APPROACH TO HAEMOLYSIS AND HAEMOGLOBINOPATHIES

By:

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LEARNING OBJECTIVES

- To be able to define haemolysis and haemolytic anaemia
- To be able to classify haemolytic anaemias into congenital and acquired types, and to know the aetiological factors in each division
- To understand the difference between intravascular and extra-vascular haemolysis, and to recognise the laboratory features of each

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- To appreciate that disorders of globin function such as sickle cell disease are subtypes of haemolytic anaemia
- To understand the role of autoantibodies in the production of haemolytic anaemias and to know the types of disease with which they are associated
- To understand some causes of non-immune acquired haemolytic anaemias

HAEMOLYSIS

- Premature destruction of RBCs.
- Hemolysis could be due to:
  a. Defect in the RBCs (intra-corpuscular) as in congenital hemolytic Anaemia.
  b. Defect in the surrounding environment (extracorpuscular) as in acquired Anaemia.

HAEMOLYTIC ANAEMIAS

Haemolysis

- describes the shortening of the lifespan of a mature red blood cell.
- increased red cell output from the marrow
- stimulated by erythropoietin
- will be sufficient to compensate for the increased red cell destruction
- more marked reductions in red cell lifespan - say to 5-10 days from the usual 120 days
- will result in ***haemolytic anaemia***
- this compensatory increase in erythroid output requires an adequately functioning bone marrow and effective erythropoiesis
- a suboptimal marrow response is seen
- haemolysis will result in anaemia more readily

Clinical Features of Hemolysis

- Pallor, lethargy
- Jaundice
- Splenomegaly
- Gall stones (Pigment – bilirubin)
- Dark urine (urobilinogen)
- Bone deformity (In some types of haemolytic anaemia)
- Leg ulcers (in some types of haemolytic anaemia).

Laboratory Features of Hemolysis

1.) Features of increased red cell breakdown.

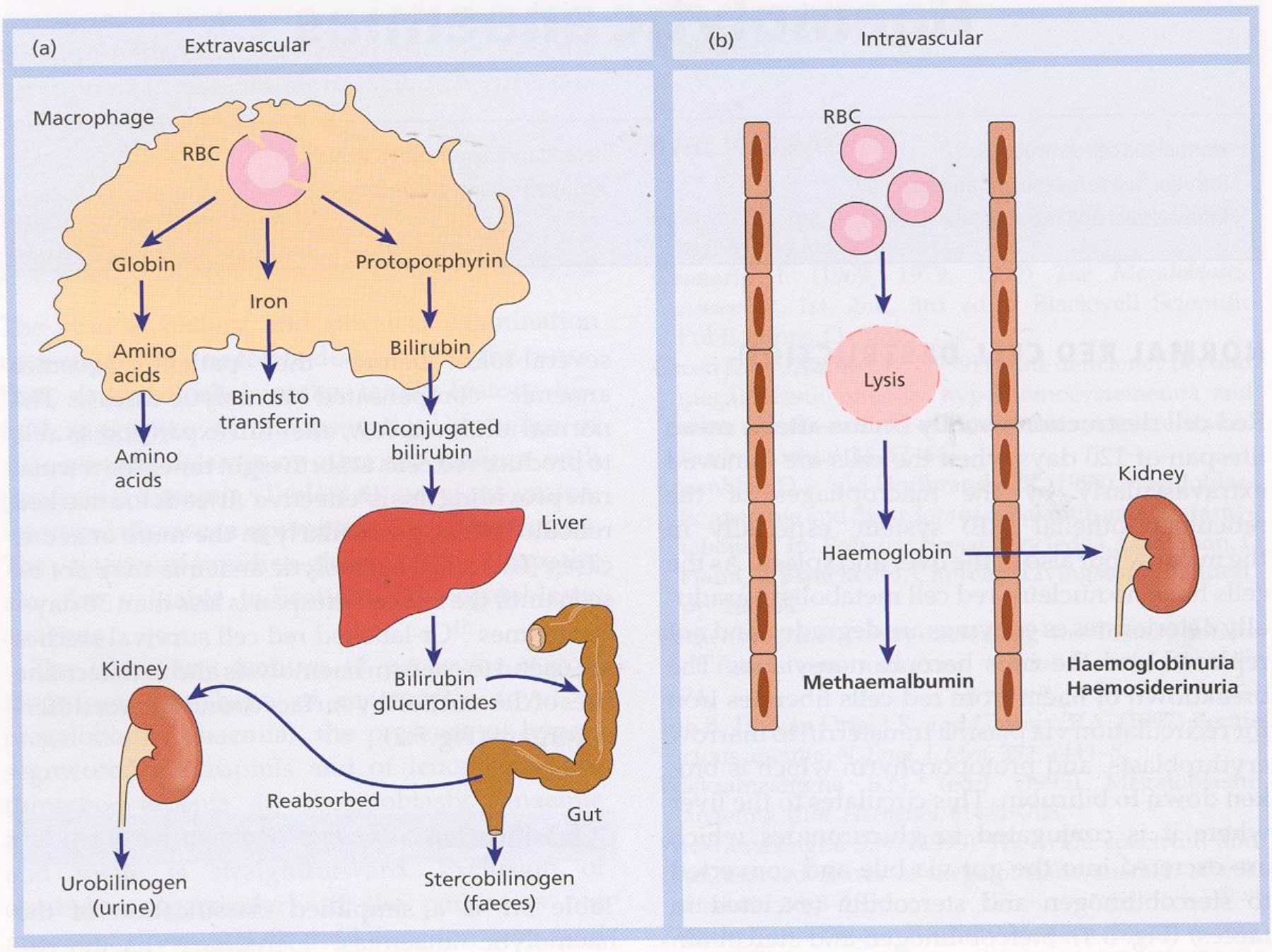
- **a. ↑ serum bilirubin is raised (unconjugated and bound to albumin).**
- **b. ↑ urine urobilinogen.**
- **c. ↑ faecal stercobilinogen.**
- **d. Absent serum haptoglobins.**
- **e. ↑ lactate dehydrogenase (LDH)**

Laboratory Features of Hemolysis

2.) Features of increased red cells production.
    a. Reticulocytosis
    b. Bone marrow erythroid hyperplasia.
3.) Damaged red cells.
    a. Morphology (e.g. microspherocytes, elliptocytes, red cells fragmentation).
    b. Increased osmotic fragility, autohaemolysis etc).
    c. Shortened red cell survival (This can be shown by ^{51}Cr labeling with study of the sites of destruction.

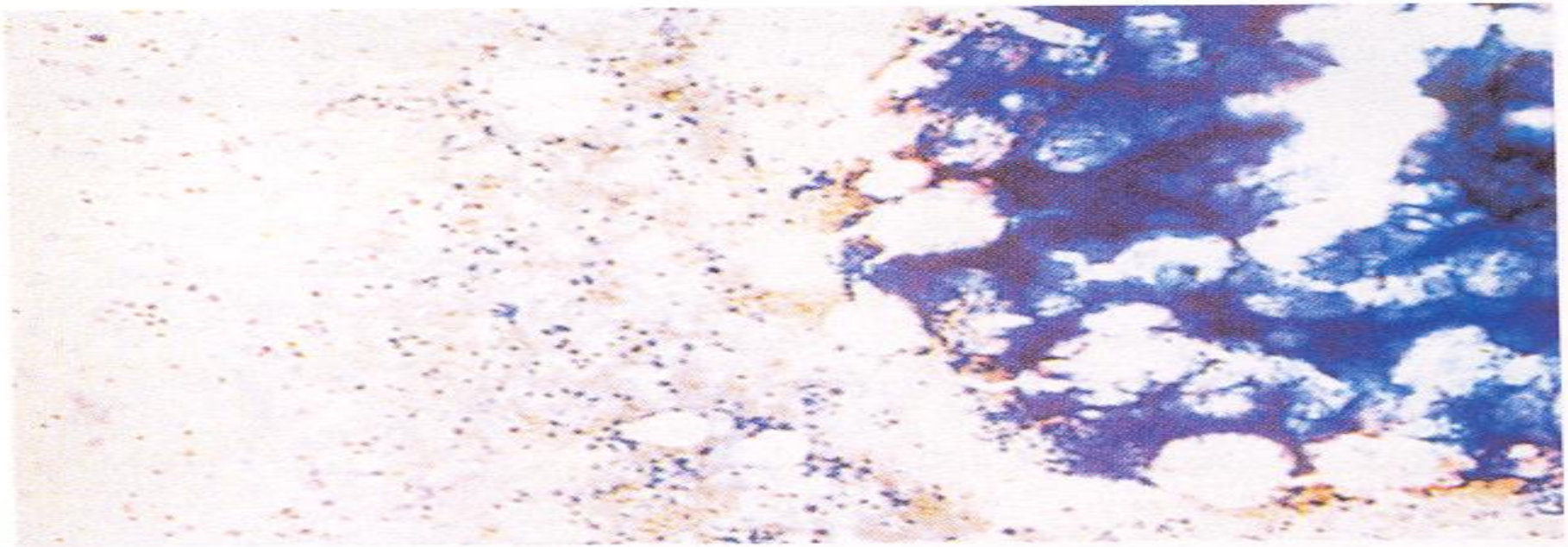
Intravascular and extravascular haemolysis

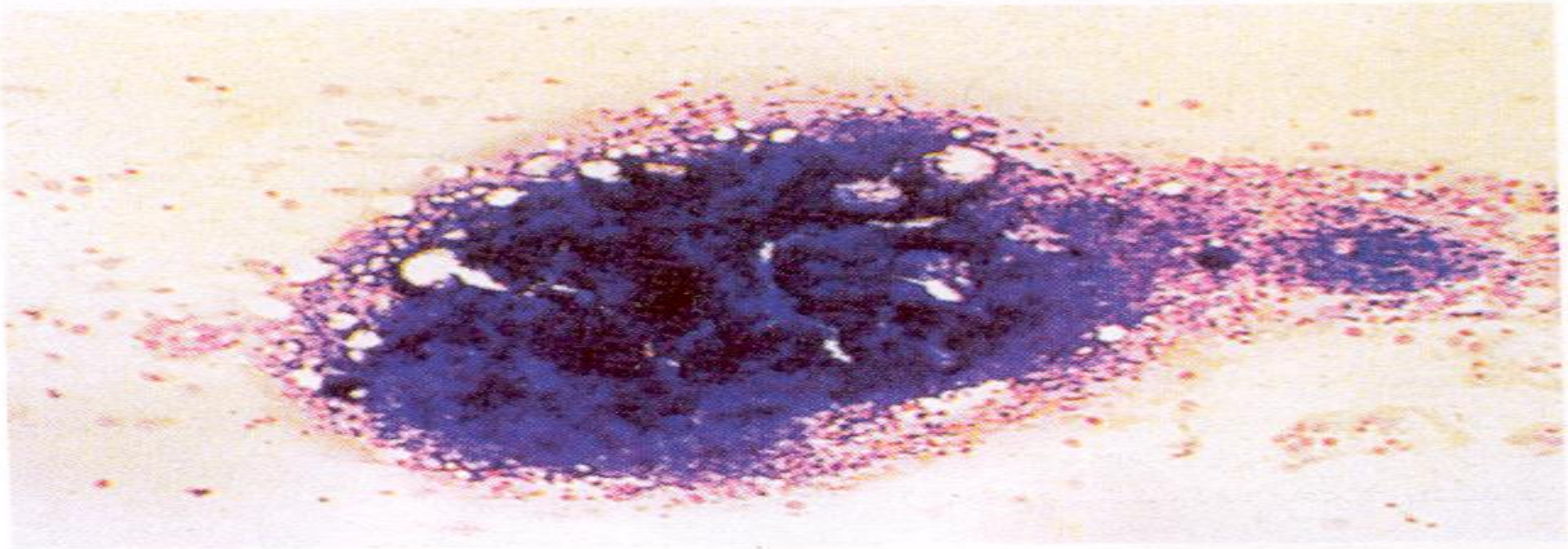
a. Intravascular haemolysis, the process of breakdown of red cells directly in the circulation.
b. Extravascular haemolysis excessive removal of red cells by cells of RE system in the spleen and liver.


(a) Extravascular
Macrophage
RBC
Globin
Iron
Protoporphyrin
Amino acids
Binds to transferrin
Bilirubin
Amino acids
Unconjugated bilirubin
Liver
Kidney
Bilirubin glucuronides
Reabsorbed
Gut
Urobilinogen (urine)
Stercobilinogen (faeces)
(b) Intravascular
RBC
Lysis
Haemoglobin
Methaemalbumin
Kidney
Haemoglobinuria
Haemosiderinuria

The main laboratory features of intravascular haemolysis are as follows:

1. **Haemoglobinaemia and haemoglobinuria.**
2. **Haemosiderinuria (Iron storage protein in the spun deposit of urine).**





Causes of intravascular haemolysis

- Mismatched blood transfusion (usually ABO)
- G6PD deficiency with oxidant stress
- Red cell fragmentation syndromes
- Some autoimmune haemolytic anaemias
- Some drug-and infection-induced haemolytic anaemias
- Paroxysmal nocturnal haemoglobinuria
- March haemoglobinuria
- Unstable haemoglobin

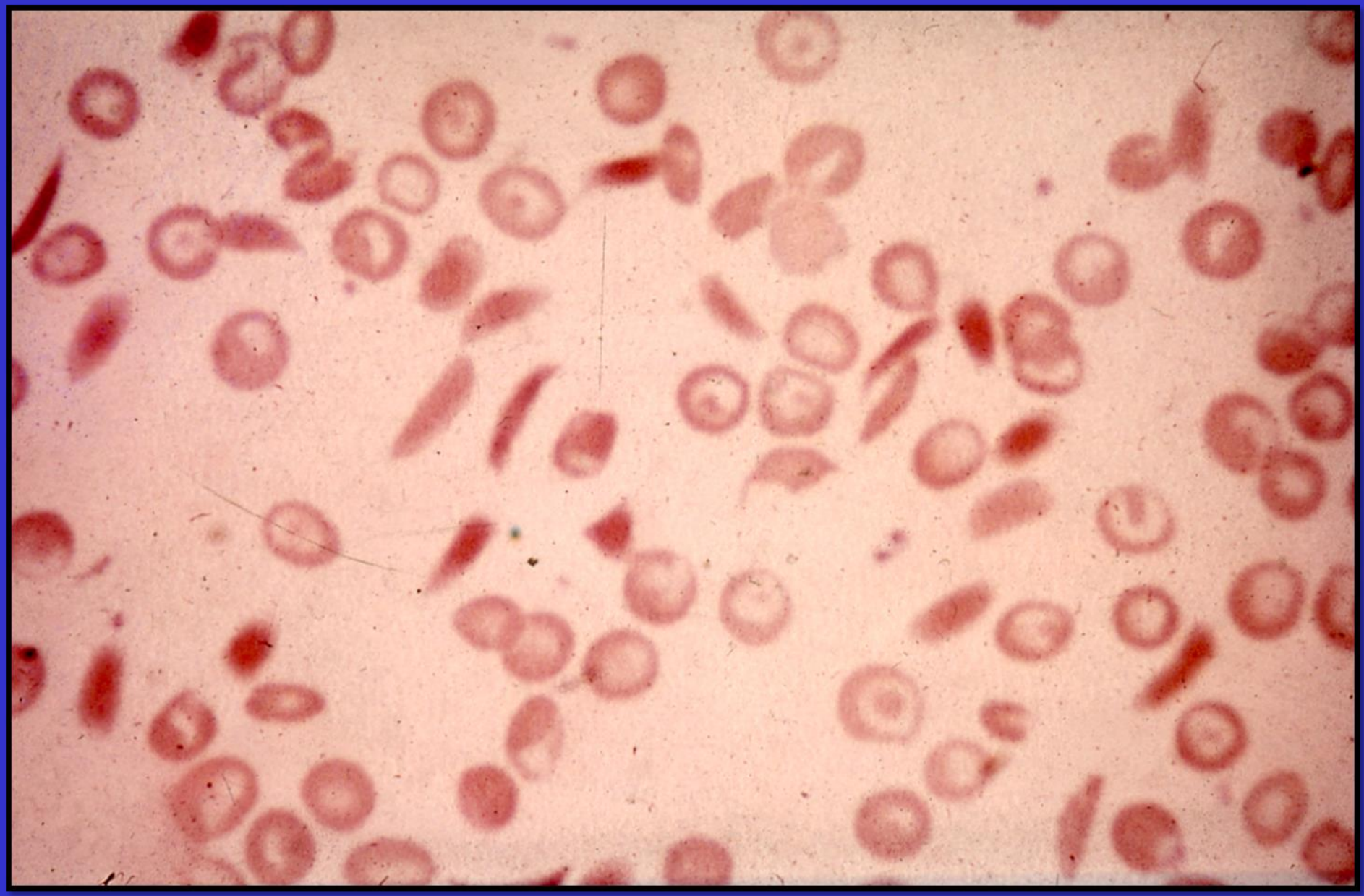
HAEMOLYTIC ANAEMIA

A . CONGENITAL

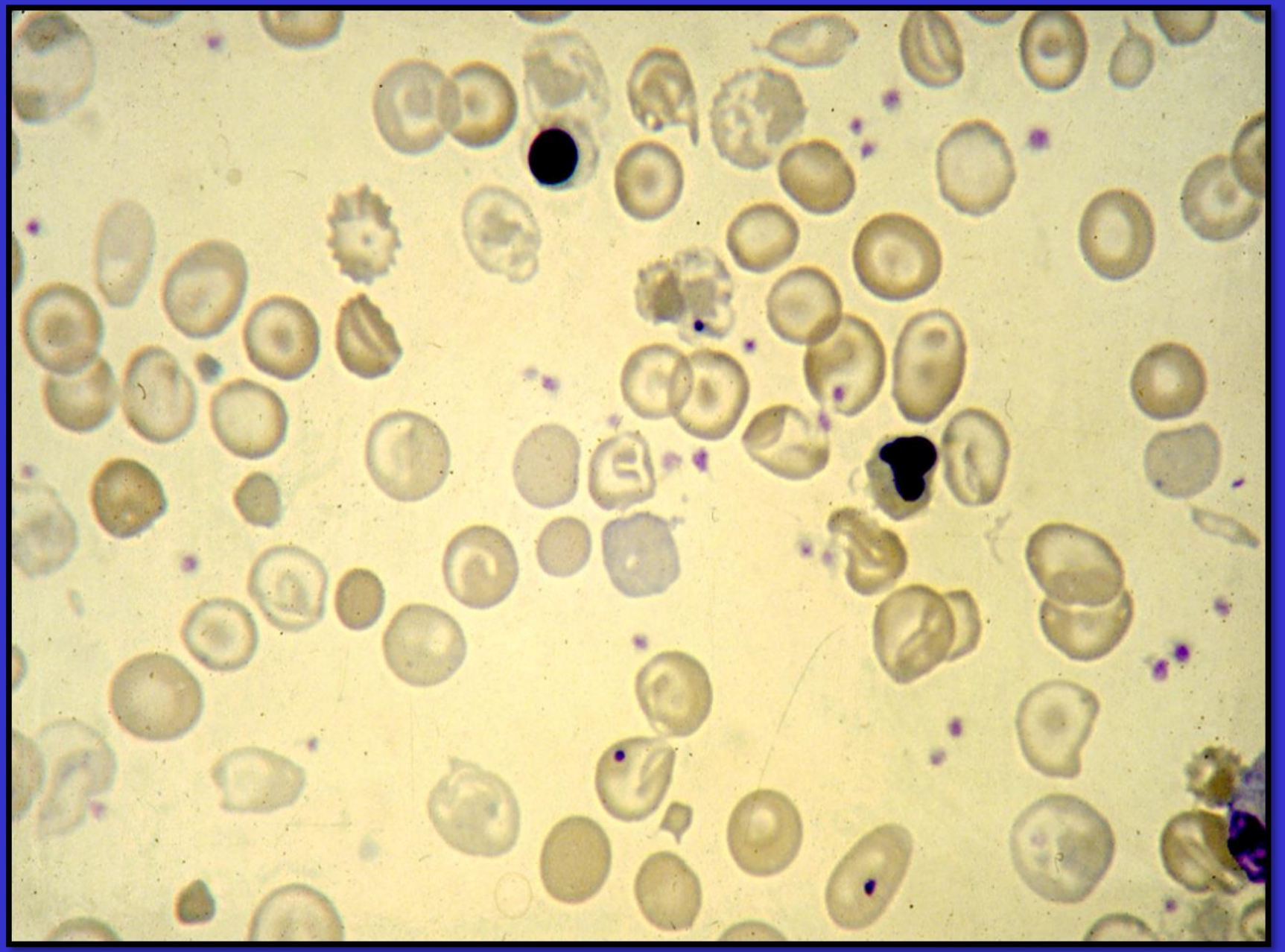
- **SICKLE CELL DISEASE & OTHER HAEMOGLOBIN DISORDERS**
- **THALASSAEMIAS**
- **ENZYMOPATHIES**
- **MEMBRANOPATHIES**

