

Cultivation of Viruses



Dr. S. A. Samant
Associate Professor,
Dept of Microbiology,
MGMMC, NM

Cultivation of Virus

- Since the viruses are obligate intracellular parasites, they cannot be grown on any inanimate culture medium
- Viruses can be cultivated within suitable hosts, such as a living cell
- The primary purposes of viral cultivation are:
 1. To isolate and identify viruses in clinical specimens
 2. To prepare viruses for vaccines
 3. And to do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells

Methods for Cultivation of Virus

□ Generally three methods are employed for the virus cultivation

1. **Inoculation of virus into animals**
2. **Inoculation of virus into embryonated eggs**
3. **Tissue culture**



Inoculation of virus into animals

- The earliest method for the cultivation of viruses causing human diseases was inoculation into human volunteers.
 - Reed and colleagues (1900) used human volunteers for their pioneering work on yellow fever.
 - Due to serious risk involved, human volunteers are used only when no other method is available and when the virus is relatively harmless.
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Contd..

- Landsteiner and Popper (1909) used monkeys for the isolation of polio virus.
 - However due to their cost and risk to handlers monkeys find only limited application in virology.
 - Theiler (1903) used white mice and extended the scope of animal inoculation greatly.
 - Mice are still the most widely employed animals in virology.
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Inoculation of Virus in Animals

- ❑ Laboratory animals play an essential role in studies of viral pathogenesis
- ❑ Live animals such as monkeys, mice, rabbits, guinea pigs, ferrets are widely used for cultivating virus
- ❑ Mice are the most widely employed animals in virology



Inoculation of Virus in Animals

- The different routes of inoculation in mice are:
 - intracerebral
 - subcutaneous
 - intraperitoneal
 - or intranasal
- After the animal is inoculated with the virus suspension, the animal is:
 - observed for signs of disease
 - visible lesions
 - or is killed so that infected tissues can be examined for virus



ANIMAL INOCULATION- DISADVANTAGES

- ❑ **Cost**
- ❑ **Maintenance**
- ❑ **Interference of immune**
- ❑ **Individual variations**
- ❑ **Difficulty in choosing of animals for particular virus**



Inoculation of Virus in Embryonated Eggs

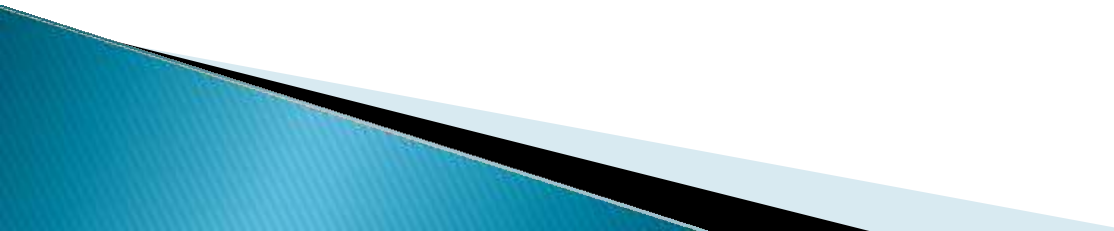
- Goodpasture and Burnet in 1931 first used the embryonated hen's egg for the cultivation of virus
- The process of cultivation of viruses in embryonated eggs depends on the type of egg being used
 - Eggs provide a suitable means for:
 - the primary isolation and identification of viruses
 - the maintenance of stock cultures and the production of vaccines



Embryonated Egg

- Terms most often refer to eggs
 - **Embryonated:** having an embryo
 - **Un embryonated:** not having an embryo
 - **De-embryonated:** having lost an embryo
- Embryonated egg, referring to an advanced stage of development and not merely after fertilisation.

