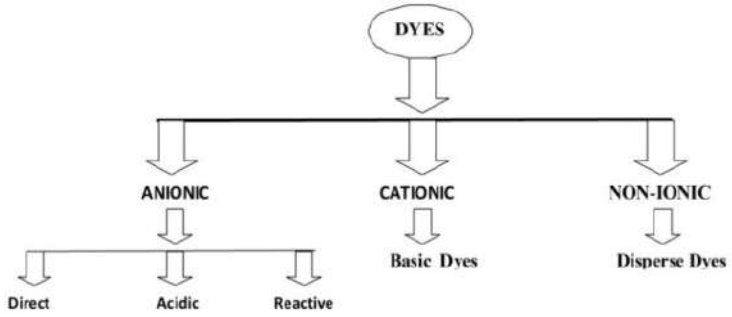
VIVEKANANDA COLLEGE
THAKURPUKUR
KOLKATA-700063

NAAC ACCREDITED ‘A’ GRADE

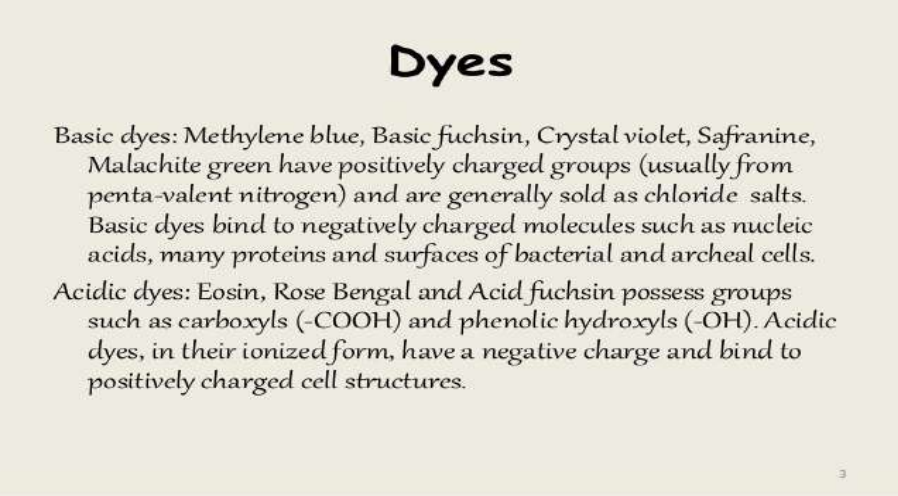
Topic	: Bacterial Growth Regulation and Control
Course Title	: Basic Microbiology and Microbial Genetics
Paper	: CC 10
Unit	: II
Semester	: 4
Name of the Teacher	: Dr. Kakali Roy
Name of the Department	: Biochemistry

Chemical Control

Dye solutions



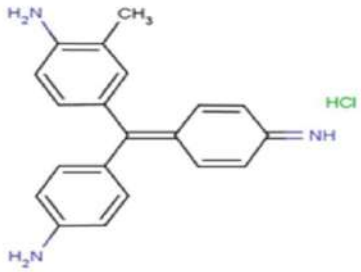
- The incorporation of dyes into culture media for the purposes of isolation and differentiation of bacteria
- Gram-negative bacteria exhibited greater resistance to dyes than gram-positive bacteria. Basic dyes are more inhibitory than acidic and neutral dyes at the same concentration.



- Crystal violet has an antibacterial action against *Escherichia coli, Staphylococcus aureus, Streptococcus faecalis* and *Bacillus subtilis*. The effect of the dye, measured as minimum inhibitory concentration or retardation of growth, increases as the pH rises from 6 to 8.

Basic Dyes

- Work best in basic pH
- Ionizes (Cl-, SO4-)
- Creates (+) Cationic chromogen
- Attracted to (-) acidic cell components [DNA, proteins]
- Examples
  - Methylene Blue
  - Crystal Violet
  - Carbol Fuchsin
  - Safranin
  - Malachite Green



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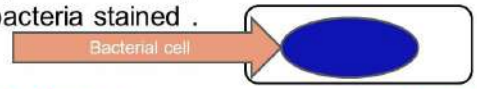
Acidic Dyes

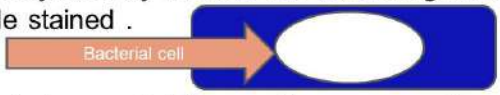
- Works best in acidic pH
- Ionizes (Na+, K+, Ca++)
- Creates Anionic (-) chromogen
- Attracted to (+) cell components [AA]
- Examples
  - Picric Acid
  - Nigrosin
  - India Ink
  - Eosin

Nigrosin

Basic Dyes : chromophore is the positive ion dye attracted by the bacteria so the cells of bacteria stained .



Acid Dyes : chromophore is the negative ion dye rejected by the cell and the background of slide stained .



