Morphology and morphogenesis of arenaviruses *

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Arenaviruses have unique structural characteristics; they are pleomorphic, have a mean diameter of 110–130 nm, and consist of a membranous envelope with surface projections surrounding an interior containing ribosomes and filaments. Virus particles bud from plasma membranes of infected cells and in many cases large intracytoplasmic inclusion bodies are formed. These characteristics allow generic identification, but not differentiation of individual viruses. Ultrastructural identification of virus particles and pathological processes in infected tissues of man and experimental animals is important in understanding the nature of arenaviral pathogenesis Such identification also contributes to our understanding of the mechanisms of viral shedding and transmission in reservoir host species.

INTRODUCTION

In several laboratories and over several years, attempts to demonstrate the virus of lymphocytic choriomeningitis (LCM) ended in frustration because particles fitting preconceived notions were not found. However, in 1968 Dalton and his colleagues (4) described novel virus particles in heavily infected cell cultures and provided cytochemical, and later immunochemical, evidence (1) that these particles were the etiological agent of LCM. Once this had been done, electron microscopic confirmation of the identification of LCM virus came from several laboratories (3, 5, 7, 8), and within a few months, morphological similarity was noted between LCM and Machupo viruses (8, 9). A new taxon was established to contain these viruses and the other members of the Tacaribe serogroup—Machupo, Junin, Amapari, Pichinde, Parana, Tamiami, Latino, and Tacaribe viruses (11); this taxon was called the *Arenavirus* genus (15). Within a year, Lassa virus was added (2, 16), but there have been no further additions.

MATERIALS AND METHODS

Viruses and host systems

Over the past 7 years all the arenaviruses have been studied in this laboratory in varying cell culture and *in vivo* systems. Identification of most of the prototype viruses has been tabulated elsewhere (9); Lassa virus strains from Nigeria, Sierra Leone, and Liberia were isolated in the CDC Maximum Security Laboratory. Studies of all the viruses in Vero cells and of Machupo virus in tissues of *Calomys callosus* were made in collaboration with K. M. Johnson, P. A. Webb, S. G. Whitfield, W. A. Chappell, and H. Wulff (9, 10; other studies of Lassa virus are unpublished). Studies of Tacaribe virus in mice and Tamiami virus in mice and *Sigmodon hispidus* (cotton rats) were made with E. C. Borden, W. C. Winn, D. H. Walker, M. R. Flemister, and S. G. Whitfield (12, 18). Studies of LCM virus in mouse tissues were made with M. S. Hirsch and D. H. Walker (17). Studies of Lassa virus in human tissues were made with W. C. Winn, T. P. Monath, and S. G. Whitfield (19, 20).

Electron microscopy

Methods of culture, infection, and harvest of Vero cells and methods relating to the preparation of experimental animal and human autopsy/biopsy tissues for electron microscopic examination have been described (8, 9). Standard methods for fixation, embedment, ultra-thin sectioning, staining (uranyl acetate plus lead citrate), and electron microscopic photography were used without alteration so that comparisons would be more meaningful. Negative contrast electron microscopic methods have been described previously also (9).

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RESULTS

Morphology of virus particles

The morphological details of all the arenaviruses are identical; no individual virus identification or subgrouping will be possible on morphological grounds. Likewise, virus particles are morphologically the same in cell culture and in human or animal tissues (Fig. 1 and 2) so the descriptions apply generally to all arenaviruses even though limited examples are shown. In ultra-thin sections, virus particles appear as round, oval, or pleomorphic forms, with mean diameters of approximately 110–130 nm and individual variations from 50 to 300 nm (9, 10). Surface projections form an outer layer, which often has a precise margin, perhaps reflecting the shape and spacing of individual projections (Fig. 1). The viral envelope is formed from the basic trilayer of the plasma membrane of the host cell (rarely intracytoplasmic membranes), but modification is evidenced by a significant increase in density of both membrane leaflets and an increase in the width and uniformity of the electron-lucent intermediary zone (Fig. 3). In fewer cases, this envelope layer may take on a more complex form, the nature of which is unknown (Fig. 4: note the viral envelopes with three dense layers). The interior of arenavirus particles has been shown to contain a highly variable number of 20–25-nm electron-dense granules (Fig. 1–4), which are easily distinguished from glycogen granules (Fig. 5) but are morphologically indistinguishable from ribosomes. These granules have been proven to be ribosomes by biochemical analysis of the RNA of purified LCM and Pichinde viruses (13, 14). We initially considered that the distribution of ribosomes within virus particles is random, but they appear to be arranged in circular fashion, particularly in larger particles beneath the viral envelope (Fig. 2, 4, 6). Likewise, it was considered that there was no further internal organization within particles, but in some particles we have found linear structures connecting ribosomes (Fig. 7, 8, 9). This organization, with ribosomes varyingly spaced upon filaments that are less than 20 nm in diameter, does not resemble the tight configuration of ribosomes in polyribosomes. Further investigation will be required to determine whether these filaments are viral ribonucleocapsids.

Negative contrast microscopy of arenaviruses has not contributed to characterization of their internal constituents, but envelope and surface projection layers may be well resolved (9, 10). Since the unit membrane envelope is extremely plastic, the shape of the larger virus particles may be bizarre; in addition, staining and drying conditions often cause osmotic blebbing of this membrane. Surface projections are closely apposed on most virus particles, but as osmotic swelling occurs, they become widely spaced (Fig. 10, left). The individual projections have been shown to be 10 nm long and club-shaped. At high magnification, this shape is evident at the periphery of particles (Fig. 10, right), and a hollow axis may be seen when projections are viewed from the end.

