Megaloblastic Anemia

Course # DL-975

by

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Approved for 1.0 CE/Contact Hour
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Level of Difficulty: Intermediate

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Please circle the one best answer for each question.

COURSE NAME: MEGALOBLASTIC ANEMIA

NAME ___________________________ LIC. # ___________________ DATE _____________

SIGNATURE (REQUIRED) _______________________________________________________________________________________________

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STREET      CITY

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1. a b c d
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10. a b c d

DISTANCE LEARNING EVALUATION FORM

According to state regulations, this form must be completed and returned in order to receive CE hours. Your comments help us to provide you with better continuing education materials in the distance learning format. Please circle the number that agrees with your assessment with, with 5 meaning you strongly agree and 1 meaning you strongly disagree.

1. Overall, I was satisfied with the quality of this Distance Learning course.
   5  4  3  2  1

2. The objectives of this Distance Learning course were met.
   5  4  3  2  1

3. The difficulty of this Distance Learning course was consistent with the number of CE hours.
   5  4  3  2  1

4. I will use what I learned from this Distance Learning course.
   5  4  3  2  1

5. The time to complete this Distance Learning course was: _________ hours

6. Please comment on this Distance Learning course on the back of this sheet. What did you like or dislike?
Megaloblastic Anemia
DL-975
1.0 CE
Level of Difficulty: Intermediate

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OBJECTIVES:
At the end of this course the participant will be able to:
1. discuss the biochemical basis of megaloblastic anemia
2. outline the requirements and dietary sources of vitamin B12
3. outline the requirements and dietary sources of folic acid
4. list the causes of megaloblastic anemias
5. outline the symptoms of megaloblastic anemias
6. describe the blood smear morphology characteristic of megaloblastic anemia
7. discuss the tests used to differentiate among the different kinds of megaloblastic anemias
8. interpret the abnormal results in the case study

CASE STUDY:
A fourteen year old white male was referred to a large medical center for profound macrocytic anemia. He was seen in the Pediatric Hematology clinic where blood was drawn for CBC and Reticulocyte count.
The CBC and differential results were as follows:

<table>
<thead>
<tr>
<th>CBC:</th>
<th>Patient</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>1.14 x 10^6/μl</td>
<td>4.7-6.1 x 10^6/μl</td>
</tr>
<tr>
<td>WBC</td>
<td>3.6 x 10^3/μl</td>
<td>4.8-10.9/μl</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>12.7%</td>
<td>42-52%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>4.4 g/dl</td>
<td>14-18 g/dl</td>
</tr>
<tr>
<td>MCV</td>
<td>111.4 fl</td>
<td>80-97 fl</td>
</tr>
<tr>
<td>MCH</td>
<td>38.7 pg</td>
<td>27-31 pg</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.7 g/dl</td>
<td>33-37 g/dl</td>
</tr>
<tr>
<td>RDW</td>
<td>22.1%</td>
<td>11.5-14.5%</td>
</tr>
<tr>
<td>Platelets</td>
<td>76 x 10^3/μl</td>
<td>130-400 x 10^3/μl</td>
</tr>
<tr>
<td>MPV</td>
<td>9.3 fl</td>
<td>7.4-10.4 fl</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Differential:</th>
<th>Patient</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN</td>
<td>29%</td>
<td>39-68%</td>
</tr>
<tr>
<td>Bands</td>
<td>1</td>
<td>2-10</td>
</tr>
<tr>
<td>Lymphs</td>
<td>67</td>
<td>16-45</td>
</tr>
<tr>
<td>Monos</td>
<td>3</td>
<td>3-14</td>
</tr>
<tr>
<td>NRBCs/100 wbc</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Suspect flags: dimorphic RBC, microcytic RBC, RBC fragments
Definitive flags: neutropenia, lymphocytosis, 2+ anisocytosis, 3+ macrocytosis, thrombocytopenia
Reticulocyte count: Patient Normal
Reticulocytes 2.6% 0.5-1.5%
Retic, Absolute $29.0 \times 10^3/\mu l$ $22.5-88.5 \times 10^3/\mu l$
Retic, Corrected 0.7% 0.4-1.7%

B12/Folate Studies: Patient Normal
B12 <100 pg/ml 200-950 pg/ml
Folate 12.7 ng/ml 3.7-19.0 ng/ml

INTRODUCTION:
Megaloblastic anemias are a heterogeneous group of disorders that have common blood abnormalities and symptoms. The characteristic blood picture consists of large oval erythrocytes, hypersegmented neutrophils and large abnormal platelets. Bone marrow RBC precursors show abnormally high nuclear to cytoplasmic ratio and abnormal megakaryocytes. Nuclear maturation is delayed while cytoplasmic development is normal.

