Fungi → Augustino Bassi, 1835, Muscardine disease of silk worm.

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→ These are the eukaryotic organisms which exists as saprophytes (Those living on dead or decaying matters).

→ Generally they are aerobic in nature.

→ The cell wall of fungi madeup of chitin, polysaccharides, mannan & their cytoplasmic membrane contains sterol.

→ They were 1st identified by "Augustino Bassi" in year 1835 from "Muscardine" disease of silkworm.

→ The study of fungi is called as Mycology.

Comparision of selected Features of Fungi & Bacteria

Characteristics	Fungi	Bacteria
1) cell type	→ Eukaryotic	Prokaryotic
2) Optimum pH	→ 4 – 6	6.5 – 7.5
3) Optimum temp.	→ (25 – 30° C Saprophytes)	32 – 37°C / 36°C
4) Cell Membrane	→ Sterols Present	Sterols Absent except mycoplasma
5) Oxygen Requirement	→ Strictly Aerobic (moulds) / Facultative Anar. (some yeasts)	Aerobic & Anaerobic
6) Light Requirement	→ None	Some P/Sic grps.
7) Carbon Source	→ Utilize organic type of carbon.	Utilize inorganic or organic type of carbon.
8) Cell wall components	→ Chitin, Polysacc, Mannan	Peptidoglycan & polysaccharides

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Classification of fungi

fungi are classified into two classes :-

(I) Morphological classification
(II) Taxonomical Classification

Morphological classification

a) Yeast
b) Yeast - like Fungi
c) Moulds
d) Dimorphic Fungi

Taxonomical classification

a) Zygomycetes
b) Ascomycetes
c) Basidiomycetes
d) Deutromycetes

Morphological Classi.

(1) Yeast

- They are round, oval, unicellular fungi.
- It contains only single cell.
- They forms circular, smooth, creamy white colonies on the surface of media
- They are aerobic & some are facultative anaerobic.
- They generally survive in 32 - 37 °C.
- example - saccharomyces, Cerevisae, Cryptococcus neoformans.
- They generally reproduce by asexual process c/d budding

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→ candida Albicans → Repeated Budding
→ Bud → mother cell
elongated chain
pseudohyphae

b) Yeast like Fungi

- Some yeast like candida Albicans, in this the bud remains attached to the mother cell & elongates, followed by repeated budding & forms chain of elongated cells of fungi called pseudohyphae.

c) Molds

- It contain multiple identical nuclei & grows in the form of mycelium or hyphae of filaments.
- It gives fuzzy appearance on the surface of media & forms black, green, brown, orange or pink colour.
- They are strictly aerobic.
- They generally grow under 22-28°C.
- They can reproduce either by sexual or asexual reproduction.

Ex → Aspergillus Niger, Aspergillus Fumigatus.

d) Dimorphic Fungi (Pathogenic)

- In this dimorphic, di means two & morphic means shape, Form
- Some fungi who ~~are~~ mainly belongs to pathogenic species ~~&~~ exhibits dimorphism.
→ Such fungi can grow either as a mould or as a yeast.
- The mould like forms produce vegetative & aerial mycelium & yeast like forms reproduce by budding or by asexual process.

Ex → Mucor Rouxii, Histoplasma Capsulatum.

Difference between Molds & Yeast

Molds	Yeast
→ It contains multiple identical nuclei	→ It contains only single cell (unicellular fungi).
→ It gives fuzzy appearance on surface of media & gives black, green, brown, orange colour on media.	- It forms white, circular, smooth, creamy colonies on the surface of media.

"Faith is the bird that feels the light when the dawn is still dark." -Rabindranath Tagore

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Molds	Yeast
→ Strictly aerobic	→ Aerobic & facultative anaerob.
– Opt. temp for growth 22-28°C	→ Temp. opt. is 32°C – 37°C temp. required.
– Reproduce by sexual or asexual process	→ They reproduce by asexual process c/d budding.
– Used for the production of enzymes & antibiotics	– Used for the production of ethanol & bakery items.
Ex → Aspergillus Niger, Penicillum Notatum, Aspergillus Fumigatus.	→ Saccharomyces Cerevisae, Triptococcus Neoformans,

Taxonomical Classification

- Fungi are placed in phylum Thallophyta.
- OTB of formation of sexual spores, they are divided into four (4) classes :-

1) Zygomycetes → sporangiospores = asexual; oospores & zygospores = sexual

They form asexual spores, Sporangiospores (Contained within sac like str. called sporangium) & sexual spores. i.e, Oospores & zygospores.

2) Ascomycetes → ascus (ascospore)

They forms ascospores in a sac called ascus by sexual process.

3) Basidiomycetes → (Basidiospores)

They form sexual spores called basidiospores on the tip or surface of basidium.

4) Deuteromycetes

- They donot produce sexual spores.
- Most of the fungi of medical importance belong to

"The letter counts not months but moments, and not time but thoughts." Rabindranath Tagore

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this class.

Reproduction of fungi (By self)

Cultivation of fungi

- Sabouraud agar → peptones → Nocardia
- Incub temp = 30°C, inspect daily
- humidified atmosphere

- Sabouraud Agar is a type of agar growth medium containing peptones, used to cultivate fungi & can also grow filamentous bacteria Nocardia.
- It was developed by raymond sabouraud in year 1892, it also contains antibacterial agent used to kill the contaminating bacterial species
- The standard temp for incubation of fungi is 30°C & cultures should be incubated in a humidified environment for 21 days.
- They should be inspected daily for a week & atleast 3 time weekly.
- After this incubation for 21 days the appearance of fungi is found.

Importance of fungi

① - fungi are imp sources of antibiotics.
ex → penicillin. (obtained from penicillium Notatum)
- Griseofulvin (obtained from Penicillium Griseofulvis)
- Cephalothin (~~Cephalothin~~ obtained from cephalosporium sp.)

