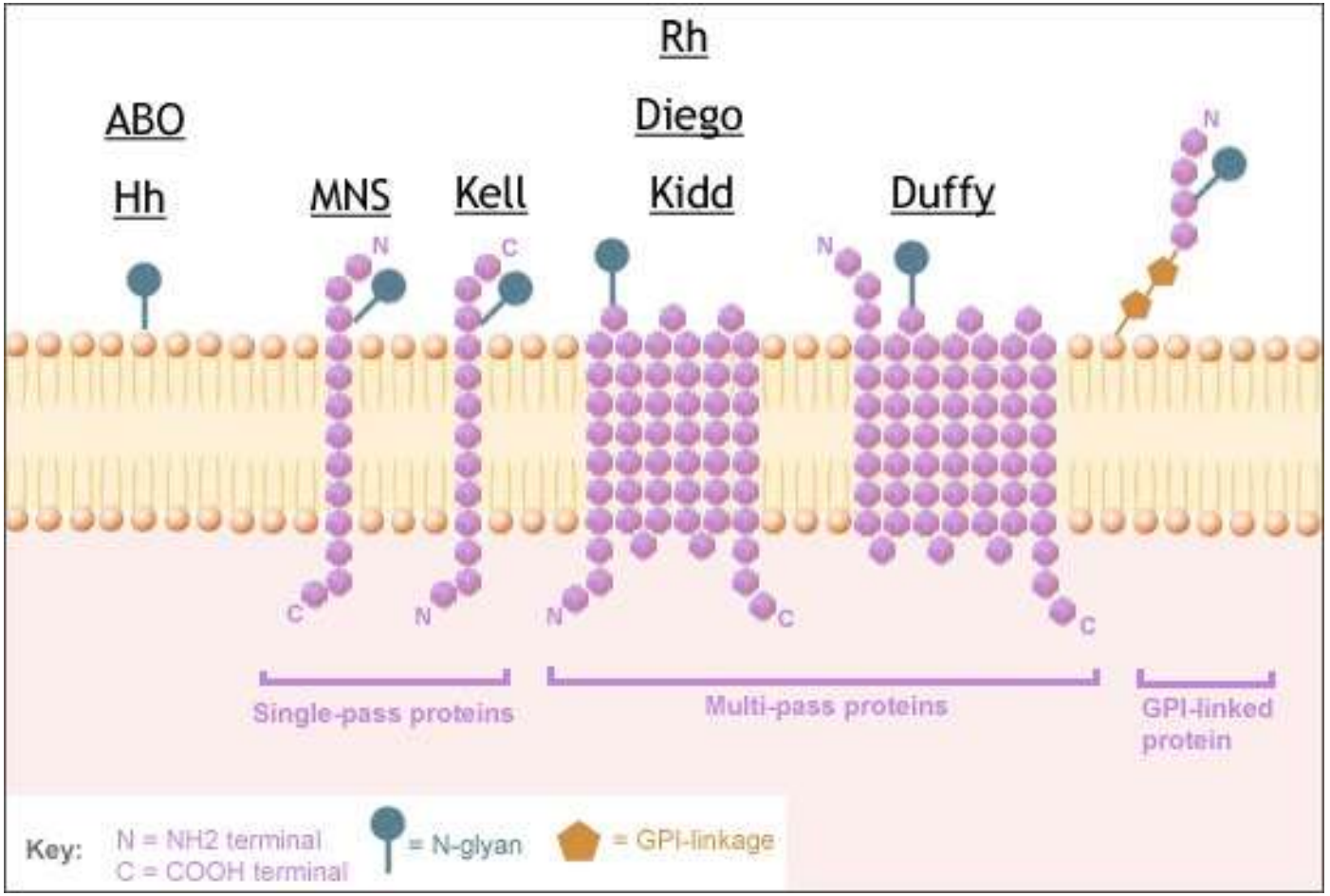
ANTIGENS - II

RAKESH SHARDA

Blood Group antigens

Blood group antigens are either sugars or proteins attached on the red blood cell membrane



ABO Blood Group System

ABO Blood Group System

- **The ABO Blood Group System was the first to be identified and is the most significant for transfusion practice**

- **It is the ONLY system in which the reciprocal antibodies are consistently and predictably present in the sera of people who have had no previous exposure to human red cells**

- **Karl Landsteiner discovered the ABO Blood Group System in 1901**

Why is it important?

- **ABO compatibility between donor cell and patient serum is the essential foundation of pre-transfusion testing**

- **It is the only system with "expected antibodies"**

- **Whether they are IgG or IgM, ABO antibodies can activate complement readily**
  - **This means that incompatibilities can cause life threatening situations (transfusion reactions)**

ABO and H Antigen - Genetics

- **Genes at three separate loci control the occurrence and location of ABO antigens.**
- **The presence or absence of the A and/or B, and H antigens is controlled by the ABO and H genes, respectively**
- **The H antigen is found on the RBC when the genotype is Hh or HH, but NOT the hh.**
- **The A antigen is found on the RBC with the Hh/ HH+A/A, A/O, or A/B genotypes**
- **The B antigen is found on the RBC with the Hh/HH+B/B, B/O, or A/B genotypes**

