Chapter 9
Absorption of Water-Soluble Vitamins and Minerals

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1 Introduction

The **nine water-soluble vitamins**, ***thiamine***, ***riboflavin***, ***niacin***, ***pantothenic acid***, ***folate***, ***biotin***, and ***vitamins B_6***, ***B_{12}***, and ***C*** are a diverse group of organic compounds that are consumed in the daily diet in microgram to milligram amounts and are essential for normal growth, development, and maintenance of the human organism. These compounds are generally metabolized to forms that serve as **coenzymes** in various biochemical reactions; *vitamin C* is an *exception*, as it functions as an essential water-soluble antioxidant.

The mechanisms of intestinal absorption of the various water-soluble vitamins share some important **general characteristics**. The vitamins are usually ***present in the diet as complex coenzyme forms*** that must be digested intraluminally or at the brush-border membrane surface into simpler forms prior to transport across the intestinal epithelium. In addition, ***dietary vitamins are often associated with proteins*** (e.g. flavoproteins), and digestion of the protein component is needed to liberate the vitamin prior to absorption. At the **low concentrations** present in the diet (typically 10^{-9}–10^{-7} mol/L), transport of the vitamins across the brush-border membrane occurs by *specialized mechanisms*, such as membrane carriers, active transport systems, and membrane binding proteins and receptors, that are specific for a particular vitamin. At **higher intraluminal concentrations** attained with pharmacologic vitamin supplementation, uptake occurs via *passive diffusion*, either transcellularly or through the paracellular pathway. Extensive metabolism of water-soluble vitamins occurs within the enterocyte, and metabolism may be coupled to the rate of uptake. Mechanisms for extrusion of the vitamins from the enterocyte are

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Table 9.1 Mechanisms of water-soluble vitamin absorption

Vitamin	Luminal Events	Transport
Thiamin	Hydrolysis of phosphorylated form	Na-independent, pH dependent and amiloride sensitive uptake; 2 carriers: one both apical and basolateral, one only apical
Folate	Hydrolysis of folate polyglutamates	Acid pH-dependent carrier; neutral pH carrier
Biotin	Digestion of protein- bound biotin	Na-dependent carrier
Vitamin C		Ascorbic acid - Na-dependent carriers, one apical and one basolateral; dehydro-L-ascorbic acid - glucose transporters, GLUT1,3,4
Vitamin B_6	Hydrolysis of phosphorylated form	Na-independent, acid pH dependent amiloride sensitive carrier
Riboflavin	Digestion of protein-bound riboflavin	Na-independent carrier
Niacin		Na-independent, acid pH-dependent carrier
Pantothenic acid	Digestion of protein-bound pantothenic acid	Same carrier as biotin
Vitamin B_{12}	Release of bound B_{12} by acid-peptic digestion; binding of B_{12} to haptocorrin; release of B_{12} from haptocorrin by pancreatic enzymes; complex with intrinsic factor	Membrane receptor (cubilin); endocytosis and processing via endosomal-lysosomal pathway; B_{12} enters transcobalamin-containing secretory vesicles, transports through basolateral membrane, and binds to transcobalamin II

less understood, but they often involve membrane carriers and specialized transport proteins. Table 9.1 summarizes some of the features of intestinal absorption of the water-soluble vitamins.

Essential mineral elements must also be absorbed to maintain normal physiological function and health. The typical diet contains several hundred milligrams per day of **macrominerals** such as *calcium*, *sodium*, *potassium*, *magnesium*, *chloride*, *phosphorus*, and *sulfur*. **Microminerals or trace elements** are present in smaller amounts, ranging from a few milligrams to micrograms per day. Essential trace elements including *chromium*, *cobalt*, *copper*, *fluoride*, *iodide*, *iron*, *manganese*, *molybdenum*, *selenium*, and *zinc* have well-established functions in human physiology. Other trace elements (*arsenic*, *boron*, *cadmium*, *nickel*, *silicone*, *tin*, *vanadium*) have physiological functions in some species, but an essential role in human metabolism had not been clearly defined. Minerals and trace elements are also present in various gastrointestinal (GI) secretions and are variably reabsorbed by the intestine. Minerals serve diverse physiological roles, including structural functions (bone minerals), components of metalloproteins (enzymes, transporters) and as ions involved in neurotransmission, muscle function, regulation of fluid and acid-base balance, energy gradients, and as second messengers.

Intraluminal factors substantially affect the efficiency of mineral and trace element absorption by altering the following processes: (**1**) intraluminal pH; (**2**) redox state of the metal; (**3**) formation of chelates that enhance solubility of the mineral; (**4**) formation of insoluble complexes that diminish absorption; and (**5**) digestion of proteins that are associated with dietary minerals. Kinetic studies of mineral uptake

Table 9.2 Common mineral interactions and antagonisms[a]

Mineral	Condition or state	Effect on net absorption
Iron	Fe deficiency	↑
	Fe excess	↓
	Mn excess	↓
	Co excess	↓
	Conditions favoring Fe^{2+}	↑
	Conditions favoring Fe^{3+}	↓
Copper	Cu deficiency	↑
	Cu excess	↓
	Zn excess	↓
	Cd excess	↓
	Ag excess	↓
	Conditions favoring Cu^{+}	↓
	Conditions favoring Cu^{2+}	↑
Zinc	Zn deficiency	↑
	Zn excess	↓
	Cu excess	↓
	Cd excess	↓
Manganese	Mn deficiency	?
	Mn excess	?
	Fe excess	↓
	Co excess	↓

From Rucker et al. [11]

[a]For given antagonists, a significant effect on absorption is often observed at intakes corresponding to 5 to ten times the normal requirements (i.e. Fe, Cu, Mn, Zn) or at concentrations >2–4 μmol/L (150–300 ng/g of intestinal content) in the case of Ag, Cd or Co when present

across the small intestine brush-border membrane generally have been consistent with ***facilitated diffusion*** or ***active transport*** when studied at **low physiological concentrations**, although many of these putative carriers have not been definitively identified. At **higher concentrations**, intestinal uptake via ***passive diffusion***, either paracellularly or transcellularly, becomes quantitatively more important. Within the enterocyte, minerals often associate with intracellular ligands that play critical roles in regulating absorption and delivery of these elements into the circulation. Mechanisms for extrusion of minerals across the basolateral membrane into the portal circulation are incompletely characterized, but associations with transport proteins and other ligands are important. Effects of one mineral on the absorption of others are commonly observed, reflecting interactions in the luminal environment and shared mechanisms for absorption (Table 9.2). For example, high doses of zinc interfere with copper absorption. Individuals chronically taking zinc supplements for prevention of colds, etc. may develop copper deficiency with anemia, neutropenia, and bone disease. Zinc is used in the treatment of Wilson's disease, a genetic disorder characterized by diminished biliary copper excretion and copper overload. Zinc treatment decreases intestinal copper absorption, contributing to a reduction in body copper.

A detailed consideration of the absorption of each of the water-soluble vitamins, minerals, and trace elements is beyond the scope of this book. In this chapter, ***two water-soluble vitamins***, **folate** and **vitamin B_{12}**, and ***two minerals***, **iron** and **calcium**, will be considered in depth as illustrations of the important principles of intestinal absorption of these classes of nutrients.

2 Folate

2.1 Structure and Biochemical Function

The term folate denotes a family of compounds with nutritional properties and biochemical structures similar to the reference compound, **folic acid** or **pteroylglutamic acid** (Fig. 9.1). Folic acid comprises **three moieties**: a ***pteridine*** linked by a methylene bridge to ***para-aminobenzoic acid*** (***PABA***) and joined by a peptide bond to ***glutamic acid***. The coenzyme forms of folate have reduced pteridine rings at positions 5, 6, 7, and 8 and one-carbon additions at N-5 or N-10. In addition, naturally occurring folates are mainly in the polyglutamate form, in which up to nine glutamate residues are conjugated via a unique γ-glutamyl bond forming a peptide chain (Fig. 9.1).

