Review Article

USE OF ELECTRON MICROSCOPY TO DIAGNOSE VIRAL ILLNESSES

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Until recently, rationale for viral diagnosis was based mainly on prevention of unnecessary antibiotic therapy for the patient and quarantine for the safety of others. However, with the advent of more and better antiviral agents, it is becoming increasingly important not only to determine the presence of viruses, but also to identify them for treatment.

Electron microscopy (EM) can be an important adjunct to conventional culture and serologic techniques in diagnosing viral illnesses. Though detection of viruses by EM requires relatively large numbers of virions, and provides no information regarding specific serotypes within a virus family, it has the distinct advantages of being simple and rapid. Also, infectious particles are not required. Some viruses do not grow in tissue culture or grow only after special manipulation, and those that do may not survive if transportation conditions to the lab are not optimal. These agents would be missed by culturing. Additionally, a wide variety of agents can be visualized by EM; because specific reagents such as antibodies, antigens, or nucleic acid and protein probes are not required, one is not limited to the availability of these reagents, and prior knowledge of the virus identity for reagent selection is not required. Finally, since the test entails the visualization of the virus itself, rather than a color change or agglutination reaction, false positive tests resulting from cross-reactions of reagents with similar materials are not likely.

Two types of preparations are primarily used for routine EM virus identification, negative staining and thin sectioning, although specialized research techniques such as scanning EM, specific antibody aggregation or labeling with electron-dense tags, *in situ* labeling, cryomicroscopy, and high-voltage microscopy have been used to classify viruses and describe virus-host relationships. Negative staining of liquid samples such as stool, urine, and cerebrospinal fluid (CSF) is very rapid, and physicians can be provided with an answer within a few minutes to a couple of hours. Solid tissue sample preparation requires more time, but results can usually be obtained within a few days.

This paper describes the characteristics of virus morphology that are used in EM identification of these agents in both liquid and solid specimens. The figures, though not inclusive of all viruses, demonstrate the major morphological characteristics by which viruses can be identified in clinical samples.

Methods

Support Films

For viewing viruses in liquid suspensions, a thin support film must be placed over the EM grid to hold the sample. These films, such as Formvar and collodion, and their preparation have been described in detail.[3] For optimal stability in the beam, films are usually coated with carbon in a vacuum evaporator. However, if an evaporator is not available, they may be viewed uncoated. The heavy metal negative stain provides some degree of electron conductivity. Coated grids are also useful in supporting thin sections if they move or break in the beam due to inadequate infiltration or polymerization. Additionally, unstable thin sections may be stabilized by applying a thin carbon coat in the vacuum evaporator.

Negative Staining

Detailed methods for collecting specimens and preparing them for EM have been described.[3-8] Briefly, liquid specimens should be transported undiluted, not placed into transport media like those sent for culture. Liquid samples are clarified of large debris by low-speed (1,000-2,000 x g) centrifugation. Fecal samples are suspended in water or volatile buffer at 10% to 20% (weight:volume) and clarified. A 20-30 µL drop of supernatant is placed on a waxy surface, such as Parafilm (American National Can, Greenwich, CT), and a grid with a carbon-coated support film is placed on the drop under a small inverted Petri dish for 5-10 minutes. The grid is drained with filter paper and immediately negatively stained by placing it, specimen side down, on a small drop of saturated aqueous uranyl acetate or 2% aqueous

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phosphotungstate for 30-60 seconds. After draining with filter paper, it can then be viewed immediately (with disinfection of the microscope holder later) or exposed to ultraviolet light (900 μW/cm^2 at a distance of 15-20 cm) on both sides for five minutes to inactivate pathogens before viewing.[6] If, after examination of this direct preparation, no viruses are found, some form of concentration should be attempted, such as ultracentrifugation, agar diffusion or, if available, antibody aggregation (see below).

Hydrophobic grids, as determined by poor spreading or staining of the sample, can be rendered hydrophilic by glow discharging the grids in a vacuum evaporator or by allowing them to incubate on various solutions, including 1% poly-L-lysine or 1% Alcian blue, for two to five minutes and then staining with water.[3]

Negatively stained specimens that contain various sized small droplets can be confusing because the droplets may resemble small icosahedral viruses; this is a problem particularly in blood and stool, which contain many lipid and proteinaceous materials. One solution is to wash the sample two to three times in water or to extract it with organic solvents. To remove lipid, we add one part of a mixture of chloroform/methanol (1:1) to one part sample, mix in an ultrasonicator or vortex mixer, and allow it to sit for several minutes. The aqueous portion comes to the top and then can be placed on a grid for negative staining. If the small droplets disappear, they were not viruses. This method will destroy enveloped viruses, but isometric and helical nucleocapsids of such viruses will survive.