Bombay Blood Group

- **The hh genotype causes NO H antigen to be produced**
- **Hence, RBCs neither possess H nor A or B antigen**
- **The patient types as O group, but with anti-A,B, and H antibodies in sera – Bombay Blood Group**
- **Bombay blood group RBCs are NOT agglutinated with anti-A, anti-B, or anti-H antibodies (no antigens present)**
- **Bombay blood group serum has strong anti-A, anti-B and anti-H antibodies, agglutinating ALL (A/B/AB/O) ABO blood groups**
- **Hence, transfuse the patient with blood that contains NO H antigen, ie only Bombay Blood Group**

ABO System

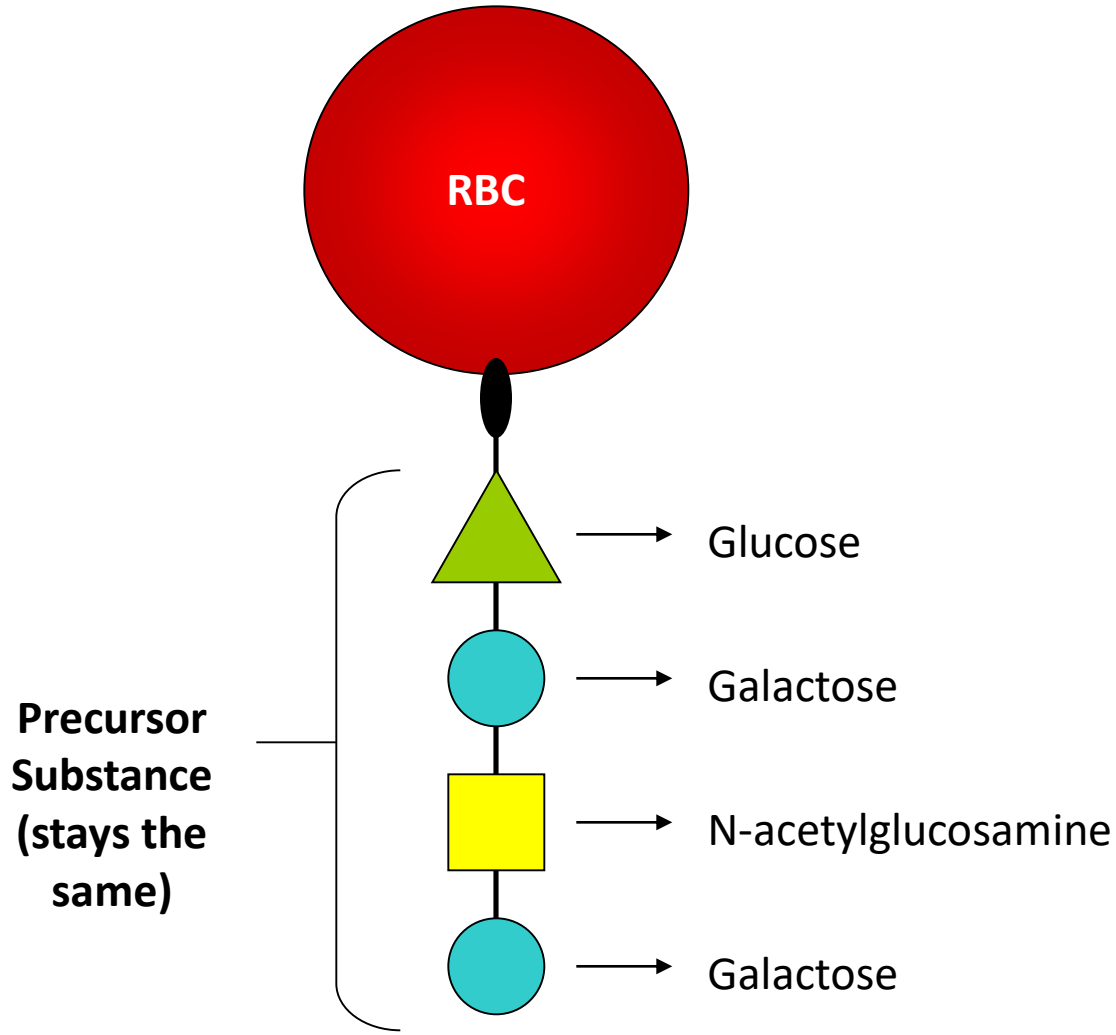
H Genotype	ABO Genotype	Antigen on RBC	Blood Group	Antibodies in serum
HH	AA/AO	H +A	A	Anti B
Hh	AA/AO	H + A	A	Anti B
HH	BB/BO	H + B	B	Anti A
Hh	BB/BO	H + B	B	Anti A
HH	AB	H + A + B	AB	None
Hh	AB	H + A + B	AB	None
HH	NIL	H only	O	Anti A + B
Hh	NIL	H only	O	Anti A + B
hh	NIL	NIL	O (Bombay Type)	Anti A + B + H

