جامعة الملك سعود
King Saud University

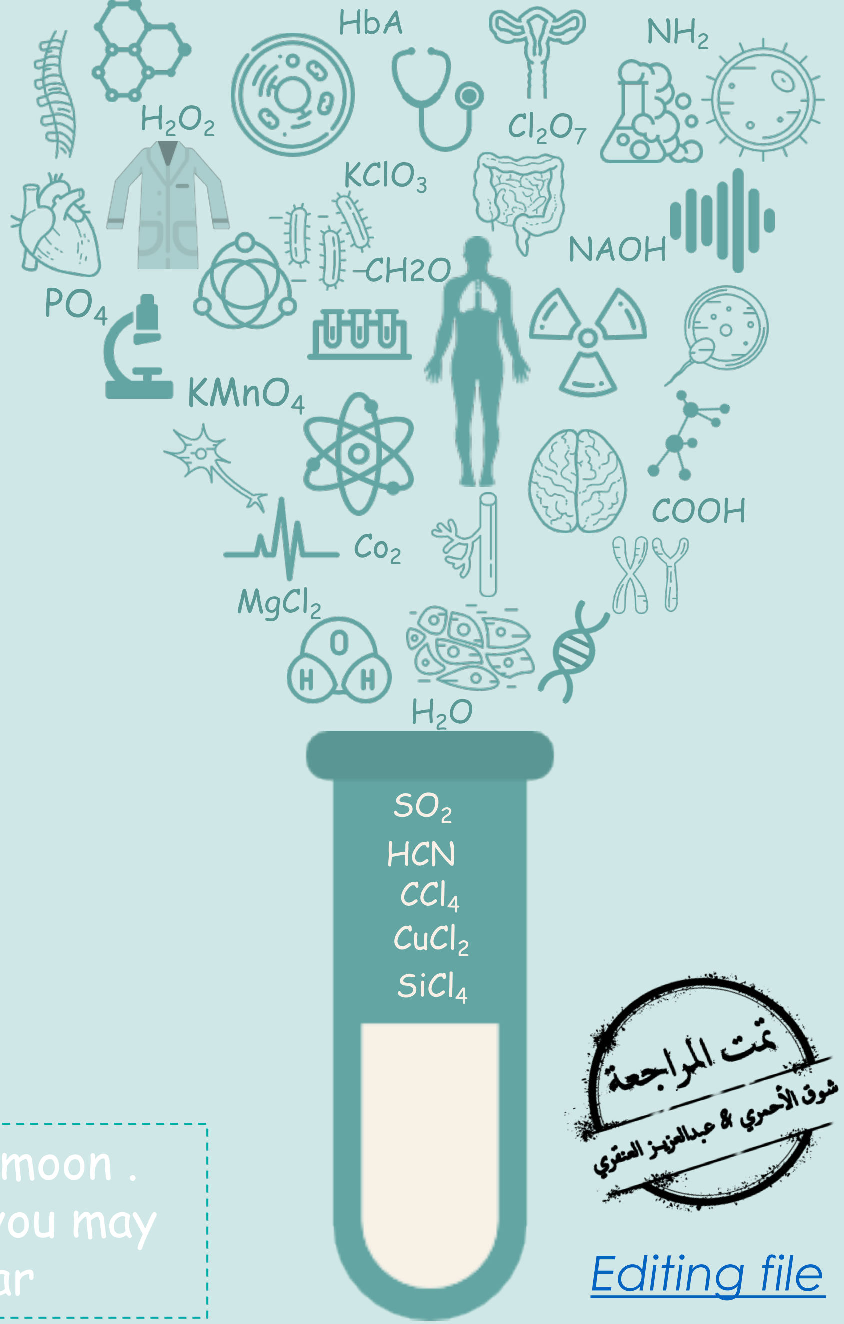
MEDICINE
KING SAUD UNIVERSITY

BIOCHEMISTRY TEAM 436

Important
Extra Information
Doctors slides
Doctors notes

Biochemistry
Plasma Proteins

Aim for the moon .
If you miss, you may
hit a star



تمت المراجعة
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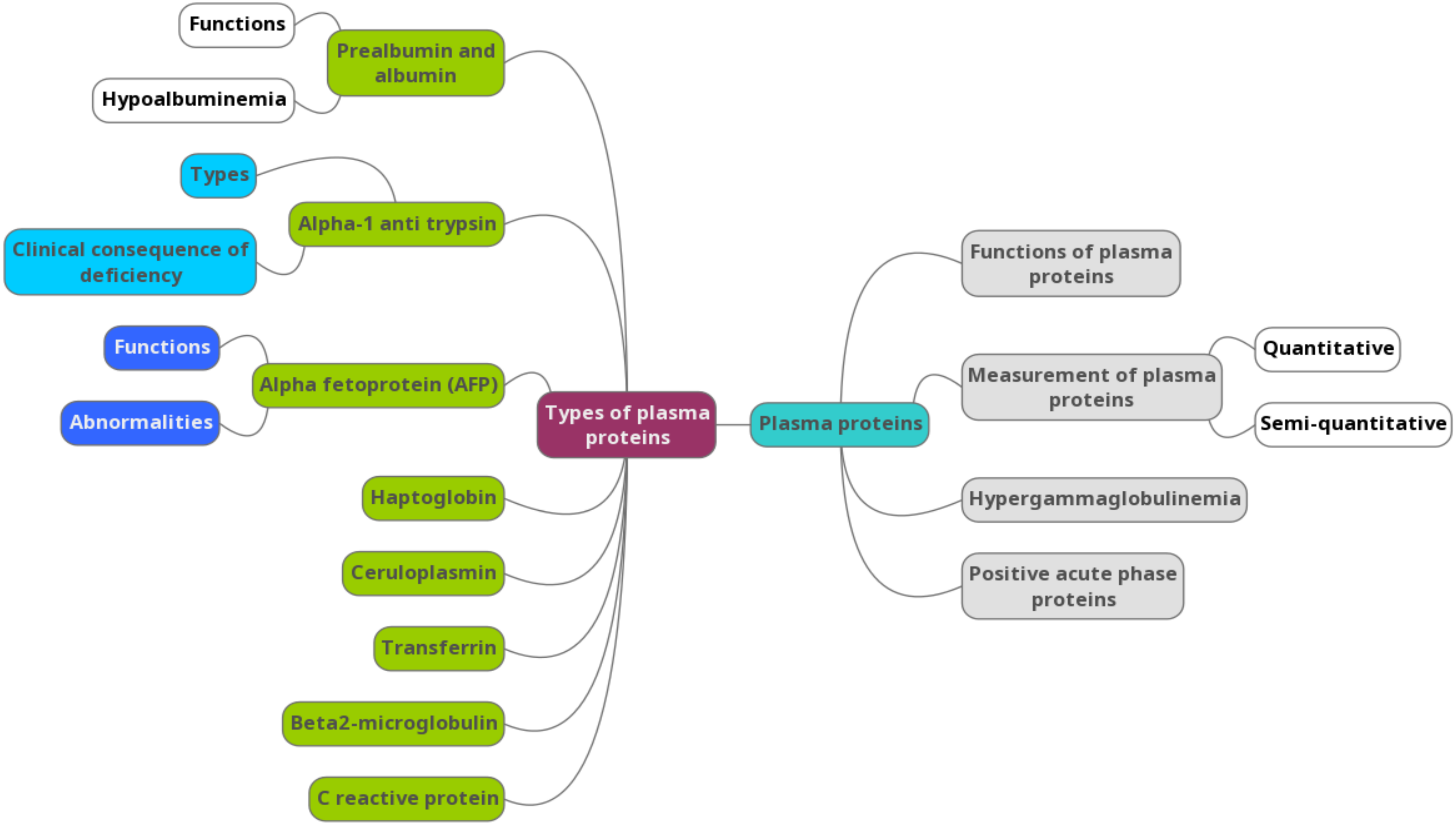
Editing file

