



IX. Proteins

Proteins

A. Introduction

1. General characteristics

- a. Specificity – while carbohydrates, lipids, etc. may be found in all animals and plants, proteins are specific
 - 1) Differing species have different proteins
 - 2) Organs/tissues have proteins that are unique
 - 3) Individuals have proteins specific for that individual
- b. All proteins contain carbon, hydrogen, oxygen, sulfur and nitrogen
- c. Nitrogen sets proteins apart from carbohydrates and fats
- d. Depending on their specific function, proteins may also contain phosphorous, iodine, iron, copper, etc.

2. Amino acids

- a. Number
 - 1) Approximately 40 are found in nature
 - 2) Of the 40, 20 seem to occur in all proteins
 - 3) Nine “essential” amino acids must be provided by diet
- b. Structure – features common to all amino acids
 - 1) Amino group (NH_2) located on a carbon adjacent to a carboxyl group (COOH)
 - 2) Peptide bonds or peptide linkage



c. Characteristics of amino acids

- 1) The part of the amino acid molecule that makes one different from another is the side chain or R group
 - a) Glycine is the simplest ($R = H$)
 - b) Alanine ($R = CH_3$)
 - c) Cystine and methionine contain sulfur in their R chains
 - d) Tyrosine, phenylalanine and tryptophane have complex R chains containing aromatic rings
- 2) Amphoteric molecules
 - a) The carboxyl group ($COOH$) is a proton donor
 - b) The amino group (NH_2) is a proton acceptor
 - c) When the molecule ionizes, it becomes a dipolar ion and is called a *zwitterion*
 - d) As a result of this characteristic, protein solutions can act as acids or bases and are used as pH buffers

d. Aminoacidopathies

- 1) Rare inherited disorders of amino acid metabolism
- 2) Often due to the failure of a specific enzyme to perform or to a membrane transport system failure
- 3) Examples
 - a) phenylketonuria



C *phenylalanine hydrolase*

b) familial tyrosinemia

c) alkaptonuria

C homogentisic acid oxidase

d) maple syrup urine disease

e) homocystinuria

C *L-serine dehydratase*

f) cystinuria

C cystine reductase?

3. Terms associated with protein chemistry

a. *Dipeptide* – two amino acids joined together by a peptide bond

b. *Polypeptide* – six to thirty amino acids linked by peptide bonds

c. *Proteosis* and *Peptones* – refers to protein breakdown products containing large numbers of polypeptides

d. *Proteins* – forty or more amino acids resulting in a chain with a molecular weight $\geq 5,000$ daltons. The chain takes on characteristics associated with proteins

4. Properties of proteins

a. *Primary structure* – refers to the sequence of the AA that make up the polypeptide



b. *Secondary structure* – refers to the twisted coil or helix structure held together by bonds formed between the different R groups

c. *Tertiary structure*

5. Denaturing of proteins

B. Functions of Plasma Proteins

1. Nutritive

2. Transport agents

a. Simple proteins

b. Conjugated proteins

- 1) Metalloproteins (apoproteins) contain a metallic ion
- 2) Chromoproteins contain an organic group with a metallic ion
- 3) Lipoproteins transport lipids
- 4) Glycoproteins contain carbohydrates
- 5) Mucoproteins

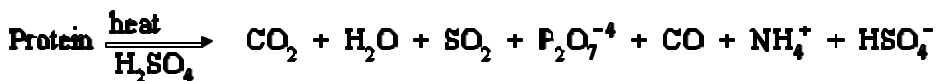


3. Nucleoproteins
4. Enzymes are functional proteins that serve as biological catalysts
5. Antibodies
6. Other functional proteins include certain hormones and certain clotting factors
7. Physiochemical function of colloidal osmotic pressure

C. Methods for Protein Determination

1. Kjeldahl technique/digestion

- a. Protein is subjected to heat and strong acid (H_2SO_4) to break it down



- b. Two of the products, NH_4^+ or HSO_4^- could be quantitated by titration or distillation processes

