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CHAPTER 12

Coronaviridae

MALCOLM R. MACNAUGHTON¹ and HEATHER A. DAVIES²

¹ Boots Celltech Diagnostics Limited, 240 Bath Road, Slough, Berkshire, U.K., and ² Clinical Research Centre, Watford Road, Harrow, Middlesex, U.K.

Family: Coronaviridae

Genus: Coronavirus

Species: Avian infectious bronchitis virus (IBV), bovine coronavirus (BCV), canine coronavirus (CCV), feline infectious peritonitis virus (FIPV), haemagglutinating encephalomyelitis virus (HEV), human coronavirus (HCV), mouse hepatitis virus (MHV), rat coronavirus (RCV), sialodacryoadenitis virus (SDAV), porcine transmissible gastroenteritis virus (TGEV).

12.1. General characteristics

Coronaviruses are spherical, lipid-containing, enveloped particles with tear-drop-shaped surface projections or peplomers. The genome is one molecule of ssRNA and the virions characteristically contain three major structural protein classes. The antigenic relationships of coronaviruses present a complex pattern. The geographic distribution of many coronaviruses is worldwide. Biological vectors of coronaviruses have not been reported, and the natural hosts form the major reservoirs for further infection.

12.2. Chemical and physical characteristics

Coronavirus particles contain three major protein classes, within which the polypeptides vary in number and molecular weight between species. The nucleocapsid protein is phosphorylated and is of molecular weight 50 000 to 60 000. The surface projection protein consists of one or two glycosylated polypeptides of molecular weight between 90 000 and 180 000. Finally, the transmembrane or 'matrix' protein is a single protein, which is glycosylated to different degrees, and has a molecular weight between 20 000 and 35 000.

The genome is a single-stranded linear molecule of RNA of positive sense, of molecular weight 5.4 to 6.9×10^6 . It is polyadenylated at the 3' terminal and capped at the 5' terminal. The average density of coronavirus particles is 1.18 g/cm^3 in sucrose and $1.23\text{--}1.24 \text{ g/cm}^3$ in CsCl.

