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# Viral Infections in the Acquired Immunodeficiency Syndrome

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*KEY WORDS* Viral diagnosis/detection, Electron microscopy/light microscopy, Antiviral therapy

*ABSTRACT* The following communication is a tripartite synopsis of the role of viral infection in the acquired immunodeficiency syndrome (AIDS). The first section describes the impact of viral opportunistic infection in AIDS; for each virus, clinical presentation and diagnosis, laboratory diagnostic approaches (with emphasis on electron microscopy), and therapeutic interventions attempted to date are discussed. The second segment explores current theories on the pathogenesis of AIDS, and describes diagnostic and therapeutic approaches to the syndrome itself. The final section catalogues ultrastructural anomalies in the cells of AIDS patients, many of which have been mistakenly identified as etiologic agents.

## INTRODUCTION

Viruses occupy a unique position in the pathogenesis of acquired immunodeficiency syndrome (AIDS), representing not only the primary etiologic agent of the disease but also a major cause of associated opportunistic infection. Antiviral therapy is thus of central importance to the palliative treatment and eventual cure of AIDS. Because of the profound deficiency of cell-mediated immunity that typifies the AIDS patient, viruses normally eliminated or kept at bay in the immunocompetent host become overwhelming. We are approaching an era in which therapy for viral agents will be as commonplace as is that for bacteria. As more and more agents are found to be effective against viral infections, precise identification of the causative viruses becomes increasingly essential. Several drugs with proven antiviral efficacy in patients without immunocompromise have been used with some success in individuals with AIDS. Though perpetual maintenance therapy is generally required in such patients to prevent recrudescence of viral infection, improved quality and prolongation of life have been attained in many instances. It is for these reasons that we present a discussion of the virus diseases seen in AIDS patients, along with their clinical presentation, diagnosis, and treatment.

## MATERIALS AND METHODS

General methods for virus isolation and laboratory diagnosis of viral diseases have been described (Hsiung, 1982; Malherbe and Strickland-Cholmley, 1980; Rothchild and Cohen, 1986; White and Fenner, 1986). Detailed methods for detection and identification of viruses by electron microscopy (EM) have been published (Miller, 1986; Doane and Anderson, 1987). Virus concentration procedures and identification of AIDS-related viruses will be described briefly here.

### *Specimen preparation*

Several of the viruses carried by AIDS patients, including hepatitis B virus (HBV) and human immunodeficiency virus (HIV) itself, are capable of infecting immunocompetent individuals. As will be discussed below, the tissue distribution of these viruses is extremely diverse; HIV has been isolated from bodily fluids from a number of sites as well as from leukocytes. Therefore, all samples from suspected AIDS cases must be treated with extreme care. Gloves should be worn routinely during all processing steps prior to

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fixation. Specimens should be processed in a biohazard hood if possible; if not, in a quiet corner away from traffic and on a piece of plastic-backed absorbent paper. Extreme care must be taken to prevent creation of aerosols. All disposables must be autoclaved before discarding, and all tools and containers must be disinfected. Easily available disinfectants are 10% Clorox or 70% ethanol (FDA, 1985).

### *Liquid samples*

If the specimen is liquid (e.g., cerebrospinal fluid [CSF], blood, urine, stool suspension, blister fluid, tears, lavages), it is centrifuged at low speed (1,500g) to remove cells and large debris. A drop of sample is placed on parafilm, and a formvar- and carbon-coated membraned grid is allowed to sit on top of the drop for 5–10 min. The grid is then drained with filter paper and transferred to a drop of 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 2–5 min; it is then washed by touching three drops of distilled water, and drained again. This fixation step is not necessary to preserve virus structure, but it is recommended for all samples from AIDS patients as a means of killing pathogenic viruses, since infectious virions can survive negative staining. In the absence of a fixation step, the grid and microscope specimen holder must be properly disinfected after viewing. Negative staining is accomplished by placing the washed, drained grid onto a drop of aqueous, saturated uranyl acetate or 1% phosphotungstate for 30–60 sec and draining. Under no circumstances should the sample be mixed with negative stain and atomized onto the grid.

Many samples contain virus at a high enough titer to allow demonstration by the simple, fast, and direct method described above. Viruses present at lower concentrations can frequently be detected by utilizing one of the liquid sample concentration techniques that we have previously described (Miller, 1986). Briefly, after low-speed debris clarification, the virus can be pelleted by ultracentrifugation for 1 hr at 100,000g or airfuge ultracentrifugation (Beckman Instruments, Fullerton, CA) at 30 lb/sq in (100,000g) for 30 min. The pellet is then resuspended in the remaining drop after decanting the supernatant into disinfectant, and the drop is placed on a grid as described above. If an EM rotor is used in the airfuge, the sample can be pelleted directly onto the grid. Low-speed centrifugation with an ultrafiltration device designed to retain particles 20 nm or greater in diameter (Schleicher and Schuell, Keene, NH) is an alternative means of concentrating virus particles.

Specific antibodies or pooled gamma globulin, which may contain virus-reactive antibodies, can be added to virus suspensions to clump and concentrate virus particles (Almeida, 1980; Kapikian et al., 1976). Final antibody dilutions should be in the range of 1:100–1:1,000. After incubation for 30 min at 37°C or overnight at 4°C, the suspension is pelleted at 15,000g for 1 hr. The pellet is then resuspended in the small drop remaining after decanting the supernatant and placed on a grid as described above.

The pseudoreplica technique, originally described by Sharp (1960), is often used with urine suspected of containing cytomegalovirus (CMV). A drop of suspension is placed on an agar block, and the liquid is allowed to diffuse into the agar. A formvar solution is then dropped onto the block, floated off onto a water surface, and picked up on a grid. A similar technique places a grid on a drop of suspension on agar, and as the solution diffuses into the agar, the virus is concentrated onto the grid (Anderson and Doane, 1972). If specific antibody is mixed with the agar, it will clump the viruses as they are concentrated onto the grid (Anderson and Doane, 1973). The utility of specific antibody addition to the specimen is realized only if the researcher has some a priori idea of the possible agents present; if a specific agent is not suspected, pooled gamma globulin may be used in hope that it contains antibody against any viruses present.

### *Tissue samples*

Biopsies for ultrathin sectioning should be processed routinely (e.g., in 2% glutaraldehyde, 1% osmium tetroxide, and 1% uranyl acetate followed by embedment) (Hayat, 1981). Rapid methods for embedment in less than 2 hr have been described (Miller, 1986).

### *Tissue culture amplification*

Occasionally, low virus concentration will preclude visualization by any of the previously discussed techniques. Allowing a round or two of virus replication in tissue culture can amplify viral numbers to a level detectable in the electron microscope (Miller and Lang, 1982). Appropriate cell lines for inoculation are discussed by Miller (1986). One must keep in mind that some viruses cannot

be grown in conventional tissue culture.

## RESULTS AND DISCUSSION

### *Viruses as opportunists in AIDS*

#### *Cytomegalovirus*

Cytomegalovirus (CMV) infection, which is often asymptomatic in normal subjects (Sommerville, 1984), is frequent and severe in AIDS patients (Quinnan et al., 1984). Indeed, most of the literature on virus infections in individuals with AIDS pertains to CMV; postmortem studies show that 30–40% of these patients are infected with the virus. Frequent sites of infection are the lungs, central nervous system (CNS), retina, gastrointestinal (GI) tract, reticuloendothelial system, kidneys, and adrenals (Guarda et al., 1984; Jensen et al., 1984; Rodrigues et al., 1983; Snider et al., 1983; Tucker et al., 1985). The virus has been isolated from blood, saliva, urine, semen, uterine secretions, breast milk, and CSF (Drew et al., 1981; Edwards et al., 1985; El-Mekki et al., 1987; Quinnan et al., 1984; Stagno et al., 1980).

CMV interstitial pneumonitis is a major cause of morbidity and mortality in AIDS patients (Macher et al., 1983). Frequently, CMV and *Pneumocystis carinii* (see Sun and Teichberg, pp. 79–103) are found together, and CMV has been found in suspected *P. carinii* pneumonia cases that were negative for the protozoan (Barbour, 1987; Gorelkin et al., 1986).

Bronchoscopic lavage has been successful in establishing the diagnosis of CMV pneumonitis in some cases (Golden et al., 1986), but open lung biopsy is recommended if bronchoscopy is negative (Barrio et al., 1987).

Unlike herpes simplex virus (HSV) and herpes zoster virus (HZV), CMV is not neurotropic and does not ordinarily cause neurological disease in immunocompetent individuals (Duchowny et al., 1979). In immunocompromised hosts, however, it can cause either encephalomyelitis or demyelination without inflammation in both the central and peripheral nervous systems (Moskowitz et al., 1984; Tucker et al., 1985). The mechanism by which demyelination occurs is unknown. Possibilities include viral cytotoxic effects on support cells, release of lytic factors from neighboring cells destroyed by the virus, and autoimmune attack secondary to crossreactivity between viral antigens and myelin or autosensitivity to damaged myelin. Other typical lesions include ventriculitis with periventricular destruction and cerebral or pituitary involvement (Hawley et al., 1983) and diffuse glial nodule encephalitis (Snider et al., 1983). Neurological symptoms include encephalopathy, often with progressive dementia, occasional focal motor deficits, seizures, and headache and fever.

Computerized tomography (CT) of the head may be unrevealing in cases in which inflammation is minimal. Contrast CT scans are of potential value in this setting. A comparison of CT findings in different viral CNS diseases is presented by Sarwar et al. (1986). Magnetic resonance imaging (MRI) studies may be useful in the diagnosis of CMV CNS infection (Levy et al., 1985; Post et al., 1986). However, definitive diagnosis rests with the identification of virus in biopsy material, occasionally in CSF, and often at autopsy (Petito et al., 1986; Tucker et al., 1985).

CMV is the major cause of blindness in AIDS patients (Palestine et al., 1984). CMV retinitis is characterized by rapidly progressive necrotic lesions, frequently with hemorrhage, which obliterate the retina within 6 months. On ophthalmoscopic examination, virus-induced white spots or granular patches are difficult to distinguish from "cotton wool spots." The latter occur in conditions of ischemia such as diabetes, hypertension, and anemia, and represent microinfarctions of nerve fibers. CMV lesions can be distinguished by their rapidly progressive nature and the presence of hemorrhage (Rodrigues et al., 1983) and by demonstration of the virus in tissue after globe removal or at autopsy (Holland, 1985; Jensen et al., 1984; Pepose et al., 1983). HSV and toxoplasma can also cause retinitis and confuse the diagnosis (Neuwirth et al., 1982). Color ophthalmoscope pictures by Rosecan et al. (1986) and De Venecia et al. (1971) demonstrate CMV retinal lesions. CMV retinitis is a poor prognostic sign in AIDS. It usually occurs late in the course of the disease; death frequently occurs within 6–8 weeks following the diagnosis of retinitis, regardless of the patient's medical status at its onset (Holland, 1985).

CMV can cause lesions throughout the GI tract, from the mouth to the anus, in AIDS patients. Alimentary CMV is often the first evidence of AIDS (Hinnant et al., 1986). Manifestations include severe debilitating diarrhea, marked weight loss, fevers, stools positive for occult blood, and occasionally massive colonic hemorrhage or perforation (Frager et al., 1986). Colonoscopy may show

yellow maculopapular lesions (Levinson and Bennetts, 1985). Radiographic and CT findings are not specific, and early lesions may not show up at all (Balthazar et al., 1985; Frager et al., 1986). The symptoms and endoscopic, colonoscopic, and radiographic findings in CMV GI disease can mimic those of other ailments, including tumors, inflammatory bowel disease with diffuse mucosal ulceration or granulomatous lesions, and other infectious processes (Elta et al., 1986; Frager et al., 1986; Freedman et al., 1985; Knapp et al., 1983). Diagnosis by biopsy must be made to rule out treatable conditions such as candidiasis.

CMV can infect the adrenal glands, causing adrenal insufficiency and hypotension (Bleiweiss et al., 1986). CMV hepatitis, cholangitis, cholecystitis, and pancreatitis have been described in AIDS patients (Galloway, 1984; Sacks and Freeman, 1984; Agha et al., 1986; Kavin et al., 1986). Endocrine problems and hypercalcemia have also been seen as a result of CMV infection of the parathyroid glands (Zaloga et al., 1985).

A final site requiring comment with regard to CMV-related disease in AIDS patients is the skin. The primary cutaneous manifestation of CMV in AIDS is its proposed role as an etiologic agent in Kaposi's sarcoma (KS), a neoplasm that presents as raised, reddish-purple lesions of skin and occasionally viscera (see Tucker, pp. 137–158). The link between CMV and KS is circumstantial but strong. In the absence of global, severe immunosuppression (as seen in late-stage AIDS), several studies have documented increased titers of antibody to CMV, but not Epstein-Barr virus (EBV) or HSV type 1 (HSV-1) or type 2 (HSV-2) in patients with KS (Drew, 1986; Giraldo et al., 1984). The virus itself has been isolated from KS tumor biopsies (Giraldo et al., 1984), and CMV DNA, mRNA, and nuclear antigen have been demonstrated in tumor tissue (Boldogh et al., 1981; Greenspan and Shillitoe, 1984; Fenoglio et al., 1982). CMV is known to be oncogenic in vitro; it can initiate cellular DNA, RNA, and protein synthesis, and its genome can induce transformation of cultured cells (Giraldo and Beth, 1986; Rapp, 1984). The virus has also been linked with adenocarcinoma of the colon (Huang and Roche, 1978), prostatic cancer (Sanford et al., 1977), and cervical cancer (Melnick et al., 1978). The exact role of CMV in KS tumorigenesis is unclear. It is not known if the virus acts directly via a viral onc gene product, recombines with or alters the host genome, or provides continuing immunosuppression enabling an emerging tumor to escape surveillance (Spector and Spector, 1984).

The argument in favor of CMV as an etiologic factor in KS is not without contradiction. Dotz and Berman (1983) have described KS patients with positive antibody titers against HSV in the absence of anti-CMV antibodies. HBV has been demonstrated in KS tissue as well as CMV (Siddiqui, 1980). However, the bulk of the evidence accumulated to date suggests that CMV plays a part in the etiology of KS.

CMV has been reported only sporadically as a causative factor in non-neoplastic cutaneous disease. In one case, the virus was demonstrated in unusual purpuric skin lesions in a patient with AIDS (Penneys and Hicks, 1985). The authors of this report stressed the importance of distinguishing such lesions from the papular lesions seen in poxvirus infections (see below).

Numerous diagnostic modalities have been applied to the detection of CMV (Doerr et al., 1985). Routine serologic analysis of circulating anti-CMV antibodies by radioimmunoassay (RIA) or enzyme-linked immunosorbant assay (ELISA) (Griffiths, 1985; Kiefer et al., 1983; McKeating et al., 1985; Nath et al., 1987) is of little value in AIDS patients. Immunosuppressed patients frequently fail to mount a serologically detectable antibody response to CMV (Doerr et al., 1977). Conversely, a majority of healthy adults possess circulating IgG antibodies to CMV, though the appearance of IgM anti-CMV antibodies may correlate with active viral disease.

