MEGALOBLASTIC ANAEMIA

BY:

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LEARNING OBJECTIVES

- To understand the mechanisms by which macrocytic anaemia may arise
- To appreciate the signs and symptoms of macrocytic anaemia
- To understand how macrocytic anaemia can be classified
- To be able to know the causes of macrocytic anaemia
- To understand the normal metabolism of vitamin B12 and folic acid, and to appreciate how megaloblastic anaemia may arise
- To suggest some normoblastic causes of macrocytosis

Normal adult red cell values

	Male	Female
Haemoglobin* (g/L)	135 – 175	115 – 155
Haematocrit (PCV) (%)	40 – 52	36 – 48
Red cell count ($x10^{12}$/L)	4.5 – 6.5	3.9 – 5.6
Mean cell haemoglobin (pg)	27 – 34	
Mean cell volume (FL)	80 – 95	
Mean cell haemoglobin concentration g/L)	300 – 350	
Reticulocyte count ($x10^{9}$/L)	25 – 125 (1.0 – 2%)	

* In children normal haemoglobin values are: newborn, 150 – 210g/L; 3 months, 95 – 125g/L; 1 year to puberty, 110 – 135g/L.

* PCV, packed cell volume.

NORMAL WHITE CELLS (WBC) COUNT IN ADULTS

TOTAL	4.0 - 11.0 X 10^9/L
Neutrophils	2.5 - 7.5 x 10^9/L
Lymphocytes	1.5 - 3.5 x 10^9/L
Monocytes	0.2 - 0.8 x 10^9/L
Eosinophils	0.04 - 0.44 x 10^9/L
Basophil	0.01 - 0.1 x 10^9/L
Platelets	150-450 x 10^9/L

* Children have higher lymphocytes count

Microcytic, Hypochromic Anaemia

MCV< 80 fL

MCH<27pg

Iron deficiency

Thalassaemia

Anaemia of chronic disease (some cases)

Lead poisoning

Sideroblastic anaemia (some cases)

Normocytic, Normochromic Anaemia

MCV 80 – 95 fL

MCH>26pg

Many haemolytic anaemias

Anaemia of chronic disease (some cases)

After acute blood loss

Renal disease

Mixed deficiencies

Bone marrow failure, e.g. post-chemotherapy, infiltration by carcinoma, etc.

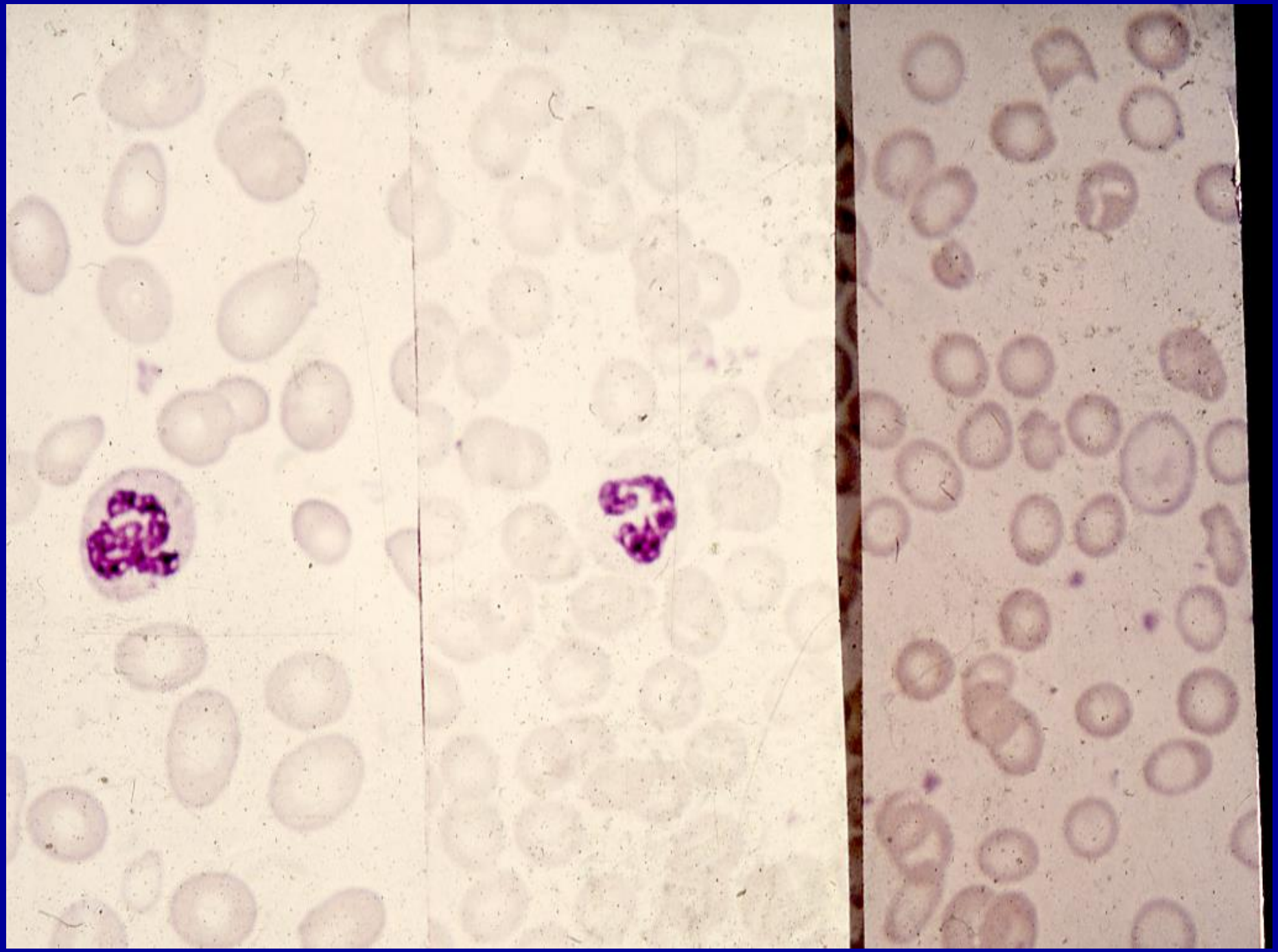
MACROCYTIC ANAEMIA

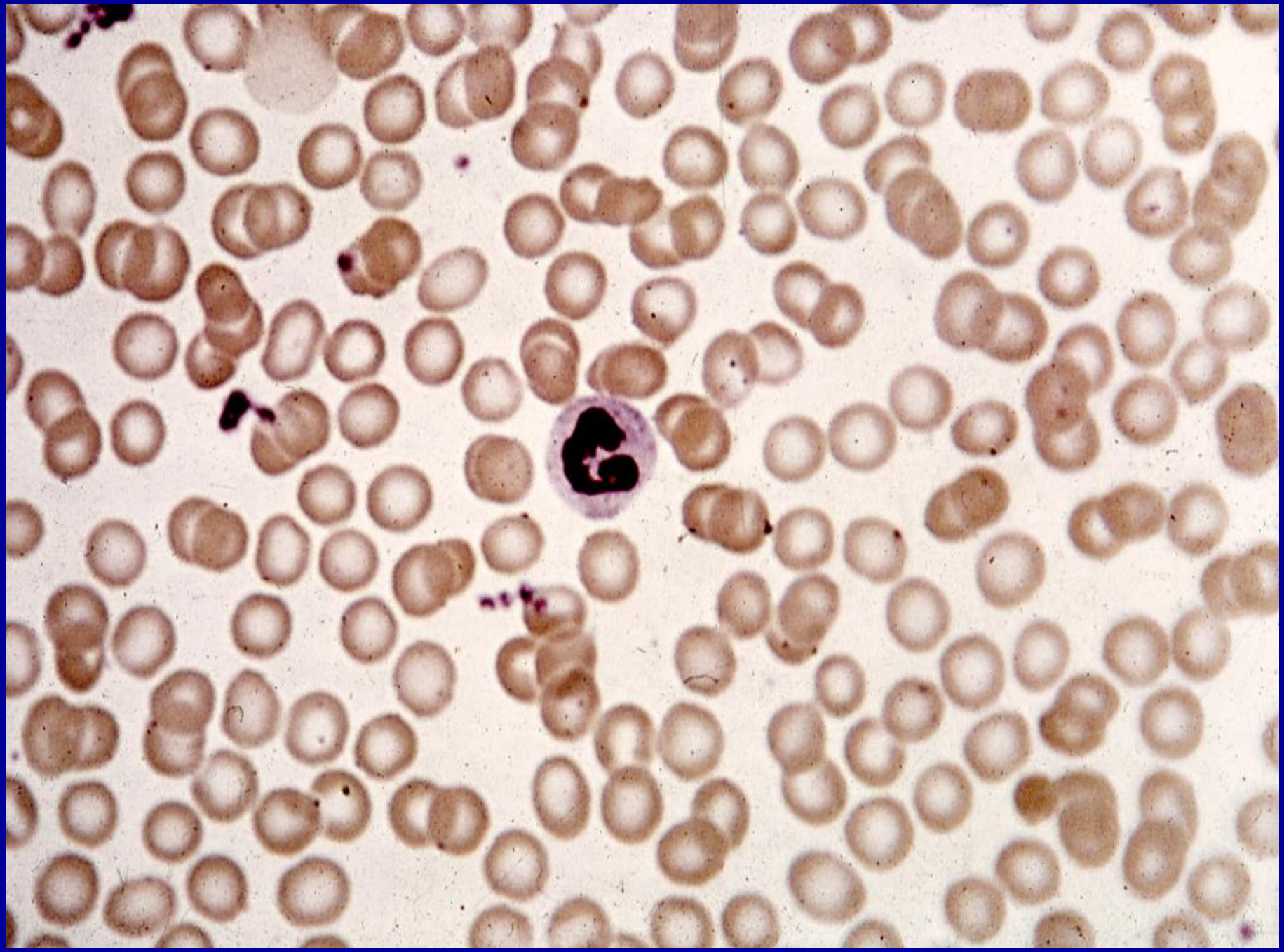
Macrocytic anaemias can be divided into those showing:

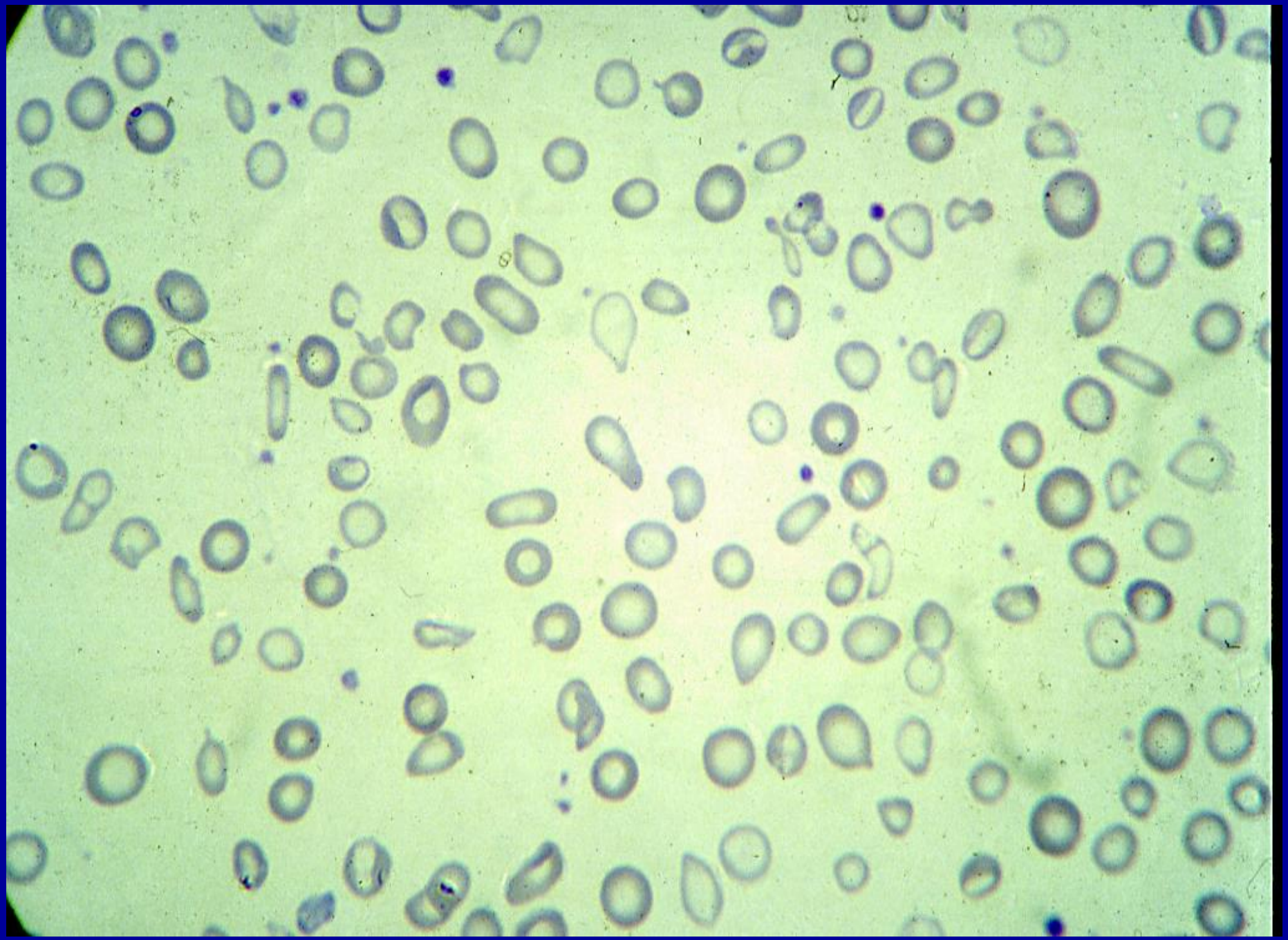
1. ***Megaloblastic erythropoiesis***
2. ***Normoblastic erythropoiesis***