OBJECTIVES

By the end of this lecture, the Second Year students will be able to:

- Identify types and various functions of plasma proteins
- Discuss the role of plasma proteins in the diagnosis of diseases and conditions
- Identify the role positive and negative acute phase proteins in various diseases
- Interpret the normal and abnormal electrophoretic patterns of plasma proteins

Lecture overview



Plasma Proteins (pps)

- Plasma contains > 300 different proteins
- Many pathological conditions affect level of pps
- **Mostly synthesized in the liver**
- Some are produced in other sites

  Such as: antibodies which are produced in lymphocytes
- A normal adult contains ~70 g/L of pps

Functions of pps

Functions of pps:

- Transport
  - (Albumin, prealbumin, globulins)
- Maintain plasma oncotic pressure
  - It's the pressure that's caused by protein to pull the water in the blood to the interstitial fluid
    - (Albumin)
- Defense
  - (Immunoglobulins and complement)
- Clotting and fibrinolysis
  - (Thrombin and plasmin)

Measurement of Plasma Proteins

Measurement of Plasma Proteins

A) Quantitative measurement of a specific protein:

Chemical:

specific way to measure specific protein.

immunological reactions:

Immunological reaction: some proteins we can't design a test sensitive enough to pick up level of the protein; we raise the antibodies against it since they are very specific they increase sensitivity.

both ways give the exact amount of wanted to protein.

B) Semiquantitative measurement by electrophoresis:

Proteins are separated by their electrical charge in electrophoresis

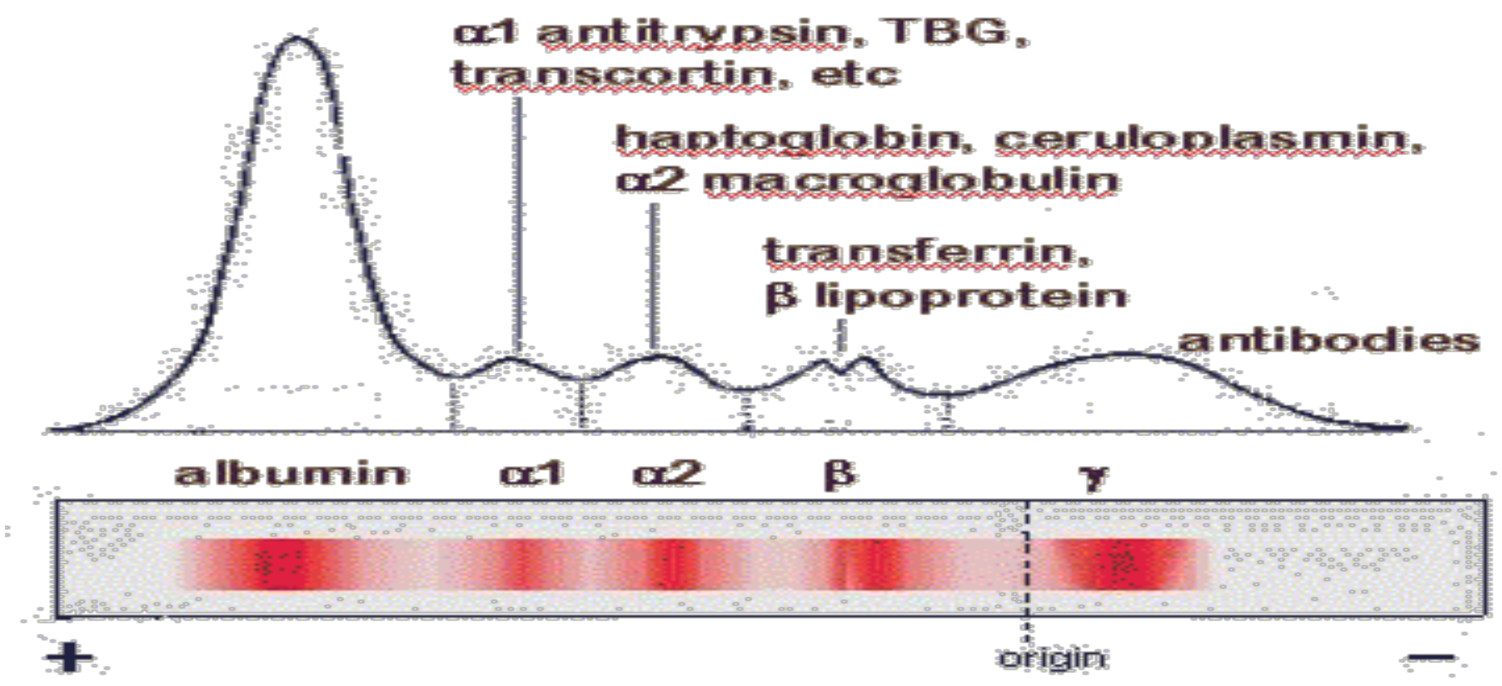
- Five separate bands of proteins are observed
- These bands change in disease

Semi quantitative is getting an idea of the quantity not an exact number.
Ex" There is protein X we study its level in a healthy population we come up with a range and then compare it to a person showing symptoms.

