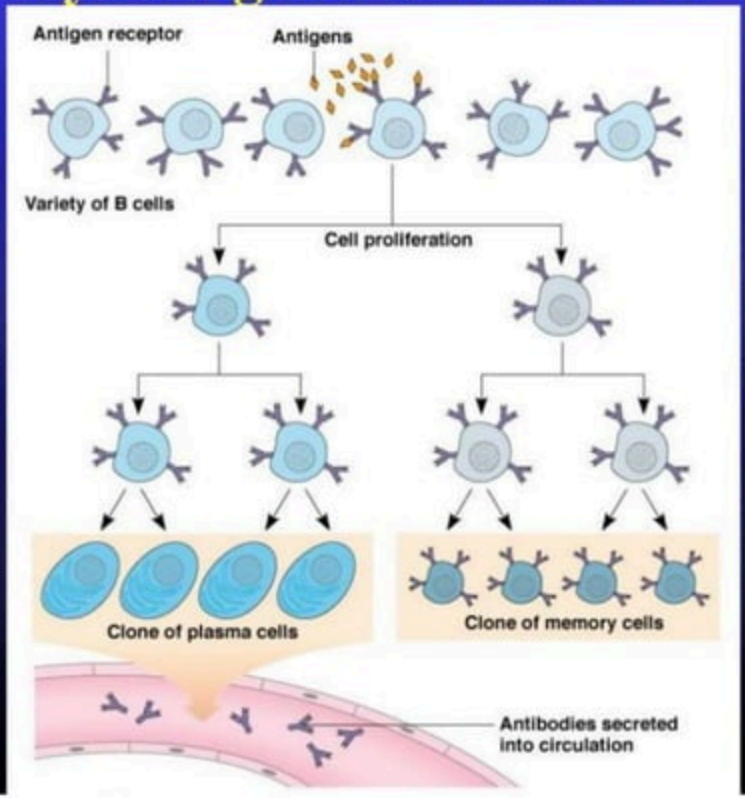
IMMUNOCHEMISTRY

How Do B Cells Produce Antibodies?

- B cells develop from stem cells in the bone marrow of adults (liver of fetuses).
- After maturation B cells migrate to lymphoid organs (lymph node or spleen).
- Clonal Selection: When a B cell encounters an antigen it recognizes, it is stimulated and divides into many clones called plasma cells, which actively secrete antibodies.
- Each B cell produces antibodies that will recognize only one antigenic determinant.

Clonal Selection of B Cells is Caused by Antigenic Stimulation



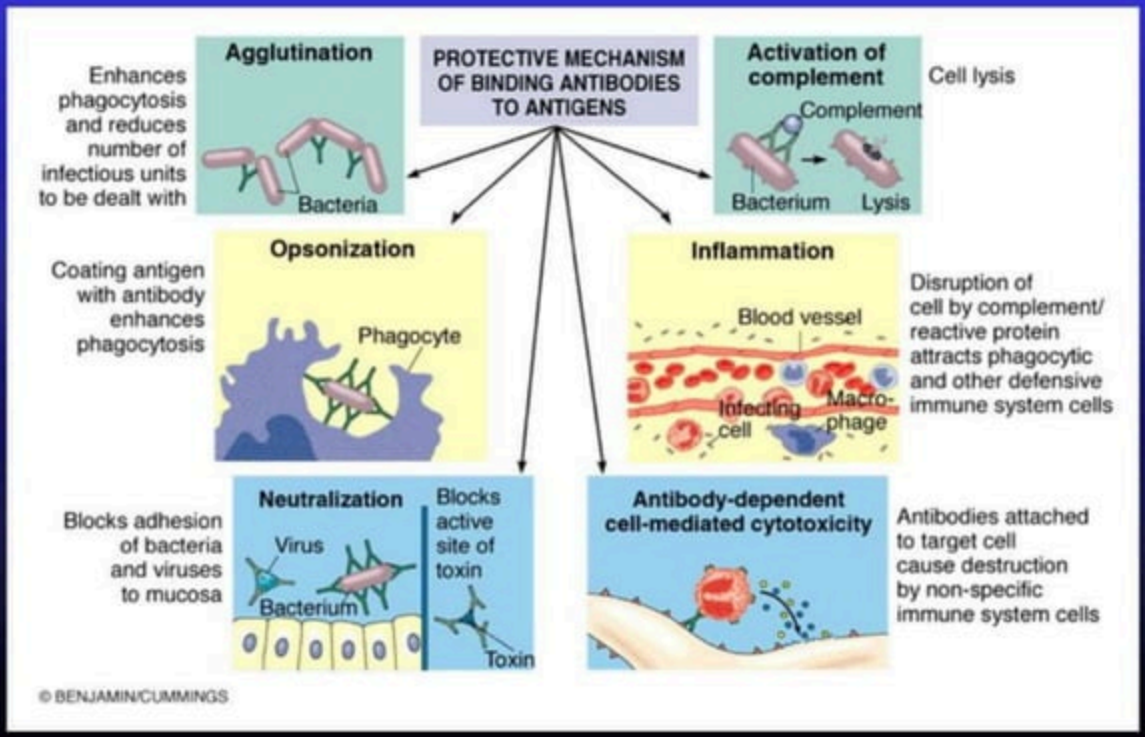
Consequences of Antigen-Antibody Binding

