VISNA VIRUS INFECTION OF SHEEP AND
HUMAN CELLS IN VITRO—AN
ULTRASTRUCTURAL STUDY

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SUMMARY

Comparison of human astrocytes (1181N1 line) and sheep choroid plexus cells (SCP) infected with visna virus (V-1181N1 and V-SCP) shows that the mode of virus assembly and the morphology and size of the free virions are identical in the 2 systems. V-SCP cells usually cytolyse soon after infection; V-1181N1 cells do not. This is considered to be due to interference with membrane assembly in the former. Syncytial giant cells from both V-1181N1 and V-SCP cultures have a much looser attachment to the growth surface than their uninfected parents. This is shown to be associated with a loss of certain microfibrillar arrays close to the plasmalemma. Uninfected SCP cultures have cilia and desmosomes and retain these after infection till cytolysis; 1181N1 cells do not have these structures, but have a well developed microtubular system, which the SCP cells lack.

INTRODUCTION

Visna virus, which causes a demyelinating disease of the brain in sheep (Sigurdsson, Pálsson & Grímsson, 1957; Sigurdsson, Pálsson & van Bogaert, 1962), proliferates in certain human astrocytes in vitro (Macintyre, Wintersgill & Thormar, 1972), producing characteristic cytopathic changes (Sigurdsson, Thormar & Pálsson, 1960). Syncytial giant cells are a constant and prominent feature of such changes in cultures both of sheep and of human cells infected with this virus (V-SCP and V-1181N1 cultures). Although the cytology of the syncytia from each species initially appears identical under light microscopy, there are major differences in their late behavioural response. Cytolysis is the usual fate of V-SCP cells; analogous human cells (V-1181N1) persist, are continuing to produce virus and to form syncytia (Macintyre et al. 1972) one year after initial infection. The 1181N1 line (Macintyre, Pontén & Vatter, 1972; Macintyre, Wintersgill, Perkins & Vatter, 1972) are cloned cells, originally derived from a brain tumour of astrocytes (Pontén & Macintyre, 1968).

The present ultrastructural studies were undertaken to seek differences among the sheep choroid plexus (SCP) cells, the human 1181N1 astrocytes, and their derivatives infected with visna virus (V-SCP and V-1181N1). Their mode of attachment to growth surfaces will be compared with that of 2 other cell types, both derived from this human astrocyte line. These are the EH-118MG cells, initially

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infected with the Engelbreth-Holm strain of Rous sarcoma virus (Macintyre, Grimes 
& Vatter, 1969), and the VRA cells infected with both Rous sarcoma and visna 
viruses (Macintyre & Wintersgill, 1972; Macintyre & Vatter, 1972). Previous reports 
on the ultrastructure of cultures infected with visna virus have concentrated upon 
the mode of maturation of the virus and the structure of the free virus (Thormar, 
1961; Thormar & Cruickshank, 1965; Coward, Harter & Morgan, 1970) in infected 
ovine choroid plexus, kidney and liver and in bovine tracheal cells. There is dis-
agreement about the nature of certain extracellular fragments (Coward et al. 1970). 
No changes in the organelles of infected cells have been reported. We have found 
ultrastructural features which distinguish SCP and 1181N1 cells, and which remain 
constant after infection. We have defined changes after infection which are common 
to both V-SCP and V-1181N1 cultures, and others which are unique to the pre-
cytophagic phase in V-SCP cells.

MATERIALS AND METHODS

Cells. The methods of primary culture, conditions for routine maintenance in vitro and 
variations employed for infection with visna virus have been reported both for the 1181N1 
human tumour astrocyte line (Macintyre, Wintersgill & Thormar, 1972; Macintyre, Pontén 
& Vatter, 1972; Macintyre, Wintersgill, Perkins & Vatter, 1972) and for SCP cultures (Sigurdsson 
et al. 1960). A detailed study of the Rous-transformed EH-118MG cells has been published 
(Macintyre et al. 1969).

