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Protein Learning Guide

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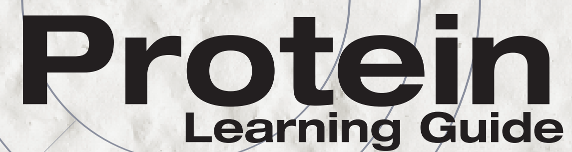
Protein
Learning Guide

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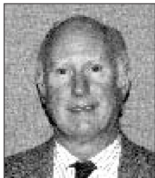
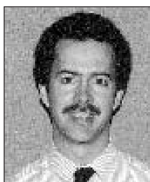


Robert F. Ritchie, M.D.

After serving in the US Navy and completing undergraduate study at Cornell University, Dr. Ritchie graduated from The University of Rochester School of Medicine in 1960. He completed his medical training at Tufts New England Medical Center and a post-graduate clinical-research fellowship at the Robert B. and Peter B. Brigham hospitals in Boston in 1965. He subsequently moved to The Maine Medical Center in Portland, Maine as a consulting clinical rheumatologist. His group developed immunofixation as a practical tool for the clinical laboratory where it has become the current "gold standard" for identifying and typing monoclonal immunoglobulins. He also introduced light-scattering immunoassays for serum proteins to clinical medicine.



Thomas B. Ledue

Mr. Ledue attended the University of Southern Maine where he obtained his baccalaureate degree in biology in 1980. Upon graduation, Mr. Ledue worked for Atlantic Antibodies, an internationally recognized manufacturer of immunodiagnostic products. Mr. Ledue oversaw the development of the company's nephelometric and turbidimetric immunoassay program. In 1988, he joined the Foundation for Blood Research (FBR). Since joining the FBR, Mr. Ledue has pursued his interests in the areas of rheumatology and protein chemistry, immunologic method development, and standardization of immunoassays. He has participated in several successful international standardization programs for apolipoproteins and plasma proteins.

About this Learning Guide

This Learning Guide is an overview of how clinically important serum proteins are used to diagnose and manage common clinical situations. In addition, it will review different techniques used to identify and quantify these proteins, including important analytical considerations. As you progress through this learning guide, review the learning objectives and complete the quizzes at the end of each section. A glossary of terms is located at the back of the guide.

Instructions for Use

This guide is organized into four chapters:

- Introduction to Plasma Proteins
- Changes in Protein Levels Associated with Disease
- Identification and Quantitation of Serum Proteins
- Laboratory Considerations in Protein Analysis

Suggestions for Further Reading (Appendix B) are general references, which can be used to expand upon the introductory discussions given here. A total of 1.0 hour CEU may be claimed upon completion of the Guide, including answering the questions.

Table of Contents

Chapter 1: Introduction to Plasma Proteins 1

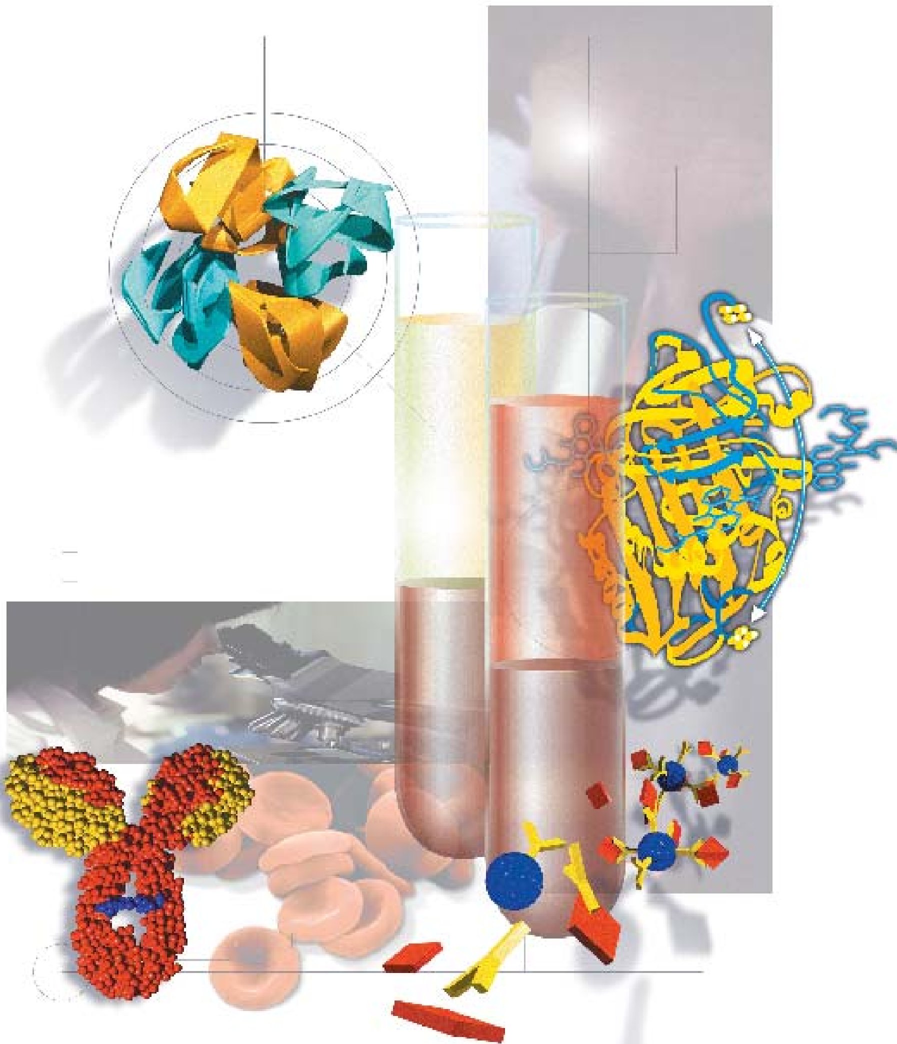
Chapter 2: Changes in Protein Levels Associated with Disease 7

Chapter 3: Identification and Quantification of Serum Proteins 23

Chapter 4: Laboratory Considerations in Serum Protein Assays 29

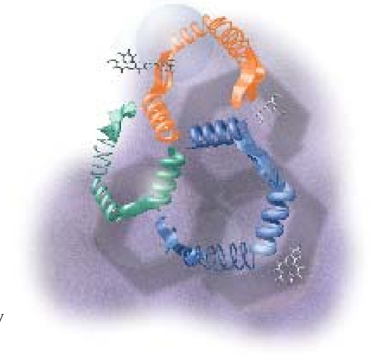
Appendix A: Glossary of Terms 34

Appendix B: Suggestions for Further Reading 37



Chapter One

Introduction to Plasma Proteins



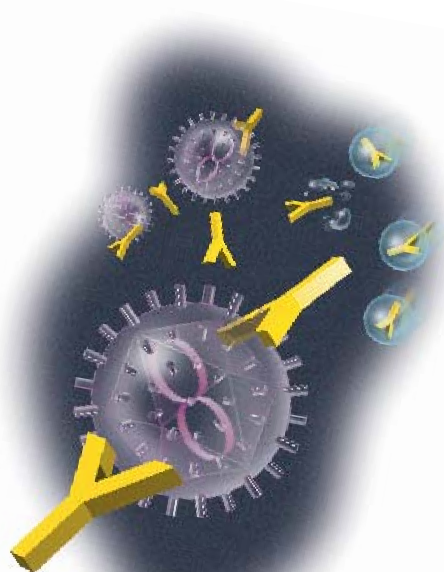
Learning Objectives:

After completion of this chapter, you will be able to:

- Describe the distribution of plasma proteins in the body
- Indicate where proteins are made in the body
- Describe suitable specimens for protein evaluation
- List the functional classifications of proteins

The human body contains countless numbers of different proteins. Literally speaking, the body's framework and substance is protein. The number of distinct proteins within one cell is estimated at 3,000 - 5,000. Not only is the variety of proteins seemingly infinite, but so are their distribution within the body, their functions, their compositions, and their structure. The proteins most amenable to routine laboratory evaluation are those in blood, urine, spinal fluid, amniotic fluid, saliva, feces, and peritoneal or pleural fluids. With a few exceptions, proteins found in the other fluids were originally contained in the blood plasma.

The plasma proteins include the immunoglobulins, enzymes, and enzyme inhibitors. Most plasma proteins, with the notable exception of immunoglobulins, are synthesized in the liver. Plasma proteins circulate in the blood and between the blood and the extracellular tissue spaces.



Functional classification of plasma proteins is useful in understanding the changes that occur in disease:

- Proteins of immune defense
  Immunoglobulins for the elimination of antigen
- Acute phase protein response
  Proteins associated with inflammation
- Transport proteins
  Proteins used to bind and transport
- Proteins of blood clotting
  Proteins involved in forming clots and acting very closely with complement
- Tissue derived proteins
  Proteins shed into the blood during cell membrane turnover or cell death
- Oncofetal proteins
  Proteins produced by tumors and/or during fetal life

Table 1: **Clinically Important Serum Proteins**

This table lists clinically important serum proteins and their primary function.

The individual proteins are listed in order of approximate electrophoretic mobility, from prealbumin to gamma: Please refer to the electrophoresis pattern graph on page 24.

