IMMUNOLOGY

TOPIC: RADIO IMMUNO ASSAY

THIRD SEMESTER M.SC ZOOLOGY

REMYA VARGHESE (ASSISTANT PROFESSOR ON CONTRACT)

RADIO IMMUNO ASSAY

RIA

RIA

- Radioimmunoassay One of the most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA).

- The technique was first developed in **1960** by two endocrinologists**, S. A. Berson and Rosalyn Yalow,** to determine levels of insulin–anti-insulin complexes in diabetics.

- In 1977, some years after Berson's death, the significance of the technique was acknowledged by the award of a Nobel Prize to Yalow.

PRINCIPLE

- The principle of RIA involves **competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody**.

- The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody

- Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts.
- The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody

- As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites.
- The decrease in the amount of radiolabeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample

I_{125}

- The antigen is generally labeled with a **gamma-emitting isotope such as I_{125}** but beta-emitting isotopes such as tritium (3 H) are also routinely used as labels.

- The radiolabeled antigen is part of the assay mixture;
- the test sample may be a complex mixture, such as serum or other body fluids, that contains the unlabeled antigen

- setting up an RIA is to determine the amount of antibody needed to bind 50%–70% of a fixed quantity of radioactive antigen (Ag^*) in the assay mixture.

- This ratio of antibody to Ag$*$ is chosen to ensure that the number of epitopes presented by the labeled antigen always exceeds the total number of antibody binding sites

- Consequently, unlabeled antigen added to the sample mixture will compete with radiolabeled antigen for the limited supply of antibody.

- Even a small amount of unlabeled antigen added to the assay mixture of labeled antigen and antibody will cause a decrease in the amount of radioactive antigen bound, and this decrease will be proportional to the amount of unlabeled antigen added.

- To determine the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from **free antigen** (antigen not bound to Ab), and the radioactivity in the precipitate is measured.