Inoculation of Virus

- ❑ Chicken, duck, and turkey eggs are the most common choices for inoculation
 - ❑ The egg used for cultivation must be sterile and the shell should be intact and healthy
 - ❑ Rigorous sterile techniques must be used to prevent contamination by bacteria and fungi from the air and the outer surface of the shell
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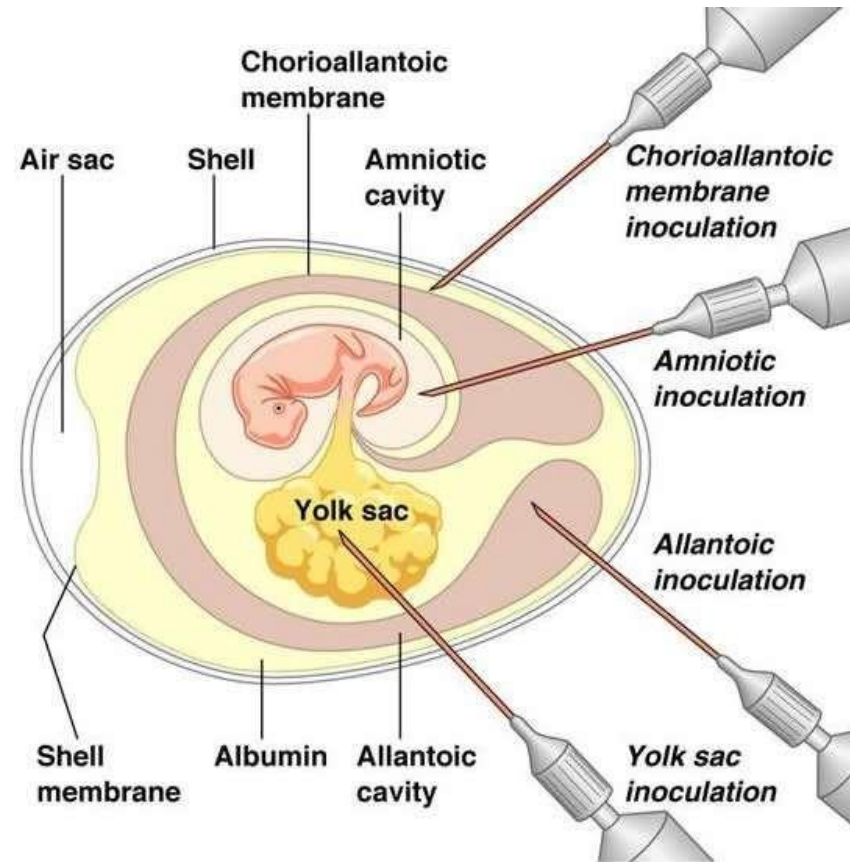
Routes of Viral Inoculation

- An embryonated egg offers various sites for the cultivation of viruses
- The different sites of viral inoculation in embryonated eggs are:

1. **Chorioallantoic membrane(CAM)**

2. **Amniotic Cavity**

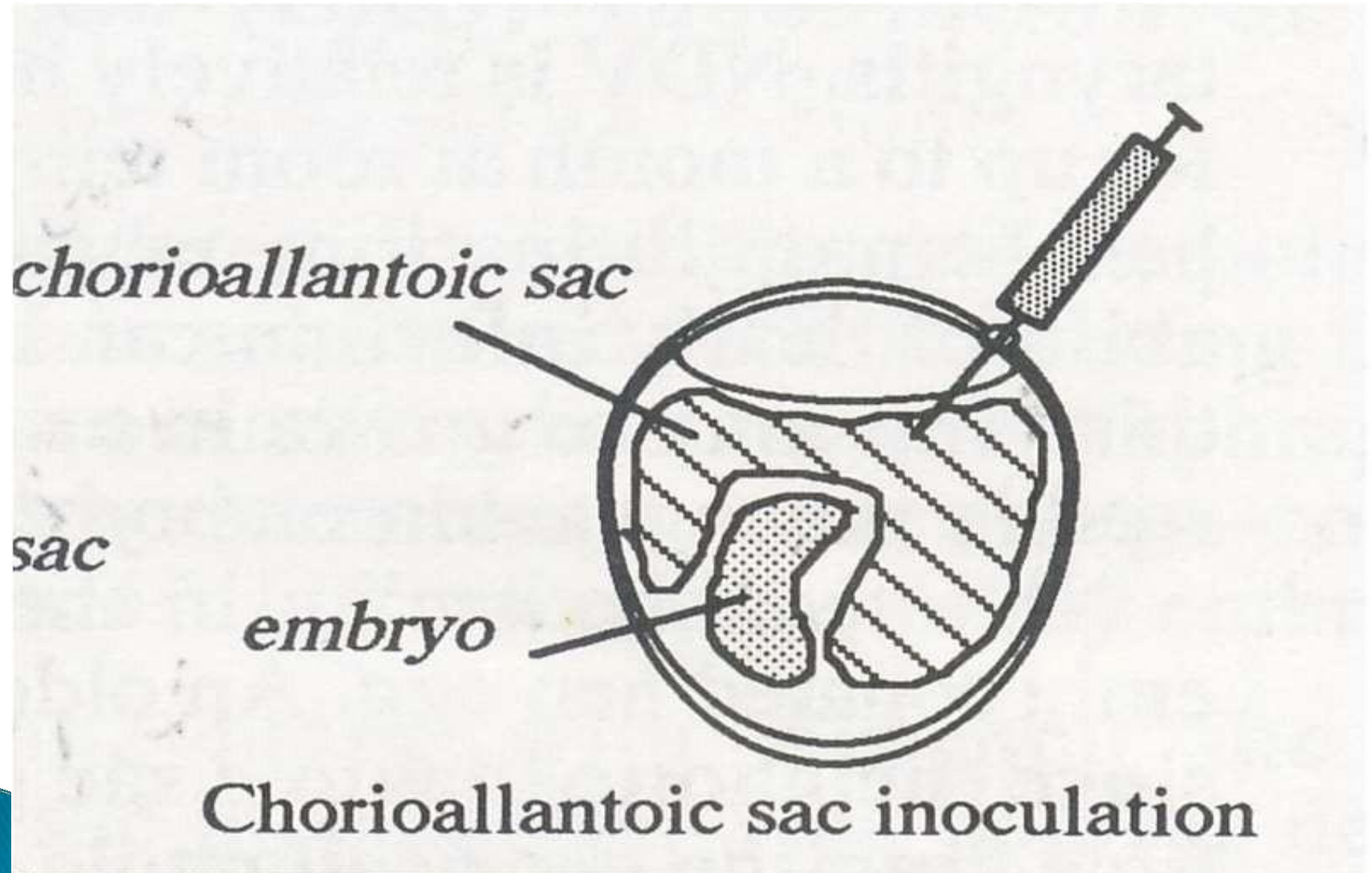
3. **Allantoic Cavity**



Chorioallantoic Membrane (CAM)

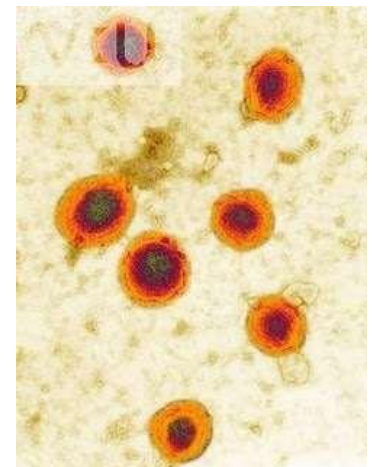
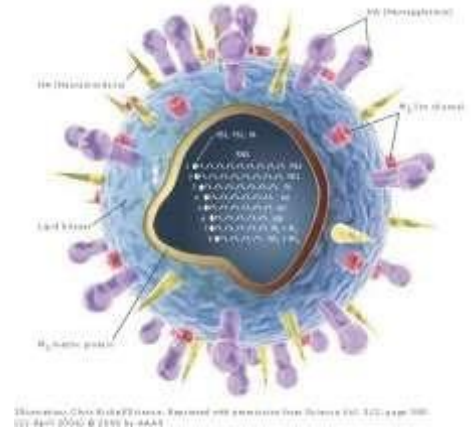
- This method has been widely used in veterinary virology
- Many viruses grow readily or can be adapted to grow on the CAM
- Viruses produce visible foci or 'pocks', inclusion bodies, oedema or other abnormalities
- Each infectious virus particle forms one pock
- Viruses which can be grown include:
 - **Herpes viruses**
 - **Poxviruses**

CHORIOALLANTOIC SAC ROUTE OF INOCULATION



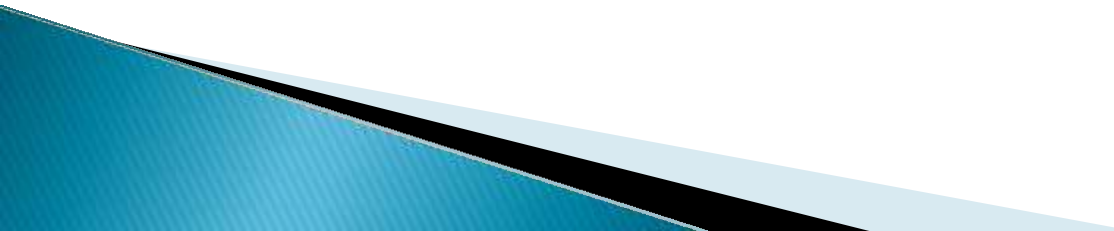
AMNIOTIC ROUTE

- **Primary isolation of influenza and mump viruses**
- **Growth of virus detected by haemagglutination**

