EVALUATION OF ELECTRON MICROSCOPIC INFORMATION AVAILABLE FROM CLINICAL SAMPLES

Sara E. Miller

Duke University Medical Center
Durham, North Carolina, USA

INTRODUCTION

Much has been written on the procedures for using electron microscopy (EM) in diagnostic virology (Almeida, 1983; Kapikian et al. 1976; Miller, 1986, 1988, 1989; Oshiro and Miller, in press) and the viruses likely to be found in clinical samples (Hsiung, 1982; Lennette, 1985). Excellent atlases are also available for identifying virions (Doane and Anderson, 1987; Palmer and Martin, 1988). This information will not be repeated here. Rather, we will elaborate on characteristics of viruses that allow them to be recognized by EM, interpretation of EM observations, i.e., what do the results tell the physician, what tests are most appropriate for which specimens, and what action can be taken based on that information.

In the past, viral diagnosis was attempted primarily to assess the necessity of quarantine to prevent spread of the disease and to prevent the administration of unnecessary antibacterial therapy. Today with the availability of several antiviral drugs and many more in clinical trials, detecting viral diseases is becoming increasingly important. Indeed, because of the specificity of many of these drugs, it is important not only to determine that an illness is of a viral origin, but also to identify which virus is present. We are now in an era with respect to viral diseases comparable to that in the 1950's when antibiotics were beginning to be used for bacterial infections. Along with drug therapy, epidemiology is another major reason today to document viral infections.

EM is a valuable tool and in some cases may be the only one for discerning the presence of viruses; however, it can and should be used in conjunction with other virological tests. EM has several advantages. The main one is that it is rapid: (a) A direct negative stain can take as little as 20 min; even with the use of concentrating methods, it can be completed in 1-2 hr. (b) Thin sectioning usually takes about 24 hr but can be speeded up to 2-4 hr, which is certainly faster than any culturing procedures, and as fast as fluorescence staining. Another advantage is that for direct examination by negative staining, specific reagents such as antibodies, nucleic acid probes, or protein standards are not required. Use of these reagents necessitates an *a priori* notion of what virus may be present to narrow the reagents employed to a manageable number. A fur-

Medical Virology 10, Edited by L.M. de la Maza and E.M. Peterson, Plenum Press, New York, 1991

ther advantage of EM is that infectious particles are not required, which is important if the sample has not been properly transported or in the case of viruses, such as the gastroenteritis viruses, that do not grow readily or at all in tissue culture. EM can also differentiate different viruses that produce similar cytopathology in tissue culture and can detect viral contaminants in cultures used for virus isolation. Because the test is a direct visualization of the agent, rather than a biochemical detection of viral components, e.g., enzyme immunoassays (EIAs) there is less chance of a false positive test.

One disadvantage of EM in diagnostic virology is that viruses within the same family most often have similar morphology and thus cannot be differentiated visually, e.g., herpes simplex virus (HSV) vs. varicella zoster virus (VZV). However, once the virus family is discerned, serology or immunoelectron microscopy (IEM) can further identify the virus. Another problem is that a fairly high number of virions (10^5-10^6/ml) must be present for any to be seen by negative staining, though several concentration methods are possible (Hayat and Miller, 1990). Other methods include clarification of debris by a low speed spin followed by ultracentrifugation, ultrafiltration, or precipitation. These drawbacks not withstanding, EM can be an important adjunct in viral diagnosis when used in conjunction with other methods.

SAMPLE COLLECTION

The collection and preparation of specimens for examination by techniques other than EM have been described in detail by Lennette (1985). To aid the physician in ordering EM virology tests, the following information will describe the kinds of samples that can be examined by EM, how much specimen should be collected, and how specimens should be sent or stored. The most frequent samples examined by EM are stools and liquids because they are more easily collected from the patient. Liquid specimens are listed in Table 1 along with an appropriate amount to send. It takes about 5 µl of liquid for a direct examination on one EM grid; however, the more sample available, the more likely it is that a viral agent could be seen after concentration procedures if it is present in low numbers.

Fecal specimens from cases of gastroenteritis are the most likely samples to be sent to the EM laboratory, not only because they are easily obtainable, but also because most of the viruses that cause gastroenteritis cannot be grown easily or at all in cell culture. Also, if one of the slide diagnostic kits for rotavirus or adenovirus is used, other viral pathogens will be missed. Stools should be sent in a cup or in the original diaper if the patient is an infant. Although a few hours at room temperature will not degrade these viruses, if there is a delay in transport, fecal samples should be refrigerated but not frozen. However, stools to be maintained for several months for epidemiological studies can either be kept at 4°C sealed tightly or under paraffin oil, or can be frozen at -70°C or in liquid nitrogen (but not in a regular refrigerator freezer).

Along with obvious liquids such as urine, cerebrospinal fluid (CSF), lavages, or synovial fluid, other specimens in this category include blister roofs that can be ground and extracted with water or volatile buffer and cytosmears on slides that can be rehydrated. If blisters do not contain enough fluid to be drawn into a syringe, they can be injected with 20-30 µl of sterile saline, and the saline can be drawn back into the syringe. Liquid specimens should be sent undiluted and unfixed in any kind of container including a syringe. Under no

TABLE 1. Amount of Various Specimens that Should be Sent to the EM Laboratory

Specimen	Amount
Blister fluid or blister roof extract	5-10 µl
Blood (not routinely examined by EM)	5-10 ml
Cerebrospinal fluid	1-5 ml
Pericardial fluid	1-5 ml
Pleural fluid, nasopharyngeal fluid, sputum (aspirates, lavages)	1-10 ml
Stool	0.5-10 ml
Tears	5-10 µl
Urine	5-10 µl
Tissue	1-3 mm^3

circumstances should they be placed into transport media as is common for delivery to the culture laboratory; this would not only dilute the viruses but also add proteins and salts that would obscure the field on the grid. As with stool, if liquid samples are to be delayed for several hours before they reach the EM lab, they should be refrigerated or shipped on wet ice but not frozen. Samples to be stored for long periods of time should be frozen at -70°C or in liquid nitrogen.

Sputum and other thick mucoid samples can be digested in the EM lab with a solution of 0.0065 M dithiothreitol in 0.1 M phosphate buffer, pH 7.0 (Stat-Pack Sputolysin Test; Behring Diagnostics, La Jolla, CA) or other reducing agents to make it manageable on the grid. A 1:1 ratio of reagent to sputum is incubated for 30-45 min at 25°C with frequent agitation until the viscosity of the sample is acceptable. After a low speed (1,000 x g) clarification, the supernatant is placed on a grid or concentrated and placed on a grid.

Blood can be examined after removing the cells in a low speed spin, and hepatitis B and parvovirus have been demonstrated by EM. However, blood is not routinely sent for EM. The number of different viral agents diagnosed from blood is small enough so that selection of specific reagents for serotyping is easy. Furthermore, serum contains proteins and lipids that are confusing by EM. Although human immunodeficiency virus type 1 (HIV-1) has been shown in thin sections of lymphocytes in early cases of acquired immunodeficiency syndrome (AIDS), it could never be identified by negative stain of serum. Thus, blood is best sent to the serology lab unless serological tests have been negative or a systemic infection by a particular agent in an immune compromised patient is suspected, and the physician wishes to test blood as well.

Tissue destined for embedment should be placed immediately upon removal into glutaraldehyde (2-5%, buffered) and transported to the EM laboratory or refrigerated until transport can be facilitated. This means that it is best to have vials of fixative available in the operating or autopsy room. If tissue must wait for a short time before being fixed, it should be kept moist with saline or buffer and cold, but never frozen. Tissue for homogenization and negative staining (see Sample Processing) should not be fixed, but maintained moist with a small amount of buffer or saline and transported on ice to the EM lab.

SAMPLE PROCESSING

A basic knowledge of specimen preparation will help the physician understand the time frame in which an answer can be expected. Liquid samples can be placed directly onto an EM grid with a support film, drained, negatively stained (Figure 1), and examined, all within 15-20 min. This direct procedure would be used for small amounts of specimens (e.g., tears and blister fluids) and as a preliminary examination for larger volumes. While rapid, this technique may not detect virions that are present in low numbers. For this reason the microscopist may use one of the several concentrating procedures that have been described in detail (Hayat and Miller, 1990). Briefly, large volumes can, after a low speed clarification (1,000 x g, 3-4 min), be ultracentrifuged (36,000 x g, 90 min). The pellet is resuspended in a small drop of water and then treated as in the direct method. Smaller volumes (100-200 µl) can be pelleted in a tube or directly onto a grid (100,000 x g, 30 min) in an Airfuge (Beckman, Fullerton, CA); this procedure requires less time. If performed in a 200 µl tube, it does not concentrate as highly as routine ultracentrifugation because less sample is pelleted. However, if 100 µl is spun directly onto a grid in the Beckman EM-90 rotor, the concentration may be as much as 10^4 X (Hammond et al. 1981). Other techniques such as agar diffusion, pseudoreplica technique, serum-in-agar, aggregation by antibody, and solid phase IEM, that require 20-30 min are described briefly in Figures 2-6. Polyacrylamide hydrogel can be used to concentrate liquid suspensions, and polyethylene glycol or ammonium sulfate

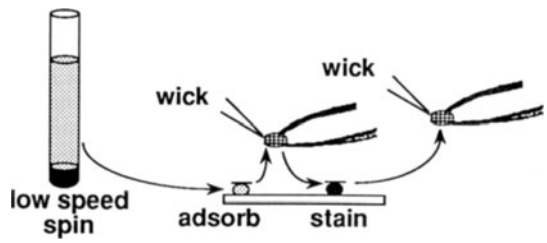

Figure 1. Direct negative stain. A water or volatile buffer extract of the sample is made, and large debris is removed by centrifugation at 1,000 x g for 3-4 min. A grid is incubated on a drop of the supernatant for 5-10 min. The grid is then drained on filter paper, placed on negative stain for 1-2 min, and drained (Miller, 1990a).

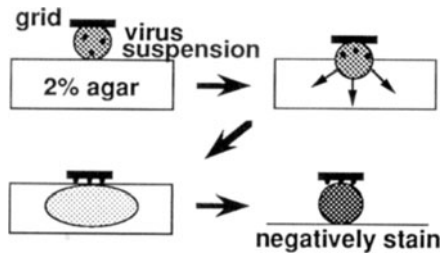

Figure 2. Agar diffusion. A drop of virus suspension is placed onto a block of 2% agar, and the liquid is allowed to diffuse into the agar (15-20 min), leaving the virus particles concentrated on the grid membrane. The grid is then negatively stained (Miller, 1990a).