Protein[1]	Protein; Approximate Molecular Mass[2]	Primary Functions	Adult Reference Intervals[3]	Decreased Levels In Serum	Increased Levels In Serum
Prealbumin	Transthyretin (TTR); thyroxine-binding prealbumin 54.98 kDa	Binds and transports ~20% of thyroid hormones (T3, T4) Binds and transports retinol binding protein (see below)	M: 20-40 mg/dL (0.2-0.42 g/L) F: 19-38 mg/dL (0.19-0.38 g/L)	Acute phase response[4] Liver disease ↑ Immunoglobulins Sieving protein loss[5] Protein calorie malnutrition Infancy	Anti-inflammatory drugs Stress; depression Androgens
	Retinol binding protein (RBP) 21 kDa	Binds and transports retinol (vitamin A)	3-6 mg/dL (0.03-0.06 g/L)	Same as transthyretin, plus vitamin A deficiency	Hypervitaminosis A
Albumin	Albumin 66.3 kDa	Maintenance of osmotic pressure Transport of metal ions, fatty acids, amino acids, drugs, hormones, bilirubin, etc. Reservoir for amino acids	3,600-5,200 mg/dL (36-52 g/L)	Acute phase response[4] Liver disease ↑ Immunoglobulins Sieving protein loss[5] Protein calorie malnutrition Genetic analbuminemia	Acute dehydration (rare)

Protein[1]	Protein; Approximate Molecular Mass[2]	Primary Functions	Adult Reference Intervals[3]	Decreased Levels In Serum	Increased Levels In Serum
α_1 Globulins	α_1-Antitrypsin (AAT); α_1-Proteinase inhibitor 51 kDa	Binding and inactivation of trypsin-like proteolytic enzymes, especially leukocyte elastase	90-190 mg/dL (0.9-1.9 g/L)	Genetic deficiency Consumption in neonatal respiratory distress syndrome and severe pancreatitis Sieving protein loss[5]	Acute phase response[4] Liver disease Estrogens (pregnancy, oral contraceptives) Androgens
	α_1-Antichymotrypsin (ACT) 68 kDa	Inactivation of chymotrypsin-like enzymes, such as leukocyte cathepsin G	30-60 mg/dL (0.3-0.6 g/L)	Acute phase response[4]	Genetic deficiency Consumption in severe asthmatic attacks Infancy
	α_1-Acid glycoprotein (AGP); Orosomucoid 40 kDa	Binds and "inactivates" progestins and neutral or cationic drugs, such as propranolol and quinidine[4]	50-120 mg/dL (0.5-1.2 g/L)	Estrogens (pregnancy, oral contraceptives) Sieving protein loss[5]	Acute phase response (with ↓ bioavailability of bound drugs)
	Apolipoprotein A1 (Apo A1) ~28 kDa	Major structural component of HDL Reverse cholesterol transport (removal from body) Activator of LCAT (cholesterol esterification)	M: 100-185 mg/dL (1-1.85 g/L) F: 110-220 mg/dL (1.1-2.2 g/L)	Male sex Androgen therapy Genetic alterations, including Tangier disease Physical inactivity	Female sex Estrogens (pregnancy, oral contraceptives) Genetic alterations Moderate alcohol intake Exercise Medications (e.g., niacin)
α_2 Globulins	Ceruloplasmin (Cp) 132 kDa	Copper oxidase, essential for oxidation of iron and incorporation into transferrin May transport some copper to tissues (primarily by albumin and transcuprein)	20-60 mg/dL (0.2-0.6 g/L); higher in F on oral contraceptives or during pregnancy	1^0 genetic deficiency (clinically similar to hereditary hemochromatosis) 2^0 deficiency in: Wilson's disease Menke's syndrome Copper deficiency Protein loss	Female sex Estrogens (pregnancy, oral contraceptives) Acute phase response[4] (mild, late increase)
	α_2-Macroglobulin (A2M) 720 kDa	General (nonspecific) inhibitor of proteolytic enzymes Transport of cytokines and growth factors	130-400 mg/dL (1.3-4.0 g/L); varies with age and sex	Pancreatitis Chronic peptic ulcer disease DIC[6] or fibrinolysis Acute phase response[4] (mild decrease) Male sex	Childhood Estrogens (pregnancy, oral contraceptives) Sieving protein loss[5] (esp. nephrotic syndrome)
	Haptoglobin (Hp) 85-840 kDa	Binds and transports free hemoglobin Minimizes renal loss of iron Peroxidase activity, especially of Hp:Hgb complexes	30-200 mg/dL 0.3-2.0 g/L	Hemolysis Ineffective erythropoiesis Estrogens (pregnancy, oral contraceptives) Sieving protein loss[5] (Hp 1-1 phenotype) Genetic deficiency	Acute phase response[4] (mild, late increase) Sieving protein loss[5] (Hp 2-1 and 2-2 phenotypes) Anti-inflammatory drugs Stress; depression Androgens
β Globulins	Transferrin (Tf) 79.6 kDa	Transport of iron (Fe^{3+}) to tissues Prevention of iron loss by kidneys Prevention of oxidation of proteins and lipids by Fe^{3+} Protection against infection by Fe-dependent bacteria	200-360 mg/dL (2.0-3.6 g/L)	Acute phase response[4] Chronic disease Protein calorie malnutrition Sieving protein loss[5] Anemia of chronic disease (with ↑ iron saturation) Variable in hereditary hemochromatosis (with high iron saturation)	Iron deficiency Estrogens (pregnancy, oral contraceptives) Variable in hereditary hemochromatosis (with high iron saturation)

Protein[1]	Protein; Approximate Molecular Mass[2]	Primary Functions	Adult Reference Intervals[3]	Decreased Levels In Serum	Increased Levels In Serum
β Globulins	Complement C3 185 kDa	Central component of both classical and alternative complement pathways Essential for complement-mediated cell lysis, opsonization, and phagocytosis	85-175 mg/dL (0.85-1.75 g/L)	Genetic deficiency (clinically similar to agammaglobulinemia) Consumption (especially in autoimmune disease and sepsis) Pregnancy (modest ↓) DIC[6] Infancy	Acute phase response[4] (mild, late increase) Mild sieving protein loss[5] Biliary obstruction
	Complement C4 206 kDa	Essential factor in classical complement pathway Clearance of immune complexes Release of a weak anaphylatoxin on activation	10-50 mg/dL (0.1-0.5 g/L)	Genetic deficiency (C4A or C4B isoforms; often associated with IgA deficiency) Consumption (especially in autoimmune disease, sepsis, hereditary angioedema) Infancy	Acute phase response[4] (mild, late increase)
	Apolipoprotein B (Apo B) 512.7 kDa	Major structural component of LDL, VLDL, IDL Transport and metabolism of lipids, including cholesterol	50-160 mg/dL (0.5-1.6 g/L); age and sex dependent	Genetic deficiency or abnormal variants Apolipoprotein E2 Medications Exercise Infancy	Decreased clearance Familial hypercholesterolemia Apolipoprotein E4 Increased synthesis
γ Globulins	Immunoglobulins A, G, and M (IgA, IgG, IgM) IgA: ~160 kDa IgG: 144-150 kDa IgM: ~970 kDa	Protection against foreign antigens, especially those of infectious agents	A: 70-400 mg/dL (0.7-4.0 g/dL) G: 700-1,500 mg/dL (7-15 g/L) M: 40-250 mg/dL (0.4-2.5 g/L) (All are age and sex dependent.)	Immunodeficiency syndromes (genetic or acquired) Infancy (nadir of IgG at 2-6 months of age) Transient hypoimmuno-globulinemia of infancy IgG (± IgA) in sieving protein loss[5]	Chronic inflammatory disease, including infection, liver disease, autoimmune disease, etc. IgM in viral disease, early bacterial disease IgM in hyper-IgM immunodeficiency Plasma cell dyscrasias (multiple myeloma, plasma cell leukemia, lymphoma, Waldenström's macroglobulinemia
	C-reactive protein (CRP) ~115 kDa	Nonspecific binding to bacterial cell walls, activating complement and opsonization Binding and transport of fibronectin, laminin, and other tissue breakdown products	<0.5 mg/dL <0.005 g/L	Infancy Anti-inflammatory therapy	Acute phase response[4] (early, marked increase)

[1]Migration on good-quality agarose gel or cellulose acetate electrophoresis at pH 8.6
[2]In kilodaltons (kDa)
[3]Recommended reference intervals for adults (~20-60 years of age), based on RPPHS/CRM 470; values condensed from Ritchie and Navolotskaia and/or Dati et al. (except for RBP, ACT, Apo A1, and Apo B, from Ritchie and Navolotskaia plus Tietz *Textbook of Clinical Chemistry*, 3rd edition)
[4]Inflammation, infection, tissue necrosis, malignancy
[5]Selective protein loss via the kidneys (nephrotic syndrome) or GI tract (e.g., in systemic lupus erythematosus or SLE)
[6]Disseminated intravascular coagulation, as seen in gram-negative sepsis and other disorders

Quiz Questions for Chapter One

Circle the correct answer or fill in the blank.