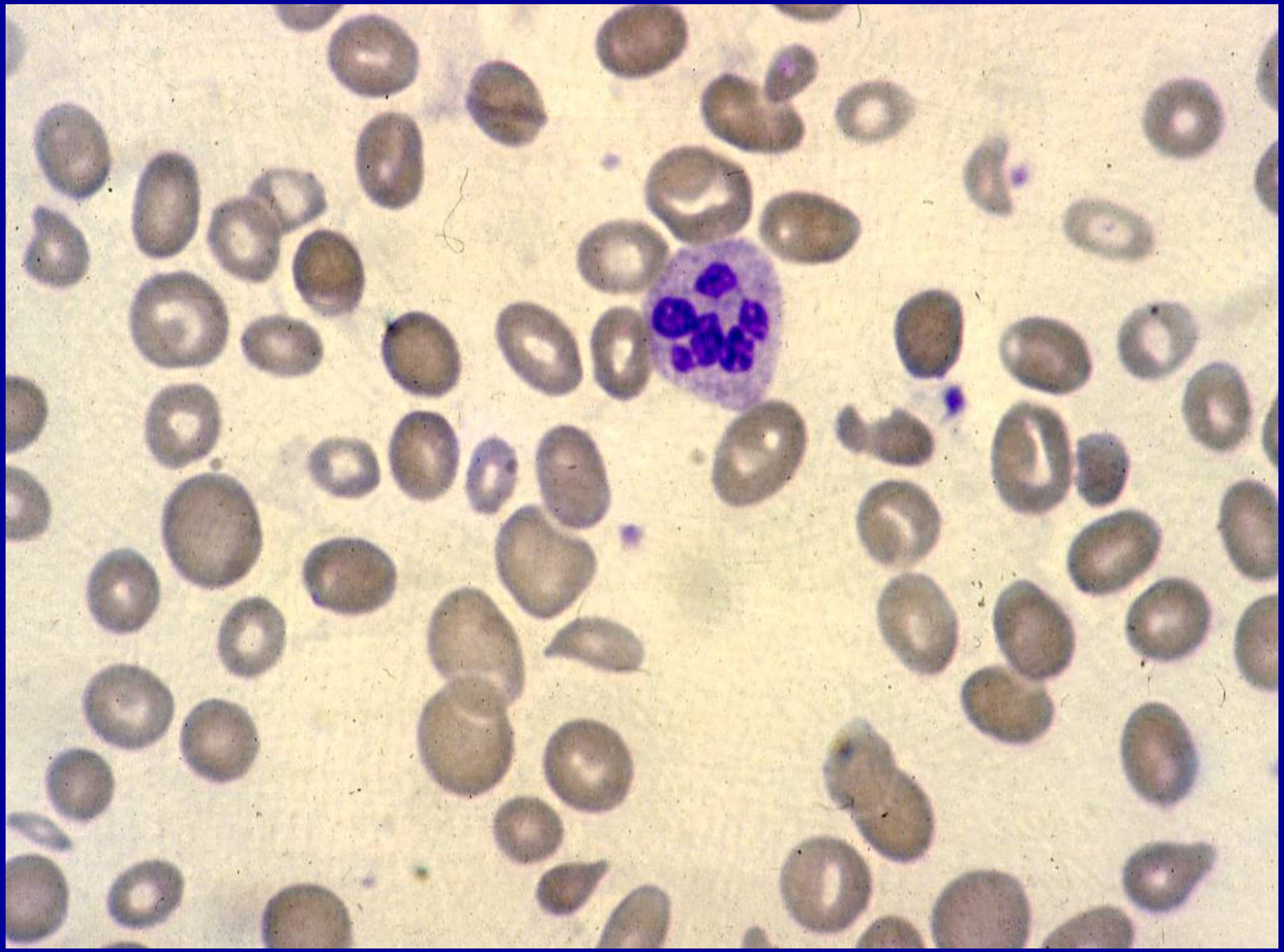
Megaloblastic erythropoiesis **-** describes abnormal red cell development characterized by a lack of synchrony between the maturation of the red cell nucleus and its cytoplasm. It arises as a consequence of disordered DNA synthesis and results in a macrocytic anaemia.

Normoblastic erythropoiesis - describes the normal appearance of red cell maturation - but may still be associated with a macrocytosis in the peripheral blood.









Conditions in which Macrocytosis or hypersegmented neutrophils may occur in the absence of megaloblastic anaemia

Macrocytosis	**Alcohol**
	Liver disease (especially alcoholic)
	Reticulocytosis (haemolysis or haemorrhage)
	Aplastic anaemia or red cell aplasia
	Hypothyroidism
	Myelodysplasia including acquired Sideroblastic anaemia
	myeloma and macroglobulinaemia
	Leucoerythroblastic anaemia
	Myeloproliferative disease
	Pregnancy
	Newborn
	? Chronic respiratory failure
Hypersegmented Neutrophils	**Renal failure**
	Congenital (familial) abnormality
	? Iron deficiency

Note:- High MCV recorded when cold agglutinins or paraproteins are present.

Macrocytosis with Normoblasts

* Normal neonates (Physiological)
* Chronic alcoholism*
* Myelodysplastic syndromes*
* Chronic liver disease*
* Hypothyroidism
* Normal pregnancy
* Therapy with anticonvulsant drugs*
* Haemolytic anaemia
* Chronic lung disease (with hypoxia)
* Hypoplastic and aplastic anaemia
* Myeloma

*Some patients show B_{12}- and folate-independent megaloblastic erythropoiesis.

Causes of megaloblastic anaemia :

1. Cobalamin deficiency or abnormalities of cobalamin metabolism

2. Folate deficiency or abnormalities of folate metabolism

3. Therapy with anitfolate drugs (e.g. methotrexate)

4. Independent of either cobalamin or folate deficiency and refractory to cobalamin and folate therapy.

    a. Some cases of acute myeloid leukaemia, myelodysplasia.
    b. Orotic aciduria (responds to uridine)
    c. Therapy with drugs interfering with synthemis of DNA (e.g. cytosine arabinoside, hydroxyurea, 6-mercaptopurine, azidothymidine (AZT)
    d. Thiamine responsive

5. Suggested but poorly documented causes of megaloblastic anaemia not due to cobalamin or folate deficiency or metabolic abnormality:

    a. Vitamin E deficiency
    b. Lesch-Nyhan syndrome (?responds to adenine)

Other causes of megaloblasts :

Abnormalities of nucleic acid synthesis
Drug therapy
- **Antipurines (mercaptopurine, azathioprine)**
- **Antipyrimidines (fluorouracil, zydovudine (AZT))**
- **Others (hydrozyurea)**

Orotic aciduria

Uncertain aetiology
Myelodysplastic syndromes, * erythroleukaemia
Some congenital dyserythropoietic anaemias

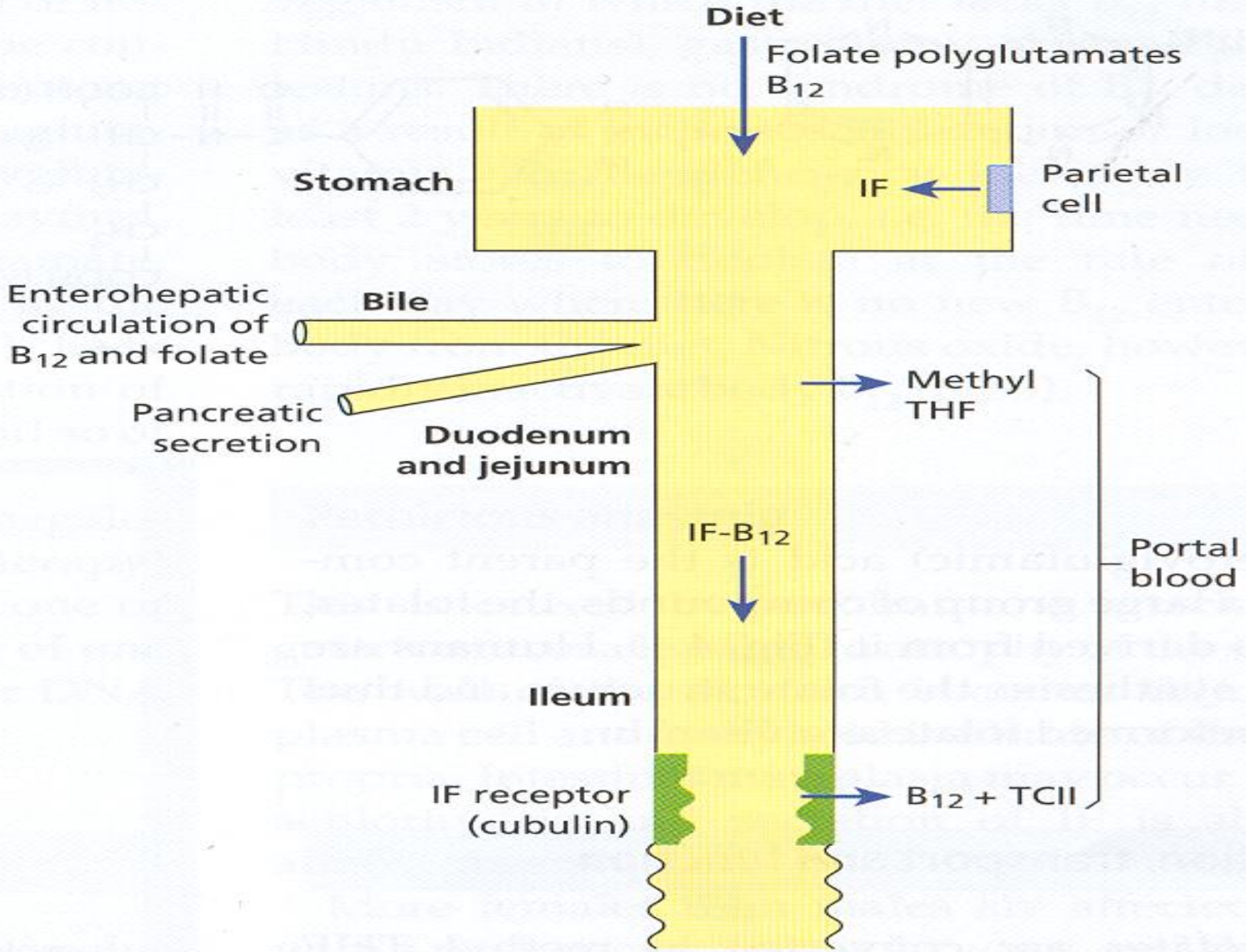
*** Some patients show normoblastic erythropoiesis.**

Vitamin B_{12} and folate nutrition and absorption

	Vitamin B12	Folate
Diterary sourse	Only food of animal origin, especially liver	Most foods, especially liver, green vegetable and yeast; destroyed by cooking
Average daily intake*	7 - 30 µg	200-250 µg
Minimum daily requirement*	1-3 µg	100-200 µg†
Body stores*	3-5 mg, mainly in the liver	8-20 mg, mainly in the liver
Time to develop deficiency in the absence of intake or absorption*	Anaemia in 2-10 years	Macrocytosis in 5 months.
Requirements for absorption	Intrinsic factor secreted by gastric parietal cells	Conversiion of polyglutamates to monoglutamates by intestinal folate conjugase
Site of absorption	Terminal ileum	Duodenum and jejunum

* In adults.

† Higher during pregnancy and lactation.


Diet
Folate polyglutamates
B_{12}
Stomach
IF
Parietal cell
Enterohepatic circulation of B_{12} and folate
Bile
Pancreatic secretion
Duodenum and jejunum
Methyl THF
$IF-B_{12}$
Portal blood
Ileum
IF receptor (cubulin)
B_{12} + TCII

Vitamin B_{12} Deficiency

- **Inadequate intake**
- **Veganism, lactovegetarianism (some cases)**
- **Inadequate secretion of intrinsic factor**
- **Pernicious anaemia**
- **Total or partial gastrectomy**
- **Congenital intrinsic factor deficiency (rare)**
- **Inadequate release of B_{12} from food**
- **Partial gastrectomy, vagotomy, gastritis, acid-suppressing drugs, alcohol abuse**
- **Diversion of dietary B_{12}**
- **Abnormal intestinal bacterial flora**
  multiple jejunal diverticula, small intestinal strictures, stagnant intestinal loops
- **Diphyllobothrium latum**
- **Malabsorption**
- **Crohn's disease, ileal resection, chronic tropical sprue, congenital selective B_{12} malabsorption with proteinuria (Imerslund-Grasbeck syndrome)**

PERNICIOUS ANAEMIA

- Severe megaloblastic anaemia due to autoimmune attack on the gastric mucosa leading to atrophy.
- More common in elderly female patients than males (1.6:1) at the age of 60 and above
- More common in Northern European and tends to be in families
- The mucosa become thin with plasma cells and lymphoid infiltration of the lamina properia.
- Intestinal metaplasia may occur.
- It maybe associated with autoimmune diseases including the autoimmune poly-endocrine syndrome

cont'd…

- Achlorhydria and absent secretion of intrinsic factor (IF).
- Progressive neuropathy is a common feature
- Absent serum vitamin B12 level or almost absent level
- Raised serum gastrin levels
- Helicobacter pylori infection may be the cause which present in younger age as iron deficiency anaemia and in the elderly as pernicious anaemia
- Increased incidence of gastric carcinoma in (2-3% of pernicious anaemia patients).

Causes of folate deficiency

Inadequate dietary intake

Malabsorption

Coeliac disease, jejunal resection, tropical sprue

Increased requirement

Pregnancy, premature infants, chronic haemolytic anaemias, myelofibrosis, various malignant diseases

