Clinical Applications: Serum Proteins

Lecture Goal(s): To describe proteins as they normally exist in biological fluids and discuss the physiologic function of these proteins. Abnormalities with these proteins will be related to disease states and disease processes.

Lecture Objectives: Upon completion of this class material each student will be able to do the following:

1. Cog/II Associate hyperproteinemia/hypoproteinemia, hyperalbuminemia/ hypoalbuminemia, and hyperglobulinemia/hypoglobulinemia with general disease states that may produce these abnormal blood levels.

2. Cog/III Correlate the following clinical conditions with electrophoretic patterns. List diseases that could cause these patterns and note individual protein abnormalities.

   a. acute inflammation
   b. subacute inflammation
   c. chronic inflammation
   d. liver cirrhosis
   e. nephrotic syndrome
   f. hypogammaglobulinemia
   g. monoclonal abnormalities
   h. polyclonal abnormalities
   i. protein losing gastroenteropathies
   j. α_1 antitrypsin deficiency

3. Cog/II Define acute phase reactants/proteins. List commonly monitored acute phase reactants/proteins along with time and magnitude of increase. Also note negative acute phase reactants/proteins.

4. Cog/II Describe the electrophoretic migration characteristics of transthyretin (pre-albumin). Note its normal physiologic function, fluids in which it is usually detected, and causes for its increase.

5. Cog/II Describe the physical characteristics of albumin and relate these characteristics to electrophoretic migration and lack of urinary excretion.

6. Cog/II Discuss the important physiologic roles of albumin and relate these to why hyperalbuminemia is usually not observed.

7. Cog/II Describe bisalbuminemia and correlate this with electrophoretic patterns. Differentiate bisalbuminemia and pseudobisalbuminemia specifically noting causes for pseudobisalbuminemia.

8. Cog/II Discuss problems resulting from hypoalbuminemia and note this as a non-specific abnormality.

9. Cog/I List the known functions of α_1-acid glycoprotein (orosomucoid) and indicate its report clinical utility.

10. Cog/II Explain the physiologic function of α_1-antitrypsin and note the significance of its decrease and increase in serum.

11. Cog/III Correlate α_1-antitrypsin deficiency with its electrophoretic pattern and explain problems that may occur with the electrophoretic mobility of α_1-antitrypsin if serum is not quickly separated from the cells following blood collection.

12. Cog/II Describe the physiologic function of α_2-macroglobulin in the body and note clinical conditions where its concentration is increased and decreased. Explain the relative concentration increase instead of an absolute concentration increase of α_2-macroglobulin in nephrotic syndrome.

13. Cog/II Describe the physiologic function of haptoglobin and note clinical conditions that may lead to increased and decreased levels of this protein.

14. Cog/II Describe the physiologic function of ceruloplasmin and note clinical conditions that may lead to increased and decreased levels of this protein.

15. Cog/II Correlate serum ceruloplasmin and copper levels with Wilson's Disease and note organs of significant copper concentration associated with this disease. Indicate the effects of copper deposition in these organs.

16. Cog/II Correlate ceruloplasmin levels with the presence of green sera.

17. Cog/II Describe the physiologic function of transferrin and note the interaction of iron in transferrin with the copper in ceruloplasmin.

18. Cog/I List the clinical conditions that may lead to increased and decreased levels of transferrin. Note the relationship between transferrin and TIBC.

19. Cog/II Describe the utility of using transferrin in determining the presence of CSF.

20. Cog/II Describe the function of hemopexin and relate hemopexin to haptoglobin.

21. Cog/I List the clinical conditions that may lead to increased and decreased synthesis of the C3 component of complement.

22. Cog/I List three conditions that may lead to hypogammaglobulinemia and explain why we only see this on electrophoresis when associated with an IgG deficiency.

23. Cog/II Discuss polyclonal abnormalities noting immunoglobulins typically present and light chain characteristics.

24. Cog/II Describe the characteristic electrophoretic and immunofixation patterns associated with polyclonal abnormalities. Explain the narrow banded pattern, how this pattern could occur, and when present, how it would be determined to be a polyclonal abnormality.

25. Cog/I List some of the causes for polyclonal abnormalities.

26. Cog/II Explain the relationship between polyclonal/monoclonal abnormalities and polyclonal/monoclonal gammopathies.

27. Cog/II Describe the electrophoretic characteristics of monoclonal abnormalities.

28. Cog/I Note the use of M-protein and paraprotein to describe the abnormal monoclonal protein.

29. Cog/II Relate the finding of a monoclonal abnormality to age and explain MGUS (Monoclonal Gammopathy of Undetermined Significance).

30. Cog/III Using the following laboratory features, provide a laboratory distinction between malignant (symptomatic) and benign (asymptomatic) states.

a. excess secretion of light chains
b. progressive rise in paraprotein level
c. suppression of normal immunoglobulins
d. high serum paraprotein levels

31. Cog/II Describe Bence Jones proteins and free light chain disease. Identify and relate electrophoresis and immunofixation electrophoresis characteristics of Bence Jones proteins.

32. Cog/I Note immunoglobulin levels suggestive of malignancy.

33. Cog/II Describe the effects of paraproteins on the following laboratory tests:

a. blood viscosity
b. coagulation factors
c. cellular components

34. Cog/I Relate the frequency of the classes and types of monoclonal gammopathies to each other.

35. Cog/I Note the following as the most common causes for laboratory detection of monoclonal gammopathies:

a. multiple myeloma
b. Waldenstrom's macroglobulinemia
c. heavy chain disease (Franklin's Disease)
d. cryoglobulinemia

36. Cog/II Describe multiple myeloma along with necessary diagnostic criteria.

a. IgG (70%)
b. Bence-Jones proteins
c. total protein and albumin
d. calcium
e. cholesterol
f. uric acid

37. Cog/II Describe Waldenstrom's macroglobulinemia and note significant laboratory tests.

38. Cog/I Explain heavy chain disease.

39. Cog/II Describe cryoglobulins and the method for detecting these proteins.

40. Cog/II Distinguish between the three types of cryoglobulins.

41. Cog/II Note C-reactive protein as a gamma globulin and as an acute phase reactant/protein. Describe its different clinical utilities.

When considering proteins, the most frequent assays performed in the clinical laboratory are total serum protein, albumin and globulins.

The following situations will lead to an **increased total serum protein** level:

- dehydration
- monoclonal diseases such as: multiple myeloma, macroglobulinemia, or cryoglobulinemia
- chronic polyclonal disease such as: liver cirrhosis, sarcoidosis (fibrous tissue development in organs), systemic lupus erythromatosus, or some chronic infections (possibly of viral origin)

The following situations will lead to a **decreased total serum protein** level:

- inadvertent overhydration
- protein loss through the kidneys such as nephrotic syndrome
- disruption of the skin such as seen with thermal burns
- decreased intake such as dietary inadequacies, starvation, or GI obstruction
- malabsorption conditions such as seen with sprue
- increased breakdown of proteins such as seen with fevers, necrosis, cancer, or steroid treatment
- situations of increased requirement of proteins such as growth, pregnancy, or hyperthyroidism

Increased albumin levels are rarely seen except in cases of acute dehydration or shock. Albumin is osmotically active meaning that where albumin is found, water will also be found. An increase in albumin concentration will only be temporary. If hyperalbuminemia were to occur, the high level of albumin in the blood stream would draw interstitial water into the vascular bed diluting albumin back to the normal level.

Decreased albumin levels will be seen in the same situations that produced a decreased total serum protein level.

Increased globulins levels will be seen in association with monoclonal diseases, some forms of liver disease, sarcoidosis, collagen diseases, and with chronic infection.

Decreased globulins levels are usually associated with hypogammaglobulinemias.

Common Protein Electrophoretic Patterns

A very good screening tool to evaluate protein levels in general in the body is electrophoresis. Protein electrophoresis pinpoints the region (alpha, beta, etc.) where an abnormality exists. From this information, specific laboratory evaluations of proteins that fall in that region are conducted to identify the specific protein(s) that is/are abnormal.

First we will consider some general protein electrophoretic patterns that are characteristic of disease, then we will continue on to specific proteins.

Acute Inflammation

The rapid breakdown of tissue is frequently found in acute inflammation and is characterized by localized biochemical response (activation of complement) and by cellular response (mobilization of phagocytes, increased synthesis of proteins). Causes for acute inflammation include burns, acute infections with fever, acute myocardial infarction, early stages of malignancy, and acute polyarthritic diseases. Clinical findings in acute inflammation include:

- fever resulting from the release of toxic substances that stimulate the central nervous system
- elevated erythrocyte sedimentation rate, increased levels of α_1 and α_2 globulins, and fibrinogen
- leukocytosis following phagocytic activity
- increased level of acute phase proteins (APP) - also known as acute phase reactants (APR)

Acute phase proteins/reactants are a family of approximately 30 plasma proteins produced in increased amounts by the liver in inflammation. Their increase in concentration reflects an important physiologic mechanism providing an increased supply of the proteins to the site of tissue injury where they either modulate the nature of inflammation or replenish proteins that are rapidly used up performing their functions.

Acute phase proteins/reactants can be seen in association with the following disorders:

- acute infection (bacterial, viral or parasitic)
- trauma of all sorts (mechanical, physical, chemical, etc.) resulting in tissue damage (contusions, surgery, thrombosis, etc.)
- cardiac failure
- metabolic coma (uremia, shock, etc.)

Acute phase proteins/reactants can be grouped into three groups based on their level of increase. Some examples of acute phase proteins/reactants in each group include:

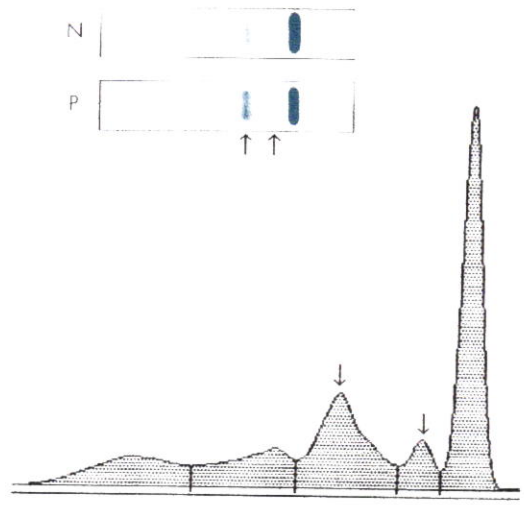
Group I (approximately 50% increase with a response time of 48 - 72 hours)
- Ceruloplasmin
- Complement C3

Group II (200 - 400% increase with a response time of 12 - 24 hours)
- α_1-acid glycoprotein
- α_1-antitrypsin

Group III (up to a 1000 fold increase with a response time of 6 - 10 hours)
- C-reactive protein
- Serum amyloid A (a relatively newly identified acute phase protein/reactant)

Typically we think of acute phase proteins/reactants increasing in response to inflammation. This increase in acute phase proteins/reactants is accompanied by a decrease in production of prealbumin, albumin, and transferrin. Thus, these proteins are known as negative acute phase proteins/reactants. As a result, during an inflammatory process, total protein levels typically remain normal or show only a slight elevation.