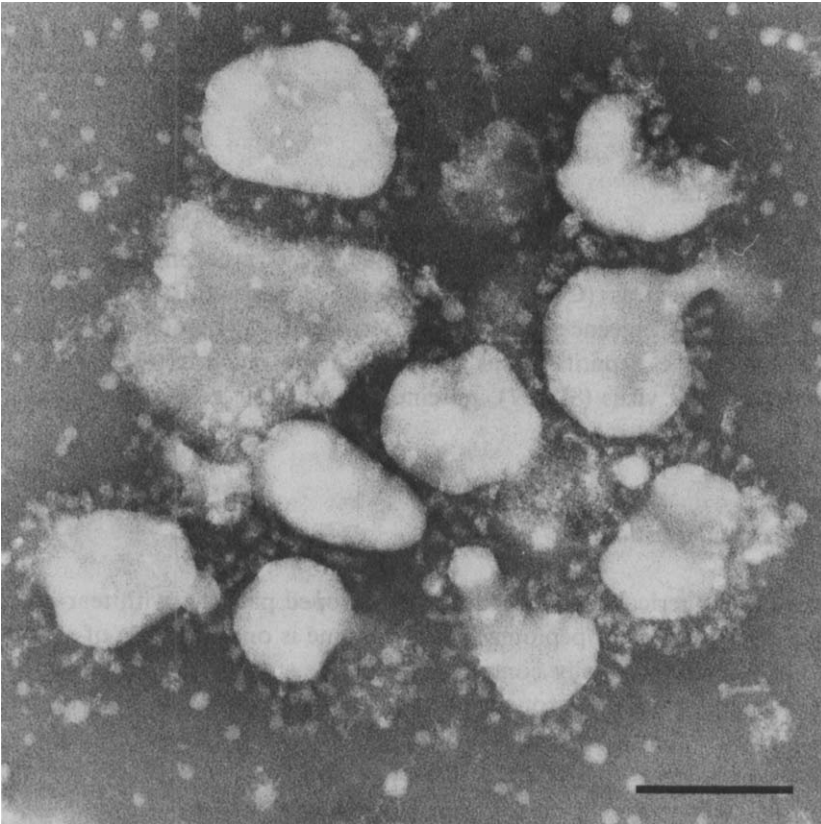


Fig. 12.1. A group of HCV 229E particles. The particles are enveloped, pleomorphic and are surrounded by a corona of surface projections. Some of the particles do not have a complete corona of surface projections. Negatively stained with 2% potassium phosphotungstate, pH 6.5. Magnification $\times 205\,200$. Bar represents 100 nm.

12.3. Structural characteristics

The apparent size and shapes of coronaviruses can vary considerably. Coronavirus particles are basically spherical, although negatively stained air-dried particles are often pleomorphic. They usually have a diameter, excluding projections, of between 80 and 120 nm, although in extreme cases the diameter can vary between 60 and 220 nm. The particles possess distinctive, tear-drop-shaped projections about 20 nm in length. Fig. 12.1 shows a preparation of typical coronavirus particles by negative staining. Particles are often seen with incomplete coronas of surface projections, which may be an artefact of preparation.

Two populations of IBV particles can be separated on equilibrium sucrose gradients. Particles with an average density of 1.18 g/cm^3 have typical coronavirus morphology and contain all the structural polypeptides and a complete genome, while less dense particles of average density 1.13 g/cm^3 have typical coronavirus morphology on negative staining but lack the nucleocapsid polypeptide and genome. These particle types can be differentiated on shadowing with carbon/platinum (Fig. 12.2). The lighter particles (Fig. 12.2a) are more flattened, producing shorter shadows than the particles with typical density of 1.18 g/cm^3 (Fig. 12.2b).

VIRION ARCHITECTURE

The virion consists of two structural complexes: the envelope (= lipid bilayer plus two glycosylated transmembrane proteins) and the ribonucleoprotein complex. The possible arrangement of structural components within the virion is presented in Fig. 12.3. It is a tentative three-dimensional model based on our present knowledge, both morphological and biochemical.

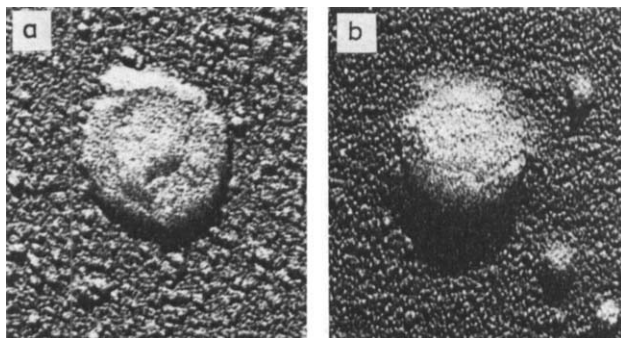


Fig. 12.2. IBV Beaudette particles from sucrose density gradients shadowed at 12° with platinum/carbon. (a) Collapsed particle from 1.13 g/cm^3 density band, showing a short shadow. (b) Particle of typical density 1.18 g/cm^3 , showing a longer shadow. Magnification $\times 120\,000$. Bar represents 50 nm.

In ultrathin sections coronavirus particles display a triple-track membrane, but the surface projections are usually not visualized. Underneath the membrane is an electron-dense layer 10–20 nm wide. The centre appears usually less dense (Fig. 12.4a). However, in some species (e.g. IBV, FIPV, TGEV, BCV and MHV) the virus interior is rather electron-dense and closely apposed to the membrane (Fig. 12.4b). Finally, virus particles with a narrow electron-dense band directly beneath the membrane have also been described (Fig. 12.4c).

The different types described may be due to variation in the arrangement of 'matrix' protein and/or nucleocapsid protein and possibly the association of these two with each other in the virus particle, which may be dependent upon the virus strain and the cell type in which the virus replicates.

