PRACTICAL HAEMOGLOBINOPATHIES

By

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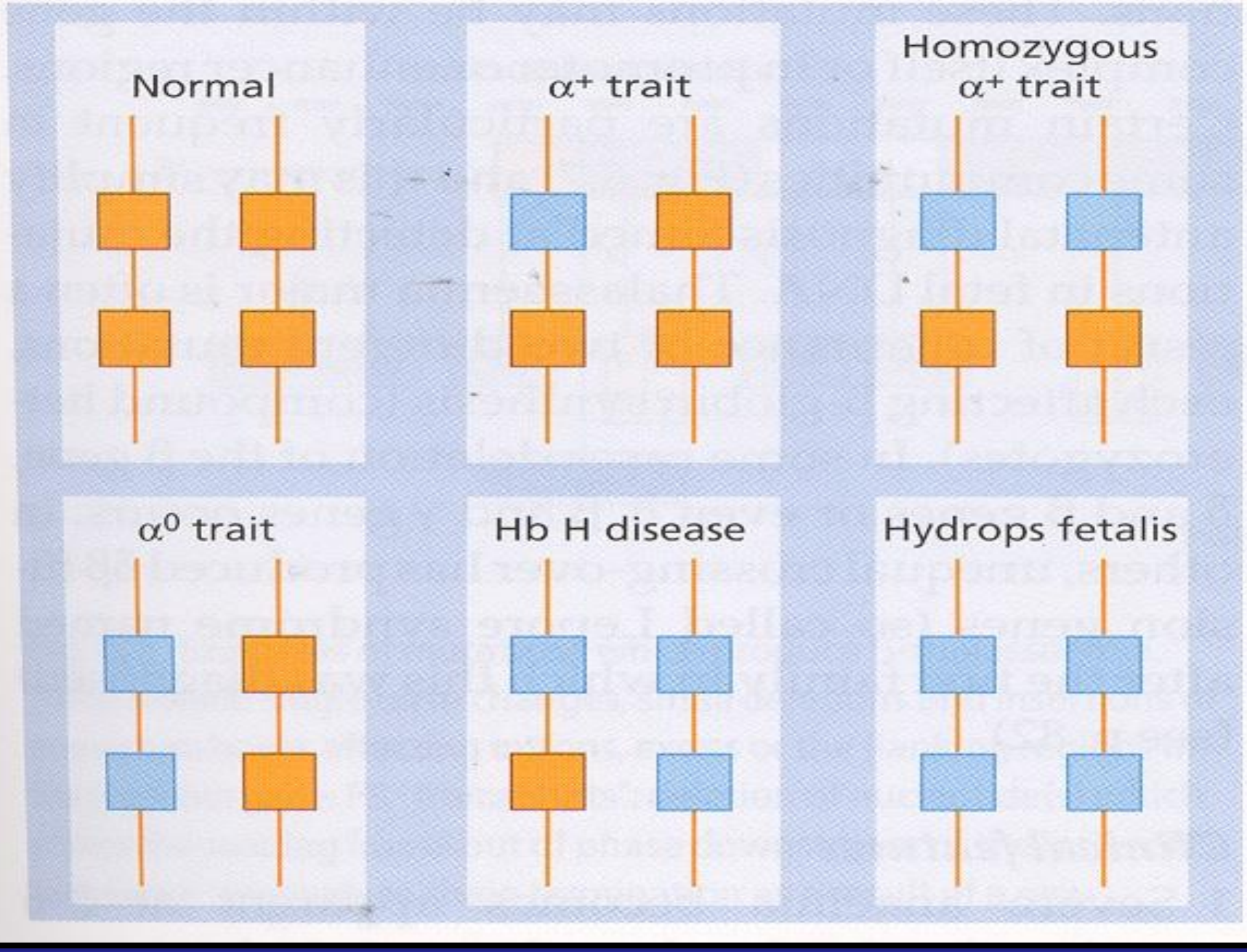
α - THALASSAEMIA

- **HETEROZYGOUS**
- **HOMOZYGOUS**


Normal
α+ trait
Homozygous α+ trait
α0 trait
Hb H disease
Hydrops fetalis

α^+-Thalassaemia trait (deletion of one or two α globin genes)

This is seen when an individual inherits the α^+-thalassaemia allele from one parent or two parents and a normal chromosome 16 from one or two parents (i.e. heterozygotes for the α^+ determinant or homozygous α^+ trait). Affected individuals are asymptomatic, although they have minor haematological changes such as slight reductions in mean cell volume (MCV) and mean cell haemoglobin (MCH).

α^0-Thalassaemia trait (deletion of both α-globin genes on one chromosome 16)

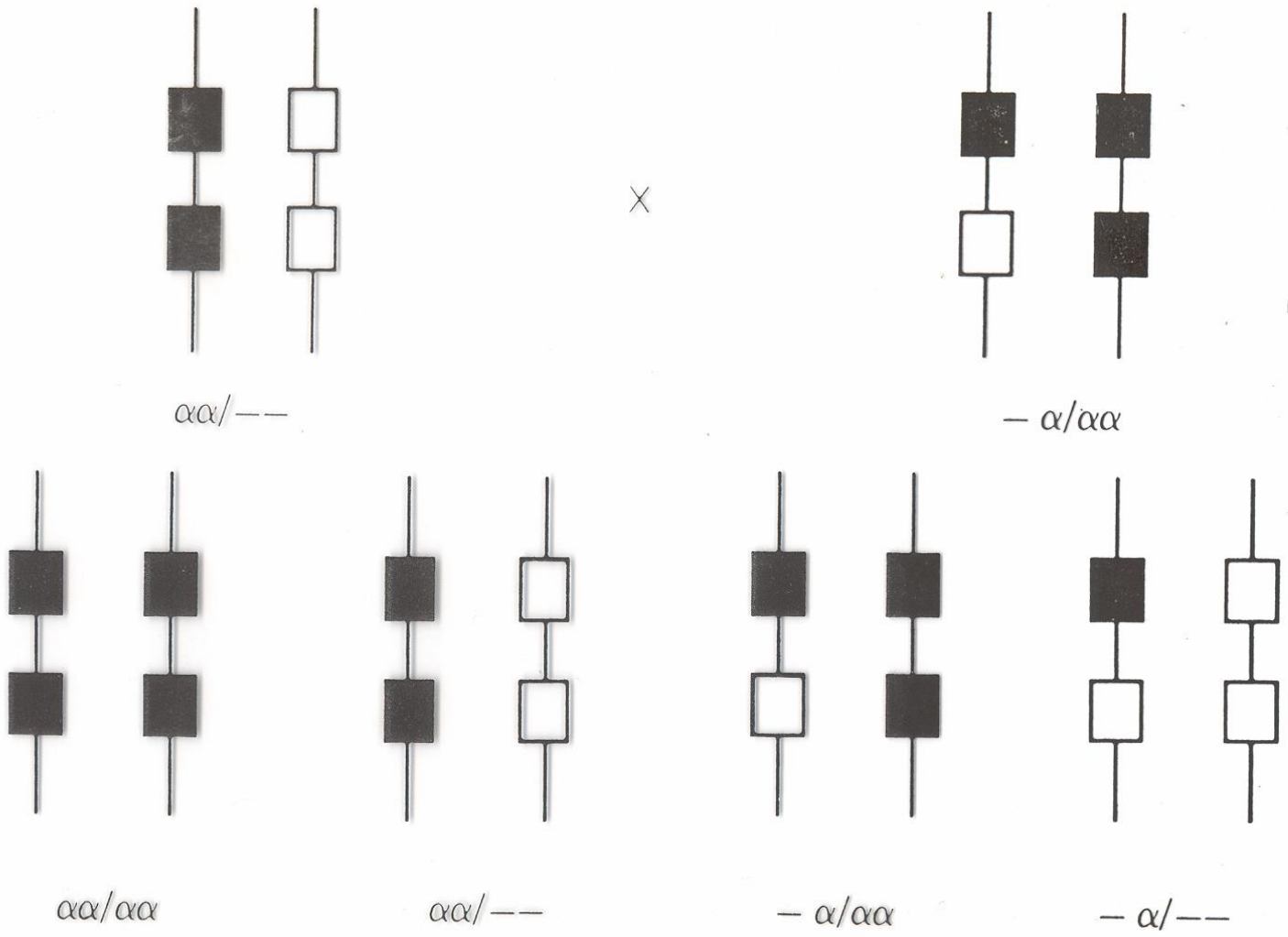
The Hb is either normal or slightly reduced and the MCV and MCH are low.

Haemoglobin H disease (deletion of three α-globin genes)

- This chronic haemolytic anaemia results from the inheritance of both the α^{+}- and α^{0}-thalassaemia alleles, leaving one functioning α-globin gene per cell. α-globin chains are produced at very low rates, leaving a considerable excess of β-chains, which combine to form tetramers (β_4). This tetramer is known as HbH.
- HbH is unstable and precipitates as the erythrocytes age, forming rigid membrane-bound inclusions that are removed during the passage of affected red cells through the spleen. The damage to the membrane brought about by this removal results in a shortened red cell lifespan.

cont'd…

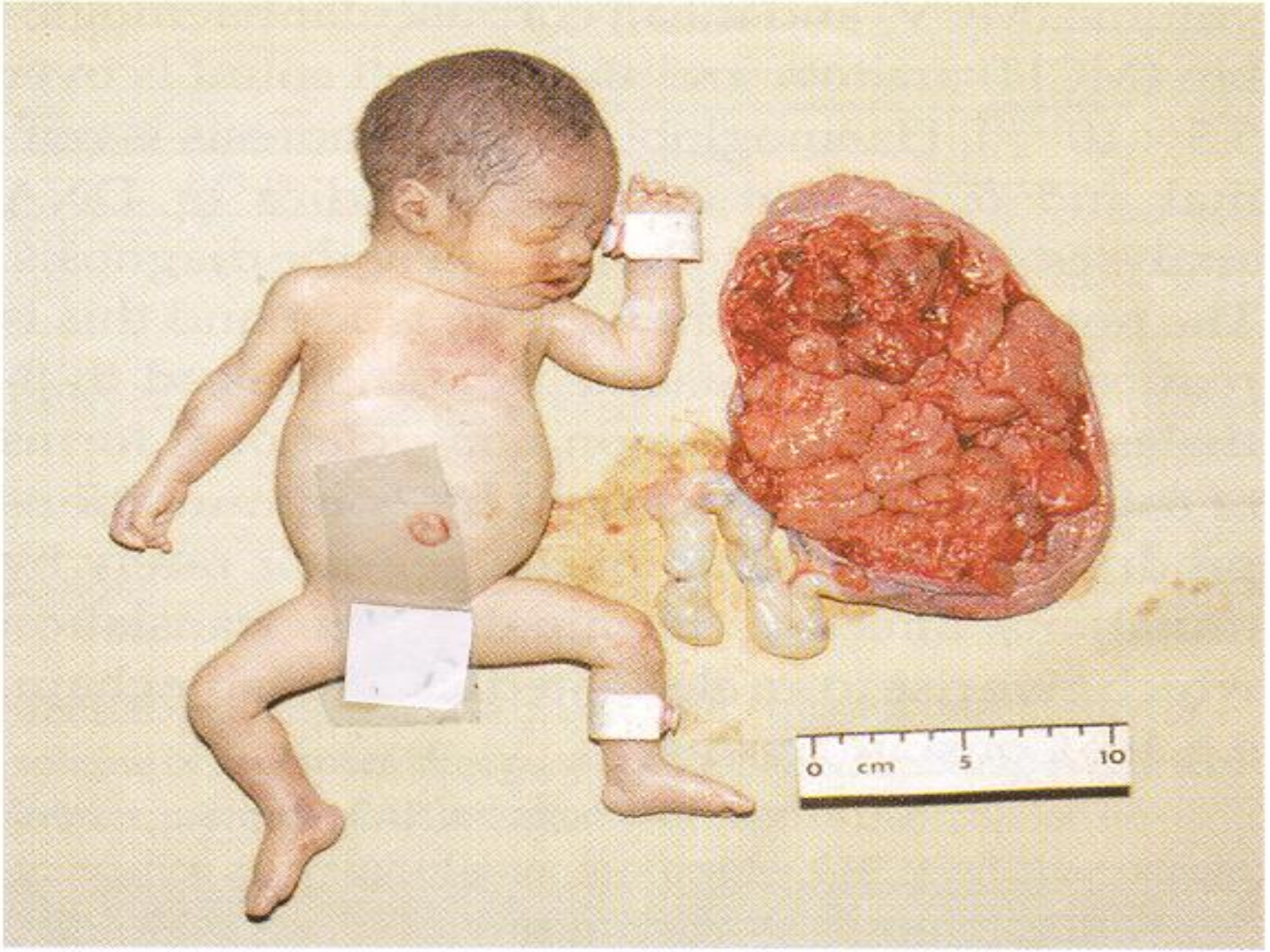
- Most patients are moderately affected, with anaemia of 7-11g/dl and markedly hypochromic, microcytic indices.
- Supravital staining of the blood film demonstrates cells with many HbH inclusions, giving a characteristic 'golf-ball' appearance.
- Most patients will be transfusion independent.
- Splenomegaly is seen in most patients.



Inheritance of HbH (−α/− −) disease. Normal α-globin genes are shown by closed boxes, and deleted or otherwise inactivated α-globin genes by open boxes.

Hb Bart's hydrops fetalis syndrome (deletion of all four α-globin genes)

No α-chains can be formed, and the fetal β-like chain γ-globin forms tetramers known as Hb Bart's. This haemoglobin is not useful for oxygen transport and, despite the persistence of the embryonic haemoglobin Hb Portland ($\zeta_2\gamma_2$), there is intrauterine or neonatal death due to hydrops.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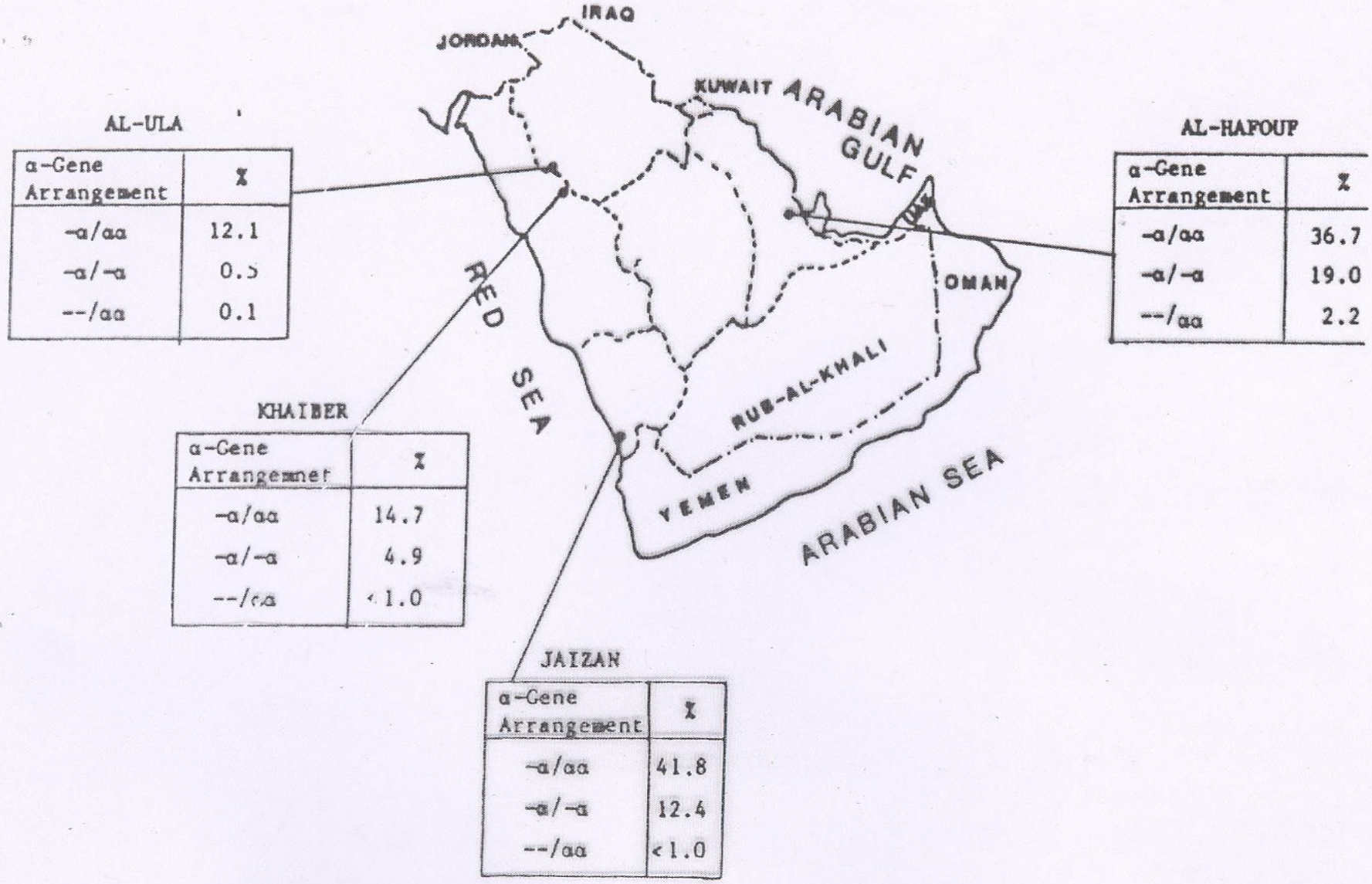

Frequency of α-thalassaemia due to α-gene deletion in different regions of Saudi Arabia (diagnosed using rest iction endonuclease Bam HI).

LABORATORY DIAGNOSIS OF ALPHA THALASSEMIA SYNDROME

- High red cell count in the trait
- Hypochromic microcytic red cells & target cells
- Normal serum iron or low in children
- Normal total iron binding capacity or high in children
- Positive Hb H inclusion bodies in the blood film preparation & positive Heinz bodies with vital stains
- Hemoglobin electrophoresis show presence of hemoglobin H (Hb H disease)
- Hemoglobin electrophoresis show low Hb A2 level
- Genetic study to confirm the diagnosis

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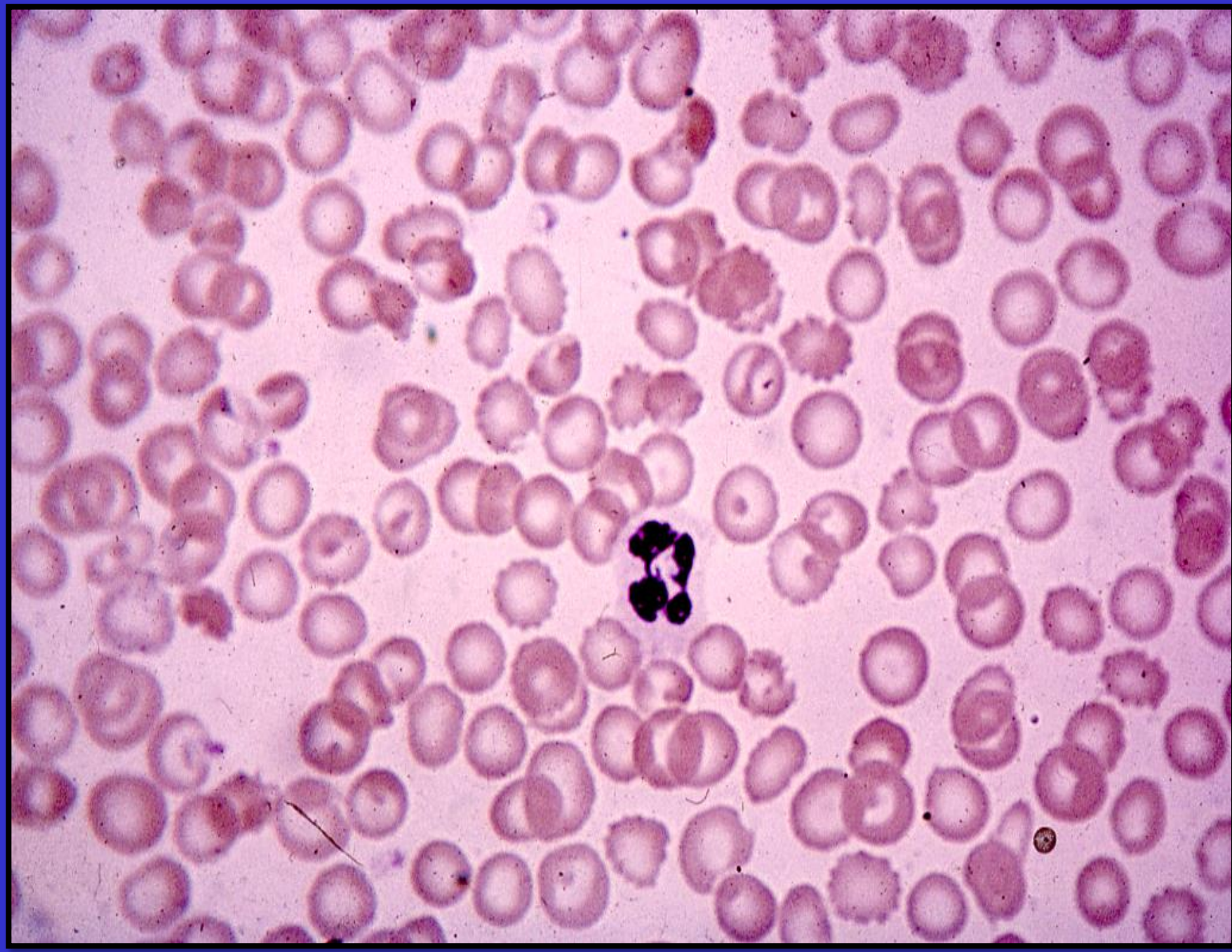
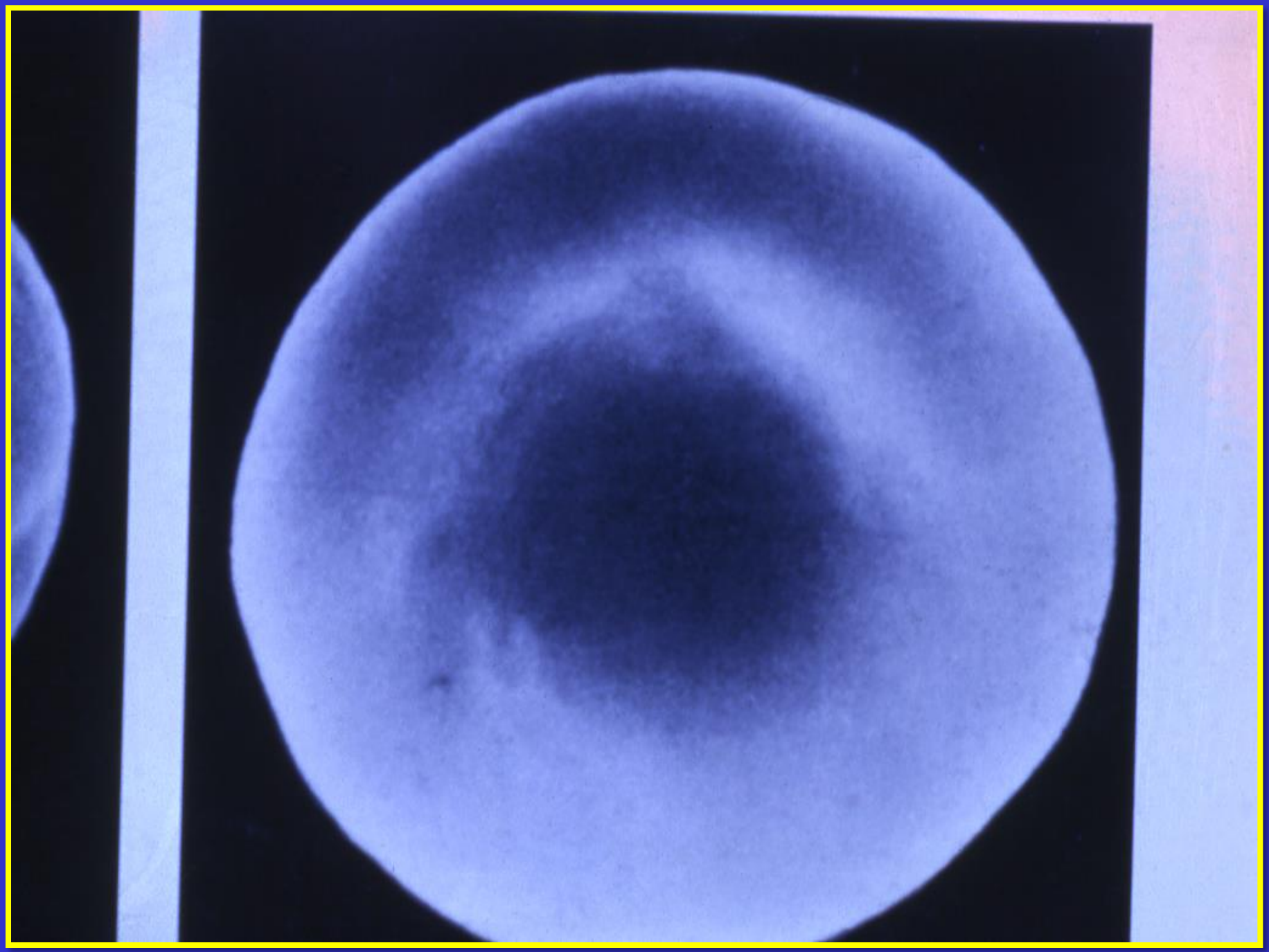
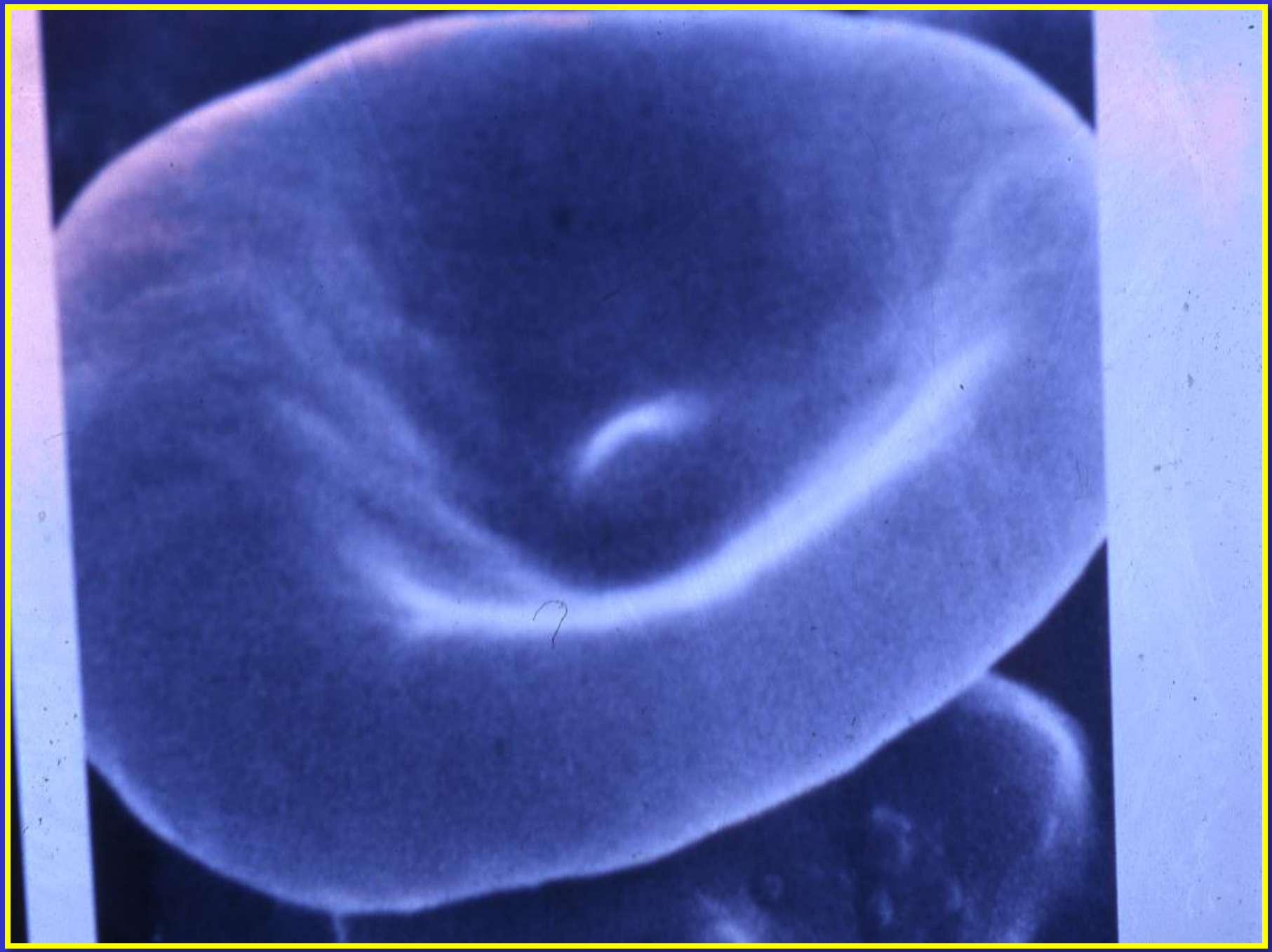
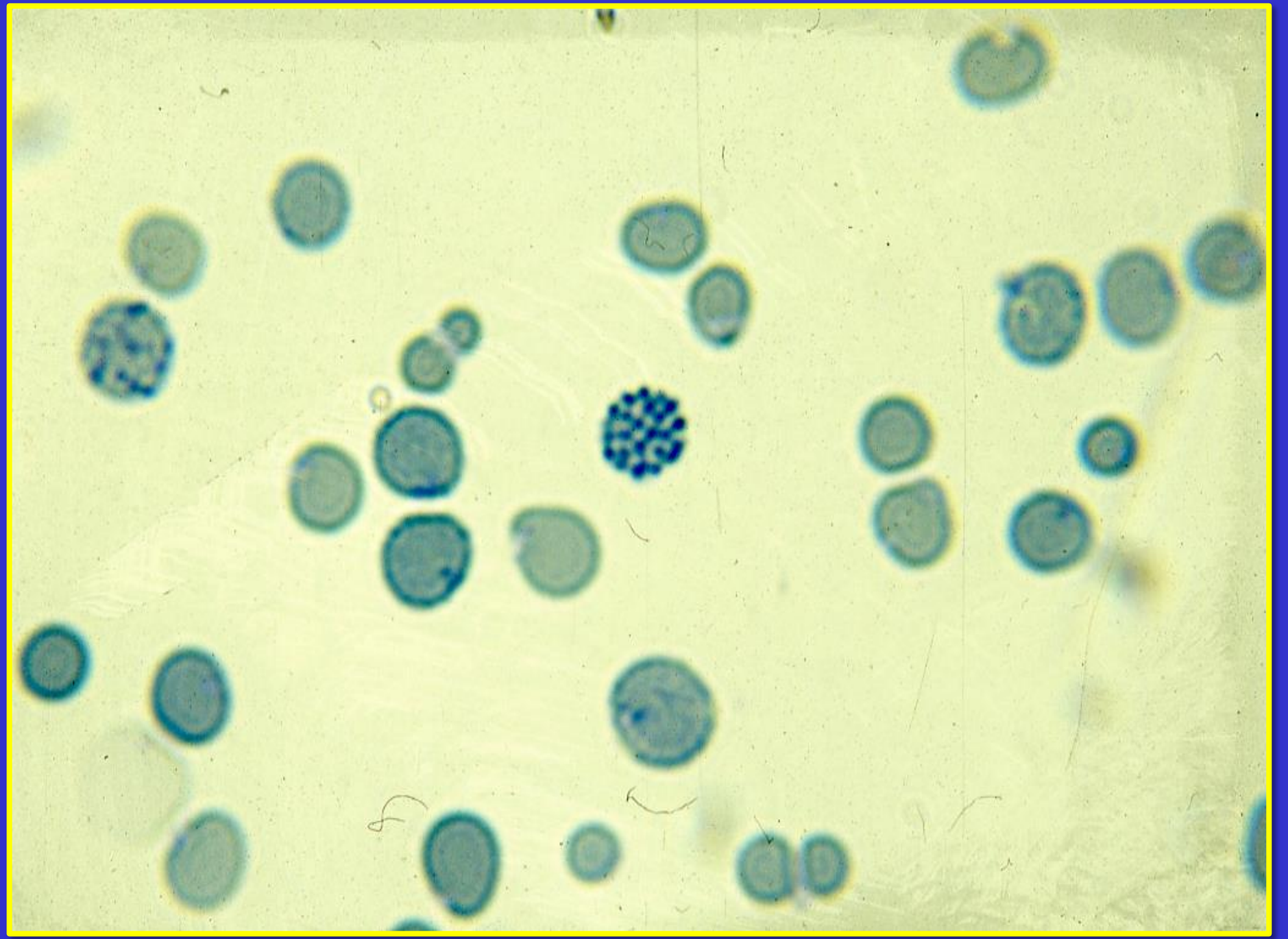
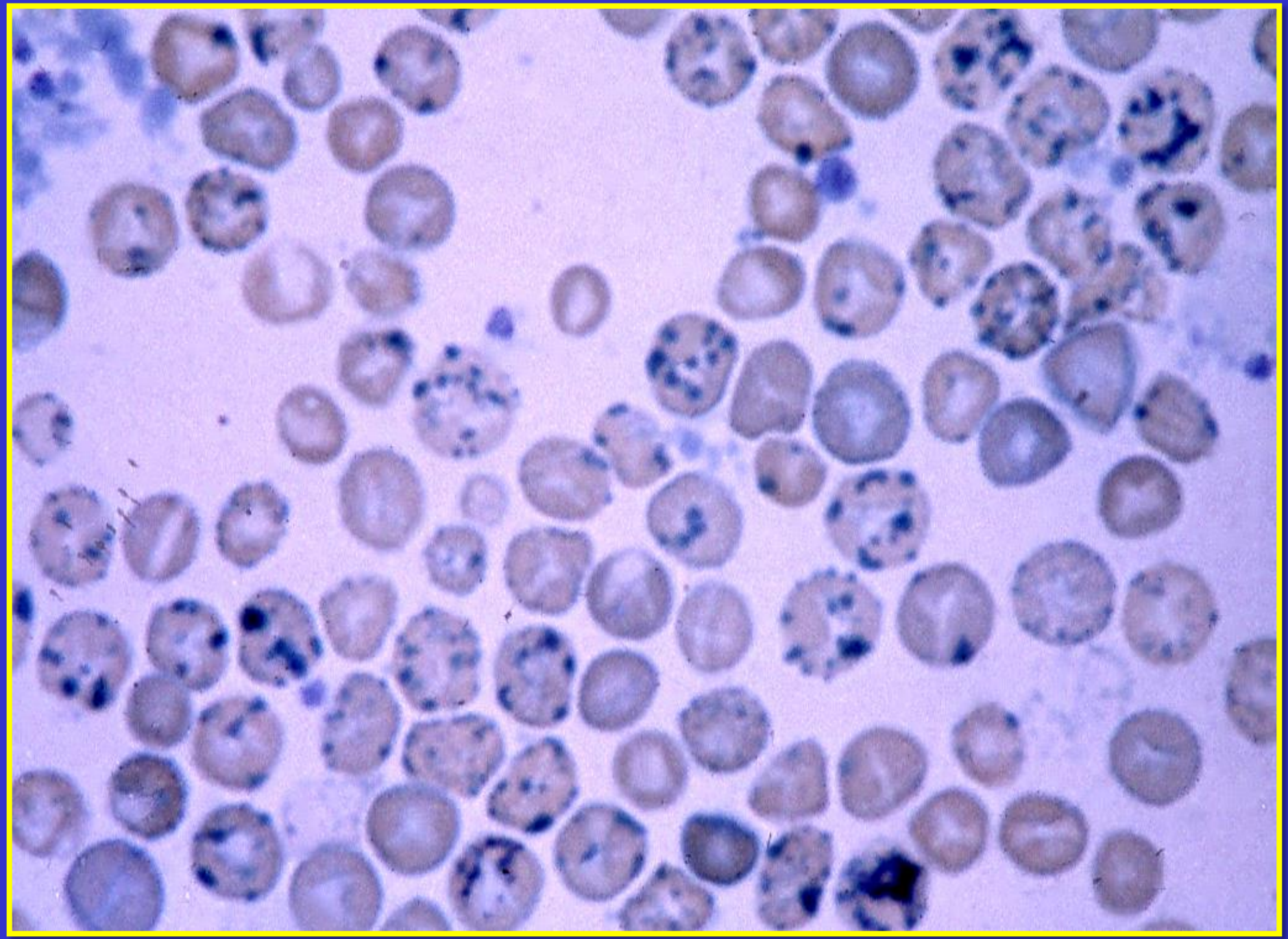
- Normal total iron binding capacity or high in children
- Positive Hb H inclusion bodies in the blood film preparation & positive Heinz bodies with vital stains
- Hemoglobin electrophoresis show raised of hemoglobin H (Hb H disease)
- Hemoglobin electrophoresis show raised Hb Bart's in newborn babies and children below 1 year of age
- Hemoglobin electrophoresis show low Hb A2 level
- Genetic study to confirm the diagnosis

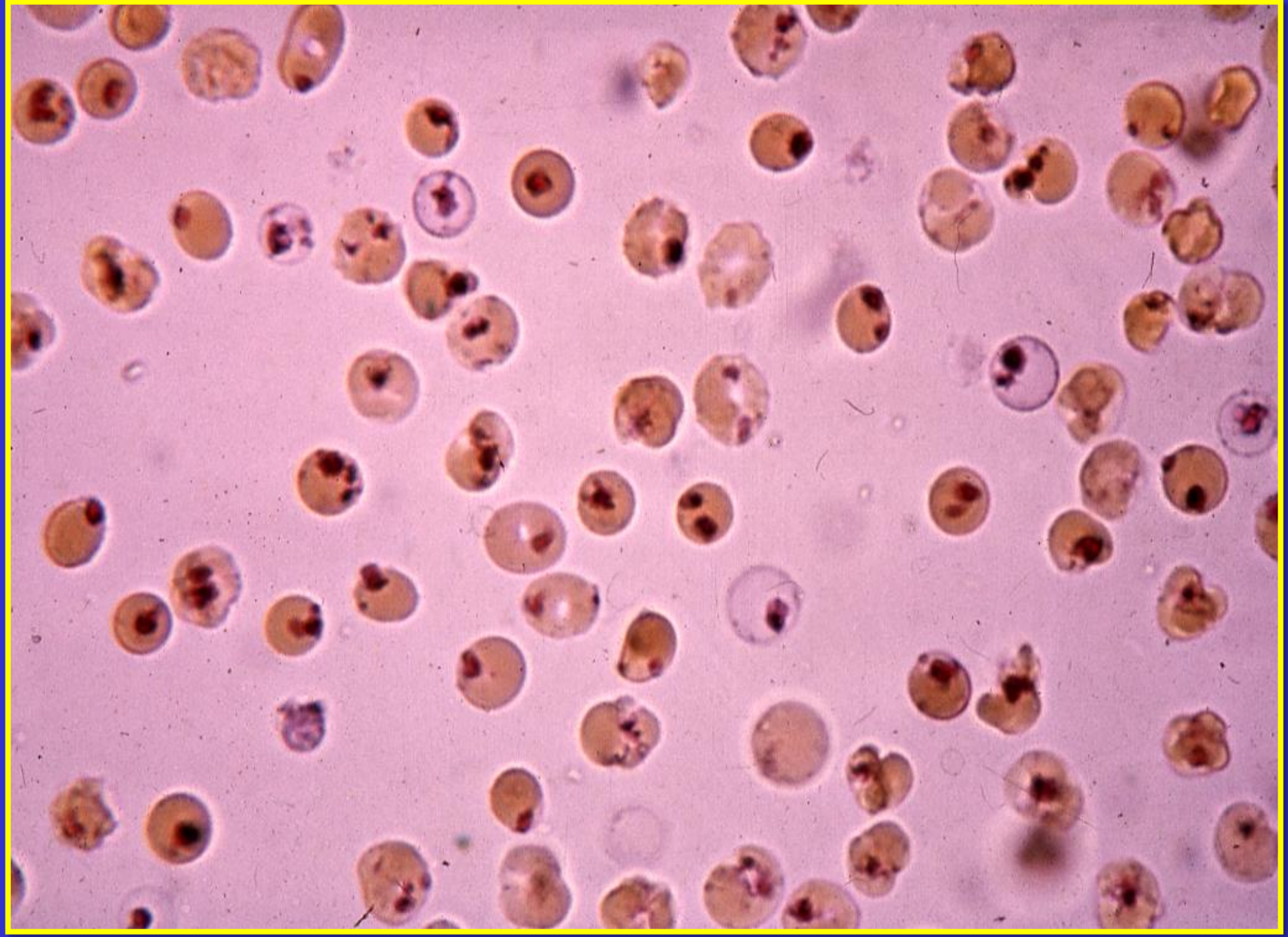


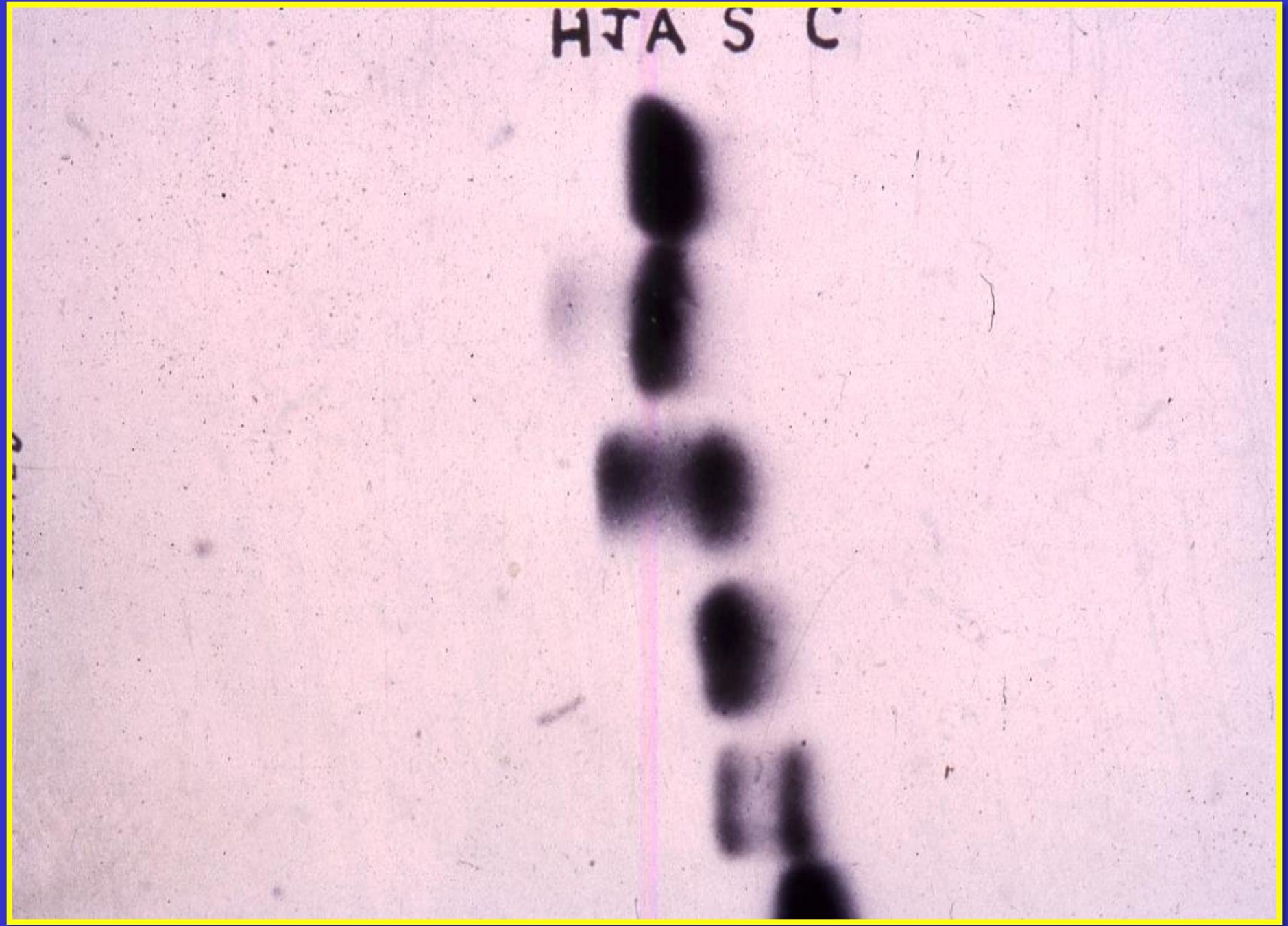






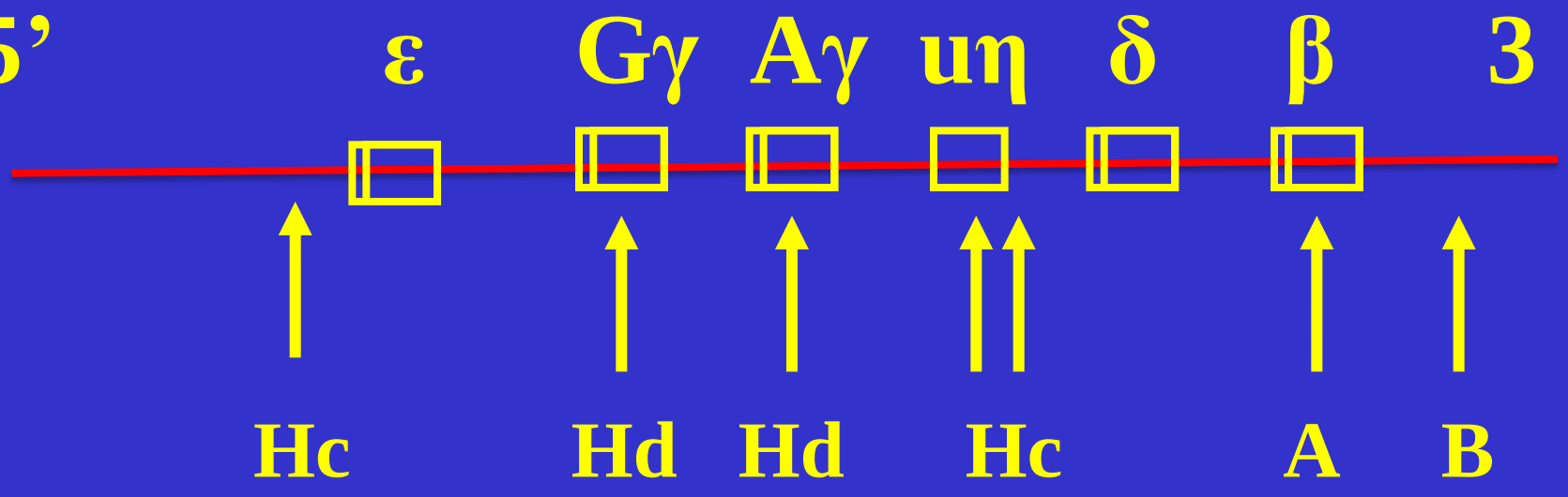





HTA S C

β-THALASSAEMIA

DR. SHIHAB AL-MASHHADANI



The –globin gene cluster showing the position of various common restriction endonuclease polymorphic sites. (Hc, Hinc II; Hd, Hind III; A, Ava II; B, Bam H1).

