# Diagnostic Electron Microscopy

## Volume 4

*Edited by*

Benjamin F. Trump, M.D.

Raymond T. Jones, Ph.D.

# Diagnostic Electron Microscopy

## Volume 4

*Edited by*

Benjamin F. Trump, M.D.
Professor and Chairman

Raymond T. Jones, Ph.D.
Associate Professor

Department of Pathology
University of Maryland School of Medicine
Baltimore, Maryland

A Wiley Medical Publication
JOHN WILEY & SONS
New York · Chichester · Brisbane · Toronto · Singapore

*Cover design: Wanda Lubelska*



***Library of Congress Cataloging in Publication Data (Revised):***

Main entry under title:

Diagnostic electron microscopy.

(A Wiley medical publication)
Includes index.
1. Diagnosis, Electron microscopic. I. Trump, Benjamin F. II. Jones, Raymond T., [DNLM: 1. Microscopy, Electron. 2. Diagnosis, Laboratory —Instrumentation. QH212.E4 D536]

RB43.5.D53 616.07'58 77-12817
ISBN 0-471-89195-9 (v. 1)
ISBN 0-471-89196-7 (v. 2)
ISBN 0-471-05150-0 (v. 3)
ISBN 0-471-05149-7 (v. 4)
ISSN 0272-3859

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

# Preface

In this final volume of the series, we cover the remaining areas of current importance in the field of diagnostic electron microscopy.

The first chapter, by Dr. Grimley and Dr. Henson, considers the application of electron microscopy to the diagnosis of virus infections. Major advances are being made in this area, especially in the area of rapid diagnosis. This subject is categorized in some detail in order to acquaint the reader with unfamiliar complexities of virus replication, morphogenesis, and classification. Also described are several new techniques for virus identification, as well as the differential diagnosis of virus infection.

Chapter 2, by Dr. Regezi, Dr. McClatchey, and Dr. Batsakis, covers the important subject of diagnosis of head and neck tumors. Many previously difficult diagnostic problems in this area can now be resolved, primarily round cell tumors, eosinophilic cell tumors, tumors composed of spindle or fusiform cells, and clear cell tumors. The ultrastructural features that permit differential diagnosis within these four major groups are discussed in great detail.

The diagnostic electron microscopy of bone lesions is considered in Chapter 3. The use of electron microscopy in this field has greatly improved the accuracy and precision of diagnosis; nevertheless, it is pointed out that perhaps more than in most other organ systems, a correlation must be made with clinical radiologic growths and light microscopic studies. In addition to reviewing the diagnostic criteria in those lesions in which electron microscopy is most helpful, Dr. Alexander reviews several special techniques that are applicable in this field, as well as in other areas of diagnostic electron microscopy.

In Chapter 4, Dr. Beals introduces a relatively new field of diagnostic electron microscopy, namely, its use in cytopathology. As in many other areas, the use of electron microscopy can substantially aid the cytopathologist in establishing a diagnosis, not only in defining the phenotype of a tumor, but also in diagnosing nonneoplastic conditions, such as the presence of microorganisms and various unknown objects. Electron microscopy applied to cytopathology requires special techniques that are considered in detail, both for transmission and scanning electron microscopy. Because of the wide variety of techniques used by the clinician to obtain cytopathologic specimens, a variety of special techniques are required for the preparation of specimens. It is probably in the area of cytopathology that application of scanning electron microscopy has found its greatest value.

In Chapter 5, Dr. Kim considers the use of electron microscopy in the identification of calculi. The use of scanning electron microscopy and microanalysis has revolutionized diagnosis of calculi. Because of their unsuitability for most light microscopic methods, the diagnosis of stones being analyzed by

chemical or crystallographic methods has been largely neglected by pathologists. Commonly used qualitative chemical reactions are insensitive for several elements, and, although x-ray diffraction is the reference method commonly used, the method is not without limitations arising from overlap of powder patterns, inability to detect amorphous or noncrystal in components, and the fact that it does not permit valuation of discrete parts of the stone of submicroscopic size. Although this discussion relates primarily to urinary stones, it develops methods including transmission electron microscopy, scanning electron microscopy, microanalysis, and selected area electron diffraction. Also described are a number of special techniques used in specimen preparation.

The use of electron microscopy in the diagnosis of neoplastic and nonneoplastic diseases of the breast is discussed in Chapter 6 by Dr. McCarty and Dr. Paull. The normal and hormone-dependent variations in morphology are reviewed, and a series of neoplastic and nonneoplastic diseases are considered.