Virus Concentration

Because viruses present in low numbers can be missed, our laboratory concentrates all negative samples except some stools by ultracentrifugation. Viruses present in fecal samples are usually present in large numbers, and concentration is unnecessary; however, if there is a suggestion that small round viruses might be present, stool samples are concentrated as well. After clarification at low speed, the supernatant is pelleted either in an Airfuge (Beckman Instruments, Inc., Palo Alto, CA) at 30 psi for 30 minutes, in a desktop ultracentrifuge at 45,000 rpm for 45 minutes, or in a floor-model ultracentrifuge at 45,000 rpm for 45 minutes, depending on the amount of sample submitted.[8] The Airfuge uses either an EM-90 rotor, which accepts filmed grids directly,[9] or a rotor that accepts 0.2 mL tubes. Grids from the EM-90 rotor are drained, negatively stained, and viewed. Because this method pellets everything in the well, it may give preparations that are too thick to see through; therefore, it is recommended that two to three twofold dilutions also be included. Pellets from tube centrifugation are resuspended in a few microliters (20-30 μL), depending on the size of the pellet, and the suspension is processed as described above for negative staining.

Another concentration method is agar diffusion.[10] A large drop (50-100 μL) of clarified liquid sample is placed onto a block of 2% agar, and a filmed grid is placed onto the drop. As the liquid diffuses into the agar, viruses are concentrated onto the grid. When the liquid is mostly gone, the grid is drained and negatively stained.

Ultrafiltration with filters having an Mr cutoff of 10,000 daltons (Amicon Corp., Danvers, MA; BioRad, Richmond, CA; Millipore, Bedford, MA; Schleicher and Schuell, Keene, NH) can be used to concentrate viruses. Liquid is forced through the unit while small particles are retained on the filter. One absolute must for success is to clarify the virus suspension of cellular debris, else the filter will become clogged. A pre-spin of about 12,000 for one to two minutes in a microcentrifuge is recommended to pellet large pieces of debris and bacteria before the supernatant is centrifuged in the filter unit.

Finally, cultivable viruses can be amplified by short-term replication in tissue culture.[11,12] Developing virions may often be seen by thin sectioning and EM before viral cytopathology is evident by light microscopy.

Immunoelectron Microscopy (IEM)

Concentration and/or specific identification of viruses in suspension can be achieved using antiviral antibodies, but as with other immunological detection systems (e.g., enzyme-linked immuno assays, serology, fluorescence microscopy, Western blots) and nucleic acid-based tests (e.g., polymerase chain reaction, nucleic acid hybridization), one must have an *a priori* notion of the virus identity to be able to select the appropriate reagents. Various methods for virus identification by IEM have been reviewed by Hayat and Miller.[3]

Antiviral antiserum added to a clarified virus suspension in low concentration (e.g., 1:1000 for specific antiserum; 1:100 for pooled gamma globulin), will cause clumping of the viruses if it recognizes them.[13,14] The mixture is incubated for one to two hours at room temperature, or overnight at 4°C, and then pelleted at 17,000 x g for 1.5 hours. The pellet is resuspended in water as with the ultracentrifuged pellet and negatively stained. This procedure can be used to concentrate viruses in dilute suspension or to serotype them. If specific antibodies are available (e.g., to different enteroviruses), a positive aggregation can determine different serotypes. Of course, appropriate negative controls should be included because sometimes viruses may tend to clump on their own. Alternatively, filmed grids can be incubated on diluted (1:1000) specific antiserum for 30-60 minutes at room temperature, washed on buffer, and then incubated on drops of the virus suspension. This technique traps and

attaches viruses recognized by the antiserum onto the grid. It is then negatively stained as described above.

If the antibody is added in sufficiently high concentration, the result will be a coating of the virus particles that will appear as a dark fuzz around them after negative staining.[13] Serotyping can be done in this way, but there is not a concentration effect.

Finally, specific antiserum can be used to coat viruses, and a colloidal gold-tagged secondary antibody produced against the animal species of the antiviral antibody can be used to label the viral particles with black dots. Immunogold labeling can be done in suspension with ultracentrifugation and washing, or directly on the grid after the viruses are attached.

Immunolabeling as well as *in situ* hybridization of viruses in thin sections has been described, but these techniques are not used for routine diagnostic virology.

Ultrathin Sectioning

Most routine methods for staining and embedding tissue in epoxy for thin sectioning[15,16] are acceptable for diagnostic virology. Tissue should be placed into 2 % to 4% glutaraldehyde immediately after removal from the patient if possible; it can also be transferred from formaldehyde into glutaraldehyde if this is the only sample available. Tissue that cannot go immediately into fixative should be transported on a saline-moistened gauze to the EM lab as soon as possible for fixation. Further processing is in osmium, often followed by uranyl acetate, dehydration in graded alcohols, and infiltration with epoxy resin. We use Spurr's resin because of its eight-hour cure rate, but others use an overnight cure of an Epon-equivalent resin.

Rapid methods for processing have been described[17-19] and essentially consist of using very small thin slivers (0.25 x 0.25 x 0.5 mm) of tissue and continual agitation in solutions, decreasing the processing time, increasing the resin catalyst (2-3X), and using a shorter and hotter baking time (e.g., 25-30 minutes at 95°C). With diligence and almost constant work, the tissue can be ready for EM exam within two to three hours.

If the only tissue available is in paraffin-embedded blocks, it can be deparaffinized with xylene, rehydrated, and processed for EM.[20-23] Also, wax sections already stained for light microscopy can be processed for EM on the slide, peeled up, and sectioned.[24,25] However, damage to ultrastructural cellular architecture will render such specimens unacceptable for diagnosing smaller viruses (e.g., picornaviruses) and many enveloped viruses (e.g., togaviruses, bunyaviruses).

