

# MEGALOBLASTIC ANAEMIA

BY:

**DR. ALIA AL-FARAEDI**

Head of Haematopathology Unit

Consultant Haematopathologist

Department of Pathology

# 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

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

\* 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 <sup>9</sup> /L
Neutrophils	2.5 - 7.5 x 10 <sup>9</sup> /L
Lymphocytes	1.5 - 3.5 x 10 <sup>9</sup> /L
Monocytes	0.2 - 0.8 x 10 <sup>9</sup> /L
Eosinophils	0.04 - 0.44 x 10 <sup>9</sup> /L
Basophil	0.01 - 0.1 x 10 <sup>9</sup> /L
Platelets	150-450 x 10 <sup>9</sup> /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 > 26 pg**

**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

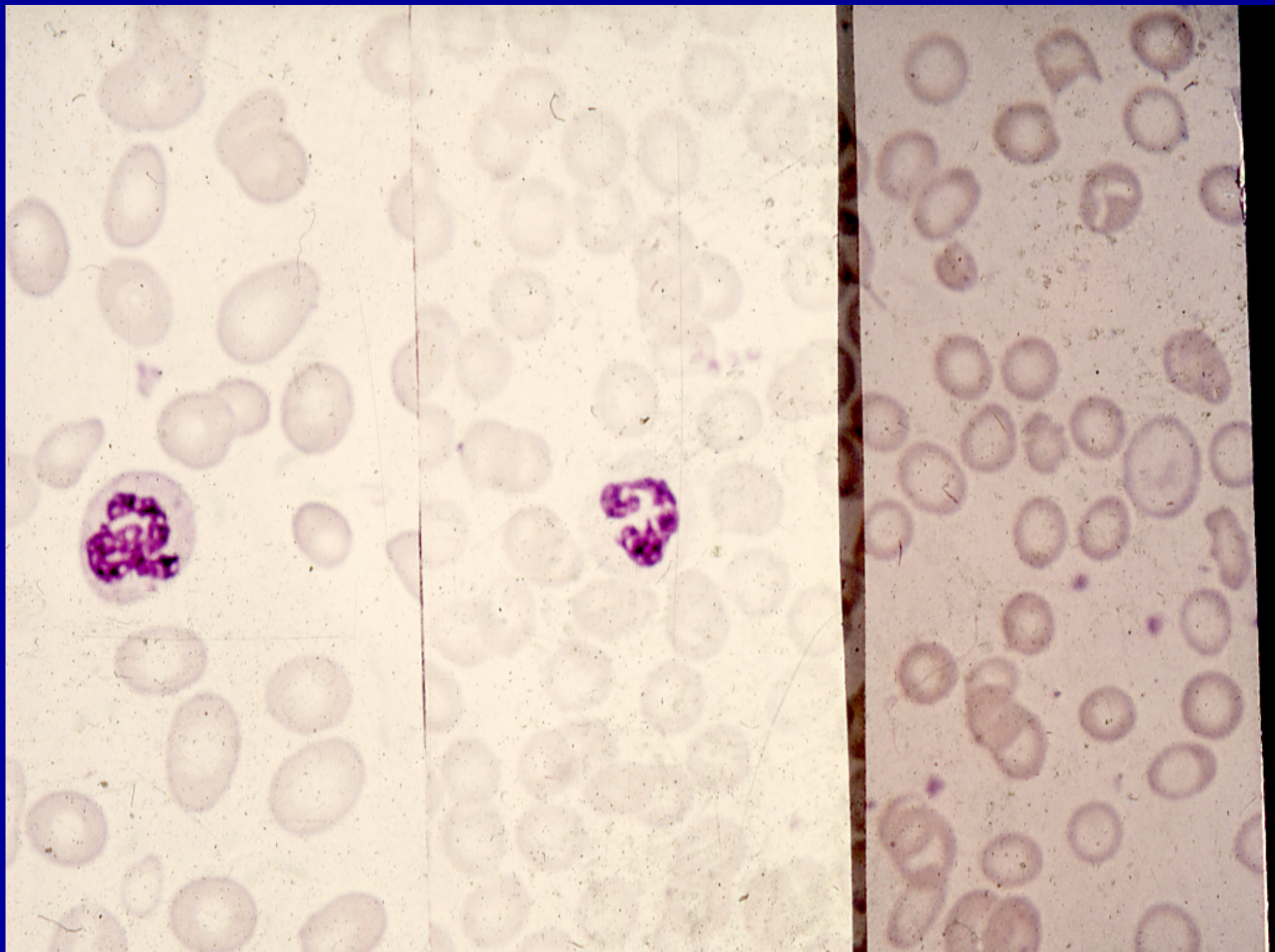
Macrocytic anaemias can be divided into those showing:

*1. Megaloblastic erythropoiesis*

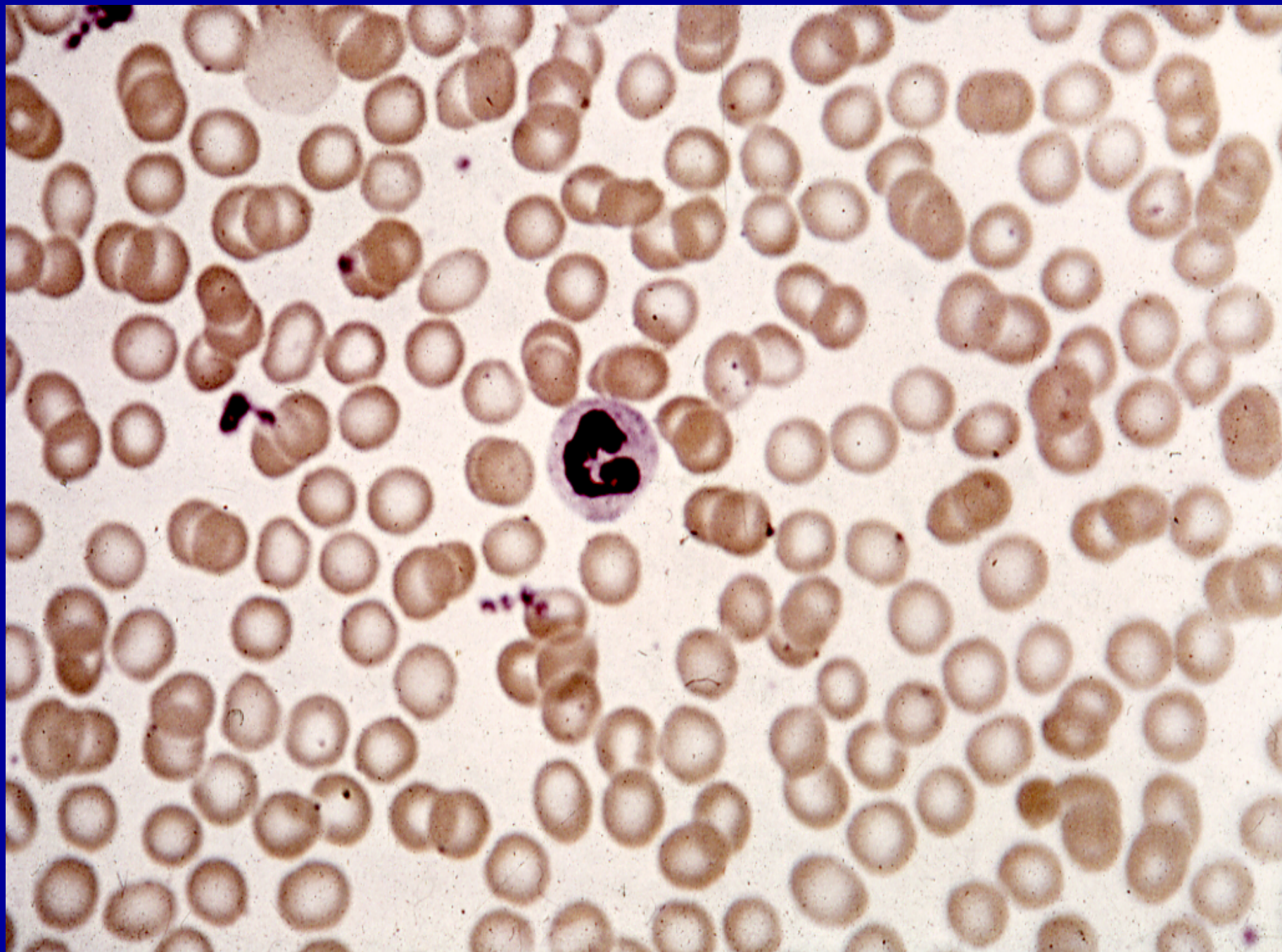
*2. Normoblastic erythropoiesis*

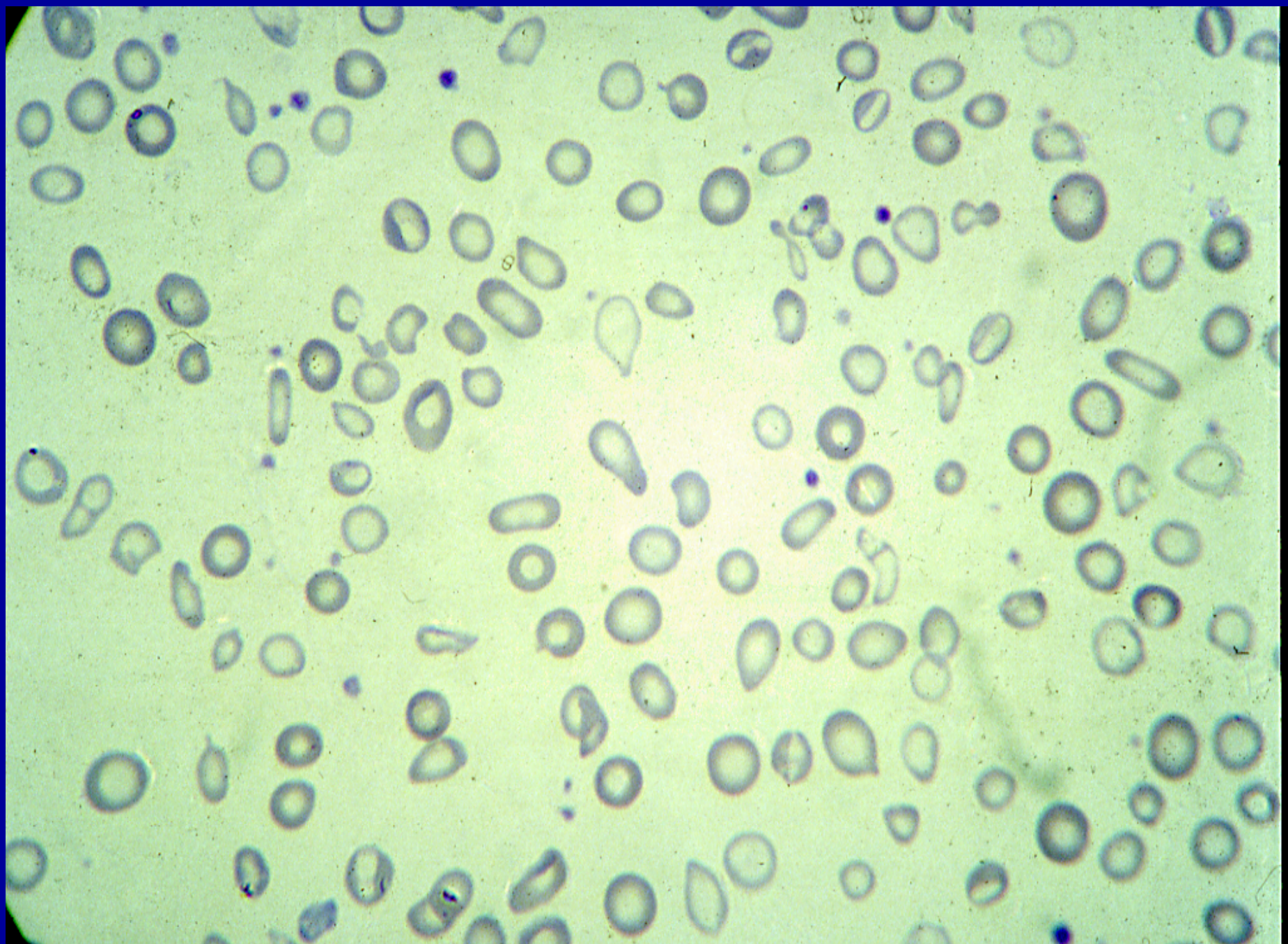
*Megaloblastic erythropoiesis* - describes abnormal red cell development characterized by a lack of synchrony between the maturation of the red cell nucleus and its cytoplasm. It arises as a consequence of disordered DNA synthesis and results in a macrocytic anaemia.