1. Where are most plasma proteins synthesized?
   a. Kidney
   b. Liver
   c. Brain
   d. Not known at this time

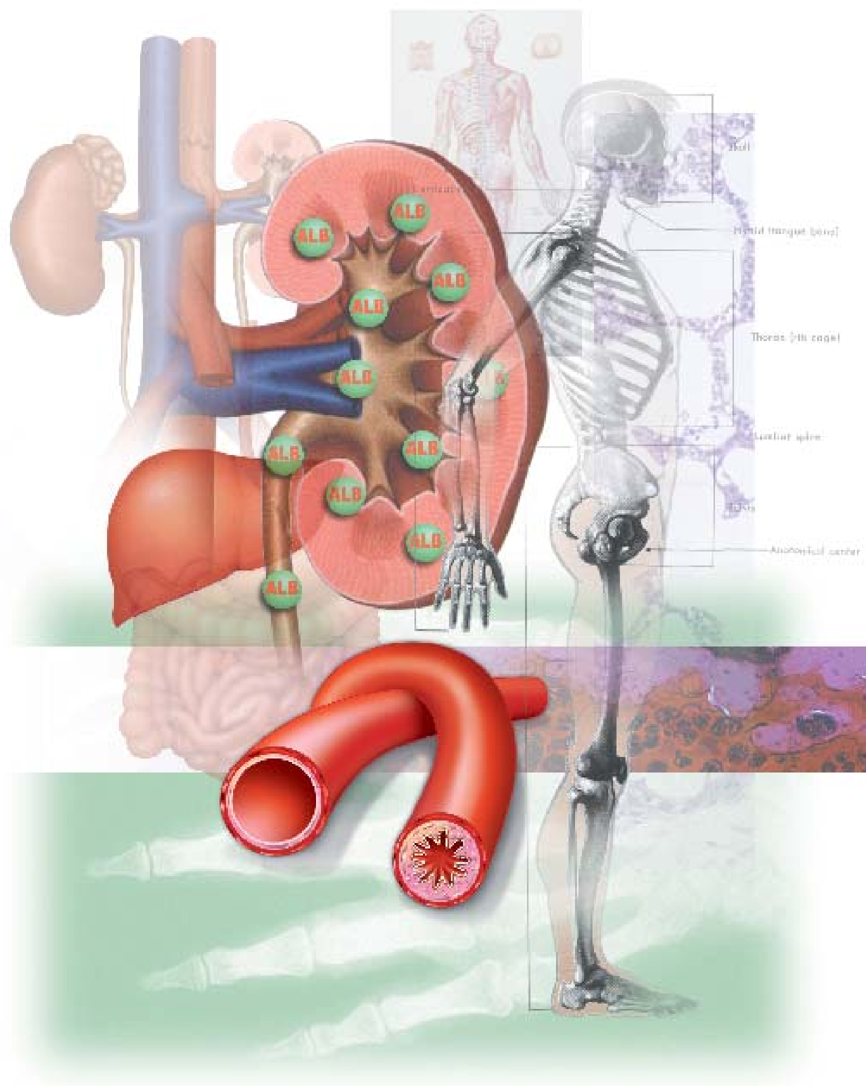
2. List three specimens that can be used to determine protein levels.

   a.________________________

   b.________________________

   c.________________________

3. The number of proteins in a distinct cell is estimated to be
   a. 1,500 - 2,000
   b. 15,000 - 20,000
   c. 3,000 - 5,000
   d. too numerous to quantify



Chapter Two

Changes in Protein Levels Associated with Disease

Learning Objectives:

After completing this section, you will be able to:

- Describe the mechanisms of protein metabolism
- Explain the difference between Positive Acute Phase Reactants (PosAPR) and Negative Acute Phase Reactants (NegAPR)
- Name eight clinical conditions that affect protein levels
- Identify changes in protein levels associated with specific diseases

Protein Metabolism and Catabolism

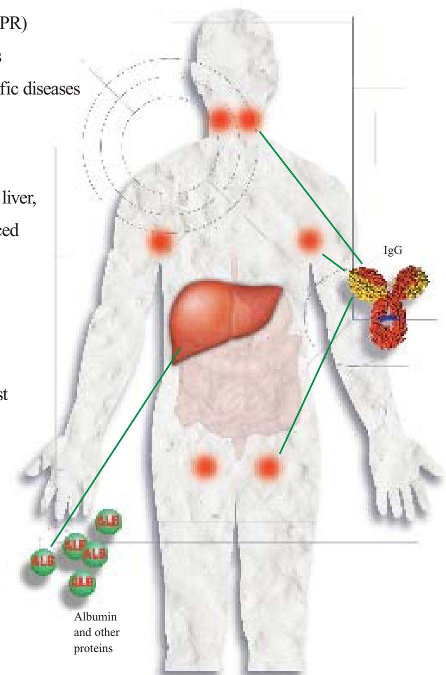
Generally, most serum proteins are synthesized (made) in the liver, with the exception of the immunoglobulins, which are produced in the lymphatic tissues, primarily in the lymph nodes.

What Happens to Proteins Once They Are Made?

Most proteins are removed from circulation and catabolized (broken down) by the liver. Up to 15 - 20% of proteins are lost in urine, feces, and other fluids. Albumin is removed by all cells in the body, which use the amino acids from the catabolism of albumin for the production of other proteins.

Why Do Concentrations Change in Disease?

In most cases, concentrations change for one or more of the following reasons associated with altered rates of synthesis and catabolism.



A. Altered rates of synthesis

Increased rates of synthesis may be caused by:

- inflammation
- anti-inflammatory drugs
- fever
- hormones
- immune response

Decreased rates of synthesis may be caused by:

- inflammation
- suppression by some diseases
- genetic deficiencies

B. Altered rates of catabolism

Increased rates of catabolism may be caused by:

- inhibition of proteolytic enzymes by inhibitors (severe pancreatitis)
- enzymatic breakdown of the proteins themselves
- loss in urine or feces or through the skin (burns)
- activation of complement due to infections or autoimmune disease

Decreased rates of catabolism may be caused by:

- blockage of liver and reticuloendothelial receptors
- changes in the distribution of proteins
  - -loss of proteins into the extracellular fluid, such as edema or pregnancy
  - -loss of proteins into body cavities, such as pleural effusion or ascites

Proteins are eliminated by one of three mechanisms: catabolized (broken down) in the blood stream, catabolized and removed by the liver or other tissues, or excreted in urine, feces, or other body fluids. Normally, proteins are eliminated by the liver when they become old and nonfunctional. Albumin is an example of a protein catabolized primarily outside the liver – it is utilized by all the cells of the body as a supply of amino acids for synthesizing their own proteins.

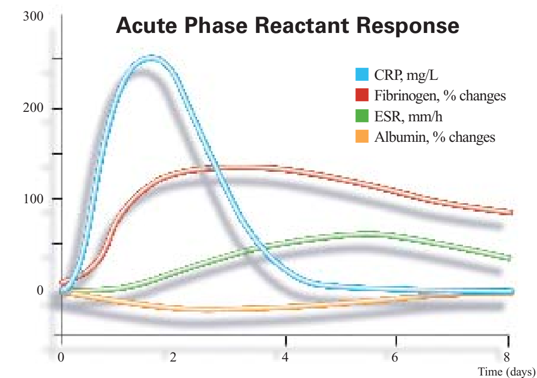
Clinical Conditions that Affect Serum Protein Levels

The following eight general clinical conditions, among others, either caused by physiological changes or induced by certain drugs, will affect serum protein levels:

- Acute inflammation, trauma, and tissue necrosis
- Chronic inflammation
- Protein loss
- Redistribution of body fluids
- Hormonal fluctuations
- In vivo hemolysis
- Iron deficiency anemia
- Drug therapy

Acute Inflammation

The most common cause of disease-related changes in serum protein concentrations is the **acute phase response** (APR). Acute phase response is a general term that refers to a number of nonspecific changes that occur as a result of many disease processes and persist as long as the disease is active. In addition to changes in serum protein concentrations, other indications of APR include fever, increased or decreased white blood cell count, and an elevated erythrocyte sedimentation rate (ESR). The acute phase response is seen in inflammation and tissue necrosis due to trauma, surgery, tumors, etc. Acute inflammation is typically caused by bacterial or viral infections.

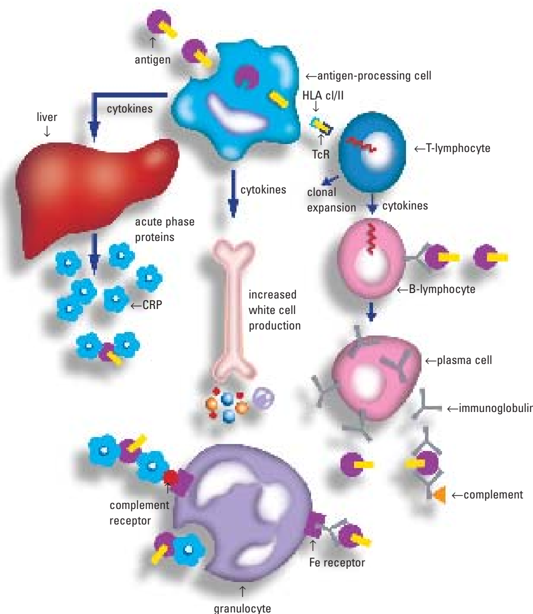


Positive and Negative Acute Phase Reactants

There are several serum proteins useful in documenting and quantifying the disease process. They can be divided into positive acute phase reactants (PosAPR) and negative acute phase reactants (NegAPR). Positive acute phase reactants are proteins that increase in concentration in response to disease. Negative acute phase reactants are proteins that decrease in concentration in response to disease.

C-reactive protein (CRP) is a positive acute phase reactant (PosAPR) that is extremely useful to the clinician in evaluating acute phase response. CRP concentrations begin to rise 6 to 12 hours after the onset of infection or trauma and may increase to more than 2,000 times normal levels. CRP concentration increases with all invasive bacterial infections, but rarely with viral infections. Once the infection begins to diminish, CRP levels begin to return to normal.

CRP Response



CRP Concentration is Helpful In

- Determining whether inflammation, tissue necrosis, or malignancy is present
- Differentiating viral from bacterial infections. Only a few viral infections, such as influenza A, result in elevated CRP concentration, whereas all invasive bacterial infections increase concentration
- Differentiating localized, noninvasive infections such as cystitis ("bladder infections") from more invasive kidney infections
- Monitoring for development of infection (e.g., after surgery or burns)
- Evaluating response to therapy (e.g., antibacterial therapy for bacterial infections)

There is one important caveat: CRP concentrations in the newborn period, and in a few adults, are much lower both in health and disease than in other individuals. For this reason, repeated or sequential determinations are often much more helpful than single assays, with rising concentration indicating clinical deterioration (worsening of the primary disease or the acquisition of a new process such as a new infection) and falling concentration indicating improvement.

