

# Electron Microscopy in Diagnosis of Infectious Diseases

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**C**



Duke Medicine

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Society for  
Ultrastructural Pathology



# Outline Part 2

## **C. Virus look-alikes**

- 1. Examples of confusing things in fluids**
- 2. Examples of cell organelles that resemble viruses in tissues**

# Things That Can Look Like Viruses But Are Not in Negative stains of Fluid Samples

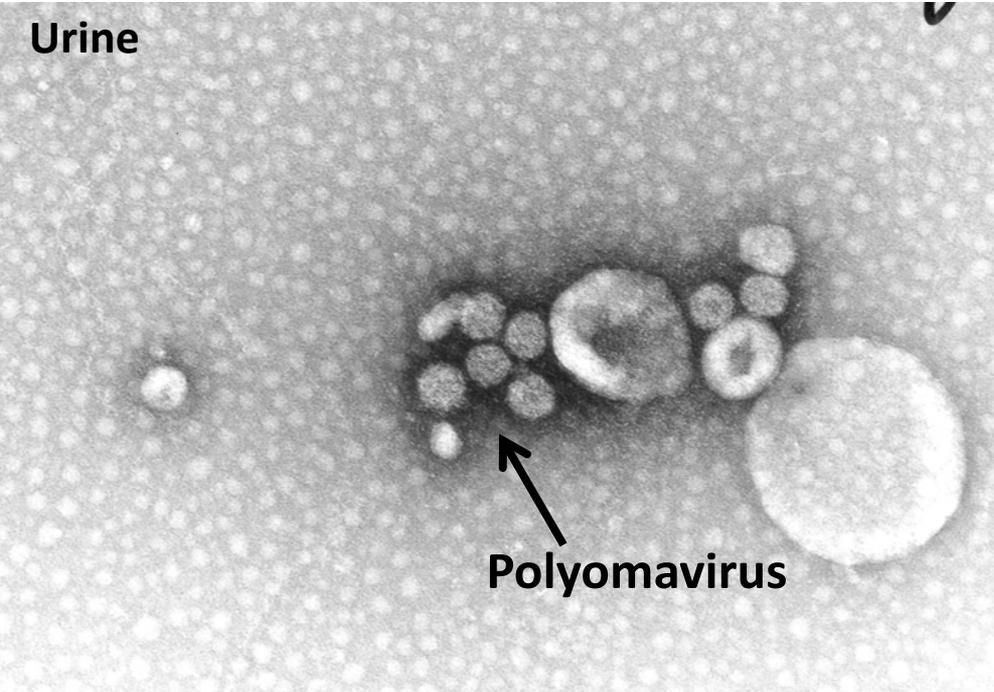


**Look-alikes**

## Things That Can Look Like Viruses But Are Not in Negative Stains of Fluid Samples

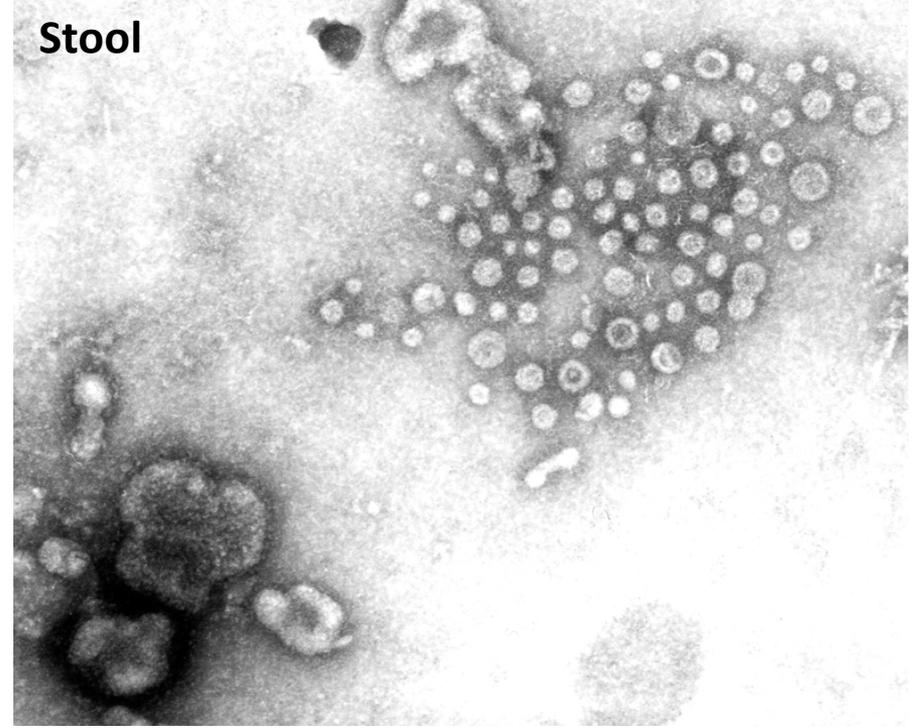
- **Resemble spherical virions or nucleocapsids**
  - **Lipid droplets**
  - **Protein droplets**
  - **Membrane vesicles**
  - **Ribosomes**
  - **Tailless bacteriophages (are viruses, but not pathogenic to humans)**