Molecular Defects in the β-Thalassaemia Syndrome

	β-Globin synthesis	β^-mRNA	β-Globin Gene	δ-Globin Synthesis	γ-Globin Synthesis
1. β^+-Thalassemia 2. β^0-Thalassemia	Decreased Absent	Decreased Absent	Present Present	Present Present	Present Present
Ferrara Variant Indian Variant	Absent Absent	Inactive Absent	Present Partially Deleted	Present Present	Present Present
3. δβ-Thalassaemia 4. HPFH	Absent Absent	Absent Absent	Deleted Deleted	Absent Absent	Increased increased

Hemoglobin Fractions in the Genotypic Variants of the β-Thalassaemia Syndromes

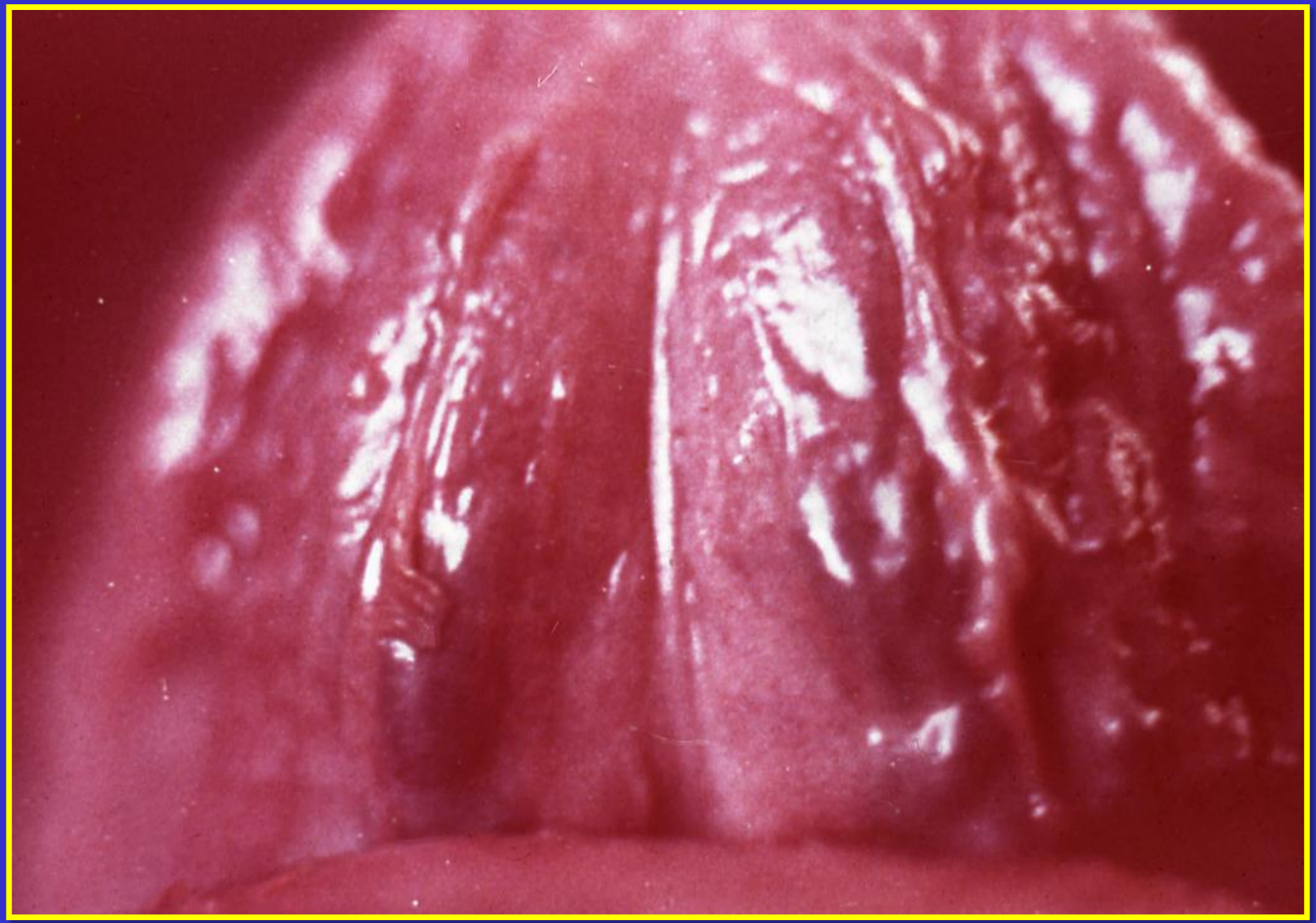
Genotype	HbA	HbA_2	HbF (%)	Other Hemoglobins
Normal				
β/β	97	2.5 – 3.2	<1	None
Thalassaemia major				
$β^0/β^0$	0	1.0 – 5.9	>94	Free α-chains
$β^+/β^+$ Mediterranean	Present	2.4 – 8.7	20 – 90	Free α-chains
$β^0/β^+$	Present	0.6 – 3.4	>75	None
$(δβ)$ Lepore/ $(δβ)$ Lepore	0	0	70 – 92	Hb Lepore (8-30%)
Thalassaemia intermedia				
$β^+/β^+$, black	Present	5.4 – 10.0	30 – 73	None
$β^0/ (δβ)^0$	0	0.3 – 2.4	60 – 99	None
$β^+/ (δβ)^0$	20 – 30	Decreased	Increased	None
$β^0/ (δβ)^0$ Lapore	0	Decreased	Increased	Hb Lepore (10%)
$β^+/ (δβ)^0$ Lepore	Present	Decreased	Increased	Hb Lepore (10%)
$β^0/β$	Present	>3.2	1.5 – 12	None
$(δβ)^0 / (δβ)^0$	0	0	100	None
$(δβ)^0/ (δβ)$Lepore	0	0	92	Hb Lepore (8%)
α/β	Present	Increased	Normal or increased	± Hb H

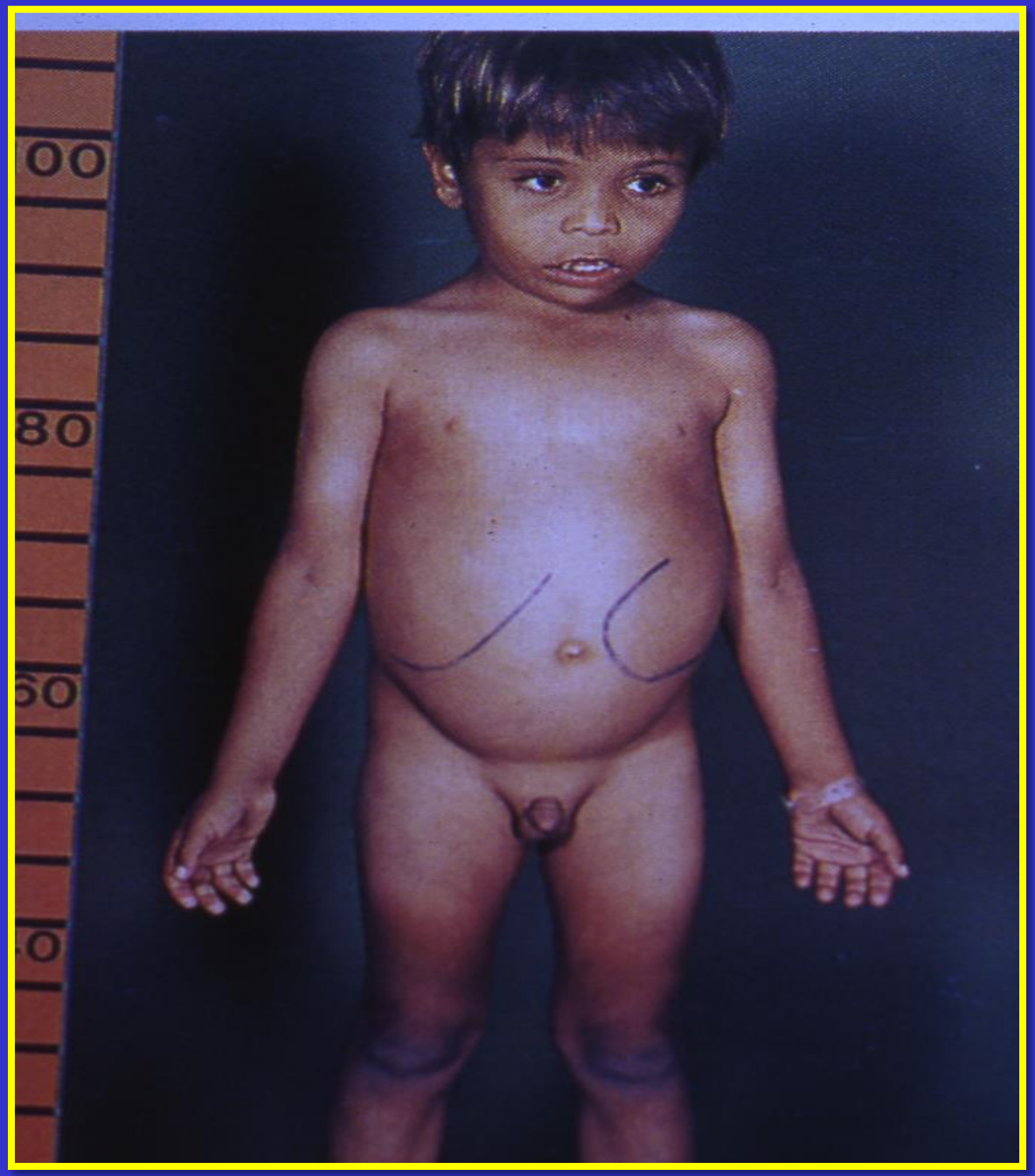
Hemoglobin Fractions in the Genotypic Variants of the β-Thalassaemia Syndromes (Continued)

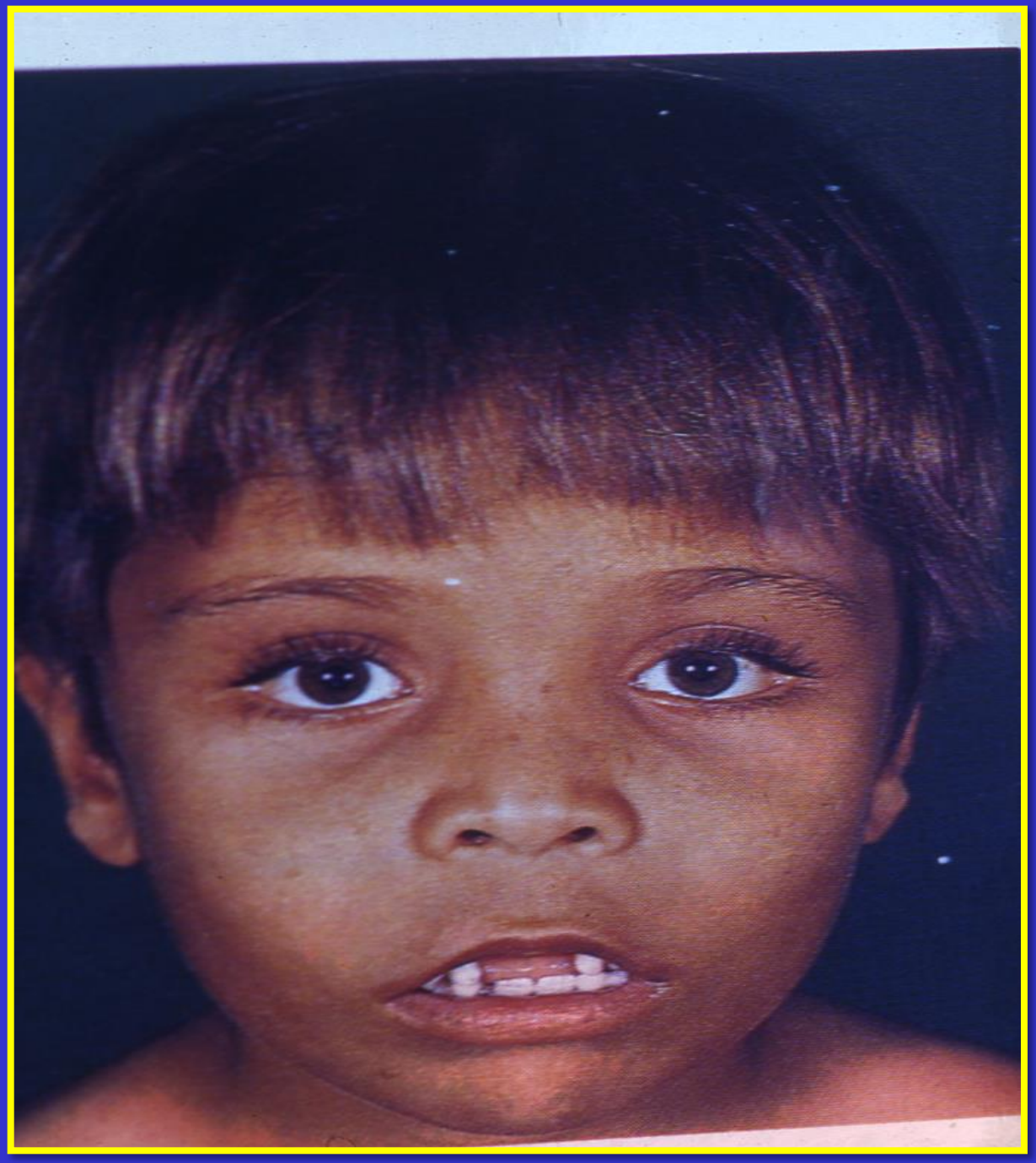
Genotype	HbA	HbA_2	HbF (%)	Other Hemoglobins
Thalassaemia minor				
β^{+}/β	>90	3.5 – 8.0	1 – 2	None
β^{0}/β	>90	3.5 – 8.0	1 – 2	None
$(\delta\beta)^{0}/\beta$	>90	2.5 – 8.0	5 – 20	None
$(\delta\beta)_{Lepore}/\beta$	Present	1.2 – 2.6	1 – 3	Hb Lepore (5 – 15%)
$(\gamma\delta\beta)^{0}/\beta$	Present	2.5 – 3.2	< 1 – 2	None
Thalassaemia minima				
β^{silent}/β	97	<3.2	<1	None

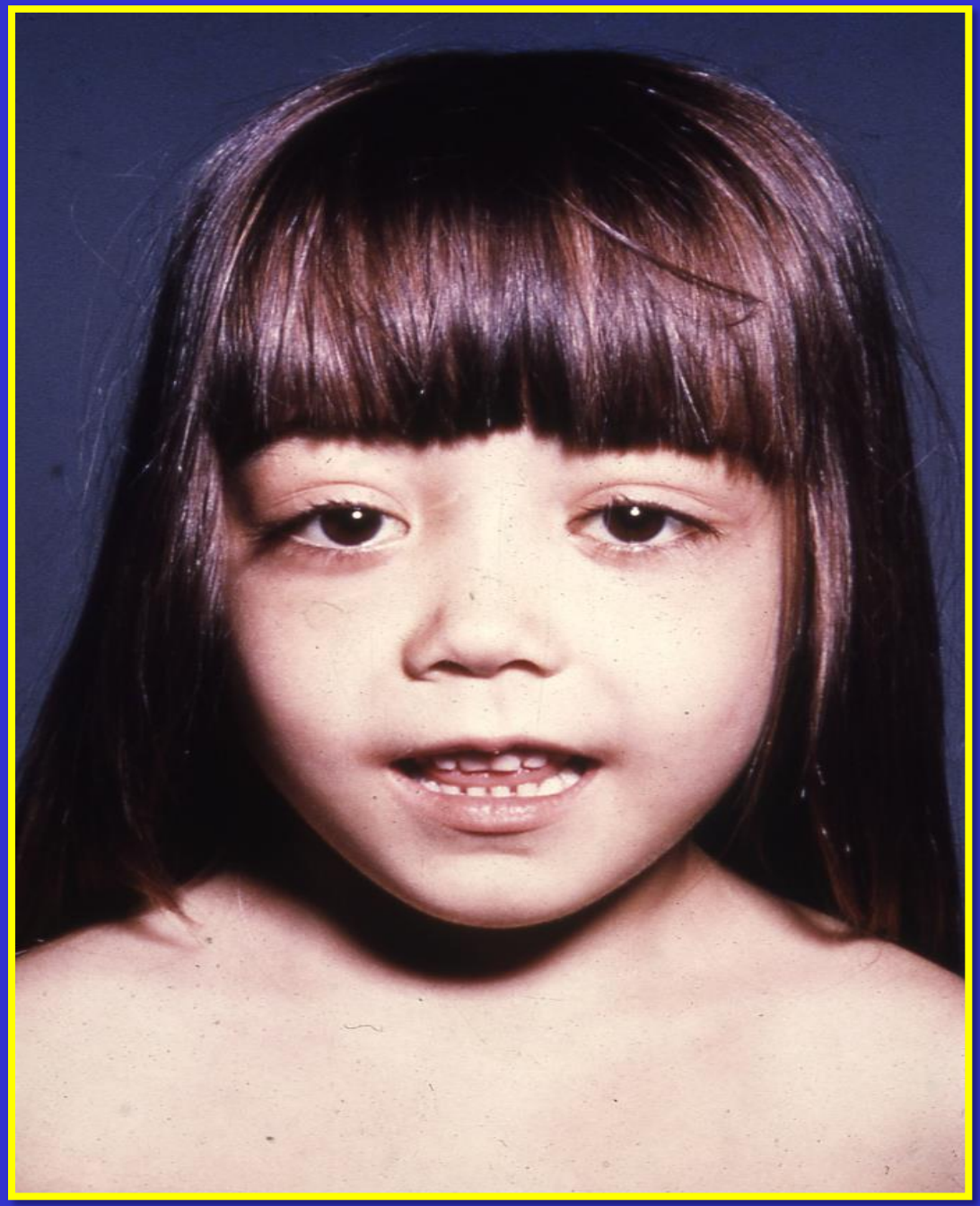
Clinical Manifestations in Thalassaemias

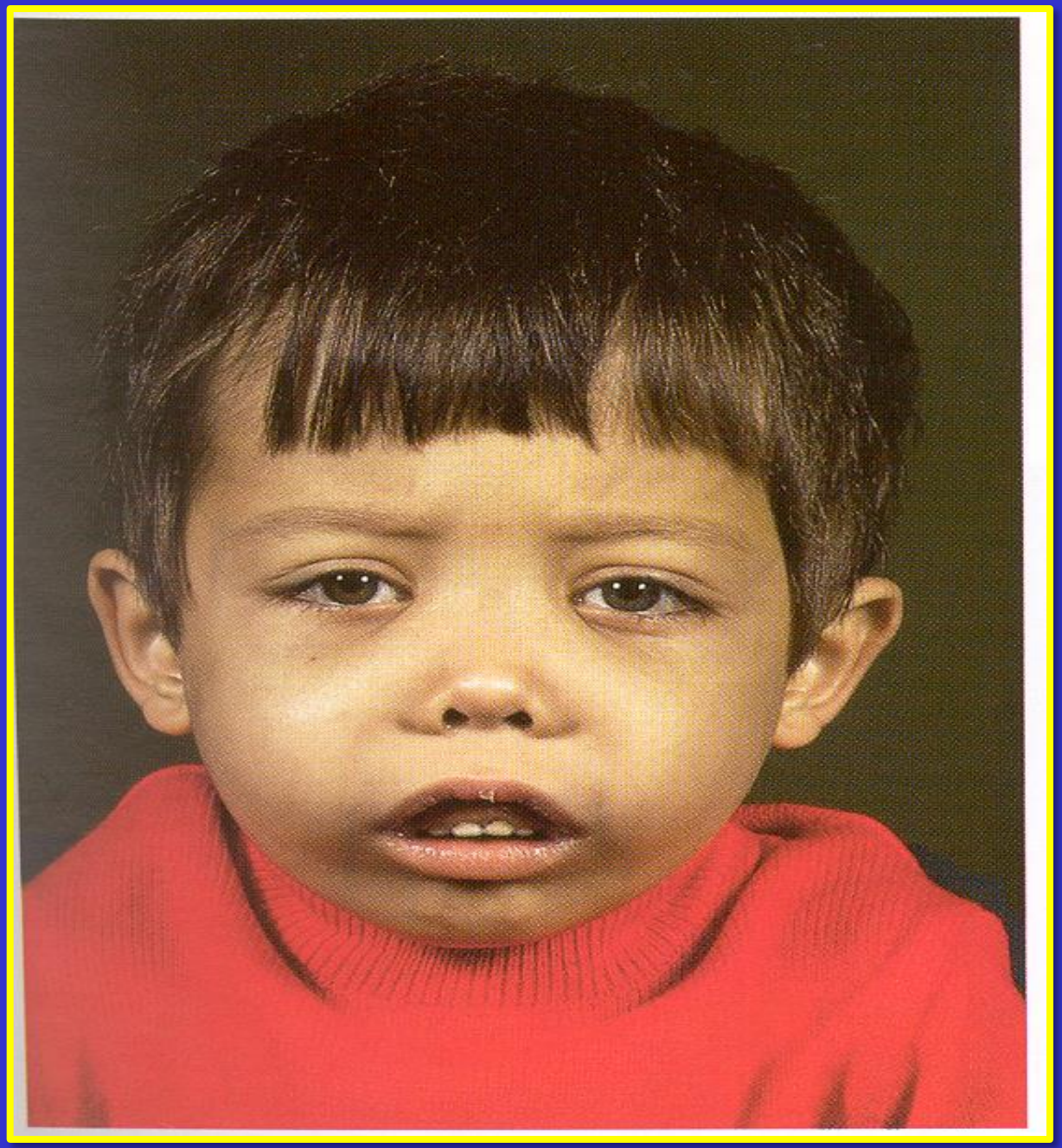
- **Pallor**
- **Jaundice**
- **Apathy and Anorexia**
- **Failure to Thrive**
- **Hepato-splenomegaly**
- **Skeletal Deformity**
- **Iron Overload mainfestations**

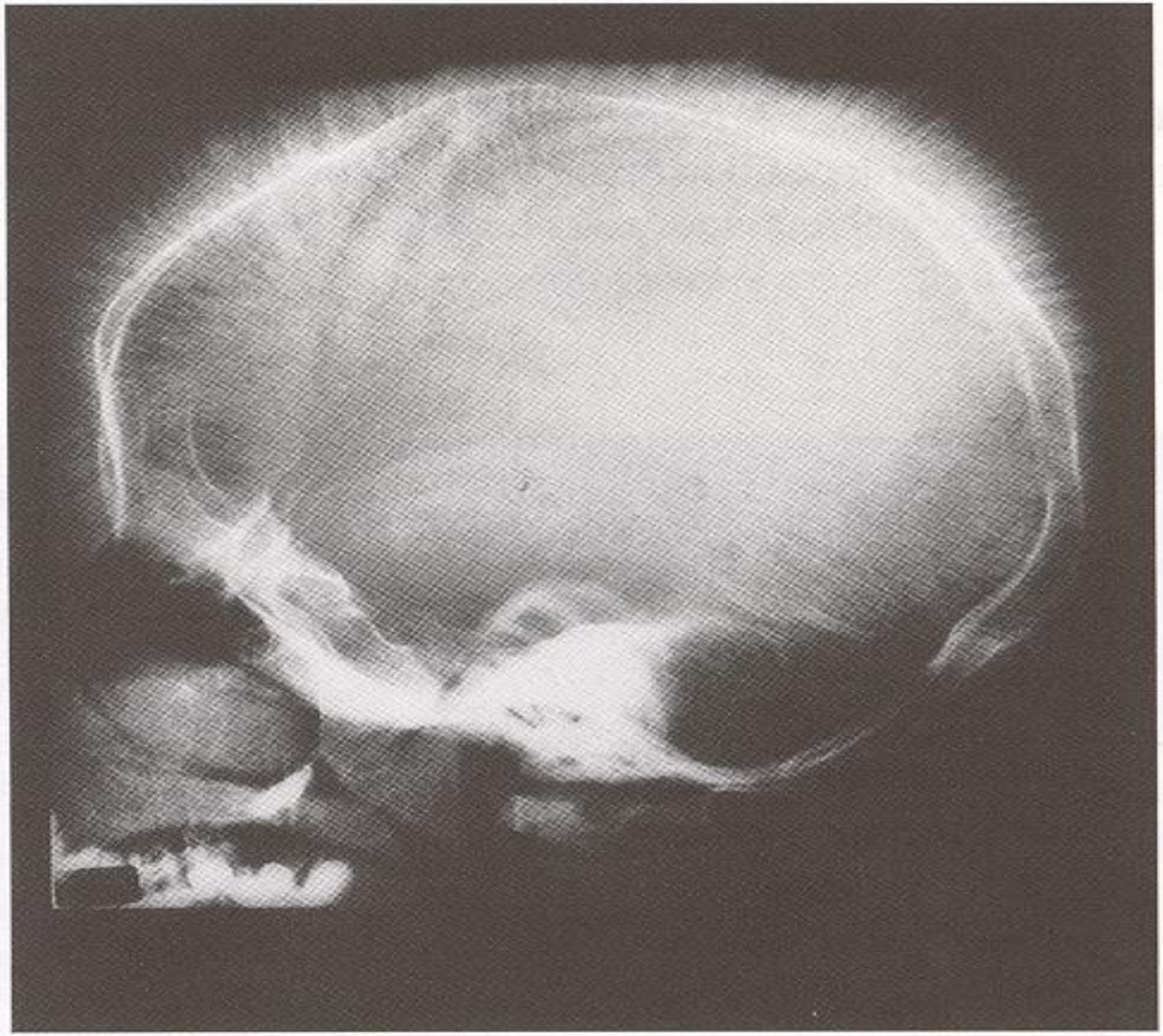


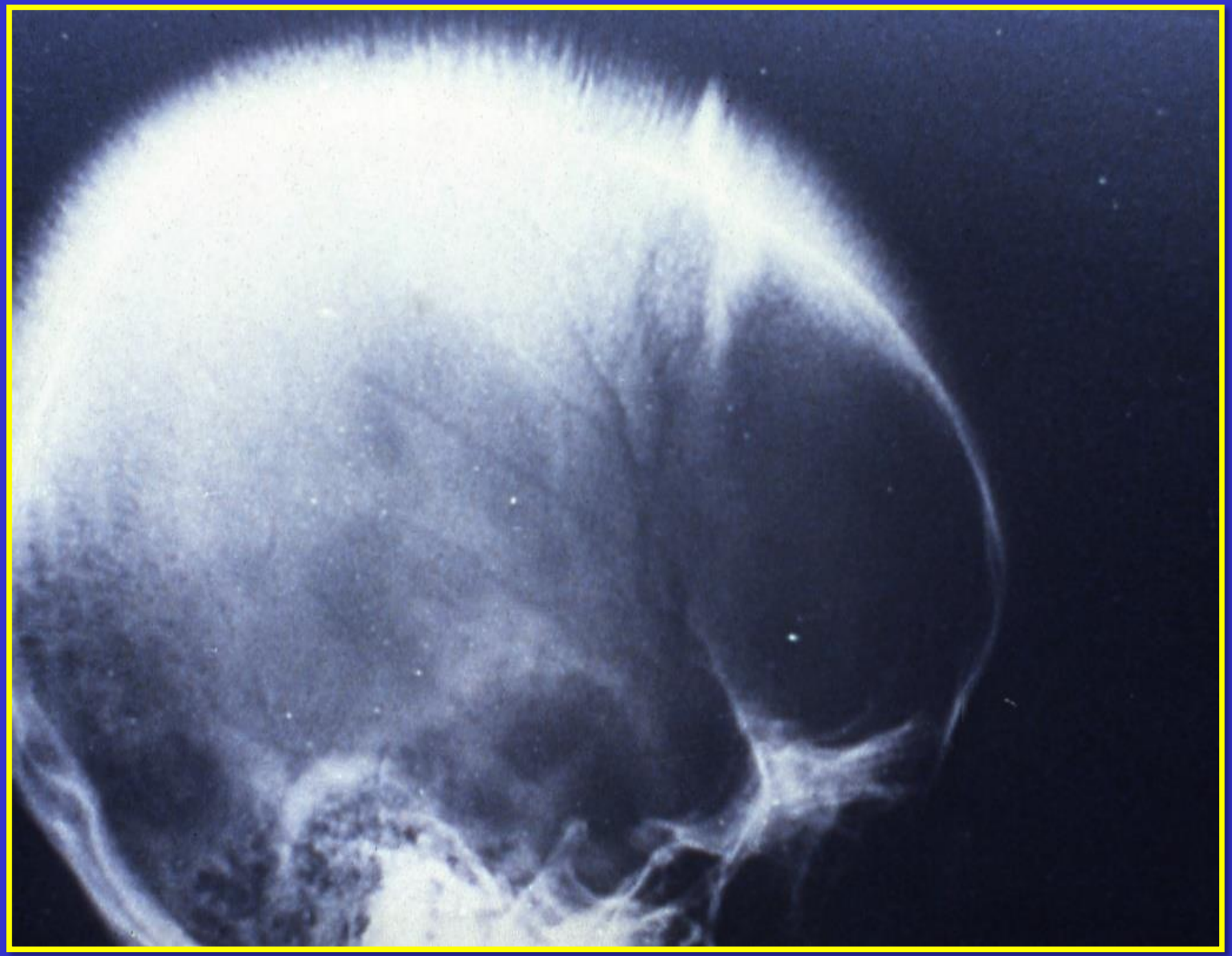


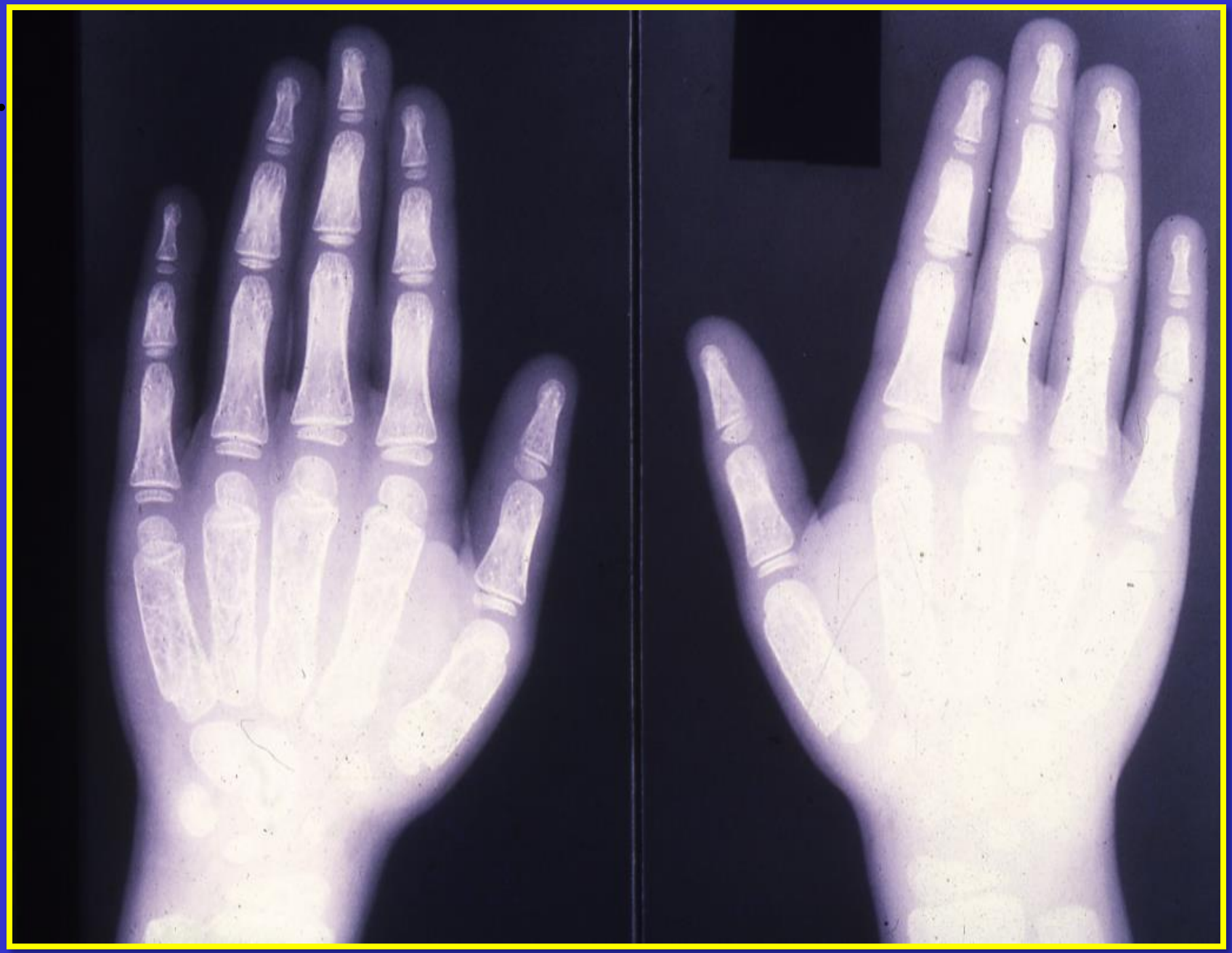


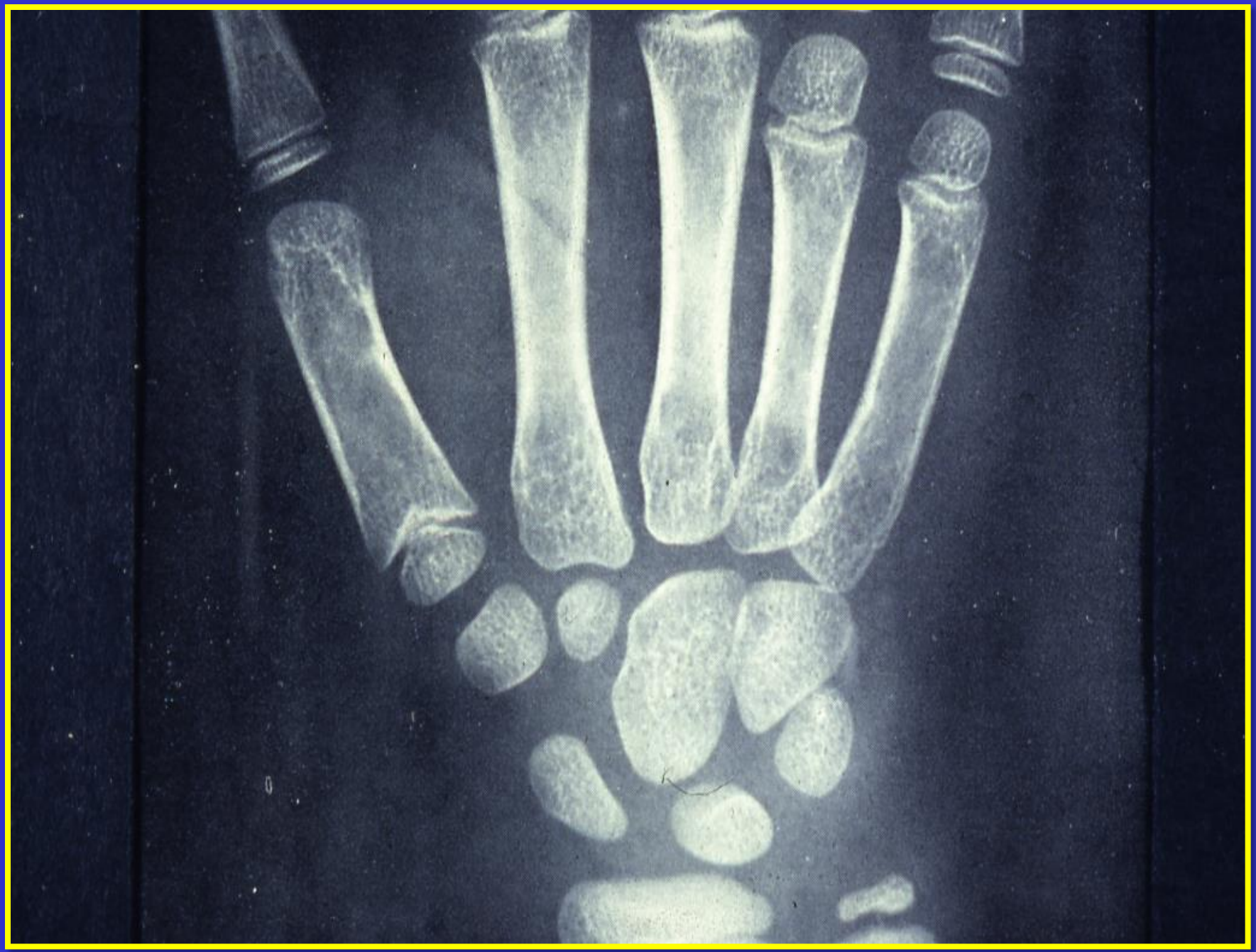












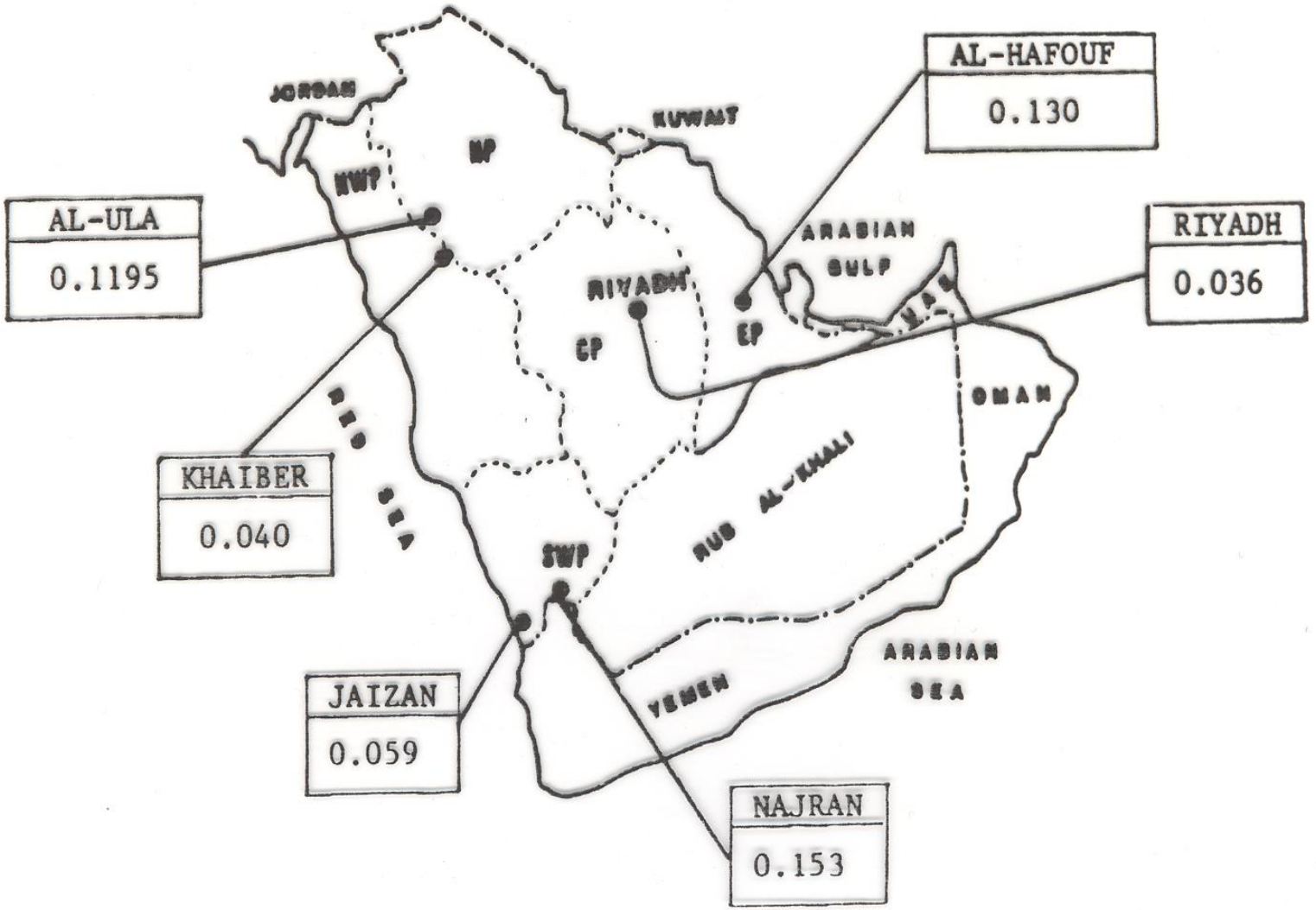
Clinical and Hematological Features of the β-Thalassemia Syndrome

	Major	Intermedia	Minor	Minima
Severity of mainfestations	++++	++	+, ±	±, 0
Genetics	Homozygotes, double heterozygotes	Homozygotes, double heterozygotes, rarely heterozygotes	Heterozygotes	Heterozygotes
Splenomegaly	++++	++,+++	+,0	0
Jaundice	+++	++,+	0	0
Skeletal changes	++++,++	+,0	+,0	0
Anemia (Hb, g/dl)	<7	7 – 10	>10	Normal

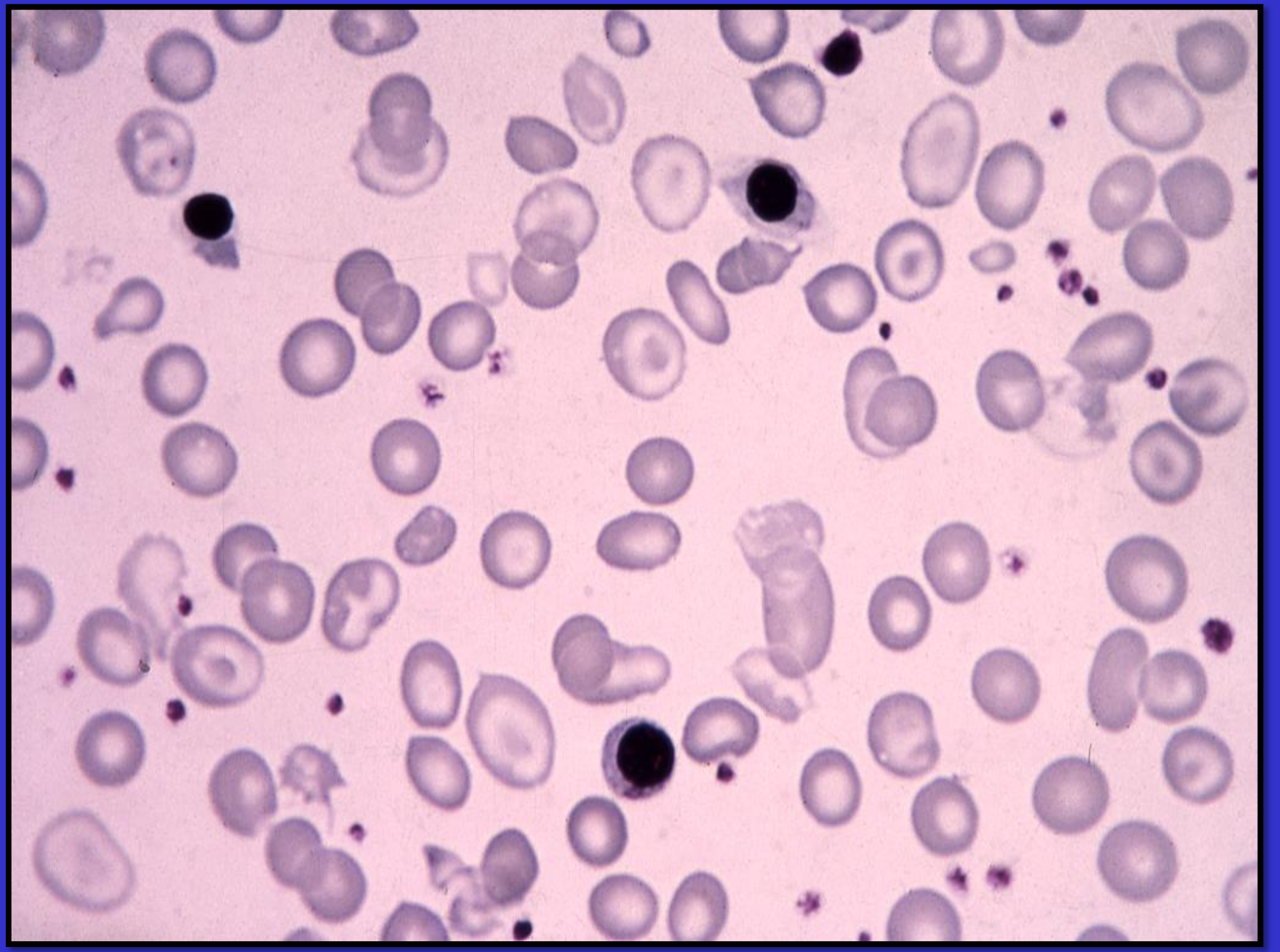
±, little or no abnormality; +, mild abnormality; ++++, prominent abnormality

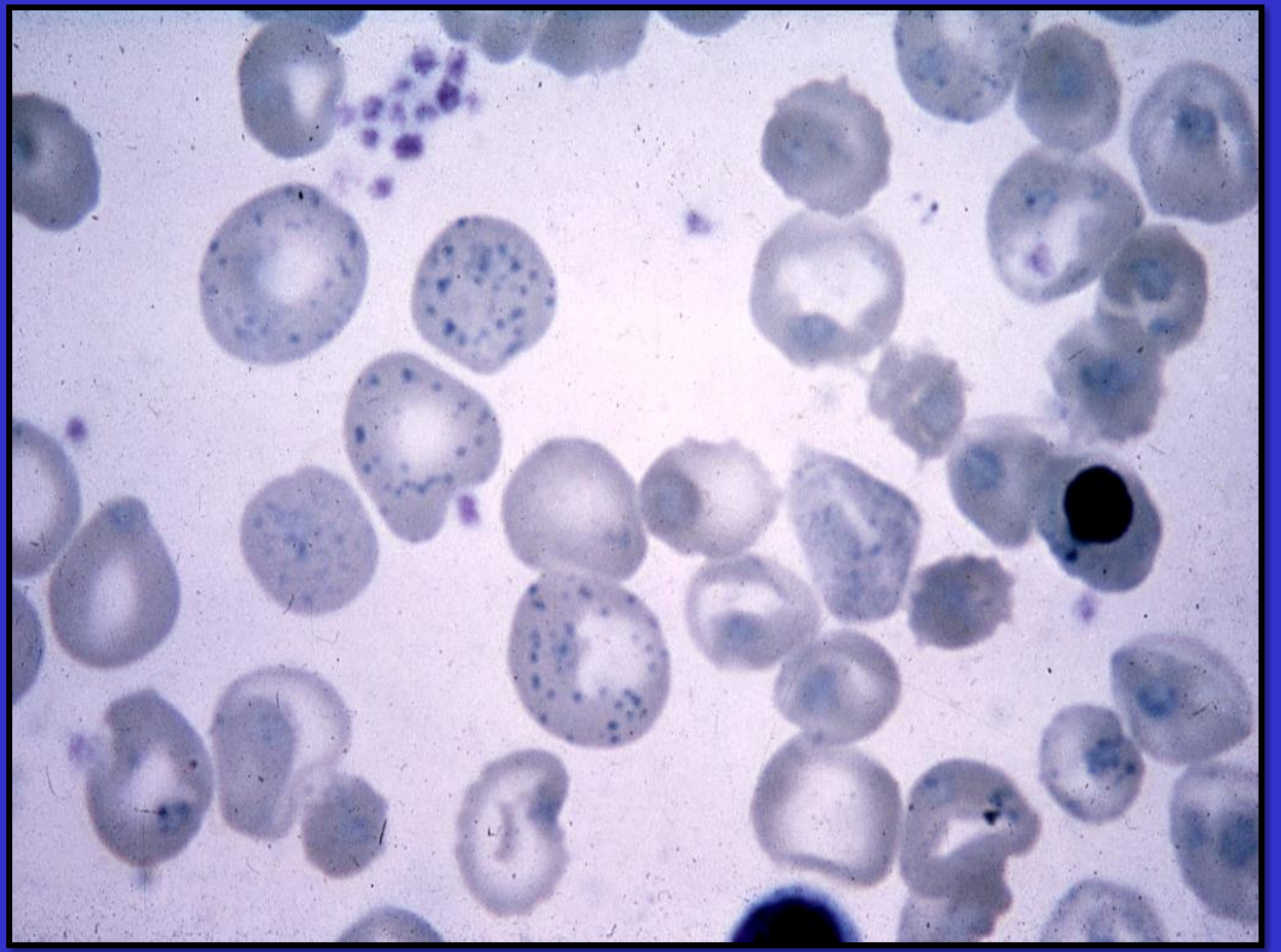
Clinical and Hematologic Features of the β-Thalassemia Syndrome (Continued)

