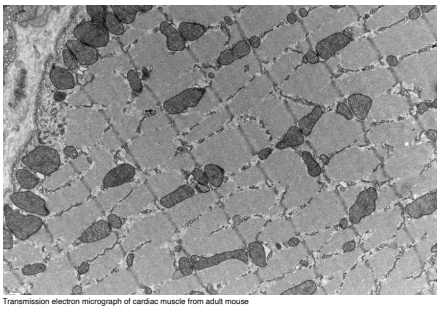
BIOENG - 518

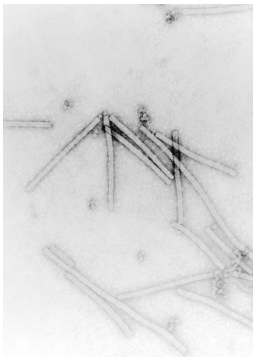
Preparing Samples for Electron Microscopy

Graham Knott, BioEM Facility, Centre of Electron Microscopy, EPFL, Lausanne



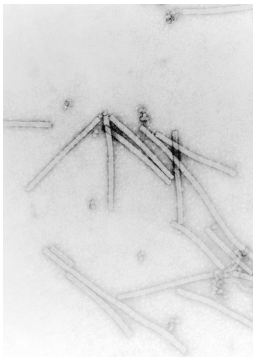
Transmission electron micrograph of cardiac muscle from adult mouse

The Earliest Imaging of Biology Using Electrons



Helmut Ruska, 1939, imaged the tobacco virus

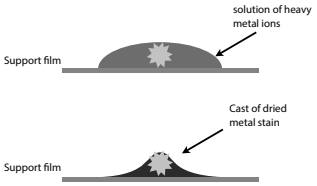
The Earliest Imaging of Biology Using Electrons



Helmut Ruska, 1939, imaged the tobacco virus

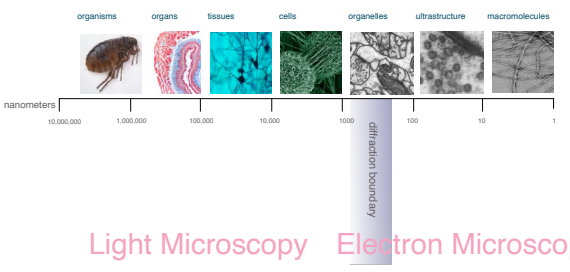
Technique - negative staining

-virus dried to a thin film
-solution of electron dense stain applied
-dried
-imaged



Scale of biological structures

Why we need electron microscopes to see biological structures



Light Microscopy Electron Microscopy

jelly fish - aequoria victoria

Considerations for EM of biological structures

- Samples contain atoms of low molecular weight
- Most biological samples contain water
- Intense heat of the e beam
- High vacuum
- Size of Specimen - only thin or very small samples can be imaged without sectioning

A STUDY OF TISSUE CULTURE CELLS BY ELECTRON MICROSCOPY

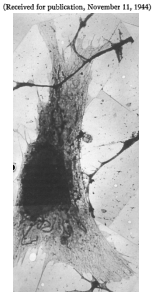
METHODS AND PRELIMINARY OBSERVATIONS

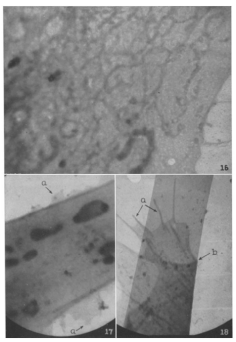
BY KEITH R. PORTER, PH.D., ALBERT CLAUDE, M.D., AND ERNEST F. FULLAM

(From the Laboratories of The Rockefeller Institute for Medical Research, and The Research Laboratories, Interchemical Corporation, New York)

PLATES 10 TO 14

(Received for publication, November 11, 1944)





A STUDY IN MICROTOMY FOR ELECTRON MICROSCOPY

KEITH R. PORTER AND J. BLUM

The Laboratories of The Rockefeller Institute for Medical Research, New York, N. Y.

1953

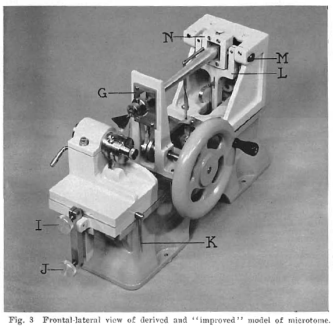
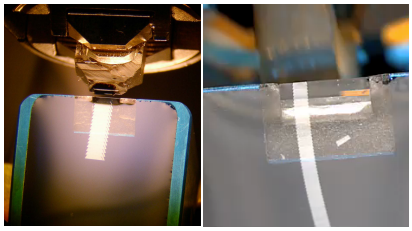


Fig. 3 Frontal-lateral view of derived and "improved" model of microtome.