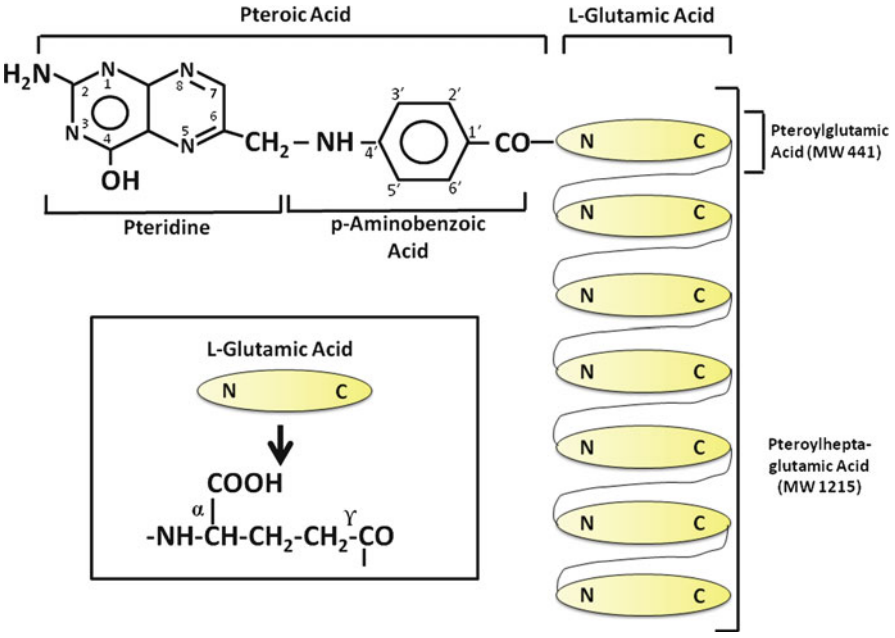

Fig. 9.1 Structure of conjugated folates (Adapted from Mason and Rosenberg [7]

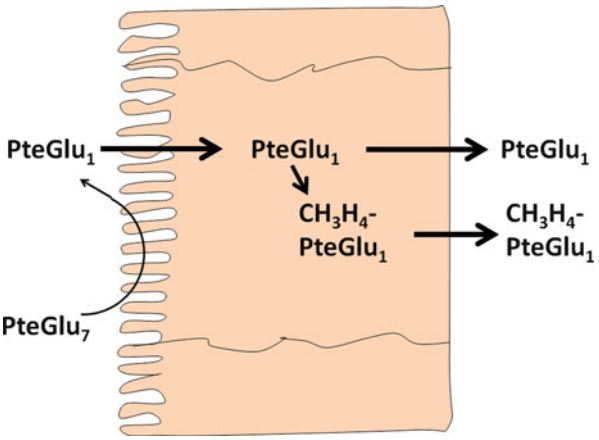

Fig. 9.2 Enterocyte metabolism and absorption of conjugated folates. *PteGlu*$_7$ heptaglutamyl folate, *PteGlu*$_1$ monoglutamyl folate, *CH*$_3$*H*$_4$*PteGlu*$_1$ 5-methyltetrahydrofolate (Adapted from Mason and Rosenberg [7])

Folate is widely distributed in foods, with liver, yeast, leafy vegetables, legumes and some fruits being especially rich sources. Only a few foods, such as milk and egg yolk, contain principally monoglutamyl forms, whereas organ meats contain mainly penta- and heptaglutamates. Folic acid is the most common form of the vitamin in pharmaceutical preparations and has been used in much of the research on intestinal folate absorption. A substantial amount of folate is also secreted in bile, mainly as **monoglutamyl 5-methyltetrahydrofolate**, and must be reabsorbed by the intestine to maintain the normal folate economy.

Folates function metabolically as **coenzymes** in biochemical reactions that transfer one-carbon units from one compound to another. These reactions are important in amino acid metabolism and in nucleic acid synthesis. **Folate deficiency** therefore impairs cell division and alters protein synthesis, with the most prominent effects noted in rapidly growing tissues. The most common clinical manifestation of human folate deficiency is a ***macrocytic anemia***, characterized by abnormally large red blood cells (**macro-ovalocytes**). ***Hypersegmentation of the chromatin of circulating neutrophils*** is also observed, and with *severe* folate deficiency ***neutropenia*** and ***thrombocytopenia*** may also be present. **Bone marrow examination** demonstrates abnormal precursors of red blood cells (megaloblasts), neutrophils, and platelets resulting from defective DNA synthesis.

2.2 Hydrolysis of Polyglutamyl Folates

In the lumen of the small intestine, **polyglutamyl folates** must be hydrolyzed to the **monoglutamyl form** before transport through the enterocyte (Fig. 9.2). Because of the unique **γ-glutamyl linkage**, polyglutamyl folates are not hydrolyzed by typical pancreatic or intestinal proteases, and specific enzymes known as **folate conjugases** are required.

In humans, folate conjugase activities are present in both the ***small intestinal brush-border membrane*** and in an ***intracellular*** fraction composed mainly of lysosomes. The brush-border membrane enzyme appears to be responsible for the hydrolysis of dietary polyglutamyl folates. This enzyme has a pH optimum of pH 6.5–7.0, the typical pH of the upper small intestine, and is activated by Zn^{2+}. The brush-border folate conjugase is an **exopeptidase** that sequentially cleaves glutamate residues from the end of the peptide chain, eventually producing the monoglutamate form. The function of the intracellular conjugase enzyme is currently unknown. This enzyme is an **endopeptidase**, cleaving the polyglutamate chain between the first and second glutamic acid residues. Intracellular conjugase has a pH optimum of pH 4.5, and no metal requirement for this enzyme has been defined. During folate digestion and absorption, monoglutamyl folates accumulate in the intestinal fluid, indicating that the ***rate-limiting step*** is the **absorption of monoglutamyl folates** rather than deconjugation of polyglutamate forms. Studies have shown that alcohol decreases brush-border conjugase activity, a factor that may contribute to the high prevalence of folate deficiency in alcoholics. Several drugs have also been shown to inhibit folate deconjugation, including the anti-inflammatory medication *sulfasalazine* used in the treatment of inflammatory bowel disease.

2.3 Absorption of Monoglutamyl Folates

Studies of the intestinal uptake of monoglutamyl folates using different experimental preparations have consistently demonstrated the presence of both **saturable** and **nonsaturable uptake mechanisms**. Saturable uptake appears to reflect carrier-mediated transport, whereas the nonsaturable component is due to diffusion through the cell membrane and/or through the paracellular pathway. At physiological concentrations, carrier-mediated transport accounts for most of the folate absorption, whereas diffusion predominates at high folate concentrations.

Two carriers involved in intestinal folate absorption have been identified, **RFC** (**reduced folate carrier**, the product of the SLC19A1 gene) and **PCFT** (the product of the SLC46A1 gene). RFC is expressed in the ***intestinal cell brush-border membrane*** and functions at neutral pH. PCFT is expressed mainly in the ***apical membrane of jejunal enterocytes*** with low expression in the ***ileum*** and ***colon***. Transport of folate through the PCFT system is proton coupled and occurs via a **folate-proton symport** using energy generated from the downhill movement of protons into the enterocyte. It is possible that PCFT is responsible for folate absorption in the proximal small bowel where the luminal pH is somewhat acidic, whereas RFC plays a role in absorption of folate in the distal small intestine and colon. **Mutation of the PCFT transporter** results in ***hereditary folate malabsorption***. Folic acid, reduced folates such as 5-methyltetrahydrofolate, and the antimetabolite methotrexate (a competitive inhibitor of the enzyme dihydrofolate reductase) all appear to share the same brush-border membrane carrier, with similar affinities for the transporter. Folate uptake, however, is structure-specific, as degradation products such as PABA

or glutamic acid and inactive diastereoisomers of 5-methyltetrahydrofolate do not interact with the brush-border membrane transporter. In addition to its effect on folate deconjugation, ***sulfasalazine*** is also a **competitive inhibitor** of intestinal monoglutamyl folate transport. The mechanism of transport of folate across the basolateral enterocyte membrane is not well understood, but some data suggest involvement of **MDR** (**multidrug resistance**) **proteins**.

