LECTURE: 18

Title **ANTIGENS AND IMMUNOGENS**

LEARNING OBJECTIVES:

The student should be able to:

- **Define the antigenicity & immunogenecity.**
- **Define the immunogen, antigen, hapten, epitope (antigenic determinant), and adjuvant.**
- **Identify the factors that affect the antigenicity (characters of the substances which make it antigenic), such as; foreignness, chemical complexity, molecular size, dose of the antigen (high & low dose), and adjuvants (Freund's adjuvants & Aluminum hydroxide).**
- **Define the relation between the epitope & the antibody specificity. Also the valence of an antigen.**
- **Explain the cross reactivity and define the antibody affinity and the avidity.**
- **Describe the types of antigens for example; bacterial, viral, and isoantigens such as; blood grouping, MHC antigens.**
- **Explain the organization & structure of MHC Genes & Products (MHC class II, III molecules, & I).**
- **Identify the role of the MHC in controlling the T-Cell response.**
- **Explain the activation of $CD8^+$ cytotoxic T cells, & what determines that whether an antigen elicits a class II or class I restricted response.**

LECTURE REFRENCE:

1. TEXTBOOK: NATIONAL MEDICAL SERIES FOR INDEPENDENT STUDY. RICHARD M. HYDE. 3RD EDITION. Chapter 2. pg 23-30.

2. TEXTBOOK: JONATHAN M. AUSTYN AND KATHRYN J. WOOD Principles of Cellular and Molecular Immunology . Chapter 2. pg 63-84.

3. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. pg 58, 216, 489.

4. TEXTBOOK: NATIONAL MEDICAL SERIES FOR INDEPENDENT STUDY. RICHARD M. HYDE. Chapter 2. pg 23-30.

ANTIGENS AND IMMUNOGENS

I. DEFINITIONS

A. Antigens. The immune response is characterized by the production of either proteins, called antibodies, or specifically reactive lymphocytes, called T cells, when an animal encounters a foreign macromolecule or cell.

1. The foreign substances that induce an immune response possess two properties;

- **a. Immunogenicity** is the inherent ability of a substance (**immunogen**) to induce a specific immune response, resulting in the formation of antibodies or immune lymphocytes.
- **b. Antigenicity**, or specific reactivity, is the property of a substance (**antigen**) that causes it to react specifically with the antibody o r lymphocyte that it caused to be produced.

2. The property of antigenicity is extremely important. It is probably the most specific reaction known in biology.
3. Immunogenic substances are always antigenic, whereas antigens are not necessarily immunogenic (e.g., Autologous serum proteins).

B. Haptens

1. Haptens are partial antigens. That is:

- **a.** Haptens are **antigenic:** they can react with immune lymphocytes or antibodies.
- **b.** However, haptens are **not immunogenic:** they can not by themselves cause the production of immune lymphocytes or antibodies.

2. Haptens are usually molecules which are too small to be immunogenic.

- **a.** Examples of haptens are antibiotics, analgesics, and other low-molecular weight compounds.
- **b. Penicillin,** for example, a clinically important hapten, has a molecular weight of 320 Daltons (0.3 kDa).

3. if a hapten of coupled to a larger **carrier molecule**, however, it becomes endowed with immunogenicity.

- **a.** The carrier molecules may be albumins, globulins, or synthetic polypeptides.
- **b.** Drugs often couple with carriers in the body and thereby acquire immunogenecity. A classic example in clinical medicine is the **allergic response** of some persons to **penicillin.**

(1) The penicilloic acid moiety of penicillin, acting as a hapten, can couple with body proteins and elicit an immune response.
(2) The immune response can be harmful, even life-threatening, thus excluding this antibiotic from use in certain individuals.

C. Epitopes

1. Definition

a. Epitopes (also called **determinant groups or antigenic determinants**) are the sites either on or within the antigen with which antibodies react.
b. Epitopes and haptens are similar, but while a hapten is artificially added to a molecule, an epitope is an integral part of the native molecule.

2. Physical properties

a. Epitopes are very small (e.g., just four or five amino acid or monosaccharide residues).
b. The epitopes on an antigen can be **linear** (i.e., continuous within the amino acid sequence of hr molecule) or **conformational** (i.e., containing amino acids that end up in the same area on the surface of the protein but are not adjacent in the peptide chain) (**Figure-1).**
c. Some antibody-binding sites (i.e., epitopes) are no the antigen's **surface** (topographic); others are **internal**.

