武漢大學
Wuhan University

ELECTROPHORESIS

Dept. of Biochemistry
Wuhan University

Development of history

Russian physicist Reuss discovered phoresis

1809

Michaelis named **Electrophoresis**

1909

Swedes Tiselius set up free electrophoresis

1937

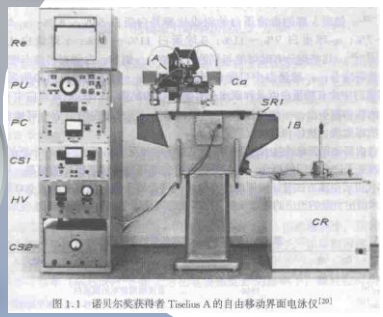


图 1.1 诺贝尔奖获得者 Tiselius A 的自由移动界面电泳仪[20]

1950s

Using different solid as supporting medium

zone electrophoresis

1980s

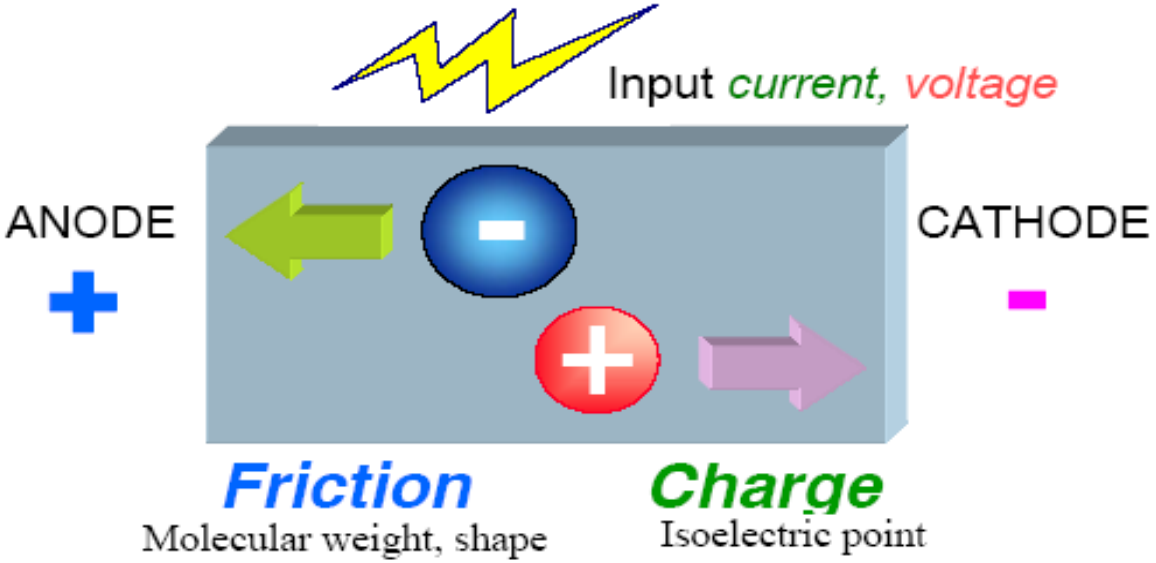
Capillary E

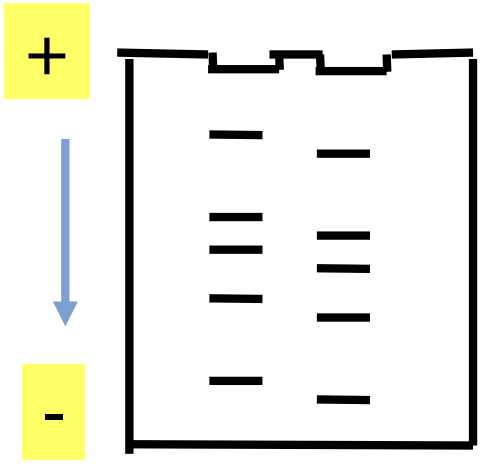
