جامعة الملك سعود
King Saud University
1957

Revised By
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Abdullah AlOmar

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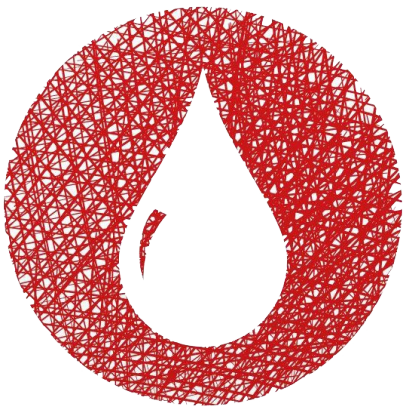

MEGALOBLASTIC

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

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Done by : Rinad Alghoraiby, Lamees Alsuwailem

Important
Notes
Doctor's slides

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 (1-2 %)	

- 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.

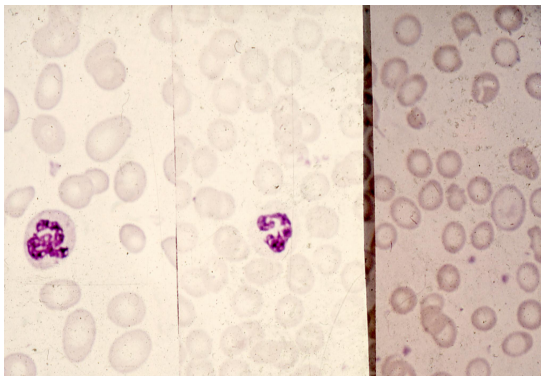
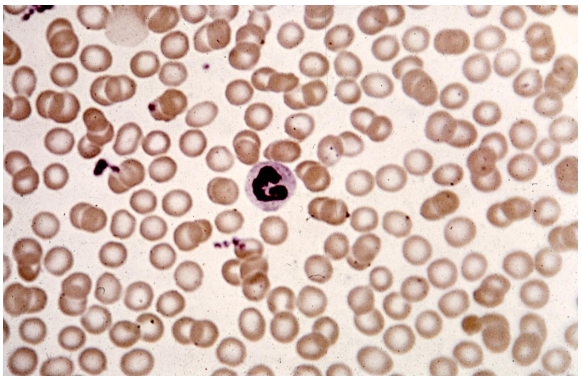
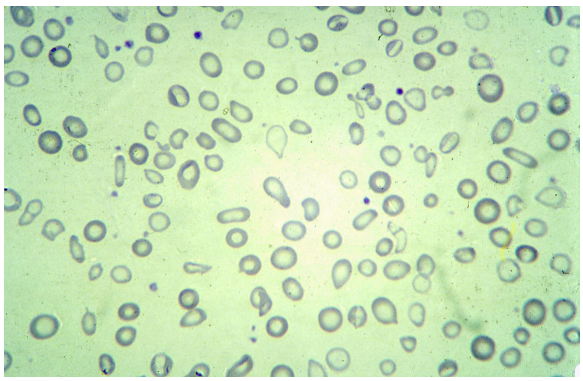
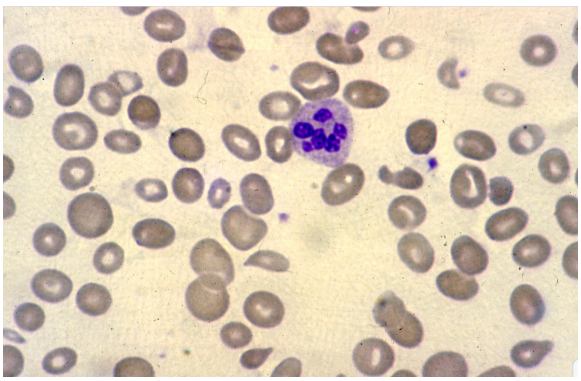
NORMAL WHITE CELLS (WBC) COUNT IN ADULTS

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 > 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	***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.

MACROCYTIC ANAEMIA

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 macroglobulinemia** - Leucoerythroblastic Anaemia - Myeloproliferative disease - Pregnancy* - Newborn* - Chronic respiratory failure
Hypersegmented Neutrophils	- Renal Failure - Congenital (familial) abnormality - Iron deficiency

*non hematological disorder.
**hematological disorder.

