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ELECTRON MICROSCOPIC DETECTION OF HUMAN PAPILLOMAVIRUS PARTICLES IN ORAL PROLIFERATIVE LESIONS

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Original Article

ELECTRON MICROSCOPIC DETECTION OF HUMAN PAPILLOMAVIRUS PARTICLES IN ORAL PROLIFERATIVE LESIONS

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Abstract

Human papilloma virus (HPV) has been demonstrated in a series of benign proliferative lesions of skin and mucosae. To prove the distribution of HPV in the oral proliferative lesions at the ultrastructural level, we performed electron microscopic analysis of 10 specimens taken from 5 patients through large excisional biopsy. All of them were diagnosed pathologically as fibropapilloma. In each patient, specimens were taken from both clinically evident proliferative lesions and clinically normal surrounding mucosa. Obtained specimens were fixed in a glutaraldehyde solution and processed for routine ultrathin sectioning. Before electron microscopic observation, the tissue sections on copper grids were subjected to amylase digestion of glycogen granules. Spherical viral particles of 40–55 nm in diameter were detected the non-keratinized epithelial cells in all specimens examined. Of particular interest were the large amounts of viral particles found in the cytoplasmic matrix and nuclei (especially on their chromatin masses) of the cells in intermediate and surface layers, which did not form a crystal array. All the membranous cell organelles of epithelial cells were, however, devoid of viral particles. Some viral particles were distributed in the extracellular spaces of an intermediate layer. Viral particles were hardly observed in the cells of a basal/suprabasal and prickle cell layers. There were no significant differences in the HPV distribution between the cells derived from the proliferative lesion and those derived from the surrounding normal mucosa.

Key words: Human papilloma virus (HPV) — Fibropapilloma — Oral mucosa — Electron microscopy

INTRODUCTION

Human papilloma virus (HPV) has been demonstrated in a variety of squamous epithelial cells in benign proliferative lesions of skin and mucosae and in squamous cell carcinomas of larynx, oral mucosa, and uterine cortex.[8] The virus has an elective tro-

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Table 1 Clinical data from cases examined

Case	Name	Age	Sex	Clinical aspect	Localization	Diagnosis
1	L.P.	51	M	verrucous, exophytic	left cheek	pseudopolyp
2	V.R.	61	F	soft, flat	tongue border	fibropapilloma
3	D.V.	37	M	verrucous, exophytic	velum	acanthosis
4	G.G.	57	F	exophytic, pedunculated	velum	fibropapilloma
5	A.C.	57	F	exophytic	velum	fibropapilloma

pism for keratinocytes, but fibroblasts do not host the virus.[23] This virus of the papovaviridae family is characterized by an eicosaedric capside with a diameter of about 55 nm and a double helix DNA of about 8000 base pairs, complexed with histones and organized in a nucleosoma.[19] The virus resides prevalently in the cellular nucleus as an episome; only in cells undergoing carcinomatous degeneration is the virus integrated into the chromosomic DNA.[2,5] There are, however, no descriptions of the virus in the cytoplasm.

HPV was recently categorized as a separate taxonomic entity. Although 42 genotypes of HPV have been discovered,[17] certain viral genotypes seem to prefer characteristic intracellular arrangements, such as the intranuclear crystalline agglomerates in verruca vulgaris.[14] Other HPV genotypes, especially those involved in oral lesions, have shown a sparse intranuclear distribution, but did not form an aggregated structure.[13,21] In verruca vulgaris and uterine cervical condylomata, the intranuclear presence of the virus has been well established by electron microscopy, but its relation with the intracellular structure of infected squamous epithelial lesion is insufficiently described.[9] Most studies of HPV in proliferative squamous epithelial lesions in the oral cavity have demonstrated the structural antigens of HPV by light microscopic immunohistochemistry.[1,6,10–12,15,16,20] In the present study, we have attempted primarily to reveal the precise intracellular distribution of HPV in oral fibropapillomatous lesions by means of electron microscopy.

