Interpretive Guide to Clinical Electrophoresis

Important Normal and Abnormal Patterns

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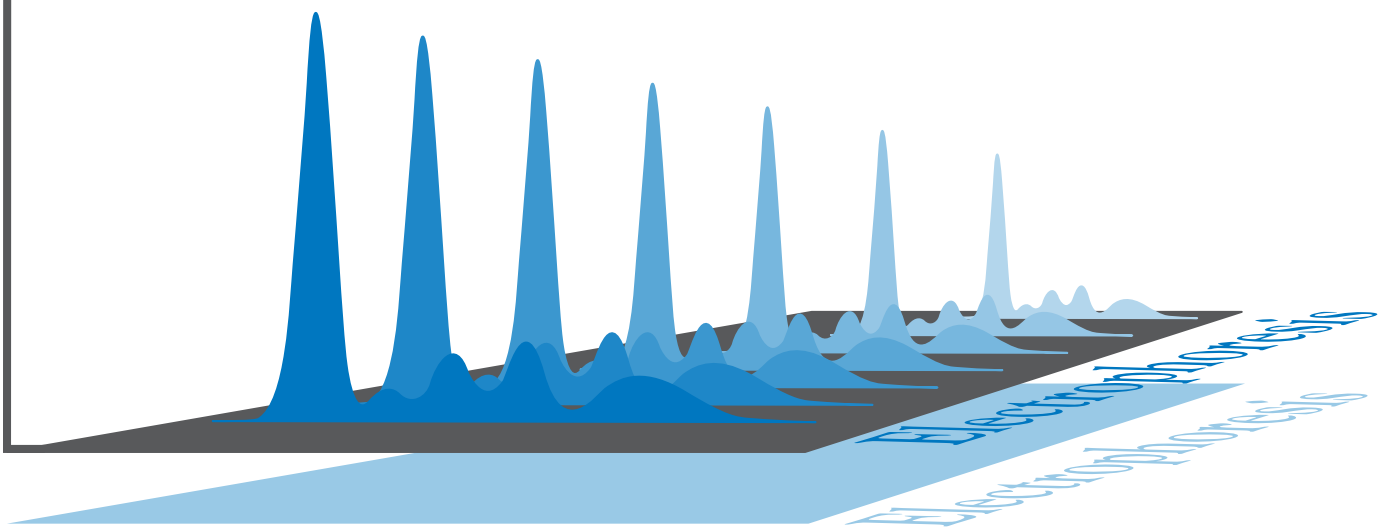

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Electrophoresis

Electrophoresis is a method for the physical separation of molecules based on their ionic charge. First used for analysis of proteins in serum and other body fluids, this technique has been adapted for other analytes, including isoenzymes and hemoglobins.

The Principles of Electrophoresis

In fluid, protein molecules (and other analytes) acquire electrical charges. When placed in an electrical field, these charged molecules are displaced. The direction of migration depends on the pH of the buffer and the isoelectric point of the protein. The degree of displacement depends primarily on the electrical charge of the protein and other factors such as potential difference, molecular weight and size, ionic strength of the buffer and the type of support media. Therefore, proteins, measured in totality by chemistry methods, can be separated into several components according to their electrophoretic mobility.

Our study is limited to results obtained by cellulose acetate electrophoresis. The patterns illustrate important pathological conditions.

Contents

1 Protein Electrophoresis

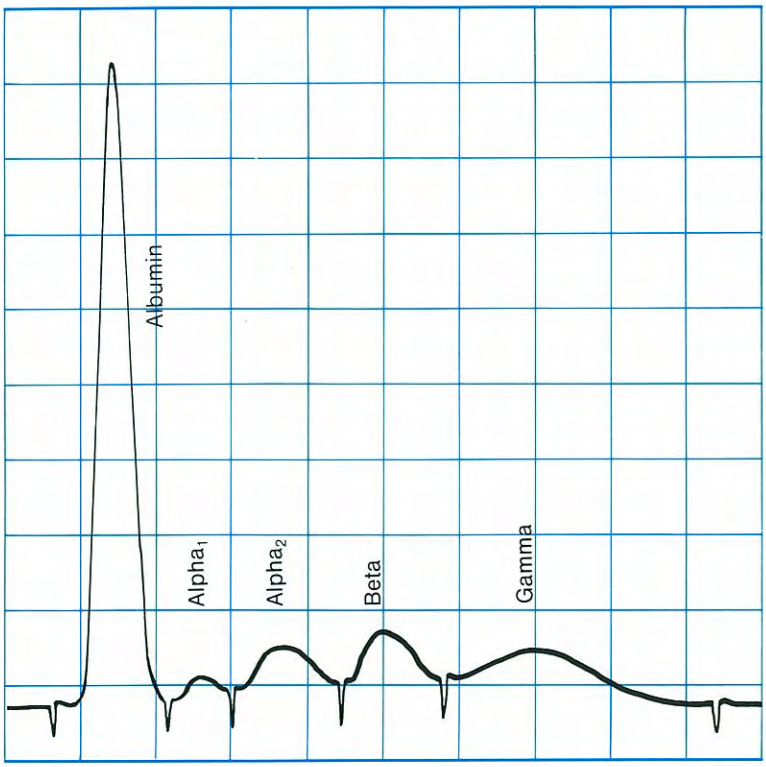
1/1 Serum Protein Electrophoresis

Electrophoresis of serum proteins is a commonly used clinical test. It provides considerable diagnostic information, especially when supplemented by complimentary tests like immunoelectrophoresis, quantitation of immunoglobulins and other specific proteins, T and B lymphocytes and lymphoblast transformation studies.

Principle

Ionized proteins, when placed in an electrical field, move toward the oppositely charged electrode. The relative migration depends on the protein. Using cellulose acetate, serum proteins can be separated into six fractions: albumin and five globulin groups — alpha 1, alpha 2, beta (often subdivided into two distinct groups – beta 1 and beta 2) and gamma.

Electrophoretic separation permits study of the biological and physical characteristics of proteins.



Normal Pattern

Total Protein	6.0 – 8.0 g/dL

Fraction	Reference (g/dL)	Reference (%)
Albumin	3.2 – 5.0	58 ± 5
Alpha 1 Globulins	0.1 – 0.4	3 ± 1.5
Alpha 2 Globulins	0.6 – 1.0	9 ± 3
Beta Globulins	0.6 – 1.3	14 ± 3
Gamma Globulins	0.7 – 1.5	16 ± 4

The characteristic appearance of the albumin fraction depends on its biochemical homogeneity. Albumin is a serum protein with a molecular weight around 70,000 daltons and an isoelectric point of 4.7. Its low molecular weight confers on it the property of migrating most anodally. Albumin forms complexes with many proteins, permitting transport of calcium and biliary pigments. Albumin plays a major role in maintaining blood volume and osmotic pressure.

Electrophoresis of serum proteins separates four groups of globulins with molecular weights much greater than albumin. Alpha and beta globulins are also transport proteins. They combine with pigments, metals, carbohydrates, and lipids. These proteins are very heterogeneous; their molecular weights range from 40,000 to over 1,000,000 daltons.

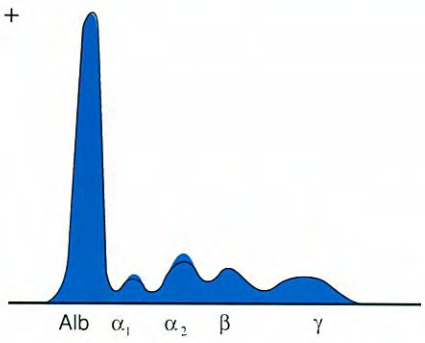
Gamma globulins, or immunoglobulins, have molecular weights from approximately 150,000 daltons to more than 1,000,000 for IgM. Immunoglobulins play an essential role in the immune defense system, providing antibodies needed for immune response to bacteria, viruses and toxins.

Electrophoretic Characteristics of Serum Proteins in Certain Clinical Conditions

	Total Protein	Albumin	Alpha 1	Alpha 2	Beta	Gamma
Acute Inflammation		↓N	↑	↑		N↓
Subacute Inflammation	N	N↓	N	↑	N	N
Chronic Inflammation		↓N	↑	↑	N↑	↑
Severe Hepatitis	↓N	↓↓	↓	↓	↓	↓
Chronic Cirrhosis	↓N or ↑	↓↓		↓	↓	↑
Acute Cirrhosis	↓N or ↑	↓↓		↓	Beta-Gamma Bridge	
Nephrotic Syndrome	↓↓	↓↓		↑↑		N↓
Hypogammaglobulinemia						↓↓↓
Paraprotein	N or ↑	↓	↓	↓	Homogeneous Peak	
Hypergammaglobulinemia	↑N	↓				↑
Hypoproteinemia (Protein Loss)	↓↓	↓↓	N↑	N↑	↓	↓N or ↑
Alpha 1 Antitrypsin Deficiency			↓↓			

↓: decrease ↑: increase N: normal

1 — Acute Inflammation



■ Abnormal
□ Normal

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).

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 alpha 1 and alpha 2 globulins and fibrinogen
— leukocytosis, following phagocytic activity

— increased level of acute phase proteins, such as alpha 1 antitrypsin, orosomucoid, haptoglobin, C reactive protein, alpha 2 macroglobulin and ceruloplasmin

Increased acute phase proteins are seen in 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.)

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. When recovery begins, there is a characteristic decrease, followed by a return to normal of the alpha 1 globulins (orosomucoids and alpha 1 antitrypsin), complement (beta 1A-beta 1C globulin) and albumin. The beginning of the immune response is marked by a slight, generally selective, increase in gamma globulins (particularly IgA). Alpha 2 globulins may remain more or less elevated (particularly haptoglobin). C reactive protein disappears or remains at a low level.

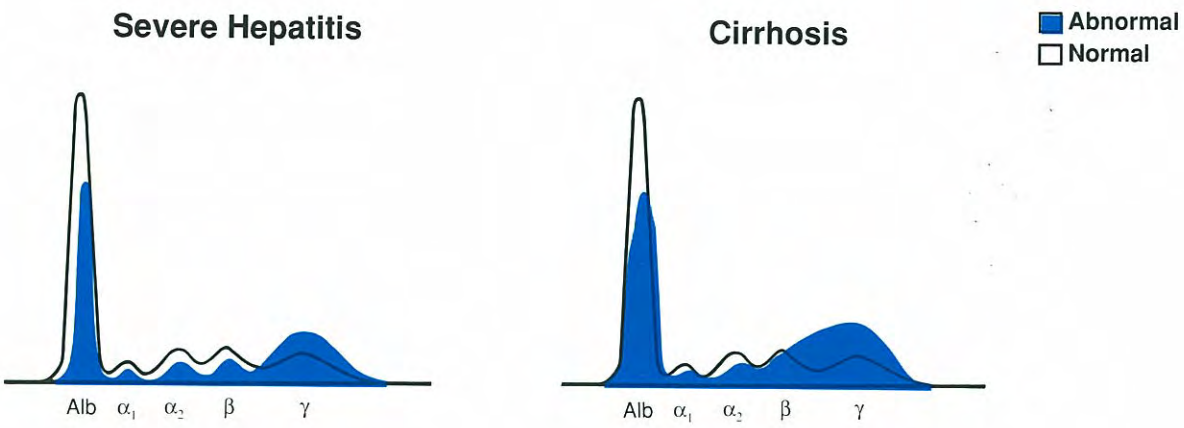
2 — Chronic Inflammation

Chronic inflammatory conditions are associated with increases of certain proteins referred to as "chronic phase proteins". Electrophoretically, this response is seen as a slight to moderate increase in the alpha 2 globulins and slight increase in the beta globulins (primarily due to complement). Albumin may be 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

3 — Liver Disease

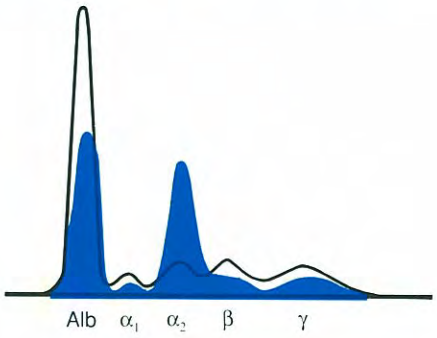


Since the liver is the site of albumin and alpha globulin synthesis, diseases affecting this organ can be expected to affect in vivo levels of albumin and alpha globulin. The liver, however, has considerable reserve synthesis capability, and decreased albumin levels are seen only in advanced hepatocellular disease.

Below are major hepatic problems and the associated serum protein electrophoresis patterns:

- — acute viral hepatitis: increased levels of IgG and IgM
- — chronic liver disease (including cirrhosis): marked increased of IgG, IgM and IgA with a decrease of albumin and transferrin
- — biliary destruction: increased levels of C_4 and beta lipoprotein.

4 — Nephrotic Syndrome



The loss of large quantities of albumin from the kidneys is consistent with nephrotic syndrome. This syndrome can be caused by diabetes mellitus, connective tissue disease, glomerular disease and circulatory disease. It is characterized by:

- — hypoproteinemia
- — hypoalbuminemia
- — edema
- — hyperlipidemia
- — proteinuria

Albumin and other low molecular weight proteins (transferrin and alpha 1 antitrypsin) are lost through the glomerular tubules. There is an associated increase in certain large molecular weight proteins (macroglobulin, IgM, lipoproteins). The electrophoresis pattern may be mimicked by acute inflammatory conditions associated with increased alpha 1 and alpha 2 globulins.

5 — Hypogammaglobulinemia and Agammaglobulinemia

Hypogammaglobulinemias are characterized by decreased amounts of most or all immunoglobulins. The majority of deficiencies are hereditary and manifest in infancy (Wiskott-Aldrich syndrome, Bruton's disease, ataxia telangiectasia).

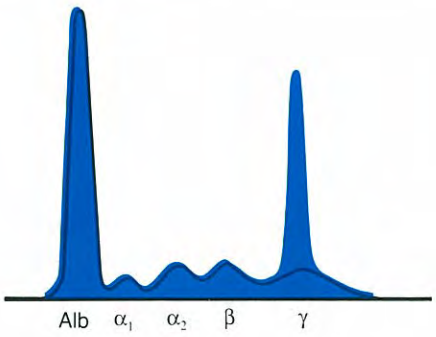
Immunoglobulin deficiencies acquired in adulthood can be secondary to disease states, such as monoclonal gammopathies, or induced by immunosuppresive therapy. Bence Jones proteins are frequently found in adults with hypogammaglobulinemia. Immunoelectrophoresis or immunofixation electrophoresis (IFE) should be performed to detect and identify monoclonal gammopathies.

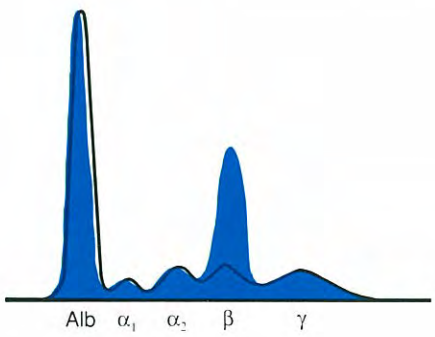
Decreases can also involve selective immunoglobulin classes like IgA, sub-classes (IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, IgA_2), or selective light chain deficiency of kappa or lambda type.

6 — Monoclonal and Biclonal Gammopathies

Monoclonal Gammopathies

Monoclonal gammopathies represent disorders of immunoglobulin synthesis associated with proliferation of clones of B lymphocytes. Electrophoresis shows one homogeneous peak (corresponding to the paraprotein) in the beta-gamma area (sometimes in the alpha 2 area), and is generally associated with decreases in normal immunoglobulins. These homogeneous paraproteins are formed from a single type of heavy chain that filters through the glomerular tubules and forms Bence Jones proteins (free light chains).

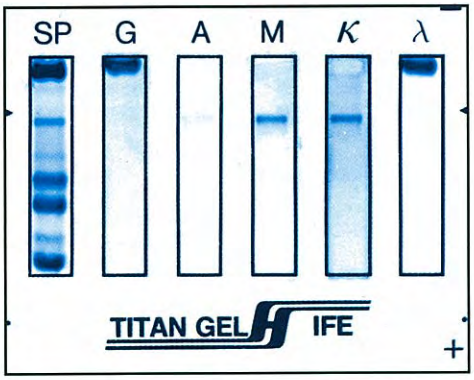




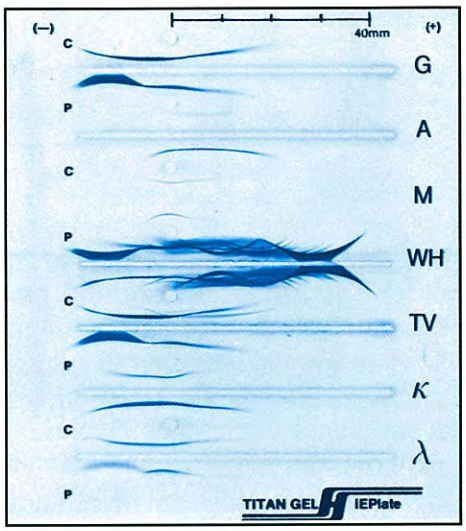
In a study of patients over the age of 70, only 8% of serum or urine monoclonal gammopathies were pathological. The majority of these monoclonal gammopathies remained benign. High monoclonal protein levels and decreased levels of other immunoglobulins may be associated with a malignancy.

Biclonal Gammopathies

Occasionally, the presence of two (and very rarely three) monoclonal proteins can be observed. Observance of these paraproteins by electrophoresis is not sufficient for complete identification. Immunoelectrophoresis (IEP) or the newer technique of immunofixation (IFE) must be performed. Immunofixation electrophoresis consists of agarose gel electrophoresis followed by immunoprecipitation by direct application of specific antisera.



Immunofixation: Gammopathy with IgG Lambda and IgM Kappa monoclonal bands



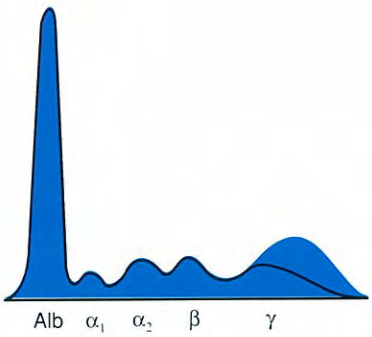
Immunoelectrophoresis: Gammopathy with IgG Lambda and IgM Kappa monoclonal bands

7 — Polyclonal Gammopathies

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

After hypoalbuminemia, polyclonal gammopathy is the most common protein abnormality 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 gamma fraction. Polyclonal gammopathies are seen in a number of disorders including:

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

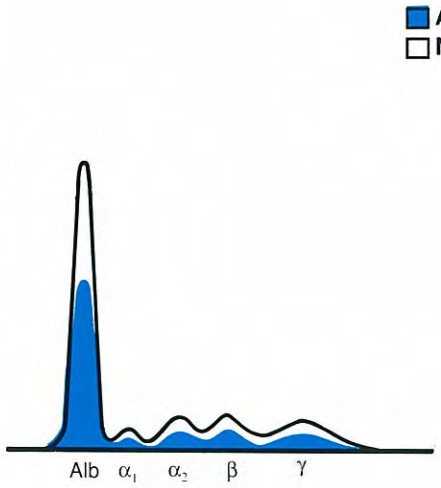


8 — Protein Losing Gastroenteropathies

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.

Protein losing enteropathies are generally secondary to pathophysiological conditions (such as those related to lymphatic abnormalities, e.g. secondary to increased blood pressure in patients with constrictive pericarditis) or to congenital abnormalities (as in primary intestinal lymphangiectasis). Decreased serum albumin may also be secondary to mucosal disease or from direct loss of serum into the intestines, as in inflammatory bowel disease.

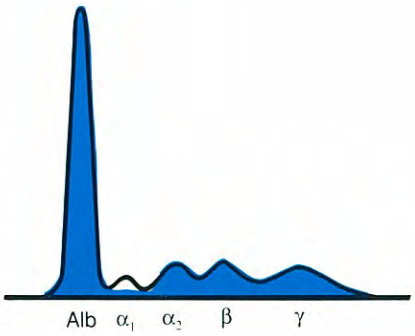
Electrophoresis results are best when supplemented by complementary investigations, like IEP or IFE when a monoclonal or biclonal gammopathy is present. Quantitation of specific immunoglobulins is also recommended. A variety of techniques are used: radial immunodiffusion, Laurell electroimmunodiffusion, nephelometry, etc. Protein quantitation permits establishment of "a protein profile" that can be associated with certain pathologic conditions.



9 — Decreased Levels of Alpha 1 Antitrypsin

Congenital Alpha 1 Antitrypsin Deficiency

Approximately 95% of all individuals have a serum alpha 1 antitrypsin concentration of 200 to 400 mg/dL. Certain hereditary abnormalities involve deficiencies in the synthesis of this globulin. In heterozygous individuals, alpha 1 antitrypsin levels are decreased to 30 - 50% of normal levels. In homozygous individuals, the alpha 1 antitrypsin level varies according to ethnic group and can be decreased as much as 80 to 90%. Homozygous individuals are predisposed to pulmonary emphysema.



Acquired Alpha 1 Antitrypsin Deficiencies

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

Alpha 1 antitrypsin phenotyping is essential for precise diagnosis of alpha 1 antitrypsin deficiencies. Phenotyping can be done by two dimensional immunoelectrophoresis or by isoelectrofocusing in polyacrylamide gel with comparison to a reference of known phenotype.

Immunoglobulin Levels in Various Disorders							
	IgG	IgA	IgM		IgG	IgA	IgM
Agammaglobulinemia	↓	↓	↓	Chronic Lymphocytic Leukemia	↓	↓	↓
Lupus Erythematous	↑	↑	↑	Acute Myelocytic Leukemia	N	N	N
Rheumatoid Arthritis	↑	↑	↑	Chronic Myelocytic Leukemia	N	↓	N
Lymphoid Aplasia	↓	↓	↓	Hodgkin's Disease	N	N	N
Selective IgG-IgA Deficiency	↓	↓	N	Laennec's Cirrhosis	↑	↑	N
Selective IgA-IgM Deficiency	N	↓	↓	Biliary Cirrhosis	N	N	↑
Anti IgA Globulinemia	N	↓	N	Acute Hepatitis	↑	↑	↑
Ataxia Telangiectasia	N	↓	N	Hepatocellular Carcinoma	N	N	↓
IgG Myeloma	↑	↓	↓	Pulmonary Tuberculosis	↑	N	N
IgA Myeloma	↓	↑	↓	Trypanosomiasis	↓	↓	↑↑↑
Waldenström's Macro-globulinemia and IgM Myeloma	↓ or N	↓ or N	↑	Gastrointestinal Protein Loss	↓	↓	↓
Acute Lymphocytic Leukemia	↓	↓	↓	Nephrotic Syndrome	↓	↓	↓

↓: decreased ↑: increased N: normal

1/2 Urine Proteins

Urinary proteins are derived primarily from plasma proteins that filter through the kidney. Normally, a system of "sieving" exists. Proteins of large molecular mass are retained as they pass through the glomerulus, but proteins of smaller molecular mass pass freely. Reabsorption or reverse filtration through the renal tubules removes all but trace amounts of these smaller molecular mass proteins from the final urine output. The small quantity of plasma protein that is excreted in normal urine amounts to about 50 to 150 mg over a 24 hour period.

The appearance of abnormal plasma proteins in the urine is of great value in evaluating renal function. With exception of conditions like the postural or orthostatic proteinuria of adolescence and certain intermittent proteinurias, permanent proteinuria is always pathological. Proteinuria can be associated with poor tubular reabsorption, but most often results from problems with permeability of the glomerular capillaries.

Appropriate study of proteinuria should include quantitative (for confirming when the threshold physiological value is exceeded) and qualitative (to verify the type of plasma protein in the urine) assessment of urine excretion. Proteinuria can be expressed in terms of the total protein excreted over a 24 hour period (g/24 hrs) and the relative protein concentration (g/L). Electrophoresis on cellulose acetate is a simple, but effective method for differentiating the types of proteinuria. It is necessary to concentrate the urine according to the total amount of protein present in the sample. Polyacrylamide gel electrophoresis permits excellent separation of the different protein fractions and with the addition of sodium dodecyl sulfate it is not necessary to concentrate the urine prior to testing.

As a guideline to the mobility of the different fractions, electrophoresis of urine proteins should be compared to that of normal serum. The results of electrophoresis or other clinical findings can be confirmed by using additional complementary tests. Urine electrophoresis, accompanied by immunoelectrophoresis of the same urine concentrate, can identify abnormal urinary globulins.

To rule out sources of error (blood, bacteria, pus, etc) microscopic examination of the urine should accompany electrophoretic testing. A number of metabolites are excreted in urine and, after concentration of the specimen, organic and mineral residues can be found in large enough quantities to impair or prevent the interpretation of electrophoretic results.

Recognizing interfering factors involves four steps before proceeding with urine electrophoresis:

1) microscopic examination
2) filtration or centrifugation
3) quantitation of urinary proteins by precipitation with strong acid (trichloroacetic or sulfosalicylic) followed by nephelometry
4) concentration of the urine to a protein level between 30 and 60 g/L.

The last step poses a number of problems. No method of concentration is selective only for proteins. Therefore, for the reasons previously outlined, it is necessary to also use dialysis to remove undesirable mineral salts, biliary salts and pigments. At the present time, several commercial systems for dialysis and concentration exist (i.e. Centricon ultrafiltration membranes). These commercial systems are not perfect, but they do permit passage of the larger molecular mass proteins. In clinical practice, this technique permits detection of the principal pathological abnormalities.

Results

Electrophoresis allows differentiation of several types of proteinuria:

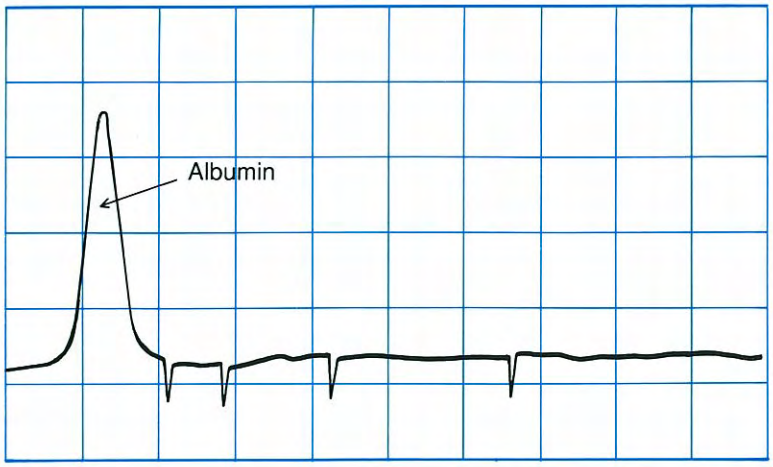
— **Physiological Proteinuria:** albumin isn't very pronounced and globulins follow an indifferent course.

— **Glomerular Proteinuria:**
  - **Selective:** the urine consists primarily of albumin (greater than 80%) and transferrin. This pattern is characteristic of nephrotic syndrome.
  - **Non-Selective:** urinary electrophoresis pattern is similar to that of serum. This pattern appears in primary and secondary glomerulonephropathies. (Glomerulonephropathy can be secondary to diseases such as diabetes, amyloidosis, dysglobulinemia, and collagen diseases).

— **Tubular Proteinuria:** albumin is not very pronounced; alpha 1, alpha 2, beta and gamma globulins dominate. This pattern is most often observed in four conditions: congenital tubular nephropathies, interstitial tubular nephritis, chronic pyelonephritis and polycystic kidneys.

— **Proteinurias Associated with Dysglobulinemias:** one or more bands in the gamma region due to intact immunoglobulin molecules, free light chains (most common), or heavy chains. Kappa light chains are seen twice as often as lambda light chains. In IgG or IgA monoclonal dysglobulinemia (non-myeloma), proteinuria is characterized by an albumin peak and an immunoglobulin peak. In Waldenström's macroglobulinemia, proteinuria can be physiological, consisting of free light chains (Bence Jones proteins).

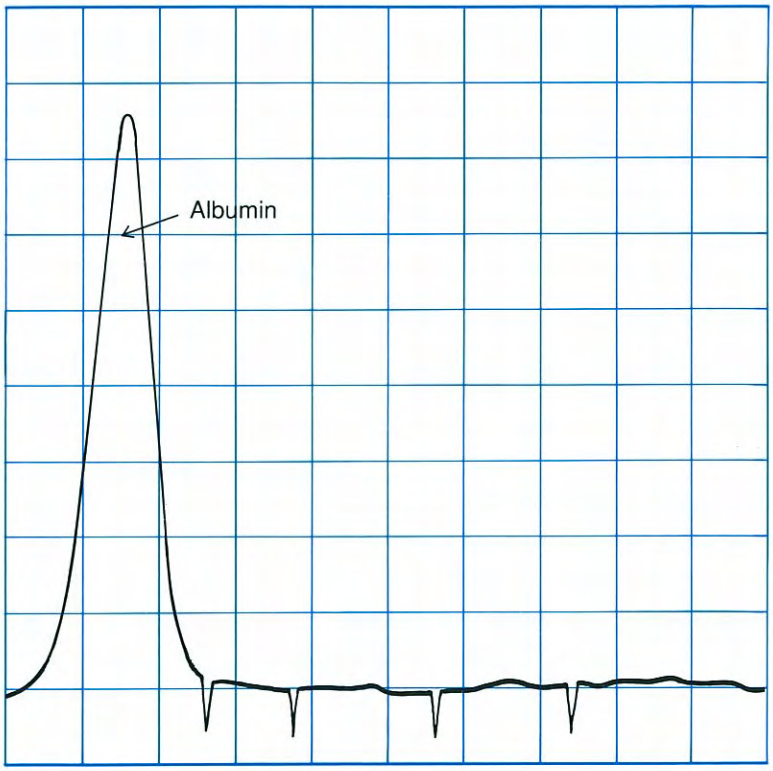
Normal Urine Protein Patterns



Urine

Protein: 65 mg/24 hrs

For electrophoresis, the urine was concentrated 50x to a relative concentration of 3.25 g/24 hrs.



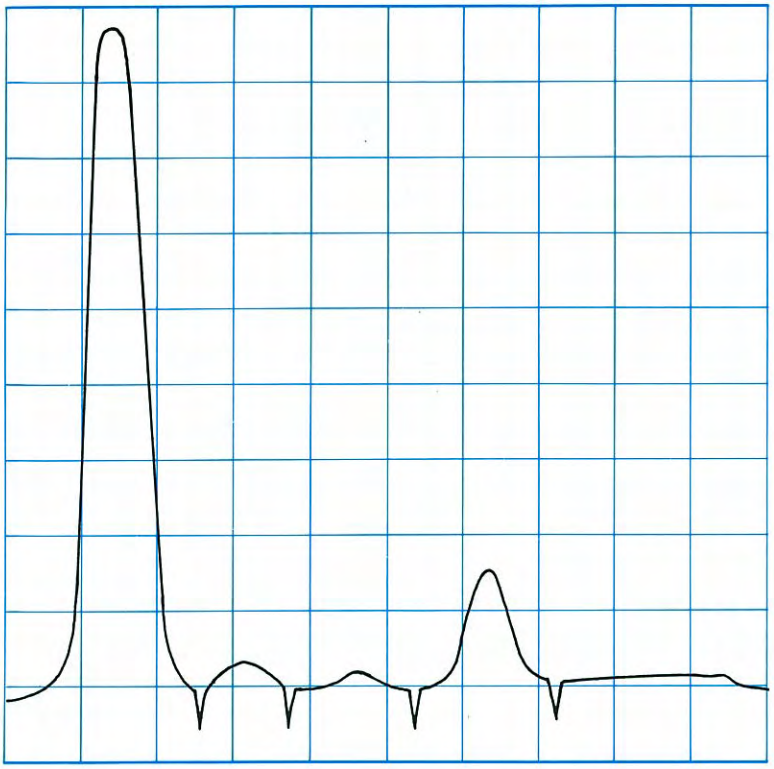
Urine

Protein: 150 mg/24 hrs

For electrophoresis, the urine was concentrated 50x to a relative concentration of 7 g/24 hrs.

