Proteins

Michael A. Pesce, Ph.D
Department of Pathology
New York-Presbyterian Hospital
Columbia University Medical Center

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

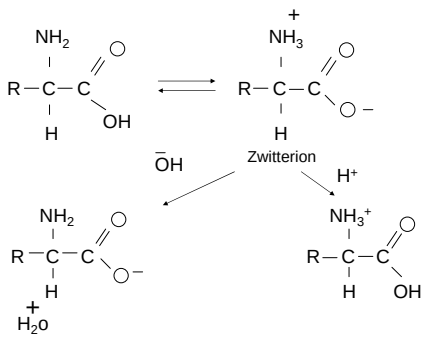
Protein Trivia

- 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 I million
- Albumin is the most abundant protein in humans and contains 550 amino acids

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.

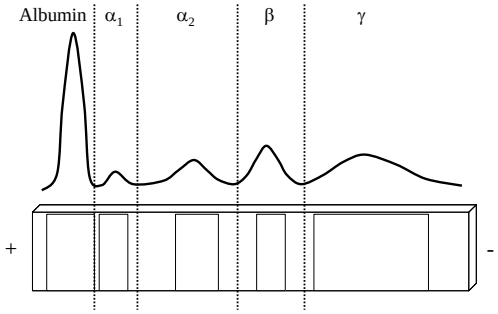
Structure of Amino Acids

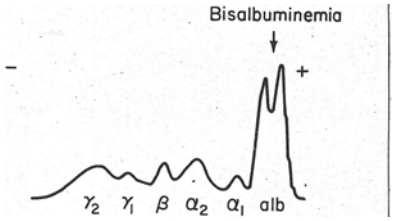


Serum Protein Electrophoresis

- 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
  - $t_{½}$=15-19 days
- Principal functions
  - Maintaining fluid balance
  - Transport Protein

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

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

Other α_1 proteins

- α_1-Acid glycoprotein (orosomucoid) and alpha- 1 anti- chromotrypsin are acute phase proteins
- α_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-immunoglogulin 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

(α_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

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

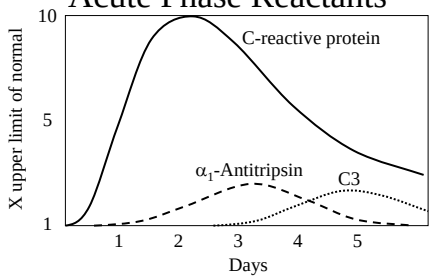
IgG migrates in the gamma and beta regions and is increased in infections, autoimmune and liver disease

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, infection0

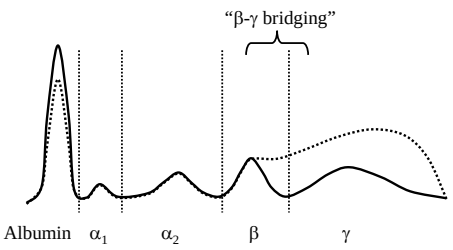
Acute Phase Reactants



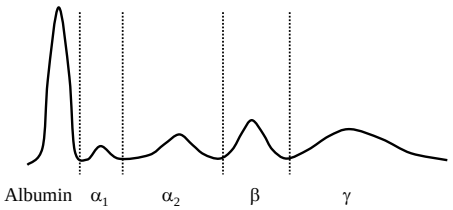
- Other ACPs include α_1-acid glycoprotein, haptoglobin, and ceruloplasmin

