

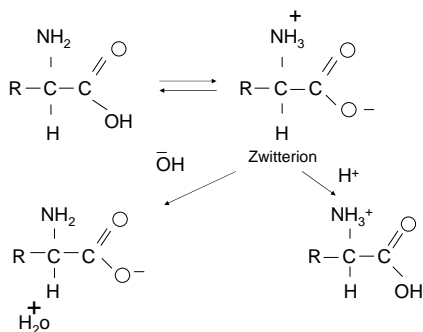
## Proteins

Michael A. Pesce, Ph.D  
Director of the Specialty  
Laboratory  
New York-Presbyterian Hospital  
Clinical professor of Pathology  
Columbia University Medical  
Center

## Learning Objectives

- Describe the electrophoresis procedure that is used to separate serum proteins and to identify a monoclonal protein
- Describe how immunofixation electrophoresis (IFE) is used to identify the heavy and light chain of the monoclonal protein
- Be able to identify a monoclonal protein from the serum protein electrophoresis and IFE patterns
- Describe the diagnostic criteria that are used to identify patients with Multiple Myeloma and MGUS

### Structure of Amino Acids



### ELECTROPHORESIS

#### Separation of a charged particle in an electric field

Rate of migration depends on:

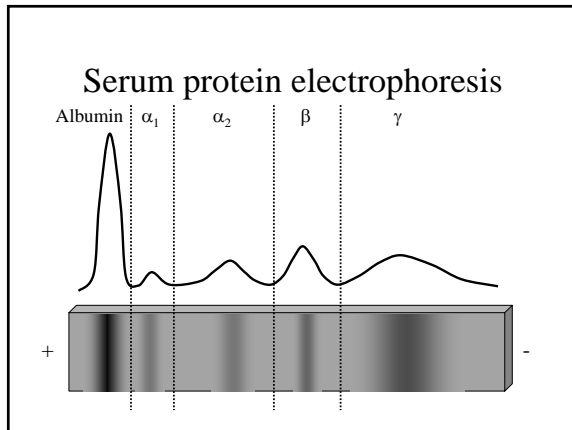
- Charge of the molecule
- Size and shape of the molecule
- Voltage
- Support medium
- pH and ionic strength of the buffer

### Optimizing electrophoresis

- Optimal electrophoretic separations must balance *speed* and *resolution*
  - Higher voltage increases speed, but heat causes evaporation of the buffer and may denature proteins
  - Higher ionic strength (buffer) increases conductivity.

### Serum Protein Electrophoresis

- Apply samples 1 uL to the agarose gel
- Electrophoresis 21°C, 650v
- Dry 54°C
- Stain - Acid Blue
- Destain - Acetic Acid
- Dry 63°C

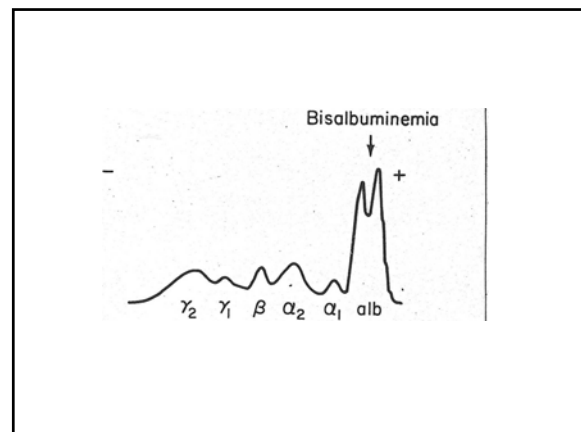


### Albumin

- Most abundant protein in plasma (approximately half of total protein)
  - Synthesized in liver
  - $t_{1/2}$ =15-19 days
- Principal functions
  - Maintaining fluid balance
  - Transport Protein

### Clinical significance of albumin

- Hyperalbuminemia is rare and of no clinical significance
- Hypoalbuminemia
  - Increased loss (nephrotic syndrome)
  - Decreased synthesis (nutritional deficit, liver failure)
- Analbuminemia markedly decreased rare
- Bisalbuminemia, dimeric albumin with equal intensities



