Purification of virus

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- **What is purification?**
- Purification is the process of separating the virus particles of host constituents and other chemical present in sap.
- Purified viral preparations help in-
- Study of physio-chemical properties of the virus
- Virus marphology (Shape & Size)

Methods of purification of virus :

1. Centrifuge
2. Chromatography
3. Electrophoresis

- **By Centrifuge**

Purification of viruses by centrifugation in gradients of inert substances. The **viruses** were separated by isopycnic **centrifugation**in "self formed" caesium chloride gradients, using a Beckman Model E analytical **centrifuge** in which a separation cell fitted with a centerpiece with two perforated partitions was used.

1. Infected leaves are thoroughly homogenized in water or preferably in phosphate, borate or citrate buffer in an electric grinder or in a mortar with pestle

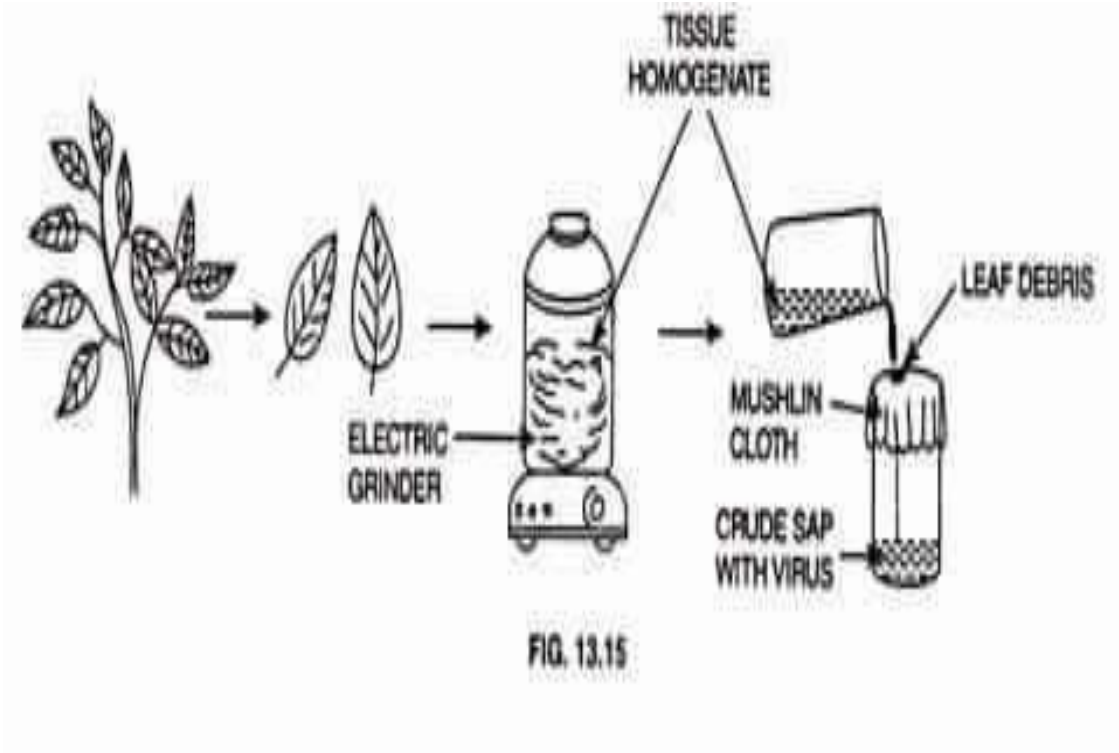

FIG. 13.15

- 2. Tissue homogenate is strained through a piece of muslin cloth (or cheese cloth). Crude sap which comes out and contains virus is collected and then poured into centrifuge tube. The tube is spun at low-speed (3000-17000 g). As a result, the crude sap differentiates into supernatant and a pellet. The pellet is discarded and the supernatant with virus is collected

