22.6.6 Megaloblastic anaemia and miscellaneous def

22.6.6 Megaloblastic anaemia and miscellaneous deficiency anaemias 5407 A.V. Hoffbrand

22.6.6 Megaloblastic anaemia and miscellaneous deficiency anaemias 5407 these conditions may mobilize iron from macrophage stores, easing functional iron deficiency and facilitating erythropoiesis. ESAs are widely used to treat patients with chronic kidney disease in whom endogenous erythropoietin production is inadequate to maintain erythropoiesis in the context of declining haemoglobin concentra- tions. However, use of ESAs for routine treatment of anaemia of in- flammation is no longer recommended, although these drugs may be valuable in selected cases where serum erythropoietin levels have been measured and are inappropriately low. The goal should be to gradually increase haemoglobin concentrations to a target range between 100 and 120 g/l, as rapid increases or higher levels have been associated with cardiovascular risk among patients with renal failure and cancer. Prognosis and outcome Anaemia of inflammation will persist for as long as erythroblasts are deprived of iron by the effects of hepcidin, or via direct cytokine-mediated suppression of erythropoiesis. Resolution or control of the underlying condition should relieve these processes and facili- tate erythropoiesis. Some emerging data suggest that following re- covery from inflammation, rebound erythropoiesis may transiently suppress hepcidin, facilitating enhanced iron absorption and release from macrophages, and optimizing recovery from anaemia. Special circumstances Anaemia of the elderly Elderly individuals have a higher prevalence of anaemia compared with the younger population. For example, in Australia 16% of

indi- viduals older than 75 years are anaemic compared with fewer than 5% of younger adults. The prevalence of anaemia increases with age and among individuals in hospital or in care. Anaemia in the eld- erly is consistently correlated with impaired physical and cognitive performance, and increased frailty. Epidemiological studies indicate that about one-third of cases of anaemia of the elderly are due to deficiencies of iron and other haematinics. A further third are due to anaemia of inflammation due to identifiable medical comorbid- ities and renal impairment. The causes of the final third are more difficult to ascertain, and it is often termed ‘unexplained anaemia of the elderly’. Some cases may also represent underlying malig- nant haematological diseases such as myelodysplasia. The mech- anisms of unexplained anaemia require further investigation, but may be multifactorial, including reduced erythroid potential of the haematopoietic stem cell compartment, hepcidin-dependent and - independent mechanisms of anaemia of inflammation, reduced erythropoietin production in response to reduced haemoglobin concentrations, and in men, hypoandrogenism. Elderly patients with anaemia should be appropriately investigated to identify po- tential therapies and exclude serious underlying conditions. Areas of uncertainty and controversy Treatment of anaemia of inflammation remains challenging, as few specific therapies are presently available. Further work is needed to assess the role, timing, and optimal regimens of intravenous iron and ESAs. The safety of and optimal targets for treatment with ESAs re- quire investigation in nonrenal- or cancer-related anaemia. The role of measurement of hepcidin in diagnosis of anaemia of inflammation and its role in guiding treatment need development. The epidemi- ology and mechanisms of anaemia of the elderly requires clarification.
Future developments The discovery that hepcidin is a key mediator of anaemia of inflam- mation has stimulated interest in development of novel therapeutics which either directly inhibit its action or potentially, prevent its expres- sion. For example, hepcidin expression can be prevented by heparin, and heparin-derived compounds (e.g. glycol-split or oversulphated heparins) have been shown to reduce hepcidin levels and alleviate an- aemia of inflammation in experimental animals. Likewise, antihepcidin monoclonal antibodies and the antihepcidin L-ribonucleic acid aptamer (NOX-H94) both neutralize circulating hepcidin, reducing the anaemia of inflammation in animal models. Finally, recombinant erythroferrone (the erythroid-derived suppressor of hepcidin) may prove to be a useful strategy for reducing hepcidin expression and treating anaemia of inflammation in the future. FURTHER READING Camaschella C, Pagani A (2018). Advances in understanding iron metabolism and its crosstalk with erythropoiesis. Br J Haematol, 182(4), 481–94. Cullis JO (2011). Diagnosis and management of anaemia of chronic disease: current status. Br J Haematol, 154, 289–300. Ganz T (2019). Anemia of inflammation. N Engl J Med, 381, 1148–57. National Blood Authority (2012). Patient blood management guide- lines: module 3 medical. National Blood Authority, Canberra. Thomas DW, et al. (2013). Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol, 161, 639–48. Weiss G (2015). Anemia of chronic disorders: new diagnostic tools and new treatment strategies. Semin Hematol, 52, 313–20. Weiss G, Ganz T, Goodnough LT (2019). Anemia of inflammation. Blood, 133, 40–50. 22.6.6 Megaloblastic anaemia and miscellaneous deficiency anaemias A.V. Hoffbrand ESSENTIALS Megaloblastic anaemias are characterized by red blood cell macrocytosis. They arise because of inhibition of DNA synthesis in the bone marrow, usually due to deficiency of one or other of vitamin B12 (cobalamin) or folate, but sometimes as a consequence of a drug or a congenital or acquired biochemical defect that disturbs vitamin B12 or folate metabolism, or affects DNA synthesis independent of vitamin B12 or folate.

section 22 Haematological disorders 5408 Biochemical and nutritional aspects of vitamin B12 and folate Vitamin B12—synthesized by bacteria; in humans the daily re- quirement of 1 to 2 µg is acquired from secondary animal sources including fish, eggs, milk, and meat. Processing within the body oc- curs as follows: (1) proteolysis of food releases dietary vitamin B12 for binding to a glycoprotein haptocorrin; (2) pancreatic trypsin degrades the glycoprotein, releasing vitamin B12 for attachment to intrinsic factor; (3) the vitamin B12–intrinsic factor complex binds to a specific receptor—cubilin amnion—expressed on the luminal brush border of the mucosal cells of the ileum, and is endocytosed; (4) after lyso- somal degradation, vitamin B12 is complexed with transcobalamin (TC)-II and secreted into the circulation; (5) the TCII–B12 complex is incorporated by cellular endocytosis in peripheral tissues and vitamin B12 released by digestion in the lysosomal compartment. Folate—occurs principally in leaves and vegetables, but is des- troyed by cooking. The daily requirement is about 100 µg, with ab- sorption occurring through a proton-coupled folate transporter in the proximal small intestine and duodenum. Attached glutamate res- idues are cleaved, releasing methyl tetrahydrofolate into the portal plasma. Biochemical basis of megaloblastic anaemia—(1) folate defi- ciency reduces the availability of the coenzyme 5,10-methylene tetrahydrofolate (THF) polyglutamate, thus inhibiting synthesis of thymidylate, which is the rate-limiting step in DNA synthesis. (2) Vitamin B12 deficiency impairs DNA synthesis indirectly because it is needed for conversion of methyl-THF entering cells from plasma to THF, the substrate of active folate coenzymes (folate polyglutamates) including 5,10 methylene-THF. Causes of megaloblastic anaemia Vitamin B12 deficiency—(1) malabsorption—including (a) gastric causes (e.g. acquired pernicious anaemia, gastrectomy); (b) intestinal causes (e.g. bacterial overgrowth, ileal resection); and (2) nutritional (e.g. vegans). Folate deficiency—(1) poor diet—e.g. poverty, alcoholism; (2) mal- absorption (e.g. gluten-induced enteropathy, tropical sprue); (3) excessive requirements (e.g. pregnancy, haemolytic anaemia); (4) excess excretion (e.g. chronic haemodialysis); (5) drugs (e.g. anti- convulsants); and (6) liver disease. Not due to vitamin B12 or folate deficiency—(1) abnormalities of vitamin B12 or folate metabolism—including (a) congenital (e.g. TCII deficiency); (b) acquired (e.g. dihydrofolate reductase inhibitors); (2) independent of vitamin B12 or folate—including (a) congenital (e.g. orotic aciduria); (b) acquired (e.g. various myeloid leukaemias); and (c) drugs (e.g. antimetabolites, hydroxycarbamide). Laboratory investigation This consists of three stages: (1) recognition that megaloblastic an- aemia is present—the mean corpuscle volume is raised to 100 to 140 fl, and the peripheral blood shows hypersegmented neutrophils. The bone marrow (if examined) is hypercellular, with megaloblastic erythroblasts and giant metamyelocytes. (2) Distinction between vitamin B12 or folate deficiency (or rarely some other factor) as the cause of the anaemia—usually achieved by assay of serum vitamin B12 and serum folate. (3) Diagnosis of the underlying disease causing the deficiency—depends on taking a dietary history, measurement of parietal cell and intrinsic factor antibodies and serum gastrin, transglutaminase antibody, and pursuing clinical clues to other pos- sible causes. Subclinical deficiency of both vitamins is more frequent than overt megaloblastic anaemia. Pernicious anaemia Antibodies in serum and gastric juice directed against parietal cells (85–90% of cases) and intrinsic factor (50%), and raised serum gastrin are associated with autoimmune gastritis and failure of absorption of vitamin B12. Clinical features—anaemia usually develops gradually, and symp- toms may not occur until it is severe. Aside from pallor, other manifestations can include (1) mild jaundice, (2) mild pyrexia, (3) psychiatric disturbance, (4) glossitis and angular cheilosis, and (5) features of an associated disorder (e.g. vitiligo, thyroid disease). Complications include (1) peripheral sensorimotor neuropathy and (2) subacute combined degeneration of the spinal cord—manifest as loss of proprioception and pyramidal weakness.

Treatment and prevention of megaloblastic anaemia Vitamin B12 deficiency—may be treated with intramuscular hydroxocobalamin (1-mg doses, six given in the first 2–3 weeks, then every 3 months). Oral therapy is practised by a minority and is un- likely to be useful in pernicious anaemia. Neurological complications are irreversible unless treated early. Folate deficiency—high-dose oral folic acid (5 mg daily) over- comes folate malabsorption, but this should not be given alone where vitamin B12 deficiency coexists because neurological disease may be precipitated or exacerbated (although the haematological abnormalities improve). Where folate metabolism is disturbed by methotrexate, oral or parenteral folinic acid is given to restore DNA synthesis. Prevention—dietary folate fortification is an accepted and highly effective public health measure in many countries (none in Europe) for reducing the incidence of neural tube birth defects. Introduction The megaloblastic anaemias are a group of disorders characterized by a macrocytic anaemia and distinctive morphological abnormal- ities of the developing haematopoietic cells in the bone marrow. In severe cases, the anaemia may be associated with leucopenia and thrombocytopenia. Megaloblastic anaemia arises because of in- hibition of DNA synthesis in the bone marrow, usually due to de- ficiency of one or other of two water-soluble B vitamins: vitamin B12 (cobalamin) or folate. Vitamin B12 deficiency may also cause a severe neuropathy. In a minority of cases, megaloblastic anaemia arises because of a disturbance of DNA synthesis due to a drug or a congenital or acquired biochemical defect that causes a disturb- ance of vitamin B12 or folate metabolism or affects DNA synthesis independent of vitamin B12 or folate. Vitamin B12 and folate are discussed first and the other rare megaloblastic anaemias are men- tioned later in this chapter. Folic acid supplements in pregnancy and food fortification with folic acid are aimed at preventing neural tube defects. Possible relations between folate and vitamin B12, and cardiovascular or

