UNIT: Proteins

Task
Electrophoresis: discussion / performance of an electrophoretic procedure, as available.

Objectives
Upon completion of this exercise, the student will be able to:
1. Review electrophoresis information as presented in class.
2. Relay characteristics of proteins and protein electrophoresis / fractionation.
3. Determine acceptability of run / procedure by analyzing and evaluating control results.
4. Provide answers to related study questions.
5. View virtual gel electrophoresis lab at: http://learn.genetics.utah.edu/units/biotech/gel/
6. Perform assigned tasks.

Introduction

Serum proteins
Plasma contains over one hundred individual proteins, each with a specific set of functions and subject to specific variations in concentration under different physiologic and pathogenic conditions. They interact with virtually all body tissues or cells and they are intimately related to protein metabolism in the liver.

Plasma proteins perform a great many physiologic functions. They serve as transport molecules for lipids, hormones, vitamins, and metals. They help maintain osmotic balance and serve as enzymes, complement components protease inhibitors, or kinin precursors. They play an important role in hemostasis (as clotting factors), the regulation of cellular activity and function, and in the defense against infection (immunoglobulins). They contribute to the nitrogen needs of the body.

The key roles which plasma proteins play in bodily function, together with the relative ease of assaying them, makes their determination a valuable diagnostic tool as well as a way to monitor clinical progress.

Separation techniques
Serum proteins have been separated into major fractions or groups for quite some time using solubility principles (“salting out” and ultracentrifugation techniques separating fractions (4.5s, 7s and 19s) based on molecular weight.

Electrophoresis
Electro - energy of electricity
Phoresis - from the Greek, ‘phoros’ - to carry across
“Moving boundary” or “free electrophoresis” employing a fluid medium for protein separation was first introduced by Tiselius in 1937. He is also credited for the majority of the resulting terminology developed. The original work resulted in separation of proteins into four major bands designated albumin, alpha, beta, and gamma globulin.

With the introduction of filter paper electrophoresis by Konig in 1937, the use of electrophoresis determinations became practical. Improved techniques resulted in greater resolution and yielded five major serum protein fractions.

The introduction of cellulose acetate in 1958 by Kohn made possible much more rapid analysis using a small sample volume. Other advantages of cellulose acetate over paper as a support media are its great tensile strength when wet, its almost pure and relatively uniform structure, minimal sample absorption, and its low affinity for dyes. Cellulose acetate also may be rendered almost crystal clear for easier quantitation. Agarose gels and polyacrylamide gels have become increasingly popular and add the effect of molecular sieving. Clinical use of electrophoretic results depends greatly on the resolution achieved for the proteins and the experience of the interpreter.
Clinical uses
Electrophoresis is best used as a screening technique. The clinical use of electrophoresis in serum / plasma protein analysis is still based on the simple electrophoretic separation of plasma proteins according to their relative mobility into: albumin, \(\alpha_1\), \(\alpha_2\), \(\beta\), and \(\gamma\) globulins. Investigations have determined that each of the classical electrophoretic zones contains two or more major plasma proteins that are often subject to independent metabolic regulation. Specific analysis of individual proteins is increasingly used as a supplement to electrophoresis analysis.

Electrophoretic techniques have been applied to a variety of protein substances in addition to total serum proteins. Applications include:

Hemoglobin Electrophoresis
Using electrophoretic separation techniques on hemoglobin proteins has identified more than 400 variations. The most common ones are HbA, HbA2, HbF, HbS, HbC, Hgb H, and Hgb M. In normal adults, only HbA and HbA2 are present at significant levels. Small amounts of HbF (which is the major Hb present in the fetus) may be present, but they are of no consequence unless levels are more than 2% of the total.

HbS is an abnormal form of hemoglobin associated with sickle cell anemia. Symptoms can be very severe.

HbC is an abnormal form of hemoglobin associated with hemolytic anemia. The symptoms are much milder than they are in sickle cell anemia.

The presence of significant levels of abnormal hemoglobins may indicate:
* Sickle cell anemia
* Hemoglobin C disease
* Rare hemoglobinopathy
* Thalassemia - an additional condition for which the test may be performed.

Normal Values
In adults, these hemoglobin molecules make up the total hemoglobin, as follows:

* Hgb A1: 95% to 98%
* Hgb A2: 2% to 3%
* Hgb F: 0.8% to 2%
* Hgb S: 0%
* Hgb C: 0%

Scans to the right:
Numbers 1, & 2 Same patient with abnormal fast moving hemoglobin fraction. Numbers 4 & 5 are same patient sample, but using different buffer. The densitometer scan at the right is of the number 5 band.
Numbers 3 & 6 are normal control sample.