Antigen-Antibody Complex: Formed when an antibody binds to an antigen it recognizes.

Affinity: A measure of binding strength.

1. **Agglutination:** Antibodies cause antigens (microbes) to clump together.
   - IgM (decavalent) is more effective that IgG (bivalent).
   - **Hemagglutination:** Agglutination of red blood cells. Used to determine ABO blood types and to detect influenza and measles viruses.
2. **Opsonization:** Antigen (microbe) is covered with antibodies that enhances its ingestion and lysis by phagocytic cells.

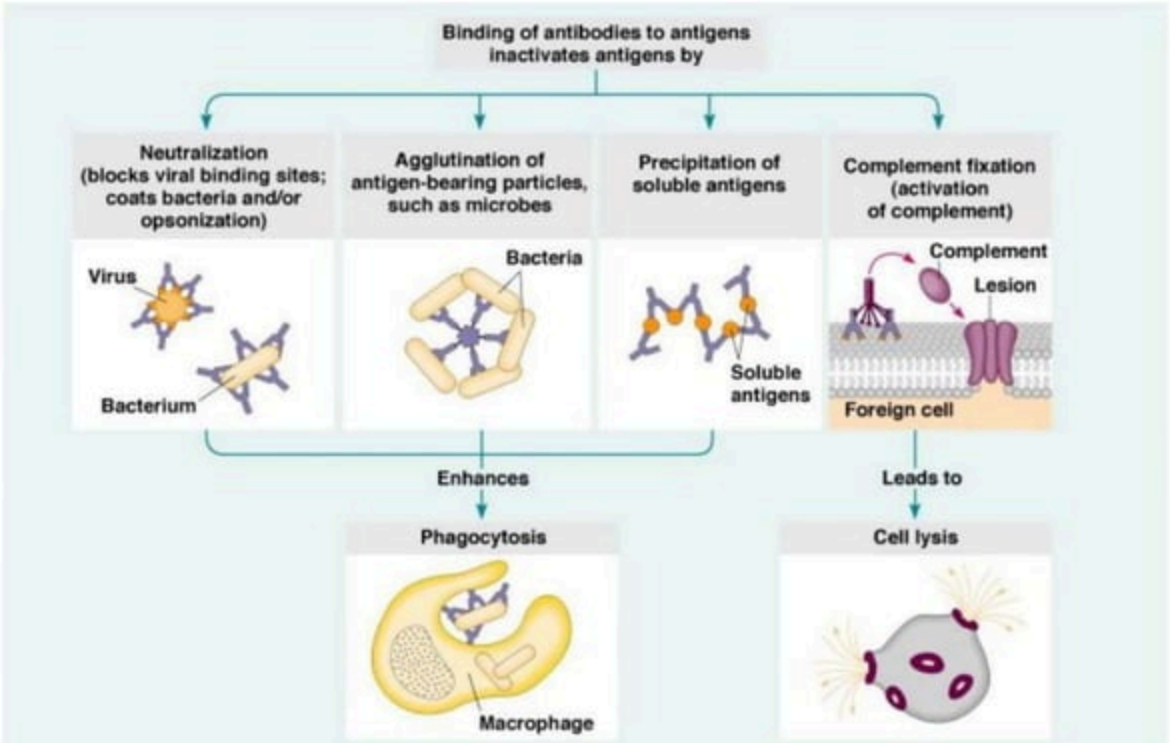
Consequences of Antibody Binding



Humoral Immunity (Continued)

3. **Neutralization:** IgG inactivates viruses by binding to their surface and neutralize toxins by blocking their active sites.