22.6.6 Megaloblastic anaemia and miscellaneous deficiency anaemias 5409 malignant diseases and cognitive defects in older people are also discussed. Biochemical and nutritional aspects of vitamin B12 and folate Vitamin B12 Biochemistry Four major forms of the vitamin exist in humans, all with the same cobalamin nucleus, which consists of a planar corrin ring (hence the term ‘corrinoids’ for vitamin B12 compounds) attached at right angles to a nucleotide portion, 5,6-dimethylbenzimidazole joined to ribose-phosphate (Fig. 22.6.6.1 and Table 22.6.6.1). 5′-Deoxyadenosyclobalamin (adocobalamin) accounts for about 80% of vitamin B12 inside mammalian cells and is located mainly in mitochondria; methylcobalamin is a minor cellular compo- nent but the main form in plasma. Both are extremely light sensi- tive and are rapidly photolysed to hydroxocobalamin by daylight; hydroxocobalamin is present in small amounts in tissues and plasma and is available commercially for therapeutic use. The fourth form, cyanocobalamin, is found only in trace amounts naturally, but is stable and used therapeutically. Hydroxo- and cyanocobalamins are converted to the two biochemically active forms. The fully re-duced compounds are termed Cob(I)alamins, and the oxidized compounds Cob(III)alamins. Analogues of vitamin B12 (pseudo- vitamin B12s) exist in nature, endogenous production of which in humans is suggested by their presence in all sera (including fetal serum) and their fall in parallel with physiologically active vitamin B12 in vitamin B12 deficiency. Vitamin B12 is known to be involved in only three reac- tions in human tissues: as adocobalamin in the isomerization of methylmalonyl CoA to succinyl CoA and of α-leucine to β-leucine, and as methylcobalamin in the methylation of homocysteine to methionine, a reaction that also requires methyltetrahydrofolate (Fig. 22.6.6.2). In some bacteria, but not in humans, vitamin B12 has a direct role in DNA synthesis by virtue of its involvement in ribonucleotide reductase. Nutrition Vitamin B12 is synthesized by

microorganisms; animals obtain it by consuming the flesh of other animals or their produce (milk, cheese, eggs, etc.)—or vegetable foods contaminated by bacteria. A healthy mixed diet contains between 5 and 30 µg daily. In some species, but not in humans, vitamin B12 is absorbed after synthesis by bacteria in the large intestine. The vitamin B12 content in humans is about 3 to 5 mg; it is found mainly in the liver (c.0.7–1.1 µg/g). Adult daily losses are related to body stores; to maintain normal body stores, daily requirements are of the order of 1 to 2 µg. It takes 3 to 4 years, on average, for deficiency to develop if supplies are totally cut off by malabsorption. There is an enterohepatic circulation for vitamin B12, variously estimated at 3 to 9 µg daily, which is intact in vegans, which may partly account for their tendency to maintain low body stores without incurring severe deficiency. The body is unable to degrade vitamin B12 and deficiency has not been shown to be due to excess utilization or loss. Absorption About 15% of dietary vitamin B12 is available for absorption. It is released from protein binding in food by proteolytic enzymes, heat, and acid, and combines one molecule to one molecule with a glyco- protein R vitamin B12-binding protein (also called haptocorrin) in gastric juice. The glycoprotein binds dietary forms of vitamin B12 but does not facilitate its absorption. Gastric pepsin and pancre- atic trypsin degrade this protein and so releases vitamin B12 for at- tachment to intrinsic factor (IF) and subsequent absorption. IF is a glycoprotein produced mainly by the gastric parietal cells (Table 22.6.6.2). The normal stomach produces a vast excess of IF, meas- ured in units (1 unit binds 1 ng vitamin B12). Vitamin B12 in bile is also attached to IF and reabsorbed through the ileum. At neu- tral pH, in the presence of calcium ions, the vitamin B12–IF com- plex attaches passively to a complex specific IF receptor, cubilin amnion, on the brush border of the mucosal cells of the terminal ileum. Cubilin is a 640-kDa peripheral membrane protein pre- sent in the epithelium of intestine and kidney. Amnionless (AMN) (50 kDa) binds to cubilin and is essential for production of mature cubilin and its transport to the apical brush border. AMN directs sublocalization and endocytosis of cubilin and the IF–B12 complex. Mutations of cubilin or AMN underlie hereditary malabsorption of vitamin B12 (discussed later in this chapter). After cubilin–AMN-mediated endocytosis, IF undergoes lyso- somal degradation. After a delay of 3 to 5 h, vitamin B12 appears in portal blood, with a peak concentration 8 h after ingestion, com- plexed with transcobalamin II (TCII) secreted into the circulation from the basolateral side of the intestinal cells. Ileal absorption of vitamin B12 is limited by the number of cubilin receptors to a few N N CH2 CH2 CH2 CH3 CO NH CH O O – P N A C B CO+ OH C D N N N N C C O O O C CH2OH CH3 CH3 Fig. 22.6.6.1 The structure of cyanocobalamin.

 micrograms daily, and although 80% of a single dose of 1 to 2 µg may be absorbed, the proportion diminishes steeply at higher doses. A small (<1%) trace of a large (≥1 mg) dose of vitamin B12 can be absorbed passively and rapidly through the buccal, gastric, and duo- denal mucosae without the involvement of the IF pathway and this forms the basis for treating malabsorption of vitamin B12 with large oral doses of cyanocobalamin. Transport Vitamin B12 in plasma is 70 to 90% attached to a glycoprotein, transcobalamin I (TCI), and 0 to 10% to transcobalamin III (TCIII), which do not enhance cell uptake of vitamin B12 (Table 22.6.6.2). TCI and III belong to a group of glycoproteins, the R binders or haptocorrins (previously mentioned), that are present in many tis- sues and fluids; these molecules have the same amino acid compos- ition but differ in the carbohydrate moiety. The haptocorrins may have the role of binding analogues of vitamin B12 derived from food or intestinal organisms and transporting them to the liver for excre- tion in the bile. Genetic mutations of the gene TCN1, which codes for TCI, cause subnormal serum vitamin B12 levels. The most important plasma vitamin B12-binding protein, TCII, is synthesized in macrophages, the liver, the ileum, and possibly the endothelium. TCII is loaded

with vitamin B12 from the ileum and by release of vitamin B12 from the liver and other organs. It is nor- mally almost completely unsaturated because it actively enhances uptake of vitamin B12 by bone marrow, placenta, and other tissues of the body that contain TCII receptors. TCII–vitamin B12 is internal- ized by endocytosis; vitamin B12 is released by proteolytic cleavage in lysosomes but TCII is not reutilized (Table 22.6.6.1). TCII has a 20% amino acid homology and greater than 50% nucleotide hom- ology with human TCI and with rat IF. It shows at least five genetic variants. Serum TCII is normally higher in women than men and in black populations compared with white. The concentration of vitamin B12 in cerebrospinal fluid is low, with a mean of 10 ng/litre in normal subjects. Most of this is attached to TCII. There is virtually no vitamin B12 in normal urine. Folate Biochemistry This vitamin exists in nature in over 100 forms, all of which are de- rivatives of folic acid (pteroylglutamic acid), which has the structure Table 22.6.6.1 Vitamin B12 and folate Vitamin B12 Folate Parent form Cyanocobalamin (cyano-B12), molecular weight 1355 Folic acid (pteroylglutamic acid), molecular weight 441.4 Crystals Dark-red needles Yellow, spear-shaped Natural forms Deoxyadenosylcobalamin Reduced (di- or tetrahydro-), methylated, formylated, other single carbon additions; mono- and polyglutamates Methylcobalamin Hydroxocobalamin Foods Animal produce (especially liver) only All, especially liver, kidney, yeast, greens, nuts Adult daily requirements 2 µg 100 µg Adult body stores 2–5 mg 6–20 mg Length of time to deficiency 2–4 years 4 months Daily diet content 5–30 µg About 200–250 µg Cooking Little effect Easily destroyed Absorption Intrinsic factor (+ neutral pH + Ca2+) via ileum Deconjugation, reduction, and methylation via duodenum and jejunum Plasma transport Tightly and specifically bound to transcobalamins One-third loosely bound albumin, other proteins;?specific protein Enterohepatic circulation 3–9 µg/day 60–90 µg/day (2) S-adenosylhomocysteine Methionine Homocysteine THF Methyl THF (3) α-leucin B-leucine adocobalamin (1) Propionyl CoA adocobalamin Succinyl CoA Methylmalonic Acid Isoleucine Odd-chain fatty acids Thymidine Valine methylmalonyl CoA mutase Methylmalonyl CoA S-adenosylmethionine Fig. 22.6.6.2 Biochemical reactions of vitamin B12 (cobalamin) in human tissues. THF, tetrahydrofolate.

 of a pteridine, a para-aminobenzoic acid moiety and L-glutamic acid (Fig. 22.6.6.3). Natural folates differ from folic acid by: • being reduced in the pteridine ring to di- or tetrahydo- forms • having a single carbon moiety attached at positions N5 or N10 (e.g. methyl, formyl, etc.) • having a chain of glutamate moieties attached by γ-peptide bonds to the L-glutamate moiety In human and other mammalian cells, the number of glutam- ates is mainly four, five, or six. Polyglutamate forms of folate are the active coenzymes; they show increased affinity or lowered Km values compared to the monoglutamate equivalent compounds, for most of the enzymes of one-carbon metabolism. In body fluids, however, folates are monoglutamate derivatives. In plasma, 5-methyltetrahydrofolate (methyl-THF) predominates. The biochemical reactions of folates are shown in Table 22.6.6.3. In each there is transfer of a single carbon group, methyl (–CH3), formyl (–CHOH), methenyl (≡CH), methylene (=CH2), or formi mino (=CHNH), from one compound to another. Three of the reactions are concerned with synthesis of DNA precursors (two purine and one pyrimidine). During thymidylate synthesis, oxidation of folate to the dihydro state occurs; the enzyme dihydrofolate reductase, the principal target for the antifolates methotrexate and pyrimethamine, returns folate to the active tetrahydro state (Fig. 22.6.6.4). During its reactions, folate is not completely reutilized, some degradation at the C9–N10 bond oc- curs to nonfolate compounds. Thus, folate utilization is increased and folate deficiency likely when cell turnover and DNA synthesis are increased. Nutrition Folate occurs in most foods, the highest concentrations (more than 30 µg/100

g wet weight) in liver (in which it is easily destroyed by cooking). Vitamin C protects folate from oxidative destruction. An average Western daily intake is about 250 µg, with 50% or more in the polyglutamate form. Body stores are about 10 to 12 mg, with a mean liver concentration of about 7 µg/g. Primitive or rapidly growing tis- sues have higher folate concentrations than corresponding mature tissues. Daily adult requirements are about 100 µg. Absorption Folates are absorbed rapidly, mainly through the duodenum and je- junum. Polyglutamates are deconjugated in the intestinal lumen, at the brush border, and possibly in lysosomes of intestinal cells by an enzyme known as folate conjugase (γ-glutamylcarboxypeptidase, pteroylpolyglutamate hydrolase). They are reduced to the tetrahydro state and methylated at the N5 position so that methyl- THF enters portal plasma whatever food folate is ingested (Table 22.6.6.1). Folic (pteroylglutamic) acid itself, which is not present in food, but is used therapeutically, enters the portal blood largely unchanged at doses of more than 200 µg, as it is a poor sub- strate for reduction by dihydrofolate reductase. A proton-coupled folate transporter (PCFT) with high affinity and a low pH op- timum is essential for absorption of reduced folates and folic acid. It is expressed particularly in the apical brush border of the en- terocytes of the duodenum and jejunum. Various mutations have been found in this transporter in patients with a specific hereditary malabsorption of folate. The protein is expressed in other tissues and may be involved in intracellular transportation of folates from endocytic vesicles. As it is also active at neutral pH for methyl- THF, it may play a role in delivering this folate to systemic cells (e.g. the liver). It may also transport antifolates (e.g. methotrexate) into the acid interior of solid tumours. The small intestine has a large capacity to absorb folate; on average 50% of natural folate is absorbed whatever the dose. If excessive amounts are fed, the excess is largely excreted in urine as folates or their breakdown products after cleavage of the C9–N10 bond. There is a substantial enterohepatic circulation for folate, estimated at up to 90 µg folate daily; if this is interrupted, plasma folate concentrations decrease to about one-third within 24 h. Table 22.6.6.2 Vitamin B12-binding proteins Intrinsic factor Transcobalamin I and IIIa Transcobalamin II Present in Gastric juice Plasma Plasma, cerebrospinal fluid Source Gastric parietal cell Granulocytes, Other organs Macrophages, liver parenchyma, ileum Molecular weight 45 000 60 000 45 500 Structure Glycoprotein (15% sugar) Glycoprotein Polypeptide Normal total binding capacity 30–110 µg/litre 700–800 ng/litre 900–1000 ng/litre Vitamin B12 content No vitamin B12 300–400 ng/litre vitamin B12 30–60 ng/litre vitamin B12 Function Vitamin B12 absorption
(not itself absorbed) ? Storage of vitamin B12 Vitamin B12 delivery to marrow, placenta, brain, and other tissues, Vitamin B12 absorption ? Protection of vitamin B12 Binding of vitamin B12 analogues a Related ‘R’ binders (haptocorrins) occur in other tissues and secretions, e.g. milk, gastric juice, saliva, and tears. N H O C COOH (α) CH COOH (γ) 1 2 3 6 7 8 9 10 N N N N N H N 5 4 Fig. 22.6.6.3 The structure of pteroylglutamic (folic) acid.