- Bacteria are slightly negative, so are attracted to the positive chromophore of the **BASIC DYE**

Dyes :

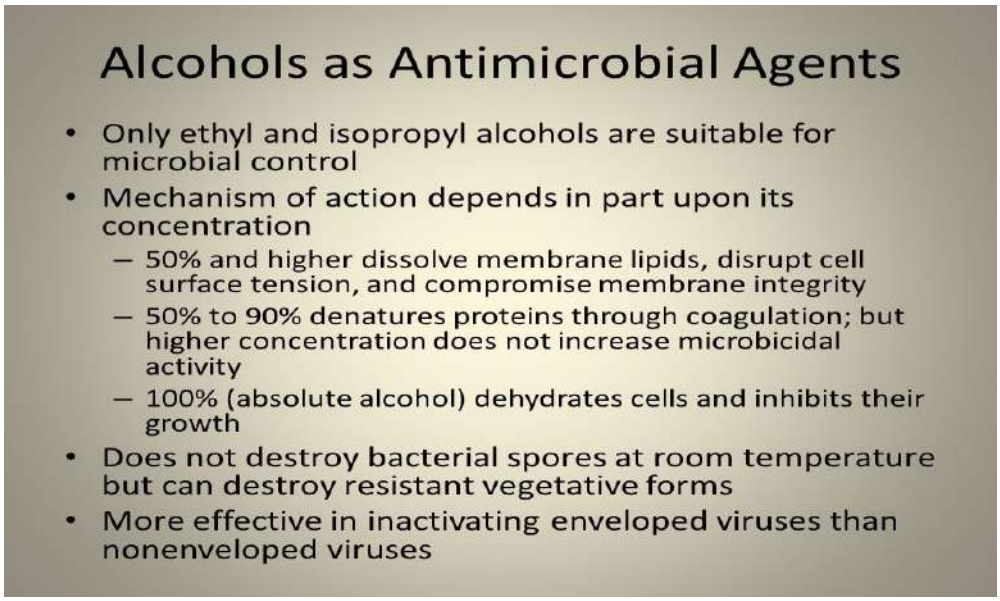
- Two groups of dyes have been used extensively as skin and wound antiseptics
  - aniline dyes
  - acridine dyes
- Both are bacteriostatic in high dilution but have low bactericidal action.
- Aniline dyes include crystal violet, brilliant green and malachite green.
- They are more active against Gram positive bacteria than Gram negative bacteria.

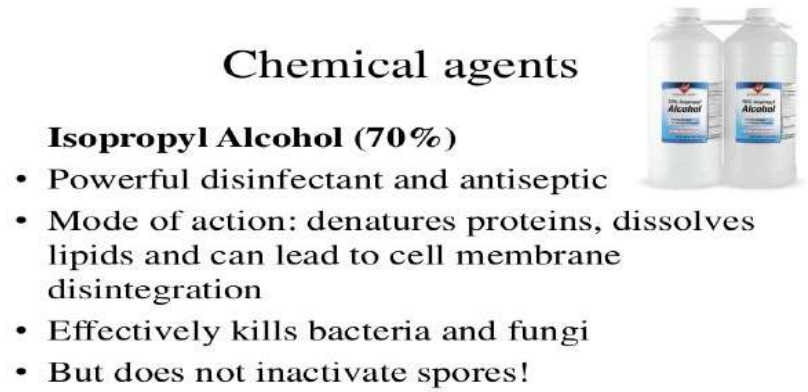
DYES:

- **Mode of action:** Acridine dyes are bactericidal because of their interaction with bacterial nucleic acids.
- **Examples:** Aniline dyes such as crystal violet, malachite green and brilliant green. Acridine dyes such as **acriflavin** and aminacrine. Acriflavine is a mixture of proflavine and euflavine. Only euflavine has effective antimicrobial properties. A related dye, ethidium bromide, is also germicidal. It intercalates between base pairs in DNA. They are more effective against gram positive bacteria than gram negative bacteria and are more bacteriostatic in action.
- **Applications:** They may be used topically as antiseptics to treat mild burns. They are used as paint on the skin to treat bacterial skin infections. The dyes are used as selective agents in certain selective media.

Alcohol

Mode of action





Application

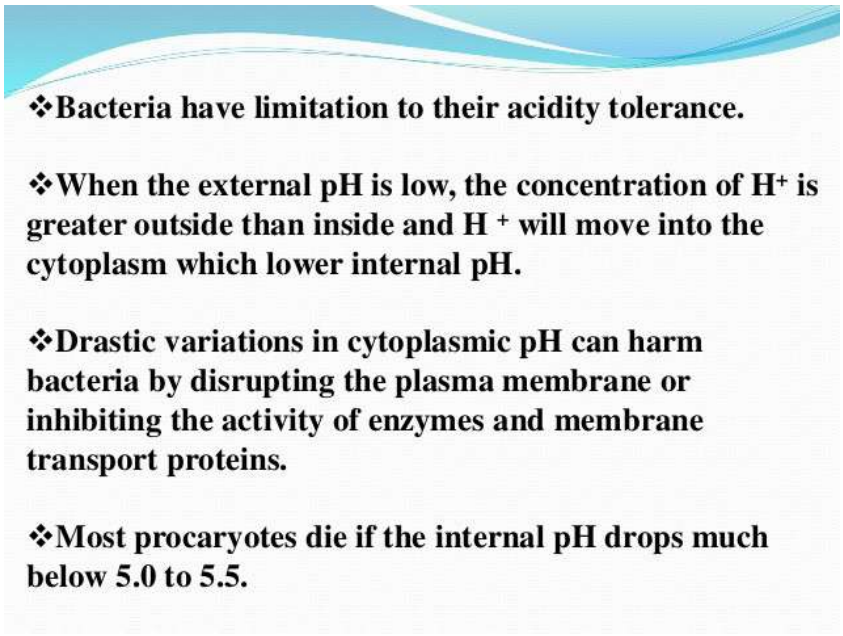
- Alcohol may cause dehydration and turn to be microbiostatic.
- Alcohol is effective in reducing the microbial flora of skin and for the disinfection of clinical oral thermometers.

