Lecture IV

Antigen – antibody interactions

Monoclonal antibodies

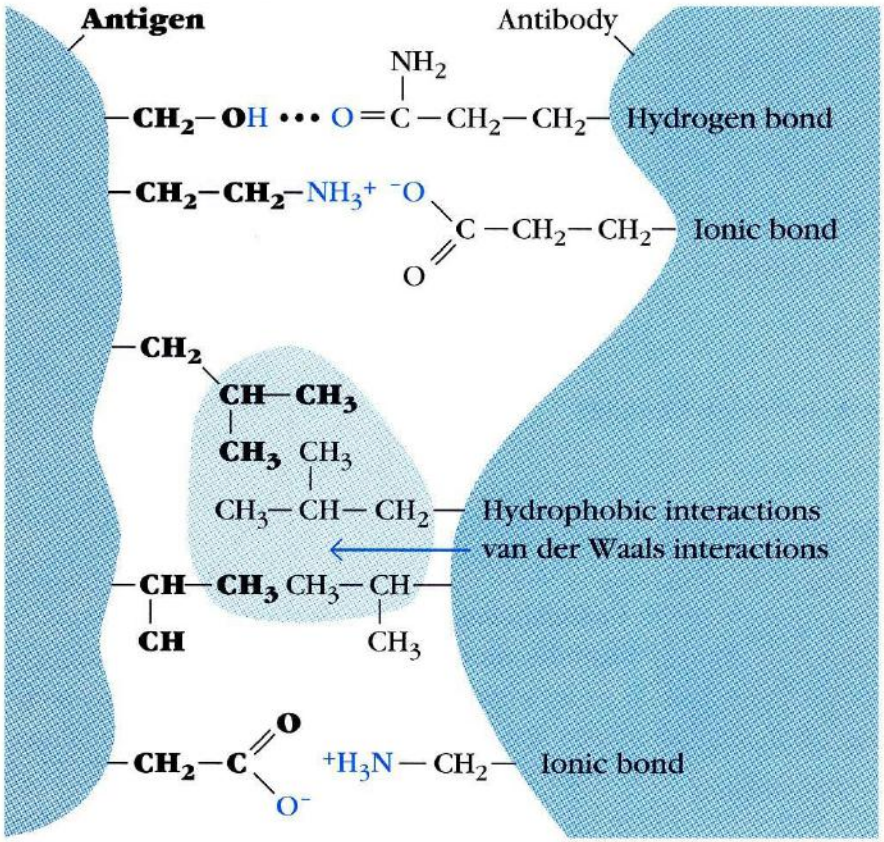


FIGURE 6-1

The interaction between an antibody and an antigen (shown here in bold type) depends on four types of noncovalent forces: (1) hydrogen bonds in which a hydrogen atom is shared between two electronegative atoms, (2) ionic bonds between oppositely charged residues, (3) hydrophobic interactions in which water forces hydrophobic groups together to maximize hydrogen bonding of water molecules, and (4) van der Waals interactions between the outer electron clouds of two atoms. In an aqueous environment noncovalent interactions are extremely weak and depend upon close structural complementarity between antibody and antigen.

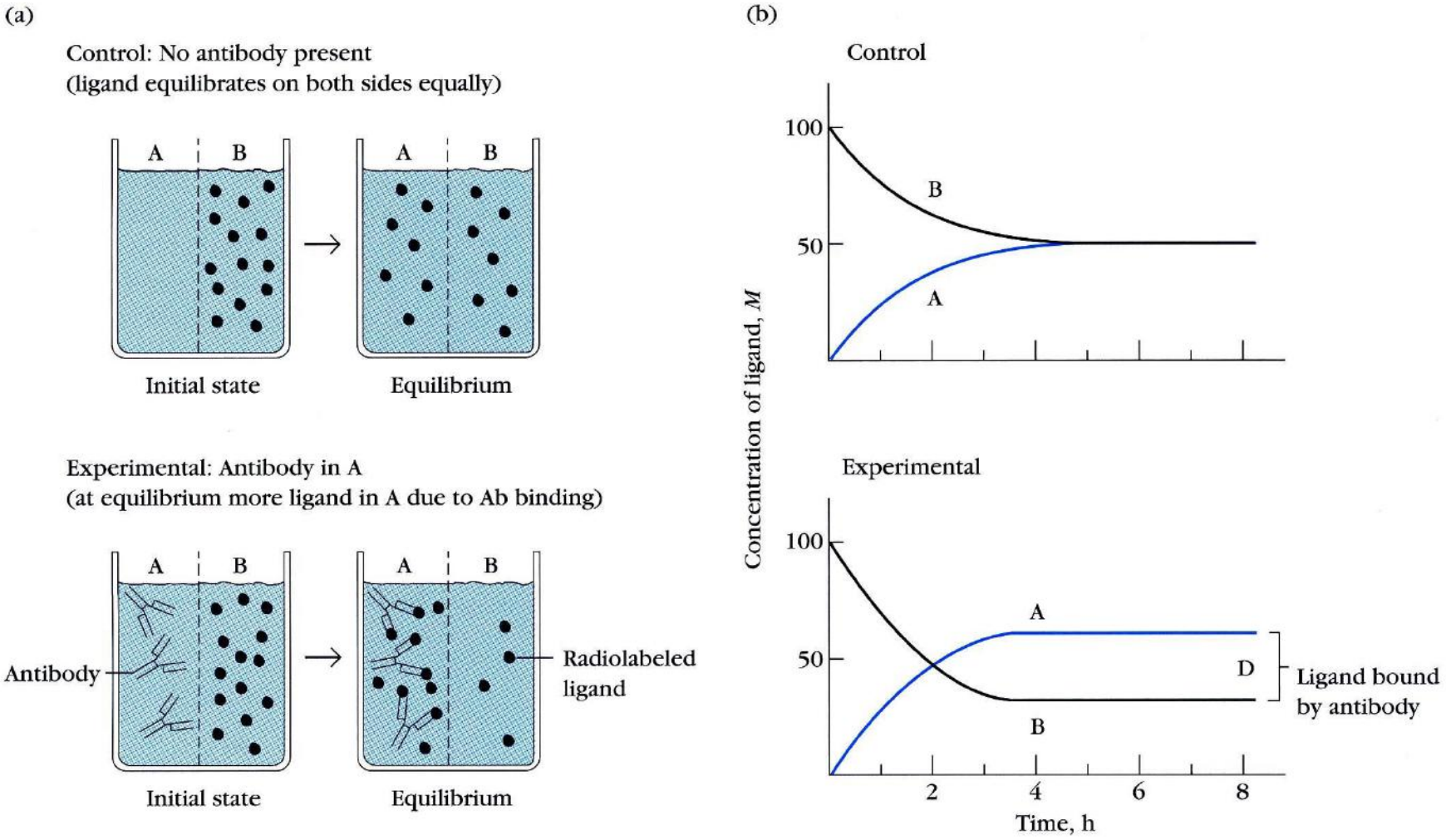


FIGURE 6-2

Determination of antibody affinity by equilibrium dialysis. (a) The dialysis chamber contains two compartments (A and B) separated by a semipermeable membrane. Antibody is added to one compartment and a radiolabeled ligand to another. At equilibrium the concentration of radioactivity in both compartments is measured. (b) Plot of concentration of ligand in each compartment with time. At equilibrium the difference in the concentration of radioactive ligand in the two compartments represents the amount of ligand bound to antibody.

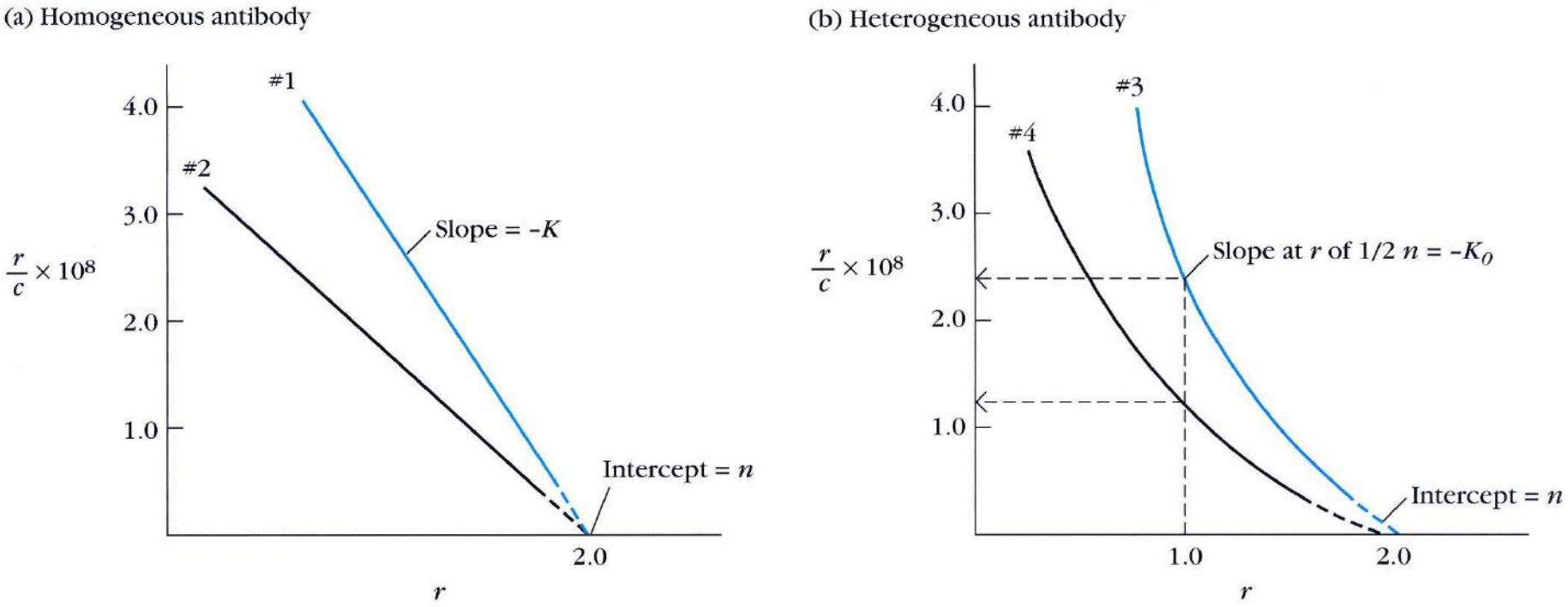


FIGURE 6-3

Scatchard plots are based on repeated equilibrium dialyses with a constant concentration of antibody and varying concentration of ligand. In these plots, r = moles bound ligand/mole antibody and c = free ligand. From a Scatchard plot, both the equilibrium constant (K) and the number of binding sites per antibody molecule (n), or its valency, can be obtained. (a) If all antibodies have the same affinity, then a Scatchard plot yields a straight line with a slope of $-K$. The Y intercept is the valence of the antibody, which is 2 for IgG. In this graph antibody #1 has a higher affinity than antibody #2. (b) If the antibodies have a range of affinities, a Scatchard plot yields a curved line, whose slope is constantly changing. The average affinity constant K_0 can be calculated by determining the value of K when one half of the binding sites are occupied (i.e., when r = 1). In this graph antisera #3 has a higher affinity ($K_0 = 2.4 \times 10^8$) than antisera #4 ($K_0 = 1.25 \times 10^8$).