(1) Internal epitopes are only expressed after the antigen has been "processed" by a phagocytic cell.
(2) Epitopes are immunoreactive only if their amino acids are spatially accessible due to tertiary protein structure.

3. Epitopes and antibody specificity

a. Epitopes determine the specificity of the antigen molecule and are what induce the antibody response.
b. Antibodies are specific for epitopes.
c. Antigens are multivalent; that is, an antigen molecule carries a number of different epitopes, sometimes hundreds of them, some specifying antibody "A", others antibody "B", and so forth, the **valence** of an antigen is equal to the total number of epitopes the antigen possesses.

4. Molecular changes. Antigen molecules can be artificially manipulated by altering, adding or taking away epitopes. With each change, antigenicity is altered.

a. New antigens are produced by altering these epitopes. This can be done by conjugation haptens to the molecule.
b. Denaturation or hydrolysis of the protein will almost always destroy conformational epitopes.

D. Adjuvants. Nonspecific stimulation of the immune response can occur via adjuvants (e.g., complete Freund's adjuvant, a mixture of killed mycobacteria and oil).

1. These substances enhance the immunogenicity of molecules without altering their chemical composition.
2. The **mechanisms** by which adjuvants exert their biological effects are multiple.

a. Adjuvants may increase the efficiency of macrophage processing of antigens.
b. Adjuvants can act as depots and prolong the period of exposure to the immunogen.
c. Adjuvants may amplify the proliferation of immunologically committed lymphocytes by enhancing the release or the action of lymphokines.

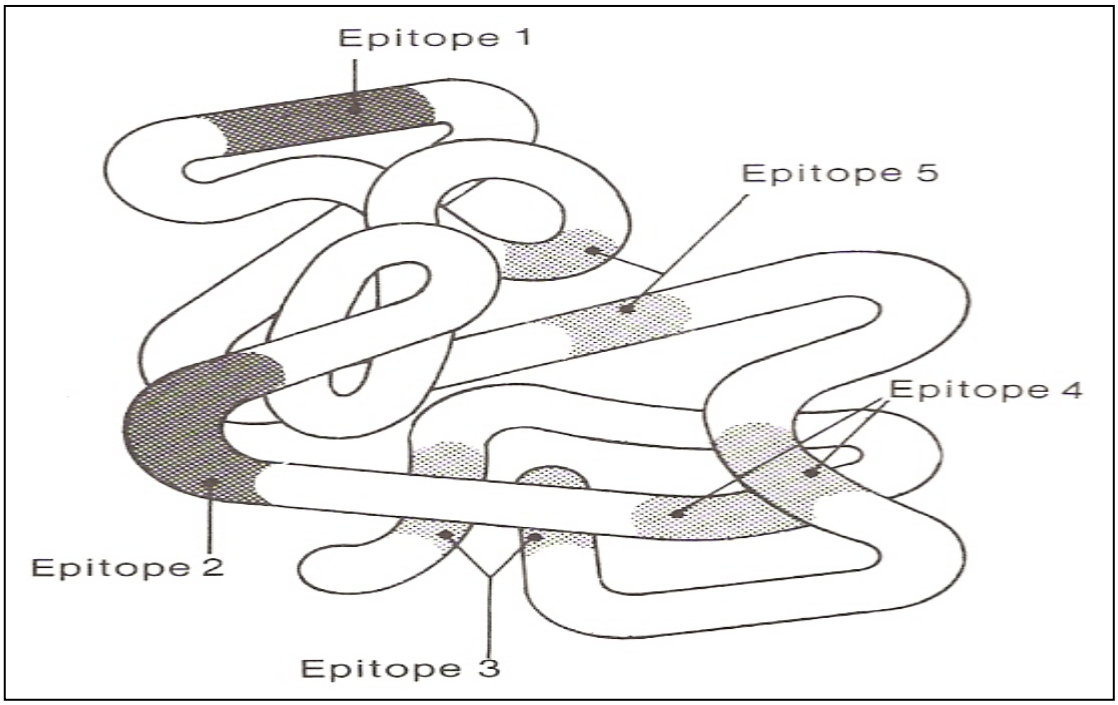


Figure-1 Model of epitopes on lysozyme**,** the shaded areas is the specific epitopes. They are composed of chain segments that are either linear (epitopes 1 and 2) or conformational (epitopes 3-5).