B. AQUIRED

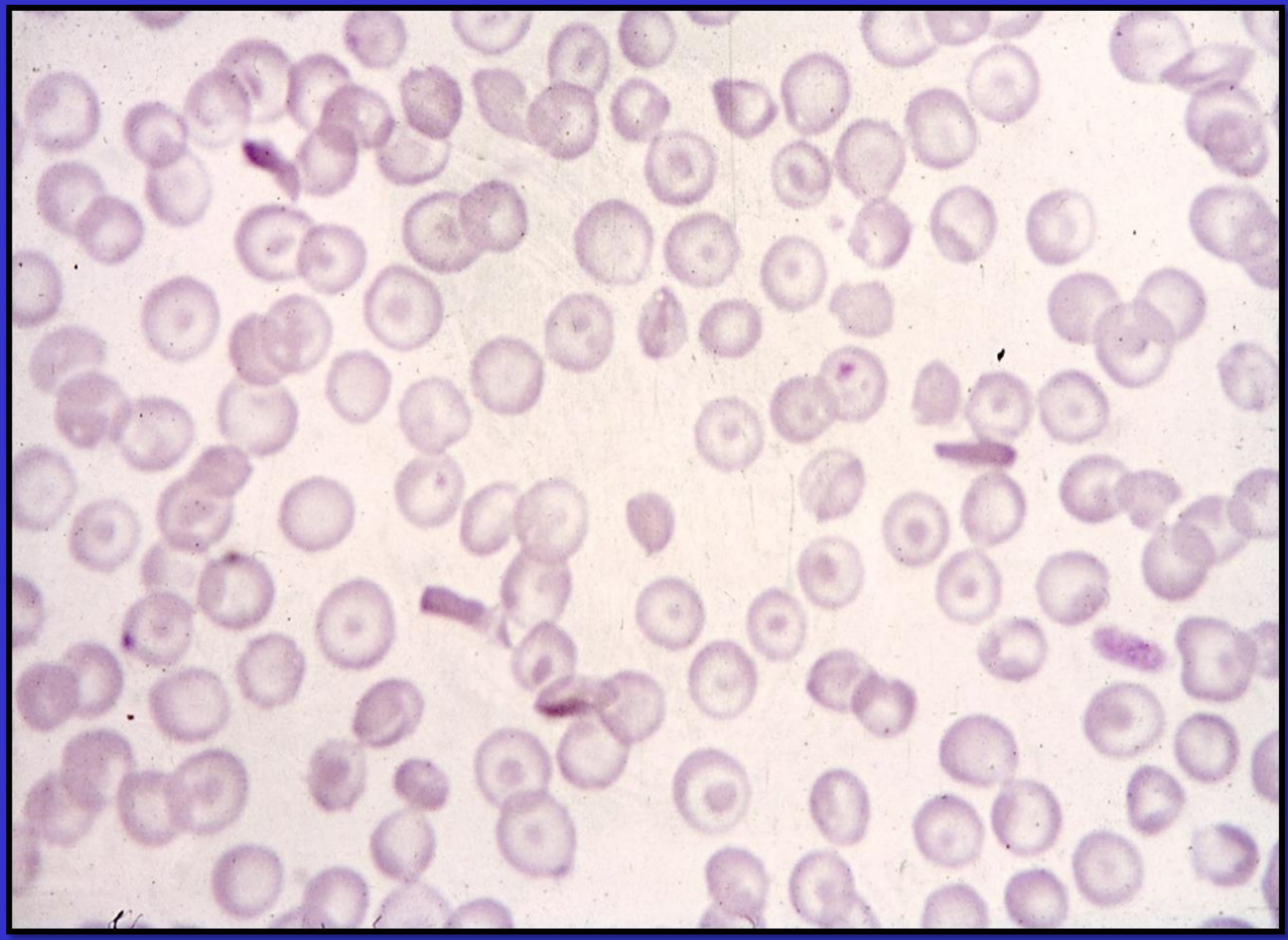
SICKLE CELL ANAEMIA



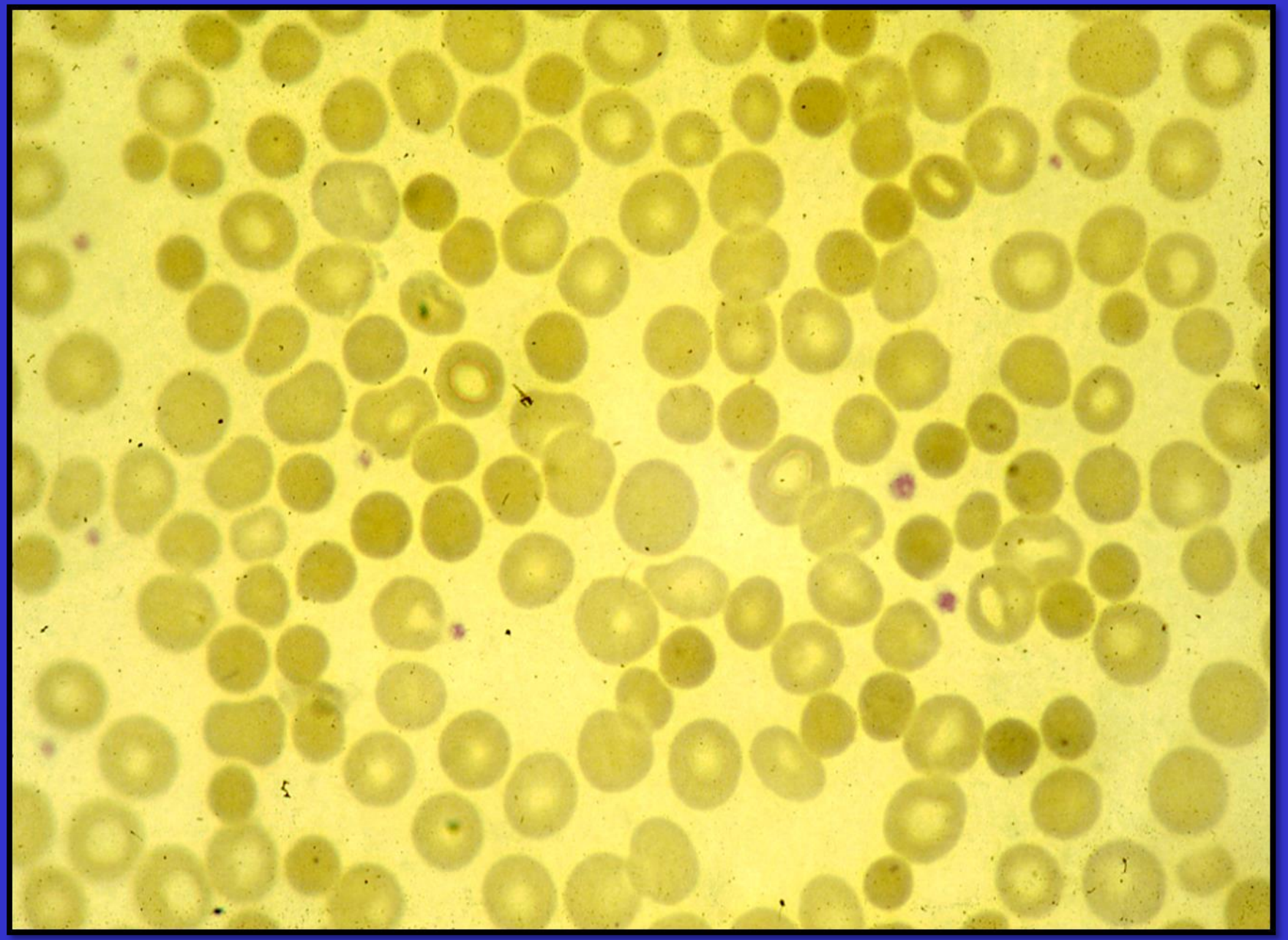
THALASSAEMIA MAJOR



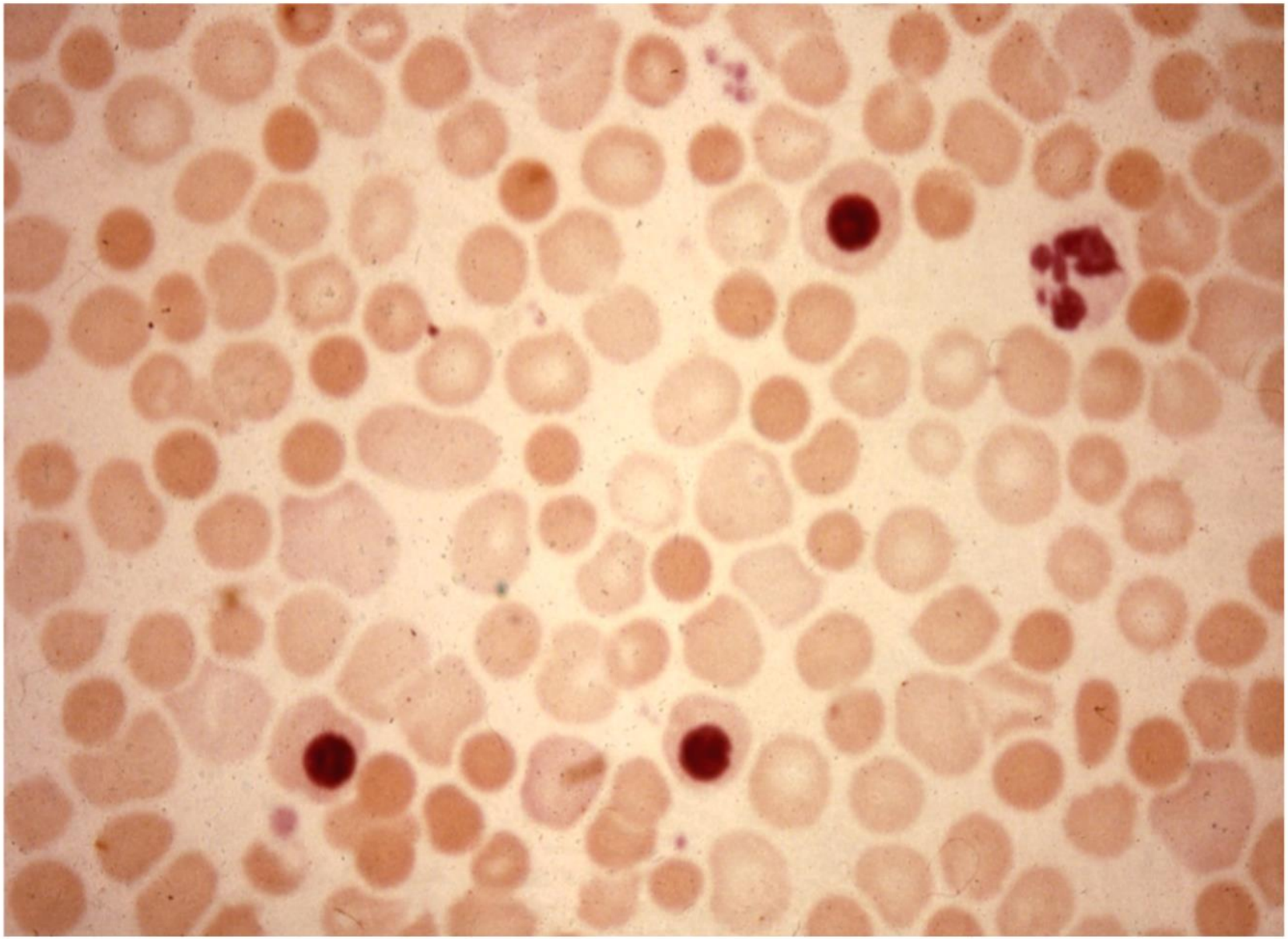
SICKLE BETA-THALASSAEMIA



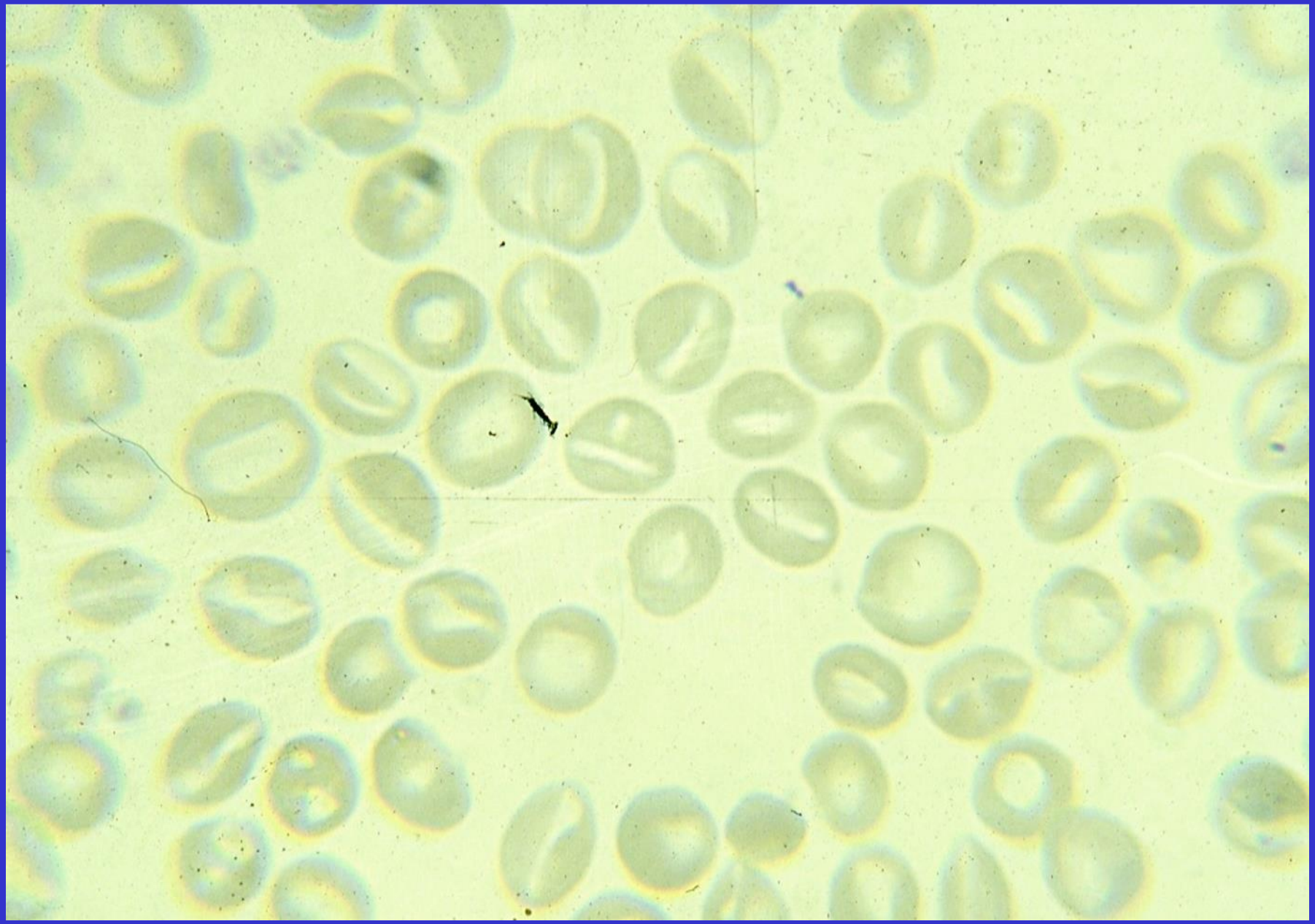
SPHEROCYTOSIS

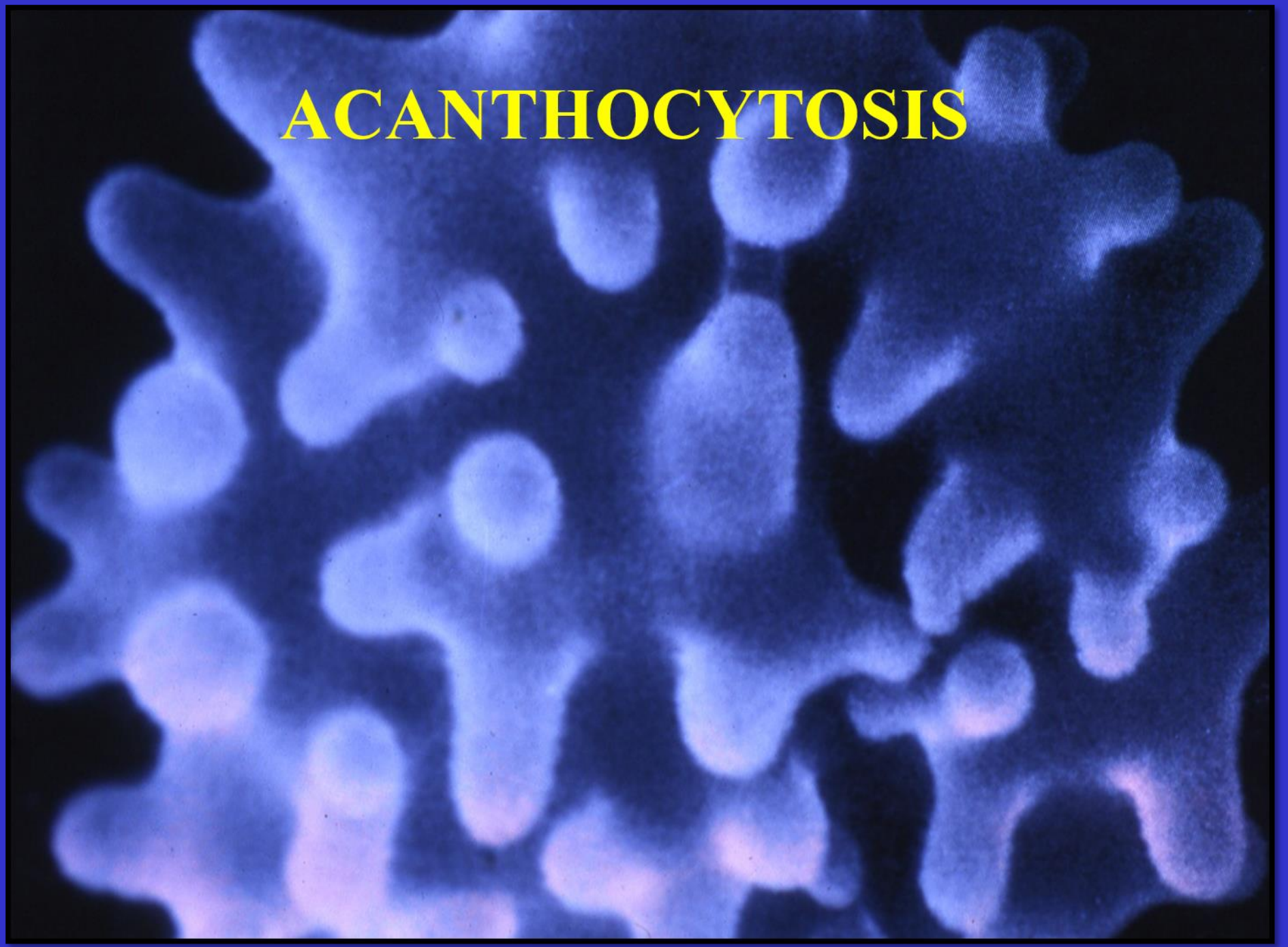


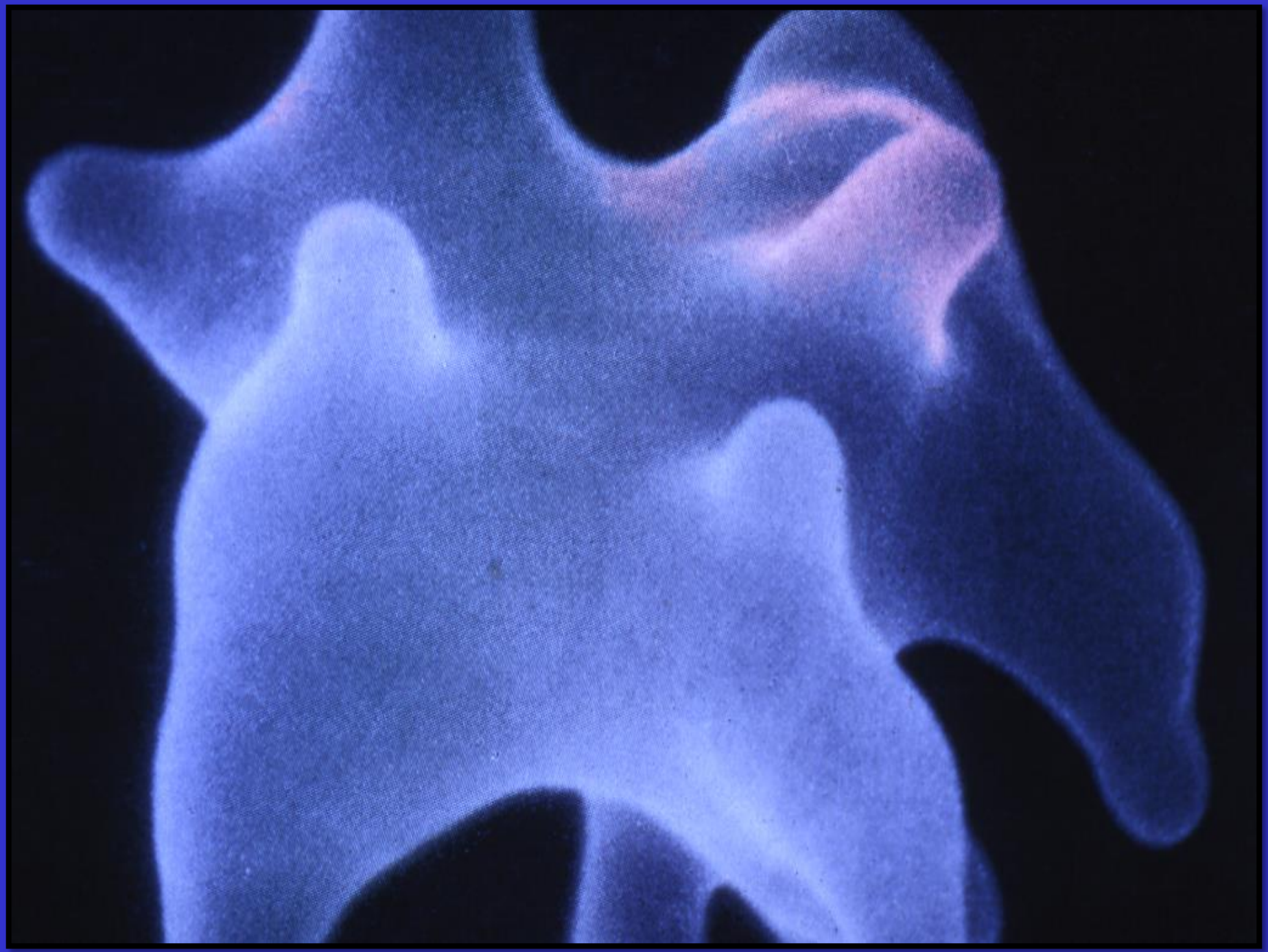
SPHEROCYTOSIS
NEW BORN



STOMATOCYTOSIS

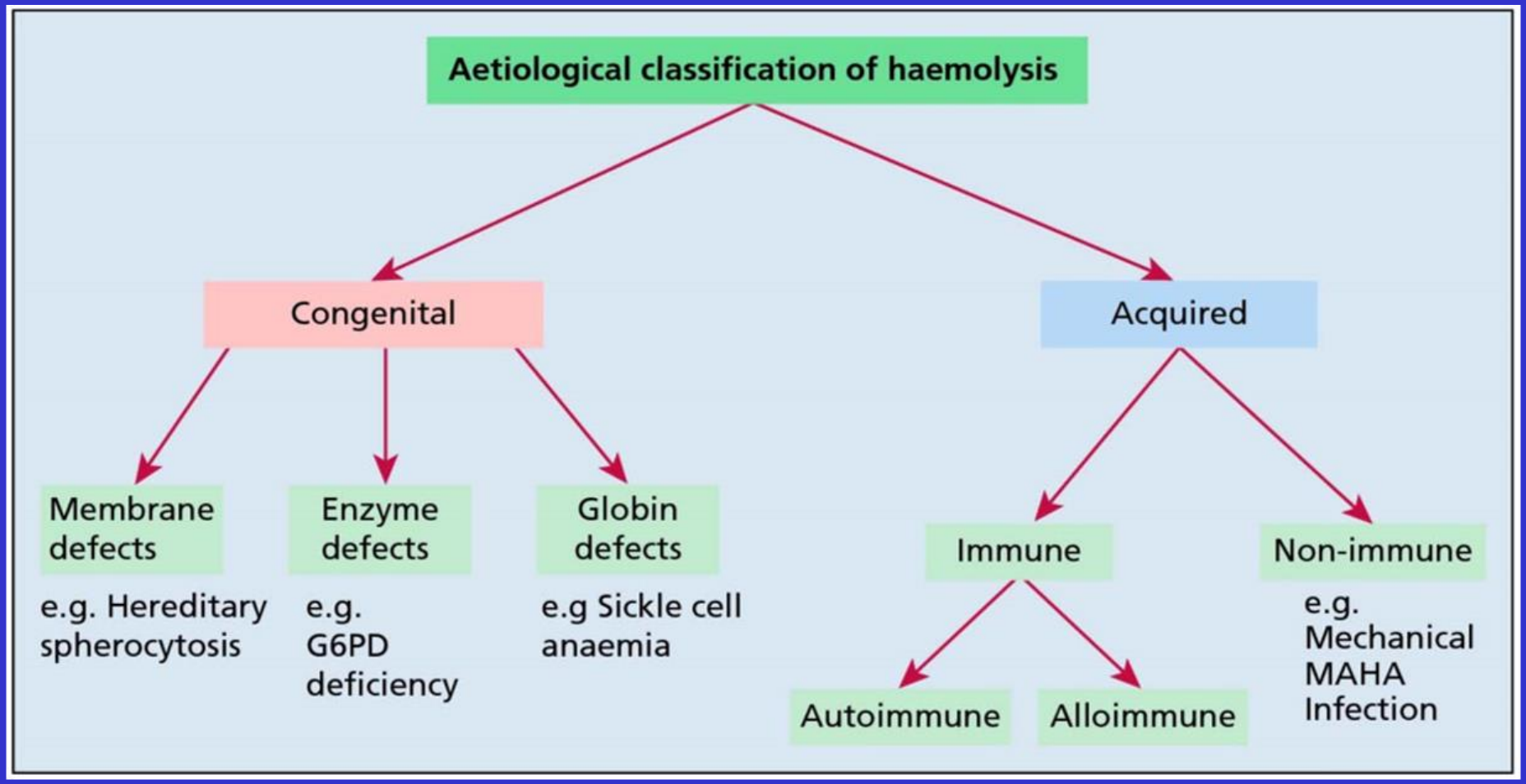



ACANTHOCYTOSIS

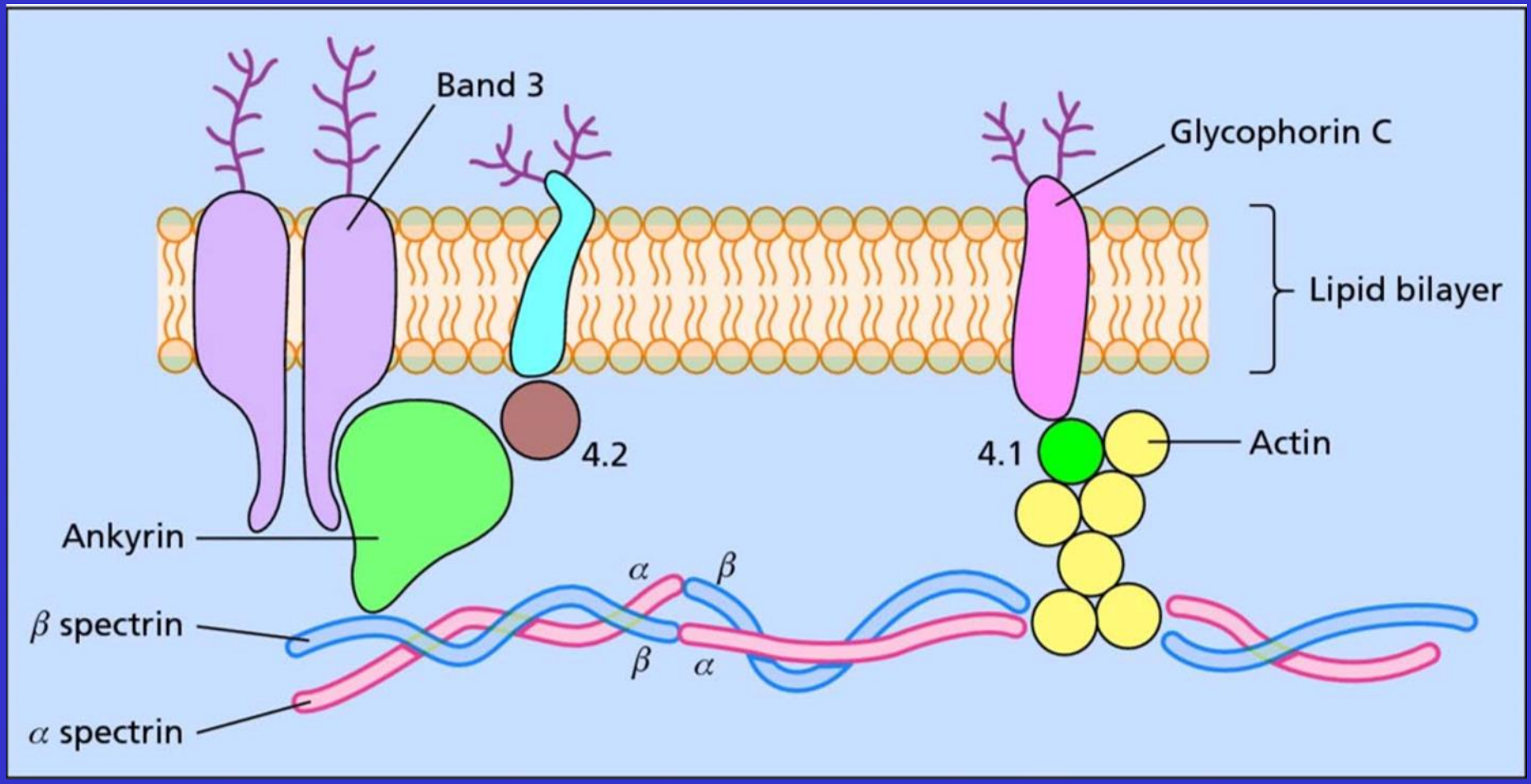


Classification Of Haemolytic Anaemias

Hereditary	Acquired
Haemoglobin	Allografts, especially marrow transplantation
Abnormal (Hb S, Hb C, unstable)	drug associated
Thalassaemia	**Red cell fragmentation syndrome**
Membranopathy	Arterial grafts, cardiac valves
Enzymopathy	Microangiopathic
	Thrombotic thrombocytopenic purpura
	Haemolytic uraemic syndrome
	Meningococcal sepsis
	Pre-eclampsia
	Disseminated intravascular coagulation
	March haemoglobinuria
	Infections
	Malaria, clostridia
	Chemical and physical agents
	Especially drugs, inductrial/domestic substances, burns
	Secondary
	Liver and renal disease
	Paroxysmal nocturnal haemoglobinuria



A classification of haemolytic anaemia by aetiology. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; MAHA, microangiopathic haemolytic anaemia.



Schematic diagram of the red cell membrane cytoskeleton.

CONGENITAL HAEMOLYTIC ANAEMIAS

Haemolysis due to defects of the red cell membrane

- Since the diameter of a normal red cell is similar to that of the smallest capillary lumen, it is essential for the red cell to be able to undergo significant deformations while traversing the circulation.
- A flexible red cell cytoskeleton, which interacts with red cell phospholipid membrane.
- Key components of the cytoskeleton include α and β spectrin, actin and protein 4.1, while connections linking the cytoskeleton to the overlying red cell phospholipid bilayer include band 3, Rh-associated glycoprotein and glycophorin C.
- Defects in any of these proteins can jeopardize the integrity of the red cell and shorten its lifespan.

Hereditary spherocytosis (HS)

- The most common haemolytic anaemia due to a membrane defect is hereditary spherocytosis (HS) in Caucasian
- About 60% of patients have mutations affecting the Ankyrin gene
- Loss of Ankyrin then leads to secondary reductions in spectrin and protein 4.1
- This leads to a loss of membrane surface area, with cells adopting a spheroid rather than biconcave shape
- Less deformable than normal red cells
- Destroyed by splenic macrophages, leading to a reduction in red cell survival by extravascular haemolysis

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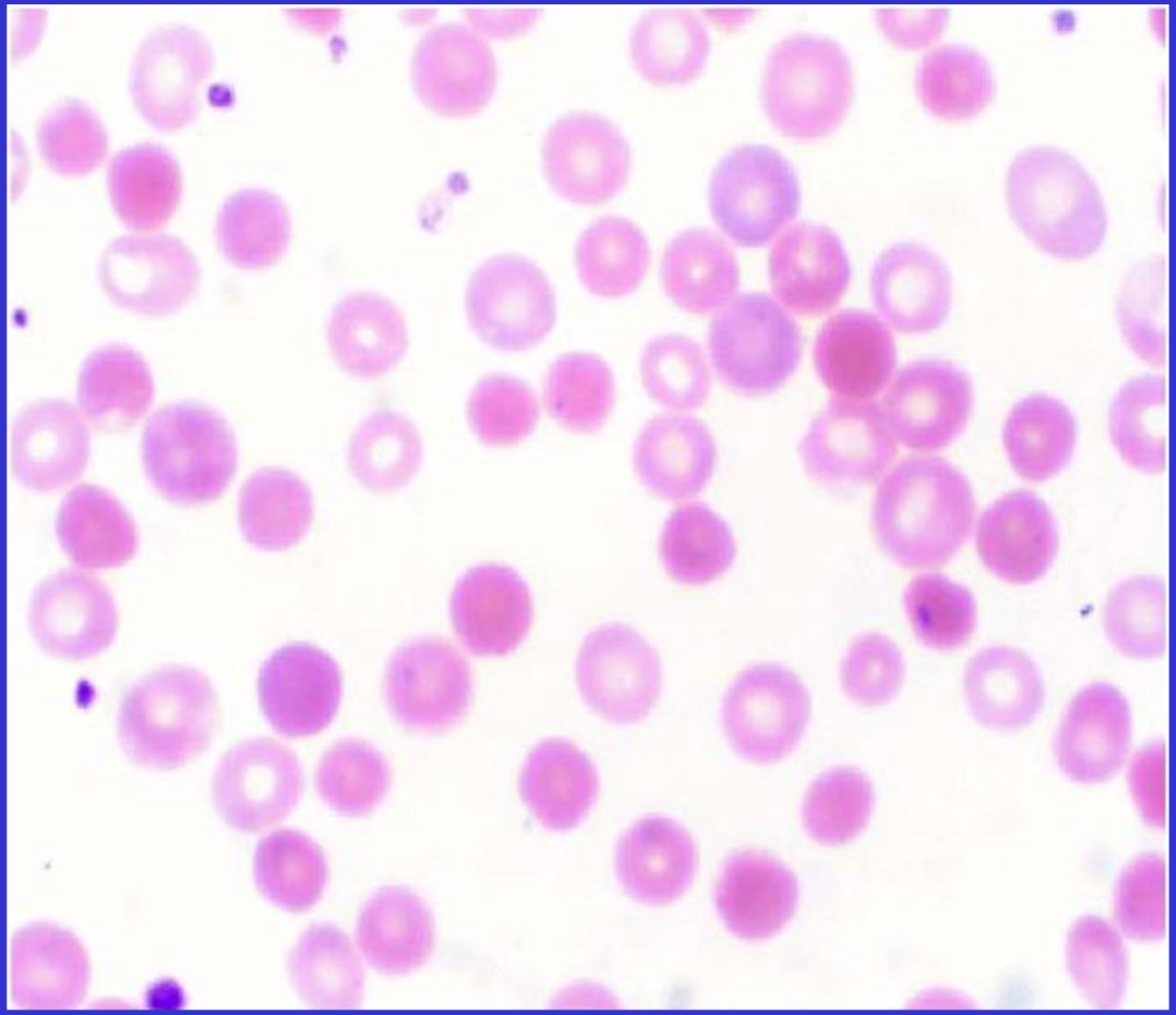
- Around 20% of all HS patients have mild disease with well-compensated haemolysis
- The majority of patients have moderate disease characterized by a Hb concentration of 8-11g/dl, while a small percentage have severe disease requiring intermittent or even regular transfusions
- Complications of the chronic haemolysis in HS include the development of pigment gallstones
- Aplastic crises may occur secondary to parvovirus B19 infection
- Megaloblastic anaemia due to folate deficiency is also occasionally found



A blood film from a patient with HS showing many spherocytes.

Diagnosis and management

- Family history
- Mild jaundice
- Pallor and splenomegaly
- Laboratory findings (anaemia, reticulocytosis and elevated plasma bilirubin
- Presence of spherocytes on the peripheral blood film
- The eosin-5-maleamide (EMA) binding test (may be used if more definitive evidence for the diagnosis is needed)
- The red cell membrane proteins study
- Electrophoresis on a denaturing polyacrylamide gel

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The treatment of HS:

- Folic acid supplementation
- Splenectomy (children with severe disease). Splenectomy will, however, increase the risk of significant infection, particularly from encapsulated organisms. This risk is especially marked in children under the age of 5, delayed until age 5-10 years.
- Administration of pneumococcal and meningococcal vaccine and *Haemophilus influenzae* type b vaccine (splenectomy preoperative preparation)
- Prophylactic penicillin V is advised lifelong (post splenectomy)

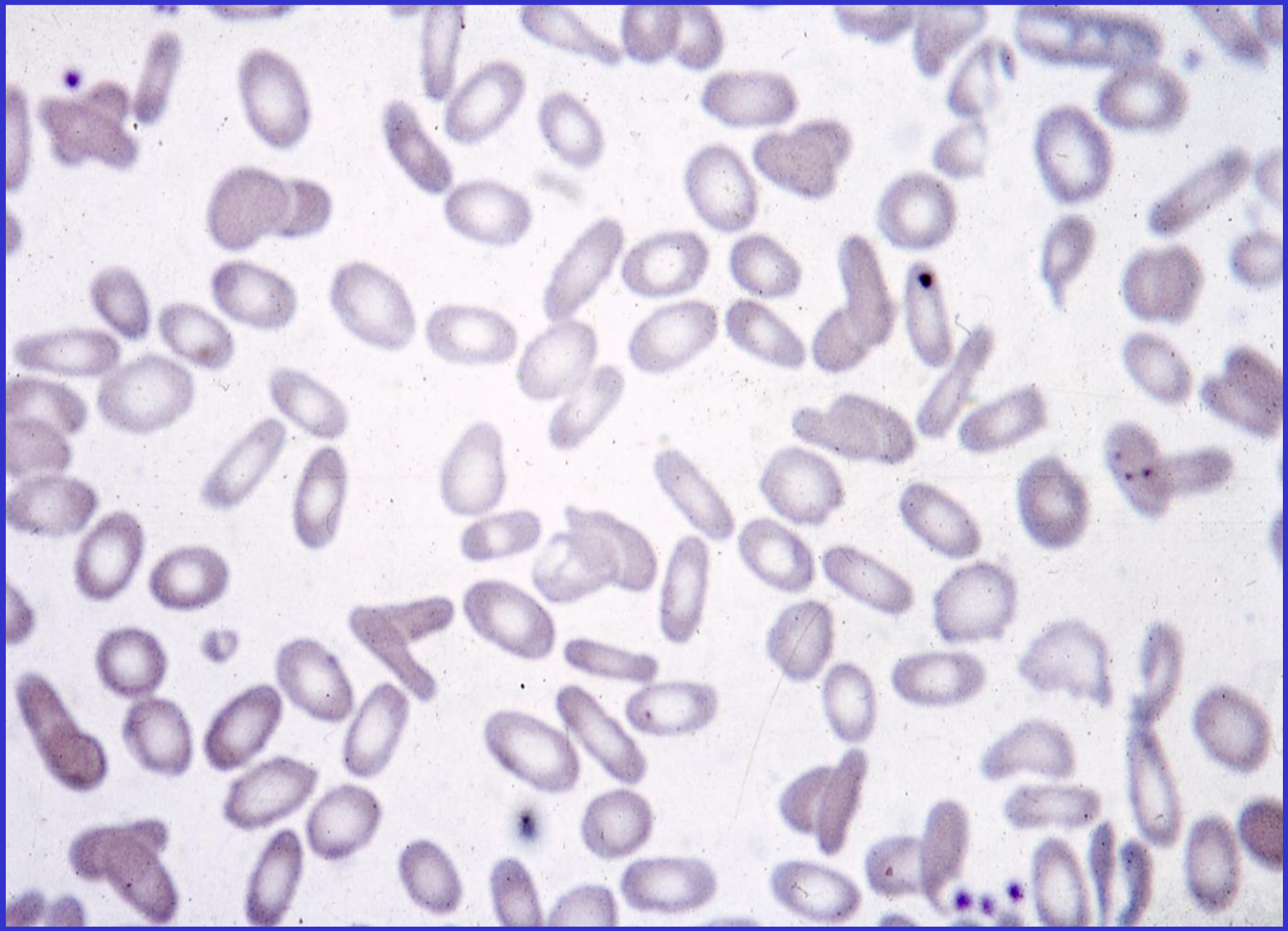
Hereditary elliptocytosis

- Hereditary elliptocytosis (HE) is also a relatively common condition
- Defects in α spectrin
- Most patients are clinically asymptomatic, some will have a chronic symptomatic haemolytic anaemia.
- All show the very characteristic red cell shape on peripheral blood films.

Hereditary pyropoikilocytosis

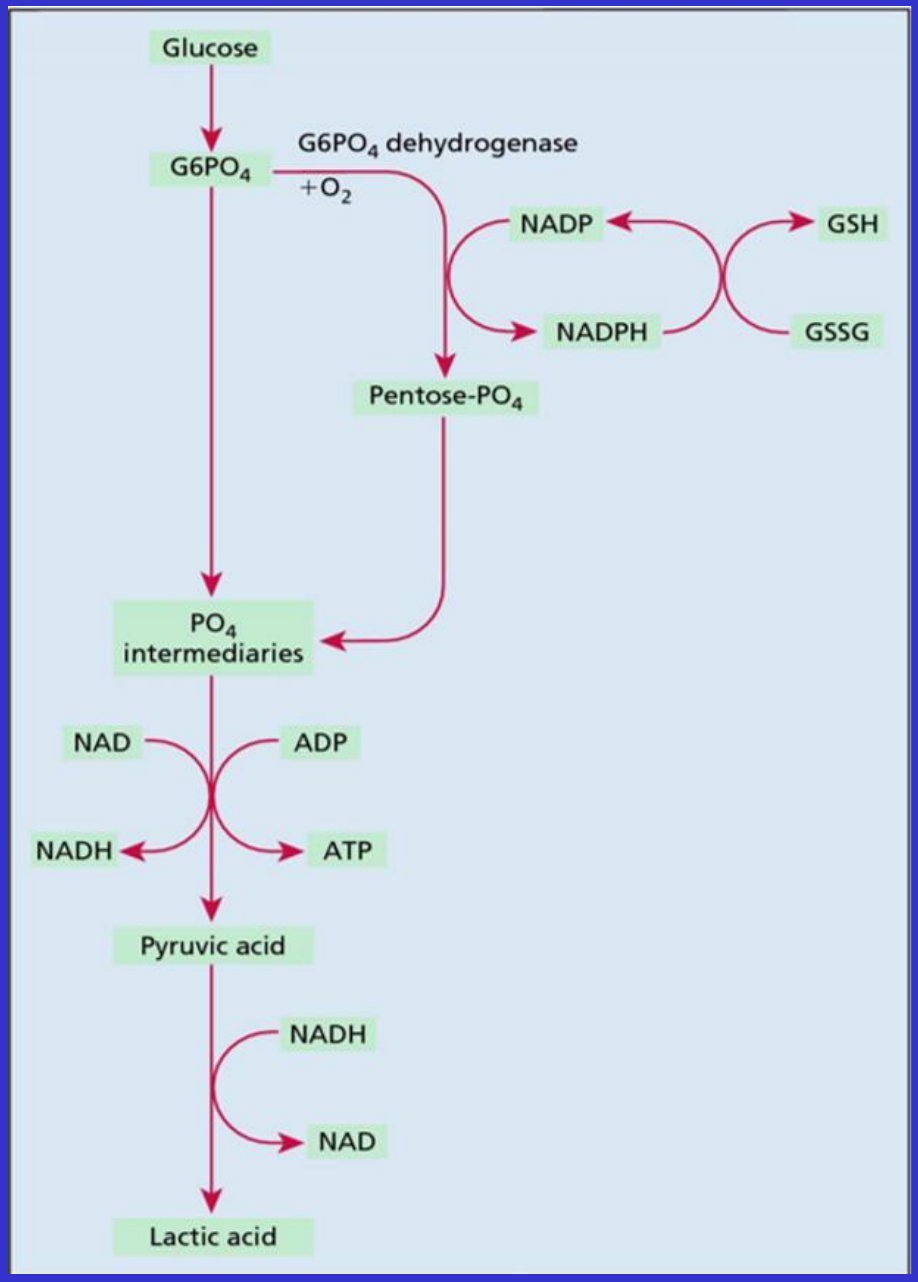
- Severe disturbance of the multimerization of spectrin
- Severe haemolytic anaemia from infancy
- Bizarre peripheral blood morphology, including microspherocytes and poikilocytes. Such patients are described as having hereditary pyropoikilocytosis.

