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PATHOMORPHOLOGICAL OBSERVATIONS ON EPIDERMAL PAPILLOMA OF FLATFISH (*LIOPSETTA OBSCURA*)*

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Morphological investigation was carried out on 10 flatfishes (*Liopsetta obscura*) which were collected in Odaito, the eastern part of Hokkaido, and they were diagnosed as epidermal papilloma.

Electron microscopically, the characteristics of so-called "X-cells" were clarified. They were considered as free cells which were completely different from epidermal cells and general inflammatory cells.

Aggregation of fine hollow particles (ca. 30 nm) in the nucleus and particles enclosed by a double membrane with a central core in the cytoplasm were found in the X-cells.

Numerous virus-like particles (ca. 100 nm) were found in the nuclei of the degenerative epithelial cells of the epidermis.

Etiological relationship between epidermal papilloma and virus-like particles are not yet unknown and further investigations are needed.

Key words: epidermal papilloma, goby, flatfish, X-cells

INTRODUCTION

Epidermal papillomas were frequently found in several kinds of flatfish: *Liopsetta obscura, Limanda punctatissima, Verasper moseri* and *Limanda schrenki*, collected in Odaito, the eastern part of Hokkaido. The ratios of fish with tumor to total catch was about 10%. Epidermal papilloma of fish has been reported in many species of fish such as various kinds of goby [1,10,12,20] and flatfish[4,7,9,11] in Japan and in many other countries. Epidermal papilloma of fish is an interesting problem in the fields of comparative oncology and etiology (theories of sea pollution by condemned materials,[15] parasitic infestation and viral infection).

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The purpose of the present report was to elucidate the pathomorphological characteristics of the tumor, especially describe the so-called "X-cells" [2,3,13,14,18,19,20] and the existence of viral particles,[5,6,8,16,17] by electron microscopy.

MATERIALS AND METHODS

Among the many kinds of flatfishes collected in Odaito, the eastern part of Hokkaido, *Liopsetta obscura* was investigated. Materials consisted of 10 flatfishes (*Liopsetta obscura*) with skin papilloma, which were collected from March 22–23, 1983.

After macroscopic observation was conducted, several pieces of tumorous growth were taken, fixed in 10% formalin and Bouin's fluid, dehydrated, embedded in paraffin, cut into thin sections and stained with hematoxylin-eosin, Azan stain and PAS reaction.

For electron microscopic observations the dissected specimens were cut into small pieces (0.5 mm^3) and fixed in 6.25% cacodilate-buffered glutaraldehyde (pH 7.4). Then, they were postfixed in 1% phosphate-buffered osmium tetraoxide and dehydrated in graded ethanol series and embedded in Epon 812. Blocks were cut by a glass knife with a Porter-Blum MT-2B ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate. Observations were made at 75 kV with an HU-12A Hitachi electron microscope. Thick sections of approximately 1 to 2 μ were stained with 1% toluidine blue for orientation by light microscopy.

For the cultivation of tumor cells, tissues were dissected into small pieces (1 mm^3) using scissors in phosphate buffered saline (pH 7.2) containing 2,000 units of penicillin, 2,000 mg of streptomycin and 5 mg of fungizone per ml. After washing the tissues with culture medium (Eagle's minimum essential medium supplemented with 1% NaCl, 10% fetal calf serum, 200 units per ml of penicillin, 200 mg per ml of streptomycin and 2.5 μg per ml of fungizone, pH 7.4), they were transferred into plastic tissue culture flask and were cultivated at 20°C.

RESULTS

I. Macroscopic changes

The weight of the flatfish was 130 ± 54 g at mean value. Tumorous lesions were observed focally from the dorsal fin to both lateral sides (fig. 1). Lesions were also observed in the ventral neck and the caudal fin. Number of the tumorous lesions was mostly one per one fish, and rarely two per one fish.

The lesions were black brown in color, some showed elevation on the surface and some showed cauliflower-like and nodular appearances. The sizes of the lesions were variable. Some of them occupied almost 1/6 of the body surface and some occupied only a small part. They were confined to the epidermis. No definite invasion of the tumorous tissues into the deeper muscular layer was found, and the lesions were sharply demarcated.