Positive acute phase reactants include CRP, α_1-antitrypsin, α_1-antichymotrypsin, α_1-acidglycoprotein, haptoglobin, C3, and C4.

Negative acute phase reactants include albumin, prealbumin, and transferrin. Most of the negative acute phase reactants have been proposed as markers of malnutrition as well, but decreases are more commonly due to an acute phase response.

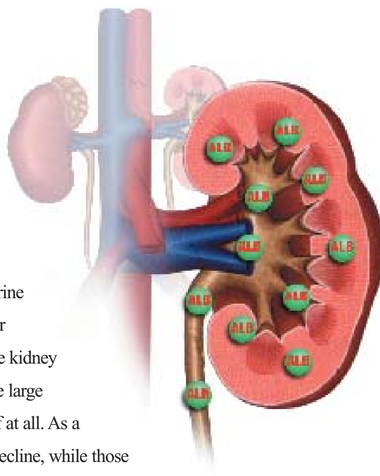
Chronic Inflammation

Chronic inflammation may be caused by infection, autoimmune diseases, early AIDS, some blood group disorders, and other conditions. In chronic inflammatory diseases, there is typically an increase in immunoglobulin concentrations in addition to a persistent acute phase response.

As a rule, chronic inflammatory changes in the immunoglobulins are as follows:

- IgM concentrations rise first, then IgG; IgM concentrations may fall or remain high, and
- IgA concentrations may rise either along with or instead of IgG, particularly in kidney, liver, and rheumatic diseases

Protein Loss



Protein loss may be divided into selective and non-selective loss. Selective protein loss is generally through a semi-permeable membrane or tight intracellular channels. Minimal change nephrotic syndrome, a type of chronic kidney disease, is a classic example of selective protein loss. Proteins are lost in the urine in concentrations inversely proportional to their molecular size. Small proteins pass through the kidney and into the urine in high concentrations, while large proteins pass through the kidneys minimally, if at all. As a result, serum concentration of small proteins decline, while those of larger proteins remain the same or increase.

Non-selective protein loss is due to either whole blood or serum loss, and all serum proteins are lost equally. Whole blood loss may be acute as with trauma, or chronic, as with types of gastrointestinal or uterine bleeding. In acute whole blood loss, there is a rapid influx of extracellular fluid into the vascular space in an attempt by the body to maintain blood pressure; this dilutes the remaining plasma and further reduces the concentration of most proteins. The extracellular fluid itself contains fairly high concentrations of the smaller serum proteins, including albumin and transferrin. Concentration of these may, therefore, not drop as dramatically as those of larger proteins such as IgM and α_2-macroglobulin. In chronic, low-grade blood loss, the liver often can replace the lost proteins as long as nutrition is adequate for new protein synthesis, so changes may be minor if seen at all. Non-selective loss of serum proteins also occurs as a result of burns, severe glomerular disease, and many forms of gastrointestinal protein loss.

Redistribution of Body Fluids

Most body spaces such as joints and peritoneum contain small amounts of fluid that act as a lubricant. These fluids contain low levels of total protein that can increase or decrease in concentration as changes in the amount of fluid occur.

The amount of fluid present may increase for any of several reasons:

- Decreased plasma osmotic pressure, such as with over hydration
- Increased intravascular hydrostatic pressure, as seen in congestive heart failure
- Lymphatic obstruction due to congenital conditions, malignancy, or parasitic infestation
- Increased vascular permeability, and
- Local disease, like malignancy or infection

In most of these, the total protein concentration is very low, with predominantly small proteins.

Hormonal Fluctuations

Certain hormones affect the synthesis or catabolism of many serum proteins, and changes in protein levels can suggest an underlying condition such as pregnancy or an endocrine tumor. For example, an increase in estrogen levels, either from pregnancy or oral contraceptives, will cause an increase in α_1-antitrypsin and ceruloplasmin levels and a decrease in α_1-acid glycoprotein and haptoglobin levels.

Hormonal effects on selected serum protein concentration

Hormones	Increased Concentration	Decreased Concentration
Estrogens (including pregnancy or oral contraceptives)	α_1-Antitrypsin, ceruloplasmin	Haptoglobin, α_1-acid glycoprotein
Androgens	Most proteins	
Glucocorticosteriods	Transthyretin, haptoglobin, α_1-acid glycoprotein	Immunoglobulins (variable); CRP, some other positive acute phase reactants

Knowledge of hormonal effects is important in the interpretation of concentration of other proteins; a few of the most important examples are shown in the Table above.

In Vivo Hemolysis

Hemoglobin within red blood cells is composed of two α-chains and two β-chains. When red blood cells become hemolyzed, hemoglobin is released and separates quickly into α,β dimers, which complex immediately with haptoglobin. This binding continues until the supply of haptoglobin is depleted. The haptoglobin-hemoglobin complexes are then removed within seconds to minutes by the liver. Because of the high affinity between haptoglobin and hemoglobin and the rapid removal of these complexes, haptoglobin levels are a sensitive indicator of hemolysis and is often referred to as a "suicidal protein."

Iron Deficiency Anemia

Transferrin is the protein responsible for transporting iron in the body. Low serum iron and ferritin concentrations and elevated transferrin concentrations are nearly diagnostic of iron deficiency anemia. Decreased hemoglobulin production results in small and pale RBCs when examined under the microscope.

Total iron binding capacity (TIBC) is often assayed instead of transferrin, and iron "saturation" is expressed as the ratio of actual serum iron to TIBC. However, free iron binds to other proteins, including albumin. Immunochemical assay of transferrin and the calculated transferrin iron saturation are much more specific. Ferritin is another serum protein associated with iron metabolism, serum levels reflecting total iron stores.

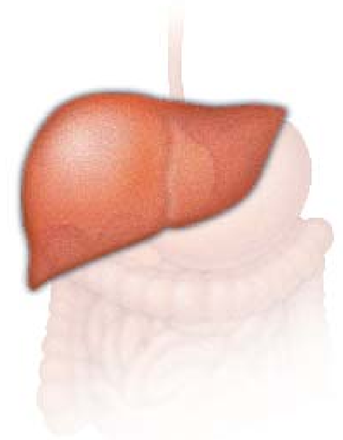
Drug Therapy

Non-steroidal anti-inflammatory agents such as aspirin or ibuprofen can affect the synthesis of acute phase protein concentrations such as prealbumin, haptoglobin, or α_1-acid glycoprotein, even if the underlying disease state is not affected. Reduction of the inflammatory response may result in a return of all acute phase proteins towards normal. Some drugs suppress the immune response and may also depress synthesis of one or all classes of immunoglobulins as well.

Changes in Protein Concentrations Associated with Specific Diseases

Liver Disease

Liver disease can be categorized into hepatocellular disease (often associated with hepatitis or cirrhosis) or biliary obstruction (which often occurs in combination with hepatocellular disease). When liver disease is suspected, the physician may order a liver panel. A suggested liver panel includes the following serum proteins: IgA, IgG, IgM, C3, α_1-antitrypsin, haptoglobin, and albumin.



Urinary Tract Disease

Changes in serum or urine protein concentrations associated with urinary tract disease may result from:

- Increased loss into the urine
- Decreased reabsorption by the renal tubules
- Local inflammation within the kidney or bladder
- Systemic effects of underlying disease processes, such as infection or autoimmune disease

Protein loss: Proteins are filtered from the blood stream across the glomerular basement membrane in inverse proportion of their molecular size:

- Very small proteins (less than 20,000 daltons – including free immunoglobulin light chains) are filtered without retardation
- IgG and A are filtered in very small amounts
- Very large proteins, such as IgM and α_2-macroglobulin, are filtered in amounts detectable only with very sensitive assays

Liver Disease

	Increased levels ↑	Variable levels ↑↓	Decreased levels ↓
Alcoholism	IgA, IgG, IgM, α_1-antitrypsin		Haptoglobin, albumin
Biliary obstruction	C3, α_1-antitrypsin, apolipoprotein B	Haptoglobin	
Cirrhosis	IgA, IgG, IgM, α_1-antitrypsin		Haptoglobin, albumin
Primary biliary cirrhosis	C3, α_1-antitrypsin	Haptoglobin	
Viral hepatitis	IgM, α_1-antitrypsin	C3	Haptoglobin, albumin

Glomerular selectivity is retained in minimal change nephrotic syndrome but may be lost to varying degrees in other forms of renal disease, including severe nephrotic syndrome. In some diseases, the classical example being acute poststreptococcal glomerulonephritis, whole blood is lost through the glomeruli. If the resulting protein in the urine is more than the renal tubules can reabsorb, the excreted urine may contain all of the serum proteins in high concentration.

Renal tubular disease: Most of the protein that passes through the glomerular basement membrane is reabsorbed by the proximal renal tubules and catabolized, again in inverse relationship to molecular size. The amino acids and ligands such as iron are then reutilized by the body. Albumin, transferrin, and other medium sized proteins are the highest concentration serum or plasma proteins in normal excreted urine (after renal tubular reabsorption).

Proximal tubular damage may occur from heavy metal poisoning or interstitial nephritis, resulting in:

- Increased excretion of very small proteins in the voided urine, due to decreased ability to reabsorb and catabolize them. Examples: β_2-microglobulin, α_1-microglobulin, retinol binding protein
- Usually normal serum levels of all proteins, in the absence of glomerular basement membrane damage or renal inflammation

Systemic disease effects: Many changes in serum protein concentrations associated with urinary tract disease result from systemic effects of the disease processes themselves, including:

- Increased immunoglobulin levels, especially in chronic infections or autoimmune diseases such as systemic lupus erythematosus (SLE)
- Acute phase responses
- Decreased complement C3 and/or C4 levels due to
  -genetic deficiency, resulting in high risk for such diseases as systemic lupus erythematous
  -decreased production as in acute post streptococcal glomerulonephritis
  -increased consumption
  -presence of circulating immune complexes

A suggested renal panel may include the following serum proteins: C3, C4, IgA, IgG, IgM, and CRP.