- c. Nesslerization – an old method for the quantitative determination of NH_3 concentration

Nessler's reagents: Alkaline mercuric iodide
 Potassium iodide
 Potassium hydroxide

- d. Protein measured as *protein nitrogen* requires a calculation

- 1) 16% of protein is nitrogen



2) Ratio $\frac{1.00}{0.16} \times (\text{nitrogen})$

OR

$$\text{protein} = 6.25 \times (\text{nitrogen})$$

2. Biuret method of protein determination
 - a. Biuret reagent
 - b. Principle – protein reacts with an alkaline copper tartrate solution to give a violet colored complex
 - c. The amount of color produced is proportional to the number of peptide bonds available

3. Total protein by refractive index
 - a. Instrument
 - b. Assumptions
 - 1) Total concentration of inorganic substances (i.e., electrolytes) do not vary appreciably from serum to serum
 - 2) Differences in refractive index reflects difference in protein concentration
 - c. Critical angle measurement theory: The light beam enters parallel to the prism and is refracted by the protein to be projected against the eye piece. The eyepiece has scales calibrated in both refractive index and gms/dL.
 - d. This procedure is excellent for clear, non-pigmented, non-turbid serums



e. Hemolysis, increased bilirubin or lipids may cause gross errors

4. Procedures for albumin determination

a. Salt fractionation

1) Albumin can be separated from globulins by using their different solubilities in water and salt solutions

a) Albumin is completely soluble in water while globulin is not

b) By adding a small amount of salt, the globulins go into solution

c) Increasing the amount of salt will cause the globulins to become insoluble

2) Determining albumin using this method

b. Dye binding – measure albumin directly by its ability to bind certain dyes

1) Methyl orange

2) HABA

3) Bromocresol green

a) Used most frequently

b) Binds with albumin at pH 4.2

4) Bromocresol purple

D. Normal Values

1. Total serum protein = 6.0-8.2 gm/dl



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2. Albumin = 3.5-5.2 gm/dl
 3. Globulin = approximately 2.0-3.0 gm/dl
 4. Albumin/globulin ratio

E. Abnormal Protein Concentrations

1. Increased serum protein
 - a. Dehydration
 - 1) Relative increase in serum protein
 - 2) Causes
 - a) Decreased water intake
 - b) Increased water loss
 - b. Multiple myeloma and related diseases
2. Decreased serum protein
 - a. Decreased liver synthesis
 - b. Extended period of low protein intake
 - c. Intestinal malabsorption
 - d. Acute and chronic infections
 - 1) Decreased concentration of albumin due to increased utilization



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- 2) Increase gamma globulin

 - e. Loss of albumin and low molecular weight globulins via kidney

 - f. Salt retention causes a relative hypoproteinemia

 - g. Severe burns

 - h. Extensive bleeding

F. Methods of Serum Protein Separation

- 1. Fractionation

- 2. Ultracentrifugation

- 3. Molecular sieves

- 4. Immunochemical methods

- 5. Serum electrophoresis
 - a. Terms
 - 1) Electrophoresis

 - 2) Electroendosmosis

 - b. Application of electrophoresis to clinical and biochemical problems is the result of the work of *Tiselius* and *Longsworth* in the development of the moving boundary technique

 - c. Uses of electrophoresis
 - 1) Protein



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- 2) Lipoprotein
 - 3) Isoenzyme
 - 4) Hemoglobin

d. Materials

- 1) Support medium
 - a) Paper – first commonly used medium
 - b) Starch
 - c) Acrylamide
 - d) Gels of agar/agarose

e) Cellulose acetate

- 2) Buffer = barbital buffer with a pH of 8.6
- 3) Electrophoresis cell

e. Process

The reason the proteins separate into the fractions is in part due to their size and weight but *mostly* due to the amount of their charge