Serial thin sectioning



50nm thick sections collected on water

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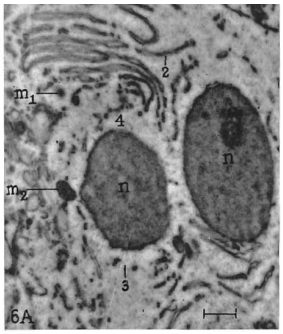

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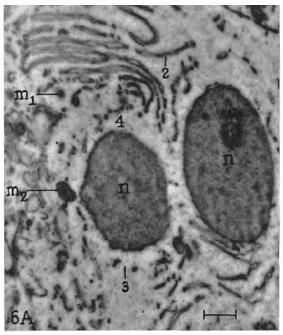

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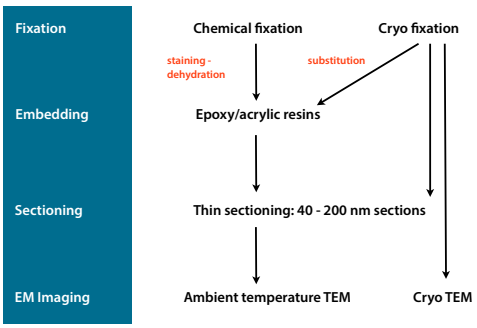
General scheme for preparing biological samples for EM



Egyptian mummy - few thousand years old

Fixation

A process used to preserve the structure of freshly killed material in a state that most closely resembles the structure of the original living state.

Chemical - coagulative/noncoagulative

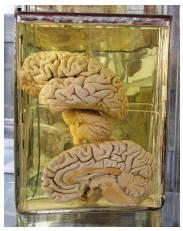
- Coagulative: original killing agents (alcohols, Bouin's), Low pH, unbuffered, coagulates cellular components.
- Non-coagulative: formaldehyde, glutaraldehyde, osmium tetroxide

Freeze Fixation

Baby mammoth, 37,000 years old

Chemically fixing biological samples

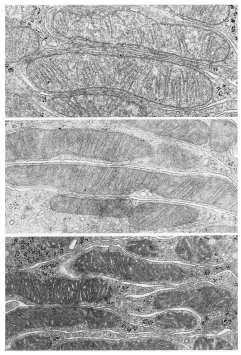
- Pieces should be small
- The volume of fixative should exceed the volume of tissue
- Insects, other invertebrates have very impermeable coating
- Larger tissues should be perfused with the fixative



Chemical fixation of biological samples - considerations

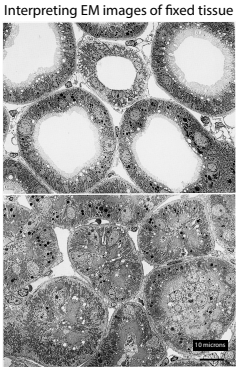
pH (Isoelectric point)
Total ionic strength of reagents
Osmolarity
Temperature
Length of fixation
Method of application of fixative

Osmolarity



in 0.1M phosphate buffer

Interpreting EM images of fixed tissue



TEM images of kidney tubules

Typical fixatives - aldehydes

Formaldehyde

Low MW - makes it one of the best penetrating of all the fixatives, widely used in fixation of resistant materials, such as seeds, spores, plant material,

Formalin 7-40% formaldehyde with methanol (up to 15%) which prevents polymerization

Glutaraldehyde

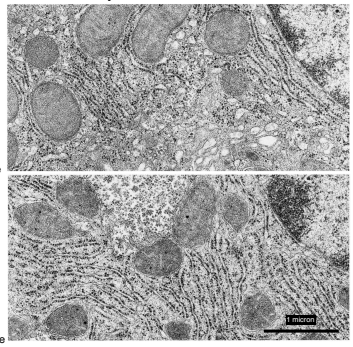
Glutaric acid dialdehyde, a 5 carbon dialdehyde, is the most widely applied fixative in both scanning and transmission electron microscopy.

Most highly cross-linking of all the aldehydes. Glutaraldehyde fixation is irreversible.

Effects of aldehydes fixatives

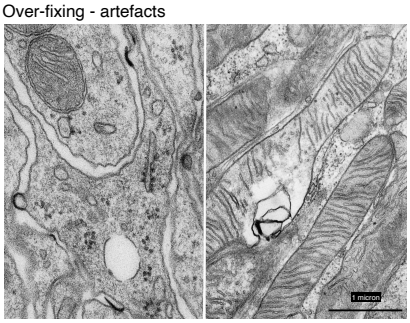
glutaraldehyde

formaldehyde



TEM images of rat liver

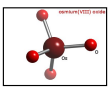
Over-fixing - artefacts

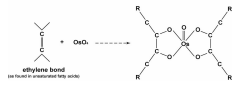


Muscle tissue fixed for 3 days at room temperature in 2.5% glutaraldehyde

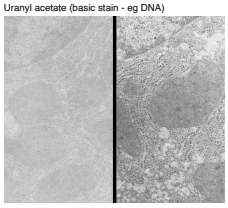
Other fixatives, and stains

Osmium Tetroxide (membranes, proteins)