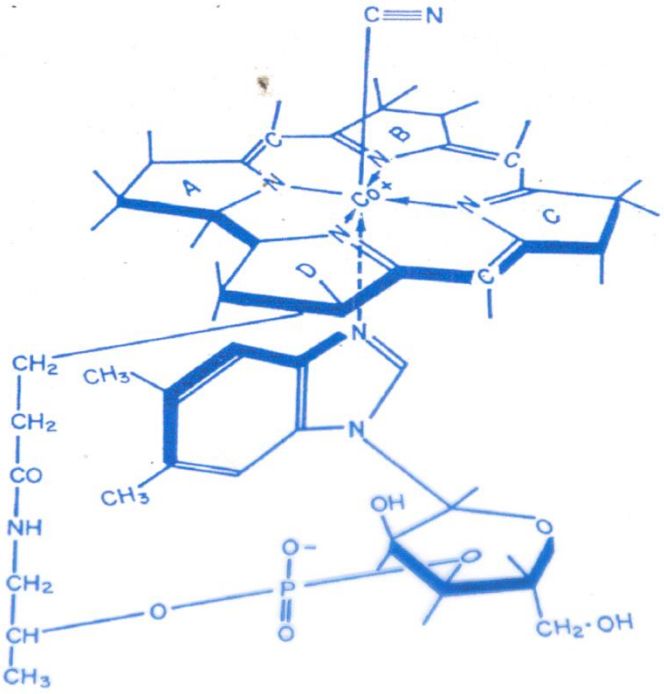
Increased loss

Long-term dialysis, congestive heart failure, acute liver disease

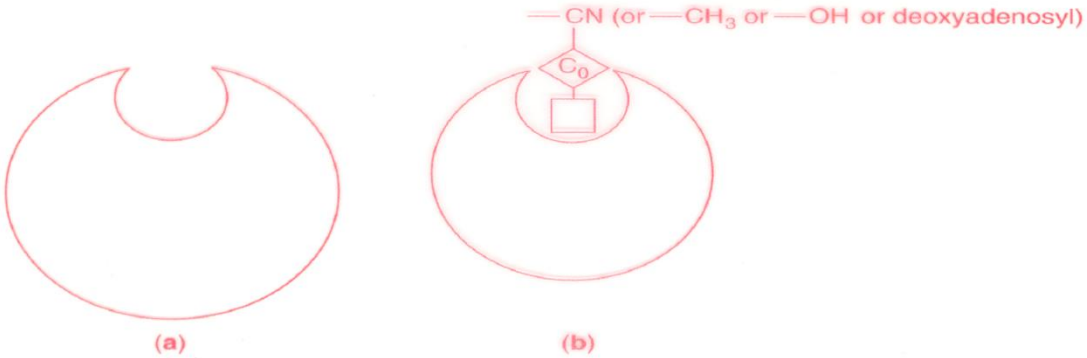
Complex mechanism

Anticonvulsant therapy, * ethanol abuse*

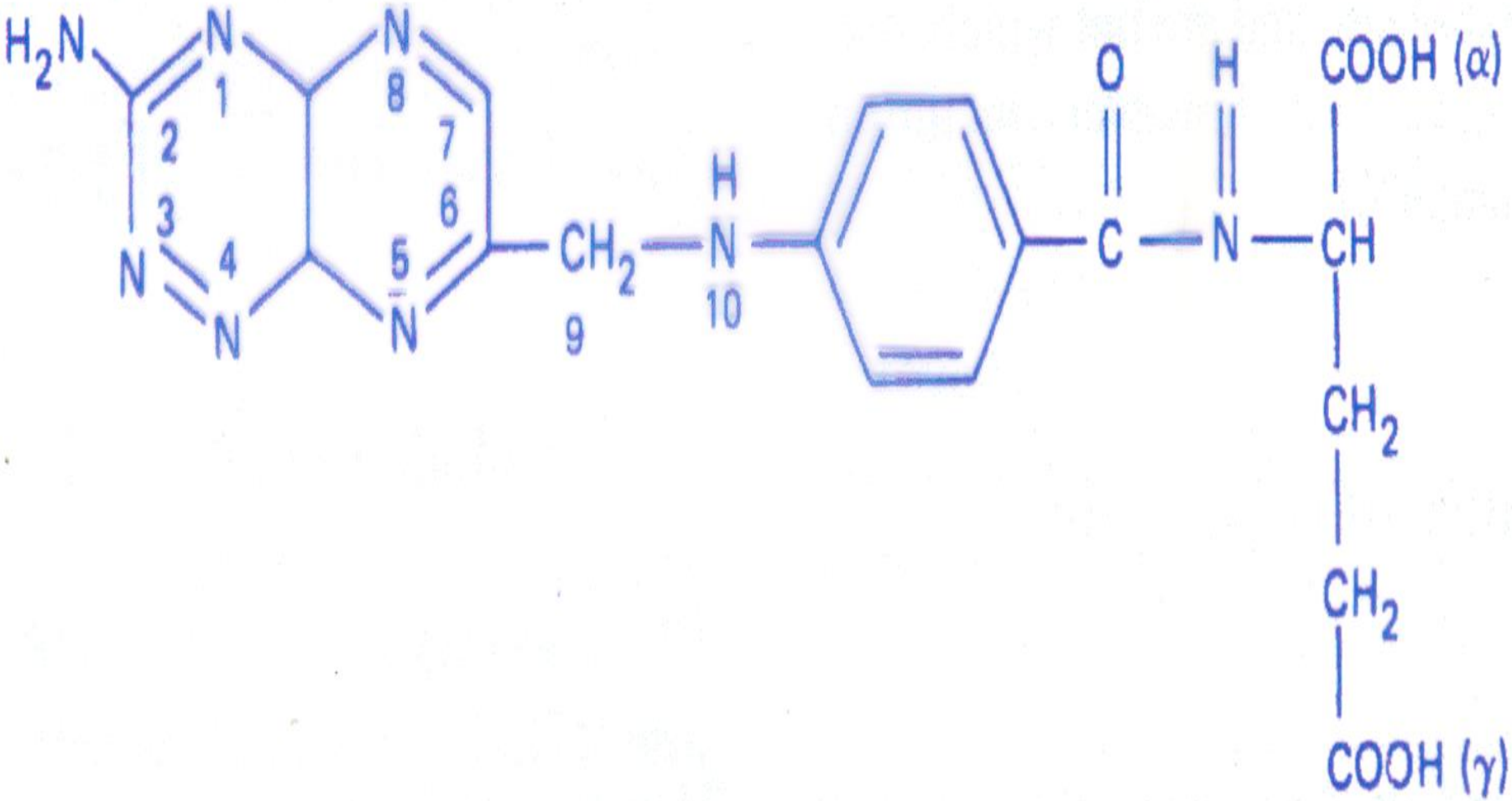
*** Only some cases with macrocytosis are folate deficient.**



The structure of vitamin B_{12} (cyanocobalamin).



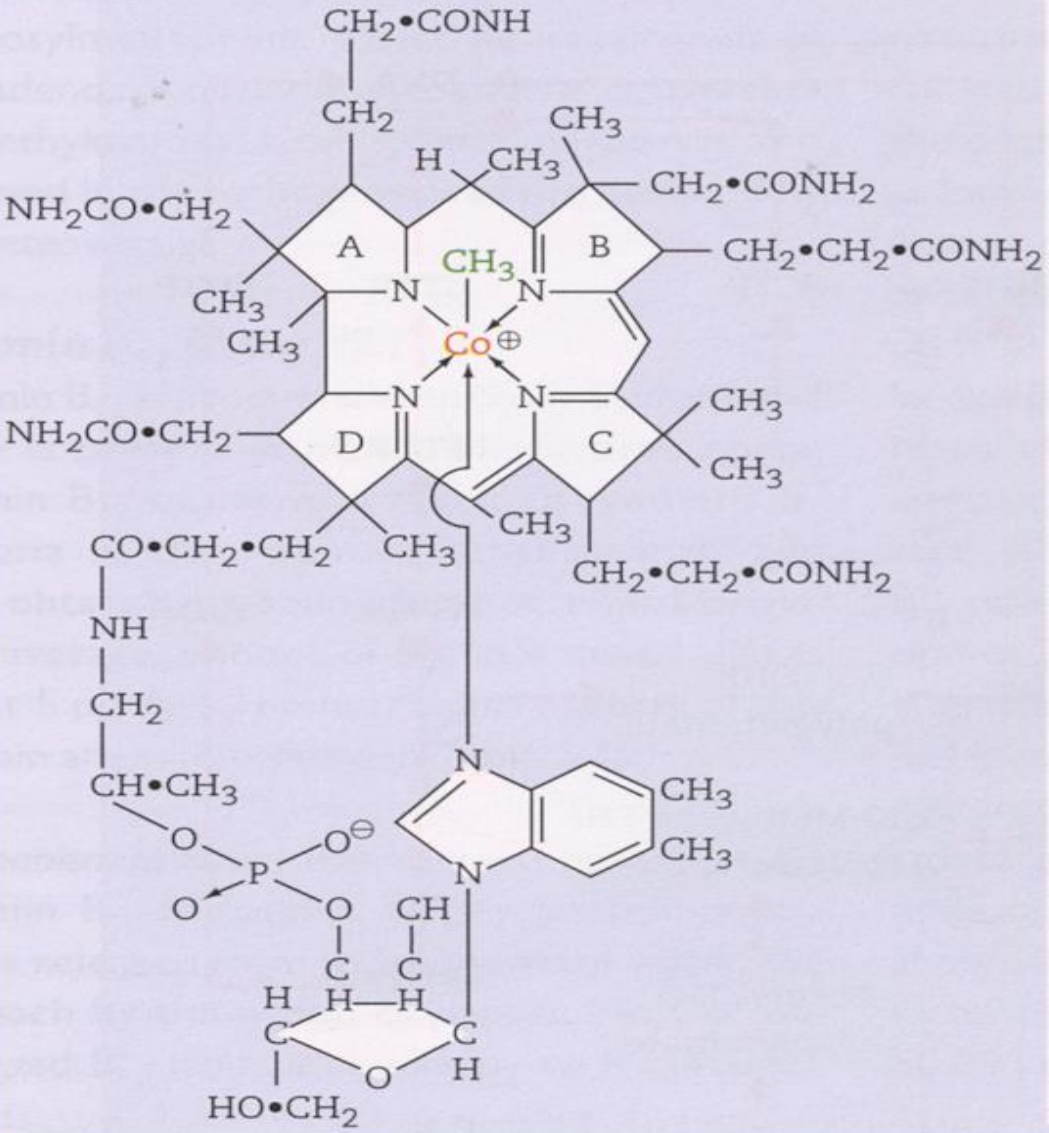
(a) Intrinsic factor and **(b)** intrinsic factor–cobalamin complex. Intrinsic factor has been estimated to have a molecular radius of 3.6 nm, vitamin B_{12} 0.8 nm, and the complex 3.2 nm.



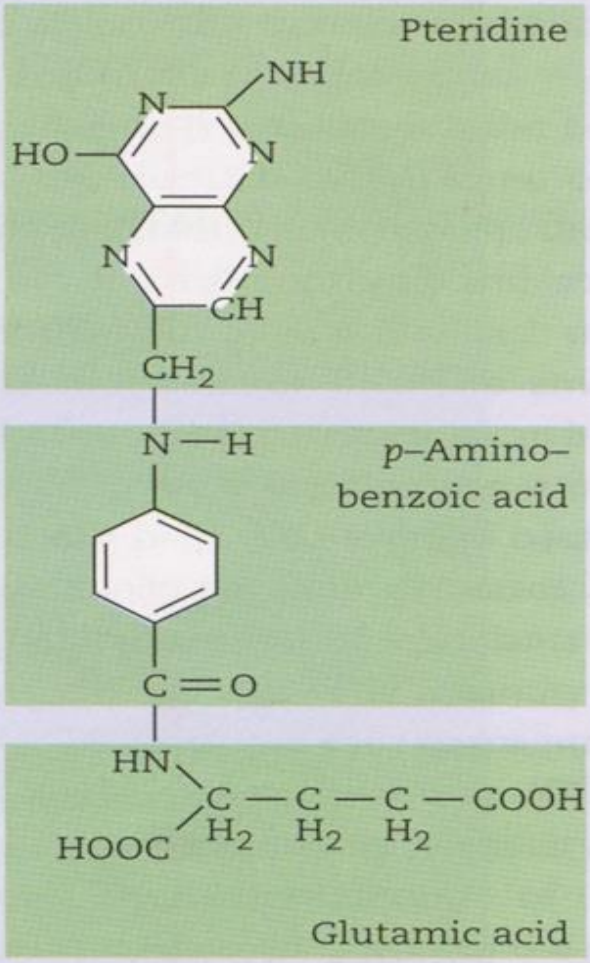
The structure of folic acid (pteroylglumatic acid).

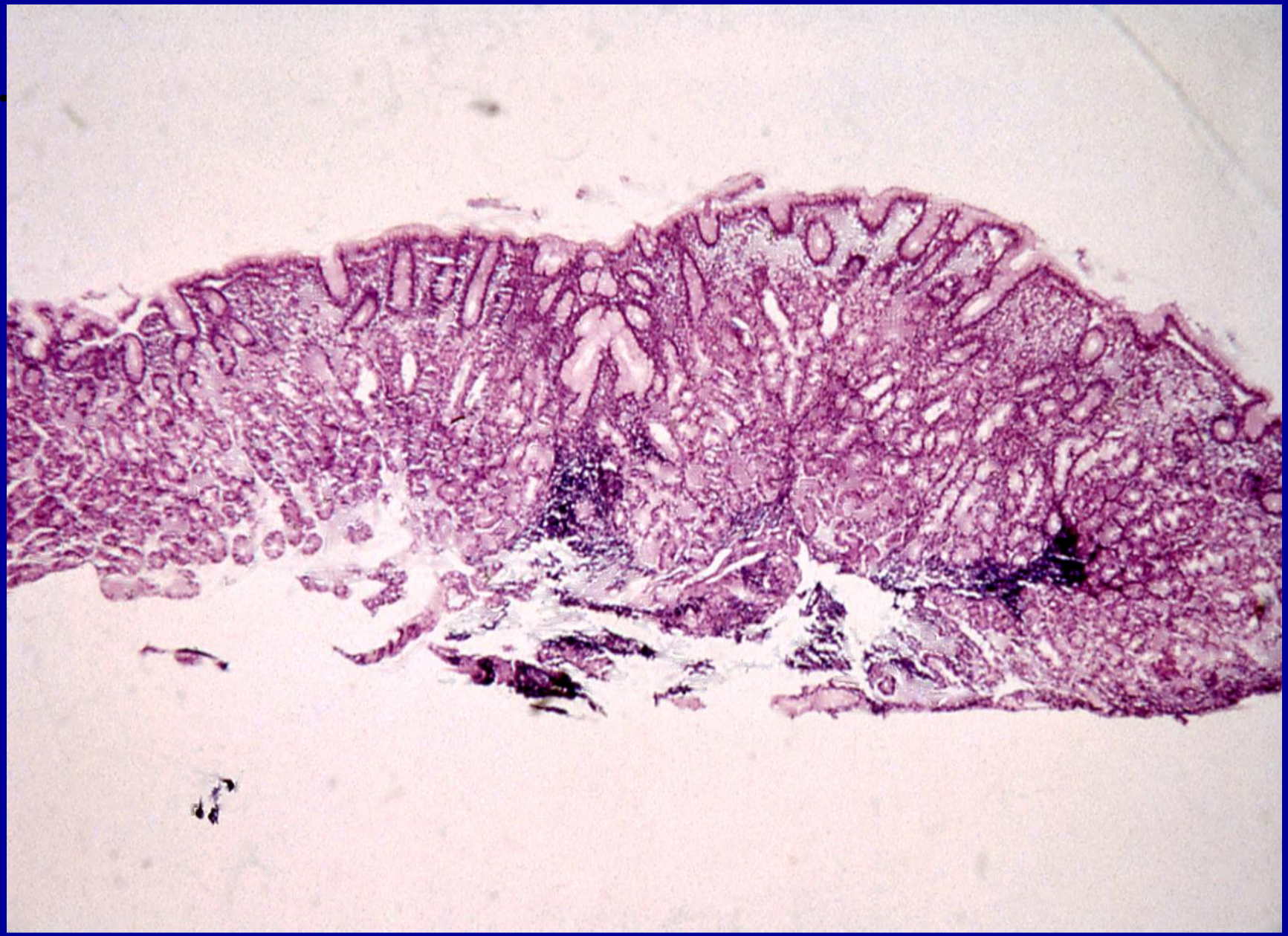
STRUCTURES OF VITAMIN B_{12} AND FOLIC ACID