Several Examples of Pathological Patterns

Acute Glomerular Nephropathy



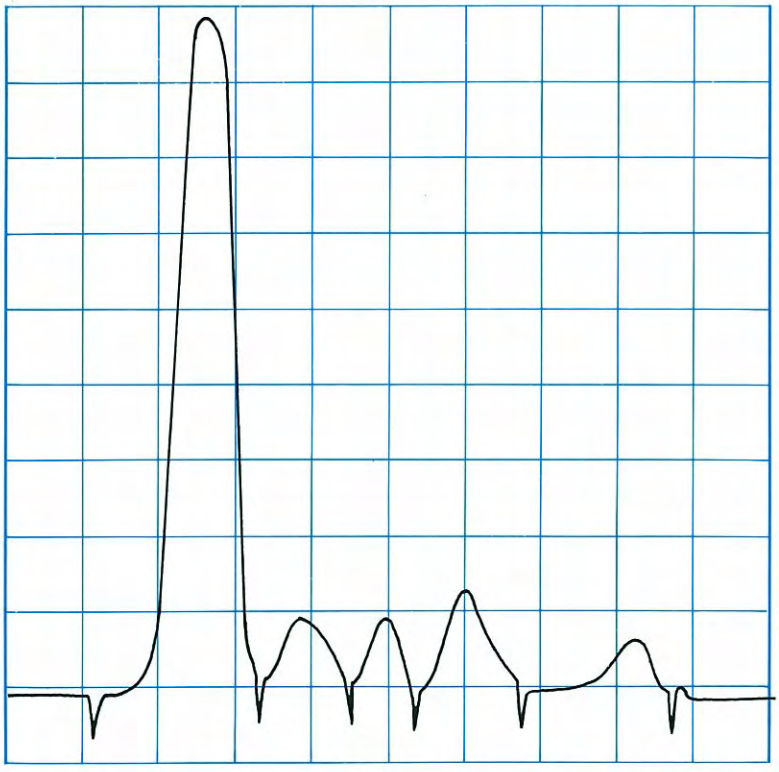
Urine

Protein: 1.60 g/24 hrs

For electrophoresis, the urine was concentrated 20x to a relative concentration of 32 g/24 hrs.

Remarks: Note the dominant albumin peak, the beta globulin (transferrin) peak and the flat gamma globulin peak.

Tubular Proteinuria



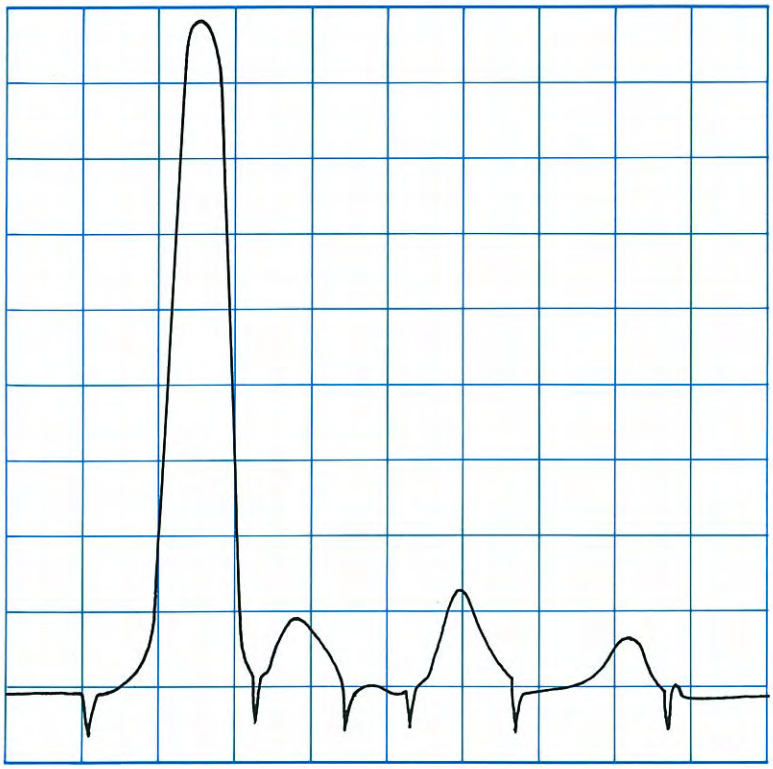
Urine

Total Protein: 1.80 g/24 hrs

For electrophoresis, the urine was concentrated 30x.

IgG Kappa* Multiple Myeloma

*The nature of the paraprotein was determined by immunoelectrophoresis.

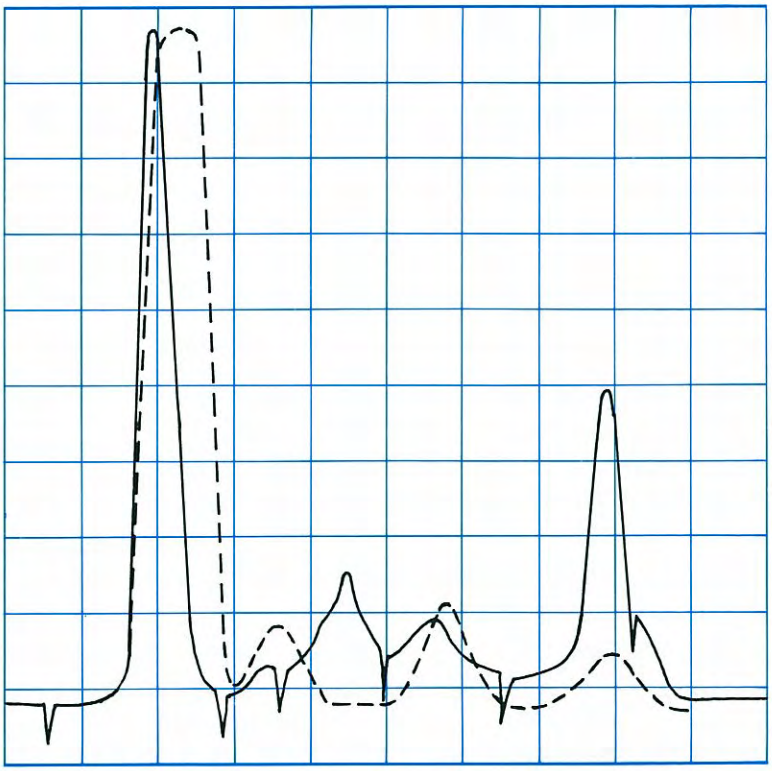


Urine

Protein: 3.2 g/24 hrs

For electrophoresis the urine was concentrated 10x.

Remarks: The large increase in the beta globulin fraction correlates to the presence of free kappa light chains (Bence Jones proteins), an indicator of renal insufficiency.



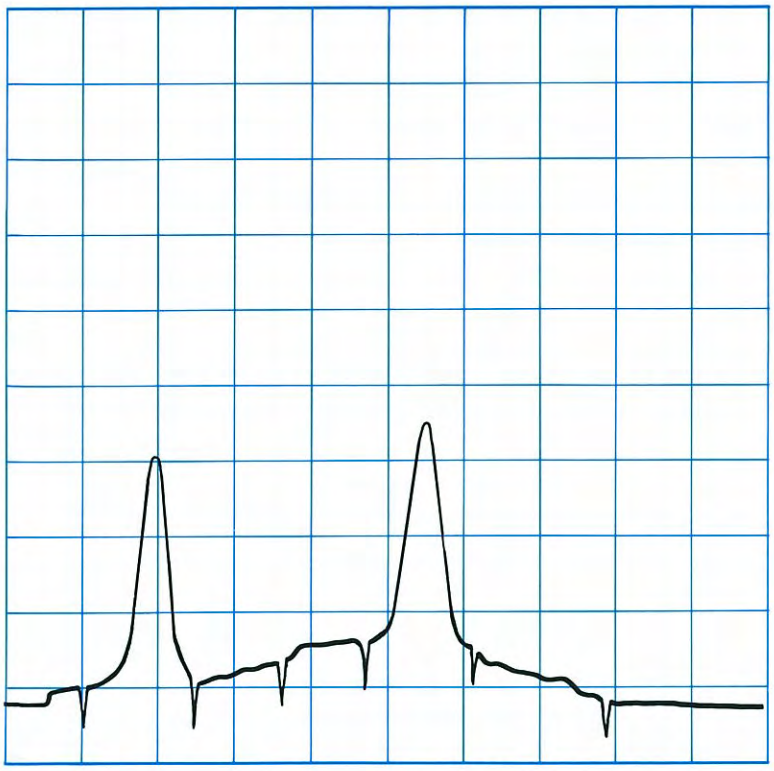
Serum

Total Protein: 88 g/L

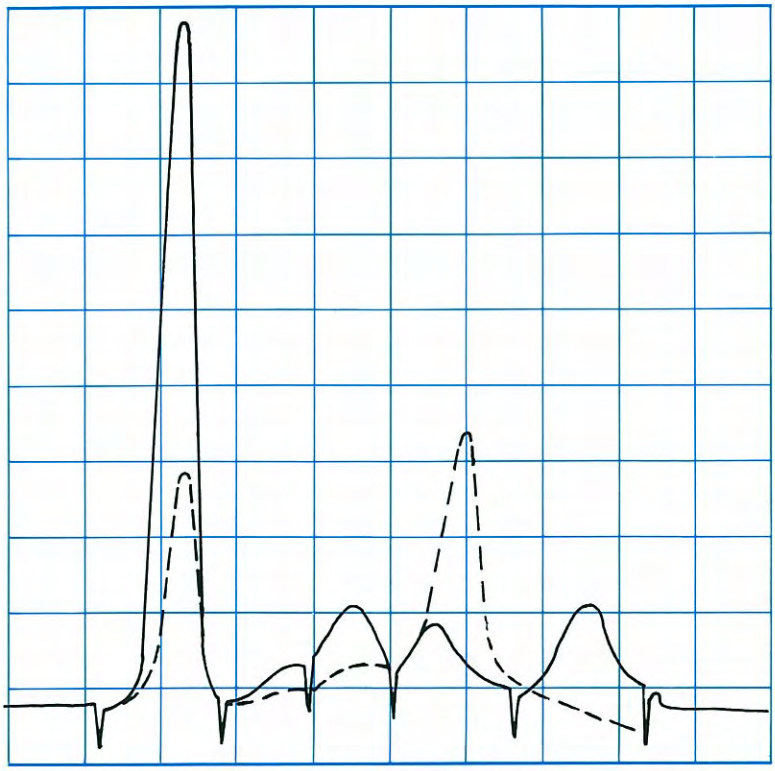
The urine pattern (represented by the dashed line) is superimposed on the serum protein pattern.

IgG Lambda* Myeloma with Bence Jones Protein

*The nature of the paraprotein was determined by immunoelectrophoresis.



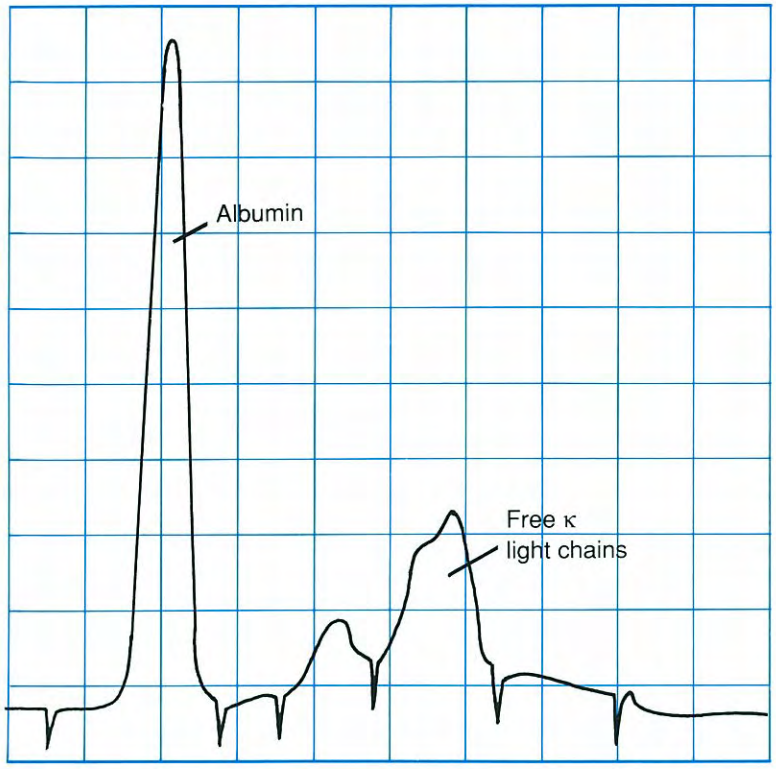
Urine
Protein: 3.9 g/24 hrs



Serum
Total Protein: 68 g/L
The urine pattern (represented by the dashed line) is superimposed on the serum protein pattern.

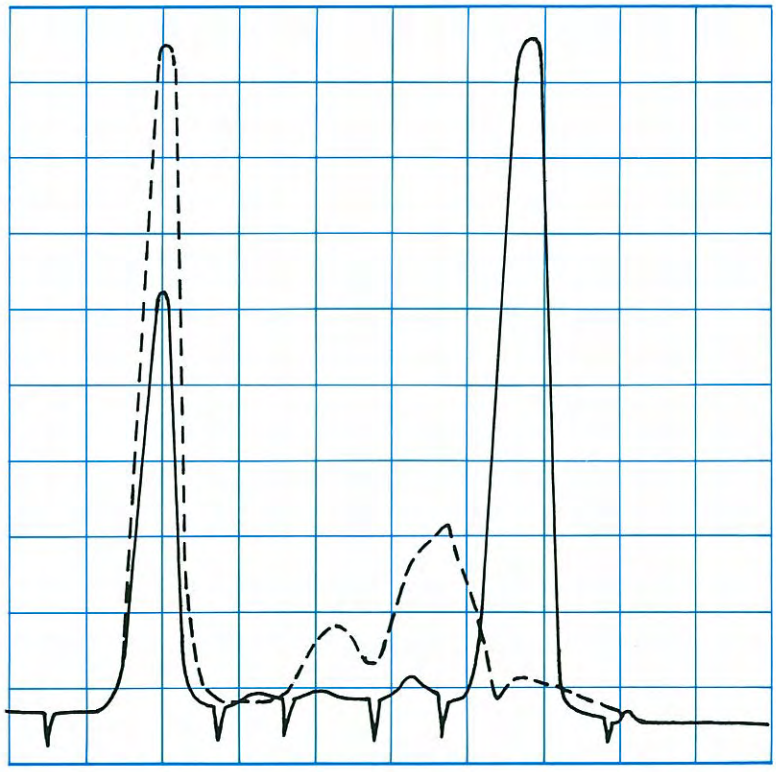
IgA Kappa* Myeloma with Bence Jones Protein

*The nature of the paraprotein was determined by immunoelectrophoresis.



Urine

Protein: 3.7 g/24 hrs



Serum

Total Protein: 110 g/L

The urine pattern (represented by the dashed line) is superimposed on the serum protein pattern.

Remarks: There is a Coomassie blue staining technique for agarose gel electrophoresis that permits visualization of unconcentrated urine at levels above 1 g of total protein.

1/3 Cerebrospinal Fluid Proteins

Cerebrospinal fluid (CSF) circulates along the central nervous system through the brain and spinal cord. CSF is produced by ultrafiltration of plasma in the choroid plexuses of the lateral, third and fourth ventricles of the brain. The volume of CSF varies with age, and in the adult reaches 140 ± 30 mL. CSF is "renewed" approximately four times per day by continuous excretion and reabsorption across the blood-CSF barrier, producing a total daily volume of 500 to 700 mL. (Reference ranges vary according to the method of measurement.)

About 95% of the protein found in CSF results from active transport of plasma proteins at the blood-CSF barrier. A small portion of CSF protein is a result of localized synthesis. Changes in the permeability of the blood-CSF barrier due to mechanical compression, extensive lesions, vascular damage or infection can result in an accumulation of protein in the CSF. The ratio of the concentration of plasma and spinal fluid proteins can be used to estimate the permeability of the blood-CSF barrier.

The study of CSF proteins should always be accompanied by that of serum in order to establish an immunological profile. This profile constitutes an important diagnostic aid for the immunopathological processes that affect the central nervous system. Protein ratios can provide important information in determining if the increase in CSF protein is due to permeability changes or to abnormal local synthesis.

Minimum requirements for the study of CSF proteins should include:

1) Quantitation of Total CSF Protein – reference range for adults is 0.40 ± 0.12 g/L (Lowry method)
2) Cellulose acetate or agarose gel electrophoresis after concentration of CSF proteins. (Polyacrylamide gel electrophoresis is also used.) Interpretation of the electrophoresis pattern is quantitative and qualitative. Seven fractions can be seen in normal CSF. The appearance of the gamma globulin fraction is of considerable importance:
   - polyclonal gamma – occurs in normal CSF, but doesn't exclude the possibility of a pathological condition
   - monoclonal peak – occurs as a result of dysglobulinemia (can be characterized by immunoelectrophoresis of the serum proteins)
   - oligoclonal bands – the presence of 2 to 5 protein fractions in the gamma region
3) Immunochemical quantitation of IgG and albumin in the CSF and serum. (In adults, the concentration of CSF albumin is equivalent to about 240 mg/L, and that of IgG is 20 – 40 mg/L.)

Altogether these results permit classification of CSF proteins into five categories: normal, inflammatory, non-inflammatory transudative, inflammatory transudative, meningitis.

Calculation of the Different Ratios

For interpreting the variations in the levels of CSF immunoglobulins, it is necessary to assess the state of the blood-brain barrier.

Ratios are calculated as follows:

Ratio 1: $\frac{\text{CSF Albumin}}{\text{Serum Albumin}} \times 100$ (N < 0.65%)

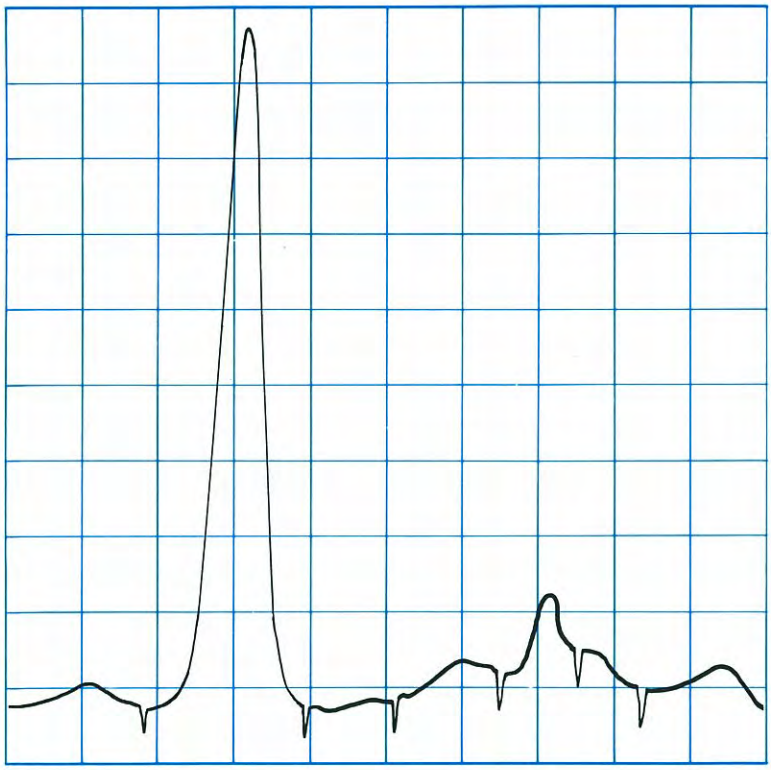
Ratio 2: $\frac{\text{CSF IgG}}{\text{CSF Albumin}} \times 100$ (N < 24%)

The ratio of IgG/Albumin varies widely among normal subjects, thus the broad reference ranges.

Ratio 3: $\frac{\text{CSF IgG/CSF Albumin}}{\text{Serum IgG/Serum Albumin}}$ (N < 0.85)

The IgG index is probably the most convincing criteria for determining if there is abnormal synthesis of IgG from within the CNS or if the increase is due to altered permeability of the blood-CSF barrier. (However, the IgG index may be normal in subjects having CSF transudate simultaneous to a large elevation of serum IgG or a decrease of serum albumin.) In determining local synthesis of IgG, it is necessary to take into consideration that the transudate of serum IgG is equal to 2/3 that of albumin.

Normal Electrophoretic Pattern



CSF

Total Protein: 0.40 g/L (average)
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

Fraction	%Normal
Prealbumin	4 ± 1
Albumin	56 ± 4
Alpha 1	5 ± 1
Alpha 2	7 ± 1
Beta 1	12 ± 2
Beta 2	6 ± 1
Gamma	10 ± 1

Radial Immunodiffusion

CSF Albumin (N = 200 mg/L)
CSF IgG (N = 35 mg/L)
CSF IgG/CSF Alb (N < 24%)
CSF Alb/Serum Alb (N < 0.65%)
Index IgG (N < 0.85%)

The Different Fractions and Their Pathological Variations

Prealbumin

Elevated in cerebral atrophy, the ventricular origin of this protein is shown by:
— an elevated percentage in CSF of cisternal or ventricular origin
— diminished or even absent in lumbar fluid as occurs in spinal blockage

Albumin

It is considered the best "marker" of the permeability of the blood-CSF barrier. Quantitation of albumin can be used to define the type of spinal fluid, the percentage of transudate, and, in particular, the precise origin of increased CSF immunoglobulins.

Alpha Globulins

Alpha 1 and alpha 2 globulins can always be observed as two fractions (sometimes 4 or 5) between the albumin and beta globulin fractions. Elevations are frequently seen in malignant processes (such as cerebral tumors) and, very rarely, in the course of some collagen diseases where it appears as an alpha-gamma bridge.

Beta 1 Globulins

Easily identifiable, it is located equidistant between the albumin and gamma peaks. Elevated percentages of beta 1 globulin are frequently seen following meningeal hemorrhage (probably bound to beta 1 lipoproteins and to fibrinogen).

Beta 2 Globulins

Rounded in appearance, this peak is less important than the peak that precedes it, beta 1 globulin. It is hardly given any significance, although, traditionally, its elevation (a capital "M" pattern) appears in certain degenerative disorders.

Gamma Globulins

— **Quantitative:** The correlation between the absolute value obtained by electrophoresis and by immunochemical quantitation of IgG is insufficient for clinical diagnosis. The percentages of the gamma globulins (IgG) uniquely reflects whether or not an immune response is present.

— **Qualitative:** the zone can display three aspects:

- **Polyclonal:** round and regular contour. This is the normal appearance and that of immunological reactions of plasma origin (inflammatory transudates, for example). Indeed, immunological reactions involve a great number of molecules (opsonins, specific antibodies, etc.). This general involvement is reflected by a heterogeneous increase of the gamma globulins, and at this point, IgG gives this polyclonal characteristic.
- **Monoclonal:** this is particularly characteristic of myelomas, with the classic pointed peak, identical to that seen in the serum. Note that in the majority of cases, the monoclonal protein is synthesized within the nervous system by abnormal plasma cells. It is not therefore made from a transudate of plasma origin.
- **Oligoclonal:** during the course of certain immunopathological nervous system disorders, two to five gamma bands may appear in a characteristic oligoclonal fashion. This is of considerable physiopathologic and diagnostic value:

  1) **Physiopathologic:** in most cases, oligoclonal banding indicates intrathecal synthesis of IgG.

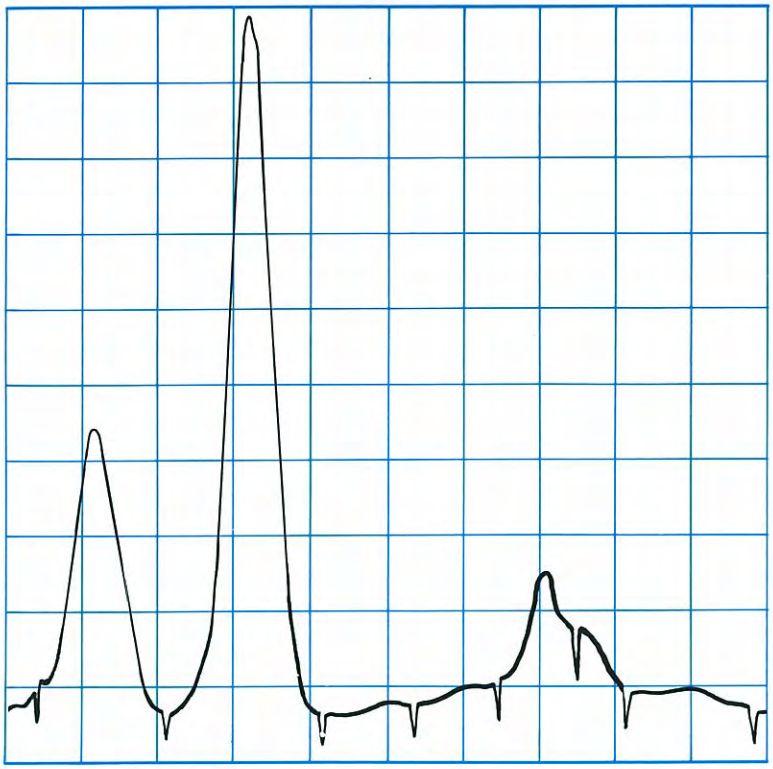
  2) **Diagnostic:** Four disorders frankly manifest this characteristic: neurosyphilis, subacute sclerosing panencephalitis, trypanosomiasis and multiple sclerosis (MS). The first three diseases can be differentiated by their particular clinical symptoms or by microbiological, parasitological or immunological (IgM for trypanosomiasis) testing. In essence, oligoclonal banding found in the CSF of a young patient is an element considerably favoring the diagnosis of MS. Generally associated with an increase in IgG, the appearance of oligoclonal bands, in otherwise normal CSF, is practically always a manifestation of MS.

     It is interesting to note that among these four diseases, only MS is not yet demonstrated with certainty to be of infectious origin. In other clinical conditions where oligoclonal bands are visibly present (meningitis, encephalitis, parasitic infections) it can be confirmed, in practically all of these cases, that the persistence of an infectious agent (bacterial, parasitic or viral), if not demonstrated, is at least strongly suspected. It is not clear if this has any bearing on the etiology of MS.

Interpretation of CSF Protein Patterns

Gamma	**Quantitative –** corresponds primarily to IgG. The percentage of gamma globulin (IgG) uniquely reflects whether or not an immune response is present. **Qualitative** 3 characteristics: — polyclonal — monoclonal — oligoclonal
Beta	Beta 1 – increased in meningeal hemorrhage (due to presence of fibrinogen and beta lipoproteins) Beta 2 – increased in some degenerative disorders
Alpha 1 **Alpha 2**	Alpha 1 antitrypsin Alpha 1 acid glycoprotein – increased due to tumorous lesions and extensive cerebral vascular damage. Alpha 2 macroglobulin – increased during infectious and inflammatory processes, in the absence of blood-CSF exchange.
Albumin	Indicator of blood-CSF barrier permeability
Prealbumin	Ventricular origin: lower levels found in lumbar fluid in spinal blockage; increased in cerebral atrophy.

Increased Prealbumin



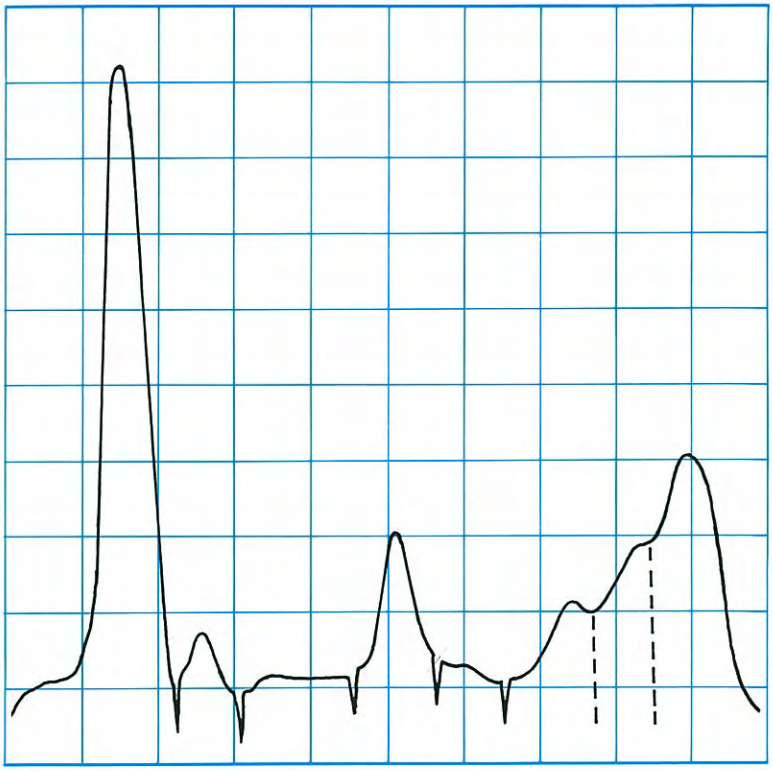
CSF
Total Protein: 0.35 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

Electrophoresis Results

Fraction	Result (%)	Reference (%)
Prealbumin	25	4 ± 1
Albumin	54	56 ± 4
Alpha 1	1	5 ± 1
Alpha 2	3	7 ± 1
Beta 1	10	12 ± 2
Beta 2	4	6 ± 1
Gamma	3	10 ± 1

Oligoclonal Banding

Multiple Sclerosis



CSF
Total Protein: 1.34 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

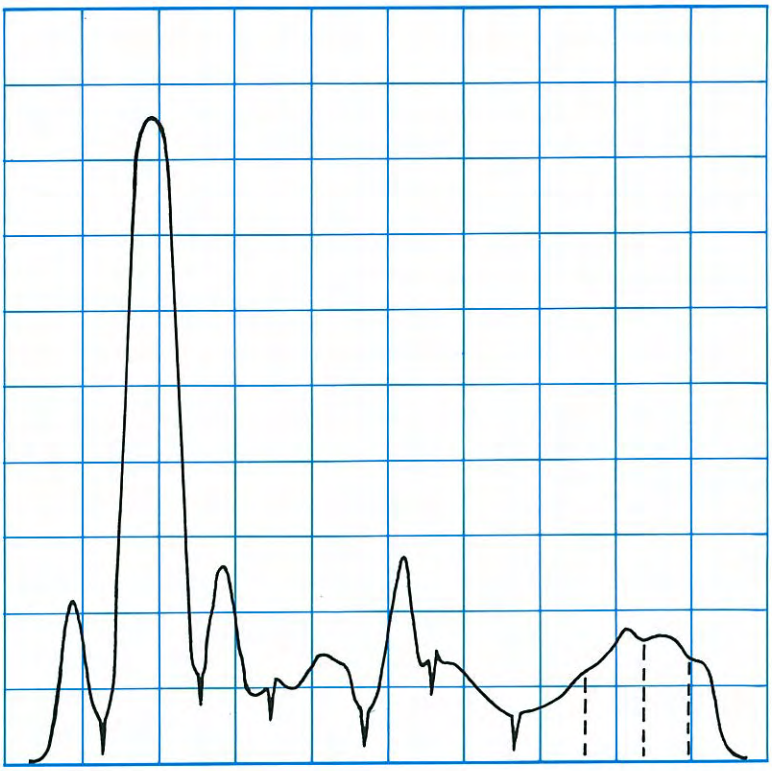
Electrophoresis Results

Fraction	Result (%)	Reference (%)
Prealbumin	4	4 ± 1
Albumin	37	56 ± 4
Alpha 1	4	5 ± 1
Alpha 2	5	7 ± 1
Beta 1	9	12 ± 2
Beta 2	4	6 ± 1
Gamma	37	10 ± 1

Radial Immunodiffusion Results

CSF Albumin	750 mg/L	(N = 200 mg/L)
CSF IgG	390 mg/L	(N = 35 mg/L)
CSF IgG/CSF Alb	52%	(N < 24%)