Hepatic cirrhosis

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

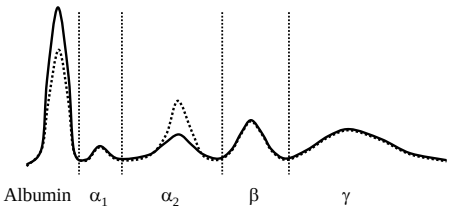


Normal SPE



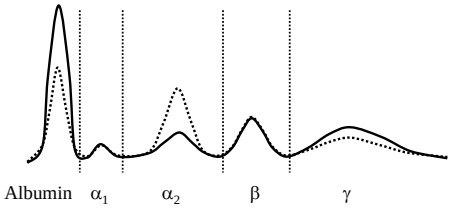
Immediate response pattern

Decrease in albumin
Increase in haptoglobin and alpha 1-proteins



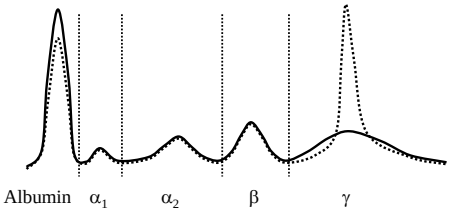
Nephrotic Syndrome

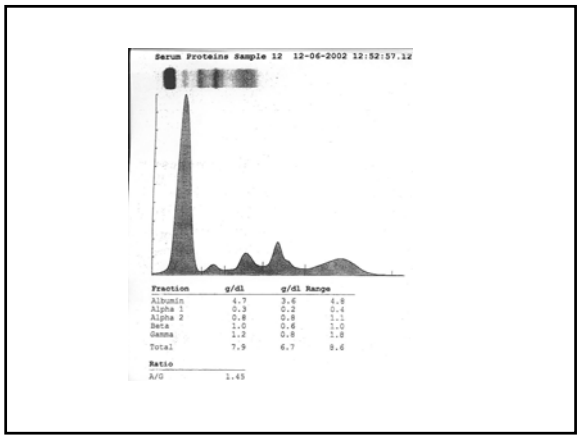
Decreased albumin
Increased α_2-macroglobulin
Decreased gamma globulins

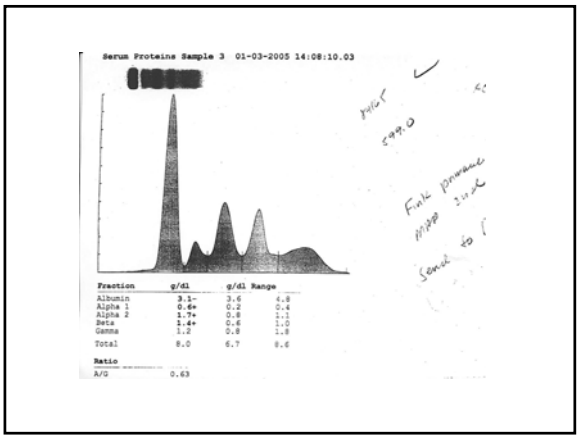


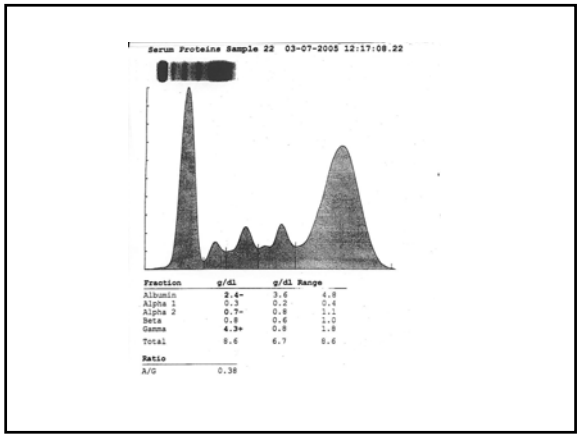
Monoclonal gammopathy

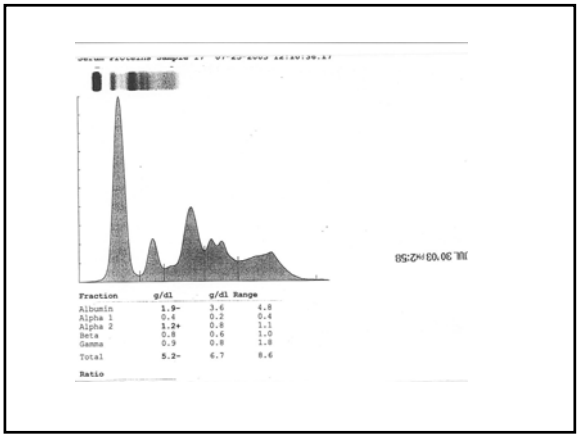
Albumin decreased
Sharp peak in gamma region