Since viral infection can be focal, and ultramicrotomy can sample only small areas, it is a good idea to cut a thick section first. A 0.5 µm thick section of the whole block face is cut with a glass knife, picked up in a loop, and heat-fixed onto a glass slide. It is then stained on the hot plate with alkaline toluidine blue[26,27] until the edge of the stain drop starts to dry and become iridescent. After gentle washing with water and drying on the heater, it is examined by light microscopy for areas of interest, such as inflammation, enlarged cells, enlarged nuclei, cells with nuclear or cytoplasmic inclusions, syncytial cells (fused cells with multiple nuclei), or necrotic tissue. Frequently, the best area to select for thin sections is the junction between normal and heavily diseased tissue. Excess tissue is trimmed away, and ultrathin sections are cut from the

FIGURE 1. Negatively stained large naked viruses. A. Adenovirus; note the bead-shaped capsomers arranged on the surface in flat triangular patterns (two triangles are delineated by arrowheads). B. Rotavirus; note the double shell (arrows) and the tubular capsomers that radiate out from the center. There is a very thin covering on the outside of most virions (arrowhead). The bars represent 100 mm.

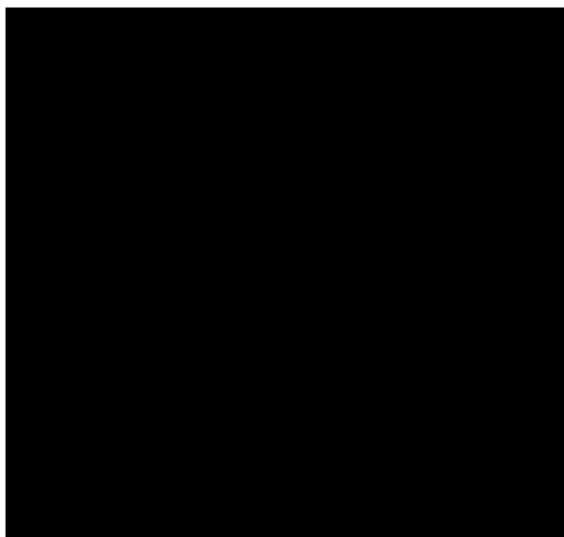

FIGURE 2. Negatively stained medium-sized naked virus. Polyomavirus is about 2/3 the size of the largest naked viruses. Note the bumps on the surface. The bar represents 100 nm.

FIGURE 3. Negatively stained small naked viruses from stool. The bar represents 100 nm.

selected area. This method saves examination time in the EM.

Alternatively, we have developed a technique whereby large pieces of tissue (up to about 2 cm in diameter) are sliced on a vibrating microtome with a razor blade, stained with propidium iodide or other fluorescent dye, and viewed with a confocal microscope. Unusual areas are cut out with a razor blade and then embedded with the confocally-viewed side outward so that this face is the first plane cut.[28] This method saves having to embed and cut thick sections of multiple random blocks of tissue.

Safety

Chemical, electrical, fire and mechanical safety in the EM lab have been addressed in a recent textbook.[16] Clinical specimens are potentially infectious and should be handled in accordance with universal precautions.[29] If potentially viable viruses are viewed by negative staining, the specimen holder should be disinfected after use with a dilute (1:10 dilution) solution of bleach for five minutes and washed thoroughly. Our procedure is to use a separate holder for clinical specimens and research specimens; a special box for contaminated forceps is kept on the microscope console.

Ethanol is not virucidal for most naked viruses. Some agents which cause brain infections are resistant to killing by fixation; thus, care should be exercised to autoclave fixative and rinse solutions and to disinfect nondisposable utensils in bleach for 20 minutes. All remaining specimen and disposable materials should be autoclaved before discarding. We store all samples, labeled with a biohazard sticker, in a refrigerator, for two weeks before discarding, and a small portion of all positive samples is stored indefinitely.

Sample Collecting

Our laboratory is constantly in touch with physicians to guide them in collection, and a hospital-wide instruction book is also available to all lab staff, nurses, and physicians. Ordering physicians are notified immediately of the receipt of duplicate, improperly collected or transported, unclearly labeled, or inappropriately ordered samples, and a solution is discussed. At the start of the year when new personnel come on staff, a notice of our services with the procedure for using them is sent to all potential users. Close communication between the ordering personnel and the EM diagnostic personnel greatly facilitates proper handling of samples.

Additionally, information about the sample, such as from what body location it was obtained, and about the patient, such as what the presenting symptoms were, are helpful in diagnosis. Some viruses have an affinity for certain organ systems, and an idea of what to look for can be helpful. Tables 1 and 2 list the most likely viruses to be visualized by EM in various samples. However, in immunocompromised individuals, such as AIDS patients and individuals receiving cancer chemotherapy, the boundaries are less clear.

Reporting

Positive specimens are reported immediately to the ordering physician by phone; then all information, including negative results, is entered into the hospital computer. For specimens sent from other institutions, results are reported by phone immediately, followed by a hard copy report, either faxed or mailed.