Preparation for electron microscopy. The general techniques used in preparing cells for 
electron microscopy have been described in part (Macintyre, Pontén & Vatter, 1972). For 
the present studies, the cells were fixed in a mixture of ice-cold glutaraldehyde and osmium 
tetroxide (Hirsch & Fedorko, 1968). The stock solutions (2.5% glutaraldehyde and 1% 
osmium tetroxide, each in 0.1 M cacodylate pH 7.4) were mixed immediately prior to use 
(1 part glutaraldehyde to 2 parts osmium tetroxide); no precipitate of osmium formed. 1 ml 
of the mixture was added directly to every 5 ml of medium immediately after removing 
the culture vessel from the incubator, without disturbing the cells, and the preparation hereafter 
kept at 0 °C. Within a minute the fluid was replaced with 5 ml of the fixative for 5-10 min, 
when the cells were scraped off with a rubber policeman, pelleted by a brief (1-2 min) centri-
fugation in the cold; and further fixative was added to a total of 30 min.

Where cell surface examination was intended, the cells were prepared in situ in the culture 
well where they had grown, either on a layer of Epon polymerized on glass or directly on 
the plastic culture dish. The cultures were fixed as above, but without removing the cells.
Variations of preparative technique for electron-microscopic examination of Rous sarcoma 
virus-transformed 118 cells (EH-118MG) have already been reported (Macintyre et al. 1969).

Subsequent procedures were identical for both pellets and in situ preparations. Several 
rinses with 0.1 M cacodylate buffer, pH 7.4, at the customary 0 °C were followed immediately 
by 2 rinses with cold 0.1 M acetate buffer, pH 6.3, containing 0.25% uranyl acetate (Hirsch 
& Fedorko, 1968). After 20 min contact with this buffer and several washes of cacodylate buffer, 
the cells were dehydrated through 70, 80 and 97% hydroxypropylmethacrylate in water, 
allowing 5 min per concentration. Dehydrating and all subsequent procedures were carried 
out at room temperature. The cells were next infiltrated with a series of hydroxypropyl-
methacrylate and Epon mixtures, and embedded in Epon. The plastic sandwich containing the 
cells of in situ preparations was removed by breaking the container and sections were cut 
both parallel to the surface of the plate (face views) and at right angles to it (side views). 
Stereomicrographs of sections from in situ preparations in areas of, for example, possible cell 
fusion, or of complicated membranal pattern, were studied using a stage tilted from 8° left 
to 8° right.

Sensitivity to ribonuclease (Sigma Biochemical Company, Missouri) was tested by des-
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Intracellular location of antigens. The presence of visna virus-specific cytoplasmic antigen in V-SCP (Harter, Hsu & Rose, 1967; Thormar, 1969) and V-1181N1 cells (Macintyre, Pontén & Vatter, 1972) has been described. Its ultrastructural location was sought using a modification of reported techniques (Nakane & Pierce, 1967). All procedures were performed at 4 °C. The cells were fixed in situ with 10% formalin (prepared from paraformaldehyde) in 0.05 M phosphate buffer, pH 7.2, then washed 3 times with phosphate-buffered saline (PBS), pH 7.2, allowing 30 min per wash. The cells were exposed with gentle agitation to specific anti-visna virus antibody (from sheep) (obtained through the courtesy of G. Petursson, Keldur, Iceland) for 60 min, and then rinsed 3 times in PBS-sucrose, allowing 30 min per rinse. One hour’s exposure to peroxidase-labelled (Nakane & Pierce, 1967) rabbit antibody to sheep gamma globulin (Meloy Laboratories, California) was followed by 3 rinses with PBS-sucrose (30 min per rinse). The cells were fixed in 5% glutaraldehyde in cacodylate buffer, pH 7.4, for 30 min, washed 4 times with PBS-sucrose (60 min per rinse), then immersed in Karnovsky’s solution (without hydrogen peroxide) for 1 h, and in complete Karnovsky’s solution for 30-60 min. Thereafter, all procedures were carried out at room temperature. The cells were rinsed in 3 changes of distilled water (to a total of 30 min), then fixed with an aqueous solution of OsO4 for 1 h. Final processing for electron microscopy was as described above. (Complete Karnovsky’s solution is a saturated solution (20-30 mg per 100 ml) of 3,3'-diaminobenzidine (either free-base or salt) in 0.05 M Tris buffer pH 7.6 with 0.001% hydrogen peroxide.)