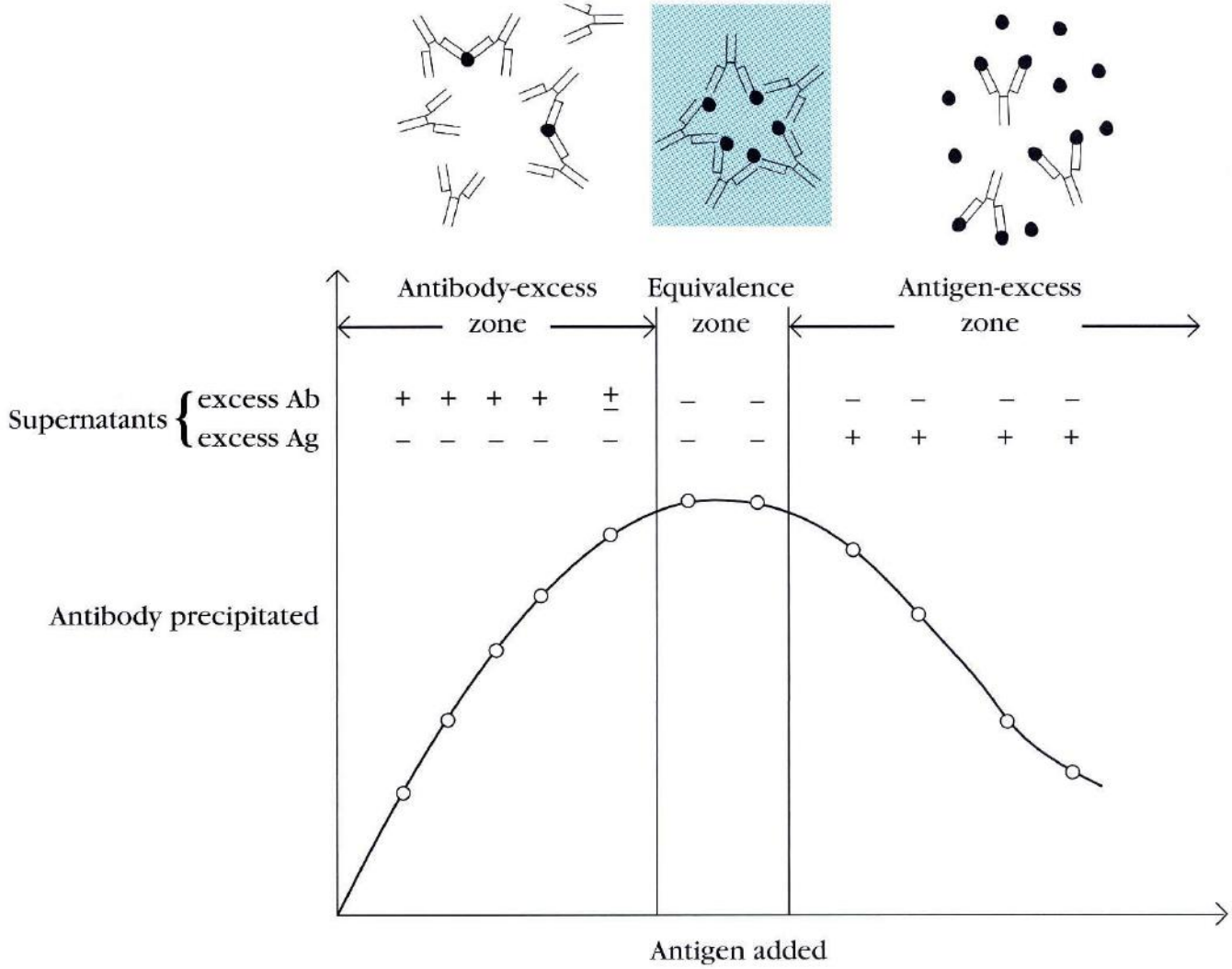
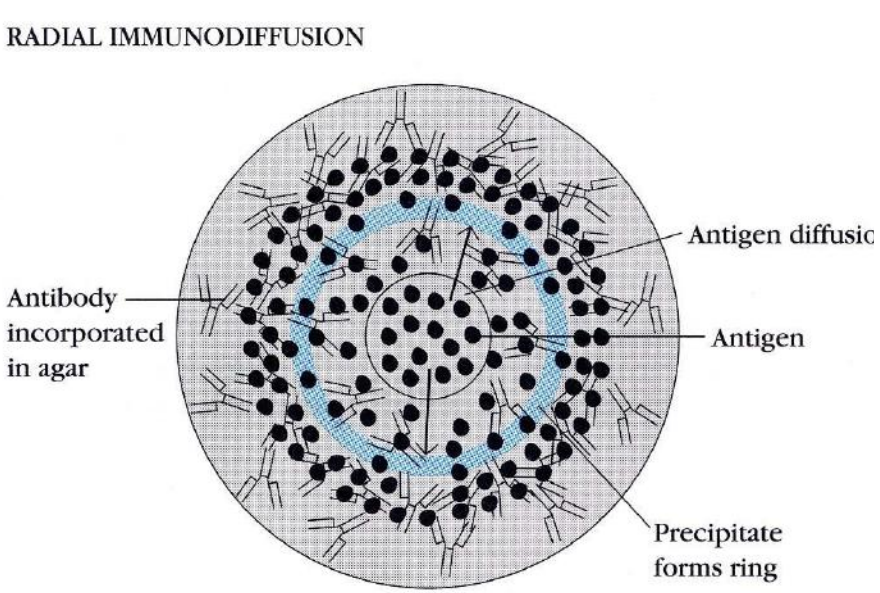


FIGURE 6-4

A precipitation curve for a system of one antigen and its antibodies. This plot of the amount of antibody precipitated versus increasing antigen concentrations (at constant total antibody) reveals three zones: a zone of antibody excess in which precipitation is inhibited and excess antibody can be detected in the supernatant; an equivalence zone of maximal precipitation in which antibody and antigen form large insoluble complexes (shaded in blue) and neither antibody nor antigen can be detected in the supernatant; and a zone of antigen excess in which precipitation is inhibited and excess antigen can be detected in the supernatant.



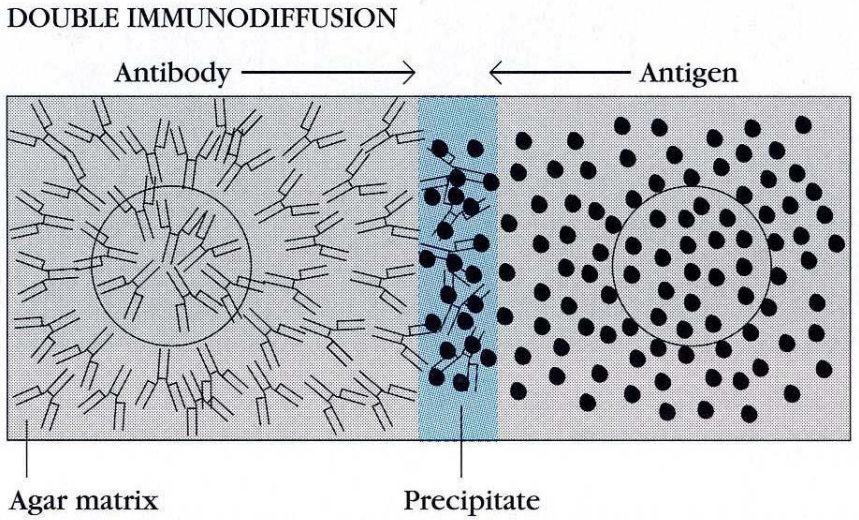
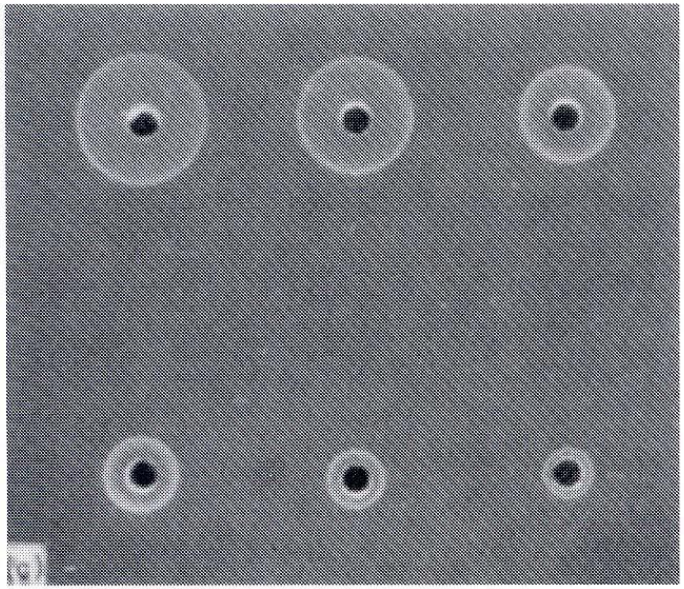
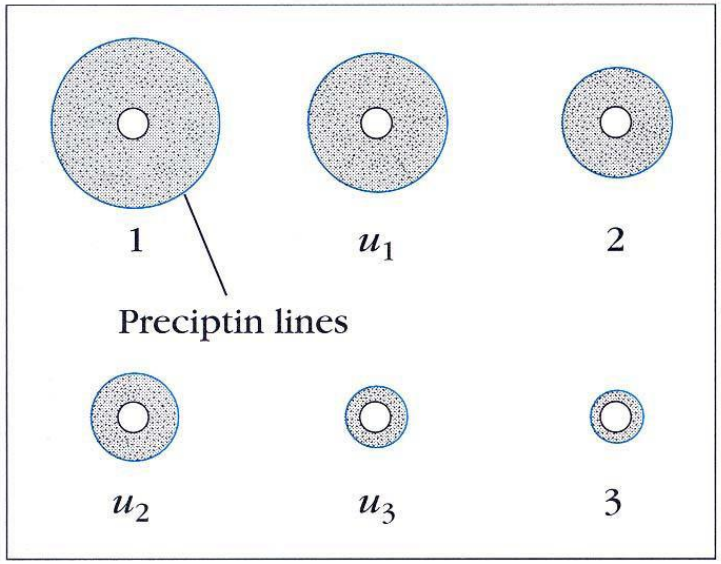


FIGURE 6-6

Diagrammatic representation of radial (Mancini) and double immunodiffusion (Ouchterlony) in a gel. In both cases, large insoluble complexes form in the agar in the zone of equivalence, which are visible as a line of precipitation (blue region). Only the antigen diffuses in radial immunodiffusion, whereas both the antibody and antigen diffuse in double immunodiffusion.