Urine

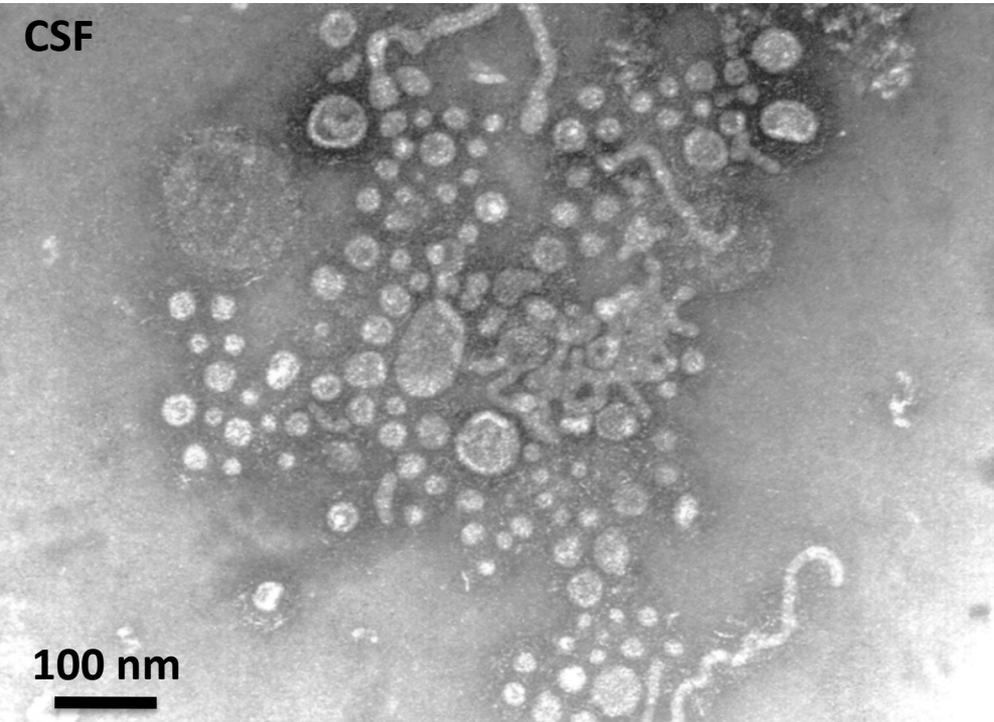


Polyomavirus

Stool

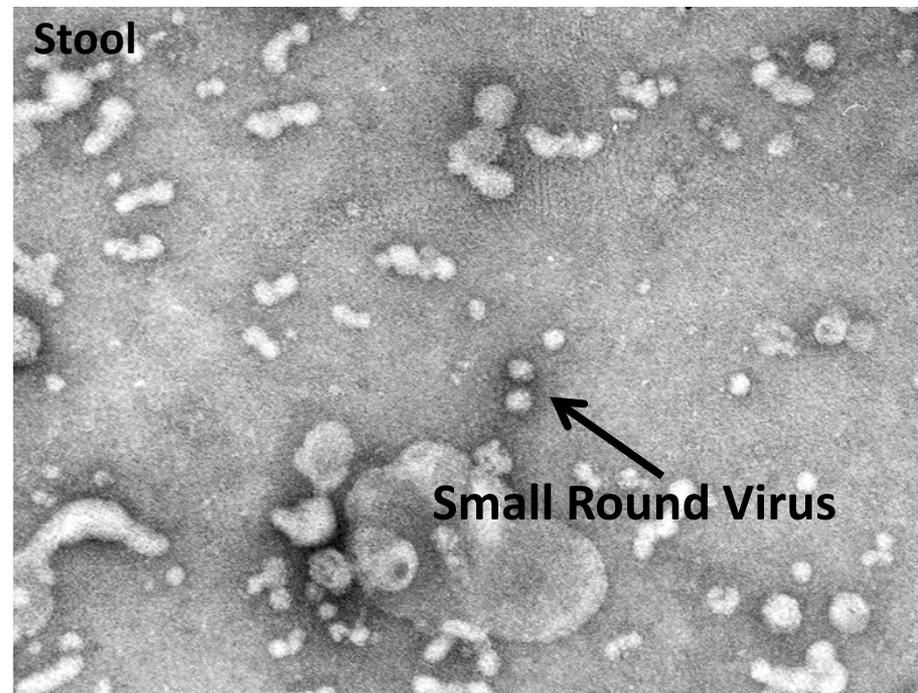


CSF

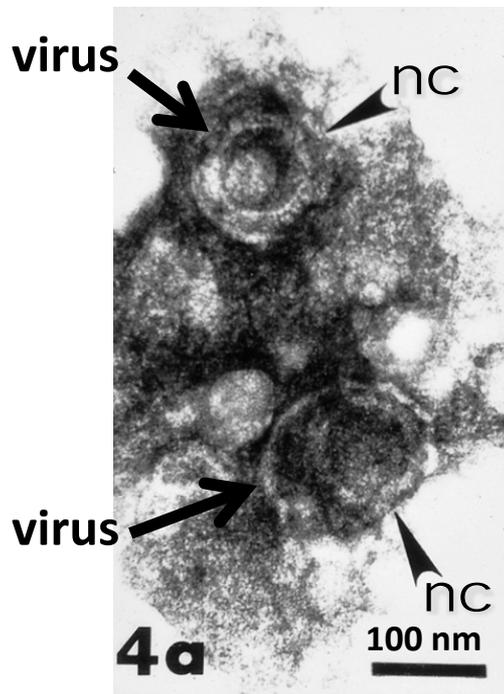


100 nm

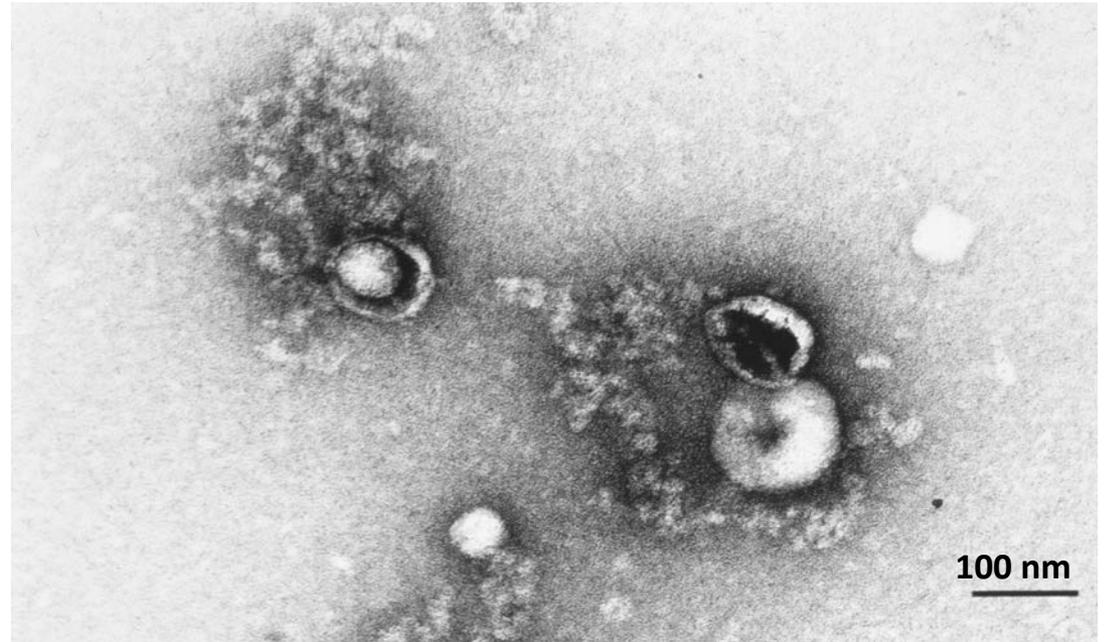
Stool



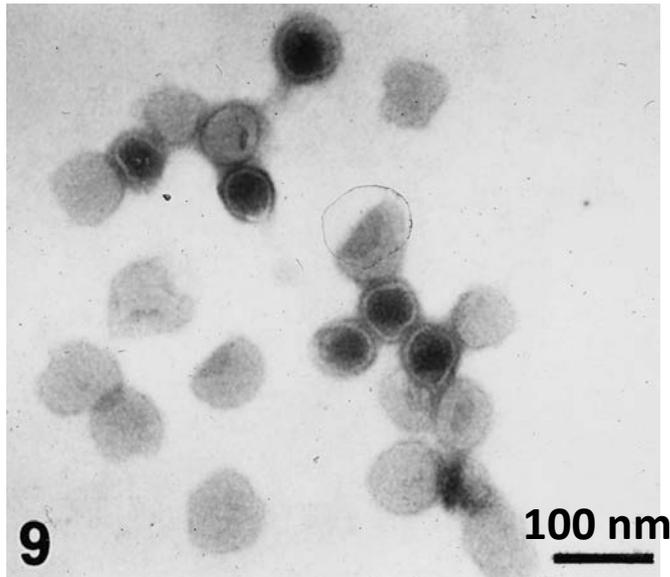
Small Round Virus



**Herpesvirus**



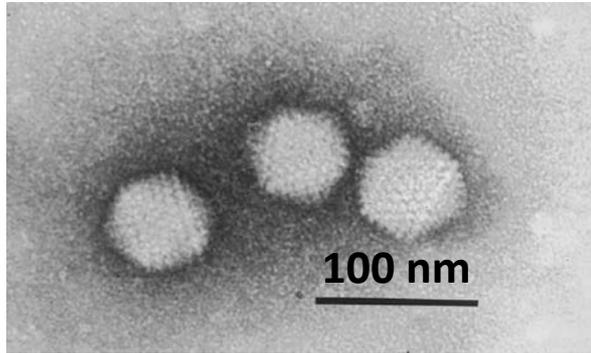
**Cell Debris**



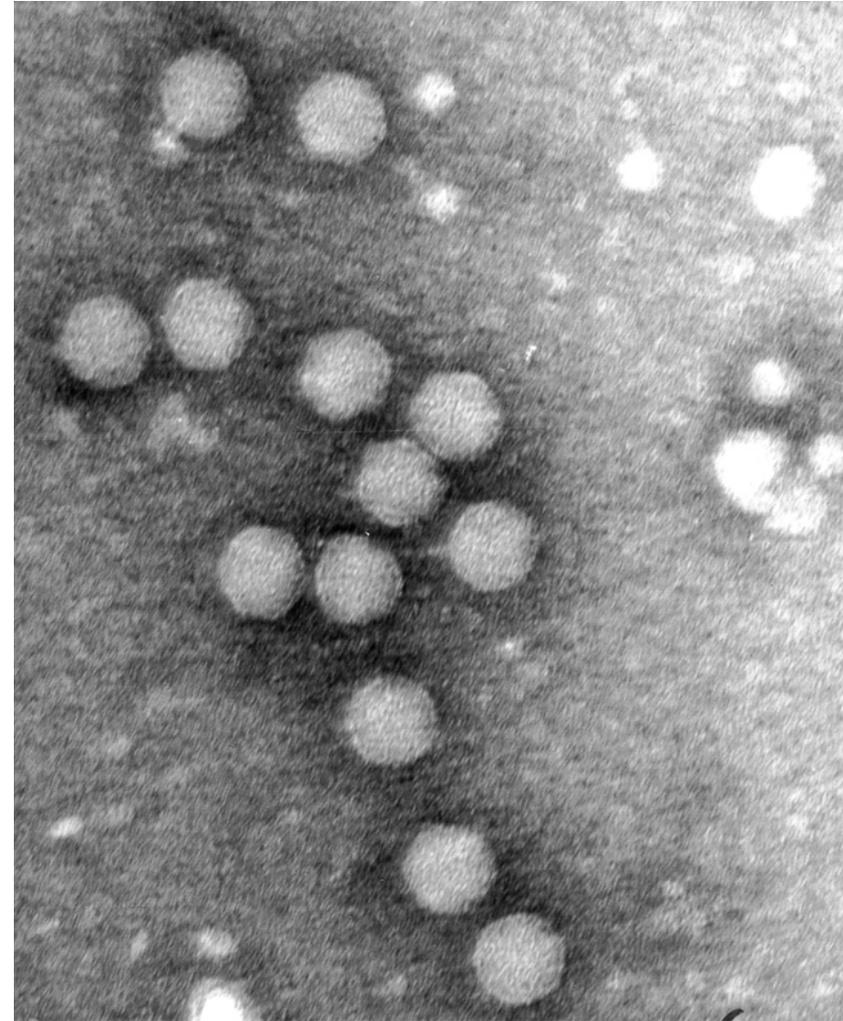
**Rubellavirus**

Courtesy of Dr. Robert Simmons

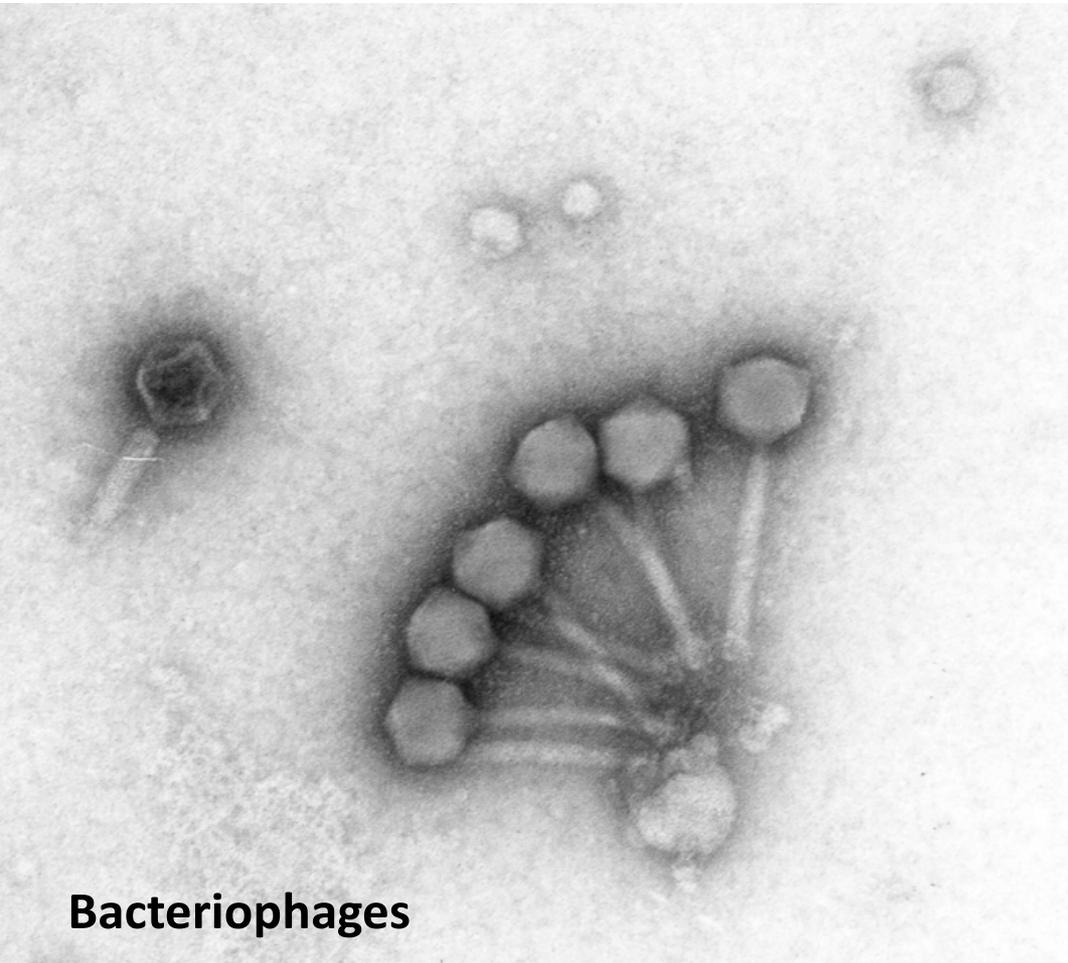
**Adenovirus**



# Hexagonal-shaped Particles



**Bacteriophages**



**Bacteriophages**

# Things That Can Look Like Viruses But Are Not in Negative Stains of Fluid Samples

- **Resemble viral capsid material**
  - **Bacteriophage neck and tail proteins**
  - **Bacterial cell wall debris**

## Small Round Viruses (SRV)

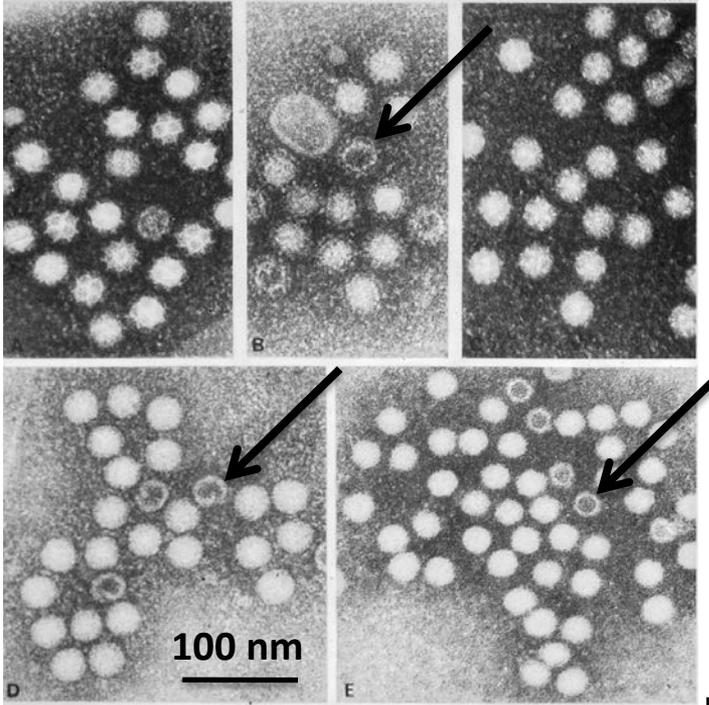
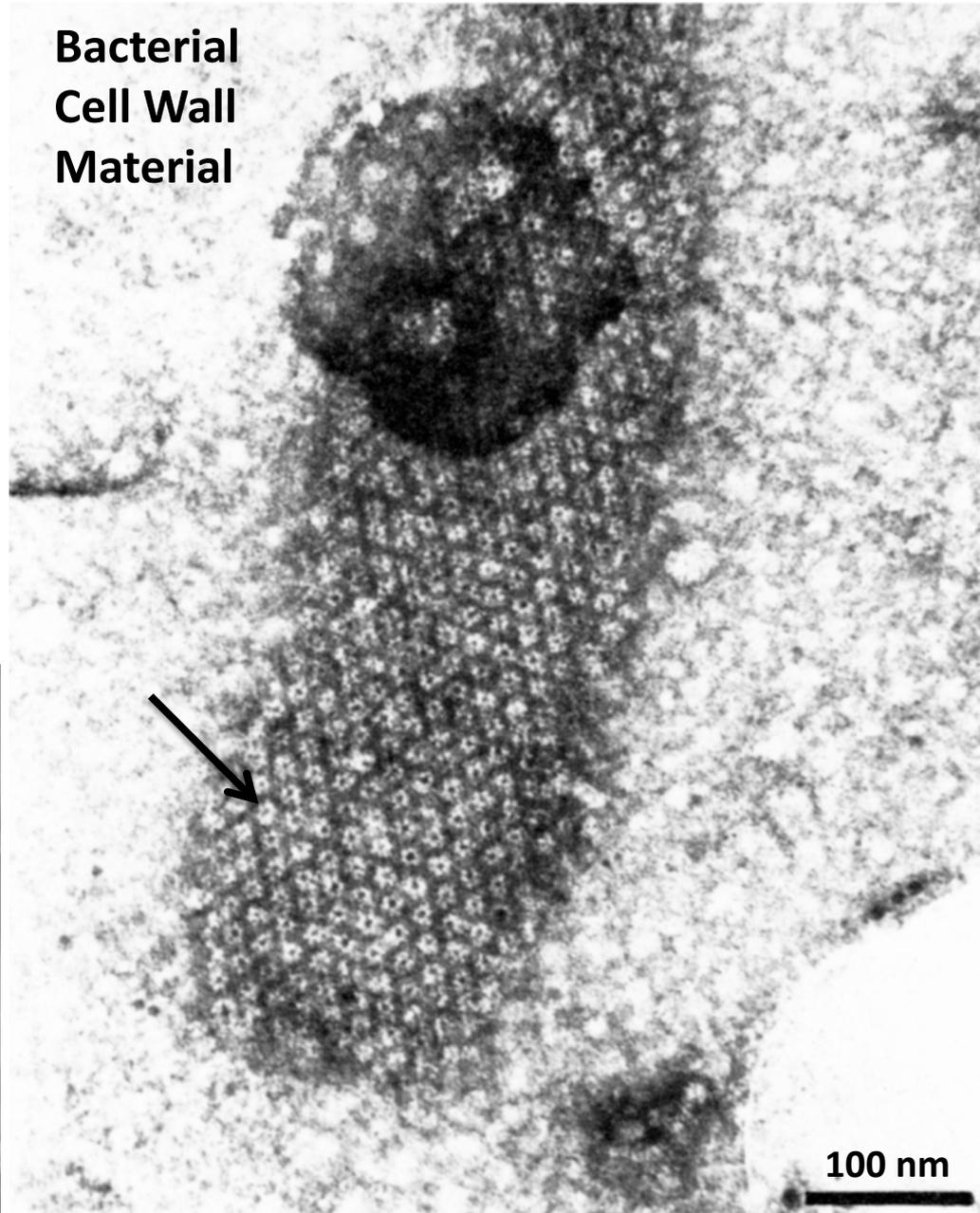
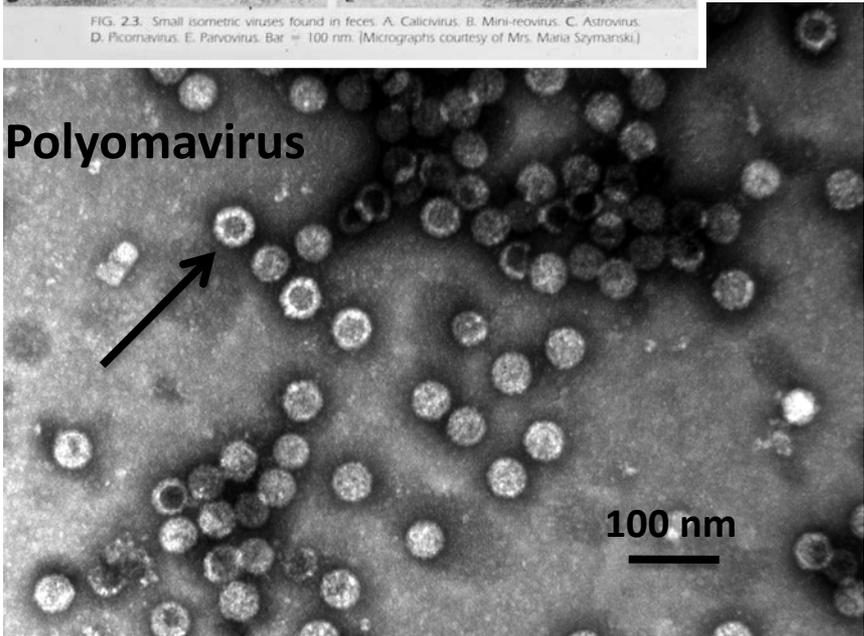


FIG. 23. Small isometric viruses found in feces. A. Calicivirus. B. Mini-reovirus. C. Astrovirus. D. Picornavirus. E. Parvovirus. Bar = 100 nm. [Micrographs courtesy of Mrs. Maria Szymanski.]

