

## Experiment 3 (103). Serum Proteins

### Background Information

Blood is a remarkable tissue containing cellular elements (erythrocytes, leukocytes and platelets) suspended in a liquid medium called plasma. Whole blood or plasma clots upon standing and if the clot is removed, the remaining straw colored fluid is called serum. Serum has basically the same composition as plasma except that it lacks certain proteins (fibrinogen and some clotting factors) that are involved in the clotting process.

Serum contains a variety of small molecular weight components as well as hundreds of different serum proteins. Serum proteins, such as the antibodies, are important in fighting disease. Other proteins in serum, such as albumin, transferrin, and the lipoproteins, function as carrier molecules for the transport of small molecular weight compounds such as metals, fatty acids, amino acids, hormones and drugs.

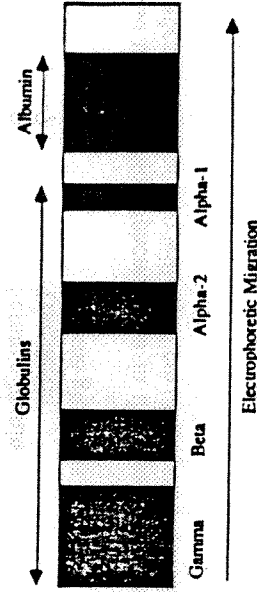
The serum proteins are usually divided into albumin and globulin classes. Serum albumin is the major protein found in serum. In normal adult humans, the serum albumin level is 3.5-4.5g/100 ml. Albumin, like many other serum proteins, is produced in liver and liver damage often results in a decrease in the level of this protein in serum. Albumin functions in the transport of many substances in blood and plays an important role in the control of plasma osmolarity.

The serum globulins can be subdivided into four major fractions. These fractions are alpha-1 ( $\alpha_1$ ) globulin, alpha-2 ( $\alpha_2$ ) globulin, beta ( $\beta$ ) globulin and gamma ( $\gamma$ ) globulin. Each globulin fraction contains a large number of different proteins. For example, the protein transferrin is only one of the proteins found in the  $\beta$  globulin fraction of serum. This protein transports iron in the circulation. The numerous antibodies in blood are found in the  $\gamma$  globulin fraction of serum. Table 3-1 lists the protein fractions of serum and the general properties of at least one specific protein found within each group.

Serum proteins are frequently separated and characterized by electrophoresis on agarose gels. In the clinical laboratory, this technique is performed in order to determine the concentration of various proteins in a patient's serum as well as to detect abnormal proteins. From the isoelectric points of the serum proteins listed in Table 3-1, it is evident that the relative rates of migration of these proteins toward the positive electrode at pH 8.6 follows the general order: albumin >  $\alpha_1$ -globulins >  $\alpha_2$ -globulins >  $\beta$ -globulins >  $\gamma$ -globulins. The electrophoretic pattern of serum proteins is illustrated on the following page.

**Table 3-1. Major Proteins in Serum.**

Fraction	Amount (g/100ml)	Example	Isoelectric Point of the Example	Site of Production	Function
Albumin	3.5-4.5	Albumin	4.7	Liver	Transport, osmotic regulation
Globulin	3.0-3.5				
$\alpha_1$ -globulins	0.3-0.6	$\alpha_1$ -Lipoprotein	5.2	Liver	Lipid transport
$\alpha_2$ -globulins	0.4-0.9	$\alpha_2$ -Macroglobulin	5.4	Liver	Protein transport
$\beta$ -globulins	0.6-1.1	Transferrin	5.9	Liver	Iron transport
$\gamma$ -globulins	0.7-1.5	The Antibodies	6.3-7.3	Lymphoid Tissue	Fight infection



**Objective** To study the electrophoretic pattern of serum proteins from cow and to identify albumin, transferrin, and the antibodies in serum.

swirl the dish to ensure that all surfaces of the blot are exposed to the antibody solution.

2. Place the lid on the dish, and float the dish in a water bath at 37° for 25 minutes.

Note: Great care should be taken not to bump the dishes during the incubation.

3. Transfer the blot to a suitable small container (e.g. a clean gel staining tray) and wash for two minutes each in 40ml of the following solutions. Manual rocking or shaking of the container should be performed during these washes.

1. TBS+NP40
2. TBS+NP40
3. TBS+NP40
4. TBS+NP40
5. TBS

4. While the blots are washing, the instructor should prepare the Color Development Solution by adding 7ml of Color Development Buffer, 0.5ml of hydrogen peroxide and 5ml chloronaphthol to 130ml of water.

5. Pour the last wash buffer off blot and then replace with 30ml Color Development Solution. Gently rock blot in Color Development Solution until purple bands appear. This should take about 10 minutes. Rinse the blot in water and examine both sides. Record your results noting the number and relative migrations of proteins that are detected in each sample. Note that the bovine albumin and bovine albumin dimer in the standard protein mixture (lanes 1 and 5) should yield a positive reaction with the antibody. Blots may be stored protected from heat and light (between 2 sheets of black construction paper, for example).

### Study Questions

1. Compare the reaction of the antibody to albumins from the various mammals. Which mammals show the greatest similarity to cow with respect to the reaction? Which show the least?
2. How does this analysis compare with the traditional taxonomic relationships reported for these animals? You may need to consult a zoology or comparative anatomy text book to determine what is known about the relationships between cow, sheep, goat, and horse.
3. Describe how your results would have been affected if the antibody had been made in rabbits against duck albumin.