Immunochemical detection of CMV and its components has been used with some success. An RIA with monoclonal antibodies has been described for detecting the agent (Goldstein et al., 1982), as have ELISAs (El-Mekki et al., 1987; McKeating et al., 1985; Yolken and Stopa, 1980). An immunofiltration technique (a modified ELISA) that is faster and simpler than, and almost as sensitive as, DNA hybridization has been described (Rossier et al., 1987). These techniques have the theoretical drawback of potentially failing to detect serological variants of the virus and depend heavily on the sensitivity and specificity of the antibodies employed.

Viral culture can be used to detect CMV, both as a diagnostic technique in its own right and as an amplification step prior to EM (Miller, 1986). Culturing of CMV, though particularly useful when virus is present at

low concentration, has the disadvantage of being extremely time-consuming; detectable growth may require 2–6 weeks (Bach, 1986; Doerr et al., 1985; Tucker et al., 1985). Detection may be enhanced by low-speed centrifugation in special tissue culture vials (Gleaves et al., 1985).

Light microscopy (LM) is a useful means of detecting the presence of CMV infection, and has the additional potential of revealing concomitant infections with other organisms, a frequent occurrence in patients with AIDS. By LM, the nuclei of CMV-infected cells often exhibit an "owl's eye" morphology, consisting of dense intranuclear viral inclusions surrounded by a clear halo and dense marginated chromatin at the nuclear membrane (Knapp et al., 1983; Nash, 1982). Cytoplasmic inclusions may also be present. The intranuclear inclusions are best demonstrated on routine hematoxylin and eosin (H & E)-stained (Meiselman et al., 1985) or Papanicolaou-stained (Schulman et al., 1982) sections. Gomori's methenamine silver and the periodic acid-Schiff reaction, frequently used in the diagnosis of *P. carinii* and fungal infection, stain cytoplasmic but not intranuclear CMV inclusions and are thus of limited value in the diagnosis of CMV infection (Gorelkin et al., 1986). Excellent color (Malherbe and Strickland-Cholmley, 1980) and black and white (Hsiung, 1982) micrographs of CMV-infected cells have been published.

Immunofluorescence microscopy has been used to demonstrate CMV in lung lavage specimens (Emanuel et al., 1986), transbronchial biopsies (Blumenfeld et al., 1984), and liver tissue (Sacks and Freeman, 1984); immunoperoxidase staining of CMV has likewise been reported for brain and spinal cord tissue (Tucker et al., 1985). These techniques, though rapid (Hackman et al., 1985), carry the same caveats mentioned in connection with immunoassays for soluble CMV antigens.

In situ hybridization with CMV nucleic acid probes has also proven to be a useful adjunct in the diagnosis of CMV infection in tissue and urine specimens. This technque is capable of detecting latently infected cells (Chou and Merigan, 1983; Myerson et al., 1984; Spector and Spector, 1985). It has been used to demonstrate CMV in colonic tissue that was negative for the virus on routine histologic and immunofluorescence examination (Roche et al., 1981). Hybridization can be performed on formalin-fixed, paraffin-embedded tissue after years of storage (Myerson et al., 1984).

EM has been used with considerable success in the diagnosis of CMV infection in both tissues and bodily fluids, especially urine. In EM examination of thin sections, spherical nucleocapsids of 100 nm are seen, mostly in the nucleus where they are constructed (Fig. 1c); some naked particles may escape into the cytoplasm before they obtain their outer membrane. Nucleocapsids receive their outer covering by budding through the nuclear membrane, sometimes through internal membranes into vesicles and sometimes through the cytoplasmic membrane. The whole virion may be 150–200 nm in diameter (Fig. 1a,b) (Muñoz et al., 1987; Rodrigues et al., 1983). Because of the pliable, nonornamented outer covering, the virion may be difficult to distinguish from cellular debris in negative stains unless the stain penetrates the membrane and outlines the icosahedral nucleocapsid (Fig. 1d,e). CMV cannot be distinguished by EM from other members of the herpes family.

Therapy for CMV infections in AIDS patients has been generally unsatisfactory. The greatest experience to date has been with 9-(1,3-dihydroxy-2-propoxymethyl) guanine (DHPG), an acyclovir analog. DHPG, also known variously as BW B759U, ganciclovir, ganciclovir, 2'NDG, and B10LF-62, has been used with some success in the treatment of CMV pneumonia (Collaborative DHPG Treatment Study Group, 1986; Laskin et al., 1987), CNS infections, and retinitis (Bach, 1986; Humphrey et al., 1986; Palestine et al., 1986; Rosecan et al., 1986). Though some AIDS patients have experienced prolonged survival and improved quality of life as a result of this therapy (Kotler et al., 1986), discontinuation of the drug has invariably led to a recrudescence of CMV infection. DHPG therapy has also been complicated by bone marrow suppression and neutropenia in some cases (Barbour, 1987; Holland et al., 1986).

Phosphonoformate (PFA, Foscarnet) has been used with some success in the treatment of CMV retinitis, and may require a less frequent maintenance regimen than DHPG (Singer et al., 1985). Other agents, such as acyclovir (acycloguanosine, Zovirax) (Bach, 1986), vidarabine (adenine arabinoside [ara-A]) (Bach, 1986), and cytarabine (cytosine arabinoside [ara-C]), alone and in combination with interferon (Furio and Wordel, 1985), have been of little therapeutic benefit in CMV infection.

Alpha interferon has been used in the treatment of KS in AIDS patients with a 40% success rate. It appears to act as an antineoplastic agent rather than as an immune modulator (Volberding et al., 1984). Other agents that have been used with some reported success include etoposide, vinblastine, and a combination of doxorubicin, bleomycin, and vinblastine (Krigel, 1984).

*Abbreviations*

| | |
|---|---|
| ara-A | adenine arabinoside |
| ara-C | cytosine arabinoside |
| ARC | AIDS-related complex |
| AZT | azidothymidine |
| BKV | BK virus |
| BVDU | bromovinyldeoxyuridine |
| CCC | cylindrical confronting cisternae |
| CCL | cylindrical confronting lamellae |
| CEDU | chloroethyldeoxyuridine |
| CMV | cytomegalovirus |
| CNS | central nervous system |
| CPE | cytopathological effect |
| CSF | cerebrospinal fluid |
| CT | computerized tomography |
| DHPG | 9-(1,3-dihydroxy-2-propoxymethyl)guanine |
| DNA | deoxyribonucleic acid |
| EBNA | Epstein-Barr nuclear antigen |
| EBV | Epstein-Barr virus |
| EEG | electroencephalography |
| ELISA | enzyme-linked immunosorbent assay |
| EM | electron microscopy |
| FIAC | fluoroiodoaracytosine |
| GI | gastrointestinal |
| H & E | hematoxylin and eosin |
| HBsAg | hepatitis B surface antigen |
| HBcAg | hepatitis B core antigen |
| HBV | hepatitis B virus |
| HIV | human immunodeficiency virus |
| HPA-23 | antimoniotungstate |
| HPV | human papillomavirus |
| HSE | herpes simplex encephalitis |
| HSV | herpes simplex virus |
| HTLV | human T-lymphotropic virus |
| HZV | herpes zoster virus |
| JCV | JC virus |
| KS | Kaposi's sarcoma |
| LAS | lymphadenopathy syndrome |
| LAV | lymphadenopathy-associated virus |
| LM | light microscopy |
| MRI | magnetic resonance imaging |
| MVB | multivesicular body |
| NCPP | noncultivable picorna- and parvovirus-like particles |
| PFA | phosphonoformate |
| PML | progressive multifocal leukoencephalopathy |
| RIA | radioimmunoassay |
| RNA | ribonucleic acid |
| RT | reverse transcriptase |
| SLE | systemic lupus erythematosus |
| TRF | test tube and ring-shaped forms |
| TRI | tubuloreticular inclusions |
| TRS | tubuloreticular structures |
| VCA | viral capsid antigen |
| VZV | varicella zoster virus |

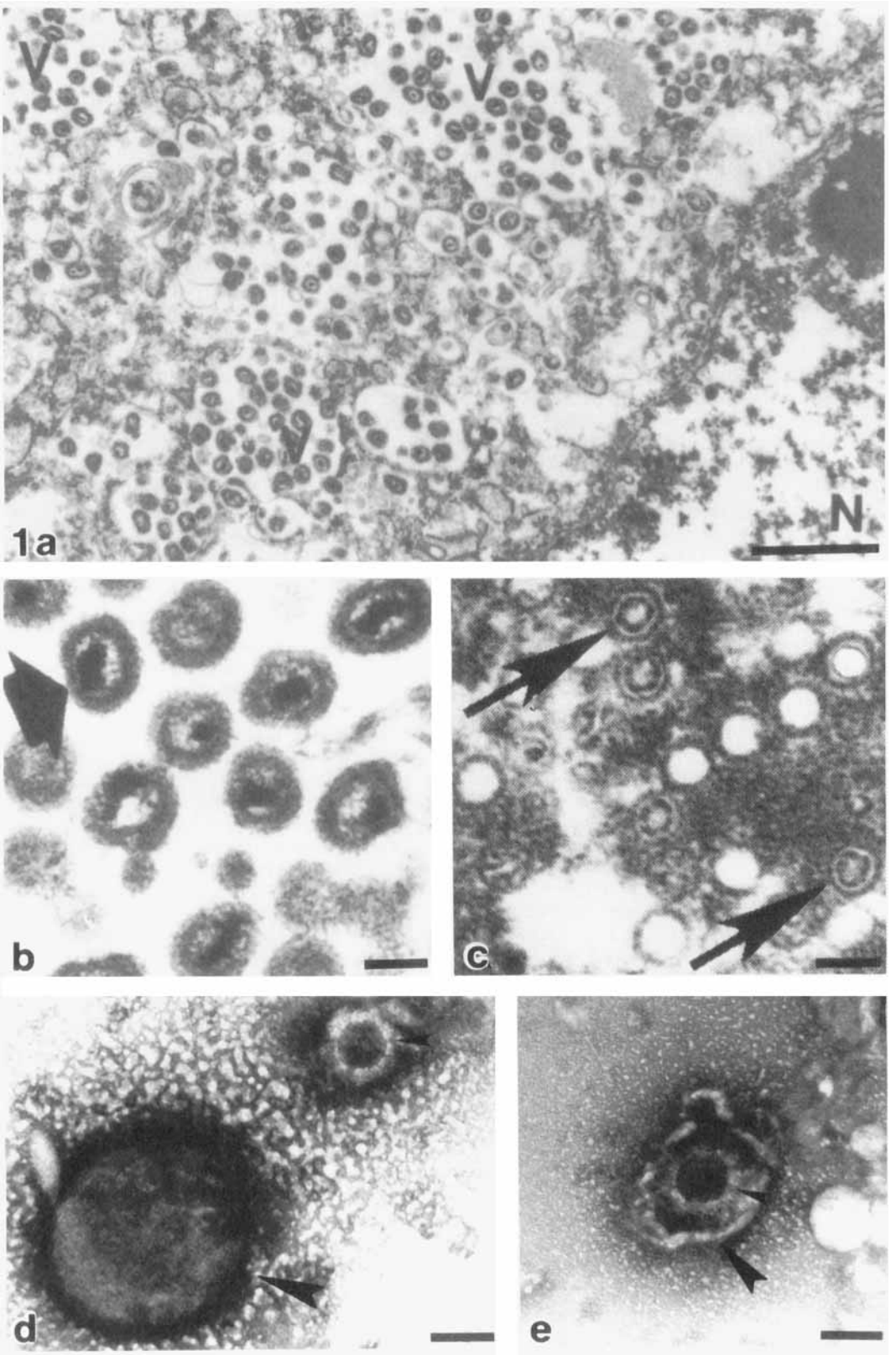
*Herpes simplex virus*

Herpes simplex virus (HSV) types 1 and 2 can both cause encephalitis, meningitis, and myelitis with immune-mediated demyelination (Britton et al., 1985; Handler and Perkin, 1982; Heller et al., 1982; Maier et al., 1986; Nahmias et al., 1982) as well as eye (Pepose et al., 1985) and skin lesions (Siegal et al., 1981). HSV hepatitis is rare but does occur in immunocompromised patients (Chase et al., 1987).

HSV encephalitis (HSE) has a propensity for involving one or both temporal lobes (Weiner and Fleming, 1984). Presentations include fever, headache, stiff neck, clouded consciousness, and focal neurologic signs, including seizures, aphasia, and focal motor and sensory deficits. Mortality from HSE is correlated with the level of consciousness at the initiation of antiviral therapy in immunocompetent hosts. In AIDS patients, the rapidity of progression of the disease is proportional to the severity and degree of immune suppression. HSE must be distinguished diagnostically from cryptococcosis, tuberculosis, and bacterial abscesses, all of which may have similar clinical presentations (Hirsch and Schooley, 1983b). CT and electroencephalography (EEG) may suggest temporal lobe lesions; radionuclide scans may

Figs. 1–11. Lower-magnification (×20,000) micrographs of virus-infected cells illustrate the relationship of the viruses to the cells. High magnification (×100,000) of both thin sections and negative stains of viruses shows virion ultrastructure; all viruses are printed at the same magnification to permit direct comparison of size. Micrographs of virus-like structures are reproduced so that they are presented here at the same final magnification for comparison. The magnification selected (×50,000) is that which is the closest to the majority of the original micrographs. They are 2½ times the size of the infected cell micrographs and ½ the size of the virion micrographs.

Fig. 1. **a:** Low magnification of complete virions of CMV (V) in cytoplasmic vesicles of an infected cell. **b:** High magnification of complete membraned virions (arrow) in the cytoplasm. **c:** High magnification of nucleocapsids (arrows) in the nucleus. **d,e:** Negatively stained CMV. d: The pleomorphic complete particle (large arrowhead) is nondescript and would not be identifiable in a field of cell membrane debris. The nucleocapsid (small arrowhead) has lost its outer covering; it is damaged so that the stain has penetrated the capsid coat. e: The membrane (large arrowhead) still surrounds the nucleocapsid (small arrowhead) but has been broken so that stain penetrated and outlined the nucleocapsid. Bar in a = 1μm; bars in b–e = 100 nm. Figures a–c are reprinted from Gorelkin et al. (1986) with permission from the W.B. Saunders Co.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provide improved detection of such lesions (Weiner and Fleming, 1984), as may MRI (Schroth et al., 1987). The presence of temporal lobe involvement is strongly suggestive of HSE, but in one study, one-half of a series of clinically suspected HSE cases were eventually shown to have other etiologies (Nahmias et al., 1982). As with CMV, definitive diagnosis requires demonstration of the virus or viral antigens in biopsy tissue, CSF, or aspirates.