RESULTS

Ultrastructural details will be given in separate sections for the SCP and the 1181N1 cells (uninfected and infected). A brief description of the light microscopy of the respective cells will open each section.

Sheep choroid plexus (SCP) cells - before and after infection with visna virus. In culture, normal SCP cells are mononucleated, elongated and spindle-shaped (Fig. 2). They show density-dependent inhibition (Fig. 3) (Stoker, 1967). 3-5 days after infection with visna virus, refractile cells appear; these cover a smaller surface area (Figs. 4, 6) than uninfected cells, and their single nucleus is difficult to discern. The attachment of the altered cells is more tenuous than that of uninfected cells (see Fig. 1). Syncytial giant cells (Fig. 5) develop 1-3 days later (depending upon virus dose) in sites previously occupied by refractile cells; the syncytia are very loosely attached to the growth surface (Fig. 1). Cell shedding increases progressively from 5 days after infection; sloughed cells take up trypan blue, i.e. are dead. Usually, by 10-12 days after infection, all V-SCP cells have undergone cytolysis and the surface of the culture vessel is bare.

Uninfected SCP cells at terminal cell density (ted) (Fig. 3) lie in regularly arranged plates (Fig. 3). Parallel arrays of morphologically identical microfilaments fill the cell tips of contiguous cells. Stereomicrography suggests a physical connexion between neighbouring cells through this region (cf. Fig. 6). A mat of microfibrils fills the extracellular area, and appears to be directly continuous with subplasmalemmal arrays. Centrioles are easily found, usually as clusters, and often associated with the origin of cilia (Fig. 26). Microtubules are rare, except in association with centrioles. There is no hyperplasia of organelles or membranes. Pinocytotic vesicles are common. The nucleus is not remarkable.

The ultrastructure of refractile V-SCP cells 5 days after infection (Fig. 6, corresponding to Fig. 4) shows dendritic cytoplasmic areas which contain broad bands of
microfibrils, rare agranular membranes and vesicles, but no microtubules. At the tip of such dendritic processes, desmosomal junctions connect contiguous cells (Fig. 6, d). Extracellular microfibrillar arrays often lie separately from the plasma-lemma of the retracted cell. Virus buds (Fig. 6, v; Figs. 7–9) and associated extracellular virus aggregates have appeared. The virions are especially common on the under surface of the cell. The nucleus (obscured by cytoplasm in light microscopy) is not remarkable. Desmosomes, cilia, centrioles associated with microtubules (Fig. 26), and intracellular bundles of microfibrils (Fig. 11, mf) persist till cytolysis, but oedema (Figs. 6, 7–11) increases the difficulty of demonstrating them. Marked
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Hyperplasia of agranular vesicles (Figs. 6, 11, 12, 14, 15), focal development of granular endoplasmic reticulum and of cisternae filled with fine granular material (Figs. 11, 13, 14), are characteristic of all V-SCP cells and become prominent 6–7 days after infection. The number of membrane-associated ribosomes appears to decrease. The plasmalemma develops numerous pseudopodia, which often carry a well-defined crescent of electron-opaque material (Figs. 7–11), sensitive to ribonuclease; these crescents are usually single, but some multiple units are seen (Fig. 9). The crescent lies 10 nm beneath the plasmalemma, often at the apex of the pseudopodium (Figs. 7–11), but sometimes in other positions (Fig. 11, single arrow). Pseudopodia are especially numerous on the upper free surface of virus-producing cells. Many agranular vesicles or cisternae (Figs. 7–9) are seen in the cytoplasm near the base of the pseudopodia, as part of the general hyperplasia of this system. Electron-opaque dots 50 nm in diameter are constant findings within mitochondria (Figs. 12, 13). Free extracellular virus particles whose nucleoid is sensitive to ribonuclease are a common finding amongst cross-sections of pseudopodia (Figs. 10, 11, p). No special feature is seen in the nuclei at this period. Fig. 5 shows a syncytium from a V-SCP culture 7 days after infection.