MATERIALS AND METHODS

Fibropapillomatous lesions with surrounding margins of clinically intact mucosa were obtained from 5 patients by excisional biopsy. Immediately after excision, the specimens were separated into the clinically evident part (fibropapillomatous lesion) and normal surrounding mucosa, and processed separately for routine histopathological examination (Table 1). Small parts of these specimens were further cut into slices of less than 1 mm thickness and fixed by immersion in 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.4) for 6 hours at 4°C. After brief washing with a 0.1 M cacodylate buffer solution, the specimens were postfixed with 1.5% potassium ferrocyanide-reduced 1% osmium tetroxide for 3 hours at 4°C. They were then block-stained with ethanolated 1% uranyl acetate, dehydrated through a graded ethanol series, and embedded in Quetol 812 (Nisshin EM, Tokyo). Ultrathin sections were cut using a diamond knife on a Reichert-Jung Ultracut OmU-4, stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-12A electron microscope at 75 kV. After thin sectioning, some grids with ultrathin sections were floated on amylase solution to digest glycogen granules in the epithelial cells.

RESULTS

Table 1 shows the results of conventional histopathologic examination. Throughout the present electron microscopic observa-

tions, the non-keratinized epithelial cell layers in the oral proliferative lesions (fibropapilloma) were clearly divided into the basal/suprabasal cell layer, prickle cell layer, intermediate layer, and surface layer (Figs. 1–5). Keratinization of epithelial cells was not observed in these cells. In all cell layers, the epithelial cells were firmly connected by well-developed desmosomes (Figs. 1–5). Because of the amylase treatment, glycogen granules in the intermediate layer cells were completely digested, forming an 'electron-lucent area' in the cytoplasm (Figs. 1, 3–5). There were, however, no degenerative structural alternations in the epithelial cells. Spherical particles were clearly distinguished from free ribosomes and glycogen granules by their size and insolubility to amylase, respectively.

In all 10 specimens examined, spherical viral particles 40–55 nm in diameter were detected in cells of the intermediate layer of the epithelia taken from the clinically-evident proliferative lesions as well as those of surrounding normal mucosa. The viral particles possessed an electron-dense core with a lateral concentric halo of lower electron density (Fig. 1). These structural features of viral particles are consistent with previous diagnoses of human papilloma virus infection by electron microscopy.[9,14,16,21] The electron-dense viral particles were clearly distinguished from free ribosomes and glycogen granules by their diameter (larger then ribosomes) and insolubility to amylase treatment, respectively (Fig. 1).

In the basal/suprabasal cell layer, viral particles were rarely observed. The viral particles were never found in the subepithelial connective tissues, including fibroblasts (Fig. 2). The prickle cells contained few viral particles. Abundant viral particles were demonstrated only in the cells of the intermediate and the surface layers (Figs. 3–5). The viral particles appeared in both the epithelial cells and narrow extracellular spaces (Figs. 3 and 4). The coexistence of already infected cells and those still without viral particles was also noted in a single cell layer (Fig. 3). The viral particles were numerous in the intermediate layer, but rather rare in the surface layer (Figs. 3 and 5). The superficial cells seldom contained viral particles (Fig. 5). In the cells of intermediate and surface layers, viral particles were distributed randomly throughout the cytoplasm, but did not form crystalline agglomerates. Viral particles were frequently aggregated, forming small clusters. On the other hand, the viral particles distributed in the nuclei usually appeared in association with euchromatin and heterochromatin, but were infrequent in the nucleoplasm (Figs. 6A and B). In several cells derived from the surrounding mucosa, large intracytoplasmic agglomerates were visible without any evidence of virus in the nucleus.

DISCUSSION

Human papilloma virus (HPV) has recently been the focus of rising scientific interest for two major reasons: 1) certain HPV genotypes are associated with carcinomas, especially those of the uterine cortex,[3,4] and 2) HPV is also associated with the Human Immunodeficiency Virus (HIV).[7] Fibropapillomatous lesions are frequently seen in the oral cavity. They have been linked to HPV, and the transmission of both viruses is thought to be mainly sexually mediated.[7] However, few comparative studies of HPV and HIV have ever been done in the oral region.

