

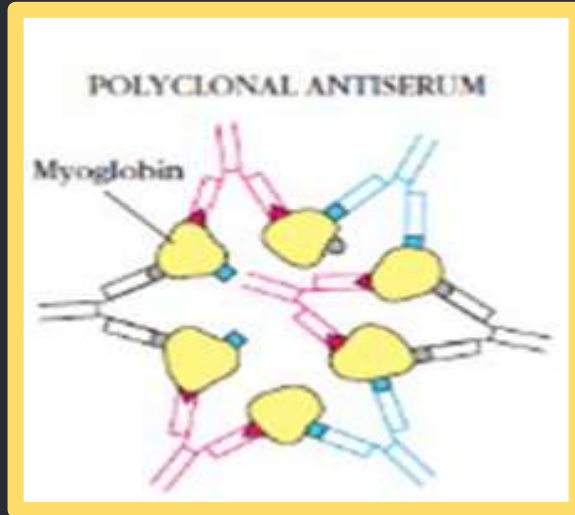


MONOCLONAL ANTIBODIES



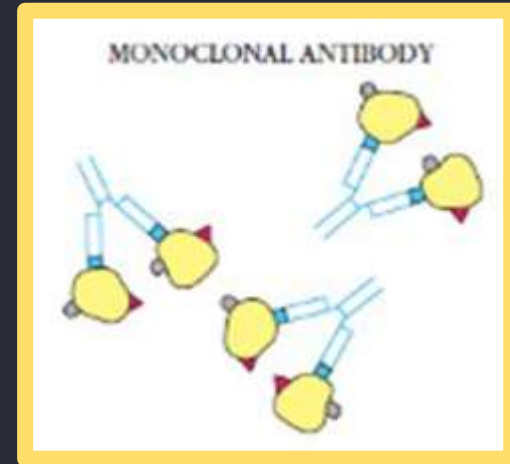
Immune Response

Polyclonal Ab (Derived from different Plasma cells)



Monoclonal Ab

Specific to single epitope
(Derived from single Plasma cell)



HYBRIDOMA TECHONOLOGY

- ✓ Normal cells are fused with a cancerous cell line
 - E.g. Myeloma
- ✓ Fusion is accomplished with PEG (polyethylene glycol)
- ✓ The new hybrid cell exhibits properties of both cell types
 - Unlimited growth
 - Secretes monoclonal antibody
 - Or secretes cytokines

