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The Ultrastructure of Multinucleate Giant Cells

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Abstract—A survey of the available ultrastructural data on physiologically and pathologically occurring and virally-induced multinucleate giant cells (MNGCs) is presented. Emphasis is initially placed upon the bone osteoclast, the skeletal muscle myotube and the placental syncytiotrophoblast. The widespread occurrence of MNGCs in a range of pathological situations is discussed, with emphasis upon the broad involvement of the macrophage in inflammatory responses. Many viruses produce cell fusion *in vivo* and *in vitro* when cell cultures are infected. Several examples are given. A clear distinction is drawn between viral fusion from “without” and viral fusion from “within” the cell. The cytopathic effect (CPE) of the animal and human retroviruses is discussed in considerable detail. The *in vivo* and *in vitro* formation of lymphocytic and macrophage MNGCs by HIV-1 is given extensive coverage. The possible significance of the presence of brain MNGCs of macrophage/microglial origin as a cellular feature of AIDS dementia is discussed. A new hypothesis is advanced relating to the possible role of endogenous C-type retrovirus in the physiological fusion of the invasive placental cytotrophoblasts to create the syncytiotrophoblast. The evolutionary and developmental significance of such an event in relation to the evolution of the placental mammals is discussed. The possible importance of MNGC formation in the depletion of the CD4⁺ population of T-lymphocytes *in vivo* in the clinical progression of the AIDS-related complex and AIDS is related to the potent fusogenic effect of HIV-1 when cell cultures are infected.

Key words: Multinucleate giant cell, syncytia, cell fusion, HIV-1, syncytiotrophoblast, endogenous retrovirus.

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LIST OF ABBREVIATIONS

| | | | |
|------|---------------------------------------|-------|---------------------------------------|
| AIDS | Acquired immune deficiency syndrome | CPE | Cytopathic effect |
| ARC | AIDS-related complex | CMV | Cytomegalovirus |
| ATLV | Adult T cell leukaemia/lymphoma virus | EBV | Epstein-Barr virus |
| BHK | Baby hamster kidney | HIV | Human immunodeficiency virus |
| BIV | Bovine immunodeficiency virus | HSV | Herpes simplex virus |
| BLV | Bovine leukaemia virus | HTLV | Human T cell leukaemia/lymphoma virus |
| BSV | Bovine syncytial virus | HSFoV | Human syncytium forming virus |
| CAEV | Caprine arthritis-encephalitis virus | LAV | Lymphadenopathy associated virus |
| | | MVV | Maedi/Visna virus |
| | | MHV4 | Corona mouse hepatitis virus type 4 |

| | |
|-------|-------------------------------------|
| MNGC | Multinucleate Giant Cell |
| MuMTV | Murine mammary tumour virus |
| PEG | Polyethylene glycol |
| PPV | Progressive pneumonia virus |
| rER | Rough endoplasmic reticulum |
| RSV | Respiratory syncytial virus |
| RT | Reverse transcriptase |
| SFoV | Simian foamy virus |
| SIV | Simian immunodeficiency virus |
| SLE | Systemic lupus erythematosus |
| SSPE | Subacute sclerosing panencephalitis |
| STLV | Simian T-lymphotropic virus |
| SV5 | Simian virus 5 |
| UV | Ultraviolet |
| VZV | Varicella zoster virus |

I. INTRODUCTION

Interest in viral adhesion to cells has been in the forefront of virological studies for many years, stemming from the widespread haemagglutinating activity of viruses (Howe and Lee, 1972) and the use of Sendai virus for the study of membrane fusion, using both mammalian and avian erythrocytes and nucleated cells (Hosaka, 1988; Ringertz and Savage, 1976). Extensive biochemical studies have been performed using chemical cell fusion agents, such as polyethylene glycol (PEG) (Kersting *et al.*, 1989; Lucy and Ahkong, 1989; Wang *et al.*, 1982) and lignin derivatives (Sorimachi *et al.*, 1990), and also by the electrofusion procedure (Gallagher *et al.*, 1990; Zheng and Chang, 1991), but these approaches will not be dealt with within this review. Although many studies have been directed towards the production of viable binucleate heterokaryons (Harris, 1970) it is clear that multinucleate giant cells (MNGCs) will also be formed, but that these are likely to be lost during subsequent cell culture since they do not remain viable for long periods of time.

In normal tissues there are very few examples of naturally occurring syncytia or MNGCs. This is undoubtedly due to the extremely specialized histological and physiological conditions that in general would have to be created to maintain their viability. Cardiac muscle is not a true syncytium, although it is considered to be a functional syncytium, with its highly ionically permeable gap junctions providing electrical communication between the cells and intercalated discs providing tissue strength. Multinucleate skeletal muscle myotubes, on the other hand, are true syncytia derived from the developmental fusion of myoblasts, leaving the nuclei spaced longitudinally and somewhat flattened, just beneath the myolemma. The cyst-like insect spermatocyte syncytium (Philips, 1970) may not be a true syncytium, whereas it is accepted that in mammalian spermatogenesis true syncytial clones are present during the development of the spermatogonia and during meiotic division yielding the spermatocytes and spermatids (Dym and Fawcett, 1971).

Similarly, the maturation of a functional group of monocyte/macrophages designated to become osteoclasts is believed to occur due to controlled fusion events (Pierce *et al.*, 1991; Marks and Popoff, 1988; Udagawa *et al.*, 1990). An interesting situation exists in insect eggs, which has been most clearly documented for *Drosophila*. The zygote nucleus undergoes several mitotic divisions within the centre of the egg, without separate cells being formed. This is followed by the progressive migration of nuclei within the ooplasm to the surface of the egg, where mitosis continues in the absence of cytokinesis. The cellular structure so formed is termed the syncytial blastoderm and it ultimately becomes the cellular blastoderm, due to infolding of the oocyte membrane between the monolayer of surface nuclei. The formation and lifespan of the placental syncytiotrophoblast, by fusion of the embryonic precursor cells of the developing mammalian embryo, termed the primitive syncytial trophoblastic cells and later the placental cytotrophoblasts or Langhans' cells, presents an extremely specialized situation (Jones and Fox, 1991). The uniquely prolific trophoblastic cells surround the blastocyst cavity and form a specialized region, the syncytial plate, the invasive precursor to the placenta. In all mammals the syncytiotrophoblast clearly has a limited lifespan, in all species correlating functionally with the length of pregnancy. Remarkably, at varying stages of the syncytiotrophoblast lifespan it will be shown to bear several ultrastructural features (Section II.B) which parallel those of the large syncytia of HIV-induced lymphoid cells (Section IV.G and H) forming several days post infection in suspension cultures.

MNGCs occur *in vivo* in a number of pathological disorders (Chambers, 1978), particularly in tumours and diseases of viral origin. Significantly, the mononuclear macrophage appears to be involved in many instances of inflammatory lung infection, and is believed to account for the microglial MNGCs found within the brains of AIDS patients expressing neurological complications (AIDS encephalopathy/dementia) (Sharer *et al.*, 1985; Takeya *et al.*, 1991). Whether or not syncytial formation *in vivo* is of importance in relation to the slow depletion of the CD4⁺ (T4) T-lymphocyte population during the progression of ARC/AIDS is not at the moment completely clear, but this remains a distinct possibility (Haseltine, 1990, 1991). MNGCs have been detected in the lymph nodes (Beneviste *et al.*, 1988), lung, spleen and brain of AIDS patients and primates infected with SIV, but not in buffy coat lymphocytes. It is reasonable to expect that any bi- or tri-nucleate cells present in the circulation would be selectively removed by passage through the spleen and thereby go undetected in samples taken from the peripheral circulation (Harris *et al.*, 1989; Haseltine, 1990). Undoubtedly, because of the very low numbers of activated viral-producing cells during the earlier stages of AIDS/ARC, there will be a vast excess (some 1:500 to 1:50,000) of non-infected or unactivated latently infected circulatory and tissue CD4⁺ T-lymphocytes available for fusion.

II. NATURALLY OCCURRING MULTINUCLEATE GIANT CELLS: DEVELOPMENT AND PATHOLOGY

A. The bone osteoclast

It is widely accepted that the multinucleate osteoclast (Fig. 1), responsible for bone resorption, is derived from extra-skeletal granulocyte-macrophage mononuclear precursors, by a process of cell fusion rather than cell division without cytokinesis (Marks *et al.*, 1983; Pierce *et al.*, 1991). Most of the evidence for this proposal comes from light microscopical data (Udagawa *et al.*, 1990), which although convincing when applied to *in vitro* culture systems is less so when derived from

in vivo samples purporting to show bone osteoclast or odontoclast formation. That the osteoclast is derived from a blood-borne precursor monocytic cell has been conclusively shown by the production of osteoclast-like cells from monocyte cultures from chicken hatchlings (Ostoby *et al.*, 1980). In this study a combination of light microscopy, scanning and transmission electron microscopy was used. Nevertheless, the available transmission electron microscopical evidence for the macrophage-macrophage fusion event is extremely limited (Sasaki *et al.*, 1989a, b; Sutton and Weiss, 1966) and not entirely convincing. Figure 2, taken from Wise *et al.* (1985), shows highly suggestive evidence for the fusion of macrophage/monocytes with a bone osteoclast. The direct contact between the monocyte plasma membrane

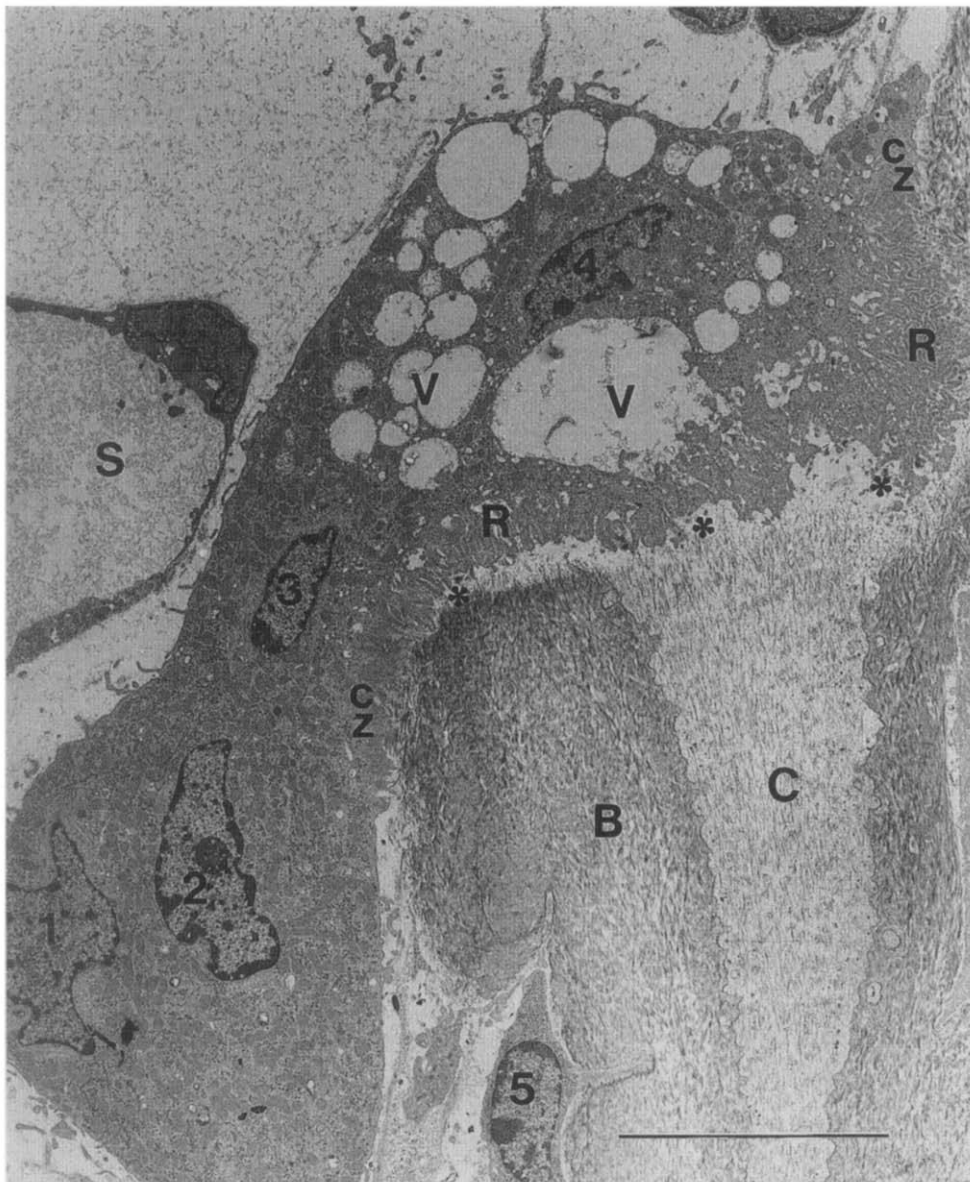


Fig. 1. An electron micrograph showing an osteoclast. Four nuclei (numbered 1 to 4) are shown in this section. This multinucleate cell is attached to both the bone (B) and calcified cartilage (C) by clear zones (CZ) and a ruffled border (R). The bone and cartilage surfaces below the ruffled border are frayed (asterisks) compared to smooth surfaces beneath the clear zones. Active osteoclasts have a vacuolated cytoplasm next to the ruffled border. These vacuoles (V) are large and indeed visible by light microscopy, and their presence is a reliable indicator of bone resorption. Large venous sinuses (S) are often seen next to osteoclasts. An osteocyte (5) is seen in the lower part of the figure. The scale bar indicates 10 μm . From Marks and Popoff (1988), with permission.

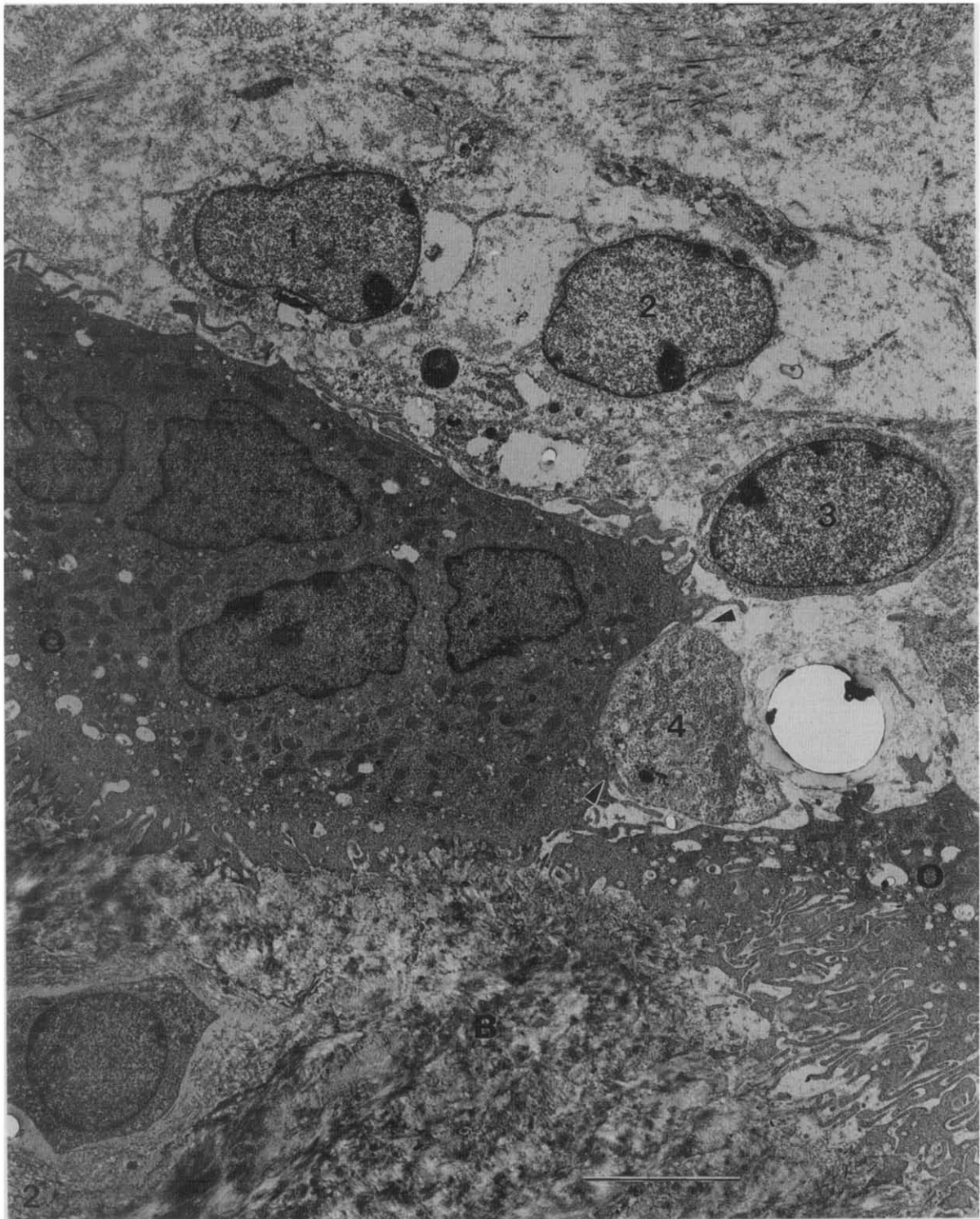


Fig. 2. An electron micrograph showing part of two osteoclasts (O) lining the bony crypt (B) of an erupting dog third premolar at 18 weeks. Closely adjacent to the osteoclasts are four monocytes (labelled by number). Monocyte number 4 appears to be in the process of fusing with one of the osteoclasts (i.e. between the arrowheads). The scale bar indicates $5\ \mu\text{m}$. Modified from Wise *et al.* (1985), with permission.

and the plasma membrane of the multinucleate osteoclast is indicated, but the differential staining density of the cytoplasm of the two cells suggests that cytoplasmic mixing has not yet occurred. Cultured monocyte/macrophages have been found to form MNGCs, when stimulated by interleukin-4 (McInnes and Renick, 1988) or insulin-like growth factor-1 (Scheven and

Hamilton, 1991). Similarly, Kassem *et al.* (1991) have produced osteoclasts in longterm bone marrow cultures in the presence of 1,25-dihydroxyvitamin D_3 . It is to be hoped that aliquots taken for electron microscopy from such osteoclast-forming cultures may clarify the early stages of macrophage fusion in the near future.

It has been shown by James *et al.* (1991) using monoclonal antibodies, that true osteoclasts can be distinguished from apparently similar MNGCs derived from a variety of different human tissues. Indeed cytochemical differences between osteoclasts and other

MNGCs were also detected by Zheng *et al.* (1991), strongly supporting derivation from the macrophage lineage yet indicating separate development and functional specialization of the osteoclast. *In vivo*, the osteoclast possesses unique structural features,

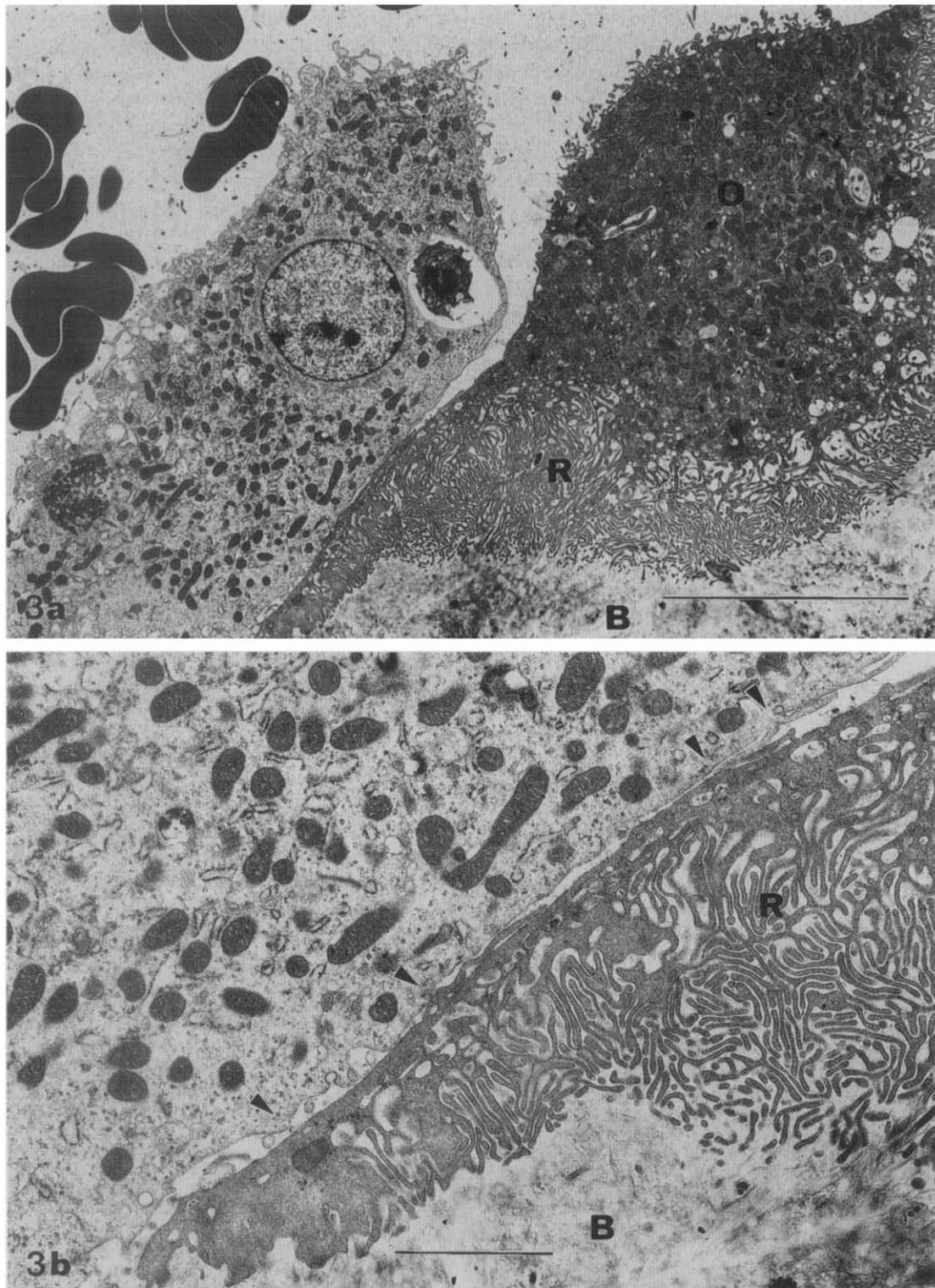


Fig. 3. (a) Part of an osteoclast (O) in the coronal portion of a dental crypt displaying an extremely pronounced ruffled border (R) adjacent to the bone (B). The identity of the cell at the LHS of the figure is uncertain, although its numerous mitochondria suggest that it may also be an osteoclast, and that areas of contact may be forming between the two cells, seen more clearly in (b) (arrowheads). Scale bars indicate 10 μm (a) and 2.5 μm (b). Modified from Marks and Cahill (1986), with permission.

intimately related to its functional role in bone resorption (Figs 1 to 3). Nuclei are distorted, with marginated heterochromatin. Facing the bone surface or calcified cartilage is a ruffled border of flexuous filopodia or microvilli, behind which is a clear cytoplasmic zone, free of organelles. Vacuolation of the osteoclast cytoplasm is a common feature, usually interpreted as being indicative of active bone resorption. The membrane surface of the osteoclast not facing the bone is smoother, with a sparse coating of short filopodia/microvilli. Detail of the undulating ruffled border of an osteoclast, actively resorbing bone during tooth eruption, is shown in Fig. 3. The creation of an acidic calcium-solubilizing environment by the osteoclast is due to the metabolic activity of the ruffled border H^+ -ATPase (for further details see Pierce *et al.*, 1991). A three-dimensional TEM study of the mouse osteoclast has been performed by Doman and Wakita (1991), who were able to distinguish ultrastructurally between migrating and bone resorbing osteoclasts. Emphasis was placed in this investigation upon the morphological classification of the clear zone between the central nucleated region of the MNGCs and the ruffled border. Knowledge of osteoclast structure and function is of vital importance for the further understanding of the hormonal control of mineral resorption in bone and teeth, as well as of bone diseases such as osteoporosis.

B. The placental syncytiotrophoblast

The ultrastructural and cytochemical changes that occur throughout the 280-day lifespan of the human placenta have been described in detail by Jones and Fox (1991). Thus, only selected ultrastructural features will be presented here, with emphasis upon the MNGC theme and comparison with the other MNGCs included within this review. The continuing growth of the syncytiotrophoblast (Fig. 4) by fusion with underlying cytotrophoblasts is the accepted mechanism for its formation, throughout approximately the first six months of pregnancy. Contrary to the osteoclast and myotube, the fusion event is much easier to demonstrate electron microscopically in placental tissue than is the case for the bone osteoclast and myoblast, and convincing evidence for cellular fusion has been available for at least 25 years (Carter, 1964; Pierce and Midgley, 1963; Pierce *et al.*, 1964). Nevertheless, it must be remembered that a vast amount of data of a histological nature, on the mammalian embryo, the normal and cancerous placenta exists at the light microscopical level (Elston, 1987; Larsen *et al.*, 1991; Kurmen, 1991; Shanklin, 1990).

Figure 5 shows part of a first trimester human placenta. The highly vacuolated syncytial layer, containing distorted nuclei with a peripheral patchy heterochromatin distribution, lies adjacent to the cellular cytotrophoblast (Langhans' cell) layer, with a cell in mitosis. Often a marked difference between the low

electron density of the chromatin in the metabolically active cytotrophoblast nuclei and that of the electron dense inactive syncytiotrophoblast nuclei is clearly apparent. This comparative feature can be somewhat variable in the first trimester placenta, as shown in Fig. 6, where the difference between the cytotrophoblast and the syncytiotrophoblast nuclei is less marked. The second trimester placental syncytiotrophoblast is characterized by possessing an extremely pronounced "scalloping" of the microvillar surface (Fig. 7), together with increased underlying cytoplasmic vacuolation, indicative of the cellular activity associated with the provision of the metabolic and nutritional requirements of the growing foetus. That cellular fusion is continuing to occur at a significant level throughout the second trimester is indicated by detection of cells of intermediate category, showing phenotypes very similar to the syncytiotrophoblast, but with desmosomal junctions still present (Fig. 8a). Thus, the process of cell fusion is unquestionably indicated by these residual desmosomal junctions, around which the plasma membrane disintegrates (Fig. 8a and b) giving complete cytoplasmic continuity, i.e. true fusion as opposed to merely cellular adherence. The physiological benefit to be gained from having a fused cell/syncytial layer immediately facing the maternal circulation is that a more complete and continually dynamic barrier to penetration by maternal cells is achieved than could be the case with a cellular layer maintained by desmosomes. Although there is little ultrastructural evidence for the presence of tight junctions (Jones and Fox, 1991), they may well play an important role in the species which possess a cellular trophoblast layer.

Towards full term, the syncytiotrophoblast nuclei progressively cluster into the so-termed syncytial "knot", with exclusion of the intervening cytoplasm and they become increasingly shrunken and electron dense (Fig. 9a). At this stage the amount of syncytiotrophoblast cytoplasm which is devoid of nuclei can become extensive (cf. Section VI.E and G). Syncytial nuclear "knots" in the post-mature placenta show varying levels of pyknosis (Fig. 9b). Nuclear breakdown may even be detected within the syncytial "knots" (Fig. 10a), an occurrence which is even more apparent under conditions of Rhesus incompatibility (Fig. 10b). (Figures 4 to 10 have been kindly made available by Dr Carolyn J. P. Jones.) The cytotrophoblast and syncytiotrophoblast appear to be susceptible to infection by HIV, although the involvement of the CD4 antigen is not decided. Thus, the placenta may provide the direct route of foetal infection by HIV from the mother (Jauniaux *et al.*, 1988).

Some considered speculations as to the possible endogenous retroviral induction of cytotrophoblast fusion and its significance for syncytiotrophoblast formation will be advanced within the framework of a new hypothesis for the evolution of placental mammals (Harris, 1991) (see Section V.A, below).

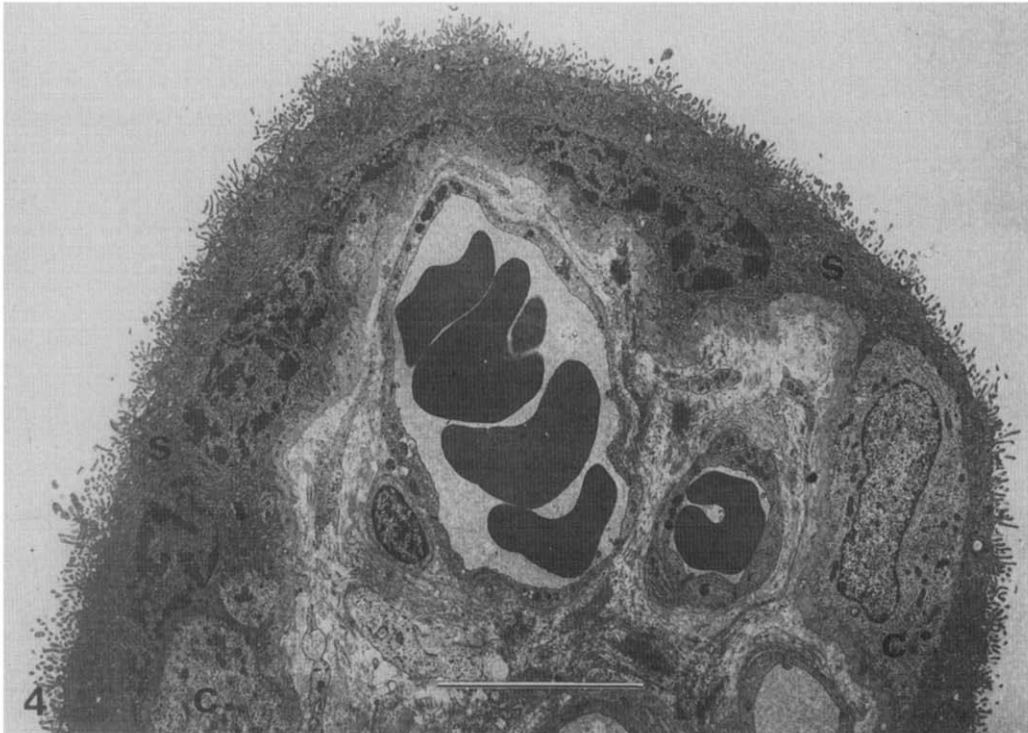


Fig. 4. Part of a human placental full term tertiary chorionic villus showing the thin syncytial/multinucleate nature of the villated trophoblast layer (S), together with the underlying cytotrophoblasts (C). The scale bar indicates 10 μm . Print kindly made available by Dr C. J. P. Jones.

C. *The skeletal muscle myotube*

During embryological development skeletal muscle is formed by the end-to-end fusion of the myoblasts.

This fusion is believed to involve cellular metalloendoproteases (Lucy and Ahkong, 1989; Pearson and Epstein, 1982). Migration of the centrally located myoblast nuclei to the surface of the myotube occurs,

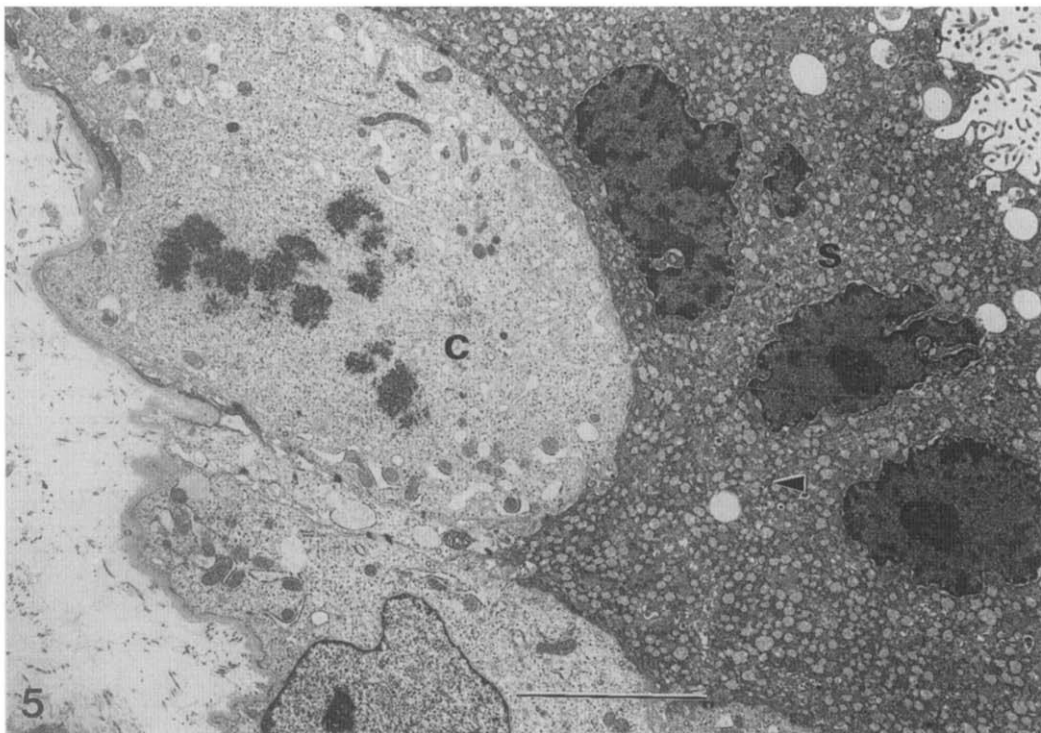


Fig. 5. Part of a first trimester human placenta, showing a cytotrophoblast in mitosis (C) and the multinucleate and vacuolated nature of the adjacent syncytiotrophoblast (S). The syncytiotrophoblast nuclei are shrunken and already slightly pyknotic. The scale bar indicates 5 μm . Print kindly made available by Dr C. J. P. Jones.

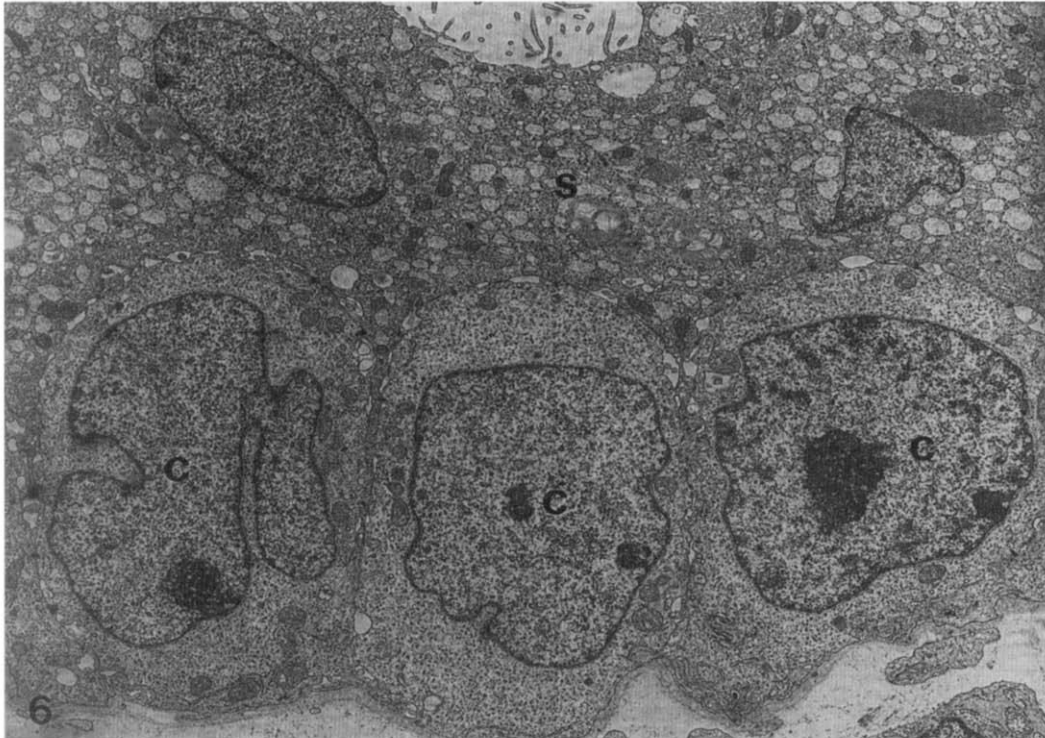


Fig. 6. Part of a third trimester human placenta showing three undifferentiated cytotrophoblasts (C), containing numerous polyribosome clusters rather than extensive rough endoplasmic reticulum, which here form an almost continuous layer adjacent to the vacuolated syncytiotrophoblast (S). Note that in this example the syncytiotrophoblast nuclei are relatively smooth and are not as electron dense as those shown in Figs 4 and 5. Print kindly made available by Dr C. J. P. Jones.

together with nuclear flattening, resulting generally in the nuclei being well spaced and located immediately beneath the myolemma (Fig. 11). Some periodic

invagination of the nuclear envelope of skeletal muscle nuclei is apparent, which sometimes appears to correlate with the surrounding actinomyosin Z-line period-

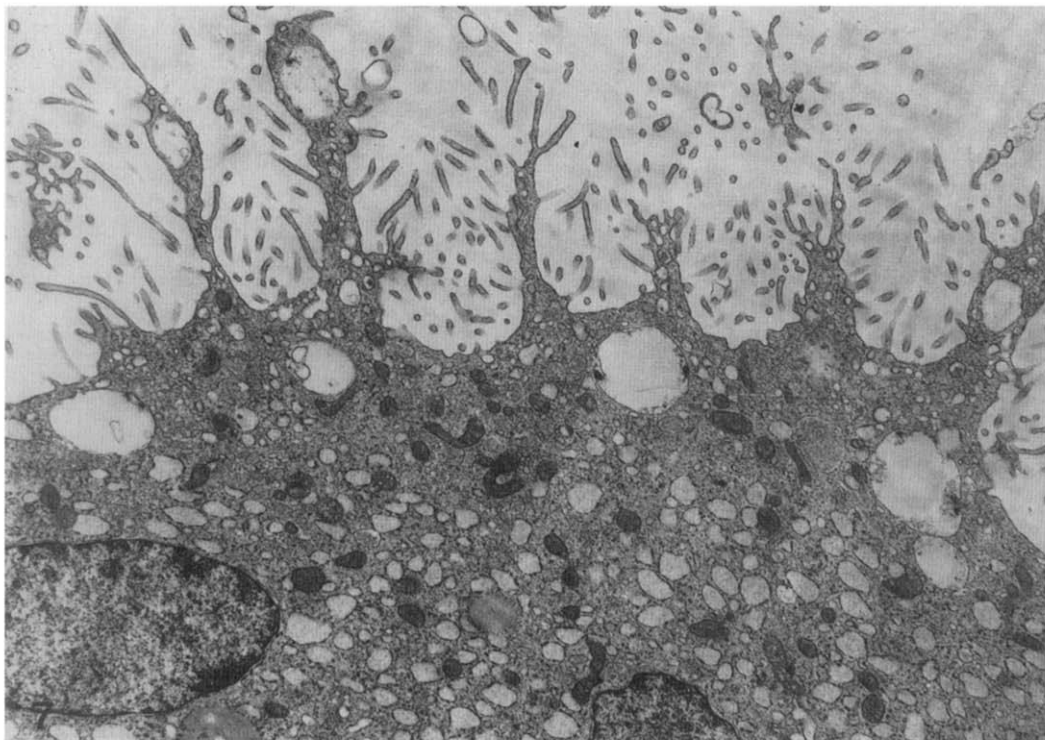


Fig. 7. Part of a first trimester human placenta showing pronounced "scalloping" and microvillation of the syncytiotrophoblast surface facing the maternal circulation. Again, vacuolation of the syncytiotrophoblast is a dominant feature. Print kindly made available by Dr C. J. P. Jones.

