

PATHOLOGY LAB

IRON

General

RDA: 10 mg/d (only 10% of iron is absorbed from the intestines)

Dietary iron

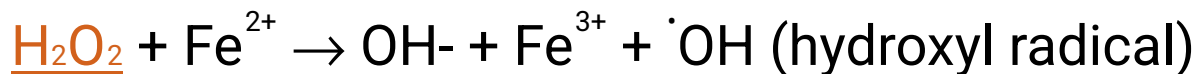
Heme iron: from meat

Non-heme iron: from plants

Ferrous iron (Fe^{2+})

Ferric iron (Fe^{3+})

Free iron: Fe^{2+} can lead to reactive oxygen species via the Fenton reaction.



Hydroxyl radicals $\rightarrow$ oxidative stress $\rightarrow$ DNA damage



~ 20% of heme iron is absorbable.

Only ~ 2% of non-heme iron is absorbable. The absorption of non-heme iron can be inhibited by phytates (in oats/bran), polyphenols (in tea), and calcium.



IRON ABSORPTION AND TRANSPORT

Iron absorption

Gastric acid leads to low pH and promotes dissolution of iron in the duodenum

Absorption occurs in the duodenum and upper jejunum

The enzyme hepcidin regulates intestinal absorption of iron.

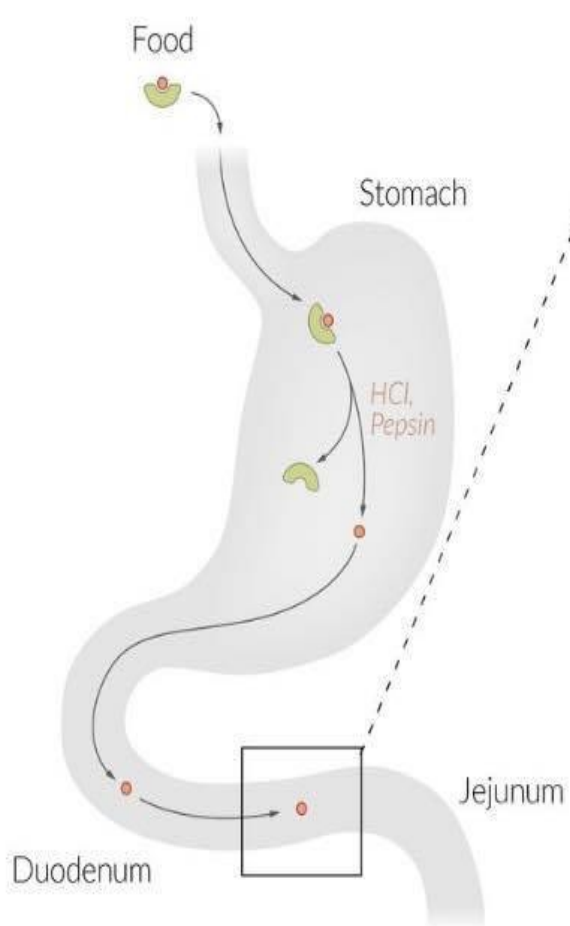
Hepcidin is synthesized in the liver

Its production is regulated by the human hemochromatosis protein (HFE protein)

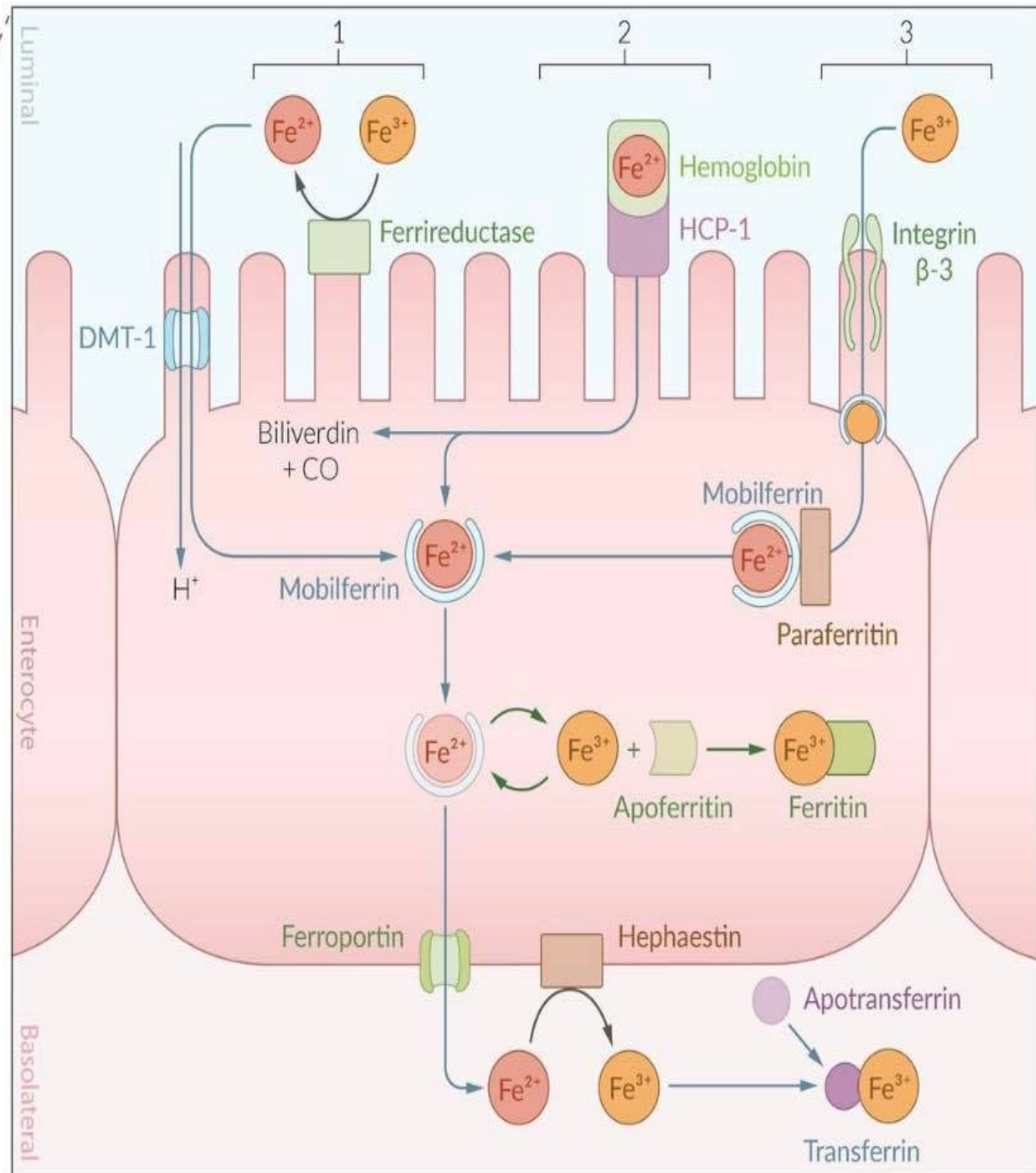
Increased body stores of iron → ↑ HFE protein → ↑ hepcidin
→ prevention of iron absorption

Iron deficiency → ↓ hepcidin → ↑ iron absorption





- Iron (Fe^{3+} , Fe^{2+})
- Protein from food



Ferric iron (non-heme iron, Fe^{3+}) is mainly reduced to ferrous iron (Fe^{2+}) and then absorbed.