4. **Antibody-dependent cell-mediated cytotoxicity:** Used to destroy large organisms (e.g.: worms). Target organism is coated with antibodies and bombarded with chemicals from nonspecific immune cells.
5. **Complement Activation:** Both IgG and IgM trigger the complement system which results in cell lysis and inflammation.

Consequences of Antibody Binding



Immunological Memory

Antibody Titer: The amount of antibody in the serum.

Pattern of Antibody Levels During Infection

Primary Response:

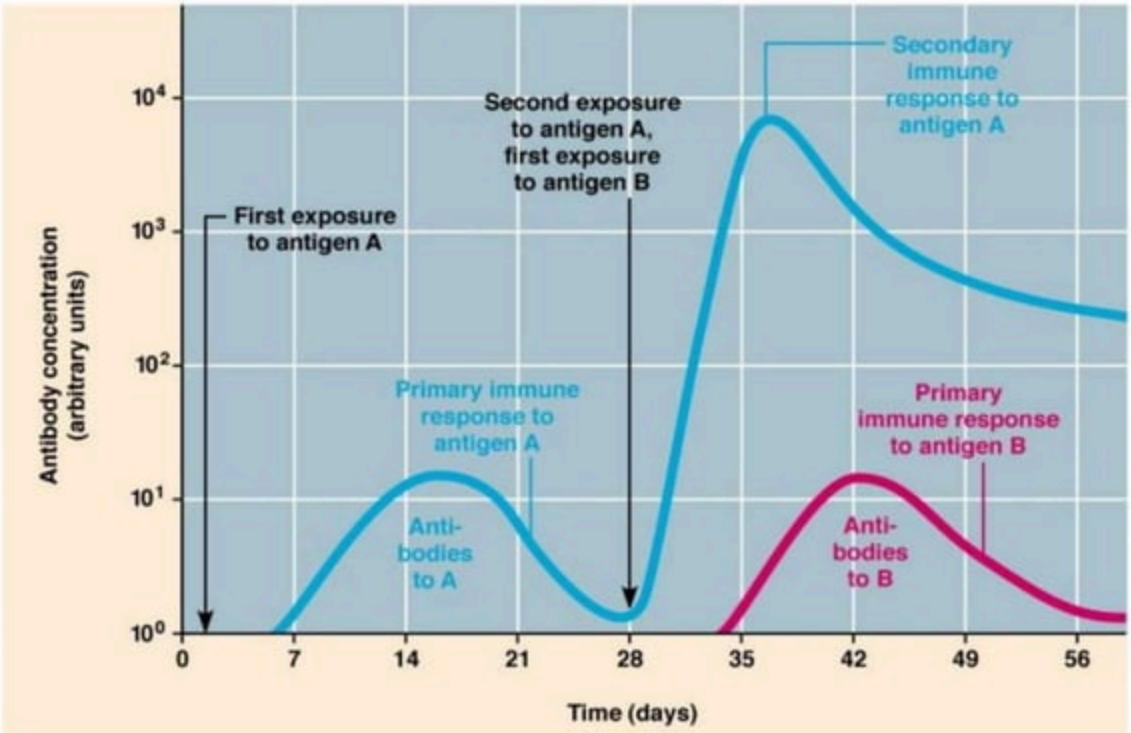
- After *initial* exposure to antigen, no antibodies are found in serum for several days.
- A gradual increase in titer, first of IgM and then of IgG is observed.
- Most B cells become plasma cells, but some B cells become long living *memory cells*.
- Gradual decline of antibodies follows.