The diseases associated with megaloblastosis are primarily pernicious anemia (associated with vitamin B12 deficiency) and folic acid deficiency. Although the number of megaloblastic anemia cases has decreased in recent years, the incidence remains between 0.25-0.5 cases per 1000 in older individuals.

HISTORY:
Paul Ehrlich in 1880 first used the term megaloblast to describe the abnormal cells in the bone marrow of a patient with pernicious anemia. He felt the very large basophilic RBC precursor was a cell type unique to the disease. We now know that this cell is a morphologically and functionally abnormal counterpart of the normal erythroid precursor cell.

Thomas Addison first described pernicious anemia, the best known of the megaloblastic anemias, in 1855. For a number of years, the disease was known as Addisonian anemia. Anton Biermer in 1872 first used the term, pernicious anemia.

Treatment for pernicious anemia was instituted in 1926 when it was found that giving patients large amounts of liver reversed the disease process. The active liver principle, vitamin B12, was discovered in 1948 but it took 25 years to develop a synthetic vitamin B12.

BIOCHEMICAL BASIS OF MEGALOBLASTIC ANEMIAS:
The etiology of the megaloblastic anemias is impaired DNA synthesis and assembly. The most common causes are vitamin B12 (cobalamin) or folic acid deficiencies. These substances are essential in the DNA synthesis pathway. Impaired DNA production causes disruption of cell division and maturation. Rapidly proliferating cells of the bone marrow and epithelial surfaces are primarily affected, leading to the characteristic blood picture and symptoms. Cobalamin is also necessary for normal function of cells of the nervous system.

Cobalamin and folate metabolisms are related and deficiency of either results in the deoxyuridylate–deoxothymidylate pathway impairment (see Figure 1). This pathway forms deoxothymidylate from deoxyuridylate and methionine from homocysteine. Folic acid is required for synthesis of thymidylate. Since thymidylate is the precursor to thymine, essential for DNA, a limitation in the supply of thymidylate impairs DNA synthesis and leads to the morphologic manifestation of megaloblastic maturation.
Cobalamin catalyzes the conversion of homocysteine to methionine. When this reaction is impaired, folate metabolism is deranged. Since folic acid is required for synthesis of thymidylate, this derangement underlies the defect in DNA synthesis and results in the megaloblastic maturation in patients who are cobalamin deficient. Impairment in the conversion of homocysteine to methionine may also be partly responsible for the neurologic complications of cobalamin deficiency. Methionine is needed for the production of choline and choline-containing phospholipids, which are required in the nervous system.

METABOLISM:
Cobalamin (Cyanocobalamin, Vitamin B12): This substance is a complex organometallic compound, in which a cobalt atom is situated within a corrin ring, a structure similar to the porphyrin ring from which heme is formed with the addition of a ferrous atom. Unlike heme, cobalamin cannot be synthesized in the human body and must be supplied by the diet. Microorganisms manufacture cyanocobalamin. These bacteria are found in the intestinal tract, but below the area where vitamin B12 is absorbed. Vitamin B12 enters the food chain through animals that eat their feces. Therefore, it is found only in animal products, not in plants. The minimum daily requirement is about 2.5 µg. Normally about 2 mg is stored in the liver and 2 mg is stored elsewhere in the body. If absorption ceases abruptly, such as when a person becomes a vegan with no supplementation, it takes about three to six years to use up the body stores and become deficient. Intrinsic factor (IF), produced by parietal cells in the stomach, is necessary for absorption of vitamin B12. The secretion of IF generally parallels that of hydrochloric acid. The cobalamin-IF complex is bound to and absorbed by specific receptors in a portion of the distal ileum.