The diagram below illustrates typical electrophoretic pattern associated with acute inflammation. Typically albumin and γ-globulins are decreased while α_1 globulins and α_2 globulins are increased.

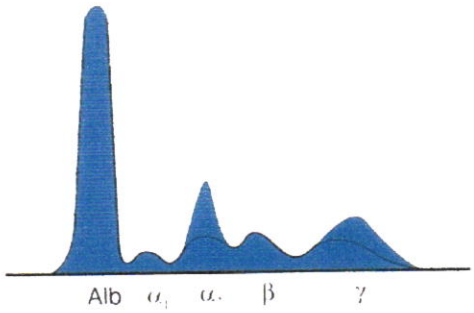


Acute inflammation

Subacute Inflammation
This condition represents an intermediate stage between the two possible courses of acute inflammation: total convalescence with a return to normal or the onset of a chronic inflammatory condition.

Severe acute inflammation is characterized by a massive increase of all acute phase proteins/ reactants. When recovery begins, there is a characteristic decrease, followed by a return to normal of the α_1 globulins, complement, and albumin. The beginning of the immune response is marked by a slight, generally selective, increase in γ globulins. α_2 globulins may remain more or less elevated.

The illustration below is a rough sketch of a subacute inflammation electrophoretic pattern. The black solid lines indicate normal α_2 and γ globulin levels.

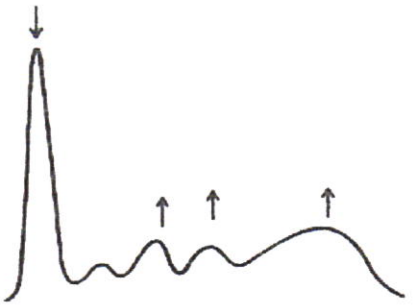


Sub-acute

Chronic Inflammation
Chronic inflammatory conditions are associated with increases of certain protein referred to as "chronic phase proteins." Electrophoretically, this response is seen as a slight to moderate increase in α_2 globulins and a slight increase in beta globulins (primarily due to complement). Albumin is slightly suppressed with a polyclonal increase of gamma globulins.

Chronic phase proteins are seen in the following disorders:
- chronic infections (brucellosis, tuberculosis, Hodgkin's Disease, etc.)
- collagen or connective tissue disease
- allergies
- malignancies
- autoimmune disorders

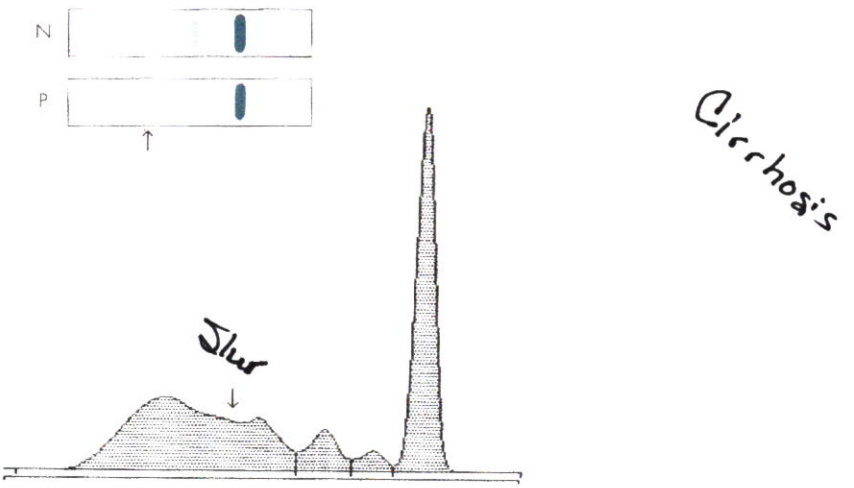
The illustration below is a rough sketch of a chronic inflammation electrophoretic pattern. The appearance of a stepwise increase from α_2 to β to γ is very characteristic of chronic inflammation. This is known as a **sarcoid-step pattern**. It is sometimes very difficult to differentiate subacute and chronic inflammation by electrophoresis alone.



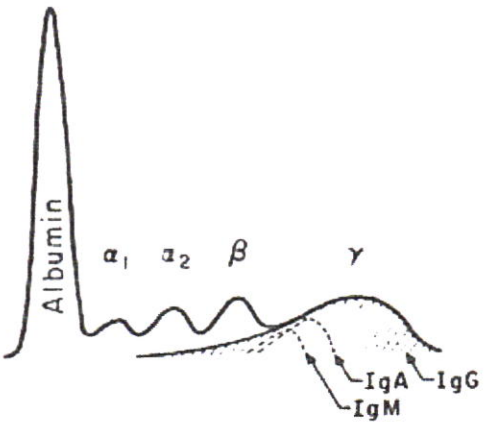
Liver Cirrhosis

Since the liver is the site of most protein synthesis in the body, diseases affecting this organ can be expected to affect *in vivo* levels of these proteins. The major proteins not synthesized by the liver include hemoglobin and immunoglobulins. The liver, however, has considerable reserve synthesis capability, and decreased levels of proteins such as albumin are seen only in advanced liver disease.

Liver cirrhosis is associated with a significant rise of IgG, IgM, and IgA along with a decrease in other proteins such as albumin and transferrin. This elevation in the three major immunoglobulins results in a very characteristic electrophoretic pattern known as β-γ **bridging** or β-γ **slurring**. This is illustrated below.



The bridging (or slurring) seen on the electrophoretic pattern above results from the location of the three immunoglobulins following electrophoresis. As illustrated below, IgM and, to a greater degree, IgA appear in the gap between the β and γ regions. Please note that in the illustration above, the anode is located on the right-hand side. In the illustration below, the anode is located on the left side. Again, the important point is the location of the major immunoglobulins.



Three possible mechanisms have been proposed to explain the increase in plasma immunoglobulin levels in liver cirrhosis.

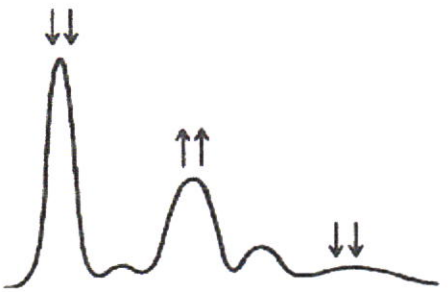
- Portal canal shunting - This alteration in liver cirrhosis allows blood from the portal circulation to directly enter the systemic circulation without passing through the liver to remove any foreign materials. Once in the systemic circulation, these foreign materials stimulate an immune response.
- Damage to Kupffer cells - Kupffer cells line the sinusoids (i.e., capillaries) from the portal vein as it passes through the liver. Kupffer cells function to remove foreign materials before portal blood is mixed with systemic blood. In this theory, liver cirrhosis includes damage to the Kupffer cells. Once again, since foreign materials are not removed as they should be, these foreign materials stimulate an immune response once they enter the systemic circulation.
- Unidentified immunologic response - Some unidentified material is released into the systemic circulation during the course of the disease which stimulates an immune response.

Nephrotic Syndrome

Nephrotic syndrome is associated with a lesion between the glomerular capillary bed and Bowman's Capsule. This lesion allows many of the plasma proteins to leave the blood stream and enter the nephron for ultimate excretion. The most significant loss is that of albumin. One of the major functions of albumin is to maintain an osmotic force to keep water in the blood stream. Normally, with albumin trapped in the blood stream, there is a constant force keeping water in the blood stream. In nephrotic syndrome, with the loss of albumin, there is a reduction in the force holding water in the blood stream which allows water to move into tissues resulting in significant edema.

Even though this lesion allows most plasma proteins to escape, larger proteins still remain trapped in the blood stream. The most prominent of these larger proteins include α_2 macroglobulin, β-lipoprotein and IgM. Of these, α_2 macroglobulin appears to show the greatest

increase. This increase, however, is relative. The main reason these larger proteins, and in particular α_2 macroglobulin, appear increased is because there is a decrease in all of the other proteins. The effect on protein electrophoresis is a decreased albumin, α_1 globulin, β-globulin, and γ-globulin. The α_2 globulin electrophoretic region shows a significant elevation, almost equaling the level of albumin. Again, the increase in α_2 globulin is due to a relative increase in α_2 macroglobulin. Although β-lipoproteins and IgM are increased, due to significant loss of other β-globulins and γ-globulins, these two electrophoretic regions appear as normal or decreased. This is illustrated in the diagram below.



Nephrotic syndrome has been caused by

- diabetes mellitus
- connective tissue disease
- glomerular disease
- circulatory disease
- toxins such as the venom from bee stings

Nephrotic syndrome is characterized by

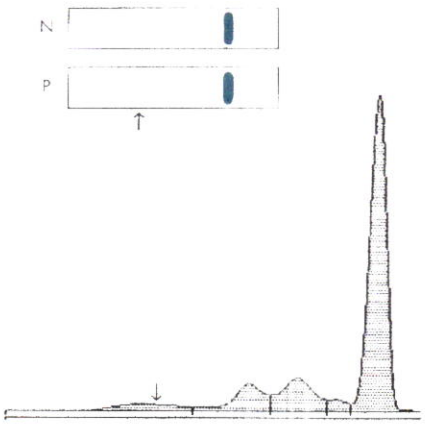
- hypoproteinemia
- hypoalbuminemia
- edema
- hyperlipidemia
- proteinuria

Hypogammaglobulinemia

Hypogammaglobulinemias are characterized by decreased amounts of most or all immunoglobulins. The majority of deficiencies are hereditary and manifest in infancy. Examples of such deficiencies that may have been considered in immunology include Wiskott-Aldrich syndrome, Bruton's disease, ataxia telangiectasia.

Immunoglobulin deficiencies acquired in adulthood can be secondary to disease states, such as monoclonal abnormalities, or induced by immunosuppresive therapy. Bence Jones proteins are frequently found in adults with hypogammaglobulinemia. Immunofixation electrophoresis is very important in the laboratory evaluation of hypogammaglobulinemias.

The following diagram illustrates the electrophoretic appearance in hypogammaglobulinemia:

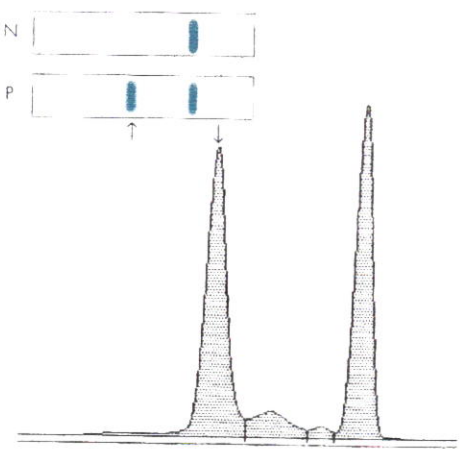


Hypogammaglobulinemia

Monoclonal Abnormalities

Monoclonal abnormalities represent disorders of immunoglobulin synthesis associated with proliferation of one clone of B lymphocytes. Electrophoresis shows one homogeneous peak which corresponds to the abnormal immunoglobulin. This homogeneous peak is relatively narrow since all of the abnormal immunoglobulins result from the same clone of B cells. This narrow electrophoretic zone may appear in the γ, β, or α_2 region. This abnormal zone is typically accompanied by a decreased production of other immunoglobulins. The reason for this will be discussed later in this unit when monoclonal abnormalities are considered in more detail.