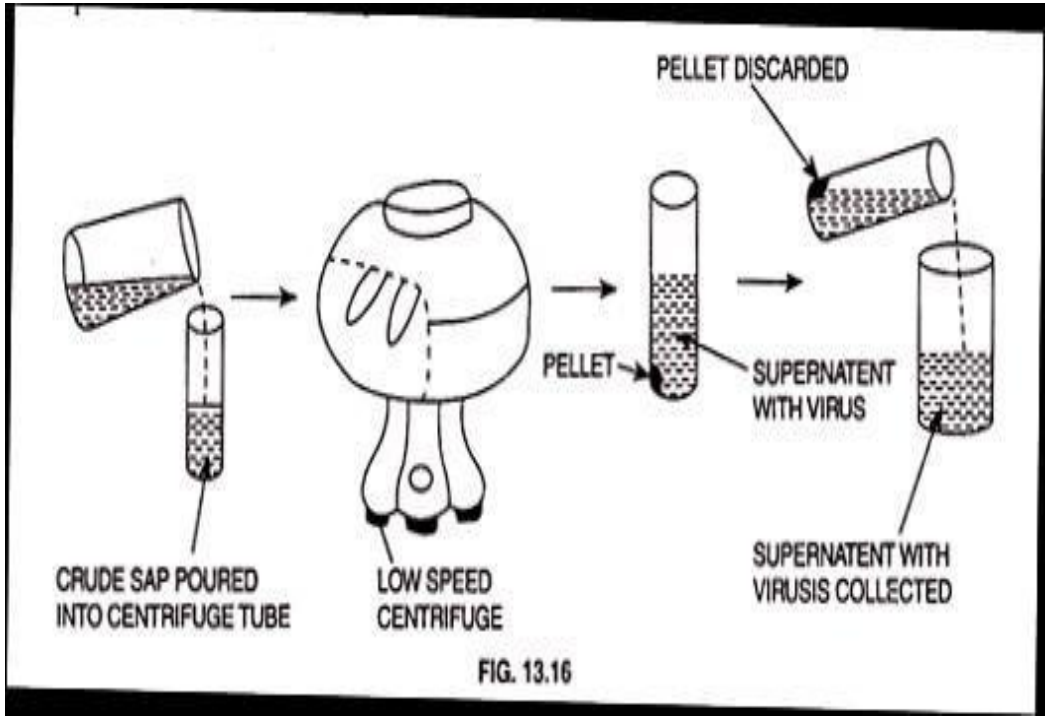

FIG. 13.16

- 3. The supernatant with virus is poured into centrifuge tube. The tube is placed in fixed-angle-rotor of ultracentrifuge and spun at high speed (40000-150000 g). After the tube settles, the virus sediments and forms tiny pellet at the bottom of the tube and a supernatant over it. Supernatant is discarded and the pellet of virus is mixed with a buffer and stirred with rod so that it re-suspends in buffer