- 1) After the serum is applied to the support media, the protein dissolves in the buffer
- 2) A specific amount of current is applied for a specific length of time
- 3) Proteins move toward the anode at a rate proportional to their mobilities



f. Staining

- 1) "Fixes" the proteins to the membrane by denaturing
- 2) Makes the fractions visible
- 3) Decolorization to remove background color
- 4) Dry membranes

g. Quantitation of the fractions

- 1) Visual
- 2) Elution
- 3) Densitometer

h. Fractions determined

- 1) Albumin
 - a) Smallest molecular weight and fastest moving fraction
 - b) Amounts to 50-65% of the total protein (3.5-5.2 gm/dl)
- 2) Alpha 1 globulin
 - a) 2-6% (0.17-0.33 gm/dl)
 - b) Increase associated with infections and inflammatory reactions
 - c) Decrease may indicate alpha 1 antitrypsin deficiency
 - d) Other alpha 1 proteins
- 3) Alpha 2 globulin
 - a) 6-13% (0.5-1.0 gm/dl)



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- b) A strong increase in this fraction (along with a decrease in albumin) is seen in nephrotic syndrome
 - c) Haptoglobin is a member of this fraction

 - d) Other alpha 2 proteins
- 4) Beta globulin
- a) 8-15% (0.5-1.0 gm/dl)

 - b) Transferrin and B lipoprotein are members of this fraction

 - c) Other B proteins

 - d) Electrophoresis “spike” possible in this area
- 5) Gamma globulin
- a) 10-20% (0.7-1.65 gm/dl)

 - b) At pH 8.6 gammas are the closest of the fractions to their isoelectric point

 - c) Decrease = hypogammaglobulinemia

 - d) Increase is seen in chronic infections (i.e., viral hepatitis), cirrhosis (fusing of the beta and gamma fractions) etc. A gamma “spike” may be seen when there is a tremendous increase in production of a particular gamma protein (as in multiple myeloma)



G. Methods for Specific Serum Proteins

H. CSF Protein Determination

1. Normal values = 14-45 mg/dl
2. The low concentration of protein in CSF generally prohibit using the Biuret method
3. Using sulfosalicylic acid (turbidimetric) will produce a fine suspension of protein particles

4. Decreased concentration
5. Increased concentration is seen in conditions of inflammation or damage to the brain meninges
6. CSF electrophoresis
 - a. Main purpose: detection of oligoclonal IgG bands
 - b. Because protein concentration in CSF is usually very low, specimen must be concentrated
 - c. Stains
 - 1) Coomassie Brilliant Blue
 - 2) Silver stain

I. Urine Proteins

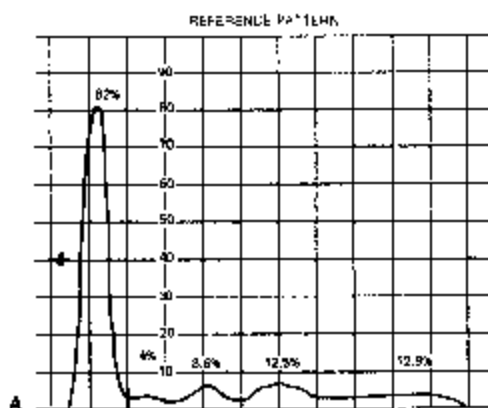
1. Review principle of urine dipstick protein



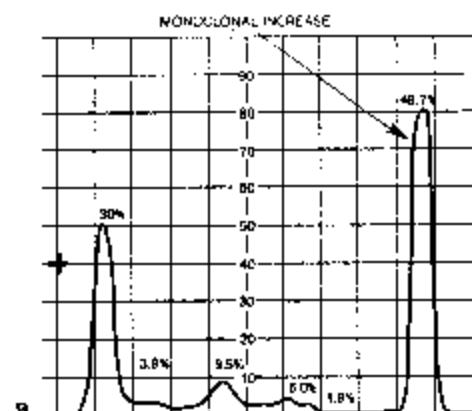
2. Significance
3. Orthostatic proteinuria
4. Bence-Jones protein

