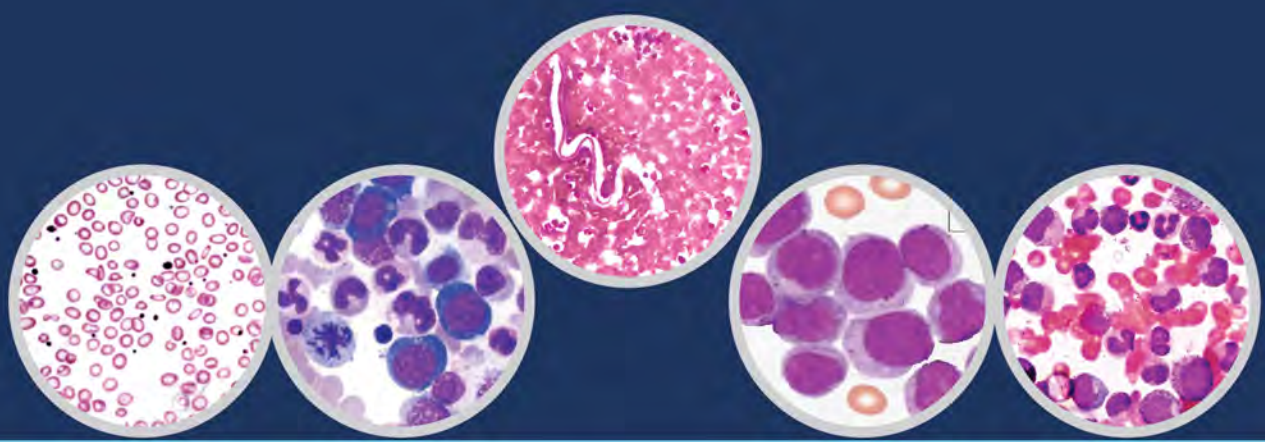
2nd Edition

Essentials in Hematology and Clinical Pathology



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Foreword
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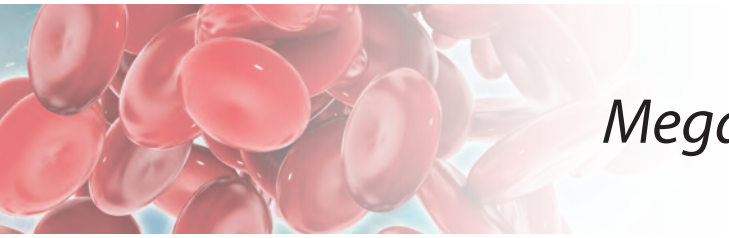


Megaloblastic Anemia

Chapter Outline

INTRODUCTION

Macrocytic anemias are characterized by large red cells with a diameter of more than 9 μ and mean corpuscular volume of more than 100 fl. The causes of macrocytic anemias may be broadly divided into megaloblastic and nonmegaloblastic depending on the appearance of developing red cell precursors in the bone marrow.

Megaloblastic anemias are characterized by the presence of abnormal red cell precursors in the bone marrow known as megaloblasts. The megaloblasts differ from their normal counterpart normoblasts in several aspects (Table 4.1).

Megaloblastic anemias are common among anemias due to impaired red cell production, being second in incidence to iron deficiency and anemia of chronic disorders.

Definition

Megaloblastic anemias are diverse group of anemias characterized by **impaired DNA synthesis** and **distinct morphologic changes** in hematopoietic cells, i.e. maturation of nucleus being delayed in relation to that of cytoplasm.

- As the name implies, there is anemia with distinct morphologically abnormally large erythroid precursors in the **bone marrow** known as **megaloblasts**.

Table 4.1: Features of megaloblast

Characteristics	*Megaloblast*
Cell size	Larger than normoblast
Nuclear chromatin	More open-sieve like (Fig. 4.7A)
Dissociation of cytoplasmic and nuclear maturation	Nuclear maturation lags behind cytoplasmic maturation
Maturation	Marrow shows increased proportion of more primitive erythroid cells

- Consequently, the red cells in the peripheral blood are also larger than normal and are termed as **macrocytes**.

Causes of Megaloblastic Change and Macrocytic Red Cells

- Megaloblastic anemia is commonly due to deficiency of **vitamin B_{12} (cyanocobalamin) or folic acid**, which are coenzymes required for the synthesis of one of the four nucleotide bases found in DNA namely thymidine.
- Deficiency of vitamin B_{12} or folic acid causes **defects in the DNA synthesis** and delayed/arrested nuclear maturation.
- Synthesis of RNA and protein is normal resulting in normal cytoplasmic maturation.
- Thus, the **nuclear maturation lags behind the cytoplasmic maturation** and hemoglobinization of cytoplasm continues for a long time. It results in abnormal cell proliferation of rapidly dividing cells in the bone marrow (erythroid, myeloid and megakaryocyte series). Impaired DNA synthesis causes delay in cell division, increased time between divisions, more cell growth and size of the cells. In erythroid series this nuclear to cytoplasmic asynchrony results in formation of large nucleated erythrocyte precursors named as **megaloblasts.** The megaloblasts have an open, stippled, lacy chromatin pattern. The megaloblastic changes are most prominent in the early nucleated red cell precursors.
- In the bone marrow, large number of megaloblastic precursors does not mature enough to be released into the blood, and are destroyed in the bone marrow **(ineffective erythropoiesis)**. There is also mild hemolysis of red cells in the peripheral blood. This releases large amounts **of lactate dehydrogenase (LDH)** resulting in **raised** levels in the blood.
- Erythroid precursor cells show reduced number of mitoses and synthesis of hemoglobin is unimpaired. The mature RBCs derived from these megaloblasts are large **(macrocytes)** and **oval** but well hemoglobinized.
- In the bone marrow, abnormal proliferation affects myeloid series producing giant metamyelocytes, and the megakaryocyte series results in dysplastic megakaryocytes.
- All rapidly dividing cells of the body (including skin, GI tract, bone marrow) exhibit megaloblastic changes and anemia is only a manifestation of a more generalized defect in DNA synthesis.