We focused on demonstrating the presence and special intracellular distribution of HPV in the oral proliferative epithelial lesions and the surrounding clinically normal mucosa. In the present study, we found the virus particles not only in the cell nucleus but also in the cytoplasm of the cells in the intermediate and surface layers of the non-keratinized epithelium. The viral particles appeared in all specimens examined, but were detected only in the cells of these two epithelial cell layers. The basal/suprabasal cells and the prickle cells seldom contained viral particles. Of particular interest is the presence of viruses in the clinically normal

mucosa which surrounds the proliferative lesions; this stresses the necessity of larger surgical excisions. The virus certainly remains present in the mucosal epithelium, without expressing its capability to induce macroscopic cell proliferation. Similar to the situation in gynaecology, where frequent colposcopic and cytologic controls are generally considered to be necessary in patients positive for cervical HPV, the possibility of malignant degeneration of the infected squamous epithelial cells in the oral region must be taken into consideration. Frequent controls should be therefore considered after the surgical removal of the fibropapillomatous lesion.

Extrachromosomal viral DNA replication was confined to the lower third of the epithelial layer; the virus remains in its latency state in the basal cell layer.[22] In fact, the HPV was seldom detected in the basal/suprabasal epithelial layer (present findings). With the differentiation of the epithelial cells, the virus was actived, and the production of the capside and the assembly of the complete viral particles were prevalent in the granular and keratinized epithelial cell layers.[18] In agreement with these observations, we found abundant viral particles in the cells of the intermediate layer, which corresponds to the granular layer in the keratinized epithelium. These results suggest that the viral infection is closely related to the differentiation state of the epithelial cells. The infection of a squamous epithelium by the HPV induces a vascular and epithelial proliferation with specific histological configurations which are an exaggregation of the normal tissue architecture.[22] Cell proliferation in the parabasal cells (acanthosis) and growth of dermal capillaries (papillomatosis) can be observed. The continuing synthesis of DNA by the cells in parabasal and intermediate layers is seen microscopically as hyperchromasia, discariosis, and retarded maturation of the surface cells.[17] In the superficial layer, the final event of the viral replication produces a characteristic cytopathic effect known as kaliocytosis, resulting in a degenerative aggregation of chromatin, nuclear collapse, and formation of intracytoplasmic vacuoli. Our present findings seem to support, at least, some of these previous observations. In addition, the viral particles were observed in the cytoplasmic matrix and in the extracellular milieu of an intermediate layer. To the best of our knowledge, the presence of HPV in these compartments has not previously been reported. Such findings are of clinical importance in determining a possible infection route for the virus in this disease.

