

# SECTION 2 - CELL FUNCTION AND BIOCHEMICAL MEASUREMENT

## EXERCISE 2.1 MEASUREMENTS OF PLASMA GLUCOSE, CHOLESTEROL, AND PROTEIN

**Approximate Time for Completion: 2-3 hours**

### **Introduction**

Three molecules that are typically measured in the blood plasma are glucose, cholesterol, and protein. Representing the three energy nutrients carbohydrates, lipids, and protein, these molecules have very important physiological roles, and so are carefully regulated. This exercise introduces students to Beer's Law and the theory of colorimetry. Students will use techniques with a colorimeter (spectrophotometer) to determine the levels of these molecules in plasma. It is important to emphasize the importance of the blank value as the basis for determining the unknown value. The unknown value can be determined by two methods: using Beer's Law proportionality equation, and by plotting the graph of the data and extrapolation from the drawn standard curve. It is recommended that students be familiar with both techniques for determining the unknown concentrations. Data collection techniques and analysis of the plotted results can be reviewed using the graph paper supplied. Students should appreciate the fact that clinical laboratories use methods today that are based on the same principles. Students should learn how easy it is to have tremendous variability in results when using the same unknown and that a single laboratory reading should never be used for a diagnosis.

### **Materials**

1. Pyrex (or Kimax) test tubes, mechanical pipettors for 40- $\mu$ l, 50- $\mu$ l, 100- $\mu$ l, and 5.0-ml volumes; and corresponding pipettes ( 0.10-ml and 5.0-ml total volume capacities)
2. Constant-temperature water bath, set at 37°C
3. Colorimeter and cuvettes
4. Glucose kit ("Glucose LiquiColor Test," Stanbio Laboratory, Inc.)
5. Cholesterol kit ("Cholesterol LiquiColor Test," Stanbio Laboratory, Inc.)
6. Total Protein Standard - human albumin standards at 2, 4, 6, 8, 10 g/dl concentrations from Sigma Diagnostic, catalog # P6529
7. Biuret reagent: To a 1.0-liter volumetric flask, add 45 g of sodium potassium tartrate and 15 g of  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ . Fill full with 0.2 N NaOH and shake to dissolve. Add 5.0 g of potassium iodide and fill to 1.0 liter volume with 0.2N NaOH.
8. Artificial serum with known values (both "normal" and "abnormal" are available from Stanbio Laboratory Inc., and other supply houses), or serum from dogs or cats (available from veterinarians)

**Textbook Correlations:** Chapter 2 – Carbohydrates and Lipids; Proteins

Chapter 14 – Exchange of Fluid Between Capillaries and Tissues

### **Answers to Questions**

1. directly
2. Beer's
3. diabetes mellitus
4. insulin
5. lipids; steroids
6. atherosclerosis
7. liver
8. hypoproteinemia, edema