Bacteria in the intestine synthesize folate, with a substantial portion in the monoglutamate form. Although the contribution of colonic absorption of bacterially-derived folate via the RFC transporter expressed in the colonocyte brush-border membrane to folate economy is uncertain, it is possible that it plays a significant role in human nutrition, particularly supplying folate for colonocytes.

Folate digestion and absorption are both regulated by the level of folate in the diet. Folate deficiency causes an increase in folate conjugase and in carrier-mediated folate transport with increased expression of PCFT and RFC. Carrier-mediated folate transport and expression of RFC and PCFT increase as enterocytes mature to villus cells.

Some data have suggested an alternative mechanism for intestinal folate absorption in the neonate. Folate in milk is largely bound to a **high-affinity folate-binding protein**. In contrast to free folate, this protein-bound folate is more avidly absorbed in the ileum than jejunum and is not inhibited by sulfasalazine.

2.4 Folate Metabolism in the Enterocyte

At physiological concentrations, folic acid is largely reduced and methylated or formylated within the enterocyte. Appearance of folate in the blood is faster after intraluminal administration of 5-methyltetrahydrofolate folate than after folic acid, suggesting that **folic acid reduction in the enterocyte via dihydrofolate reductase** may be ***rate-limiting***. At pharmacological concentrations, unmodified folic acid appears in the portal blood.

3 Vitamin B_{12}

3.1 Structure and Biochemical Function

The basic structure of vitamin B_{12} is illustrated in Fig. 9.3. A **central cobalt atom** is surrounded by a **planar corrin nucleus** comprising **four reduced pyrrole rings** linked together. Below the corrin nucleus is a nucleotide moiety (1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole-3-phosphate) that lies at a right angle to the corrin nucleus and is joined to the rest of the molecule at two points: (**1**) via a **phosphodiester bond** to a 1-amino-2 propanol group and (**2**) through coordination to the central cobalt via one of its nitrogens. The various forms of

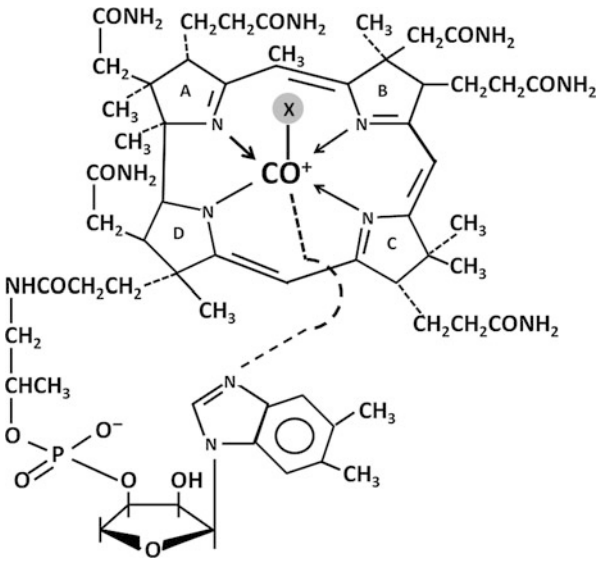

Fig. 9.3 Structure of vitamin B_{12}. X, axial ligand in coordinate linkage with cobalt

vitamin B_{12} have different anionic groups in coordinate linkage with the cobalt. The coenzyme forms of the vitamin are **methylcobalamin** and **adenosylcobalamin** (**5′-deoxyadenosylcobalmin**), formed via a unique carbon-cobalt bond. Other important forms of vitamin B_{12} are **hydroxocobalamin** and **cyanocobalamin**.

Vitamin B_{12} is synthesized *only* by microorganisms, and in the human diet it is almost entirely by animal products. Strict vegetarians are therefore at increased risk for vitamin B_{12} deficiency. The daily losses of vitamin B_{12} are, however, very small compared with the body pool size, and 10–20 years of a deficient diet is required to produce clinically significant depletion. Meat contains mainly adenosyl- and hydroxocobalamin, whereas dairy products have predominantly methyl and hydroxocobalamin. Cyanocobalamin is a stable form of the vitamin used in pharmaceutical preparations. Cyano- and hydroxocobalamin are readily converted to the coenzyme forms by enzyme systems found in the cytoplasmic and mitochondrial fractions. In **human plasma and tissue**, the predominant forms of vitamin B_{12} are ***methylcobalamin***, ***adenosylcobalamin***, and ***hydroxocobalamin***. Bile contains a significant amount of vitamin B_{12} that is reabsorbed by the small intestine. The importance of this enterohepatic circulation in the maintenance of the vitamin B_{12} pool is indicated by the observation that patients with vitamin B_{12} malabsorption become deficient in only 2–3 years compared with the 10–20 years needed for deficiency to develop in individuals who lack dietary vitamin B_{12}, but normally conserve biliary cobalamins.

Vitamin B_{12} serves as a **coenzyme** for two important enzymatic reactions. Methylcobalamin is required for the conversion of homocysteine to methionine (Fig. 9.4). This reaction is catalyzed by the cytoplasmic enzyme **5-methyltetrahydrofolate-homocysteine methyltransferase**, which utilizes 5-methyltetrahydrofolate as a methyl donor. This pathway is therefore important to maintain the

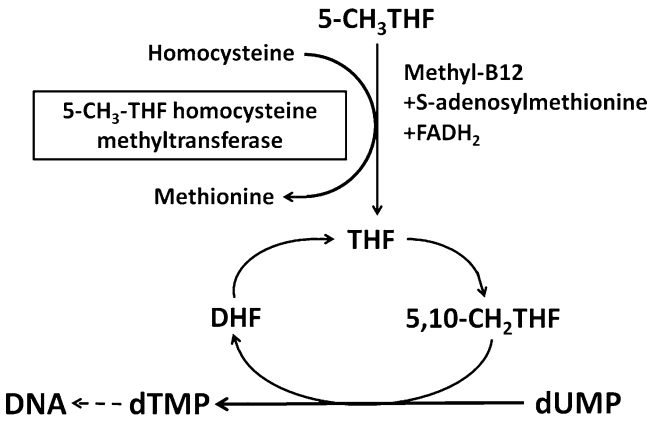

Fig. 9.4 Role of methylcobalamin in the conversion of homocysteine to methione. *5-CH_3THF* 5-methyltetrahydrofolate, *THF* tetrahydrofolate, *CH_2THF* methylenetetrahydrofolate, *DHF* dihydrofolate, *dUMP* uridylate, *dTMP* thymidylate (Adapted from Kano et al. [5])

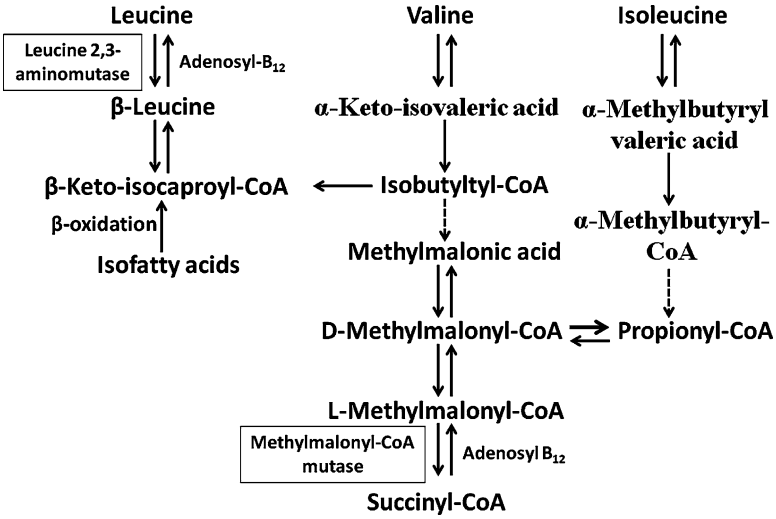

Fig. 9.5 Function of adenosylcobalamin in the mitochondrial isomerase reaction that converts methylmalonyl CoA to succinyl CoA (Adapted from Kano et al. [5])

supply of both methionine and tetrahydrofolate. Tetrahydrofolate is subsequently converted to 5,10-methylenetetrahydrofolate, which donates its one-carbon unit to deoxyuridylate, forming thymidylate and contributing to DNA synthesis. **Adenosylcobalamin** is required for the isomerase reaction in mitochondria that converts methylmalonyl coenzyme A (CoA) to succinyl CoA (Fig. 9.5). Methylmalonyl CoA is derived from propionate and amino acids such as

valine, isoleucine, and threonine. In **vitamin B_{12} deficiency**, propionate and methylmalonate accumulate and result in impaired fatty acid synthesis.