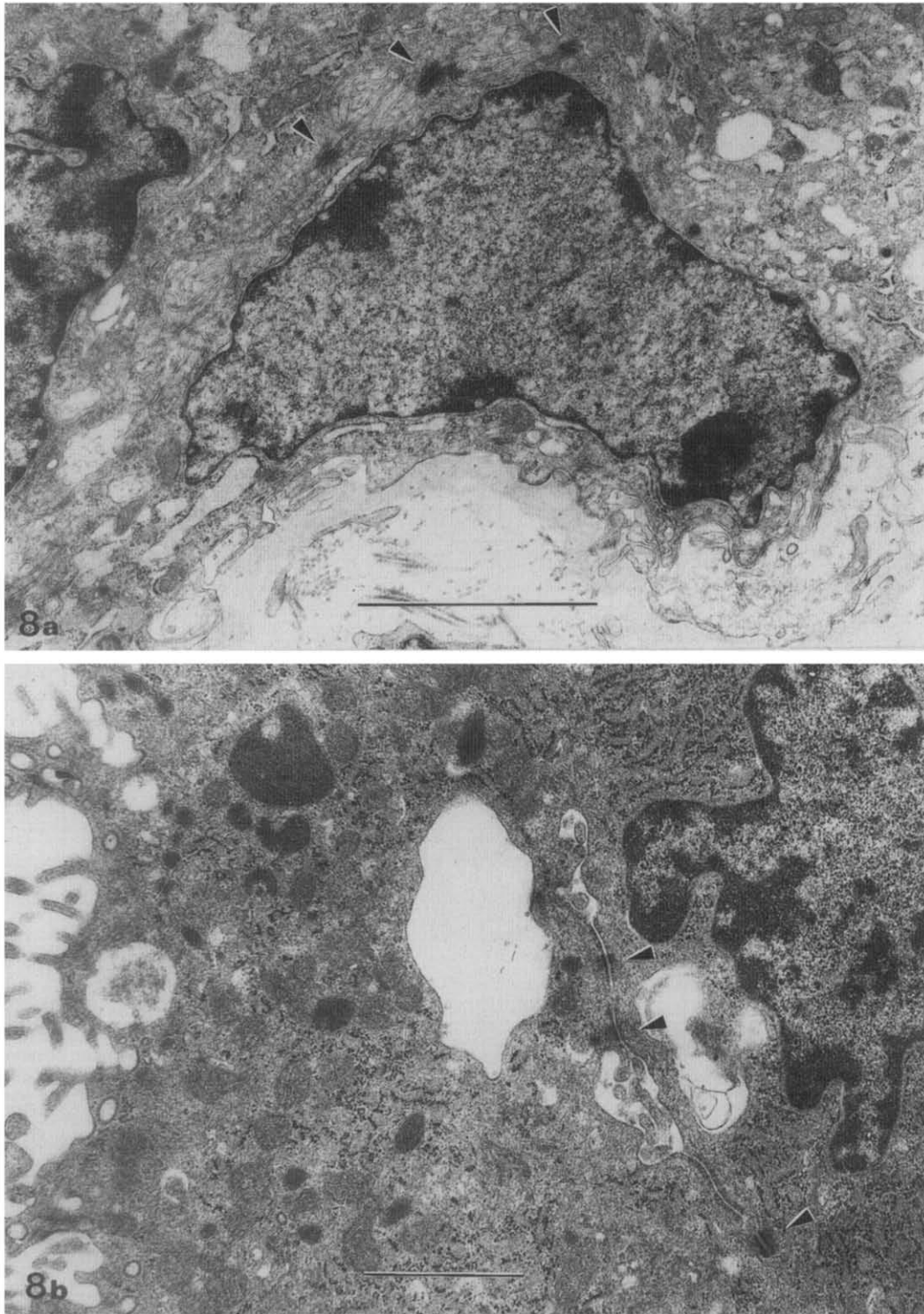


Fig. 8. Second trimester human intermediate placental trophoblastic cells, similar to syncytiotrophoblast, showing the presence of desmosomal junctions (arrowheads) and disintegrating plasma membrane between two cell nuclei (a) and of intracellular residual plasma membrane and desmosomes (arrowheads) at a further stage of cell fusion (b). The scale bars indicate $2\ \mu\text{m}$ (a) and $1\ \mu\text{m}$ (b). Prints kindly made available by Dr C. J. P. Jones.

icity. Occasionally two or more nuclei may be closely apposed (Fig. 12), but this is not generally the case. In mature skeletal muscle, "satellite" myoblast-like cells lie alongside the myotubes, beneath the basal lamina and may continue to be incorporated into the myotube bundle; undoubtedly they represent a source of primitive cells for muscle regeneration (Moraczewski

et al., 1988). Ultrastructurally, myoblast fusion has not been conclusively demonstrated, but convincing evidence has recently come from the *in vivo* studies of Robertson *et al.* (1990) who performed investigations on the regeneration of experimentally damaged skeletal muscle. The breakdown of the regenerative myogenic cell plasma membrane within the cytoplasm of the

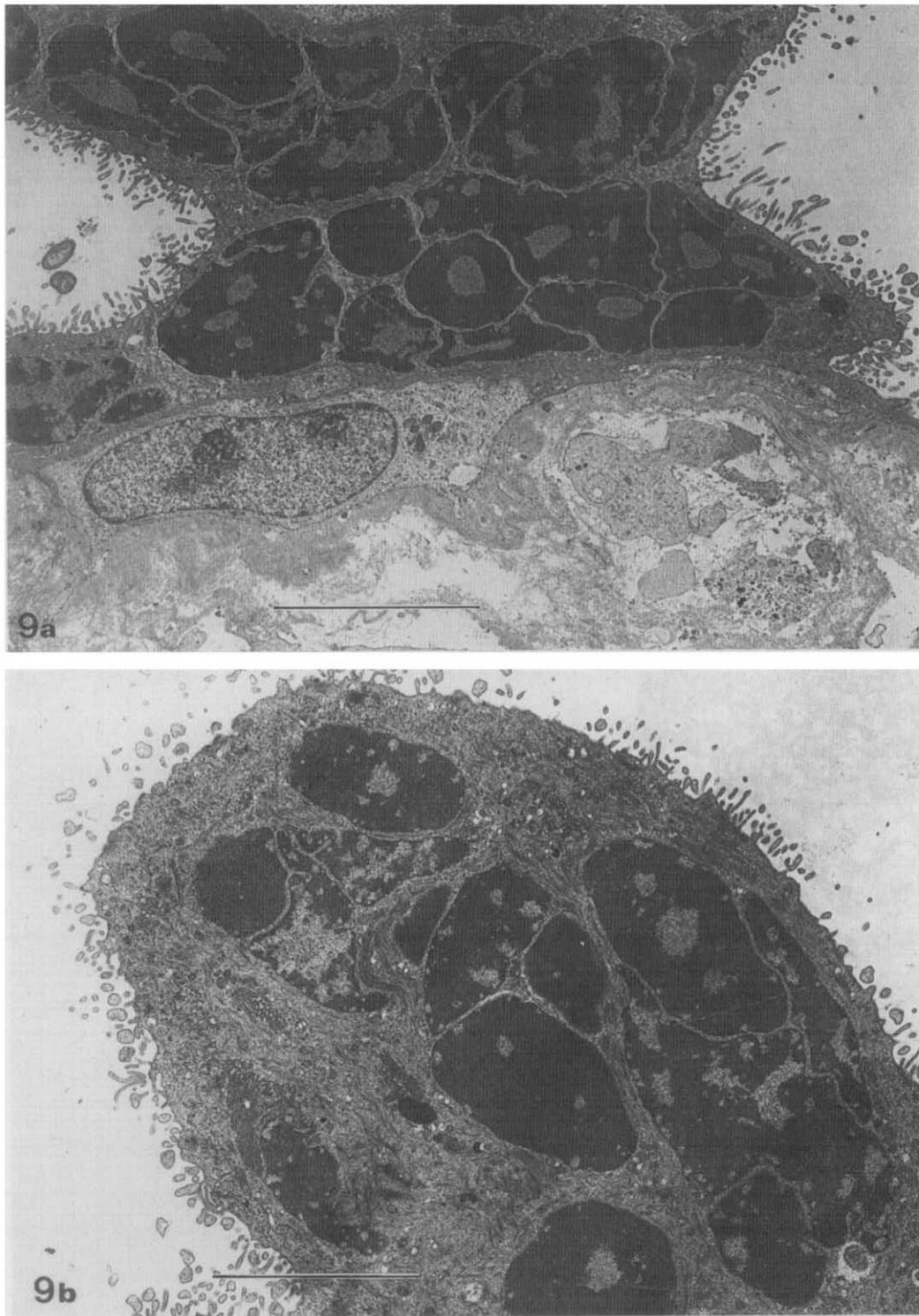


Fig. 9. Typical human placental syncytiotrophoblast nuclear "knots" at full term (a) and in a post-mature placenta (b). In (a) an apparent syncytial bridge between two villi is shown. In (b) the nuclei are at a more advanced stage of pyknosis than those in (a). The scale bars indicate $5\ \mu\text{m}$. Prints kindly provided by Dr C. J. P. Jones.

fused cells was clearly shown (Fig. 13), together with the laying down of organized myofibrils. The absence of glycocalyx and basal lamina appeared to be necessary to enable plasma membrane-membrane contact to occur at the early stage of the fusion process. The somewhat specialized situation in the developing and regenerating lymph-heart muscles in avian and amphibian embryos (Runyantsev and Krylova, 1990)

serves to indicate the developmental fusion of satellite myoblasts.

In skeletal muscle atrophies, degeneration of the myofibrils occurs without depletion of the number of nuclei per myotube (Tomé and Fardeau, 1986). Under these conditions (e.g. atrophied muscle from an AIDS patient) marked clustering of the nuclei does, however, occur (Figs 14 and 15), with pronounced myofibrillar

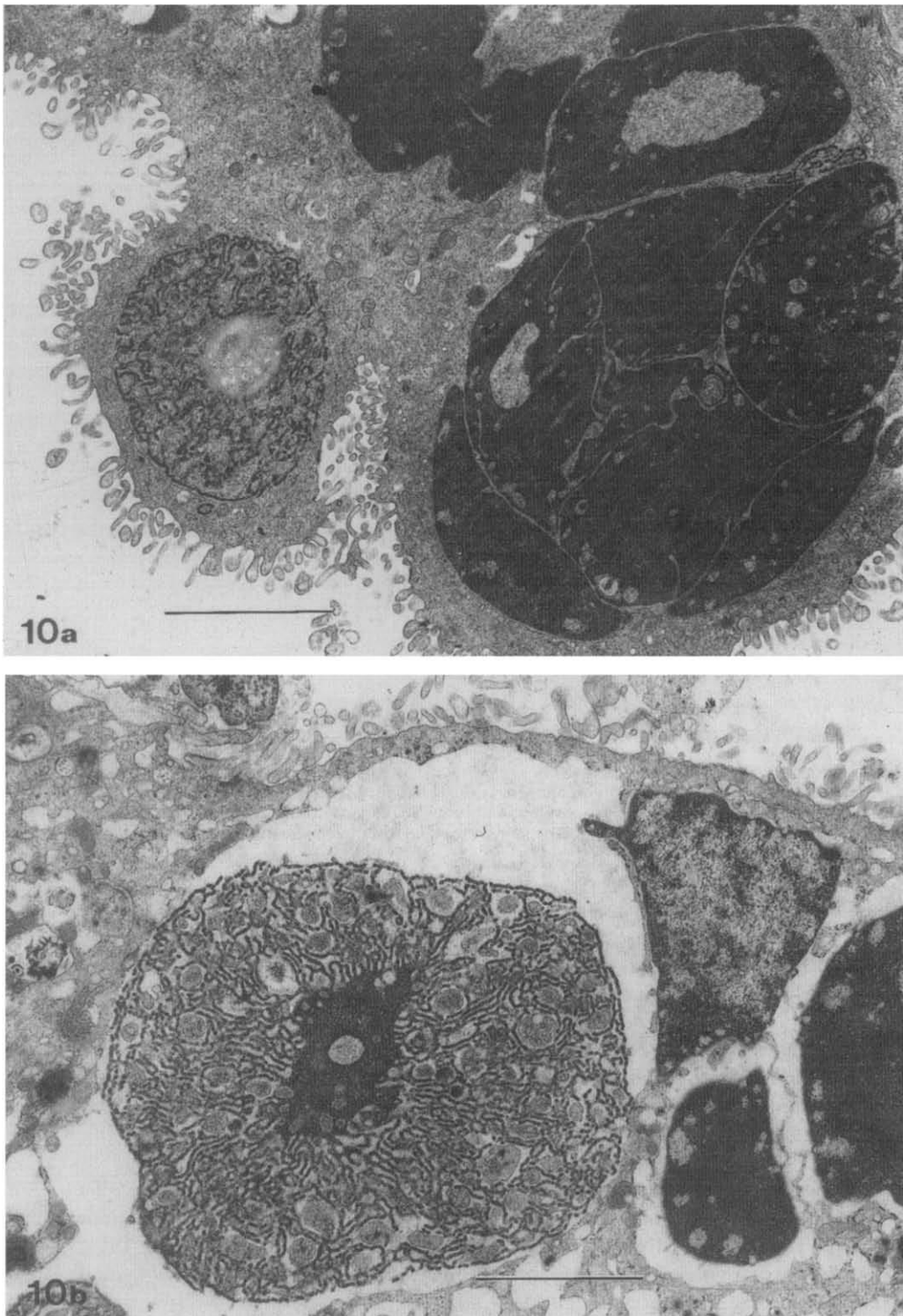


Fig. 10. The pyknotic degeneration of nuclei in a placental syncytial "knot", with almost complete exclusion of cytoplasm from between the nuclei (a) and in the case of Rhesus incompatibility when more marked nuclear breakdown occurs (b). The scale bars indicate $2.5 \mu\text{m}$. Prints kindly provided by Dr C. J. P. Jones.

disorganization. As with the full term syncytiotrophoblast and cultured MNGCs, there may be almost complete exclusion of cytoplasm from between the clustered nuclei (Fig. 15). An account of muscular changes in the AIDS patient has recently been presented by Buskila and Gladman (1990), but does not place significant emphasis upon ultrastructural aspects of muscle wasting.

D. Pathology

In pathological tissues there are numerous examples of MNGCs, ranging from the binucleate Reed-Sternberg cell in Hodgkin's disease, the Langhans' tuberculous lesion and the foamy lipid-containing bone marrow binucleate and multinucleate macrophage in Gaucher's disease, through to the multinucleate giant epithelial



Fig. 11. Part of a mouse skeletal muscle myotube, showing a single flattened nucleus located immediately beneath the myolemma (arrowheads). Note the infoldings of the nuclear envelope, indicating the capacity of the nucleus to readily adjust to the contractile state of the adjacent myofibrils. The scale bar indicates 2 μm .

cells detected in sarcoidosis (also due to infection with the syphilitic spirochaete). Lung diseases, in particular the pneumonias (viral and non-viral), are common conditions in which MNGCs have been detected (Dail and Hammar, 1988).

Many tumours express MNGCs and they have also been detected in granulomatous inflammatory conditions such as rheumatoid arthritis (Bardosi *et al.*, 1989), giant cell arteritis (temporal arteritis) (Wawryk *et al.*, 1991) and the cardiac Aschoff cell in acute rheumatic fever. In giant cell myocarditis (Komada *et al.*, 1991), which is also associated with various types of inflammatory cells, the MNGCs can closely resemble degenerate cardiac muscle cells. Giant cell carcinomas, characterized by the presence of MNGCs, occur within epithelial tumours (Black and Epstein, 1974) and at several visceral sites, such as breast, thyroid, pancreas, lung, ovary and kidneys. The pleomorphic giant cell carcinoma of the lung (Wang *et al.*, 1976) has been shown to contain unique ultrastructural features, which may represent extreme anaplasia. Giant cell carcinomas containing histiocyte or osteoclast-like giant cells often bear a strong structural resemblance to the giant cell osteoclastomas of bone, as is the case with MNGCs in many primary visceral giant cell tumours. Thus, it is clear that the monocyte/macrophage appears to have a pronounced tendency to be involved

with the production of pathological osteoclast-like tumour MNGCs *in vivo*. Such examples have been shown in tuberculoid granuloma, osteogenic sarcoma (osteosarcoma) (Ghadially and Mehta, 1970), sarcoid granuloma (Ghadially, 1982; Kirkpatrick *et al.*, 1988), sarcoidosis (Carr, 1980), tumour of the urinary bladder (Amir and Rosenmann, 1990), giant cell tumour of the pancreas (Goldberg *et al.*, 1991), fibrous histiocytoma (Smith *et al.*, 1990) and a malignant giant cell tumour of the uterus (Magni *et al.*, 1991). Foreign-body subcutaneous granulomas containing MNGCs have been studied by conventional thin sectioning (Cain *et al.*, 1981; Papadimitriou and Archer, 1974; Papadimitriou and Rigby, 1979; van der Rhee *et al.*, 1979) and also using the quick freezing deep etching method (Baba *et al.*, 1991). Emphasis has been placed in these studies upon the cytoskeletal distribution and organization within the MNGCs, which in general appear to retain features characteristic of the macrophage. In breast carcinoma there have been numerous reported cases of the presence of MNGCs with an osteoclast-like nature (Oemus and Timmel, 1990; Trojani *et al.*, 1989). Figure 16 shows an example of a MNGC within a breast carcinoma. Such MNGCs can also occur in areas remote from the tumour and in non-tumour-bearing breasts, so it is reasonable to propose that growth factor(s) released by the tumour

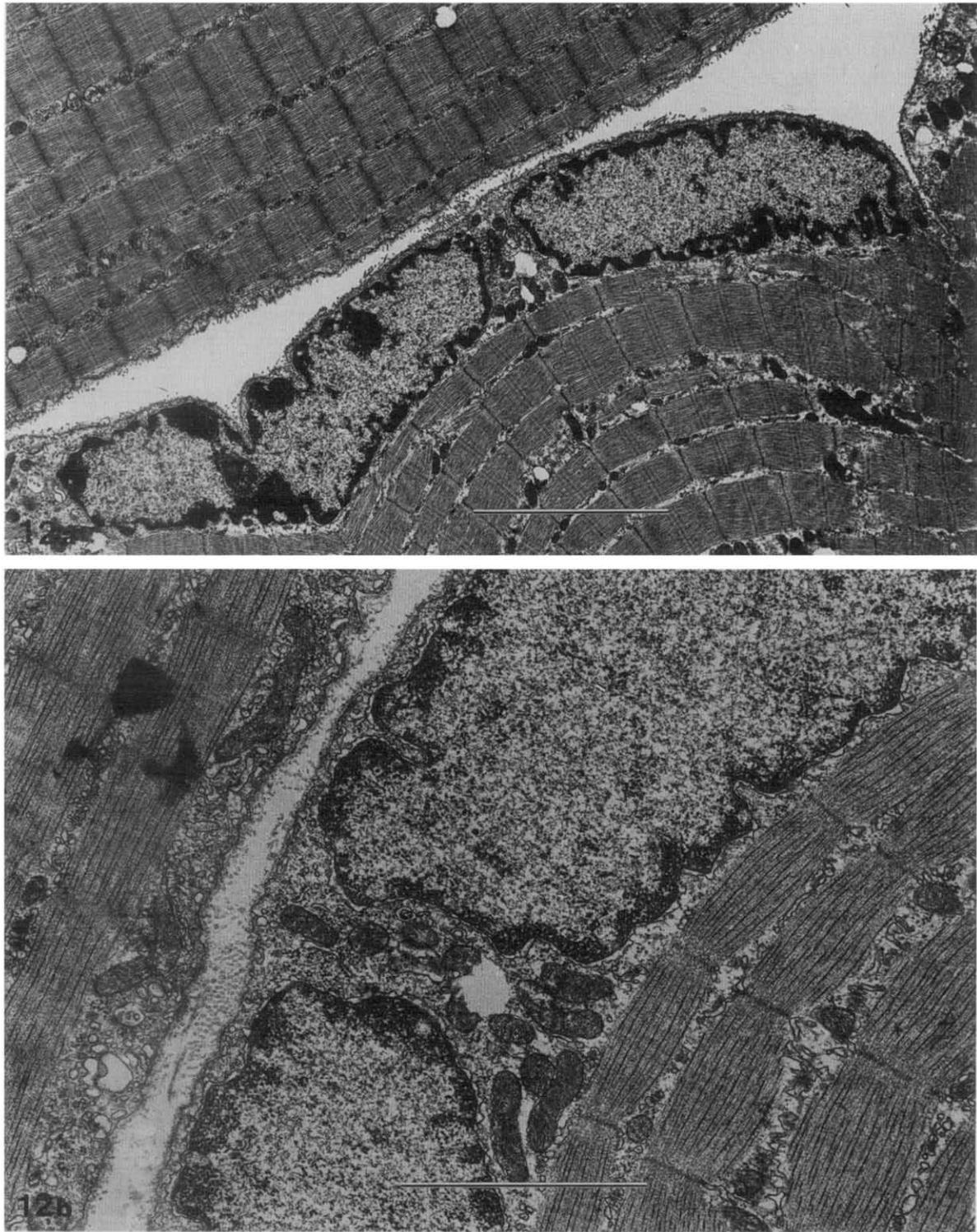


Fig. 12. Part of a mouse muscle myotube where two nuclei are located in close apposition to one another, immediately beneath the myolemma (a). At higher magnification (b) the undulating nuclear envelope is clearly presented, together with the thin rim of heterochromatin adjacent to the inner nuclear membrane/nuclear lamina. The scale bars indicate $5\ \mu\text{m}$ (a) and $2\ \mu\text{m}$ (b).

mass have had a secondary stimulatory action on the fusion of monocyte/macrophages. The role of cytokines in the fusion of macrophages (Möst *et al.*, 1990) indicates that interferon-gamma is essential and that cellular expression of the adhesion molecule LFA-1 (CD18) is also involved. Athanasou *et al.* (1989) consider that these breast carcinoma MNGCs are distinct from both osteoclasts and other types of inflammatory foreign

body giant cells. Tumours of the bone, which involve macrophage polykaryons in bone resorption have been defined (Kanchisa *et al.*, 1991; Mie *et al.*, 1991), and also the giant cell tumour of the tendon sheath (Athanasou *et al.*, 1991). Other bone tumours which display MNGCs, such as some osteogenic sarcomas and tumours of the tendon sheath may, however, also be of osteoblastic and fibrous histiocytic origin, since

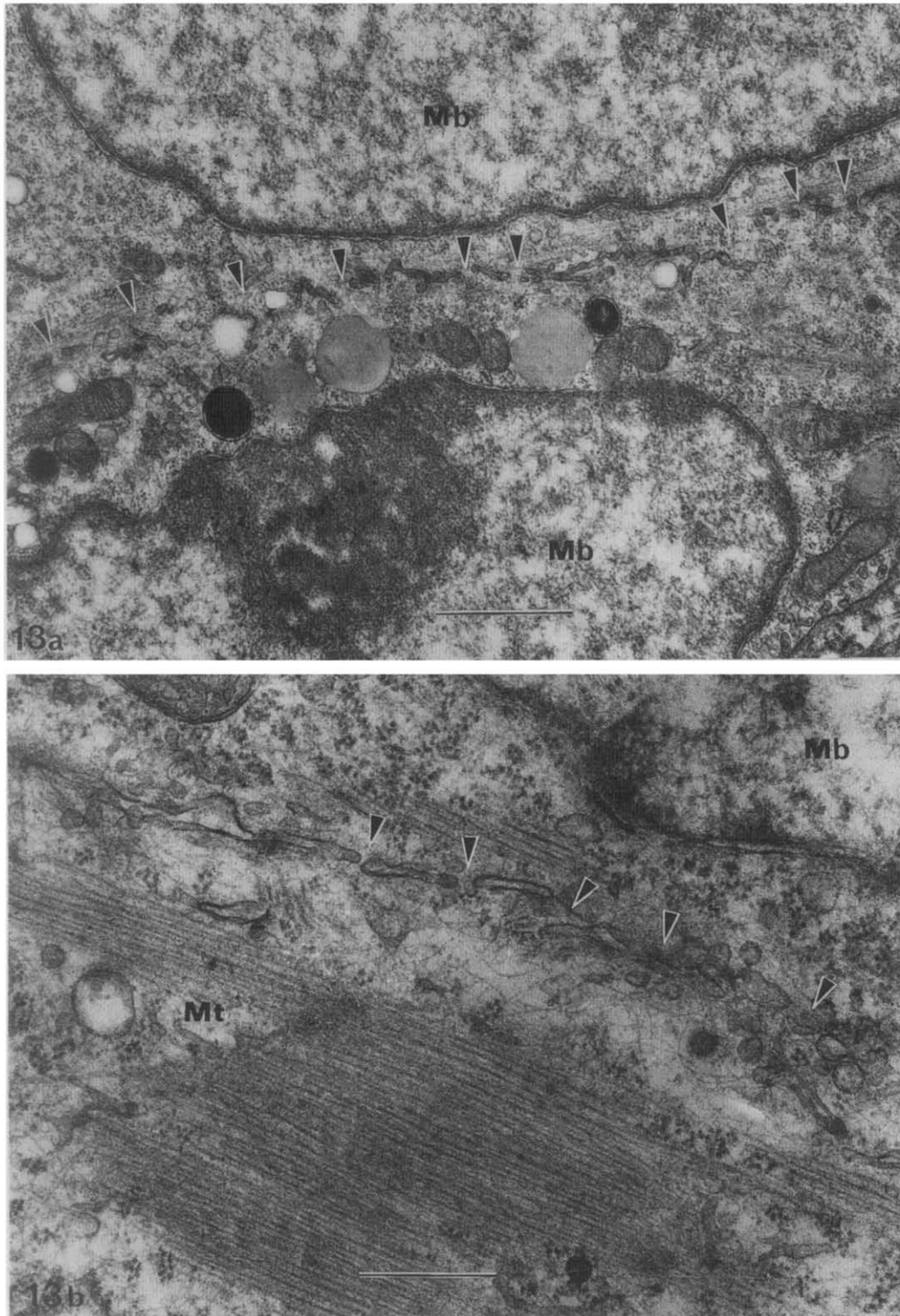


Fig. 13. The regeneration of mouse skeletal muscle following injury, showing in (a) the fusion between two myoblasts (Mb). Arrowheads indicate multiple fusion foci. In (b) fusion is occurring between a myoblast (Mb) and a myotube (Mt). Again multiple plasma membrane fusion foci are clearly apparent (arrowheads). The scale bars indicate $1\ \mu\text{m}$ (a) and $0.5\ \mu\text{m}$ (b). Micrographs kindly supplied by Dr T. A. Robertson. From Robertson *et al.* (1990) with permission.

they do not always display clear osteoclastic characteristics (Marks and Chambers, 1991; Stark *et al.*, 1983). Somewhat like the macrophage, the cytotrophoblast also appears to be involved in the formation of syncytiotrophoblastic-like giant cell tumours (cf. gonadal and extragenital germ cell tumours such as the teratocarcinomas and choriocarcinomas) (Zimmerli

and Hedinger, 1991) which can also form metastatic carcinomas of the gastrointestinal tract, where there is focal trophoblastic differentiation (Saigo *et al.*, 1981). Indeed, the placental site trophoblastic tumour can be highly aggressive, with extensive infiltration of the myometrium and metastasis to the lungs (Orrell and Sanders, 1991). Although there are reported cases

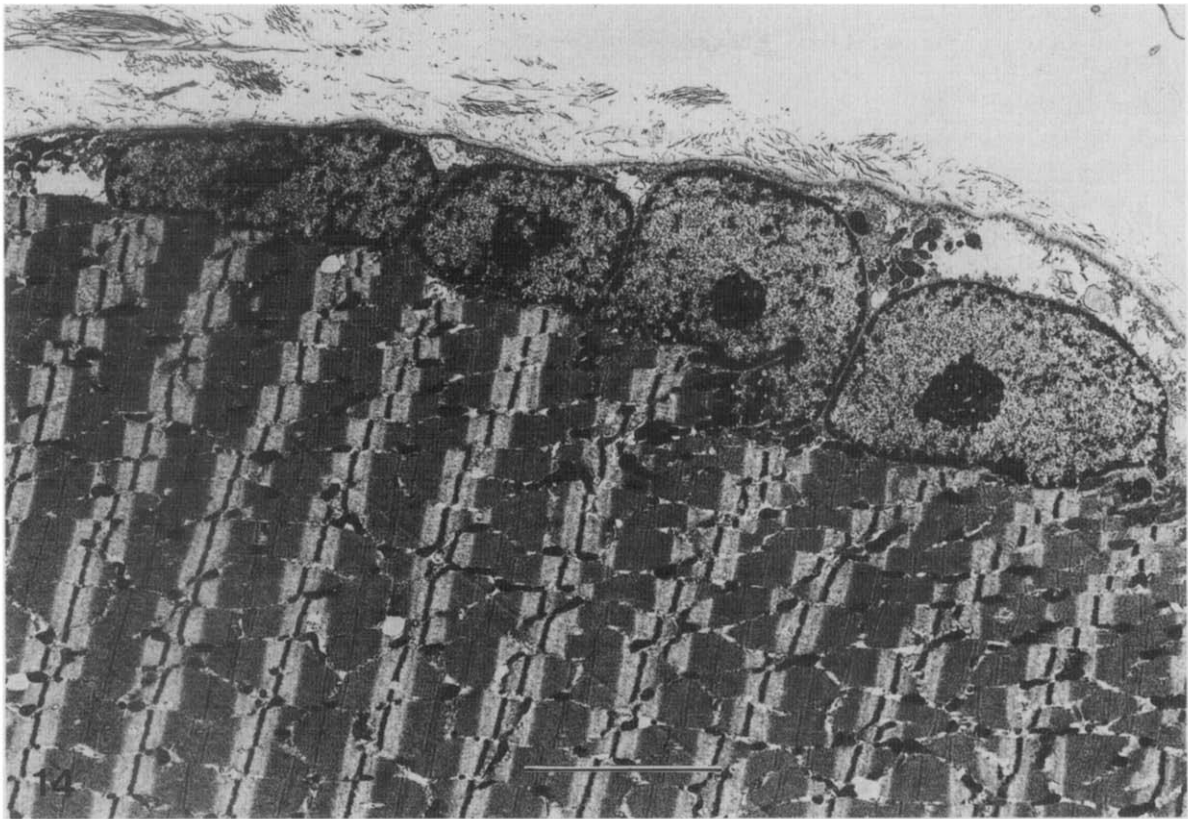


Fig. 14. The surface of a myotube present in a muscle biopsy sample taken from the atrophied skeletal muscle of an AIDS patient. Clustering of four abnormally rounded nuclei immediately beneath the myolemma can be seen. Collagen fibrils are present out with the basement membrane. The myofibrils are relatively normal in this section, but cf. Fig. 15. The scale bar indicates 5 μ m.

of multinucleated cells being present in certain haematological conditions (Kurabayashi *et al.*, 1989), it is not always clear whether a multi-lobulated nucleus within a single enlarged cell is being discussed rather than a truly MNGC.

An interesting *in vivo* investigation of methylcholanthrene-induced primary tumours (Fortuna *et al.*, 1989) has suggested that cell fusion is of significance in relation to primary tumour formation, but that the fused cells were progressively lost.

III. MULTINUCLEATE GIANT CELLS IN VIRAL DISEASES

A. General considerations and the cytopathic effect

In the case of virally-induced MNGCs it is important to emphasize that in all instances a major contribution has been made by light microscopy of human and animal biopsy samples and of virally infected cell culture lines (Malherbe and Strickland-Cholmley, 1980). In most cases, electron microscopy is unfortunately lacking, but it is clear that the cytopathic action of fusogenic viruses when applied to cell cultures often conforms very well with the *in vivo* actions of the virus. The term Cytopathic Effect (CPE) of a particular virus originally had a rather broad meaning, but has come to refer predominantly to

the fusogenic action of a virus in producing MNGCs *in vivo* and *in vitro*; it will be used in this more restricted context throughout this review. Thus, the CPE should be distinguished from the overall cytolytic or cytotoxic action of a virus, such as the cellular destruction of the anterior horn neurones by poliovirus or production of lysis of cultured cells. It is also important to clearly distinguish between virally-induced cell fusion from "without" and from "within" the cell.

All fusogenic viruses need to be able to bind to a cell surface receptor, usually on the specific cell type to which the virus has an affinity. Then follows fusion of the virus with the plasma membrane or endocytosis, internalization and acidic endosomal fusion of the viral membrane, infection of the cell, viral replication and subsequent release. The fusogenic action of a membrane-enveloped virus may occur directly when one or more viruses fuse with the plasma membrane of two cells, thereby providing a cytoplasmic bridge between the cells (fusion from "without"). Alternatively, newly synthesized viral protein may need to be expressed on the cell surface to enable fusion to take place, in this case by attachment to receptors on non-infected cells or latently infected cells not yet expressing viral proteins in their plasma membrane (fusion from "within"). The former situation has been elegantly described for the Sendai virus-induced fusion of chicken erythrocytes with HeLa cells in the classical studies of Henry Harris (1970,

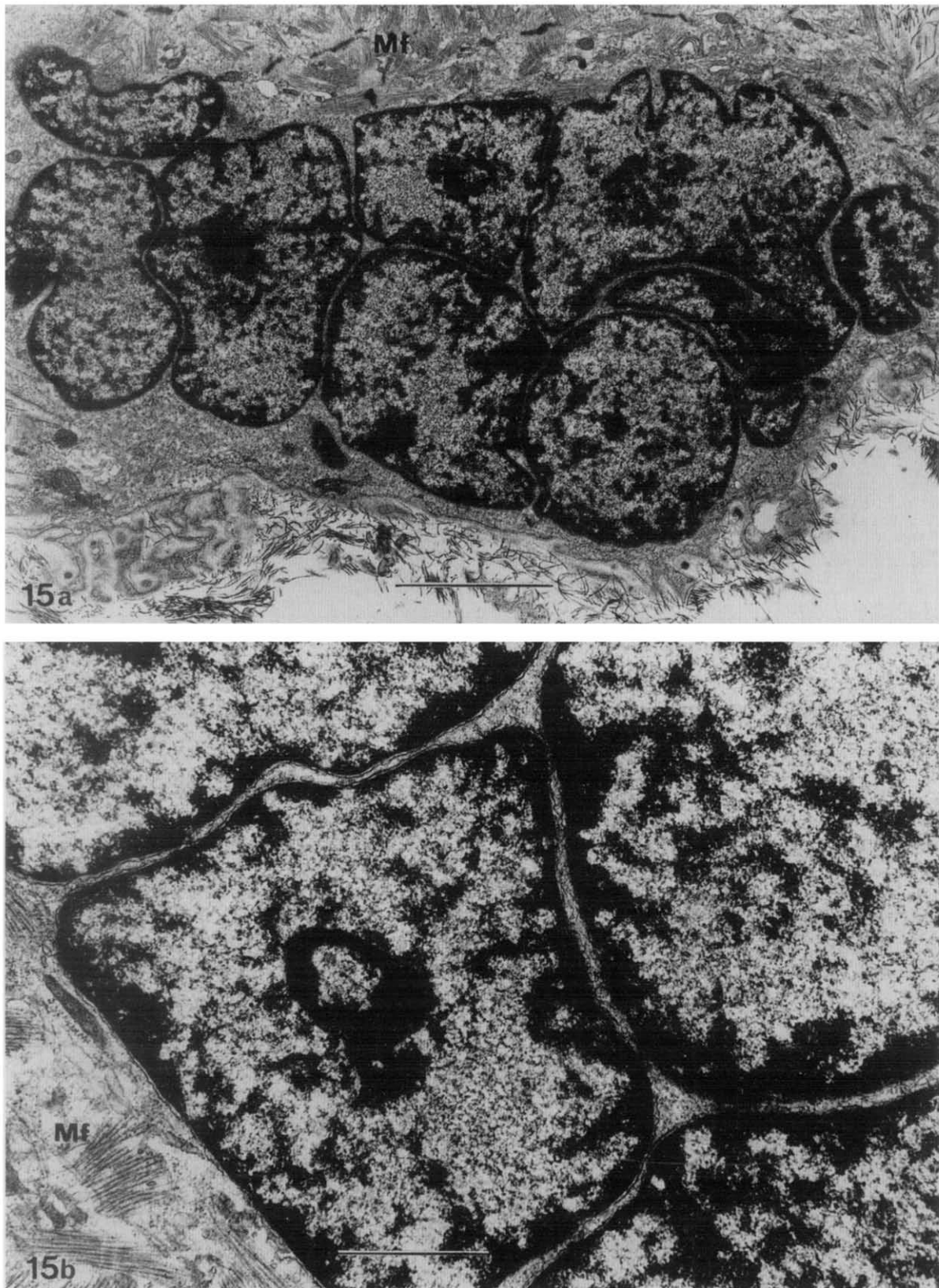


Fig. 15. A cluster of nuclei within a myotube showing marked myofibrillar (Mf) disorganization, in a muscle biopsy sample taken from the atrophied skeletal muscle of an AIDS patient. The nuclei show no indication of flattening and have clustered as a "knot" with almost total exclusion of cytomembranes, mitochondria and myofibrils. The scale bars indicate $5\ \mu\text{m}$ (a) and $1\ \mu\text{m}$ (b).

1974) and also for chicken erythrocytes and hamster melanoma cells (Zakai *et al.*, 1974). Both viable and UV irradiated Sendai virus have this fusogenic capacity,

indicating that viral replication is not required. This approach provided the foundation for extensive studies on heterokaryon formation and the whole field of

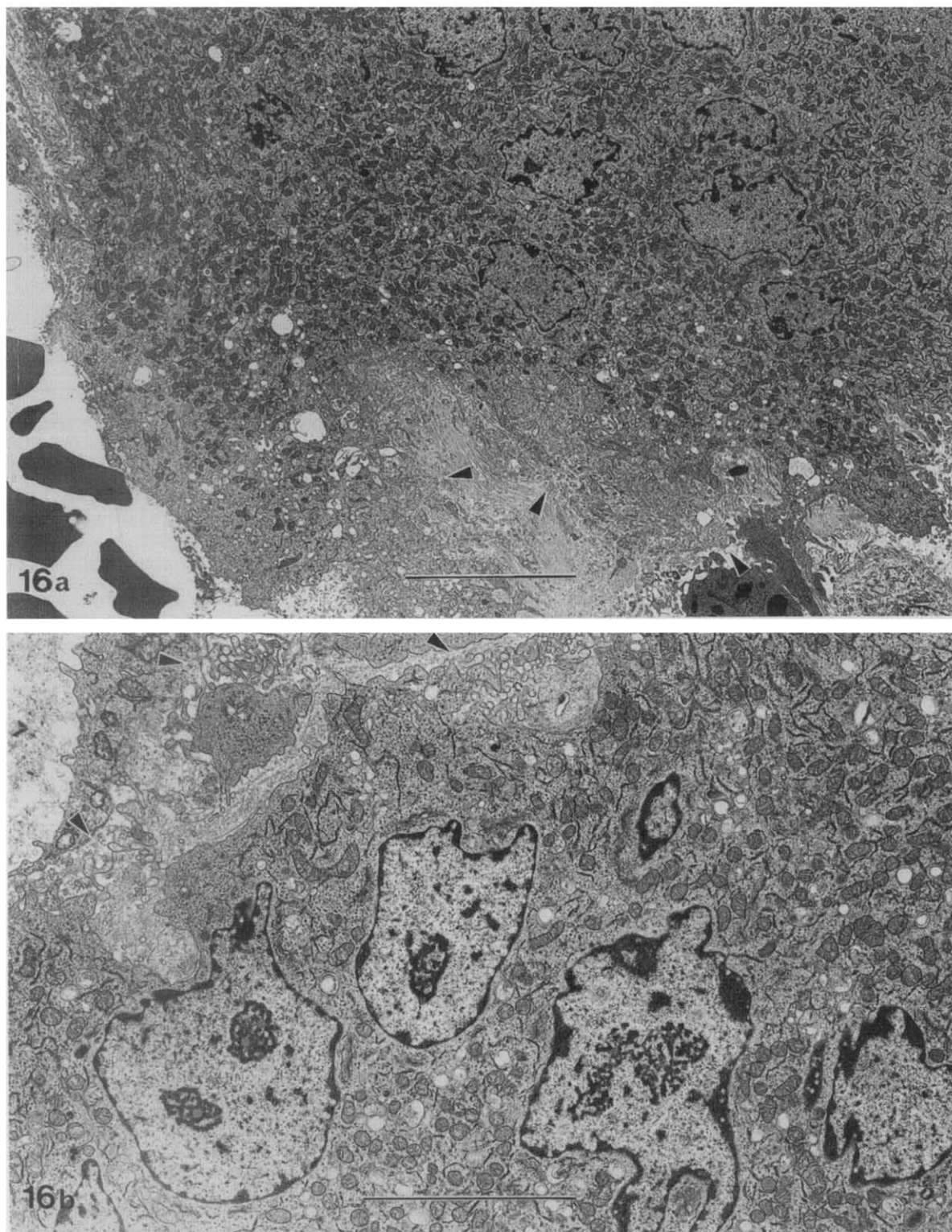


Fig. 16. A multinucleate osteoclast-like cell, detected within a mammary tumour. Note the marked undulations of the nuclei and the proliferation of mitochondria, typical of the osteoclast, together with pronounced surface villation (arrowheads). Scale bars indicate $10\ \mu\text{m}$ (a) and $5\ \mu\text{m}$ (b). Micrographs kindly provided by Mr C. Wells, St. Bartholemew's Hospital.

monoclonal antibody production, together with the extensive field of viral-induced membrane-membrane fusion from without (White and Blobel, 1989). The second situation appears to be of particular importance in the case of herpes- and retroviral-induced cell fusion, where viral integration, replication and expression of viral proteins at the cell surface (Desportes *et al.*, 1989)

is required to induce MNGC formation (Kalgemaraman *et al.*, 1990). In this situation viral inactivation by UV or gamma irradiation (Chatterjee and Hunter, 1980; Kitchen *et al.*, 1989) prevents the fusion event, as assessed by the *in vitro* cellular syncytium-formation assay (e.g. Nagy *et al.*, 1983). It should be emphasized that this cellular assay for retroviral fusogenicity

(Hoshino *et al.*, 1983; Klement *et al.*, 1969) has also become an extremely valuable tool for the investigation of anti-HIV agents (including antibodies, viral surface antigens, peptides homologous to the attachment epitope(s) of viral surface glycoproteins, cloned and synthetic analogues to the cellular CD4⁺ receptor, and drugs) (Baba *et al.*, 1990; Bremermann and Anderson, 1990; Gruters *et al.*, 1987; Lifson *et al.*, 1991; Nara *et al.*, 1987; Owens *et al.*, 1990; Pal *et al.*, 1991; Tochikura

et al., 1988). Of great significance, within the present context, has been the demonstration that the incorporation of the HIV envelope glycoprotein gene into the cellular genome results in the fusion of these genetically modified cells with CD4⁺ unmodified cells (Ashorn *et al.*, 1990; see also Aoki *et al.*, 1991 and Kost *et al.*, 1991). Biochemical aspects of virally-induced cell fusion from without have been thoroughly reviewed by Gallagher *et al.* (1980); Kielian and Jungerwirth (1990); Spear (1987);

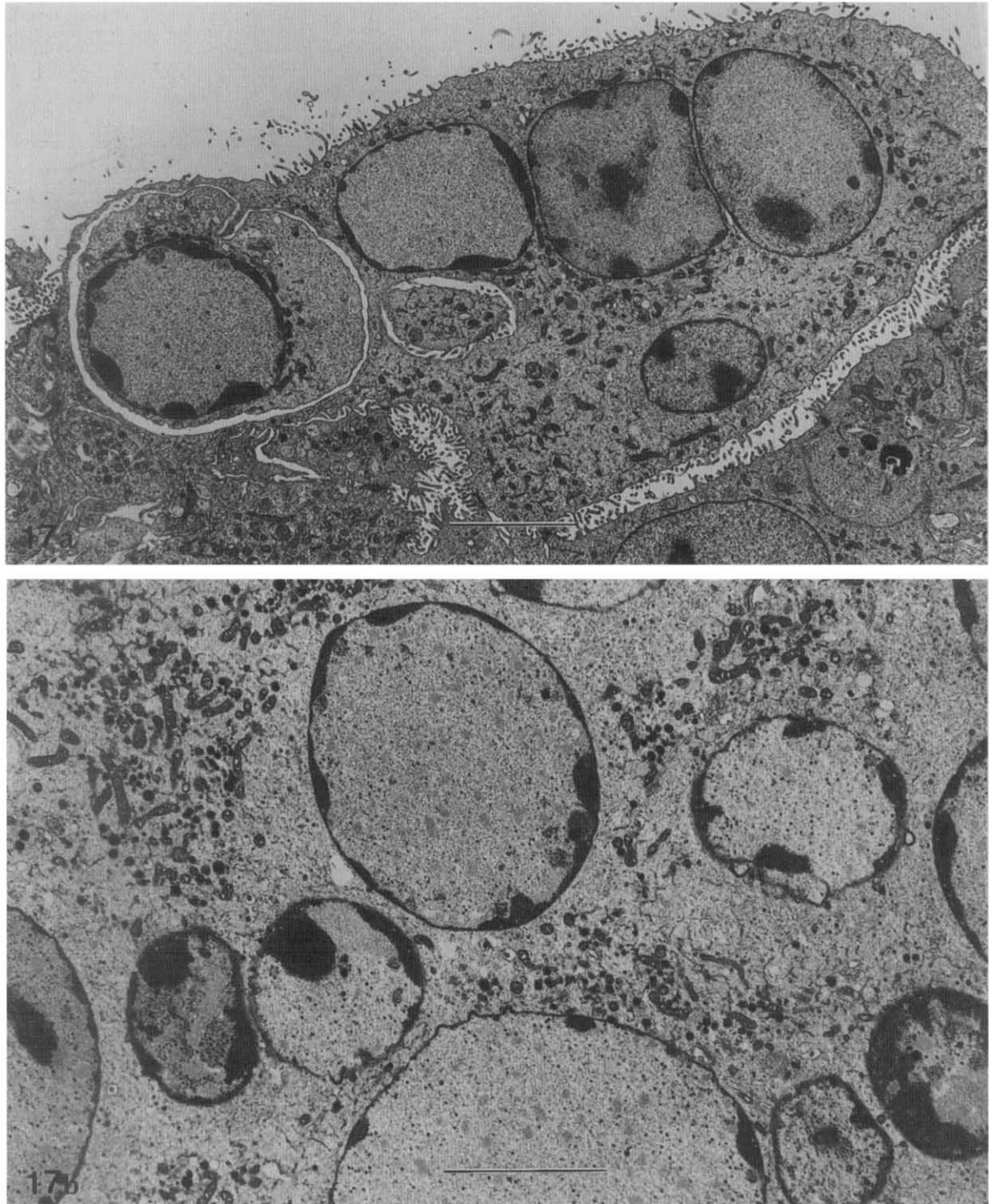


Fig. 17. Multinucleate giant cell formation within a cell culture infected by herpes simplex virus. Note the surface villation, the organelle free zone between the clustered nuclei and the intranuclear viral particles (b). The scale bars indicate 5 μ m. Micrographs kindly provided by Mr G. Tovey, London School of Hygiene and Tropical Medicine.