II. PROPERTIES OF IMMUNOGENICITY

A. General considerations. The degree of immunogenicity of a molecule is influenced by several factors. The relationship can be expressed algebraically by the following formula:

Immunogenicity = (foreignness) (chemical complexity) (molecular size)

B. Foreignness

1. An antigen must be foreign or alien to the host with which it makes contact.
2. The greater the phylogenetic difference, the more foreign something becomes. The use of transplant terminology helps to clarify this concept.

a. Autologous antigens are found within the same individual; that is, they are not foreign to that individual. For example, a skin graft from an individual's thigh to his chest is an **autograft,** and is not foreign.
b. Syngeneic antigens are found in genetically identical individuals (e.g., individuals from an inbred strain of mice of identical twins). A graft between members of an inbred strain is a **syngeneic graft** or an **isograft,** and is not foreign.
c. Allogeneic antigens (alloantigens) are found in genetically dissimilar members of the same species. For example, a kidney transplant from mother to daughter is called an **allograft** or a **homograft,** and it is foreign.

(1) Some alloantigens, called isoantigens, are found in some members of a species and not in others.
(2) The A and B blood group antigens are examples of isoantigens.

D. Xenogeneic (heterogenic) antigens are found in different species. For example, a transplant of monkey kidney to humans is called a heterograft or xenografts, and it is foreign. The term heterologous is also sometimes used as a synonym for xenogeneic.

3. Special types of antigens

a. Heterogenetic (heterophile or heterophile) are a type of xenogeneic antigen.

(1) Heterophile antigens occur in different species and have several particular characteristics:

(a) They are **cross-reacting** (i.e., they combine with antibody induced by a different but closely related antigen).
(b) They occur in phylogeneetically unrelated species.

(2) The principle of the **cross-reacting heterophile antibody response** has a practical application as it provides the basis for several **clinical tests.**

(a) The spirochete that causes **syphilis** has a heterophile antigen similar to a hapten called **cardiolipin** that is found in beef heart muscle. This heterophile antigen forms the basis of a diagnostic test for syphilis.
(b) The heterophile antibody response can also be used in the diagnosis of **infectious mononucleosis** caused by the **Epstein-Barr virus,** as most of the patient have present in their serum an antibody that reacts with sheep red blood cells (SRBCs).
(c) Serum from patients with **rickettsial** infections agglutinates certain strains of proteus vulgaris (OX19, OX 2, OXK). This heterophile immune response is termed the **Weil-Felix reaction.**

b. Sequestered antigens

(1) Antibodies are not ordinarily made to Autologous brain or cornea protein because these substances do not come in contact with antibody-producing cells since they are inaccessible to antibody-forming lymphoid tissues (i.e., they are "sequestered"). For example, the central nervous system and cornea are devoid of lymphatics, and the cornea is also nonvascularized; both lymphatics and blood vessels are required for an immune response.
(2) If sequestered antigens are released (i.e., if sequestered tissue is exposed to the antibody-producing lymphoreticular system), then an immune response may result.
(3) Experimental allergic encephalomyelitis can be produced in animals by infection of homologous or heterologous brain tissue in Freund's adjuvant. The antigen is a basic protein in myelin.

4. Tissue-specific (organ-specific) antigens

a. Various organs have in their makeup certain antigens unique to those organs.

(1) Thyroid has an organ-specific antigen, **thyroglobulin.**

(a) Any thyroid from any species contains this unique thyroid antigen.
(b) An immune response to this antigen is seen in patients with Hashimoto's thyroditis.

(2) Basic proteins exist in brain tissue regardless of species, and it does not exist in any other organ. Basic protein has been implicated in experimental allergic encephalomyelitis.

(a) A sequence of amino acids in basic protein resembles a sequence in hepatitis B viral polymerase; the viral peptide elicits an immune response that causes inflammation of the central nervous system.
(b) This type of immunologic damage due to cross-reacting epitopes is referred to as **antigenic mimicry.**

b. Certain epitopes are found on selected cells. For example, mature thymus-derived lymphocytes (T cells) bear the CD3 marker in the in membranes.