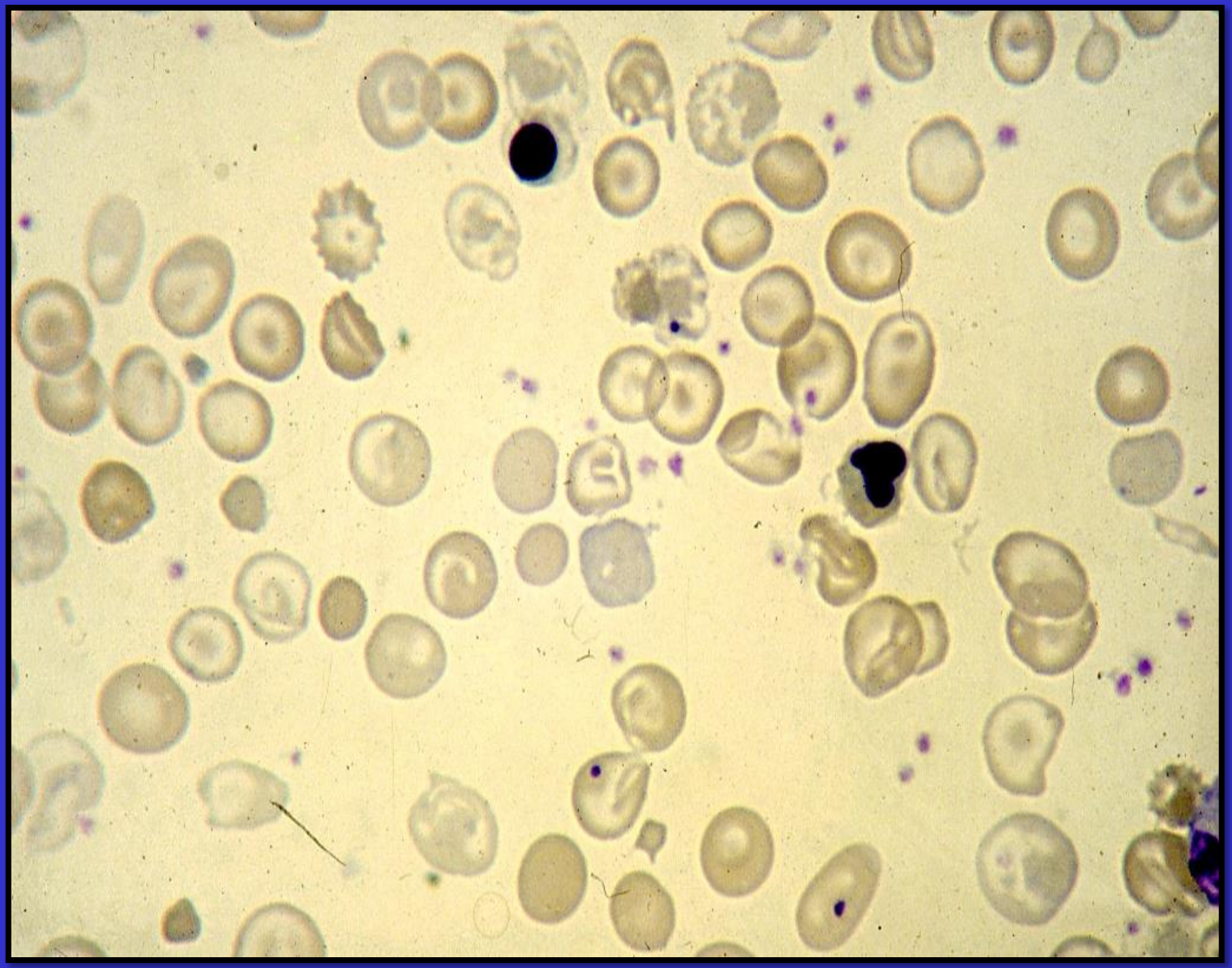
	Major	Intermedia	Minor	Minima
Hypochromia	++++	+++	++	+
Microcytosis	+++	++	+	0
Target cells	10 – 35%	++	+	±
Basophilic stippling	++	+	+	0, +
Reticulocytes (%)	5 – 15	3 – 10	2 – 5	1 – 2
Nucleated red cells	+++	+, 0	0	0
±, little or no abnormality; +, mild abnormality; ++++, prominent abnormality				

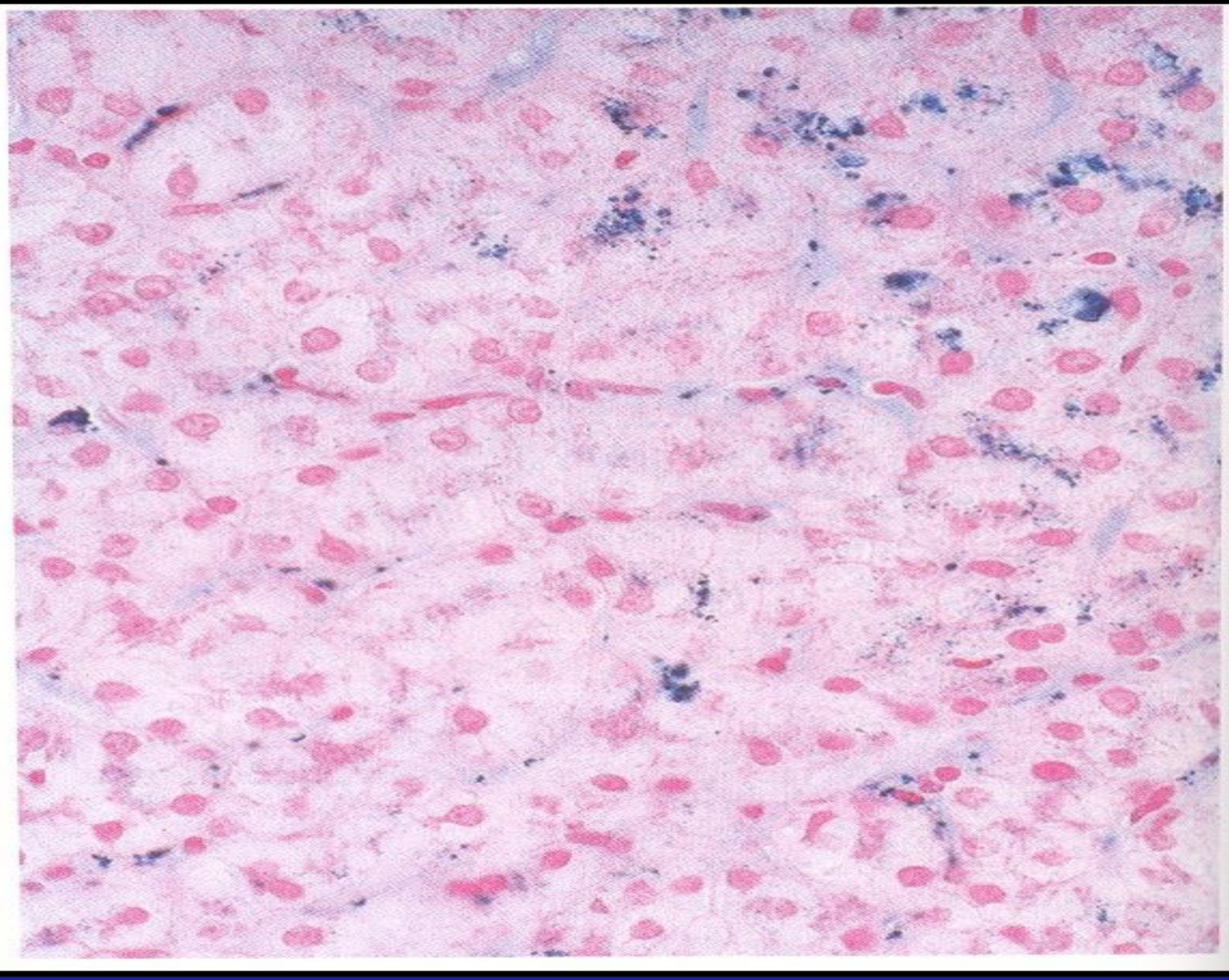


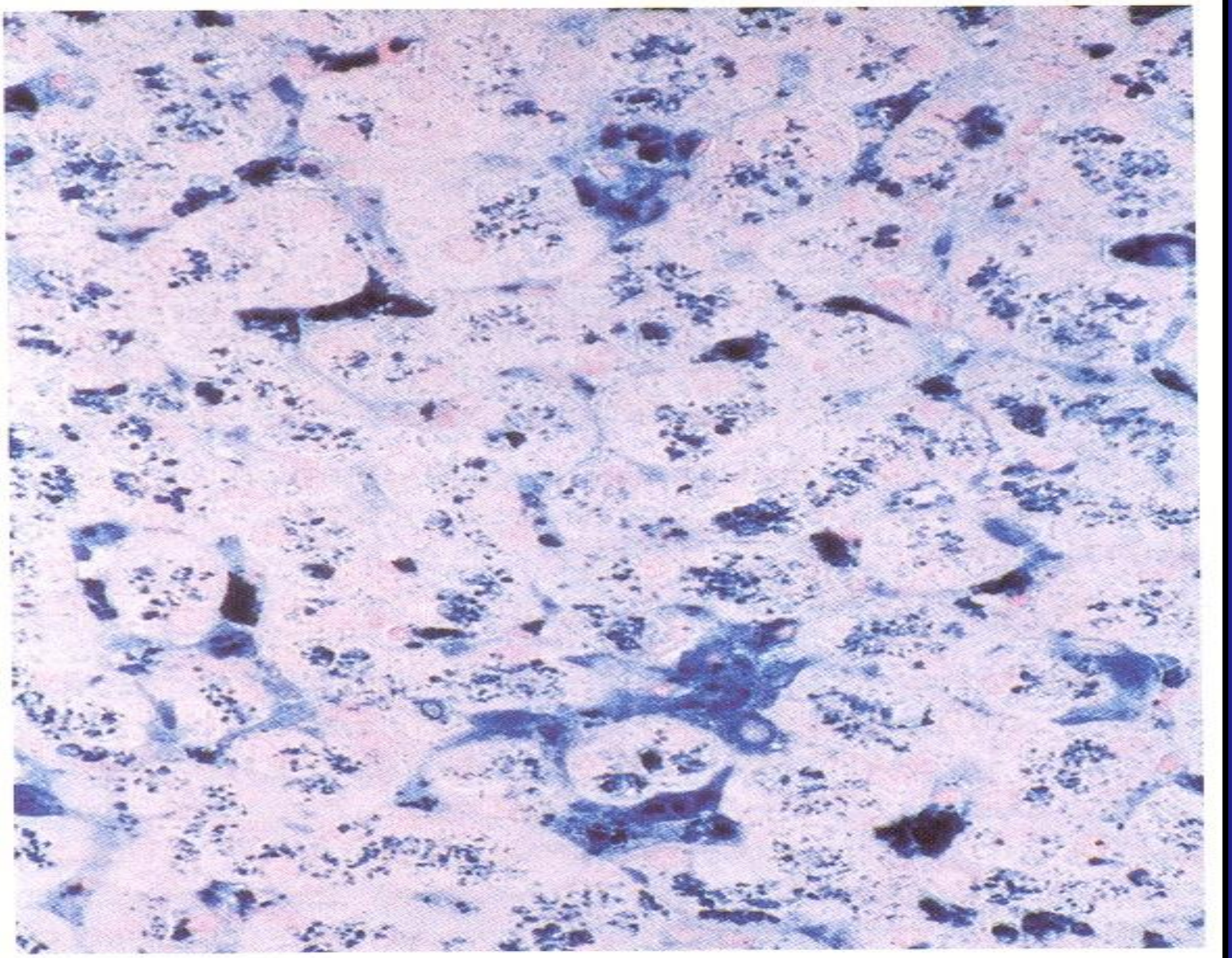
Frequency of β-thalassaemia in different regions of Saudi Arabia. (From Ref. No. 20.) (No. investigated: Al-Hafouf 300; Riyadh 250; Al-Ula 427; Khaiber 500; Jizan 1271; Najran 301.) $f = 8.8353$; $df = 10$; $p < 0.01$





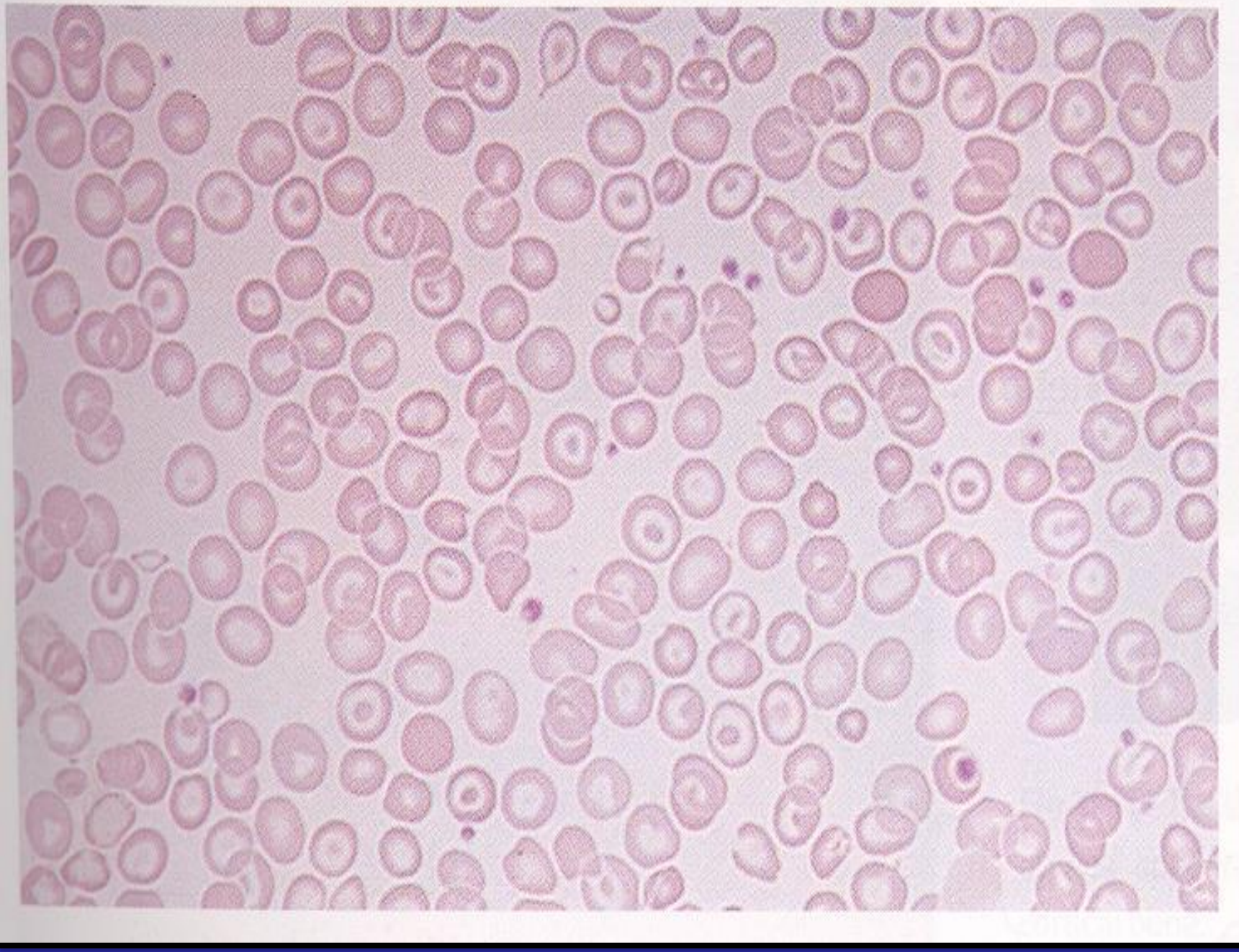






Laboratory Features of Beta Thalassemia Trait

- Mild hypochromic microcytic anemia with target red cells in the blood film.
- Raised red cells count.
- Raised Hb A2 level.
- Normal serum iron or low in children.
- Normal TIBC or raised in children.
- Normal red cell distribution width (RDW)
- Genetic study is required in difficult cases



ING KHALID HOSP.
O BOX 7805 RIYADH

HEMATOLOGY UNIT

Page No.:1

Pat.No [redacted]
Name: [redacted]
Hospital:KING KHALID UNIVERSITY HOSPITA DOB: [redacted]
Location: (PCF01) PCC (Female)
Doctor:UNKNOWN *

ref:
eq No.:H02022419 Date Coll.:04/01/23(18/03/02) Date Recd.:04/01/23(18/03/02)
rinted:09/01/1423(23/03/02)08:32 Time Recd.:10:30

DTA Whole Blood

	Full Blood Count				
[*]	WBC	5.60		4 - 11	x10.e9/L
[ ]>	RBC	5.67	H	4.2 - 5.5	x10.e12/L
<[ ]	HGB	98	L	120 - 160	g/L
<[ ]	HCT	31.0	L	37 - 47	%
<[ ]	MCV	54.6	L	80 - 94	fl
<[ ]	MCH	17.3	L	27 - 32	pg
<[ ]	MCHC	315	L	320 - 360	g/L
[ ]>	RDW	15.6	H	11.5 - 14.5	%
[*]	PLT	426		140 - 450	x10.e9/L
[*]	MPV	7.9		7.2 - 11.1	fl
<[ ]	PDW	15.6	L	20 - 70	%
[ ]>	PCT	0.339	H	0.150 - 0.32	%
	Differential				
[*]	%NEUT	74		40 - 75	%
<[ ]	%LYMP	19	L	20 - 45	%
<[ ]	%MONO	2	L	3 - 9	%
[*]	%EOS	5		0 - 6	%
[*]	#NEUT	4.14		2 - 7.5	x10.e9/L
[*]	#LYMP	1.06		1 - 5	x10.e9/L
<[ ]	#MONO	0.11	L	0.2 - 0.8	x10.e9/L
[*]	#EOS	0.28		0.0 - 0.8	x10.e9/L
	Morophology				
	Flag Comments	3+ ,3+			
	Flag Comment 1				
	ANISO				
	MICRO	MK			
	MACRO				
	POIKILO				
	HYPO	MK			
	Polychromasia				
	LSHIFT				
	TARGET CELLS	SL			
	Ovalocytes	SL			
[*]	Retic Count	1.4		0.2 - 2.0	%
[ ]>	ESR	35	H	3 - 9	mm/hr

NG KHALID HOSPITAL | DEPARTMENT OF SPECIAL BIOCHEMISTRY
BOX 7805 RIYADH |==========

|Hosp No. Page No.:1
|Patient: AA
|Hosp Srce:KING KHALID UNIVERSITY HOSPIT DOB: 1
|Location: (EHC) Employee Health Clinic
ef: |Doctor:UNKNOWN *
No.:S0202265 Date Coll.:04/01/23(18/03/02) Date Recd.:04/01/23(18/03/02)
inted:09/01/1423(23/03/02)08:34 Time Recd.:10:51

ious Blood

Hemoglobin Electrophoresis

95 - 99	%	<[ ]	Hemoglobin A	93.5	L
0 - 2.0	%	[*]	Hemoglobin F	2.0	
2.0 - 3.5	%	[ ]>	Hemoglobin A2	4.5	H
			Hemoglobin S	0.0	
			Hemoglobin E	0.0	
			Hemoglobin C	0.0	
	%		Hemoglobin O	0.0	

PREMARITAL SCREENING

- CBC and Differential count
- Reticulocytes count
- Sickle cell solubility test
- Hb electrophoresis
- Virology study for hepatitis B, C, HIV by PCR

Kingdom of Saudi Arabia
Ministry of Higher Education
King Saud University
College of Medicine
& king Khalid University Hospital

المملكة العربية السعودية
وزارة التعليم العالي
جامعة الملك سعود
كلية الطب
ومستشفى الملك خالد الجامعي

نموذج فحص ما قبل الزواج

تاريخ سحب العينة : / / ١٤هـ رقم الملف الطبي: ..... رقم المختبر .....

اسم الطبيب المعالج: ..... رقم التحويلة/النداء ..... العيادة .....

البيانات الشخصية:

الاسم ..... الجنسية: ..... العمر: ( ) الجنس : ☐ ذكر ☐ أنثى.

رقم السجل المدني/الإقامة: ..... العنوان: ..... الهاتف: .....

الفحوصات المطلوبة:

١- تعداد الدم الكامل (CBC). ٢- اختبار الخلايا المنجلية (Sickling).

٣- الرحلان الكهربي لخضاب الدم (Hb Electrophoresis). ٤- اختبارات أخرى (Other Tests).

LABORATORY RESULT

TEST	NORMAL RANGE	RESULT	REMARKS
RBCX10^{12}/L	M:4.7 – 6.1 ….F:4.2-5.5		
HBg/dL	M:13 –18 ….F:12-16		
Het%	M:42 – 52 ….F:37- 47%		
MCV fL	80 – 94		
MCH pg	27 – 32		
MCHCg/dL	32 – 36		
RDW	11.5 – 14.5%		
Retic	0.5 – 2%		
Sicking Test	Positive or Negative		
Hb A	95 – 97%		
Hb A2	2.0 – 3.5%		
Hb F	<1.5%		
Abnormal Hemoglobin			
TEST	PATIENT RESULT	HEMOGLOBIN	PATIENT RESULT
Hb S		Hb J	
Hb C		Hb O – Arab	
Hb D		Hb H	
Hb E		Hb Barts	
Hb G		Other Test	
Other Hb			

المشرف الفني بالوحدة: .....

ملاحظات: ..... COMMENTS:.....

.....

.....

استشاري أمراض الدم بالمختبر: ..... التوقيع: .....

✱ **ملاحظة هامة:** هذه الشهادة تبين نتيجة الفحص المخبري لمرضي الأنيميا المنجلية والثلاسيميا فقط، ولا تشمل أي أمراض وراثية أخرى للطرفين المعنيين.

Kingdom of Saudi Arabia
Ministry of Higher Education
King Saud University
College of Medicine
& king Khalid University Hospital

المملكة العربية السعودية
وزارة التعليم العالي
جامعة الملك سعود
كلية الطب
ومستشفى الملك خالد الجامعي

نموذج فحص ما قبل الزواج

تاريخ سحب العينة : / / ١٤ هـ رقم الملف الطبي: رقم المختبر .....

اسم الطبيب المعالج: .. رقم التحويلة/النداء ..... العيادة .....

البيانات الشخصية:

الاسم .. الجنس .. العمر: ( ) الجنس : ☐ ذكر ☐ أنثى

رقم السجل المدني/الإقامة: ..... العنوان: ..... الهاتف: .....

الفحوصات المطلوبة:

١ – تعداد الدم الكامل (CBC). ٢ – اختبار الخلايا المنجلية (Sickling).

٣ – الرحلان الكهربي لخضاب الدم (Hb Electrophoresis). ٤ – اختبارات أخرى (Other Tests).

LABORATORY RESULT

TEST	NORMAL RANGE	RESULT	REMARKS
RBCX10^{12}/L	M:4.7 – 6.1 ....F:4.2-5.5	4.5	
HBg/dL	M:13 –18 ....F:12-16	12.9	
Het%	M:42 – 52 ....F:37- 47%	37.8	
MCV fL	80 – 94	83.9	
MCH pg	27 – 32	28.6	
MCHCg/dL	32 – 36	34.1	
RDW	11.5 – 14.5%	13.6	
Retic	0.5 – 2%	–	
Sicking Test	Positive or Negative	Negative	
Hb A	95 – 97%	96.9	
Hb A2	2.0 – 3.5%	2.6	
Hb F	<1.5%	<0.5	
Abnormal Hemoglobin			
TEST	PATIENT RESULT	HEMOGLOBIN	PATIENT RESULT
Hb S		Hb J	
Hb C		Hb O – Arab	
Hb D		Hb H	
Hb E		Hb Barts	
Hb G		Other Test	
Other Hb			

المشرف الفني بالوحدة: .....

ملاحظات: سالمة من أمراض الدم الوراثية

COMMENTS: .....

استشاري أمراض الدم بالمختبر: ..... التوقيع: 6-2-26

✱ **ملاحظة هامة:** هذه الشهادة تبين نتيجة الفحص المخبري لمرضي الأنيميا المنجلية والثلاسيميا فقط، ولا تشمل أي أمراض وراثية أخرى للطرفين المعنيين.

Prenatal diagnosis of the haemoglobinopathies (Including thalassaemia)

DNA Analysis

A. Chorionic villus sampling

Transcervical approach (9 – 11 weeks of pregnancy)

Transabdominal approach (up to 15 weeks of pregnancy)

B. Amniotic fluid cell analysis (16 – 20 weeks gestation)

C. Fetal blood sampling (> 20 weeks gestation)

DNA analysis

Haematological parameters

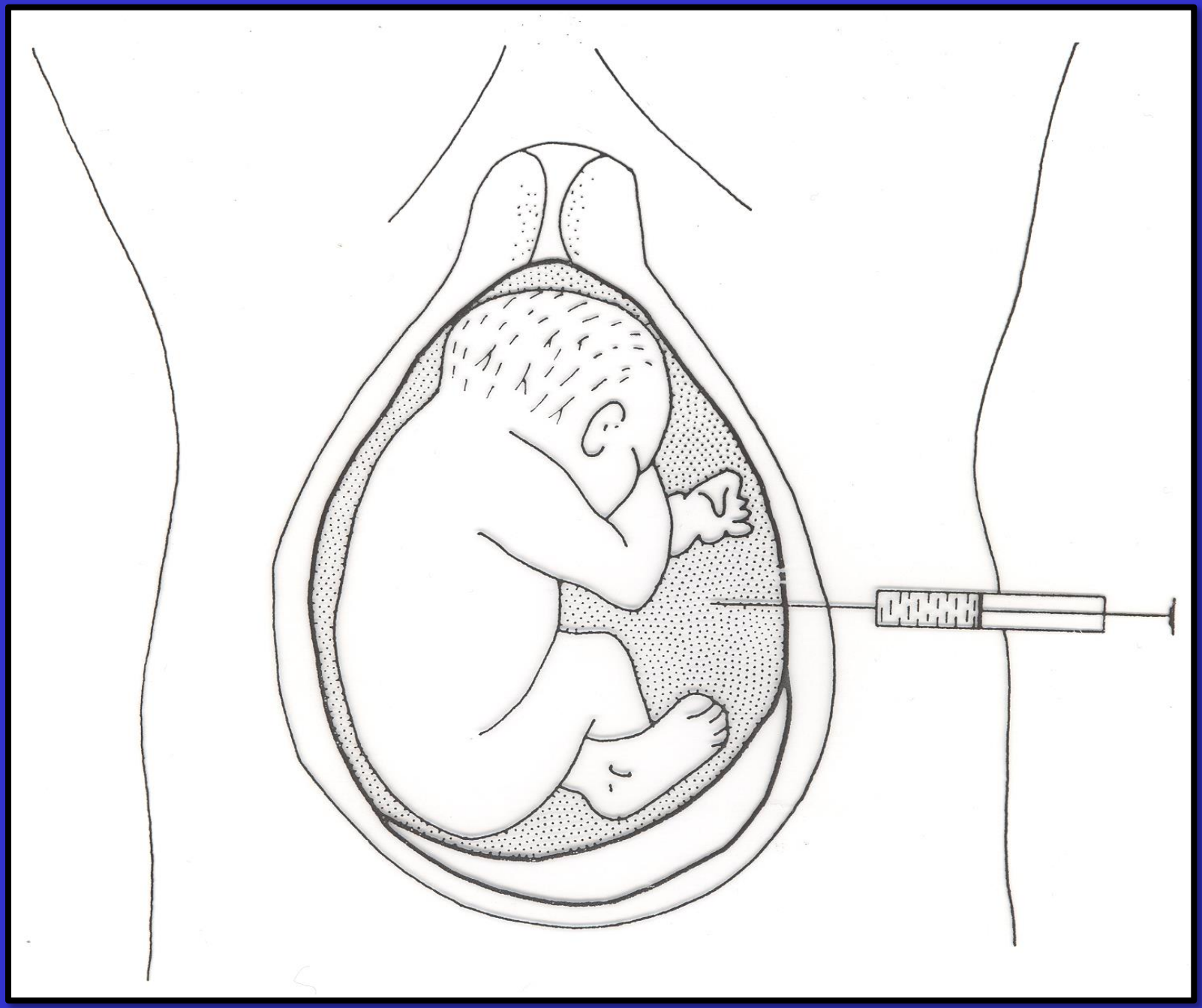
Biochemical analysis

Globin chain synthesis

α/β Ratio

α/γ Ratio

α/δ Ratio



DNA ANALYSIS

1. Gene mapping
2. RFLPs linkage analysis
   (Restriction fragment length polymorphisms)
3. Oligonucleotide probes
   (Using short gene probes 17 – 19 Nucleotide)
4. Gene amplification
   (Enzymatic amplification of DNA sequences)

   DNA polymerase chain reaction technique.

MANAGEMENT OF THE THALASSEMIAS

- **Blood Transfusion**
- **Iron chelation therapy**
- **Splenectomy**
- **Hormone replacement**
- **Bone marrow transplantation**
- **Gene therapy**

SUMMARY OF RECOMMENDATIONS FOR THE TREATMENT OF THALASSEMIA MAJOR

TRANSFUSION

Transfusion, in the absence of cardiopathy:

- **Blood-type the patient completely;**
- **Vaccinate hepatitis B negative patients against hepatitis;**
- **Transfuse when the Hb remains consistently below 8 g/dL, or earlier if there are other indications;**
- **Keep the pretransfusion Hb between 10.5 and 11 g/dL;**
- **Give 10-15 mL/kg of blood preparation in 2 h;**
- **Do not raise the posttransfusion Hb above 16 g/dL;**
- **Choose a 3-4 week transfusion interval.**

SUMMARY OF RECOMMENDATIONS FOR THE TREATMENT OF THALASSEMIA MAJOR (Continued)

TRANSFUSION

Transfusion in the presence of cardiopathy, or when the Hb is less than 5 g/dL:

- **Inject furosemide 1-2 mg/kg;**
- **Preferably use fresh blood;**
- **Do not transfuse more than 5 mL/kg of blood;**
- **Do not transfuse faster than 2 mL/kg, or for more than 4 h;**
- **If necessary, divide the blood among 2 or more bags;**
- **Use very short intertransfusion intervals.**

IRON CHELATION THERAPY

1) Desferrioxamine S.C. 20-60 mg/kg/day in 8 h (average 40 mg/kg/day, or 280 mg/kg/ week).
2) In selected subjects, give desferrioxamine i.v. in high dose, maximum 100 mg/kg over 8 h, only on the days of transfusion.

SPLENECTOMY

1) Is indicated when the blood consumption is more than 1.5 times normal.
2) Give anti-pneumococcal vaccine to children more than 2 years old prior to splenectomy.
3) Inform the patients and their family doctors of increased risk of serious infections.
4) Give prophylactic penicillin, and a platelet anti-aggregant when there is thrombocytosis.

INVESTIGATIONS

Prior to treatment:	**Study the case, and do complete red cell typing.**
Before each transfusion:	**Hb, cross-match and red cell antibody detection, serum transminases (in areas with a high incidence of hepatitis). Record the date of transfusion, net weight and mean hematocrit of the blood preparation, and the Hb of the patient**
After each transfusion:	**Measure the posttansfusion Hb.**
Every 3 months:	**Measure height and weight**
Every 6 months:	**Ferritin estimation.**
Every year:	**Evaluate growth and development.**
	Calculate the transfusion indices.
	Evaluate iron balance.
	Complete evaluation of the case.
Variable intervals:	**Cardiac and endocrinological investigations according to the clinical state of the patient.**

Diagnosis of Haemoglobinopathies including Thalassaemias

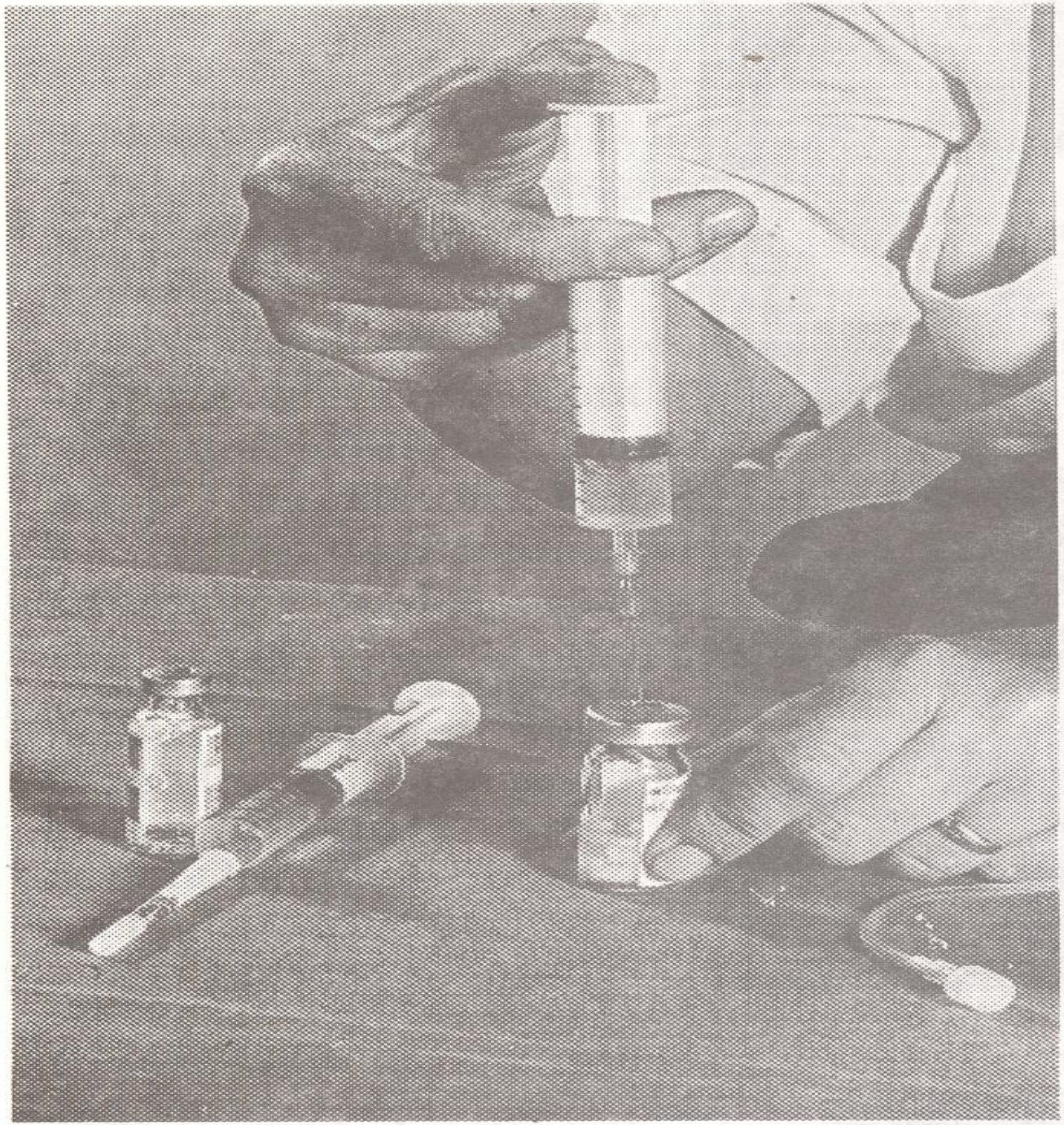
A. **Personal & Family History**

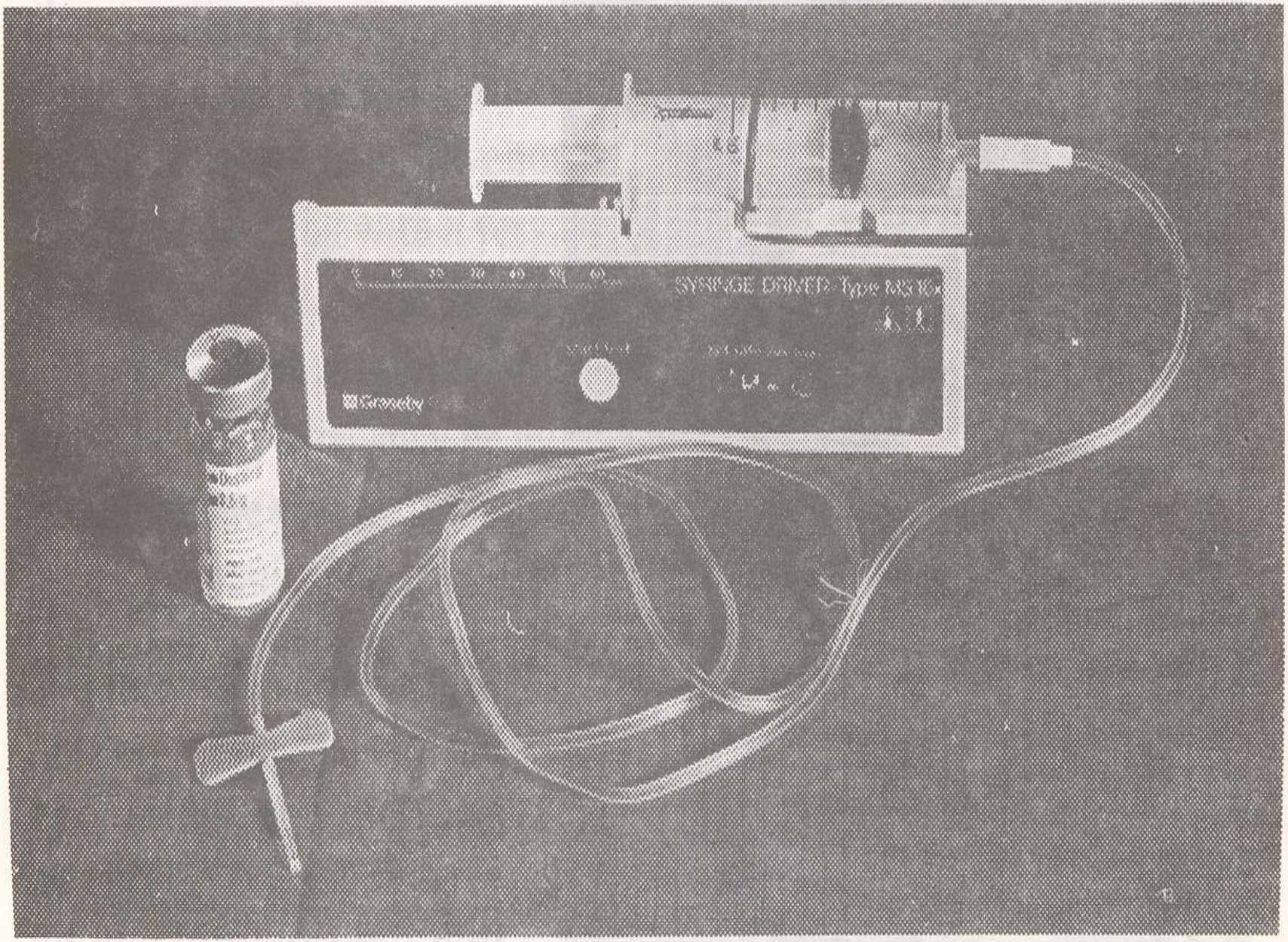
B. **Physical Examination**

C. **Laboratory Investigation**

1. Haematological Tests – CBC, Red cell indices, blood film Morphology, reticulocyte count.
2. Sickling Tests – Sickle cell test, Sickle cell solubility test.
3. Hb Electrophoresis at alkaline/acidic pH and quantitation.
4. Quantitation of HbA2 and HbF
5. Osmotic fragility test
6. Serum iron total iron binding capacity and ferritin level
7. Biochemical tests:

   Liver functions tests, renal function tests, blood gases and acid-base status, bone profile and urine analysis.
8. Special Tests
   - A. Family studies (Laboratory Investigations)
   - B. Measurement of Alpha/Non-Alpha chain ratio
   - C. Gene Studies





ORAL IRON CHELATION THERAPY

- **Deferiprone [Ferriprox]**
- **Oral Tablet or Syrup 5 to 10 mg /kg /day divided in 3 doses.**
- **More effective than desferoxamine in chelating cardiac iron.**
- **Total iron excretion with deferiprone is less than with desferoxamine.**
- **Major adverse effect especially in children include - Gastrointestinal symptoms, joint pain, liver disfunction, neuropenia in 27% of patients.**

ORAL IRON CHELATION THERAPY (*cont'd…*)

- ✓ **Deferasirox (EXJADE, NOVARTIS)**
- ✓ **The dose is 20-30 mg/kg/day once daily.**
- ✓ **Approved by FDA.**
- ✓ **Reduction of liver iron to 50%, reduction of serum ferritin to 70% after 1 year treatment.**

Side effects:

- **Nausea, vomiting, diarrhea, abdominal pain, skin rash.**
- **Mid increase in serum cratinine in 30% of patients as with Desferoxamine ocular and auditory disturbance have been reported.**
- **Increase in serum transaminases in 10% of patients.**
- **Reduction of the dose in steps 5-10mg/kg/day every 3-6 months depending on serum ferritin level.**

Assessment of Iron Stores

- Serum ferritin
- Serum iron and percentage saturation of transferrin (iron-binding capacity)
- Bone marrow biopsy (Perl's stain) for reticuloendothelial stores
- DNA test for mutation resulting in Cys282 Tyr in the HFE gene
- Liver biopsy (parenchymal and reticuloendothelial stores)
- Liver CT scan or MRI
- Cardiac MRI
- Desferrioxamine iron excretion test (chelatable iron)
- Repeated phelobotomy until iron deficiency occurs

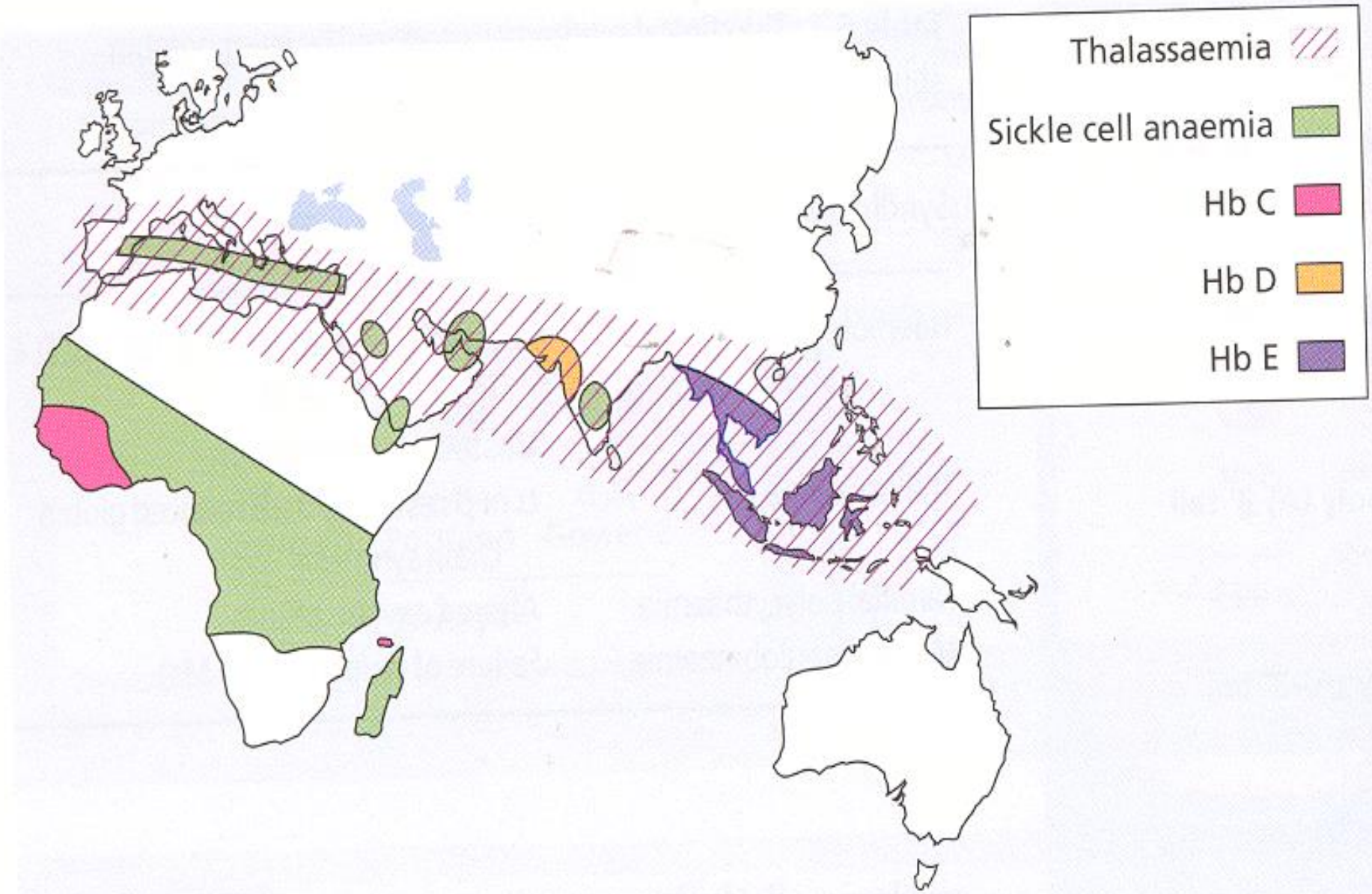
Assessment of tissue damage caused by iron overload

Cardiac	**Clinical; chest X-ray; ECG; 24-h monitor; echocardiography; radionuclide (MUGA scan to check left ventricular ejection fraction at rest and with stress**
Liver	**Liver function tests; liver biopsy; CT scan**
Endocrine	**Clinical examination (growth and sexual development) glucose tolerance test; pituitary gonadotrophin release tests; thyroid, parathyroid, gonadal, adrenal function, growth hormone assays; radiology for bone age; isotopic bone density study**

CT, computed tomography; ECG, electrocardiography; MRI, magnetic resonance imaging; MUGA, multiple gated acquisition.