9. protein
10. "All fats are lipids" because fats are organic molecules that are relatively insoluble in water. Fats (triglycerides) represent a subcategory of lipids; "not all lipids are fats" because the term lipids includes other subcategories such as phospholipids and steroids, that are not triglycerides.
11. Plasma proteins contribute to the colloid osmotic pressure of the blood, which is needed for fluid balance. Plasma proteins also serve as circulating enzymes, hormones, transport molecules, and antibodies. Although the liver is a major source of plasma proteins, these molecules can originate from various tissue cells located around the body. Protein synthesis is a complex process that starts with a gene (DNA) that becomes transcribed and translated at the ribosomes to form new proteins. With the help of endoplasmic reticulum and Golgi complexes, newly formed polypeptides are released into the plasma.
12. The blank tube (B) contains all of the chemicals present in the other tubes except the molecule that is being measured. By setting the absorbance of the blank tube to zero (zero concentration = zero absorbance), the absorbance of the test solutions is made to be due entirely to the concentration of the test molecule. The blank also establishes the first data point (reference) for drawing the standard curve.
13. No. Protein molecules (polypeptides) that are eaten are completely digested in the gastrointestinal tract. Only the simple amino acids (or dipeptides, occasionally) are absorbed into the blood. Proteins present in the blood, therefore, are made by cells of the body following the DNA genetic code, whereas amino acids in the blood may have been derived from either ingested proteins or from the catabolism of existing body proteins.  
No. Glycogen (animal starch) is a polymer of glucose molecules that is assembled from free plasma glucose molecules that have entered the liver cells. Food starch (plant starch) from the diet is completely hydrolyzed to simple sugar molecules before absorption in the digestive tract. Furthermore, the chemical nature of the bonds between glucose molecules in plant starch is distinct from that found between glucose molecules in glycogen.
14. A linear standard curve is drawn because, according to Beer's law, concentration and absorbance are directly proportional. Deviations from a straight line are thus due to experimental error, and a straight line that averages the location of the data points allows the random errors in one direction to offset those in the opposite direction. The line goes through the origin because zero concentration was set to zero absorbance using the blank solution, as in question 12.

## **EXERCISE 2.2 THIN-LAYER CHROMATOGRAPHY OF AMINO ACIDS**

**Approximate Time for Completion: 1-2 hours**

### **Introduction**

This exercise is designed to introduce students to amino acids and to thin-layer chromatography (TLC). Thin-layer chromatography is performed in many research laboratories to detect and analyze the presence of different amino acids. It takes approximately 20 minutes to spot the plates and place them in the developing chamber. The class must wait about an hour for the plates to develop. During this time, other exercises may be performed such as exercise 2.3 or 2.4. When the plates are removed from the developing chamber, they should be sprayed with ninhydrin in a well-ventilated location, preferably within a fume hood.

### **Materials**

1. Silica gel plates (F-254 rapid, adhered to plastic or glass), capillary tubes, chromatography (or hair) dryers, rulers (F = Fluorescent). Note: Alternatively, chromatography *paper* can be used.
2. Developing chambers, oven set at 60°C
3. Amino acid solutions: arginine, cysteine, aspartic acid, phenylalanine - 1.0 mg/ml each dissolved in 0.1N HCl:isopropyl alcohol (9:1); "unknown" solution of amino acids containing two of these amino acids in the same solution
4. Developing solvent: 20 ml of 17% NH<sub>4</sub>OH (dilute concentrated NH<sub>4</sub>OH with an equal amount of water), 40 ml of ethyl acetate, and 40 ml of methanol per developing chamber
5. Ninhydrin spray

**Textbook Correlations:** Chapter 2 – Proteins

Chapter 4 – Inborn Errors of Metabolism

### Answers to Questions

1. The functional residual or R group
2. Inborn errors of metabolism
3.  $R_f$  means “relative to the front.” This is the ratio of the distance an amino acid spot has traveled from its origin relative to the distance the solvent front has traveled from the same origin.
4. Different amino acids have different  $R_f$  values because their R groups are chemically different; this gives each amino acid its own unique solubility in the developing solvent and, therefore, its own unique migration distance.
5.  $8/12 = 0.67$
6. The maximum  $R_f$  value an amino acid spot can have is 1.0, when the spot has migrated a distance equal to that of the solvent front.
7. The structure of amino acid “A” must be more polar than that of amino acid “B.” Since A traveled farther in solvent system 1 that is more polar, A must be more polar (“like dissolves like”). Conversely, amino acid “B” is less polar.