The morphology of coronavirus *surface projections* can vary considerably between different strains. The conventional structure on negative staining consists of tear-drop-shaped projections (Fig. 12.5a), although cone-shaped projections (Fig. 12.5b) are also observed. In all these cases the projections have the same length of about 20 nm. Other coronaviruses have short (5–10 nm) as well as 20-nm projections (Fig. 12.6). Projections with blebs on thin stalks have been reported for other coronaviruses.

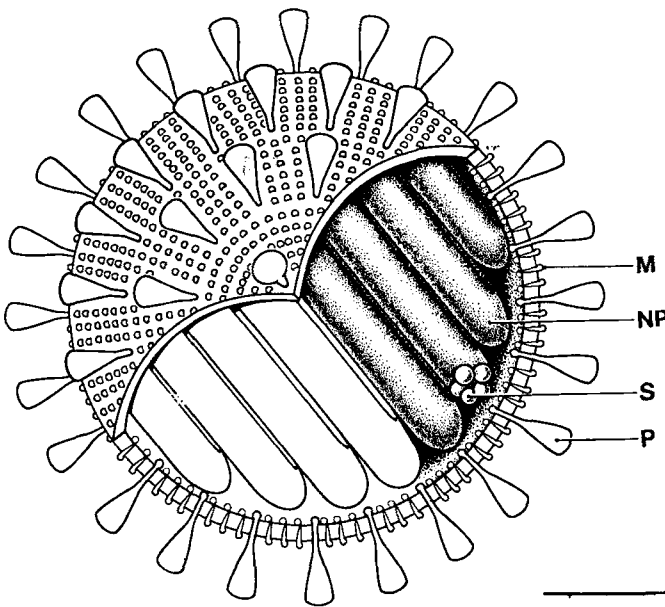


Fig. 12.3. Three-dimensional model of a coronavirus, drawn to scale. M, transmembrane protein; P, surface projection protein; NP, nucleocapsid protein; S, subunit of nucleocapsid protein. The shape of the transmembrane protein is not known. Bar represents 50 nm.

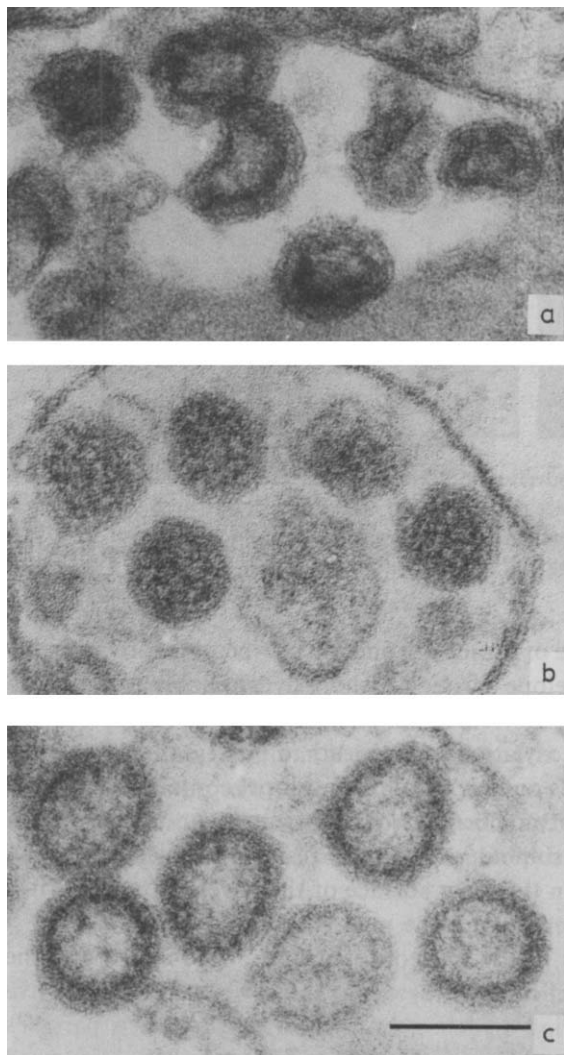


Fig. 12.4. Thin sections of purified pelleted coronaviruses grown in vitro. (a) HCV 229E grown in human macrophages, 24 hours post-infection. (b) MHV3 grown in human macrophages, 20 hours post-infection. (c) IBV Beaudette grown in the chorioallantoic membrane of a 10-day-old fertile chicken egg, 18 hours post-infection. Magnification $\times 185\,000$. Bar represents 100 nm.