② Yeast & moulds are good sources of diff. enzymes.
ex → Amylase produced from Aspergillus species.

③ Moulds (aspergillus species) are used in the production of citric acid, oxalic acid & gluconic acid.

④ fungi has been also used to alter the texture, improve the flavour, increase palatibility (taste) & digestibility of natural or processed food.
"Faith is the bird that feels the light when the dawn is still dark." —Rabindranath Tagore
ex → Penicillium Species is used for ripening of varity of cheese.

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(5) Yeast are used for fermentation process in the production of beverages & juices.

ex → Saccharomyces Cerevisiae

(6) Moulds are also used for production of industrial alcohol by fermentation.

ex → Fusarium Species.

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Virus

They are → obligate parasites

→ These are the micro-organisms which are the smallest infectious agent that can be seen only by the help of electron microscope.

→ They are usually 10 to 100 times smaller than bacteria.

→ The smallest known virus is approx 0.002 μm in diameter & the largest ones are about 0.8 μm in diameter.

→ They multiply only within their living host cells

→ Viruses are actually nucleoproteins. The proteinaceous coat i.e, capsid surrounds the nucleic acid which forms the central core of the virus particle

→ The viral genetic material consists maybe either DNA or RNA

→ The two nucleic acids are never present in a given virus.

→ They vary in size from 10 – 300 nm. They are so minute that they can easily pass through bacterial filters

→ Viruses are easily transmitted from infected host to the healthy ones through various agencies.

→ Viruses are so effective that even their smallest amount can cause infection on the host successfully.

→ Since the viruses have no metabolic activities of their own & utilize the metabolism of host cells, antibiotics have no effect on them generally.

→ Study of virus is c/d virology.

→ Viruses multiply only in cells of particular species & thus they are divided into 3 main classes –

(a) Bacterial viruses – or Phaginae, DNA, Bacterio phages

(b) plant virus – Phytophaginae, RNA

(C) Animal viruses – Zoophaginae

Bacterial viruses

- They are also c/d phaginae.
- They have DNA & are called bacteriophages, or

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Simply phages.

Plant viruses

→ They are also c/d phytophaginae. They have RNA and infect potato, sugarcane, tobacco & other higher plants.

Animal viruses

→ Also called as zoophagineae.

→ They usually have DNA but may also have RNA & infect man, piegon, parrot, dog, cow, orthropods.

Classificatn

1. O.T.B of Morphology
2. O.T.B of their host
3. O.T.B of genetic material

(1) OTB of Morphology

Virus
- RNA Containing
  - isohedral symmetry
    - Envelope
    - Non-envelope
  - Helical symmetry
    - Envelope
    - e.g → Influenza Virus, Measles, Mumps.
- DNA Containing
  - Icosahedral symmetry
    - No envelope
    - envelope (e.g chicken pox)
  - Complex Symm
    - Envelop
    - ex ↓ smallpox

(2) OTB of their host

→ Same
- → animal virus
- → Plant virus
- → Bacterial virus

"Birds is the bird that feels the light when the dawn is still dark." - Rabindranath Tagore

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Virions → single & fully developed form of virus.

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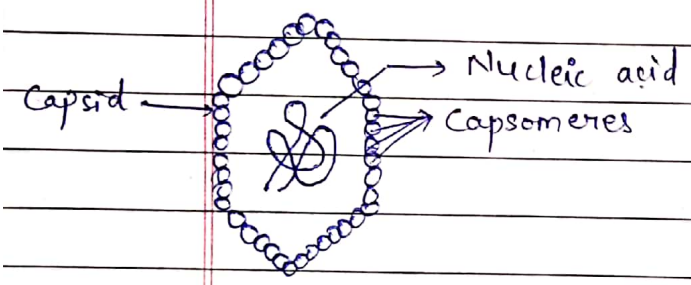
③ OTB of Genetic Material

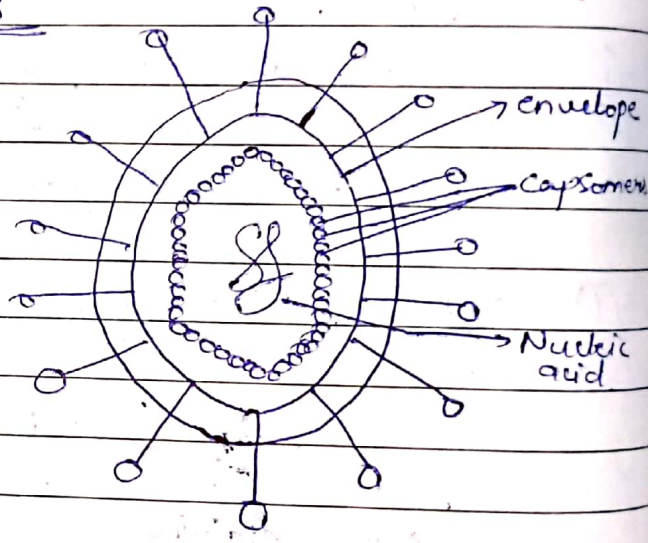
Virus

DNA Virus	RNA Virus
→ Pox Varicella	→ AIDS
→ Variola	→ Mumps
→ Adenovirus	→ Rubeiella
→ Herpes Virus	→ Tagovirus
→ Hepatitis Virus	→ Polio (Picrona Virus)

Structure of Virus



(a) Naked Virus



(b) Enveloped Virus

Virions

- These are individual particles of completely developed viral particle composed of nucleic acid & surrounded by protein coat c/d. capsomeres which combine & form capsid which protect it from environment.
- Simplest virions consists of two basic components :-
  (a) Nucleic acid (DNA & RNA)
  (b) Protein coat (capsid) → Protection from environment
  → Helps in attachment to the host cell.

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→ Capsid is made up of large no. of protein subunits k/a capsomeres.

→ Some virus have additional covering envelope c/d enveloped virus.