## **EXERCISE 2.3 ELECTROPHORESIS OF SERUM PROTEINS**

**Approximate Time for Completion: 40 minutes**

### Introduction

This exercise is designed to introduce students to the variety of proteins found in serum. There are different classes of proteins found in serum that can be separated from each other and identified by electrophoresis. Due to the equipment required however, this exercise is often performed as a demonstration. Serum from this exercise can be obtained from the same samples used in exercise 2.1. Alternatively, fingertip blood in capillary tubes can be centrifuged, as in the determination of a hematocrit, to obtain the serum. Only a drop of serum is required per test, such that one hematocrit tube can contain enough serum for about four tests. The Ponceau S stain should be treated with care since it contains trichloroacetic acid. This stain can be reused semester after semester if it is poured from the trough back into the bottle. Results are better in this exercise when the buffer is kept cold and when the membrane is blotted with filter paper to remove excess buffer.

### Materials

1. Plastic troughs, buffer chamber, sample applicator, power supply, Sepharose strips (the Septra Tek system, Gelman), forceps
2. High-resolution buffer, Ponceau S stain (Gelman), 5% acetic acid (v/v)
3. Sterile lancets, 70% ethanol, and a biohazard disposal receptacle for blood-contaminated objects
4. (alternatively, test tubes containing previously prepared serum samples or artificial serum may be used)
5. Non-heparinized capillary tubes, micro rubber bulbs (to expel solutions from the capillary tubes), and microhematocrit centrifuge

**Textbook Correlations:** Chapter 2 – Proteins

Chapter 13 – Plasma Proteins

Chapter 15 – Antibodies

## Answers to Questions

1. An amphoteric molecule is a molecule that contains at least one ionizable group that can become negatively charged and at least one ionizable group that can become positively charged. The actual net charge at a particular time depends on the pH of the solution.
2. zwitterion formula [draw one]
3. a. lysine and arginine  
b. aspartic acid and glutamic acid
4. positive; negative
5. fibrinogen
6. fibrin
7. albumins
8. liver
9. gamma globulins
10. Serum is identical to plasma except the protein fibrinogen is lacking. Serum is obtained from clotted blood. In the formation of a clot plasma fibrinogen is converted to fibrin. The resulting fluid is serum.
11. The rates at which proteins travel away from the negative pole (cathode) and toward the positive pole (anode) is influenced by their net negative charge, their size, and their shape.
12. The albumin band is dark because it contains the highest concentration of proteins of any class of plasma proteins; the band is narrow because albumins are structurally homogenous. The major function of the albumins is to provide colloid osmotic pressure; this function is dependent on the concentration, not on the structure, of the proteins. The gamma globulin band contains antibodies. Since antibodies with different specificities have different structures, antibodies are structurally heterogeneous and produce a wide band in the electrophoresis pattern.
13. Since the liver is the primary source of plasma proteins the density of various protein bands would decrease depending upon the severity of the disease. The bands most affected would include albumin, fibrinogen, alpha globulins, and beta globulins. The decrease in these plasma protein levels could lead to a drop in colloid osmotic pressure, disturbances in the solubility of plasma lipids such as hormones and bile pigments, and prolongation of the blood clotting time.

## **EXERCISE 2.4 MEASUREMENTS OF ENZYME ACTIVITY**

**Approximate Time for Completion: 1-2 hours**

### **Introduction**

This exercise is designed to introduce students to the concept of enzyme activity. In this exercise, students must carefully monitor time, temperature, and concentration. Further, this exercise offers an opening for a discussion of enzymes and methods of enzyme assays in perhaps more detail than would be available in lecture. Part A works best with fresh raw chicken liver (beef and frog livers do not seem to work as well). Part B measures the concentration of alkaline phosphatase in serum (normal and abnormal, if desired). Part C measures the concentration of lactate dehydrogenase in serum (normal and abnormal, if desired). To save time, students in small groups of two or three can perform only part B or only part C of the exercise, with data exchanged at the end of the exercise.