section 22 Haematological disorders 5412 Transport Folate is transported in plasma, two-thirds unbound and about one- third loosely bound to albumin and possible other proteins. There are two highly specific mammalian folate transporters. SLC19A1 is a facilitative transporter with the characteristics of an anion ex- changer. The gene is located at chromosome 21q 22.2. The protein has 12 transmembrane domains and both N- and C-termini are dir- ected to the cytoplasm. It is ubiquitously expressed on normal tis- sues and tumours. Its affinity for folic acid and methotrexate is one to two orders less than for reduced folates. The second is a group of high-affinity binding proteins (FRs) encoded by three genes, des- ignated α, β, and γ, localized to chromosome 11q13.3 to 11q13.5. FRα and FRβ are both glycosylphosphatidylinositol (GPI)-anchored proteins. The physiological role of the FRs is not clear. They are ex- pressed in the apical brush border of the

renal tubular epithelial cells so may have a role in renal reabsorption of folates. Folate taken up by membrane-bound FRs is thought to enter endocytic vesicles. FRα (but not FRβ) knockout mice show fatal morphological abnormalities, suggesting a critical role in mouse development. The FRs have enhanced expression on certain tumour cells and this has prompted studies aimed at developing tumour- specific antifolates or folate-conjugated radiopharmaceuticals or other molecules. Plasma folate is filtered by the glomerulus and mostly reabsorbed unless the renal tubular maximum is exceeded. Normal urine folate is 0 to 13 µg in 24 h. Folate is secreted into cerebrospinal fluid (which has a mean concentration of 24 µg/litre) and is present in bile. Human milk has a folate concentration of 50 µg/litre. Prostate- specific membrane antigen is a folate hydrolase carboxypeptidase which can release glutamates in either α or γ linkages. The physio- logical significance of this is unknown. Biochemical basis of megaloblastic anaemia All known causes of megaloblastic anaemia, whether drugs, de- ficiencies, or inborn errors of metabolism, inhibit DNA synthesis by reducing the activity of one of the many enzymes concerned in
Table 22.6.6.3 Biochemical reactions of folates Reaction Enzyme 1. Conjugation or deconjugation Hydrolysis of poly- to monoglutamates Folate ‘conjugase’ (α-glutamylcarboxypeptidase; pteroylpolyglutamate hydrolase) Conjugation of monoglutamates to polyglutamates Folate-polyglutamate synthase 2. Oxidation–reduction Oxidized or dihydrofolates converted to tetrahydrofolates Dihydrofolate reductase 3. Amino acid interconversions (a) Homocysteine → methioninea 5-Methyl-THF methyltransferase (methionine synthase) Methyl THF →THF (b) 5-Formiminoglutamic acid (Figlu) → glutamic acid Figlu transferase THF → formimino THF Serine–hydroxymethyltransferase (c) Serine → glycine THF → 5,10-methylene THF 4. DNA synthesis Purine synthesis: (a) GAR → formyl GAR GAR transformylase 5,10 Methenyl THF →THF (b) AICAR → inosinic acid AICAR transformylase 10-Formyl THF →THF Pyrimidine synthesis: Deoxyuridine monophosphate (dUMP) → thymidine monophosphate (TMP) Thymidylate synthase 5,10-Methylene THF → THF 5. Formate fixation Formic acid + ATP + THF → 10-formyl-THF + ADP THF formylase 6. ? Methylation of biogenic amines E.g. dopamine → epinine ? Dopamine methyltransferase Methyl THF → THF THF, tetrahydrofolate; DHF, dihydrofolate; GAR, glycinamide ribotide; AICAR, 5-amino-4-imidazolecarboxamide ribotide. a See Figs. 22.6.6.2 and 22.6.6.4. Reaction (6) has been demonstrated only in vitro and may not take place in vivo.

 purine or pyrimidine synthesis or by inhibiting DNA polymeriza- tion from its precursors. Folate deficiency, by reducing supply of the active coenzyme form, 5,10-methylene-THF, inhibits thymidylate synthesis, a rate-limiting reaction in DNA synthesis. Vitamin B12 does not have a direct role in this or any other reaction in mamma- lian DNA synthesis. Vitamin B12 deficiency inhibits DNA synthesis indirectly because of the requirement for methylcobalamin in the conversion of methyl-THF that has entered cells from the plasma to THF. Deficiency of vitamin B12 is considered to decrease the intra- cellular supply of THF, from which the natural folate coenzymes, folate polyglutamates, are made. Methyl-THF cannot act as a sub- strate for synthesis of folate polyglutamates in human cells. When vitamin B12 is deficient there is reduced activity of all reactions re- quiring folate coenzymes, including those involved in DNA syn- thesis (Fig. 22.6.6.4). Misincorporation of the base uracil, because of the accumulation of dUMP (Fig. 22.6.6.4) and hence of dUTP, has been proposed to contribute to the DNA abnormality. Clinical features and causes of megaloblastic anaemia Although pernicious anaemia (PA) is only one of the many causes of megaloblastic anaemia (Tables 22.6.6.4–22.6.6.6), it is convenient to describe the general clinical features of the anaemia under this heading; PA is the most frequent cause of megaloblastic anaemia in Western countries. The laboratory findings

and treatment of PA and other megaloblastic anaemias are discussed later. Pernicious anaemia (Addisonian pernicious anaemia, Biermer's anaemia) Definition An autoimmune disease in which there is atrophy of the stomach with severely reduced or absent IF and acid secretion with consequent malabsorption of vitamin B12 and vitamin B12 deficiency. There is an autoimmune gastritis caused by pathological CD4 T cells reacting against gastric H/K-ATPase. Aetiology PA is a disease of older people: less than 10% of patients are under the age of 40 years. There is a female:male ratio in most (but not all) series of about 1.6:1. There is a slightly higher prevalence (c.44 vs 40%) of blood group A in patients with PA compared with con- trols in the United Kingdom. No overall association between PA and HLA type has been found, but those with an endocrine disease have a greater incidence of HLA B8, B12, and BW15. An association between autoimmune gastritis and HLA DRB103 and DRB104 has been reported in Finnish and Italian populations. PA occurs in all ethnic groups including African, Indian, Native American, and Chinese, as well as white Europeans. There is a higher incidence in close relatives, of either sex, of an affected person. DNA sequence variants of a gene NLRP1, located at chromosome 17p13, encoding NACHT, a leucine-rich repeat protein which is a regulator of the innate immune response, have been associated with vitiligo and its associated diseases including PA. About 55% of patients have serum thyroid antibodies and 33% with primary myxoedema have parietal cell antibody. There is probably no association with diabetes mellitus. Other evidence for an immune aetiology of the gastritis of PA is the improvement in mucosal appearance and function with corticosteroid therapy, the presence of antibodies in serum and gastric juice dir- ected against parietal cells and IF, and of cell-mediated immunity to IF. Parietal cell antibody is present in the serum of 85 to 90% of patients. The autoantigens are the α- and β-subunit of the gas- trin proton pump (H+,K+ ATPase). Two antibodies to IF exist in serum. Type I ('blocking') occurs in about 50% of patients and is directed against the vitamin B12-binding site. Type II (to the ileal binding site) occurs in 30 to 35% but only if type I antibody is also present. Antibodies to IF in gastric juice may neutralize the action of remaining IF. The incidence of parietal cell and IF antibodies in serum in PA may be different in different groups of patients, younger patients having a lower incidence of parietal cell antibody while black patients and Hispanic patients may have a higher inci- dence of IF antibodies. The antibodies to IF are virtually specific for PA but parietal cell antibodies occur in many subjects with atrophic gastritis without PA. An autoantibody to the gastrin receptor may also occur in serum in PA. PA may be associated with hypogammaglobulinaemia or with se- lective IgA deficiency when it tends to present at an early age. Serum gastrin concentrations are raised (>200 μg/litre) in 90% of patients with PA, and serum pepsinogen (PG) concentrations are less than 30 μg/litre in 92% of such patients with a low PGI/PGII ratio. DNA dATP dGTP dTTP dCTP dTDP thymidine kinase Thymidine dTMP thymidylate synthase dUMP DHF-polyglutamate dihydrofolate reductase Methylation of myelin, basic proteins, lipids, DNA, amines S-adenosyl- methionine S-adenosyl homocysteine THF-polyglutamate THF Methionine Homocysteine Methyl THF (synthesized by small intestine from dietary folates) 5,10 methylene THF – polyglutamate Deoxyuridine (dU) Fig. 22.6.6.4 Suggested mechanisms by which vitamin B12 deficiency affects folate metabolism and interferes with DNA synthesis. Indirect involvement of vitamin B12, as methylcobalamin, in DNA synthesis is suggested by the 'methylfolate' trap ('tetrahydrofolate starvation') hypothesis. Methylcobalamin is involved in formation of intracellular THF from plasma methyl-THF. THF and/or its formyl derivative, but not methyl-THF, are the 'ground substances' from which all folate coenzymes are made by glutamate addition and single carbon unit transfer. 5,10-Methylene-THF polyglutamate is involved in thymidylate synthesis. A, adenine; C, cytosine; D, deoxyribose; DP, diphosphate; G, guanine; T, thymine; THF, tetrahydrofolate; TP, triphosphate; U, uridine.

section 22 Haematological disorders 5414 The relationship of PA, autoimmune gastritis, and Helicobacter pylori infection is not clear. Young subjects (<40 years) with gastritis, hypergastrinaemia, and positive antiparietal cell antibody in serum will usually show iron deficiency anaemia whereas older (>60 years) with these features more frequently have macrocytic red cells and low serum vitamin B12 levels. H. pylori infection occurs in up to 40% of such subjects less than 20 years old but in only 10% of those older than 60 years. It has been proposed that H. pylori is an infective trigger to autoimmune gastritis by molecular mimicry. Pathology There is a gastritis in which all layers of the body and fundus of the stomach are atrophied with loss of normal gastric glands, mucosal architecture, and absence of parietal and chief cells, but mucous cells lining the gastric pits are well preserved. An infiltrate of plasma cells and lymphocytes with an excess of CD8 cells occurs and intestinal metaplasia may be present. The antral mucosa is well preserved ex- cept in hypogammaglobulinaemia, and, like the fundus, shows an increased number of gastrin-secreting cells. Clinical features The general features of megaloblastic anaemia are similar, whatever the underlying cause. Particular clinical features may point to the underlying disease, whether PA or some other cause. In PA, the an- aemia usually develops gradually, perhaps over several years, and symptoms may not occur until it is severe. The most common com- plaints are due to the anaemia, but loss of mental and physical drive, numbness, or difficulty in walking suggest neurological complica- tions. Psychiatric disturbances are common and range from mild neurosis to severe organic dementia. They may occur in the absence of anaemia or macrocytosis. Mild jaundice, loss of appetite and weight, indigestion, and episodic diarrhoea are frequent. An inter- current infection may precipitate severe anaemia and thus symp- toms. Older patients may present with congestive heart failure. In a few patients, bruising due to thrombocytopenia is marked. Many symptomless patients are diagnosed because a routine blood test is made. Physical signs, if present, are those of anaemia, perhaps with mild jaundice, giving the patient a so-called lemon-yellow tint. A few patients with deficiency of either vitamin B12 or folate de- velop a widespread brown pigmentation, affecting nail beds and skin creases particularly, but not mucous membranes. This is re- versible with the appropriate therapy. The tongue may be red, smooth, and shiny, occasionally with ulcers. A mild pyrexia up to 38°C is common in patients with moderate to severe anaemia. Table 22.6.6.4 Causes of vitamin B12 deficiency and malabsorption of vitamin B12 Causes of severe vitamin B12 deficiency (a) Nutritional: Vegans Long-continued extremely poor diet (rarely) (b) Malabsorption: Gastric causes: (Addisonian) pernicious anaemia Congenital intrinsic-factor deficiency or abnormality Total and partial gastrectomy Destructive lesions of stomach Intestinal causes: Gut flora (associated with jejunal diverticulosis, ileocolic, fistula, anatomical blind loop, stricture, Whipple’s disease, scleroderma, HIV disease) Ileal resection and Crohn’s disease Chronic tropical sprue Selective malabsorption with proteinuria Fish tapeworm Transcobalamin II deficiency Causes of malabsorption of vitamin B12 usually without severe vitamin B12 deficiency Malabsorption of food vitamin B12 (due to simple atrophic gastritis, gastric bypass, proton pump inhibitors, H. pylori infection); severe chronic pancreatitis, Zollinger–Ellison syndrome, adult gluten-induced enteropathy, giardiasis, HIV disease, graft-versus-host disease Drugs: p-aminosalicylic acid, colchicine, neomycin, slow K, ethanol, metformina, phenformin, anticonvulsants a Recent studies suggest metformin lowers serum vitamin B12 by reducing the level of TCI. Table 22.6.6.5 Causes of folate deficiency Poor diet Especially poverty, psychiatric disturbance, alcoholism, dietary fads, scurvy, kwashiorkor, goats’ milk anaemia Malabsorption Gluten-induced enteropathy (child or adult or associated with dermatitis herpetiformis) Tropical sprue Congenital specific malabsorption Minor factors: jejunal resection, inflammatory bowel disease, systemic infections Drugs: cholestyramine, sulphasalazine, methotrexate, ? others (see