The major clinical manifestations of vitamin B_{12} deficiency are **hematologic** and **neuropsychiatric abnormalities**. The hematologic changes are identical to those seen in folate deficiency (see above) and are thought to be due impaired generation of tetrahydrofolate and altered DNA synthesis. The neuropsychiatric abnormalities may be a consequence of alterations in brain and peripheral nerve fatty acid synthesis due to deficient methylmalonyl-CoA mutase activity, but disordered folate and methionine metabolism may also play important roles.

3.2 Intraluminal Events in Vitamin B_{12} Absorption

In the diet, vitamin B_{12} is predominately protein-bound, either to transport proteins or to the enzyme systems described above. Acid and pepsin play important roles in the digestion of these proteins and in the release of vitamin B_{12} into the gastric fluid. Individuals with reduced gastric acid and pepsin secretion are often able to absorb pure crystalline vitamin B_{12} normally, but have impaired absorption of vitamin B_{12} contained in food.

Gastric juice contains **two important vitamin B_{12} binding proteins**, ***haptocorrin*** (***R protein-type binder***) and ***intrinsic factor*** (***IF***). Haptocorrin is a 60–66-kd glycoprotein present in many digestive secretions, although the haptocorrin in gastric juice is mainly derived from the salivary gland. Vitamin B_{12} has a **much higher affinity for haptocorrin** than for IF, particularly at low pH. The vitamin B_{12} liberated from food therefore binds preferentially to haptocorrin in gastric juice. In addition, bile contains a substantial amount of vitamin B_{12} bound to haptocorrin. Both haptocorrin and IF are unaffected by acid-peptic digestion. In contrast, pancreatic proteases do not alter IF, but modify haptocorrin to a smaller molecular weight form that has a markedly decreased affinity for vitamin B_{12}. In the small intestinal fluid, therefore, vitamin B_{12} is rapidly and essentially completely transferred from haptocorrin to IF (Fig. 9.6).

IF is a 48–50-kd glycoprotein produced by the **gastric parietal cells**, the same cell type that is responsible for acid secretion. Within the parietal cell, immunoreactive IF can be found in the endoplasmic reticulum, Golgi apparatus, and in tubulovesicles. IF secretion is stimulated by **gastrin**, **histamine**, and **cholinergic agonists**. Following stimulation, tubulovesicles containing IF migrate to the periphery of secretory canaliculi, and IF is then observed on the secretory microvilli. These findings indicate that IF secretion occurs via membrane-associated vesicular transport and fusion of the tubulovesicles with the secretory canalicular membrane. Although IF secretion is stimulated by the same agents that induce gastric acid secretion, these two events are regulated differently. Following stimulation, IF secretion is rapid, perhaps due in part to wash out of preformed IF, and subsequently declines to a much lower plateau value. The IF-mRNA level in the parietal cells is not affected by secretagogues. In contrast, gastric acid secretion has a slower

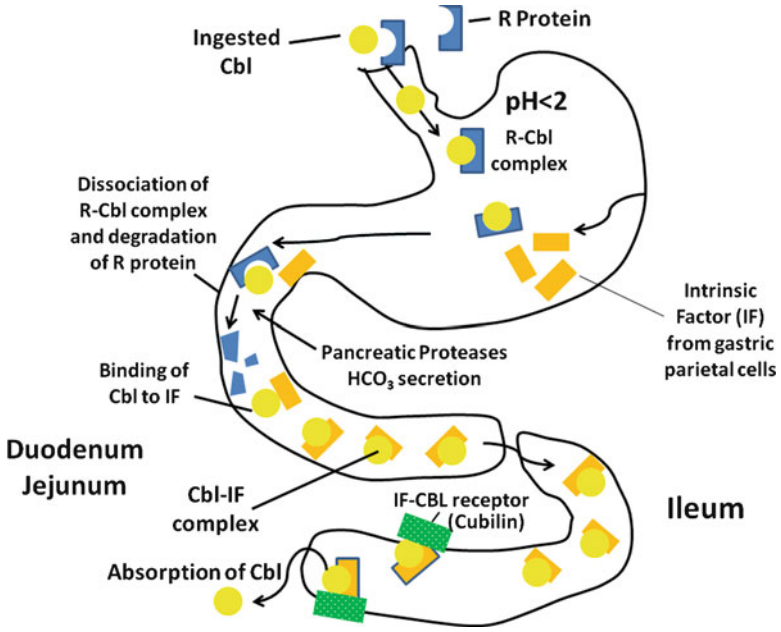

Fig. 9.6 Absorption of cobalamin. Vitamin B_{12} is associated with binding proteins in the gastric and intestinal lumen. *Cbl* vitamin B_{12}, *IF* intrinsic factor, *R* haptocorrin

time course and is sustained at a high level. Some data suggest that gastric acid inhibits secretion of IF, accounting for the different secretory patterns. **Histamine H_2-receptor blockers**, such as *cimetidine*, substantially decrease gastric acid but have only a slight inhibitory effect on IF secretion. The hormone **secretin** decreases acid secretion, but has no effect on IF. Agents, such as *omeprazole*, that block acid secretion by inhibiting the gastric H^+/K^+-ATPase do not alter IF secretion.

Vitamin B_{12} appears to fit into a hydrophobic pit of the IF protein, with the nucleotide portion in the interior of the pit and the anionic moiety coordinated to cobalt facing outward. Binding of vitamin B_{12} to IF exposes hydrophilic regions of IF that increase binding of the IF-vitamin B_{12} complex to brush-border membrane receptors.

3.3 Mucosal Events in Vitamin B_{12} Absorption

The **IF-vitamin B_{12} complex** binds to a specific receptor present in the brush-border membrane of ileal enterocytes, but not in the proximal intestine (Fig. 9.7). The receptor for the IF-vitamin B_{12} complex in the distal ileum is a 460 kDa protein cubilin. Binding of IF-vitamin B_{12} to cubilin is enhanced by Ca^{2+} and

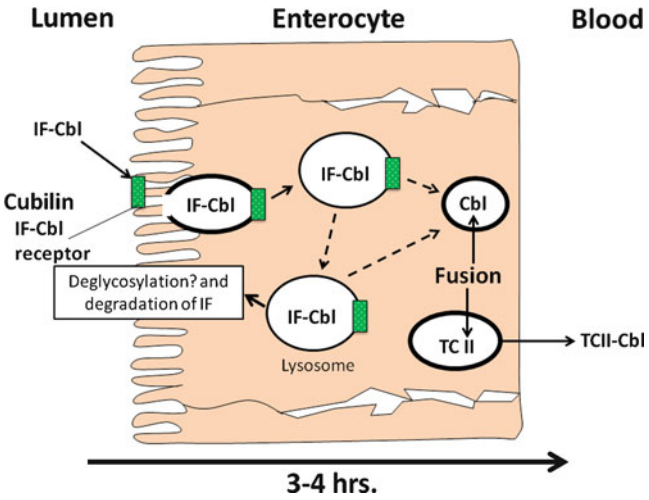

Fig. 9.7 Transport of IF- bound vitamin B_{12} across the ileal enterocyte. *IF* intrinsic factor, *Cbl* vitamin B_{12}, *TCII* transcobalamin II (Adapted from Seetharam [14])

possibly bile salts. **Cubilin** is associated with another protein **amnionless** (**AMN**) that is involved in the localization of cubilin to the apical membrane surface and in internalization by endocytosis of the IF-vitamin B_{12} complex. **Mutations in the genes coding for cubilin or amnionless** result in ***congenital vitamin B_{12} malaborption*** (***Imerslund-Grascbeck syndrome***). Other proteins can associate with cubilin and may also be involved in vitamin B_{12} absorption. Within the enterocyte, the IF-vitamin B_{12} complex dissociates from cubilin in the endosome and reaches the lysosome were IF is degraded to forms that poorly binding vitamin B_{12}. The vitamin B_{12} leaves the lysosome and enters the cytoplasm probably mediated by a protein LMBD1.