METABOLISM OF VITAMIN B_{12} AND FOLIC ACID

Metabolism of vitamin B_{12} and folic acid are closely related and both are essential for normal DNA synthesis and nuclear maturation. Other disorders may be associated with macrocytosis but megaloblastic hematopoiesis is most commonly due to deficiency of vitamin B_{12} or folic acid.

Vitamin B_{12} Metabolism

Human beings are totally dependent on animal products in the diet for vitamin B_{12} requirement. Vitamin B_{12} is not present in food from vegetable sources. Therefore, strict vegetarians do not get an adequate quantity of vitamin B_{12}. A balanced diet (not rigid vegetarian!) contains significantly large amounts of vitamin B_{12} which accumulates in the body (liver) and is enough for several years. Due to this adequate storage, if there is any dietary deficiency or

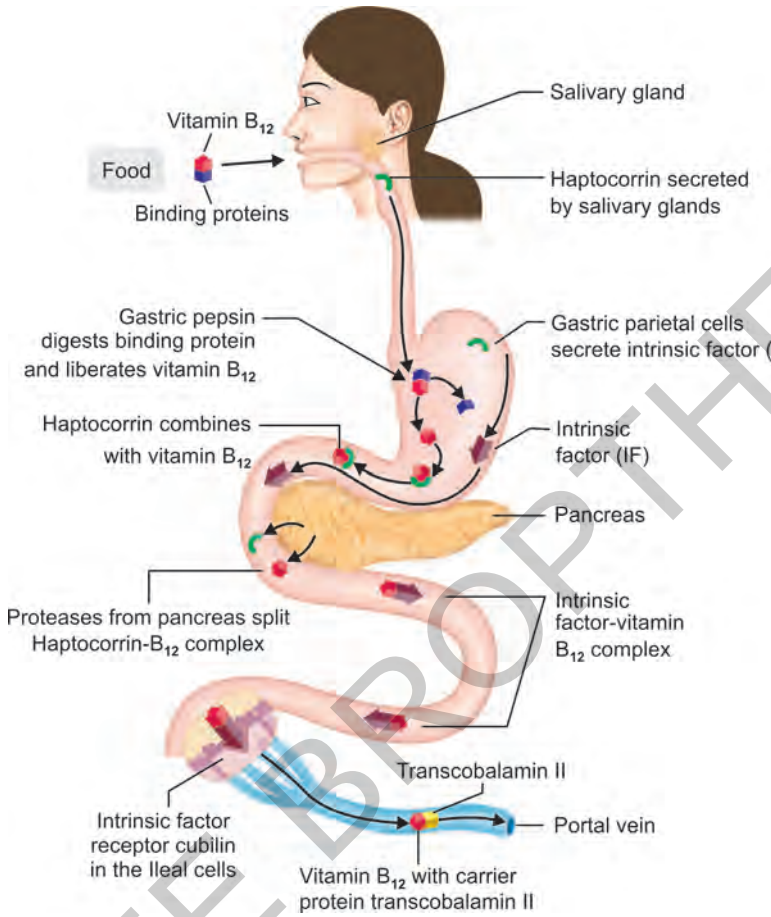


Fig. 4.1: Mechanism of vitamin B_{12} absorption

malabsorption of vitamin B_{12}, its clinical manifestations appear only after about 2 to 4 years. Vitamin B_{12} is a complex compound known as cobalamin and daily requirement is about 2 to 3 µg.

Absorption, Transport and Storage (Fig. 4.1)

- Vitamin B_{12} in food is usually in coenzyme form (as deoxyadenosylcobalamin and methylcobalamin) and bound to binding proteins in the diet.
- In the stomach, peptic digestion at low pH is required for release of vitamin B_{12} from binding protein in the food. The released vitamin B_{12} binds with salivary protein called **haptocorrin**, which is secreted in salivary juices.
- These haptocorrin-B_{12} complexes leave the stomach along with unbound special protein called **intrinsic factor** (IF), which is produced by gastric (fundus and cardia) parietal (oxyntic) cells (intrinsic factor is also called as Castle intrinsic factor).
- As the haptocorrin-B_{12} complexes pass into the second part of the duodenum, pancreatic proteases release vitamin B_{12} from haptocorrin. Vitamin B_{12} then associates with the intrinsic factor and forms IF-B_{12} complex.

- This stable IF-vitamin B_{12} complex is transported to the ileum, where it is endocytosed by ileal enterocytes. These ileal enterocytes express a receptor on their surfaces for the intrinsic factor. These receptors are called **cubilin**.
- In the ileal epithelium, vitamin B_{12} combines with a major carrier protein, transcobalamin II, and is actively transported into the mucosal cells and then into the blood.
- Transcobalamin II-vitamin B_{12} complex delivers vitamin B_{12} to the liver and other cells of the body, particularly rapidly proliferating cells in the bone marrow and mucosal lining of the gastrointestinal tract.

Role of Vitamin B_{12}

Vitamin B_{12} is indirectly required for DNA synthesis in various metabolic steps and its deficiency impairs DNA synthesis.

- Methylcobalamin is the main form of vitamin B_{12} in plasma, and is an essential coenzyme for conversion of homocysteine to methionine and formation of tetrahydrofolate (THF) from methyl THF (Fig. 4.2). During the former reaction, vitamin B_{12} loses its methyl group and this is replaced from methyl THF, the principal form of folic acid in plasma. Tetrahydrofolate is essential for the generation of a precursor of DNA known as deoxythymidine monophosphate (dTMP).