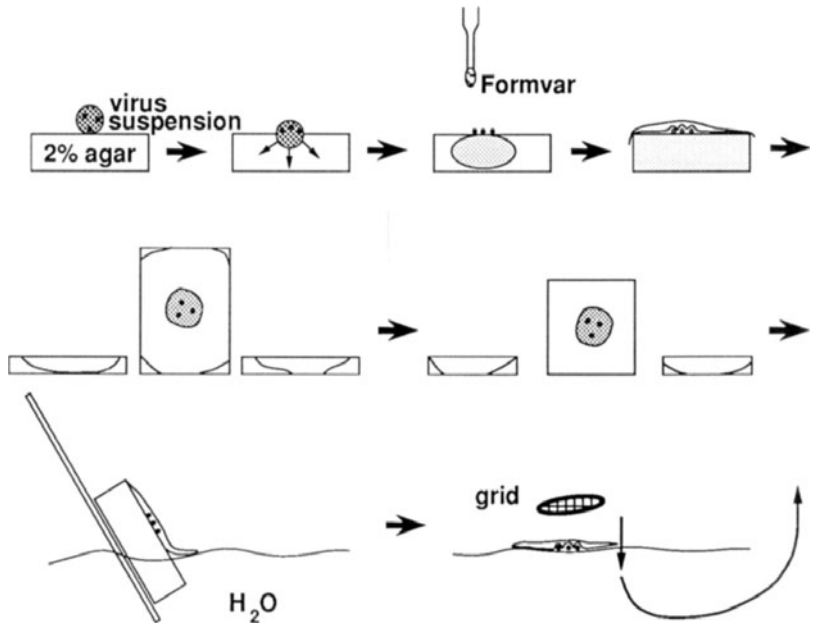

Figure 3. Pseudoreplica technique. After clarification at 1,000 x g, a virus suspension is placed onto 2% agar, and the liquid is allowed to be absorbed. A drop of Formvar (0.5%) is dropped onto the agar block. To remove excess Formvar that has run over the edge, the block is turned onto its side, and the two vertical edges are trimmed with a razor blade. The block is then turned 90°, and the other two edges are trimmed. This method of trimming prevents the Formvar from being pushed into the agar by the blade from the top and permits easier separation. The block is laid on a microscope slide, and the Formvar film is floated onto a water surface. It is then picked up on a grid in the same manner as coating grids with a plain Formvar film. (Miller 1990a; Hayat and Miller, 1990)

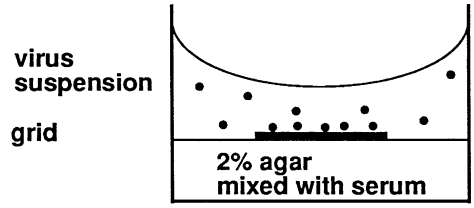

Figure 4. Serum-in-agar. Appropriate viral antiserum or pooled gamma globulin is mixed with cooled, but still molten, 2% agar and poured into a 96-welled plate. (Different concentrations or different antisera can be placed into the various wells.) A grid, film side up, is laid onto the surface, and virus suspension is added to the well. After the virus suspension has been almost absorbed into the agar (15-20 min), the grid is removed, drained, and negatively stained (Hayat and Miller, 1990; Miller, 1990a).

can be used to precipitate viruses from large volumes (e.g., several liters). These latter methods, also described in Hayat and Miller (1990), require several hours to overnight.

IEM is a term that is used variously to denote a concentrating step and/or a specific virus identification step. Immunological reactions have the advantage that they permit visualization of viruses at more dilute concentrations, and the disadvantage that they require an *a priori* notion of what virus might be present for selecting the proper antiserum to use. While these manipulations require a longer time than the direct method, they are still simple and take only a few hours. Various immunological techniques such as aggregation, coating, and gold labeling are explained in Figures 5, 7, and 8, and are described in detail in Hayat and Miller (1990).

Tissue samples usually require fixation, epoxy embedment, and ultrathin sectioning for virus examination. These procedures routinely take at least 24 hr for processing, although in the hands of an experienced microtomist who has the time and willingness to devote full attention to an emergency specimen, it can be accomplished in 2-4 hr (Doane et al. 1974; Miller and Lang, 1982; Miller and Nielsen, 1975). If a large amount (>1 g) of tissue is available, as in autopsy cases, some of it can be embedded and the rest ground in a tissue homogenizer, extracted with water, and examined rapidly by negative staining. Homogenization and negative staining works better for naked virions but also can demonstrate nucleocapsids of enveloped viruses if the nucleocapsids have a recognizable morphology; however, this technique is generally of low yield.

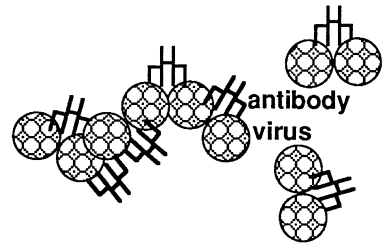

Figure 5. Virus aggregation by antibody. Clarified sample and antiserum are mixed (specific antibody, 1:100-1:1,000; pooled gamma globulin, 1:10-1:25) and incubated (overnight at 4°C or 2-3 hr at room temperature). The mixture is centrifuged at 17,000 x g for 1.5 h, and the pellet is resuspended in a small drop of water and negatively stained (Hayat and Miller, 1990; Miller, 1990a).

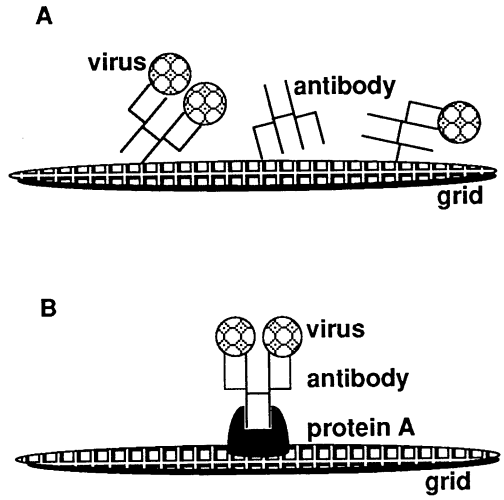

Figure 6. Solid phase immunoelectron microscopy (SPIEM). a. Antibody-coated grid. A grid is incubated for 30 min at room temperature on antibody (specific antibody, 1:1,000-1:2,000; pooled gamma globulin, 1:100-1:200). It is then washed on 20 drops of buffer and incubated for 30-60 min on a drop of virus suspension; finally, it is negatively stained. b. Protein A/antibody coated grid. First, the grid is incubated on a drop of protein A (10 μg/ml) for 10-15 min; it is then washed on drops of buffer and incubated on antibody and virus suspension as described in "a" above, except that the antibody concentration can be 10-100 times higher. This method attaches the antibody to the grid so that its virus-reactive sites are outward, thus preventing the antibody molecules from landing on the grid with their reactive sites down and blocking the binding of the virus. It also permits the use of more concentrated antiserum because the nonspecific inhibition of virus-binding by high protein concentration is overcome by the proper orientation of the virus-reactive sites (Hayat and Miller, 1990; Miller, 1990a).

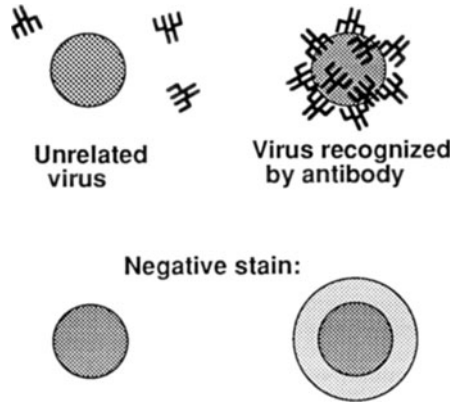

Figure 7. Virus serotyping by coating with antiserum. Viruses are attached to grids in any of the previously described methods. The grids are incubated on drops of specific antiserum (1:1,000-1:2,000) for 30 min at room temperature, washed in water, and negatively stained. If the antiserum has recognized the virus, a coat of fuzz will appear around the virus, permitting specific identification or serotyping. (Hayat and Miller, 1990).

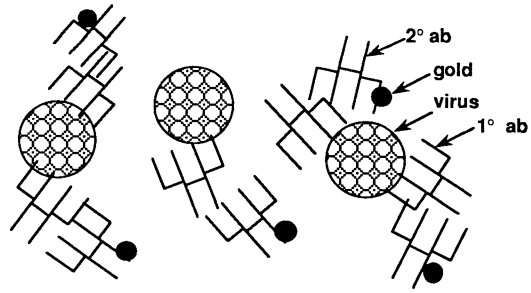

Figure 8. Immunogold labeling. Method 1: Virus is attached to a grid by one of the previously described methods. The grid is incubated with antiviral antibody (1:10-1:100) for 30 min at room temperature. After an extensive wash (at least 6 drops for 5 min each drop), the grid is incubated for 20 min at room temperature on a drop of secondary reagent (antibody against the primary antibody or protein A) (1:10-1:20) conjugated to 10 nm colloidal gold. It is then washed as before on buffer, rinsed in 3 drops of water, and negatively stained. Method 2: The virus suspension and primary antibody are incubated together 30 min at room temperature. The mixture is ultracentrifuged, and the pellet is resuspended in a small amount of buffer. Protein A- or antibody-gold is added and incubated for 20 min at room temperature. The suspension is again ultracentrifuged, and the pellet is resuspended in water and negatively stained (Hayat and Miller, 1990; Miller, 1990a).

A procedure for amplifying virus numbers in tissue is first to mince it and inoculate it into cell culture to permit a round or two of replication. Virions or forming virions can sometimes be seen in thin sections of the tissue culture before cytopathological effect is evident by light microscopy or fluorescence microscopy (Miller and Lang, 1982).

VIRUS IDENTIFICATION

To recognize the presence of a virus in a clinical sample, it is important for the electron microscopist to know the morphological characteristics by which viruses can be identified. A schematic of virus morphology as seen by negative staining is shown in Figure 9 and by thin sectioning in Figure 10. In negative stains, naked viruses are isometric and have a rigid outer coat that is not easily deformable; their size (20-80 nm) and the organization of their capsomers may render them identifiable (Figures 11, 12). Many of the small icosahedral viruses simply look like round fuzzy balls in patient samples and hence are called small round viruses (SRV) (Figure 13). Some of the small viruses have a rough surface; and a few can be identified in clinical samples (Figure 14). If not, they are referred to as small round structured viruses (SRSV). Enveloped viruses have a pliable membrane surrounding the core that is usually derived from cell membranes containing viral proteins; these virions are often pleomorphic due to drying artifacts. Occasionally enveloped virions have visible spikes on their outsides (Figure 15), but sometimes the spikes are so short that they are not easily discernable, and the virus cannot be distinguished from cellular debris. However, if the stain penetrates the membrane, and if the nucleocapsid has a characteristic shape, identification can be made even if the spikes are so short as to be indistinguishable (Figure 16). The nucleocapsid inside the membrane may be isometric like the naked viruses (Figure 16), helical (Figure 15), complex (Figure 17), or morphologically nondescript (Figure 18).

In thin sections, the location of viruses within the cells is important in identification. As a general rule, DNA viruses are usually seen in the nucleus (Figure 19), while RNA viruses are found in the cytoplasm (Figure 20). However, there are some exceptions. For example, poxviruses (DNA viruses) are constructed in the cytoplasm (Figure 21); herpesvirus nucleocapsids (DNA viruses) originate in the nucleus, but can make their way to the cytoplasm, enveloped or unenveloped (Figure 22); and nucleocapsids (Figure 15) of the measles virus-like agent sometimes found in subacute sclerosing panencephalitis (an RNA virus) have been seen in nuclei. Naked particles lyse their host cells to get out, and in late infection, unenveloped DNA virions can be seen in both the nucleus and cytoplasm. Naked viruses can sometimes be seen in matrices or paracrystalline arrays (Figure 19b). The nucleocapsids of enveloped viruses may bud into or out of cell membranes to obtain their outer covering (Figures 20, 22, 23, 24). They can be spherical (Figure 22), helical (Figure 20), or nondescript (Figure 23).

