DISORDERS OF THE BLOOD AND BONE MARROW RESULT IN SOME OF THE COMMONEST SYMPTOMS AND SIGNS FOR WHICH PATIENTS SEEK MEDICAL ADVICE. A CAREFULLY TAKEN HISTORY AND METICULOUS PHYSICAL EXAMINATION, COMPLEMENTED BY THOUGHTFUL AND JUDICIOUS USE OF LABORATORY TESTS, PROVIDES THE CLINICAL BASIS FOR A WORKING DIAGNOSIS. SPECIALISED HEMATOLOGY MAY BE NEEDED TO EXTEND EVALUATION TO THE BONE MARROW AND PLASMA WHILE SUPPLEMENTARY IMAGING OR RADIOMOLECULAR TECHNOLOGY FURTHER LINK PRIMARY CARE PRACTITIONER OR SPECIALIST TO THE EXPERIENCED HEMATOLOGIST. SUCH A, MULTIDISCIPLINARY AND FULLY INTERACTIVE APPROACH OFFERS THE PREFERRED WAY TO PROBLEM SOLVING AND OPTIMUM MANAGEMENT.

A GENERAL COMMENTARY

MAINTENANCE OF GOOD HEALTH IS CRITICALLY DEPENDENT ON OPTIMAL PROVISION OF TRACE ELEMENTS SUCH AS IRON AS WELL AS A VARIETY OF VITAMINS. NUTRITIONAL INADEQUACY, IN CERTAIN CASES, SUCH AS WITH FOLIC ACID, CAN BE LINKED WITH INCREASED INCIDENCE OF MALIGNANCY. IN STARK CONTRAST IS WORLDWIDE PREOCUPPATION WITH DIETARY SUPPLEMENTS, MORE OFTEN THAN NOT IN AN IRRATIONAL ENDENV TO MAINTAIN OR PROMOTE A SENSE OF WELL-BEING.

INTRODUCTION

VITAMIN B12 AND FOLATE DEFICIENCIES ARE THE MAIN CAUSE OF MEgaloblastic hematopoiesis. BOTH DISORDERS ARE CHARACTERISED BY SPECIFIC MORPHOLOGIC FEATURES IN ALL CELL LINES READILY EVIDENT IN THE BONE MARROW. COMPARABLE DISTURBANCES IN CYTOMORPHOLOGY, REFLECTING DEFECTIVE DNA SYNTHESIS BUT HAVING NORMAL LEVELS OF THESE TWO ESSENTIAL NUTRIENTS, ARE THE PRELEUKAEMIC OR MYELODYSPLASIA SYNDROMES AND RETROVIRAL INFECTIONS.

MAINTENANCE OF GOOD HEALTH IS CRITICALLY DEPENDENT ON OPTIMAL PROVISION OF TRACE ELEMENTS SUCH AS IRON AS WELL AS A VARIETY OF VITAMINS.
**PHYSIOLOGY**

**VITAMIN B12 (COBALAMIN)**

**Biologic importance**

A unique characteristic is the presence of cobalt in the molecule, giving rise to the alternative name of cobalamin. There are two main naturally occurring forms, 5'-deoxyadenosylcobalamin, which is located in the mitochondria, functions as a co-factor for the enzyme methylmalonyl-CoA mutase in the conversion of methylmalonyl-CoA to succinyl-CoA, which then enters the citric acid cycle. Methylcobalamin is found in plasma and the cytoplasm, accepts a methyl group from N5-methyl-tetrahydrofolate (5-MTHF). The latter is an essential step in the folate pathway, and acts as the cofactor for the enzyme methionine synthase in the conversion of homocysteine to methionine. This pathway is the first step by which folate enters the bone marrow and other cells from the plasma (Figure 1). Methionine donates methyl group to various reactions in the body. Recall that other forms, cyanocobalamin and hydroxycobalamin exist.

**Distribution and requirements**

Cobalamin is exclusively produced by micro-organisms and therefore is found only in meat, fish and dairy products. Unlike folate, this molecule is heat stable and is not destroyed during cooking. Hepatic stores are relatively large at 2-3 mg, considering that only a small daily amount of 1-3 μg is required in a healthy diet. An average balanced diet provides 5 to 30 μg, primarily derived from animal origin, with small additions via bacterial synthesis in the gastrointestinal tract.

**Figure 1: The effect of cobalamin and folate on nucleic acid synthesis**

- **N-5-METHYL THF**
- **METHIONINE SYNTHASE**
- **COBALAMIN**
- **METHYL-CEB**
- **POLYGLUTAMATED-THF**
- **DNA synthesis and repair**
- **TS = thymidylate synthase**
- **THF = Tetrahydrofolate**
- **CEB = Cobalamin**

The primary role of the intracellular metabolic routes of cobalamin and folate is the sequential transfer of single carbon units for the synthesis and repair of DNA as well as DNA methylation through the metabolism of methionine.