## Bacterial Cell Wall Material



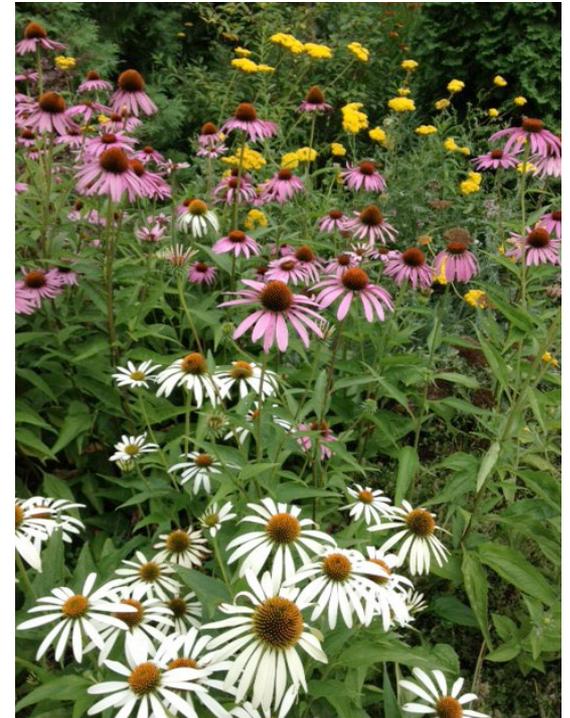
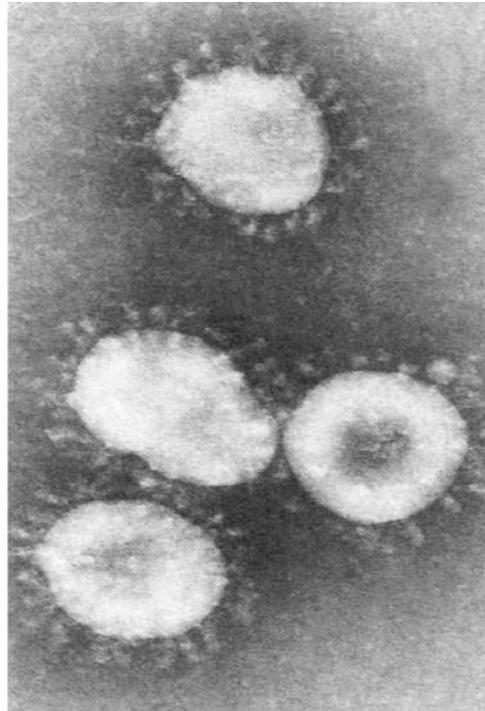
## Polyomavirus



## Things That Can Look Like Viruses But Are Not in Negative Stains of Fluid Samples

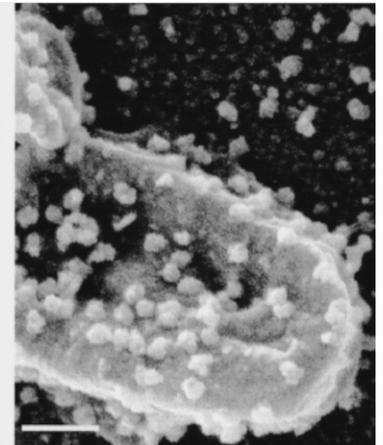
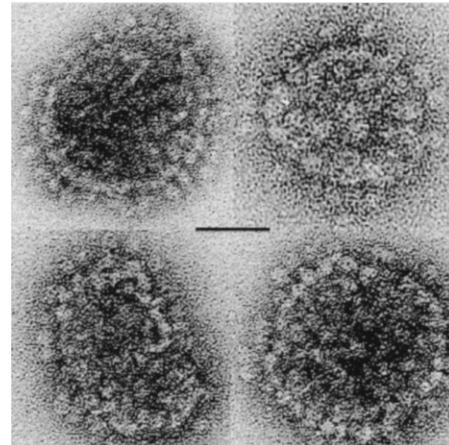
- **Resemble spiked/fuzzy enveloped viruses**
  - **Inside-out mitochondrial membranes**

# Fringed Particles



**Coronaviruses**

Courtesy of Dr. Fred Murphy, CDC



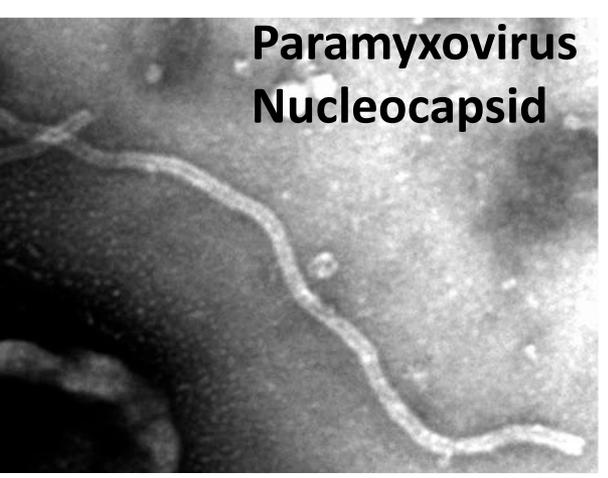
**Inside-out Mitochondrial Membranes**

[JCB.org/content/278/14/12305](http://JCB.org/content/278/14/12305)

## **Things That Can Look Like Viruses But Are Not in Negative Stains of Fluid Samples**

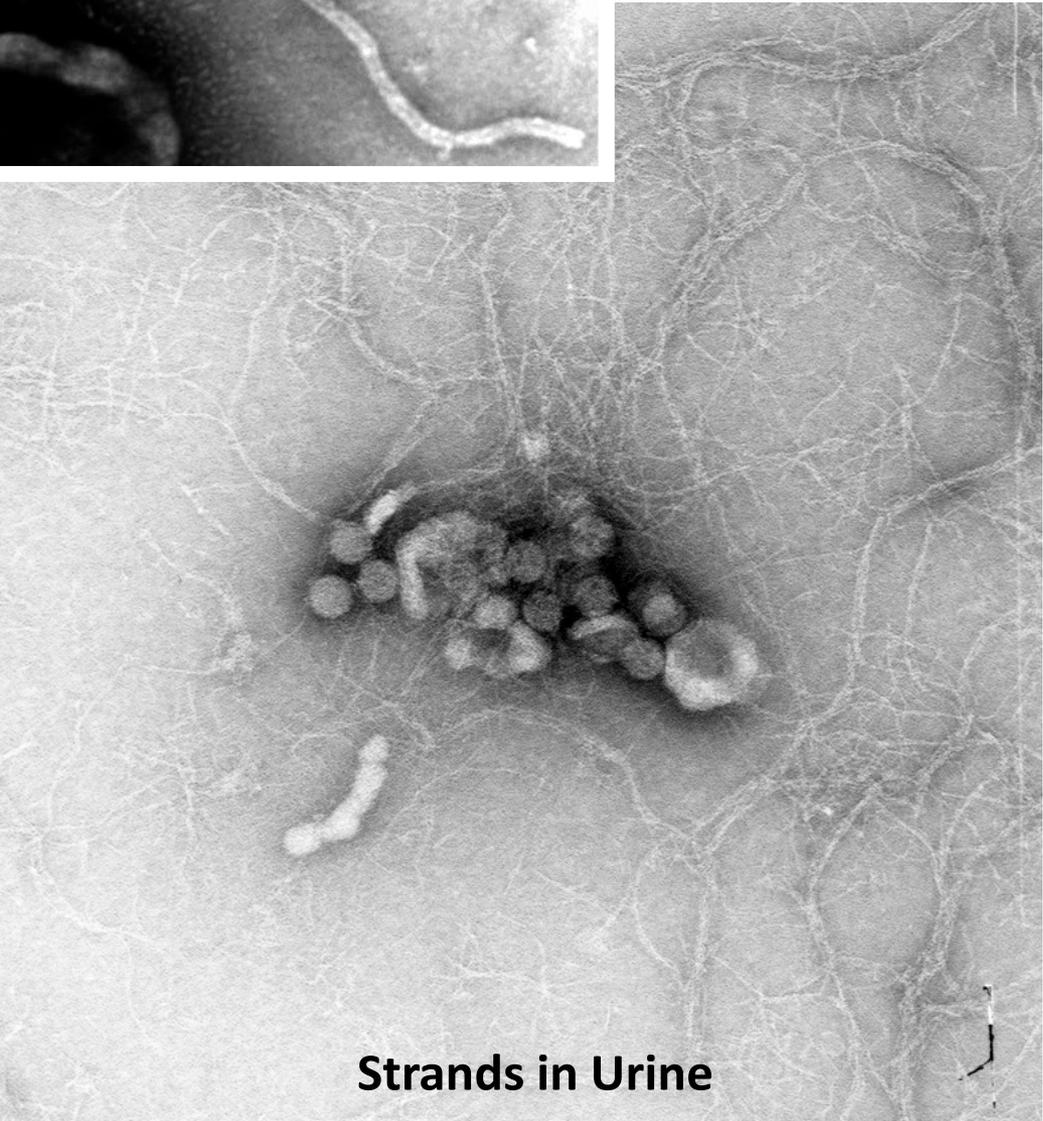
- **Resemble filamentous nucleocapsids**
  - **Crystalline structures such as salt precipitates**
  - **Flagella, pili**
  - **Fibers from clothing, gauze, food, filters**
  - **Strands or liquid boundaries in sputum, urine**

**Paramyxovirus  
Nucleocapsid**

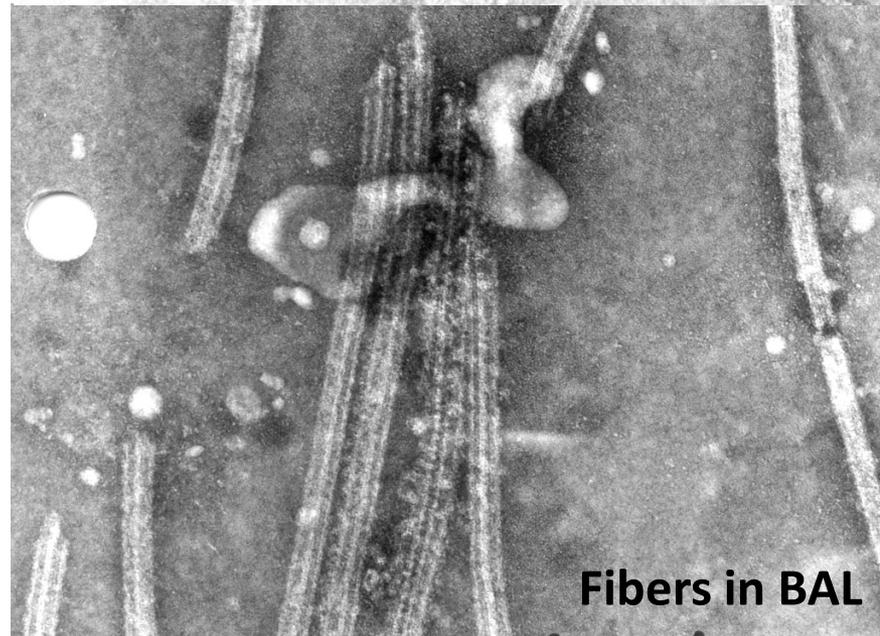


# Filaments

**Flagella in Stool**



**Strands in Urine**

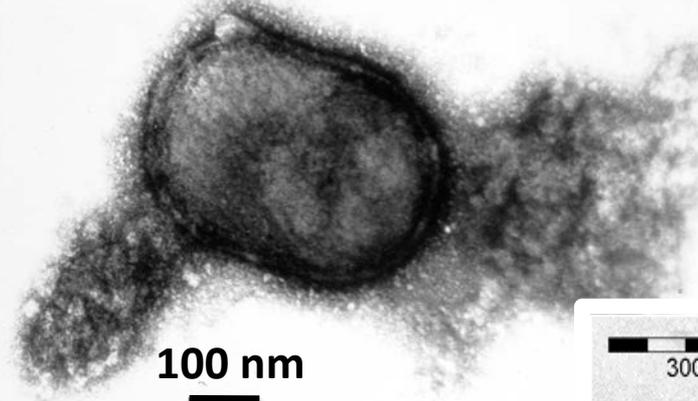


**Fibers in BAL**

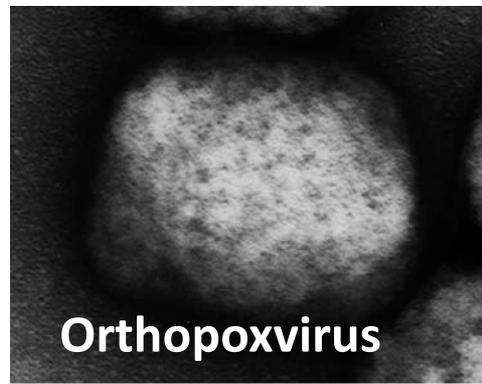
# Things That Can Look Like Viruses But Are Not in Negative Stains of Fluid Samples

- **Resemble poxviruses**
  - **Cell debris**
  - **Melanosomes**

**Orthopoxvirus**

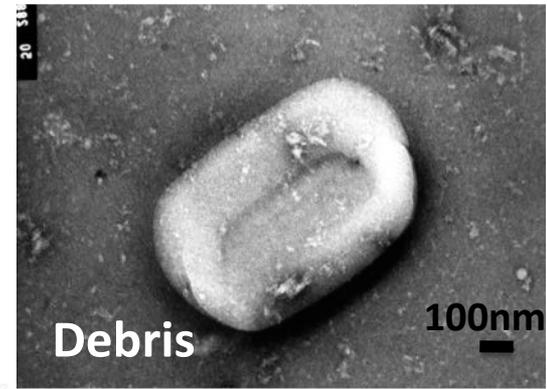


100 nm



**Orthopoxvirus**

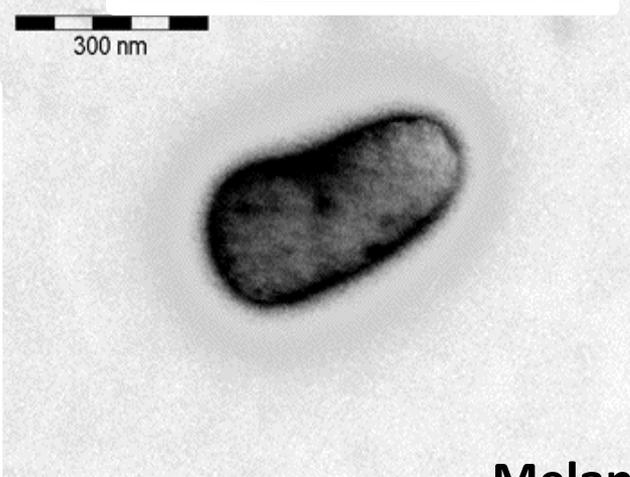
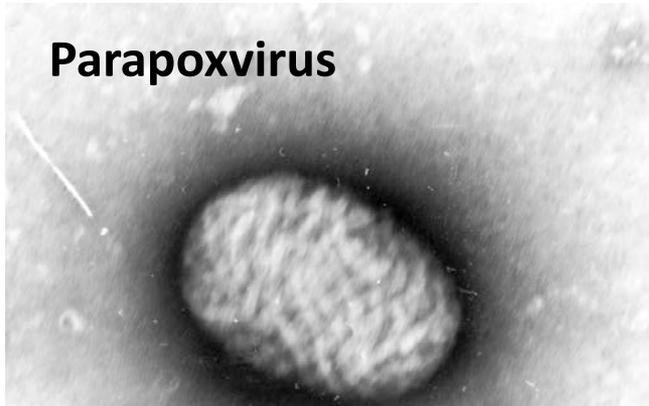
300 nm



