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

cont'd…

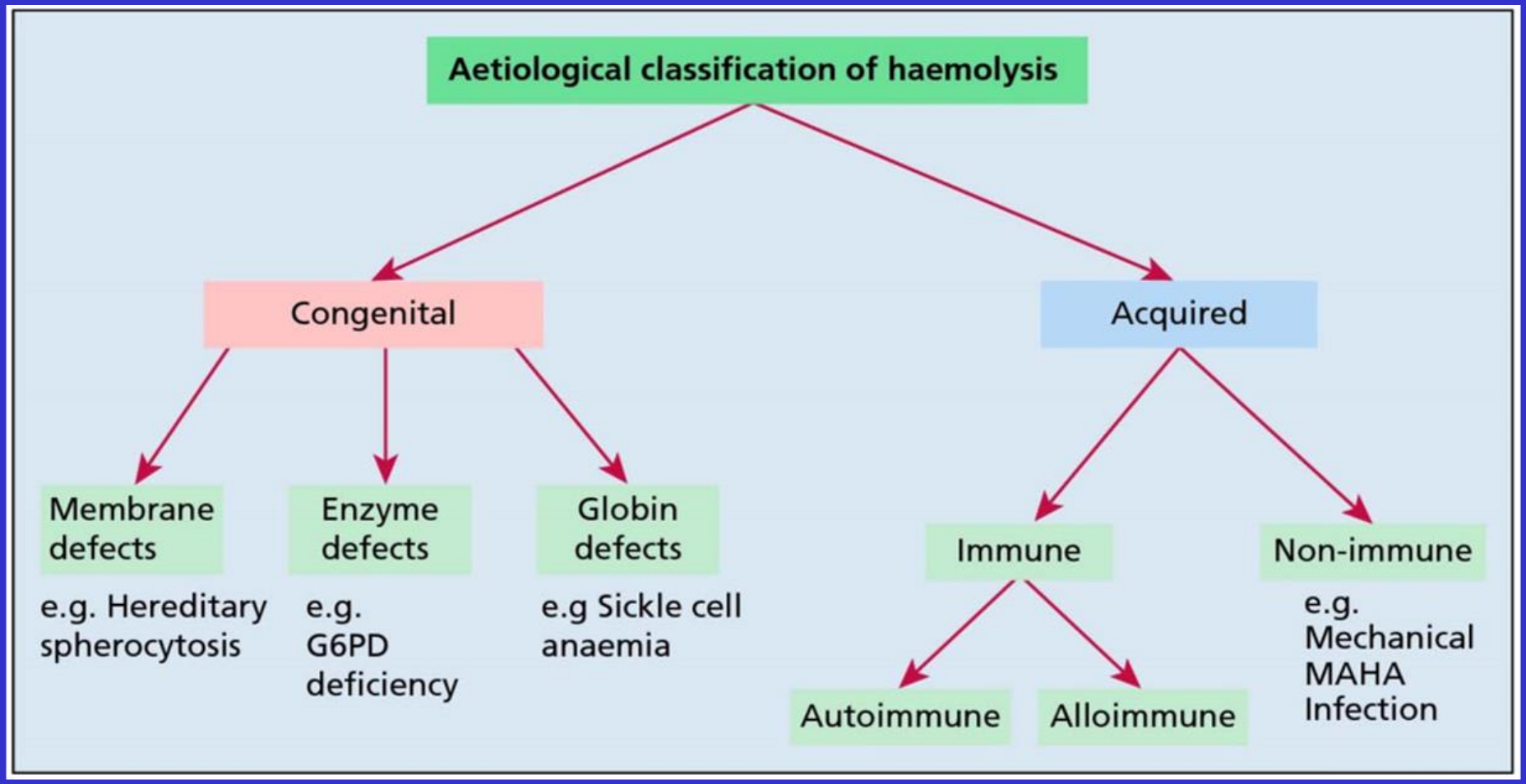
- To appreciate that disorders of globin function such as sickle cell disease are subtypes of haemolytic anaemia
- To understand the role of autoantibodies in the production of haemolytic anaemias and to know the types of disease with which they are associated
- To understand some causes of non-immune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

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.

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.

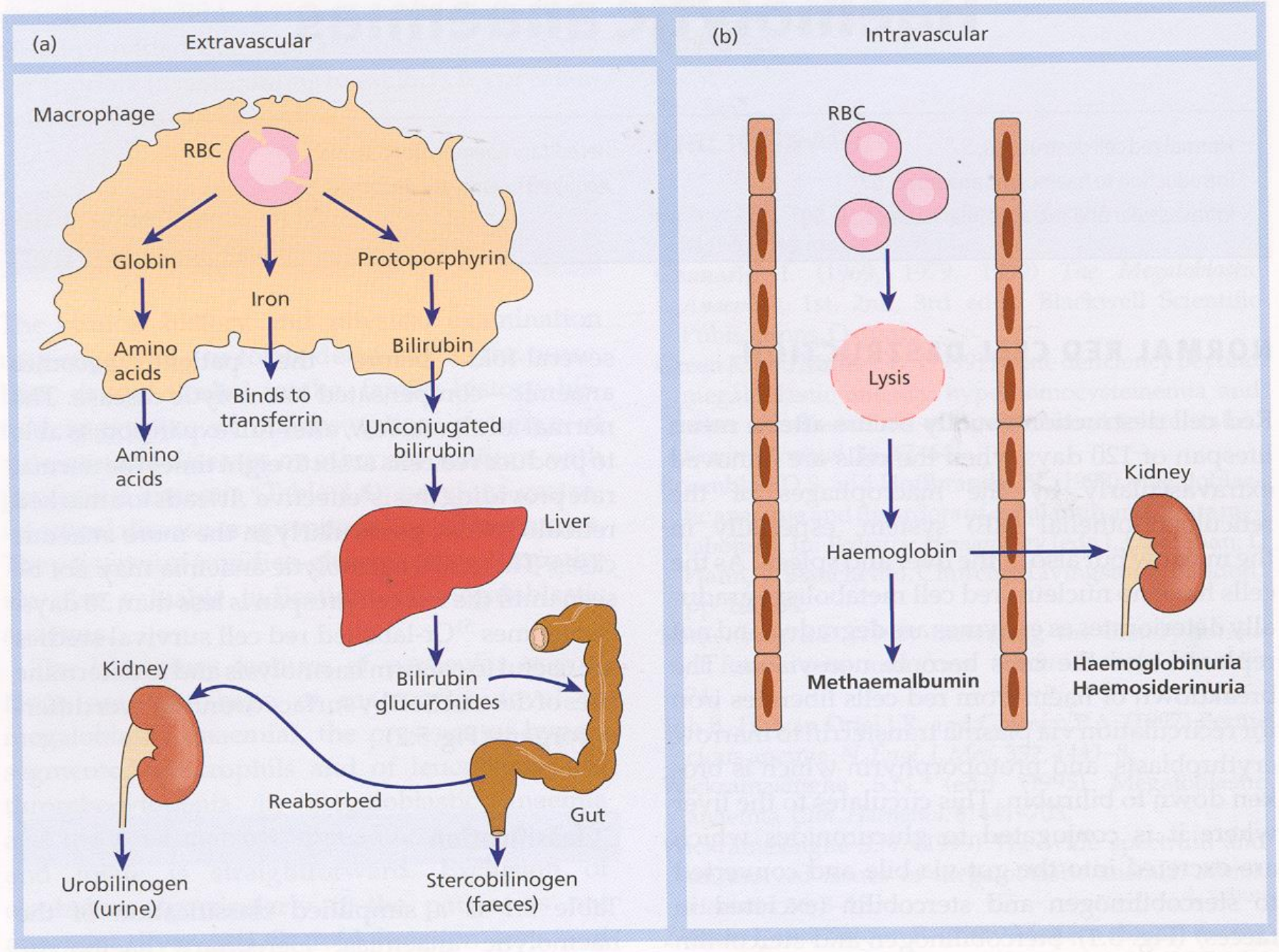
- **a. ↑ serum bilirubin is raised (unconjugated and bound to albumin).**
- **b. ↑ urine urobilinogen.**
- **c. ↑ faecal stercobilinogen.**
- **d. Absent serum haptoglobins.**
- **e. ↑ lactate dehydrogenase (LDH)**

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.

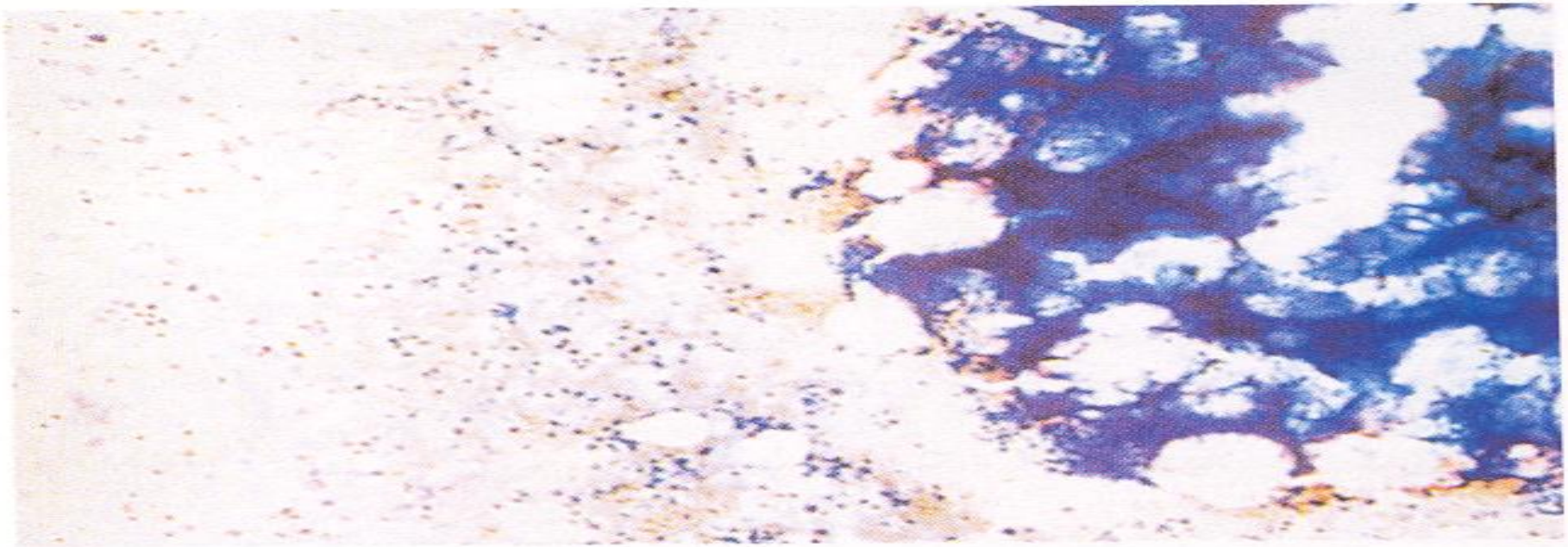
Intravascular and extravascular haemolysis

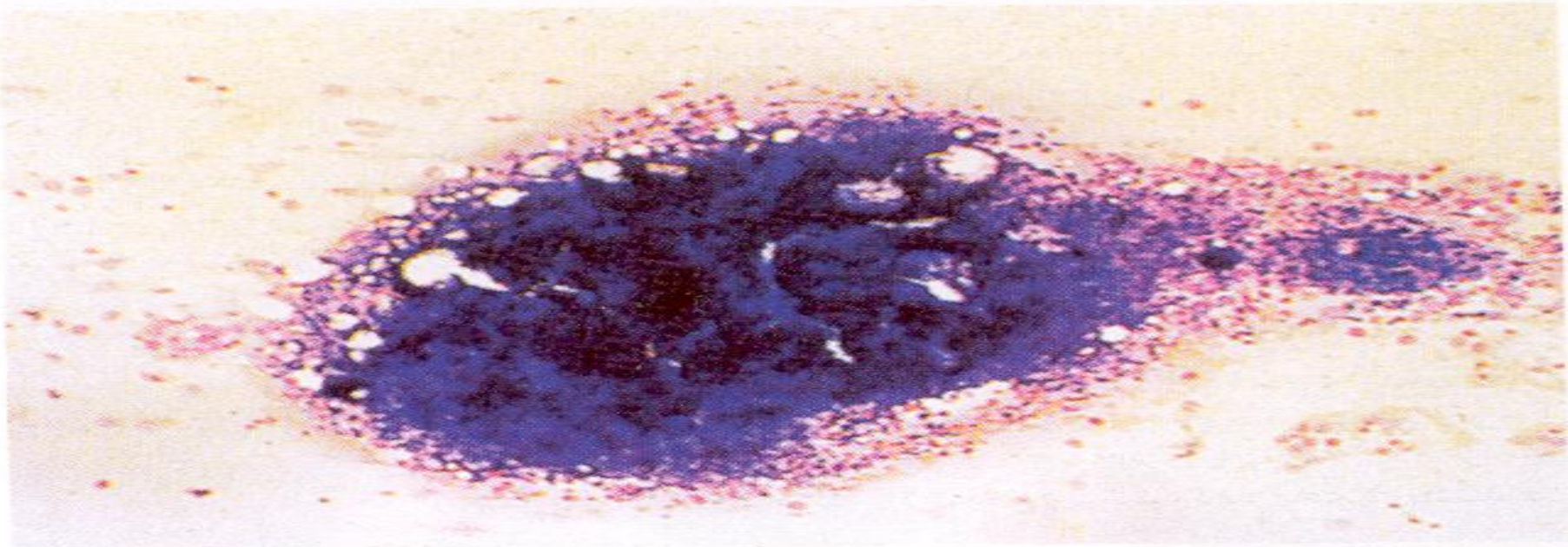
a. **Intravascular haemolysis, the process of breakdown of red cells directly in the circulation.**
b. **Extravascular haemolysis excessive removal of red cells by cells of RE system in the spleen and liver.**


(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

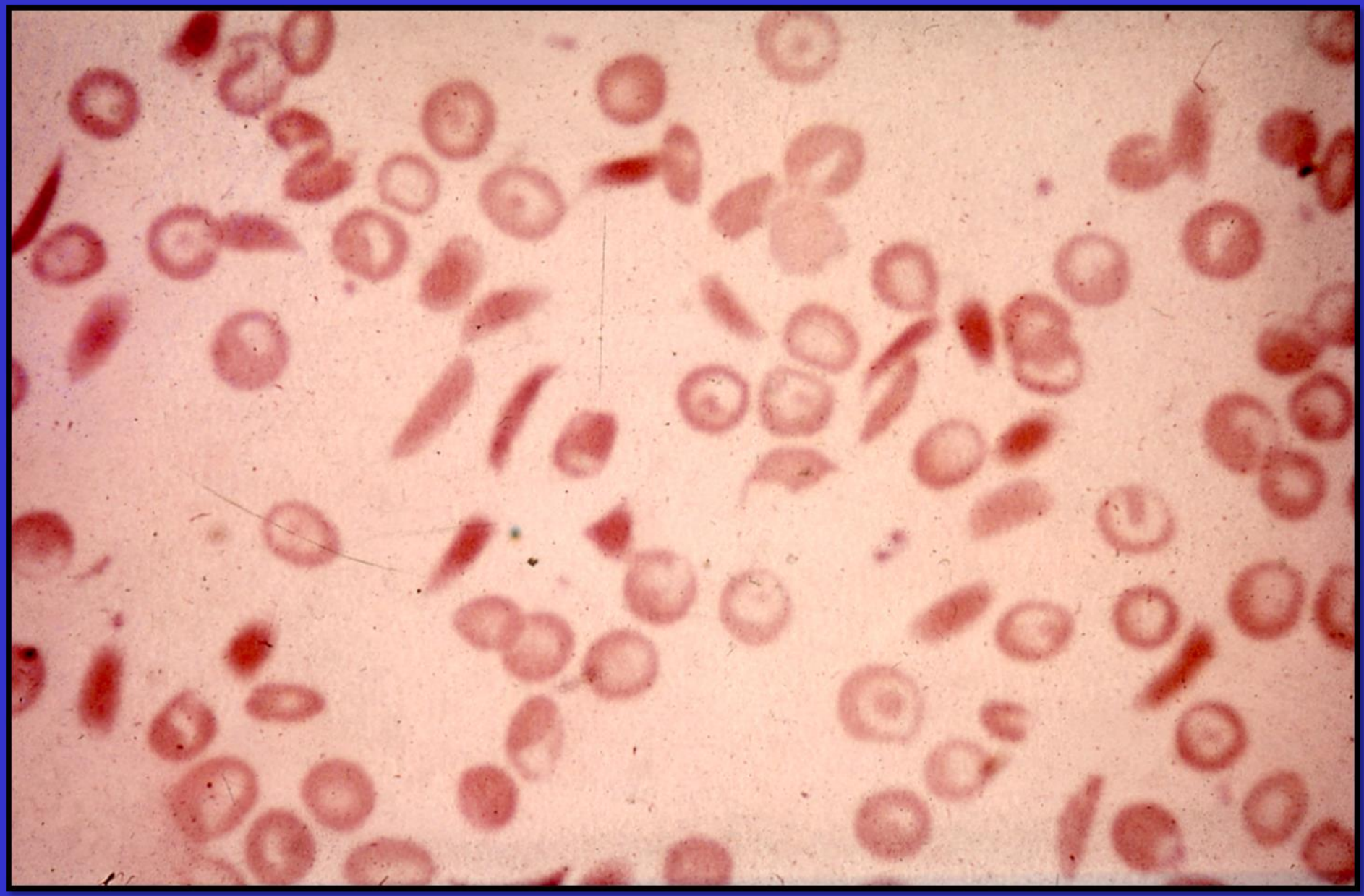
HAEMOLYTIC ANAEMIA

A . CONGENITAL

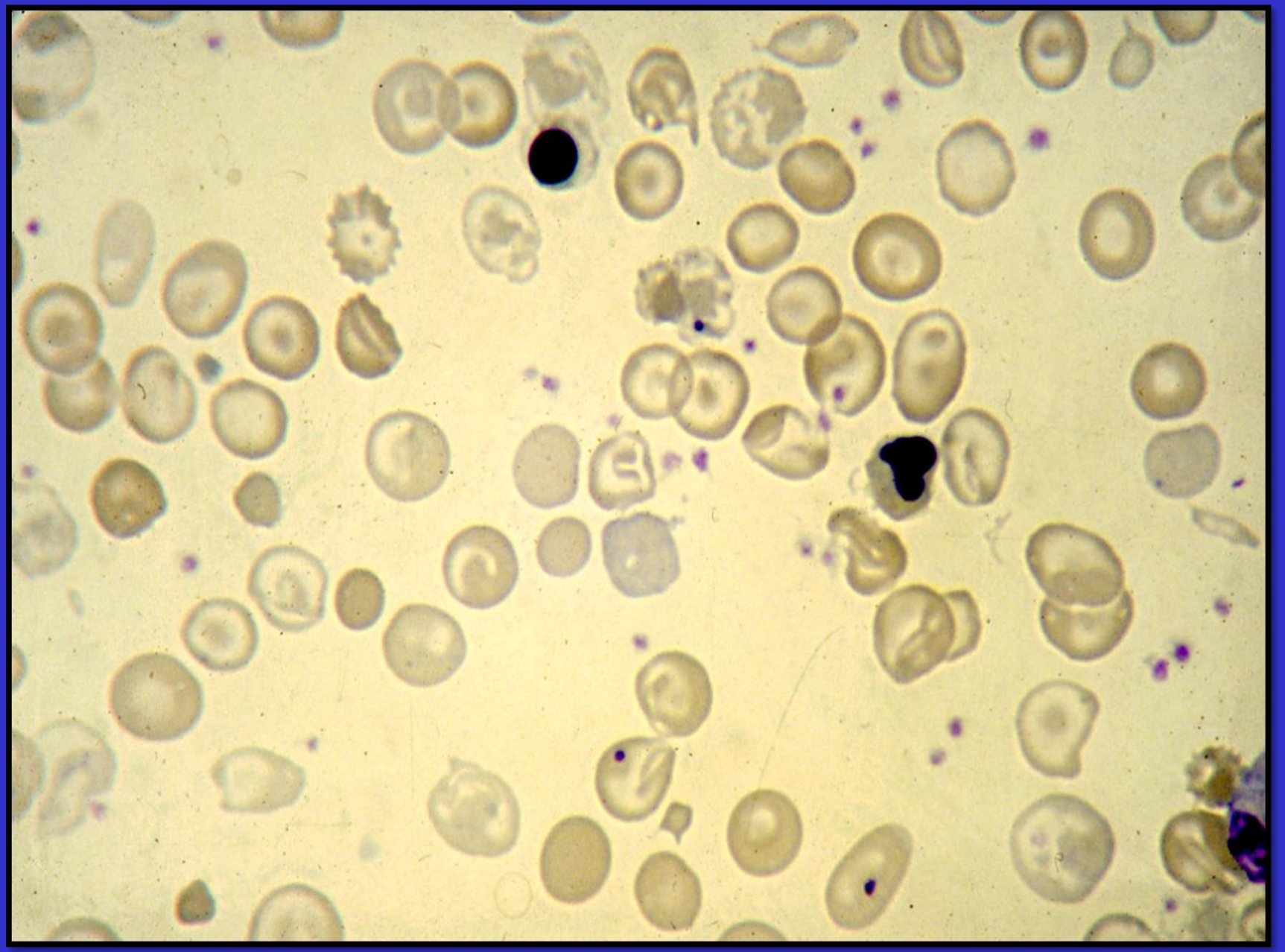
- **SICKLE CELL DISEASE & OTHER HAEMOGLOBIN DISORDERS**
- **THALASSAEMIAS**
- **ENZYMOPATHIES**
- **MEMBRANOPATHIES**