Immunological Memory (Continued)

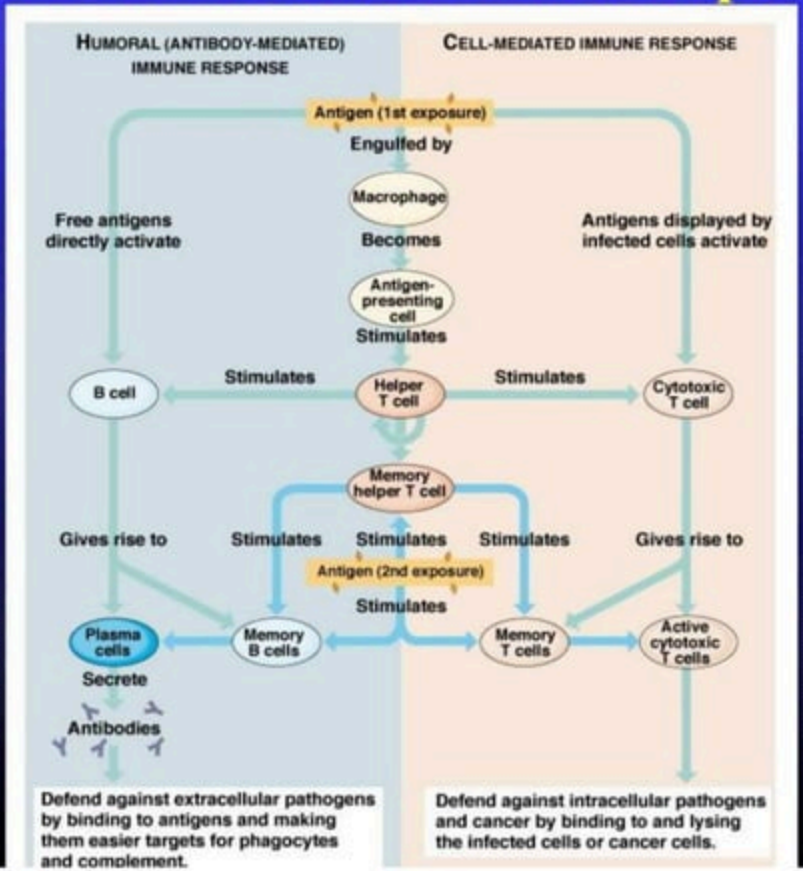
Secondary Response:

- Subsequent exposure to the same antigen displays a faster and more intense antibody response.
- Increased antibody response is due to the existence of memory cells, which rapidly produce plasma cells upon antigen stimulation.

Antibody Response After Exposure to Antigen



Overview of the Immune Response



FREE RADICALS

DEFINITION : A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outermost orbital.

Free radical is generally represented by superscript dot.

HOW DO THEY GENERATE?

- Oxygen atom has two parallel spin electrons in its outermost orbital.
- For a bond to occur the other molecule should also have two opposite spin electrons in its outer orbital , so here Oxygen accepts only one electron which is opposite in its spin and hence the other electron is unpaired thus forming free radical and it wanders in search of its compatibility.
- Free radicals that are formed by the oxidation reactions are termed as Reactive Oxygen Species.