ELLIPTOCYTOSIS



Haemolysis due to abnormalities of red cell enzymes

Haemolytic anaemias may also result from congenital abnormalities of the enzymes required for energy transfer in glucose metabolism. The red cell needs a continuous supply of energy for the maintenance of membrane flexibility and cell shape, the regulation of sodium and potassium pumps, and the maintenance of Hb in the reduced ferrous form.



A schematic diagram of the pathway of glucose metabolism in the red cell, to show the important role of G6PD. A decreased activity of the enzyme leads to a deficiency of the reducing compounds NADPH and GSH.

Glucose-6-phosphate dehydrogenase deficiency

- ✓ Deficiency of glucose-6-phosphate dehydrogenase (G6PD), the first enzyme of the pentose-phosphate shunt, will prevent the normal generation of NADPH, with subsequent erythrocyte sensitivity to oxidative stress.
- ✓ Various point mutations in the G6PD gene on the X chromosome resulting in enzymes with altered activity.
- ✓ The normal G6PD enzyme is designated type B and is the prevalent form worldwide; G6PD type A is a normal variant found in approximately 20% of healthy individuals of African ancestry.
- ✓ Defective forms of G6PD include the A- African variant and the Mediterranean variant.
- ✓ Since the gene for G6PD is found on the X chromosome, affected individuals are male.

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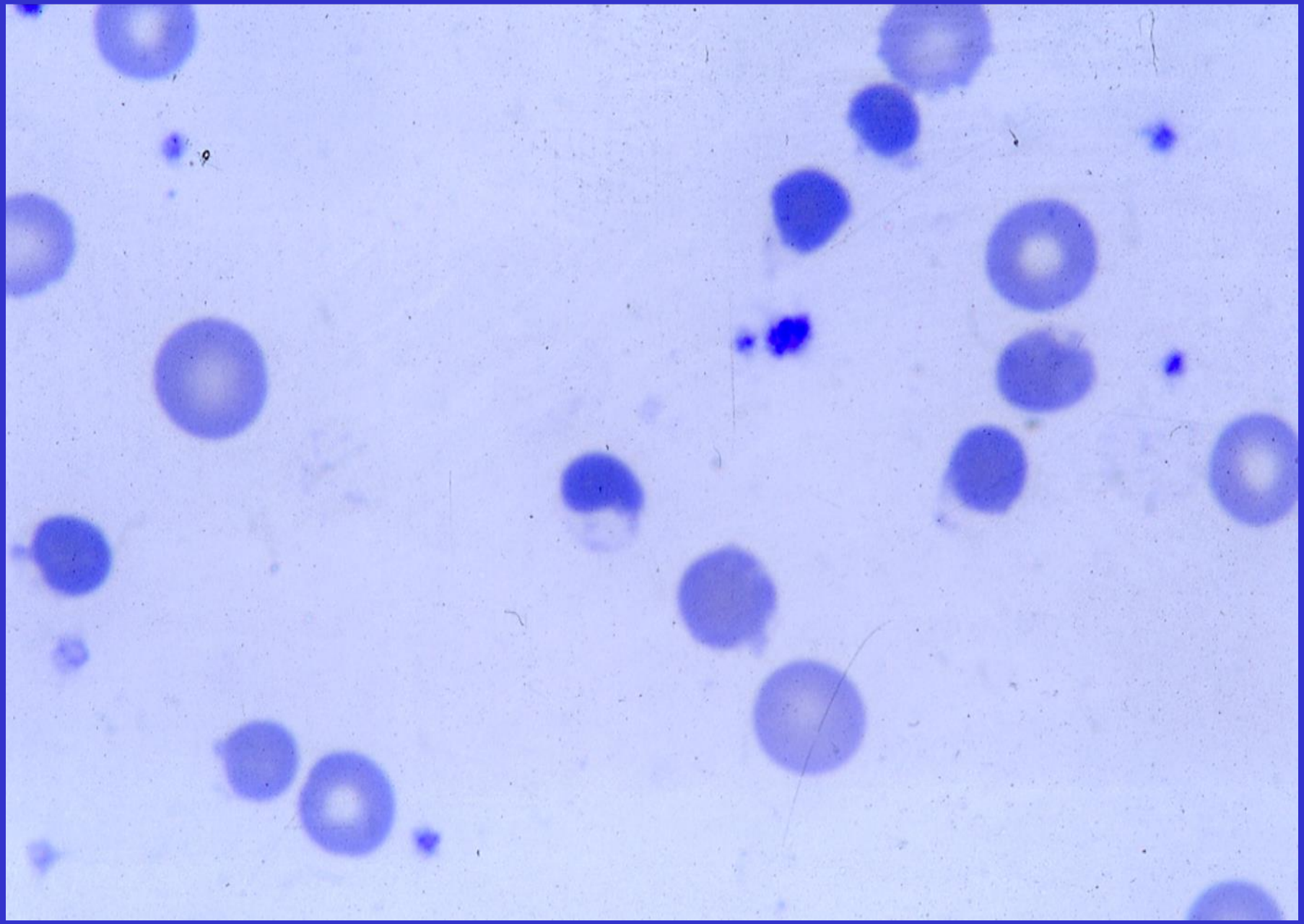
- ✓ Patients with low levels of the enzyme are poorly protected against oxidative challenge - with some medications and even foods resulting in marked oxidative damage to the red cell.
- ✓ When the red cell is exposed to oxidants, haemoglobin is converted to methaemoglobin and denatured.
- ✓ Denatured haemoglobin then precipitates forming inclusions in the red cell (termed Heinz bodies and detected by supravital staining, as in Figure 3.7).
- ✓ Heinz bodies, and the portion of the red cell membrane to which they become attached, are removed by splenic macrophages as the red cells pass through the spleen; the resulting inclusion-free cells display unstained areas at their periphery ('bite' cells, seen in Figure 3.8).

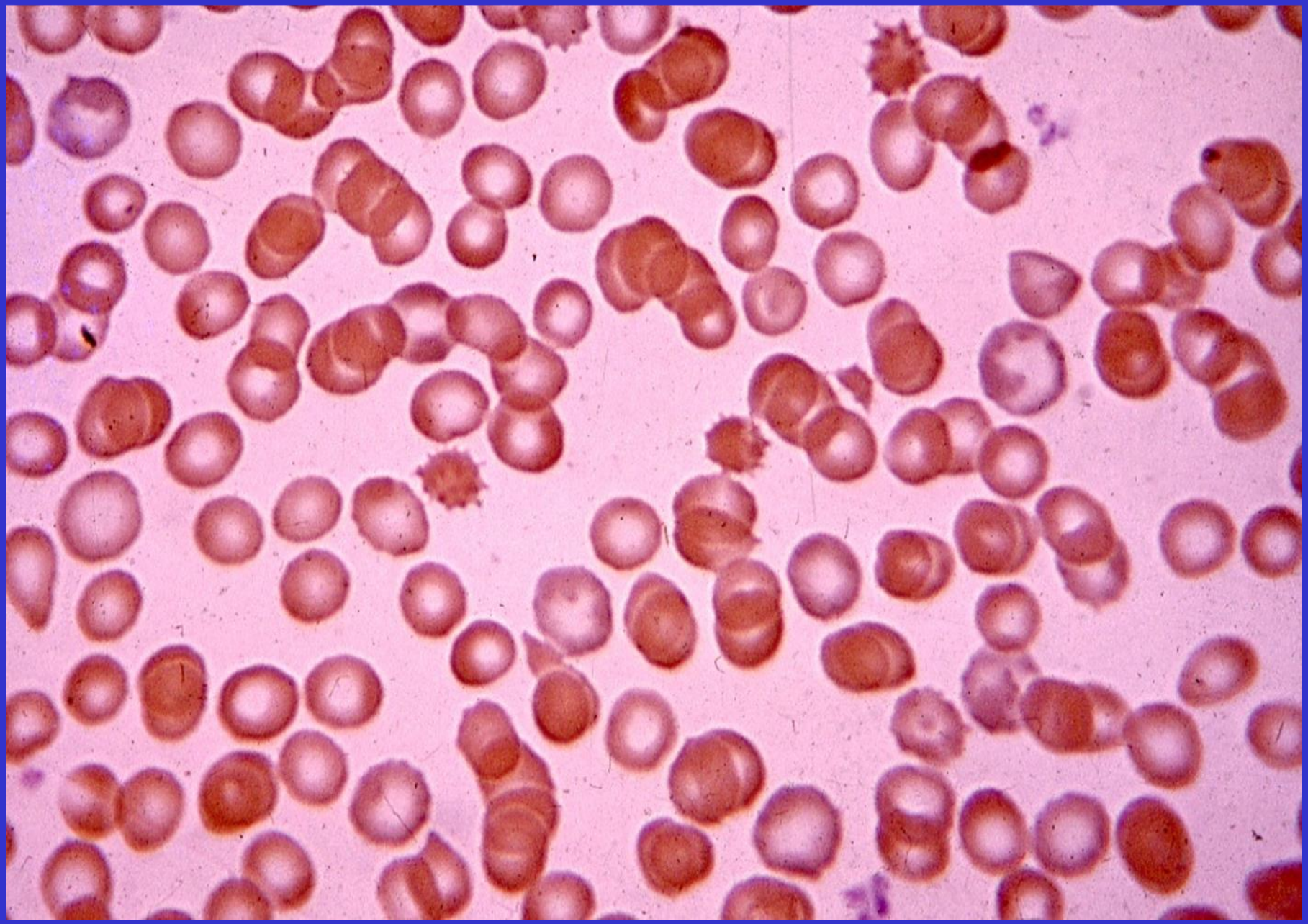
cont'd…

- ✓ Screening tests and assays for detecting G6PD deficiency are available.
- ✓ Haemolysis typically begins 1-3 days following exposure to the oxidative stressor, with anaemia being maximal about 7-10 days after exposure.
- ✓ Patient may report dark urine due to haemoglobinuria.
- ✓ ***Favism*** **-** a syndrome in which an acute haemolytic anaemia occurs after the ingestion of the broad bean (*Vicia fava)* in individuals with a deficiency of G6PD (commonly of the Mediterranean type). Favism usually affects children; severe anaemia develops rapidly and is often accompanied by haemoglobinuria.

G6PD DEFICIENCY



PYRUVATE KINASE DEFICIENCY



Treatment of G6PD deficiency

- Treatment generally focuses on the avoidance of oxidative precipitants to haemolysis. In many cases, haemolysis is self limiting.
- Packed red cell transfusion may be required in cases of severe haemolysis.

Other red cell enzyme deficiencies causing haemolysis

- Pyruvate kinase deficiency is another relatively common example.
- There is usually a chronic haemolytic anaemia and some patients may benefit from splenectomy.

Haemolysis due to haemoglobin defects

- Defects in the structure of haemoglobin.
- Structural variants of the globin chains may affect the lifespan of the red cell, with sickle cell anaemia being the best-described example.
- A tendency of the HbS variant to polymerize under conditions of low oxygen tension leads to distortion of the erythrocyte in the well-recognized sickle shape.

Acquired haemolytic anaemias

In the acquired haemolytic anaemias, red cells may be destroyed either by immunological or by non-immunological mechanisms.

Immune haemolytic anaemias

- In these conditions, antigens on the surface of red cells react with antibodies sometimes with complement activation. IgG-coated red cells interact with the Fc receptors on macrophages in the spleen, and are then either completely or partially phagocytosed. When the phagocytosis is partial, the damaged cell will return to the circulation as a spherocyte. Red cells that are also coated with the activated complement component C3 may interact with C3 receptors on macrophages and are usually completely phagocytosed. In most instances where complement is activated, the cascade sequence only proceeds further and permits deposition of the membrane attack complex (C5-C9) with resultant intravascular haemolysis.

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- The immune haemolytic anaemias may be due to autoantibodies; that is, antibodies formed against one or more antigenic constituents of the individual's own tissues. These include autoimmune haemolytic anaemia (AIHA) and some drug-related haemolytic anaemias. It is also possible to develop alloimmune haemolytic anaemia, consequent on the production of antibodies against red cells from another individual, as in haemolytic transfusion reactions and haemolytic disease of the newborn.

Autoimmune haemolytic anaemias

‘Warm’ autoantibodies react best with the red cell antigen at 37°C and are usually of IgG subtype.

‘Cold’ antibodies react best at temperatures below 32ºC (usually below 15ºC) and, since they are usually of IgM subtype, are capable of agglutinating red cells.

Classification of AIHAs

Caused by warm-reactive antibodies

Idiopathic

Secondary (chronic lymphocytic leukaemia, Lymphoma, systemic lupus erythematosus (SLE), some drugs)

Caused by cold-reactive antibodies

Cold haemagglutinin disease

Idiopathic

Secondary (*Mycoplasma pneumoniae* infection, infectious mononucleosis, lymphomas)

Paroxysmal cold haemoglobinuria

Idiopathic

Secondary (some viral infections, congenital and tertiary syphilis)

Warm AIHA

1) In idiopathic warm AIHA, haemolysis dominates the clinical picture and no evidence can be found of any other disease.
2) In secondary AIHA, the haemolysis is associated with a primary disease such as chronic lymphocytic leukaemia or systemic lupus erythematosus (SLE).

- The antibody-coated red cells undergo partial or complete phagocytosis in the spleen and by the Kupffer cells of the liver. There may be partial activation of the complement cascade.

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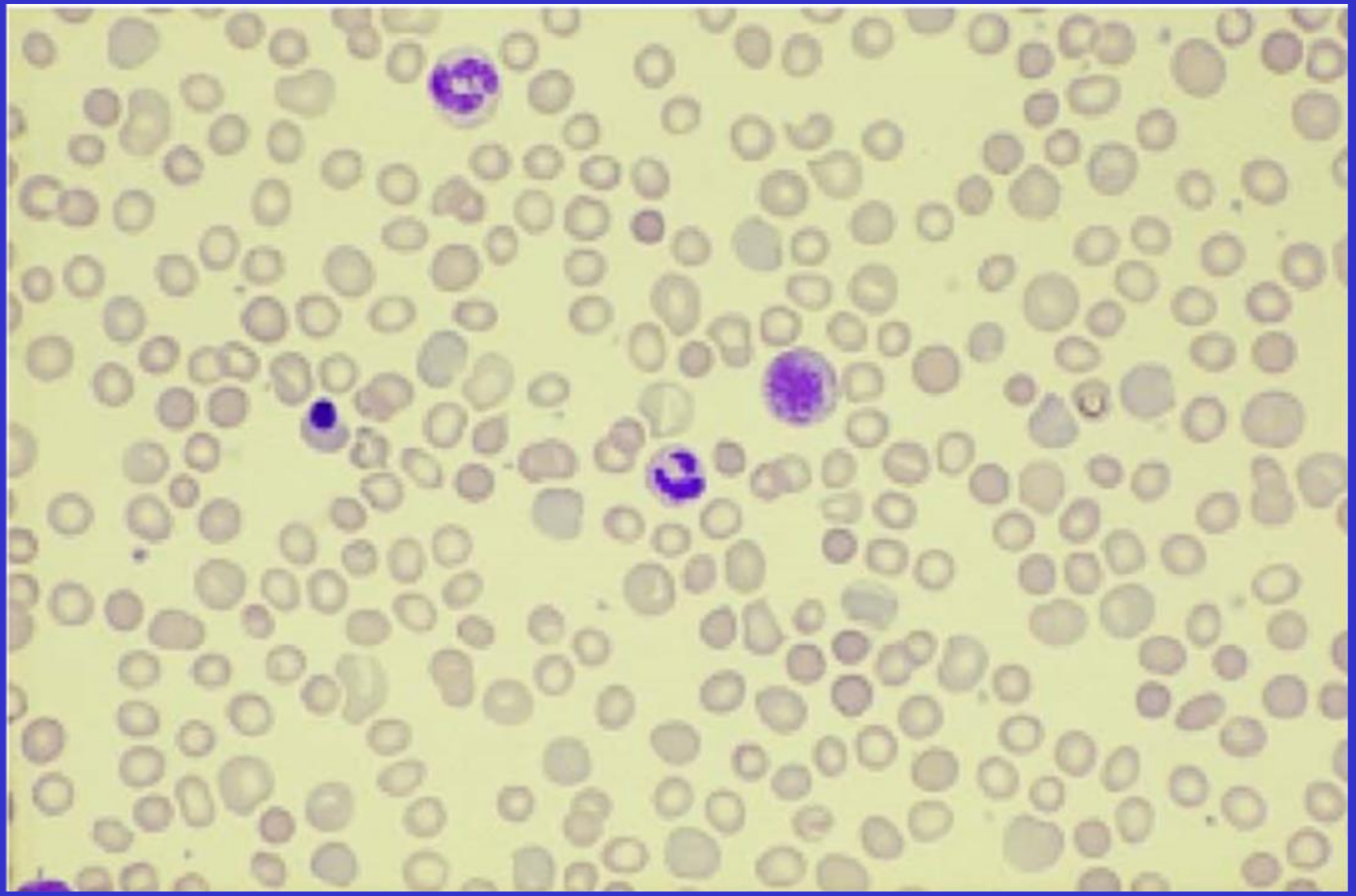
- Haematological findings include anaemia, spherocytosis, reticulocytosis and occasional nucleated red cells in the peripheral blood. The critical diagnostic investigation is the direct antiglobulin test.
- Haemolysis can be limited by treatment with prednisolone.
- If reduction in haemolysis is not maintained when the dose of steroids is lowered, splenectomy or alternative immunosuppressive therapy should be considered.
- The anti-CD20 monoclonal antibody rituximab, as well as immunosuppressants such as azathioprine or cyclophosphamide, may be beneficial in reducing autoantibody production.



Blood film from a patient with idiopathic AIHA (warm-reactive antibody) showing prominent spherocytosis and polychromasia

Cold haemagglutinin disease (CHAD)

- Since cold antibodies react with red cells only at temperatures below about 32ºC, they typically bind to the red cell surface in the cooler superficial blood vessels of the peripheries.
- Since the cold antibodies are typically of the IgM subtype, their pentameric structure permits direct agglutination of red cells coated with antibody; they are therefore sometimes termed cold agglutinins.
- Symptoms due to cold AIHA are worse during cold weather. Exposure to cold provokes acrocyanosis, due to the formation of agglutinates of red cells in the vessels of the skin.

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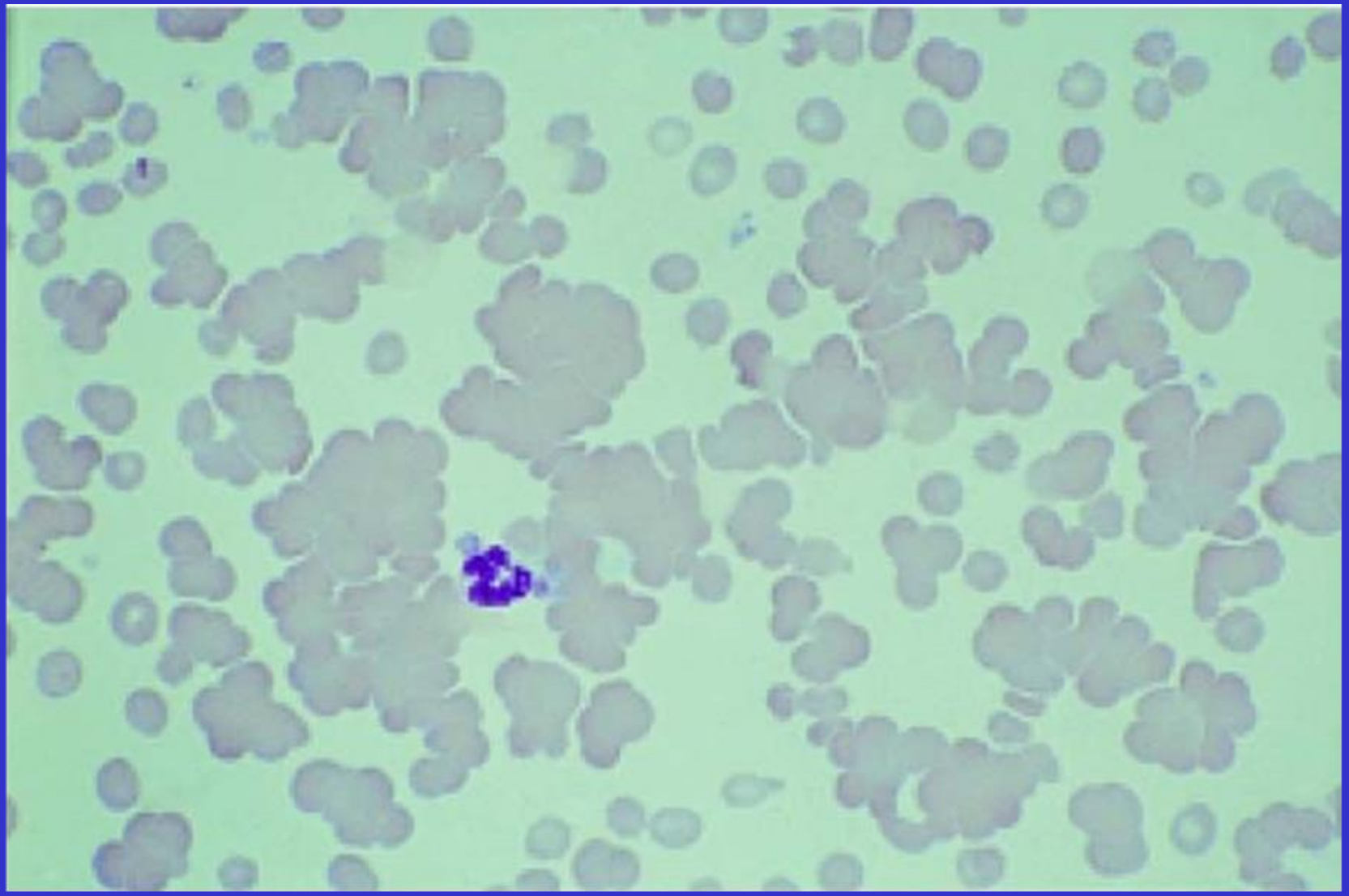
- The direct activation of the complement system leads to red cells lysis and, consequently, to haemoglobinaemia and haemoglobinuria.
- Chronic idiopathic CHAD is managed initially simply by keeping the patient warm.
- Treatment with rituximab may be effective.
- Other causes of haemolytic anaemia with an immune element include: 1) paroxysmal nocturnal haemoglobinuria; 2) paroxysmal cold haemoglobinuria; 3) drug-related haemolytic anaemias.



Numerous red cell agglutinates on a blood film from a patient with idiopathic CHAD.

Causes of acquired non-immune haemolytic anaemias.

Mechanical trauma to red cells

Abnormalities in the heart and large blood vessels

Aortic valve prostheses (Figure 3.11), severe aortic valve disease

Microangiopathic haemolytic anaemia

Haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura, metastatic malignancy, malignant hypertension, disseminated intravascular coagulation

March haemoglobinuria

Burns

Infections

Clostridium perfringes (welchii), malaria (Figures 3.12 and 3.13), bartonellosis

Drugs, *chemicals and venoms

Oxidant drugs and chemicals, arsine, acute lead poisoning, copper toxicity, venoms of certain spiders and snakes

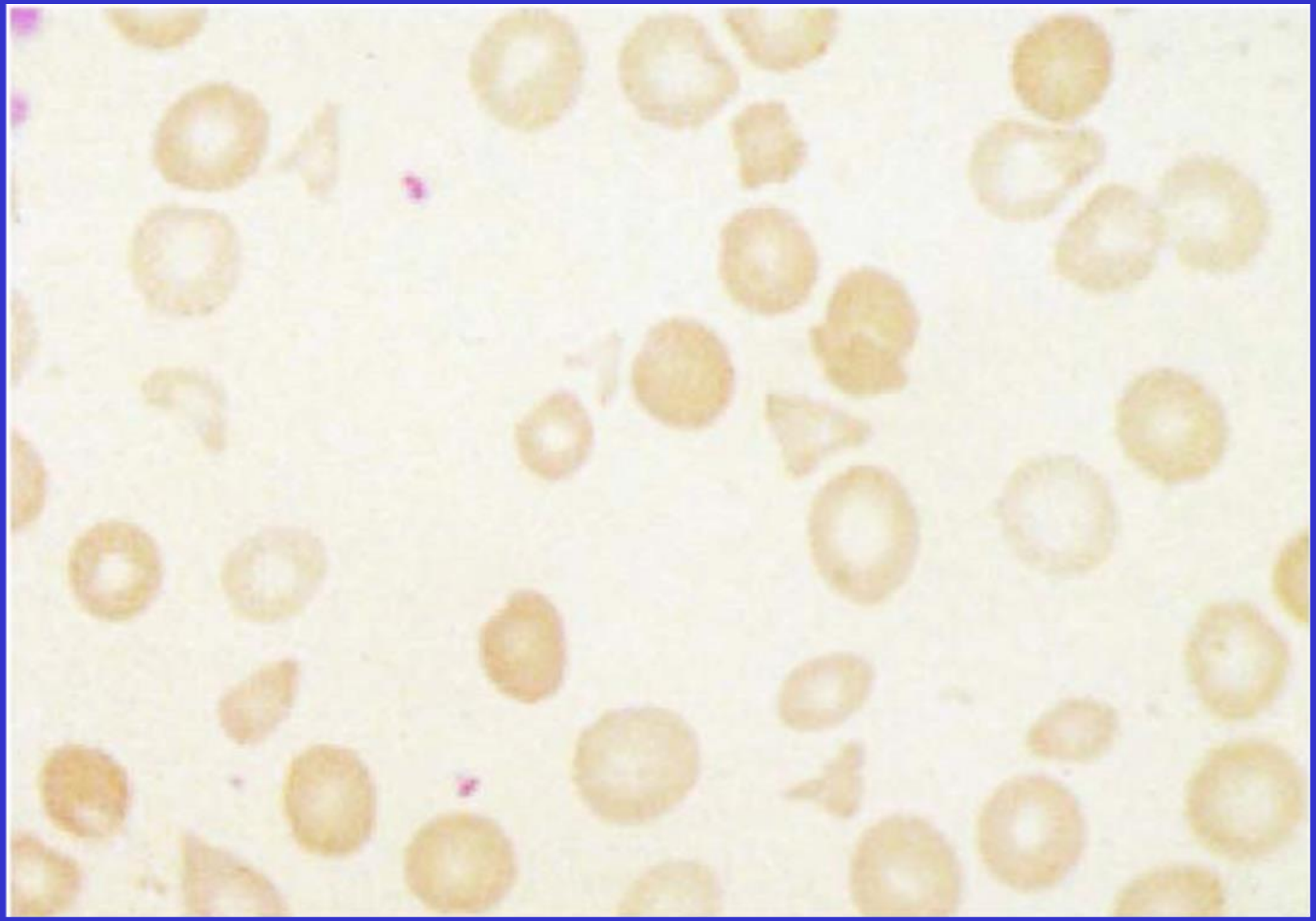
Hypersplenism

Note: ***Some drugs cause haemolysis by immune mechanisms.**

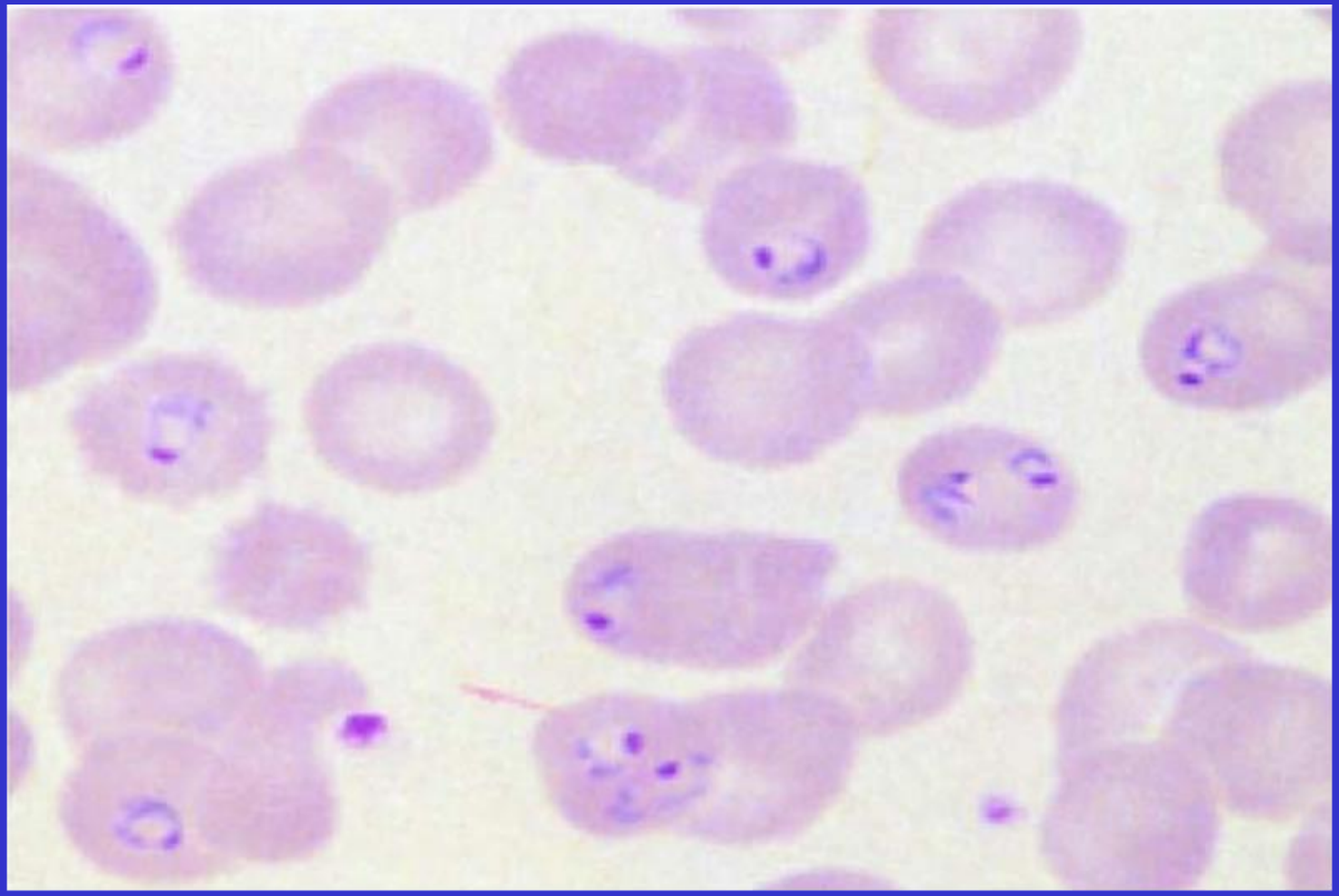
Non-immune haemolytic anaemias

Mechanical damage to red cells

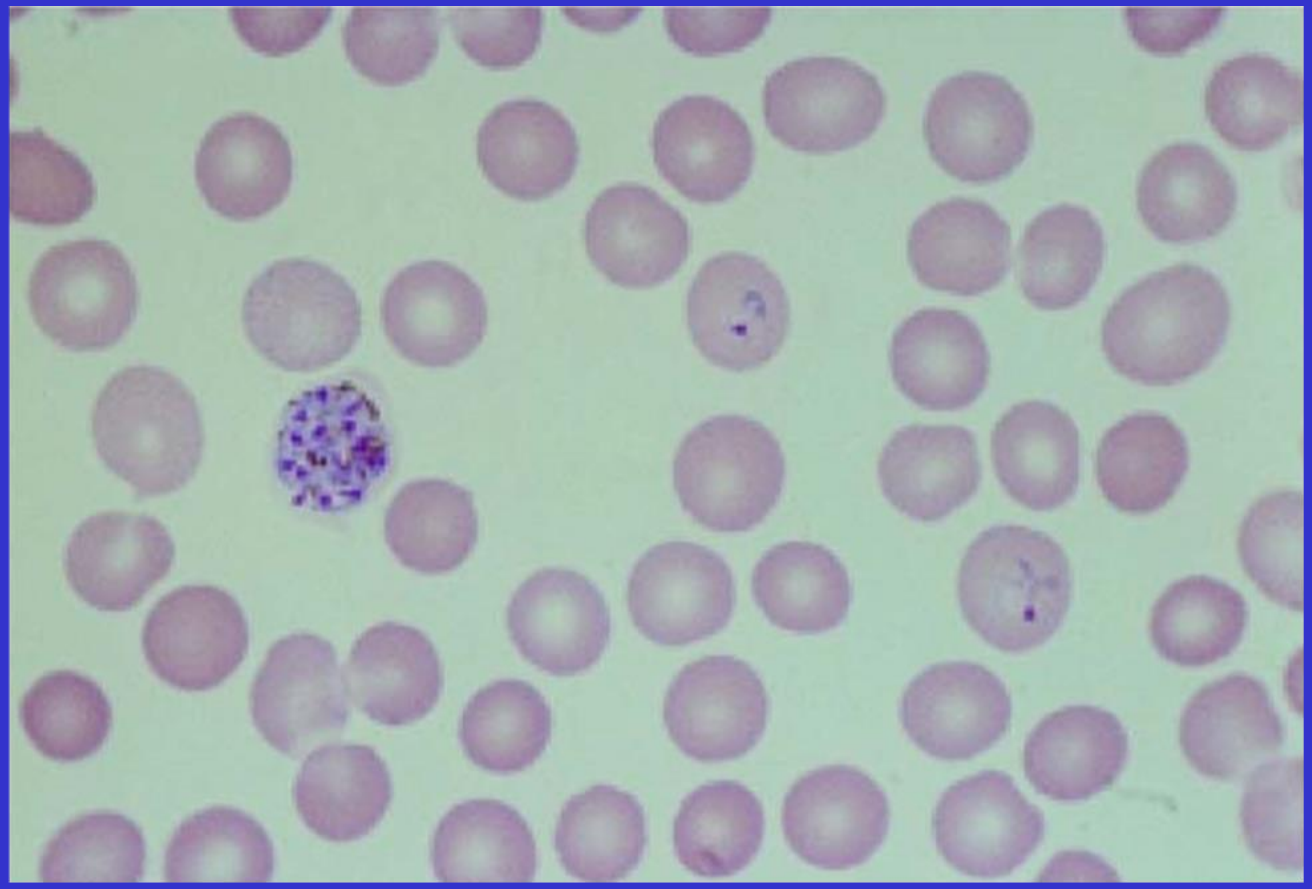
Several of the mechanical causes of acquired non-immune haemolytic anaemia are summarized in Table 3.3. Red cells are mechanically damaged when they impact upon abnormal surfaces. In disseminated intravascular coagulation inappropriate activation of the coagulation cascade produces fibrin strands which are thought to cause mechanical destruction of red cells. Such damage usually results in the presence of red cell fragments in the blood film.