  In vitamin B_{12} deficiency, the main cause of impaired DNA synthesis is that methyl THF is not converted into THF. Methyl THF accumulates in the cell and is known as **methyl THF trap.**
- Vitamin B_{12} is also required for conversion of methylmalonyl CoA to succinyl malonyl CoA (Fig. 4.3). Deficiency of vitamin B_{12} causes increased levels of methylmalonic acid in plasma

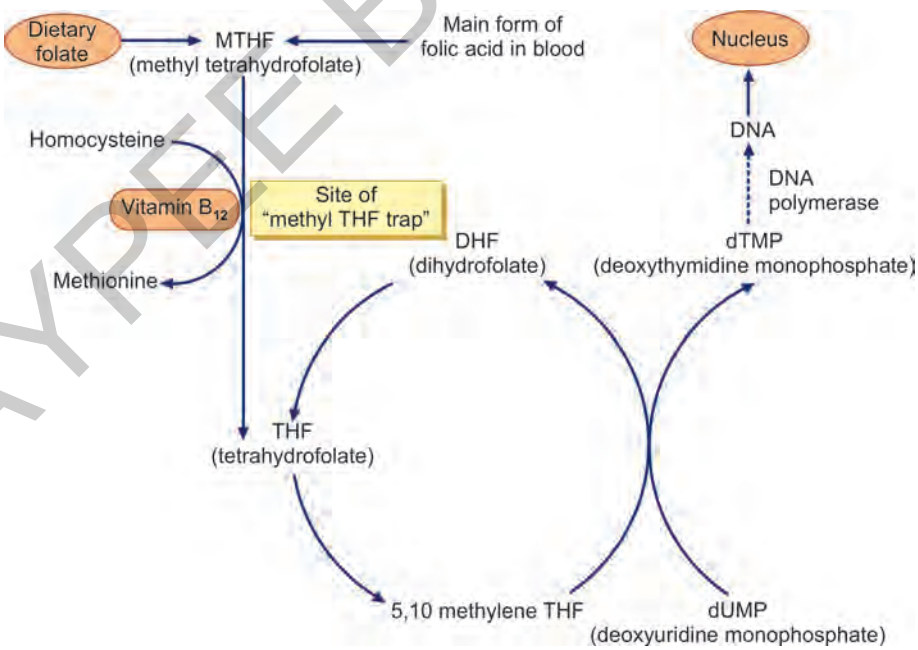


Fig. 4.2: Interrelation and role of vitamin B_{12} and folic acid in DNA synthesis

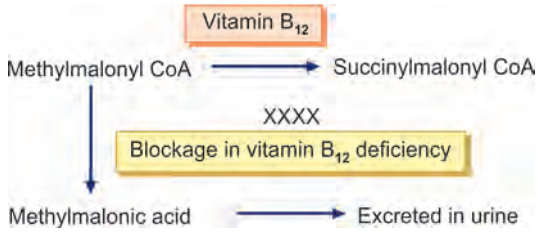


Fig. 4.3: Role of vitamin B_{12} in methylmalonyl CoA metabolism

and urine. This results in the formation of abnormal fatty acids which get incorporated into neuronal lipids. Consequently, this predisposes to myelin breakdown and is probably responsible for neurologic complications of vitamin B_{12} deficiency.

Folic Acid Metabolism

Humans are entirely dependent on dietary sources for their folic acid requirement. The daily requirement is 50–200 mg. Green vegetables, yeast, legumes, fruits and animal proteins are the richest sources and most normal diets contain sufficient amounts of folic acid. The folic acid in these foods is largely in the form of polyglutamates. Polyglutamates are sensitive to heat (thermolabile); boiling, steaming or frying and cooking destroys most of the folic acid. Intestinal conjugases split the polyglutamates into monoglutamates that are readily absorbed in the proximal jejunum. During intestinal absorption, they are modified to 5 methyltetrahydrofolate, the normal transport form of folic acid.

Role of Folic Acid (FA)

The active form of folic acid is tetrahydrofolate (THF) which is the biologic "middleman" involved in metabolic processes which synthesize DNA. The various reactions in which folic acid plays a main role are:

- Synthesis of purine (required for DNA and RNA).
- Conversion of homocysteine to methionine, a reaction also requiring vitamin B_{12}.
- Conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP): 5,10-methylene THF polyglutamate is required for conversion of dUMP to dTMP and DNA, a rate limiting step in pyrimidine synthesis (Fig. 4.2).
- Metabolism of histidine: Histidine is metabolized to formiminoglutamic acid (FIGLU) which combines with THF to form glutamic acid (Fig. 4.4). In FA deficiency, this reaction cannot take place and therefore FIGLU accumulates and is excreted as such in urine. This is used as a test to measure folic acid deficiency.

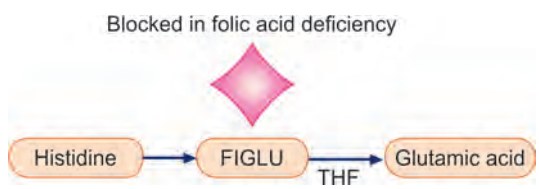


Fig. 4.4: Role of folic acid in metabolism of histidine

ETIOLOGY OF MEGALOBLASTIC ANEMIA

Megaloblastic anemia is commonly due to deficiency of **vitamin B_{12} (cobalamin) or folic acid** (Table 4.2).