B. AQUIRED

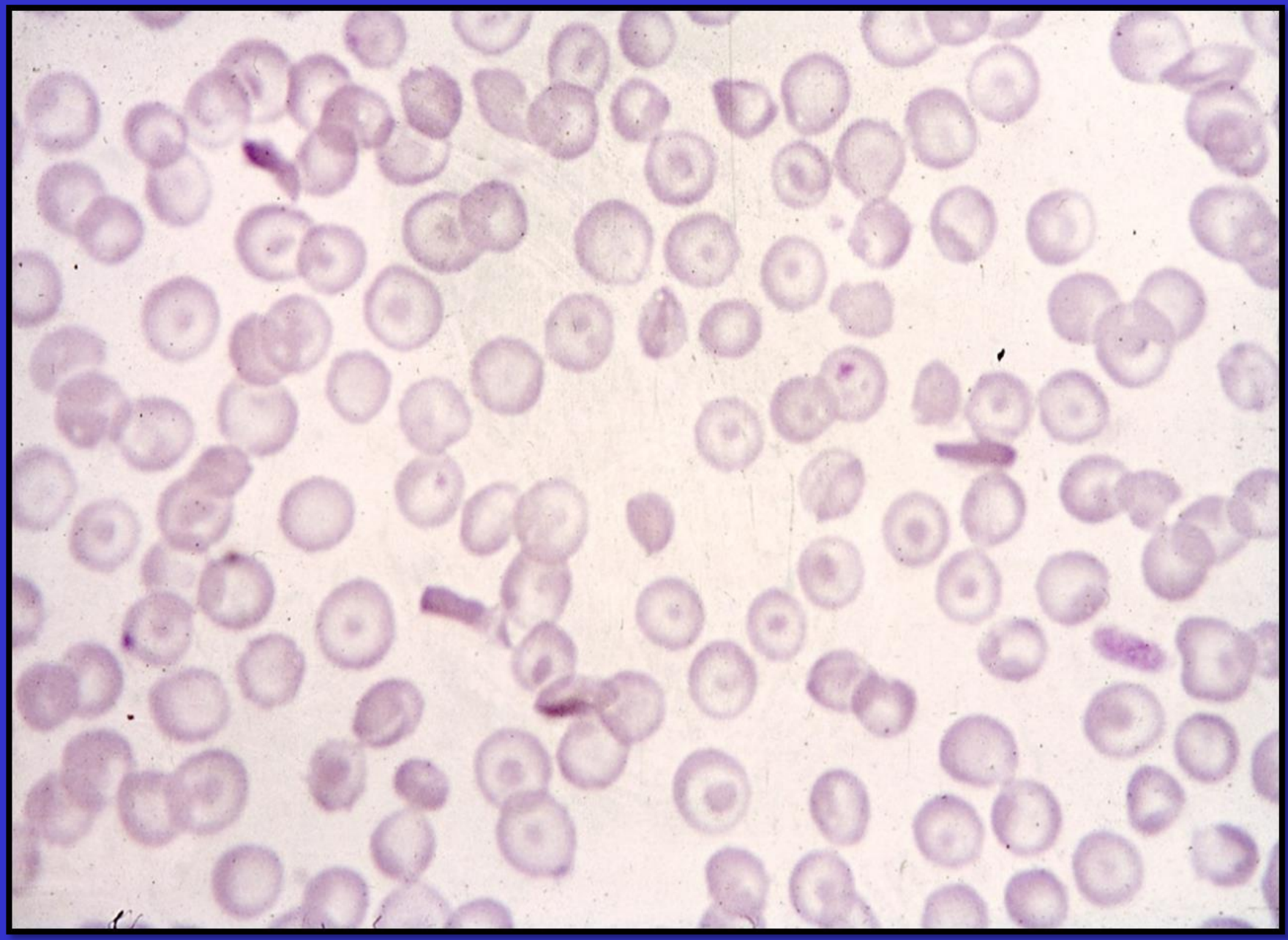
SICKLE CELL ANAEMIA

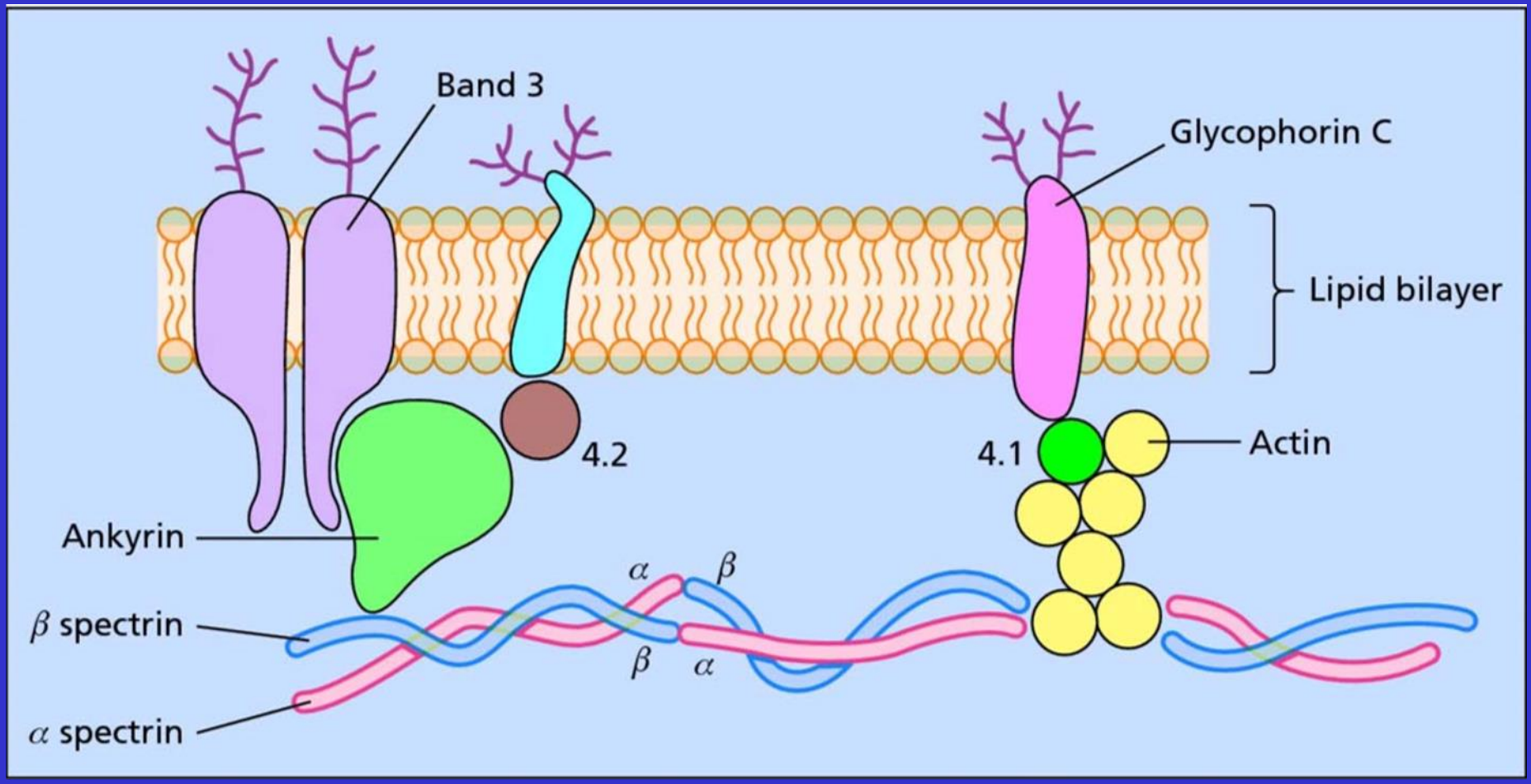


THALASSAEMIA MAJOR



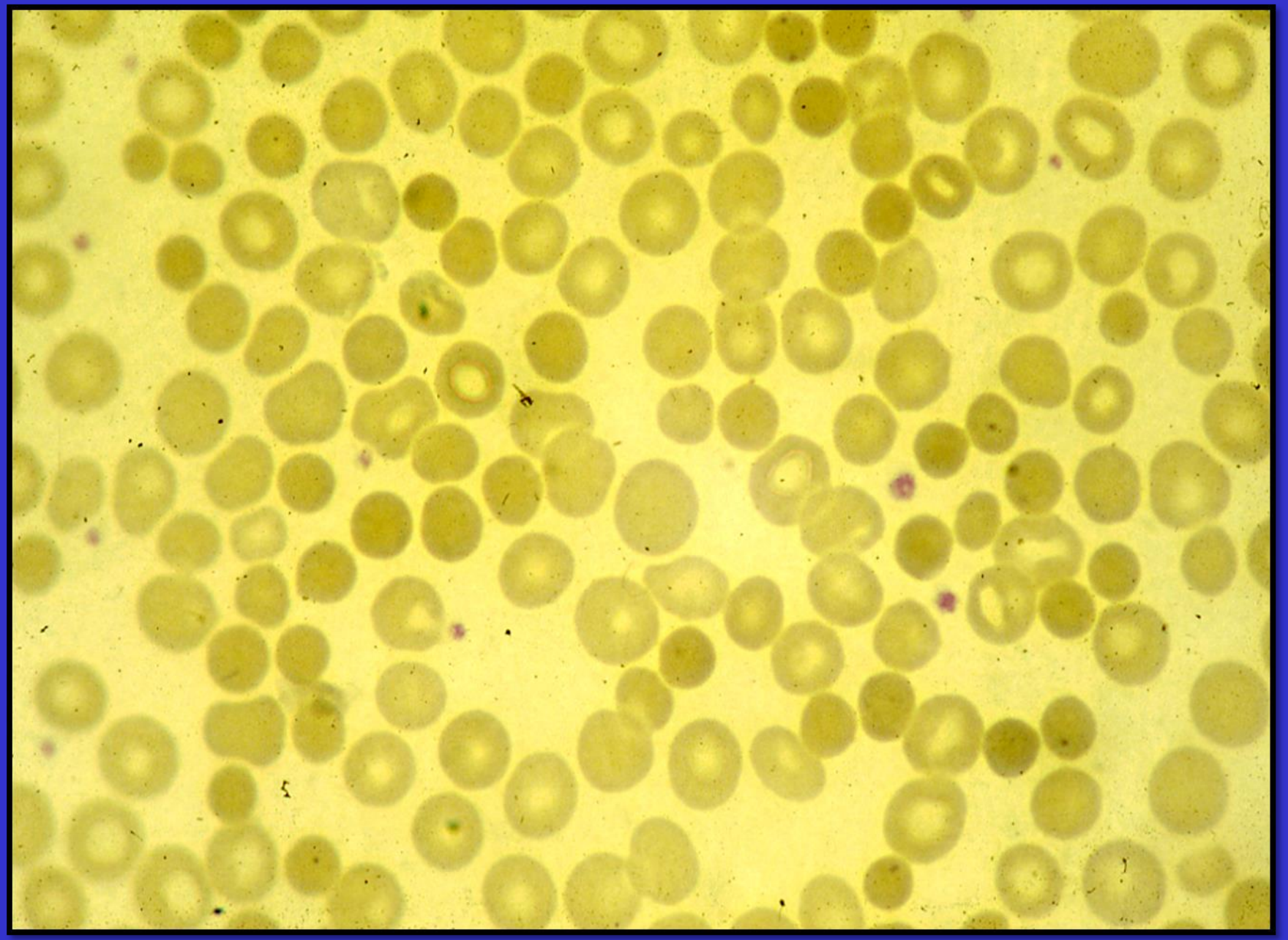
SICKLE BETA-THALASSAEMIA



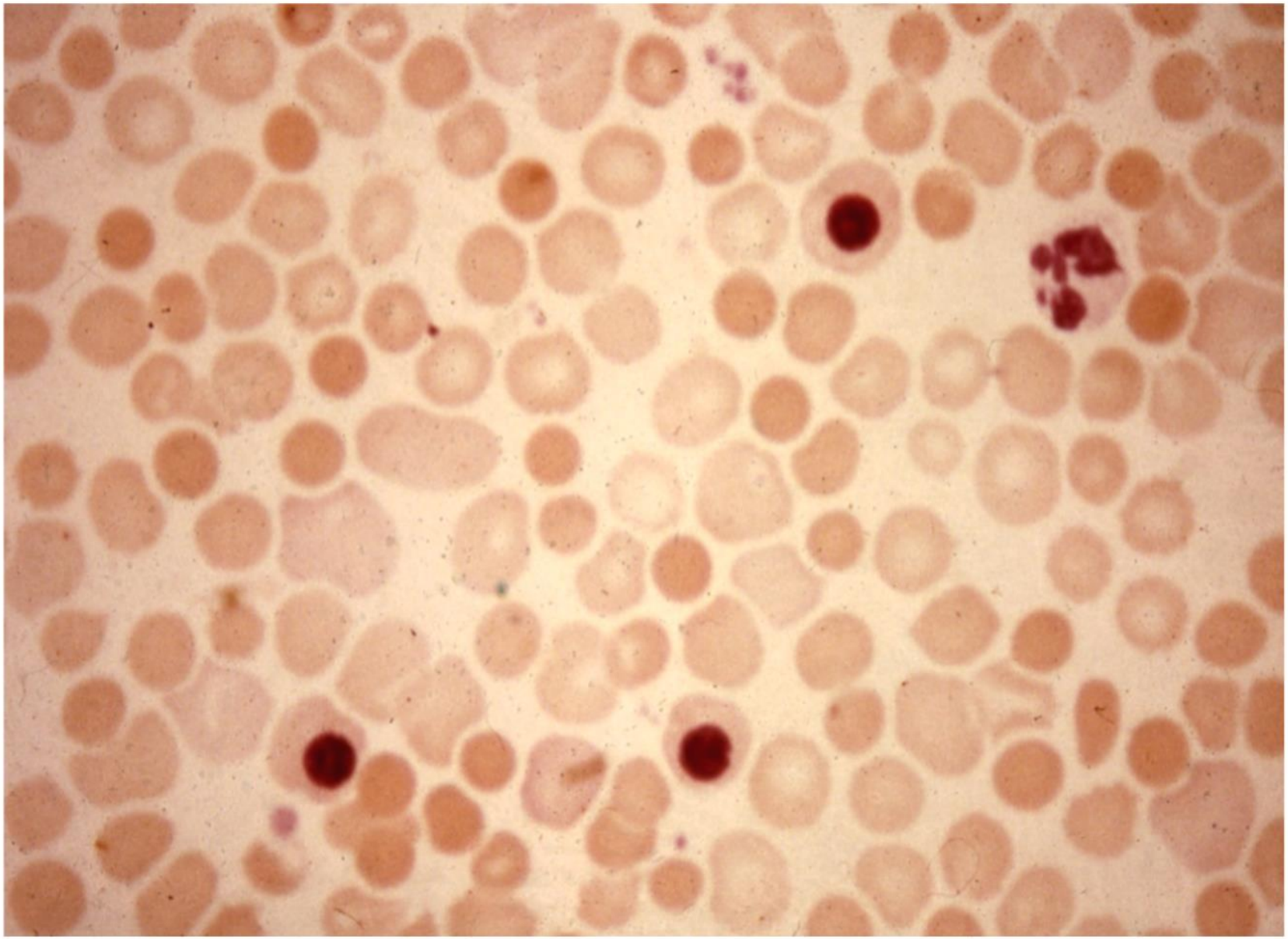


Schematic diagram of the red cell membrane cytoskeleton.

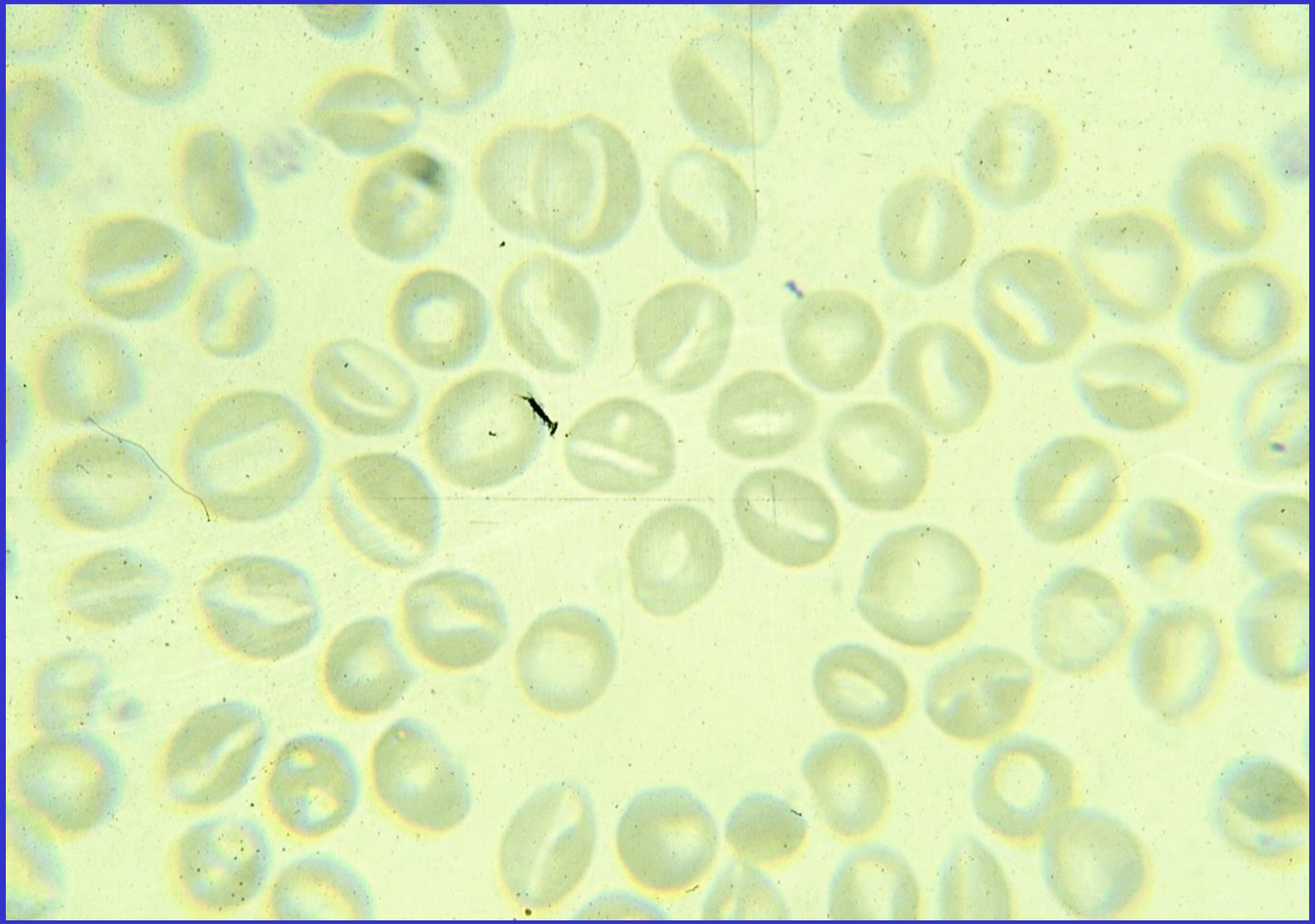
SPHEROCYTOSIS

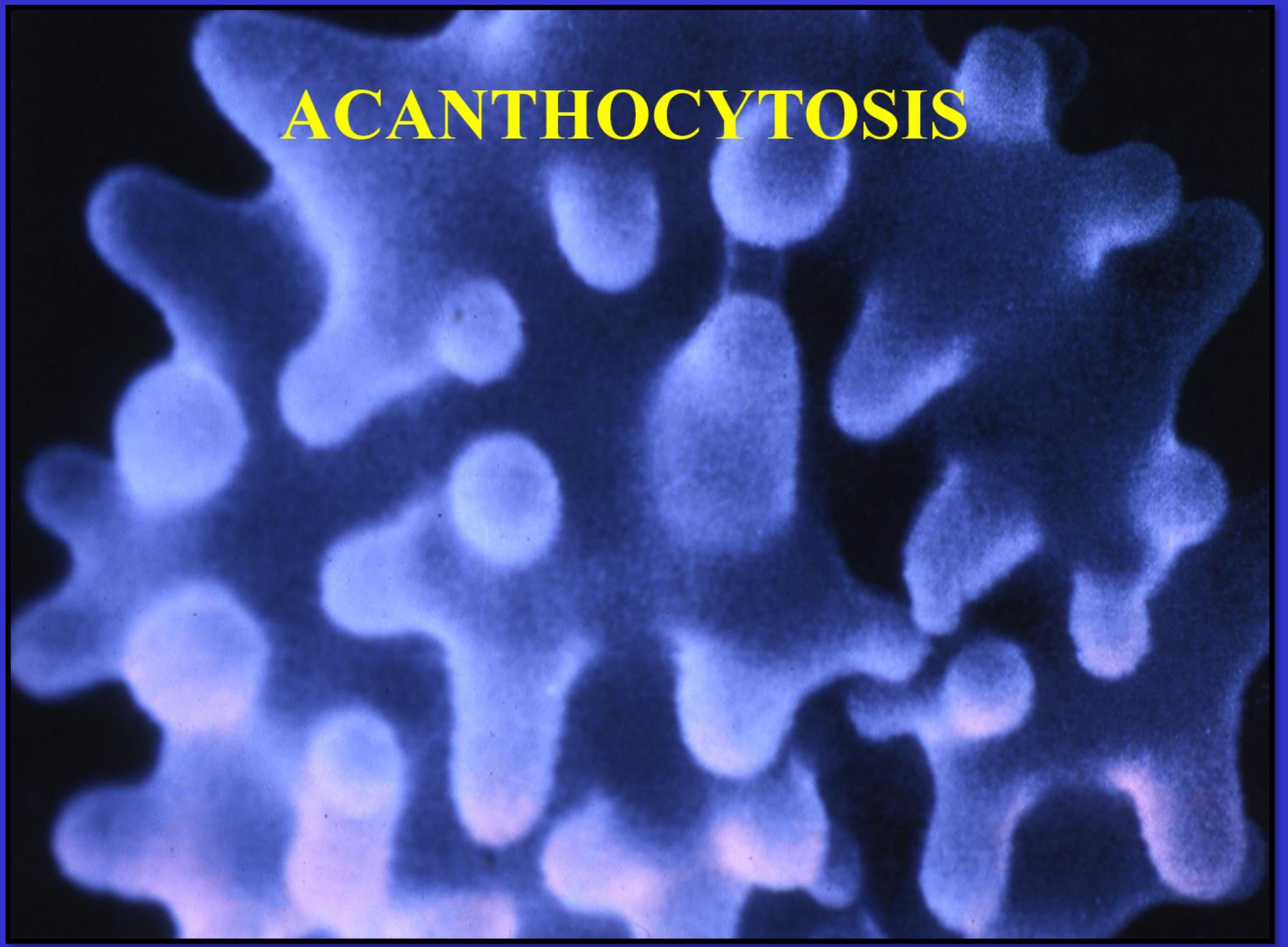


SPHEROCYTOSIS
NEW BORN



STOMATOCYTOSIS

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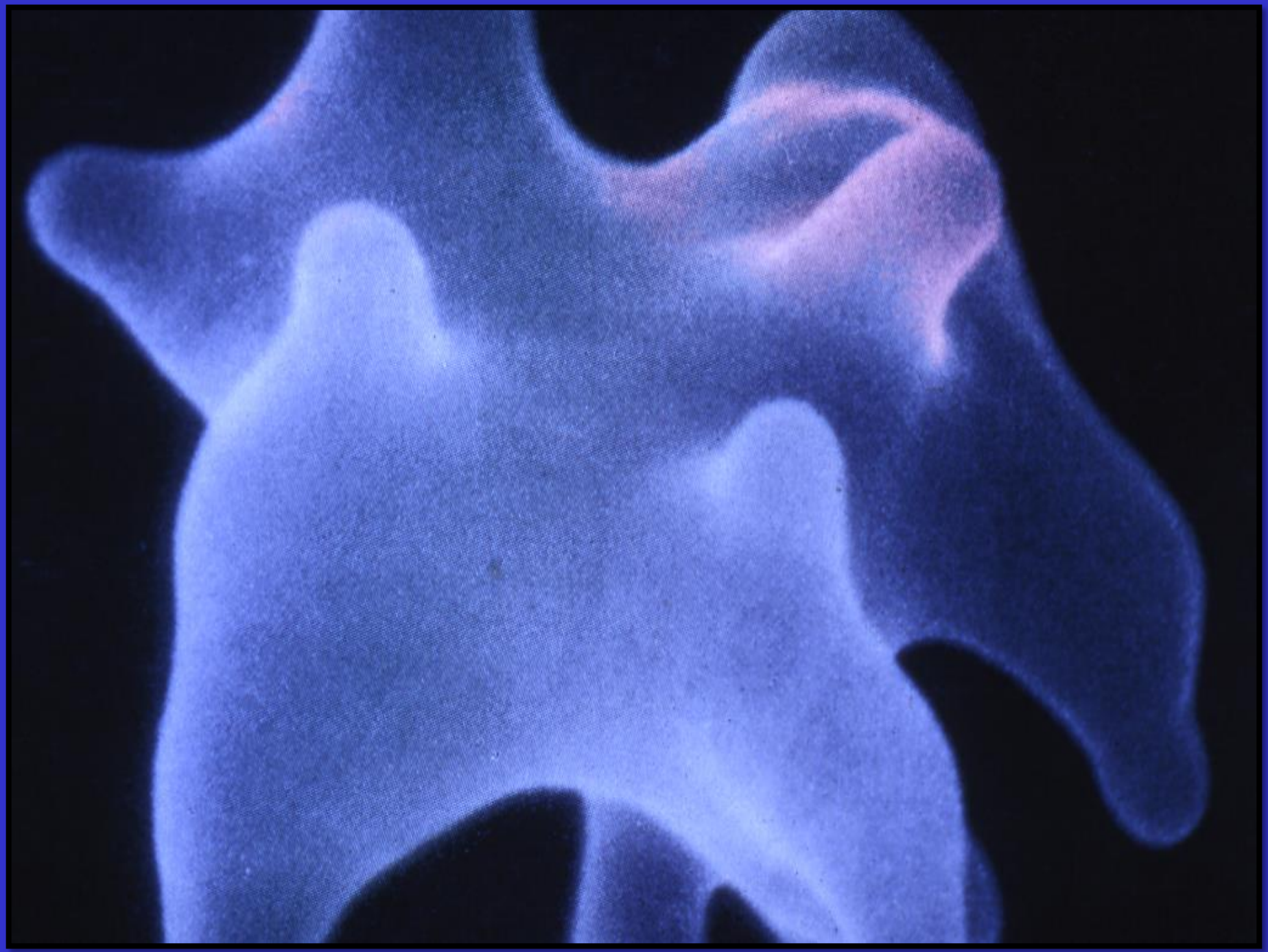