Most HSV retinitis is associated with encephalitis. In contrast to CMV retinitis, the lesions of which resemble ischemic cotton wool spots, HSV retinitis is characterized by retinal edema, hemorrhage, and perivasculitis. In advanced stages, a hazy yellow-white necrotic retina progresses to exudative retinal detachment (Pepose et al., 1985).

Serological methods for detecting active HSV infection have been described (Doerr et al., 1987); however, as in the case of CMV, they are of limited value in AIDS patients, since a majority of healthy individuals are seropositive for HSV, and immunosuppressed patients with HSV infections are frequently seronegative.

LM of H & E-stained sections of HSV-infected tissue shows diffuse basophilic and contracted eosinophilic intranuclear inclusions, sometimes with marginated chromatin (Malherbe and Strickland-Cholmley, 1980). Immunoperoxidase staining of tissue sections fixed with Bouin's fixative rather than formalin has also been used to demonstrate the inclusions (Pepose et al., 1985). HSV- and CMV-infected cells are frequently similar in appearance, though the latter are often larger, with inclusions occupying a greater percentage of the nucleus.

Unlike CMV, HSV causes characteristic cytopathologic effects (CPE) in tissue culture, consisting of a shift to rounded cellular morphology followed by rapid destruction of the cell monolayer within 1–2 days (Hsiung, 1982). A rapid identification technique has been described for HSV-1 and HSV-2 that makes use of centrifugation in shell vials (originally described for CMV by Gleaves et al., 1985) and staining with fluoresceinated monoclonal antibodies. Detection and typing can be accomplished in 1–2 days (Winter et al., 1987).

A method for rapid identification of HSV has been described using amplification in tissue culture and identification by EM (Miller and Lang, 1982). In the electron microscope, thin sections of HSV-1 (Fig. 2) and HSV-2 (Fig. 3) resemble CMV (Fig. 1). Nucleocapsids of 100 nm may be seen in the nucleus or cytoplasm (Fig. 2a,b), and complete membraned virions (Fig. 2c) can be seen budding from the nuclear membrane, into cytoplasmic vesicles, or from the cytoplasmic membrane (Fig. 2a). Redundant membrane production is characteristically seen in herpesvirus-infected cells (Fig. 2a). Tubular structures are occasionally seen within the nuclei of cells infected with HSV-2 (Fig. 3) (Fong and Hsiung, 1978), but not HSV-1. A negative stain of blister fluid may reveal the 100-nm icosahedral nucleocapsid if the membrane has been broken (Fig. 2d). Though HSV-1 and HSV-2 cannot generally be differentiated from each other or from HZV in negatively stained preparations, herpesviruses and poxvirus, the agent of molluscum contagiosum, can be distinguished readily.

Acyclovir is currently the drug of choice for the treatment of HSV infections (Hirsch and Schooley, 1983b; Whitley et al., 1987). Intravenous acyclovir has been used with success in the treatment of HSE in immunocompetent patients (Levy et al., 1985) and has also been used to manage HSV infections in patients with AIDS (Barbour, 1987). Intravenous vidarabine decreases the mortality of HSE from 70% to 28% in nonimmunocompromised hosts. Since the large volume of intravenous fluid required for vidarabine administration may engender brain edema, brain biopsy is recommended to establish the necessity for treatment (Furio and Wordell, 1985; Pepose et al., 1985). Oral acyclovir may prove beneficial as prophylaxis for HSV infection in immunocompromised hosts (Fiddian, 1987; Hirsch and Schooley, 1983b). Topical acyclovir for HSV mucocutaneous lesions shortens the time to resolution in first-episode infections but is less effective for recurrent disease (Mindel et al., 1987). Intravenous acyclovir is also effective for serious skin lesions (Hirsch and Schooley, 1983b). Ocular HSV keratitis can be treated with topical trifluorothymidine and vidarabine (Hirsch and Schooley, 1983b). Other nucleic acid analogs, such as bromovinyldeoxyuridine (BVDU), chloroethyldeoxyuridine (CEDU), and fluoroiodoaracytosine (FIAC) are promising new topical drugs for herpetic keratitis (De Clerq, 1986).

*Herpes zoster virus*

Herpes zoster virus (HZV) (varicella zoster virus [VZV]) causes chickenpox in children, becomes latent in dorsal root (spinal) gan-

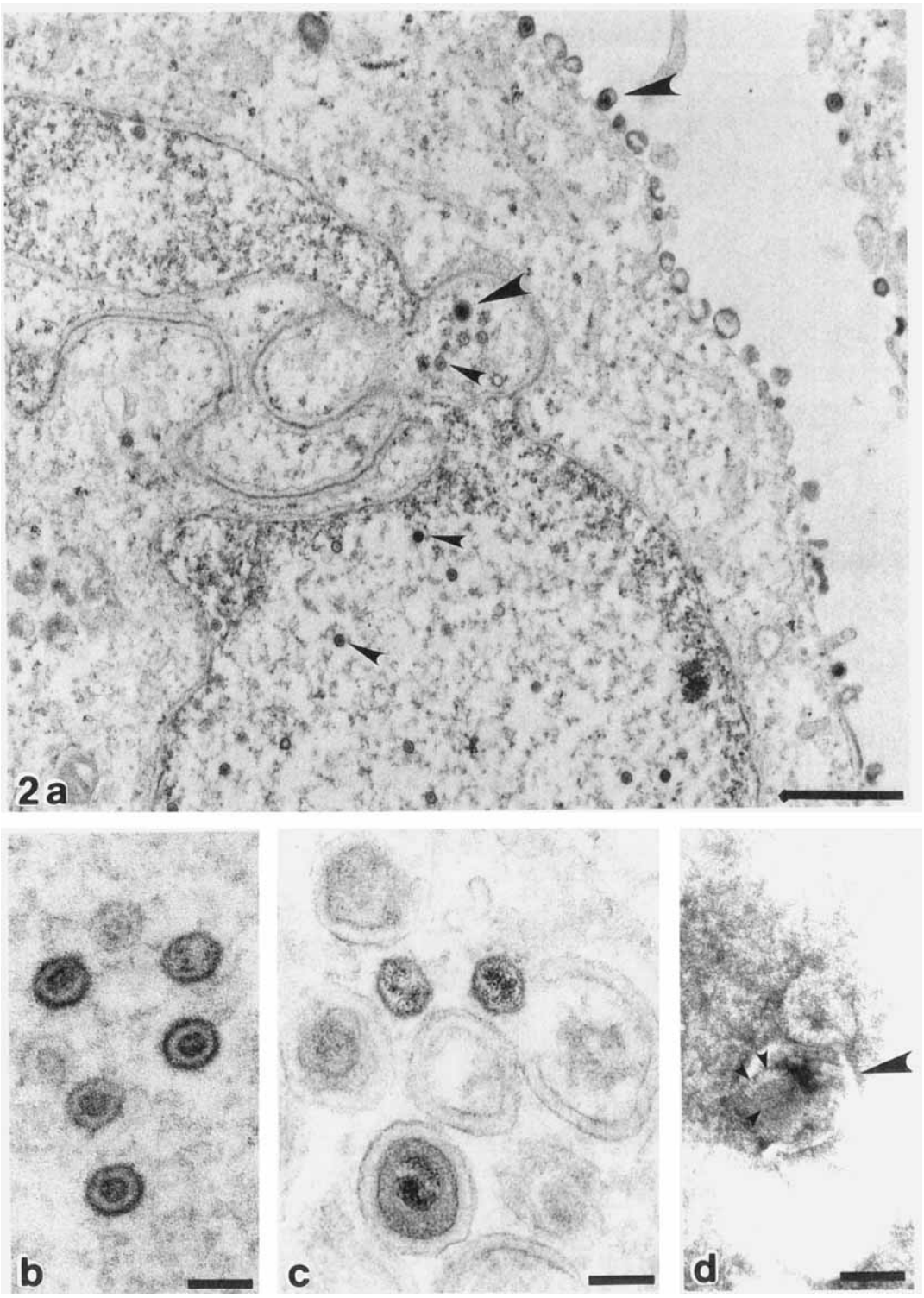


Fig. 2. **a:** HSV-1 nucleocapsids (small arrowheads) in the nucleus and cytoplasm of an infected cell; mature, membraned virions (large arrowheads) in the cytoplasm and at the cell surface. **b:** Higher magnification of nuclear nucleocapsids. **c:** Higher magnification of cytoplasmic nucleocapsids and membraned virus. **d:** Negatively stained virion; note that the stain has penetrated the membrane (large arrowhead) allowing visualization of the capsomers (petite arrowheads) but has not penetrated the interior of the nucleocapsid. Compare to negatively stained CMV. Bar in a = 1 $\mu$m; bars in b–d = 100 nm.

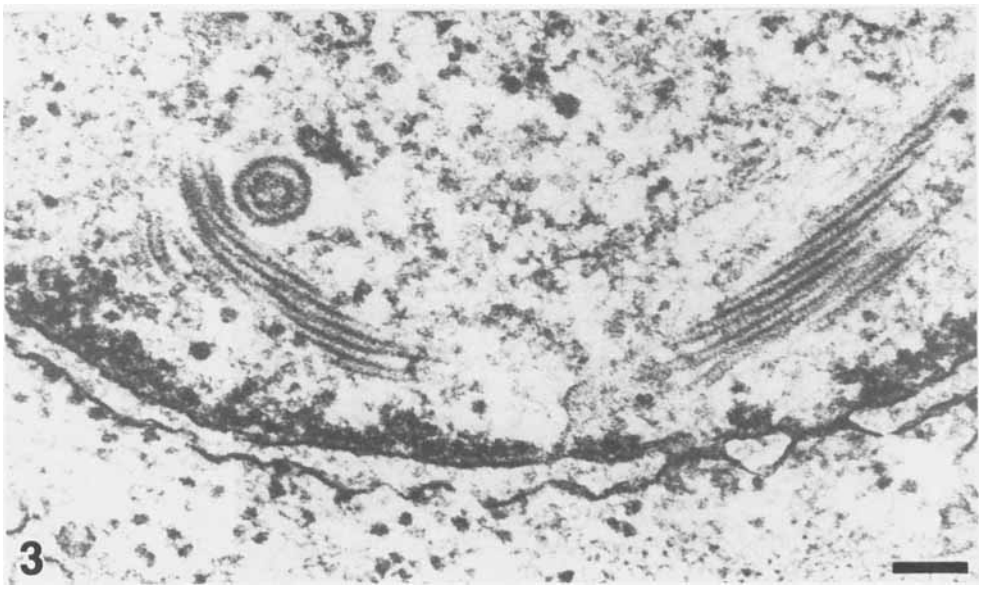


Fig. 3. One intranuclear HSV-2 nucleocapsid and filaments characteristic of HSV-2, but not HSV-1 infection. Bar = 100 nm. Reprinted from Fong and Hsiung (1978), with permission from the CRC Press, Inc.

glia, and may reactivate later. Reactivation often takes the form of shingles, a painful vesicular skin rash resulting from a deficiency in T cell or killer cell activity that is frequently seen in elderly patients. Shingles may be accompanied by neurological complications, including postherpetic neuralgia, paresis, and meningoencephalitis.

In immunocompromised patients, disseminated HZV infection may occur, with GI, pulmonary, and nervous system involvement (Friedman-Kien et al., 1986). HZV CNS infection in individuals with AIDS may take the form of a hemorrhagic, necrotizing encephalitis with early symptoms of headache, fever, seizures, and aphasia. HZV also causes shingles in AIDS patients, and it may be an early clinical indicator of impending AIDS in those at risk (Cone and Schiffman, 1984; Payne et al., 1984; Friedman-Kien et al., 1986). HZV infection of the eye in young adults is a marker for AIDS (Cole et al., 1984; Levy et al., 1985; Sandor et al., 1986). HZV has also been reported in biopsies of hairy leukoplakia (HL) lesions (Greenspan et al., 1984), and it has been isolated from lung tissue without histological evidence of pulmonary infection (Ryder et al., 1986).

Laboratory diagnostic approaches to HZV infection are similar to those utilized in other herpesvirus infections, including culture, LM with and without immunoperoxidase staining, Southern blot hybridization, and EM (Doerr et al., 1987; Gilden et al., 1987; Ryder et al., 1986). LM examination may reveal intranuclear and cytoplasmic inclusions difficult to distinguish from those seen in HSV infection (Hsiung, 1982; Malherbe and Strickland-Cholmley, 1980); immunostaining may aid in this differentiation. By EM, typical herpes family nucleocapsids and membraned virions are evident in thin sections. HZV in negative stain resembles HSV but can be distinguished from poxvirus. Differentiation of HZV from other herpetic infections is important for the selection of proper drug therapy. DHPG, active against CMV infections, does not affect HZV; a higher concentration of acyclovir is required in HZV than in HSV infections (Murray, 1987).

Intravenous vidarabine, acyclovir, and FIAC have been used in the treatment of HZV infection. These agents decrease cutaneous spread, accelerate pain resolution, and may prevent fatal dissemination, particularly if therapy is initiated within 72 hours of appearance of cutaneous lesions (Chang et al., 1987). The efficacy of acyclovir is reportedly greater than that of vidarabine (Furio

and Wordell, 1985; Shepp et al., 1986). Additional therapeutic approaches currently under study include oral acyclovir, intramuscular vidarabine (Wood et al., 1987; Sherlock and Corey, 1985), and new drugs, including BVDU and 6-deoxycyclovir (De Clercq, 1986). In immunocompetent patients, prednisone decreases the severity and pain of HSV-induced lesions and reduces the likelihood of postherpetic neuralgia. However, corticosteroids may induce dissemination of HZV and are thus contraindicated in immunocompromised patients (Murray, 1987). Corticosteroids may also predispose to disseminated CMV infection in AIDS patients (Armstrong et al., 1985; Gold, 1985).

*Epstein-Barr virus*

Epstein-Barr virus (EBV) has been implicated as an etiologic agent in Burkitt's lymphoma (a B cell lymphoma) in Africans (Epstein and Achong, 1977; Purtilo et al., 1985). In Orientals, EBV has been associated with nasopharyngeal carcinoma (Klein, 1979). In Americans and Europeans, its primary clinical importance is as the causative agent of infectious mononucleosis (Henle et al., 1968). However, Burkitt's-like lymphomas have been reported in AIDS patients (see Tucker, pp. 137–158), and EBV has been demonstrated in such lymphomas (Berman et al., 1985; Rosenberg et al., 1986; Whang-Peng et al., 1984). Chromosomal translocations similar to those in Burkitt's lymphoma (Purtilo et al., 1985) have been demonstrated in AIDS B cell lymphomas (Gyger et al., 1985; Lipscomb et al., 1983–1984; Petersen et al., 1985; Whang-Peng et al., 1984). Possible factors predisposing patients with AIDS to EBV-related malignancies include profound T cell immunodeficiency and abnormally high numbers of EBV-infected B cells (Birx et al., 1986; Purtilo et al., 1985; Sumaya et al., 1986).