The following diagram illustrates the electrophoretic appearance of a monoclonal abnormality. This abnormality is an IgA monoclonal gammopathy which appears in the β-region. Notice that the other immunoglobulins are absent.



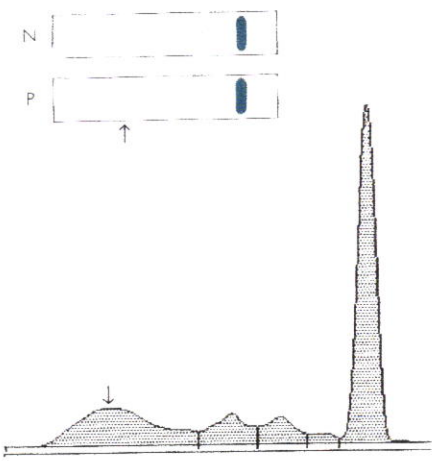
Polyclonal Abnormalities

Polyclonal abnormalities are characterized by broad, diffuse increases in the gamma region of the serum protein electrophoretic pattern. The three major immunoglobulins (IgG, IgA, IgM) are increased in variable relative concentrations.

After hypoalbuminemia, polyclonal abnormalities are the most common protein abnormalities seen. Continued evaluation of polyclonal patterns has some prognostic value. For example, clinical improvement in a primary disease state is marked by a decrease of the γ-region. Polyclonal abnormalities are seen in a number of disorders including:

- chronic liver disorders
- collagen disorders
- chronic infections
- metastatic carcinoma
- cystic fibrosis
- thermal burns during recovery stage

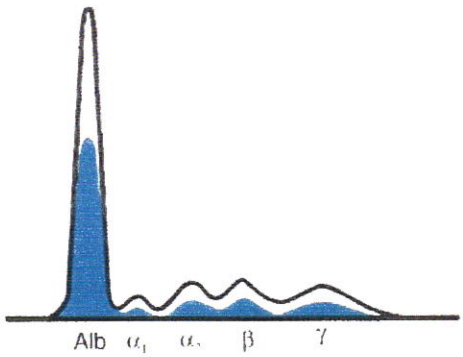
The following diagram illustrates the electrophoretic appearance of a polyclonal abnormality.



Protein Losing Gastroenteropathies

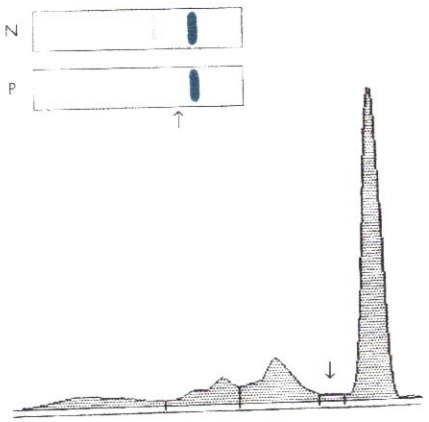
Excessive gastrointestinal loss of albumin and other proteins is seen in a variety of gastrointestinal disorders. Proteins are affected to various degrees in relation to the severity of the protein losing disorder.

The following diagram illustrates the across-the-board decrease seen on serum protein electrophoresis in the presense of protein losing gastroenteropathies. In the diagram, the black line represents normal levels of protein fractions and the blue indicates what is found in this condition.



α_1-Antitrypsin Deficiency

Approximately 95% of all individuals have a serum α_1-antitrypsin concentration of 200 - 400 mg/dL. Certain hereditary abnormalities involve deficiencies in the synthesis of this globulin. In heterozygous individuals, α_1-antitrypsin levels are decreased to 30 - 50% of the normal level. In homozygous individuals for this deficiency, α_1-antitrypsin reductions vary by ethnic group and can decrease as much as 80 - 90%. Since α_1-antitrypsin is the major α_1 globulin, this level of reduction will have a significant impact on the α_1 region of the electrophoretic pattern. This is illustrated below.



Alpha 1 anti-trypsin deficiency

Acquired deficiencies of α_1-antitrypsin are seen in nephrotic syndrome due to urinary loss of this low molecular weight protein and in liver disease.

Specific Plasma Proteins

Up to this point we have considered some of the commonly encountered serum protein electrophoretic patterns that you should be able to recognize. Now we are ready to consider individual proteins. In Lecture 2 on the laboratory analysis of proteins, the individual proteins that fall into the major electrophoretic regions were summarized in a diagram. While we will not

consider each protein presented in that diagram, the major proteins will be considered in the order of their electrophoretic migration, beginning with the closest to the anode.

Pre-albumin or Transthyretin

A newer name for pre-albumin is transthyretin which you may see abbreviated as TTR. Pre-albumin appears on the anodal side of albumin on electrophoresis, i.e., it migrates faster than albumin.

Pre-albumin serves as one of the transport proteins for thyroxine (T_4) in the blood stream. Thus, pre-albumin may also be referred to as thyroxine-binding pre-albumin. Pre-albumin also appears to play a role in the transport of Vitamin A.

Generally, pre-albumin is below the level of detection in serum and is not observed on serum protein electrophoresis. Pre-albumin does appear, however, on spinal fluid protein electrophoresis. The reason pre-albumin is seen with spinal fluid electrophoresis is the spinal fluid is concentrated prior to electrophoresis. The rationale for this concentration step will be discussed in the next lecture. Following spinal fluid concentration, pre-albumin will typically be seen. Pre-albumin is able to enter the spinal fluid because it is a very compact protein allowing it to easily cross the blood-brain barrier.

Peripherally (i.e., outside of the central nervous system), pre-albumin levels are found decreased in association with protein malnutrition. This, however, is not very commonly evaluated by the clinical laboratory. There may be a peripheral increase in pre-albumin associated with increased synthesis by the liver following stimulation by drugs like aspirin and glucocorticoids.

Centrally (i.e., in the central nervous system) pre-albumin levels are found increased in cerebral atrophy.

Albumin

Although albumin is not a particularly large protein, its low isoelectric point (pI) confers a substantial net negative charge at physiologic pH (7.4), and anodal migration on electrophoresis. This charge is important because the glomerular basement membrane also has a net negative charge, resulting in repulsion of albumin at the glomerulus and helping to minimize excretion. The albumin that normally passes through the glomerulus is almost totally reabsorbed by healthy proximal tubules. When there is injury to the renal tubules, substantial amounts of albumin and other proteins that are normally reabsorbed are excreted into the urine.

Usually we think of albumin as the protein fraction that migrates closest to the anode (once again, pre-albumin is not normally seen on serum protein electrophoresis).

Albumin serves many important functions in the body. The primary function of albumin is generally considered to be the maintenance of colloid osmotic pressure (COP) within the blood stream. Although albumin makes up only about 50% of the proteins in the blood stream, it accounts for about 80% of the colloid osmotic pressure in the blood stream. Thus, albumin is the major force that holds water in the blood stream. If albumin levels were to drop significantly,

such as in nephrotic syndrome, water would leave the blood stream and enter tissues leading to edema.

A second important function of albumin is as a transport molecule. The presence of many surface charged groups plus many specific binding sites, both ionic and hydrophobic, give albumin the ability to bind and transport a large number of compounds. A third function of albumin is to serve as an amino acid reservoir, delivering amino acids to tissues for the synthesis of proteins. A fourth function of albumin is as an important component of total plasma antioxidant activity. Albumin removes potentially hazardous oxidizing materials away from tissues and transports these materials to the liver for removal from the body.

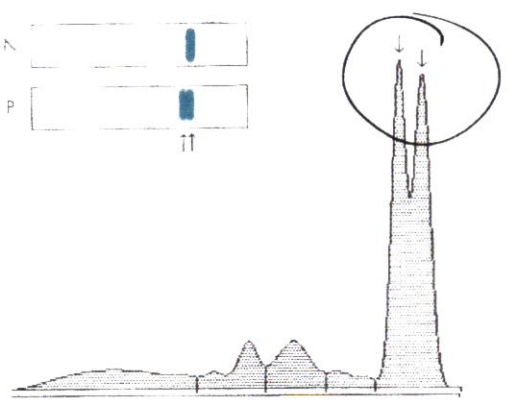
Albumin is synthesized in the liver. In an average 70 kg adult, approximately 10-18 grams of albumin are synthesized each day. This level of albumin production is considered to by only one third of the liver's capacity.

A naturally occurring hyperalbuminemia is not seen. This is because of the osmotic activity of albumin. Wherever albumin is found in the body, it will always attract an osmotic equivalent of water. Therefore, albumin naturally dilutes itself. Transient hyperalbuminemia may be seen in the initial stages of acute dehydration. Albumin rapidly dilutes itself in the blood stream at the expense of tissue water. One related note regarding blood collection for albumin is that prolonged application of a tourniquet will falsely elevate serum albumin levels.

Hypoalbuminemia is the most frequently encountered serum protein abnormality. Serum albumin levels below 2.0-2.5 g/dL (normal range – 3.5-5.2 g/dL) are considered significant because of the potential for the development of hypoalbuminemic edema (decreased albumin, therefore, there is reduced pressure to hold water in the vessels). The problem with finding hypoalbuminemiais that it is a non-specific abnormality. At the beginning of this lecture pathologic states associated with hypoalbuminemia were presented. One important concern hypoalbuminemia is related to the fact that albumin is a very important transport protein. Typically, materials being transported by albumin are not toxic to the body as long as they are bound to albumin. In hypoalbuminemia, with a decrease in albumin, there is the potential for multiple toxicities to develop.

Another abnormality of albumin is bisalbuminemia. True bisalbuminemias are believed to be congenital and show a split albumin peak on electrophoresis. This is illustrated below.

Bisalbuminemia



It has been shown that single amino acid substitutions in the albumin molecule are responsible for the differing electrophoretic rates. The primary physiologic problem associated with bisalbuminemias is based on the fact that albumin serves as a major transport molecule within the body. Problems occur when there are differences in binding capacities for various substances such as thyroxine (T_4). True bisalbuminemias are rare.

Pseudo-bisalbuminemias, on the other hand are more frequent. Most of these are of the fast variety, i.e. the abnormal protein has greater electronegativity than albumin causing the abnormal band to migrate closer to the anode.

Pseudo-bisalbuminemias may be seen in association with disease states such as:

- Acute pancreatitis
- Chronic uremia
- Acute thermal burns

In these three cases, the abnormal protein band is due to proteolysis – i.e. a splitting, say, of the α_1 globulin. The result of this splitting is the formation of a protein fragment which migrates closer to the anode than albumin.