**Intake, absorption and internal exchange**

Recovery from external environment occurs in two ways. Small amounts are passively transported through the mucous membranes including the nasal mucosa, duodenum, ileum and sublingually. The other main mode is active. Here peptic digestion occurs in the stomach releasing the vitamin from food for uptake by a low affinity or R-binder, which is related to transcobalamin I and III. On reaching the alkaline environment of the duodenum, the protein is degraded by the pancreatic trypsin and the liberated vitamin binds to an intrinsic factor, which is generated by the parietal cells in the fundus and body of the stomach. Internalisation takes place in the ileum via enterocytes having special receptors on the microvillus membranes (Figure 2).

In plasma, there are 3 specific binding proteins, transcobalamin I, II, and III. Transcobalamin II is the essential protein for transferring vitamin B12 into the cells through association with specific cell membrane receptors. The amount bound to transcobalamin II is normally very low (about 25%), because most serum B12 is bound to transcobalamin I which does not transfer readily across cell membranes.

**Loss to the environment**

There is a continuous secretion into the biliary system, most of which is recovered and reused with excess found in faeces.

**Cobalamin is exclusively produced by microorganisms and therefore is found only in meat, fish and diary products**
**Figure 2: Physiological steps in cobalamin absorption**

Intake, absorption and internal exchange

Recovery from the diet is mostly in the polyglutamate form, which is hydrolyzed to a monoglutamate by the enzyme hydrolase for the purpose of recovery occurring in the brush border, mostly in the upper small intestine, with declining capacity in the ileum and jejunum. Actual movement is via simple diffusion or by binding to folate transport proteins. Further enzymatic breakdown of monoglutamate takes place in the enterocyte before it is released in the route of renal (N5-methyl-THF) into the portal circulation. Most of this vitamin then circulates loosely bound to albumin or to a lesser degree to a high-affinity binding protein. Cellular uptake is facilitated by folate receptors (Figure 3).

Less to the environment

Unbound plasma folate is filtered and then reabsorbed by the proximal renal tubules with urinary loss reflecting only a small percentage of the dietary intake. Bilithal excretion is estimated to be 100 μg/day, most of which is reabsorbed in an enterohepatic circulation. Presence in faeces appears to be similar in type and quantity to urinary losses.

**THE FOLATES**

**Biologic importance**

Folic acid is the trivial name for polyglutamic acid, which is the parent compound of a large group of compounds, the folates, that occurs in nature conjugated to polyglutamate chains. Humans are unable to synthesize these structures, and therefore require preformed naturally occurring vitamin for many functions in the body, including purine and pyrimidine synthesis and integrity of the homeocysteine pathway. Deficiency results in defective DNA synthesis that impairs the ability of all proliferating cells to synthesize enough nucleic acid during the cell cycle, with subsequent asynchronous nuclear and cytoplasmic maturation. This manifests with ineffective hematopoiesis and impaired cell proliferation.

**Distribution and requirements**

The main sources of folate are liver, spinach and other dark green, leafy vegetables. In most countries cereal fortification often becomes the major dietary source. Adults require 100–200 μg/day, with increased requirements of up to 500 μg/day during pregnancy. Children and infants have lower recommended daily requirements of 25–100 μg.
Methylcobalamin acts as a coenzyme in the remethylation cycle, where single carbon units are transported to other proteins, with the reconversion of homocysteine back to methionine.

Due to the large size, the polyglutamated folate form cannot diffuse across membranes, thereby retaining it for physiological intracellular function. Specifically, a methylene group is added to form N-5,N10-methylene-THF, which is involved in the nucleic synthesis. This compound provides the methyl group needed in the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), one of the building blocks of DNA. Folate is conserved intracellularly by two mechanisms. It can be reduced back to polyglutamated THF after demethylation, or N-5,N10-methylene-THF can enzymatically be reduced to 5-methyl-THF (Figure 1).

During cobalamin deficiency, intracellular folate concentration falls due to the lack of polyglutamate formation, as 5-MTHF demethylation cannot occur. The plasma folate rises as the monoglutamate form diffuses out of the cell. Homocysteine accumulates, as it is dependent on cobalamin for its conversion to methionine. Similarly a folate deficiency would cause failure of the methyl group donation to cobalamin and therefore accumulation of homocysteine in the cells. Folate and cobalamin are biochemically intertwined and impaired DNA synthesis will occur when either vitamin is deficient. Distinctions do exist and can be assessed biochemically.