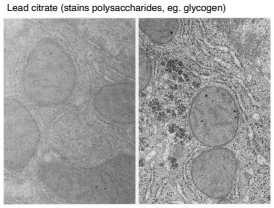




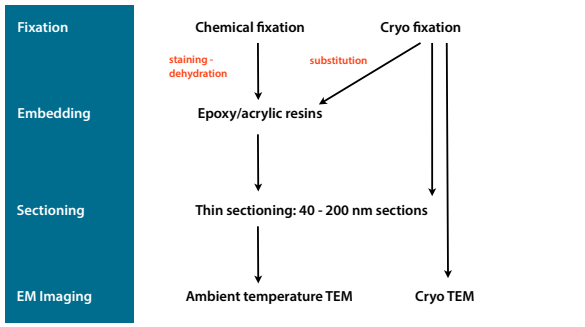
Uranyl acetate (basic stain - eg DNA)



Lead citrate (stains polysaccharides, eg. glycogen)



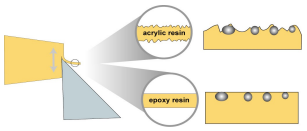
General scheme for preparing biological samples for EM



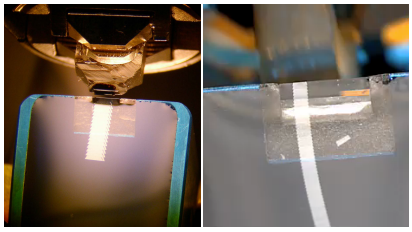
Embedding in resin

Epoxy resin - araldite

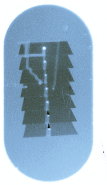
Acrylic resin - methyl methycrylate



Serial thin sectioning



50nm thick sections collected on water

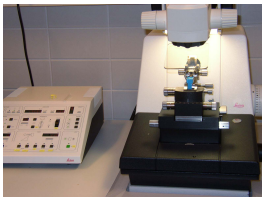


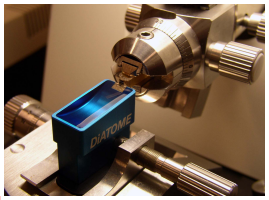
beam damage

DISADVANTAGES OF SSTEM FOR 3D STRUCTURAL ANALYSIS

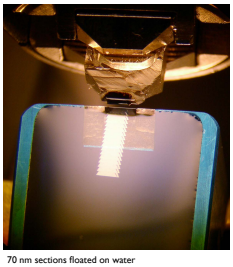
- labour intensive
- slow
- section loss and damage
- thickness of section

Tissue blocks are sectioned with diamond or glass knives using ultramicrotomes

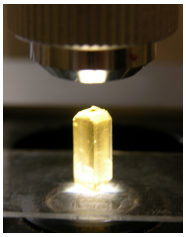




Serial thin sectioning

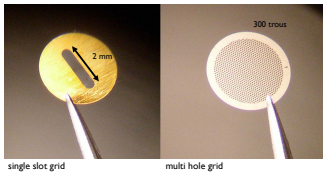


70 nm sections floated on water

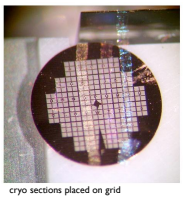



Resin embedded tissue blockUltramicrotome - for block preparation and sectioning

Typical EM grids for holding sections



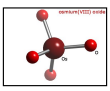
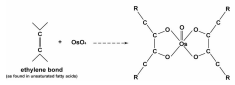
single slot grid multi hole grid



cryo sections placed on grid

Other fixatives, and stains

Osmium Tetroxide (membranes, proteins)