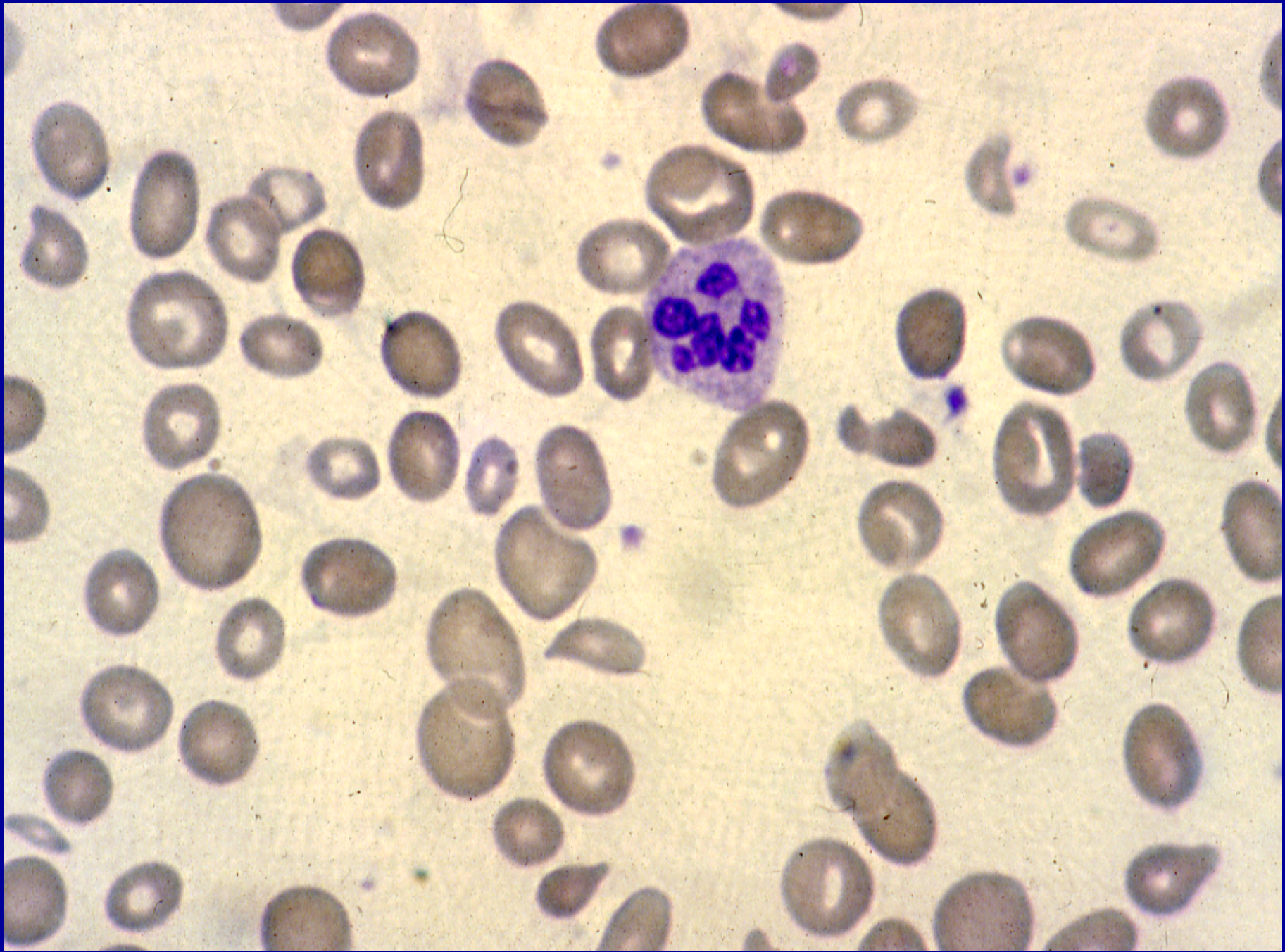
*Normoblastic erythropoiesis* - describes the normal appearance of red cell maturation - but may still be associated with a macrocytosis in the peripheral blood.











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

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### 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

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**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**

# Causes of megaloblastic anaemia :

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1. **Cobalamin deficiency or abnormalities of cobalamin metabolism**
  2. **Folate deficiency or abnormalities of folate metabolism**
  3. **Therapy with antifolate 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 synthesis 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)**
- 
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# Other causes of megaloblasts :

## **Abnormalities of nucleic acid synthesis**

### **Drug therapy**

**Antipurines (mercaptopurine, azathioprine)**

**Antipyrimidines (fluorouracil, zydovudine (AZT))**

**Others (hydroxyurea)**

### **Orotic aciduria**

### **Uncertain aetiology**

**Myelodysplastic syndromes, \* erythroleukaemia**

**Some congenital dyserythropoietic anaemias**

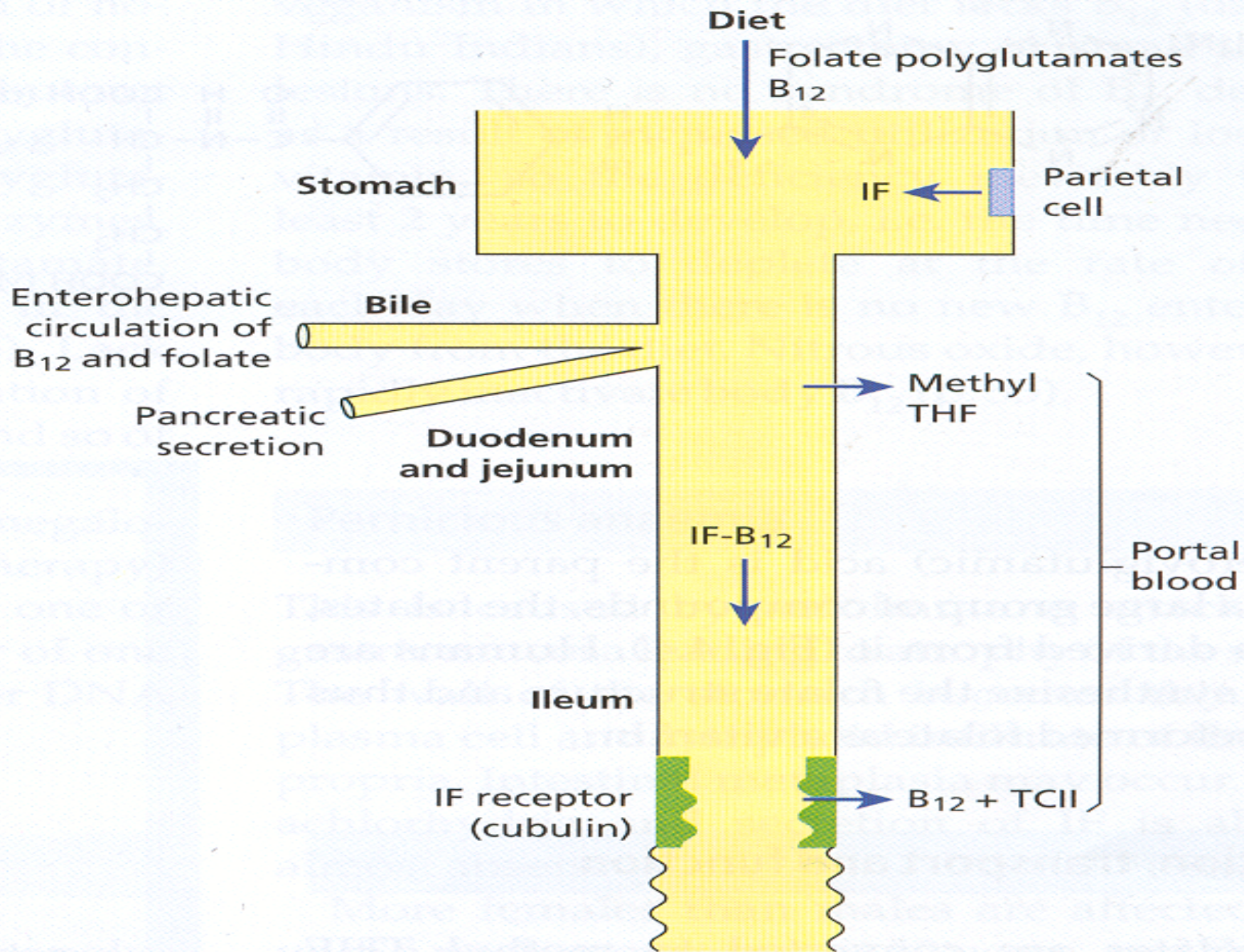
# Vitamin B<sub>12</sub> and folate nutrition and absorption

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

\* In adults.

† Higher during pregnancy and lactation.





## Vitamin B<sub>12</sub> Deficiency

- **Inadequate intake**
- **Veganism**
- **Inadequate secretion of intrinsic factor**
- **Pernicious anaemia**
- **Total or partial gastrectomy**
- **Congenital intrinsic factor deficiency (rare)**
- **Partial gastrectomy, vagotomy, gastritis, acid-suppressing drugs, alcohol abuse**
- **Abnormal intestinal bacterial flora, multiple jejunal diverticula, small intestinal strictures**
- **Diphyllobothrium latum**
- **Malabsorption**
- **Crohn's disease, ileal resection, chronic tropical sprue**