**PATHOLOGY**

**VITAMIN B12 (COBALAMIN)**

**Congenital**

Defects can be due to single gene mutations and are transmitted as recessive traits affecting absorption, transport, or intracellular metabolism of cobalamin. Imerslund-Gr?ebbeck syndrome is one such example where whole families present with selective intestinal malabsorption uninfuenced by intrinsic factor function. It is associated with proteinuria and structural genitourinary abnormalities. Found typically in infancy, biochemical disturbances, especially methylmalonic aciduria, result in neurologic defects.

**Acquired**

Gastric disease accounts for most deficiencies including gastritis, pancreatitis and small bowel diseases exemplified by Crohn's disease. Infrequently occur in entire populations, and may be found in groups that practice veganism or where poor diet is found, as among the institutionalised elderly. More usually, autoimmune destruction of foetogut mucosa leads to decreased production of intrinsic factor in Addisonian Pernicious Anaemia: extensive resection of stomach or terminal ileum may have a similar result. Competition for vitamin B12 by fish tapeworm, or intestinal stasis syndrome and sprue are alternatives that should not be overlooked. Numerous medications can limit absorption, and include neomycin, biguanides and proton pump inhibitors.

**THE FOLATES**

**Congenital**

May be due to defective transmembrane transport or suboptimal utilisation, resulting in early appearance of severe megaloblastic anaemia, which responds to treatment. Unfortunately, the associated mental retardation improves less satisfactorily because of inability to maintain adequate folate levels in the spinal fluid. Malfunction of the enzyme methylene-tetrahydrofolate reductase is characterised by mental retardation, seizures, and schizophrenic syndromes, often with vasculopathy, but not necessarily with any haematologic abnormality.

**Acquired**

Daily attract more attention as a result of the high incidence of developmental abnormalities of the neural tube in neonates and children. In addition, a variety of neurologic, metabolic, cardiovascular, neoplastic, and epithelial lesions are becoming more prominent in the elderly, with increasing concern about general poor nutrition, drug and alcohol abuse. Malabsorptive states, as seen with inflammatory bowel disease, sprue or short bowel syndrome and certain drugs, like methotrexate, trimethoprim and phenytoin also hinder absorption. Furthermore failure to meet increased demands, as in pregnancy, exfoliative dermatitis and chronic haemolysis, also leads to folate deficiency.

**CLINICAL FEATURES**

With the exception of neurological disease, the clinical features of both deficiencies are similar, causing megaloblastic anaemia. Vitamin B12 deficiency, however, develops less rapidly than folate due to the larger reserve stores often taking years to manifest. In contrast, folate depletion can occur within months when intake is disturbed. Presentation is with non-specific symptoms and signs due to tissue hypoxia and compensatory mechanisms. Mild jaundice due to increased haemoglobin reflects breakdown secondary to the ineffective haematoapoiesis. Rapidly growing cells, like epithelium, exhibit abnormal growth and lead to glositis, angular stomatitis and malabsorption with weight loss. Melanin pigmentation and purpura, due to secondary thrombocytopoiesis, are less frequent skin manifestations.

**Cobalamin deficiency**

Neurological problems are usually seen in severe deficiency, including subacute combined degeneration of the cord and a progressive, symmetrical neuropathy.

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The brunt falls on the lower limbs initially as paresthesia and ataxia, but can progress to spasticity and paraplegia. Rarely, optic atrophy or psychiatric disorders arise, among which are depression, mania or even psychosis, although may include memory loss, irritability and dementia. It is important to note that normal peripheral full blood count does not exclude clinically important deficiency and, particularly in the elderly, levels should be interpreted in this context.

The skeleton is frequently overlooked when osteoblast function is suppressed in the face of cobalamin deficiency, increasing risk for osteoporosis and hip and spine fractures.

Pernicious anaemia is more commonly associated with elderly females of northern European descent, but can also occur in younger, and especially, African women. This condition carries increased risk for other autoimmune diseases and stomach carcinoma. The coincidence of iron deficiency can mask the macrocytosis, and affected individuals may not necessarily be anaemic. Hypersegmented neutrophils documented on peripheral smear should alert clinicians to this condition.

Folate deficiency
Although clinically similar to cobalamin deficiency, there is usually a stronger association with substance abuse and poor diet upon presentation. There are strong implications for occurrence of neural tube and other congenital defects during pregnancy. It also carries increased risk of epithelial and other cancers including colon as well as neuroblastoma in the young age group.

In the older population cardiovascular disease is correlated with hyperhomocysteaemia, with increased incidence of myocardial infarcts, peripheral and cerebral vascular disease such as venous thrombosis and atherosclerosis. The benefits of folate supplementation in reducing the rate of myocardial infarction or stroke have not yet been established. Less clearly defined are disturbances in platelet function that may accelerate thrombosis in parallel with hyperhomocysteaemia.

References are available on request.