### **Materials**

1. Beakers, rusty nails, chicken liver
2. Hydrogen peroxide
3. Test tubes, mechanical pipettors, automatic microliter pipettors, and pipettes
4. Constant-temperature water bath set at 37°C
5. Colorimeter (spectrophotometer) and cuvettes

6. Serum or reconstituted commercially obtained normal and abnormal serum - available, for example from: SigmaBio-Analytic Laboratories, Inc.; or Bio-Data Abnormal Serum - cat# 10500 or 10501; or Stanbio - Ser-T-Fy I cat# G425-86 (normal); and II cat# G428-86 (abnormal)  
*Note:* If permitted on your campus, students can derive their own enzyme concentrations from sera obtained from a finger lance, microcapillary pipette, and microcentrifugation.
7. Alkaline Phosphatase Test and Lactate Dehydrogenase Test - available, for example from: Biotron Diagnostics, Inc.; Bio-Analytic Laboratories, Inc. - cat# 21701 (Alkptase); cat# 22701 (LDH) both colorimetric tests; or Stanbio Laboratory, Inc. - cat# 0905-100 (Alkptase); 0945-100 (LDH)

**Textbook Correlations:** Chapter 4 – Enzymes as Catalysts; Effects of Temperature and pH; Cofactors and Coenzymes

### Answers to Questions

1. An international unit (IU) of enzyme activity is the amount of enzyme required to convert one micromole of substrate into product per minute under standardized conditions. To assay the enzyme activity, the appropriate cofactors or coenzymes must be provided, the pH and temperature must be standardized, and the concentration of the substrate must be very large relative to the concentration of the enzyme.
2. & 3. An enzyme displays maximum activity at some particular pH optimum and at some particular temperature optimum. If the pH or temperature is either increased or decreased from this optimum, the rate of the enzyme-catalyzed reaction will decrease due to adverse structural changes in the tertiary structure of the enzyme.
4. Cofactors and coenzymes are specific, smaller, nonprotein molecules that are required for an enzyme to work optimally. Cofactors are specific inorganic ions (such as magnesium), whereas coenzymes are organic compounds derived from water-soluble vitamins.
5. (a) hydrolyze the phosphate group from the substrate molecule  
 (b) synthesize glycogen molecules from glucose monomers by dehydration synthesis  
 (c) remove a hydrogen atom from lactic acid  
 (d) assemble a polymer of DNA from nucleotide monomers (DNA synthesis)
6. A large amount of substrate must be added to the sample of plasma to ensure the availability of substrate does not limit the rate of enzyme activity. To measure the activity of a different enzyme in the same plasma sample the investigator must use a reagent that will produce a differently colored product. With a differently colored product it will absorb optimally at a wavelength other than that required for the first enzyme assay.
7. When the pH or temperature deviates from the optimal for an enzyme (or other important protein), the tertiary or 3-dimensional bending and folding of the enzyme is altered. When structural changes occur, especially at the active site region, the activity of the enzyme will be adversely affected.
8. Lactate dehydrogenase catalyzes the conversion of pyruvic acid to lactic acid in the skeletal muscle during anaerobic respiration. In these cases, the coenzyme NADH is oxidized to NAD. In the liver and cardiac muscle, by contrast, lactate dehydrogenase catalyzes the reverse reaction whereby lactic acid is converted to pyruvic acid. This involves the reduction of NAD to NADH. The ability of the liver to reform pyruvic acid from lactic acid is a crucial part of the Cori cycle. In this pathway, lactic acid produced by the anaerobic metabolism of glucose in working skeletal muscles can be recycled into pyruvic acid and ultimately used to reform glucose by cells of the heart and the liver.
9. Under ideal conditions, one international unit (IU) is defined as the quantity of enzyme required to convert one micromole of substrate per minute into products. In this way, the amount of product formed will double when the reaction time is doubled. Therefore, measurement of the reaction time has to be controlled and be less than the time all substrate would be converted to product. If the tubes were allowed to incubate overnight before finally stopping the reaction, even the tubes with low enzyme activity would have had time to convert all substrate to product. In this case, the enzyme units of activity as stated in the definition could not be determined.