Contents

Definition

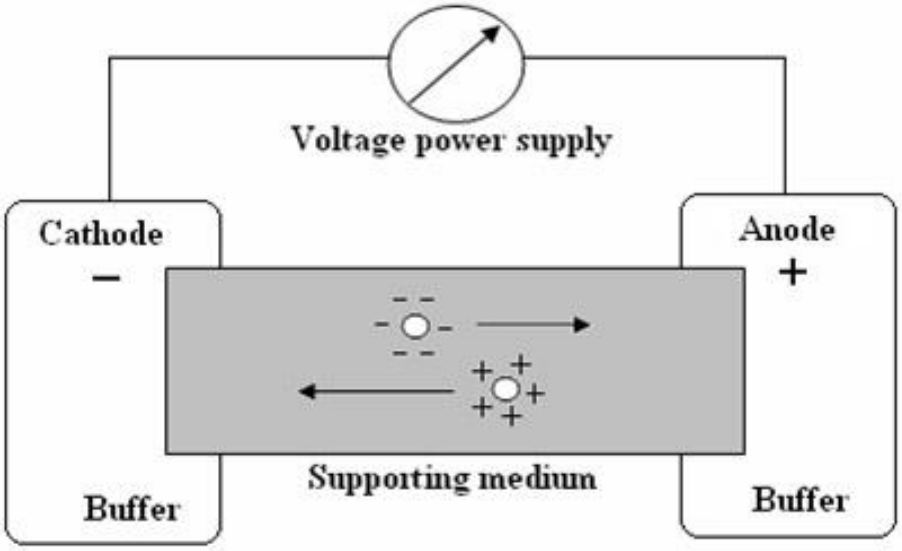
- **Electrophoresis** is the forced migration of **charged particles** such as **macromolecules**, in an electric field.

Cations move toward the cathode and anions move toward the anode.





An Electrophoretic System



a voltage power supply, electrodes, buffer, and a supporting medium

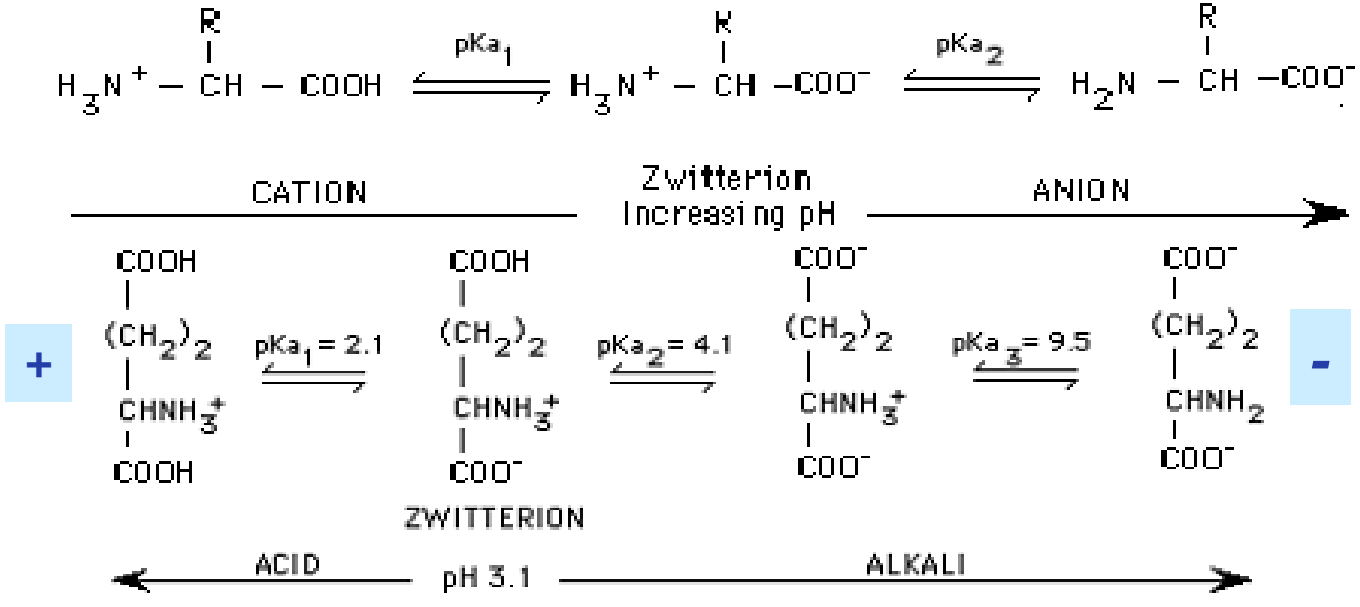
Application of Electrophoresis

- To separate and purify the charged biomolecules:

Protein, amino acids; Nucleic acid (DNA, RNA)

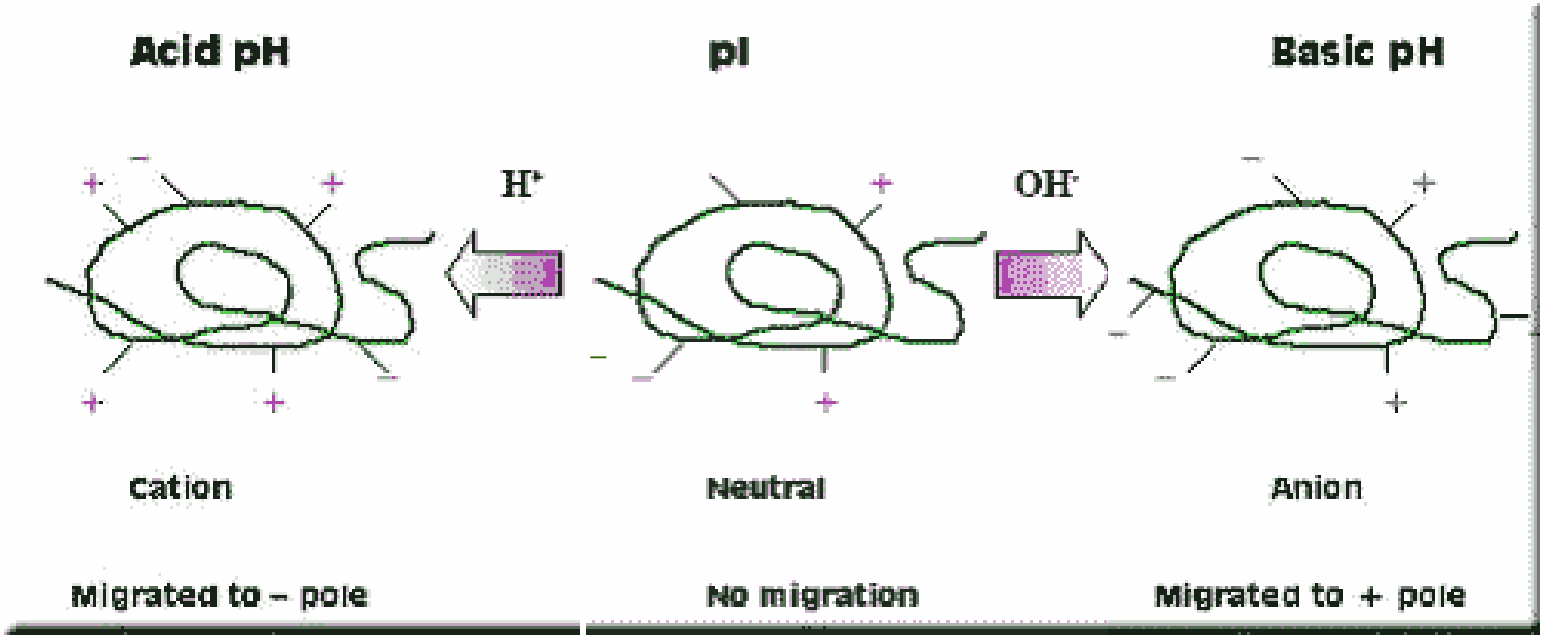
For Example

amino acids



Principle of Protein Electrophoresis

For example: protein Electrophoresis



SEPARATION OF SERUM PROTEINS BY CELLULOSE ACETATE MEMBRANE (CAM) ELECTROPHORESIS

Objective

P31

Master the principle and method of electrophoresis

How to separate and identify serum proteins using cellulose acetate membrane (CAM) as a supporting medium --*CAM electrophoresis.*

Separation of Serum Proteins by CAM Electrophoresis

Principle:

In alkaline buffer solution, serum proteins are negatively charged. They will migrate toward anode in electric field.