**Debris**

100nm

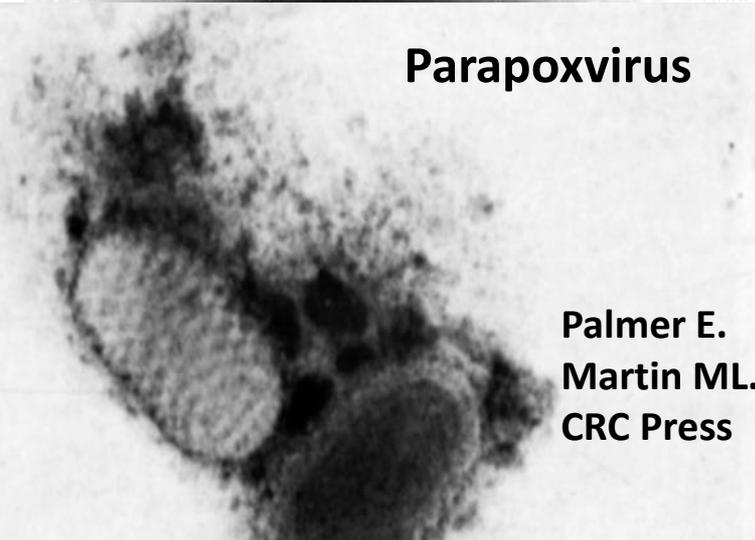
**Parapoxvirus**



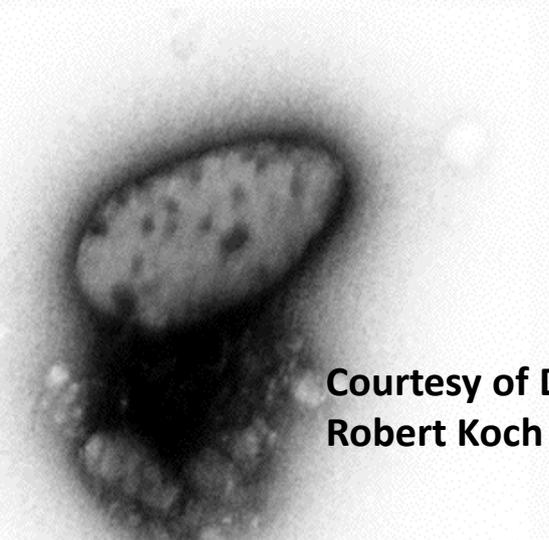
**Melanosomes**

300 nm

**Parapoxvirus**

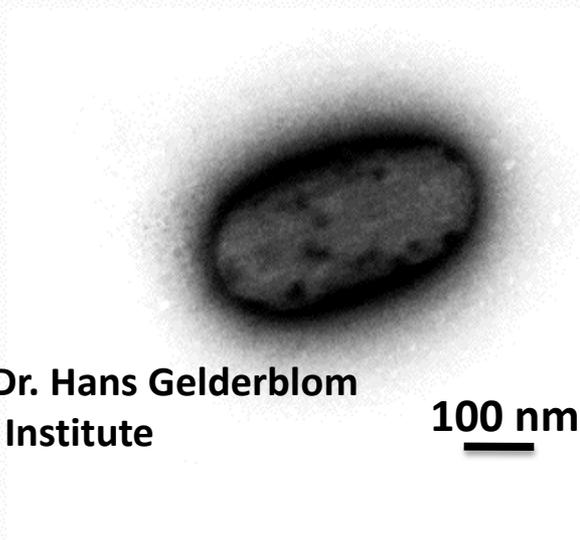


Palmer E.  
Martin ML.  
CRC Press



Courtesy of Dr. Hans Gelderblom  
Robert Koch Institute

100 nm



## **Problems of Negative Stains in Virus Identification and Diagnosis**

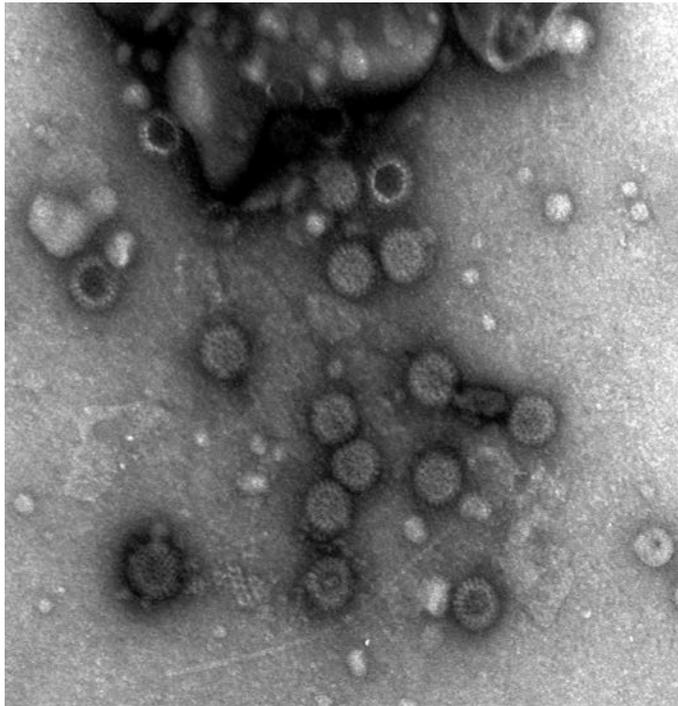
- **Viruses may be present but few in number**
- **Grid film properties may prevent spreading**
- **Dried or frozen samples will be distorted**
- **Bacteriophages can look like viruses**
- **Cell debris can resemble viruses**

## Ways to get around limitations

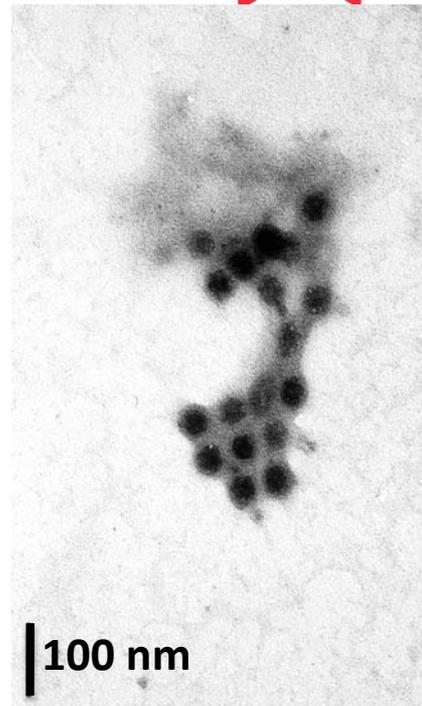
- **Ultracentrifugation**
- **Antibody concentration**
- **Grid treatment to render them hydrophilic**
- **Low speed centrifugation to reduce debris (<2000 x g)**

## Staining Characteristics

Finely granular



~~Slick/slimey~~



## Grid Treatments

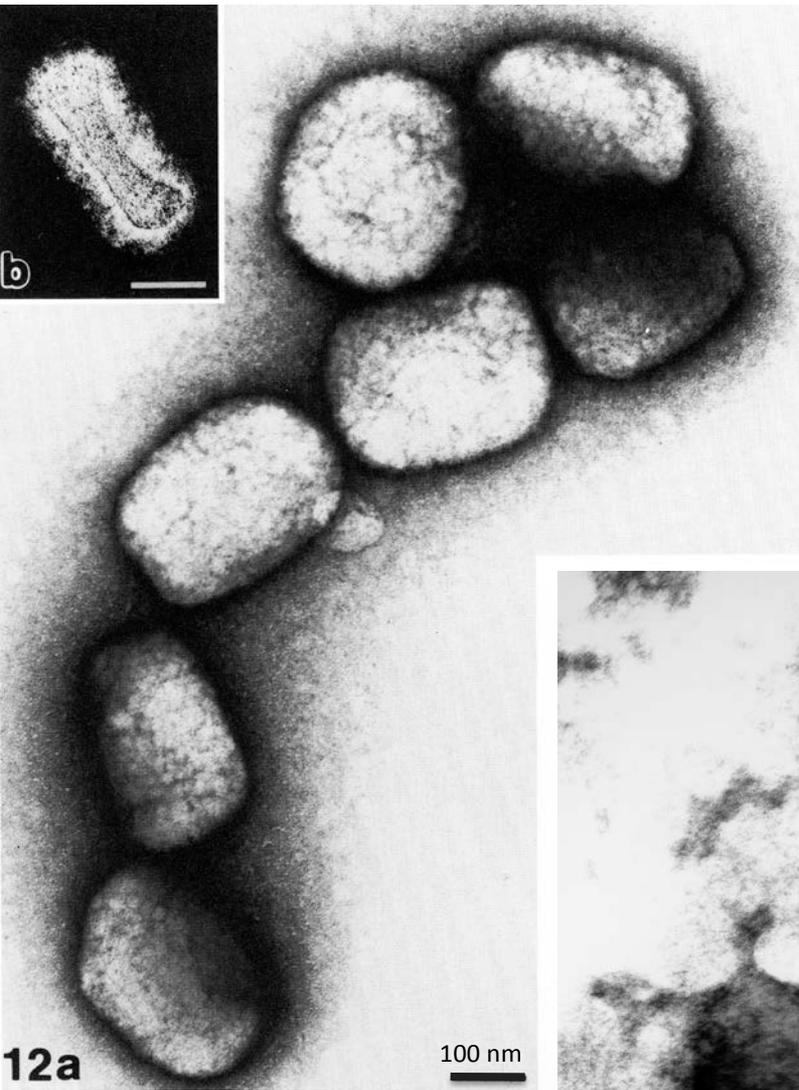
Glow Discharge

Alcian Blue

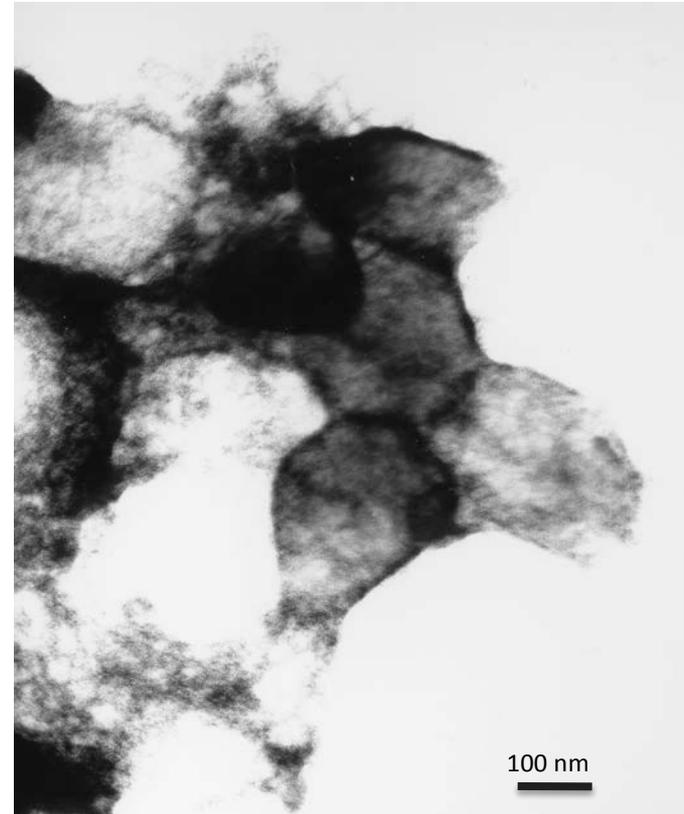
Bacitracin

Poly-L-Lysine

# Negatively Stained Poxvirus



## Frozen sample



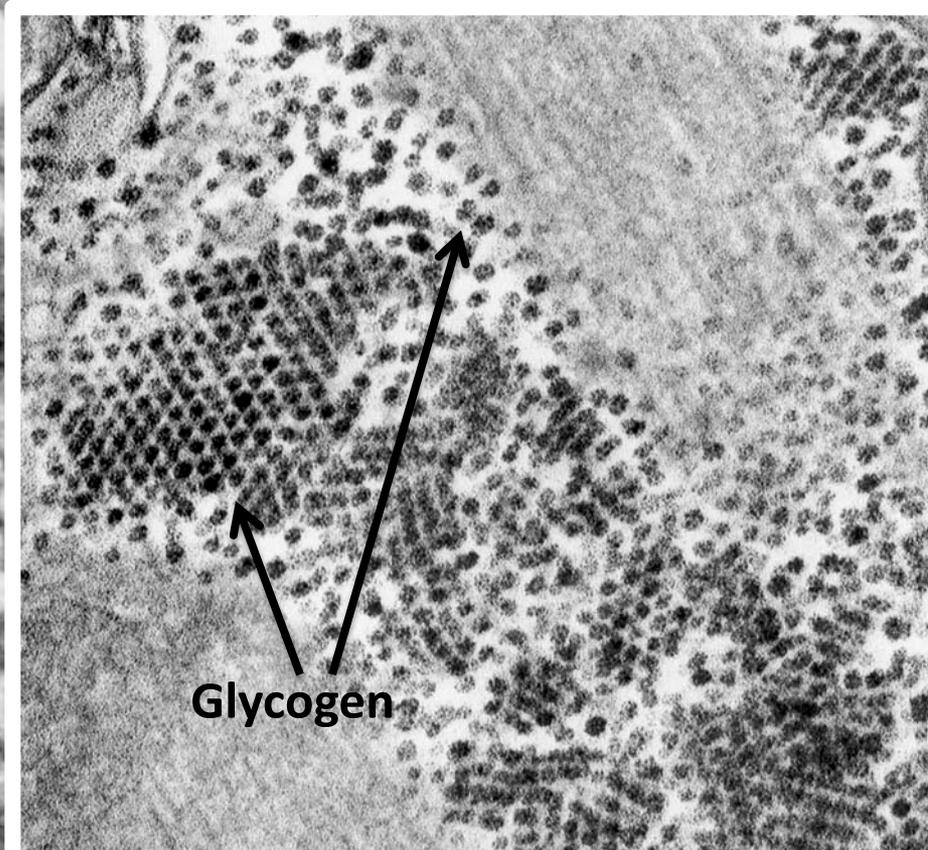
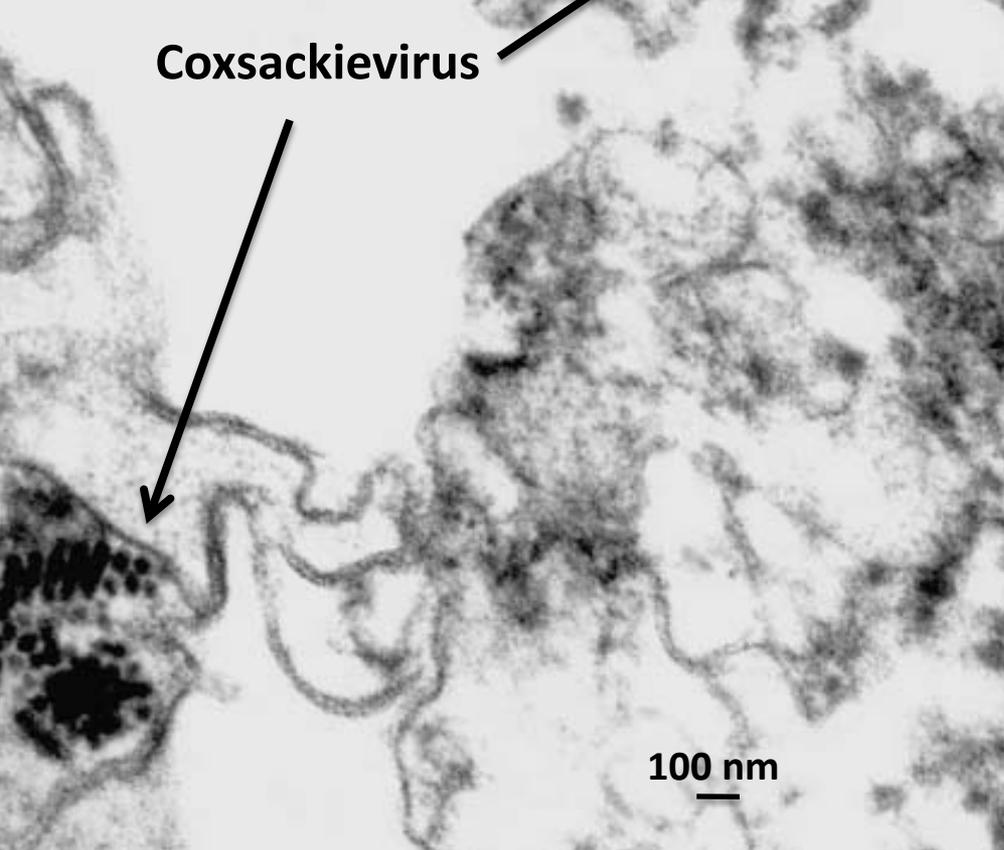
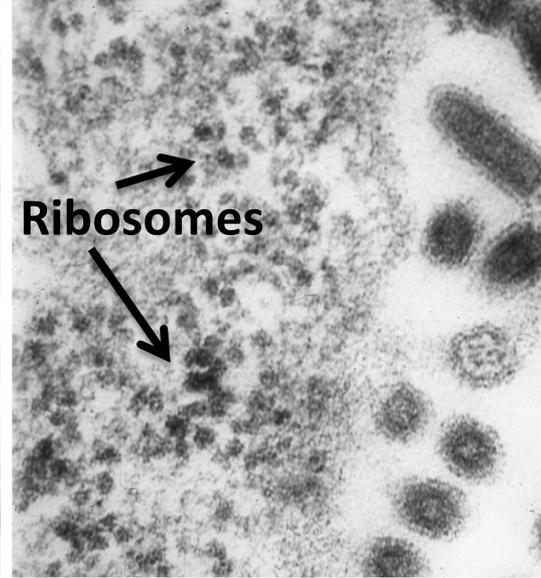
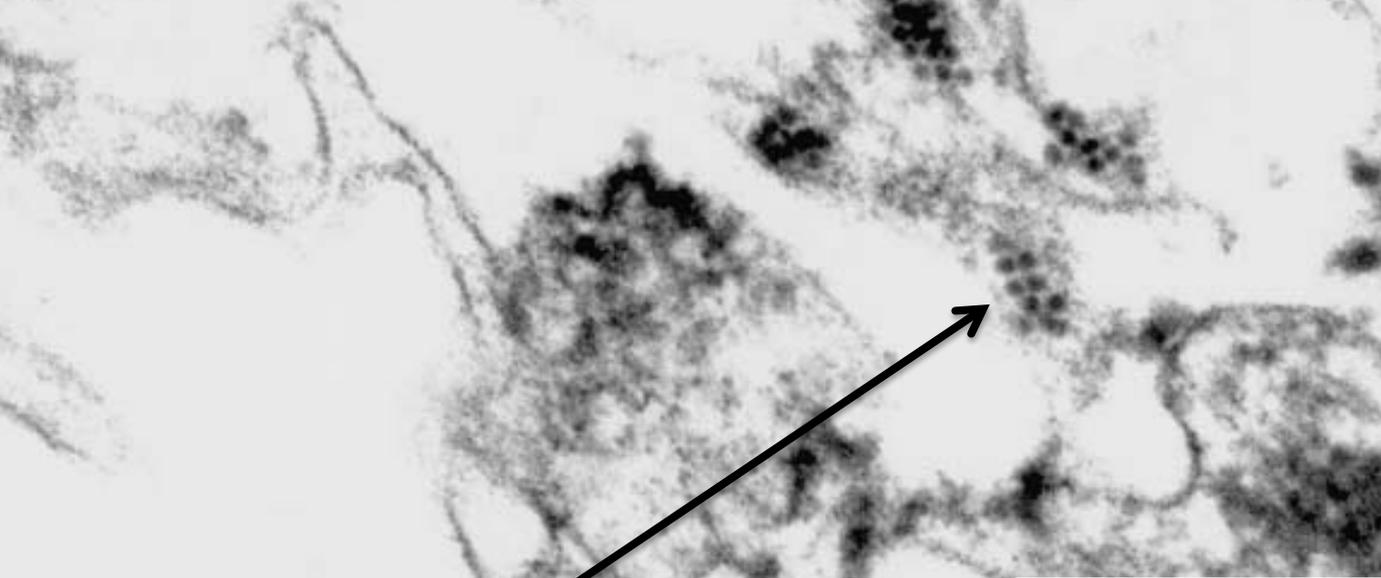
# Things That Can Look Like Viruses But Are Not In Thin sections of Infected Tissues