→ Some virus lacks in envelope c/d naked virus.

→ They donot have nucleus, cytoplasm & cell membrane.

Symmetry in Virus

(1) Helical symmetry
(2) Icosahedral symmetry
(3) Complex symmetry

Helical symmetry (rod or cylindrical = virus)

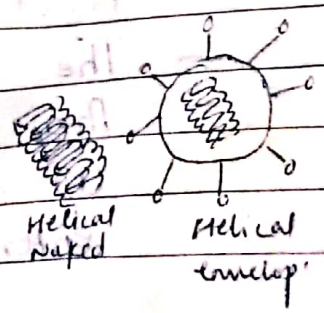
- Virus are made up of single kind of capsomere.
- When this single type of capsomere & nucleic acid combines together & forms a spiral or helical tube & results in rod or cylindrical shape of virus.
- They are highly rigid, long & flexible.

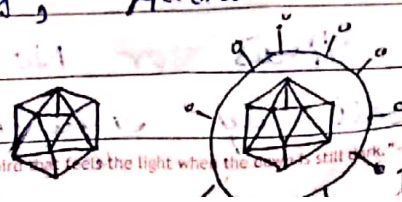
ex → Helical virus, Rabbies virus, Tobacco Mosaic Virus.

Icosahedral Symmetry (animal virus) (20△, 12 corner & 30 edges)

- Generally animal viruses are Icosahedral.
- An Icosahedral is a polygon with 20 △ faces, 12 corners & with 30 edges.
- The icosahedral capsids are of two types :-
  (a) Pentagonal capsomeres c/d pentones
  (b) Hexagonal capsomeres c/d hexones.

ex → Polio virus, Adeno virus





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③ Complex Symmetry

This type of capsid is neither having helical shape nor having icosahedral shape because of its complexicity.

eg → Bacterial Virus (Bacteriophages)

Replication In Virus

— Virus having a lack of biosynthetic enzymes, so they replicate by utilizing ~~ere~~ biochemical activity of host cell to synthesize their macromolecules required for the production of a new virion or virus particle.

— The replication in virus divided into 6 stages :-

a) Adsorption or attachment
b) Penetration
c) Uncoating
d) Biosynthesis
e) Virion Assembly
f) Release

~~Replication / P~~

Adsorption

→ It is the 1st event of replication in virus.

→ In this the virus comes in contact with cells of host and binds with the specific receptor of the host cell

Penetration

→ naked = engulfment by phagosomes
→ enveloped : releases its nucleocapsid cytoplasm

— The penetration of virus occurs by two ways.

— They are –

(1) Naked Virus or Non- enveloped Virus, enter the cell by engulfment of the whole virus.

"The butterfly counts not months but moments, and has time enough." Rabindranath Tagor

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by the means of phagocytosis, mechanism c/d viropexis.

(ii) Enveloped Virus → The envelope of virus used with host cell plasma membrane and releases its nucleocapsid into the cytoplasm.

c) Uncoating

In this virus seprates its nucleic acid & capsid inside the host cell by the action of lysosomal enzymes.

d) Biosynthesis

- After the process of uncoating there is a synthesis of viral nucleic acid & capsid protein by using enzyme present in the host cell.
- The site of viral synthesis depends on the type of virus.

ex → 1) DNA virus synthesize their components in the host cell nucleus

DNA = nucleus

RNA = cytoplasm 2) RNA virus synthesize their components in the cytoplasm of host cell.

e) Virion Assembly (encapsidation)

The newly produced capsomere proteins combines or enclose with the nucleic acid to form a viral nucleocapsid & the process is called encapsidation

f) Release

It is a final event in the replication of virus & results in the release of mature virions from their host cell.

Naked → Reverse phagocytosis, w/o affecting host cell ~~exp~~ pelowm

Enveloped → Envelopment

"Faith is the bird that feels the light when the dawn is still dark." —Rabindranath Tagor

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Cultivation of Virus

→ Viruses are obligate parasites & totally depends on host cell to survive.
→ They can multiply only in living cells.
→ There are 3 methods for the cultivation of virus, are as follows –

a) Animal Inoculation
b) Embryonated Eggs
c) Tissue Culture

Animal Inoculation

→ It is one of the oldest method for the cultivat^n of virus.
→ Reed & Collea gues, in the year 1900 used human volunteers. Due to the serious risks involved human volunteers are used only when no other method is available.
→ Landsteiner & Popper in the year 1909 used monkeys for the isolation of polio virus.
→ Other animals such as rabbits, guinea pigs are used now-a-days.
Example → Pox virus inoculated in the cornea of rabbits. The growth of virus indicated by death or disease of animal.
→ Animal inoculation also used for the study of pathogenesis & immune response.

→ These are identified by microscopy (inclusion body), neutralisation (NT) & haemagglutination inhibition (HI) Tests.

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Embryonated Eggs

→ Good Pasture in the year 1931, he used 1st time Embryonated hen's egg.

→ This method was further developed by 'Burnett'.

→ The embryonated egg offers several sites for the cultivation of viruses.