Folic acid: This is the common name for pteroylmonoglutamic acid. It is formed of three basic components: a pteridine derivative, para-aminobenzoic acid, and L-glutamic acid. Many different plants and bacteria synthesize it. Fruits and vegetables form the primary dietary source of the compound, but prolonged cooking destroys it. The minimum daily requirement is about 50 µg but this may be increased several-fold during times of increased metabolic demand such as pregnancy or in hemolytic anemias. Normally body stores range from 5 to 20 mg, with half stored in the liver. The large minimum daily requirement means that a deficiency will occur within months if dietary intake or intestinal absorption is curtailed. It is readily absorbed in the duodenum and proximal jejunum.
FIGURE 1. Roles of Vitamin B12 and Folic acid in DNA synthesis

Deoxyuridylate \(\rightarrow\) Thymidylate \(\rightarrow\) DNA

\(\text{CH}_2\text{-tetrahydrofolate}\) \(\rightarrow\) dihydrofolate \(\leftrightarrow\) Folic acid

Tetrahydrofolate

\(\text{Methionine}\)

\(\text{Vitamin B12}\)

\(\text{Homocysteine}\)

5-methyl tetrahydrofolate

SYMPTOMS OF MEGALOBLASTIC ANEMIA:

The anemia symptoms of both folic acid deficiency and vitamin B12 deficiency are the same. In both there are often gastrointestinal tract symptoms such as anorexia and diarrhea because the epithelial cells lining the intestines, in addition to RBCs, have a rapid turnover. Frequently the patient does not seek medical help until some of the following conditions occur: weakness, light-headedness, palpitations, angina, and symptoms of congenital heart failure. Folic acid deficiency results in anemia and intestinal manifestations but not neurological symptoms.

In pernicious anemia neurologic manifestations also occur. The onset is insidious as destruction of parietal cells increases, and the body’s vitamin B12 reserves are gradually consumed. Neurologic symptoms begin after demyelination followed by axonal degeneration. The most common manifestations are numbness and parasthesias in the extremities, weakness, and ataxia. The patient experiences tingling of the extremities and has an unsteady gait. Some have deranged mental states ranging from irritability and forgetfulness to severe dementia and psychosis. Although administering vitamin B12 by injection may cure the anemia, the neurologic symptoms may persist if there has been death of neurons. Differentiation between folic acid deficiency and vitamin B12 deficiency is important. If large amounts of folic acid are given to a pernicious anemia (vitamin B12) patient, the anemia may be improved but the neurologic symptoms become worse.

DEFICIENCY STATES: (See Table I, Classification of Deficiency States)

Causes of megaloblastic anemia differ in various parts of the world. In the temperate zones, folate deficiency in alcoholics and pernicious anemia predominate. In the equatorial areas tropical sprue is an important cause. In Scandinavia the fish tapeworm, *Diphyllobothrium latum*, may contribute to the incidence.

In the United States, supplementation of bread and cereal products with folic acid and vitamin B12 has decreased the incidence of megaloblastic anemia due to inadequate intake. Only
people with very poor diets, such as alcoholics who get most of their calories from drinks, have inadequate intake of folic acid. Defective absorption, increased folic acid requirements and use of drugs that impair DNA metabolism continue to cause megaloblastosis in the U.S.

**Cobalamin (Vitamin B12) Deficiency:**
Deficiency of vitamin B12 in the U.S. is almost always due to malabsorption. Several different conditions may cause malabsorption—decreased production of Intrinsic Factor (IF) (pernicious anemia), disorders of the terminal ileum, and competition in the intestinal tract for vitamin B12.

**Pernicious Anemia:**
This disease is caused by inadequate secretion of IF by the parietal cells in the stomach—due to atrophy of the gastric mucosa or autoimmune destruction of the parietal cells. It is found most often in older individuals, particularly in those of northern European descent or in African Americans. It is less common in southern Europeans and Asians. There is an increased incidence of pernicious anemia in patients with autoimmune diseases such as Hashimoto’s thyroiditis, Grave’s Disease, myxedema, or hypoparathyroidism. About 90% of pernicious anemia patients have abnormal circulating antibodies against parietal cells. About 60% have anti-intrinsic factor antibodies. Relatives of pernicious anemia patients have increased incidence of the disease.