II. Light microscopic changes

The normal epidermal layers around the tumorous lesions consisted of 6–8 layers of squamous epithelial cells. There were numerous mucous-cysts in the superficial layers of the epidermis. Eosinophilic cell infiltration was marked in the epidermis. In the tumorous lesions, thickening and hyperplasia of the epidermis were clearly seen. There was also proliferation of the slender branching fibrovascular stroma which supported a thick layer of hyperplastic cells of the epidermis (figs. 2 & 3). Various types of lesions were seen in the epidermis, such as thickening of the epidermis due to the epithelial cell proliferation, fold-formation and papillary growth of the epithelial cells. Histopathologically, they were diagnosed as "epidermal papilloma".

In the superficial layers of the epidermal papilloma, there were some mucous cysts, and eosinophilic cell infiltration and macrophagic activities were observed. On the other hand, melanophores were seen in the basal cell layer of the epidermis and the dermis (fig. 4).

As to the other characteristic changes, numerous "X-cells", which were not found in the normal epidermis, were observed in the tumorous lesions of the epidermis. The X-cells were large round or ovoid cells (4–20 μm), and they were located in the spaces of the epidermis. Especially, the X-cells had characteristic pale large nuclei with prominent nucleoli (1–1.5 μm). The cytoplasm of these cells showed a weak stainining nature and had a granular appearance (fig. 5). The prominent nucleoli were stained scarlet red by Azan stain (fig. 6).

In the dermis, there were no changes in the *stratum compactum*; on the other hand, in the *stratum spongiosum*, there were some proliferation of the loose connective tissue and occasionally congestion of the blood capillaries. Fibroblastic or histiocytic proliferation and lymphocytic or melanophore infiltration were seen occasionally in the dermis.

Parasitic infestation (trematodes) was found in the connective tissue of the dermis in one case.

III. Electron microscopic changes

The papillomatous lesions consisted of thickening of the squamous epithelial cells, which comprised the normal coating of the epithelium.

Epidermis

The superficial layer The superficial layer of the epidermis consisted of a single layer of surface cells having broadly based microvilli. Numerous desmosome-like structures and interdigitation between the superficial cells and the cells of the intermediate layer were seen (fig. 7). Apparent terminal bars were observed at the outer aspect of the lateral membranes of adjacent superficial cells. The shapes of the nuclei were irregular and the nucleoplasm showed low electron density. The cytoplasm was also pale. The Golgi apparatus was well developed in the perinuclear zone. Numerous vesicles and vacuoles, which composed the smooth surfaced endoplasmic reticulum (sER), were diffusely distributed throughout the cytoplasm. In some of the

vacuoles, electron dense particles were contained. The rough surfaced endoplasmic reticulum (rER) was poorly developed and the mitochondria were scattered throughout the cytoplasm. In some of the cytoplasm of these cells, tonofilaments were clearly seen and formed a desmosome-tonofilament complex (fig. 7). Mucous cells were often found under the single layer of surface cells. Most of the cytoplasm of these cells was occupied by a large cyst containing numerous mucous droplets. Therefore, the nuclei or cytoplasmic organellae often could not be seen in the sections (fig. 8).

The intermediate cell layer The intermediate cell layer consisted of several cell layers which were similar to those of *stratum spinosum* in Mammalia. Interdigitation of the cellular junction and numerous desmosome-like structures (desmosome-tonofilament complex) were seen in these cells. There were numerous tonofilaments in the cytoplasm of these cells. The shapes of the nuclei were irregular and had deep indentation and some showed lobulation. In the perinuclear zone, there were a few rER, numerous vesicles of sER, mitochondria and small Golgi apparatus (fig. 9).

The basal cell layer The epithelial cells, comprising a single layer of the basal cells, had a cylindrical form and their long axes were placed perpendicular to the skin surface. Their nuclei were round or ovoid and their long axes coincided with those of the cells. They had one or two small nucleoli and tonofilaments running through the peripheral area of the cytoplasm. The cytoplasmic organellae were similar to those of the upper layers, but sER were conspicuously seen. The dermis was distinctly separated from the epithelial cells of the epidermis by an amorphous electron dense basement membrane. The half desmosome, which is seen in Mammalia, was not clearly seen (fig. 10).

Dermis Electron microscopically, the findings of the dermis were the same as the histological findings. Infiltrative cells consisted of fibroblasts, lymphocytes, histiocytes, macrophages and melanophores (fig. 11).

