Chapter 43

Cobalamin (B_{12})

Overview

- Vitamin B_{12} is an antipernicious anemia factor.
- Vitamin B_{12} is generally absent from plant and vegetable foods unless they are contaminated by microbes.
- Liver is a good source of the three endogenous forms of vitamin B_{12} (methylcobalamin, 5'-deoxyadenosylcobalamin, and hydroxocobalamin).
- Ileal absorption of B_{12} requires intrinsic factor, which is synthesized by gastric parietal cells, as well as by pancreatic ductular cells in dogs and cats.
- The association of Co^{+++} with B_{12} is the primary recognized action of this trace element in mammalian metabolism.
- Entry of propionate into hepatic gluconeogenesis requires 5'deoxyadenosylcobalamin.
- The metabolism of vitamin B_{12} is intimately entwined with that of folic acid.
- Common symptoms of vitamin B_{12} deficiency include homocystinuria and methylmalonuria.
- A secondary intestinal dysfunction may develop from persistent cobalamin deficiency.

It was recognized in the early 1800's that **pernicious (or megaloblastic) anemia** may (in part) be due to a disorder of the digestive tract and assimilative organs. It was determined in the early 1900's that this condition could be reversed and controlled by eating raw or **mildly-cooked liver**. Therefore, investigators postulated that a gastric "**intrinsic factor (IF),**" combined with an "**extrinsic factor**" from ingested liver, would bring about absorption of an "**antipernicious anemia factor**." The extrinsic factor was later found to be the antipernicious anemia factor, **cobalamin (vitamin B_{12})**, and the IF was determined to be an important **glycoprotein**, secreted into gastric or abomasal juice by parietal cells, and additionally by pancreatic ductular cells in dogs and cats.

This water-soluble vitamin, which is thought to be the **largest essential nutrient absorbed intact through the distal ileal mucosa**, is generally absent from plant and vegetable foods, unless they are contaminated by microbes. Herbivores generally obtain cobalamin from symbiotic microbes in their digestive tracts (providing sufficient cobalt (Co^{+++}) is available for microbial B_{12} biosynthesis; see Chapter 52). Vitamin B_{12} is conserved by the healthy liver, a particularly good source of this vitamin for ominvores and carnivores.

Cobalamin is a complex molecule, consisting of a "**corrin ring**," which is a more hydrogenated form of the porphyrin ring associated with heme (see Chapter 32), with differences as well in the side chains of the ring. Cobalamin contains **Co^{+++}** rather than Fe^{++}, and a **5,6-dimethylbenzimidazole grouping** attached to the corrin

ring through a complex linkage, involving an unusual **ribose-phosphate** moiety (**Fig. 43-1**). The 5,6-dimethylbenzimidazole grouping is also chelated to Co^{+++} at the active center of the corrin ring. Vitamin B_{12} exists in four forms that differ in the nature of additional **R groups** attached to Co^{+++}. The cyano derivative, commonly known as **cyanocobalamin**, is the commercially available form. After transport in blood, cobalamin is usually taken-up by target cells as **hydroxocobalamin**, where it can be converted to **methylcobalamin** in the cytoplasm, or **5'-deoxyadenosylcobalamin** in mitochondria. In the liver, it is found in all three forms. The association of Co^{+++} with vitamin B_{12} is the primary recognized function for this trace element in mammalian metabolism (although other secondary actions are also known; see Chapter 52).