‘Drugs’). Excessive requirements Physiological: Pregnancy Prematurity and infancy Pathological: (a) Malignancies—leukaemia, carcinoma, lymphoma, myeloma, etc. (b) Blood disorders—haemolytic anaemia (especially sickle-cell anaemia, thalassaemia major), myeloproliferative diseases (c) Inflammatory—tuberculosis, malaria, Crohn’s disease, psoriasis, exfoliative dermatitis, rheumatoid arthritis, etc. (d) Metabolic—homocystinuria (some cases) Excess urinary excretion Congestive heart failure, acute liver damage, chronic dialysis Drugs Mechanism uncertain Anticonvulsants (diphenylhydantoin, primidone, barbiturates) ? Alcohol Also drugs causing malabsorption of folate (see ‘Malabsorption’) Liver disease Mixed causes as above, and poor storage

22.6.6 Megaloblastic anaemia and miscellaneous deficiency anaemias 5415 The liver may be enlarged while the cardiovascular system shows changes due to anaemia. Patients with PA may also have features of an associated disorder on presentation, most commonly myxoe- dema. Other thyroid disorders, vitiligo, carcinoma of the stomach (incidence three times that of controls), Addison’s disease, and hypoparathyroidism, may precede, occur simultaneously with, or follow the onset of the anaemia. A few cases of PA with gastric at- rophy, achlorhydria, and IF antibodies have occurred in children. They may show associated autoimmune conditions, for example, myxoedema, hypoparathyroidism, Addison’s disease, or chronic mucocutaneous candidiasis. Neurological complications of vitamin B12 deficiency Vitamin B12 deficiency may cause a symmetrical neuropathy, af- fecting the lower limbs more than the upper, which usually pre- sents with paraesthesiae or with ataxia, particularly in the dark. In some cases, loss of cutaneous sensation, spastic paraparesis, muscle weakness, urinary or faecal incontinence, an optic neuropathy, or psychiatric disturbance dominates. The nervous system disease is due to severe deficiency judged by serum vitamin B12 levels or methylmalonic acid (MMA) excretion, but may occur with mild or no anaemia. A similar neurological syndrome with paraparesis has been described in dentists and others repeatedly exposed to nitrous oxide (N_2O), which inactivates methionine synthase. The biochem- ical explanation for the neurological disease is not clear. A defect in fatty acid metabolism in myelin tissue has been suggested. Studies in N_2O-treated monkeys have also suggested that the neuropathy re- sults from accumulation of S-adenosyl homocysteine (caused by the block in conversion of homocysteine to methionine) with inhibition of transmethylation of biogenic amines, proteins, phospholipids, and neurotransmitters in the spinal cord and brain. Methionine has been shown to prevent the neurotoxicity caused by N_2O in experi- mental animals. General tissue effects of vitamin B12 and folate deficiencies Both deficiencies cause macrocytosis and related cytopathic effects in proliferating epithelial cells throughout the body (e.g. bronchial, bladder, buccal, and uterine cervix), with glossitis and angular cheilosis, a mild malabsorption syndrome, and reduced regen- eration of damaged liver cells. In both sexes, sterility (reversible with vitamin B12 or folate therapy) may result from effects on the gonads. It is possible that the deficiencies in children affect overall body growth. Nutritional vitamin B12 deficiency in infants long term causes failure to thrive and poor brain growth with poor intellectual outcome. Generalized, reversible melanin pigmentation occurs in a few patients with vitamin B12 or folate deficiency, the cause of which is uncertain. Defective bactericidal activity of phagocytes due to im- paired intracellular killing has been described in vitamin B12 but not in folate deficiency. Vitamin B12 deficiency reduces serum concen- trations of the osteoblast-related proteins alkaline phosphatase and osteocalcin, but whether clinically important bone disease occurs is unknown. Neural tube defects Folic acid supplements at the time of conception and in early (first weeks) of pregnancy reduce the incidence of neural tube defects (NTDs) (anencephaly, encephalocoele, and spina bifida) in the first and subsequent pregnancies when such a malformation has occurred

previously. Folic acid fortification of the diet has led to a substantial reduc- tion of incidence of NTDs (e.g. in North America). The explanation for the effect of folic acid on NTDs is not certain. Women carrying affected fetuses have on average lower serum folate and vitamin B12 concentrations and higher serum homocysteine levels than matched controls. There is a linear relationship when plotted on logarithmic scales between the birth incidence of NTDs and maternal red cell folate, indicating that an increase in red cell folate even within normal range is associated with a constant, proportional decrease in the birth frequency of NTDs. Folic acid prevention of NTDs (and in some studies cleft lip and palate), despite apparently normal serum and red cell folate concentrations, suggests that folic acid is over- coming a metabolic abnormality in folate metabolism. Only one such defect, a mutated 5,10 methylene tetrahydofolate reductase (MTHFR) enzyme, has been identified. Periconceptional use of vita- mins or supplements containing folic acid is also associated with a reduced incidence of birth defects associated with maternal diabetes mellitus. Mutated MTHFR, a common thermolabile variant (677C→T) (Ala225Val) is associated with lower serum and red cell folate con- centrations and with higher plasma homocysteine than in control subjects in the general population. The prevalence of the homozy- gous state in the population is approximately 5% and in parents of fetuses with NTDs the prevalence is approximately 13%. The pres- ence of this mutation can therefore account for only a small propor- tion of NTDs. Mutations of other genes (e.g. VANGL1) not related to folate metabolism, have been found in NTD families. Serum vitamin B12 levels are also lower in sera of mothers with NTD infants than in controls. Also, TCII receptor polymorphisms are associated with Table 22.6.6.6 Megaloblastic anaemia not due to vitamin B12 or folate deficiency Abnormalities of vitamin B12 or folate metabolism Congenital: Transcobalamin II deficiency or functional abnormality Inborn errors of folate metabolism, e.g. methylfolate transferase deficiency Homocystinuria and methylmalonic aciduria (some cases) Acquired: Nitrous oxide Dihydrofolate reductase inhibitors: methotrexate, pyrimethamine, trimethoprim, ?pentamidine, triamterene Independent of vitamin B12 or folate Congenital: Orotic aciduria (responds to uridine) Lesch–Nyhan syndrome, ? responds to adenine Thiamine-responsive Some cases of congenital dyserythropoietic anaemia Acquired: Erythroleukaemia, other myeloid leukaemias (some cases) Myelodysplasia Drugs: Antimetabolites: 6-mercaptopurine, cytosine arabinoside, hydroxycarbamide, 5-fluorouracil, azathioprine, etc.

section 22 Haematological disorders 5416 increased risk for NTD. There are, however, no studies showing that vitamin B12 therapy or dietary fortification with vitamin B12 reduces the incidence of NTDs. Mental deterioration There is a more rapid decline in cognitive function in subjects with low serum vitamin B12 levels, serum holotranscobalamin, and raised serum MMA concentrations. Studies suggest that low serum folate levels are not associated with cognitive loss or depression in the eld- erly. Meta-analysis suggests administration of folic acid and vitamin B12 may have a mild effect on memory but does not improve or sta- bilize cognitive function in older people with or without low serum vitamin B12 or folate concentrations. Although low serum vitamin B12 levels and raised serum homocysteine and methylmalonate levels have been reported to be more frequent in subjects with de- mentia, including Alzheimer’s disease, than in controls, trials of vitamin B12 therapy have generally shown no benefit in treating the dementia or slowing its progression. Vitamin B12 has been used to treat chronic fatigue syndrome but there are no controlled trials which validate this. Cardiovascular disease and stroke McCully (1969) first implicated homocysteine as a cause of ath- erosclerosis. This was based on pathological studies of children or young adults with congenital homocystinuria, whether due to a defect of cystathionine

synthase, methionine synthase, or MTHFR (Fig. 22.6.6.5). In these children, plasma homocysteine concentra- tions are raised to 10 to 100 times normal. It is now apparent that milder rises in plasma homocysteine are associated with coronary or peripheral arterial disease, stroke, and deep vein thrombosis. Homocysteine can directly injure endothelial cells, activate platelets and leucocytes, stimulate vascular smooth muscle proliferation, oxi- dize low-density lipoprotein (LDL), and disturb collagen and extra- cellular matrix formation. Determinants of plasma homocysteine include age, sex, renal function, protein intake, vitamin B6, folate, and vitamin B12 status, the presence of the thermolabile variant MTHFR, smoking, and alcohol consumption, as well as intake of various drugs. Folate deficiency assessed by serum or red cell folate or by dietary folate intake is also associated with coronary vascular disease, myo- cardial infarct, and peripheral vascular disease. Meta-analysis of prospective trials shows that a 25% lower starting homocysteine level is associated with 11% lower coronary heart disease risk (and 19% lower stroke risk). There is also an association of the MTHFR homozygous state TT, which is associated with a higher homocyst- eine level in serum than the wild type CC state, and ischaemic heart disease. Meta-analysis of 75 studies showed an increased risk of is- chaemic heart disease in TT compared to CC homozygotes, odds ratio 1.16 (1.04–1.29). However, meta-analysis of 26 randomized control trials enrolling 58 804 participants showed that folic acid supplementation was not associated with a significant change in car- diovascular disease or all-cause mortality, although it was linked to a decreasing trend in stroke risk. This was more marked in popu- lations without mandatory fortification of the diet with folic acid (Yang et al. 2012). Huo et al. (2014) in the most recent meta-analysis of 15 randomized trials (N = 55 764) found an overall reduction in stroke (relative risk 0.92; P = 0.04). This was more marked in those followed for more than 3 years, those without background fortifica- tion of breakfast cereals with folic acid and those not taking statins. It may be relevant that a reduction in incidence of stroke occurred in the United States of America and Canada coinciding with the intro- duction of dietary folic acid fortification, whereas no reduction in incidence occurred over the same period (1998–2002) in England and Wales without fortification. A recent Chinese randomized con- trolled study over 4.5 years has confirmed that folic acid 0.8 mg daily is effective in reducing primary occurrence of ischaemic stroke, par- ticularly in those starting with lower folate levels, with the MTHFR TT mutation and for longer receiving folic acid (Huo et al. 2015). Those with the TT mutation had the highest stroke risk. Wald et al. (2011) have suggested that in trials of prevention of secondary cor- onary disease, aspirin may be negating or reducing the effect of lowering homocysteine and folic acid prophylaxis. On this basis, folic acid would have a role in primary prevention of ischaemic heart disease in those not taking aspirin. Malignancy Positive and negative associations between the occurrence of various types of lymphoblastic or myeloblastic leukaemias in infancy and childhood and polymorphisms of folate-metabolizing enzymes have been reported. Folic acid prophylactically in pregnancy has been reported to reduce the incidence of a subsequent childhood acute lymphoblastic leukaemia and of brain tumours. In Canada, food for- tification with folic acid has been associated with a 60% reduction in incidence of neuroblastoma. Epidemiological studies show an inverse risk of colorectal cancer or adenoma and folate status and a less clear-cut relation exists with other gastrointestinal, lung, breast, ovary, and cervical carcinomas. Also small randomized and nonrandomized trials suggest a benefit of supplemental folic acid on incidence of colorectal cancer. A large randomized trial has shown no difference in overall incidence of co- lonic adenomas in women between controls and subjects receiving folic acid, vitamin B6, and vitamin B12 supplements. It has also been suggested that an increased incidence of colorectal cancer in the United States of America and Canada is associated with the fortification of the diet with folic acid, but the temporal disasso- ciation between fortification and

risk in colorectal cancer incidence Methionine synthase Methionine Homocysteine Cystathionine synthase Cystathionine Tetrahydrofolate 5-Methyl tetrahydrofolate 5,10-Methylene tetrahydrofolate reductase 5,10-Methylene tetrahydrofolate Fig. 22.6.6.5 The role of three enzymes (cystathione synthase, methionine synthase, and MTHFR) and three vitamins (vitamin B12, vitamin B6, and folate) in homocysteine metabolism.

