B. Sc (P) Life Science III year Semester VI

DSE-1 Analytical Techniques in Plant Sciences

Practical- Study of Microscopic Techniques-2

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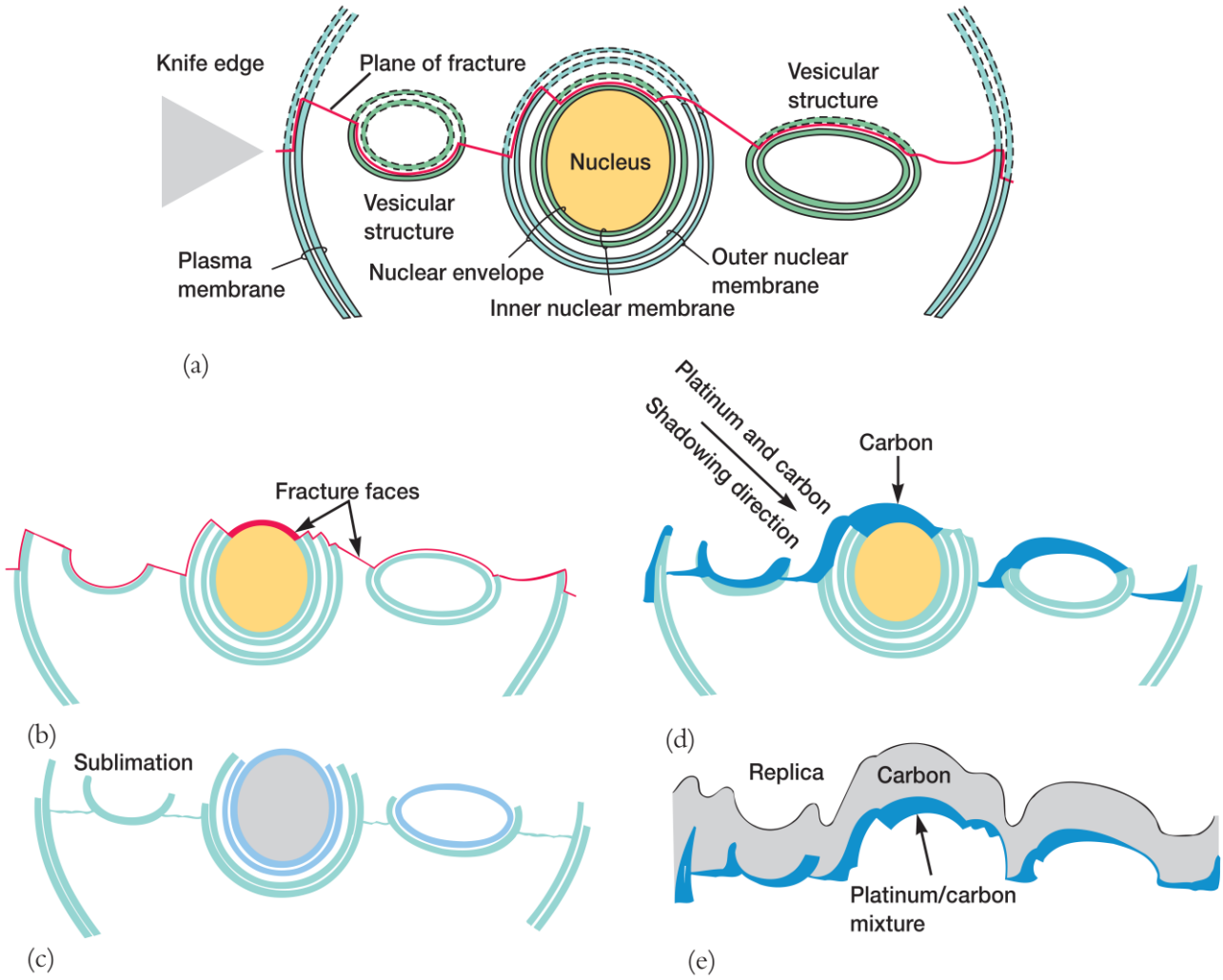
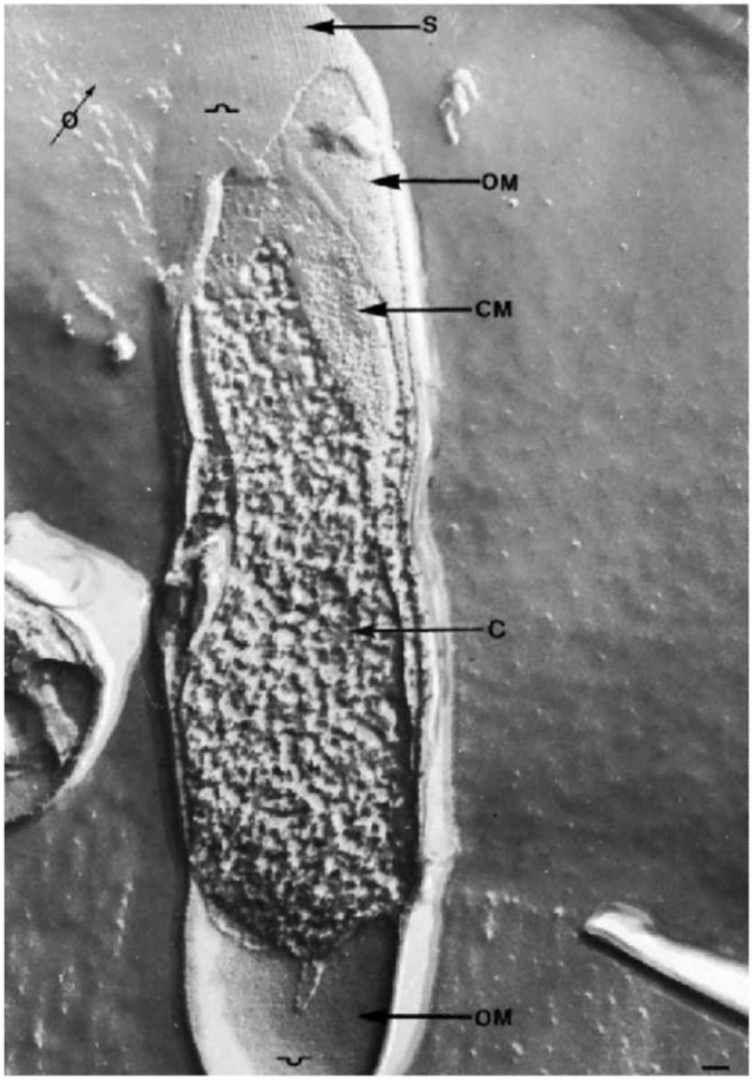
Freeze Etching Technique