C. Chemical complexity

1. With the exception of pure lipids, most macromolecular organic chemical grouping can be immunogens.

a. Proteins. The majority of immunogens are proteins.

(1) Proteins are the strongest antigens, because they have the largest array of potential building blocks (amino acids).

(a) This diversity imparts epitopes of differing specificities to the molecule.
(b) The total immune response will be the sum of all the individual antibodies that are produced.
(2) Immunogenicity can be enhanced by adding haptens (i.e., epitopes) to the molecule.
(3) Lipoproteins are a complex type of protein immunogen that exist as part of many cell membranes.

b. Polysaccharides

(1) Most polysaccharides are haptens or incomplete immunogens.

(a) They do not possess sufficient chemical diversity for full immunogenicity.
(b) In addition, they are usually rapidly degraded when they enter a host; thus they are not in contact with the immune apparatus long enough to induce a response
(2) How ever, polysaccharides can be immunogens, occurring in two forms:

(a) Pure polysaccharide substances (e.g., the capsular polysaccharides that are responsible for the protective immune response to the pneumococcus).
(b) Lipopolysaccharides (e.g., the endotoxins that occur within the cell membranes of gram-negative bacteria).

c. Glycoproteins

(1) The immunogenicity of glycoproteins is best illustrated by the A and B blood group antigens and the Rh antigens.
(2) The A and B substances are strong immunogens, and the immune response they induce is to the carbohydrate epitope of the molecule.

d. Polypeptides

(1) Polypeptide immunogens include hormones (e.g., insulin, growth hormone) and synthetic compounds (e.g., polylysine).
(2) Polypeptides are usually weakly immunogenic.

e. Nucleic acids and nucleoproteins

(1) Nucleic acids are considered to be nonimmunogenic; however, when single-stranded, they can act as immunogens.
(2) Nucleoproteins are stronger immunogens because the nucleic acid is coupled to protein. In patients with systemic lupus erythematosus (SLE), antibodies to autologous nucleoproteins are produced.

f. Lipids. These are also nonimmunogenic, although a few (e.g., cardiolipin) can function as haptens.

2. Bacterial and mammalian cells are strong immunogens, and present a vast array of different epitopes to the host.

D. Molecular size. Usually, the larger the molecule, the better the immunogen (Table-1), although there are exceptions.

Molecule	Size	Relative Immunogenicity
Hemocyanin	**1000**	+ + + +
Gamma globulin	**160**	+ + +
Diphtheria toxin	**58**	+ +
Insulin	**6**	+
Vasopressin	**1**	+ / -
Aspirin	**0.18**	-

Table-1 Relationship of Molecular Size to Immunogenicity

1. As a general rule, molecules below 5 kDa will not be immunogenic. Reasonable immune responses will be induced by molecules like serum albumin (40 kDa).
2. Size is important for several reasons:

a. The number of epitopes increases proportionately with the size of the protein.
b. Larger size means that the molecule will be phagocytized.

(1) Antibodies to most antigens are formed much more efficiently if the antigen is first "processed" by a macrophage; this involves phagocytosis of the antigen.
(2) Antigens that are difficult or impossible to phagocytize are not immunogenic at times.

III USE OF IMMUNOGENS IN VACCINATION

A. Purpose. The result of active immunization is the production of protective antibodies or specifically sensitized lymphocytes.
B. Examples of bacterial immunogens

1. All gram-negative flagellated bacteria (e.g., Salmonella typhi) contain two types of antigens to which the host organism makes different antibodies.
a. The **H antigens,** referring to the **flagella**
b. The **O antigens,** referring to the **body** of the organism

2. Bacterial virulence factors as immunogens

a. Any bacterial cell is potentially a good immunogen, since hundreds of different antibodies are formed in response to the bacterial cell.
b. However, the only protective antibodies are those directed against the cell's **virulence factors,** the factors of the bacterial cell that give it the ability to cause disease.

(1) The polysaccharides of ht **pneumococcal capsule** must be neutralized if the virulence of the pneumococcus is to be overcome, since the capsule is antiphagocytic.
(2) Protection from **diphtheria and tetanus toxins** can be obtained if antibodies to these toxins are produced, since the toxins are what cause the diseases.
(3) **Lipopolysaccharide endotoxins** are a major virulence factor for gram-negative bacteria, and antibody against this endotoxin is needed for protection.