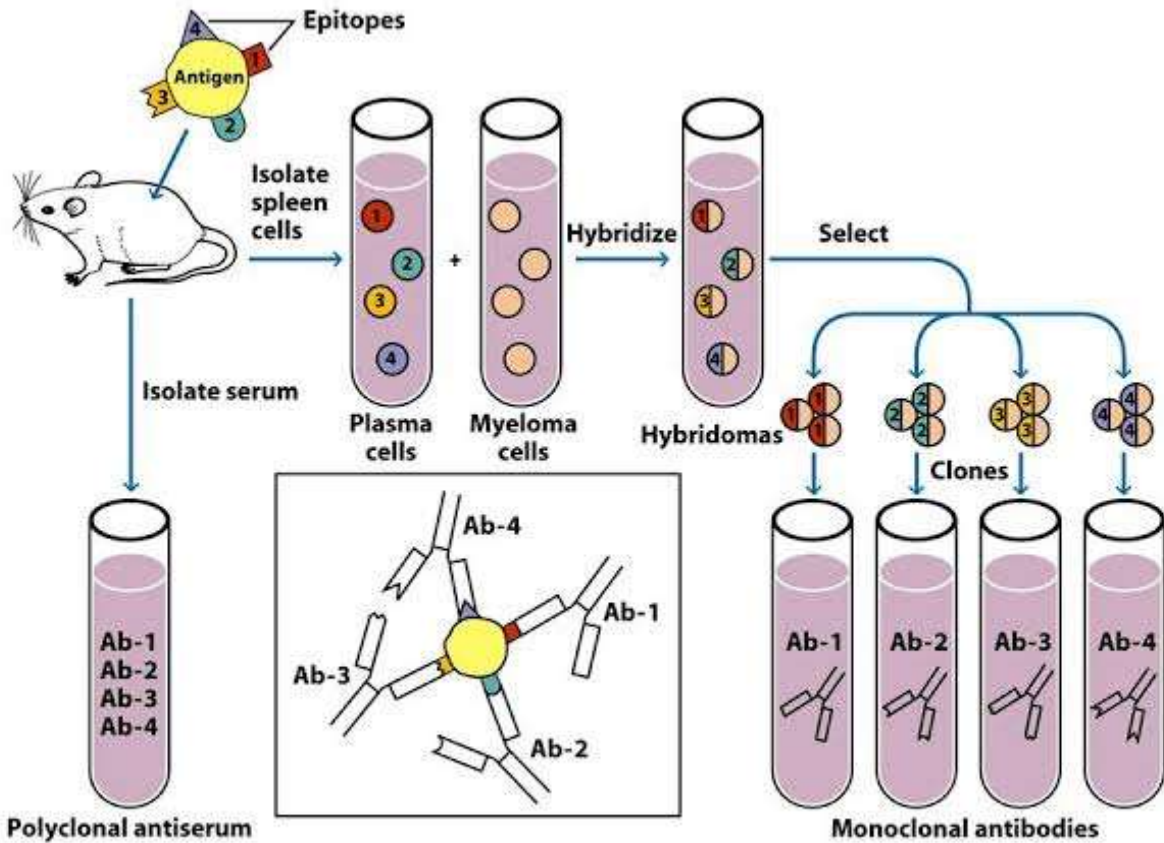


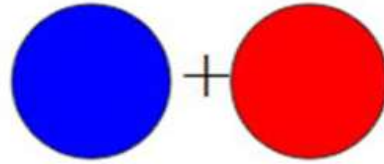


Figure 4-25
 Ruby IMMUNOLOGY, Sixth Edition
 © 2007 W.H. Freeman and Company

- 
- **For monoclonal antibody production**
 1. - **Animal is immunized with antigen**
 2. - **Spleen cells are isolated**
 3. **Fused with myeloma cells using PEG**
 - **This cell is deficient in HGPRT (hypoxanthine guanine phosphoribosyl transferase)**
 - **Alternatively TK (thymidine kinase deficient)**
 - **Fails to survive in selection medium**
 - **Aminopterin inhibits "De novo Pathway", "Salvage Pathway" is not possible due to HGPRT or TK deficiency**
 - **Also Ig Deficient**
 1. - **It can not secret any immunoglobulins**
- 

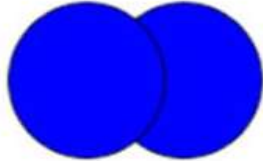
Plasma Cells From
Immunized Animal



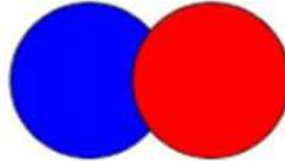
Myeloma HGPRT Deficient
And Ig Deficient



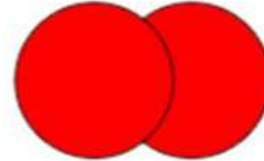
Senescence



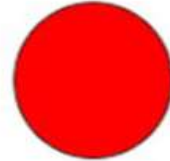
Senescence



Can Use Salvage
Pathway
No Senescence



HAT Medium



HAT Medium

Chromosomes



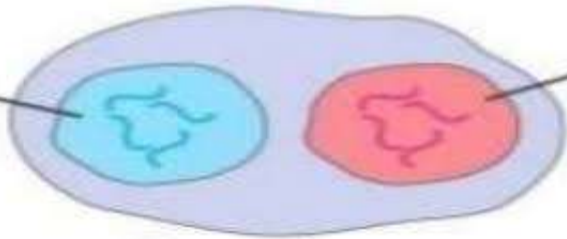
Normal T or B cell
(dies after 7-10
days in culture)

Cancerous T or B cell
(grows continuously
in culture)