H Antigen

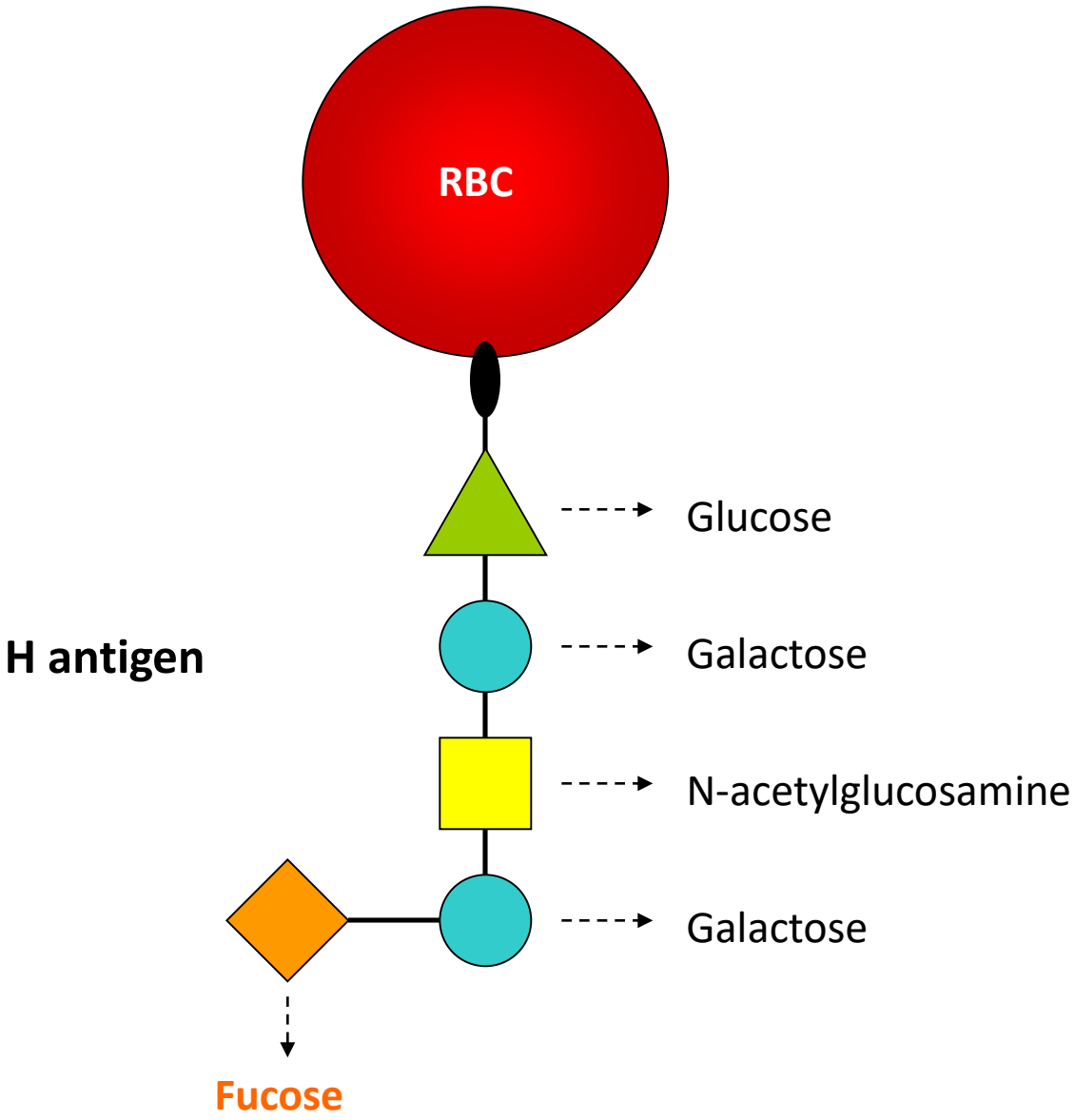
- **The *H* gene codes for an enzyme that adds the sugar fucose to the terminal sugar of a precursor substance (PS).**

- **The precursor substance (proteins and lipids) is formed on an oligosaccharide chain (the basic structure)**