Other situations associated with bis-albuminemias include:

- This may be seen with heparin treatment, which alters lipoproteins
- This may be result from the chemical makeup of albumin. Albumin has 35 cysteine residues. 34 of these form intramolecular disulfide bonds leaving one free. Upon prolonged storage (many days), albumin will form covalently linked dimers by interaction of these free cysteines between albumin molecules. These dimers show a slight electrophoretic difference from the albumin monomer, hence a pseudo-bisalbuminemia.
- Drugs or metabolites bound to albumin may alter the charge on albumin thus causing some albumin to migrate separately from other albumin.

α_1-acid glycoprotein

α_1-acid glycoprotein (AAG) is a globulin that migrates in the α_1 region on serum protein electrophoresis. Another name for α_1-acid glycoprotein is **orosomucoid**. This protein is produced by the liver and in granulocytes and monocytes. The physiologic function is poorly understood, but is does appear to be important as a transport protein. α_1-acid glycoprotein is known to transport hormones and drugs in the blood stream. AAG is an acute phase reactant and it has recently been reported as particularly useful for monitoring the clinical course of ulcerative colitis.

α_1-antitrypsin

α_1-antitrypsin is a globulin the migrates in the α_1 region on serum protein electrophoresis. This globulin makes up approximately 90% of the α_1-globulin fraction on serum protein electrophoresis. α_1-antitrypsin functions as a protease inhibitor; it inhibits the action of the naturally occurring proteolytic enzymes such as:

Trypsin	Leukocyte proteases
Elastase	Plasmin
Chymotrypsin	Thrombin
Collagenase	

All of these may be released during inflammatory reactions.

In the absence of α_1-antitrypsin, inhibition of these enzymes does not take place allowing these enzymes to metabolize or digest parenchyma (the essential functional element of an organ; the framework of the lung).

An α_1-antitrypsin deficiency appears to be hereditary and has been associated with two different diseases: pulmonary emphysema in adults and liver cirrhosis in children. Pulmonary emphysema is much more common than cirrhosis. Emphysema resulting from an α_1-antitrypsin deficiency is characteristically more severe in the lower lobes of the lung. Individuals who are heterozygous for this deficiency are referred to as having a predisposition to emphysema (i.e., they may or may not develop the clinical symptoms of emphysema. Individuals who are homozygous have a high tendency of developing emphysema. Avoidance of cigarette smoking by homozygous individuals is essential, since cigarette smoke is a major source of irritants that trigger leukocytes in the lung to release proteases.

In the case of α_1-antitrypsin deficiency, protein electrophoresis is used as a screening technique. The electrophoretic pattern has been described in the above section on common electrophoretic patterns. Since the total α_1-globulins are present in such a small quantity, it is sometimes difficult to say with any confidence that they are decreased. Therefore, to confirm α_1-antitrypsin deficiency, a quantitative assay, such as nephelometry, should be performed to specifically measure α_1-antitrypsin levels.

α_1-antitrypsin may also also be associated with a hyper-α_1-globulinemia. This is seen in the case of a non-specific acute phase increase of α_1-antitrypsin. Hyper-α_1-globulinemia is usually due to an increase of α_1-antitrypsin as a result of acute, sub-acute, or chronic inflammatory disorders.

One final note regarding α_1-antitrypsin. If serum is not separated from cells soon after clot formation, leukocytes may release elastase and other proteases that can bind with α_1-antitrypsin and change its electrophoretic mobility.

α_2-macroglobulin

This globulin is produced primarily in the liver and it major function is inhibition of enzymes such as plasma proteinase. It also serves as a transport protein. α_2-Macroglobulin is a very large molecule.

As seen in the preceding section on general electrophoretic patterns, the α_2-globulin fraction may be greatly increased in nephrotic syndrome. This is primarily due to an increase in α_2-macroglobulin, whose concentration rises tenfold or more. Recall that nephrotic syndrome is a

lesion in the membrane connecting the capillary bed (glomerulus) with the nephron (Bowman's capsule). As a result of this lesion, lower molecular weight proteins such as albumin leave the blood stream. α_2-Macroglobulin remains in the bloodstream because of its very large size.

In severe cases of nephrotic syndrome, the level of α_2-macroglobulin has reached, or even exceeds, the level of albumin. The importance of this is, as albumin is being lost, α_2-macroglobulin appears to begin to play a key role in maintaining some degree of osmotic pressure in the blood stream, thereby holding water in the blood stream. This function primarily belongs to albumin when albumin is present in normal levels in the blood stream.

Based on the discussion of α_2-macroglobulin thus far, the increase seen in nephrotic syndrome would be considered relative increase. The actual level of α_2-macroglobulin appears elevated because of the loss of other proteins, primarily albumin. There is also some evidence that α_2-macroglobulin synthesis may be increased as well.

The combination of hypoalbuminemia and hyper-α_2-macroglobulinemia is typically associated with nephrotic syndrome.

One additional situation where α_2-macroglobulin is commonly found elevated is in patients with acute thermal burns. These patients may show a similar pattern to that of nephrotic syndrome during recovery.

These two cases (nephrotic syndrome and thermal burns) are the only situations where the α_2-globulin fraction is increased as a result of an increase in α_2-macroglobulin.

α_2-Macroglobulin synthesis is decreased in acute phase reactions. Thus, this globulin is considered a negative acute phase reactant protein.

Haptoglobin

Usually an increase in the α_2-globulin region of serum protein electrophoresis is associated with a haptoglobin increase. Haptoglobin is commonly known as the hemoglobin-carrying protein. It binds irreversibly with hemoglobin that is released into the circulation by intravascular hemolysis and prevents loss of hemoglobin into the urine. This in turn protects the renal tubules from hemoglobin-induced damage. Also, since haptoglobin prevents the loss of hemoglobin, it could be said that haptoglobin functions to conserve the iron found in hemoglobin.

Free hemoglobin binds with haptoglobin to form hemoglobin-haptoglobin complexes. These complexes are usually very quickly cleared from the circulation (within minutes) by hepatocytes, where hemoglobin is broken down into its amino acids. The iron released is transported to the bone marrow by another transport protein, transferrin. Transferrin is a β-globulin discussed below.

Significant and rapid decreases in serum haptoglobin levels reflect the rates of intravascular hemolysis. Monitoring haptoglobin levels as a means to evaluate intravascular hemolysis is a major clinical use of haptoglobin.

A secondary role of haptoglobin is as an acute phase protein/reactant where it serves as a protease inhibitor.

The greatest clinical utility of haptoglobin is associated with serum/plasma deficiencies. These deficiencies can be divided into two categories.

- Acquired haptoglobin deficiencies – Acquired deficiencies refer to any disorder which is associated with significant intravascular hemolysis. In this case, due to the accelerated removal of hemoglobin-haptoglobin complexes, serum levels of haptoglobin will decrease.
- Secondary hypohaptoglobinemia – An individual may suffer from a permanent state of secondary hypohaptoglobinemia as a result of a congenital defect in the erythrocytes which leads to significant and continual intravascular hemolysis. This may be associated with G6PD deficiency, hereditary spherocytosis, or thalassemia.

Ceruloplasmin

This α_2-globulin functions to carry copper throughout the body. It is capable of reversibly binding eight atoms of copper per molecule, and it carries more than 95% of all of the copper in the body, therefore, very little copper remains free in the blood stream. Each molecule of ceruloplasmin can bind six atoms of copper. When there is a full complement of copper, this protein has a blue appearance. The combination of this blue color with the straw/yellow color from chromagens in serum/plasma will produce a greenish appearance in serum/plasma when ceruloplasmin levels are elevated.

Ceruloplasmin is important in iron metabolism. Ceruloplasmin is believed to oxidize ferrous iron (Fe^{+2}) to ferric iron (Fe^{+3}) which is important in situations where the iron is being transferred from ferritin to transferrin. It is most likely the copper in ceruloplasmin that is actually responsible for the oxidation.

As long as copper is bound to ceruloplasmin in the body, it is considered to be non-toxic. Unbound copper, however, does appear to be toxic. In its unbound form, copper freely leaves the blood stream and concentrates in areas such as the cerebellum, eyes, and liver. Once in these areas, copper interferes with the normal function of these tissues.

The most clinically significant ceruloplasmin abnormality is a ceruloplasmin deficiency. This deficiency may either be acquired or congenital. An acquired deficiency of ceruloplasmin is primarily associated with liver disease (such as cirrhosis). This is because ceruloplasmin is produced by the liver.

A congenital deficiency of ceruloplasmin is known as **Wilson's disease** (hepatolenticular degeneration). The clinical features of this disease are a result of free copper diffusing into tissues. As copper deposits accumulate in the susceptible tissues, the following clinical features appear:

- copper in the liver leads to liver cirrhosis
- copper in the brain (particularly the cerebellum) leads to Parkinsonism (bizarre) type movements
- copper deposits in the eye results in a golden-brown pigmentation around the cornea

There are three diagnostic features of Wilson's disease.

- golden-brown rings encircling the cornea
- a copper content in excess of 250 mg/g of dry liver (biopsy specimen)
- increased urinary excretion of copper, decreased ceruloplasmin, and, generally, a decreased serum copper

Ceruloplasmin is an acute phase protein/reactant. Thus, its levels may be increased in periods of inflammation. In addition, oral contraceptives and pregnancy appear to double ceruloplasmin levels. Considering the effects of ceruloplasmin described above, it would not be considered unusual to find a green serum in these situations.

β-lipoprotein

β-lipoprotein is one of the major β-globulins. β-lipoprotein is also known as low density lipoprotien (LDL). The characteristics of β-lipoprotein have already been discussed when considering lipids and will not be considered further here.

Transferrin

Transferrin is a second major β-globulin. The function of transferrin is to transport free iron in the blood stream. The phrase "free iron" refers to non-heme iron. Transferrin is capable of binding two iron atoms per molecule. Iron transported by transferrin is in the ferric state (Fe^{+3}). If iron is presented to transferrin in the ferrous state (Fe^{+2}), it will be oxidized to the ferric state by the copper bound in ceruloplasmin prior to binding to transferrin.

Fe 3 = ferric
Fe 2 = ferrous

Transferrin is capable of picking iron up from the liver, the GI tract, or ferritin (the tissue storage form of iron), and carrying it to the bone marrow for hemoglobin synthesis and to other tissues which require the presence of iron-containing enzymes.

Transferrin is normally 30% saturated with iron. Total transferrin levels are typically estimated by measuring the "total iron binding capacity" (TIBC). The TIBC refers to the total amount of iron that can be bound by the transferrin available in the blood stream. This is commonly utilized as an index of iron metabolism and erythropoiesis.

Iron metabolism, including transferrin, will be discussed at a later time. The focus here is on the protein transferrin.