Vitamin B_{12} is released from the ileum into the portal blood bound to another binding protein, **transcobalamin II** (**TC-II**). The mechanisms for transfer of vitamin B_{12} to TC-II in the intestine are incompletely understood. TC-II is synthesized by enterocytes, and a complex of vitamin B_{12} with TC-II could traverse the enterocyte basolateral membrane. Evidence exists, however, for transport of free vitamin B_{12} across the basolateral membrane, perhaps mediated by **multidrug resistance protein1** (**MDR1**). The vitamin B_{12} would then associate with TC-II outside the enterocyte. The vitamin B_{12}-TC-II complex is taken up by peripheral tissues by receptor-mediated endocytosis.

Vitamin B_{12} absorption in neonates may occur through a different mechanism. Neonates have relatively low levels of IF, and in milk vitamin B_{12} is bound to haptocorrin. Neonatal small intestine expresses a receptor protein known as the **asialoglycoprotein receptor**. Haptocorrin-bound vitamin B_{12} is taken up into other cell types, such as hepatocytes, via this receptor, and it is possible that uptake of

the haptocorrin-vitamin B_{12} complex in the neonatal intestine also occurs through this pathway. High oral doses of vitamin B_{12} (about 1–2 mg) can effectively treat patients with pernicious anemia, an auto-immune gastritis that causes IF deficiency and vitamin B_{12} malabsorption. In ***pernicious anemia***, high dose vitamin B_{12} could be absorbed to a limited extent via the asialoglycoprotein receptor or an alternative paracellular route.

3.4 Schilling Test

The Schilling test is used clinically to **assess vitamin B_{12} absorption**. A tracer dose (0.5–1.0 mcg) of radioactive vitamin B_{12} is given orally, and 1–2 h later a large flushing dose (1,000 mcg) of unlabeled vitamin B_{12} is administered intramuscularly to saturate plasma binding sites so that most of the absorbed vitamin B_{12} is excreted in the urine. If the urinary excretion of radioactivity is low (less than 8 % of the administered dose in 24 h), a second-stage test is done in which IF is given orally along with the radioactive vitamin B_{12}. Patients with diseases causing diminished IF secretion, such as *pernicious anemia*, have **reduced radioactive vitamin B_{12} absorption that normalizes when given with IF**. In contrast, those with *ileal disease* or *resection* and reduced absorption of the IF-vitamin B_{12} complex have **diminished absorption in both parts of the Schilling test**. A singlestage absorption test has been developed in which the patient is given both ^{58}Cocobalamin and IF-bound ^{57}Co-cobalamin orally, and the urinary excretion of the two isotopes compared to distinguish between deficient IF secretion and ileal dysfunction. Tests utilizing radioactive cobalamin incorporated into food proteins have also been devised to more accurately assess the patient's capacity to absorb vitamin B_{12} from food sources.

4 Iron

4.1 Biochemical Function

Iron is an essential trace element because of its crucial roles in **cellular oxidative energy metabolism**. Iron is a component of the oxygen-transporting proteins ***hemoglobin*** and ***myoglobin*** and of specific redox enzymes. A **microcytic hypochromic anemia** (small red blood cells with reduced hemoglobin) is the most important clinical consequence of **iron deficiency**; however, diminished work and school performance may be observed in depleted individuals even before the development of anemia. **Cellular iron overload**, as occurs with the genetic disorder ***hemochromatosis***, results in oxidant damage to many tissues.

4.2 Dietary Sources of Iron

Iron is present in the diet in **two forms**, as a component of ***heme*** (***heme iron***) and as various ***nonheme iron compounds***. Heme iron represents about 40 % of the total iron in animal foods, whereas essentially all of the iron in plant foods is nonheme iron. In a typical mixed American diet, about 10 % of total iron is heme iron. The intestinal absorption of heme iron is considerably more efficient than that of nonheme iron, and uptake of these two forms into the enterocyte occurs via distinct pathways (see below). From a typical daily dietary intake of 10–20 mg of mixed heme and nonheme iron, about 10 % is absorbed by an iron sufficient individual, replacing the daily losses of 1–2 mg and maintaining the body iron content. In iron deficiency, the intestinal absorption of iron significantly increases. The regulation of body iron therefore occurs principally by adjusting intestinal absorption according to tissue requirements rather than by altering iron excretion.

4.3 Intraluminal Factors in Intestinal Iron Absorption

Nonheme iron is present in the diet mainly in the **ferric** ($\mathbf{Fe^{3+}}$) **state**. Ferric iron is soluble at an acidic pH but precipitates above pH 3. Subjects with reduced gastric acid secretion may therefore develop ***iron deficiency*** because the ferric iron is not solubilized by an acidic pH within the stomach. **Ferrous** ($\mathbf{Fe^{2+}}$) **iron salts** are used in pharmaceutical preparations, as ferrous iron is soluble at the nearly neutral pH of the small-intestinal luminal fluid and is therefore more efficiently absorbed than is ferric iron. Various compounds present in the diet or secreted into the intestine, such as certain sugars (e.g. fructose), amino acids (e.g. histidine), amines, and polyols, form unstable iron chelates by binding only a few of the six coordinating bonds of iron. These complexes help keep iron soluble in the intestinal luminal fluid. **Vitamin C** (**ascorbic acid**) is a well-known facilitator of iron absorption. Ascorbic acid forms an iron chelate that increases iron solubility but, more importantly, reduces iron to the more soluble Fe^{2+} state. The unstable iron chelates serve as iron donors to mucins produced by the upper GI tract. One molecule of macromolecular mucin can bind many iron atoms, and other transitional metals also bind mucins competitively with iron. The iron complexes with mucins, which keep the iron soluble and available in an acceptable form, and iron then undergoes uptake into the enterocyte. Bile increases intestinal nonheme iron absorption, probably by several mechanisms. Bile contains iron chelators and has reductants that convert Fe^{3+} to the more soluble Fe^{2+}. At concentrations below the critical micellar concentration, the bile salt **taurocholate** forms a soluble complex with Fe^{2+}. It has also been suggested that taurocholate may induce the formation of ion-permeable channels in the brush-border membrane, facilitating iron uptake.

Other components of the diet decrease nonheme iron absorption by precipitating iron or by forming stable chelates that interfere with the binding of iron to mucins. **Inhibitors of iron absorption** include *oxalates*, *phytates*, *tannates*, and *carbonates*. The efficiency of iron absorption from different foodstuffs may vary tenfold because

of the presence of various enhancers and inhibitors. Fe^{3+}complexed with mucin is also reduced to Fe^{2+}prior to intestinal membrane transport. Reductants are present in the diet and in bile, and the small intestinal brush-border membrane also contributes to this process. Reductants of systemic or enterocyte origin (e.g. ascorbate, glutathione, etc.) are secreted into the luminal fluid, and the brush-border membrane enzyme reductase **duodenal cytochrome b** (**Dcytb**) acts to reduce $Fe^{3+}$to Fe^{2+}at the apical surface.