Most authors suggest that the surface glycoprotein projection polypeptide is a high molecular weight glycoprotein of about 180 000 which may be an association of two non-identical subunits of about 90 000 or which can readily be cleaved into two molecules of about 90 000. IBV may be an exception to this in that the higher molecular weight form has not been observed. The surface projections can be

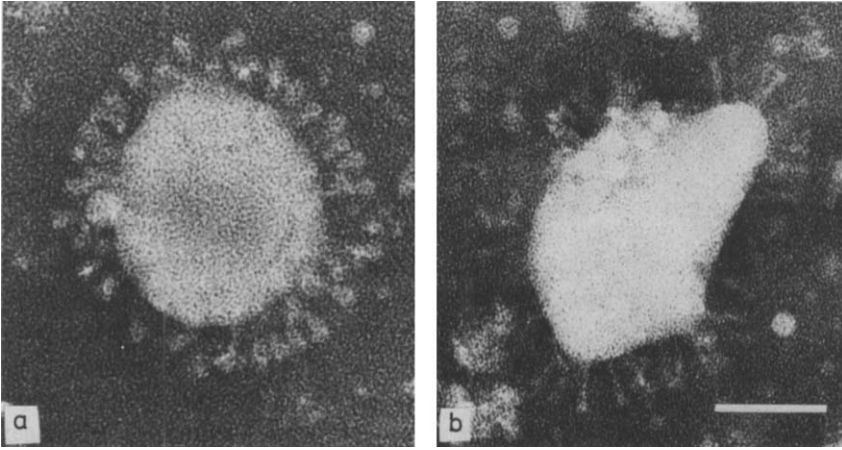


Fig. 12.5. Single coronavirus particles negatively stained with 2% potassium phosphotungstate, pH 6.5. (a) HCV 229E with tear-drop-shaped surface projections. (b) MHV3 with cone-shaped surface projections. Magnification $\times 307\,800$. Bar represents 50 nm. (Panel (a) is from Arch. Virol. 70 (1981) 301–313; panel (b) is from Arch. Virol. 59 (1979) 25–33 with permission.)

quantitatively removed by pronase or bromelain and the polypeptides are probably anchored to, or in, the viral envelope through a short hydrophobic region.

The '*matrix*' glycoprotein has a molecular weight of between 20 000 and 35 000 depending upon its degree of glycosylation and appears to possess three domains. A small hydrophilic region extends outside the viral envelope, contains all the carbohydrate of the molecule and can be removed by pronase or bromelain. A hydrophobic domain contains disulphide bonds and resides within the lipid bilayer. The third domain resides on the inner surface of the envelope, and in vitro evidence suggests that it may interact with the nucleocapsid.

The *nucleocapsid* is a complex of the nucleoprotein (M_r 50–60 $\times 10^3$) and the RNA genome. Single-stranded helical structures have been observed by negative staining in spontaneously disrupting or detergent-disrupted coronavirus particles (Fig. 12.7). Diameters of the helices up to 14–16 nm have been reported. Shadowed nucleocapsids that have been released from disrupting virus particles reveal filaments (Fig. 12.8) similar to those observed on negative staining (Fig. 12.7).