RBC Precursor Structure



Formation of the H antigen

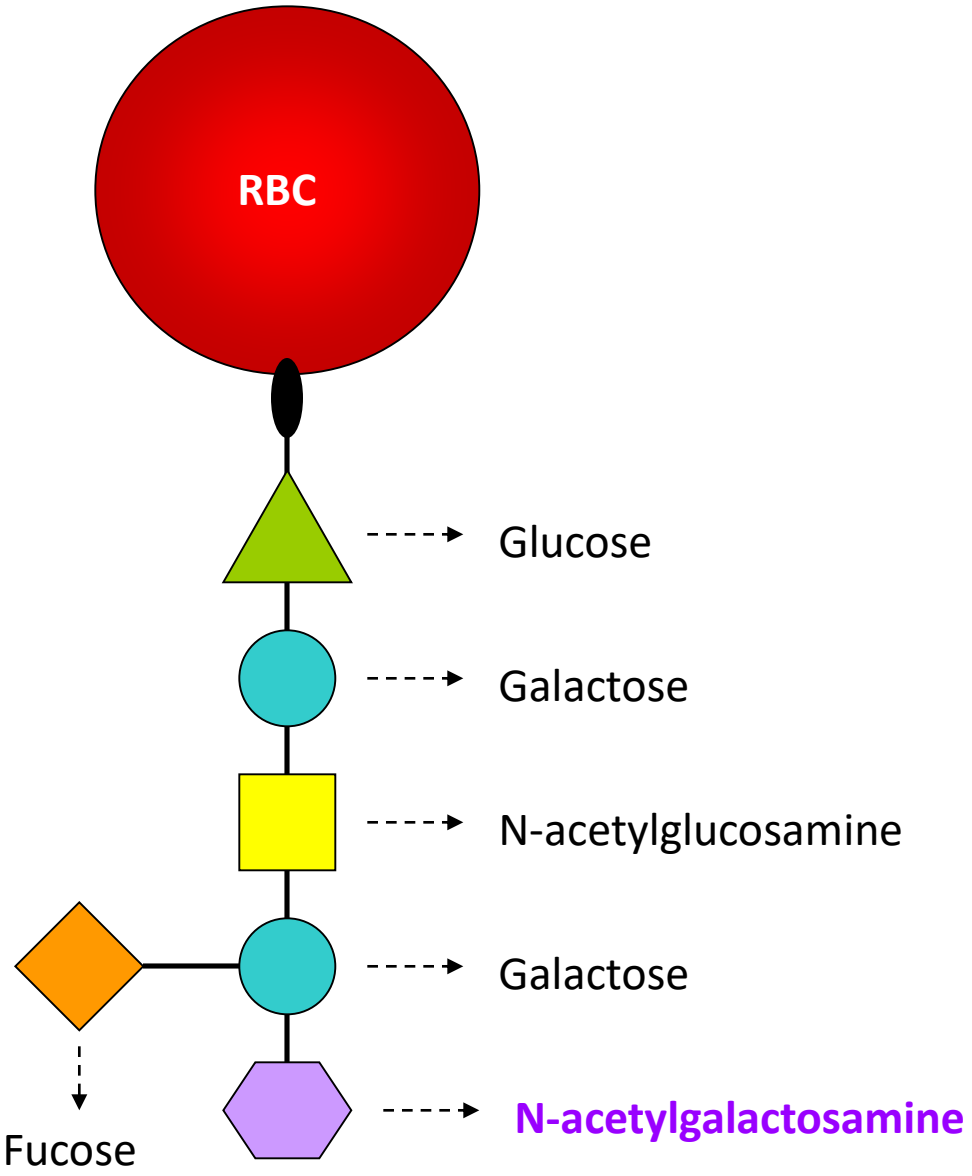


A and B Antigen

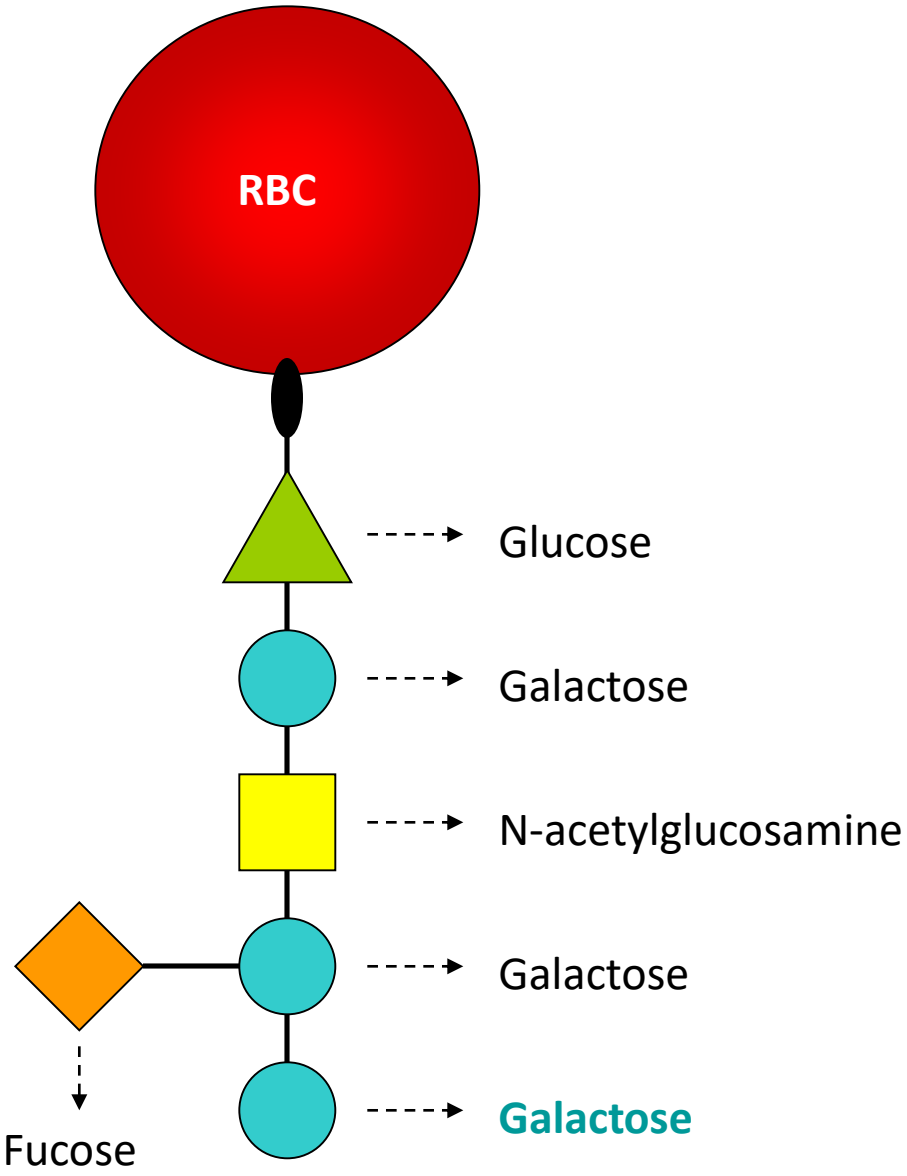
- **The "*A*" gene codes for an enzyme N-acetyl galactosaminyl transferase that adds N-acetyl galactosamine to the terminal sugar of the H antigen**