Table 4.2: Causes of megaloblastic anemia

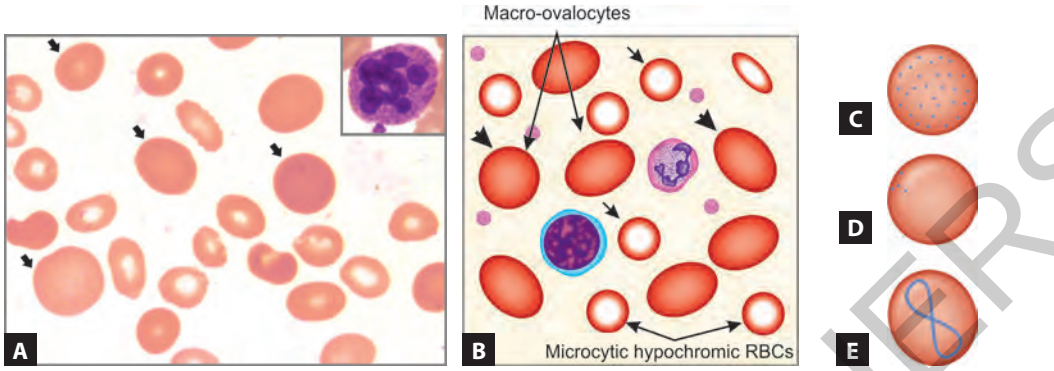
Vitamin B_{12} Deficiency
Decreased intake Inadequate diet, "pure vegetarians"
Impaired absorption Deficiency of gastric acid or pepsin or intrinsic factor • Pernicious anemia • Post-gastrectomy Intestinal • Loss of absorptive surface – Malabsorption syndromes – Diffuse intestinal disease (e.g. lymphoma, systemic sclerosis) – Ileal resection, Crohn disease • Bacterial or parasitic competition for vitamin B_{12} – Bacterial overgrowth in blind loops and diverticula of bowel – Fish tapeworm infestation (*Diphyllobothrium latum*) Damage to exocrine pancreas
Increased demand Pregnancy, hyperthyroidism, disseminated cancer
Folic Acid Deficiency
Decreased intake Inadequate diet: Alcoholism, malnutrition
Impaired absorption Malabsorption states: Nontropical and tropical sprue Diffuse infiltrative diseases of the small intestine (e.g. lymphoma) Drugs: Phenytoin and oral contraceptives
Increased loss Hemodialysis
Increased demand Pregnancy, infancy, disseminated cancer, markedly increased hematopoiesis
Impaired utilization Folic acid antagonists, such as methotrexate

LABORATORY FINDINGS OF MEGALOBLASTIC ANEMIA

Certain features common to all forms of megaloblastic anemia are discussed together. Blood findings in vitamin B_{12} and/or folic acid deficiency are similar and are characterized by macrocytosis of red cells and megaloblastosis of bone marrow.

Peripheral Blood

- **Hemoglobin:** Hemoglobin levels are decreased and usually in the range of 5 to 10 g/dL.
- **Hematocrit:** Decreased.
- **Red cell indices:**
  - **Mean cell (corpuscular) volume (MCV) above 100 fl (normal 82 to 98).**
  - Hemoglobin content in the red cell is proportionately increased and therefore **mean corpuscular hemoglobin concentration (MCHC)** remains **normal.**
  - **Mean cell (corpuscular) hemoglobin (MCH) is increased.**



Figs 4.5A to E: Peripheral blood smear showing macro-ovalocytes (short arrows) and hypersegmented neutrophil (inset in A and long arrow in B); (C) Basophilic stippling; (D) Howell-Jolly bodies; (E) Cabot ring

- **Peripheral smear:**
  - **RBCs: Red blood cells** are of variable size with majority being **macrocytic and oval (macro-ovalocytes)** and are diagnostic of megaloblastic anemia. Macrocytes are larger in diameter, thickness and volume. Because macrocytes are thicker and well-hemoglobinized, most **macrocytes lack the central pallor** of normal red cells and can even appear hyperchromic (Figs 4.5A to E). There is marked variation in the size and shape of red cells (anisopoikilocytosis).
  - Evidences of dyserythropoiesis (refer page 32) may be present like:
    - Basophilic stippling (Fig. 4.5C) appear as blue black cytoplasmic inclusions and represents precipitated ribosomal RNA.
    - Howell-Jolly bodies (Fig. 4.5D) are nuclear remnants and may be observed in few red cells.
    - Cabot ring (Fig. 4.5E) which stains pink blue and represents nuclear remnants.
  - **WBCs: White cells** are decreased (leukopenia) and show **hypersegmented neutrophils (with five to six or more nuclear lobes)**. Their presence is the **first** and specific morphologic **sign** of megaloblastic anemia. Such **neutrophils are** also **larger than normal (macropolymorphonuclear).**
  - **Platelets:** Decreased (thrombocytopenia) and the count varies.

  Dimorphic anemia: In cases of combined vitamin B_{12}/folic acid and iron deficiency, peripheral smear demonstrates dual population of macrocytes and hypochromic microcytes (Fig. 4.6). However, bone marrow shows normoblastic reaction with giant metamyelocytes and complete absence of marrow iron.