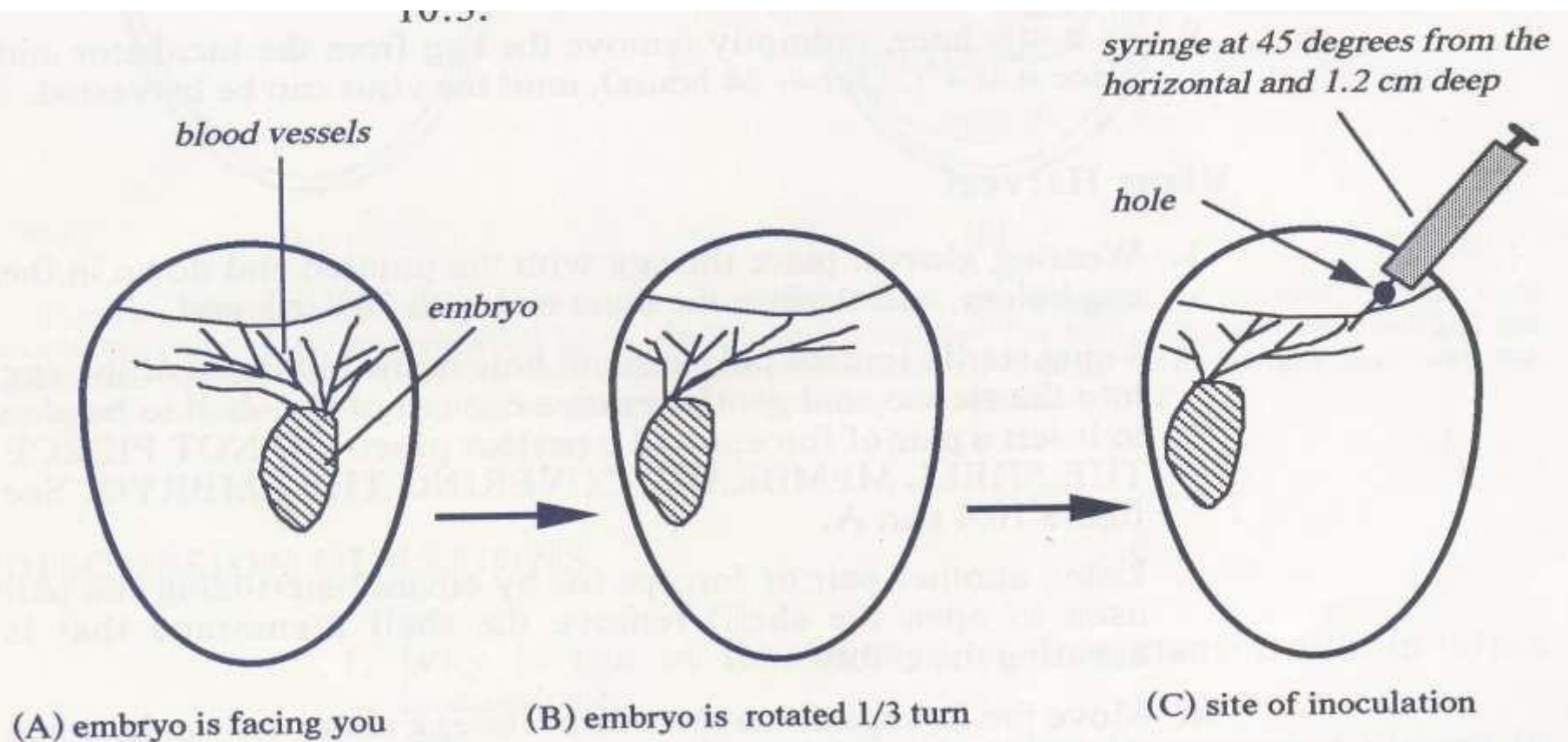


Mumps Virus

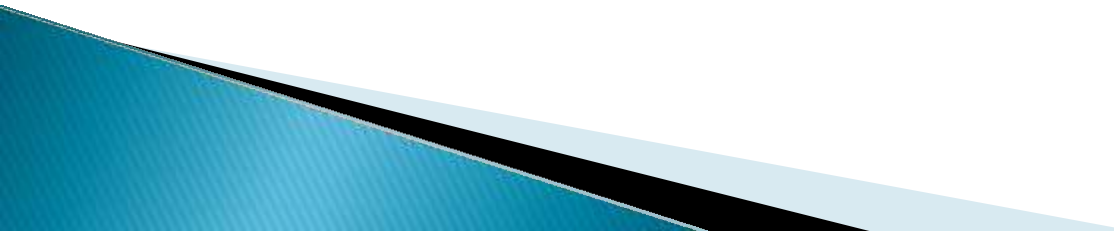
ALLANTOIC ROUTE

- **Most popular**
 - **Most of avian viruses**
 - **High titered virus**