# 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

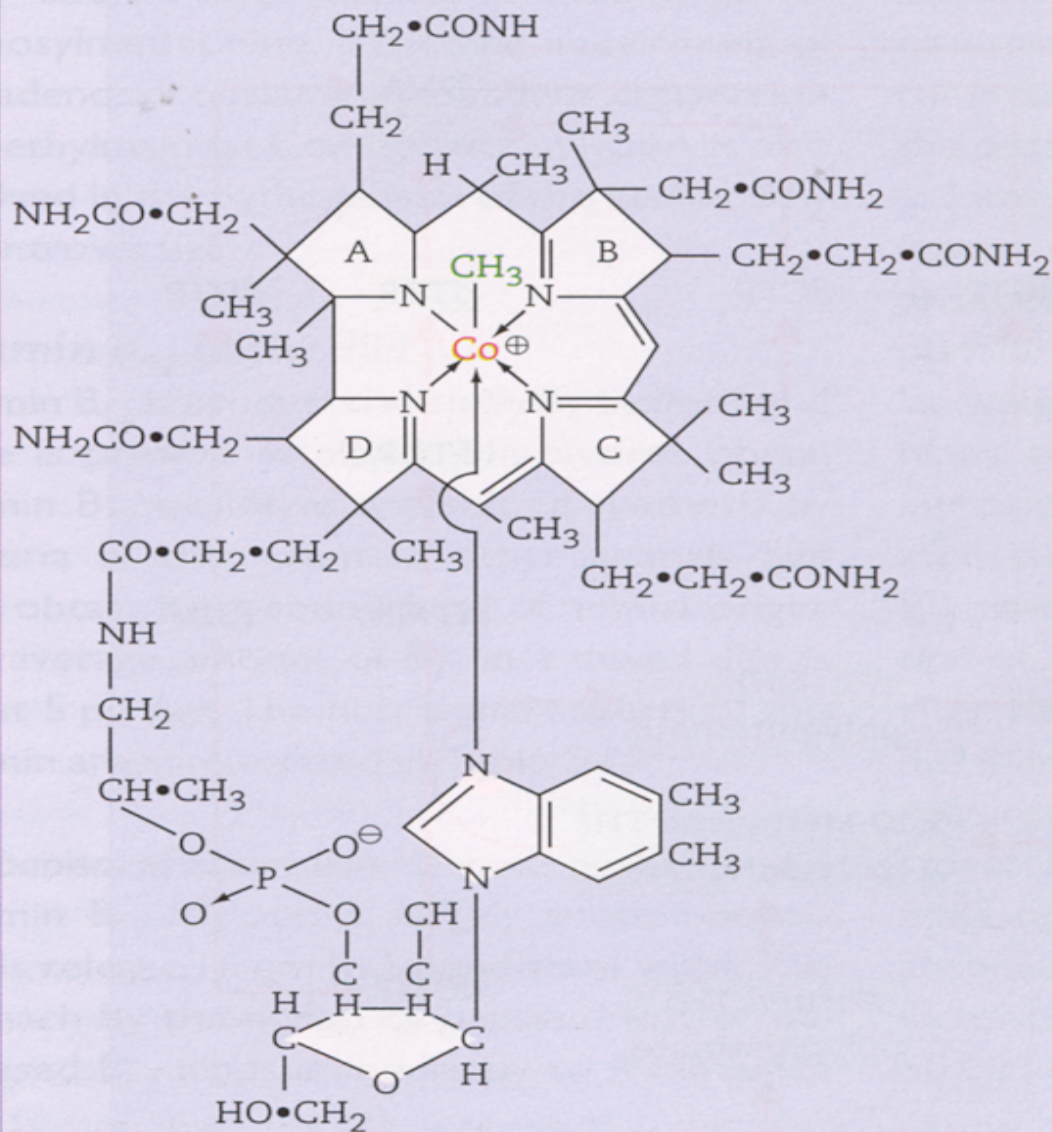
## **Complex mechanism**

Anticonvulsant therapy, \* ethanol abuse\*

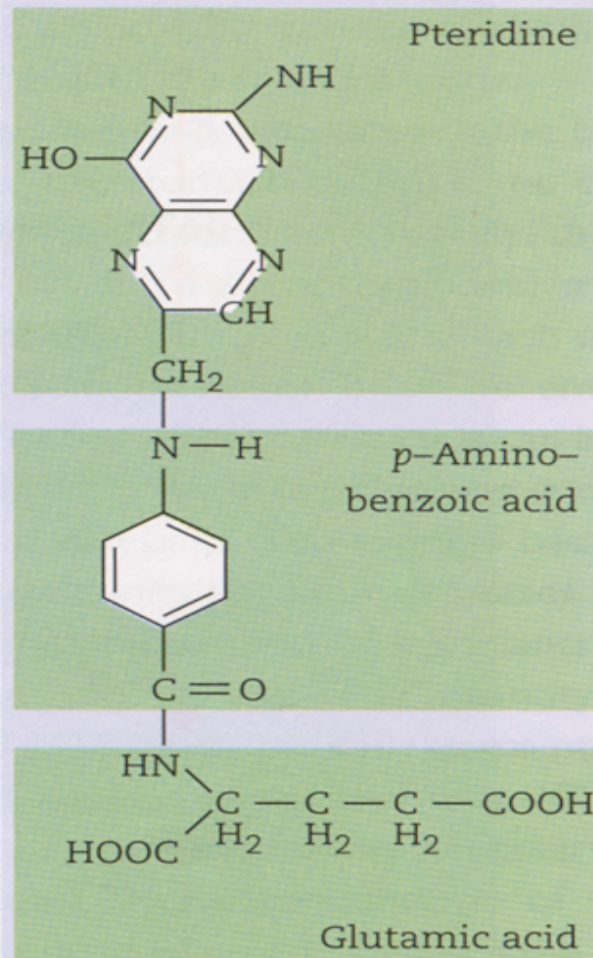
\* Only some cases with macrocytosis are folate deficient.

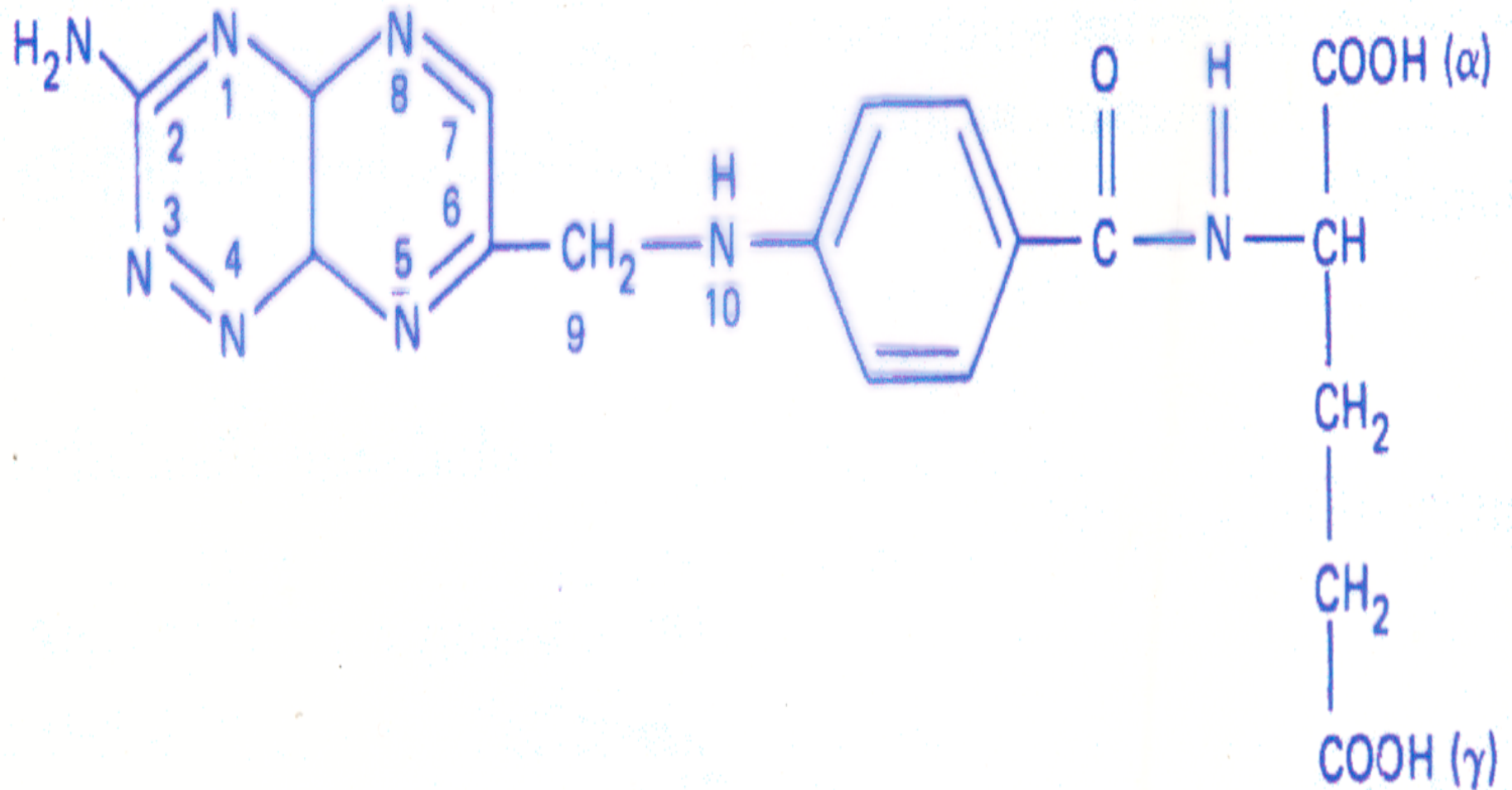
# STRUCTURES OF VITAMIN B<sub>12</sub> AND FOLIC ACID

Methylcobalamin

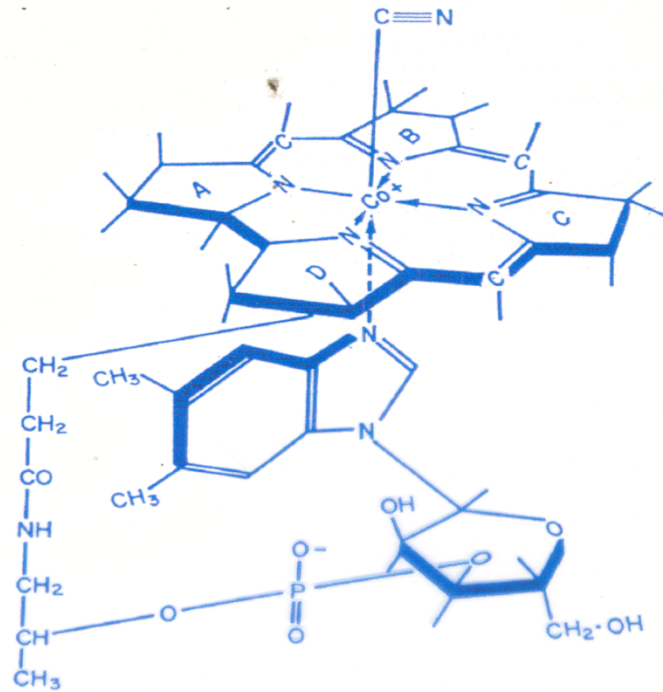


Folic acid

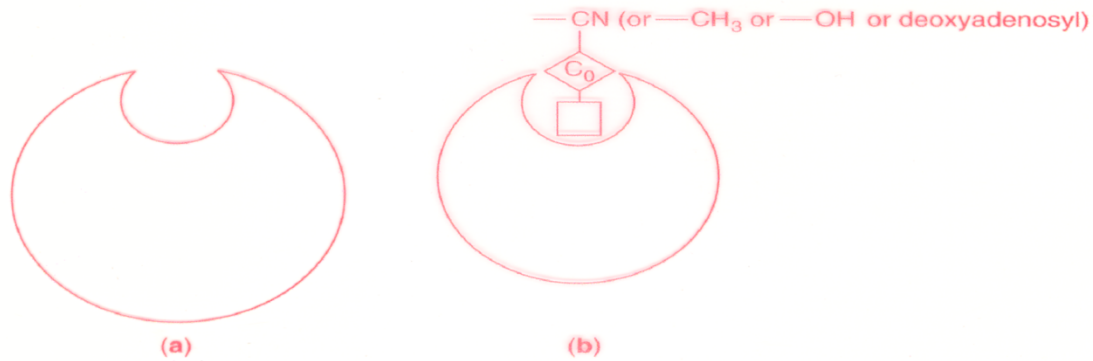




The structure of folic acid (pteroylglumatic acid).



The structure of vitamin B<sub>12</sub> (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<sub>12</sub> 0.8 nm, and the complex 3.2 nm.

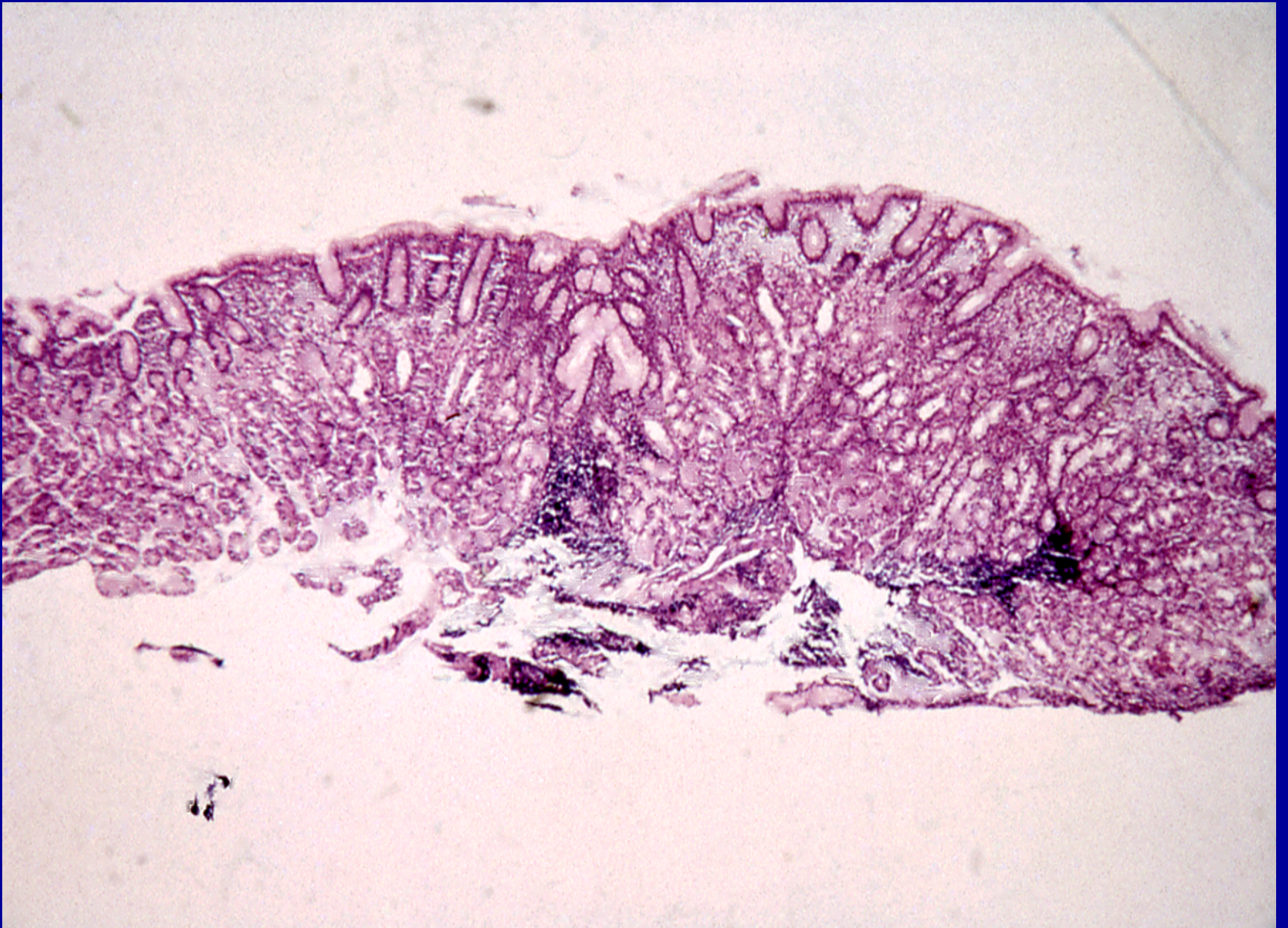
# PERNICIOUS ANAEMIA

- 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 propria.
- 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).

