**HAEMATOLOGY 6  
B12 and FOLATE DEFICIENCY**

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# Learning Objectives

# The student should be able to

1. Describe the role of vitamin B12 and folic acid in haemopoiesis, dietary sources and absorption of these vitamins, causes of deficiency, clinical and haematological features of vitamin B12 and folic acid deficiency and the diagnosis, further investigation and management of these deficiencies

**And to be able to explain that**

1. Synthesis of DNA requires both vitamin B12 and folate
2. Integrity of the nervous system requires vitamin B12
3. Deficiency of either causes anaemia, which is both **macrocytic** and **megaloblastic**

**MACROCYTIC ANAEMIA**

This is defined as anaemia with an increase in the mean cell volume (MCV) of the red cells, e.g. as measured by an automated full blood count machine. A raised MCV means that the red cells produced are larger than normal. Causes of a raised MCV are as follows:

1. Vitamin B12 or folate deficiency
2. Liver disease
3. Hypothyroidism
4. Excessive alcohol consumption
5. Drugs e.g. azathioprine, zidovudine
6. Haematological disorders

a) myelodysplasia

b) aplastic anaemia

c) reticulocytosis e.g. chronic haemolytic anaemias

**Megaloblastic anaemia**

This is defined by an abnormal but distinct morphological appearance of early and developing red cells. As the nucleus and cytoplasm of normal red cells mature, they demonstrate characteristic morphological features, readily discernible by light microscopy. The earliest recognisable erythroid cell in the bone marrow is the proerythroblast. This is a large cell with dark blue cytoplasm, reflecting the high RNA content. The nucleus contains only slightly condensed chromatin, which can have a lacy appearance. The proerythroblast gives rise to a series of progeny called erythroblasts, which contain progressively less RNA and more haemoglobin; late erythroblasts have pink rather than blue cytoplasm and are normally confined to the bone marrow. Meanwhile the nuclear chromatin becomes more condensed as cells mature from proerythroblasts to early, intermediate and late erythroblasts and the nucleus is extruded completely from the latter to form reticulocytes. These may be found in the peripheral blood and are the precursors of mature red blood cells. In megaloblastic anaemias, there is asynchrony between the maturation of the nucleus and cytoplasm and thus a nucleus with a unclumped chromatin and lacy appearance may be seen even in late (pink) erythroblasts. These abnormal cells are called megaloblasts, whereas erythroblasts showing normal maturation are called normoblasts. As a result of the delayed maturation of the nuclei, many red cells die in the bone marrow and the activity of red cell production increases to compensate. This is referred to as ineffective erythropoiesis.

White cells also show characteristic morphological abnormalities. In the bone marrow, myeloblasts successively give rise to promyelocytes, myelocytes, metamyelocytes and neutrophils. In megaloblastic anaemias the metamyelocytes may be 2-3 times the normal size (“giant metamyelocytes”) and neutrophils have hypersegmented nuclei.

**Peripheral blood**

Although the term megaloblastic refers to changes visible in the bone marrow, certain associated abnormalities are visible in the peripheral blood as follows:

a) Red blood cells often show variation in size (anisocytosis)

b) The mean cell volume (MCV) is high.

c) The haemoglobin concentration (Hb) may be low as a result of ineffective erythropoiesis.

d) Hypersegmented neutrophils can be seen.

e) The white count &/ or platelet count may also be low

**Causes of megaloblastic anaemia** include vitamin B12 and/or folate deficiency. Drugs which interfere with DNA synthesis directly, or with the metabolism of vitamin B12 or folate (e.g. methotrexate) will also cause a megaloblastic change.

**Causes of haematinic deficiencies**

Always consider the following:

1. inadequate intake
2. increased demand
3. inadequate absorption
4. excessive losses or utilization

**Vitamin B12**

1. Inadequate intake is rare

\* Vitamin B12 is found in animal products, so vegans are at risk

\* Abnormal bacterial flora in the small bowel (e.g. associated with stagnant loops) can consume vitamin B12

2. Increased demands are usually readily covered by the vitamin B12 stores, which are relatively large in relation to daily needs and usually sufficient to last for many years.

3. Absorption of B12 is complicated and failure of absorption is the commonest cause of B12deficiency

B12 is absorbed in the ***small bowel*** following combination with intrinsic factor.   
Intrinsic factor is made in the ***stomach***.

B12 absorption may be impaired in the following situations:

1. reduction in active intrinsic factor

post gastrectomy

autoimmune gastric atrophy **(“pernicious anaemia”)**

1. small bowel disease

surgical resection

Crohn’s disease

coeliac disease

4. Excessive losses: this is not a common cause of B12 deficiency

**Consequences of vitamin B12 deficiency**

* Megaloblastic anaemia
* Neurological problems:

a) peripheral neuropathy

b) subacute combined degeneration of the spinal cord

1. optic neuropathy
2. dementia

**Laboratory diagnosis of B12 deficiency**

* blood count and film
* serum B12 level

The **Schilling test** may be necessary to determine the CAUSE of the B12 deficiency.

Radiolabelled B12 is given orally and its excretion in the urine is measured, after first having saturated the serum B12-binding proteins by giving an intramuscular injection of non-radio-active B12. Clearly, any radiolabelled B12 detected in the urine must have been successfully absorbed in the small intestine. If the excretion is low then the test is repeated with the addition of intrinsic factor. If this restores the excretion of B12 to normal it is possible to conclude that the defect lies with a lack of intrinsic factor secretion. The detection of anti-parietal cell and anti-intrinsic factor antibodies in the blood, particularly the latter, would be additional evidence that a patient had pernicious anaemia.

**Folate**

1. Inadequate intake is common. Folate is found in animal and plant products but is readily destroyed by cooking, canning and processing. Poor nutrition, for example in the elderly, alcoholics or those living in poverty is a common cause of folate deficiency

2. Increased demand is also a common cause of deficiency

* Physiological: pregnancy, lactation, adolescence, premature babies
* Pathological: an excessive turnover of cells as may occur with haemolytic anaemias, malignancy, erythroderma.

3. Absorption of folate occurs in the duodenum and jejunum. This is rarely a cause of folate deficiency unless there is widespread disease of the small bowel such as coeliac disease.

4. Excessive losses: this is not a common cause of folate deficiency.

**Consequences of folate deficiency**

* megaloblastic anaemia
* neural tube defects in developing fetus
* ? increased risk of coronary artery disease if associated with variant enzymes in folate metabolic pathway.

**Laboratory diagnosis of folate deficiency**

* FBC and film
* Serum folate and red cell folate (red cell folate gives a better indication of body stores of folate whereas serum folate reflects recent intake)