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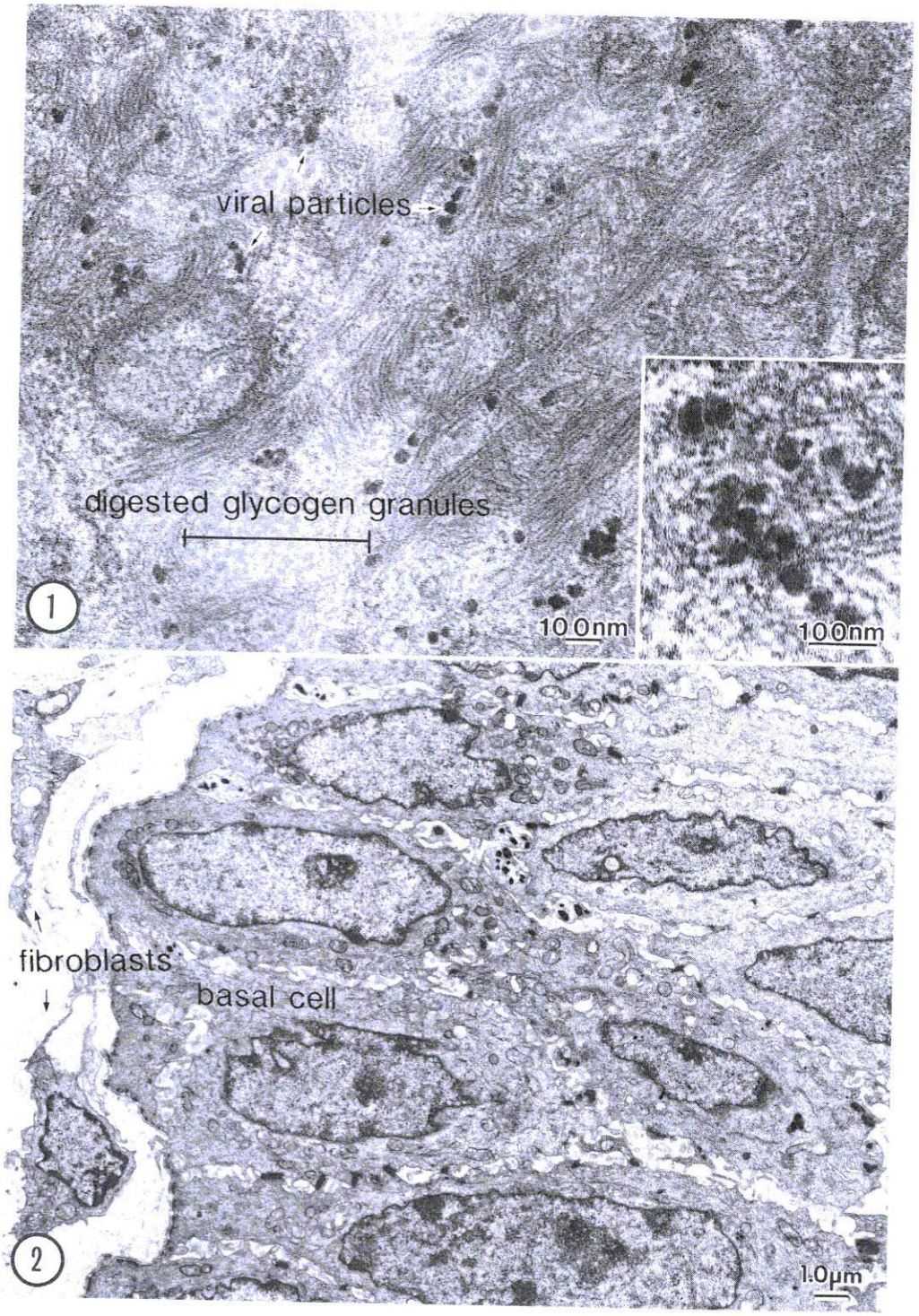
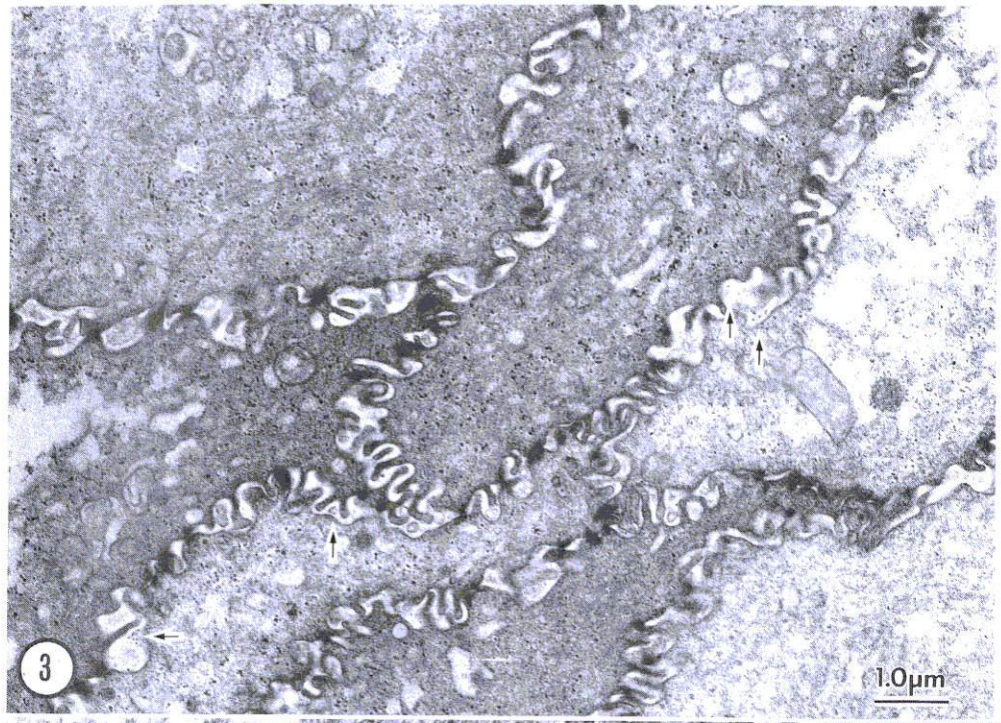