Abnormal Haemoglobins (Haemoglobinopathies)

HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION



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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
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LYS-VAL-GLY-ALA-HIS-ALA-GLY-GLU-TYR-GLY-ALA-GLU-ALA-LEU-GLU

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LEU-THR-SER-LYS-TYR-ARG
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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

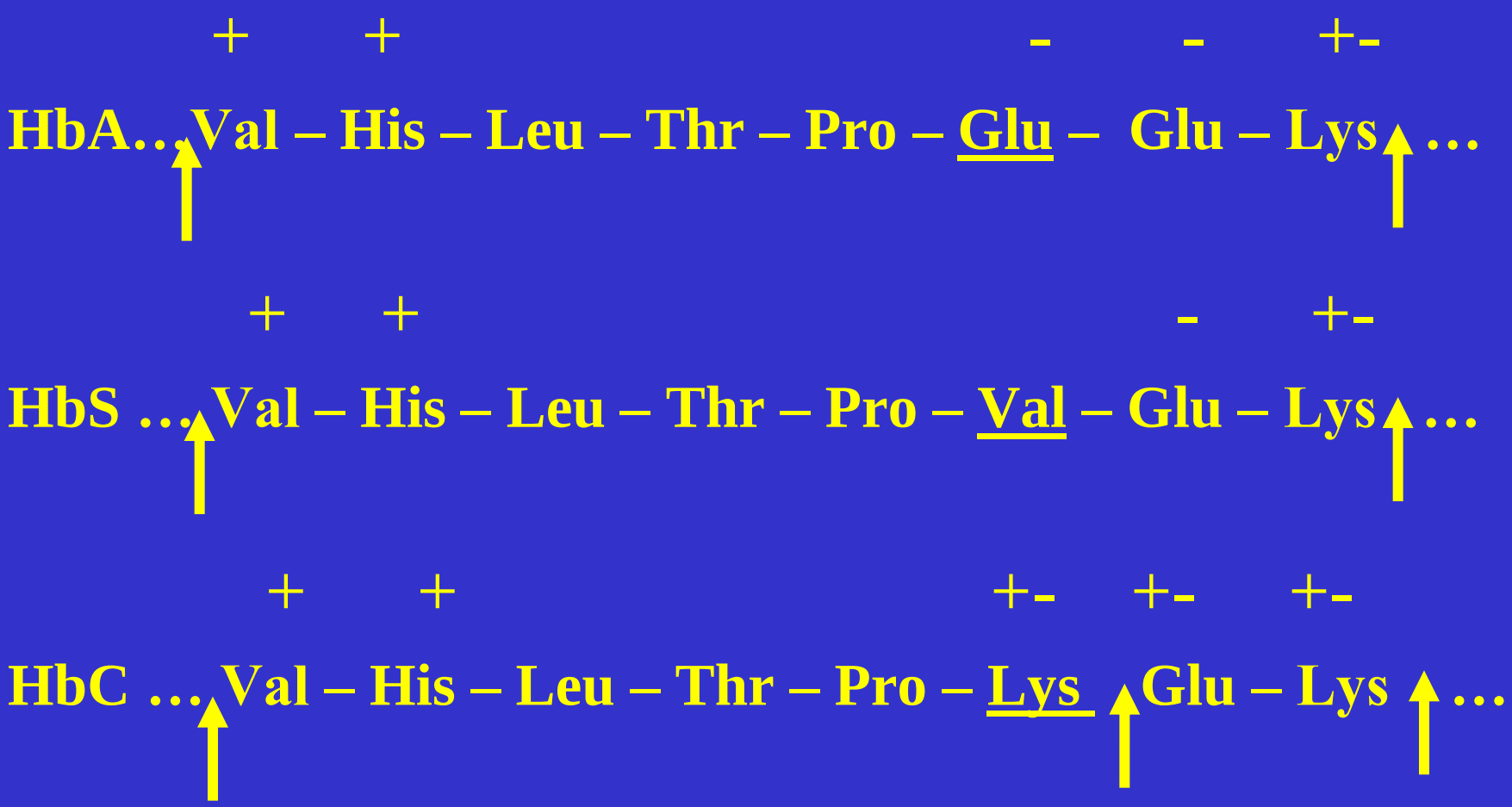
DNA Coding for the Amino-Acid in the sixth position in the β-chain

Normal

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

Sickle

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



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

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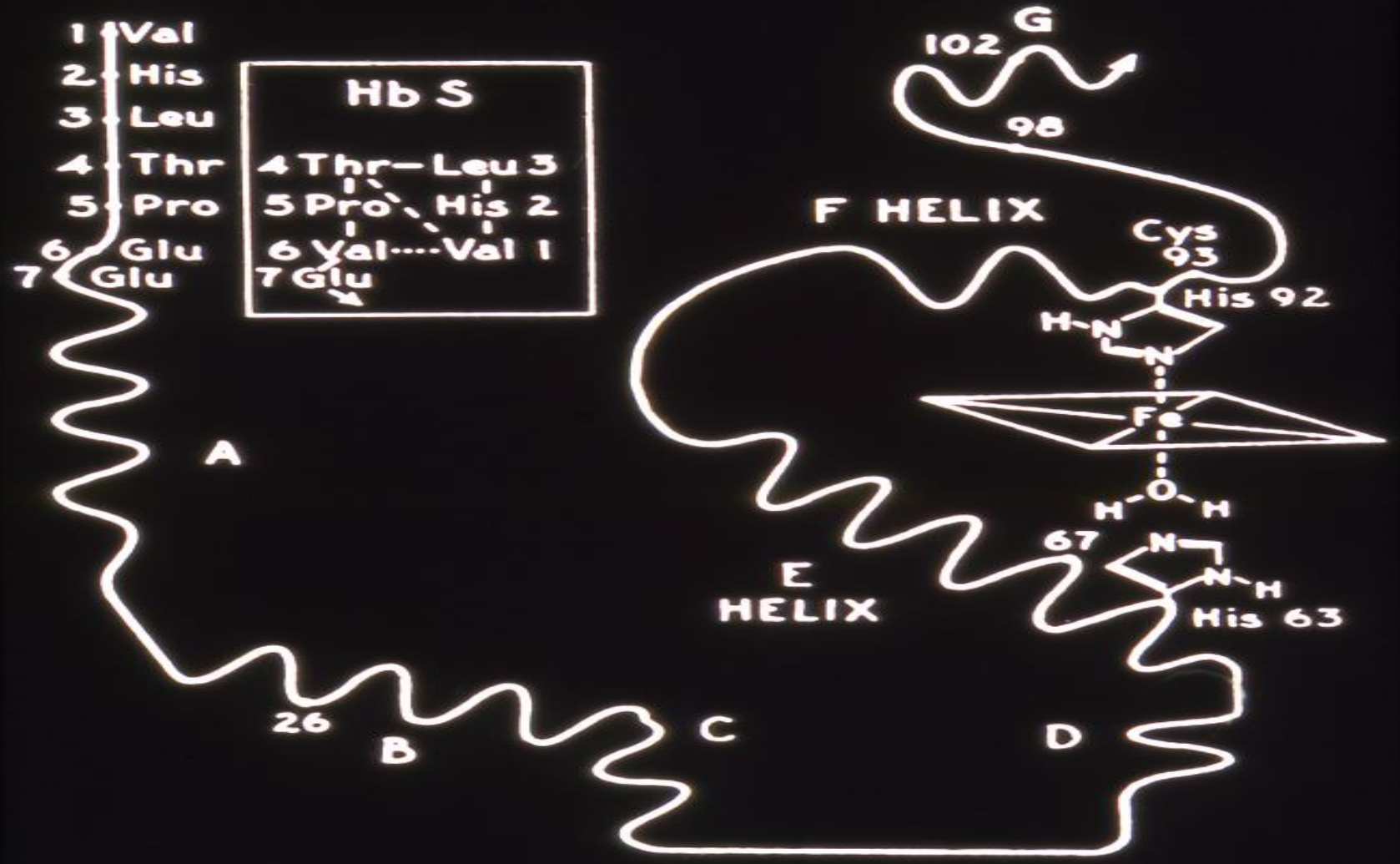
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2 His
3 Leu
4 Thr
5 Pro
6 Glu
7 Glu
Hb S
4 Thr — Leu 3
5 Pro — His 2
6 Val ···· Val 1
7 Glu
A
26
B
C
D
E
HELIX
F HELIX
G
102
98
Cys
93
His 92
Fe
67
His 63

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

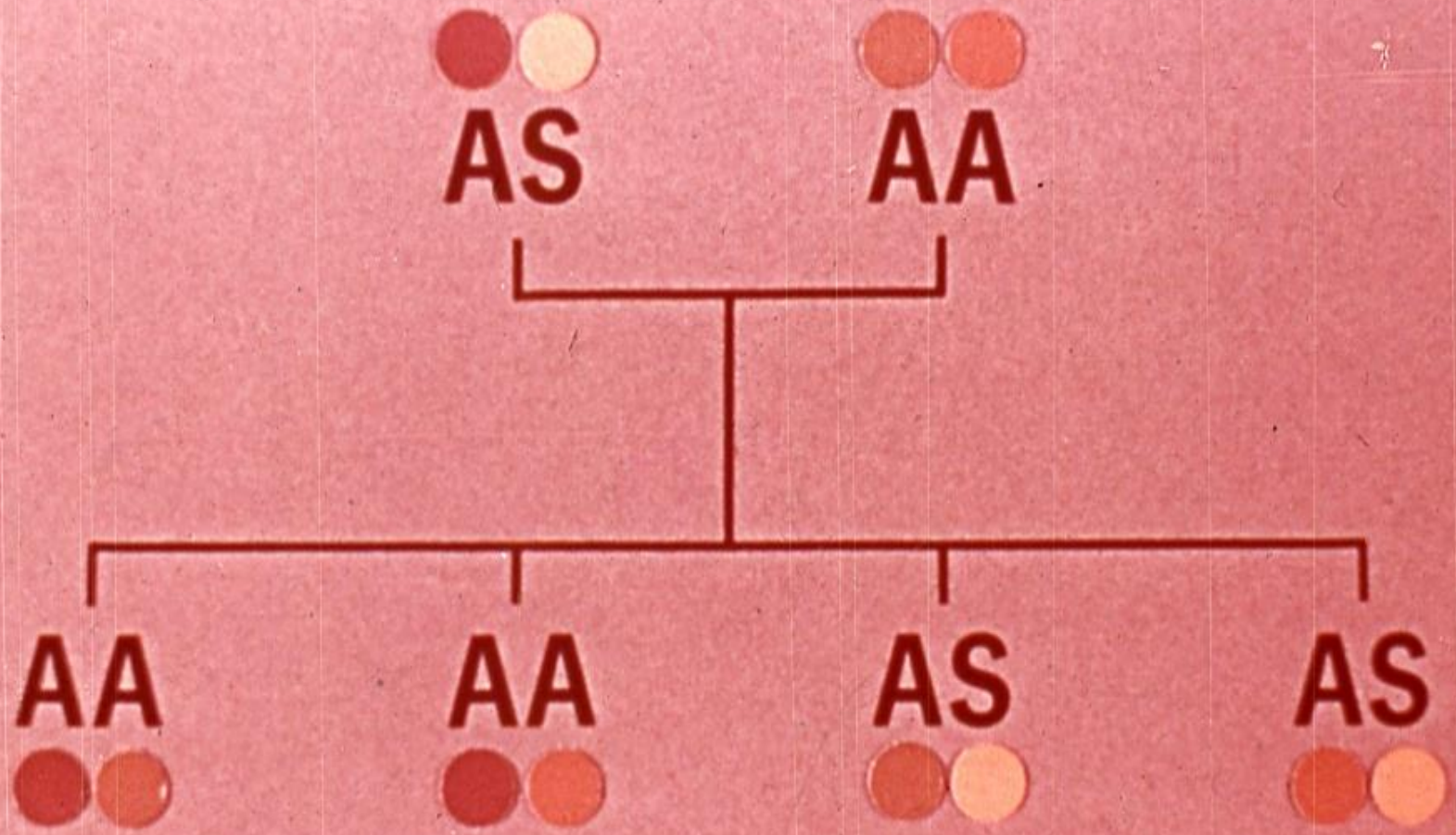
DNA Base composition	CCT	G T G	G A G
Amino Acid	pro	val	glu
	5	6	7

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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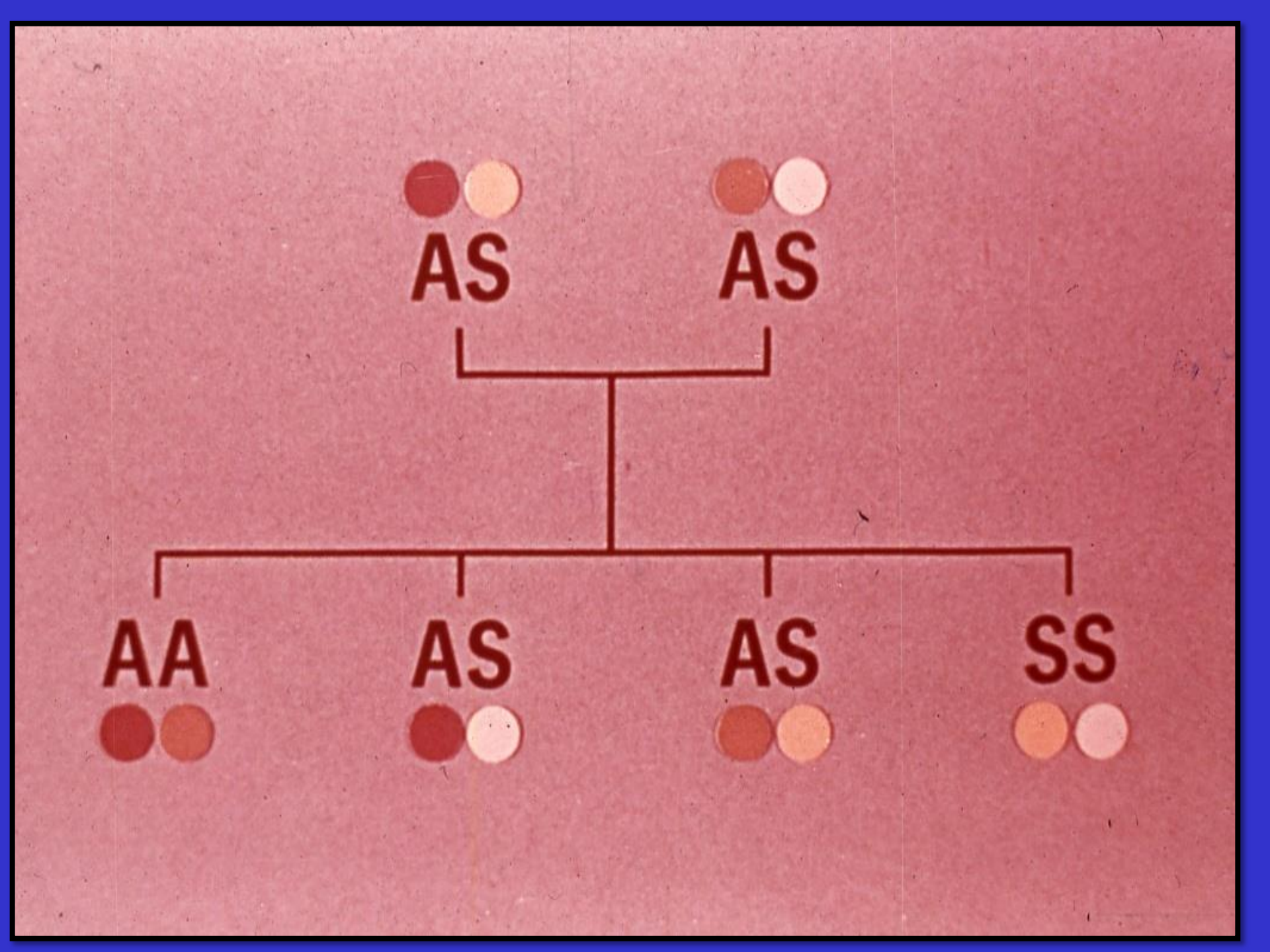
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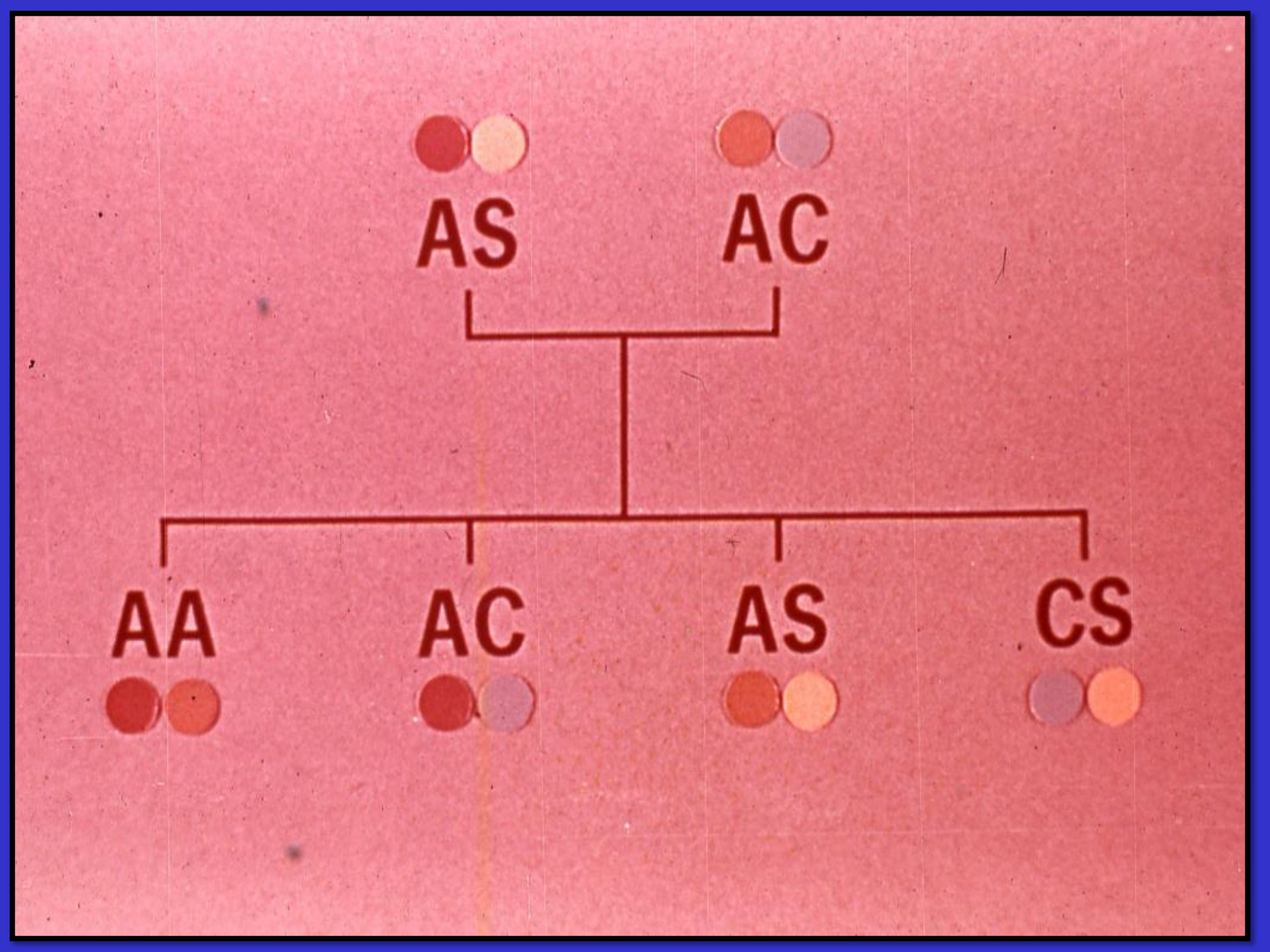
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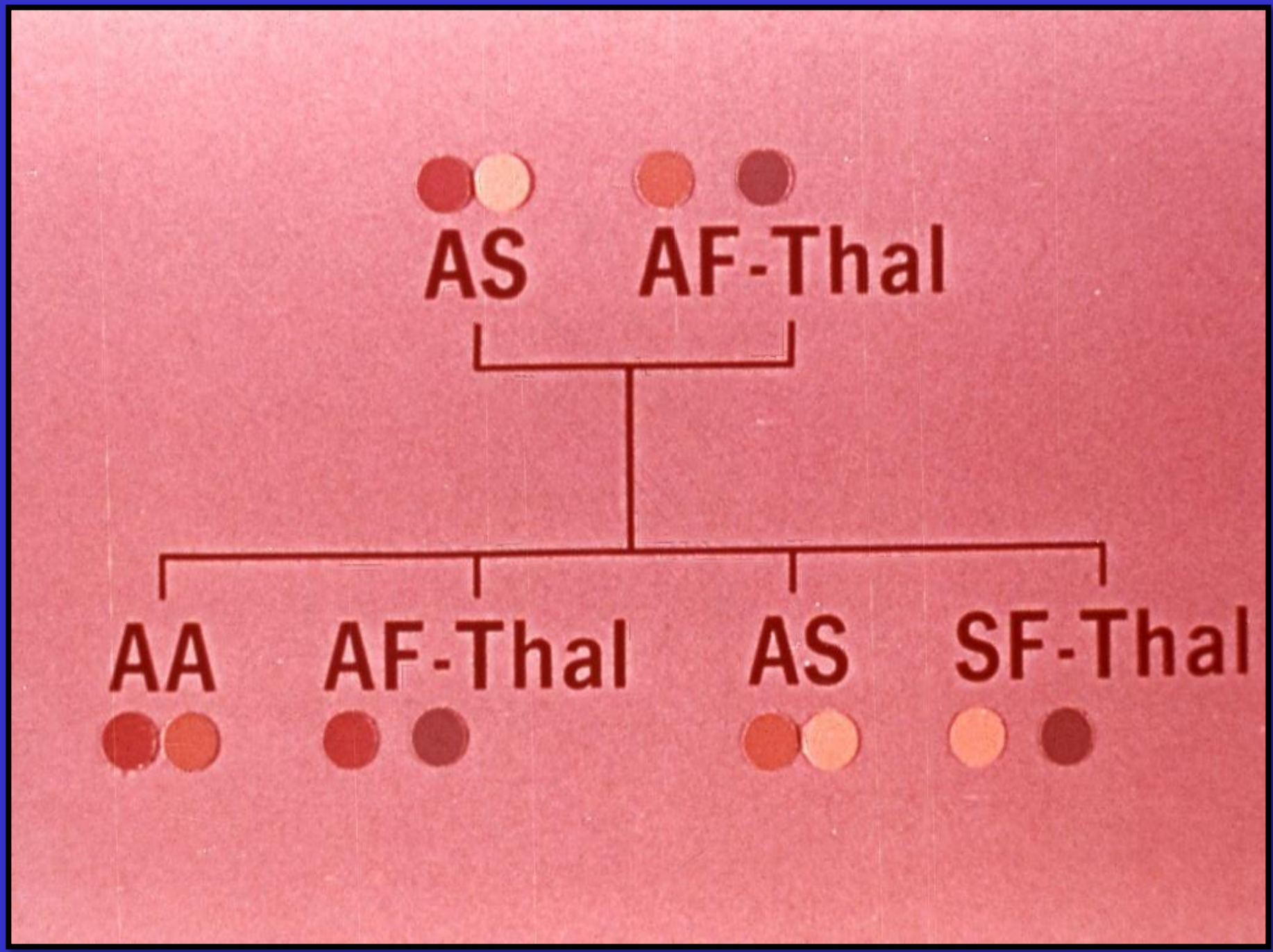
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AA
AF-Thal
AS
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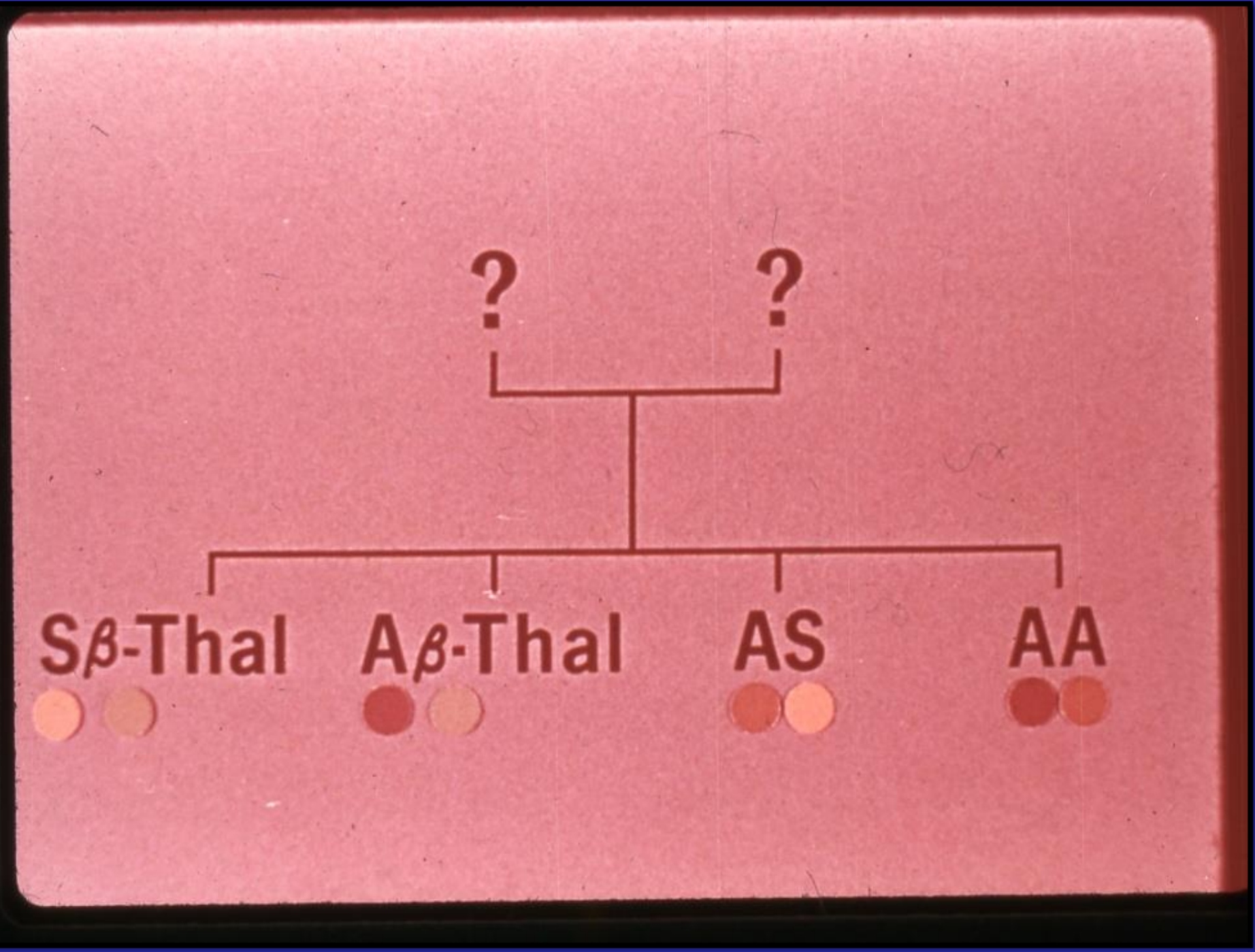
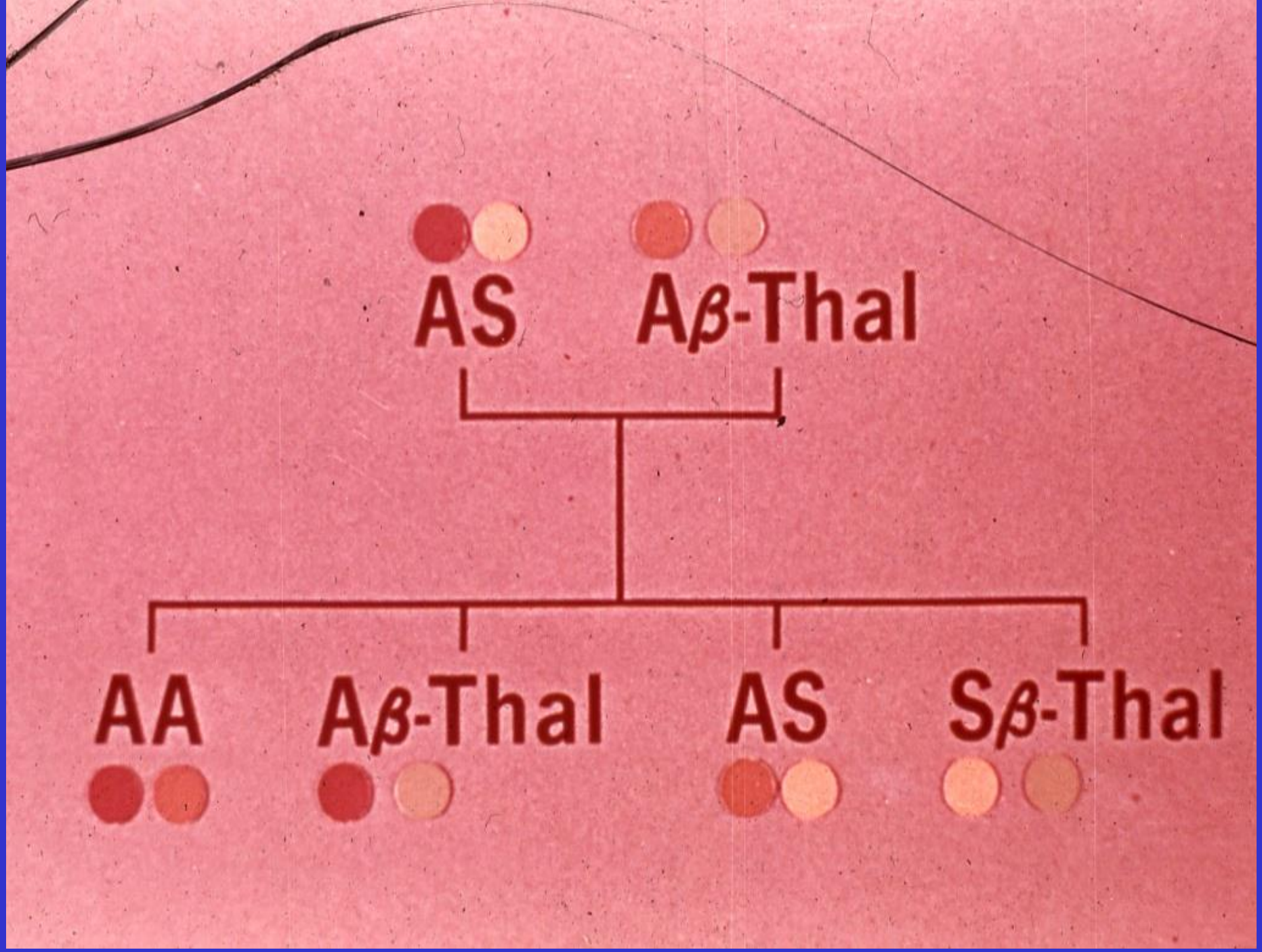

? ?
Sβ-Thal
Aβ-Thal
AS
AA


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

CRISES IN SICKLE CELL DISEASE

HYPERHAEMOLYTIC

AREGENERATIVE OR APLASTIC

SMALL VESSEL OCCLUSION

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

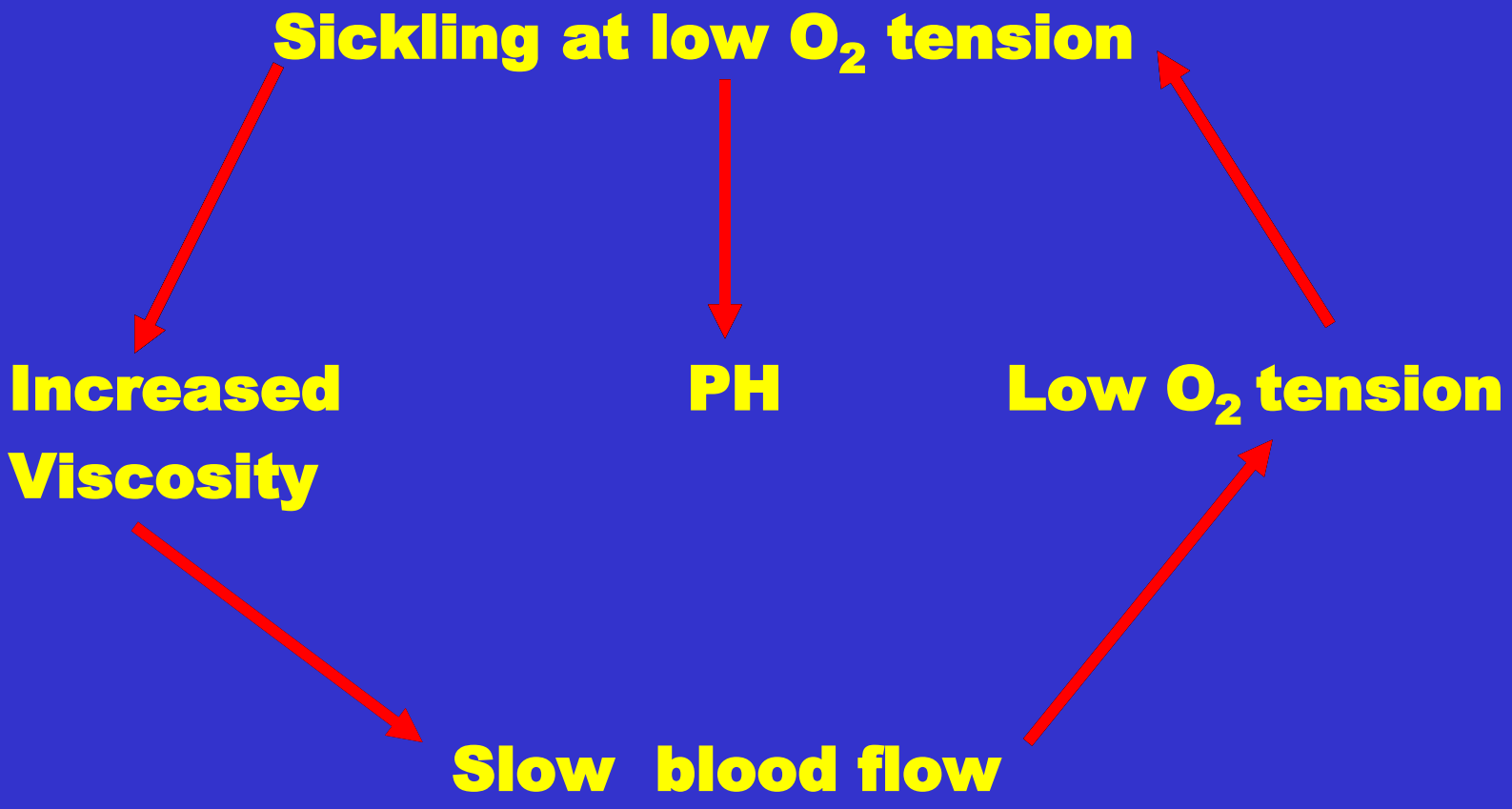
Concentration of Hb S

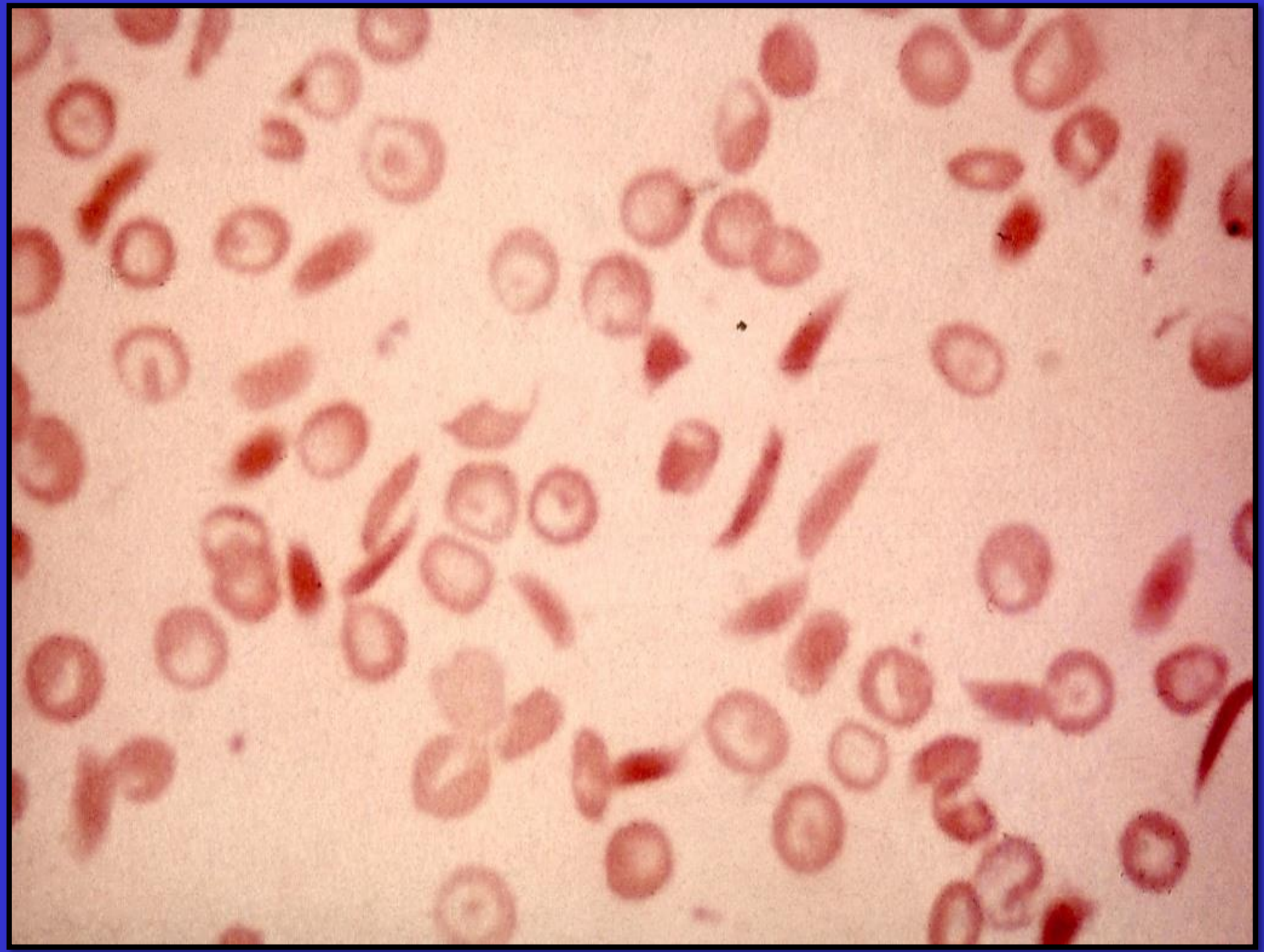
Presence of other haemoglobins

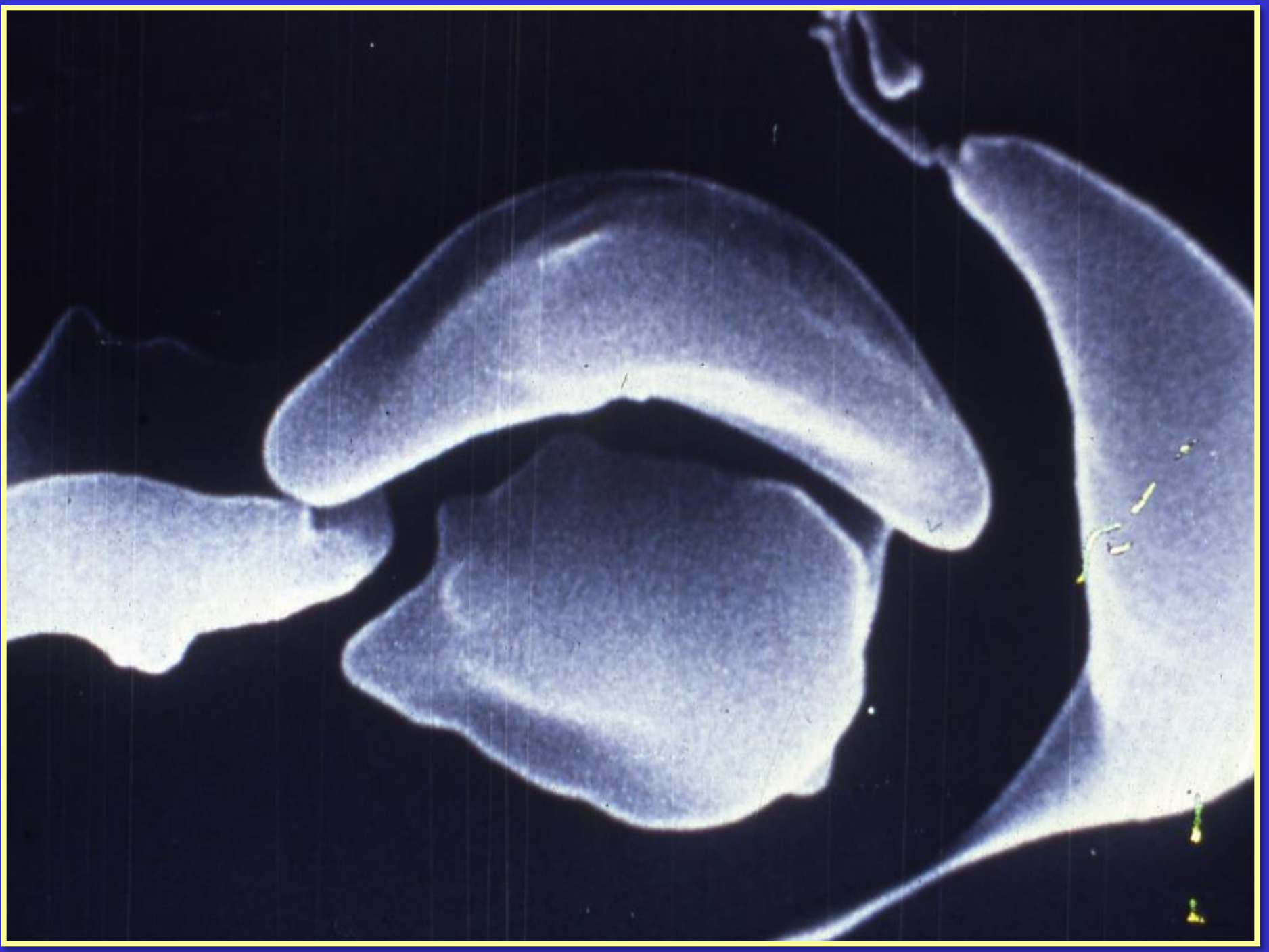
polymerisation : S > D > C > J = A > F

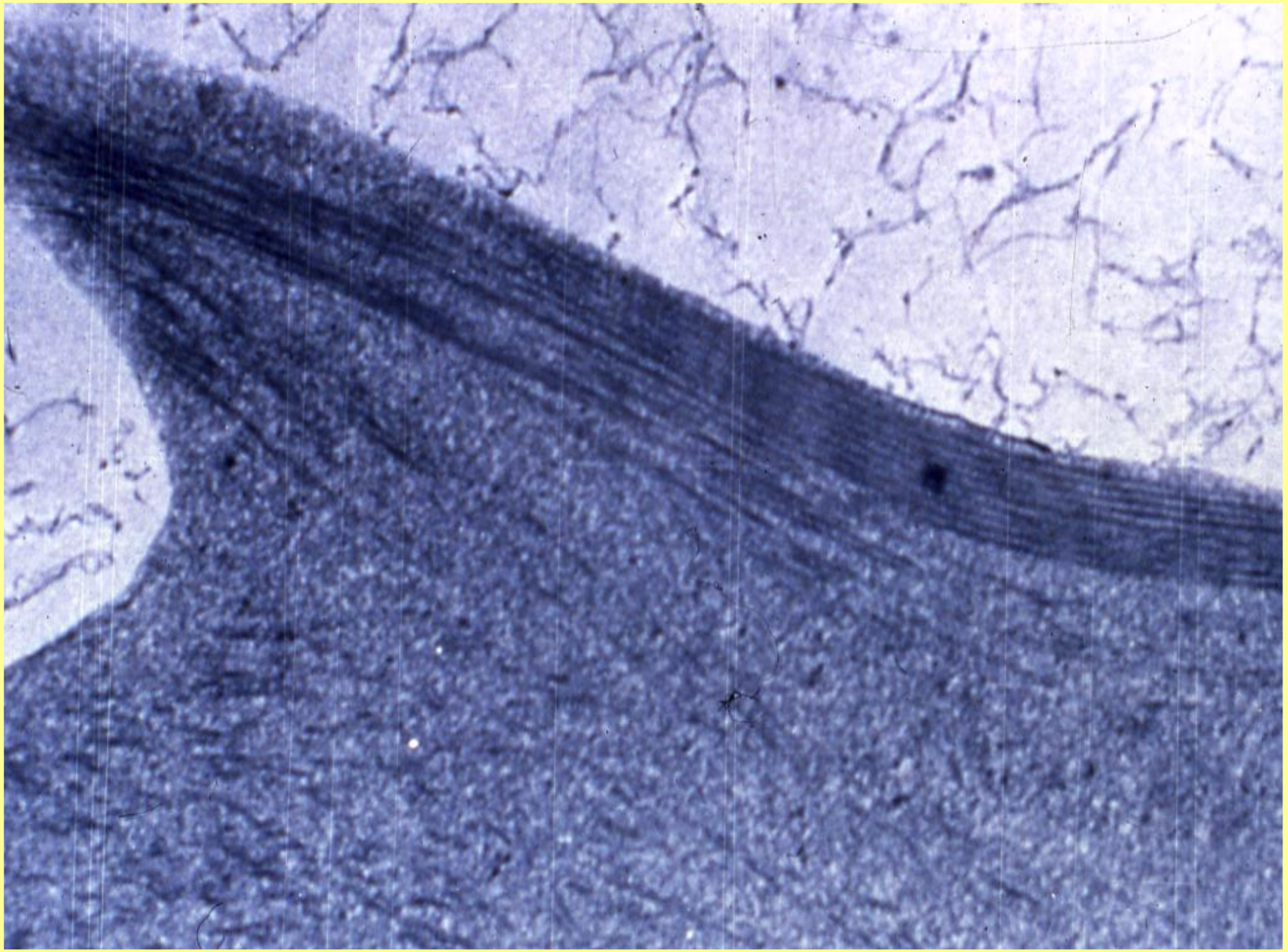
FACTORS PRECIPITATING CRISES IN SICKLE CELL DISEASE

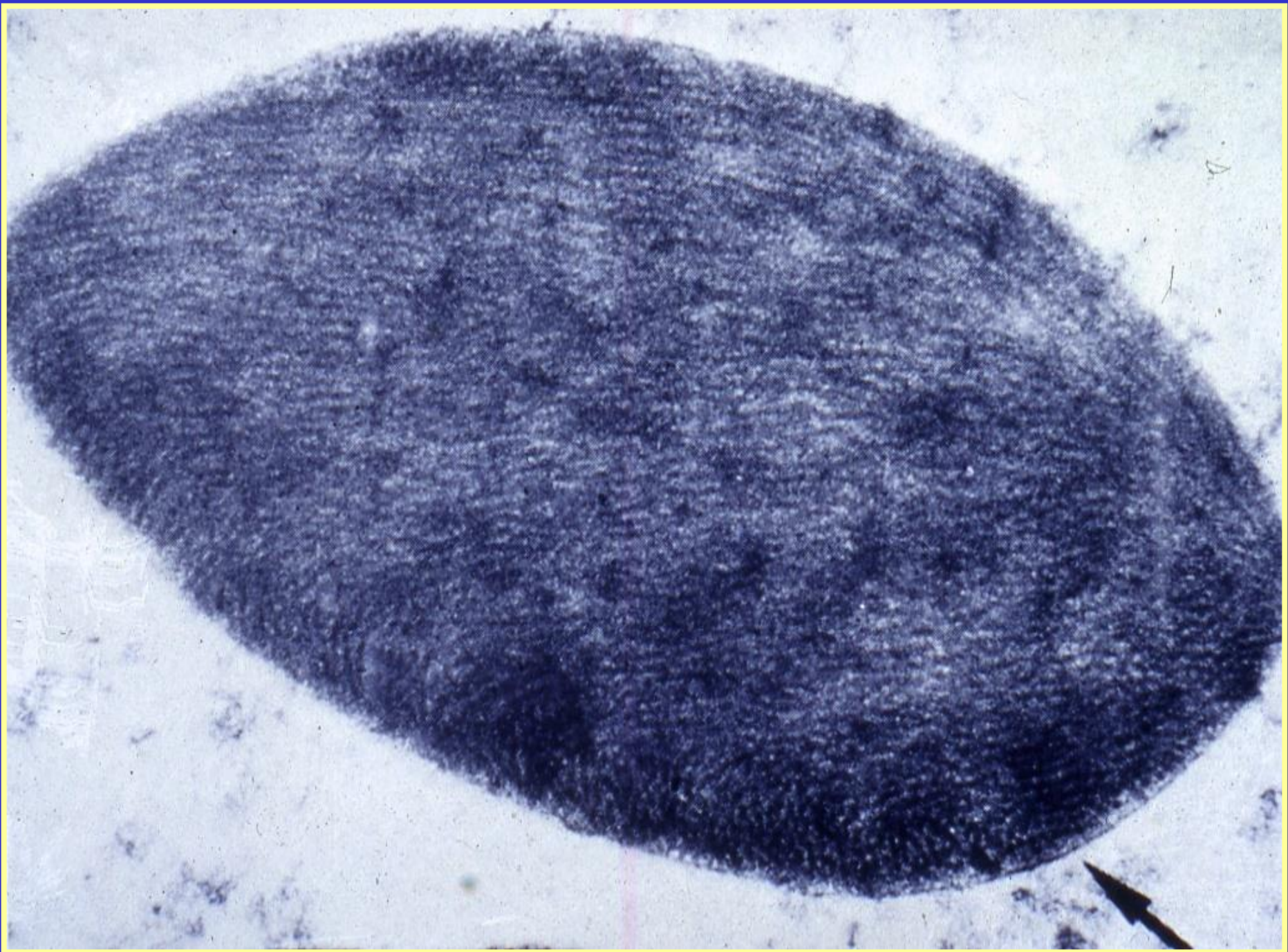
- INFECTIONS (especially malaria)
- PYREXIA
- EXPOSURE TO COLD
- DEHYDRATION
- PREGNANCY