Polyethylene glycol

Nucleus
of normal
lymphocyte

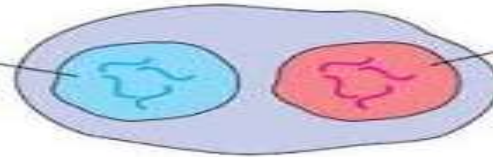
Nucleus of
cancer cell



Heterokaryon

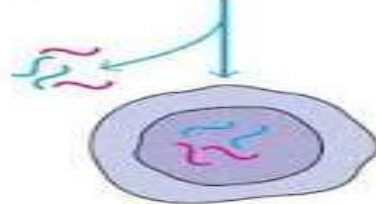
Nucleus of normal lymphocyte

Nucleus of cancer cell



Heterokaryon

Random chromosomal loss



Hybridoma

(expresses some normal B-cell or T-cell genes but grows indefinitely like a cancer cell)

Monoclonal antibody



B-cell hybridoma

Interleukin 2 (IL-2)



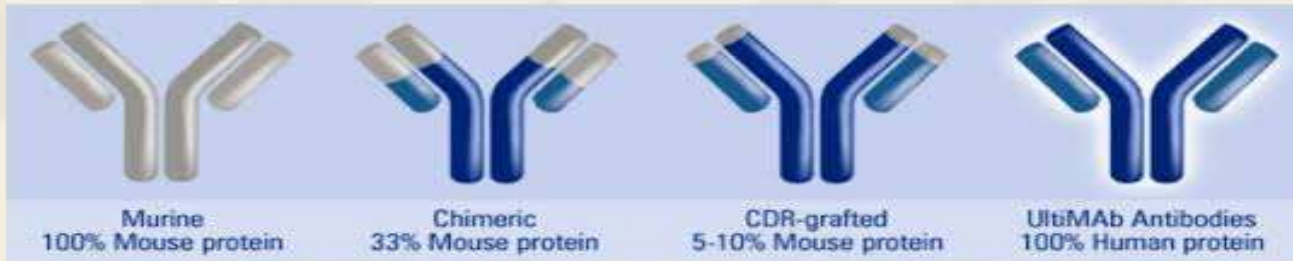
T-cell hybridoma

The types of mAb designed

1. **A. Murine source mAbs: rodent mAbs with excellent affinities and specificities, generated using conventional hybridoma technology. Clinical efficacy compromised by HAMA(human anti murine antibody) response, which lead to allergic or immune complex hypersensitivities.**
2. **B. Chimeric mAbs: chimeras combine the human constant regions with the intact rodent variable regions. Affinity and specificity unchanged. Also cause human anti chimeric antibody response (30% murine resource)**
3. **C. Humanized mAbs: contained only the CDRs of the rodent variable region grafted onto human variable region framework**

