9b. OVERVIEW OF HEMOLYSIS

Lecture Objective

- At the end of the lecture you should be able to:
  - Define the terms hemolysis and hemolytic disorders
  - Pathophysiology of hemolysis
  - Describe the clinical features associated with hemolysis
  - Outline the investigation of a hemolytic condition

Definition

- Destruction of erythrocytes with the release of red cell contents
- In hemolytic conditions, there is premature destruction of the BCs with consequent shortening of the lifespan of red cells
- Haemolysis leads to hemolytic anemia when bone marrow activity is unable to compensate for the red cell loss
- Normally, the bone marrow can expand up to 6-8 times

Consequences of hemolysis

- Increased catabolism of Hb
- Compensatory bone marrow hyperplasia
- These form the basis for laboratory diagnosis tests for hemolysis

Pathophysiology

- A larg number of hereditary acquired disorders cause hemolysis

  Increased RBC destruction may be due to **intrinsic/intra-copurscular** RB disorders - mostly hereditary or **extrinsic/extra-copurscular** factors - mostly acquired
- Hemolysis may occur **extravascularly** or **intravascularly** or both
- Hemolysis may be acute, chronic or episodic

Extravascular hemolysis

- More common
- Removal and destruction of damaged RBCs by the macrophages of the spleen and liver (may occur in the BM)
- Damaged/abnormal RBCs are phagocytosed and destroyes by macrophages
- The iron released is recycled; no IDA development.

Intra-vascular hemolysis

- The destruction of RBCs occurs in circulation with the release of cell contents into the plasma
- Released hemoglobin in plasma is bound by haptoglobin and hemopexin
- Mechanical trauma from a damaged endothelium, complement fixation and activation on the cell surface, infectious agents may cause direct cell membrane degradation and cell destruction in

- In intravascular hemolysis Hb released is lost through kidneys resulting in hemoglobinuria
- Haemosiderin

  Excess Hb released from RBCs in circulation is filtered by the kidney and iron removed and stored as hemosiderin in cells of the proximal tube

  When the cells slough off with the hemosiderin they are excreted into the urine, producing a brownish/dirty color

Classification

- Intracorpuscular Defects/ Hereditary
  - Membrane defects
  - Haemoglobin defects
  - Enzyme defects
- Extracopuscular Defects/Acquired
  - Immune causes
  - Non-immune causes

Intracorpuscular causes (Mostly hereditary)

- Red cell membrane defects:
  - Hereditary spherocytosis
  - Hereditary elliptocytosis and ovalocytosis
  - Hereditary stomatocytosis
  - PNH (acquired)
- Red cell ~~membrane~~ defects:
  - G6PD deficiency
  - Pyruvate kinase deficiency
  - Other enzymes of the PPP

- Hemoglobin disorders
  - Structural Hb abnormalities
    - HbS, HbC, HbE, HbD etc.
    - Unstable hemoglobins and others
  - Imbalance in globin chain synthesis
    - Thalassaemia syndromes (alpha & beta thal)

Extra-corpuscular causes:

- Non-immune causes

  Infections -> Parasitic diseases (malaria bacterial sepsis - Clostridial infections)

  Toxins, chemicals, drugs

  Red cell fragmentation syndromes e.g. DIC. prosthetic cardiac valves etc.

  Hypersplenism

- Immune mediated hemolysis

  Autoimmune hemoltyic anemia (AIHA)

  Alloiumune

  Haemolytic transfusion reaction; Haemolytic disease of the new born;

  allograft associated HA

  Drug induced immune hemolytic anemia

Clinical features

- Hx and PE can provide important clues
- Mild hemolysis may be assymptomatic
- Gestures of aaemia:

  Symptoms: Dyspnea, fatigue, weakness, edema, angina and cardiopulmonary decompensations

  Sign: Pallor of the mucous membranes

  Jaundice of skin and mucous membrane

  Enlarged spleen; intravascular hmolysis - Dark urine; hemoglobinuria and heomisiderinuria

Features reflecting the underlying cause for hemolysis

- painful occlusive crises - SCD
- Leg ulcer - (SCD)
- Skull and skeletal deformities e.g. skull bossing (SCD, Thal)
- Gall stones
- Fava beans, oxidant drugs can induce hemolysis in G6PD deficiency

Lab evaluation

- Aim:
  - Test for hemolysis and anemia
  - Determine mechanisms of hemolysis and precise diagnosis
  - Initial tests: evidence of hemolysis, anemia
  - Specific confirmatory tests
  - Additional studies

Laboratory features: Initial tests

- CBC count
  - Low Hb, Hct/PCV, RBC if anemia is present
  - RBC indices (MCV, MCH, MCHC)
  - WBC counts and differentials
  - Platelet count
  - Reticulocyte count is increased

- Peripheral smear morphology
- Can provide important clues as to cause of haemolysis
  - Demonstrates sickle cells, spherocytes
  - fragments target cells
  - Red cell inclusions
  - Polychromasia (reticulocytosis)
  - Nucleated red cells
  - Parasites

Biochemical tests for hemolysis

- Serum LDH is raised
- Serum haptoglobin is decreased, serum hemopexin is low
- Methaemalbumin (intravascular hemolysis)
- Indirect bilirubin is raised is raised, urobilirubinogen in urine
- Urine hemoglobin present (hemoglobinuria)
- Urine hemosiderin

Specific studies

- Directed by clinical features; intial lab test findings
  - Direct antiglobulin test (DAT)/Coombs test
  - Sickling test, Hb electrophoresis, Hb solubility
  - G6PD screening tests
  - Enzyme assays
  - Osmotic fragility testing for spherocytosis
  - Ham`s test (PNH)
  - Others e.g. RBC membrane studies etc.
- Bone marrow examination is not necessary for diagnosis of straight

Conclusion

- Hemolysis is the destruction of RBCs
- Haemolytic disorders are caused by various hereditary or acquired conditions
- Clinical features include anaemia, jaundice, haemoglobinuria (intravascular haemolysis) and features attributable to particular disorder
- Diagnostic tests aim at:
  Detecting hemolysis and anaemia
  Determining the mechanism of hemolysis and the precise diagnosis.

HAEMOLYTIC ANAEMIA.

- MBchB III.
- Dr. J. RAJAB
- August 2009

ENZYMOPATHIES.

- G6PD – Deficiency
- Pyruvate kinase deficiency
- Glutathione reductase deficiency
- Other enzymes in the Embden Myerhof pathway & Hexose Monophosphate shunt.

ENZYMOPATHIES.

- Glucose-6-Phosphate Dehydrogenase Deficiency
- Mediterranean countries and blacks
- Sex linked of intermediate dominance- long arm of X chromosome
- >200 structural variants of the enzyme. Commonest: B+, A+, A-, Mediterranean, canton, etc
- Acute haemolysis when exposed to certain **drugs:** primaquine(blacks), sulfonamides,nitrofurantoin, about 200 substances assoc.
- **Infections** -bacteria,viral

-**DKA,** neonatal jaundice assoc. with vit.K

Vicia fava (beans) (favism)-severe lVhaemolysis,haemoglobinuria

ENZYMOPATHIES.

- Pyruvate kinase –common in Europeans. rare
- Hexokinase
- Glucose phosphate isomerase
- Phosphofructokinase
- Glutathione reductase

MEMRANOPATHIES.

- Congenital Hereditary Spherocytosis

Reduction in amount of spectrin

Reduced SA to volume ratio-spherocyte,less deformable,splenic phagocytosis

-Autosomal dominant,M=F

-Haemolytic crisis ppt. by infection,anaemia, jaundice, splenomegaly,gallstones(50%)

Aplastic crisis - parvovirus B19

- Hereditary elliptocytosis

-autosomal dominant trait varied expression severity varies.

- Hereditary xerocytosis inc.membrane permeability to cations
- Hereditary hydrocytosis.

IMMUNE HAEMOLYTIC ANAEMIAS.

- **Autoimmune haemolytic Anaemia**

Warm ab type 1gG, 1gG+c^1

–idiopathic (30%)

–2º SLE, AI dx, CLL, Lymphomas, Drugs (methyldopa), ulcerative colitis,ovarian teratoma

Cold ab type 1gM+c^1

–idiopathic (Cold HaemAgglutinin Disease CHAD)

– 2º Mycoplasma, infectious mononucleosis,lymphoma PCH(rare)

ALLOIMMUNE.