X-cells Numerous large round cells, which were considered as X-cells, were observed in the epidermis. A large round and pale nucleus with electron dense prominent round granular nucleolus was characteristic of the X-cells. The cell membranes of the X-cells were smooth but occasionally showed deep indentation. As enveloped cells, the cytoplasm of the epithelial cells of the epidermis occasionally showed deep invagination into the X-cells. Desmosome-like structures and interdigitation were seen between X-cells and adjacent epithelial cells. X-cells were observed as free cells. In the cytoplasm of X-cells, various sized fat droplets with varied electron density and small granules with high electron density were seen. Vacuolized degenerative mitochondria were also distributed throughout the cytoplasm of the X-cells. Fine vesicular sER, a few rER, small Golgi apparatus and microtubuli were seen in the perinuclear area (figs. 12, 13 & 14).

Aggregation of fine hollow particles (ca. 30 nm) was rarely found in the nucleus of the X-cells (fig. 15).

In the perinuclear area of the cytoplasm of the X-cells, aggregation of particles, which was enclosed by a double membrane with a central core, was also found occasionally. The sizes of these particles were 60–80 nm on the inside and 130–160 nm on the outside (figs. 16 & 17). Tangential section of the nuclear membrane revealed numerous nuclear pores and microtubuli (figs. 13 & 14).

Numerous macrophages were seen between the epithelial cells of the epidermis and their phagosomes had phagocytized degenerated cells.

Virus-like particles In one case of the present investigation, numerous virus-like particles were seen in the nuclei of the epithelial cells of the epidermis. The cells which contained these particles showed degenerative changes such as vacuolization and a pale appearance. The sizes of the particles were ca. 100 nm. They were enveloped by a double membrane and some of the particles had a central core, but some had hollow spaces. These particles were similar to the structures of herpesvirus in Mammalia (figs. 19 and 20).

IV. Tissue culture

Two types of cells grew in the periphery of tumor tissues. Cell growth was initiated between one to two weeks after cultivation. Fibroblastic cells contained a large number of pigment granules in the cytoplasm. Epithelioid cells were round in shape and had pale cytoplasm. Both types of cells, however, fell off gradually from the plastic vessel and therefore it was difficult to maintain or to subculture of the cells.

Discussion

From the results of the present investigation, the existence of so-called "X-cells" was confirmed in the epidermal papillomas which were frequently found in the flat fishes collected in Odaito, the eastern part of Hokkaido. These findings were similar to those of previous reports.

This type of tumor has been classified into angioepithelial nodule, epidermal papilloma and angioepithelial polyp according to the characteristics of the lesions.[9] Therefore, the intermediate form among these types of tumor is often observed. The present investigated cases were all classified as epidermal papilloma, though the grade had some variation.

The epidermal papilloma was found in both lateral sides and was black brown in color. This finding seems to be caused by the presence of melanophores.

As new findings, aggregation of hollow particles (ca. 30 nm) in the nucleus and particles with a double membrane in the cytoplasm were found in the X-cells. These findings were different from the report by Peters et al.[13] On the other hand, the numerous virus-like particles in the nuclei of the epithelial cells of the epidermis were apparently similar to those seen in herpesvirus of mammalia.

However, hitherto many workers have reported the existence of virus-like parti-

cles in the epidermal papilloma in various kinds of gody[5,6,8] and flatfish,[6,16,17] but no one has succeeded in the experimental production of papilloma by these particles. The structures of these particles were inconsistent and different from each other. Therefore it is doubtful whether these particles are real virus particles or not. The virus-like particles of our present case were completely different from those of previous reports and they showed some similarity to herpesvirus. Therefore, further investigations of the oncogenicity of these particles and their existence in other normal fishes are warranted.

On the other hand, the origin of the X-cells in the skin papilloma is still disputed.[2,3,13,14,18-20] A general agreement has not yet been reached as to whether they are fish cells that have undergone a transformation or are amoebalike parasitic protozoa. Perters et al.[13] considered that the X-cells in the skin papillomas of Pacific flatfish might be virus-transformed cells. Brooks et al.[2] suggested the possibility that the X-cells did not originate from the fish itself but rather from an unknown monocellular parasite. From the results of the present investigation, the X-cells were considered as free cells which were completely different from the epidermal cells and general inflammatory cells. Tissue culture study may provide useful informations on the origin and nature of X-cells and the participation of the cells in the development of epidermal papilloma.