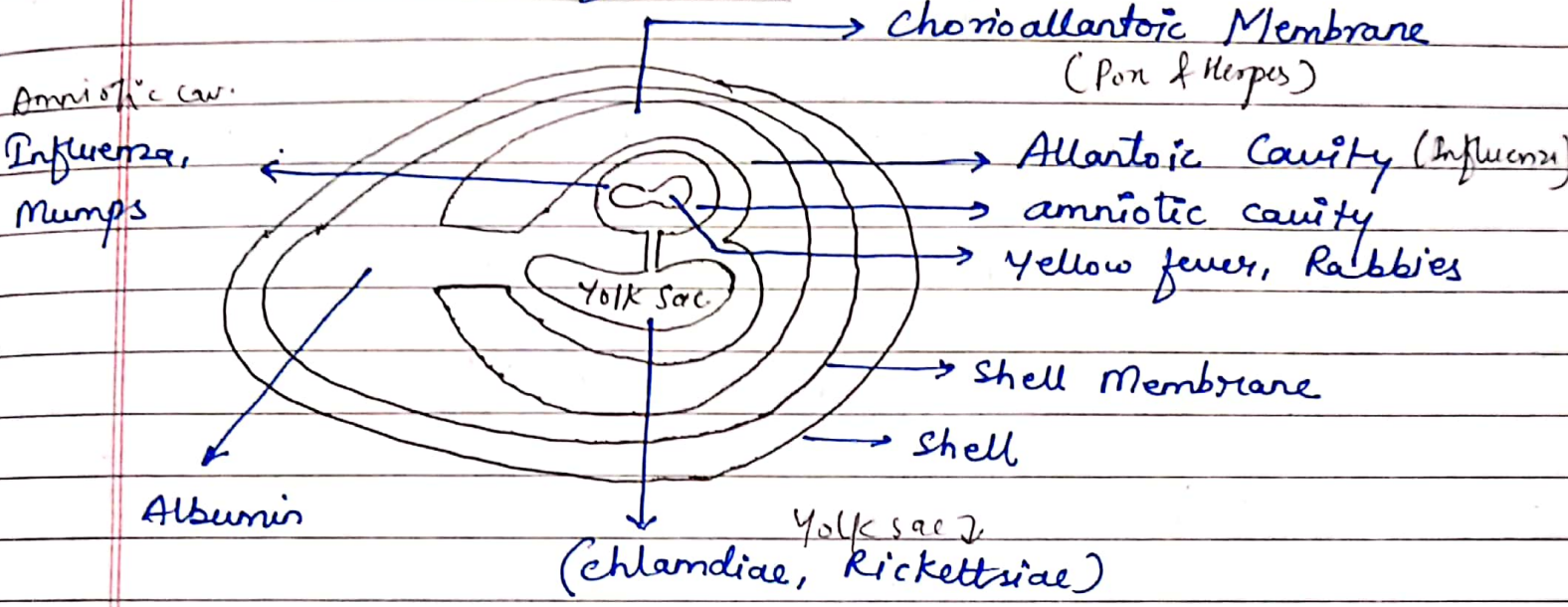

fig:- A chick Embryo Showing the Inoculation Roots for Virus Cultivation.

Advantages of Embryonated Eggs

1. The eggs are much simpler to handle than animals.
2. Eggs are very economical & easily available.
3. They are clean & bacteriologically sterile.
4. Chick Embryo offers several sites for the cultivatn of virus.

Tissue Culture

→ The 1st application of tissue culture in virology was given by "Stein Hardt" and Colleague in the year 1930-1913 who maintained the vaccine virus in fragments of Rabbit Cornea.

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- In year 1928, 'Maitland' used chopped tissues in nutrient media for the cultivation of Vaccinea virus.
- There are mainly 3 types of tissue cultures
  (1) Organ Culture
  (2) Ex-plant Culture
  (3) Cell culture.

Pg/ 135

Organ → Tracheal ring organ culture → Corno virus (an respiratory pathogen)

Explant culture → Adenoid tissue explant → Adeno virus.

cell culture → fibroblast, muscle epithelial cells are used.

tissue removed → broken/cut small pieces by homogenization → washed with salt soln (Hank's or Eagle's sol) → Converted to Constituent cell by dispersion of cells by tissue process → By breaking proteinaceous cohesive fibrous by trypsin (trypsin soln) → putted in flask → cultivated → Incubated

Disinfection

→ It is a process of removal or destruction of microbes that are capable of producing an infection by the use of chemical agents.

→ In this generally the spores are not killed.

Disinfactants

→ These are the chemical substances or agents that are used to kill microbes like bacteria, fungi, etc

Antiseptic

– It is applied to living tissue to destruct microbes.

Sanitarzation

– It is the process of cleaning pathogenic microbes from public utensils, hands, and other objects.

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Ideal properties of disinfectant

→ Should be easily available.
→ Should have pleasant odour.
→ Should not stain tissue.
→ Should be non-irritant
→ Should be non-toxic.
→ Should be able to destroy infectious agent.
→ Should provide fast action.
→ Should be long-lasting.
→ Should be eco-friendly.
→ Should be easily dissolved.
→ Should have a high penetration power to penetrate inside the microbial cell to destruct them.

Classification of Disinfectant

1. Alcohol
2. Heavy metals
3. Halogens
4. Phenol & it's derivatives.
5. Aldehydes
6. Dyes
7. Detergents & Soaps

1. Alcohol

– There are three types of alcohol that are used as a disinfectant.

a) Ethyl Alcohol → virus
Used in conc. b/w 50 – 70% & effective against virus

b) Methanol
They are toxic to eyes & effective against fungal spores.
fungal

c) isopropyl alcohol → bacteria

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c) Isopropyl Alcohol

- Used in conc. b/w 50-70 %.
- Better than ethanol.
- Having Bactericidal property.

Mechanism

It denatures protein & damage lipid complex

(2) Heavy Metals

→ It includes the salts of Cu, Hg, etc. are used as weak disinfectant.

→ 1% $AgNO_3$ is also be used as a disinfectant.

→ Mechanism

It denatures protein of microbial cell.

(3) Halogen (Germicidal action)

→ Under this chlorine, Bromine, Iodine & Fluorine in the free-state work as a germicidal agent.

→ Bromine & Fluorine are the irritants and difficult to handle.

→ Chlorine & Iodine are used as a strong germicidal disinfectant.

→ Iodine

→ Used as skin-disinfectant & is biocidal & amoebicidal

→ Chlorine → Bacteria ↓

- The organic, inorganic & gaseous form of chlorine are mainly used to reduce bacterial population.
- Due to the formation of hypochlorous acid when free chlorine reacts with water.

$$Cl_2 + H_2O \longrightarrow HCl + HClO$$

Hypochlorous

"The butterfly counts not months but moments, and has time enough."