TABLE 1. *Most likely viral agents in clinical samples visualized by negative staining and electron microscopy.**

Blisters	herpes simplex virus (HSV/HHV 1, 2); varicella zoster virus (VZH/HHV 3); poxviruses (orf, vaccinia, molluscum contagiosum)
Blood/serum	SRVs (small round viruses): hepatitis A virus (HAV, HEV); parvovirus B19 (a very small SRV); hepatitis B virus (HBV); HCV (hep C: unrecognizable by neg. st. + EM); toga-, bunya-, arenaviruses (rare, unrecognizable from cell debris); filoviruses (rare)
Cerebrospinal fluid	SRVs (enteroviruses, Coxsackie viruses); adenovirus; herpesviruses; paramyxoviruses (measles, mumps); toga-, arenaviruses (unrecognizable)
Nasopharyngeal aspirates/lung lavages	ortho- (influenza), paramuxoviruses (respiratory syncytial virus/RSV, para-influenza, measles, mumps: indistinguishable from each other); SRVs (rhino-, enteroviruses); adenovirus; herpesviruses (CMV, HSV, VZV); coronavirus; reovirus; SRVs
Pleural/pericardial fluids	SRVs
Stool	rotavirus; adenovirus; calicivirus; astrovirus; SRVs; SRSVs (small round structured viruses); coronavirus
Tears	adenovirus; SRVs; herpesviruses (HSV, CMV, VZV)
Urine	CMV; polyomavirus; adenovirus; picornavirus; paramyxoviruses (mumps, measles); bunya-, arenaviruses (unrecognizable)

*reprinted from reference # 8.

TABLE 2. *Most likely viral agents in clinical samples visualized by thin sectioning and electron microscopy.**

Brain	herpesviruses (HSV, CMV); adenovirus; papovavirus; measles virus (SSPE); SRVs; *rare*: togaviruses (rubella, yellow fever, EEE, WEE, Powassan fever); bunyaviruses (LCM, LaCrosse, several hemorrhagic fever viruses); HIV; rabies; prions: not visible
Eye	adenovirus; herpesviruses (HSV, CMV, VZV); SRVs; molluscum contagiosum (immunosuppressed patients); rubella (congenital)
Heart	adenovirus; SRVs (enteroviruses, especially Coxsackie); bunya; arenaviruses (EM unnecessary in diagnosis)
Intestine	rotavirus; adenovirus; SRVs; herpesviruses (HSV, CMV); coronavirus
Kidney	polyomavirus; adenovirus; herpesviruses (HSV, CMV); *rare*: Hantaan virus (bunya-)
Liver	SRVs (HAV, HEV); adenovirus; herpesviruses (CMV, HSV); HBV; HCV (unrecognizable); *rare*: togaviruses; hemorrhagic fever viruses (filo-, arena-, bunyaviruses)
Lung	ortho- (influenza); paramyxoviruses (RSV, parainfluenza, measles, mumps); SRVs; adenovirus; coronavirus; herpesviruses (HSV, CMV, VZV)
Pancreas	(EM not usually necessary); paramyxoviruses (measles, mumps); SRV (Coxsackie); HSV
Skin	herpesviruses (HSV, VZV); poxviruses (orf, vaccinia, molluscum contagiosum); measles virus; rubella virus; SRVs (enteroviruses, esp. Coxsackie); parvovirus B19; papilloma virus (EM not necessary); *rare*: bunya-, arenaviruses; rabies (nerve endings)

*reprinted from reference # 8.

Shipping

Outside cases are shipped to the EM lab on ice (in wet ice or gel-pack, not frozen in dry ice) by overnight carrier. Liquids are sent *unfixed* and *undiluted* in a sealed container inside a zip-lock bag containing absorbent material in case of leakage. Potentially infectious material should be labeled with a biohazard sticker. Tissues should be fixed in glutaraldehyde immediately upon removal from the patient and sent in small vials completely filled with glutaraldehyde to prevent the tissue from sticking on a side of the container out of solution and becoming dehydrated.

Results

Molecular biologists describe viruses in two large categories: DNA viruses and RNA viruses; indeed , all viral atlases are divided this way. However, electron microscopists must be concerned with appearance for identification. There are two major morphological categories of viruses, naked ones and enveloped ones. Identification of viruses based on morphology will be described in both negative stains and thin sections.

Identification of Viruses in Liquid Samples

Naked Viruses

Naked viruses are all icosahedral; they have protein shells that are fairly rigid and maintain their spherical shape when air-dried with stain; they can be compared to a baseball, a rigid sphere. Human naked viruses come in three size ranges: 75-90 nm, 45-55 nm, and 22-35 nm.

Figure 1 demonstrates the large naked viruses. Adenovirus (Figure 1A) has small bead-shaped capsomers grouped in flat triangular facets like a geodesic dome, with six beads per side; it is single shelled. It is clearly distinct from rotavirus (Figure 1B), which has tubular capsomers that radiate out from the center like the spokes of a wheel (hence, the name "rota"). Rotavirus is mostly double-shelled, and when the stain penetrates a virion, this double structure can be seen. Occasionally, smaller single-shelled variants can be found within the population of complete virions. A third large naked virus, reovirus, shares many ultrastructural features with rotavirus. Rotavirus differs morphologically from reovirus in having a thin rim of material around the outer shell (Figure 1B); however, this rim can be lost, rendering distinction of the two viruses

FIGURE 4. Negatively stained enveloped viruses. A. Herpesvirus. Note that the stain has penetrated the outer membrane to outline the nucleocapsid (NC) inside. The membrane appears smooth on the outside because the exterior proteins do not project very far. B. Measles virus (V). Note the spikes or fuzz on the surface (arrowheads), making this virus recognizable from cellular debris, and the filamentous or helical nucleocapsid (NC). C. Vaccinia, a poxvirus. The overall shape is oval or brick-shaped, and the surface has deep crevasses. The bars represent 100 nm.

difficult.