Abnormal Haemoglobins (Haemoglobinopathies)

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 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

 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

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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

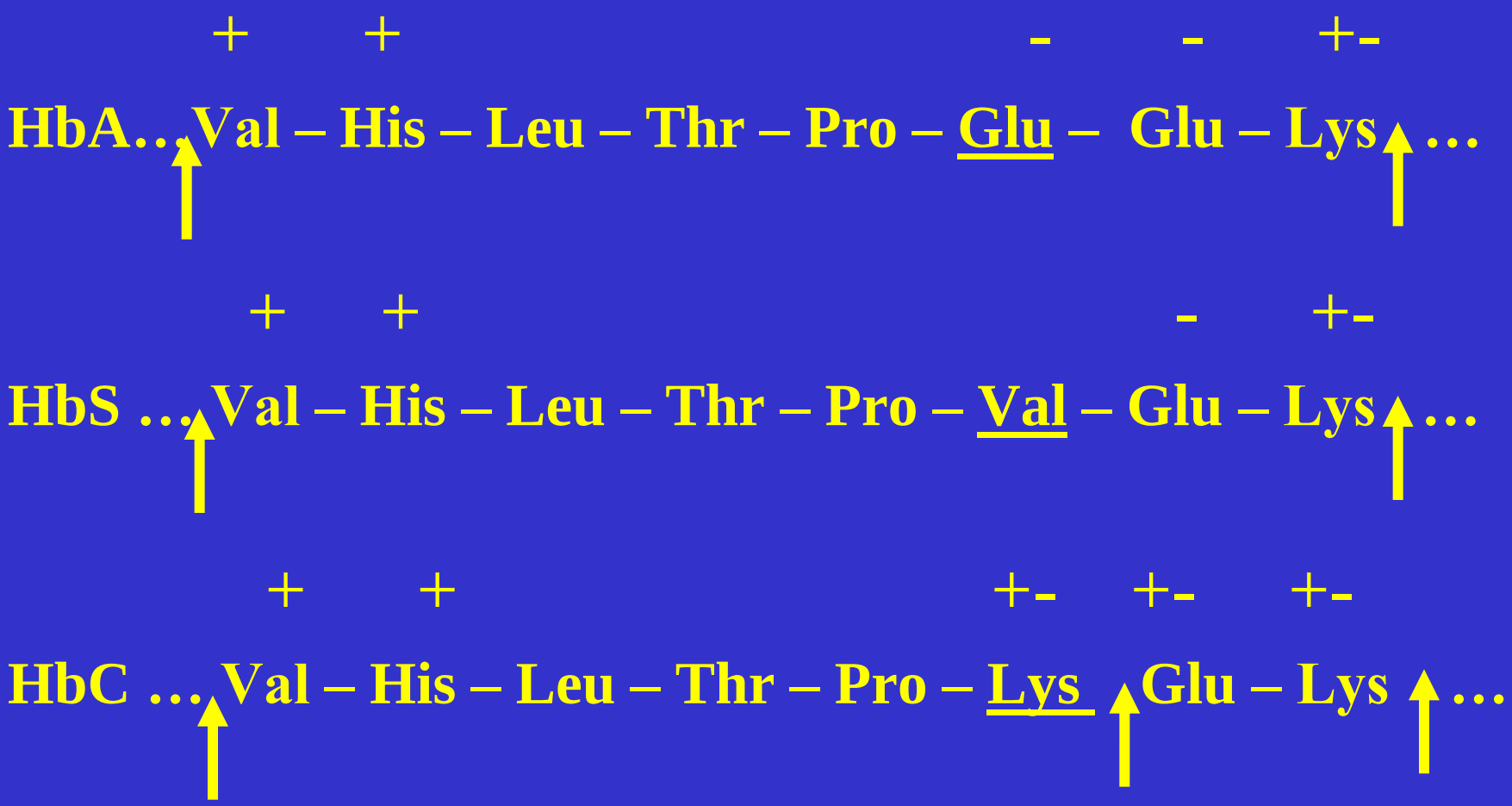
DNA Coding for the Amino-Acid in the sixth position in the β-chain

Normal

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

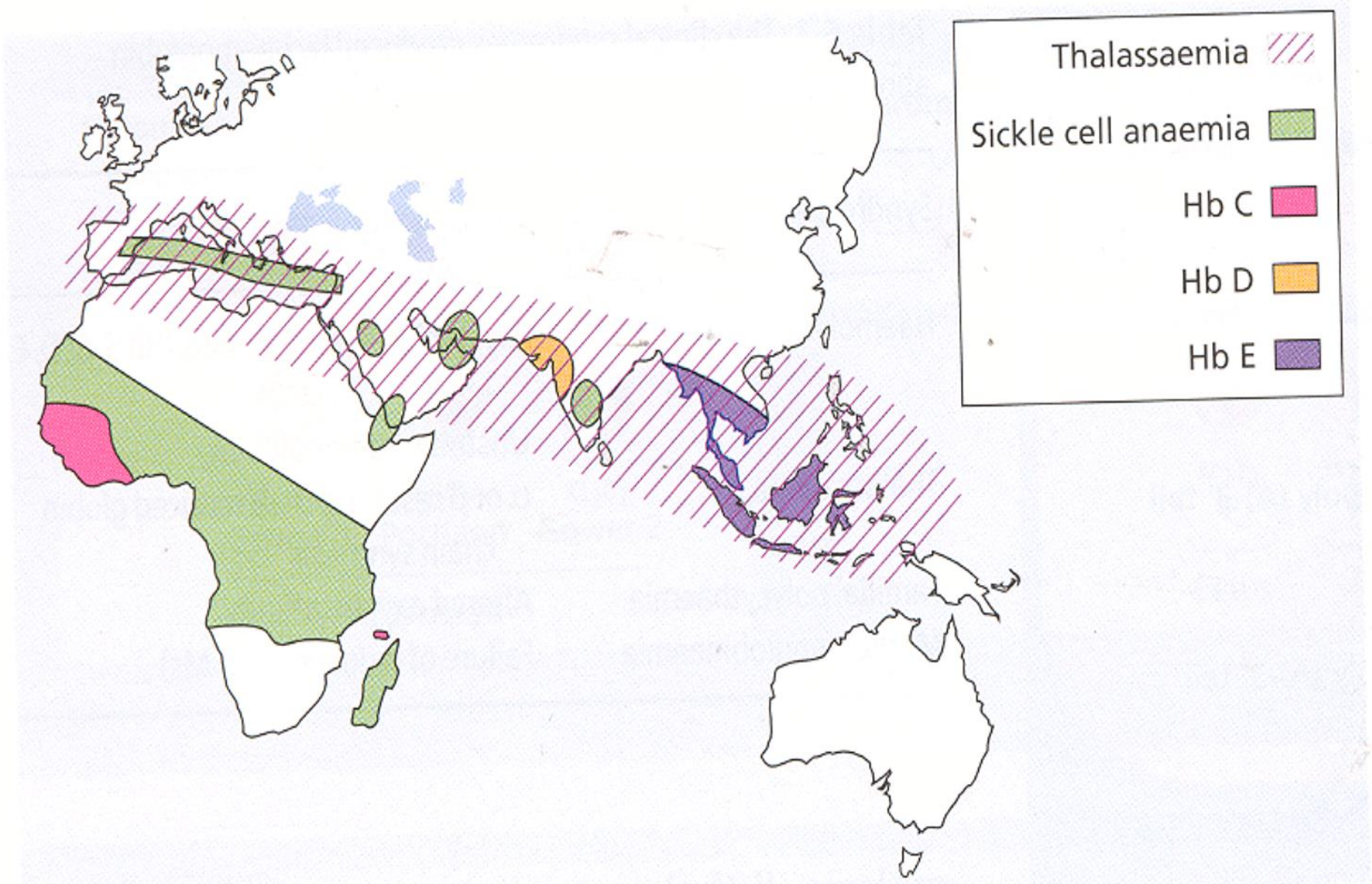
Sickle

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

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 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

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ARG-MET-PHE-LEU-SER-PHE-PRO-THR-THR-LYS-THR-TYR-PHE-PRO-HIS

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PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VAL-LYS-GLY-HIS-GLY-LYS

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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
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GLU-PHE-THR-PRO-PRO-VAL-GLN-ALA-ALA-TYR-GLN-LYS-VAL-VAL-ALA

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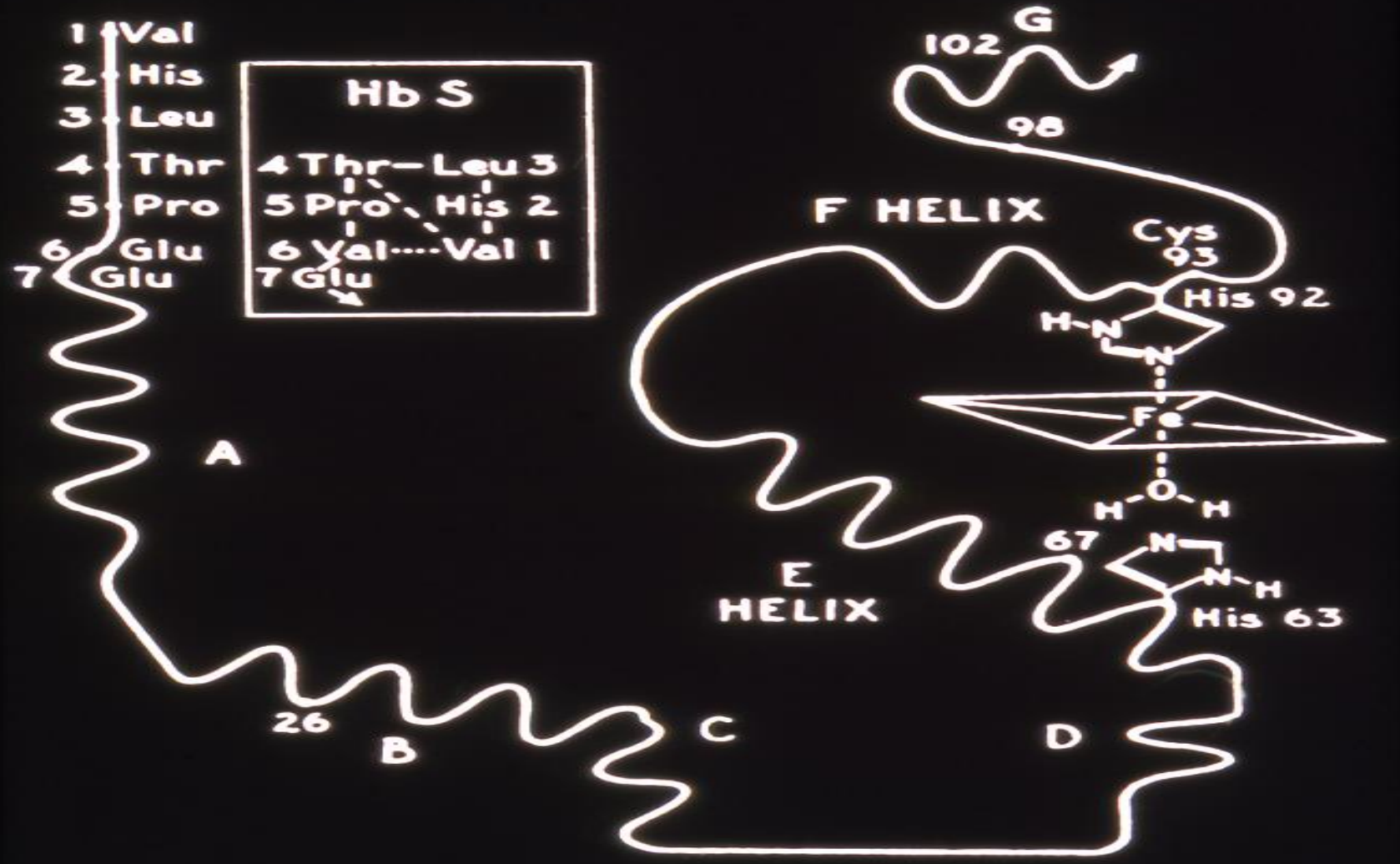
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2 His
3 Leu
4 Thr
5 Pro
6 Glu
7 Glu
Hb S
4 Thr — Leu 3
5 Pro — His 2
6 Val ···· Val 1
7 Glu
A
26
B
C
D
E
HELIX
F HELIX
G
102
98
Cys
93
His 92
Fe
67
His 63

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

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Sickle

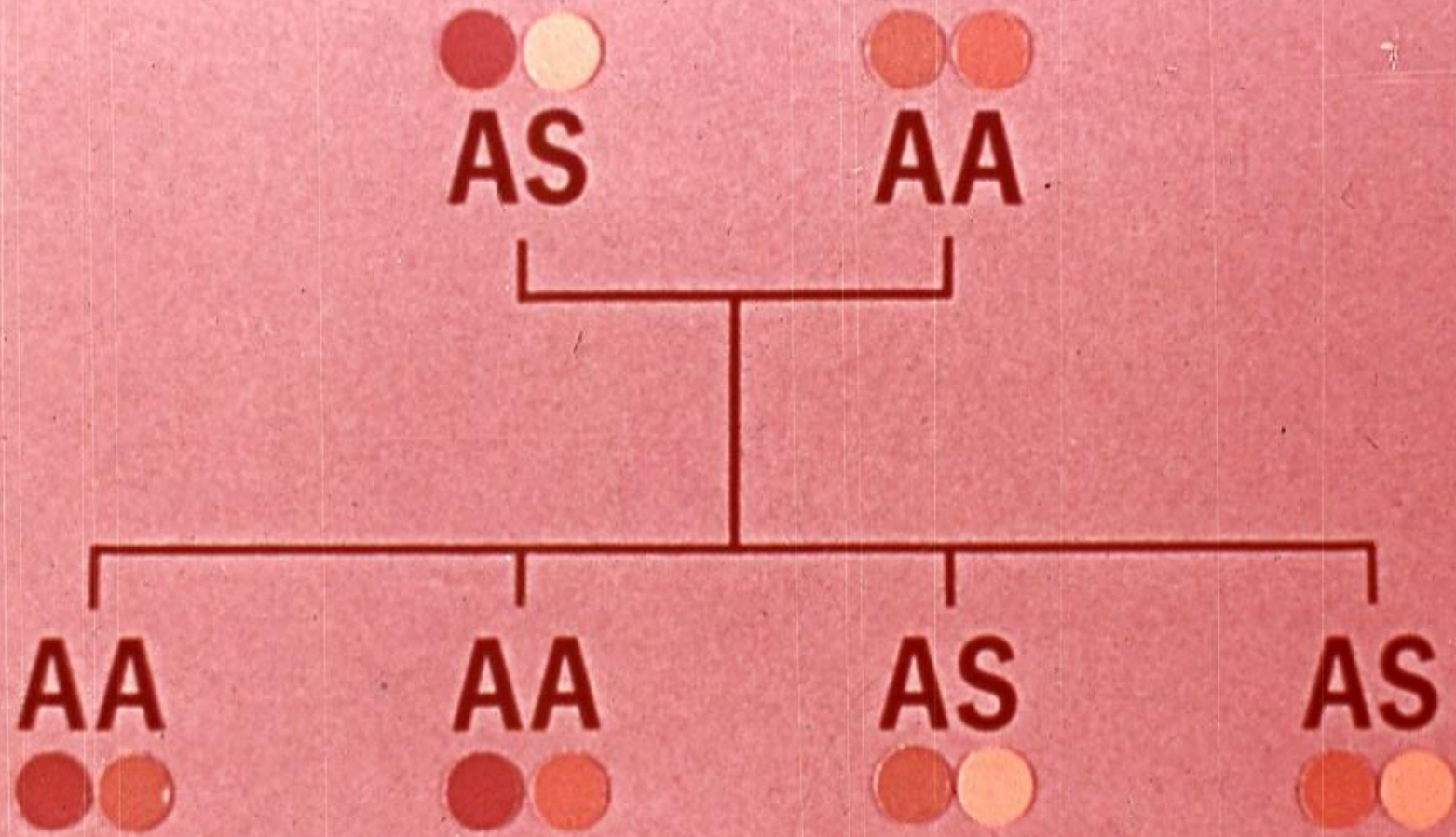
DNA Base composition	CCT	G T G	G A G
Amino Acid	pro	val	glu
	5	6	7