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

Macrocytosis with Normoblasts

Dr. said this list is enough

- Normal neonates and pregnancy (Physiological)
- Chronic alcoholism*
- Myelodysplastic syndromes* [1]
- 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 : Important!

Dr. said forget about this list and just remember the ones in red!

1. **Cobalamin (B_{12}) deficiency or abnormalities of cobalamin metabolism**
2. **Folate deficiency or abnormalities of folate metabolism**
3. Therapy with **antifolate drugs** (e.g. methotrexate)[2]
4. Independent of either cobalamin or folate deficiency and **refractory**[3] 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 anemia not due to cobalamin or folate deficiency or metabolic abnormality:
    a. Vitamin E deficiency
    b. Lesch-Nyhan Syndrome (?responds to adenine)

[1] bone marrow is not making enough blood cells].
[2] methotrexate is chemotherapy effect DNA synthesis.. and any drugs affect absorption of folate.
[3] they don't response to treatment.

Other causes of megaloblasts :

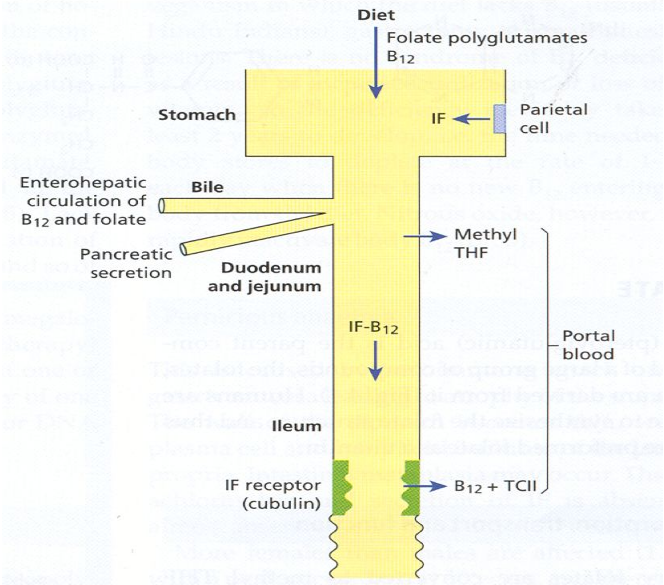
- Abnormalities of nucleic acid synthesis
- Drug therapy
  - Antipurines (mercaptopurine, azathioprine)
  - Antipyrimidines (fluorouracil, zydovudine (AZT))
  - Others (hydrozyurea)
- Orotic aciduria
- Uncertain aetiology
- Myelodysplastic syndromes, * erythroleukemia
- Some congenital dyserythropoietic anaemias

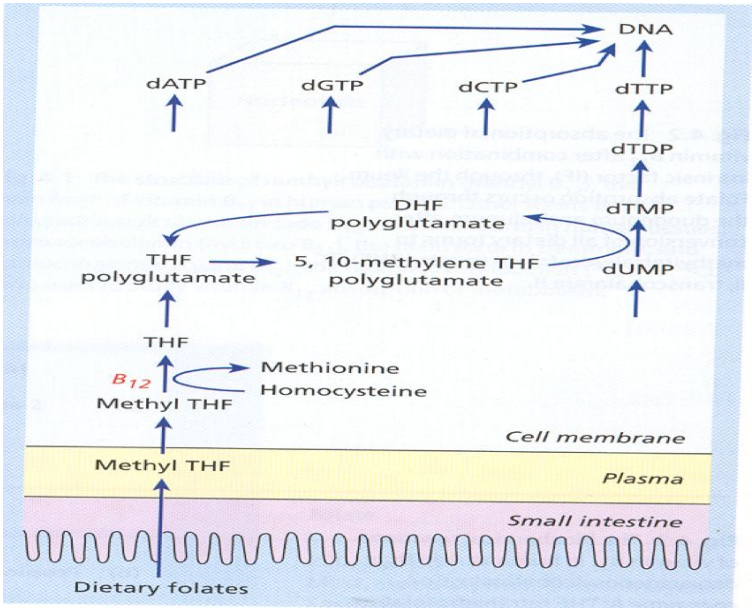
Vitamin B_{12} and folate nutrition and absorption Important!