Electrophoresis: An electrical field is applied to a mixture of proteins to separate it. Separation is based on the size of the molecule. The smallest is the fastest, the heaviest is the slowest. And all of them move to the positive charge.
We'll also need a medium for the proteins: gel or paper but usually a gel is used. After separation there a couple of ways to visualize them: by staining them or by binding them to antibodies.

Measurement of Plasma Proteins

Normal Pattern of Plasma Protein Electrophoresis:



5 distinct bands with specific positions for each protein and a specific thickness showing the quantity of it.

- Prealbumin is the fastest but it's not shown in the chart so it's albumin.
- We can call them albumin and the globulins.
- Intensities are different so we make a density analysis to show the amount of plasma proteins.
- Albumin 50-55%, gamma 20%, a1 and b 7-14%, A1 is the remaining.
- Prealbumin is not shown in the classical electrophoresis, we need a more sensitive test (immunoelectrophoresis).

Types of Plasma Proteins

- Types of Plasma Proteins
  - Prealbumin
  - Albumin
  - α1-Globulins:
    - a1-Antitrypsin
    - α-fetoprotein
  - α2-Globulins:
    - Ceruloplasmin,
    - haptoglobin
  - β-Globulins:
    - CRP
    - transferrin
    - β2-microglobulin
  - γ- Globulins

Prealbumin is not a precursor of albumin it's a different protein. It's a good indication of nutrition status it has a half life of 2 days not like albumin which is 20 days.

Because of the loss of blood. Also any condition that increases catabolism in the body.

Types of Plasma Proteins:

1-Prealbumin (transthyretin):

- A transport protein for:
  1. Thyroid hormones
  2. Retinol (vitamin A)
- Migrates faster than albumin in electrophoresis
- Separated by immunoelectrophoresis
- Lower levels found in:
  - liver disease, nephrotic syndrome, acute phase inflammatory response, malnutrition
- Short half-life (2 days)

2-Albumin:

- Most abundant plasma protein (~40 g/L) in normal adult
- Synthesized in the liver as preproalbumin and secreted as albumin
- Half-life in plasma: 20 days
- Decreases rapidly in injury, infection and surgery

Functions

It means it attracts water which maintains homeostasis, so for ex. If albumin decreases in blood → edema → like in nephrotic syndrome.

If a person is in shock and needs proteins he goes through pinocytosis where the cell membrane stretches around the molecule and takes it in into a vesicle .

Functions:

- **Maintains oncotic pressure:**
  - The osmotic pressure exerted by plasma proteins that pulls water into the circulatory system
  - Maintains plasma volume and fluid distribution in and outside cells
  - 80% of plasma oncotic pressure is maintained by albumin
- **A non-specific carrier of :**
  - hormones, calcium, free fatty acids, drugs, etc.
- **Tissue cells can take up** albumin by pinocytosis where it is hydrolyzed to amino acids
- **Useful in the treatment**
  - of liver diseases, hemorrhage, shock and burns
  - because if there's a lot of blood loss or skin burn causing hypovolemia so albumin can maintain oncotic pressure.

Hypoalbuminemia

Causes:	Effects:
1-Decreased albumin synthesis (liver cirrhosis, malnutrition)	1-Edema due to low oncotic pressure: Albumin level drops in liver disease causing low oncotic pressure Fluid moves into the interstitial spaces causing edema
2-Increased losses of albumin:	2-Reduced transport of drugs and other substances in plasma
A-Increased catabolism in infections	When you take a drug, it goes all around the body by blood and get metabolized and cleared . If protein is less then the time it stays in the body increases.
B-Excessive excretion by the kidneys (nephrotic syndrome)	3-Reduced protein-bound calcium Total plasma calcium level drops Ionized calcium level may remain normal
C-Excessive loss in bowel	No clinical conditions are known that cause the liver to produce large amounts of albumin
D-Severe burns (plasma loss in the absence of skin barrier)	The only cause of hyperalbuminemia is DEHYDRATION,
Another case of low amount of albumin or any other plasma protein is when the nurse wants to draw blood she'll use a tonica to make the veins more visible, so if she forgot to remove it after inserting the needle and she drew blood, albumin amount would be lower than normal because blood flow is occluded.	

3- α1-Antitrypsin

- Synthesized by the liver and macrophages
- An acute-phase protein that inhibits proteases
- Proteases are produced **endogenously** and from leukocytes and bacteria
  - Digestive enzymes (trypsin, chymotrypsin)
  - Other proteases (elastase, thrombin)
- Infection leads to **protease release** from bacteria and leukocytes

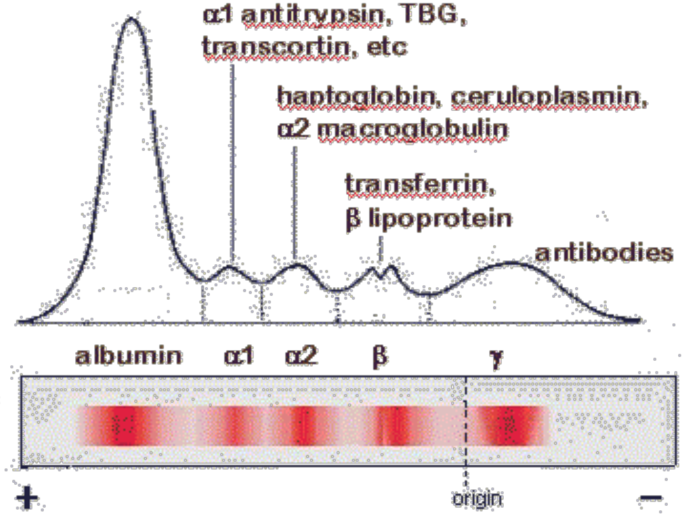
Types of α1-Antitrypsin

- Over 30 types are known
- The most common is M type
- Genetic deficiency of α1-Antitrypsin
  - ✓ Synthesis of the defective α1-Antitrypsin occurs **in the liver** but it cannot secrete the protein
  - ✓ α1-Antitrypsin accumulates in **hepatocytes** and is deficient in plasma

- Trypsin = protease = digesting protein normally produced by cells in the body. Or proteins that is part of leukocytosis.
- Antitrypsin is used when proteins are synthesized excessively.
- Acute phase protein → the body's respond is within 24hrs. If increased then its positive if it decreases then its negative.

