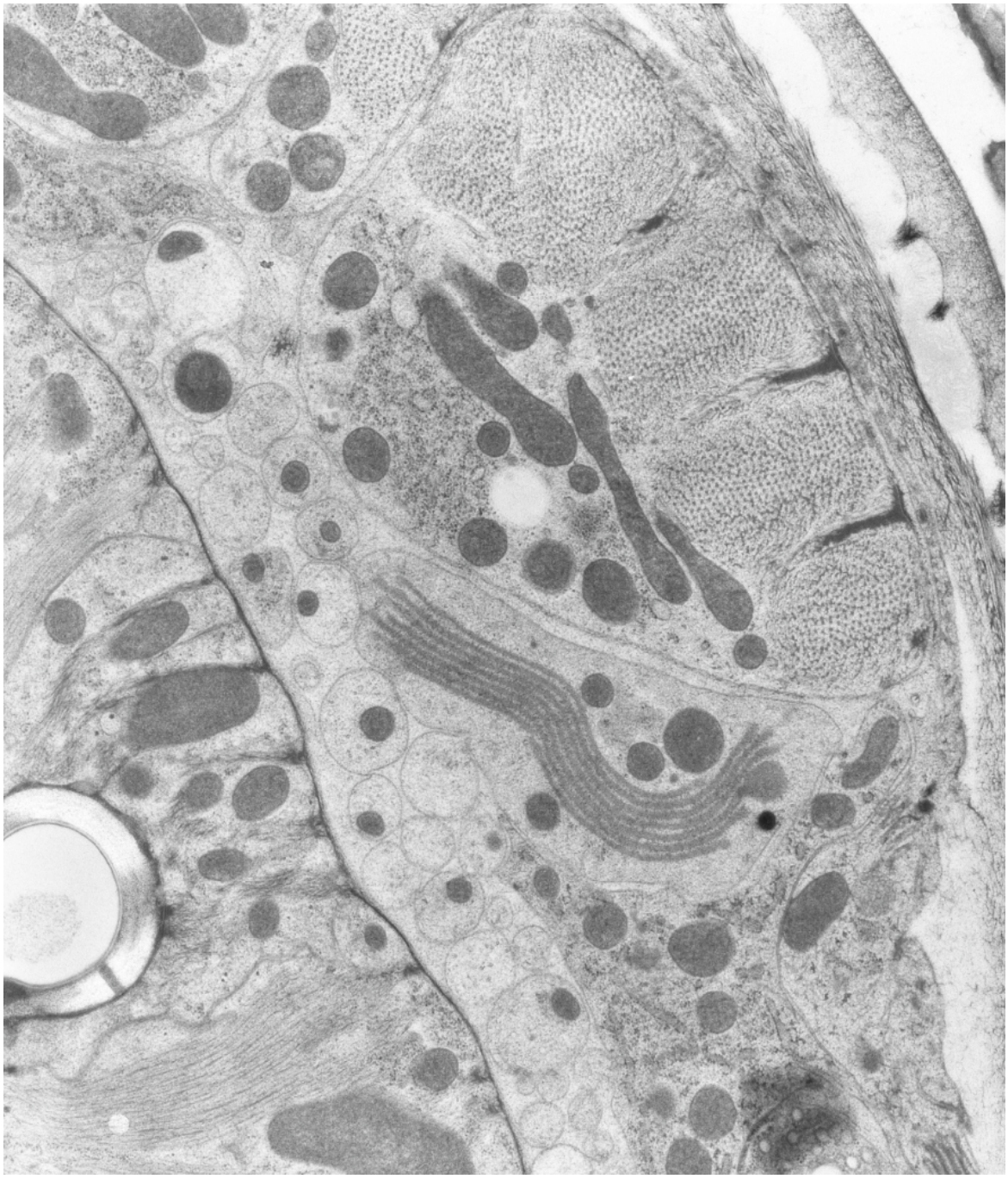
Freeze substitution with tannic acid/osmium (short incubation) January 2004