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Mechanism

It oxidises the protein & enzyme of microbial cell.

(4) Phenol & Its derivative → Joesph Lister 1954

→ It is a first chemical agent which is used as an antiseptic & introduced by 'Joesph Lister' in 1854.

→ 1% phenol posseses bactericidal action

→ It has a drawback that it is rapidly absorbed by the skin & mucous membrane & causes toxicity.

→ It can be replaced by similar related compounds like cresol, chloroxylenol, Chlorohexadiene used as antiseptic.

Mechanism

→ It destruct / disrupt the cell membrane & precipitation of cell protein.

(5) Aldehydes

→ There are two types of aldehydes that are generally used for disinfection :-

1. formaldehyde
2. Gluteraldehyde

Formaldehyde

→ It can be used in gaseous form or in aqueous solution,

→ it is sporicidal, bactericidal & virucidal.

→ The gaseous form of formaldehyde used to disinfect room & operation theaters.

→ 10% formalin used to kill bacterial culture, clean, contaminated surface, instruments & also used to preserve tissues.

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Gluteraldehyde

- It is more effective than formaldehyde.
- It is less toxic.
- It is used to kill bacterial spores, fungi & various types of virus, i.e, HIV & enterovirus.

Mechanism

Denatures proteins of microbial cell.

(6) Dyes

→ A no. of dyes used to inhibit the bacterial growth like Acridine & Triphenylmethane & are used as anti-microbial agent.

Mechanism

It inhibit the cellular oxidation process.

(7) Detergents & Soaps

→ They are the surface active agents or emulsifiers that are classified into four groups :-

(a) Anionic
(b) Cationic
(c) Non-ionic
(d) Amphoteric

→ The most imp. antibacterial agent belongs to the cationic surface active agents.

Ex → Cetrimide & Benzalkonium Chloride

8) Oxidising Agents

1. Hydrogen Peroxide (H_2O_2)
2. Potassium permagnate ($KMnO_4$)

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H_2O_2

→ 3% solution of H_2O_2 is used to clean wounds, remove pus & also used as mouthwash.

$KMnO_4$

→ used in the treatment of urethritis.

(9) Ozone Sterilization

- It is used as a disinfectant for food & water. ~~tus~~
- Used in gas or liquid form as an antimicrobial agent.
- Used in the storage & processing of foods.

Factors affecting / influencing disinfection

1) Concentration
2) Time of contact
3) Temp.
4) pH
5) Type or no. of micro-organ. present
6) Surface Tension
7) Chemical str. of disinfectant
8) Formulation of disinfectant

1) Concentration

→ The rate of killing or micro-org. is directly prop. to the conc. of disinfectant.

→ A concentrated disinfectant should act rapidly & a dilute disinfectant should take more time to kill microbe.

ex → 1% phenol is generally used as opt. conc. & below this conc. disinfectant effectiveness goes down

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2) Time of Contact

→ Sufficent time of contact must be allowed to exert it's action. & also depends on the nature of disinfectant, conc, pH, temp, etc.

~~→ Conc., pH~~

3) Temperature

→ The rate of disinfection is increased with the temp.

→ The effect of temp. is expressed by →

$$\theta(T_2 - T_1) = \frac{t_1}{t_2}$$

Where, θ = temp. coefficent

T_1 & T_2 = temp. diff by 10°C

t_1 & t_2 = time req. to kill / lethal time

4) pH

The bacterial growth is optimal in the pH range of 6-8 & outside the range, the growth declines.

5) Type & No. of microbes present

→ Larger the microbe population, more the conc. of disinfectant required.

→ The efficency of disinfectant also based on the species of microbes.

ex → Disinfectants & Antiseptics may have greater effect on gram positive bacteria.

6) Surface Tension

→ If the disinfectant having a surfactant property then it posses a good disinfectant action.

→ It helps in adsorptn of surfactant on the cell of microbes.

"The butterfly counts not months but moments, and has time enough." –Rabindra

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ex → Combination of soap with phenol have excellent disinfectant property (lysol)

7) Chemical Structure of Disinfectant

** → The chemical str. of compound also affects the disinfectant activity.

ex → substitution of an alkyl chain upto 6 carbons in length at para position to phenolic group increases the activity & if greater than 6-C in length decreases the disinfectant activity

8) Formulation of disinfectant

formulation also affects the disinfectant action.

ex → disinfectants like chlorhexadiene, citrimide, Benzal konium chloride shows good effect with alcohol instead of water.

Evaluation of Disinfectants

The effectiveness of disinfectants can be evaluated by two ways :-

1. Bacteriostatic Test
2. Bactericidal Test

Bactericidal Test → water soluble disinfectant

→ It includes phenol co-efficent type tests.

→ Phenol co-efficent test is suitable for water soluble disinfectant & possess anti-microbial action similar to phenol.

- → Rideal walker Test
- → Chick Martin Test
- → AOAC Test.

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Rideal Walker Test

- The test was first introduced by Rideal & Walker in year 1903.

Principle → Salmonella Typhi

In this the dilution of test disinfectant is compared with the standard dilution of phenol & check activity against (Salmonella Typhi.)

Procedure

→ Take salmonella Typhi & produce a culture of it in media in two different plates.

→ Now test disinfectant solution & phenol is added about (0.2ml) in culture plate

↓

At different intervals 2.5mins, 5mins, 7.5mins & 10mins, the sub-culture are taken & transfered to the fresh broth media

↓

Now the broth tubes are incubated at 37°C for 48 - 72 hrs & growth is measured

Chick Martin Test (Organic matter +ve), Salmonell typhi

- The test is given by chick & martin in the year 1908
- The test is used for testing disinfectant in which organic matter is present.
- In this method the test organism is salmonella Typhi

Procedure

In this serial dilution of test disinfectant sol^n & phenol is prepared seprately in distilled water

↓

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To this add 3% yeast suspension also added

↓

Now add the test organism, i.e, Salmonell typhi

↓

After contact time of 30 mins the mixture is transferred to freshly prepared broth

↓

Incubate at 37°C for 48 hrs

↓

Presence or Absence of growth calculated.