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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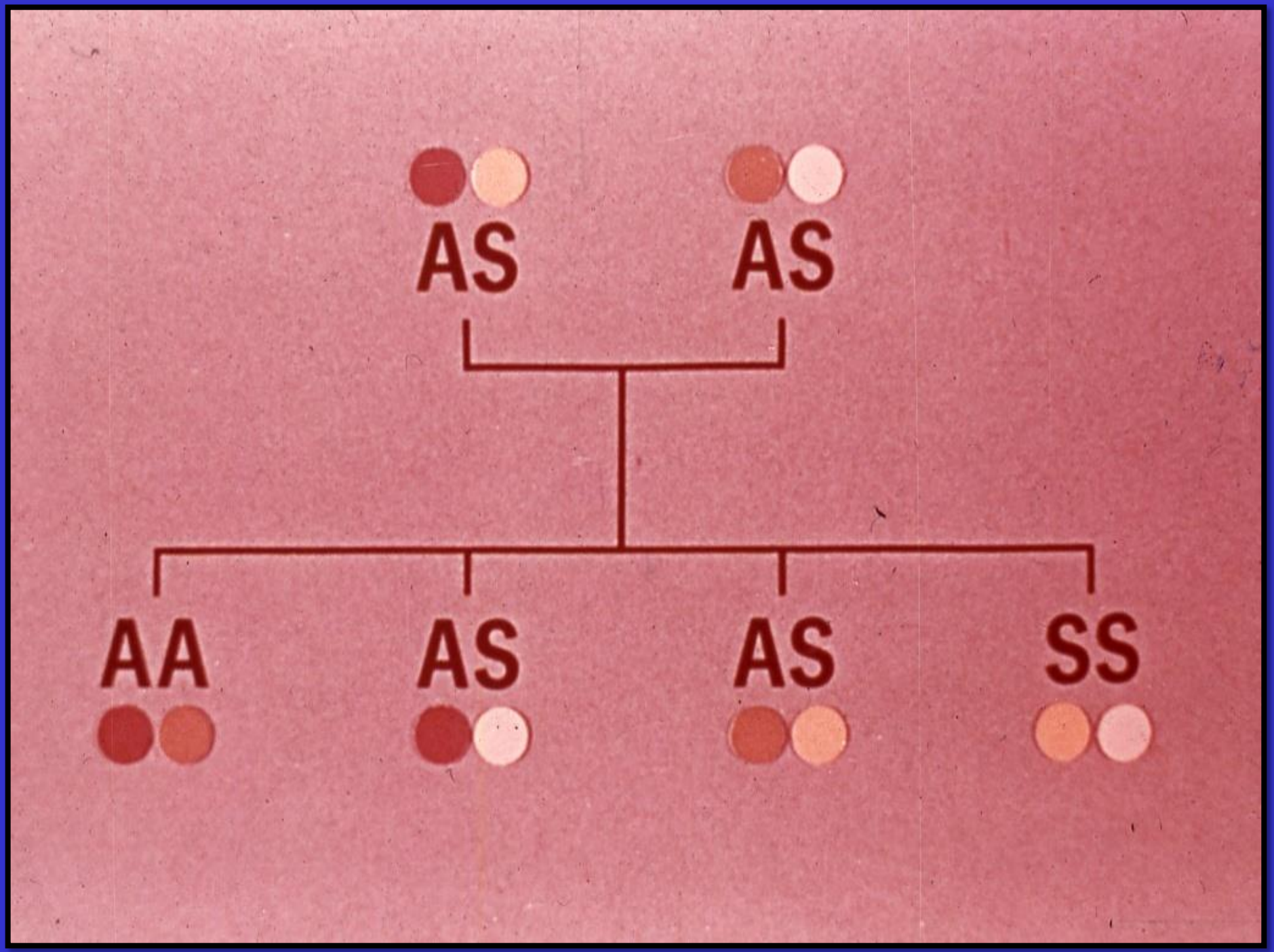
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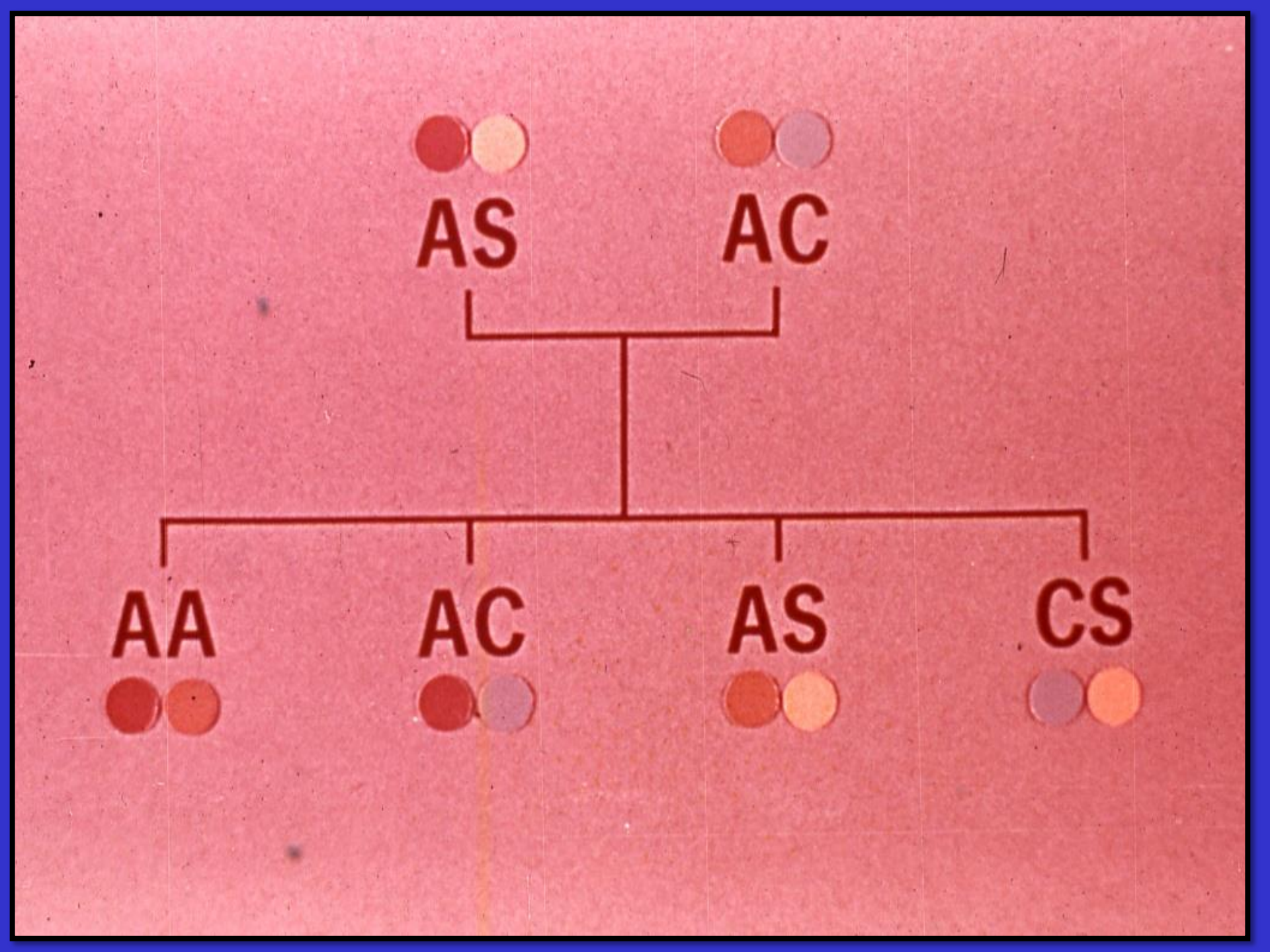
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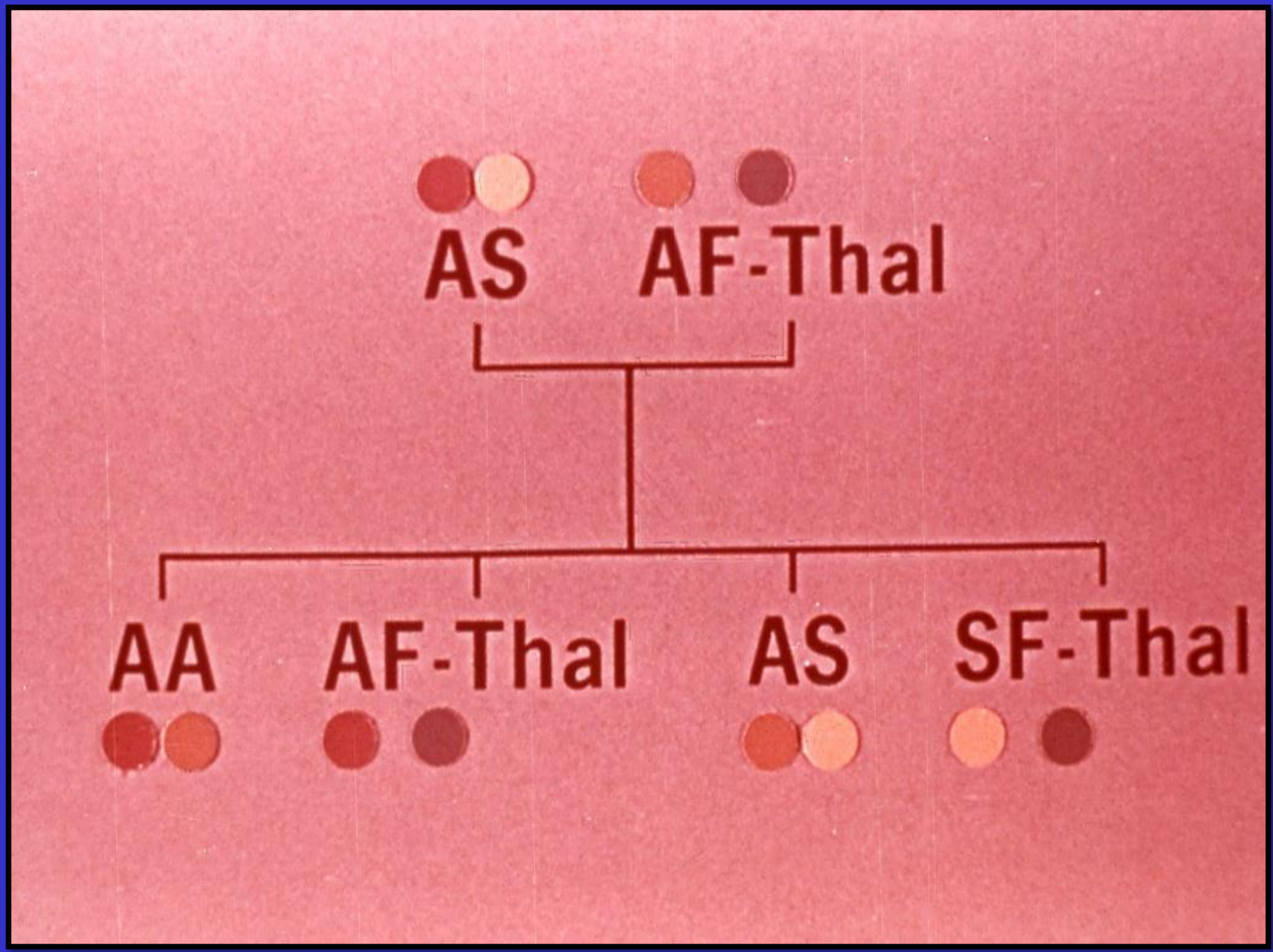
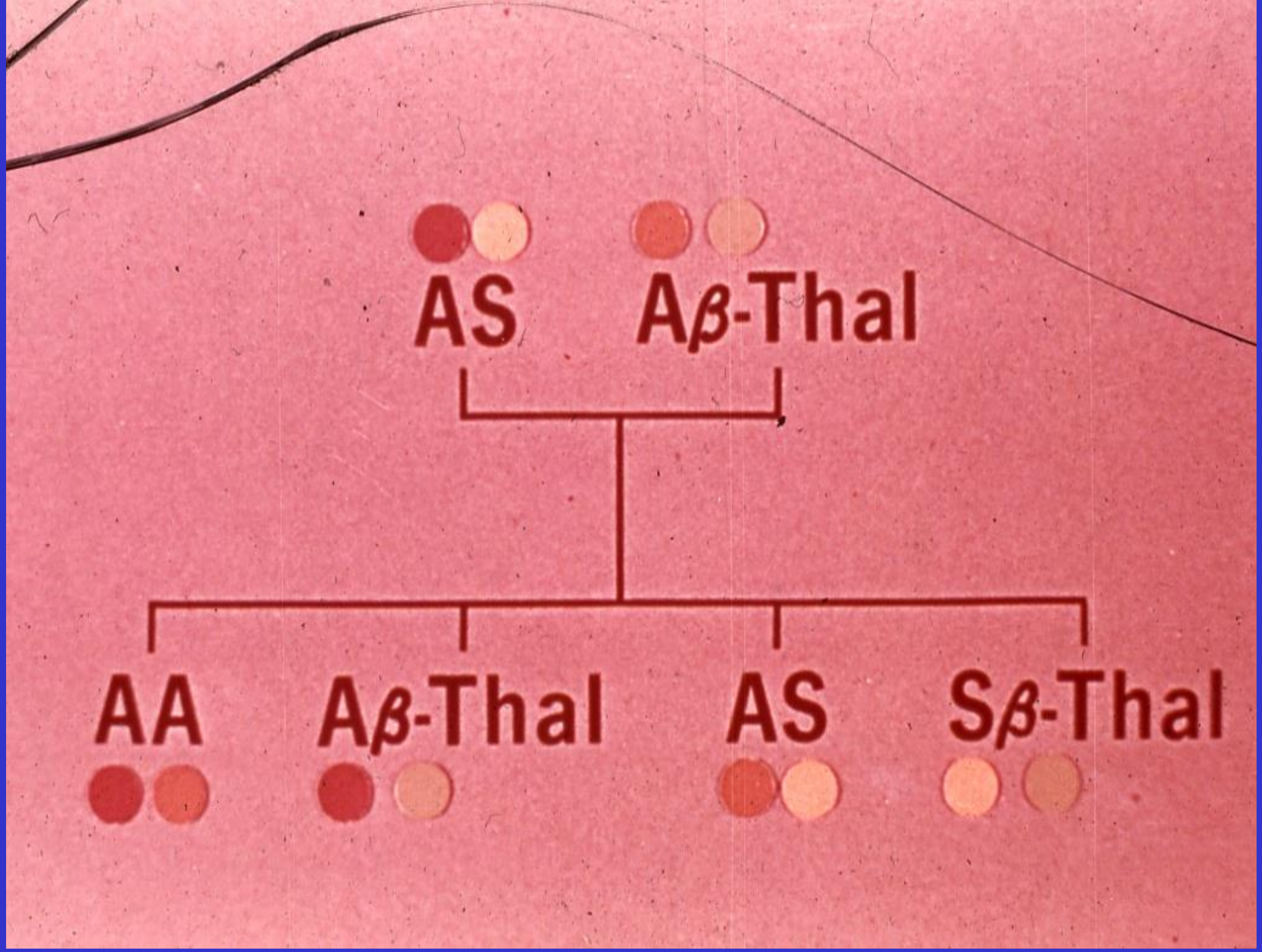

AS
AF-Thal
AA
AF-Thal
AS
SF-Thal


? ?
Sβ-Thal
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

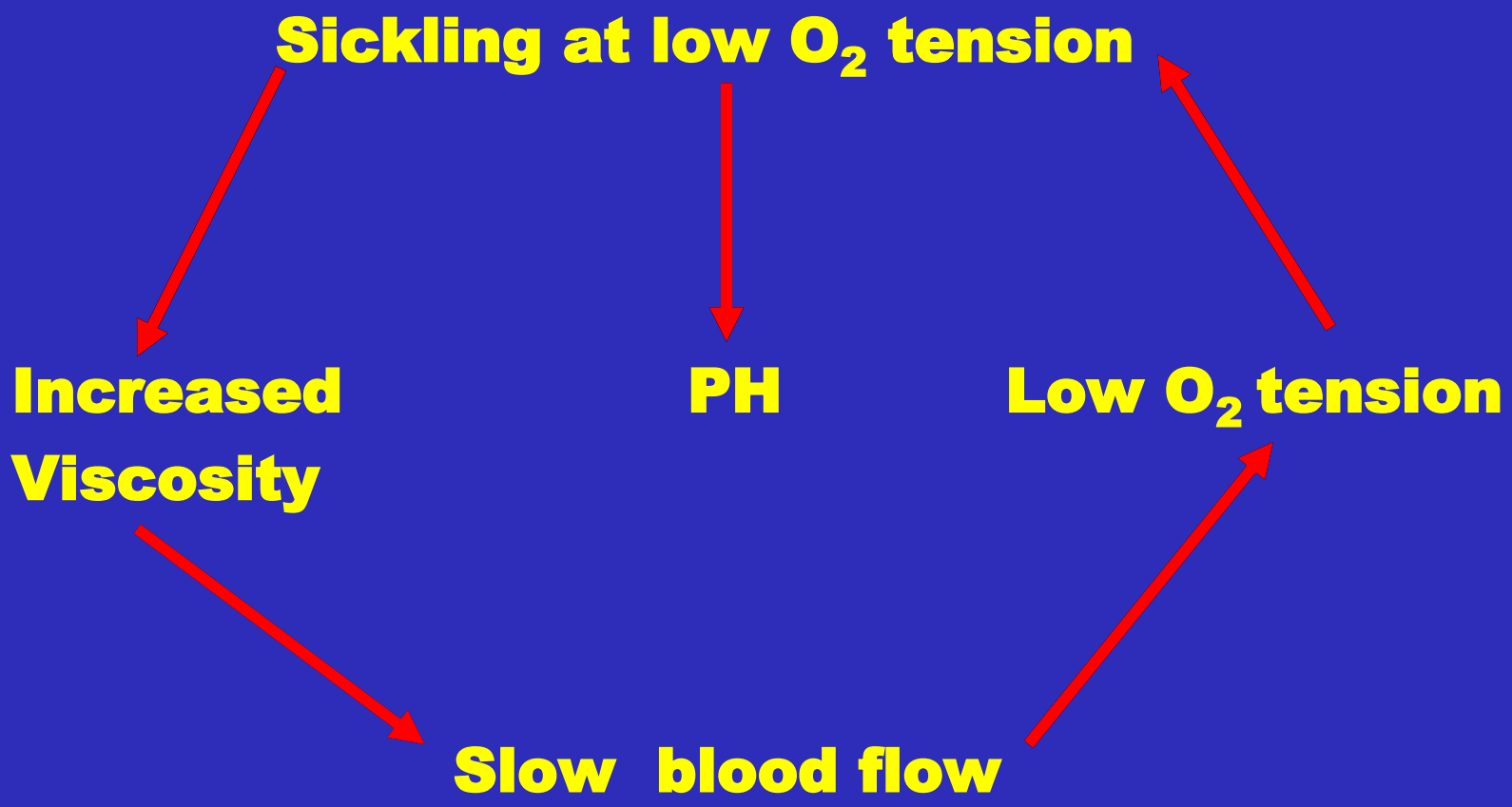
Oxygen tension — 50–60 mm Hg for SS
20–30 mm Hg for AS

pH — inhibited at alkaline pH
exacerbated by acidification

Concentration of Hb S

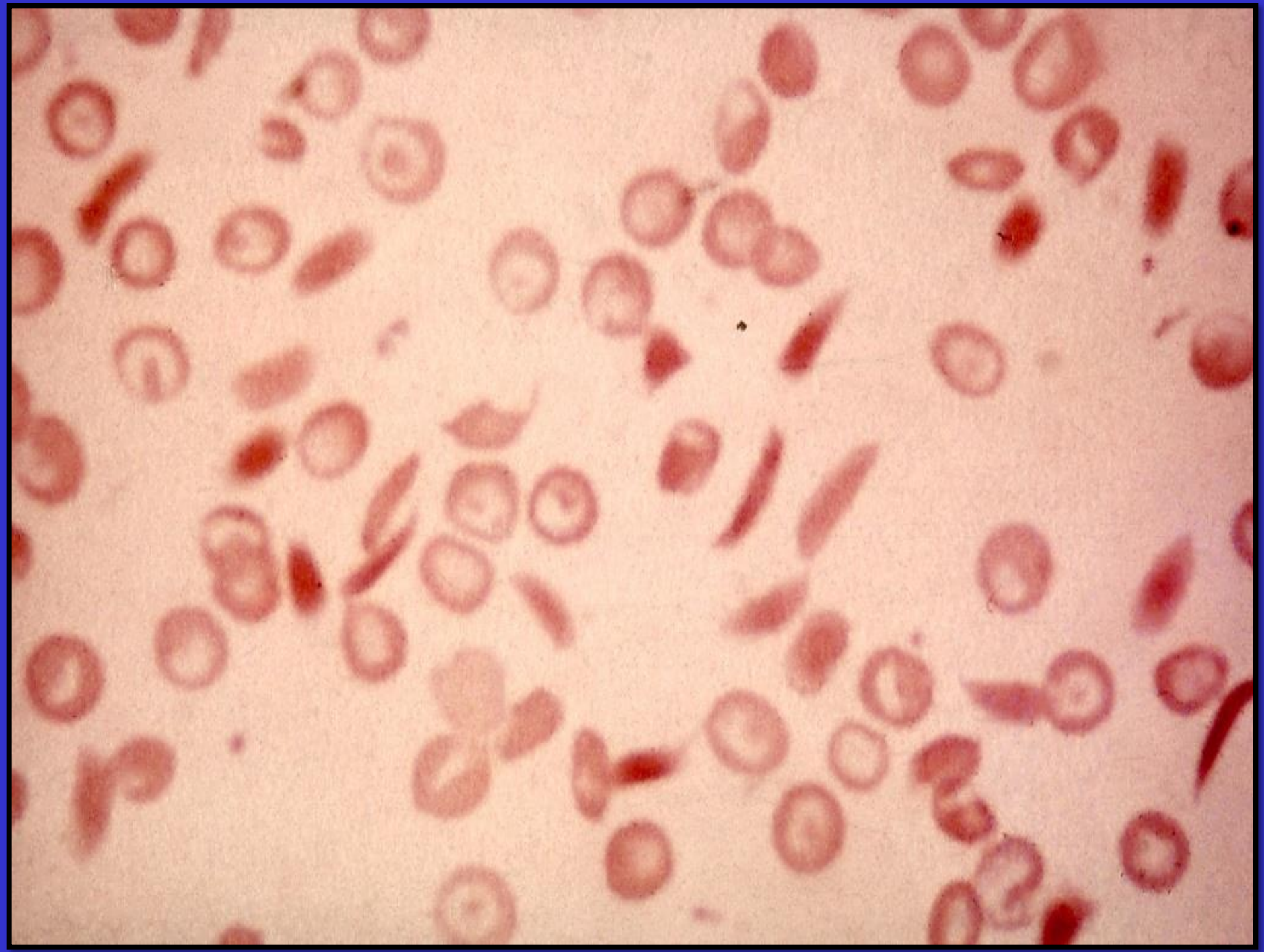
Presence of other haemoglobins

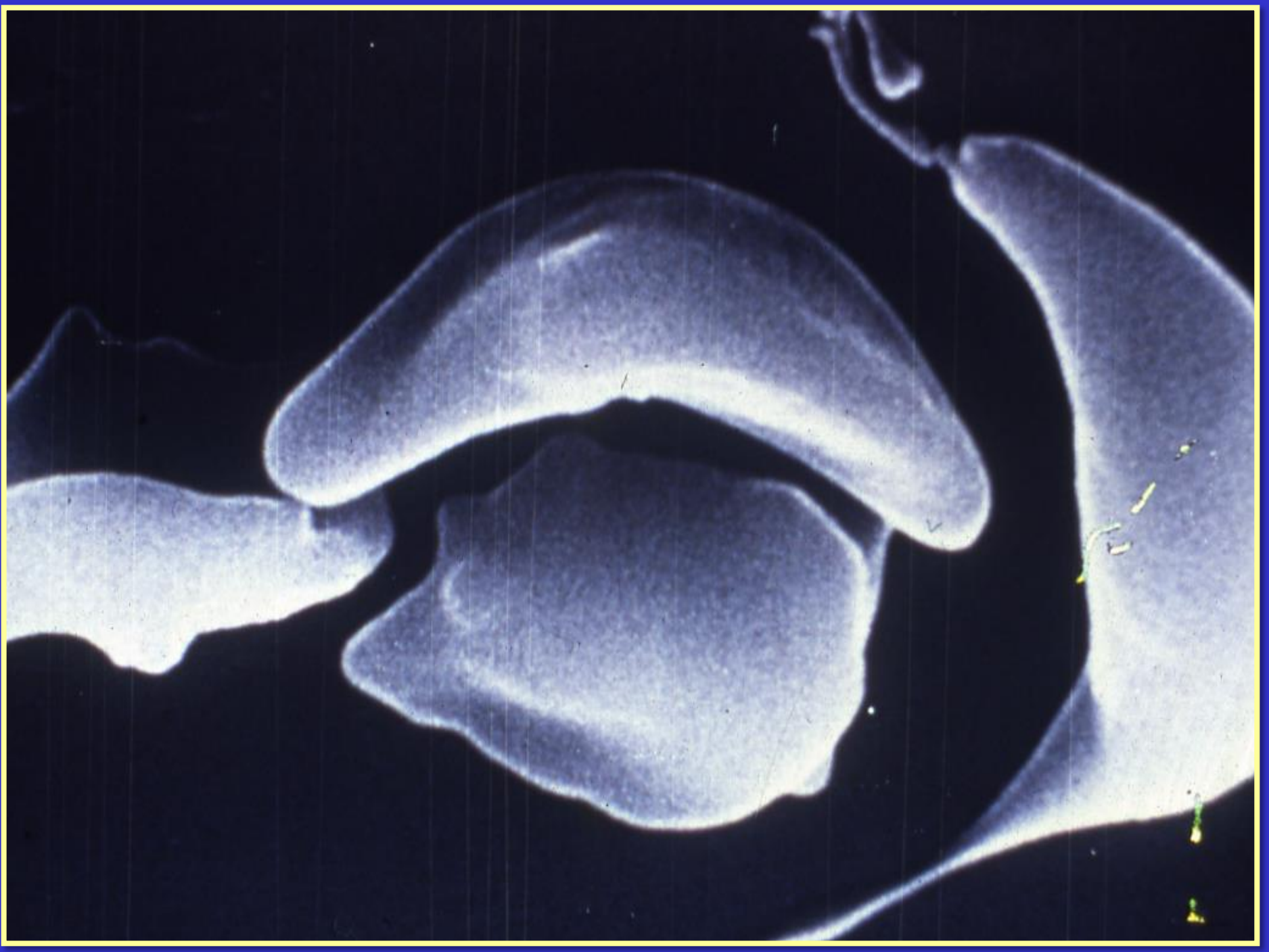
polymerisation : S > D > C > J = A > F

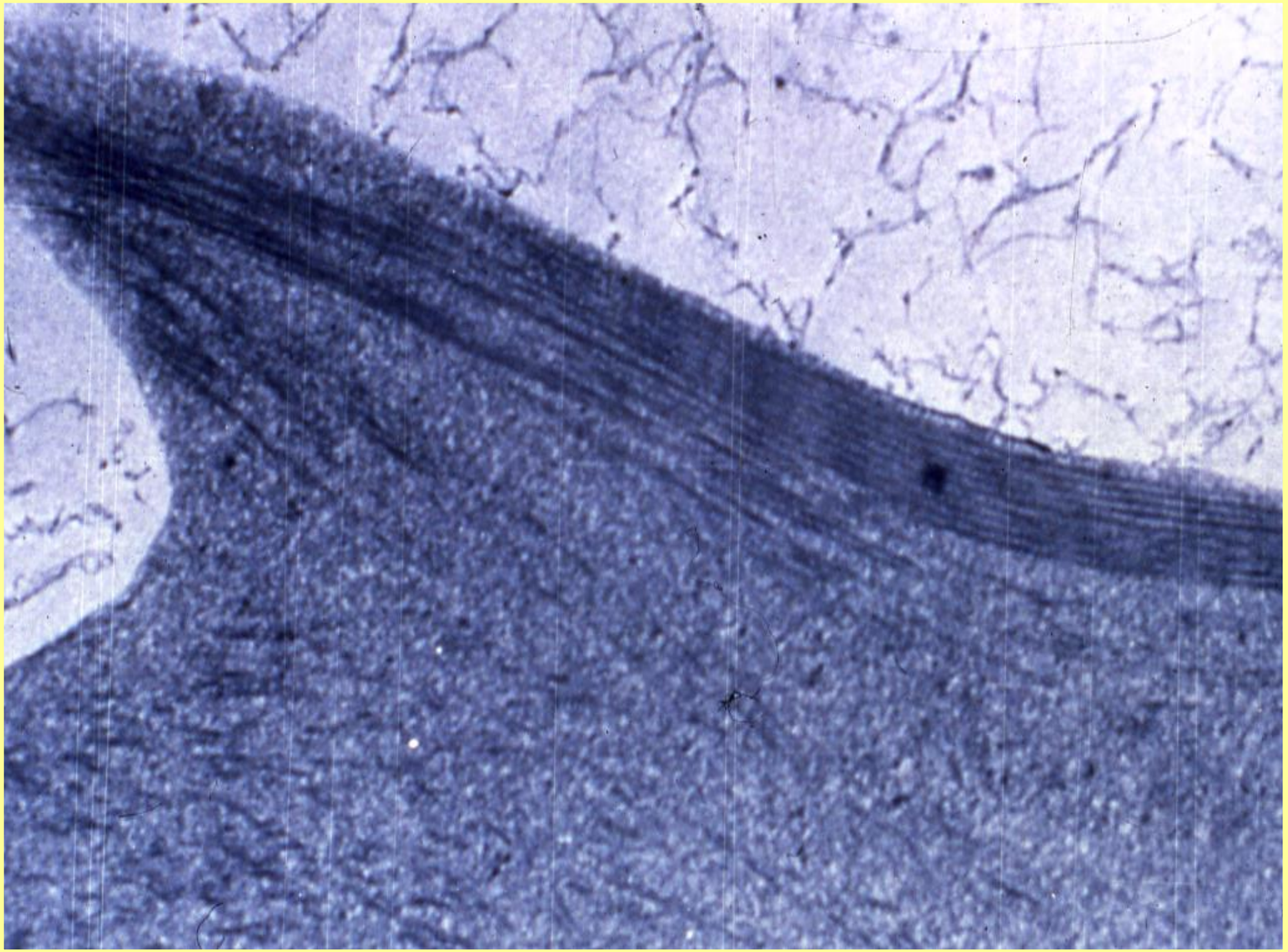

Sickling at low O_2 tension
Increased Viscosity
PH
Low O_2 tension
Slow blood flow

