SECTION 6 - BLOOD: GAS TRANSPORT, IMMUNE, AND CLOTTING FUNCTIONS

EXERCISE 6.1 RED BLOOD CELL COUNT, HEMOGLOBIN, AND OXYGEN TRANSPORT

Approximate Time for Completion: 2-3 hours

Introduction

This exercise is designed to introduce students to the composition of blood with emphasis on the physiological importance and clinical measurements of red blood cells and hemoglobin. By performing a red blood cell count, measuring the hematocrit, and calculating corpuscular volume and hemoglobin concentration, students will become more familiar with red blood cells. Since this exercise involves handling blood, your students should use sterile technique and touch only his/her own blood. Avoid milking the finger to squeeze out blood since the risk of hemolysis and possible contamination of your sample will increase the variability in your results. Take proper measures to dispose of anything contaminated with blood in specifically marked biohazard containers. Although somewhat long, this exercise can be combined with one or both of the other blood-related exercises in this section. If necessary the students can be divided into smaller groups working independently on each exercise, and then reconvene to exchange data and complete the laboratory reports.

Materials

1. Hemocytometer
2. Unopettes (Becton-Dickinson) for manual red blood cell count and hemoglobin measurements
3. Heparinized capillary tubes, clay capillary tube sealant (Seal-ease), microcapillary centrifuge, hematocrit reader
4. Microscope
5. Sterile lancets and 70% alcohol for preparing fingertip blood. Alternatively, dog or cat blood (obtained from a veterinarian) may be used.
6. Colorimeter and cuvettes
7. Biohazard containers for disposal of blood-contaminated items

Textbook Correlations: Chapter 13 – The Formed Elements of Blood

Chapter 16 – Hemoglobin and Oxygen Transport

Answers to Questions

1. four, oxygen
2. erythropoietin; kidney
3. reticuloendothelial; spleen, liver, bone marrow
4. bilirubin; jaundice
5. Hematocrit is the ratio of the volume of packed red blood cells to the total blood volume.
6. oxyhemoglobin
7. methemoglobin
8. carboxyhemoglobin
9. anemia
10. inadequate amounts of iron in the diet

11. Anemia is a condition characterized by an abnormally low red blood cell count and/or hemoglobin concentration. It may be caused by a high rate of red blood cell destruction or insufficient red blood cell production. The latter situation may be due to dietary conditions such as iron deficiency, or deficiencies in vitamin B_{12} or folic acid; or by bone marrow disease. This condition is dangerous because the O_2 carrying capacity of the blood is significantly reduced, leading to hypoxemia and cellular hypoxia.

12. During labor and delivery, a newborn undergoes a rapid rate of red blood destruction. This results in the liberation of excessive amounts of heme from newly released hemoglobin. These free heme molecules are converted by the newborn liver into bilirubin that circulates in the blood. If the liver cannot get rid of the excess bilirubin by conjugating it and excreting it in the bile, then the blood concentration of bilirubin can rise to a sufficient level to produce yellowing of the skin in visible areas of the body, such as the sclera of the eyes. Babies often develop this jaundice because their livers are not fully functional at the time of birth. *Note:* Since bilirubin is fragmented by ultraviolet light, newborn nurseries use a bili-light to reduce the circulating levels of bilirubin. Prudent, but brief exposure to daylight accomplishes the same result.

13. Yes. If the person's plasma volume is larger than normal, the measurement of a lower hematocrit value would erroneously suggest that the packed red cell volume is too low yet, when evaluated, the red blood cell count may be normal. The increase in plasma volume is most likely due to iatrogenic causes (excessive fluid intake).

