Immunoelectrophoresis

BCH462 [practical]

Objective:

- To learn the technique of immunoelectrophoresis.

Precipitation Reactions:

-Precipitation reactions are based on the interaction of antibodies and antigens. They are based on two soluble reactants that come together to make one insoluble product, the precipitate (Figure).

-These reactions depend on the formation of lattices (cross-links) when antigen and antibody exist in optimal proportions.[it is known as zone of equivalence and appears to us as precipitation].

-Excess of either component reduces lattice formation and subsequent precipitation.

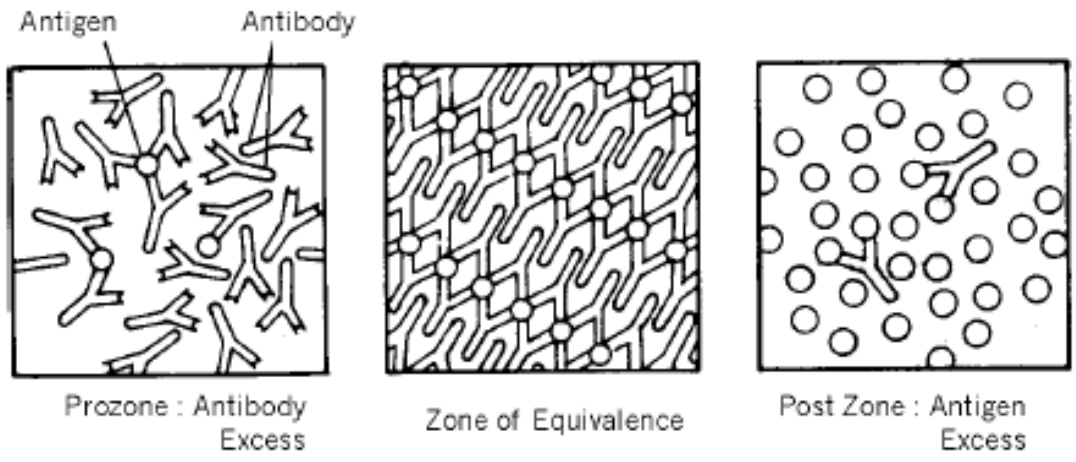
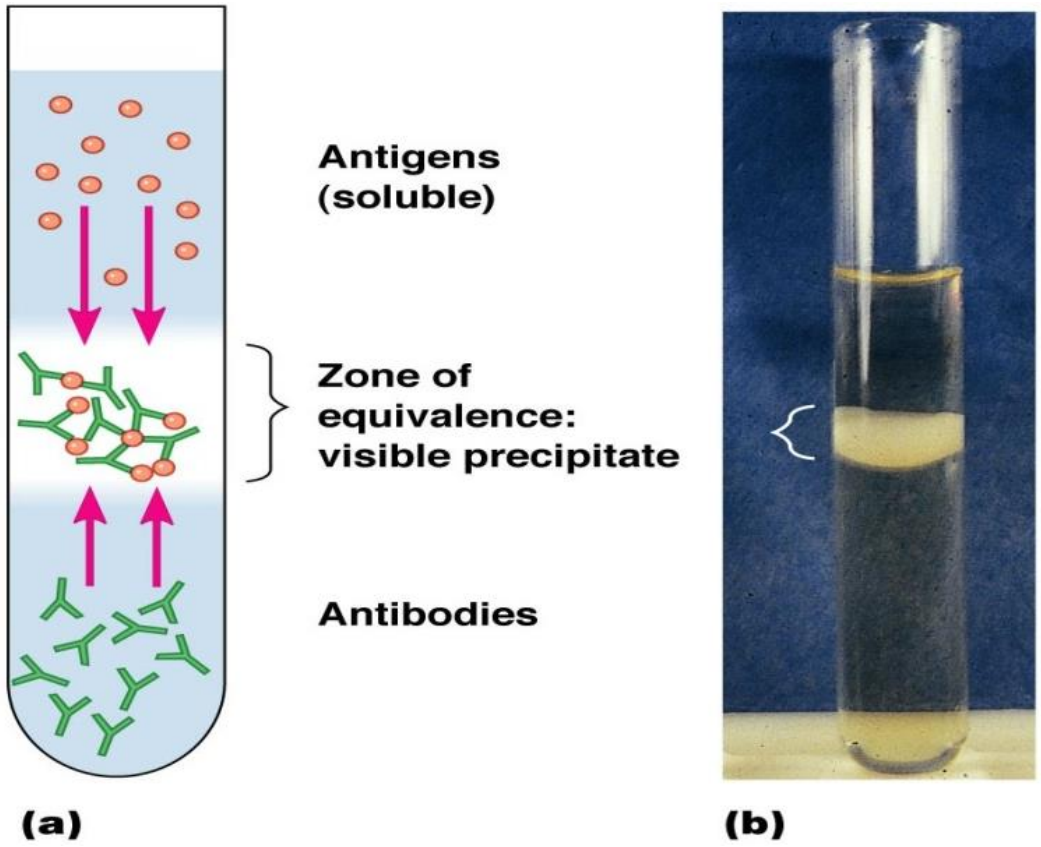


Figure 8.2. The optimal ratio of antigen to antibody (middle) yields an insoluble precipitate in the classic precipitation test. Extreme antibody excess (left) or antigen excess (right), however, does not lead to lattice formation.