Methylcobalamin

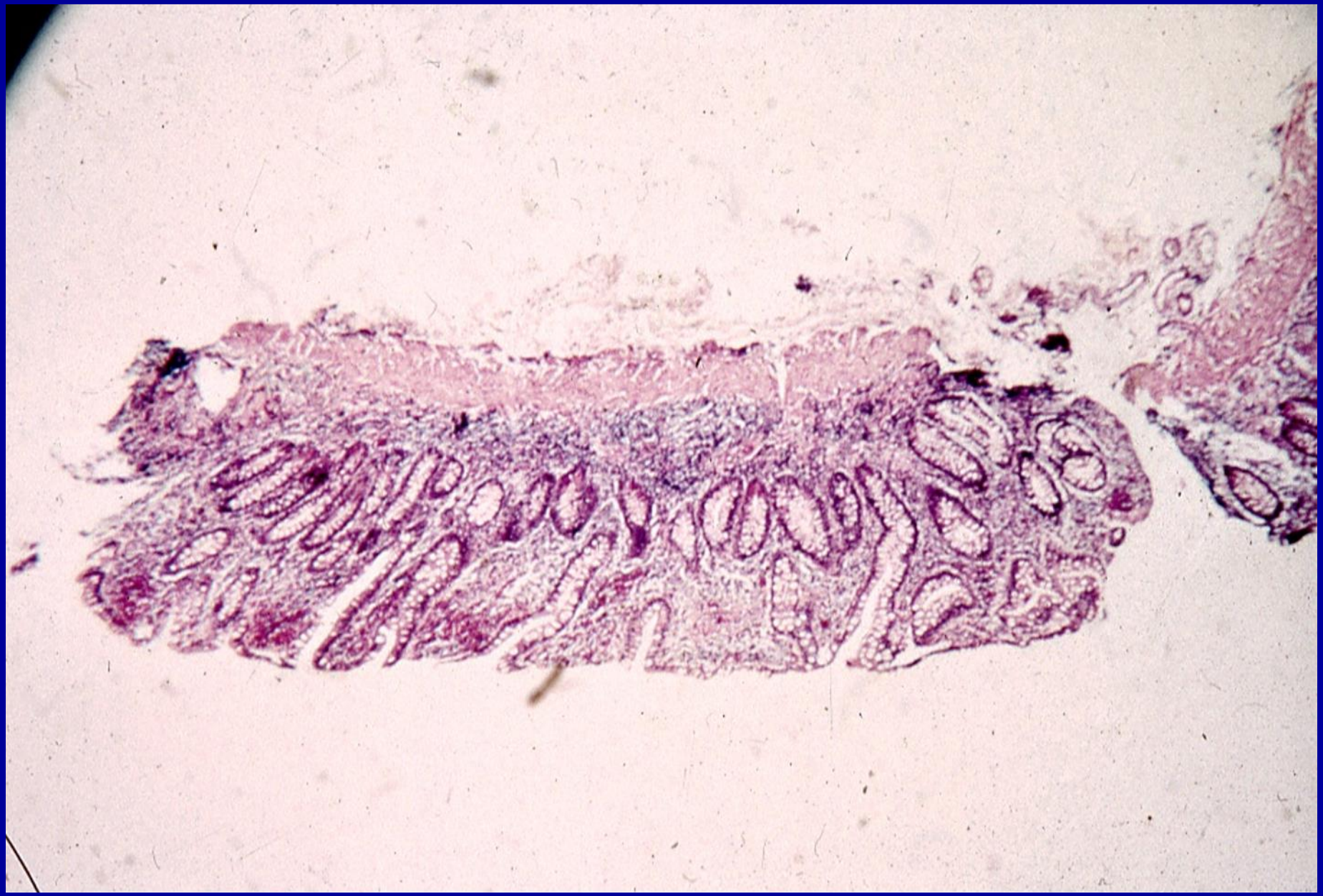


Folic acid

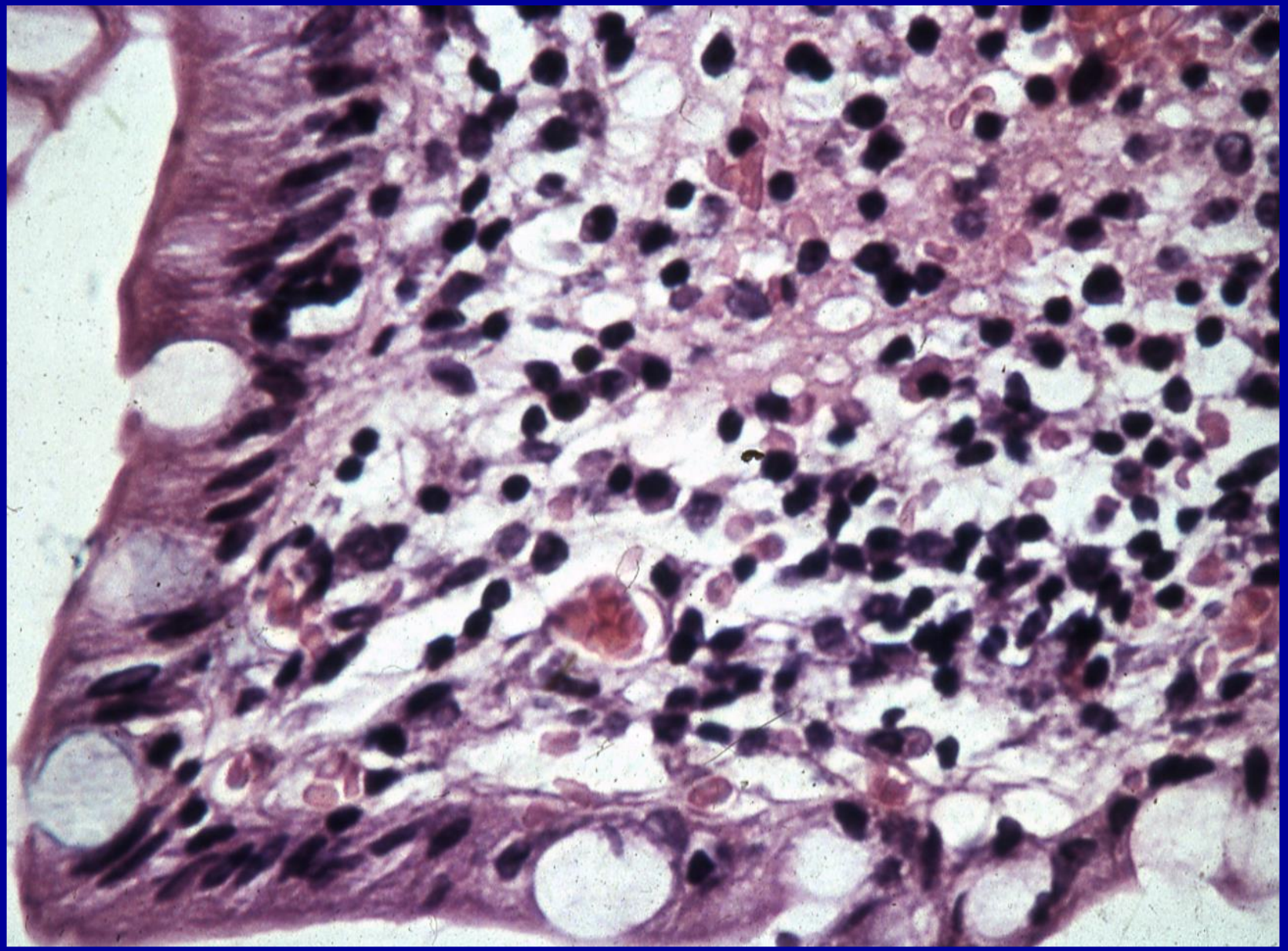




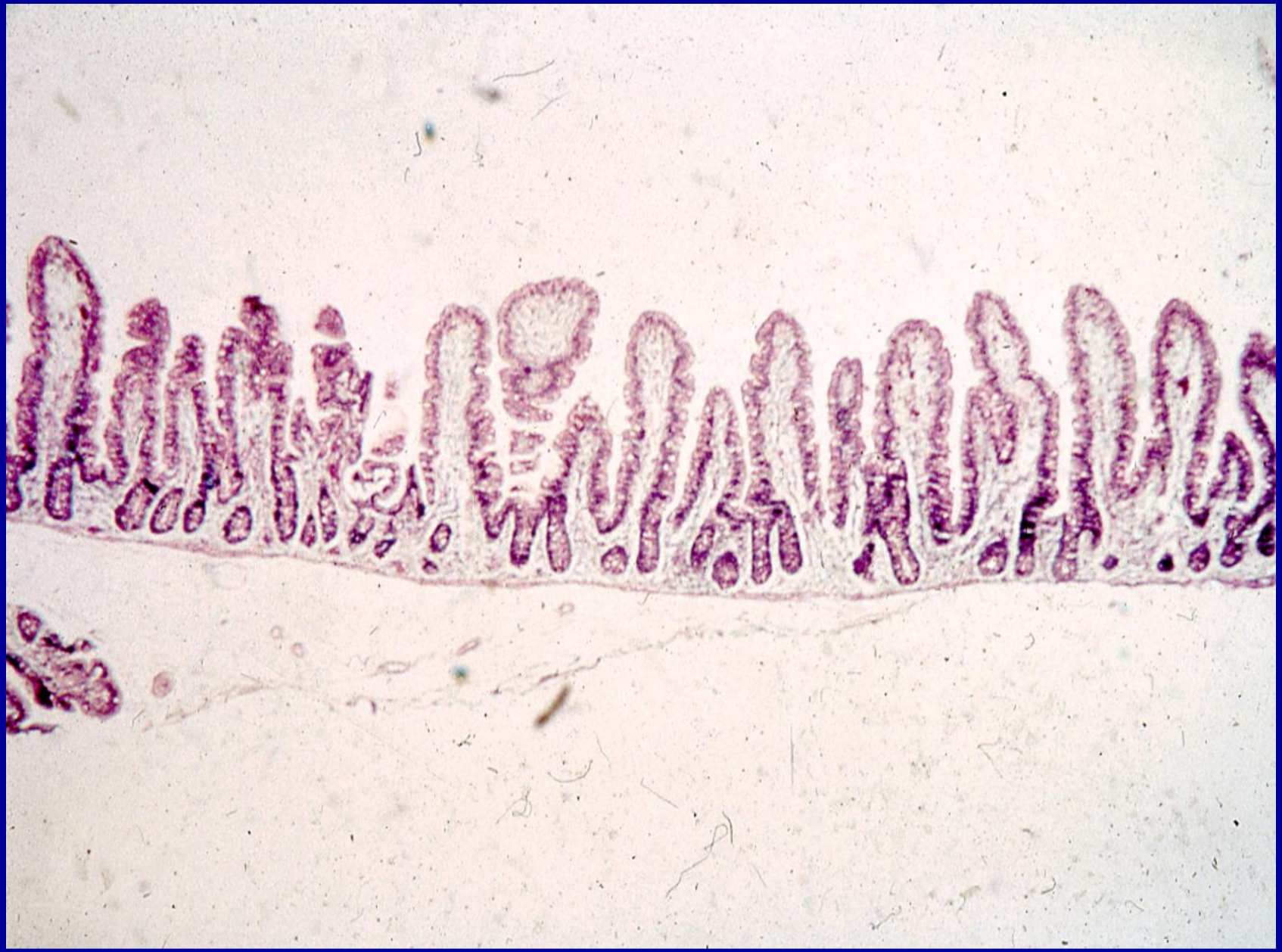
Normal gastric mucosa



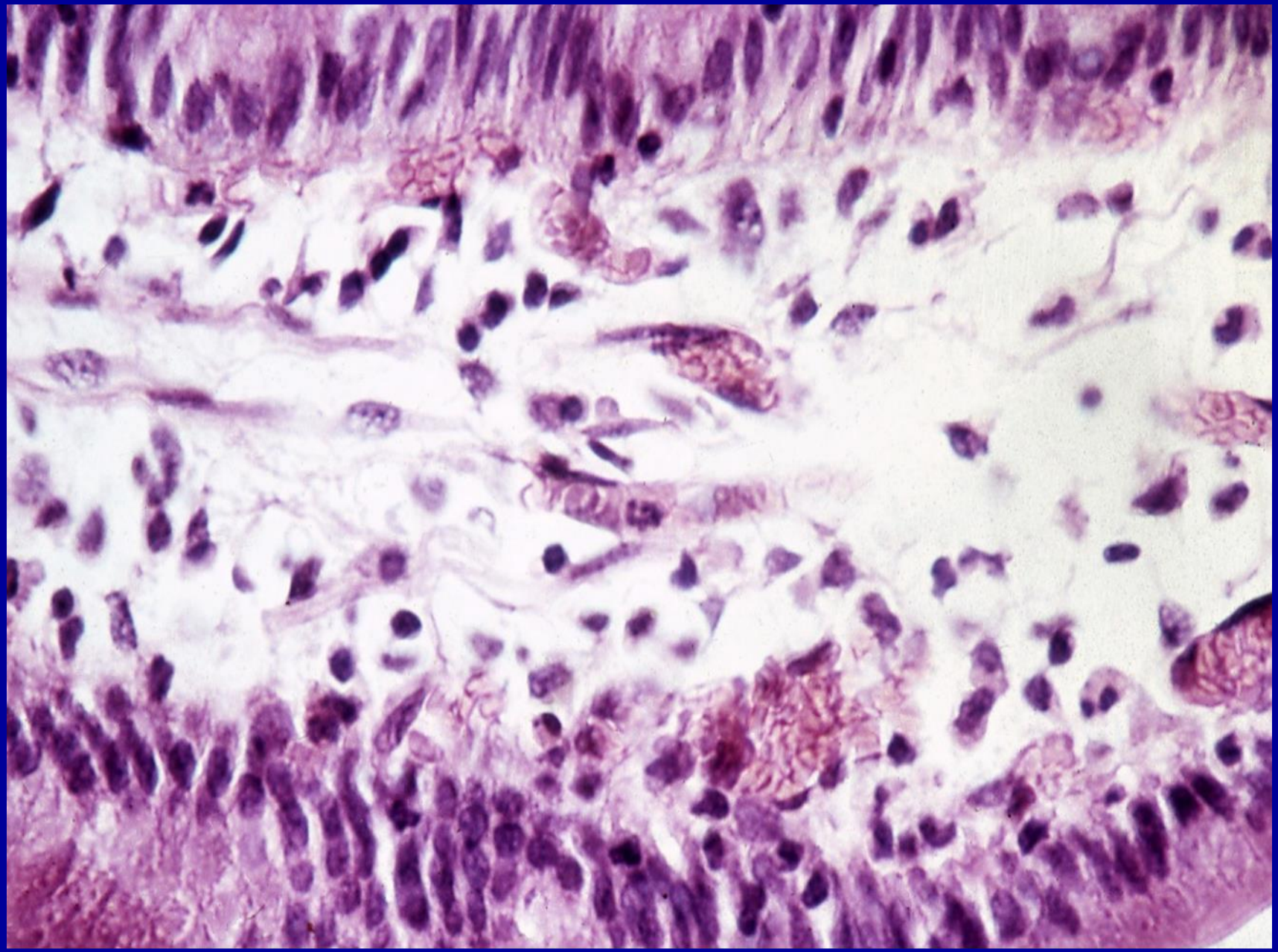
Gastric atrophy in patients with pernicious anaemia



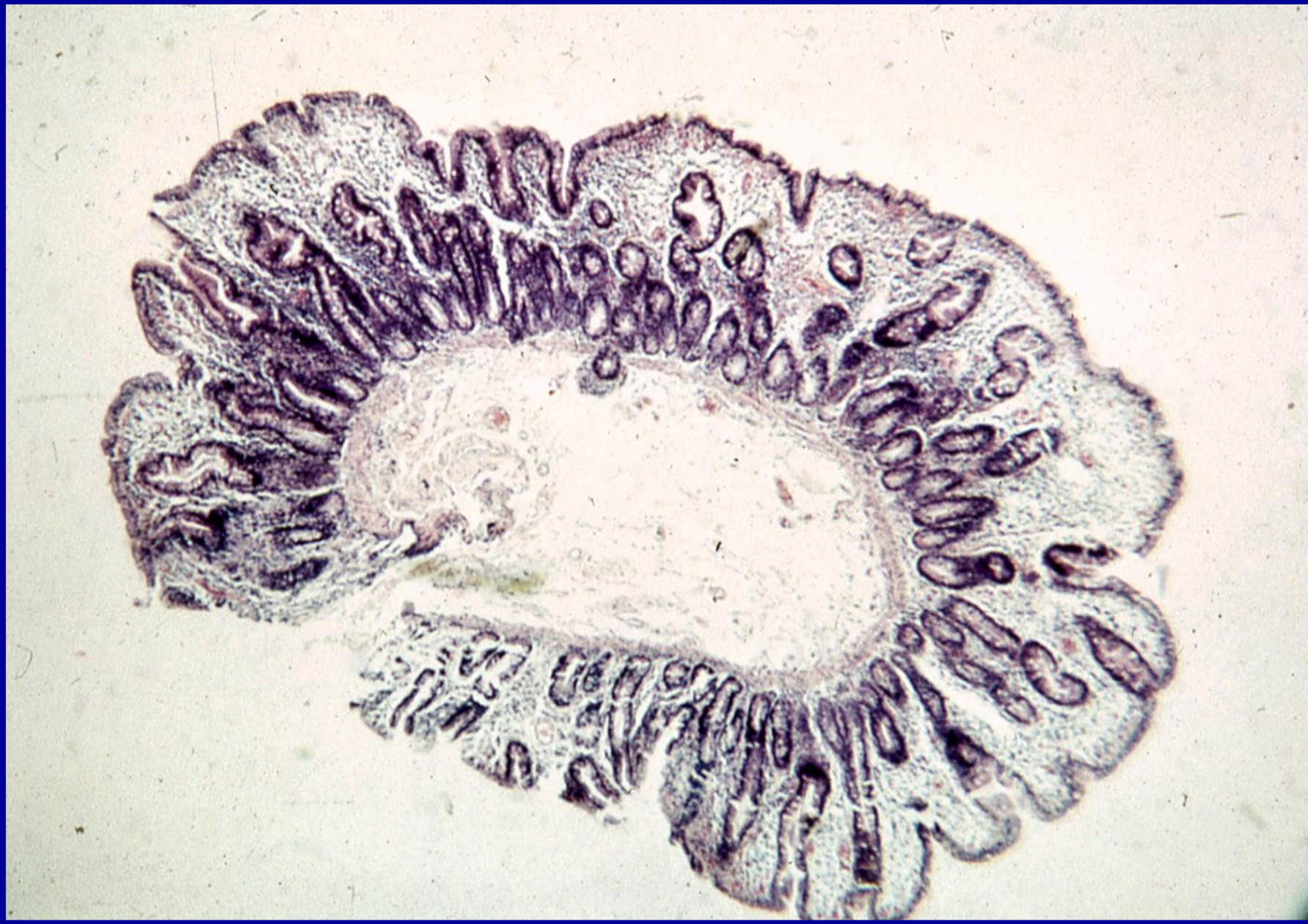
Heavy infiltration of lamina properia with plasma cells and lymphocytes in patients with pernicious anaemia