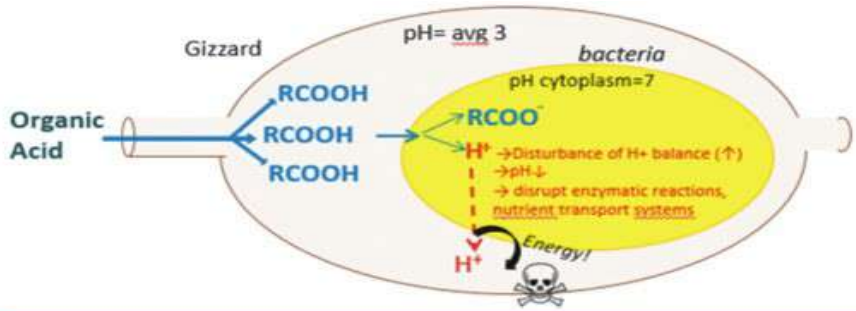
- Alcohol concentrations above 60% are effective against viruses
- Due to its cleansing or detergent action, alcohol has an effective surface disinfection for removal of microorganisms

Acid

Mode of action

- Acidic disinfectants function by destroying the bonds of nucleic acids and precipitating proteins.
- Acids also change the pH of the environment making it detrimental to many microorganisms.





the **antibacterial effect of organic acids** is by

- ✓Modification of bacteria's internal pH,
- ✓Inhibition of bacteria's fundamental metabolic functions,
- ✓Accumulation of toxic anions in the bacteria and
- ✓Distruption of bacteria's cellular membrane.

Organic Acids

Organic Acids - various organic acids and their salts are common antimicrobials in foods

- preservatives to control mold growth

- sorbic acid (Ca, Na, K) used in cheeses, baked goods, soft drinks, fruit juices, jams, jellies

- benzoates (sodium benzoate, methy-p-hydroxybenzoate [methylyparaben]) fruit juices, jam, jellies, soft drinks, salad dressings, margarine, and many pharmaceutical products

- boric acid used in eye washes

- calcium propionate-prevents mold growth in bread

Mode of Action of Organic Acids

- The mode of action of organic acids in animal diets has not been clearly elucidated; this incomplete understanding has limited the application of organic acids in broiler diets.
- Several possible mechanisms have been proposed and most of them have been associated with:
  1. Decreased pH in diets and subsequent reduction of the pH in the GIT
  2. Improved nutrient utilization in diets by increasing nutrient retention
  3. Inhibition of pathogenic bacterial growth

Lactic Acid

Lactic acid and its salts are used in food more for flavor enhancement than for their antibacterial effect, especially when used above pH 5.0.

They have antibacterial effects in concentrations 1-2%.

Growth of Gram-positive and Gram-negative bacteria is reduced, indicating increased bacteriostatic action.

Below pH 5.0, lactic acid has a bactericidal effect, especially against Gram-negative bacteria.

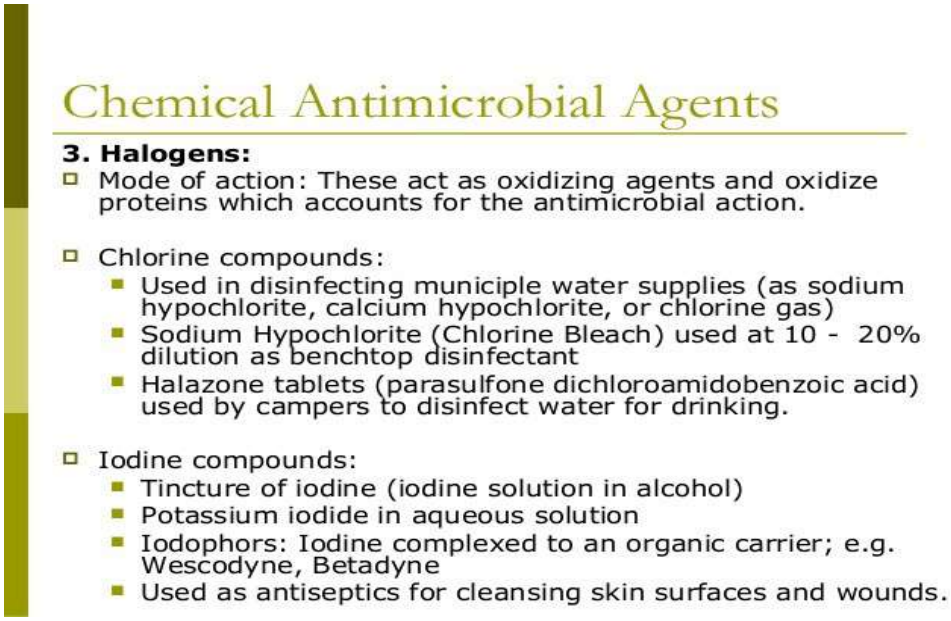
Antimicrobial Agents

- **Benzoic acid**
- Disrupts cell membrane function/inhibits enzymes (moulds, yeasts, some bacteria)
- to preserve fruit juices, fruit beverages, pickled vegetables, olives