In addition to its posited etiologic role in B cell lymphoma, EBV has been linked circumstantially to several other conditions in AIDS patients. EBV antigens have been demonstrated in oral neoplasms, including squamous cell carcinoma and oral KS (Greenspan and Shillitoe, 1984; Ziegler et al., 1982). The virus itself has been found in lungs of patients with lymphocytic interstitial pneumonia, in the brains of children with AIDS (Andiman et al., 1985), and in biopsies of oral HL, a condition manifested by white flattened plaques on the sides and ventral surface of the tongue. The role of EBV in the pathogenesis of HL is unclear; by immunoperoxidase staining, tissue from HL plaques has also been shown in many cases to contain papillomavirus, and in some cases, HSV (Greenspan et al., 1984, 1985). The course of HL is variable; there may be spontaneous regression and recurrence. It is not known if the lesion is a premalignant condition, but since both herpesviruses and papillomaviruses have been implicated as possible causative agents, and since both have oncogenic capabilities, regular observation and biopsy is recommended (Youngs et al., 1986). HL must be distinguished clinically from oral candidiasis, which may respond favorably to antifungal therapy.

The morphologic and biochemical identification of EBV, in contrast to the other members of the herpes family, plays a relatively minor role in the clinical diagnosis of EBV-related complications in AIDS. The diagnosis of B cell lymphoma relies mainly on histology, immunohistochemistry, and occasionally, cytogenetics. Similarly, the presence of HL is generally confirmed histologically; by LM, the lesions resemble flat warts, with superficial whorls of fine keratin that are responsible for their "hairy" appearance.

EBV serology suffers from the same drawbacks as serological analysis of the other herpesviridae. High-titered anti-EBV antibodies are present in many healthy individuals and pregnant women; approximately one-half of all normal individuals tested exhibit some degree of seropositivity, presumably as a result of latent viral infection. Anti-EBV antibodies are also frequently found in recipients of organ transplants and patients with lymphoproliferative diseases. In contrast, some virus-positive individuals produce no serological response (Fackler et al., 1985). Almost all AIDS patients exhibit elevated anti-EBV titers (Ciobanu and Wiernik, 1986; Rinaldo et al., 1986), suggesting that many may harbor infections that are due to viral reactivation facilitated by immunosuppression.

Certain EBV antigens can be detected by immunohistologic analysis. Viral capsid antigen (VCA) is present during viral replication, and the Epstein-Barr nuclear antigen (EBNA) is also detectable in latently infected cells (Lindahl et al., 1974; Pearson et al., 1983). Nucleic acid hybridization can also be used to detect the presence of EBV genomes in productively and latently infected cells (Andiman et al., 1983; Brandsma and Miller, 1980; Pi et al., 1983).

EM of cells productively infected with EBV reveals virus particles morphologically similar to the other herpesviruses. Virus particles have been demonstrated by EM in the lesions of HL, but EM is not a routinely useful diagnostic procedure for this disorder. EBV morphology and virus-induced CPE are described by Epstein and Achong (1979).

In vitro, actively replicating EBV is sensitive to several antiviral agents, including $\alpha$ IFN, vidarabine, phosphonoacetic acid, and acyclovir; but these drugs have no effect on latently infected cells. Acyclovir has been used with some success in the treatment of polyclonal B cell lymphoma, but not the monoclonal B cell lymphoma that often develops subsequently (Andersson et al., 1987; Hirsch and Schooley, 1983a).

*Hepatitis B virus*

Hepatitis B virus (HBV) is widespread among homosexual men, intravenous drug abusers, and patients with AIDS, the latter of whom are generally seropositive for the virus (Noonan et al., 1986). In spite of this fact, clinically overt liver disease is unusual in AIDS patients, a finding in keeping with the current concept that antiviral cellular immune responses play a major role in the pathogenesis of HBV hepatitis (Ray et al., 1976; Rustigi et al., 1984). Hepatocellular carcinoma, which has been linked clinically (Romet-Lemonne et al., 1983) and experimentally (Griffiths, 1983) with HBV infection, has likewise not been seen with increased frequency in AIDS patients.

HBV infection is generally diagnosed by the serological detection of circulating viral antigens or antiviral antibodies (Omata et al., 1978; Barr and Mitchell, 1987; Hoar et al., 1985). The virus can also be detected by nucleic acid hybridization techniques (Noonan et al., 1986; Romet-Lemonne et al., 1983; Scotto et al., 1983; Siddiqui, 1983; Yoffe et al., 1986), sometimes in the absence of detectable HBV antibody or antigen (Hoar et al., 1985; Laure et al., 1985).

Routine histologic analysis is useful in the clinical assessment of hepatitis in both its acute and chronic phases, though many of the histologic findings in HBV hepatitis are indistinguishable from those encountered in hepatitis caused by other viruses, drugs, and toxins (Huang and Fisher, 1987). Histologic examination can be augmented by immunofluorescence analysis for hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) (Ray et al., 1976).

HBV cannot be grown in conventional tissue culture. However, the levels of viremia often attained in acute infections (up to $10^{13}$ particles/ml; Griffiths, 1983) permit demonstration by EM, particularly after ultracentrifugation or immunoaggregation (see "Materials and Methods"). The infectious virus, or Dane particle, measures 42 nm in diameter and is roughly spherical; it is frequently accompanied by noninfectious 27-nm-diameter spherical and filamentous particles composed of viral capsid antigen (HBsAg) (Fig. 4c). In liver tissue, aggregates of nondescript intracytoplasmic particles can be seen (Fig. 4a,b).

Several antiviral agents, including vidarabine, acyclovir, and human leukocyte interferon, have been employed with variable success in the treatment of HBV infection. Response rates ranging from negligible to as high as 50% have been reported for vidarabine and interferon in studies involving immunocompetent patients (Thomas et al., 1987).

*Papovavirus*

Papovaviruses are associated with progressive multifocal leukoencephalopathy (PML), a subacute demyelinating disease seen in patients with lymphoproliferative, chronic granulomatous and immunosuppressive disorders. Symptoms of the disease include blurred vision, blindness, numbness, dizziness, hemiparesis, and ataxia (Levy et al., 1985); affected individuals are generally afebrile.

Two related papovaviruses have been implicated in the etiology of PML: JC virus (JCV), which has been found in most of the cases studied to date, and SV-40, a simian papovavirus, which has been isolated in a small number of cases (Zu Rhein, 1969; Coleman et al., 1978; Narayan et al., 1973). JCV and BK virus (BKV), a third papovavirus whose etiologic role in PML has not been firmly established and which, like JCV, derives its name from the initials of the patient from whom it was first isolated, are frequently shed in the urine of transplant patients (Andrews et al., 1983; Hogan et al., 1980) and in the urine in late pregnancy or puerperium (Coleman et al., 1983). A majority of healthy individuals have been exposed to these viruses by adolescence (Padgett and Walker, 1983).

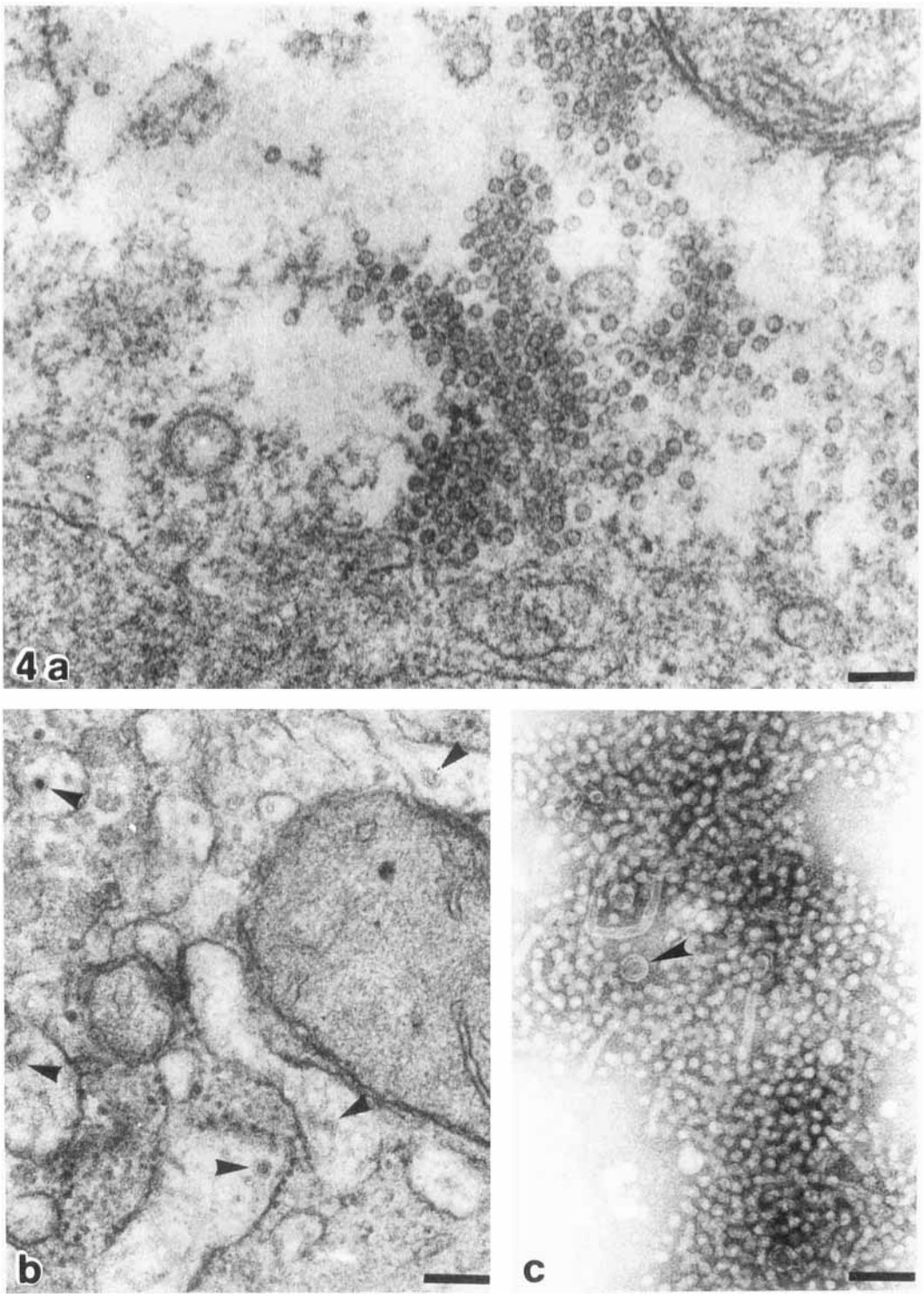


Fig. 4. **a:** HBcAg particles in the cytoplasm of an infected hepatic cell. **b:** Complete HBV virions (arrowheads) in the cytoplasm of an infected cell. **c:** Negative stain showing infective Dane particles (arrowhead) and HBsAg in tubular and pleomorphic spherical forms. Bars = 100 nm. Reprinted from Huang and Fisher (1987), with permission from the Williams and Wilkins Co.

The pathogenesis of PML is incompletely understood. The ubiquity of the associated papovaviruses in normal individuals suggests that the disease may represent a reactivation of latent infection rather than a primary infection (Krupp et al., 1985; Chesters et al., 1983). PML occurs more frequently in AIDS patients, 2–4% of whom develop the disease, than in individuals with other immunosuppressive disorders. It has been proposed that HIV in the brain may have a direct effect on reactivation of latent papovaviruses (Stoner et al., 1986).

On CT scans, the lesions of PML appear as hypodense areas in the white matter with cortical sparing. They frequently originate in one cerebral hemisphere, often with localization to the occipital lobe, and subsequently spread to involve the remainder of the brain. The presence of such lesions in the setting of a normal CSF examination is diagnostic for PML. Similar CT findings are occasionally encountered in toxoplasmosis, but cerebral infection with *Toxoplasma* is invariably associated with CSF perturbations (Weiner and Fleming, 1984). In cases in which the diagnosis is uncertain, CT-directed needle biopsy can provide tissue for virological examination (Snider et al., 1983).

Papovavirus infection can be detected by a number of techniques. Serology is not useful, since 70% of adults have antibodies against JCV, and patients with PML have no increase in titer over normal individuals (Brooks and Walker, 1984; Miller et al., 1982; Padgett and Walker, 1983). However, both BKV and JCV have been detected by nucleic acid hybridization in renal tissue of PML patients (Chesters et al., 1983). JCV is fastidious, but has been isolated in primary human fetal glial cells and urine-derived epithelial cells; BKV is less restrictive for host cells (Beckman and Shah, 1983).

In spite of the availability of these techniques, the diagnosis of PML generally requires examination of involved brain tissue. Advanced lesions have necrotic centers containing numerous foamy phagocytic cells. At the peripheries of such lesions, enlarged, deformed astrocytes resembling malignant cells are found, and glial cells with intranuclear viral inclusions may be seen. A detailed description of the histopathology of PML is provided by Richardson et al. (1983) and McKeever et al. (1983).

Fluorescence microscopy has been used to detect papovavirus antigens in brain tissue (Budka and Shah, 1983). The immunohistology of PML is somewhat complex. Infected tissue is often reactive with antisera against JCV (Miller et al., 1982; Narayan et al., 1973). However, antibodies against the SV-40 T antigen also stain the nuclei of JCV-infected cells, occasionally in the absence of staining for other viral antigens. Both oligodendrocytes and astrocytes appear to be infected (Stoner et al., 1986). The T antigen is destroyed by formaldehyde fixation, though the virus itself is stable. The antigen is preserved in acetone-fixed frozen sections.

By EM, the PML agents are 35- to 40-nm spherical viruses, frequently associated with filaments 16–27 nm in diameter (Fig. 5a–c) (Miller et al., 1982; Zu Rhein, 1969). In negatively stained urine specimens, the viruses appear as 35- to 40-nm icosahedra (Fig. 5d,e).

Treatment of PML has been attempted with vidarabine and cytarabine with some initial improvement but is unsatisfactory in AIDS patients (Krupp et al., 1985; Peters et al., 1980; Snider et al., 1983; Walker, 1978).

### *Papillomavirus*

Human papillomaviruses (HPV) cause condyloma acuminatum (venereal warts), a highly infectious, sexually transmitted disease (Ferenczy et al., 1985), and have been associated with malignant transformation (Banks et al., 1987; Gupta et al., 1987a; McCance, 1987). Some strains may give rise to dysplasia without warts (Frazer et al., 1986). Perianal condylomata are common in homosexuals, and anorectal carcinoma has been reported in AIDS patients (Croxson et al., 1984; Frazer et al., 1986). Along with viruses in the herpes family, HPV has been reported in lesions of oral HL (see "Epstein-Barr virus" above) (Greenspan et al., 1985).