### Alpha 1 Proteins

Alpha-1-Lipoprotein-HDL

Alpha-1-Antitrypsin-

protease inhibitor that binds to and inactivates trypsin

- Deficiency leads to destruction of the alveolar walls and is associated with pulmonary deficiency
- Deficiency also seen in cirrhosis
- Alpha-1-antitrypsin is an acute phase protein and is increased in acute episodes of tissue damage

### Other $\alpha_1$ proteins

- $\alpha_1$ -Acid glycoprotein (orosomucoid) and alpha-1 anti-chromotrypsin are acute phase proteins
- $\alpha_1$ -Fetoprotein (AFP)
  - Principal fetal protein, used to screen for fetal abnormalities (neural tube defects)

### Alpha-2-Proteins

Alpha-2-Macroglobulin - 720 Kda –

Large non-immunoglobulin in plasma

Synthesized in the liver

Increased levels in nephrosis because its large size prevents passage into the urine. Also there is an increase in synthesis.

– It is not an acute phase protein

### ( $\alpha_2$ ) Haptoglobin

- Synthesized in the liver
- Binds to, and preserves, hemoglobin
- Low Haptoglobin levels in intravascular hemolysis
- Increased haptoglobin levels because it is an acute phase

### BETA PROTEINS

Transferrin - 77 Kda –

Iron transport protein, also binds copper

Increased in iron deficiency anemia, pregnancy and estrogen therapy

– Decreased in acute inflammation due to decrease synthesis of transferrin by the liver

– Negative acute phase protein

### Other ( $\beta$ ) proteins

- Beta-1 Lipoprotein 2750Kda
- Increased in nephrosis and Type II hypercholesterolemia
- C3 and C4 migrate in the  $\beta$  region
- Complement proteins are decreased in genetic deficiencies, and increased in inflammation. C3 is a late acute phase protein. C3 may not be detected if the sample is kept at room temperature
- IgA

### $\gamma$ Region

- Includes immunoglobulins (IgG, IgA, IgM, IgD and IgE)
- Single sharp peak indicates a paraprotein and is associated with a monoclonal gammopathy
- A small band is indicative of MGUS