IV. BASIS OF ANTIGEN SPECIFICITY

The exquisite sensitivity of the immune response has its basis in molecular differences in antigenic structure. Several examples follow.

A. Differences in position within the molecule

1. This aspect of the specificity of the immune response has been studied in relation to haptens, as illustrated in F**igures 2, and 3**.

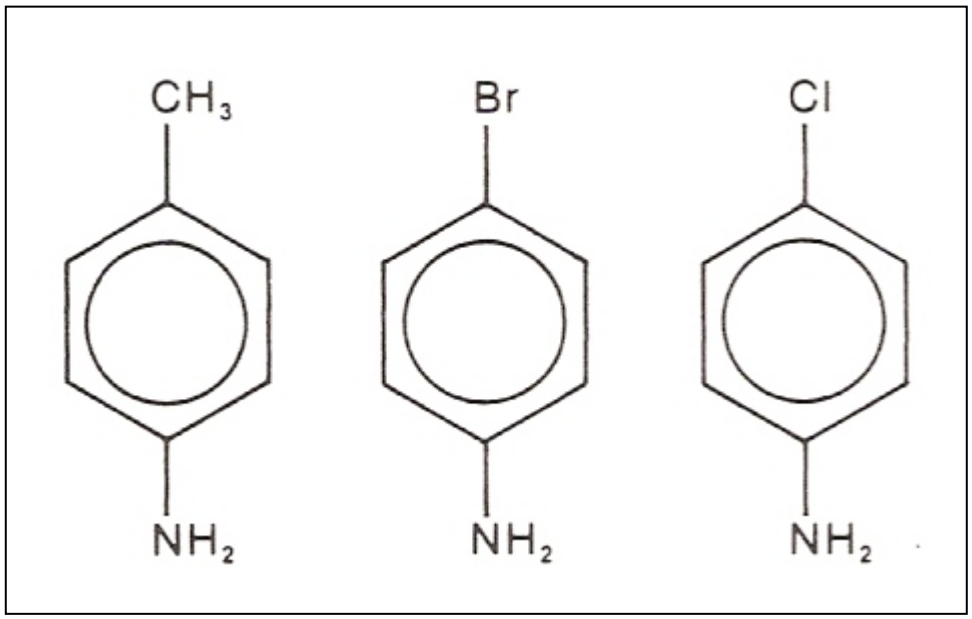


Figure-2 Examples of cross-reactive substituted benzene haptens. Antibody raised against any one of the haptens shown will cross-react with the other two haptens. However, a hapten with a carboxyl group in the paraposition would not react with antibody against the illustrated haptens.

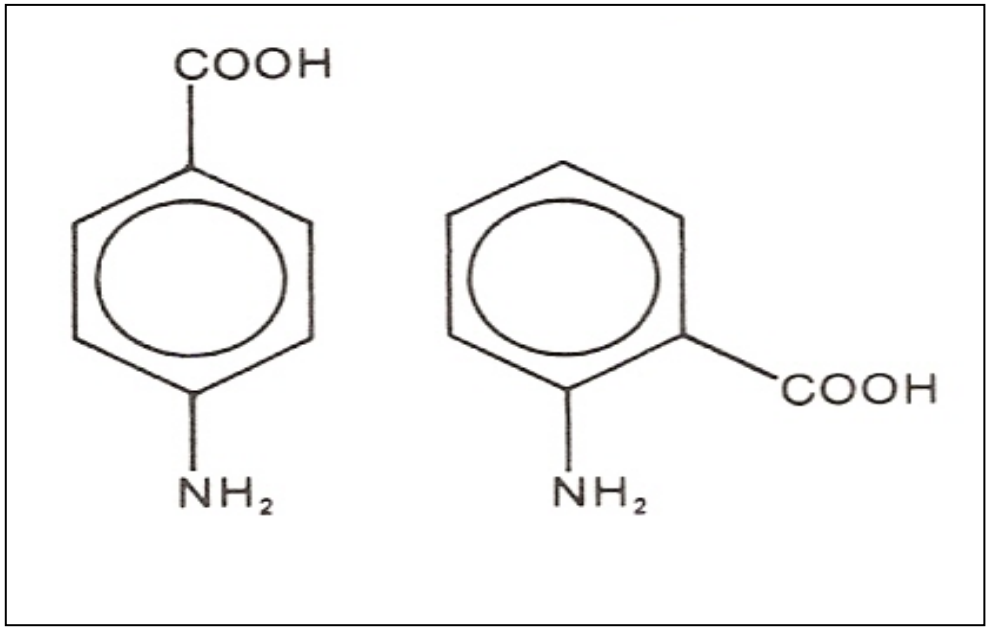


Figure-3 Examples of non-cross-reactive substituted benzene haptens. The change from para to ortho substitution produced a new specificity.