A few unusual viruses do not conform to the above generalities and deserve separate mention. Poxviruses are complex structures, and although enveloped, they have definite recognizable shape, either oval or brick-shaped (Figures 17 and 21). EM has been particularly useful in diagnosing viral skin lesions because poxviruses and herpesviruses can be rapidly and easily differen-

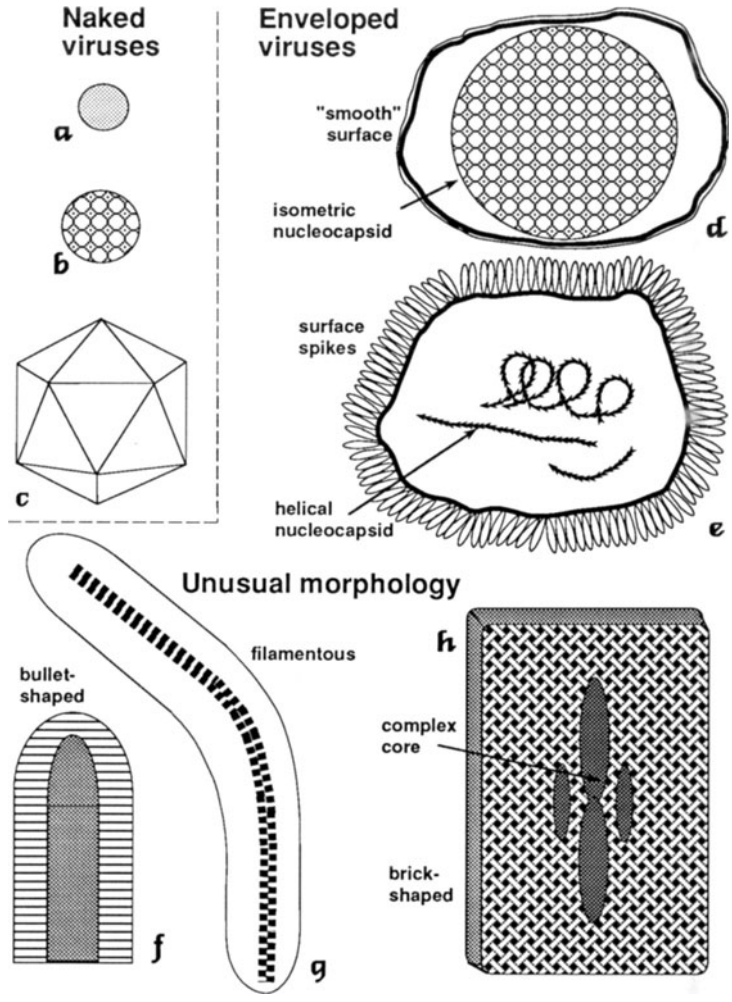

Figure 9. Schematic of the major morphological categories of viruses as seen by negative staining. a. small round featureless virus (e.g., parvovirus); b. small round structured virus (e.g., calicivirus); c. large virus with identifiable capsomeric structure (e.g., adenovirus); d. enveloped virus with short indistinguishable spikes that make the membrane appear smooth; this one is shown with a nucleocapsid of icosahedral symmetry (e.g., herpesvirus); some do not have a morphologically recognizable nucleocapsid; e. enveloped virus with spikes forming a fringe, and a helical nucleocapsid (e.g., measles virus); f. bullet-shaped virus (e.g., rabies virus); g. long filamentous virus (e.g., Marburg virus); h. brick-shaped virus (poxviruses) with a complex core (Miller, 1990a). The sizes are roughly to scale with the diameter of the nucleocapsid in *d* equal to 100 nm.

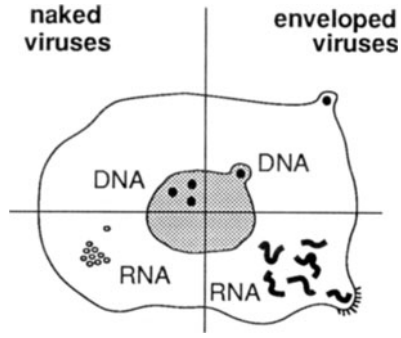

Figure 10. Schematic of viruses as seen by thin sectioning. Cellular location, shape of the nucleocapsid, and whether or not the virus is enveloped are important in identification (Miller, 1990a).

tiated by negatively staining blister fluid. Rhabdoviruses (Figure 24), e.g., rabies and LeDantec viruses, are also membraned, but routinely have a bullet shape where one end is rounded, and the other end is blunt. Though rhabdovirus infection is rare and thus the diagnostic opportunity is infrequent, the shape of the virion makes it easily recognizable in negative stains or thin sections. Finally, the filoviruses, though enveloped, are distinguishable by EM. Morphologically, they resemble the rhabdoviruses somewhat, except that they can be very long (sometimes up to 1,400 nm) filamentous virions with a diameter of 70-80 nm. In negative stains, they often are bent or curved and may appear in the shape of a shepherd's crook or a numeral 6. Fortunately, infection with filoviruses, e.g., Marburg and Ebola viruses, is rare.

Once the existence of a viral agent is determined, one can consult an atlas (Doane and Anderson, 1988; Palmer and Martin, 1989). Viruses can be identified morphologically with respect to families. Though specific characterization may rely on serological techniques, some important differentiations can be made. Finally, and very importantly, artifacts, cell components, and cell debris can resemble viral particles (Miller, 1986, 1989; Oshiro and Miller, in press). If an electron microscopist is unsure of a diagnosis, it is better to record the sample as negative or questionable. A false positive is potentially more detrimental than a false negative report because it might lead to the cessation of the search for an etiological agent.

DIAGNOSIS

Some viruses are usually restricted to certain organ systems, thus the origin of the specimen can yield clues to the identification of viral agents, and should be communicated to the microscopist. This information has been described in detail (Hayat and Miller, 1990; Miller, 1986; Oshiro and Miller, in press). Table 2 is a summary of likely viral pathogens in various systems that can be visualized by EM; diseases caused by viral agents have been described in detail elsewhere (White and Fenner, 1986). In the immunocompromised host,

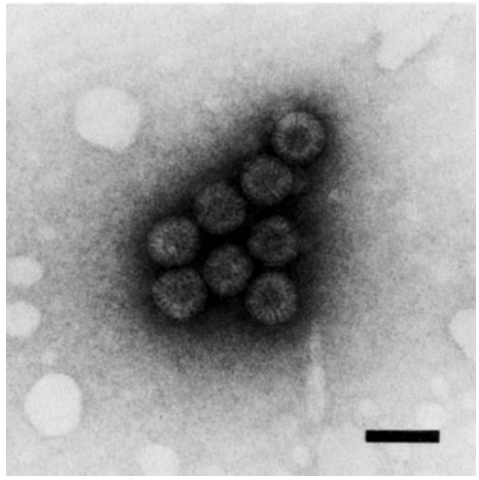

Figure 11. Rotavirus. This is one of the largest naked viruses infecting humans. Its size (70-75 nm) and the arrangement of its capsomers make it clearly identifiable in stool samples. Rotavirus is named from the fact that its capsomers appear like the spokes of a wheel. Group A rotavirus is responsible for 30-50% of the cases of infantile gastroenteritis, and infections with it are more prevalent in the winter months. Adult diarrhea rotavirus (ADRV) (Group B) can be seen, but is not more prevalent in children. Bar represents 100 nm.

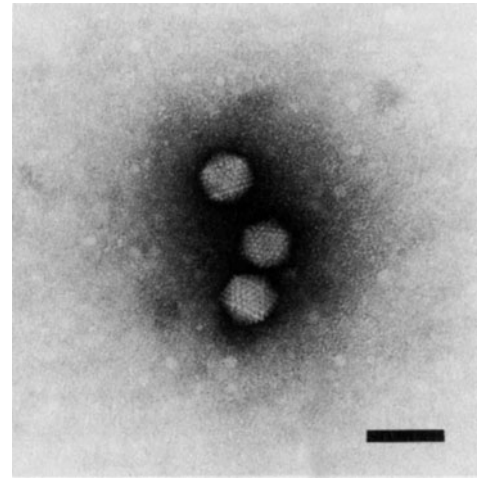

Figure 12. Adenovirus seen in stool but also in other parts of the body. Enteric adenoviruses cannot be distinguished morphologically from other strains. The 75-80 nm icosahedral virion has flat triangular facets made up of marble-shaped capsomers. Depending on its orientation on the grid film, its circumference may appear hexagonal unlike the spherical rotavirus. Bar represents 100 nm.

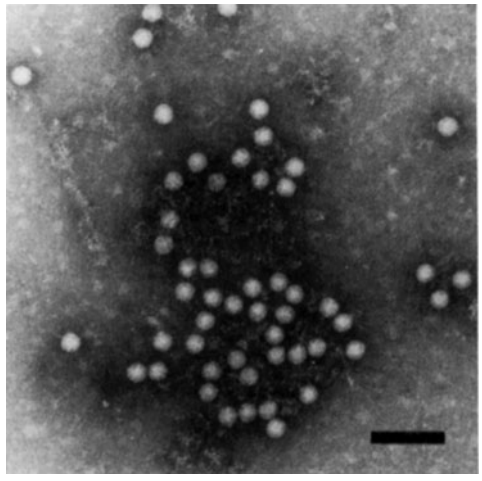

Figure 13. A small round virus without distinguishing morphological characteristics seen in stool from a patient with diarrhea. Bar represents 100 nm.

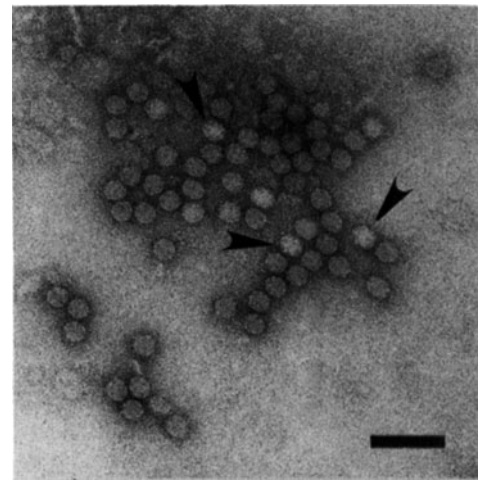

Figure 14. A small round structured virus seen in stool from a patient with diarrhea; it was later identified after printing the micrograph as astrovirus. Arrows denote three particles that show the star-shaped pattern. Bar represents 100 nm.

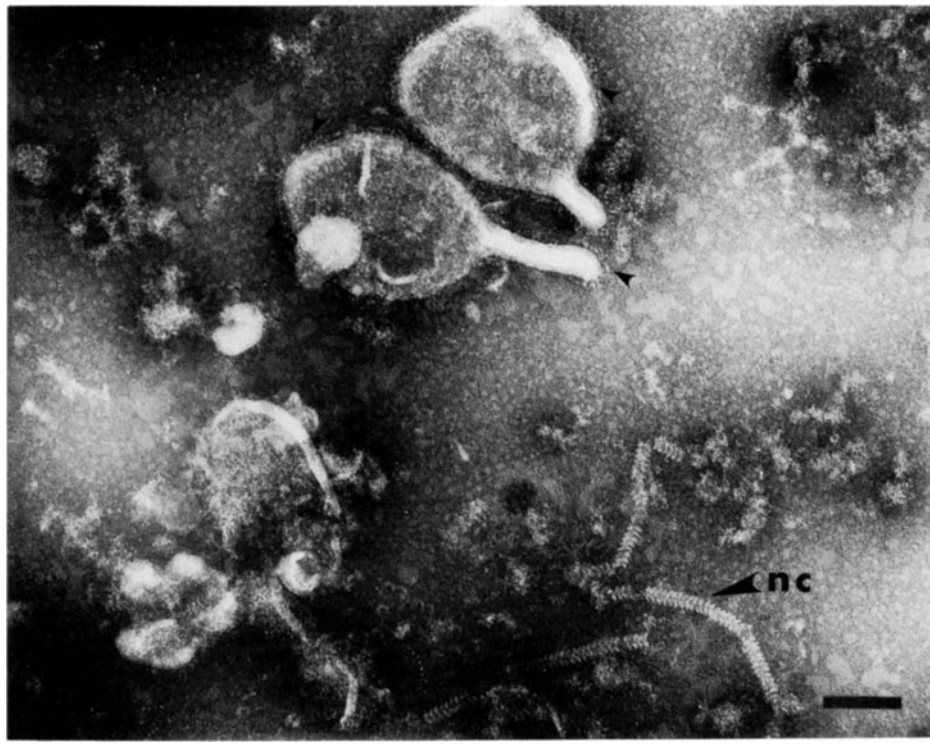

Figure 15. Measles virus. The complete particles are enveloped with spikes (small arrows) on the outside. The 18-nm helical nucleocapsids (nc) appear in a herringbone pattern, and may show circles (bottom center) if they become broken. Bar represents 100 nm.