- **The "*B*" gene codes for an enzyme D-galactosyl transferase that add D-galactose to the terminal sugar of the H antigen**

Formation of the A antigen



Formation of the B antigen



ABO Antigens in Secretions

- **Secretions include body fluids like plasma, saliva, synovial fluid, etc**
- **Blood Group Substances are soluble antigens (A, B, and H) that can be found in the secretions.**
- **This is controlled by the *H* and *Se* genes**

ABO Antibodies

- **The ABO Blood Group System does NOT require the presence of a foreign red blood cell for the production of ABO antibodies**

- **ABO antibodies are "non-red blood cell stimulated", probably induced due to environmental exposure and/or gut microflora.**

- **Referred to as "expected antibodies"**

- **Titer of ABO Abs is often reduced in elderly and in patients with hypogammaglobulinemia**

ABO antibodies

- **IgM is the predominant antibody in Group A and Group B individuals**
  - **Anti-A**
  - **Anti-B**

- **IgG (with some IgM) is the predominant antibody in Group O individuals**
  - **Anti-A,B (with some anti-A and anti-B)**
  - **Anti-A,B is <u>one</u> antibody, it is not a mixture of anti-A and anti-B antibodies**

Rh Blood Group System

Rh (D) Antigen

- **Of next importance is the Rh type.**
  - **Term "Rh" is a misnomer.**
  - **There are two genes - RHD and RHCE- that are closely linked.**
  - **Numerous genetic rearrangements between them has produced hybrid Rh genes that encode a myriad of distinct Rh antigens.**
  - **Rh is a blood group system with many antigens, (49 todate)one of which is D.**

- **Rh + or - refers to the presence or absence of the (Rh) D antigen on the red blood cell.**

Rh (D) Antigen (continued)

- **Production of antibody to D requires exposure to the antigen unlike ABO antigens**

- **The D antigen is very immunogenic, ie, individuals exposed to it will very likely make an antibody to it if they encounter the D antigen on transfused RBCs (causing a haemolytic transfusion reaction, HTR) or on fetal RBCs (causing HDN).**

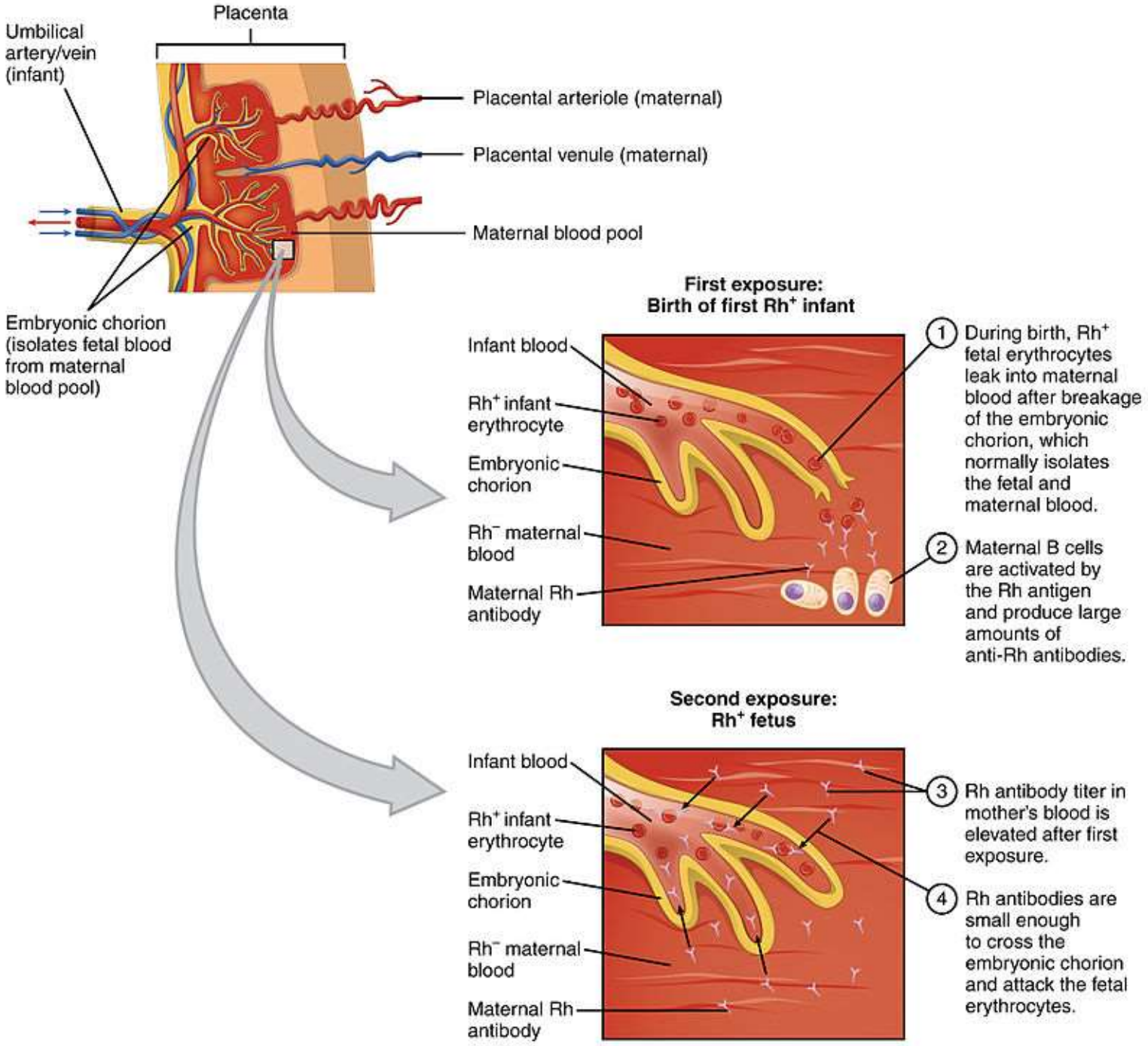
- **For this reason all individuals are typed for D, if negative must receive Rh (D) negative blood.**