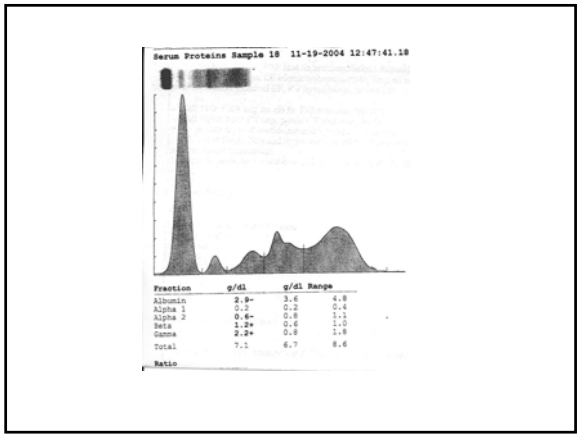


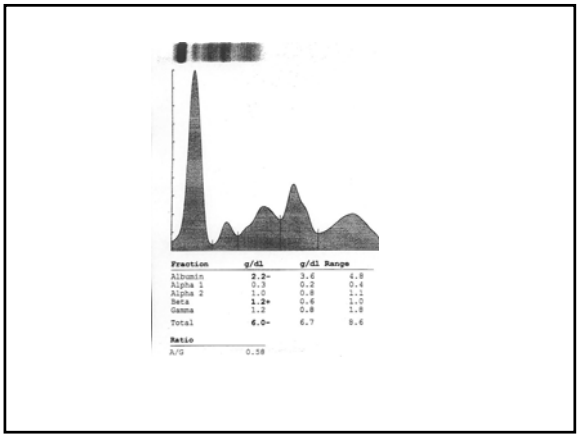

Serum Proteins Sample 12 12-06-2002 12:52:57.12
Fraction g/dl g/dl Range
Albumin 4.7 3.6 4.8
Alpha 1 0.3 0.2 0.4
Alpha 2 0.8 0.8 1.1
Beta 1.0 0.6 1.0
Gamma 1.2 0.8 1.8
Total 7.9 6.7 8.6
Ratio
A/G 1.45

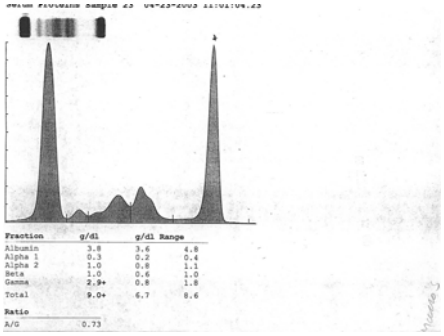

Serum Proteins Sample 3 01-03-2005 14:08:10.03
Fraction g/dl g/dl Range
Albumin 3.1- 3.6 4.8
Alpha 1 0.6+ 0.2 0.4
Alpha 2 1.7+ 0.8 1.1
Beta 1.4+ 0.6 1.0
Gamma 1.2 0.8 1.8
Total 8.0 6.7 8.6
Ratio
A/G 0.63


Serum Proteins Sample 22 03-07-2005 12:17:08.22
Fraction g/dl g/dl Range
Albumin 2.4- 3.6 4.8
Alpha 1 0.3 0.2 0.4
Alpha 2 0.7- 0.8 1.1
Beta 0.8 0.6 1.0
Gamma 4.3+ 0.8 1.8
Total 8.6 6.7 8.6
Ratio
A/G 0.38


Fraction g/dl g/dl Range
Albumin 1.9- 3.6 4.8
Alpha 1 0.4 0.2 0.4
Alpha 2 1.2+ 0.8 1.1
Beta 0.8 0.6 1.0
Gamma 0.9 0.8 1.8
Total 5.2- 6.7 8.6
Ratio