- **HaemolyticTransfusion Reaction**
- Immediate 1gG, 1gM of ABO, Rhesus
- Life threatening- Massive IV Haemolysis

❑ **Haemolytic Disease of the Newborn**

- Maternal 1gG to fetus(anti A from gp O mother –gp A fetus)
- Rhesus HDN- rarer
- Others anti B, anti C, anti E, anti kell

❑ **Allogeneic transplantation.(**renal, hepatic, cardiac BM)

DRUG INDUCED HA.

- Drug absorption mechanism/hapten carrier (penicillin, ampicillin, cephalosporin, TC)
- Immune complex mechanism(innocent bystander phenacetin, quinine, quinidine,rifampicin
- Membrane modification mechanism (cephalosporins)
- True AIHA (methyldopa, fludarabine)

PNH.

- Rare acquired clonal disorder of stem cells
- Deficiency of GP1 anchor (link proteins on rbc membrane eg CD55,DAF,MIRL
- Sensitive to lysis by c^1 –Chronic IV haemolysis.–haemosidenuria
- Associated with thrombosis of large veins, BM hypoplasia.
- +ve Hams test

OTHERS.

- Severe Burns- acanthocytes,Spherocytes
- Secondary HA
  -Renal disease
  -Liver disease

Red cell fragmentation syndromes.

- Physical damage to rbc on abnormal surfaces
- MAHA -fibrin strands, artificial valves
  endothelial damage, arterial grafts
- DIC
- Malignant hypertension.
- TTP
- HUS
- Pre-eclampsia
- Meningococcal sepsis.

OTHERS.

Parasites – malaria, babesia
Toxoplasmosis, Bartonella

- Bacterial – meningococcal/pneumococcal septicaemia (MAHA),Cl. perfringes ppt. of haemolysis in G6PD deficiency
- Viral – dengue, yellow fever
- Chemicals – oxidative IV haemolysis (Dapsone, salazopyrine)
- Wilson's disease
- Heavy metals & Chemicals – Lead, chlorate, arsine poisoning.

Clinical features

1. anaemia, can be severe
2. Jaundice
3. Splenomegaly
4. Bossing of skull
5. S&S of underlying condition
6. Acrocyanosis-CHAD
7. Dark urine

LABARATORY MANAGEMENT.

- **Anaemia** –?haemolytic

Increased Hb breakdown – FBC
–PBF–poikilocytes,polychromasia,nrbc
–Reticulocyte count,heinz bodies ,basophilic stippling
–serum bilirubinaemia

?site of rbc destruction

IV –urobilinogen
–Free Hb
–Haemosiderinuria
–Haemopexin
–Haptoglobulins

EV–(splenomegaly cf)

LABARATORY MANAGEMENT.

? Aetiology

Hx

Clinical Features

-associated disease

-drug hx

-? idiopathic

-HTR, HDN, allograft associated.

Specific Investigations.

- Immune – DAT
  - –IAT, ab specificity(warm, cold, c^1
- Hams test, flow cytometry – PNH
- Osmotic fragility, flow cytometry– membrane disorders.
- Enzyme Assays
- Assays – CU, lead, arsine in suspected chemical HA.

Principles of Treatment.

- Supportive

-red cell transfusion support,mx underling ppt. factor

- Warm ab AIHA - spontaneous recovery
  steroids
  red cell transfusion support.
  splenectomy
  Immunosuppressants(azathioprine, cyclophosphomide, cyclosporin)

Principles of Treatment.

- Cold ab – keep warm
  - folate supplements
  - steroids, ?splenectomy
  - chlorambucil
  - plasmapheresis
- Drugs – withdrawal
- Management of Underlying Dx

•Mx

- PNH – Difficult

  anticoagulants, immunosuppressants, allogeneic BM stem cell transplant as some may progress to BM hypoplasia.

Hemolytic anaemias
enzyme and membrane defects

Table 5.1 Classification of haemolytic anaemias.

Hereditary	Acquired
Membrane Hereditary spherocytosis, hereditary elliptocytosis	**Immune** *Autoimmune* Warm antibody type (see Table 5.5) Cold antibody type
Metabolism G6PD deficiency, pyruvate kinase deficiency	*Alloimmune* Haemolytic transfusion reactions Haemolytic disease of the newborn Allografts, especially marrow transplantation
Haemoglobin Genetic abnormalities (Hb S, Hb C, unstable); see Chapter 6	*Drug associated* **Red cell fragmentation syndromes** See Table 5.6
	March haemoglobinuria
	Infections Malaria, clostridia
	Chemical and physical agents Especially drugs, industrial/domestic substances, burns
	Secondary Liver and renal disease
	Paroxysmal nocturnal haemoglobinuria

Hereditary spherocytosis

- Congenital haemolytic disorder
- Inherited defect in the red cell membrane cytoskeleton
- Formation of spherocytic red cells
- Less deformable than normal red cells
- Trapped and destroyed in the spleen
- Reduced surface area to volume ratio
- Osmotically fragile
- Autosomal dominant
- Northern European decent highest prevalance

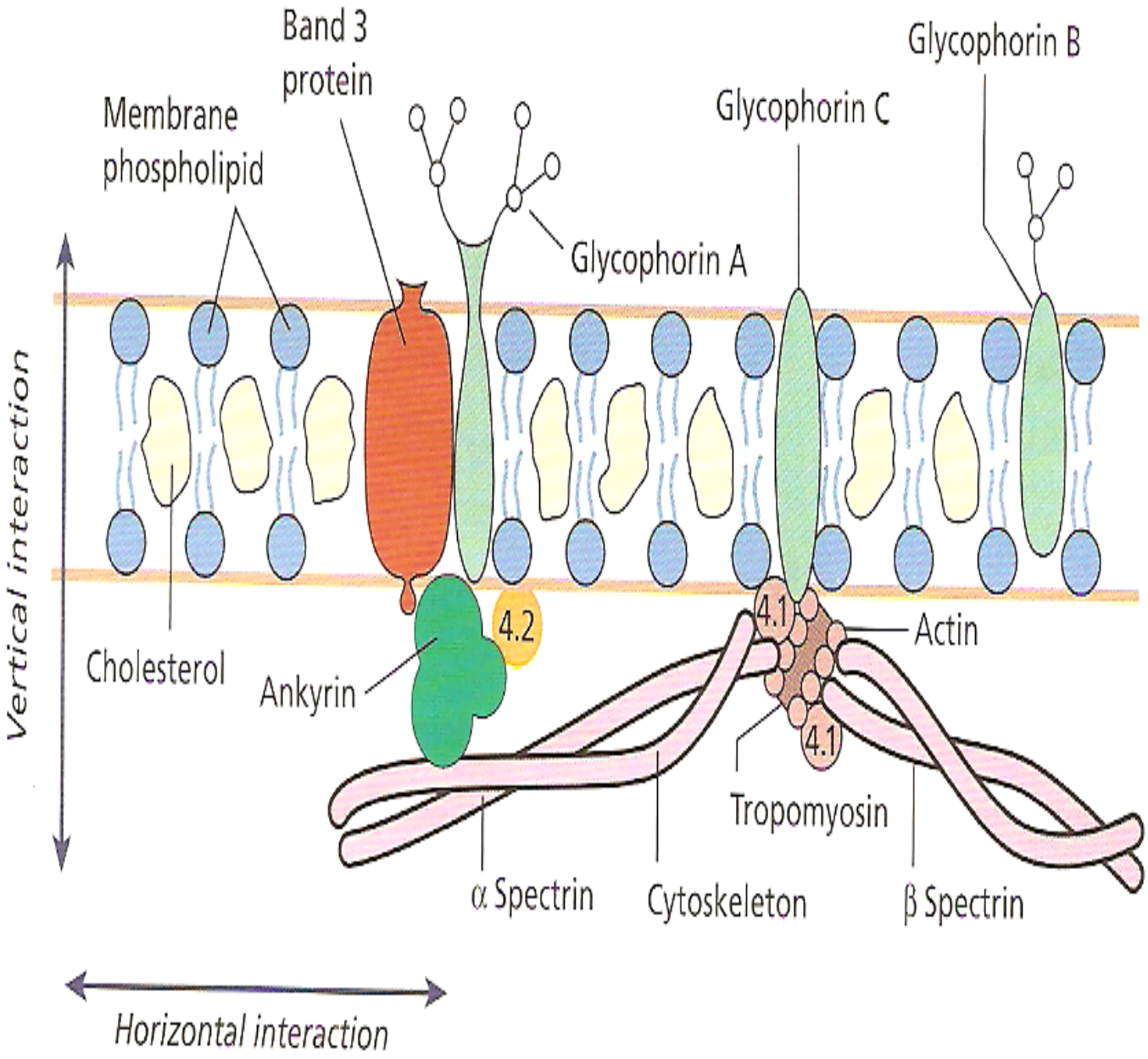


Fig. 2.12 The structure of the red cell membrane. Some of the penetrating and integral protein carry carbohydrate antigens; other antigens are attached directly to the lipid layer.