Rh (D) Antigen (continued)

- **The most important patient population to consider is females of child-bearing age.**

- **If immunized to Rh (D) antigen (as during first pregnancy), the anti-Rh antibodies can cross the placenta and destroy Rh (D) positive fetal cells resulting in death (HDN/HDFN/erythroblastosis fetalis).**

- **The American College of Obstetricians and Gynecologists (ACOG) recommends that all RhD negative mothers, regardless of fetal blood type, receive RhIG (RhoGAM) at about 28 weeks gestation, and again shortly after delivery in the case of an RhD positive or RhD unknown baby.**


Placenta
Umbilical artery/vein (infant)
Placental arteriole (maternal)
Placental venule (maternal)
Maternal blood pool
Embryonic chorion (isolates fetal blood from maternal blood pool)
First exposure: Birth of first Rh+ infant
Infant blood
Rh+ infant erythrocyte
Embryonic chorion
Rh− maternal blood
Maternal Rh antibody
1 During birth, Rh+ fetal erythrocytes leak into maternal blood after breakage of the embryonic chorion, which normally isolates the fetal and maternal blood.
2 Maternal B cells are activated by the Rh antigen and produce large amounts of anti-Rh antibodies.
Second exposure: Rh+ fetus
Infant blood
Rh+ infant erythrocyte
Embryonic chorion
Rh− maternal blood
Maternal Rh antibody
3 Rh antibody titer in mother's blood is elevated after first exposure.
4 Rh antibodies are small enough to cross the embryonic chorion and attack the fetal erythrocytes.

MITOGENS

- **Mitogens are lectins of plant/animal origin which induce mitosis, and hence proliferation, in all lymphocytes simultaneously without interaction with an antigen or superantigen.**

- **Mitogens can induce proliferation of either T-cells or B-cells or even both.**

- **Examples**
  - **T cell inducers – PHA (Phyto Haemagglutinin A) from red kidney beans, ConA (Concalavin A) from jack beans, PWM (Poke Weed Mitogen)**
  - **B cell inducers - LPS**

IMMUNOLOGICAL SPECIFICITY

ANTIGENIC OR IMMUNOLOGICAL SPECIFICITY

- Refers to the ability of immunological molecules (Ab/BCR/TCR) to differentiate minor strucutural differences between antigens or epitopes.
- Derives mainly from the reason that the immunological molecules recognizes the shape of an antigen or epitope.
- The binding between antigen and immunological molecules is due to non-covalent bonds, not covalent bonds.
- Hence as close approximation of two reactants as possible is must for a firm, irreversible binding.
- **ANY MINOR ALTERATIONS IN THE ANTIGENIC STRUCTURE WILL AFFECT THE BINDING STRENGTH OF AN ANTIBODY TO AN ANTIGEN**

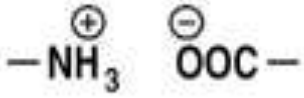
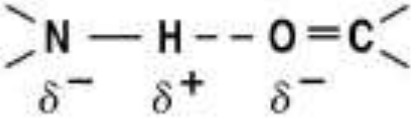
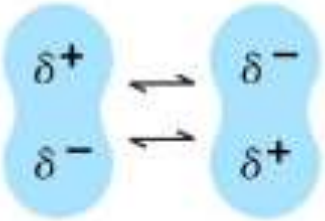
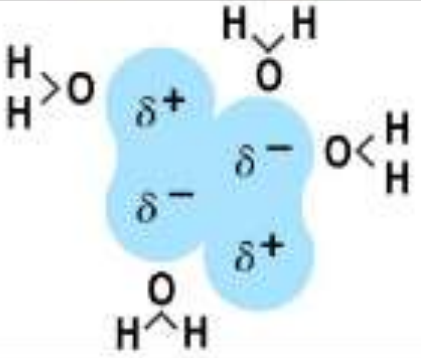
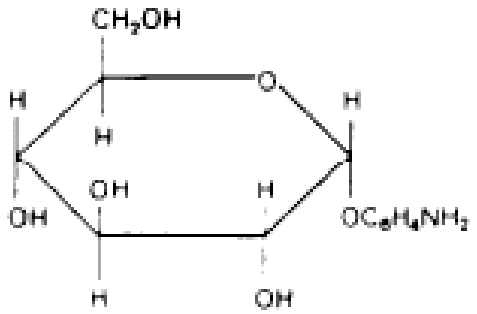
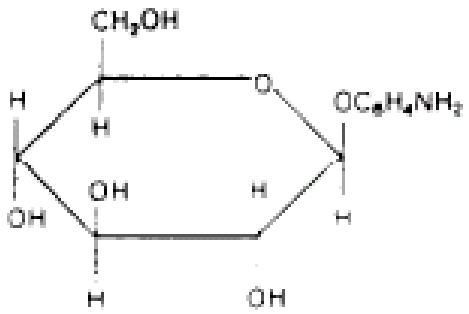
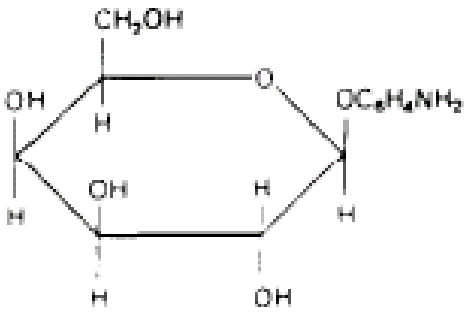
Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	
Hydrogen bonds	Hydrogen shared between electronegative atoms (N,O)	
Van der Waals forces	Fluctuations in electron clouds around molecules oppositely polarize neighboring atoms	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	