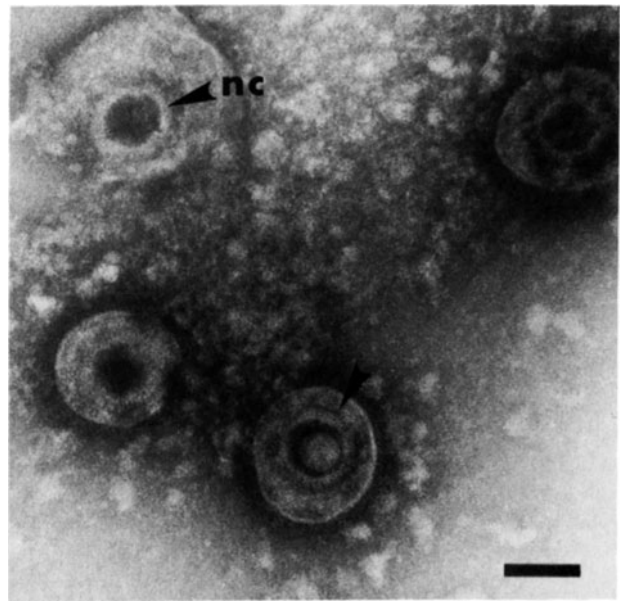

Figure 16. Herpes simplex virus. The enveloped particle has projections that are so short that they are not recognizable. Within the virion, 100-nm nucleocapsids (nc) are visible because the negative stain has penetrated the broken membrane. Some nucleocapsids here are damaged, and appear empty, but the one at the bottom center shows the nucleic acid core inside the nucleocapsid. Bar represents 100 nm.

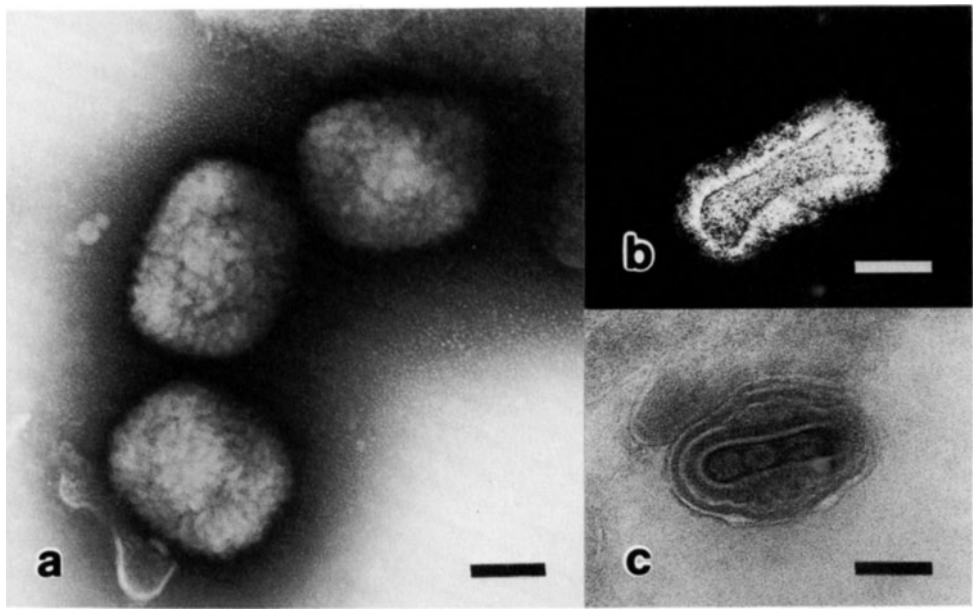

Figure 17. Vaccinia virus. a. Virions are brick-shaped or oval; the outer membrane surface is rough. b. The print is considerably overexposed to show the dump bell-shaped core that has been penetrated slightly by the negative stain (reprinted from Miller, 1986). c. An ultrathin frozen section of a vaccinia virus showing the complex core; contrasted with uranyl acetate. Bars represent 100 nm.

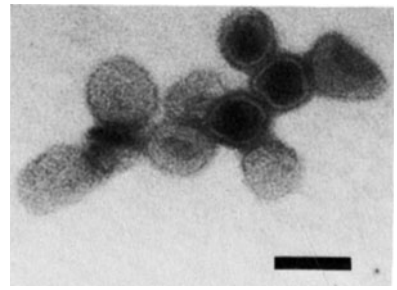

Figure 18. Rubella virus. An enveloped virus with projections so short as to be indistinguishable and a nucleocapsid that does not have identifying characteristics (reprinted from Miller, 1986). It would be impossible to identify this type of virus in an unknown sample because of its similarity to membranous cell debris. Bar represents 100 nm.

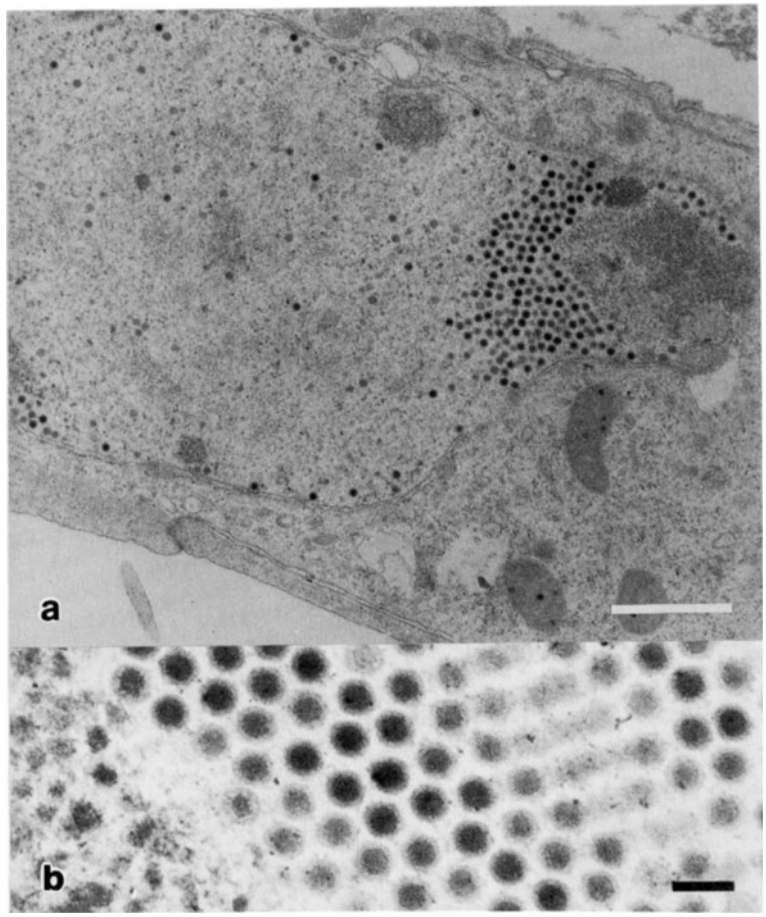

Figure 19. Adenovirus-infected cell. a. The DNA virions are formed in the nucleus. Late in infection, virions may also be seen in the cytoplasm as they lyse the host cell to get out. b. The icosahedral viruses can form paracrystalline arrays as seen here at high magnification. Bar in *a* represents 1 μm; bar in *b* represents 100 nm.

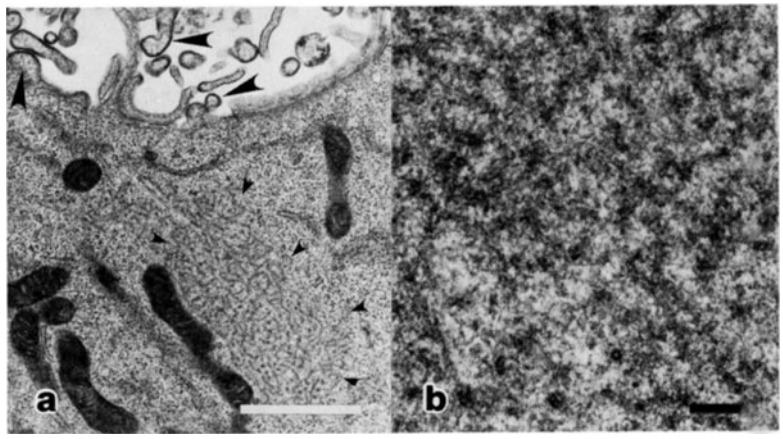

Figure 20. Measles virus-infected cell. a. Low magnification showing the helical RNA nucleocapsids (small arrows) in the cytoplasm and budding virions (large arrows) at the plasma membrane. The virions have an outer membrane that appears dense, or fuzzy at high magnification in thin section; this fuzz corresponds to the spikes in negative stain (Figure 15). Bar represents 1 μm. b. High magnification of the 18-nm worm-like nucleocapsids. Bar represents 100 nm.

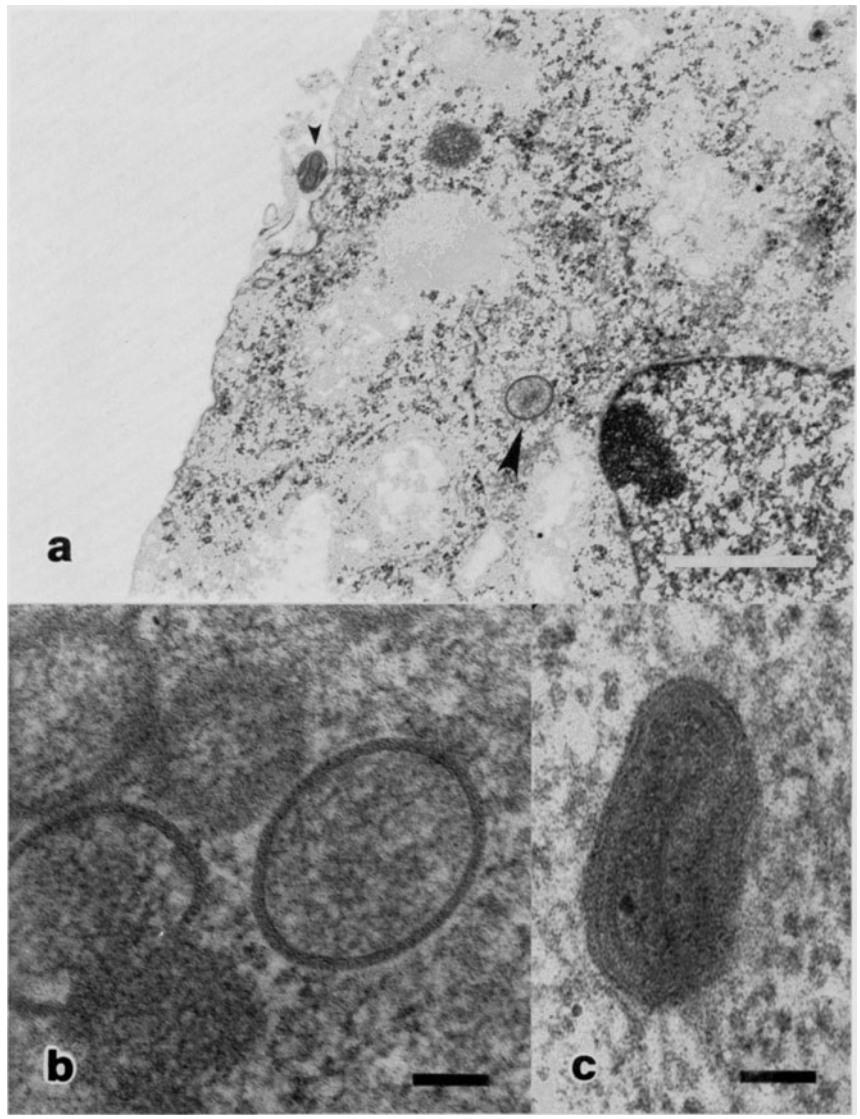

Figure 21. Poxvirus (vaccinia)-infected cell. a. Though the nucleic acid is DNA, this virus is constructed in the cytoplasm, and the envelope is created *de novo*, rather than by budding from cell membranes; immature particle (large arrow); mature particle (small arrow). Bar represents 1 μm. b. High magnification of immature particles. Bar represents 100 nm. c. High magnification of mature particle. Bar represents 100 nm.