Heterogeneity characterizes V-SCP cultures from 5 days onwards, and by 10–11 days after infection it is maximal, seen even within individual syncytia (Fig. 14). In this particular cell, the granular endoplasmic cisternae appear to carry the normal complement of ribosomes. V-SCP cells approaching cytolysis, i.e. 12–13 days after infection, show 2 unique features: (1) the accumulation of sheaves (Fig. 15, s) of

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**Fig. 1.** A. The uninfected SCP cell has broad pedicles of attachment to the vessel surface. Subplasmalemmal and extracellular microfibrillar arrays are associated with such pedicles (cf. Fig. 2).

B. 3–5 days after infection with visna virus, the V-SCP cell is attached by narrower pedicles and the distal borders of the cell have separated from the vessel surface. Extracellular microfibrillar mats are associated with the plasmalemma only at the pedicles (cf. Figs. 4, 6).

C. 7–9 days after infection, the V-SCP cells have many polykaryotic forms and still fewer pedicles. Microfibrillar arrays have disappeared from the areas which have separated from the vessel surface (cf. Figs. 16, 20, 21). V-1181N1 syncytia are similar.

D. The visna virus syncytia (V-SCP) in the later stages of infection carry most of the cell body on a stalk, which often contains microfilaments. Attachment pedicles are now very few. Nuclei and organelles cluster in the upper part of the cell. V-1181N1 syncytia are similar (cf. Fig. 11). Finally, the microfibrils disappear from the remaining pedicle (cf. Fig. 22, arrow) and the cell loses its attachment to the vessel.

E. The uninfected 1181N1 cell is firmly attached to the vessel through its length by well-defined subplasmalemmal arrays and a firm external microfibrillar mat.

F. After infection with Rous sarcoma virus (EH-118MG cells), the firm attachment with its microfibrillar arrays persists. Fused nuclei are common in this cell type.

G. This depicts the giant cell of the VRA group (infected by both visna and Rous sarcoma viruses). One part of the cell is firmly attached throughout its length, and has microfibrils arrayed in such areas. Associated nuclei are often fused (cf. EH-118MG, f). The other part of the same cell extends from a broad stalk as an irregular hydra-like mass with discrete nuclei (cf. V-1181N1, V-SCP, d, f). The gross architecture can be seen in vitro under light microscopy.
microfilamentous material amidst polysomes and ribosomes; and (2) of granular, electron-opaque aggregates (which appear to be RNA) (Fig. 16, g), both situated close to the nuclei of the syncytia. Nuclei are often pleomorphic; chromatin aggregation is common. Occasional autophagocytic vacuoles are observed in viable cells. Dead cells are frequent in these cultures.

**Localization of visna virus-specific cytoplasmic antigen.** Paranuclear granular endoplasmic cisternae (Fig. 18 cf. Fig. 13) contain visna virus-specific cytoplasmic antigen, as adjudged by the peroxidase-labelling technique. This finding holds for both V-SCP and V-1181N1 cells, and supports previous observations from fluorescence microscopy (Fig. 17) (Macintyre, Wintersgill & Thormar, 1972).