Adenoviruses are frequently found in the gastrointestinal and respiratory tract of immune competent patients. Other locations are shown in Tables 1 and 2. Rotaviruses are seen only in the intestinal tract, while reoviruses are seen (infrequently) in lung preparations. The presence of reoviruses in intestinal specimens is controversial; in general, double-shelled particles in stool specimens, even those lacking an outer rim, are most likely rotavirus.

Medium-sized naked viruses, measuring 45 or 55 nm, belong to the papovavirus family. A polyomavirus (45 nm) (Figure 2) can be seen frequently in urine of transplant patients and pregnant women. Another has been described in extracts of homogenized brain from patients with progressive multifocal leukoencephalopathy, although the best way of demonstrating this infection is by thin sectioning of brain biopsy. The larger of these two types of viruses is papillomavirus (55 nm), which causes warts; it has been demonstrated by negative staining of homogenized lesions, but the best diagnosis is by routine histology of biopsied material. It is often difficult to observe virions in thin sections of skin lesions by EM because the tissue can contain integrated viral DNA while not producing complete virions.

The smallest naked virus group contains a number of different organisms. Many are so small and morphologically indistinct that they have been referred to in the literature as small round viruses (SRVs) or small round structured viruses (SRSVs). The very smallest ones, 22 nm in diameter, are parvoviruses which may be seen in amniotic fluid or in blood in “fifth disease,” an erythematous disease in small children; they may also be found in blood of patients with anemia during erythroblast crises. Other parvoviruses, seen in stool, are defective, nonpathogenic viruses sometimes accompanying

FIGURE 5. Thin sections of a large naked virus. A. Adenovirus, a DNA virus in the nucleus (N) of a cell. The bar represents 1 μm. B. A higher magnification of adenovirus showing a paracrystalline array of particles. The bar represents 100 nm.

adenovirus; they are designated adenovirus-associated virus (AAV).

Slightly larger particles, 25-35 nm in diameter, may be seen in fecal samples of patients with gastroenteritis, in respiratory secretions, and occasionally in cerebrospinal fluid; refer to Tables 1 and 2 for a more complete listing. They are members of the picornavirus or calicivirus families. Some of these small icosahedral viruses are morphologically indistinct, i.e., they appear simply as fuzzy balls (Figure 3), while others, such as astrovirus and calicivirus, may have some exterior structure that is specifically identifiable. If small round viruses found in stool are morphologically unidentifiable, the possibility that they may be tail-less bacteriophages must be considered. Pathogenic viruses are often present in large numbers, sometimes in clumps, and are not usually associated with bacterial debris such as flagella or cell walls. Convalescent serum can be examined by IEM, as described above, for antibodies reactive with viruses in stool samples obtained during the acute phase of illness; this technique can conclusively determine the role of the virus particles in gastroenteritis.

Confusion can also arise if there are spherical droplets present in negative stains, particularly in serum and stool samples; these resemble small viruses. Virus particles within a given type are relatively uniform in size, may be present in large numbers, and often occur in aggregates. Contaminating lipid drops can be removed by an organic solvent extraction, as described above. Other structures that may be confused with viruses have been described elsewhere.[6]

Enveloped Viruses

Enveloped viruses (Figure 4) usually obtain their coating as they bud through cellular membranes that have been programmed to contain viral proteins; poxvirus is an exception. In many cases, this coating is pliable and covers the nucleocapsid loosely; thus, when air-dried, it may take any shape. These pleomorphic viruses may be compared to a soft plastic beach ball which, when deflated, is no longer round. The size of enveloped viruses varies with the type; some are as small as 40-60 nm (e.g., togaviruses, flaviviruses), while others may be as big as 200-300 nm (e.g., herpesviruses [Figure 4A], measles virus [Figure 4B]). A few of the enveloped viruses retain their shape during drying. The poxviruses (Figure 4C) are ovoid or