10. No. The presence of an intracellular enzyme in the blood plasma would suggest damage to those pancreatic cells that synthesize the enzyme pancreatic amylase (such as pancreatitis). The enzyme activity would depend upon the pH and temperature optima for pancreatic amylase. Given that this enzyme has an optimal pH range of 6.7 to 7.0 (see Table 18.8) pancreatic amylase would not be as active in the blood (pH of 7.4) as it normally is in the small intestine. It may, however, have some activity since the temperature optimum is the same in both locations at 37 degrees centigrade.

## **EXERCISE 2.5 GENETIC CONTROL OF METABOLISM**

**Approximate Time for Completion: 30-60 minutes**

### **Introduction**

This exercise is designed to introduce students to the concept whereby gene products can become involved in the regulation of metabolism. Phenylketonuria, alkaptonuria, and cystinuria are examples of metabolic diseases caused by defective genes. In this exercise, students will measure the quantities of the gene products phenylpyruvic acid and homogentisic acid. Caution is advised since silver nitrate must be stored in brown bottles in order to maintain its reactivity, and lead acetate is toxic. Students should be given appropriate warnings and guidance about proper handling and disposal of these potentially toxic substances.

### **Materials**

1. Test tubes, Pasteur pipettes (droppers), urine collection cups, biohazard waste receptacles
2. Phenistix (Ames Laboratories), silver nitrate (3 g/dl), 10% ammonium hydroxide, 40% sodium hydroxide, lead acetate (saturated)

*Note:* I have had trouble locating Ames Laboratories recently and no other vendor appears to carry the Phenistix test strips at the moment. Also, synthetic urine is becoming more available with specific tests including normal and abnormal values, check with large vendors such as Ward's Natural Science Establishment, Inc..

**Textbook Correlations:** Chapter 3 – Cell Nucleus and Gene Expression

Chapter 4 – Metabolic Pathways

### **Answers to Questions**

1. (a). *genetic transcription*—DNA-directed RNA synthesis  
(b). *genetic translation*—the synthesis of protein using the codons of mRNA as a template.
2. The genotype is a description of the genetic constitution of an organism. The phenotype is a physical description of the effects of the genotype on the organism. The genotype controls the phenotype through genetic transcription (DNA-directed RNA synthesis) and genetic translation (RNA-directed protein synthesis), since it is the action of proteins that directly produces the phenotype.
3. A metabolic pathway is a sequence of reactions in which the product of one enzymatic reaction becomes the substrate of the next enzyme in the sequence. If part of an unbranched pathway, A, B, C, D, and E molecules would be produced in order along a straight line. If molecule C branches to form D and E, then an alternate pathway such as to form new molecules D and E must be shown to branch from the C molecule. An inborn error of metabolism is a disease or condition that results when one of the enzymes that catalyzes a step in the metabolic pathway is missing or defective, causing an inheritable (genetic) defect. If a defect occurs along one branch of a metabolic pathway (such as from D to E), then the alternate pathway (D to E) would be favored – leading to a lack in the production of E and an overproduction of E, with potentially clinical implications.
4. Phenylketonuria (PKU) is an inborn error of metabolism that is produced as a result of defective enzyme responsible for the conversion of the amino acid phenylalanine into tyrosine. As a consequence, accumulations of phenylalanine cause the excessive production of an alternate product called phenylpyruvic acid. Abnormally large amounts of phenylpyruvic acid can affect the developing central nervous system and can cause mental retardation.

- In order to correct an inborn error of metabolism would require gene replacement. The defective gene would have to be replaced by a healthy gene. Since these pathways are critical for daily metabolism, any defect would be expressed in all cells of the body that use this part of the genome and therefore could not be permanently corrected by the administration of a simple pill or injection.
- Once the complete human genome is sequenced the individual genes representing the defective genes can be identified and synthesized. One day, treatment will consist of gene replacement therapy during which the defective gene will be replaced with the “good” gene.