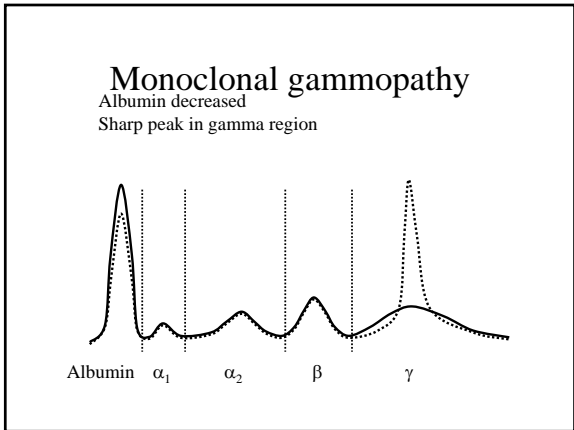
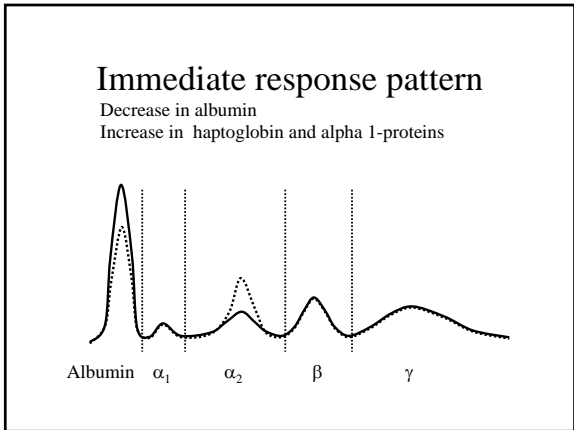
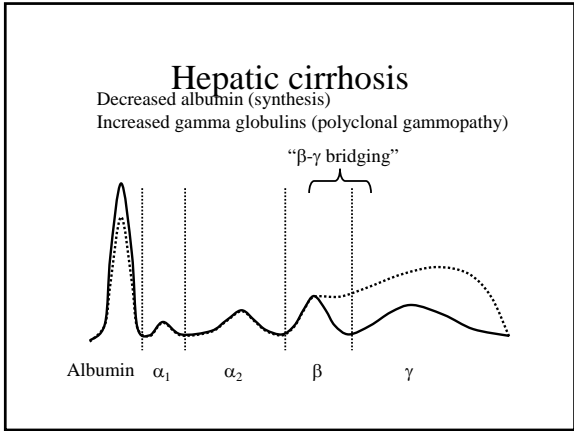
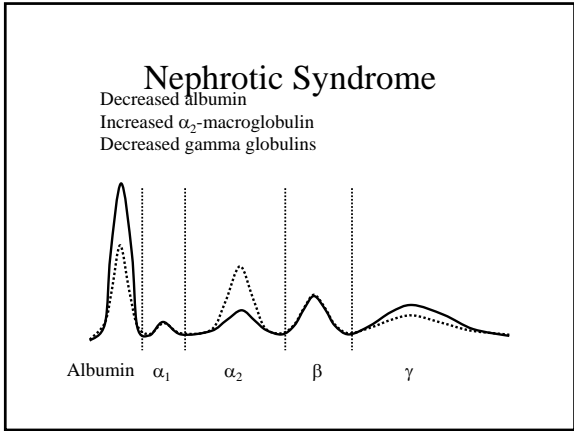
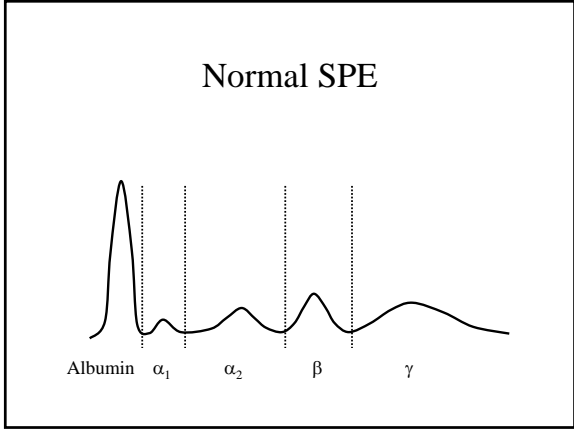
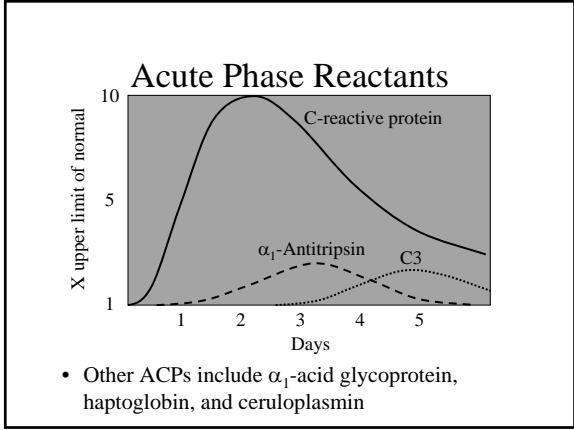
### Gamma Region

IgG migrates in the gamma and beta regions and is increased in infections, autoimmune and liver disease

IgM migrates in the gamma region

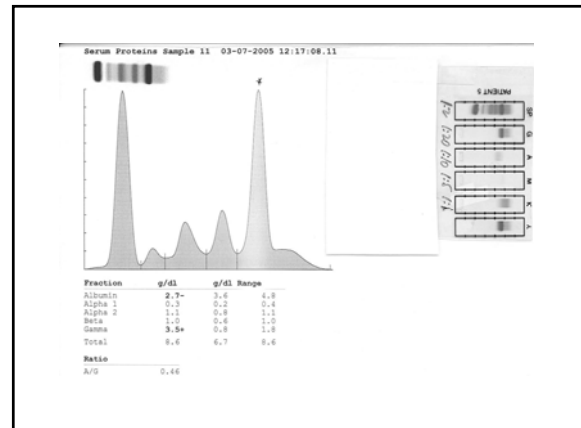
IgA migrates in the alpha-2, beta and gamma regions

