

<특별강연II>

CRYOTECHNIQUES IN ELECTRON MICROSCOPY

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Diverse cryotechniques have been used in electron microscopy. For example, rapid freezing as a fixation method of biological materials was introduced. This method has several advantages to the ordinary chemical fixation. Namely, the morphology of living cell and tissue is fixed instantly, and all chemical components including their biological activity can be preserved in situ. therefore, the method seems to be ideal to study morphology as well as cytochemistry. However, serious disadvantage of this method prevents its wider use, namely the limited surface zone ca 10 um deep can be used. Rest of the specimen is damaged due to the ice-crystal formation.

After rapid freezing, the specimens are prepared for TEM either by thin sectioning through freeze substitution followed by plastic embedding, or by freeze-fracture replica method.

For XMA, cryosection are made and are freeze-dried on the grid.

For immunocytochemistry, the material is usually fixed with aldehyde fixative lightly and immersed in cryoprotectant such as sucrose solution.

Cryosections are made and reacted with antibody on the grid.

By using low-temperature cryostage, the frozen specimen can be observed under TEM or SEM directly. In this case, the material is embedded in vitrified ice and protected from the electron beam.