Note location of (-) anode.
Lipoprotein Electrophoresis

Lipoproteins are protein molecules that transport lipids such as cholesterol and triglycerides throughout the body. There are four major classes of lipoproteins:

* Chylomicrons, which circulate in the blood and deposit the triglycerides in fatty tissue
* Very low-density lipoproteins, which like chylomicrons also circulate in the blood and deposit triglycerides in fatty tissue but contain a higher concentration of cholesterol
* Low-density lipoproteins, also called “bad cholesterol,” form 60 to 70 percent of a person’s total cholesterol
* High-density lipoproteins, also called “good cholesterol,” removes excess cholesterol from tissues in the body

Various works of researchers, Fredrickson, Levy, & Lee, have demonstrated through epidemiologic studies the correlation of atherosclerosis, cardiovascular disease and stroke with abnormal levels of lipoproteins. Electrophoretic separation of lipoprotein fractions is one method of evaluating lipoprotein fractions.

Isoenzyme Electrophoresis

Isoenzymes are multi-molecular forms of an enzyme. They are structurally different enzymes, often with differing biochemical or immunological characteristics, but can catalyze the same reaction. They often are found in varying amounts in different tissues throughout the body. Detecting and quantifying isoenzymes is used as a means to determine the extent of tissue damage. Electrophoresis one such method of doing this.

Enzymes of interest with known iso forms include alkaline phosphatase, creatine phosphokinase, and lactate dehydrogenase.

Lactate dehydrogenase (LDH) or (LD) is used as an example.

The LD molecule has two polypeptide units, identified as “H” and “M”. They can exist in five combinations:

<table>
<thead>
<tr>
<th>designation</th>
<th>peptides</th>
<th>tissue(s) associated with</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD&lt;sub&gt;1&lt;/sub&gt;</td>
<td>(HHHH)</td>
<td>heart, RBCs, &amp; kidney</td>
</tr>
<tr>
<td>LD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>(MHHH)</td>
<td>also heart</td>
</tr>
<tr>
<td>LD&lt;sub&gt;3&lt;/sub&gt;</td>
<td>(MMHH)</td>
<td>lung, spleen, pancreas</td>
</tr>
<tr>
<td>LD&lt;sub&gt;4&lt;/sub&gt;</td>
<td>(MMMH)</td>
<td>not specific - many tissues</td>
</tr>
<tr>
<td>LD&lt;sub&gt;5&lt;/sub&gt;</td>
<td>(MMMM)</td>
<td>liver &amp; skeletal muscle</td>
</tr>
</tbody>
</table>

Clinical usefulness of LD isoenzymes include evaluation of liver and heart disease. The most significant use of LD isoenzyme analysis is when LD<sub>2</sub> levels exceed LD<sub>1</sub>. This is known as a “flipped pattern” and is seen in acute myocardial infarction.

General Procedures, protein electrophoresis on cellulose acetate

Principles

The principle of electrophoresis is based on the fact that a charged particle placed in an electrical field will migrate toward one of the electrodes of the field depending on the:

- electrical charge on the particle
- size of the particle
- strength of the electrical field
- nature of the medium used to support the particle during the migration process
The following lists would be applicable for protein electrophoresis using cellulose acetate as the support medium.

**Supplies and Equipment**
1. applicator
2. sample well plate
3. aligning base
4. cellulose acetate support medium
5. chamber and wicks
6. staining set
7. evaporation hood
8. densitometer

**Reagents**
1. Barbitol Buffer pH 8.6
2. Ponceau S stain
3. 5% acetic acid
4. Clearing solution: 125 mL of reagent grade methanol plus 50 mL DI water

**Specimens**
1. Fresh unhemolyzed serum is the specimen of choice. Plasma can be used but an extra peak will appear in the beta zone.
2. Urine can be used, but usually concentration procedures must be done to assure the presence of protein in adequate amounts.
3. CSF specimen. Concentration may be required.

**Results**
1. **Relative Position of the Protein Fractions**
   The fastest moving band, and normally the most prominent, is the albumin band found closest to the anodic edge of the plate. The faint band next to this is Alpha, Globulin, followed by Alpha, Globulin, Beta and Gamma Globulins.

2. **Basic Evaluation**
   A. Determine relative and absolute amount of serum albumin. While a decreased amount is often associated with renal diseases, it may also be due to increased utilization.