**Look-alikes**

## Things That Can Look Like Viruses But Are Not in Thin Sections of Tissue Samples

- **Resemble spherical virions or nucleocapsids**
  - **Ribosomes**
  - **Microtubules cut in cross section (look like 20-nm parvovirus)**
  - **Glycogen**
  - **Membrane vesicles (caveolae, pinocytotic vesicles, lysosomes, lysosomes, phagosomes, peroxisomes, Golgi vesicles, synaptic vesicles, cell debris from lysed cells)**

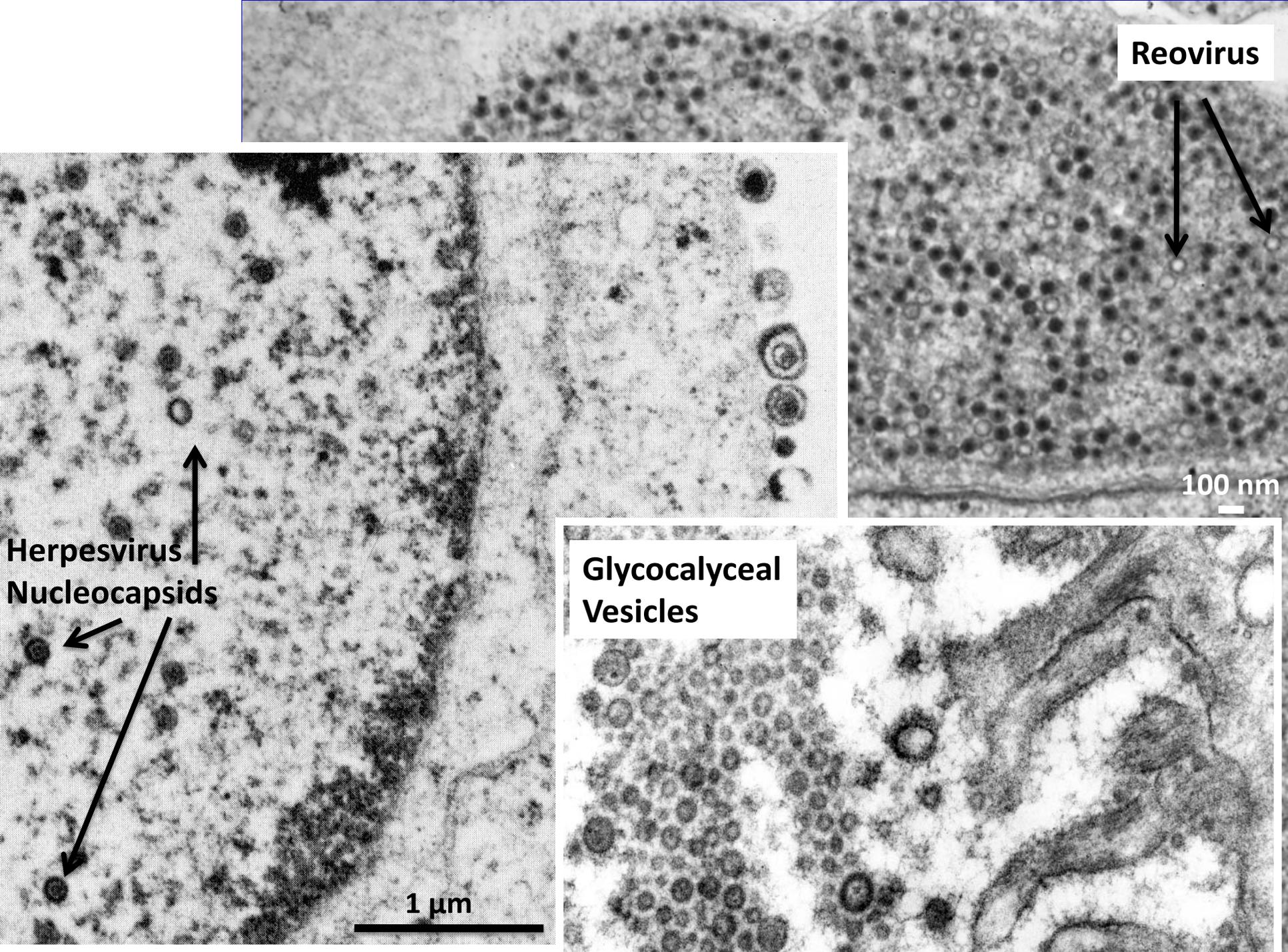


**Cocksackievirus**

**Ribosomes**

**Glycogen**

100 nm



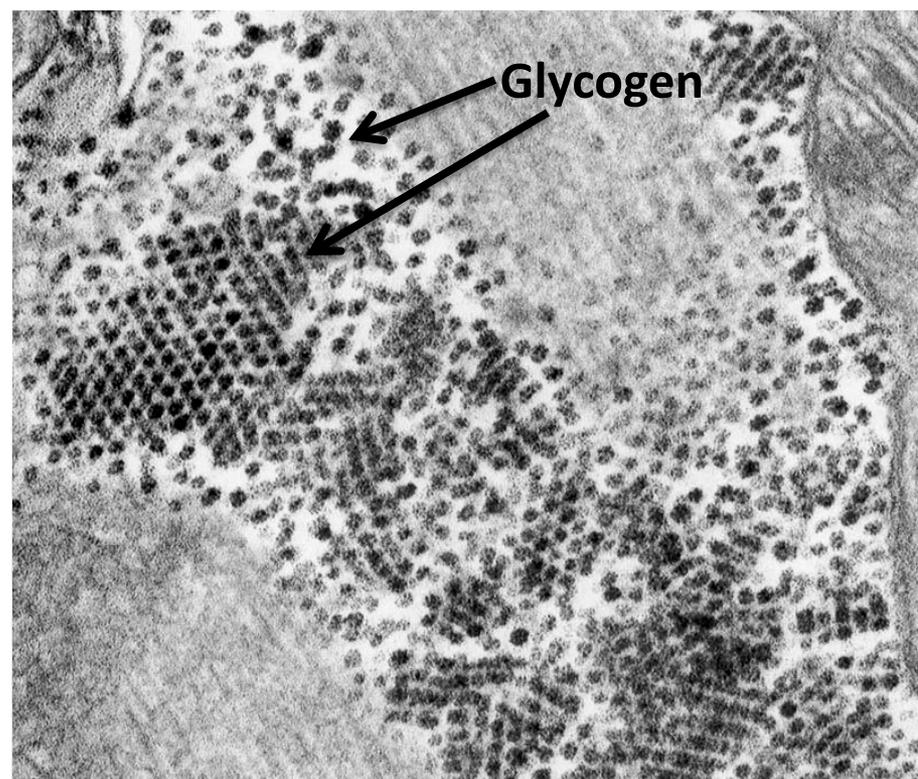
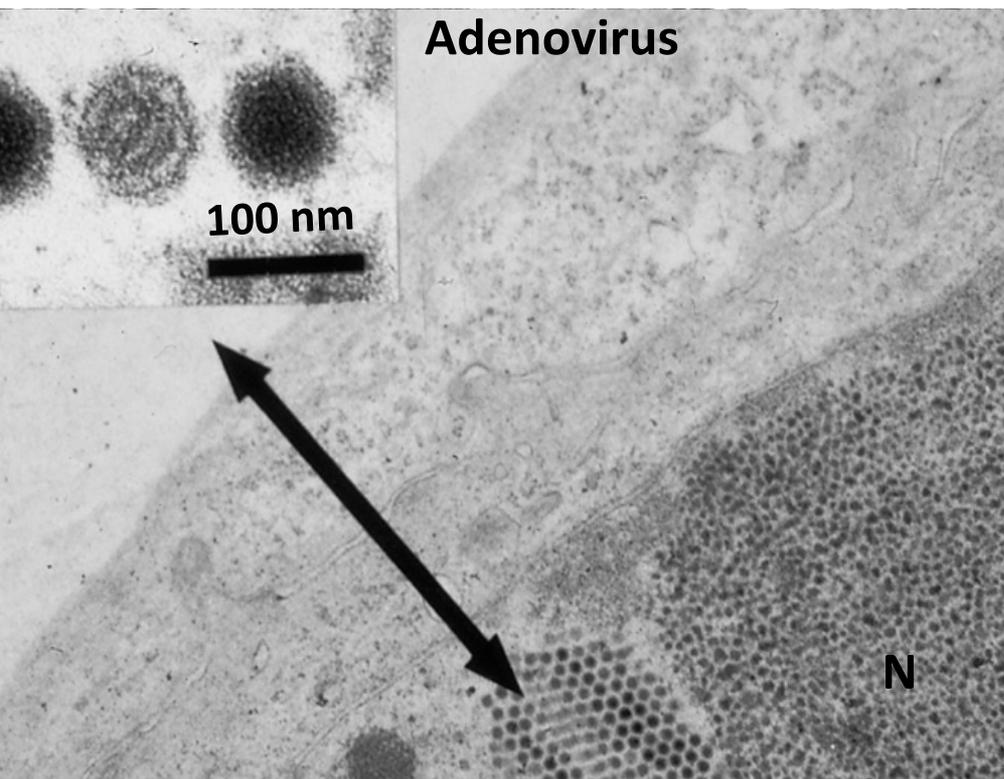
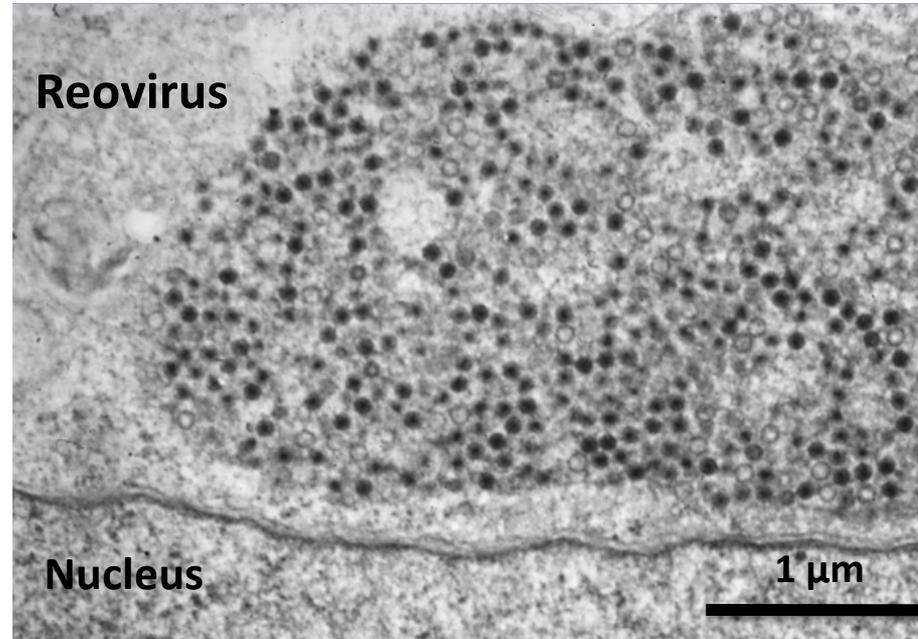
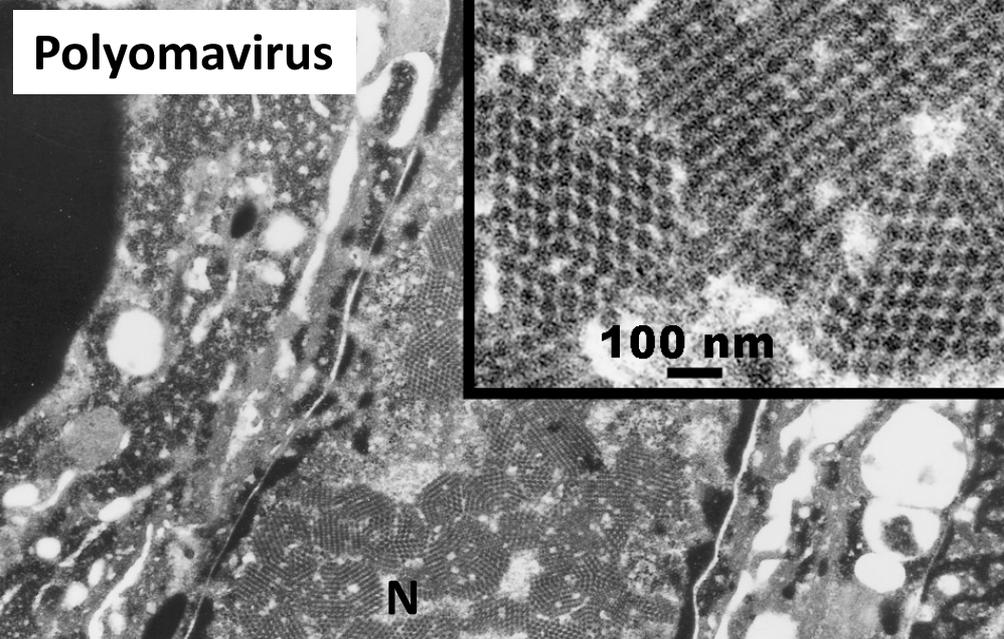
Reovirus