It has been suggested that the coronavirus nucleocapsid consists of the linear genome of molecular weight 5.4–6.9 $\times 10^6$ associated with phosphorylated nucleocapsid protein of molecular weight 50 000–60 000. The globular subunits of the nucleocapsid are 5–7 nm in the long axis and it is thought they are helically arranged and that five subunits, as shown in Fig. 12.3, represent one turn of the helix. In the virus particle, the nucleocapsid is coiled into a single-stranded helix which has been visualized inside virus particles, but is more readily observed when released from disrupted particles (Figs. 12.7 and 12.8).

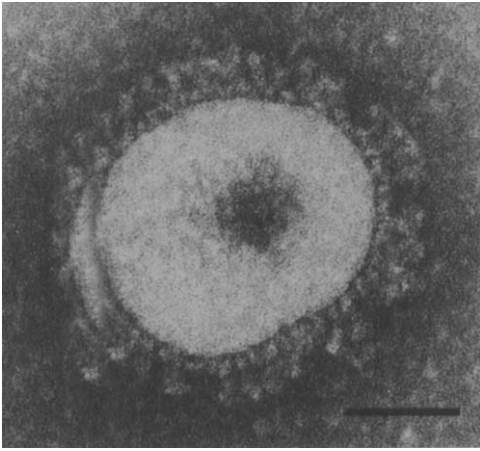


Fig. 12.6. A single particle of BCV, negatively stained with 2% potassium phosphotungstate, showing surface projections of 20 nm and the shorter ones of 5–10 nm. Magnification $\times 300\,000$. Bar represents 50 nm. (From *Arch. Virol.* 70 (1981) 301–313 with permission.)

12.4. Antigenic properties

Three distinct antigenic molecules are found in the virion, corresponding to each class of virion protein, i.e., surface projections, matrix and nucleocapsid protein. The surface projection antigen is involved in neutralization, virus attachment, cell-to-cell fusion activity and, where appropriate, haemagglutination. The nucleocap-

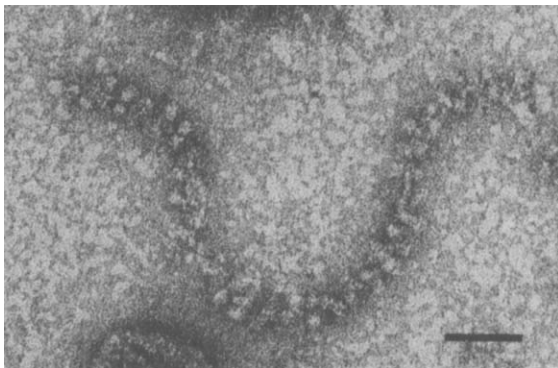


Fig. 12.7. Nucleocapsid of HCV 229E negatively stained with 1% uranyl acetate, pH 4.5. Magnification $\times 312\,000$. Bar represents 30 nm. (From *J. Gen. Virol.* 39 (1978) 545–550 with permission.)



Fig. 12.8. Nucleocapsids from spontaneously disrupted IBV Beaudette particles shadowed at 12° with platinum/carbon. Large arrows indicate the direction of shadowing. Magnification $\times 82080$. Bar represents 200 nm. (From *J. Gen. Virol.* 53 (1981) 67–74 with permission.)

sid protein antigens appear common to all coronaviruses. The main antigenic determinants reside in the surface projections and this antigen is used for serological grouping of coronaviruses. One avian and two mammalian serological groups have been identified (Table 12.1). HCVs are found in both mammalian serological groups, and all HCV strains belong to either the HCV 229E or the HCV OC43 serological groups.

TABLE 12.1.