Stegman *et al.* (1989) and White and Blobel (1990). Emphasis has generally been placed upon the specific viral membrane fusion proteins and the peptide epitopes responsible for attachment to cellular surface receptors, together with the environmental pH required for the viral-cell and cell-cell fusion process. This biochemical progression has been followed more recently throughout investigations on the retroviral surface glycoproteins and cellular CD4 receptor.

B. Herpes viruses

The family Herpesviridae contains a significant number of animal and human viruses of veterinary and clinical importance. Three subfamilies have been defined: (1) the Alphaherpesvirinae, which contain the genera Simplex viruses (e.g. herpes simplex virus HSV

and varicella zoster virus VZV of man), (2) the Betaherpesvirinae, which contain the genus Cytomegaloviruses (e.g. cytomegalovirus CMV of man) and (3) the Gammaherpesvirinae, with the genus Lymphocryptoviruses (e.g. Epstein-Barr virus, EBV, of man). All herpes viruses are enveloped DNA viruses and are responsible for numerous conditions in man. It is significant that both CMV and EBV are extremely common viruses, with a high proportion of the population processing circulatory antibody, and that they both infect lymphoid cells. In the immunocompromised transplant patient, CMV has been shown to cause cellular fusion and may actively promote pneumonia. HSV and VZV, on the other hand, infect primarily epithelial cells and have been found to produce enlarged vacuolated (balloon) cells, which fuse into syncytial masses within the HSV or VZV vesicular lesion. The genome of the herpesvirinae is integrated into the cellular genome, a feature sometimes expressed by

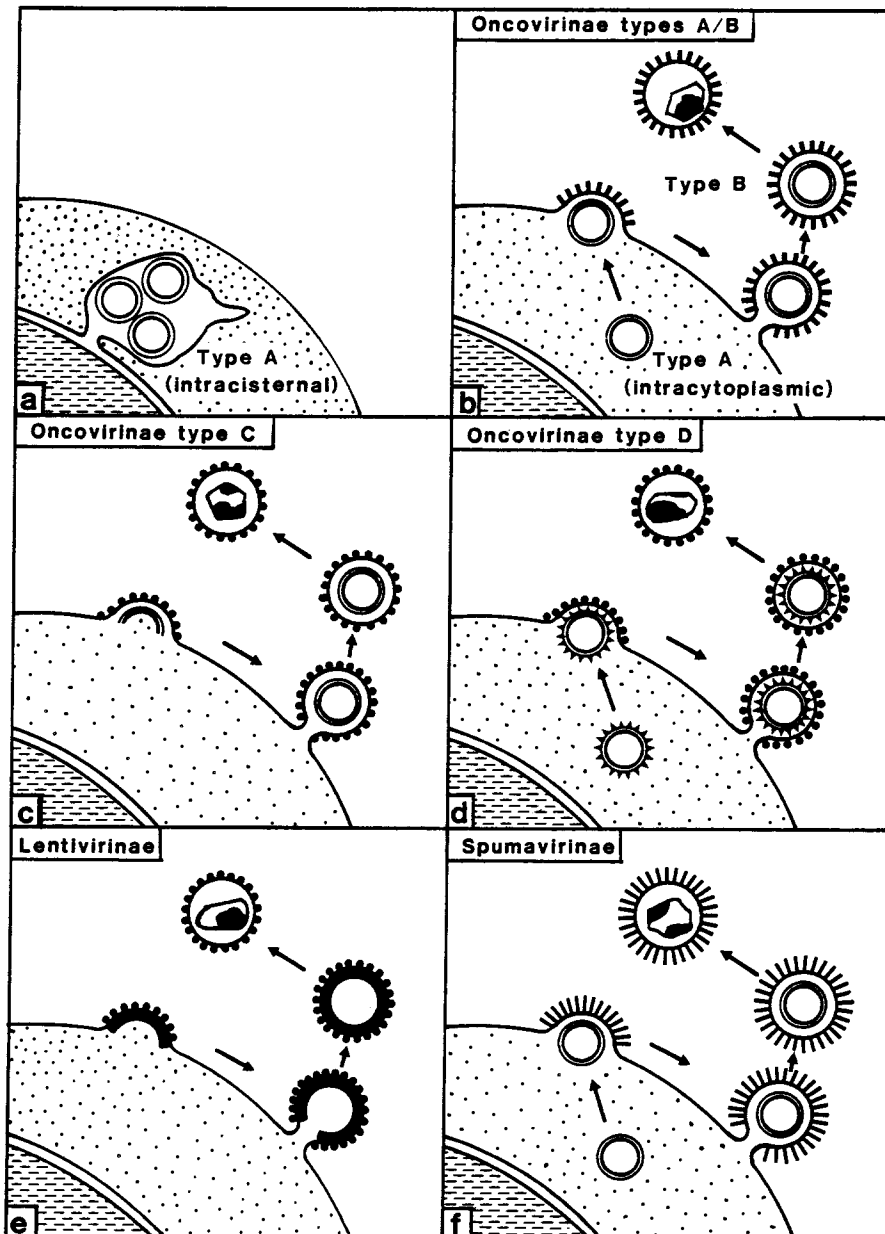


Fig. 18. A schematic representation of the formation and release of the different animal and human retroviral classes. (From H. Frank, 1987a, with permission.)

recurrent episodes of viraemia throughout life (particularly with EBV and the Simplex viruses), despite the presence of circulatory antibody. Examples of a MNGCs induced by HSV infection of cultured cells are shown in Fig. 17.

C. Paramyxoviruses

Members of the family Paramyxovirinae are renowned for their ability to produce cell fusion *in vivo* and *in vitro*. Three genera have been defined: (1) the Paramyxoviruses (e.g. human parainfluenza types 1 to 4, mumps virus, Sendai virus, Newcastle disease virus and Simian virus 5 SV5), (2) the Morbilliviruses (e.g. measles, canine distemper and rinderpest) and finally (3) the Pneumoviruses (e.g. respiratory syncytial virus RSV and pneumonia virus of man).

All paramyxoviruses are enveloped RNA viruses, but no integration of the viral genome into the cellular genome occurs, since these viruses lack reverse transcriptase. *In vivo*, measles virus is known to be responsible for the Warthin-Finkeldy "mulberry giant cells"

(Warthin, 1931) and is also the causative virus in subacute sclerosing panencephalitis (SSPE) (Brown and Thormar, 1976), involving skin, mucosal and respiratory surfaces, urinary, intestinal tract and lymphoid inflammations, all with detectable MNGCs. Respiratory syncytial virus is responsible for "giant cell pneumonia" (Dail and Hammar, 1988) with very large MNGCs detectable in the respiratory tract, these being particularly frequent in young children. A recent report of syncytial giant cell hepatitis (Phillips *et al.*, 1991) has emphasized the severe clinical consequences of paramyxovirus-induced hepatitis in man, and the need for more careful viral classification in severe sporadic hepatitis. Parainfluenza virus Type 3 has been found to occur as an opportunistic infection in the immunodeficient AIDS patient, and was thought to potentiate the pronounced presence of MNGCs detected at autopsy. In the case of Sendai virus (Haemagglutinating virus of Japan) and all other members of the paramyxovirinae, it is clear that a direct cellular fusogenic activity is expressed by the viral membrane glycoprotein, which does not require biosynthesis and expression of new viral proteins at the surface of the infected cell for fusion to occur (Hosaka, 1988).

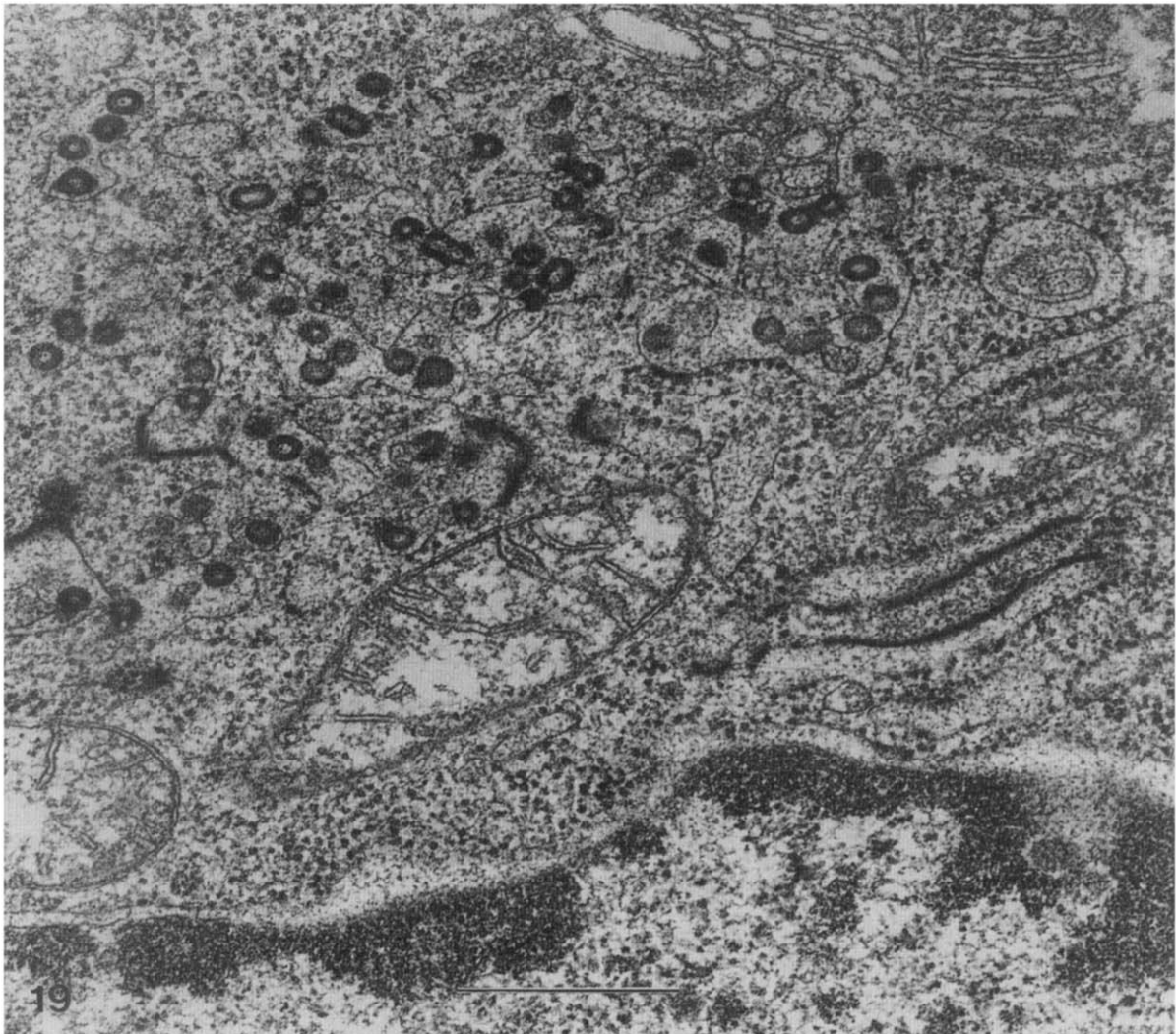


Fig. 19. An example of a mouse myloid leukemia cell line infected with an intracisternal type A oncovirus. Numerous electron dense viral particles can be seen within the cytoplasmic cisternae. The scale bar indicates 0.5 μm .

An early, yet elegant, electron microscopical study of SV5 (Compans *et al.*, 1964) demonstrated pronounced syncytium formation when a baby hamster kidney cell line (BHK21-F) was infected, yet no such CPE was detected with monkey kidney cells. This variation in CPE was interpreted as being indicative of differing viral release mechanisms. Within the BHK21-F syncytia, nuclei became markedly arranged in rows, rather than clusters, and cytoplasmic vacuolation was not pronounced (cf. Section VI.E and F). A detailed, but primarily light microscopical study of the organization of cytoplasmic fibers within BHK-21 cells following fusion with SV5 and also PEG has been presented by Wang *et al.* (1982). In general the two forms of syncytium induction gave rise to similar MNGC features although the central alignment of the nuclei was more pronounced in the virally-induced syncytia.

D. Retroviruses

The large family of animal and human Retroviridae (synonymous with the Reoviridae, Oncoviridae, Leucoviridae and Oncornavirinae) contains three subfamilies (Fig. 18): (1) The Oncovirinae, with the genera Oncovirus B (e.g. murine mammary tumour virus [MuMTV]), Oncovirus C (e.g. murine leukaemia and sarcoma viruses, avian leukaemia and sarcoma viruses, bovine leukaemia virus [BLV], human T cell leukaemia/lymphoma virus type I [HTLV-I] which is equivalent

to Adult T-cell leukaemia/lymphoma virus [ATLV], and [HTLV-II]), Oncovirus D (e.g. Mason-Pfizer monkey virus, squirrel monkey retrovirus and Lamgmar type D retrovirus). (2) The Lentivirinae, with the genus Lentivirus (e.g. Maedi/Visna virus [MVV], progressive pneumonia virus [PPV], caprine arthritis-encephalitis virus [CAEV], equine infectious anaemia virus [EIAV], human immunodeficiency virus types 1 and 2 [HIV-1/-2] (formerly termed HTLV-III/LAV), simian immunodeficiency virus [SIV], simian T-lymphotropic virus-3 [STLV-III], feline immunodeficiency virus and bovine immunodeficiency-like virus [BIV]). (3) The Spumavirinae, with the single genus Spumavirus (e.g. simian foamy virus [SFoV], bovine syncytial virus [BSV] and human syncytium forming virus [HSFoV]). The spumaviruses have recently been reviewed, from a molecular genetics point of view, by Flügel (1991) and Mergia and Luciw (1991).

The cellular pathology of the different retrovirus subfamilies and their genera presents a rather variable picture, with some overlap of characteristics. The overall retroviral classification is also based upon molecular biological and morphological criteria, such as the conformation of the mature virion core, the clarity with which the surface glycoprotein is revealed and the cellular location of the forming virion within infected cells, as described diagrammatically in Fig. 18 (Bouillant and Becker, 1984; Frank, 1987a). The Oncovirus B does not induce cell fusion *in vivo*, but is characterized by its pronounced tumorigenicity. The incomplete Oncovirus B

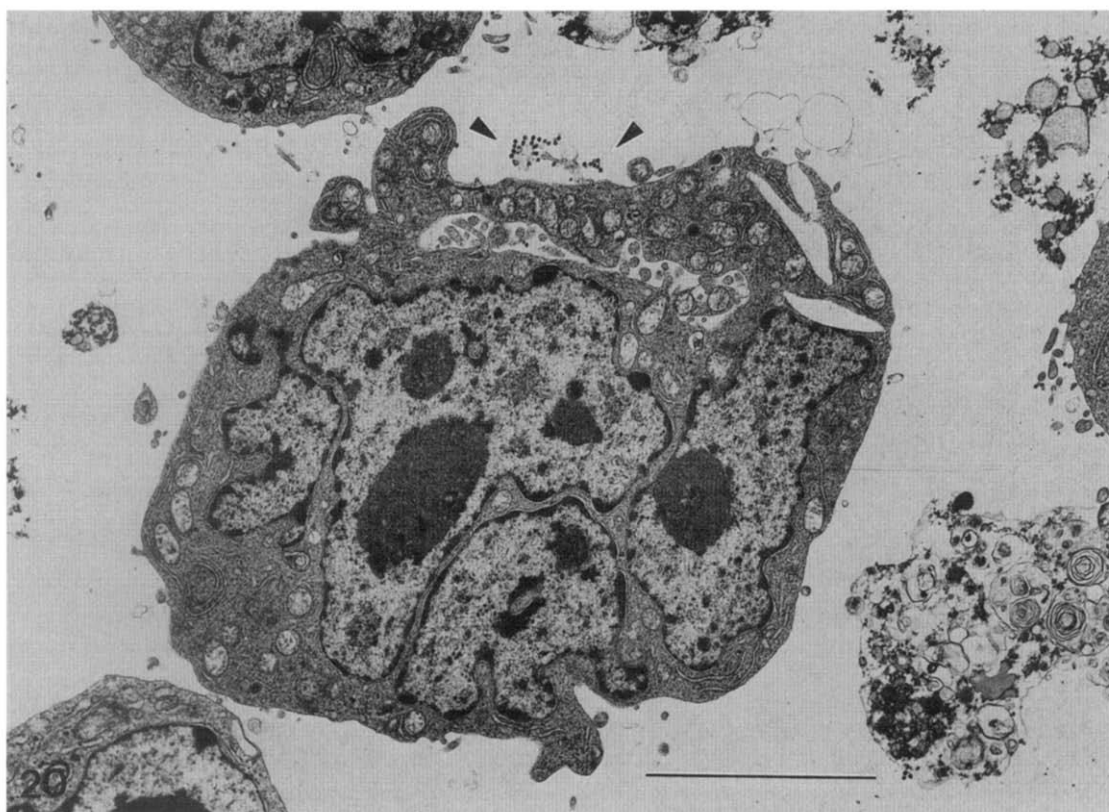


Fig. 20. A multinucleate cell within a mouse myeloid leukemic cell line infected with a type A/B oncovirus. A cluster of extracellular viral particles is detectable (arrowheads). The scale bar indicates 5 μm . Micrograph kindly provided by Mr G. Tovey, London School of Hygiene and Tropical Medicine.

lacking the glycoprotein membrane (also termed the Oncovirus A, is released and remains intracisternally, as shown in Fig. 19), whereas the complete Oncovirus B is produced by budding/exocytosis of the Oncovirus A through the plasma membrane (Fig. 18a, b). Having enveloped the immature viral core with a glycoprotein membrane, maturation of the core follows after exocytosis (Ohtsuki *et al.*, 1987). Cultures infected with Oncovirus B do not generally have a tendency to exhibit cell fusion and MNGC formation (Sarkar, 1989) although Okoi *et al.* (1990) found this to occur with murine mammary tumour virus. The genus Oncovirus

C, on the other hand, has a strong tendency to induce malignancies *in vivo*, and in cell cultures infected cells fuse with non-infected cells (Bouillant *et al.*, 1975, 1980; Frank, 1987b; Hoshino *et al.*, 1983; Klement *et al.*, 1969; Monozaki *et al.*, 1990; Nagy *et al.*, 1983; Ogura *et al.*, 1977; Timar *et al.*, 1987). Figure 20 shows a cultured mouse myeloma cell line in which a MNGC has been induced by the presence of murine leukaemia virus. A group of free virions can be seen adjacent to the MNGC, but there is little evidence of viral budding. Part of a MNGC induced by bovine leukaemia virus (BLV) is shown in Fig. 21.

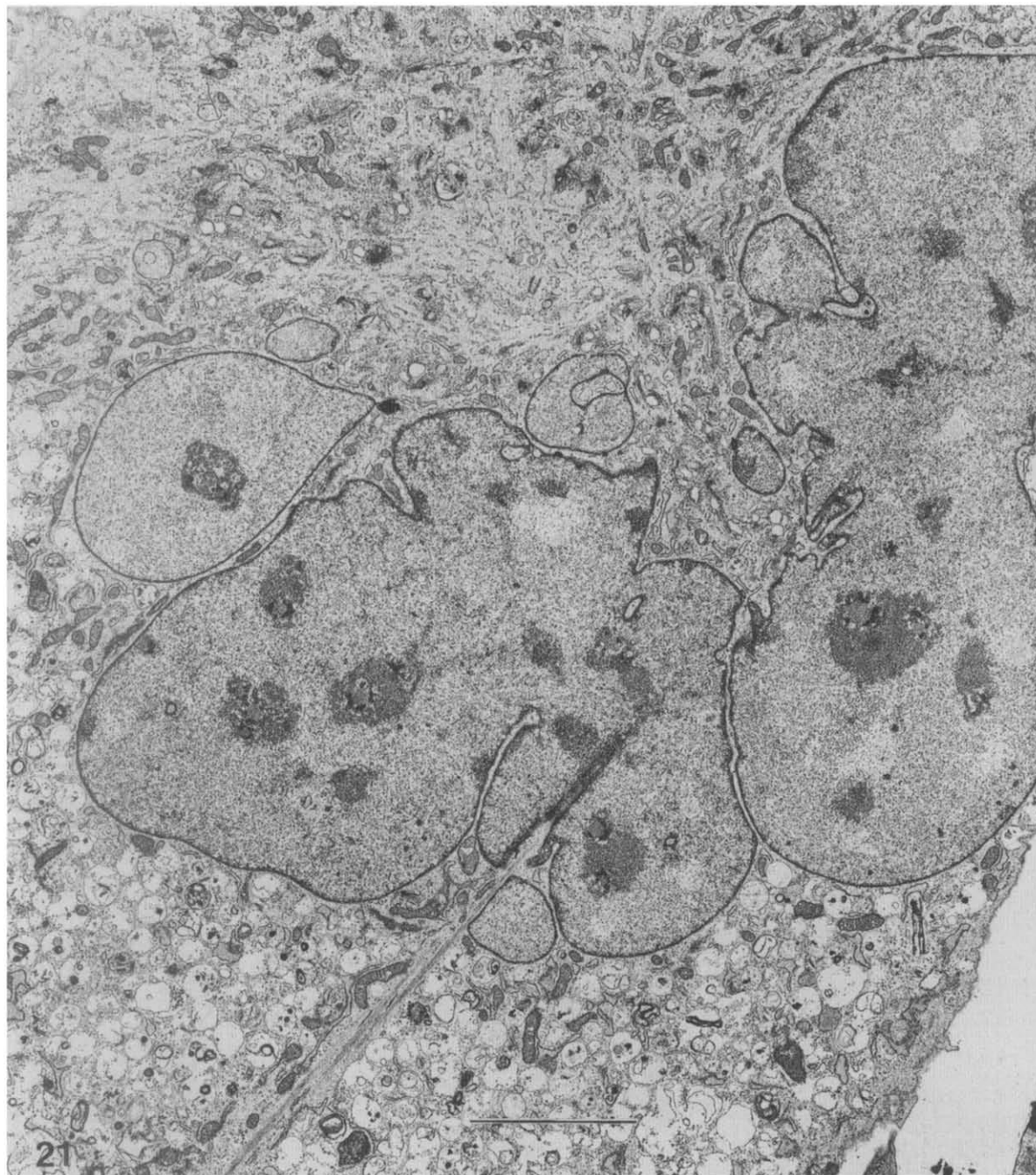


Fig. 21. Part of a MNGC induced in culture by bovine leukaemia virus (48 hr post infection). Considerable lobulation of the nuclei is apparent together with localized infolding of the nuclear envelope. The pronounced nucleoli show exceptionally clearly the structural demarcation within this subnuclear component. The scale bar indicates $5\mu\text{m}$. Previously unpublished electron micrograph provided by Drs Bouillant and Becker, Nepean, Ontario.

(Figures 21 to 24 were kindly provided by Dr A. M. P. Bouillant and Dr S. A. W. E. Becker, Animal Diseases Research Institute, Nepean, Ontario, Canada.) The ribonucleoprotein core of type C oncoviruses usually forms only at the plasma membrane of infected cells, with subsequent budding and maturation (Fig. 18c). An interesting report by Aziz *et al.* (1989) indicated that a defective mouse leukaemia virus had the ability to cause a severe immunodeficiency disease, indicating

strong parallel with the lentiviruses. Viruses of the genus oncovirus D do not induce tumours, but syncytia are detected *in vitro* (Bohannon *et al.*, 1991) and *in vivo* together with a persistent non-lytic infection. The spherical immature core of type D oncoviruses, which possesses a marked subunit structure, initially forms in the cytoplasm and buds through the plasma membrane (Fig. 18d) (Chatterjee and Hunter, 1980; Gelderblom, 1987). The Lentivirinae are all characterized by their

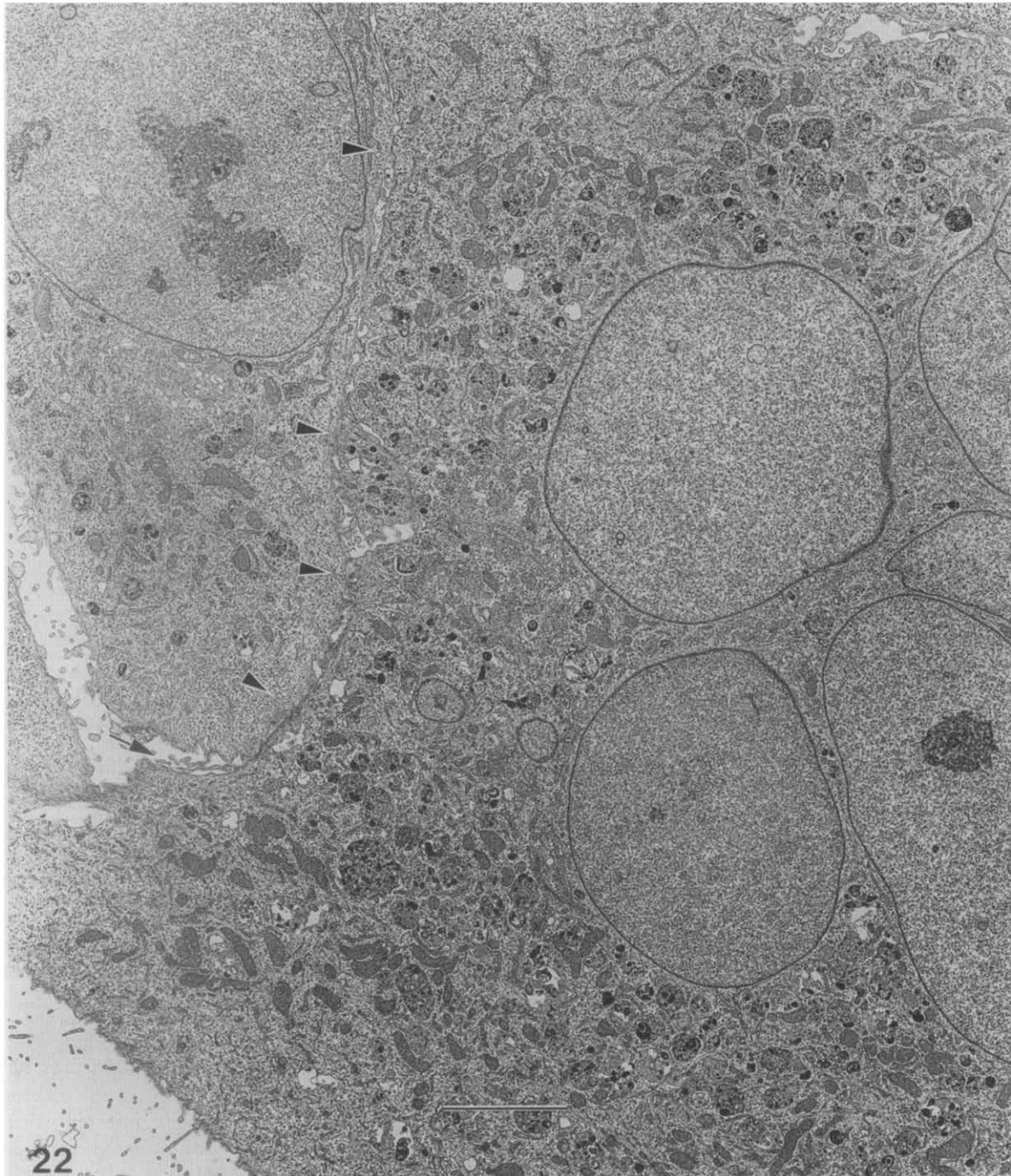


Fig. 22. Part of a MNGC induced in culture by bovine immunodeficiency virus (48 hr post infection). Arrowheads indicate zones of fusion between a single cell and the MNGC. Mitochondria and large lysosomes containing electron-dense granules are clustered around the nuclei, leaving a surface zone depleted in these organelles but containing disorganized rough endoplasmic reticulum and cytoskeletal filaments. Very little virus can be detected budding from the MNGC surface (arrow) and none into internal vacuoles. The scale bar indicates 5 μ m. Previously unpublished electron micrograph provided by Drs Bouillant and Becker, Nepean, Ontario.

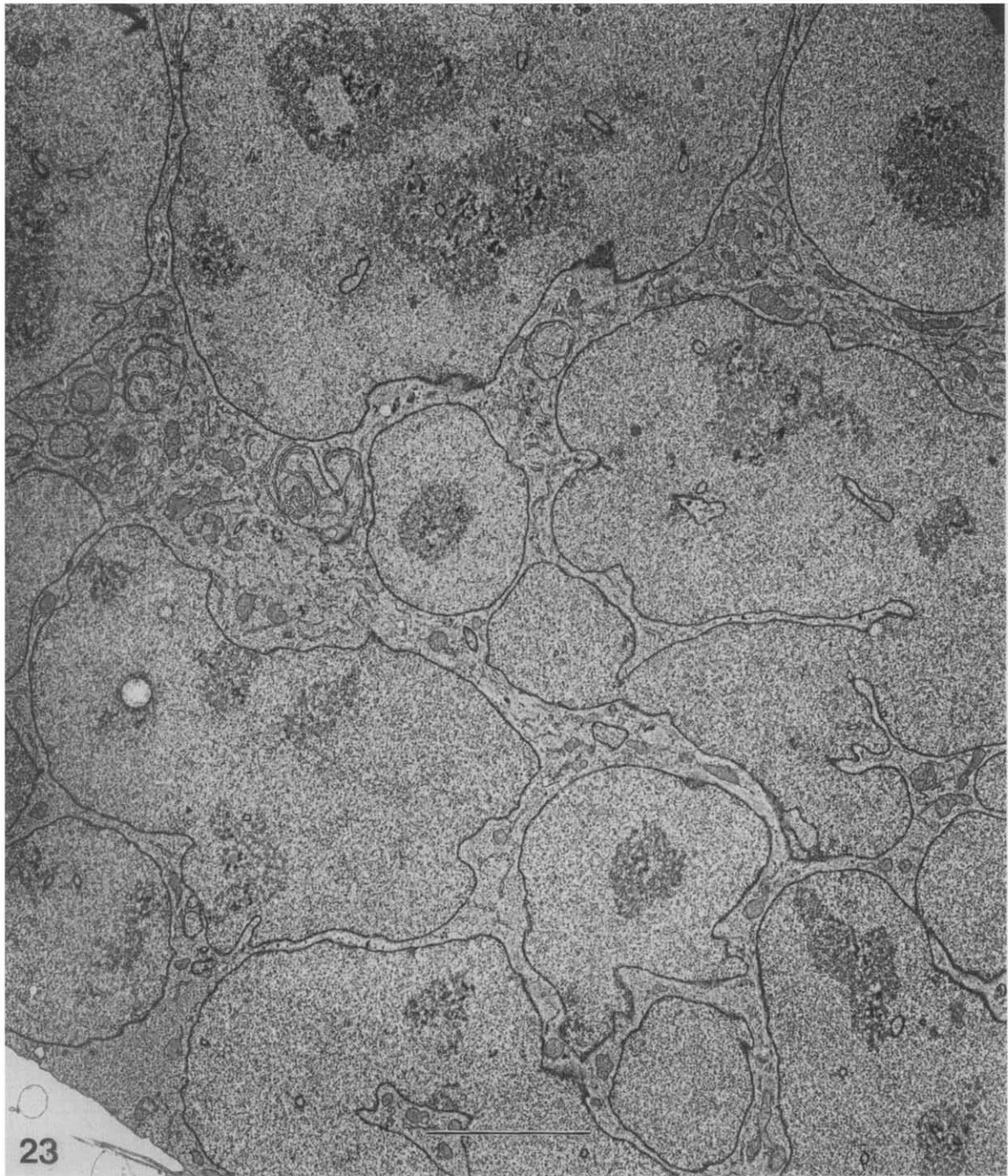


Fig. 23. Part of a MNGC induced in culture by bovine immunodeficiency virus (48 hr post infection). Clustered nuclei are shown, with almost total exclusion of mitochondria, lysosomes and cytomembranes. Considerable infolding of nuclear membrane is apparent, resulting in lobulation of the nuclei. The scale bar indicates 5 μ m. Previously unpublished electron micrograph provided by Drs Bouillant and Becker, Nepean, Ontario.

slow *in vivo* pathogenesis, following integration via reverse transcriptase (RT) into the cellular genome as proviral DNA. It appears that very often some additional lymphocytic activation/stimulation (cytokine/immunological/viral) is required to induce lentiviral replication with subsequent budding and release from infected lymphoid cells, but it is now believed that the macrophage may indeed be the prime host cell of the lentivirinae (Narayan and Clements, 1989). Considerable evidence is accumulating to indicate that

in vivo both animal and human lentiviruses have the property of inducing cell fusion of both macrophages and lymphocytes. *In vitro*, cells infected with lentiviruses all have the property of inducing cell fusion and syncytiogenesis with non-infected cells carrying the appropriate receptor for the viral surface glycoprotein (Daniel *et al.*, 1984, 1985; Gonda *et al.*, 1987). Indeed, the early studies on HIV (then termed HTLV-III/LAV) were to some extent slowed by this extreme cytopathic effect, which is more pronounced for HIV than for

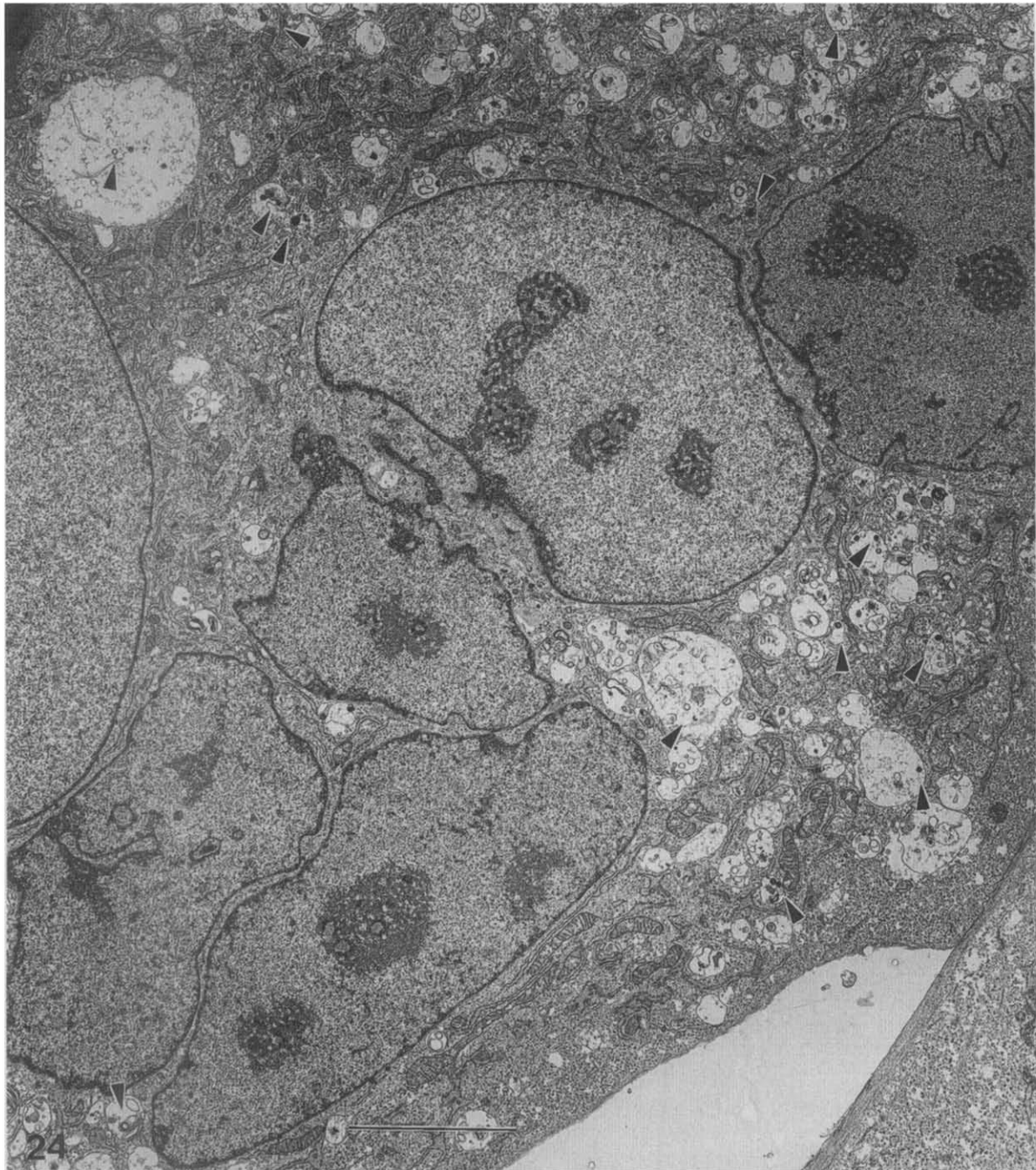


Fig. 24. Part of a MNGC induced in culture by bovine syncytial virus (36 hr post infection). Note the pronounced vacuolation of the cytoplasm and the release of incomplete spuma virus internally (arrowheads). Nuclei show less invagination and lobulation than is present in MNGC induced by BLV and BIV. The scale bar indicates $5\ \mu\text{m}$. Previously unpublished electron micrograph provided by Drs Bouillant and Becker, Nepean, Ontario.