CRP is the most sensitive indicator of an acute phase reaction (inflammation, trauma, infection)



### IMMUNOFIXATION ELECTROPHORESIS

- Dilute samples with saline
- Apply sample 1 uL to the agarose gel
- Electrophoresis 21°C, 650 v
- Apply antisera
- Blot and dry 50°C
- Stain - Acid Violet
- Destain - Acetic Acid
- Dry 60°C



### MULTIPLE MYELOMA

Multiple Myeloma - proliferation of a single clone of plasma cells that produces a monoclonal protein

Annual Incidence - 4 in 100,000

Number of cases per year - 13,000

Represents 1% of all malignant diseases

Median age at diagnosis - 65 years

Median survival - 3 years

### DIAGNOSTIC CRITERIA FOR MULTIPLE MYELOMA

Bone Marrow Plasmacytosis >10% of Plasma Cells  
Serum Monoclonal Protein

End Organ Damage

Lytic Bone Lesions

Renal Insufficiency

Anemia

Increased Calcium

### Clinical Laboratory in Multiple Myeloma

-Biochemical -

Serum monoclonal proteins

Polyclonal Immunoglobulin Decreased

Proteinuria, Bence-Jones Protein present in urine

BUN, Creatinine ↑

Calcium ↑, N

- Hematological -

Hemoglobin Decreased

Anemia - Normochromatic, Normocyte

ESR Increased

Rouleaux Formation

### Frequency of Monoclonal Proteins in Multiple Myeloma

IgG-58%

IgA- 24%

Light Chains- 15%

Biclonal- 2%

IgD- 1%

### Monoclonal Gammopathy of Undetermined Significance

Defined as the presence of a serum monoclonal protein at low levels

Number of cases per year - 750,000-1,000,000

54% Men 46% Women

Occurs in 2% of persons over 50 years, 3% over 70 years

Median age at diagnosis - 72 years

Median survival - 12 years

### Monoclonal Gammopathy of Undetermined Significance

Serum monoclonal protein <3.0 g/dL

Stability of monoclonal protein during long term follow-up

<10% Plasma cells in bone marrow

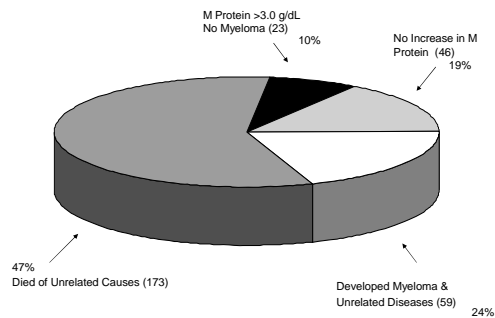
None or a small amount of Bence-Jones protein in urine

Absence of lytic bone lesions

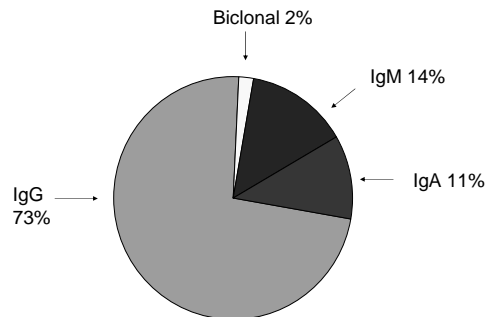
Serum calcium, BUN, creatinine - Normal

Hemoglobin - Normal

### CLINICAL COURSE OF 241 PATIENTS WITH MGUS



### Distribution Frequency of Monoclonal Proteins in MGUS



### Summary

- Serum protein electrophoresis and IFE are used to identify a monoclonal protein in the serum of patients with Multiple Myeloma and MGUS.
- Patients with Multiple Myeloma and MGUS are followed by measuring the concentration of the monoclonal protein using serum protein electrophoresis.