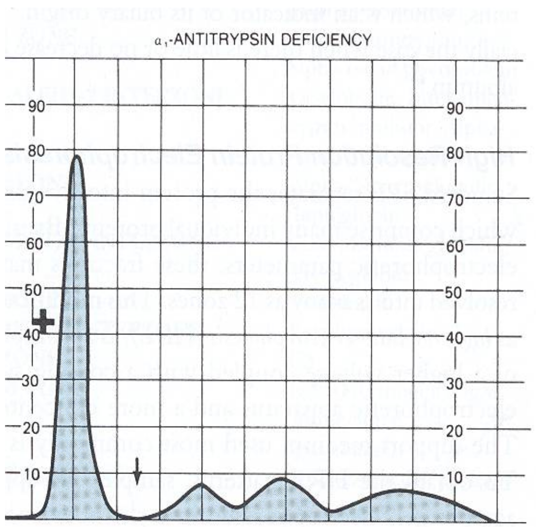
Clinical Consequences of α1-Antitrypsin Deficiency

- Neonatal **jaundice** with evidence of **cholestasis**
- Childhood **liver cirrhosis**
- Pulmonary emphysema in young adults



Laboratory Diagnosis

1. Lack of α1-globulin band in protein electrophoresis
2. Quantitative measurement of α1-Antitrypsin by:
   - Radial immunodiffusion, isoelectric focusing or nephelometry



If α1-Antitrypsin is diffient excessive cleavage/digestion of proteins happen. Especially in smokers an inflammatory reaction happens where neutrophils produce elastase, and since there's no α1-Antitrypsin to cleave it, elastase would accumulate causing emphysema

α-Fetoprotein (AFP)

- Synthesized in the **developing embryo** and **fetus** by the **parenchymal cells of the liver**
- AFP levels decrease gradually during intra-uterine life and reach adult levels at birth
- **Function** is unknown but it may protect fetus from immunologic attack by the mother
- No known physiological function in adults

- AFP has different isoforms associated to different types of cancers for ex. L3 → liver cancer, so we measure L3 isoform AFP and we measure total AFP if L3 is more than 10% then it means that in the next 21 months the person would develop cancer.

ABNORMALITIES

1. Elevated maternal AFP levels are associated with:
   - Neural tube defect (spina bifida), anencephaly
2. Decreased maternal AFP levels are associated with:
   - Increased risk of Down syndrome
3. AFP is a tumor marker for:

   Hepatoma and testicular cancer

Haptoglobin

- Synthesized by the **liver**
- Binds to free hemoglobin to form complexes that are metabolized in the RES
- **Limits iron losses** by preventing Hb loss from kidneys
- Plasma level decreases during hemolysis

- Prevent hemoglobin loss especially in people w/ hemolytic disease.
- If hemoglobin went to the kidney first there will be loss of it and it'll cause damage to the kidney and then hemoglobin and iron are lost.
- Haptoglobin attaches to hemoglobin + iron complex making it bigger and prevent it being transferred to kidney and takes it to be degraded in reticuloendothelial system, after that hemoglobin and iron are taken back in body.

RES : Reticulo Endothelial System

Ceruloplasmin

- Synthesized by the **liver**
- Contains >90% of serum copper
- An oxidoreductase that **inactivates ROS** causing tissue damage in acute phase response
- **Important for** iron absorption from the intestine

❖ Wilson's disease:

  - Due to **low** plasma levels of ceruloplasmin
  - Copper is accumulated in the liver and brain

- It is called apoceruloplasmin.
- It carries 90% of copper and the albumin carries the rest 10%.
- Diffiency causes oxidative damage.

Transferrin

- A major iron-transport protein in plasma 30% saturated with iron
- Plasma level drops in:
  Malnutrition, liver disease, inflammation, malignancy
- **Iron deficiency** results in increased hepatic synthesis
- A negative acute phase protein
- 1 molecule transferrin binds to 2 molecules of iron
- Iron ↓ liver produces ↑ transferrin

β2–Microglobulin

- A component of human leukocyte antigen (HLA)
- Present on the surface of lymphocytes and most nucleated cells
- Filtered by the renal glomeruli due to its small size but most (>99%) is reabsorbed
- Elevated serum levels are found in:
  - Overproduction in disease
- May be a tumor marker for:
  - Leukemia, lymphomas, multiple myeloma

Not a tumor marker for diagnosing, rather it helps determining the size of tumor

- Elevated serum levels also in :
  ✓Inflammation , infections , rheumatoid arthritis and SLE

CRP

C-Reactive Protein (CRP)

- An acute-phase protein synthesized by the liver
- Important for phagocytosis
- High plasma levels are found in many inflammatory conditions such as rheumatoid arthritis
- A marker for ischemic heart disease

- Binds on the surface of the cells to make them more susceptible to phagocytosis by macrophages

- Ultrasensitive c-reactive protein is the marker for ischemic heart disease and not normal CRP