Evolution of Therapeutic Antibodies

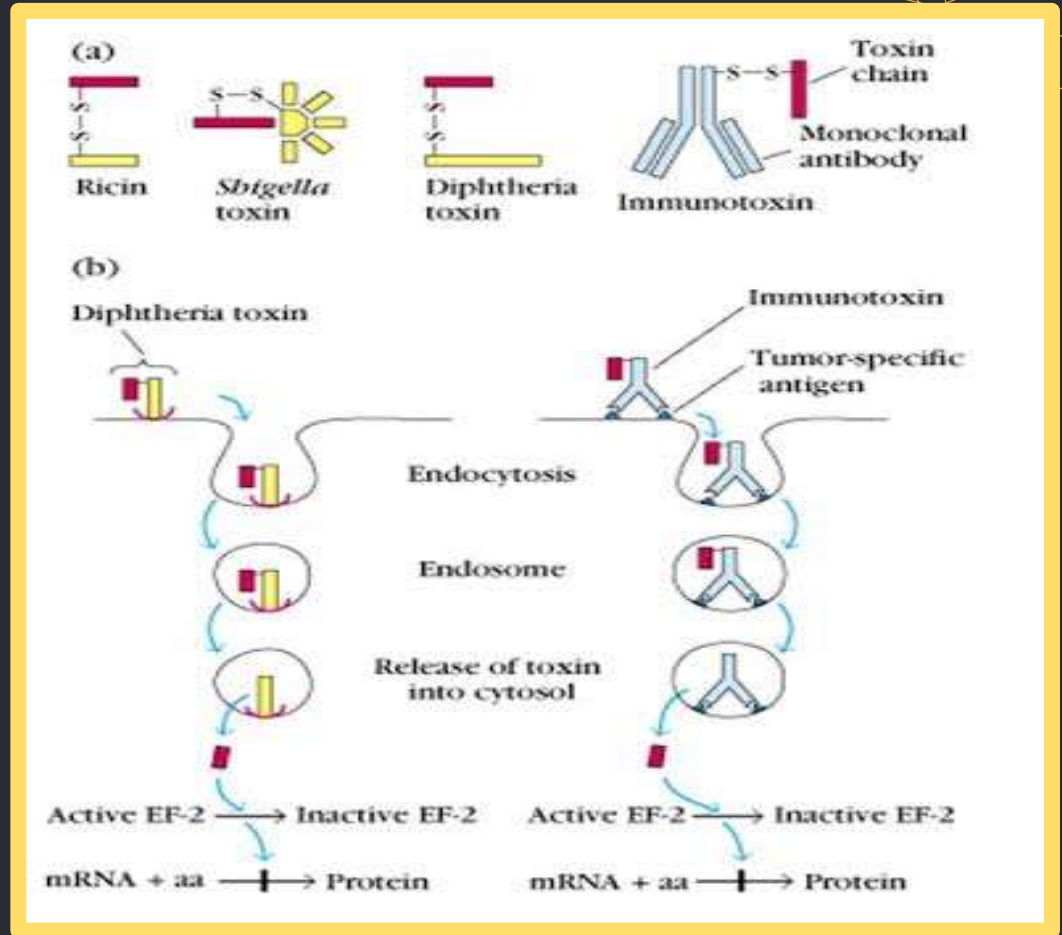
Evolution of Therapeutic Antibodies



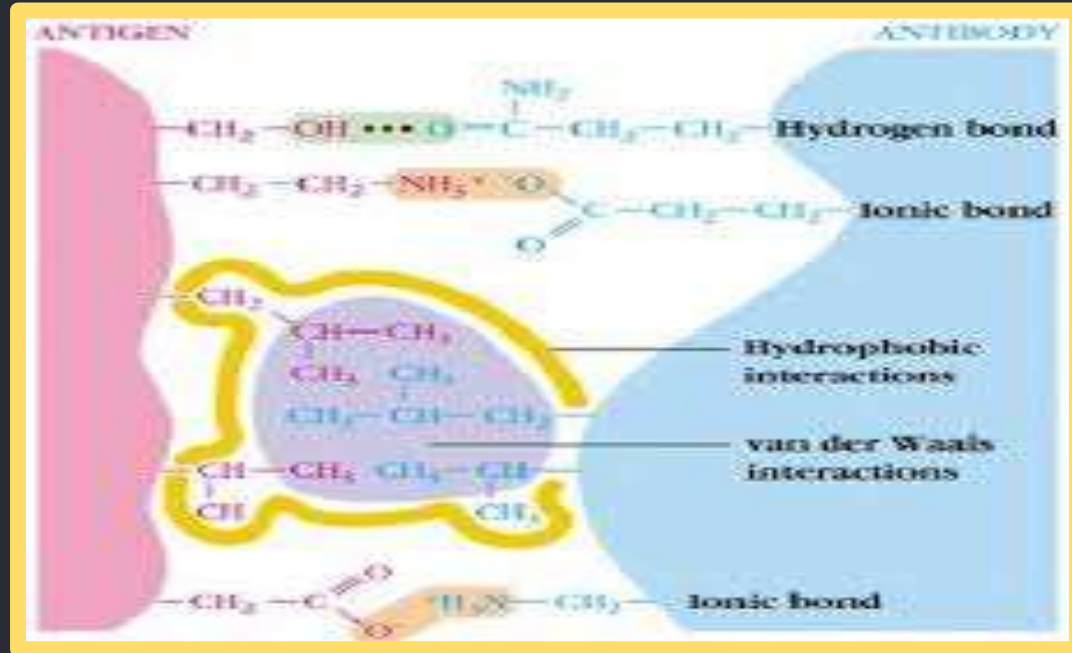
APPLICATION OF MONOCLONAL ANTIBODIES

✓ Diagnostics

✓ Immunotoxin

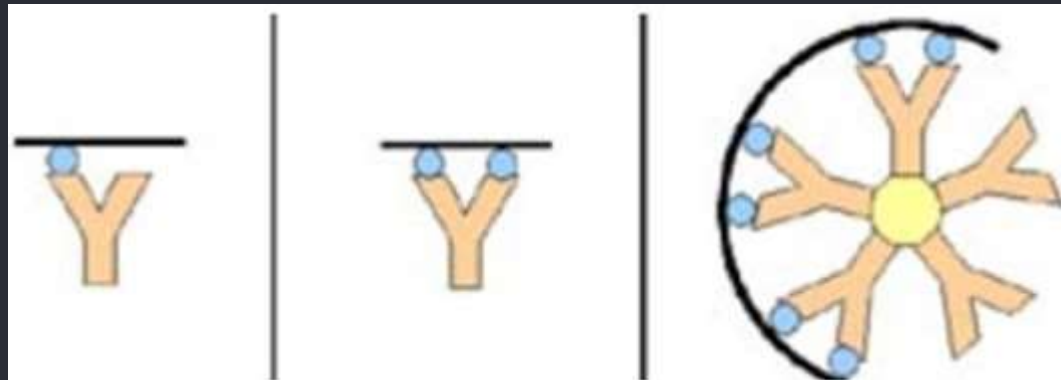


ANTIGEN ANTIBODY INTERACTION

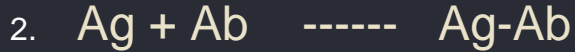


Important terms:

1. Affinity- The combined strength of the non covalent interactions between a single antigen-binding site on an antibody and a single epitope.
2. Avidity-The strength of such multiple interactions between a multivalent antibody and antigen
3. Cross reactivity- antibody elicited by one antigen can cross-react with an unrelated antigen



1. The association between a binding site on an antibody (Ab) with a monovalent antigen (Ag) can be described by the equation:



3. • K_a , equilibrium constant for association: $K_a = [Ag-Ab] / [Ab][Ag]$

4. • Low-affinity Ag-Ab complexes have K_a values between 10^3 and 10^4 L/mol:
high-affinity complexes can have K_a values as high as 10^8 L/mol.

5. • The equilibrium constant for dissociation reaction is K_d , the reciprocal of K_a



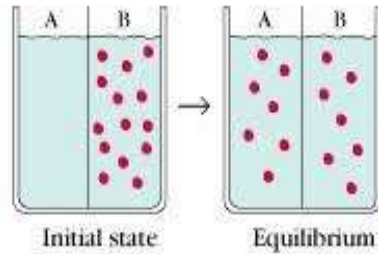
$$K_d = [Ab][Ag] / [Ag-Ab] = 1/K_a$$

• A quantitative indicator of the stability of an Ag-Ab complex

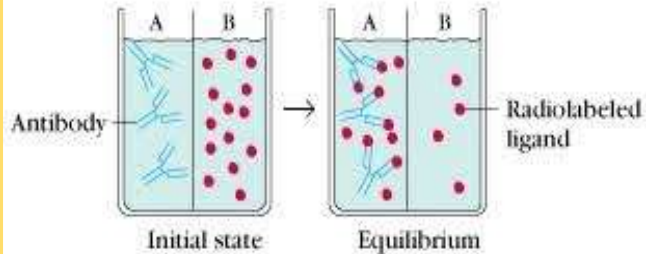
Ka, can be determined by equilibrium dialysis

(a)

Control: No antibody present
(ligand equilibrates on both sides equally)

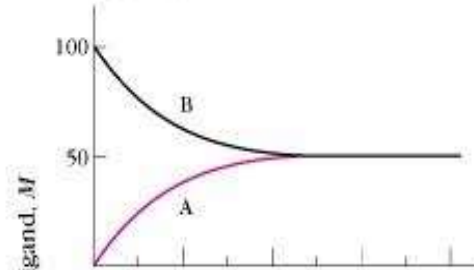


Experimental: Antibody in A
(at equilibrium more ligand in A due to Ab binding)

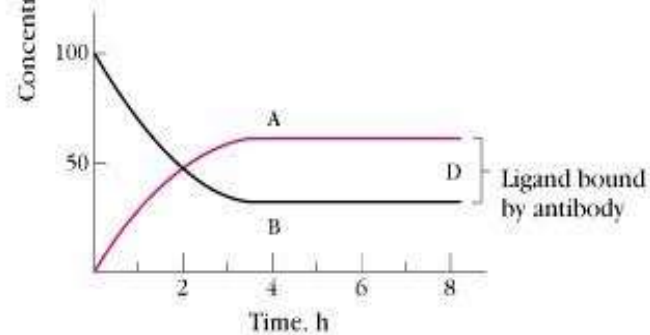


(b)