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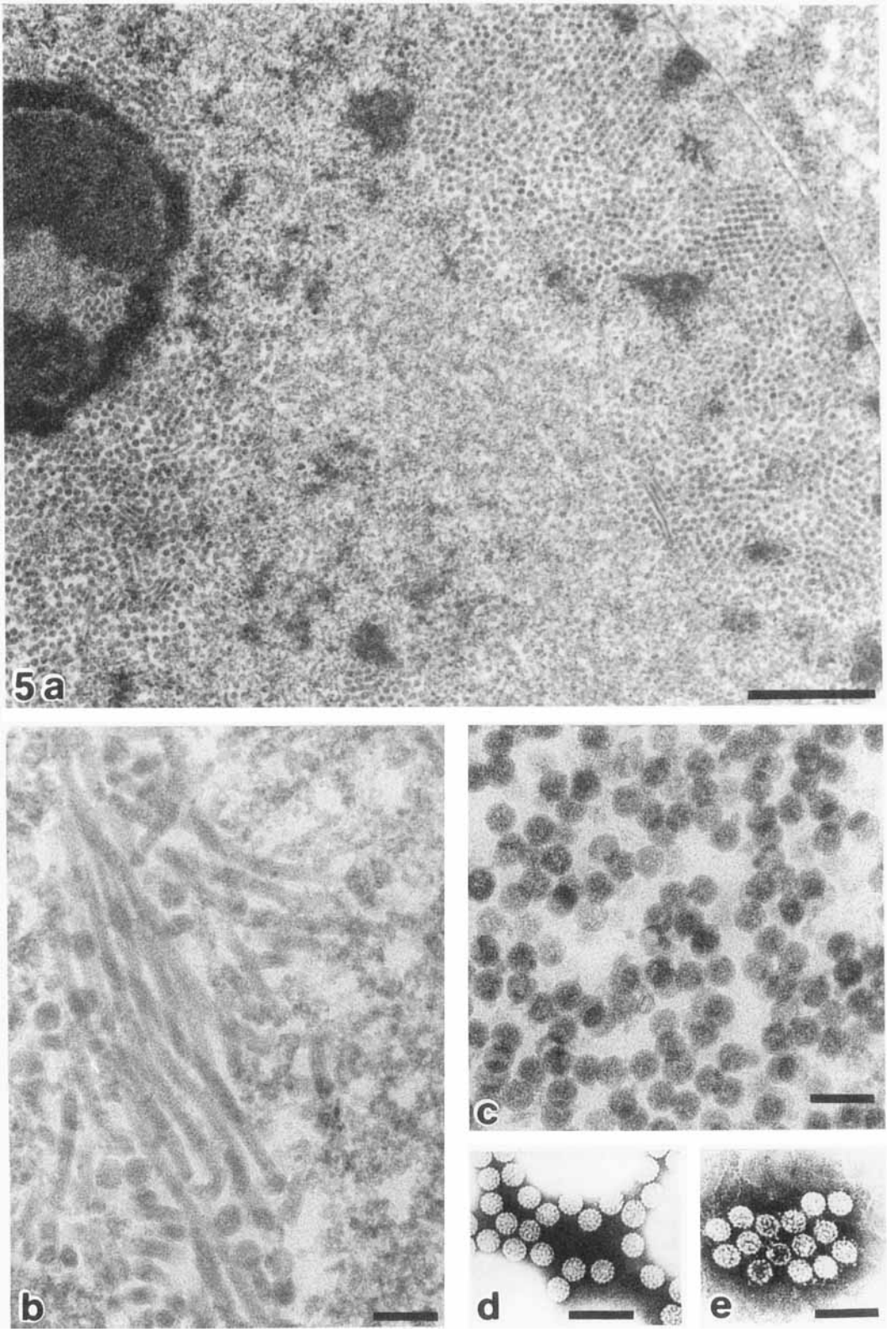
Fig. 5. **a:** Polyomavirus in the nucleus of a cell from a case of PML. The virus is most likely JCV. Many particles are randomly dispersed; some areas show crystalline arrays, others show filaments. **b:** Higher magnification of the filaments. **c:** Higher magnification of the nondescript icosahedrons. **d:** Negative stain of a papovavirus isolated from the brain of an immunodeficient patient. **e:** Negative stain of virus from urine of the same patient. Bar in a = 1 $\mu$m. Bars in b–e = 100 nm. Micrographs a–c are courtesy of Dr. Douglas Anthony, Department of Pathology, Duke Medical Center, Durham, NC. Micrographs d and e are reprinted from Takemoto et al. (1974), with permission from the *Journal of the National Cancer Institute.*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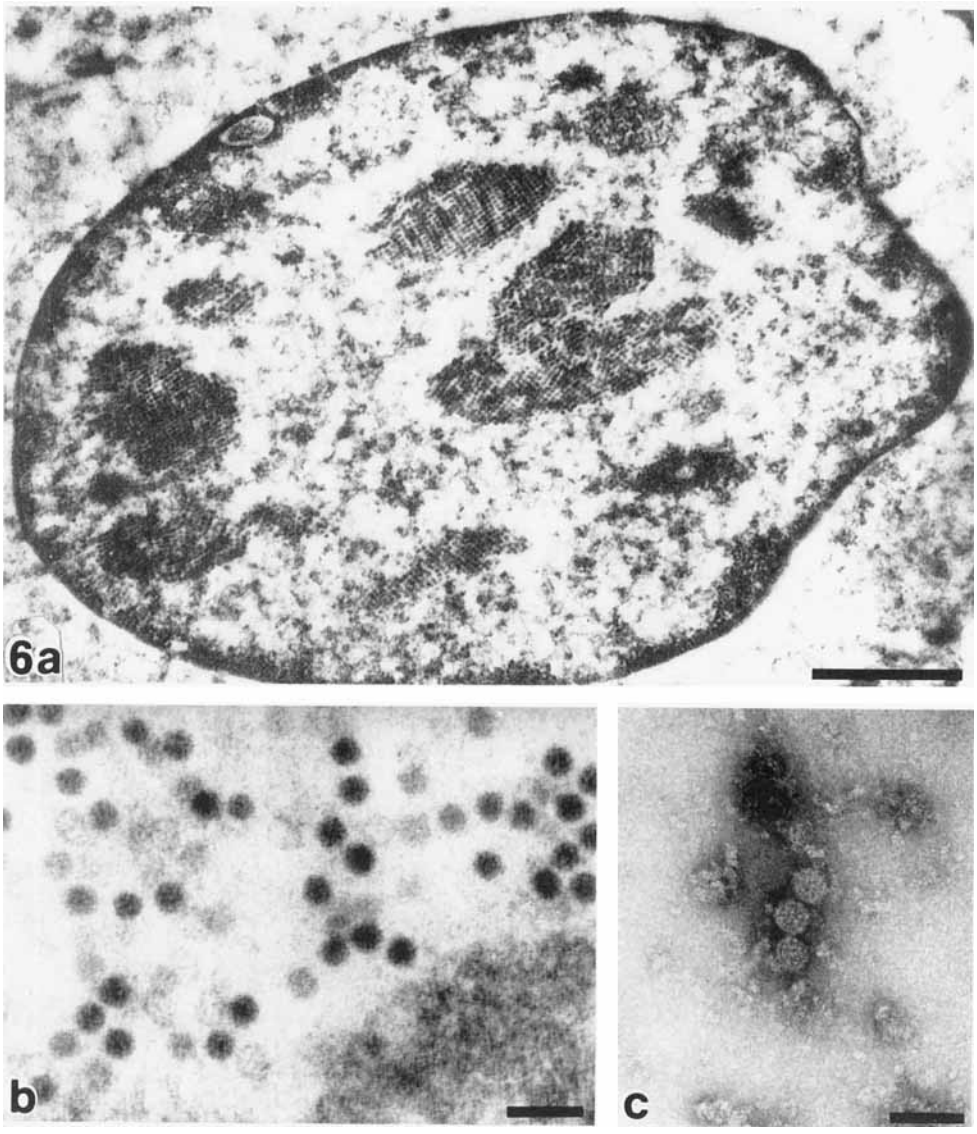



Fig. 6. **a:** HPV in the nucleus of an infected cell. Small nondescript particles occasionally align in a crystalline array. **b:** High magnification of nondescript nuclear particles. **c:** Negative stain of bovine papillomavirus; the virion appears knobby or warty. Note the similarity to JCV and BKV. Bar in a = 1$\mu$m. Bar in b = 100 nm. a is reprinted from Almeida et al. (1962), with permission from the Williams and Wilkins Co. Infected human cell in b and virus in c were kindly provided by Dr. Susan Watts, Department of Surgery, UNC School of Medicine, Chapel Hill, NC.

Virus components can be detected by immunolabeling (Banks et al., 1987) or in situ hybridization (Gupta et al., 1987a; Wickenden et al., 1985). However, diagnosis is usually made by routine histology of the excised lesion. Histological characteristics of warts include epidermal hyperplasia, hyperkeratosis, cytoplasmic vacuolization with eosinophilic inclusions, and excessive nuclear basophilia (Murphy et al., 1984).

Papillomaviruses can be detected by EM, though it is not generally used in the clinical diagnosis of warts. EM of negatively stained wart viruses shows knobby, 45-nm icosahed-

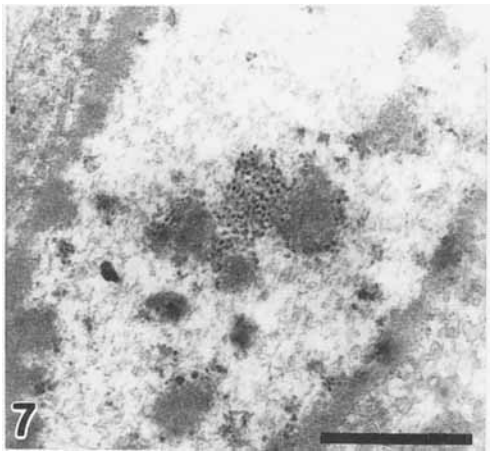



Fig. 7. Nuclear granules in a degenerating cell, not to be confused with small nuclear viruses. Bar = 100 nm.

rons (Fig. 6c). In thin sections, the virus appears in the nuclei of cells as 45-nm dense spherical particles, sometimes in crystalline arrays (Fig. 6a,b). Nuclear virus particles must be differentiated from nuclear granules, a nonspecific clumping of chromatin resulting from cell injury (Fig. 7). Viruses are uniform in size and have a distinct round shape; nuclear granules are not as uniformly sized or shaped. The ultrastructure of warts and inclusion bodies is described by Almeida (1962). LM and EM of malignant lesions are described by Kovi et al. (1974); however, the nuclear densities described are of questionable viral nature. Warts caused by papillomavirus should be differentiated from the "carcinoma-like" Buschke-Löwenstein tumor, as the latter is not responsive to podophyllin (Kovi et al., 1974).

Because of their potential for malignancy, venereal warts should be removed; this can be achieved by topical treatment with 25% podophyllin in benzoin. Cancer treatment must be directed at the tumor, rather than the virus, and includes surgery and radiation (Kovi et al., 1974).

*Adenovirus*

Adenoviruses can cause a number of respiratory illnesses and conjunctivitis in immunocompetent patients; they can also be found in individuals without evidence of disease (Taylor, 1977). They are not usually seen in the urine of healthy individuals (Horwitz et al., 1984). Adenoviruses have been isolated from patients with AIDS (Gotlieb et al., 1981; Horwitz et al., 1984; Siegal et al., 1981). In one case of encephalitis in an AIDS patient with dementia and spastic quadriparesis, adenovirus was isolated from CSF (Horoupian et al., 1984), but the causal relationship of the virus to brain degeneration is unknown. Virus can be isolated from urine in up to 20% of AIDS patients (De Jong et al., 1983) and has also been isolated from stool (Armstrong et al., 1985). The significance of adenovirus infection to disease or immunodeficiency in AIDS is not known, but strains 34 and 35 have been suggested to be cofactors in the syndrome (Horwitz et al., 1984).

Many of the human adenovirus strains are common, and infection early in life is frequent (Hierholzer et al., 1982; De Jong et al., 1983), making serology of no use in diagnosis. Cultural isolation is difficult from urine, which provides a hostile environment for virus survival, but some strains can be isolated from other locations. Certain strains are difficult to culture under any circumstances. Hybridization techniques (Horwitz et al., 1984; Engler and Kilpatrick, 1981) and EM can be used to detect the virus. By LM, inclusions in the nuclei of infected cells stain densely (Malherbe and Strickland-Cholmley, 1980). Adenovirus-infected cell cultures exhibit a CPE consisting of cellular rounding and clumping (Hsiung, 1982). By EM, the virus is a 75-nm naked icosahedron; triangular facets resembling a geodesic dome can often be seen in negative stains (Fig. 8b). In thin sections, the flat, faceted sides sometimes align, creating a crystalline array. A DNA virus, adenovirus is found in the nucleus (Fig. 8a,c).

No treatment has been described for adenovirus infections.