Because of the differences of net charge and molecular weight, the migration velocities of proteins are distinct.

Proteins separated on film are fixed in position and stained with a protein-specific dye, such as amido black. After staining and destaining, five zones indicating fractions including albumin, a_1-globulin, a_2-globulin,β-globulin, and γ-globulin of serum proteins can be visualized.

Their percentages can be estimated by scanning with densitometry.

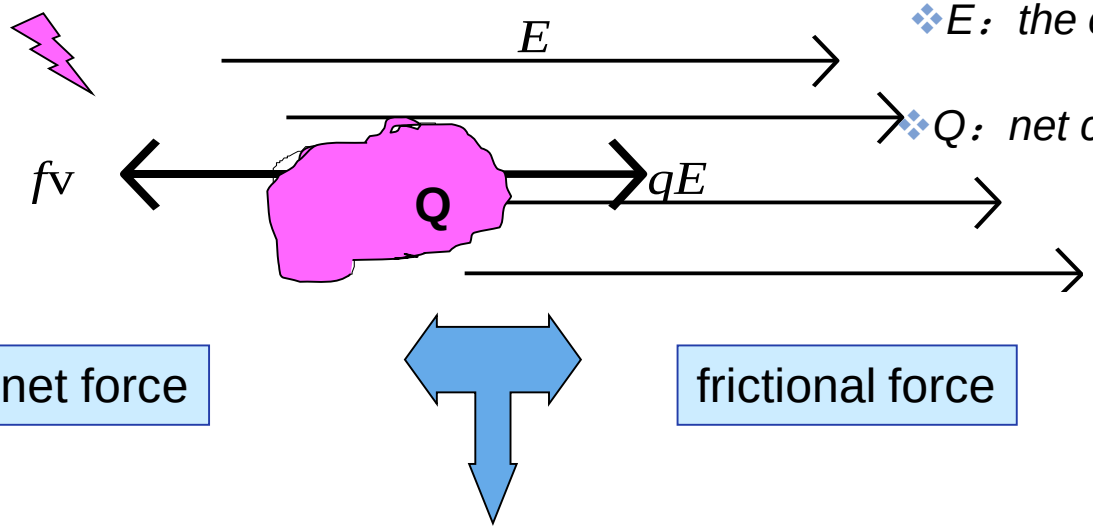
Procedure

P32

1. Immerse a CAM strip into the electrophoretic buffer, take it out and absorb water with filter paper.

2. Add 3~5μl sample on the rough side of membrane at the site 2 cm from cathode, and put the membrane on the electrophoretic device with the rough side of membrane down, connect the electrode and the film with filter paper. Electrophoresis is carried out at a constant voltage of 80V for 50~60 min.

Principle of Electrophoresis



- E：the electric field strength
- Q：net charge of the molecule

- electric force $F = E\,Q$
- frictional force $f = f v$

Equilibrium (migrate with a constant velocity ,

electric force = frictional force

So the migration velocity: $v = E\,Q / f$

Electrophoretic mobility, m

(an intrinsic property)

$$m = v / E = EQ / fE = Q / f$$

- **Q** (decided by Charge)

  Charge varies as a function of **building block molecule composition** and **buffer pH** .