Viral morphogenesis and infected cell characteristics

Arenaviruses mature via budding from the plasma membrane of infected cells in culture and *in vivo* (Fig. 3). Ribosomes are contained in the nascent buds, and early in infection the membrane changes involved in viral envelope construction are precisely limited to bud sites. Late in infection, large areas of plasma membrane become dense, seem to have viral surface projections, and may accumulate a thick layer of amorphous material in patches (Fig. 11). Infected Vero cells undergo cytopathic changes that include rarefaction, condensation, and lysis as stages in the common terminal necrotic pathway. We have not observed the massed convolutions of cytoplasmic membranes as described in L-cells infected with LCM virus (7).

Cytopathology is variable in *in vivo* arenavirus infections. In many cases (usually natural virus-host pairings), virus particles may form in the absence of any cytopathic change in host cells. In other cases, necrosis similar to that described for infected Vero cells may occur at sites of viral production. An example of the latter in human Lassa virus infection has been described by Winn and his colleagues (19, 20).

Intracytoplasmic inclusion bodies are prominent in many arenavirus-infected cells in culture and *in vivo*; it has been shown that they are made up of masses of ribosomes (usually monoribosomes) in a moderately electron-dense matrix. The relative balance between ribosomes and matrix material varies. Inclusions seem to start as rather dispersed accumulations, which are hardly discernible from normal ribosome concentration (Fig. 12), but condensation and continued synthesis result in rather uniformly marginated masses, which are large (Fig. 13). One additional feature of early inclusions (seen repeatedly in LCM and Tamiami virus infections *in vivo*) is the presence of fine filaments inter-

Fig. 1. Lassa virus in first Vero cell passage after isolation from a patient's pharynx in Sierra Leone. The envelope and surface projection layers of virus particles are well resolved. × *121 000.*

Fig. 2. Lassa virus in liver biopsy tissue from the same case yielding the isolate illustrated in Fig. 1. Although surface layers of particles are less distinct, there are no structural differences between viruses in cell culture and *in vivo.* × *157 000.*

Fig. 3. Junin virus budding from the plasma membrane of a Vero cell. The viral envelope is denser and more distinct than the host cell membrane from which it is derived. Ribosomes are present in the nascent bud. × *173 000.*

Fig. 4. Parana virus in Vero cell culture. The viral envelopes have extradense layers in this case, but the nature of this variation is unknown. × *146 000.*

Fig. 5. Lassa virus in human liver. Distinction between ribosomes (within the virus particles) and other granules, such as glycogen (arrows), is necessary for virus identification in a background of pathological tissue. × *105 000.*

Fig. 6. Lassa virus in human liver. Although the position of ribosomes in arenavirus particles is usually random, in many larger particles they appear in a circle beneath the envelope, which suggests some linkage. × *129 000.*

Fig. 7. Lassa virus in Vero cell culture. A filamentous structure links the ribosomes within the virus particle (arrow). × *173 000.*

Fig. 8. Pichinde virus in Vero cell culture. Intraviral filament (arrow) is less than 20 nm in diameter and ribosomes are irregularly associated with it. × *173 000.*

Fig. 9. Tamiami virus in Vero cell culture. Before filaments (arrow) within virus particles can be definitely classed as nucleocapsids, further investigation will be necessary. × *137 000.*

Fig. 10. Composite of LCM virus (left) and Parana virus (right) stained with sodium silicotungstate to show widely spaced and closely apposed projection, each of which is a hollow club-shaped structure, 10 nm long. × *260 000.*

Fig. 11. Amapari virus in Vero cell culture. Large areas of the surface of infected cells may change and accumulate a thick amorphous layer (arrows); cytoplasmic rarefaction is the commonest evidence of cytonecrotic processes. × *60 000.*

Fig. 12. LCM virus in mouse choroid plexus epithelium. Early inclusion body with dispersed accumulation of ribosomes and rather dense matrix material free in cytoplasm. × *35 000.*