Fragmented red cells (schistocytes) in the blood film of a patient with a malfunctioning aortic valve prosthesis.



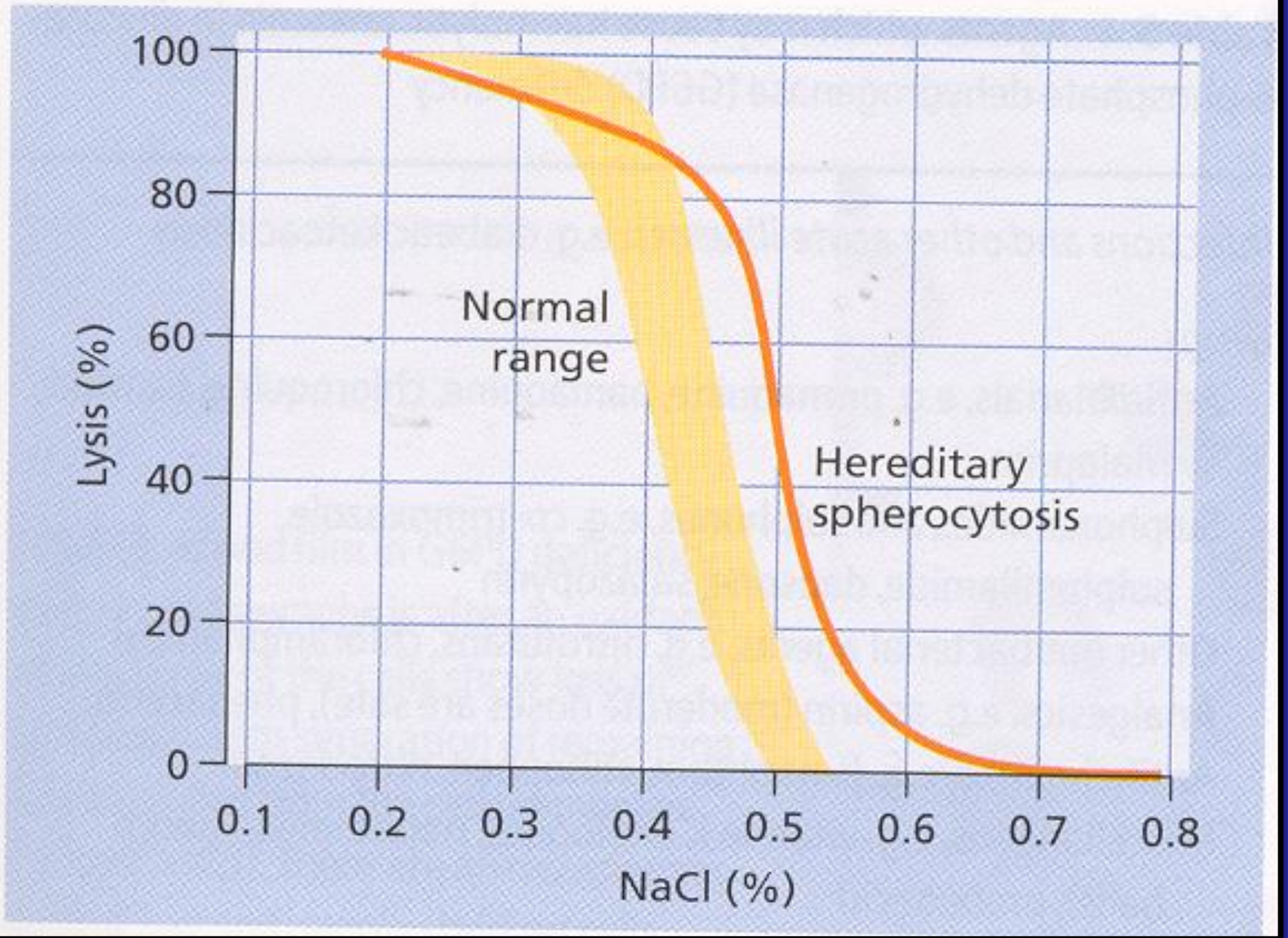
Blood film from a patient with *Plasmodium falciparum* malaria showing several parasitized red cells. Red cells heavily parasitized with malaria may be subject to intravascular lysis.



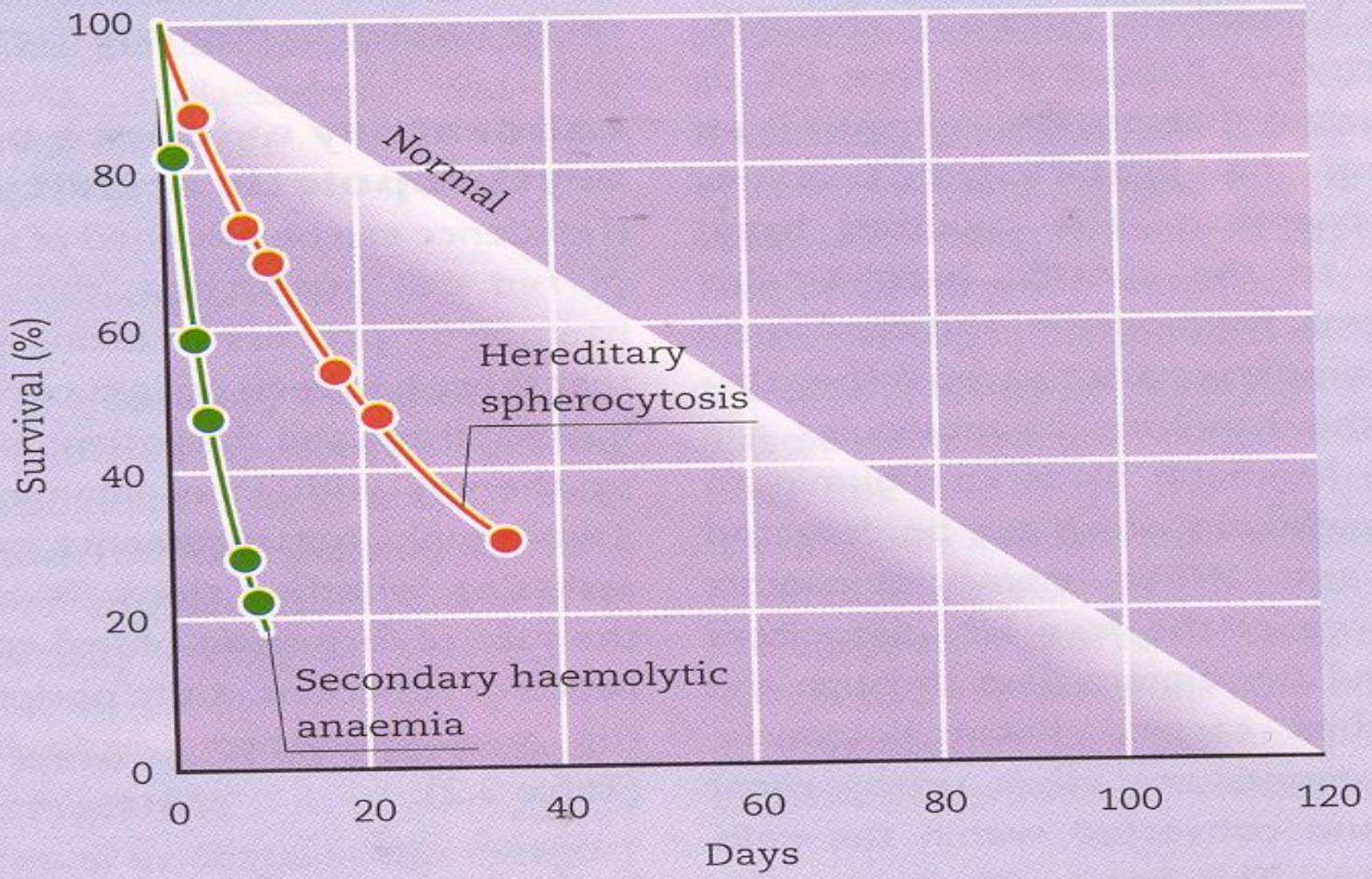
Blood film from a patient with *Plasmodium vivax* malaria showing two parasitized red cells, each containing a single parasite (ring form or early trophozoite and an ameboid late trophozoite). Another red cell contains a schizont. Some of the parasitized cells are slightly enlarged.

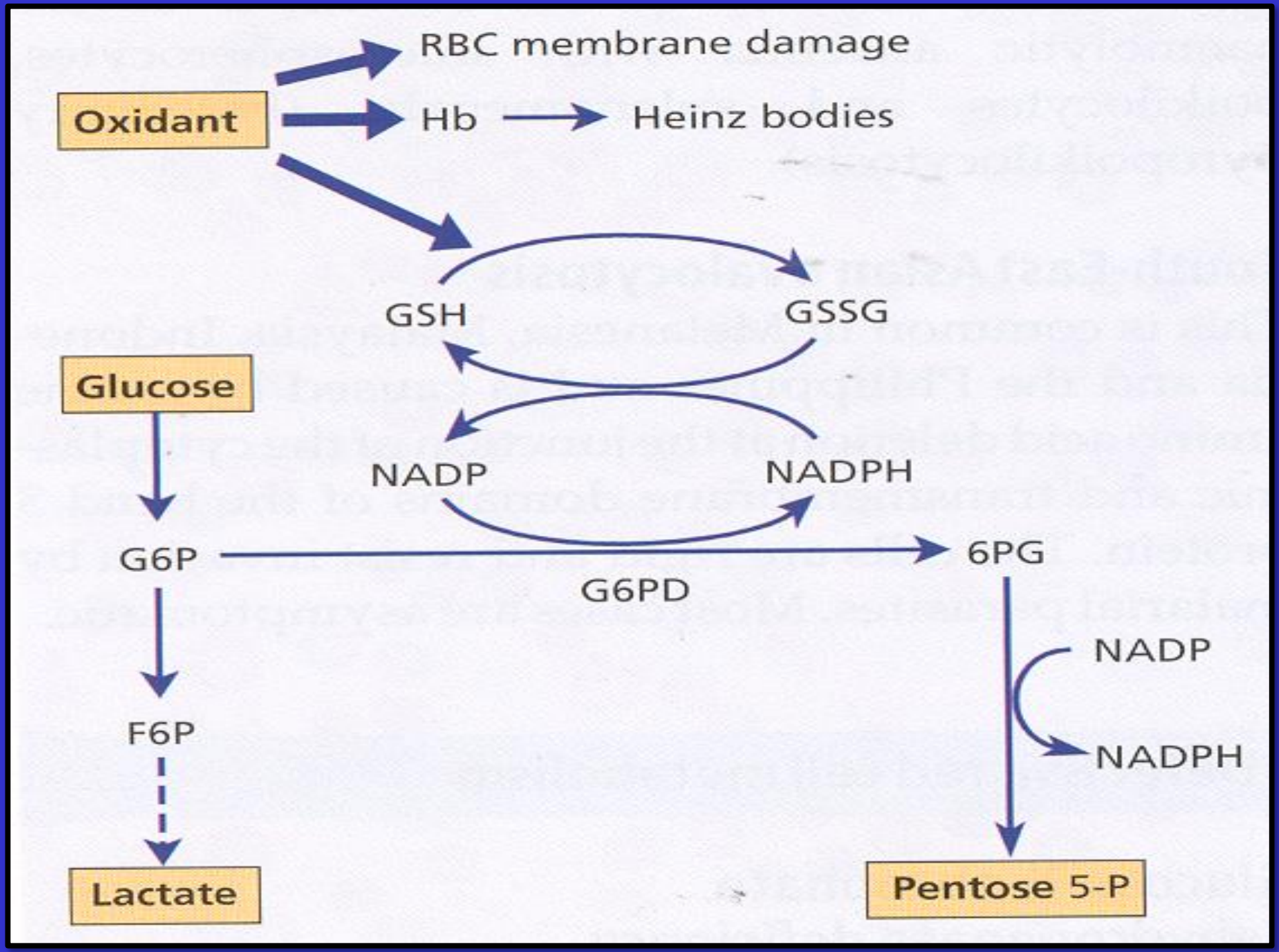
Hypersplenism

Hypersplenism describes the reduction in the lifespan of red cells, granulocytes and platelets that may be found in patients with splenomegaly due to any cause. The cytopenias found in patients with enlarged spleens are also partly caused by increased pooling of blood cells within the spleen.


100
80
60
40
20
0
Lysis (%)
Normal
range
Hereditary
spherocytosis
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
NaCl (%)

RED CELL SURVIVAL MEASUREMENTS




RBC membrane damage
Oxidant
Hb
Heinz bodies
GSH
GSSG
Glucose
NADP
NADPH
G6P
6PG
G6PD
NADP
NADPH
F6P
Lactate
Pentose 5-P

Abnormal Haemoglobins (Haemoglobinopathies)

```
 1   2   3   4   5   6   7   8   9   10  11  12  13  14  15
VAL-HIS-LEU-THR-PRO-GLU-GLU-LYS-SER-ALA-VAL-THR-ALA-LEU-TRY

 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
GLY-LYS-VAL-ASN-VAL-ASP-GLU-VAL-GLY-GLY-GLU-ALA-LEU-GLY-ARG

 31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
LEU-LEU-VAL-VAL-TYR-PRO-TRY-THR-GLN-ARG-PHE-PHE-GLU-SER-PHE

 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
GLY-ASP-LEU-SER-THR-PRO-ASP-ALA-VAL-MET-GLY-ASN-PRO-LYS-VAL

 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
LYS-ALA-HIS-GLY-LYS-LYS-VAL-LEU-GLY-ALA-PHE-SER-ASP-GLY-LEU

 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
ALA-HIS-LEU-ASP-ASN-LEU-LYS-GLY-THR-PHE-ALA-THR-LEU-SER-GLU

 91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
LEU-HIS-CYS-ASP-LYS-LEU-HIS-VAL-ASP-PRO-GLU-ASN-PHE-ARG-LEU

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
LEU-GLY-ASN-VAL-LEU-VAL-CYS-VAL-LEU-ALA-HIS-HIS-PHE-GLY-LYS

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
GLU-PHE-THR-PRO-PRO-VAL-GLN-ALA-ALA-TYR-GLN-LYS-VAL-VAL-ALA

136 137 138 139 140 141 142 143 144 145 146
GLY-VAL-ALA-ASN-ALA-LEU-ALA-HIS-LYS-TYR-HIS
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 1   2   3   4   5   6   7   8   9   10  11  12  13  14  15
VAL-LEU-SER-PRO-ALA-ASP-LYS-THR-ASN-VAL-LYS-ALA-ALA-TRY-GLY

 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
LYS-VAL-GLY-ALA-HIS-ALA-GLY-GLU-TYR-GLY-ALA-GLU-ALA-LEU-GLU

 31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
ARG-MET-PHE-LEU-SER-PHE-PRO-THR-THR-LYS-THR-TYR-PHE-PRO-HIS

 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VAL-LYS-GLY-HIS-GLY-LYS

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LYS-VAL-ALA-ASP-ALA-LEU-THR-ASN-ALA-VAL-ALA-HIS-VAL-ASP-ASP

 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
MET-PRO-ASN-ALA-LEU-SER-ALA-LEU-SER-ASP-LEU-HIS-ALA-HIS-LYS

 91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
LEU-ARG-VAL-ASP-PRO-VAL-ASN-PHE-LYS-LEU-LEU-SER-HIS-CYS-LEU

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
LEU-VAL-THR-LEU-ALA-ALA-HIS-LEU-PRO-ALA-GLU-PHE-THR-PRO-ALA

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
VAL-HIS-ALA-SER-LEU-ASP-LYS-PHE-LEU-ALA-SER-VAL-SER-THR-VAL

136 137 138 139 140 141
LEU-THR-SER-LYS-TYR-ARG
```

Some Known Haemoglobin Mutants

NAME	SUBSTITUTION
Hb. S	α2 β2 6 GLU → VAL
Hb. C	α2 β2 6 GLU → LYS
Hb. E	α2 β2 26 GLU → LYS
Hb. O ARAB	α2 β2 121 GLU → LYS
Hb. D PUNJAB	α2 β2 121 GLU →GLN
Hb RIYADH	α2 β2 120 LYS → ASN
Hb. HAMMERSMITH	α2 β2 42 PHE → SER
Hb. N. BALTIMORE	α2 β2 95 LYS → GLU
Hb. KORLE-BU	α2 β2 73 ASP → ASN
Hb. K. WOOLWICH	α2 β2 132 LYS → GLN
Hb. K. IBADAN	α2 β2 46 GLY → GLU
Hb. KÖ LN	α2 β2 98 VAL → MET
Hb. J. BALTIMORE	α2 β2 16 GLY → ASP

Some Known Haemoglobin Mutants

NAME	SUBSTITUTION
Hb. G. PHILADELPHIA	α2 68 ASN → LYS β2
Hb. ZAMBIA	α2 60 LYS → ASN β2
Hb. G. CHINESE	α2 30 GLU → GLN β2
Hb. HASHARON	α2 47 ASP → HIS β2
Hb. J. TONGARIKI	α2 115 ALA → ASP β2
Hb. J. OXFORD	α2 15 GLY → ASP β2
Hb. NORFOLK	α2 57 GLY → ASP β2

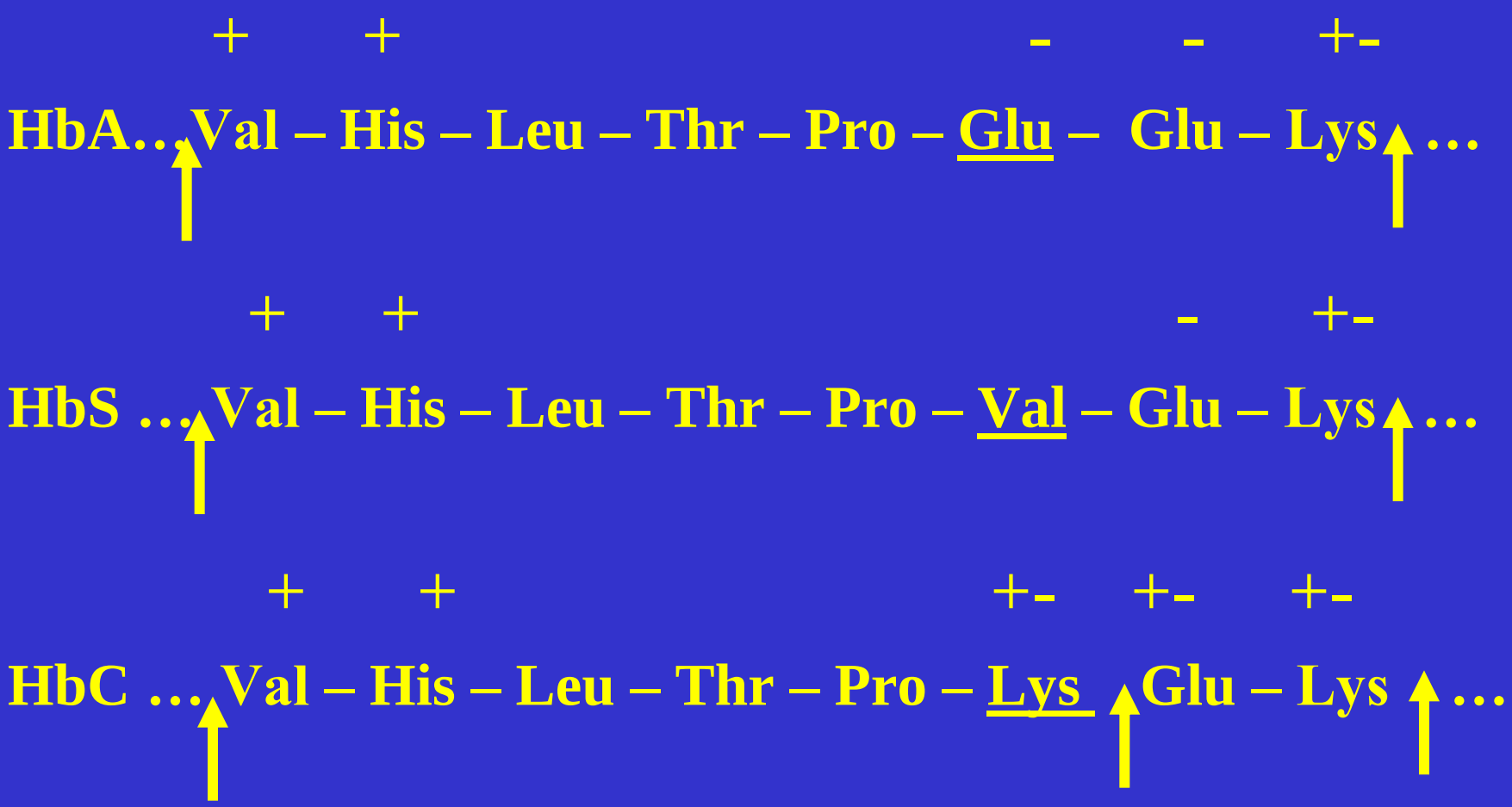
DNA Coding for the Amino-Acid in the sixth position in the β-chain

Normal

	5	6	7
Amino Acid	pro	glu	glu
DNA Base Composition	CCT	G A G	G A G

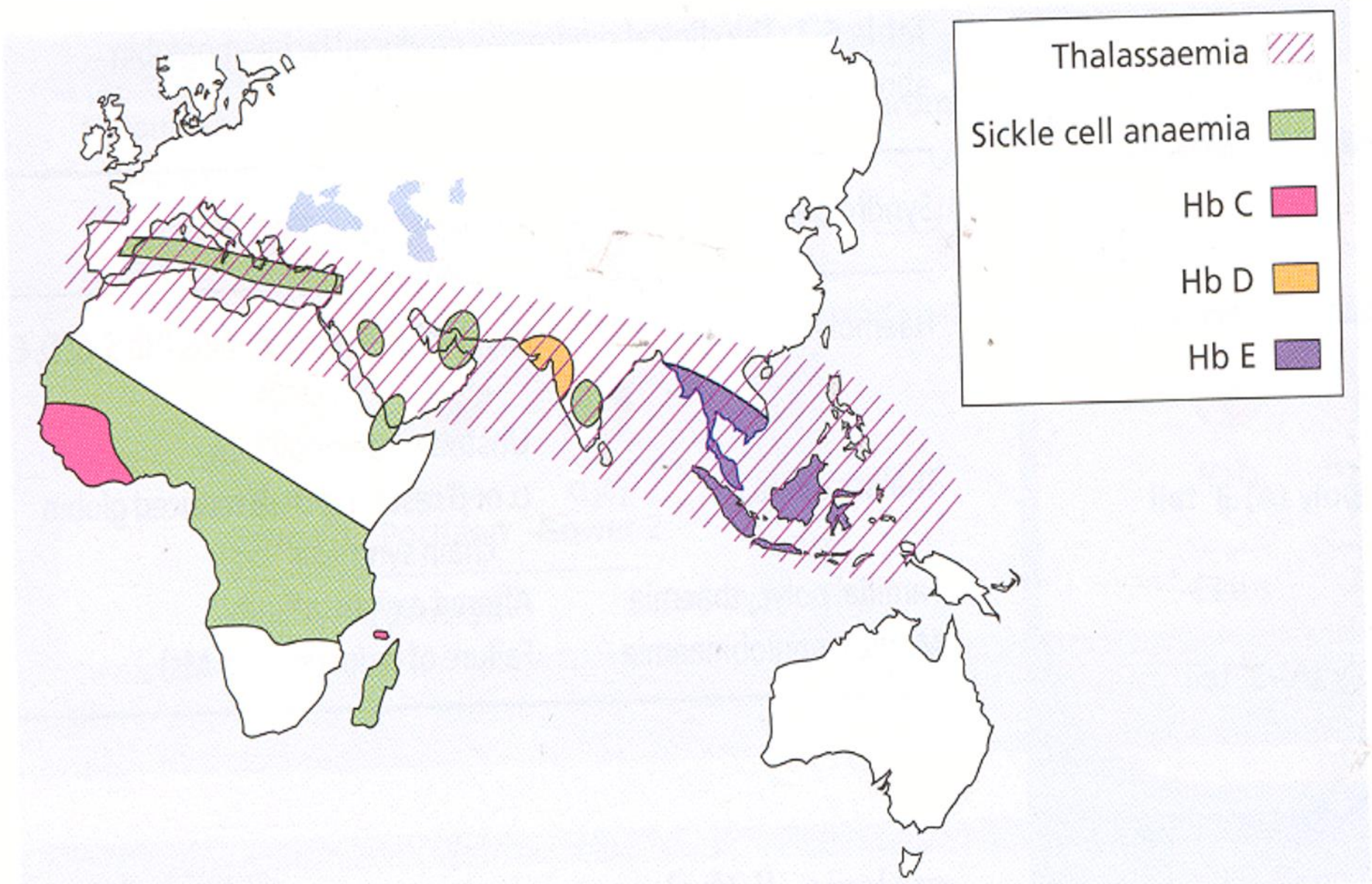
Sickle

DNA Base composition	CCT	G T G	G A G
Amino Acid	pro	val	glu
	5	6	7



Amino acid sequences of the peptides 4 in haemoglobins A, S and C.

HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION



SICKLE CELL DISEASE

By:

DR. SHIHAB AL-MASHHADANI
Consultant Haematologist
Head of Haematology Division
Associate Professor
Department of Pathology
College of Medicine
King Saud University

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 1   2   3   4   5   6   7   8   9   10  11  12  13  14  15
VAL-LEU-SER-PRO-ALA-ASP-LYS-THR-ASN-VAL-LYS-ALA-ALA-TRY-GLY

 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
LYS-VAL-GLY-ALA-HIS-ALA-GLY-GLU-TYR-GLY-ALA-GLU-ALA-LEU-GLU

 31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
ARG-MET-PHE-LEU-SER-PHE-PRO-THR-THR-LYS-THR-TYR-PHE-PRO-HIS

 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VAL-LYS-GLY-HIS-GLY-LYS

 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
LYS-VAL-ALA-ASP-ALA-LEU-THR-ASN-ALA-VAL-ALA-HIS-VAL-ASP-ASP

 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
MET-PRO-ASN-ALA-LEU-SER-ALA-LEU-SER-ASP-LEU-HIS-ALA-HIS-LYS

 91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
LEU-ARG-VAL-ASP-PRO-VAL-ASN-PHE-LYS-LEU-LEU-SER-HIS-CYS-LEU

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
LEU-VAL-THR-LEU-ALA-ALA-HIS-LEU-PRO-ALA-GLU-PHE-THR-PRO-ALA

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
VAL-HIS-ALA-SER-LEU-ASP-LYS-PHE-LEU-ALA-SER-VAL-SER-THR-VAL

136 137 138 139 140 141
LEU-THR-SER-LYS-TYR-ARG
```

```
 1   2   3   4   5   6   7   8   9   10  11  12  13  14  15
VAL-HIS-LEU-THR-PRO-GLU-GLU-LYS-SER-ALA-VAL-THR-ALA-LEU-TRY

 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
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LEU-LEU-VAL-VAL-TYR-PRO-TRY-THR-GLN-ARG-PHE-PHE-GLU-SER-PHE

 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
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 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
LYS-ALA-HIS-GLY-LYS-LYS-VAL-LEU-GLY-ALA-PHE-SER-ASP-GLY-LEU

 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
ALA-HIS-LEU-ASP-ASN-LEU-LYS-GLY-THR-PHE-ALA-THR-LEU-SER-GLU

 91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
LEU-HIS-CYS-ASP-LYS-LEU-HIS-VAL-ASP-PRO-GLU-ASN-PHE-ARG-LEU

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LEU-GLY-ASN-VAL-LEU-VAL-CYS-VAL-LEU-ALA-HIS-HIS-PHE-GLY-LYS

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
GLU-PHE-THR-PRO-PRO-VAL-GLN-ALA-ALA-TYR-GLN-LYS-VAL-VAL-ALA

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GLY-VAL-ALA-ASN-ALA-LEU-ALA-HIS-LYS-TYR-HIS
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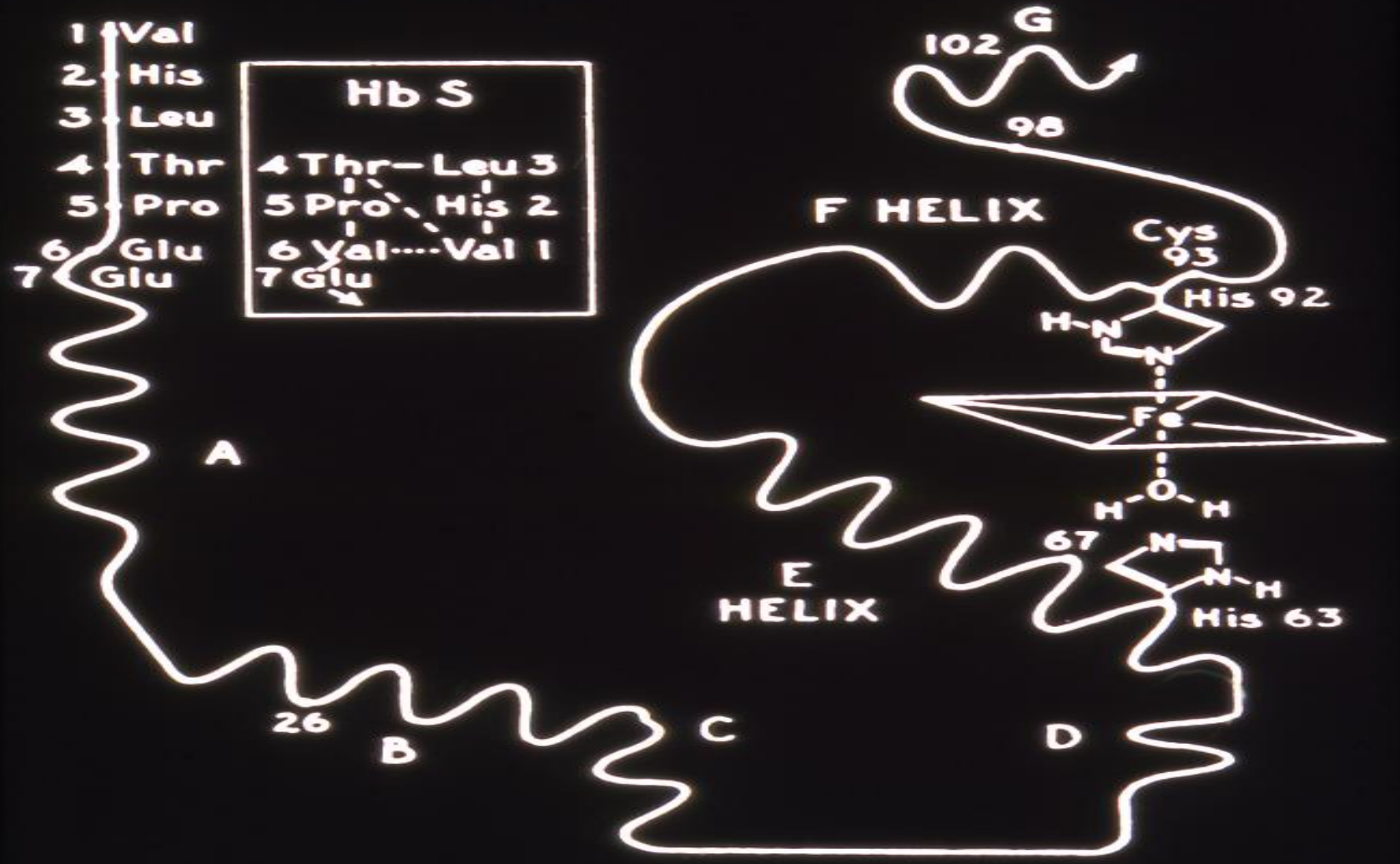
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2 His
3 Leu
4 Thr
5 Pro
6 Glu
7 Glu
Hb S
4 Thr — Leu 3
5 Pro — His 2
6 Val ···· Val 1
7 Glu
A
26
B
C
D
E
HELIX
F HELIX
G
102
98
Cys
93
His 92
Fe
67
His 63

DNA Coding for the Amino-Acid in the sixth position in the β-chain

Normal

	5	6	7
Amino Acid	pro	glu	glu
DNA Base Composition	CCT	G A G	G A G

Sickle

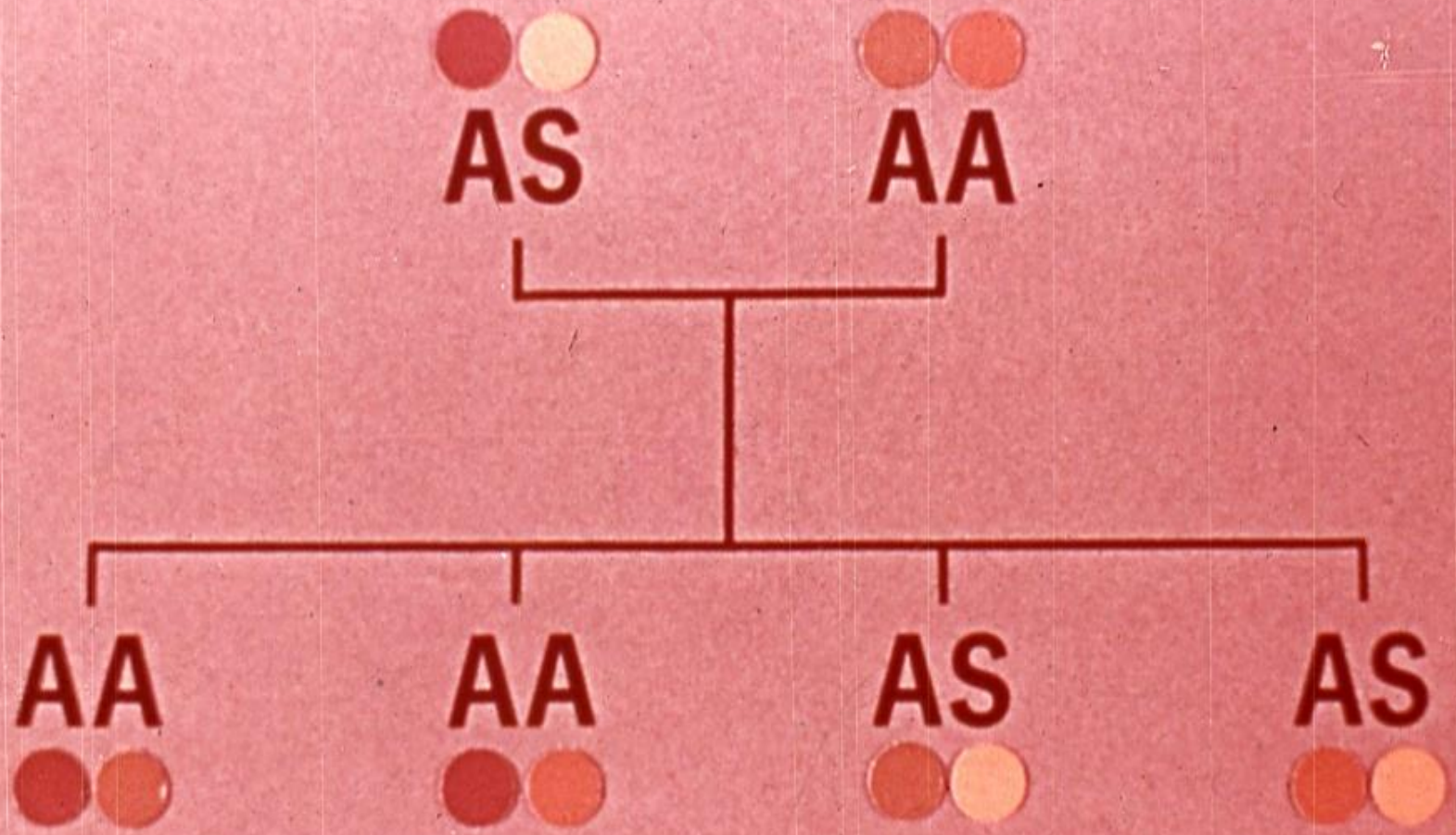
DNA Base composition	CCT	G T G	G A G
Amino Acid	pro	val	glu
	5	6	7

1910 1st published report of sickle cell anaemia (Herrick)

1949 Pauling et al : chemical difference between HbA and HbS

1956 Ingram: Fingerprinting

βglu → val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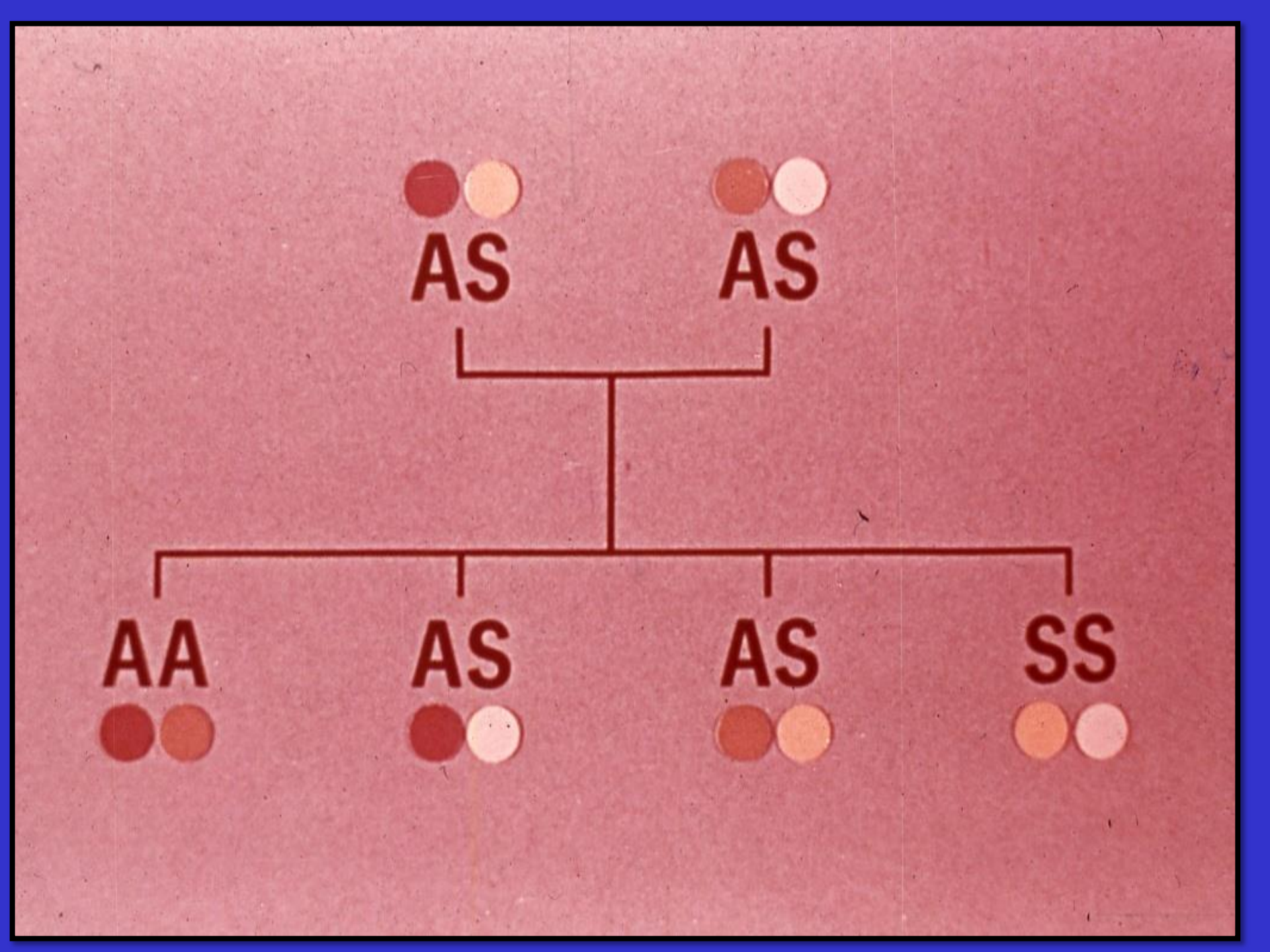
AA
AA
AA
AS
AS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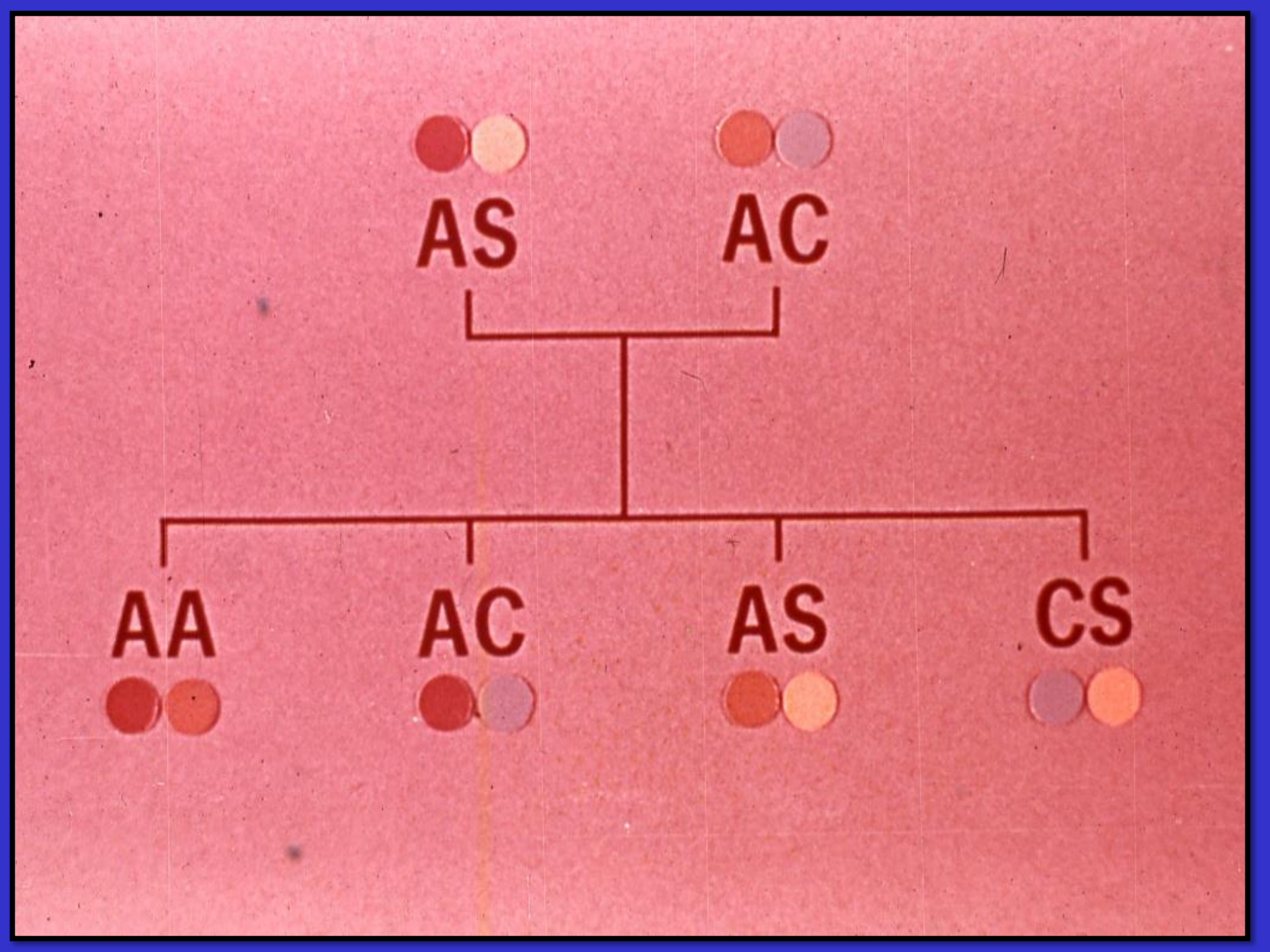
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AC
AA
AC
AS
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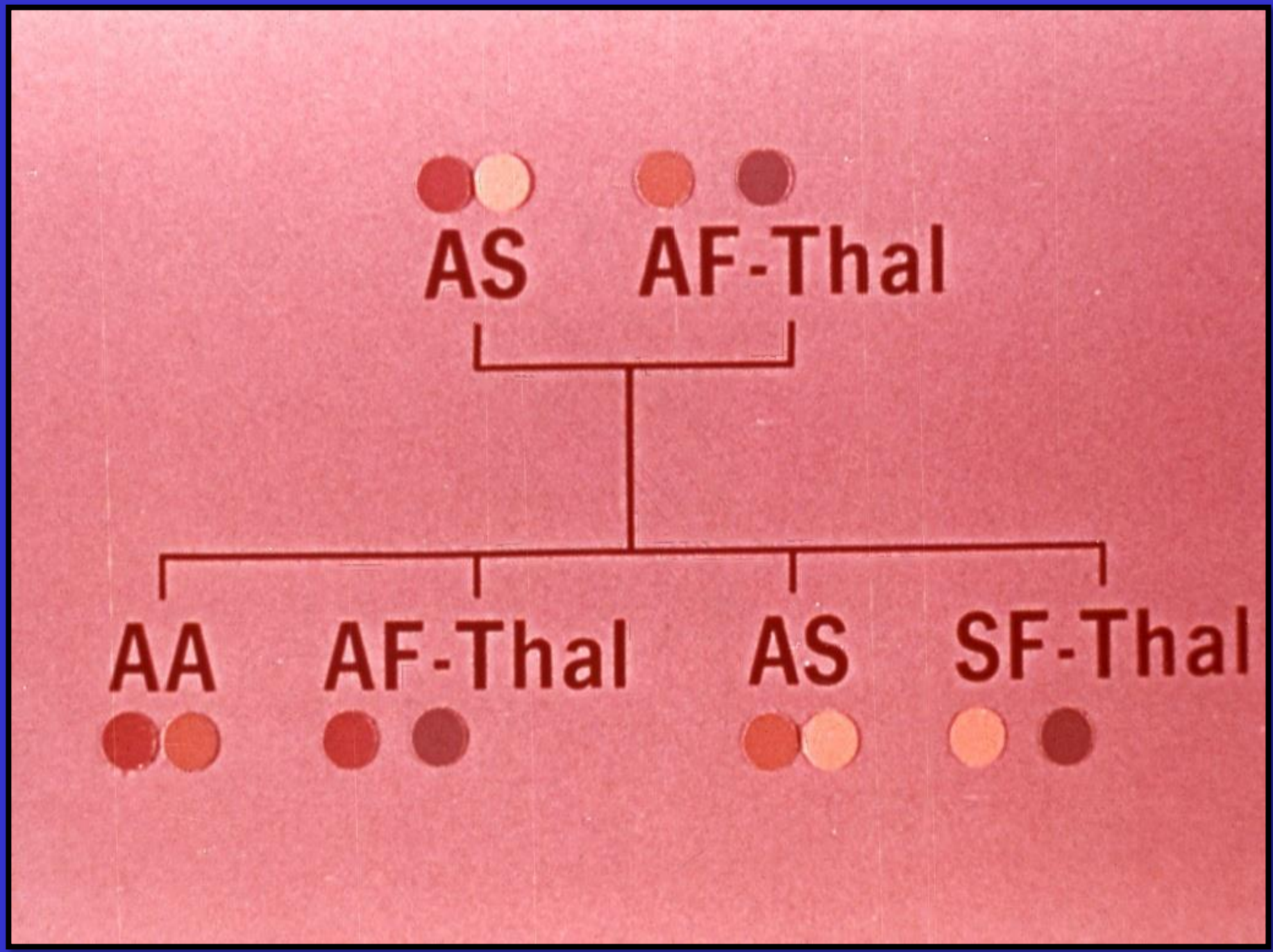
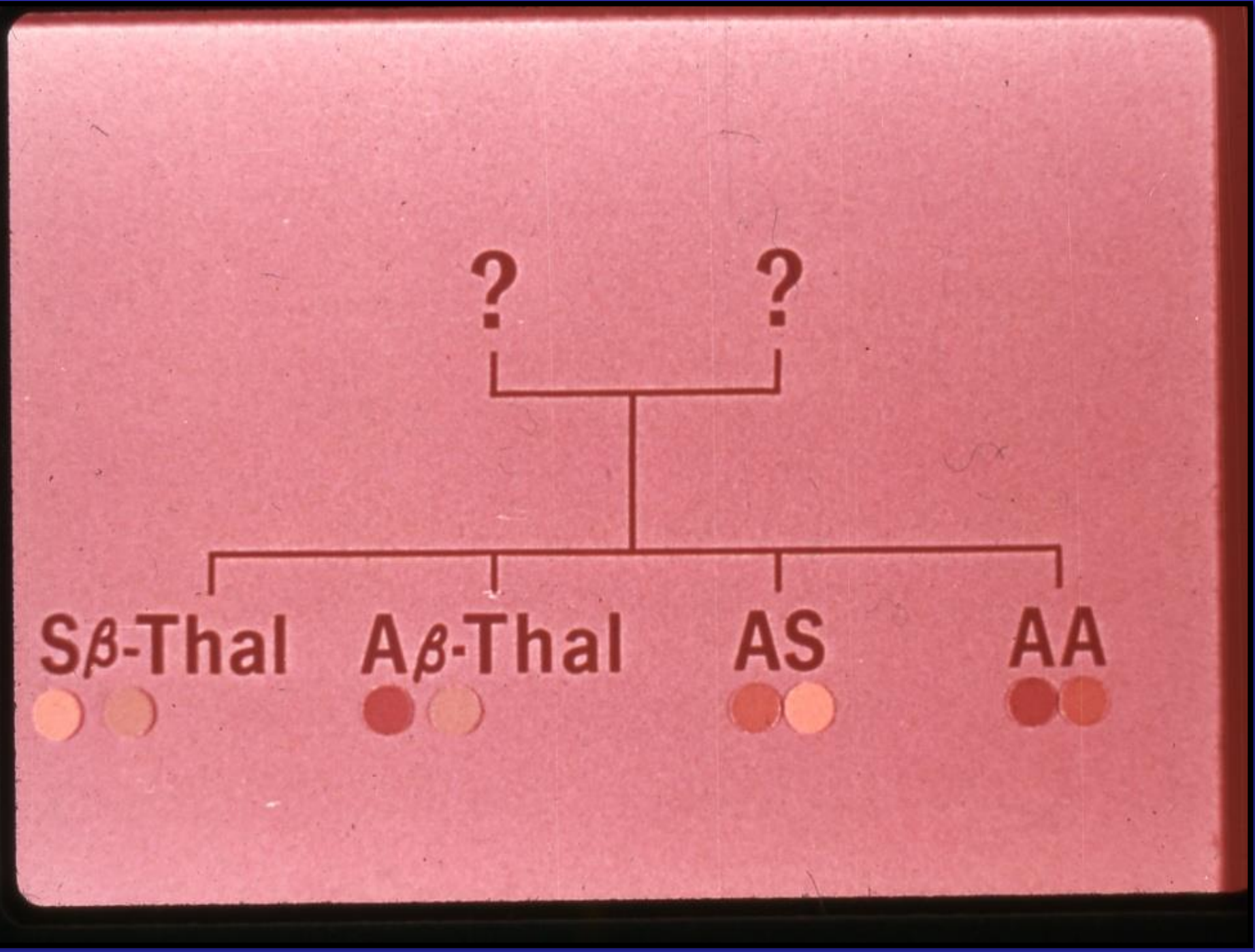

AS
AF-Thal
AA
AF-Thal
AS
SF-Thal


? ?
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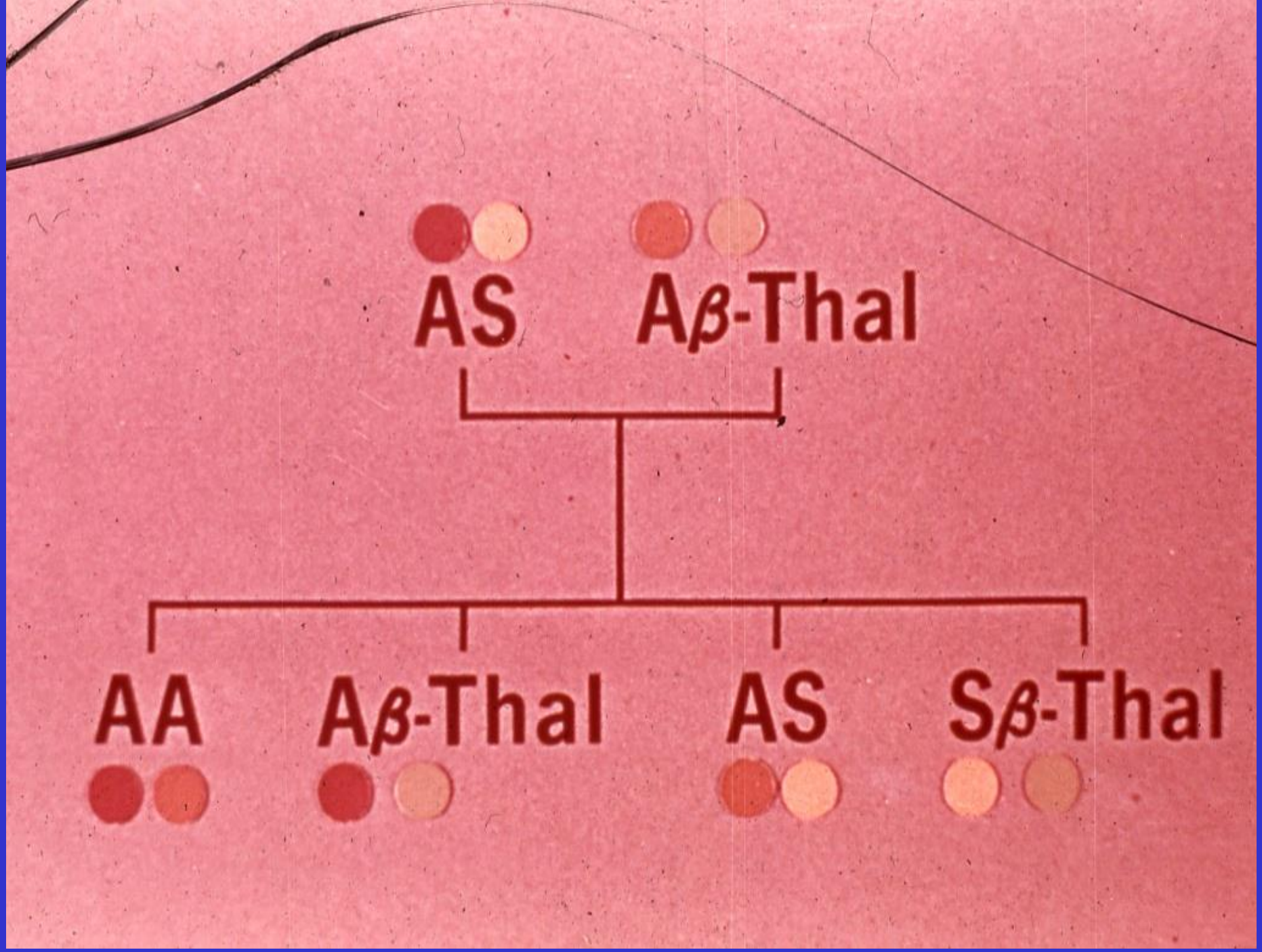
Aβ-Thal
AS
AA


AS
Aβ-Thal
AA
Aβ-Thal
AS
Sβ-Thal

SICKLE CELL DISEASE

THE SICKLE CELL TRAIT

HOMOZYGOUS SICKLE CELL DISEASE (SS)

Sickle cell anaemia

DOUBLY HETEROZYGOUS SICKLE CELL DISEASE

Sickle cell / haemoglobin C disease

Sickle cell / thalassaemia

PROPERTIES OF HbS

Solubility ↓

Conformational changes – "tactoid formation"

→ sickled cells

→ irreversibly sickled cells

↑ mechanical fragility → haemolysis

↑ viscosity → organ infarction

FACTORS AFFECTING SICKLING

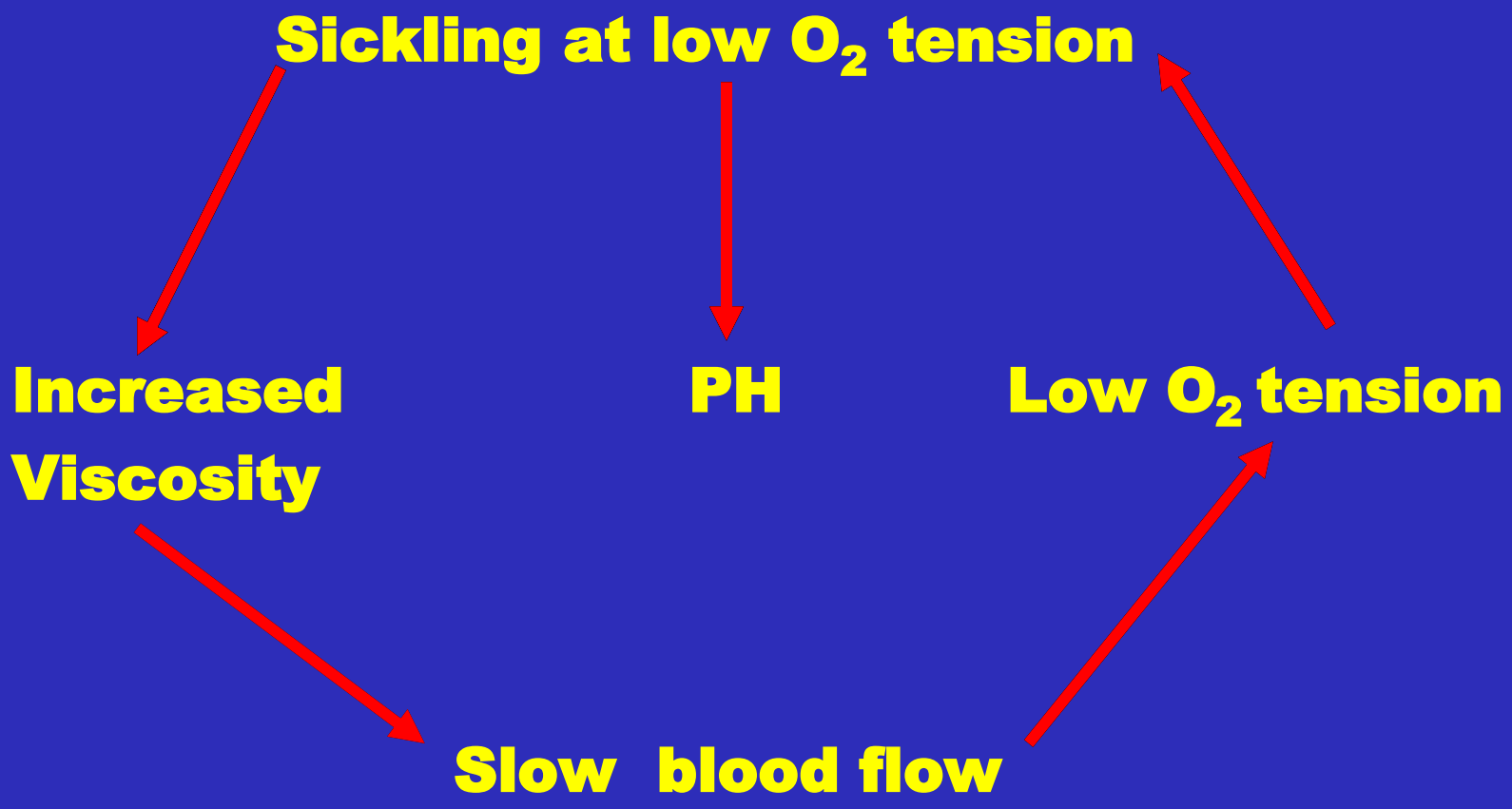
Oxygen tension — 50–60 mm Hg for SS
20–30 mm Hg for AS