HTLV-I and HTLV-II, until continuous non-fusing high producer lymphoid cell lines were established (i.e. H9, CEM, JM, GB8). That animal lentiviruses can infect mononuclear macrophages with viral release from the cell surface and also in a more pronounced manner into intracellular vacuoles, is also indicated by the study of Lairmore *et al.* (1987) (cf. Sections IV.B and V.B below). A number of ultrastructural studies have been devoted to animal lentiviruses (Booth and van der Maeten, 1974; Bouillant *et al.*, 1989; Thormar, 1961; Weiland and Bruns, 1980; Weiland *et al.*, 1977), which provided a sound basis from which the more

recent studies on the human lentiviruses have progressed. Bovine immunodeficiency virus (BIV)-induced MNGC formation occurs in lymphoid cell cultures (Fig. 22). The cell fusion process is indicated (arrowheads), despite the fact that very little virus is budding from the surface of the MNGC. Large lysosomes containing electron dense granules are particularly abundant and considerable disorganization of the cytomembranes and cytoskeleton is apparent. Under these conditions marked clustering of the nuclei occurs (Fig. 23), with almost total exclusion of mitochondria and cytomembranes. Lentiviral release occurs via a

very pronounced localized arc-like thickening of the plasma membrane where the core precursor is forming, with subsequent budding of the electron dense, spherical, immature virion and maturation of the core (Fig. 18e) after release from the cell (Frank, 1987c). The exact stages of the lentiviral core maturation are, as yet, not precisely defined in ultrastructural terms (Goto *et al.*, 1990; Katsumoto *et al.*, 1987, 1988, 1990), although much progress has recently been achieved (Hoglund *et al.*, 1992).

The spumaviruses do not induce malignancies, but induce a persistent infection, despite circulatory antibody. A lytic *in vivo* cytopathic effect is observed, initially expressed by the presence of highly vacuolated "foamy" MNGCs, which subsequently become necrotic and die. Similar features are often observed in infected cell cultures. Bovine syncytial virus (BSV) readily induces MNGC formation in culture, as shown in Fig. 24. Characteristic vacuolation of the cytoplasm is apparent, with formation/budding of incomplete virions occurring internally. Indeed the immature spumavirus is believed to form in the cytoplasm; exocytosis then imparts a viral membrane which contains particularly pronounced surface glycoproteins (Fig. 18f) (Gelderblom and Frank, 1987).

E. Other viruses

Although numerous enveloped RNA viruses not mentioned above, including the orthomyxoviruses, togaviruses and rhabdoviruses all produce acid pH-dependent cell fusion from without (White and Blobel, 1989), the available EM data is sparse. An example of a recent LM and molecular study comes from Gallagher *et al.* (1991) who investigated the broad pH dependency of cell fusion induced by corona mouse hepatitis virus type 4 (MHV4). Variant viruses containing genetically altered fusion-active surface glycoprotein, produced at a specific region of the polypeptide, were found to require acidic pH.

The pox viruses have been known for many years to induce cell fusion (e.g. Ichikashi and Dales, 1971). Whilst viral envelope proteins are again involved in the fusion event, the precise role of the pox virus surface tubules and their p58 protein (Stern and Dales, 1976), which may represent an excessive production of membrane protein, remains unclear.

Other viral groups, such as the alphaviruses (Koblet, 1990), are also known to produce cell fusion, but the lack of published electron microscopical data precludes further discussion.

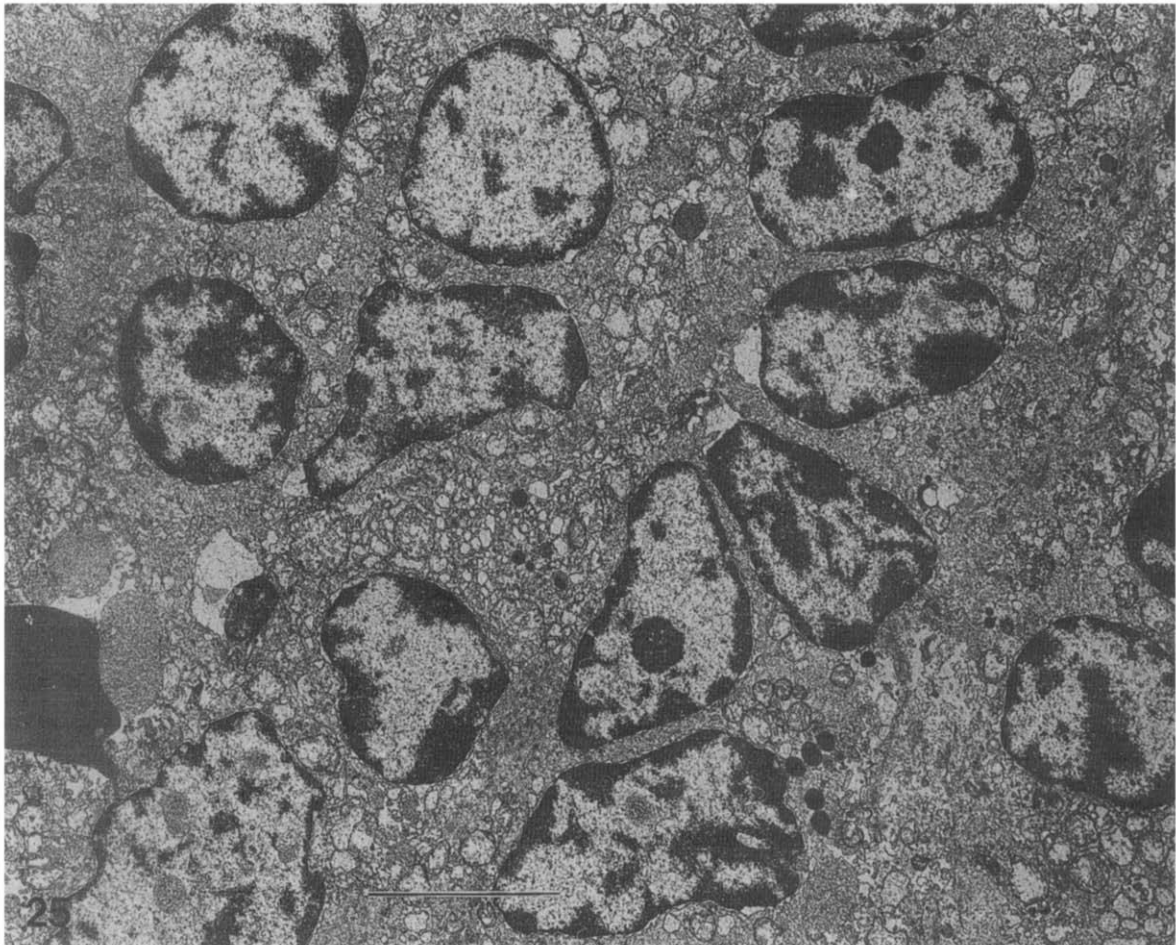


Fig. 25. A possibly artefactual "multinucleate zone", detected within a lymph node biopsy sample from an AIDS patient. The surface of the syncytium is not detectable, suggesting that plasma membrane breakdown may have occurred due to sample autolysis prior to fixation. The lymphoid nuclei show no signs of activation within a germinal centre, a feature usually indicative of HIV infection, and the mitochondria appear to be extremely swollen. The scale bar indicates 5 μ m.

IV. HUMAN IMMUNODEFICIENCY VIRUS-1

A. Multinucleate giant cells in vivo and in vitro

The two most marked and possibly significant clinical features which have emerged from the study of biopsy and autopsy samples taken from patients with ARC or AIDS are (1) the marked diminution in the population of circulatory CD4⁺ (helper) T-lymphocytes as the disease progresses from the latent infection to the more active phase of HIV replication and release (Lucey *et al.*, 1991; de Wolf *et al.*, 1988) and (2) the presence of

MNGCs in a number of tissues, most particularly the brain, lymph nodes, spleen and lungs. Whilst the demonstration of these MNGCs, of macrophage/monocyte (microglial) and lymphocyte origin, has been based primarily upon their detection by light microscopy (Budka *et al.*, 1987; Byrnes *et al.*, 1983; Dickson *et al.*, 1990; Joshi *et al.*, 1984; Sharer *et al.*, 1985; Wiley *et al.*, 1986), an increasing amount of data is becoming available utilizing TEM to assess the presence of MNGCs together with viral particles (Felice *et al.*, 1987; Gendelman *et al.*, 1989; Lindboe and Froland, 1988; Naito *et al.*, 1989; Orenstein and Jannotta, 1988). One problem with the EM of biopsy and autopsy samples

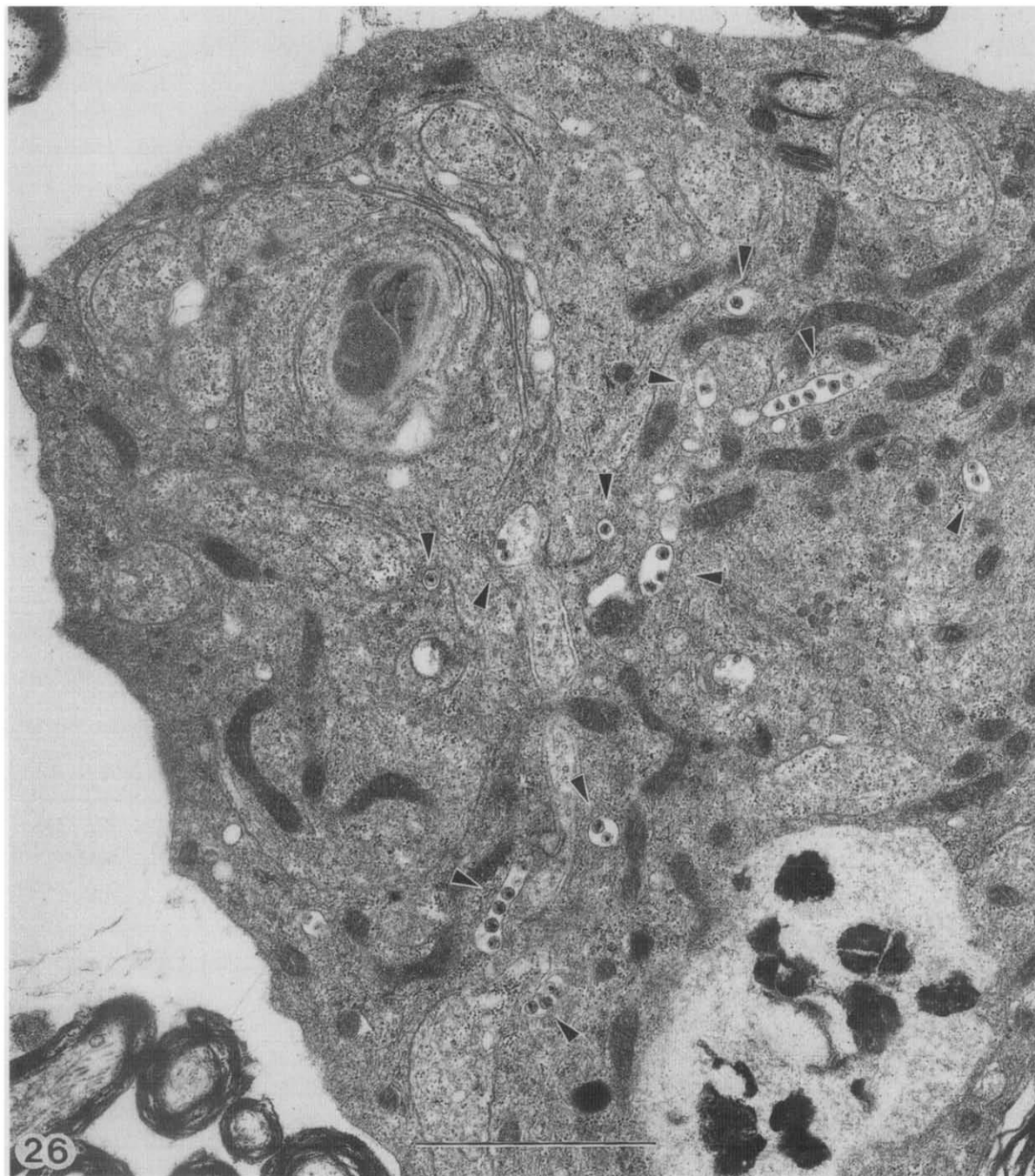


Fig. 26. Part of a human central nervous system macrophage containing numerous cytoplasmic vacuoles containing mature HIV particles (arrowheads). The scale bar indicates 2.0 μ m. Print kindly provided by Dr J. M. Orenstein. From Orenstein *et al.* (1988), with permission.

often relates to the quality of the tissue and its fixation, which can result in the apparent presence of MNGCs simply by the loss of plasma membrane due to autolysis, but with retention of nuclear integrity. Degeneration or swelling of other cellular organelles, such as mitochondria, will usually be an indication of this cellular damage (Fig. 25). In the situation shown, it was not possible to define the surface of a MNGC, thus, indicating apparent multinucleation due to tissue autolysis. Indeed, the EM screening of lymph node and spleen biopsy samples from AIDS

patients for activated viral-producing lymphocytes, i.e. with viral budding and the presence of immature and mature virions, has proved to be an extremely difficult task, unless activated germinal centres have been previously defined by light microscopy (le Tourneau *et al.*, 1986). Even though the number of infected lymphocytes is somewhat higher than in the periferal circulation it still remains a very low ratio. However, the detection of virus-producing monocytes from brain biopsies has proved to be somewhat less difficult (Fig. 26).

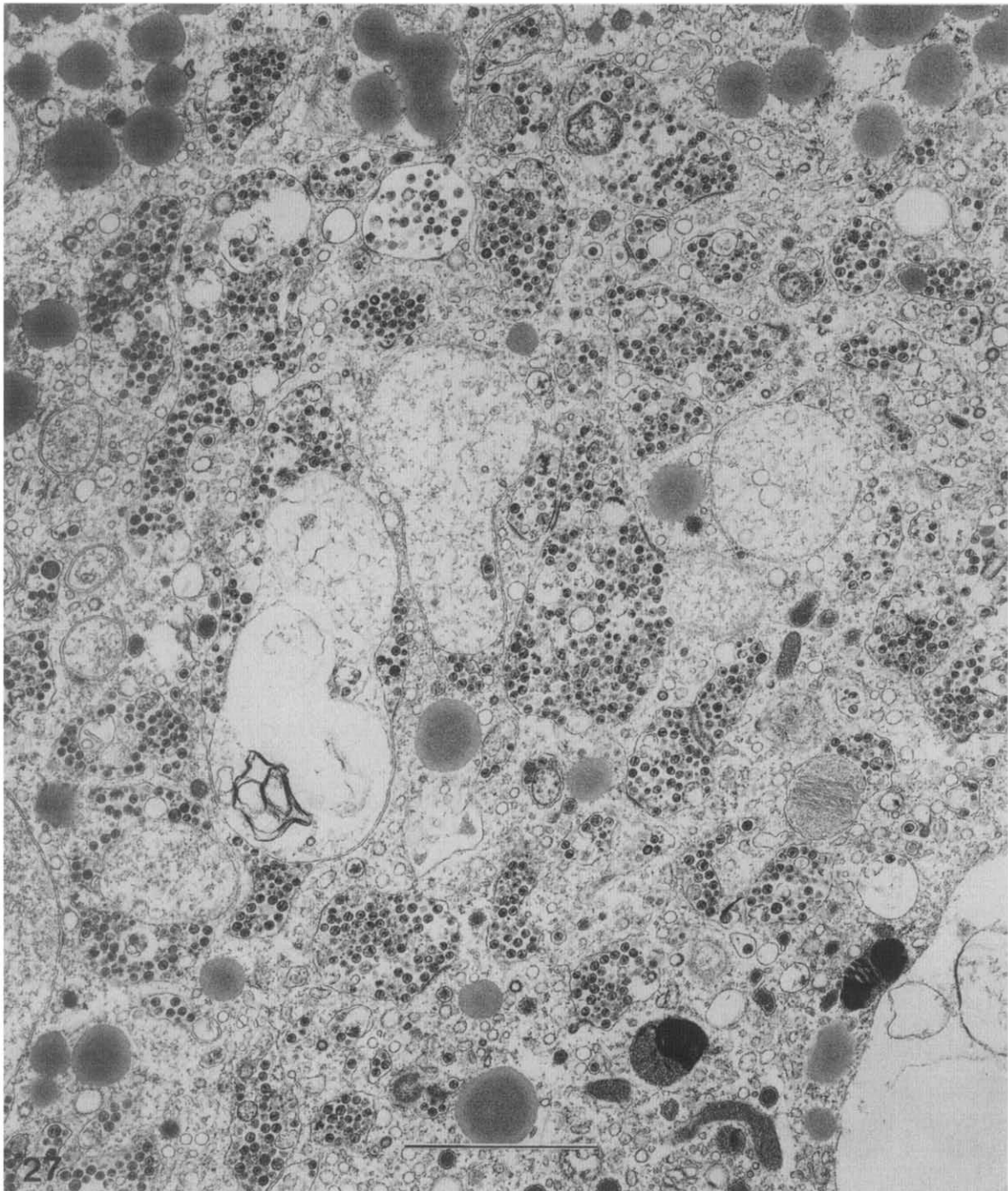


Fig. 27. Part of an HIV-1-infected (14 day) multinucleated cell of macrophage origin, containing very large numbers of cytoplasmic vacuoles laden with mostly mature HIV-1 particles. The scale marker indicates $2.5\ \mu\text{m}$. Print kindly provided by Dr J. M. Orenstein. Modified from Orenstein *et al.* (1988), with permission.

The natural extension of the above *in vivo* observations was the production of primary cell cultures from lymphocytes and macrophages taken from HIV-positive people and AIDS/ARC patients (Bigi *et al.*, 1990; Gendelman *et al.*, 1989; Ho *et al.*, 1986; Klatzmann *et al.*, 1984; Orenstein *et al.*, 1988; Watkins *et al.*, 1990) together with the establishment of the HIV producer cell lines (Popovic *et al.*, 1984). A significant observation has been that HIV-infected quiescent lymphocytes generally require additional cellular stimulation with a mitogen such as PHA in order for viral production to be detected. Similarly, cultured primary macrophages on stimulation (Gendelmann *et al.*, 1988; Ho *et al.*, 1986; Pautrat *et al.*, 1990) will readily produce and release virus, within single cell and MCGCs (Fig. 27). The receptivity/permissiveness of HTLV-I or HTLV-II transformed (transactivated) lymphoid cells to HIV infection followed by rapid viral replication and release, combined with the formation of MNGCs as the culture time progresses (often together with a population of mitotic single uninfected and infected cells) has yielded valuable information on the nature of viral glycoprotein Gp120, the cellular CD4 antigen and CD4-Gp120 interaction (Clapham *et al.*, 1987; Harada *et al.*, 1985; Hussey *et al.*, 1988; Ho *et al.*, 1991; Lifson *et al.*, 1986a, 1986b; Montefiori and Mitchell, 1987; Nara *et al.*, 1987; Sodroski *et al.*,

1986; Yoffe *et al.*, 1987). There is here a clear parallel between the C-type retrovirally-transformed cultured lymphoid cells and the large transformed single lymphocytes seen in lymph node biopsy samples from AIDS patients (Byrnes *et al.*, 1983), which often lie alongside MNGCs. It is an apparent physiological contradiction that mitotic and immunostimulation may actually make T-lymphocytes more receptive to HIV infection! The similarity of the HIV-syncytium forming assay and the MNGC/syncytium formation by cells infected with other retroviruses (human and animal) should be noted. The fusion of infected cells with non-infected cells, provides the technical basis for many investigations directed towards immunological or pharmaceutical prevention of the cytopathic action of HIV, as a possible target for future clinical treatment (Bremermann and Anderson, 1990; Fund *et al.*, 1987; Gruters *et al.*, 1987; Hanada *et al.*, 1991; Lifson *et al.*, 1991; Matsui *et al.*, 1990; Momota *et al.*, 1991; Owens *et al.*, 1990; Pal *et al.*, 1991; Srinivas *et al.*, 1991; Stein *et al.*, 1991; Suzuki *et al.*, 1989; Tochikura *et al.*, 1988; Wells *et al.*, 1991).

In many of the above mentioned *in vitro* studies, emphasis has been placed upon the assessment of MNGC formation by light microscopy. The Montagnier group (Klatzmann *et al.*, 1984; Montagnier *et al.*, 1984) were the first to include electron micrographs of a HIV- (then LAV) induced syncytia in lymphoid cell

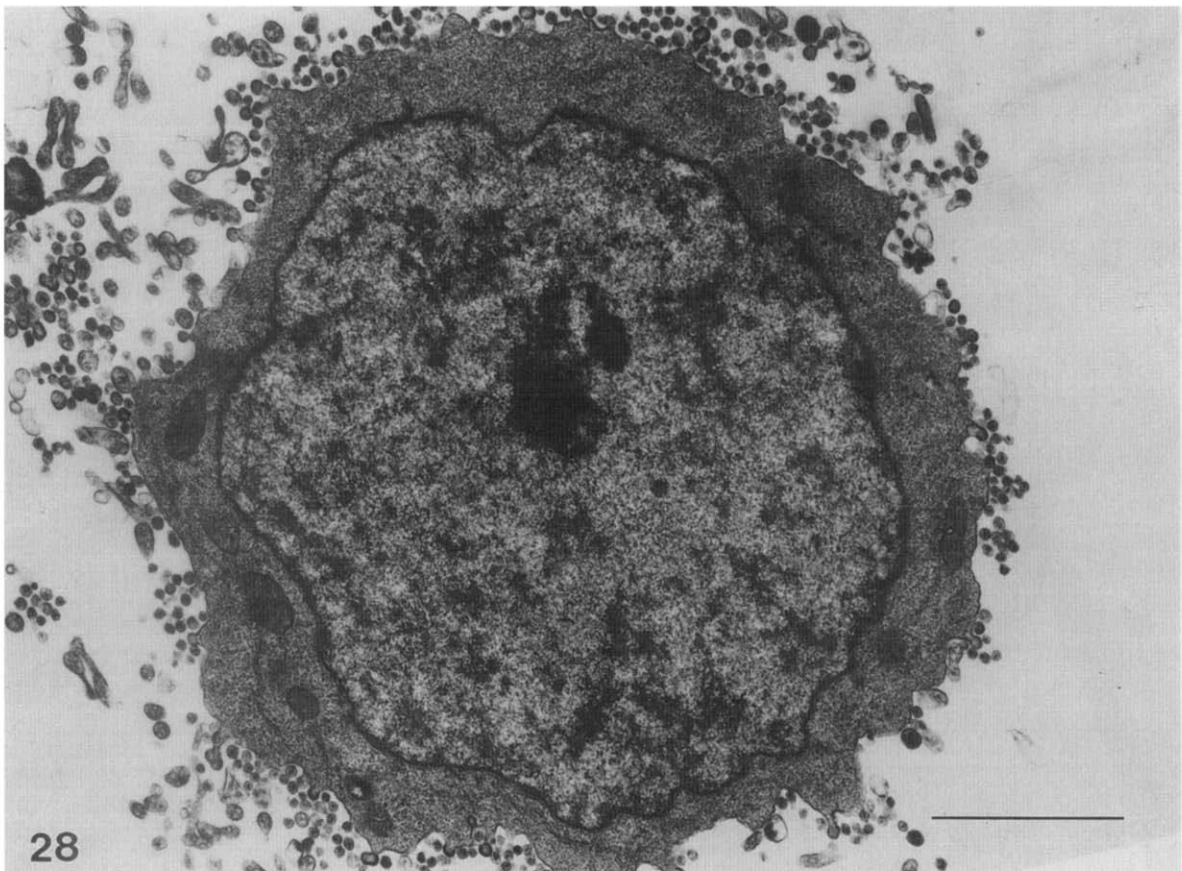


Fig. 28. A single C8166 lymphoblastoid cell infected with HIV-1 (day 5 post infection). The cell is releasing large numbers of virions over the entire surface. The scale bar indicates 2.5 μ m.

cultures, but they overlooked the distinct fact that viral release also occurs within cytoplasmic vacuoles in such MNGCs. This point was perhaps over emphasized in the EM study of Dowsett *et al.* (1987) who neglected the continued yet slower surface release of HIV. The fact that HIV release occurs at both the surface of lymphoid MNGCs as well as into the intra-syncytial vacuoles was clearly demonstrated by Harris *et al.* (1989) (see below, Section IV.E and IV.F). A distinction should, however, be firmly drawn between the mechanism of HIV release by single lymphoid cells and macrophages, the former liberate virus only at the cell surface, whereas the single and multinucleate macrophage tends to liberate virus primarily into cytoplasmic vacuoles (Gendelman *et al.*, 1988, 1989; Orenstein *et al.*, 1988; Orenstein and Jannotta, 1988; Pautret *et al.*, 1990) (Figs 26 and 27), but Watanabe *et al.* (1991) observed viral budding at the macrophage surface.

B. The single HIV-1 infected lymphoid cell in culture

Electron microscopical studies on the HIV-1 infection of suspension cultures of the permissive human C8166 HTLV-I transformed lymphoblastoid cell line have been performed by Harris *et al.* (1989). From approximately two days post infection of the cell culture, viral-producing cells can be detected. This HIV-1 release is seen to

occur only at the cell surface (Fig. 28); compare with the intravacuolar viral release in the macrophage (Figs 26 and 27). The ultrastructural detail of viral budding and maturation, will not be dealt with further and the reader is referred to the recent articles by Christie and Almeida (1988), Clavel and Orenstein (1990), Gelderblom (1991), Gelderblom *et al.* (1992), Goto *et al.* (1990), Hoglund *et al.* (1992) and Katsumoto *et al.* (1987, 1988, 1990). It should be noted that although viral release apparently occurs evenly over the surface of infected cells in some instances, in many cases discrete patches containing large numbers of viruses at varying stages of release can be encountered (Fig. 29), with large regions of plasma membrane devoid of virus. Released virions have a pronounced tendency to remain close to the plasma membrane following budding (Fig. 30), where HIV protease-dependent core maturation then proceeds.

C. The fusion event in HIV-1 infected lymphoid cell cultures

The major difficulty facing those who wish to make a precise assessment of the early events of cell fusion by transmission electron microscopy, centres around the problem of being able to distinguish between close contact of adjacent cells and true adherence with commencement of breakdown/fusion of the two plasma

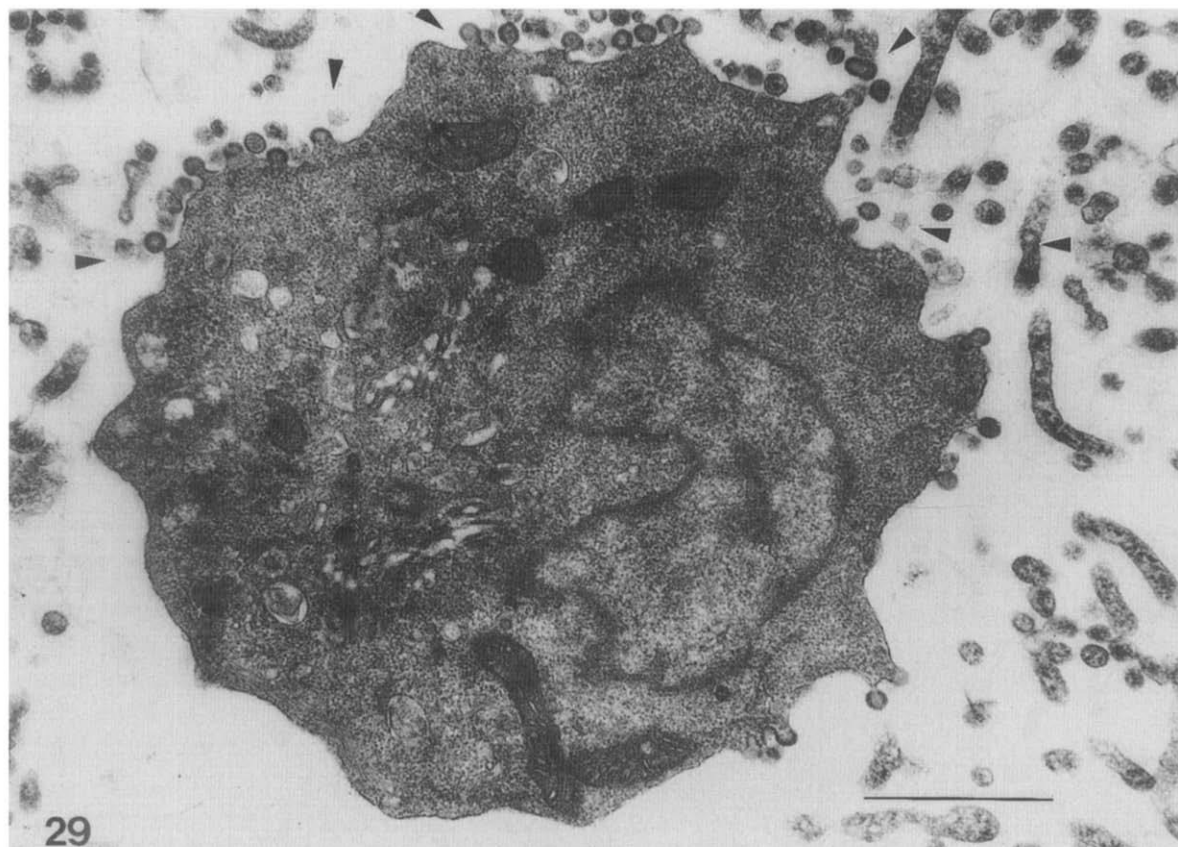


Fig. 29. A single C8166 lymphoblastoid cell infected with HIV-1 (day 3 post infection). Virions are budding predominantly from the upper region of the cell surface (arrowheads), with a considerable portion of the surface devoid of virions. The scale bar indicates 1.0 μm .

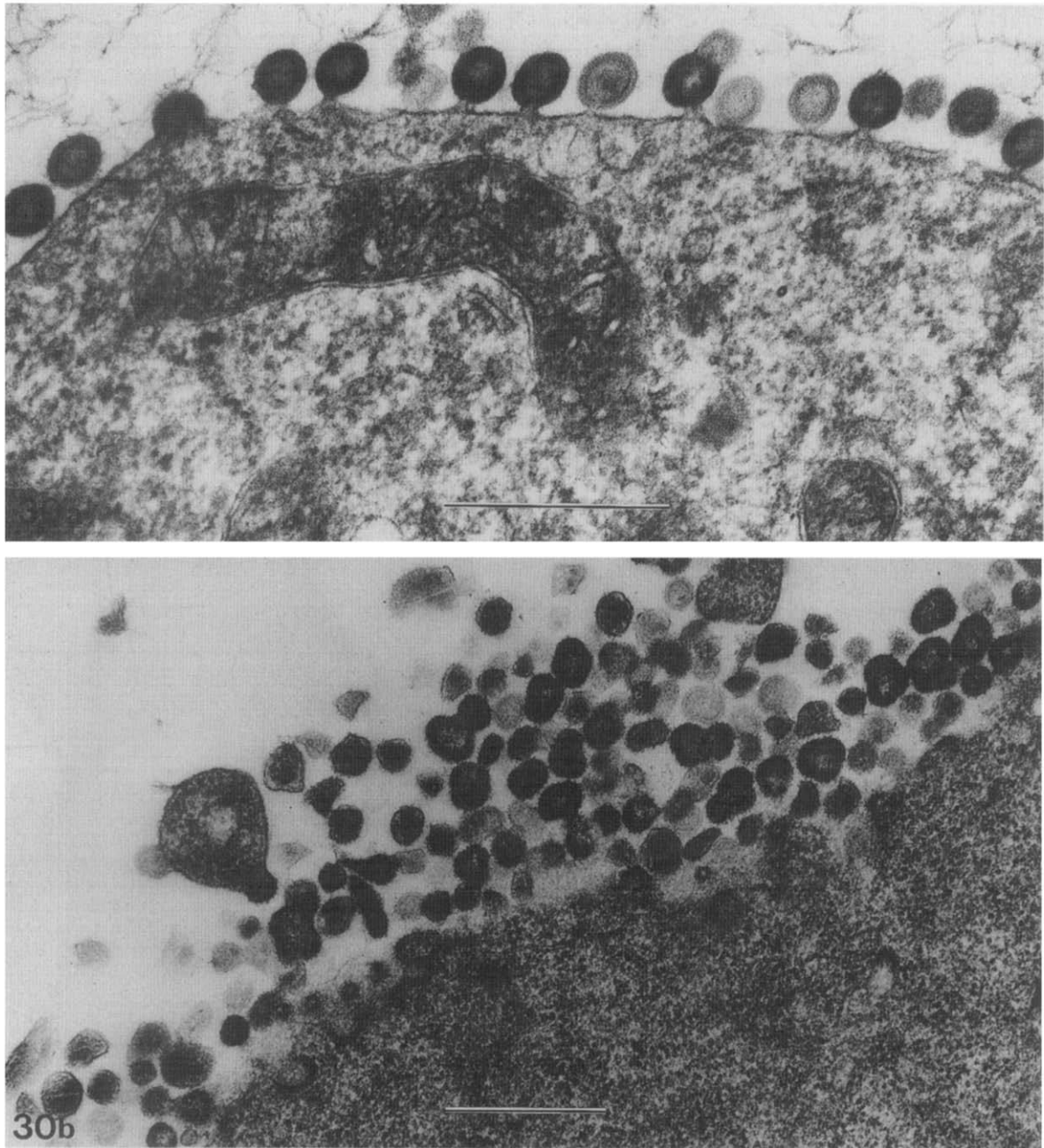


Fig. 30. Higher magnification images of HIV-1 budding from the plasma membrane of infected C8166 lymphoblastoid cells. In (a) all the viral particles are immature, with many still attached to the cell by a slender stalk. In (b) a much larger number of immature virions are being released from the cell surface, many exhibiting deviant shapes. Further away from the cell surface some of the virions have matured and formed electron-dense cores, whereas all those close to the cell surface are immature. Note the surface glycoprotein coat of the immature virions. The scale bars indicate $0.5 \mu\text{m}$.

membranes, thereby creating cytoplasmic continuity between the cells. The problem is somewhat worse for monolayer cultures than for suspension cultures, since the latter often possess a surface microvillar coat and do not usually have any spontaneous tendency to aggregate. Dispersion and slow centrifugation of fixed cells in low melting temperature agarose is desirable for EM processing, rather than centrifugal pelleting, since the latter can induce artificial close contact between cells. Even very large fragile syncytia are well preserved and dispersed by this agarose procedure.

Thus, with the extensively microvillated C8166 lymphoblastoid cells, processed in agarose, it can be

safely assumed that when two cell surfaces are in close proximity, with one or both cells exhibiting viral production, these cells may well be in the process of undergoing fusion. The presence of large numbers of single cells alongside the fusing cells provides further justification for the validity of this interpretation. The detection of lesions in the two plasma membranes then provides direct evidence for this fusion process, which does not require the presence of viral particles at the fusion site. Rather, it does require the presence of newly synthesized viral glycoprotein Gp120 (Deportes *et al.*, 1989) to attach to available CD4^+ receptors. Figures 31 and 32 show varying stages of the cell fusion process

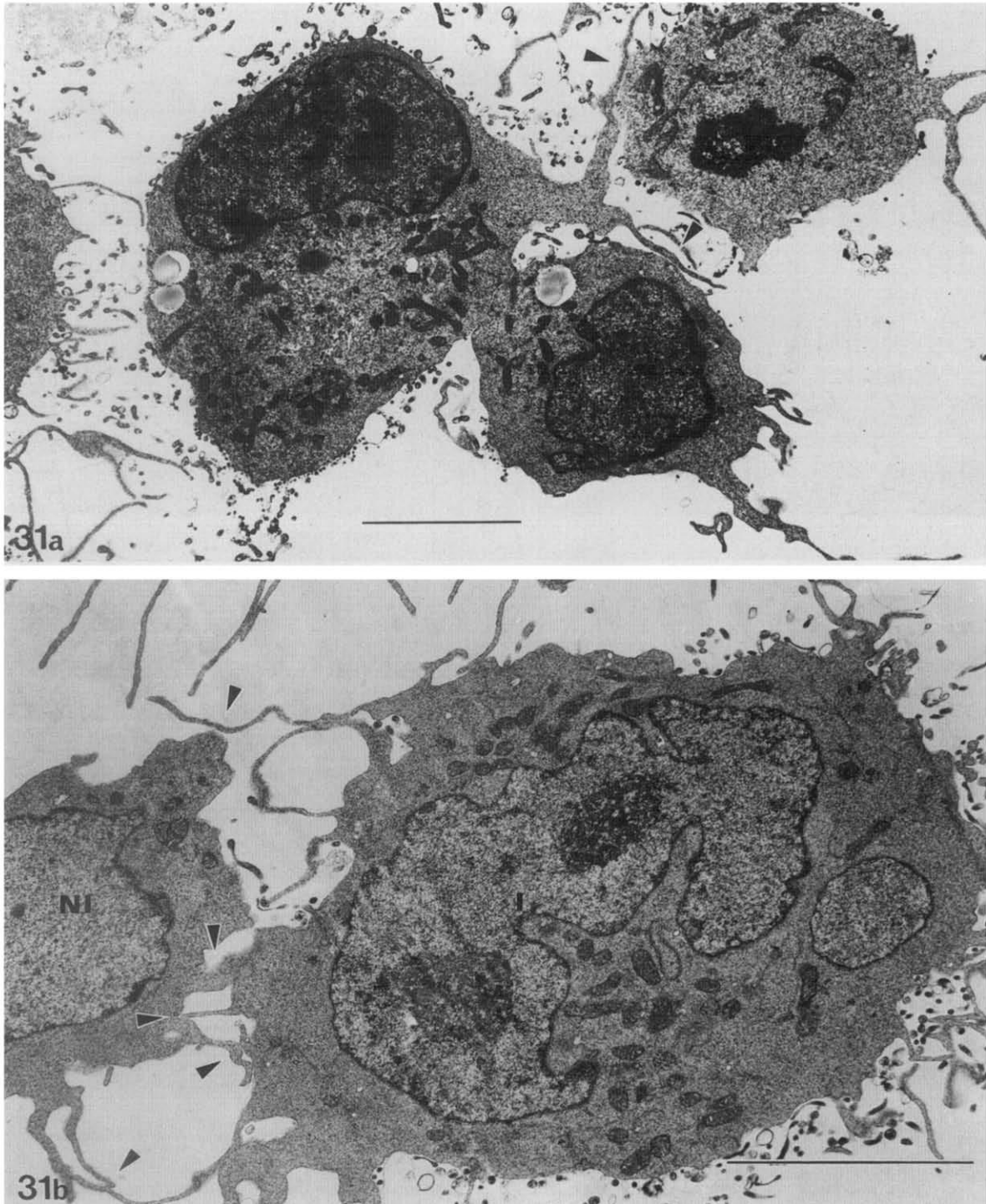


Fig. 31. The early stages of cell fusion in a HIV-1-infected C8166 suspension culture (day 5). Cells initially make contact with their cell processes (arrowheads) (a and b), usually without any clear bridging by surface virions, as expected for fusion from "within". Although it is sometimes clear (b) that one cell is infected (I) and releasing virus and the other cell is not releasing virus (NI), in many cases this distinction is by no means simple. Scale bars indicate 5 μm .

and in Fig. 33 the breakdown of the two plasma membranes at the zone of contact is indicated at higher magnification. By five days post infection, C8166 cultures are found to contain a complete range of multinucleate cells, (i.e. binucleate to MNGC containing many tens of nuclei) together with large numbers of actively dividing mononuclear cells. This diversity of cellular material can best be demonstrated by light microscopy of Toluidine blue-stained semi-thin plastic

sections (Fig. 34), which does indeed provide a low magnification overview of much of the data presented in Section IV.