Transferrin deficiencies are usually acquired and nonspecific. These deficiencies are typically associated with liver disease since transferrin is synthesized in the liver. Transferrin levels in the blood stream have also been found to decrease in chronic infections. This observation causes transferrin to be classified as a negative acute phase reactant/protein. In nephrotic syndrome, hypotransferrinemia is typically seen due to loss of transferrin into the urine

Increased transferrin levels in the blood stream are characteristically seen in iron-deficient states. Hypertransferrinemia has become almost synonymous with iron-deficiency anemia.

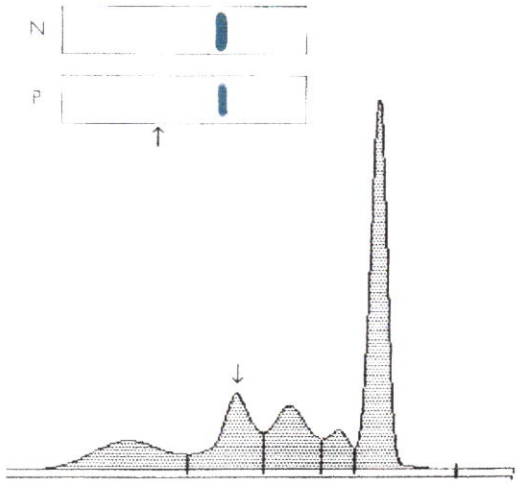
CSF contains two forms of transferrin.

- the form normally found in the blood stream
- the form found only in spinal fluids named **Tau protein**

Tau = CSF

These two forms of transferrin migrate differently on agarose gel electrophoresis and can thus be differentiated. In patients with leakage of fluid from locations such as the ear or nose, identification of Tau protein by electrophoresis can help distinguish between CSF and other fluids.

Transferrin is normally a single molecular species with a tight electrophoretic mobility. Thus, when transferrin is elevated, such as with iron-deficiency anemia, this abnormally tight zone can take on the appearance of a monoclonal abnormality. This can be especially misleading when iron-deficiency anemia is severe. This is illustrated in the diagram below.



Hemopexin

Hemopexin is a β-globulin that acts in a similar manner to haptoglobin. Recall that haptoglobin serves to bind hemoglobin. Also, recall that haptoglobin is present in the blood stream in only limited quantities. Once haptoglobin levels are depleted in hemolysis, hemopexin begins to serve a very important function; specifically binding heme (the iron-containing core of the hemoglobin molecule).

C3 Component of Complement

C3 in the last β-globulin that we will consider. C3 is present in the highest concentration of all of the complement components in plasma. The function of C3 has been previously discussed in other classes.

Increases and decreases in C3 both have clinical significance.

Increases in C3 are associated with:

- Acute phase reactant/protein nature of C3
- Biliary obstruction – levels of C3 are elevated in direct proportion with bilirubin. Why this is true is still unclear
- Focal glomerulosclerosis – increased C3 levels are associated with a favorable prognosis, decreased levels increase risk of infection

Decreases in C3 are associated with:

- Genetic deficiency in the production of C3 – increased risk of infection
- Acquired deficiency – usually autoimmune diseases that actually consume C3

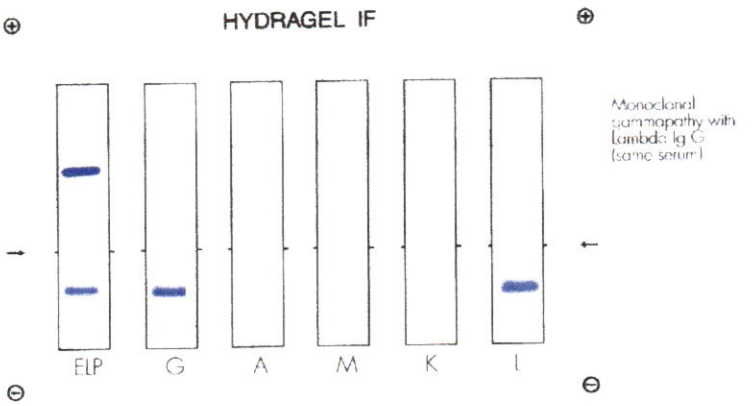
The γ Electrophoretic Region

This region is made up of immunoglobulins (which are also known as γ-globulins). Electrophoretic evaluation of the γ-region is best when supplemented by complementary investigations like immunoelectrophoresis or immunofixation electrophoresis. The typical goal

is to distinguish between monoclonal and polyclonal abnormalities. Quantitation of specific immunoglobulins is also recommended. A variety of techniques may be used for quantitation, some of which include: radial immunodiffusion, Laurell electroimmunodiffusion, nephelometry, etc. Protein quantitation permits establishment of a "protein profile" that can be associated with certain pathologic conditions.

In the clinical laboratory there has been a general shift away from immunoelectrophoresis toward immunofixation electrophoresis. The primary reason for this transition is ease of interpretation. Immunofixation electrophoresis is much easier to interpret. A comparison of these two methods is illustrated below.

Immunoelectrophoresis is depicted as arcs whose interpretation relies on the morphologic characteristics of the arc. The immunoelectrophoresis pattern shown below is from a patient with a lambda IgG monoclonal abnormality.



If an IgM monoclonal abnormality were suspected, mercaptoethanol reduction would be necessary to show clearly the distorted arc corresponding to the light chain involved. Mercaptoethanol reduction serves to break the disulfide bonds binding IgM monomers. Please do not try to memorize the information in this paragraph. I give you this to illustrate the difficulty in using immunoelectrophoresis. This precautionary reduction step is not necessary with immunofixation electrophoresis which does not generate any umbrella (i.e., arc characteristic) effect.

Immunofixation electrophoresis is depicted as homogenous bands whose interpretation is much easier than immunoelectrophoresis. Easy to perform, immunofixation electrophoresis provides results within one or two hours. This techniques also offers many advantages such as good sensitivity and easy interpretation (especially for biclonal or oligoclonal abnormalities). The immunofixation electrophoresis results illustrated below resulted from the same patient used to illustrate the immunoelectrophoresis characteristics of a lambda IgG monoclonal abnormality above.

Immunoglobulin abnormalities are typically classified by using three categories:

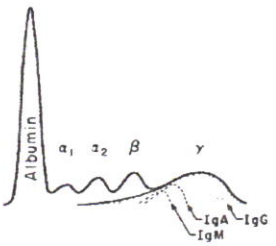
- Hypo-gamma-globulinemias
- Polyclonal abnormalities (also called polyclonal gammopathies)
- Monoclonal abnormalities (also called monoclonal gammopathies)

Hypo-gamma-globulinemia

Hypo-gamma-globulinemia has already been introduced in the section entitled "Common Protein Electrophoretic Patterns." Hypo-gamma-globulinemia may result from any one of three conditions:

- Hypercatabolism, i.e. increased breakdown of proteins
- Pathological loss of proteins from skin, GI tract, or kidneys
- Defective synthesis of these proteins

As with all protein abnormalities, electrophoresis is typically used as a screening tool. Keep in mind that a decreased γ-globulin is apparent by electrophoresis only when the patient has an IgG deficiency. This is because IgG accounts for ~75% of the γ-globulin region on electrophoresis as illustrated in the below diagram. IgA and IgM deficiencies may not be easily recognized on serum protein electrophoresis.



Also, in adults, the finding of hypo-gamma-globulinemia requires the exclusion of Bence-Jones proteins or other B-cell malignancies as the underlying cause. This will be considered below with monoclonal abnormalities.

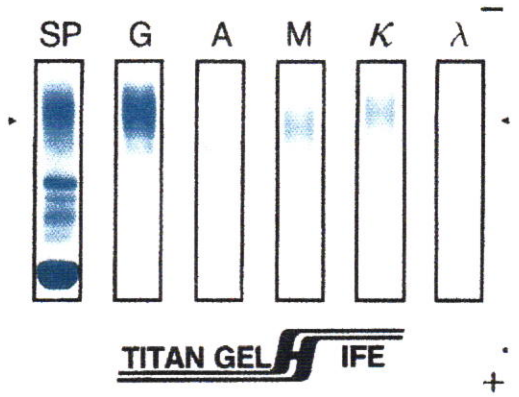
Polyclonal Abnormalities (Gammopathies)

Polyclonal abnormalities have already been introduced in the section entitled "Common Protein Electrophoretic Patterns." Polyclonal abnormalities are usually associated with a broad diffuse increase of the electrophoretic gamma globulin fraction. Usually all three major classes of immunoglobulins are increased in polyclonal abnormalities, i.e. IgG, IgA, and IgM. So we see an increase in the serum of several different immunoglobulins that are products of many different clones of plasma cells. This polyclonal increase is usually in response to antigenic stimulation.

In most cases, hyper-gamma-globulinemia of the polyclonal variety is characterized by a broad, diffuse and heterogeneous increase, mainly of the electrophoretic gamma globulin fraction. This electrophoretic pattern was illustrated in the previous section. This increase is a result of increased proliferation of numerous plasma cell clones. As already stated, in most cases, all three major immunoglobulin classes are increased here. Even though these immunoglobulin classes are elevated, the normal kappa to lambda ratio is preserved.

There are some cases, however, where the polyclonal abnormality results in a narrow banded zone. This type of pattern is due to a polyclonal increase of immunoglobulins located primarily at one electrophoretic region. These types of patterns are seen in individuals with chronic active hepatitis and in certain patients with large amounts of circulating immune complexes for whatever reason (such as rheumatoid arthritis).

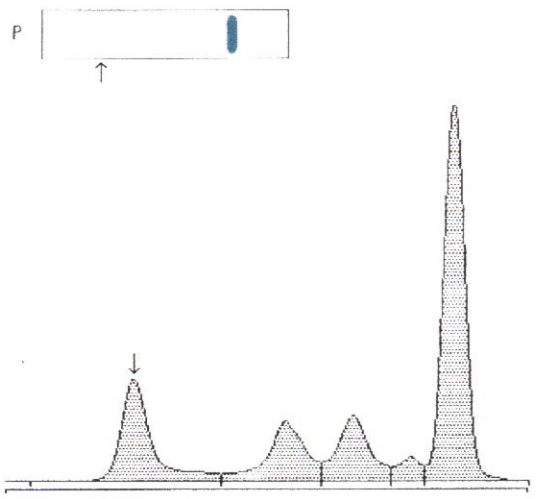
The problem with a narrow banded polyclonal abnormality is that it can be easily confused with monoclonal abnormality (which, as discussed below, is typically very narrow banded). In this case, immunofixation would play a crucial role in properly identifying what initially appeared as a monoclonal abnormality. Again, for a polyclonal abnormality, we would find all three major immunoglobulin present with a normal kappa to lambda ratio. A polyclonal abnormality is illustrated below.



