low-temperature SEM, see under "Other Imaging and Analytical Techniques"). As the SEM is an electric device, the sample has to be an electrical conductor.

Since biological specimens are usually wet and subject to alteration, they require first a fixation with glutaraldehyde and osmium tetroxide (5), followed by dehydration in graded acetones and, finally, a critical point drying (6) to avoid deformations.

The main difficulties in the preparation of lyophilized material for SEM observation are due to two physical properties:

1. The very brittle nature of the material. Even cutting with ultrasharp blades is very difficult and usually alters the structure. Embedding in resins such as Epon®, Araldite®, or paraffin is difficult due to poor infiltration. Sticking on specimen stubs is also a problem as the material is likely to break easily. Very fluid glues and extremely cautious handling are required. Freezing the material followed by cryofracture (freeze fracture) can be a good alternative to cutting, but poor thermic conductivity of the material can be a problem. A carbon-metal replica of cell wall surface can then be made and studied in TEM for higher resolution imaging.
2. Very high electric insulation due to their organic nature and their cellular structure. This requires a heavy (5–10 nm) sputter coating with gold eventually after carbon coating. This means that high-resolution imaging is not realistic and artefacts due to electric charging are very frequent.

## CASE EXAMPLE: THE STRUCTURE OF FREEZE-DRIED PLUGS

The samples described are lyophilized mannitol freeze-dried plugs produced by freeze-drying in glass vials.

Fracture of the vials was initialized with a glazier's diamond and continued by contact with a red-hot iron rod. The plugs were carefully removed with sharp sickle-shaped tweezers and cut on a hard Teflon® surface with a new stainless steel scalpel. Pieces, each showing surface, cross section, or bottom, were glued with fast-setting epoxy glue on aluminum sample stubs.

A carbon layer was first deposited by evaporation under a vacuum of carbon threads to set a preliminary electric conductivity that facilitates sputter coating. Approximately 10 nm of gold was then deposited by sputtering (physical vapor deposition) in a low-pressure argon atmosphere. Conductive bridges between the sample and aluminum stub were made with silver paint. Special caution should be taken to prevent absorption of the silver paint by capillarity in the material.

Observation was made in a Leica S430 SEM, in secondary electron mode, with an acceleration voltage of 10 or 20 kV.

The pictures shown are a selection from several different sources and, therefore, are not necessarily taken on the same plug. The magnification figure has no sense since reproduction factors are not clearly known. However, the scale bar, usually in microns, is accurate and gives a good idea of the sizes.

The plugs are usually biconcave (Fig. 4) with a membrane-like free surface (Fig. 5). This "skin" is partially torn revealing the subjacent lamellar structure. In the medium internal region, the cell structure is rather regular, showing little perturbation of cell walls (Fig. 6). The region underneath the surface has a lamellar structure (Fig. 7) that may be related to high pressure effects during