Proteins

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Protein Trivia

- The most abundant organic molecule in cells (50% by weight)
- About 300 proteins have been identified in plasma
- Proteins can have a MW of greater than 1 million
- Albumin is the most abundant protein in humans and contains 550 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.

Structure of Amino Acids

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
Serum protein electrophoresis

**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
- Hyoalbuminemia
  - 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 α₁ 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

### Other (β) Proteins

- Beta-1 Lipoprotein 2750Kda
- Increased in nephrosis and Type II hypercholesterolemia
- C3 and C4 migrate in the β region
- Compliment 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

### (α₂) 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

### γ 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

### 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

### 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)
Acute Phase Reactants

- Other ACPs include α1-acid glycoprotein, haptoglobin, and ceruloplasmin

Hepatic cirrhosis
Decreased albumin (synthesis)
Increased gamma globulins (polyclonal gammopathy)

Immediate response pattern
Decrease in albumin
Increase in haptoglobin and alpha 1-proteins

Nephrotic Syndrome
Decreased albumin
Increased α2-macroglobulin
Decreased gamma globulins

Monoclonal gammopathy
Albumin decreased
Sharp peak in gamma region
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

- 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

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

Distribution Frequency of Monoclonal Proteins in MGUS

- IgG 73%
- IgM 14%
- IgA 11%

CLINICAL COURSE OF 241 PATIENTS WITH MGUS

- M Protein >3.0 g/dL No Myeloma (23) 10%
- No Increase in M Protein (46) 19%
- Developed Myeloma & Unrelated Diseases (59) 24%
- Died of Unrelated Causes (173) 47%

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BANDS MISTAKEN FOR MONOCLONAL IMMUNOGLOBULINS

<table>
<thead>
<tr>
<th>BAND</th>
<th>CONDITION</th>
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</thead>
<tbody>
<tr>
<td>Alpha-2-Macroglobulin</td>
<td>Nephrotic syndrome</td>
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<tr>
<td>Hemoglobin-haptoglobin</td>
<td>Hemolysis</td>
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<tr>
<td>Beta-1-Lipoprotein</td>
<td>Hyperlipidemia</td>
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<tr>
<td>Fibrinogen</td>
<td>Inadequate clot</td>
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<tr>
<td>C-Reactive Protein</td>
<td>Acute inflammation</td>
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<tr>
<td>Immune complex pattern</td>
<td>Inflammation</td>
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**CONDITION**

- Nephrotic syndrome
- Hemolysis
- Hyperlipidemia
- Inadequate clot
- Acute inflammation
- Inflammation