Vitamin C increases absorption (converts $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$).

Calcium decreases absorption (due to chelation of iron).

A minority of iron is absorbed as ferric iron (Fe^{3+}).

Heme iron can be directly absorbed into intestinal cells.

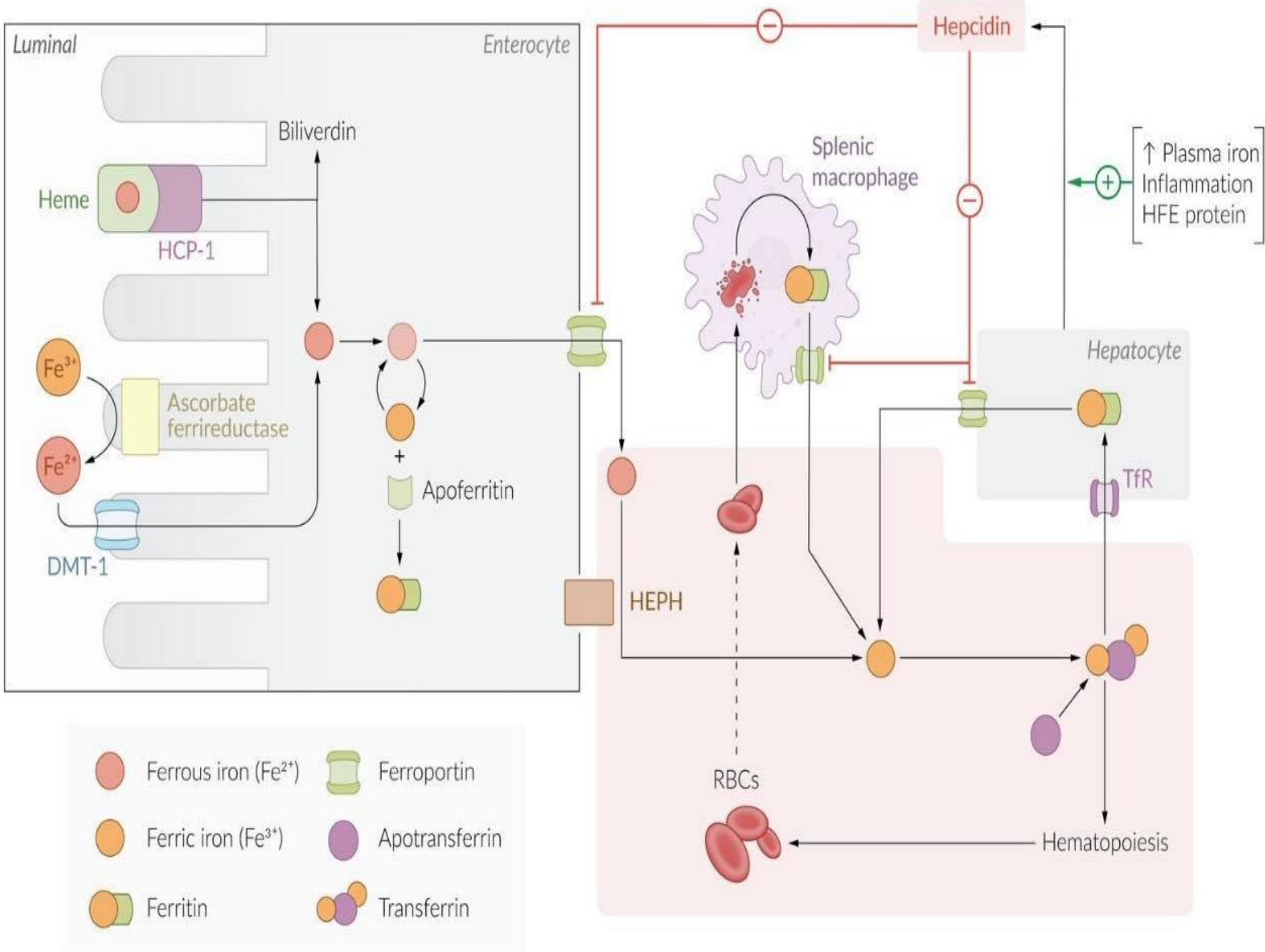


Iron transport

Ferroportin (inhibited by hepcidin) transports ferrous iron (Fe^{2+}) from the enterocytes to the bloodstream. The enzyme ferroxidase (also known as ceruloplasmin) oxidizes ferrous iron back to ferric iron (converts $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$).

Transferrin: binds and transports the ferric iron (Fe^{3+}) to the erythroid precursor cells (in bone marrow) for hemoglobin synthesis.





IRON STORAGE, RECYCLING, AND LOSS

Iron storage

Total body iron content: ~ 3 g ()/~ 6 g ().

Iron is present in the body in two forms:

Functional iron (80%): hemoglobin (largest proportion),
myoglobin, cytochrome enzymes

Storage iron (20%): as Fe^{3+}

Ferritin: hepatocytes, macrophages, enterocytes

Hemosiderin: hepatocytes

Ferritin levels correlate strongly with that of total body iron. However, ferritin is an acute phase reactant, and its levels also increase during inflammation.



About two third of the total body iron is in the circulation as heamoglobin.

Iron is stored in reticuloendothelial cells, hepatocytes and skeletal muscle cells.

About two third of this is stored as ferritin and one third as haemosidrein in normal individuals

Small amounts of iron are also found in plasma(bound to transferrin), with some in myoglobin and enzymes



FERRITIN VS. HAEMOSIDERIN

Ferritin: water soluble complex of iron and protein. It is more easily mobilized than hemosidein for Hb formation.

It is present in small amounts in plasma

Haemosiderin: is an insoluable iron-protein complex found in mcrophage in bone marrow, Liver and spleen.

Unlike ferritin it is visible by light microscopy in tissue sections and bone marrow films after staining by perls reaction



Iron recycling

Reticuloendothelial macrophages (in the spleen and liver) phagocytose senescent RBCs and release iron from hemoglobin.

Transferrin binds the released iron and transports it to the bone marrow for erythropoiesis.

Iron loss

Shedding of skin and mucosal epithelial cell → daily loss of 1–2 mg of iron

Any source of bleeding (e.g., menstruation, occult GI bleed) increases iron loss.



Function

Integral component of hemoglobin and myoglobin

Cofactor for:

Cytochrome C, cytochrome P450

Peroxidases

Metalloproteases (e.g., NADH dehydrogenase)

Phosphoenolpyruvate carboxykinase

(gluconeogenesis), aconitase (Krebs cycle)

Ribonucleotide reductase (DNA and RNA synthesis)



IDA- EPIDEMIOLOGY

Most common form of anemia worldwide
~ 3% of the general population in the United States is affected.

African-American and Mexican-American populations in the US are at increased risk.

Prevalence highest in:

- Children up to 5 years of age

- Young women of child-bearing age (due to menstrual blood loss)

- Pregnant women



ETIOLOGY

The most common causes of IDA can be divided by age group and pathophysiologic mechanism.