Figure 3-9 Immunobiology, 6/e. (© Garland Science 2005)

Stereochemical specificity example

Table 3–2. Reactions of antisera with isomeric glucoside protein conjugates.*
(+ represents precipitation)

	p-Aminophenol α-glucoside	*p*-Aminophenol β-glucoside	*p*-Aminophenol β-galactoside
			
Antisera against:			
α-Glucoside	+++	++	0
β-Glucoside	++	+++	0
β-Galactoside	0	0	+++

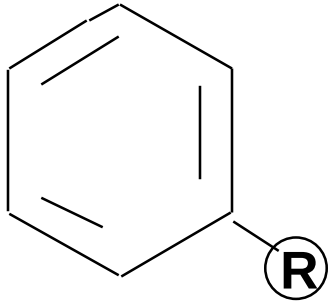
*Reproduced, with permission, from Humphrey JH, White RG: *Immunology for Students of Medicine.* Davis, 1970.

Minor Changes in an Antigen will affect its binding with an antibody

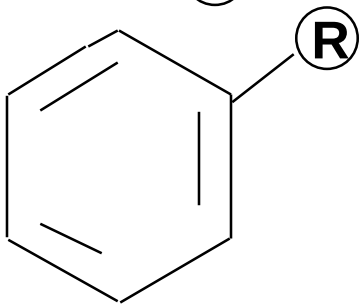
Change of Ⓡ position

R= Sulfa (SO_3H)

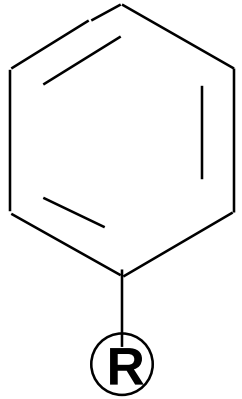
Meta-Ⓡ



Ortho-Ⓡ



Para-Ⓡ

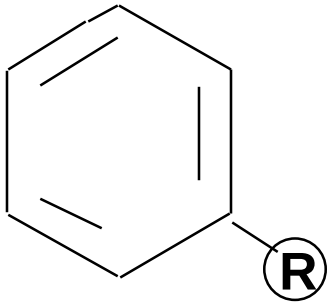


Q: Level of response for Antibody Against Meta-Ⓡ ?

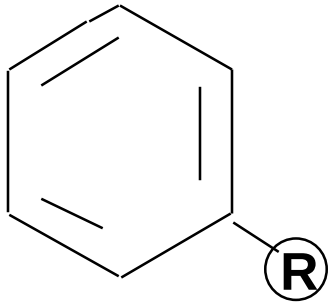
+++	**±**	**±**

Minor Changes in Antigen: (continued)

No change in position of Ⓡ but a minor change of Ⓡ [a single atom from sulfur to arsenic AsO_3H]



Sulfur + Anti-Meta (sulfur) ⟶ +++



Arsenic + Anti-Meta (sulfur) ⟶ [no binding]

CROSS REACTING ANTIGNES

CROSS REACTING ANTIGENS

- **The antigens to which antibodies formed against another antigen reacts.**

- **The antigen that induces antibody formation is called as 'homologous antigen' and the antigen that cross react is called as 'heterologous antigen'.**

- **Examples-**
  - **Rickettsia and *Proteus* OX-19 strain (Typhus diagnosis)**
  - ***Treponema pallidum* and Cardiolipn (VDRL test)**

Cross-reactivity

Antibody can react with two similar but non-identical antigens

The two may share one or more identical epitopes, e.g., toxoids; diphtheria, tetanus