Serum Proteins Sample 18 11-19-2004 12:47:41.18
Fraction g/dl g/dl Range
Albumin 2.9- 3.6 4.8
Alpha 1 0.2 0.2 0.4
Alpha 2 0.6- 0.8 1.1
Beta 1.2+ 0.6 1.0
Gamma 2.2+ 0.8 1.8
Total 7.1 6.7 8.6
Ratio


Fraction g/dl g/dl Range
Albumin 2.2- 3.6 4.8
Alpha 1 0.2 0.2 0.4
Alpha 2 1.0 0.8 1.1
Beta 1.2+ 0.6 1.0
Gamma 1.2 0.8 1.8
Total 6.0- 6.7 8.6
Ratio
A/G 0.58



Fraction	g/dl	g/dl Range	
Albumin	3.8	3.6	4.8
Alpha 1	0.3	0.2	0.4
Alpha 2	1.0	0.8	1.1
Beta	1.0	0.6	1.0
Gamma	2.9+	0.8	1.8
Total	9.0+	6.7	8.6
Ratio			
A/G	0.73		

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

IMMUNOFIXATION ELECTROPHORESIS

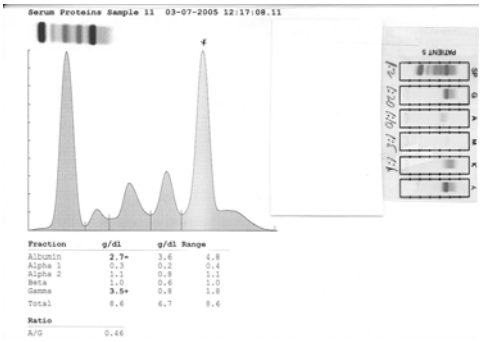
- Dilute samples with saline
- Apply sample I uL to the agarose gel
- Electrophoresis 21ºC, 650 v
- Apply antisera
- Blot and dry 50ºC
- Stain - Acid Violet
- Destain - Acetic Acid
- Dry 60ºC

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



Fraction	g/dl	g/dl Range	
Albumin	2.7-	3.6	4.8
Alpha 1	0.3	0.2	0.4
Alpha 2	1.1	0.8	1.1
Beta	1.5	0.6	1.0
Gamma	3.5+	0.8	1.8
Total	8.6	6.7	8.6
Ratio			
A/G	0.46		