Coronavirus serological groups

| Group No: | 1 | 2 | 3 |
|----------------|---------------------------------|--|-----|
| Virus serotype | CCV FIPV HCV 229E TGEV | BCV HCV OC43 HEV MHV RCV SDAV | IBV |

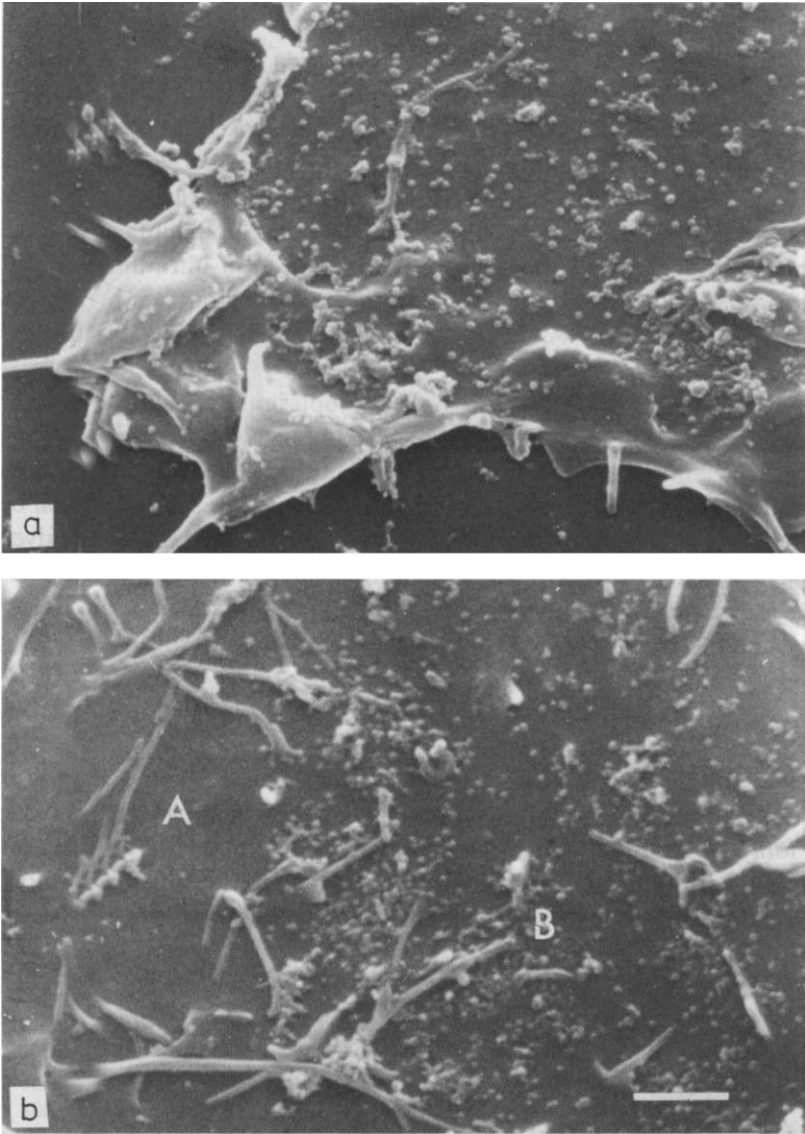


Fig. 12.9. Scanning electron micrographs of HCV 229E particles distributed on the surface of MRC continuous cells grown as a monolayer. (a) Cell prefixed with paraformaldehyde to immobilize membrane receptors and then absorbed with virus. The virus particles are randomly distributed. (b) After absorption at 0°C and then warming to 33°C for 20 min, virus particles redistribute. Region A is a peripheral area of the cell and has fewer particles than region B. Magnification $\times 125\,000$. Bar represents $1\ \mu\text{m}$. (From *J. Gen. Virol.* 53 (1981) 267–273 with permission.)

12.5. Biological properties

Coronaviruses bind to specific cell surface receptors via the surface projection glycoproteins (Fig. 12.9a) and redistribute to specific areas of cells (Fig. 12.9b). The marked host and tissue tropisms exhibited by coronaviruses are thought to be due to possible host cell receptor specificities. The entire replicative cycle occurs in the cytoplasm. Indeed, replication proceeds normally in enucleated cells.

Uptake of virus particles occurs by endocytosis. After 1–2 hours infection small endocytic vesicles with one or two virus particles are seen in ultrathin sections. Release of nucleocapsid probably occurs after fusion of the viral membrane with that of the endocytic vesicle. RNA is copied to produce mRNAs and subsequently protein products.