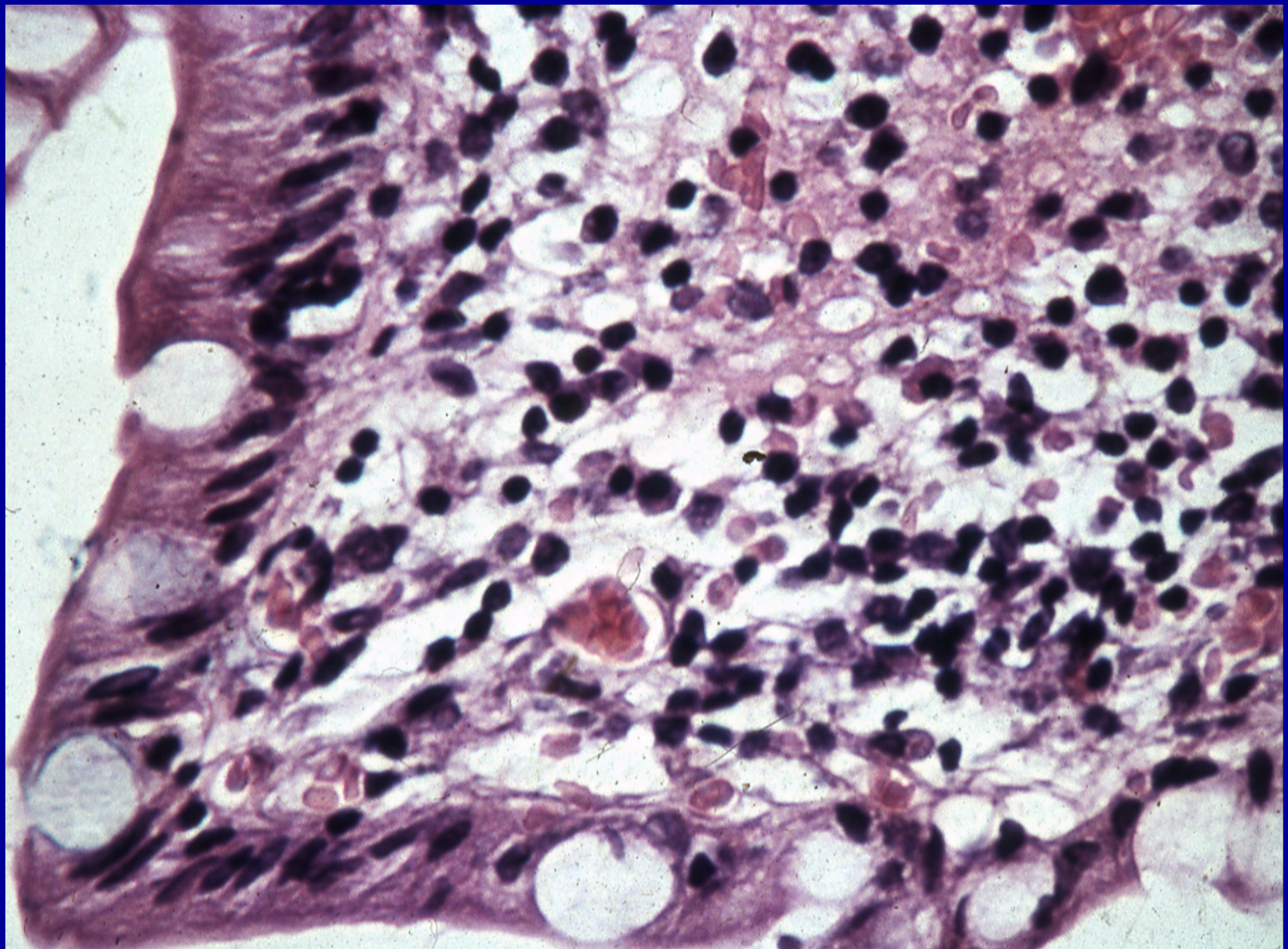




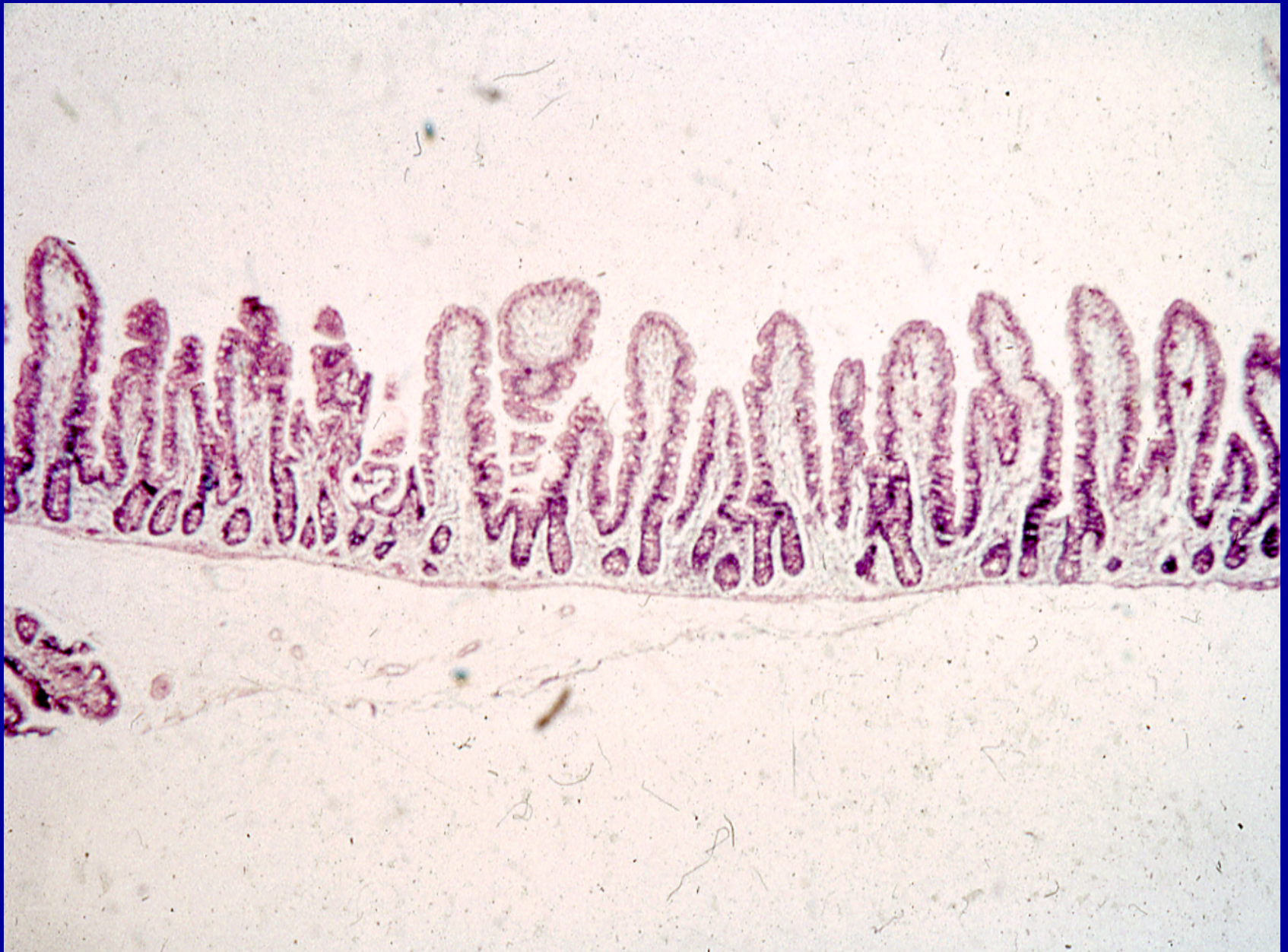
**Normal gastric mucosa**



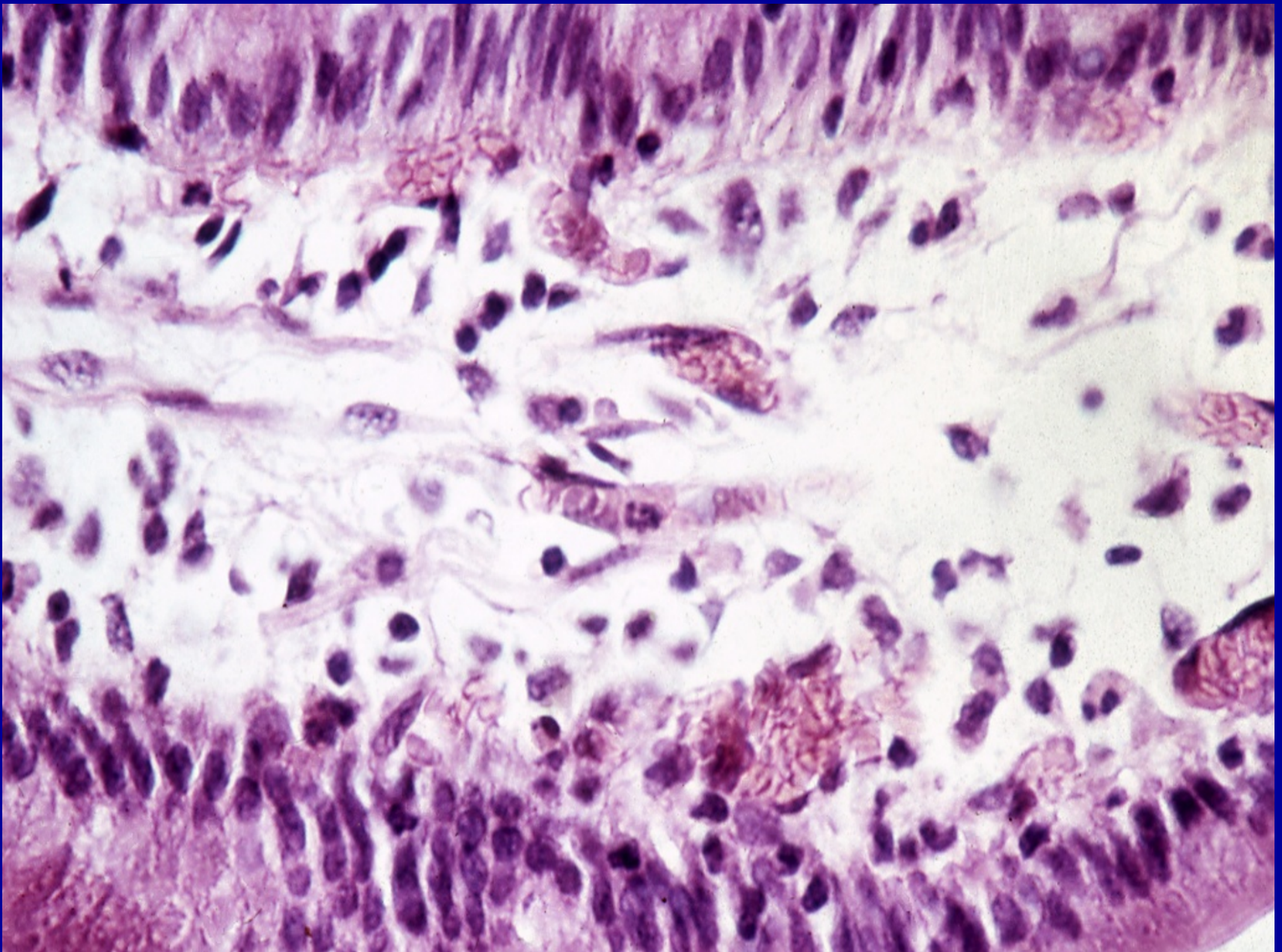
**Gastric atrophy in patients with pernicious anaemia**