Hypergammaglobulinemia

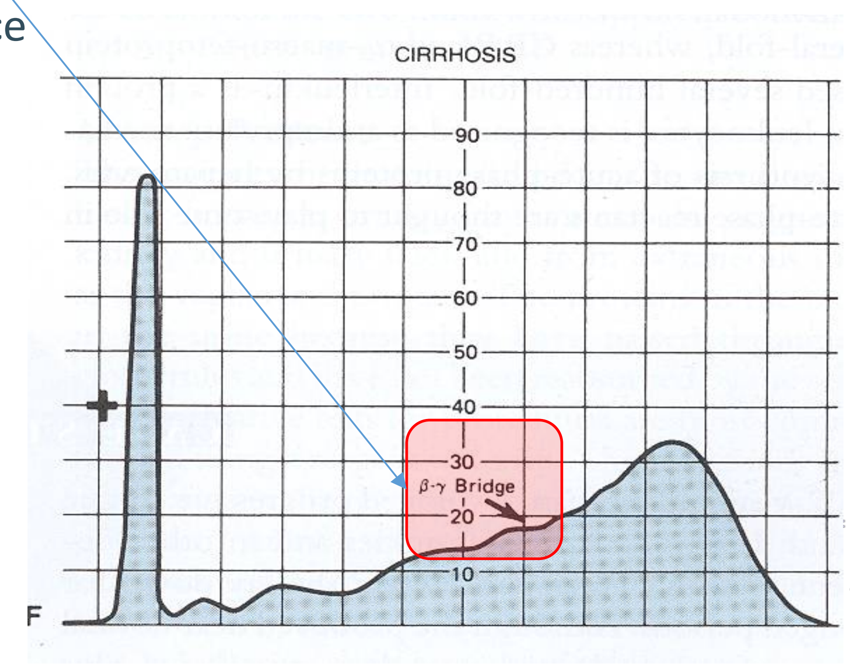
❖ **May result from stimulation of:**

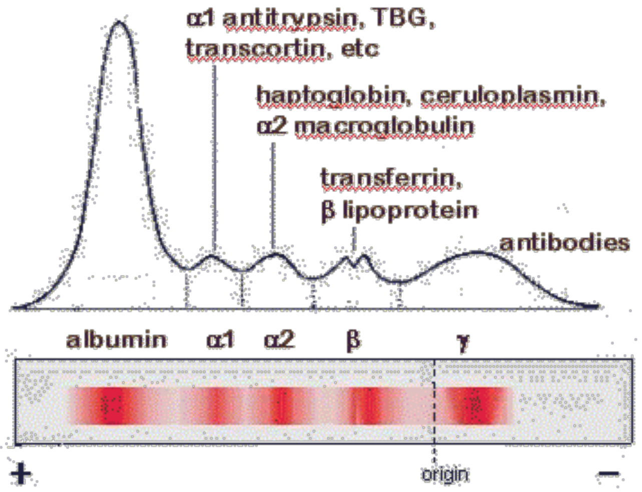
- **B cells** (Polyclonal hypergammaglobulinemia)
- **Monoclonal proliferation** (Paraproteinemia)

- Polyclonal → mixture of antibodies from different plasma cells
- Monoclonal → specific type of antibody from one type of plasma protein

Polyclonal hypergammaglobulinemia:

- Stimulation of many clones of B cells produce a wide range of antibodies
- γ-globulin band appears large in electophoresis
- **Clinical conditions:** acute and chronic infections, autoimmune diseases, chronic liver diseases





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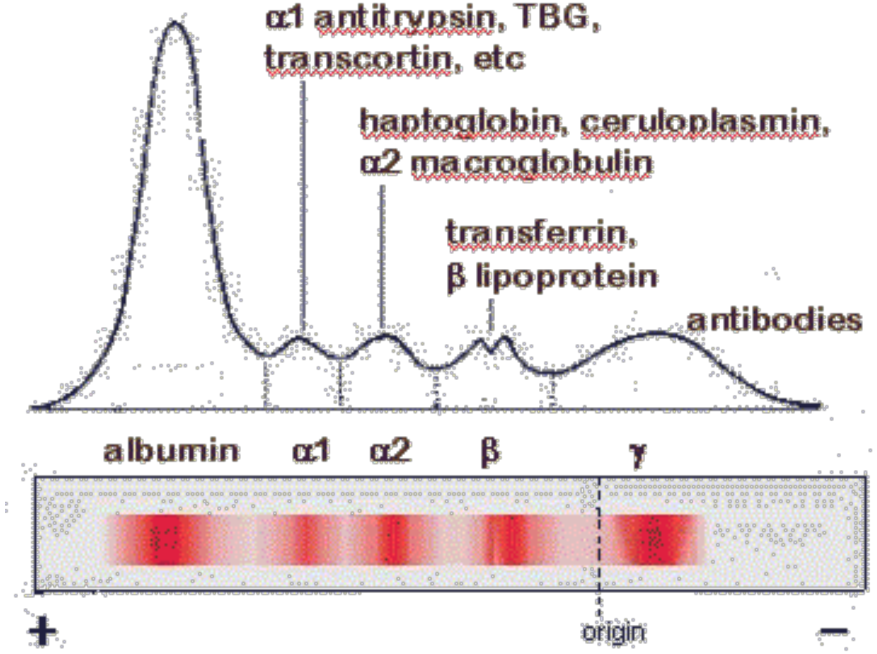
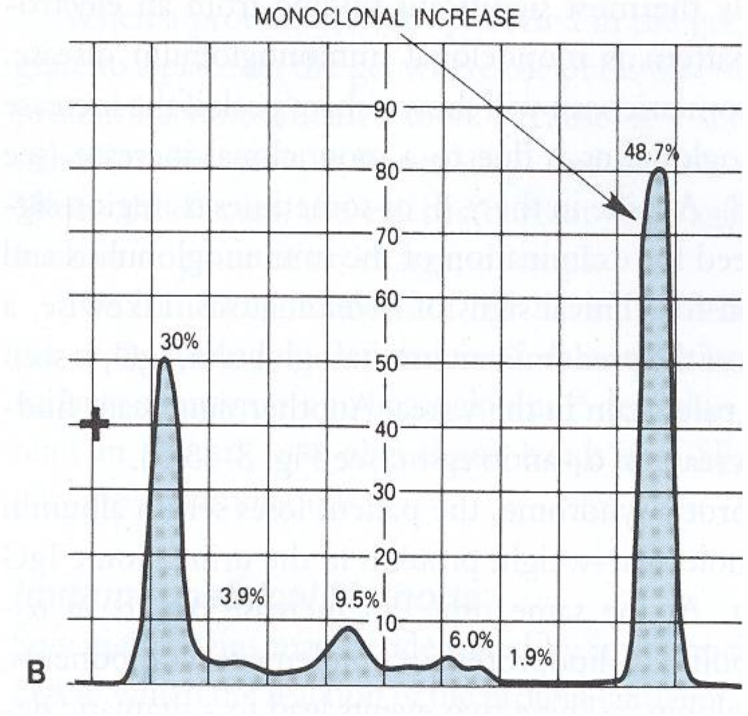
Monoclonal Hypergammaglobulinemia