Complete virions are assembled in the rough endoplasmic reticulum (RER) or Golgi apparatus. Nucleocapsid is deposited beneath a length of membrane of the RER or Golgi vesicle in which surface projections are inserted. The vesicle then invaginates and is finally pinched off. Virus particles are released from the cell by 'reverse endocytosis' i.e. fusion of smooth-walled vesicles (Fig. 12.10, derived from the Golgi apparatus or RER) with plasma membrane, although a few reports suggest that cell lysis might occur (Fig. 12.11). Late in the infection period, virus particles may line the plasma membrane of a heavily infected cell.

PATHOGENICITY

Coronaviruses cause a wide range of disease in animals, including bronchitis in chickens, enteritis in calves, rats, mice and pigs, hepatitis in mice, peritonitis in cats, and encephalomyelitis in pigs. In man, disease is restricted to respiratory tract infections. These infections are generally mild and confined to the upper respiratory tract, although more serious lower respiratory tract infections can occur in children.

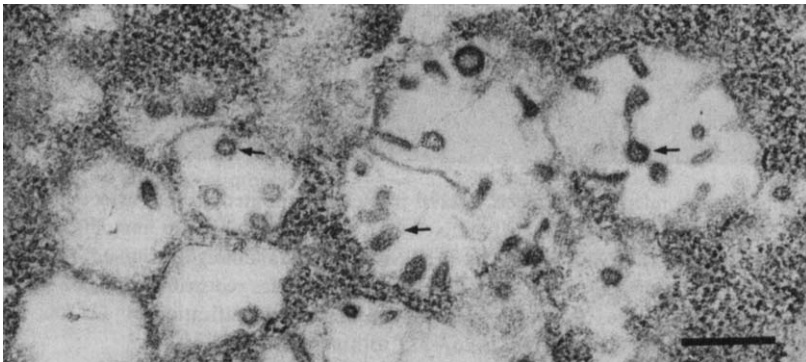


Fig. 12.10. HCV 229E particles (arrowed) in vesicles of an infected MRC continuous cell (MRCc cell line). Magnification $\times 41\,040$. Bar represents 300 nm.

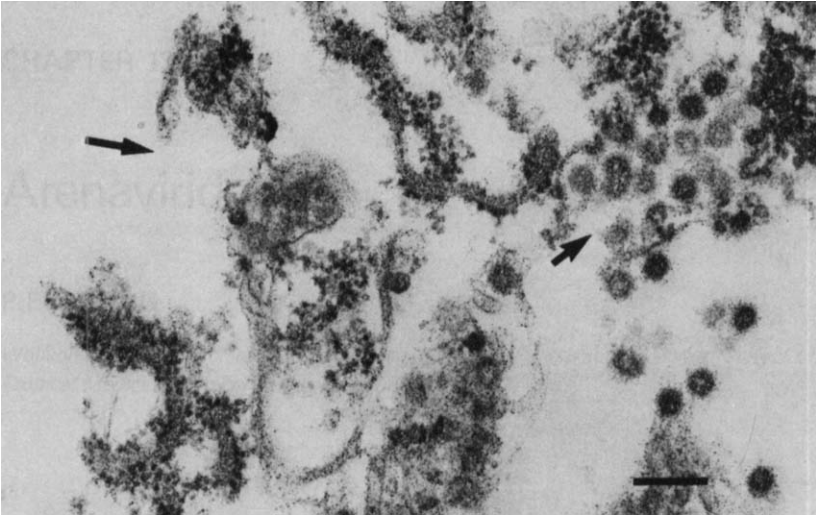


Fig. 12.11. Release of the coronavirus CV Paris from a lysed epithelial cell of calf colon. Note the absence of a limiting membrane at the edge of the cell (long arrow) and the virus-containing vesicle that has broken open (short arrow). Magnification $\times 47\,880$. Bar represents 200 nm.

Acknowledgements

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Suggested reading

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