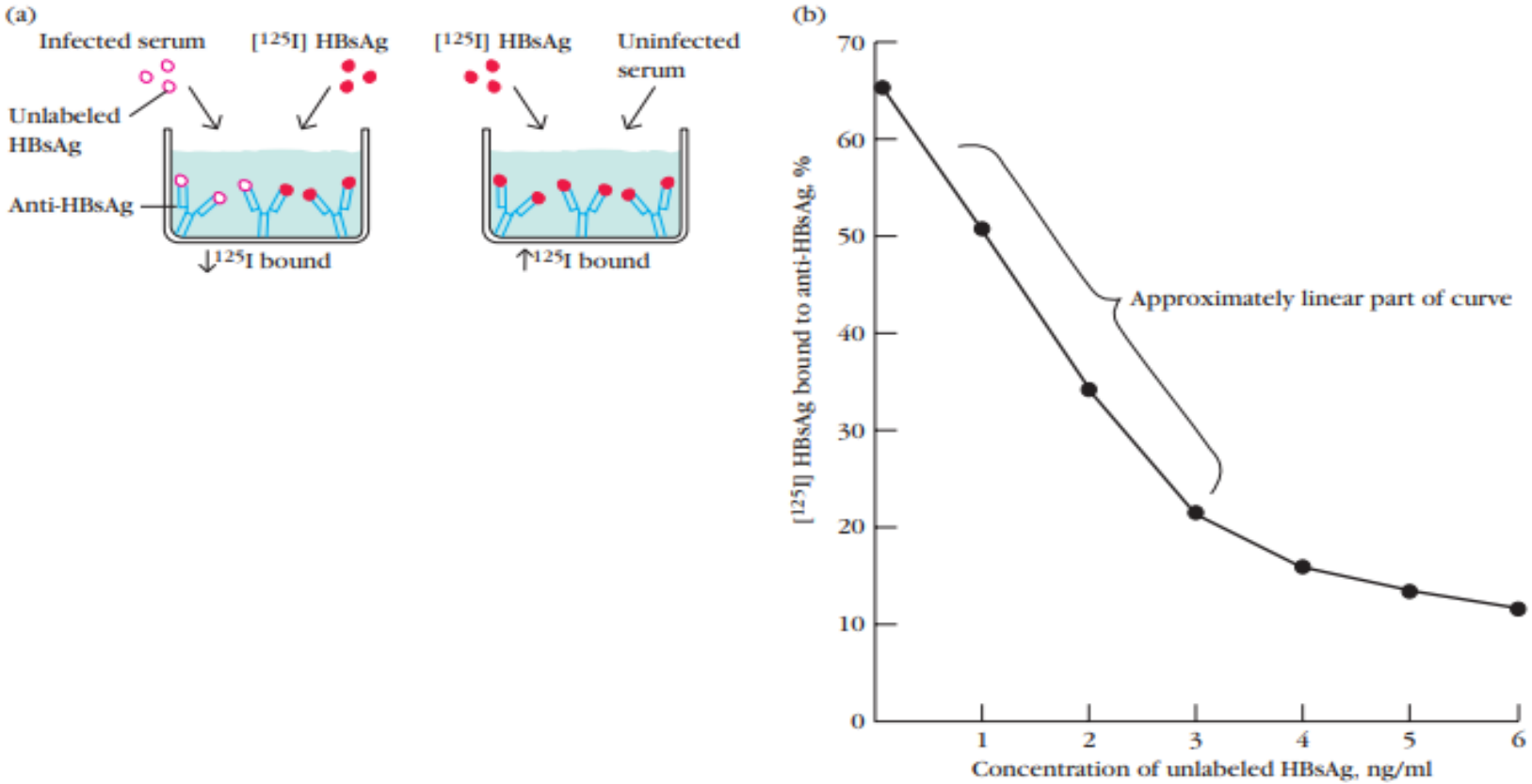

FIGURE 6-9 A solid-phase radioimmunoassay (RIA) to detect hepatitis B virus in blood samples. (a) Microtiter wells are coated with a constant amount of antibody specific for HBsAg, the surface antigen on hepatitis B virions. A serum sample and [^{125}I]HBsAg are then added. After incubation, the supernatant is removed and the radioactivity of the antigen-antibody complexes is measured. If the sample is infected, the amount of label bound will be less than in controls with uninfected serum. (b) A standard curve is obtained by adding increasing concentrations of unlabeled HBsAg to a fixed quantity of [^{125}I]HBsAg and specific antibody. From the plot of the percentage of labeled antigen bound versus the concentration of unlabeled antigen, the concentration of HBsAg in unknown serum samples can be determined by using the linear part of the curve.

- A standard curve can be generated using unlabeled antigen samples of known concentration (in place of the test sample), and from this plot the amount of antigen in the test mixture may be precisely determined.

- Several methods have been developed for separating the bound antigen from the free antigen in RIA.
- One method involves precipitating the Ag-Ab complex with a secondary anti-isotype antiserum.

- For example, if the Ag-Ab complex contains rabbit IgG antibody, then goat anti-rabbit IgG will bind to the rabbit IgG and precipitate the complex.
- Another method makes use of the fact that protein A of Staphylococcus aureus has high affinity for IgG

- If the Ag-Ab complex contains an IgG antibody, the complex can be precipitated by mixing with formalin-killed S. aureus.

- removal of the complex by either of these methods

- **The amount of free labeled antigen remaining in the supernatant can be measured in a radiation counter; subtracting this value from the total amount of labeled antigen added yields the amount of labeled antigen bound**.

- In another approach, the antibody is immobilized on the walls of **microtiter wells** and the amount of bound antigen determined.
- Because the procedure requires only small amounts of sample and can be conducted in small 96-well microtiter plates,
- This procedure is well suited for determining the concentration of a **particular antigen in large numbers of samples**

- For example, a microtiter RIA has been widely used to screen for the presence of the hepatitis B virus.

- RIA screening of donor blood has sharply reduced the incidence of hepatitis B infections in recipients of blood transfusions.