D. Formation of multinucleate giant cells

It is a somewhat arbitrary decision as to when a multinucleate cell becomes a multinucleate giant cell. A

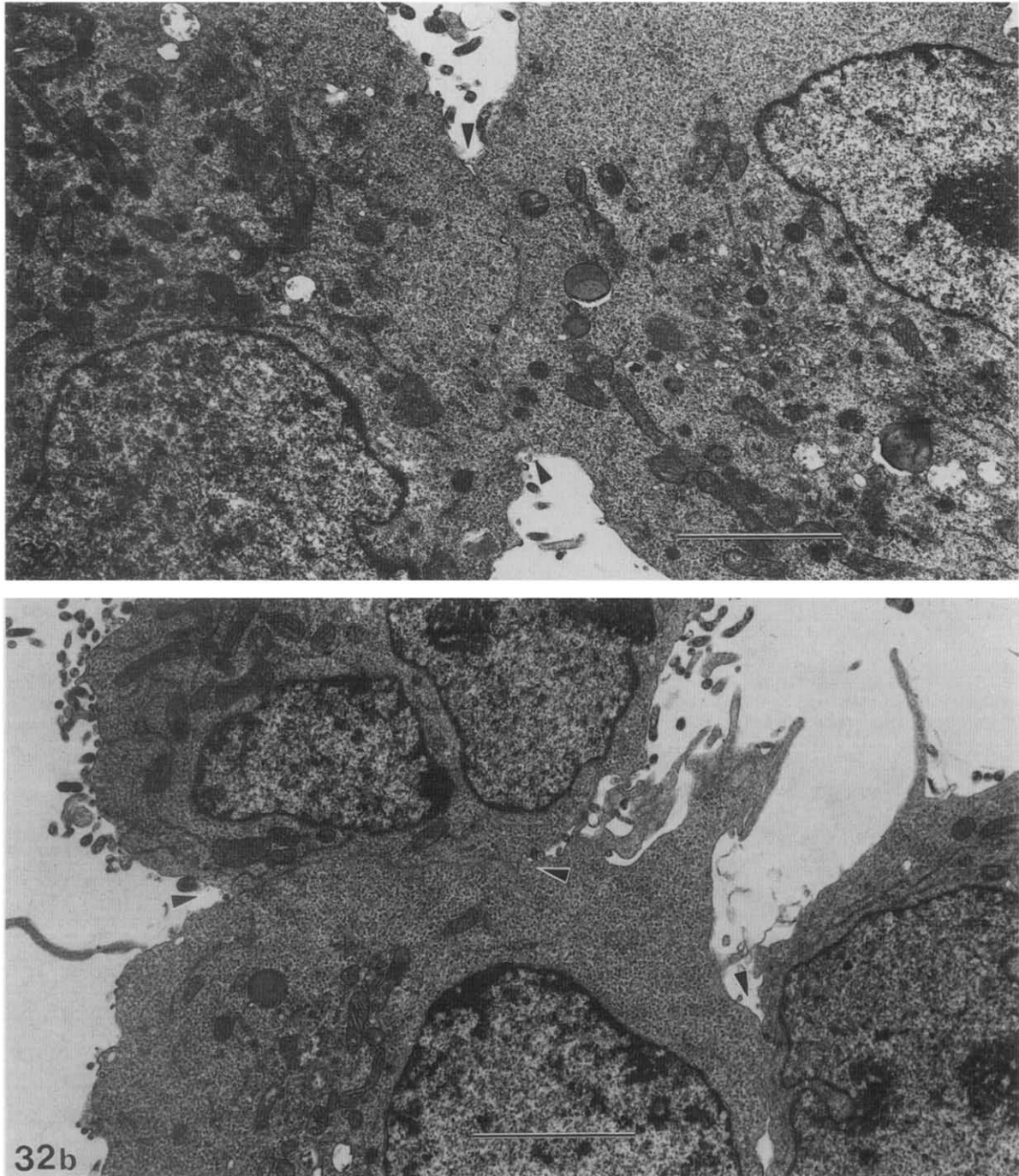


Fig. 32. A slightly later stage of cell fusion between HIV-1-infected C8166 cells. The region of plasma membrane contact (a and b) has extended (arrowheads) as a prelude to complete fusion. The scale bars indicate $2.5 \mu\text{m}$.

reasonable proposition might be when the fused cell contains upwards to 10 nuclei, but this fails to emphasize the extremely large MNGCs that can be formed under appropriate culture conditions, and which maintain “viability” for several days. Examples of individual multinucleate cells containing, two, three, four, five and six nuclei etc., have been readily obtained (Figs 35 and 36). Undoubtedly the continued fusion of single cells and small multinucleate cells is the mechanism by which the larger MNGCs are formed (Fig. 37). It is likely, however, that there is an active phase of cell fusion, followed by progressive metabolic changes within the MNGCs

which reduces the capacity for further fusion and ultimately leads to loss of viability. This statement is based upon the observation that the electron- and toluidine blue-dense MNGCs containing pyknotic nuclei do not apparently continue to fuse with single cells or small multinucleate cells (see below, Section IV.H).

One of the most characteristic features of HIV-1 induced MNGCs is their pronounced vacuolation. This can be detected within multinucleate cells containing upwards of approximately 10 nuclei (Figs 38 and 39) through to those containing upwards of 100 nuclei, which then may contain very large numbers of small

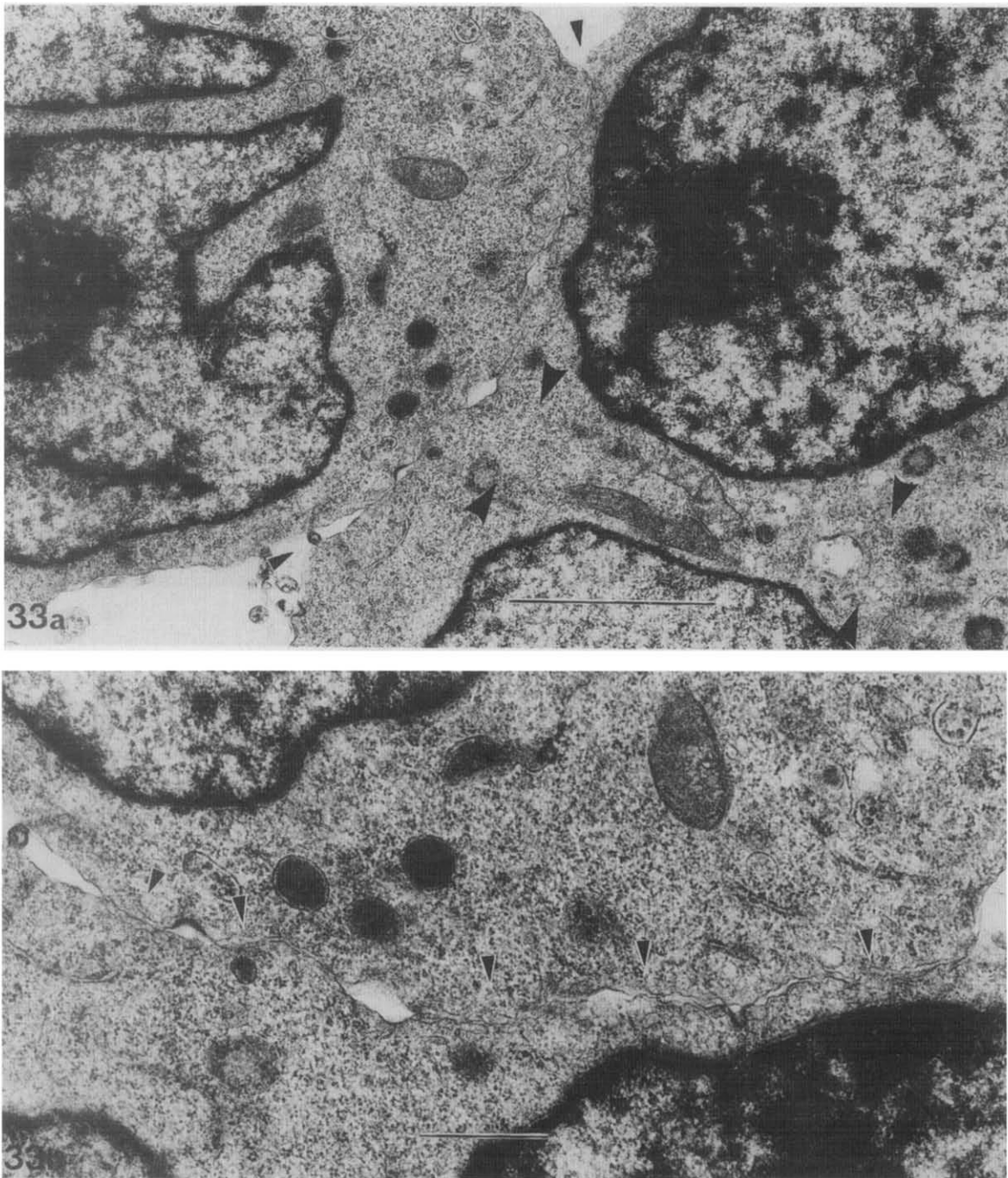


Fig. 33. Higher magnifications (a and b) showing zones of membrane fusion between adjacent C8166 cells (small arrowheads). Although breakdown of the plasma membrane is not rapid, as fusion progresses it becomes impossible to detect the former plasma membranes (between large arrowheads). Scale bars indicate $2\ \mu\text{m}$ (a) and $0.5\ \mu\text{m}$ (b).

vacuoles or enormous vacuoles which exhibit a “ballooning” effect (Fig. 34 and see below, Section IV.E). This vacuolation should be contrasted with the known CPE of the Spuma/Foamy viruses, which produce marked vacuolation within single cells and MNGCs. The relatively small MNGCs (Figs 38 and 39) continue to release HIV-1 in very large quantities from the syncytial surface, at discrete patches. The microvillation of the surface diminishes to some extent with MNGC growth, indeed part of the surface may become almost smooth, whilst other regions retain microvilli.

E. Cytoplasmic organization within multinucleate giant cells

As the number of nuclei incorporated into the multinucleate C8166 lymphoblastoid cells increases, certain clearly definable cytoplasmic changes occur, in addition to vacuolation and surface microvillation. The nuclei have a pronounced tendency to cluster, centrally in the smaller MNGCs and then in groups located some distance from the surface, in the larger MNGCs (Fig. 40). Mitochondria also cluster more centrally, in

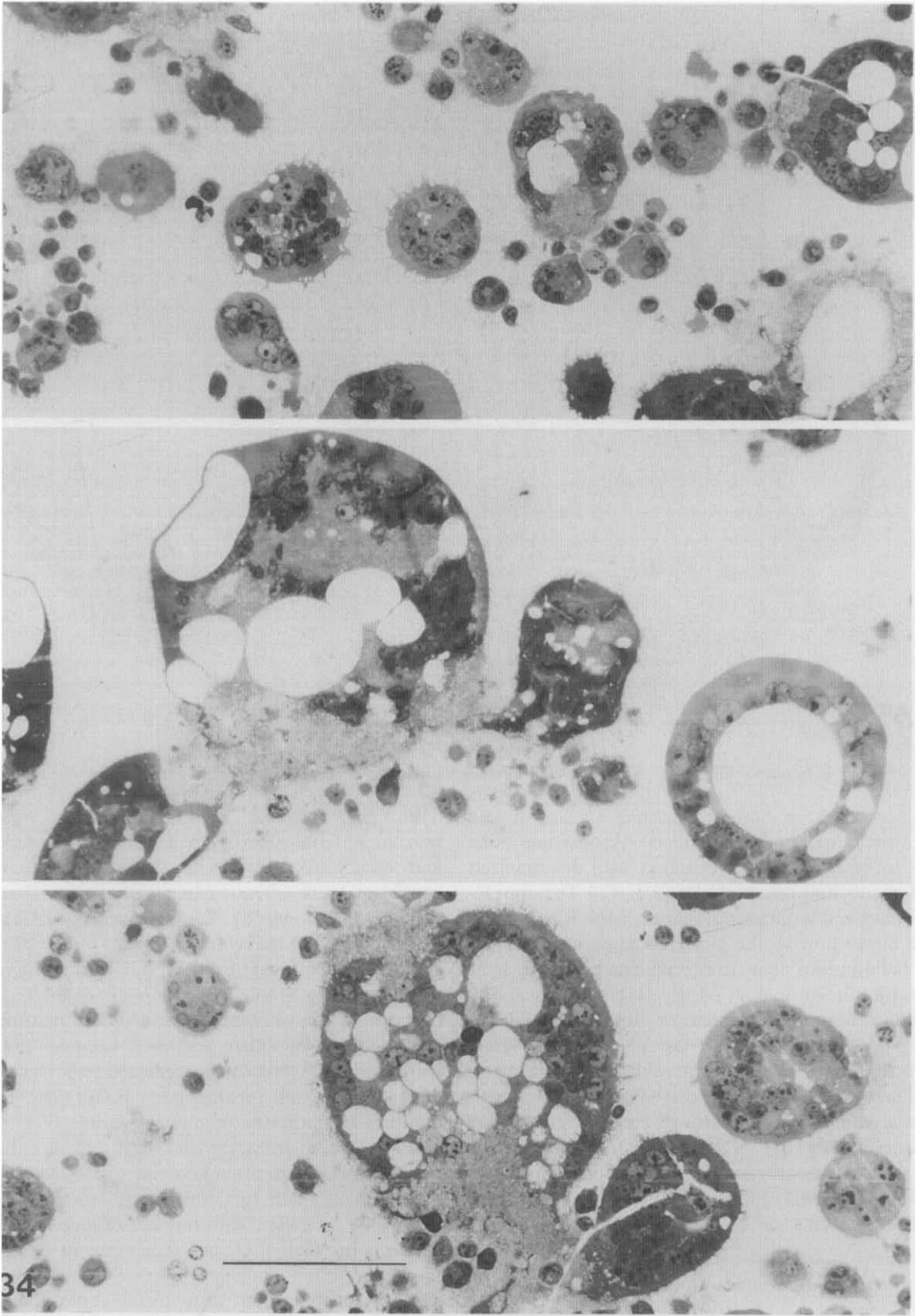


Fig. 34. Light micrographs of semi-thin plastic sections of a C8166 cell culture 5 days after infection with HIV-1. Note the wide range of MNGC size and the presence of many single cells. The larger MNGCs exhibit extensive vacuolation and "ballooning". The scale bar indicates 50 μ m.

a zone immediately outside the nuclear cluster. This produces a marked depletion of mitochondria in a broad zone of cytoplasm extending to the MNGC surface (Fig. 41). Single HIV-infected cells appear to

contain a normal complement of rough endoplasmic reticulum (rER), but organized cytomembranes appear to be lost in the MNGCs, except in close vicinity to the nuclei, where rER membranes can be seen (Fig. 41b).



Fig. 35. A binucleate C8166 cell. Very little HIV-1 release is occurring at the surface. The scale bar indicates 2.5 μ m.

Indeed, the mitochondrion-depleted cytoplasmic zone appears to be rich in free ribosomes with disorganized microfilaments and microtubules (Fig. 42). The appearance of small and large vacuoles in the MNGCs, initially in close apposition to the clustered nuclei, creates the foamy balloon-like structures, indicated by the light micrographs shown in Fig. 34. By electron microscopy it is readily apparent whether or not these vacuoles contain HIV. Clearly, the process of cell fusion must give rise to a gross excess of internalized plasma membrane. There is, therefore, the possibility that this plasma membrane could be re-utilized to create the vacuolar membranes within the MNGCs, possibly along with Golgi and/or smooth endoplasmic reticulum membrane. Immunolabelling data is required to answer these important questions.

F. Viral release from and within multinucleate giant cells

That single HIV-1 infected C8166 cells and small MNGCs release virus by budding from the surface has already been emphasized, in contradistinction to the single macrophage which releases primarily into internal vacuoles. We have found that medium sized MNGCs will very often be found to continue liberating virus at the surface (Harris *et al.*, 1989), whilst also budding virus

into the internal vacuoles (Fig. 43). With increasing size and vacuolation, by far the larger quantity of virus continues to be liberated internally rather than at the surface, as shown by Dowsett *et al.* (1978). These workers failed to make any comment on the progressive change of HIV release from MNGC surface to vacuoles, with respect to MNGC size. Sometimes the large vacuoles can be almost completely filled with mature virions (Fig. 44) and on other occasions vacuoles are almost empty (Fig. 43). Highly disorganized regions containing a network of small vacuoles can contain extremely large numbers of immature or mature virions (Fig. 45). The massive level of intrasyncytial viral release indicated in Figs 44 and 45 can often give rise to the overlapping of budding sites at the vacuolar membranes. This results in the release of a significant number of larger multi-cored viruses with deviant shapes. The cores in these deviant virions are often elongated tubes with a constant diameter, rather than the more usual conical shape, but cf. Klimkait *et al.* (1990), Christie and Almeida (1988) and Clavel and Orenstein (1990).

G. Nuclear changes within multinucleate giant cells

In the HIV-1 infected C8166 cell and the MNGCs that are actively liberating virus, at their surface or into vacuoles, it can be assumed that large amounts of viral



Fig. 36. A tetranucleate C8166 cell, showing the first signs of the "rounding-up" phenomenon that occurs to the MNGC as more nuclei are incorporated (cf. Fig. 34). The scale bar indicates 2.5 μm .

proteins and envelope glycoprotein are being synthesized by the cell. This follows from the initial insertion of the retroviral provirus within this transactivated/permissive cell line followed by the production of viral mRNAs. This production of viral mRNA is under the control of the two HIV activators of viral production, the *tat* and *rev* proteins. Under appropriate cell culture conditions, this can result in production of very large amounts of viral mRNAs, in turn responsible for the massive viral production described above.

It has been claimed by Koga *et al.* (1990), from immunolabelling data, that accumulations of Gp160 occur at the nuclear pore complexes of CD4⁺ cells transfected with the Gp160 gene. This is a rather difficult observation to reconcile with viral budding and also with the known mRNP nuclear-cytoplasmic translocation process that is believed to occur at the nuclear pore complexes. Our own data (Harris, 1990; Harris *et al.*, 1990) have shown that in the single cells and MNGCs actively producing large quantities of virus, a morphological change is detectable at the nuclear envelope. The nuclear pore complexes increase in prominence, this being expressed by a thickening, i.e. extension into the cytoplasm and an increase in electron density (Fig. 46), compared to cells not liberating HIV-1. It is suggested that this is an ultrastructural indication of the increased viral mRNA production in the nucleus and mRNP translocation at

the nuclear pore complexes. Clearly, further detailed ultrastructural and immunolabelling studies are required to obtain supportive data for this proposal, which is nevertheless, reasonably in accord with current ideas on the nucleocytoplasmic translocation of mRNP complexes (Mehlin *et al.*, 1991).

Following the phase of active viral production and release within the MNGCs, progressive nuclear changes occur, in parallel with those in the cytoplasm, both being an indication of the gradual loss of viability. The initially relatively smooth surfaced nuclei within the MNGCs become progressively shrunken and misshapen, this being shown firstly by nuclear envelope invaginations (Fig. 47a) and an increase in electron density with spongy reticulation of the nucleoli (Fig. 47b). Although all the nuclei may not have entered the MNGC at exactly the same time, there is remarkable uniformity in nuclear morphology within any one MNGC, suggesting that a short-lived active/dynamic phase of cell fusion terminates relatively quickly (hours rather than days). Shrinkage of the nuclei creates profiles very similar to those often seen within tumour or degenerate tissues (Fig. 48). At this later stage an increase in both nuclear and cytoplasmic electron density is noticeable. It should be emphasized that these metabolically inactive nuclei no longer exhibit the pronounced nuclear pore complexes mentioned above.

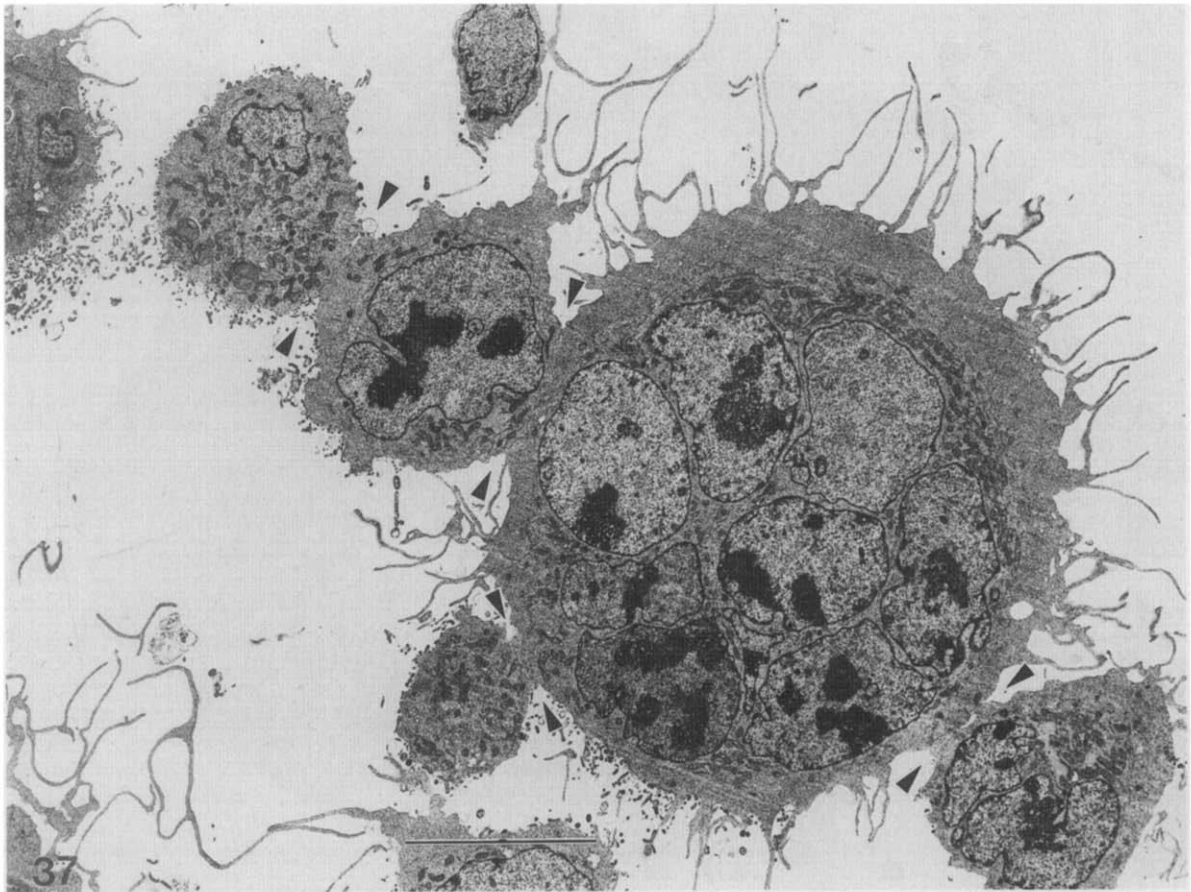


Fig. 37. The continued fusion of single cells (arrowheads) with a MNGC, thereby yielding a progressively larger MNGC. It is clear that some of the single cells are themselves already infected with HIV-1 and are releasing virus at the time of the fusion event. The scale bar indicates 10 μm .

H. Degeneration of multinucleate giant cells in culture

The long-term viability of the HIV-1 induced MNGCs in suspension culture is clearly limited to a few days, but the continued presence of single cells can give rise to a steady production of smaller MNGCs, as indicated by the range of cellular material present (Fig. 34). The marked increase in cytoplasmic electron density that occurs as the older, generally larger, MNGCs appears to be due to a progressive ageing phenomenon rather than rapid cytolysis. Electron microscopical specimens reveal increasing nuclear clustering and pyknosis (Figs 49 and 50), which parallels in a remarkable manner that occurring in the syncytiotrophoblast at the later stages of pregnancy (Figs 9 and 10). It should, of course, be mentioned that nutritionally, the relatively thin yet extensively spread syncytiotrophoblast is in a much superior situation, since it is in direct contact with the maternal blood supply. Also, other metabolic and hormonally controlled causes for syncytiotrophoblast degeneration which limit its survival, will be functionally linked to normal foetal development.

The ultimate degeneration of the MNGCs in suspension culture occurs when the sites of internal vacuolation disperse as a massive "foamy mass" (Fig. 51). The other, non-vacuolated, electron-dense cytoplasmic regions exhibit marked surface folding and abnormal

villation. This can give rise to a multiplicity of surface microvilli (Fig. 51), which undoubtedly indicate the rapidly approaching syncytial "death".

The somewhat limited data presented above serves to indicate that the HIV-1 infected C8166 lymphoblastoid suspension culture system expresses a complex dynamic situation within which cellular events relating to: (1) viral integration, replication and release, (2) cell fusion, (3) MNGC formation and (4) MNGC ageing and death can be investigated at the ultrastructural level. The survey presented represents only a small fraction of the available data, some of which has already been published elsewhere (Harris *et al.*, 1989, 1990; Harris, 1990).

V. GENERAL DISCUSSION AND SPECULATIONS

A. The embryological origin of the placenta and the evolution of placental mammals

Embryologists working at the end of the last century, such as Kölliker, recognized that the blastocyst was the tissue or organ from which the unique development of all mammals proceeds. Today we can be a little more precise, within the context of the extensive knowledge of embryological histology, placental ultrastructure and

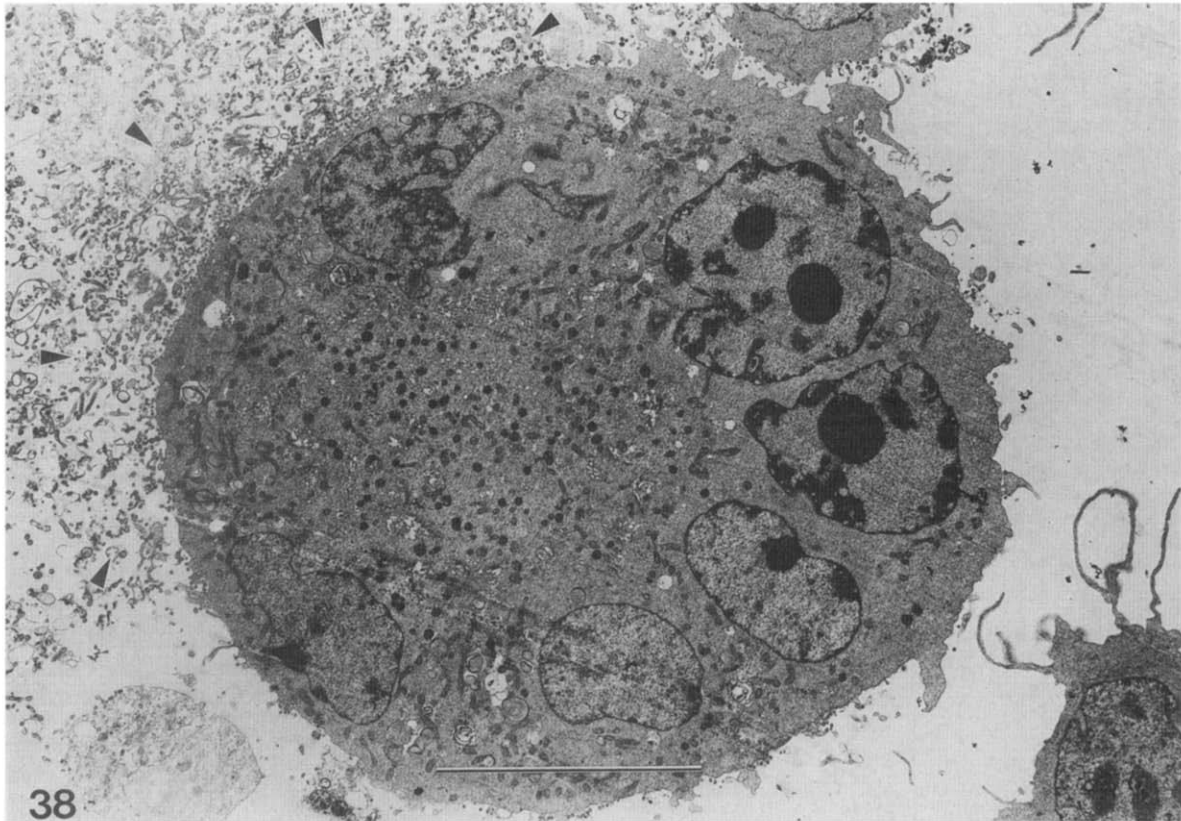


Fig. 38. A well-rounded MNGC, already exhibiting considerable variation in nuclear morphology. Massive HIV-1 release is occurring only at one region of the surface (arrowheads) and to a lesser extent elsewhere. Very early signs of vacuolation are present. The scale bar indicates 10 μ m.

pathology, by saying that it is reasonable to define the trophoblast, the cellular layer of the blastocyst cavity enclosing the truly formative embryonic cells, as representing the most distinctive mammalian characteristic at this early stage of development. To quote Davies and Hesseldahl (1971) "There is no homologue to the trophoblast in submammalian forms. The trophoblastic shell must be regarded as a peculiar mammalian structure developed solely in response to the demands of viviparity and prolonged gestation in the uterus".

The important question that must be posed is, why did the rapidly growing trophoblast develop, presumably at some critical stage in animal evolution just prior to the divergence of the mammalia (Aitken *et al.*, 1979; Pierce and Midgley, 1963; Pierce *et al.*, 1964)? All early stage trophoblasts are multicellular, but in the majority of mammals a discrete trophoblastic pole or syncytial plate develops, which becomes the forming placenta and site of uterine attachment. That the trophoblast has a remarkable growth rate and a "tumour-like" invasive nature in the region of the syncytial plate is believed to account for the continual expansion of the placenta by fusogenic incorporation of the underlying trophoblast cells, which are later defined as the Langhans' cells or cytotrophoblasts. The fact that certain mammals (ungulates and some rodents) do not have a fused-cell syncytiotrophoblast apparently does not preclude the function of the invasive trophoblast or its role in the creation of the placenta and the foetal/maternal barrier (Wynn,

1971), but clearly more up-to-date histological and ultrastructural studies are required in this comparative sphere. In the large majority of mammals, trophoblastic cell fusion does occur and we must ask why it happens and what physiological benefits the animal gains from the presence of the syncytiotrophoblast rather than a multicellular trophoblast.

From the early 1970s through to the present day there have been repeated electron microscopical observations claiming the presence of retroviral particles and reverse transcriptase in animal and human placental tissue (Feldman, 1979; Feldman *et al.*, 1983; Johns and Renager, 1990; Imamura *et al.*, 1976; Nelson *et al.*, 1978; Panem, 1979; Smith and More, 1988; Ueno *et al.*, 1983). In most cases these are C-type retrovirus particles, which bud from the plasma membrane, although occasionally A-type intracisternal particles have also been detected (Enders, 1971). Most of these data were produced within the context of the intensive search throughout the 1970s for human tumour-forming viruses and has been greatly overshadowed by the more recent and highly productive work on the animal leukaemia viruses and human leukaemia/lymphoma viruses HTLV-I and HTLV-II, and indeed that on the animal and human lentiviruses. The observation must, nevertheless, be considered to be correct and highly indicative of the presence of one or more endogenous retroviruses, expressed selectively within placental tissue. More specifically, retrovirus-like particles have been found to be budding from the

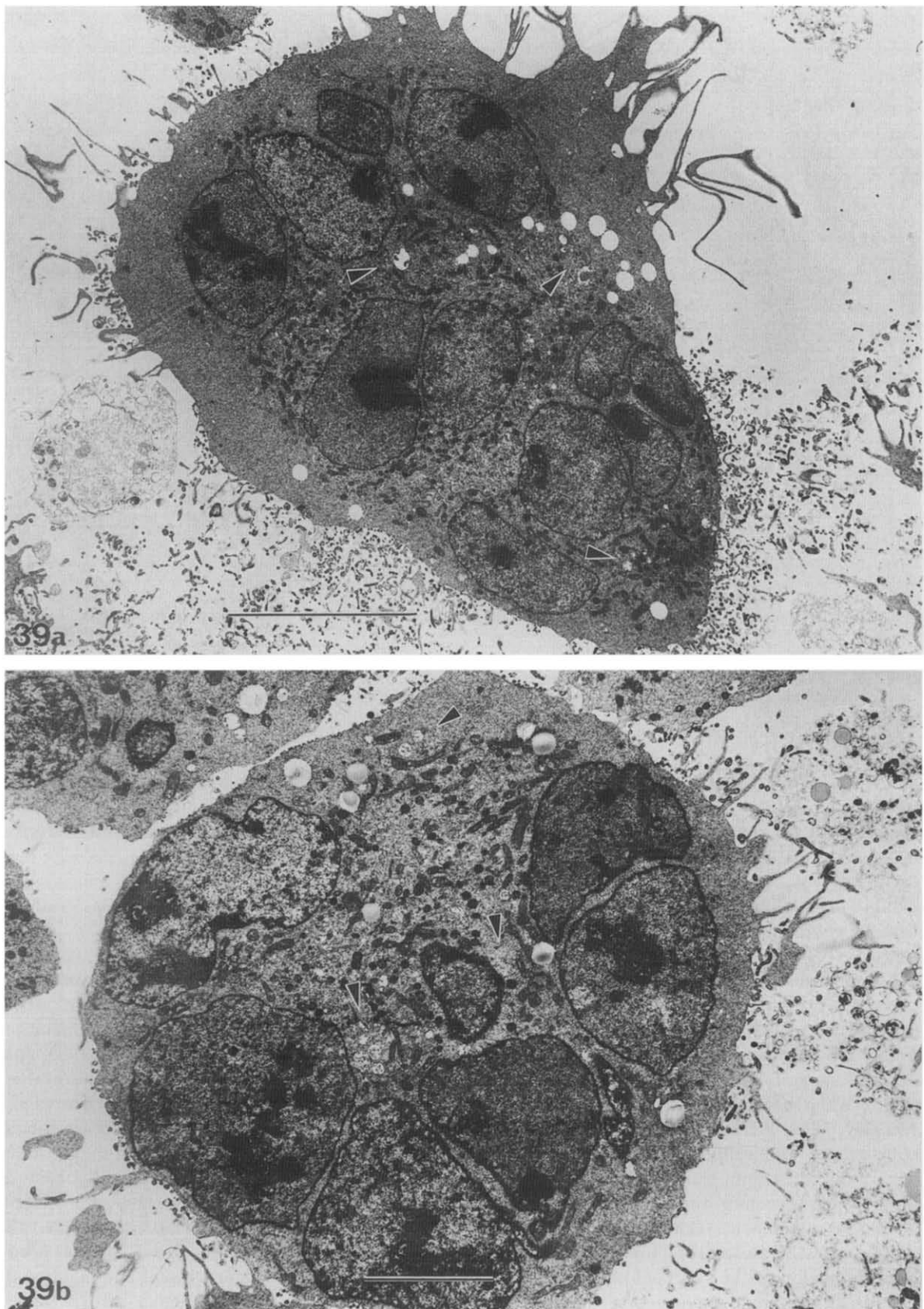


Fig. 39. C8166 MNGCs showing the continued release of HIV-1 from the syncytial surface, with early stages of cytoplasmic vacuolation (arrowheads) but with no internal HIV release. In (a) viral release is restricted to specific surface zones, whereas in (b) it appears to be occurring over most of the syncytial surface. The scale bars indicate 10 μm (a) and 5 μm (b).

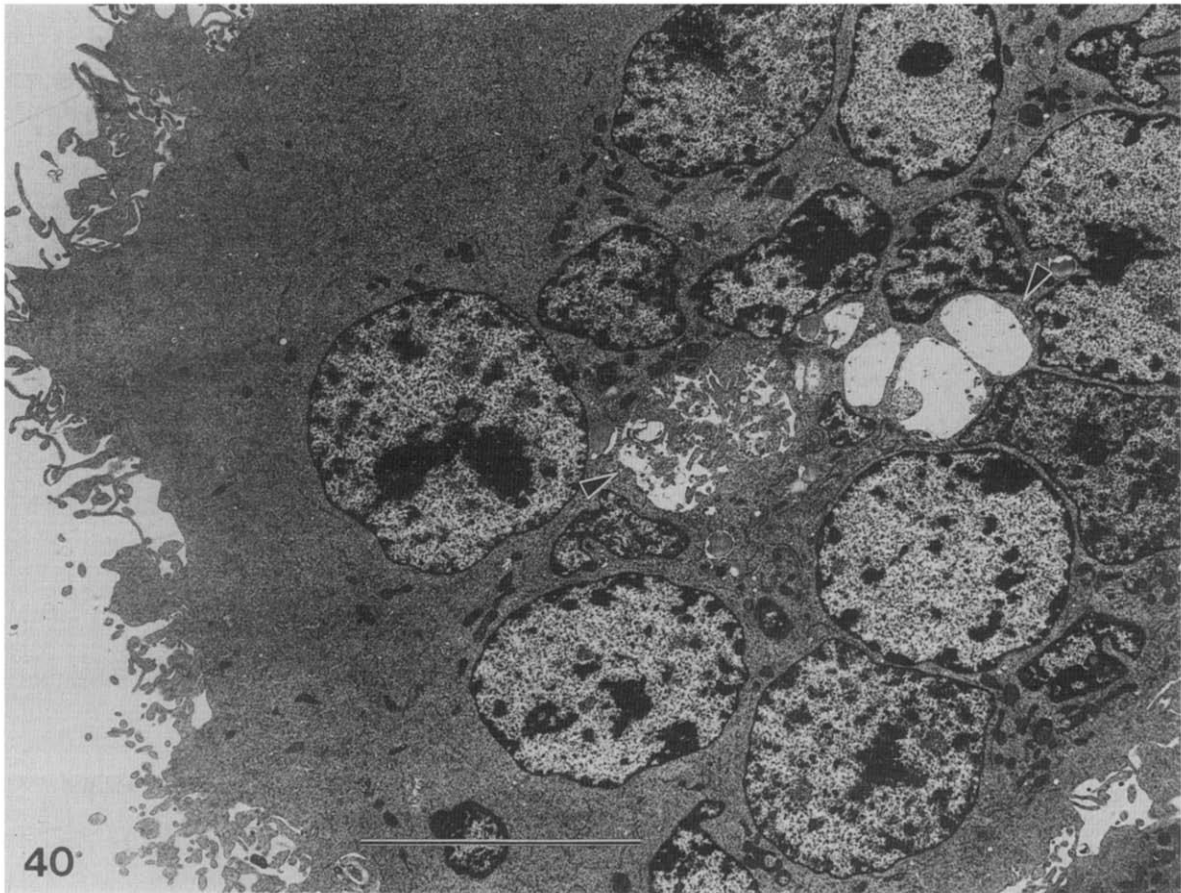


Fig. 40. Part of a medium sized MNGC, showing pronounced nuclear and mitochondrial clustering. Early vacuolation and internal HIV-1 release (arrowheads). The scale bar indicates 10 μm .

syncytiotrophoblast, particularly at the border with the underlying cytotrophoblasts, the histological zone at which cellular fusion is postulated to occur. Isolation of infectious virions from placental tissue has proved to be difficult (see comments by Weiss, 1982), but Stromberg and Beneviste (1983) claimed to be able to isolate an endogenous retrovirus from rhesus monkey trophoblast. Supplementary to this morphological evidence for the presence of endogenous retrovirus in placental cytotrophoblasts and syncytiotrophoblast, much data of a biochemical, immunological and molecular biological nature has accumulated which supports the overall implications of the ultrastructural data. Reverse transcriptase has been detected together with a number of viral antigens, located particularly within the syncytiotrophoblast, using immunofluorescence microscopy (Suni *et al.*, 1981, 1988a, b). It is eminently reasonable to propose that if the integrated provirus of such an endogenous virus is indeed replicating, the production of intracellular trophoblastic viral proteins could occur at the early stages of embryological development. Rather than remaining latent, as is also an inherent property of many retroviruses, there could be biosynthesis of the complete range of viral gene products or selected gene products. These may include the viral envelope glycoprotein, which will be incorporated into the plasma membrane of the infected cell, prior to viral budding. This could, in turn, have the capacity, as do other C-type

oncoviruses and lentiviruses discussed above (Section III.D), to induce cell fusion by interaction with an appropriate receptor exposed on the surface of the plasma membrane of neighbouring cells (see also Davies and Chilton, 1978). It has been suggested by Montagnier *et al.* (1984) that the extreme ability of certain members of the retroviridae to produce cytopathic cell fusion may indicate a general effect of retroviral glycoproteins at the plasma membrane of infected cells, which might deeply affect their specialized biological functions. Thus, a clear parallel can be drawn between the *in vitro* cell culture situation and *in vivo* situation, where MNGCs are formed following retroviral infections, and that are here postulated to occur in the trophoblast and placenta as the growing syncytiotrophoblast incorporates more cytotrophoblasts. Indeed the morphological features of the syncytiotrophoblast throughout its growth phase and degeneration are remarkably similar to those shown by cultured MNGCs (i.e. surface microvillation, cytoplasmic vacuolation, nuclear clustering and pyknosis).

Furthermore, early attempts at growing the blastocyst/trophoblast in organ culture (Blandau, 1971; Lopta *et al.*, 1982; Mohr and Trounson, 1982) and more recently the cell culture of cytotrophoblast-derived cell lines, indicates that they possess an inherent aggregative and fusogenic potential, and in many cases spontaneously form multinucleate syncytiotrophoblasts.