Alkali

- Sodium or ammonium hydroxide, sodium carbonate, calcium oxide are effective antimicrobial agent
- Alkaline agents work by saponifying lipids within the envelopes of microorganisms.
- It destroys proteins by hydrolysis
- The activity of alkali compounds is slow but can be increased by raising the temperature. Alkalis have good microbicidal properties

Halogen



Chemical Methods of Microbial Control

- **Halogens**
  - Intermediate-level antimicrobial chemicals
  - Believed to damage enzymes via oxidation or by denaturation
  - Widely used in numerous applications
    - Iodine tablets, iodophores, chlorine treatment, bleach, chloramines, and bromine disinfection

Halogens

- A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VII of the periodic table.
- They exist as diatomic molecules in the free state and form salt like compounds with sodium and most other metals.
- The halogens iodine and chlorine are important antimicrobial agents. Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores.
- Iodine often has been applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide. Although it is an effective antiseptic, the skin may be damaged, a stain is left, and iodine allergies can result.
- More recently iodine has been complexed with solubilizing agent or surfactants to form an **iodophor.**
- **Iodophors** are water soluble, stable, and non staining agents, release iodine slowly to minimize skin burns and irritation. They are used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfecting.

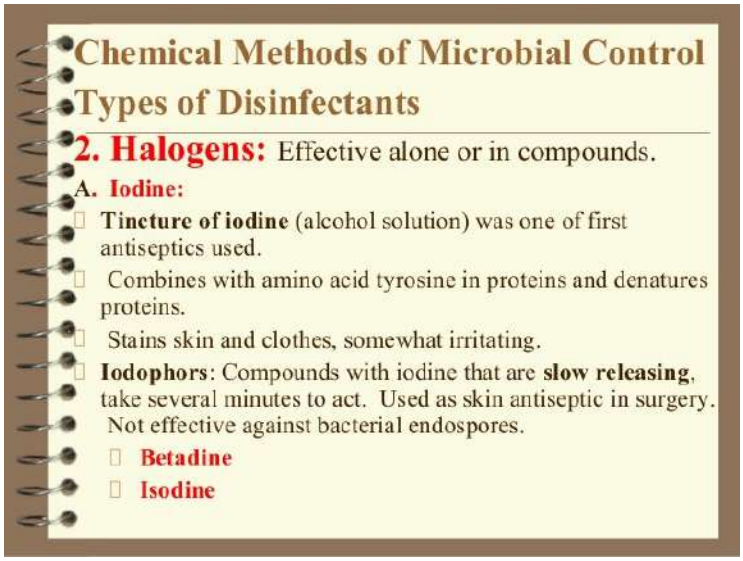
Cholorophores

(1) Chlorinated lime (bleaching powder)

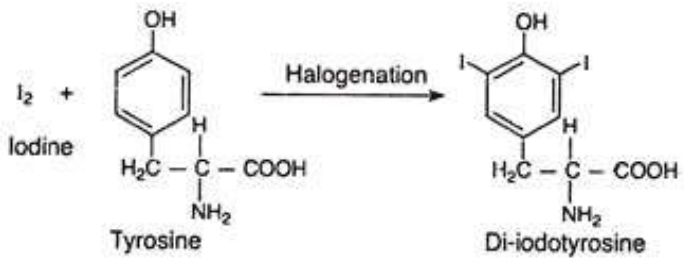
- obtained by action of chlorine on lime.
- used to disinfect drinking water

(2) Sodium hypochlorite

- Powerful disinfectant used in dairies for milk cans.
- Too Irritant to be used as antiseptic.
- Root canal therapy in dentisry



Tincture of iodine is applied as an antiseptic in households and hospitals to disinfect wounds, cuts, scratches.



Inactivate enzymes and cellular proteins that require tyrosine for activity

Iodine and Iodophors

- Iodine has been recognized as an effective antiseptic since the 1800s.
- Iodine molecules rapidly penetrate the cell wall of microorganisms and inactivate cells by forming complexes with amino acids and unsaturated fatty acids, resulting in impaired protein synthesis and alteration of cell membranes.
- Iodophors are composed of elemental iodine, iodide or triiodide, and a polymer carrier (i.e., the complexing agent) of high molecular weight. The amount of molecular iodine present (so-called "free" iodine) determines the level of antimicrobial activity of iodophors.
- Combining iodine with various polymers increases the solubility of iodine, promotes sustained release of iodine, and reduces skin irritation.