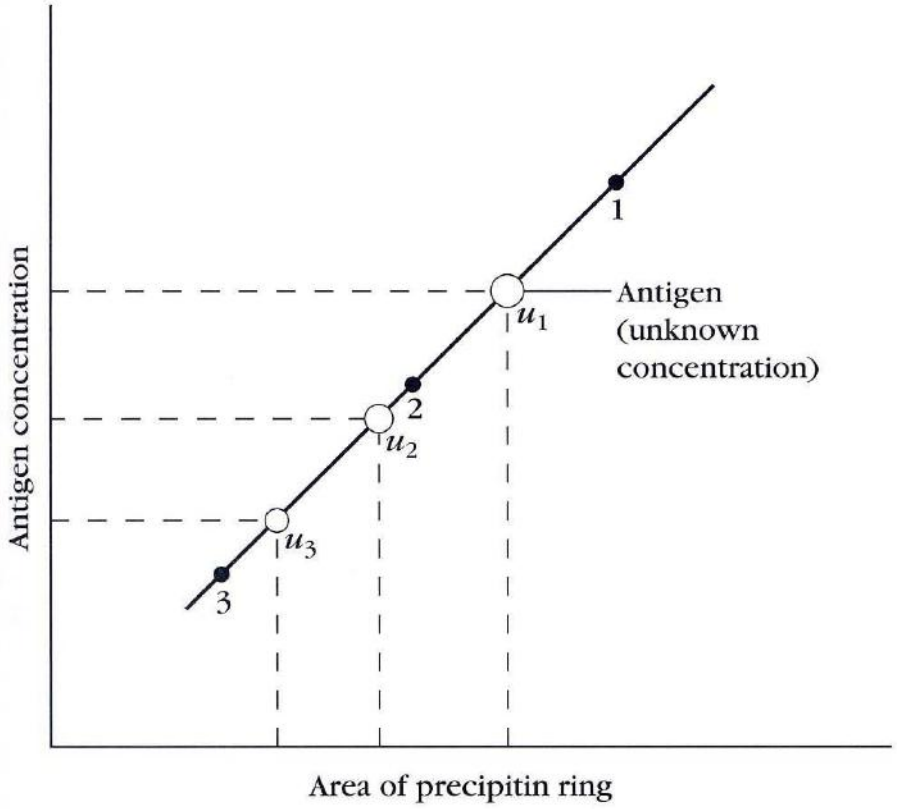


FIGURE 6-7

Determination of antigen concentration by radial immunodiffusion. The area of the ring of precipitation is proportional to the concentration of antigen. A standard curve can be obtained from the results with known concentrations of antigen (wells 1–3). From the standard curve, the antigen concentration can be determined in samples of unknown concentration (wells u_1, u_2, and u_3). [Photograph from D. M. Weir (ed.), 1986, *Handbook of Experimental Immunology,* Blackwell Scientific Publications.]

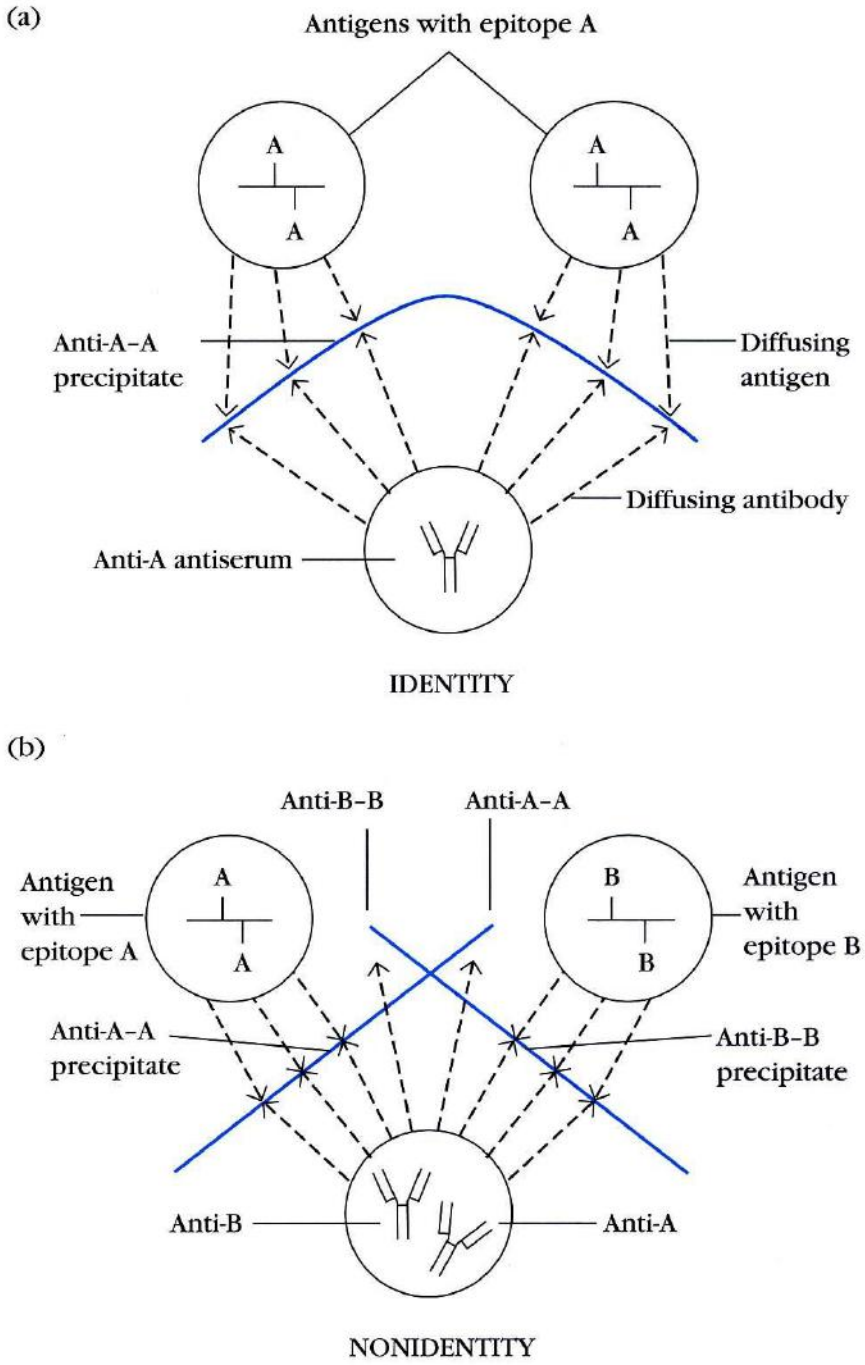


FIGURE 6-8

Diagrams of possible precipitin patterns obtained in double immunodiffusion (Ouchterlony method) of antiserum with two different antigen preparations. The pattern of lines (blue) indicates whether the two antigens have identical epitopes (identity), partially identical epitopes (partial identity), or no epitopes in common (nonidentity).