- HS results from deficiency of
  - spectrin (relatively common)
  - ankyrin
  - band 3
  - protein 4.2
- Weakining of contact between lipid bilayer and skeleton
- Loss of membrane in these contact areas
- Increase SA to Vol ratio
- Spherocytes
- More loss of membrane in the spleen more spherical and less deformable in the splenic enviroment

Clinical features

- Presentation in childhood with anaemia
- Intermittent jaundice
- Enlarged spleen
- Family history
- Gall stones
- Chronic leg ulcers in an occasional patient
- Transfusion is seldom required except in a few cases

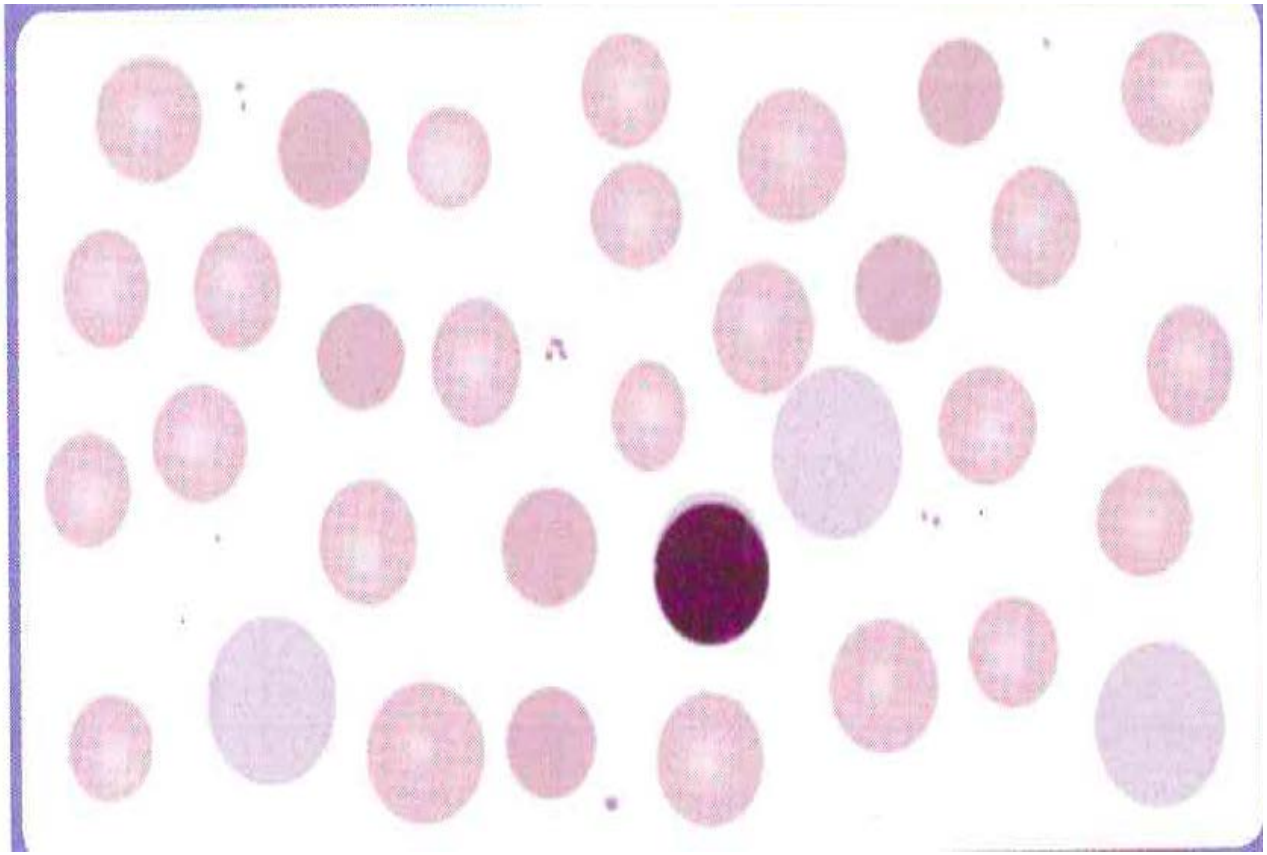
Laboratory features

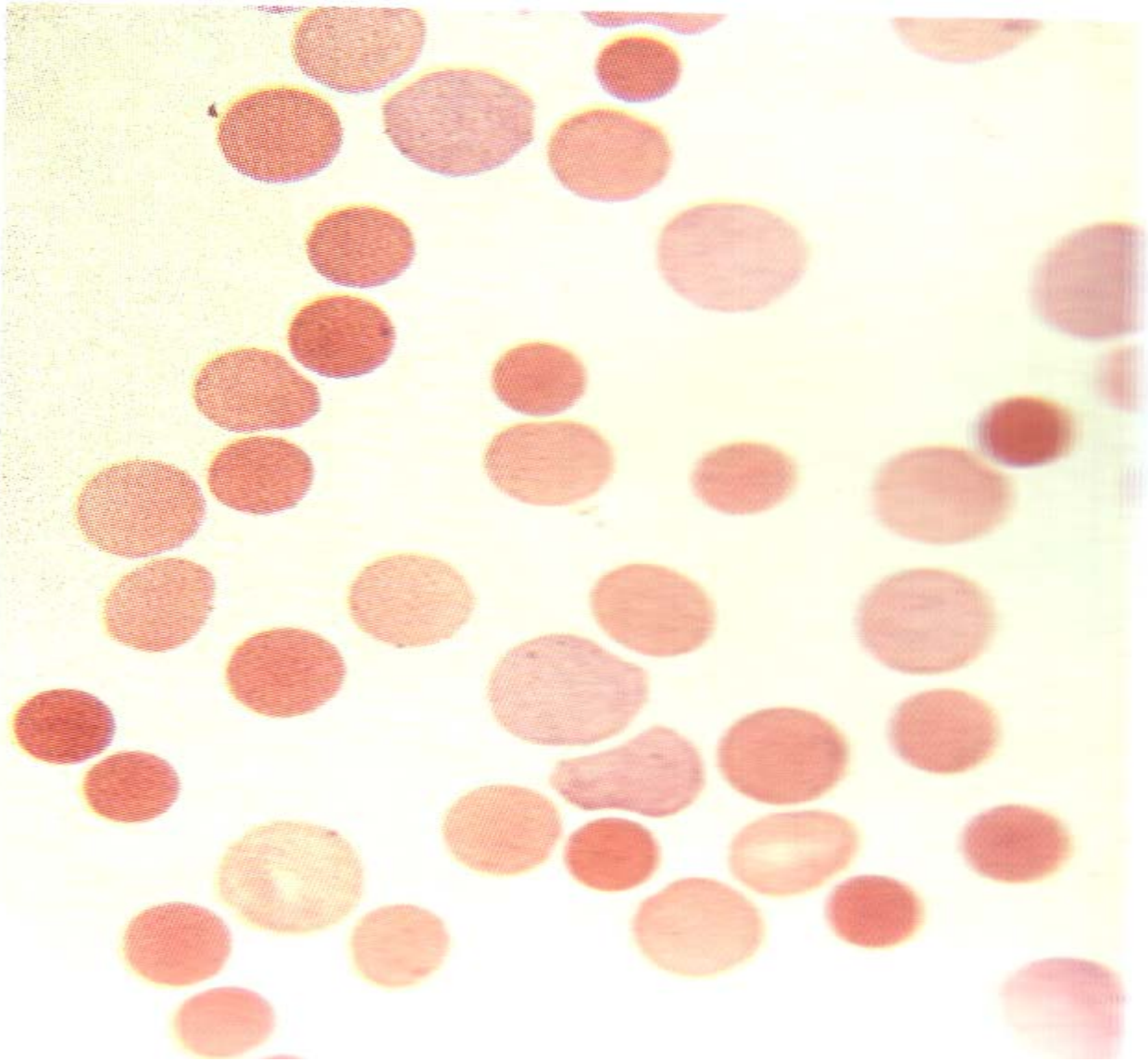
- Mild to moderate anaemia
- Increased MCHC
- Normal MCH
- Normal MCV
- Reticulocyte count is increased
- Spherocytes on PBF
- Increased fragility