Control

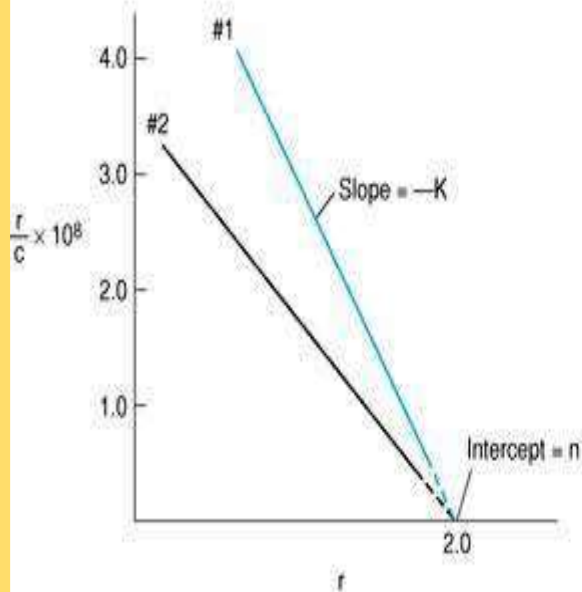


Experimental

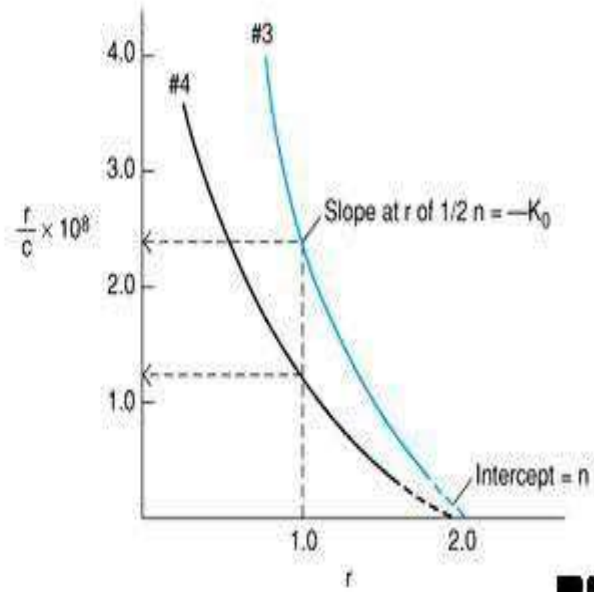


Scatchard equation: $r/c = K_a n - K_a r$

(a) Homogeneous antibody