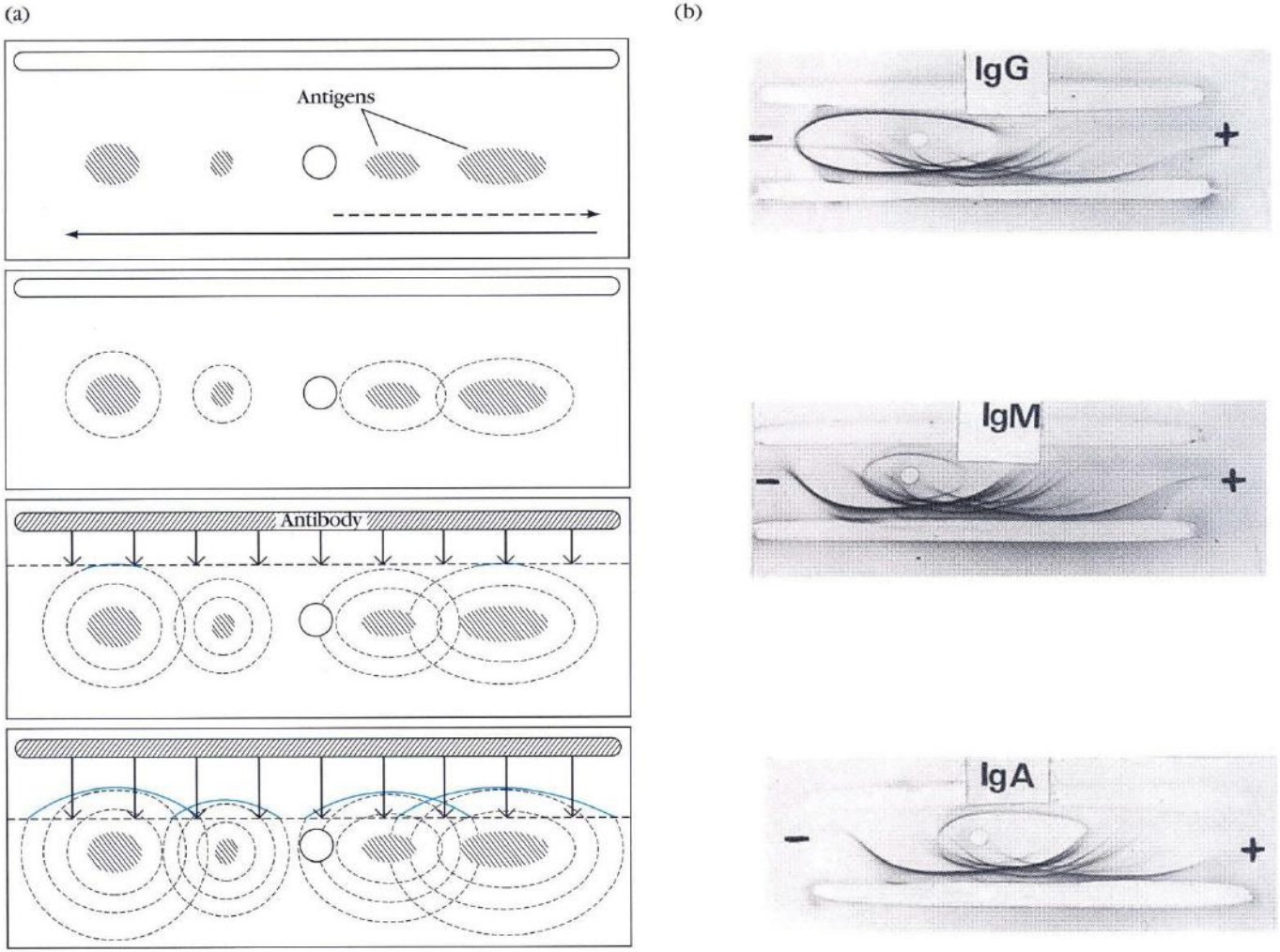
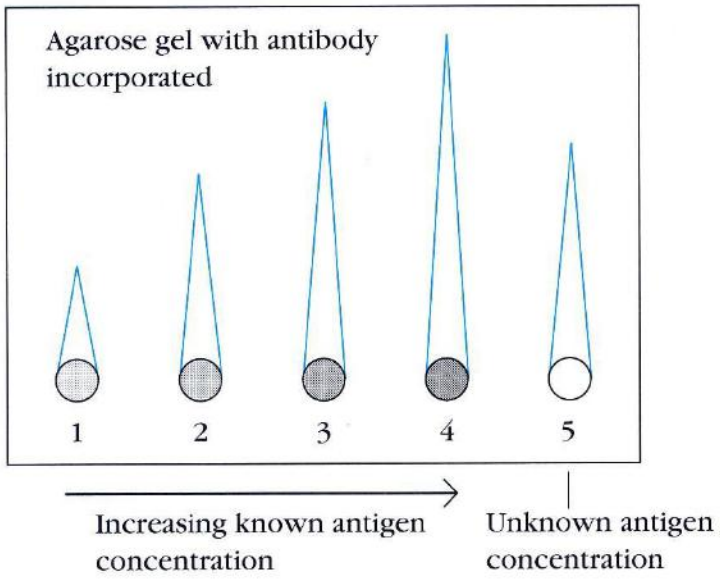
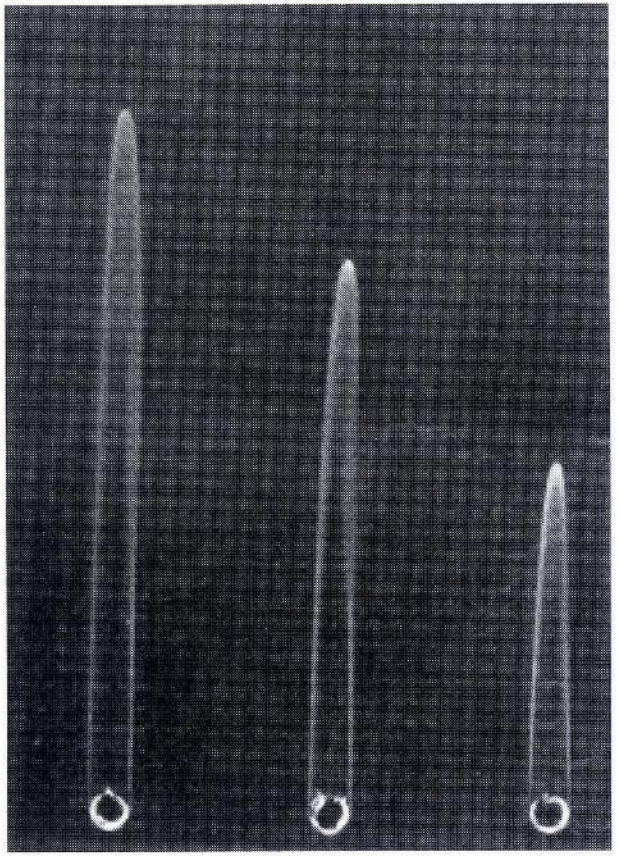


FIGURE 6-9

Immunoelectrophoresis of an antigen mixture. (a) An antigen preparation is first electrophoresed, which separates the component antigens on the basis of charge. Antiserum is then added to troughs on one or both sides of the separated antigens and allowed to diffuse; in time, lines of precipitation (blue curves) form where specific antibody and antigen interact. (b) Immunoelectrophoretic patterns of human serum. Goat antibody to whole human serum was placed in the bottom trough of each slide; goat antibody to human IgG, IgM, or IgA was placed in the top trough of each slide. After electrophoresis, the IgG, IgM, and IgA antibodies form a single line of precipitation with their respective antisera. The position of the human IgG, IgM, and IgA in the electrophoresed whole human serum sample can be determined by comparing the position of the single band at the top of each slide with the complex band pattern of the whole serum sample. [Part (b) J. S. Garvey et al., 1977, *Methods in Immunology*, 3d ed., W. A. Benjamin Inc., Advanced Book Program.]





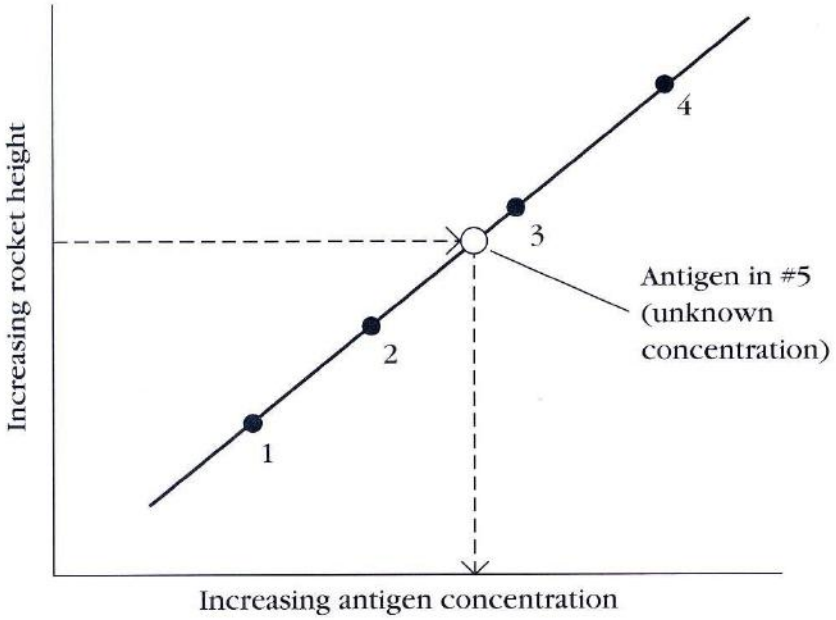
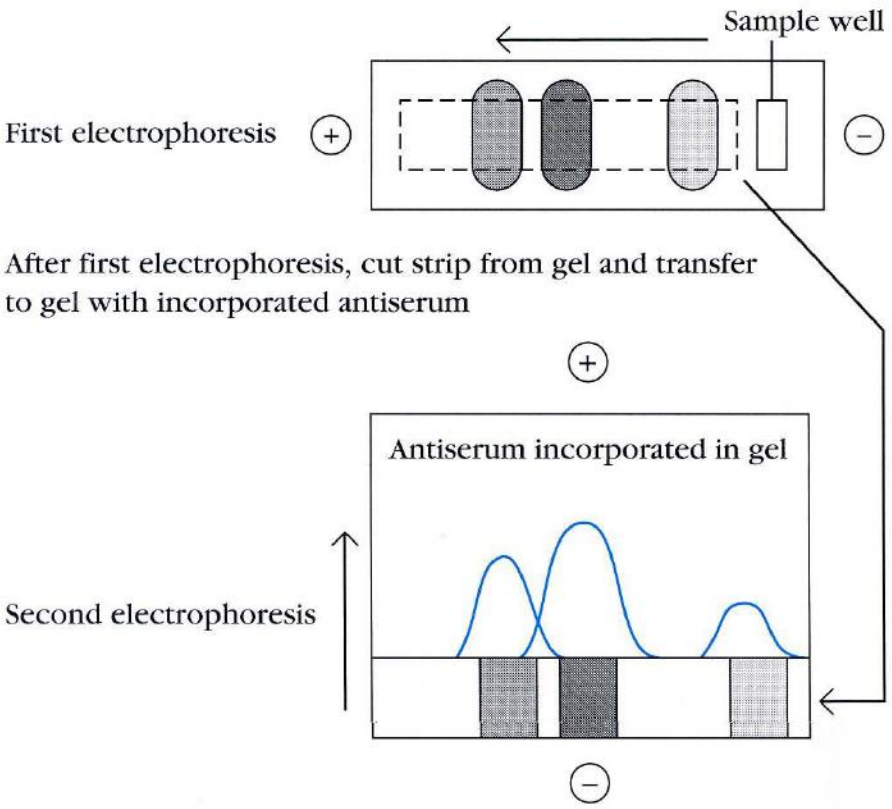


FIGURE 6-10

In rocket electrophoresis, antigen is electrophoresed in an agarose gel in which antibody has been incorporated. The height of the rocket-shaped line of precipitation that forms following electrophoresis for 2–3 h is proportional to the concentration of antigen. A sample of unknown antigen concentration (well 5) can be quantitated by reference to a standard curve. [Photograph from D. M. Weir (ed.), 1986, *Handbook of Experimental Immunology,* Blackwell Scientific Publications.]