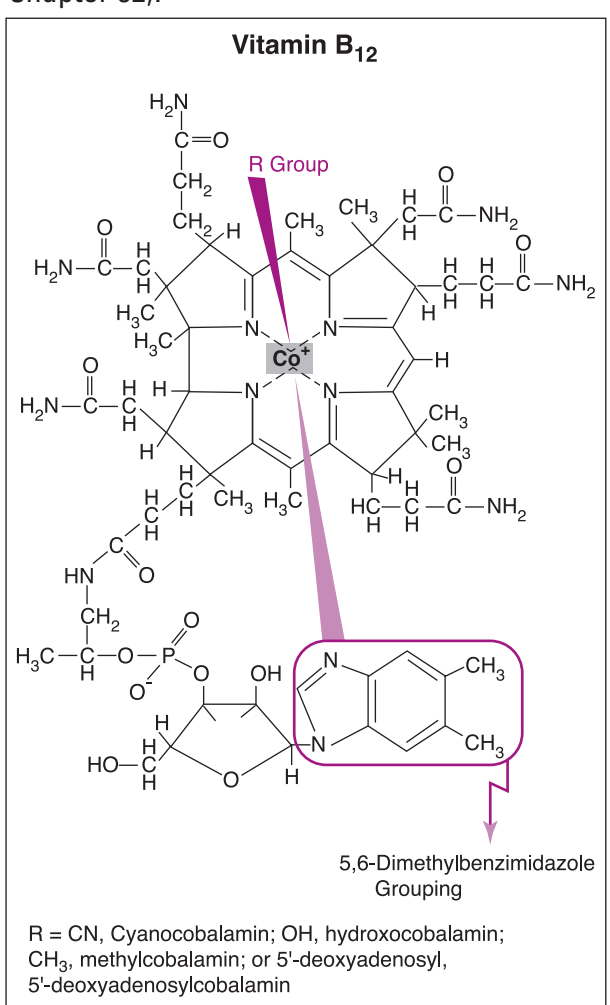


Figure 43-1

There are **two** important enzymatic reactions in animals that require **vitamin B_{12}** (**Fig. 43-2**):

1) Rearrangement of **methylmalonyl-CoA** to **succinyl-CoA** (which requires **5'-deoxyadenosylcobalamin**); and
2) Transfer of a **methyl group** from **N^5-methyltetrahydrofolate** (**N^5-methyl-H_4 folate**) to **homocysteine** in the formation of **methionine** (which requires **methylcobalamin**).

The first reaction is important in the sequential conversion of **propionate** to **succinyl-CoA**, an intermediate of the TCA cycle. Propionate is formed from microbial cellulose and starch digestion, from the terminal 3 carbons of odd-chain fatty acids during mitochondrial β-oxidation, from β-aminoisobutyrate during pyrimidine degradation, and from several amino acids during protein degradation (see Chapters 37 and 42). It is of particular significance in the process of **hepatic gluconeogenesis**.

Through the second reaction, which requires methylcobalamin, **H_4 folate** is made available to

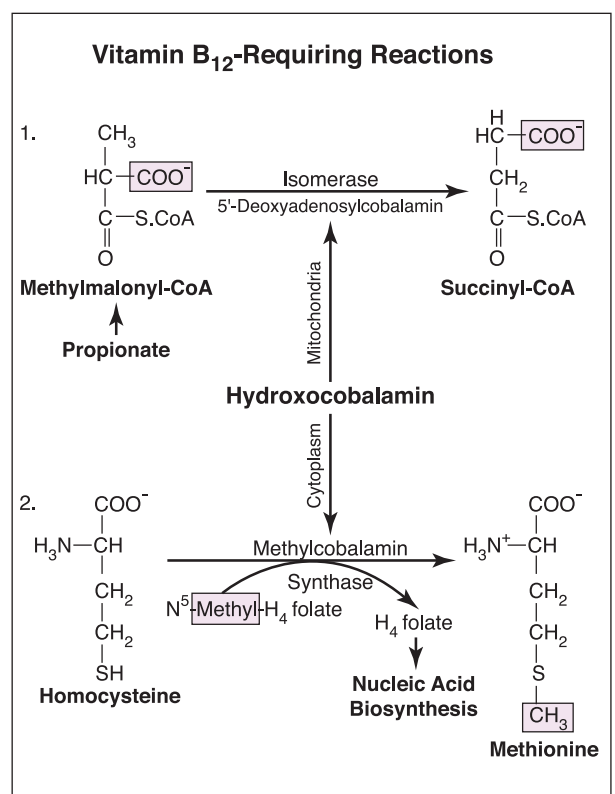


Figure 43-2

participate in **purine**, **pyrimidine**, and **nucleic acid biosynthesis**. The metabolism of vitamin B_{12} is thus intimately entwined with that of another water-soluble vitamin, **folic acid**, and both are fundamental to one-carbon metabolism (see Chapter 16).