Heme iron is precipitated in an acid environment, but it is soluble at the more alkaline pH of the small intestinal fluid. Chelation is therefore not needed to facilitate solubility, and many of the substances that enhance or inhibit nonheme iron absorption have no effect on the absorption of heme iron. Iron is more poorly absorbed from a dose of heme, compared with an equivalent amount of hemoglobin, and it has been demonstrated that globin degradation products and certain amino acids, amines, and amides inhibit heme polymerization within the intestinal lumen and facilitate absorption.

4.4 Enterocyte Iron Transport

Iron is absorbed predominantly in the ***duodenum*** (Fig. 9.8). Fe^{2+} iron is transported across the brush-border membrane by the **divalent metal transporter DMPT1**, and loss of function mutations of this transporter results in very low rates of iron absorption and severe microcytic anemia. Following uptake into the enterocyte the iron can have **two fates**. (**1**) It can be sequestered in the iron storage protein **ferritin** that is expressed in intestinal epithelial cells. Iron bound to ferritin is lost back into the intestinal lumen as senescent epithelial cells are sloughed from the villus tips. (**2**) Alternatively, iron can be transported across the basolateral membrane. This occurs via the Fe transporter **ferroportin 1** (**FP1**). During the process of iron transport across the basolateral membrane, Fe^{2+} must be oxidized to Fe^{3+} prior to binding to the circulating iron transport protein transferrin. The oxidation of Fe^{2+} to Fe^{3+} at the basolateral membrane is facilitated by the protein **hephaestin** (**HP**). In a state of ***iron deficiency***, duodenal levels of Dcytb, DMPT1, and FP1 are increased and the content of ferritin is reduced, favoring iron absorption. In contrast, ***iron sufficiency*** results in increased duodenal ferritin and decreased levels of Dcytb, DMPT1, and FP1 that would favor sequestration of iron in the mucosa and limit absorption.

Heme iron is taken up into the enterocyte as the intact **metalloporphyrin**. Evidence exists for uptake of heme both through a specific carrier and via diffusion through the lipid bilayer. Within the cell, iron is released from the porphyrin by the action of the enzyme **heme oxygenase** and enters the circulation as inorganic iron. Although there is no competition between heme and nonheme iron for uptake into the intestine, there is competitive inhibition in the overall process of intestinal absorption. This observation indicates that the inorganic iron released from heme intracellularly binds to the same cytosolic proteins and follows the same pathway through the cell and across the basolateral membrane as absorbed nonheme iron.

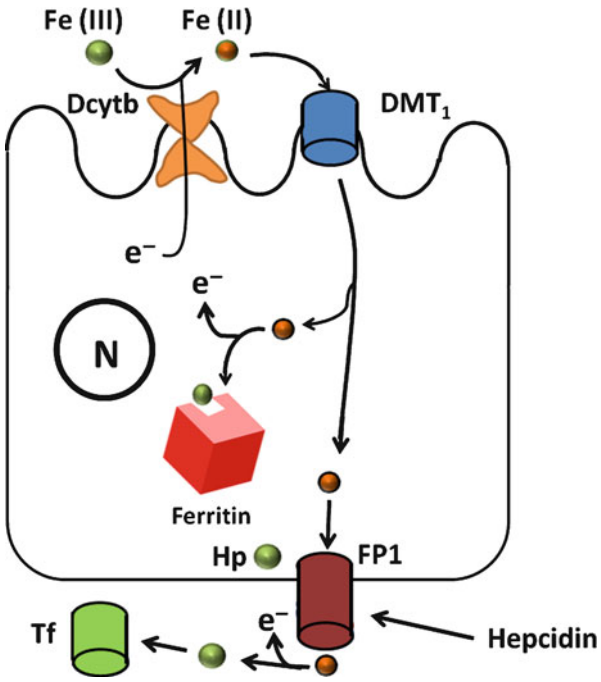

Fig. 9.8 Enterocyte iron transport. Fe^{3+} is reduced to Fe^{2+} by duodenal cytochrome b (Dcytb) in the brush-border membrane. Fe^{2+} is transported across the brush-border membrane by the divalent metal transporter DMPT1. Within the enterocyte, Fe^{2+} can bind to ferritin and be lost as cells are sloughed into the intestinal lumen, or transported across the basolateral membrane by the Fe transporter ferroportin 1 (FP1). During transport across the basolateral membrane, Fe^{2+} is oxidized to Fe^{3+} by hephaestin (HP) and binds to transferrin (Tf). Hepcidin secreted by the liver is the major systemic regulator of iron absorption. Hepcidin binds to FP1 and induces its degradation. Hepcidin secretion is inhibited by iron deficiency, favoring intestinal iron absorption

Several **iron-regulatory proteins** (**IRPs**) have been identified in duodenal enterocytes that interact with specific sequences in the mRNA transcripts of genes (or iron-responsive elements, **IREs**), coding for the proteins involved in intestinal iron transport. The IRP levels and binding to IREs are altered by cellular iron content. Interaction of IRPs with IREs alters the transcription or mRNA degradation rates of the different proteins involved in iron transport.

4.5 Systemic Regulation of Iron Absorption

The status of tissue iron stores in the most well-known systemic regulator of iron absorption. In addition to iron deficiency, however, iron absorption is also increased in ***chronic hypoxia*** and in diseases associated with ***ineffective erythropoiesis*** (**thalassemia**, **chronic hemolytic disease**). In contrast, various inflammatory states

result in diminished iron absorption. **Hepcidin** is a protein secreted by the **liver** that is the **principal regulator of iron absorption**. Hepcidin secretion is inhibited by iron deficiency and increased by iron overload. Hypoxia and anemia also decrease hepatic hepcidin secretion. Hepcidin binds to FP1 in the duodenal enterocyte basolateral membrane, inducing endocytosis of FP1 and its proteolysis in lysosomes. The reduction of FP1 caused by hepcidin decreases iron export from the enterocyte and limits overall intestinal iron absorption.

The regulation of hepcidin secretion by iron is complex and incompletely understood. Extracellular iron is sensed by hepatocytes via binding of the circulating Fe^{3+}-transferrin complex to transferrin receptors in the hepatocyte membrane. The protein HFE is also a component of this complex, and **mutation of HFE** is the most common cause of the genetic disease ***hemochromatosis***, which causes excessive iron absorption, iron overload and oxidative damage to many tissues. The regulation of hepcidin occurs at the transcriptional level. In addition to the extracellular iron-sensing mechanism, other proteins involved in the regulation of hepcidin mRNA synthesis by iron include **bone morphogenic protein 6** (**BMP6**) that responds to intracellular iron, BMP receptor, down-stream signaling elements, hemojuvelin, and others. Mutations in hepcidin, transferrin receptor 2, ferroportin, and ferritin are rare causes of iron overload.

The mediators of altered hepcidin secretion in hypoxia and anemia are not well-understood, but likely involve **signaling molecules** derived from the ***bone marrow***. **Interleukin-6** and other inflammatory mediators have been demonstrated to induce hepcidin secretion.

Duodenal enterocytes express the **transferrin receptor** on the basolateral membrane. The circulating transferrin-Fe^{3+} may form a complex with HFE and the transferrin receptor that is taken up by endocytosis. At the acidic pH of the endocytic vesicle, iron is released, reduced to Fe^{2+}, and transported into the cytoplasm probably by DMPT1. The transferrin receptor and other proteins cycle back to the cell membrane. It is possible that iron sensing by this mechanism also regulates duodenal iron absorption.

5 Calcium

5.1 Functions and Dietary Sources of Calcium

The adult body contains approximately 1,200 g of calcium, with about 99 % present as a **structural element of bones and teeth**. The remaining 1 % of body calcium plays crucial **regulatory roles** in processes such a *nerve conduction*, *muscle contraction*, *blood clotting*, *membrane permeability*, *Ca^{2+}-dependent protein kinases*, and so on. Dairy products contribute more than 55 % of the average dietary calcium intake in the United States. Other sources include leafy green vegetables, soft fish bones, and calcium-fortified foods.