Chapter 7 discusses the ultrastructural pathology of the heart. This field has expanded very rapidly during recent years, especially with regard to the development of techniques for safely obtaining myocardial biopsies, hence the rapid development of knowledge in this field. Dr. Ferrans and Dr. Butany consider special methods needed to minimize artifacts in myocardial biopsies and overall methods used for evaluation. Many previously obscure features in the myocardium can now be diagnosed with relative ease. The chapter is well documented with numerous references.

In Chapter 8, Dr. Shelburne and co-workers review the ultrastructural characteristics of nonneoplastic diseases of the lung—another subject neglected until recent years. With the advent of methods for studying both biopsy and autopsy specimens, the pathologist is now able to approach the diagnoses and characterization of previously obscure conditions. This area has shown increasing application of new types of electron microscopy, such as electron microanalysis.

BENJAMIN F. TRUMP, M.D.
RAYMOND T. JONES, PH.D.

*Baltimore, Maryland*

# Contents

# 1
# Electron Microscopy in Virus Infections*

*Philip M. Grimley, M.D.*
*Professor*
*Department of Pathology*
*Uniformed Services University of the Health Sciences,*
*School of Medicine*
*Bethesda, Maryland*

*Donald E. Henson, M.D.*
*Senior Scientist*
*Laboratory of Pathology*
*National Cancer Institute*
*Bethesda, Maryland*

Ultrastructural examination of tissues, innoculated cell cultures, or body fluids and excretions offers direct and accurate means for the diagnosis of many human virus infections. The rapidity of direct electron microscopy (EM) is a major advantage for clinical pathologists, virologists, and epidemiologists (1–8). Surprisingly, considering the public health magnitude of virus infections and the broad spectrum of related disease processes, many opportunities for application of diagnostic electron microscopy have not been fully realized. There are practical reasons for this, including the initial capital expenses and technical training requirements (6). In addition, prospective clinical investigators might be intimidated by unfamiliar complexities of virus replication, morphogenesis, and taxonomy. Much confusion has been engendered by normal or pathologic cell components that resemble viruses in size, shape, or location (9–11).

Major aims of this chapter are to categorize key events in the morphogenesis of known or suspected virus pathogens and to explore general problems in identifying specific virions or viral inclusions. A number of excellent atlases, reviews, and medical texts offer background reference material and illustrations (9,10,12–20).

* The opinions or assertions contained herein are private views of the authors and are not official. They do not necessarily reflect views of either the Uniformed Services University of the Health Sciences or the Department of Defense.

## SPECTRUM OF VIRAL DISEASE IN HUMANS

Viruses have been implicated in an extensive spectrum of human diseases (20). Several hundred distinct species are potentially infectious. Fortunately, most viruses produce subclinical infections, mitigating the consequences of disease. Many serious forms of virus disease have been controlled by public health measures; nevertheless, such agents as the respiratory syncytial myxoviruses remain a leading cause of morbidity and mortality in infants and young children. Adverse effects of virus infection on the fetus, including congenital damage induced by cytomegalovirus (CMV), rubella, or varicella have not been eradicated (16,21). Outbreaks of fatal influenza continue to afflict susceptible adult populations, and new pathogens, such as the Lassa fever arenavirus, threaten to disseminate with expanded international travel (22).

Paradoxically, general improvements in the quality of medical care also set the stage for opportunistic viral infections, particularly herpesviruses. These infections can involve transplant recipients, other immunosuppressed patients, and burn or cancer victims (23–28). Cytomegalovirus is an especially ubiquitous agent that tends to manifest itself during the perinatal period (21); it can be reactivated after a debilitating illness or by immunosuppressant drugs (25). Blood-transmitted viral disease remains a diagnostic and therapeutic problem despite recognition of the hepatitis B antigens (29).

Discovery of viruses and recognition of their pathogenetic capacity at the turn of the century focused attention on acute illnesses with toxic clinical manifestations, such as smallpox, rabies, and yellow fever. In such infections, the virus is cytocidal, and the ultimate stage of its life cycle involves cell necrosis with release of virions. Virions are biologically discrete, environmentally resistant particles that serve to transmit infectious units of viral genetic material from cell to cell or from host to host. Repeated cycles of virus growth and maturation thus conform to the classic Koch's postulates; that is, infectious agents can be recovered between cycles and shown to reinfect. This process is known as productive infection.

During the past two decades, experimental and clinical evidence has broadened the concept of viral infection beyond the traditional framework. Viral genetic systems are known to employ complex strategies for replication and propagation of their genetic material (18,19). These include subtle mechanisms through which genetic material of some viruses can associate or integrate with genetic material of the host (30). Formation of virions is suppressed or deferred, making the infections nonproductive and persistent (30–32). Replication and transmission of viral genetic information to daughter cells occurs during host cell division without external release of virions (see *Detection of Occult Viral Infections*).