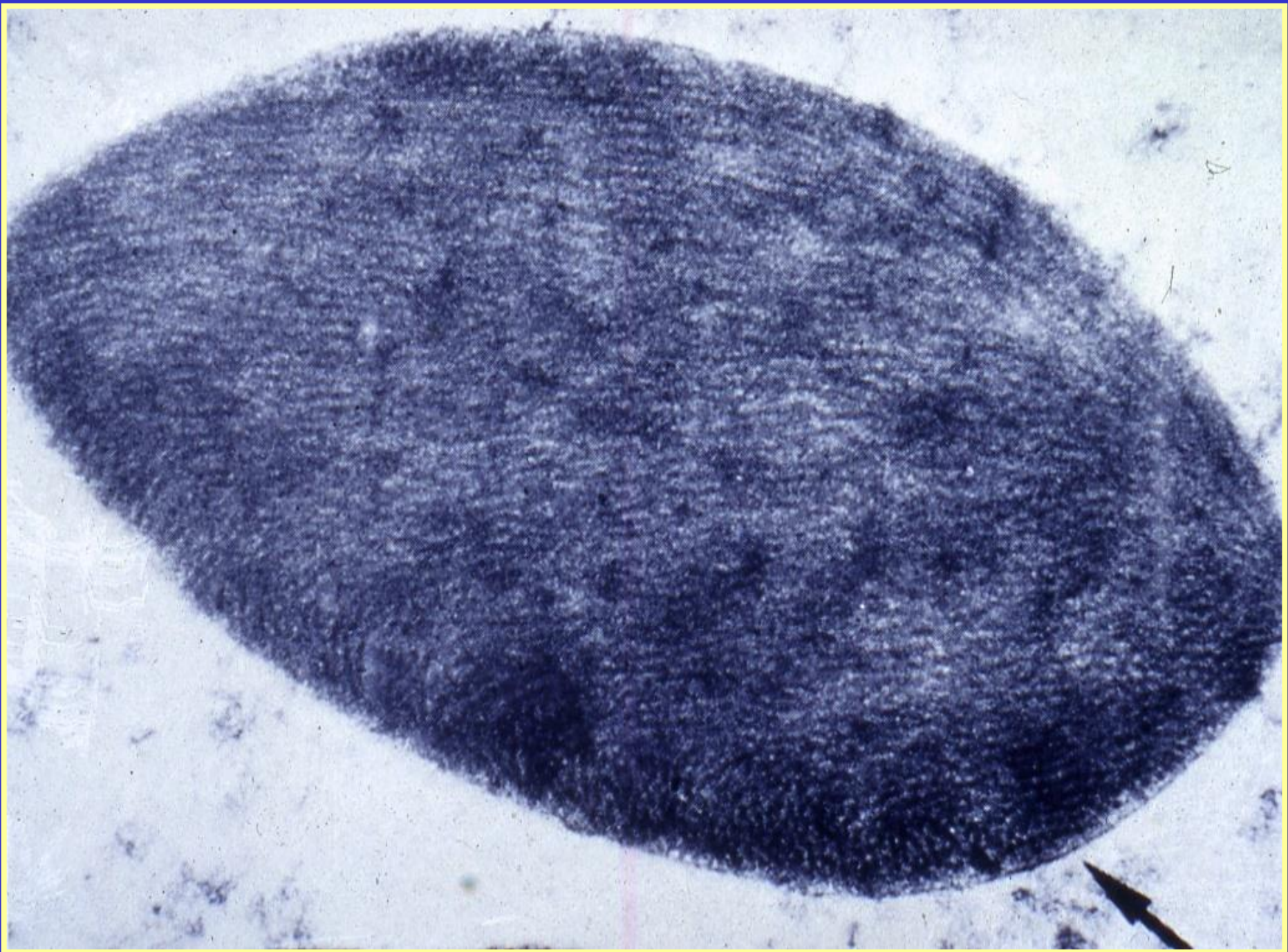
FACTORS PRECIPITATING CRISES IN SICKLE CELL DISEASE

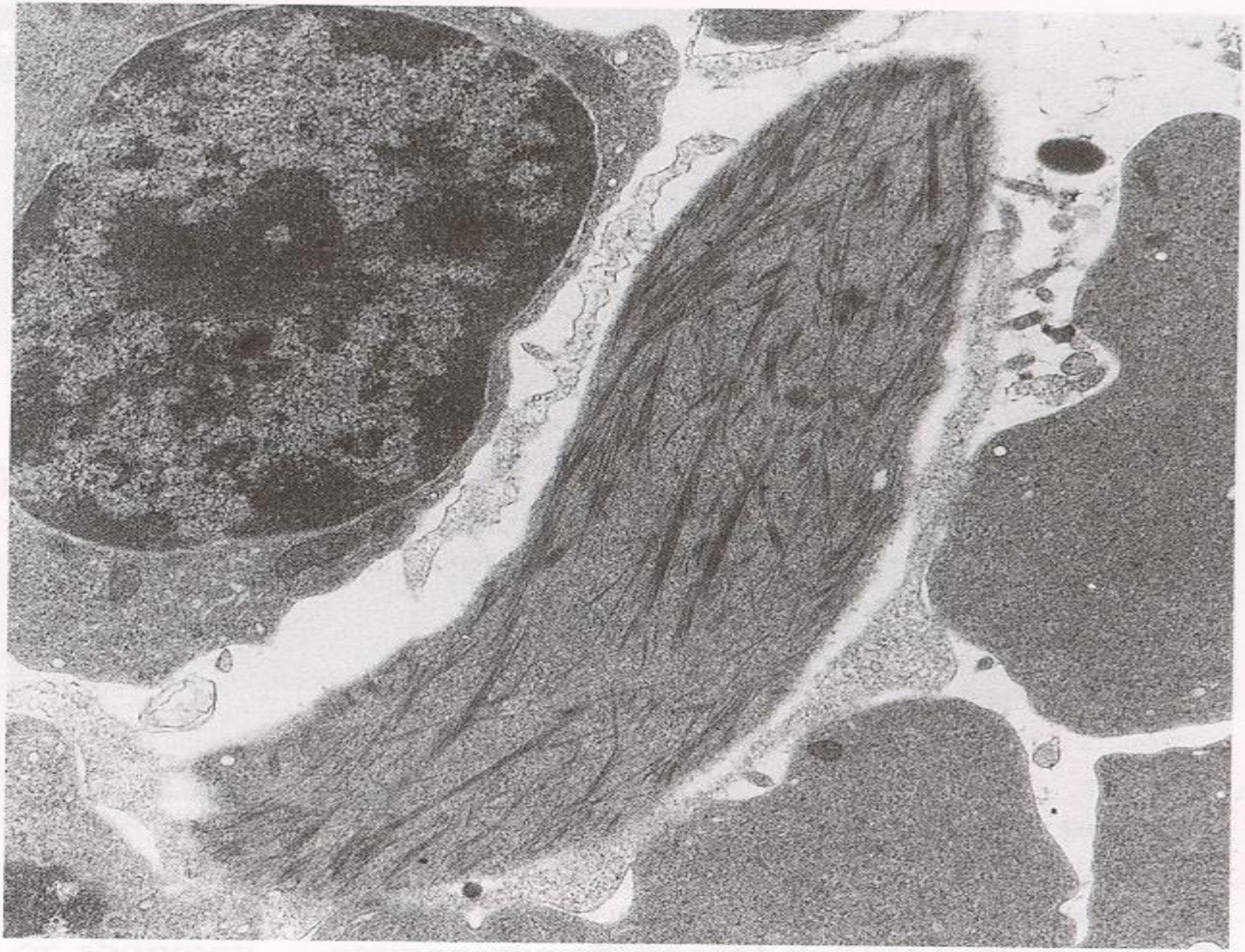
- INFECTIONS (especially malaria)
- PYREXIA
- EXPOSURE TO COLD
- DEHYDRATION
- PREGNANCY



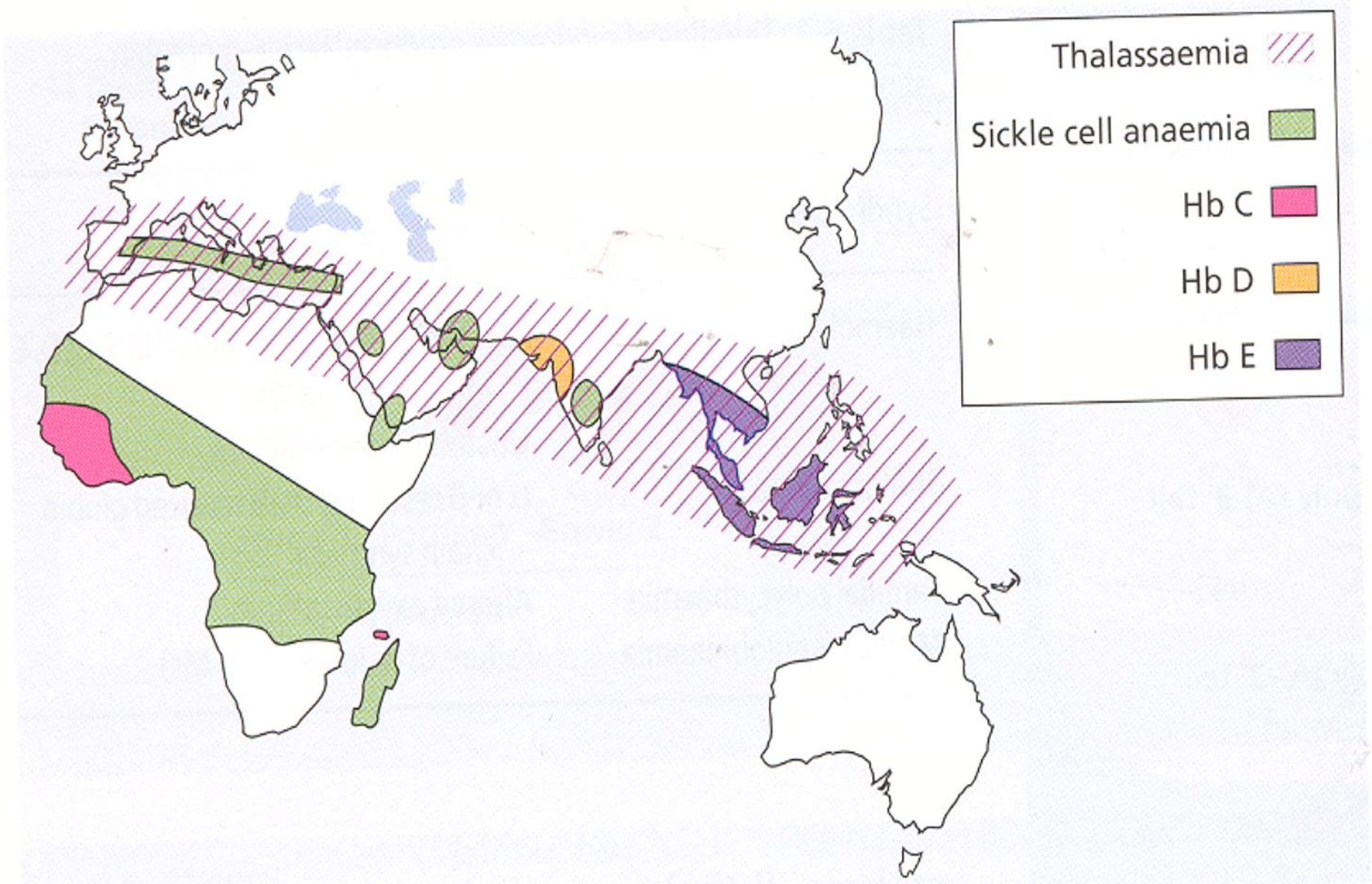


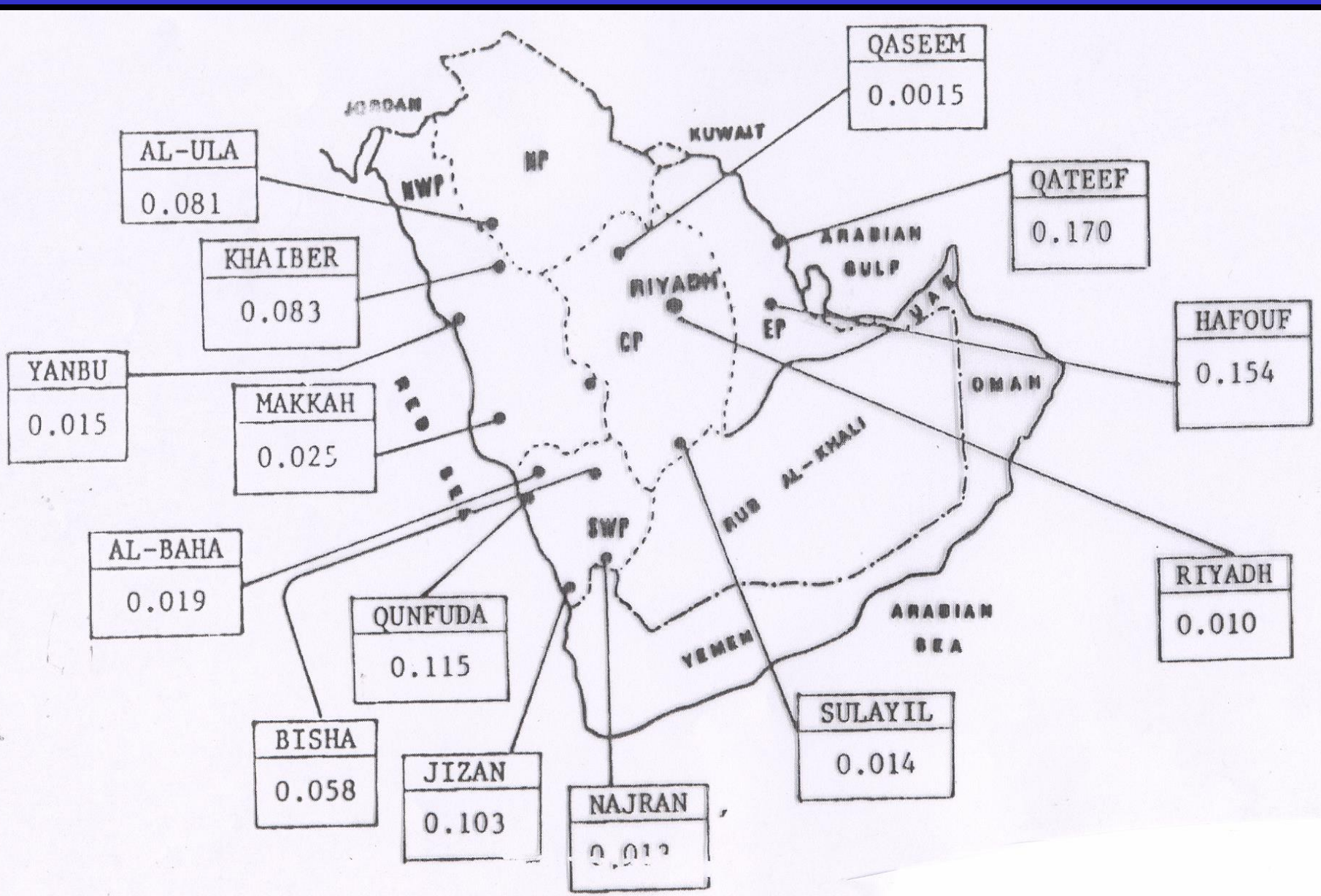






HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION





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

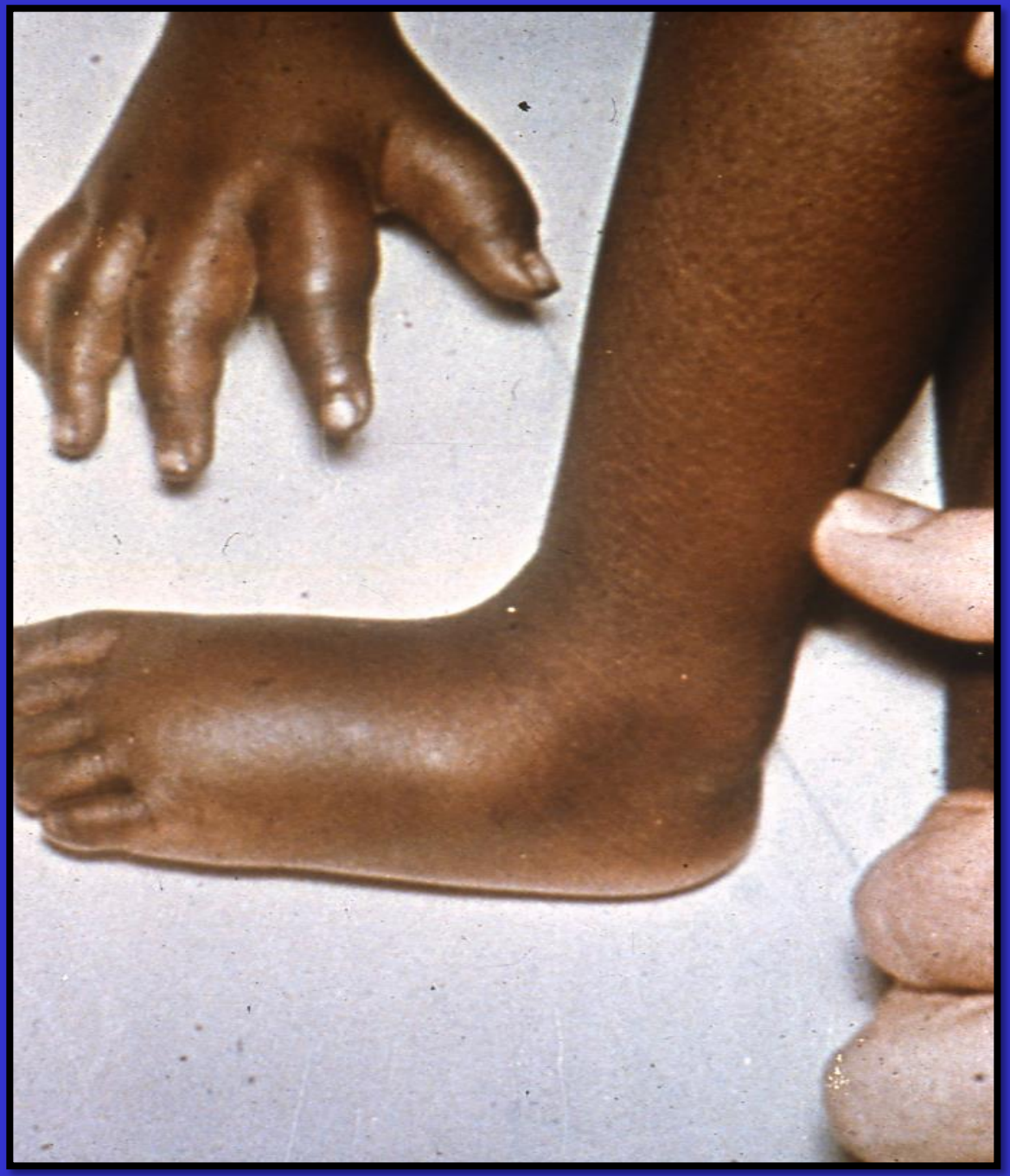
TISSUE INFARCTION

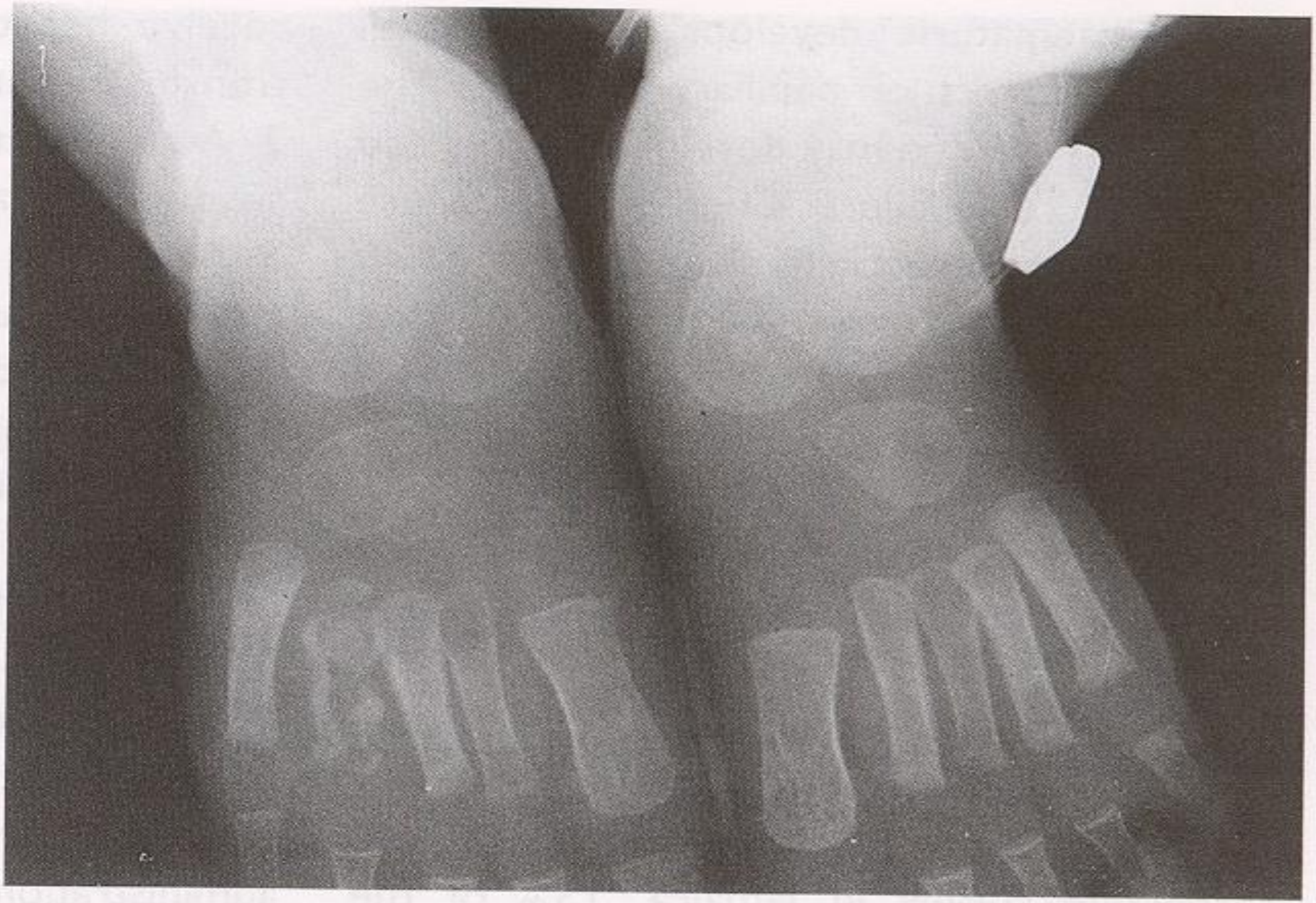
Clinical Manifestations in Sickle Anaemia