5. Waldenstrom's macroglobulinemia
 - a. Also a malignant myeloma-type of disease
 - b. Cells secrete heavy chain IgM macroglobulins that are *not* excreted in the urine
 - c. Electrophoresis spike occurs in serum samples but not in urine samples
 - d. Test: Sia water test

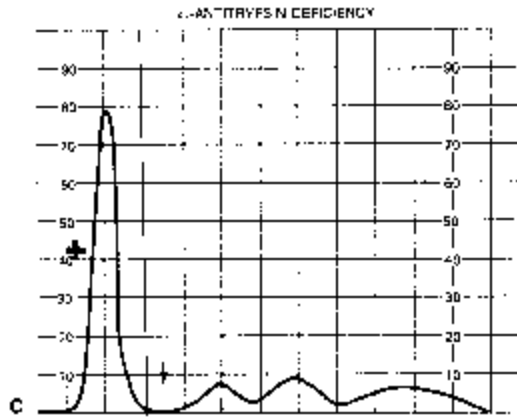
Selected densitometric patterns for protein electrophoresis. Albumin is at the anodal (+) end followed by α_1 , α_2 , β , and γ -globulin fractions. Arrows indicate decrease or increase in fractions.



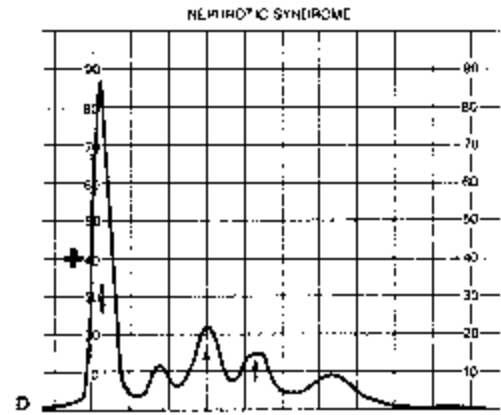
(A) Reference pattern (agarose).



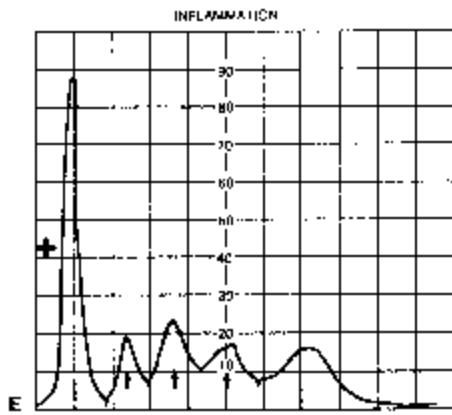
(B) Monoclonal increase in γ area (agarose).



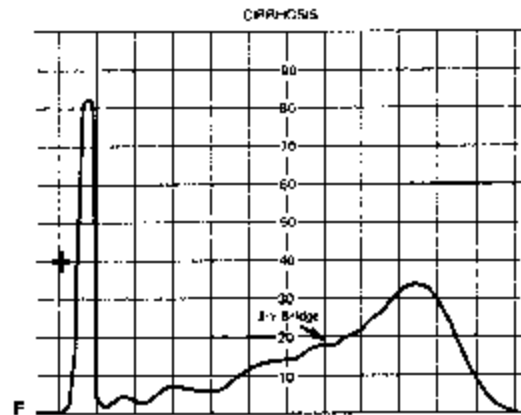
(C) α_1 -Antitrypsin deficiency (cellulose acetate).



(D) Nephrotic syndrome (cellulose acetate).



(E) Inflammation (cellulose acetate).



(F) Cirrhosis (cellulose acetate).

(A and B are courtesy of Drs. Liu, Fritsche, and Jose Trujillo, Director, and Ms. McClure of the Department of Laboratory Medicine, The University of Texas M.D. Anderson Hospital. Others are courtesy of Dr. Wu of the Hermann Hospital Laboratory/The University of Texas Medical School.