Iodophores are used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfecting.

Heavy Metal

- Heavy metals (like mercury, silver) and heavy metal compounds have some degree of toxicity for microorganisms.
- They act as germicides.

HEAVY METALS:

- **Mode of action**: Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic.
- **Examples:** Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome merthiolate)
- **Applications:** these heavy metals are biocidal.
- **Disadvantages:** Mercuric chloride is highly toxic, are readily inactivated by organic matter.

1% silver nitrate solution can be applied on eyes as treatment for opthalmia neonatorum

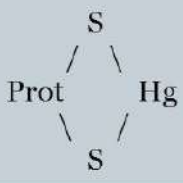
Protein Precipitant Antimicrobial Agents

3. Mercury Compounds

- The action of mercury on microorganisms and body tissues is primarily due to the **mercury ion**.
- The mercury ion reacts with many polar groups on the amino acids of the protein but has a particular affinity for sulfhydryl (-SH) groups.
- The mercuric ion reacts with the cysteine sulfhydryl groups on the protein of enzymes to form mercaptides that may be represented by any of the following structures (where X= Cl, OH):

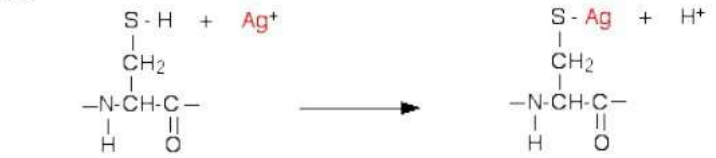
Prot-S-Hg-X

Prot-S-Hg-S-Prot



Examples :

- Silver ions (heavy metal) react with -SH groups in the side groups of cysteine residues in the protein chain:



cysteine residue in protein chain

- If the cysteine residue is somewhere on the protein chain which affects the way it folds into its tertiary structure, then altering this group could have an effect on the shape of the active site, and so stop the enzyme from working.

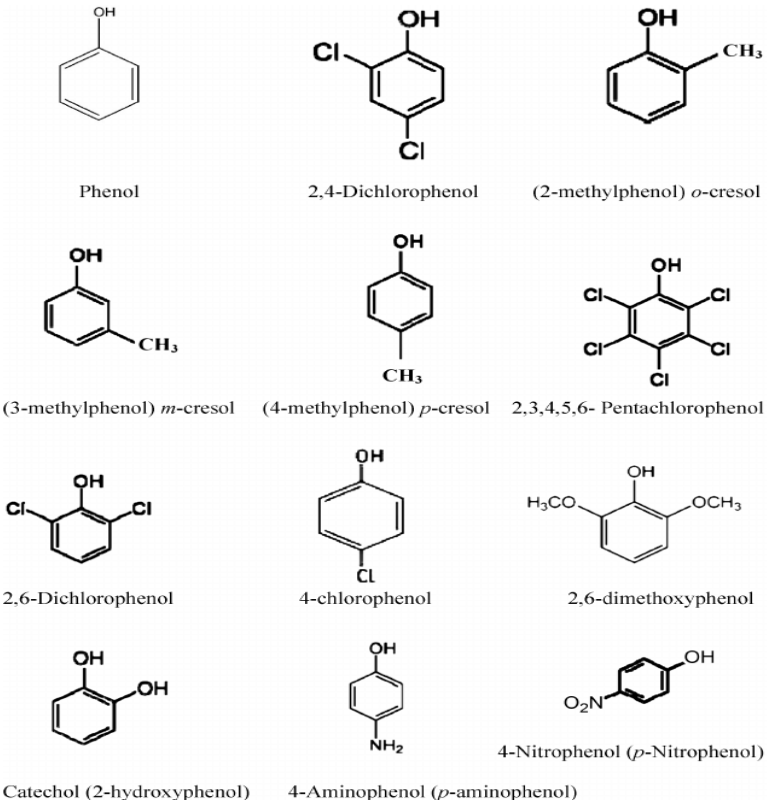
Protein Precipitant Antimicrobial Agents

Uses of Ammoniated Mercury :

- It is mildly antiseptic and is used primarily in ointments.
- Used in the treatment of impetigo contagiosa and fungal infections of the skin (dermatomycoses).
- Used in the treatment of crab louse infestations.
- Available as 5% and 3% ophthalmic ointment.

Toxicity : Overuse of the product has produced chronic toxicities so applications to large areas for prolonged periods of time are certainly not recommended.

Phenol and Phenol derivatives



Chemical Antimicrobial Agents

1. **Phenols:**

- Mode of action: depending on the concentration of the phenolic compound, the activity may result in disruption of cells, precipitation of cell protein, inactivation of enzymes and leakage of amino acids from the cells.
- Examples: Phenol, orthocresol, orthophenylphenol, hexachlorophene
- These are either bactericidal or bacteriostatic depending upon the concentration used.
- Solution of phenol is also known as carbolic acid.

❑ Phenols

- Phenols are widely used as disinfectants and preservatives.
- Advantages:
- As disinfectants they have good antimicrobial activity and are rapidly bactericidal but generally are not sporicidal. They are more active at acid pH.
- Disadvantages: The main disadvantages of phenols are their caustic effect on skin and tissues and their systemic toxicity. Their activity is markedly diminished by dilution and is also reduced by organic matter.
- The more highly substituted phenols are less toxic and can be used as preservatives and antiseptics.