Antigens
(soluble)
Zone of
equivalence:
visible precipitate
Antibodies
(a)
(b)

Precipitation reactions (Immunology}

- Simple Immunodiffusion (ID)

1.Single Radial ID (RID) (Mancini)

2.Double ID (Ouchterlony)

- Electro-Immnodiffusion

1. Immunoelectrophoresis (IEP)
2. Immunofixation
3. Rocket Electroimmunodiffusion (EID)
4. Counterimmunoelectrophoresis (CIEP)

Immunoelectrophoresis:

-Technique based on the principles of electrophoresis of antigens and immunodiffusion of the electrophoresed antigens with a specific antiserum to form precipitin bands.

-It is used to detect the presence of antibodies.

- Used mainly to determine the blood levels of three major immunoglobulins: immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA).

Immunoelectrophoresis:

A gel is prepared with alternating wells.

1. The antigen mixture is first electrophoresed to separate its components by charge.

2. Troughs are then cut into the agar gel parallel to the direction of the electric field.

3. Antiserum is added to the troughs.

4. Antibody and antigen then diffuse toward each other.

5. Lines of precipitation [arcs] will be produced where they meet in appropriate proportions [at the zone of equivalence].

6. The precipitin line indicate the presence of the antigen- antibody complex. While the absence of precipitin line indicates the absence of antigen- antibody complex.