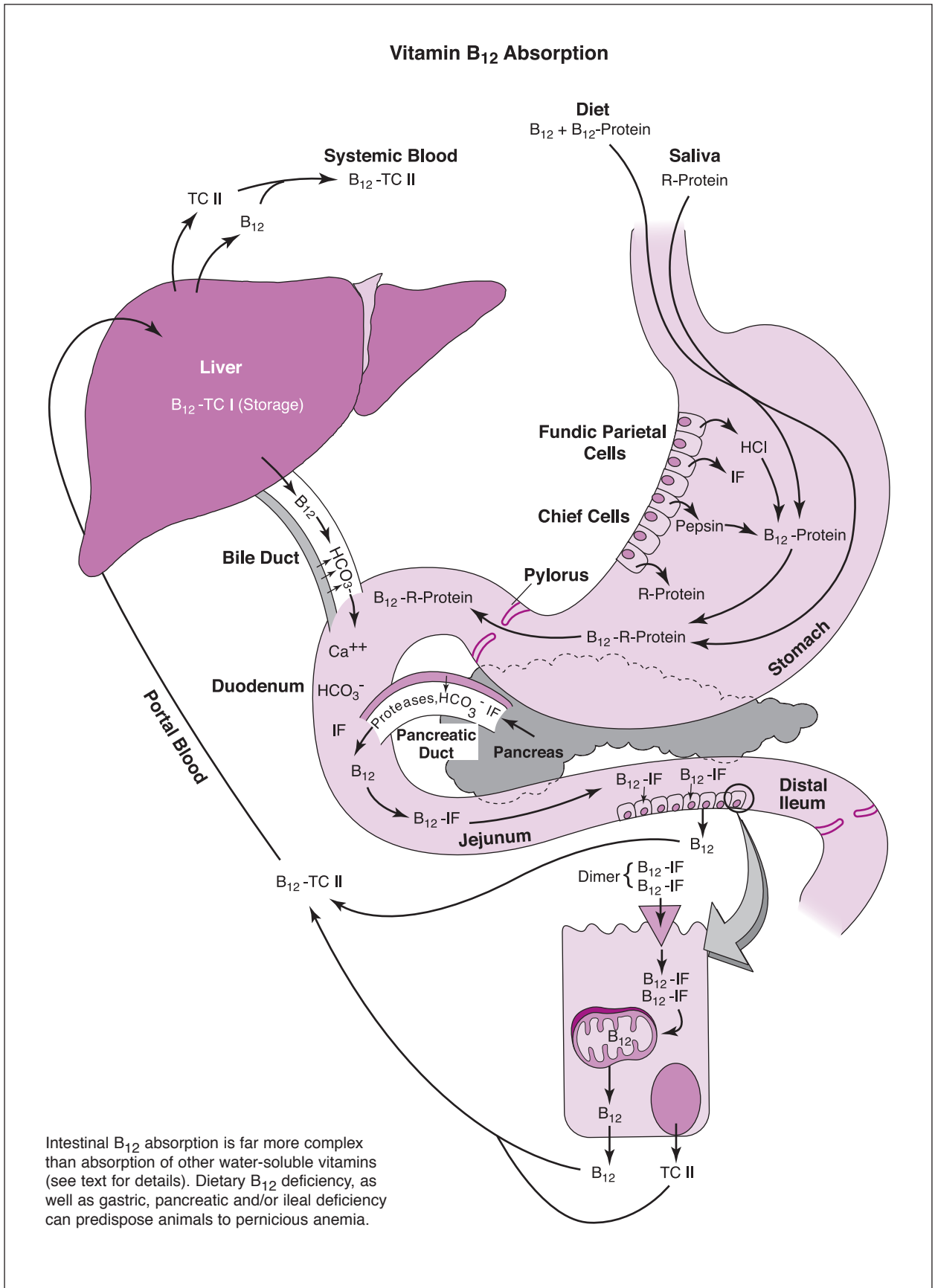
Other reactions involving this vitamin, as a coenzyme or otherwise, cannot be excluded at this time. It has proposed involvement in the synthesis of either the lipid or protein components of **myelin** (see Chapter 59), independent of the methylmalonyl-CoA reaction. This would help to explain the demyelination (or lack of myelination), and nerve degeneration observed in B_{12} deficiency. Investigators have proposed that neurologic disorders associated with B_{12} deficiency may be secondary to a relative deficiency of methionine. A deficiency of this vitamin also appears to result in a loss of tissue **carnitine**, perhaps in the form of a propionic acid adduct entering blood and urine. However, this could occur because of methylmalonate and propionate accumulation due to their lack of conversion to succinyl-CoA via methylmalonyl-CoA isomerase (or mutase). Less carnitine would have consequences for the shuttling of long-chain fatty acids across mitochondrial membranes (see Chapter 55).

Intestinal absorption of B_{12} is far more complex than absorption of other water-soluble vitamins. Dietary B_{12} is frequently bound to protein (B_{12}-protein; **Fig. 43-3**). Gastric (or abomasal) HCl and pepsin help to free B_{12} from ingested protein, which allows it to become immediately bound to a number of endogenous **R-proteins**. These proteins are present in saliva and gastric juice, they bind vitamin B_{12} tightly over a wide pH range, and they are both structurally and functionally related to the transcobalamins found in blood and in the liver. These R-proteins have a high affinity for vitamin B_{12}.