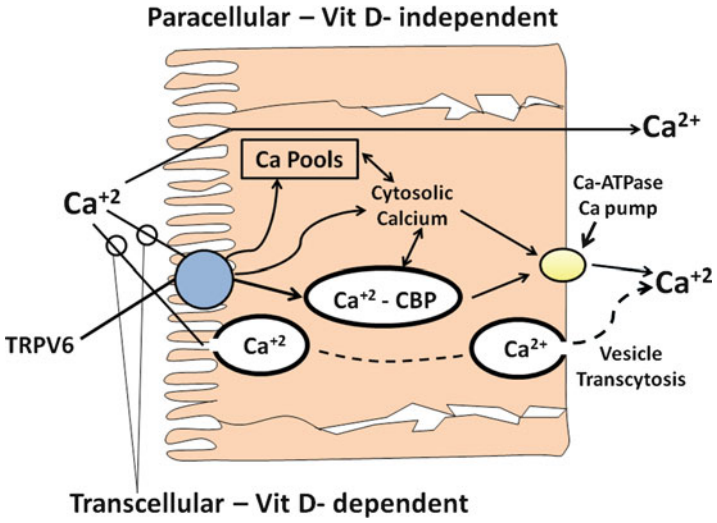

Fig. 9.9 Calcium absorption

5.2 *Pathways of Intestinal Calcium Absorption*

Calcium is absorbed throughout the small intestine and colon, but the **highest rate of absorption** is in the ***duodenum***. At **high dietary intakes**, calcium is absorbed mainly via passive diffusion through a paracellular pathway. At **low levels of intake**, however, efficient calcium absorption is achieved by a saturable, energy-dependent transcellular pathway through the absorptive cells that is dependent on the hormonal form of vitamin D, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] and is most active in the duodenum (see Chap. 8 for a brief description of vitamin D metabolism). Transcellular calcium transport involves **three separate steps**: (**1**) entry into the cell through the brush-border membrane down an electrochemical gradient; (**2**) translocation of calcium through the enterocyte; and (**3**) extrusion from the enterocyte at the basolateral membrane surface against an electrochemical gradient (Fig. 9.9). $1,25(OH)_2D_3$ influences each of these processes, although the detailed mechanisms of these steps remain controversial.

5.3 *Calcium Entry into Enterocytes*

Intracellular Ca^{2+} concentration is approximately 100 nmol/L, whereas the luminal-fluid Ca^{2+} concentration is about 1–10 mmol/L. In addition, the electrical potential within the enterocyte is approximately −55 mV relative to the luminal fluid. Both **electrical** and **chemical gradient forces** therefore favor calcium entry into the cell, and no metabolic energy is needed for this step.

Much of the information concerning calcium uptake has come from studies using highly purified brush-border membrane vesicles prepared from experimental animal or human intestine. Calcium entry into these vesicles is not energy-dependent, but shows saturation kinetics consistent with uptake via calcium transporters or channels. The initial rate of entry of calcium into intestinal brush-border membrane vesicles prepared from vitamin D-deficient animals is reduced compared with vitamin D-sufficient animals; however, there is no effect on the final equilibrium concentration, indicating that vitamin D alters the rate of, but not the capacity for, uptake. **Vitamin D-inducible calcium selective ion channels *CaT1*** (***TRPV6***) and ***CaT2*** have been identified in the duodenal brush-border membrane. CaT1 knockout mice demonstrate reduced vitamin D-dependent intestinal calcium absorption, but it is not completely eliminated, indicating several pathways of calcium uptake. Evidence exists for calcium entry into the enterocyte via a mechanism involving endocytic vesicles. The relative importance of these different pathways for calcium entry into the enterocyte remains uncertain.

5.4 Transcellular Calcium Transport

Absorbed calcium must move from the brush-border membrane across the enterocyte to the basolateral surface for extrusion from the cell. The rate of free Ca^{2+} flux across the enterocyte has been estimated from the cell size, the presumed transcellular concentration gradient, and the diffusion constant of Ca^{2+} in water. The estimated value is two orders of magnitude slower than the observed absorption rate. **Three possible mechanisms** have been suggested for facilitating movement of calcium across the enterocyte: (**1**) ***intracellular facilitated diffusion*** involving binding of calcium to a calcium-binding protein, calbindin-D; (**2**) calcium transport in ***fixed intracellular organelles***; and (**3**) ***vesicular transport*** involving endosomes, lysosomes, and the cytoskeleton.

In the intestine, $1,25(OH)2D_3$ induces the synthesis of the vitamin D-dependent calcium-binding protein **calbindin-D**, In mammals, calbindin-D is a 9-kd protein, whereas in avian intestine it is present as a 28-kd protein. In general, there is a linear relationship between intestinal calbindin-D content and the calcium absorption rate. In addition, calbindin-D is more abundant in the proximal small bowel than in the ileum, paralleling the calcium absorption rates. It is postulated that calbindin-D acts as an **intracellular carrier** to facilitate movement of calcium through the cell by **greatly increasing the transcellular calcium gradient**. Based on a calbindin-D concentration of 100 μmol/L, an intracellular Ca^{2+} concentration of 100 nmol/L, a diffusion coefficient for calbindin-bound calcium equal to 0.15 of the value for free calcium, and two calcium-binding sites on calbindin-D, it has been calculated that this binding protein would cause a 75-fold increase of transport beyond that of free Ca^{2+} ion, a value nearly identical to the experimentally measured rate. Recent studies of calbindin-D_{9K} knockout mice, however, have shown no significant

reduction of calcium absorption compared to wild type controls, indicating that calbindin-D_{9K} is not essential for vitamin D-dependent calcium absorption.

Some studies reported vitamin D-stimulated binding of calcium to intracellular organelles such as Golgi apparatus, rough endoplasmic reticulum, or mitochondria, but these remain controversial. Vitamin D-dependent calcium transport has been observed in isolated Golgi membranes.

Recent research has suggested that much of vitamin D-dependent calcium absorption occurs via a **vesicular pathway** involving sequestration of calcium in endocytic vesicles, fusion of these endosomes with lysosomes, movement of vesicles and lysosomes along microtubules to the basolateral cell surface, and exocytosis from the enterocyte. Calbindin-D has been found within vesicles in the enterocytes, perhaps explaining their avidity for calcium. During calcium absorption, calbindin-D has been reported to decrease in the enterocyte and to appear in the core of the villus, suggesting extrusion from the cell along with the calcium. Some researchers have suggested that this vesicular transport pathway is initiated by a $1,25(OH)_2D_3$-induced rise in intracellular Ca^{2+} concentration and activation of cellular protein kinases.

5.5 Calcium Extrusion

Calcium is extruded from the basolateral enterocyte surface against a steep electrochemical gradient requiring metabolic energy. As in many cell types, enterocytes and colonocytes contain an ***ATP-dependent calcium pump*** in the basolateral membrane called the **calcium-transporting ATPase**. This transport activity is correlated with intestinal calcium absorption, as it is greater in the proximal than distal bowel and greater in villus than crypt cells, and declines with aging. The calcium-binding affinity of the calcium-transporting ATPase is about 2.5 times that of calbindin-D, providing a gradient of binding affinities from brush-border membrane to basolateral membrane that favors vectorial transport. Studies with basolateral membrane vesicles have shown that calcium pump activity is **stimulated by $1,25(OH)_2D_3$**. Kinetic analyses showed that this was due to an ***increase in transport capacity***, ***with no change in affinity***. In **vitamin D-replete animals**, the calcium-pumping rate is greater than the calcium absorption rate, indicating that pump activity can accommodate the active calcium transport process. In **vitamin D deficiency**, however, pump activity could be rate-limiting, and treatment of vitamin D-deficient animals with $1,25(OH)_2D_3$ has been shown to increase immunodetectable pumps in the basolateral membrane.