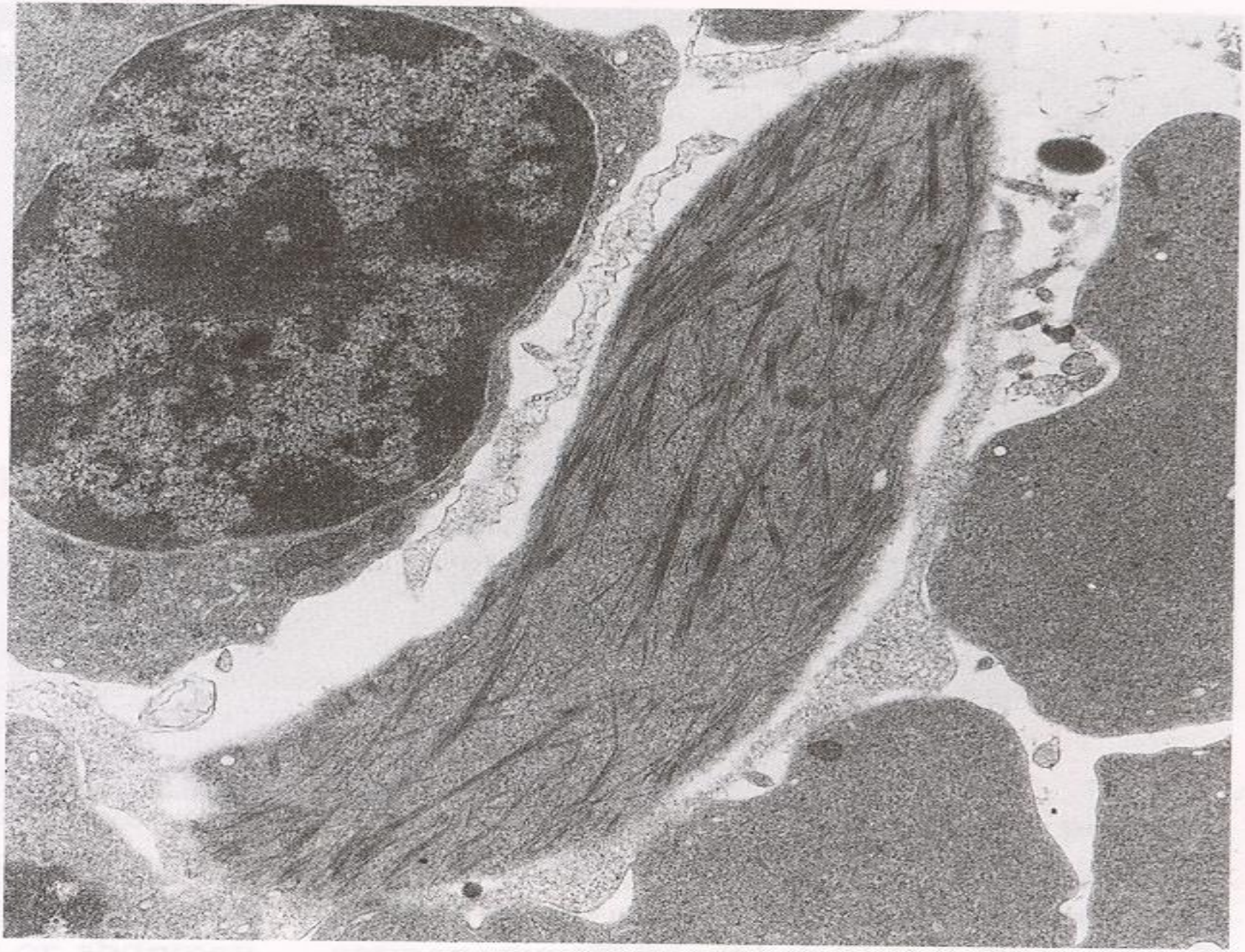

Sickling at low O_2 tension
Increased Viscosity
PH
Low O_2 tension
Slow blood flow



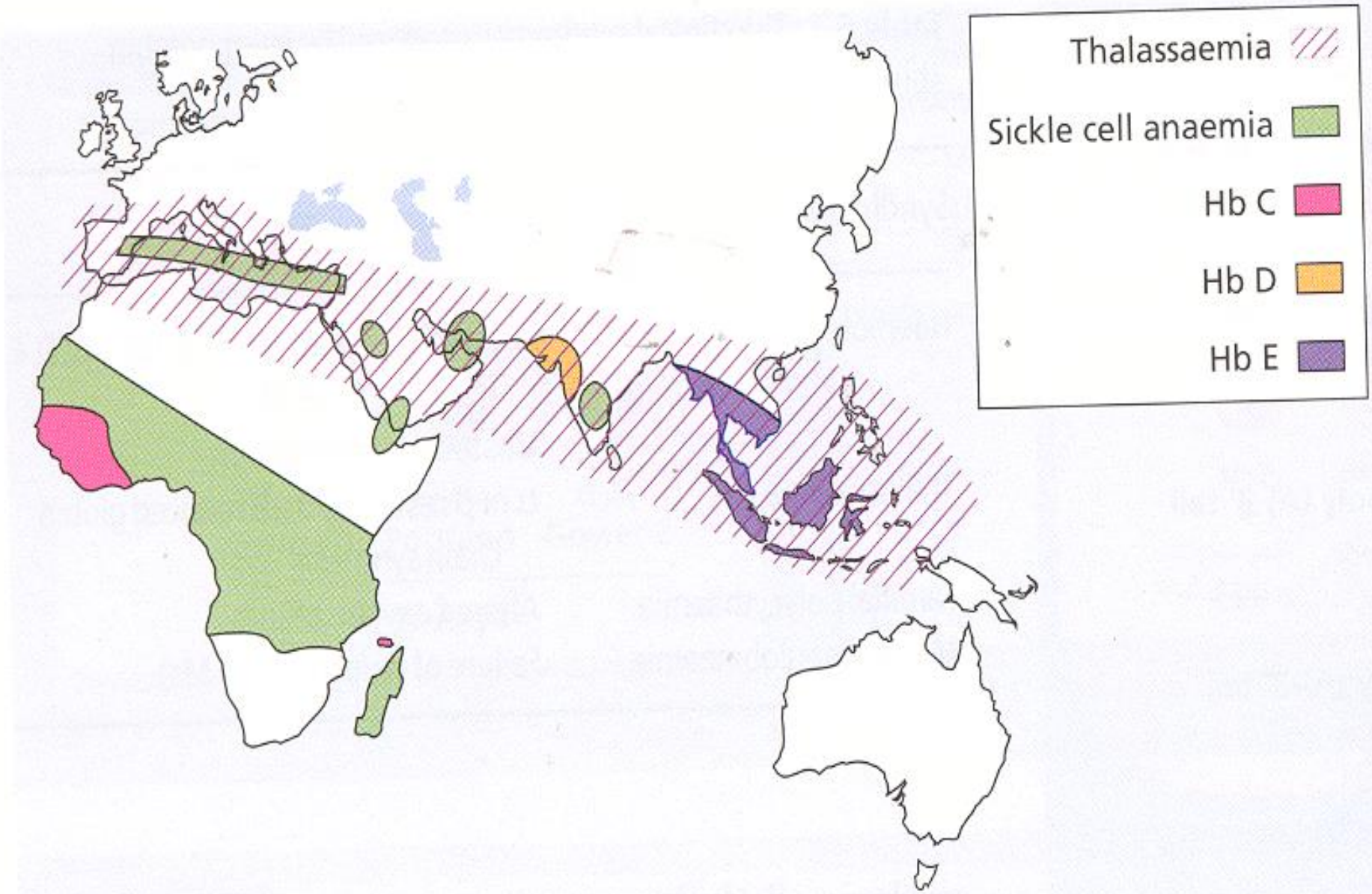


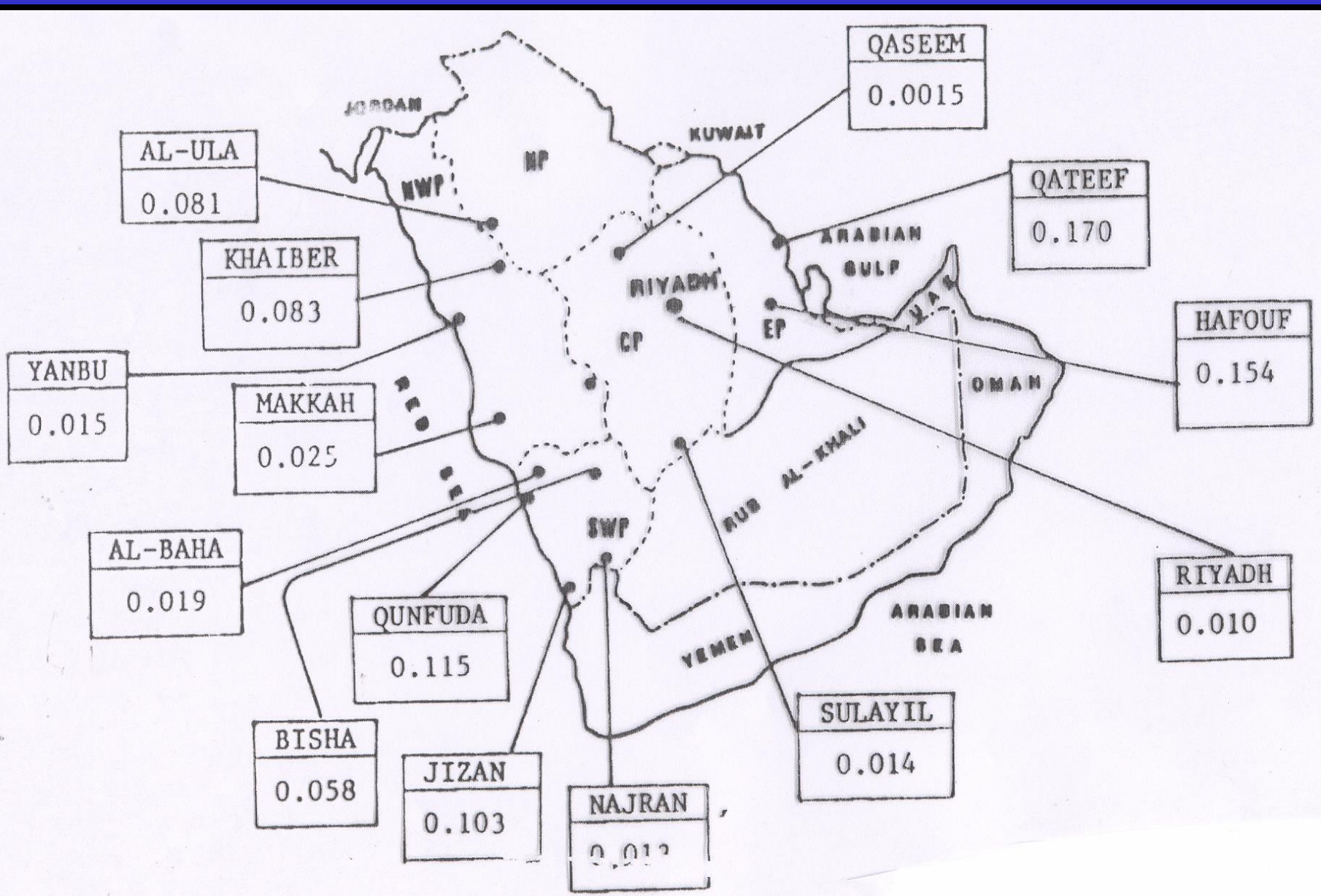






HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION





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

CLINICAL MANIFESTATIONS OF SICKLE CELL DISEASE

HAEMOLYTIC ANAEMIA

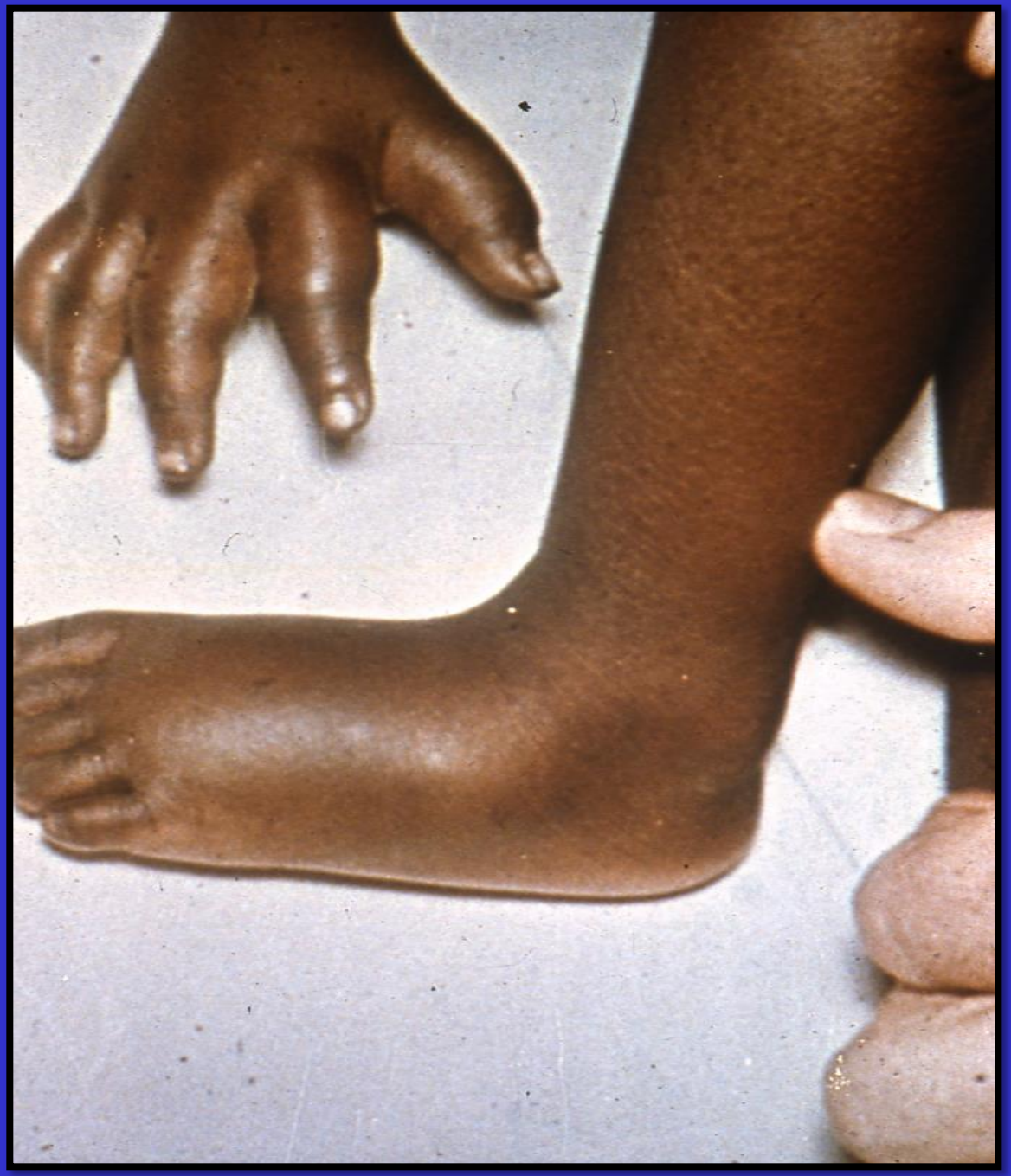
TISSUE INFARCTION

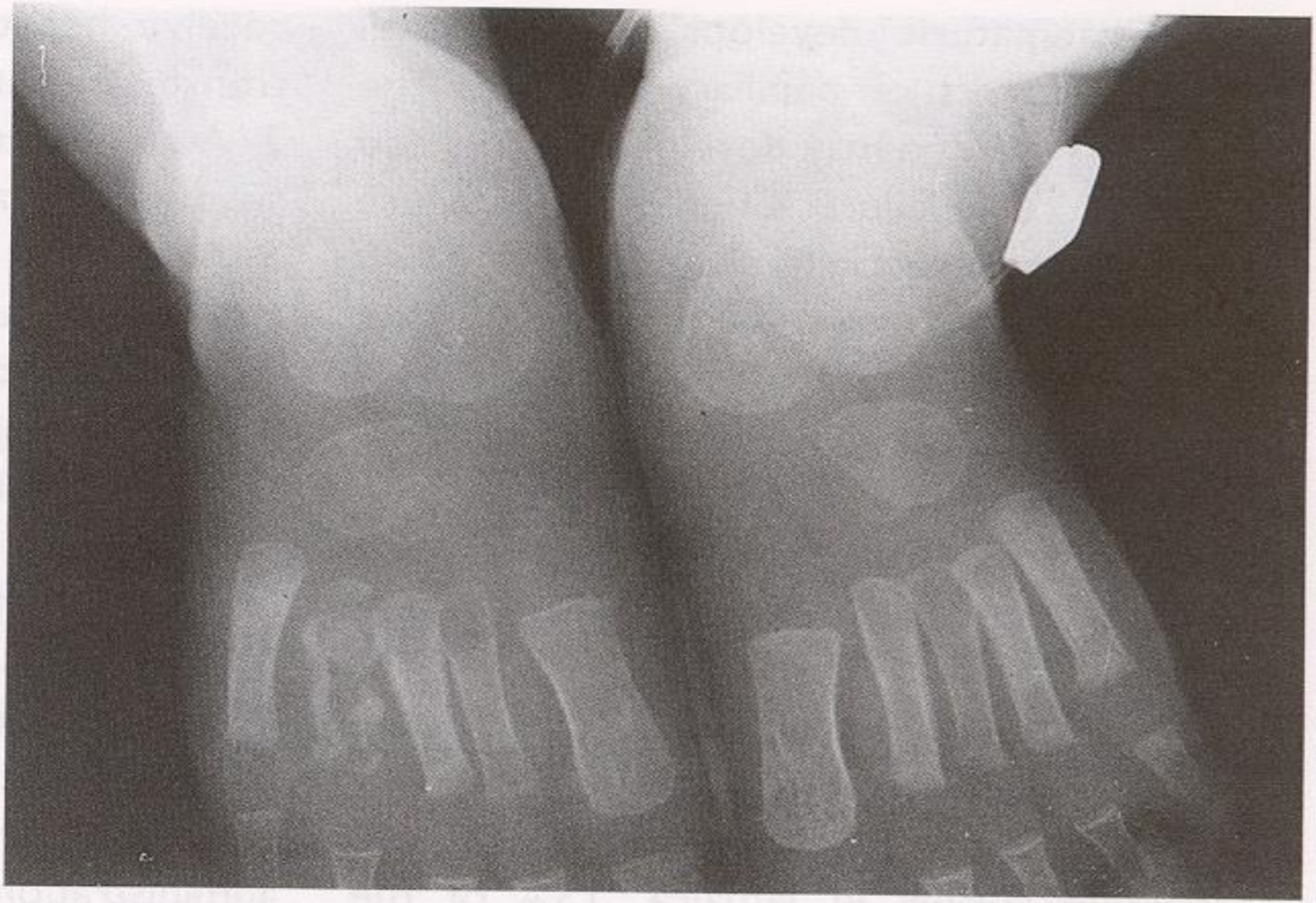
Clinical Manifestations in Sickle Anaemia

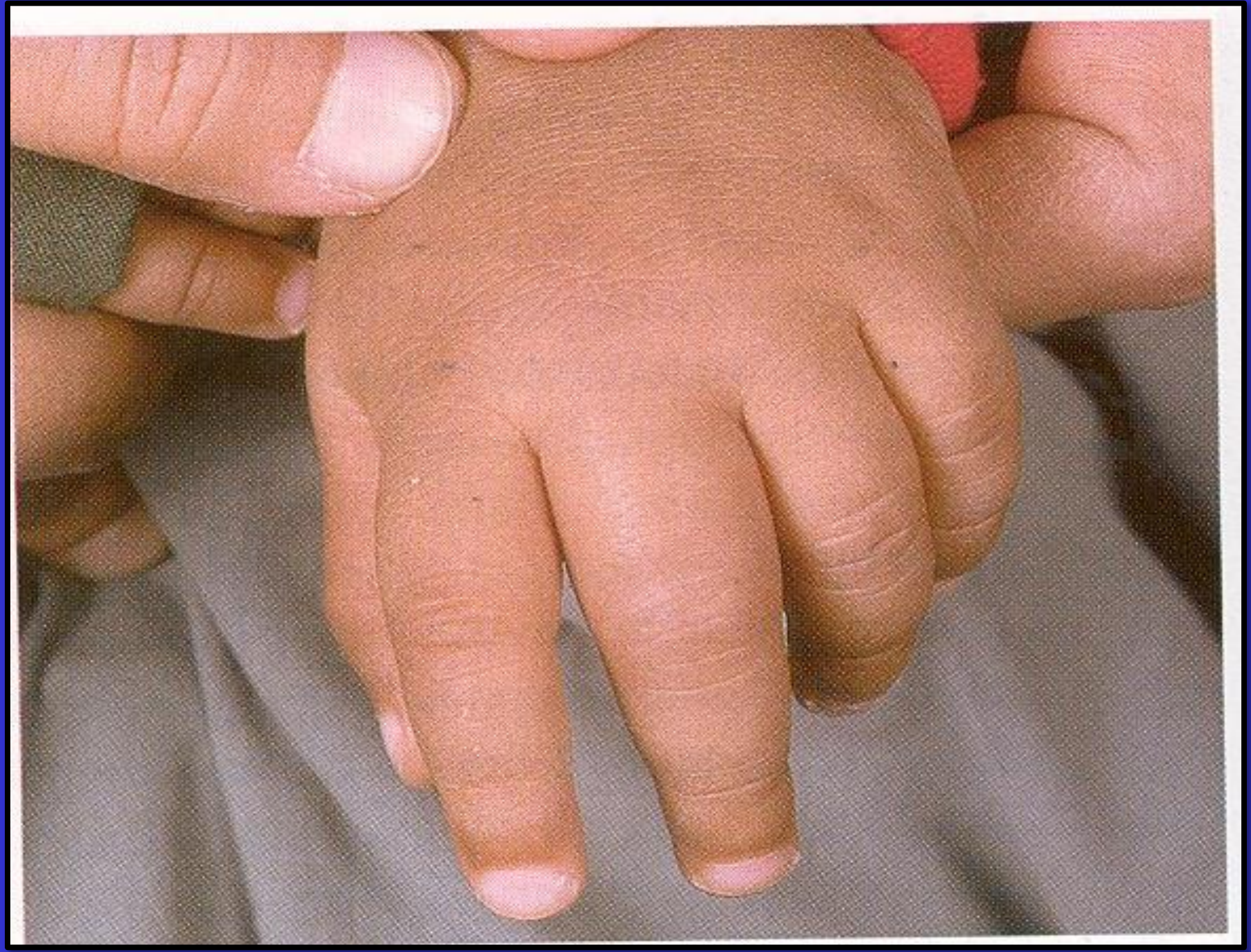
- Pallor (Anaemia)
- Jaundice & Dark Urine
- Apathy & Anorexia
- Hand-Foot Syndrome (Young Children)
- Splenic sequestration (Young children) Hepatic Sequestration
- Bones and Joints Pain
- Abdominal Pain

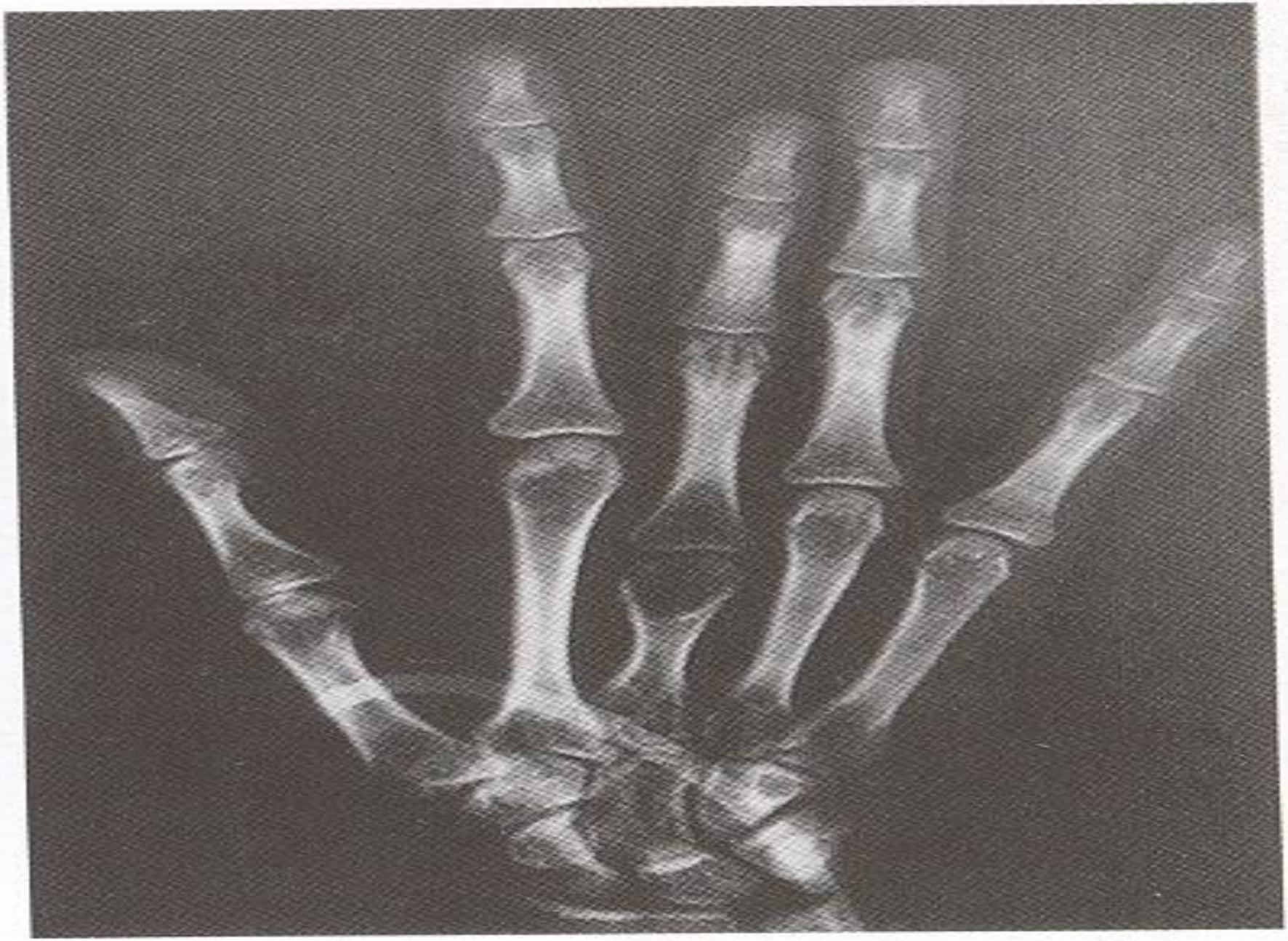
Clinical Manifestations in Sickle Anaemia

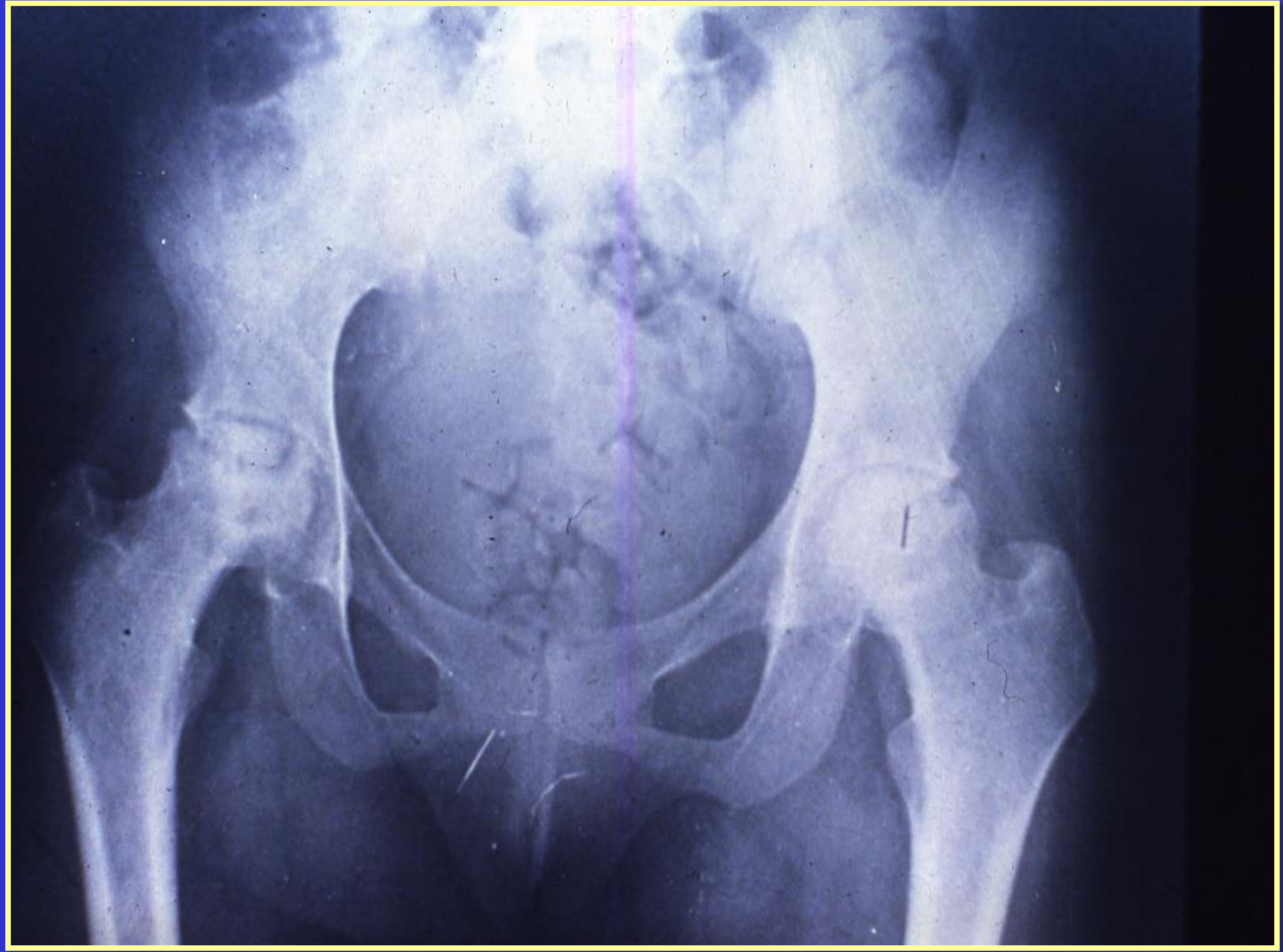
- Recurrent Infections & Chest Symptoms (Acute Chest Syndrome)
- Hepato-Splenomegaly
  - → (Early Childhood)
  - → (Association with Thalassaemias)
- CNS Presentations
- Leg Ulceration
- Skeletal Deformity