**Disorders of the terminal ileum:**
Any abnormality that compromises the absorptive capacity of the terminal ileum can result in cobalamin deficiency. The conditions include ileitis, surgical resection of the small intestine, and sprue.

**Competition for Vitamin B12 by intestinal organisms:**
*D. latum*, the fish tapeworm, may cause vitamin B12 deficiency by competition for vitamin B12 in the intestinal tract. Elimination of the parasite cures the problem.

**Blind loops and other intestinal anatomic lesions** may harbor bacteria that consume cobalamin before absorption.

**Folic Acid Deficiency:**
Folic acid deficiency can be due to dietary deficiency, increased requirement, or defective absorption.

**Dietary deficiency:**
Dietary supplementation has decreased the incidence of dietary deficiency since the required addition of folic acid to enriched grain products was instituted in 1998. However, it can still occur in individuals with markedly inadequate diets such as alcoholics and the elderly who eat no fresh food.

**Increased demand:**
Pregnant women, hemolytic anemia patients, and some infants and teenagers undergoing growth spurts have increased demand for folic acid. Folic acid deficiency in the first weeks of pregnancy, usually before the pregnancy is known, can cause neural tube defects in the fetus. Dietary supplementation has decreased the incidence of this defect by 50%. Patients with markedly increased hematopoiesis as occurs in hemolytic anemias require additional folic acid.

**Defective absorption:**
Tropical sprue, a poorly understood disease with malabsorption, and non-tropical (celiac) sprue (gluten sensitivity) may result in decreased absorption of folic acid. Other small bowel disorders may also cause malabsorption.

**Other causes of Deficiency States:**
Drugs that impair DNA synthesis or metabolism may cause megaloblastic anemia. These are primarily drugs used in the treatment of malignancies, particularly leukemias, including DNA inhibitors (purine and pyrimidine analogues) and folate antagonists (methotrexate). Zidovudine, an
anti-viral drug used in HIV treatment, the ribonuclease reductase inhibitor (hydroxyurea), and anticonvulsants (phenytoin) may also cause megaloblastic anemia.

TABLE I: CLASSIFICATION OF DEFICIENCY STATES:

**Cobalamin Deficiency:**

I. Inadequate intake (vegans—strict vegetarians)
II. Defective absorption
   a. Inadequate production of intrinsic factor
      i. Pernicious anemia
   b. Disorders of terminal ileum
      i. Tropical sprue
      ii. Non-tropical sprue
      iii. Ileal resection
      iv. Regional enteritis
   c. Competition for Cobalamin
      i. Fish tapeworm (*Diphyllobothrium latum*)
      ii. Bacteria (blind loop syndrome)
   d. Drugs: para amino salicylic acid, colchicines, neomycin

III. Impaired utilization of cobalamin
   a. Nitrous oxide
   b. Inborn errors of metabolism (rare)

**Folic Acid Deficiency**

I. Inadequate intake
II. Increased requirements
   a. Pregnancy
   b. Infancy
   c. Malignancy
   d. Increased hematopoiesis (hemolytic anemias)

III. Defective absorption
   a. Intestinal disorders (Tropical sprue, Non-tropical sprue)
   b. Drugs: phenytoin, barbiturates, alcohol

IV. Impaired metabolism
   a. Folic acid antagonist therapy (methotrexate, trimethoprim, pyrimethamine)
   b. Enzyme deficiencies

**Other:**

I. Drugs that impair DNA metabolism
   a. Purine and pyrimidine antagonists, acyclovir, zidovudine, hydroxyurea

II. Marrow stem cell disorders
   a. Dysmyelopoietic syndromes
   b. Erythroleukemia (DiGuglielmo’s syndrome)

III. Metabolic disorders (rare)

**LABORATORY TESTS:**
The finding of significant macrocytosis (MCV>100fL) suggests the presence of a megaloblastic anemia. Other causes of macrocytes are hemolysis with increased reticulocytes, liver disease, alcoholism, hypothyroidism, and aplastic anemia; however, the macrocytes in these conditions are round, not oval. If the MCV is over 110fL, the patient is most likely to have a megaloblastic anemia. Other findings in the blood count are decreased hemoglobin and RBC count, decreased numbers of WBC and platelets.