pH — inhibited at alkaline pH
exacerbated by acidification

Concentration of Hb S

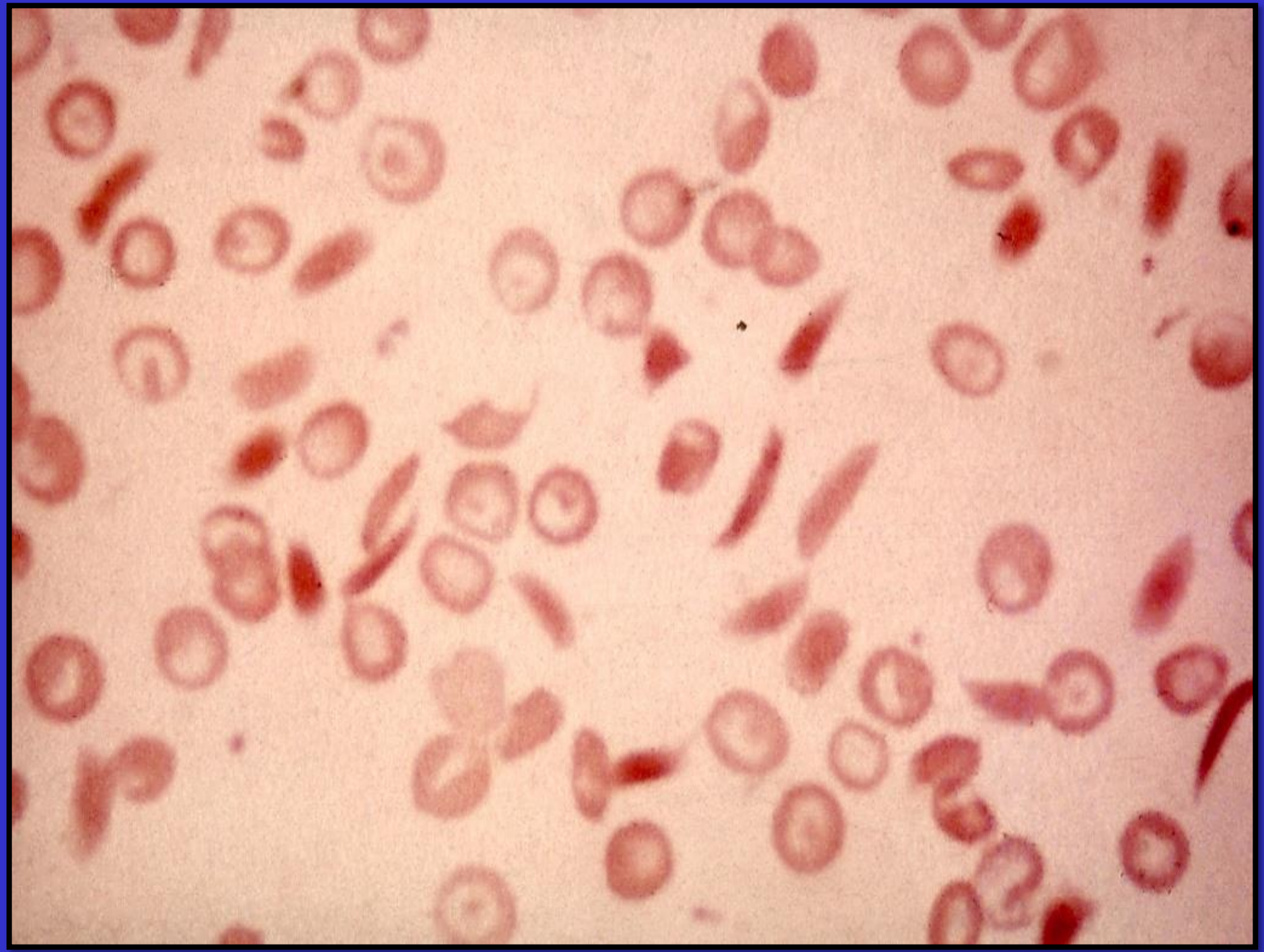
Presence of other haemoglobins

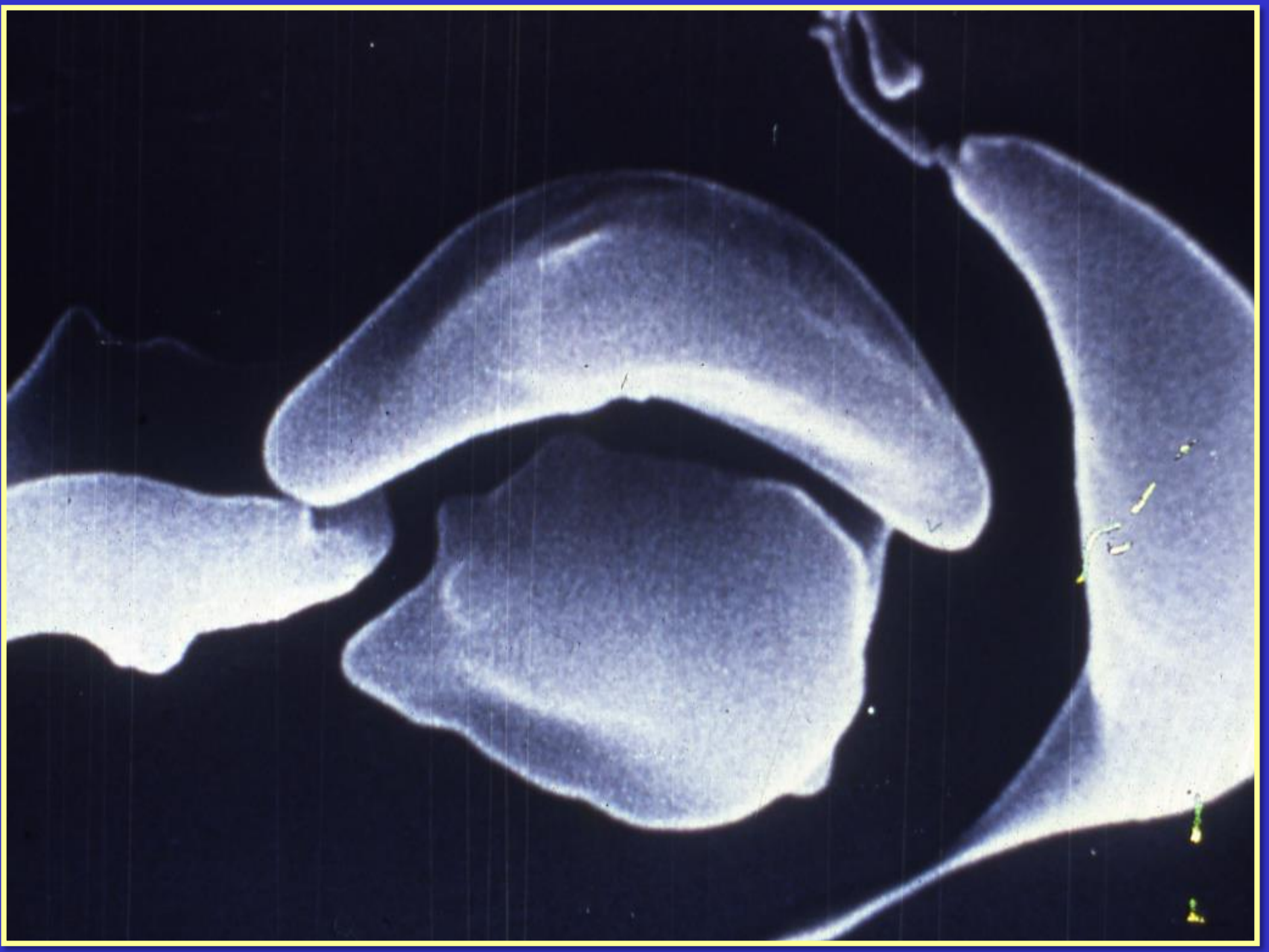
polymerisation : S > D > C > J = A > F

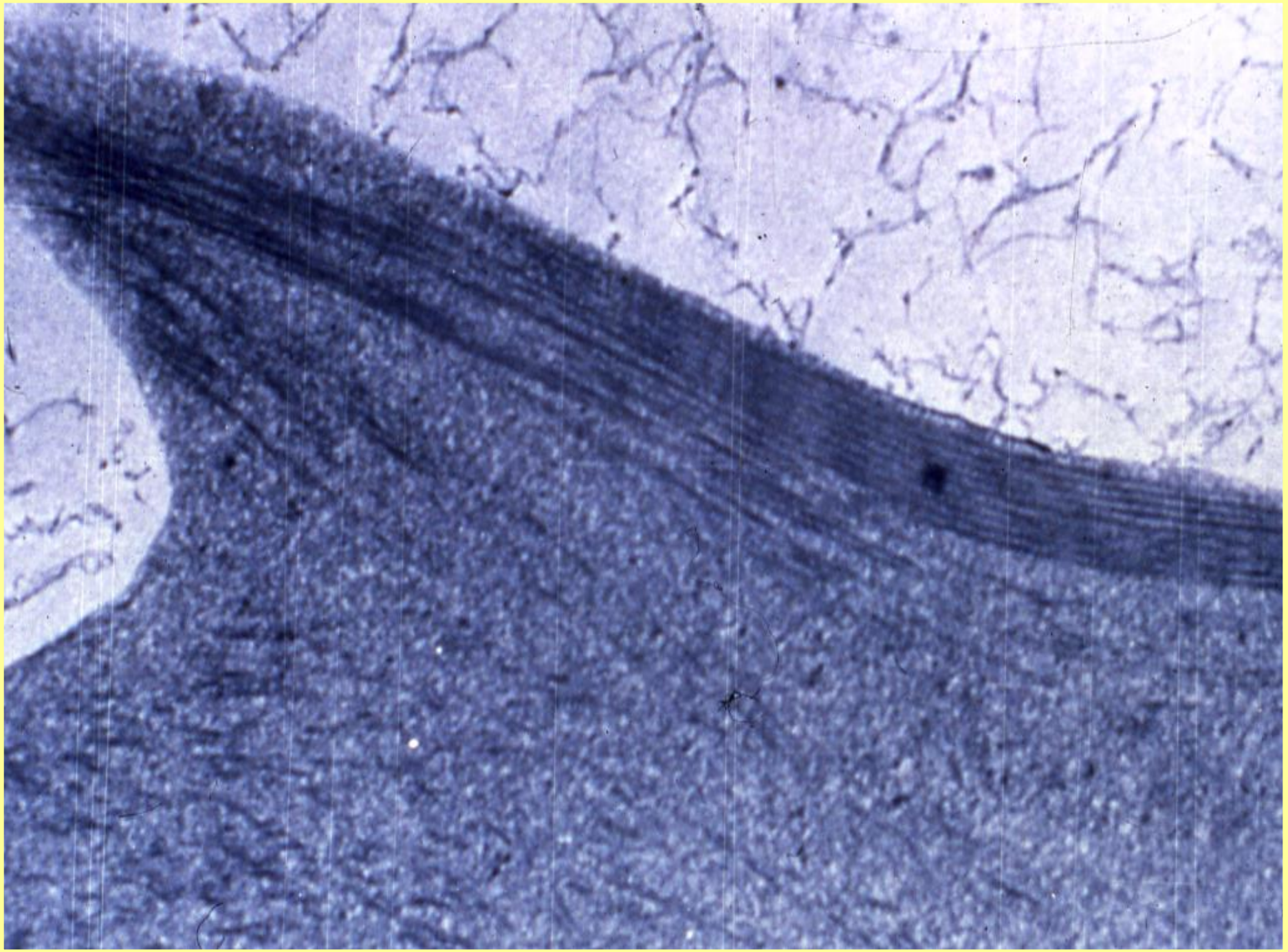

Sickling at low O_2 tension
Increased Viscosity
PH
Low O_2 tension
Slow blood flow

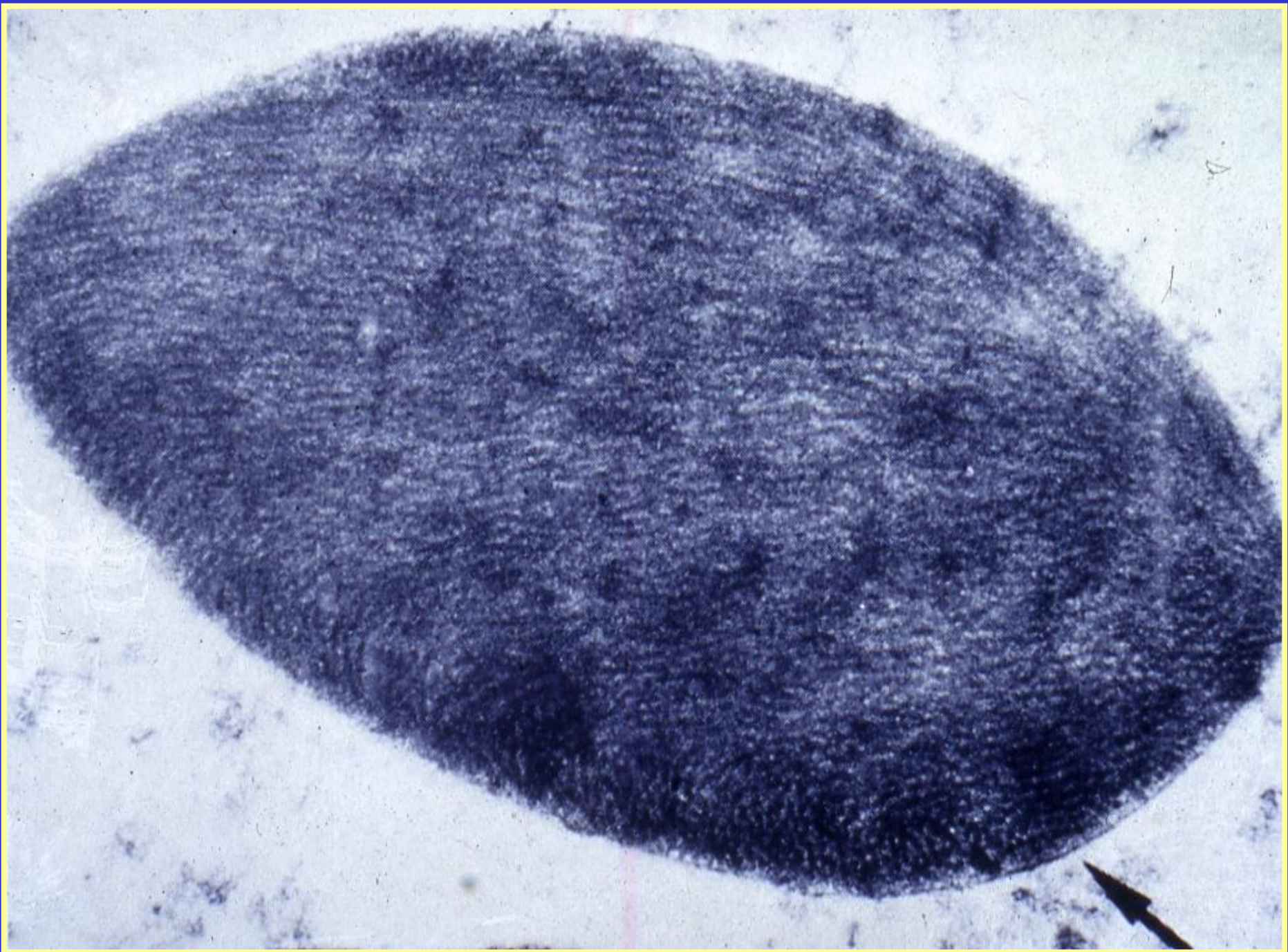
FACTORS PRECIPITATING CRISES IN SICKLE CELL DISEASE

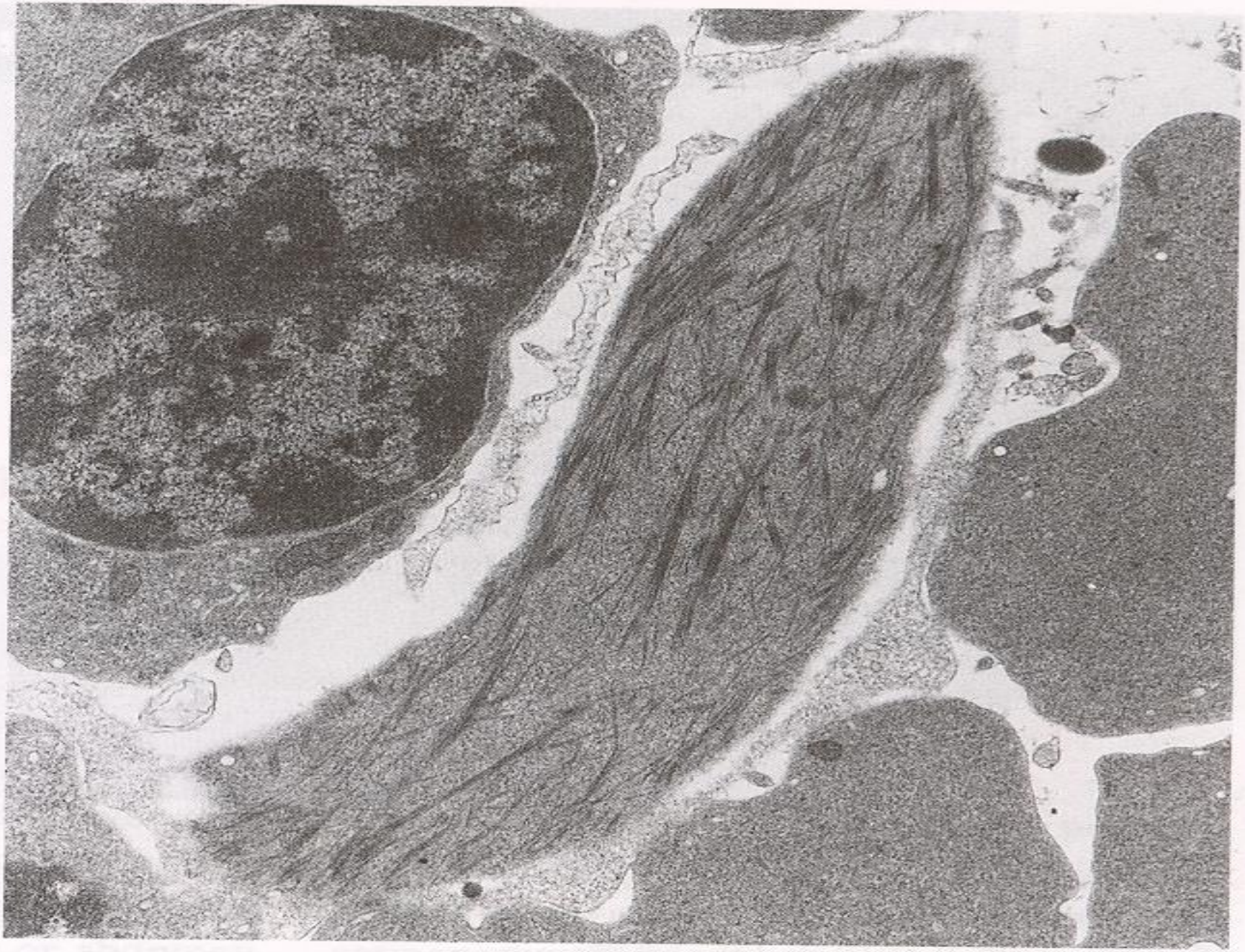
- INFECTIONS (especially malaria)
- PYREXIA
- EXPOSURE TO COLD
- DEHYDRATION
- PREGNANCY



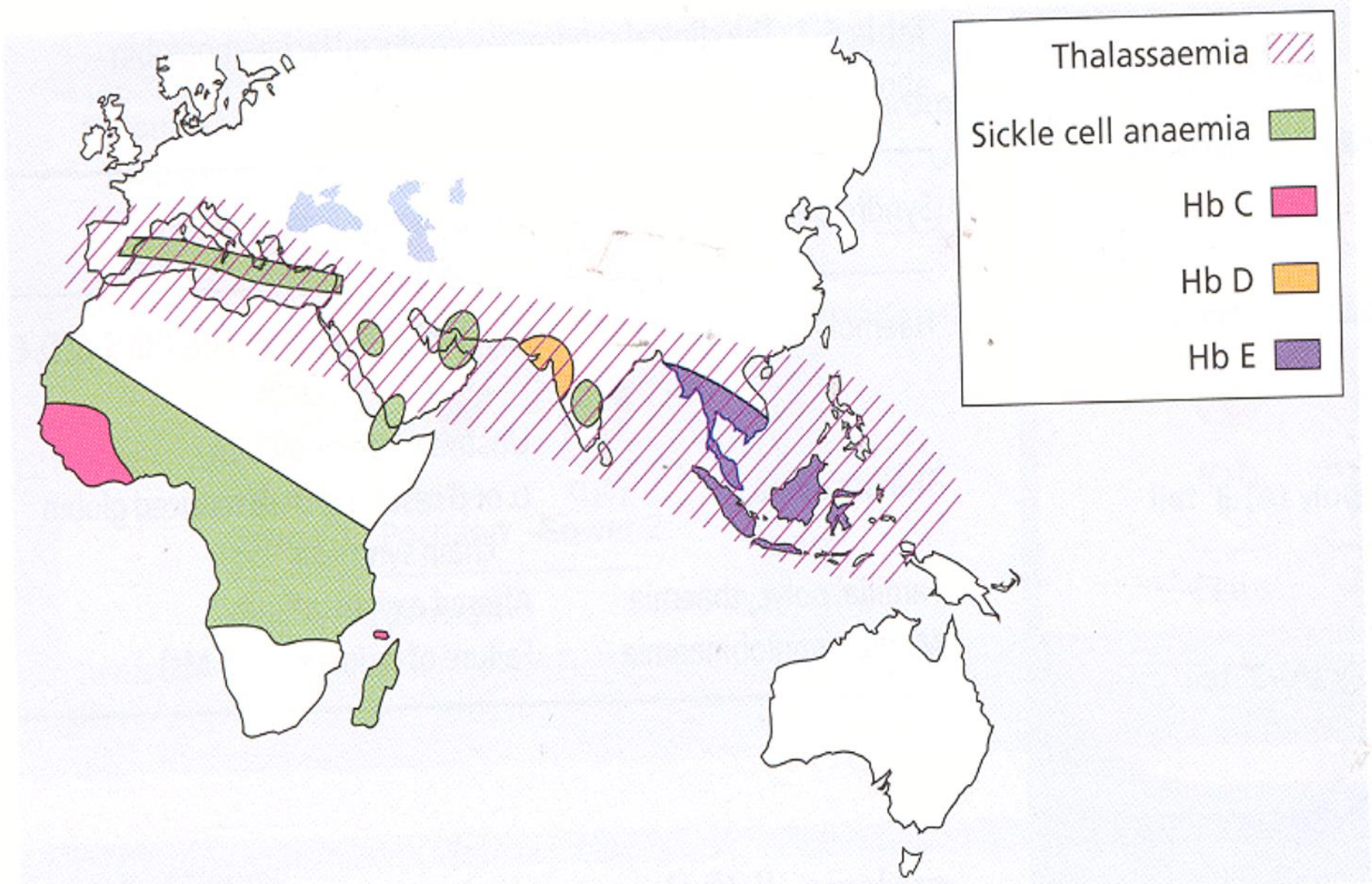


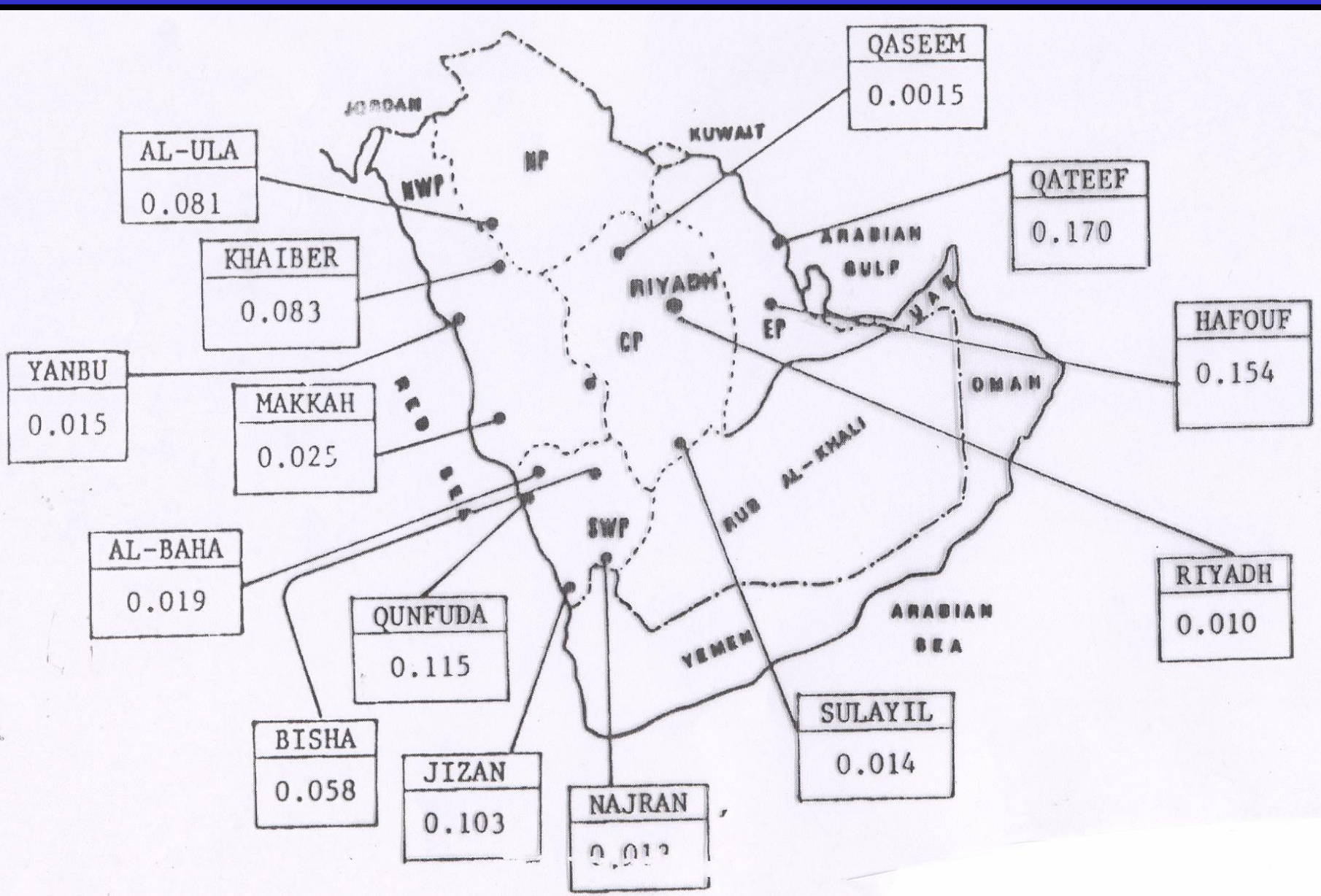






HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION





Frequency of sickle cell (Hb S) gene in different regions of Saudi Arabia

CRISES IN SICKLE CELL DISEASE

HYPERHAEMOLYTIC

AREGENERATIVE OR APLASTIC

SMALL VESSEL OCCLUSION

CLINICAL MANIFESTATIONS OF SICKLE CELL DISEASE

HAEMOLYTIC ANAEMIA

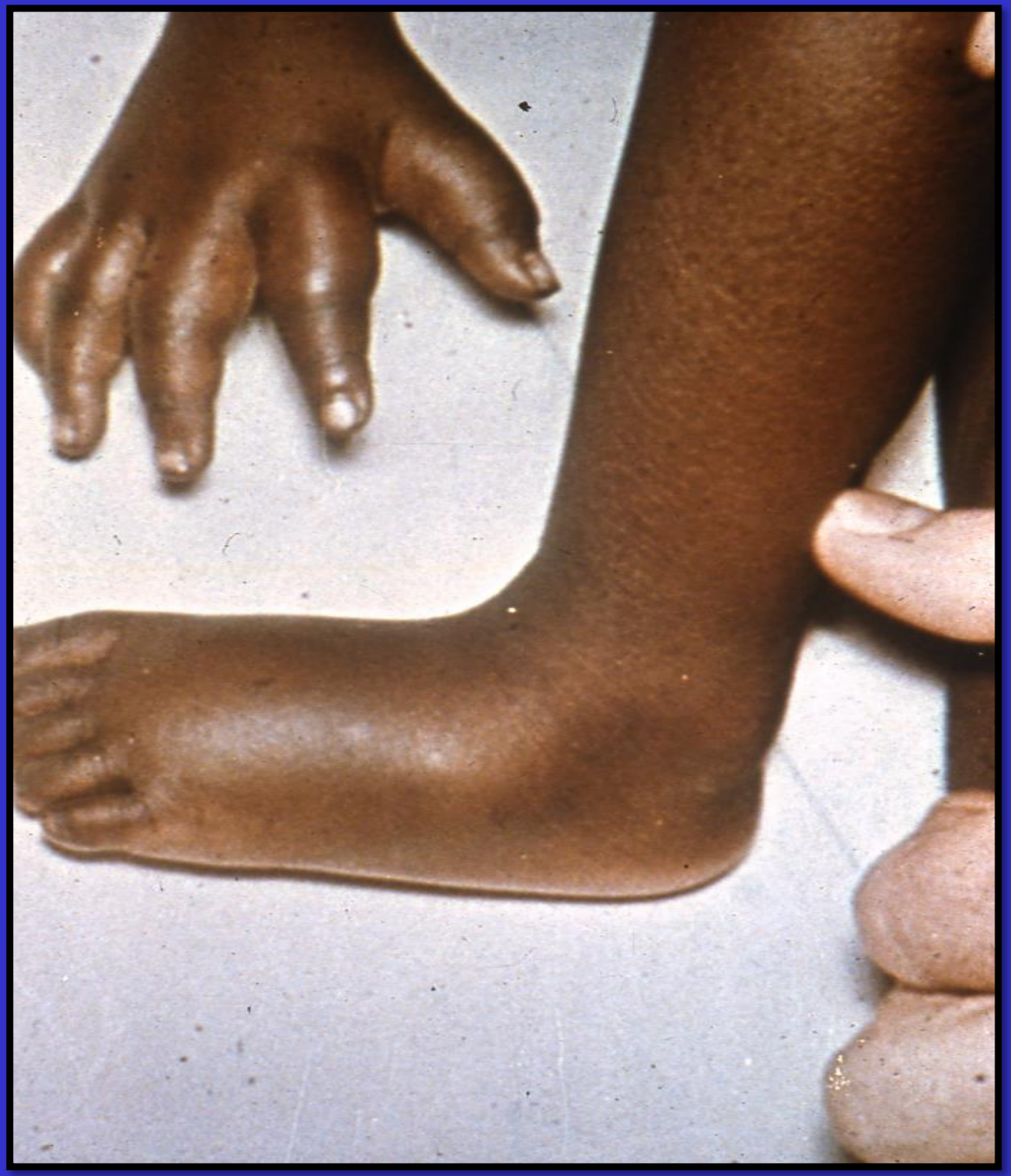
TISSUE INFARCTION

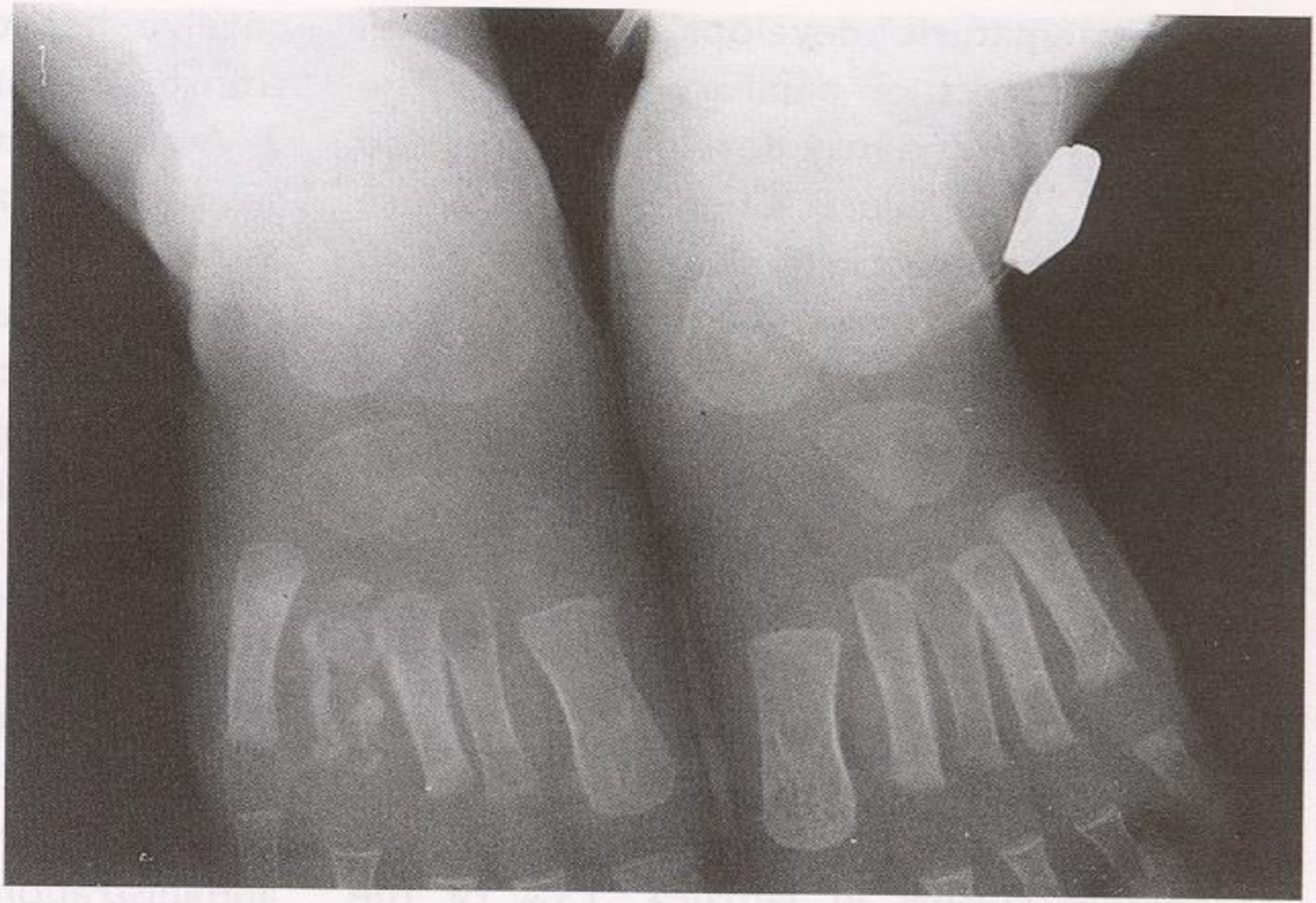
Clinical Manifestations in Sickle Anaemia