## **EXERCISE 2.6      DIFFUSION, OSMOSIS, AND TONICITY**

**Approximate Time for Completion: 2-3 hours**

### **Introduction**

This exercise is designed to introduce students to osmosis and osmolality. Students will first experiment with the solubility of substances in polar and nonpolar solvents and then observe a thistle tube to see the movement of water caused by osmosis. Finally, students will place red blood cells in solutions of different tonicity to see the effects of osmolality on red blood cell appearance and diameter. Red blood cells placed in the two hypertonic solutions will crenate while the cells placed in the two hypotonic solutions will swell and eventually hemolyze.

### **Materials**

- Test tubes, thistle tubes, dialysis tubing
- Beakers, ring stands, burette clamps
- Lancets, alcohol swabs, or blood from veterinary or other appropriate source; biohazard receptacles
- Microscopes, slides, cover slips, and transfer pipettes
- Two dilutions of molasses, 200 mL (a 12 oz. jar) diluted with water to 1 L (20% solutions); and 250 mL of molasses diluted with water to make 1 L (25% solution)
- Sodium chloride solutions in dropper bottles in these concentrations: 0.20 g, 0.45 g, 0.85 g, 3.5 g, and 10 g per dl each
- Toluene, potassium permanganate crystals, vegetable oil, laboratory detergent

**Textbook Correlations:** Chapter 6 – Osmosis; Regulation of Blood Osmolality

### **Answers to Questions**

- Osmosis is the passage of solvent (water) from a more dilute to a more concentrated solution through a membrane that is more permeable to water than to the solute.
- Osmotic pressure is a measure of the ability of one solution to “pull in” water from another solution across a semipermeable membrane. Osmosis is simple diffusion of water through a membrane as a result of the higher-to-lower water concentration gradient. Since osmotic pressure is proportional to its solute concentration, the osmotic pressure of distilled water is zero.
- Two solutions are said to be isotonic to each other when osmosis will not occur between two solutions that are separated by a membrane permeable to water. When a single solution is said to be isotonic, it is understood that net osmosis will not occur between that solution and human plasma, the reference solution.
- crenate
- hypertonic
- |          |          |
|----------|----------|
| NaCl     | Glucose  |
| 0.133 M  | 0.200 M  |
| 133 mM   | 200 mM   |
| 266 mOsm | 200 mOsm |

Osmosis will occur from the glucose to the NaCl solution because the NaCl solution is hypertonic to the glucose solution (assuming the membrane is not permeable to either solute). Since the NaCl solution is more concentrated than the glucose solution (less water), solvent will move into the NaCl solution.

7. When urine is hypertonic, it has a higher concentration of solutes than plasma. Since urine is formed initially as an isotonic filtrate with the same osmolality as plasma, a hypertonic urine output means that water was reabsorbed from the filtrate along the nephron and back into the blood, thus conserving water in the body.
8. The sugar content of molasses was osmotically active because the dialysis membrane was not permeable to the sugar, but was permeable to water. Therefore, there was a net movement of water from the beaker into the sugar solution represented by the molasses.
9. As the man loses water, through urine, sweat, and breathing, he becomes dehydrated if he does not drink. His blood volume decreases as solvent (water) is lost causing the blood to become more concentrated. This rise in blood osmolality stimulates osmoreceptors in the hypothalamus, which causes a sensation of thirst and the release of ADH. From the table in Part C. of the lab report, seawater is hypertonic to the blood. Drinking seawater will, in fact, raise the osmolality. Since the receptors for thirst are stimulated by a high blood osmolality, drinking seawater cannot satisfy the sense of thirst.
10. Salting meat creates a local hypertonic environment at the interface between salt and meat. When decomposing organisms (bacteria and fungi) come in contact with the salted meat, water will be drawn out of these cells, thus killing or inactivating the organisms and rendering the underlying meat sterile. Before preparing however, the pioneers must remove the salt and rehydrate the meat.