a. Haptens can be, for example, side chains of benzene rings, or substituted benzene rings. Attaching these to protein molecules renders them immunogenic; the immune response can then be mounted against both the carrier protein and the hapten.
b. All of the substituted groups shown in **figure-2** are in the para position; however, the para-substituted group is different in each case.

(1) The immune response generated by any one of the conjugated hapten groups in **Figure-2** is completely cross-reactive against the others.
(2) Apparently, the host can recognize the para position but not the subsistent groups (as long as these remain chemically similar).

c. On the other hand, the host can distinguish between ortho and para positions (**Figure-3)** and the antibodies formed are not cross-reactive.

3. The extreme specificity of the immune response is used in the identification of several biologically important molecules in human serum **Table-2.**

B. Differences in glycosidic linkages.

A clinically relevant example is provided by the capsular antigens of the pneumococcus, one of the major causes of lobar pneumonia.

1. The pneumococcus has more than 80 different immunologic types of capsular polysaccharide, and an antibody to one type does not react with an antigen of another type.
2. The capsular polysaccharides are structurally different, which accounts for their antigenic differences.

a. The type II pneumococcal capsule has as its disaccharide building block glucose in 1→ 4, 1→ 6 linkages. The antibody directed against pneumococcus type II is directed against this glucose polymer.
b. This specificity is known because the antibody will react with glucose 1→ 4, 1→ 6 linkages regardless of where they are found (e.g., it will react with those found in glycogen).
c. The reaction is so specific that it can be used to determine if the 1→ 4, 1→ 6 linkages exists in unknown polysaccharides.

C. Differences in amino acid residues

Another clinically relevant example of the specificity of the immune response is its ability to distinguish very small changes in compounds, such as the various insulin molecules.

1. Diabetics, who use, for example, bovine insulin for maintenance, sometimes become **insulin tolerant**, and the insulin is no longer effective.
2. **Insulin,** a weak antigen, has a molecular weight of approximately 6 kDa and is composed of two chains, an chain and β chain. Bovine and human insulin vary by three amino acid residues sitting side by side on the α chain.
3. In insulin tolerance, antibodies are formed to these three amino acids and inactivate the molecule.
4. The treatment for this antibody inactivation of the insulin is to switch from beef to pork insulin, since the three amino acids would be different.

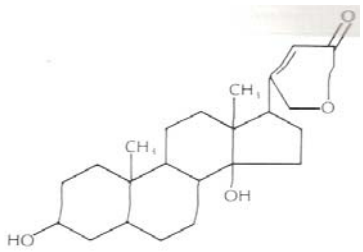
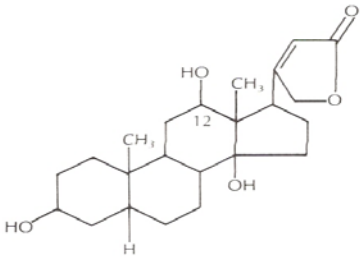
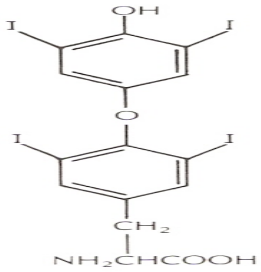
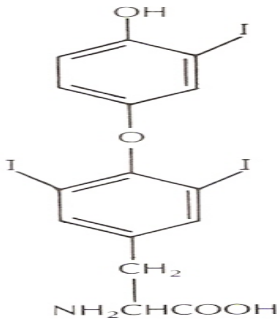
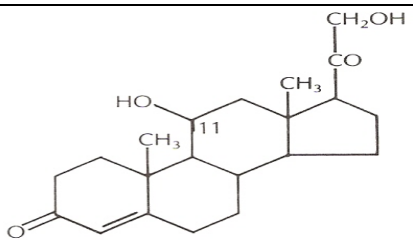
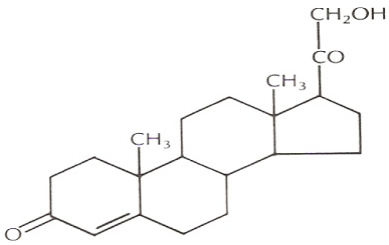
Molecular Pair	Structure	Identifying Difference
Digitoxin		
Digoxin		**Presence of hydroxyl at carbon 12**
Thyroxine		**Presence of iodine at 5" position**
Triiodothyronine		
Corticosterone		**Presence of hydroxyl at carbon 11**
Deoxycorticosterone		