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



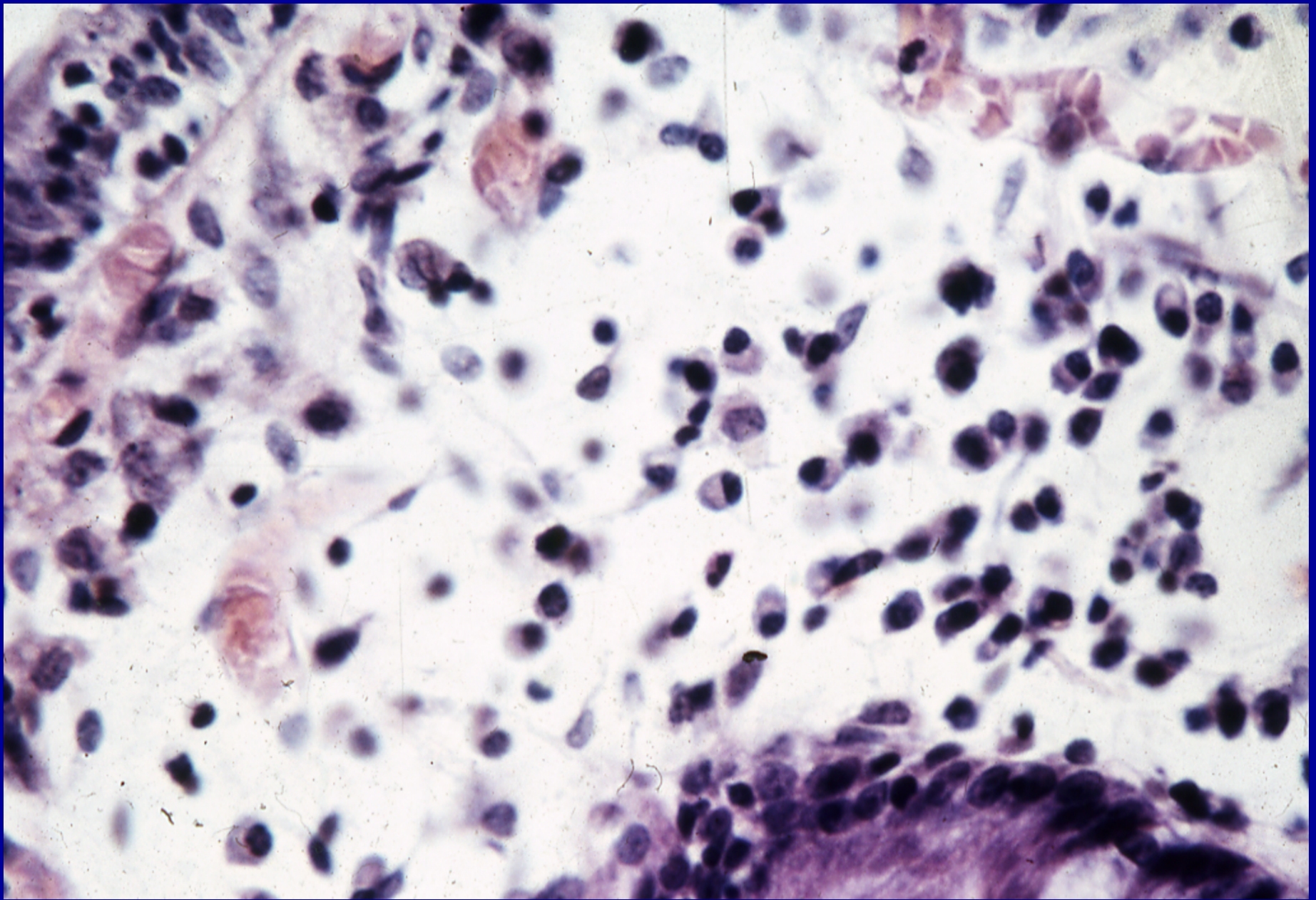
**Normal small intestinal mucosa**



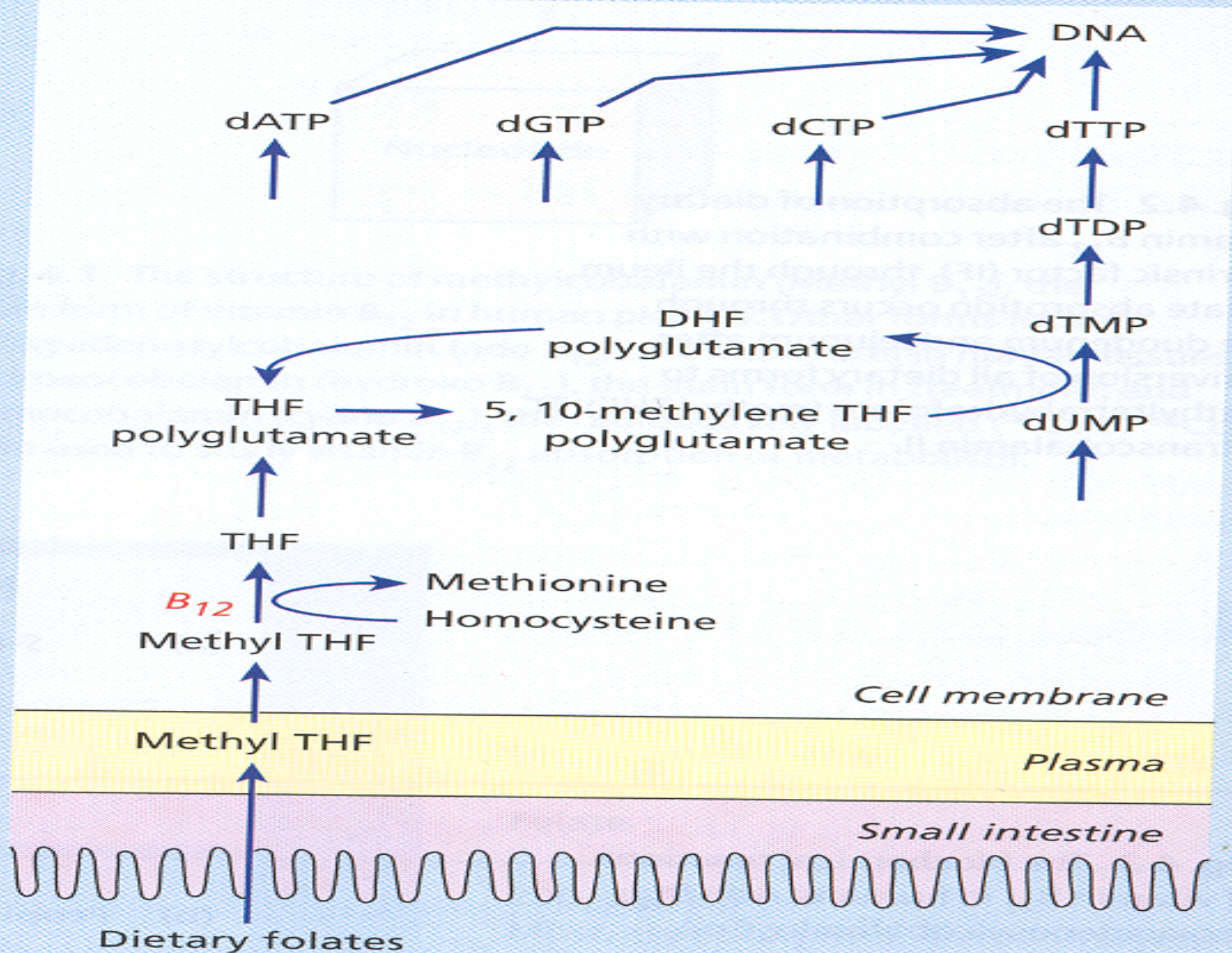
**Normal histology of small intestinal mucosa**



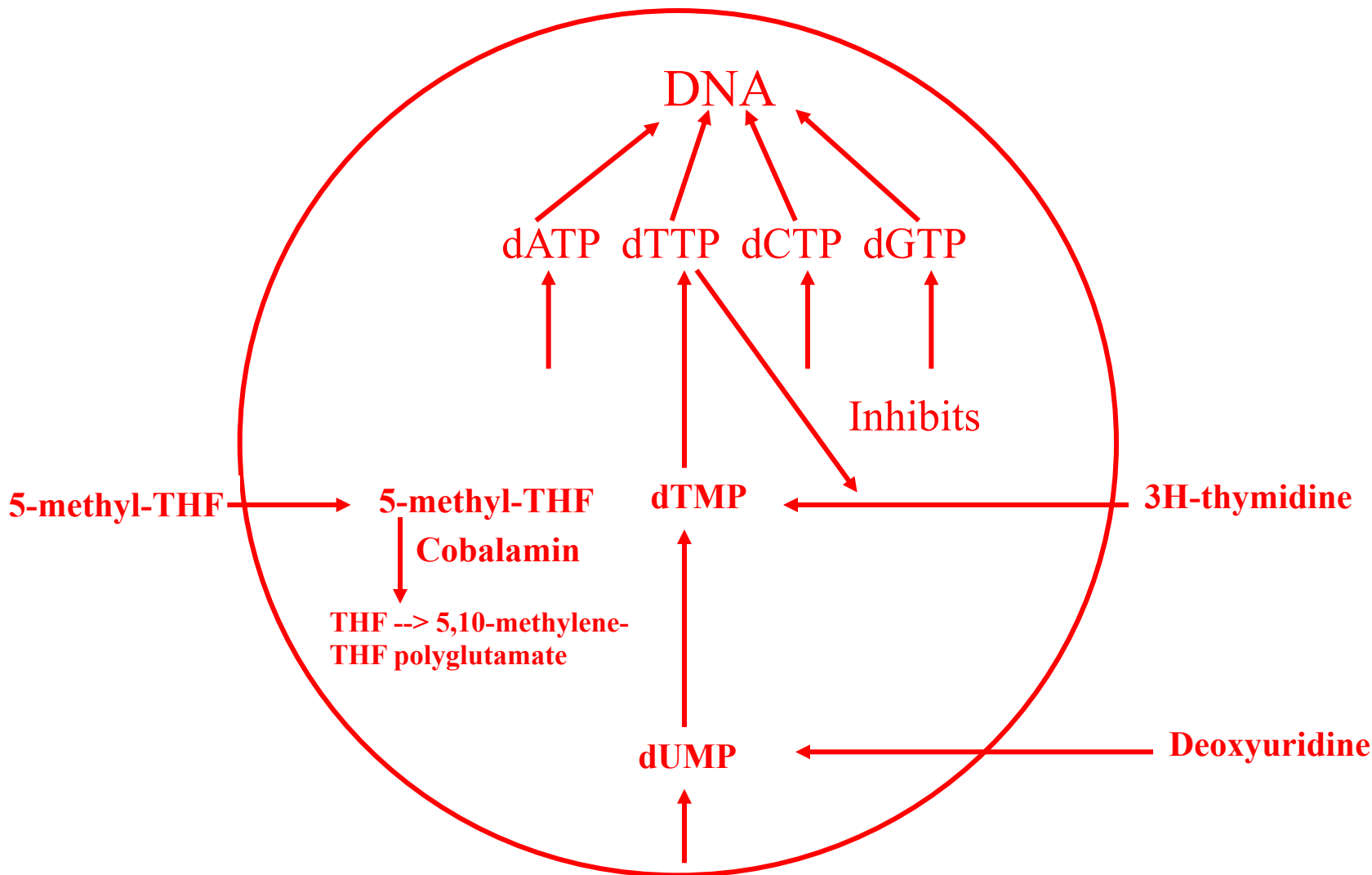
**Flattening of small intestinal mucosa in malabsorption syndrome**