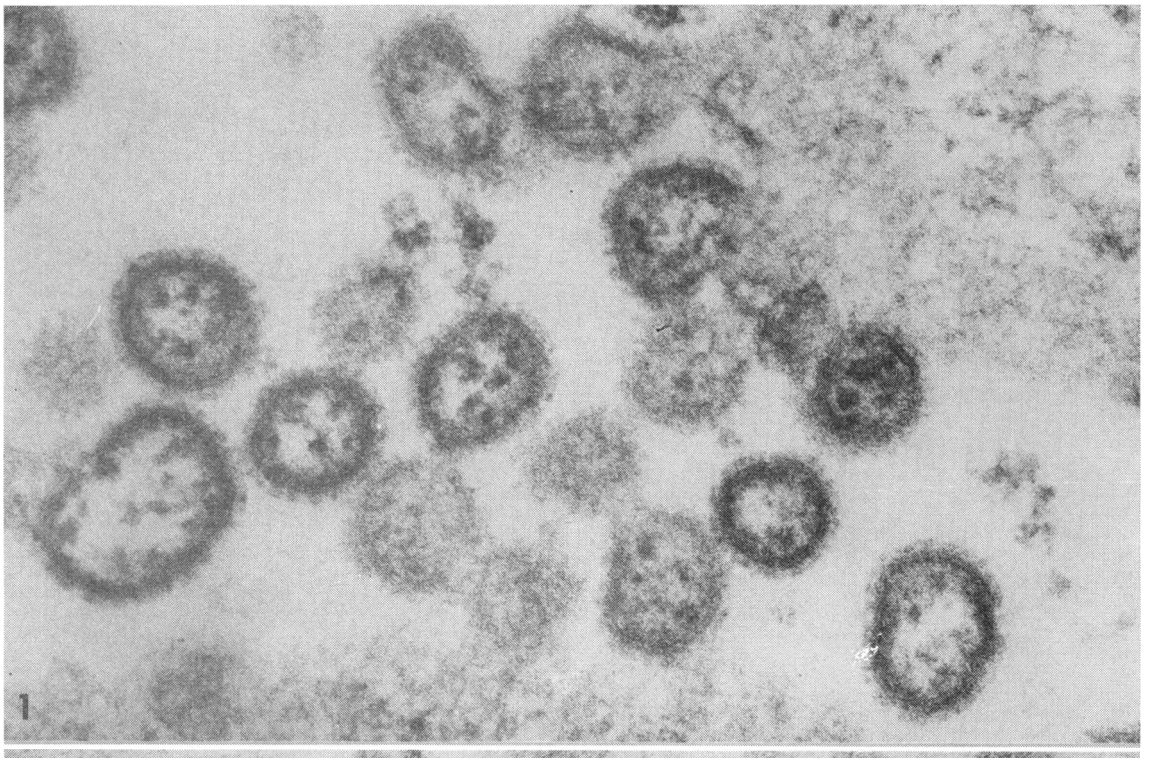
Fig. 13. Tamiami virus in the brain of *Sigmodon hispidus* 150 days after infection. Inclusion bodies persist in neuronal and glial cytoplasm as condensed ribosome-matrix masses with uniform margins. × *40 000.*

Fig. 14. Tamiami virus in *Sigmodon hispidus* brain 23 days after inoculation. Fine filaments are intermixed with ribosomes and amorphous matrix in some early inclusions. × *40 000.*

Fig. 15. Tamiami virus in *Sigmodon hispidus* spleen 9 days after inoculation. Virus is present within the platelet demarcation channels of a megakaryocyte; the effect of this infection upon platelet function is unknown. × *40 000.*

Fig. 16. LCM virus in mouse choroid plexus. Virus particles (nascent and formed) located among the microvilli of this epithelium are the likely target for the cell-mediated immune activity. × *53 000.*

Fig. 17. Machupo virus in salivary gland of *Calomys callosus.* Virus produced by mucous acinar cells is filling an acinar lumen and is free to contaminate saliva. × *46 000.*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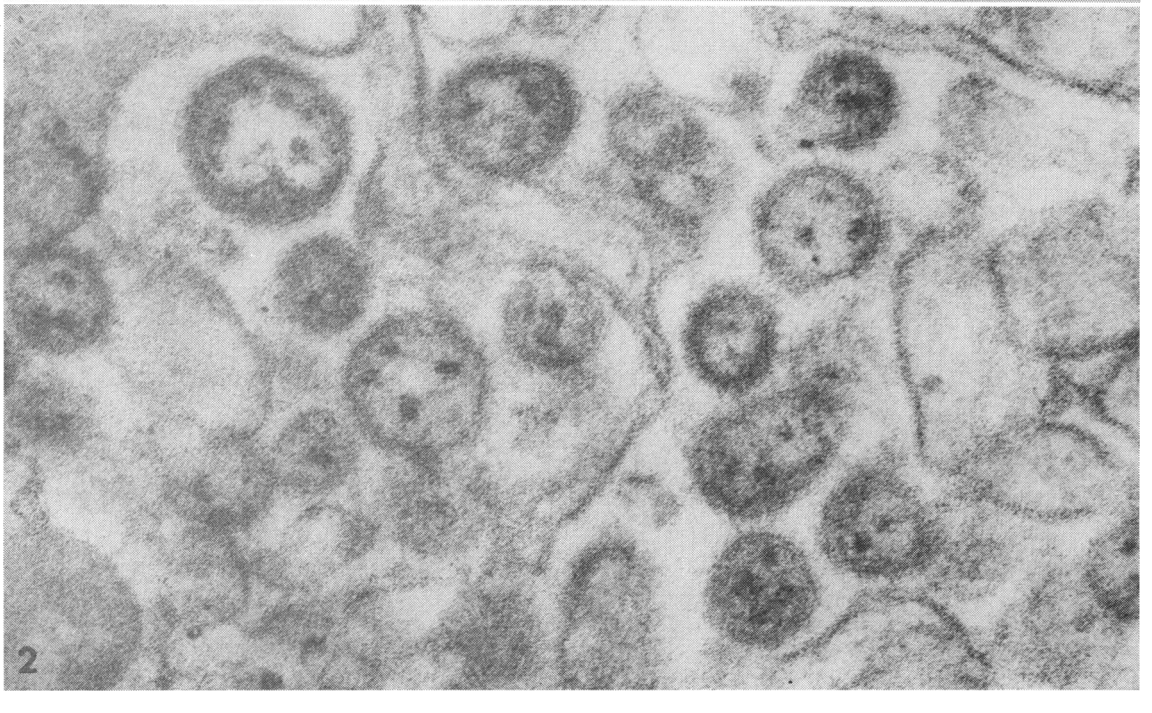
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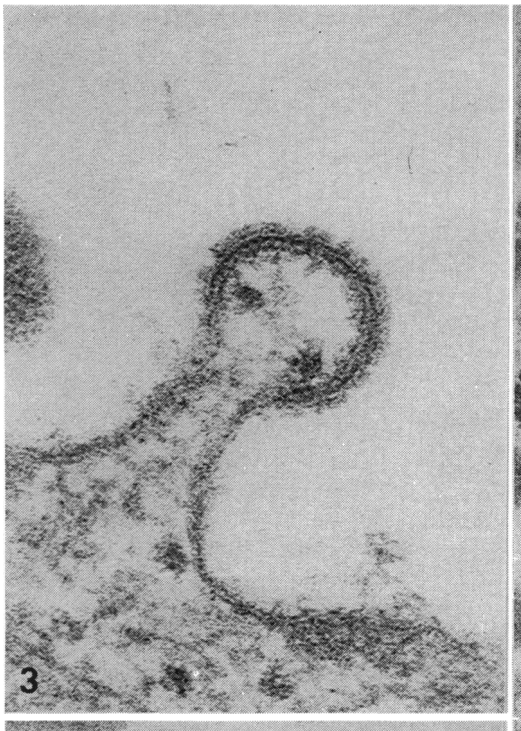