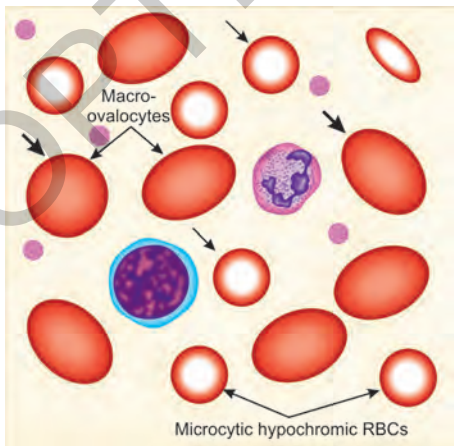


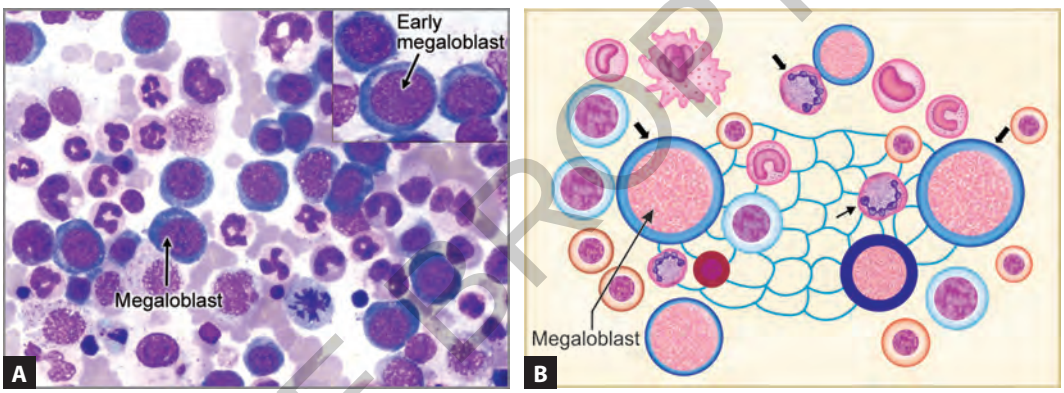
Fig. 4.6: Peripheral blood smear of dimorphic anemia showing both microcytic hypochromic cells (narrow arrow) and macrocytes (thick arrow)

- **Reticulocyte count:** It is normal or low. Reticulocytosis does not occur since it is a dyserythropoietic anemia. Nucleated red cells occasionally appear in the circulating blood with severe anemia.

Bone Marrow

Bone marrow shows characteristic morphological features.

- **Cellularity:** Marrow is moderately to **markedly hypercellular** mainly due to proliferating erythroid precursors which may completely replace the fatty marrow.
- **M : E (Myeloid : Erythroid) ratio:** Because of marked erythroid hyperplasia, M:E ratio is reversed ranging from 1:1 to 1:6 (normal 2:1 to 4:1).
- **Erythropoiesis:** It is of **megaloblastic type** (Figs 4.7A and B) in contrast to normal normoblastic type.
  - **Megaloblasts:** These are **large, abnormal counterparts of normal normoblasts** and their characteristic features are presented in Table 4.1 and Figure 4.8. Megaloblastic change is detected in all stages of red cell development. They demonstrate **asynchrony of nuclear and cytoplasmic maturation**, nuclear chromatin failing to mature because of impaired DNA synthesis while cytoplasm gets normal hemoglobinization.



Figs 4.7A and B: Bone marrow aspirate showing megaloblastic precursors (short arrows) in varying stages of maturation (inset in A shows early megaloblasts). B also shows hypersegmented neutrophils (long arrows)

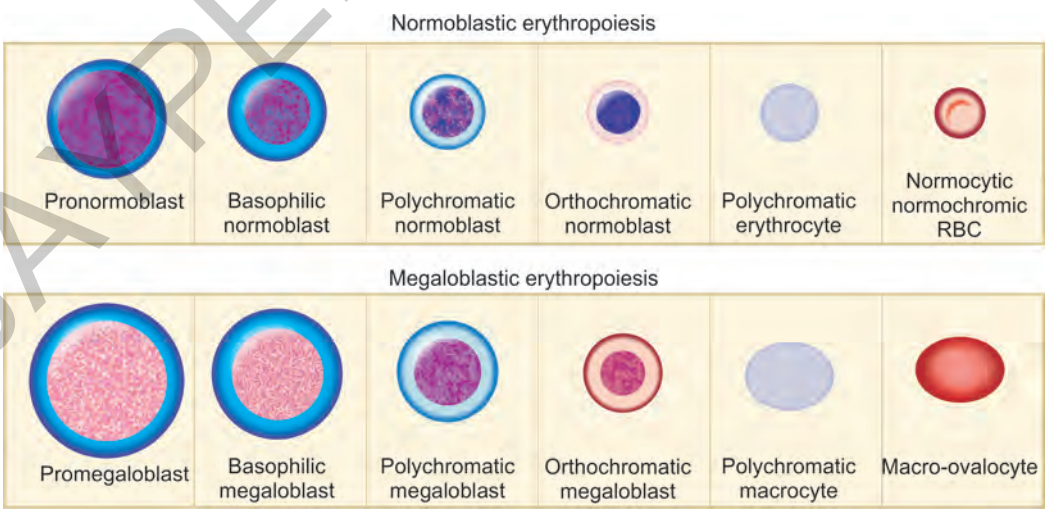


Fig. 4.8: Comparison of normoblastic erythropoiesis with megaloblastic type

Table 4.3: Differences between normoblast and megaloblast

Characteristics	*Normoblast*	*Megaloblast*
Cell size	Normal	Larger than corresponding normoblast
Nuclear chromatin	Normal	Open sieve-like
Nuclear maturation	Normal	Lags behind cytoplasmic maturation
Mitosis	Normal	Increased and abnormal
Maturation in bone marrow	Normal (Late > intermediate > early normoblast)	Increased proportion of more primitive erythroid cells (Late < intermediate < early normoblast)
Evidence of dyserythropoiesis	Absent	Present (irregular nuclei, Howell-Jolly bodies)
Myelopoiesis	Normal	Show giant metamyelocytes
Found in	Normal bone marrow	Bone marrow of megaloblastic anemia

  - **Ineffective erythropoiesis:** In an untreated megaloblastic anemia there is preponderance of more primitive cells. The late stage megaloblast forms die in marrow and are known as ineffective erythropoiesis (or intramedullary hemolysis).
    Note: Ineffective erythropoiesis is the term used for erythropoiesis in which there is death of developing erythroid cells at the site of production and/or production of non-viable red cells.
  - **Dyserythropoiesis:** Dyserythropoiesis means abnormal erythropoiesis with bizarre bone marrow morphology and ineffective erythropoiesis. The dyserythropoiesis in the megaloblasts demonstrates the following morphological features:
    - In the cytoplasm: Howell-Jolly bodies (see Fig. 4.5D) and abnormal hemoglobinization
    - In the nucleus: Irregular nuclear borders due to nuclear budding, nuclei joined by bridge (internuclear bridging) and abnormal mitosis.
- **Myelopoiesis:**
  - Myeloid cells appear adequate in number, but patients with severe anemia show neutropenia.
  - Because DNA synthesis is impaired in all proliferating cells, granulocytic precursors also display nuclear-cytoplasmic asynchrony in the form of **giant metamyelocytes and band forms**.
- **Megakaryopoiesis:** Megakaryocytes are normal or increased in number. They may be abnormally large and may have bizarre, multilobate nuclei with open nuclear chromatin.
- **Bone marrow iron:** In pure vitamin B_{12}/folic acid deficiency bone marrow iron is moderately increased.