   B. Determine the A/G ratio.

   C. Evaluate for decreased fractions, such as alpha 1 which is associated with alpha 1 antitrypsin.

   D. Evaluate gamma fraction. The gamma fraction is of special interest because most immunoglobulins migrate to this area. A decrease could indicate defective production (hypogammaglobulinemia), and general increased levels are associated with infection. The gamma region is most often region where abnormal, malignant proteins would be found. A spike or sharply increased level found in this area is seen in a number of rare malignant conditions where there is over production of immunoglobulin proteins. In fact, serum protein electrophoresis is most often done to screen for multiple myeloma, a rare cancer that causes overproduction of IgM proteins.
3. Calculations

Calculate from the integrator scale on the scan the relative percent of the various fractions in the sample.

Determine the total protein in the sample in grams per deciliter by a standard laboratory method.

Multiply the relative percent of each band times the total protein value. Divide this by 100. The result is the value in grams per deciliter for the various fraction.

**Example:**

Total Protein = 8.2 gms/dL

- **Albumin** = 59% \( \frac{59}{100} \times 8.2 = 4.8 \text{ gms/dL} \)
- **Alpha\textsubscript{1}** = 5% \( \frac{5}{100} \times 8.2 = 0.3 \text{ gms/dL} \)
- **Alpha\textsubscript{2}** = 8% \( \frac{8}{100} \times 8.2 = 0.7 \text{ gms/dL} \)
- **Beta** = 10% \( \frac{10}{100} \times 8.2 = 0.8 \text{ gms/dL} \)
- **Gamma** = 19% \( \frac{19}{100} \times 8.2 = 1.6 \text{ gms/dL} \)

Relative percent and grams per deciliter may be computed automatically using a computer accessory with the densitometer.

**Expected Values**

The expected values for serum protein electrophoresis on cellulose acetate stained with Ponceau S were determined from an in-house study of 51 normal subjects. These values are for illustrative purposes only. Each laboratory should establish its own range.

<table>
<thead>
<tr>
<th>Mean (gm/dL)</th>
<th>S.D.</th>
<th>Range (+2 S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>4.27</td>
<td>0.32</td>
</tr>
<tr>
<td>Alpha\textsubscript{1}</td>
<td>0.23</td>
<td>0.06</td>
</tr>
<tr>
<td>Alpha\textsubscript{2}</td>
<td>0.91</td>
<td>0.13</td>
</tr>
<tr>
<td>Beta</td>
<td>1.00</td>
<td>0.13</td>
</tr>
<tr>
<td>Gamma</td>
<td>1.16</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**Interpretation of Patterns**

Below is a normal serum protein graph showing the location of some of the more commonly known proteins.

**Record Your Results**

- Total
- Albumin
- Alpha\textsubscript{1}
- Alpha\textsubscript{2}
- Beta
- Gamma
Study Questions

Instructions: Based on information presented in lecture and lab notes, answer the following questions. Legibly write your answers in the space provided. Unless otherwise indicated, each question is worth one point.

1. What is the charge of a cathode? Anode?
2. Which ions travel toward the anode? The cathode?
3. What property of proteins in the buffer makes electrophoresis possible?
4. Identify the buffer referred to in the previous question. What is its pH?
5. List four (4) variables that affect electrophoretic separation of proteins. (½ point each)
   a. 
   b. 
   c. 
   d. 
6. Identify the five (5) major protein classes, listing them in order of migration from the anode to the cathode.
7. The introduction of “moving boundary” technique of electrophoresis is credited to
8. List two (2) support mediums, other than cellulose acetate, that have been used in moving boundary electrophoresis.
9. List two (2) methods, other than electrophoresis, that have been used to separate proteins into fractions.
11. Which protein fraction is most affected by it? (½ point)
12. Why is this fraction influenced by electroendosmosis? (½ point)
13. The lab says that a plasma can be used for electrophoresis, but an extra peak will be detected. What would cause this extra protein peak to occur in electrophoresed normal plasmas?

14. Calculate the concentration in gm/dL for each electrophoretic fraction of a specimen having a total protein of 6.4 gm/dL with 47% albumin, 4% alpha_1, 9% alpha_2, 11% beta, and 29% gamma.

15. What rare malignancy produces a distinct serum protein electrophoretic pattern?

16. List three (3) applications of electrophoresis, other than for serum proteins.

17. What hemoglobins can be identified by electrophoresis of a ‘normal specimen’?

18. What is the value of lipoprotein electrophoresis?

19. What is the value of isoenzyme electrophoresis?

20. To whom is the original work on electrophoresis credited?