## CLINICAL EXPRESSIONS OF VIRAL INFECTION

The clinical expression of viral infection represents the outcome both of natural tropism and physical dissemination (16,34), often modulated by host immune responses (35,36). Clinical manifestations can show a pattern of tropism for

one or more organ systems, which can characterize a particular virus genus or even a discrete species, such as herpes simplex, varicella, measles, or mumps. Infections such as hepatitis, diabetogenic pancreatitis, and poliomyelitis exhibit a high degree of tissue tropism (29,37,38), which can reflect tissue distribution of virus receptors (38). In infectious mononucleosis, the Epstein-Barr herpesvirus selectively infects the subpopulation of B lymphocytes. Immune response of the T lymphocytes creates the characteristic hematologic picture (36). Immunologic mechanisms also define the histopathogenesis of persistent hepatitis and lymphocytic choriomeningitis (14,20).

The progression of clinical manifestations, organ tropism, and routes of dissemination must be intelligently considered in selecting tissues for morphologic detection of virus pathogens (20). In measles, for example, Koplik's spots of the oral mucosa herald the exanthem following an initial phase of respiratory tract involvement (39). Viral disease of the nervous system can involve a long period of latency (16). Herpesviruses remain latent in paraspinal ganglia for decades (40). Several diseases of the brain are characterized as slow infections with insidious onset or lethargic progression. These infections can be produced by "unconventional" virus agents or can result from immunologic impairments in the presence of virus infections (10,16). Search for pathogens in such circumstances requires intensive efforts with combined morphologic, tissue culture, and immunodiagnostic techniques.

## LABORATORY DIAGNOSIS OF VIRAL INFECTIONS

The role of EM in viral diagnosis is best appreciated in the perspective of other clinical laboratory methods (41). Three direct and rapid approaches are (*a*) to detect virions, (*b*) to observe viral morphogenesis, or (*c*) to identify virion antigens. Additional approaches, essential for the diagnosis of nonproductive or persistent infections, are to locate nonstructural viral gene products or to detect viral genes themselves (see *Detection of Occult Viral Infections*). Selection of techniques will be dictated by the clinical setting, the differential diagnosis, and the urgency of diagnosis.

### Viral Antigens

Detection of viral antigens, circulating or in tissues, is an expanding frontier of virologic diagnosis (42,43). This is particularly important in cases of restricted expression of viral genes or when infected tissues are not directly accessible to biopsy. In recent years, for example, reliance has been placed on serologic detection of circulating virus antigens for laboratory diagnosis of hepatitis B (29) and applications of the enzyme-linked immunosorbent assay (ELISA) are increasing (42). Many viruses produce distinct cytologic alterations recognizable under the light microscope (see below), but sensitivity and specificity of cytohistologic observations can be improved by introduction of immunoperoxidase or immunofluorescent techniques for identification of discrete viral antigens (29,42–46), as well as by EM.

## Virus Culture

Biologic amplification of virus numbers can be time consuming (42) but improves the statistical probability for detection and identification when levels of virus production are low. This is most conveniently accomplished in tissue culture: A range of mammalian or arthropod cells with susceptibility to various virus families is well known and appropriate cell lines are readily available from central resources, such as the American Type Culture Collection. Innoculation of embryonated eggs, suckling mice, or other laboratory animals also remains useful for specific virus families (41).

The growth of virus in vitro is often recognized by the development of specific lesions or cytopathic effects (CPE), which can be confirmed by EM. In the absence of characteristic CPE, EM is often essential (3,8), although many immunodiagnostic techniques are now becoming available (42,43). After biologic isolation and ultrastructural identification of a virus family, additional studies such as neutralization of infectivity with specific antisera permit generic or strain identification (41,46).

In some cases direct growth of infected tissue explants can facilitate discovery of latent agents such as herpesviruses (40,47), but this is not a rapid diagnostic approach. Other latent viruses are revealed by cocultivation (see *Detection of Occult Viral Infections*).

## Serology

Once viruses produce cell-associated or structural proteins during infection, the host can be stimulated to produce virion or nonvirion antibodies. A fourfold or greater rise of specific antibodies in conjunction with appropriate physical findings is commonly accepted clinical evidence of specific infection, but such information often arrives too late to influence therapeutic decisions during the acute phase of illness (42). Antibody levels can be measured by a variety of standard immunologic techniques, such as complement fixation, neutralization, hemagglutination inhibition, or radioimmunoassay, (RIA) (41,42). Tests of cell-mediated immunity can be useful in evaluating previous exposure to a virus (48).

## Role of Electron Microscopy

It is now almost impossible to imagine the era before 1940, when the essential morphologic forms of viruses remained shrouded in mystery, and they were known only by physical size, growth characteristics, or biologic manifestations. A major excitement during the early years of biologic EM was the new ability to resolve individual virus particles, to classify them, and to penetrate the secrets of their life cycles (49,50).