	Vitamin B12	Folate
Diterary sourse	Only food of animal origin, especially liver Our bodies are not able to synthesize it Not destroyed by cooking	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† This value and the previous one are higher comparing to vitamin B12
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 Important!	Intrinsic factor (To modify the absorption) secreted by gastric parietal cells	Conversiion of polyglutamates to monoglutamates by intestinal folate conjugase
Site of absorption Important!	Terminal ileum	Duodenum and jejunum

* In adults.

† Higher during pregnancy and lactation.





Vit. B12 absorption:

1. Vit. B12 from the diet
2. Vit. B12 binds to Intrinsic Factor (secreted by parietal cells) in the stomach
3. IF-B12 complex pass to terminal ileum
4. Attach to receptor (cubulin)
   Important for acquiring vit.B12 to plasma
5. Bind to Transcobalamin II (TCII) which is a carrier in the plasma

Important!

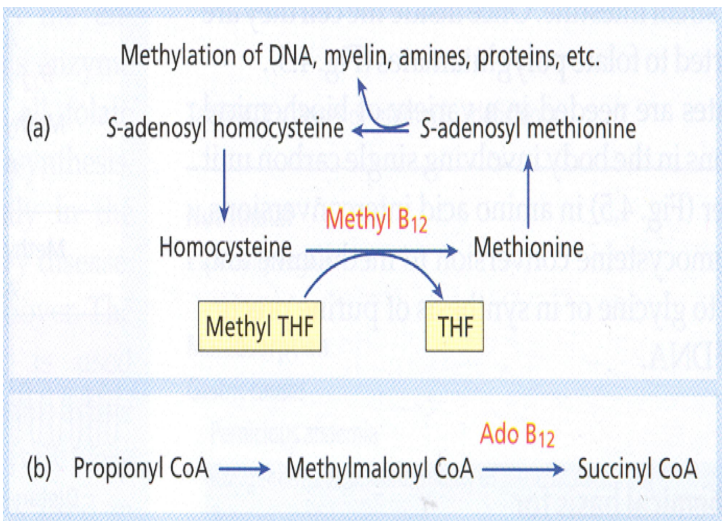
What is important for Vit. B12?

IF, Cubilin receptor, transporter

~ Anything that interferes with this conjugation can lead to Vit. B12 deficiency.

Folate:

- Folic acid in the gut and food is in the form of Methyl tetrahydrofolate
- Methyl THF is circulating in the small intestine and plasma
- Methyl THF converted to THF (require B12 as a cofactor)
- We get 5,10-methylene THF polyglutamate (most important form of folic acid) which is integrated in the DNA synthesis



Homocysteine
Methyltransferase
Methylcobalamin

Homocysteine → **Methionine**

5-Methylterahydrofolate **Tetrahydrofolate**

Causes of :

IF is usually released from parietal cells in the fundus and body of the stomach (NOT the antrum), so histological changes in the stomach won't involve the antrum!

Vitamin B_{12} Deficiency	- Inadequate intake - Veganism[1] - Inadequate secretion of intrinsic factor - Pernicious anaemia[2] - Total or partial gastrectomy - Congenital intrinsic factor deficiency (rare) - Partial gastrectomy, vagotomy, gastritis, acid-suppressing drugs, alcohol abuse - Abnormal intestinal bacterial flora[3] ,multiple jejunal diverticula, small intestinal strictures - Diphyllobothrium Latum - Malabsorption - Crohn's disease, ileal resection, chronic tropical sprue
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.

PERNICIOUS ANAEMIA

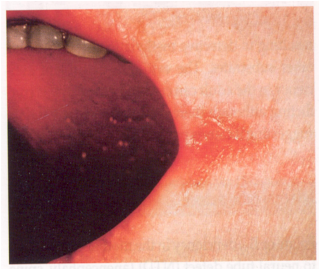
Important! [clinical features]

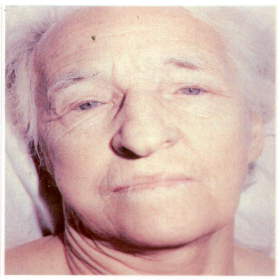
- Severe megaloblastic anaemia due to **autoimmune**[4] attack on the gastric mucosa leading to atrophy.
- More common in elderly female patients than males (1.6:1) at the age of 60 and above.
- More common in Northern European and tends to be in families.
- The **mucosa become thin with plasma cells and lymphoid infiltration of the lamina propria.**
- **Intestinal metaplasia** may occur.[5]
- It may be associated with autoimmune diseases including the autoimmune poly-endocrine syndrome.
- 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).

