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PRECIPITATION REACTIONS IN IMMUNOLOGY

Amol S. Deshmukh[1]*, Pravin R. Dighe[1], Kundan J. Tiwari[2] and Supriya T. Garud[2]

[1]S.M.B.T College of Pharmacy, Dhamangaon, Nashik, Maharashtra, India.
[2]S.M.B.T Institute of D. Pharmacy, Dhamangaon, Nashik, Maharashtra, India.

***Corresponding Author: Mr. Amol S. Deshmukh**

Assistant Professor, Department of Pharmaceutics, S.M.B.T College of Pharmacy, Dhamangaon, Nashik, Maharashtra, India.

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ABSTRACT

Precipitation reactions in immunology are based on the interaction between antigens and antibodies. These are based on two reactants which are soluble that combine to make one product which is insoluble, and that product is called precipitate. The interaction of the antigen and antibody is a chemical reaction and is specific. When antigen and antibody exist in optimal proportions there is a formation of lattices (cross-links). The molecules are held together by intermolecular forces which are effective only when the antibody combining site and the antigenic determinant group are able to make close contact. Present review gives detail study about the precipitation reactions in immunology.

KEYWORDS: Antigen, Antibody, Precipitation, Immunology, Serology.

INTRODUCTION

An antigen is defined as any substance when introduced into the body, stimulates the production of an antibody with which it reacts specifically and in a manner that reaction is observable. Antibodies are specific substances in the bodies of the vertebrate secreted in the tissue fluids from the lymphoid cells that have been stimulated by foreign substances (antigens) with which they react specifically. The interaction of the antigen and antibody is a chemical reaction and is specific.[1,2] The reaction between an Ab and an Ag involves numerous non-covalent interactions between the epitope and paratope. Epitope is the binding site on the Ag, and the paratopes is the binding site on the Ab.[3] The study of reaction between the antigen and antibody is known as Serology. Serology is classified as precipitation, agglutination, complement fixation, neutralization, immobilization and intra-dermal reaction. Serology is also defined as a branch of science which deals with the study of serum.[1,4] A basic immunological reaction involves in Antigen-antibody reactions and it describes a specific response among antigen and antibody.[5]

PRECIPITAION REACTIONS

Antigens and antibodies are both complexes of amino acids and also have positive and negative polar groups distributed over their surfaces in specific but reciprocal patterns. When the antigen and corresponding antibody molecules are mixed, electrical attraction and repulsion takes place and results in orientation of the corresponding antigen and antibody molecules with respect to their molecular forms and electrical charges, so that an absolute 'fit' (mold and cast) is obtained.[6]

Precipitation reactions involve the reaction of soluble antigen with IgG or IgM antibodies to form large interlocking molecular aggregates called lattices. Precipitation reactions occur in two distinct stages. First, the antigens and antibodies rapidly form small antigen-antibody complexes. This interaction occurs within seconds and is followed by a slower reaction, which may take minutes to hours, in which the antigen-antibody complexes form lattices that precipitate from solution. Precipitation reactions occur only when the ratio of antigen to antibody is optimal.[7]

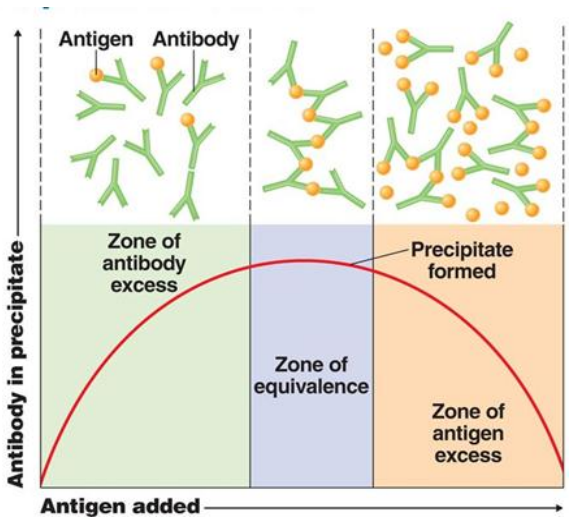

Figure 1: Precipitation curve.

Above figure 1 shows that no visible precipitate forms when either component is in excess. The best possible

ratio is formed when separate solutions of antigen and antibody are sited adjacent to each other and permitted to diffuse together. The equivalence zone represents the concentration of antigen and antibody where complete precipitation occurs.[4]

The following types of precipitation and flocculation tests are used commonly.

1. **Ring test:** The detection of antigens is very simple by using this test. In this test a layer of antigens is formed over layer of serum in a narrow tube. The line of precipitate is observed at the junction of two clear fluids as shown in figure 2. C-reactive protein test, Ascoli's thermo precipitin test, diagnosis of anthrax and typing of streptococci are some of the important applications of the ring test. Detection of adulteration of food stuffs is also done by using ring test.