*Poxvirus*

A poxvirus causes molluscum contagiosum, a sexually transmitted papular skin lesion, which in immunocompetent individuals is usually self-limiting and resolves spontaneously in 3–12 months. In AIDS patients, the disease is more extensive and often occurs on the face (Redfield et al., 1985). In the absence of exogenous immunosuppression, extensive molluscum lesions in unusual locations with unusually rapid recurrence despite diligent therapy suggest AIDS (Katzman et al., 1985; Lombardo, 1985; Sarma and Weilbaecher, 1985). The disease must be differentiated from cutaneous cryptococcosis with hypopigmented papules (Rico and Pen-

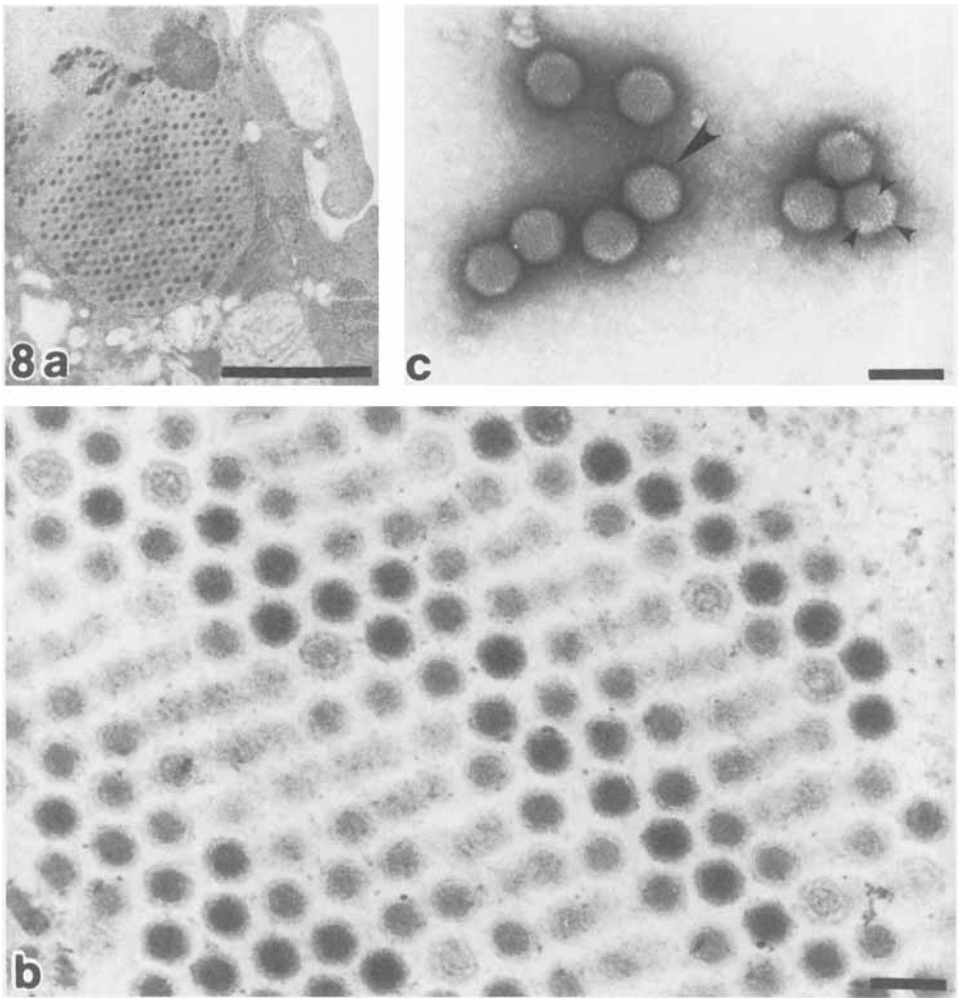
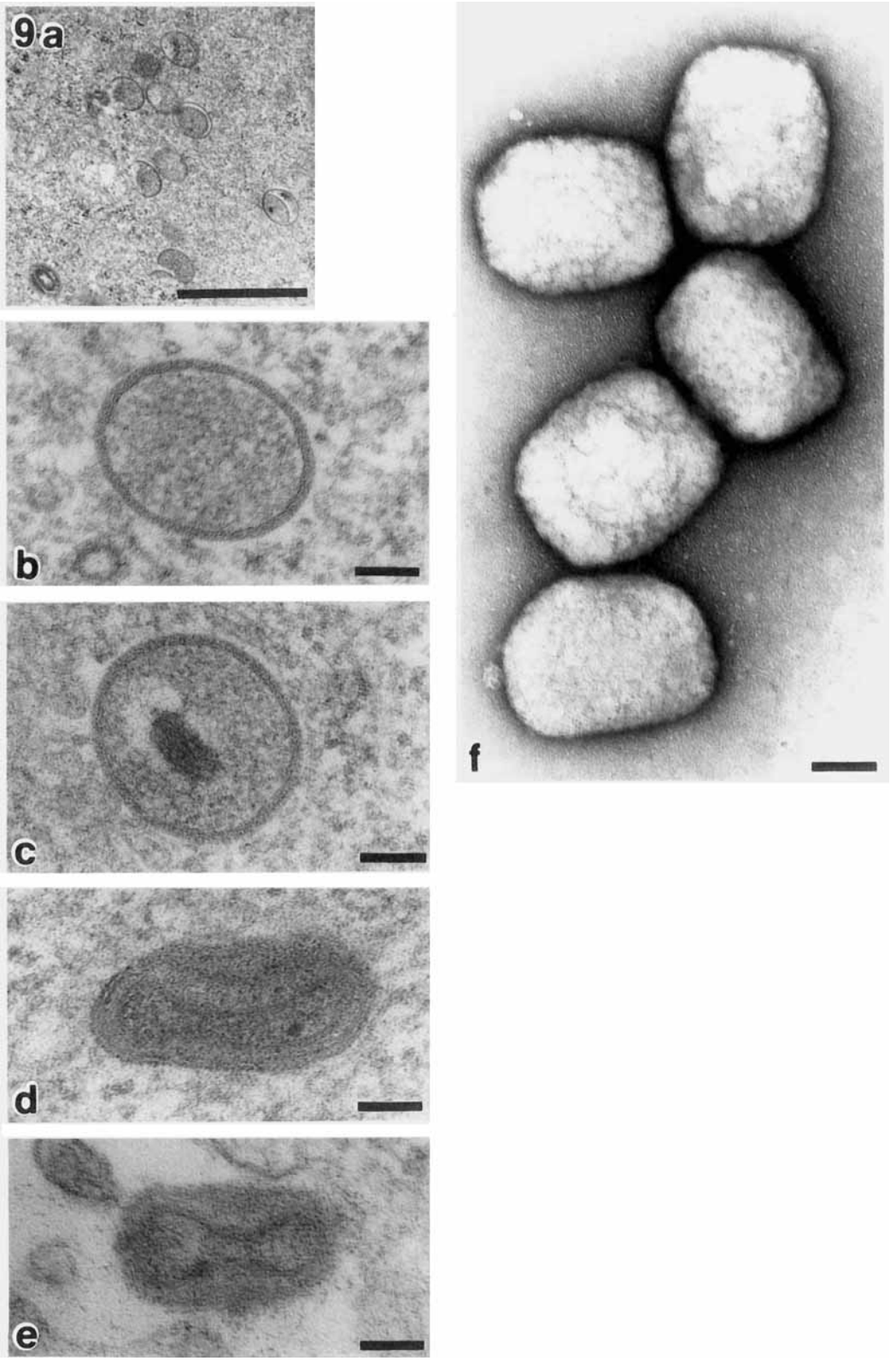



Fig. 8. **a:** Adenovirus in the nucleus of an infected cell. **b:** Higher magnification showing crystalline array of virions. **c:** Negative stain of icosahedral virions. Depending on their orientation on the grid, virions may appear hexagonal (large arrowhead). Flat, triangular facets may also be distinguishable on their surfaces (apices of two triangles are marked by petite arrowheads). Bar in a = 1 $\mu$m. Bars in b and c = 100 nm.

neys, 1985) and cutaneous CMV infection (Penneys and Hicks, 1985).

Characteristic histology includes epithelial hyperplasia and downward lobular growth of epidermal cells. The "molluscum body" in the stratum granulosum and stratum corneum is a large homogeneous cytoplasmic inclusion containing virus (Katzman et al., 1985). In H & E-stained sections, molluseum bodies within deeper epithelial strata are eosinophilic, while those in more superficial cells are basophilic. The CPE of poxvirus-infected cell cultures includes multinucleated cells (Hsiung, 1982). By EM of negatively stained preparations, the agent is a large brick-shaped virus measuring 200–250 nm by 200–250 nm by 300–350 nm; a representative poxvirus is shown in Figure 9. It is

Fig. 9. **a:** Cowpox virions in the cytoplasm of an infected cell. **b–e:** High magnification of different stages of development showing oval virus with amorphous center and condensation of the nucleoid into a "dumbell"-shaped structure. **f:** Negative stain of the "brick-shaped" virus. Bar in a = 1 $\mu$m. Bars in b–f = 100 nm. Virus and infected cells were kindly provided by Dr. David Pickup, Department of Microbiology and Immunology, Duke Medical Center, Durham, NC.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easily distinguishable from the spherical herpesviruses, a fact of potential value in the differentiation of molluscum lesions from HSV blisters or the lesions of shingles. In thin sections, poxvirus appears in the cytoplasm of cells as a large oval body with a dense coat (Fig. 9a–e); mature particles have a condensed core in the shape of a dumbbell (Fig. 9d,e). Negative staining shows a rough brick-shaped virion (Fig. 9f).

Treatment of molluscum contagiosum is by curettage or cryotherapy of lesions. There is no drug treatment presently available, though acyclic and carbocyclic adenosine analogues such as neoplanocin A have shown promise against poxvirus as well as other viruses (De Clercq, 1986).

*Coronavirus*

Coronaviruses have been seen associated with enteric infections (MacNaughton, 1982; Chany et al., 1982; Vaucher et al., 1982), but they have also been demonstrated in some apparently healthy individuals (Maass and Baumeister, 1983). Some strains have been associated with cold symptoms (McIntosh, 1974; Larson et al., 1980). Coronavirus particles have been demonstrated in stool samples from AIDS patients and in the serum of one patient with lymphadenopathy syndrome (LAS) and diarrhea. They were seen in only 3 of 20 healthy homosexual males, and were not seen in healthy heterosexuals (Kern et al., 1985). Although coronaviruses have been associated with disease in both normal individuals and AIDS patients, they do not appear to be a major contributor to morbidity and mortality in either group.

An ELISA has been described for detection of coronavirus (MacNaughton, 1982). In negative stains for EM, coronaviruses are membraned pleomorphic particles studded with 20-nm club-like spikes (Fig. 10c). The nucleocapsid is nondescript. In thin sections, virions can be seen within or budding into the cisternae of endoplasmic reticulum (Fig. 10a,b) (Becker et al., 1967).

No treatment has been described for coronavirus infection.

*Parvovirus*

Parvovirus or noncultivable picorna- or parvovirus-like (NCPP) particles have been seen in children with AIDS; detection was by hybridization and serology (Wainberg and Mills, 1985). However, no role in AIDS has been suggested for these agents (Anderson et al., 1985).

*Human T-lymphotropic virus type I*

Human T-lymphotropic virus type I (HTLV-I) is a lymphotropic retrovirus that causes malignant transformation, rather than depletion, of helper T cells. It is the etiologic agent of a form of T cell leukemia endemic in portions of Japan and the Caribbean, but not frequently encountered elsewhere (Catovsky et al., 1982; Haynes et al., 1983; Poiesz et al., 1980; Tajima et al., 1979). HTLV-I has been isolated from several AIDS patients (Getchell et al., 1987; Harper et al., 1986; Kanner et al., 1987; Palmer et al., 1983). Gallo suggests that the virus may advance the progression of AIDS (Edwards, 1987). The morphology of HTLV-I is described by Palmer and Goldsmith (pp. 3–15) and Shamoto et al. (1981).

*Viruses as etiologic agents in AIDS*

Since its initial description, human immunodeficiency virus (HIV) has been established as a necessary factor in the pathogenesis of AIDS (Gallo et al., 1986; Montagnier et al., 1984; Montagnier, 1986; Sarngadharan et al., 1985). The central etiologic role of HIV notwithstanding, numerous questions remain regarding other potential contributing factors in the development of the disease. Chief among these are the following:

1. Are additional external influences, particularly other viral infections, necessary prior or subsequent to exposure to HIV for the development of AIDS?
2. Do genetic factors, both in the virus and the host, influence susceptibility to AIDS?
3. Will all individuals infected with HIV subsequently develop overt AIDS, or do some eliminate the virus or maintain it in a perpetual equilibrium similar to that seen for the herpesviruses?

*Exogenous cofactors in the pathogenesis of AIDS*

In addition to HIV, numerous exogenous factors have been suggested to be necessary for, or at least conducive to, the development of AIDS. Several modes of action have been proposed for these cofactors, including induction of activated lymphocytes, the primary target cells of HIV infection (Montagnier et al., 1984); facilitation of HIV infection via immunosuppression; and reactivation of latent HIV infection. The evidence for cofactors in the etiology of AIDS is derived largely from epidemiologic and in vitro studies. Firm establishment of an etiologic role for any given factor has been hampered by the lack of a suitable animal model for AIDS; chim-

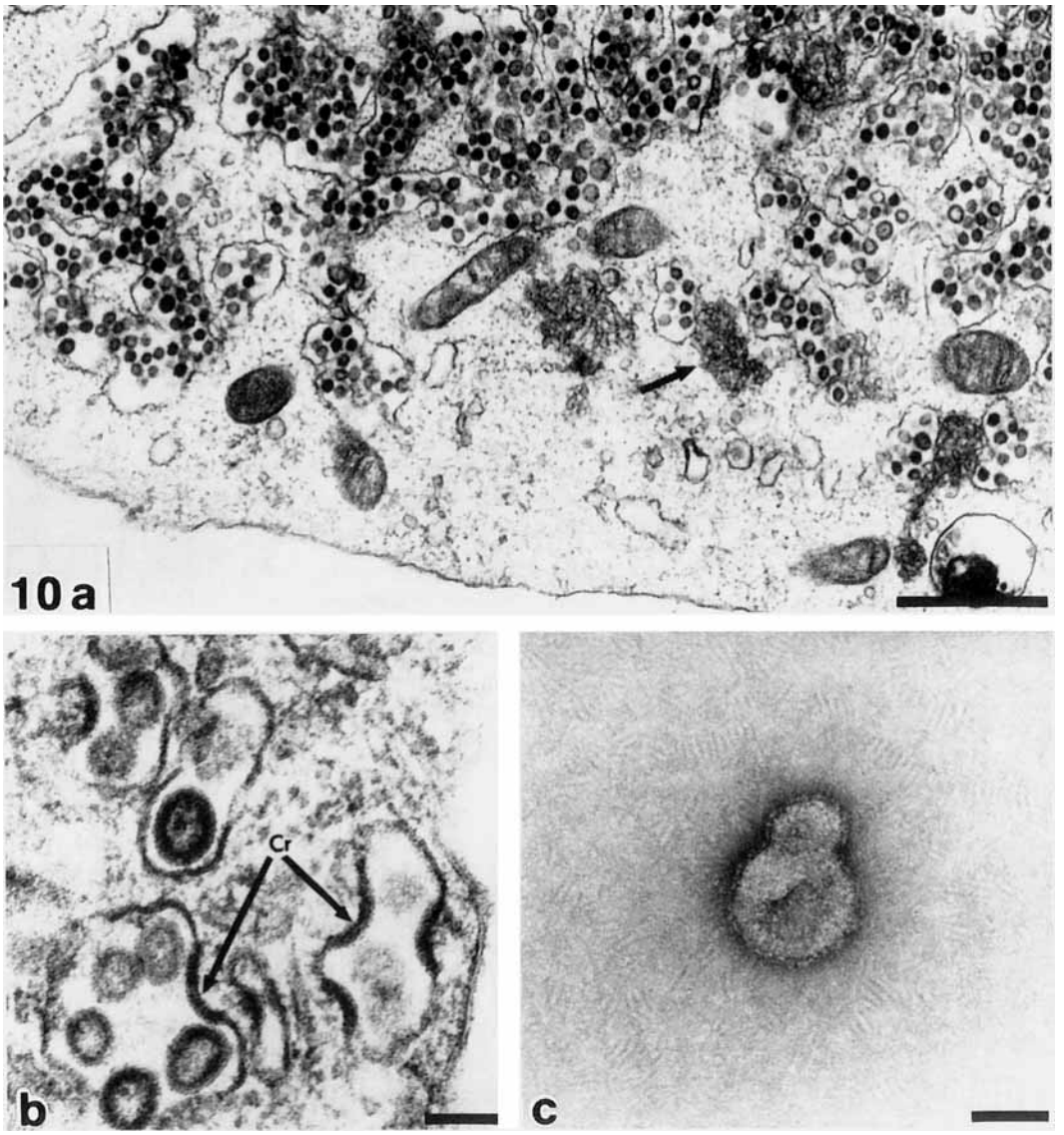



Fig. 10. **a:** Coronavirus particles within the cisternae of endoplasmic reticulum of a cell; dense material associated with coronavirus infection (arrow). **b:** Complete virions inside cytoplasmic vesicles and thickened membranes of particles budding into the vesicles (arrows). **c:** Negatively stained coronavirus-like agent from stool; 20-nm spikes surround the virion. Bar in a = 1$\mu$m. Bars in b and c = 100 nm. a and b are reprinted from Becker et al. (1967) with permission from the American Society for Microbiology Publications Department.

panzees can be infected with HIV and make antibodies to the virus, but they fail to develop subsequent immunodeficiency.