The blood smear characteristically shows well-hemoglobinized oval macrocytes and anisopoikilocytosis. Occasional nucleated RBC may be present. The neutrophils may show hypersegmentation. The finding of one neutrophil with 6 lobes signifies megaloblastic anemia (normal neutrophils have 2-4 lobes). Large or bizarre misshapen platelets are usually present.

Bone marrow shows hypercellularity with decreased myeloid:erythroid ratio. The erythroid precursors are abnormally large with the nucleus less mature than the cytoplasm. The nuclear chromatin is more dispersed than normal and shows a characteristic megaloblastic pattern. Granulocyte precursors are larger than normal, particularly bands and metamyelocytes. Megakaryocytes are decreased with abnormal morphology.

Erythroid precursors show increased destruction (ineffective erythropoiesis). This increased destruction results in increased unconjugated bilirubin and lactic acid dehydrogenase in the plasma. The patient may show jaundice from the bilirubin.

The reticulocyte count helps differentiate between other causes of macrocytosis and megaloblastosis. If the reticulocyte count is sufficiently increased to be the cause of macrocytosis, a possible hemolytic anemia needs to be investigated. Reticulocytes may show polychromatophilia on the blood smear, and are round, not oval. If the reticulocyte count is decreased/normal, then tests need to be done to determine which deficiency is present. Serum folic acid and vitamin B12 levels are performed. If the serum folate is less than the normal range, this is the cause of the megaloblastic anemia. If the serum cobalamin level is decreased below 100pg/ml, then further tests need to be done to find the cause. The Schilling test can determine whether the cause is pernicious anemia, some other cause of defective absorption, or dietary deficiency.

The Schilling test: A patient is given radioactive cobalamin by mouth, followed shortly thereafter by an intramuscular injection of unlabeled cobalamin. This unlabeled cobalamin is given to fill the deficient body sites so that absorbed radioactive B12 will be excreted in the urine rather than be retained to fulfill the deficiency. The proportion of the administered radioactivity excreted in the urine during the next 24 hours provides a measure of absorption of cobalamin (making sure the urine collection is complete). Since vitamin B12 deficiency is almost always due to deficient absorption, this stage of the Schilling test should be abnormal, i.e., small amounts of radioactivity in the urine. Next the patient is given radiolabeled cobalamin bound to intrinsic factor. Absorption of the radiolabeled B12 bound to intrinsic factor will be normal if the patient has pernicious anemia. If cobalamin absorption is still decreased, the patient may have ileal disease or bacterial overgrowth (blind loop) syndrome. Bacterial overgrowth may be corrected by antibiotic administration. Ileal disease and pernicious anemia require regular vitamin B12 injections.

Today, the Schilling test has largely been replaced by serum homocysteine and methylmalonic acid (MMA) tests. Both are increased in cobalamine deficiency, but only homocysteine is increased in folate deficiency. To confirm the diagnosis of pernicious anemia, intrinsic factor antibodies can be tested as they occur in 50% of patients and are specific for this disorder. Other tests used to diagnose pernicious anemia include serum gastrin, which is raised; serum pepsinogen 1, which is low; and gastric endoscopy. Parietal cell antibody occurs in 90% of patients with pernicious anemia, but this indicator must be used with other tests as it is not specific for pernicious anemia.
CASE STUDY: Refer to the case at the beginning of the article.

The patient’s cell counts reflected a slight pancytopenia. The histograms produced by an electrical impedance / optical analyzer showed a relatively normal WBC histogram. (The histograms are not shown in this article).

The RBC histogram showed a very broad, distorted bell-shaped curve. The mode is shifted to the right, which correlates with the MCV of 111. The left side of the histogram, which normally comes down to the baseline, showed a significant population of small cells, which are most probably schistocytes. The histogram is very wide. This is reflected by the red cell distribution width (RDW) of 22.1.

The platelet histogram, which usually shows a normal distribution, does not come down to the baseline on the right side, which correlates with the presence of schistocytes. These RBC fragments are in the size range of platelets and are included in the platelet count.

The Definitive and Suspect Flags indicated all of these abnormalities.

On the manual differential, RBC morphology was markedly abnormal with marked aniso-poikilocytosis including macro-ovalocytes, target cells, teardrop RBCs and schistocytes. Nucleated RBCs were present. A few hypersegmented neutrophils were also seen. The reticulocyte % and the absolute reticulocyte count were slightly elevated and the corrected reticulocyte count was normal, but inappropriately low for the degree of anemia present.