Fig. 1 Viral particles and digested glycogen granules in the cytoplasm of an intermediate layer. Inset is a higher magnification view of viral particles. U-Pb staining. (×50,000; inset: ×100,000)

Fig. 2 The basal/suprabasal cell layer. (×5,000)



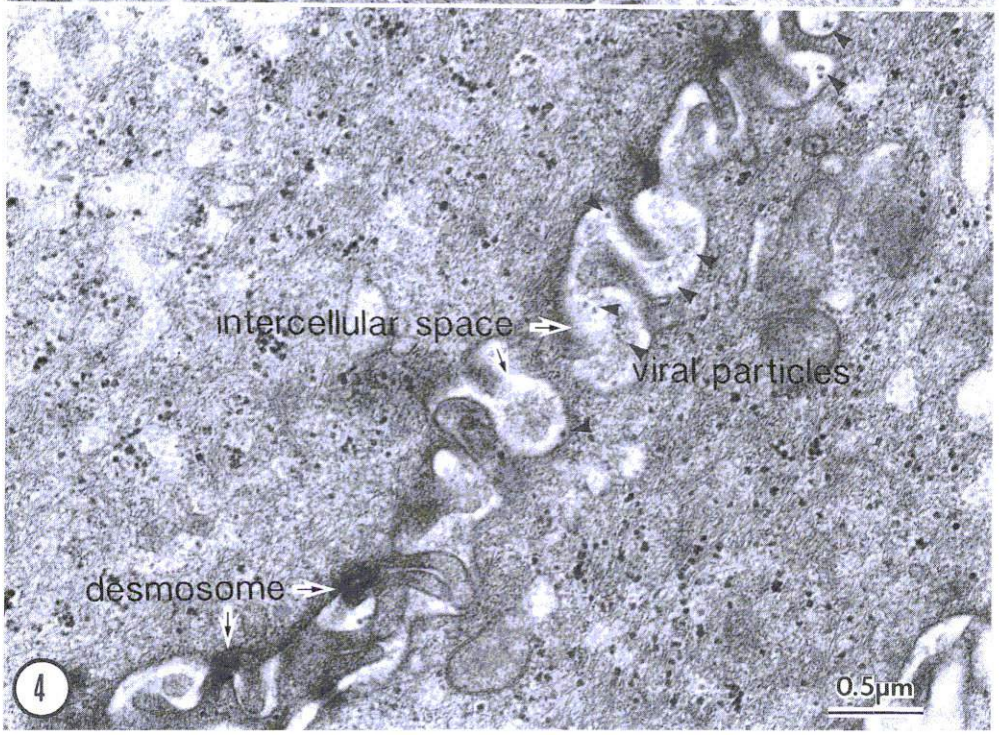


Fig. 3 The cells in an intermediate layer. Numerous viral particles are distributed over the cytoplasm. Some viral particles are also seen in the extracellular spaces (arrow indicated). (×10,000)

Fig. 4 Higher magnification view of two cells in an intermediate layer. Note the presence of many viral particles (arrowheads) in the extracellular space as well as in the cytoplasm. (×25,000)