CHARACTERISTICS OF FREE RADICAL

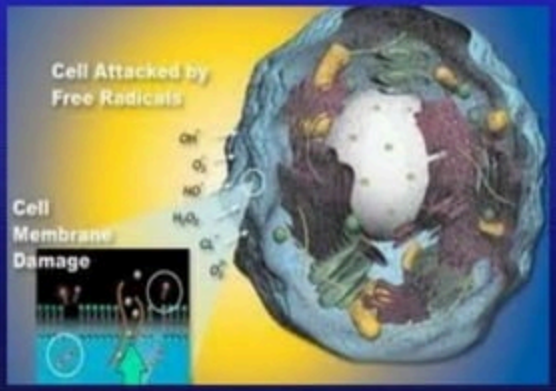
Characteristics of Free radical :

- It is highly reactive
- Short life span
- Causes Damage to various Tissues by generation of new reactive oxygen species by chain reaction.

EXAMPLES OF FREE RADICALS

EXAMPLE S OF FREE RADICALS :

- Superoxide anion
- Singlet oxygen
- Hydrogen peroxyl radical
- Hydroxyl radical
- Hydrogen peroxide
- Nitric Oxide
- PeroxyNitrite
- Lipid peroxide radical.
- Hypochlorous acid



Out of these H2O2 and Singlet oxygen are not free radicals but due to extreme reactivity they are included in Reactive oxygen Species.

Harmful effects of free radicals

- **Proteins**: Free radicals cause oxidation of sulfhydryl groups & modification of certain amino acids (e.g. methionine, cysteine, histidine, tryptophan).
- ROS may damage proteins by fragmentation, cross-linking & aggregation.
- **Lipids**: PUFA are susceptible to damage by free radicals.

- **Carbohydrates:**
- **Oxidation of monosaccharides (e .g. glucose) can produce H_2O_2 & oxoaldehyde.**
- **Glycation increases the susceptibility of proteins to the attack by free radicals. E.g. DM.**
- **Nucleic acids:**
- **Free radicals may cause DNA strand breaks, fragmentation of bases & deoxyribose.**

Clinical significance

- **Chronic Inflammation**
- **Chronic inflammatory diseases such as rheumatoid arthritis are self-perpetuated by the free radicals released by neutrophils.**
- **ROS induced tissue damage appears to be involved in pathogenesis of chronic ulcerative colitis, chronic glomerulonephritis,**

- **Cardiovascular diseases (CHD):**
- **Oxidized low density lipoproteins (LDL), formed by the action of free radicals, promote atherosclerosis & CHD.**
- **Cancer:**
- **Free radicals can damage DNA & cause mutagenicity and cytotoxicity & play a key role in carcinogenesis.**

- **Respiratory diseases:**
- **Direct exposure of lungs to 100% oxygen for a long period is known to destroy endothelium & cause lung edema.**
- **This is mediated by free radicals.**
- **ROS are also responsible for adult respiratory distress syndrome (ARDS), a disorder characterized by pulmonary edema.**

- **Diabetes:**
- **Destruction of islets of pancreas due to the accumulation of free radicals is one of the causes for the pathogenesis of IDDM.**
- **Cataract:**
- **Increased exposure to oxidative stress contributes to cataract formation.**
- **Male infertility: Free radicals reduce sperm motility & viability & male infertility.**

- **Aging process:**
- **Reactive oxygen metabolites (ROM) play a pivotal role in the degenerative brain disorders such as Parkinsonism, Alzheimer's dementia and multiple sclerosis.**
- **Cumulative effects of free radical injury cause gradual deterioration in aging process.**

ANTIOXIDANTS

- ***DEFINITION*** **: An antioxidant is a molecule capable of inhibiting the oxidation of other molecules.**
- **Oxidation reactions can form free radicals and these start chain reactions that damage cells .**
- **Antioxidants terminate these chain reactions by removing**

Antioxidants

- **Antioxidants in relation to lipid peroxidation:**
- **Preventive antioxidants that will block the initial production of free radicals e.g. catalase, glutathione peroxidase.**
- **Chain breaking antioxidants that inhibit the propagative phase of lipid peroxidation e.g. Superoxide dismutase, vitamin E, uric acid.**

- **According to location:**
- **Plasma antioxidants: e.g. β-carotene, ascorbic acid, bilirubin, uric acid, ceruloplasmin, transferrin.**
- **Cell membrane antioxidants: e.g. α-tocopherol.**
- **Intracellular antioxidants: e.g. SOD, catalase, glutathione peroxidase.**