Polyclonal Hyperimmunoglobulinemia

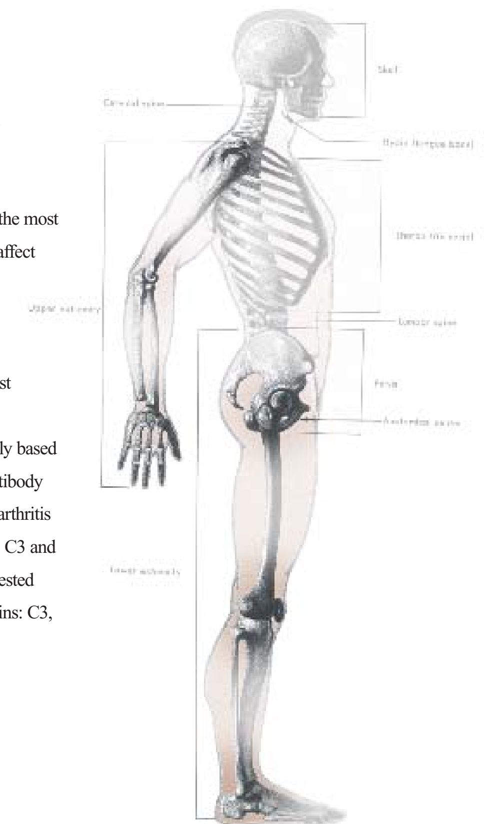
Polyclonal increases in immunoglobulins (multiple types) are common in most immune responses to infections and in autoimmune disease. Typically all three major immunoglobulin classes are elevated with one or two types predominant in specific diseases:

- IgA: skin, gastrointestinal, respiratory, urinary tract
- IgA and IgG: liver disease
- IgM: blood stream infections
- IgE: asthma and other allergies
- IgG: autoimmune and immune complex diseases

Diseases of the Immune System

Autoimmune disease and immunodeficiency represent the most significant categories of immune-related disorders that affect serum protein concentrations.

Autoimmune diseases display varying combinations of acute and chronic inflammatory responses although most of these disorders are associated with elevated levels of immunoglobulins, especially IgG. Diagnosis is ultimately based on the clinical profile of the patient, but specific autoantibody studies such as Rheumatoid Factor (RF) in rheumatoid arthritis can be very helpful in a differential diagnosis. Typically, C3 and C4 levels are decreased in autoimmune disease. A suggested autoimmune profile includes the following serum proteins: C3, C4, IgA, IgG, IgM, CRP, and haptoglobin.



Immunodeficiency may be genetic or acquired as the result of disease or therapy (such as immunosuppressive drugs following organ transplant). Immunodeficiency seriously compromises the body's ability to fight against infectious agents such as viruses, bacteria, or fungi. As you would expect, some or all immunoglobulin levels are severely decreased with most immune deficiency disorders.

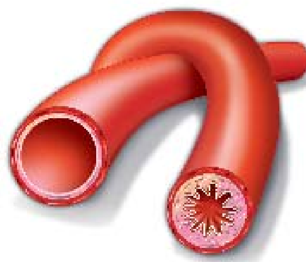
Malignancies: Malignancies may be categorized into B cell and non-B cell types. Malignant transformation of the B lymphocytes usually results in the formation of an excessive amount of a single immunoglobulin class and subclass. These immunoglobulins are monoclonal (meaning that they are identical in structure and electrophoretic mobility) and commonly known as M components or paraproteins. The associated diseases are called monoclonal gammopathies, including:

- Waldenström's macroglobulinemia
- Multiple myeloma
- Lymphoma
- Plasma cell leukemia

Non-B cell malignancies include solid tumors of other organs and some forms of lymphoma. Large organ tumors can cause changes related to that organ's specific function. For example, liver tumors may exhibit signs and symptoms of other forms of liver disease and biliary obstruction and may exhibit the same changes in protein concentrations. In addition, an acute phase response is usually present if the tumor is large.

Arteriosclerotic Cardiovascular Disease (ASCVD)

Many factors increase susceptibility to arteriosclerotic cardiovascular disease, commonly referred to as hardening of the arteries. However, abnormalities in the lipid transport system of the blood, including the lipoproteins, play a major role. Lipoproteins consist of several "packages" of lipids including cholesterol, triglyceride, phospholipids, and apolipoproteins. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) contain apolipoprotein B. High density lipoprotein (HDL) contains apolipoprotein A-1.



Recent studies have shown that elevated levels of Apo B are predictive of ASCVD and subsequent myocardial infarction, similar to elevated levels of LDL cholesterol or total cholesterol.

Apo A1, in contrast, is associated with HDL and is involved with the removal of cholesterol from cells in the body. Decreased levels of Apo A1 are predictive of ASCVD and subsequent myocardial infarction, similar to low levels of HDL cholesterol. Thus, Apo B and Apo A1 assays can be used in addition to or instead of the more traditional LDL and HDL cholesterol assays to assess risk of developing cardiovascular disease.

Assays for the apolipoproteins are now rapid and precise, and new international standards have substantially decreased interlaboratory variance, which was a problem in the past. Assays following clinical events, such as MIs or strokes, are best performed after several weeks or months, since concentrations tend to change dramatically post-event and slowly return to baseline concentration.

Concentrations of the apolipoproteins are affected by several other factors, including exercise, inflammation, and hormones or drugs.

Protein Energy Malnutrition (PEM)

Protein Energy Malnutrition is associated with inadequate protein intake. Although synthesis of all proteins may be decreased, prealbumin is the protein most commonly used to evaluate protein energy malnutrition because its half-life is relatively short. It must be remembered, however, that low levels are seen with a multitude of other disorders including acute phase responses, which are more common than protein energy malnutrition in developed countries.

Many patients who are being evaluated for possible protein energy malnutrition are on anti-inflammatory therapy which significantly increases prealbumin and haptoglobin concentrations. Therefore, caution must be used in interpreting prealbumin concentration, whether low, normal, or elevated. Clinical evaluation should be given at least as much priority as protein assays in determining protein energy malnutrition status.

Table 2: **Implications of Changes in Some Clinically Important Proteins**

Specific Protein	Decreased Levels ↓	Increased Levels ↑
Apolipoprotein A	Arteriosclerosis	Low risk of coronary disease
Apolipoprotein B	Severe hepatic dysfunction	Arteriosclerosis, hyperlipidemias
C-reactive Protein (CRP)		Inflammation, acute and chronic tissue destruction, tumors
Complement 3 (C3)	Autoimmune disease, chronic hepatitis, lupus	Inflammatory disease
Complement 4 (C4)	Autoimmune disease, chronic hepatitis, lupus, acute glomerular nephritis	Acute inflammatory process
Haptoglobin	Hemolytic anemia, sickle cell anemia, liver disease	Acute and chronic inflammatory disease
Immunoglobulin A (IgA)	Immune deficiency states, non-IgA myelomas	Chronic cirrhosis, chronic liver disease, IgA myeloma
Immunoglobulin G (IgG)	Immune deficiency states, non-IgG myelomas	Chronic infections, liver disease, IgG myeloma
Immunoglobulin M (IgM)	Immune deficiency states, non-IgM myelomas	Chronic infections, liver disease, Waldenström's macroglobulinemia
Prealbumin	Malnutrition, liver disease, acute inflammation	Hodgkin's disease, corticosteroid therapy
RF		Rheumatic disease
Transferrin	Inflammation, chronic hepatitis	Iron deficiency, acute hepatitis, pregnancy

Quiz Questions for Chapter Two

Circle the correct answer.

1. Which group of proteins is synthesized in the lymphatic tissues?
   a. Positive Acute Phase Reactants
   b. Negative Acute Phase Reactants
   c. Immunoglobulins

2. Indications of an Acute Phase Response include:
   a. Changes in serum protein levels
   b. Fever
   c. Increased or decreased white blood cell count
   d. Elevated ESR
   e. All of the above

3. Positive acute phase reactants are proteins that increase in concentration in response to disease.
   a. True
   b. False