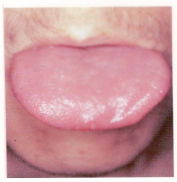
[1]shortest of sources of vit B12.
[2] absence of IF, or presence of any antibodies that can destroy it.
[3] interfere with absorption in ileum.
[4] antibodies against IF.
[5] Usually lead to stomach carcinoma.

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 [1]
- **Purpura, melanin pigmentation**
- Infections[2]







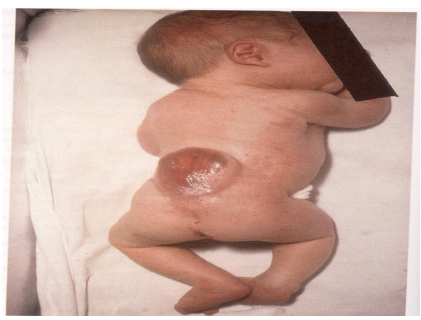
Neuropathy due to Vit B_{12} and folate deficiency

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-adenosylhomocysteine and reduced level of S-adenosylmethionine in nervous tissue resulting in defective methylation of myelin and other substrates.
- **Neuropathy is mostly due to vitamin B_{12}deficiency**[3]

Neural tube defect (NTD)

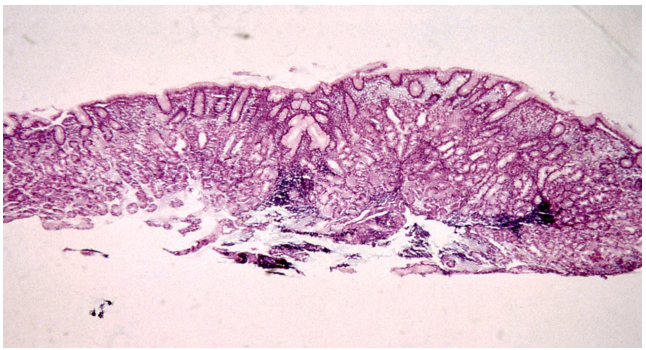
- (Anencephaly, spina bifida or encephalocoele) in the fetus due to folate or VitB_{12}deficiency in the mother. This result in build-up of homocysteine and S-adenosyl homocysteine in the fetus which impair methylation of various proteins and lipids.
- Polymorphism in the enzyme 5,10 methylene tetrahydrofolate 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.



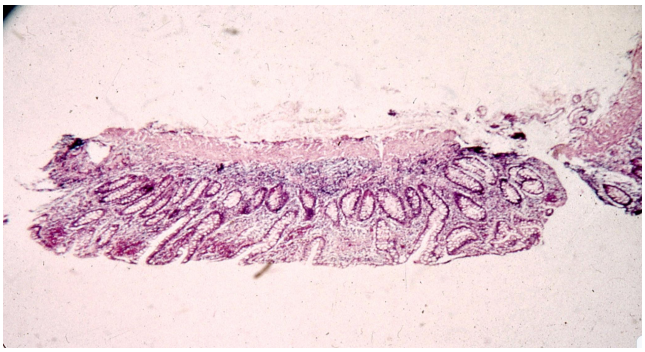
[1]Because the vit.B12 usually affects the epithelial tissues of the blood

[2] if they have thrombocytopenia or leukopenia .

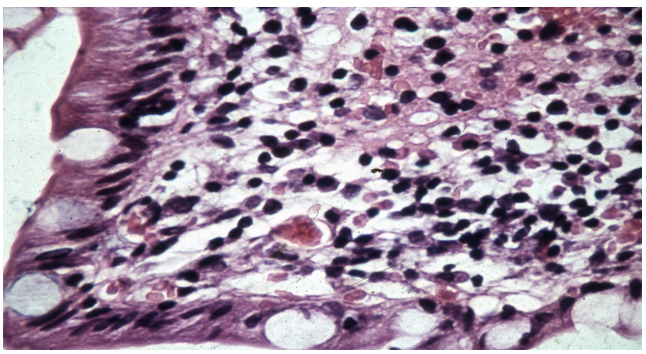
[3] rather than folic acid def.