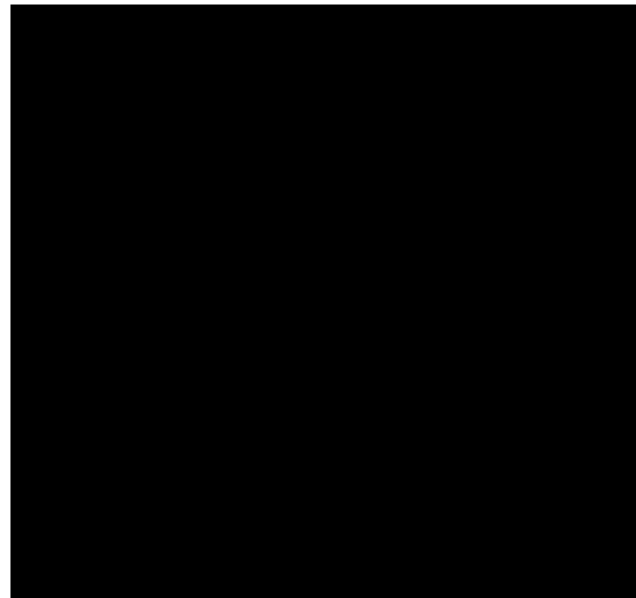

FIGURE 6. Thin section of a small RNA virus. Nodamura virus in mouse muscle, shown to demonstrate paracrystalline arrays of small viruses in the cytoplasm of a cell. The bar represents 100 nm. Micrograph supplied by Cynthia Goldsmith, Centers for Disease Control and Prevention, Atlanta GA.

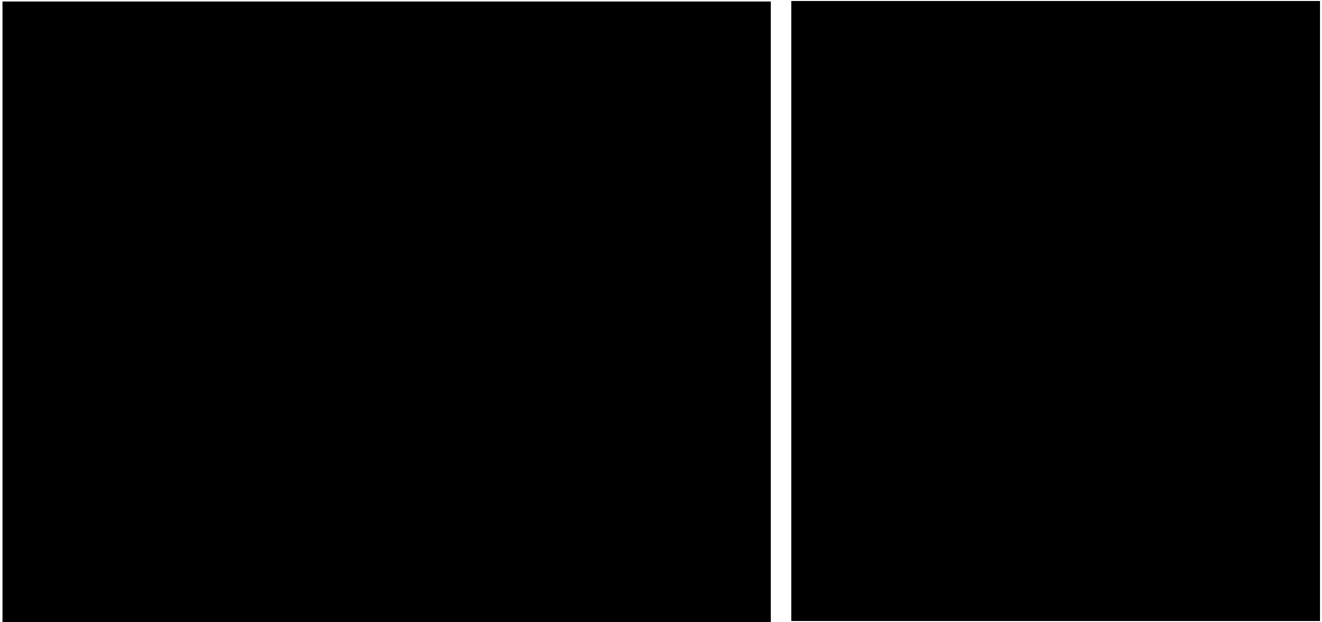

FIGURE 7. Thin sections of an enveloped DNA virus with a spherical nucleocapsid. A. Low magnification of herpesvirus. Nucleocapsids (NC) are formed in the nucleus; nucleocapsids and complete virions (V) can be seen in the cytoplasm. The bar represents 1 µm. B. High magnification of an enveloped particle. The bar represents 100 nm.