- Freeze fracture replication by itself is an extremely valuable technique but Russell Steer (early 1970s) made even more informative by including a step called **freeze etching**.
- In this step, the frozen fractured specimen is exposed to vacuum at an elevated temperature for a few minutes while still in the cold chamber.
- As result a layer of ice evaporates (sublimates) from the exposed surface, preserving the structure of the specimen.
- Now the surface of the structure is coated with ultrathin (2-7 nm) heavy metal coating followed by thick (15-20 nm) carbon coating to stabilize metal film and to elate a metallic replica that reveals the external and internal surfaces of the cellular membrane.
- The technique delivers very high resolution and can be used to reveal the structure and distribution of macromolecular complexes such as those of cytoskeleton.
- This technique has been successfully employed to determine the presence of tight or occluding junctions where membrane glycoproteins bind cells together. The technique serves as the only way to determine the presence of such junctions.
- This technique has also been employed to study the inter-membrane structures.
- It also allows the visualization and detailed analysis of the function of specific proteins in bacteria and viruses.
- The technique is further improved by John Heuser as **deep-etching technique** with even higher resolution (about 2 nm). Greater amount of surface ice is removed which enables the visualization of cellular organelles.
- This technique has also been widely used for ultrastructural investigation in many areas of cell biology and holds promise as an emerging imaging technique for molecular, nanotechnology, and materials science studies.



The Freeze-Etching Technique. In steps (**a**) and (**b**), a frozen eucaryotic cell is fractured with a cold knife. Etching by sublimation is depicted in (**c**). Shadowing with platinum plus carbon and replica formation are shown in (**d**) and (**e**).



A freeze-etched preparation of the bacterium *Thiobacillus kabobis.* Note the differences in structure between the outer surface, S; the outer membrane of the cell wall, OM; the cytoplasmic membrane, CM; and the cytoplasm, C. Bar _ 0.1 _m.

SOURCES

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