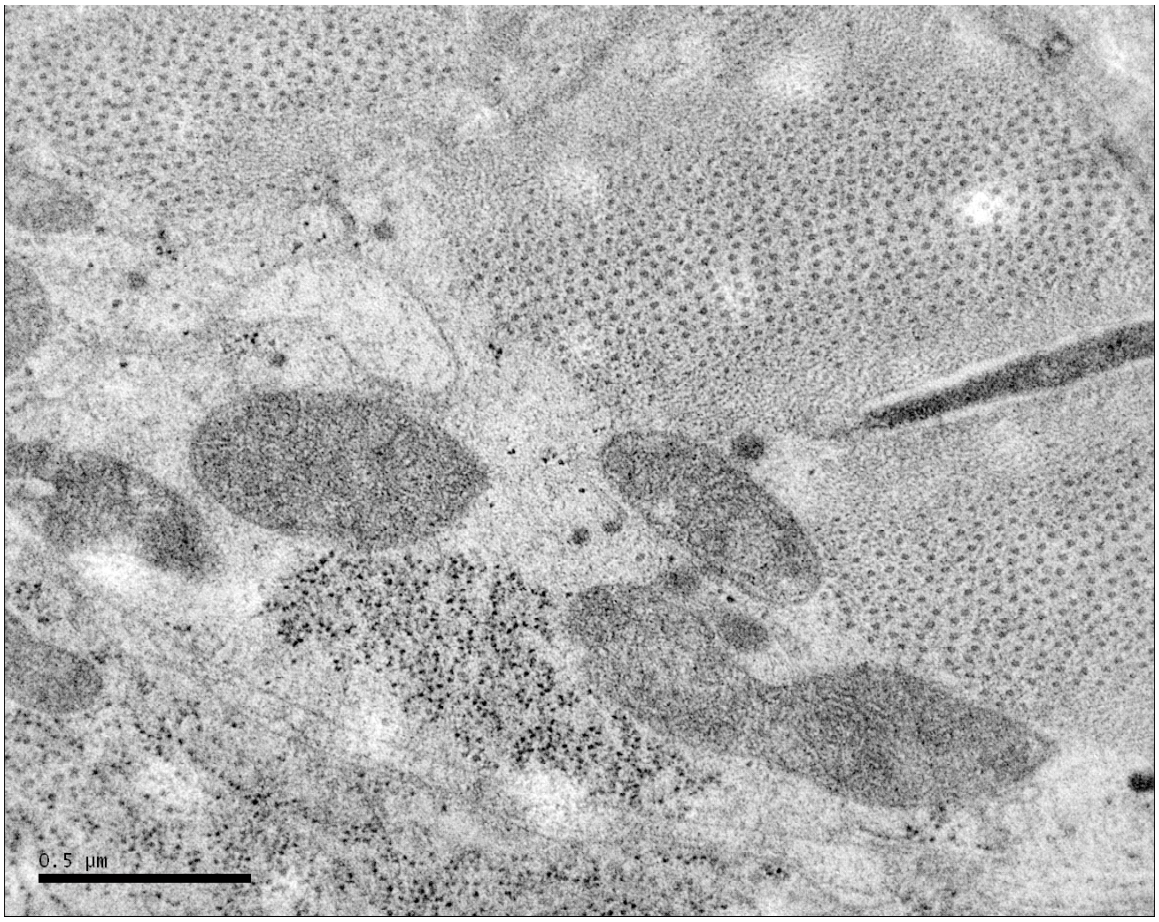
Freezing:
Several animals (~10) were loaded into a 100um deep chamber surrounded by *E. coli* and frozen in a BalTech HPM 010.