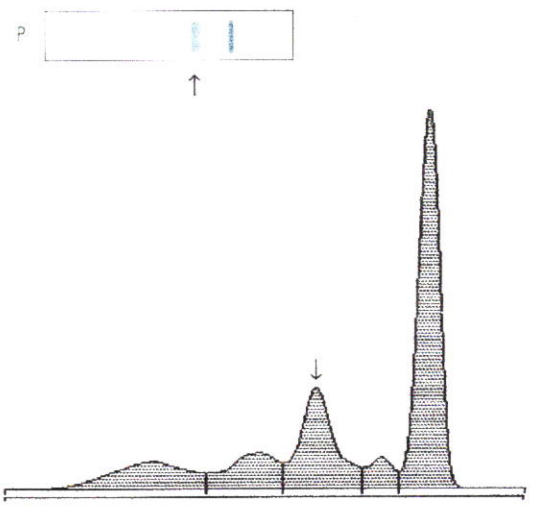
At this time there is need to address some confusing terminology: this is polyclonal/monoclonal **gammopathy vs.** polyclonal/monoclonal **abnormality**.

The term γ-globulin was initially equated with those proteins possessing antibody characteristics because it was first thought that all antibodies migrated in the γ electrophoretic region. With the development of immunoelectrophoresis and then immunofixation, it was determined that the electrophoretic distribution of antibody proteins covered not only the γ electrophoretic region, these antibody protein also appeared in the α and β regions. This is illustrated by the two electrophoretic patterns below, both of which were confirmed with immunofixation.

Electrophoresis showing monoclonal component



Electrophoresis with monoclonal component in α2 zone



In order to address this problem, the name **immunoglobulin** was introduced to describe proteins possessing either antibody characteristics or the characteristic structure of antibodies. Therefore, since these abnormalities may appear in regions other than just the γ-region, the correct way to refer to related abnormalities is as **monoclonal/polyclonal abnormalities**. Even though this is true, the old name (gammopathy) is still frequently used.

Monoclonal abnormalities

Immunofixation electrophoresis plays a very important role in the laboratory identification of monoclonal abnormalities. Thus, at the outset we will review some characteristics of immunofixation electrophoresis. Immunofixation electrophoresis (IFE) is a technique that takes advantage of both antibody specificity for antigens and the ability of antibodies to fix (immunoprecipitate) proteins in a gel matrix. IFE is performed after the specimen is subjected to electrophoresis (with application of the same specimen in multiple lanes). After electrophoresis is complete, a template cutout is placed on the gel such that it isolates the area of sample migration in each lane. Each isolated electrophoretic lane is then overlaid with specific antisera for different protein-antigens of interest. The antiserum reacts with the specific protein of interest, fixing it in the gel matrix. After washing away unreacted reagent and unfixed proteins, the gel is stained and the immune precipitates identified visually. The ability to visualize a specific protein is enhanced several fold with IFE compared to conventional electrophoresis. This is because the additional protein from the antibody-antigen reaction magnifies the intensity of antigen staining.

Overview of Monoclonal Abnormalities

Monoclonal abnormalities are characterized by an uncontrolled proliferation of a single clone of plasma cells at the expense of other clones. This dysfunction often leads to the synthesis of large amounts of one homogeneous immunoglobulin or immunoglobulin subunit with decreased level of normal immunoglobulins. The stimulus for proliferation is unknown but is probably not antigenic (the stimulus noted above for polyclonal abnormalities).

Since normal immunoglobulins are often suppressed in these disorders, there can be a functional immunodeficiency in association with increased γ-globulin levels. This immunosuppression can have life-threatening consequences in a patient whose condition is already compromised by the primary disease.

Electrophoretic patterns and immunoglobulin test results can be strikingly abnormal in patients with multiple myeloma and other B-cell related neoplasms. As a result, protein analysis has been a valuable tool in the diagnosis and monitoring of these lymphoproliferative diseases.

Immunochemical methods can quantitate the abnormal protein production that marks these disorders, but only electrophoresis can demonstrate its monoclonal nature. The γ-region of the electrophoretic pattern can show a dense, highly restricted band from uncontrolled proliferation of one cell clone, with decreased background staining due to the shutdown of normal immunoglobulin synthesis.

Even though monoclonal abnormalities can have a dramatic pattern of protein change in some instances, their clinical interpretation can be difficult. It is important to consider that one-third of the patients with immunochemical evidence of monoclonal abnormality are asymptomatic.

These could be patients with benign or transient monoclonal proteins, especially in an older population. They could also represent an early or relatively dormant stage of the disease that might later accelerate.

Some patients show symptoms of a plasma cell dyscrasia or other lymphoproliferative disorder, but do not exhibit the characteristic monoclonal band or spike in their serum protein electrophoretic patterns. This is often the case with light chain disease where only kappa or lambda monoclonal light chains are synthesized by the clone. These low molecular weight immunoglobulin fragments are filtered through the glomerulus and into the urine, giving a serum electrophoresis pattern that shows hypogammaglobulinemia with either a very faint monoclonal band or no band at all. There is also the possibility of a non-secretory clone which produces no monoclonal immunoglobulin. The serum protein patterns in patients with non-secretory clones frequently show hypogammaglobulinemia due to the inhibition of normal clones.

Suggested Protocol for Monoclonal Abnormality Evaluation

1. Serum/Urine protein electrophoresis. For urine, a 24-hour urine specimen is preferable but a random specimen is adequate to characterize the monoclonal protein. Electrophoresis is an important first step because only electrophoresis can demonstrate the monoclonal nature of the protein.
2. Quantitative serum immunoglobulins. This is used to assess general immune competence and provide baseline values of immunoglobulin concentrations for follow-up.
3. Serum/Urine immunofixation electrophoresis. This provides definitive identification of the monoclonal protein.

Discussion of Monoclonal Abnormalities

As noted above, monoclonal abnormalities are usually characterized by a narrow, restricted immunoglobulin increase typically in the γ electrophoretic region. This narrow band is referred to as a monoclonal "spike." This monoclonal spike is said to be due to a M-protein. A M-protein structurally consists of one immunoglobulin class and one light chain type. The letter M is used because of the frequent association of this monoclonal spike with myeloma (a tumor in the bone marrow) and macroglobulinemia.

Multiple Myeloma

Monoclonal abnormalities are the result of disordered immunoglobulin synthesis and is associated with three characteristics:

- Excessive proliferation of B-cell clones
- The formation of electrophoretically, structurally, and antigenically homogeneous immunoglobulins or light chain types which are present in serum and/or urine
- A deficiency of background immunoglobulins

Monoclonal abnormalities are **not** rare. For instance, large medical centers (~1000 beds) typically encounter one new case of a monoclonal abnormality on the average of every 2.5 days.

The incidence of a monoclonal abnormality increases with advancing age. Sporadic cases of asymptomatic monoclonal abnormality may be encountered in immunodeficient newborns and infants. Beyond 40 years of age there appears to be an exponential increase in incidence.

These asymptomatic, i.e. non-malignant, cases are also known as **Monoclonal Gammopathy of Undetermined Significance (MGUS)**, also termed "Benign Monoclonal Gammopathy" (BMG). MGUS is recognized when:

- The M-protein does not exceed 2 g/dL if IgG, or 1 g/dL if IgA or IgM
- Polyclonal immunoglobulins are within the normal reference interval and stable
- No Bence-Jones proteinuria is present
- No lytic lesions are seen on radiographic studies
- There is no increase in plasma cells in the bone marrow
- Stable concentrations of the M-protein is seen over time

MGUS accounts for many of the M-proteins uncovered. Patients demonstrating MGUS require periodic monitoring with serum and urine studies because their disease may progress. Treatment of patients with MGUS is not recommended until laboratory or other abnormalities progress or symptoms of multiple myeloma develop, because these patients may remain stable for years.

M-proteins are also referred to as paraproteins. Many use these two names interchangeably. A paraprotein is defined as a protein (other than fibrinogen) that appears as an abnormal narrow band on the electrophoretic pattern anywhere between the α- and γ-regions. Most frequently these abnormal bands appear in the γ-region.

A paraprotein is not a disease entity in itself, but it is a sign of an underlying disease. When a cell becomes malignant, and it and its offspring (i.e., a clone of cells) produce increased amounts of a single protein, then the term monoclonal abnormality is used. Monoclonal abnormalities result in the production of paraproteins.

Demonstration of a M-protein in the serum or urine by some laboratory technique only allows for the laboratory diagnosis of monoclonal abnormality. The clinical diagnosis of monoclonal abnormality requires the differentiation between the malignant (or symptomatic) state and the benign (or asymptomatic) state. Approximately $^2/_3$ of the patients with immunochemical evidence of M-proteins are symptomatic or will become symptomatic; in other words, these individuals will develop either:

- Multiple Myeloma
- Waldenstrom's Macroglobulinemia
- Heavy Chain Disease (Franklin's Disease)
- Cryoglobulinemia

Approximately $^1/_3$ of the individuals with M proteins are asymptomatic and they are either apparently healthy or they may have disorders not directly related to B-cell diseases. The incidence of asymptomatic monoclonal abnormalities increase disproportionately at the higher age ranges.

The laboratory plays a very important role in differentiating symptomatic (malignant) and asymptomatic (benign) monoclonal abnormality. There are 4 biochemical features that assist in this differentiation:

Feature	Symptomatic	Asymptomatic
Excess secretion of light chains	84%	0%
Progressive rise in paraprotein level	99%	1%
Suppression of normal immunoglobulins	98%	10%
"High" serum paraprotein level	92%	15%

The significance of this differentiation is that symptomatic cases tend to become progressively worse, possibly to a point of death, while asymptomatic cases tend not to progress.

Considering some the characteristics in the table above, excessive secretion of light chains is seen in 84% of individuals who are symptomatic (presence of malignancy) while light chains are not seen in asymptomatic cases. (Compare this with MGUS.) These light chains are found in excess in the urine when the malignant cell builds up an imbalance in light and heavy chain synthesis. These light chains show up in the urine because they have a relatively low molecular weight (~25,000 daltons). The only time you would see an accumulation of these light chains in the serum would be during renal failure. These can be detected with sensitive methods such as immunofixation electrophoresis. These light chain proteins are known as **Bence-Jones proteins**.

Bence Jones proteins are monoclonal kappa or lambda immunoglobulin light chains which are not attached to the heavy chain portion of the immunoglobulin molecule. Bence Jones proteins are seen in two types of syndromes: (1) in conjunction with a typical monoclonal abnormality or (2) in free light chain disease.

Light chain disease is a monoclonal abnormality in which only kappa or lambda monoclonal light chains, or Bence Jones proteins, are produced. Light chain disease comprises approximately 14% of monoclonal abnormalities. It ranks behind IgG myeloma and IgA myeloma. Its laboratory diagnosis presents various difficulties not associated with other common monoclonal abnormalities and requires considerable clinical skill and sophisticated analytic techniques.

Patients with B-cell malignancies other than light chain disease may be asymptomatic until serum levels of monoclonal proteins are very high (about 2 g/dL for IgG abnormalities). On the other hand, very small amounts of Bence Jones protein in serum can be associated with significant clinical problems, especially pathologic renal changes. Free light chains filter through the glomerulus almost without obstruction due to their small molecular size and accumulate in the tubules. Renal impairment can result from the toxicity of the light chains. Pathological changes can range from relatively benign tubular proteinuria to acute renal failure or amyloidosis.