AOAC Test

→ In this AOAC stands for (Association of Official Analytical Chemist)

→ In this test three bacterias are used, i.e, Pseudomonas ~~Aueruginosa~~ Aeruginosa, Staphylococcus Aureus, Salmonella Choleraesuis.

Procedure

→ In this the metal carry a rings dipped in culture of test bacteria in liquid media

↓

The metal carry a ring is removed & dried at 37°C for short time

↓

The dried culture are then placed in disinfectant solution for 10mins at 20°C

↓

Now the carrier rings are transffered to fresh broth media

↓

Now the efficiueness of disinfectant can be determined by no. of culture grown.

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II) Bacteriostatic Test

1. Tube dilution test
2. Agar Cup Test
3. Ditch plate Test / Method
4. Kelsey Sykes Test

1. Tube dilution Test

→ In this the disinfectant is incorporated in nutrient broth or agar medium

↓

Now add the test micro-organism

↓

Now this tubes are incubated at 35 °C for 2-3 days (48 - 72 hrs)

↓

After that the result in the form of turbidity & colonies were observed.

2. Agar Cup Test

In this the agar is melted & cooled at 45 °C

↓

Inoculate with test micro-organism

↓

Now poured ~~cold~~ whole media into a petri plate

↓

Make it solidify

↓

Now make holes about 9mm in diameter

↓

Now put the disinfectant soln directly on the hole

↓

Incubate it at 35 °C for 48 - 72 hrs or 2-3 days

↓

"The butterfly counts not months but moments, and has time enough." —Rabindranath Tagor

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The Zone of Inhibition is observed.

③ **Ditch plate method**

Prepare an agar plate
↓
make it solidify & make a ditch
↓
Now put a disinfectant solⁿ on a ditch
↓
The micro-organism that are resistant to disinfectant solⁿ grow near with it & the micro-organ. that are susceptible grow outside the area of ditch.

④ **Kelsey Sykes Test**

→ The method was introduced by Kelsey & Sykes in the year 1960

→ In this method, four test organisms are used – Staphylo coccous aureus, Pseudomonas aeruginosa, E. Coli, Proteus Vulgaris.

→ The test is used to determine the conc. of disinfectant effective in clean & dirty condition.

Procedure

The test organism are cultivated in a medium containing Tween 80 (as an surfactant or emulsifier is used) in a broth.
↓
The reaction is carried under clean & dirty condition for maintaining dirty condⁿ dried yeast is used
↓
Incorporated disinfectant solⁿ
↓
The dilution of disinfectant made in hard water in clean or yeast condⁿ in interval of 0, 10 & 20 mins
↓
Now prepare 3 batches of this diff. interval & each batch consists of 5 tubes containing above medium
↓
Incubate at 32°C for 48 hrs → observe for presence of negative cell culture passes

→ Quality → kill of pathogen not compared with phenol

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Sterility Testing Methods

→ It is used to test microbes in pharmaceutical products like opthalmic products, powders, etc to make the substance free from pyrogens.

Culture Media Used

(I) fluid Thioglycolate media
(II) Alternate thioglycolate media
(III) Soyabean casein digest media

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Method for sterility test

I) Membrane filteration Method
II) Direct Inoculation method

Membrane filteration method

→ The method is used to examine - oil, an ointment, that can be put in solution, a solid that is insoluble in culture medium, a solid powder that is soluble in water or aqueous media, for parenteral products.

- A membrane filter consists pore size of not greater than 0.45 μm & diameter of 50mm.
- The (cellulose nitrate filters) are used for aqueous, oily & weakly alcoholic solution.
- (Cellulose acetate filters) are used for strong alcoholic solutions.

Procedure

drug + vehicle → filtered with membrane → microbes trapped & cultured in cultured media → further tested

II) Direct Inoculation Method

The quantity of substance is diluted & inoculated in culture media
↓
Incubation is done for 14 days
↓
And observe the growth of microbes

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Definatn

- Decaying or deterioration of pharmaceutical product by the contamination of microbe is c/d microbial spoilage.

- The microbes can enter the product at :-
  (1) Time of manufacturing
  (2) Time when the patient used product or drug
  (3) From raw materials
  (4) Packaging of product in a wrong way

Types of spoilage

(1) Infection induced by contaminated pharmaceutical product.
(2) Physical & chemical deterioration or decaying of product.
(3) Observable effects of spoiled products
(4) Ingredients capable of microbial attack

(1) Infection induced by contamination pharmaceutical product
In this contamination of harmul microbes in the product may be caused at the time of manufacturing & also be from raw materials used.
- This contaminated pharmaceutical product gives serious infection to the patient.

(2) Physical
- The microbial growth may occur durn storage of product & results in a toxic product.
ex → (1) Severe eye infection occurs in patient by using multidose eye drop contaminated with pseudomonas aeruginosa.

(2) Contaminated ointment & cream causes burn & skin infectn problem.

manufacturing → raw material
Storage
Toxic product

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→ specialized micro-environment

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② Physical & chemical deterioration or decayn of product

→ Some naturally occurn ingredient ~~product~~ of pharmaceutical operatn were obtained from animal origin (animal ~~origin~~ extrat) & also frm crude vegetables that contains microbial nutrients.

→ The microbe intakes this nutrient & grows & cause decaying of product by affecting its physico-chemical ppts of drugs.

ex → POD depends upon chemical str., physical chemical ppt & level of microbial contaminant present

③ Observable Effect of spoiled product

→ Spoilage of pharmaceutical product becomes visible if there is a high content of microbes present.