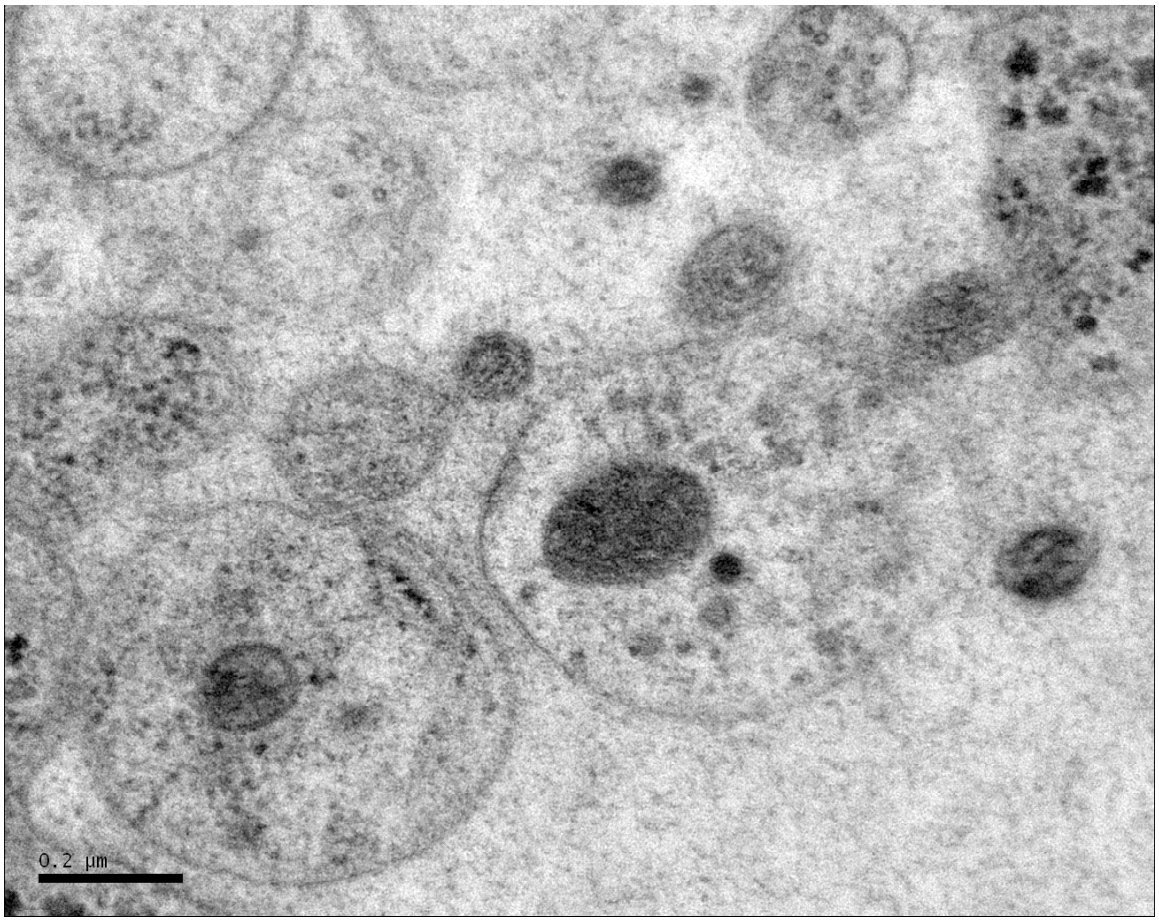
Freeze substitution and embedding:
The freeze substitution was carried out in an Reichart AFS.
-90C for 4 days in 0.1% tannic acid in anhydrous acetone
-90C for 2 hours in anhydrous acetone, changing the acetone several times
-90C for 4 hours in 2% osmium in anhydrous acetone
-90C for 2 hours in anhydrous acetone, changing the acetone several times
-90C to –45C at 4C/hr, total of 11.25 hours
removed the sample from the AFS and placed it at –20C overnight, then
4C for 1 hour, then room temperature for 1 hour and embedded in Araldite 502.

Sectioning:
40nm sections were collected on formvar covered grids and counterstained (4 minutes in 2.5% uranyl acetate in 70% methanol, 2 minutes in Reynolds lead). Micrographs were collected using a Gatan digital camera while the specimen was being viewed in a Techni 12 electron microscope at 80kV.

Observation:
Most tissues were well preserved, however, some membranes (specifically, synaptic vesicles and mitochondrial membranes) are poorly contrasted (see following micrographs).




0.5 µm


0.2 µm