- Proliferation of a single B-cell clone produces a single type of Ig
- Appears as a separate dense band (paraprotein or M band) in electrophoresis
- Paraproteins are characteristic of malignant B-cell proliferation
- Clinical condition: multiple myeloma



- A single thin peak thin peak



Positive Acute Phase Proteins

- Plasma protein levels **increase** in:

  *Infection, inflammation , malignancy, trauma, surgery

- These proteins are called acute phase reactants

- Synthesized due to: body's response to injury

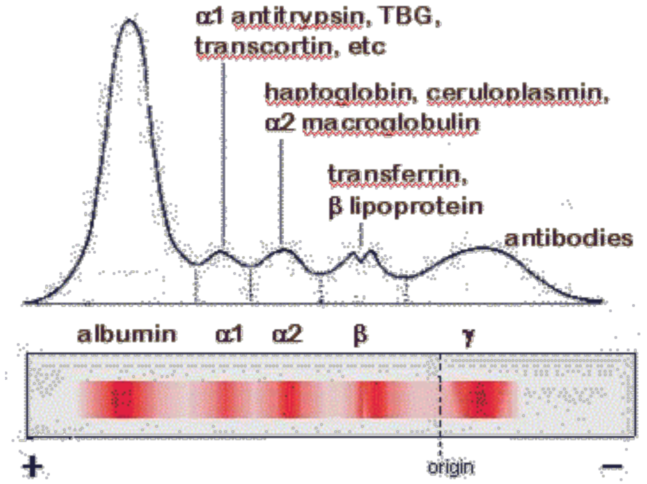
- Examples: α1-Antitypsin, haptoglobin, ceruloplasmin, fibrinogen, c-reactive protein

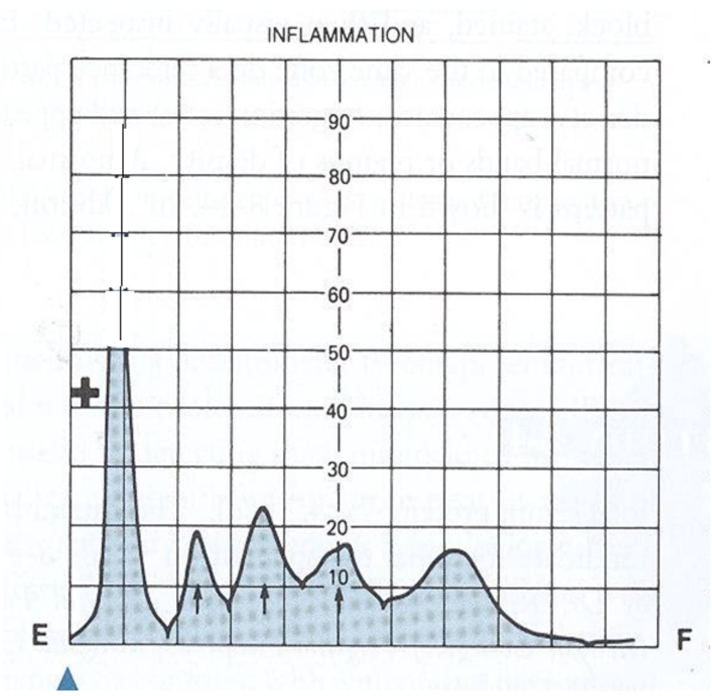
- <u>Mediators cause these proteins to increase after injury</u>

- **Mediators:** Cytokines (IL-1, IL-6), tumor necrosis factors α and β , interferons, platelet activating factor

- **Functions:**

  1. Bind to polysaccharides in bacterial walls
  2. Activate complement system
  3. Stimulate phagocytosis

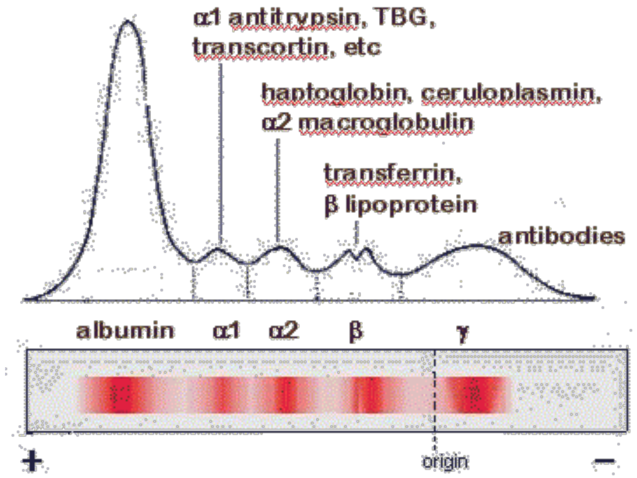


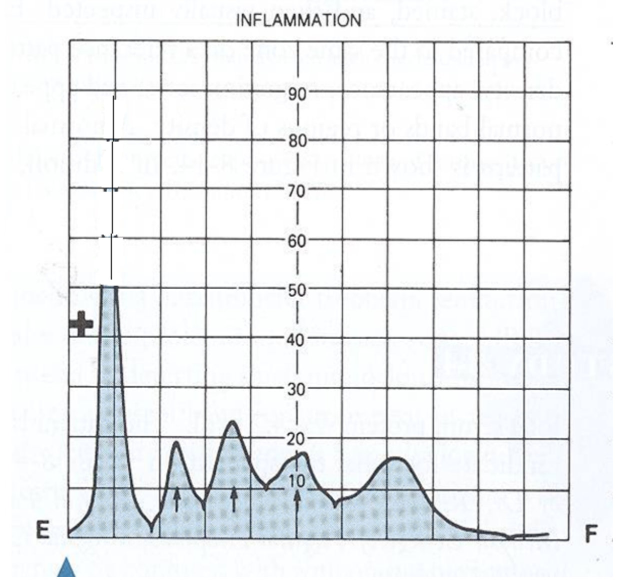


Positive Acute Phase Proteins

Negative Acute Phase Proteins

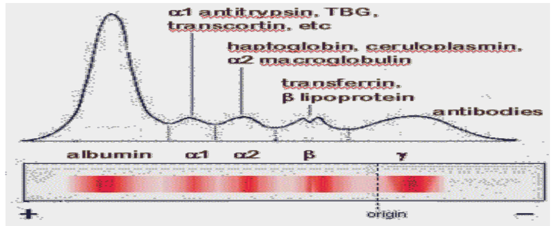
- **These proteins decrease in inflammation:**
  - Albumin, prealbumin, transferrin
- Mediated by inflammatory response via cytokines and hormones
- Synthesis of these proteins decrease to save amino acids for positive acute phase proteins