- *f*

  based on **Size** (decided by **molecular weight**), **Shape**

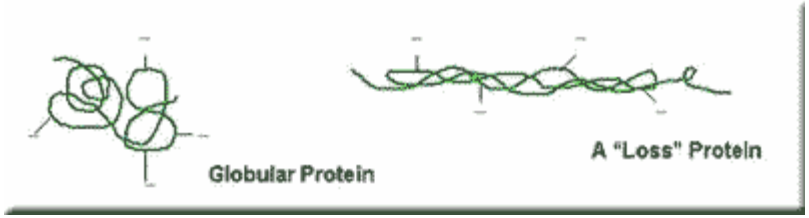
Impact Factors of Electrophoresis

1.Properties of the Molecules

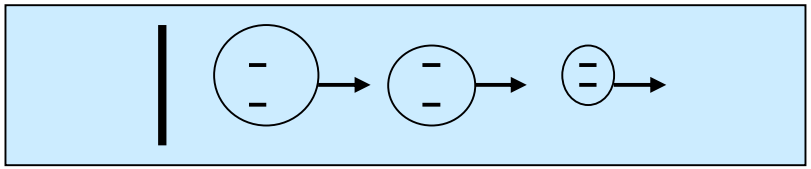
Molecular Shape

Molecular Size

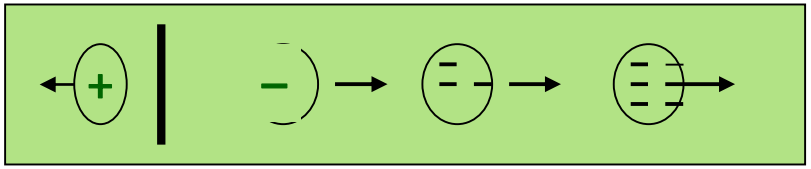
Net charge



A long, loose protein travels at a slower rate than a globular protein in a gel.



A smaller molecular has a faster U



Impact Factors of Electrophoresis

2. Properties of the electrophoretic System

- Electric field strength: E

- Property of the support medium

influence separation in 3 aspects:

- restrictions on mobility (adsorbing 吸附)
- electroendosmosis 电渗
- effect of molecular Sieve 凝胶的分子筛

Impact Factors of Electrophoresis

3、 **Electrophoretic buffer（缓冲系统）**

Solution pH

Ionic Strength

$$I = \frac{1}{2}\sum c_i z_i^2$$

Adherence of buffer

The most suitable Ionic Strength is about 20~200 mM.

Support Mediums

- Paper or Starch
- Cellulose Acetate Membrane
- Agarose Gel
- Polyacrylamide Gel (PAG)

Staining after electrophoresis

1. Proteins

Special protein dye: **Amido Black-10B;**

Coomassie brilliant Blue R-250 or G250;

Silver staining;

fluorescence labelled (Cy2, Cy3 and Cy5) and Radiolabelled Proteins (C-14, H-3, P-32)

Immunochemical detection: Western Blotting

Staining after electrophoresis

2. **Enzymes**

Appropriate enzymatic methods

3. **Glycoproteins**

Schiffs Reagent (Neutral Glycoprotein);

Alcian blue (Acidic mucopolysaccharides)

4. **Lipid**

Sudan black, Oil red O

5. **Nucleic acids**

Ethidium Bromide or SYBR gold

Special Electrophoretic Techniques and Applications

- Isoelectric focusing (IEF)
- Capillary electrophoresis (CE)
- Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE, or 2DE)
- Pulsed-Field Gel Electrophoresis (PFGE)

SEPARATION OF SERUM PROTEINS BY CELLULOSE ACETATE MEMBRANE ELECTROPHORESIS

Procedure

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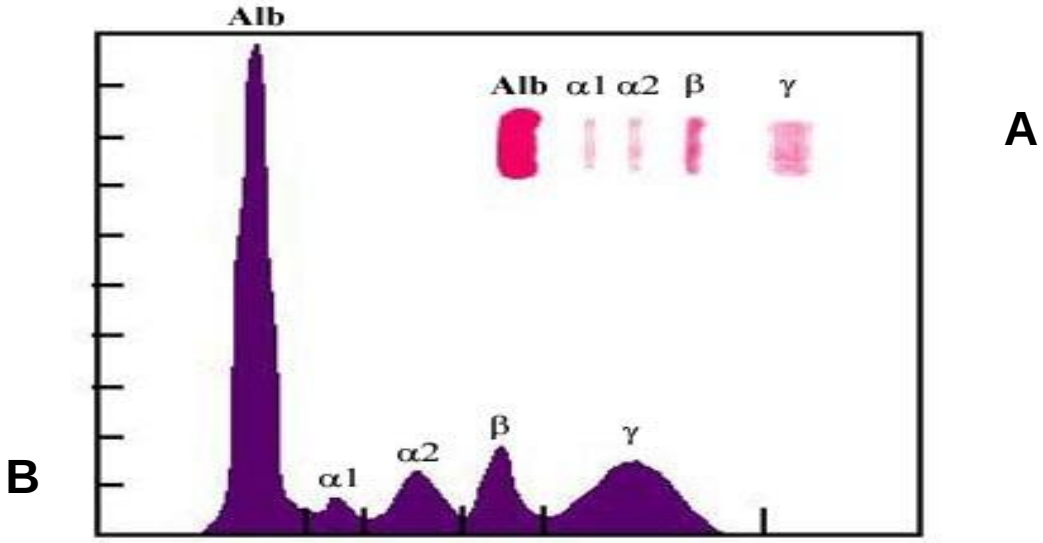
2. Electrophoresis is carried out at a constant voltage of 80V for 50~60 min.
3. After electrophoresis, take out membrane, immerse into the dye (Amido Black-10B) solution for 1 min. Then put it into destaining solution for rinsing.
4. Observe and judge the shade of color and width of the bands to estimate their quantity.