14. The one respect in which the laboratory results of carbon monoxide poisoning may be similar to that of anemia would occur if using the spectrophotometer to measure the concentration of hemoglobin in a solution of hemolyzed blood. Colorimetry measures the intensity of the unknown hemoglobin concentration (absorbance) relative to that of a standard hemoglobin solution of known concentration. To accurately measure hemoglobin concentration in a solution, all hemoglobin must be converted into its ferric form (oxidized, methemoglobin). In both cases, the absorbance of both anemia and CO-bound hemoglobin blood samples would be very low. In the anemia sample, the low hemoglobin absorbance is usually due to the low red blood cell count, while that seen in CO poisoning occurs because CO does not readily dissociate from carboxyhemoglobin such that the required methemoglobin conversion would not occur resulting in a low absorbance value.

15. High-altitude polycythemia is caused by an increase in the secretion of the hormone, erythropoietin by the kidneys. The increase in the rate of red blood cell production by the bone marrow would raise the hematocrit value. It also increases the oxygen-carrying capacity of the individual. Athletes commonly train at high altitudes (as a form of natural blood doping) in the attempt to increase cardiovascular endurance. Adverse effects would include an increase in the viscosity of the blood that raises the resistance to blood flow and raises blood pressure, disorders that could lead to cardiovascular problems such as stroke, hemorrhage, clotting problems, and other vascular problems related to the increased viscosity of blood.

EXERCISE 6.2 WHITE BLOOD CELL COUNT, DIFFERENTIAL, AND IMMUNITY

Approximate Time for Completion: 2-3 hours

Introduction

This exercise is designed to introduce students to the physiological importance and clinical measurements of white blood cells. By performing total and differential white blood cell counts students will become familiar with the five types of white blood cells, representing two general categories. Since this exercise involves handling blood, your students should use sterile technique and touch only his or her own blood. Take proper measures to dispose of anything contaminated with blood in specifically marked biohazard containers. As explained in exercise 6.1, this exercise can be combined with the other exercises from section 6 for an entire laboratory period devoted to blood.

Materials

1. Microscopes, hemocytometer slides
2. Thoma diluting pipettes (calibrated for WBC counts), lancets, alcohol swabs
3. Lancets and alcohol swabs, for preparing fingertip blood. Alternatively, dog or cat blood (obtained from a veterinarian) may be used.
4. For total white blood cell count - methylene blue in 1% acetic acid; for differential count - Wright's stain (or Harleco Diff-Quik or VWR Statstain)
5. Heparinized capillary tubes, glass slides
6. Biohazard containers for disposal of blood-contaminated items

Textbook Correlations: Chapter 13 – The Formed Elements of Blood

Chapter 15 – Defense Mechanisms; Functions of B Lymphocytes; Functions of T Lymphocytes

Answers to Questions

1. neutrophil (b)
2. lymphocyte (d)
3. eosinophil (a)
4. basophil (e)
5. monocyte (c)
6. B; T
7. diapedesis
8. neutrophils
9. antigens
10. T cells (lymphocytes) provide cell-mediated immunity, with some secreting lymphokines whereas others either serve as helper, suppressor, or killer T cells in the immune response. T cells are either derived from or had an ancestor cell that came from the thymus gland. By contrast, B cells originate from the bone marrow (B as in bursa). When activated, B cells can be transformed into antibody-secreting plasma cells that provide humoral immunity.
11. The clonal selection theory proposes that a specific antigen molecule activates the immune system by recognizing and binding with a type of specific receptor protein located on the membrane of a circulating lymphocyte. This bonding stimulates the lymphocyte to divide numerous times, producing a large population of genetically identical cells (a clone). Such a lymphocyte clone is produced, therefore, by prior exposure to the antigen and takes weeks or months to occur. Upon subsequent exposure to that particular antigen causes B cell clones develop into plasma cells that secrete large numbers of antibody molecules in the plasma. These specific antibodies then may rapidly destroy the invading bacteria, thus providing specific humoral immunity against those bacteria. By contrast, T cells require moving into close proximity with their victim cells in order to destroy them, thus providing specific cell-mediated immunity.
12. The virulence, or ability to cause disease, of an organism depends upon the agent's ability to invade and reproduce inside the host. Any method that would kill the pathogenic organism or prevent it from multiplying would work. Exposing the pathogenic organisms to ionizing radiation or to hypertonic solutions are examples of methods that may be used to kill pathogenic organisms. The integrity of the specific antigen protein molecules present within the membranes of the organism, however, must be preserved intact. We have learned that exposure to a pathogen with reduced or no virulence has the same antigenicity as the fully virulent pathogen and thus allows the exposed immune system to become sensitized to those antigens. These antigens, when introduced into the host could then stimulate the immune system and provide a strong defense against any subsequent exposure to that pathogen. The dangers of this procedure lie in the fact that not all pathogens treated would die and that administration to individuals as an immunization may introduce live pathogens, causing illness.