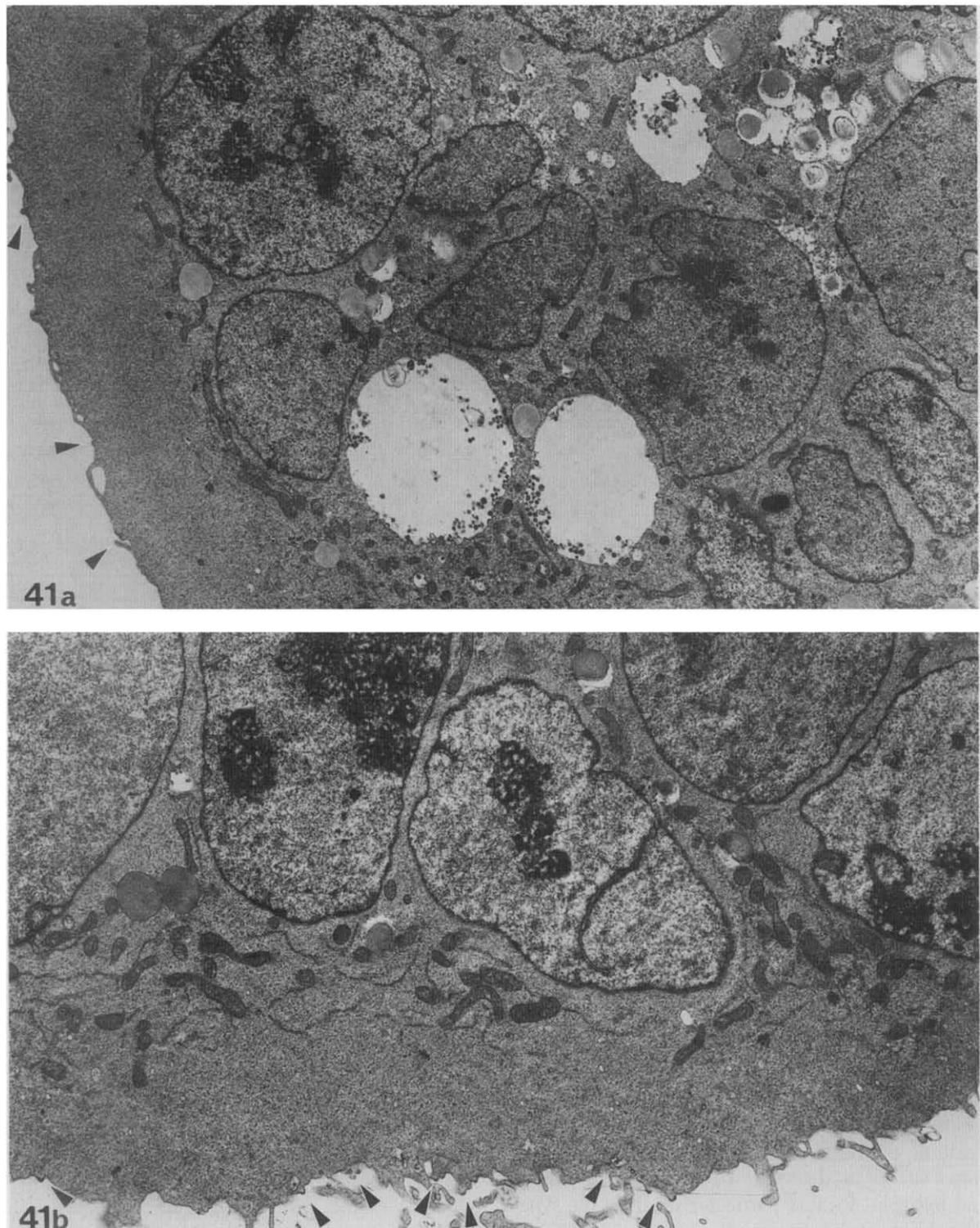


Fig. 41. Electron micrographs showing small sectors of larger MNGCs, which emphasize the central clustering of mitochondria and nuclei, leaving an essentially organelle-free cytoplasmic zone. In (a) vacuolation with internal HIV release is clearly shown and in (b) ER membranes can be seen in close association with the mitochondria. Some virus budding at the surface can be detected (arrowheads).

Inexplicably, Friedman and Skehan (1979) appeared to retain the even then outdated concept that nuclear division in cytotrophoblast cultures occurred by mitosis without cytokinesis and was responsible for the formation of MNGCs in cultures treated with methotrexate, an error carried even further in parts of the more recent developmental biology literature (Gilbert, 1988).

It is clear that in evolutionary and biological terms something extremely significant occurred at the time when the placental mammals diverged from all other animal species, including the marsupial mammals. A hypothesis has been advanced (Harris, 1991) which proposes that at some stage in animal evolution, prior to the divergence of the placental mammals, one or more

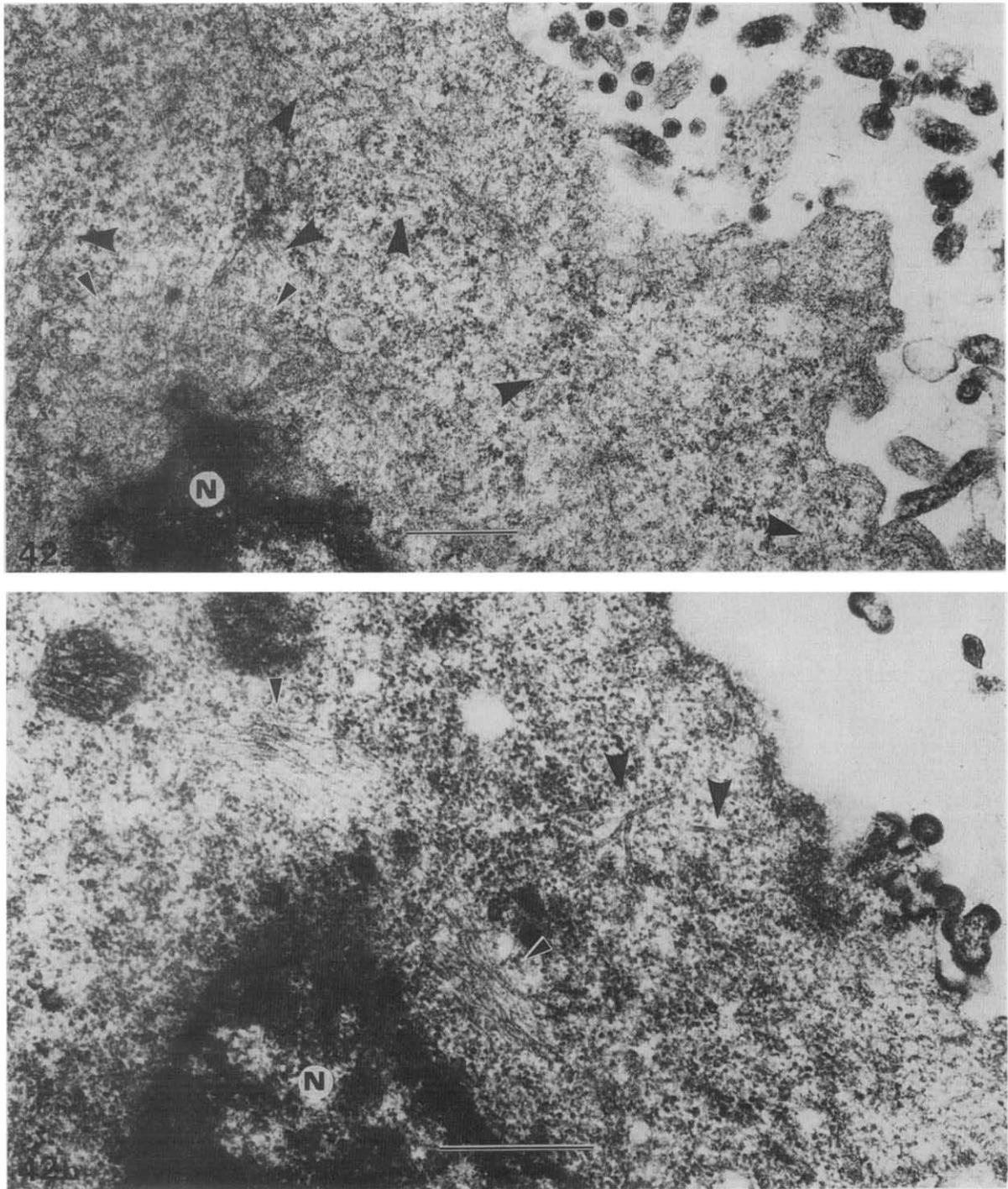


Fig. 42. Electron micrographs showing the MNGC organelle-free cytoplasmic zone. Disorganized intermediate filaments (small arrowheads) can be seen in the vicinity of the nuclei (N). Likewise the microtubules appear to be highly disorganized (large arrowheads). Numerous free ribosomes and polysomes are present in this organelle-free cytoplasmic zone, there being a notable absence of rER membranes. The scale bars indicate 0.5 μ m.

embryos became infected at a very early intrauterine stage with a "primitive" retrovirus. The immediate effect of this infection may have been (1) the integration of the viral progene into the formative embryo cells, including the germline cells, (2) the induction of a tumour-like proliferative growth of certain embryonic cells, thereby creating a primitive rapidly growing trophoblast, possessing a blastocyst cavity within which the truly embryo-forming cells are situated and protected. Whether or not a generalized trophoblastic myometrial

invasiveness was present at this stage can only be speculated upon, but the creation of the early syncytial plate, in direct contact with the myometrium, by a cell fusion process is proposed. In the light of the known fusogenic potential of certain retroviral progene glycoprotein products, it is reasonable to speculate that this specific trophoblastic fusion could have occurred at this early developmental stage, a feature subsequently retained by all placental mammals. It is worth bearing in mind that in the early mammalian embryo of some

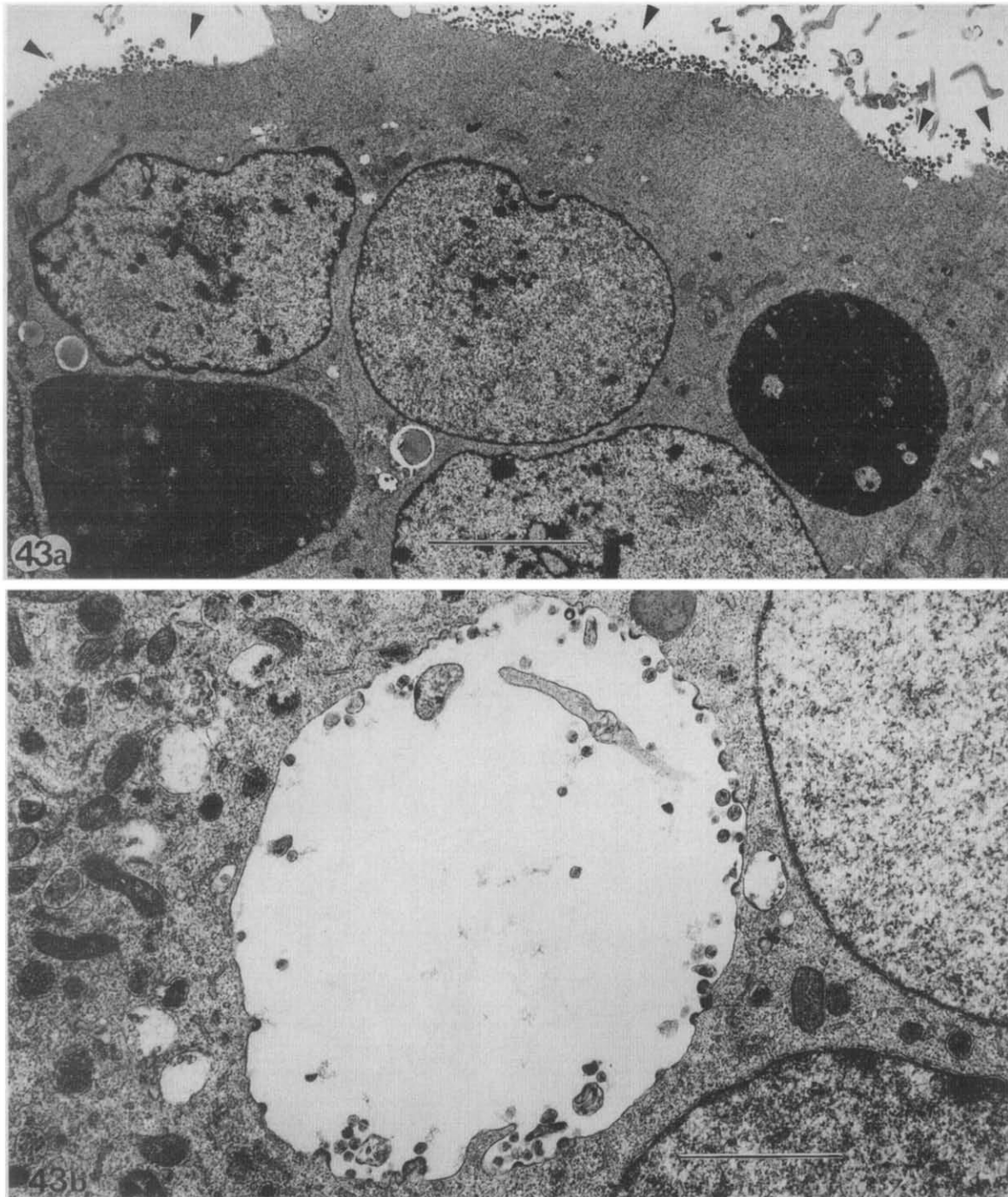


Fig. 43. Examples of HIV-1 budding at the surface of a MNGC (arrowheads) (a), and into an internal vacuole (b), indicating that both routes of viral release operate in large MNGCs that are showing little sign of degeneration and nuclear pyknosis. The scale bars indicate $5\ \mu\text{m}$ (a) and $2.5\ \mu\text{m}$ (b).

100 plus cells, approximately 90% contribute to the rapidly growing trophoblast, whereas only about 5% represent the formative (embryo) and 5% the primary endoderm (Davies and Hesseldahl, 1971).

In the main, the parallel drawn between the tumour-like nature of the invasive trophoblast and indeed the placenta, is widely accepted by pathologists (Aitken *et al.*, 1979; Shanklin, 1990). When the placenta is discarded at full term of pregnancy, it may well do so primarily because of the degenerative metabolic and hormonal changes that occur within

the syncytiotrophoblast resulting in a loss of its viability (cf. cultured MNGCs). Gynaecologists define various invasive pathological conditions of the myometrium, generally termed placental site tumours, nodular pseudotumour or trophoblastoma, where deep infiltration of the myometrium occurs. Clusters of MNGCs of trophoblastic origin can be detected, together with inflammatory cells (Shanklin, 1990). The presence of C-type retroviruses in a variety of germ cell ovarian and testicular tumours cells (Anderson *et al.*, 1991; Bronson *et al.*, 1984; Beilby, 1985;

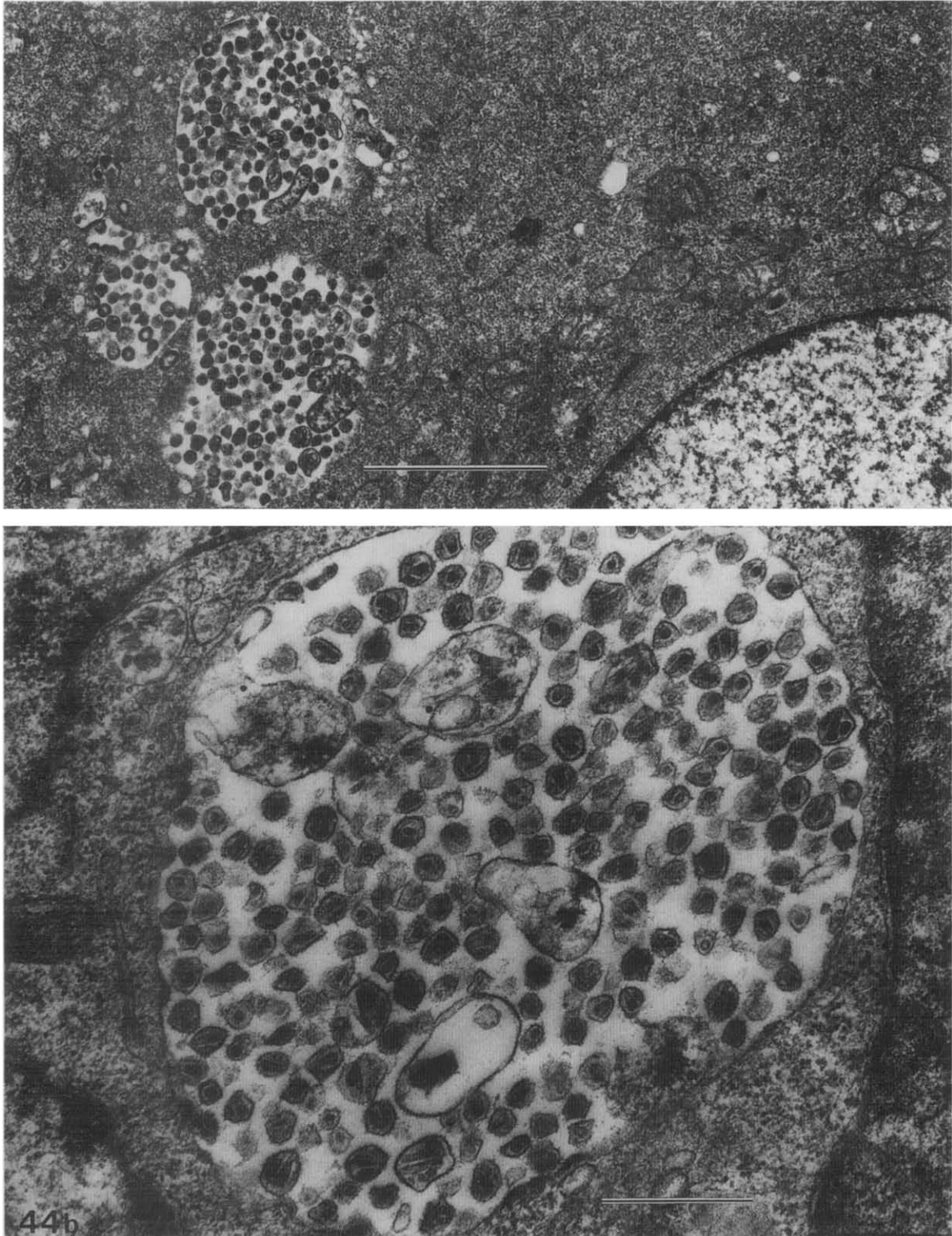


Fig. 44. MNGC vacuoles that are almost completely filled with HIV-1. In (a) many of the virions are newly released and immature, whereas in (b) most of the virions are mature and budding appears to have almost terminated. A number of multi-cored deviant virions are present. The scale bars indicate $2\ \mu\text{m}$ (a) and $0.5\ \mu\text{m}$ (b).

Boller *et al.*, 1983; Löwer *et al.*, 1984, 1987), which in many cases also have a considerable tendency towards cell fusion, provides peripheral support for the above proposal. Nevertheless, the control of trophoblast metalloproteinases and invasiveness, and possibly syncytiotrophoblast formation, has been suggested by Graham and

Lala (1991) to be influenced by transforming growth factors β and β_1 .

In terms of biological symbiosis, an endogenous virus or proto-oncogene (Adamson, 1987) needs to be perpetuated from generation to generation. This would indeed need to be the case for the germline integrated

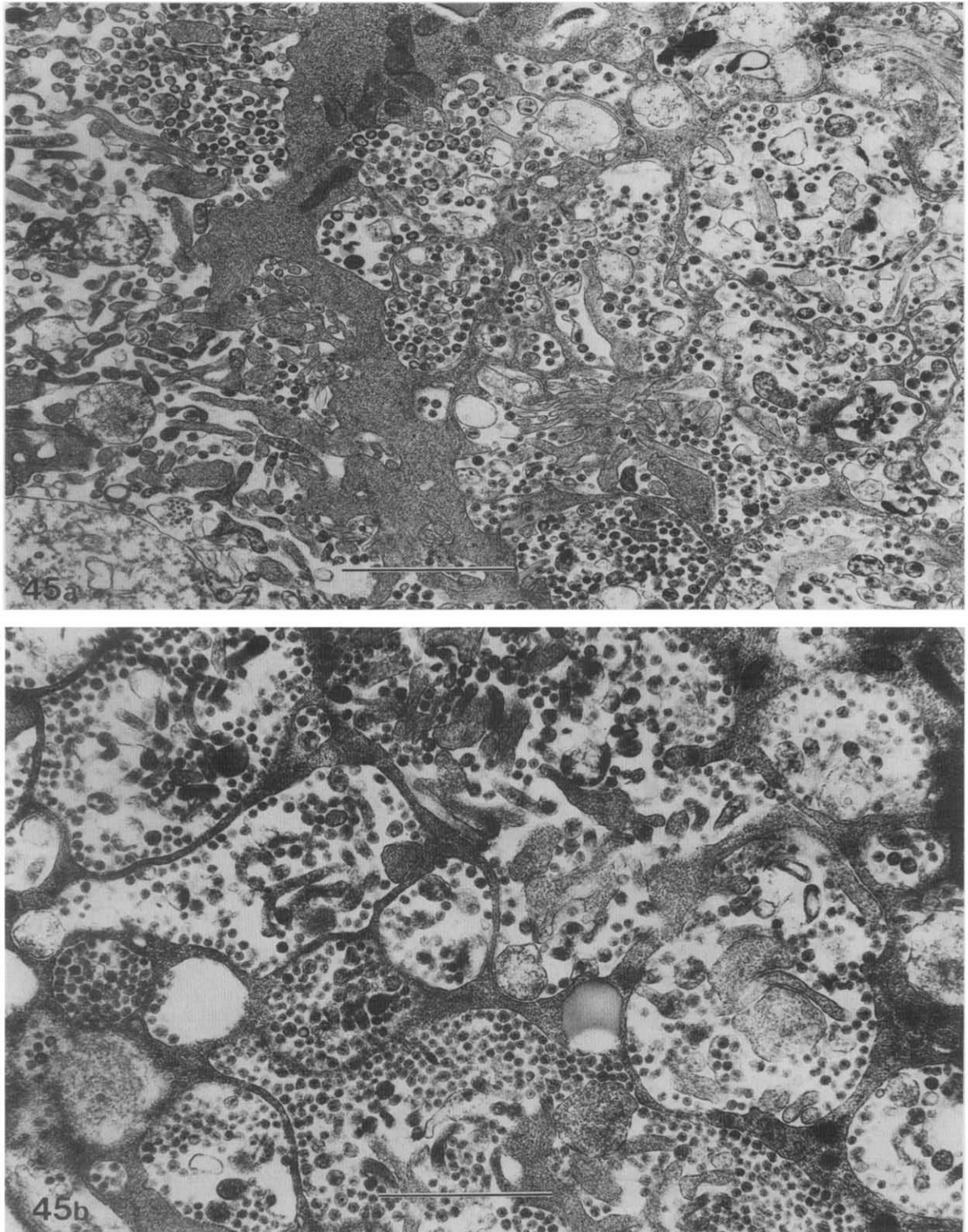


Fig. 45. Regions within large MNGCs where numerous small vacuoles appear to be interconnected (cf. Fig. 34) and contain very large amounts of virus. In (a) active budding of HIV-1 is occurring, with accumulation of immature and mature virions. In (b) most of the virions are mature. The scale bars indicate $2\ \mu\text{m}$.

Fig. 46. (a) A single nucleus within a MNGCs which is actively releasing HIV-1. The nuclear envelope contains extremely pronounced nuclear pore complexes (arrowheads) which extend as electron-dense structures significantly further than the outer nuclear membrane. It is proposed that this is a morphological indication of the activated production of viral mRNP, under the control of the *tat* and *rev* proviral gene products. In (b) a nucleus within a single HIV-1-producing cell is shown at higher magnification. Arrowheads indicate the electron dense nuclear pore complexes. The scale bars indicate $2.0\ \mu\text{m}$ (a) and $0.5\ \mu\text{m}$ (b).

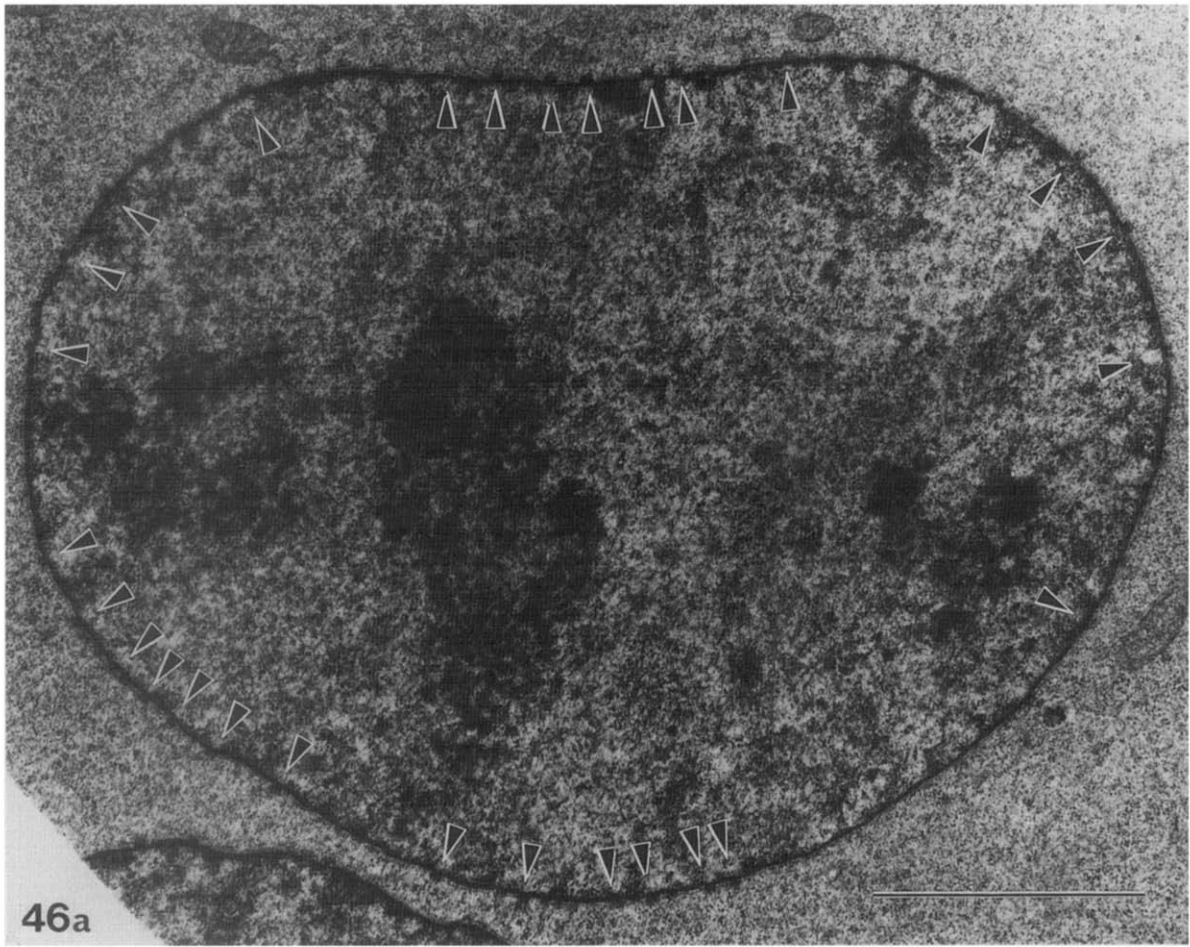


Fig. 46(a).

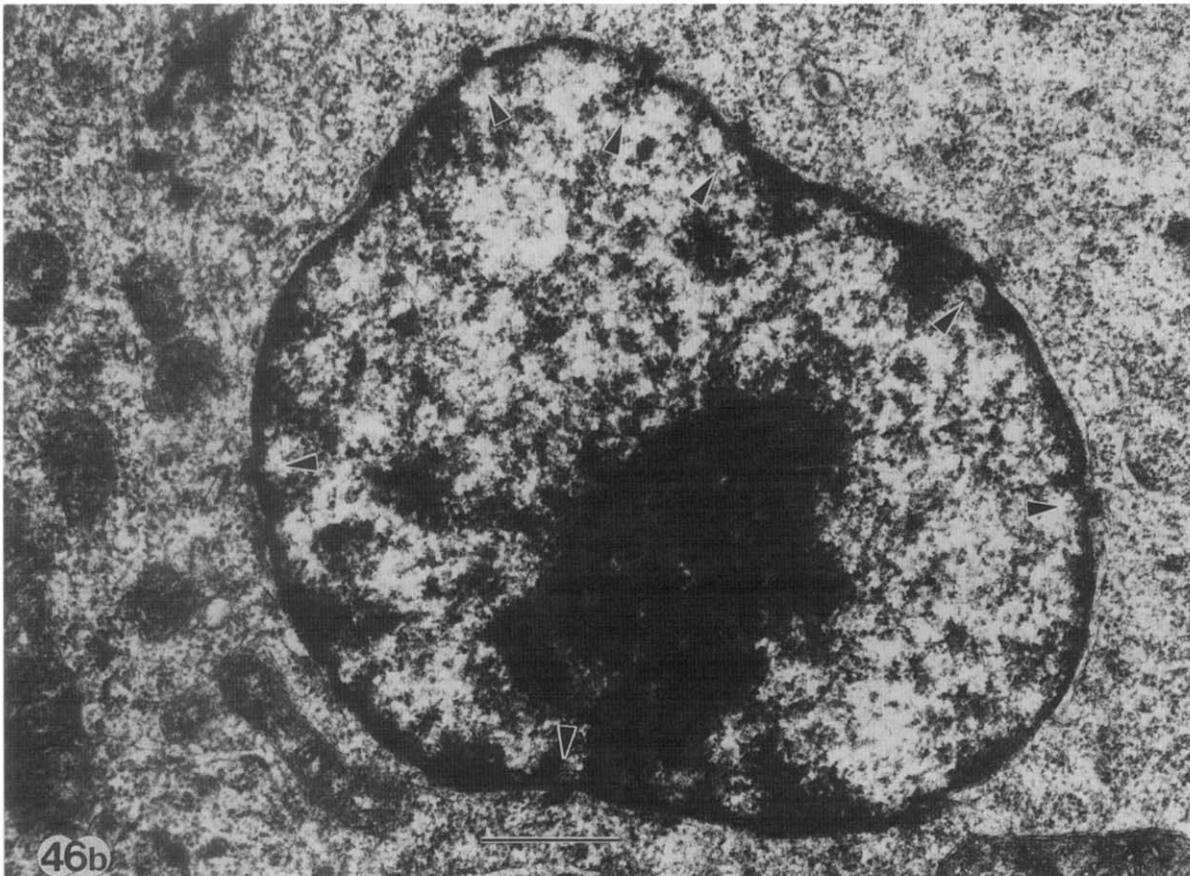


Fig. 46(b).

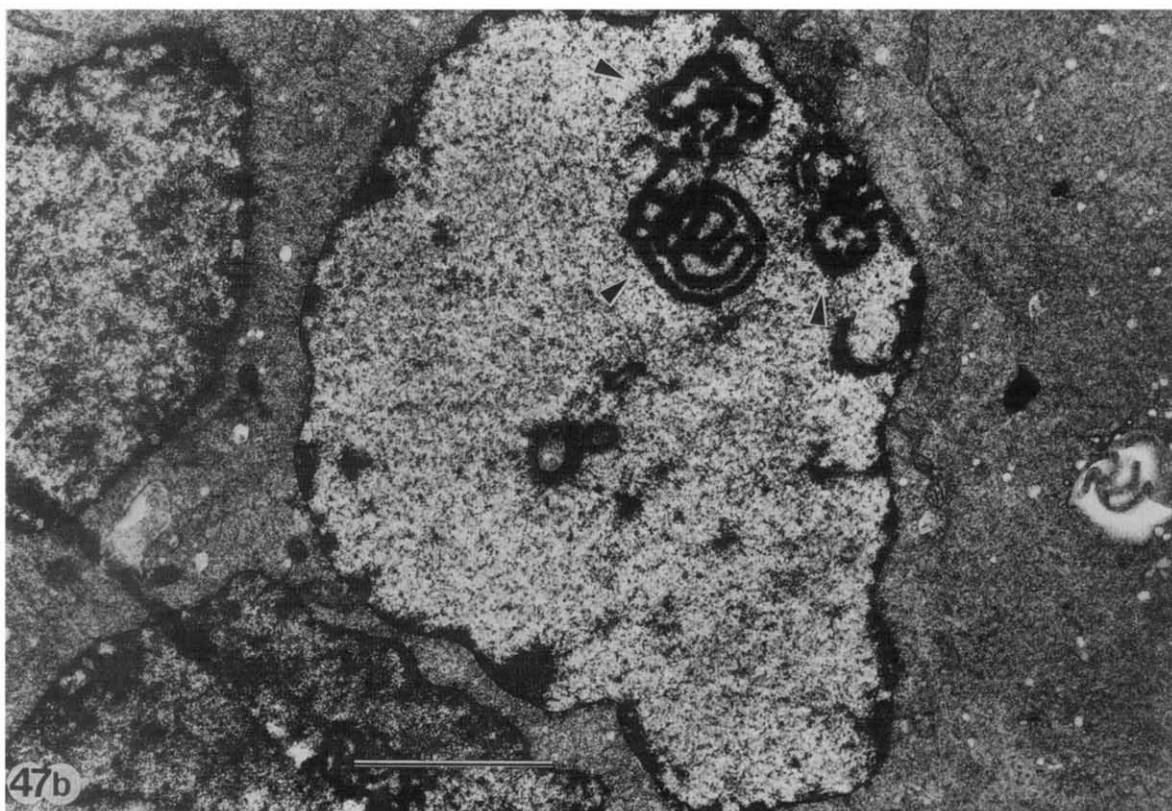
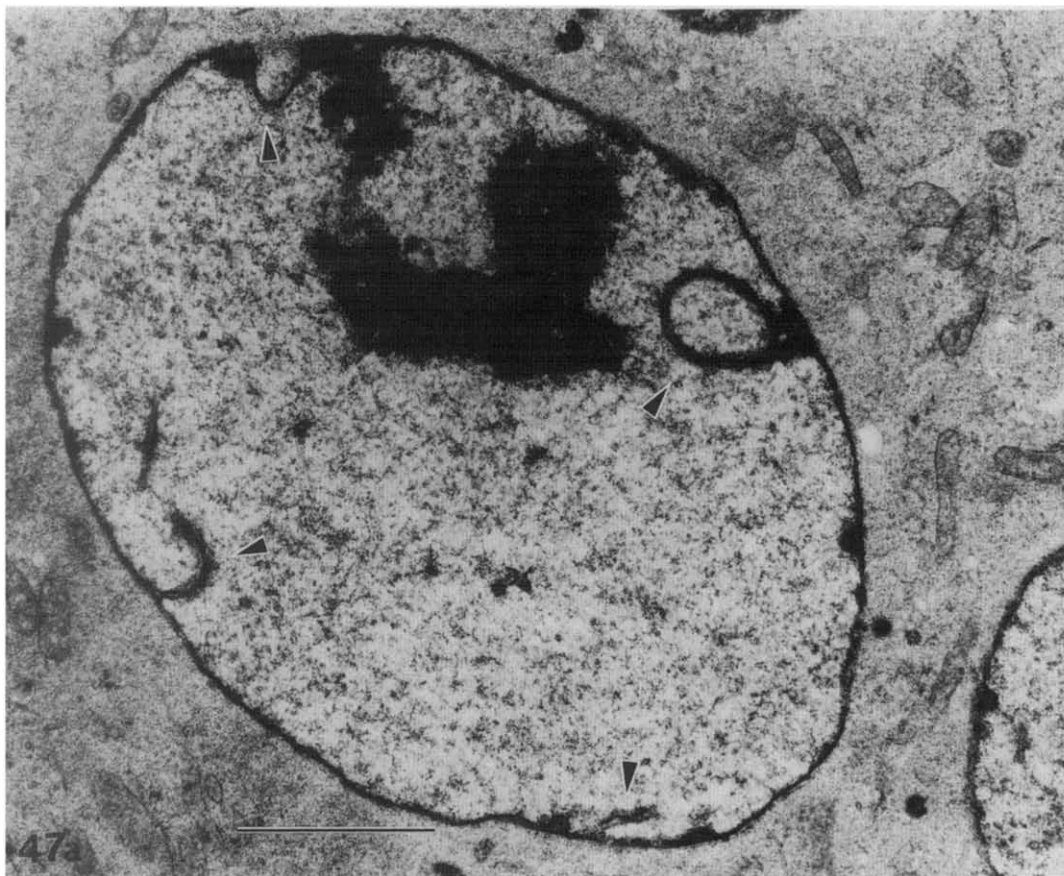


Fig. 47. (a) Shows a nucleus within a large MNGC showing infolding of the nuclear membrane (arrowheads), an early morphological indication of degeneration. The nucleus is only slightly mis-shapen, the nucleolus is relatively normal and the chromatin has not yet become condensed. Note the absence of pronounced nuclear pore complexes (cf. Fig. 46). In (b) a nucleus at a slightly further stage of degeneration is shown. The undulating surface contour of the nucleus indicates some shrinkage and the nucleolus is exhibiting marked reticulation (arrowheads). Heterochromatin blocks are beginning to appear at the nuclear surface (arrows). The scale bars indicate $2.0 \mu\text{m}$.

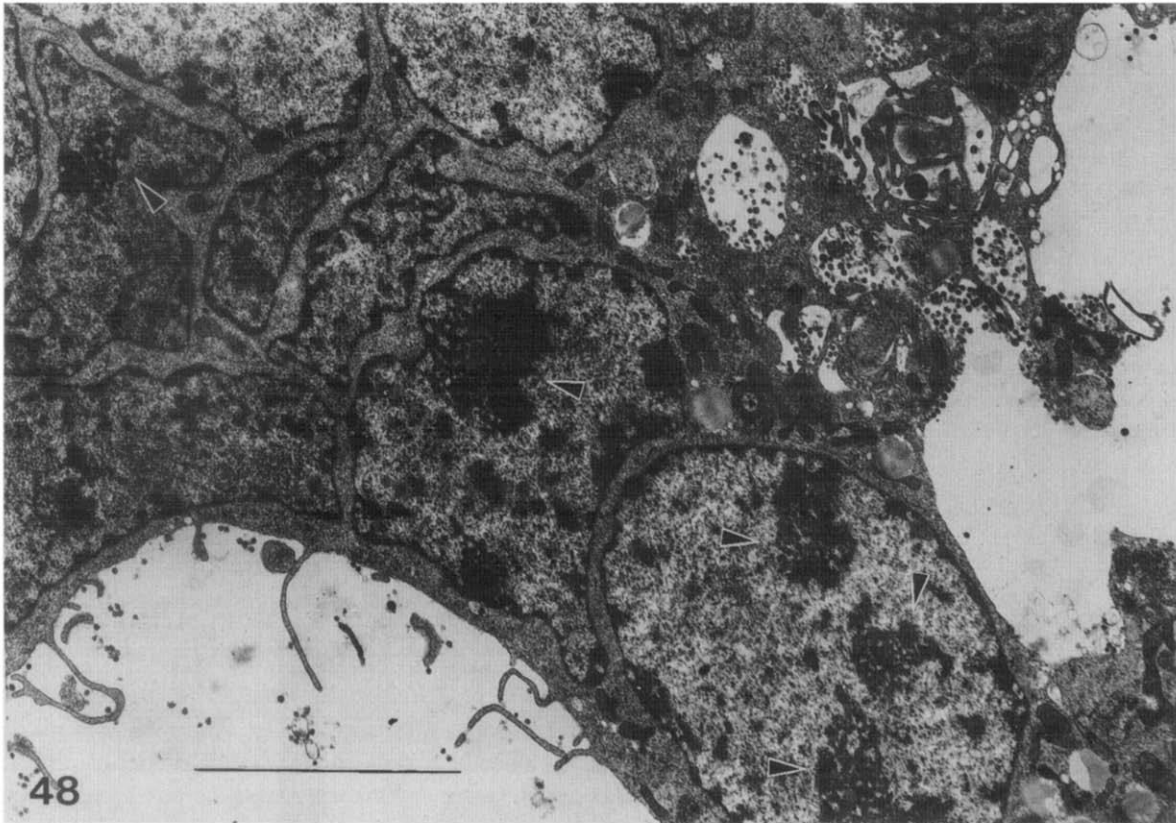


Fig. 48. Markedly shrunken and distorted nuclei, containing reticulated nucleoli (arrowheads), within a large vacuolated MNGC. HIV-1 release is apparent within the vacuoles. The scale bar indicates 5 μ m.

provirus DNA of the postulated retrovirus, despite the fact that the extra-embryonic placental tissue which most markedly expresses the presence of the retroviral genome and its products and is significantly influenced by their presence (i.e. trophoblast growth, invasiveness and fusogenic potential) is (fortunately) discarded at the end of each pregnancy. Fertilization may indeed provide the stimulation for the developmental expression of the postulated endogenous virus/viruses within each early embryo and impart untold benefit by enabling one or more embryos to progress through the first stages of growth within the protective and nutritive confines of the uterus. Ultimately the animal then sheds the histologically organized, yet undoubtedly tumour-like, vascular and endocrinal placental growth. Thus, a situation of true symbiosis is proposed, having benefits for both the perpetuation of the retroviral genome and the survival potential of the embryos of all placental mammals.