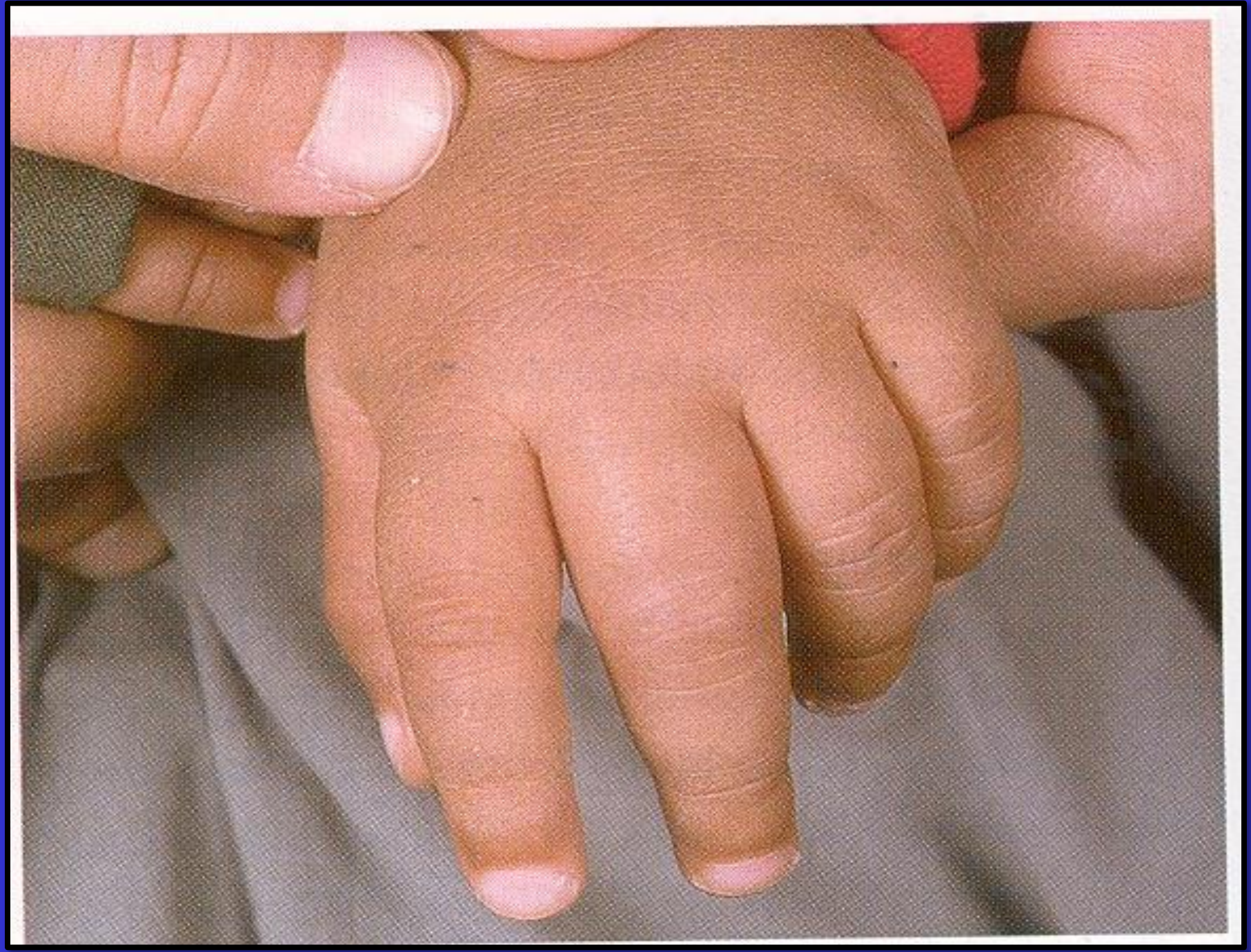
- Pallor (Anaemia)
- Jaundice & Dark Urine
- Apathy & Anorexia
- Hand-Foot Syndrome (Young Children)
- Splenic sequestration (Young children) Hepatic Sequestration
- Bones and Joints Pain
- Abdominal Pain

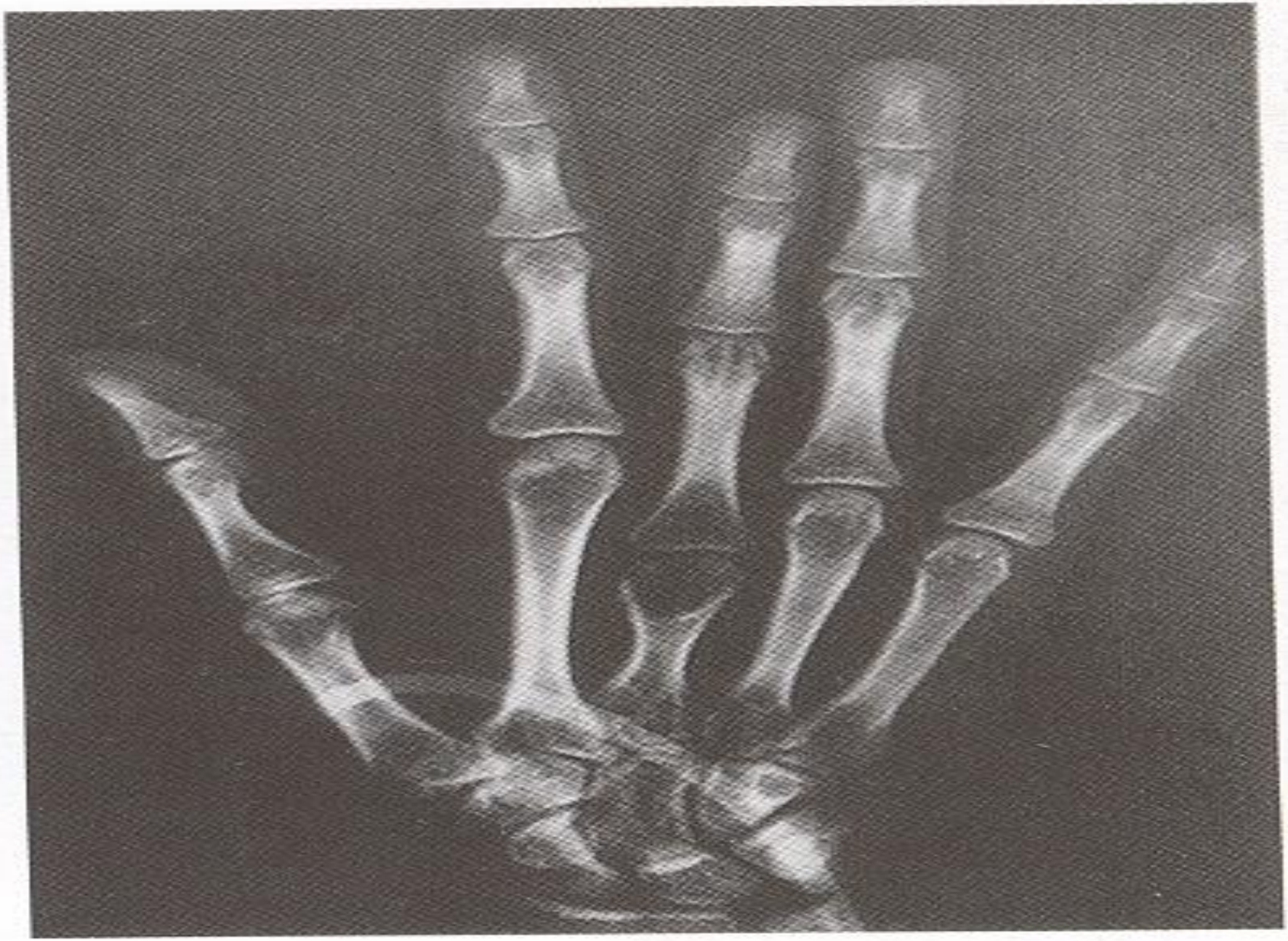
Clinical Manifestations in Sickle Anaemia

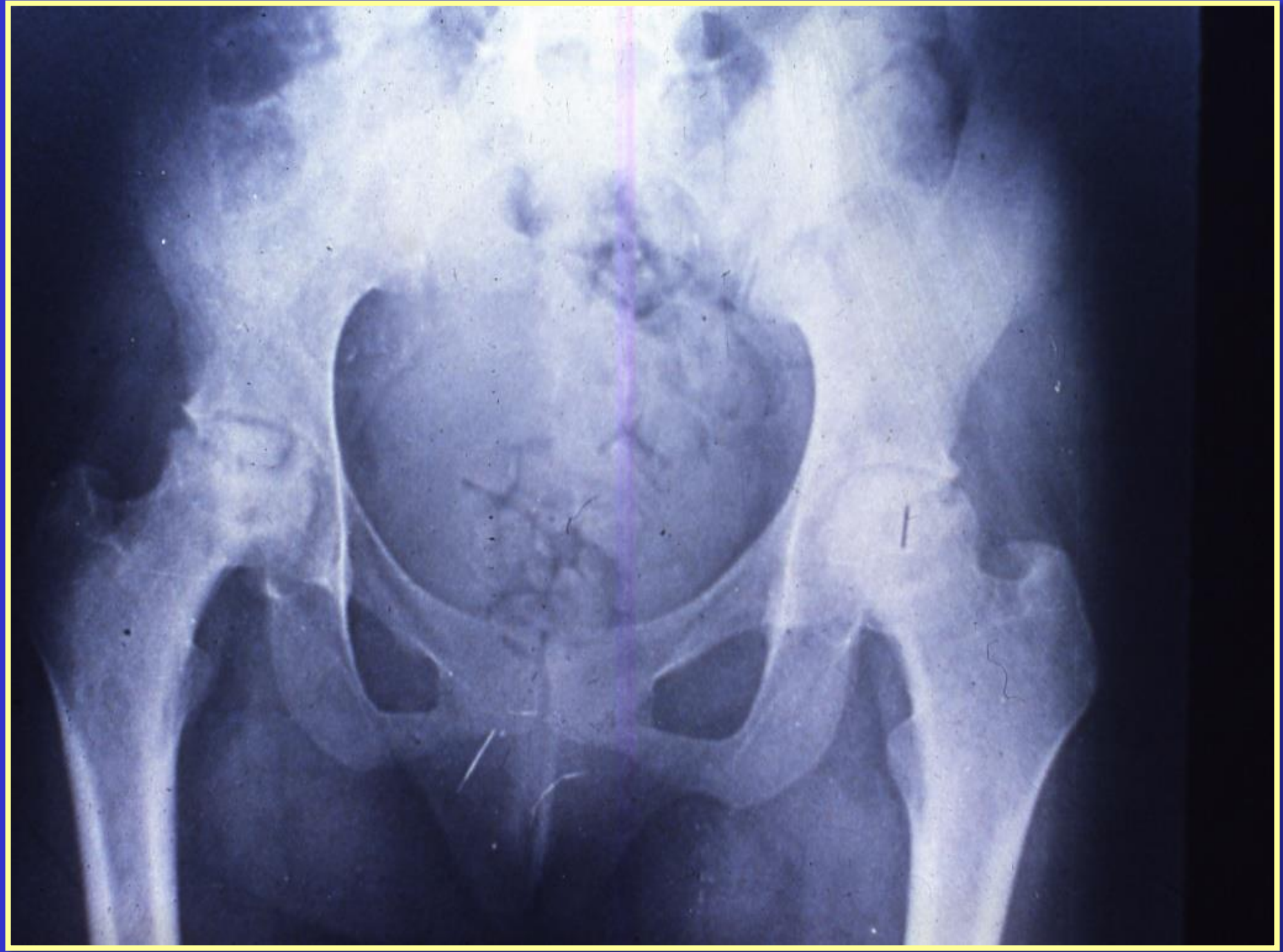
- Recurrent Infections & Chest Symptoms (Acute Chest Syndrome)
- Hepato-Splenomegaly
  - → (Early Childhood)
  - → (Association with Thalassaemias)
- CNS Presentations
- Leg Ulceration
- Skeletal Deformity

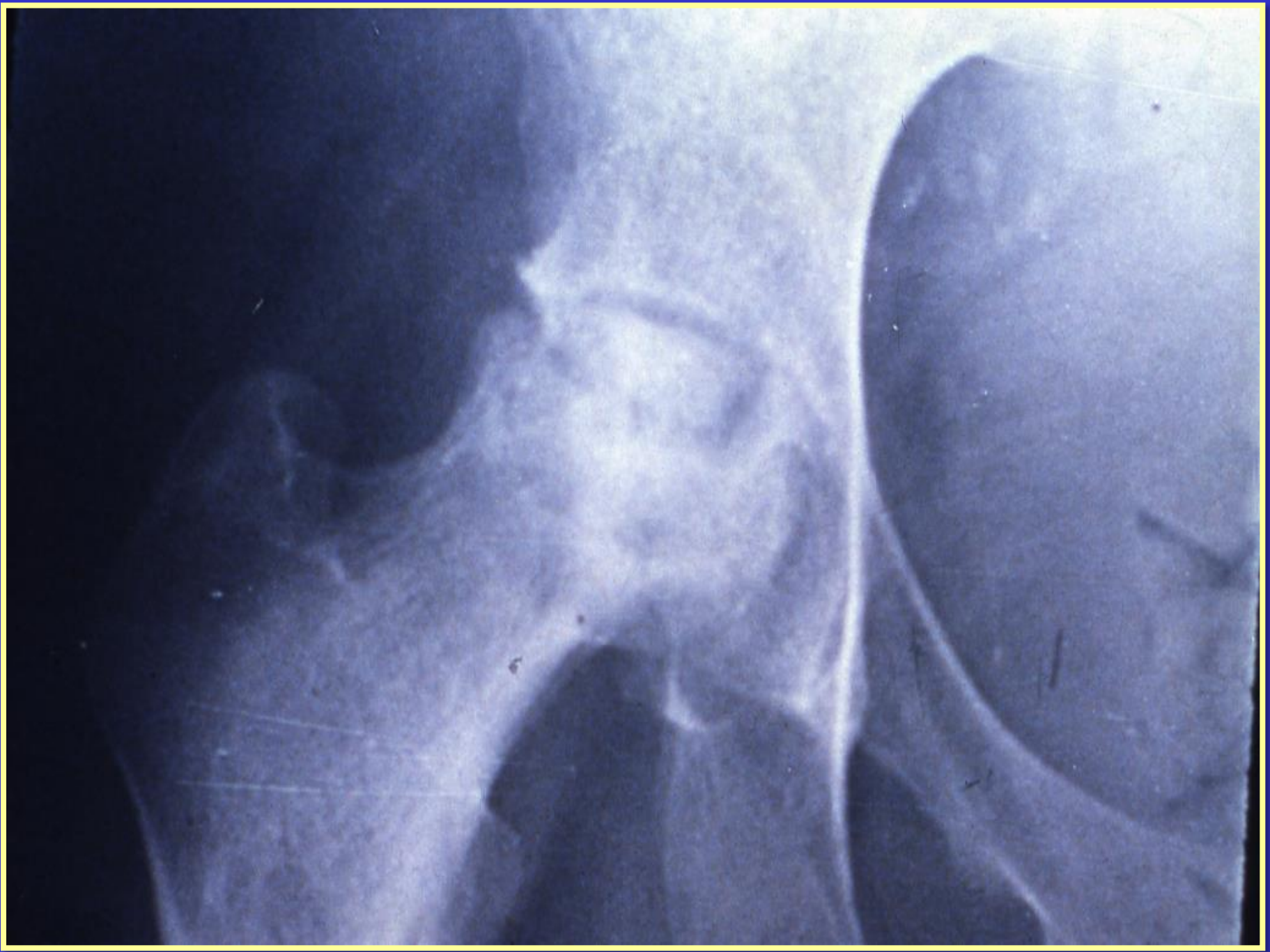


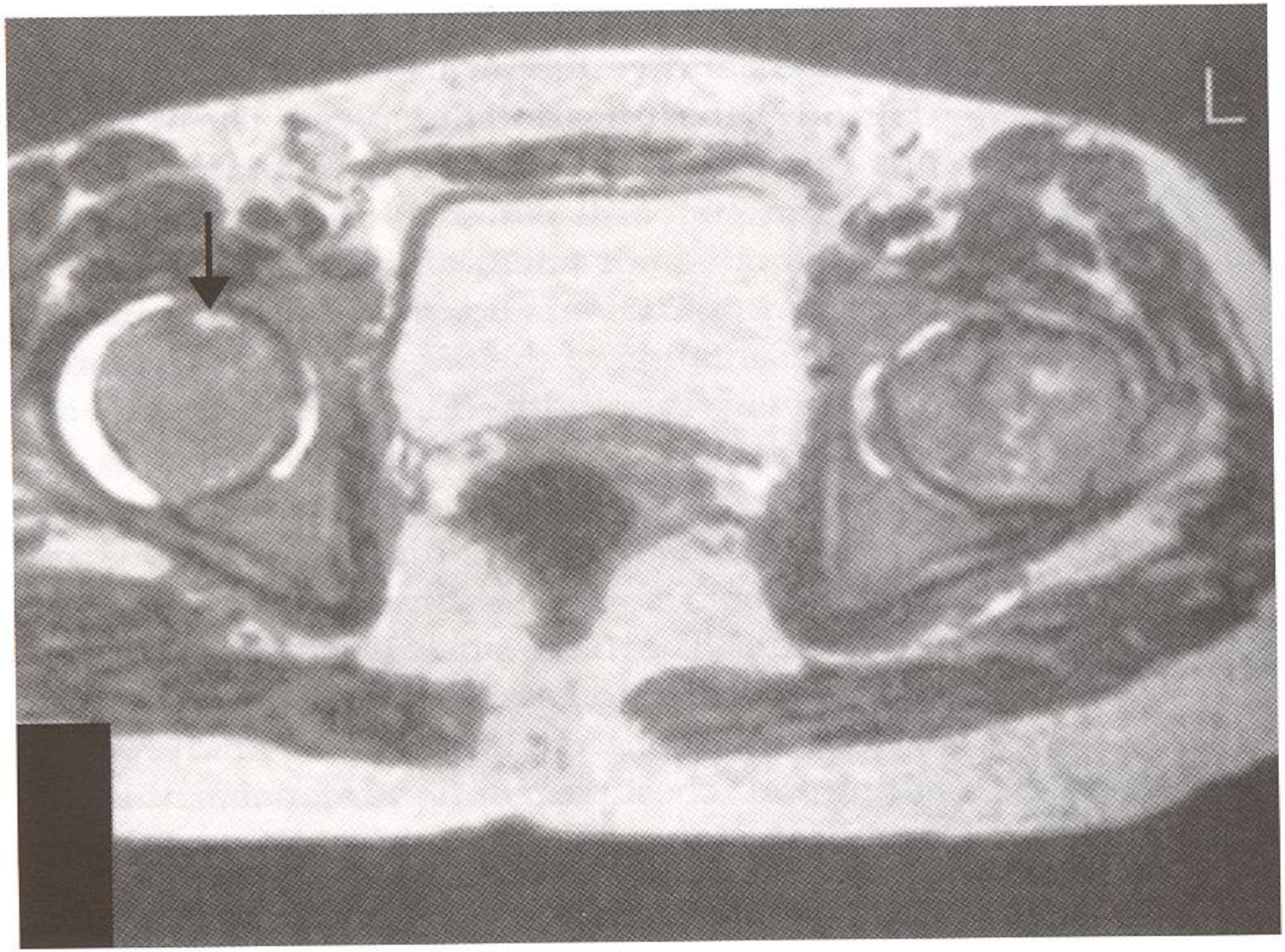








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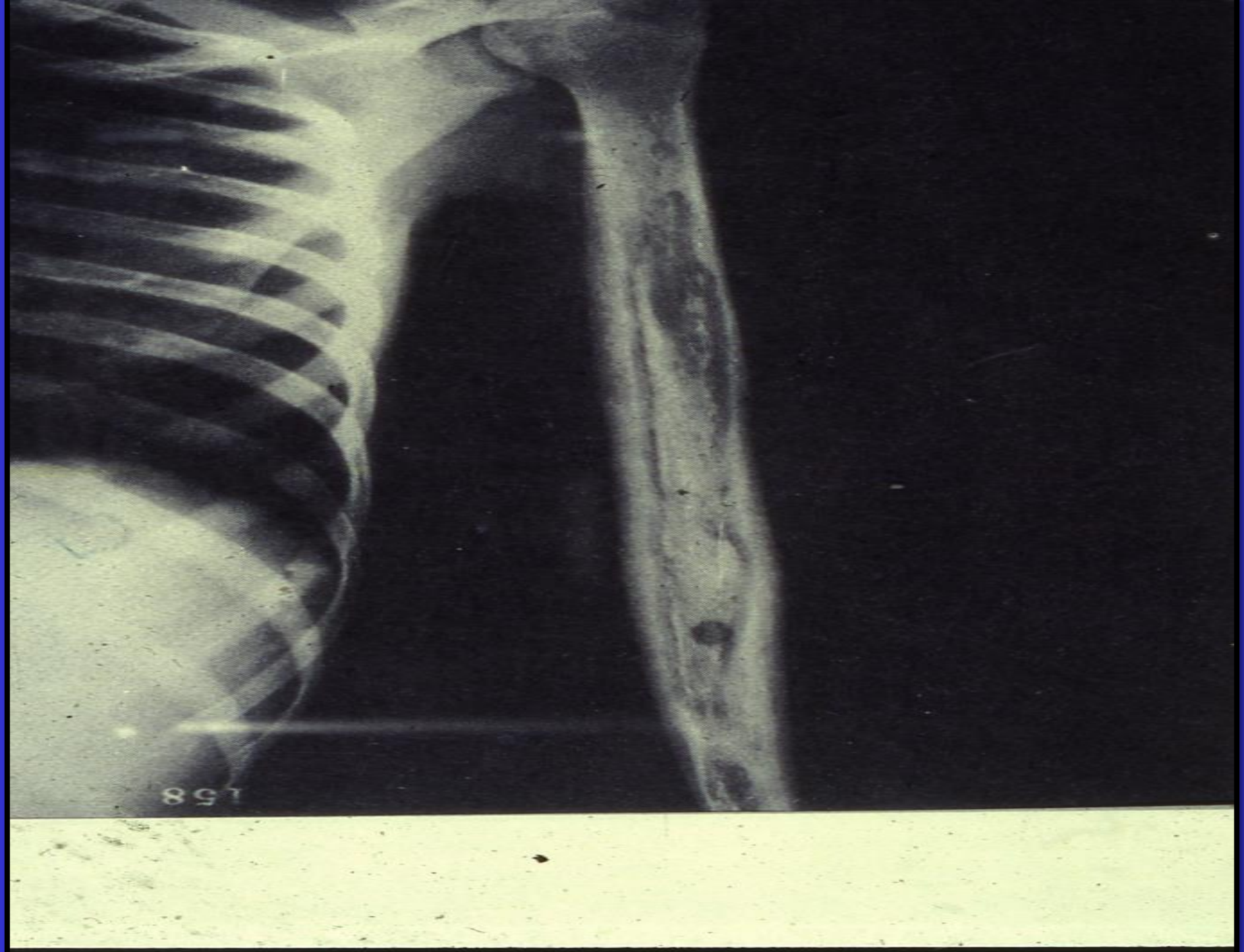
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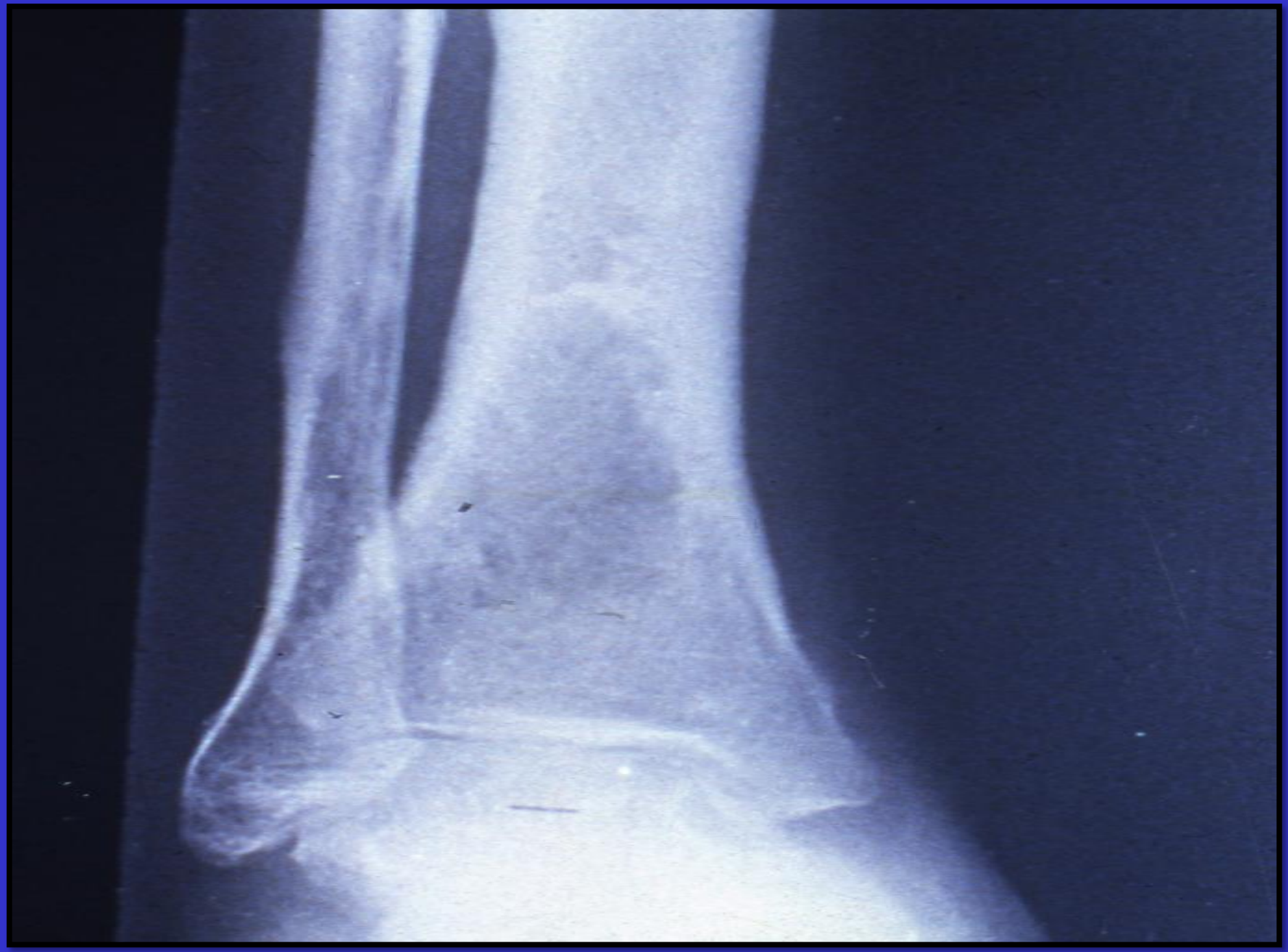
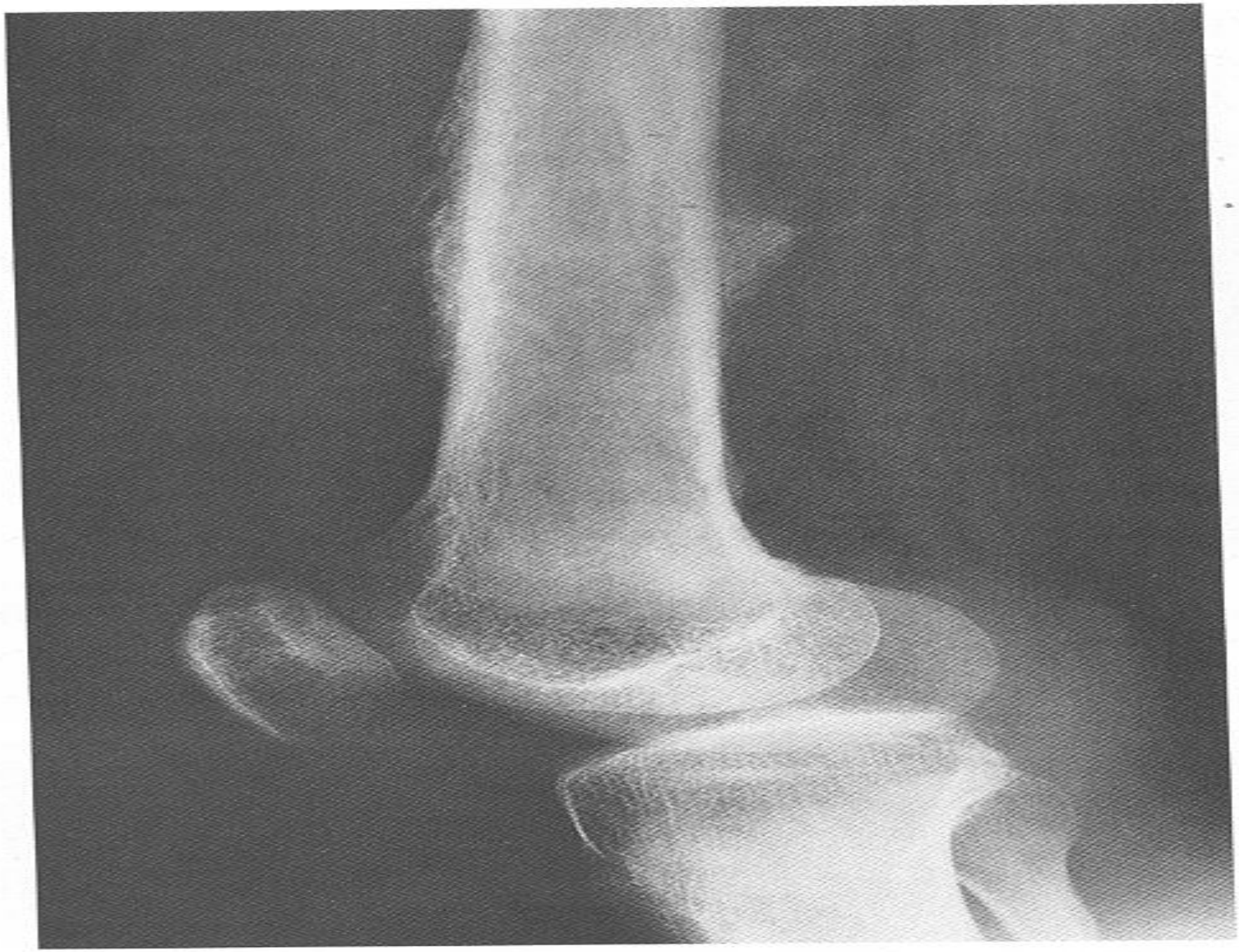
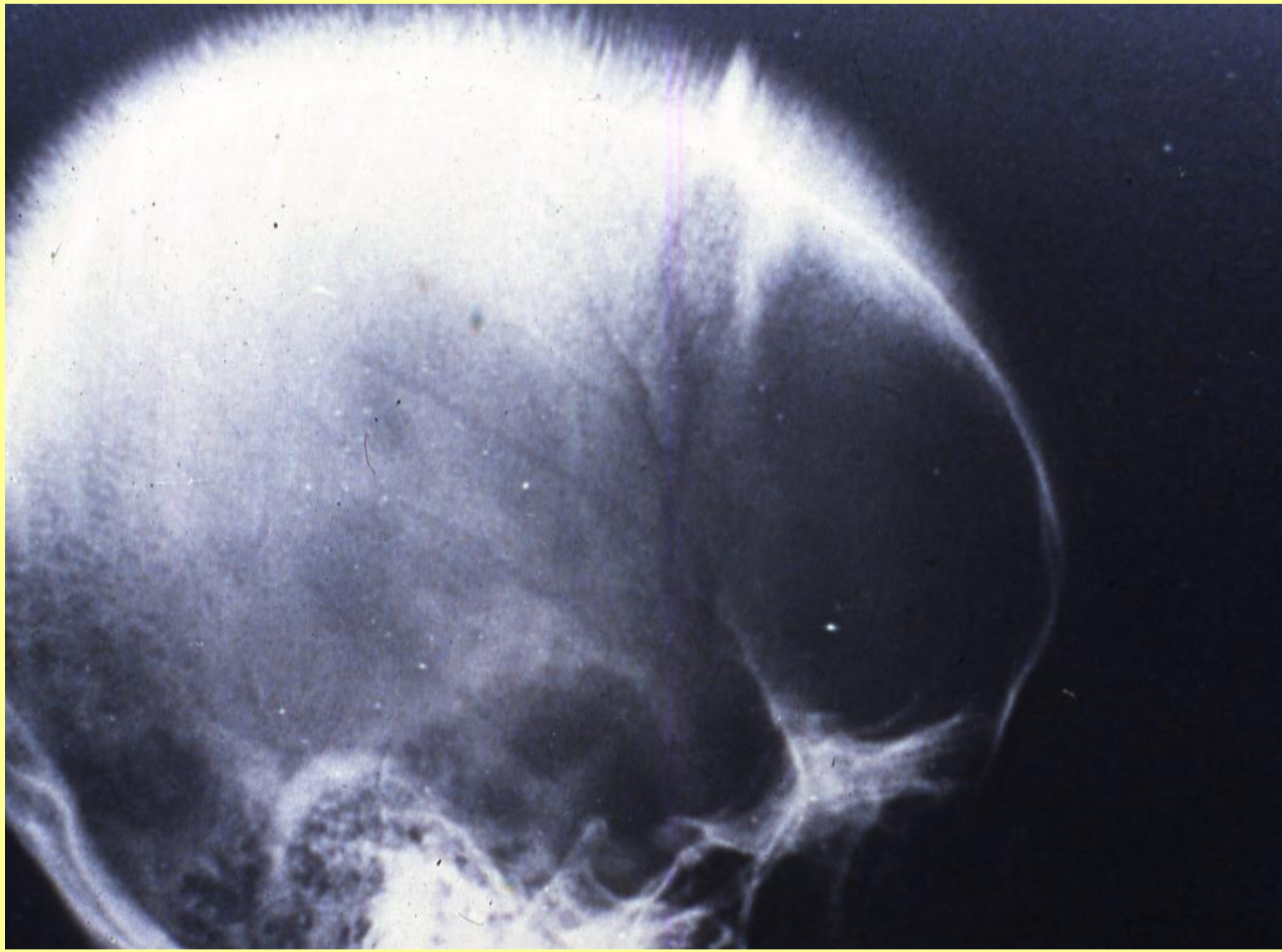
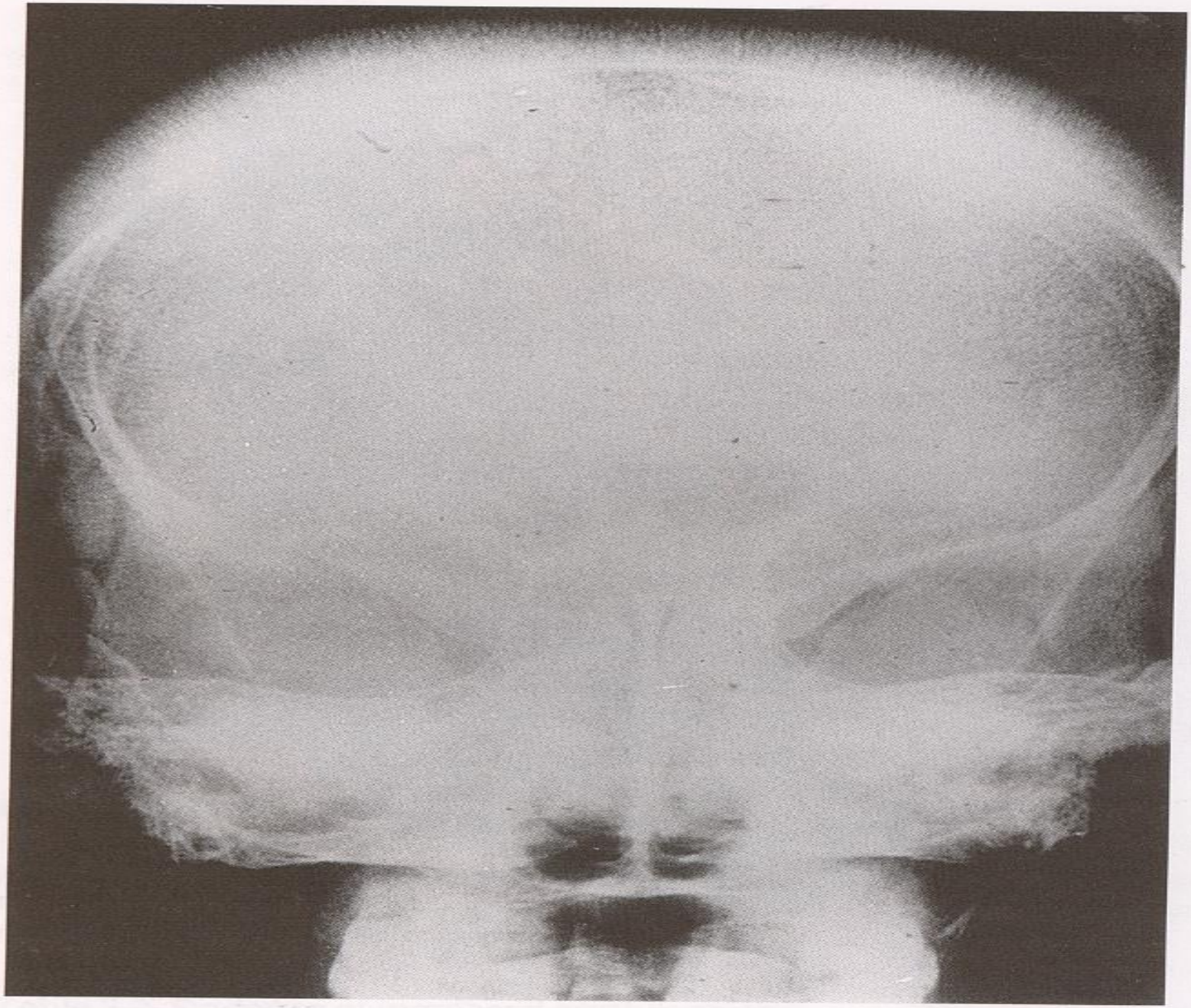
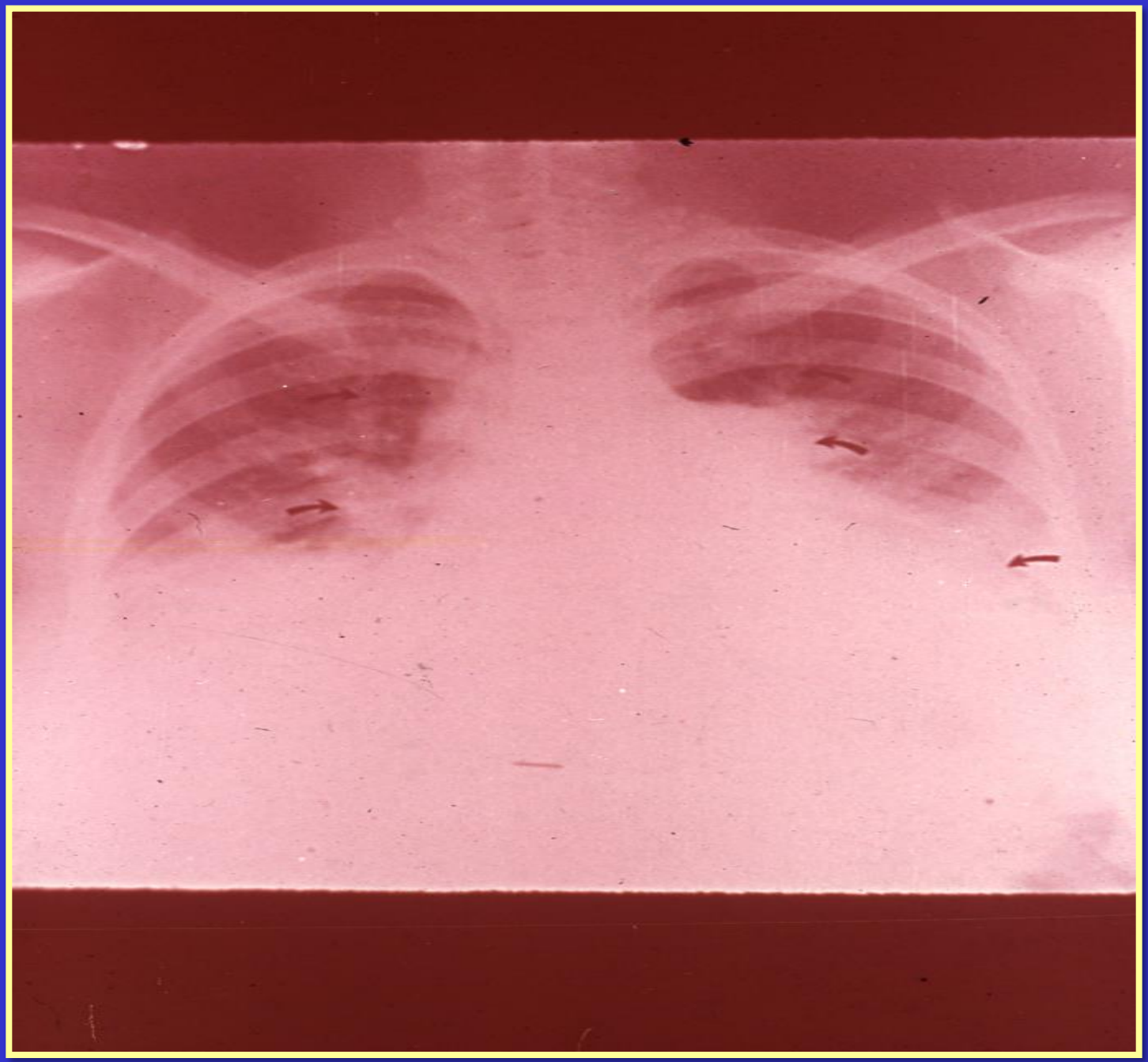
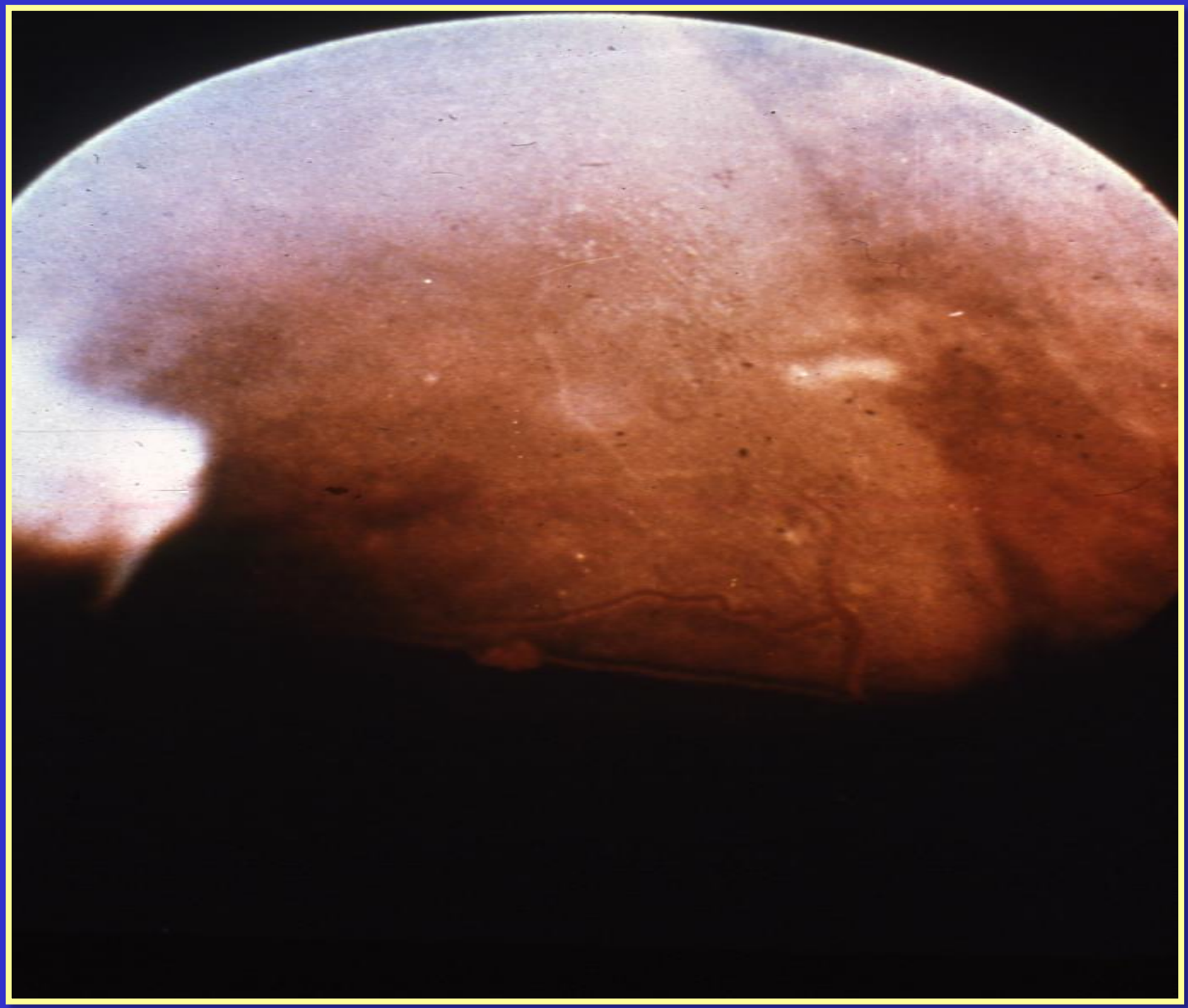