Numerous studies have implicated HBV as a cofactor in AIDS. Most patients with AIDS are serologically positive for HBV (Elfassi et al., 1984; Rustigi et al., 1984; Noonan et al., 1986), and the epidemiologies of AIDS and HBV infection are similar (Noonan et al., 1986). Newly diagnosed AIDS victims frequently possess high-titered antibodies to HBcAg and HBsAg, suggesting that HBV infection antedated the development of AIDS.

HBV is lymphotropic; HBV DNA has been demonstrated in peripheral blood cells and bone marrow from numerous patients (Ro-

met-Lemonne et al., 1983; Elfassi et al., 1984; Yoffe et al., 1986), including patients with AIDS (Laure et al., 1985; Lie-Injo et al., 1985; Noonan et al., 1986). Indeed, peripheral blood mononuclear cells from AIDS patients contain HBV DNA more frequently than those from hepatitis patients without AIDS, though it is not known whether this fact reflects a predisposition to AIDS in individuals infected with HBV or an increased susceptibility to HBV infection in individuals with AIDS.

Most homosexuals in high-risk groups for AIDS have been exposed to CMV, EBV, and HSV; these agents have likewise been proposed as possible AIDS cofactors. CMV infection can cause extended immunosuppression and is frequently associated with lowered helper/suppressor T cell ratios (Giraldo and Beth, 1986; Rapp, 1984; Schechter et al., 1985). In addition to a possible etiologic role in AIDS itself (Drew, 1986), CMV has been strongly implicated as a cofactor in Kaposi's sarcoma (see above).

EBV has been reported to decrease helper/suppressor T cell ratios (Schechter et al., 1985) as well as T cell cytotoxicity against EBV-infected target cells (Ragona et al., 1986). A role for EBV in AIDS-related complex (ARC) has been proposed (Ragona et al., 1986; Rinaldo et al., 1986; Schechter et al., 1985); symptoms of ARC, including anorexia, malaise, fatigue, and night sweats, frequently resolve in parallel with declining anti-EBV antibody titers (Gervais et al., 1983–1984). EBV is also a potent transforming agent, and has been implicated as a possible etiological factor in several malignancies (see above).

HSV, JCV and other DNA viruses have been shown to augment HIV gene expression in vitro (Gendelman et al., 1986; Martin et al., 1986; Mosca et al., 1987). The disparate nature of these viruses has led to speculation that their common inductive effects on HIV may be mediated indirectly via host cellular mechanisms.

In spite of these observations, the role of viruses other than HIV as cofactors in AIDS is by no means firmly established. The epidemiologic evidence, in particular, is complex and occasionally contradictory. CMV and HSV, though commonly encountered in patients with sexually acquired AIDS, are less prevalent in hemophiliacs and children who contract the disease by hematogenous exposure to HIV and are uncommon in tropical areas where AIDS is nonetheless endemic (Blaser and Cohn, 1986). Proponents of viral cofactor theories counter that many hemophiliacs have serologic evidence of exposure to herpesviruses and HBV (Landay et al., 1983; Sullivan et al., 1986); that in others, factor VIII injections may have immunosuppressive properties which provide an alternative predisposition to HIV infection (Verani et al., 1986); and that HIV-seropositive hemophiliacs, including some with high anti-HIV antibody titers (Volsky et al., 1986), may indeed have a lower frequency of overt AIDS than seropositive homosexual men (Sullivan et al., 1986). Likewise, many children with AIDS, including infants, have evidence of EBV infection (Martin et al., 1986; Rubinstein, 1983; Scott et al., 1984); and the immunological immaturity of neonates could conceivably provide an alternative to viral cofactors in the facilitation of HIV infection (Rubinstein, 1983). The inability of the immune system in childhood to mount a full response to HIV infection is illustrated by the observation that some asymptomatic children who harbor HIV lack serologically detectable anti-HIV antibodies (Borkowsky et al., 1987; Pahwa et al., 1986), and that the seropositive infants with ARC lose antibody response to HIV by age 10 months (Mok et al., 1987).

Other exogenous factors initially suggested as cofactors in AIDS, such as illicit drug use, increased number of male sexual partners, and frequent receptive anal intercourse, do not seem to have a direct role in the pathogenesis of the disease. These factors appear to operate by increasing the probability of exposure to HIV (Schechter et al., 1985).

### *Genetic factors in the pathogenesis of AIDS*

Development of overt AIDS may be a function of genetics—both of the infecting HIV strain and of the human host. Several AIDS-causing viruses with considerable genetic and virulence variation have been isolated (Barin et al., 1985; Coffin, 1986; Edwards, 1987; Ratner et al., 1985; Shaw, 1984). A relationship between clinical severity of HIV infection and in vitro replication potential of the virus has been demonstrated (Åsjö et al., 1986). Different types of HIV varying with respect to growth rate and severity of cytopathological effect (CPE) have been reported (Rübsamen-Waigmann et al., 1986). Two HIV variants with different DNA restriction maps

were seen in one patient, suggesting either antigenic variation or superinfection by one of the two strains. Sodroski et al. (1986) suggest a mechanism for regulation of latent and lytic phases of the virus life cycle. See Gonda (pp. 17–40) for a review of HIV genetics and the contribution of EM to that knowledge.

Regarding host genetic susceptibility, one study suggests that some individuals have lymphocytes that can suppress HIV activity (Walker et al., 1986). Genetic studies of AIDS patients with KS have shown an increase in histocompatibility antigen DR5 and a decrease in DR3 (Giraldo et al., 1984). It has also been speculated that HIV, which adheres to T lymphocytes via the CD4 molecule, induces the production of antibodies that crossreact with the endogenous CD4 ligand, thus interfering with lymphocyte communications necessary for normal immune responses (Stricker et al., 1987; Ziegler et al., 1986).

*HIV infection and AIDS*

As of yet, it is unclear whether HIV infection is occasionally compatible with survival for a normal life span or invariably leads to premature demise. The factors that delimit HIV-positive asymptomatic individuals from victims of overt AIDS have not been clearly identified (Valle et al., 1985), though the wide variation in health status of HIV-seropositive individuals suggests that some may be capable of existing in equlibrium with the agent. It is estimated that approximately 30% of individuals infected with HIV develop AIDS within 5 years. Statistics are unavailable for the development of AIDS following longer latencies; estimates range as high as 70% or greater (M. Gonda, personal communication).

In addition to its etiologic role in immunodeficiency, HIV is capable of directly infecting the CNS. Neurological HIV disease may precede immunodeficiency and is estimated to occur in 30–90% of AIDS victims (Berger, 1987; De la Monte et al., 1987; Purtilo et al., 1985). Initial manifestations include acute or chronic meningitis, encephalitis, dementia, myelopathy, and peripheral neuropathy.

Encephalopathy is the most common HIV-associated neurologic disorder. Early symptoms are subtle and include mild progressive memory loss, generalized weakness with motor disturbances, and occasionally, seizures (Berger, 1987). The presentation of HIV encephalopathy is frequently similar to those of opportunistic encephalitides, such as HSV and CMV encephalitis, PML, and CNS toxoplasmosis. Since some of these disorders are potentially treatable, every effort should be made to establish a definitive diagnosis. MRI is the noninvasive study of choice, and CSF can be examined for virus or antiviral antibodies.

Alternative presentations of HIV CNS disease are seen less frequently. HIV aseptic meningitis is occasionally encountered, sometimes in association with a mononucleosis-like syndrome. Peripheral neuropathies may also occur; their manifestations include a motor disturbance resembling Guillain-Barré syndrome, pain, weakness, and autonomic neuropathy. The CSF is generally normal in HIV peripheral neuropathy. An associated myopathy of uncertain etiology, with weakness and electromyographic abnormalities, is occasionally seen (Berger, 1987).

The current Centers for Disease Control surveillance case definition of AIDS relies partially, though by no means exclusively, on the presence of laboratory evidence for HIV infection (Centers for Disease Control, 1987). A number of laboratory procedures for the detection of HIV have proven useful in the diagnosis of AIDS. However, a number of additional laboratory diagnostic procedures may be used to confirm the presence of AIDS and to detect HIV-associated CNS infection.

Many tests are available to detect antibody to HIV. Several ELISAs are commercially available and are relatively simple to perform; sensitivity is 97% or better, and specificity ranges from 70% to 100% (Gurtler et al., 1987; Sarngadharan et al., 1984; Weiss et al., 1985). Western blot analysis is generally used for confirmation of the presence of anti-HIV antibodies, though it is more labor intensive than routine serological tests (Towbin et al., 1979). Western blot tests generally correlate with the presence of virus, and antibody titers correlate with the progression of AIDS (Casareale et al., 1984–1985). However, the value of serological testing for HIV is decreased somewhat by the fact that some virus-positive individuals are antibody negative and that many presently healthy individuals are known to have anti-HIV antibodies (Groopman et al., 1985; Salahuddin et al., 1984).

Biochemical assays can detect the presence of HIV by identifying a specific viral enzyme, reverse transcriptase (RT), not seen in unin-

fected cells (Poiesz et al., 1980); assays for proviral DNA are also available (Gelmann et al., 1983). Other detection systems for viral components include dot-blot hybridization, antigen capture, and radioimmunoassay (Monroe et al., 1987; Gupta et al., 1987b). The virus itself has been isolated from peripheral blood cells after activation or cocultivation with susceptible cells (Gallo et al., 1984; Levy et al., 1984; Popovic et al., 1984) and has been isolated from CSF (Ho et al., 1985).

LM of HIV-infected brain tissue shows multinucleated giant cells; foamy macrophages, necrosis, perivascular inflammation, and demyelination may also be present (Berger, 1987; De la Monte et al., 1987). Immunoperoxidase staining shows viral antigens in many different cell types (Ward et al., 1987). LM can also be used to demonstrate CPE of HIV on leukocyte cultures (Rübsamen-Waigmann et al., 1986).

HIV is not seen routinely by EM in lymphocytes from patients with AIDS unless they are first activated or cocultivated with susceptible tissue culture cells (Gallo et al., 1984) or stimulated with growth factors (Markham et al., 1985; Montagnier et al., 1984). The virus has been seen directly by EM in brain biopsies in oligodendroglia, astroglia, macrophages, and macrophage-like multinucleated cells, as well as extracellular spaces, capillary lumens, and endothelial gaps (Epstein et al., 1984–1985; Gyorkey et al., 1987; Koenig et al., 1986; Wiley et al., 1986); and it has been seen in germinal centers of lymph nodes (Hammar et al., 1984; Warner et al., 1986). EM techniques that have been used to detect HIV include examination of thin sections of infected tissue, scanning electron microscopy of infected cells, and negative staining of purified virus (Tsuchie et al., 1986), as well as immunogold labeling of HIV-infected cell cultures (Pekovic et al., 1986). Electron microscopic morphology of the virus is discussed in detail by Palmer and Goldsmith (pp. 3–15) and Schüpbach et al. (1984).

Treatment of AIDS has been attempted with a number of antiviral agents. Indeed, the AIDS epidemic has done much to spur the rapid development of drug therapies for both AIDS itself and its opportunistic complications. Several drugs have been shown to have in vitro activity against HIV, but their clinical efficacy has been variable.

Azidothymidine (AZT), which inhibits HIV replication and CPE, is the only drug currently on the market for AIDS. The molecular mechanism of action of AZT is described by Yarchoan and Broder (1986). Side effects are headache, neutropenia, and anemia. Acyclovir, an antiherpetic agent, can potentiate the antiretroviral action of AZT, but the mechanism of potentiation is unknown (Yarchoan and Broder, 1986).

Suramin (Germanin), in human use already for trypanosomiasis and onchocerciasis, inhibits HIV in vitro. However, its efficacy in vivo is equivocal, and its use is associated with a high incidence of adrenal insufficiency, rash, fever, and liver and other toxicities (FDA, 1985; Tuazon and Labriola, 1987).

Ribavirin has been used successfully in the treatment of some RNA virus infections, including influenza A, and shows in vitro activity against HIV. In vivo, it decreases viral RT activity in lymphocytes, but does not eliminate the virus. Reversible side effects include anemia, nausea, headache, and mental status changes. Humal trials are continuing (FDA, 1985; Tuazon and Labriola, 1987).

Antimoniotungstate (HPA-23) is active in vivo in animals against a broad range of RNA and DNA viruses and inhibits the RT of HIV. Initial studies showed virus elimination in some patients with a reappearance of viremia after discontinuation of the drug. Other patients had no clinical improvement. Thrombocytopenia, liver dysfunction, and fever are side effects. Further clinical trials are in progress (FDA, 1985; Tuazon and Labriola, 1987).

Phosphonoformate inhibits the DNA polymerases of human herpesviruses and has been used topically in the treatment of CMV retinitis and herpes mucocutaneous lesions. It also inhibits the RT of retroviruses. Clinical trials in AIDS and ARC patients are underway in Denmark and Sweden. The main side effect of phosphoformate is kidney toxicity (FDA, 1985; Tuazon and Labriola, 1987).

DHPG, effective against CMV, also has some in vitro activity against HIV and is in clinical trials. Ansamycin and several other drugs that have in vitro activity against HIV are being prepared for clinical trials (FDA, 1985; De Clercq, 1986).