Neurosyphilis



CSF
Total Protein: 0.88 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

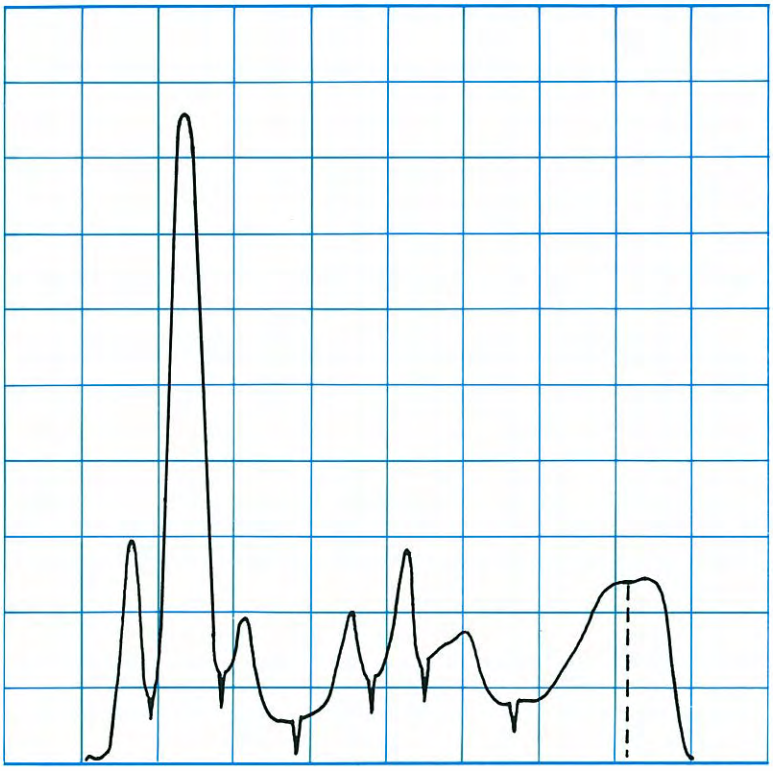
Electrophoresis Results

Fraction	Result (%)	Reference (%)
Prealbumin	6	4 ± 1
Albumin	42	56 ± 4
Alpha 1	9	5 ± 1
Alpha 2	8	7 ± 1
Beta 1	8	12 ± 2
Beta 2	7	6 ± 1
Gamma	20	10 ± 1

Radial Immunodiffusion Results

CSF Albumin	440 mg/L	(N = 200 mg/L)
CSF IgG	140 mg/L	(N = 35 mg/L)
CSF IgG/CSF Alb	32%	(N < 24%)

Neurosyphilis



CSF
Total Protein: 0.68 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

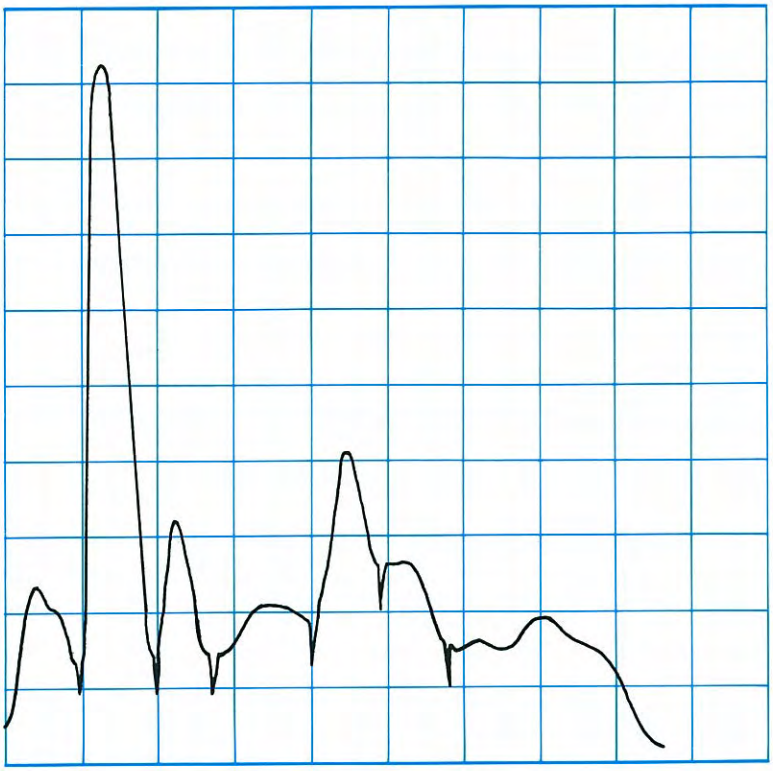
Electrophoresis Results

Fraction	Result (%)	Reference (%)
Prealbumin	6	4 ± 1
Albumin	34	56 ± 4
Alpha 1	7	5 ± 1
Alpha 2	7	7 ± 1
Beta 1	9	12 ± 2
Beta 2	10	6 ± 1
Gamma	26	10 ± 1

Radial Immunodiffusion Results

CSF Albumin	270 mg/L	(N = 200 mg/L)
CSF IgG	140 mg/L	(N = 35 mg/L)
CSF IgG/CSF Alb	52%	(N < 24%)

Meningitis due to Cytomegalovirus



CSF
Total Protein: 0.47 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

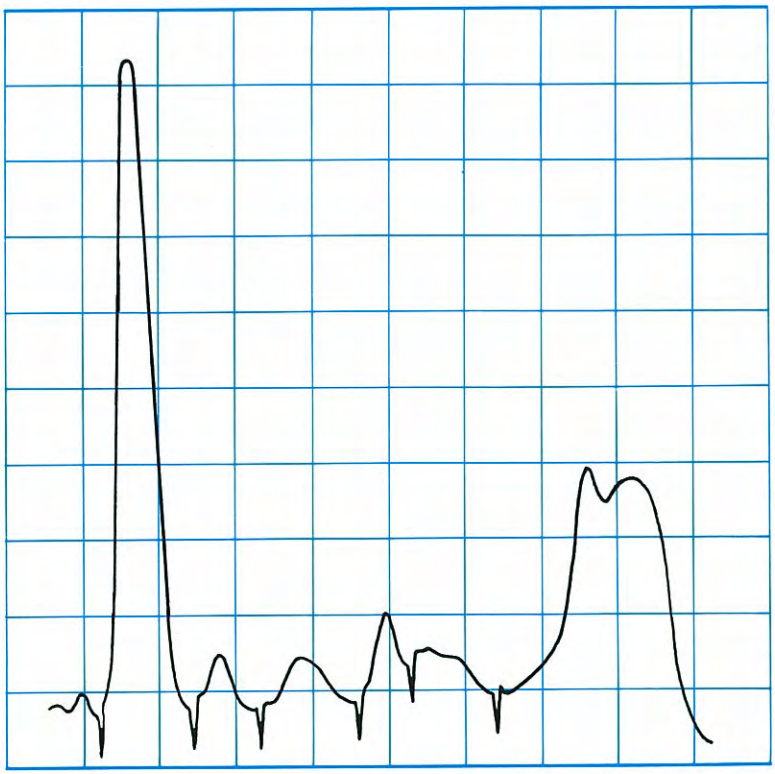
Electrophoresis Results

Fraction	Result (%)	Reference (%)
Prealbumin	9	4 ± 1
Albumin	29	56 ± 4
Alpha 1	9	5 ± 1
Alpha 2	12	7 ± 1
Beta 1	14	12 ± 2
Beta 2	10	6 ± 1
Gamma	17	10 ± 1

Radial Immunodiffusion Results

CSF Albumin	170 mg/L	(N = 200 mg/L)
CSF IgG	52 mg/L	(N = 35 mg/L)
CSF IgG/CSF Alb	30%	(N < 24%)

Cerebral Toxoplasmosis



CSF
Total Protein: 1.05 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

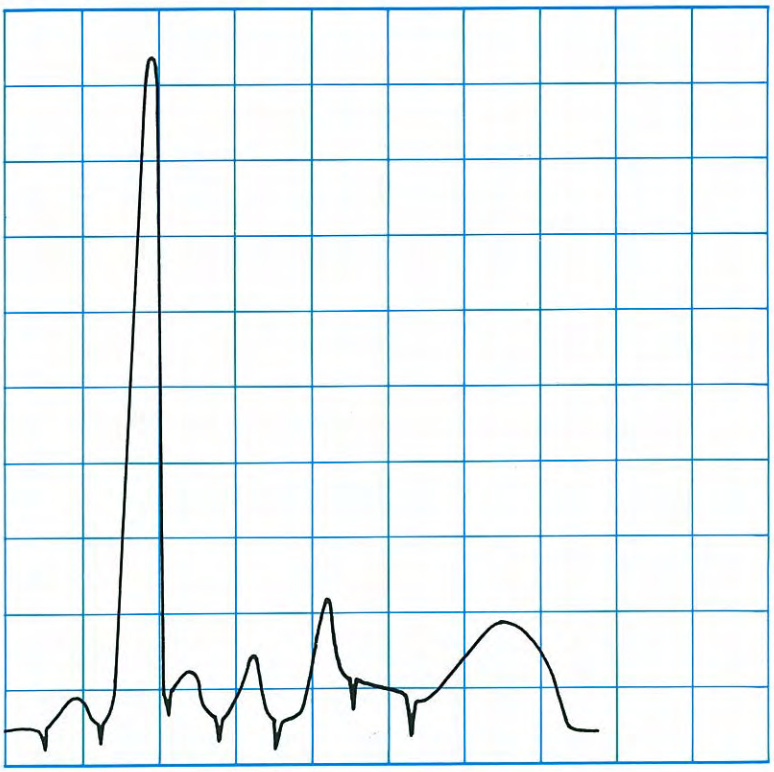
Electrophoresis Results

Fraction	Result (%)	Reference (%)
Prealbumin	3	4 ± 1
Albumin	34	56 ± 4
Alpha 1	5	5 ± 1
Alpha 2	7	7 ± 1
Beta 1	6	12 ± 2
Beta 2	8	6 ± 1
Gamma	37	10 ± 1

Radial Immunodiffusion Results

CSF Albumin	530 mg/L	(N = 200 mg/L)
CSF IgG	430 mg/L	(N = 35 mg/L)
CSF IgG/CSF Alb	81%	(N < 24%)

Polyclonal Gamma Globulin Increase



CSF
Total Protein: 1.50 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: xanthochromic

Electrophoresis Results

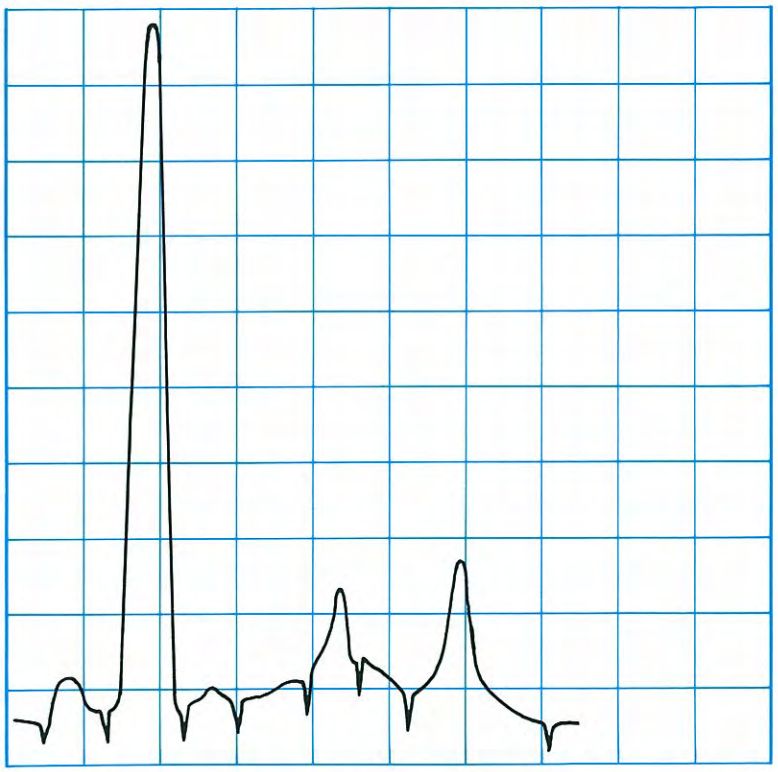
Fraction	Result (%)	Reference (%)
Prealbumin	4	4 ± 1
Albumin	39	56 ± 4
Alpha 1	4	5 ± 1
Alpha 2	7	7 ± 1
Beta 1	10	12 ± 2
Beta 2	8	6 ± 1
Gamma	28	10 ± 1

Radial Immunodiffusion Results

CSF Albumin	580 mg/L	(N = 200 mg/L)
CSF IgG	410 mg/L	(N = 35 mg/L)
CSF IgG/CSF Alb	70%	(N < 24%)

Monoclonal Peak in the Gamma Region Corresponding to an IgM Protein with Free Lambda Light Chains

(Also present in serum)



CSF
Total Protein: 0.67 g/L
(Reference range = 0.25 to 0.52 g/L)
Appearance: clear, colorless

Electrophoresis Results

Fraction	Result (%)	Reference (%)
Prealbumin	4	4 ± 1
Albumin	56	56 ± 4
Alpha 1	4	5 ± 1
Alpha 2	7	7 ± 1
Beta 1	11	12 ± 2
Beta 2	8	6 ± 1
Gamma	13	10 ± 1

Radial Immunodiffusion Results

CSF Albumin	510 mg/L	(N = 200 mg/L)
CSF IgG	330 mg/L	(N = 35 mg/L)
CSF IgG/CSF Alb	10%	(N < 24%)
CSF Alb/Serum Alb	1.4%	(N < 0.65%)

1/4 Other Body Fluid Proteins

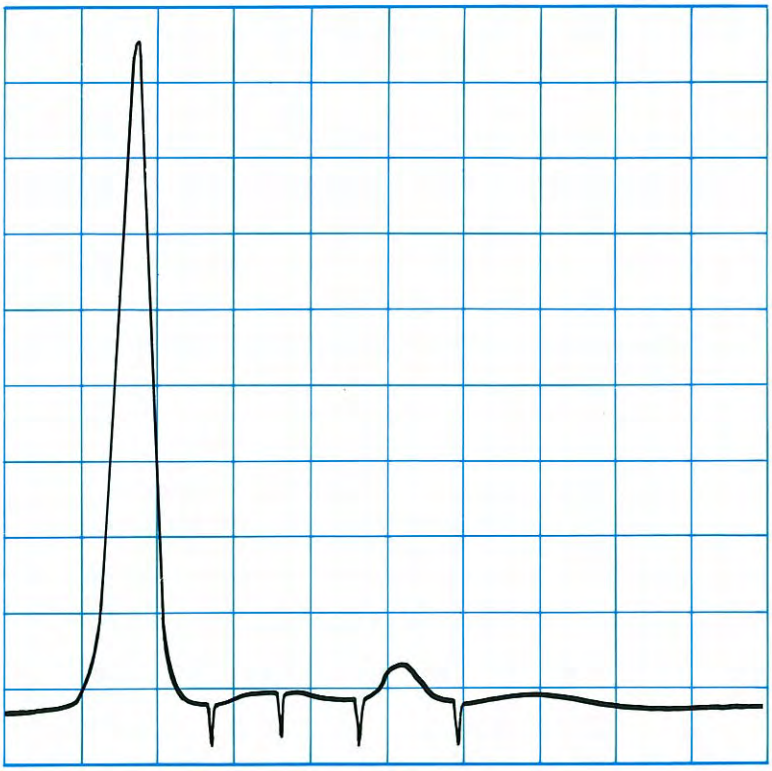
Under normal conditions, body fluids found in the pleura, pericardium, pertitoneum and synovia are contained by two thin epithelial membranes. The body cavities these membranes separate contain little or no liquid. In certain pathological conditions, usually involving mechanical compression, excess liquid is formed. The volume is variable, ranging from a small amount to several liters, as in cirrhotic dropsy. The exudate contains all the elements of serum, the concentration of which varies according to the fluid volume.

Liquids are aspirated by puncturing the site, preferably using an anticoagulant. Cytological examination and electrophoresis of the fluid should be performed. Parallel testing of the patient's serum is also recommended.

In the electrophoretic analysis of these fluids it is important to determine:
— the concentration of the proteins
— the albumin/globulin ratio
— the tracing characteristic or shape of the gamma globulin fraction

Note: Some body fluids, such as synovial fluid, contain serum constituents, specific enzymes and other substances, which confer viscous properties to the exudate. This viscosity can affect the electrophoretic separation and may require several dialyses to pre-treat the sample.

Pleural Fluid in Inflammation



Total Protein: 15 g/L (average)

A/G Ratio: 3.48 (very elevated)

Gamma Globulin: Curve is flat; low level of immunoglobulins

Fractions	%
Albumin	77.76
Alpha 1	2.70
Alpha 2	3.23
Beta	8.24
Gamma	8.07

1/5 Electrophoresis of Tears

Electrophoresis of tears is an important element of opthamological diagnosis. It is currently being used in specialized centers.

Protein Composition of Tears

The total protein concentration is about 4 to 7 g/L. Protein composition is complex; separation by polyacrylamide gel electrophoresis followed by isoelectrofocusing has permitted the isolation of about 60 fractions. These proteins can be divided into several groups:

— **Serum proteins** that filter across the blood-lacrimal barrier comprise about 1% of the total protein. Included are albumin, IgG, IgA, IgE, haptoglobin, alpha 2 macroglobulin, transferrin, alpha 1 antichymotrypsin and zinc alpha 2 glycoprotein.
— **Proteins synthesized within the lacrimal gland** account for approximately 99% of the total protein and include secretory IgA, lysozyme and lactotransferrin. About 25 to 30% of the lacrimal proteins are attributed to a heterogenous mixture of Rapid Migrating Proteins designated RMP 1 and RMP 2.
— **Beta 2 microglobulin**, enzymes of intermediate metabolism
— **Proteins synthesized within the conjunctiva,** almost always associated with pathological conditions

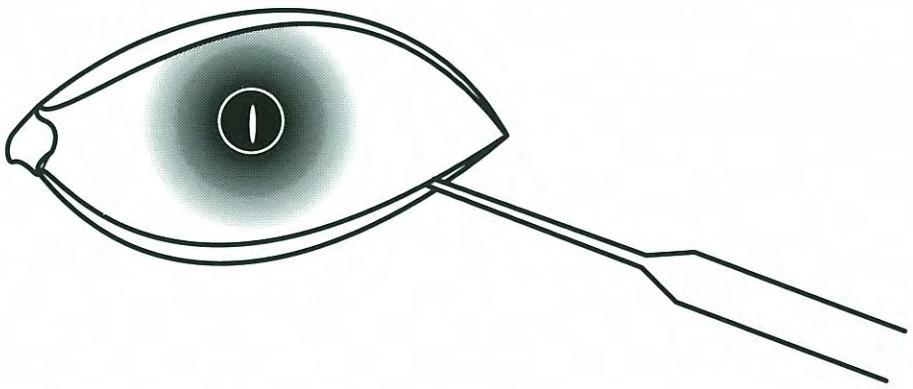
Directions for Sample Collection

Materials:

— Pasteur pipette, tapered and polished at its end
— polystyrene microcentrifuge tubes
— onion or other tear inducing agent

Procedure:

•The patient should be seated. Pull gently on the lower eyelid to form a pocket. Place the pipette on the outer third of the eyelid (see drawing). To prevent or at least limit the amount of "reflexive crying", care should be taken to collect the tears with the least amount of trauma possible.

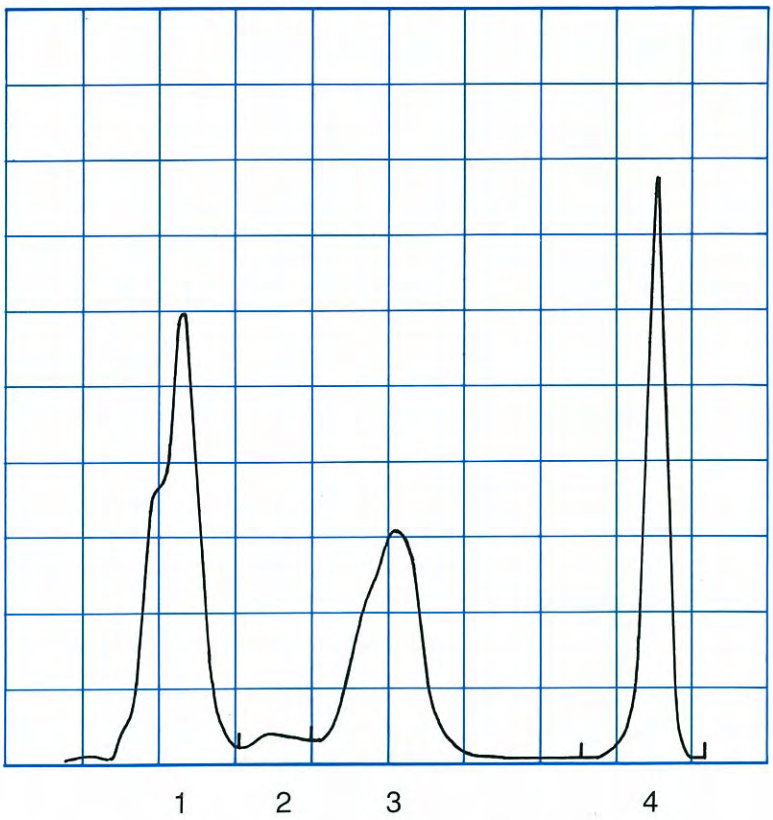


- The act of gently placing the end of the pipette in the lower conjunctivial pocket may suffice to induce lacrimal secretion. If the eye is dry, tears may be induced by the use of an onion or other agent.
- Collect the tears by aspirating the pipette. The tears of the two eyes should be collected separately.
- Place the aspirated tears in a microcentrifuge tube and centrifuge at a low speed. The supernatant is used for electrophoresis and can be stored at 4°C.

Electrophoresis

The principle is the same as for protein electrophoresis on cellulose acetate. The pH of the buffer is between 8.6 and 9.0. Ponceau S is used to visualize the proteins.

"Normal" Electrophoresis Pattern



- **Fraction 1** 25 - 35%
  Albumin and a heterogeneous mix of Rapid Migrating Proteins (RMP 1, RMP 2)

- **Fraction 2** 4 - 10%
  Lacrimal and serum proteins

- **Fraction 3** 35 - 45%
  Lactotransferrin (Ltf) and immunoglobulins

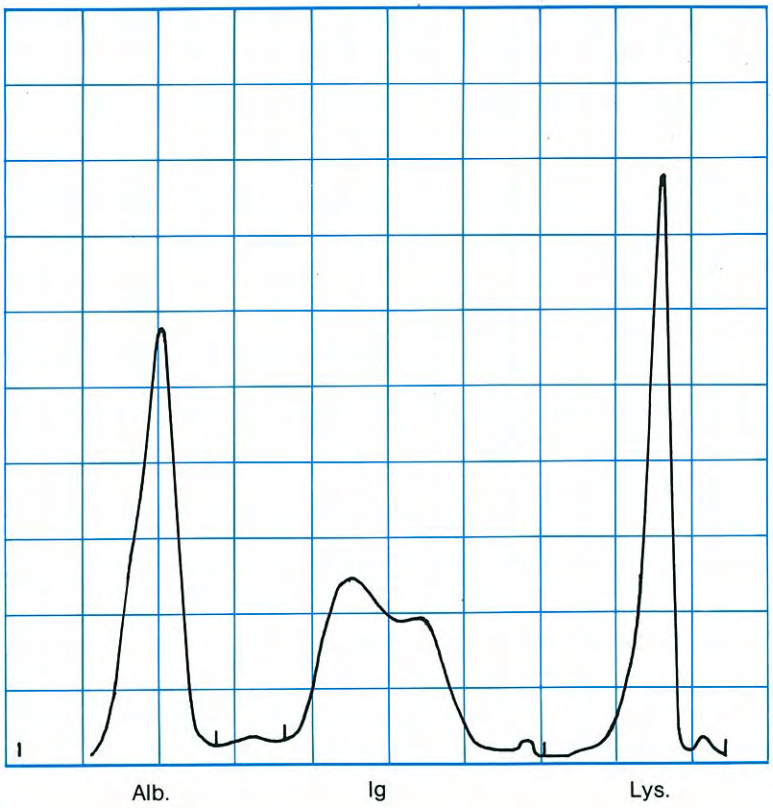
- **Fraction 4** 25 - 35%
  Lysozyme (Lys)

The rapidly migrating proteins (RMP), lactotransferrin and lysozyme are synthesized by the lacrimal gland and correspond to the three major peaks seen on cellulose acetate electrophoresis. A dysfunction of the lacrimal gland can result in diminished synthesis of one or more of these proteins.

Albumin and IgG can be increased as a result of changes in the permeability of the blood-lacrimal barrier, particularly in association with inflammatory reactions. An isolated increase of IgG can occur when there is local synthesis in the conjunctiva.

Abnormal Electrophoresis Patterns

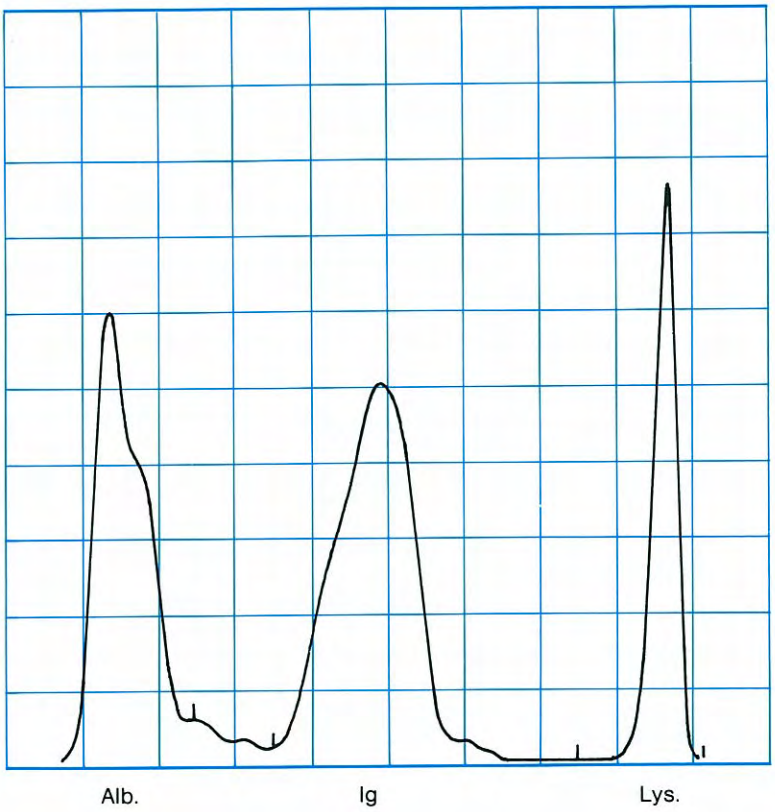
1 – Inflammation



Slight Inflammation

Little change in the RMP level, but a slight increase in albumin

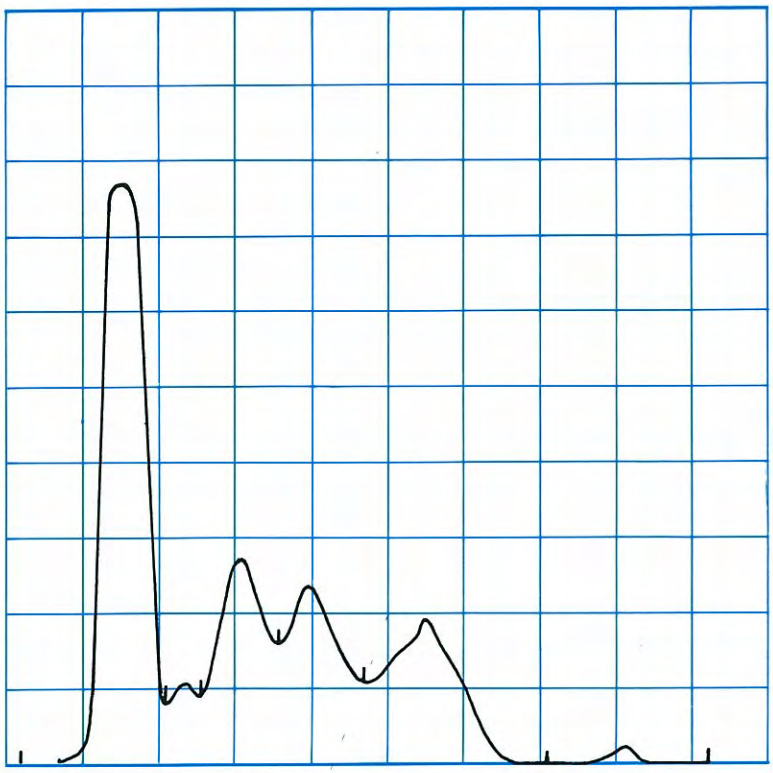
Fraction	%	Reference (%)
RMP 1	33.7	25 - 35
RMP 2	1.9	4 - 10
Ltf	36.1	35 - 45
Lys	28.3	25 - 35



Moderate Inflammation

Significant increase in albumin, relative decrease in lysozyme

Fraction	%	Reference (%)
RMP 1	33.7	25 - 35
RMP 2	2.4	4 - 10
Ltf	44.2	35 - 45
Lys	10.7	25 - 35

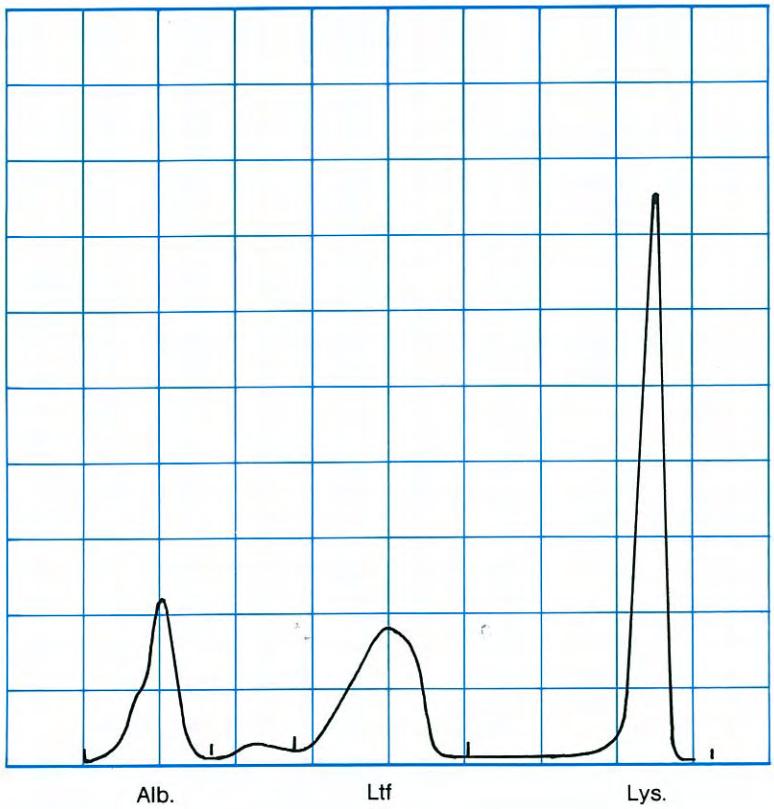


Severe Inflammation

The pattern is very nearly like that of serum. The relative % and absolute concentration of lysozyme is greatly diminished.