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

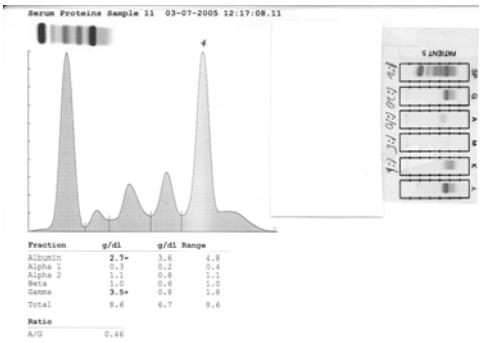
<10% Plasma cells in bone marrow

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

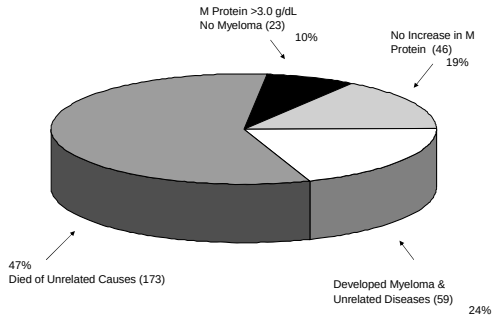
Absence of lytic bone lesions

Serum calcium, BUN, creatinine - Normal

Hemoglobin - Normal



CLINICAL COURSE OF 241 PATIENTS WITH MGUS



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

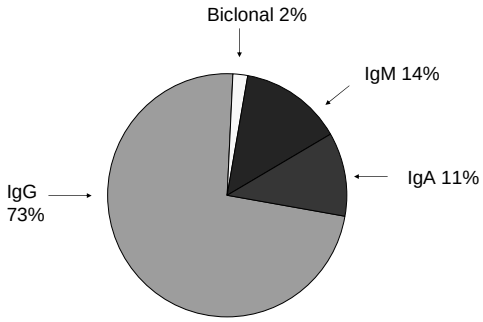
54% Men 46% Women

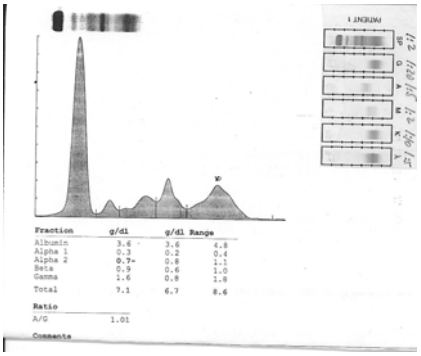
Occurs in 2% of persons over 50 years, 3% over 70 years

Median age at diagnosis - 72 years

Median survival - 12 years

Distribution Frequency of Monoclonal Proteins in MGUS



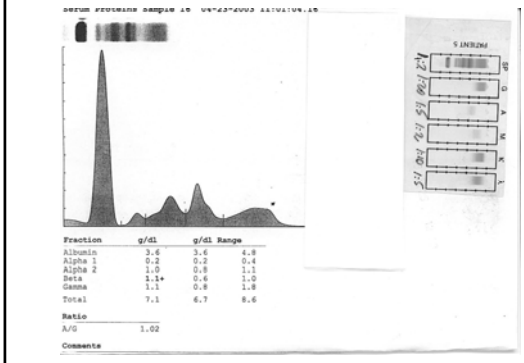


Fraction	g/dl	g/dl Range	
Albumin	3.6 -	3.6	4.8
Alpha 1	0.3	0.2	0.4
Alpha 2	0.7-	0.8	1.1
Beta	0.9	0.6	1.0
Gamma	1.6	0.8	1.8
Total	7.1	6.7	8.6

Ratio

A/G 1.01

Comments

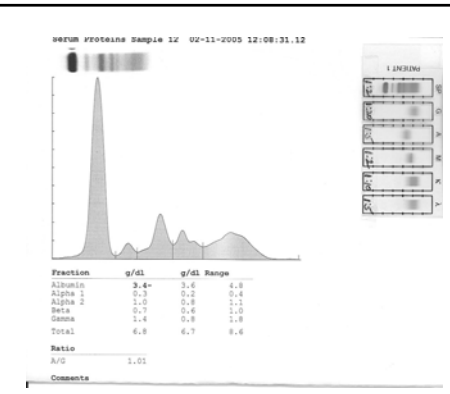


Fraction	g/dl	g/dl Range	
Albumin	3.6	3.6	4.8
Alpha 1	0.2	0.2	0.4
Alpha 2	1.0	0.8	1.1
Beta	1.1+	0.6	1.0
Gamma	1.1	0.8	1.8
Total	7.1	6.7	8.6

Ratio

A/G 1.02

Comments



Serum Proteins Sample 12 02-11-2005 12:08:31.12

Fraction	g/dl	g/dl Range	
Albumin	3.4-	3.6	4.8
Alpha 1	0.3	0.2	0.4
Alpha 2	1.0	0.8	1.1
Beta	0.7	0.6	1.0
Gamma	1.4	0.8	1.8
Total	6.8	6.7	8.6