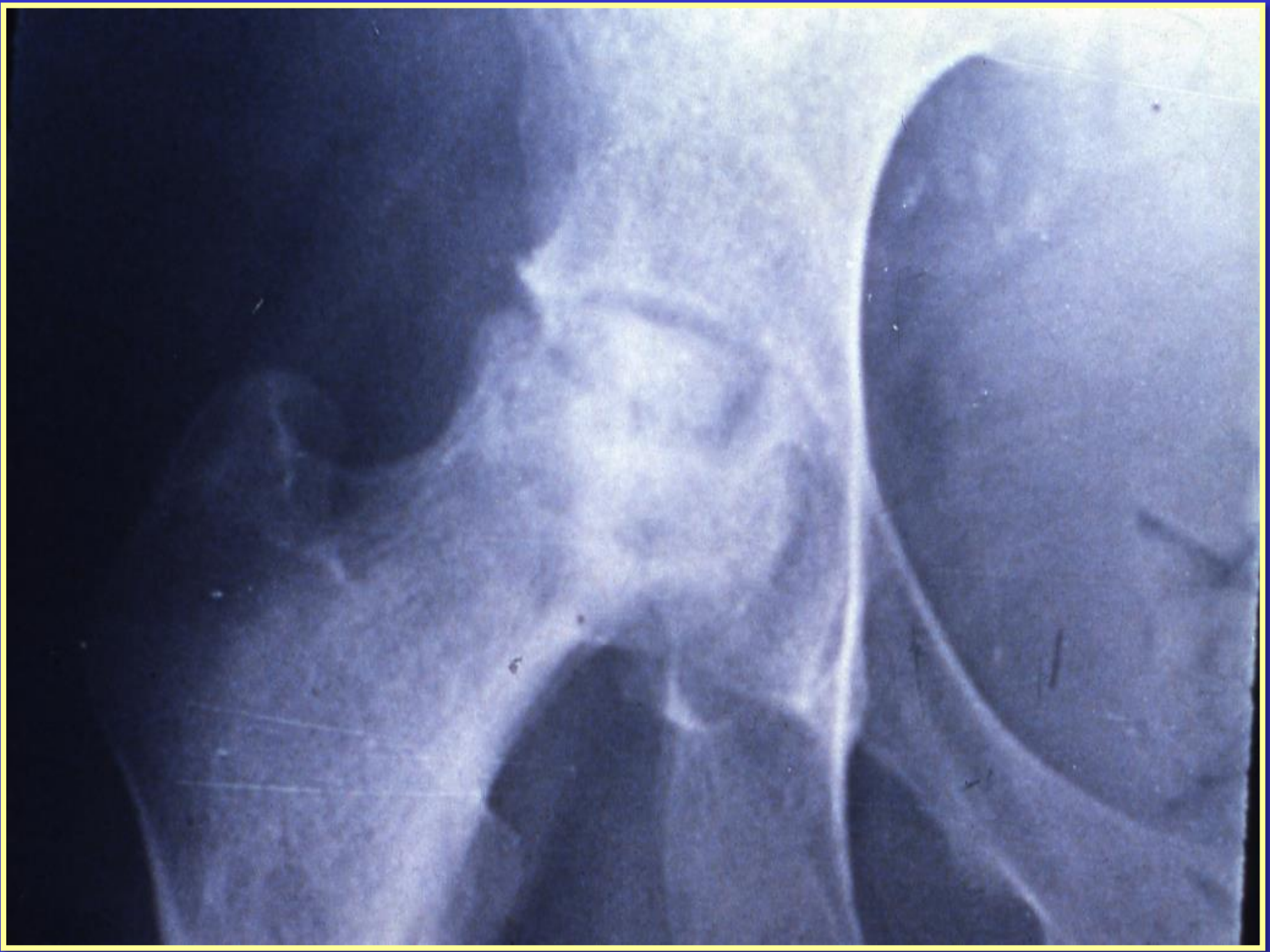


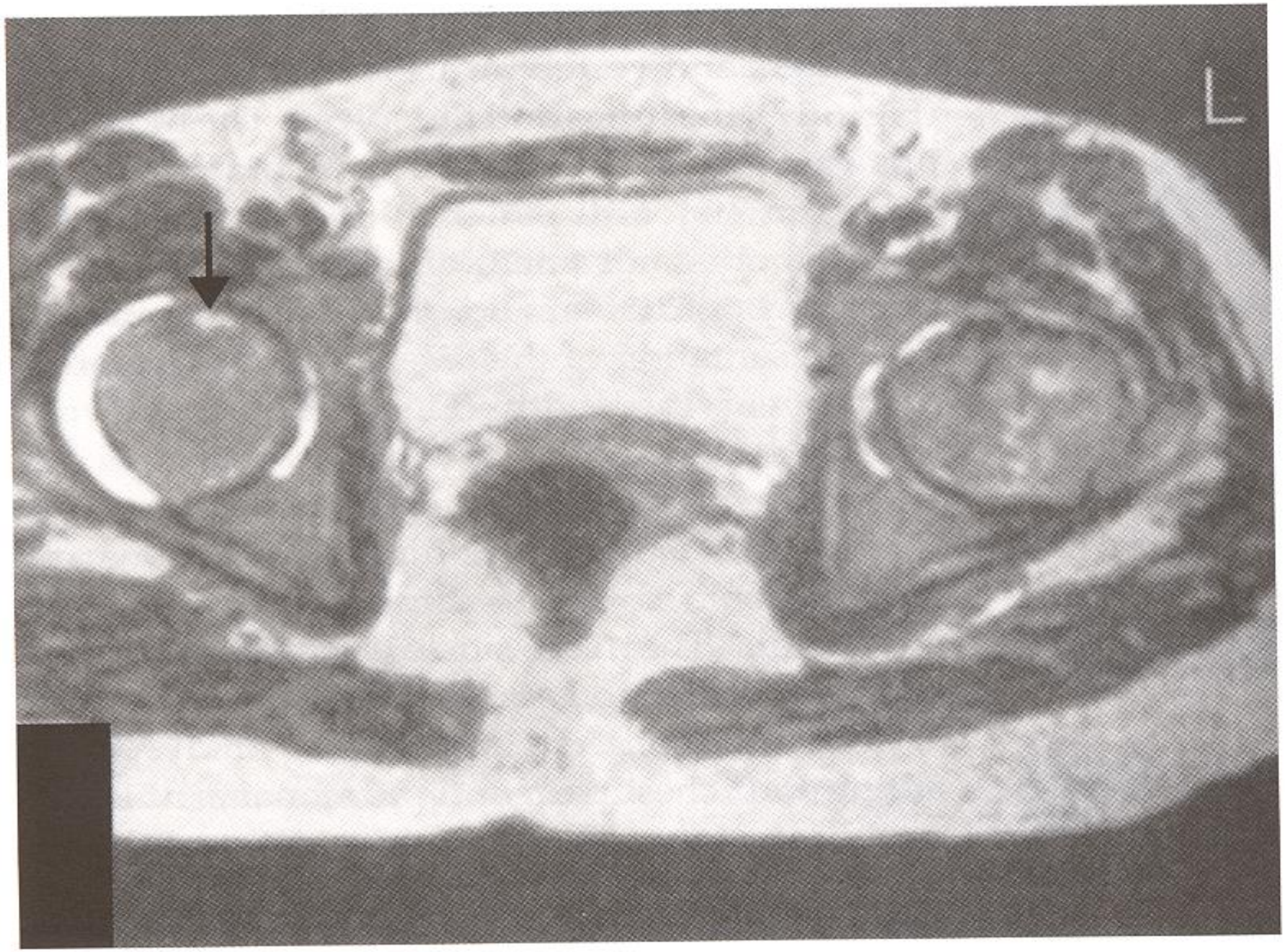








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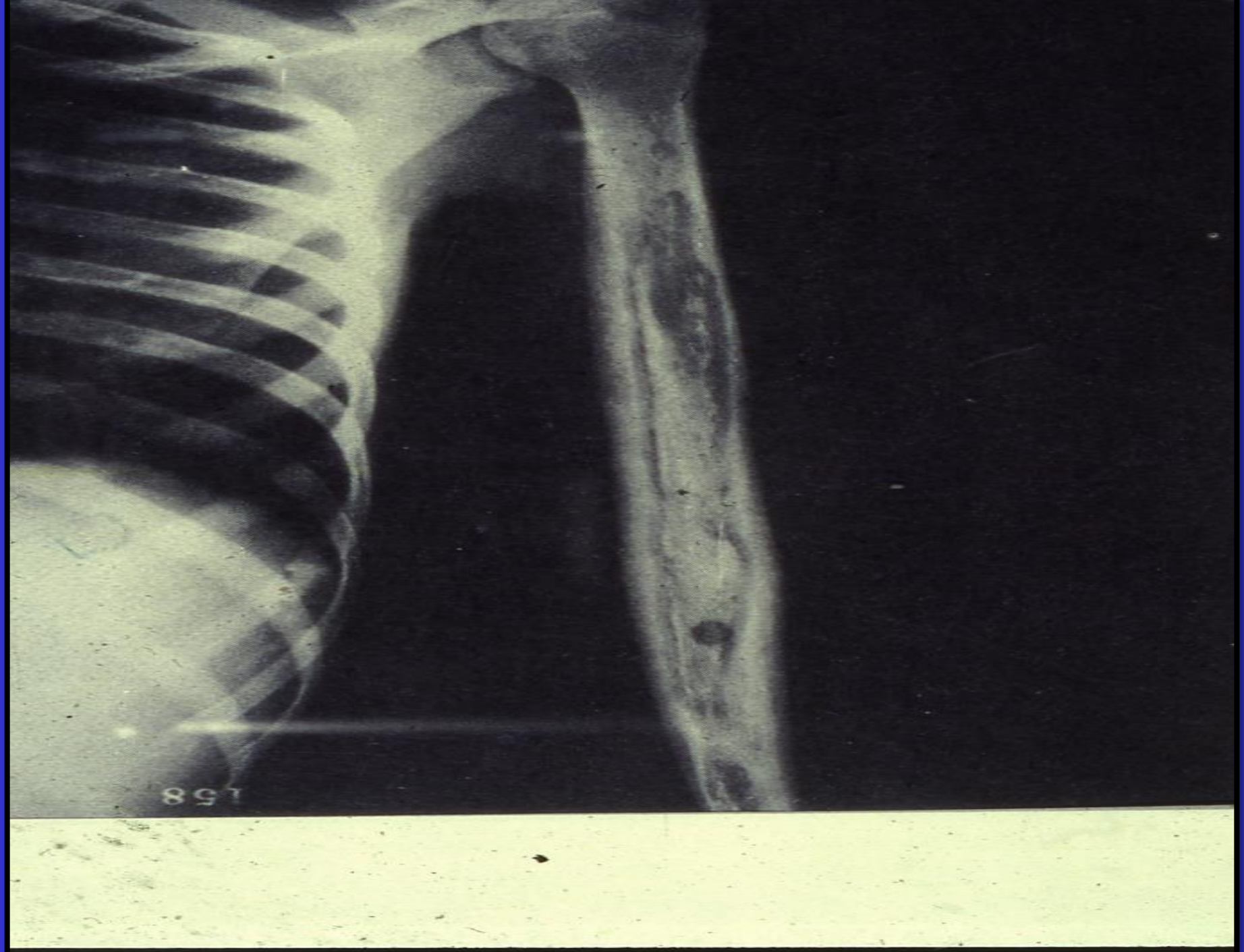
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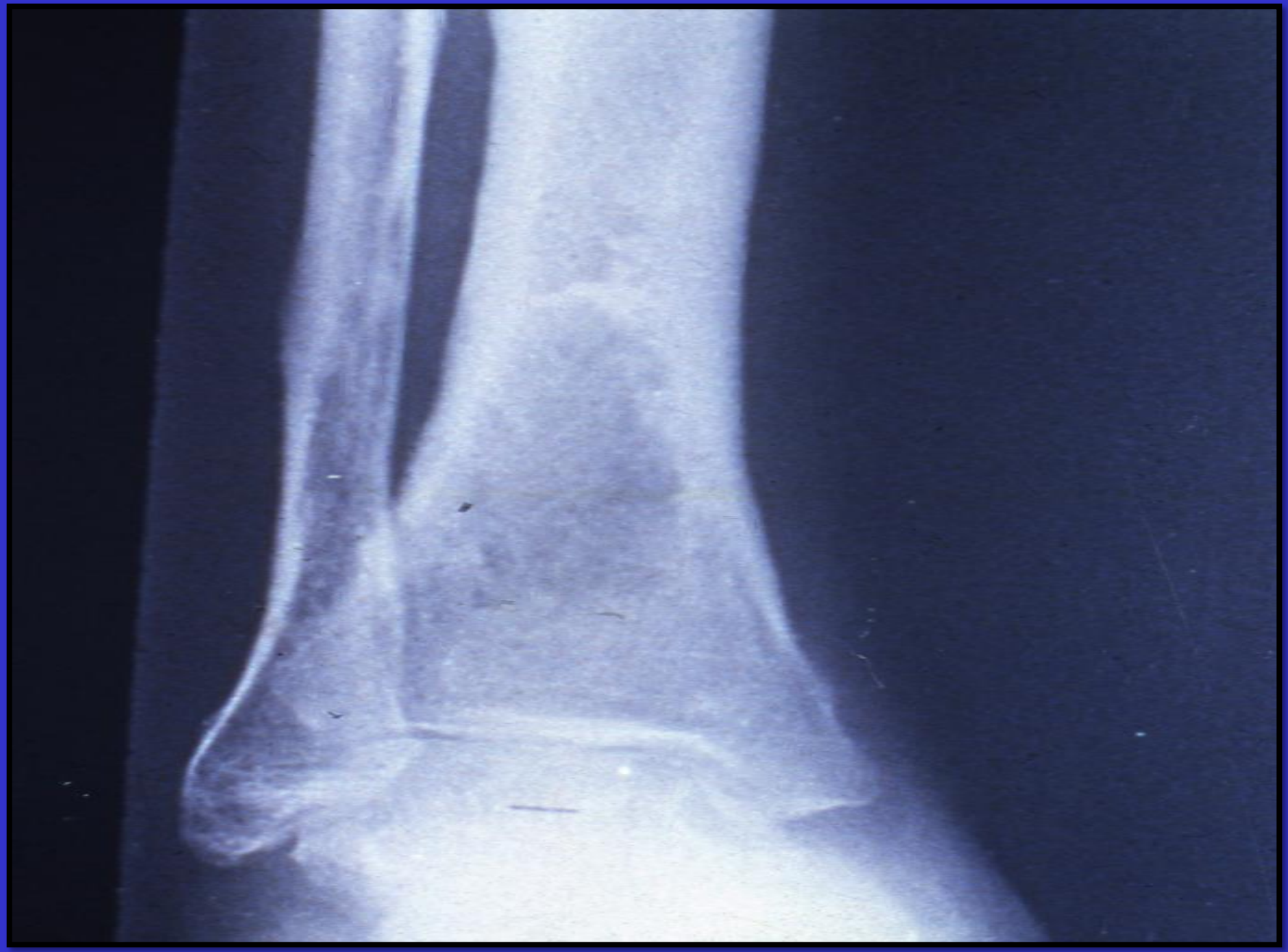
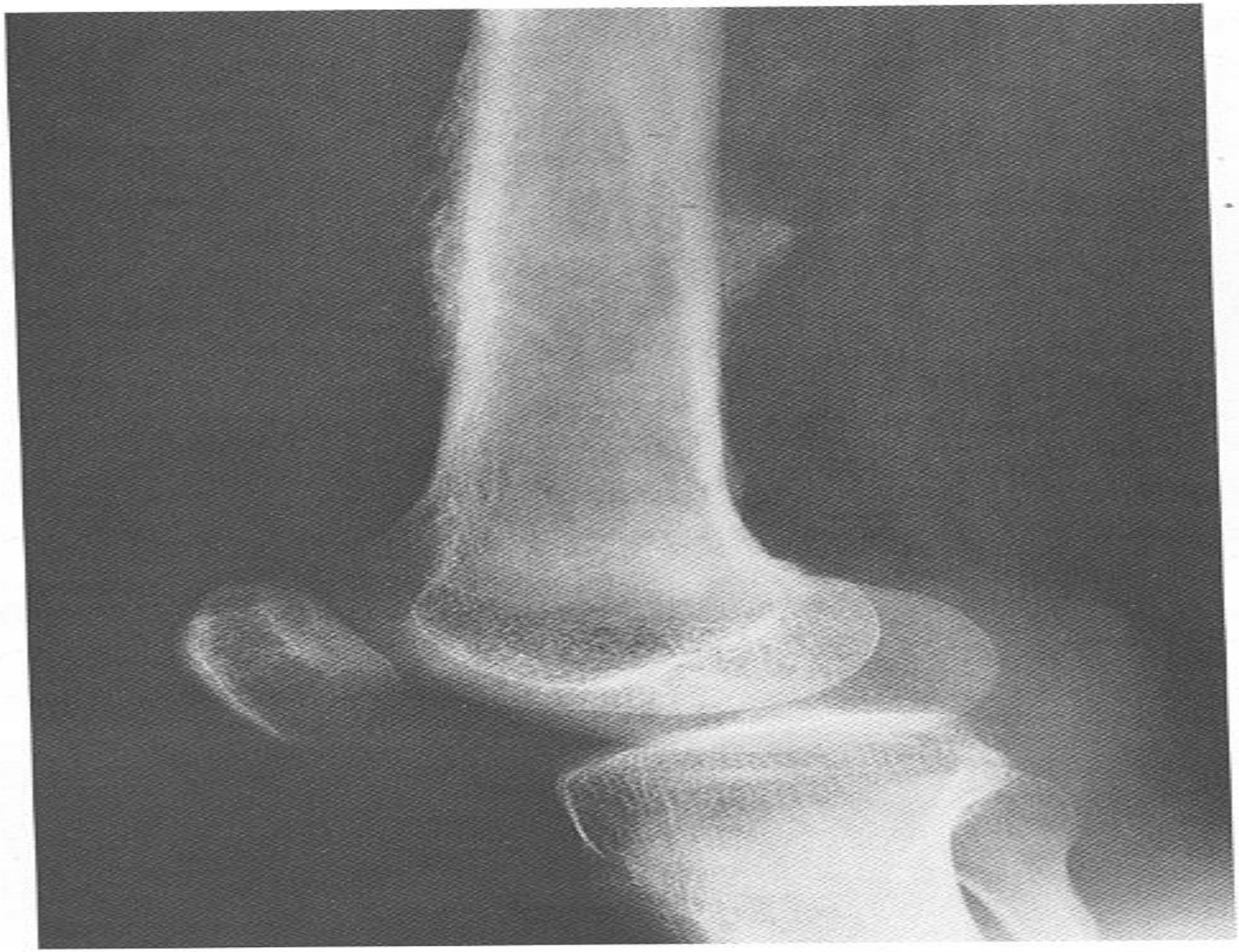
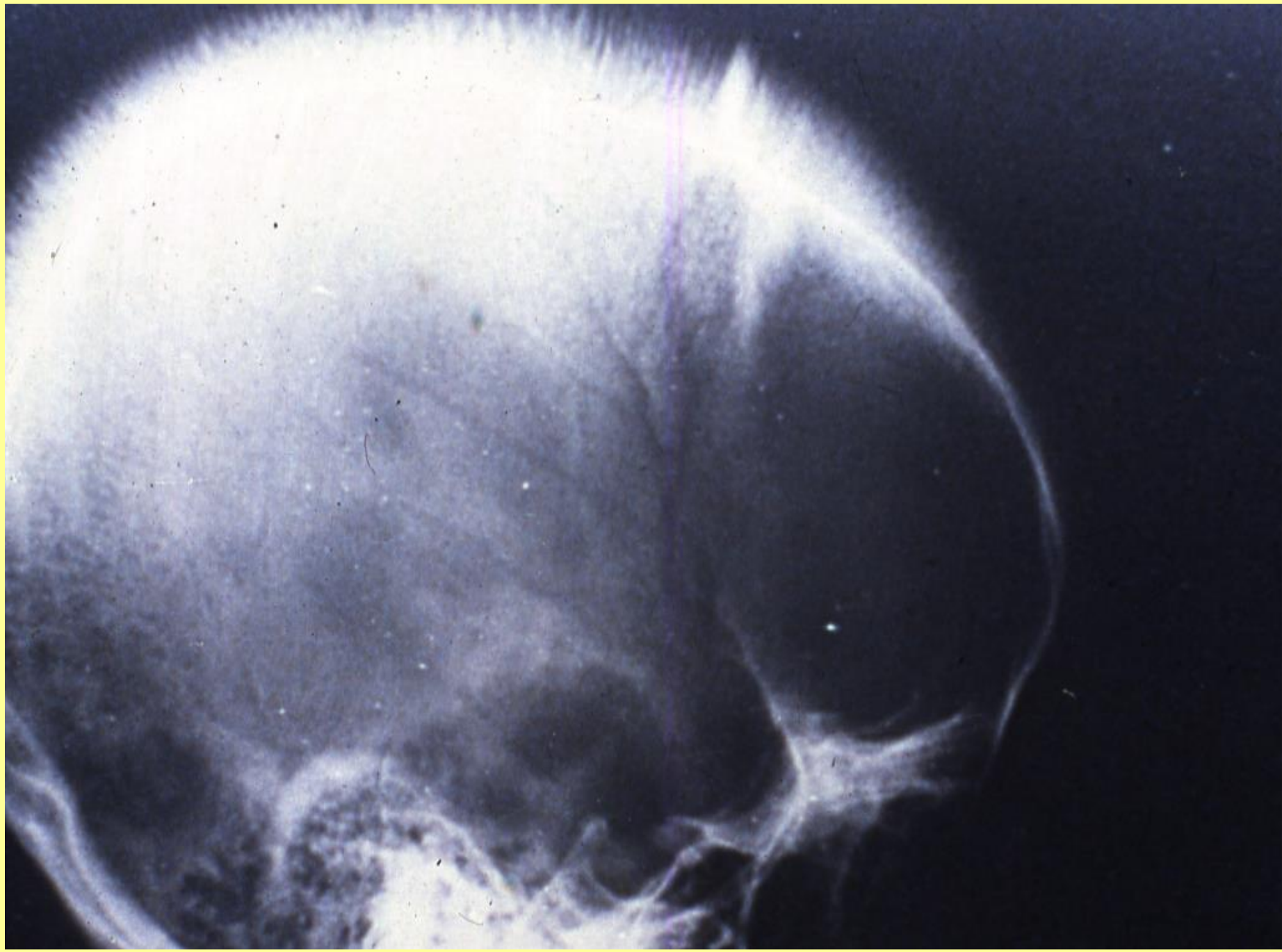
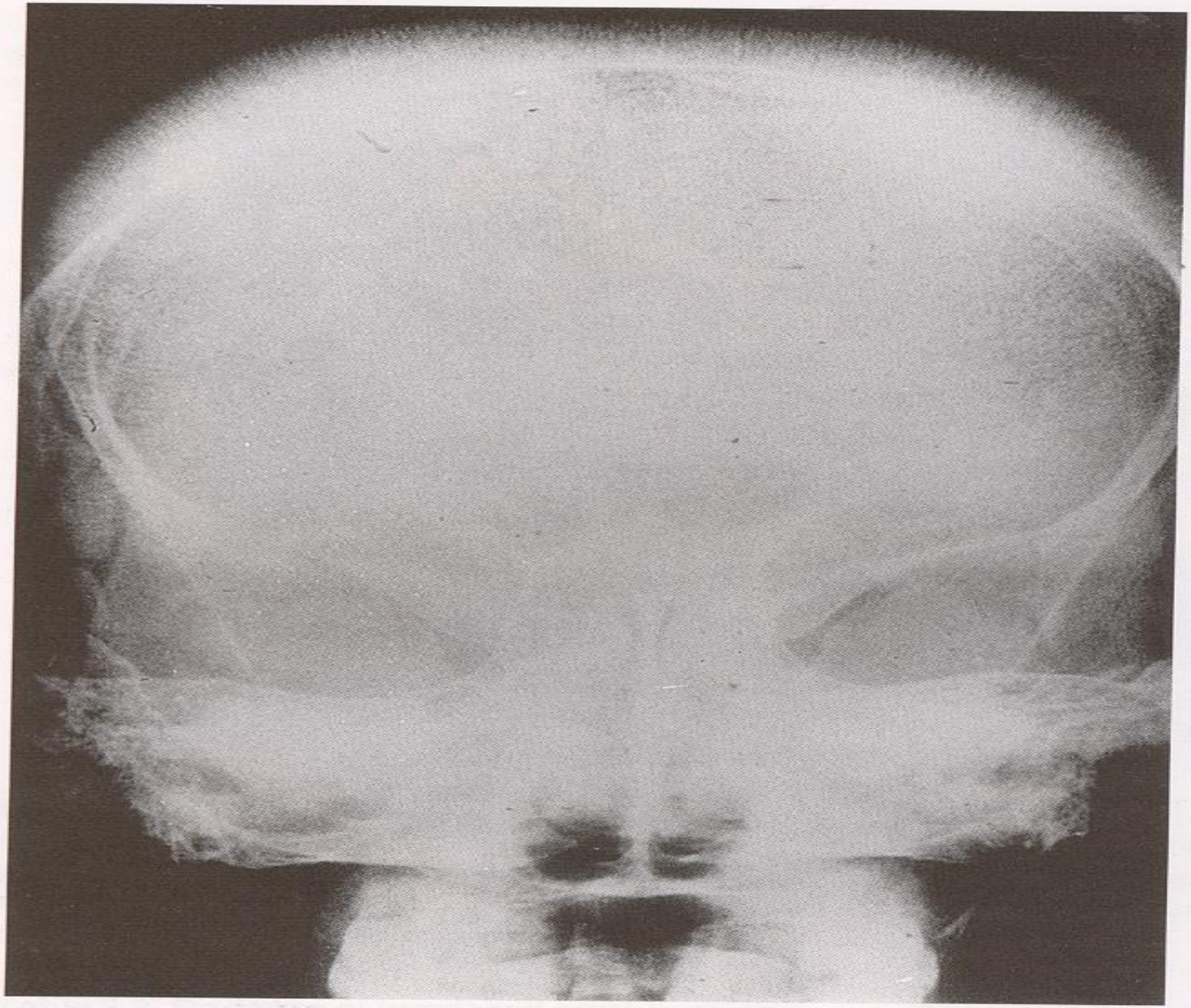
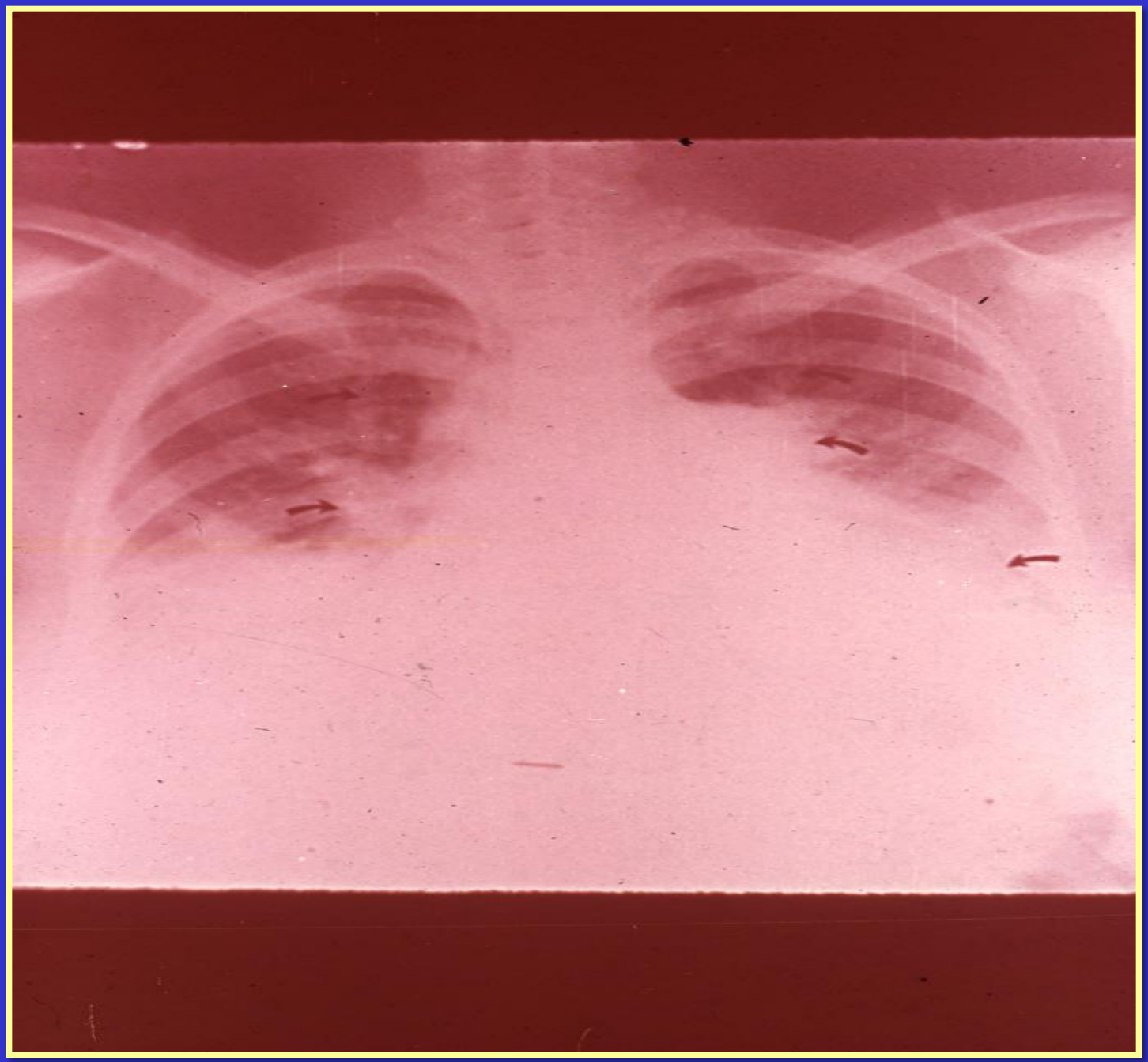
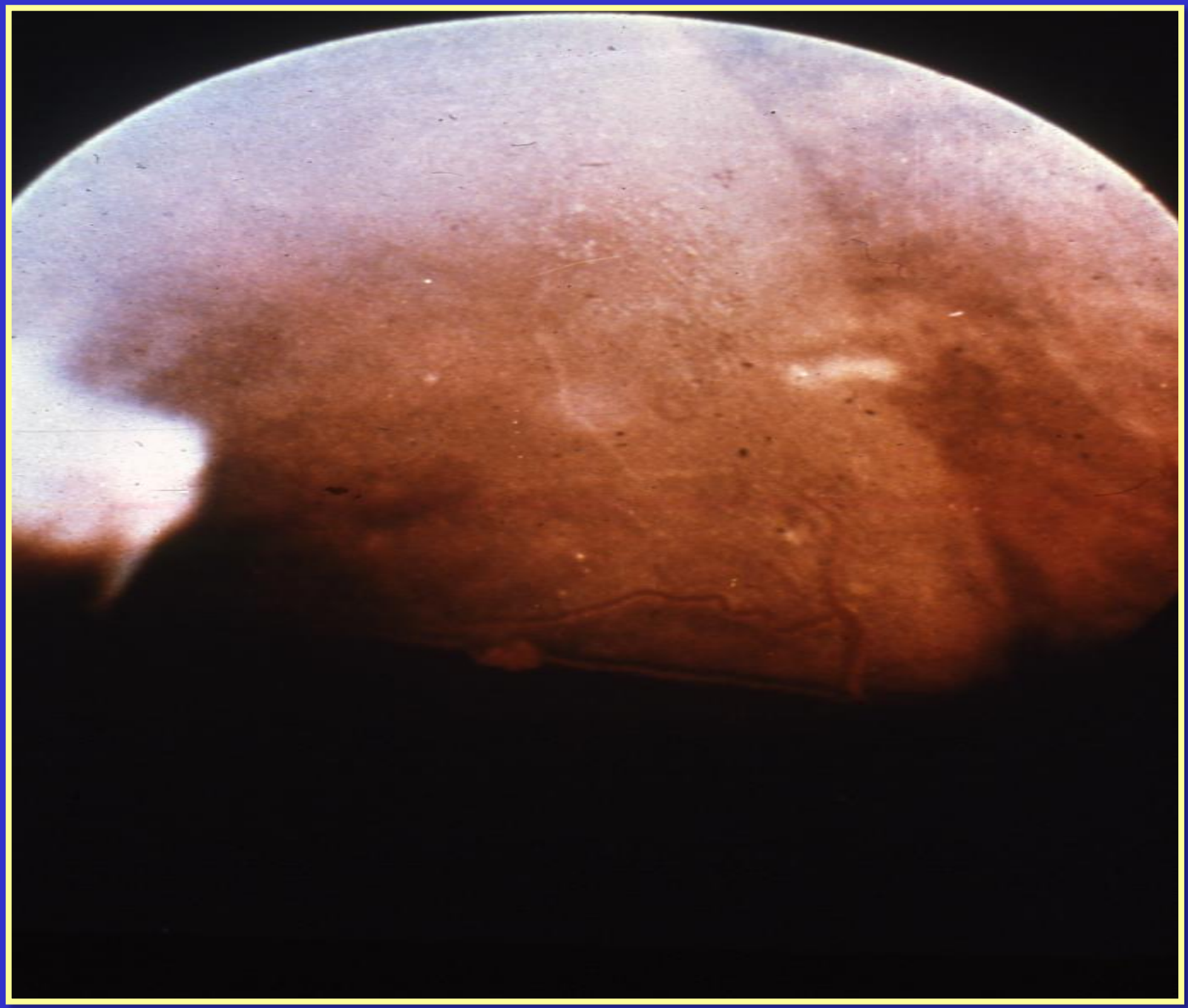


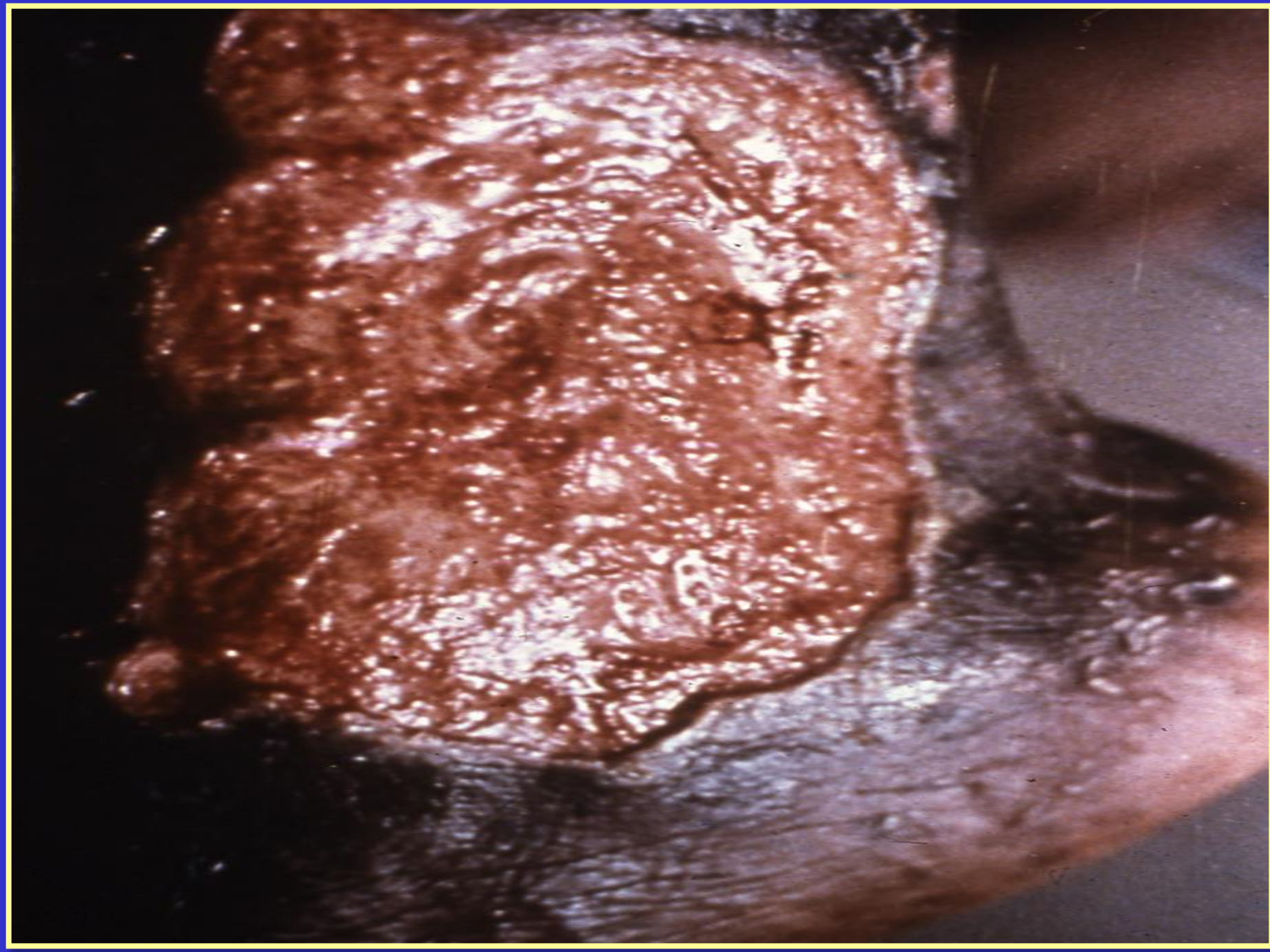


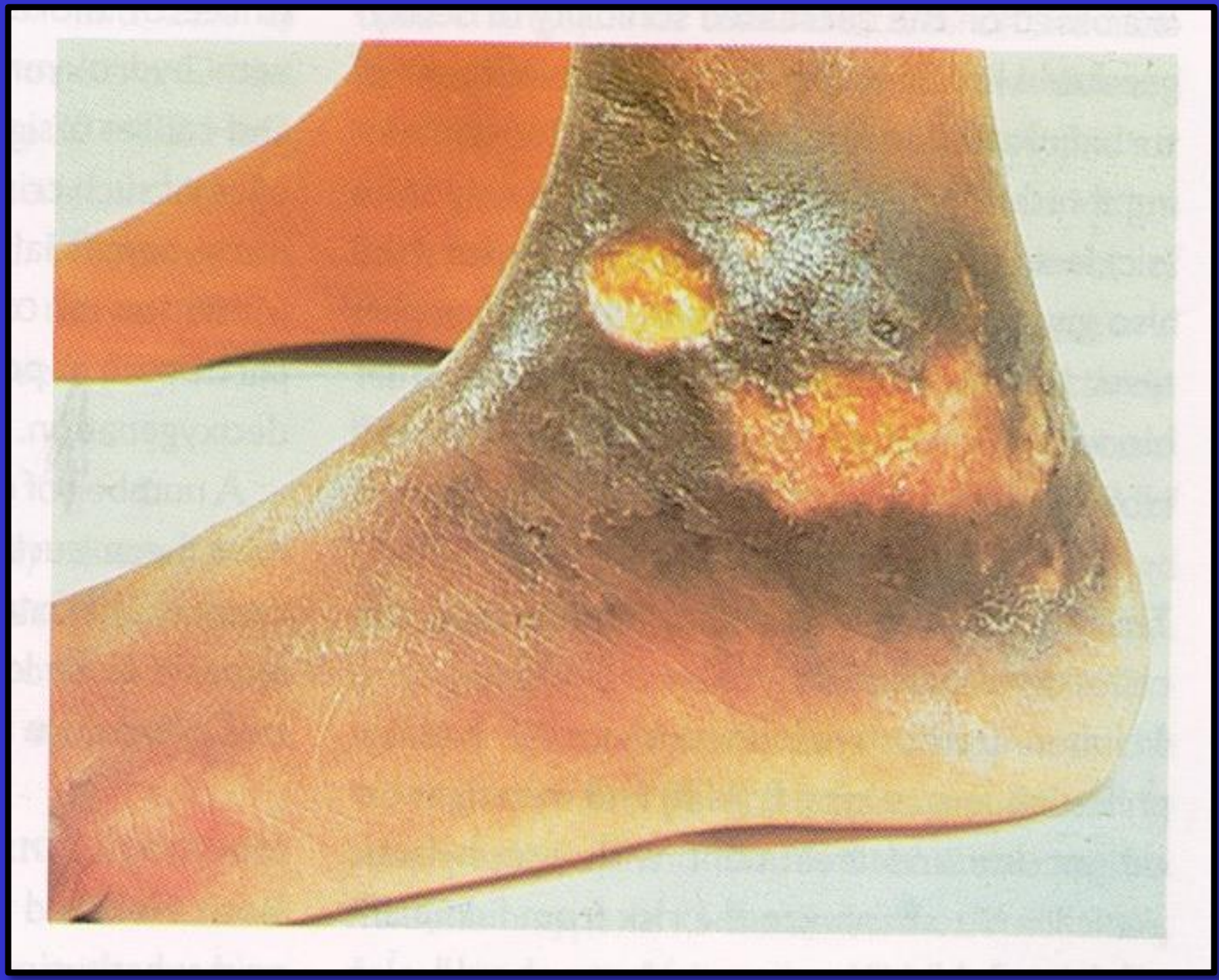


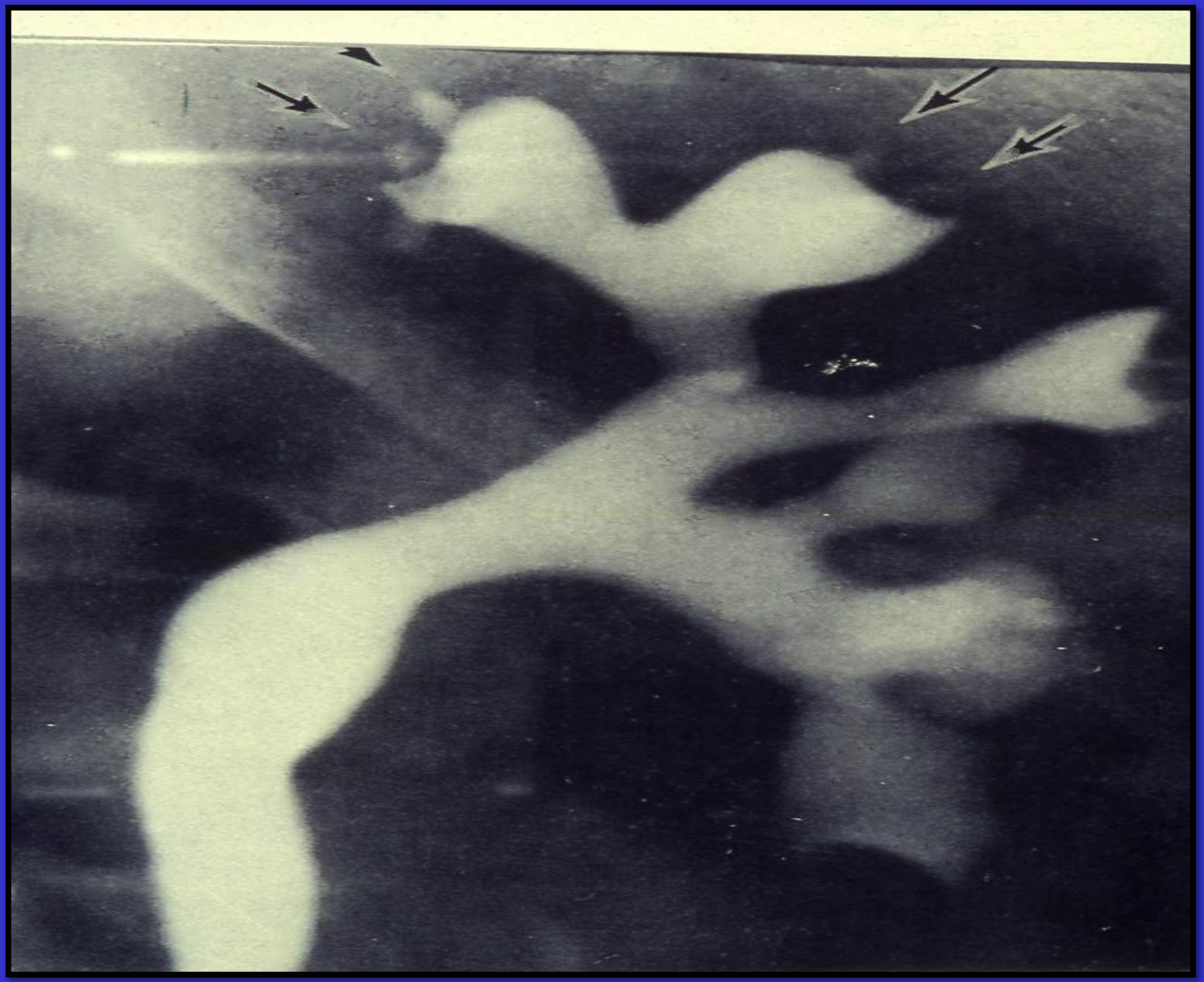






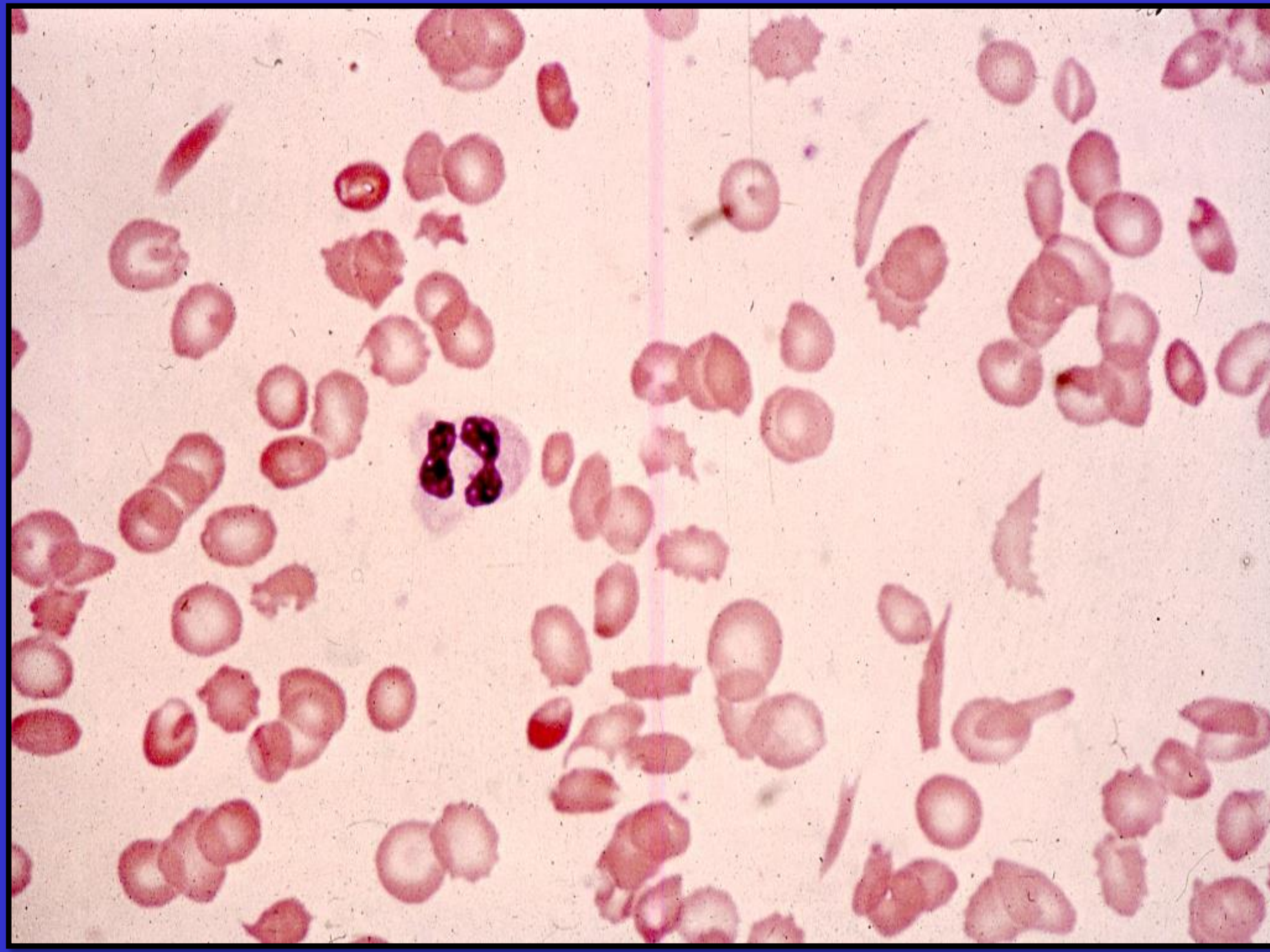


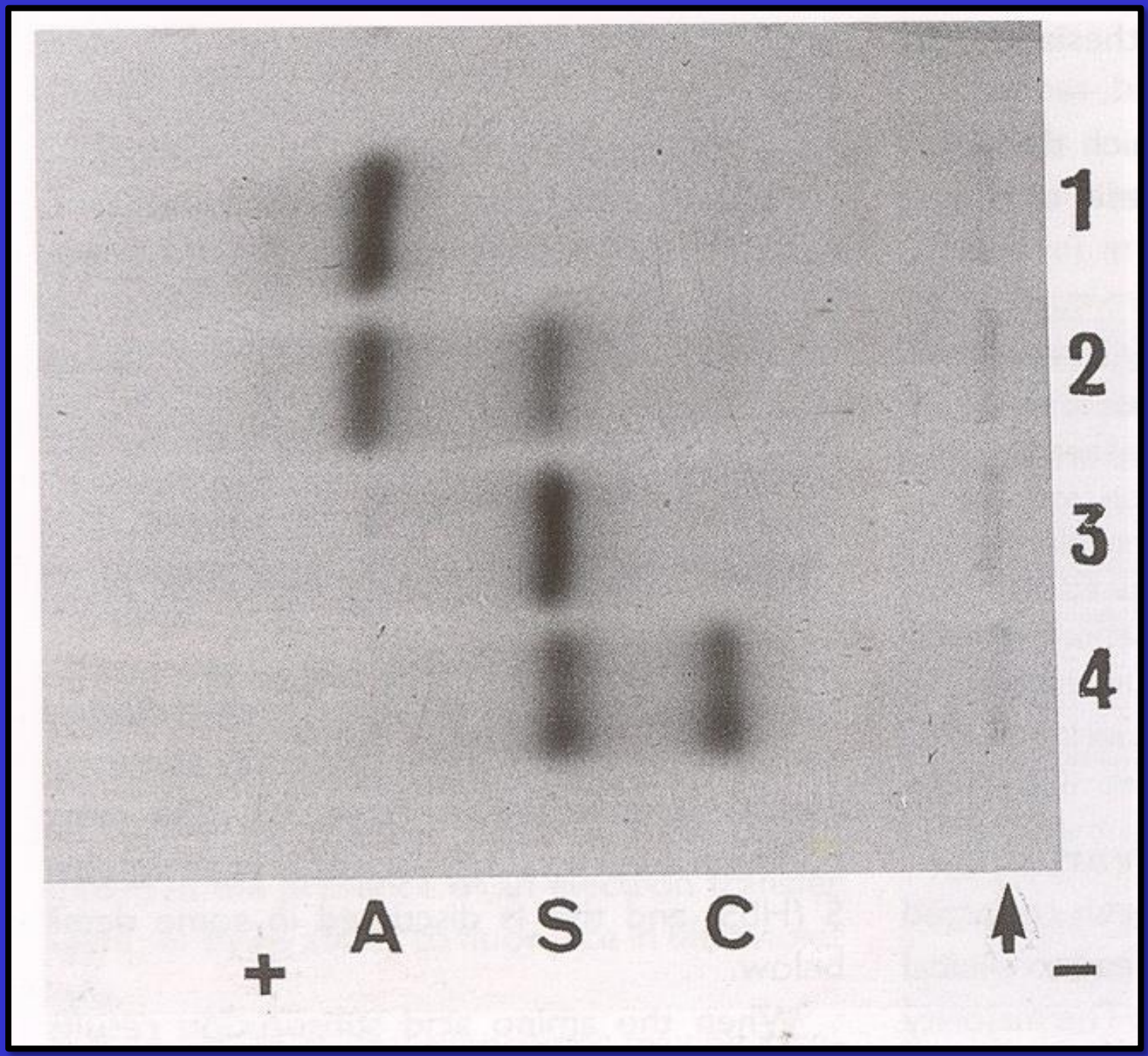




Laboratory Diagnosis of Sickle Cell Disease

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

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+
–

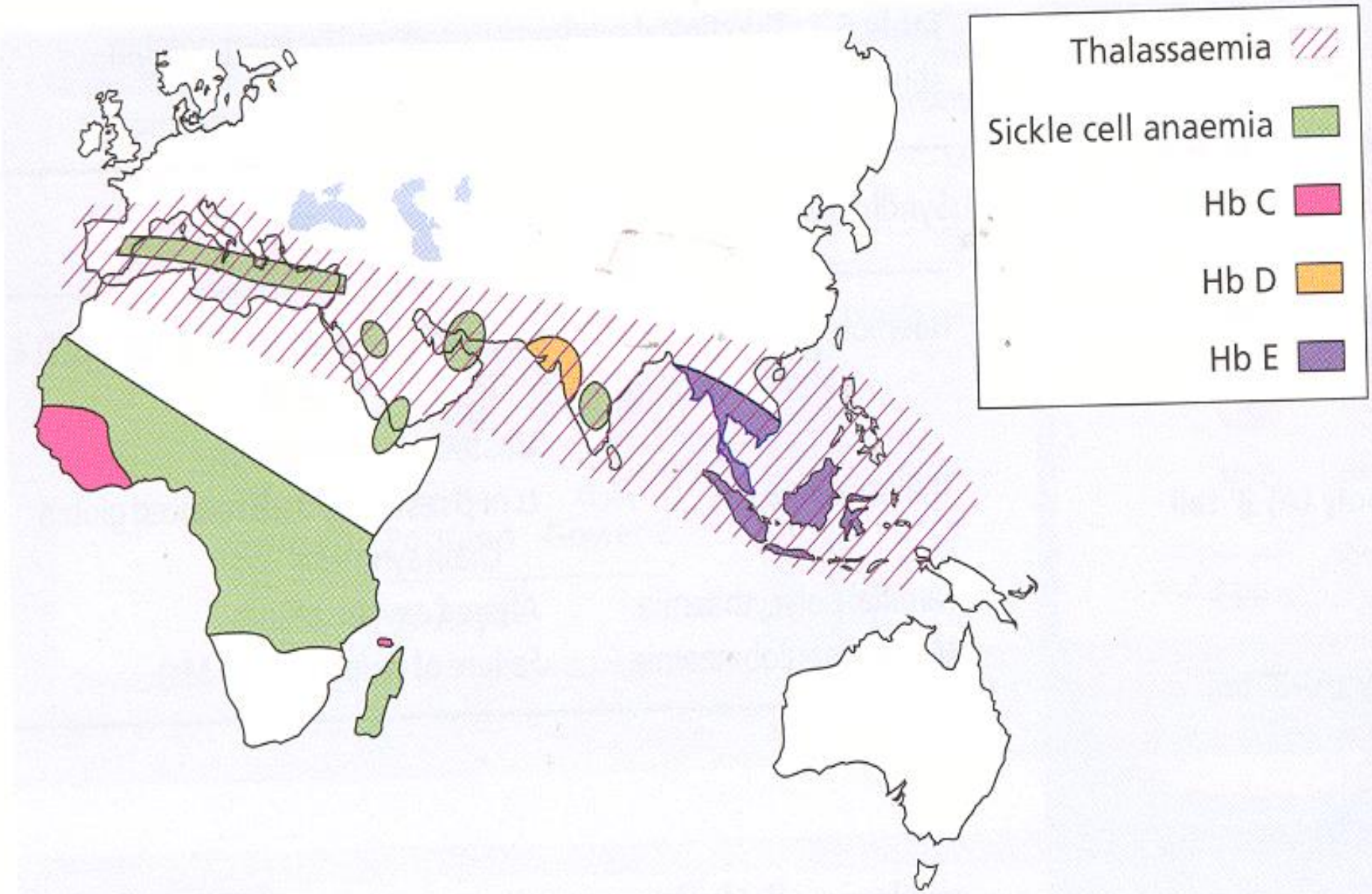
Indications for Blood Transfusion in Sickle Cell Anaemia

- 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

HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION



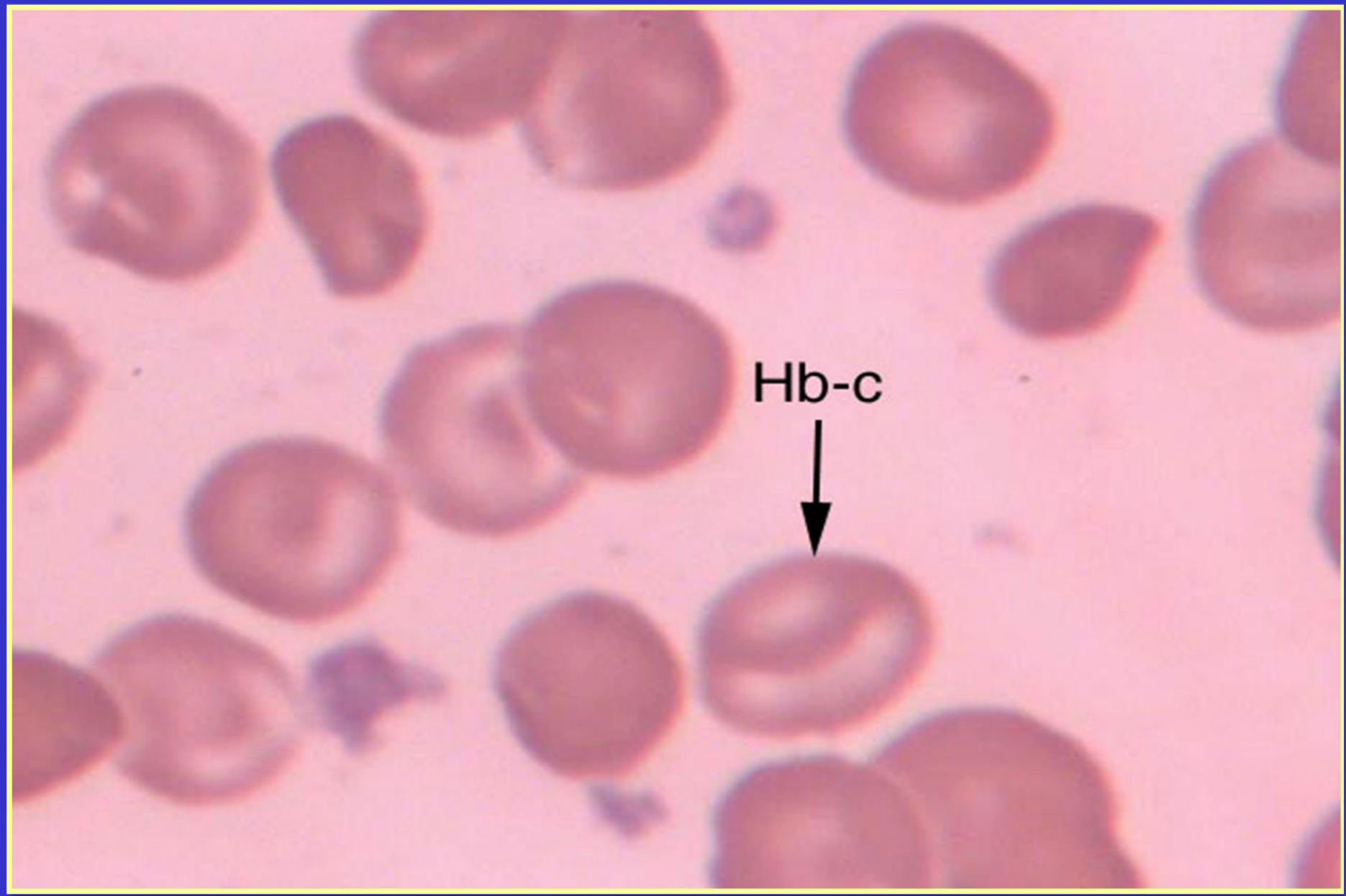
Abnormal Haemoglobin Variants

Hb C:-

- Is due to replacement of glutamic acid in position 6 of the beta chain by lysine ($\alpha_2\beta_2$ 6-GLU→ LYS).
- About 7-22% of people of West Africa are hetrozygotes especially Nigeria and North Ghana
- Homozygotes are rare and have mild to moderate hemolytic anaemia with many thick target RBCs in the blood film and mild to moderate splenomegaly.
- The chronic hemolytic anaemia is due to reduced red cell deformability on deoxygenation.

  Deoxygenated HbC is less soluble than deoxygenated HbA.
- Double heterozygotes with sickle Hb S/C give moderate to severe anaemia with symptoms of sickle cell disease.

HAEMOGLOBIN C DISEASE



Hb D Punjab

($\alpha_2\beta_2$-121 GLU → GLN)

- Prevalent in Indian and Pakistani in every 100 persons about 1 trait (1% of the population).
- Trait are usually healthy.
- Homozygous D/D have mild to moderate anaemia.
- Combined double heterozygotes Hb S/D can give rise to moderate to a severe anaemia and symptoms of sickle cell disease.

Hb E:

- ($\alpha_2\beta_2$ 26 GLU → LYS) is one of the most common beta-chain variants.
- It is very prevalent in South East Asia (50%) of the population are heterozygotes.
- Patients who are homozygous generally have mild haemolytic anaemia, microcytic hypochromic red cells and mild enlargement of the spleen.
- Carriers are symptomless unless they have combined other mutations such as the one for alpha thalassemia, or beta-thalassemia trait.

Hb O Arab

($\alpha_2\beta_2$-121 GLU → LYS)

- Heterozygotes are not symptomatic.
- Double heterozygous with sickle S/O are clinically severe.
- Hb O- Arab enhance the polymerization of HbS.

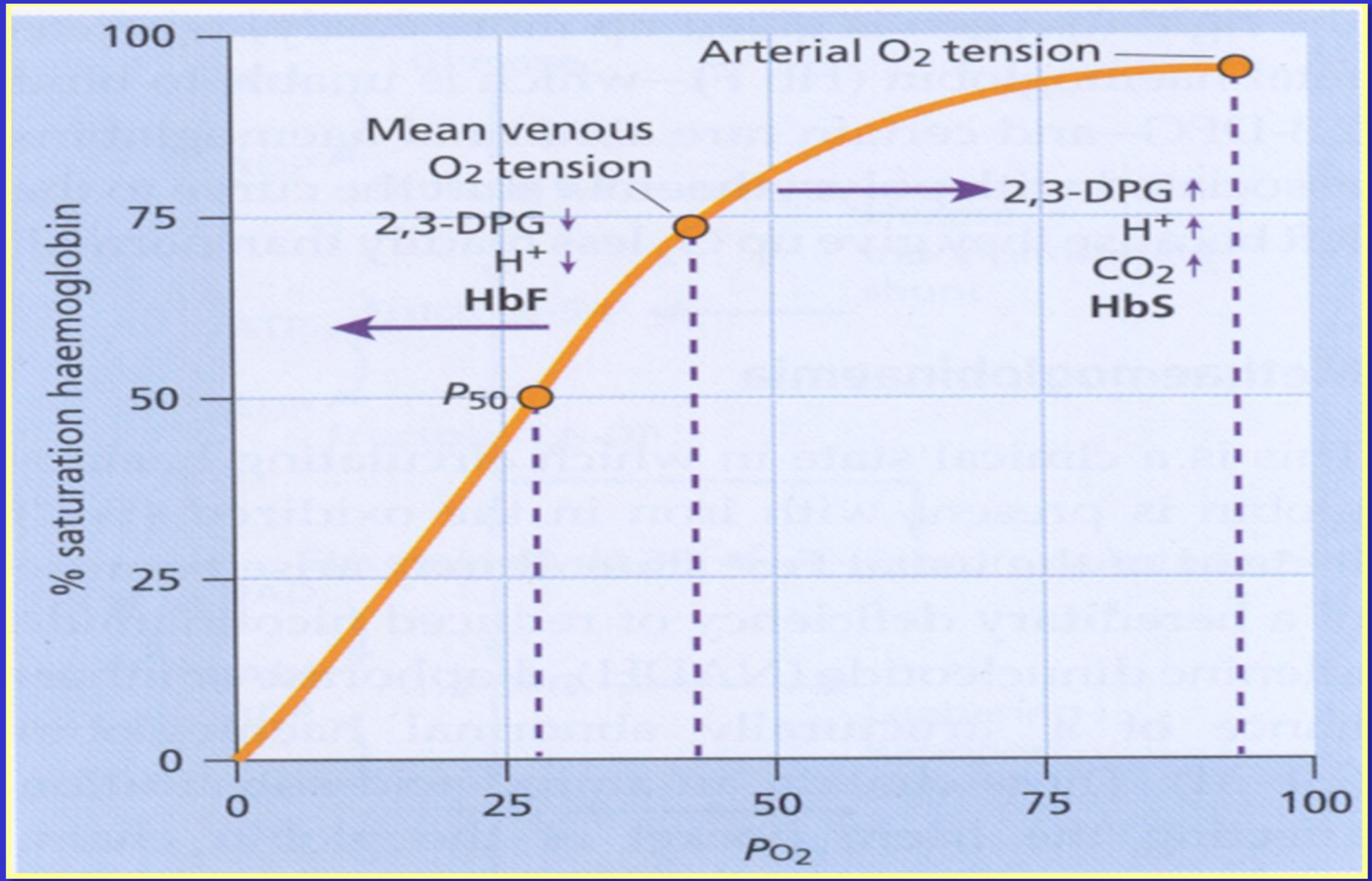
High Oxygen affinity haemoglobins

Hb Chesapeake:

(α_2-92 ARG → LEU β_2).