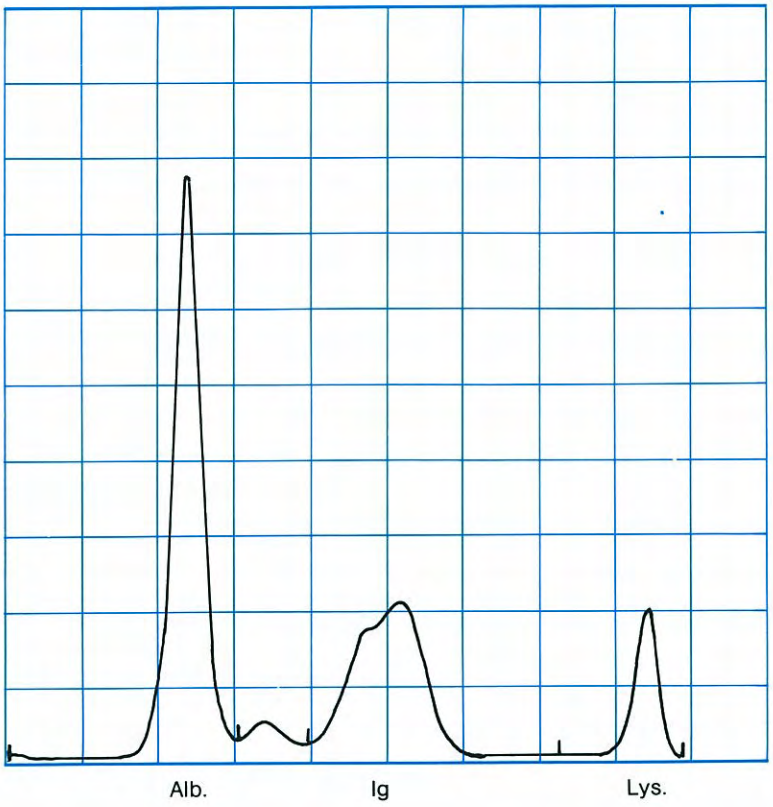
Fraction	%	Reference (%)
Alb,	42.7	25 - 35
RMP 1	3.9	
	17.1	
	17.1	
	18.3	
Lys	0.9	25 - 35

2 – Dry Eye Syndrome Caused by Lacrimal Gland Disease



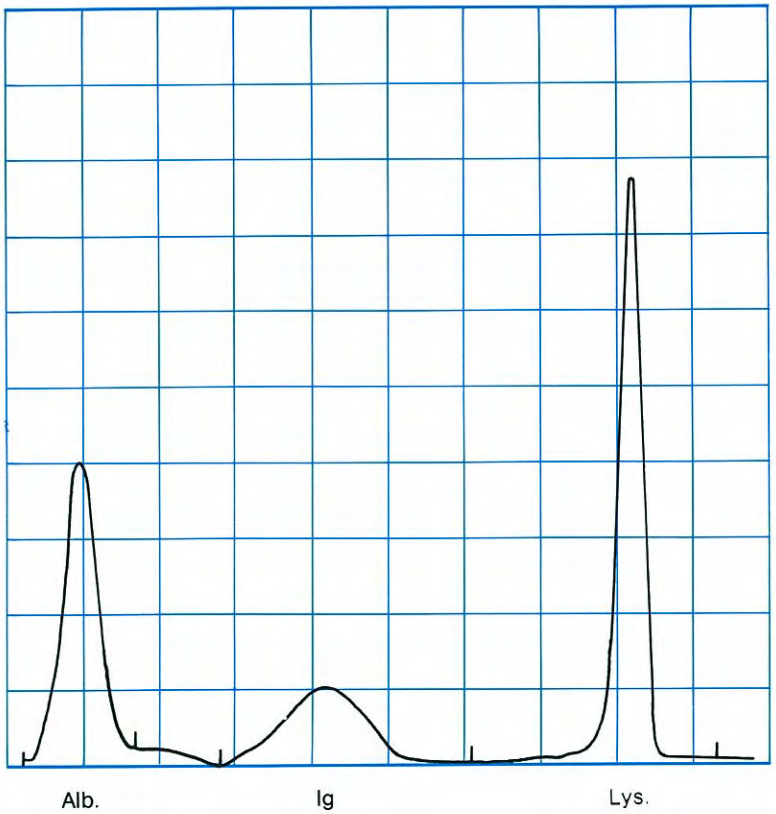
Disease of the lacrimal gland is characterized by decreases in RMP 1 and other fractions.

Fraction	%	Reference (%)
RMP 1	19.5	25 - 35
RMP 2	3.1	4 - 10
Ltf	33.1	35 - 45
Lys	44.3	25 - 35



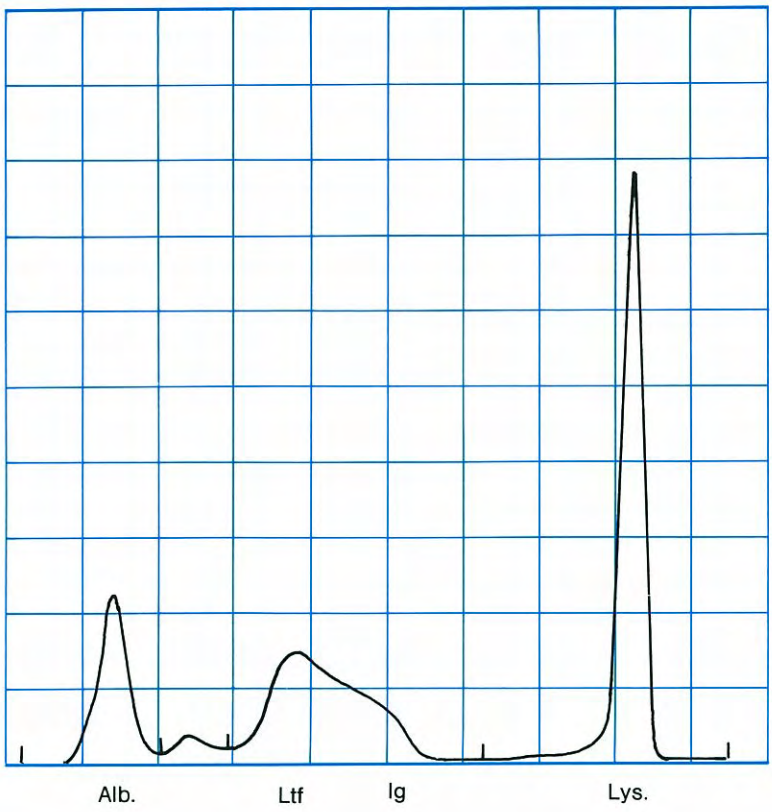
Decreased Lysozyme

Fraction	%	Reference (%)
RMP 1	51.4	25 - 35
RMP 2	4.5	4 - 10
Ltf	33.6	35 - 45
Lys	10.5	25 - 35



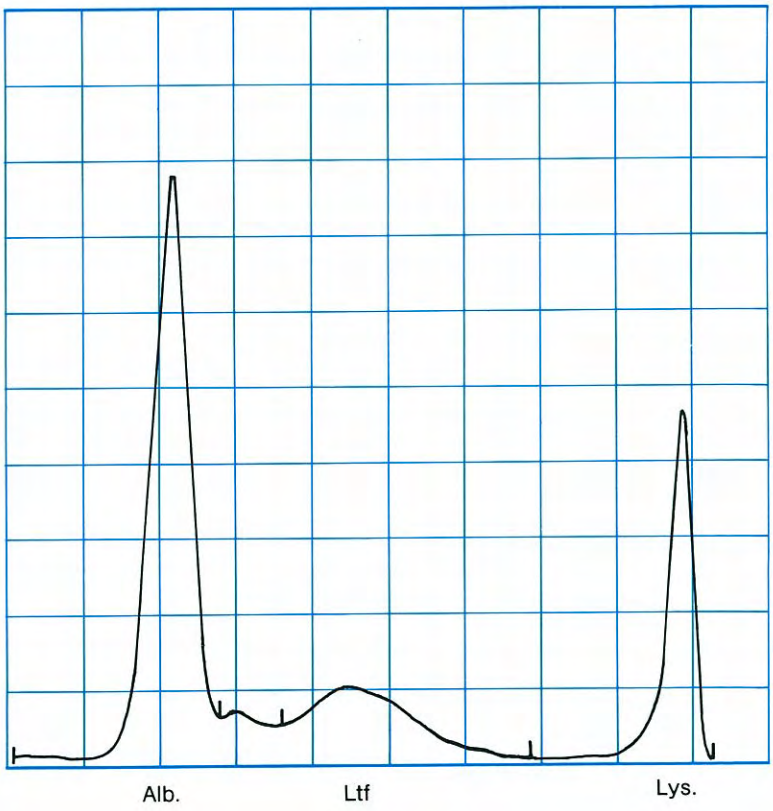
Decreased Lactotransferrin

Fraction	%	Reference (%)
RMP 1	35.1	25 - 35
RMP 2	2.7	4 - 10
Ltf	19.2	35 - 45
Lys	43.0	25 - 35



Decreases in RMP and Lactotransferrin

Fraction	%	Reference (%)
RMP 1	19.1	25 - 35
RMP 2	3.5	4 - 10
Ltf	35.8	35 - 45
Lys	41.6	25 - 35



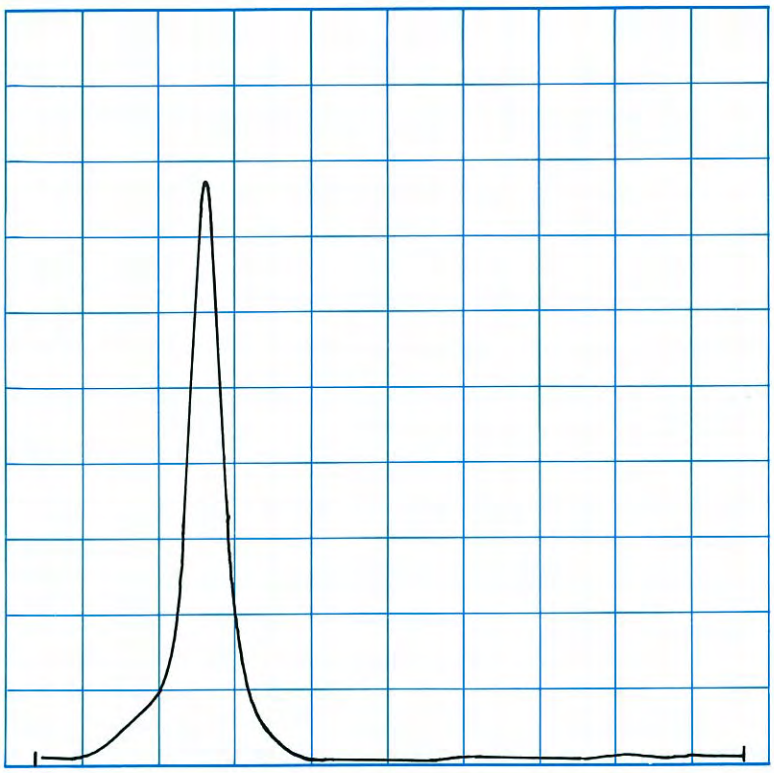
Decreases in Lysozyme and Lactotransferrin

Fraction	%	Reference (%)
RMP 1	54.1	25 - 35
RMP 2	5.6	4 - 10
Ltf	19.8	35 - 45
Lys	20.5	25 - 35

3 – Protein Anomaly

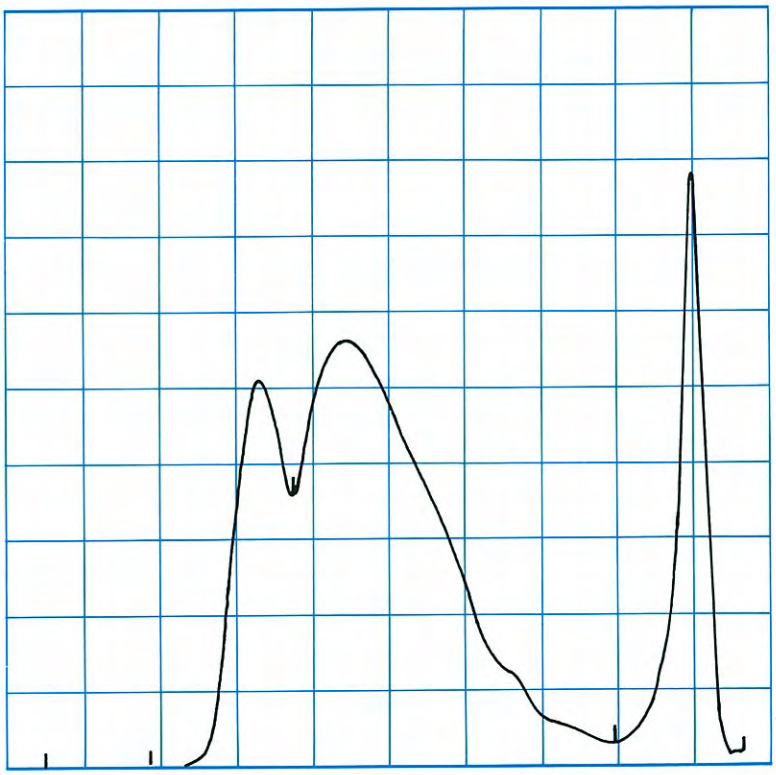
The disappearance of normal fractions and appearance of one or more new fractions can occur unexpectedly in a normal subject. It may frequently be associated with dirty contact lenses. The pattern returns to normal after a certain time.

Two types exist:
– **Type 1:** No antibacterial factors are present (lactotransferrin and lysozyme)
– **Type 2:** Intermediate to type 1 and normal



Type 1

Fraction	Reference (%)
RMP 1	25 - 35
RMP 2	4 - 10
Ltf	35 - 45
Lys	25 - 35



Type 2

Fraction	Reference (%)
RMP 1	22.0
RMP 2	
Ltf	60.4
Lys	17.6

Fraction	Reference (%)
RMP 1	25 - 35
RMP 2	4 - 10
Ltf	35 - 45
Lys	25 - 35

1/6 High Resolution Protein Electrophoresis and Silver Staining

The technique of high resolution electrophoresis is so called because it permits separation of a greater number of fractions than classical 5-banded protein electrophoresis on cellulose acetate. The number of fractions separated depends on the support media and the staining techniques employed.

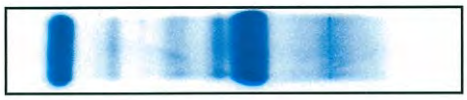
Agarose Gel Electrophoresis with Silver Staining

The use of agarose gels is more and more common place in the clinical laboratory (serum proteins, lipoproteins, hemoglobins, isoenzymes). These gels are particularly useful for biological fluids with low levels of protein such as urine and cerebrospinal fluid.

Stains like Coomassie blue (Chapter 1/3) require that the cerebrospinal fluid sample be concentrated prior to separation. Silver staining techniques used with agarose gel electrophoresis permit visualization of the sample without preliminary concentration and a only few microliters of CSF are needed. Silver staining on agarose gels is 100 times more sensitive than Coomassie blue on polyacrylamide gels. Densitometric quantitation of proteins stained with silver stain is of uncertain value. What is important is that silver staining permits visualization of cerebrospinal fluid proteins without concentrating the specimen. With this technique, oligoclonal banding in the gamma region is readily observed. In addition, pre-albumin and two transferrin bands are also observed with this technique.

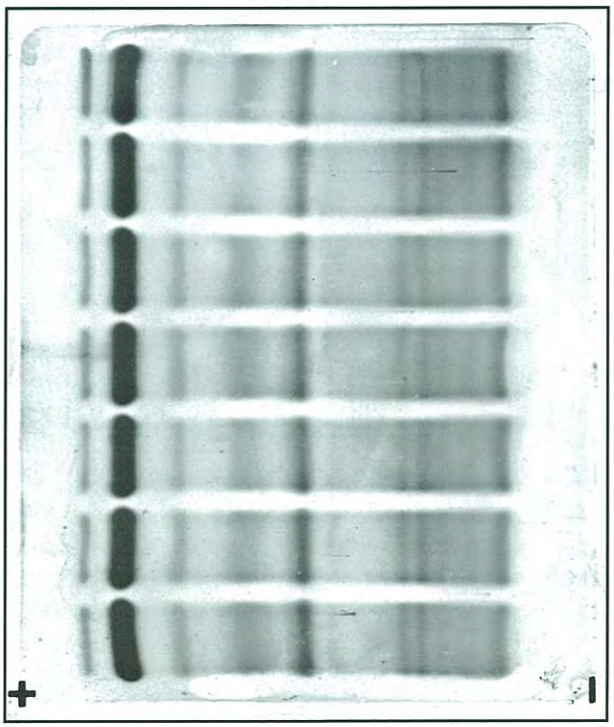
Agarose Gel Electrophoresis with Coomassie Blue Stain – Serum

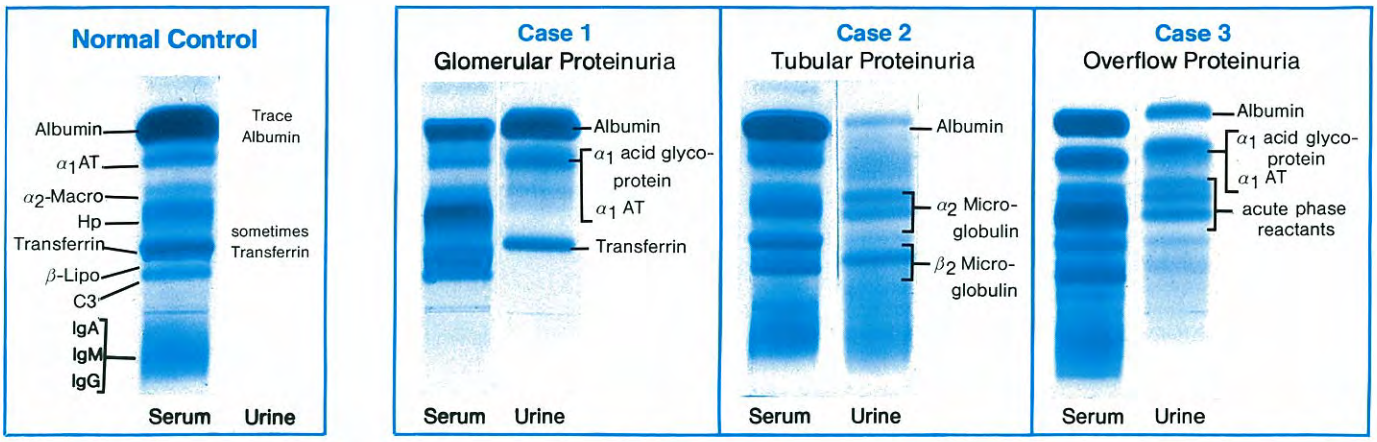
Serum protein pattern shows a monoclonal peak in the beta globulin region.



Agarose Gel Electrophoresis with Silver Stain – CSF

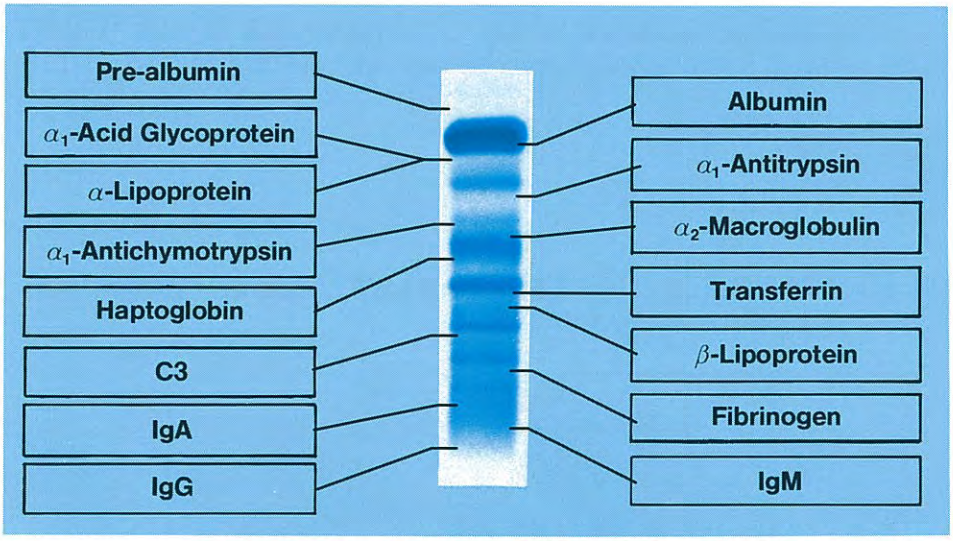
The gel shows oligoclonal banding in the gamma region.





Three cases illustrating the differences in protein electrophoresis patterns in glomerular, tubular and overflow proteinuria.

Agarose Gel Electrophoresis with Coomassie Blue Stain



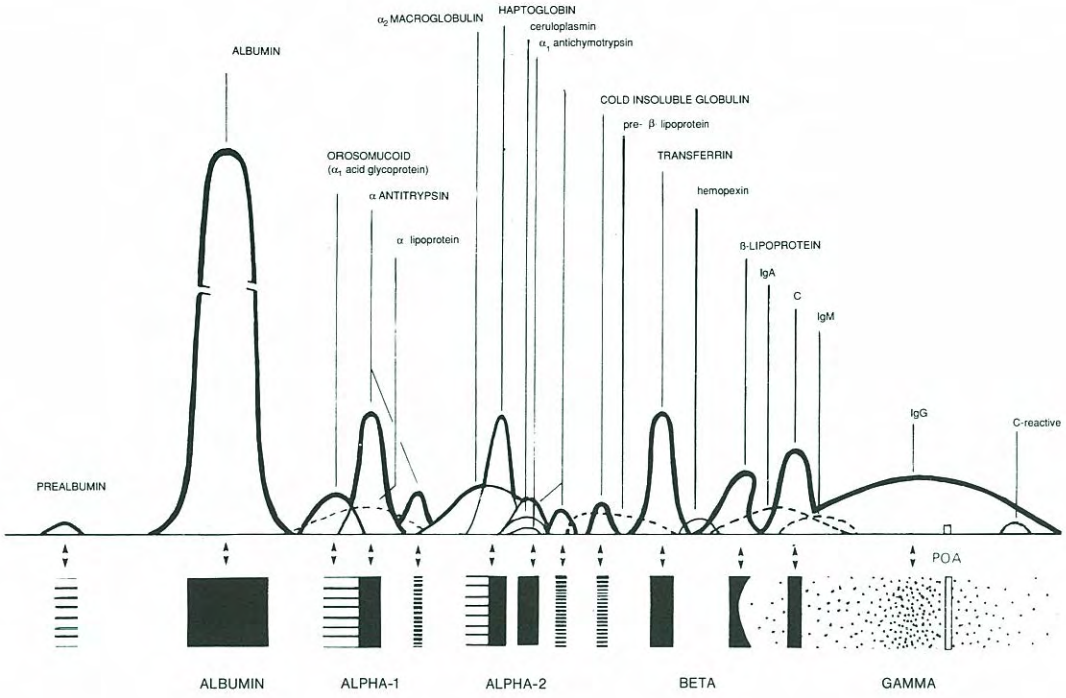
This agarose gel electrophoresis pattern of normal human plasma indicates fifteen major proteins.

Polyacrylamide Gel Electrophoresis

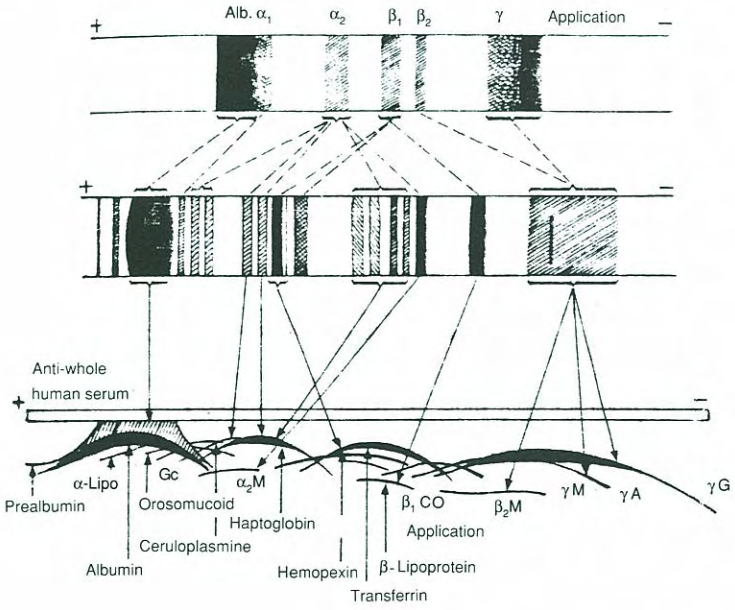
Polyacrylamide gel electrophoresis takes much longer and is more delicate than high resolution protein electrophoresis. The pore sizes and resolving properties of the gel varies according to the preparation and polymerization of the acrylamide. Separation is related to the size and the electrical charge of the molecule at a given pH. Electrophoresis may be carried out in a continuous or discontinuous buffer solution, in a glass tube or in a slab, in a vertical or horizontal position.

This technique has many applications in research. New simplified techniques may have application for lipoproteins, urine and spinal fluid proteins and phenotyping of haptoglobins and alpha 1 antitrypsin.

Relative Electrophoretic Mobility of Proteins on Agarose Gel



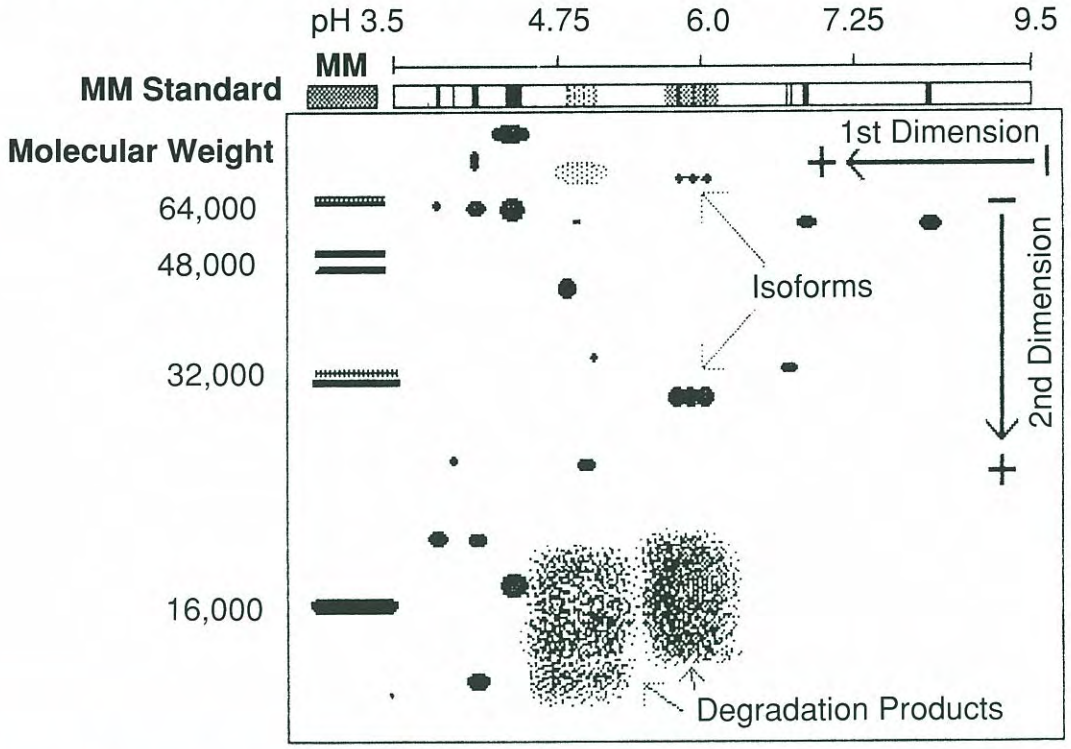
Relative Electrophoretic Mobility of Proteins on Paper (top), Acrylamide Tube Gels (middle) and Immunoelectrophoresis (bottom)



1/7 Two Dimensional Electrophoresis

In 1969 Giddings demonstrated that simple, one dimensional electrophoretic techniques could not completely separate all the components of a complex mixture of proteins. This is particularly true of biological fluids where concentration and molecular mass varies in ratio from 1 to 1000 with very little difference in relative mobility or net charge. Two dimensional electrophoresis permits separation of proteins using optimal electrophoresis conditions for two different physical or chemical properties.

Usually isoelectrofocusing is used in the first dimension to separate proteins based on isoelectric point of the molecule. It is frequently followed by polyacrylamide gel electrophoresis using sodium dodecyl sulfate to separate the proteins by molecular mass (MM).



1 – Isoelectrofocusing

The technique of isoelectric focusing (IEF) is based on the fact that at a given pH each protein has a neutral electrical charge where the number of negatively charged groups (–COOH, –SH) is equal to the positively charged groups (–NH2). This is called the isoelectric point (pI).

Thus pI can be used to separate proteins by electrophoresis in a gel where there is a pH gradient from cathode (alkaline pH) to anode (acid pH). The pH gradient is created by a mixture of ampholytes that are partitioned in order of pI. The strong buffering capacity of the ampholytes provides a pH gradient in the gel that corresponds to the isoelectric point of the ampholyte. The protein sample is applied to the gel in the region where the pH is equal to 7. At this pH, proteins with a pI less than 7 have a net negative charge, while those with a pI greater than 7 have a net positive charge.

An electrical field is applied to the two ends of the gel: negatively charged molecules migrate toward the more alkaline cathode and inversely, positively charged molecules move toward the more acidic anode. When the molecule reaches the pH gradient equivalent to its pI, the molecule attains a neutral electrical charge and loses its electrophoretic mobility, becoming focalized in a very narrow band. IEF separates serum proteins into about 40 fractions. The resolving power of IEF is greater than 0.01 pH units.This technique is capable of separating two proteins or isoforms between which the only difference is the substitution of a basic amine for one neutral or acidic amino acid.

2 – SDS Electrophoresis

Sodium dodecyl sulfate (SDS) attaches to protein at the rate of one SDS molecule for every two amino acids. This attachment changes the helical structure of the molecule and imparts a strongly negative net charge that masks the initial charge of the molecule. Attachment of the SDS affixes a constant charge per unit of weight to the molecule, thus electrophoretic mobility becomes a function of molecular mass. Separation on polyacrylamide gels is directly related to the resistance caused by molecular size; the bulkier the molecule the slower it moves. The molecular mass of an unknown protein is derived by comparing it to the electrophoretic mobility of proteins of known molecular mass.

3 – Visualization Techniques

Polyacrylamide Gels
After fixation of the proteins and removal of the SDS, proteins can be visualized with Coomassie blue stain (sensitivity: 0.5 to 1 mg) or silver stain (sensitivity: 5 to 30 ng). The technique of silver staining is similar to the photographic process: Ag+ ions attach to the proteins and are transformed to visible Ag° by a reducing agent. Unlike Coomassie blue, silver does not stain proteins stoichiometrically making quantitative interpretation difficult: the sensitivity depends on the concentration and nature of the protein.

Transfer to Nitrocellulose
After electrophoresis, the gel used for the second dimension is placed on a sheet of nitrocellulose. When pressure is applied perpendicular to this "sandwich", the proteins migrate toward the sheet of nitrocellulose and are adsorbed there. Two types of visualization are used: (1) visualization of all proteins with colloidal gold (sensitivity equivalent to silver stain) or (2) visualization of particular proteins using specific antisera. The specific antiserum is tagged with peroxidase or colloidal gold. When the tagged antiserum is placed on the nitrocellulose, the antiserum reacts with a specific protein and can then be precipitated with an oxidizing agent (diaminobenzidine, chloro-napthol). The antiserum tagged with colloidal gold gives a red tint to the proteins. This can be enhanced by the addition of Ag+ ions, which in the presence of a reducing agent, precipitates as Ag° around the gold.

4 – Interpretation

Densitometric scanning of a two dimensional gel is difficult, for each spot or protein is defined by three criteria: its pI (x), its MM (y) and its relative intensity (z). More than 1,000 spots may be visible on a single gel. This complexity demands an "expert system" for numerization and interpretation of the spots. Expert systems are already used in the study of some pathologies: nephrotic syndromes, monoclonal and polyclonal gammopathies, dysbetalipoproteinemias (phenotyping of apolipoprotein E). Some authors – Righetti, Radola, Catsimpoolas, Anderson – are beginning a systematic cartography of all the human proteins, including erythrocytic, lymphatic, hepatic, salivary, urinary and serum proteins. The total number of human proteins is estimated to be somewhere between 30,000 and 50,000.

The evolution, especially the simplification and automation of two dimensional electrophoresis, may make this technique available to non-specialized laboratories within the next ten years. Two dimensional electrophoresis shows great potential for permitting early detection of certain anomalies and for monitoring disease processes.

1/8 Lipoproteins and HDL Cholesterol

Since Fredrickson and Lees proposed a system for classifying hyperlipoproteinemias, the concept of detecting and preventing coronary artery disease utilizing lipoprotein electrophoresis has become a reality.

Accurate determination of lipoprotein phenotypes is necessary because hyperlipemia is symptomatic of a group of disorders that are dissimilar in clinical features, prognosis and responsiveness to treatment. Since treatments of the disorders vary with the different phenotypes, it is absolutely necessary that the correct phenotype be established before treatment is begun.