13. An animal injected with a pathogen is exposed directly to the pathogen and will develop lymphocyte clones (active immunity) that will produce large concentrations of specific antibodies circulating in the plasma portion of its blood. The antibody-enriched plasma (antitoxin or antiserum) can then be removed from the animal and administered to a person exposed to this pathogen, passively immunizing the victim. The advantages are that the antibodies will immediately go to work destroying the invading pathogen and that a prior exposure to sensitize the immune system is not required. The shortcomings are that this passive immunity is short-lived in that the person does not develop long-term immunity to subsequent exposure to the same pathogen, as one would if actively immunized; and that the victim may develop a serious sensitivity to other components of the animal's serum, adding to the distress.

EXERCISE 6.3 BLOOD TYPES

Approximate Time for Completion: 30 minutes-1 hour

Introduction

This exercise is designed to introduce students to the ABO and Rh blood typing systems. Since blood cells have characteristic antigens on their cell membranes, it is possible to type blood. A discussion of antigens and antibodies helps the students understand the concepts and importance of blood typing before they perform this exercise. Since this exercise involves handling blood, your students should use sterile technique and touch only his/her own blood. Take proper measures to dispose of anything contaminated with blood in specifically marked biohazard containers. Take proper measures to dispose of anything contaminated with blood. This exercise can be combined with other exercises from section 6 for an entire laboratory period devoted to blood.

Materials

1. Sterile lancets, 70% alcohol
2. Anti-A, anti-B, and anti-Rh sera (Hardy Diagnostics)
3. Slide warmer, glass slides, toothpicks
4. Container for the disposal of blood-containing objects

Textbook Correlations: Chapter 13 – Red Blood Cell Antigens and Blood Typing

Answers to Questions

1.	antigens present	antigens absent
a.	A	B, Rh
b.	Rh	A, B
c.	A, B	Rh

2. AA or AO
3. AO or OO
4. O
5. AB (Rh negative)
6. O (Rh positive)
7. Rh negative; Rh positive
8. The danger of mixing blood types that don't match is red blood cells may clump together, or agglutinate. This clumping will plug blood vessels and prevent the normal flow of blood to the tissues.

9. Hemolytic disease of the newborn occurs only if the mother is Rh negative, with both the father and the baby Rh positive. At delivery, the mother's blood may be exposed to the Rh antigen from the baby's RBCs and, over time, develop antibodies against it. If a second developing child is also Rh positive, antibodies from the mother may cross the placenta and destroy the fetal red blood cells. The development of maternal antibodies against the Rh factor may be prevented by administration of Rh antibodies (RHOgam) usually once during pregnancy and again shortly after delivery. The injected anti-Rh antibodies will seek out, agglutinate, and destroy any Rh-positive fetal red blood cells that may have entered the mother's blood supply before her immune system could produce antibodies against them.
10. Since blood type is the phenotypic expression of genotype, blood types cannot be used to prove fatherhood but can be used to disprove fatherhood. For example, if a type O woman claims that a type A man is the father of her type O baby, the blood types in this case can neither prove nor disprove her claim. This is because the man who is type A may have either the genotype AA or the genotype AO. If he has the AA genotype, he cannot have fathered a type O child, but if he has the AO genotype, there is a 50% chance that he could have fathered the type O child.
11. The safety depends on the amount of donor plasma. The donor blood type O will have RBCs with no antigens so no agglutination will occur, but the donated plasma will contain antibodies against the A antigens on the RBCs of the recipient. If some donor plasma is given, some agglutination of the recipient RBCs with anti-A antibodies from the donated plasma will occur. This agglutination by whole blood is often overlooked in emergency situations since the prime reason for the transfusion is to supply the victim with RBCs. Donated blood is usually centrifuged and the packed cells separated from the plasma so that when needed in an emergency, only the packed cells are given in a transfusion.