→ A spoiled product may gives uppleasant smell or odour, sour taste & also gives change in colour of the product like green, pink, yellow, black, brown microbial pigments.

• Organoleptic Test
• Sour fatty acid
• Fishy amines
• Sickly taste & smell
• lumpy or gritty
• Thickening & suspension agent produce mucoid
• reduction in viscosity by depolymerisation

④ Ingredients capable of microbial attack

(i) Humectants → ↑ conc. of glycerol & sorbital used in pharmaceutical formulation supports microbial growth

(ii) preservative & disinfectant

mostly the organic preservatives & disinfectants are metabolised by various bacteria & fungi & may serves as growth substrate at conc. below its effective level.

(iii) Sweetining, flavouring, colouring agents

Some agents in the pharmacy that are the substrate for microbial growth in aqueous media are as follows :- sweetness, flavouring agent in aqueous stalk like peppermint water & colouring agent like amaranth or tartrazin

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(iv) Therapeutic agents

Those agents used to alter body functions. some therapeutic agents were metabolised & serve as Substrate for microbial growth. They are as follows -

→ analgesic (aspirin, paracetomol)
→ alkaloids (sec. metabolites of plants → morphine, atropine)
→ Barbiturates (sedative actn & inhibit excitatory stimulatn)

Factors affecting Microbial spoilage

1. Size of Inoculum
2. Nutritional factor
3. Moisture Content
4. Temperature
5. pH
6. Package design
7. Protective components (preservative, disinfectants)
8. Redox potential

Sources / Types of Microbial Contamination

Atmosphere
water
Raw material
Process → equipments, Packaging, Buildings
operators

Assessment of microbial contamination & spoilage Evaluation/

1. Physical & Chemical change
2. Sterility Test
3. Assessment of viable micro-organism in non-sterile products.
4. Estimation of pyrogens

(1) Physical & chemical change

The physical & chemical changes in pharmaceutical operations can be identified by -

- Change in viscosity

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- Change in - colour
  - pH
  - amount of O_2 consumed by microbes

② Sterility Test

- All the opthalmic prepration & injectables were tested by sterility test.
- The sterility testing methods are of two types:-
  a) Membrane filteration method
  b) Direct inoculation method
- The sterility test is performed under asptic condition (in a clean room & under the laminar flow cabinet).

Evaluation/ Live/

③ Assessment of viable micro-organism in non-sterile products

The detection of viable micro-organism in non-sterile products is done by using methods like :-
(1) Plate count method
(2) Membrane filter count method

④ Estimation of pyrogens

- Pyrogens are the agents that causes rise in body temp.
- It contains lipopolysaccharides & lipoproteins as a chick constituent in their cell wall of gram negative bacteria & called as endotoxins.
- These endotoxins are commonly known as pyrogens.
- The pyrogens were detected by 2 methods :-
  (1) SHAM TEST
  (2) LAL TEST

(1) SHAM TEST

→ The test is based on the rise in body temp. of rabbit
→ Test Animals :- The test is performed by using a group of 3 rabbits.

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Sample prepration :- Dissolve the test substance with pyrogen free saline solution or other solution & warm it upto 38°C before injection.

Procedure

- The sample substance or solution injected through marginal ear vein of rabbit.
- The vol. of injection is not more than 10ml/kg body wt. of rabbit

↓

Now record the temperature of each animal at hourly intervals for 3 hours

↓

If the sum of response of group of three rabits exceed 1.4°C & if the response of any individual rabbit is 0.6°C the prepration under examination passes the test.

3 rabbit sum > 1.4°C & anyone indi = 0.6°C → sample passes

(2) LAL TEST

- LAL stands for Limulus Amoebocyte Lysate.
- In this the pyrogen containing sample causes the gel formation in the lysis product of amoebocyte cells,
- In this test, LAL reagent is used.
- LAL reagent is an aqueous extract of blood cells (Amibocytes obtained from atlantic horse shoes crab.
- In this LAL reacts with the bacterial endotoxins lipopolysaccharide in the the sample which is a membrane component of gram negative bacteria.
- The pyrogen (endotoxin bacteria) containing sample causes formation of gel.

Preservation of pharmaceutical products using antimicrobial agents

→ To reduce the risk of spoilage & also to kill the contamination of microbes in pharmaceutical products & an antimicrobial agent i.e, preservative included at the time of manufacturing.

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→ The formulations or products, preservatives were added to protect it from microbial spoilage.
The products are -
- Tablets, capsules
- Injections
- semisolids (cream, ointment)
- Opthalmic products (eye drops)
- liquids or mixtures (suspension, syrup)

→ A single preservative is not suitable for preservat of ~~all~~ all pharmaceutical formulation. So the combination of 2 or more preservative are used to extend the range of preservation.
- The selection of a preservative system must be based on the nature of formulation.
- The preservatives are added to the product to enhance their shelf-life (the length of time that a product can be stored & remains suitable for use)
- The preservatives may be natural or synthetic chemical to prevent the undesirable decomposition & changes in the various products like biological samples, pharmaceuticals, etc.

Natural preservatives
ex → Vinegar, salt, sugar, etc.

Synthetic preservatives

Formulation	Preservative	Concentration in w/v
→ Tablets or Capsules	methyl paraben	0.1 %
→ Injections	benzyl alcohol	0.2 - 0.6 %
→ ~~Thiomersat~~ "	Thiomersal	0.01 %
"	methyl hydroxybenzoate	0.1 %
→ Eyedrops	Benzal konium chloride	0.01 %
	Phenyl mercuric nitrate	0.002 %
→ liquids or mixtures	Bronopol	0.02 %

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liquids & mixtures	Alcohol	15 - 20 %
	methyl paraben	0.1 %
	Benzalkonium chloride	0.005 – 0.02 %
	Chlorocresol	0.1 %
Semi-solids	Chlorocresol	0.2 %
	Dichlorobenzyl alcohol	0.1 – 0.2 %

Ideal properties for Anti-microbial agent

- It should be safe to use.
- It should be non-irritant.
- It should not ~~be~~ have any effect on product pH.
- It should not have any adverse effect on product
- It should be economical.
- It should be stable
- It should possess high water solubility.
- It should be stable on various range of temperature & pH
- It should be efficent against various microbes.