Differences between normoblast and megaloblast are presented in Table 4.3.

Common Biochemical Tests for Vitamin B_{12} and Folic Acid Deficiency

- The deoxyuridine suppression test: Deoxyuridine suppression test is a sensitive measure of deficiency of 5,10-methylene THF, which occurs in both folic acid and vitamin B_{12} deficiency.
- Serum homocysteine: Their levels are increased.

- Serum bilirubin: Mild increase of serum bilirubin causes mild jaundice. This is due to hemolysis resulting from intramedullary death of megaloblasts in the bone marrow.
- Serum iron and ferritin: Both are increased in pure vitamin B_{12}/folic acid deficiency anemias.
- Plasma lactate dehydrogenase (LDH): Elevated often markedly.

ANEMIAS OF VITAMIN B_{12} DEFICIENCY

Causes of Vitamin B_{12} Deficiency (Table 4.2)

Decreased Intake

Vitamin B_{12} (cyanocobalamin) is mainly present in animal products. Vegetables and fruits contain very little vitamin B_{12}. Strict vegetarians who do not consume meat, eggs and milk are likely to develop vitamin B_{12} deficiency. Inadequate diet presents features of anemia after many years because of adequate vitamin reserves. Milk is a good source of vitamin B_{12}.

Impaired Absorption

The absorption of vitamin B_{12} can be impaired by disruption of any one of the steps involved in its absorption.

Gastric factors

- **Achlorhydria (deficiency of gastric acid) and loss of pepsin secretion:** The release of vitamin B_{12} from bound proteins in food requires pepsin and low pH produced by HCl.
- **Deficient IF:** Gastrectomy and in **pernicious anemia**, the intrinsic factor is inadequate or absent.

Intestinal factors

- **Loss of absorptive surface:** Conditions like ileal resection, diffuse ileal disease or Crohn disease result in defective absorption of intrinsic factor-vitamin B_{12} complex. Tropical sprue and nontropical sprue may lead to malabsorption of vitamins.
- **Bacterial or parasitic competition for vitamin B_{12}.**
  - **Bacterial competition:** Abnormal intestinal anatomy or surgical sequelae that results in stenotic areas known as blind loop or diverticulosis. In these conditions stasis of intestinal contents results in bacterial overgrowth which compete for vitamin B_{12}.
  - **Parasitic competition:** Fish tapeworm *Diphyllobothrium latum* infestation can induce a deficiency state by competing for vitamin B_{12}.

Damage to the exocrine pancreas

With loss of exocrine pancreatic function, vitamin B_{12} cannot be released from R-binder-vitamin B_{12} complexes.

Increased Demand

The requirement for vitamin B_{12} can be so great as to produce a relative deficiency even with normal absorption. This may be observed in circumstances like pregnancy, hyperthyroidism, disseminated cancer and chronic infections.

Pernicious Anemia

Definition

Pernicious anemia (PA) is a chronic disease resulting from deficiency of intrinsic factor causing impaired absorption of vitamin B_{12} and eventually megaloblastic anemia.

Incidence

Pernicious anemia may occur in all racial groups but is very rare in India. A genetic predisposition is suspected, because of tendency to form antibodies against multiple self-antigens.

Age

Pernicious anemia is a disease of older age and generally presents in the fifth to eighth decades of life.

Sex

Females are more involved than males (F:M is 1.5: 1).

Etiopathogenesis

Pernicious anemia is an **autoimmune** disease which develops due to destruction of gastric mucosa. The evidences for autoimmune etiology are:

- Its association with other autoimmune diseases like Graves disease, Hashimoto thyroiditis and adrenalitis is well established.
- Microscopic examination of stomach shows damage to gastric parietal cells accompanied by dense infiltration by lymphocytes and plasma cells. These changes are mediated both by cellular and humoral immune reactions and cause ***chronic atrophic gastritis***.
- Response to steroids.
- Presence of autoantibodies in most of the patients. **Two major types** of autoantibodies are found.
  - **Anti-intrinsic factor (IF) antibody**
    - Type I (blocking) antibody: This antibody blocks the binding of vitamin B_{12} to IF and are present in 50–75% of the cases. It can be detected in both plasma and gastric juice.
    - Type II (binding) antibody: It attaches to the IF–vitamin B_{12} complex and prevent its binding to receptors in the ileum. It is present in about 40% of patients.
  - **Parietal cell (Type III) antibody:** It is directed against ATPase pump in parietal cells but is neither specific for PA nor other autoimmune disorders. It is found in 90% of patients with PA as well as in older patients with chronic nonspecific gastritis.

The autoimmune process (Fig. 4.9) starts with activation of CD4+ T cells which initiates injury to the gastric mucosa. The mucosal damage secondarily triggers the formation of autoantibodies, which exacerbate the damage to IF secreting parietal cells. Anemia develops when sufficient number of parietal cells are damaged (resulting in IF deficiency) along with depletion of vitamin B_{12} reserves. Thus, autoantibodies are not the primary cause of chronic atrophic gastritis but they are of diagnostic value.