Laboratory evaluation

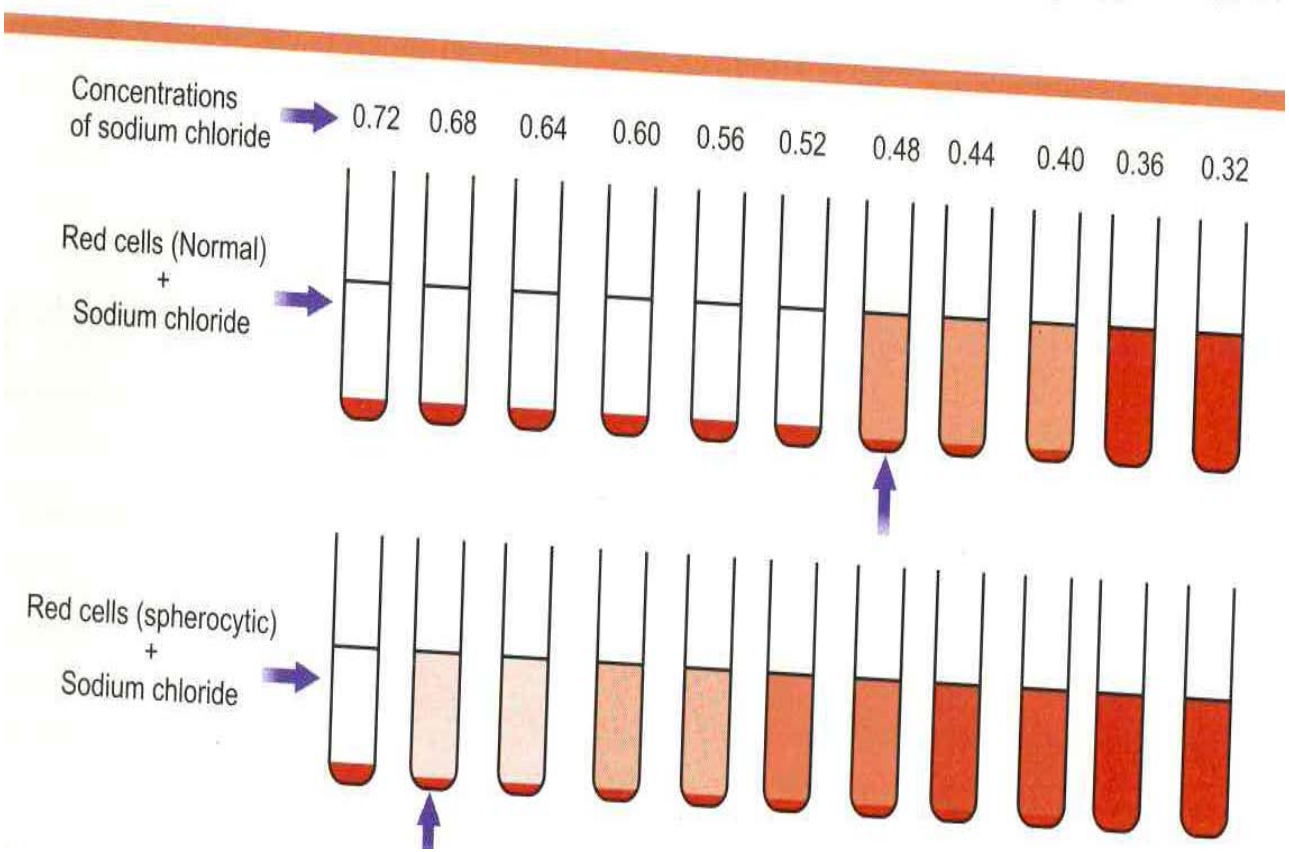
- FBC
- PBF
- Reticulocyte count
- Osmotic fragility test
- Gel electrophoresis

SPHEROCYTES





OSMOTIC FRAGILITY TESTING



Appendix F. **OSMOTIC FRAGILITY OF ERYTHROCYTES**

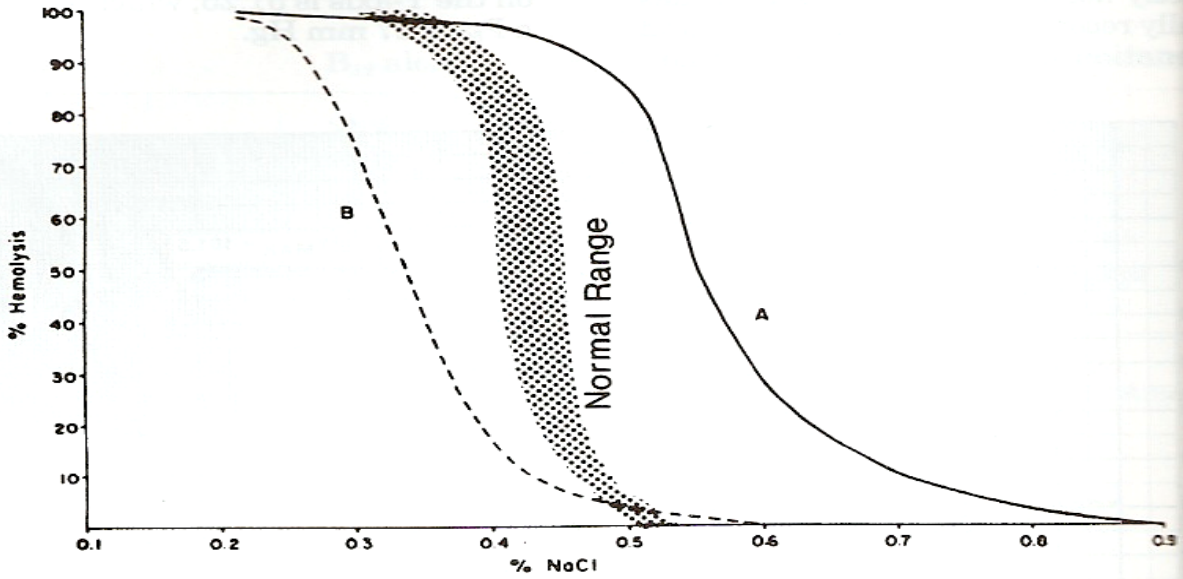
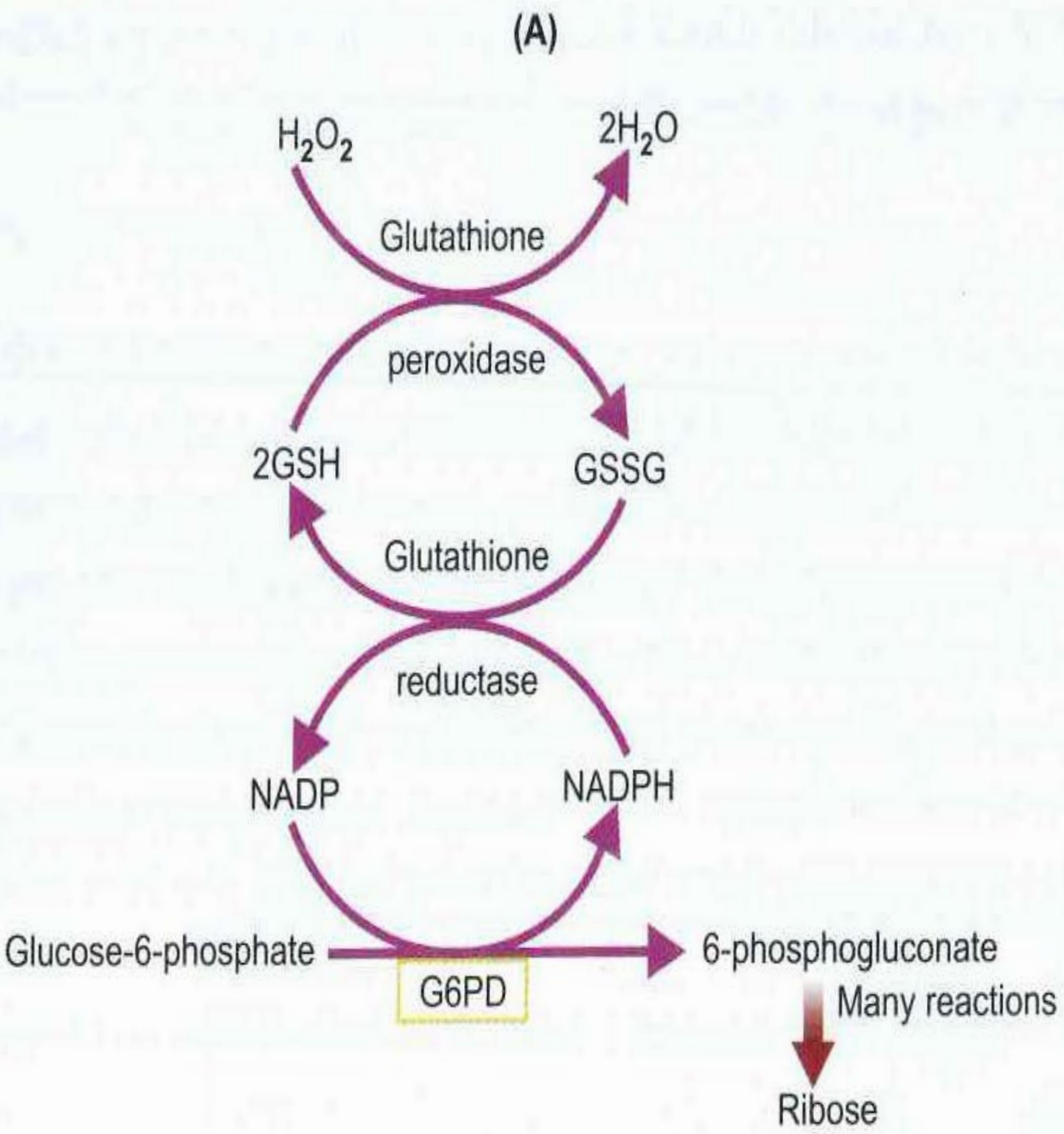