B. The possible significance of T-lymphocyte depletion in AIDS produced by HIV-induced cell fusion

The relatively slow progression of the ARC and AIDS, indicated clinically by the inversion of the T-lymphocyte CD4/CD8 (T4/T8) helper/suppressor ratio, is difficult to account for in terms of an immunological cytolytic response. Recent concepts may indicate an autoimmune (Weimer *et al.*, 1991) or

immunosuppressive (Habeshaw *et al.*, 1990) response directed against CD4⁺ cell production and function may be involved rather than an immunolytic reaction. A hypothesis for T-cell dysfunction and depletion within the AIDS patient has recently been advanced by Ameisen and Capron (1991), within the framework of "programmed cell death" or apoptosis (Laurent-Crawford *et al.*, 1991). On the other hand, it is certainly not beyond the bounds of possibility that the slow incorporation of activated viral-producing CD4⁺ lymphocytes, available from the pool of latently infected cells (McElrath *et al.*, 1991; Zack *et al.*, 1991), into binucleate or trinucleate cells following fusion with uninfected lymphocytes could account for the CD4⁺ (helper) T-lymphocyte depletion (Fauci, 1988; Haseltine, 1990, 1991; Harris *et al.*, 1989). This would be very difficult to detect histologically, for the reasons previously advanced (Section I), except when larger MNGCs are formed. That the level of CD4⁺ T-lymphocyte activation is an important factor is implicit and is clearly likely to be increased in the immunocompromised practising homosexual and drug addict. In addition, secondary lymphocytic viral infection with CMV, EBV (Bigi *et al.*, 1990) or human Herpes 6 virus (Lusso *et al.*, 1991), may potentiate HIV replication and release. That CD4⁺ T-cell stimulation and phosphokinase C activation is indeed important in the fusogenic response to cells expressing the HIV-1 envelope glycoprotein has been emphasized by the work of Mohaghighpour *et al.* (1991), and

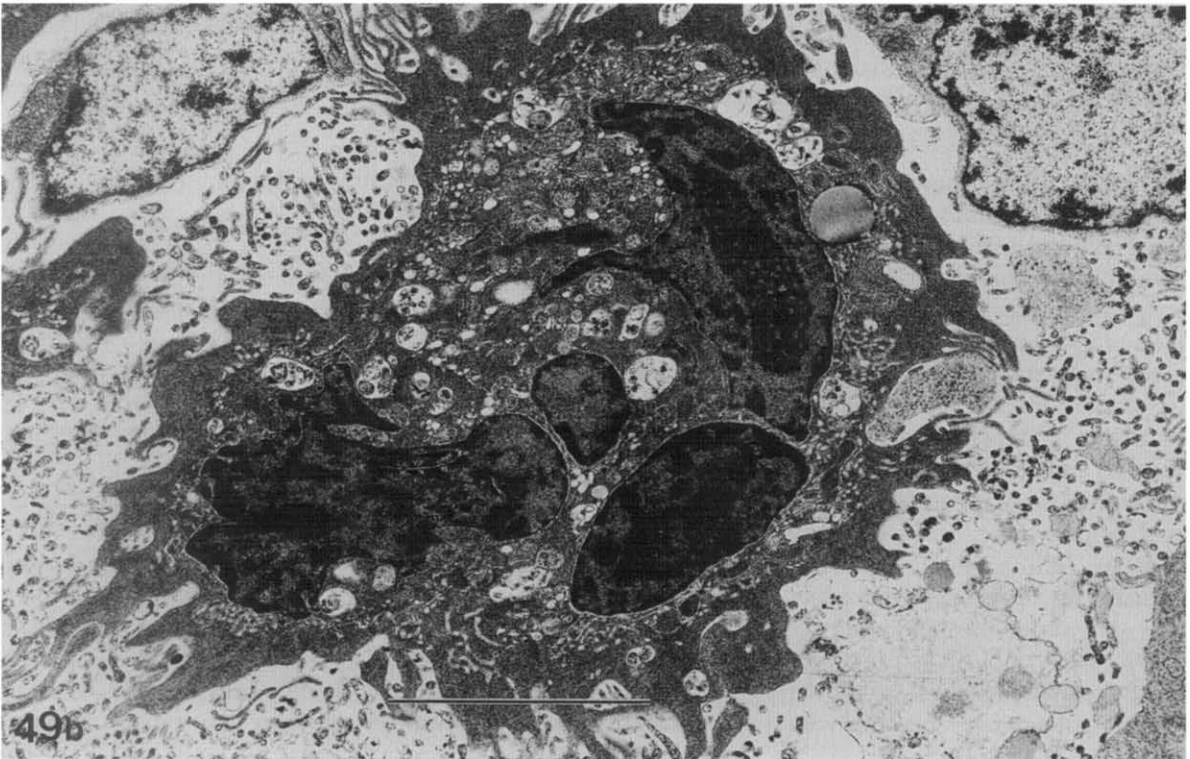
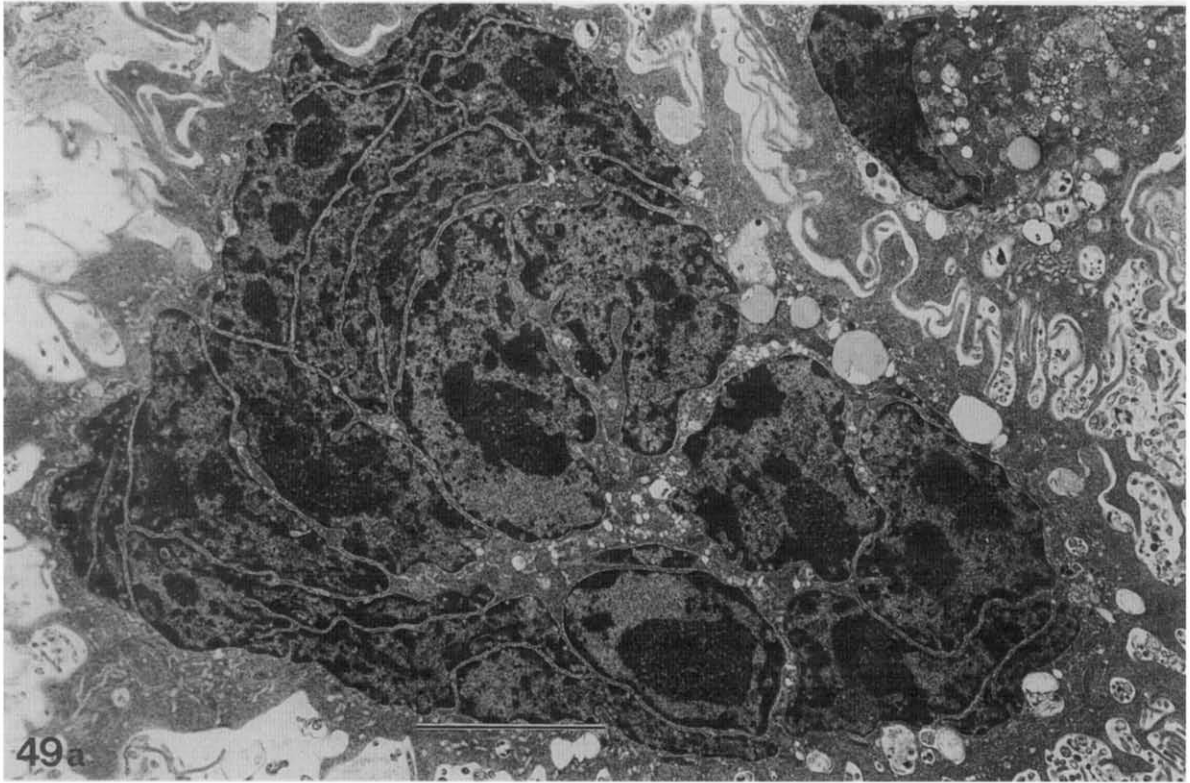


Fig. 49. Sections of electron-dense degenerate MNGCs containing shrunken pyknotic nuclei with large "spongy" reticulated nucleoli. The cytoplasm has undergone extensive vacuolation and there is grossly abnormal surface villation. Compare closely with the nuclear "knots" present in full term placental syncytiotrophoblast (Figs 9 and 10). The scale bars indicate $5\ \mu\text{m}$.

Fig. 50. A single nucleus within a degenerate MNGC, exhibiting marked surface undulation, a large "spongy" reticulated nucleolus and expanded nuclear envelope intracisternal space. The surrounding cytoplasm is grossly abnormal, indicating the approach of syncytial death. The scale bar indicates $1.0\ \mu\text{m}$.

Fig. 51. The massive "foamy" degeneration of a MNGC into a large network of HIV-1 containing vacuoles (cf. Figs 34 and 45). The scale bar indicates $10\ \mu\text{m}$.

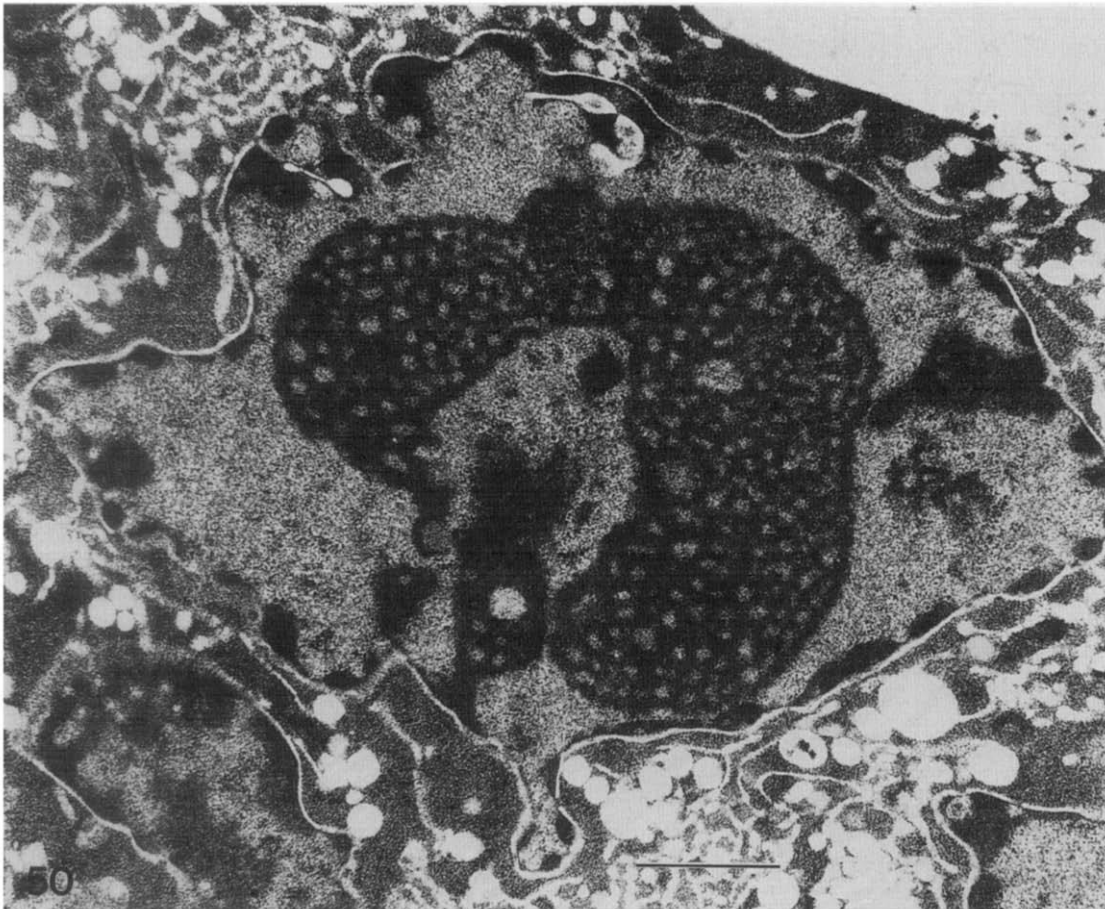


Fig. 50

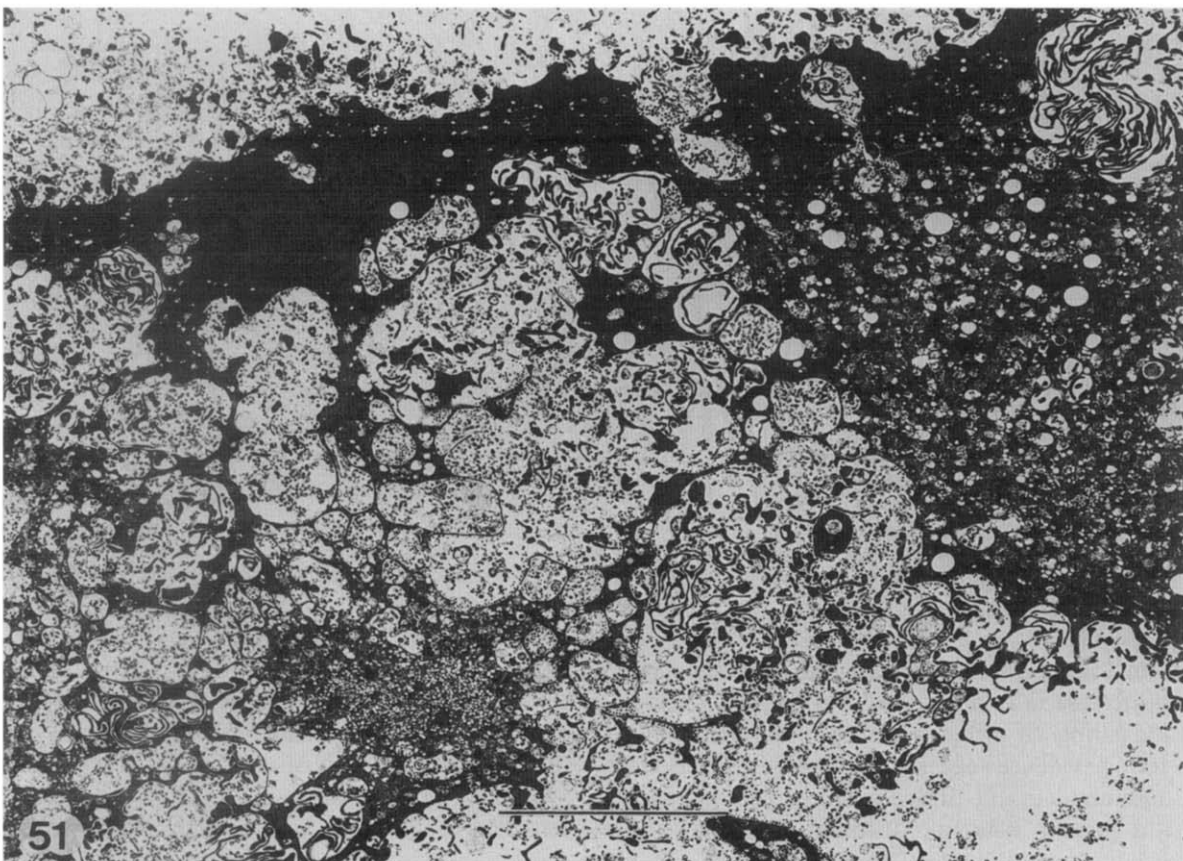


Fig. 51

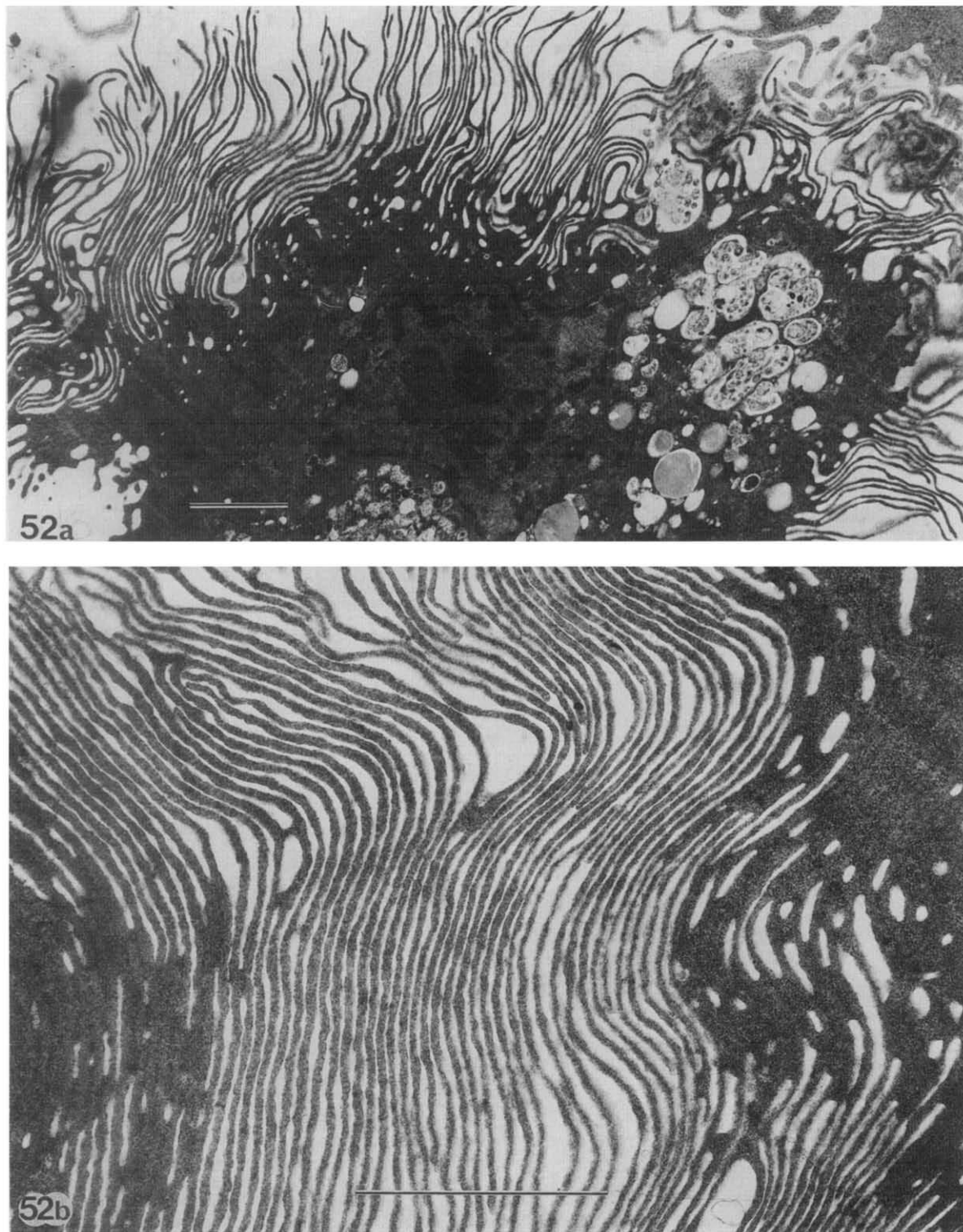


Fig. 52. The villated surface of degenerate MNGCs, showing grossly abnormal surface microvilli which form at the final stages of syncytial survival in suspension culture. Note the electron dense cytoplasm and pyknotic nuclei, and the extensive vacuolation. The scale bars indicate 2.5 μm .

syncytium formation has been shown to be associated with an increase in cellular oleic acid (Apostolov *et al.*, 1989) indicating an increase in membrane fluidity.

Mounting evidence suggests that virulent HIV isolates from immunodeficient individuals expressing AIDS possess a greater syncytium-forming CPE than those from earlier stages of the ARC and AIDS (Cheng-Mayer *et al.*, 1988; Fenyo *et al.*, 1988; Fiore *et al.*, 1990;

Tersmette *et al.*, 1988, 1989). The rapid evolution of HIV *in vivo* appears to continually generate subpopulations of virions exhibiting an increasing cytopathic potential throughout the progression of AIDS (Habeshaw *et al.*, 1990). A note of caution has, however, come from Pantaleo *et al.* (1991) who consider that there may be some dissociation of HIV syncytium-forming capacity and HIV spreading, and that the cellular assay for

inhibition of syncytium formation may not necessarily always be an indicator of the suppression of infection. Furthermore, the envelope glycoprotein of an infectious noncytopathic strain of HIV-2 has been found to be unable to induce syncytium formation (Mulligan *et al.*, 1990). Contrary to this, Watanabe *et al.* (1991) have detected a chimpanzee-passaged HIV-1 isolated which is cytopathic to chimpanzee CD4⁺ cells *in vitro* and *in vivo*, but does not cause development of disease. A differential discrimination between HIV-1 infection and syncytium formation has also been advanced by Lifson *et al.* (1991), based upon the CD4 (81-92) amino acid sequence and its interaction with GP120. Nevertheless, it is very significant that the V3 loop of the HIV-1 envelope glycoprotein (GP120) has been found to be intimately involved with both syncytium formation and viral replicative capacity (de Jong *et al.*, 1992). Also, mutation of the transmembrane glycoprotein GP41 of HIV-1 has been claimed to interfere with cell fusion and infectivity (Freed *et al.*, 1992).

Despite the above emphasis upon the possible role of cell fusion in CD4⁺ T-lymphocyte depletion, it must be remembered that the macrophage lineage is also a major target for HIV (Gendelman *et al.*, 1989; Gendralt *et al.*, 1991; Pautret *et al.*, 1990; Wiley *et al.*, 1986) and that an increasing body of evidence shows the presence of neuronal multinucleate macrophage/microglial cells in AIDS dementia. A similar situation exists in the brain of Rhesus monkeys infected with SIV (Lackner *et al.*, 1991). At the moment there is no suggestion that such macrophage derived MNGCs in the brain of AIDS patients have a direct role in the creation of encephalopathy (Dickson *et al.*, 1991; Takeya *et al.*, 1991; Vazeux, 1990). Rather, it is thought that the release of certain inflammatory compounds or neurotoxins from HIV-infected macrophages (Guilian *et al.*, 1990; Pulliam *et al.*, 1991) may be responsible for the neurological complications of AIDS.

C. Final comments

The diverse content of this review serves to highlight the widespread significance of MNGCs in normal tissues and in both viral and non-viral pathology. An attempt has been made to survey the principal areas of interest and overall comparisons have been made, where possible. Although the involvement of one or more early retroviruses in the evolution of the placental mammals might be considered to be a highly speculative suggestion, the hypothesis advanced here and elsewhere (Harris, 1991), serves to emphasize this important evolutionary question, that has for too long been neglected. Modern cellular and molecular biology have between them the available tools to answer some of the questions posed and provided answers to the possible involvement of integrated retroviral proDNA in the processes of evolution, development and oncogenesis.

It is hoped that greater appreciation of the existence and importance of MNGCs will follow from the publication of this review. Also, that further interest will be shown in the application of transmission electron microscopy to the study of the cytopathic effects of retroviruses. In combination with immunolabelling, this approach has an even greater potential, largely unexplored in this field of study. The possible involvement of C-type retroviruses in cell fusion and the formation of the Reed–Sternberg cell in Hodgkin's disease has been recently advanced by Sinkovics (1991), a phenomenon that appears to parallel the syncytium-forming capacity of many the retroviruses discussed throughout this review. It should, perhaps be stressed finally, that no suggestion is advanced above for the involvement of retroviruses in myoblast fusion during myotube formation or in macrophage fusion to yield the osteoclast, although for the latter in certain pathological situations (i.e. HIV-induced encephalopathy and mammary cancer) some role for such viruses in macrophage fusion may be implied.

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REFERENCES

- Adamson, E. D., 1987. Expression of proto-oncogenes in the placenta. *Placenta* **8**, 449–466.
- Aitken, R. J., 1979. Origin and formation of the placenta. In: *Placenta—A Neglected Experimental Animal*, Beackonsfield, P. and Vilee, C. (eds), Pergamon Press, 152–163.
- Ameisen, J. C. and Capron, A., 1989. Cell dysfunction and depletion in AIDS. The programmed cell death hypothesis. *Immun. Today* **12**, 102–104.
- Amir, G. and Rosenmann, E., 1990. Osteoclast-like giant cell tumor of the urinary bladder. *Histopathology* **17**, 413–418.
- Anderson, K. P., Low, M.-A. L., Lie Y. S., Keller, G.-A. and Dinowitz, M., 1991. Endogenous origin of defective retrovirus-like particles from a recombinant chinese hamster ovary cell line. *Virology* **181**, 305–311.
- Aoki, N., Shioda, T., Satch, H. and Shibuta, H., 1991. Syncytium formation of human and non-human cells by recombinant vaccinia viruses carrying the HIV env gene and human CD4 gene. *AIDS* **5**, 871–876.
- Apostolov, K., Galpin, S. A., Habib, N. A., Barker, W., Wood, C. B. and Kinchington, D., 1989. Syncytia formation in HIV-1 infected cells is associated with an increase in cellular oleic acid. *FEBS Lett.* **250**, 241–244.
- Ashorn, R. A., Berger, E. A. and Moss, B., 1990. Human immunodeficiency virus envelope glycoprotein/CD4-mediated fusion of nonprimate cells with human cells. *J. Virol.* **64**, 2149–2156.
- Athanasou, N. A., Wells, C. A., Quinn, J., Ferguson, D. J. P., Heryet, A. and McGee, J. O'D., 1989. The origin and nature of stromal osteoclast-like multinucleated giant cells in breast carcinoma: implications for tumour osteolysis and macrophage biology. *Br. J. Cancer* **59**, 491–498.
- Athanasou, N. A., Quinn, J., Ferguson, D. J. P. and Magee, J. O., 1991. Bone resorption by macrophage polykaryons of giant cell tumors of tendon sheath. *Br. J. Cancer* **63**, 527–533.

- Aziz, D. C., Hanna, Z. and Jolicœur, P., 1989. Severe immunodeficiency disease induced by a defective leukaemia virus. *Nature* **338**, 505–508.
- Baba, M., Schols, D., Panwels, R., Nakashima, H. and De Clerq, E., 1990. Sulphated polysaccharides as potent inhibitors of HIV-induced syncytium formation: A new strategy towards AIDS chemotherapy. *J. acq. Immun. Def. Syndr.* **3**, 493–499.
- Baba, T., Shiozawa, N., Hotchi, M. and Ohno, S., 1991. Three-dimensional study of the cytoskeleton in macrophages and multinucleate giant cells by quick-freezing and deep-etching method. *Virchows Arch. B Cell Path.* **61**, 39–47.
- Bardosi, A., Dimitri, T. and Gabius, H.-J., 1989. Are glycoconjugates and their endogenous receptors involved in the fusion of mononuclear macrophages resulting in multinucleate giant cells? *J. Submicrosc. Cytol. Path.* **22**, 229–238.
- Beilby, J. O. W., 1985. Germ-cell tumours of the ovary. In: *Ovarian Cancer*, Hudson, C. N. (ed.), Oxford University Press, Oxford, 94–112.
- Benveniste, R., Morton, W., Clark, E., Tsai, C.-C., Ochs, H. D., Ward, J. M., Kuller, L., Knott, W. B., Hill, R. W., Gale, M. J. and Thouless, M. E., 1988. Inoculation of baboons and macaques with SIV/Mne, a primate lentivirus closely related to HIV-2. *J. Virol.* **62**, 2091–2101.
- Bigi, G., Sone, G., Cerri, A., Syun, D. and Sarin, P. S., 1990. High expression of multinucleate giant cells in cultures of peripheral cells HIV patients. *Haematologica* **75**, 212–219.
- Black, M. M., and Epstein, W. L., 1974. Formation of multinucleate giant cells in organized epithelioid cell granulomas. *Am. J. Path.* **74**, 263–274.
- Blandau, R. J., 1971. Culture of guinea pig blastocyst. In: *The Biology of the Blastocyst*, Blandau, R. J. (ed.), The University of Chicago Press, Chicago, 59–69.
- Bohannon, R. C., Donehower, L. A. and Ford, R. J., 1991. Isolation of a type D retrovirus from B-cell lymphomas of a patient with AIDS. *J. Virol.* **65**, 5663–5672.
- Boller, K., Frank, H., Löwer, J., Löwer, R. and Kurth, R., 1983. Structural organization of unique retrovirus-like particles budding from human teratocarcinoma cell lines. *J. gen. Virol.* **64**, 2549–2559.
- Boothe, A. D. and van der Maaten, M. J., 1974. Ultrastructural studies of a visna-like syncytia producing virus from cattle with lymphocytosis. *J. Virol.* **13**, 197–204.
- Bouillant, A. M. P. and Becker, S. A. W. E., 1984. Ultrastructural comparison of oncovirinae (Type C), spumavirinae, and lentivirinae: three subfamilies of retroviridae found in farm animals. *J. natn. Cancer inst.* **72**, 1075–1084.
- Bouillant, A. M. P., Bundza, A., Genest, P. and Greig, A. S., 1980. Production of tumors in hamsters by cells of a pig uterine tube line. *Can. J. comp. Med.* **44**, 77–86.
- Bouillant, A. M. P., Greig, A. S., Lieber, M. M. and Todaro, G. J., 1975. Type C virus production by a continuous line of pig oviduct cells (PFT). *J. gen. Virol.* **27**, 173–180.
- Bouillant, A. M. P., Ruckerbauer, G. M. and Nielsen, K. H., 1989. Replication of the bovine immunodeficiency-like virus in diploid and aneuploid cells: permanent, latent and virus-productive infections *in vitro*. *Res. Virol.* **140**, 511–529.
- Bremermann, H. J. and Anderson, R. W., 1990. The HIV cytopathic effect—potential target for therapy. *J. acq. Immun. Def. Syndr.* **3**, 1119–1128.
- Bronson, D. L., Saxinger, W. C., Ritz, D. M. and Fraley, E. E., 1984. Production of virions with retrovirus morphology by human embryonal carcinoma cells *in vitro*. *J. gen. Virol.* **65**, 1043–1051.
- Brown, H. R. and Thormar, H., 1976. Non-productive subacute sclerosing panencephalitis (SSPE) virus of human and ferret: an ultrastructural study. *Acta Neuropath. (Berlin)* **34**, 339–351.
- Budka, H., Costanzi, G., Cristina, S., Lechi, A., Parravicini, C., Trabatttoni, R. and Vago, L., 1987. Brain pathology induced by infection with the human immunodeficiency virus (HIV). *Acta Neuropath. (Berlin)* **75**, 185–198.
- Buskila, D. and Gladman, D., 1990. Musculoskeletal manifestations of infection with human immunodeficiency virus. *Rev. Infect. Dis.* **12**, 223–235.
- Byrnes, R. K., Chan, W. S., Spira, T. J., Ewing, E. P. and Chandler, F. W., 1983. Value of lymph node biopsy in unexplained lymphadenopathy in homosexual men. *J. Am. Med. Ass.* **250**, 1313–1317.
- Cain, H., Ktsud, B., Firinges, B., Osborn, M. and Weber, K., 1981. Centrioles, microtubules and microfilaments in activated mononuclear and multinucleate macrophages from rat peritoneum: Electron-microscopic and immunofluorescence microscopic studies. *J. Path.* **133**, 301–323.
- Carr, I., 1980. Sarcoid macrophage giant cells. Ultrastructure and lysosome content. *Virchows Arch. B Cell. Path.* **32**, 147–155.
- Carter, J. E., 1964. Morphological evidence of syncytial formation from the cytotrophoblast cells. *Obstet. Gynec.* **23**, 647–656.
- Chambers, T. J., 1978. Multinucleate Giant Cells. *J. Path.* **126**, 125–148.
- Chatterjee, S. and Hunter, E., 1980. Fusion of normal primate cells: a common biological property of the D-type retroviruses. *Virology* **107**, 100–108.
- Cheng-Mayer, C., Seto, D., Tateno, M. and Levy, J. A., 1988. Biological features of HIV-1 that correlate with virulence in the host. *Science* **240**, 80–82.
- Christie, I. L. and Almeida, J. D., 1988. Further studies of HIV morphology by negative staining. *AIDS* **2**, 459–464.
- Clapham, P. R., Weiss, R. A., Dalgleish, A. G., Exley, M., Whitby, D. and Hogg, N., 1987. Human immunodeficiency virus infection of monocytic and T-lymphocytic cells: Receptor modulation and differentiation induced by phorbol ester. *Virology* **158**, 44–51.
- Clavel, F. and Orenstein, J. M., 1990. A mutant of human immunodeficiency virus with reduced RNA packaging and abnormal particle morphology. *J. Virol.* **64**, 5230–5234.
- Compans, R. W., Holmes, K. V., Dales, S. and Choppin, P. W., 1964. An electron microscopic study of moderate and virulent virus-cell interactions of the parainfluenza virus SV5. *Virology* **30**, 411–426.
- Dail, D. H. and Hammer, S. P., 1988. *Pulmonary Pathology*. Springer-Verlag, New York.
- Daniel, J. C. and Chilton, B. S., 1987. Virus-like particles in embryos and female reproductive tract. In: *Development in Mammals*, Vol. 3, Johnson, M. H. (ed.), North-Holland, Amsterdam, 131–187.
- Daniel, M. D., King, N. W., Letvin, N. L., Hunt, R. D., Sehgal, P. K. and Desrosiers, R. C., 1984. A new type D retrovirus isolated from macaques with an immunodeficiency syndrome. *Science* **223**, 602–605.
- Daniel, M. D., Letvin, N. L., King, N. W., Kannaigi, M., Sehgal, P. K., Hunt, R. D., Kanki, P. J., Essex, M. and Desrosiers, R. C., 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* **228**, 1201–1204.
- Davies, J. and Hesseldahl, H., 1971. Comparative embryology of mammalian blastocysts. In: *The Biology of the Blastocyst*, Blandau, R. J. (ed.), The University of Chicago Press, Chicago, 27–48.
- Desportes, I., Bonnet, D., Nicol, I., Snart, R. and Sarin, P., 1989. Expression of HIV antigens at the surface of infected T4 cells: Immunoelectron microscopic evidence of an immunogenic phase prior to the viral release. *AIDS Res. Human Retroviruses* **5**, 107–114.
- Dickson, D. W., Mattiace, L. A., Kure, K., Hutchins, K., Lyman, W. D. and Brosnan, C. F., 1991. Biology of Disease: microglia in human disease, with an emphasis on acquired immune deficiency syndrome. *Lab. Invest.* **64**, 135–156.
- Domon, T. and Wakita, M., 1991. The three-dimensional structure of the clear zone of a cultured osteoclast. *J. Electron Microsc.* **40**, 34–40.
- Dowset, B. A., Roff, M. A., Greenaway, P. J., Elphic, E. R. and Farrar, G. H., 1987. Syncytia—a major site for the production of human immunodeficiency virus. *AIDS* **1**, 147–150.
- Dym, M. and Fawcett, D. W., 1971. Further observations on the numbers of spermatogonia, spermatocytes and spermatids connected by intracellular bridges in the mammalian testis. *Biol. Reproduct.* **4**, 195–215.
- Elston, C. W., 1987. Gestational trophoblastic disease. In: *Haines and Taylor Obstetrical Gynaecology and Pathology*, Fox, H. (ed.), 3rd edn, Vol. 2, Churchill Livingstone, 1045–1078.
- Enders, A. C., 1971. The fine structure of the blastocyst. In: *The Biology of the Blastocyst*, Blandau, R. J. (ed.), The University of Chicago Press, Chicago, 71–94.
- Fauci, A. S., 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science* **239**, 617–622.
- Feldman, D., 1979. Virus particles in the basal plate of rhesus monkey and baboon placenta. *Cancer Res.* **39**, 1772–1783.
- Feldman, D., Valentine, T., Niemann, W. H., Hoar, R. M., Cukierski, M. and Hendrickx, A., 1989. C-type virus particles in placentas of rhesus monkeys after maternal treatment with recombinant leukocyte A interferon. *J. exp. Path.* **4**, 193–198.

- Felice, G., Carnevale, G., Lanzarini, P., Orsolini, P., Soldini, L. and Cereda, P. M., 1987. Human immunodeficiency virus (HIV): An ultrastructural study. *Microbiologica* **10**, 209–216.
- Fenyó, E. M., Morfeldt-Mason, L., Chiodi, F., Lind, B., von Gegerfelt, A., Albert, J., Olausson, E. and Asjo, B., 1988. Distinctive replicative and cytopathic characteristics of human immunodeficiency virus isolates. *J. Virol.* **62**, 4414–4419.
- Fiore, J. R., Calabro, M. L., Angarano, G., de Rossi, A., Fico, C., Pastore, G. and Bianchi, L. C. 1990. HIV-1. Variability and progression to AIDS. *J. med. Virol.* **32**, 252–256.
- Flügel, R. M., 1991. Spumaviruses: A group of complex retroviruses. *J. acq. Immun. Def. Synd.* **4**, 739–750.
- Fortuna, M. B., Dewey, M. J. and Furmanski, P., 1989. Cell fusion in tumor development and progression: Occurrence of cell fusion in primary methylcholanthrene-induced tumorigenesis. *Int. J. Cancer* **44**, 731–737.
- Frank, H., 1987a. Retroviridae. In: *Animal Virus Structure (Perspectives in Medical Virology, Volume 3)* Nermut, M. V. and Steven, A. C. (eds), Elsevier, Amsterdam, 253–256.
- Frank, H., 1987b. Oncovirinae: type C retroviruses. In: *Animal Virus Structure (Perspectives in Medical Virology, Volume 3)* Nermut, M. V. and Steven, A. C. (eds), Elsevier, Amsterdam, 273–287.
- Frank, H., 1987c. Lenivirinae. In: *Animal Virus Structure (Perspectives in Medical Virology, Volume 3)* Nermut, M. V. and Steven, A. C. (eds), Elsevier, Amsterdam, 295–304.
- Freed, E. O., Delwart, E. L., Buchschacher, G. L. and Panganiban, A. T., 1991. A mutation on the HIV type 1 transmembrane glycoprotein gp41 dominantly interferes with fusion and infectivity. *Proc. natl. Acad. Sci. U.S.A.* **89**, 70–83.
- Friedman, S. J. and Skehan, P., 1979. Morphological differentiation of human choriocarcinoma cells induced by methotrexate. *Cancer Res.* **39**, 1960–1967.
- Fund, M. S. C., Sun, C., Sun, N.-C., Chang, N. T. and Chang T. W., 1987. Monoclonal antibodies that neutralize HIV-1 virions and inhibit syncytium formation. *Biotechnology* **5**, 940–945.
- Gallagher, T., Chignol, M. C., Chardonnet, Y., Guerret, S. and Schmitt, D., 1990. Ultrastructure of hybrids derived from electric pulse fusion of human HeLa cells and murine 3T3 4E cells. *J. Submicrosc. Cytol. Path.* **22**, 147–152.
- Gallagher, T. M., Escamis, C. and Buchmeier, M. J., 1991. Alteration of the pH dependence of coronavirus-induced cell fusion: effect of mutations in the spike glycoprotein. *J. Virol.* **65**, 1916–1928.
- Gallagher, W. R., Levitan, D. B., Kirwin, K. S. and Blough, H. A., 1980. Molecular and biological parameters of membrane fusion. In: *Cell Membranes and Viral Envelopes*, Vol. 1, Blough, H. A. and Tiffany, J. M. (eds), Academic Press, London, 395–457.
- Gelderblom, H. R., 1987. Oncovirinae: type D oncovirus. In: *Animal Virus Structure (Perspectives in Medical Virology, Volume 3)* Nermut, M. V. and Steven, A. C. (eds), Elsevier, Amsterdam, 289–293.
- Gelderblom, H. R., 1991. Assembly and morphology of HIV—potential effect of structure on viral function. *AIDS* **5**, 617–638.
- Gelderblom, H. R. and Frank, H., 1987. Spumavirinae. In: *Animal Virus Structure (Perspectives in Medical Virology, Volume 3)*, Nermut, M. V. and Steven, A. C. (eds), Elsevier, Amsterdam, 305–311.
- Gelderblom, H. R., Bauer, P. G., Özel, M., Höglund, S., Niedrig, M., Renz, H., Morath, B., Lundquist, P., Njolsson, A., Mattowm J., Grund, C. and Pauli, G. 1992. Morphogenesis and morphology of HIV. In: *Membrane Interactions of Human Immunodeficiency Virus*, Aloia, R. C. (ed.), Academic Press, New York. (in press).
- Gendelman, H. E., Orenstein, J. M., Martin, M. A., Ferrua, C., Mitra, R., Phipps, T., Wahl, L. A., Lane, H. C., Fauci, A. S., Burke, D. S., Skillman, D. and Meltzer, M. S., 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. *J. exp. Med.* **167**, 1428–1441.
- Gendelman, H. E., Orenstein, J. M., Baca, L. M., Weiser, B., Burger, H., Kalter, D. C. and Meltzer, M. S., 1989. The macrophage in the persistence and pathogenesis of HIV infection. *AIDS* **3**, 475–495.
- Gendrault, J. L., Steffan, A. M., Schmitt, M. P., Jaeck, D., Aubertin, A. M. and Kirn, A., 1991. Interaction of cultured human Kupffer cells with HIV-infected CEM cells: an electron microscopic study. *Pathobiology* **59**, 223–226.
- Ghadially, F. N., 1982. *Ultrastructural Pathology of the Cell and Matrix*, 2nd edn, Butterworths, London, 506–507.
- Ghadially, F. M. and Mehta, P. N., 1970. Ultrastructure of osteogenic sarcoma. *Cancer Res.* **25**, 1457–1467.
- Gilbert, S. F., 1988. *Developmental Biology*, 2nd edn, Sinauer Associates, Sunderland, MA, 145.
- Goldberg, R. D., Michelassi, F. and Montase, A. G., 1991. Osteoclast-like giant cell tumor of the pancreas immunophenotypic similarity to giant cell tumor of bone. *Human Path.* **22**, 618–622.
- Gonda, M. A., Braun, M. J., Carter, S. G., Kost, T. A., Bess, J. W. Jr, Arthur L. O. and van der Maaten, M. J., 1987. Characterization and molecular cloning of a bovine lentivirus related to human immunodeficiency virus. *Nature* **330**, 388–395.
- Goto, T., Ikuta, K., Zhang, J. J., Morita, C., Sano, K., Komatsu, M., Fujita, H., Kto, A. and Nakai, M., 1990. The budding of defective HIV-type 1 particles from cell clones persistently infected with HIV-1. *Arch. Virol.* **111**, 87–102.
- Graham, C. H. and Lala, P. K., 1991. Mechanism of control of trophoblast invasion in situ. *J. Cell Physiol.* **148**, 228–234.
- Gruters, R. A., Neefjes, J. J., Tersmette, M., de Goede, R. E. Y., Tulp, A., Huisman, H. G., Miedema, F. and Ploegh, H. L., 1987. Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. *Nature* **330**, 74–77.
- Guilian, D., Vaca, K. and Noonan, C. A., 1990. Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science* **250**, 1593–1595.
- Habeshaw, J. A., Dalglish, A. G., Bountiff, L., Newell, A. L., Wilks, D., Walker, L. C. and Manca, F., 1990. AIDS pathogenesis. HIV envelope and its interaction with cell proteins. *Immun. Today* **11**, 418–425.
- Hanada, A., Hoshimo, H., Kakajima, K., Adachi, M., Ikeda, K., Achiwa, K., Itoh, T. and Suzuki, Y., 1991. Inhibition of infection with HIV type-1 by sulfated gangliosides. *Biochem. biophys. Res. Commun.* **175**, 1–9.
- Harada, S., Koyanagi, Y. and Yamamoto, N., 1985. Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. *Science* **229**, 563–566.
- Harris, H., 1970. *Cell Fusion*, Clarendon Press, Oxford.
- Harris, H., 1974. *Nucleus and Cytoplasm*, 3rd edn. Clarendon Press, Oxford.
- Harris, J. R., 1990. Ultrastructural changes in nuclei within HIV-1-induced cultured cell syncytia. In: *Nuclear Structure and Function*, Harris J. R. and Zbarsky, I. B. (eds), Plenum Press, New York, 483–488.
- Harris, J. R., 1991. The evolution of placental mammals. *FEBS Lett.* **295**, 3–4.
- Harris, J. R., Kitchen, A. D., Harrison, J. F. and Tovey, G., 1989. Viral release from HIV-1-induced syncytia of CD4⁺ C8166 Cells. *J. med. Virol.* **28**, 81–89.
- Harris, J. R., Tovey, G. and Kitchen, A. D., 1990. Ultrastructural changes in HIV-induced syncytia of cultured cells. In: *Proceedings of EMAG-MICRO 89, Institute of Physics Conference Series, No. 98*, IOP Publishing Ltd, London, 723–726.
- Haseltine, W. A., 1990. Molecular biology of HIV-1. In: *AIDS and the New Viruses*, Dalglish, A. G. and Weiss, R. A. (eds), Academic Press, London and New York, 11040.
- Haseltine, W. A., 1991. Molecular biology of the human immunodeficiency virus type 1. *FASEB J.* **5**, 2349–2360.
- Ho, D. D., Rota, T. R. and Hirsch, M. S., 1986. Infection of monocyte/macrophages by human T lymphotropic virus type III. *J. clin. Invest.* **77**, 1712–1715.
- Ho, D. D., McKeating, J. A., Li, X. L., Moudgil, T., Daar, E. S., Sun, N.-C. and Robinson, J. E. 1991. Conformational epitope on Gp120 important in CD4 binding and HIV-1 neutralization identified by a human monoclonal antibody. *J. Virol.* **65**, 489–493.
- Höglund, S., Ofverstedt, L. G., Nilsson, A., Lundquist, P., Gelderblom, H., Özel, M. and Sköglund, U., 1992. Spatial visualization of the maturing HIV-1 core and its linkage to the envelope. *AIDS Res. Human Retroviruses* **8**, 1–8.
- Hosaka, Y., 1988. Sendai virion structure and its interaction with cellular membranes. *Electron Microsc. Rev.* **1**, 17–38.
- Hoshino, H., Shimoyama, M., Miwa, M. and Sugimura, T., 1983. Detection of lymphocytes producing human retrovirus associated with adult T-cell leukemia by syncytia induction assay. *Proc. natl. Acad. Sci. U.S.A.* **80**, 7337–7341.
- Howe, C. and Lee, L. T., 1972. Virus-erythrocyte interactions. *Adv. Virus Res.* **17**, 1–50.
- Hussey, R. E., Richardson, N. E., Kowalski, M., Brown, N. R., Chang, H.-C., Siliciano, R. F., Dorfman, T., Walker, B., Sodroski, J. and Reinherz, E. L., 1988. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. *Nature* **331**, 78–81.