At least four genes coding for plasma membrane calcium-transporting ATPases have been identified, and the diversity of pump proteins is further increased by alternative splicing of these gene transcripts. Different isoforms of the calcium-transporting ATPase are found in the small intestine and colon. Enterocytes contain a **basolateral Na^+/Ca^{2+} exchange system**, but this system is not regulated by

vitamin D, does not vary along the length of the small intestine, and has much less activity than the calcium-transporting ATPase. The possibility of vesicular calcium transport has been discussed above.

5.6 Other Factors Affecting Calcium Absorption

In addition to vitamin D, a number of other dietary constituents have been suggested to alter intestinal calcium absorption. **Phosphate**, **oxalate**, **phytate**, and **fatty acids** can precipitate soluble calcium, and certain types of **dietary fiber** can bind calcium. In some studies, **lactose** has been shown to increase calcium absorption, probably by influencing the paracellular pathway. Overall, at intakes of the typical American diet, these factors appear to influence calcium absorption only modestly. **Bile salts** increase calcium absorption by similar mechanisms to those previously described for iron absorption.

Clinical Correlations

Case Study 1

A 40-year-old woman with long-standing Crohn's disease had a resection of 150 cm of terminal ileum. Two years later she develops recurrent disease and is placed on the treatment of sulfasalazine. After 1-year treatment, she presents with complaints of fatigue and numbness and tingling in her feet. She reports eating a normal, unrestricted diet and does not take vitamin supplements. Laboratory evaluation shows a megaloblastic anemia and reduced serum folate and vitamin B_{12} levels.

Questions

1. **What is the pathogenesis of vitamin B_{12} deficiency in this patient, and what treatment would you recommend?**
   Answer: Vitamin B_{12} bound to intrinsic factor (IF) is absorbed via binding to cubilin and then taken up by endocytosis in the ileum. This patient with a **large ileal resection** would have ***vitamin B_{12} malabsorption***. **Large doses of oral vitamin B_{12}** may be absorbed in the proximal small intestine by passive diffusion or other mechanisms and could be tried as replacement therapy; however, **intramuscular or intranasal vitamin B_{12}** will correct the deficiency.
2. **What is the pathogenesis of this patient's folate deficiency?**
   Answer: The patient reports eating an unrestricted diet thus inadequate folate intake is unlikely. Many Crohn's disease patients, however, avoid eating fruits and vegetables, which are important dietary sources of folate. Folate is absorbed mainly in the proximal intestine, which is likely to be functioning well in this patient. She is, however, taking the medication sulfasalazine as treatment for her Crohn's disease, and **sulfasalazine** is an **inhibitor of folate conjugase**

(the enzyme that deconjugates polyglutamyl folates) and **of monoglutamyl folate uptake**. It is likely that her folate deficiency is due to this drug-nutrient interaction.

3. **What would be the results of a Schilling test in this patient?**
   Answer: The Schilling test would demonstrate **reduced urinary excretion** of **orally administered radioactive vitamin B_{12}** given either alone or with IF.

Case Study 2

A 65-year-old man undergoes a total gastrectomy for a gastric adenocarcinoma. After surgery, he is not given any vitamin or mineral supplements. Four years later, he presents to his doctor with marked fatigue. He is found to be severely anemic. The average size of his red blood cells is normal, but examination of a blood smear shows that some cells are abnormally large, whereas others are very small.

Questions

1. **Which nutritional deficiencies are likely to be causing this patient's anemia?**
   Answer: The patient is likely to be **vitamin B_{12}-deficient**, since total gastrectomy eliminates secretion of IF needed to bind vitamin B_{12} prior to receptor-mediated ileal uptake. He is also likely to be **iron-deficient**, as gastric acid is needed to solubilize dietary ferric iron and he has not received iron supplementation. **Folate absorption** could be somewhat impaired since folate is optimally absorbed in the proximal intestine at a slightly acidic pH, and the lack of gastric acid will cause the intraluminal pH to rise. **Patients who have had total gastrectomy**, **however**, **are *not* commonly folate-deficient**, probably because the lack of gastric acid permits the proliferation of ingested folate-producing bacteria in the upper intestine and that folate is absorbed.
2. **How do you explain the findings regarding the size of his red blood cells?**
   Answer: Vitamin B_{12} and **folate deficiency** cause a ***macrocytic anemia***, whereas **iron deficiency** causes a ***microcytic anemia***. In this patient with both vitamin B_{12} and iron deficiency, the average red blood cell size may be normal, but individual erythrocytes may be either macrocytic or microcytic.
3. **Why did this patient become vitamin B_{12}-deficient in only 4 years?**
   Answer: The daily turnover rate of vitamin B_{12} is normally very low compared with body stores. Vitamin B_{12} depletion will therefore not occur until 10–20 years of dietary lack. This patient, however, will have malabsorption of both dietary vitamin B_{12} and vitamin B_{12} present in bile. The **lack of reabsorption** and **conservation of biliary vitamin B_{12}** results in ***accelerated depletion***.

Case Study 3

A 45-year-old woman has a 15-year history of primary biliary cirrhosis, a disease characterized by progressive destruction of intrahepatic bile ducts and reduction of bile flow. She presents with a fractured humerus after lifting a small bag of groceries. She is found to have markedly reduced bone density. Laboratory testing

reveals reduced serum calcium, phosphorus, and 25-hydroxyvitamin D_3 levels, markedly reduced urinary calcium excretion, and 30 g/day of steatorrhea (normal, less than 7 g/day). She is noted to eat few dairy products because they produce gas and diarrhea.

Questions

1. **What factors contribute to calcium malabsorption in this patient?**
   Answer: The most important cause of calcium malabsorption in this patient is **vitamin D deficiency**, as vitamin D is necessary for efficient calcium transport through the transcellular pathway (see Chap. 8 for a discussion of vitamin D absorption). In addition, bile salts directly enhance intestinal calcium absorption, and **reduced bile flow** in this patient would diminish the intraluminal bile salt concentration. The patient has steatorrhea, and calcium complexes with unabsorbed fatty acids, forming soaps that are excreted in the stool.
2. **What nutritional therapy would you prescribe for her metabolic bone disease?**
   Answer 2: The patient should receive **supplementation** to correct her vitamin D deficiency. **25-Hydroxyvitamin D_3** is absorbed better than vitamin itself in patients with severe cholestatic liver disease and may be the better form of therapy (Chap. 8). She should be placed on a **low fat diet** to decrease her steatorrhea and be given **calcium supplementation** in the form of low-lactose, low-fat dairy foods or pharmaceutical preparations.
3. **How does her avoidance of dairy products contribute to her bone disease?**
   Answer 3: The patient has symptoms of lactose intolerance and avoids dairy products (see Chap. 6 for a discussion of lactose absorption). **Dairy foods** are the ***major dietary sources*** of both **vitamin D** and **calcium**. In addition, **lactose** may ***enhance the efficiency of calcium absorption*** through the ***paracellular pathway***.

Case Study 4

A 55-year-old man is found on routine testing to have abnormal liver function tests (elevated serum transaminases and alkaline phosphatase). His father died of cirrhosis and congestive heart failure. Laboratory testing shows that his serum iron is 300 mcg/dl, serum iron-binding capacity is 350 mcg/dl, and serum ferritin is 4,500 ng/ml.

Questions

1. **What additional tests would you order?**
   Answer: The patient likely has hereditary hemochromatosis. Genetic testing can be performed for certain mutations causing this disorder. The most common mutation is in the HFE gene (mutation C282Y). The HFE protein is involved in the regulation of hepcidin secretion from the liver by iron, which in turn controls intestinal iron absorption. The patient should also have a liver biopsy to assess the degree of liver damage from hepatic iron overload.

2. **What other gene mutations could cause hemochromatosis?**
   Answer: Mutations in the genes coding for hepcidin, transferrin receptor 2, ferroportin, and ferritin, all genes involved in the regulation of iron absorption, can also cause hemochromatosis.
3. **What is the appropriate treatment for this patient?**
   Answer: The patient needs to be placed on a regular schedule of blood donation until the body iron burden is reduced, assessed by a fall in the serum ferritin level to 50–100 ng/ml.

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