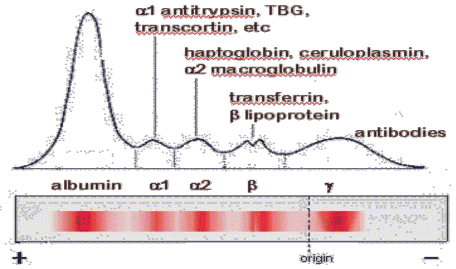


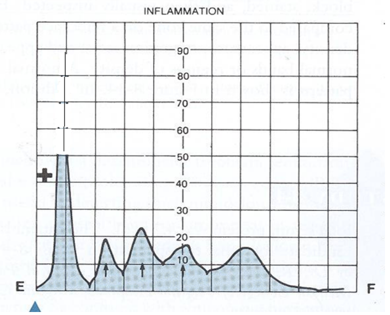
Plasma proteins summary

Overview:
•Functions and characteristics of plasma proteins
•Measurement of plasma proteins and diagnosis of
•diseases
•Electrophoretic patterns of plasma proteins
•Acute phase proteins
•**Plasma Proteins:**
•Plasma contains >300 different proteins
•Many pathological conditions affect level of plasma
•proteins
•Mostly synthesized in the liver
•Some are produced in other sites
•A normal adult contains ~70 g/L of pps
•**Functions of plasma proteins:**
•Transport (Albumin, prealbumin, globulins)
•Maintain plasma oncotic pressure (Albumin)
•Defense (Immunoglobulins and complement)
•Clotting and fibrinolysis (Thrombin and plasmin)
•**Measurement of Plasma Proteins:**
•A) Quantitative measurement of a specific protein:
• Chemical or immunological reactions
B) Semiquantitative measurement by electrophoresis:
•Proteins are separated by their electrical charge in electrophoresis
•Five separate bands of proteins are observed
•These bands change in disease
•**Normal Pattern of Plasma Protein Electrophoresis:**



Types of Plasma Proteins:
Prealbumin Albumin α1-Globulins:
• a1-Antitrypsin, α-fetoprotein α2-Globulins:
• Ceruloplasmin, haptoglobin β-Globulins:
• CRP, transferrin, β2-microglobulin γ- Globulins
Positive Acute Phase Proteins:
• Plasma protein levels increase in:
→ Infection, inflammation, malignancy, trauma, surgery
• These proteins are called acute phase reactants
• Synthesized due to body's response to injury
• Examples: α1-Antitypsin, haptoglobin, ceruloplasmin, fibrinogen, c-reactive protein
• Mediators cause these proteins to increase after injury
• Mediators: Cytokines (IL-1, IL-6), tumor necrosis factors α and β, interferons, platelet activating factor • ***Functions:***
1. Bind to polysaccharides in bacterial walls 2. Activate complement system
3. Stimulate phagocytosis





Negative Acute Phase Proteins:
• These proteins decrease in inflammation → Albumin, prealbumin, transferrin
•Mediated by inflammatory response via cytokines and hormones
•Synthesis of these proteins decrease to save amino acids for positive acute phase proteins

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Plasma proteins summary

Prealbumin (Transthyretin)

A transport protein for:

- Thyroid hormones
- Retinol (vitamin A)

Migrates faster than albumin in electrophoresis
Separated by immunoelectrophoresis
Lower levels found in:

- liver disease, nephrotic syndrome, acute phase inflammatory response, malnutrition
  Short half-life (2 days)

Albumin

- Most abundant plasma protein (~40 g/L) in normal adult
- Synthesized in the liver as preproalbumin and secreted as
- albumin
  Half-life in plasma: 20 days
  Decreases rapidly in injury, infection and surgery
- **Functions:**
- Maintains oncotic pressure:
- The osmotic pressure exerted by plasma proteins that
- pulls water into the circulatory system
- Maintains fluid distribution in and outside cells and plasma volume
- 80% of plasma oncotic pressure is maintained by albumin
- A non-specific carrier of
- hormones, calcium, free fatty acids, drugs, etc.
- Tissue cells can take up albumin by pinocytosis where it is hydrolyzed to amino acids
- Useful in treatment of liver diseases, hemorrhage, shock and burns.

Hypoalbuminemia

- Decreased albumin synthesis (liver cirrhosis, malnutrition) Increased losses of albumin
- Increased catabolism in infections
- Excessive excretion by the kidneys (nephrotic
- syndrome)
- Excessive loss in bowel (bleeding)
- Severe burns (plasma loss in the absence of skin barrier)

Effects:

- Edema due to low oncotic pressure
  Albumin level drops in liver disease causing low
- oncotic pressure
- Fluid moves into the interstitial spaces causing edema
- Reduced transport of drugs and other substances in plasma
- Reduced protein-bound calcium
  Total plasma calcium level drops
  Ionized calcium level may remain normal

Hyperalbuminemia

- No clinical conditions are known that cause the liver to produce large amounts of albumin
- The only cause of hyperalbuminemia is dehydration

alpha1 and alpha2 globulins summary

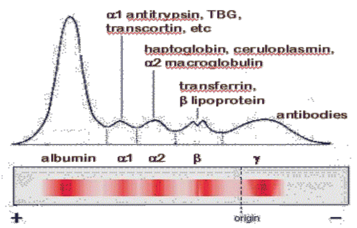
α1-Antitrypsin

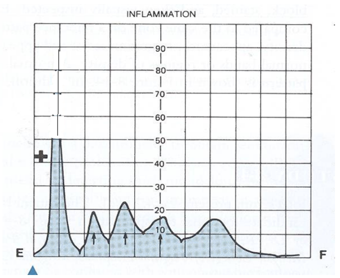
Synthesized by the liver and macrophages
An acute-phase protein that inhibits proteases
Proteases are produced endogenously and from leukocytes and bacteria
- Digestive enzymes (trypsin, chymotrypsin)
- Other proteases (elastase, thrombin)

Infection leads to protease release from bacteria and from leukocytes
Over 30 types are known
The most common is M type
Genetic deficiency of a1-Antitrypsin
- Synthesis of the defective a1-Antitrypsin occurs in the liver but it cannot secrete the protein
- α1-Antitrypsin accumulates in hepatocytes and is deficient in plasma