Plasma lipids do not circulate freely in the plasma, but are transported bound to proteins and can thus be classified as lipoproteins. Lipoproteins are soluble complexes composed of a lipid part (cholesterol, triglycerides, phospholipids) linked by hydrophobic bonds to a protein part (apoprotein).

Lipoproteins are divided into four principal categories:

Classification by Technique and Composition of Lipoprotein Fractions

Classification According To:	Approximate Composition:			
Lipoprotein Electrophoresis	**Protein**	**Triglyceride**	**Cholesterol**	**Phospholipids**
Chylomicrons	2%	98%		
Beta	21%	12%	45% (LDL)	22%
pre-Beta	10%	55%	13% (VLDL)	22%
Alpha	50%	6%	18% (HDL)	26%
Albumin	Weakly binds free fatty acids			

Electrophoresis separates lipoproteins into alpha (HDL), pre-beta (VLDL), beta (LDL) and sometimes chylomicrons which remain at the application point. The separation of these fractions differs according to the type of support media used (cellulose acetate, polyacrylamide or agarose gels). The lipid quantitation is affected by the type of stain used: traditional lipoprotein phenotyping (alpha, beta, pre-beta, chylomicrons) uses a total lipid stain, while HDL, VLDL, LDL quantitations employ a cholesterol specific stain.

In addition to the four major lipid fractions, a few minor classes are easily detected using agarose or polyacrylamide gels:

- **IDL – intermediate Density Lipoproteins –** blockage of the intravascular catabolism of VLDL results in an accumulation of these intermediary lipoproteins. This fraction is found to various degrees in normal serum, but a marked increase is characteristic of a type III phenotype. IDL migrates between pre-beta and beta, but closer to the pre-beta.

- **Lipoprotein Lp (a)** – polymorphic form of beta lipoprotein (LDL) that migrates between pre-beta and beta, but closer to the beta. It can be found in normal serum. It plays an atherogenic role, but at what point an increase is pathologically significant is not clear.

- **Lipoprotein-X** – an abnormal lipoprotein that is found in cholestasis or cases of parenteral nutrition rich in lipids.

Fredrickson and Lees Classification of Lipid Phenotypes

1 – Primary Hyperlipoproteinemias

Type I: Hyperchylomicronemia

Chylomicrons can be seen at the application point on electrophoresis. The beta, pre-beta and alpha lipoproteins are diminished or even absent.
Cholesterol is normal or elevated.
Triglycerides are very elevated, reaching levels as high as 117 mmol/L (100 g/L).
Serum is creamy and forms a superficial layer upon standing 12 hours refrigerated.

Type IIa: Primary Hypercholesterolemia

No chylomicrons present.
Beta lipoproteins are grossly elevated.
Pre-beta and alpha lipoproteins are normal.
Cholesterol is very elevated; triglycerides are normal.
The serum is clear.

Type IIb: Mixed Hyperlipidemia

No chylomicrons present.
Beta and pre-beta lipoproteins are increased.
Alpha lipoproteins are normal. Cholesterol is elevated; triglycerides are normal or slightly elevated.
The serum is opalescent.

Type III: Mixed Hyperlipidemia

An IDL fraction appears between the beta and pre-beta fractions.
Beta lipoproteins are diminished.
Pre-beta lipoproteins may be more or less elevated.
Cholesterol and triglycerides may be slightly or grossly elevated.
The serum may be clear or slightly opalescent.

Type IV: Carbohydrate Induced Endogenous Hypertriglyceridemia

A faint band of chylomicrons may be present.
Pre-beta lipoproteins may be slightly to grossly elevated.
Beta and alpha lipoproteins may be decreased.
Cholesterol is normal or slightly elevated; triglycerides are slightly to grossly elevated.
The serum can be clear or opalescent.

Type V: Endogenous and Exogenous Hyperglyceridemia

Faint to intense, well-defined band of chylomicrons present. A well-defined chylomicron band best distinguishes type IV and type V lipoproteinemias.

Beta lipoproteins are normal to slightly elevated.

Pre-beta lipoproteins are slightly to grossly elevated and alpha lipoproteins are diminished.

Triglycerides are normal to slightly elevated.

The serum is creamy, with a floating layer appearing after 12 hours in refrigeration.

2 – Secondary Hyperlipoproteinemias

There are a number of lipid changes resulting from various diseases and are termed secondary hyperlipoproteinemias. Some typical secondary hyperlipoproteinemias are:

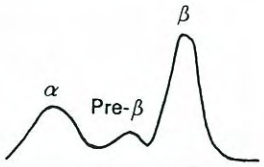
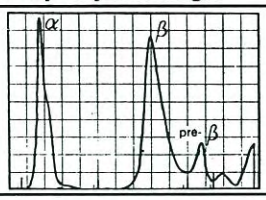
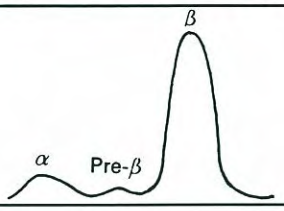
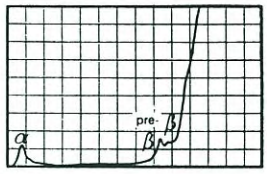
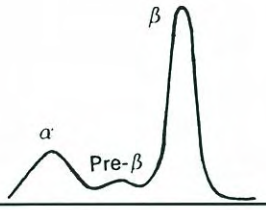
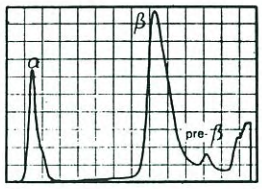
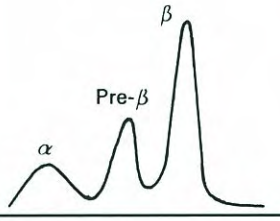
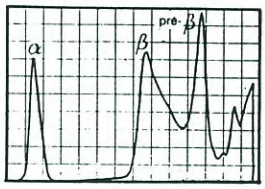
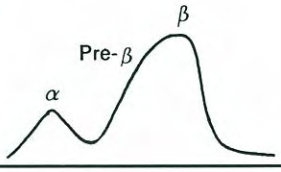
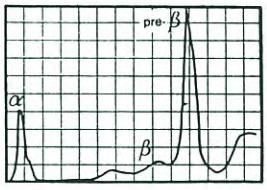
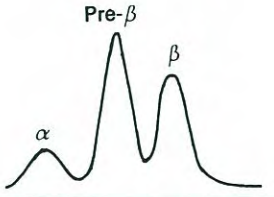
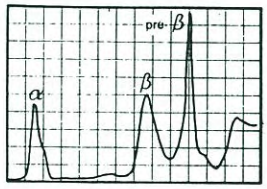
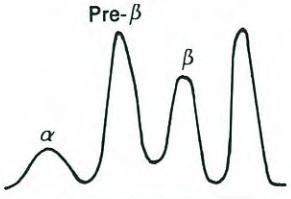
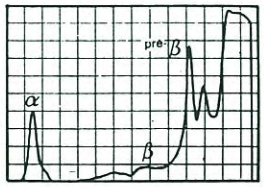
Diabetes – is generally accompanied by an excessive and rapid mobilization of fatty acids from body stores. There is an increase in total serum lipids and in particular triglycerides. During episodes of diabetic coma and in acidosis, there is a significant decrease in cholesterol and beta lipoprotein levels. Lipoprotein electrophoresis can detect a type I or IV. Insulin therapy brings about a rapid dissipation of the lipidemia.

Nephrotic Syndrome – Protein loss from the kidney results in increased production of protein by the liver. Plasma lipids bind to the protein resulting in hyperlipoproteinemia. The lipoprotein pattern resembles a type IV.

Alcoholism – Triglycerides are increased resulting in a type IV lipoprotein pattern.

Liver Diseases –

- **Obstructive Liver Disease –** Beta and alpha lipoproteins are increased. In severe cases, the alpha protein migration may be retarded, resulting in a broad alpha band.
- **Liver Cell Failure –** A very low alpha protein level can be found.
- **Acute Viral Hepatitis –** Increased alpha lipoproteins are generally associated with this condition.

Type	Lipoprotein Electrophoresis Densitometric Tracings			Serum Appeara and Lipid Valu
	Cellulose Acetate	Polyacrylamide gel	Fraction %	
Normal			α = 33 ß = 57 Pre-ß = 10	Serum –Fresh: Clear –After 24 hrs: Clear Cholesterol: Normal Triglycerides: Normal
I			α = 28 ß = 32 Pre-ß = 40 Chylomicrons	Serum –Fresh: Cloudy –After 24 hrs: Creamy Cholesterol: N or + Triglycerides: +++
IIa			α = 20 ß = 73 Pre-ß = 7	Serum –Fresh: Clear –After 24 hrs: Clear Cholesterol: +++ Triglycerides: N
IIb			α = 17 ß = 51 Pre-ß = 32	Serum –Fresh: Clear or slight turbid –After 24 hrs: Clear or slightly turbid Cholesterol: +++ Triglycerides: N or +
III			α = 20 ß = 20 Pre-ß = 60	Serum –Fresh:Clear or slightl turbid –After 24 hrs: Clear or slightly turbid Cholesterol: ++ Triglycerides: ++
IV			α = 15 ß = 24 Pre-ß = 61	Serum –Fresh: Milky or turbid –After 24 hrs: Milky or turbid Cholesterol: + Triglycerides: + or ++
V			α = 24 ß = 22 Pre-ß = 54 Chylomicrons	Serum –Fresh: Cloudy –After 24 hrs: Creamy or cloudy Cholesterol: + Triglycerides: + or ++

ication of Hyperlipidemias

ce s	Frequency and Mode of Transmission	Clinical Manifestations	Secondary Causes	Suggested Therapy
	96%	N/A	N/A	Diet: Normal Treatment: None
	0.01% (very rare) Autosomal recessive Onset in childhood	Eruptive xanthomas Hepatosplenomegaly Abdominal pain Pancreatitis	Dysgammaglobulinemia Diabetes Hypothyroidism	Diet: Severe restriction of carbohydrates and fats Treatment: None
	1.6% (common) Autosomal dominant; accounts for 60-70% of infantile hyperlipidemias	Tendon xanthomas (occasionally associated with polyarthritis) Macroglobulinemia Xanthelasma Juvenile corneian arc Tuberous xanthomas	Hypothyroidism Acute intermittent porphyruria Multiple myeloma Nephrotic syndrome Increased arteriosclerosis	Diet: Foods low in cholesterol and saturated fats Treatment: Cholestyramine
	1.4% (common)			Diet: Reduced total caloric intake with less saturated fat, cholesterol, alcohol and carbohydrate Treatment: Cholestyramine
	0.19% (rare)	Palmar xanthomas Tuberous xanthomas Tendon xanthomas Juvenile corneian arc Xanthelasma (rarely) Lipid arc	Hypothyroidism Dysgammaglobulinemia Diabetes Multiple myeloma	Diet: Reduced total caloric intake with fewer carbohydrates, saturated fats and cholesterol Treatment: Clofibrate
	0.7% (less common) Autosomal dominant	Eruptive xanthoma Tuberous xanthoma Hepatosplenomegaly	Stress Nephrotic syndrome Juvenile diabetes Multiple myeloma Alcoholism Obesity Vernes syndrome Oral contraceptives Von Gierke's syndrome	Diet: Reduced total caloric intake with fewer carbohydrates and less alcohol Treatment: Secondary
	0.1% (rare)	Eruptive xanthoma Abdominal pain Pancreatitis Hepatosplenomegaly Lipid arc Tuberous xanthoma Paresthesis	Hypothyroidism Nephrotic syndrome Diabetes Multiple myeloma Pancreatitis Alcoholism Oral contraceptives	Diet: Reduce total caloric intake with fewer carbohydrates and saturated fats Treatment: Monitoring of the latent diabetic

Determination of HDL Cholesterol by Electrophoresis

For many years the notion has existed that high cholesterol is closely associated with atherosclerosis. However, there are numerous discrepancies between total cholesterol levels and clinical manifestations of atherosclerosis. Gradually, as light was shed on the metabolism and role of cholesterol, an explanation for the discrepancies has emerged. The numerous studies of Castelli and his collaborators have shown that cholesterol molecules, once synthesized, attach to a carrier protein. The role, either beneficial or detrimental, is determined by the density of the lipoprotein carrier molecule.

Cholesterol associated with low density lipoproteins (LDL and VLDL) has an adverse tendency to penetrate the tissues, forming a deposit or atherogenic plaque. This is not true for cholesterol associated with high density lipoproteins (HDL). There is, therefore, a significant inverse relationship between the level of HDL cholesterol and the risk of atherosclerosis.

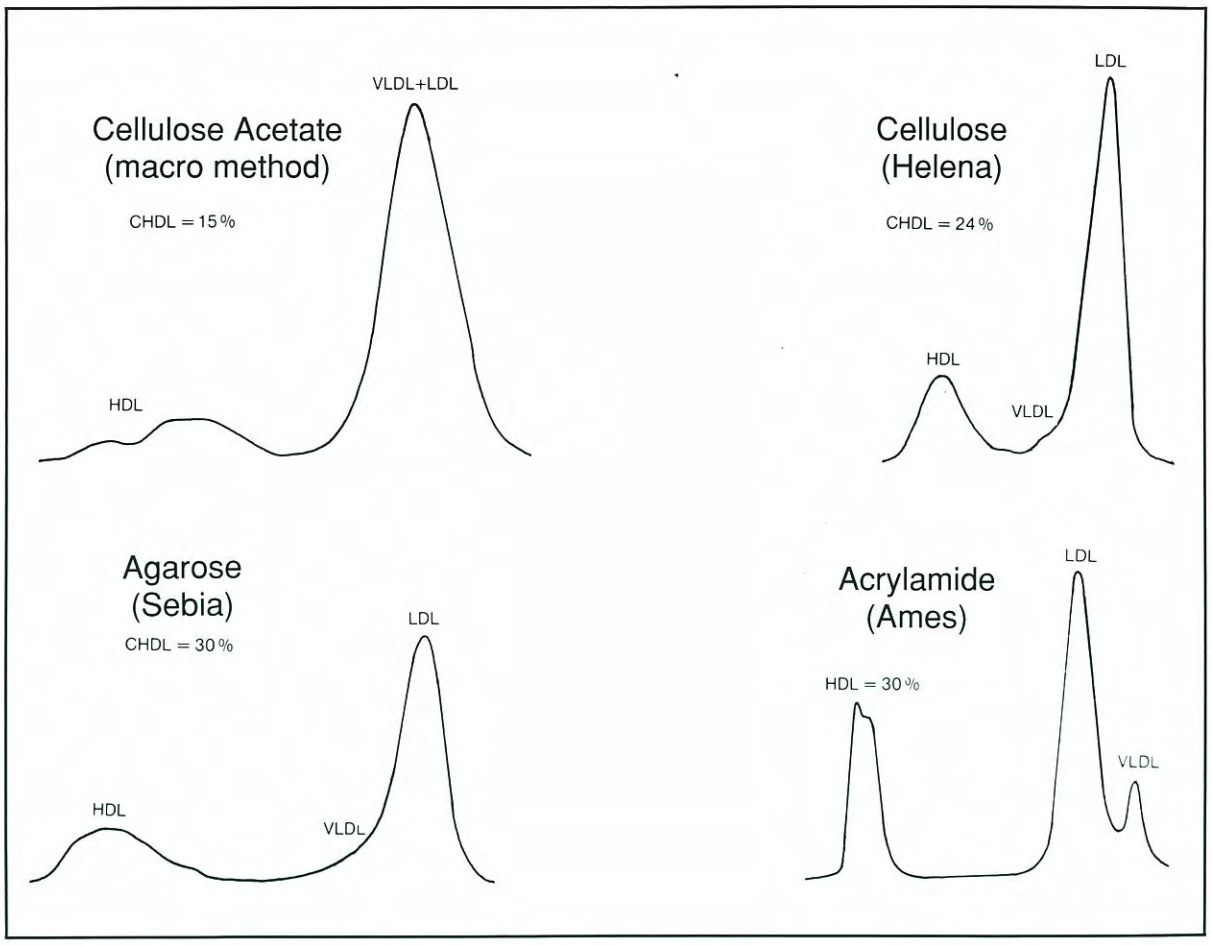
Methods for estimating the level of HDL cholesterol are either based on the cholesterol carried by the lipoprotein or its coupled protein (apolipoprotein A). Other than ultracentrifugation, which is generally reserved for the research domain, the different methods include:

— Selective precipitation of LDL and VLDL, so that HDL remains on the surface
— Immunological quantitation of apolipoproteins
— Electrophoretic separation of LDL, VLDL and HDL, visualized by coloration of the cholesterol and cholesterol esters.

Electrophoresis appears to be the method of choice. It is less costly than precipitation, yet gives the same information. Support media include cellulose acetate, agarose and polyacrylamide gels. Densitometry reveals the distribution of cholesterol on the different lipoproteins.

HDL Cholesterol (mg/dL) = %HDL x Total Cholesterol (mg/dL)

Electrophoretic Methods



From A. Sassolas

Interpretation of HDL Cholesterol Values

There are numerous factors that may influence the HDL cholesterol value. These factors must be taken into consideration when interpreting HDL cholesterol.

Factors that may increase HDL cholesterol:

— Estrogen therapy
— Anti-epileptic medications
— Intense physical exercise
— Alcohol consumption

Factors that may decrease HDL cholesterol:

— Heavy smoking (nicotine addiction)
— Lack of physical exercise

Most importantly, HDL cholesterol values vary according to age and sex.

Editor's Note: Data for the following charts were taken from Helena's HDL Cholesterol Report Form (Form No. 418) rather than converting data to mg/dL from mmol/L as reported in the original manuscript.

Variation in HDL, Total Cholesterol and Total Cholesterol/HDL Ratio by Age and Sex

Age Group	No. Subj.	TOTAL* Mean (mg/dL)	SD	Total C/HDL Mean	ALPHA HDL Mean (mg/dL)	SD	PRE-BETA (VLDL) Mean (mg/dL)	SD	BETA (LDL) Mean (mg/dL)	SD
Males:										
16-19 yrs.	21	169	28	3.8	44	8	25	13	100	24
20-29	26	202	32	4.0	50	15	27	17	125	29
30-39	24	204	38	5.0	41	12	34	21	131	28
40-49	26	217	35	4.4	49	13	33	14	136	33
50-59	21	212	37	4.9	43	12	36	15	134	33
Females:										
17-19 yrs.	11	164	26	3.3	50	11	8	7	106	23
20-29	22	179	22	3.0	59	14	14	8	107	20
30-39	32	197	41	3.3	59	11	19	11	121	36
40-49	36	211	34	3.2	66	18	23	14	124	29
50-59	22	221	34	3.5	64	16	22	11	136	32

* Total cholesterol was determined using an enzymatic method which has a normal range of 130 - 300 mg/dL

Reporting Results

HDL Cholesterol can be expressed as an absolute value or as a ratio to LDL cholesterol or total cholesterol. In considering normal values, in addition to those delimited above, myocardial infarction or similar traumatic episodes may have a statistically significant affect. A 4 – 8 week wait is recommended before HDL values are assessed.

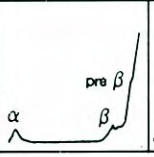
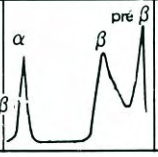
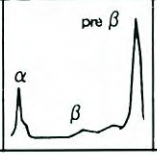
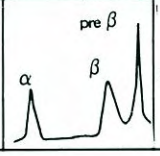
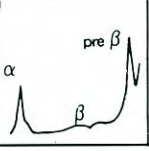
Interpretation of the Risk of Atherosclerosis

Incidence of Coronary Heart Disease by HDL Cholesterol Level
(Framingham Study)

HDL Cholesterol Level (mg/dL)	MEN CHD Incidence	MEN Population At Risk	MEN Rate/ 1000	WOMEN CHD Incidence	WOMEN Population At Risk	WOMEN Rate/ 1000
All levels	79	1025	77.1	63	1445	43.6
< 25	3	17	176.5	0	4	0.0
25-34	17	170	100.0	11	67	164.2
35-44	35	335	104.5	12	220	54.5
45-54	15	294	51.0	19	386	49.2
55-64	8	134	59.7	14	353	39.7
65-74	1	40	25.0	3	216	13.9
75+	0	35	0.0	4	199	20.1

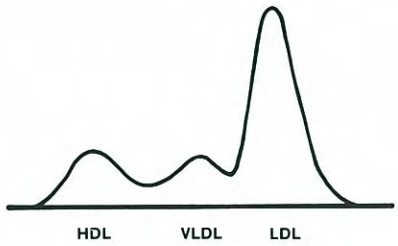
HDL Cholesterol Ratio to LDL Cholesterol or Total Cholesterol

Risk	LDL Cholesterol / HDL Cholesterol		Total Cholesterol / HDL Cholesterol	
	Men	Women	Men	Women
Average Risk	3.55	3.22	5.00	4.40
2X Average Risk	6.25	5.03	9.51	7.00
3X Average Risk	7.99	6.14	23.00	11.00

	Fredrickson Classification System							
	Normal	**I**	**IIA**	**IIB**	**III**	**IV**	**V**	
Serum Appearance at 0°C	Clear	Cloudy	Clear	Clear or Cloudy	+ or – Cloudy	Opalescent	Cloudy	
Serum Appearance after 12 hrs. at 14° C	Clear	Creamy on top Clear on bottom	Clear	Clear or Cloudy	+ or – Cloudy	Opalescent	Creamy on top Cloudy	
Triglycerides	Normal	+ + +	Normal	Normal or +	+ +	+ or + +	+ + or + + +	mmol/l (N = 0.60-1.70)
Cholesterol	Normal	N or +	+ + +	+ + +	+ +	+ +	+	mmol/l (N = 3.90-6.00)
Electrophoresis on polyacrylamide tube gel								

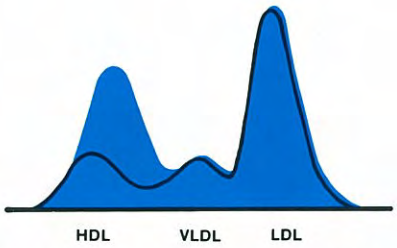
HDL Cholesterol Electrophoresis Patterns

In the patterns that follow, the normal pattern is outlined in black and the patient is in blue.

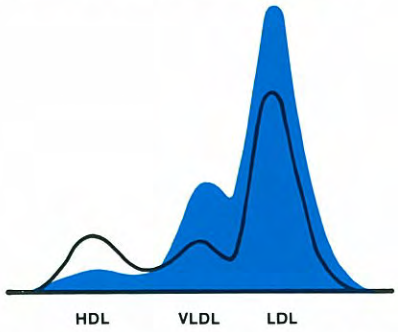


Abnormal
Normal

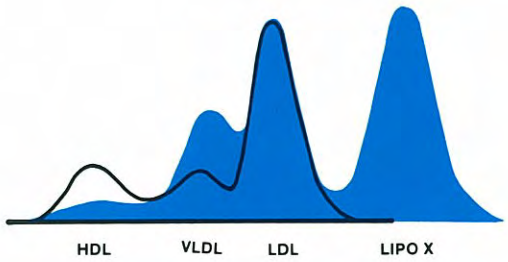
AVERAGE RISK
TOTAL C/HDL RATIO = 5.0



INCREASED HDL - LOW RISK
TOTAL C/HDL RATIO = 3.0



DECREASED HDL - HIGH RISK
TOTAL C/HDL RATIO = 13.0



DECREASED HDL - LIPOPROTEIN X
CHOLESTASIS

2 Isoenzyme Electrophoresis

Definition of an Isoenzyme

Isoenzymes are molecular variations of the same enzyme. They have identical catalytic activity, but different spatial configurations. These molecules differ by certain biochemical properties, such as electrophoretic mobility.

Although these isoenzymes are different, they catalyze the same reaction and, in general, are quantitated together when measuring chemical activity.

For the most part, different organs or tissues possess a single molecular form of the enzyme. Therefore when the isoenzymes are separated by electrophoresis, the specific organ responsible for the increase in total enzyme activity can be identified. That is the reason for doing isoenzyme electrophoresis.

Interpretation of isoenzyme electrophoresis is particularly significant in the case of:

– Creatine Kinase (CK)

– Lactate Dehydrogenase (LD)

– Alkaline Phosphatase (ALP)

2/1 Creatine Kinase (CK)

Creatine kinase (CK) is an enzyme made up of many molecular forms and is present in a great number of tissues. Creatine kinase catalyzes the reversible dephosphorylation of creatine phosphate to form ATP.

$$\text{Creatine phosphate} + \text{ADP} \overset{\text{CK}}{\rightleftharpoons} \text{Creatine} + \text{ATP}$$

CK is a dimer formed by the association of two types of polypeptide chains, traditionally called M and B. Each of these associations results in a molecule with different isoelectric points, permitting them to be differentiated by electrophoresis.The study of the serum creatine kinase isoenzymes provides a number of diagnostic elements to the clinician.

The Three CK Isoenzymes

- CK3 or CK-MM is present in skeletal muscle; it does not migrate on electrophoresis, but remains at the application point.
- CK2 or CK-MB is found in high concentrations in the heart, colon and placenta; it has an intermediate migration between CK-MM and CK-BB.
- CK1 or CK-BB is found in the brain and all organs; it migrates anodically.

Mitochondrial Isoenzyme (CKm)

CKm migrates cathodically and is not formed from M or B subunits. It is characterized by its affinity for creatine phosphate and its heat stability (5 minutes at 56°C). Treatment with 2 mmol/L urea at 25°C for 30 minutes yields a subunit with an isoelectric point close to that of CK-MM.

Significant amounts of the cathodally migrating mitochondrial CK isoenzyme are found in the serum of the majority of patients with neoplastic diseases. The presence of mitochondrial CK is considered a marker for carcinoma in the metastatic stage. In adults, it is a poor prognostic indicator (intestinal cancer or serious pathological conditions). This isoenzyme is also found in the serum of neonates, indicating mitochondrial hyperactivity and tissue anoxia (following surgical procedures).

Atypical Forms (Macro CK)

- A CK-BB/IgA or IgM complex that migrates between CK-MM and CK-MB
- A CK-MM or CK-BB/IgA complex that migrates toward the cathode

In normal individuals, the total enzymatic activity of CK is between 20 – 75 IU/L at 30°C. Electrophoresis shows that this normal activity is due almost completely to CK-MM. The other CK isoenzymes are present in negligible amounts.

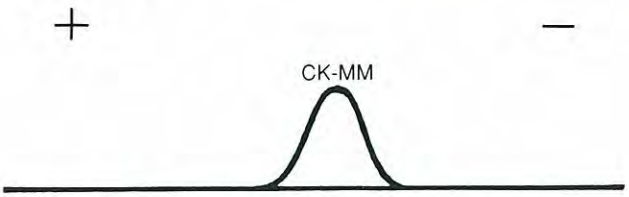
At normal protein levels (7.0 g/dL), CK-BB is the most heat sensitive fraction (37°C). The presence of CK-BB in the serum indicates a significant alteration in the injured organ such as infarct, cranial trauma, venous or arterial lesions, and carcinomas of diverse origins. The CK-BB isoenzyme accounts for a significant proportion of the total CK in isolated cases: acute pulmonary edema, heroin addiction.

Increased CK-MB activity (> 5% of the total) is diagnostic for myocardial, intestinal and placental infarcts. In the early phase of myocardial infarction, monitoring the CK-MB isoenzyme activity every 3 hours during the first 24 post-infarct hours indicates efficacy of therapy and relapse. The Electrophoretic profile can vary according to the time the sample was drawn, how soon electrophoresis was performed and the size of the infarct. The early appearance of CK-BB, CK-MB and CKm isoenzymes indicates very severe myocardial damage.

An increase in the total CK activity (> 20,000 IU/L) due solely to CK-MM can be found in rhabdomyolysis and muscle trauma from multiple causes.

CK Isoenzyme Patterns on Agarose Gel Electrophoresis

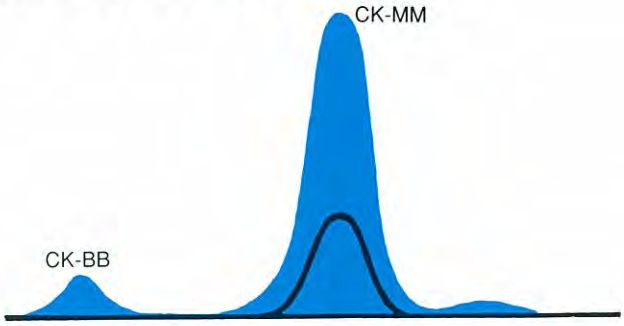
Normal



■ Abnormal □ Normal

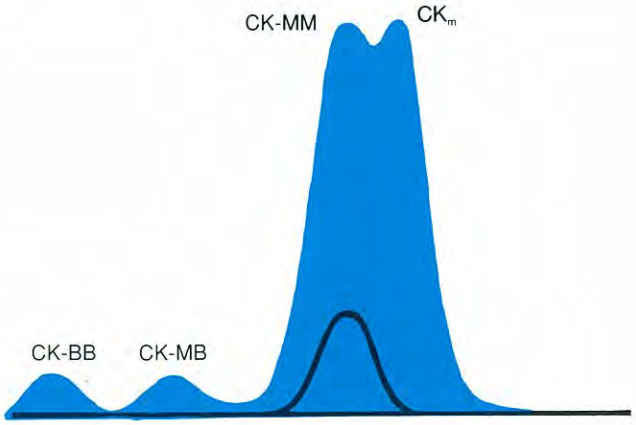
Total CK	up to 70 IU/L
CK-MM	95 – 99%
CK-MB	nondetectable
CK-BB	nondetectable

Cranial Trauma



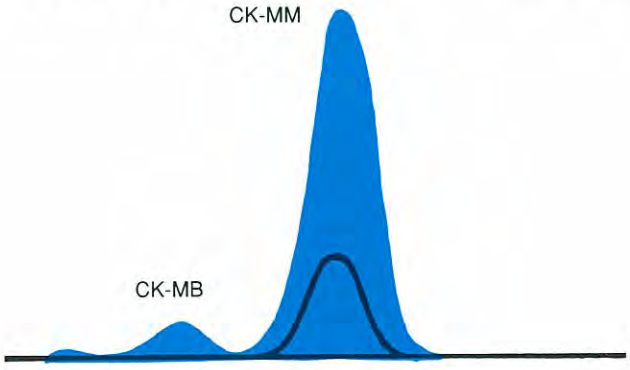
Total CK	> 70 IU/L
CK-MM	95 – 99%
CK-MB	nondetectable
CK-BB	> 2%

Massive Myocardial Infarction



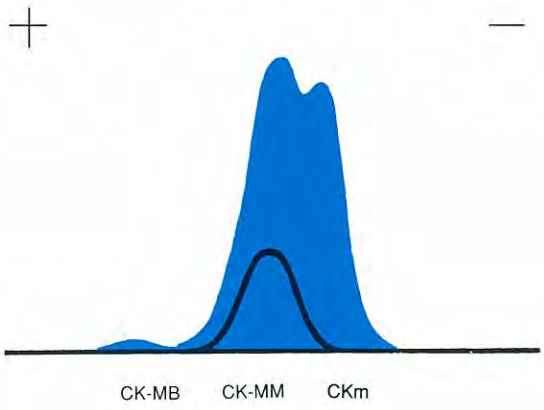
Total CK	> 70 IU/L
CK-MM	88 – 95%
CK-MB	up to 5 – 12%
CK-BB }	detectable as a function
CKm }	of the time of sample collection and the degree of necrosis

Moderate Myocardial Infarction



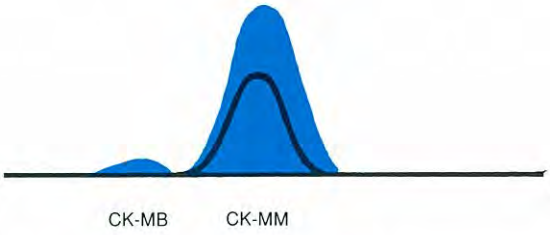
Total CK	> 70 IU/L
CK-MM	88 – 95%
CK-MB	up to 5 – 12%
CK-BB	nondetectable
CKm	nondetectable

Liver and Lung Cancer

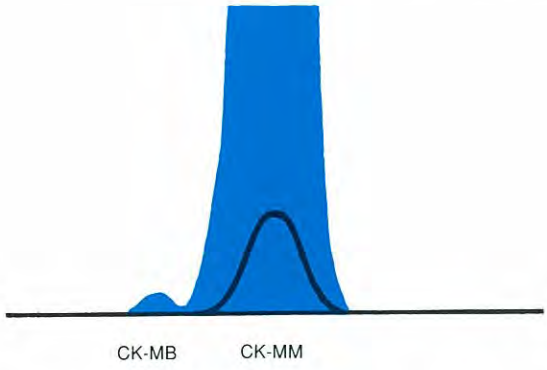


	Abnormal	Normal
Total CK		> 70 IU/L
CK-MM		up to 20 – 30%
CKm		70 – 80%
CK-MB		nondetectable
CK-BB		nondetectable

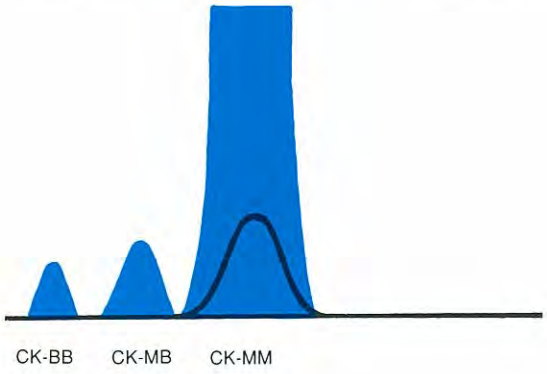
Excessive Muscular Activity



Duchenne's Muscular Dystrophy



Malignant Hyperpyrexic Shock (Renal Shock)



CK Isoenzyme Profile Following Cardiac Surgery

Time after surgery	Total CK IU/L	CK-MM IU/L	CK-MB IU/L	CK-BB IU/L	Electrophoresis Pattern		
					CK-BB	CK-MB	CK-MM
3 hrs	1,446	1,151	245	50			
8 hrs	2,100	1,771	329	0			
16 hrs	2,046	1,847	199	0			
20 hrs	1,770	1,698	72	0			
24 hrs	1,690	1,597	93	0			
32 hrs	1,642	1,586	56	0			
48 hrs	514	514	0	0			

The disappearance of CK-BB and the progressive decrease of CK-MB is a favorable prognostic indicator.

