Immunological Techniques

For PG. Sem II Students

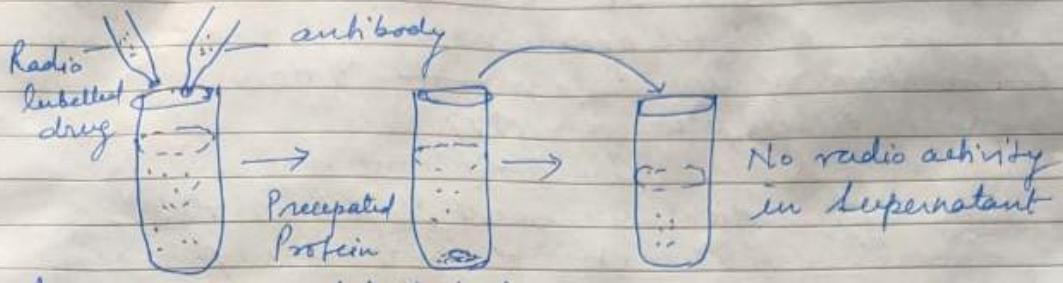
Radio immunoassay for Immunoglobulins

Radioimmunoassay (RIA) technique was developed by two endocrinologists Berson and Yalow. This technique is used to measure and detect very small fractions of Vitamins, hormones, drugs antigens and antibodies having 0.001 micrograms per milliliter or less. Rosalyn ~~Yalow~~ Yalow was awarded Nobel prize in 1977.

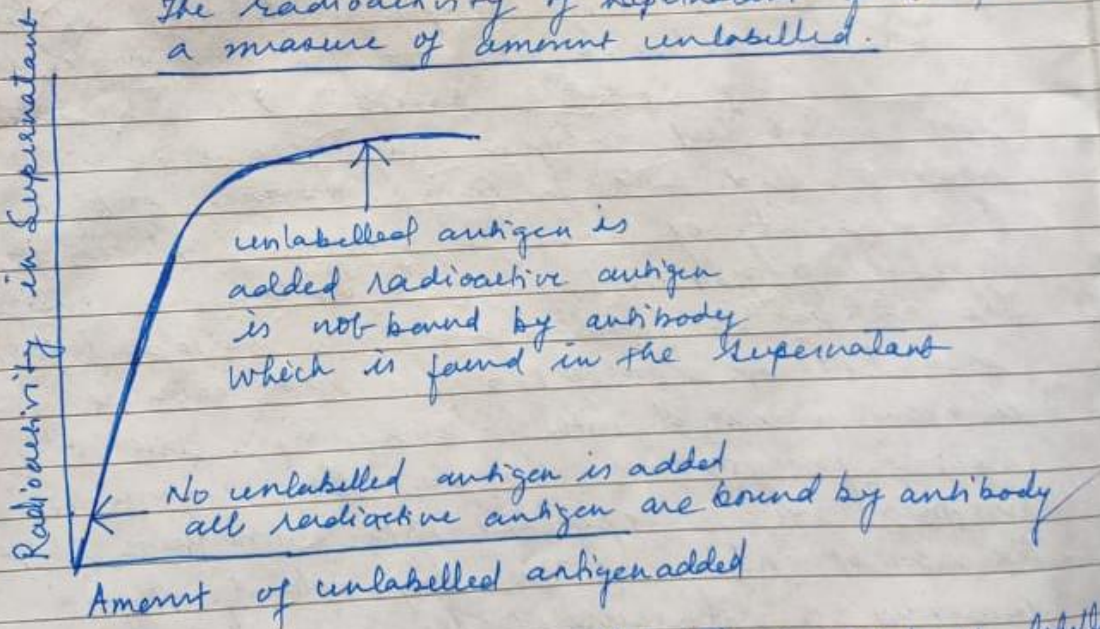
The Radioimmunoassay for antibody radioallergensorbent test (RAST) is done. The test consists of antigen impregnated cellulose disc immersed in test serum for binding of antibody with antigen. The disc is washed with PBS saline and then immersed in a solution having radio labeled antiglobulin. This radiolabelled antiglobulin bind with only antigen bound but serum antibody in disc in case of specific single class of antibodies. This facilitates activity of specific antibodies of test serum. This technique is good for immunoglobulin IgE

It is based on the principle that unlabelled antigen may displace the radiolabelled antigen from immune complex. The quantity of labelled antigen displaced is directly proportional to the unlabelled antigen. The antigen is labelled ~~and~~ with isotope tritium Carbon (14) or iodine 125. The radiolabelled antigen is mixed with its specific antibody raised for immune complex. The immune complex is precipetated with ammonium sulphate The radioactivity of the supernatant fluid provides a measure of the quantity of unbound labelled antigen. If unlabelled antigen is added to the mixture it competes with epitope of labeled antigen, consequently some labeled antigen is unable to bind antibody and the amount of

radioactivity in supernatant fluid increases. A standard curve is drawn using known amount of antigen via test sample may be measured with reference to standard curve



The radioactivity of supernatant fluid provides a measure of amount unlabelled.



The standard curve shows that the amount of labelled antigen displaced is directly related to the amount of antigen added.