**Human astrocytes of the 1181N1 line before and after infection with visna virus.** In culture, the 1181N1 cells are long, tapering and spindle-shaped (Fig. 19). They show neither contact- nor density-dependent inhibition (Abercrombie & Heaysman, 1953; Stoker, 1967). The pattern of cell alterations after infection with visna virus mimics that just described for the V-SCP cells, with the development of refractile, dendritic cells followed by syncytia but there is no cell death (Macintyre, Wintersgill & Thormar, 1972). The most prominent cell in the altered culture is the syncytial giant cell (Figs. 1, 20, 21). The syncytium grows to a finite size, covers a predictable surface area, then retracts (Fig. 1), rounds up (Fig. 22, arrow) and floats off as a viable cell (as adjudged by its response to vital dyes). At the stage of growth depicted in Fig. 20, the cell will reattach after trypsinization; at the stage reached in Fig. 22, it will not.

The uninfected 1181N1 cell (Fig. 19) has no desmosomes, no cilia, centrioles are difficult to find and pinocytosis rare. There is marked hyperplasia of the granular endoplasmic reticulum, its cisternae and the Golgi complex. Lysosomes, polyribosomes, glycogen granules and microtubules (compare with Fig. 26) are numerous. The narrow extracellular space contains fine microfibrillar bands, which communicate with subplasmalemmal bundles (Macintyre, Pontén & Vatter, 1972).

After infection, the membrane systems remain well organized and generally hyperplastic in the V-1181N1 cells (Fig. 25). There is an increase in the agranular endoplasmic arrays. Occasional electron-opaque intramitochondrial dots are found. No paranuclear sheaves of microfilaments nor collections of granular material have been seen in the V-1181N1 cells. There is, however, irregularity of the cell surface, associated with the development of pseudopodia and buds containing electron-opaque crescents (Fig. 23), associated externally with clusters of virions identical in morphology with those produced by the V-SCP cultures (Fig. 24). The nucleoids are sensitive to ribonuclease. Subplasmalemmal oedema and proliferation of smooth membranal vesicles and cisternae (Fig. 23) are likewise associated with the areas of budding just described. The nuclei of the syncytia are not remarkable. Dead cells are not a feature of V-1181N1 cultures.

**Comparative attachment modes of uninfected (SCP, 1181N1) cells with derivates infected with visna virus (V-SCP, V-1181N1), Rous sarcoma virus (EH-118MG) and both viruses (VRA).** It is clear from Fig. 1 that the areas of attachment to the vessel surface differ among the above cell types. At one extreme are the 1181N1 astrocytes...
and the EH-118MG giant cells, which maintain a firm attachment along their entire length; at the other extreme are the visna virus-infected cells, which attach by ever-narrowing pedicles and eventually roll off. VRA cells infected by both Rous sarcoma and visna viruses show mixed features. All areas of attachment have sub-plasmalemmal bands and extracellular arrays of microfibrils; on stereomicroscopic examination, these appear to be continuous through the plasmalemma. When the subplasmalemmal arrays disappear and the external microfibrils lose their connexion with the plasmalemma (as in visna virus infection) the attachment with the surface is lost. The stalk (Fig. 1) of a visna virus syncytium often contains microfibrils; its nuclei and organelles accumulate above the stalk region; no cell membranes lie between these nuclei. Loss of adhesion to the vessel surface is therefore a separate phenomenon from cytolysis.

**DISCUSSION**

The above reports compare the ultrastructure of human and sheep cells infected in vitro with visna virus (V-1181N1 and V-SCP cells). It is clear that the final steps in production of viral RNA and assembly of the virion, as well as the virion itself, are morphologically identical in the 2 species. We subscribe to the theory (Thormar, 1961; Thormar & Cruickshank, 1965) that visna virus RNA assembles as crescents under the plasmalemma and subsequently buds off as a virion from the cell surface. The use of our in situ technique has demonstrated that short pseudopodia are frequent in such areas; we believe that the structures described as a new type of virus particle (Coward et al. 1970) are rather sections of such pseudopodia. On rare occasions, we have seen, under stereomicroscopy, 3-nm diameter strands in parallel within the crescent region as well as in the virion; this is consistent with a helical structure as suggested for the oncornaviruses (Sarkar, Nowinski & Moore, 1971) with which visna virus has many features in common (Thormar, 1971). Additional analysis is needed.