2/2 Lactate Dehydrogenase (LD)

Lactate dehydrogenase (LD) is found in virtually every human tissue with the liver, skeletal muscles and the heart having the highest concentration. The red blood cells, the kidney, the lung, the pancreas and the brain all have about equal amounts of LD.

1 – Serum LD Isoenzymes

Five LD isoenzymes can be separated by routine electrophoresis (cellulose acetate, agarose). Each isoenzyme has been numbered according to its electrophoretic mobility. The fastest migrating fraction (most anodal) is designated LD1 and is found predominantly in cardiac muscle and in the brain. The slowest (most cathodic) fraction is LD5 and is found primarily in the liver, skeletal muscle and saliva. The others: LD2, LD3 and LD4 are found to varying degrees with LD1 and LD5 in all tissues.

Each isoenzyme is a tetramer (composed of 4 polypeptide chains) and differences in electrophoretic mobility are due to the combination of polypeptide chains. There are only two different polypeptide chains, but it is the combination of these two chains that determines the structure of each isoenzyme.

The H polypeptide chain is so named because it has been isolated from the heart. The M chain has been found in skeletal muscle. LD1 is composed of four identical H chains and LD5 is composed of four M chains. The polypeptide configuration of each isoenzyme is as follows:

LD1	H	H	H	H	H_4
LD2	H	H	H	M	H_3M
LD3	H	H	M	M	H_2M_2
LD4	H	M	M	M	HM_3
LD5	M	M	M	M	M_4

All LD isoenzymes catalyze the same reversible reaction:

$$\text{L-lactate} + NAD^+ \underset{}{\overset{LD}{\rightleftharpoons}} \text{Pyruvate} + NADH + H^+$$

However, there is a difference among the isoenzymes as to substrate preference and direction of the reaction. Tissues engaged primarily in aerobic metabolism contain more H than M chains and prefer the lactate → pyruvate reaction while tissues more involved in anaerobic metabolism have more M chains and favor the pyruvate → lactate reaction.

Normal human serum contains a small amount of circulating LD due to normal tissue breakdown. LD levels increase significantly following tissue damage. It is possible in many cases to determine the organ(s) affected by performing LD isoenzyme studies.

In normal human serum, LD2 is the most prevalent isoenzyme and the ratio of LD1/LD2 is less than 1. Following myocardial infarct, there is a noticeable increase in LD1 to the extent that the ratio of LD1/LD2 is generally equal to or greater than 1. This "flipped" LD1/LD2 ratio is used to confirm myocardial infarction.

The LD level begins to rise approximately 12 – 24 hours after necrosis, frequently reaching a level two to three times (or greater) the upper limit of normal. Maximum activity is reached between the third and fourth day and can remain elevated two weeks after infarction. In 80% of the patients who have had myocardial infarction, the "flipped" LD1/LD2 ratio will be found 48 hours after necrosis.

Liver and muscle diseases can be detected by an elevated LD5.

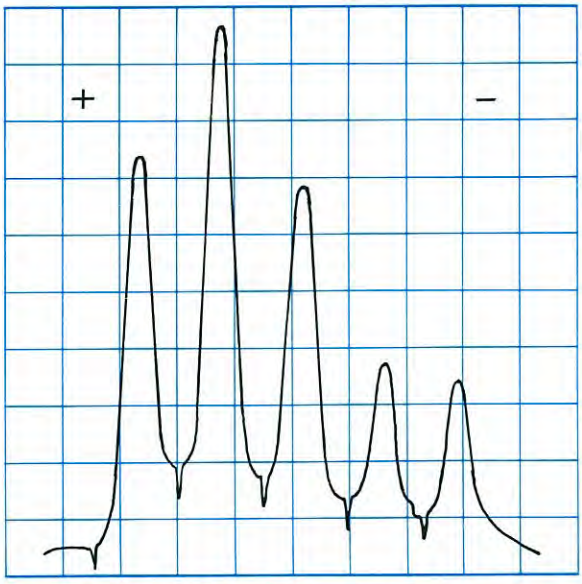
Pathological Significance of the Different LD Isoenzymes*

Increased LD1, LD2	Increased LD3	Increased LD4, LD5
Myocardial infarct or surgery	Rapid destruction of lymphocytes	Liver disease
In vivo or in vitro hemolysis	Infectious mononucleosis	Skeletal muscle infections
Cellular tumors	Infection of the lymphoid tissue	Prostate cancer
Renal infarct	Splenic necrosis	Malignancies
Muscular dystrophy	Malignancies	Septic shock
	Pulmonary embolism	

*From McKenzie

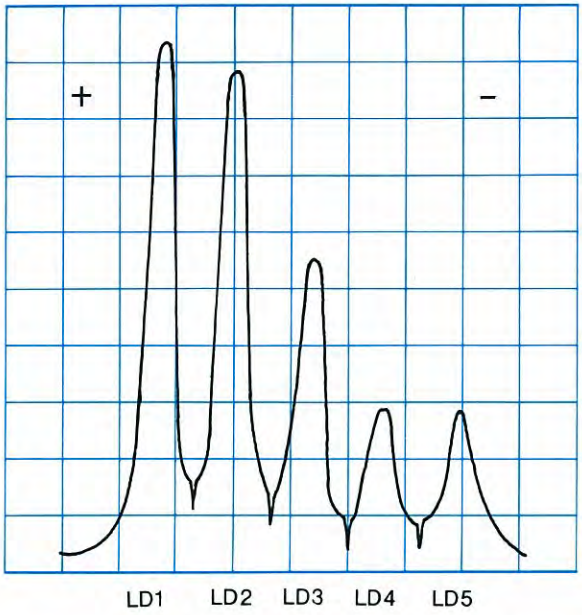
Typical Isoenzyme Results

Normal Pattern



Total LD = 100 – 300 IU/L at 30°C

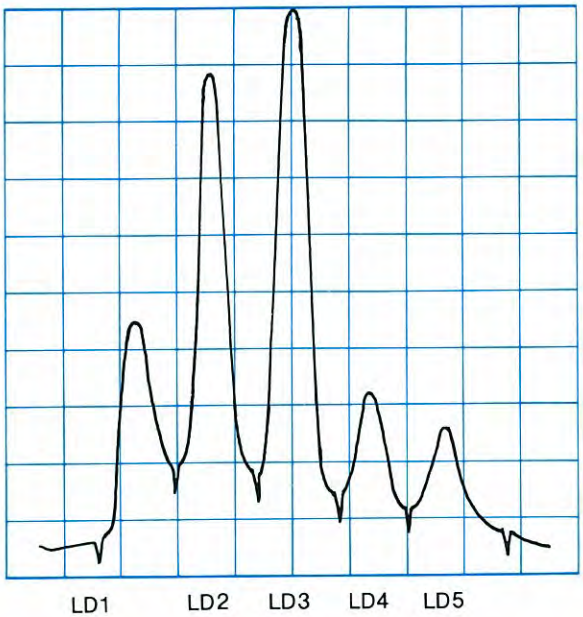
Fraction	%
LD1	20 – 30
LD2	32 – 35
LD3	20 – 25
LD4	7 – 10
LD5	7 – 10



Myocardial Infarction

Total LD = 1,640 IU/L

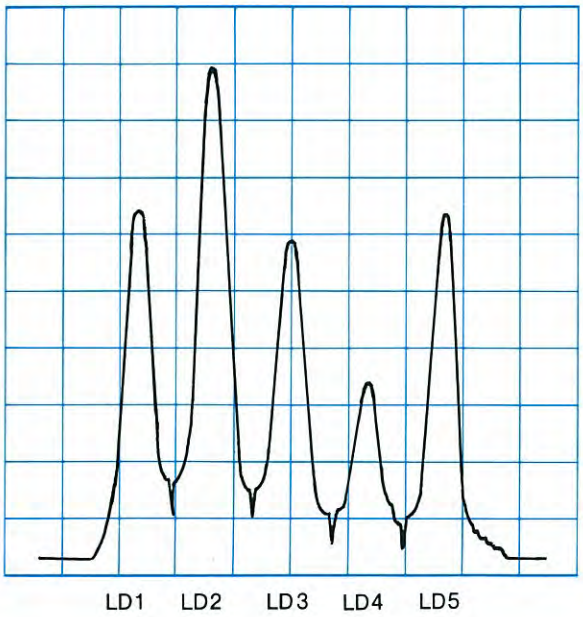
Fraction	%
LD1	35
LD2	31
LD3	18
LD4	8
LD5	8



Pulmonary Embolism

Total LD = 470 IU/L

Fraction	%
LD1	15
LD2	32
LD3	40
LD4	8
LD5	5



Liver Disease

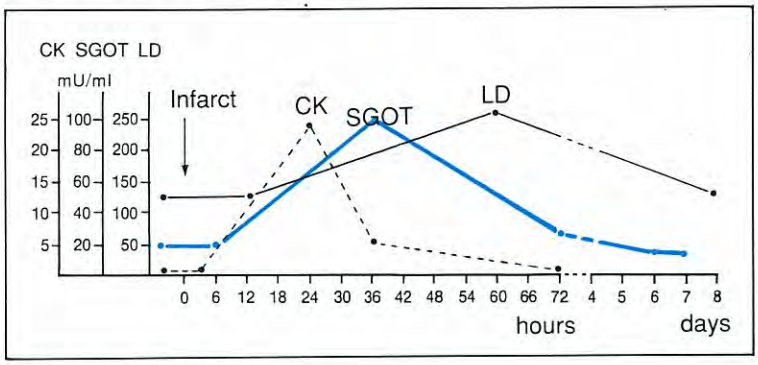
Total LD = 750 IU/L

Fraction	%
LD1	20
LD2	31
LD3	20
LD4	11
LD5	18

Interpretation of the CK/LD Isoenzyme Profile

It is now evident that the isoenzymes of CK and LD are the tests necessary to confirm or rule out myocardial infarction. Whereas CK-MB (heart fraction) appears early after the start of an infarct, the "flipped" LD1/LD2 ratio doesn't appear until sometime later.

Typical Cardiac Enzyme Patterns as a Function of Time*



*From F.W. Schmidt, The Principles of Diagnostic Enzymology

If an increase of CK-MB is not observed during the first 24 hours, it is almost certain that the patient has not suffered a myocardial infarction. The presence of an increased CK-MB and a "flipped" LD1/LD2 ratio in the first 48 hours is proof of a myocardial infarction.

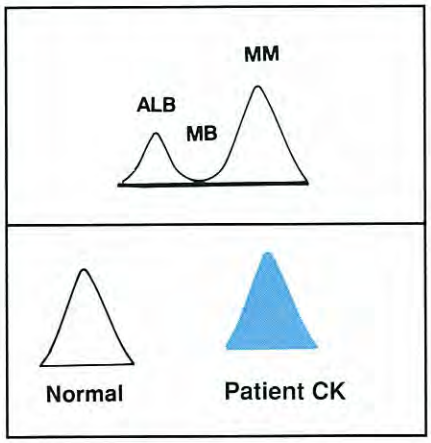
An increase in CK-MB activity, but with a normal LD pattern indicates coronary insufficiency and intermediate syndrome, angina pectoris, and mild MI.

Cardiac Isoenzyme Profiles

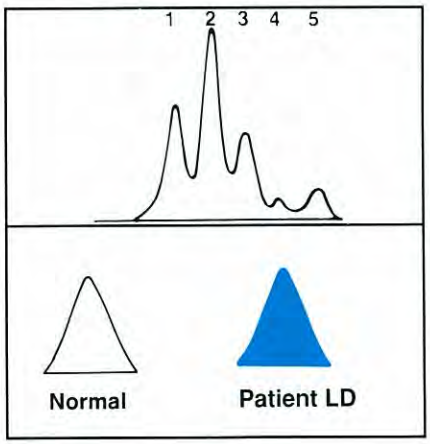
Normal Patterns

In the patterns that follow, the normal pattern is outlined in black. The patient's CK isoenzymes are in light blue and the darker blue represents the patient's LD isoenzymes.

CK Isoenzymes



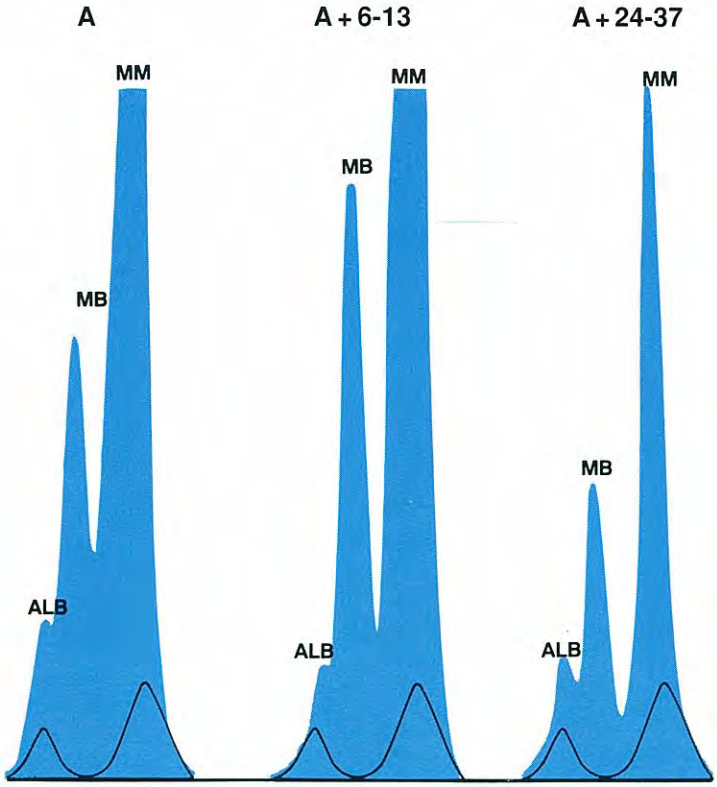
LD Isoenzymes



These patterns show the isoenzyme levels over time:
A = Sample drawn at time of admission to CCU
A + 6-13 = Sample drawn 6 to 13 hours after admission
A + 24-37 = Sample drawn 24 to 37 hours after admission