Based on age

Infants

Exclusive intake of nonfortified cow's milk (cow's milk has a very low concentration of iron and also disrupts iron absorption)

Exclusive breastfeeding after 6 months of age

Children

Malnutrition (mainly resource-limited countries)

Excessive intake of cow's milk (> 24 ounces/700 mL per day)

Meckel diverticulum



Adolescence: menarche/menstruation

Adults (20–50 years)

Menorrhagia or pregnancy (females)

Peptic ulcer disease (males)

Adults > 50 years

Colon polyps/carcinoma in high-income countries

Hookworm (Ancylostoma duodenale, Necator americanus) in resource-limited countries

In resource-rich countries, adults > 50 years that present with IDA should have colon polyps/carcinoma ruled out as a potential underlying etiology.



Based on underlying mechanism

Iron losses

Bleeding

Gastrointestinal bleeding

Occult gastrointestinal malignancy (e.g., colon cancer)

Hookworm infestation (e.g., *Ancylostoma* spp., *Necator americanus*)

Peptic ulcer disease

Increased risk with NSAID use

Menorrhagia

Hemorrhagic diathesis (e.g., hemophilia, von Willebrand disease)

Meckel diverticulum

Dialysis-dependent renal failure

Frequent blood donation



Decreased iron intake

Chronic undernutrition

Cereal-based diet

Strict vegan diet

Decreased iron absorption

Achlorhydria/hypochlorhydria (e.g., due to autoimmune or H. pylori infection-induced atrophic gastritis)

Inflammatory bowel disease, celiac disease

Surgical resection of the duodenum

Bariatric surgery



- Increased demand
 - Pregnancy
 - Lactation
 - Growth spurt
 - Erythropoietin (EPO) therapy



PATHOPHYSIOLOGY

Iron deficiency → ↓ binding of iron to protoporphyrin (last reaction in heme synthesis) → ↓ production of hemoglobin



CLINICAL FEATURES

Signs and symptoms of anemia

Fatigue, lethargy

Pallor (primarily seen in highly vascularized mucosa, e.g., the conjunctiva)

Cardiac: tachycardia, angina, dyspnea on exertion, pedal edema, and cardiomyopathy in severe cases

Brittle nails, koilonychia (spoon-like nail deformity) ,
hair loss

Pica, dysphagia

Angular cheilitis : inflammation and fissuring of the corners of the mouth

Atrophic glossitis : erythematous, edematous, painful tongue with loss of tongue papillae (smooth, bald appearance)







IDA can be associated with Plummer-Vinson syndrome (PVS)

Triad of iron deficiency anemia, post cricoid dysphagia, and upper esophageal webs

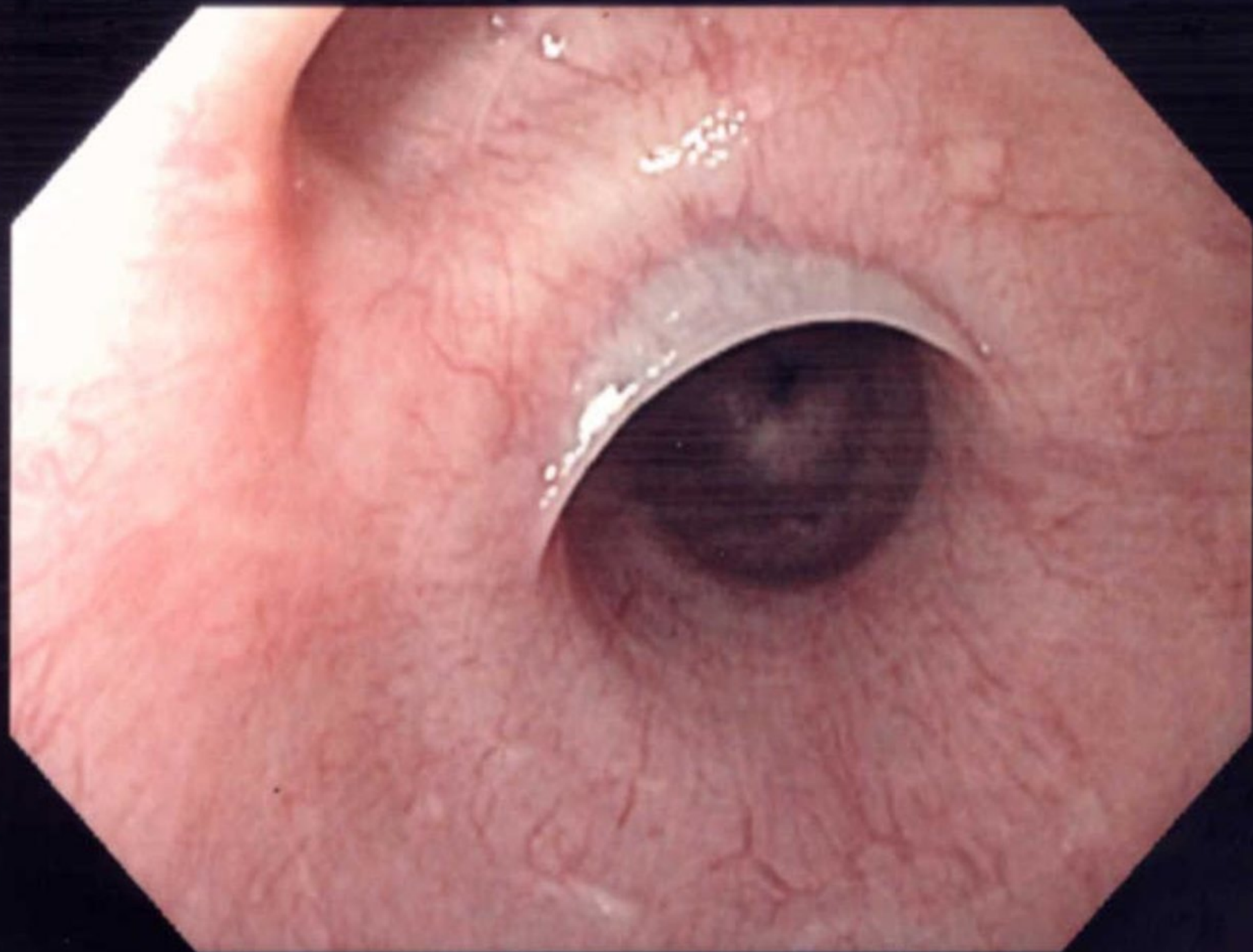
Thin membranes of normal esophageal tissue protrude into the esophagus causing symptoms such as dysphagia, odynophagia, and food impaction.

Acquired esophageal webs are much more common than congenital webs and are mainly observed in Plummer–Vinson syndrome.

Associated with an increased risk of esophageal squamous cell carcinoma and glossitis

Etiopathogenesis unknown





DIAGNOSTICS

Routine studies

CBC

↓ Hemoglobin: Anemia is typically defined as a hemoglobin level less than two standard deviations below the mean (adjusted for age and sex).