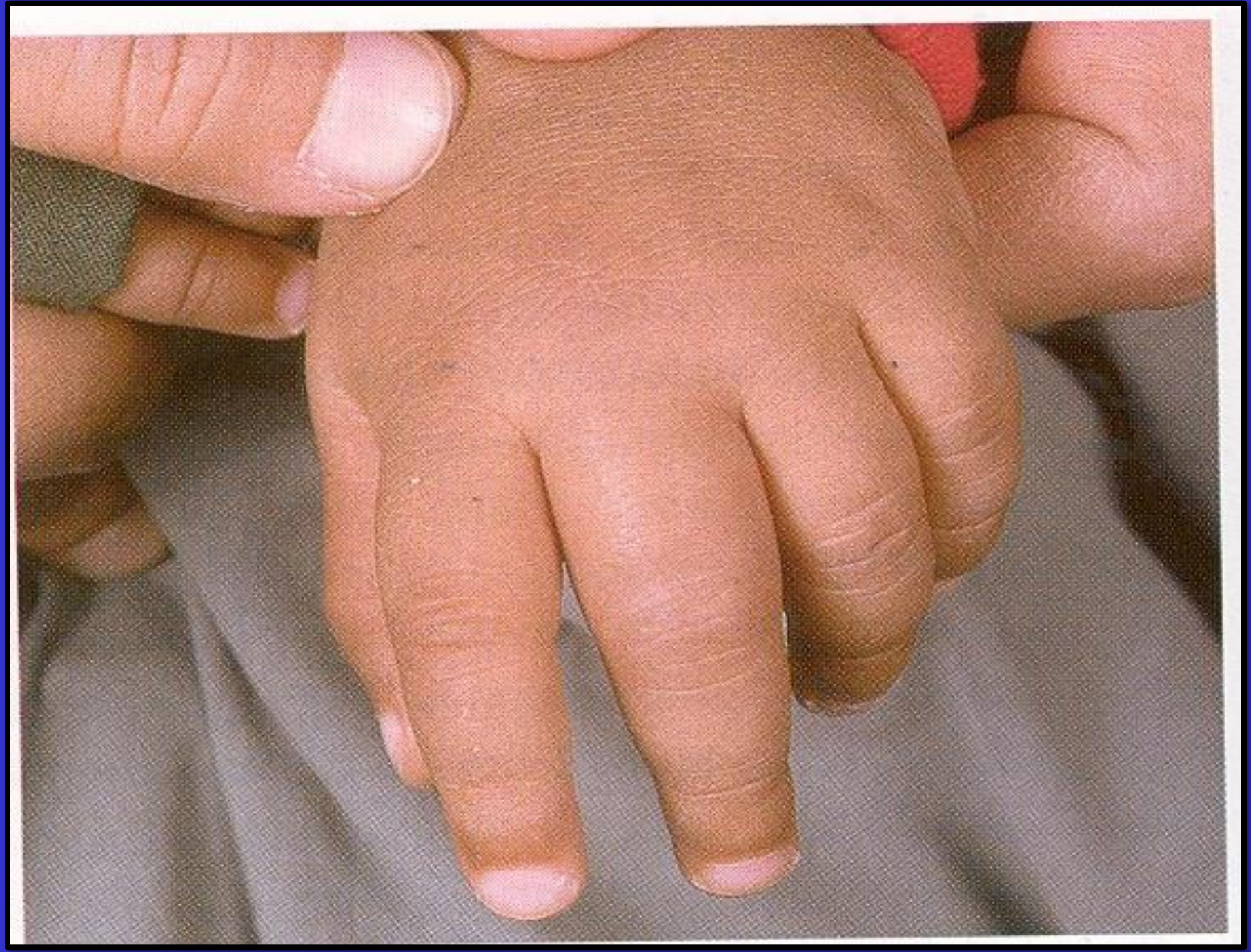
- Pallor (Anaemia)
- Jaundice & Dark Urine
- Apathy & Anorexia
- Hand-Foot Syndrome (Young Children)
- Splenic sequestration (Young children) Hepatic Sequestration
- Bones and Joints Pain
- Abdominal Pain

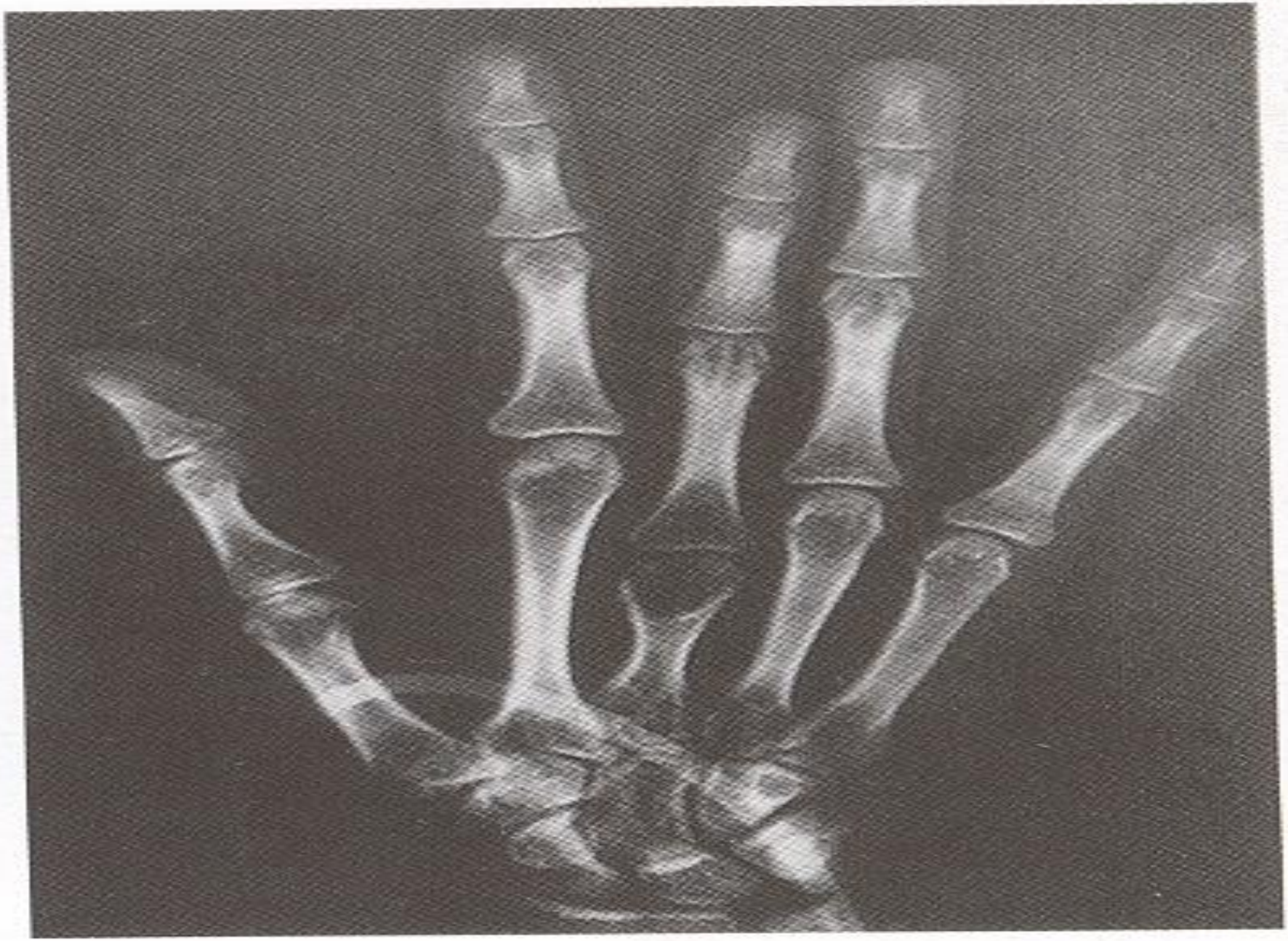
Clinical Manifestations in Sickle Anaemia

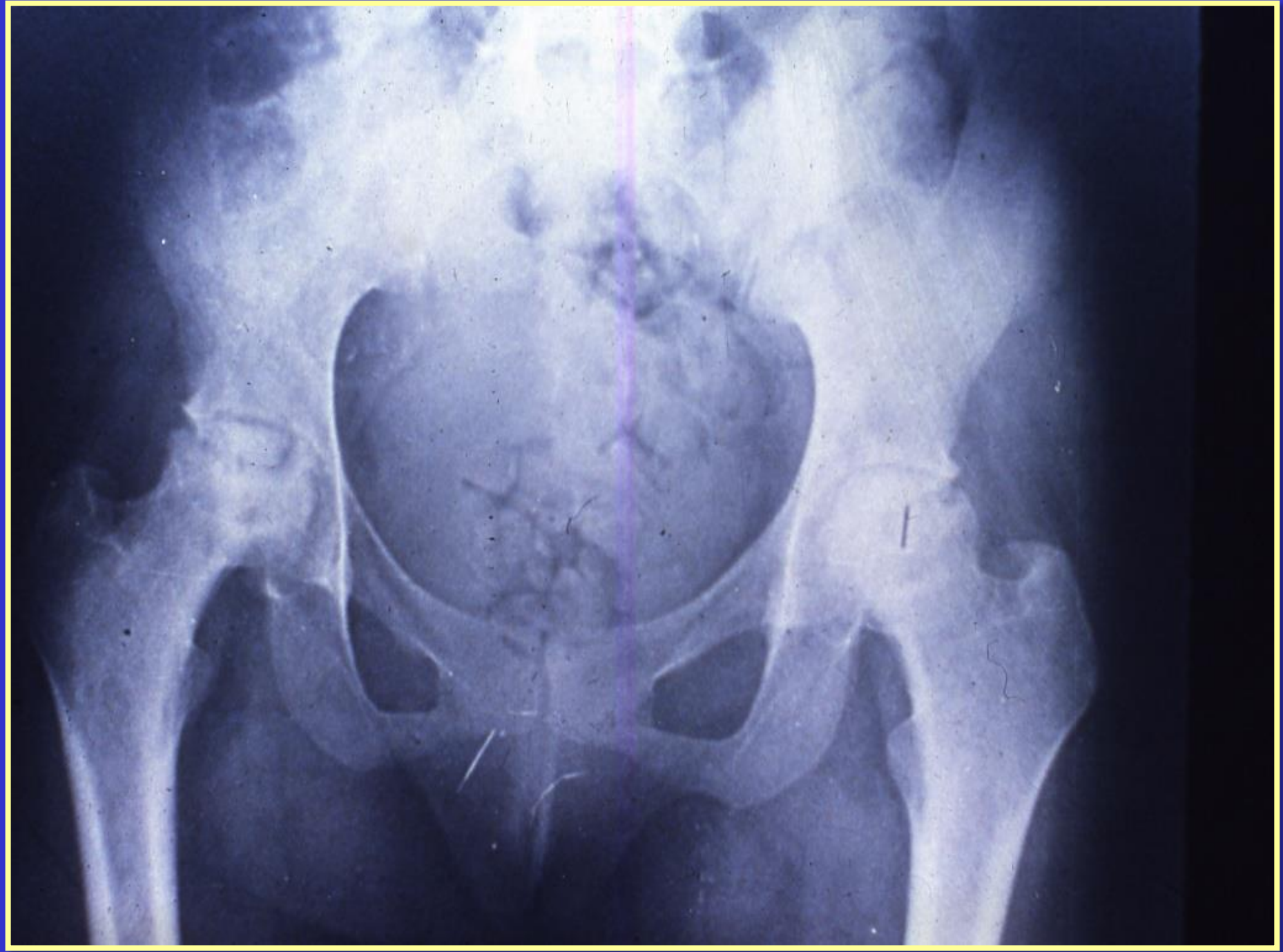
- Recurrent Infections & Chest Symptoms (Acute Chest Syndrome)
- Hepato-Splenomegaly
  - → (Early Childhood)
  - → (Association with Thalassaemias)
- CNS Presentations
- Leg Ulceration
- Skeletal Deformity