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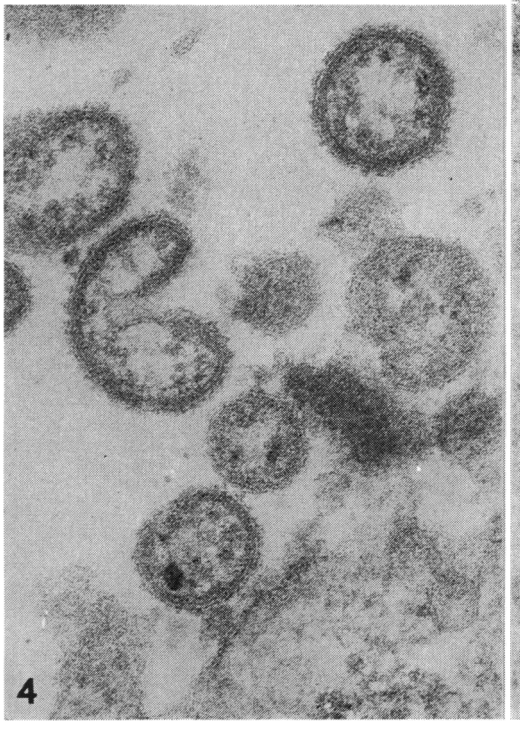

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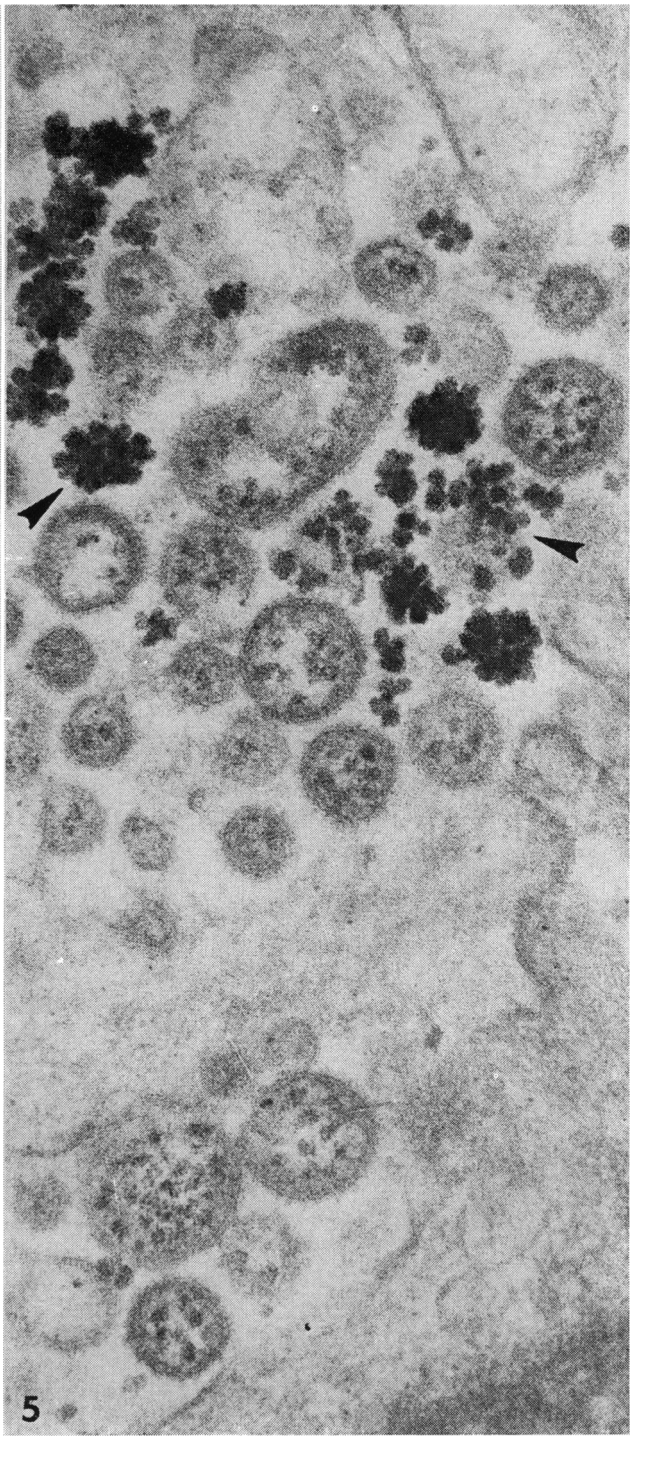