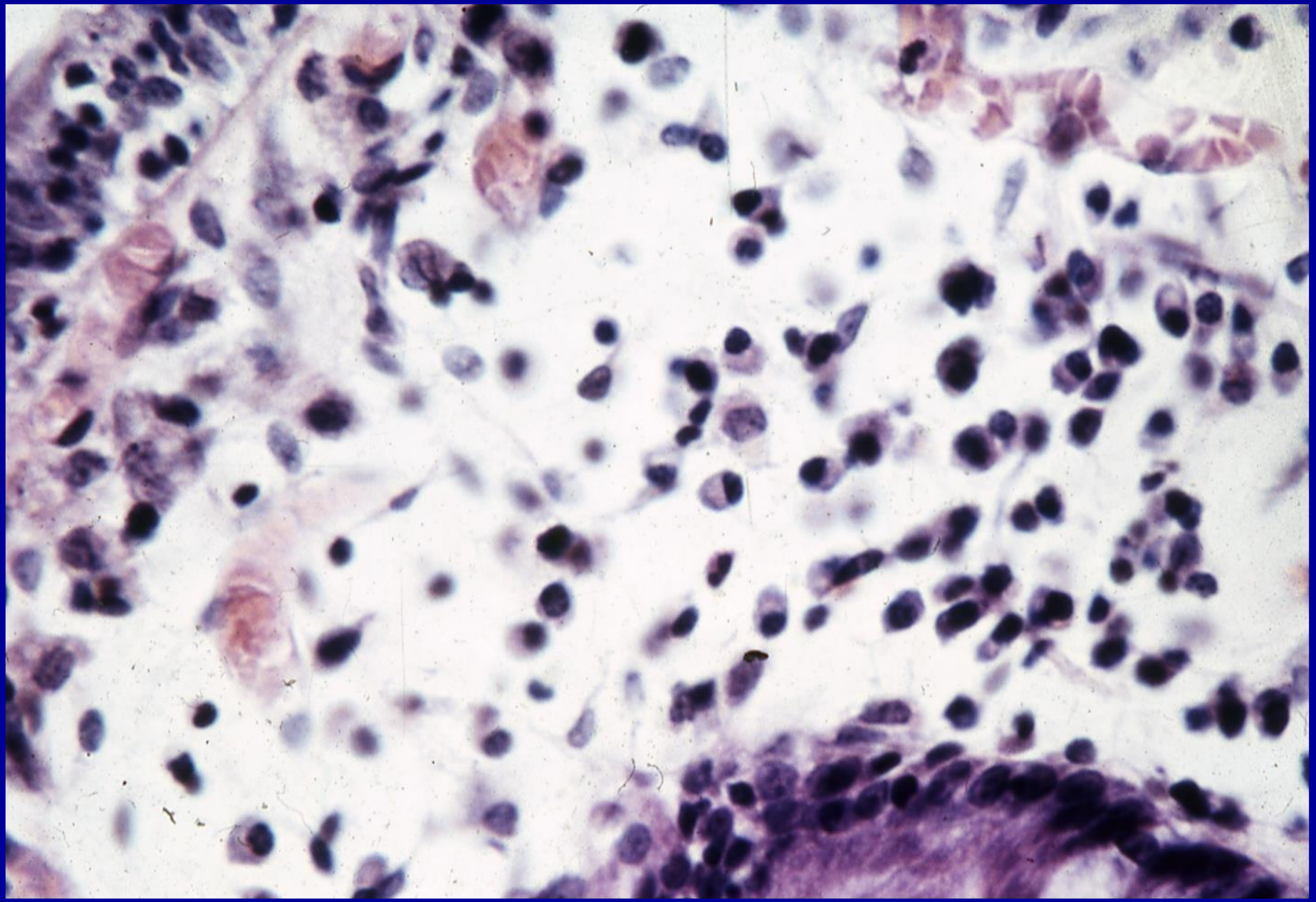
Normal small intestinal mucosa



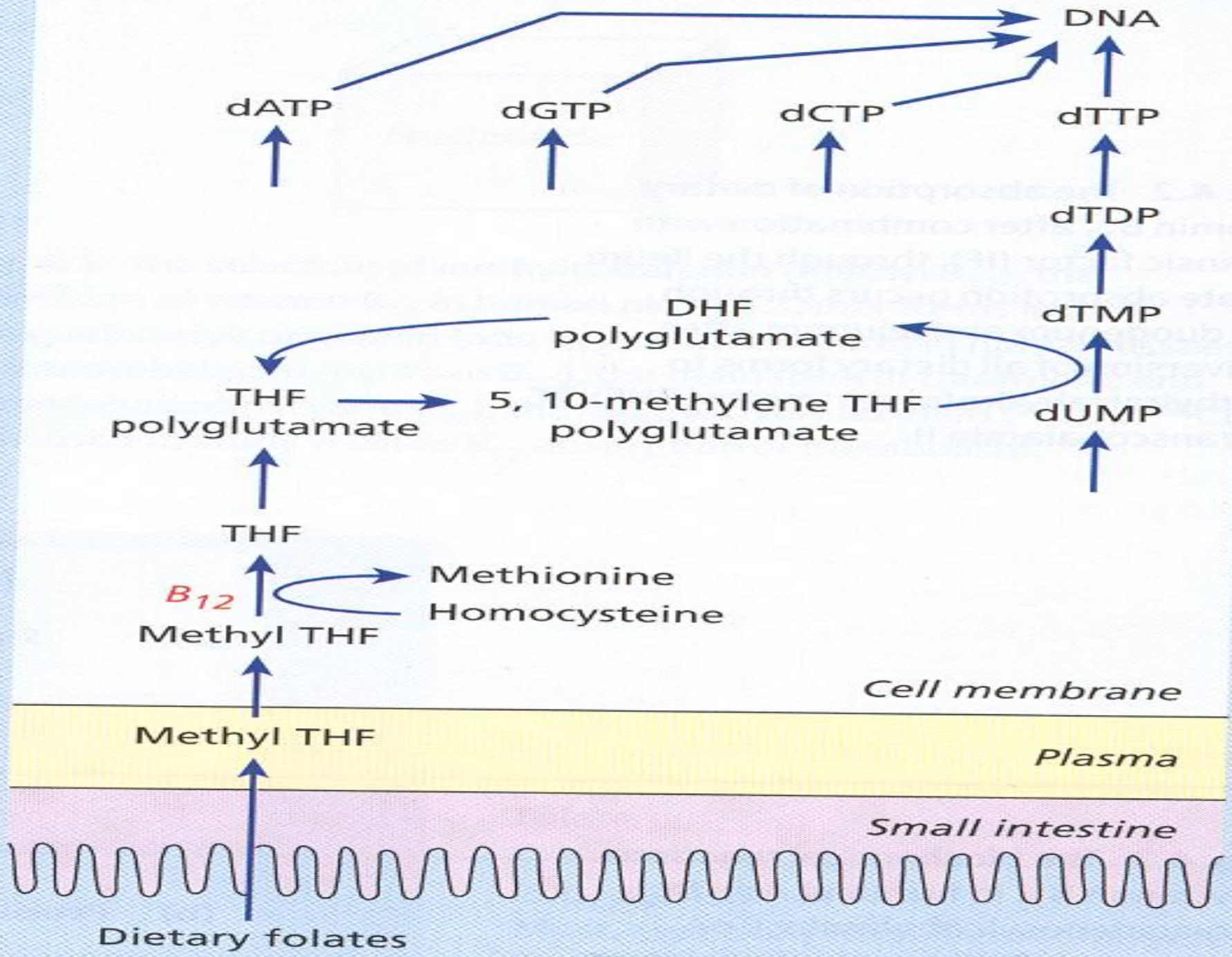
Normal histology of small intestinal mucosa

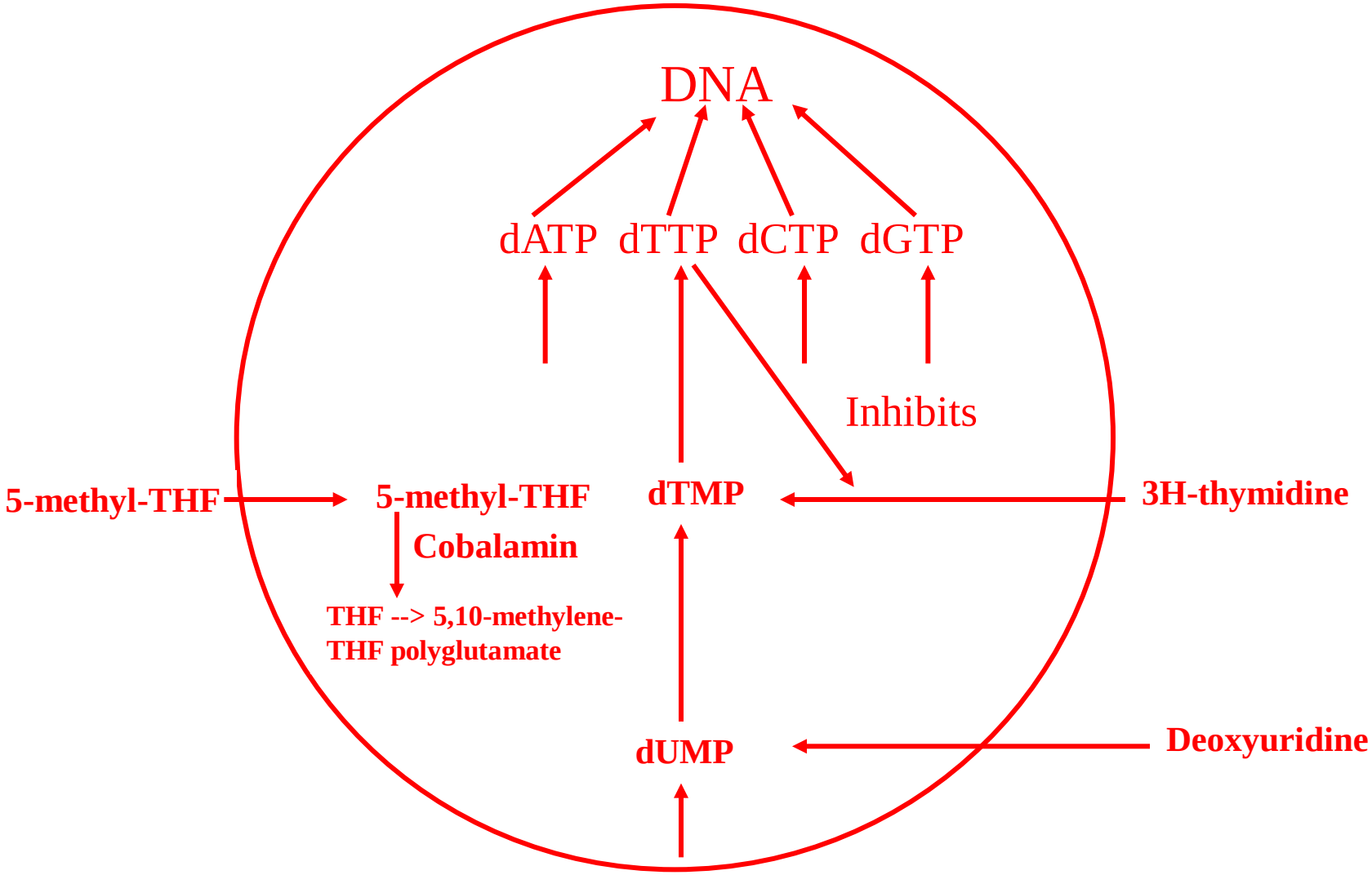


Flattening of small intestinal mucosa in malabsorption syndrome



Histopathology of small intestinal mucosa in malabsorption syndrome


DNA
dATP
dGTP
dCTP
dTTP
dTDP
dTMP
dUMP
DHF
polyglutamate
THF
polyglutamate
5, 10-methylene THF
polyglutamate
THF
B_{12}
Methionine
Homocysteine
Methyl THF
Cell membrane
Methyl THF
Plasma
Small intestine
Dietary folates



Deoxyuridine suppression test. The circle represents a bone marrow or other haemopoietic cell. THF = tetrahydrofolate; MP = monophosphate; TP = triphosphate; d= deoxyribose; A – adenine; T= thymine ; C = cytosine; G= guanine.

