). Heinz bodies are oxidized denatured haemoglobin. Siderotic granules (Pappenheimer bodies) contain iron. They are purple on conventional staining but blue with Perls' stain. The Howell–Jolly body is a DNA remnant. Basophilic stippling is denatured RNA.

Clinical picture of H.A:

1- pallor
2- jaundice
3-↑ urobilinogen in urine (turns dark on standing)
4- gall stones
5- splenomegaly, hepatomegaly (HSM)
6-leg ulcers
7-associated congenital defects
8- neonatal jaundice
9-iron overload in severe an.
10-growth retardation.
11-bony changes (e.g: frontal bossing of skull)
12-complications:

i- Haemolytic crisis:
ppt by infection:
↑ pallor, jaundice, bilirubin, retics, BM erythroid hyperplasia.

ii- Aplastic crisis:
in pure red cell aplasia due to parvo virus B19 infection.
↓Erythropoiesis, ↓Hb, N retics, N bilirubin, self limited.

iii- folate def.: gradual ↓ in Hct .

Types of Haemolysis:

1- According to site of Haemolysis:

A- Extravascular Haemolysis:

- Most common.
- RBCs destruction occurs inside MQ of spleen, BM or liver.
- Hb transforms to bilirubin → pass to blood.
- e;g: physiological haemolysis
  most of chronic H.A.

B- Intravascular Haemolysis:

- Less common
- RBCs r destroyed in circulation.
- RBCs lysed → Hb free in plasma (**Haemoglobinaemia**)
  saturation of plasma Haptoglobin → complex removed by RES
  ↓**haptoglobin**
- Excess free Hb → filtered by the glomerulus → saturate renal tubular reabsorptive capacity → **Haemoglobinuria**
- Hb enters renal tubules → iron binds haemosiderin → sloughing e' renal cells into urine → **Haemosiderinuria**

- Oxidation of excess plasma Hb → bind to albumin → met Hb albumin & to haemopexin & removed by liver **(Schumm's test)**

Lab features of IVH:

1- Haemoglobinaemia
2-Haemoglobinuria
3-Haemosiderinuria
4-↓Haptoglobin
5- Methaemalbuminaemia: detected by **Schumm's test:** by spectrophotometer → shift of absorption band from 630 → 558 upon adding ammonium sulphate to plasma.

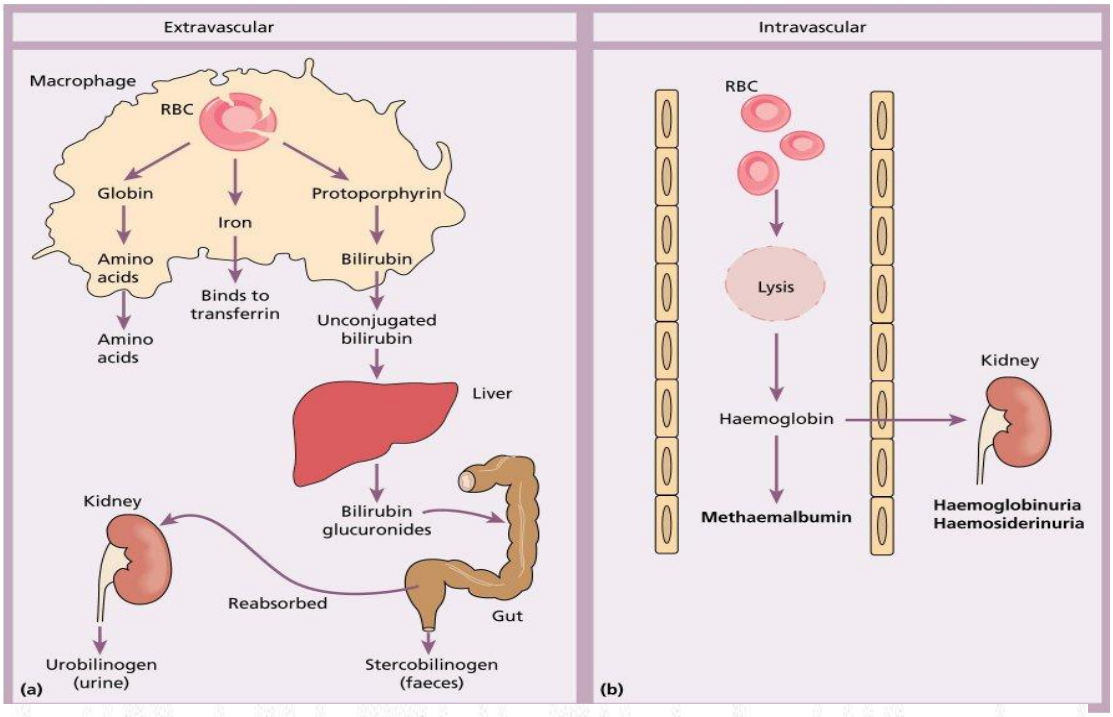
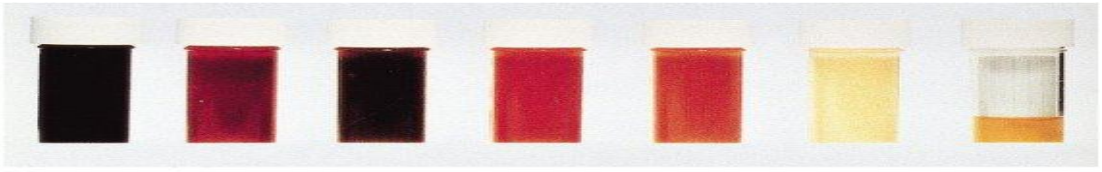
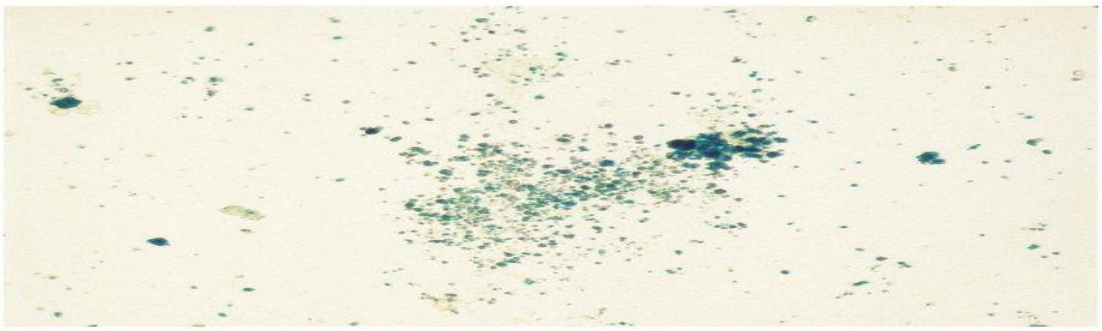