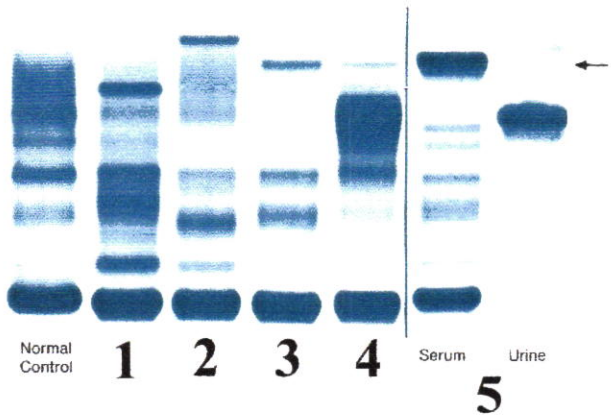
Bence Jones proteins may be detected in serum, urine or both. The level of monoclonal light chain in serum or urine is related to filtration, reabsorption or catabolism of the protein by the kidney. During the early stages of the disease when the kidney is only mildly affected, excretion and reabsorption continue normally but only partial catabolism occurs. At this point, Bence Jones proteins may be detected in the serum but not urine. Progressive renal involvement impairs reabsorption so that diminished reabsorption with decreased catabolism results in free

light chains in both serum and urine. Later, as reabsorption is totally blocked, light chains are present in urine only. In terminal stages of the disease, uremia occurs and renal clearance is affected and Bence Jones proteins again appear in serum.

Serum protein electrophoresis patterns from patients with monoclonal free light chains may show:

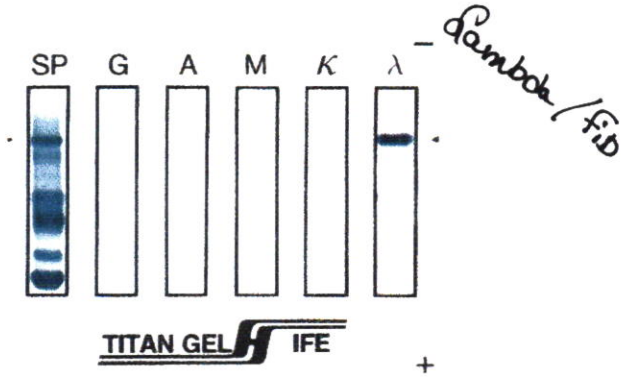
- the typical well defined monoclonal band
- a somewhat broad diffuse band which results from polymerization of monoclonal proteins
- a normal γ-region
- hypogammaglobulinemia

Consider the following electrophoresis and immunofixation electrophoresis results.

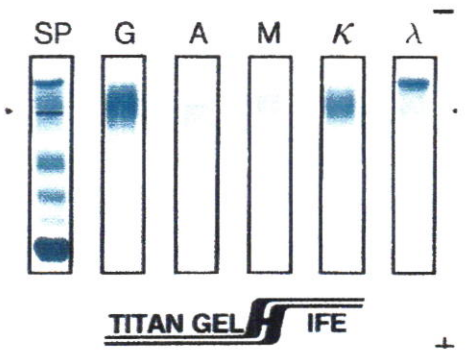


The normal control is serum. Sample 1 through 4 are from serum. Sample 5 pattern shows both urine and serum electrophoresis results. The arrow shows the point of application.

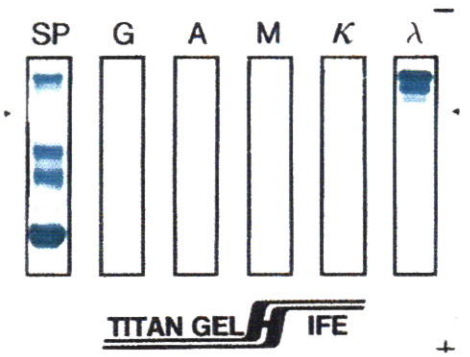
The pattern below shows the immunofixation results for sample 1 above. The monoclonal immunoglobulin is identified as free lambda light chain. This is laboratory evidence of **Free Lambda Light Chain Disease**. Notice that the serum electrophoresis result shows a second prominent band slightly anodic to the free lambda band which did not react with IgG, IgA, IgM, kappa or lambda antisera. The band was identified as fibrinogen



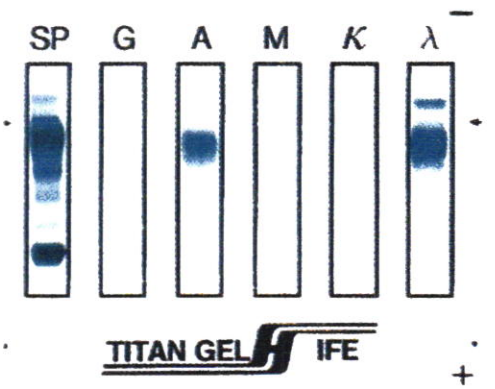
The pattern below shows the immunofixation results for sample 2 above. The small black arrow on the right of the SP lane and the left of the λ lane show the point of sample application. The very narrow dark line at the point of application in the SP lane is an artifact. Continuing with the γ-region, we see normal IgG, IgA and IgM, kappa and lambda. In addition, just above the IgG region, we see a very tight band in the SP lane and a corresponding tight band in the lambda lane. This indicates the presence of free lambda light chains. This is laboratory evidence of **Free Lambda Light Chain Disease**.



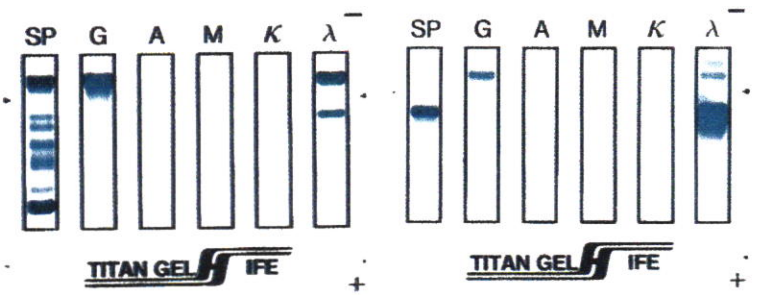
The pattern below shows the immunofixation results for sample 3 above. The monoclonal immunoglobulin is identified as free lambda light chain. Once again, this is laboratory evidence of **Free Lambda Light Chain Disease**. The multiple bands are probably due to the polymerization of the lambda chains. The monomer, dimer, and trimer of the lambda light chain all have different electrophoretic migration patterns.



The pattern below shows the immunofixation results for sample 4 above. IgA appears to be the only immunoglobulin present. This is characteristic of monoclonal abnormalities where background immunoglobulin synthesis does not occur. There is also only one light chain type which is characteristic of monoclonal abnormalities. Thus, there appears to be an IgA lambda monoclonal abnormality. In addition, there is an additional zone cathodal to the abnormal monoclonal immunoglobulin. Since there is no reaction with IgG, IgA, IgM, or kappa lanes in this area, this is evidence of the presence of lambda light chains. This is laboratory evidence of **IgA Lambda Monoclonal Abnormality with Free Lambda Light Chains**.



The pattern below shows the immunofixation results for sample 5 above. The serum is on the left and the urine is on the right. The serum sample shows **IgG Lambda Monoclonal Abnormality with Free Lambda Light Chains**. The urine contains IgG lambda monoclonal immunoglobulin with a very large band of free lambda light chain. Multiple lambda bands in the urine are probably due to polymerization of free lambda light chains. Appearance of the intact IgG molecule in the urine is indicative of kidney damage which allows large proteins to filter through the glomerulus.



Now returning to the table above, a progressive rise in paraprotein level is seen in 99% of symptomatic patients and only 1% of asymptomatic patients. A great deal of clinical evidence now appears to indicate that paraprotein serum levels increase with time and is directly related to the mass of the tumor. This increase serum paraprotein level appears to be exponential.

Still considering the table above, 98% of the symptomatic patients will show a suppression of normal immunoglobulins. As malignant cells begin to overproduce their product, the remaining normal immunoglobulins are suppressed, therefore, their serum level drops. As seen in the immunofixation patterns above, the level of normal (sometimes referred to as background) immunoglobulins are so low they are not even detected with this very sensitive technique. This suppression of background immunoglobulins is seen in only 10% of the asymptomatic cases.

Finally, 92% of symptomatic patients show a high serum paraprotein level while this is seen in only 15% of asymptomatic patients. Essentially, the higher the immunoglobulin level, the greater the indication of malignancy. Consider the following immunoglobulin levels:

Ig	Normal (g/dL)	Level suggestive of malignancy (g/dL)
G	0.9-1.5	>2
A	0.14-0.22	>1
M	0.08-0.12	>1

When these paraproteins are elevated in the blood stream, they produce significant effects on three laboratory tests.

- The first effect of elevated paraproteins is on blood viscosity. With an increase in paraprotein levels there is an increase in the viscosity of blood. Multiple myeloma and Waldenstrom's macroglobulinemia are the two major myelomas (tumor of the bone marrow) and both result in an increased viscosity of the blood. It is important, however, to keep in mind other causes of hyperviscosity.
    - One place where you may see this is with an increased number of blood cells such as polycythemia (an increased erythrocyte count) and leukemia (an increased white cell count)
    - Another condition where blood viscosity will be increased is with certain abnormalities of red blood cells. Here certain abnormal red blood cells are resistant to deformation. Examples include the sickle cells of sickle cell anemia and the spherocytes of spherocytosis.
- The second effect of elevated paraproteins is seen on blood components, such as coagulation factors. Specifically, excessive paraproteins tend to inhibit thrombin formation, therefore, preventing clotting.
- The third clinical effect of elevated paraproteins is on cellular components of the blood: red blood cells, white blood cells, and platelets. Paraproteins tend to cause rouleaux of red cells (stacking one on another). They also tend to suppress phagocytosis by white blood cells. They also inhibit the adhesiveness of platelets. Platelet adhesion is important in the clotting process.

Disease States Associated With Monoclonal Abnormalities

As we begin to consider some of the commonly encountered disease states associated with monoclonal abnormalities, I first want to list the frequency of the classes and types of monoclonal abnormalities.

Classes and types	Frequency (%)
IgG	57
IgA	24
Light chains only	14
IgD	2
IgM	<1
IgE	Rare

Multiple Myeloma

Multiple myeloma is probably the most common underlying cause of monoclonal abnormalities. Multiple myeloma includes all of the abnormalities above except IgM. The IgM abnormality has distinct characteristics and is associated with Waldenstrom's Macroglobulinemia.

Multiple myeloma is a malignancy of plasma cells involving the bone marrow. This disease has its highest incidence in the 60-year-old age group. The mean age of diagnosis is 62. At some stage during the disease the plasma cells infiltrate the bone marrow.