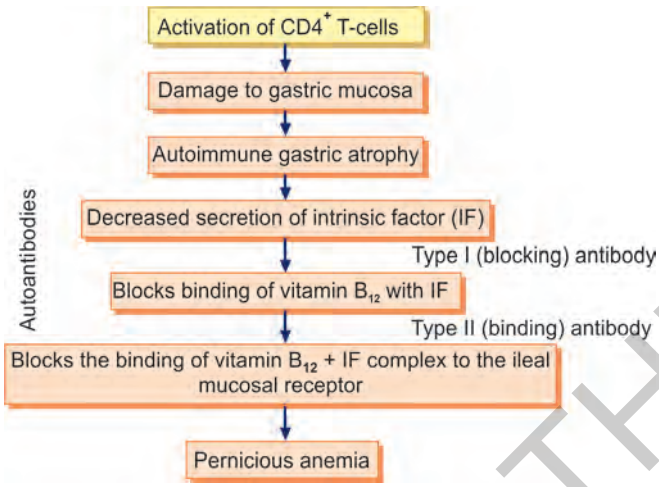


Fig. 4.9: Pathogenesis of pernicious anemia

Clinical Features *(Fig. 4.10)*

- **Onset:** Insidious and progresses slowly unless halted by therapy.
- **Classic triad of presentation:** Weakness, sore throat and paraesthesias.
- **Tongue:** Painful red **"beefy"** tongue due to glossitis and atrophy of papillae. The patient complains of loss of taste and appetite.
- **Peripheral neuropathy:** Glove and sock distribution of numbness or paresthesia. This tingling begins in tips of toes and progresses proximally and is bilateral and symmetric.
- **Ataxia:** Lack of voluntary coordination of muscle movement, uncoordinated gait, impairment of vibration and position sense.
- **Atherosclerosis:** Serum homocysteine level is raised and is a risk factor for atherosclerosis and thrombosis.

Laboratory Findings *(Fig. 4.10)*

Blood and bone marrow

The changes in the bone marrow and blood are similar to those described earlier for all megaloblastic anemias (Refer page 31–32).

Morphology

Alimentary system: Abnormalities are regularly found in the tongue and stomach.

- **Atrophic glossitis:** The tongue appears shiny, glazed and beefy.
- **Stomach:**
  - **Diffuse chronic atrophic gastritis** is associated with impaired secretion of hydrochloric acid, pepsin and intrinsic factor.

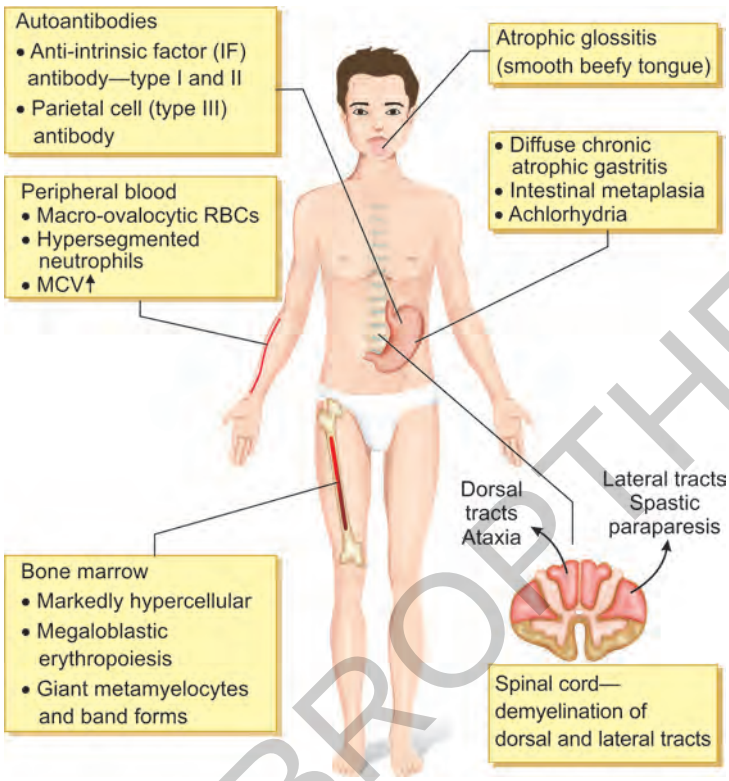


Fig. 4.10: Clinical features and laboratory findings in pernicious anemia

- The characteristic histological feature is the atrophy of the glands (mainly in the fundus), with loss of both chief cells and parietal cells. As the disease advances parietal cells disappear completely.
- The nuclei of mucosal cells are increased in size and look similar to that of megaloblasts.
- There is dense infiltration by lymphocytes and plasma cells. Severity of gastritis worsens with advancing age.

– **Intestinal metaplasia**: The epithelium lining the glands is replaced by mucus-secreting goblet cells which resemble those lining the large intestine. This is a type of metaplasia and is known as intestinal metaplasia/**intestinalization**.

The gastric atrophy and metaplastic changes are due to autoimmune reaction and not due to deficiency of vitamin B_{12}. So, parenteral administration of vitamin B_{12} corrects the changes in the bone marrow, but not the gastric changes. Incidence of gastric cancer is higher in patients with pernicious anemia.

Central nervous system: The lesions are found in 75% of all cases of severe pernicious anemia.

- **Demyelination in the dorsal and lateral tracts:** The spinal cord shows demyelination in the dorsal and lateral tracts. Demyelination of **dorsal** tracts causes sensory ataxia and that of **lateral** tracts gives rise to spastic paraparesis, and severe paresthesia in the lower limbs.
- Because both sensory and motor pathways are involved, the term "subacute combined degeneration" is used to describe these neurologic changes found in vitamin B_{12} deficiency.