Women: nonpregnant < 12 g/dL; pregnant < 11 g/dL

Men: < 13 g/dL

↓ Hematocrit

↑ Platelet count (reactive thrombocytosis)



Red blood cell indices

RBC: initially normal (decreased with prolonged deficiency)

Mean corpuscular volume

Typically ↓ (microcytic)

May be normal (normocytic)

Children: age-specific lower limit of normal for MCV values is (70 + age in years)

Mean corpuscular hemoglobin

Typically ↓ (hypochromic)

May be normal (normochromic)

Normal or ↓ reticulocyte count

Red cell distribution width (RDW)

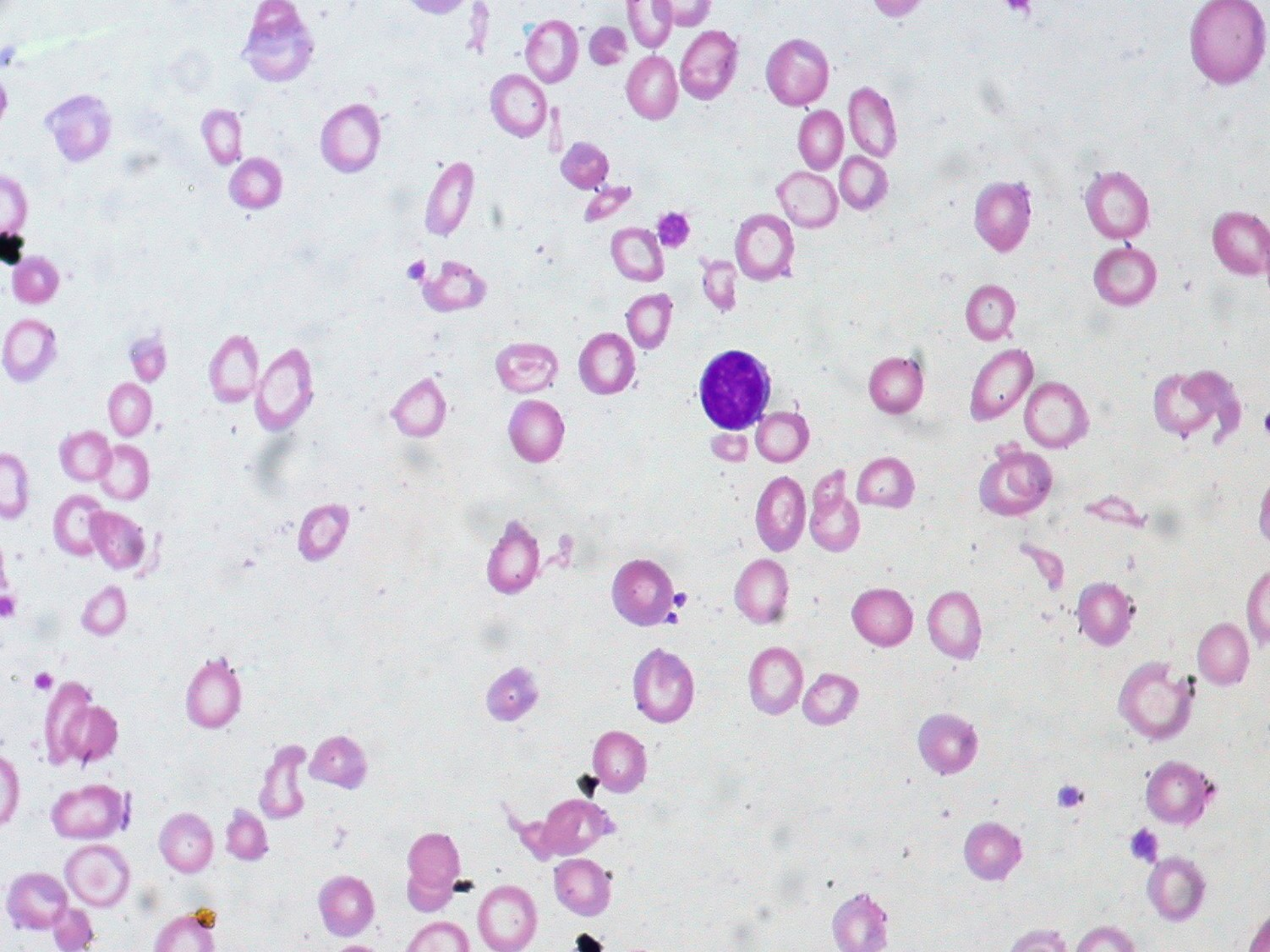
Increases in established IDA

Can help distinguish IDA from anemia of chronic disease and certain types of thalassemia (in which the RDW is usually normal).



Peripheral blood smear: anisocytosis and
hypochromasia (increased zone of central pallor)





Iron studies

Best initial test: ↓ serum ferritin (Reference ranges vary (e.g., 15–200 ng/mL in men, 12–150 ng/mL in women). Consult local laboratory standards.)

< 45 ng/mL: IDA highly likely

45–100 ng/mL: Consider further evaluation (e.g., transferrin, TSAT).

Comorbid chronic inflammatory conditions, CKD, CHF: further evaluation even if ferritin is normal

- ✓ Ferritin is an acute phase reactant and may be falsely elevated in these patients. Additional tests (e.g., CRP, transferrin, and others) are often necessary.



Further evaluation

↓ Serum iron

↑ Serum transferrin and total iron binding capacity (TIBC)

↓ Transferrin saturation (TSAT)

↑ Serum soluble transferrin receptor (sTfR)

✓ Unlike ferritin, this parameter is not sensitive to inflammation.

Additional tests to consider

Serum free erythrocyte protoporphyrin: elevated

✓ In IDA, iron is low and protoporphyrin is normal. FEP is protoporphyrin that is not bound to iron in RBCs.

EPO: normal or elevated



Bone marrow biopsy

Gold standard, but only indicated in patients with suspected IDA and equivocal iron studies.

Findings: decreased or absent stainable iron stores

- ✓ Unreliable in patients who have received a blood transfusion or parenteral iron, in which case stores will be normal or high



In combination with an elevated TIBC, low ferritin and iron levels are diagnostic of iron deficiency anemia.

Increased ferritin does not rule out iron deficiency anemia. It can be increased in response to simultaneous inflammation.



TREATMENT

Iron therapy

Oral supplementation is effective and inexpensive, however, adherence is often poor due to side effects. Parenteral iron therapy is beneficial in select cases.

Adverse effects

Gastrointestinal discomfort, nausea, constipation, black discoloration of stool



VITAMIN B12 DEFICIENCY (COBALAMIN DEFICIENCY)

Vitamin B₁₂ deficiency

Epidemiology

Young adults: 5–7%
Adults: 10–30%

Etiology

Decreased intake
(e.g., malnutrition,
vegan or restricted diet)

Decreased absorption
(e.g., in pernicious anemia)

Increased requirement
(e.g., during pregnancy)

Prognosis

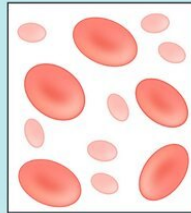
Without treatment:
irreversible neurological damage

With timely treatment:
complete remission possible

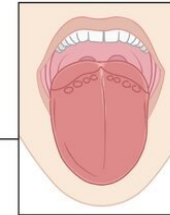
Anemia-related findings

Scleral icterus

Pallor



Megaloblastic
anemia



Glossitis

Symptoms of pernicious anemia may occur

Hashimoto thyroiditis

Gastritis
↑ Risk of gastric cancer

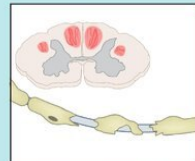
Vitiligo

Infertility

Hyperpigmentation

Neurological symptoms

Depression, psychosis
Decreased visual acuity
Spinocerebellar ataxia



Subacute combined
degeneration of spinal cord

Bladder dysfunction

Erectile dysfunction

Peripheral neuropathy
(distal paresthesia, hypoesthesia, pain)



VITAMIN B12

Sources

Synthesis: Only microorganisms can produce vitamin B₁₂.