Current knowledge of the viral disease spectrum represents a fruition of ultrastructural and conceptual advances made during the past three decades. Electron microscopy and improved tissue culture techniques have been crucial catalysts. Table 1 summarizes the most significant fundamental functions of EM in virology with some typical examples. Potential diagnostic applications will be exposed more fully as this chapter unfolds.

**Table 1.** Major Functions of Electron Microscopy in Virology

| |
|---|
| Resolution of purified virions or subunits (49–54) |
| Classification of virions (50,55) |
| Quantitation of infectious particles (56–58) |
| Examination of nucleic acid strands (59) |
| Observation of virion uptake (17) |
| Localization of virus replication sites (17,59–62) |
| Differentiation of events in morphogenesis (60,62–65) |
| Identification of new or unexpected pathogens (5,44,47,66–68) |
| Definition of subcellular antigens (43–45,69–71) |
| Confirmation of antiserum specificity (43,53,72,73) |
| Characterization of virion assembly defects (60,74) |
| Exploration of virus–host interactions (15,16,34,63–65,75–77) |

## MOLECULAR ORGANIZATION AND TAXONOMY OF VIRIONS

### Basic Structural Components of Virions

Complete virions are the most complex level of integrated structural development in the cycle of a productive virus infection. Each virion is a physically stable packet of viral genetic material surrounded by polypeptide molecules and, in many cases, is enclosed within a lipoprotein envelope (Figs. 1, 2). The virion core consists primarily of nucleic acid (RNA or DNA) that can be associated with one or two proteins, such as polymerase enzymes required for initiating early viral gene functions (19,78). The core macromolecules are compactly organized and relatively electron dense. Native electron density is enhanced by usual application of heavy metal stains, particularly uranyl acetate and lead citrate (Fig. 2).

Viral polypeptides delimiting the virion core are aggregated into repeating macromolecular subunits, or capsomeres. These capsomeres assemble an intact

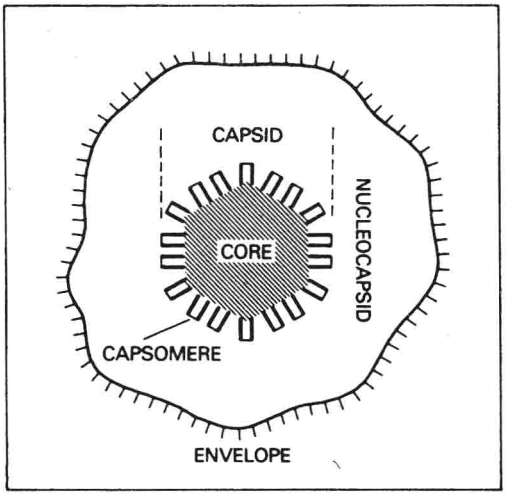


**Figure 1.** Schematic representation of a virion showing the compact core surrounded by capsomeres that form the capsid. Regular spikes on the outer envelope symbolize glycoprotein peplomers. (Adapted from Horne, RW: *Sci. Am* 208:48, 1963.)

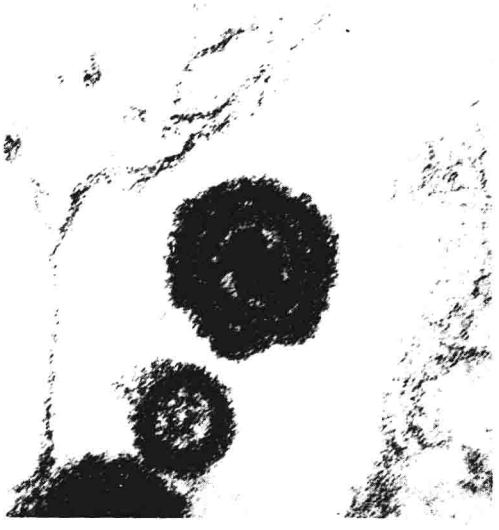

**Figure 2.** Ultrathin section of a herpes virion. Uranyl acetate and lead citrate stains. Electron-dense core, hexagonal capsid, and externally coated envelope correspond to Figure 1 schematic. (×150,000)

shell or capsid. Individual capsomeres can be resolved by transmission electron microscopy (TEM) (Figs. 3, 4; see *Direct Identification of Virions by Negative Staining*). The entire unit of the capsid with a nucleic acid core is referred to as the nucleocapsid and the assembly process is known as encapsidation.