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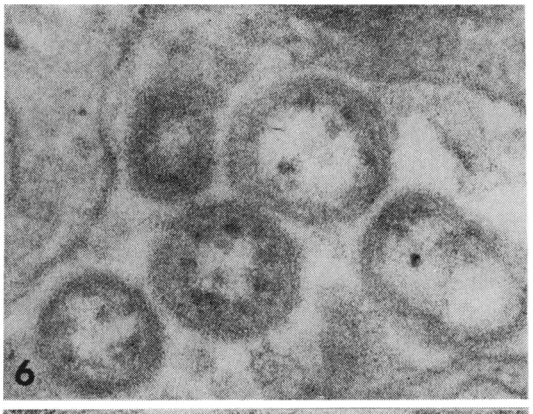
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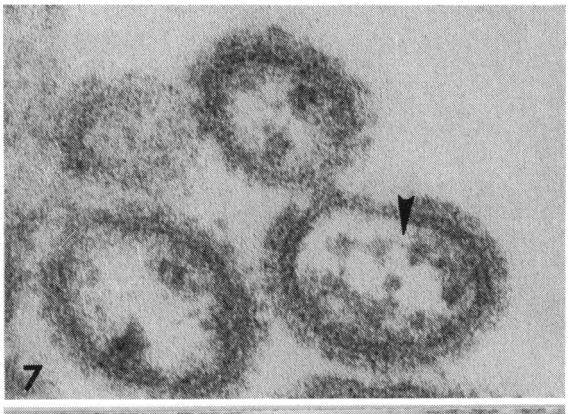
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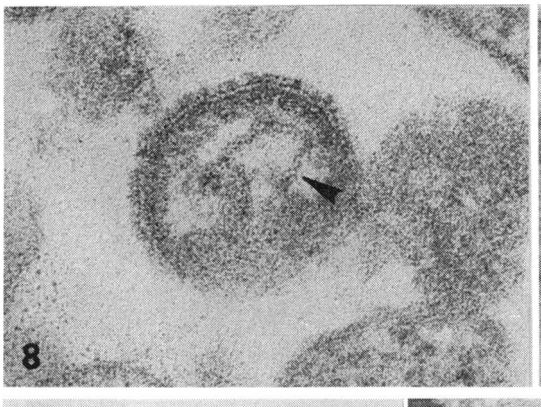
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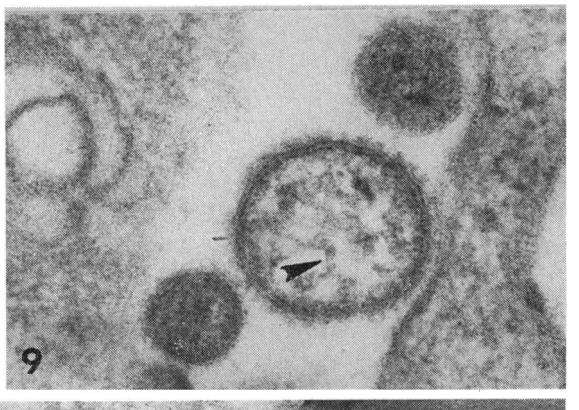
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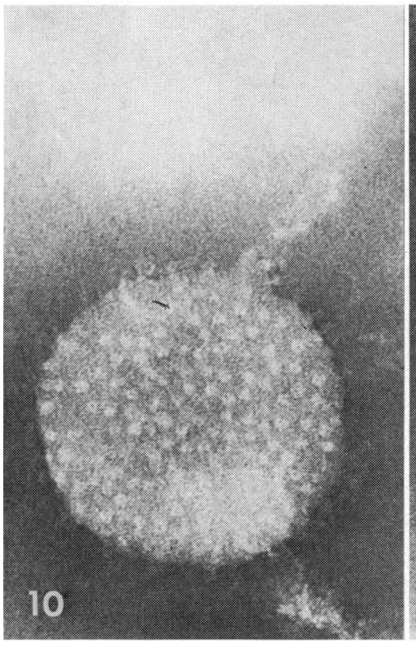
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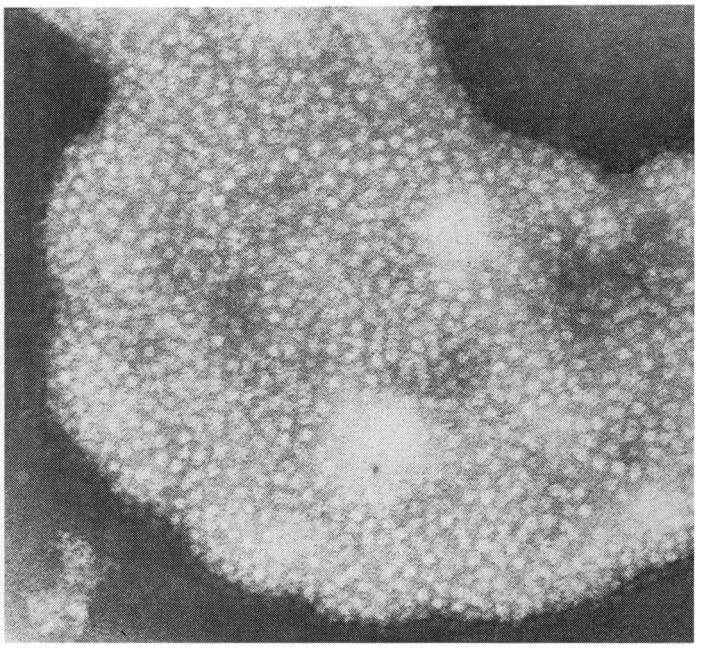