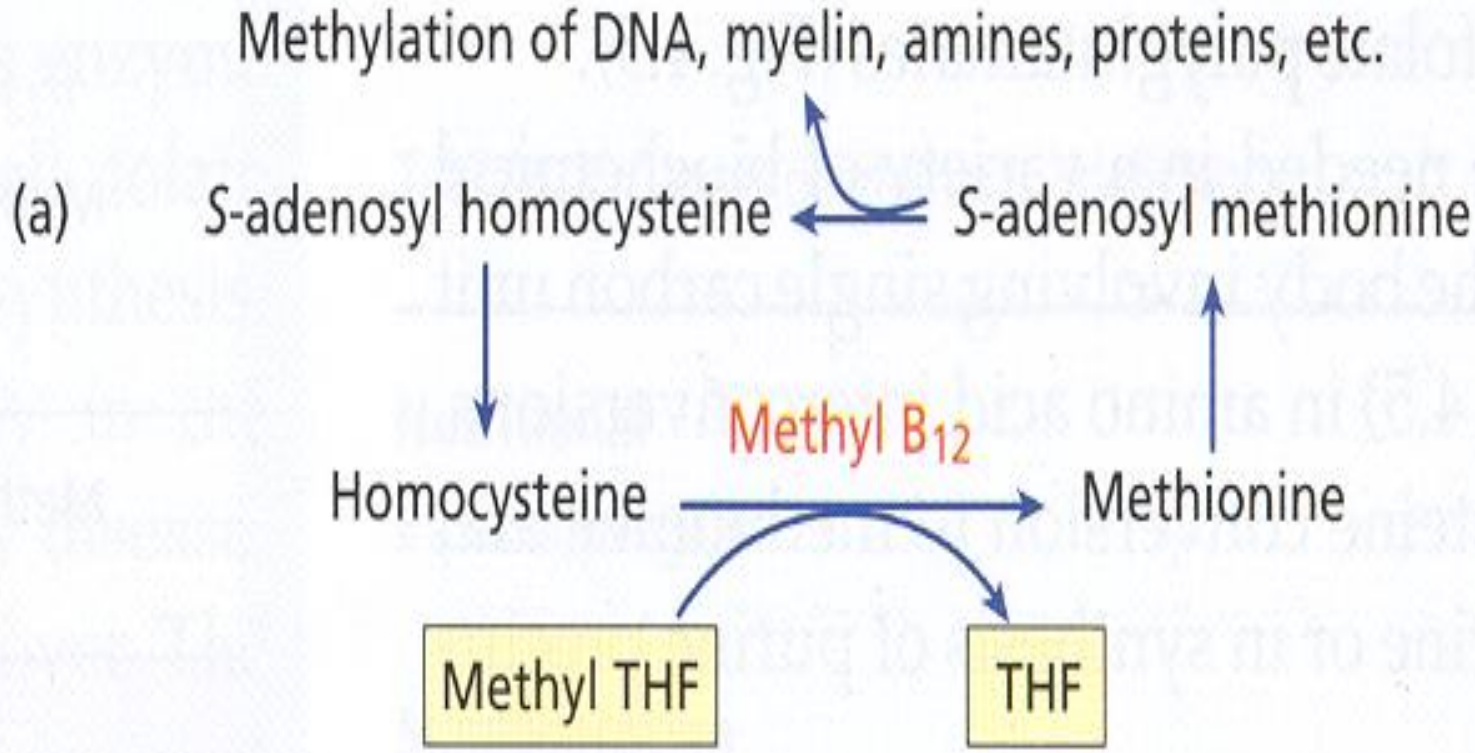
Homocysteine

Methyltransferase

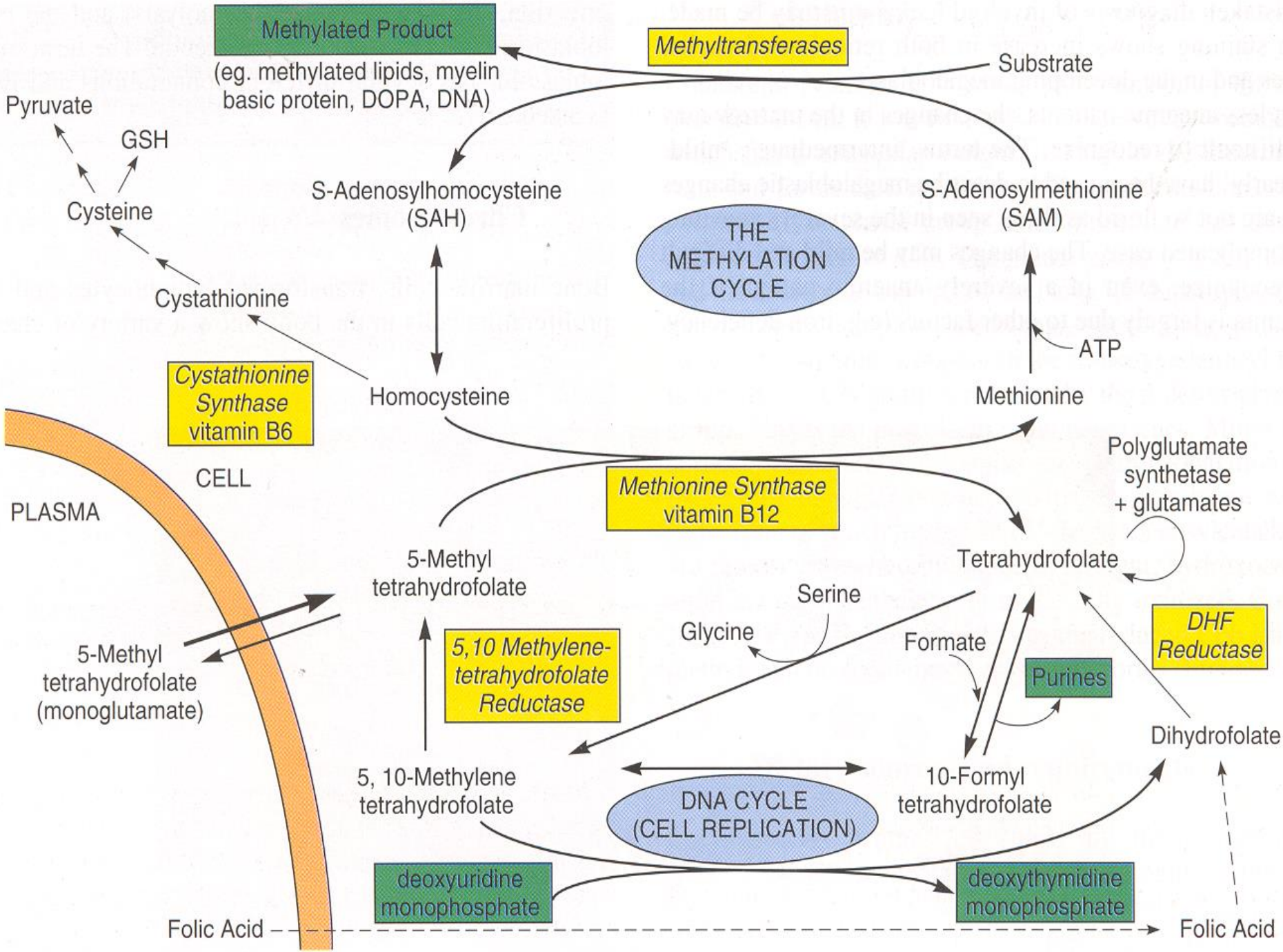
Methylcobalamin

5-Methylterahydrofolate

Tetrahydrofolate

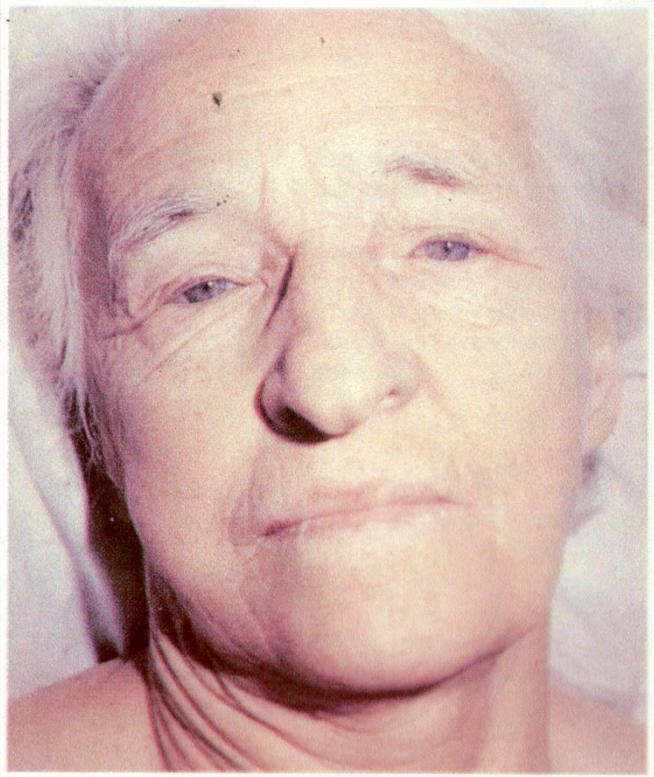

Methylation of DNA, myelin, amines, proteins, etc.
(a)
S-adenosyl homocysteine
S-adenosyl methionine
Methyl B12
Homocysteine
Methionine
Methyl THF
THF

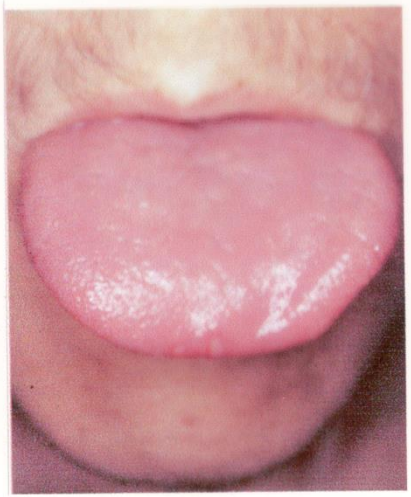

Ado B12
(b)
Propionyl CoA
Methylmalonyl CoA
Succinyl CoA

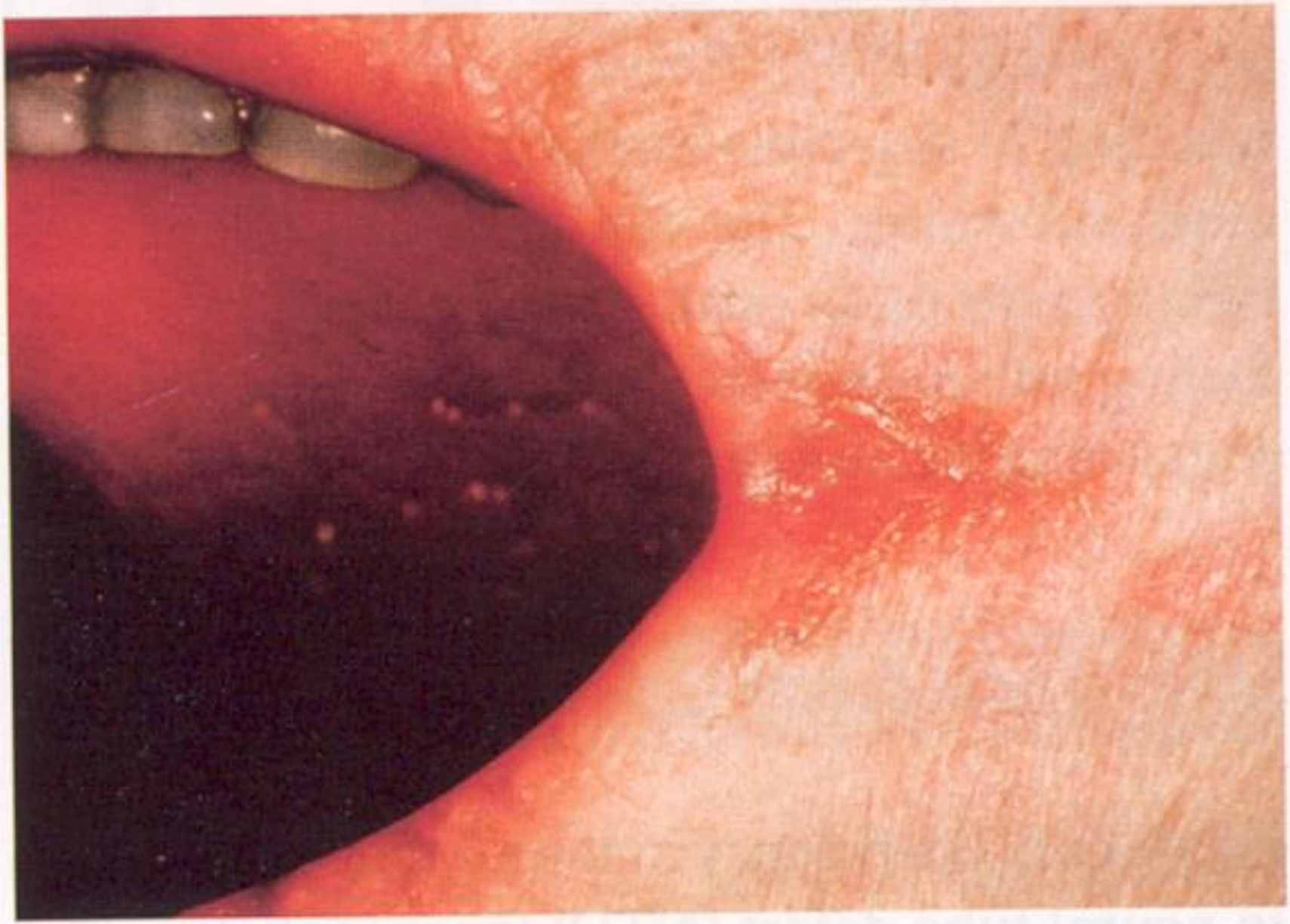

Methylated Product
(eg. methylated lipids, myelin basic protein, DOPA, DNA)
Methyltransferases
Substrate
Pyruvate
GSH
Cysteine
S-Adenosylhomocysteine (SAH)
S-Adenosylmethionine (SAM)
THE METHYLATION CYCLE
Cystathionine
ATP
Cystathionine Synthase vitamin B6
Homocysteine
Methionine
CELL
PLASMA
Methionine Synthase vitamin B12
Polyglutamate synthetase + glutamates
5-Methyl tetrahydrofolate
Tetrahydrofolate
Serine
Glycine
Formate
DHF Reductase
5-Methyl tetrahydrofolate (monoglutamate)
5,10 Methylene-tetrahydrofolate Reductase
Purines
Dihydrofolate
5, 10-Methylene tetrahydrofolate
10-Formyl tetrahydrofolate
DNA CYCLE (CELL REPLICATION)
deoxyuridine monophosphate
deoxythymidine monophosphate
Folic Acid
Folic Acid

Clinical Features of Megaloblastic Anaemia – Progressive symptoms and signs of anaemia

- **Weakness, anorexia, weight loss, diarrhoea or constipation, tiredness, shortness of breath, angina of effort, heart failure**
- **Mild jaundice, glossitis, stomatitis, angular cheilosis.**
- **Purpura, melanin pigmentations.**
- **Infections**







Neuropathy due to Vit B_{12} and folate deficiency

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Progressive neuropathy affecting

- The peripheral sensory nerves
- Posterior and lateral columns of the spinal cord (subacute combined degeneration of the cord)
- Optic atrophy
- Psychiatric symptoms
- The neuropathy is likely due to accumulation of S-adenosyl homocysteine and reduced level of S-adenosyl methionine in nervous tissue resulting in defective methylation of myelin and other substrates.
- Neuropathy is mostly due to vitamin B_{12} deficiency.