 - **Simple technique**
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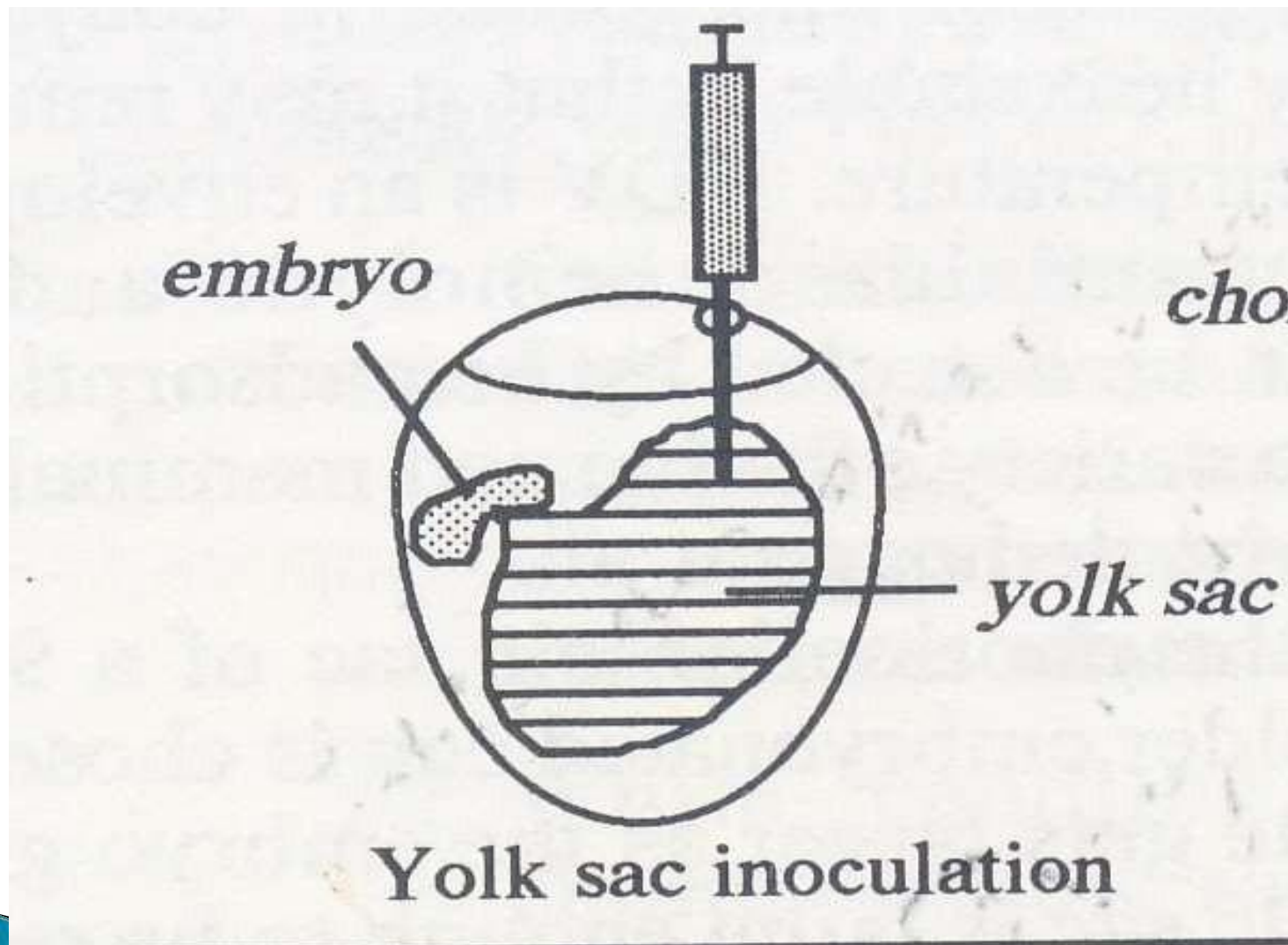
ALLANTOIC ROUTE – INOCULATION SITE DETERMINATION