- Carriers are without clinical symptoms.
- Homozygous of erythrocytosis (polychemia) due to increased O_2 affinity.
- The patients have no splenomegaly. (except for patient's with concomitant β-thalassemia).
- They have normal WBC, and normal platelets.

* High Hb, High RBCs count and high haematocrit. (HCT).



The haemoglobin oxygen (O_2) dissociation cruve. 2,3-DPG, 2,3-diphosphoglycerate.

Unstable Haemoglobins

Hb koln ($\alpha_2\beta_2$-98 VAL → MET)

Hb Hammersmith ($\alpha_2\beta_2$ 42 PHE → SER)

Hb Hasharon (α_2-47 ASP → HIS β_2).

- **These abnormal haemoglobin cause haemolysis in the newborn (congenital non-spherocytic haemolytic anaemia).**
- **Heinz body hemolytic anaemia with sensitivity to oxidant drugs, such as sulfonamides.**
- **Reticulocytosis out of proportion to the level of Hb.**
- **Increased formation of methemoglobin.**
- **Spontaneous or drug induced haemolytic anaemia due to instability of the haemoglobin and consequent intracellular precipitation.**
- **Thalassaemia – like peripheral blood picture.**

  ***Clinically:** The patient have anemia, jaundice, splenomegaly / hepatomegaly and gall stones.*

Low oxygen affinity haemoglobins

- ❖ More than 50 variants with reduced oxygen affinity have been identified.

- Hb kansas ($\alpha_2\beta_2$102 ASN → THR)
- Hb Aukland ($\alpha_2\beta_2$ 25 GLY → ASP)
- Rare as homozygotes.
- Patients have anaemia and congenital cynosis due to reduced oxygen affinity.

Congenital Methaemoglobinaemia

- **Hb M Boston (α_2 58 HIS → TYR - β_2)**
- **Hb M Saskatoon (α_2-β_2-63 HIS → TYR)**
- **Hb M Hyde park ($\alpha_2\beta_2$ 92 HIS → TYR)**
- **Hb M IWATE ($\alpha_2$87 HIS → TYR-β_2)**

Cynosis in homozygotes due to congenital methaemoglobinaemia as a consequences of substitution of amonoacids near or in haem pocket.

Hb Indianapolis

- **(α_2-$\beta_2$112 CYS – ARG)**
- **Is a rare and slightly unstable beta-globin variant.**
- **Carriers are clinically normal with only mild reticulocytosis.**
- **Homozygons have haemolytic anaemia and renal failure in severe cases.**
- **Thalassaemia-like syndrome due to marked instability of the Hb.**

EFFECTS OF HAEMOGLOBIN VARIANTS

Variant	Clinical and haematological abnormalities
HbS	Recurrent painful crises (in adults) and chronic haemolytic anaemia; both related to sickling of red cells on deoxygenation*
HbC	Chronic haemolytic anaemia due to reduced red cell deformability on deoxygenation, * deoxygenated HbC is less soluble than deoxygenated HbA.
Hb Köln, Hb Hammersmith	Spontaneous or drug-induced haemolytic anaemia due to instability of the Hb and consequent intracellular precipitation.
HbM Boston, HbM Saskatoon	Cyanosis due to congenital methaemoglobinaemia as a consequence of a substitution near or in the haem pocket.
Hb Chesapeake	Hereditary polycythaemia due to increased O_2 affinity.
Hb Constant Spring, Hb Lepore, HbE	Thalassaemia-like syndrome due to decreased rate of synthesis of normal chains.
Hb Indianapolis	Thalassaemia-like syndrome due to marked instability of Hb

** Only in homozygotes*

KKUH

Heamatology Unit

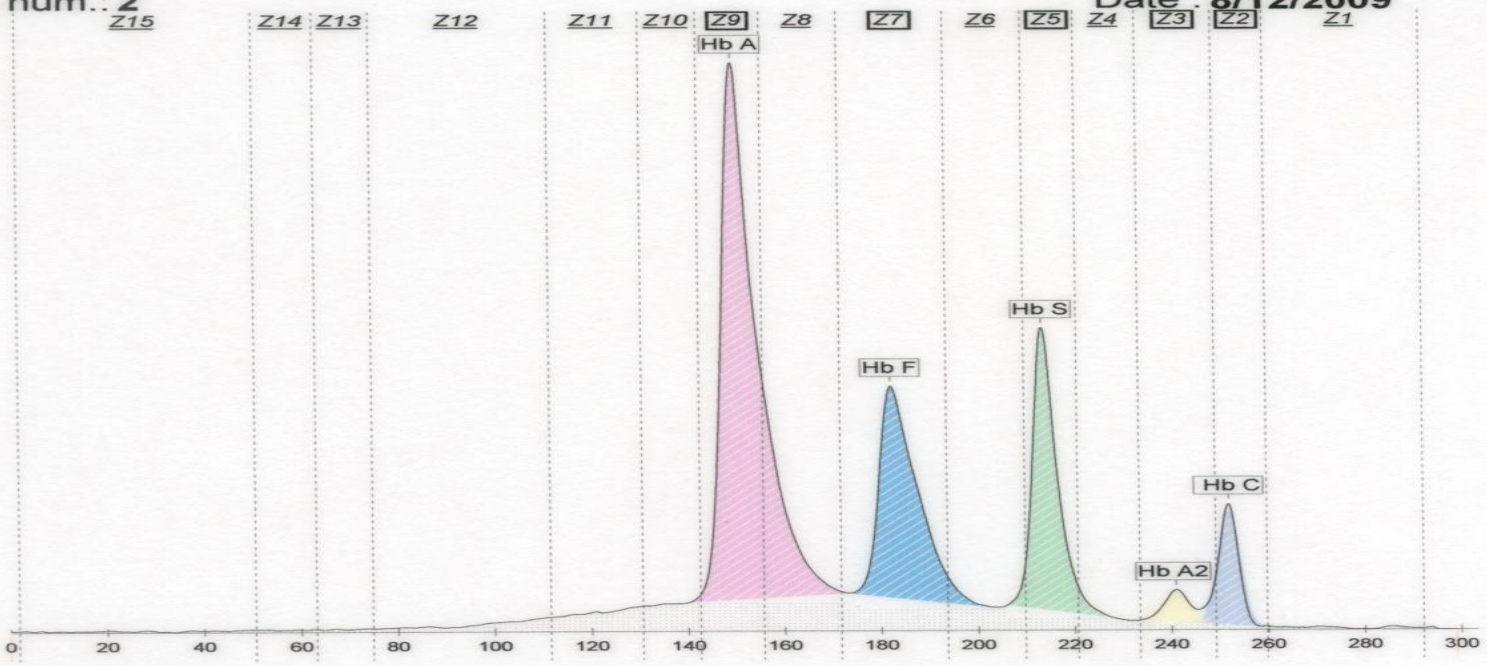
Hb Electrophoresis

Hospital No.: **QC Hb AFSC CONTROL-**

ID : **Hb AFSC CONTROL-2**

Sample num.: **2**

Date : **8/12/2009**



Hb Electrophoresis

Fractions	%	Ref. %
Hb A	**51.3**	46.7 - 56.9
Hb F	**21.4**	17.4 - 22.4
Hb S	**18.3**	17.3 - 22.3
Hb A2	**2.3**	2.1 - 3.3
Hb C	**6.7**	4.6 - 7.0

KKUH

Heamatology Unit

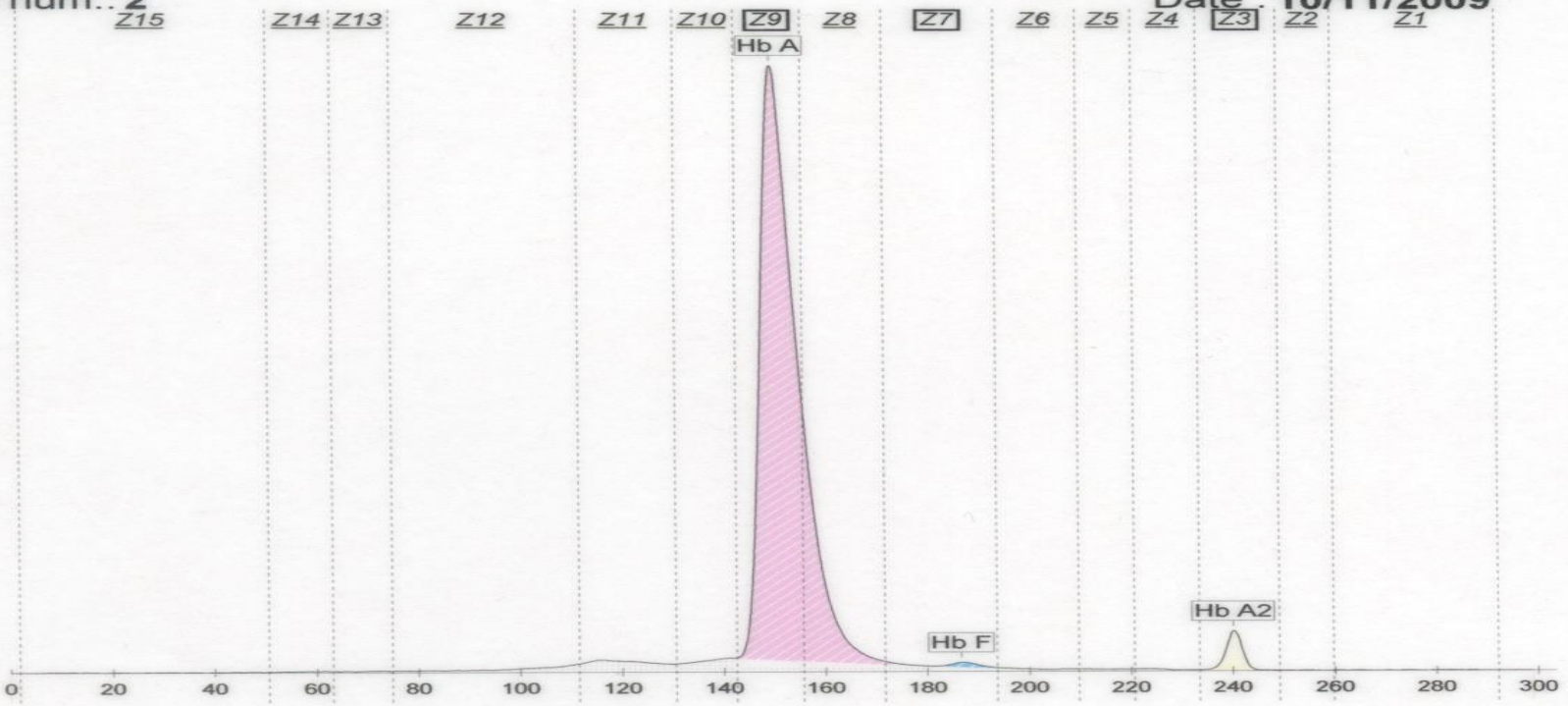
Hb Electrophoresis

Hospital No.: **933376**

ID : **061773**

Sample num.: **2**

Date : **10/11/2009**



Hb Electrophoresis

Fractions	%	Ref. %	
Hb A	**96.7**	96.8 - 97.8	
Hb F	**0.5**	=< 2.0	<
Hb A2	**2.8**	1.5 - 3.5	

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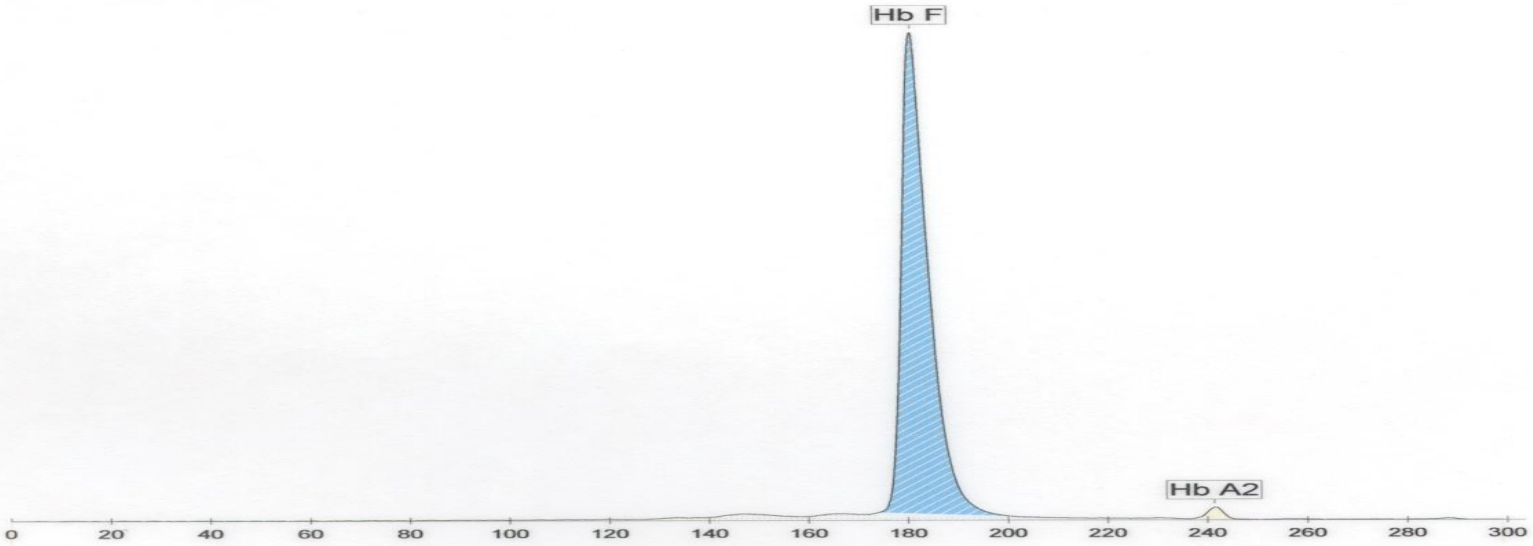
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: 921107 ID : 063761

Sample No 54 Date : 09/05/2010



Fractions	%	Ref. %
Hb F	98.5	
Hb A2	1.5	

Comment :

28/3/2010

CBC Hb 98

MCV 73

NRBC 34

KKUH

Heamatology Unit

Hb Electrophoresis

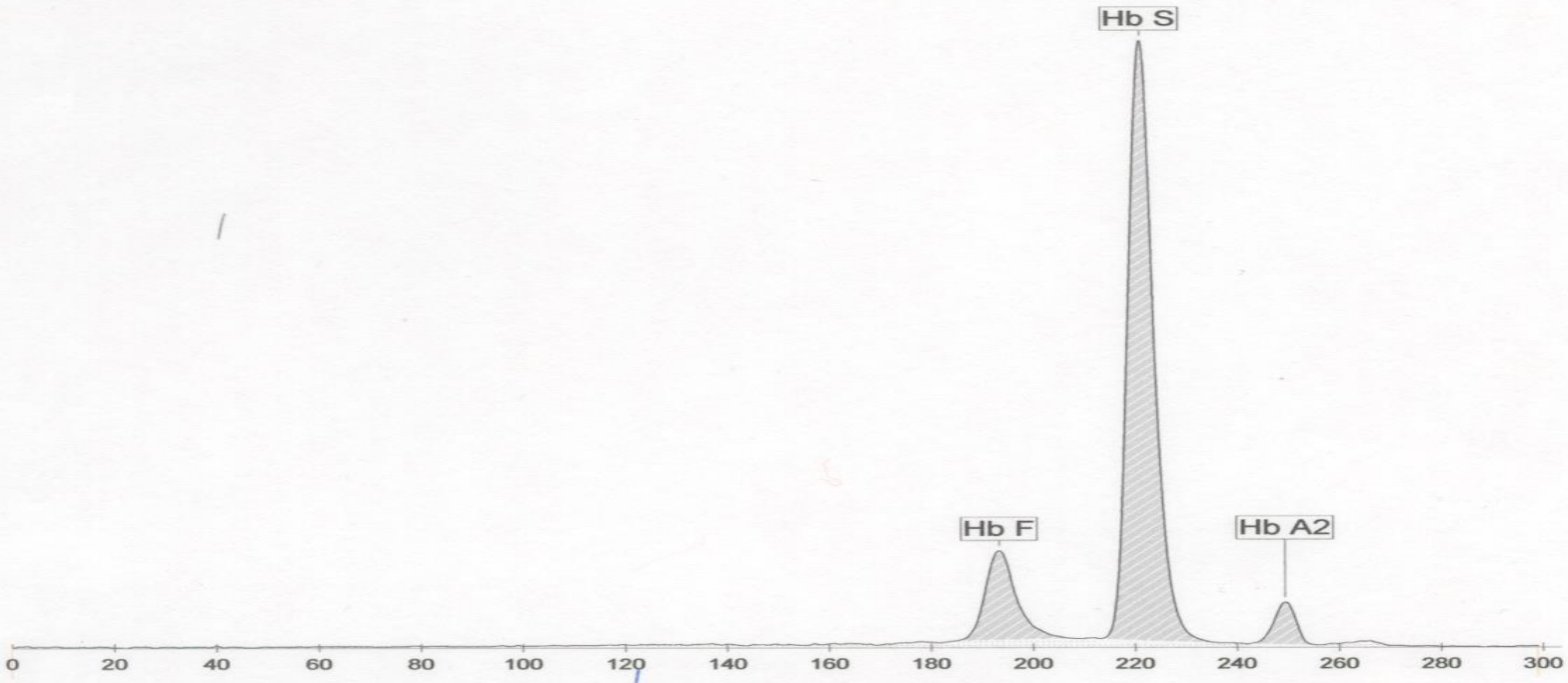
INSTRUMENT ID : KKUH : 24509

Hospital No.: 233095

Sample No 20

ID : **063478**

Date : **17/04/2010**



Fractions	%	Ref. %
Hb F	14.7	
Hb S	80.5	
Hb A2	4.8	

KKUH

Heamatology Unit

Hb Electrophoresis

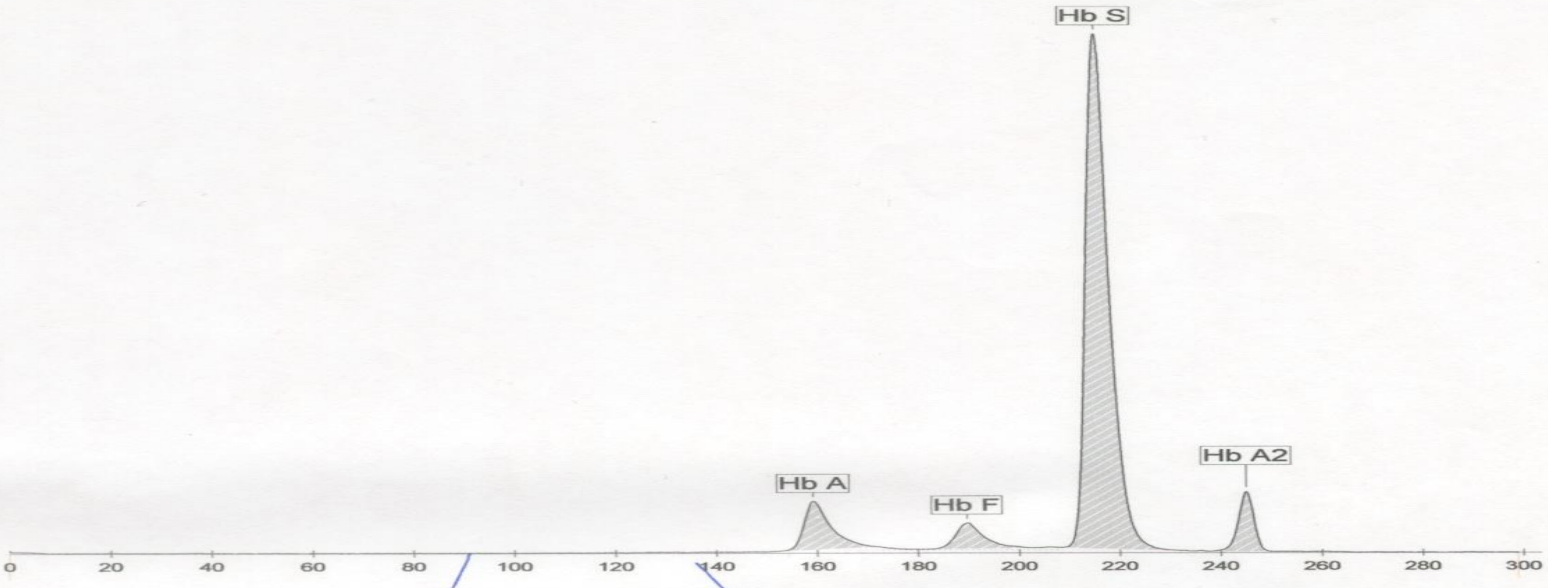
INSTRUMENT ID : KKUH : 24509

Hospital No.: **913628**

Sample No **34**

ID : **063511**

Date : **19/04/2010**



Fractions	%	Ref. %
Hb A	**8.7**	
Hb F	**4.9**	
Hb S	**80.1**	
Hb A2	**6.3**	

Comment :

Homozygous sickle cell thal

KKUH

Heamatology Unit

Hb Electrophoresis

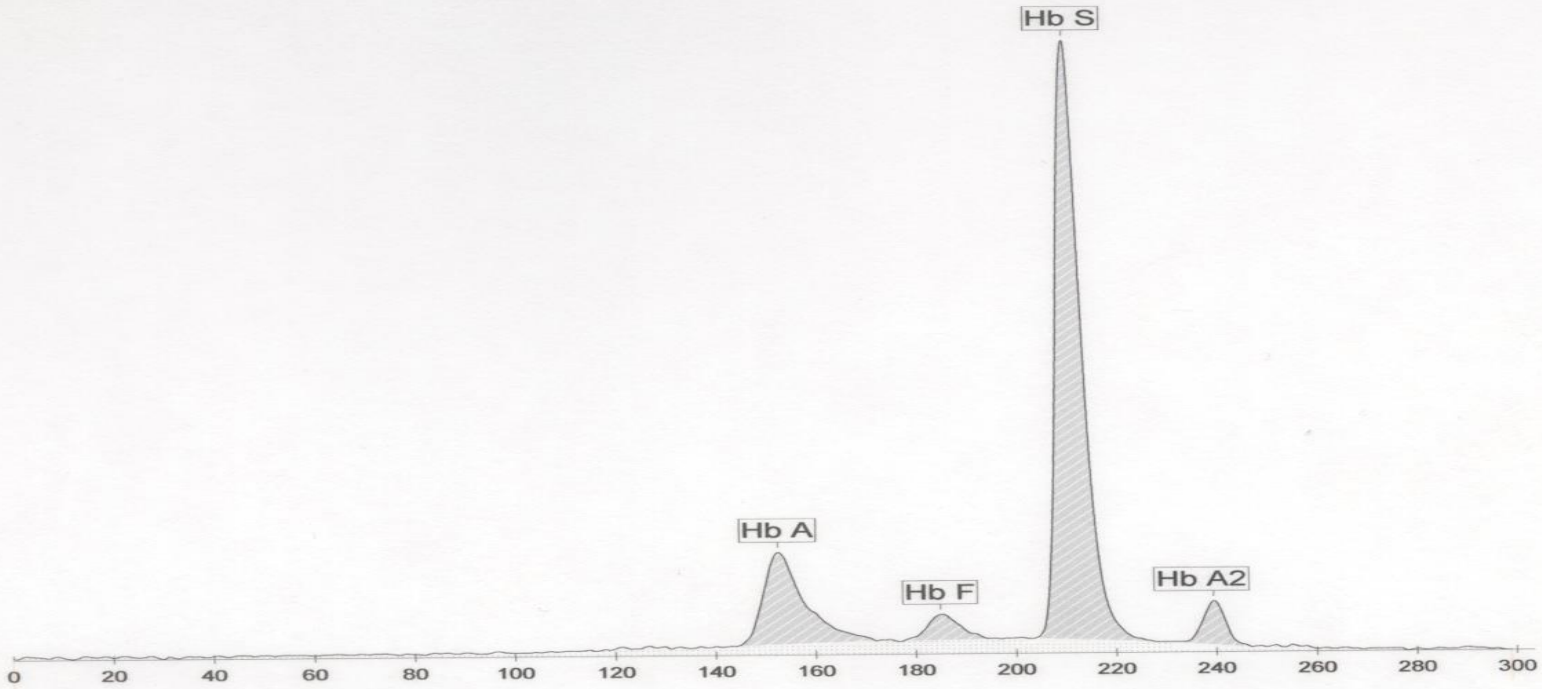
INSTRUMENT ID : KKUH : 24509

Hospital No.: **010755**

Sample No **19**

ID : **064209**

Date : **28/06/2010**



Fractions	%	Ref. %
Hb A	18.0	
Hb F	4.0	
Hb S	73.3	
Hb A2	4.7	

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Heamatology Unit

Hb Electrophoresis

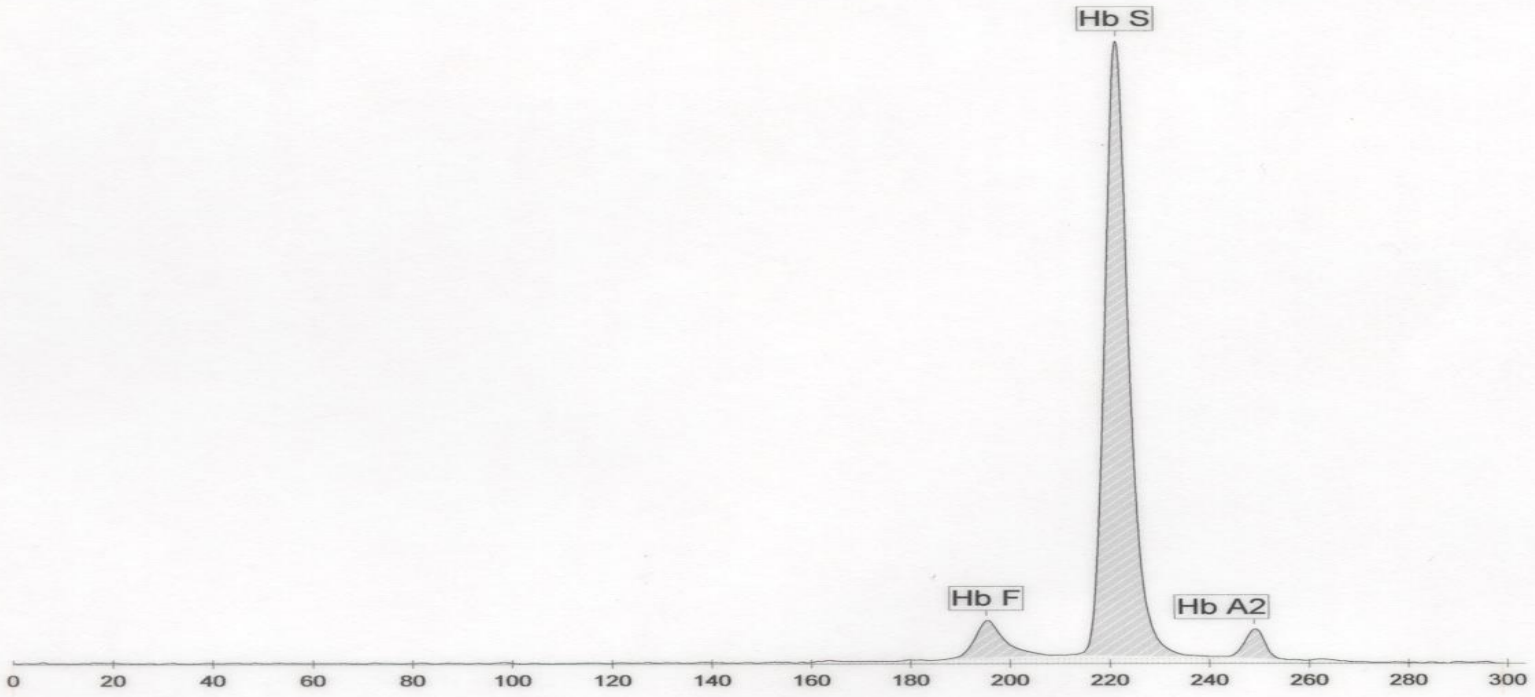
INSTRUMENT ID : KKUH : 24509

Hospital No.: 594729

Sample No 37

ID : **064199**

Date : **27/06/2010**



Fractions	%	Ref. %
Hb F	6.5	
Hb S	89.9	
Hb A2	3.6	

KKUH

Heamatology Unit

Hb Electrophoresis

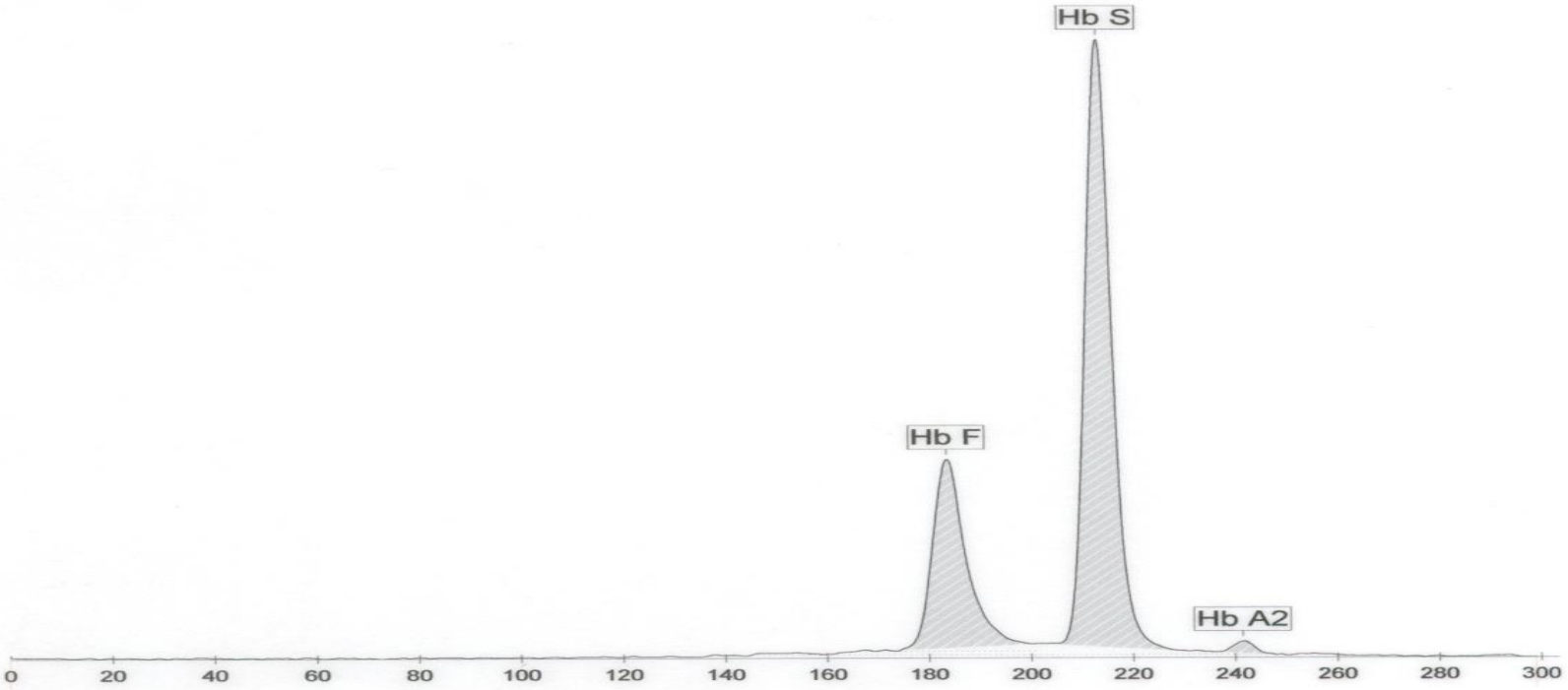
INSTRUMENT ID : KKUH : 24509

Hospital No.: **610043**

Sample No **52**

ID : **064229**

Date : **29/06/2010**



Fractions	%	Ref. %
Hb F	28.1	
Hb S	70.8	
Hb A2	1.1	

KKUH

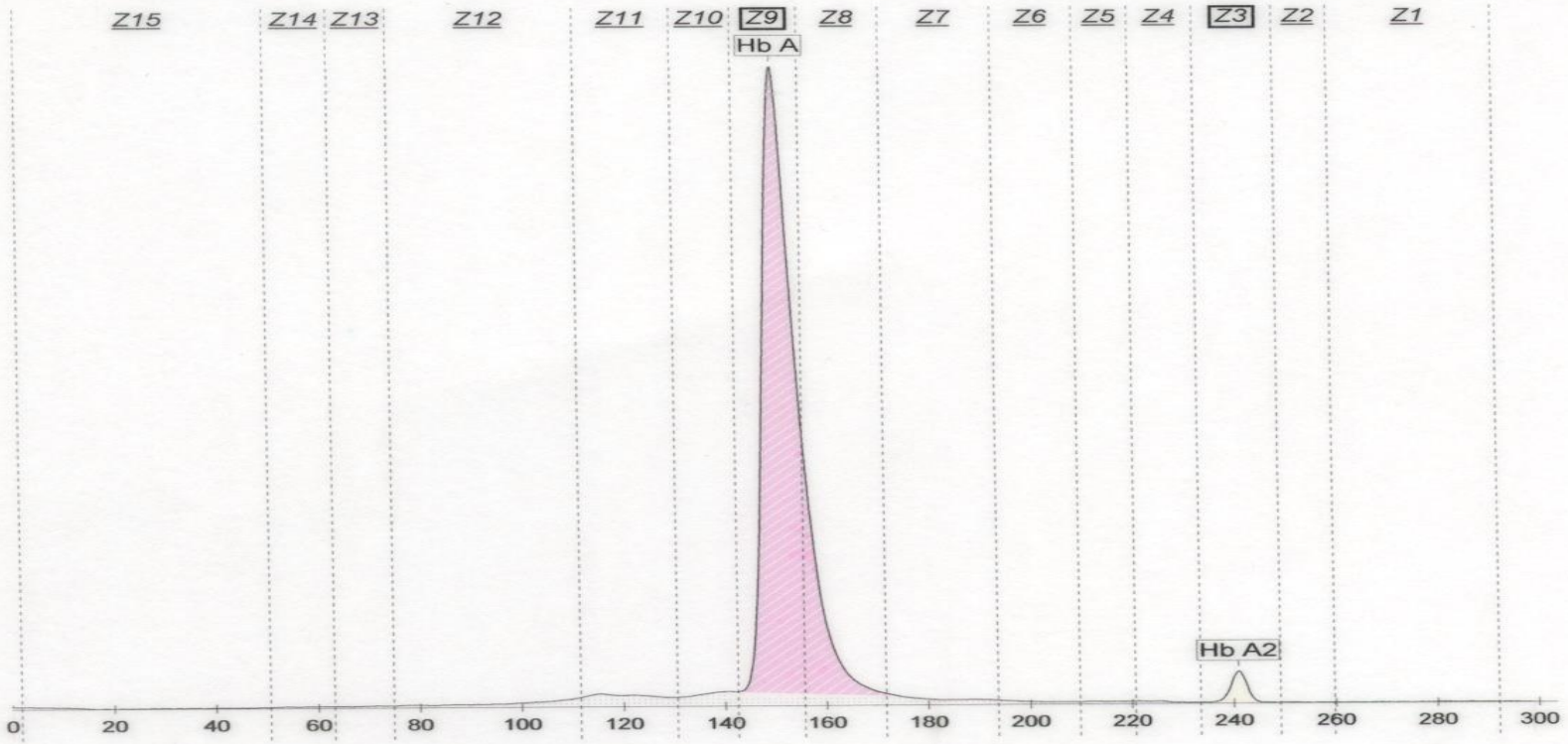
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: **Rack: SEBIA Pos.: 2** ID : **ABDULLAH**

Sample No **20** Date : **19/05/2010**



Fractions	%	Ref. %
Hb A	**97.7**	95.0 - 99.0
Hb A2	**2.3**	1.5 - 3.5

KKUH

Heamatology Unit

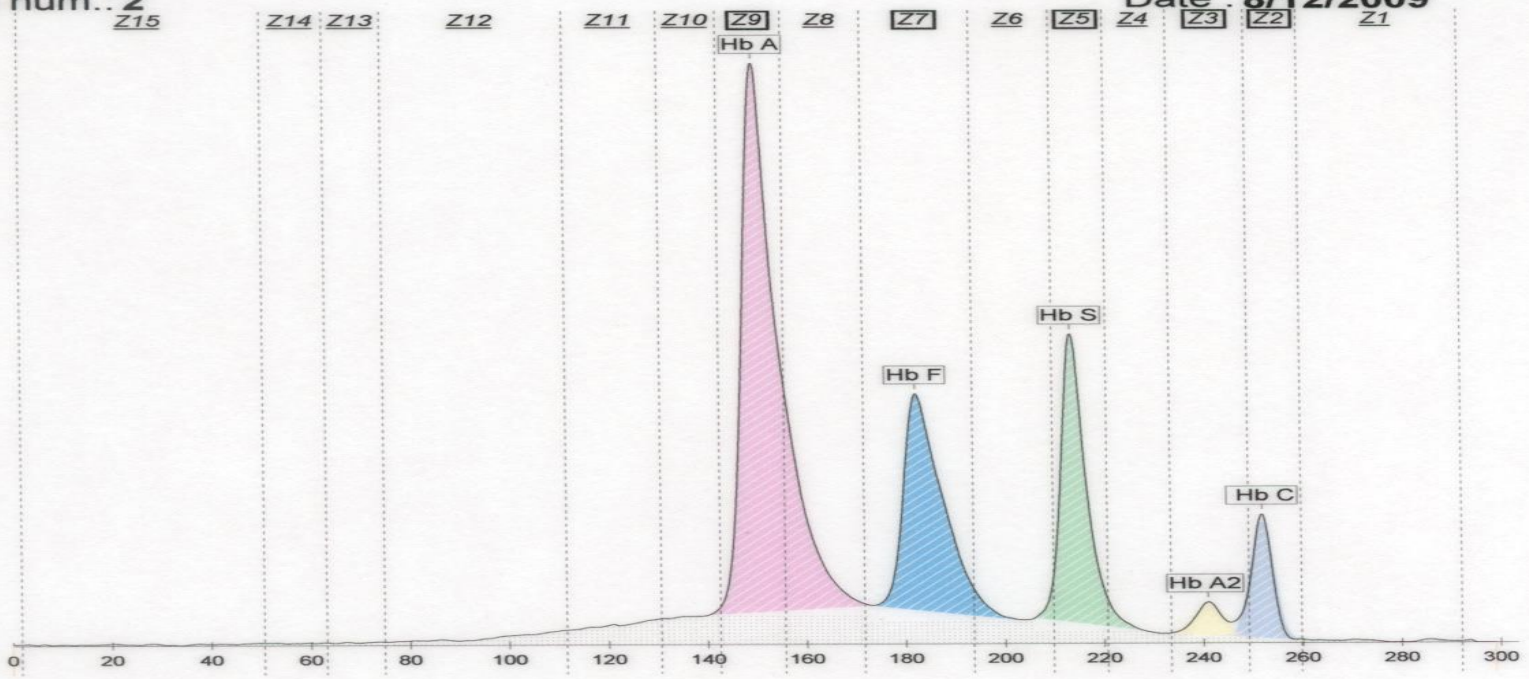
Hb Electrophoresis

Hospital No.: **QC Hb AFSC CONTROL-**

ID : **Hb AFSC CONTROL-2**

Sample num.: **2**

Date : **8/12/2009**



Hb Electrophoresis

Fractions	%	Ref. %
Hb A	**51.3**	46.7 - 56.9
Hb F	**21.4**	17.4 - 22.4
Hb S	**18.3**	17.3 - 22.3
Hb A2	**2.3**	2.1 - 3.3
Hb C	**6.7**	4.6 - 7.0

KKUH

Heamatology Unit

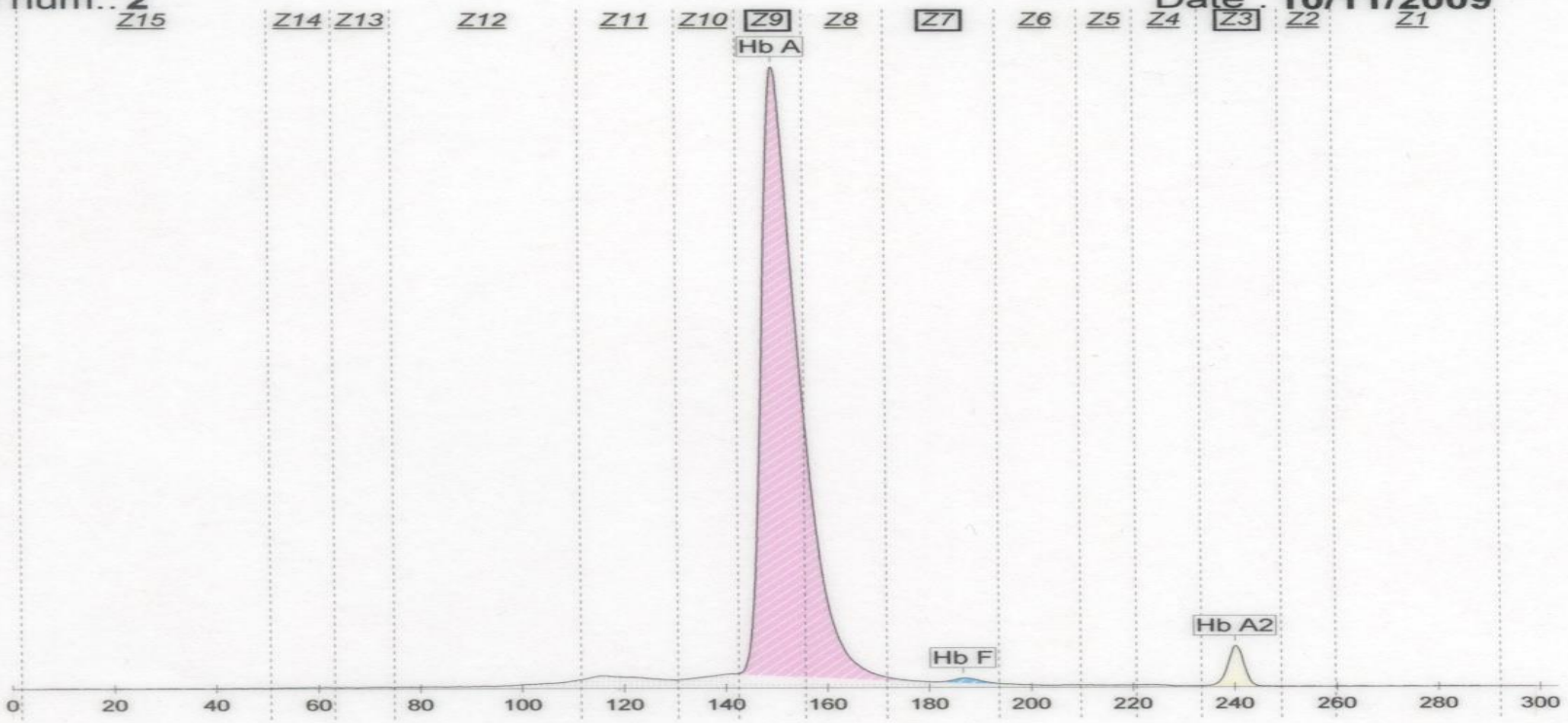
Hb Electrophoresis

Hospital No.: **933376**

ID : **061773**

Sample num.: **2**

Date : **10/11/2009**



Hb Electrophoresis

Fractions	%	Ref. %	
Hb A	**96.7**	96.8 - 97.8	<
Hb F	**0.5**	=< 2.0	
Hb A2	**2.8**	1.5 - 3.5	