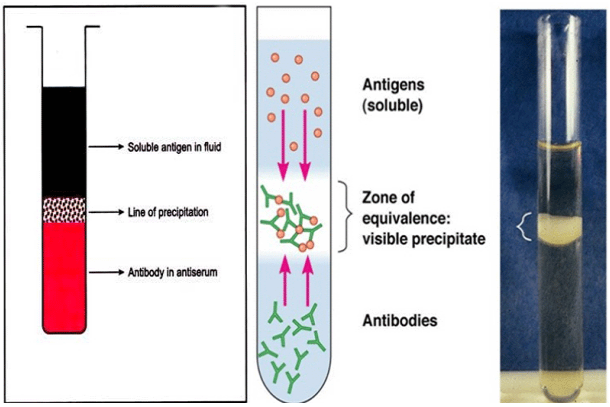

Figure 2: Ring test.

2. **Slide test:** In this test a drop of each of the antigen and the antiserum are added on a slide, then both antigen and antiserum are mixed by shaking. The reaction observed in the form of floccules formation. The VDRL test for syphilis is one of the examples of slide flocculation.
3. **Tube test:** In this test a fixed quantity of antitoxin is added in the tubes. Then serial dilutions of the toxoid or toxin are added to those tubes. The amount of toxoid or toxin which flocculates optimally with a one unit of the antitoxin is known as Lf dose. One of the example of a tube test is the Kahn test for syphilis. For the standardization of toxoids and toxins a quantitative tube flocculation test is used.
4. **Immunodiffusion (Precipitation in Gel):** The important characteristic property of this method is that the precipitation reaction is observable in the form of distinct band which is more stable and if necessary can be stained for preservation. The number of different antigens in the reacting mixture can be readily visualized, as each antigen-antibody reaction gives rise to a line of precipitation. Immunodiffusion also indicates identity, non-identity and cross-reaction between different antigens. Immunodiffusion is usually performed in a soft agar (1%) or agarose gel. Precipitation in gel is more advantageous than precipitation in liquid medium. There are different types of immunodiffusion tests are as follows:

a) ***Single diffusion in one dimension (Oudin Procedure):*** Agar gel having antibody is taken in a tube and antigen solution is layered over it. The antigen diffuses downwards and wherever it reaches in optimum concentration with antibody a line of precipitation is formed. The number of bands indicates the number of different antigens present.

b) ***Double diffusion in one dimension (Oakley-Fulthorpe procedure):*** In a test tube antibody is incorporated in agar gel. Above this layer a column of plain agar is placed which in turn is superimpose with antigen, either as liquid or incorporated into agar. The antigen-antibody forms a band of precipitate where they meet at optimum proportion by moving towards each other through the intervening agar.

c) ***Single diffusion in two dimensions (Radial immunodiffusion):*** A slide but generally petri plate is used for this method. Agar gel is poured on a slide or in a petriplate and antiserum is then incorporated in agar gel. Well were prepared on the agar surface and antigen is added to a well. Bands of precipitate of ring shape are formed around wells. The concentration of antigen is estimated by measuring the diameter of ring.

d) ***Double diffusion in two dimensions (Ouchterlony procedure):*** Agar gel is poured on a slide or petri plate and different wells were prepared on surrounding and one at center on agar surface. In the central well the antiserum is added and different antigens are added in the surrounding well. If in observation the two precipitin bands found totally combine, then the pattern is called as reaction of identity. This indicates that the antigens in the adjacent wells are identical. If in observation the precipitin bands form separately and cross each other, then the pattern is known as reaction of non-identity. This indicates that the unrelated antigens are present. If in observation the precipitation bands fuse but form a spur like projection, in this case the antigens are cross-reacting, this is known as reaction of partial identity. For the diagnosis of small pox this method was a routine technique. For toxigenicity of *Diphtheria bacilli* a special variety of this test is used called as Elek test. Double diffusion in two dimensions is used for comparison of different antigens and antisera.[2,8]

5. **Immunoelectrophoresis:** **A**n electrochemical process is a process in which colloidal particles (suspended particles) or macromolecules which having a net electric charge are travels in a solution or agar gel under the influence of an electric current is known as electrophoresis. A characteristic of living cells in suspension and biological compounds (such as protein antigens) in solution or in gel is that in an electric field they travel to the positive or negative electrode, depending on the charge on the

substance. Positively charged substances travel to the cathode while negatively charged ones go to the anode, this movement is called as electrophoretic mobility. When electrophoresis is applied to the study of antigen-antibody reactions, it is called immunoelectrophoresis. When a fluid containing antigens in the form of proteins is placed in a well which was prepared in a thin layer of buffered agar. If an electric current is applied to this, the antigens will be travels and distributed in separate spots along a line passing throughout the well and that is parallel to the current flow direction. When the current is shut off, diffusion will begin from each of these spots. By placing antiserum in a trench cut in the agar parallel to the electroporetic distribution of the antigens, the precipitin reaction can be used to demonstrate the nature of the diffusing molecules. In this case, a broad band of antibody diffuses toward the antigens from the linear antibody trench, while the antigens diffuse as expanding discs. This results in a complex pattern of arc-shaped zones of precipitate.[4]

CONCLUSION

Antigens and antibodies are playing an important role in immunological reactions. Different reactions are involved in immunology with special reference to serology are precipitation, agglutination, complement fixation, neutralization, immobilization and intra-dermal reaction etc. among them precipitation reaction and its subtypes were described in this review.

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