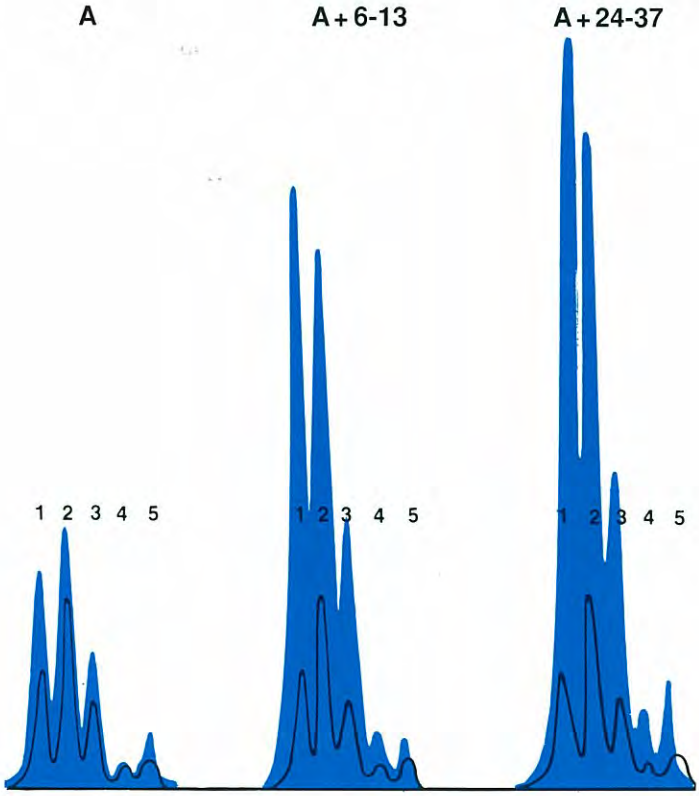
Massive Myocardial Infarction

CK Isoenzymes



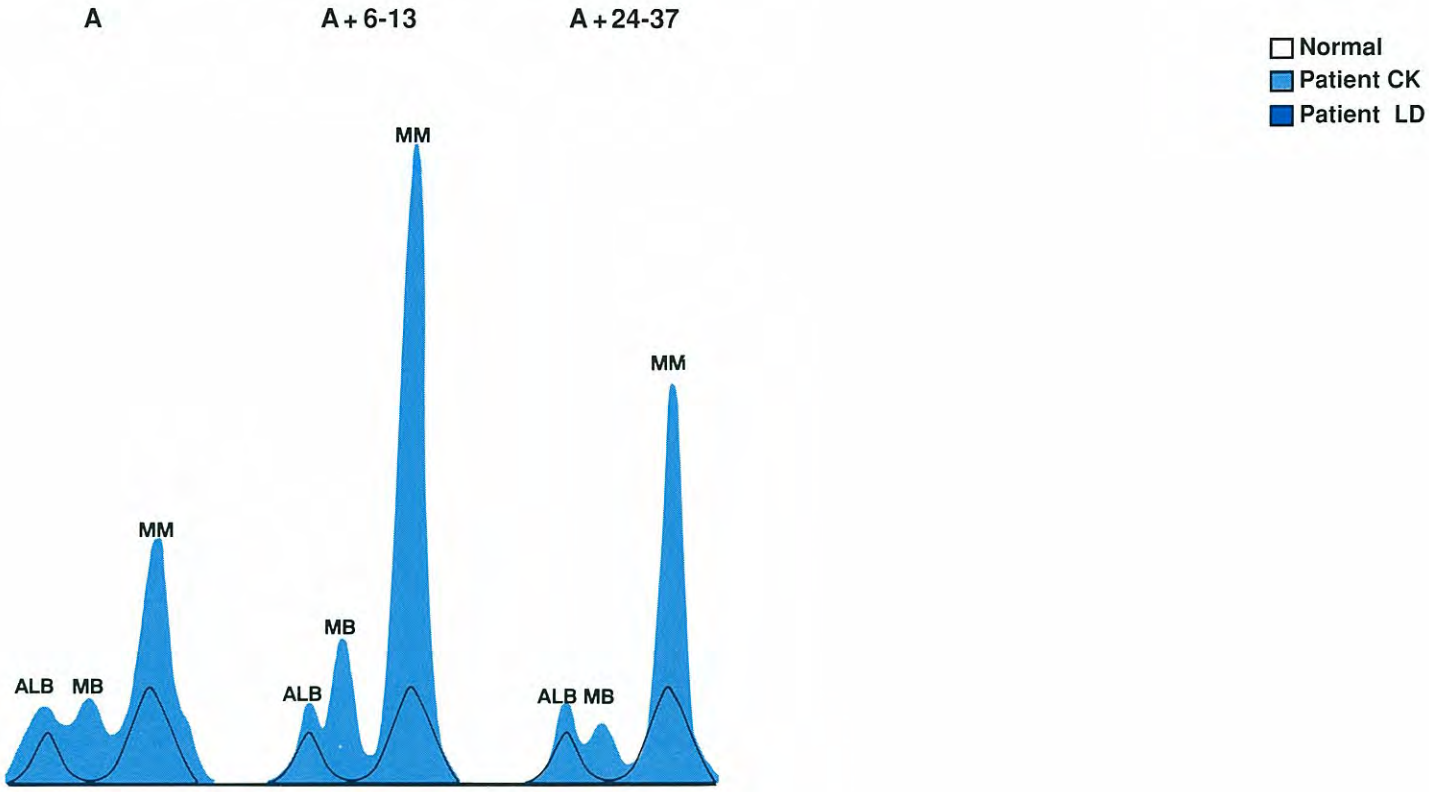
Normal
Patient CK
Patient LD

LD Isoenzymes

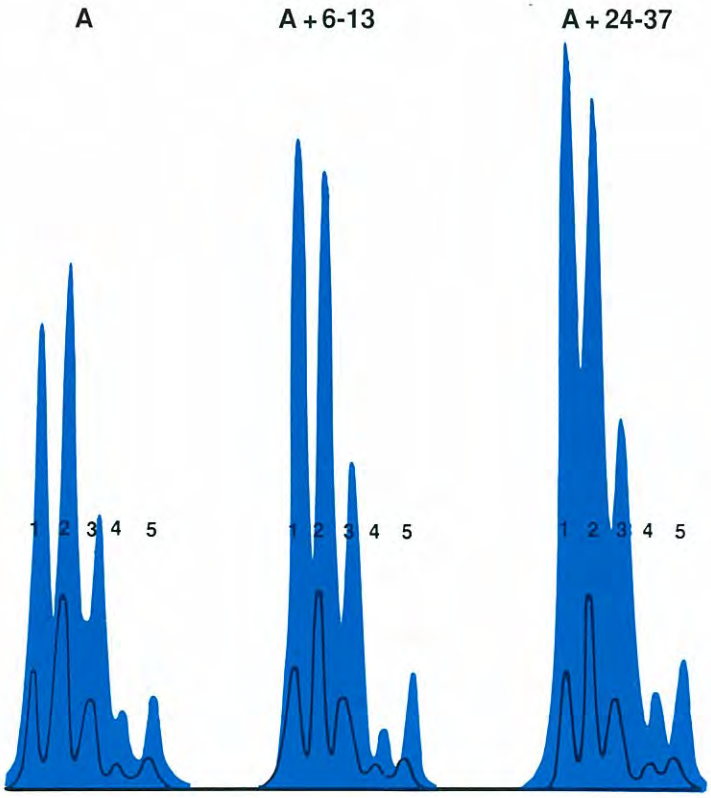


Moderate Infarction

CK Isoenzymes

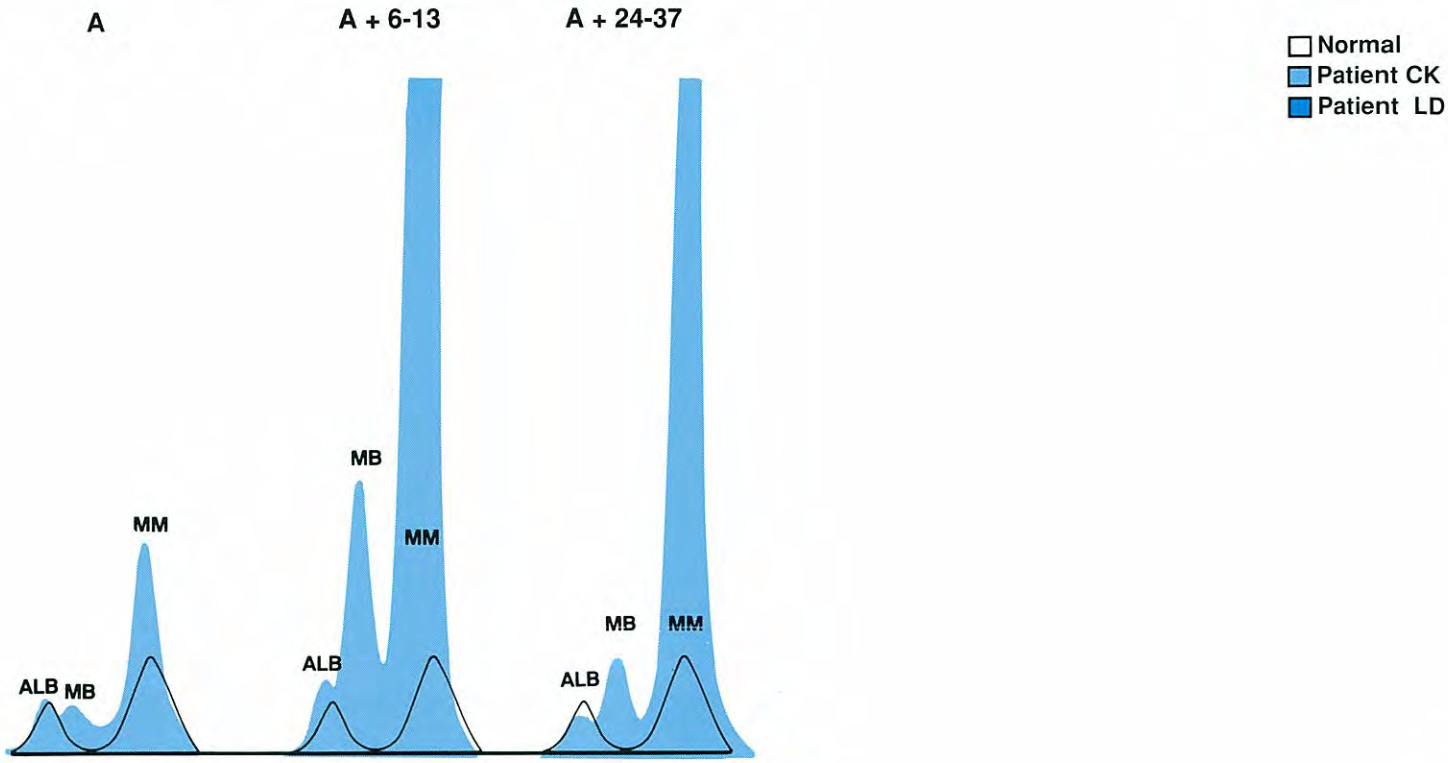


LD Isoenzymes

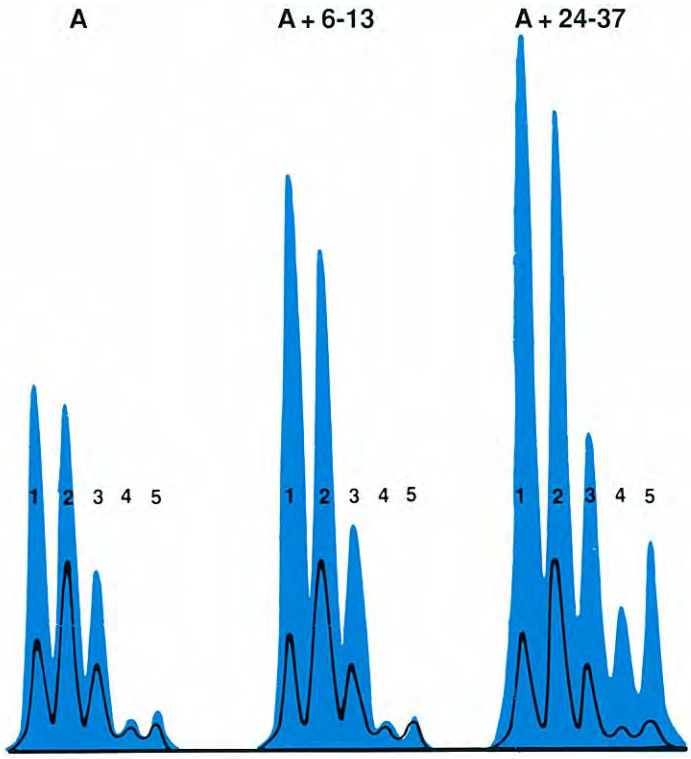


Moderate Infarction – Possible Reinfarction

CK Isoenzymes



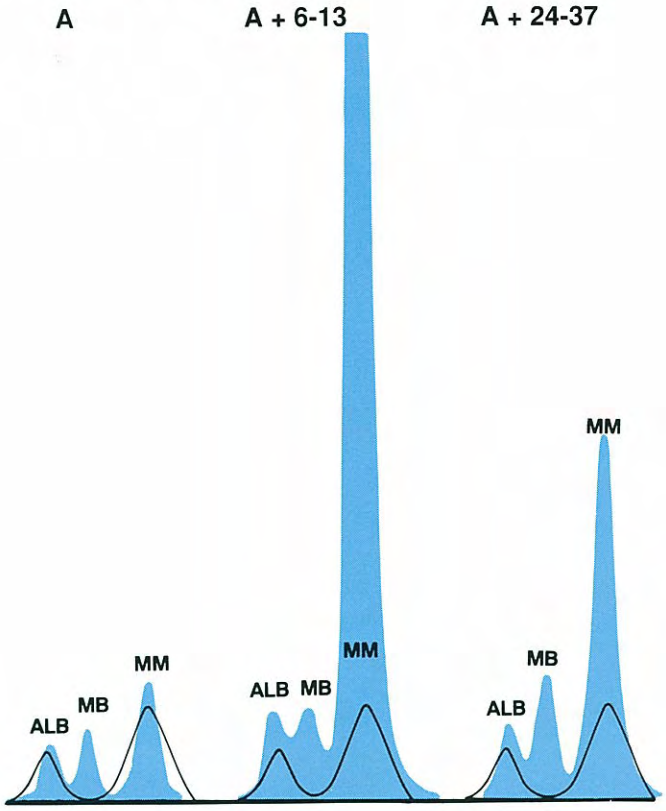
LD Isoenzymes



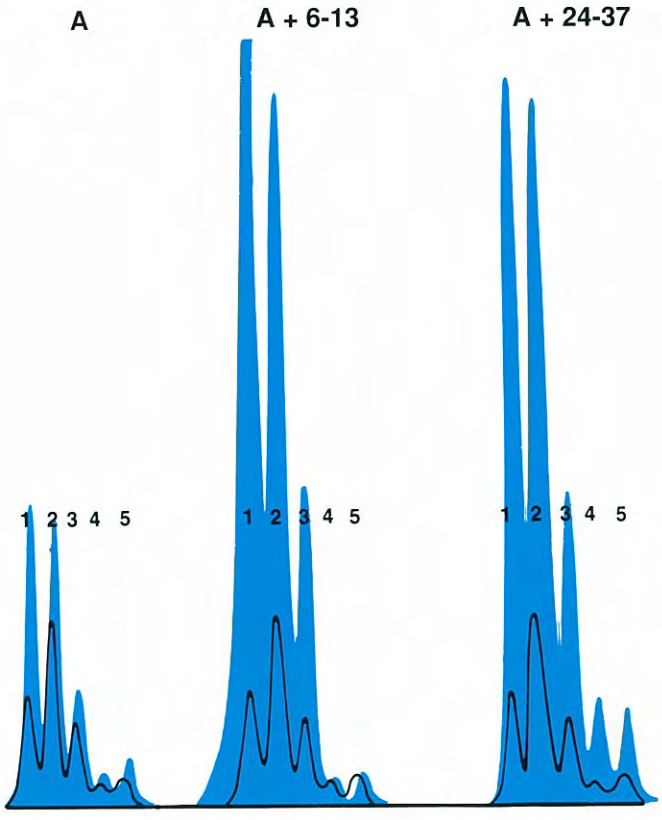
Infarction with Extension

Normal
Patient CK
Patient LD

CK Isoenzymes

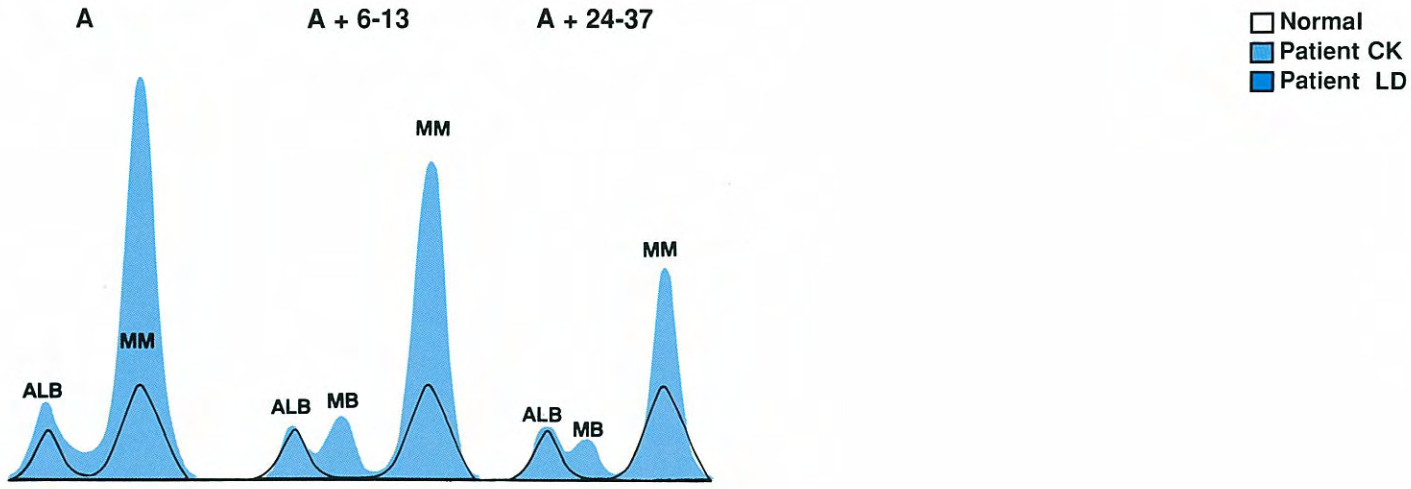


LD Isoenzymes

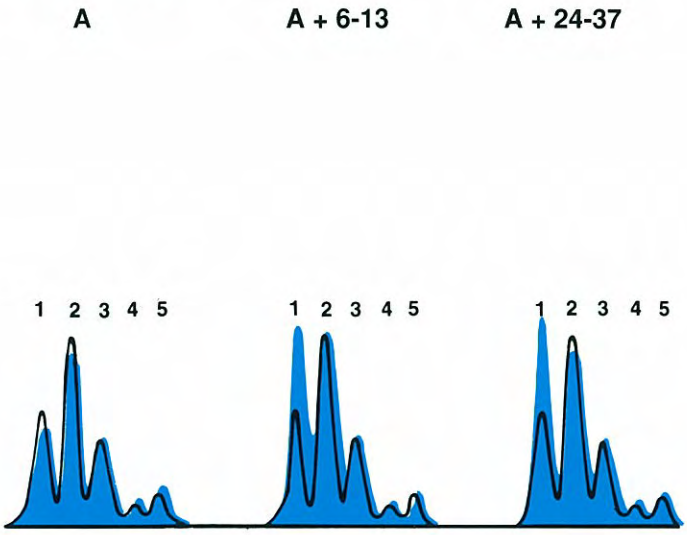


Mild Infarction

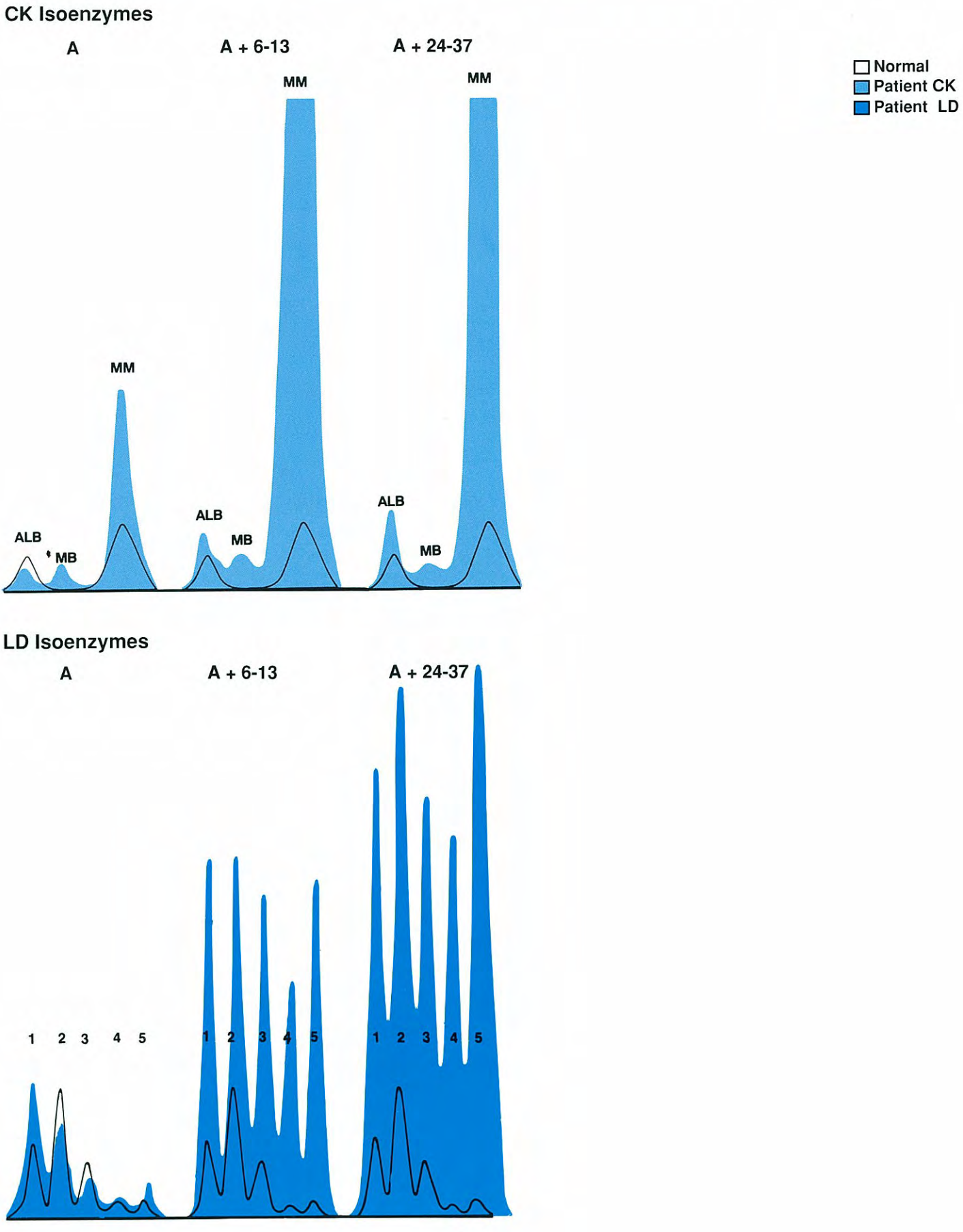
CK Isoenzymes



LD Isoenzymes

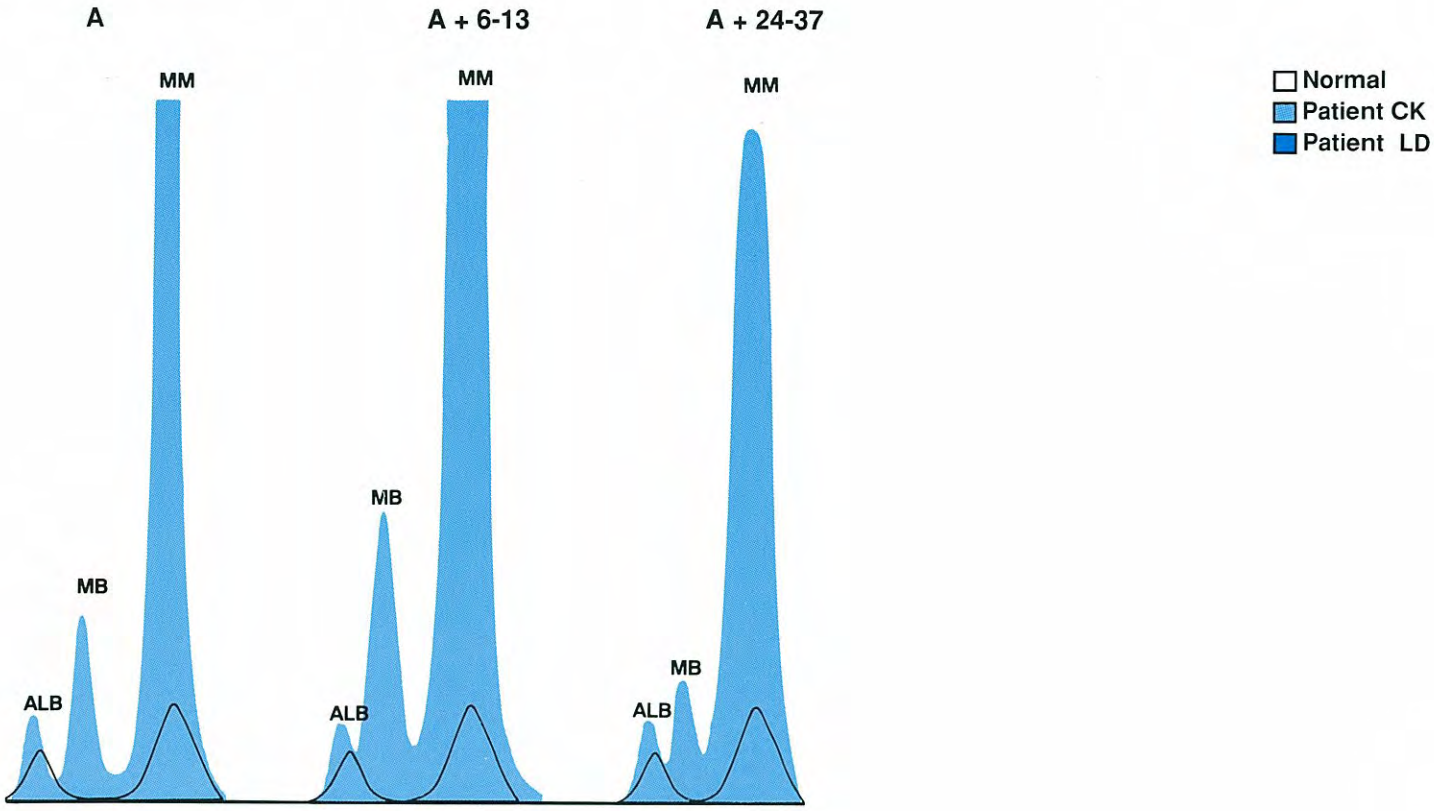


Myocardial Infarction Associated with Heart Failure

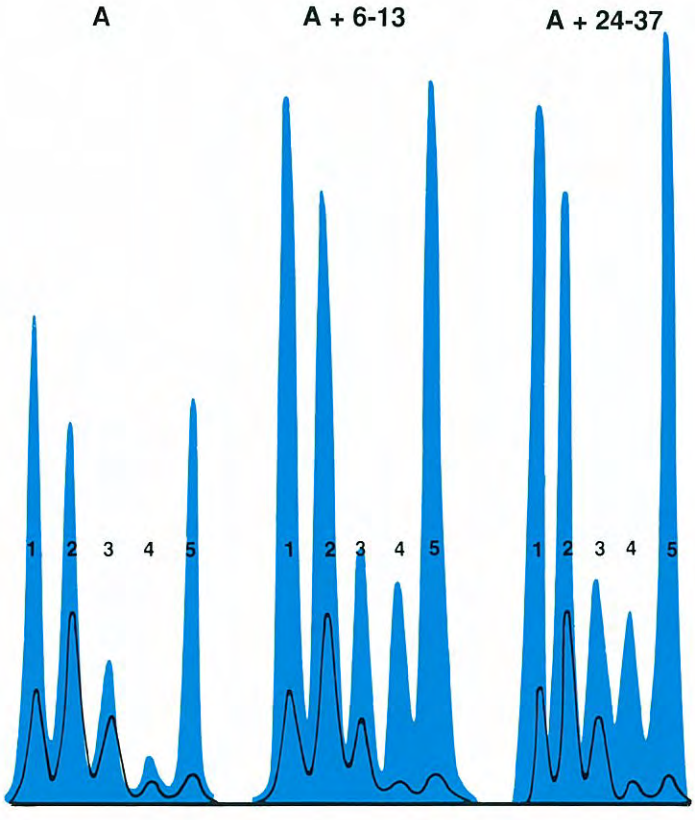


Myocardial Infarction Associated with Hepatic Congestion

CK Isoenzymes

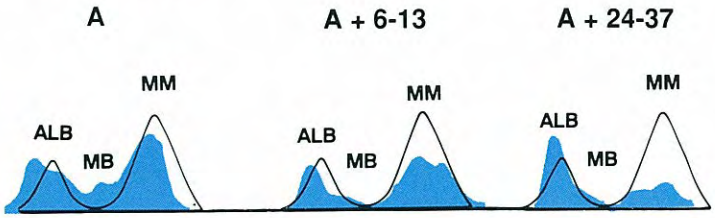


LD Isoenzymes



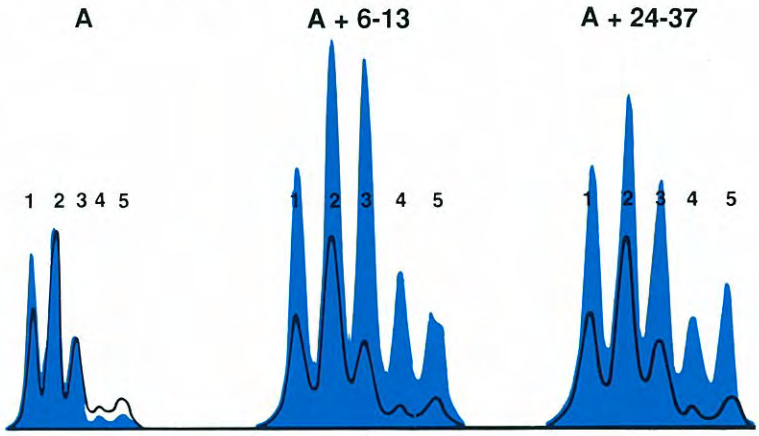
Pulmonary Infarct

CK Isoenzymes



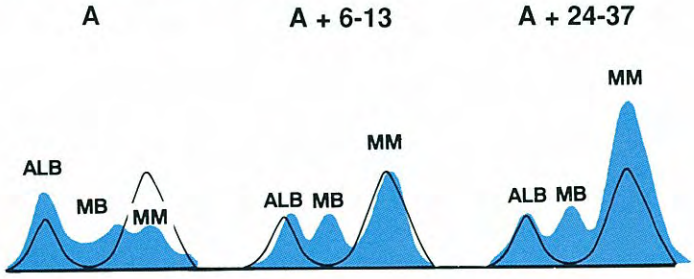
Normal
Patient CK
Patient LD

LD Isoenzymes

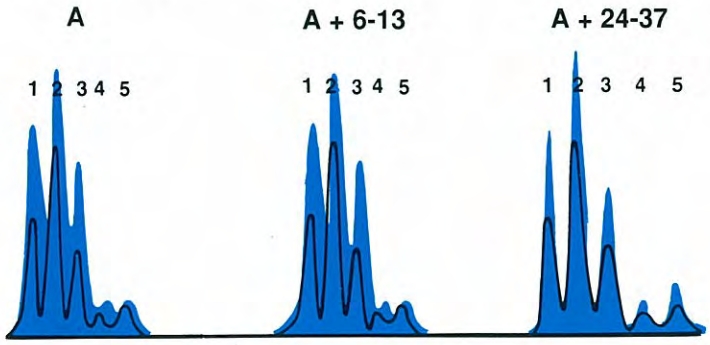


Myocardial Ischemia

CK Isoenzymes



LD Isoenzymes



2 – LD Isoenzymes in Saliva

The role of lactate as the mechanism for acidification of dental plaque is well established. Plaque is the major cause of buccal pathology, i.e. caries and periodontal disease. In a healthy adult, salivary LD isoenzyme activity is normally around 300 IU/L. In pathological conditions affecting the buccal cavity, values vary from 100 to 600 IU/L.

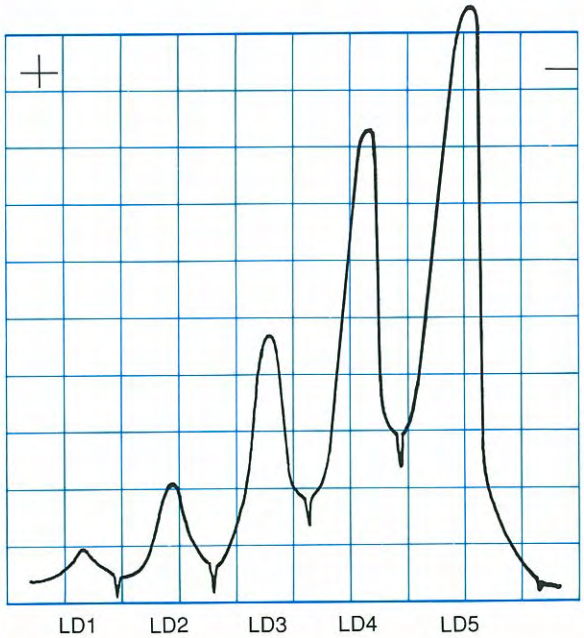
Salivary LD isoenzymes have two origins: endogenous and exogenous. Endogenous LD originates from the salivary glands, leukocytes, gingival fluid and the buccal epithelium. Exogenous LD originates from the microorganisms that make up the normal flora of the buccal cavity, primarily *streptococcus mutans* and *lactobacillus casei*.

The five LD isoenzymes found in saliva have the same electrophoretic mobility as those found in serum, but the distribution is very different (see normal pattern below). Most of the LD isoenzyme activity in saliva can be attributed to LD4 and LD5. These are the isoenzymes that are found in anaerobic tissue metabolism and are responsible for catalyzing the reduction of pyruvate to lactate.

In periodontal disease, total salivary LD enzyme activity is on the average seven times greater than normal, that is 2,000 IU/L or more. The distribution of the isoenzyme activity is the same as in normal saliva. (The serum LD pattern remains normal.)

Because periodontal disease is not evident until it reaches an irreversible stage, analysis of salivary LD activity is important as a good early marker of the pathological process.

Normal Pattern



Total LD 300 IU/L

Fraction	%
LD1	2
LD2	8
LD3	15
LD4	30
LD5	45

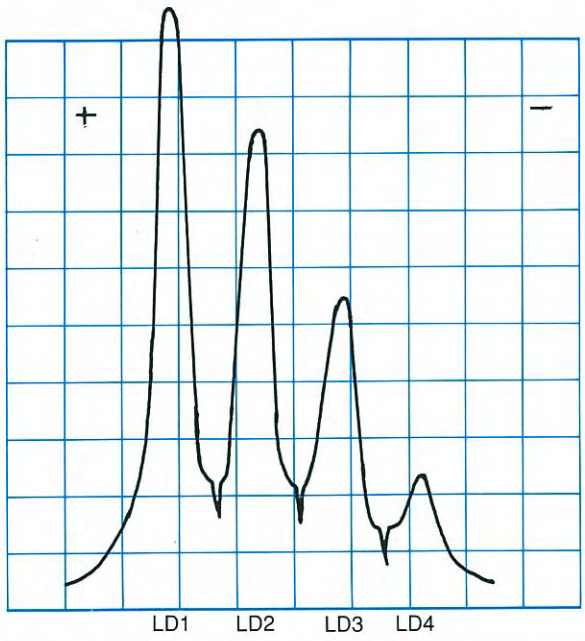
3 – LD Isoenzymes in CSF

Assessing total LD activity and the distribution of the LD isoenzymes in CSF is of interest in meningitis, coma due to trauma and central nervous system tumors. CSF LD activity is independent of serum LD activity and, in a healthy adult, is ≤ 30 IU/L.

After concentration of the CSF specimen, the normal electrophoretic pattern on cellulose acetate shows the presence of 3 to 4 isoenzymes: LD1, LD2, LD3, and, sometimes, LD4. The presence of these isoenzymes corresponds to aerobic cerebral metabolism.

Assessing the isoenzyme profile can be very useful in differentiating bacterial, fungal and viral forms of infectious meningitis. Total LD and lactate are very elevated in bacterial and fungal meningitis. Acidosis is more or less severe, favoring anaerobic metabolism of bacteria and leukocytes. Increased CSF pressure diminishes cerebral circulation and oxygenation. All five LD isoenzyme are present. Typically, in viral meningitis, LD activity is slightly elevated (≤ 50 IU/L) and only three isoenzymes are found (LD1, 2 and 3).

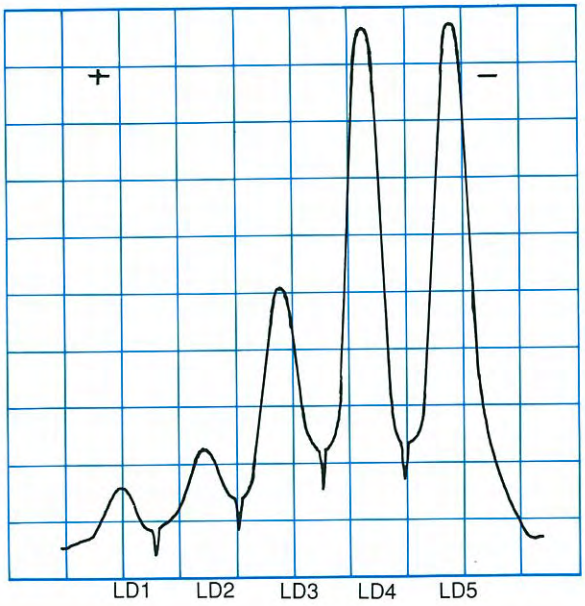
Normal Pattern



Total LD 20 IU/L

Fraction	%
LD1	45
LD2	30
LD3	20
LD4	5

Pattern in Bacterial Meningitis



Total LD 209 IU/L

Fraction	%
LD1	3
LD2	7
LD3	18
LD4	35
LD5	37

2/3 Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is an enzyme that exists in all tissues and organs. When tissue necrosis occurs, lysed cells release a large amount of alkaline phosphatase into the circulation. In normal serum, a small amount of alkaline phosphatase is present due to normal destruction of tissue. Abnormal cellular damage results in increased total alkaline phosphatase activity.

Alkaline phosphatase, like most other enzymes, is composed of multiple molecular forms or isoenzymes. Although their molecular structures are different, the isoenzymes all catalyze the same reaction – the hydrolysis of phosphate esters. Some organs have only one major isoenzyme rather than multiple isoenzyme forms. The liver, bone and intestine each have different isoenzyme forms.

Serum alkaline phosphatase isoenzymes can be separated by electrophoresis, permitting identification of the specific organ causing the increase in total enzyme activity.

In normal subjects, total alkaline phosphatase enzyme activity (measured at 30° C) varies according to the age of the subject:

0 – 2 months	100 – 230 IU/L
2 – 6 months	80 – 280 IU/L
6 mos – 3 yrs	100 – 230 IU/L
3 – 15 years	90 – 300 IU/L
> 15 years	30 – 100 IU/L

Electrophoretic separation of alkaline phosphatase on cellulose acetate allows visualization of several distinct isoenzyme fractions.

Interpretation of Results

Liver Isoenzyme

The major liver isoenzyme of alkaline phosphatase migrates to a position correlating to the alpha 2 serum protein fraction. Liver isoenzyme is found in the serum of all normal individuals. Elevations occur in a wide variety of conditions including drug induced liver disease, neoplastic cholestasis of primary and secondary cancers of the liver or bile duct, intrahepatic cholestasis due to viral hepatitis, cirrhosis, parasitic or inflammatory liver disease, and bile duct stricture. Increased liver isoenzyme may or may not be accompanied by an increased bilirubin.

Bone Isoenzyme

Migration of bone ALP isoenzyme correlates to the beta 1 serum protein position. Low levels of this isoenzyme are found in normal individuals. Physiological increases are normal in growing children. In pathological states, increases correlate to hyperosteoblastic activity. This hyperactivity manifests itself each time destruction of bone is accompanied by reconstruction, as seen in children with rickets and in adults with osteomalacia, hyperparathyroidism with osteodystrophy, Paget's diseases, bone tumors (osteogenic sarcoma, Ewing's sarcoma) and metastatic bone tumors treated with calcitonin.

Although its appearance is rare, a second bone isoenzyme that migrates in the alpha 1 – alpha 2 region has been reported in children. This isoenzyme, unlike the usual bone isoenzyme, is thermal stable and disappears with age without any affect on the child's development.

Fast Liver

This isoenzyme, also known as the biliary or macrohepatic fraction, appears in the alpha 1 region. It is never found in normal individuals. It is a very sensitive and early marker of intra and extra hepatic cholestasis, even when total alkaline phosphatase activity remains normal.

This isoenzyme is useful in oncology for following patient progress. Its appearance in serum indicates the possibility of liver metastisis and the need for specific follow up testing. Compared to other enzymes used for diagnosing hepatic disorders (total alkaline phosphatase, gamma GT, 5'nucleotidase), this fraction appears to be more specific and sensitive. It has been found in 96% of cancer patients with confirmed liver metastisis. It has also been found in about one-third of patients with liver cirrhosis and occasionally in patients with non-cancerous liver diseases (hepatitis, lithiasis).

Intestinal Isoenzyme

This isoenzyme, which migrates in the beta 2 area, is seen in normal, healthy individuals with type B or O blood, especially following a meal. In pathological states, it is associated with cirrhosis, sugar diabetes and cancerous diseases of the intestinal tract. When accompanied by the presence of fast liver, it can be an indicator of intra-hepatic cholestasis.

Placental Isoenzyme

Does not exist physiologically, except in pregnant women beginning with the 16th week and increasing until term. It disappears approximately 3 to 6 days following delivery. Although significantly increased in cases of hypertension and preeclampsia, the level is often variable and, therefore, of mediocre value in monitoring pregnancy. This isoenzyme is remarkably thermal stable at 65°C. This property permits it to be easily differentiated from other ALP isoenzymes.

Oncogenic Isoenzymes

A number of oncogenic isoenzymes have been identified: Nagao, Regan, Timberly. Their electrophoretic mobility, antigenicity and thermostability are identical to placental isoenzyme. These oncogenic isoenzymes appear inconsistently in certain types of neoplasms (ovarian cancer, broncho-pulmonary cancers). The band disappears following successful treatment of the cancer.

Alkaline Phosphatase and Immune Complexes

Electrophoresis shows a weak, diffuse pattern caused by complexing of IgG (or more rarely, IgA) to bone or liver ALP isoenzyme. The complex can be disassociated by treating the serum with trypsin. The significance of this complex in autoimmune diseases is debatable. It has been observed in nervous system disorders and in neoplasias with or without secondary hepatitis or the presence of the fast liver band. The disappearance of the complex correlates well with clinical amelioration of disease, while its persistence is associated with grave prognostic outcome. These immune complexes have not be observed in healthy individuals.

Ultra-Fast Liver

This fraction migrates ahead of the alkaline phosphatase isoenzymes and is thought to be an albumin-bilirubin complex rather than an isoenzyme. It is found in icteric patients. This band is visualized as the result of a diazo reaction with the reagent used for visualization of the alkaline phosphatase isoenzymes. It can be excluded from the densitometric calculations.

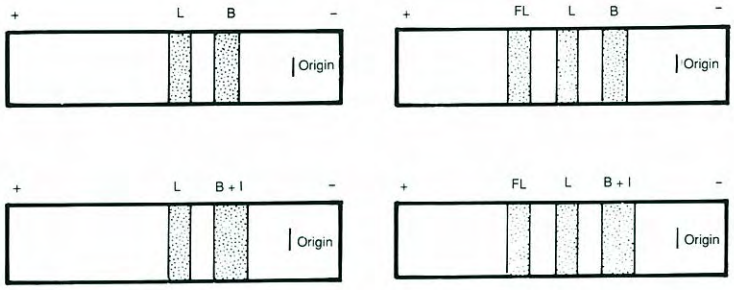
Normal Values

The following results were obtained in a study of 130 healthy male and female individuals between the ages of 20 and 90 years:

Fast Liver Isoenzyme .....0 %
Liver Isoenzyme .....50 %
Bone Isoenzyme.....50 %
Intestinal Isoenzyme..... 0 - 10 %

Notes

Identification of alkaline phosphatase isoenzymes can be complemented by heat denaturation of the serum sample. Heat denaturation at 56°C enhances the differentiation of liver and bone isoenzymes. Placental and oncogenic isoenzymes are characterized by thermostability at 56°C. Electrophoresis is performed by running the heat treated and untreated samples side by side on the cellulose acetate plate.

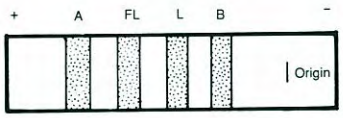


Legend

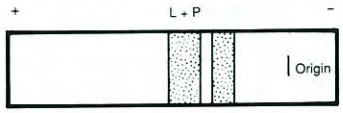
A = Albumin
FL = Fast Liver
L = Liver
B = Bone
I = Intestine
P = Placenta
IC-ALP = Alkaline Phosphatase with Immune Complex

Particular Cases

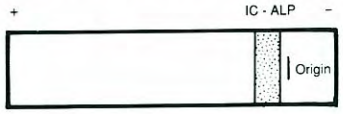
Hyberbilirubinemia



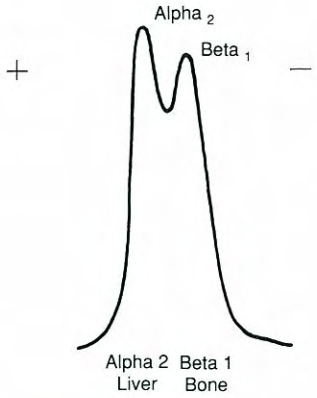
Placental Isoenzyme



Immune Complexes

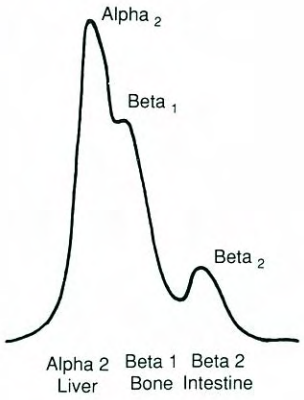


Normal Pattern



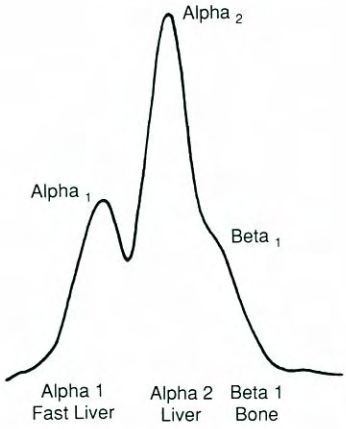
Avg. Total ALP:	**70 IU/L**
	(Normal < 120 UI/L)
Fraction	**%**
Alpha 1	0
Alpha 2	52
Beta 1	48
Beta 2	0

Pathological Profiles



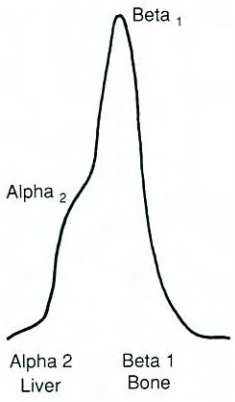
Liver Cirrhosis

Total ALP:	**175 IU/L**
Fraction	**%**
Alpha 1	0
Alpha 2	63
Beta 1	22
Beta 2	15



Biliary Obstruction

Total ALP:	**380 IU/L**
Fraction	**%**
Alpha 1	22
Alpha 2	61
Beta 1	17
Beta 2	0



Hyperosteoblastic Activity

Total ALP:	**320 IU/L**
Fraction	**%**
Alpha 1	0
Alpha 2	22
Beta 1	78
Beta 2	0

3 Hemoglobin Electrophoresis

Hemoglobinopathies are genetic anomalies characterized by qualitative or quantitative variation of the hemoglobin.