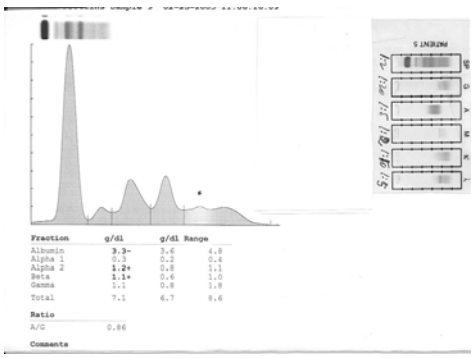
Ratio

A/G 1.01

Comments

BANDS MISTAKEN FOR MONOCLONAL IMMUNOGLOBULINS

BAND	CONDITION
Alpha-2-Macroglobulin	Nephrotic syndrome
Hemoglobin-haptoglobin	Hemolysis
Beta-1-Lipoprotein	Hyperlipidemia
Fibrinogen	Inadequate clot
C-Reactive Protein	Acute inflammation
Immune complex pattern	Inflammation

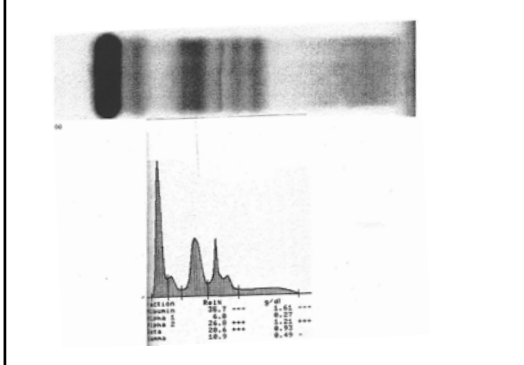


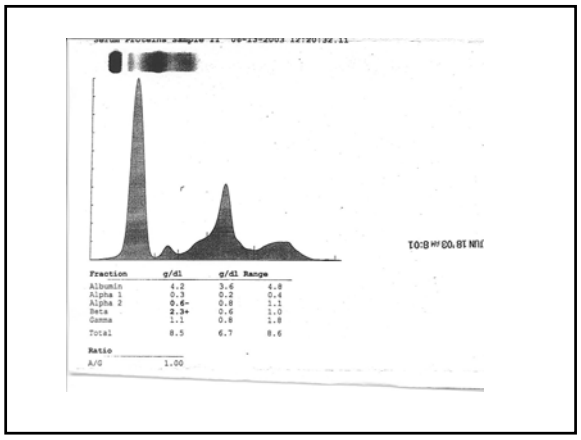
Fraction	g/dl	g/dl Range	
Albumin	3.3-	3.6	4.8
Alpha 1	0.3	0.2	0.4
Alpha 2	1.2+	0.8	1.1
Beta	1.1+	0.6	1.0
Gamma	1.1	0.8	1.8
Total	7.1	6.7	8.6

Ratio

A/G 0.86

Comments

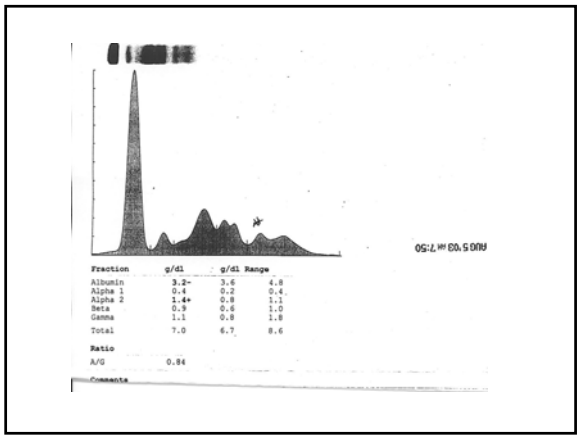




Fraction	g/dl	g/dl Range	
Albumin	4.2	3.6	4.8
Alpha 1	0.3	0.2	0.4
Alpha 2	0.6-	0.8	1.1
Beta	2.3+	0.6	1.0
Gamma	1.1	0.8	1.8
Total	8.5	6.7	8.6

Ratio

A/G 1.00



Fraction	g/dl	g/dl Range	
Albumin	3.2-	3.6	4.8
Alpha 1	0.4	0.2	0.4
Alpha 2	1.4+	0.8	1.1
Beta	0.9	0.6	1.0
Gamma	1.1	0.8	1.8
Total	7.0	6.7	8.6

Ratio

A/G 0.84

Comments