100 nm

Herpesvirus  
Nucleocapsids

1 µm

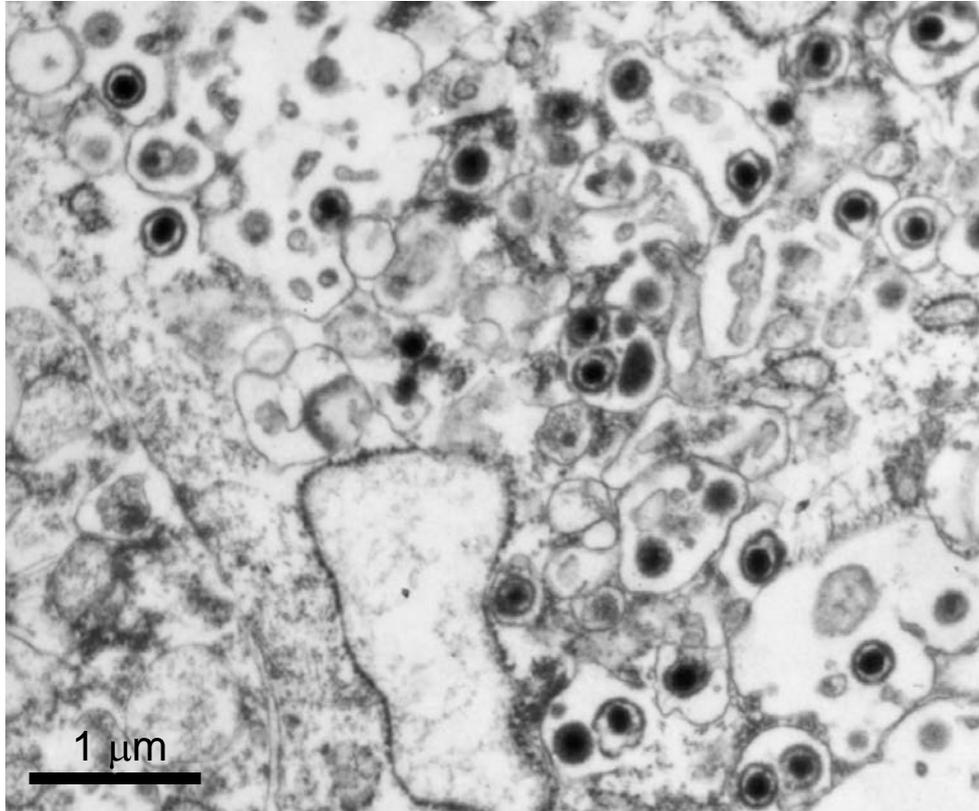
Glycoalyceal  
Vesicles



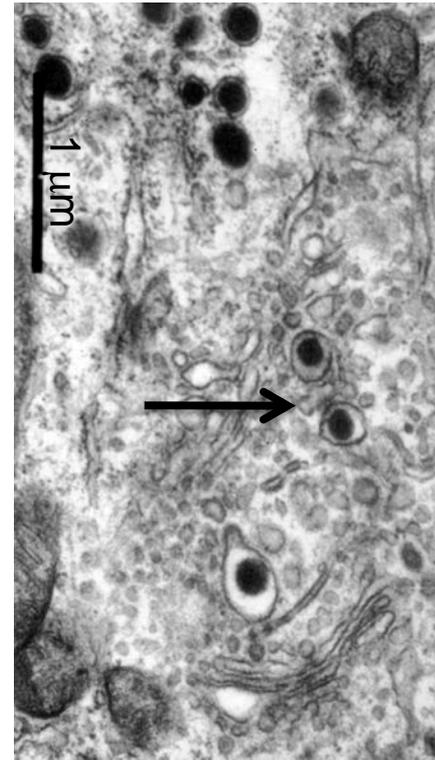
# Things That Can Look Like Viruses But Are Not in Thin Sections of Tissue Samples

- **Resemble spherical virions or nucleocapsids**
  - **Neurosecretory vesicles**
  - **Nuclear granules**
  - **Nuclear bodies**
  - **Nuclear pores**
  - **Mitochondrial dense granules**
  - **Microvilli and collagen in cross section**