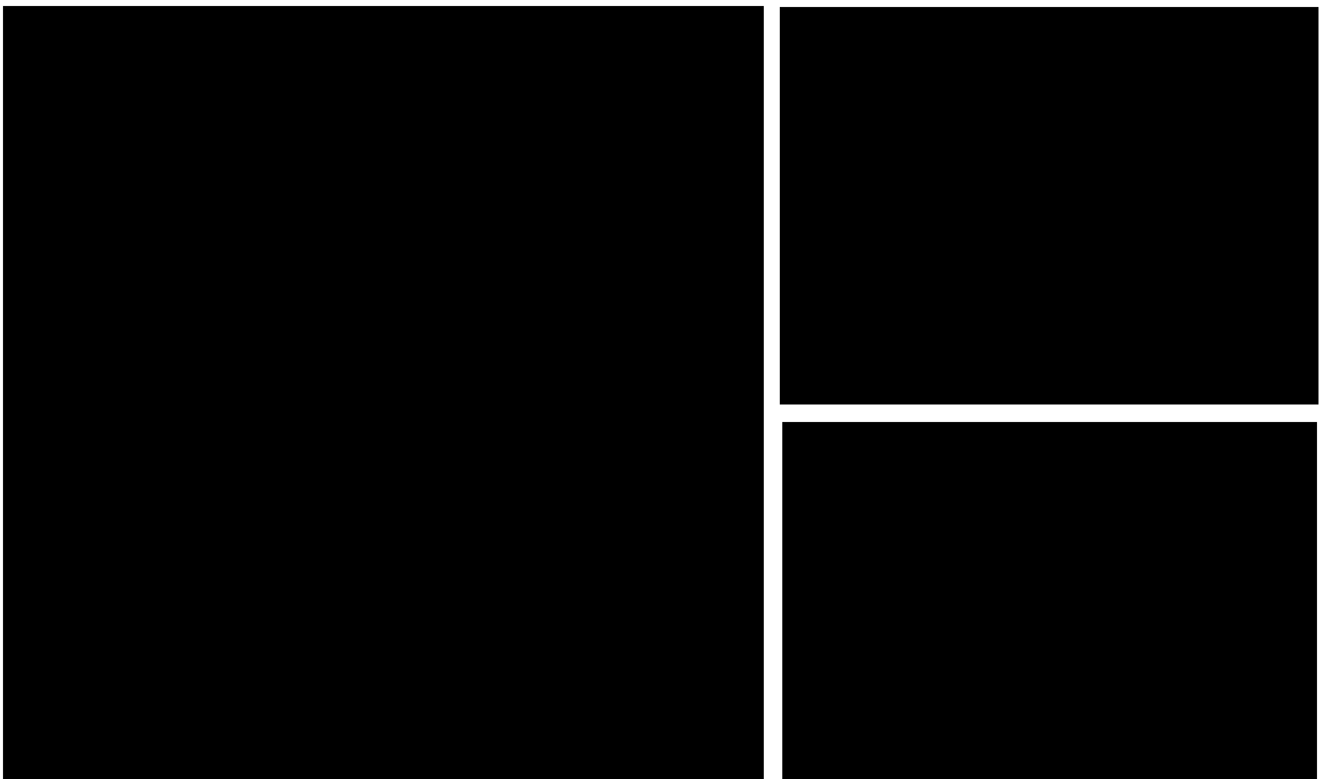

FIGURE 8. Thin section of a poxvirus-infected cell. A. Low magnification of vaccinia in various stages of development in the cytoplasm of a cell; I, immature virus particle just beginning to form its shell; D, developing particle in the process of condensation; M, mature particle. The bar represents 1 µm. B. High magnification of a developing particle (bottom) in the process of condensing and possibly an immature particle or a different plane through a condensing particle (top). C. High magnification of a mature particle; note the complex dumbbell-shaped core. Bars in B and C represent 100 nm.

brick-shaped, 150-250 x 200-250 x 300-350 nm in size; their envelope is synthesized *de novo* in the cytoplasm, rather than obtained through budding. The rhabdoviruses are bullet-shaped, with a usual diameter of about 80 nm and a length of 200-300 nm. The filoviruses look like long rhabdoviruses; the diameter is about the same, but their length may be up to 1400 nm. Sometimes they are bent or have curved ends; they may even curl into figures like 6's or 9's.

The membrane has outer projections which may or may not be long enough to be visible as fuzz or spikes on the exterior. Viruses with spikes, such as the orthomyxoviruses, paramyxoviruses, and coronaviruses, can be more easily distinguished from membranous cell debris in negative stains than smoother ones such as bunyaviruses, arenaviruses, and retroviruses. In the case of virions with short, indistinguishable projections, sometimes the stain penetrates the envelope, making the nucleocapsid visible inside (Figure 4A).

Inside the envelope is a nucleocapsid: a core composed of nucleic acid and some proteins. It can be spherical (Figure 4A), like the naked icosahedral viruses; helical (Figure 4B), like a coiled phone wire; complex (poxvirus); or morphologically nondescript.

Identification of Viruses in Thin Sections of Infected Cells

As a general rule, DNA viruses are usually found in the nucleus, and RNA viruses are found in the cytoplasm, but there are a few exceptions. Naked viruses are seen as dense round particles usually clustered together in virus "factories" within cells and may be arranged in paracrystalline arrays. Naked DNA viruses (e.g., adenoviruses [Figure 5], papovaviruses) are constructed in the nucleus, and may be seen in the cytoplasm late in infection when the nuclear membrane has deteriorated. Naked RNA viruses (e.g., picorna-viruses [Figure 6]) are constructed in the cytoplasm and are not found in the nucleus.

The nucleocapsids of enveloped DNA viruses (e.g., herpesvirus [Figure 7], cytomegalovirus) are formed in the nucleus (except for poxviruses). They may bud through the nuclear membrane to obtain their outer covering or may escape through nuclear pores into the cytoplasm, where they bud into vesicles or through the plasma membrane to receive their outer envelope. Poxviruses (DNA viruses) (Figure 8) are produced in the cytoplasm, and their envelope is formed *de novo* without budding. These virions are oval or brick-shaped.

Isometric nucleocapsids appear round in thin sections (e.g., herpesviruses [Figure 7]), while helical ones appear as curvy filaments like "worms" going in and out of the plane of section (e.g., measles virus [Figure 9], influenza virus). The poxviruses have a complex core seen in different forms, depending on their maturation state (Figure 8). Some nucleocapsids do not take a specific shape and are simply dark, unrecognizable, or morphologically indistinct (e.g., retroviruses [Figure 10], flaviviruses).