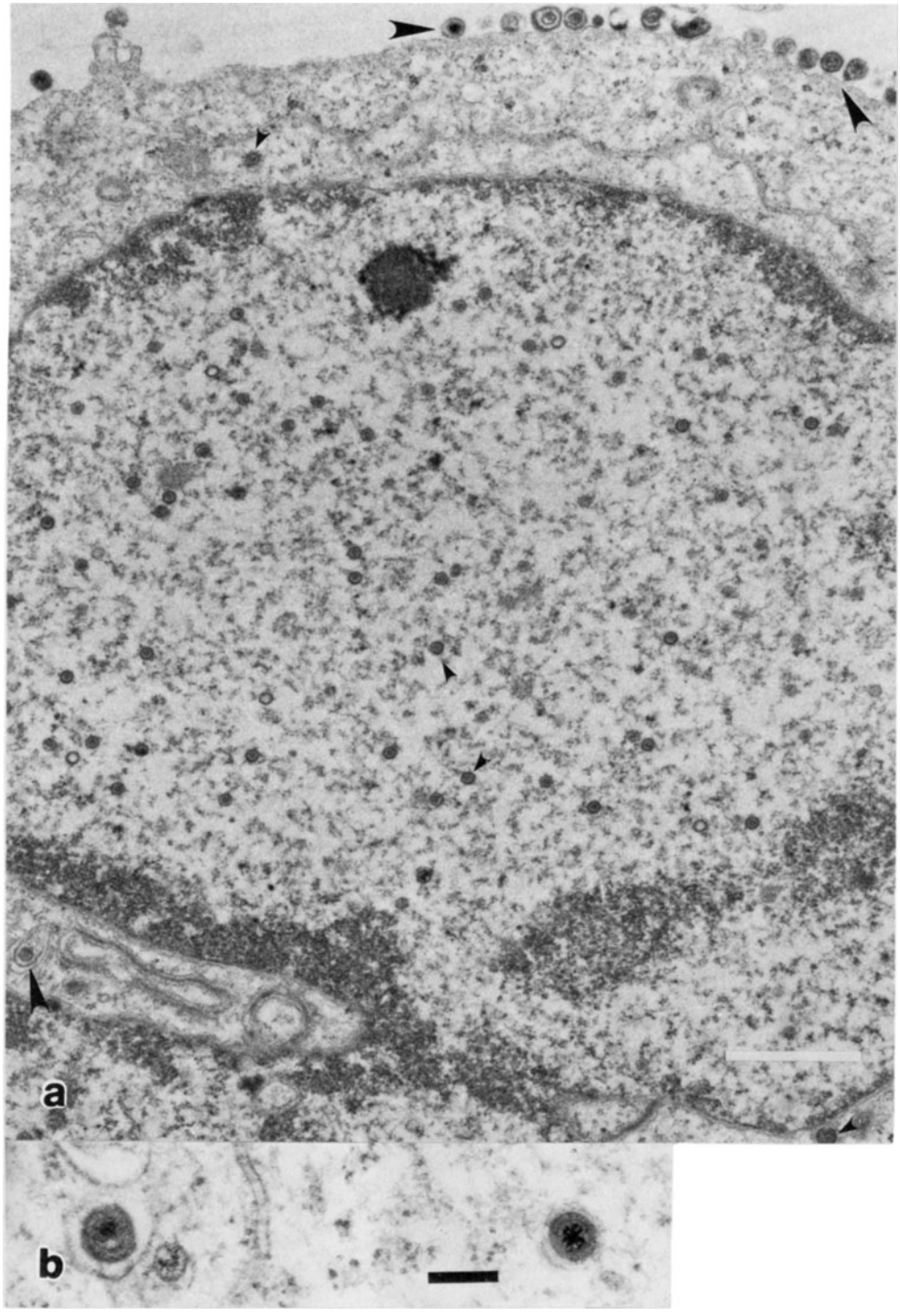

Figure 22. Herpes simplex virus-infected cell. a. Nucleocapsids (small arrows) are seen in the nucleus and cytoplasm; complete virions (large arrows) are seen in the cytoplasm and budding from the plasma membrane. The envelope can be obtained from the nuclear membrane, plasma membrane, or internal cell membranes. HSV often causes cells to over-produce membranes as seen in the lower left. Bar represents 1 μm. b. High magnification of cytoplasmic particles. Bar represents 100 nm.

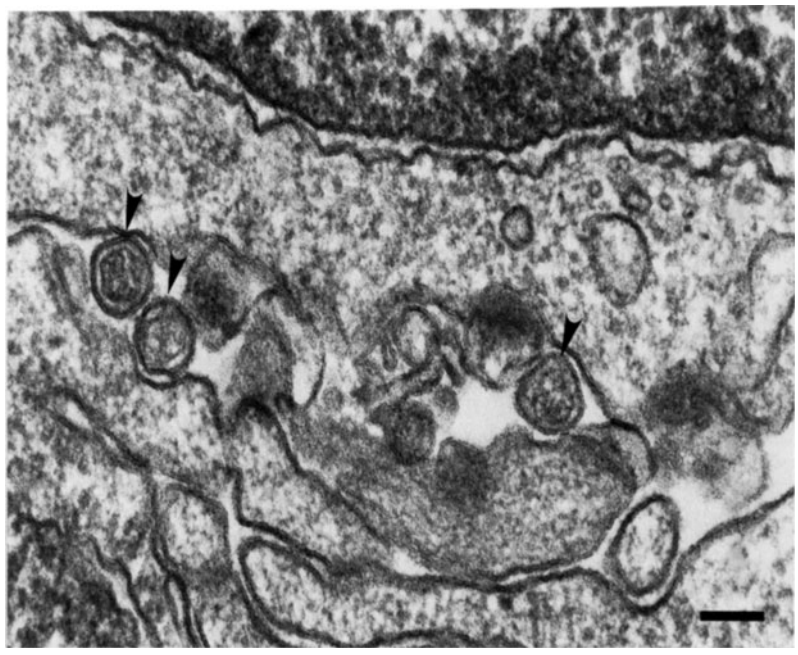

Figure 23. HTLV-1 (arrows), an RNA virus that buds from the cytoplasmic membrane to obtain its outer covering. Its nucleocapsid, although dense, and its short surface projections are not readily distinguishable amid cell debris in negative stains (reprinted from Miller, 1986). Bar represents 100 nm.

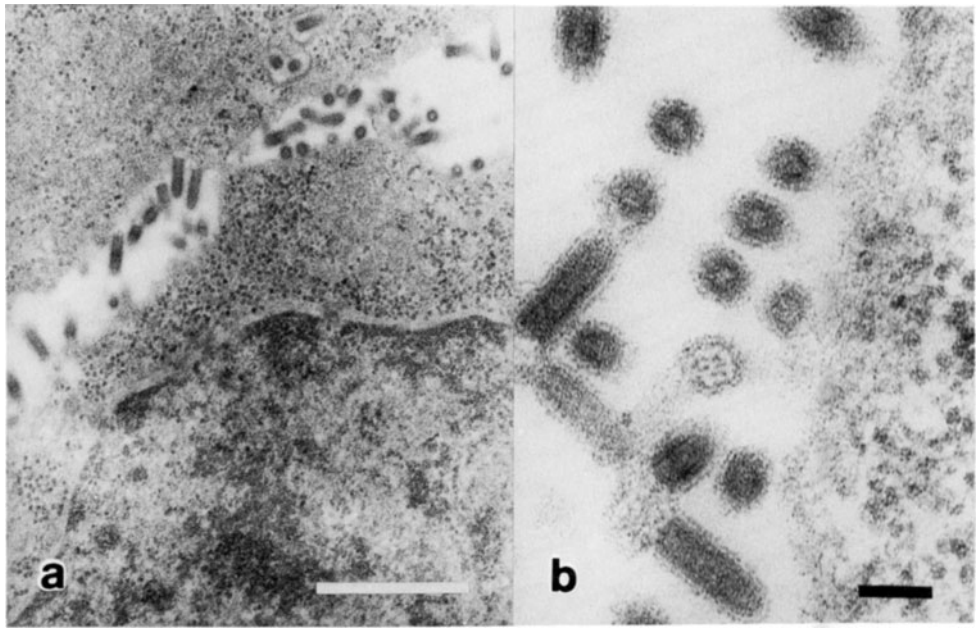

Figure 24. Cell infected with vesicular stomatitis virus, a rhabdovirus. In rabies-virus-infected cells, matrices of ribonucleoprotein form in the cytoplasm and can be identified by fluorescence microscopy as Negri bodies. Rhabdoviruses bud from the cytoplasmic membrane to form bullet-shaped virions shown at higher magnification in *b*. Bar in *a* represents 1 μm; bar in *b* represents 100 nm.

TABLE 2. Likely Viral Pathogens in Specimens Visualized by EM

BLISTERS
Herpes simplex virus/Varicella zoster virus
Poxviruses (e.g., Orf/Vaccinia viruses)
CEREBROSPINAL FLUID
SRV (e.g., Coxsackie virus)
Herpes simplex virus
Measles virus
Mumps virus
Rubella virus
PLEURAL/PERICARDIAL FLUIDS
SRV (eg., Coxsackie virus)
SERUM (not usually processed by EM)
Hepatitis A virus
Hepatitis B virus
Non-A, non-B hepatitis viruses
Parvovirus
Filovirus (eg., Ebola virus)
SPUTUM/NASOPHARYNGEAL SECRETIONS
Respiratory syncytial virus
Influenza/parainfluenza viruses
SRV (e.g., rhinovirus, enterovirus)
Adenovirus
Mumps virus
Cytomegalovirus
Measles virus
Reovirus
Coronavirus
STOOL
Rotavirus
Adenovirus
Calicivirus
Astrovirus
SRV (small round viruses); featureless
SRSV (small round structured viruses); rough surface (Norwalk virus-like)
Coronavirus
SYNOVIAL FLUID
SRV (e.g., parvovirus, Coxsackie virus)
TEARS
Adenovirus
SRV
Herpes simplex virus
THROAT WASHES
Influenza/parainfluenza
SRV (e.g., enterovirus)
Adenovirus
Mumps virus
Measles virus
Rabies virus
URINE
Cytomegalovirus
Papovavirus

the systemic demarcation is not as distinct; however, certain types of viral infections are more prominent in these patients (Miller and Howell, 1988). In AIDS patients, the most commonly seen viral infection, and second in frequency only to *Pneumocystis carinii*, is cytomegalovirus (CMV). Other viruses in the herpes family are also frequent; these include HSV-1 and HSV-2, VZV and Epstein Barr virus (EBV), although the latter is not usually visualized by EM. Hepatitis B virus, polyomaviruses, and papillomaviruses are also common pathogens in these patients; adenoviruses and poxviruses are not uncommon. Coronaviruses, parvoviruses and human T-lymphotropic virus type 1 (HTLV-1) have also been reported.

EM RESULTS

Stool

For several reasons previously noted, EM is the method of choice for viral diagnosis from fecal specimens (Table 3). The recommendation from a recent workshop on gastroenteritis viruses at the Centers for Disease Control (Lew et al. submitted), for reporting results is that the agents listed in Table 4 be named when present. Their prevalence and seasonality as seen at 10 different EM viral laboratories in the US and Canada are also shown. Of 52,691 specimens examined, 16% were positive for a viral agent. Rotaviruses are the most prevalent, particularly in infants and especially in the winter months. Adenovirus and SRV tie for second in prevalence.

Quite often when a stool sample is received in the EM laboratory, the instructions on the requisition are for a "Rotazyme test" (Abbott Laboratories, Abbott Park, IL), as many physicians are aware of the commercially available slide kits for rotavirus detection (not all of which are named "Rotazyme", e.g., "Rotaclone", Cambridge Bioscience, Worcester, MA). There are also kits for adenoviruses. However, use of one of these kits would not detect any of the other potential agents. One laboratory in Canada with a heavy load screens samples with a rotavirus slide kit and performs EM on those that are negative (Lew et al. submitted). This procedure is sufficient for most specimens, but in the case of immunodeficient patients where a second virus may be present, it would miss anything but the one against which the antiserum was directed. We routinely perform direct EM on all stool specimens, and for those that are negative, we perform one of the concentrating procedures previously mentioned. Cell culture is inappropriate for fecal specimens because the viral agents found there either have not be propagated in tissue culture or grow slowly and with difficulty in the research but not the diagnostic laboratory.