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1% phenol solution used as skin disinfectant and 0.5% as gargle in glycerol

2. Phenols and Phenolics:

- **Phenol** (carbolic acid) was first used by Lister as a disinfectant in 1867
- Destroy plasma membranes and denature proteins
- Rarely used today because it is a skin irritant and has strong odor.
- 1% of concentration act as bactericidal
- **Phenolics** are chemical derivatives of phenol
  - **Cresols (Lysol)**:
    - Derived from coal tar
    - Uses for contaminated glassware, cleaning floors, disinfection of excreta
  - Chlorhexidine –comercially savlon
    - Member of biguanide group
    - Widely used in wounds, preoperative disinfection of skin

PHENOL DERIVATIVES

- denaturing bacterial proteins.
  - Phenol (carbolic acid).
  - Resorcinol: 1/3 as potent as phenol - mouth wash, lozenges & as antifungal
  - Chloroxylenol : 4.8% sol. (DETTOL) surgical antisepsis.
  - Hexachlorophene
    - inhibiting bacterial enzyme and ↑conc - bacterial lysis.
    - soap & other cleansing antiseptics
    - Highly active against gram +ve
    - disinfect urine, faeces, pus, sputum of patients
    - antipruritic preparation - mild anaesthetic action. drdkg07@gmail.com

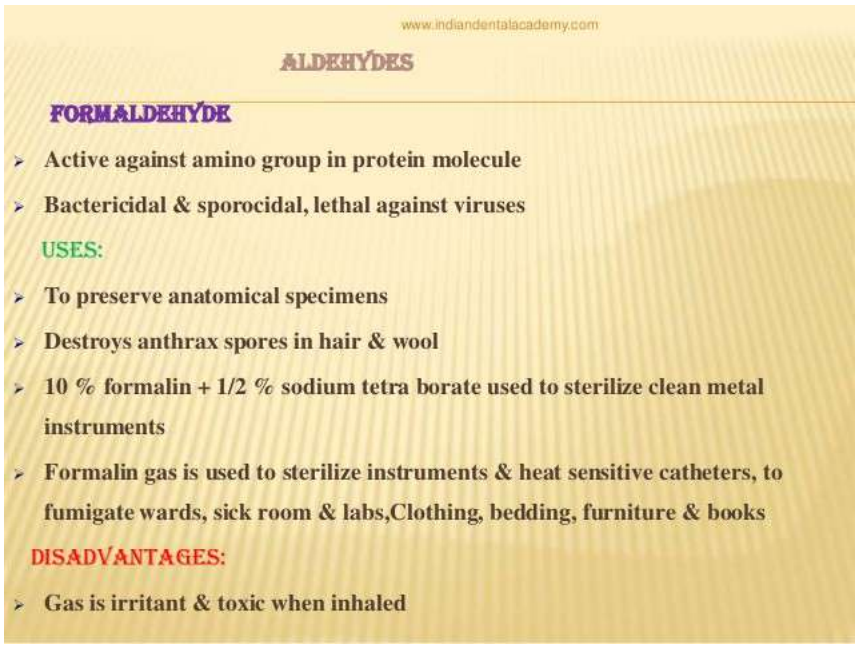
Formaldehyde

Formaldehyde acts by alkylation of chemical groups in proteins and nucleic acids.

- **Mode of Action**
- Formaldehyde inactivates microorganisms by alkylating the aminoacid and sulfhydryl groups of proteins and ring nitrogen atoms of purine bases.
- **Disadvantages**
- It acts as a potential carcinogen
- Toxic
- Irritant

ALDEHYDES (FORMALDEHYDE)

- It denatures proteins,
- general protoplasmic poison (but acts slowly).
- Broad spectrum germicide.
- Use as antiseptic is restricted
  - irritating nature & pungent odor.
  - 4% sol - hardening & preserving dead tissues.
- 37% sol. FORMALIN.
  - Occasionally used to disinfect instruments & excreta.
- Glutaraldehyde
  - less volatile, less pungent,
  - less irritating.
  - 2% sol - disinfect surgical instruments & endoscopes



Ethylene Oxide

- Ethylene oxide (C_2H_4O), sometimes called oxirane, is an organic alkylating gas with very potent and highly penetrating properties.
- Ethylene oxide is capable of destroying most viruses, bacteria, and fungi, including bacterial spores.
- It reacts with protoplasm, DNA, and proteins and deactivates enzymes and other biologically important components of a living organism.
- Ethylene oxide is more effective in killing microorganisms under dry conditions.