**Histopathology of small intestinal mucosa in malabsorption syndrome**







**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.**

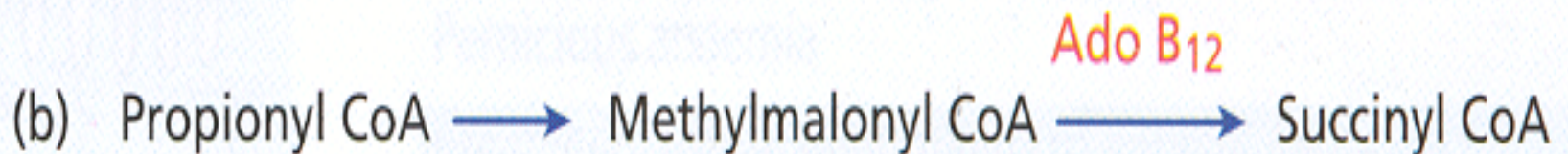
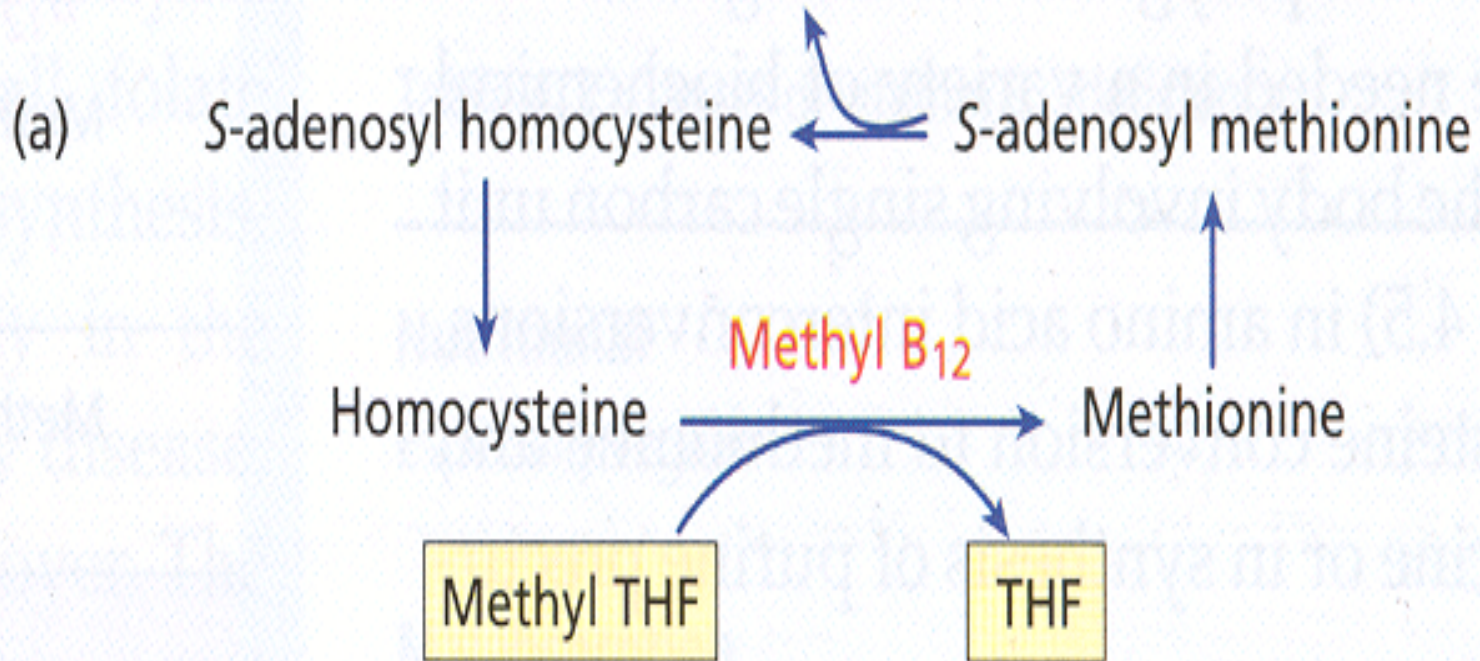
**Homocysteine**  
**Methyltransferase**  
**Methylcobalamin**

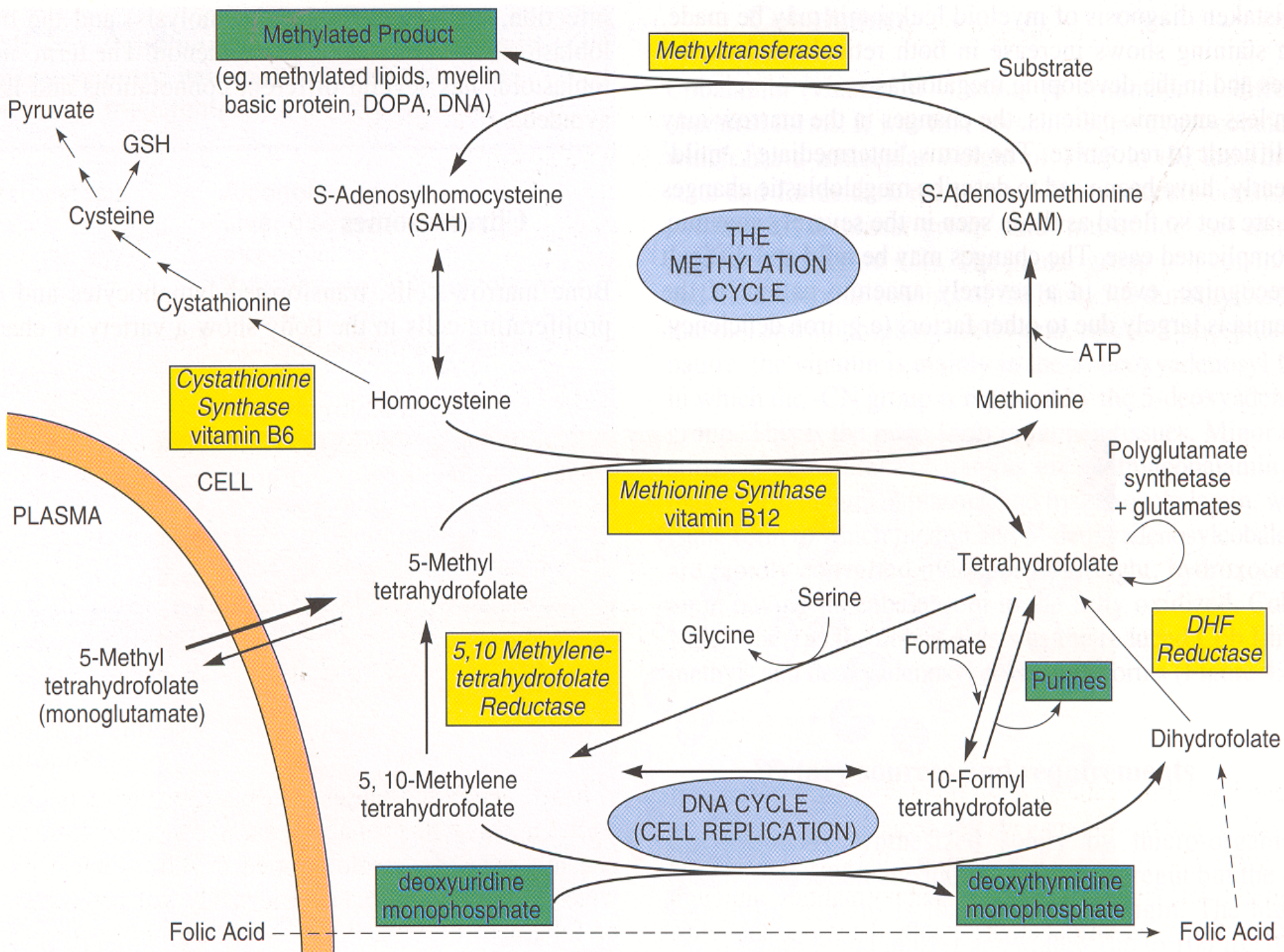


**5-Methyltetrahydrofolate**

**Tetrahydrofolate**

Methylation of DNA, myelin, amines, proteins, etc.





**Methylated Product**  
(eg. methylated lipids, myelin basic protein, DOPA, DNA)

**Methyltransferases**

Substrate

S-Adenosylhomocysteine (SAH)

S-Adenosylmethionine (SAM)

**THE METHYLATION CYCLE**

ATP

Methionine

Homocysteine

**Methionine Synthase**  
vitamin B12

Polyglutamate synthetase + glutamates

Tetrahydrofolate

**DHF Reductase**

**Purines**

Dihydrofolate

Formate

10-Formyl tetrahydrofolate

**DNA CYCLE (CELL REPLICATION)**

**deoxyuridine monophosphate**

**deoxythymidine monophosphate**

Folic Acid

Folic Acid

Glycine

Serine

5-Methyl tetrahydrofolate

**5,10 Methylene-tetrahydrofolate Reductase**

5, 10-Methylene tetrahydrofolate

5-Methyl tetrahydrofolate (monoglutamate)

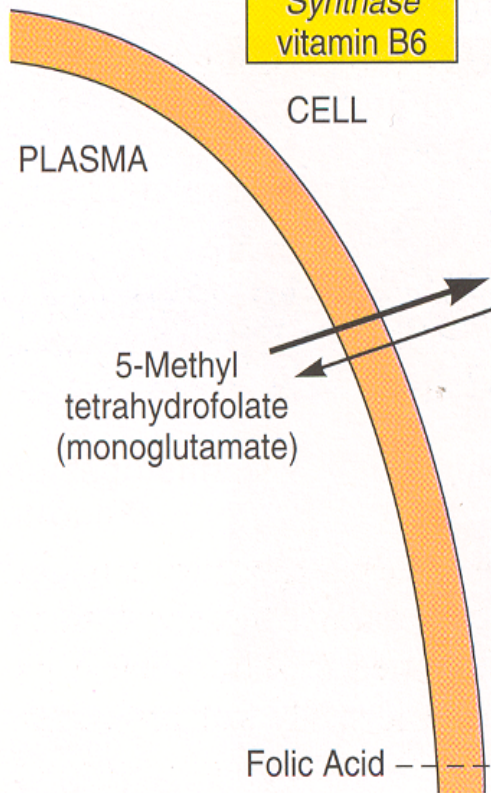
**Cystathionine Synthase**  
vitamin B6

Cystathionine

Cysteine

GSH

Pyruvate



PLASMA

CELL

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

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- ❖ **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**
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FIGURE 1. Large, dark, ulcerated lesion on the buccal mucosa, likely a squamous cell carcinoma.

# Neuropathy due to Vit B<sub>12</sub> and folate deficiency

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## Progressive neuropathy affecting