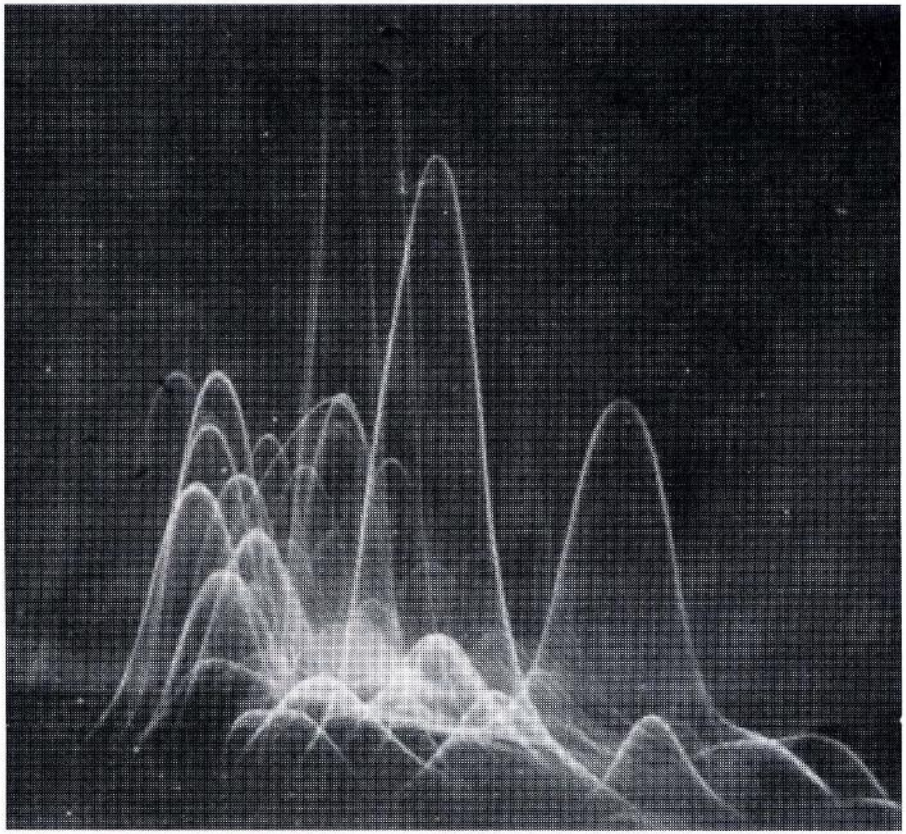
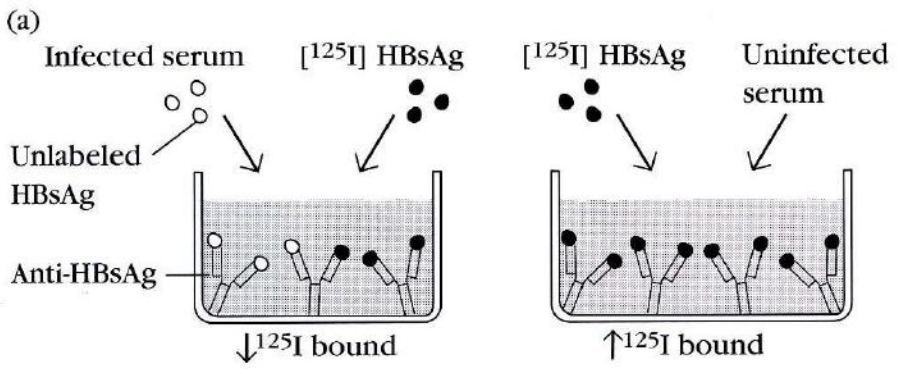


FIGURE 6-11

Several antigens in a complex antigen mixture can be quantitated by two-dimensional immunoelectrophoresis. The antigen sample is first electrophoresed; after the gel is laid over another gel containing antiserum, it is electrophoresed at right angles. The heights of the precipitin peaks (blue curves) in the second electrophoresis are proportional to the antigen concentrations, which can be determined by reference to standard curves. [Photograph from D. M. Weir (ed.), 1986, *Handbook of Experimental Immunology,* Blackwell Scientific Publications.]



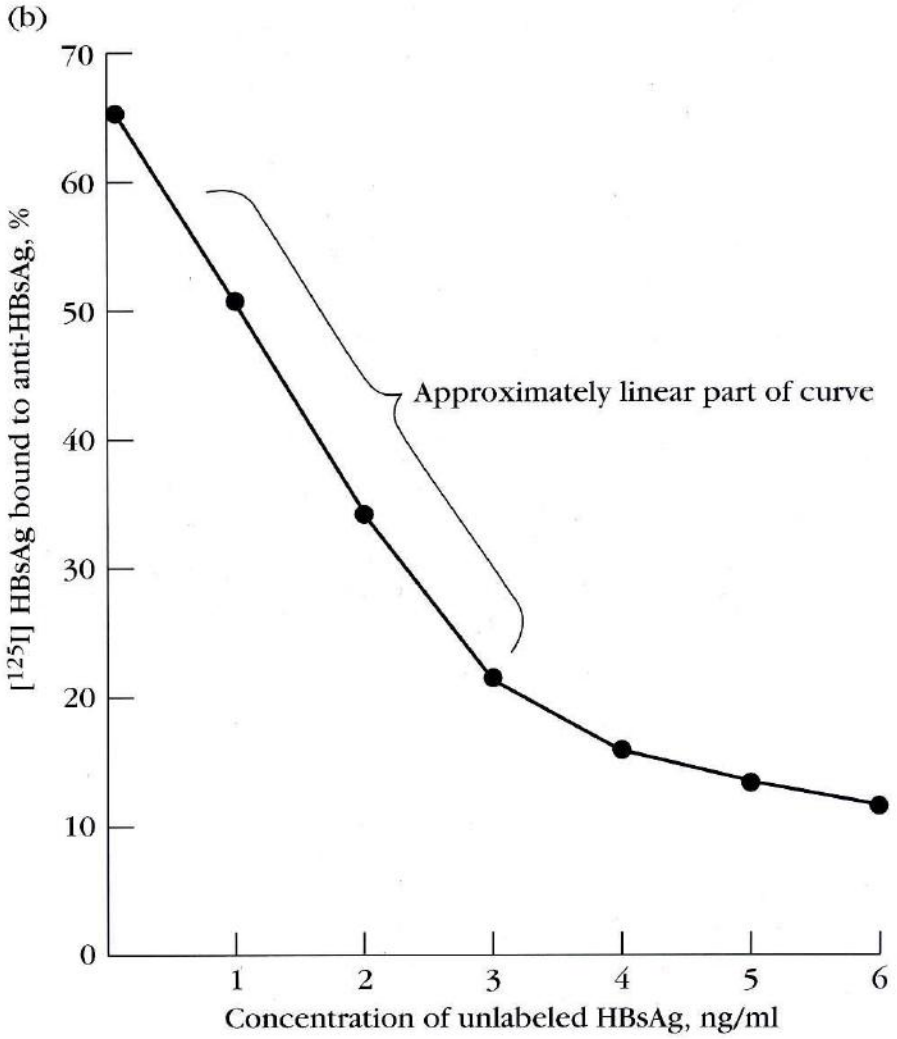


FIGURE 6-13

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 amount of radioactivity bound to the antibody is determined. 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 from the linear portion of the curve.

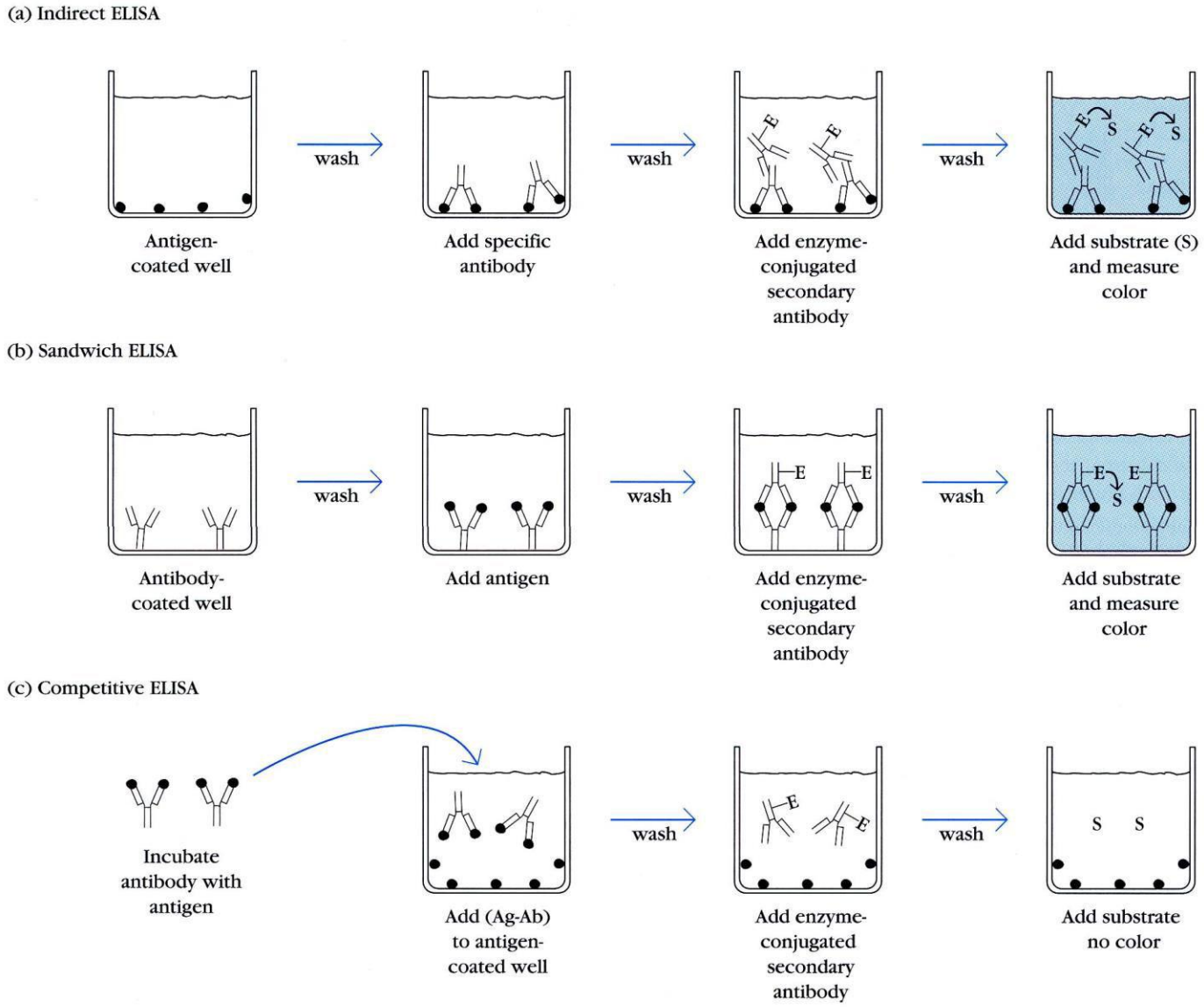


FIGURE 6-14

Variations in the enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined with an indirect ELISA (a), whereas antigen can be determined with a sandwich ELISA (b) or competitive ELISA (c). In the competitive ELISA, which is an inhibition-type assay, the concentration of antigen is inversely proportional to the color produced.