The implications of a viral diagnosis depend on the virus. The presence of even a few rotaviruses in the stool of a patient with gastroenteritis is significant in the cause of disease. Rotavirus infection should be taken seriously, particularly in the very young; indeed in third world countries, it is responsible for many deaths. Fluid replacement with restoration of electrolyte balance should be initiated as soon as possible as dehydration can occur very rapidly. Further, since rotavirus is very contagious, stringent quarantine measures should be adopted to prevent spread throughout the nursery. In immunocompetent hosts, the infection is self limiting with supportive therapy, but in immunodeficient babies, therapy with immune globulin and ribavirin has been attempted.

TABLE 3. Procedures for Most Likely Virus Detection

Sample	Desired Diagnosis (presence or absence)	Technique
Stool	virus[a]	EM best/only
Urine	virus	EM, culture
Blisters	herpes vs. pox	EM, culture
Sputum	RSV	immunofluorescence
	virus	culture, EM
Nasopharyngeal secretions, lavages	virus	culture, EM
Pleural/ pericardial fluids	virus	culture, EM
CSF	virus	culture, EM less efficient
Tears	virus	culture, EM
Blood	hepatitis A, B	serology
	hepatitis, non-A/B	serology, EM, culture
	parvovirus	EM
Synovial fluid	virus	culture, EM
Tissue	papilloma	*in situ* hybridization
	EBV	*in situ* hybridization
	retroviruses	EIA, Western blot, *in situ* hybridization
	virus	culture, EM-thin sections

[a] Any virus

TABLE 4. Viruses Detected from Stool at Ten EM Viral Laboratories

Virus	Prevalence (%) Ave/Range[a]	Seasonality	Patient's age (yrs)
Rotavirus	48/26-83	Winter	< 1 (54 %) 1 - 4 (46 %)
Adenovirus	17/8-27	-	all
SRV/SRSV	10/0-40	-	infants (48%) older children & adults
Astrovirus	2/0-10	Winter	< 1 (64 %) 1 - 4 (36 %)
Calicivirus	1/0-4	-	≤ 1
Coronavirus	7/0-13[b]	-	?

[a] Range among the different centers
[b] One center reported 67%

From Lew et al. (submitted)

The detection of only a few adenoviruses in stool may not indicate intestinal infection. Adenoviruses can infect the respiratory tract, and enteric adenoviruses cannot be distinguished morphologically from respiratory adenoviruses that may have been swallowed. However, if there are numerous virions in a clinical setting of diarrhea, they probably are significant. In the case of cold or flu-like symptoms, the significance of intestinal adenoviruses is equivocal, but once such viruses are detected by EM, an immunological test (e.g., Adenoclone, Cambridge Bioscience, Worcester, MA) can be run to determine the strain if necessary. On the other hand, respiratory viruses can replicate in the intestinal mucosa and elicit pathologic effects; thus, differentiation between an enteric and other adenoviruses may be moot in the case of obvious diarrhea.

There is a problem with the interpretation of the presence of SRV in fecal samples. In North America, no one system of classification has been accepted for small isometric viruses. In Britain, Caul and Appleton (1982) define SRV as small (22-26 nm) round "featureless" viruses that do not induce an immune response and are not considered to be pathogenic. They seem to appear in stool after a diarrheal illness caused by one of the SRSV (Caul, personal communication). However, parvoviruses are 18-26 nm in diameter, and parvovirus-like agents have been seen by EM in feces of enteritis patients; these particles could be clumped with homologous serum (Paver et al. 1973). Furthermore, correct EM calibration is essential to classify one of these SRV as less than or more than 26 nm if the size is close to that. Thus, in this author's opinion, the issue of pathogenicity of SRV in stool has not yet been resolved. The SRSV include

Norwalk agent, mini-reovirus and other viruses whose surface is rough; most of these have been shown to be pathogenic. Astrovirus, calicivirus, and some small isometric viruses have been shown to cause important nosocomial infections (Blacklow, 1990; Lew et al., submitted; Riepenhoff-Talty, 1982). Since infection with these agents is often mild, and since precautions against the spread of diarrhea are most likely when the disease is severe, appropriate precautions may not be taken. This can result in higher treatment costs and possible severe ramifications for immunocompromised patients.

Coronaviruses and coronavirus-like particles (CVLP) have been reported in stool from both gastroenteritis patients and normal, asymptomatic individuals (MacNaughton and Davies, 1981). Furthermore, identification of these viruses is difficult, since they can be confused with membranous debris with spikes (e.g., mitochondrial membrane). A single or few isolated particles, unless they are diagnosed by an experienced microscopist, should not be considered conclusive. If there are numerous, similarly-sized particles present, their significance increases.

As with rotavirus, infection of the immunocompetent patient with any of the enteric viruses is self-limiting as long as homeostasis is maintained with supportive treatment. However, in the immunodeficient patient, diagnosis is a must for the administration of specific immune globulin and perhaps trial therapy with antiviral drugs. Little is known about the efficacy of drugs in this case, but there may be no other hope for some of these patients. The prospects for antiviral chemotherapy of gastrointestinal illnesses have been discussed by Babiuk et al. (1985).

Urine

Urine is another specimen for which EM can be very useful. Although viruses are not usually seen in urine during infections of other systems, they can be found there in certain cases. In congenital CMV infections, virion numbers are quite high in urine. Papovaviruses have been demonstrated in urine from pregnant women in the absence of disease and from kidney transplant patients. Parvoviruses have been seen in high numbers in urine from patients with aplastic anemia. Several different viruses have been isolated from urine in disseminated viral infections of immunocompromised hosts. In these instances, EM may be the method of choice because it is rapid and virus concentration in the sample is high. Papovaviruses and parvoviruses are not grown in the diagnostic culture laboratory, and growth of CMV requires 2-3 weeks. A fluorescence test is commercially available for CMV, but not for papova- or parvoviruses.

Respiratory Secretions/Pleural and Pericardial Fluids

In respiratory infections, if the physician suspects respiratory syncytial virus (RSV) (e.g., during a winter outbreak of lower respiratory tract illness), (s)he can request a fluorescent microscopy examination for that particular virus. However, if the fluorescence exam is negative, there would be no further information on the etiology, whereas EM may be able to demonstrate the presence of adenovirus or a rhinovirus. In the case of RSV, EM might be able to show the helical nucleocapsids, but would not be able to distinguish between RSV and the other paramyxoviruses. If EM had been performed first and showed helical nucleocapsids, the patient's symptoms and the epidemiology might suggest a differential diagnosis; then fluorescence microscopy or one of

the enzyme immunoassays could confirm it. Fluorescence assays are also available for parainfluenza virus 1, 2, and 3; adenovirus; influenza virus A and B; HSV; VZV; and CMV. Rapid enzyme immunoassays are available for RSV, adenovirus, HSV, and VZV. Rhinoviruses, enteroviruses and parvoviruses have also been seen in throat washings and respiratory secretions. These agents appear by EM simply as SRV. Some do not grow in culture or require extended culture time (days to weeks) with multiple passages. EM is in some cases the only option for identifying such noncultivable agents. Since the ability of an agent to grow in tissue culture is generally not known *a priori*, inoculation of cultures and EM examination should both be undertaken. Drug therapy is available for some of these agents, a factor which increases the importance of viral identification.

The viruses most likely to be seen in pleural and pericardial fluids are those of the SRV variety (entero- and rhinoviruses). Other than demonstrating their presence and size, EM could not further differentiate them, unless a particular agent were suspected and antiserum against that agent were available.

Blister Fluid/Skin

For blisters, if the question is herpesvirus vs. poxvirus, the method of choice is EM; then fluorescence microscopy or IEM could further delineate the agent. If the symptoms (e.g., genital lesions vs. thoracic lesions) suggest a particular disease, EM could substantiate it. For example, in the first case, an EM finding of a herpesvirus would suggest HSV-2, (possibly HSV-1), while in the second case it would suggest VZV. Various poxvirus infections are seen in animal handlers, laboratory workers, and immunocompromised individuals. Differentiating these agents is important for therapy. Viruses have been demonstrated by EM, both with negative staining of scrapings and with thin sections (e.g., measlesvirus, papillomavirus). *In situ* hybridization (Chesselet, 1990; Metcalf et al. 1988; Norval and Bingham, 1987) is becoming more widely used for papilloma virus, particularly since the genome can be present in normal-appearing tissue at the lesion margin in the absence of complete virions. However, this procedure is not routinely available in all diagnostic virology laboratories.

Cerebrospinal Fluid

Viruses have been seen in CSF, but when present, they usually are not numerous; thus, culturing is the diagnostic method of choice. However, if several milliliters (2-5) are available, enough for culture and EM, it may be possible to demonstrate virus by EM after ultracentrifugation. Many enteroviuses cannot be readily grown, or require a long time and several blind passages. The EM diagnosis, if positive, would be of a small round virus of a given size; the size could narrow the possibilities, but precise identification could not be made directly. If a particular virus were suspected (e.g., poliovirus vaccine strain in an immunodeficient infant), and antiserum were available, identification could be made by IEM or by antibody neutralization in tissue culture.

Synovial Fluid

Parvovirus B19, rubellavirus, Coxsackie B virus, EBV, and arboviruses have been associated with arthritis. Parvovirus (which does not grow in cul-

ture) and Coxsackie viruses can be demonstrated by EM. Other viruses that are enveloped would be difficult to identify by EM because of their similarity to membranous debris, unless specific antiserum were available for IEM.

Blood

As previously mentioned, EM is generally not the method of choice for blood samples, but if serological tests are negative, EM as well as culture may be useful. Many different non-A, non-B hepatitis viruses have been described (referenced in Hayat and Miller, 1990), some of which may be demonstrated by EM. Serum samples including convalescent serum should be collected and preserved for IEM. Some of these agents have not been grown in tissue culture, and thus, may not be demonstrable by culturing. Also, parvovirus B19 (an SRV) has been seen by EM in blood from children with erythema infectiosum (fifth disease), in cases of aplastic crisis in sickle cell anemia, and in asymptomatic viremic volunteers (Cossart et al. 1975; Versteeg and Salimans, 1988). It does not grow in culture; thus, with exception of research procedures with molecular probes, EM and IEM are the procedures of choice for diagnosis.

Care must be taken when examining serum by negative staining because droplets of lipids and proteins present can appear as round spots and may resemble SRV. Clues to the viral nature of these structures are uniformity of size and shape and possibly a 3-dimensional appearance. Sometimes, but not always, stain collects around the underside of viruses producing a darker background immediately around the virion, while droplets often flatten out like a fried egg and appear simply as clear "holes" in the stain. Additionally, enveloped viruses such as *Retroviridae, Arenaviridae, Togaviridae,* and *Bunyaviridae,* that do not have long identifiable spikes or recognizable nucleocapsids cannot be distinguished from cell membrane debris by negative staining.

ANTIVIRAL DRUG THERAPY

One of the main reasons for pursuing a viral diagnosis today is the increasing availability of antiviral agents. Except for ribavirin, most currently available antiviral drugs do not have wide spectrum activity; accurate identification of viral infectious agents is therefore crucial for proper drug selection. The viruses for which some antiviral agent has been described are listed in Table 5. Detailed information on mechanism of action, licensed and investigational drugs, dosages, and research studies has been published (American Society of Hospital Pharmacists, 1990; De Clerq and Walker, 1987; Mills and Corey 1985, 1989; Olin, 1990). A brief summary of the more common ones follows.