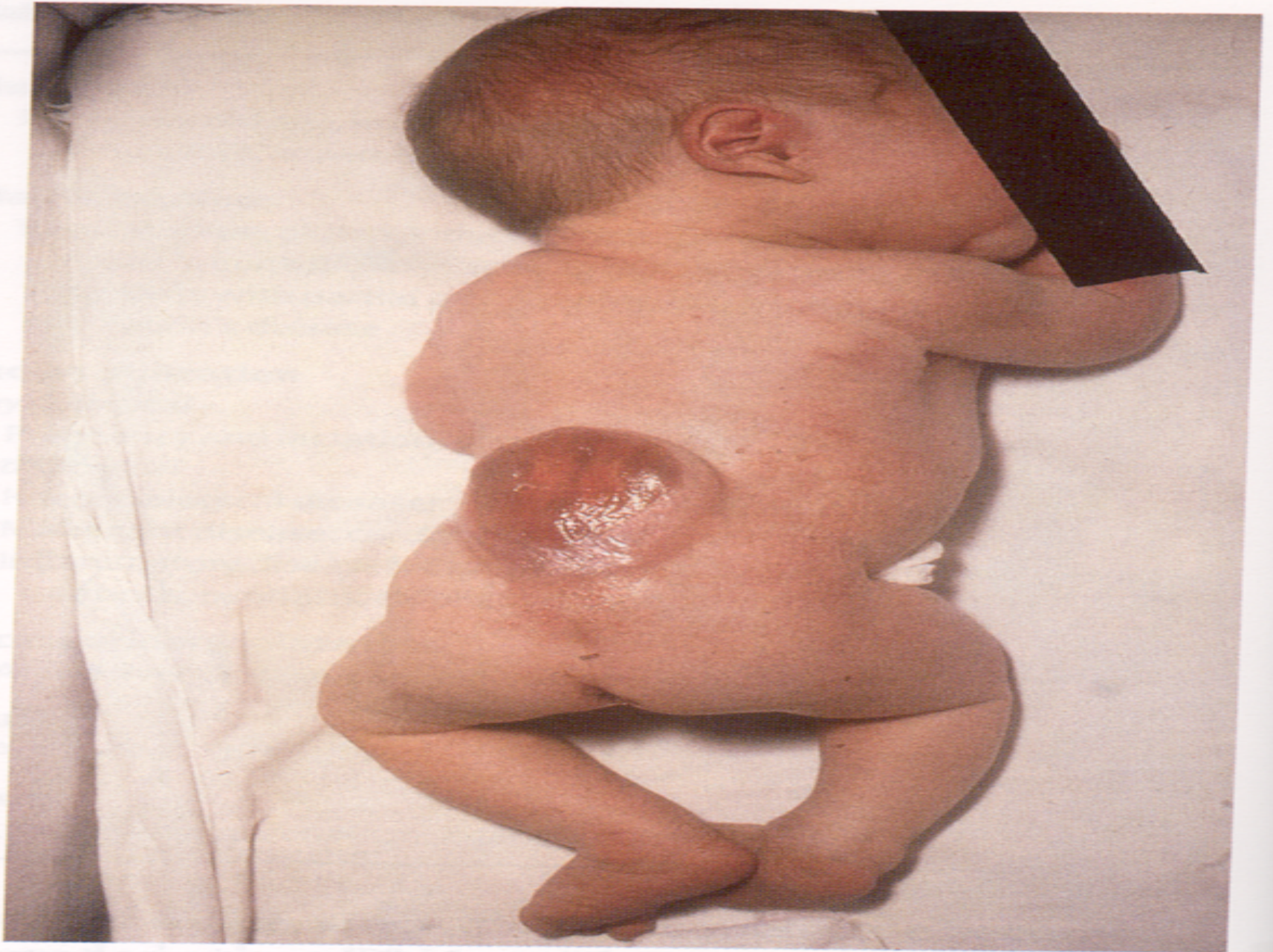
- **The peripheral sensory nerves**
  - **Posterior and lateral columns of the spinal cord (subacute combined degeneration of the cord)**
  - **Optic atrophy**
  - **Psychiatric symptoms**
  - **The neuropathy is likely due to accumulation of S-adenosyl homocysteine and reduced level of S-adenosyl methionine in nervous tissue resulting in defective methylation of myelin and other substrates.**
  - **Neuropathy is mostly due to vitamin B<sub>12</sub> 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<sub>12</sub> deficiency in the mother. This results in build-up of homocysteine and S-adenosyl homocysteine in the fetus which impairs 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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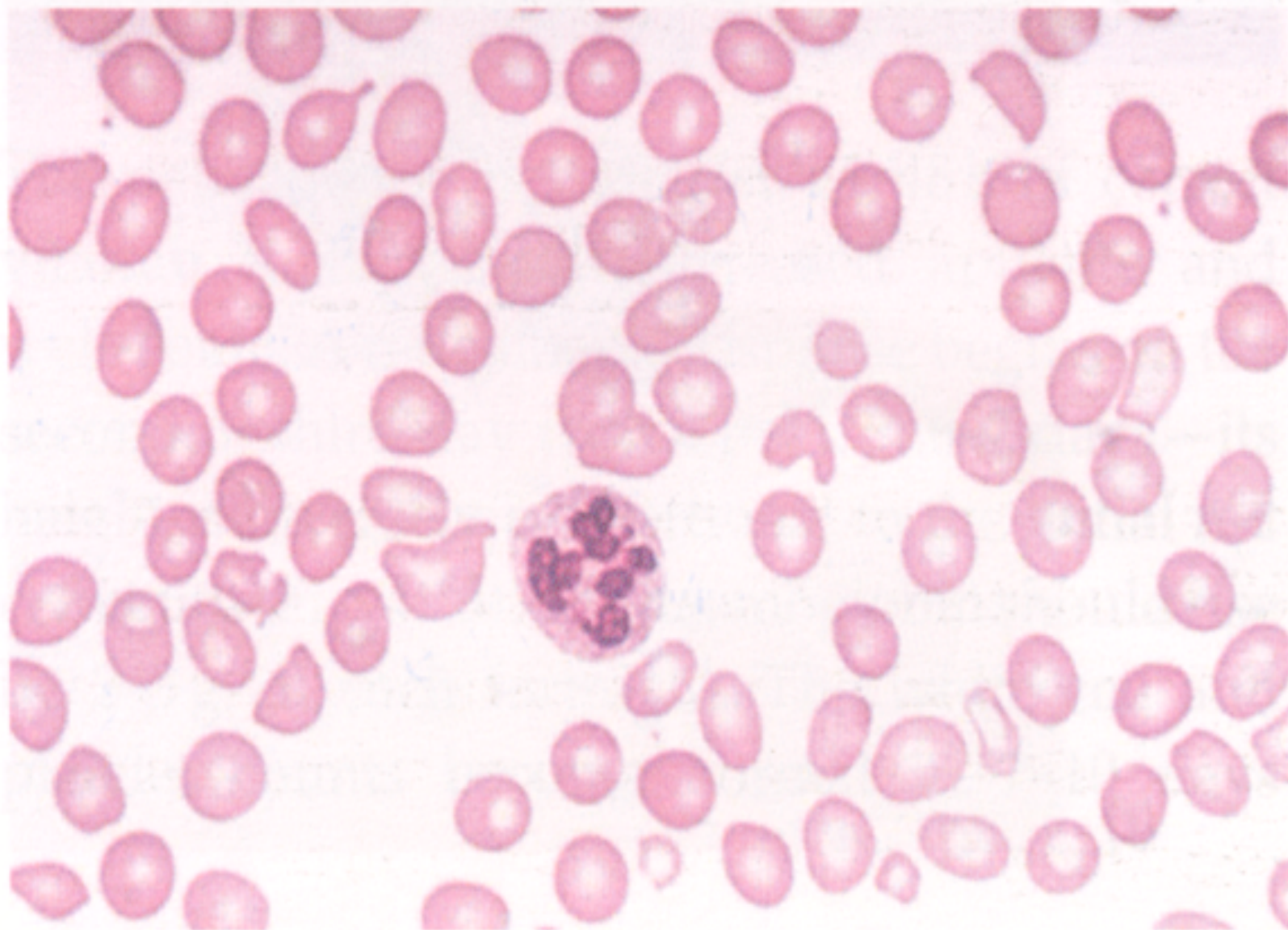


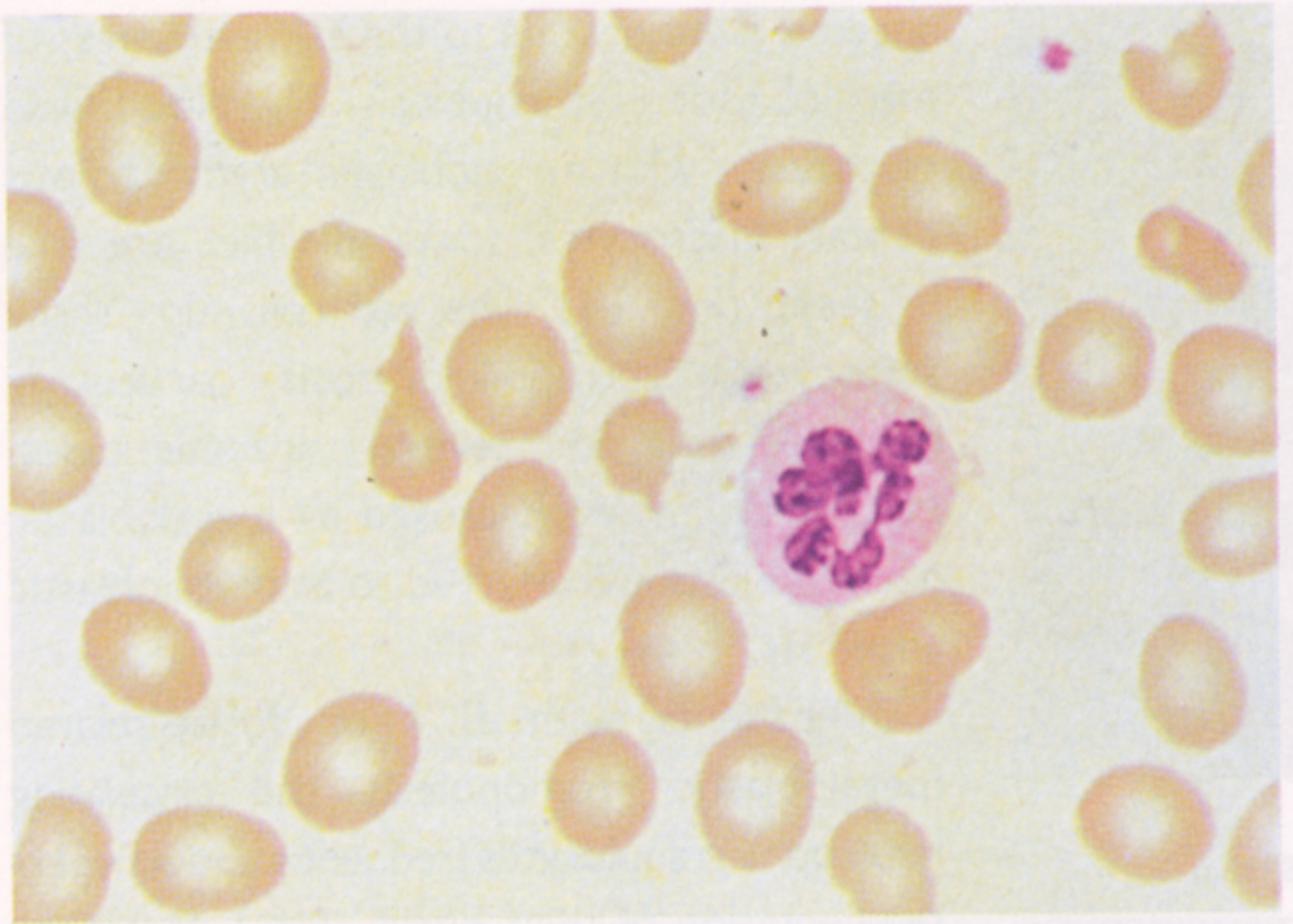
# Haematological findings in Megaloblastic Anaemia

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## 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**
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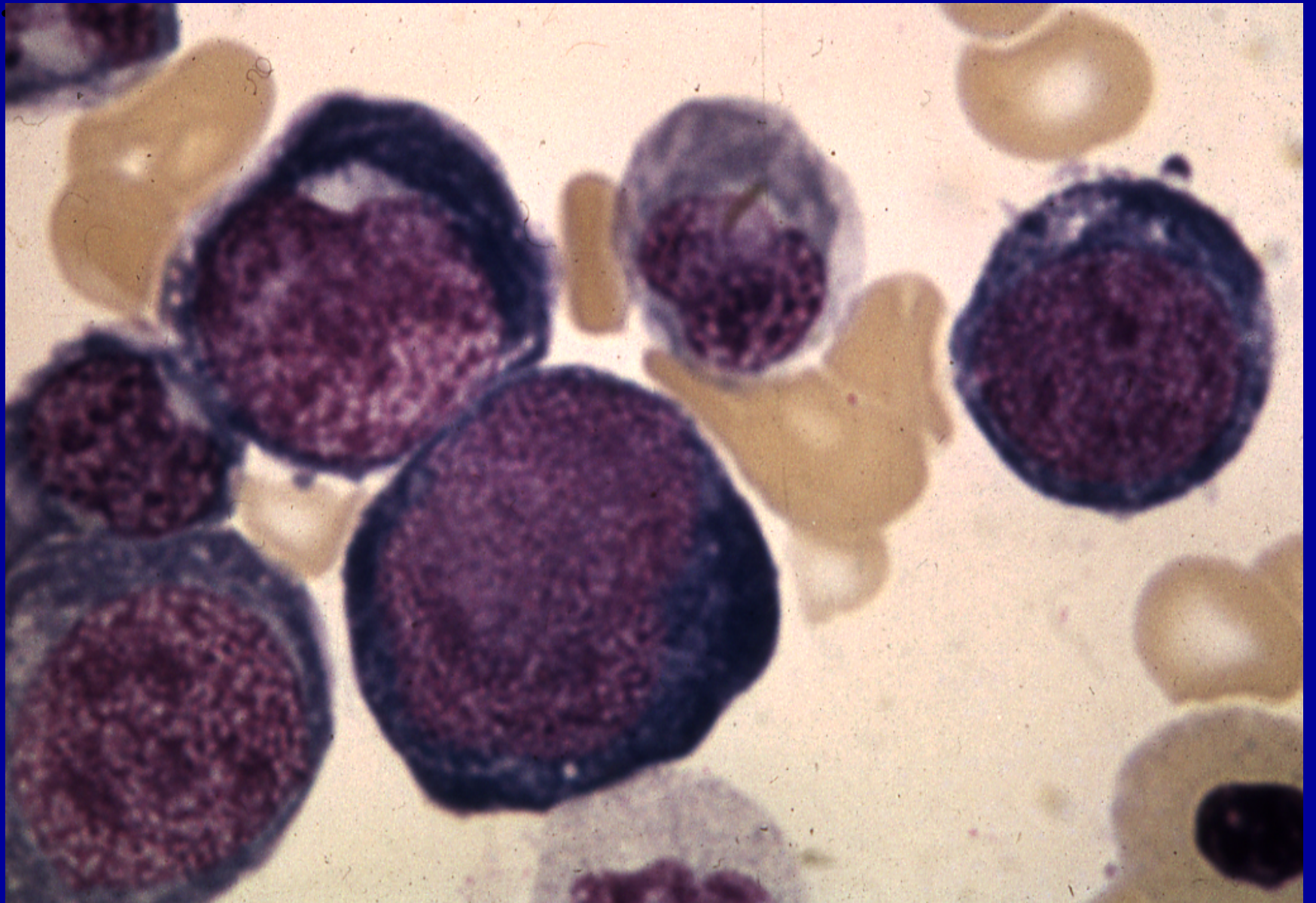