KKUH

Heamatology Unit

Hb Electrophoresis

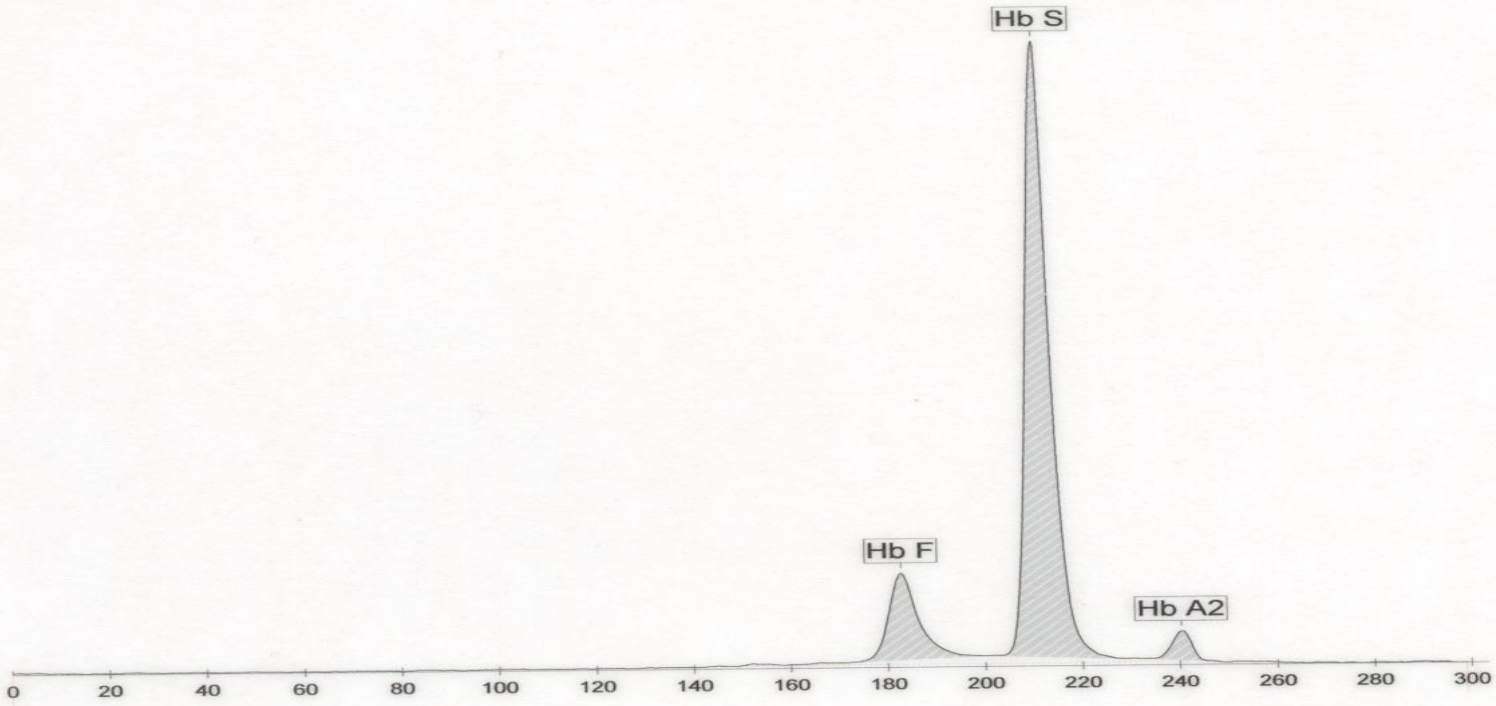
INSTRUMENT ID : KKUH : 24509

Hospital No.: **873506**

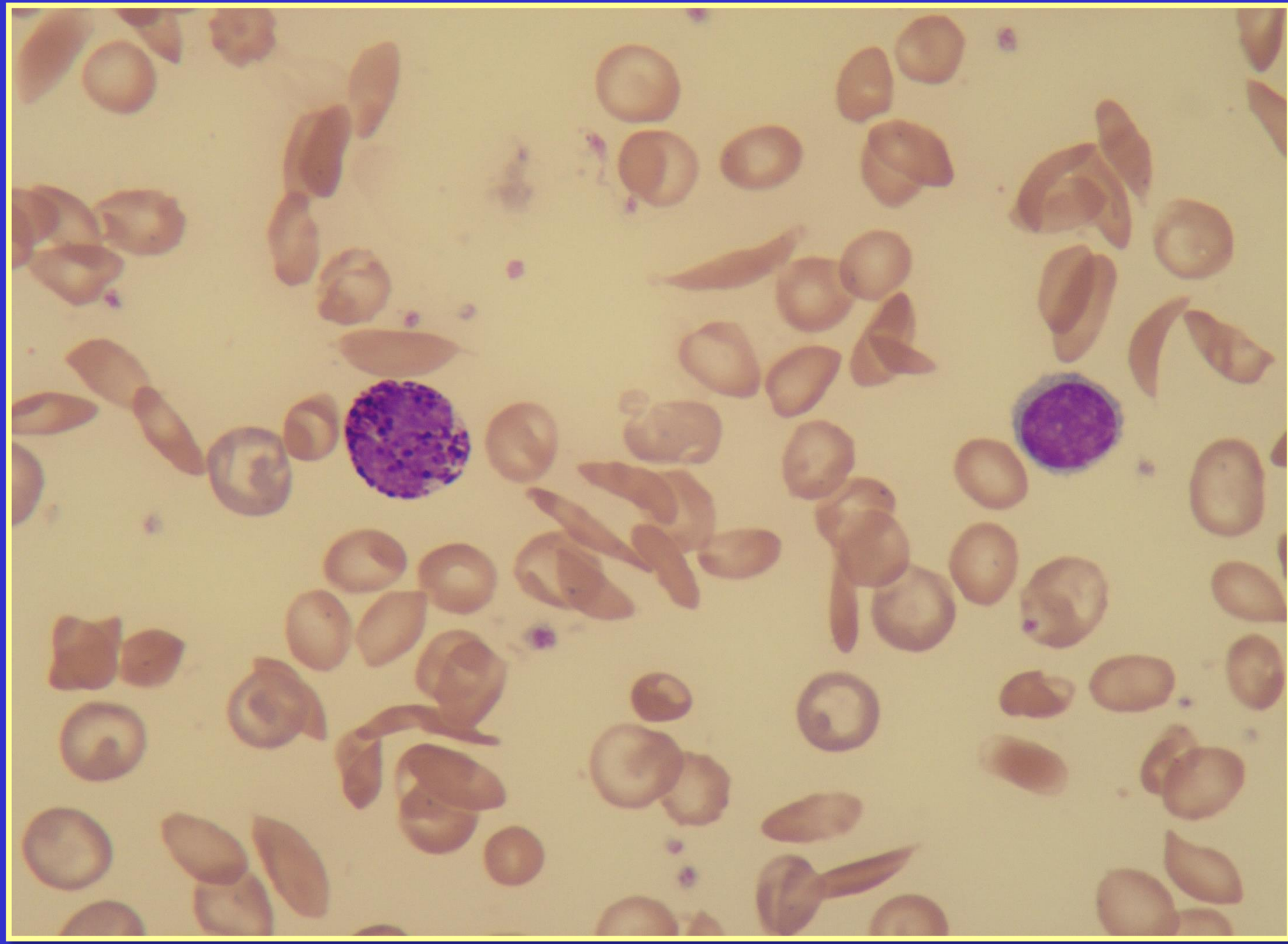
Sample No **53**

ID : **064230**

Date : **29/06/2010**



Fractions	%	Ref. %
Hb F	14.5	
Hb S	82.2	
Hb A2	3.3	



KKUH

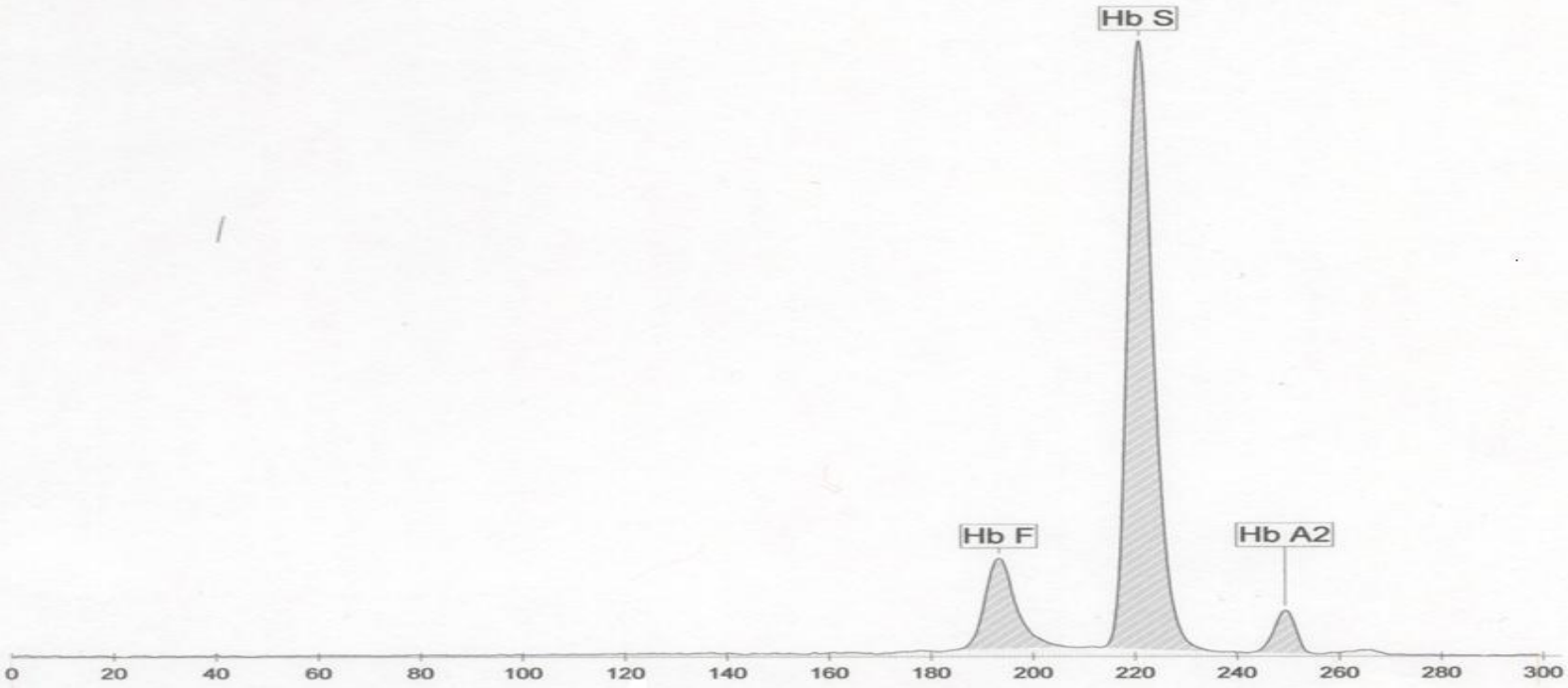
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: 233095 ID : 063478

Sample No 20 Date : 17/04/2010



Fractions	%	Ref. %
Hb F	14.7	
Hb S	80.5	
Hb A2	4.8	

KKUH

Heamatology Unit

Hb Electrophoresis

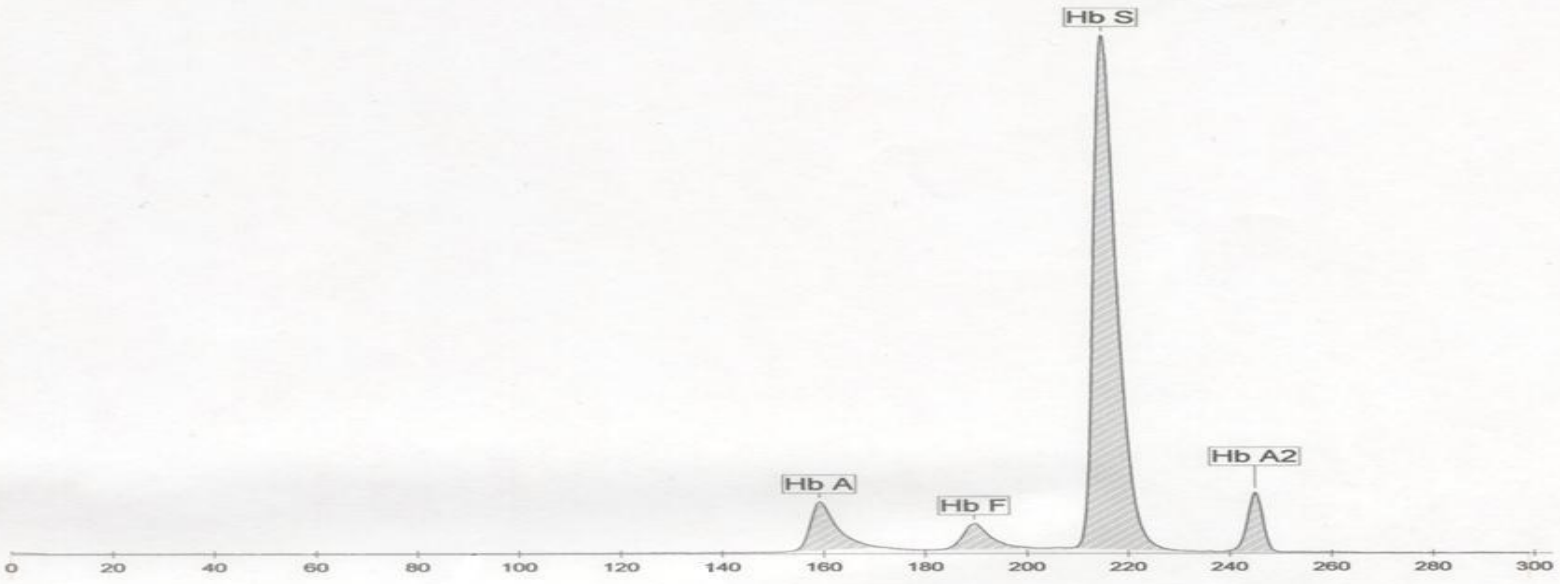
INSTRUMENT ID : KKUH : 24509

Hospital No.: 913628

Sample No 34

ID : 063511

Date : 19/04/2010



Fractions	%	Ref. %
Hb A	8.7	
Hb F	4.9	
Hb S	80.1	
Hb A2	6.3	

Comment :

KKUH

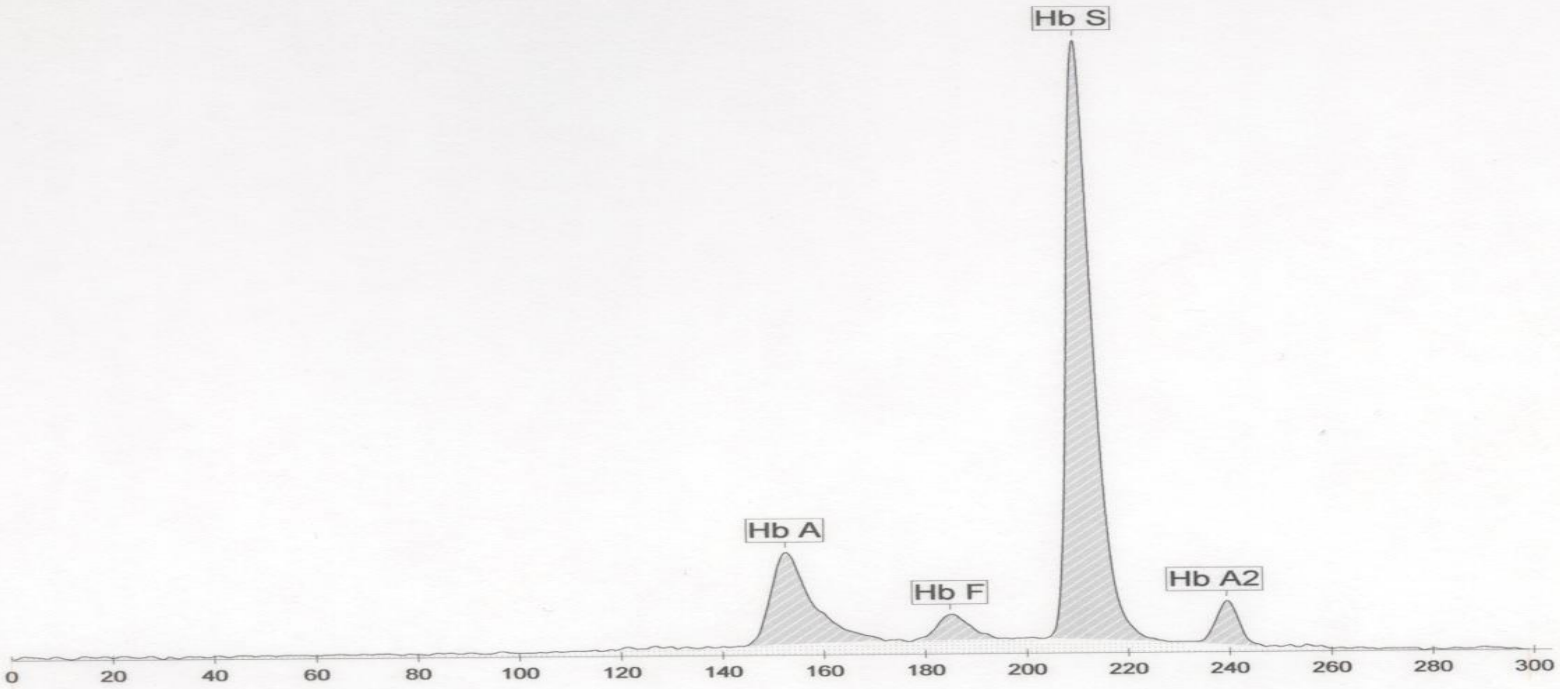
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: 010755 ID : 064209

Sample No 19 Date : 28/06/2010



Fractions	%	Ref. %
Hb A	18.0	
Hb F	4.0	
Hb S	73.3	
Hb A2	4.7	

KKUH

Heamatology Unit

Hb Electrophoresis

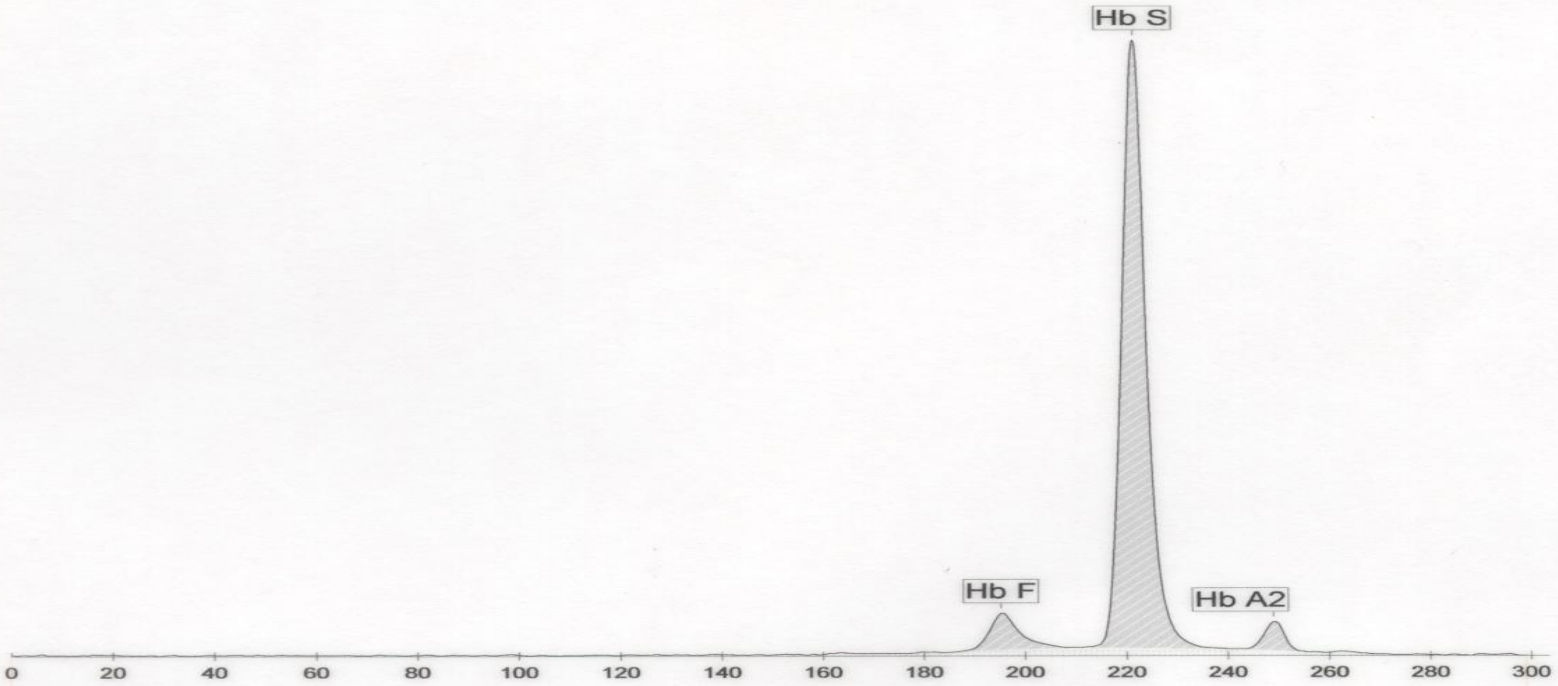
INSTRUMENT ID : KKUH : 24509

Hospital No.: 594729

Sample No 37

ID : **064199**

Date : **27/06/2010**



Fractions	%	Ref. %
Hb F	**6.5**	
Hb S	**89.9**	
Hb A2	**3.6**	

KKUH

Heamatology Unit

Hb Electrophoresis

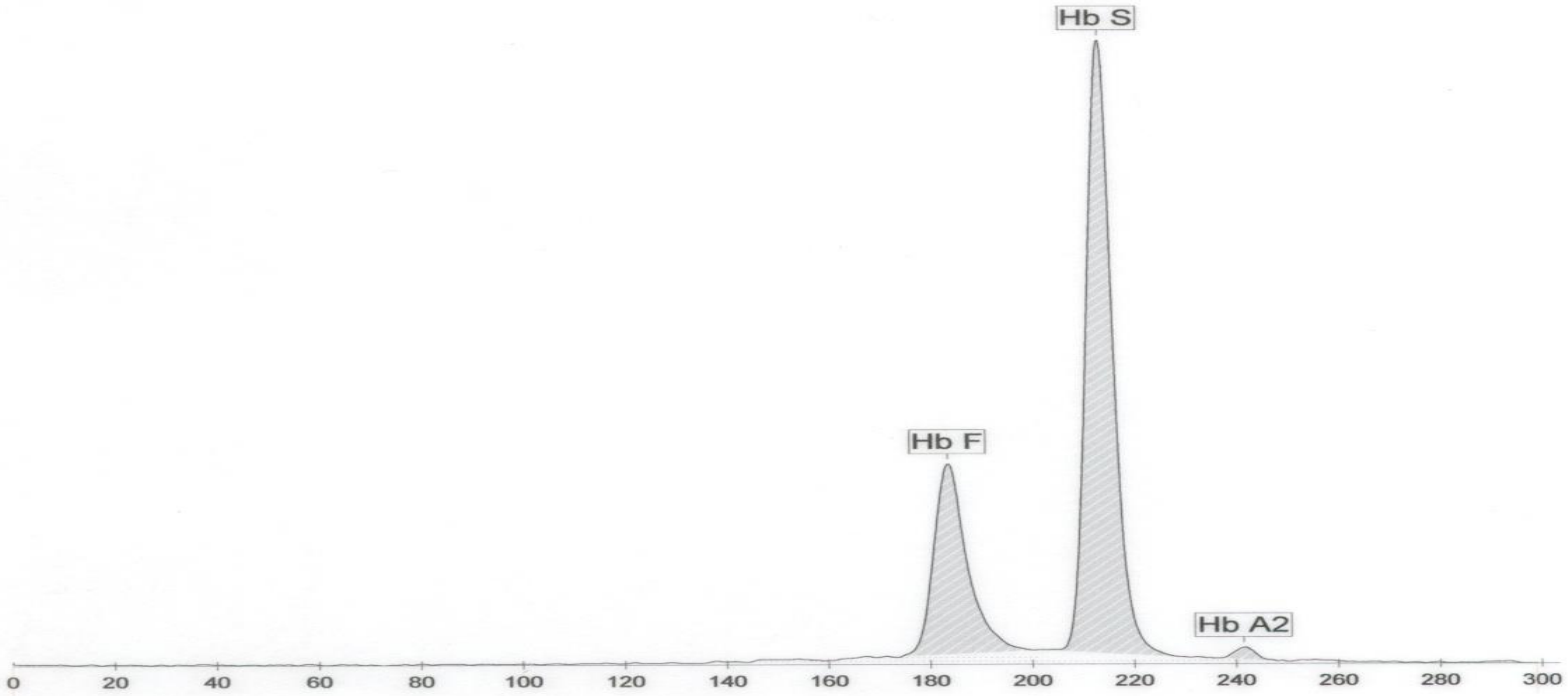
INSTRUMENT ID : KKUH : 24509

Hospital No.: 610043

Sample No 52

ID : 064229

Date : 29/06/2010



Fractions	%	Ref. %
Hb F	28.1	
Hb S	70.8	
Hb A2	1.1	

KKUH

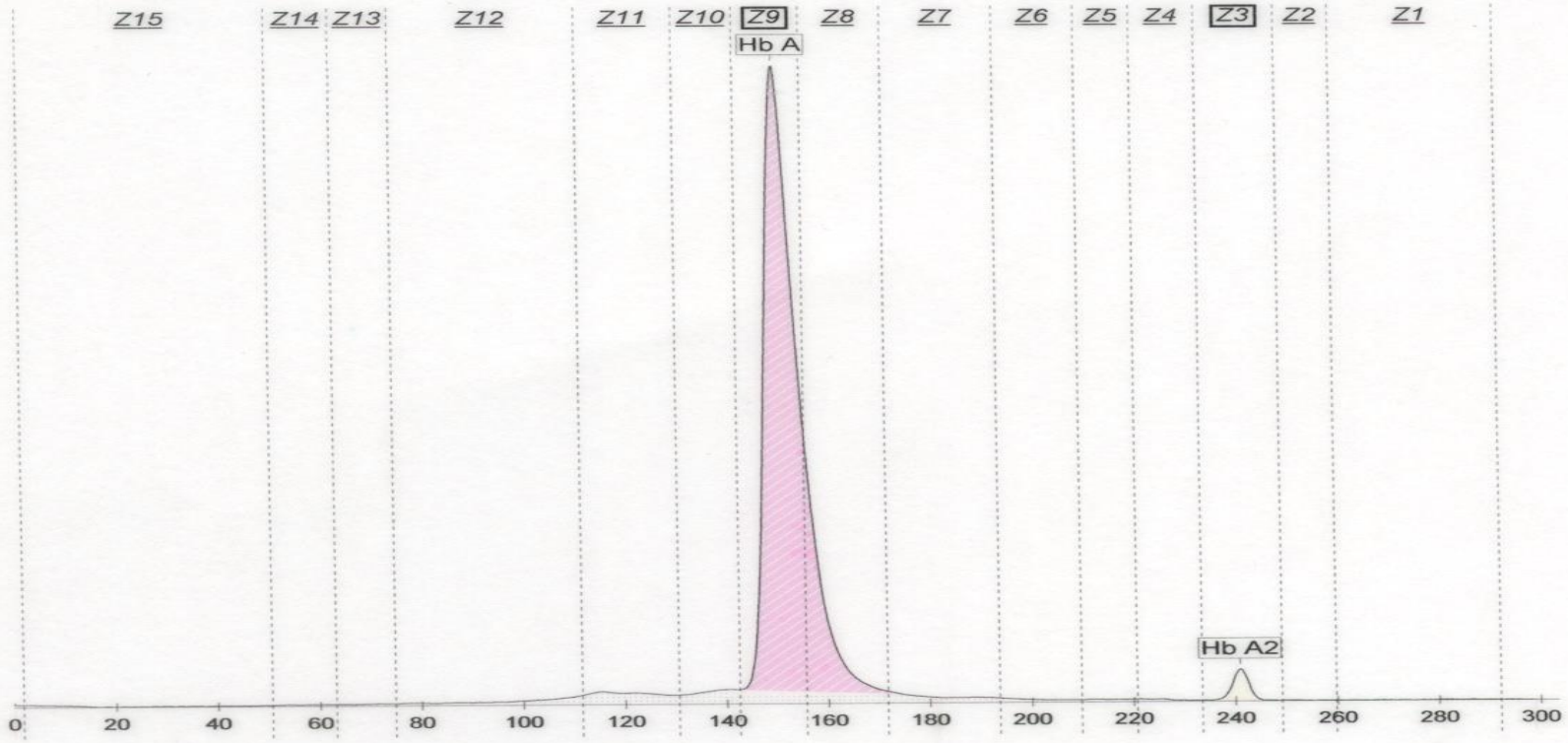
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: **Rack: SEBIA Pos.: 2** ID : **ABDULLAH**

Sample No **20** Date : **19/05/2010**



Fractions	%	Ref. %
Hb A	**97.7**	95.0 - 99.0
Hb A2	**2.3**	1.5 - 3.5

KKUH

Heamatology Unit

Hb Electrophoresis

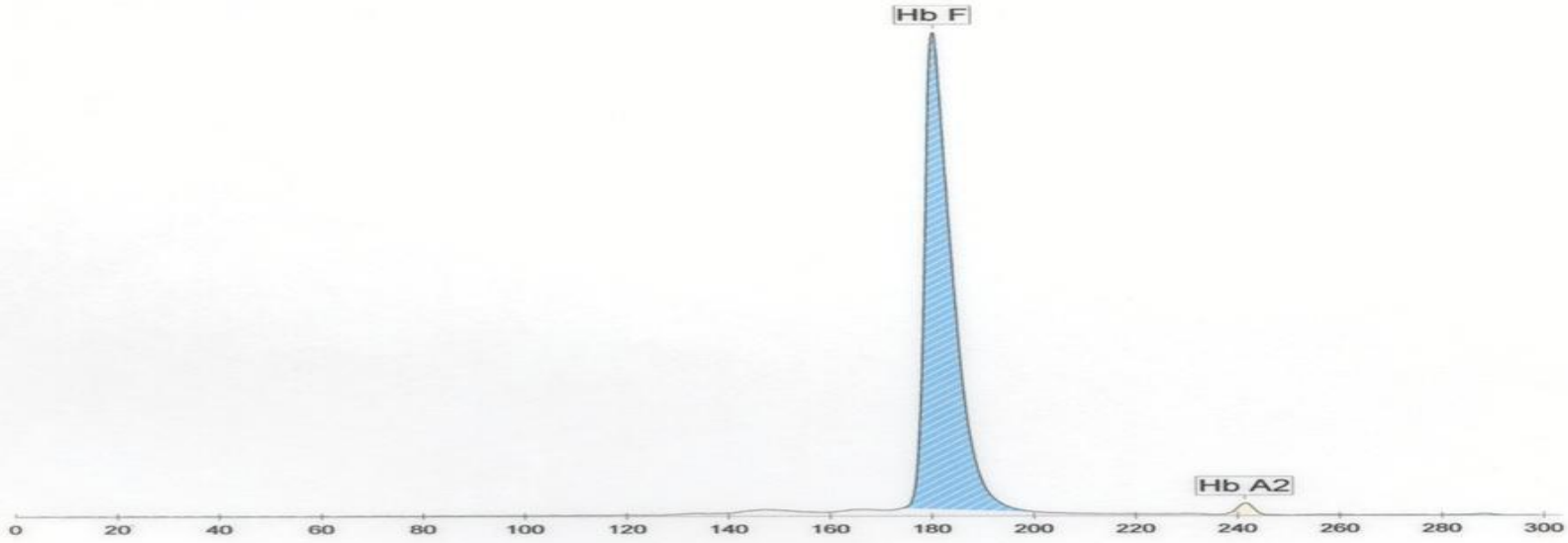
INSTRUMENT ID : KKUH : 24509

Hospital No.: 921107

Sample No **54**

ID : **063761**

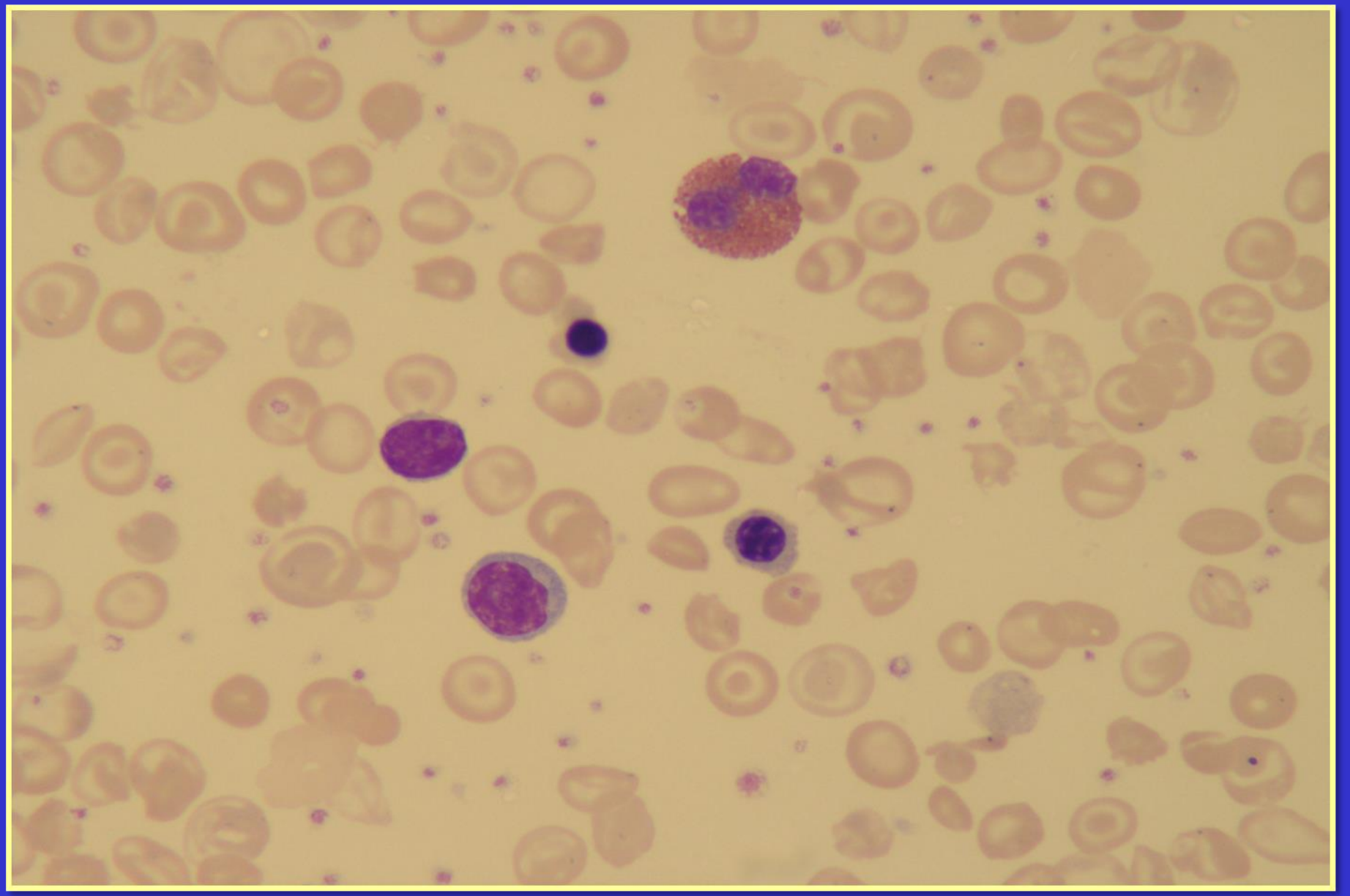
Date : **09/05/2010**



Fractions	%	Ref. %
Hb F	**98.5**	
Hb A2	**1.5**	

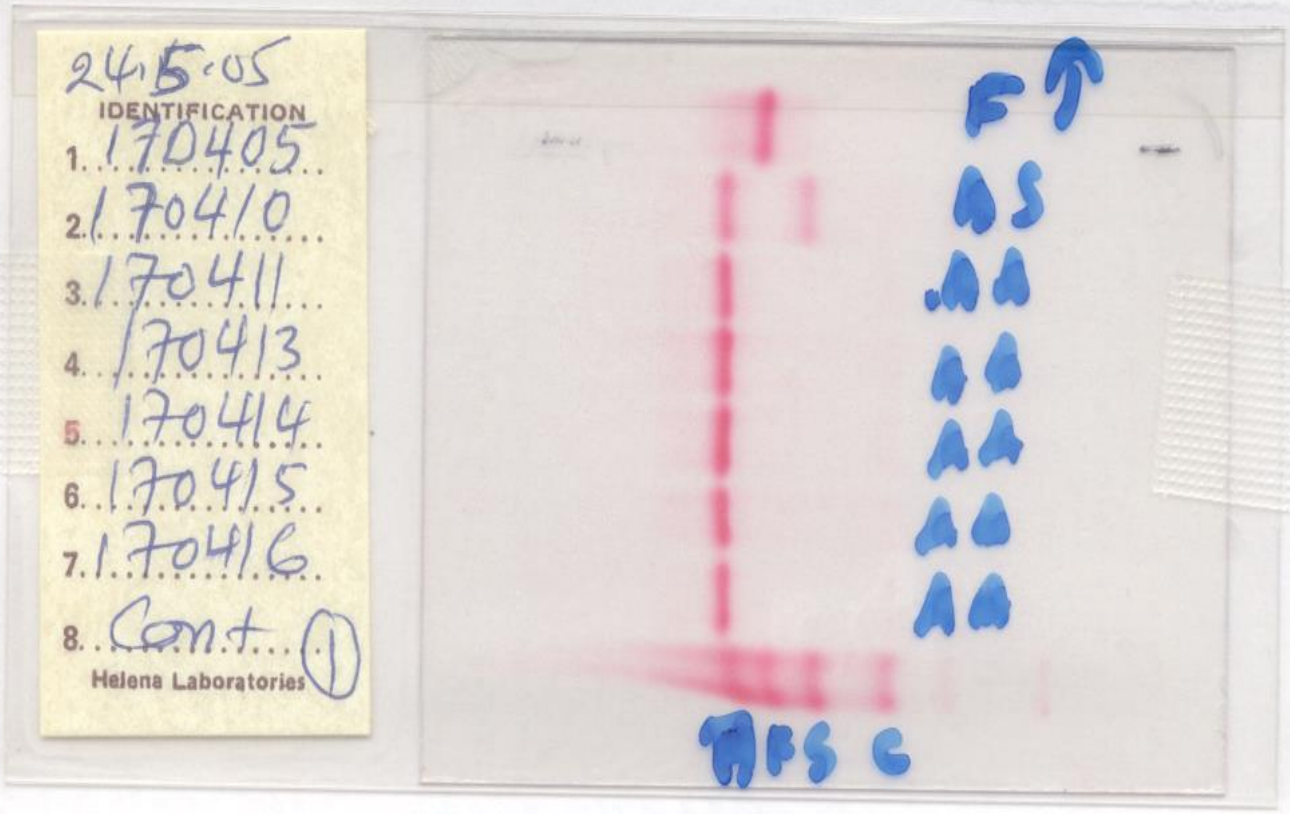
Comment :

Beta Thalassaemia Major



(+)

(−)


24.5.05
IDENTIFICATION
1. 170405
2. 170410
3. 170411
4. 170413
5. 170414
6. 170415
7. 170416
8. Cont. (1)
Helena Laboratories
F
AFS C

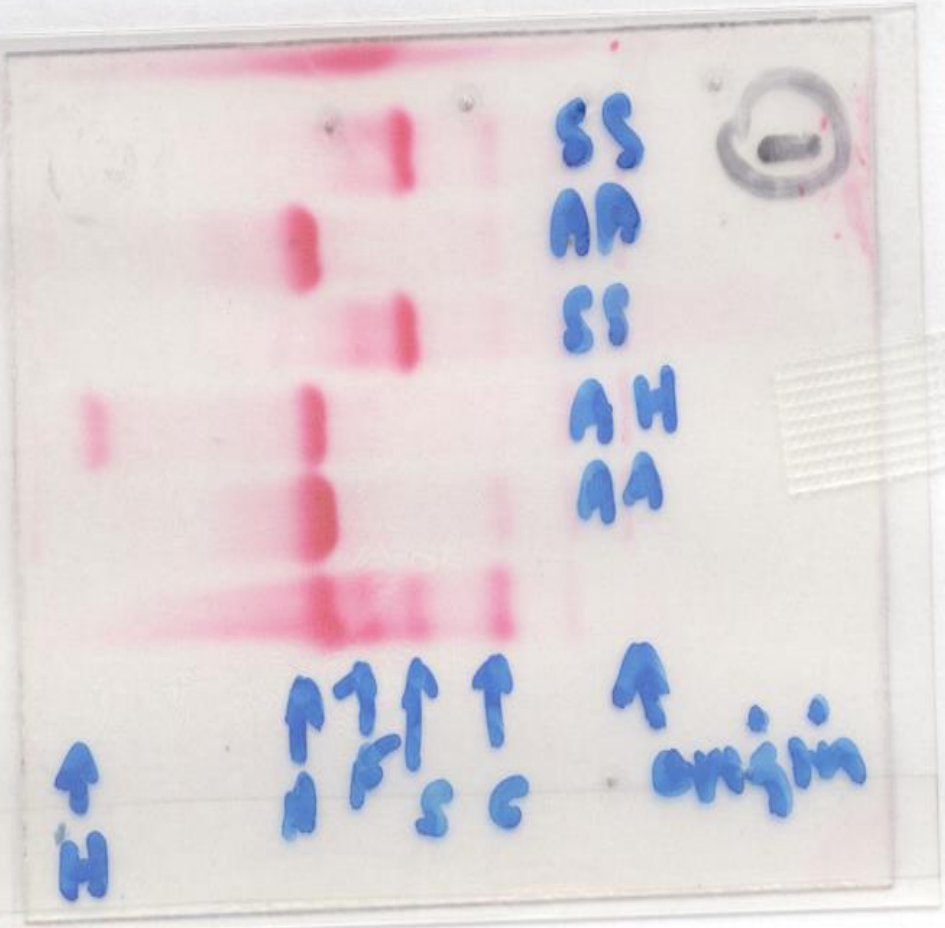
(+) ⟵ (−)

9.5.05

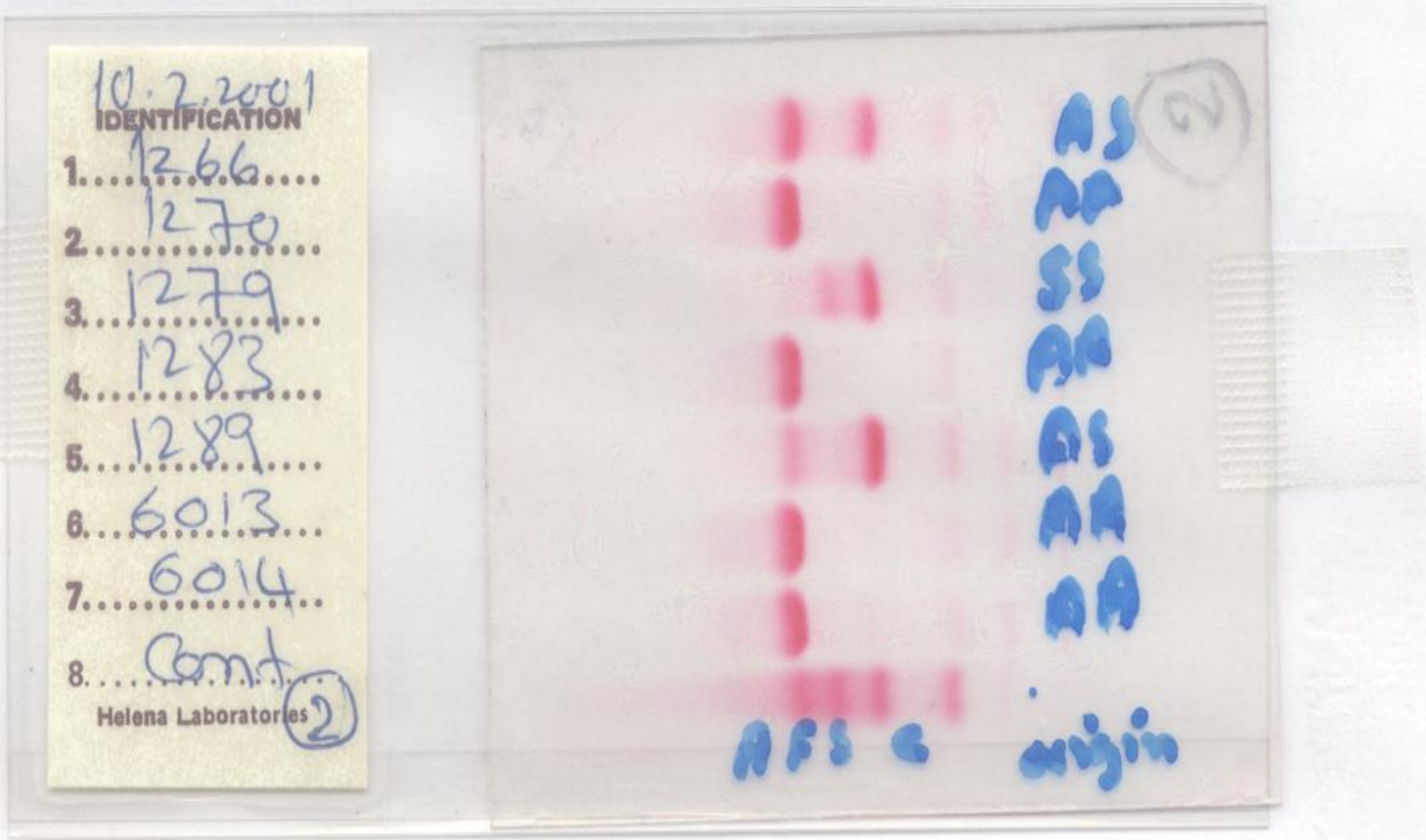
IDENTIFICATION

1. 170368
2. 170369
3. 684245
4. Cont...
5. .....
6. .....
7. ..... (2)
8. .....

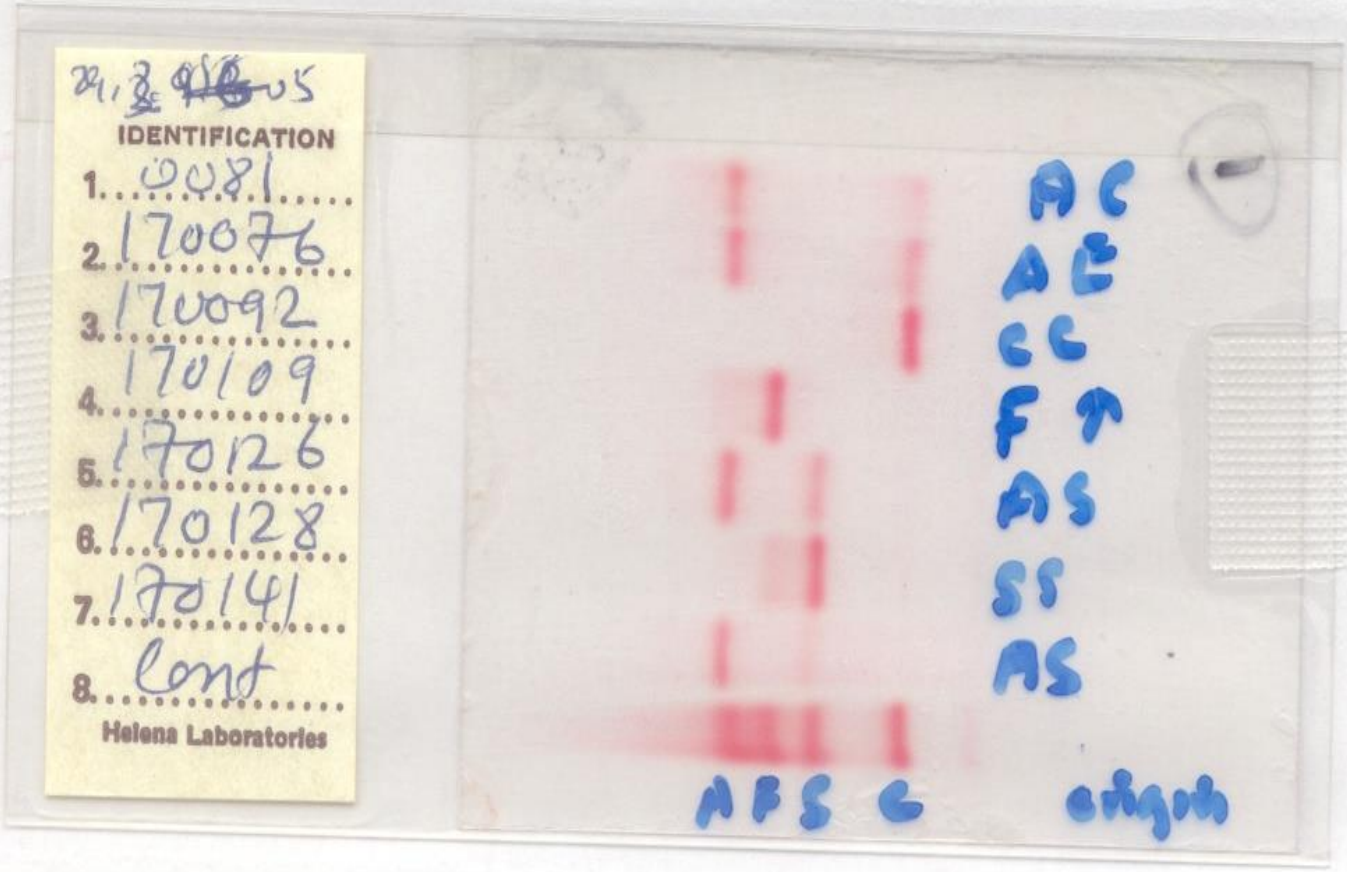
Helena Laboratories



(+) ⟵ (−)



(+) ←——— (-)


IDENTIFICATION
1. 0081
2. 170076
3. 170092
4. 170109
5. 170126
6. 170128
7. 170141
8. cont
Helena Laboratories
AC
AE
CC
FA
AS
SS
AS
origin
A F S C


Thank you!
TESCHIO 99