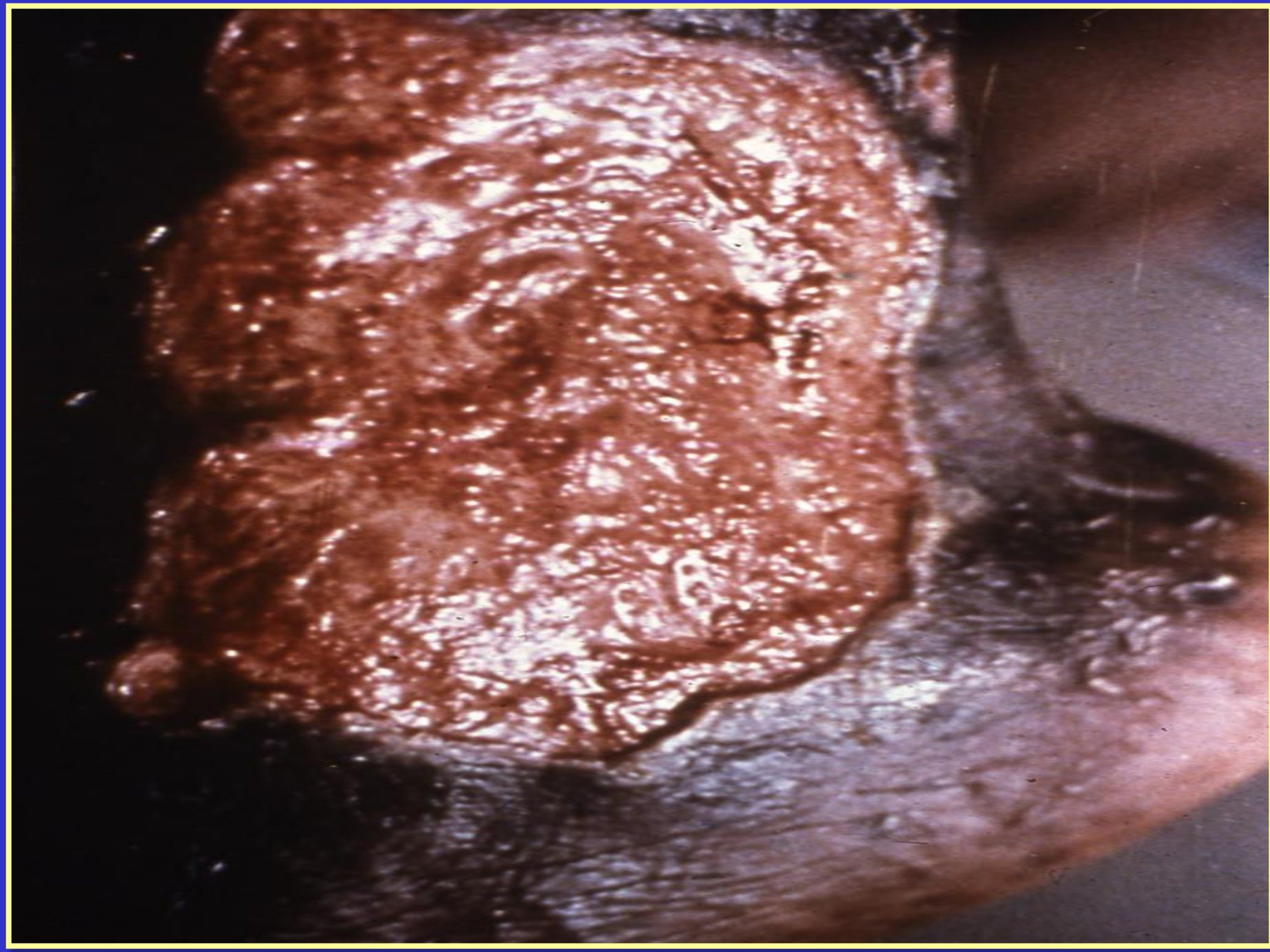


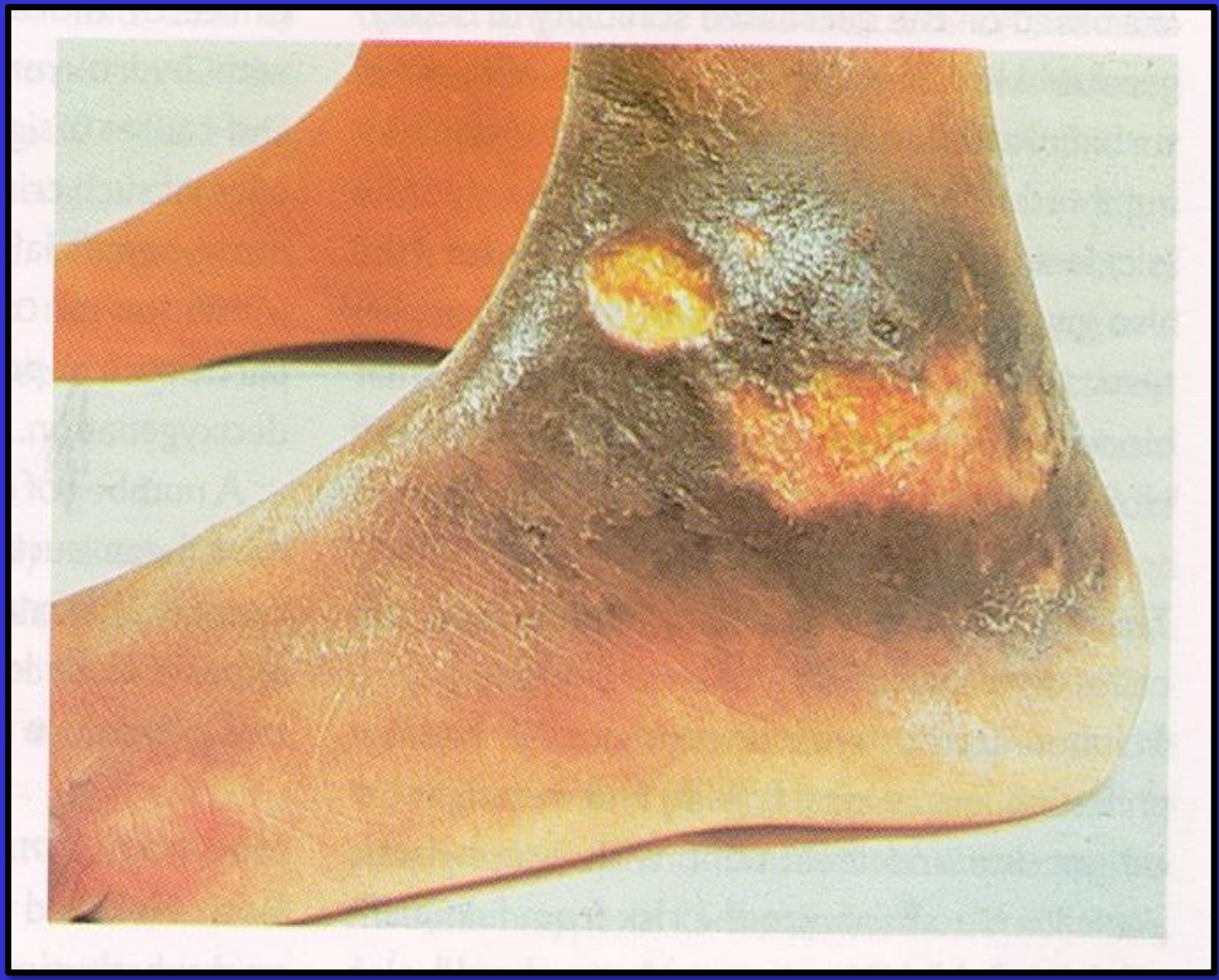


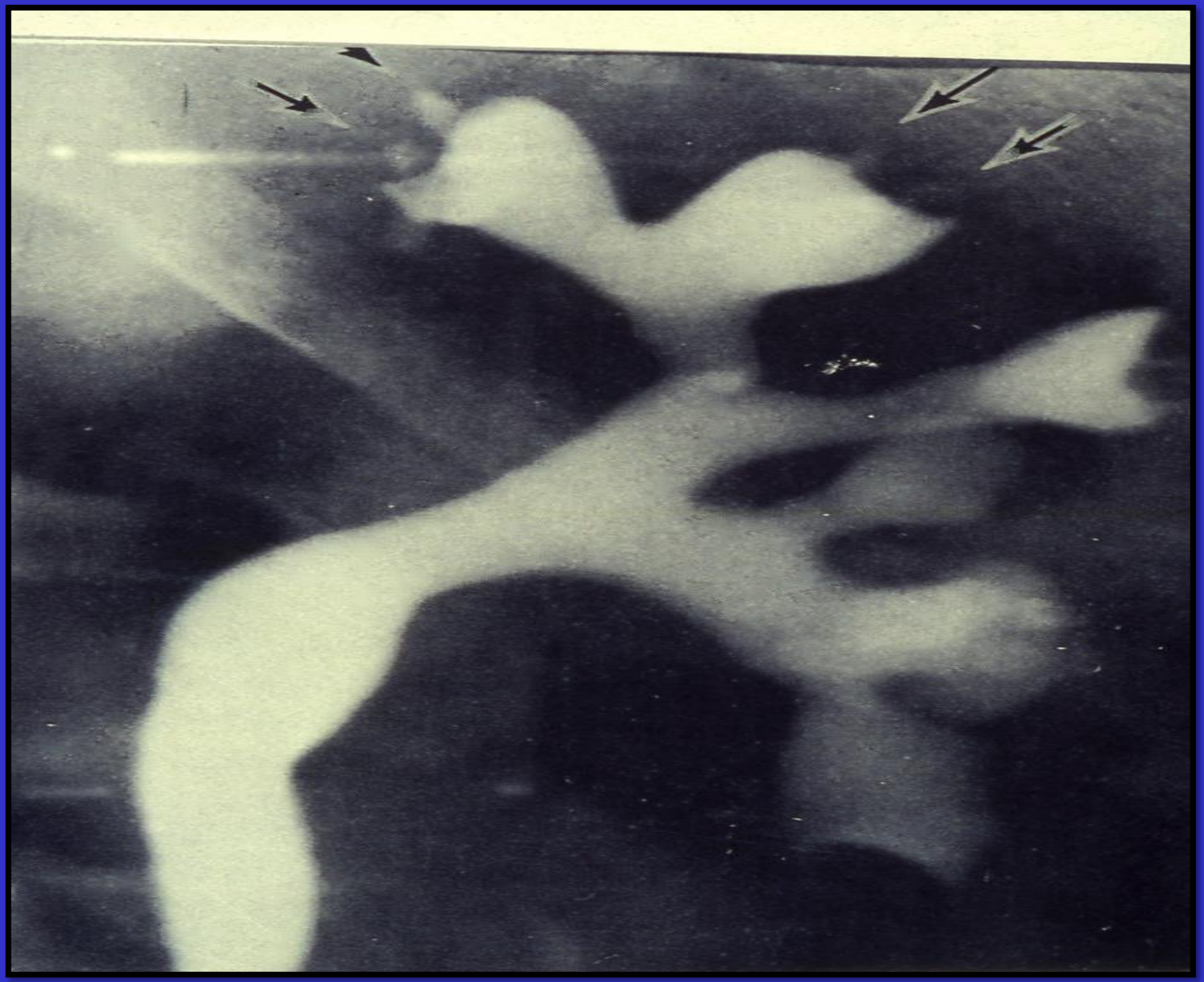






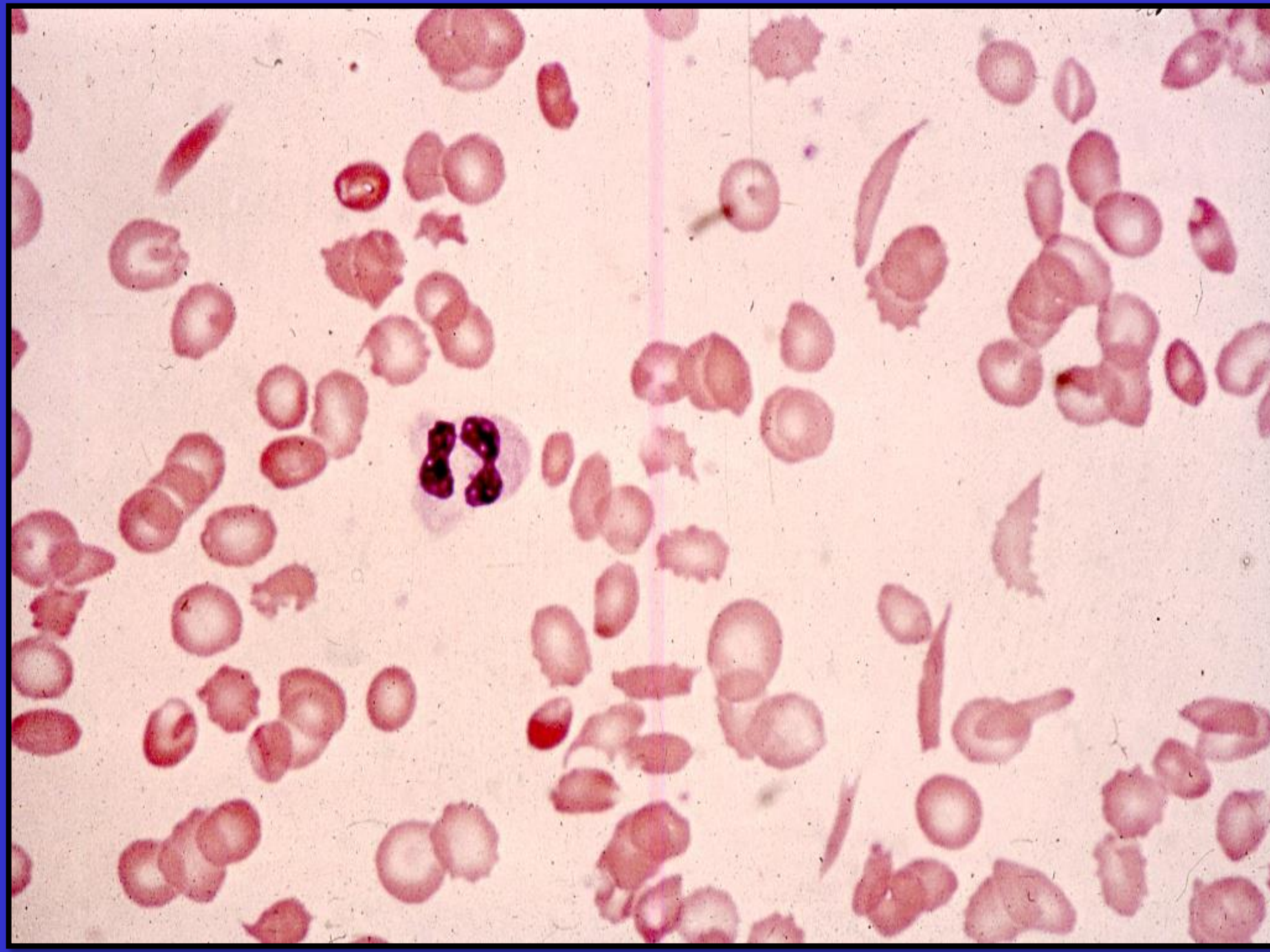


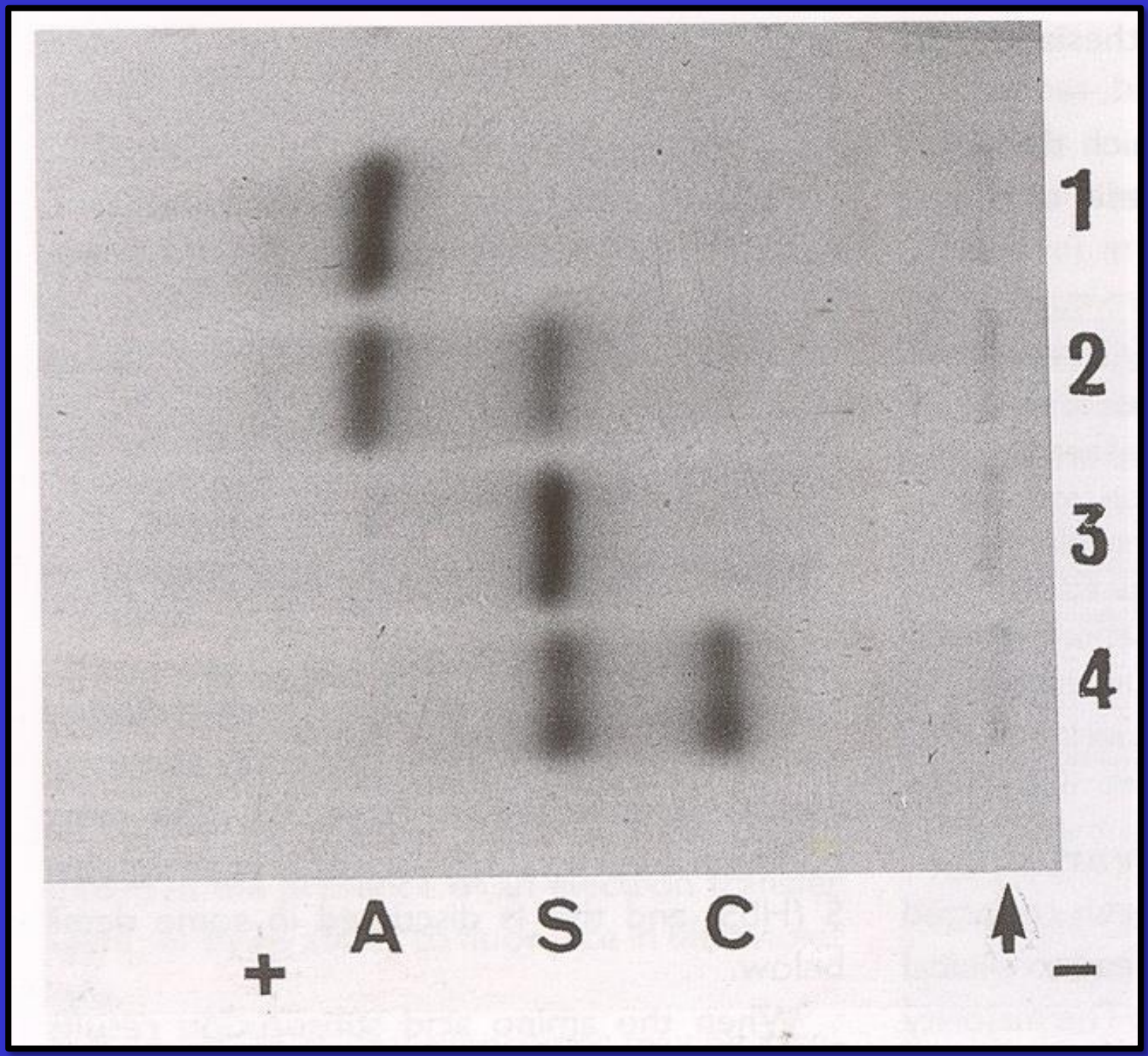




Laboratory Diagnosis of Sickle Cell Disease

- CBC
- Blood Film
- Sickle Solubility Test
- Hb Electrophoresis
- Genetic Study

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Indications for Blood Transfusion in Sickle Cell Anaemia

- Splenic sequestration
- Hepatic sequestration
- Aplastic crisis
- Overwhelming infections
- Elective or emergency surgical operation
- Severe painful crisis associated with severe haemolysis
- Pregnancy

Indications for exchange transfusion

- Strokes
- Pulmonary infarcts with infection
- Pregnancy (Severe persistent painful crisis)
- Priapism
- Preparation for major surgery


Thank you!
TESCHIO 99