 makes this unlikely—increased detection by screening endoscopy is a more likely explanation. There has been no increase in mortality rate from colorectal cancer in the United States or Canada since for- tification. Most recent analysis has shown no significant effect of folic acid at large doses over prolonged periods on cancer incidence. Meta-analysis of 13 trials carried out before 2011, 10 for cardiovas- cular disease prevention and 3 for colonic adenoma and cancer in- cidence, involving almost 50 000 subjects in the supplemented and control groups and lasting for a mean of 5.2 years, did not show an effect on cancer incidence at doses of folic acid daily of 2 to 5 mg. This was true for individual cancers of breast, prostate, lung, or large bowel, as well as for rarer cancers. No overall increased incidence of cancer was found in the analysis of 14 trials of B vitamin supplemen- tation (some included in the previous study). Other effects Complications of pregnancy that have been ascribed to folic acid, including miscarriage and multiple pregnancy, have no sound basis. Malabsorption of vitamin B12 Congenital deficiency or structural abnormality of intrinsic factor Fewer than 100 cases have been reported of a child being born with absent or nonfunctioning IF due to a mutation of the IF gene. There is an otherwise normal stomach on biopsy and normal secretion of acid. Inheritance is autosomal recessive. In different cases, IF may be present in the gastric juice but susceptible to acid degradation or cannot bind vitamin B12, or binds it but cannot attach it to ileal receptors. These children tend to present with irritability, vomiting, diarrhoea, and loss of weight, and are found to have megaloblastic anaemia. The usual age of diagnosis is about 2 years, although a few have been diagnosed as early as 4 months and others only in their teens. Gastrectomy All patients who have total gastrectomy will develop vitamin B12 deficiency, which usually presents between 2 and 6 years postoperatively. They should be treated with prophylactic vitamin B12 injections from the time of the operation. Iron deficiency usually accounts for the anaemia that occurs after partial gastrectomy. A mi- nority develop megaloblastic anaemia due to vitamin B12 deficiency. In most of these patients, malabsorption of vitamin B12 is due to an abnormal jejunal flora. The exact incidence of vitamin B12 deficiency depends mainly on the size of the gastric remnant. After gastric pli- cation or Roux-en Y surgery for obesity, vitamin B12 deficiency may occur and oral vitamin supplementation is often used. Monitoring for vitamin B12 deficiency is advisable. Small-intestinal lesions Colonization of the upper small intestine with colonic bacteria, if sufficiently heavy as in the stagnant-loop syndrome, leads to mal- absorption of vitamin B12. The most common causes are listed in Table 22.6.6.4. It appears that the bacteria destroy IF. Infestation with the fish tapeworm (Diphyllobothrium latum) has a similar ef- fect but is now almost completely eradicated; infestation is only suf- ficiently marked in Finland and Russian lake regions to suggest a possible cause of megaloblastic anaemia. Resection of 1 m or more of terminal ileum This causes severe malabsorption of vitamin B12. Other diseases that may affect ileal structure and function include tropical sprue, in which severe vitamin B12 deficiency with anaemia or, rarely, neur- opathy is a manifestation only in the chronic phase; gluten-induced enteropathy in which megaloblastic anaemia, if it occurs, is always due to folate deficiency (and vitamin B12 deficiency, if it occurs, is mild); and in Crohn’s disease, malabsorption of vitamin B12 is fre- quent but severe vitamin B12

deficiency is unusual unless there is an ileal resection, fistula, or stagnant loop. Selective malabsorption of vitamin B12 with proteinuria (Imerslund's disease, Imerslund–Gräsbeck syndrome, recessive megaloblastic anaemia, MGA1) (OMIM 261100) This congenital disorder with autosomal recessive inheritance is the most common cause of megaloblastic anaemia due to vitamin B12 deficiency in nonvegan children. The child secretes IF normally but is unable to transport vitamin B12 across the ileum to portal blood. Most Finnish patients with MGA1 carry the disease-specific mu- tation P1297L (FM1) in cubilin. A second less frequent mutation (FM2) activates a cryptic splice site with insertion of multiple stop codons in the CUB6 domain. Other mutations in cubilin have been described. In Norway at least six different mutations of the AMN gene have been reported in affected families. The proteinuria, pre- sent in over 90% of cases, is benign, nonspecific, and persists after vitamin B12 therapy. The clinical presentation of the disease is iden- tical to that of congenital IF deficiency. Other causes of malabsorption of vitamin B12 The most frequent cause of subclinical vitamin B12 deficiency in the United Kingdom and United States of America, shown by a border- line or low serum vitamin B12 level, and normal blood count with or without a raised serum homocysteine and methylmalonate levels, is malabsorption of dietary vitamin B12. This is thought to be due to failure of release of dietary vitamin B12 from its protein binding in food. It is usually due to an atrophic gastritis resulting in reduced pepsin activity. The gastritis may be associated with a positive par- ietal cell antibody test or with H. pylori infection. The incidence is slightly more common in the elderly and the deficiency rarely pro- gresses to megaloblastic anaemia or vitamin B12 neuropathy. Several other conditions and drugs may cause malabsorption of food vitamin B12, for example, proton pump inhibitors which very rarely cause deficiency of clinical severity. Other causes of vitamin B12 malabsorption by unidentified mechanisms include p-aminosalicylate, colchicine, neomycin, and 'slow' potassium tablets. Recent studies suggest the fall in serum vitamin B12 level with metformin is due to a reduced serum level of TCI rather than malabsorption. In chronic pancreatitis and the Zollinger–Ellison syndrome, there is failure to release vitamin B12 from gastric haptocorrin due to absence or inactivation of pancre- atic trypsin. Serum vitamin B12 concentrations fall progressively in untreated HIV-infected patients and subnormal serum values occur in 10 to 35% of individuals with AIDS; increased concentrations of TCII are usual and malabsorption of vitamin B12, not corrected by IF, has been found in some of these patients. An abnormal small-intestinal flora is the most likely cause of the vitamin B12 malabsorption.

section 22 Haematological disorders 5418 Malabsorption of vitamin B12 also occurs in inherited TCII defi- ciency and temporarily after total-body irradiation before stem cell transplantation. In chronic graft-versus-host disease affecting the gut, malabsorption of vitamin B12 is usual, due to the abnormal gut flora as well as to an ileal defect. Irradiation to the ileum during radiotherapy treatment for carcinoma of the cervix has also been reported to cause vitamin B12 malabsorption. Dietary vitamin B12 deficiency This occurs most commonly in vegans. The incidence of overt meg- aloblastic anaemia is much lower than the incidence of subclinical deficiency assessed by the serum vitamin B12 assay. These individ- uals have low vitamin B12 stores. Babies have been born vitamin B12 deficient with megaloblastic anaemia caused by severe vitamin B12 deficiency (due to poor diet or tropical sprue) in the mother. Breast milk may also be vitamin B12 deficient if the mother's stores are low. Dietary deficiency of vitamin B12 also occurs rarely in nonvegetarian people living on inadequate diets because of poverty. Folate deficiency Clinical features The main clinical features of megaloblastic anaemia due to folate deficiency are similar to those seen when the anaemia is due to vitamin B12 deficiency, except that a neuropathy does not occur and the

underlying aetiology tends to be different. Cognitive changes and depression may be caused by the deficiency, and neurological abnormalities do occur with inborn errors of folate metabolism and may be precipitated by antifolate drugs. Folate de- ficiency may develop rapidly in a few months, and although many mildly deficient patients do not progress for months or years, in some patients the deficiency may lead to a severe pancytopenia (‘arrest of haematopoiesis’) over a short period, particularly if an infection supervenes. Nutritional folate deficiency Minor degrees of nutritional folate deficiency are frequent in most countries. Potentially millions of people in northern China, Bangladesh, Burma, Malaysia, Africa, or India have low levels of folate due to a poor dietary intake and nutritional folate deficiency is the main cause of megaloblastic anaemia, often presenting in pregnancy. In many countries—for example, Caribbean islands, Sri Lanka, and South-East Asia—tropical sprue (see Chapter 15.10.8) is an important cause of both deficiencies and is difficult to distinguish from ‘pure’ nutritional deficiency. Severe folate deficiency has been estimated to account for about 17% of all cases of megaloblastic anaemia in the United Kingdom, where it occurs mainly in the context of a poor diet and/or alco- holism. In some cases, barbiturates or consumption of spirits or cough mixtures or a physical abnormality such as rheumatoid arth- ritis, or tuberculosis may aggravate the effect of a poor diet. A few cases have developed because a special diet is taken, such as for phenylketonuria or for slimming. Scurvy is usually accompanied by severe folate deficiency. Goats’ milk anaemia is a nutritional folate deficiency due to the low (6 µg/litre) folate content of goats’ milk. Malabsorption (Also see Diseases of the gastro-intestinal tract described in Section 15.) Gluten-induced enteropathy Folate deficiency due to malabsorption of folates occurs in virtually all untreated patients, the serum folate being subnormal in virtu- ally 100% and red cell folate subnormal in 80% or more. Anaemia occurs in about 90% of adult cases, due to folate deficiency alone in 30 to 50%, and to mixed iron and folate deficiency in the remainder. Mild vitamin B12 deficiency may also occur, but it is not a cause of anaemia in uncomplicated cases. Spontaneous atrophy of the spleen occurs in most of the patients; in about 10 to 15% of cases; the blood film shows the presence of Howell–Jolly bodies, and other features of hyposplenism. A gluten-free diet produces a spontaneous rise in serum and red cell folate in those patients who respond. In chil- dren with gluten-induced enteropathy, anaemia is most often due to combined iron and folate deficiency. Patients with dermatitis herpetiformis almost all show some degree of gluten-induced duo- denal and jejunal abnormality; the severity of folate malabsorption and deficiency correlates with the severity of the intestinal lesion. Tropical sprue (Also see Chapter 15.10.8). Malabsorption of folate occurs in all severe, untreated patients in the acute phase and megaloblastic an- aemia due to folate deficiency may develop within a few months. Not only does the anaemia respond to folate therapy but in many pa- tients all the clinical features, and malabsorption of fat, vitamin B12, and other substances, improves on folate therapy alone. In the first year, about 60% of patients appear to be cured by folic acid alone. Long-standing cases are more likely to be vitamin B12 deficient and thus to require vitamin B12 as well as folate and antibiotic therapy. Congenital specific malabsorption of folate This is a rare, autosomal recessive abnormality. Affected children show features of damage to the central nervous system (mental re- tardation, fits, athetotic movements) and present with megaloblastic anaemia responding to physiological doses of folic acid given paren- terally but not orally. Folate levels in cerebrospinal fluid are low. It is due to inherited mutations of the proton-coupled folate transporter (PCFT) affecting protein stability or its membrane trafficking. Other causes Absorption of folate is impaired by systemic infections. Mild de- grees of folate malabsorption have also been reported after jejunal resection or partial gastrectomy, with Crohn’s disease, and with lymphoma. In the intestinal stagnant-loop syndrome, folate levels tend to be high due to absorption of bacterially produced

folate. Alcohol, anticonvulsants, oral contraceptives, antituberculous drugs, nitrofurantoin, and sulfasalazine have been suggested, on variable evidence, to cause malabsorption of folate in some subjects but none is definitely established except sulfasalazine. Increased folate utilization A general mechanism of increased folate utilization in conditions of increased cell turnover has emerged. This consists of partial degrad- ation of folate at the C9–N10 bond rather than complete recycling of the folate coenzymes required in DNA synthesis. Pregnancy This, associated with poor nutrition, is probably the most common cause of megaloblastic anaemia world-wide, unless folic acid