Figure 6.1 **(a)** Normal red blood cell (RBC) breakdown. This takes place extravascularly in the macrophages of the reticuloendothelial system. **(b)** Intravascular haemolysis occurs in some pathological disorders.



(a)



(b)

Figure 6.3 (a) Progressive urine samples in an acute episode of intravascular haemolysis showing haemoglobinuria of decreasing severity. **(b)** Prussian blue-positive deposits of haemosiderin in a urine spun deposit (Perls' stain).

Causes of IVH:

- Mismatched blood transfusion (ABO).
- In acute attacks of H.A.
- G6PD def. e' oxidant stress.
- In haemoglobinuria (PCH, PNH & march haemoglobinuria).
- RBCs fragmentation syndrome.
- Some AIHA.
- Some drug & infection induced H.A.
- Malaria.
- Unstable Hb.

2- According to the defect:

- Intracorpuscular (in RBCs).
- Extracorpuscular (in environment).

3-According to inheritance:

- Hereditary H.A
- Acquired H.A.

All I.C r hereditary except PNH (acquired).
All E.C r acquired

Classification of H.A:

I- Corpuscular causes:

A- Congenital:

1-Disorders of RBCs membrane:

spherocytosis
ovalocytosis
stomatocytosis

2-Disorders of RBCs metabolism (Enzymopathies):

G6PD def.
Pyruvate Kinase (PK) def.

3- Disorder of Hb: (Haemoglobinopathies):

Quantitative: e.g: thalassaemia
Qualitative : e.g: HB M, altered affinity, etc…

B-Acquired membrane defect:

PNH.

II- Extra corpuscular causes: (all r aquired)

A- Abnormal plasma constituents:

1- Immune H.A:

i-Auto immune:

warm Ab type
cold Ab type

ii- Allo immune:

Haemolytic transfusion reaction
Haemolytic disease of the newborn (HDN)
post marrow transplantation.

iii- Drug immune

2-Drugs & toxins

3-Lipid disorders;

Abetalipoproteinaemia
Liver dis.
Vit E def.

B- Abnormal physical environment:

1- Blood vessel abnormalities:

Microangiopathic H.A e.g: thrombotic thrombocytopenic purpura (TTP), haemolytic uraemic syndrome (HUS).
Marsh haemoglobinuria.
Red cell fragmentation syndrome: in arterial grafts, cardiac valves.
Malignant hypertension, pre-eclampsia, DIC.

2- Hypersplenism

3-severe burns

4- Infections:

Malaria, bartonella bacilli