Table-2 Detection of Hormones in Human Serum Based on Antibody Recognition of Specific Epitopes

V. FORCES OF ANTIGEN-ANTIBODY ATTRACTION

The antigen-antibody complex is not bound firmly together. It may even dissociate spontaneously. However, the equilibrium is far to the right, with a very large association constant ($K_{\circ}$) or 10^6 to 10^8.

A. Types of attracting forces. Various forces act to hold the antigen-antibody complex together.

1. **Van der walls forces** act because of spatial fit (**Figrue-4**). These forces hold antigen to antibody when the two molecules have corresponding shapes (**Figure-4 A)** but are less effective when the correspondence is poorer (**Figure-4 B**).
2. **Coulombic forces** are patterns of complementary electrical charge on the molecule. The electrostatic interactions tend to hold the molecules together (**Figure-5**).
3. Antigen-antibody complexes are probably held together by a mixture of Van der Waals and Coulombic forces.

B. Affinity and avidity

1. The strength of attraction between a single epitope and its matching paratope (the antigen binding site on the antibody molecule) is referred to as an **affinity** of the reaction between the two reactants. Antigen-antibody complexes of low affinity dissociate readily.
2. A related term, **avidity,** refers to the strength of the interaction between multivalent antigens and the population of antibodies which they have induced. Avidity is influenced by the affinity of individual antibodies for their epitopes, the valence of the antigen, and the valence of the antibodies.

C. Accessibility of the epitope. Studies using synthetic polypeptides have shown that only those amino acids that are spatially accessible due to **tertiary protein structure** are immunoreactive.

1. Proteins can exist as globular or fibrous proteins or mixtures of the two; the nature of the structure is important.
2. The ability of antibody to bind to antigenic sites can be affected by **altering the tertiary structure.**

a. The antigenic sites would then no longer be spatially arranged so that antibody-antigen coupling could occur.

b. Insulin molecules provide an illustration.

(1) Insulin is composed of A and B chains. Antibody to either one of these chains can be produced by splitting the chains, purifying them, and injecting them into a foreign host. The host will produce antibody to the particular chain injected.

(2) If these antibodies are infected back into the animal species that supplied the original insulin, the antibodies will not react with intact insulin molecules.

(3) The explanation is that the tertiary structure of insulin must be such that the determinant groups on the molecule are accessible.