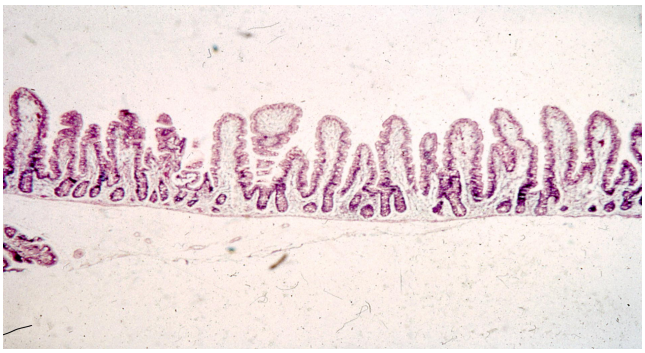
Normal gastric mucosa



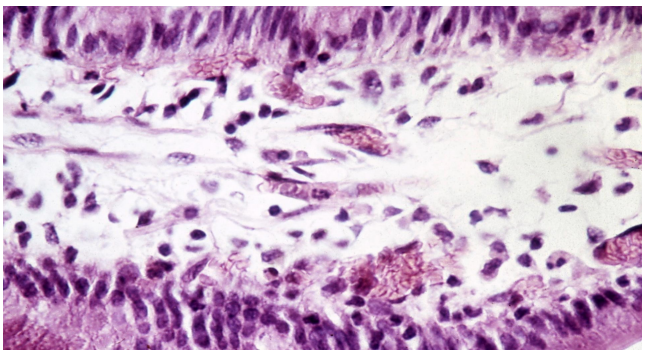
Gastric atrophy in patients with pernicious Anemia



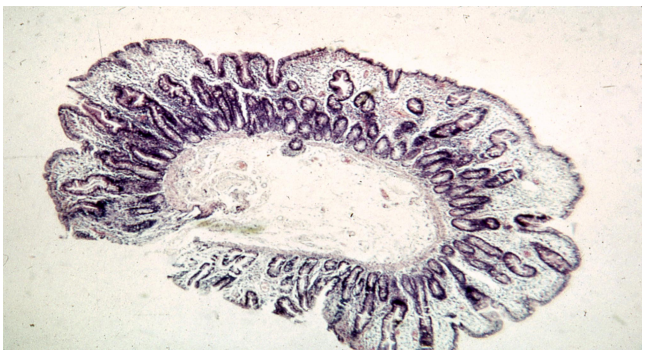
Heavy infiltration of lamina properia with plasma cells and lymphocytes in patients with pernicious Anemia