22.6.6 Megaloblastic anaemia and miscellaneous deficiency anaemias 5419 supplements are taken. The frequency of the anaemia was about 0.5% in most Western cities and up to 50% in some areas of Asia and Africa until the introduction of prophylactic folic acid. The incidence increases with parity and is higher in twin pregnan- cies. Folate requirements in a normal pregnancy are increased to about 300 to 400 µg daily. Serum and red cell folate tend to fall as pregnancy progresses, and to rise spontaneously about 6 weeks after delivery. Lactation may prove an additional cause of folate deficiency, however, which may precipitate megaloblastic anaemia postpartum. The cause of the deficiency in pregnancy is increased degrad- ation of folate. Folate transfer to the fetus may play a minor part; in a few, megaloblastic anaemia of pregnancy is the first sign of gluten- induced enteropathy. The statistical association of iron and folate de- ficiencies in pregnancy is probably due to a poor quality of the diet in certain women. Prophylactic folic acid should now be given routinely in preg- nancy; 400 µg/day is recommended (mentioned previously) and intake in women who may become pregnant should be at least this amount daily from food or supplements. Larger doses (4– 5 mg/day) should be used if there has been a previous infant with an NTD. Prematurity Newborn infants have higher serum and red cell folate concentra- tions than adults. These fall to a nadir at about 6 weeks of age. In pre- mature infants, the decline is particularly steep and megaloblastic anaemia may develop, particularly if infections, feeding difficulties, or haemolytic disease with exchange transfusion have occurred. Prophylactic folic acid (e.g. 1 mg/week for the first 3–4 weeks of life) may be given, particularly to those babies weighing less than 1.5 to 1.8 kg at birth. Malignant diseases Mild folate deficiency is frequent in patients with cancer (Table 22.6.6.5). In general, the severity correlates with the extent and degree of dissemination of the underlying disease. However, pa- tients with megaloblastic anaemia due to folate deficiency are un- usual and, supplementation tends to be avoided in the absence of a categorical indication for its use (e.g. anaemia, leucopenia, etc.) due to concerns regarding promoting tumour growth. Blood disorders Chronic haemolytic anaemia Requirements for folate are in- creased in patients with increased erythropoiesis, particularly when there is ineffective erythropoiesis with a high turnover of primitive cells. Occasional patients, presumably those with a poor folate in- take, develop megaloblastic anaemia, particularly in sickle cell anaemia, thalassaemia major, hereditary spherocytosis, and warm- type autoimmune haemolytic anaemia; prophylactic folic acid is usually given in these disorders. Primary myelofibrosis Megaloblastic haematopoiesis has been reported in as many as one-third of patients. Circulating meg- aloblasts, increased transfusion requirements, severe thrombo- cytopenia, or pancytopenia may be the first indication that folate deficiency has developed. Polycythaemia vera is not a typical cause of folate deficiency. Inflammatory diseases Folate deficiency has been described in patients with tubercu- losis, malaria, Crohn’s disease, psoriasis, widespread eczema, and rheumatoid arthritis. The degree of deficiency is related to the extent and severity of the underlying disorder. Increased demand for folate probably is a factor but reduced appetite is also important in those who develop

megaloblastic anaemia. Metabolic Homocystinuria Patients with the most common form of this dis- order, due to cystathionase deficiency, may show folate deficiency, possibly due to excess conversion of homocysteine to methionine and thus excess utilization of the folate coenzyme concerned (see Chapter 12.2). Excess urinary loss of folate Urine folate excretion of 100 µg a day or more occurs in some pa- tients with congestive cardiac failure or active liver disease causing necrosis of liver cells. It is presumed that losses are due to release of folate from damaged liver cells. Haemodialysis and peritoneal dia- lysis remove folate from plasma. Folic acid (e.g. 5 mg/week) is now usually given prophylactically to patients with renal failure who re- quire long- term dialysis. Drugs Dihydrofolate reductase inhibitors Methotrexate, aminopterin, pyrimethamine, and trimethoprim all inhibit dihydrofolate reductase (DHFR) but have different rela- tive activities against the human, malarial, and bacterial enzymes. Methotrexate is converted to polyglutamate forms, which increases its activity against DHFR and also increases its retention in cells. These methotrexate derivatives invariably impair human folate me- tabolism. Trimethoprim, used as an antibacterial agent, may aggra- vate pre-existing folate or vitamin B12 deficiency but does not in itself cause megaloblastic anaemia. Alcohol Folate deficiency may occur in alcoholism. The main factor is poor nu- trition and it is likely that alcohol interrupts the enterohepatic circu- lation for folate. It also has a direct effect on haematopoiesis, causing vacuolation of normoblasts, impaired iron utilization, sideroblastic changes, macrocytosis, megaloblastosis, and thrombocytopenia, even in the absence of folate deficiency. Beer drinkers usually avoid folate de- ficiency because of the high folate content of beer. The usual red cell macrocytosis in nonanaemic alcoholics is not related to folate deficiency. Anticonvulsants and barbiturates Diphenylhydantoin, primidone, and barbiturate therapy may be as- sociated with folate deficiency. The more severe deficiency is associ- ated with poor dietary intake of folate and prolonged drug therapy at high doses. The mechanism of the deficiency is unknown and double-blind trials have shown no effect of folic acid supplementa- tion on the frequency of seizures. Other drugs Nitrofurantoin, triamterene, proguanil, and pentamidine have been reported to cause folate deficiency.

 Liver disease Folate deficiency occurs most commonly in alcoholic cirrhosis where alcohol, poor nutrition, and release of stored folate with ex- cess urine losses may all be important. The deficiency is less frequent in other types of liver disease. Laboratory investigation of megaloblastic anaemia This consists of three stages: (1) recognition that megaloblastic an- aemia is present; (2) distinction between vitamin B12 or folate de- ficiency (or rarely some other factor) as the cause of the anaemia; and (3) diagnosis of the underlying disease causing the deficiency (Table 22.6.6.7). Recognition of megaloblastic anaemia Peripheral blood The mean corpuscle volume (MCV) is raised to between 100 and 140 fl. Oval macrocytes are seen in the blood film. In mild cases, macrocytosis is present before anaemia has developed. Cabot rings (composed of arginine-rich histone and nonhaemoglobin iron) and occasional Howell–Jolly bodies (DNA fragments) may occur due to extramedullary haematopoiesis in the liver and spleen. The MCV may be normal if there is associated iron deficiency, when the blood film appears dimorphic, or if the anaemia (usually due to folate de- ficiency or antimetabolite drug therapy) develops acutely over the course of a few weeks. The MCV is also normal in some severely an- aemic cases involving excess red cell fragmentation. The reticulocyte count is low for the degree of anaemia, usually of the order of 1 to 3%. The peripheral blood also shows hypersegmented neutrophils (which have nuclei with more than five lobes; Fig. 22.6.6.6) and the leucocyte count is often moderately reduced in both neutrophils and lymphocytes, although the total leucocyte count rarely falls to less than 1.5 × 109/litre. The platelet count may be moderately

reduced but rarely falls below 40 × 109/litre. Biochemical changes These are confined to the anaemic patient and include a mild rise in serum bilirubin (up to 50 µmol/litre), mainly unconjugated, a rise in serum lactic dehydrogenase of up to 10 000 IU/litre. The serum iron and ferritin are also raised and fall with effective treatment. The serum cholesterol is low and alkaline phosphatase mildly reduced. Absence of haptoglobins is usual. In severe cases, free haemoglobin may be present in plasma, Schumm's test for methaemalbumin in serum is positive, and haemosiderin and fibrin degradation prod- ucts are present in urine. The direct antiglobulin test is weakly posi- tive in some patients, due to complement. Bone marrow The bone marrow is hypercellular in moderate or severely anaemic cases. The myeloid–erythroid ratio is often reduced or reversed. The erythroblasts are larger than normal and show asynchronous mat- uration of nucleus and cytoplasm, nuclear chromatin remaining primitive with an open, lacy, fine granular pattern despite normal maturation and haemoglobinization of the cytoplasm. Excessive numbers of dying cells and nuclear remnants including Howell– Jolly bodies, mitoses, and multinucleate cells may be present. Because of death (by apoptosis) of later cells, there is a dispropor- tionate accumulation of early cells. Giant and abnormally shaped metamyelocytes and megakaryocytes with hypersegmented nuclear lobes are also usually present (Fig. 22.6.6.7). Table 22.6.6.7 Laboratory diagnosis of megaloblastic anaemia

1. General tests Peripheral blood film and count Bone marrow Serum bilirubin, iron, lactate dehydrogenase
2. Tests for vitamin B12 or folate deficiency Serum vitamin B12 and folate; red cell folate Serum homocysteine and methylmalonic acid levels
3. Tests for cause of vitamin B12 or folate deficiency Vitamin B12 deficiency: Serum antibodies to parietal cell, intrinsic factor Serum gastrin Gastric secretion; intrinsic factor, acid Endoscopy, gastric biopsy Upper gastrointestinal endoscopy Proteinuria, fish tapeworm ova, intestinal flora, etc. Folate deficiency: Transglutaminase, endomysial antibodies Small-intestinal function Duodenal biopsy Barium follow-through Tests for many underlying conditions Fig. 22.6.6.6 Megaloblastic anaemia. Hb 40 g/litre MCV 120 fl. Hypersegmented neutrophil, oval macrocytes, and a small lymphocyte to show size of macrocytes. The fragmentation of advanced megaloblastosis is present. Thrombocytopenia is marked. Fig. 22.6.6.7 Megaloblastic anaemia. Bone marrow aspirate showing megaloblasts at different stages and giant metamyelocytes.

 The severity of these changes parallels the degree of anaemia. In milder cases, changes, described as 'intermediate', 'transitional', or 'moderate', are principally in the size and nuclear chromatin pattern of the erythroblasts, with giant metamyelocytes present; hypercellularity and gross dyserythropoiesis may be absent. In very mild cases, megaloblastic changes are difficult to recognize. In pa- tients with severe anaemia but only mild megaloblastic changes, some additional cause for the anaemia should be sought. Chromosomes Changes found in marrow and other proliferating cells include (1) random chromatin breaks; (2) exaggeration of centromere con- striction; and (3) thin, elongated, uncoiled chromosomes. Ineffective haematopoiesis The increased cellularity of the marrow with degenerate forms, and the low reticulocyte count suggest that many developing cells are dying in the marrow. This occurs by apoptosis, especially of late erythroblasts. The raised unconjugated serum bilirubin, lactic de- hydrogenase, and lysozyme are all due to ineffective haematopoiesis. Differential diagnosis Other causes of macrocytosis include a high reticulocytosis

(e.g. haemolytic anaemia or regeneration of blood after haemorrhage), aplastic anaemia, red cell aplasia, liver disease, alcoholism and myxoedema, the myelodysplastic syndromes, myeloid leukaemias, cytotoxic drug therapy, chronic respiratory failure, plasma cell mye- loma, and other paraproteinaemias. If a bone marrow biopsy has been performed, the principal differentiation is from other causes of megaloblastosis, particularly myelodysplasia. Other causes of meg- aloblastic anaemia not due to vitamin B12 or folate deficiency are listed in Table 22.6.6.6. Some patients with rapidly developing megaloblastic anaemia, particularly due to folate deficiency, may develop almost complete aplasia of the red cell series, and the peripheral blood and bone marrow may resemble that of acute myeloid leukaemia. Diagnosis of vitamin B12 or folate deficiency The peripheral blood and bone marrow appearances are identical in folate or vitamin B12 deficiency. Special tests are, therefore, needed to distinguish between the two deficiencies. Vitamin B12 deficiency The assay of serum vitamin B12 content of serum is by competi- tive binding of intrinsic factor and immunochemiluminescence- based assays. The reference range, depending on the assay, is from 160 to 200 mg/litre to 960 to 1200 ng/litre. Some report in pmol/ litre (1 pmol = 1.355 ng/litre). It is difficult to determine a normal range and each laboratory should establish this independently. The concentrations are low in vitamin B12 deficiency, being extremely low in patients with neurological disease. Unfortunately, using competitive-binding assays, false-normal results have been reported in some patients with untreated pernicious anaemia and intrinsic factor antibodies in serum, which interfere with the test. Subnormal serum vitamin B12 concentrations in the absence of tissue vitamin B12 deficiency have been reported in pregnancy, in inherited mu- tations of TCI (haptocorrin), in severe nutritional folate deficiency, in subjects taking large doses of vitamin C, and occasionally in iron deficiency. In the elderly, low serum vitamin B12 concentrations usu- ally in the range 100 to 200 ng/litre may occur in the absence of an- aemia or macrocytosis. In some research studies, serum holo-TCII levels have been meas- ured to diagnose vitamin B12 deficiency. Raised serum vitamin B12 concentrations, if not due to therapy, are most commonly caused by a rise in TCI as in a leucocytosis due to a myeloproliferative disease. Raised haptocorrin also occur in as- sociation with some tumours, especially hepatoma and fibrolamellar tumour of the liver. In benign leucocytosis, the rise is mainly of TCIII and this is often not accompanied by a high serum vitamin B12. Raised serum TCII concentrations occur in conditions where macrophages are stimulated, for example, autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, in Gaucher disease, and in some monocytic or monoblastic leukae- mias, and in inflammatory bowel disease. In active liver diseases, vitamin B12 leaks from the liver with saturation of the serum vitamin B12 binders. A second and less widely used test for vitamin B12 deficiency is serum MMA. Serum MMA levels are raised in vitamin B12 defi- ciency but not in folate deficiency. Raised levels may also occur in renal failure. Rare cases of congenital methylmalonic aciduria have been described, due to a variety of enzyme defects. A sensitive method of measuring MMA in serum was introduced and combined with serum homocysteine assay for the diagnosis of vitamin B12 or folate deficiency. The minor increases in serum MMA concentration found particularly in older people in the absence of macrocytosis or anaemia, with or without borderline vitamin B12 concentrations, may suggest ‘biochemical’ vitamin B12 deficiency which does not progress to megaloblastic anaemia, but the normal ranges for MMA for different age groups are difficult to determine. Randomized trials are needed to assess the value of preventing or treating putative vitamin B12 deficiency in these subjects. Folate deficiency Direct tests include the serum and red cell folate assay. The serum folate is always low (<3 µg/litre, 7 nmol/litre) in folate deficiency (and is normal or raised in vitamin B12 deficiency unless folate de- ficiency is also present). Raised levels occur after folate therapy