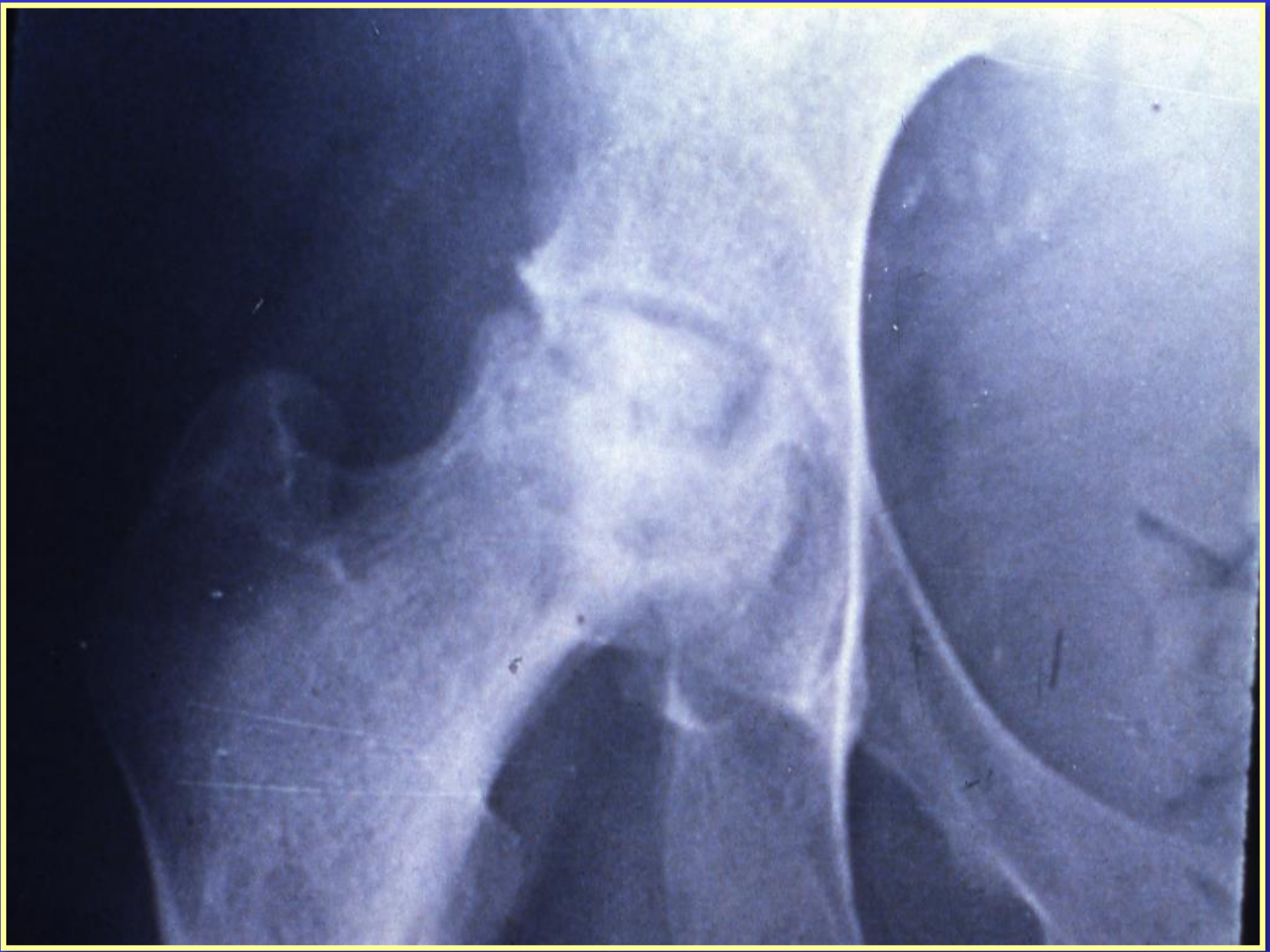


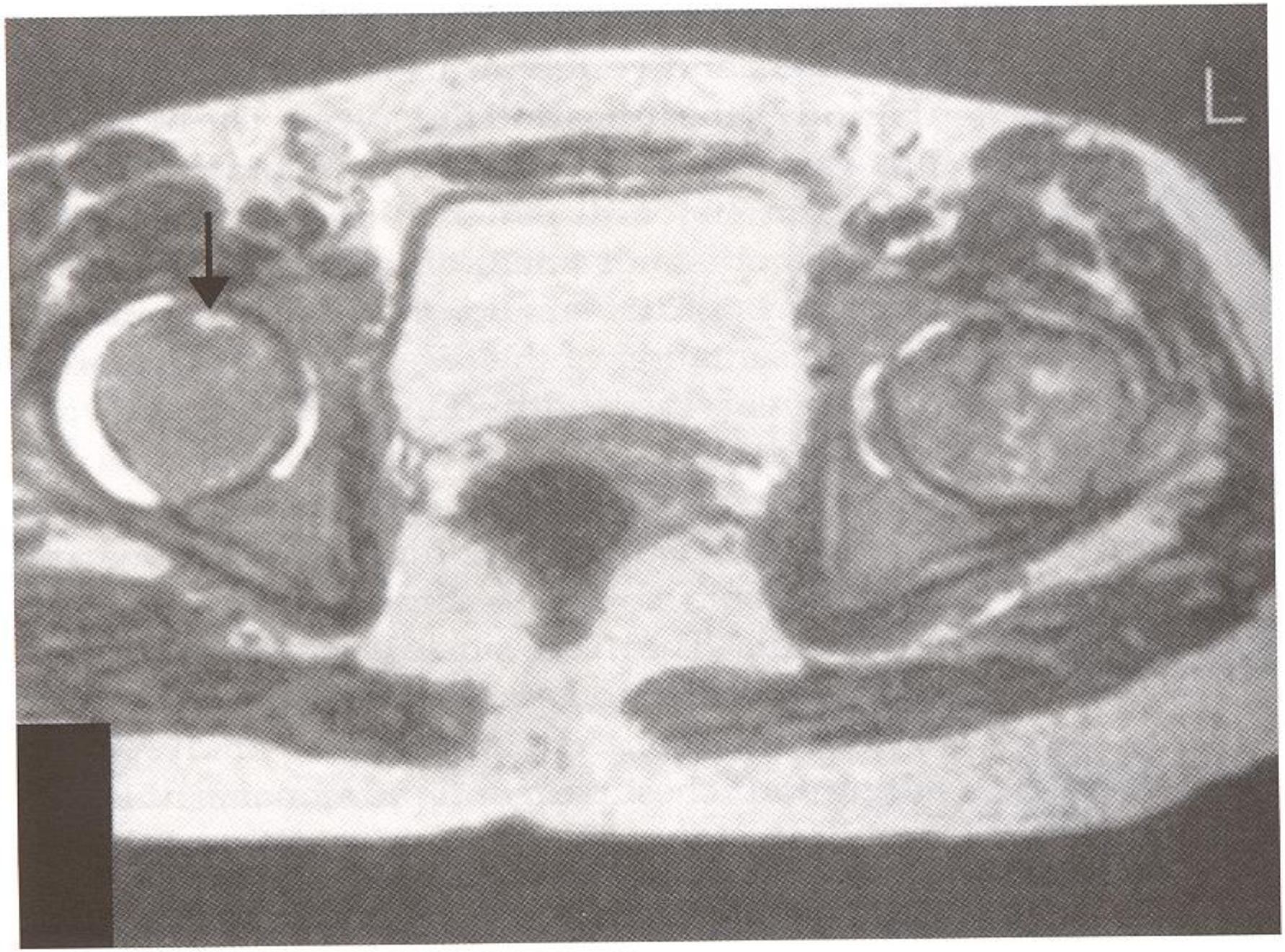








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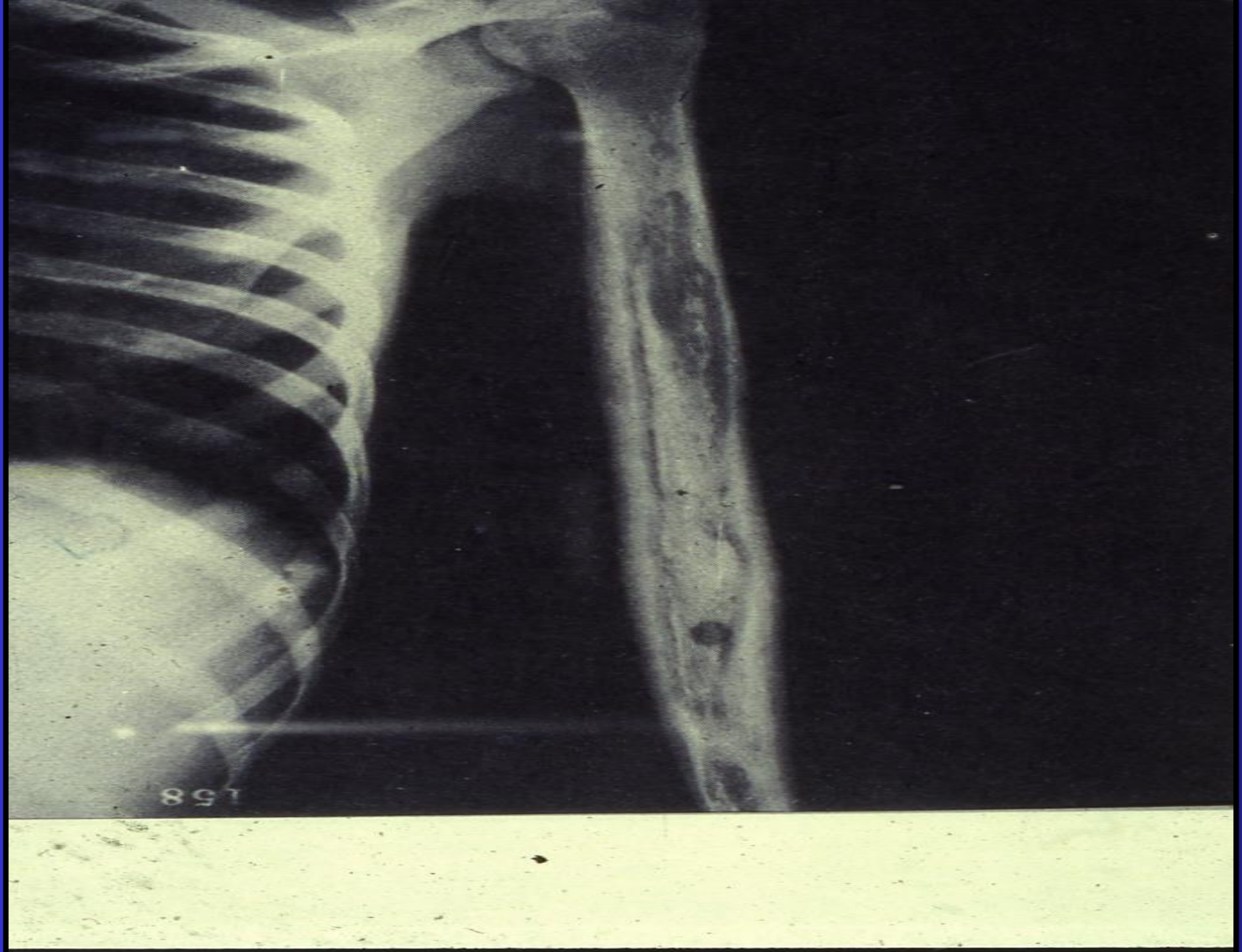
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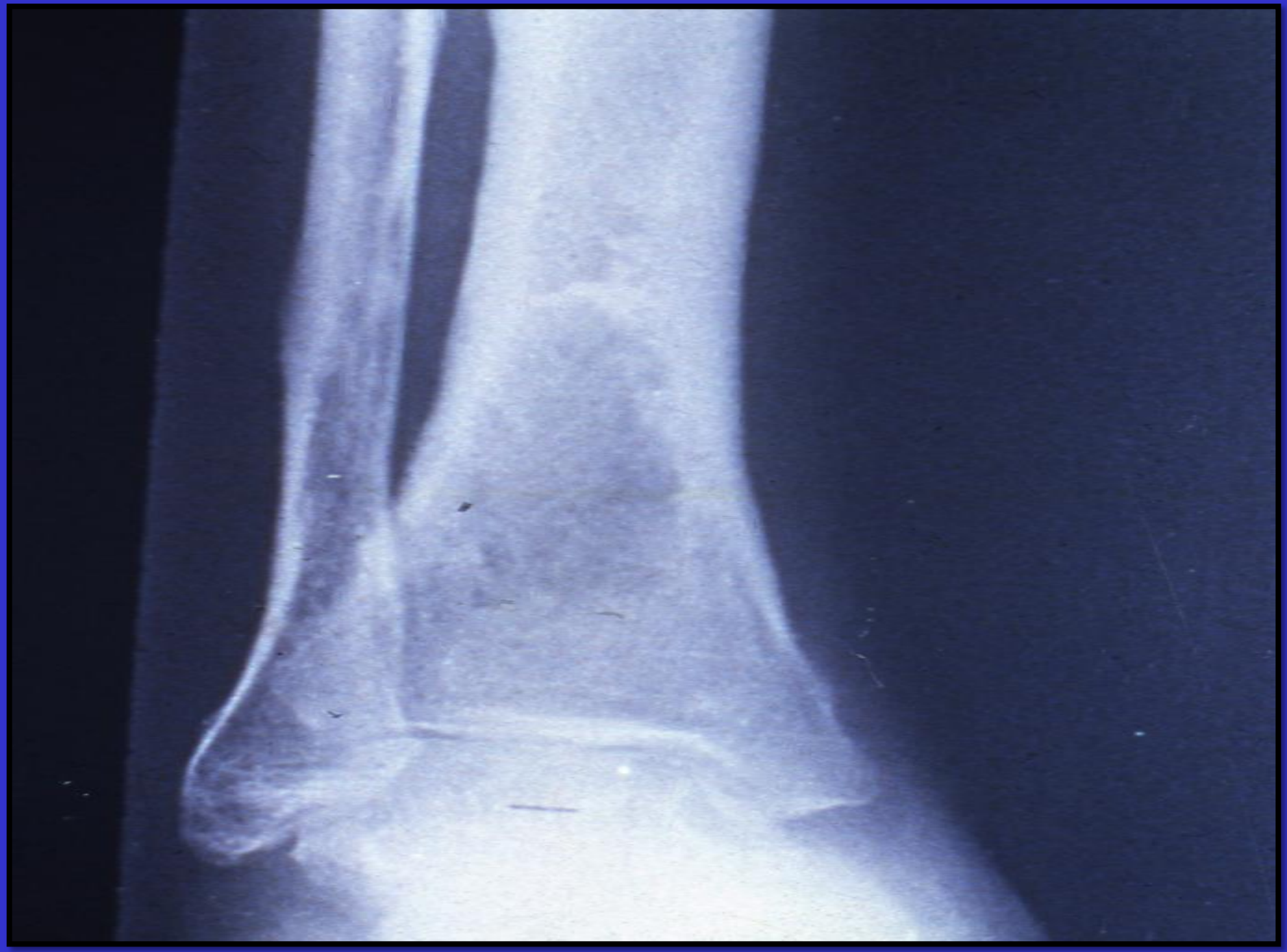
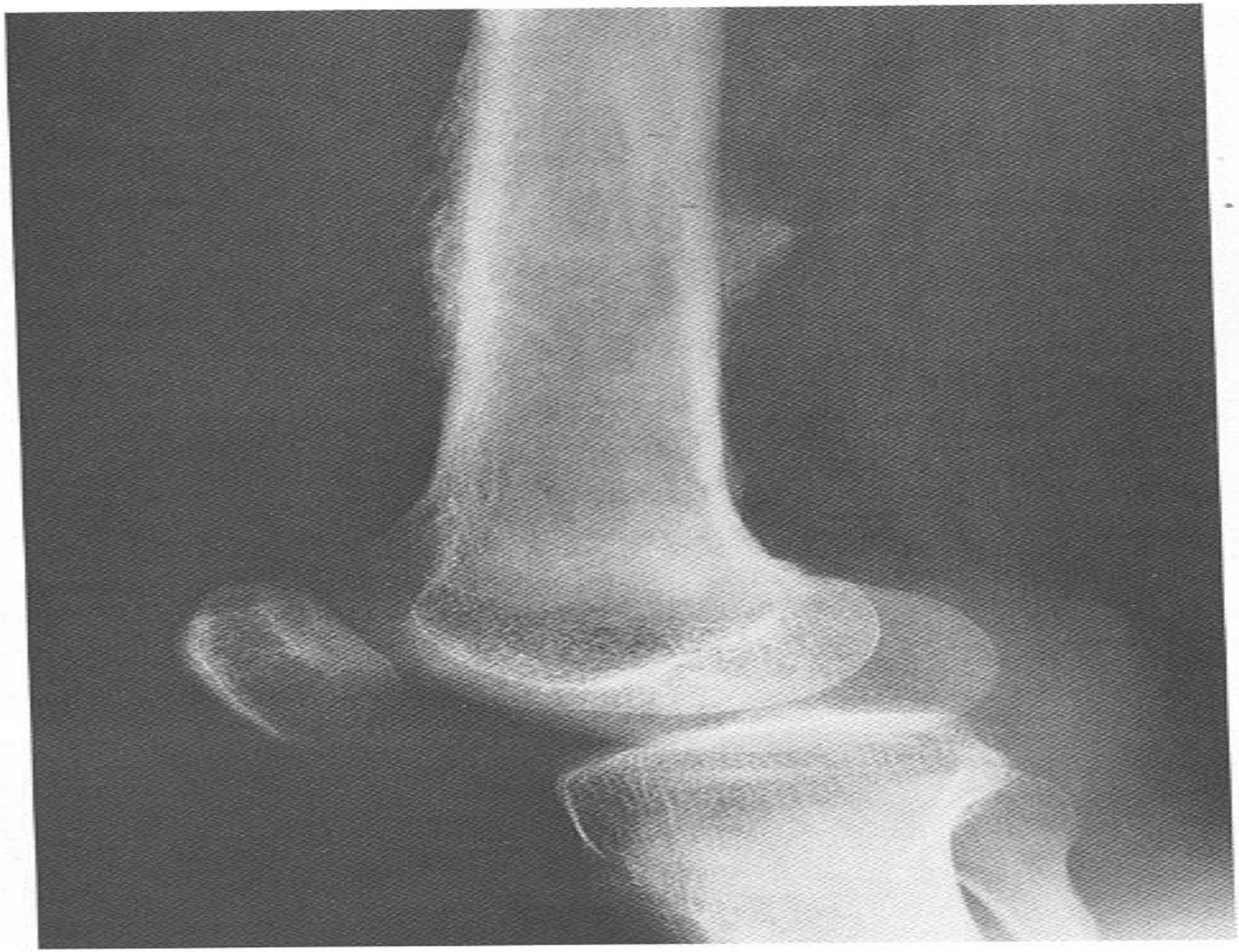
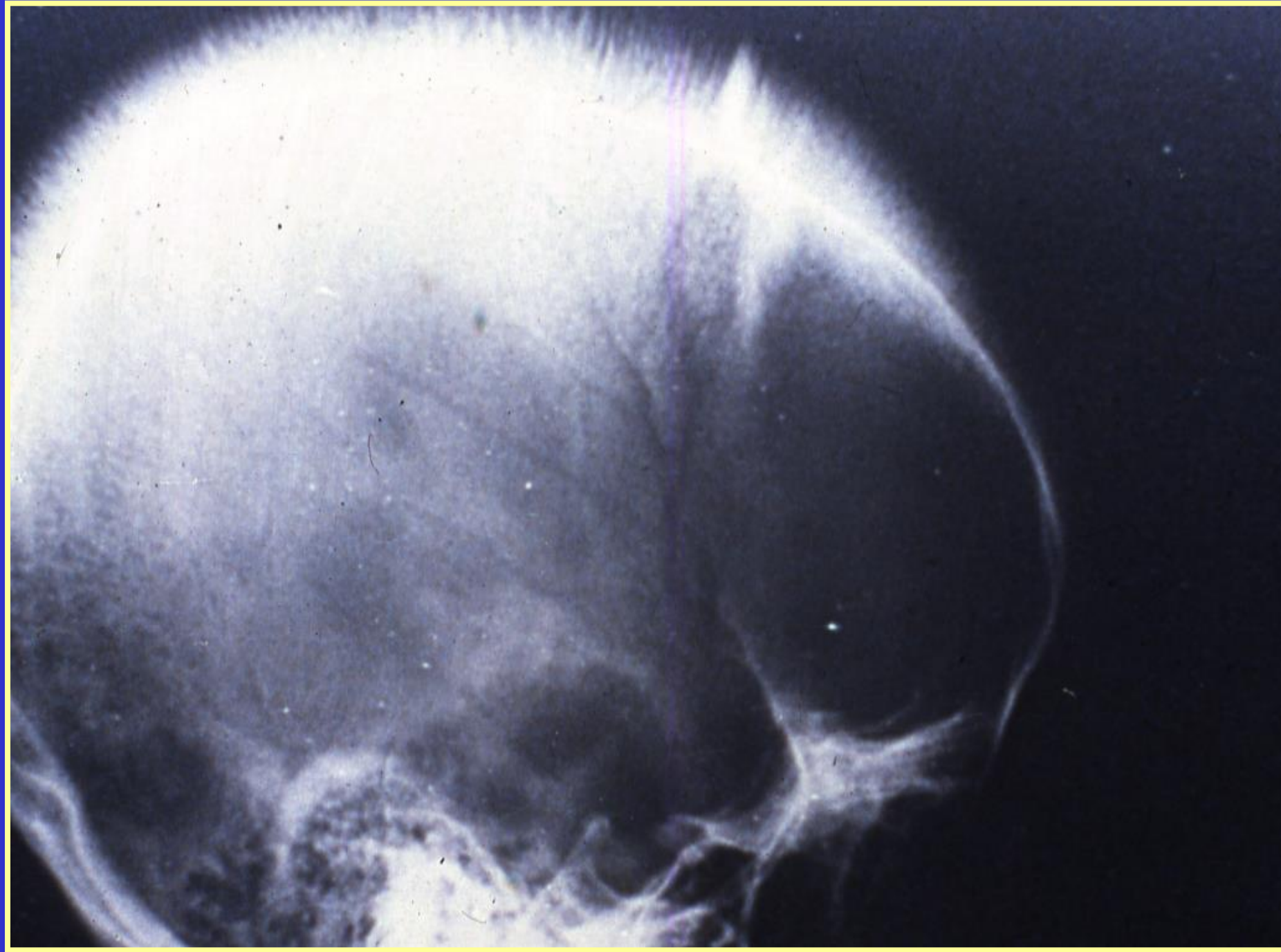
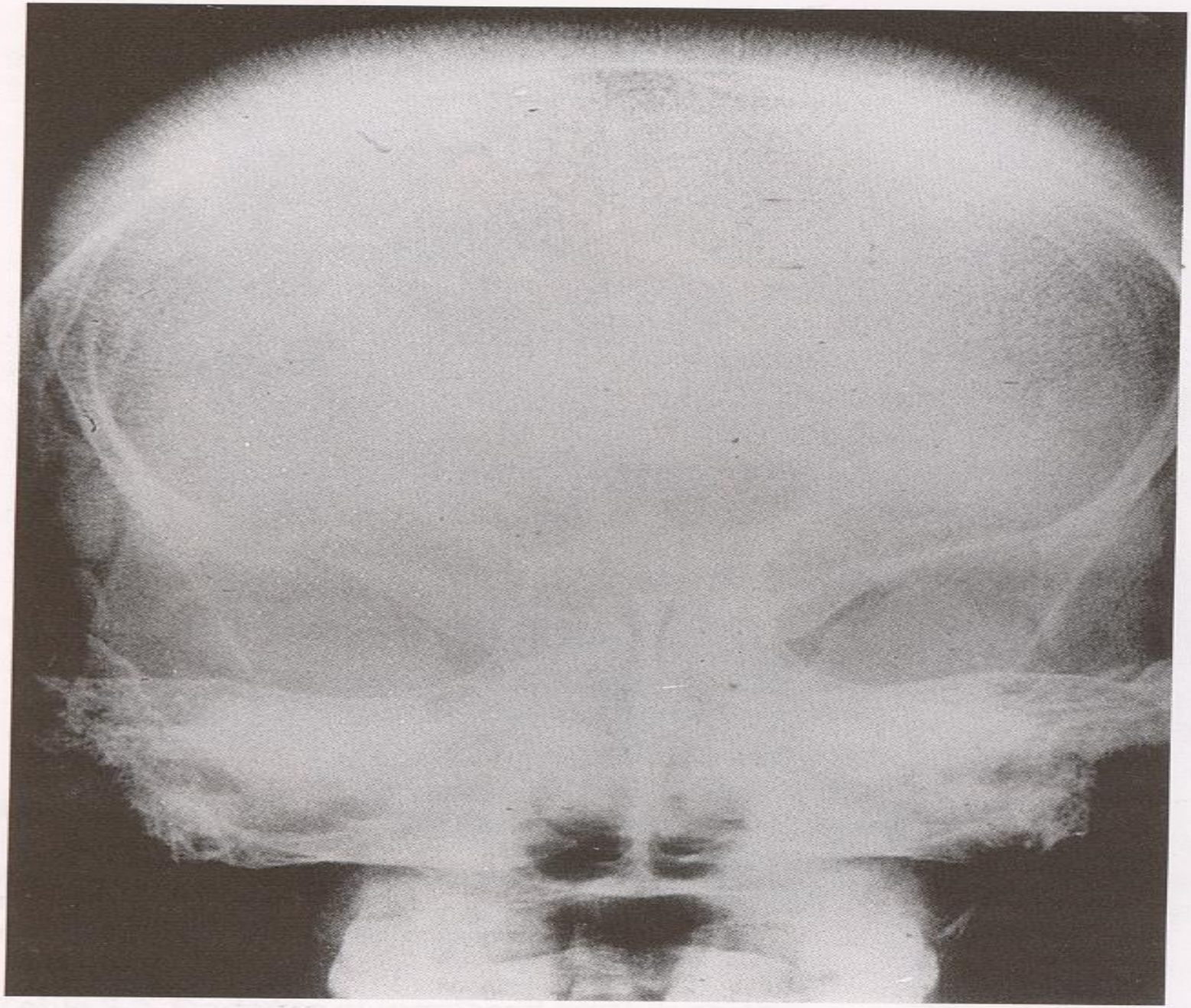
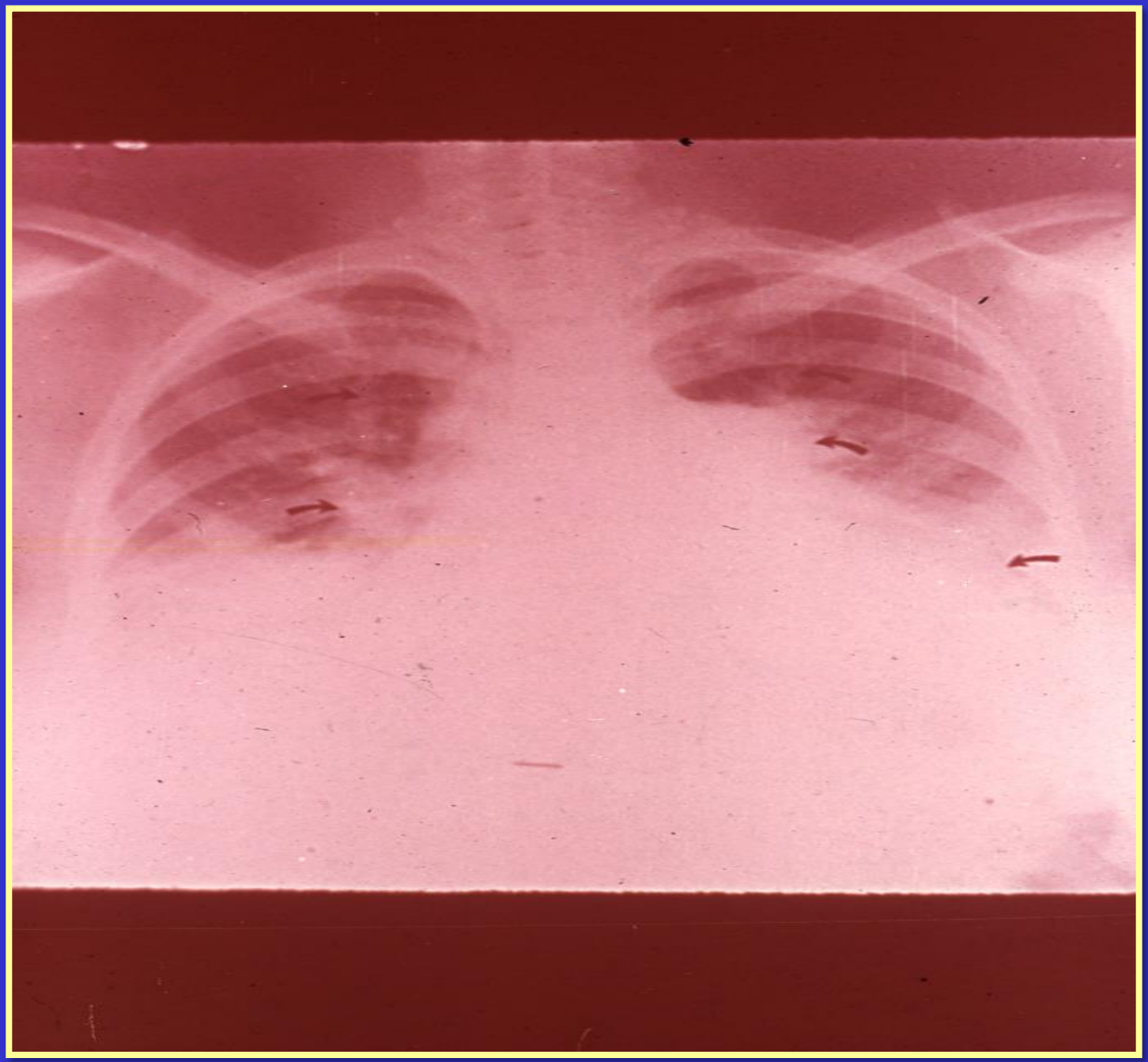
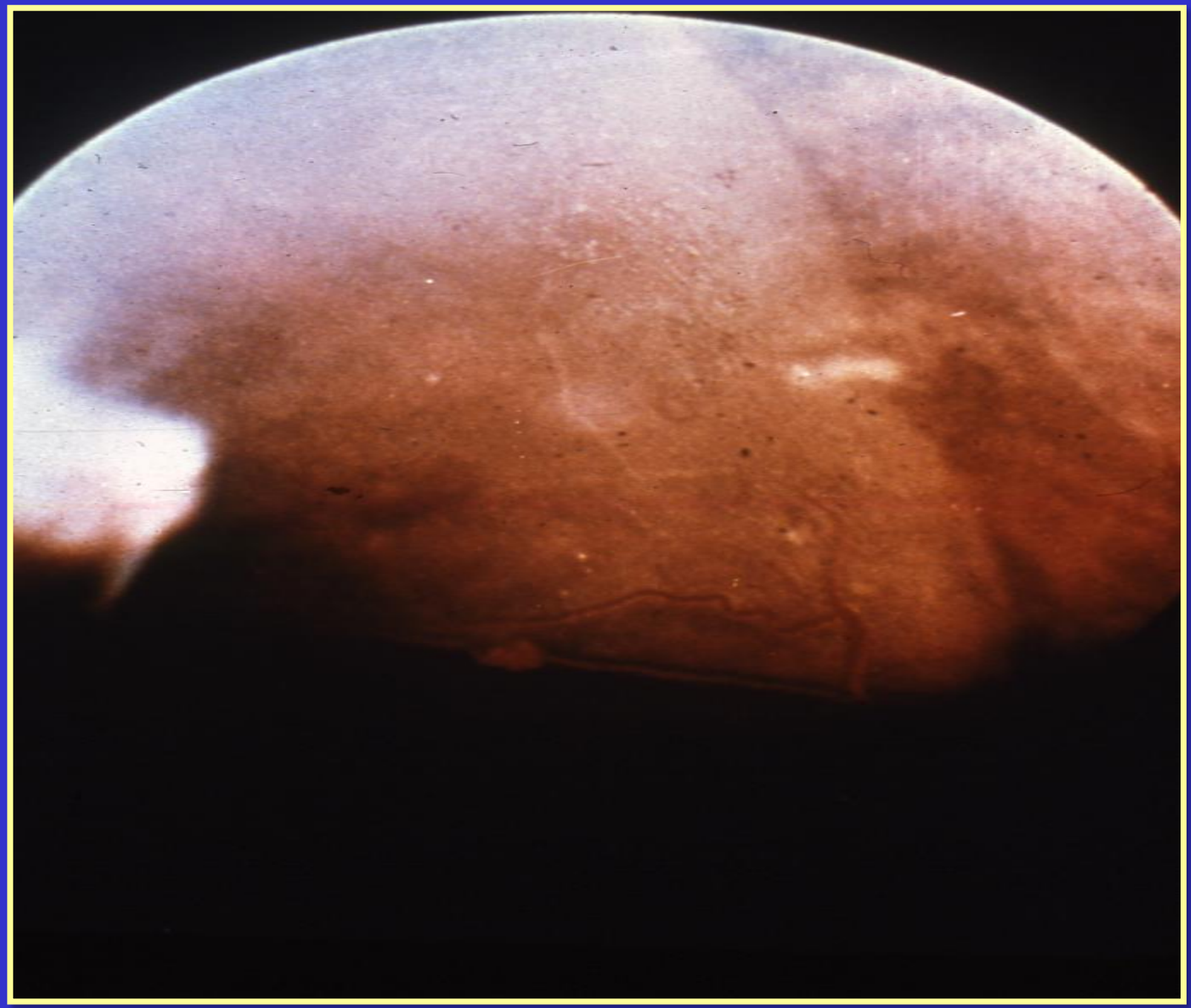