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Neural tube defect (NTD)

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- **(Anencephaly, spina bifida or encephalocoele) in the fetus due to folate or Vit B_{12} deficiency in the mother. This result in build-up of homocysteine and S-adenosyl homocysteine in the fetus which impair methylation of various proteins and lipids.**

- **Polymorphism in the enzyme 5,10 methylene tetrahydrofolate reductase (5,10-MTHFR). This mutation (677 C→T) in the MTHFR gene results in low serum and red cell folate and high serum homocysteine in the parents and fetus with NTD.**

- **Cleft palate and hair lip.**

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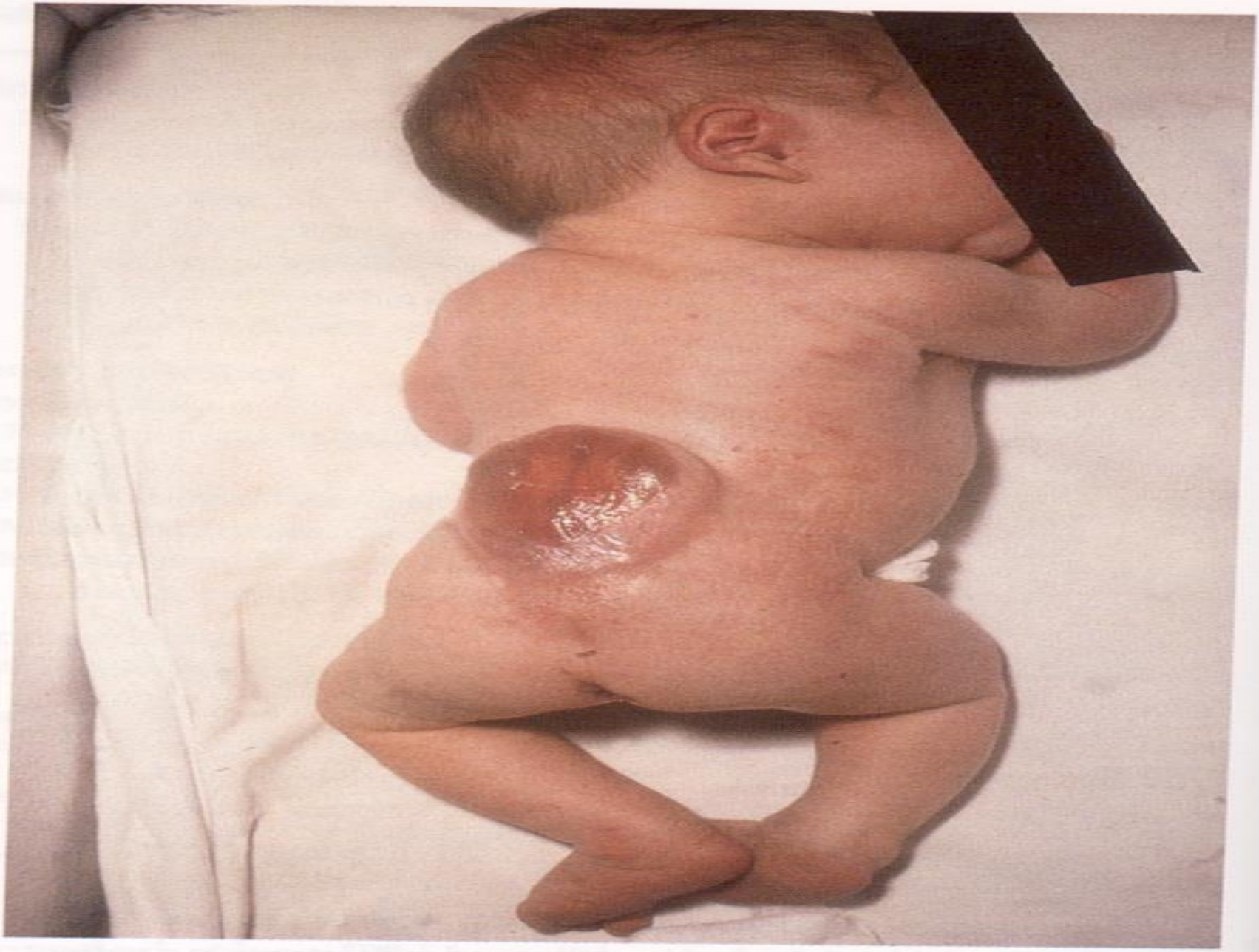
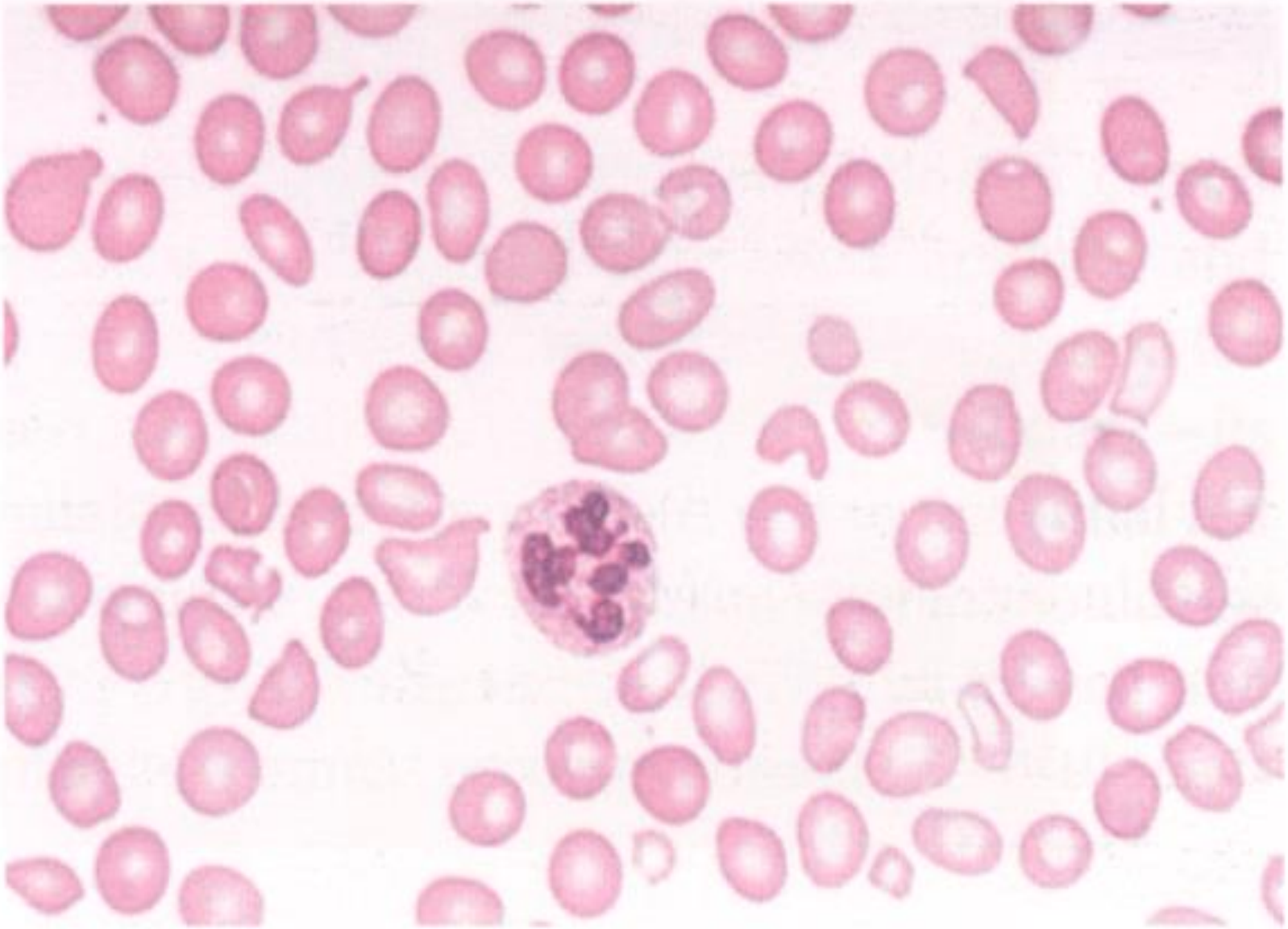
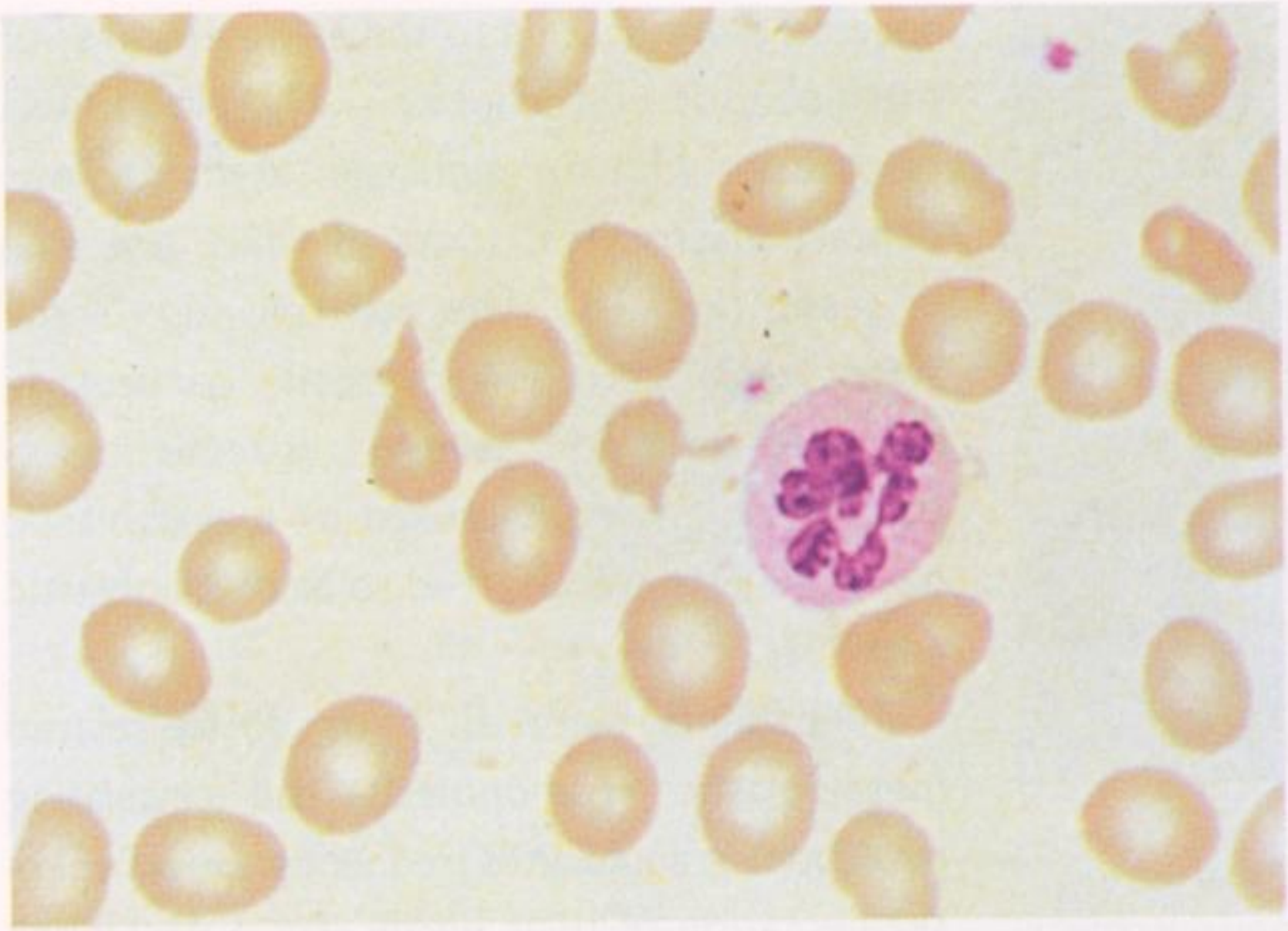


Haematological findings in Megaloblastic Anaemia

Peripheral Blood:

- Macrocytic anaemia, oval macrocytes, anisocytosis, poikilocytosis high MCV.
- Dimorphic anaemia when it is associated with iron deficiency or with thalassaemia trait.
- Hypersegmented neutrophils.
- Leucopenia and thrombocytopenia