A vitamin B12 assay was performed which showed the patient to be markedly deficient. Folate levels were normal.

History: The patient was born in 1977 at 28 weeks gestation, weighing 2 lbs., 7 oz. He had problems with hyaline membrane disease and developed necrotizing enterocolitis at five days of age. The bowel perforated on the eighth day and 100 cm. of his bowel, including the ileocecal valve, was removed. This was estimated to be approximately 50% of his ileum.

He was eventually released from the hospital and placed on multi-vitamins with folate and on vitamin B12 replacement therapy by intramuscular injection. The family had numerous employment and insurance difficulties during the ensuing years and the vitamin B12 injections were not given regularly.

A Schilling test (without Intrinsic Factor) had been performed in 1984, which established that the patient was definitely unable to absorb vitamin B12 normally, as excretion of radiolabelled Co-58 was less than 0.6% (normal >7%). The next step of the Schilling test was not done, since the patient history indicated that a large portion of the ileum had been removed, thus the remaining area was shown to be unable to absorb the vitamin.

Finally, vitamin B12 injections were discontinued 2-3 years ago because the family lacked medical insurance and could not afford the treatments. This time frame correlates with the amount of time necessary to deplete stored vitamin B12 in a child this age and with the onset of his clinical symptoms.

Case Study Conclusions:

The patient was given an injection of vitamin B12 and re-tested a week later. There was marked improvement in his clinical symptoms, CBC, and a significant reticulocyte response.
This patient requires ongoing vitamin B12 replacement therapy for the rest of his life. Failure to comply will result in reappearance of symptoms and possible irreversible damage to his nervous system.

REFERENCES
1. Case study furnished by Cannon J, UC Davis Medical Center

REVIEW QUESTIONS
Course #DL-975
Choose the one best answer

1. A patient consults his physician because of weakness and shortness of breath. He has gluten sensitivity and has been a vegan vegetarian for ten years. His MCV was 110fL. The most probable cause of his megaloblastic anemia is
   a. Folic acid deficiency
   b. Diphyllobothrium latum
   c. vitamin B12 deficiency
   d. tropical sprue

2. All but which of the following are causes of megaloblastic changes in RBCs?
   a. hemolytic anemia
   b. Diphyllobothrium latum
   c. pernicious anemia
   d. treatment with methotrexate

3. Which of the following foods is not a source of vitamin B12?
   a. rice
   b. eggs
   c. milk
   d. fish

4. A complication of vitamin B12 deficiency that is not found in folic acid deficiency is
   a. shortness of breath
   b. palpitations
   c. ataxia
   d. angina

5. A blood smear of a patient with a megaloblastic anemia characteristically shows
   a. hypochromic RBC
   b. polychromatophilic cells
   c. increased numbers of small platelets
   d. oval macrocytes
6. A Schilling test was used in the diagnosis of megaloblastic anemias to
   a. rule out folic acid deficiency
   b. identify the ova of *Diphyllobothrium latum*
   c. separate pernicious anemia from ileal disease
   d. separate ileal disease from sprue

7. The biochemical cause of megaloblastic anemia is
   a. a decrease in thymine for DNA synthesis
   b. a decrease in uridine for DNA synthesis
   c. a defect in the hemoglobin biosynthetic pathway
   d. a decrease in purine for RNA synthesis

8. In the Case Study the Schilling test with intrinsic factor was not done because
   a. it was too expensive
   b. the patient was already shown to be lacking intrinsic factor
   c. the cause of malabsorption was known from his history
   d. intrinsic factor is not needed for absorption of vitamin B12

9. Megaloblastic maturation is characterized by
   a. delayed cytoplasmic maturation and normal nuclear maturation
   b. decreased hemoglobin in the RBC cytoplasm
   c. normal cytoplasmic maturation and delayed nuclear maturation
   d. hypossegmentation of neutrophils

10. Absorption of folic acid
    a. is done by the parietal cells in the stomach lining
    b. occurs passively in the jejunum
    c. requires combination with intrinsic factor
    d. occurs only in a section of the distal ileum