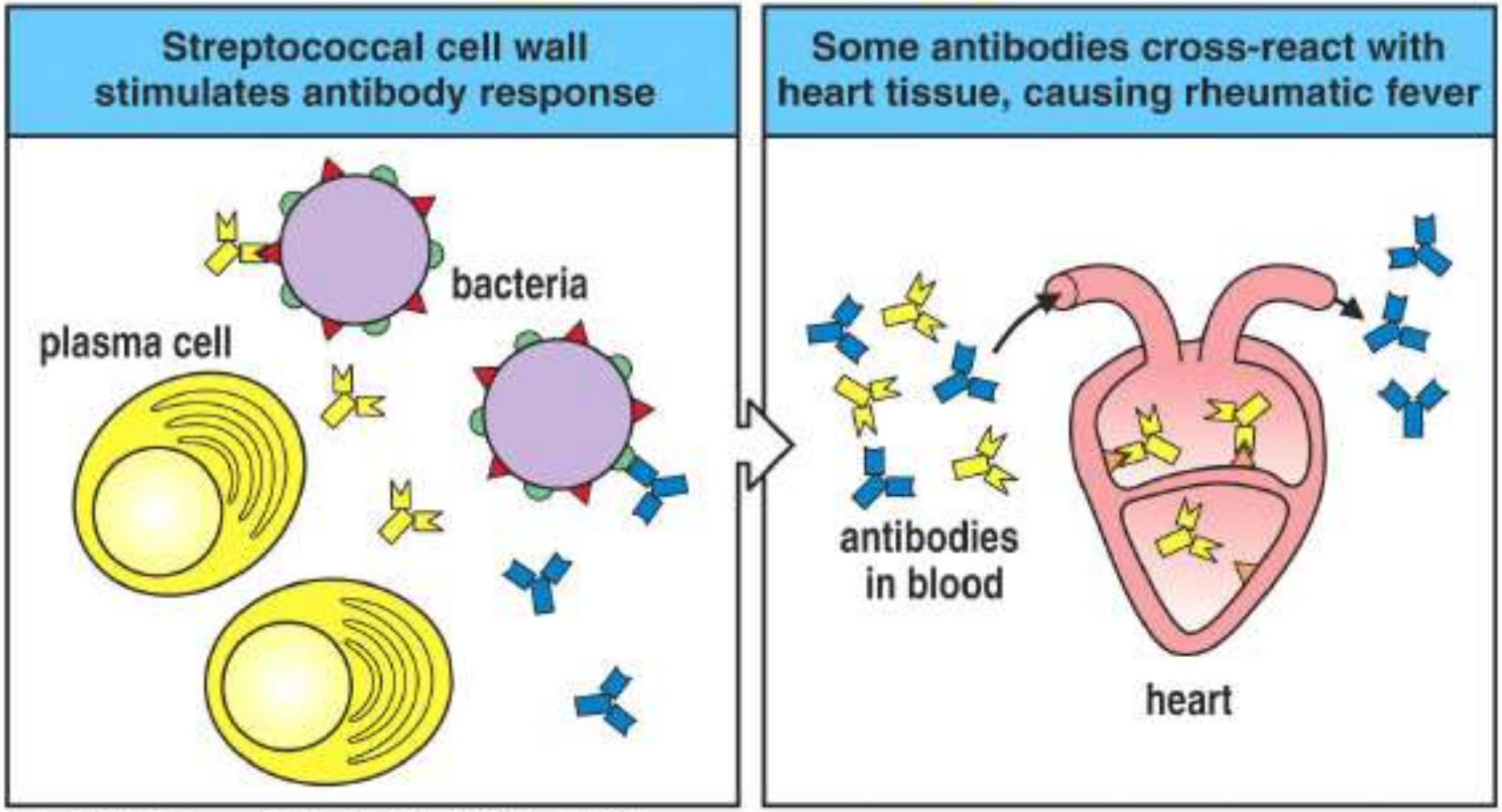
The two may share one or more structurally similar but non-identical epitopes, e.g., bacterial antigens and autoantigens

CROSS REACTIVE EPITOPES

COMMON EPITOPES FOUND ON DIVERSE ANTIGENS

- **Cross reactive epitopes found in pathogenic *Brucella* and non-pathogenic *Yersinia.***
- **Gut Bacteria and Blood group antigens**

HETEROPHILE ANTIGENS


Streptococcal cell wall stimulates antibody response
bacteria
plasma cell
Some antibodies cross-react with heart tissue, causing rheumatic fever
antibodies in blood
heart

HETEROPHILE ANTIGENS

- **Cross-reacting antigens which are found cells and/or fluids of widely unrelated living beings due to presence of identical epitopes.**

- **Example – FORSSMAN ANTIGEN**
  - **Forssman antigen is a glycolipid heterophile antigen present on red blood cells of horse, sheep, dog, cat, mouse and fowl; kidney cells of guinea pigs and; bacteria, such as *Streptococcus pneumoniae, Clostridium welchii, Salmonella parartyphi,* etc.**

ADJUVANTS

Adjuvants

Substances that are non-immunogenic alone but enhance the immunogenicity of other molecules by: (a) inducing Ag aggregation; and (b) stimulating non-BCR, non-TCR receptors

Adjuvants that enhance immune responses

Adjuvant name	Composition	Mechanism of action
Incomplete Freund's adjuvant	Oil-in-water emulsion	Delayed release of antigen; enhanced uptake by macrophages
Complete Freunds adjuvant	Oil-in-water emulsion with dead mycobacteria	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators in macrophages
Freunds adjuvant with MDP	Oil-in-water emulsion with muramyldipeptide (MDP), a constituent of mycobacteria	Similar to complete Freund's adjuvant
Alum (aluminum hydroxide)	Aluminum hydroxide gel	Delayed release of antigen; enhanced macrophage uptake
Alum plus *Bordetella pertussis*	Aluminum hydroxide gel with killed *B. pertussis*	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators
Immune stimulatory complexes (ISCOMs)	Matrix of Quil A containing viral proteins	Delivers antigen to cytosol; allows induction of cytotoxic T cells