Clinical Consequences of a1-Antitrypsin Deficiency
Neonatal jaundice with evidence of cholestasis
Childhood liver cirrhosis
Pulmonary emphysema in young adults
Laboratory Diagnosis
Lack of ✔ 1-globulin band in protein electrophoresis
Quantitative measurement of ✔ 1-Antitrypsin by:
- Radial immunodiffusion, isoelectric focusing or nephelometry

α1-Antitrypsin





-Fetoprotein (AFP)

Synthesized in the developing embryo and fetus by the parenchymal cells of the liver
AFP levels decrease gradually during intra-uterine life and reach adult levels at birth
Function is unknown, but it may protect fetus from immunologic attack by the mother
No known physiological function in adults
Elevated maternal AFP levels are associated with:
- Neural tube defect, anencephaly

Decreased maternal AFP levels are associated with:
- Increased risk of Down's syndrome

AFP is a tumor marker for:
- Hepatoma and testicular cancer

Ceruloplasmin

- Synthesized by the liver
  Contains >90% of serum copper
  An oxidoreductase that inactivates ROS causing tissue
- damage in acute phase response
  Important for iron absorption from the intestine Wilson's disease:
  - Due to low plasma levels of ceruloplasmin
  - Copper is accumulated in the liver and brain

Haptoglobin

- Synthesized by the liver
  Binds to free hemoglobin to form complexes that are
- metabolized in the RES
  Limits iron losses by preventing Hb loss from kidneys
- Plasma level decreases during hemolysis

β and Gamma Globulins summary

Transferrin

A major iron-transport protein in plasma 🎭 30% saturated with iron

Plasma level drops in:

🎭 Malnutrition, liver disease, inflammation, malignancy

🎭 Iron deficiency results in increased hepatic synthesis

A negative acute phase protein

β_2–Microglobulin

A component of human leukocyte antigen (HLA)

Present on the surface of lymphocytes and most nucleated cells

Filtered by the renal glomeruli due to its small size but most (>99%) is reabsorbed

Elevated serum levels are found in

🎭 Overproduction in disease

May be a tumor marker for:

🎭 Leukemia, lymphomas, multiple myeloma

C-Reactive Protein (CRP)

An acute-phase protein synthesized by the liver Important for phagocytosis

High plasma levels are found in many inflammatory conditions such as rheumatoid arthritis

A marker for ischemic heart disease

Hypergammaglobulinemia

May result from stimulation of

B cells (Polyclonal hypergammaglobulinemia)

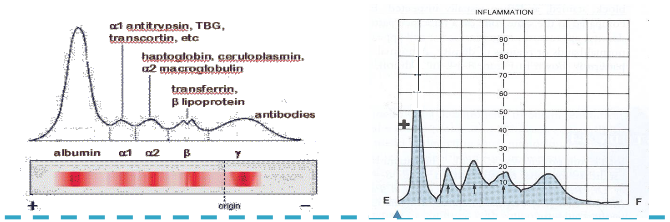
Monoclonal proliferation (Paraproteinemia)

Polyclonal hypergammaglobulinemia:

Stimulation of many clones of B cells produce a wide range of antibodies

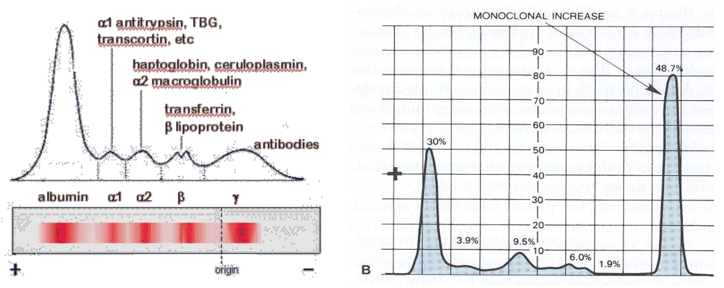
γ -globulin band appears large in electophoresis

Clinical conditions: acute and chronic infections, autoimmune diseases, chronic liver diseases



Monoclonal Hypergammaglobulinemia

- Proliferation of a single B-cell clone produces a single type of Ig
- Appears as a separate dense band (paraprotein or M band) in electrophoresis
- Paraproteins are characteristic of malignant B-cell proliferation
- Clinical condition: multiple myeloma



QUIZ

Q1 : low levels of prealbumin is found in ?

A- liver disease

B- Arthritis .

C- Vitamin A deficiency .

D- All of the above

Q2 : Which ONE of the following is an effect of hypoalbuminemia ?

A- Edema .

B- inflammation .

C- Increase transport of drugs .

D- Plasma Ca Increase .

Q3 : the most common type of alpha1-Antitrypsin is ?

A- L .

B- N .

C- M .

D- both A & B .

Q4 : Which ONE of the following is the cell that synthesize alpha-Fetoprotein ?

A- parenchymal cells of kidney .

B- parenchymal cells of liver .

C- lumen cells of kidney .

D- lumen cells of liver .

Q5 : What is the function of Haptoglobin ?

A- protect against infection .

B- prevent HB loss from liver .

C- limits iron loss related to kidney .

D- Non of the above

Q6 : Which of the following is true regarding CRP ?

A- Important for phagocytosis .

B- synthesized by kidney .

C- marker for heart failure .

D- All of them .

QUIZ

Q7 : What are the abnormalities associated with impaired alpha-fetoprotein ?

1- Elevated maternal AFP levels are associated with:
Neural tube defect (spina bifida), anencephaly
2- Decreased maternal AFP levels are associated with:
Increased risk of Down syndrome
3- AFP is a tumor marker for:
Hepatoma and testicular cancer

Q8 : Determine two causes and associated conditions of hypoalbuminemia ?

1- Decreased albumin synthesis (liver cirrhosis, malnutrition)
2- Increased losses of albumin
- Increased catabolism in infections
- Excessive excretion by the kidneys (nephrotic syndrome)

Suggestions and recommendations

1) A 2) A 3) C 4) B 5) C 6) A

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THANK YOU

FOR CHECKING OUR WORK

PLEASE CONTACT US IF YOU HAVE ANY ISSUE

- Lippincott's Illusrated Reviews Biochemistry 6th E

Review the notes

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