FIG. F-1. Normal and abnormal osmotic fragility curves, plotted from photoelectric obtained by Dacie's method. A. Increased osmotic fragility. B. Decreased osm fragility. (From Miale JB, *Laboratory medicine: hematology*, 6th ed. St. Louis: M Year Book, 1982, with permission.)

Red Cell Enzyme Disorders
Glucose 6 PD deficiency

- G6PD most common red cell enzyme disorder
- Affects 400 million people world wide
- Characterized by reduced activity of G6PD and haemolysis on exposure to certain drugs
- Many different variants
- X linked disorder

- G6PD required in a reaction that generates NADPH from NADP (HMP shunt)
- NADPH required for continous supply of reduced glutathione (GSH)
- GSH detoxifies hydrogen peroxide
- In G6PD deficiency reduced glutathione causes accumulation of Hydrogen peroxide causing oxidation of haemoglobin with denaturation and precipitation of globin chains (Heinz bodies)


(A)
H_2O_2
$2H_2O$
Glutathione
peroxidase
2GSH
GSSG
Glutathione
reductase
NADP
NADPH
Glucose-6-phosphate
G6PD
6-phosphogluconate
Many reactions
Ribose
(B)
Deficiency of G6PD
Decreased NADPH
Decreased GSH
Accumulation of H_2O_2
Oxidant damage to red cells
Haemolysis in spleen

- Red cells with denatured haemoglobin are removed by the spleen
- Other red cells rupture in circulation (intravascular haemolysis) due to peroxidation of membrane lipids

Clinical features

- Haemolysis following oxidant stress
- Drugs
- Infections
- Anaemia, jaundice and dark urine 1-3 days after exposure to drug
- Ingestion of fava beans (favism)

Table 4.6: Common drugs and chemicals causing haemolysis in G6PD deficiency

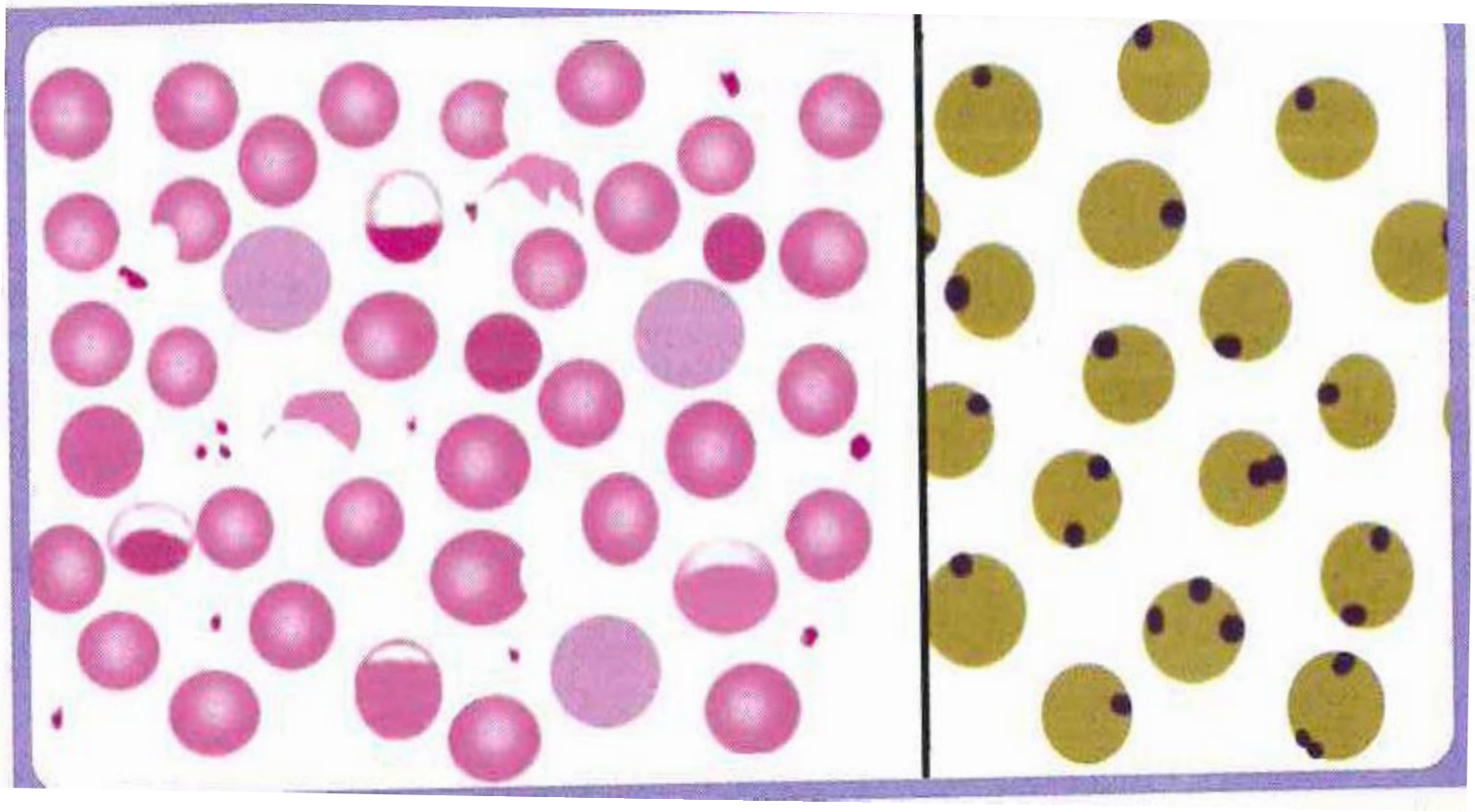
- **Antimalarials: Primaquine**, Chloroquine, Quinacrine, **Pamaquine**
- **Antibacterials:** Sulfacetamide, **Sulfamethoxazole**, Sulfanilamide, Sulfapyridine, **Nalidixic acid**, **Nitrofurantoin**, Furazolidone, **Dapsone**
- **Analgesics:** Acetanilid, **Aspirin**, **Phenacetin**
- **Others:** Phenylhydrazine, Ascorbic acid, Vit K (water-soluble), **Methylene blue**, Naphthalene (moth balls)