For most DNA viruses and a few RNA viruses, the nucleocapsid stage is equivalent to the naked virion (Fig. 5, Table 2). In other infections, the nucleocapsid is loosely or tightly enveloped by a membrane during emergence from the host cell, and only the enveloped stage is designated as the virion (Figs. 2, 6). With exception of poxviruses, the virion envelope originates as a piece of host cell, derived from either the nuclear envelope (Fig. 6), the

**Figure 3.** Herpesvirus nucleocapsids negatively stained with 2% PTA at pH 5.3. *Top:* Surface pattern of individual capsomeres (×60,000). *Bottom:* Profile of a capsid with characteristic 24 peripheral capsomeres. (×100,000)

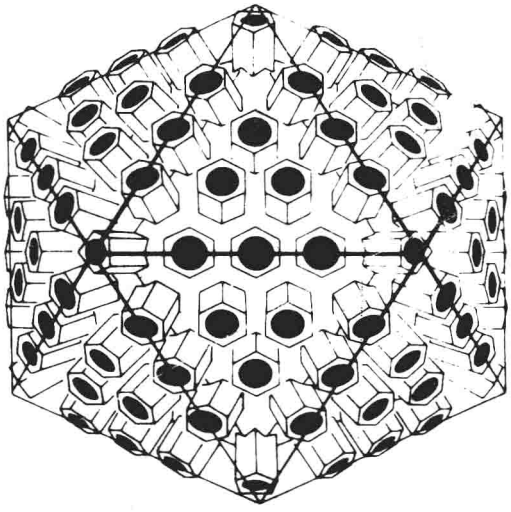

**Figure 4.** Schematic representation of an icosohedral capsid constructed from hexons and pentons. Edge axes of twofold symmetry drawn in heavy lines. (Redrawn from Horne, RW: *Sci Am* 208:48, 1963.)

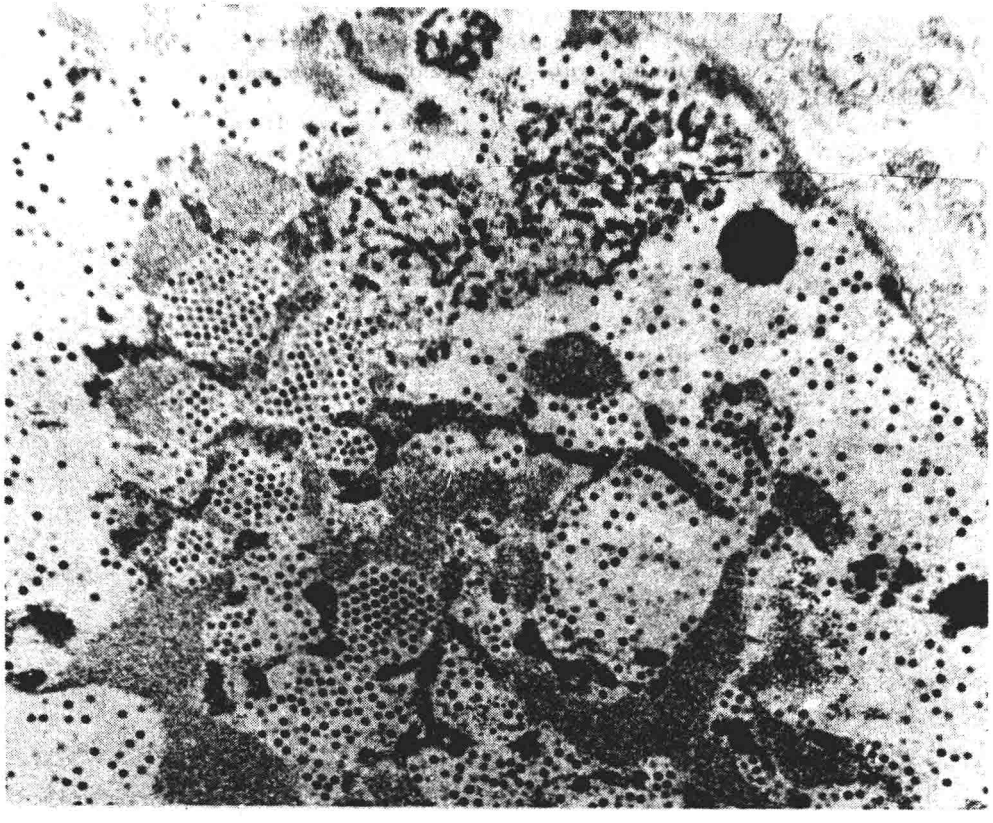

**Figure 5.** Nucleus of human AH-1 cell infected by adenovirus. Paracrystalline packing of naked nucleocapsids reflects their individual icosohedral symmetry. Note characteristic patchwork of granular and fibrillar chromatin material. (×22,000)