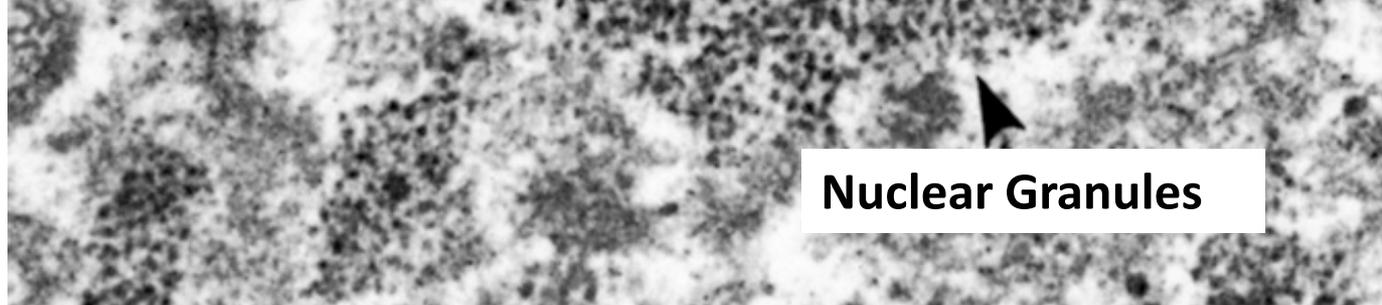
**Herpesvirus**



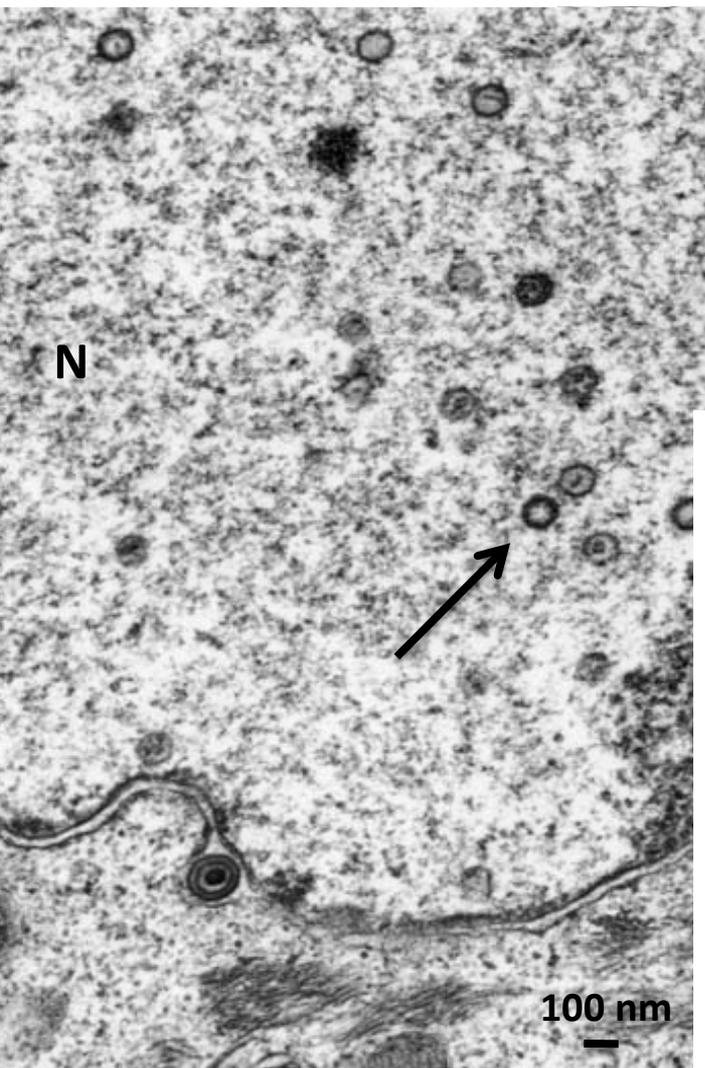
**Neurosecretory  
Granules**



**Herpesvirus  
Nucleocapsids**

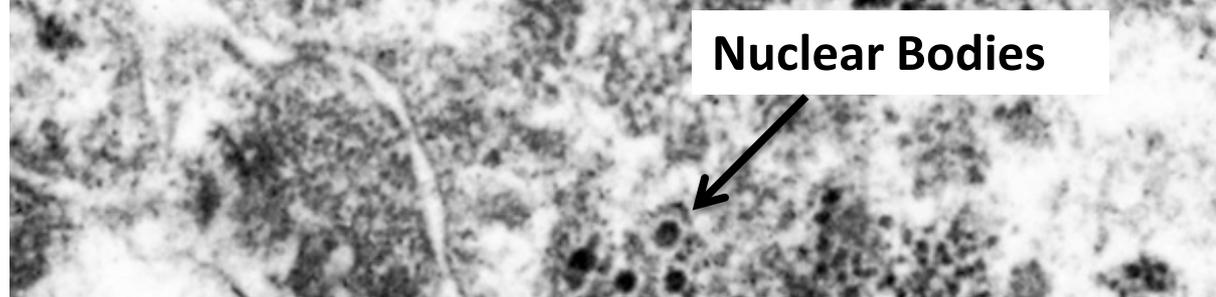


**Nuclear Granules**

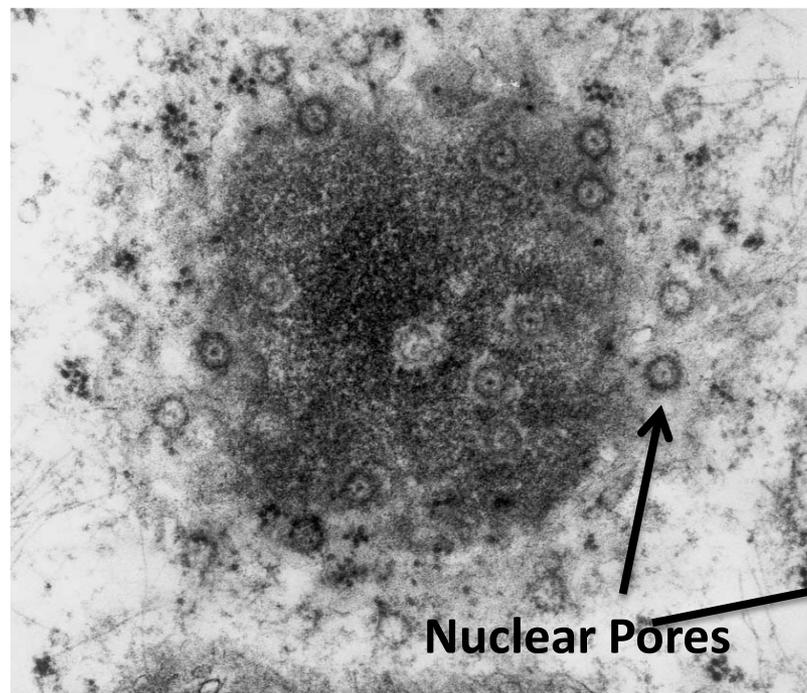


**N**

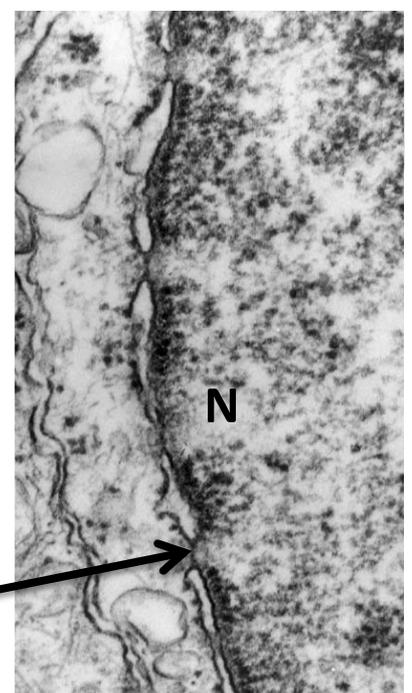
**100 nm**



**Nuclear Bodies**



**Nuclear Pores**

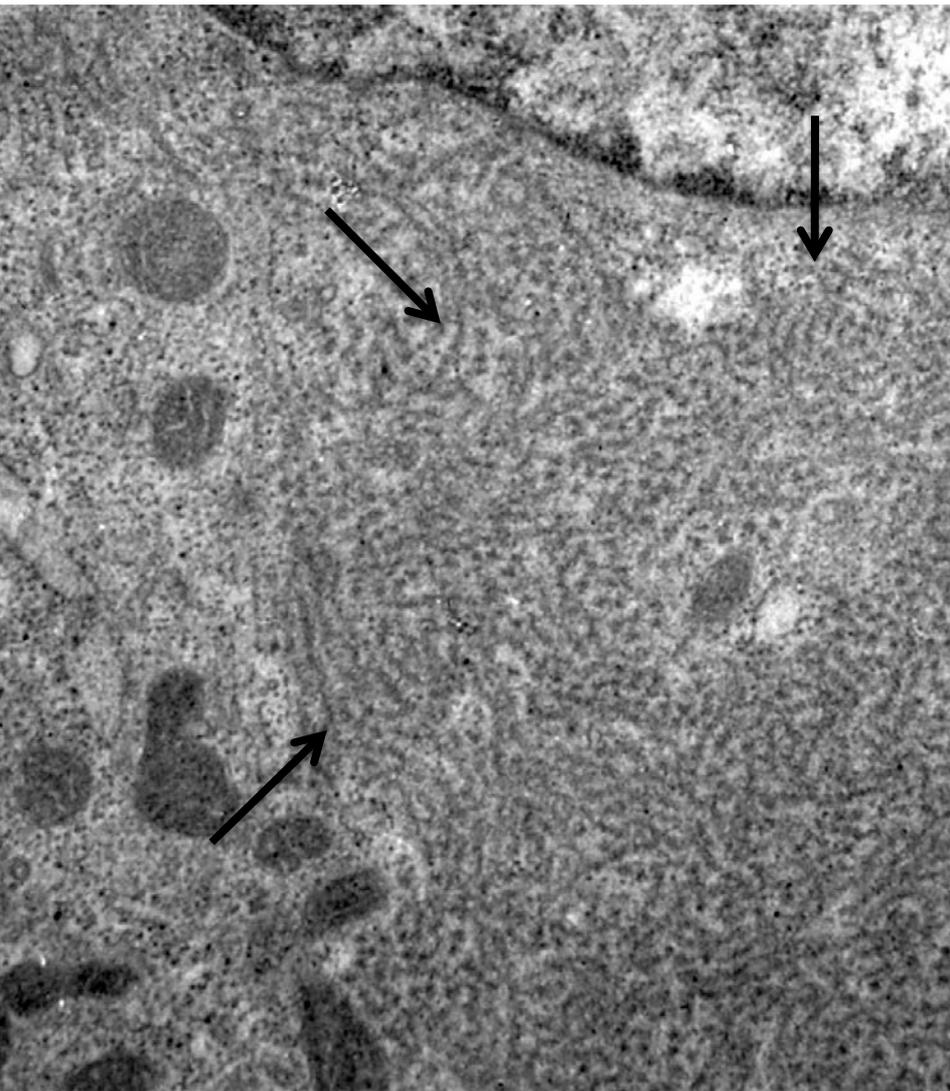


**N**

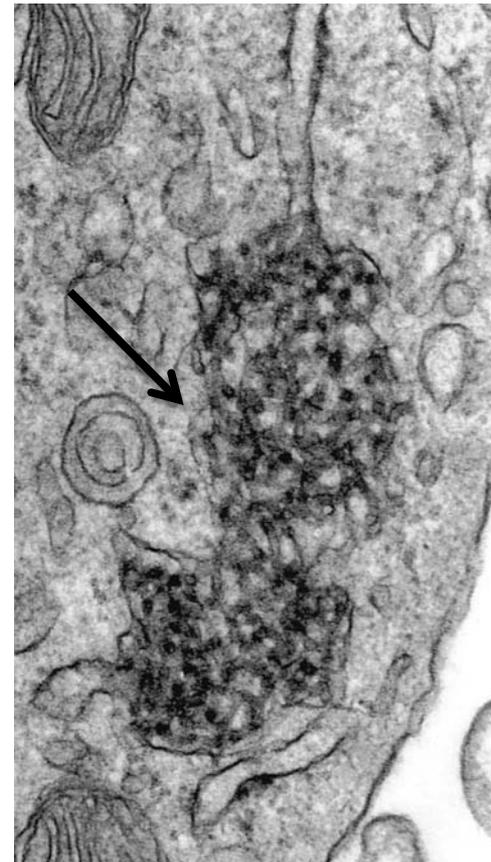
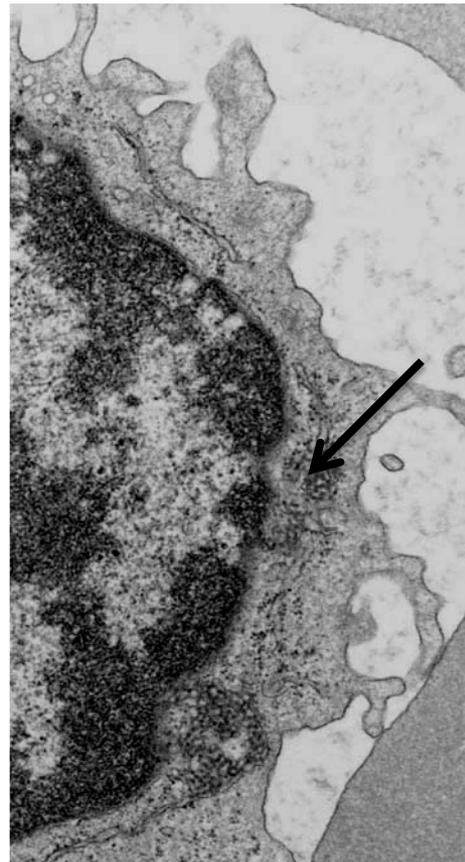
## Things That Can Look Like Viruses But Are Not in Thin Sections of Tissue Samples

- **Filaments may resemble helical nucleocapsids**
  - **Microtubules**
  - **Intermediate filaments**
  - **Tubular reticular inclusions (TRI) (seen in lupus, HIV infection, interferon production or therapy)**

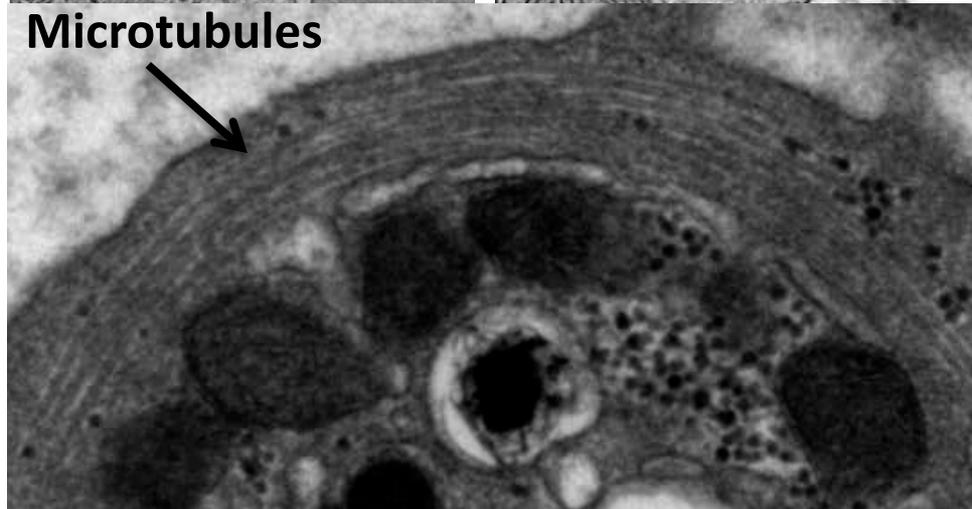
**Paramyxovirus Nucleocapsids**



**Tubuloreticular Inclusions (TRIs)**



**Microtubules**



## Things That Can Look Like Viruses But Are Not in Thin Sections of Tissue Samples

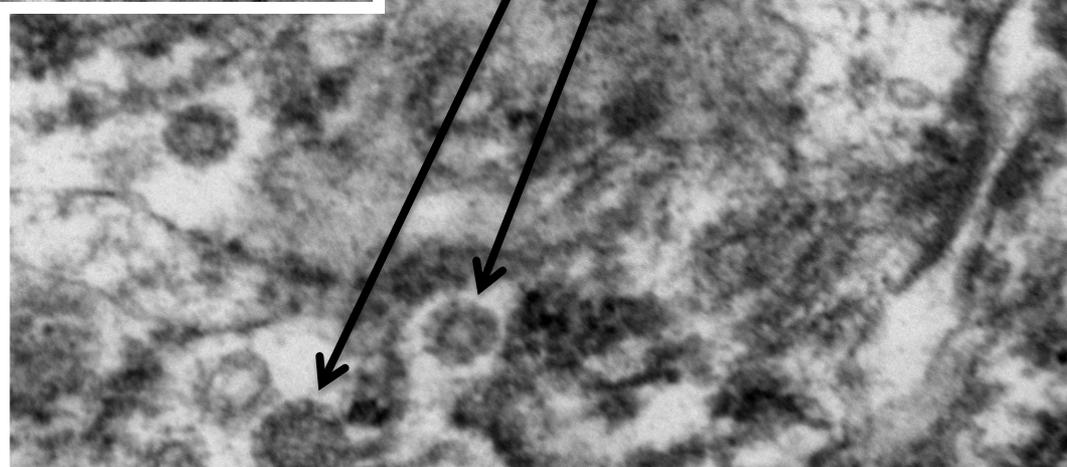
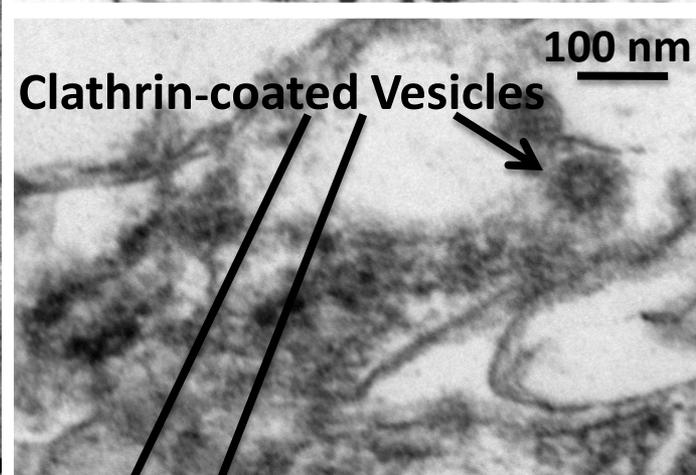
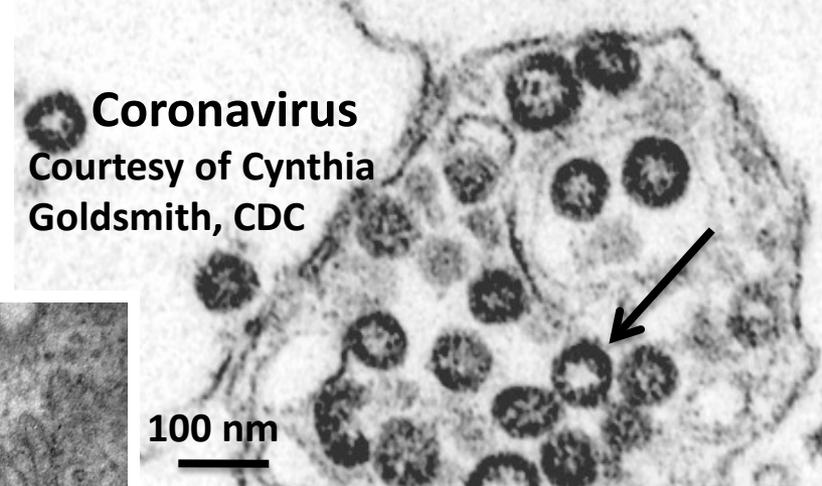
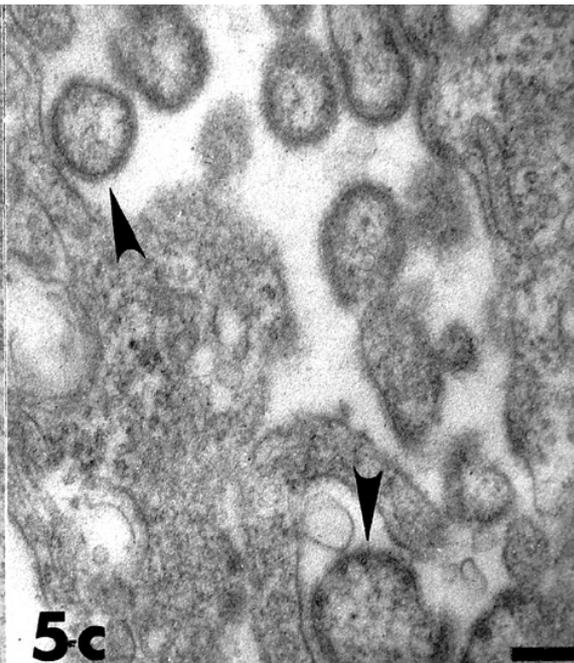
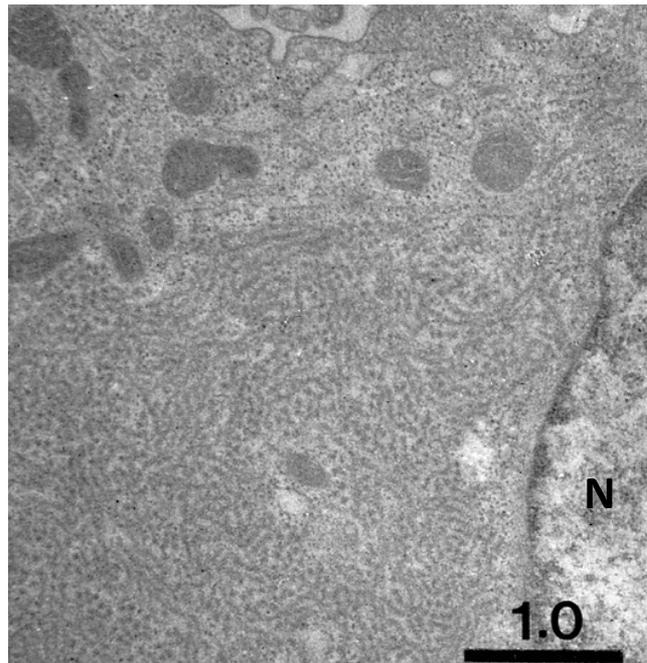
- **Spiked organelles resemble enveloped viruses**
  - **Clathrin-coated vesicles**
  - **Microvilli (cross sections)**

# Paramyxovirus

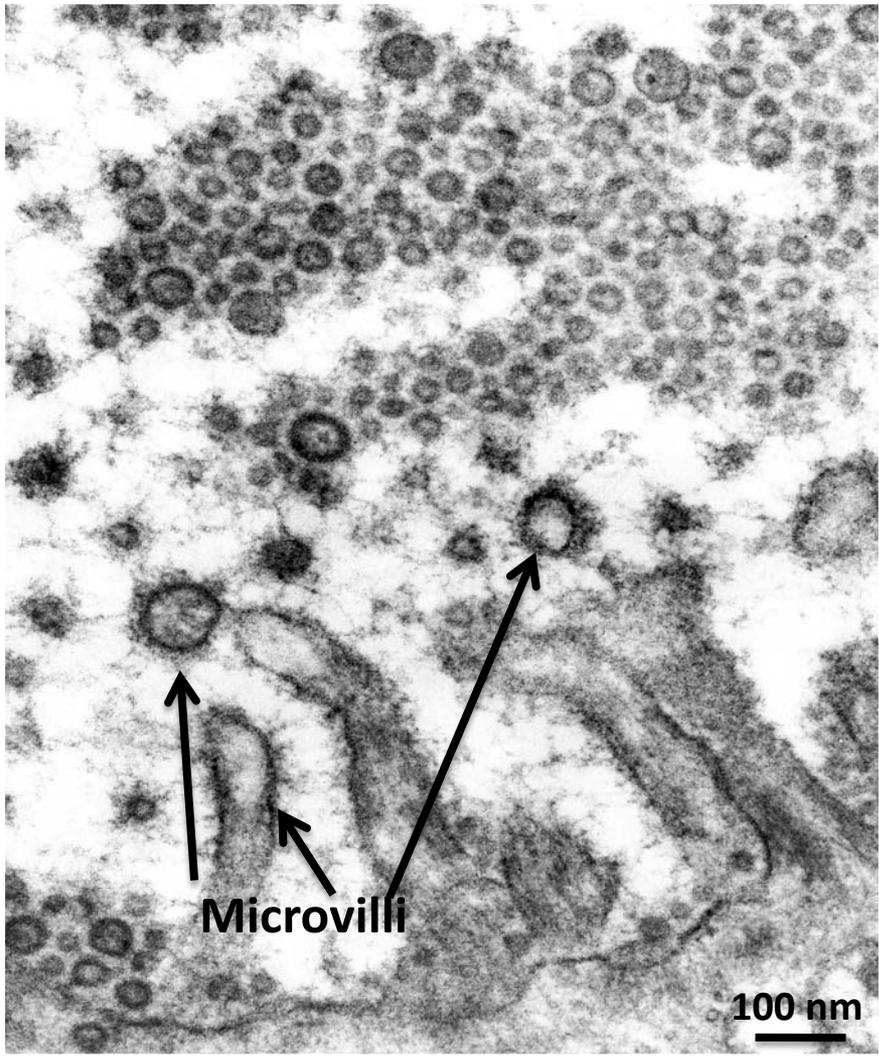
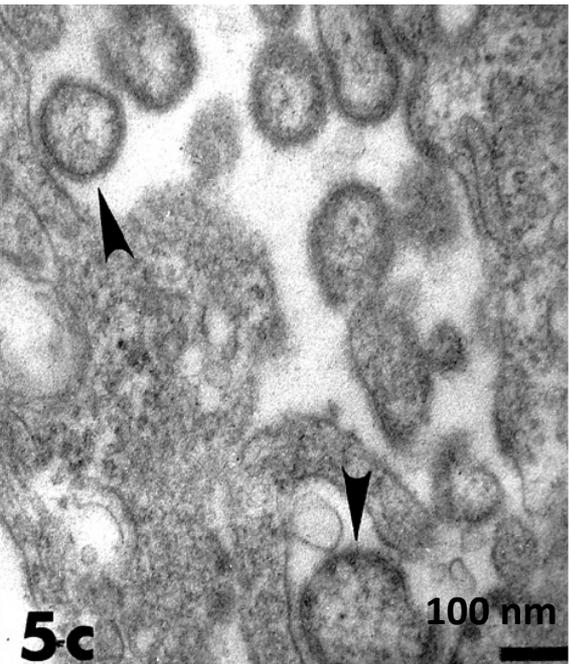
## Nucleocapsids