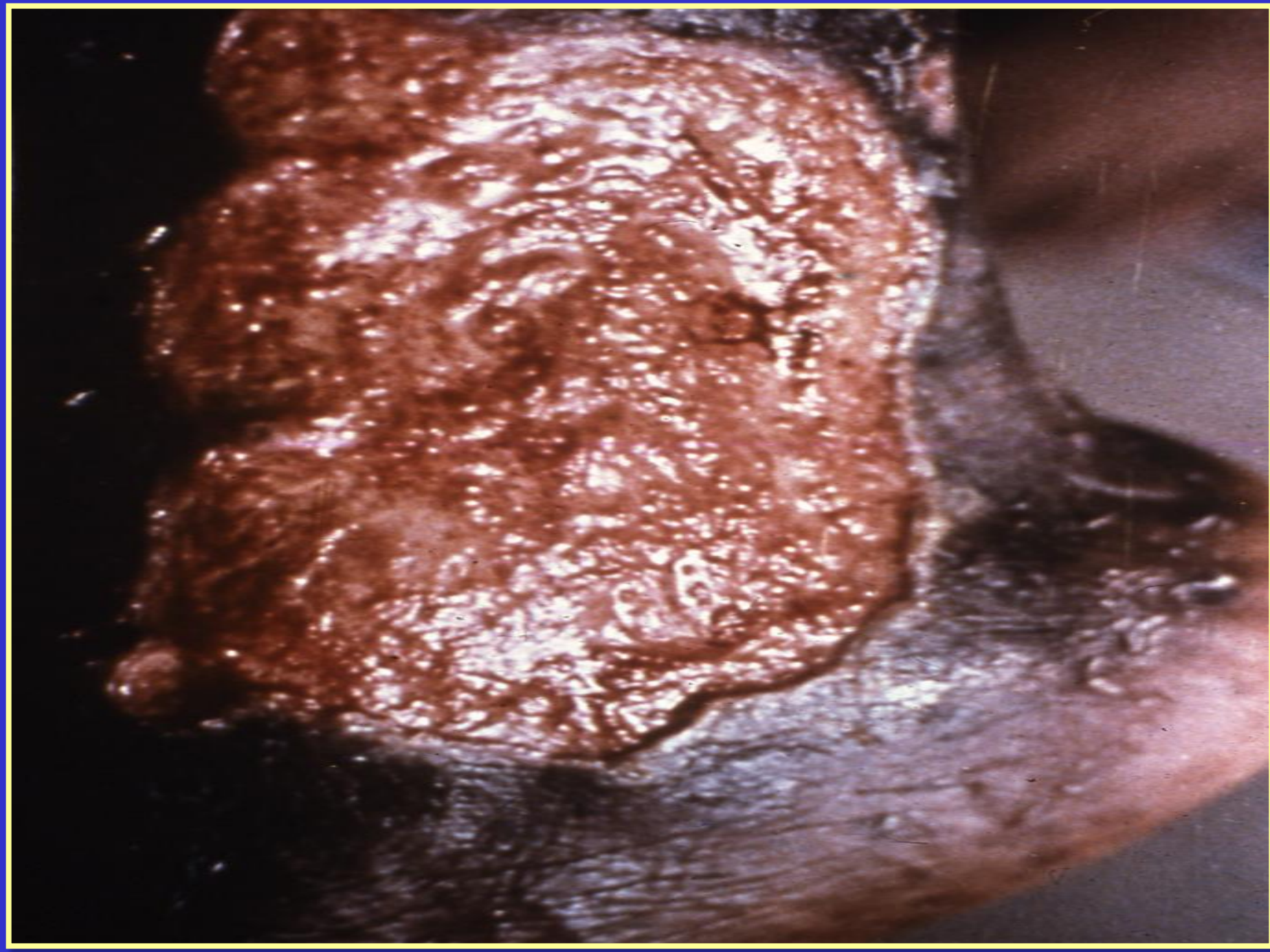


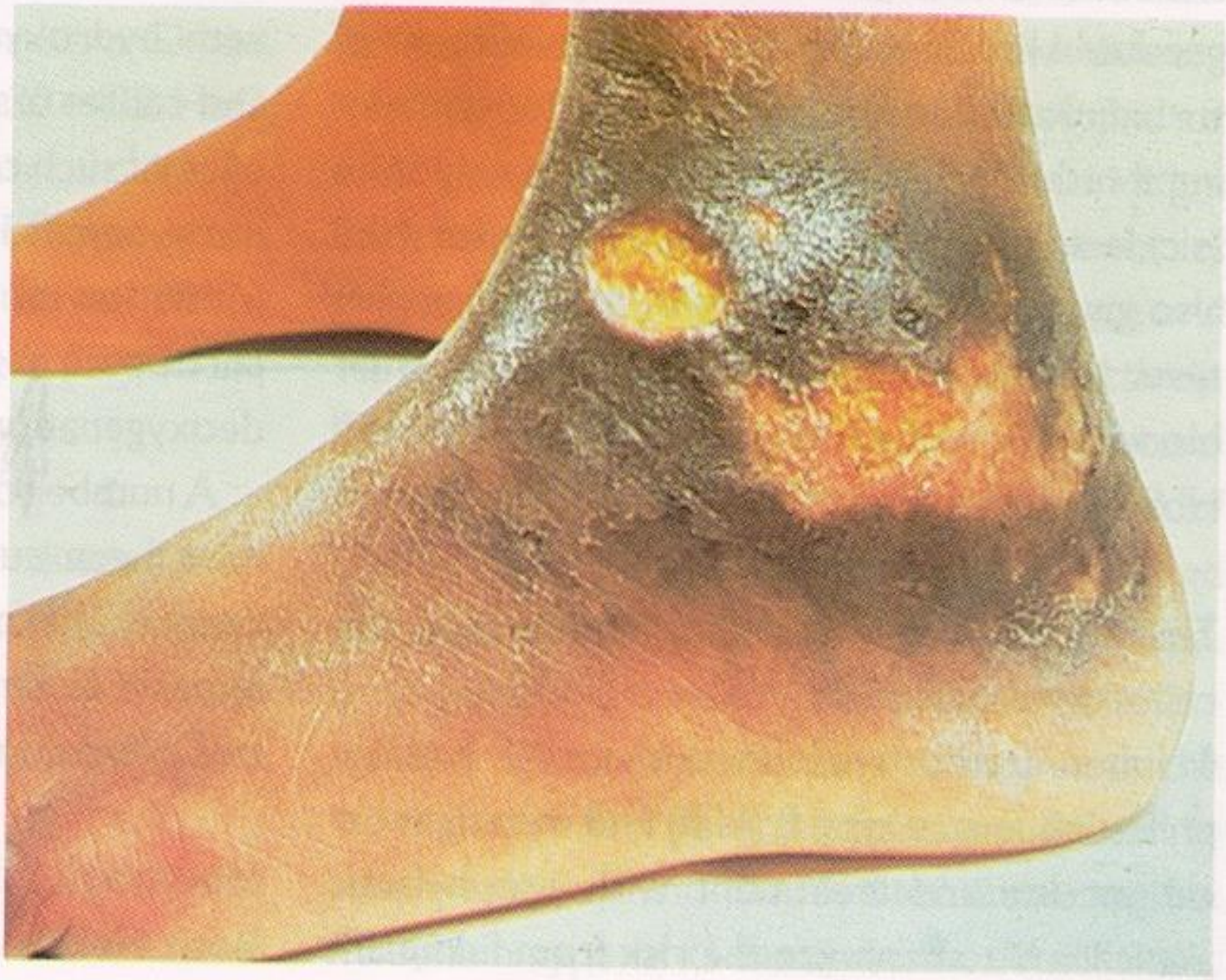


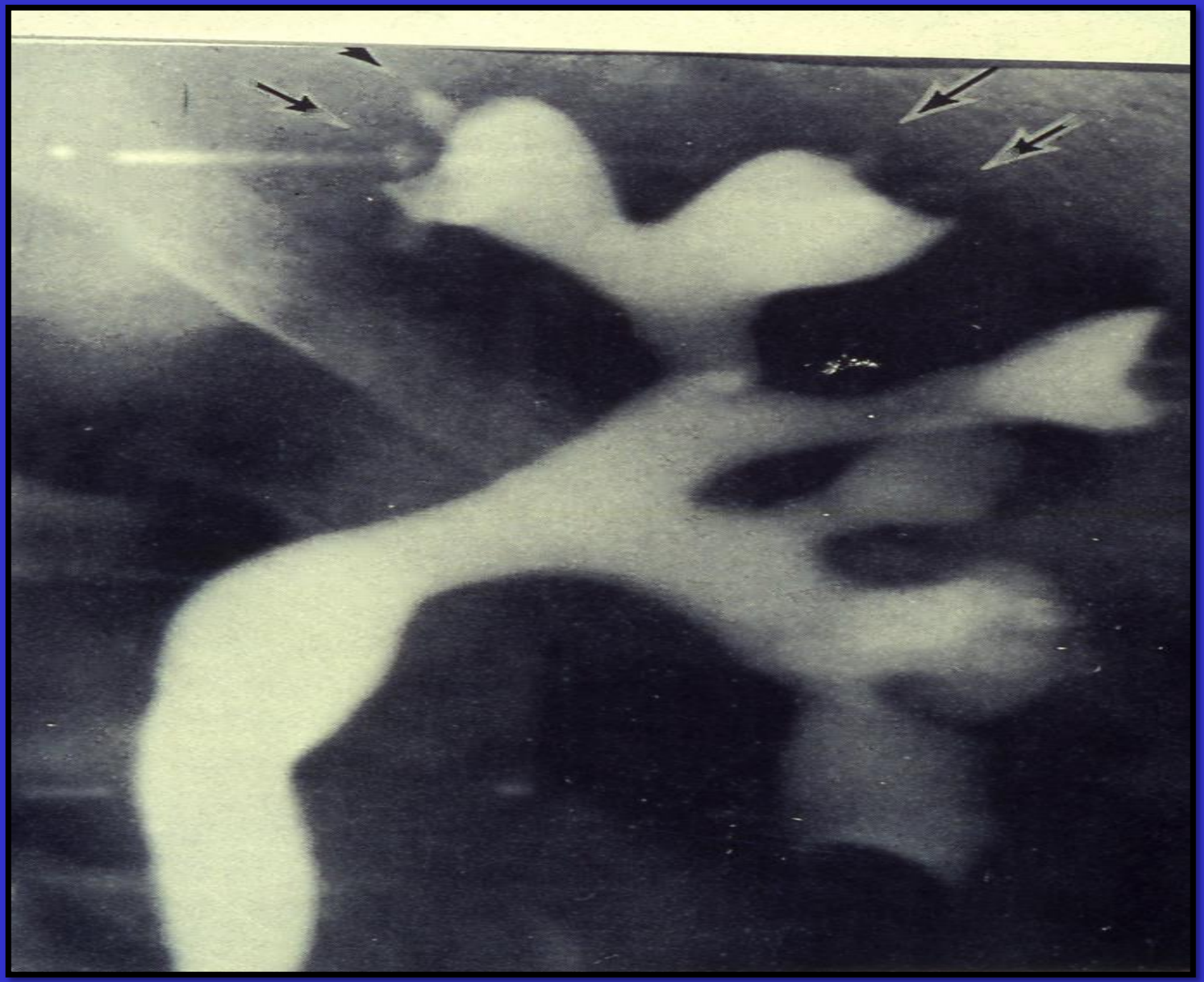






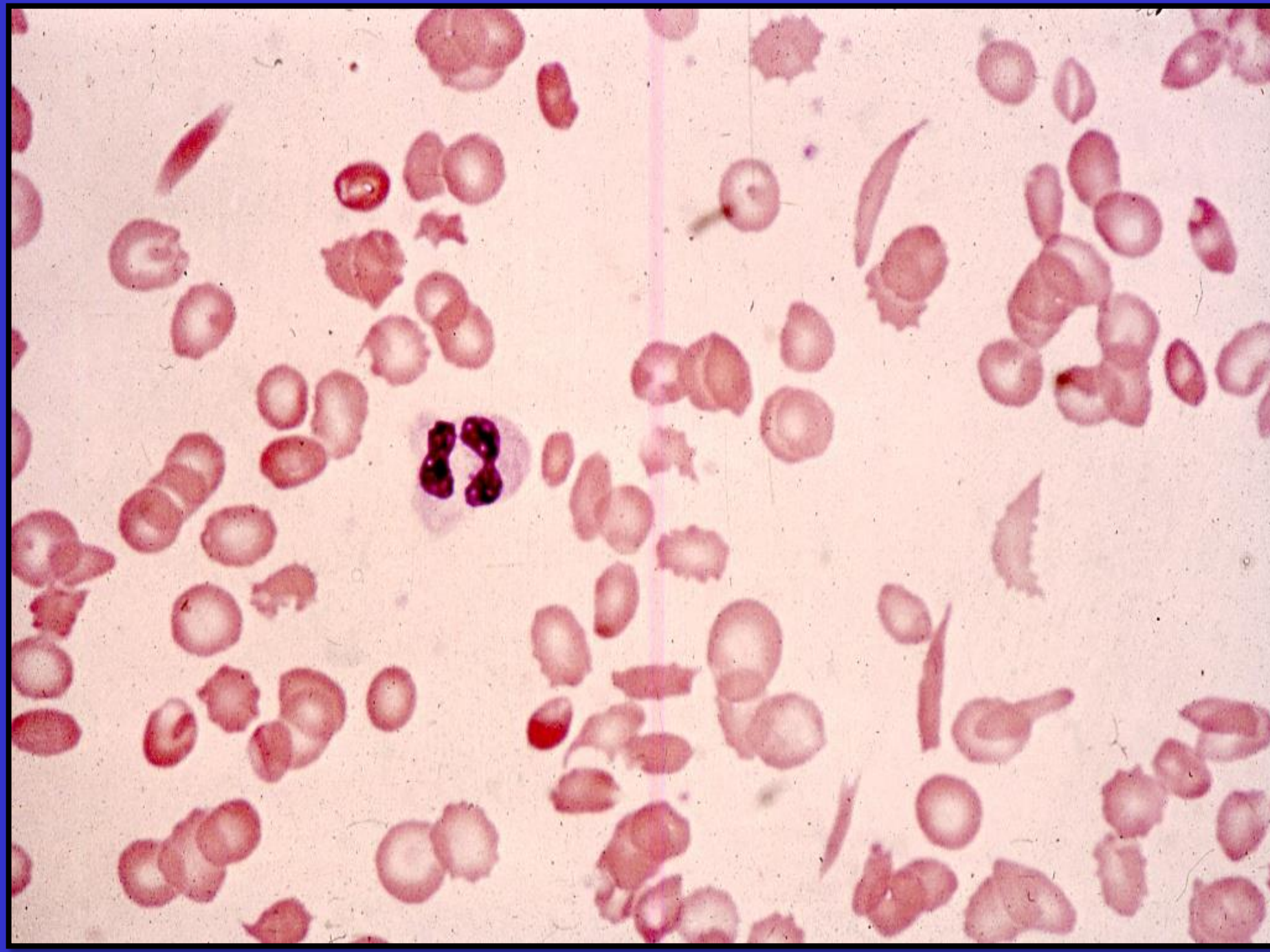


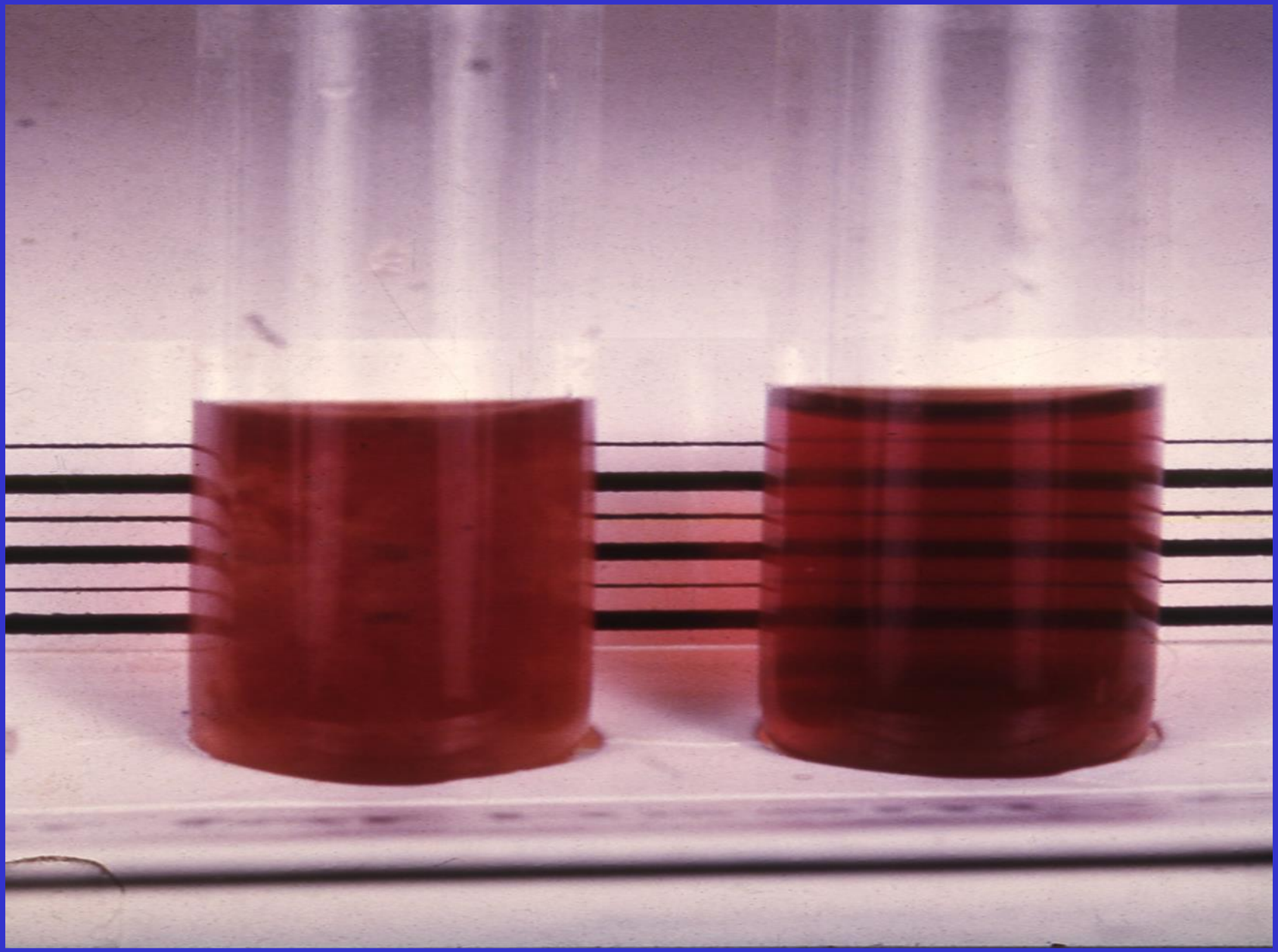




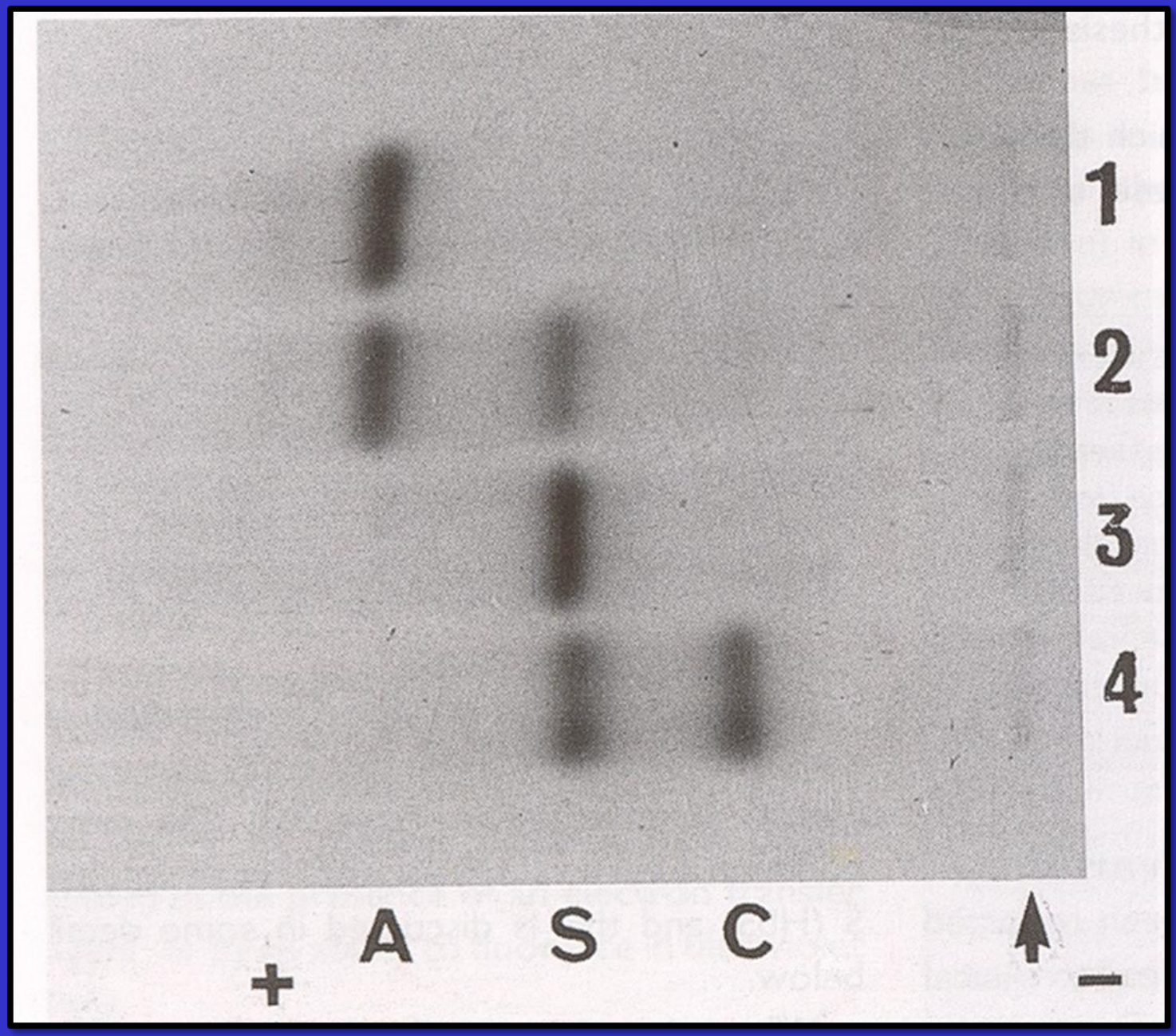
Laboratory Diagnosis of Sickle Cell Disease

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





SICKLE CELL SOLUBILITY TEST



Hb ELECTROPHORESIS

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