Acyclovir

Acyclovir (ACV, Acyclovir sodium, acycloguanosine, Zovirax) is a well known anti-*Herpesviridae* drug. Suppressive therapy has been given orally in some patients with severe HSV infections to reduce the severity or frequency of symptomatic recurrences. The use of topical ACV in first episodes reduces the time for virus shedding, duration of pain and itching, and time for crusting and healing, but it is not as effective as oral and intravenous ACV. In the case of recurrent genital herpes, topical treatment is not effective, while oral ACV is, especially when started at the first sign of the prodrome; however, the benefit

TABLE 5. Human Viruses Against Which Drugs Are Available

CMV	Acyclovir	Ganciclovir[a]	Vidarabine[b]		Ribavirin[c]
EBV	Acyclovir				
HSV-1	Acyclovir	Ganciclovir[a]	Vidarabine		Ribavirin
HSV-2	Acyclovir	Ganciclovir[a]			Ribavirin
Herpes simae	Acyclovir	Ganciclovir[a]			
VZV	Acyclovir	Ganciclovir[a]	Vidarabine	Zidovudine[c]	

HIV-1	Zidovudine	Ribavirin
Arboviruses		Ribavirin[c]
Colorado tick fever virus		Ribavirin[c]
Crimean-Congo hemorrhagic fever virus		Ribavirin
Dengue fever virus	Amantadine[d]	
Encephalomyocarditis virus (EMC)		Ribavirin[c]
Hantaan virus (Korean hemorrhagic fever)		Ribavirin
Japanese encephalitis virus		Ribavirin[c]
Junin virus (Argentine hemorrhagic fever)		Ribavirin
Lassa fever virus		Ribavirin
Lymphocytic choriomeningitis virus	Amantadine[d]	
Machupo virus (Bolivian hemorrhagic fever)		Ribavirin
Pichinde virus		Ribavirin[c]
Rift Valley fever virus		Ribavirin
Rubella virus	Amantadine[d]	
Semliki Forest virus	Amantadine[d]	
Venzuelan equine encephalitis virus (VEE)		Ribavirin[c]
Yellow fever virus		Ribavirin[c]
Influenza A virus	Amantadine	Ribavirin
Influenza B virus	Amantadine[d]	Ribavirin
Influenza C virus	Amantadine[d]	
RSV	Amantadine[d]	Ribavirin
Parainfluenza virus	Amantadine[d]	Ribavirin
Measles virus		Ribavirin
SSPE virus		Ribavirin
Mumps virus		Ribavirin
Rhinoviruses virus		Ribavirin[c]
Coxsackie virus B1		Ribavirin
Enterovirus 72 (hepatitis A virus)		Ribavirin
Reovirus 1, 2, 3		Ribavirin
Rotavirus		Ribavirin[c]
Adenovirus		Ribavirin[c]
Hepatitis B virus		Ribavirin[c]
Poxvirus		Ribavirin[c]

[a] Principle use against CMV, but active against other *Herpesviridae*

[b] Less effective against HSV encephalitis than acyclovir

[c] *In vitro* activity. *In vivo* activity may not exist or may not have been shown.

[d] *In vitro* activity at high concentrations (> 10 μg/ml)

From American Society of Hospital Pharmacists, 1990a; Mills and Corey, 1985; Olin, 1990

is not as great as in first episodes. Because oral ACV is less expensive and easier to give than intravenous ACV, it is the therapy of choice for first episode genital herpes. ACV appears to be more effective than vidarabine for treating HSV encephalitis and also for treating chickenpox in the immunocompromised host.

Both oral and intravenous ACV are effective in speeding healing and shortening the period of acute VZV infections when started within 48-72 hr after onset of symptoms. It may also reduce the occurrence of post-herpetic neuralgia. The efficacy of parenteral ACV in CMV treatment is unclear, although it has produced improvement in some immunocompromised patients with CMV pneumonia. ACV inhibits EBV replication in active production *in vitro* but has no effect on the episomal EBV DNA. It has been shown to reduce virus shedding in acute infectious mononucleosis but does not substantially affect the course of the disease. It has been used against EBV infection in AIDS patients to reduce the clinical progression of hairy leukoplakia, and to treat several patients with fever, interstitial pneumonitis, panleukopenia, and high levels of anti-EBV antibodies. However, it has had little effect in nonimmunocompromised hosts, including attempts to treat chronic fatigue syndrome. Variations in therapeutic efficacy with respect to virus type, lesion location, and mode of drug delivery are discussed in detail by Mertz (1989). ACV has been used concomitantly with AZT without increased toxicity, and preliminary *in vitro* data suggest that it may potentiate the antiretroviral activity of AZT.

ACV is a purine nucleoside analog that is preferentially absorbed by herpesvirus-infected cells and converted to the triphosphate form that is a toxic analog of deoxyguanosine triphosphate. It also interferes with HSV DNA polymerase by forming a complex with the enzyme and the DNA template. Without the 3' OH group for the 5'- to 3'-phosphodiester linkage, it acts as a chain terminator. It is inactive against the other DNA viruses such as vaccinia virus and adenovirus 5 as well as several RNA viruses against which it has been tested.

Several other antiherpesviral drugs are being examined. FIAC (2'-fluoro-5-iodoarabinosyl-cytosine), a pyrimidine analog, is very active *in vitro* against herpesviruses. Clinical trials in the immunocompromised host show dramatic decreases in time to last lesions, crusting, pain, and dissemination; however, there is mild liver toxicity. BVDU (bromovinyldeoxyuridine) has a much increased activity over ACV in VZV infections. BW-A515U is a prodrug of ACV that produces high levels of plasma ACV after oral administration. BVaraU (1-b-D-arabinofuranosyl-E-5[2-bromovinyl] uracil), FMAU (fluoromethylarabinosyluridine), and FIAW (fluoroiodoarabinosyluridine) are active *in vitro* against herpesviruses and resemble ACV in their mechanism of action. All are nucleoside analogs.

Amantadine Hydrochloride

Amantadine hydrochloride (amantadine HCl) (Symadine, Symmetrel) is used primarily in the prophylaxis of influenza A after exposure of high risk persons; however, early immunization is still the method of choice for prevention. If administered 24-48 hr after onset of influenza A, amantadine reduces the duration of the disease and provides a more rapid return to activities and improvement in lung function. It is not effective *in vivo* against influen-

za strains other than type A nor parainfluenza viruses, rhinoviruses, adenoviruses, RSV, and others.

Amantadine HCl is a synthetic cyclic primary amine that is administered orally. Its mechanism of action is not completely understood, but it appears to inhibit membrane-associated events such as penetration of the virus into the host cell and release of infectious viral nucleic acid.

Rimantidine is a derivative of amantadine that is used in the United Soviet Socialist Republic against influenza A and may become available in the United States soon. Animal studies show it to be somewhat more effective than amantadine.

Ansamycin

Ansamycin (rifabutine), a derivative of the antimycobacterial rifamysin binds retroviral reverse transcriptase *in vitro*. Clinical trials in AIDS patients have not been rewarding.

Antimoniotungstate

Antimoniotungstate (HPA-23) has anti-reverse transcriptase activity in a number of animal retroviruses both *in vitro* and *in vivo* and has been tested in AIDS patients. Although a transient reduction in peripheral blood virus was seen in some patients, others had no improvement. The drug causes thrombocytopenia, liver dysfunction and fever.

Foscarnet

Foscarnet (phosphonoformate trisodium, PFA) is active against all herpesviruses; it may be considered for ACV-resistant herpesvirus isolates and for CMV infection in AIDS patients on AZT. It is being tested as a topical anti-HSV drug and as a systemic anti-CMV drug. Clinical trials of intravenous foscarnet have shown some nephrotoxicity and abnormalities of serum calcium and phosphorus levels.

Foscarnet and the related phosphonoacetic acid (PAA) are pyrophosphate analogs. They interfere with the viral polymerase of some DNA and RNA viruses. The reverse transcriptase of retroviruses is inhibited by PFA but not PPA.

Ganciclovir

Ganciclovir sodium (BW B759U, BIOLF-62, DHPG sodium, GCV sodium, Cytovene, nordeoxyguanosine) is active *in vitro* against all herpesviruses, but its principle use is against CMV retinitis in immunocompromised patients; it is being investigated in gastrointestinal CMV infections in AIDS patients and bone marrow transplant recipients. Because of high toxicity, the use of ganciclovir should be weighed against its side effects including mutagenicity, carcinogenicity, adverse reproductive potential, renal and hematological toxicity, psychosis, and phlebitis. Ganciclovir should not be used in immunocompetent hosts, and combined use with AZT results in profound, prolonged neutropenia. A synergistic effect on CMV is seen with ganciclovir and interferon α or β.

Ganciclovir is a purine nucleoside analog of guanine that interferes with DNA synthesis by competing with deoxyguanosine. Its increased activity over

ACV in CMV inhibition is thought to result from slower catabolism of ganciclovir triphosphate by intracellular phosphatases.

Ribavirin

Ribavirin (ICN-1229, Virazole, RTCA, Tribavirin) has the widest spectrum of all the antiviral drugs. Its principle use is against RSV in severe lower respiratory tract infections, and it has been used in influenza A and B infections. Although not currently included in U.S. Food and Drug Administration approved labeling, it has been effective against Lassa and Crimean-Congo fevers as well as measles virus, HSV-1 and 2, enterovirus 72 (hepatitis A), and adenovirus, and is under investigation for management of HIV-1 infection. However, it is antagonistic to AZT and should not be used concomitantly. It is inhibitory *in vitro* to a number of different RNA and DNA viruses including various *Arenaviridae, Bunyaviridae, Orthomyxoviridae, Paramyxoviridae, Reoviridae, Retroviridae, Togaviridae, Adenoviridae, Herpesviridae, and Poxviridae*. In some cases, though, the virus inhibition *in vivo* is less than that *in vitro*.

Ribavirin interferes with viral RNA and DNA synthesis and subsequently, protein synthesis by a mechanism that, like those of many other antiviral drugs, is not completely known yet. It exhibits a greater effect on viral DNA and RNA synthesis than cellular nucleic acid synthesis except in a few cases such as HSV-1 and enterovirus 72. Its activity depends on its intracellular conversion to ribavirin-5'-triphosphate and -monophosphate; the triphosphate competes with ATP and GTP for viral RNA polymerase and inhibits the enzymes responsible for capping the 5' viral mRNA with guanosine. The monophosphate inhibits IMP dehydrogenase, the enzyme that synthesizes GTP. Ribavirin inhibits phosphorylation of thymidine but unlike ACV is not readily incorporated into DNA or RNA.

Selenazole and tiazofurin are selenium- and sulfur-containing nucleoside analogs related to ribavirin. *In vitro*, they are active against most of the viruses that are sensitive to ribavirin, and in some cases, their interaction with ribavirin is additive or synergistic. Their efficacies and toxicities *in vivo*, particularly that of the selenium compound, have not been established.

Suramin

Suramin (Germanin), is one of the longest known drugs that has been considered as an antiviral agent. It has been used for many years against African trypanosomiasis and onchocerciasis. Suramin, a sodium salt derivative of napthalenetrisulfonic acid, is active *in vitro* against retroviruses and was used in clinical trials against HIV-1. However, it causes considerable side effects including adrenal, urinary, and hepatic abnormalities; fevers; rashes; as well as rare idiosyncratic reactions. It is no longer seriously considered as a therapy in AIDS.

Vidarabine

Vidarabine (adenine arabinoside, Ara-A) has been used to treat HSV encephalitis, resulting in reduced mortality, but appears to be less effective than ACV; comparative studies are underway. It has been used to treat shingles and chickenpox in immunocompromised patients but is ineffective in treatment of

VZV encephalitis. In both immunocompromised and immunocompetent hosts, vidarabine has been used to treat CMV infections.

Vidarabine is a purine nucleoside produced from fermentation by a streptomycete. Its mode of action is unclear, but it may block viral DNA polymerase; it is not significantly incorporated into the viral DNA.

Adenine arabinoside monophosphate (ara AMP) inhibits HBV replication and can be given by intramuscular injection. Its efficacy in clearing one of the internal antigens of hepatitis B (HBeAg) from chronically infected individuals has been variable.

Zidovudine

Zidovudine (azidothymidine, AZT, Compound S) is probably the most widely known antiviral agent due to its popularity in the AIDS press. Studies have shown that when initiated early, AZT reduces the progression of the disease; further study is necessary to determine whether length of survival is increased if the drug is administered to symptomless HIV-positive individuals. In addition to HIV-1, it is active against some other mammalian retroviruses and has *in vitro* activity against EBV.

AZT is a synthetic thymidine analog that, when incorporated into DNA, results in the inability to form phosphodiester linkages. *In vitro* it interferes with viral reverse transcriptase. The U.S. Food and Drug Administration has designated it as an orphan drug for management of HIV-1. An orphan drug is one whose cost of research and production surpasses the revenue expected from sales, but for humanitarian reasons, the producer makes it available in return for certain monetary advantages (such as tax breaks).