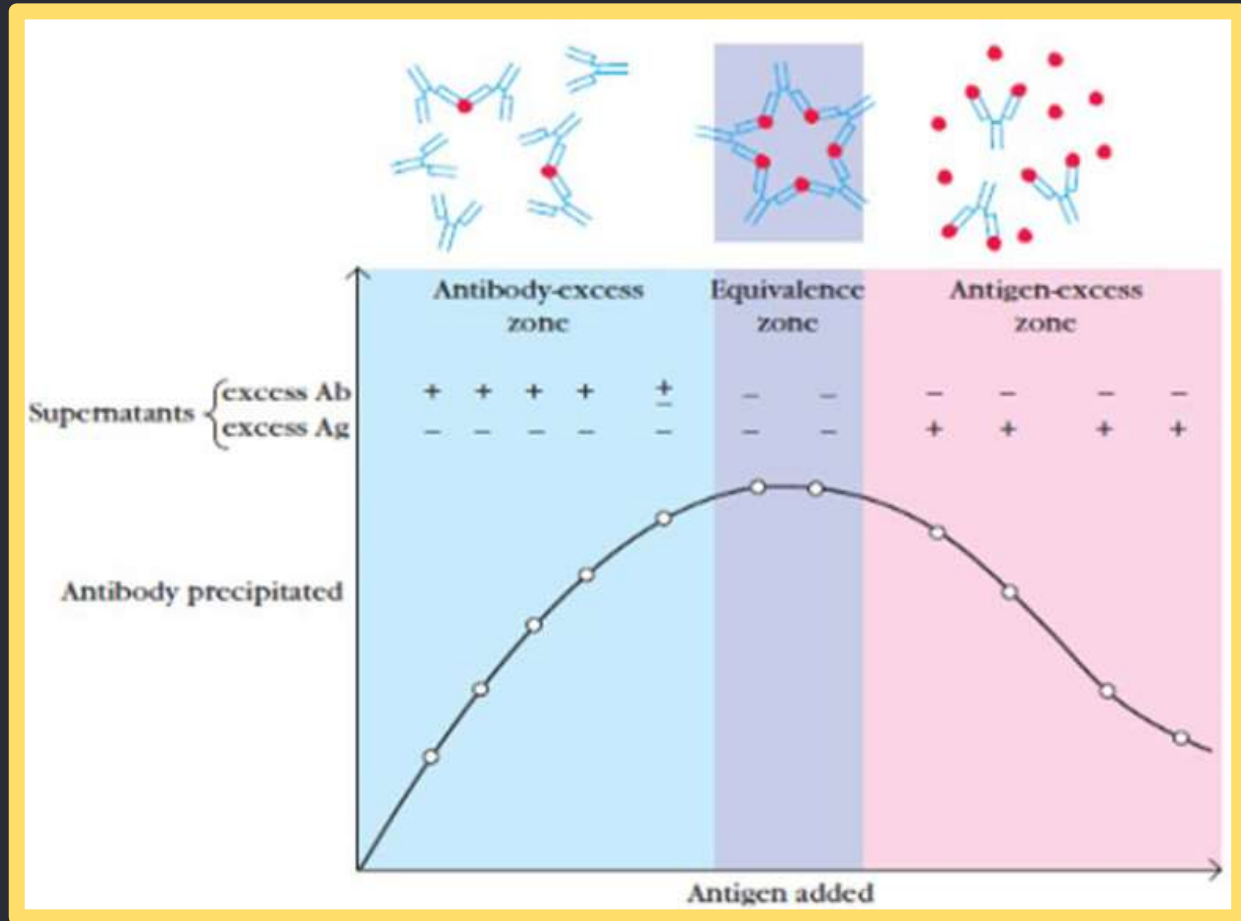


(b) Heterogeneous antibody



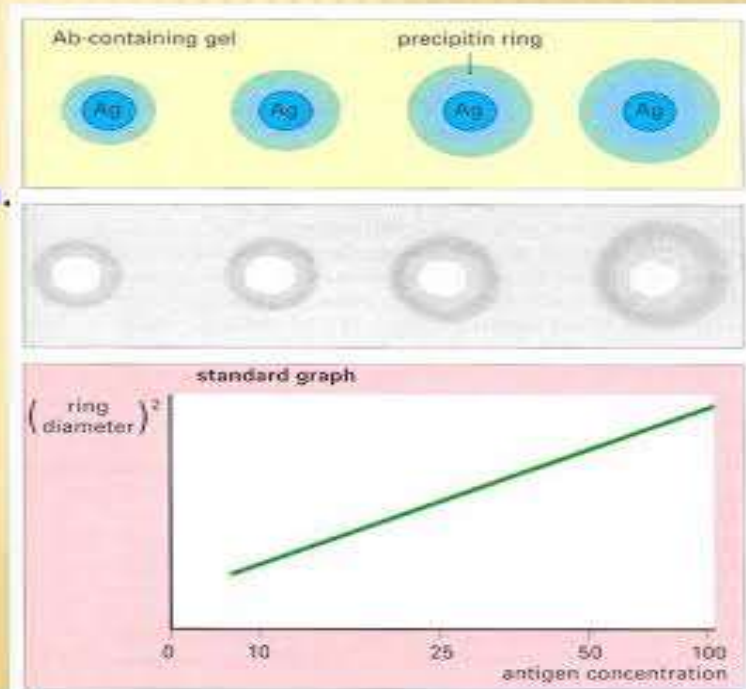
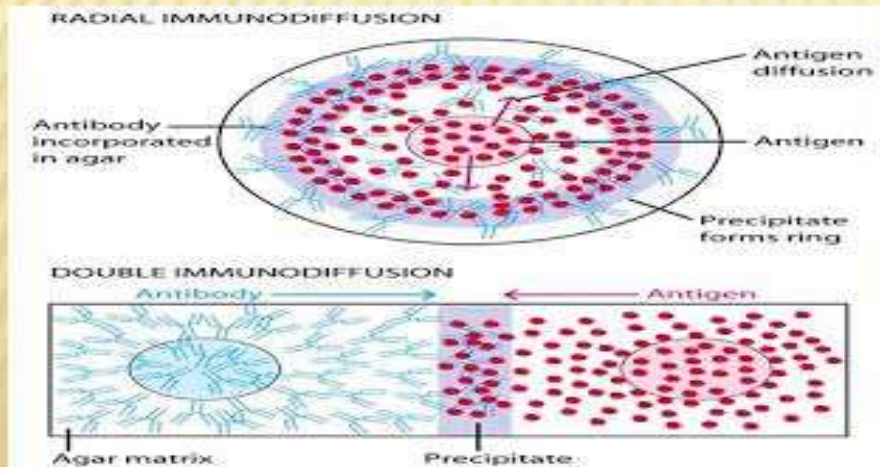
Precipitation reaction