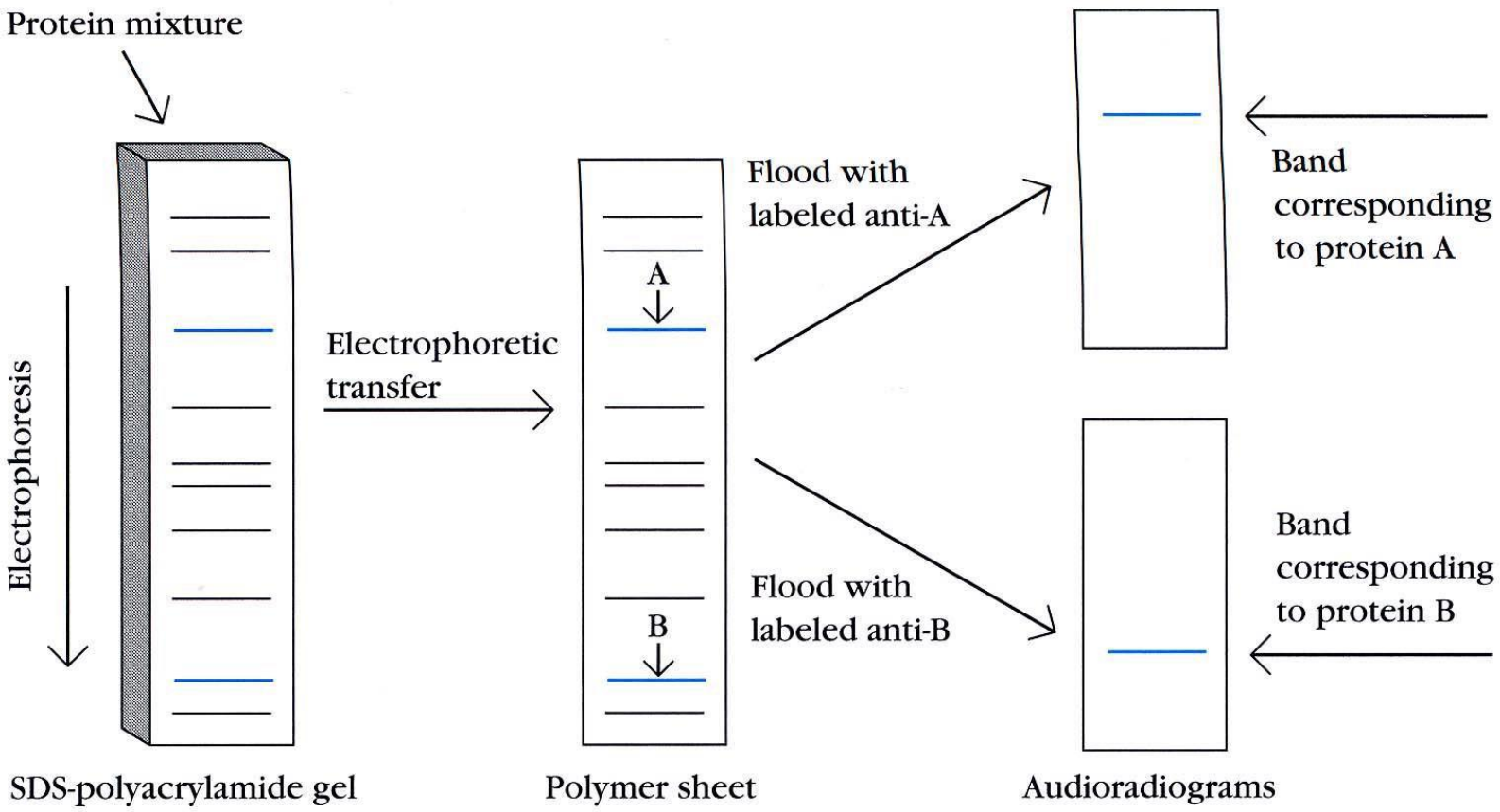


FIGURE 6-15

In Western blotting a protein mixture is separated by electrophoresis, and the protein bands are transferred by electrophoresis onto a nitrocellulose or other polymer sheet. After the sheet is flooded with radiolabeled specific antibodies, the various protein bands can be visualized by autoradiography.

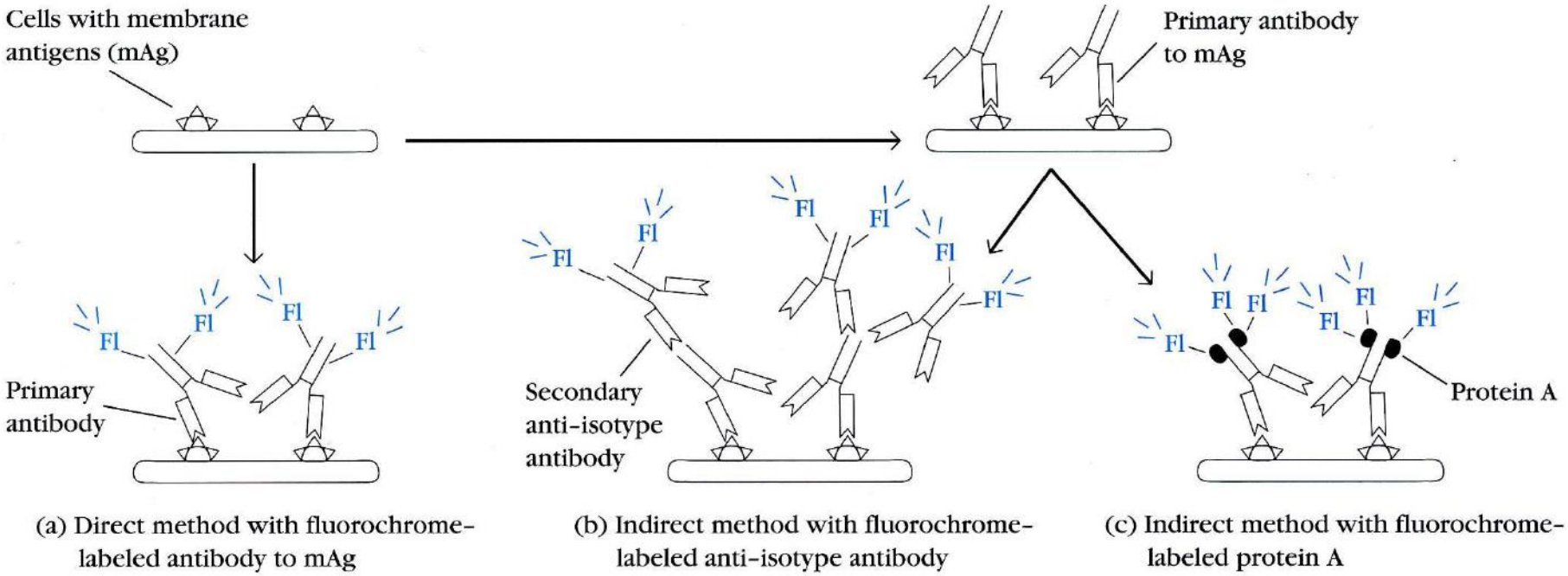


FIGURE 6-16

Direct and indirect immunofluorescence staining of membrane antigen (mAg). Cells are affixed to a microscope slide. In the direct method (a), cells are stained with anti-mAg antibody that is labeled with a fluorochrome (Fl). In the indirect methods (b and c), cells are first incubated with unlabeled anti-mAg antibody and then stained with a fluorochrome-labeled secondary reagent that binds to the primary antibody. Observation under a fluorescence microscope indicates whether the cells have been stained.

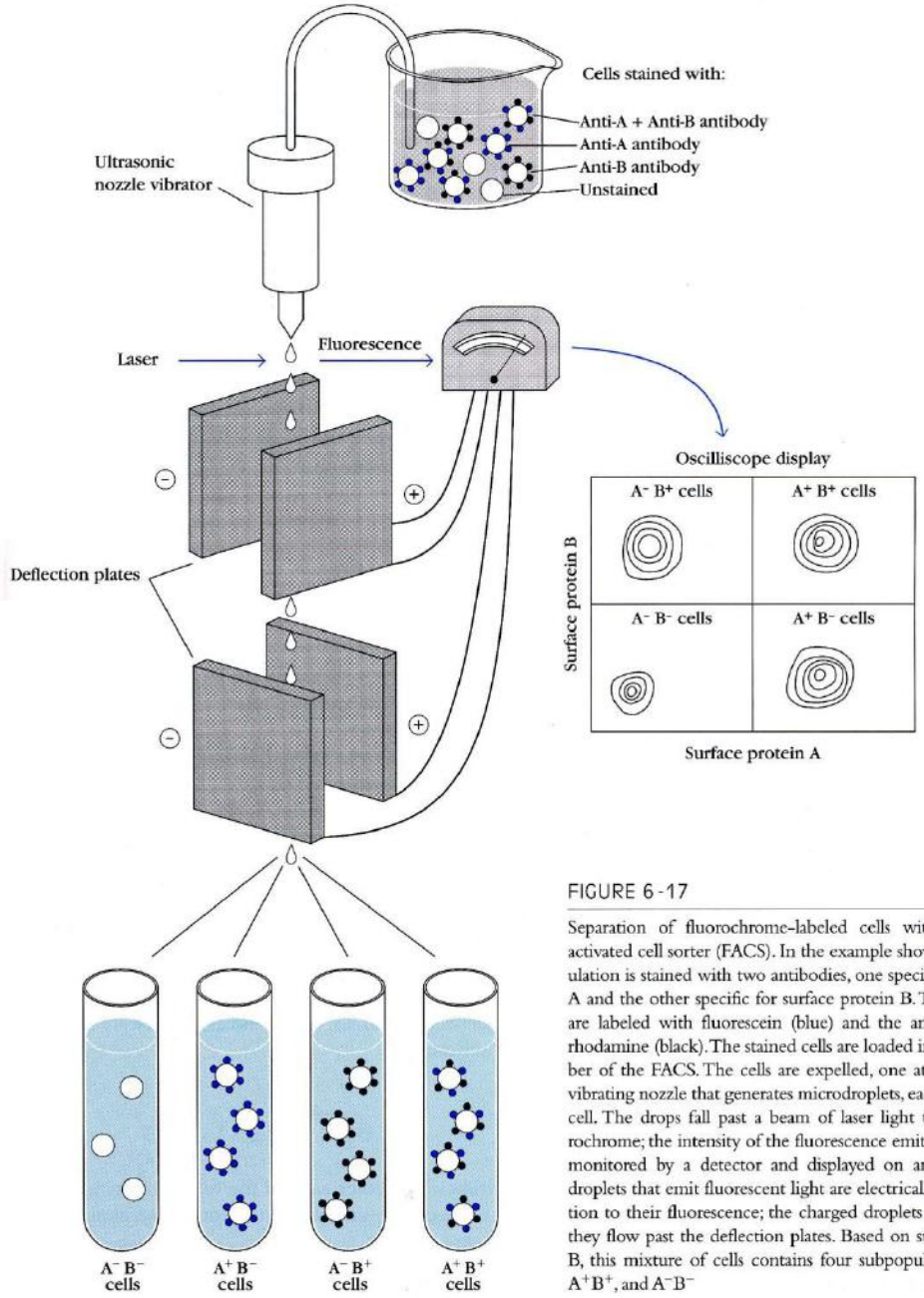


FIGURE 6-17

Separation of fluorochrome-labeled cells with the fluorescence-activated cell sorter (FACS). In the example shown, a mixed cell population is stained with two antibodies, one specific for surface protein A and the other specific for surface protein B. The anti-A antibodies are labeled with fluorescein (blue) and the anti-B antibodies with rhodamine (black). The stained cells are loaded into the sample chamber of the FACS. The cells are expelled, one at a time, from a small vibrating nozzle that generates microdroplets, each containing a single cell. The drops fall past a beam of laser light that excites the fluorochrome; the intensity of the fluorescence emitted by each droplet is monitored by a detector and displayed on an oscilloscope. Those droplets that emit fluorescent light are electrically charged in proportion to their fluorescence; the charged droplets then are separated as they flow past the deflection plates. Based on surface proteins A and B, this mixture of cells contains four subpopulations: A^+B^-, A^-B^+, A^+B^+, and A^-B^-