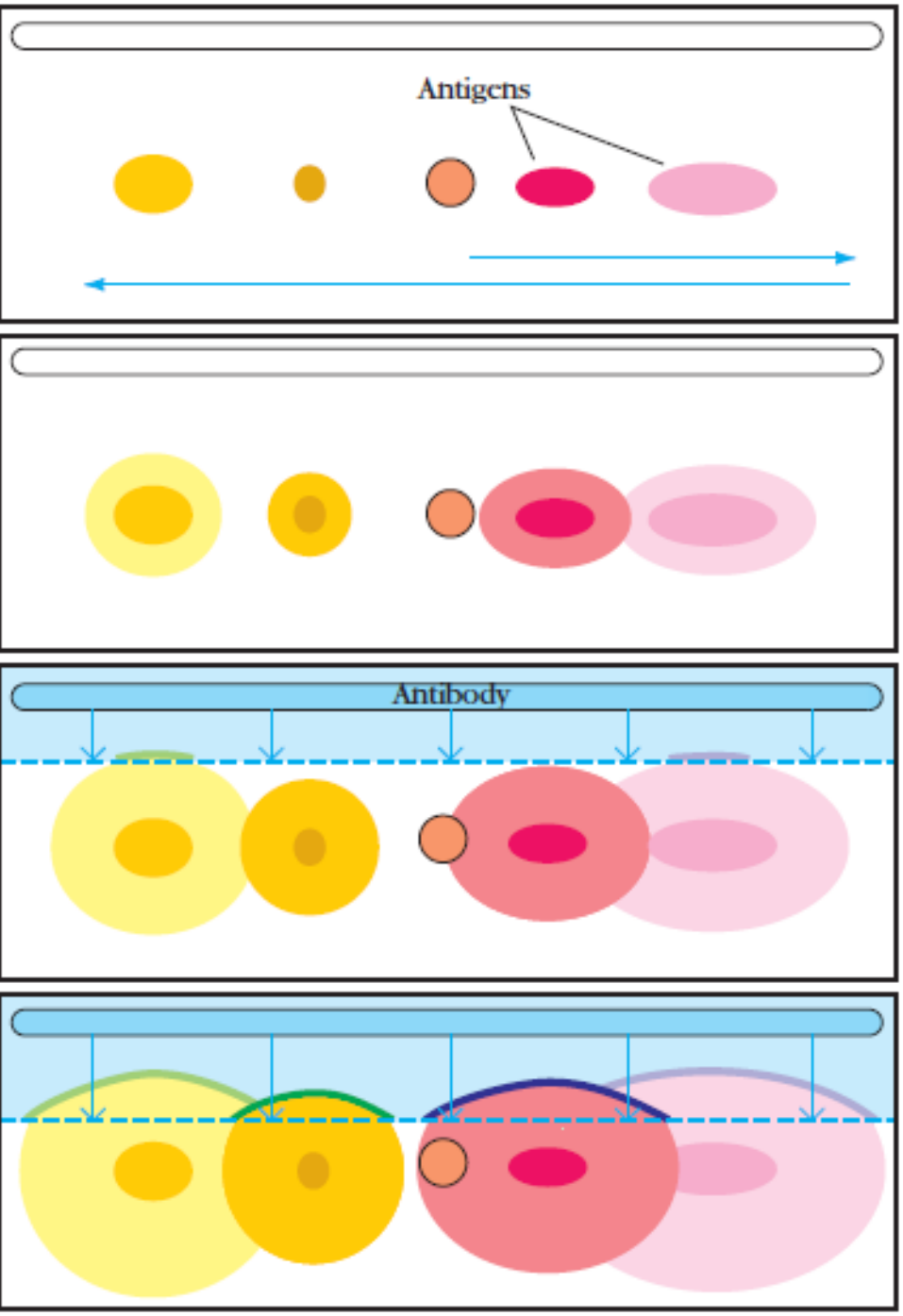
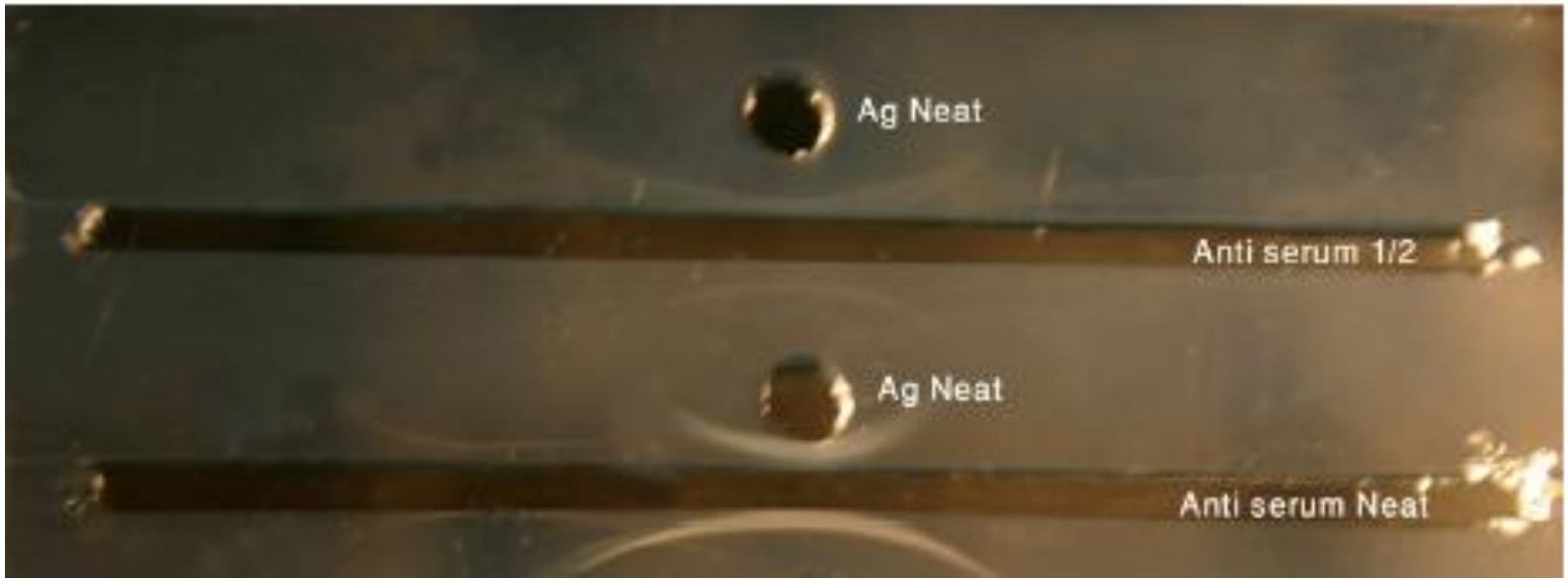


Figure:
Immunoelectrophoresis of an antigen mixture.

-An antigen preparation (orange) is first electrophoresed, which separates the component antigens on the basis of charge.

-Antiserum (blue) is then added to troughs on one or both sides of the separated antigens and allowed to diffuse.

-In time, lines of precipitation (colored arcs) form where specific antibody and antigen interact.


Ag Neat
Anti serum 1/2
Ag Neat
Anti serum Neat

During:

electrophoresis, molecules placed in an electric field acquire a charge and move towards appropriate electrode. Mobility of the molecule is dependent on a number of factors:

-Size of molecules to be separated.
-concentration of agarose gel.
-Voltage applied.
-The buffer used for electrophoresis.