Reference

- Serum Protein Electrophoresis--Normal Pattern



A: Pattern of separated serum proteins visualized in the CAM strip.

B: Densitometric scanning of the CAM strip converts bands to characteristic peak of 5 fractions.

Serum Protein

Proteins		PI	MW
albumin	54~73%	4.8	69 kD
α_1 – globulin	2.8~5.1%	5.06	200 kD
α_2 – globulin	6.3~10.6%	5.06	300 kD
β – globulin	5.2~11%	5.12	90~150 kD
γ – globulin	12.5~20%	6.85~7.5	156~300 kD

Clinical Significance

1. The concentration of total plasma proteins is about 60~80 g/L. The plasma proteins are a complicated mixture that includes not only simple proteins but also mixed or conjugated proteins (glycoproteins and lipoproteins).
2. The CAM electrophoresis can separate the plasma proteins into 5 fractions. Albumin is the major protein of human plasma (38~48 g/L, more than 50% of the total plasma proteins. The liver produces about 12 g of albumin per day.

3. Albumin has two main functions:

(1) maintaining colloid osmotic pressure of blood;

(2) transportation: acting as a carrier molecule for bilirubin, fatty acids, trace elements and many drugs.

4. As an indicator of liver and kidney disease, immune deficiencies, malignancies of the immune system, acute and chronic infection, genetic deficiencies, central nervous system disease and numerous other pathologies.