- **According to nature & action:**
- **Enzymatic antioxidants:**
- **E.g. SOD, catalase, glutathione peroxidase & glutathione reductase.**
- **Non-enzymatic antioxidants:**
- **Nutrient: carotenoids, α-tocopherol, selenium & vitamin C.**
- **Metabolic: glutathione, ceruloplasmin, albumin, bilirubin, transferrin, ferritin, uric acid.**

TYPES OF ANTIOXIDANTS

- These are three types

I) Enzymatic and Non enzymatic antioxidants:

They are present in both Extracellular and Intracellular Environment.

Enzymatic Antioxidants -

S.No	Antioxidant	Location	Function
A	Glutathione peroxidase	Mitochondria & Cytosol	Removal of H_2O_2 and Organic Hydro peroxide
B	Catalase	Mitochondria & Cytosol	Removal of H_2O_2

Non Enzymatic Antioxidants

S. No	Antioxidant	Location	Function
A	Carotenoids	Lipid soluble antioxidants in membrane tissue	Removal of ROS
B	Bilirubin	Product of heme metabolism in blood	Extracellular antioxidants
C	Glutathione	Non-protein thiol in cell	Cellular oxidant defense
D	α- Lipoic acid	Endogenous thiol	Serves as substitute for Glutathione,

E	Viamin C	Aqueous phase of cell	Free scavenger, recycle vit- E
F	Vitamin E	Cell	Chain breaking Antioxidant
G	Uric acid	Product of Purine Metabolism	Scavenging of OH radical

Antioxidant enzymes

- **Superoxide dismutase:**
- **It converts superoxide (O_2) to hydrogen peroxide & O_2.**
- **This is the first line of defense to protect cells from the injurious effects of superoxide**
- **Catalase:**
- **H_2O_2 is metabolised by catalase.**

- **Glutathione peroxidase:**
- **It detoxifies H_2O_2 to H_2O, while reduced glutathione (G-SH) is converted to oxidized glutathione (GS-SG).**
- **The reduced glutathione can be regenerated by glutathione reductase utilizing NADPH.**
- **HMP shunt is the major source of NADPH.**

Functions of Antioxidants :

- Antioxidants such as vit- C & vit- E boost our immune system.
- Certain phytochemicals have beneficial effect on heart diseases.
- Antioxidants lower the level of LDL-cholesterol, thus preventing plaque deposition in the blood vessels.
- It is beneficial in cancer prevention.
- Antioxidants neutralize substances that can damage the genetic material by oxidation.

Collagen:-

- Major Structural protein found in connective tissue.
- It is abt 25-30% of the total weight of protein in the body.
- It serves to hold together the cells in yhe tissues.
- It is the major fibrous element of tissues like bone, teeth, tendons, cartilage & blood vessels.

Functions of Collagen:-

I. To give support to organs.

II. To provide alignment of cells, so that cell anchoring is possible.This in turn, helps in proliferation & differentation of cells.

III. In blood vessels, if collagen is exposed, platelets adhere & thrombus formation is initiated.

Elastin:-

- Protein found in connective tissue & major component of elastic fibers.
- Elastic fibers can stretch & then resume their original length.
- They have high tensile strength.
- They are found in the ligements as well as in the walls of the blood vessels, especially large blood vessels like aorta.

- It is rich in glycine & proline residues just like collagen.
- It is highly insoluble & extremely stable.
- It exhibits a variety of random coil conformations that permit the protein to stretch & recoil during physiological processes.

Keratin:-

- Proteins present in hair, skin, nails, horns etc.
- Fibers present are called alpha keratins & matrix as keratohyalin.
- They mainly have the alpha helical structure.
- Keratohyalin matrix has cysteine rich polypeptide chains which are held together by disulfide bonds.