The colonic flora produces vitamin B₁₂.

However, vitamin B₁₂ produced by colonic flora cannot be absorbed by the body because the terminal ileum (absorption site) lies prior to the colon.

Found almost exclusively in animal products



Absorption

Stomach: Cobalamin is released from ingested proteins by pepsin and binds to the glycoprotein haptocorrin (R-protein), which protects it from gastric acid.

Duodenum: Cobalamin is released from haptocorrin by trypsin and binds to intrinsic factor (IF), a protein produced by the parietal cells of the stomach that facilitates cobalamin absorption in the ileum.

Terminal ileum: cubilin receptor-mediated endocytosis of the intrinsic factor-cobalamin complex → breakdown of IF in enterocytes, release of cobalamin, followed by binding to carrier protein transcobalamin II and then enters the plasma → cobalamin is either delivered to metabolically active tissues or stored in the liver



Transport in blood: via transcobalamin

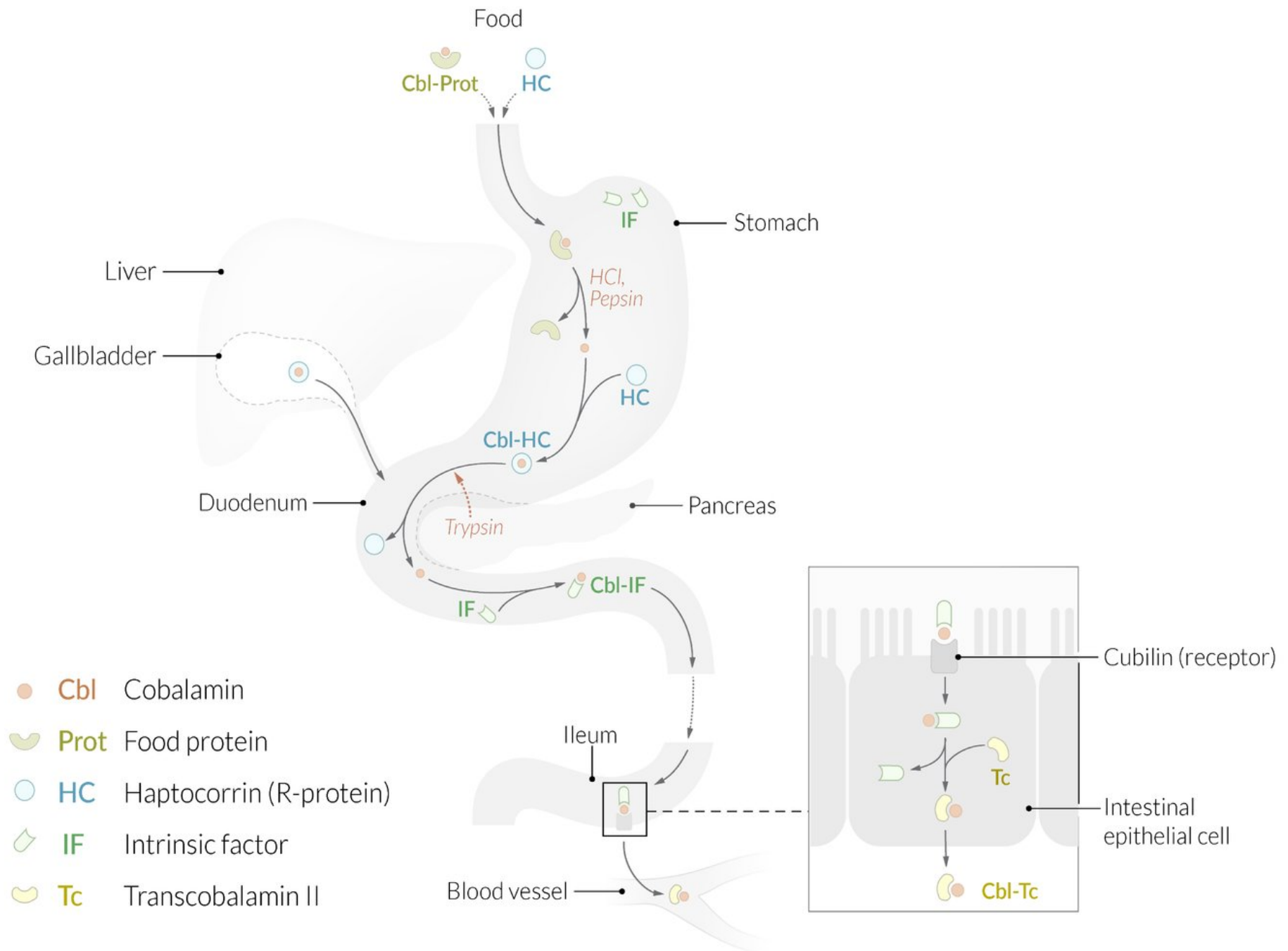
Storage: large reserve pool

60% in the liver (enough for approx. 3–4 years)

30% in muscle tissue

Vitamin B₁₂ is the only water-soluble vitamin that is stored in the body in significant amounts.