The nucleocapsids of enveloped RNA viruses bud through internal cytoplasmic membranes into vesicles (e.g., bunyaviruses, arenaviruses) or through the plasma membrane into the extracellular space (e.g., retroviruses [Figure 9]). Rarely, filamentous (helical) nucleocapsids of paramyxoviruses can be found in the nucleus late in infection, as sometimes in cases of measles or subacute sclerosing panencephalitis, but complete, enveloped RNA virions are not seen in the nucleus.

Some viruses become integrated into the host DNA and may not produce complete virions; these are unrecognizable by conventional EM. Examples are many retroviruses and some herpesviruses.

Discussion

This discourse is meant to be an introduction to virus morphology, to show the major characteristics by which viruses can be identified as such. Once a structure is suspected of being viral, one may consult an atlas[30-32] for specific identification. One of the obligations in virus identification is to be able to distinguish viruses from normal cellular organelles and nonspecific artifacts caused by cell degradation and death. This necessitates a good familiarity of normal cell architecture,[33-36] and pathological changes due to other causes.[37,38] Such things as dense core granules, peroxisomes, microbodies, coated vesicles, nuclear pores, microtubules in cross section, intermediate filaments in longitudinal and cross section, ribosomes, glycogen, lipid, and nonspecific alterations like nuclear granules and nuclear bodies can pose as viruses.

Electron microscopy, because of its rapid procedures, and lack of need for infective particles or specific viral reagents, can be an important instrument in the diagnosis of viral illnesses. One of the most beneficial areas is in the identification of gastroenteritis viruses, many of which do not have available biochemical reagents for detection, and most of which do not readily grow in tissue culture. The same is true for polyomaviruses in urine. Another case where EM is the best modality for diagnosis is in differentiating herpesvirus from poxvirus in skin lesions.

Electron microscopy is also of great benefit in the management of the growing population of patients with immune deficiencies. Included in this group are patients undergoing cancer chemotherapy and individuals with

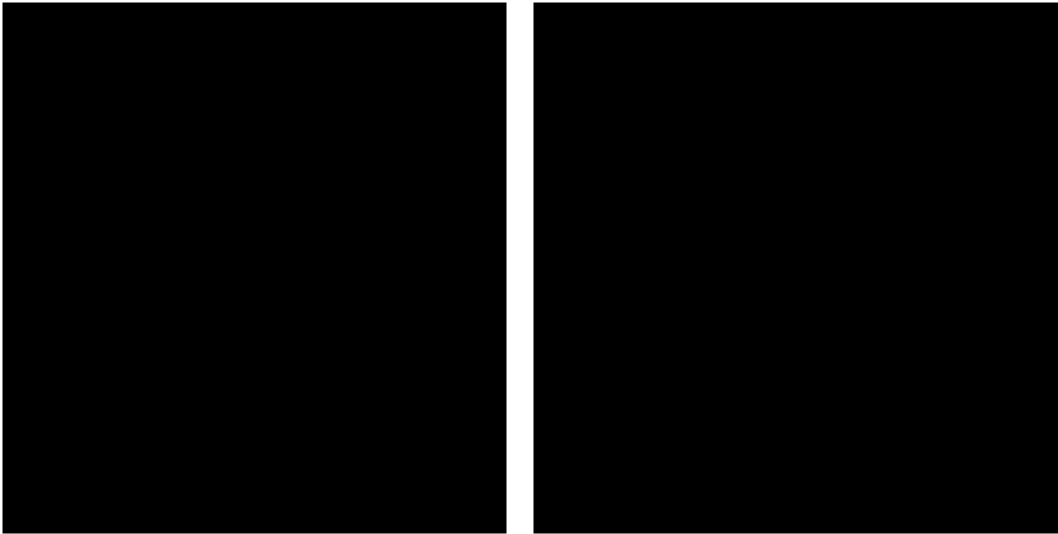

FIGURE 9. Thin section of an enveloped virus with a helical nucleocapsid. A. Low magnification of a measles virus-infected cell. Helical nucleocapsids form in the cytoplasm and appear as curvy filaments weaving in and out of the plane of section (arrowheads). Mature virus particles (V) are seen at the periphery of the cell, budding from the plasma membrane. The bar represents 1 µm. B. High magnification of budded particles. A cross-section of a nucleocapsid can be seen inside (NC), and the virion projections show up as fuzz on the exterior (arrowheads). The bar represents 100 nm.

FIGURE 10. Thin section of an enveloped virus with a morphologically nondescript core. A. Low magnification of human T-cell leukemia/lymphoma virus showing particles that have budded from the cytoplasmic membrane (arrowheads). The bar represents 1 µm. B. High magnification of the enveloped virions (V); the icosahedral nucleocapsids (NC), although basically round, do not have a uniform shape; compare these to herpesvirus (Fig. 7B). The bar represents 100 nm.

congenital or acquired immunodeficiencies. Such patients are surviving longer because of better management, but are frequently plagued with viral infections.[39] Rapid diagnosis by electron microscopy, coupled with prompt antiviral therapy, may be life-saving in these patients.

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