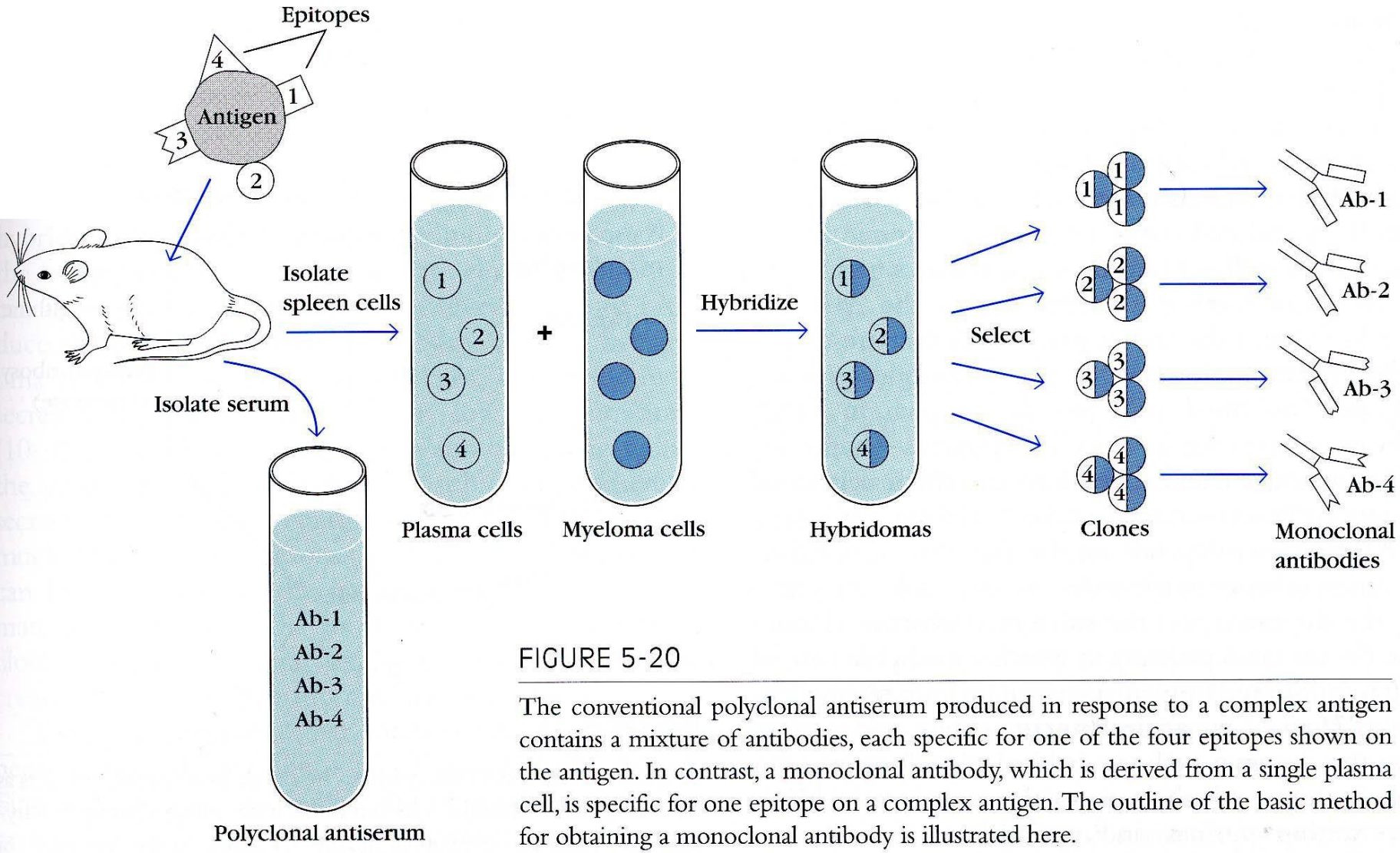


FIGURE 5-20

The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of antibodies, each specific for one of the four epitopes shown on the antigen. In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated here.

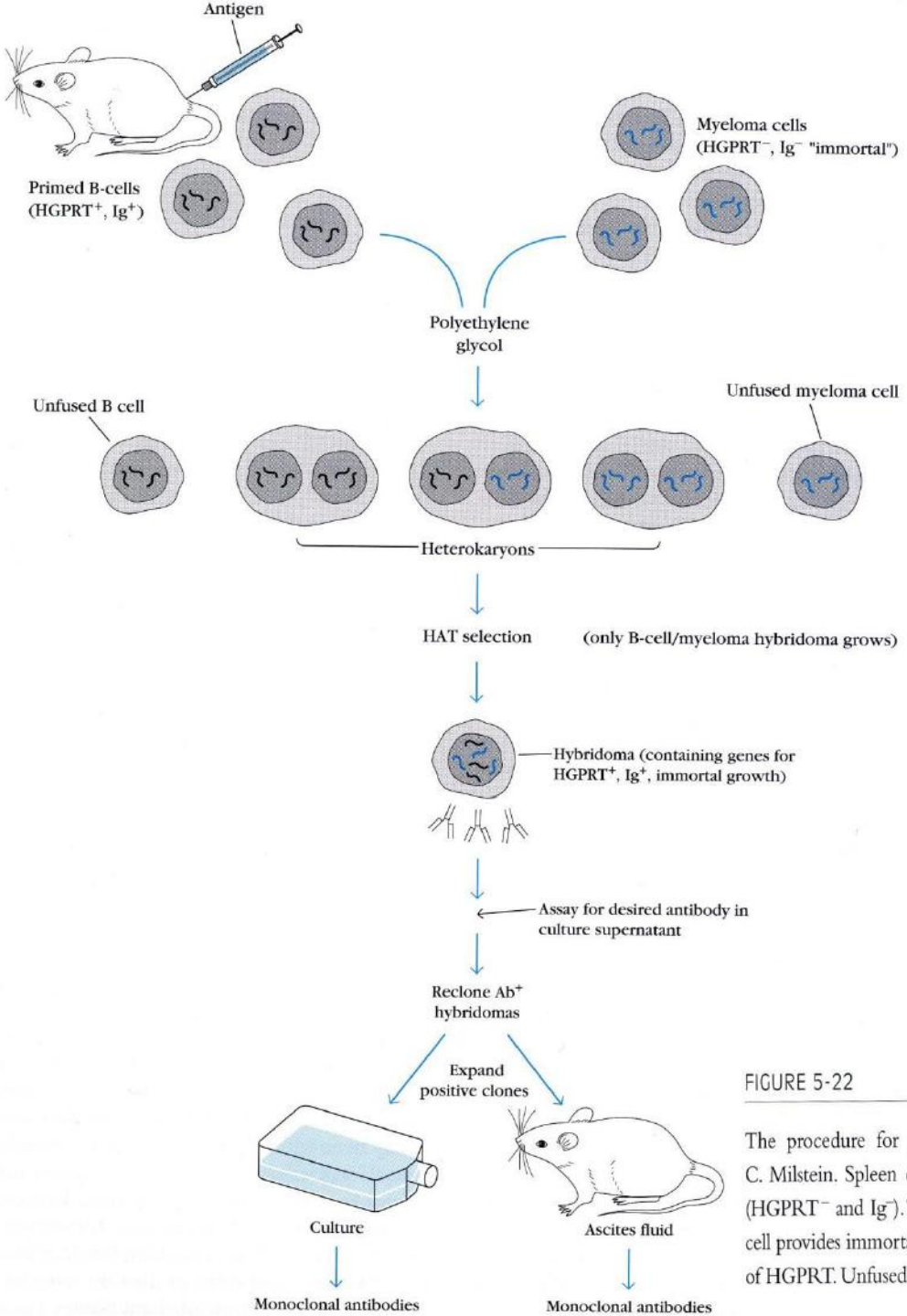


FIGURE 5-22

The procedure for producing monoclonal antibodies specific for a given antigen developed by G. Kohler and C. Milstein. Spleen cells ($HGPRT^+$ and Ig^+) from an antigen-primed mouse are fused with mouse myeloma cells ($HGPRT^-$ and Ig^-). The spleen cell provides the necessary enzymes for growth on HAT medium, while the myeloma cell provides immortal-growth properties. Unfused myeloma cells or myeloma/myeloma fusions fail to grow due to lack of HGPRT. Unfused spleen cells have limited growth capabilities in vitro and will die within a few days.