Functions

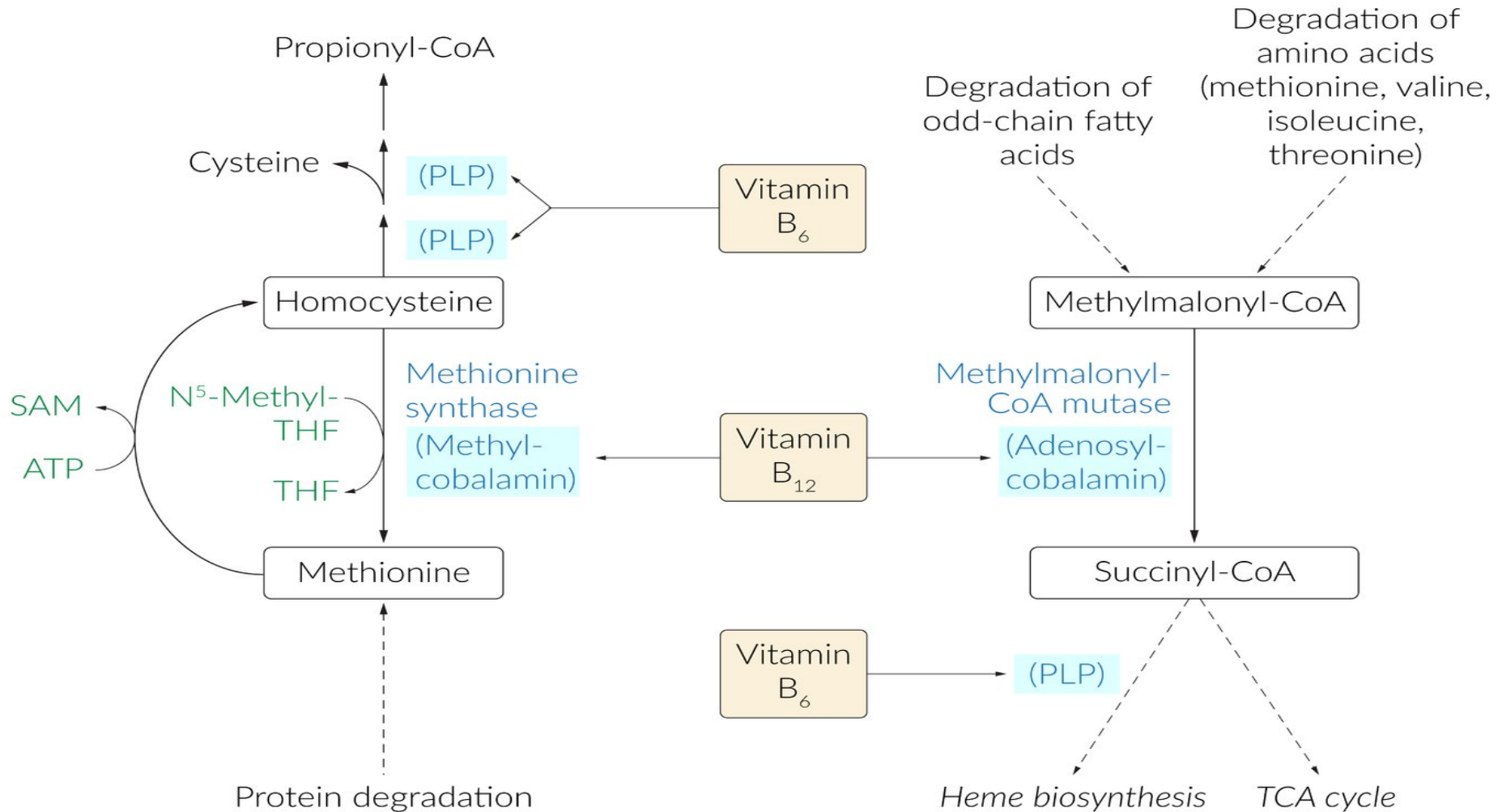
Cofactor for enzymes:

Methionine synthase: Methylcobalamin is required for the transfer of methyl groups (e.g., in DNA synthesis).

Methylmalonyl-CoA mutase (e.g., in odd-chain fatty acid metabolism)



METABOLIC FUNCTIONS OF VITAMINS B6 AND B12



ETIOLOGY

Malabsorption

↓ Intrinsic factor (IF)

Atrophic gastritis due to

Autoimmune atrophic gastritis: most common cause of
vitamin B₁₂ deficiency

H. pylori infection

Gastrectomy



Reduced uptake of IF-vitamin B₁₂ complex in terminal ileum due to:

Alcohol use disorder

Crohn disease, celiac disease

Pancreatic insufficiency

Surgical resection of the ileum

Diphyllobothrium latum (tapeworm) infection

Bacterial overgrowth

Enteritis

Achlorhydria



Malnutrition

Anorexia nervosa

Strict vegan diets: occurs only after years of a strict diet that excludes all animal products (unlike folate deficiency, which occurs within a few months of insufficient intake)

Increased demand: e.g., during pregnancy, breastfeeding, fish tapeworm (Diphyllobothrium latum)^[1] infection, and leukemia

Drugs: metformin, proton-pump inhibitors, colchicine



PATHOPHYSIOLOGY

Physiological function :

Vitamin B₁₂ is a water-soluble cofactor for enzymatic reactions of DNA synthesis (via methionine synthase) and odd-chain fatty acid metabolism (via methylmalonyl CoA mutase). A deficiency of vitamin B₁₂ leads to enzyme dysfunction.



Dysfunctional methionine synthase (normally converts homocysteine to methionine, thereby demethylating N5-methyl-THF to THF)

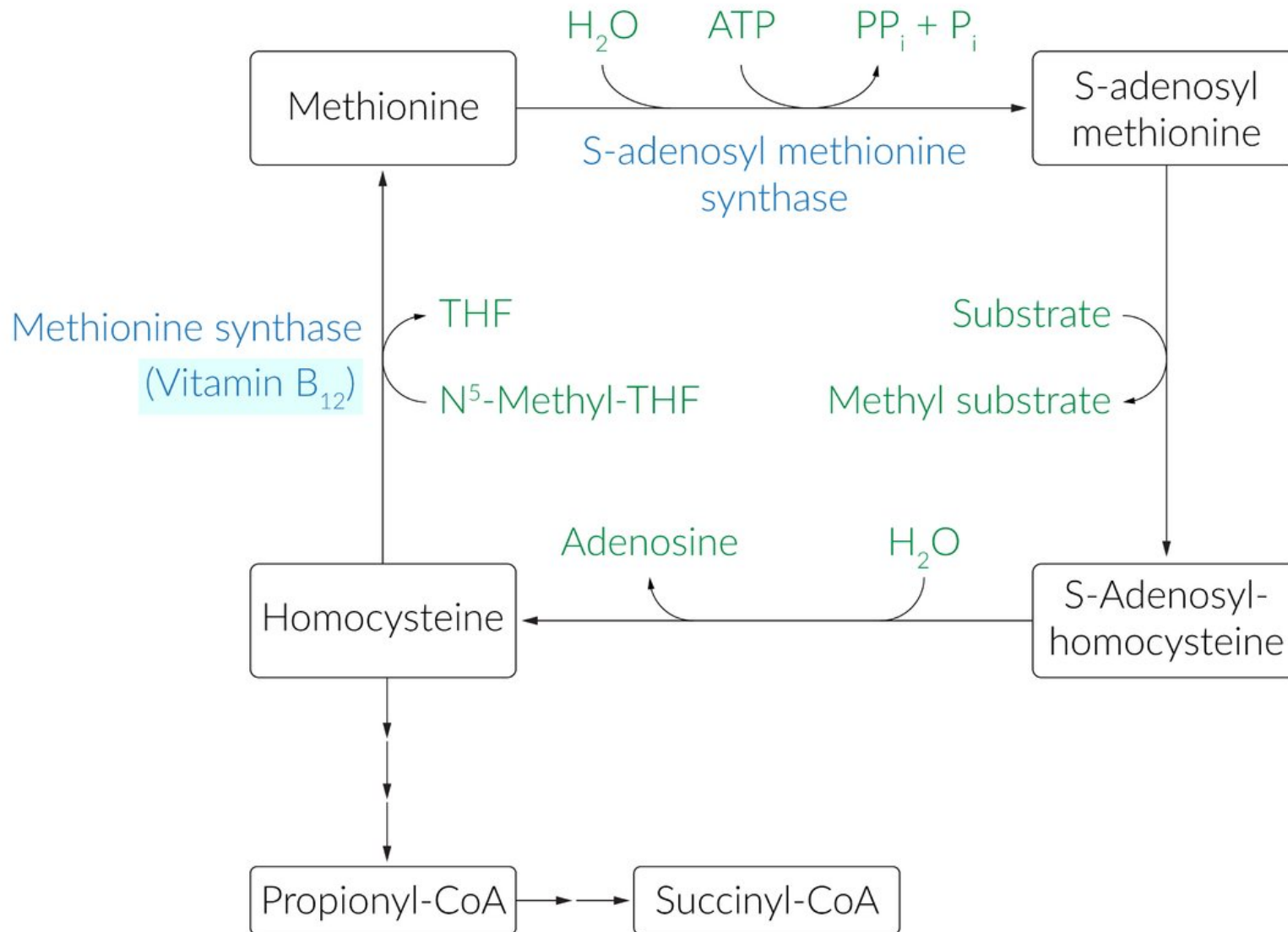
↓ Tetrahydrofolate (THF; cofactor in purine synthesis)
→ ↓ DNA synthesis → large, nucleated hematopoietic cells, including megaloblasts (Inhibition of DNA synthesis results in the formation of megaloblasts, which are large, nucleated, immature RBCs.) → megaloblastic precursors undergo apoptosis or are phagocytosed by macrophages → pancytopenia (including megaloblastic anemia)