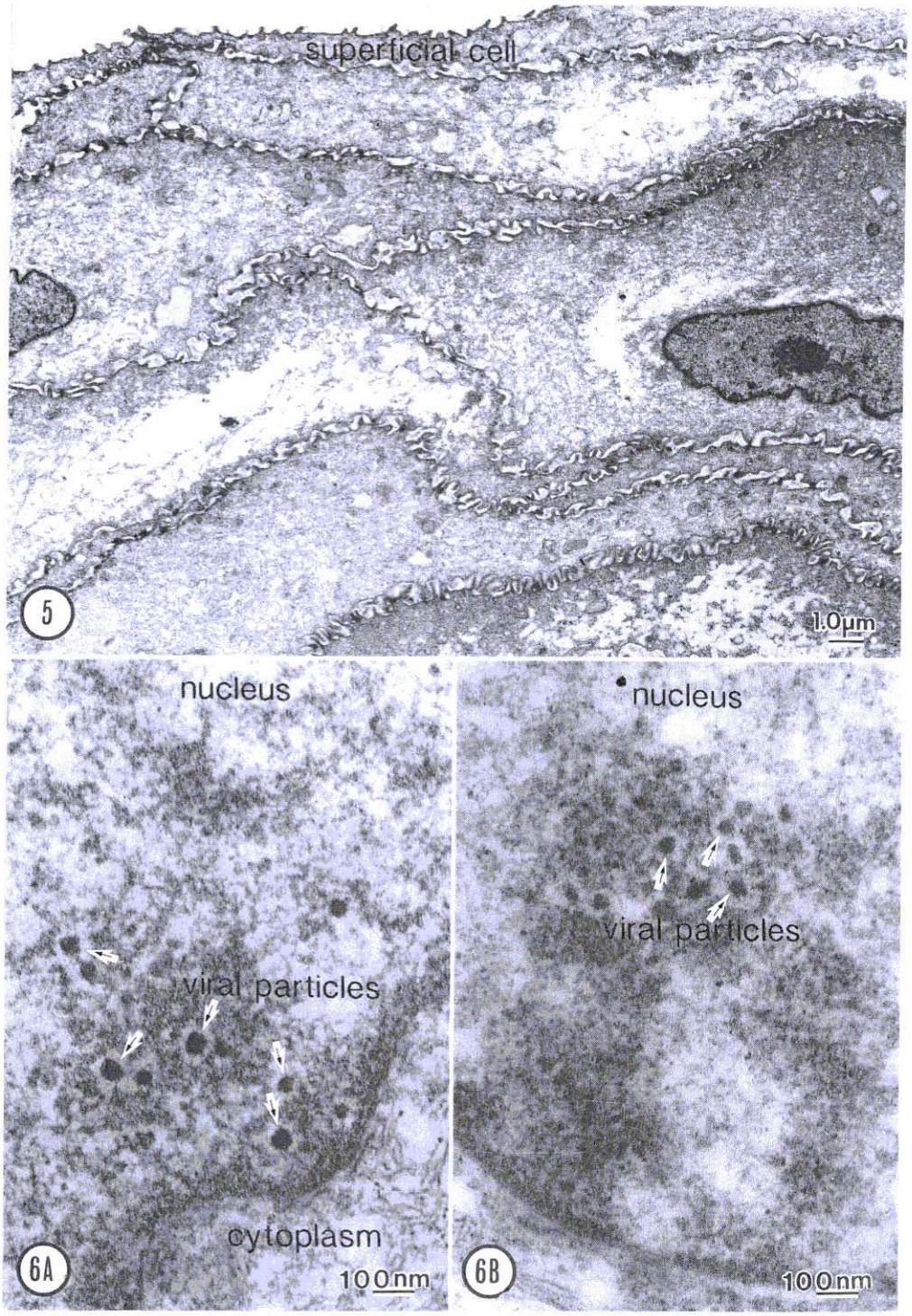


Fig. 5 The cells in a surface layer. (×5,000)

Fig. 6 Intranuclear viral particles in association with chromatin masses. (×60,000)