We should like to single out the following 4 'artifacts' for comment. More virions appear to be produced on the lower surface of cells, probably because fluid movement is less free in this area; secondly, the variability of ultrastructural changes seen within one culture after infection stems partly from the fact that the virus is added at terminal cell density (Macintyre, Pontén & Vatter, 1972), therefore the topmost cell layers are infected first; thirdly, the 'hyperchromaticity' of the nucleus of the early altered cell is due to the accumulation of retracted cytoplasm on top of the nucleus, and not to chromatin condensation; fourthly, centrioles seem more abundant in the SCP than in any of the numerous other cell types examined by us (Macintyre et al. 1969; Macintyre, Pontén & Vatter, 1972; Macintyre, Wintersgill, Perkins & Vatter, 1972); this is probably attributable to their plate-like growth pattern in vitro as well as to the cell type.

We should like to postulate that there is interference with membrane formation in the V-SCP cells. The continuing decrease in ribosomes associated with the granular endoplasmic reticulum in V-SCP cells, the accumulation of sheaves of membrane-
like material amidst ribosomes and polysomes and of aggregates of RNA material in precytolytic V-SCP cells support this concept. The opaque granules within mitochondria of the V-SCP cells resemble polyphosphates (Jensen, 1968), which are a recognized component of many bacteria, accumulate when nucleic acid synthesis decreases (Harold, 1963, 1966), and contain energy-rich phosphate (Lipmann, 1965). They have been reported in the mitochondria of rat cells (Lynn & Brown, 1963), but this work has not been confirmed (Stahl, Felter & Ebel, 1967; Harold, 1966). These granules are rare within the mitochondria of uninfected SCP and V-1181N1 cells; but common in V-SCP mitochondria.

Microfibrils appear to be important for attachment of cells to growth surfaces. For example, disruption of either the external or the internal arrays after infection with visna virus is accompanied by loss of attachment to the growth surface (Fig. 1). The location of the visna virus-specific cytoplasmic antigen is identical in both species, and there is hyperplasia of the smooth endoplasmic reticulum system.

The differences between the fine structure of uninfected SCP and 1181N1 cells result from their cells of origin. Choroid plexus cells are a specialized form of ependyma, a surface lining cell, and produce cerebrospinal fluid. Desmosomal linkages, cilia and pinocytosis are notable features. Astrocytes lie within the brain, intimately associated with neurons; their function is still obscure. The 1181N1 line of human tumour astrocytes has many of the morphological characteristics of normal astrocytes (Macintyre, Pontdn & Vatter, 1972; Macintyre, Wintersgill, Perkins & Vatter, 1972), with well-developed arrays of microtubules, no desmosomes and no cilia. The 1181N1 cells have in addition generalized hyperplasia of membranes and organelles. However, both choroid plexus cells and astrocytes are easily infected by visna virus.

In conclusion, we have demonstrated that the virus produced from V-1181N1 and V-SCP cultures is identical as regards morphology and mode of assembly. The V-SCP cells die because of apparent interference with assembly of membranous components; membrane integrity is preserved in the V-1181N1 cells. The decreased adhesiveness to vessel surfaces of cells infected with visna virus is associated with loss of certain microfibrillar arrays and is probably a separate phenomenon from cytolysis.

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REFERENCES


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Fig. 2. Sheep choroid plexus (SCP) cells grow as an orderly array of mononucleated, spindle-shaped cells. Wright Giemsa, × 400.