↓ Methionine → neuropathy ^[2]

↑ Homocysteine → endothelial damage → predisposes to cardiovascular disease

Can also cause secondary folate deficiency





Dysfunctional methylmalonyl CoA mutase

Methylmalonyl CoA cannot be converted to succinyl CoA → accumulation of methylmalonyl CoA and its precursor propionyl CoA, as well as their associated odd-chain fatty acids, which cannot be completely metabolized

Propionyl CoA replaces acetyl CoA in neuronal membranes → demyelination → neurological manifestations

Folate deficiency also leads to low levels of THF, resulting in megaloblastic anemia.



PERNICIOUS ANEMIA

A type of vitamin B₁₂ deficiency caused by autoantibodies against intrinsic factor and/or gastric parietal cells (type II hypersensitivity reaction)

Antiparietal cell antibodies : target gastric parietal cells

Causes ↓ acid production and atrophic gastritis

↓ Intrinsic factor production → ↓ vitamin B₁₂ absorption in terminal ileum

Anti-IF antibodies : bind intrinsic factor and block the vitamin B₁₂ binding site

Associated with other autoimmune diseases (e.g., hypothyroidism, vitiligo)

Increases the risk of gastric cancer



CLINICAL FEATURES

Signs of anemia (e.g., fatigue, pallor)

Mild scleral icterus and/or jaundice (Caused by elevated indirect bilirubin, which results from increased hemolysis of erythrocyte precursors)

Neurological disturbances are generally symmetrical Peripheral neuropathy : tingling, numbness, pins-and-needles sensation, coldness (especially in the lower extremities)

Neuropsychiatric disease (e.g., reversible dementia, depression, paranoia)

Worsening vision



GLOSSITIS



DIAGNOSTICS

Hematological findings

Signs of megaloblastic anemia

↓ Hb

↑ MCV (macrocytic), ↑ MCH (hyperchromic)

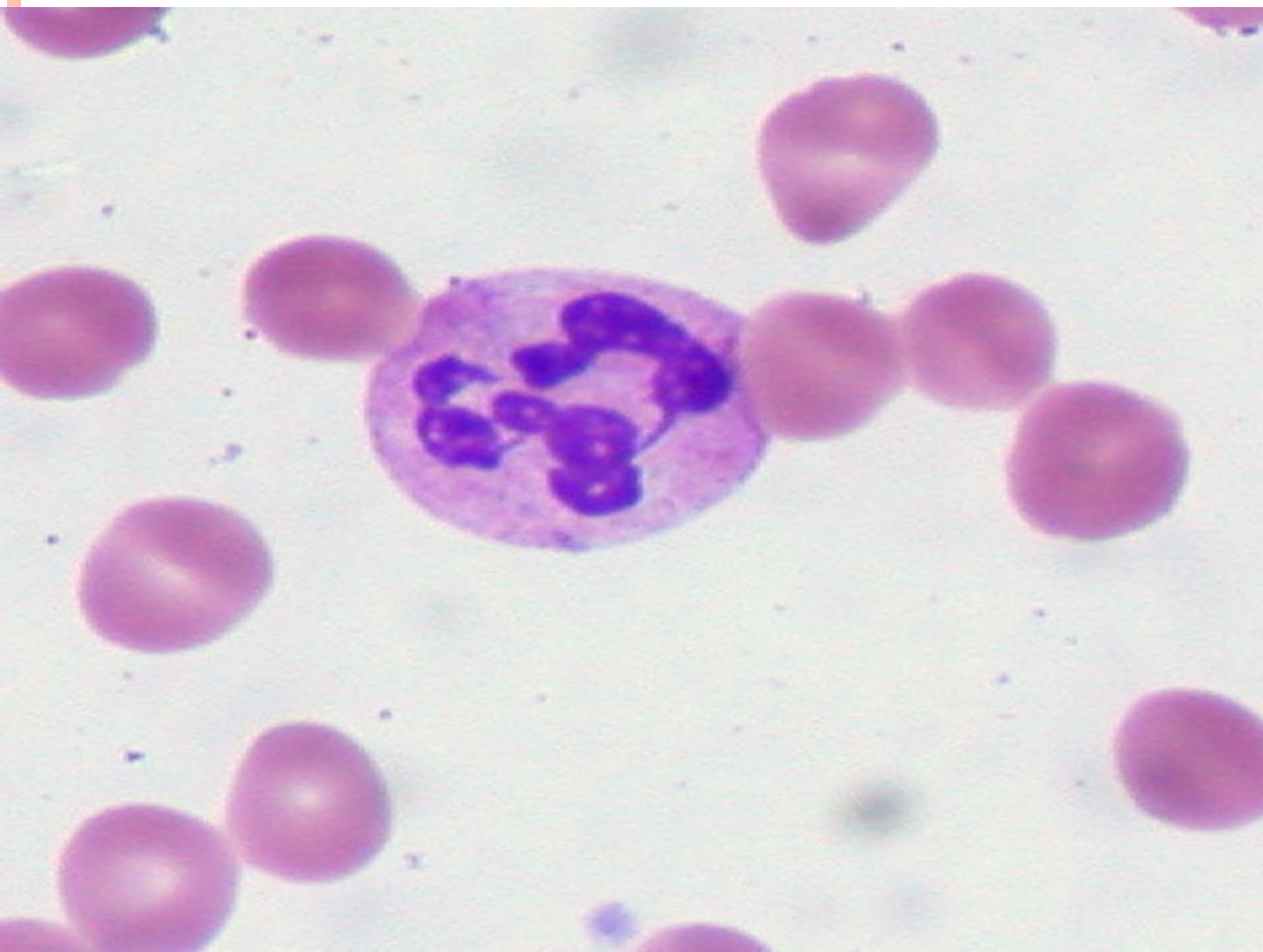
Hypersegmented neutrophils

↓ Reticulocytes

Frequently thrombocytopenia and leukopenia
(possibly pancytopenia)



HYPERSEGMENTED NEUTROPHILS



If vitamin B₁₂ serum levels are decreased,
determine the underlying cause

1-Test for autoantibodies

Anti-IF antibodies

Antiparietal cell antibodies

2-If autoantibodies are negative, perform: Schilling
test: a test to determine the cause of vitamin B₁₂
deficiency

Consists of four stages that assess impaired vitamin
B₁₂ uptake

No longer routinely used in clinical practice

Gastroscopy



If vitamin B₁₂ serum levels are normal:

Measure homocysteine: elevated in both vitamin B₁₂ deficiency and folate deficiency

Measure methylmalonic acid (MMA) to help rule out folate deficiency (MMA is normal in folate deficiency and elevated in vitamin B₁₂ deficiency)-

Without vitamin B₁₂, methylmalonyl CoA mutase cannot break down methylmalonyl CoA, which leads to the accumulation of MMA.