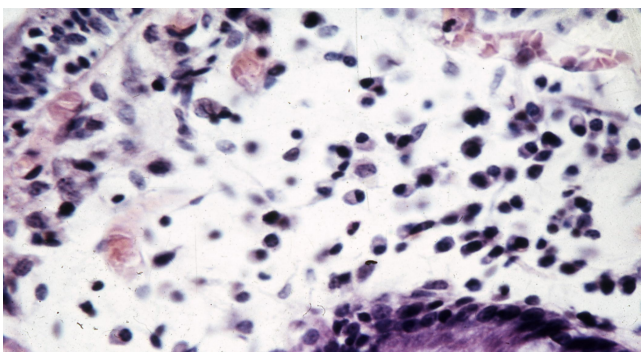
Normal small intestinal mucosa



Normal histology of small intestinal mucosa



Flattering of small intestinal mucosa in malabsorption syndrome

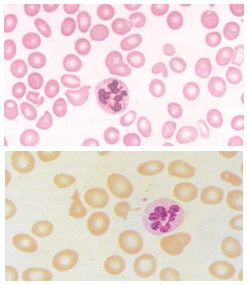


Histopathology of small intestinal mucosa in malabsorption syndrome

Haematological findings in Megaloblastic Anaemia

Peripheral Blood

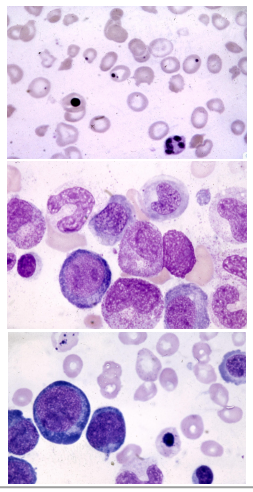
- Macrocytic anaemia, oval macrocytes, anisocytosis, poikilocytosis high MCV.
- Dimorphic anaemia when it is associated with iron deficiency or with thalassaemia trait.
- Hypersegmented Neutrophils. (seen in iron def. and megaloblastic anemia)
- Leukopenia and thrombocytopenia (in anything affecting the bone marrow)



Bone Marrow

Important!

- 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.

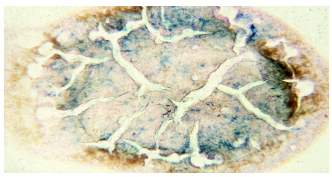


Other laboratory abnormalities

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

The ones in bold are seen in both hemolytic and Megaloblastic anemias

Dr. said forget about the last two



Treatment of megaloblastic 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 Important!	Total gastrectomy Ileal resection	Pregnancy, severe haemolytic anaemias, dialysis, prematurity

In hemolytic anemias they have combined problems, hemolysis and sometimes folic acid deficiency.

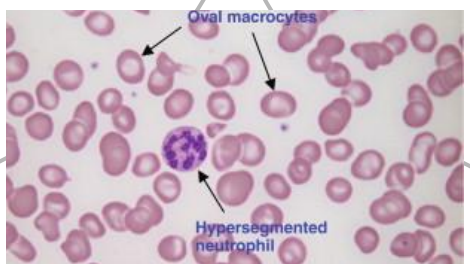
Summary

Megaloblastic Anemia

High MCV

Normal MCH

Due to **impaired DNA synthesis** which results in **enlarged RBCs**



Vitamin B12 Deficiency

Essential for: DNA synthesis, nuclear maturation, and healthy myelin production for neurons.

Found in: food of animal origin

Average daily intake: less than folate

Time to develop deficiency: years

Absorption: requires **IF**, Cubilin, TCII

Site of absorption: Terminal ileum

Causes of deficiency:

- Inadequate intake
- Vegans
- Malabsorption
- Gastrectomy
- Ileal resection
- Pernicious anemia

Manifest as:

- Neuropathy as a result of deranged myelin.
- Neural tube defect

Treatment:

- Hydroxocobalamin, IM

Folic Acid Deficiency

Found in: green vegetables, yeast

Average daily intake: more than B12

Time to develop deficiency: months

Absorption: 5,10-methylene THF polyglutamate (most important form of folic acid) which is integrated in the DNA synthesis

Site of absorption: duodenum and jejunum

Causes of deficiency:

- Inadequate intake
- Increased requirement (pregnancy)
- Malabsorption
- Increased loss
- Alcoholism

Manifest as:

- Neural tube defect

Treatment:

- Folic acid, oral

Pernicious anemia:

- Severe megaloblastic anaemia due to autoimmune attack on the gastric mucosa leading to atrophy
- Absent secretion of intrinsic factor (IF)

Quiz

Q1- Which one of the following is NOT a cause of megaloblastic anemia?
A. Hypothyroidism
B. Cobalamin deficiency
C. Therapy with methotrexate
D. Acute myeloid leukemia

Q2- Which one of the following, when deficient, takes a longer time for the patient to develop megaloblastic anemia?
A. Vitamin B12
B. Folate
C. Vitamin A
D. Vitamin C

Q3- Which of the following is the site of absorption for folate?
A. Duodenum
B. Jejunum
C. Terminal Ileum
D. A and B

Q4- Which one of the following is a laboratory finding of megaloblastic anemia?
A. Decreased LDH levels
B. Increased haptoglobins
C. Increased urobillinogen and stercoblilinogen
D. Decreased CO levels

Q5- Which one of the following should receive a prophylactic dose of vitamin B12?
A. Pregnant women
B. Patient on renal dialysis
C. Severe hemolytic anemia
D. Patient who had a total gastrectomy

1) A
2) A
3) D
4) C
5) D