# Haematological Findings in Megaloblastic Anaemia

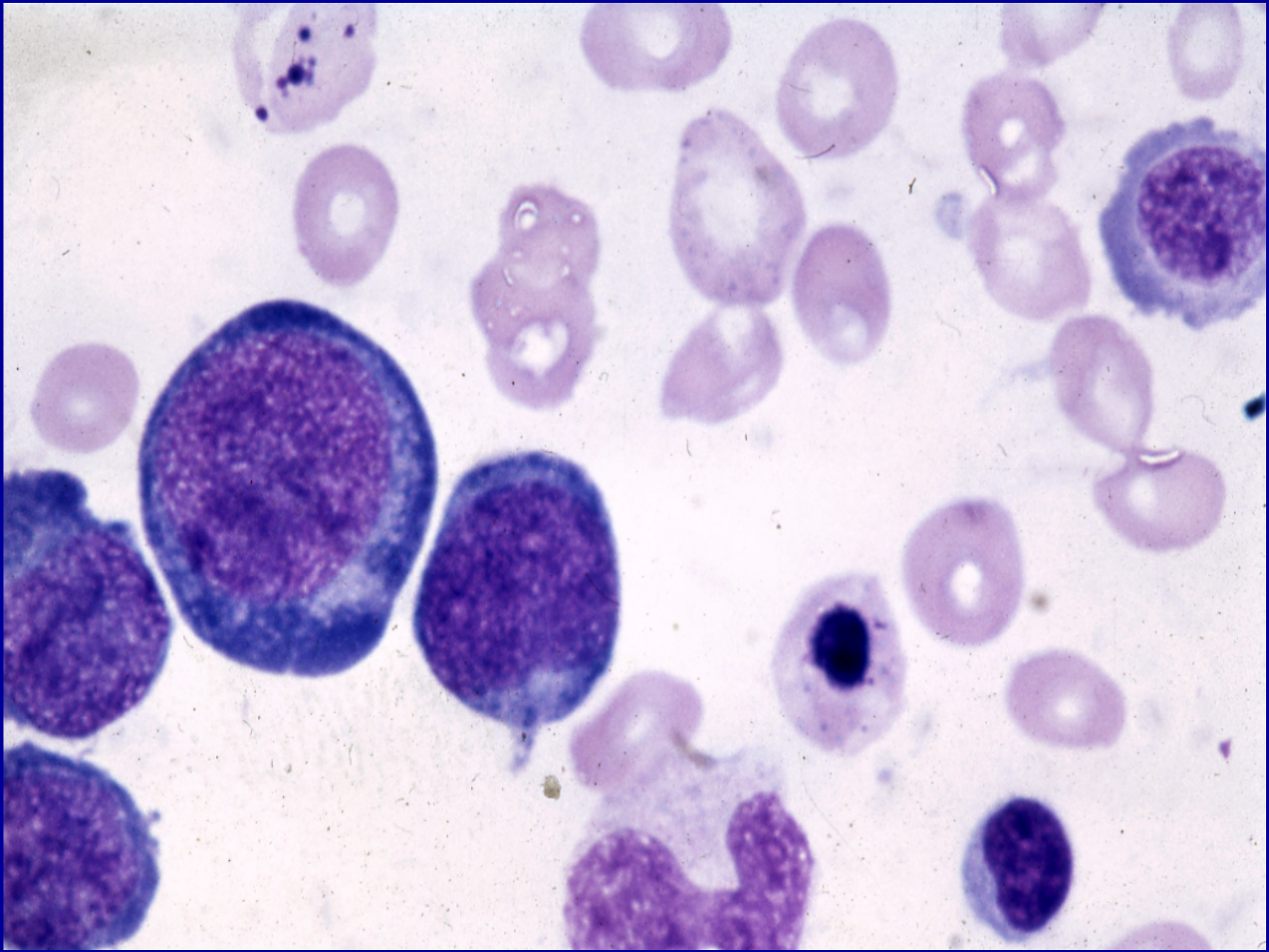
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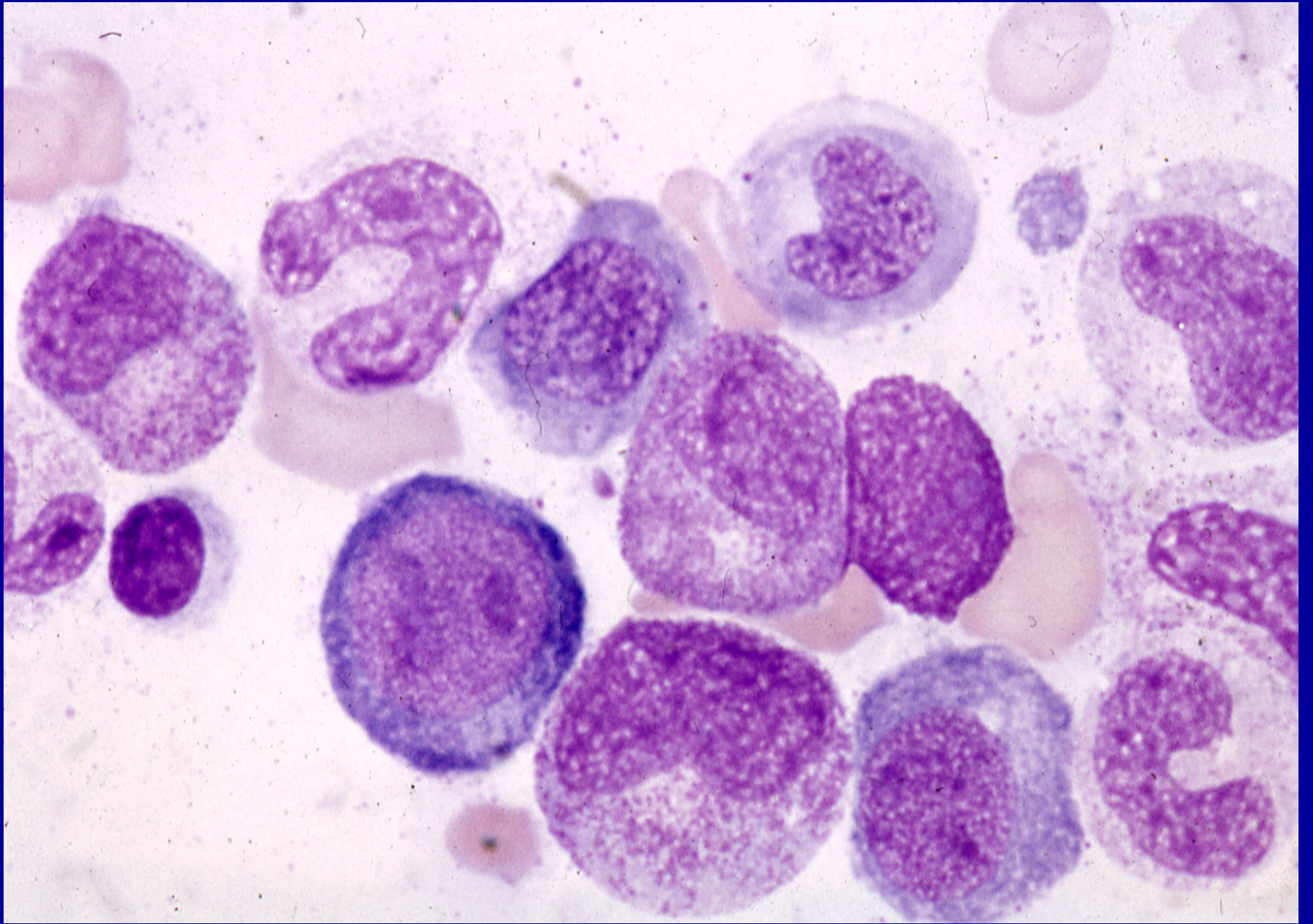
## Bone Marrow:

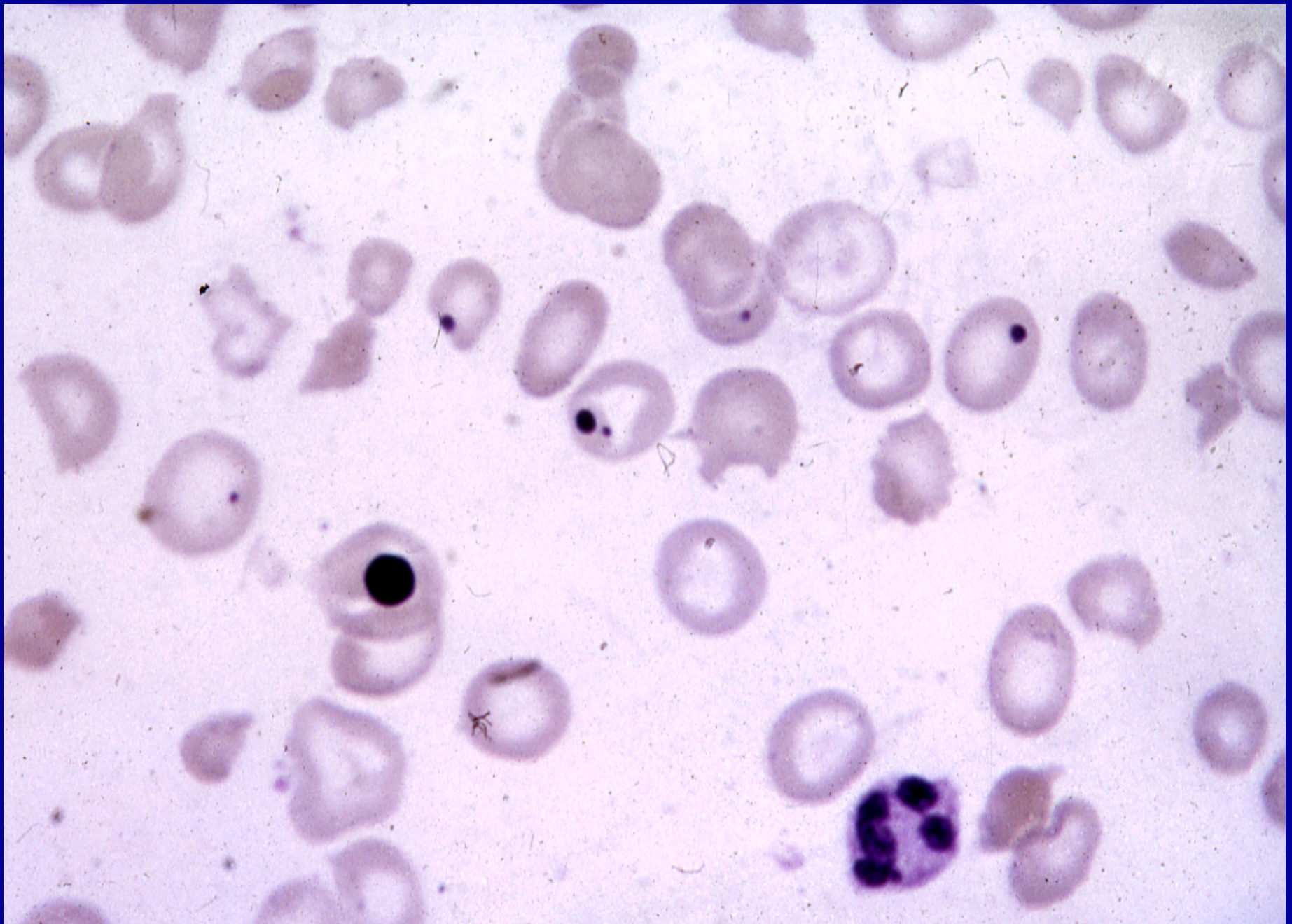
- ❖ **Hypercellular marrow with M:E ratio in normal or reduced.**
- ❖ **Accumulation of primitive cells due to selective death of more mature cells.**
- ❖ **Megaloblast (large erythroblast which has a nucleus of open, fine, lacy chromatin).**
- ❖ **Dissociation between the nuclear and cytoplasmic development 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

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

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

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A tropical beach scene at sunset. The sky is a mix of deep blue and purple, with a bright orange and yellow sun setting behind a line of clouds. The ocean is a deep blue, and the beach is a light tan color. Several palm trees are silhouetted against the sky. The text "Thank you !!!" is written in a yellow, cursive font at the bottom of the image.

*Thank you !!!*