and also in vitamin B12 deficiency and in the stagnant-loop syndrome. Red cell folate is not now recommended for routine diagnostics but may be useful in some patients with probable folate deficiency in whom the serum folate is found to be normal. It is low in a propor- tion of patients with megaloblastic anaemia solely due to vitamin B12 deficiency. Serum homocysteine levels are usually raised in folate and vitamin B12 deficiency and many other situations. Diagnosis of the cause of vitamin B12 deficiency Although the clinical and family history and the clinical findings may point to PA or some other cause of vitamin B12 deficiency, it is important to establish this for certain. A brief dietary history will rapidly establish whether or not the patient is a vegan or takes a very inadequate diet. Endoscopy and gastric biopsy will show features of gastric atrophy and help to exclude gastric carcinoma. Follow- through radiographic examination of the small intestine will help to exclude a small-intestinal lesion (e.g. duodenal or jejunal diver- ticulosis). The serum gastrin concentration is raised in patients with PA and the serum is tested for antibodies to IF, parietal cells, and

section 22 Haematological disorders 5422 thyroid; serum immunoglobulins are measured in view of the asso- ciation with hypogammaglobulinaemia. Diagnosis of the cause of folate deficiency An inadequate diet is usually at least partly implicated, but an exact estimate of dietary intake from the clinical history is impossible be- cause of variation in folate content of foods, losses in cooking, and size of portions. Often it is the general social circumstances that sug- gest a poor intake. Drug intake, particularly of barbiturates, is im- portant. Many underlying inflammatory or malignant diseases may exaggerate the tendency to folate deficiency in patients with inad- equate diets. The main cause of malabsorption of folate is gluten- induced enteropathy; in patients with severe folate deficiency, tests for transglutaminase and endomysial antibodies and a duodenal bi- opsy are usually necessary. In certain tropical countries, sprue may cause a generalized malabsorption syndrome in which folate defi- ciency commonly occurs. Treatment of megaloblastic anaemia Therapy is aimed at correcting the anaemia, completely replenishing the body of whichever vitamin is deficient, treatment of the under- lying disorder, and prevention of relapse. In most cases, it is possible to diagnose which deficiency is present before starting therapy. Vitamin B12 deficiency Hydroxocobalamin 1 mg intramuscularly given six times at several days' interval over the first few weeks will restore normal vitamin B12 stores. There is no evidence that patients with vitamin B12 neur- opathy derive greater benefit from more frequent doses, although many physicians use these for 6 months or so. Response to therapy The patient feels better within 24 to 48 h, and the mild fever, if not due to infection, abates. A painful tongue and an uncooperative, dis- orientated state may also be improved in 48 h. The white cell count becomes normal by 3 to 7 days and the platelet count rises and may reach levels of 500 to 1000 × 109/litre before falling to normal at about 10 to 14 days. The bone marrow reverts to normoblastic by 36 to 48 h, although giant metamyelocytes persist for 10 to 12 days. The neuropathy always improves with therapy but residual deficits remain in some patients; this applies usually to those with the longest histories or the most severe manifestations, particularly where there is subacute combined degeneration of the spinal cord and spastic paraparesis. Maintenance Hydroxocobalamin, 1 mg intramuscularly, is given once every 3 months for life in PA and most other causes of vitamin B12 de- ficiency to prevent relapse. The life expectancy in PA once treated is as good as that in the general population in women, and slightly lower in men, probably due to the increased incidence of carcinoma of the stomach. In a few patients with vitamin B12 deficiency, the underlying cause can be reversed; for example, expulsion of the fish tapeworm, improvement of vegan diet, surgical correction of an in- testinal stagnant loop. A few micrograms of vitamin B12 can be ab- sorbed each day in PA from

oral doses of 1 mg or more by passive diffusion, but this maintenance therapy is usually reserved for those who cannot have injections—for example, those with a bleeding dis- order, or who refuse them—and for the extremely rare individual who is allergic to all injectable forms of vitamin B12. Vegans may be maintained on much smaller oral doses of vitamin B12 each day, such as 50 µg as a tablet or syrup. Prophylaxis Vitamin B12 therapy should be given from the time of operation after total gastrectomy or ileal resection. Patients with PA tend to develop iron deficiency anaemia and they may also develop thyroid disorders or carcinoma of the stomach. It is advisable that a regular blood count be made once a year. Routine endoscopy is not war- ranted but these diseases must be particularly borne in mind if rele- vant symptoms or signs develop. It is unclear whether vitamin B12 should be given orally or par- enterally to those with biochemical (subclinical) vitamin B12 de- ficiency without anaemia or macrocytosis or clinical symptoms. Trials are needed to clarify this. Folate deficiency This is corrected by giving 5 mg folic acid by mouth daily. It is essen- tial to first exclude vitamin B12 deficiency so that precipitation of a neuropathy is avoided. It is usual to continue for at least 4 months until there is a completely new set of red cells, although body stores will theoretically be normal within a few days of therapy. In patients with severe malabsorption of folate, larger oral doses of folic acid (e.g. 5 mg three times a day) may be used but it is not necessary to give parenteral folate except for those unable to swallow tablets. The response to therapy is as described for vitamin B12. The decision whether or not to continue folic acid beyond 4 months depends on whether or not the cause can be corrected. In practice, long-term folic acid is usually needed only in patients with severe haemo- lytic anaemias (e.g. sickle cell anaemia and thalassaemia major), myelofibrosis, and in gluten-induced enteropathy when a gluten- free diet is either unsuccessful or not feasible. Prophylactic folic acid This should be given to all pregnant women to prevent megaloblastic anaemia and reduce the incidence of NTDs (doses of 300–400 µg/ day are used). Only 19% of women in a Northern Ireland study had taken folic acid preconception, resulting in a lower red cell folate and thus increased risk of NTDs, during the first trimester, than in women who had taken preconception folic acid. Studies in the United States of America show that black and Hispanic women have a lower dietary intake of folate than white non-Hispanic women and, therefore, particularly need folic acid supplements periconception. Doses of 5 mg/day would have a greater effect but currently need a medical prescription in the United Kingdom. They are given if there has been a previous infant with an NTD. Folic acid is given to pa- tients undergoing regular haemodialysis or peritoneal dialysis, to premature infants weighing less than 1.5 kg at birth, and to selected patients in intensive care units or receiving parenteral nutrition. In young children exposed to a high risk of malaria, combined iron and folic acid supplements may be harmful and should be avoided. Folate therapy has been shown to improve chromosomal stability in the fragile X syndrome, even though these patients do not have folate deficiency or a demonstrable defect of folate metabolism.

22.6.6 Megaloblastic anaemia and miscellaneous deficiency anaemias 5423 Food fortification Mandatory fortification of cereals and grains with folic acid (140 µg/ 100 g cereal grain) aimed at reducing the incidence of NTDs began in the United States of America in 1998 and is now also practised in Canada, Chile, and 70 other countries amounting to about 20% of the world’s population. Median serum folate in clinical specimens in United States of America rose from 12.6 to 18.7 µg/litre between 1997 and 1998. There was also a fall in serum homocysteine levels. The theoretical side effects of fortification are largely in patients with unsuspected vitamin B12 deficiency who, it has been suggested, might present with neuropathy if the extra folate consumed prevents the development of anaemia due to vitamin B12 deficiency. However, the small amounts

of folic acid with each meal would be largely if not entirely converted to methyl-THF by the small intestine and this folate is not able to affect haematopoiesis in vitamin B12 deficiency. In keeping with this, there is no evidence for an increased incidence of nonanaemic subjects with low serum vitamin B12 levels in the United States of America since fortification. In the United Kingdom, fortification of flour with folic acid (240 µg/100 g flour) has been re- commended but not implemented. As discussed previously, there is no evidence that fortification would affect the incidence in the population of any type of cancer. Fortification of grain with vitamin B12 has also been suggested to reduce the incidence of NTDs, but this has not been implemented in any country. Folinic acid (5-formyl-THF) This reduced folate is used to prevent or treat toxicity due to metho- trexate or other dihydrofolate reductase inhibitors. Severely ill patients Some patients, usually elderly, are admitted to hospital severely ill with megaloblastic anaemia, perhaps in congestive heart failure or with pneumonia. In this case, it is necessary to commence therapy immediately after obtaining blood for vitamin B12 and folate assay, before it is known which deficiency is present. Both vitamins should be given simultaneously in large doses. Heart failure and infection should be treated in conventional fashion but blood transfusion should be avoided, except in cases of extreme anaemia, when 1 to 2 units of packed cells may be given slowly. Other therapy Hypokalaemia has been reported to occur during initial therapy but is, rarely, if ever, clinically important. An attack of gout has been reported on the days 6 to 7 of therapy. Most patients develop hyperuricaemia at this stage but the clinical disease probably only occurs in those with a strong gouty tendency. Iron deficiency com- monly develops in the first few weeks of therapy and this should be treated initially with oral ferrous sulphate in the usual way. Megaloblastic anaemia due to inborn errors of folate or vitamin B12 metabolism Folate A number of babies have been described with congenital de- ficiency of one or other enzyme concerned in folate metab- olism: 5-methyltetrahydro-folate, methylene THF-reductase, FIGLU-transferase, methenyl-THF cyclohydrolase. Some of the ba- bies had multiple congenital defects including the heart and cerebral ventricles and nearly all showed impaired mental development. In the methylfolate transferase deficiency, megaloblastic anaemia was present. Vitamin B12 Congenital deficiency of TCII was first reported as an autosomally recessive disease in 1971 in two siblings who developed megalo- blastic anaemia requiring therapy with large daily doses of vitamin B12 at 3 and 5 weeks of age. Similarly affected families have been de- scribed. A spectrum of mutations in the gene for TCII have been de- tected. In some cases, TCII is undetected; in others, often presenting later in life, functionally inactive TCII has been detected. The serum vitamin B12 concentration is normal, vitamin B12 being bound to TCI. Absorption of vitamin B12 is impaired. Treatment is with mas- sive doses of vitamin B12 (e.g. 1 mg intramuscularly, three times each week). Delay in treatment may allow a neuropathy to occur. In con- trast, in subjects with rare, inherited, mutations of TCI, low serum vitamin B12 concentrations occur, but haematopoiesis is normal. Children with one form of congenital methylmalonic aciduria, which responds to vitamin B12 therapy in large doses, have been shown to have a defect in conversion of hydroxocobalamin to adocobalamin. They do not show megaloblastic anaemia. In a few, this defect has been associated with a defect of formation of methylcobalamin and with homocystinuria, but some of the chil- dren have also surprisingly not shown megaloblastic anaemia. Neurological abnormalities are usual. Homocystinuria and meg- aloblastic anaemia without methylmalonic aciduria have also been reported. In some cases, the defect appears to be in maintaining vitamin B12 bound to methionine synthase in the reduced state. Megaloblastic anaemia due to acquired disturbances of folate or vitamin B12 metabolism Folate Therapy with dihydrofolate reductase inhibitors may cause megalo- blastic anaemia. This is usual with methotrexate and less likely with pyrimethamine