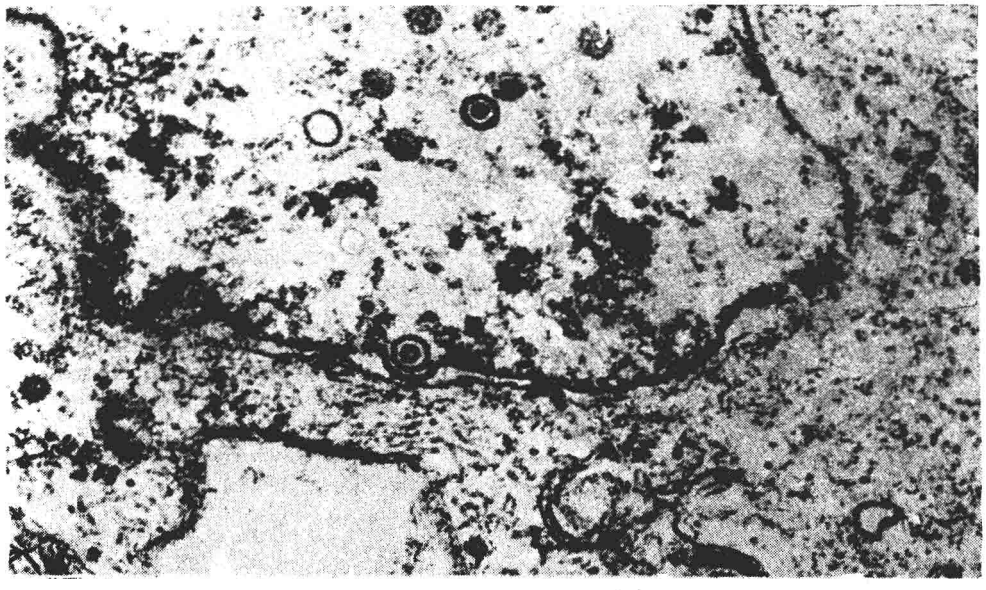

**Figure 6.** Virus of herpes family in process of budding through a nuclear membrane in a rat kidney cell infected with cytomegalovirus. Note hexagonal symmetry of nucleocapsids. (×47,500)

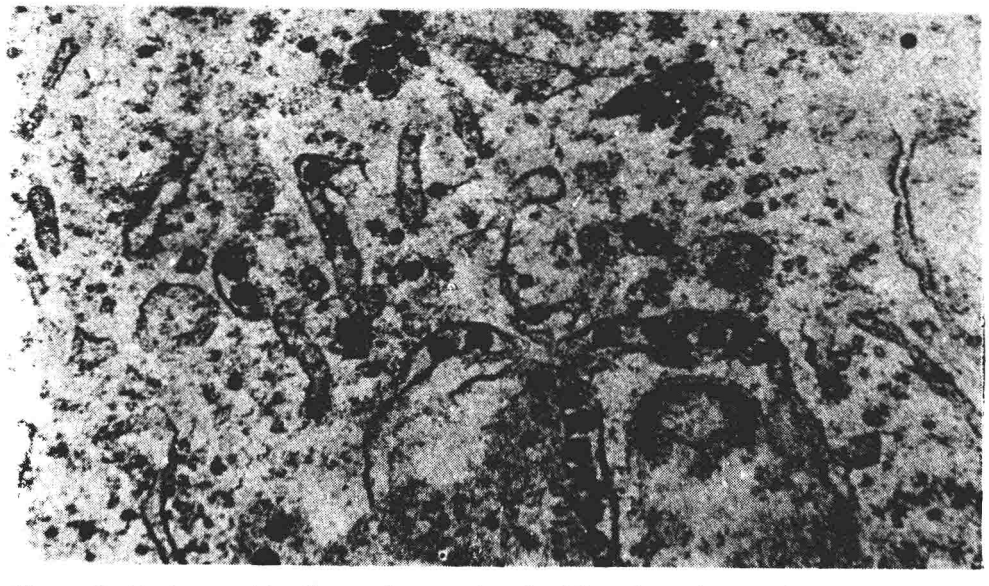

**Figure 7.** Nucleocapsids of an alpha togavirus budding through smooth cytomembranes of the Golgi region to become mature virions. Mouse neuron infected with Semliki Forest virus, ref. 75. (×60,000)

endoplasmic reticulum (ER) (Fig. 7), cytoplasmic vacuoles (Fig. 8), or the plasma membrane (Fig. 9). This host membrane serves as a fluid-mosaic scaffold into which polypeptide macromolecules or peplomers of virus gene origin are selectively concentrated (see *Envelopment*). The envelope peplomers as well as the nucleocapsid capsomeres serve protective and receptor functions (17).