Biochemical Parameters

- **Diagnostic tests for vitamin B_{12} deficiency:**
  - Serum vitamin B_{12} levels: **Decreased**
  - Serum methylmalonic acid: Increased
  - Urinary excretion of methylmalonic acid: Increased.
- **Schilling test for vitamin B_{12} absorption** (Fig. 4.11)

  Use: Schilling test helps in distinguishing megaloblastic anemia due to intrinsic factor (IF) deficiency (Pernicious anemia) from other causes of vitamin B_{12} deficiency. It is diagnostic of PA but now very infrequently performed.

  Method and interpretation: Radioactive vitamin B_{12} (1 µg) is given orally to a fasting patient. This is followed by non-radioactive 1,000 µg of vitamin B_{12} intramuscularly. The injected vitamin B_{12} saturates vitamin B_{12} binding proteins and flush out the ingested radioactive vitamin B_{12} which will be excreted in urine. The urine is collected for 24 hours.
  - **Stage 1:** Normal persons excrete more than 10% of oral radioactive dose in 24 hour urine. Patients with pernicious anemia excrete less than 5% of the oral dose.

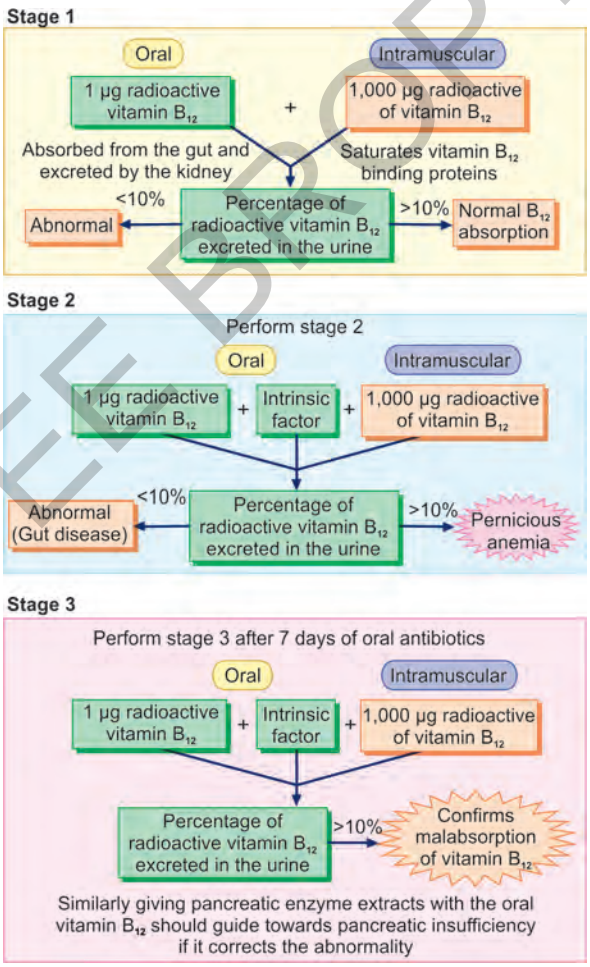


Fig. 4.11: Stages of Schilling test

Essentials in
Hematology and Clinical Pathology

Salient Features

- Provides knowledge of hematology and clinical pathology in a simple, lucid and easily understandable and reproducible format
- Highly illustrated in multicolor, easy-to-understand, and student-friendly book
- Contemporary concepts on hematological diseases
- Concise text in bullet form for easy review and recollection
- Key points are provided in bold words so that it will help the student to just brush through the entire book within a few hours before the examination or viva voce
- Molecular basis of common hematological disorders
- Laboratory findings of hematological diseases presented in simplified manner
- A summary of important points at the end of each chapter
- Provided with essay questions, short answer questions and 351 MCQs to encourage self-assessment
- Text enhanced by 135 illustrations, 27 photomicrographs, 18 photographs, radiographs, and more than 146 tables and text boxes
- Facilitates learning and preparing for practical examination in pathology
- Book is divided into four sections—Section 1: Disorders of Red Cells (Chapters 1 to 17), Section 2: Disorders of White Cells (Chapters 18 to 32), Section 3: Disorders of Hemostasis (Chapters 33 to 37), and Section 4: Clinical Pathology (Chapters 38 to 67) deals with laboratory investigations done in routine practice
- Includes Appendix 1: Recent WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues, and Appendix 2: Reference Values of Commonly Performed Important Laboratory Tests.

Ramadas Nayak MBBS MD graduated from Mysore Medical College, Mysuru, Karnataka, India in the year 1979 and completed his postgraduation in Pathology from Kasturba Medical College, Manipal University, Mangaluru, Karnataka. Presently, he is working as Professor and Head, Department of Pathology, Yenepoya Medical College, Yenepoya University, Mangaluru. In his illustrious teaching career of 35 years, he was Head, Department of Pathology at Kasturba Medical College, Mangaluru for a span of 5 years. Apart from publishing over 84 scientific papers in both national and international journals, he is reviewer of the article for *Journal of Oral and Maxillofacial Pathology*. He serves as an examiner for both undergraduate and postgraduate examinations in several universities. He has worked as Project Officer for Development of Cancer Atlas in India—a project conducted by Indian Council of Medical Research (ICMR) sponsored by WHO. He was the editorial Committee Member of South Asia Edition of *British Medical Journal*. He is also an author of seven books entitled *Essentials in Hematology and Clinical Pathology, Textbook of Pathology and Genetics for Nurses, Rapid Review of Hematology, Exam Preparatory Manual for Undergraduates in General and Systemic Pathology, Exam Preparatory Manual for Undergraduates—Pathology for Dental Students, Textbook of Pathology for BPT Students,* and *Exam Preparatory Manual for Undergraduates—Medicine*. Twice he secured "Good teacher award" and eight times "Best audiovisual award" in Kasturba Medical College, Mangaluru. Above all, he is loved and admired by his students.

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