Other avenues of potential therapeutic strategy include passive treatment with immune globulin; reconstitution of the immune system by bone marrow transplantation or lymphocyte transfusion; and enhancement of the immune system with immunomodulators, including endogenous substances such

as α and γ interferon, interleukin-2, thymosin, and transfer factor, and exogenous agents such as ampligen, inosine pranobex (isoprinosine), and imuthiol (Bolognesi and Fishinger, 1985; Carter, et al., 1987; Lane et al., 1985; Sheridan et al., 1984; FDA, 1985; Hopkins, 1985; Pompidou et al., 1985; Tuazon and Labriola, 1987). Reconstitution has thus far been unsuccessful; before this treatment will work, measures must be developed to prevent the transplanted cells from becoming infected with HIV. Successful therapy of AIDS may eventually rely upon a combination of immunomodulatory and antiviral approaches.

### *Virus-like structures described in AIDS patients*

Prior to the identification of HIV, ultrastructural research on the etiology of AIDS yielded a number of candidates for the causative agent. The identity of many of these particles has since been elucidated. Some are actually virions present as opportunistic infections; others are either subcellular organelles or artifacts; still others are viral-induced but nonspecific changes.

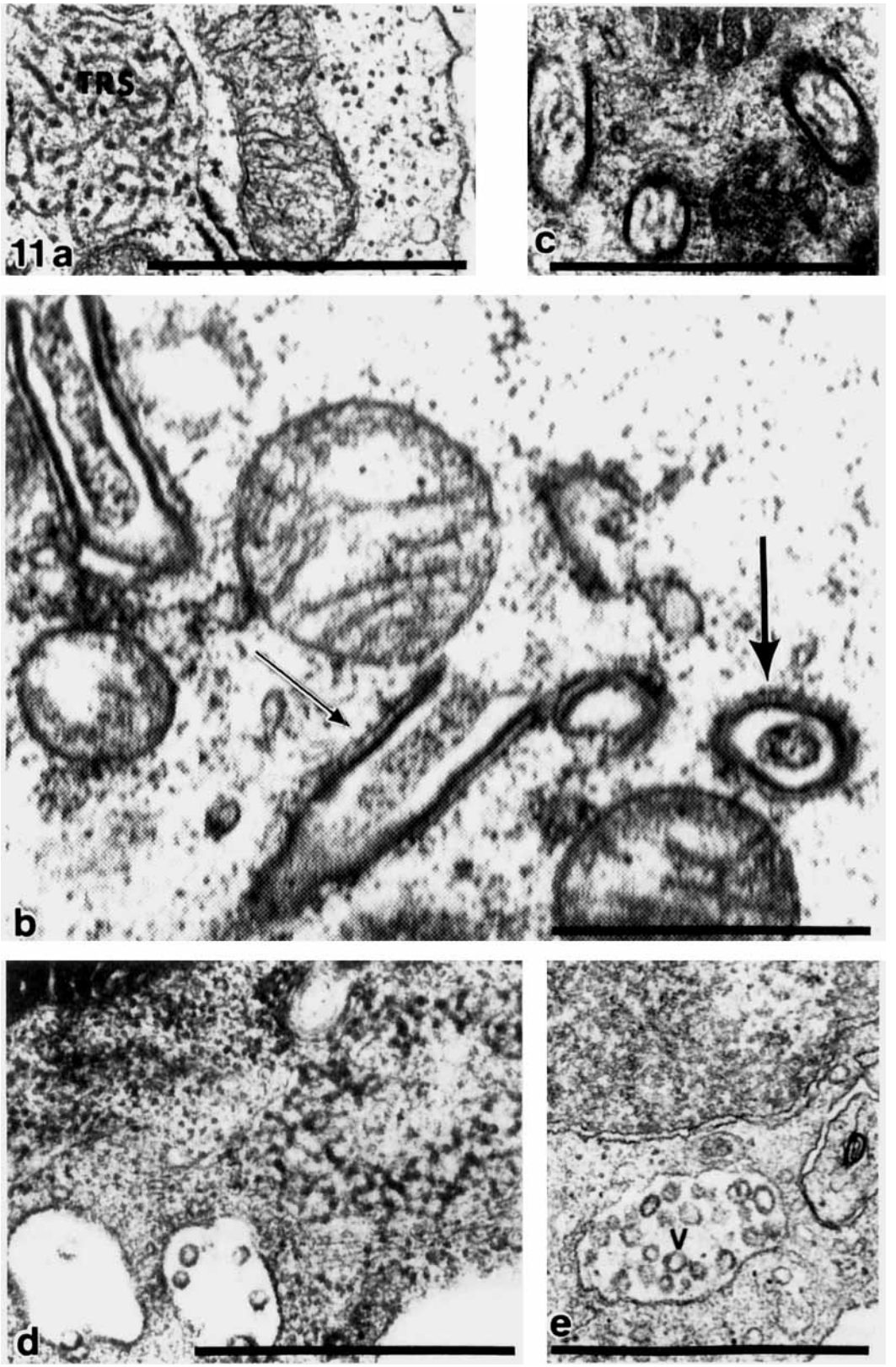
The most frequently reported observation has been of tubuloreticular structures (TRS) or tubulorecticular inclusions (TRI) see Tucker, pp. 137–158), seen mainly in lymphocytes (Feremans et al., 1983; Orenstein, 1983; Schaff et al., 1983; Schenk et al., 1986; Sidhu et al., 1983; Thune et al., 1983; Watanabe et al., 1984; Zucker-Franklin, 1983), but also in the choroid of the eye (Jensen, 1985), endothelial cells in Kaposi's sarcoma, and fibroblasts (Zucker-Franklin, 1985). These "wormlike" inclusions resemble nucleocapsids of myxoviruses, though TRS are slightly larger in diameter (25 nm vs. 9–18 nm) (Fig. 11a,c,d). Similar structures have been described in the kidneys of patients with systemic lupus erythematosus (SLE), and have been seen in other diseases and in drug-treated cells (Sinkovics et al., 1969; Grimley et al., 1973; Grimley and Schaff, 1976; Schaff et al., 1982). Larger rod-like structures measuring 65 × 143 nm have been described in a lymphoid line from an infant with AIDS (Burrage et al., 1984). TRS contain lipid and acidic glycoprotein, but not nucleic acid (Sidhu and Waisman, 1985).

The factors that predispose to the development of TRS are obscure. Antibodies against HTLV-I and (perhaps crossreacting) antibodies against HTLV-III have been identified in individuals with SLE (Olsen et al., 1987), raising the possibility of retroviral infection in such patients and providing a plausible explanation for ultrastructural similarities between AIDS and SLE. TRS have also been related to interferon exposure (Rich, 1981).

Membranous structures designated as test tube and ring-shaped forms (TRF), cylindrical confronting cisternae (CCC), or cylindrical confronting lamellae (CCL) have been seen in many AIDS patients (Fig. 11b,c) (see Tucker, pp. 137–158). They appear to be concentric arrangements of endoplasmic reticulum with a layer of electron-dense material interposed between them; they may be up to 3.5 μm long and 250 nm in diameter (Kostianovsky et al., 1987; Sidhu et al., 1983). Similar inclusions have been seen in other diseases, such as adult T cell leukemia and other neoplasms, multiple sclerosis, and SLE, as well as virus-infected cells (Shamoto et al., 1981; Prineas, 1978; Hammar et al., 1984). TRF can be differentiated from the simple paired cisternae seen in stimulated cells (Kostianovsky et al., 1987). Continuity between TRS and TRF has been seen (Fig. 11c), and both are probably the result of membrane proliferation, perhaps caused by viral infection or other stimulation (Feremans et al., 1983; Hammar et al., 1984; Kostianovsky et al., 1984; Schaff et al., 1983).

Another membranous structure composed of 70-nm vesicles within a larger membrane has been described in lymphocytes of AIDS patients (Fig. 11e) (Feremans et al., 1983). Gardiner et al. (1983) have suggested that these organelles represent multivesicular bodies (MVB) similar to those seen in normal (Brown et al., 1983; Ghadially, 1982) and activated (Grossi et al., 1983) lymphocytes. However, in a later publication, Feremans et al. (1984) described the immunolabeling of large cytoplasmic vacuoles resembling MVB in 1% of mononuclear cells from an AIDS patient; the antibodies used were against HTLV-I and bovine leukemia virus followed by secondary antibodies conjugated to colloidal gold. The authors speculated that the labeled material consisted of HIV antigens that cross-reacted with antibodies against core proteins of related viruses.

Vesicular rosettes have been described in lymphocytes of AIDS patients. These bulbous membrane structures are 300–500 nm in diameter and contain 30- to 60-nm vesicles (Fig. 11f) (Ewing et al., 1983). They have also been seen in other situations (Miller and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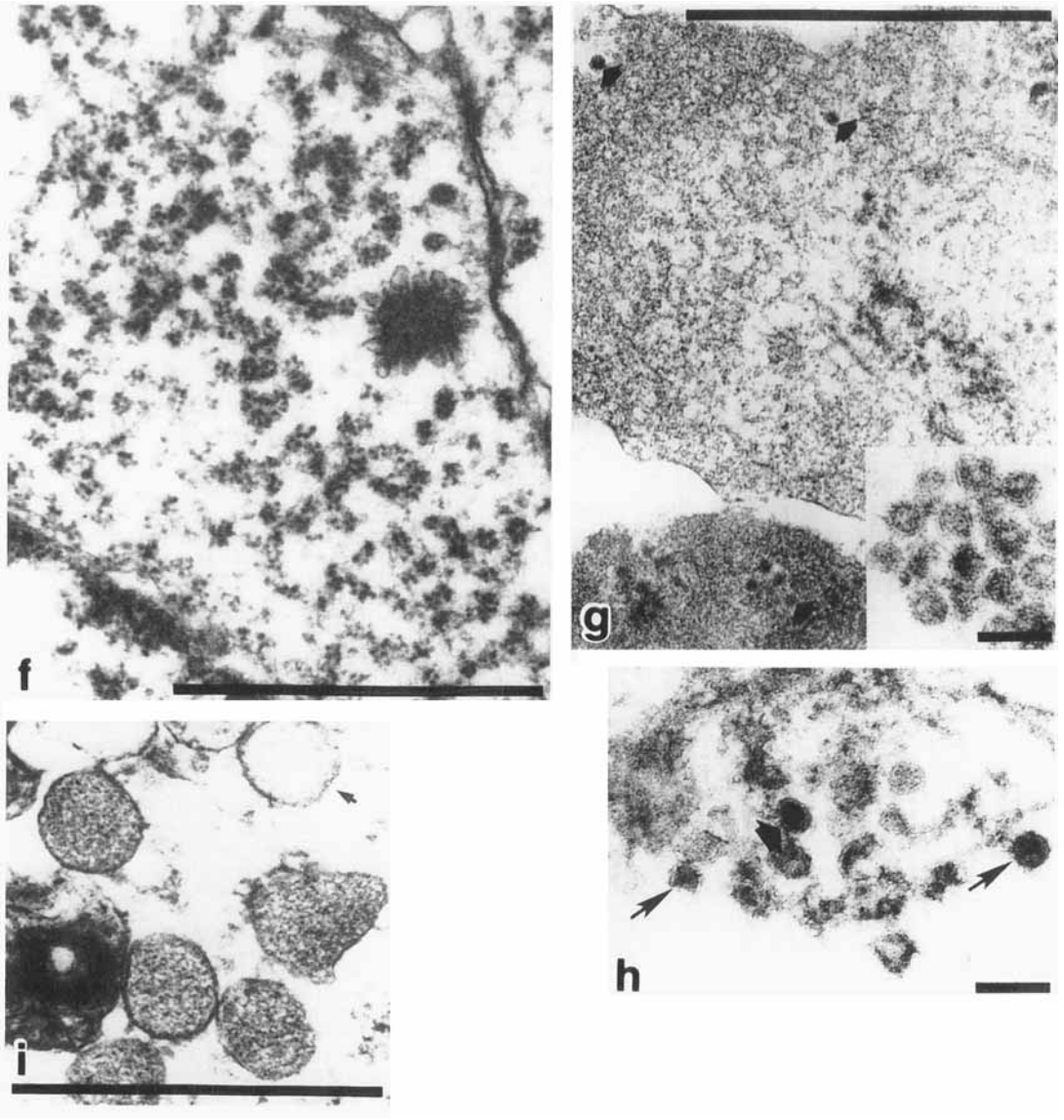


Fig. 11. Virus-like structures and unusual ultrastructure reported in AIDS patients. **a:** TRS 20 nm in diameter **b:**TRF composed of rough endoplasmic reticulum enclosing dense material, cut in longitudinal (small arrow) and cross (large arrow) section. **c:**TRF with TRS in the lumens. **d:** TRS together with vacuoles resembling MVB. **e:**"Virus-like" structures (V) inside a cytoplasmic vesicle. This structure resembles MVB and the vesicle in d. **f:** Vesicular rosette in the cytoplasm composed of smaller vesicles. **g:** Virus-like particles in phagocytosed debris within a histiocyte. **h:** Intracytoplasmic particles in an epithelial cell from the same patient as that in g. **i:** Extracellular "virus-like" vesicles from tissue culture cells cocultivated with AIDS material. All bars = 1 $\mu$m except the inset in g and h, which = 100 nm. a and c are reprinted from Sidhu et al. (1983), with permission from *The Lancet*; b and d are from Orenstein (1983), with permission from *The Lancet*; e is from Feremans et al. (1983), with permission from *The Lancet*; f is from Ewing et al. (1983), with permission from *The New England Journal of Medicine*; g and h are from Chandler et al. (1984), with permission from the *Annals of Internal Medicine*; i is from Lo (1986), with permission from the *American Journal of Tropical Medicine.*

Rogers, 1984), and their distinction from multivesicular bodies or lysozomes has been questioned (Zucker-Franklin, 1983).

Unidentified particles of 45–55 nm with an electron-dense core have been reported in intestinal epithelial cells of AIDS patients (Fig. 11g,h). They were seen predominantly within membrane-bound cytoplasmic vacuoles and in phagocytosed debris, but occasionally were observed extracellularly. The authors speculated that they differed from previously reported breakdown products (Chandler et al., 1984).

Virus-like particles 140–280 nm in diameter enclosing 8- to 9-nm diameter tubular structures resembling nucleocapsids (Fig. 11i) have been seen both intra- and extracytoplasmically in a mouse cell line transfected with DNA from a patient with AIDS, but not in nontransfected cells (Lo, 1986). These structures do not resemble known viruses, and in the single published micrograph, it is difficult to distinguish them from cell processes.

## CONCLUSIONS

The importance of an understanding of viral processes in patients with AIDS cannot be overemphasized. For the present time, opportunistic viral infections account for a large portion of the morbidity and mortality experienced by such patients. The tempo of viral infection in individuals with AIDS is often swift, and demands rapid and accurate diagnostic approaches. Therapy for viral infections in AIDS patients, though currently far from satisfactory, is of some benefit, and is the focus of intense investigation.

In the future, elucidation of the pathogenesis of AIDS and the ultimate conquest of the disease will rest at least in part on an understanding of viral mechanisms. It is anticipated that the investigative modalities described above will play a major role in this understanding.

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