## Virion Classification

A rational taxonomy of microorganisms must be predicated on genetic relationships. Schemes for classifying virions continue to evolve with growth of molecular biology. Characteristics of nucleic acid in the virion core are a primary

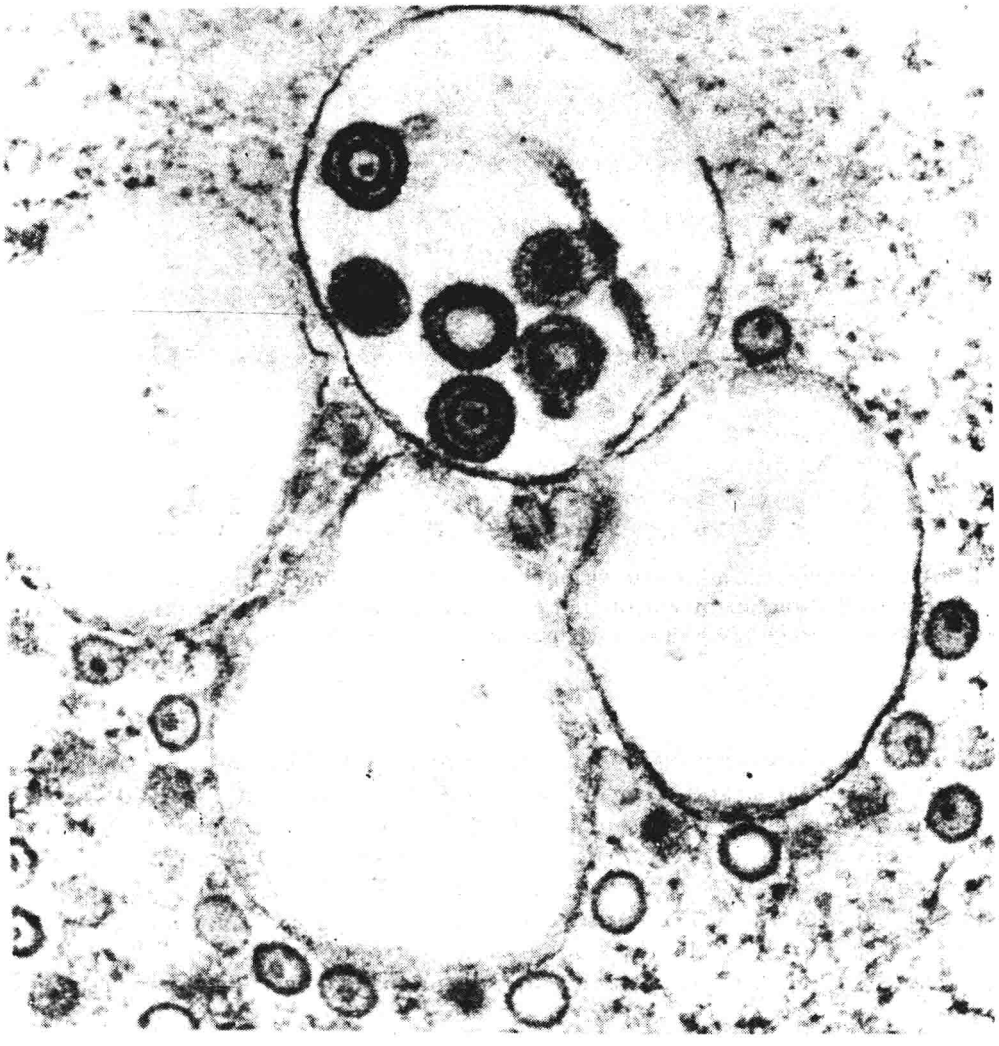

**Figure 8.** Intracytoplasmic nucleocapsids of a herpesvirus compared with mature virions within a cytoplasmic vacuole. Note several empty capsids. Human WI-38 cell. (×90,500)