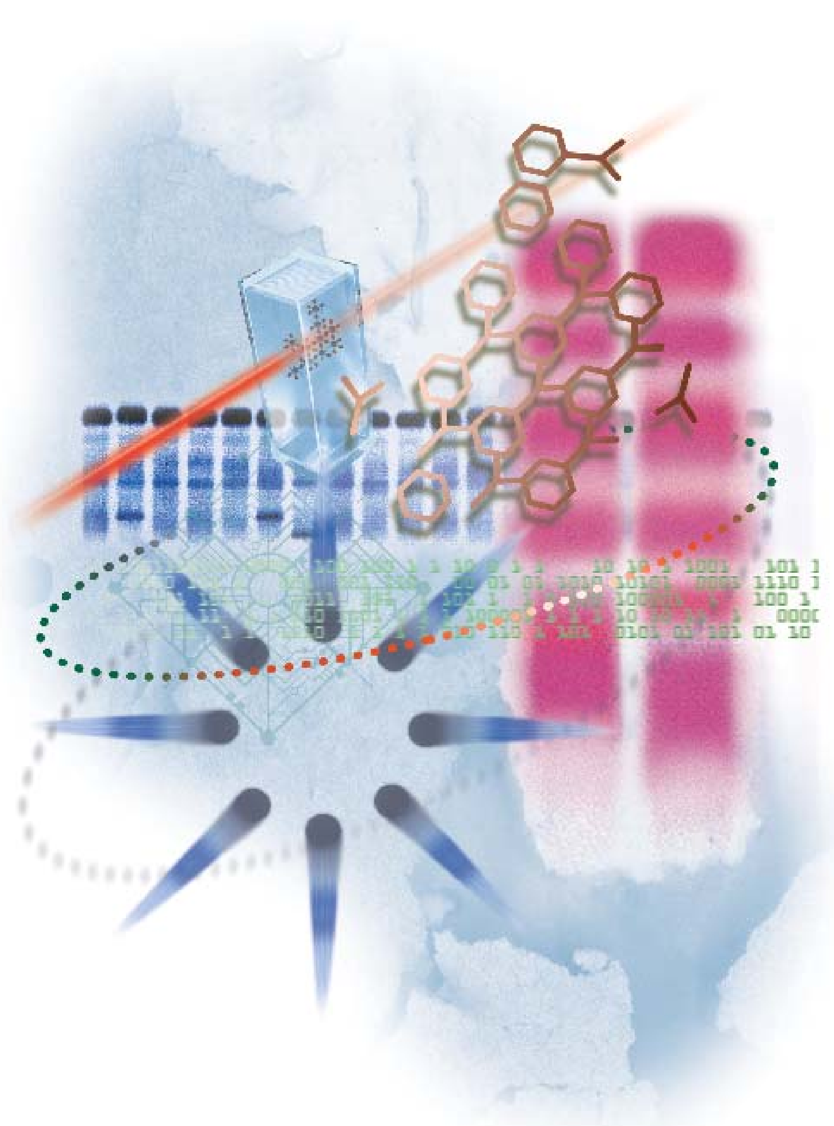
4. Which serum protein is most useful in determining if an acute phase response is due to bacterial or viral infection?
   a. C3
   b. C-reactive protein
   c. IgG

5. What category of protein loss is a result of whole blood loss, burns, or severe glomerular disease?
   a. Selective protein loss
   b. Non-selective protein loss

6. Haptoglobin is known as a/an ________ protein.
   a. Aggressive
   b. Suicidal
   c. Incomplete

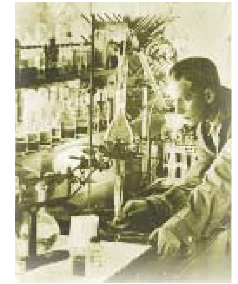
7. The most common cause of low serum prealbumin concentration is
   a. Anti-inflammatory therapy
   b. Estrogens
   c. Malnutrition
   d. Inflammatory disease

8. Which of the following is associated with increased risk of ASCVD?
   a. Elevated serum Apo A1 and Apo B
   b. Decreased serum Apo A1 and Apo B
   c. Decreased serum Apo A1 and increased Apo B



Chapter Three

Identification and Quantification of Serum Proteins



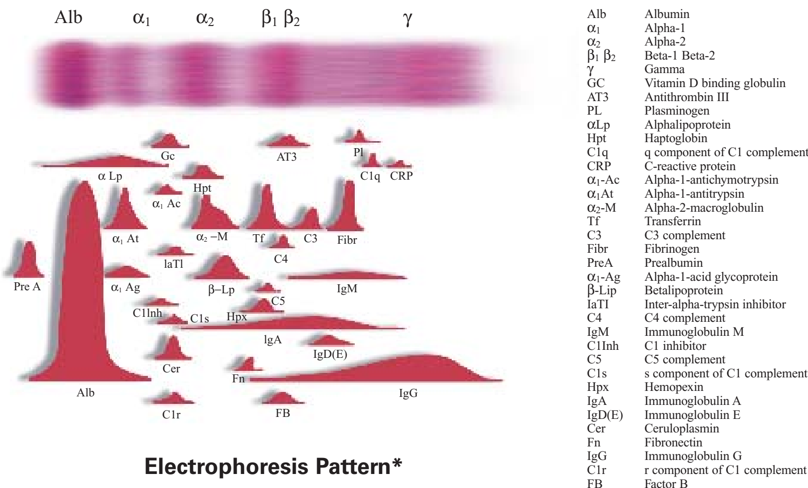
Learning Objectives:

After completion of this chapter, you will be able to:

- List 5 technologies used to identify and quantify serum proteins
- Describe each of these 5 analytical techniques

Early Methods of Serum Protein Analysis

A few assays for serum proteins have been in use for decades, including total serum protein, albumin, and the albumin/globulin ratio. The development of practical serum electrophoresis on filter paper strips in the 1950's permitted visualization and estimation of α-, ß-, and γ-globulins, in addition to albumin. Later, the immunoelectrophoretic method of Grabar and Williams permitted qualitative and semiquantitative evaluation of individual proteins. This method is still used by some laboratories to identify so-called monoclonal immunoglobulins (commonly called "MC," for Monoclonal Components). This and other methods have permitted the identification of at least 150 individual serum proteins.



Alb	Albumin
α_1	Alpha-1
α_2	Alpha-2
β_1 β_2	Beta-1 Beta-2
γ	Gamma
GC	Vitamin D binding globulin
AT3	Antithrombin III
PL	Plasminogen
αLp	Alphalipoprotein
Hpt	Haptoglobin
C1q	q component of C1 complement
CRP	C-reactive protein
α_1-Ac	Alpha-1-antichymotrypsin
α_1At	Alpha-1-antitrypsin
α_2-M	Alpha-2-macroglobulin
Tf	Transferrin
C3	C3 complement
Fibr	Fibrinogen
PreA	Prealbumin
α_1-Ag	Alpha-1-acid glycoprotein
β-Lip	Betalipoprotein
IaTI	Inter-alpha-trypsin inhibitor
C4	C4 complement
IgM	Immunoglobulin M
C1Inh	C1 inhibitor
C5	C5 complement
C1s	s component of C1 complement
Hpx	Hemopexin
IgA	Immunoglobulin A
IgD(E)	Immunoglobulin E
Cer	Ceruloplasmin
Fn	Fibronectin
IgG	Immunoglobulin G
C1r	r component of C1 complement
FB	Factor B

Electrophoresis Pattern*

*Adapted from Laurell, C-B (11).

In the 1960's and 1970's, as high quality antisera specific to the individual proteins became available, truly quantitative methods of assay were developed. The prototype of these was radial immunodiffusion (RID), in which a sample of serum or other fluid is placed into a well and allowed to diffuse passively into a gel (usually agar or agarose) containing antibodies to the protein of interest. The diameter of the resulting precipitin ring is proportional to the concentration of the protein of interest. This method revolutionized protein assays. However, it is slow, taking several hours to several days, and demands careful measurement of the relatively small rings.

Laurell and coworkers later used an electrical current to "drive" proteins into the antibody-containing gels. This method, which Laurell called *electroimmuno assay*, results in cone or "rocket" shaped precipitin peaks in the gels. The use of the electrical current greatly speeds up the diffusion and precipitin formation. Usually, only a few minutes are required for completion. In addition, the peaks can be measured with greater precision than can the small rings of RID.

Immunoturbidimetric and Immunonephelometric Methods

Over the past three decades, improved techniques of protein purification and the production of higher affinity and avidity antiserum have made it possible to automate many protein assays using immunonephelometric and immunoturbidimetric methods. These automated methods offer excellent accuracy and precision with the ability to provide rapid turnaround of results at a relatively low cost. At least 25 proteins have concentrations high enough to be assayed by immunoturbidimetric or immunonephelometric methods, using either dedicated instruments or routine clinical chemistry analyzers.

Immunonephelometric and Immunoturbidimetric assays are based on the same reaction principle. When a soluble antigen (the protein you want to measure) is mixed with the corresponding antibody (contained in the reagents), immune complexes are formed, making the solution turbid. The amount of turbidity is proportional to the amount of antigen present. When light passes through the reaction cuvette, the immune complexes that are formed reduce the amount of directly transmitted light that is able to pass through the cuvette and increase the amount of scattered light.

Nephelometric assays measure the intensity of scattered light as shown in the Figure below:

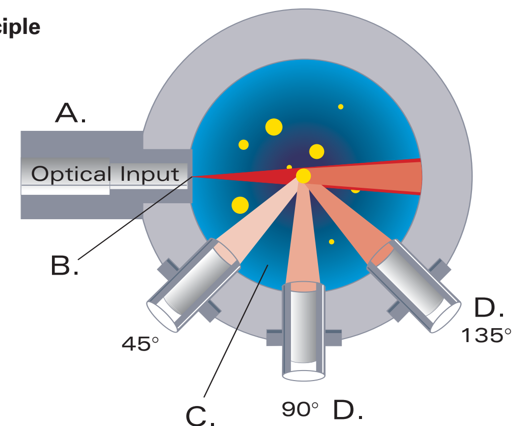
Nephelometry Principle

A. Incoming Light

B. Slit

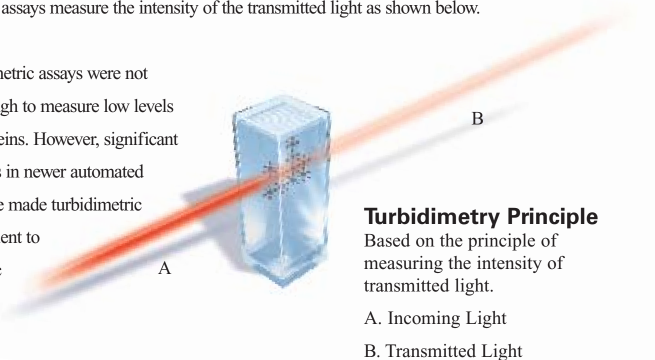
C. Reaction Cuvette

D. Photo Detectors



Turbidimetric assays measure the intensity of the transmitted light as shown below.