Starting folate treatment before excluding vitamin B₁₂ deficiency may correct anemia, but it can worsen neuropathy!

In contrast to vitamin B₁₂ deficiency, folic acid deficiency is generally not associated with neurological symptoms.



Treatment

IM supplementation of vitamin B₁₂



FOLATE DEFICIENCY (FOLIC ACID DEFICIENCY, VITAMIN B9 DEFICIENCY)

Physiology

Folate : coenzyme in single-carbon transfers and methylation reactions → DNA synthesis and amino acid metabolism ^[1]

Active form: tetrahydrofolate (THF), obtained after reduction of folate by dihydrofolate reductase

Sources

Leafy green vegetables , fruits, and liver

Fortified foods (e.g., bread, flour, and cereal)

Absorption: in the jejunum

Storage: liver (stores folate for up to three months, after which time signs and symptoms of deficiency begin to appear)



ETIOLOGY

Malnutrition

Insufficient intake, malnutrition (e.g., “tea and toast” diet)
Chronic alcohol use

Malabsorption

Small bowel disease (e.g., tropical sprue, celiac disease,
inflammatory bowel disease)

Surgical resection of the small intestine

Increased requirement

Pregnancy/lactation

Severe hemolytic anemia

Drug-related ^[2]

Methotrexate

Antiepileptic drugs (e.g., phenytoin)

Sulfonamides

Trimethoprim



PATHOPHYSIOLOGY

Decreased folate levels leads to decreased levels of tetrahydrofolate. This, in turn, leads to the following effects:

↓ DNA synthesis → megaloblastic erythropoiesis → megaloblastic anemia (and ↓ in other cell lines)

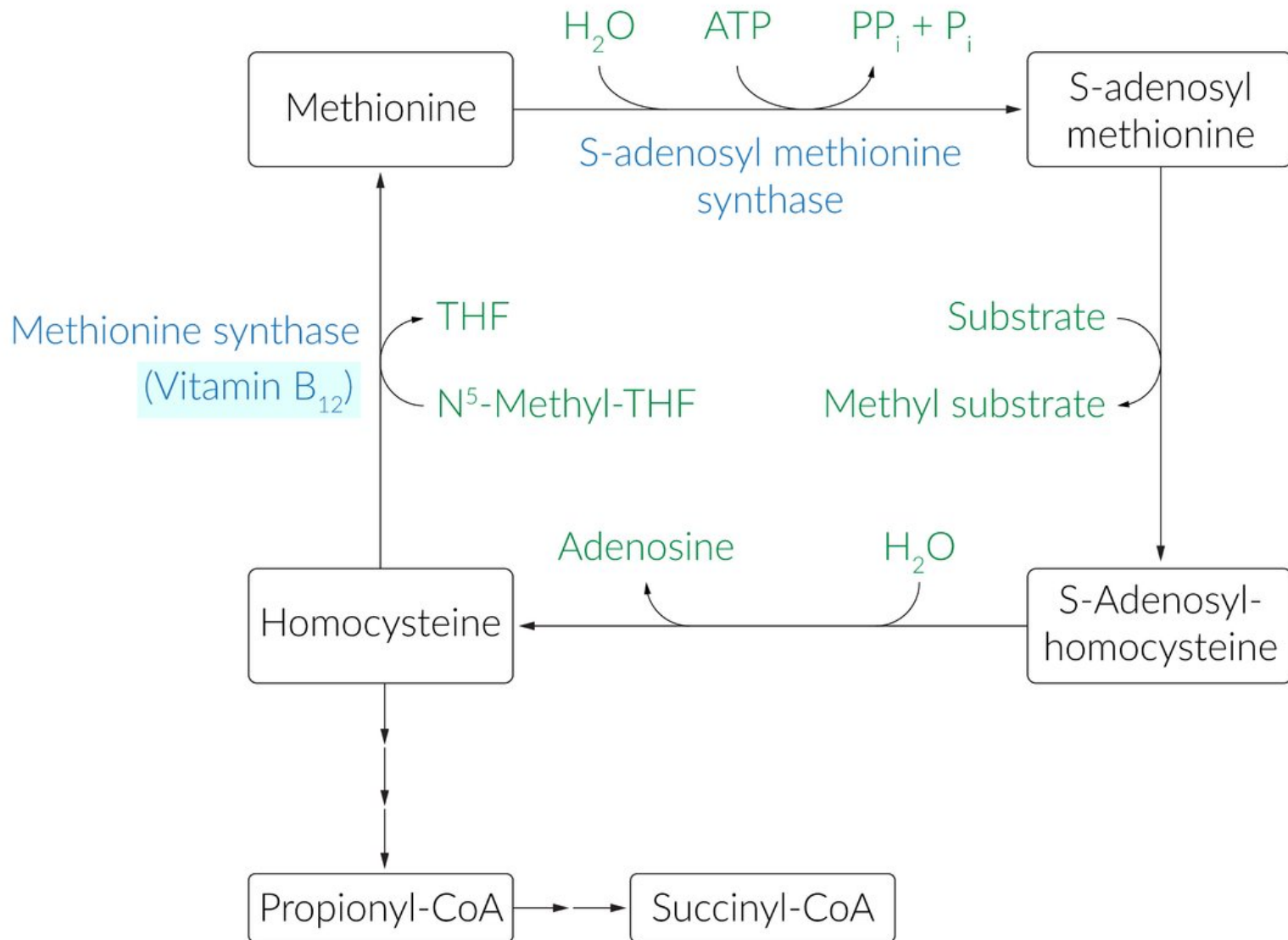
↓ Methionine and ↑ homocysteine → endothelial damage → ↑ risk of cardiovascular disease and thromboembolic events

During fetal development: nucleotide synthesis impairment → neural tube defects



Methionine synthase, also referred to as homocysteine methyltransferase, is the enzyme responsible for regenerating methionine from homocysteine, which requires methylated THF and vitamin B₁₂. With THF decreased, methionine cannot be synthesized causing homocysteine to accumulate.





CLINICAL FEATURES

Signs of anemia (e.g., fatigue, pallor)

Glossitis

Maternal deficiency: fetal spina bifida/anencephaly

Unlike vitamin B₁₂ deficiency, folate deficiency does not result in neurological symptoms.



DIAGNOSTICS

Macrocytic, megaloblastic anemia: $\uparrow$ MCV ($> 100 \mu\text{m}^3$)

$\uparrow$ Homocysteine

Methylmalonic acid (MMA) is normal (unlike in vitamin B₁₂ deficiency, where MMA is $\uparrow$)

Hypersegmented polymorphonuclear cells (PMNs)

Folate serum levels are not reliable

If folate deficiency is suspected, always exclude the possibility of vitamin B12 deficiency.



TREATMENT

Oral folate supplementation for four months or until hematologic recovery takes place

Nutritional counseling to increase folate intake

Because of the risk of neural tube defects, it is recommended that women planning to become pregnant initiate folate supplementation before conception.