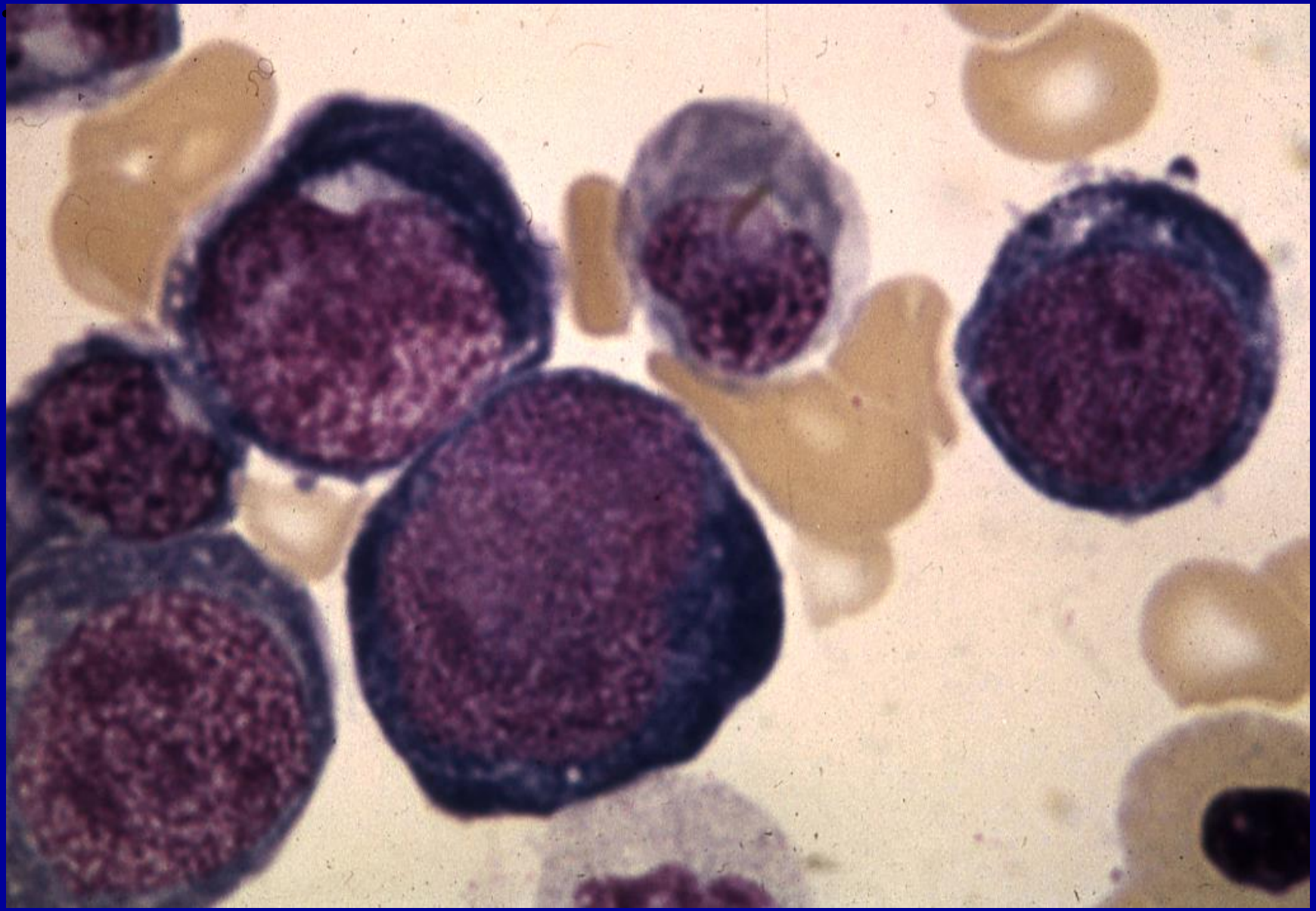


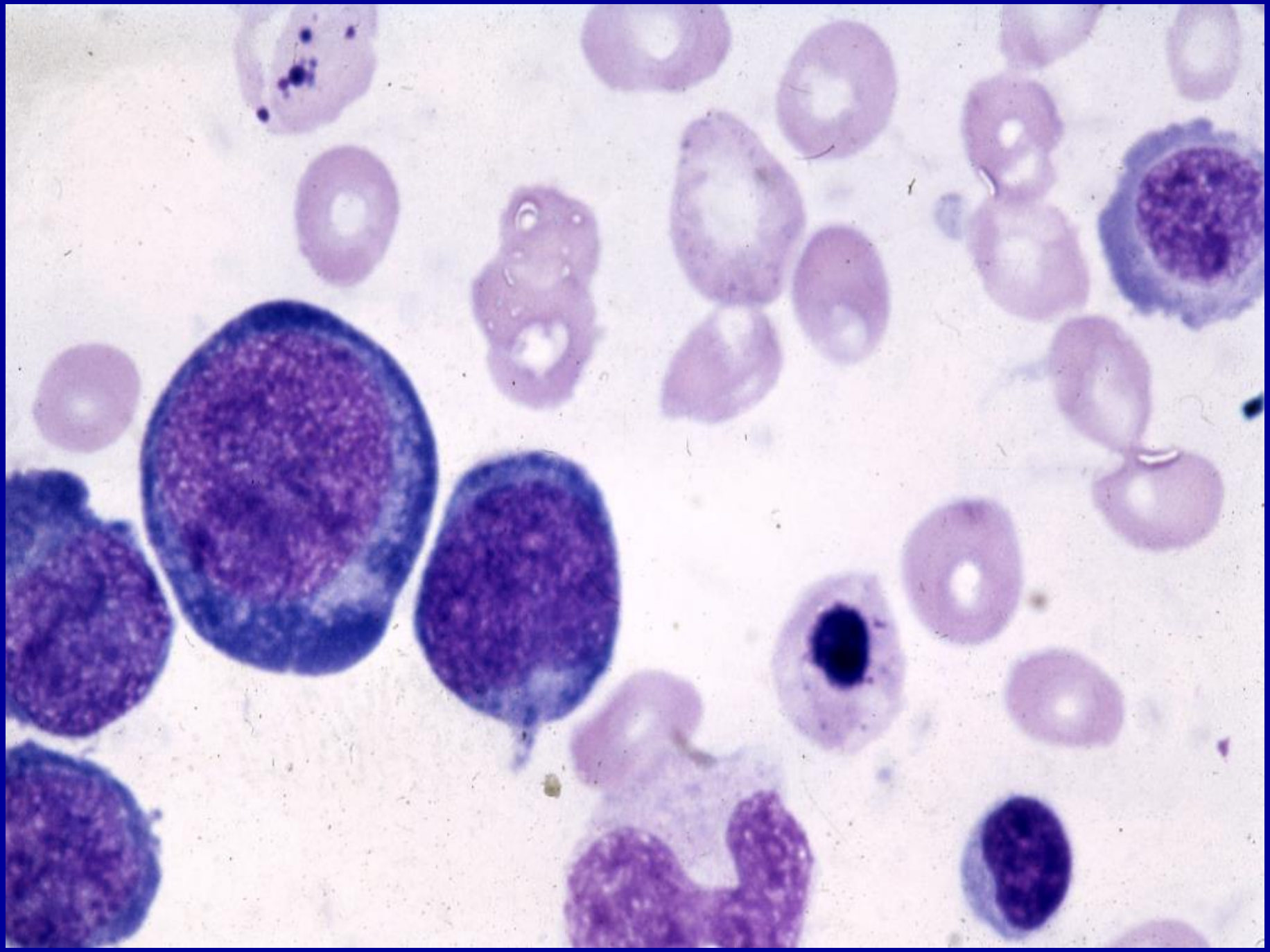


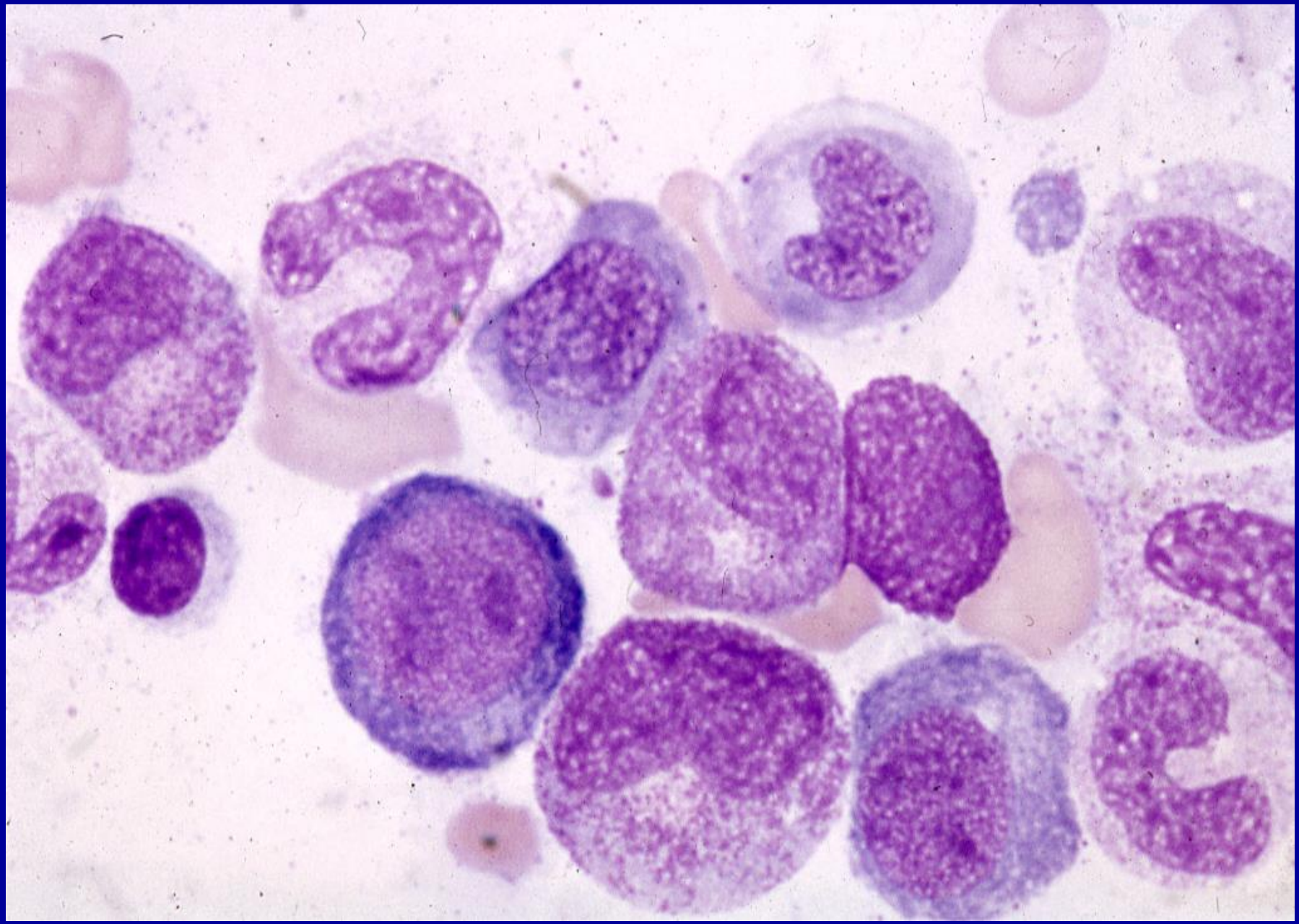
Haematological Findings in Megaloblastic Anaemia

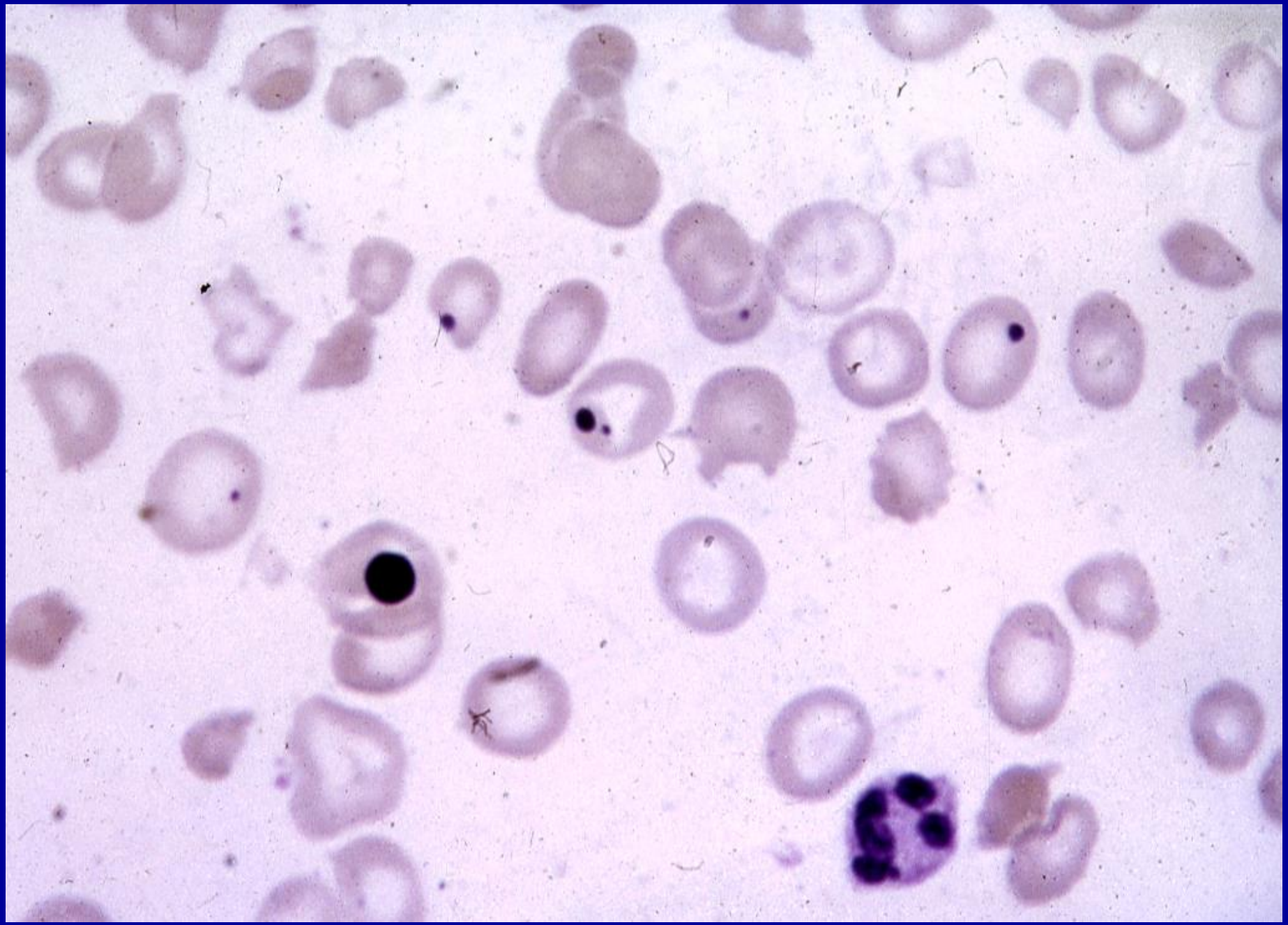
Bone Marrow:

- Hypercellular marrow with M:E ratio in normal or reduced.
- Accumulation of primitive cells due to selective death of more mature cells.
- Megaloblast (large erythroblast which has a nucleus of open, fine, lacy chromatin).
- Dissociation between the nuclear and cytoplasmic development in the erythroblasts.
- Mitosis and dying cells are more frequent than normal.
- Giant and abnormally shaped, metamyelocytes, polypoid megakaryocytes.
- Increased stainable iron in the macrophage and in the erythroblasts.



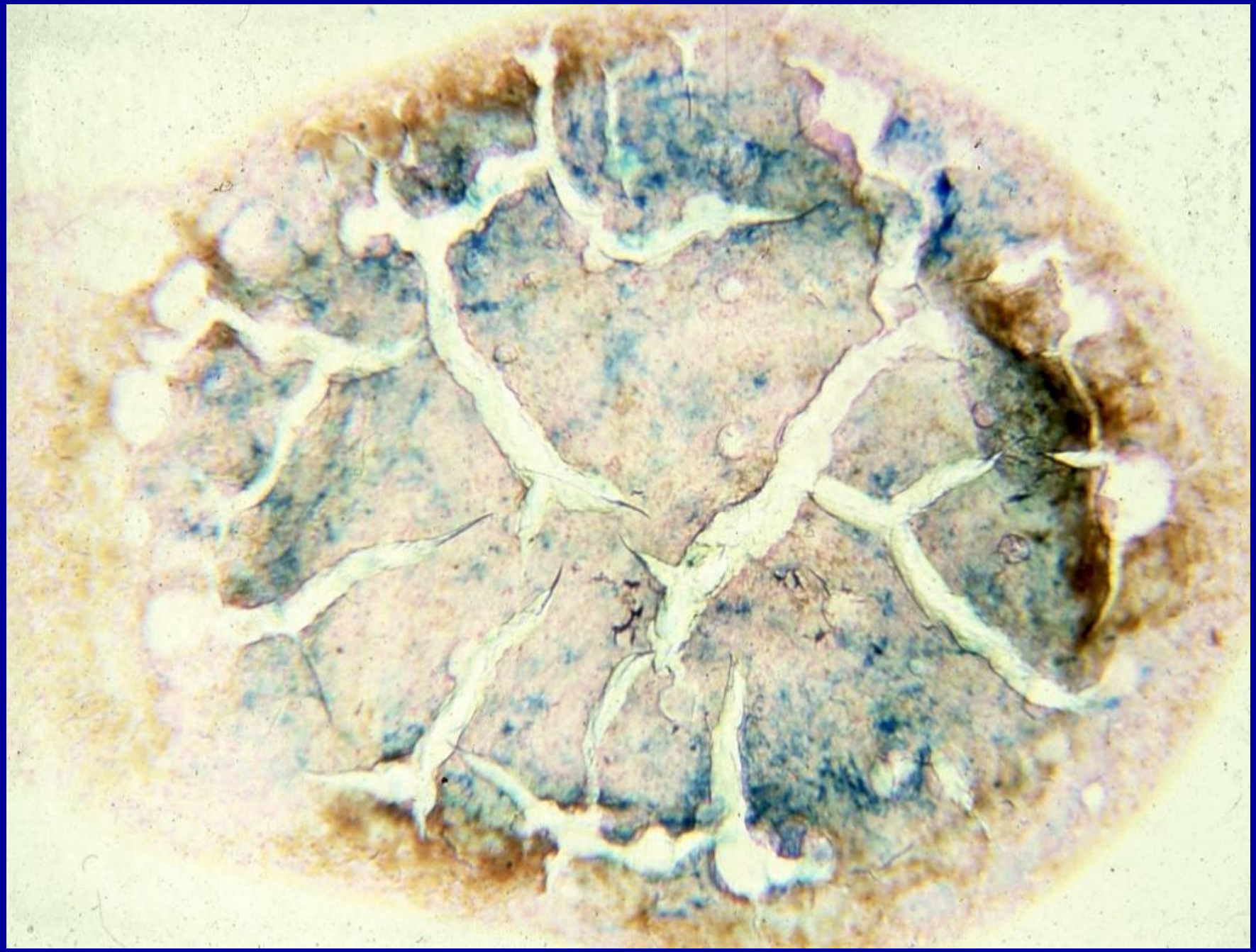






Other laboratory abnormalities

- Chromosomal abnormalities
- Ineffective haemopoiesis. (Intramedullary cell death by apoptosis) associated with increased serum indirect bilirubin.
- ↑ urobillinogen and faecal stercobillinogen.
- ↑ LDH ↑ serum iron ↑ blood carbon monoxide.
- ↑ serum lysozyme
- ↓ reduced haptoglobins
- Positive schumm's test
- Positive urine haemosiderin.



Treatment of megaloblatic anaemia

	Vitamin B_{12} deficiency	Folate deficiency
Compound	Hydroxocobalamin	Folic acid
Route	Intramuscular	Oral
Dose	1000 µg	5mg
Initial dose	6X1000 µg over 2-3 weeks	Daily for 4 months
Maintenance	1000 µg every 3 months	Depends on underlying disease; life-long therapy may be needed in chronic inherited haemolytic anaemia, myelofibrosis, renal dialysis
Prophylactic	Total gastrectomy Ileal resection	Pregnancy, severe haemolytic anaemias, dialysis, prematurity


Thank you !!!