In recent years, the correlation between sea pollution by condemned materials and the occurrence of epidermal papilloma has attracted public attention, but no reliable conclusions have been reached as yet. Therefore, further investigations on the etiological causes of epidermal papilloma in fish should be conducted.

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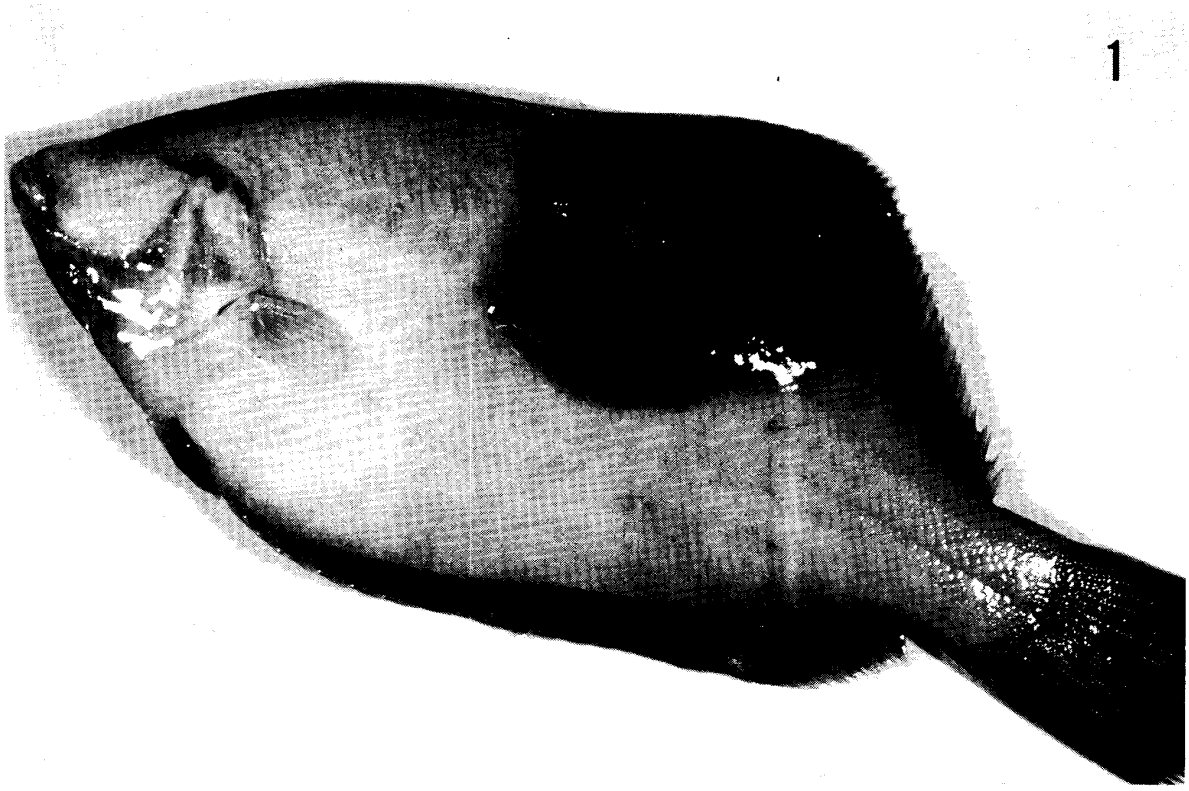
EXPLANATION OF PLATES

PLATE I

Fig. 1 *Liopsetta obscura*, Papillomatous lesion from the dorsal fin to the lateral side of the body.