Fig. 3. Side view of an in situ preparation of uninfected SCP cells at terminal cell density. The surface of the culture vessel is indicated by an arrow; the upper layer of the culture is represented by the cell at the top of Fig. 3. The cells grow in regular layered plates, attached by narrow pedicles to the growth surface. The extracellular area is filled by a dense matrix of microfibrils. Broad bands of microfibrils fill the tips of the cells, and are also seen beneath the plasmalemma and deeply within the cytoplasm. Micronemes seem to be absent. Organelles are normal; lysosomes (l) are rare and polysomes (p) are not abundant. Electron micrograph, in situ preparation, × 22,000.

Fig. 4. This V-SCP culture was infected at terminal cell density 5 days previously with 6TCID₆₀ visna virus/cell. This was the usual amount of virus used. The cells of the upper layers of the culture have retracted cytoplasm; their borders appear dendritic and their nucleus hyperchromatic. The nuclei and cytoplasm of cells which lie deeper in the culture appear normal. Wright Giemsa, × 400.

Fig. 5. This is a syncytium from a V-SCP culture, 7 days after infection. The cytoplasm is voluminous, thinly spread and covers a surface area some 20 times greater than does an uninfected SCP cell. Its multiple nuclei are almost always discrete and similar to those of uninfected cells. Wright Giemsa, × 500.
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Fig. 6. This is a face view of an in situ preparation of a V-SCP culture, taken 5 days after infection with visna virus. It depicts the fine-structural features of the refractile cells of Fig. 4. Contiguous cells connect at desmosomes (d). The elaborate dendritic processes contain microfibrils but no microtubules. Virus budding (v) and associated clusters of extracellular virus are common. At this early stage, the viruses are frequently associated with subplasmalemmal microfibrillar bundles as seen in the lower part of this micrograph. The cytoplasm is loosely textured and contains many agranular vesicles. In situ preparation, × 14,000.

Figs. 7–9. These are 3 areas of surface budding with associated external clusters of virions, from a V-SCP culture infected for 5 days. The presence of agranular endoplasmic reticular forms and their cisternae (arrows) is common at the base of the pseudopodial stalk in which the budding virion develops. The cytoplasm is very loosely textured. The crescent of electron-opaque material of the bud is 7 nm thick and lies 10 nm beneath the inner plasmalemmal leaflet. Most of the virions are between 85 and 90 nm in diameter; a few are larger (120 nm). The nucleoid is eccentrically located within the envelope and 40 nm in diameter. Some particles are empty; most carry one nucleoid; some have double nucleoids. Fig. 7, × 90,000; Fig. 8, × 80,000; Fig. 9, × 104,000.

Fig. 10. This is part of the upper surface of a V-SCP cell 7 days after infection. Cross-sections of pseudopodia (ps), virions, surface budding with crescent formation, and marked hydropism of the cell cytoplasm with perhaps a decrease of membrane-associated ribosomes are features of this micrograph. (See also Fig. 1.) × 37,000.
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Fig. 11. This is a side view of part of the upper surface of a V-SCP syncytium 9 days after incubation. Numerous pseudopodia (ps) of many sizes extend from the cell surface. They often carry electron-opaque crescents beneath the plasmalemma (arrow), and are sometimes packed with agranular vesicles. Microfibrils (mf) are perinuclear. There is marked hyperplasia of the agranular vesicles; the granular endoplasmic reticulum appears to have fewer associated ribosomes. (See also Fig. 1.) In situ preparation, × 25,000.