Early turbidimetric assays were not sensitive enough to measure low levels of serum proteins. However, significant improvements in newer automated analyzers have made turbidimetric assays equivalent to nephelometric analysis.



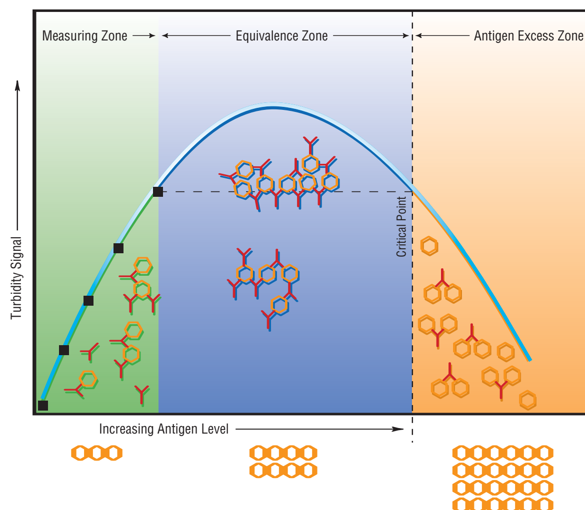
Turbidimetry Principle

Based on the principle of measuring the intensity of transmitted light.

A. Incoming Light

B. Transmitted Light

Heidelberger Curve



The Antibody Excess Zone: The amount of precipitate increases as more antigen is added. The supernatant still contains free antibody.

The Equivalence Zone: Maximum precipitation occurs. The supernatant contains neither free antigen nor free antibody at the peak of the curve.

The Antigen Excess Zone: Due to high antigen concentration, the formation of small soluble immune complexes is favored rather than real precipitate. When insufficient amounts of antibody are available to bind with antigen, an erroneously low result can occur. Therefore, it is imperative that each assay system have some way to flag or test for antigen excess.

Quiz Questions for Chapter Three

Circle the correct answer or fill in the blank.

1. What laboratory procedure has been utilized since the early 1950's to separate, visualize, and estimate serum proteins?

   ____

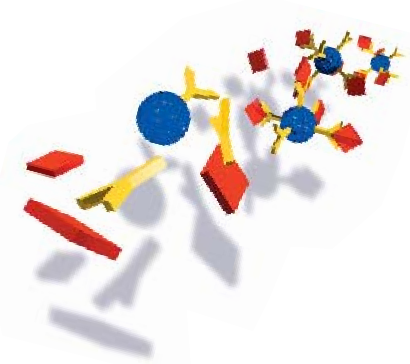
2. The use of ______________________________ in the 1960's and 1970's made it possible to quantify proteins accurately.

3. The use of automated procedures, improved means of protein purification and higher affinity and avidity of antiserum to quantify proteins has resulted in
   a. Direct measurement of proteins
   b. More accurate measurement of proteins
   c. More precise measurement of proteins
   d. Rapid results
   e. Lower cost
   f. All of the above

4. Which immunoassay method of serum protein analysis measures the intensity of directly transmitted light? ____________________________

Chapter Four

Laboratory Considerations in Serum Protein Assays



Learning Objectives:

After completing this section, you will be able to:

- Describe pre-analytical factors that affect serum protein concentrations
- State the optimum time to collect a blood sample for protein analysis
- Indicate how to store samples prior to assay
- List 7 analytical considerations specific to protein analysis
- Explain antigen excess and how it can affect patient results

Pre-Analytical Factors that Affect Serum Protein Concentration

There are several factors which may affect serum protein concentrations. These factors must be considered when interpreting results.

Time of day: Serum concentrations of proteins (and those of other analytes such as cholesterol) vary as much as 10 to 20 percent over the course of the day. If possible, samples should be drawn early in the morning for consistency.

Position: Sitting (or lying in bed) for as little as 15 minutes before drawing blood results in lower values. It is recommended that ambulatory patients sit for 15 minutes before phlebotomy.

Exercise: Vigorous physical exercise results in dehydration. In addition, traumatic exercise such as jogging results in hemolysis in the small blood vessels of the feet and subsequent depletion of haptoglobin.

Pre-Analytical Factors that Affect Serum Protein Concentration

- Time of day
- Position
- Exercise
- Fasting vs. non-fasting
- Medications
- Time of year (season)
- Age and gender
- Geographic location
- Venipuncture technique
- Sample handling and storage

Fasting vs. non-fasting: Eating increases serum triglycerides, resulting in turbidity. In assays which depend upon optical clarity, such as turbidimetry and nephelometry, fasting before sampling is recommended.

Medications: Many medications, such as anti-inflammatory drugs and hormones, can affect protein synthesis and catabolism.

Time of year (season): Some protein concentrations vary between hot (usually higher values) and cold weather.

Age and gender

Geographic location: The major geographic variation is in immunoglobulin concentrations, which are significantly higher in individuals living in semitropical and tropical zones (possibly due to parasitism and/or increased numbers of bacterial and fungal infections).

Venipuncture technique: Prolonged tourniquet application in venipuncture or squeezing the fingers for finger stick samples, can significantly dilute samples and give spurious results. Tourniquets should not be applied for more than 3 minutes, and finger-stick blood should be flowing freely before collecting a sample.

Sample handling and storage: For serum, blood should be allowed to clot quickly at room temperature. Serum should be separated from the cells as soon as possible. This is particularly important for complement factor assays and for phenotyping of α_1-antitrypsin. Serum should be assayed immediately or stored at 4° C (for up to 2-5 days).

Prolonged storage should be at -20 to -70° C in small freezer-safe tubes with tight caps and minimal air space over the serum. Frozen samples should be thawed slowly (e.g., overnight in the refrigerator) for assays of fragile proteins such as C3, C4, and Ceruloplasmin. After complete thawing, all samples must be thoroughly mixed by inversion.

Analytical Considerations

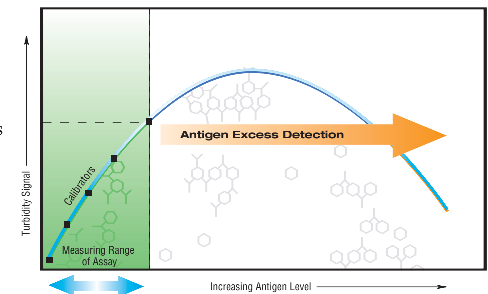
Methods of assay: For solid-phase assays, such as enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays (RIA), either monoclonal or polyclonal antisera can be used as long as affinity and avidity of the antibodies is high. For liquid-phase and gel assays, such as radial immunodiffusion (RID), electroimmuno assay, immunonephelometry (IN), and immunoturbidimetry (IT), polyclonal antisera usually give better and faster precipitate formation. For particle-enhanced immunonephelometry and immunoturbidimetry, monoclonal antibodies often work well.

Assay parameters: Assays should always be performed according to the manufacturer's directions. This includes temperature, blanking method, time of incubation, and calibration.

Instrument calibration: Calibrators and controls should be those supplied by the kit manufacturer or checked for accuracy of assigned values against a primary standard, such as RPPHS/CRM 470 (available from the College of American Pathologists, Chicago, and from the Bureau Communitaire de Référence, Brussels).

Antigen excess: High dose "hook effect" or "pro-zone" is a phenomenon that is inherent in all immunoprecipitin assays. Due to high antigen concentration small soluble immune complexes are formed instead of true precipitate. The supernatant fluid may contain free antigen. This results in an apparent decrease in analyte (antigen) concentration.

If the sample signal becomes higher than the signal of the highest standard, automated analyzers are equipped with parameters to signal a warning (e.g., "too high") – indicating that re-analysis using diluted samples should be performed.



Interpretation of results: If possible, protein assays and electrophoretic patterns should always be interpreted in view of clinical and demographic information. Only in this way can the proper reference intervals be utilized and the clinician's question adequately answered.

Reference intervals: Reference intervals vary by age and sex and, in some cases, by geographic location and racial or ethnic group. The Committee on Plasma Proteins of the International Federation for Clinical Chemistry is currently evaluating serum protein reference intervals based on RPPHS/CRM 470. Meanwhile, the Committee has published interim values for Caucasian adults aged 20-60 years (Dati et al.). Also, 5th through 95th percentile ranges by age and gender can be found in the 2-volume reference edited by Ritchie and Navolotskaia. It is strongly recommended that each laboratory determine reference intervals appropriate for its own population(s).

Profiles: In many cases, combinations of assays or profiles can be substantially more helpful than single assays. Two examples include the evaluation of Haptoglobin and α_1-acid glycoprotein concentration in detecting in vivo hemolysis and of CRP and Transthyretin concentration in evaluating protein nutritional status vs. inflammation.

Reading principles: Automated instruments operate according to one of four different principles regarding mixing of reagents and reading of the signals.

True sample blanking: This involves taking a reading just prior to the addition of antibody. This preferred method helps to minimize spectral interferences from the sample, e.g., lipemia, icterus, and hemolysis and corrects for initial turbidity, and results in maximum signal.

Blanking immediately after mixing all reagents: The reaction buffer, the sample and the antibody are mixed. Then as fast as possible the first reading is taken and stored to memory. When the reaction has reached the end point the second reading is taken and stored into memory. The final result is obtained by subtracting the first from the second reading.

Reaction velocity as a function of time: The result is calculated from the increase in signal per unit of time. The rate is the signal measured. Use of this rate principle assumes that actual patient protein samples react with the same velocity as the protein used to calibrate the assay on the instrument.

End point: This method utilizes signal development as a function of time.
The reaction between an antigen and an antibody needs a period of time to reach end point. The sample signal obtained prior to the addition of antibody is subtracted from the end point signal; the antigen concentration can then be calculated.

Quiz Questions for Chapter Four

Circle the correct answer.