Yolk Sac Inoculation

- It is also a simplest method for growth and multiplication of virus
 - Mostly mammalian viruses are isolated using this method
 - Immune interference mechanism can be detected in most of avian viruses
 - This method is also used for the cultivation of some bacteria like **Chlamydiae and Rickettsiae**
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YOLK SAC ROUTE



Tissue Culture

- There are three types of tissue culture:
 - **Organ culture.**
 - **Explant culture.**
 - **Cell culture.**
- **A. Organ culture:**
 - Small bits of organs can be maintained in vitro for days and weeks, preserving their original architecture and function. Organ culture are useful for the isolation of some viruses which appear to be highly specialised parasite of certain organs.

For example, the tracheal ring organ culture is employed for the isolation of coronavirus, a respiratory pathogen.

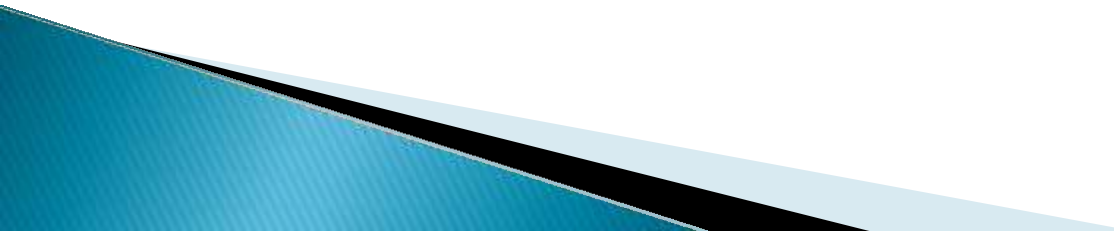
- B. **Explant culture:**

Fragments of minced tissue can be grown as 'explants' embedded in plasma clots.

They may also be cultivated in suspension.

This method is now seldom employed in virology.

Adenoid tissue explant culture were used for the isolation of adenoviruses.



C. Cell culture: -This is the type of culture routinely employed for growing viruses.

- Tissue are dissociated into the component cell by the action of proteolytic enzyme.



- The cells are washed , counted and suspended in a growth medium.



- Such media will enable most cell types to multiply with a division time of 24-48 hrs.



- The cell suspension is dispensed in bottles, tubes or petridishes



- The cell adhere to the glass surface and on incubation, divide to form a confluent monolayer sheet of cells covering the surface within about a week.

Based on their origin, chromosomal characters and the number of generation through which they can be maintained, cells cultures are classified into three types.



Primary cell culture

- These are normal cells obtained from fresh organs of animals or human being and cultured.
 - They are capable of only limited growth in culture and cannot be maintained in serial culture.
 - e.g. monkey kidney cell culture.
 - human embryonic kidney.
 - chick embryo cell culture.
 - They are commonly employed for primary isolation of viruses and in preparation of vaccine.
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Diploid cell culture.

- These are cells of single type, contain the same number of chromosome as the parent cells and are diploid.
 - The diploid cell strains can be subculture for limited number of times.
 - After about 50 serial passage they undergo senescence.
 - They are also employed for the production of viral vaccine. Eg. human embryonic lung cell strain WI-38
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Continuous cell line

- These are cells of single type capable of infinite growth in vitro.
- They are derived from cancer cells.
- They are termed continuous cell lines as they can be serially cultivated indefinitely.
- The continuous cell line have been derived from human cancer such as **HeLa, Hep 2 and KB cells(human carcinoma of nasopharynx)**
- Continuous cell line are maintained either by serial subculture or by storing in deep freeze at -70 so that these can be used when necessary.

Thank You!