Other Considerations for Antiviral Therapy

In addition to antiviral agents, drugs that afford various forms of symptomatic relief are occasionally employed in the treatment of viral illness. Awareness of the manifestations of different viral infections can influence symptomatic therapy. For example RSV and parainfluenza virus precipitate the release of histamines into nasopharyngeal secretions and thus contribute to wheezing. However, this is not true in rhinovirus or coronavirus infections. Therefore, H1 antihistamines would not be effective therapy for common colds. On the other hand, rhinoviruses cause the production of high kinin levels in secretions, which suggests that antiinflammatory drugs might be a useful therapy.

Obviously, the best antiviral therapy is prevention with vaccines (American Society of Hospital Pharmacists, 1990c; Jordan, 1988; Quinnan, 1990), but unfortunately they are not available for all viruses. A few antisera are available for passive immunization when exposure has not permitted vaccination (American Society of Hospital Pharmacists, 1990b); but again, not all viral infections can be treated in this manner. Considerable research is in progress on vaccine production, and this subject is too vast to be covered here. We have focused on a synopsis of drugs currently available or presently in clinical trials. Other possibilities are stimulation of the host response to viral infections with immunomodulators such as interferons, interleukins, thymic humoral factors, cyclosporin A, ampligen, or isoprinosine (Mills and Corey, 1989), alone or in combination with drug therapy. Some antiretroviral drugs have *in vitro* synergistic interaction with others or with interferon or soluble CD4; prelimi-

nary studies suggest that alternating therapy or combination therapy may decrease toxicity or increase efficacy. However, immunotherapy could be counterproductive, for example, if it produces activated cells that viruses use for replication or activates latent virus. Investigational drugs active *in vitro* against viruses must be thoroughly studied to determine such things as their toxicity *in vivo*, their ability to produce adequate serum levels, and their interaction with other therapeutic agents.

SUMMARY

EM is a very advantageous procedure for diagnostic virology and should be used in conjunction with culture and other specialized techniques that require specific probes. It is a very rapid procedure and is particularly good for viral agents that cannot be cultured and for specimens that are liquid. If specific antiserum is available, viruses can be serotyped by EM.

The morphological characteristics that should be noted for virus identification by negative staining are whether the viruses are naked or enveloped, and if enveloped whether they have recognizable spikes. Also, the shape of the nucleocapsid in enveloped virions is important. In thin sections the virus location in infected cells is a clue to the nucleic acid type. Other characteristics just mentioned for negative stains are important in thin sections as well.

Specimens to be examined by negative staining (liquids and stools) should be sent to the EM laboratory unfixed and undiluted; tissue samples should be fixed immediately upon removal in buffered glutaraldehyde. Specimens for EM should never be frozen in a regular refrigerator freezer. Long term storage of stool specimens can be accomplished in liquid nitrogen, at -70°C, or in sealed containers at 4°C. Tissue specimens, if they are not processed through epoxy resin, should be stored at 4°C in glutaraldehyde.

Other procedures for the laboratory diagnosis of viral infections are reviewed by Spector and Lancz (1986), Yolken (1990), and White and Fenner (1986), and rapid laboratory techniques are discussed by Henshaw (1988; submitted).

The current armamentarium of antiviral drug therapy includes ACV, amantadine, ganciclovir, ribavirin, vidarabine, and AZT; many other agents are being developed. The availability of such therapeutic options makes the diagnosis of viral infections increasingly important.

REFERENCES

Almeida JD (1983) Uses and abuses of diagnostic electron microscopy. Curr Top Microbiol 104:147-158.

American Society of Hospital Pharmacists (1990a) Antivirals. In AHFS Drug Information. Bethesda: American Society of Hospital Pharmacists, Inc., pp. 353-388.

American Society of Hospital Pharmacists (1990b) Serums. In AHFS Drug Information. Bethesda: American Society of Hospital Pharmacists, Inc., pp. 1880-1920.

American Society of Hospital Pharmacists (1990c) Vaccines. In AHFS Drug Information. Bethesda: American Society of Hospital Pharmacists, Inc., pp. 1921-1073.

Babiuk LA, Sabara MI and Frenchick P (1988) Prospectives for the treatment of gastrointestinal tract virus. In De Clercq E (ed), Clinical Use of Antiviral Drugs. Boston: Martinus Nijhoff Publishing, pp. 341-359.

Blacklow NR (1990) Medical virology of small round gastroenteritis viruses. In de la Maza LM and Peterson EM (eds), Medical Virology, Vol. 9, New York: Plenum Press, pp. 111-128.

Caul EO and Appleton H (1982) The electron microscopical and physical characteristics of small round human fecal viruses: an interim scheme for classification. J Med Virol 9:257-265.

Chesselet M-F (1990) *In Situ* Hybridization Histochemistry. Boca Raton: CRC Press, Inc..

Cossart YE, Field AM, Cant B and Widdows D (1975) Parvovirus-like particles in human sera. Lancet 1:72.

De Clerq E and Walker RT (eds) (1987) Antiviral Drug Development. A Multidisciplinary Approach. New York: Plenum Press.

Doane FW and Anderson N (1987) Electron Microscopy in Diagnostic Virology. New York: Cambridge University Press.

Hammond GW, Hazelton PR, Chuang I and Klisko B (1981) Improved detection of viruses by electron microscopy after direct ultracentrifuge preparation of specimens. J Clin Microbiol 14:210-221.

Hayat MA and Miller SE (1990) Negative Staining. New York: McGraw-Hill Publishing Co.

Henshaw NG (1988) Identification of viruses by methods other than electron microscopy. ASM News 54:482-485.

Henshaw NG (submitted) Diagnostic virology. In Joklik WK, Willet HP, Wilfert CM and Amos DB (eds), Zinsser Microbiology. New York: Appleton-Century-Crofts.

Hsiung GD (1982) Diagnostic Virology Illustrated by Light and Electron Microscopy. New Haven: Yale University Press.

Jordan WS Jr. (1988) Program for accelerated development of new viral vaccines. Prog Med Virol 35:1-20.

Kapikian AZ, Dienstag JL and Purcell RH (1976) Immune electron microscopy as a method for the detection, identification, and characterization of agents not cultivable in an in vitro system. In Rose NR and Friedman H (eds), Manual of Clinical Immunology. Washington: American Society for Microbiology, pp 467-480.

Lennette DA (1985) Collection and preparation of specimens for virological examination. In Lennette EH, Balows A, Hausler WJ, Jr, Shadomy HJ (eds) Manual of Clinical Microbiology, 4th Ed., Washington, D.C., American Society for Microbiology, pp. 687-693.

Lew J, Glass R, Petric M, LeBaron C, Hammond G, Miller SE, Robinson C, Boutillier J, Talty M, Payne C, Franklin R, Oshiro L and Jaqua M-J (submitted) Six year retrospective surveillance of gastroenteritis viruses identified at ten electron microscopy centers in the United States and Canada.

MacNaughton MR and Davies HA (1981) Human enteric coronaviruses. Arch Virol 70:301-313.

Mertz GJ (1989) Mucocutaneous herpes simplex virus infections: prophylaxis and treatment. In Mills J and Corey L (eds), Antiviral Chemotherapy. New Directions for Clinical Application and Research. New York: Elsevier Science Publishing Co., Inc., pp. 1-22.

Metcalf TG, Jiang X, Estes MK and Melnick JL (1988) Nucleic acid probes and molecular hybridization for detection of viruses in environmental samples. Prog Med Virol 35:186-214.

Miller SE (1986) Detection and identification of viruses by electron microscopy. J Electron Microsc Tech 4:265-301.

Miller SE (1988) Diagnostic virology by electron microscopy. ASM News 54:475-481.

Miller SE (1989) Electron microscopy in rapid viral diagnosis. EMSA Bull 19:53-59.

Miller SE (1990a) Electron microscopy in diagnostic virology. I. Specimen handling and procedures for identifying viruses in samples. In Mukherjee TM (ed), National Training Course on the Application of Electron Microscopy in Biomedical Research and Diagnosis of Human Diseases. Adelaide, So. Australia: World Health Organization, pp. 100-105.

Miller SE (1990b) Electron microscopy in diagnostic virology. II. Virus morphology and confusing structures. In: Mukherjee TM (ed), National Training Course on the Application of Electron Microscopy in Biomedical Research and Diagnosis of Human Diseases. Adelaide, So. Australia: World Health Organization, pp. 106-114.

Miller SE and Howell DN (1988) Viral infections in the acquired immunodeficiency syndrome. J Electron Microsc Tech 8: 41-78.

Miller SE and Lang DJ (1982) Rapid diagnosis of herpes simplex infection: amplification for electron microscopy by short term in vitro replication. J Infect 4:37-41.

Miller SE and Nielsen RA (1979) Rapid embedding: tissue to TEM in less than 2 hours. Proc. Southeastern Electron Microsc Soc 2:27.

Mills J and Corey L (1985) Antiviral Chemotherapy. New Directions for Clinical Application and Research, Vol. 1. New York: Elsevier Science Publishing Co., Inc.

Mills J and Corey L (1989) Antiviral Chemotherapy. New Directions for Clinical Application and Research, Vol. 2. New York: Elsevier Science Publishing Co., Inc.

Norval M and Bingham RW (1987) Advances in the use of nucleic acid probes in diagnosis of viral diseases of man. Arch Virol 97:151-165.

Olin BR (ed) (1990) Antiviral agents. Drug Facts and Comparisons. St. Louis: JB Lippincott Co.

Oshiro LS and Miller SE (in press). Electron microscopy. In Lennette EH (ed), Laboratory Diagnosis of Viral Infections, 2nd Ed. New York: Marcel Dekker.

Palmer EL and Martin ML (1988) Electron Microscopy in Viral Diagnosis. Boca Raton: CRC Press, Inc.

Paver WK, Caul EO, Ashley CR and Clarke SKR (1973) A small virus in human faeces. Lancet 1: 237-240.

Quinnan GV Jr (1990) Immunization against viral diseases. In Galasso GJ, Whitley RJ and Merigan TC (eds), Antiviral Agents and Viral Diseases of Man. New York: Raven Press, pp. 727-969.

Riepenhoff-Talty M, Saif LJ, Barret HJ, Suzuki H and Orga PL (1982) Potential spectrum of etiological agents of viral enteritis in hospitalized infants. J Clin Microbiol 17:352-356.

Specter S and Lancz GJ (1986) Clinical Virology Manual. New York: Elsevier Science Publishing Co., Inc.

Versteeg J and Salimans MM (1988) The human parvoviruses. In de la Maza LM and Peterson EM (eds), Medical Virology, Vol VII. New York: Elsevier Science Publishers, pp. 33-56.
Yolken RH (1990) Laboratory diagnosis of viral infections. In Galasso GJ, Whitley RJ and Merigan TC (eds), Antiviral Agents and Viral Diseases of Man. New York: Raven Press, pp. 141-181.
White DO and Fenner FJ (1986) Medical Virology. Orlando: Academic Press, Inc.

DISCUSSION

de la Maza L (University of California Irvine Medical Center, Orange, CA):

Are you offering these types of diagnostic tests on a stat basis? If somebody comes with a specimen, are you willing to perform the test in the middle of the night, with a turn-around time of 20 minutes?

Miller S:

Absolutely, and I've been called at home. We have gotten to know the pediatricians, so that when they order a test and want it stat, they call me up and say, "how do I get there." The problem with some of the larger hospitals (we are a tertiary hospital) is that there is a single collecting laboratory. The nurse collects the sample, and it gets shuffled off to Central Collection along with all the other things that get sent there: the bacteria, the fungi, the parasites, and everything else. Then they sort everything, and the courier picks it up. If you have just missed the last courier for the day, it can sit around somewhere and get lost for a while. If they physician wants something stat, (s)he calls up, and I say, "I'm in room 339 Jones; collect the sample and send it over by medical student." We pretty much can stop what we're doing and perform the test. Again, the "quick and dirty" direct method is the one that is 20-30 minutes. If we do the direct procedure and it's negative, then we start playing with the sample and will run one of the concentration procedures that takes more time. Almost always, we use the Airfuge, and then sometimes we use some of these other techniques as well.