Epoxides(Ethylene Oxide, Propylene Oxide)

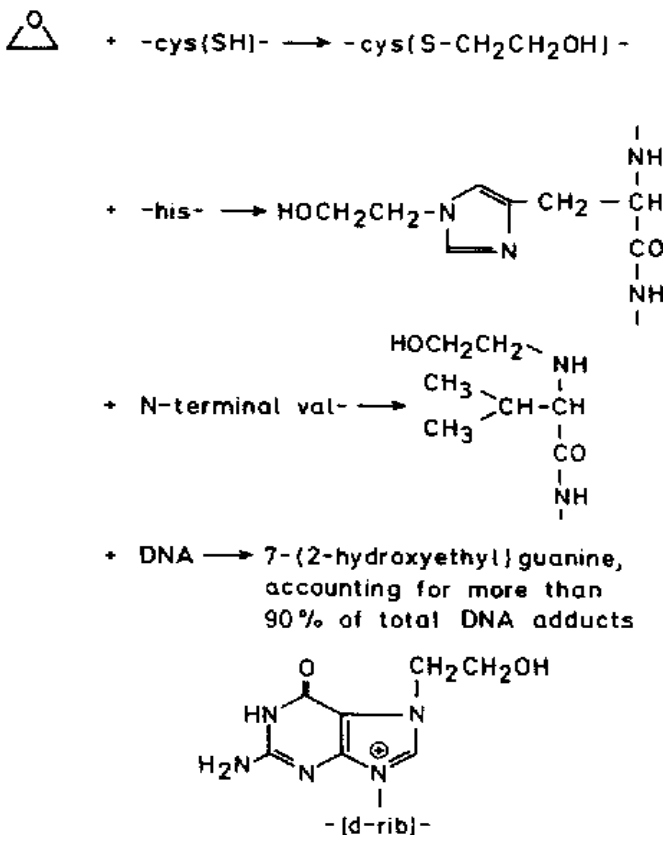
Ethylene oxide and propylene oxide are used as fumigants to destroy microorganisms and insects in grains, cocoa powder, gums, nuts, dried fruits, spices, and packaging materials.

They are effective against cells, spores, and viruses.

Epoxides are alkylating agents and react with various groups(SH, NH2, OH) in cellular macromolecules e.g., enzymes adversely affecting their functions.

They react with chlorides and form toxic compounds.

Alkylation of macromolecular targets by ethylene oxide



7- Ethylene Oxide

- Ethylene oxide gas is used extensively in hospitals for the sterilization of heat-sensitive materials such as surgical instruments and plastics.
- Mode of action:

It kills by alkylating both proteins and nucleic acids, i.e., the hydroxyethyl group attacks the reactive hydrogen atoms on essential amino and hydroxyl groups.

- Examples:

Ethylene oxide, peracetic acid, formaldehyde and H_2O_2 gases.

Detergents

Chemical Antimicrobial Agents

6. Synthetic Detergents:

- These are surface-tension depressants which are used for cleaning surfaces.
- They are extensively used in laundry and dish-washing powders, shampoos and other washing preparations.
- Some are highly bactericidal.
- There are three types of detergents: Anionic detergent (sodium lauryl sulphate), Cationic detergent (cetylpyridinium chloride) and Nonionic detergents (these do not possess any antimicrobial activity).
- Cationis detergents are more germicidal than anioinis compounds.

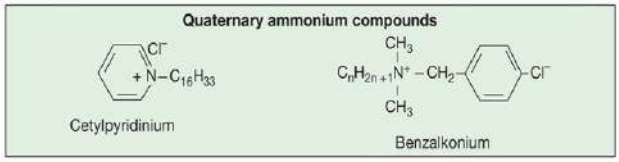
Cationic detergents :

quaternary ammonium compound :

benzalkonium chloride 0.1%

- highly bactericidal in the absence of contaminating organic matter
- react with lipid of cell membrane of bacteria
- alter membrane surface properties and its permeability led to loss of essential cell component and death