- Precipitation in fluids
- Bivalent Ab
- Bivalent/ Multivalent Ag



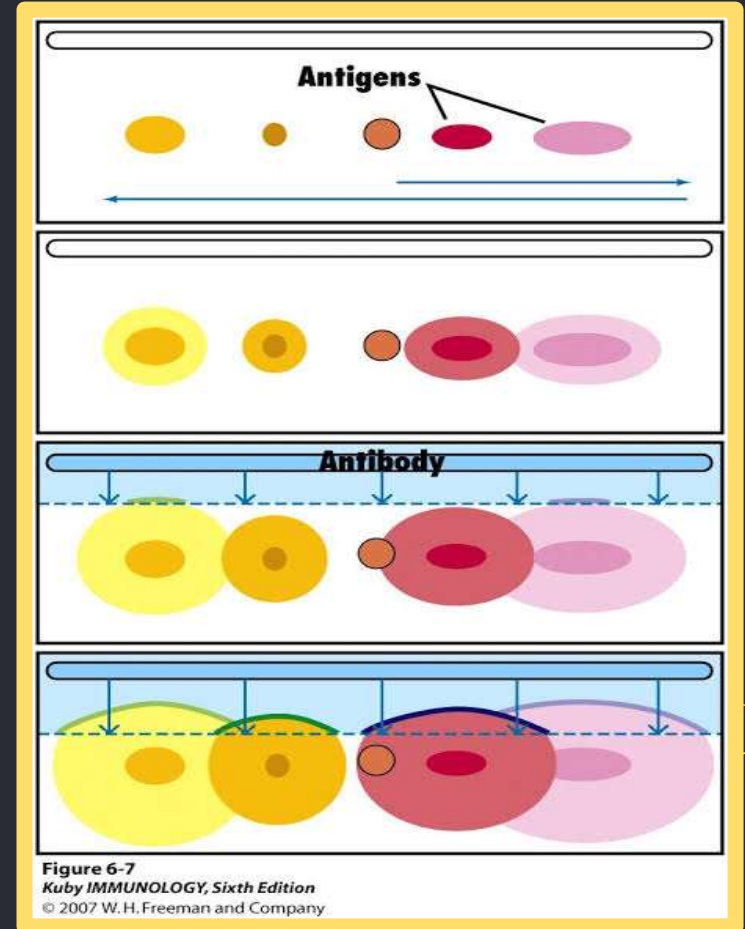
RADIAL IMMUNODIFFUSION

- ✖ A quantitative technique based upon the reaction between an Ag placed in a well diffuses into an agar containing the Ab.
- ✖ During diffusion period the Ag & Ab continue to react until the zone of equivalence is reached with formation of a well-defined ring of precipitation around the Ag well which is **proportional to the Ag concentration.**



Immuno-electrophoresis

- Qualitative technique
 - Antigen mixture is first electrophoresed
- ↓
- Antiserum is added to the troughs which are parallel to the direction of the electric field
- ↓
- Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropriate proportions



THANKS