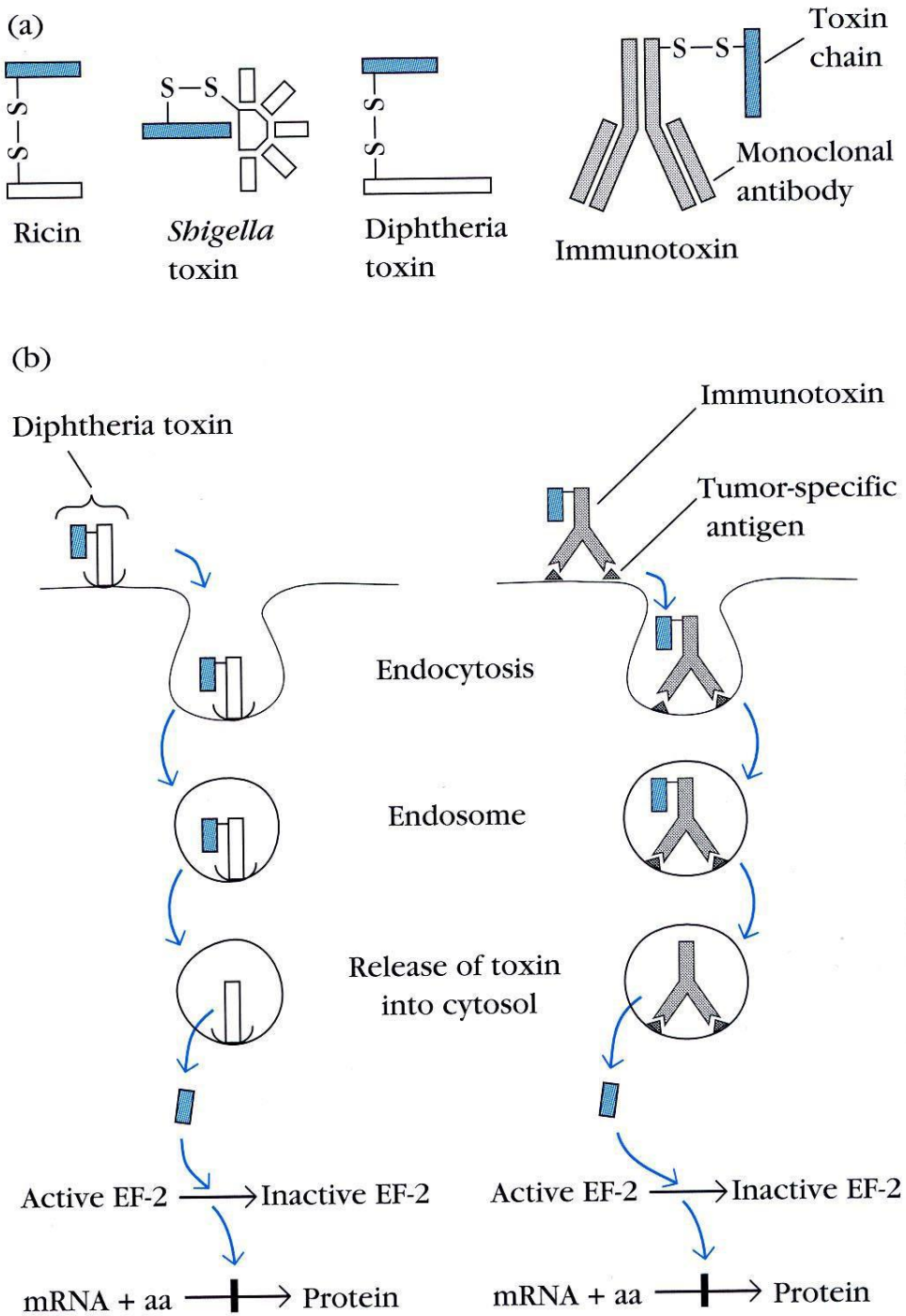


FIGURE 5-23

(a) Toxins used to prepare immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin. Each toxin contains an inhibitory toxin chain (blue) and a binding component (white). To make an immunotoxin, the binding component of the toxin is replaced with a monoclonal antibody (gray). (b) Diphtheria toxin binds to a cell-membrane receptor (*left*) and a diphtheria-immunotoxin binds to a tumor-associated antigen (*right*). In either case the toxin is internalized in an endosome. The toxin chain is then released into the cytoplasm, where it inhibits protein synthesis by catalyzing the inactivation of elongation factor 2 (EF-2).

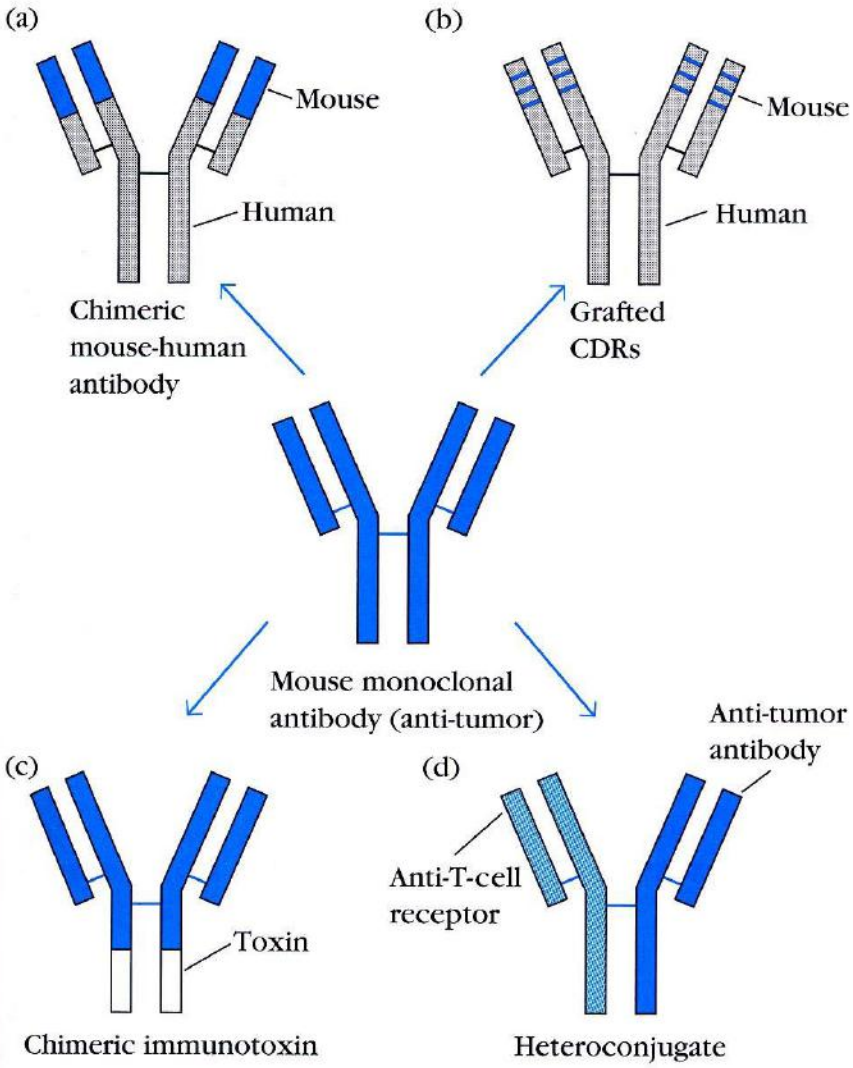


FIGURE 5-25

Chimeric and hybrid monoclonal antibodies engineered by recombinant DNA technology. (a) Chimeric mouse-human monoclonal antibody containing the V_H and V_L domains of a mouse monoclonal antibody (blue) and the C_L and C_H domains of a human monoclonal antibody (gray). (b) A chimeric monoclonal antibody containing only the CDRs of a mouse monoclonal antibody (blue bands) grafted within the framework regions of a human monoclonal antibody. (c) A chimeric monoclonal antibody in which the terminal Fc domain is replaced by a toxin chain (white). (d) A heteroconjugate in which one-half of the mouse antibody molecule is specific for a tumor antigen and the other half is specific for the CD3/T-cell receptor complex.

antibody has specificity for a tumor and the other half has specificity for a surface molecule on an immune effector cell, such as an NK cell, an activated macrophage, or a cytotoxic T lymphocyte (CTL). The heteroconjugate thus serves to cross-link the immune effector cell to the tumor. Some heteroconjugates have been designed to activate the immune effector cell when it is cross-linked to the tumor cell so that it begins to mediate destruction of the tumor cell.

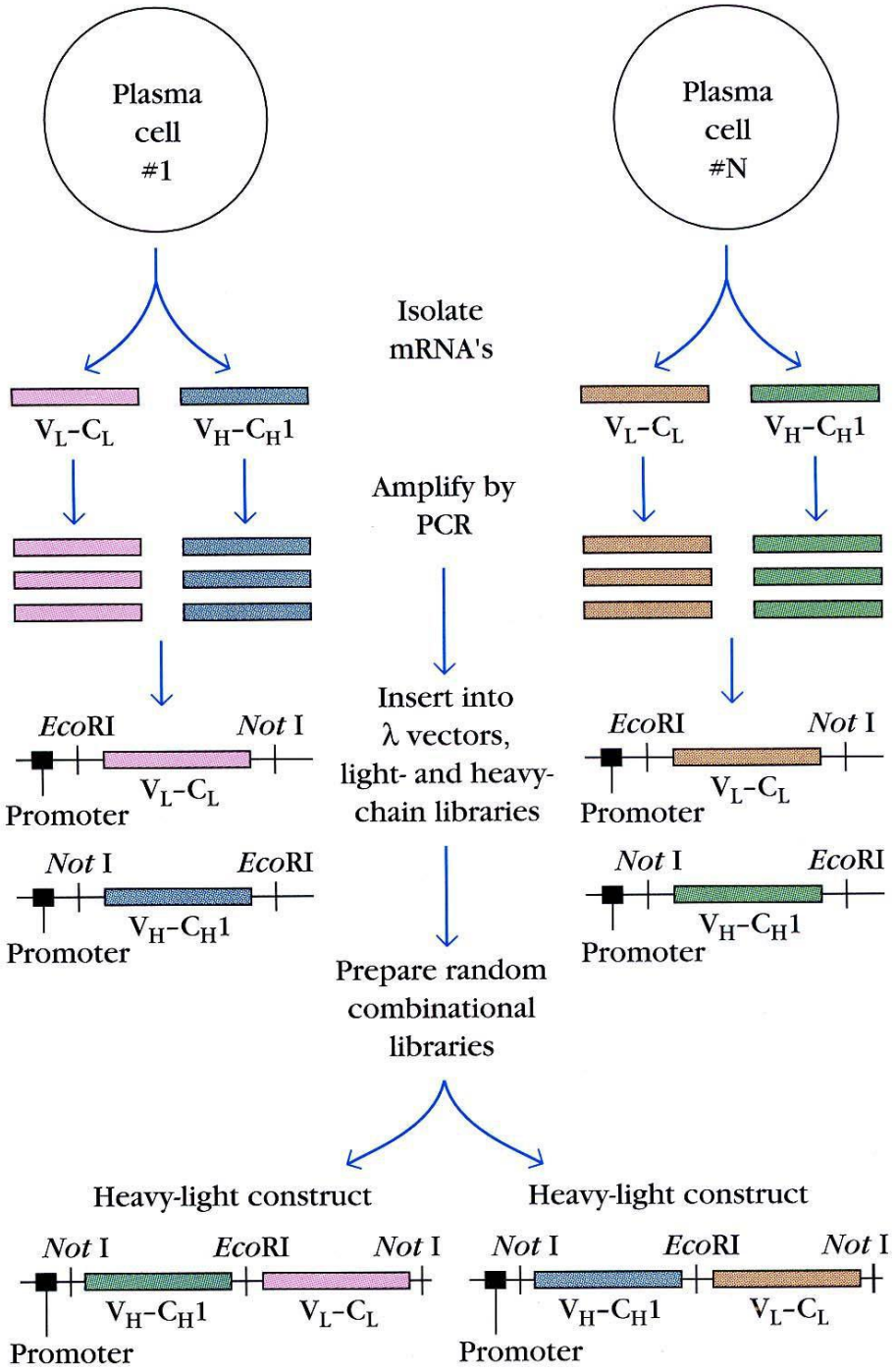


FIGURE 5-26

General procedure for producing gene libraries encoding Fab fragments. In this procedure isolated mRNA heavy and light chains are amplified by the polymerase chain reaction (PCR) and cloned in λ vectors. Random combinations of heavy and light chain genes generate an enormous number of heavy-light constructs encoding Fab fragments with different antigenic specificity. [Adapted from W. D. Huse et al., 1989, *Science* **246**:1275.]