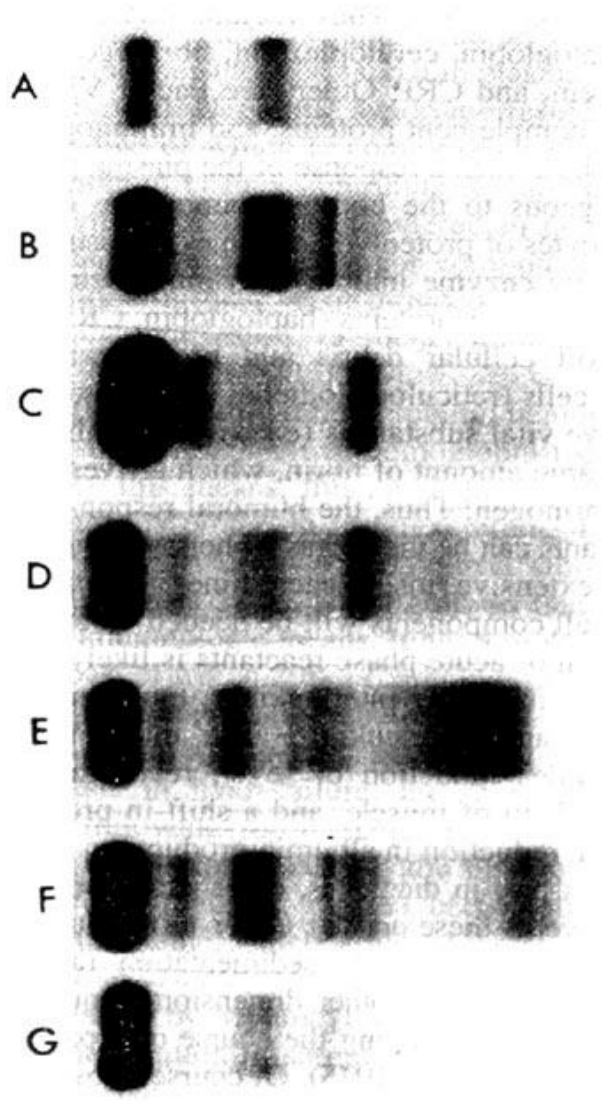


Figure 11–3. Abnormal protein patterns by agarose gel electrophoresis. Anode is to the left; fractions are as labeled in Figure 11–1; all samples are serum except for C. *A*, Inanition in an elderly patient with low total protein and markedly depressed albumin. *B*, Nephrotic syndrome with elevated α_2-macroglobulin and beta lipoprotein. *C*, Urine from protein-losing nephropathy. *D*, Iron deficiency with elevated transferrin. *E*, Broadly elevated gamma and low albumin due to liver disease. *F*, Oligoclonal gamma fraction in patient with renal disease. *G*, Hypogammaglobulinemia.

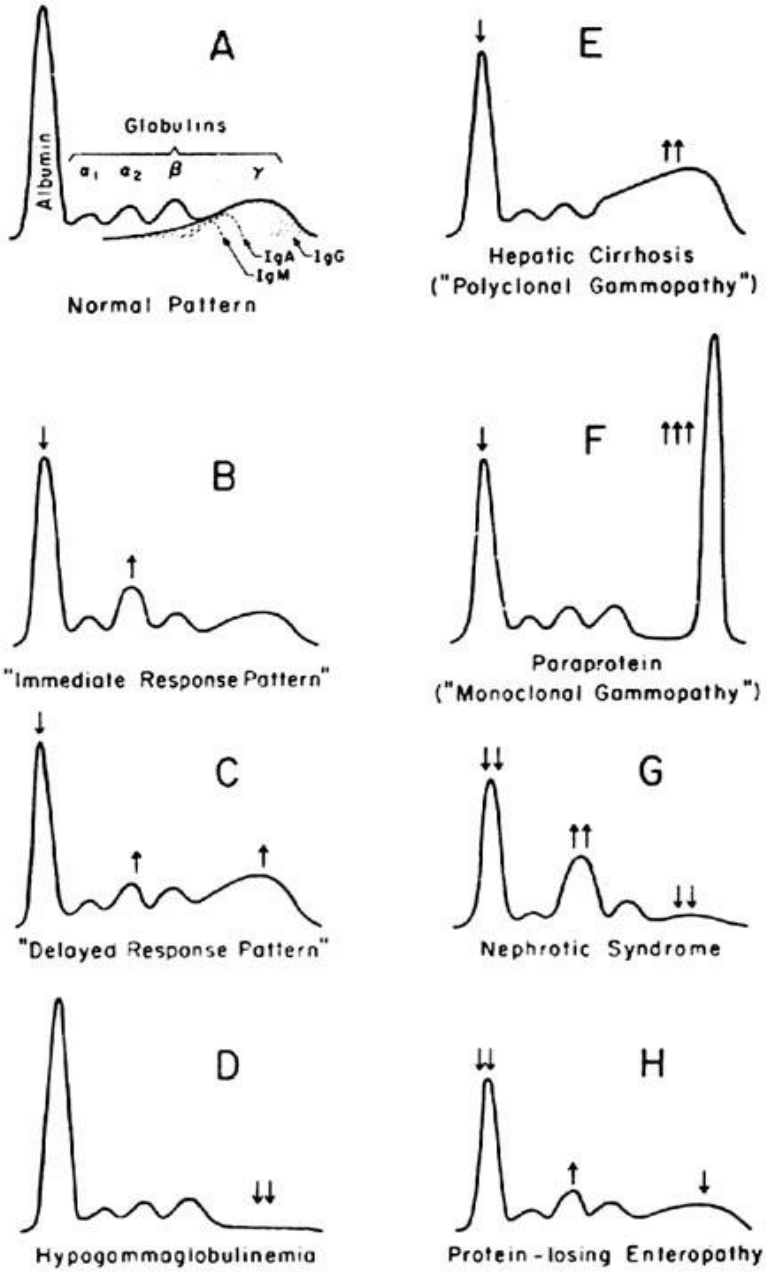


Figure 11–4. Serum protein electrophoresis: clinicopathologic correlations. (Courtesy of Dr. A. F. Krieg.)