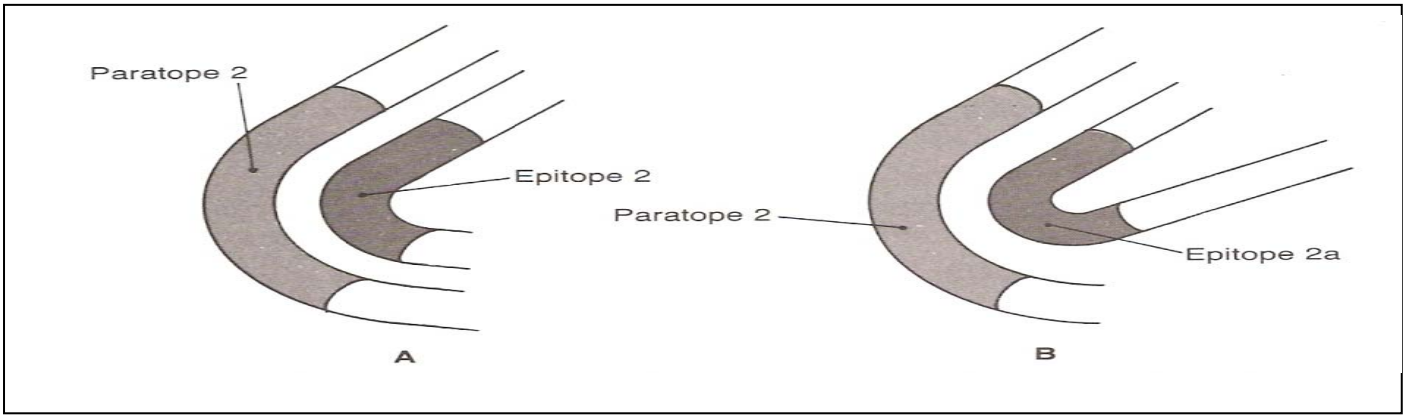


Figure-4 Van der Waals forces: influence of spatial fit on antigen-antibody reactions. A significant modification in the shape of epitope 2 of lysozyme precludes its interaction with the matching antigen-binding site (paratope) of the original antibody molecule.

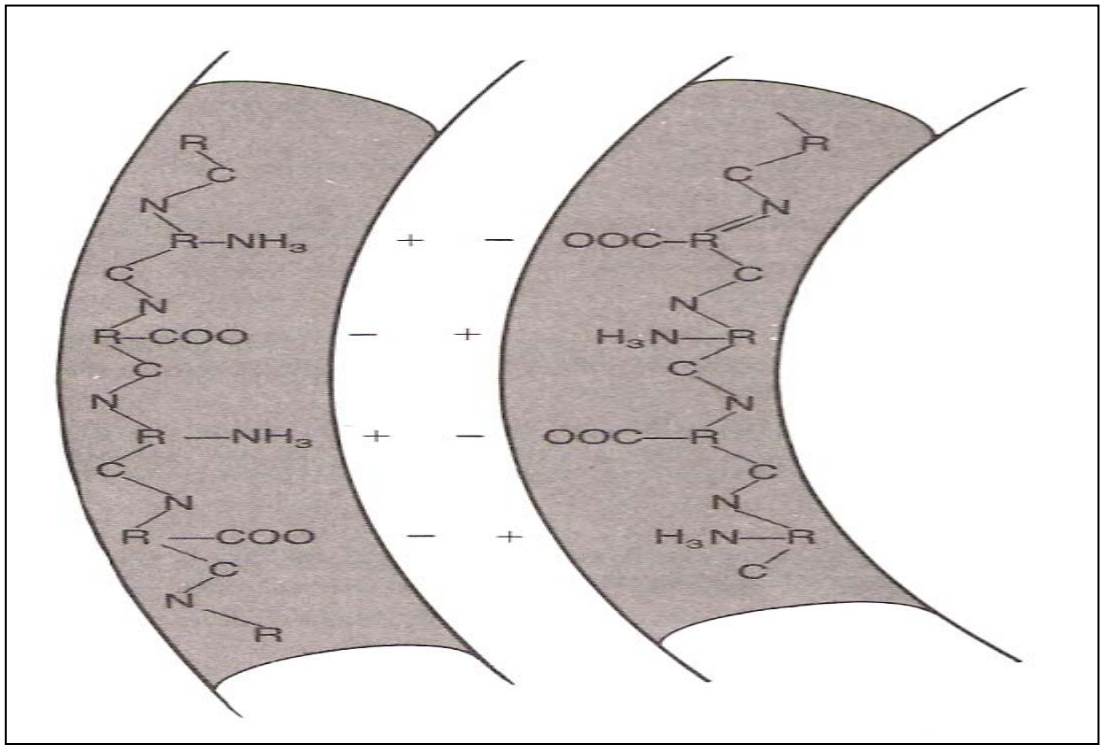


Figure-5 Coulombic forces, the pattern of electrical charges influences the interaction between antigen and antibody. As the pattern becomes more complementary, the force of attraction becomes proportionately stronger, and the affinity of the two reactants increases.

VI THYMUS DEPENDENCE AND IMMUNOGENICITY

A. Most humoral immune responses are **thymus-dependent,** in that there is a need for a thymus derived "helper" cell in the induction of antibody synthesis.
B. A very small number of responses are **thymus-independent.**

1. The immunogens in these responses are characterized by having monotonously repeating epitopes. Most are polysaccharides or polymerized proteins such as flagellin.
2. They induce only IgM antibodies.
3. There is no anamnestic response on secondary challenge with the same immunogen.