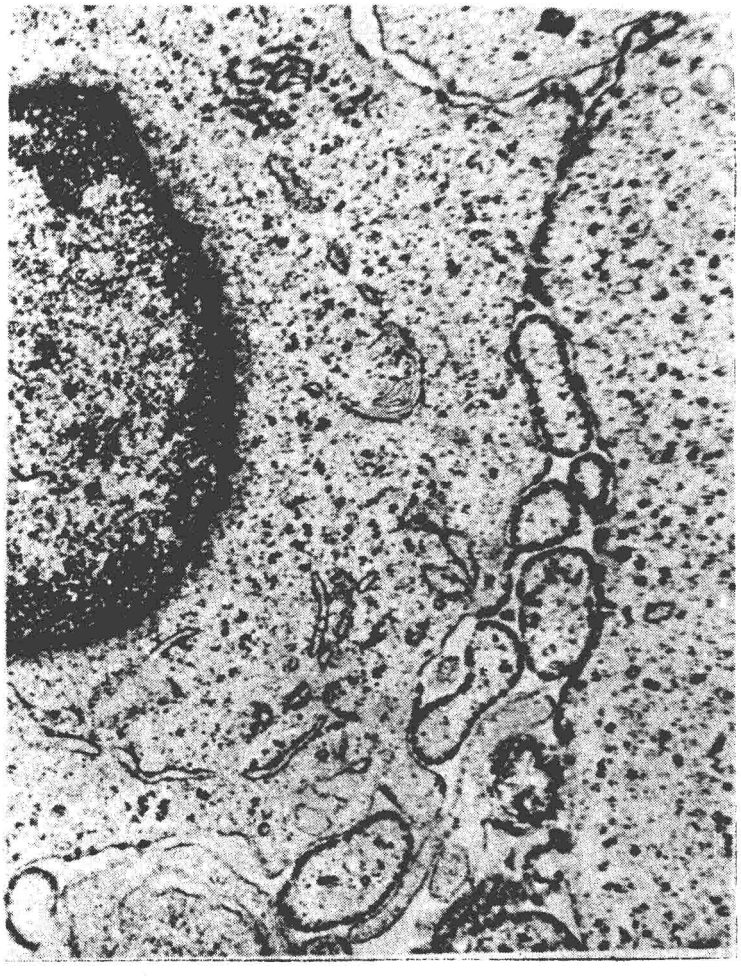

**Figure 9.** Pleomorphic myxovirus virions in process of budding from an infected human lymphoid cell. Note alignment of transected helical nucleocapsids just beneath virion envelope membrane, as well as increased membrane density in regions of budding. (×23,000)

distinction between virus families: The core nucleic acid can be a single or double molecular strand, and molecular weights range from 1.5–160 × $10^6$ daltons for the DNA viruses to 2.5–15 × $10^6$ daltons for the RNA viruses (Table 2). The large DNA viruses (adenoviruses, herpesviruses, poxviruses) thus possess a significantly greater genetic capacity to code for polypeptides than do any of the RNA viruses. At least 100 separate polypeptides were identified in a poxvirus by two-dimensional electrophoresesis (79).

Virions within each virus family are distinguished by regular shapes and uniform sizes (Fig. 10). Remarkably, these external forms are practically sufficient to recognize most families of human pathogens (50,55). Phenotypic features of the virus nucleocapsid and the presence or absence of an envelope

**Table 2.** Selected Features of Pathogenic Virions

| *Family Name* | *Core Nucleic Acid (mol wt × $10^6$)* | *Nucleic Acid Replication Site* | *Site of Nucleocapsid M phogenesis* | *Site of Virion Envelopment* | *Nucleocapsid Symmetry* | *Virion Shape* | *Average Major Dimensions of Virion (nm)* |
|---|---|---|---|---|---|---|---|
| DNA | | | | | | | |
| Parvovirus | SS (2) | N | N | Naked | Icosohedral | Spherical | 18–26 |
| Papovavirus | DS (5) | N | N (C) | Naked | Icosohedral | Spherical | 40–55 |
| Adenovirus | DS (23) | N | N | Naked | Icosohedral | Spherical | 70–90 |
| Herpesvirus | DS (90) | N | N (C) | NE, CM | Icosohedral | Spherical | 100–120 |
| Poxvirus | DS (160) | C | C | (CM, PM) | Complex | Brick | 300–450 |
| RNA | | | | | | | |
| Reovirus | DS (15) | C | C | Naked | Icosohedral | Spherical | 68–80 |
| Picornavirus | SS (3) | CM | C | Naked | Icosohedral | Spherical | 25–30 |
| Togavirus | SS (4) | CM | CM | CM, PM | Icosohedral | Spherical | 40–70 |
| Retrovirus | SS (10) | C (N) | PM | PM | Combined | Spherical | 100 |
| Orthomyxovirus | SS (5) | C | C | PM (CM) | Helical | Pleomorphic | 80–120 |
| Paramyxovirus | SS (6) | C (N) | C (N) | PM (CM) | Helical | Pleomorphic | 150–300 |
| Rhabdovirus | SS (4) | C | CM, PM | PM, CM | Helical | Bullet | 130–300 |
| Arenavirus | SS (7) | C | PM | PM | Helical | Pleomorphic | 90–250 |
| Coronavirus | SS (6) | C | PM | CM | Helical | Spherical | 80–160 |
| Bunyavirus | SS (7) | | C | CM | Helical | Spherical | 60–120 |

*Abbreviations*: C = cytoplasm, CM = cytomembranes, DS = double stranded, N = nucleoplasm, NE = nuclear envelope, SS = single stranded, PM = plasma membrane, ( ) = alternate pathway or site.