The rate of **IF** secretion by parietal cells usually parallels their rate of HCl secretion. Intrinsic factor binds B_{12} with less affinity than do the R-proteins. Thus, most B_{12} released from food in the stomach is bound by R-proteins. Pancreatic **trypsin** and other **proteases** begin degradation of complexes between R-proteins and B_{12} in the duodenum. This degradation greatly lowers the affinities of the R-proteins for B_{12}, so that this vitamin can now be appropriately transferred to IF. **Calcium** (**Ca^{++}**) and **bicarbonate** (**HCO_3^-**) from pancreatic and biliary ductular cell secretions, also help to provide conditions necessary for the binding of B_{12} to IF in the duodenum.

When IF binds with B_{12}, IF undergoes a conformational change favoring formation of dimers. The brush border plasma membranes of mucosal cells in the distal ileum, a similar site of **bile acid absorption** (see Chapter 62), contain receptor proteins that recognize and bind these **B_{12}-IF dimers**. However, they do not recognize and bind the B_{12}-R-protein complexes, nor do they bind free B_{12}. Consequently, in pancreatic insufficiency, particularly in dogs and cats, the B_{12}-R-protein complexes may not be properly degraded, and pancreatic ductular IF secretion may be inadequate, therefore leaving B_{12} tightly bound in these R-protein complexes. Since these complexes are not available for absorption, B_{12} deficiency may ensue.

Following uptake of the B_{12}-IF dimer complex by mucosal cells in the distal ileum, B_{12} is slowly released into portal blood. It does not normally appear there until about 4 hours following ingestion, leading some investigators to suggest that the delay may involve mitochondrial incorporation during absorption. Once in portal blood, B_{12} is bound to **transcobalamin II** (**TC II**), a β-globulin synthesized largely by the liver, but also by ileal epithelial cells. This B_{12}-TC II complex is rapidly cleared from portal blood by the liver through receptor-mediated endocytosis. Within hepatocytes B_{12} becomes bound to a closely related **TC I**, and stored (50-90% of total). Small amounts of free B_{12} are secreted into bile (0.1-0.2% per day), with about



Intestinal B_{12} absorption is far more complex than absorption of other water-soluble vitamins (see text for details). Dietary B_{12} deficiency, as well as gastric, pancreatic and/or ileal deficiency can predispose animals to pernicious anemia.

Figure 43-3

70% of this normally being reabsorbed again by the distal ileum. Because only about 1% of the body store is lost daily in animals, if intestinal B_{12} absorption totally ceases, it generally takes several weeks-months before symptoms of deficiency appear.

In the complete absence of IF, only about 1-2% of an ingested load of B_{12} can be absorbed. When **B_{12} is deficient in the diet**, when **pancreatic insufficiency** ensues, or when **ileal absorption is compromised** (perhaps due to **gastrectomy** and thus **IF deficiency**, to **overgrowth of cobalamin-binding bacteria** in the small bowel, or to several other forms of **intestinal disease**), the most common condition resulting is **pernicious** (**or megaloblastic**) **anemia**. This condition develops largely because of impairment of the **methionine synthase** reaction, which causes impaired DNA synthesis. This further prevents cell division, and formation of the nucleus of new erythrocytes. Therefore, megaloblasts accumulate in bone marrow. Since folate becomes trapped as **N^5-methyl-H_4 folate**, purine and pyrimidine biosyntheses are impaired (see Chapter 16). Reduced activity of reactions one and two in **Fig. 43-2** lead to **methylmalonuria** and **homocystinuria**, respectively, common symptoms of **vitamin B_{12} deficiency**. Severely subnormal serum cobalamin concentrations may also adversely affect normal proliferation of rapidly-dividing crypt cells in the intestinal mucosa, which generally replicate themselves on a weekly basis. Hence, the specific activities of jejunal digestive enzymes may deteriorate (see Chapters 7, 38, and 60) , therefore adding a **secondary intestinal dysfunction** to persistent cobalamin deficiency.

Although vitamin B_{12} is absorbed in the terminal ileum, **folate**, another water-soluble vitamin, is absorbed in the jejunum. Low plasma folate can indicate proximal bowel dysfunction (see Chapter 16), whereas low plasma cobalamin can indicate dysfunction of the terminal ileum. On the other hand, high plasma folate concentrations may be due to intestinal bacterial overgrowth.