1. Which of the following patient behaviors is (are) known to affect serum protein concentration?
   a. Exercise
   b. Amount of sleep
   c. Position
   d. Posture
   e. Both a and c
   f. All of the above

2. Fasting before blood sampling is recommended because eating may
   a. increase triglycerides and interfere with assays
   b. increase levels of certain proteins

3. Protein concentrations in a given individual are consistent year-round.
   a. True
   b. False

4. Geographic location is most often associated with variation in concentration of
   a. complement components
   b. ceruloplasmin
   c. acute phase reactants
   d. immunoglobulins
   e. all of the above
   f. none of the above

5. Serum samples may safely be stored at room temperature for up to one week before assaying proteins.
   a. True
   b. False

6. Checking for antigen excess is important for
   a. results that approach the high end of the assay range
   b. albumin, since concentrations are so high in most patients
   c. polyclonal IgG, even if concentrations are normal

Appendix A

Glossary of Terms

The definitions for this glossary are modified from various NCCLS documents, the OVID Technology Medline Database, and miscellaneous textbooks.

Affinity: A measure of the attraction between a single antigenic site and a single antibody to that site.

Amino acid: Simple nitrogenous organic compounds which make up all peptides, polypeptides, and proteins.

Analyte: The substance, set of substances, or "factor" to be assayed.

Antibody: A glycoprotein produced by B cells in response to exposure to an antigen and reacting specifically with that antigen; a part of humoral immunity.

Antigen: A substance that is capable, under appropriate conditions, of inducing a specific immune response and of reacting with the product of that response (antibody or specifically sensitized T-lymphocyte).

Antigen excess: The presence of excess antigen in relationship to antibody concentration, resulting in increased solubility of immune complexes and underestimation of antigen concentration (sometimes called "prozone effect").

Antiserum: A serum containing antibodies.

Autoantibody: An antibody directed against a self-antigen, i.e., against a normal tissue constituent.

Avidity: The combined intensity of reactivities of an antibody and antigen, representing the net affinity of all binding sites in the antiserum.

B lymphocytes (B cells): The bone marrow-derived lymphocytes that produce immunoglobulins.

Calibrator: A material of known characteristics (concentration, activity, reactivity) used to calibrate or adjust an assay procedure. The material must have the same performance characteristics as the test samples in that procedure.

Certified Reference Material (CRM): A reference material certified by a recognized organization, such as the World Health Organization (WHO).

Cirrhosis: The end stage of various chronic liver diseases, characterized by extensive fibrosis in association with the formation of regenerative nodules.

Clearance: The rate of removal or loss of a protein by all routes of utilization and excretion.

Electroimmuno assay: An immunoassay in which antigen and antibody are forced into association by an electrical current; "rocket immunoassay" or "electroimmunodiffusion."

Electrophoresis (agarose gel): A form of electrophoresis in which protein molecules move through buffer suspended in an agarose gel matrix.

Electrophoretic mobility: A description of the movement of a protein in an electric field, usually in pH 8.6 buffer of a given ionic strength. The term can also be qualitative, e.g., α-, β-, γ-, etc.

ELISA (Enzyme-linked immunosorbent assay): An immunoassay utilizing an antibody labeled with an enzyme marker. The change in enzyme activity as a result of the enzyme-antibody-antigen reaction is proportional to the concentration of the antigen and can be measured.

Half-life: The time required for half of a given substance to be altered, excreted, or destroyed- i.e., to "disappear" from the circulation.

H (heavy) chains: The longer polypeptide chain of the immunoglobulin molecule.

Hemolysis: Disruption of the integrity of the red cell membrane causing release of hemoglobin.

Hodgkin's disease: A malignant disease characterized by progressive enlargement of the lymph nodes, spleen and general lymphoid tissue and the presence of large, usually multinuclear cells of unknown origin.

Immune complexes: The complexes formed by the binding of antigen and antibody molecules, with or without complement fixation.

Immunodiffusion: Technique involving the diffusion of antigen or antibody through a semi-solid medium, usually agar or agarose gel.

Immunofixation: A method of identifying proteins or genetic variants thereof by immunoprecipitation in the gel following electrophoresis or isofocusing.

Immunoglobulin: A protein composed of heavy and light chains and functioning as an antibody.

L (light) chains: The shorter polypeptide chain of the immunoglobulin molecule.

Lipoprotein: A family of particles combining water insoluble lipids with proteins that allow for their dissolution in plasma and uptake by cells.

Malignancy: The condition in which normal cell differentiation, control, growth, and morphology regress towards a more ancestral state.

Monoclonal antibodies: Antibodies produced in vitro by a cell line arising from a single cell. All molecules are of a single class and subclass and have a single antigenic specificity.

Monoclonal gammopathies: Conditions characterized by the presence of a monoclonal serum (or urine) protein.

Multiple myeloma: A malignant tumor of plasma cells usually arising in the bone marrow.

Necrosis: Death of tissue, either individual cells or groups of cells.

Nephelometry: A means of analyzing the amount of an analyte in suspension in an optically clear fluid by measuring the amount of light refracted by the suspended particles, in this case immune complexes.

Nephrotic syndrome: Clinical association of heavy proteinuria, hypoalbuminemia, and generalized edema.

Paraprotein: An immunoglobulin (or fragment) produced by a single clone of plasma cells and thus having a single structure.

Plasma cell: A mature B cell that actively secretes a specific antibody.

Precision: The extent to which replicate analyses of a sample agree with each other, usually expressed as imprecision (the coefficient of variation of a population of values, equal to the standard deviation x 100 divided by the mean).

Radial Immunodiffusion (RID): An assay in which antigen diffuses passively from a central well into an open-pored gel matrix forming a circle of immune complex.

Radioimmunoassay (RIA): A quantitative assay for the detection of antigen-antibody reactions using a radioactively labeled substance to measure the binding of the unlabeled substance to a specific antibody or other receptor system.

Reference material: A material or substance in which more properties are sufficiently well established to permit its use in calibrating or verifying a measurement, method, or apparatus.

Reference interval: The range of values, generally the 95% "confidence limit" (2.5 – 97.5 centile), found in an apparently healthy population.

Reticuloendothelial system: An extensive network of macrophages that exist throughout the body, clearing foreign matter, immune complexes, etc.

Rheumatoid arthritis: A chronic systemic disease, primarily of the joints, marked by inflammatory changes in the synovial membranes and articular structures, widespread fibrinoid degeneration of collagen fibers, and atrophy and rarefaction of bony structures.

Rheumatoid Factor (RF): Antibody directed against antigenic determinants in the Fc region of IgG.

Standard: A material against which other materials may be measured or compared.

Systemic Lupus Erythematosus (SLE): A chronic, relapsing, inflammatory and often febrile multisystemic disorder of connective tissue, characterized by involvement of the skin, joints, kidney, and serosal membranes.

Subclass: A subdivision of an immunoglobulin class based on structural and antigenic differences in the heavy chains of the molecules.

Turbidimetry: A means of analyzing the amount of an analyte in suspension in an optically clear solution by measuring the amount or percentage of light that passes through the solution.

Waldenström's macroglobulinemia: A malignant neoplasm of cells with lymphocytic, or plasmacytic, or intermediate morphology that secrete an IgM component ("macroglobulin").

Appendix B

Suggestions for Further Reading

1. Ritchie RF, Navolotskaia O, eds. *Serum Proteins in Clinical Medicine*. Vol. I. Laboratory Section. Foundation for Blood Research, Scarborough, ME, 1996.

   (This volume discusses the individual proteins and assay methods; it was intended for laboratorians but is of interest to clinicians who have an interest in specific proteins, such as α_1-antitrypsin or ceruloplasmin.)

2. Ritchie RF, Navolotskaia O, eds. *Serum Proteins in Clinical Medicine*. Vol. II. Clinical Section. Foundation for Blood Research, Scarborough, ME, 1999.

   (This volume contains discussions of the changes in proteins associated with pathologic processes and specific disease states and should be of interest to both laboratorians and clinicians.)

3. Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). *Eur J Clin Chem Clin Biochem*. 1996;34:517-520.

   (This paper gives suggested international reference intervals for adult Caucasians, pending the determination of more definitive intervals.)

4. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: CA Burtis and ER Ashwood, eds. Tietz *Textbook of Clinical Chemistry*. 3rd edition. Philadelphia: W.B. Saunders; 1999:477-540.

   (This recently revised chapter is a discussion intermediate in level between the current Guide and the volumes edited by Ritchie and Navolotskaia–references 1 and 2 above.)

5. Whicher JT. Abnormalities of plasma proteins. In: Williams DL and Marks V, eds. *Biochemistry in Clinical Practice*. London: William Heinemann; 1983:221-250.

6. Morgan BP. Complement: *Clinical Aspects and Relevance to Disease*. San Diego: Academic Press; 1990.

7. Roitt IM, Brostoff J, Male DK. *Immunology*. 3rd edition. St. Louis: Mosby; 1993.

8. Laurell C-B. Determination and interpretation of plasma proteins. *Aust Fam Physician*. 1976;5:36-41.

9. Jeppsson J-O, Laurell C-B, Franzén B. Agarose gel electrophoresis. *Clin Chem*. 1979;25:629-638.

10. Putnam FW, editor. *The Plasma Proteins*. Vol. 1, 2nd edition. San Diego: Academic Press; 1975.

    (This volume has general discussions and summaries of the major proteins. There are 4 other volumes in the series, with more detailed discussions of many proteins.)

11. Laurell, C-B. Electrophoresis, specific protein assays, or both in measurement of plasma proteins? *Clin Chem.* 1973;19:99-102.

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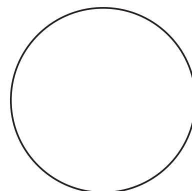

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