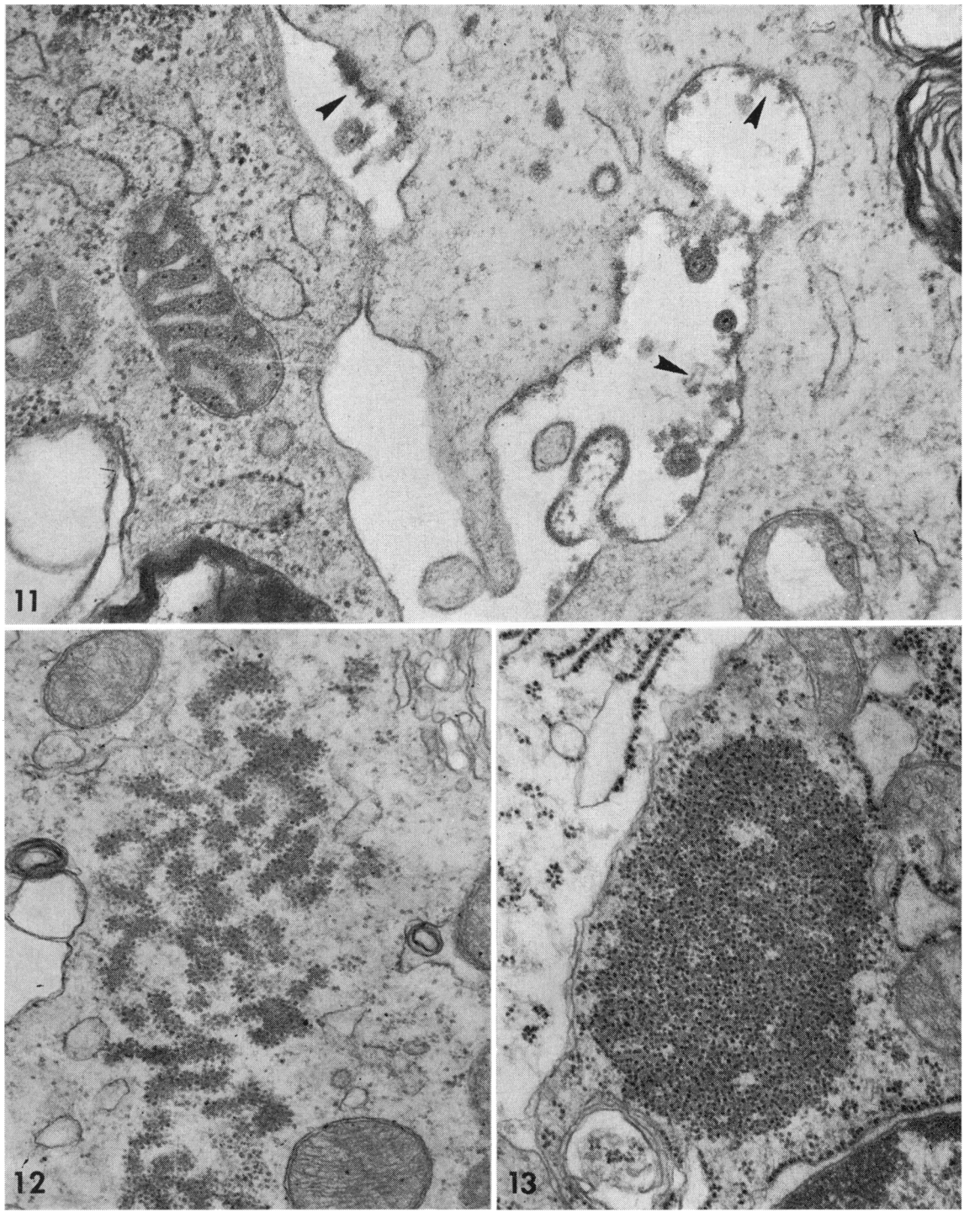

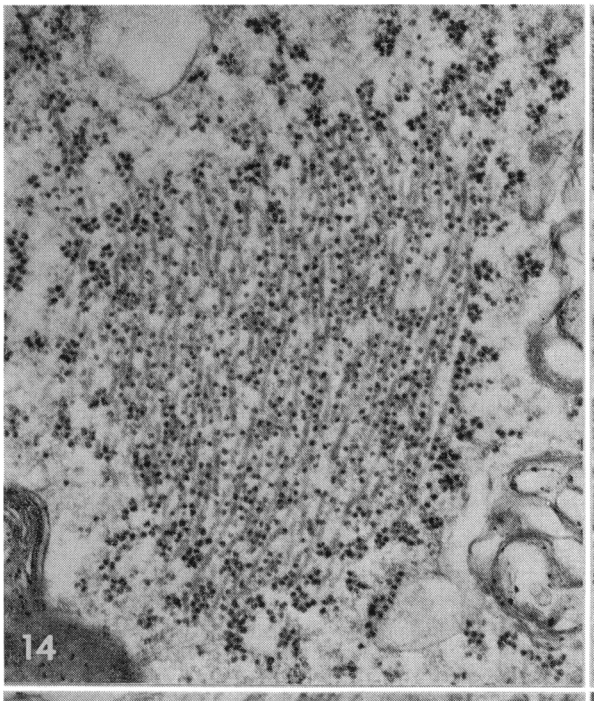
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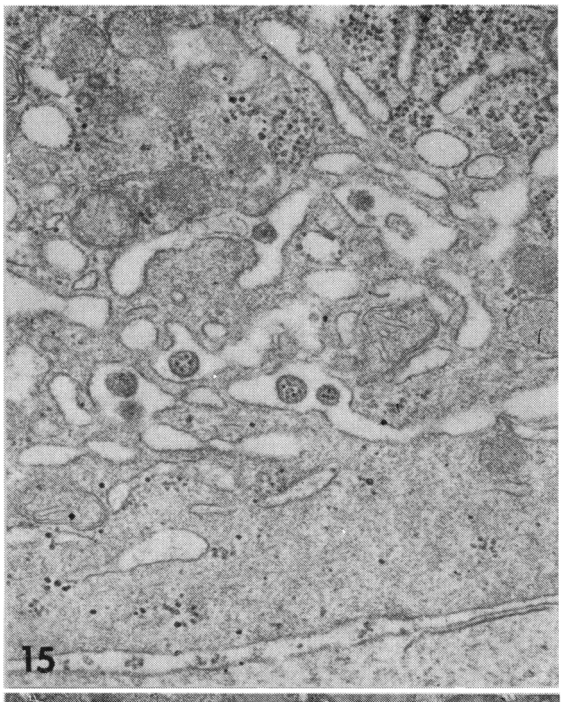
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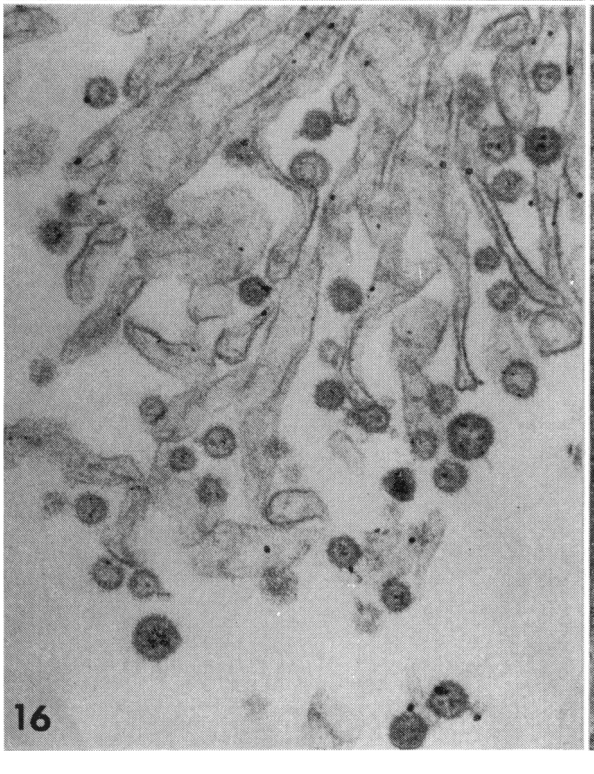
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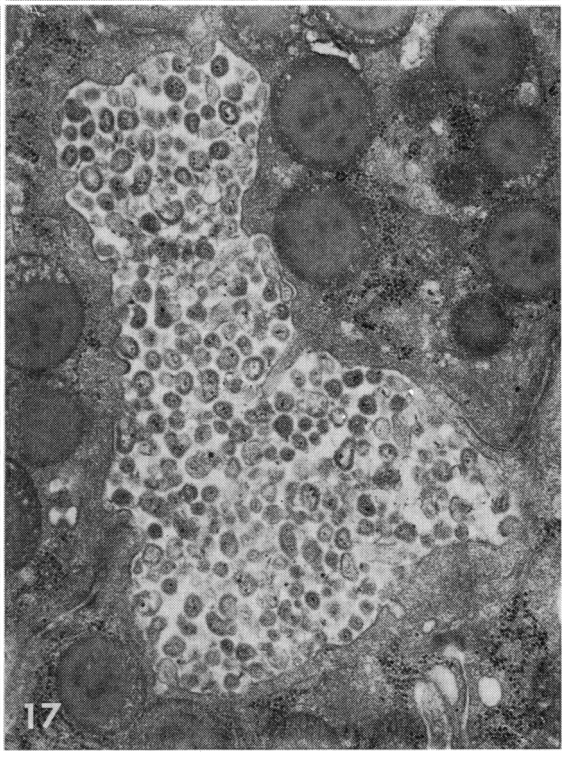
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mixed with the ribosomal aggregates (Fig. 14); the nature of these remains unknown.

In vivo sites of viral proliferation

Although many organs and tissues support the growth of arenaviruses in human and animal infections, it is the course of the disease in a few key targets that determines the clinical severity of disease and, in reservoir hosts, the persistence of viral carriage and shedding. Our knowledge in this area is fragmentary, but a few examples follow in which ultrastructural observations contribute to understanding of viral natural history.