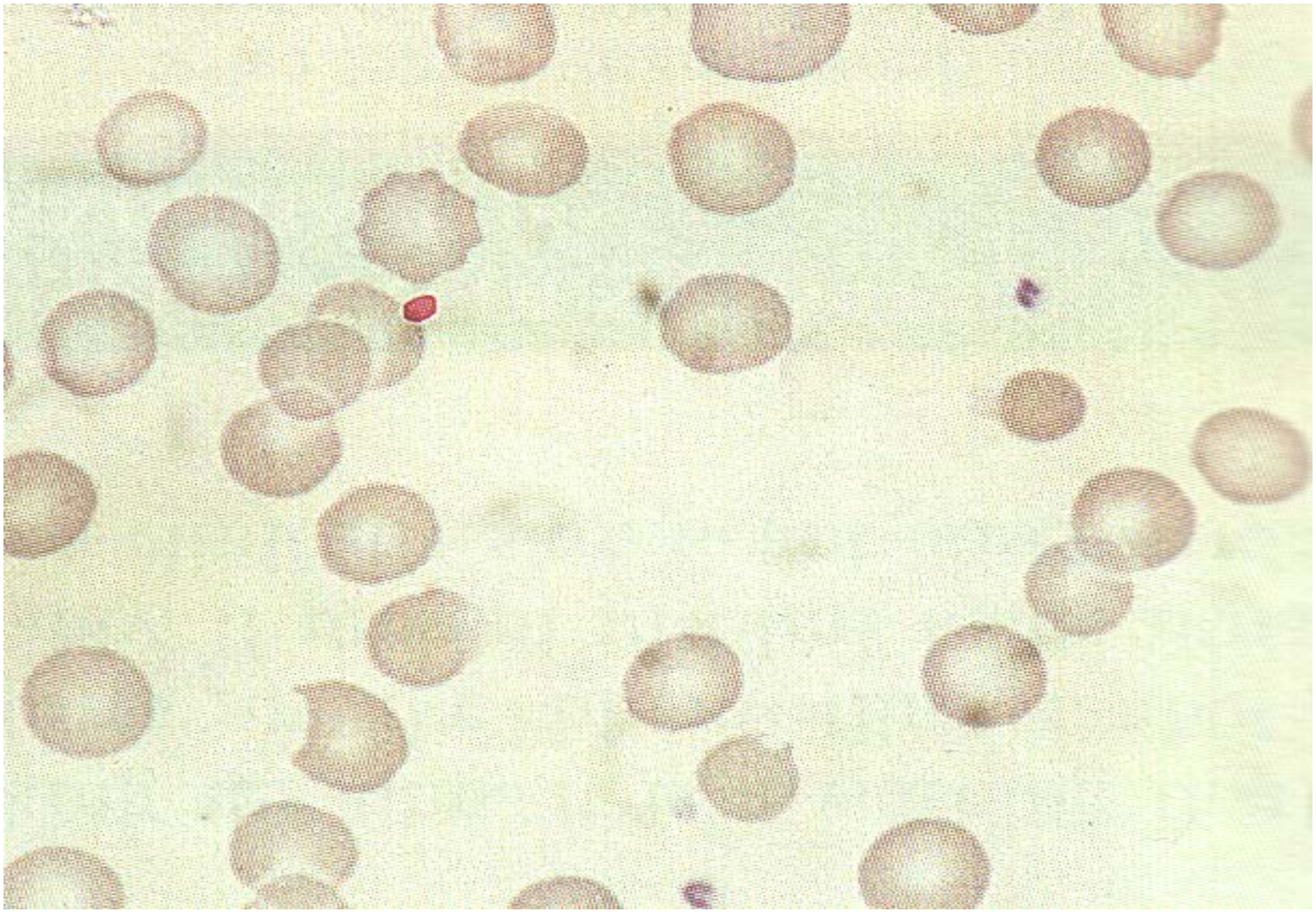
Agents marked in bold: Definite risk of haemolysis

Laboratory evaluation

- Evidence of haemolysis
- PBF fragmented cells , bite cells, blister cells(ghost cells)
- Haemoglibinuria
- Reduced haptoglobins
- Heinz bodies demonstrated by special stains

FRAGMENTS, HEINZ BODIES





tests

- Screening tests
- fluorescent spot tests (detects NADPH)
- methaemoglobin reduction test
- Quantitation of G6PD

Acquired Hemolytic Anemia

Dr Peter Maturi Mwamba
Lecturer
Hematology and Blood Transfusion

Lecture outline

- Introduction:
  Categories of acquired HA
  Mechanisms

- Immune haemolytic anaemia
  Mechanisms

- Non immune HA
  Causes

- Hypersplenism

Learning objectives

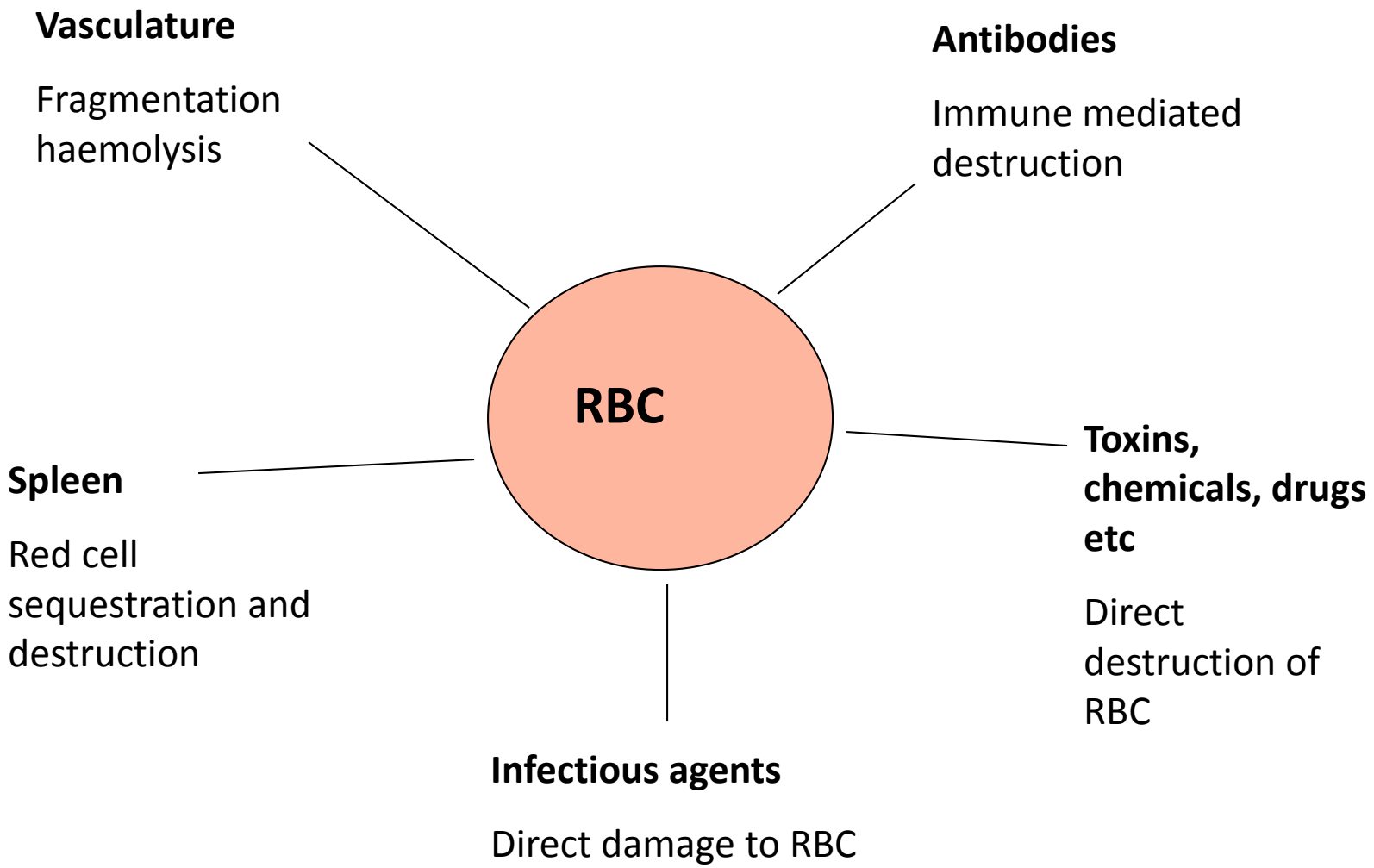
- At the end of this lecture you should be able to:
  - Discuss the acquired red cell defects causing haemolysis
  - Outline the investigations for an acquired haemolytic condition

Acquired causes of haemolysis

- Acquired haemolytic conditions are due to extracorpuscular causes of haemolysis,
  Due to abnormal environmental factors acting on normal RBC