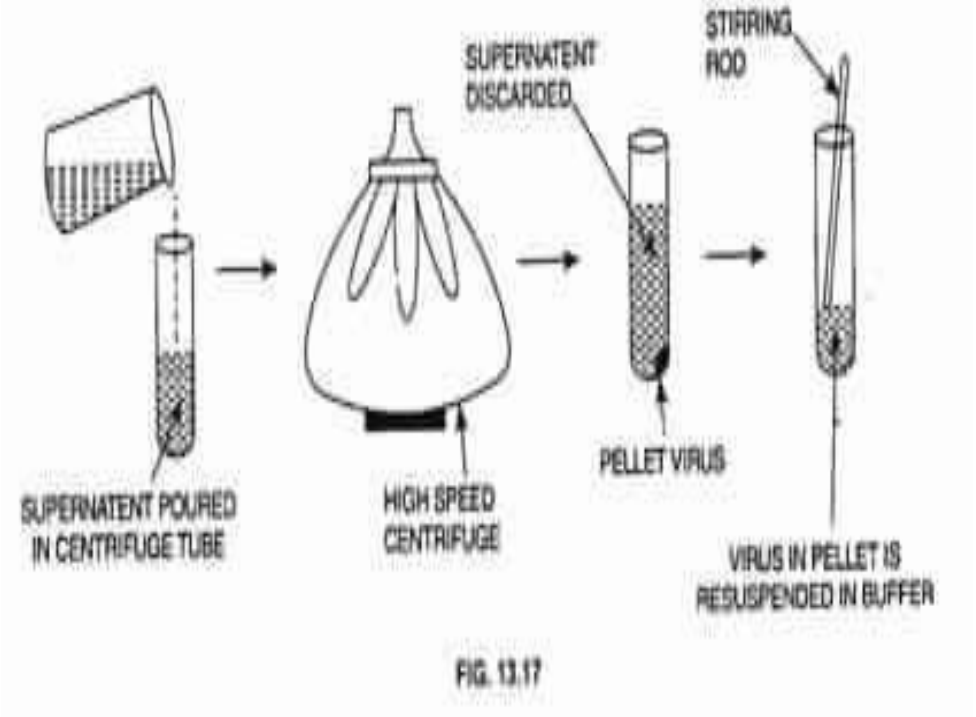

FIG. 13.17

- 4. Low and high speed centrifugation steps are repeated 2-3 times and the virus is purified by density gradient centrifugation, the most frequently used technique.
- A tier of layer of sucrose solutions of different concentrations (e.g., 10-40%), and hence densities, is formed in the centrifuge tube; layer at the bottom being the most dense and one at the top the least dense with layer of intermediate concentrations. Virus suspension is placed at top of the top-most layer and centrifuge tube centrifuged in swimming-bucket rotors at high speed ultracentrifuge

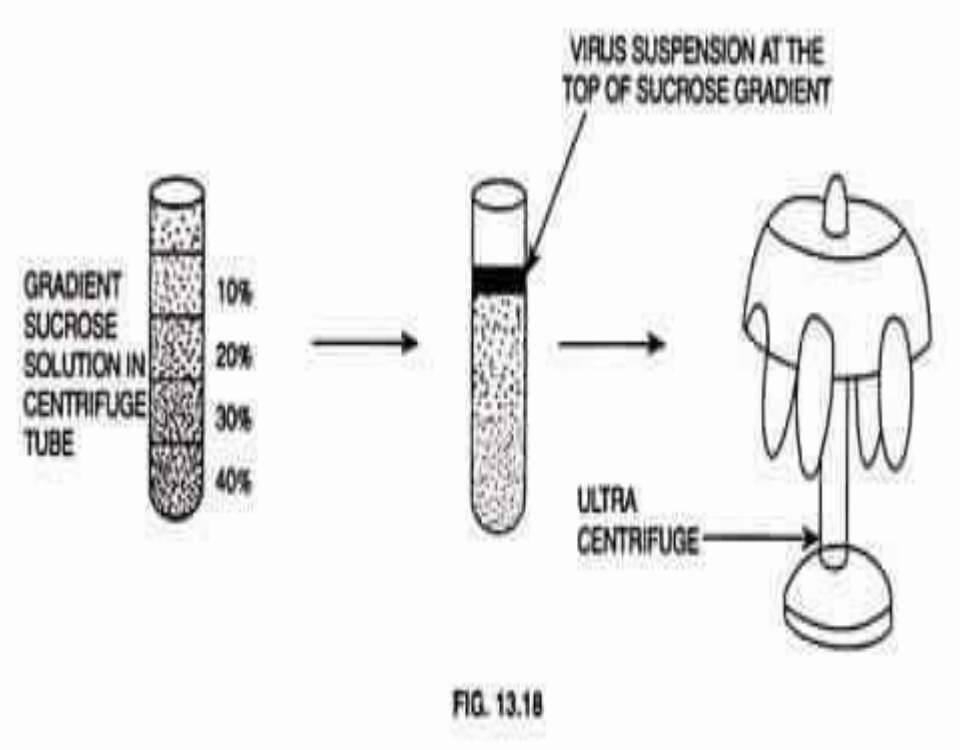

FIG. 13.18

- 5. When settled, plant virus particles move together as a band in gradient solution of sucrose. The virus-band is collected as separate fraction through puncture at the bottom of the centrifuge tube. The virus-fraction is placed in cellulose dialysis tubing and sucrose is removed by dialysis in buffer solution or water. Thus, the virus is obtained in pure form (Fig. 13.19).
- The concept of purity of plant viruses is an optional one because the virus preparation obtained after purification is, however, rarely absolutely pure as it usually contains some impurities.