Immunodiffusion:

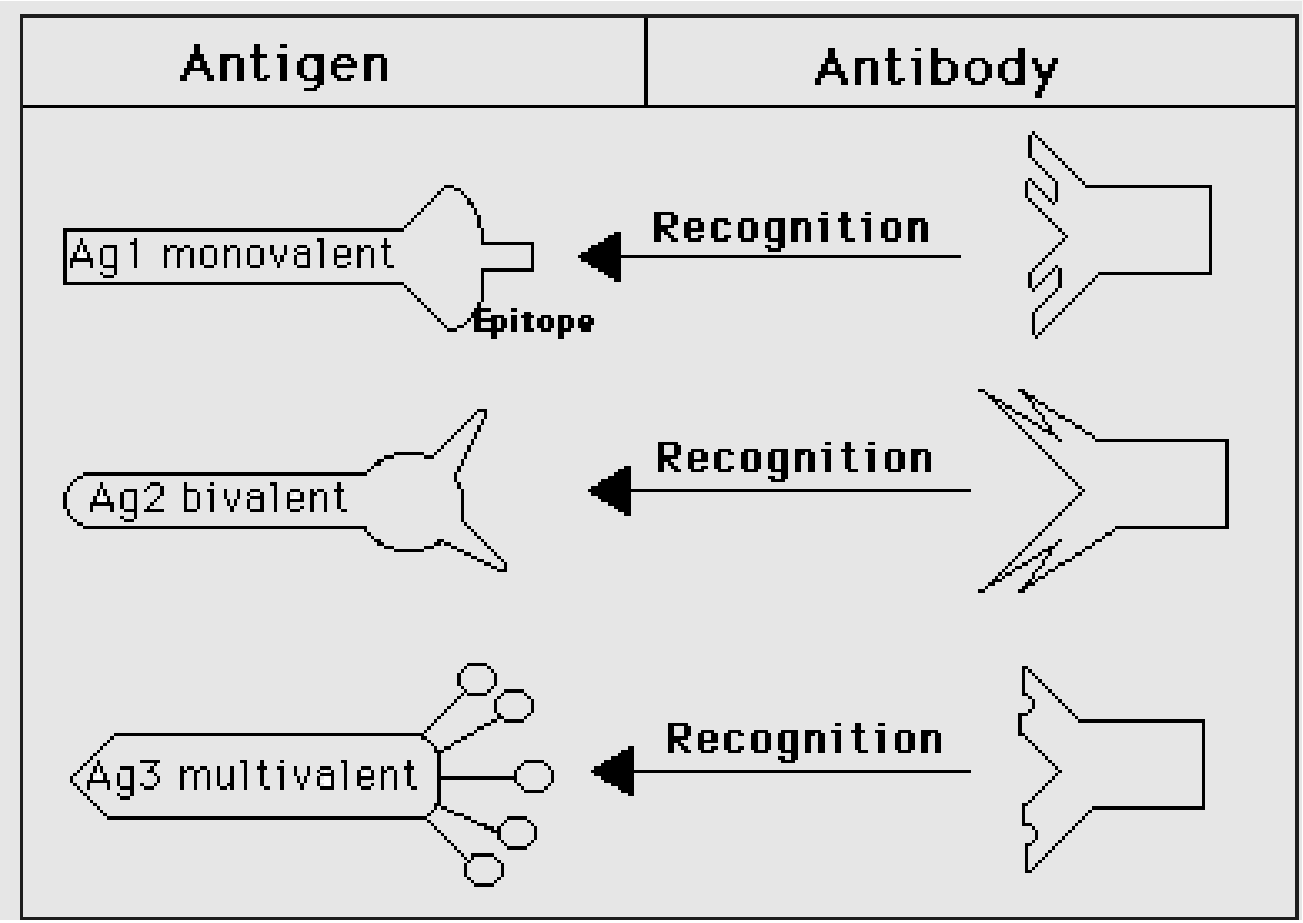
Antigens resolved by electrophoresis are subjected to immunodiffusion with antiserum added in a trough cut in the agarose gel. antigen-antibody complex precipitates at the zone of equivalence to form an opaque arc shaped line in the gel.

Applications:

-Immunoelectrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum.

- This technique is useful in determining whether a patient produces abnormally low amounts of one or more isotypes of Ig , characteristic of certain immunodeficiency diseases.

It can also show whether a patient overproduces some serum protein, such as albumin, immunoglobulin, or transferrin.



Antigen [Ag] molecules each have a set of antigenic determinants or epitopes.

Monoclonal Antibody	Polyclonal Antibody
Consists of one antibody class/subclass which is selective for a single epitope on the antigen	Contains a mixture of antibodies (mainly IgGs), often recognizing multiple epitopes on the antigen
Because of their specificity, they are less likely to cross-react with other proteins, giving lower background than polyclonal antibodies	May contain non-specific antibodies resulting in background staining