EXERCISE 6.4 BLOOD CLOTTING SYSTEM

Approximate Time for Completion: 15-30 minutes

Introduction

This exercise is designed to introduce students to the complex events involved in blood clotting. A watch with a second hand (or better, a stopwatch) and good lighting are needed for this exercise. The appearance of the end point in the prothrombin time test is different from the appearance in the APTT test, so practice is required for accurate results. Plasma that is not fresh will have abnormally long times for these tests.

Materials

1. Pipettes (0.10-0.20 ml), small test tubes
2. Constant-temperature water bath set at 37°C
3. 0.02 M calcium chloride, activated thromboplastin, activated cephaloplastin (Dade), fresh plasma

Textbook Correlations: Chapter 13 – Blood Clotting

Answers to Questions

1. tissue thromboplastin release
2. extrinsic pathway
3. thrombin (factor II)
4. prothrombin
5. Vitamin K
6. chelates or removed calcium ions from the plasma (necessary cofactor in the activation of many clotting factors)
7. prothrombin time; activated partial thromboplastin time (APTT)
8. adenosine diphosphate (ADP)

9. hemophilia

10. Factors X, V, II, and I. A deficiency in factor VIII describes classical hemophilia (recessive trait carried on the X chromosome; results in delayed formation of fibrin) that reflects an interruption of the intrinsic clotting pathway. Therefore, the hemophiliac's activated partial thromboplastin time (APTT) would be abnormally long. Since the extrinsic pathway is not affected, the hemophiliac would have a normal prothrombin time.

11. The gene responsible for making factor VII would be defective. Factor VII is tissue thromboplastin, a clotting factor required in the extrinsic pathway and thus results in the prolonged prothrombin time. The activated partial thromboplastin test (APTT) is not sensitive to this factor so the results will appear normal with this test.

12. Heparin acts as an anticoagulant by activating antithrombin II, which in turn, combines with and inactivates thrombin. Inactivating thrombin directly prevents the conversion of fibrinogen to fibrin and, in this way, prevents the normal formation of a clot by either pathway. Both tests would result in abnormally long times. Citric acid and oxalic acid inhibit clotting by binding to Ca^{2+}. During the clotting tests Ca^{2+} can then be added to the mixture at time zero to initiate the activation of clotting factors. Since heparin inhibits the action of thrombin, the addition of Ca^{2+} would have no ability to reverse the anticoagulant action and the test would have no starting point.

13. Vitamin K is needed by the liver for the production of a number of clotting factors. If a person were deficient in this vitamin an abnormally slow clotting time could be treated with exogenous vitamin K. Administration of vitamin K would not immediately hasten the clotting time because an interval after the vitamin was given would be required for the biosynthesis of the deficient factors.

14. Classic hemophilia (defective factor VIII) and Christmas disease (defective factor IX) are inherited as a sex-linked recessive trait. Since the defect is located on the X chromosome, mostly males would express this defect that appears in 50% of males born to a heterozygous mother. Females with this defect is much less common because females would have receive a defective X chromosome from both parents (be homozygous for the defect). Hemophilia due to factor XI or XII deficiencies, however are the result of genetic defects inherited as autosomal traits. Consequently, this form of hemophilia would then be expressed equally in males or females without regard for the sex of the individual.