Myosins:-

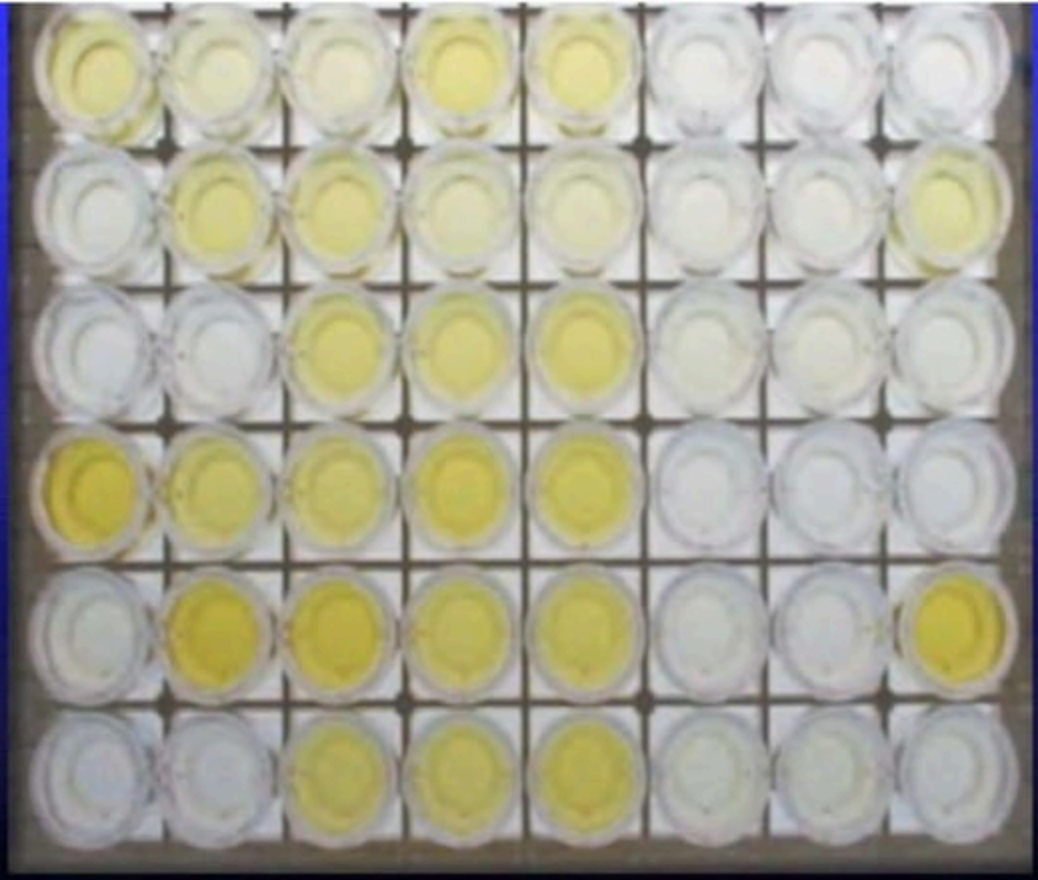
- These are actually a family of proteins with about 15 members.
- Myosin i.e predominantly present in muscle is myosin-II.
- It constitute about 45% of muuscle protein & found in thick filaments.
- Composed of 6 polypeptide chains i.e hexamer.
- Contains one pair of H-chain & two pair of L-chains.

Enzyme Linked Immuno-Sorbent Assay(ELISA):-

- It is based on the immunochemical principles of Ag-Ab reaction.
- ELISA techniques are widely used not only for hormone measurements but also for detecting any other growth factors, tumor markers, bacterial or viral antigens, antibodies against microbes & any other antigens or antibodies in biological fluids.
- This test is commonly employed to detect Ags or Abs present in very small quantities in tissues or blood.

Applications:-

- ELISA is widely used for the determination of small quantities of proteins(hormones, Ags,Abs) & other biological substances.
- Most commonly used pregnancy test for the detection of human chorionic gonadotropin (hCG) in urine is based on ELISA.
- By this test, Pregnancy can be detected within few days after conception.
- ELISA is also been used used for the diagnosis of AIDS.



ELISA