To date, there are more than 300 abnormal hemoglobins that can be identified in man. Of these, hemoglobins S, C, D and E are of greatest clinical significance. This discussion primarily addresses hemoglobins S and C, and the thalassemias. Electrophoresis is indispensable for diagnosing these disorders.

The Genetics of Hemoglobins

All hemoglobinopathies are governed by the laws of genetics. The vast majority of abnormal hemoglobins result from the mutation of a single polypeptide chain. These anomalies are transmissible, hereditary, autosomal traits.

In the heterozygous subject, an abnormal gene is inherited from one parent and it directs the formation of an abnormal hemoglobin. Theoretically, one part of the hemoglobin is abnormal and the other is normal. In the homozygous subject, identical abnormal genes are inherited, one from each parent, and the majority of hemoglobin is abnormal.

It is also possible to inherit two different hemoglobin abnormalities, one from each parent. Even with two abnormal hemoglobins, a small portion of the hemoglobin may be normal.

Only about a fourth of these mutations are detected by frequently used methods, such as hemoglobin electrophoresis at various pH values. Other methods are necessary for pursuing complete identification of the abnormal hemoglobin.

In the heterozygous state, the majority of hemoglobinopathies are only rarely accompanied by overt clinical symptoms. In contrast, homozygous states are almost always clinically and hematologically apparent.

Values for Normal and Physiological Hemoglobin Variants

Total hemoglobin is generally expressed as grams of hemoglobin per 100 mL of venous blood. In males, normal values are between 13.6 and 17.2 g/100 mL of blood (average value: 15.4); in females, values are slightly lower and are between 11.5 and 15.0 g/100 mL (average value: 13.3).

Significant variation can be observed as a function of age. At birth, total hemoglobin is very elevated (21 g/100 mL). But the total rapidly decreases to a minimum level (12 g/100 mL) that persists until about 12 years of age, then slowly increases to normal adult levels.

The Structure of Hemoglobins

The hemoglobin molecule consists of two parts: a porphyrin group or heme, and the protein or globin portion. Globin is made up of four polypeptide chains attached to the porphyrin ring. In the normal subject these chains can be of four types: alpha, beta, delta and gamma. In normal and abnormal hemoglobins (with the exception of hemoglobin H and Bart's), two sets of identical polypeptide chains make up the globin (i.e. α_2 $ß_2$ or α_2 δ_2).

The structure of the globin chain, like all proteins, is genetically controlled.

Normal Hemoglobins

In a normal adult, one finds hemoglobin A or A_0 (α_2 $ß_2$) and hemoglobin A_2 (a_2d_2).

Normal Values

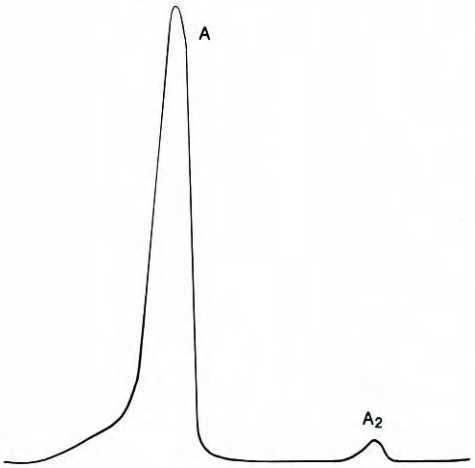
HbA	96.6 – 98.5%
HbA_2	1.5 – 3.0%

Electrophoresis is done on cellulose acetate at an alkaline pH. In old blood samples, a light, diffuse band, called HbA_3, can often be seen in front of HbA.

In the newborn, hemoglobin F (α_2 γ_2) represents nearly all the pigment during uterine life. It is slowly replaced by hemoglobin A.

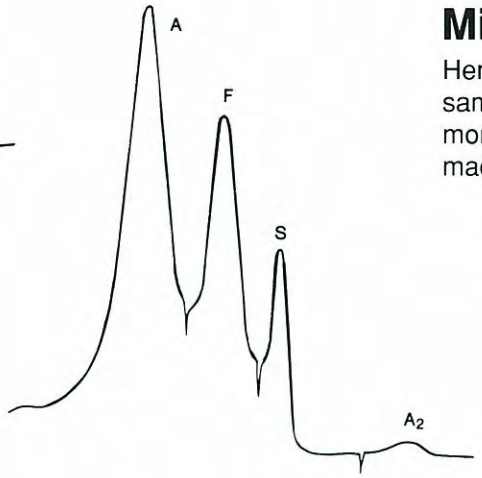
For a few children, the persistence of fetal hemoglobin requires careful interpretation and only the study of the parents' hemoglobins can bring a solution in some cases.

Levels of HbA_2	
Cord Blood	0 – 1.8%
Birth – 6 months	0 – 3.5%
After 6 months	1.5 – 3.5%
Beta thalassemia minor	3.7 – 6.5%
Beta thalassemia major	Normal or Elevated
Hemoglobin H	Normal or Decreased
Heterozygous depranocytosis	1.7 – 4.5%



Normal Hemoglobin

Hemoglobin A = 98.6%
Hemoglobin A_2 = 3.2%



Migration Marker

Hemoglobins D and S migrate in the same position, as do C and A_2. A more conclusive identification can be made following additional testing.

Hemoglobin Anomalies

Some anomalies are qualitative, that is to say that the structure of the hemoglobin chain is abnormal due to the substitution of one or more amino acids (hemoglobinopathy). Other anomalies are quantitative, that is there is a deficiency in the synthesis of one of the globin peptide chains (thalassemia).

In all cases, workup for diagnosis should include
– Ethnic background
– Hemoglobin electrophoresis on cellulose acetate (alkaline pH) and agarose (acid pH).

3/1 Qualitative Evaluation of Hemoglobinopathies

A large number of mutations have been observed. Only a few are of serious biological consequence. The three most important, for the practitioner, are those that involve hemoglobin S (depranocytes), hemoglobin C and hemoglobin E.

Sickle Cell Anemia

Depranocytosis or sickle cell anemia is present almost exclusively in the black race. The disorder is found in 10% of black Americans and in almost 40% of black Africans in certain regions. Worldwide there are more than 10 million carriers of the disorder.

In sickle cell anemia, the characteristic sickled appearance of the red cells, also called depranocytes, is due to the presence of hemoglobin S.

Homozygous S disease is characterized by the almost exclusive presence of hemoglobin S on electrophoresis. HbA_2 is present in variable quantities (1 to 5%). HbA is notably absent.

In sickle cell trait (heterozygous S), hemoglobin electrophoresis shows a band of HbA, a band of HbS and normal production of HbA_2. The concentration of HbS varies from 20 to 50%.

Hemoglobin S is also found in association with other hemoglobinopathies.

- Hemoglobin S Thalassemia: The clinical severity of this double heterozygous mutation is related to the percentage of normal and abnormal hemoglobins produced.
- Hemoglobin S/C Disease: No hemoglobin A is synthesized. The condition is serious, approaching that of homozygous SS disease.
- Hemoglobin S and the Hereditary Persistence of Fetal Hemoglobin (HPFH): The presence of hemoglobin F inhibits sickling, which decreases the severity of the disease.

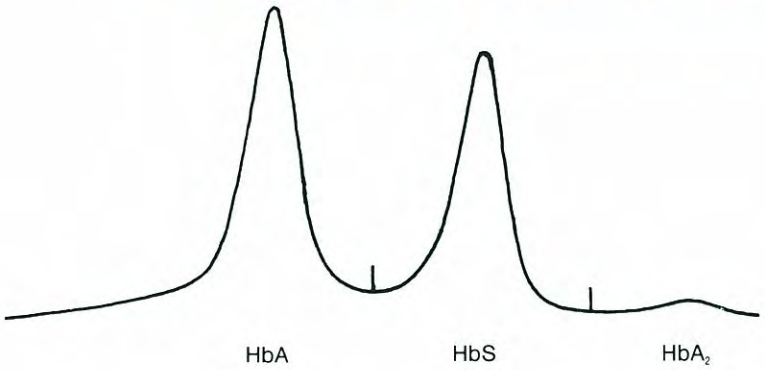
Hemoglobin C Disease

The homozygous form is rare and primarily found in West Africa. The A/C heterozygote does not manifest clinical or hematological symptoms. Hemoglobin C is a slow migrating hemoglobin on alkaline cellulose acetate electrophoresis.

Hemoglobin E

Hemoglobin E is very common in the Far east. The heterozygote has no clinical manifestations. A double heterozygote for hemoglobin C and E or HbC/thalassemia, greatly worsens the prognosis. Electrophoresis shows hemoglobin E migrates with HbC and HbA_2.
Many abnormal hemoglobin variants have been described, but only a few occur with sufficient frequency to cause a significant incidence of disease and many do not cause any recognizable clinical manifestation.

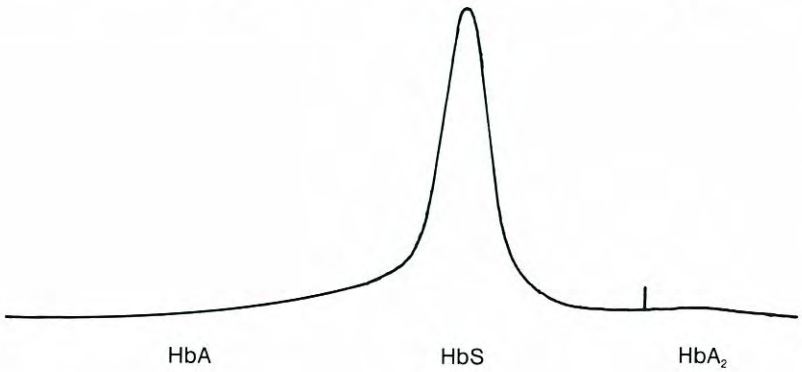
Hemoglobin Electrophoresis at pH 8.2 – 8.6



Sickle Cell Trait

(Heterozygous A/S)

HbA = 56.48%
HbS = 40.85%
HbA_2 = 2.67%



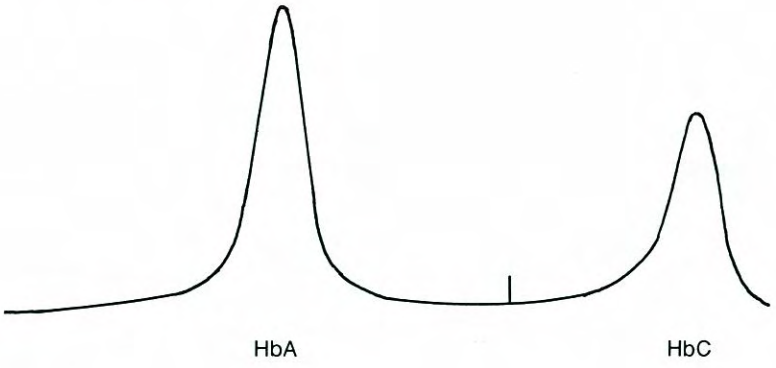
Sickle Cell Anemia

(Homozygous S/S)

HbA = 0%
HbS = 96.85%
HbA_2 = 3.15%

Heterozygous Hemoglobin C Disease

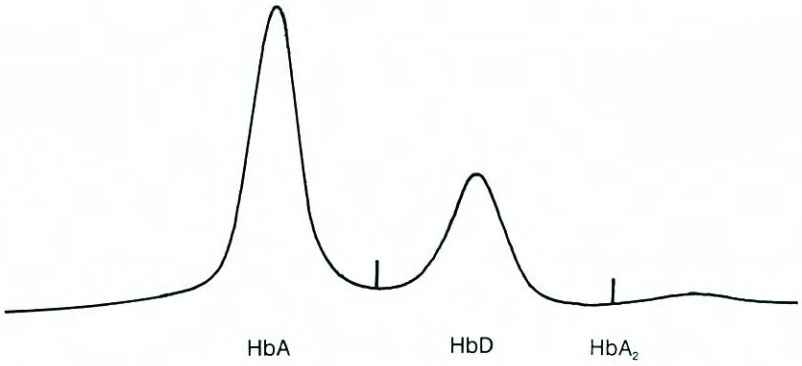
(HbC – HbA)



HbA = 63.91%
HbC = 36.09%

Heterozygous Hemoglobin D Disease

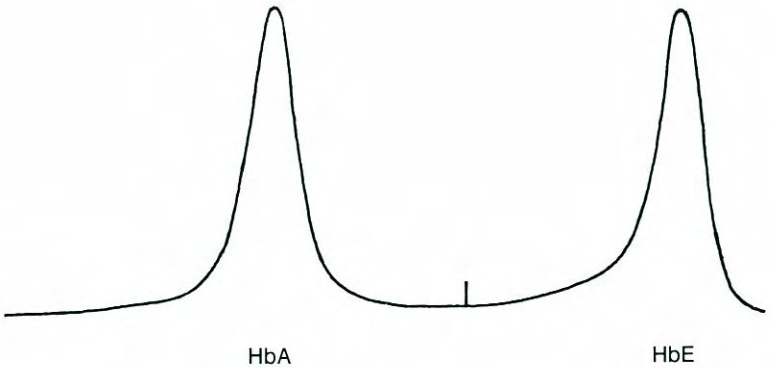
(HbD – HbA)



HbA = 66.11%
HbD = 31.00%
HbA_2 = 2.82%

Heterozygous Hemoglobin E Disease

(HbE – HbA)



HbA = 52.53%
HbE = 47.47%

3/2 Quantitative Evaluation of Thalassemias

Thalassemia is a group of hemoglobinopathies in which a mutation results in the absence or decreased production of one or more globin chains. It is a default not in the structure of the chain but rather in deficient chain synthesis.

Insufficient synthesis occurs most frequently in the beta chain and less frequently in the alpha chain.

In beta thalassemia, insufficient synthesis of the beta chain is accompanied by increased synthesis of the gamma and delta chains. Alpha chains, present in excess, form alpha 4 tetramers that precipitate and are responsible for the destruction of erythroblasts in the bone marrow and of the red cells in the blood.

In homozygotes, severe anemia exists. Hemoglobin F is very elevated, hemoglobin A_2 moderately so. In accordance with the severity of the mutation, there is little or no synthesis of hemoglobin A because little or no beta chains are produced. Hemoglobin F is unequally distributed in the red blood cells.

In heterozygotes, production is normal or moderately diminished and the normal gene compensates. It is usually compensated by hemoglobin F (3 to 6%) and hemoglobin A_2.

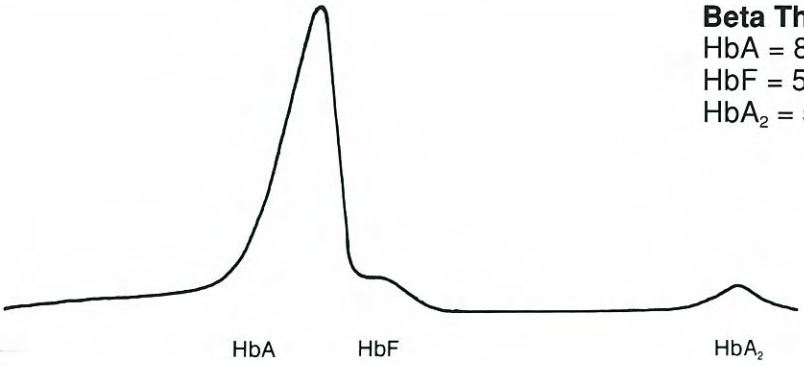
There are many thalassemia variants. The most important are the $beta_2$ and $delta_2$ thalassemias ($delta_2$ affects Greeks). The disorders affect not only the synthesis of beta chains but also delta chains. Compensation is uniquely accomplished by hemoglobin F. Hemoglobin A_2 cannot be synthesized.

The hereditary persistence of fetal hemoglobin is due to non-synthesis of beta chains. These subjects have a very elevated level of hemoglobin F, reaching 100% in the homozygote. There are usually no clinical complications.

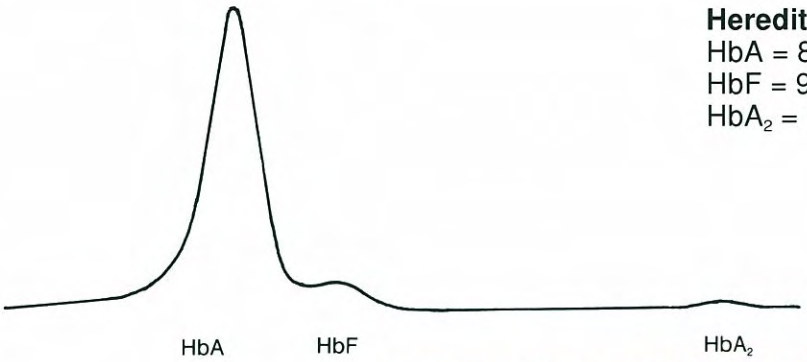
Hemoglobin Lepore results from a crossing over of beta and delta genes. Symptoms are similar to other thalassemias, but with the presence of hemoglobin Lepore.

Alpha thalassemias occur frequently in Orientals, but also exist in negroes and in Europeans. The homozygous form can be lethal in utero. Heterozygotes have an excess of beta chains and form beta 4 tetramers: hemoglobin H. At birth, all of the tetramers are gamma 4 or Bart's hemoglobin.

Frequently, due to the existence of a double heterozygote, thalassemia is associated with other hemoglobinopathies. Sickle thalassemia is the most common of these.



Beta Thalassemia Minor
HbA = 89.21%
HbF = 5.39%
HbA_2 = 5.40%



Hereditary Persistence of Fetal Hemoglobin (HPFH)
HbA = 89.08%
HbF = 9.07%
HbA_2 = 1.84%

Profile of Various Thalassemia Syndromes*

	HbA	HbA_2	HbF	Other Hb	Anemia	Microcytosis
ß – thalassemias						
ßo	0	↑		0	+++	+
Homozygotes ß+	↓	Variable	↑	0	++	+
ß-δ	0	↑		0	++	+
ßo	≠N	↑	N	0	± or 0 (1)	+
Heterozygotes ß+	≠N	↑	N	0	± or 0 (1)	+
ß-	≠N	N	↑	0	± or 0 (1)	+
α – thalassemias						
Major (2)	0	0	0	Hb Bart's Hb H	+++	
Hemoglobin H	↓	N or ↓	N	Hb H Hb Bart's	+	+
Minor	≠N	N	N	Hb Bart's (3)	±	
Silent	N	N	N	Hb Bart's (4)	0	0
Hemoglobin Lepore						
	↓	↓	↑	Hb Lepore	Variable	+
Hereditary Pesistance of Fetal Hemoglobin						
Homozygote	0	0	(5)	0	0	0
Heterozygote	↓	Variable	↑ (5)	0	0	0

* From Gentalini, Tropical Medicine.

Notes:

(1) Frequent false polycythemia

(2) incompatible with life

(3) 5-6% at birth

(4) 1-2% at birth

(5) Homogeneous distribution of Hemoglobin F

Notes

1) It is essential to work with a fresh specimen, because hemoglobin is labile and disappears after prolonged refrigeration. Spurient denatured hemoglobin bands may appear in aged specimens.
2) Some unstable hemoglobins are electrophoretically neutral and can only be detected by stability tests.
3) It is always necessary to evaluate hemoglobin studies in the context of clinical and physiological symptoms, and family and geographical studies. The electrophoresis results of the specimen should be compared to that of a known normal, cord blood and a known HbS.

Disorders	**Clinical and Physiological Signs**	**Characteristics**
Hemoglobin S and Sickle Cell Anemia (Central Africa, India, Saudi Arabia)	Splenic infarction Severe anemia with 5 –10% reticulocytes, normoblasts, anisocytes Polychromatophilic poikilocytosis Leukocytosis (10 – 20,000) Hemolysis, subicteric to 30 mg/L bilirubin	Hemoglobin S Blood smear shows sickling
Beta thalassemia major (Homozygous) (Blacks, Orientals and Mediterraneans)	Cooley's Anemia: severe hypochromic anemia Anisocytes, spherocytes,polychromatophilic, poikilocytosis, target cells, reticulocytes and nucleated red blood cells Leukocytosis (10 – 25,000) Hemolysis, bilirubin, increased osmotic fragility, splenomegaly	Hemoglobins A, F and A_2 Kleihauer test (HbF is up to 90% of the total)
Beta thalassemia minor (Heterozygous)	Hypochromic, polycythemia	Hemoglobins A, F and A_2
Hemoglobin Lepore Lepore (Homozygous)	Severe "thalassemia" syndrome	Hemoglobins F, A, A_2 and Lepore
Hereditary Persistence of Fetal Hemoglobin (HPFH) (F trait)	Minor "thalassemia" syndrome	Hemoglobin F Kleihauer test results characteristic of F hemoglobin
Hemoglobin C, D and E (Far East, Ghana)	Hemolytic anemia, recurrent jaundice Eventual splenomegaly, arthralgia	Characteristic electrophoresis pattern

RELATIVE ELECTROPHORETIC MOBILITIES OF HUMAN HEMOGLOBINS ON CELLULOSE ACETATE AT pH 8.4.

Normal Hemoglobins

Hemoglobin	Structure	Mobility
		(−) C A_2 S F A_1 J (+) I
Adult hemoglobins (A_2 A)	($\alpha_2\delta_2$), ($\alpha_2\beta_2$)	
Hemoglobin A_2 or Hb-A_2	$\alpha_2\delta_2$	
Fetal hemoglobin or HbF	$\alpha_2\gamma_2$	
Embryonic Hb-Gower 1		
Embryonic Hb-Gower 2		

Hemoglobin A Variants

Hb-G/C	
Hb-C	β 6 Glu → Lys
Hb-Porto Alegre (polymer)	
Hb-E	β 26 Glu → Lys
Hb-SG (hybrid)	
Hb-O	
Hb-Ube 1	
Hb-Koln	β 98 Val → Met
Hb-St. Mary's	
Hb-S	β 6 Glu → Val
Hb-Zurich	β 63 His → Arg
Hb-Stanleyville 2	α 78 Asn → Lys
Hb-D	
Hb-Lepore	δ-β fusion
Hb-L	
Hb-P	β 117 His → Arg
Hb-Stanleyville 1	α 68 Asn → Lys
Hb-Beilinson	
Hb-G	
Hb-Fukuoka	
Hb-Kokura	
Hb-Q	α 74 Asp → His
Hb-Shimonoseki	α 54 Gln →Arg
Hb-Seattle	
Hb-Porto Alegre (tetramer)	
Hb-M	
Hb-Hope	β 136 Gly → Asp
Hb-K	
Hb-Hopkins 2	α 112 His → Asp
Hb-J	
Hb-Hikeri	
Hb-Norfolk	α 57 Gly → Asp
Hb-N	
Hb-I	

Hemoglobin A_2 Variants

Hb-B_2 (A'_2)	δ 16 Gly → Arg
Hb-Flatbush	δ 22 Ala → Glu
Hb-Sphakia	δ 2 His → Arg
Hb-A_2 Babinga	δ 136 Gly → Asp
Hb-A_2 Coberg	δ 116 Arg → His
Hb-A_2 Indonesnia	δ 69 Gly → Arg
Hb-NYU	

Hemoglobin F Variants

Hb-Alexandra	γ 12 Thr → Lys
Hb-Aegena	
Hb-F Roma	
Hb-Texas	

Hemoglobins Without α - Chains

Hb-δ	δ
Hb-Bart's	γ_4
Hb-H	β_4

Hemoglobins With Only α - Chains

Hb-α^G	
Hb-α^A	

Frequently Encountered Hemoglobinopathies

Control A_1 FSC	
HPFH (A_1 F_1 A_2)	
Sickle Trait (A_1SA_2)	
S-C Disease (SC)	
Sickle Disease (S)	
C-Disease (C)	
S-β Thal (A_1 FSA_2)	

RELATIVE MOBILITIES OF α CHAIN MUTANTS ON CITRATE AGAR ELECTROPHORESIS

Alpha Chain Mutants

Hemoglobin	Structure	Mobility in Citrate Agar pH 6.0
		(+) C S A F (−)
Hb-J Paris	α 12 Ala → Asp	
Hb-J Oxford	α 15 Gly → Asp	
Hb-I	α 16 Lys → Glu	
Hb-Fort Worth	α 27 Glu → Gly	
Hb-Hasharon (Sealy)	α 47 Asp → His	
Hb-Montgomery	α 48 Leu → Arg	
Hb-Russ	α 51 Gly → Arg	
Hb-Shimonoseki	α 54 Gln → Arg	
Hb-G-Philadelphia	α 68 Asn → Lys	
Hb-Winnipeg	α 75 Asp → Tyr	
Hb-Inkster	α 85 Asp → Val	
Hb-Broussais	α 90 Lys → Asn	
Hb-Titusville	α 94 Asp → Asn	
Hb-G-Georgia	α 95 Pro → Leu	
Hb-Rampa	α 95 Pro → Ser	

RELATIVE MOBILITIES OF β CHAIN MUTANTS ON CITRATE AGAR ELECTROPHORESIS

Beta Chain Mutants

Hb-S	β 6 Glu → Val
Hb-C	β 6 Glu → Lys
Hb-C Harlem	β 6 Glu → Val
	β 73 Asp → Asn
Hb-G-San Jose	β 7 Glu → Gly
Hb-J-Baltimore	β 16 Gly → Asp
Hb-G-Coushatta	β 22 Glu → Ala
Hb-E	β 26 Glu → Lys
Hb-Alabama	β 39 Gln → Lys
Hb-Austin	β 40 Arg → Ser
Hb-G-Galveston	β 43 Glu → Ala
Hb-Williamette	β 51 Pro → Arg
Hb-Osu Christianborg	β 52 Asp → Asn
Hb-N-Seattle	β 61 Lys → Glu
Hb-Korle Bu	β 73 Asp → Asn
Hb-Mobile	β 73 Asp → Val
Hb-D-Ibadan	β 87 Thr → Lys
Hb-Gun Hill	β 91-95 deleted
Hb-N-Baltimore	β 95 Lys → Glu
Hb-Malmo	β 97 His → Gln
Hb-Koln	β 98 Val → Met
Hb-Kempsey	β 99 Asp → Asn
Hb-Richmond	β 102 Asn → Lys
Hb-Burke	β 107 Gly → Arg
Hb-P	β 117 His → Arg
Hb-D L.A. (Punjab)	β 121 Glu → Gln
Hb-O-Arab	β 121 Glu → Lys
Hb-Camden	β 131 Gln → Glu
Hb-Deaconess	β 131 Gln → O
Hb-K-Woolwich	β 132 Lys → Gln
Hb-Hope	β 136 Gly → Asp
Hb-Bethesda	β 145 Tyr → His
Hb-Cochin Port Royal	β 146 His → Arg

3/3 Glycosylated Hemoglobins

Introduction

The quantity of glycosylated hemoglobin (G-Hb) is an effective measure of overall glucose metabolism.

For reference, normal hemoglobins in the adult are as follows:
HbA ($alpha_2$, $beta_2$) = 90 – 97%
HbA_2 ($alpha_2$, $delta_2$) ≤ 2.5%
HbF ($alpha_2$, $gamma_2$) ≤ 0.5%

HbA_1, composed of five fractions (HbA_{1a}, HbA_{1b}, HbA_{1c}, HbA_{1d}, and HbA_{1e}). HbA_1 normally constitutes 5 to 8% of the total hemoglobin and has the same polypeptide structure as HbA, but with an attached hexose.

HbA_{1c} is glycosylated by the attachment of a glucose molecule to the N-terminal amino acid of the hemoglobin molecule. HbA_{1c} is the largest portion of the glycosylated fractions (HbA_{1a_1} = 0.2%, HbA_{1a_2} = 0.2%, HbA_{1b} = 0.5%, HbA_{1c} = 4 – 6%, HbA_{1d} = 0.2 – 0.6%).

Glycosylation is a slow, irreversible, non-enzymatic process that occurs after synthesis of the erythrocyte during its 120 day life span. The G-Hb value is lower in young erythrocytes and in hemolysis.

Thus, proportional to time (the life span of the erythrocyte) and the concentration of glucose, the G-Hb value reflects the glucose situation of 60 days preceding testing. Glucose measurement, on the other hand, can only reflect glycemia at the exact moment the blood is drawn.

Methods of Quantitation

The methods are diverse: ion exchange chromatography, affinity chromatography, HPLC, agarose gel electrophoresis, isoelectrofocusing, colorimetry with thiobarbituric acid.

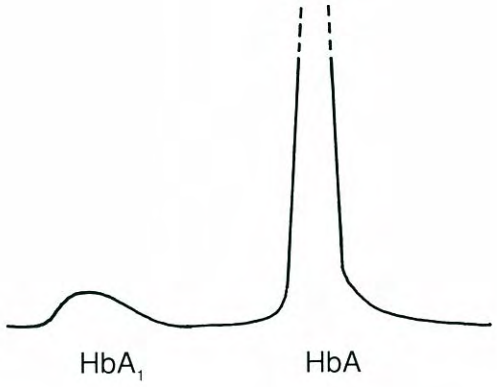
The expected values vary according to the method used. With ion exchange mini-column chromatography, abnormal hemoglobins (HbS, D, C, E, F) affect the glycosylated hemoglobin value. Hemoglobin F results in an overestimation of the glycosylated hemoglobin level by mini columns and by gel electrophoresis. Glucose also attaches to the beta chains of abnormal hemoglobins (HbS, HbC, etc). It is necessary to add the percentages of the different glycosylated fractions separated by electrophoresis. Affinity chromatography allows an estimation of the amount of glycated hemoglobin regardless of the hemoglobin abnormality.

Values in the Normal Adult

Values are interpreted according to the method used. Techniques specific for HbA_{1c} give a percentage equal to 5.5%. Techniques that measure all of the HbA_1 fractions (in particular, agarose electrophoresis at an acid pH, ion exchange mini-columns, and affinity mini-columns) give a higher value: 7 – 8%.

Normal Electrophoretic Pattern

HbA = 93.0%
HbA_1 = 7.0%



Glycosylated hemoglobin values, reflecting the glycemic state, in a diabetic in control should be 1 to 2% above the expected value for a non diabetic. In fact, a diabetic on insulin with a normal glycosylated hemoglobin value is at risk of hypoglycemia.

HbA_1 gives an objective reflection of the state of glucose control. It permits the physician to effectively evaluate the patient's treatment and the risk of hypoglycemia. It has gained acceptance as a good standard for assessment of diabetic control.

Normal Isoelectrofocusing Electropherogram of Hemoglobin

Component	Abbreviation
Hemoglobin A_{1a}	HbA_{1a}
Hemoglobin A_{1b}	HbA_{1b}
Hemoglobin-glutathion	HbA_3
Hemoglobin-glucopyranosyl	HbA_{1d}
Hemoglobin deoxy-froctose	HbA_{1c}
Hemoglobin A_1	HbA_1
Hemoglobin F	HbF
Hemoglobin α^{++} β^{+++}	Met Hb
Hemoglobin α^{+++} β^{++}	Met Hb
Hemoglobin α^{+++} β^{+++}	Met Hb
HbA_2 glycated	glyc-HbA_2
HbA_2	HbA_2

Nature of the Sugars Bound to Hemoglobin A

Fraction	% of Total Hb	N-terminal Ligand
HbA_{1a1}	0.2	Hexose-diphosphate: Fructose-diphosphate Glucose, mannose, galactose-diphosphate
HbA_{1a2}	0.2	Hexose-monophosphate: Glucose-6-Phosphate Glucose, mannose, galactose-monophosphate
HbA_{1b}	0.5	Hexose non-phosphorylated Glucose, mannose, galactose
HbA_{1c}	4 – 6	1-deoxy-ß-D-fructosyl
HbA_{1d}	0.2 – 0.6	ß-D-glucopyranosyl

Notes

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