## Complete Virions

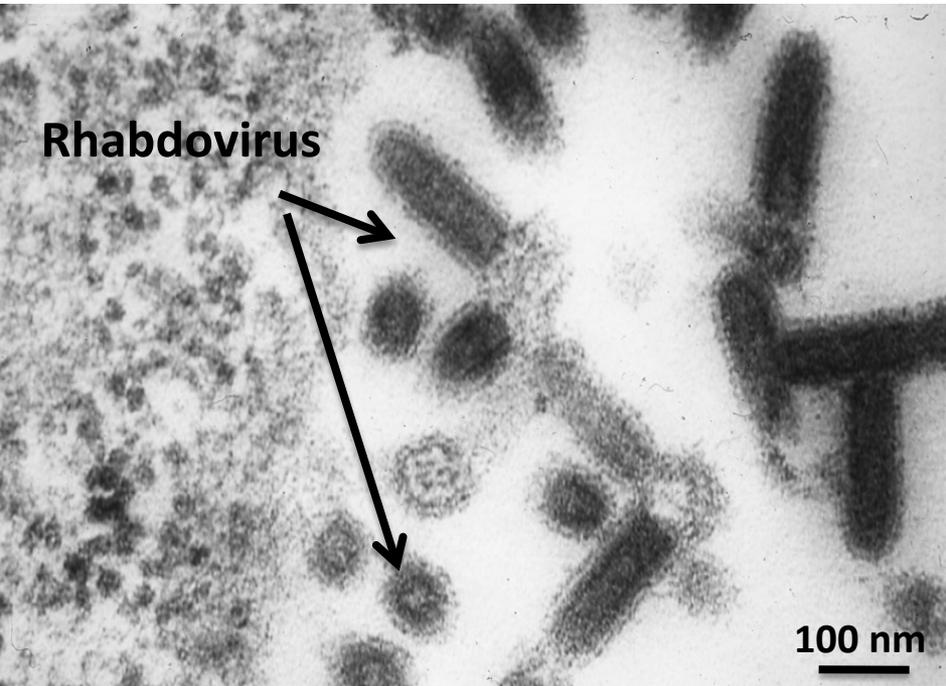
**Coronavirus**  
Courtesy of Cynthia Goldsmith, CDC



# Paramyxovirus

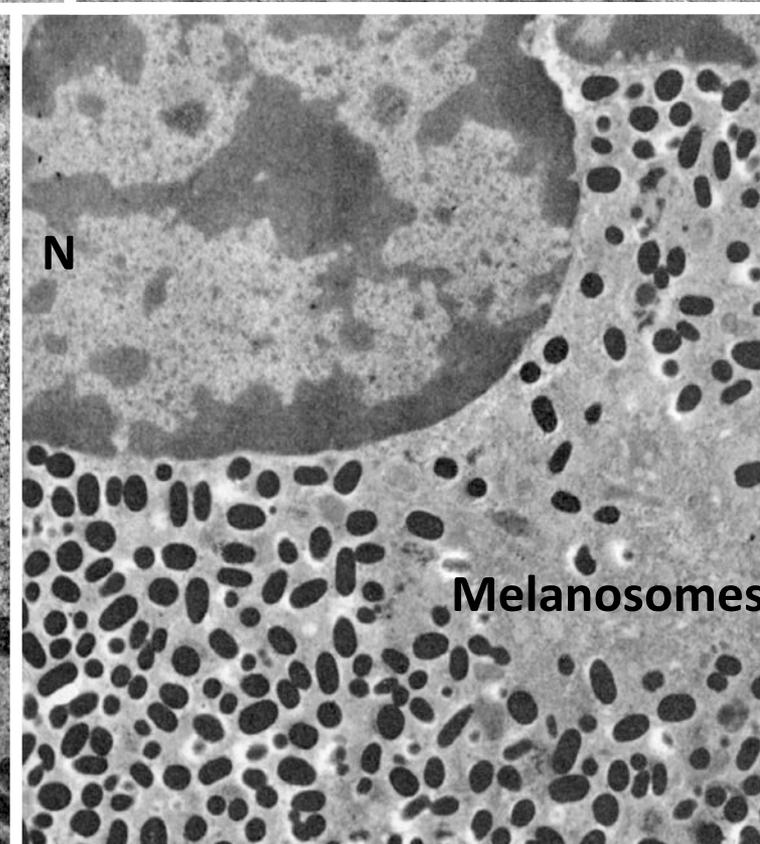
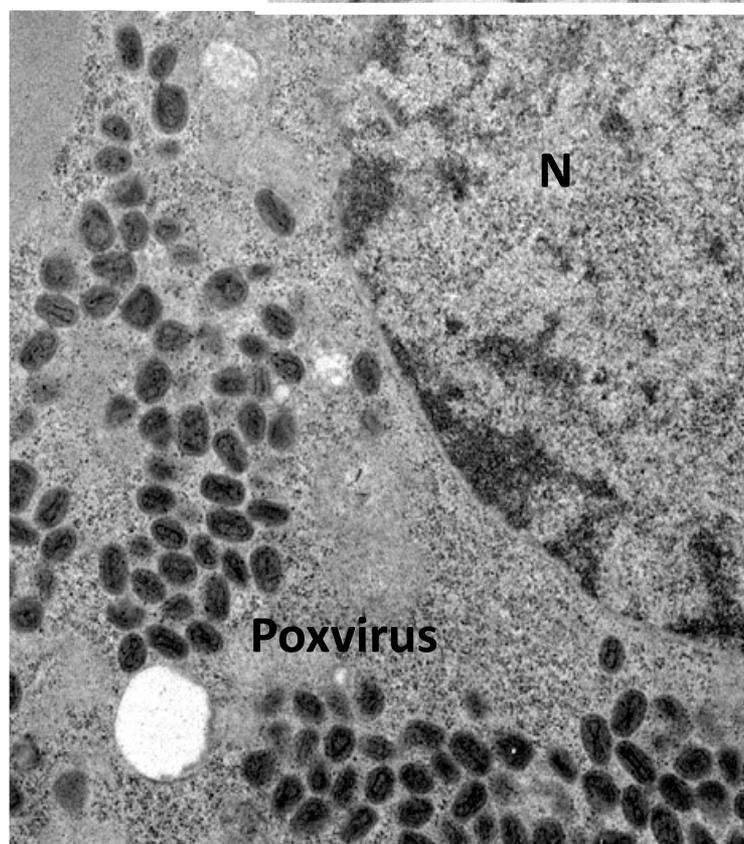
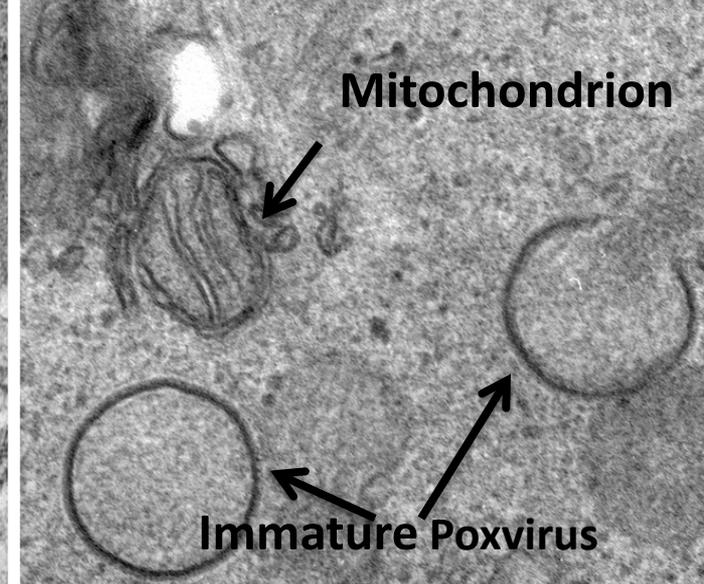
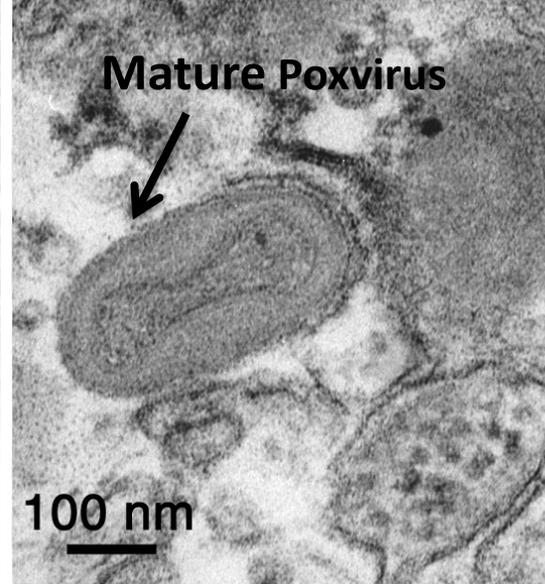
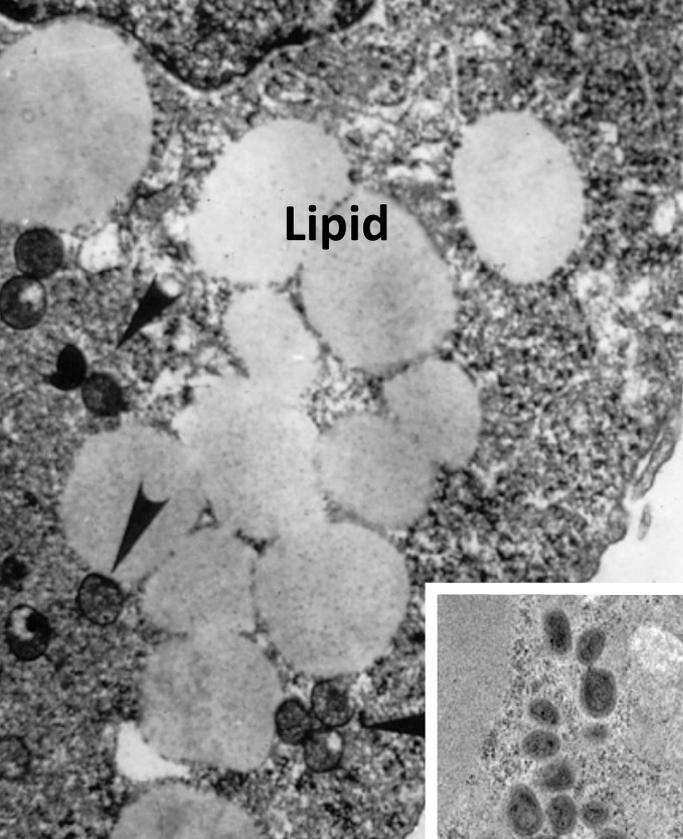


# Rhabdovirus



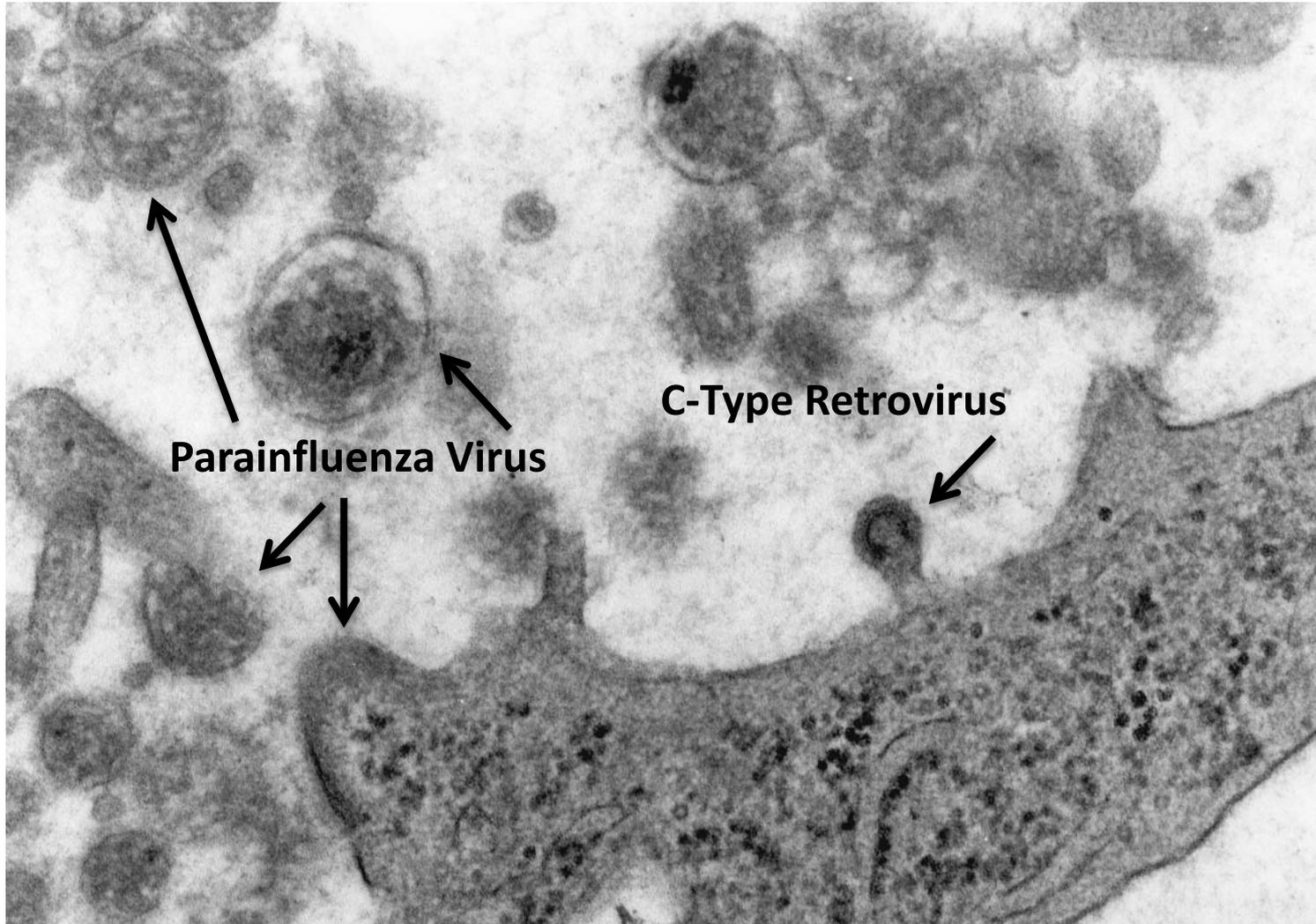
# **Oval or oblong inclusions resemble poxviruses**

- **Round/oval organelles resemble poxviruses**
  - **Lipid inclusions**
  - **Melanosomes**
  - **Mitochondria**



## Dual Infection

When you find one agent, do not stop looking;  
there may be others.



## **Summary of Confusing Inclusions**

**Many things including normal organelles can look like infectious organisms.**

**A good knowledge of normal cellular architecture is essential to recognize unusual things that should not be in patient specimens.**

**Be able to describe the unusual structure, even if you cannot name it.**

**Narrow down the field of possibilities if possible (virus, bacteria, higher organism).**

**Seek expert help.**