unless high doses are used or the patient is already folate deficient. Trimethoprim and triamterene are very weak folate antagonists in humans, but may precipitate megaloblastic anaemia in patients already B12 or folate deficient (mentioned earlier). Vitamin B12 Nitrous oxide (N2O) This anaesthetic gas oxidizes vitamin B12 from the active fully reduce cob(I)alamin form to the inactive cob(II)alamin and cob(III)alamin forms, inactivating methylcobalamin and hence methionine synthase. Megaloblastosis develops within several hours in humans. This recovers over several days when exposure to N2O is discontinued. After many weeks of exposure to N2O, monkeys develop a neuropathy resembling vitamin B12 deficiency neuropathy in humans; peripheral neuropathies and more severe neurological disease have also been described in humans (e.g. dentists and anaesthetists) repeatedly ex- posed to the gas. When N2O is used as anaesthetic for patients with low vitamin B12 stores, megaloblastic anaemia or neuropathy may

section 22 Haematological disorders 5424 be precipitated months later, due to failure to replenish vitamin B12 stores by absorption. Recovery from N2O exposure needs new co- balamin and also synthesis of new apoenzyme (methionine synthase) because this protein is also damaged by active oxygen derived from the N2O–cobalamin reaction. Methylmalonic aciduria has not been found in animals or humans exposed for short periods to N2O, as methylmalonic CoA mutase does not need reduced B12. Megaloblastic anaemia not due to folate or vitamin B12 deficiency or metabolic defect Congenital Orotic aciduria This is a very rare, recessive disorder involving two consecutive en- zymes (orotidsylic pyrophosphatase and orotidylic decarboxylase) in pyrimidine synthesis and presents with megaloblastic anaemia in the first few months of life. The diagnosis is made if needle-shaped, colourless crystals of orotic acid are found in the urine, daily ex- cretion ranging from 0.5 to 1.5 g. Heterozygotes excrete slightly raised amounts of orotic acid but show no haematological disorder. Treatment with uridine (1–1.5 g/day) leads to a haematological re- sponse, restoration of normal haematopoiesis and growth, and re- duction in orotic acid excretion. Lesch–Nyhan syndrome A few patients with this rare disorder of purine synthesis have shown megaloblastic change but whether this was due to associated folate deficiency or a direct result of reduced purine synthesis is not certain (see Chapter 12.4). Vitamin E deficiency This has been reported to cause megaloblastosis in a group of chil- dren with kwashiorkor. However, many were also folate deficient. Vitamin C deficiency Megaloblastic appears to be due to associated folate deficiency. Thiamine responsive About 12 cases have been well documented. They have also shown sideroblastic change and a defect in phosphorylation of thiamine has been implicated. Diabetes mellitus and sensorineural deafness are additional features. There is a fault in thiamine phosphorylation due to a genetic defect of the phosphorylase enzyme. Responding to large doses of vitamin B12 and folate A single patient has been reported who needed both vitamins in large doses, but the site of the defect was not elucidated. Congenital dyserythropoietic anaemia Some cases of congenital dyserythropoietic anaemia show megalo- blastic changes not due to vitamin B12 or folate deficiency. Acquired Megaloblastic changes are often marked in acute myeloid leukaemia and less commonly in other forms of acute myeloid leukaemia. They also occur in the myelodysplastic syndromes. Drugs that directly inhibit purine or pyrimidine synthesis (e.g. cytosine arabinoside, 5-fluorouracil, hydroxycarbamide, 6-mercaptopurine, or azathioprine and azidothymidine (AZT)) may cause megaloblastic anaemia. Alcohol has also been found to have a direct effect on the bone marrow, causing megaloblastosis in some cases even in the absence of vitamin B12 or folate deficiency. On the other hand, drugs that inhibit mitosis (e.g. colchicine or daunorubicin) or alkylate preformed DNA (e.g. cyclophosphamide, chlorambucil, or busulfan) do not cause megaloblastosis. Other deficiency anaemias Vitamin C Anaemia is usual in scurvy but the

pathogenesis is complicated. It is likely that vitamin C has a direct effect on erythropoiesis but folate and iron deficiencies, haemorrhage, or haemolysis often complicate the picture. Biochemical and nutritional aspects Vitamin C is needed for collagen synthesis by its involvement in the hydroxylation of protein and for maintenance of intercellular substance of skin, cartilage, periosteum, and bone. It may also have a general role in oxidation–reduction systems, for example, gluta- thione, cytochromes, pyridine, and flavin nucleotides. Although vitamin C is also thought to be needed for maintaining body fol- ates in the reduced active state, the exact reactions involved are unclear. Vitamin C has a particular role in iron metabolism, iron excess causing increased utilization of vitamin C and in extreme cases clinical scurvy, whereas iron deficiency is associated with a raised leucocyte ascorbate concentration. Vitamin C is needed for incorporation of iron from transferrin into ferritin and for iron mobilization from ferritin. Vitamin C therapy increases iron excre- tion in patients receiving subcutaneous desferrioxamine infusions and also, at least in experimental animals, affects iron distribution by increasing parenchymal relative to reticuloendothelial iron. Minimum adult daily requirements for vitamin C are about 10 mg but 30 to 70 mg is recommended; utilization, and therefore require- ments, are relatively higher in infants, children, and pregnant and lactating women. Vitamin C may be excreted as such but is also broken down to oxalate. Vitamin C is present in food as its reduced (ascorbic acid) and oxidized (dehydroascorbic acid) forms, the highest concentrations occurring in green vegetables, fruits, liver, and kidney. Potatoes are not a rich source but provide a substantial proportion of normal dietary intake. Cooking, particularly in alkaline conditions with large volumes of water, destroys the vitamin, which is also lost on storage with exposure to the air. Absorption occurs through the length of the small intestine and deficiency is never solely due to malabsorption. The anaemia of scurvy is typically normochromic, normocytic with a slightly raised reticulocyte count (to 5–10%) and a normoblastic marrow with erythroid hyperplasia. This suggests a direct role for vitamin C in erythropoiesis but not all patients with clinical scurvy are anaemic. Extravascular haemolysis with mild jaundice and increased urobilinogen excretion occurs in many of the patients. Moreover, in many the anaemia is complicated by folate deficiency (due to inadequate folate intake) with a megaloblastic

 marrow, or in a few by iron deficiency due to external haemorrhage, reduced diet intake, and possibly reduced iron absorption. In a few patients placed on a low-folate diet, response of megaloblastic haematopoiesis to vitamin C alone has been described. In others, response of the megaloblastic anaemia to folic acid alone on a diet low in vitamin C has occurred; but in most such cases, both vitamin C and folic acid have been found necessary. Vitamin B6 This, as its coenzyme form pyridoxal-5-phosphate, is involved in many reactions of the body, especially transaminases and decarboxylases. It is also a cofactor in the important rate-limiting reaction in haem synthesis, δ-aminolaevulinic acid (ALA)-synthase. It occurs in natural tissues in three major forms: pyri- doxine, pyridoxamine, and pyridoxal phosphate. Red cells are capable of interconverting them. Anaemia due purely to vitamin B6 deficiency has been produced in animals. It is hypochromic and microcytic with a raised serum iron and increased iron in erythroblasts, with some partial or complete ring sideroblasts. A similar anaemia has occurred in humans with malabsorption, pregnancy, or haemolysis but has not been fully documented to respond to physiological doses of vitamin B6 alone. Vitamin B6-responsive anaemia is, however, well documented among patients with sideroblastic anaemia of all types. Pyridoxine re- sponses occur particularly in the inherited form (when it is as- sumed that a fault in one or other enzyme of haem synthesis, e.g. ALA-synthase, increases the need for pyridoxal phosphate as cofactor) and when sideroblastic anaemia occurs in patients receiving pyridoxine

antagonists, such as antituberculous drugs. The value of pyridoxine dietary supplements in lowering serum homocysteine and reducing the incidence of cardiovascular dis- ease has yet to be proven. Riboflavin On the basis of studies in experimental animals and humans fed a deficient diet together with a riboflavin antagonist, deficiency of this vitamin is known to cause a normochromic, normocytic an- aemia associated with a low reticulocyte count and red cell aplasia in the marrow, sometimes with vacuolated normoblasts. The exact biochemical basis is undecided. Clinically, a similar anaemia may occur in pure form but is usually associated with the anaemia due to protein deficiency, as in kwashiorkor or marasmus. Other clinical features of riboflavin deficiency—dermatitis, angular cheilosis, and glossitis for example—may be present. Thiamine For discussion, see under megaloblastic anaemia not due to folate or vitamin B12 deficiency or metabolic defect. Nicotinic acid, pantothenic acid, and niacin Deficiencies of these vitamins cause anaemia in experimental ani- mals, but anaemia purely due to one or other of these deficiencies has not been established to occur in humans. Vitamin E This vitamin is needed for preventing peroxidation of cell mem- branes. A haemolytic anaemia responding to vitamin E has been reported in premature infants. Less well documented is a macrocytic anaemia due to vitamin E deficiency in protein–calorie-deficient infants and aggravation of anaemia in patients with thalassaemia major because of vitamin E deficiency. Protein deficiency Anaemia is usual in both ‘pure’ protein deficiency (kwashiorkor) and in protein–calorie malnutrition (marasmus). It has been re- ported in many parts of the world where malnutrition, especially in children and pregnant women, is common. The anaemia also occurs in patients with gastrointestinal disease and severe malab- sorption. The anaemia is typically normochromic, normocytic, and of the order of 80 to 90 g/litre. The reticulocyte count is usu- ally reduced and the marrow may show a selective reduction in erythropoiesis. Experimental studies in animals suggest that the anaemia is largely due to reduced serum erythropoietin levels con- sequent on a lack of stimulus for erythropoietin secretion. Lack of amino acids for synthesis of erythropoietin or globin is not the cause. In many patients, the anaemia is complicated by infection, folate or iron deficiency, and possibly other vitamin deficiencies (e.g. riboflavin, vitamin E) and then it may be more severe and show additional morphological abnormalities in the blood and marrow. FURTHER READING General Devalia V, Hamilton MS, Molloy AM (2014). Guidelines for the diag- nosis and treatment of cobalamin and folate disorders. Br J Haem, 166, 496–513. Longo DL (2015). Drug-induced megaloblastic anemia. N Engl J Med, 373, 1649–58. McLean E, de Benoist B, Allen LH (2008). Review of the magnitude of folate and vitamin B12 deficiencies worldwide. Food Nutr Bull, 29 Suppl 2, 538–51. Vitamin B12 Carmel R (2011). Biomarkers of cobalamin (vitamin B12-12) status in the epidemiologic setting: A critical overview of context, applica- tions, and performance characteristics of cobalamin, methylmalonic acid and holotranscobalamin 11. Am J Clin Nutr, 97, 541–56. Carmel R (2012). Subclinical cobalamin deficiency. Curr Opin Gastroenterol, 28, 151–8. Green R (2017). Vitamin B12 deficiency from the perspective of the practising hematologist. Blood, 129, 2603–11. Green R, et al. (2017). Vitamin B12 deficiency. Nat Rev Dis Primers, 3, 17040. Hershko C, et al. (2006). Variable hematological presentation of auto- immune gastritis: age related progression from iron deficiency to cobalamin depletion. Blood, 107, 1673–9. Lewerin C (2008). Serum biomarkers for atrophic gastritis and anti- bodies against Helicobacter pyloric in the elderly: Implications for vitamin B12, folic acid and iron status and response to oral vitamin therapy. Scand J Gast, 143, 1502–8. Mills JL (2011). Do high blood folate concentrations exacerbate meta- bolic abnormalities in people with low vitamin B12 status. Am J Clin Nutr, 94, 495–500.

Revision #1
Created 2026-01-22 16:42:43 UTC by Omar Ayman

Updated 2026-01-22 16:42:43 UTC by Omar Ayman