Figs. 12, 13. These are representative portions of cells from the same culture as in Fig. 11. Electron-opaque dots 50 nm in diameter are common within mitochondria. There is a remarkable hyperplasia of both the agranular and the granular endoplasmic reticular systems, together with hydropism of associated cytoplasm. Membrane-associated ribosomes again appear to be fewer. The nuclei are not remarkable. Electron micrographs, Fig. 12, × 13,500; Fig. 13, × 14,000.
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Fig. 14. This is a peripheral area of a V-SCP syncytium 11 days after infection. Free virions and surface budding are shown. The cytoplasm has numerous plates of granular endoplasmic reticular elements in parallel stacks. The surrounding region is filled with a finely filamentous meshwork, ribosomes and rare agranular vesicles. Two other areas within the same cell have large clusters of agranular cysternae and rare lysosomes. The nucleus of the cell (not shown) had patches of chromatin aggregation. x 32000.

Fig. 15. The paranuclear cytoplasm of V-SCP cells 12-13 days after infection contains in some areas an accumulation of filamentous structures (12 nm in diameter) in sheaves (s). These structures lie amidst numerous ribosomes. There is a marked accumulation of agranular endoplasmic vesicles. x 40000.

Fig. 16. This cell from the same culture as Fig. 15 shows varying sized aggregates (g, 100-130 nm in diameter) of irregular electron-dense material close to the pleomorphic nucleus. Ribosomes and polyribosomes neighbour these aggregates. There is moderate hydropism and agranular endoplasmic reticular hyperplasia. There is some lipid accumulation (li). x 14000.

Fig. 17. The fluorescence of the visna virus-specific cytoplasmic antigen in this V-1181N1 cell is most prominent in the perinuclear region. Indirect fluorescence, x 1000.

Fig. 18. Paranuclear cysternae of the granular endoplasmic reticulum contain a marked accumulation of the cytochemical product 'osmium black' (arrows), indicative of the presence of visna virus-specific cytoplasmic antigen. Peroxidase-labelling technique, x 25000.
Fig. 19. The 1181N1 line of human tumour astrocytes consists of very elongated spindle-shaped cells which grow in irregular mutilayers and do not stop mitosing, synthesizing DNA or moving with either contact or high density. Wright Giemsa, × 300.

Fig. 20. This V-1181N1 syncytium was taken from a culture 9 months after initial infection. The size and the numerous discrete nuclei are identical with V-SCP syncytial characteristics (Fig. 5). Wright Giemsa, × 500.

Fig. 21. This phase photograph of a smaller V-1181N1 syncytium from the same culture as Fig. 19 shows a horseshoe-shaped arrangement of individual nuclei and tenuous cytoplasm. Live culture, phase contrast, × 500.

Fig. 22. The arrow marks a syncytial giant cell which has rounded up and is about to roll off (see also Fig. 1). Live culture, phase contrast, × 500.

Fig. 23. This is a portion of a V-1181N1 cell, 5 months after infection with visna virus. The plasmalemma is irregular; buds containing well defined electron-opaque crescents are identical with those in V-SCP cells (Figs. 7–11). These develop at the surface of the cell and are especially prominent in the pseudopodia (not shown in this micrograph). Supporting cytoplasm is oedematous, carries agranular vesicles, cysternae and some polysomes. × 55 000.

Fig. 24. This cluster of extracellular particles was seen in a V-1181N1 culture 5 months after infection. It includes typical visna virions of 85 and 120 nm diameter, with usually a single eccentric nucleoid 40 nm in diameter. There are also some apparently empty capsids. Longitudinal (arrow) and cross-sections of pseudopodia are seen among the virions. × 61 000.

Fig. 25. This appearance is characteristic of V-1181N1 cells and was seen in a culture 7 months after infection. All membrane systems stay intact. The microtubules are well defined. There is no decrease in membrane-associated ribosomes. The agranular endoplasmic vesicles and cysternae are hyperplastic. Electron-opaque dots are rarely seen in V-1181N1 mitochondria. Electron micrograph, × 50 000.

Fig. 26. This depicts an area of centriolar activity with cilia production in a V-SCP cell 3 days after infection. The findings in this area are identical with those of an uninfected cell. This is the one area in the sheep choroid plexus cells where microtubules are always found. × 30 000.