OBJECTIVES

- Explain why either folic acid or vitamin B_{12} deficiency could lead to megaloblastic anemia (MA; see Chapter 16).
- Recognize why folic acid supplementation can partially offset the MA caused by vitamin B_{12} deficiency, yet have no effect on the homocystinuria.
- Identify the essential trace element required by microbes in the biosynthesis of hydroxocobalamin, and know why liver is a good dietary source of cobalamin.
- Name the commercial, mitochondrial and cytoplasmic forms of cobalamin.
- Identify and discuss the importance of two important enzymatic reactions in animals requiring vitamin B_{12}.
- Explain why neuronal demyelination is sometimes seen in B_{12} deficiency (see Chapters 57 & 59).
- Understand the relationship between gastric (and abomasal) HCl secretion, and pancreatic/biliary HCO_3^- secretion to vitamin B_{12} absorption.
- Explain the interactions between dietary protein, R-proteins, gastric and pancreatic proteases and intrinsic factor (IF) in B_{12} gastrointestinal transport.
- Indicate why the rate of gastric IF secretion usually parallels the rate of gastric HCl secretion.
- Recognize the anatomic relationship between intestinal bile acid and intestinal B_{12} absorption (see Chapter 62).
- Explain the process of intestinal B_{12} absorption, transport in blood and storage in the liver.
- Identify various causes of vitamin B_{12} deficiency, and explain why homocystinuria, methylmalonuria and intestinal dysfunction may become pathophysiologic signs.
- Explain why high or low circulating levels of vitamin B_{12} or folate can indicate intestinal bacterial overgrowth, or intestinal dysfunction.

QUESTIONS

1. **Vitamin B_{12} is intimately entwined with the action of which water-soluble vitamin?**
   a. Vitamin C
   b. Niacin
   c. Pantothenic acid
   d. Pyridoxine
   e. Folic acid

2. **Vitamin B_{12} helps which of the following substrates gain access to the TCA cycle?**
   a. Lactate
   b. Aspartate
   c. Propionate
   d. Leucine
   e. Medium-chain fatty acids

3. **All of the following are associated with vitamin B_{12} deficiency, EXCEPT:**
   a. Gastrectomy.
   b. Pancreatic insufficiency.
   c. Ileal disease.
   d. Rumen bacterial overgrowth.
   e. Dietary cobalt deficiency.

4. **Cobalamin receptor proteins in the distal ileum normally bind which of the following with the greatest affinity?**
   a. Vitamin B_{12}-R-Protein complexes
   b. Vitamin B_{12} in the free form
   c. Vitamin B_{12}-IF dimers
   d. Vitamin B_{12}-transcobalamin I complexes
   e. Vitamin B_{12}-transcobalamin II complexes

5. **Which one of the following is attached to the corrin ring of vitamin B_{12} through a complex linkage involving ribose-phosphate?**
   a. N^5-Methyltetrahydrofolate
   b. 5'-Deoxyadenosylthiamine
   c. Homocysteine
   d. 5,6-Dimethylbenzimidazole
   e. Iron

6. **Which one of the following is the best source of cobalamin?**
   a. Kidney
   b. Liver
   c. Skeletal muscle
   d. Brain
   e. Egg yolk

7. **The largest B-complex vitamin absorbed intact through the adult intestine is thought to be:**
   a. Thiamin.
   b. Riboflavin.
   c. Niacin.
   d. Pantothenic acid.
   e. Cobalamin.

8. **Which one of the following aids in the conversion of homocysteine to methionine?**
   a. Methylcobalamin
   b. 5'-Deoxyadenosylcobalamin
   c. Hydroxocobalamin
   d. Cyanocobalamin
   e. Pyridoxine

9. **Vitamin B_{12} deficiency is best associated with:**
   a. Hyperglycemia.
   b. Hypertension.
   c. Gallstones.
   d. Anemia.
   e. Inflammation.

10. **Vitamin B_{12} deficiency would be expected to lead to which of the following?**
    a. Polycythemia and clotting abnormalities
    b. Amino aciduria
    c. Intrinsic factor deficiency
    d. Uremia
    e. Methylmalonuria and homocystinuria

11. **Transcobalamin II is synthesized by:**
    a. The stomach.
    b. The pancreas.
    c. Hepatocytes.
    d. Biliary ducts.
    e. Salivary glands.

12. **Intrinsic factor (IF) is a:**
    a. Glycoprotein.
    b. Eicosanoid.
    c. Porphyrin.
    d. Lipoprotein.
    e. Nucleotide.

13. **R-proteins that bind B_{12} are produced:**
    a. In salivary glands.
    b. By gastric secretory cells.
    c. By pancreatic ductular cells.
    d. All of the above.
    e. A and B above.

ANSWERS

13. e
12. a
11. c
10. e
9. d
8. a
7. e
6. b
5. d
4. c
3. d
2. c
1. e