One key to arenavirus pathogenesis is lymphotropism. As lymphoreticular cells are infected early, there is a mechanistic basis for host hyporesponsiveness, tolerance, and persistent contamination of the environment with virus. This early lymphoreticular tropism has been studied ultrastructurally in *Calomys callosus* infected with Machupo virus (10) and *Sigmodon hispidus* infected with Tamiami virus (12). In these infections, virus grows in lymphoreticular cells, but there is no apparent cytopathology. The mechanisms by which the viruses interfere with host defences must be subtle, but the slow or incomplete termination of viral carriage proves that such mechanisms are effective. In the lymphoreticular organs of these animals, megakaryocytes are also infected early (Fig. 15); this infection site suggests a relationship between platelet function and the pathogenesis of arenavirus haemorrhagic fevers.

Host responsiveness, which is delayed or absent in some arenavirus infections, may be overexuberant in others. In LCM virus infection of mice, cell-mediated immune damage to infected choroid plexus, ependyma, and meninges is lethal. Ultrastructural study of this interaction indicates that the antigenic target on the surface of infected cells is virus itself (Fig. 16); the dysfunction of choroid plexus epithelium after T-cells attack the viral target is subtle and not seen morphologically, but the breakdown of the blood-brain barrier at this site is enough to cause death by ionic imbalance (18). In other rodent arenavirus infections, central nervous system involvement may be limited to the brain parenchyma (6, 12), and neuronal foci may become targets for T-cell attack.

Infection in several organs contributes to arenaviral transmission patterns. Infection in kidney yields the virus transmitted among animals and to man via urine contamination. Infection of salivary gland yields the virus that contaminates saliva; from ultrastructural studies of Machupo virus in *Calomys callosus* (10) and Tamiami virus in *Sigmodon hispidus* (12), it is clear that very large amounts of virus may be produced by mucous acinar cells without damage (Fig. 17). Although saliva volume is certainly less than urine volume, the hazard to man from either source may be considerable.

DISCUSSION

The unique structure of arenaviruses should make it easy to identify new isolates and potential new members of the group. For example, Lassa virus was confirmed as a new arenavirus in 1970 by thin-section electron microscopy (16), and arenaviral hepatitis was found to be the cause of death in a suspected Lassa fever patient from Sierra Leone in 1972 (19, 20). However, it must be emphasized that because of the fragility and rather indistinct morphology of arenavirus particles in a tissue background, only very well preserved materials are suitable for such identification, and even then a conservative approach to distinguishing virus from cell organelles and breakdown products is necessary. Thin-section electron microscopy fits well in a comprehensive identification scheme, but we must continue to explore means of employing negative contrast microscopy, which is the only electron microscopic method suitable for large numbers of specimens.

In studies of the mechanisms by which arenaviruses infect, persist, and cause severe disease in different animals and man, electron microscopy has particular merit when used in combination with light microscopic histology, organ and tissue titration, frozen-section immunofluorescence, and immunological manipulations. By using this integrated approach we can hope to bring our understanding of all arenaviral diseases up to the level exemplified by our knowledge of LCM virus infection of the mouse. In particular, electron microscopy is needed because infected cells differ in their accumulation of viral antigen (as studied by immunofluorescence) and their production of virus particles. This dichotomy seems to be a factor in viral pathogenesis and in experimental " late diseases ", which may yet be found in natural arenaviral infections.

RÉSUMÉ

MORPHOLOGIE ET MORPHOGENÈSE DES ARÉNAVIRUS

Les arénavirus possèdent des caractéristiques structurales particulières; les particules virales sont rondes ou pléomorphiques et ont un diamètre moyen de 110 à 130 nm. Elles sont constituées d'une enveloppe membraneuse couverte d'appendices et contiennent à l'intérieur un nombre variable de ribosomes et, dans des cas plus rares, de fins filaments. Les particules virales se fixent sur les membranes plasmiques des cellules infectées et forment très souvent de vastes inclusions intracytoplasmiques constituées d'un amas de ribosomes, de filaments, et d'une matrice. Ces caractéristiques permettent l'identification générique mais non la différenciation individuelle des virus; c'est sur elles que se fonde la définition du taxon « arénavirus ».

L'identification ultrastructurale des particules virales dans les tissus infectés de l'homme et des animaux d'expérience est indispensable à la compréhension de l'interaction virus/hôte et de la pathogénèse des infections à arénavirus. Pour être en mesure d'évaluer convenablement les processus de ces infections, il faut que la pathologie ultrastructurale soit connue. L'article offre des exemples d'observation ultrastructurale *in vivo* mais nos connaissances en ce domaine sont fragmentaires et appellent un supplément d'études.

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DISCUSSION

OLDSTONE: In instances where LCM virus particles are seen budding from the choroid plexus of animals that have received a T-cell transfer, do you find the T-cells alongside the viral particles themselves?

MURPHY: In the unmanipulated infection, infiltrating cells are not seen focalized on virus particles and damage is not associated with the presence of lymphocytes or virus particles. In fact, at the time of death damage to the choroid plexus epithelium is not apparent—it is extremely subtle. *In vivo* cytolysis, the " kiss of death ", is not morphologically discernable.

OLDSTONE: What about the " kiss at introduction "? Do you find the T-cell recognizes the budding virion and lines up against the virion?

MURPHY: The cell does not sit on the viral target site, but from the number of round cells (T-cells and macrophages) that move from the choroid capillary through the basement lamina and through the epithelium and then attack from the surface where most of the virus particles are budding, one would conclude that a general " lining up " is occurring. We are unlikely to see the " kiss of death ".

OLDSTONE: Do you ever find virus inside resting lymphocytes?

MURPHY: No.

OLDSTONE: Have you measured any function in these proliferating lymphocytes, such as the release of MIF or lymphotoxin, to see if it is depressed by infection?

MURPHY: We have not done that.