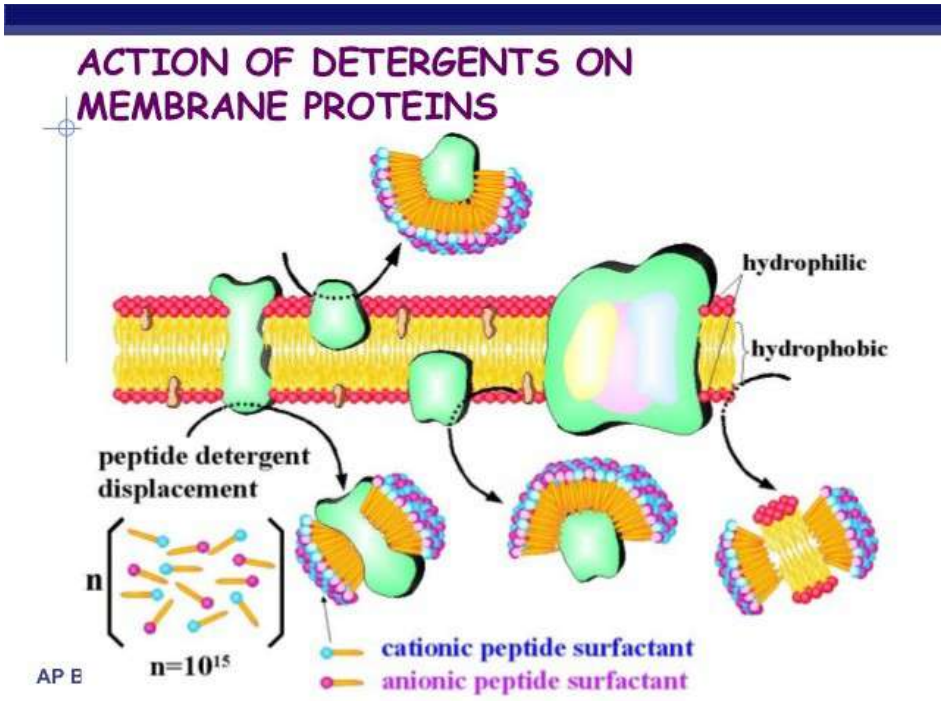
Quaternary Ammonium Compounds



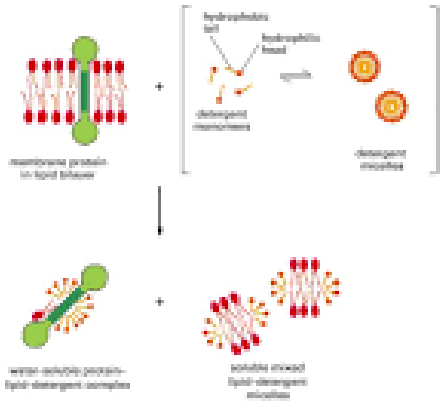
- detergents that have antimicrobial activity and are effective disinfectants
  - amphipathic organic cleansing agents
- **most likely disrupts cell membrane**
- cationic detergents are effective disinfectants
  - kill most bacteria, but not *M. tuberculosis* or endospores
  - safe and easy to use, inactivated by hard water and soap

Anionic surfactants

- **Sodium Lauryl Sulphate BP**
- Mixture of sodium alkyl sulphates, the chief of which is sodium dodecyl sulfate, $C_{12}H_{25}SO_4^-Na^+$
- It is very soluble in water at room temperature, and is used
- pharmaceutically as a preoperative skin cleaner, having
- Bacteriostatic action against gram-positive bacteria, and also in medicated shampoos
- Component of emulsifying wax.

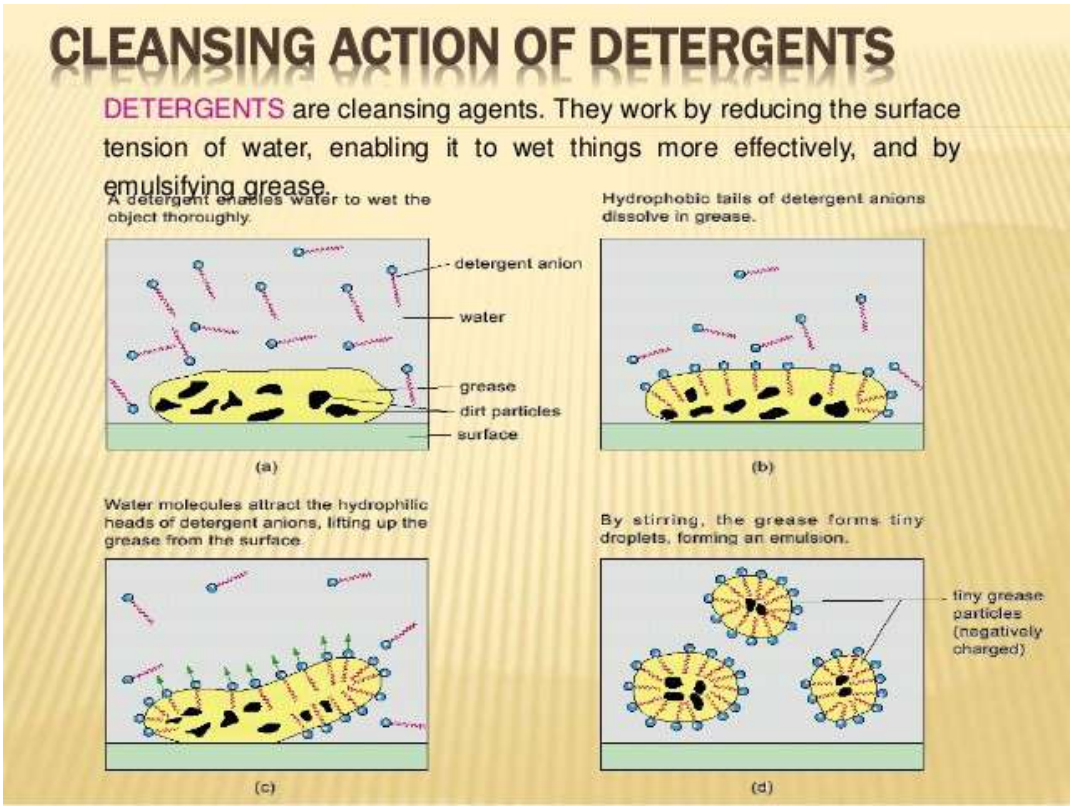


Solubilizing membrane proteins with a mild detergent



CLEANSING ACTION OF DETERGENTS

DETERGENTS are cleansing agents. They work by reducing the surface tension of water, enabling it to wet things more effectively, and by emulsifying grease.



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