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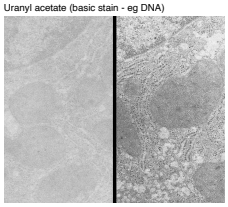
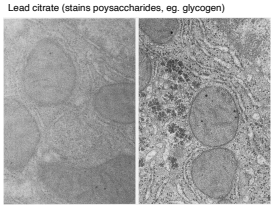
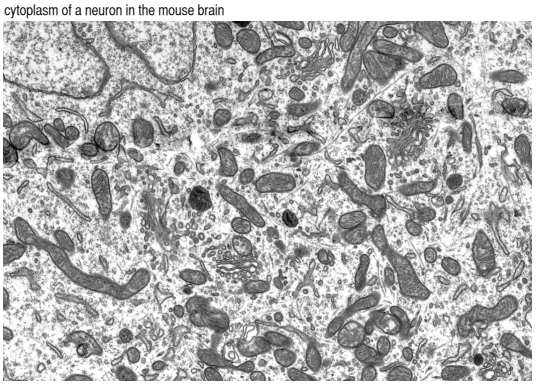
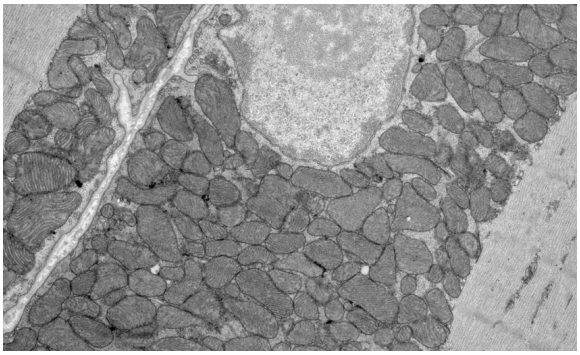


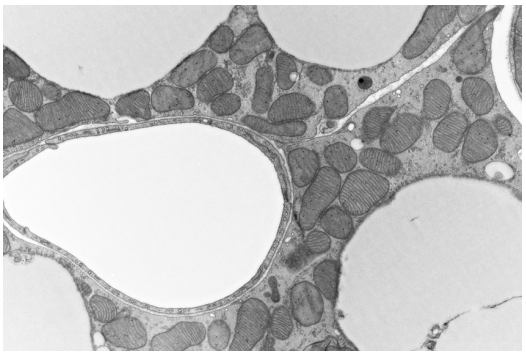
Lead citrate (stains poysaccharides, eg. glycogen)



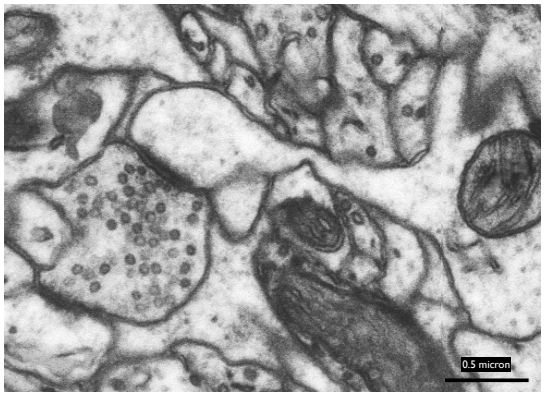
cytoplasm of a neuron in the mouse brain







TEM of adult mouse S1 layer IV



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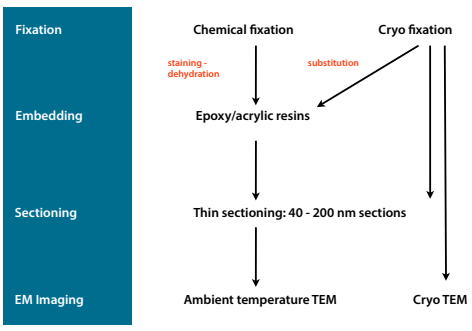
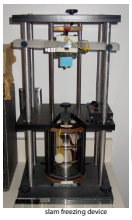


Cryogens	Melting pt	Boiling pt
Freon 13	-181	- 81
Isopentane	-160	28
Propane	-189	-42
Nitrogen	-209	-196
Ethane	-183	-88
Helium	-272 (1° K)	-269

Freezing Equipment

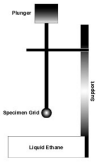
Device	Freezing depth (microns)	cost
Plunge freezer	10-20	2D
Spray freezer	10-20	10-50
Slam freezer	20-40	2K
Propane Jet	40	10K
High Pressure	50-100	400K

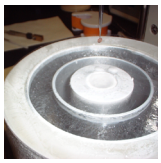
plunge freezing bath

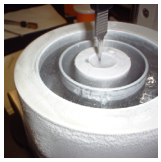

slam freezing device

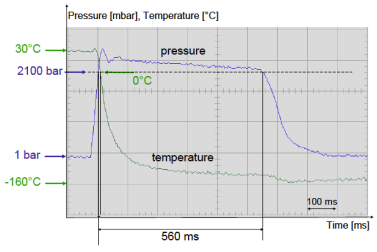

high pressure freezer

Plunge freezing a sample of protein complex in solution





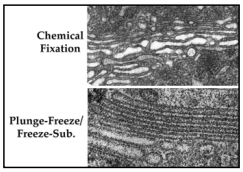




Freeze fixation

Reasons for freeze fixation

- halt rapid events
- structures are fixative sensitive.
- removal of water changes topography/morphology



Disadvantages

- Specialized equipment required
- Freeze Damage
- Limited view of specimen or difficulty manipulating frozen material
- Hazards of using some cryogens