- Ichikawa, Y. and Dales, S., 1971. Biogenesis of poxviruses: inter-relationship between hemagglutinin production and polykaryocytosis. *Virology* **46**, 533-543.
- Imamura, M., Phillips, P. E. and Mellors, R. C., 1976. The occurrence and frequency of Type C virus-like particles in placentas from patients with systemic lupus erythematosus and from normal subjects. *Am. J. Path.* **83**, 383-393.
- James, I. E., Walsh, S., Dodds, R. A. and Gowen, M., 1991. Production and characterization of osteoclast-selective monoclonal antibodies that distinguish between multinucleate cells derived from different human tissues. *J. Histochem. Cytochem.* **39**, 905-914.
- Juniaux, E., Nessmann, C., Imbert, C., Meuris, S., Puissant, F. and Hustin, J., 1988. Morphological aspects of the placenta in HIV pregnancies. *Placenta* **9**, 633-642.
- Johns, T. C. and Renagar, R. H., 1990. Ultrastructural morphology and relaxin immunolocalization in giant trophoblast cells of the golden hamster placenta. *Am. J. Anat.* **189**, 167-178.
- Jones, C. J. P. and Fox, H., 1991. Ultrastructure of the normal human placenta. *Electron Microsc. Rev.* **4**, 129-178.
- de Jong, J.-J., Goudsmit, J., Keulen, W., Klaver, B., Krone, W., Tersmette, M. and de Ronder, A., 1992. Human immunodeficiency virus type 1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity. *J. Virol.* **66**, 757-765.
- Joshi, V. V., Oleske, J. M., Minnefo, A. B., Singh, R., Bokhari, T. and Rapkin, R. H., 1984. Pathology of suspected acquired immune deficiency syndrome in children: A study of 8 cases. *Pediatric Path.* **2**, 71-87.
- Kalganaraman, V. S., Rusch, D. M., Osborne, J., Padgett, M., Hwang, K. M., Lifson, J. D. and Eiden, L. E., 1990. Evidence by peptide mapping that the region CD4 (81-92) is involved in gp120/CD4 interaction leading to HIV infection and HIV-induced syncytium formation. *J. Immun.* **145**, 4072-4078.
- Kanehisa, J., Izumo, T., Takeuchi, M., Yamamako, T., Fujii, T. and Takeuchi, H., 1991. *In vitro* bone resorption by isolated multinucleate giant cells from a giant cell tumor of bone-light and electron microscopical study. *Virch. Archiv. A* **419**, 327-338.
- Kassem, M., Mosekilde, L., Rungby, J., Mosekilde, L., Melson, F. and Eriksen, E. F., 1991. Formation of osteoclasts and osteoclast-like cells in long-term human bone marrow cultures. *Acta path. Mic. Immun. scan.* **99**, 262-268.
- Katsumoto, T., Hattori, N. and Kurimura, T., 1987. Maturation of human immunodeficiency virus, strain LAV, *in vitro*. *Intervirology* **27**, 148-153.
- Katsumoto, T., Hattori, N., Yamada, O. and Kurimura, T., 1988. Intermediate virions in maturation of human immunodeficiency virus, strain LAV. *J. Electron Microsc.* **39**, 205-207.
- Katsumoto, T., Asanaka, M., Kageyama, S., Kurimura, T., Nakajima, K., Noto, A., Tanaka, H. and Sato, R., 1990. Budding process and maturation of human immunodeficiency virus examined by means of pre- and post-embedding immunocolloidal gold electron microscopy. *J. Electron Microsc.* **39**, 33-38.
- Kersting, U., Joha, H., Steigner, W., Gassner, B., Gstraunthaler, G., Pfaller, W. and Oberleithner, H., 1989. Fusion of cultured dog kidney (MDCK) cells: I. Technique, fate of plasma membranes and of cell nuclei. *J. Memb. Biol.* **111**, 37-48.
- Kielian, M. and Junderwirth, S., 1990. Mechanisms of enveloped virus entry into cells. *Molec. Biol. Med.* **7**, 17-32.
- Kirkpatrick, C. J., Curry, A. and Bisset, D. L., 1988. Light- and electron-microscopic studies on multinucleated giant cells in sarcoid granuloma: new aspects of asteroid and Schaumann Bodies. *Ultrastruct. Path.* **12**, 581-598.
- Kitchen, A. D., Mann, G. F., Harrison, J. F. and Zuckerman, A. J., 1989. The effect of gamma irradiation on the human immunodeficiency virus and human coagulation proteins. *Vox Sang* **56**, 223-229.
- Klatzmann, D., Barré-Sinoussi, F., Nugeyre, M. T., Dauguet, C., Vilmer, E., Griscelli, C., Brun-Vézinet, F., Rouzioux, C., Gluckman, J. C., Cherman, J.-C. and Montagnier, L., 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* **228**, 59-63.
- Klement, V., Rowe, W. P., Hartley, J. W. and Pugh, W. E., 1969. Mixed culture cytopathogenicity: a new test for growth of murine leukemia virus in tissue culture. *Proc. natn. Acad. Sci. U.S.A.* **63**, 753-758.
- Klimkait, T., Strebel, K., Hoggan, M. D., Martin, M. A. and Orenstein, J. M., 1990. The human immunodeficiency virus type 1-specific protein vpu is required for efficient virus maturation and release. *J. Virol.* **64**, 621-629.
- Koblet, H., 1990. The "merry-go-round": alphaviruses between vertebrate and invertebrate cells. In: *Advances in Virus Research*, Vol. 38, Maromorosch, K., Murphy, F. A. and Shatkin, A. J. (eds), Academic Press, New York, 343-402.
- Koga, Y., Sasai, M., Nakamura, K., Kimura, G. and Nomoto, K., 1990. Intracellular distribution of the envelope glycoprotein of HIV and its role in the production of the cytopathic effect in CD4⁺ and CD4⁻ human cell lines. *J. Virol.* **64**, 4661-4671.
- Komada, M., Matsumoto, Y., Fujiwara, M., Zhang, S. S., Hanawa, H., Itoh, E., Tsuda, T., Izumi, T. and Shibata, A., 1991. Characteristics of giant cells and factors related to the formation of giant cells in myocarditis. *Circulation Res.* **69**, 1042-1050.
- Kost, T. A., Kessler, J. A., Patel, I. R., Gray, J. G., Overton, L. K. and Carter, S. G., 1991. HIV infections and syncytium formation in HeLa cells expressing glycopospholipid-anchored CD4. *J. Virol.* **65**, 3276-3283.
- Kurabayashi, H., Miyawaki, S., Murakami, H., Nemoto, K., Sawamura, M., Kubota, K., Naruse, T. and Tsuchiya, J., 1989. Ultrastructure of multinucleated myeloma cells: Report of one case. *Am. J. Hemat.* **31**, 284-285.
- Kurman, R. J., 1991. The morphology, biology and pathology of intermediate trophoblast: A look back to the present. *Human Path.* **22**, 847-855.
- Lackner, A. A., Smith, M. O., Munn, R. J., Martfield, D. J., Gardner, M. B., Marx, P. A. and Dandekar, S., 1991. Localization of Simian immunodeficiency virus in the central nervous system of Rhesus monkeys. *Am. J. Path.* **139**, 609-620.
- Lairmore, M. D., Akita, G. Y., Russel, H. I. and de Martini, J. C., 1987. Replication and cytopathic effects of ovine lentivirus strains in alveolar macrophages correlate with *in vivo* pathogenicity. *J. Virol.* **61**, 4038-4042.
- Larsen, L. G., Theilade, K., Skibsted, L. and Jacobsen, G. K., 1991. Malignant placental site trophoblastic tumor. *AMPIS (Suppl.)* **23**, 138-145.
- Laurent-Crawford, A. G., Krust, B., Muller, S., Rivière, Y., Rey-Cuillé, M.-M., Béchet, J.-M., Montagnier, L. and Hovanessian, A. G., 1991. The cytopathic effect of HIV is associated with apoptosis. *Virology* **185**, 829-839.
- Lifson, J. D., Feinberg, M. D., Reyes, G. B., Rabin, L., Banaspour, B., Chakrabarti, S., Moss, B., Wong-Staal, F., Steimer, K. S. and Engelman, E. G., 1986a. Induction of CD4-dependent cell fusion by the HTLC-III/LAV envelope glycoprotein. *Nature* **323**, 725-728.
- Lifson, J. D., Reyes, G. R., McGrath, M. S., Stein, B. S. and Engelman, E. G., 1986b. AIDS retrovirus induced cytopathology: Giant cell formation and involvement of the CD4 antigen. *Science* **232**, 1123-1127.
- Lifson, J. D., Rausch, D. M., Kalyanaraman, V. S., Hwang, K. M. and Eiden, L. E., 1991. Synthetic peptides allow discrimination of structural features of CD4(81-92) important for HIV-1 infection versus HIV-1 induced syncytium formation.
- Lindboe, C. F. and Froland, S. S., 1988. Unusual nuclear inclusions in CNS macrophages of a man with encephalomyelitis and acquired immunodeficiency. *Ultrastruct. Path.* **12**, 145-150.
- Lopata, A., Kohlman, D. J. and Kellow, G. N., 1982. Fine structure of human blastocysts in culture. In: *Embryonic Development, Part B: Cellular Aspects*, Burger, M. M. and Weber, R. (eds), Alan R. Liss, New York, 69-85.
- Löwer, R., Löwer, J., Frank, H., Harzman, R. and Kurth, R., 1984. Human teratocarcinomas cultured *in vivo* produce unique pertovirus-like viruses. *J. gen. Virol.* **65**, 887-898.
- Löwer, J., Wondrak, E. M. and Kurth, R., 1987. Genome analysis and reverse transcriptase activity of human teratocarcinoma-derived retroviruses. *J. gen. Virol.* **68**, 2807-2815.
- Lucey, D. R., Hensley, R. E., Word, W. W., Hensley, R. E. and Bosell, R. N., 1991. CD4⁺ monocyte counts in persons with HIV-1 infection. An early increase is followed by a progressive decline. *J. acq. Immun. Def. Syndr.* **4**, 24-30.
- Lucy, J. A., Ahkong, Q. F., 1989. Membrane Fusion: Fusogenic agents and osmotic forces. In: *Subcellular Biochemistry* Vol. 14 Harris, J. R. and Etemadi, A.-H. (eds), Plenum, New York, 189-228.
- Lusso, P., de Maria, A., Malnati, M., Lori, F., DeRocco, S. E., Baseler, M. and Gallo, R. C., 1991. Induction of CD4⁺ susceptibility to HIV-1 infection of CD8⁺ lymphocytes by human herpes virus 6. *Nature* **349**, 533-535.
- Magni, E., Lairitzen, A. F., Wilkenjensen, C. and Horn, T., 1991. Malignant giant cell tumour of the uterus. *APMIS* **99**, 23, 113-118 (Suppl.).

- Malherbe, H. H. and Strickland-Cholmley, M., 1980. *Viral Cytopathology*, CRC Press, Boca Raton.
- Marks, S. C., 1983. The origin of osteoclasts: evidence clinical implications and investigative challenges of an extraskeletal source. *J. Oral Path.* **12**, 26–256.
- Marks, S. C. and Cahill, D. R., 1986. Ultrastructure of alveolar bone during tooth eruption in the dog. *Am. J. Anat.* **177**, 427–438.
- Marks, S. C. and Chambers, T. J., 1991. The giant cells recruited by subcutaneous implants of mineralized bone particles and slices in rabbits are not osteoclasts. *J. Bone Min. Res.* **6**, 401–410.
- Marks, S. C. and Popoff, S. N., 1988. Bone cell biology: the regulation of development, structure, and function in the skeleton. *Am. J. Anat.* **183**, 1–44.
- Matsui, T., Kobayashi, A., Yoshida, O., Ishii, S., Abo, Y. and Yamamoto, N., 1990. Effects of succinylated concanavalin A on infectivity and syncytial formation of human immunodeficiency virus. *Med. Microbiol. Immun.* **179**, 225–236.
- McElrath, M. J., Steinman, R. M. and Cohn, Z. A., 1991. Latent HIV-1 infection in enriched populations of blood monocytes and T-cells from seropositive patients. *J. clin. Invest.* **87**, 27–30.
- McInnes, A. and Renick D. M., 1988. Interleukin 4 induces cultured monocyte/macrophages to form giant multinucleated cells. *J. exp. Med.* **167**, 598–611.
- Mehlin, H., Skoglund, U. and Daneholt, B., 1991. Transport of the Balbiani ring granules through nuclear pores in *Chironomus tentans*. *Expl Cell Res.* **193**, 72–77.
- Mergia, A. and Luciw, P. A., 1991. Replication and regulation of primate foamy viruses. *Virology* **184**, 475–482.
- Mie, Y., Miyauchi, Y., Morishita, T., Miura, S., Honoki, K., Aoki, M. and Tamai, S., 1991. Osteoclast origin of giant cells in giant cell tumors of bone. Ultrastructural and cytochemical study of six cases. *Ultrastruct. Path.* **15**, 623–630.
- Mohaghaghpour, N., Chakraborti, R., Stein, B. S., Gowda, S. G. and Engelman, E. G., 1991. Early activation events render T-cells susceptible to HIV-1-induced syncytia formation—role of protein kinase C. *J. biol. Chem.* **266**, 7233–7238.
- Mohr, L. R. and Trounson, A. O., 1982. Comparative ultrastructure of hatched human, mouse and bovine blastocysts. *J. Reprod. Fert.* **66**, 499–504.
- Momota, K., Kaneko, I., Kimura, S., Mitamura, K. and Shimada, K., 1991. Inhibition of HIV type-1-induced syncytium formation and cytopathicity by complestatin. *Biochem. biophys. Res. Commun.* **179**, 243–250.
- Monozaki, N., Ogura, H., Matsushashi, S., Joh, K., Tabuchi, K., Kimura, G. and Hori, K., 1990. Selective syncytium formation by murine leukemia virus in rat 3Y1 fibroblasts transformed by adenovirus type-12 or its E1A gene. *Arch. Virol.* **115**, 123–126.
- Montagnier, L., Cherman, J. C., Barré-Sinoussi, F., Chamaret, S., Gruest, J., Nugeyre, M. T., Rey, F., Dauguet, C., Axler-Blin, C., Vézinet-Brun, F., Rouzioux, C., Saimot, G. A., Rozenbaum, W., Gluckman, J. C., Klatzman, D., Vilmer, E., Griscelli, C., Foyer-Ganzengel, C. and Brunet, J. B., 1984. A new human T-lymphotropic retrovirus: characterization and possible role in lymphadenopathy and acquired immune deficiency syndromes. In: *Human T-cell Leukemia/Lymphoma Viruses*, Gallo, R. C., Essex, M. E. and Gross, L. (eds), Cold Spr. Harb. Lab., New York, 363–379.
- Montefiori, D. C. and Mitchell, W. M. (1987) Persistent coinfection of T lymphocytes with HTLV-II and HIV and the role of syncytium formation in HIV-induced cytopathic effect. *Virology* **160**, 372–378.
- Moraczewski, J., Martelly, I. and Gautron, J., 1988. Phorbol ester binding in isolated muscle satellite cell compared to fetal myogenic cells from rat. In: *Monographs Developmental Biology*, Vol. 21, Anton, H. J. (eds), Karger, Basel, 78–83.
- Möst, J., Neumayer, H. P. and Dierich, M. P., 1990. Cytokine-induced generation of multinucleated giant cells *in vitro* requires interferon and expression of LFA-1, *Eur. J. Immun.* **20**, 1661–1668.
- Mulligan, M. J., Kumar, P., Hui, H. X., Owens, R. J., Ritter, G. D., Halin, B. H. and Compans, R. W., 1990. The *env* gene of an infectious noncytopathic HIV-2 is deficient in syncytium formation. *AIDS Res. Human Retroviruses* **6**, 707–720.
- Nagy, K., Clapham, P., Cheingsong-Popov, R. and Weiss, R. A., 1983. Human T-cell Leukemic virus type I: Induction of syncytia and inhibition by patients sera. *Int. J. Cancer* **32**, 321–328.
- Naito, M., Jogasaki, M., Takashashi, K., Matsumi, S., Hattori, T. and Takatsuki, K., 1989. Ultrastructural behaviour of human immunodeficiency virus (HIV) in multinucleated giant cells in the brain of a Japanese hemophilic presenting AIDS encephalopathy. *Ultrastruct. Path.* **13**, 433–441.
- Nara, P. L., Hatch, W., Dunlop, N. M., Robey W. G., Arthur, L. O., Gonda, M. A. and Sischinger, P., 1987. Simple, rapid, quantitative syncytium forming microassay for the detection of HIV neutralizing antibody. *AIDS Res. Human Retroviruses* **3**, 283–302.
- Narayan, O. and Clements, J. E., 1989. Biology and pathogenesis of lentiviruses. *J. gen. Virol.* **70**, 1617–1639.
- Nelson, J., Leong, J. A. and Levy, J. A., 1978. Normal human placentas contain RNA-directed DNA polymerase activity like that in viruses. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6263–6267.
- Oemus, K. and Timmel, H., 1990. Chondroosteoid breast tumor with multinucleate giant cells. *Pathology* **11**, 307–312.
- Ogura, H., Paulson, J. and Bauer, H., 1977. Cross neutralization of ovine and bovine C-type leukemia virus-induced syncytia formation. *Cancer Res.* **37**, 1486–1489.
- Ohtsuki, Y., Fujimoto, S., Takata, M., Aoyama, A., Hoshino, N. and Akasi, T., 1987. Ultrastructural and immunological studies of virus particles observed in a T-cell line derived from a mouse spontaneous lymphoma. *Arch. Path. Jpn* **37**, 201–206.
- Orenstein, J. M. and Janotta, F., 1988. Human immunodeficiency virus and papovavirus infections in acquired immunodeficiency syndrome: an ultrastructural study of three cases. *Human Path.* **19**, 350–361.
- Orenstein, J. M., Meltzer, M. S., Phipps, T. and Gendelman, H. E., 1988. Cytoplasmic assembly and accumulation of human immunodeficiency viruses types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastructural study. *J. Virol.* **62**, 2578–2586.
- Orrell, J. M. and Sanders, D. S. A., 1991. A particularly aggressive placental site trophoblastic tumour. *Histopathology* **18**, 559–560.
- Ostoby, P., Oursler, M. J. and Anderson, F., 1986. The osteoblast and osteoclast cytodifferentiation. In: *Progress in Developmental Biology, Part. B.*, Slavkin, H. C. (ed.), Alan R. Liss, Inc., New York, 409–411.
- Owens, R. J., Tanner, C. C., Mulligan, M. J., Srinivas, R. V. and Compans, R. W., 1990. Oligopeptide inhibitors of HIV-induced syncytium formation. *AIDS Res. Human Retroviruses* **6**, 1289–1296.
- Pal, R., Mumbauer, S., Hoke, G., Larocca, R., Myers, C., Sarngadharan, M. G. and Stein, C. A., 1991. Effect of Evans Blue and Typan Blue on syncytia formation and infectivity of human immunodeficiency virus type I and type II *in vitro*. *AIDS Res. Human Retroviruses* **7**, 537–543.
- Panem, S., 1979. C-type virus expression in the placenta. *Curr. Top. Path.* **66**, 175–189.
- Pantaleo, G., Poli, G., Butini, L., Fox, C., Dayton, A. I. and Fauci, A. S., 1991. Dissociation between syncytia formation and HIV spreading—suppression of syncytia formation does not necessarily reflect inhibition of HIV infection. *Eur. J. Immun.* **21**, 1771–1774.
- Papadimitriou, J. M. and Archer, M., 1974. The morphology of murine foreign body multinucleate giant cells. *J. Ultrastruct. Res.* **49**, 372–386.
- Papadimitriou, J. M. and Rigby, P. J., 1979. The detection of a contractile apparatus in murine multinucleate giant cells. *J. Path.* **129**, 91–96.
- Pautret, G., Suzan, M., Saluan, D., Corbeau, P., Allasia, C., Morel, G. and Filippi, P., 1990. HIV type-1 infection of U937 cells promotes cell differentiation and a new pathway of viral assembly. *Virology* **179**, 749–758.
- Pearson, M. L. and Epstein, H. F., 1982. Muscle development. In: *Molecular and Cellular Control*, Cold Spr. Harb. Lab., New York.
- Philips, D. M., 1970. Insect sperm: their structure and morphogenesis. *J. Cell Biol.* **44**, 243–277.
- Phillips, J. M., Blendis, L. M., Poucell, S., Patterson, J., Petric M., Roberts, E., Levy, G. A., Superina, R. A., Greig, P. D., Cameron, R., Langer, B. and Purcell, R. H., 1991. Syncytial giant-cell hepatitis. *New Eng. J. Med.* **324**, 455–460.
- Pierce, A. M., Lindskog, S. and Hammarström, L., 1991. Osteoclasts: structure and function. *Electron Microsc. Rev.* **4**, 1–45.
- Pierce, G. B. and Midgley, A. R., 1963. The origin and function of human syncytiotrophoblast giant cells. *Am. J. Path.* **43**, 153–173.
- Pierce, G. B., Midgley, A. R. and Beals, T. F., 1964. An ultrastructural study of differentiation and maturation of trophoblast in the monkey. *Lab. Invest.* **13**, 451–464.

- Poppovic, M., Sarngadharan, M. G., Read, E. and Gallo, R. C., 1984. Detection, isolation, and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**, 497-500.
- Pulliam, L., Hendier, B. G., Tang, N. M. and McGrath, M. S., 1991. HIV-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. *J. clin. Invest.* **82**, 503-512.
- van der Rhee, H. J., van der Burgh-de Winter, C. P. M. and Daems, W. Th., 1979. The differentiation of monocytes into macrophages, epithelial cells, and multinucleated giant cells in subcutaneous granulomas. I. Fine structure. *Cell Tiss. Res.* **197**, 355-378.
- Ringertz, N. R. and Savage, R. E., 1976. *Cell Hybrids*. Academic Press, New York.
- Robertson, T. A., Grounds, M. D., Mitchell, C. A. and Papadimitriou, J. M., 1990. Fusion between myogenic cells *in vivo*—An ultrastructural study in regenerating murine skeletal muscle. *J. Struct. Biol.* **105**, 170-182.
- Rumyantsev, P. P. and Krylova, M. I., 1990. Ultrastructure of myofibers and cells synthesizing DNA in the developing and regenerating lymph-heart muscles. *Int. Rev. Cytol.* **120**, 1-52.
- Saigo, P. E., Rosen, P. P., Brigati, D. J., Turnbull, A. D. and Sternberg, S. S., 1981. Primary gastric carcinoma. An immunological study. *Am. J. Surg. Path.* **5**, 333-342.
- Sarkar, N. H., 1989. Oncovirinae: type B oncovirus. In: *Animal Virus Structure (Perspectives in Medical Virology, Vol. 3)* Nermut, M. V. and Steven, A. C. (eds), Elsevier, Amsterdam, 257-272.
- Sasaki, T., Takahashi, N., Higashi, S. and Suda, T., 1989a. Multinucleated cells formed on calcified dentine from mouse bone marrow cells treated with $1\alpha,25$ -dihydroxyvitamin D_3 , have ruffled borders and resorb dentine. *Anat. Rec.* **224**, 379-391.
- Sasaki, T., Shimizu, T., Suzuki, H. and Watanabe, C., 1989b. Cytodifferentiation and degeneration of odontoclasts in physiologic root resorption of kitten deciduous teeth. *Acta Anat.* **135**, 330-340.
- Scheven, B. A. A. and Hamilton, N. J., 1991. Stimulation of macrophage growth and multinucleate cell formation in rat bone marrow cultures by insulin-like growth factor-I. *Biochem. biophys. Res. Commun.* **174**, 647-653.
- Shanklin, D. R., 1990. *Tumors of the Placenta and Umbilical Cord*. B. C. Decker, Inc., Philadelphia.
- Sharer, L. R., Cho, E.-S. and Epstein, L. G., 1985. Multinucleated giant cells and HTLV-III in AIDS encephalopathy. *Human Path.* **16**, 760.
- Sinkovics, J. G., 1991. Hodgkin's disease revisited: Reed-Sternberg cells as natural hybridomas. *Crit. Rev. Immun.* **11**, 33-63.
- Smith, C. A. and Moore, H. D., 1988. Expression of C-type viral particles at implantation in the marmoset monkey. *Human Reprod.* **3**, 395-398.
- Smith, N. M., Davies, J. B., Shrimankar, J. S. and Malcolm, A. J., 1990. Deep fibrous histiocytoma with giant cells and bone metaplasia. *Histopathology* **17**, 365-367.
- Sodroski, J., Goh, W. C., Rosen, C., Cambell, K. and Haseltine, W. A., 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature* **322**, 470-474.
- Sorimachi, K., Akimoto, K., Yamazaki, S., Niwa, A., Toda, S. and Yasumura, Y., 1990. Multinucleation of macrophages with water-solubilized lignin derivatives *in vitro*. *Cell Struct. Funct.* **15**, 317-322.
- Spear, P. G., 1987. Virus-induced cell fusion. In: *Cell Fusion* Sowers, A. E. (ed.) Plenum, New York, 3-43.
- Srinivas, R. V., Venkatachalapathi, Y. V., Rui, Z., Owens, R. J., Gupta, K. B., Srinivas, S. K., Anantharmaiah, G. M., Segrest, J. P. and Compans, R. W., 1991. Inhibition of virus-induced cell fusion by apolipoprotein A-I and its amphipathic peptide analogs. *J. cell. Biochem.* **45**, 224-237.
- Starka, A. S., Aparisi, T. and Ericsson, J. L. E., 1983. Human osteogenic sarcoma: Fine structure of the osteoblastic type. *Ultrastruct. Path.* **4**, 311-329.
- Stegman, T., Doms, R. W. and Helenius, A., 1989. Protein-mediated membrane fusion. *A. Rev. Biophys. Chem.* **18**, 187-211.
- Stein, C. A., Pal, R., De Vico, A. L., Hoke, G., Mumbauer, S., Kistler, O., Sarngadharan, M. G. and Letsinger, R. L., 1991. Mode of action of 5'-linked cholesteryl phosphorothioate oligodeoxynucleotides inhibiting syncytia formation and infection by HIV-1 and HIV-2 *in vitro*. *Biochemistry (U.S.A.)* **30**, 2439-2443.
- Stern, W. and Dales, S., 1976. Biogenesis of vaccinia: Isolation and characterization of a surface component that elicits Ac suppressing infectivity and cell-cell fusion. *Virology* **75**, 232-241.
- Stromberg, K. and Beneviste, R., 1983. Efficient isolation of endogenous rhesus retrovirus from trophoblast. *Virology* **128**, 518-523.
- Suni, J., Wahlström, T. and Vaehri, A., 1981. Retrovirus p30-related antigen in human syncytiotrophoblasts and immunoglobulin antibodies in cord-blood sera. *Int. J. Cancer* **28**, 559-566.
- Suni, J., Närvänen, A., Wahlström, T., Aho, M., Pakkanen, R., Vaehri, A., Copeland, T., Cohen, M. and Oroszlan, S., 1984a. Human placental syncytiotrophoblast M, 75,000 polypeptide defined by antibodies to a synthetic peptide based on a cloned human endogenous retroviral DNA sequence. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6197-6201.
- Suni, J., Närvänen, A., Wahlström, T., Lehtovirta, P. and Vaehri, A., 1984b. Monoclonal antibody to human T-cell leukemia virus p19 defines polypeptide antigen in human choriocarcinoma cells and syncytiotrophoblasts of first-trimester placentas. *Int. J. Cancer* **33**, 293-298.
- Sutton, J. S. and Weiss, L., 1966. Transformation of monocytes in tissue culture into macrophages, epithelioid cells and multinucleated giant cells. An electron microscopical study. *J. Cell Biol.* **28**, 303-332.
- Suzuki, H., Okubo, A., Yamazaki, S., Suzuki, K., Mitsuya, H. and Toda, S., 1989. Inhibition of the infectivity and cytopathic effect of human immunodeficiency virus by water-solubilized lignin in an extract of the culture medium of *Lentinus edodes mycelia* (LEM). *Biochem. biophys. Res. Commun.* **160**, 367-373.
- Takeya, M., Naito, M., Eto, K. and Takahashi, K., 1991. Immunohistochemical characterization of multinucleated giant cells in the brain of a Japanese AIDS patient. *Acta Path. Jap.* **41**, 212-216.
- Tersmette, M., de Goede, R. E. Y., Al, B. J. M., Winkel, I. N., Gruters, R. A., Cuypers, H. T. and Miedema, H. G., 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: Frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J. Virol.* **62**, 2026-2032.
- Tersmette, M., Gruters, R. A., de Wolf, F., de Goede, R. E. Y., Lange, J. M. A., Schellekens, P. T. A., Goudsmit, J., Huisman, H. G. and Miedema, F., 1989. Evidence for a role of virulent HIV variants in the pathogenesis of AIDS: studies on sequential HIV isolates. *J. Virol.* **63**, 2118-2125.
- Thormar, H., 1961. An EM study of tissue cultures infected with visna virus. *Virology* **14**, 463-475.
- Timar, J., Nagy, K., Robertson, D., Clapham, P. and Weiss, R., 1987. Ultrastructure and immunoelectron microscopy of human T-cell leukaemia virus type I-producing lymphoid and non-lymphoid human tumour cells. *J. gen. Virol.* **68**, 1011-1020.
- Tochikura, T. S., Nakashima, H., Tanabe, A. and Yamamoto, N., 1988. Human immunodeficiency virus (HIV)-induced cell fusion: quantification and its application for the simple and rapid screening of anti-HIV substances *in vitro*. *Virology* **164**, 542-546.
- Tomé, F. M. S. and Fardeau, M., 1986. Nuclear changes in muscle disorders. In: *Methods and Achievements in Experimental Pathology*, Vol. 12. Jasmin, G. and Simard, R. (eds), Karger, Basel, 261-296.
- le Tourneau, A., Audouin, J., Diebold, J., Marche, C., Tricottet, V. and Reynes, M., 1986. LAV-like viral particles in lymph node germinal centers in patients with the persistent lymphadenopathy syndrome and the acquired immunodeficiency syndrome-related complex. *Human Path.* **17**, 1047-1053.
- Trojani, M., De Mascarel, I., Caquet, M., Coindre, J. M. and De Mascarel, A., 1989. Carcinome mammaire à cellules géantes de type ostéoclastique. *Ann. Path.* **9**, 189-194.
- Udagawa, N., Takahashi, N., Akatsu, T., Tanaka, H., Sasaki, T., Nishihara, T., Koga, T., Martin, R. J. and Suda, T., 1990. Origin of osteoclasts: Mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow derived stromal cells. *Proc. natn. Acad. Sci. U.S.A.* **87**, 7260-7264.
- Ueno, H., Imamura, M. and Kikuchi, K., 1983. Frequency and antigenicity of type C retrovirus-like particles in human placenta. *Virechows Arch. A* **400**, 31-41.
- Vazeux, R., 1990. AIDS encephalopathy and tropism of HIV for brain monocytes/macrophages and microglial cells. *Pathology* **59**, 214-218.

- Wang, E., Ross, D. S., Heggeness, M. H. and Chopin, P. W., 1982. Function of cytoplasmic fibers in syncytia. *Cold. Spr. Harb. Symp. Quant. Biol.* **46**, 997-1012.
- Wang, N. S., Seemayer, T. A., Ahmed, M. N. and Knaak, J., 1976. Giant cell carcinoma of lung, A light and electron microscopical study. *Human Path.* **7**, 3-16.
- Warthin, A. S., 1931. Occurrence of numerous giant cells in the tonsils and pharyngeal mucosa in the prodromal stage of measles. *Arch. Path.* **11**, 864-874.
- Warwryk, S. O., Ayberk, H., Boyd, A. W. and Rode, J., 1991. Analysis of adhesion molecules in the immunopathogenesis of giant cell arteritis. *J. clin. Path.* **44**, 497-501.
- Watanabe, M., Ringler, D. J., Fultz, P. N., MacKey, J. J., Boyson, J. E., Levine, C. G. and Letvin, N. L., 1991. A chimpanzee-passaged human immunodeficiency virus isolate is cytopathic for chimpanzee cells but does not induce disease. *J. Virol.* **65**, 3344-3348.
- Watkins, B. A., Dorn, H. H., Kelly, W. B., Armstrong, R. C., Potts, B. J., Michaels, F., Kufta, C. V. and Dubois-Dalcq, M., 1990. Specific tropism of HIV-1 for microglial cells in primary human brain cultures. *Science* **249**, 549-553.
- Weiland, F. and Bruns, M., 1980. Ultrastructural studies on medi-visna virus. *Arch. Virol.* **64**, 277-285.
- Weiland, F., Matheka, H. D., Coggins, L. and Härtner, D., 1977. Electron microscopical studies on equine infectious anemia virus (EIAV). *Arch. Virol.* **55**, 335-340.
- Weimer, R., Daniel, V., Zimmermann, R., Schimpf, K. and Opelz, G., 1991. Autoantibodies against CD4 cells are associated with CD4 helped in HIV-infected patients. *Blood* **77**, 133-140.
- Weiss, R. A., 1982. Human T-cell retroviruses. In: *RNA Tumor Viruses: Molecular Biology of Tumor Viruses*, Weiss, R., Teich, N., Varmus, H. and Coffin, J. (eds), Cold Spring Harbor Laboratory, New York, 405-485.
- Wells, D. E., Chatterjee, S., Mulligan, M. J. and Compans, R. W., 1991. Inhibition of human immunodeficiency virus type 1-induced cell fusion by recombinant interferons. *J. Virol.* **65**, 6325-6330.
- White, J. M. and Blobel, C. P., 1989. Cell-to-cell fusion. *Curr. Opin. Cell Biol.* **1**, 934-939.
- Wiley, C. A., Schrier, P. D., Nelson, J. A., Lampert, P. W. and Oldstone, M. B. A., 1986. Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc. natn. Acad. Sci. U.S.A.* **83**, 7089-7093.
- Wise, G. E., Marks, S. C., Jr and Cahill, D. R., 1985. Ultrastructural features of the dental follicle associated with formation of the tooth eruption pathway in the dog. *J. Oral Path.* **14**, 15-26.
- de Wolf, F., Roos, M., Lange, J. M. A., Houweling, J. T. M., Coutinho, R. A., van der Noordaa, J., Schellekens, P. T. and Goudsmit, J., 1988. Decline in CD4⁺ cell numbers reflects increase in HIV-1 replication. *AIDS Res. Human Retroviruses* **4**, 433-440.
- Wynn, R. M., 1971. Immunological implications of comparative placental ultrastructure. In: *The Biology of the Blastocysts*, Blandau, R. J. (ed.), The University of Chicago Press, Chicago, 495-514.
- Yoffe, B., Lewis, D. E., Petrie, B. L., Noonan, C. A., Melnick, J. L. and Hollinger, F. B., 1987. Fusion as a mediator of cytolysis in mixtures of uninfected CD4⁺ lymphocytes and cells infected by human immunodeficiency virus. *Proc. natn. Acad. Sci. U.S.A.* **84**, 1429-1433.
- Yokoi, T., Aoyama, A., Nagoyoshi, S., Tsutsui, Y., Saga, S., Iida, K., Takashi, M., Kimura, Y. and Hoshino, M., 1990. Characterization of cell fusion in XC cells induced by Sancur murinus mammary tumor virus. *Arch. Virol.* **115**, 267-276.
- Zack, J. A., Arrigo, S. J., Weitsman, S. R., Go, A. S., Haislip, A. and Chen, I. S., 1991. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* **61**, 213-222.
- Zakai, N., Loyter, A. and Kulka, R. G., 1974. Fusion of erythrocytes and other cells with retention of erythrocyte cytoplasm. Nuclear activation in chicken erythrocyte-melanoma heterokaryons. *J. Cell Biol.* **61**, 241-248.
- Zheng, Q. and Chang, D. C., 1991. Reorganization of cell structures during cell fusion. *J. Cell Sci.* **100**, 431-442.
- Zheng, M. H., Papadimitriou, J. M. and Nicholson, G. C., 1991. A quantitative cytochemical investigation of osteoclasts and multinucleate giant cells. *Histochem. J.* **23**, 180-188.
- Zimmerli, U. U. and Hedinger, C. E., 1991. Hyperplasia and hypertrophy of Leydig cells associated with testicular germ cell tumors containing syncytiotrophoblastic giant cells. *Virch. Archiv. A* **419**, 469-474.