- Main categories:
  Immune haemolytic anaemias
  Non-immune acquired HA

Environment and red cell damage



IMMUNE HAEMOLYTIC ANAEMIA

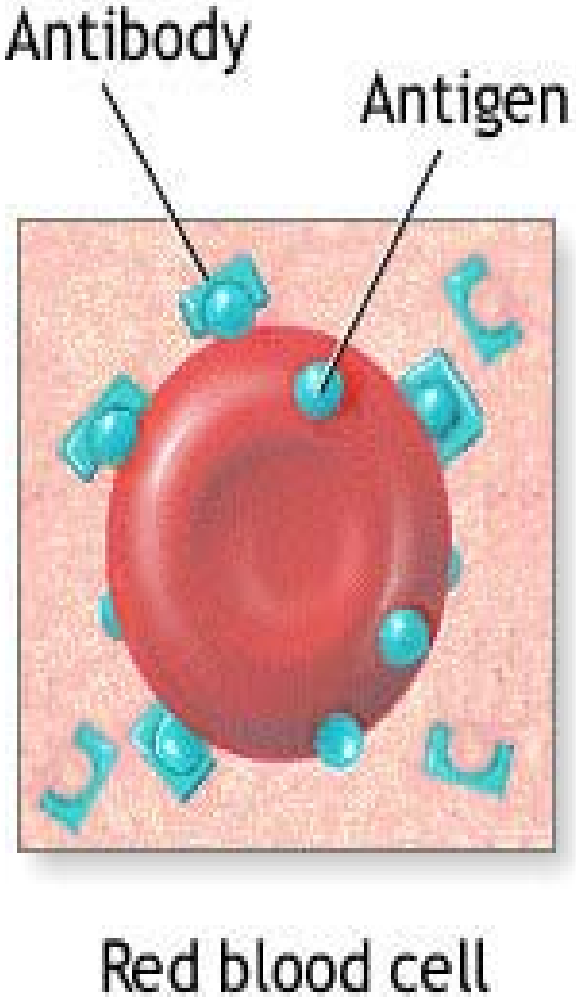
- Due to antibodies produced against red cell antigens
  - Auto-immune HA
  - Allo-immune HA
  - Drug-induced HA

Mechanisms of RBC destruction:

- Cell-mediated immune (CMI) destruction leading to **extravascular** destruction
- Complement mediated destruction – may lead to **intravascular** haemolysis

- CELL MEDIATED IMMUNITY

  RBC sensitized by IgG is destroyed by cells of the RES



- Antibody against a specific red cell antigen latches onto the antigen
- The antibody may be IgG or IgM.
- IgG coated red cells usually removed intracellularly.
- IgM antibodies can cause compliment activation and red cell lysis

1. Autoimmune haemolysis

- Warm antibody type
- Cold antibody type

A: **Warm antibody autoimmune hemolytic anemia**

Idiopathic

Associated with:

- Lymphoproliferative disorders and other malignancies
- Associated with autoimmune diseases – eg SLE

IgG mediated

B: **Cold antibody autoimmune hemolytic anemia**

Idiopathic (cold hemagglutinin syndrome)

Associated with infections eg Infectious mononucleosis and mycoplasma (atypical) pneumonia

Paroxysmal cold hemoglobinuria (rare)

IgM mediated

2. **Alloimmune hemolytic anemia**

Haemolytic disease of the newborn (HDN)

- Rh disease (Rh D)
- Other blood group incompatibility (RhC, Kell, Rhe, Kidd, Duffy, MN, P and others)

Blood transfusion reactions (ie from a non-compatible blood type

Transplantation-associated haemolytic disease

3. **Drug induced immune mediated HA**

Membrane modification mechanism

Drug absorption mechanism: (Hapten type)

Immune complex mechanism: (Innocent bystander mechanism)

Membrane modification mechanism

- Modifying the red cell membrane components

- Examples: Cephalosporin

Drug absorption mechanism: (Hapten type)

- Drugs molecules attach into the cell membranes through non-immune mechanisms to the membrane.

- Examples here include eg for penicillin, cephalosporins and tetracyclines

Immune complex mechanism: (Innocent bystander mechanism)

- Here drugs form hapten-carrier complexes with plasma proteins which enhance drug-specific antibody production.

- Once drug antibodies are present, reintroduction of the drug causes immune complexes to form which are absorbed on to the red cell membrane and complement activated.

- Examples of drug:

  Rifampicin, phenacetin, quinine, quinidine,

Non-immune mediated HA

Causes:

Drugs
Toxins
Trauma
MAHA
Infections

- Direct Coombs test is negative

1. Drugs

Some drugs and other ingested substances lead to haemolysis by direct action on RBCs, e.g. ribavirin

Via oxidative mechanisms. This is particularly likely to occur when there is an enzyme deficiency in the antioxidant defence system of the redcell eg G6PD deficiency where antimalarial oxidant drugs like primaquine damage red blood cells

Some drugs cause RBC (red blood cell) lysis even in normal individuals. These include dapsone and sulfasalazine.

2. Toxins

Snake venom;
Plant poisons such as aesculin

3. Trauma

Mechanical eg heart valves

Extensive vascular surgery

Microvascular disease

4. MAHA

- Microangiopathic hemolytic anemia
  Thrombotic Thrombocytopenia Purpura (TTP)

  Hemolytic Uremic Syndrome (HUS)

  Disseminated Intravascular Coagulation(DIC)

5. Infections

Malaria – Falciparum:
Babesiosis
Bacterias

Here hemolysis involves extravascular destruction of parasitized cells in the spleen and liver and intravascular lysis when the spozoites break out of the cells.

Hypersplenism

- Hypersplenism
  Spleen sequesters red cells and destroys abnormal cells and aged cells
  Hypersplenism associated with increased sequestration and destruction
  Hypersplenism associated with splenomegaly

Paroxysmal Nocturnal Haemoglobinuria

- Uncommon acquired disorder due to an acquired somatic mutation
- Acquired membrane defect of RBC rendering cell sensitive to C^{I} mediated lysis

  deficiency of glycophosphatidylinositol leading to absence of protective proteins on the membrane

Laboratory Workup

Initial Investigations:

- FBC

  Hb, Hct, Rbc MCH, MCHC
  WBC, Plt

- Reticulocyte count

  Raised indicating increased erythropoietc activity

Peripheral Blood film (PBF)

- Important morphologic changes e.g.
  Spherocytes (Immune haemolytic anaemias, toxins, burns)
  Red cell fragments (fragmentation anaemias)
  Polychromasia
  Nucleated red cells
  Parasites etc

Biochemical tests for haemolysis

- Bilirubin levels: increased indirect bilirubin
- LDH increased
- Haptoglobulin,
- Hemopexin decreased
- Urinalysis
  - Haemosiderin
  - Haemoglobinuria
  - Urobilinogen

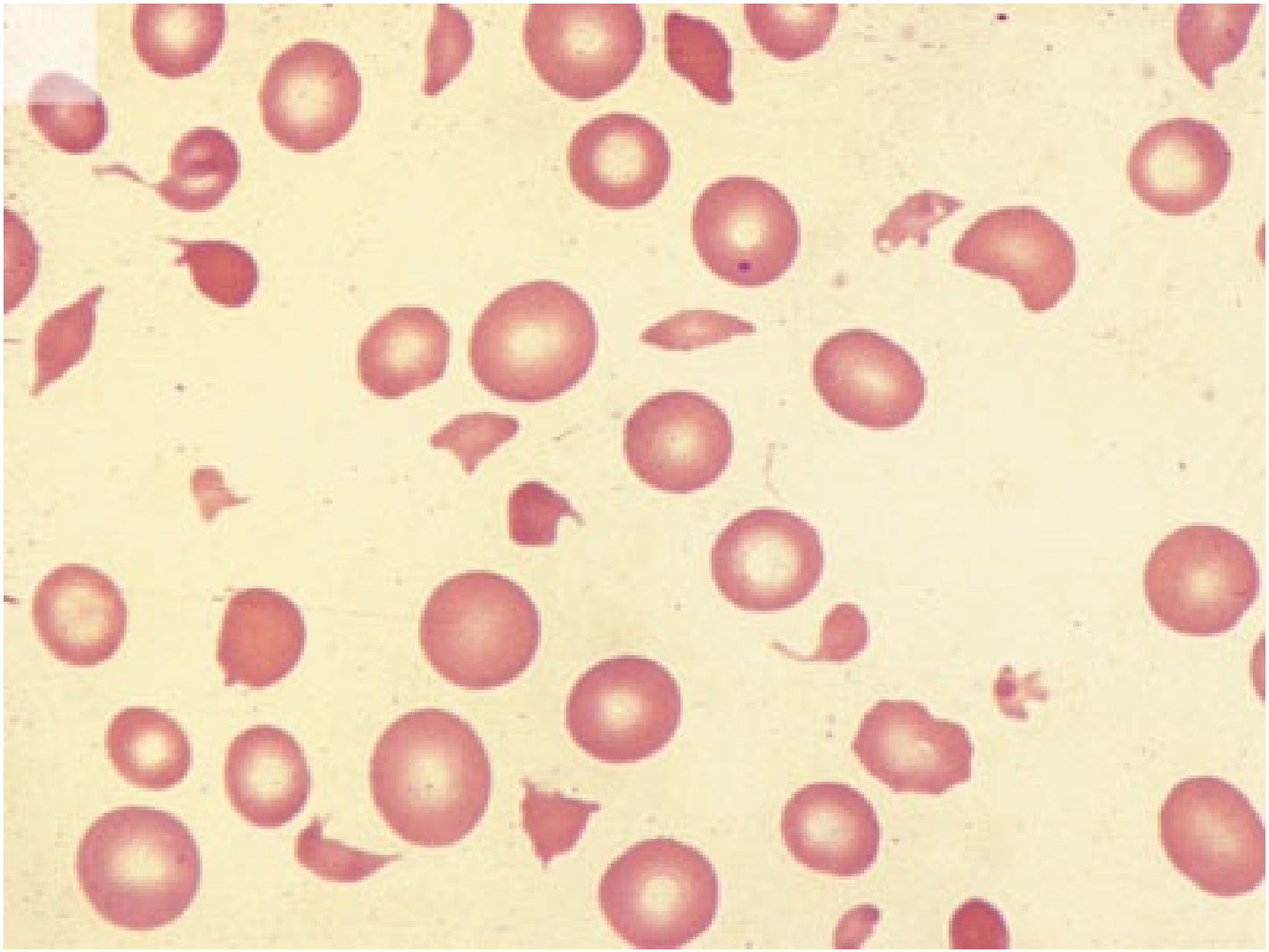
Specific studies:

Directed by history and PE and preliminary tests:

- Direct antiglobulin test (Immune mediated haemolysis)

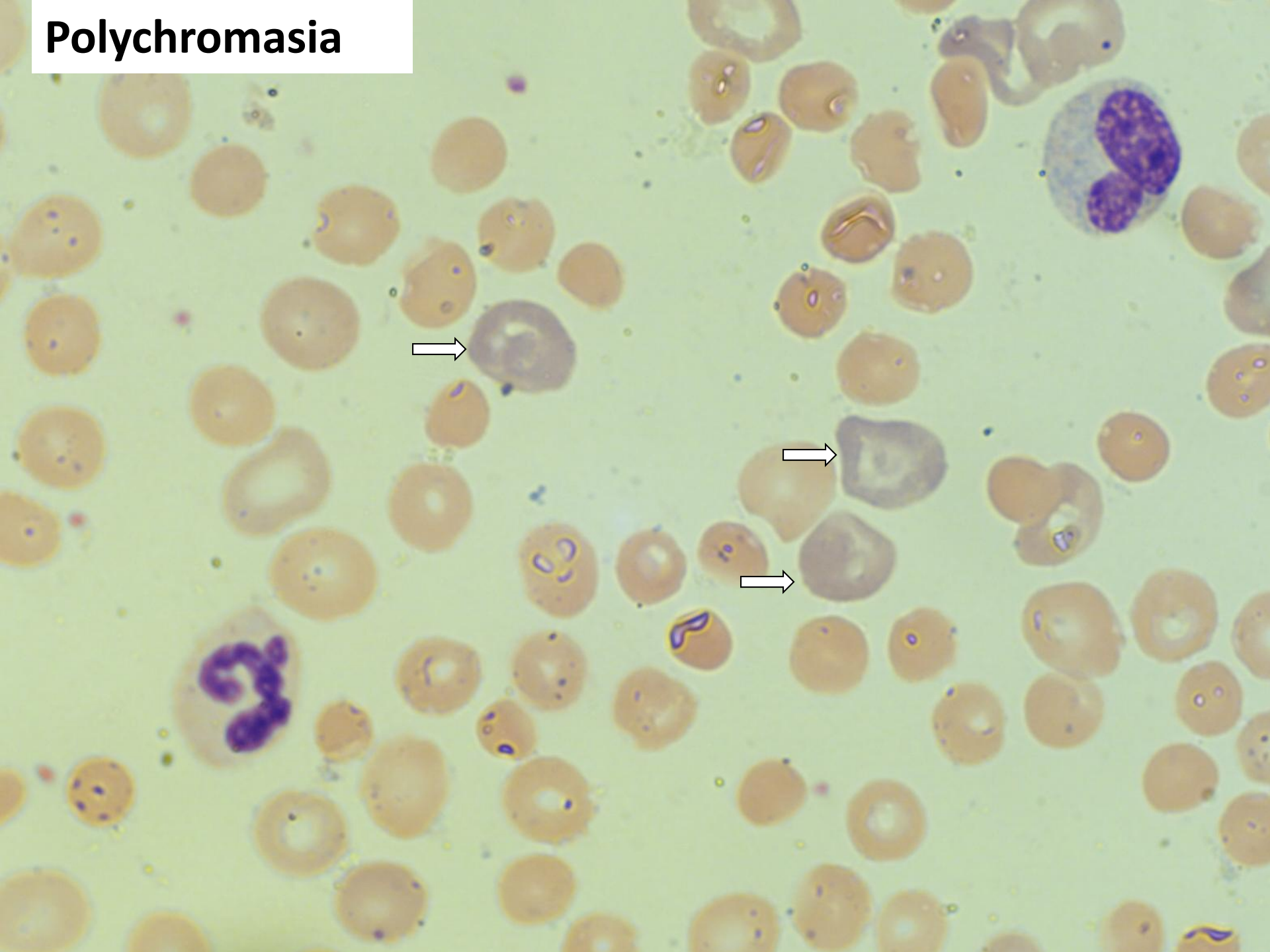
- Tests for drug mediated haemolysis

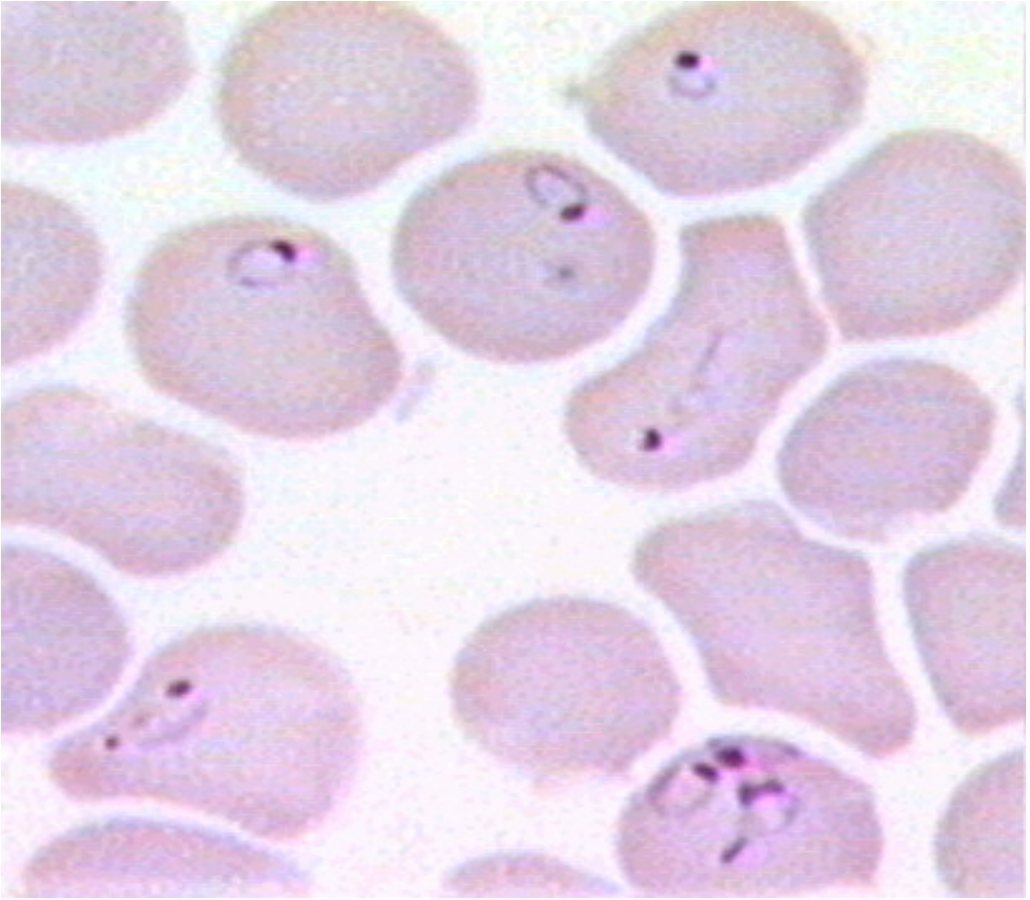
- Hams-Dacie/Hams test – PNH



FRAGMENTS/SCHISTOCYTES

Polychromasia





Malaria parasites