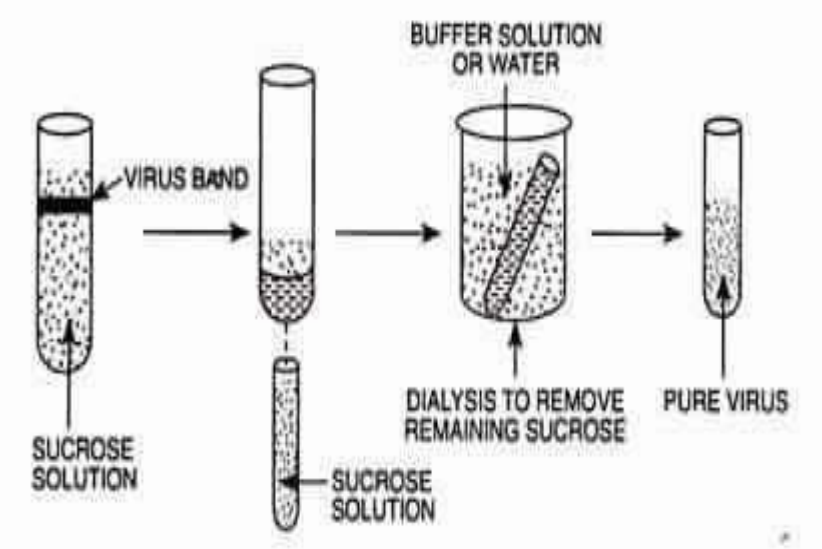

FIG. 13.19

- For practical purposes a virus preparation is considered to be pure if its properties (e.g., amino acid composition, nucleotide composition, percentage of protein, sedimentation profile etc.) do not change upon further purification. However, the purification of a virus is always done with some particular experimental work in mind so that the degree of purity is tested with reference to that work.

- By Chromatography

- **Chromatography is useful for purifying both enveloped and non-enveloped viruses. Most viruses are enveloped which mean that they have their nucleic acid (DNA and RNA) covered in a protein cover called the capsid which further has a membrane envelope on it.**
- **Ex. chickenpox virus and the influenza virus**
- **Non-enveloped viruses do not have the envelope.**
- **Ex. parvovirus and adeno virus**

- **Chromatography methods based on size :**

1. *Size- exclusion chromatography (SEC)*
2. *Ion – exchange chromatography*
3. *Affinity chromatography*

Size- exclusionchromatography

- Size-exclusion chromatography (SEC) efficiently use for purification of retroviruses.
- Using this chromatographic method, retroviruses are excluded from internal pores of the gel due to their large size while low molecular contaminants are retarded by the resin.
- The method produces very high yields of biologically active vectors due to the mild conditions used during this procedure.
- SEC is likely to be useful only as a final step due to its limited resolution and inherent low capacity.
- This type of chromatography tends to operate at low linear flow rates and typically results in product dilution.

Ion-exchange chromatography

- Anion and cation exchange media or charged membranes can be used to adsorb selectively virus particles in the presence of other charged contaminants.
- For example, at neutral pH, the surface of retrovirus particles has predominately negative net charge and therefore virions will selectively bind to anion exchange media.
- Membrane chromatography is an effective alternative to traditional column chromatography which is well suited for particles similar to retrovirus virions in size.
- It combines advantages of membrane technology (high flow rates) and liquid chromatography (high selectivity).
- . Anion exchange membrane absorbers have been tested for purification of lentiviral vectors showing excellent results.

Affinity chromatography

- Affinity chromatography is a separation method based on specific and reversible binding between two molecules.
- This procedure can render high viral yield in a single purification step, thus simplifying the downstream process.
- Another possibility is to explore the natural ability of viruses to bind commercially available affinity ligands.
- This kind of chromatography technique is often called pseudo affinity chromatography.
- Several types of resins based on this principle were used to purify retroviruses.

These are different type of ligands is provided below.

1. Hydroxyapatite
2. Heparin
3. Cellufine Sulfate

Hydroxyapatite

- The mechanism of interaction of hydroxyapatite resins with viruses is not completely understood, but it appears to be combined effect of anion exchange, cation exchange and calcium coordination.
- Hydroxyapatite chromatography matrices, originally used to purify wild-type MoMLV, were found to bind oncoretroviral vectors..
- MoMLV is Moloney murine leukemia virus.
- Ceramic hydroxyapatite, which can provide faster flow rates and larger pore diameters more suitable for purification of virus particles.

Heparin

- Heparin belongs to the family of glycosaminoglycan.
- Heparin affinity chromatography offers the possibility to selectively and efficiently purify retroviruses.
- It was found that soluble heparin inhibit retrovirus vector transduction, indicating a possible interaction between retrovirus envelope protein and heparin molecule.
- The recovery of VSV-G pseudotyped lentiviral vectors reached 53%, while 94% of the impurities have been removed.

Cellufine Sulfate

- The Cellufine Sulfate resin has been shown to be effective for purification of several DNA and RNA enveloped viruses, including human immunodeficiency virus.
- Cellufine Sulfate is produced by the chemical modification of cellulose beads with a low concentration of sulfate ester and mimics sulphated polysaccharides.
- Importantly, Cellufine Sulfate chromatography was successfully used recently for purification of alphaviruses.


Thank You