Growth of animal cells in culture

- The process in which the cells are grown under controlled condition generally outside from its natural environment (artificial environment) called as cell culture
- In the cell culture technique, the cells may be directly removed or taken by mechanical or enzymatic action from an experimental animal.
- The examples of cell which are obtained from experimental animal for culture are –
  - lymphocytes
  - cells from cardiac & skeletal tissues.
  - liver cells
  - skin
  - Kidney
  - diff type of tumour cells

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- The culture media for the growth and maintainence of in vitro (outside) animal cells should supply –
  - essential nutrients
  - Growth factors
  - Harmones
  - Gases like oxygen & CO_2
  - Physico-chemical environment like pH buffer, osmotic pressure, temperature, etc.

Types of cells involved in the animal cell culture

(1) Primary cells
(2) Transformed cells

Primary cells

- In this the cells are derived from animal tissues & when it transfer to culture medium, they grows limitedly

Transformed cells

- This kind of cells are derived from animal tumours & grows indefinitely in culture.
- They have an unstable & abnormal chromosomes.

Conditional Requirement for cell's growth

- The conditions need to be maintained for animal cell's growth are :-

(1) Balenced salt solution
- Used to maintain the salt concentration & osmotic pressure.
- It is made up of inorganic salts like Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, etc.

(2) Buffering System
- Used to maintain the pH in media.

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- Generally bicarbonates are used to make buffer.

(3) - pH

- Neutral pH value i.e., 7 is provided to facilitate the growth of cells

(4) Temperature

- It varies on the type of host or animal cells.
- Mammalian cells maintained at 37°C for optimal growth.
- While cold-blooded animals maintained at 15-26°C.

(5) Energy Source

- The major source of energy is carbohydrate.
- The most commonly used are :-
  - Glucose
  - Galactose
  - Sucrose
  - Fructose
  - Maltose
  - Mannose, etc

(6) Amino Acids

- These are the building block material for cells.
  ex → Arginine, Histidine, Cysteine, lysine, etc.

(7) Vitamins

Ex → Biotine, Cholic Acid, Nicotinic acid, Riboflavin, pentothanic acid (Vit B5), etc, thiamine, etc.

(8) Hormones & Growth Factors

- They are very essential for the survival & proliferation of cells.
- Most commonly used ones are insulin, hydrocortisone.

General procedure for Cell culture

(1) Prepration & sterilisation of culture vessel
(2) Prepration & sterilization of culture medium.
(3) Isolation of cells or tissues.
(4) Disaggregation of tissue
(5) Transfer of disaggregated cells into media.
(6) Subculturing of cells.

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① Prepration & sterilization of culture vessel

- Before starting the procedure, the culture vessel like glassware & plasticwares used for culturing should be soaked overnight in non-toxic detergent, followed through washing with tap water & then with distilled water.
- Now the containers were sterilized by hot air oven.

② Prepration & Sterilization of Medium

- The constituents of culture media & the reagents used for culturing should be sterilized either by autoclaving or by filteration.
- Autoclaving is generally preffered as it is uniformly effective & it is economic.

③ Isolation of cells & tissues

- Before isolation of the tissue the organ site of experimental animal is sterilised with 70% alcohol & tissues are removed aseptically.
- The isolated tissues are transffered to balanced salt solution
- Now the isolated tissue should be stored under refrigerator before transffering them to a culture medium.

④ Disaggregation of tissues

A suspension of cell can be obtained by disaggregating or breakdown of tissue either by mechanically or by enzymatically.

a) Mechanical disaggregation

- It involves slicing the tissue into pieces & collecting the cells.

b) Enzymatic disaggregation

- It involves the use of enzymes either protolytic

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enzyme or other enzyme.
ex → Trypsin, Collagenase, etc. to breakdown & convert the tissue into cell.

(5) Transfer of disaggregated cell into media
- The disaggregated cells are transferred to the culture medium aseptically.
- The culture medium contains nutritional requirement that facilitate the cell growth.
- Now the whole assembly were stored in incubator in a proper temperature, pH, etc. & incubation is done.
- After incubation the observence of newly formed clones / cells occur limitedly & are called as primary cells.

(6) Subculture

(6) Subculturing of cells
- After the cells formed in primary culture, they were removed with the help of enzymes (those used in obtaining primary culture).
- The removed cells were transferred to the newly or fresh culture medium for sub-culturing.

Advantages of Animal Culture
- It provides controlled physio-chemical environment like pH, temperature, osmotic pressure, etc.
- It achieves homogenity of cells types.
- It requires smaller quantities of reagents.
- It is economical.

Transformed Cell Culture
- Transformation means a phenotypic change which depends on the uptake of new genetic material.
- Transformation of cultured cells means spontaneous

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or permanent phenotypic change due to a genetic change in DNA or gene expression.

- Transformed cells can be grown easily at a faster rate and can be often grown in suspension & have extra or abnormal chromosomes.
- Transformation of cells results in the following change

(1) Genetic Instability

(2) Immortalisation :- The normal cells usually have a life span of 20-100 generations but in this the cells can produce continous cell lines with an infinite life span.

3) Melignancy :-
The cells having the capacity to generate (tend to spread quickly) invasive tumour indicate the condition of melignancy.

Application of cell culture in pharmaceutical Industry & research

- Toxicity Testing
- Cancer research
- Virology
- Cell based manufacturing
- Genetic Engineering
- Gene Therapy
- Model System.
+ Drug effect on cell.
+ Nutritional Study.

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