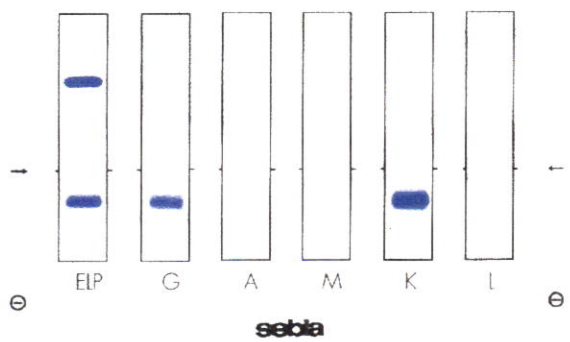
Approximately 75-80% of the patients with this disease show an abnormal monoclonal serum protein. About 70% of the time this can be categorized at IgG. In addition to this serum monoclonal protein, many of the patients with multiple myeloma excrete Bence-Jones proteins in the urine.

A patient with multiple myeloma usually has some significant changes in their blood chemistries.

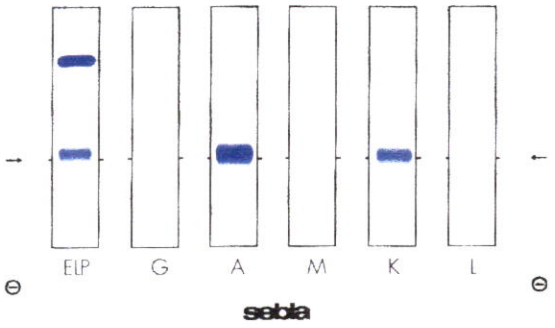
- About 60% of the patients with multiple myeloma have an elevated total serum protein and a decreased serum albumin. In many cases the total protein level may increase to 10-12 g/dL.
- The calcium level is increased in approximately 50% of patients with multiple myeloma. This is both calcium in the serum and urine. This is primarily due to bone destruction during the disease process.
- Serum cholesterol levels are usually below 200 mg/dL. For some reason there tends to be a rapid clearing of neutral lipids from the plasma.
- Uric acid levels are increased. This is because of increased cell destruction. As a result, increased plasma uric acid levels may lead to attacks of gout.

Focusing on electrophoretic results in mutiple myeloma, the monoclonal band shown on the electrophoretogram of patients with multiple myeloma confirms the clinical diagnosis of the disease.

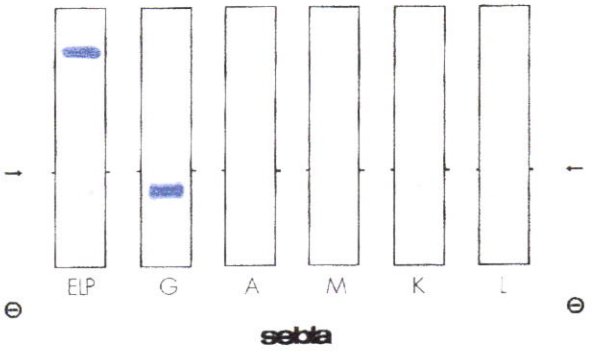
This first immunofixation pattern illustrates an IgG Kappa monoclonal component with decreased concentrations of other classes of immunoglobulins.



This next immunofixation pattern illustrates an IgA Kappa monoclonal component in the β-globulin zone with decreased concentration of other classes of immunoglobulins. Note, even though these other immunoglobulins are decreased, you can still see some evidence of IgG.



Advanced stages of malignancy are often associated with protein patterns that are more difficult to interpret. The next pattern is a **biclonal gammopathy** consisting of a predominant IgG Kappa component and a less concentrated, more anodal IgG Lambda component.



Note in all cases associated with multiple myeloma, a urine should be evaluated for the presence of Bence Jones proteins.

Waldenstrom's Macroglobulinemia

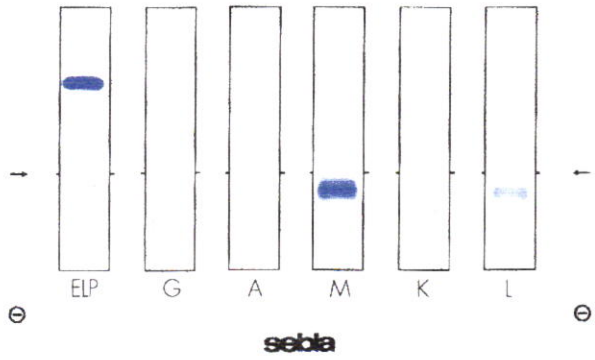
Whereas myeloma can be associated with the presence in serum of monoclonal components of several types (IgG, IgA, IgD, IgE, or free light chains), Waldenstrom's macroglobulinemia is pathologically characteristized by a serum monoclonal IgM in high concentration. Its designation as macroglobulinemia is related to the high molecular weight of the IgM pentamer. Thus, we can say that Waldenstrom's macroglobulinemia is a lymphoproliferative (proliferation of lymphoid tissue such as lymphocytic leukemia or malignant lymphoma) malignancy associated with the secretion of monoclonal IgM.

Two laboratory characteristics of this disease that were also observed with multiple myeloma are rouleaux formation and hyperviscosity. Also, anywhere up to 90% of the individuals with this disease excrete Bence-Jones proteins in the urine.

A screening test that may be used for Waldenstrom's macroglobulinemia is the **Sia Water Test**. In this test, a drop of serum is allowed to fall into a tube of boiled water. Normally a faint haze is seen. In the case of Waldenstrom's macroglobulinemia, a distinct precipitate is seen.

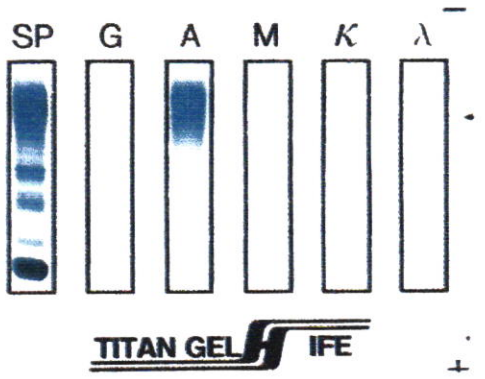
In the lab, serum protein electrophoresis would show a paraprotein. The next step would be to perform serum immunofixation, which would show an increase in IgM. Then nephelometry or radial immunodiffusion could be used to quantitate the abnormal immunoglobulin.

Below is an illustration of an IgM Lambda monoclonal component seen in Waldenstrom's macroglobulinemia. Note that the background immunoglobulins are actually decreased in concentration (although that may be hard to see without comparing the diagram with a normal sample to compare intensity of staining).



Heavy Chain disease (Franklin's disease)
Heavy chain diseases are rare with less than 100 cases reported in the literature. Heavy chain diseases are characterized by the presence of monoclonal proteins comprised of the heavy chain portion of the immunoglobulin molecule. In this disease there is the overproduction of either gamma, alpha, mu, or delta heavy chains which suggests a deletionary defect of the Fc fragment. These monoclonal heavy chains may be detected in serum and/or urine depending on the class of heavy chain involved.

Franklin reported the first case of heavy chain disease, which involved a gamma chain. Franklin's disease is synonymous with gamma heavy chain disease. Alpha heavy chain disease is the most frequently encountered form of this protein abnormality. The pattern below illustrates immunofixation electrophoresis characteristics of IgA Heavy Chain Disease.



Cryoglobulinemia

Cryoglobulinemia is the fourth finding that may be associated with symptomatic monoclonal abnormalities. Cryoglobulins are immunoglobulins which undergo insolubilization at temperatures below 37°C and dissolve on rewarming. Cryoglobulins have been found mostly in association with lymphoproliferative and autoimmune disorders, chronic infections, liver and kidney diseases, and following organ transplantation. Cryoglobulins have the ability to fix complement and initiate an inflammatory reaction.

Three types of cryoglobulins have been defined. The mixed cryoglobulins are antigen-antibody complexes with both elements being required for precipitation. Precipitation of Type I cryoglobulins does not involve antigen-antibody reactions.

Type I Monoclonal cryoglobulins consist of monoclonal immunoglobulins only. They account for 5 - 10% of cryoglobulins and are found almost exclusively in patients with malignant B-cell tumors. The most common immunoglobulin to be found is IgM followed by IgG, IgA and rarely Bence Jones protein.

Type II Mixed cryoglobulins that include two classes of immunoglobulins, one of which is monoclonal. The monoclonal component always has rheumatoid factor (RF) activity and usually is an IgM with kappa light chains. The second component is polyclonal IgG. Type II cryoglobulins account for 50 - 65% of cryoglobulins. Type II cryoglobulins are associated with lymphoproliferative and autoimmune disorders, but the majority (about 80%) are idiopathic.

Type III Mixed cryoglobulins formed by two types of polyclonal immunoglobulins. Most commonly IgM bound to either IgG or IgA. These comprise about 30% of cryoglobulinemias. Type III cryoglobulins are seen in a variety of autoimmune, systemic rheumatic diseases and persistent infections with immune complexes (such as bacterial endocarditis).

Cryoglobulinemia is more frequent in Waldenstrom's Macroglobulinemia than in Mutiple Myeloma.

Procedure to screen for cryoglobulins:

- Collect blood in a pre-warmed container and keep it at 37°C.
- Following centrifugation, incubate serum at 4°C.
- If a precipitant appears, heat serum to 37°C; cryoglobulins will redissolve.
- Confirm and type by immunofixation electrophoresis.

Monoclonal cryoglobulins generally will precipitate within 24 hours, but mixed cryoglobulins often are present in low concentrations and require prolonged incubation at low temperatures for detection.

Failure to detect a cryoglobulin may be due to loss of cryoglobulins with erythrocytes if clotting of blood is allowed to occur at temperatures below 37°C, or failure to allow sufficient time for cryoglobulin formation to occur.

C-reactive protein (CRP)

C-reactive protein is the final γ-globulin to be considered. This protein was initially found in the sera of patients who had recovered from *Streptococcus pneumoniae* infections. This protein was found to bind to the C-polysaccharide on the cell wall of *Streptococcus pneumoniae*. C-reactive protein was later found to react with many other substances such as DNA, nucleotides, various lipids, and other polysaccharides. Thus, it appears to serve as a general scavenger molecule resulting it in being classified as an acute phase reactant/protein. C-reactive protein was the first acute phase reactant/protein to be discovered, and, as already discussed, this protein shows the most dramatic increases in concentration during acute phase reactions.

By electrophoresis, C-reactive protein is a γ-migrating protein that may form a distinct monoclonal-appearing band in patients having a severe inflammatory response.

C-reactive protein is sometimes used as a rapid test for presumptive diagnosis of bacterial infection (high CRP) versus viral infection (low CRP). C-reactive protein is often used by rheumatologists to monitor the progression or remission of autoimmune disease. There has also been recent interest in the use of C-reactive protein in conjunction with serum lipids for the identification of individuals at risk for cardiovascular events. This use of C-reactive protein is presumably due to the role that inflammation plays in atherogenesis. Persons with high normal CRP are at greater risk for stroke or myocardial infarction than those with low normal values.