Fig. 2 Epidermal papilloma. There are proliferation of a slender branching fibrovascular stroma which supports a thick layer of hyperplastic cells of the epidermis. Azan stain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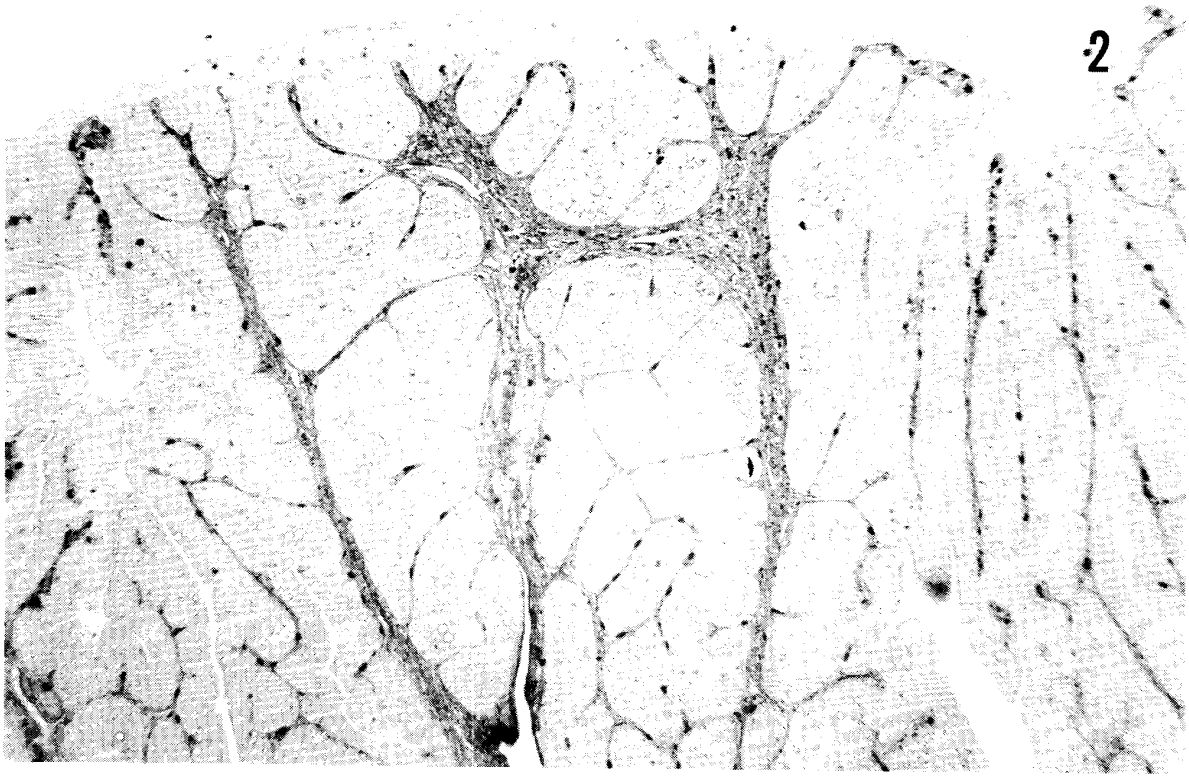
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PLATE II

Fig. 3 Epidermal papilloma. Trematodes are seen in the connective tissue of the dermis. hematoxylin-eosin (H–E) stain

Fig. 4 Epidermal papilloma. Mucous cyst formation and melanophores are seen in the dermis. H–E

Fig. 5 Epidermal papilloma. Numerous X-cells are seen in the epidemis. H–E

Fig. 6 Epidermal papilloma. Numerous X-cells with prominent nucleoli. Azan stain


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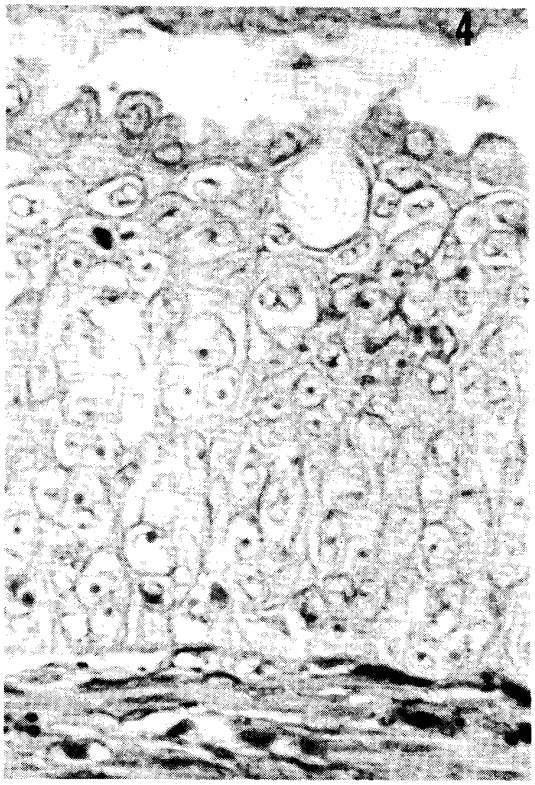
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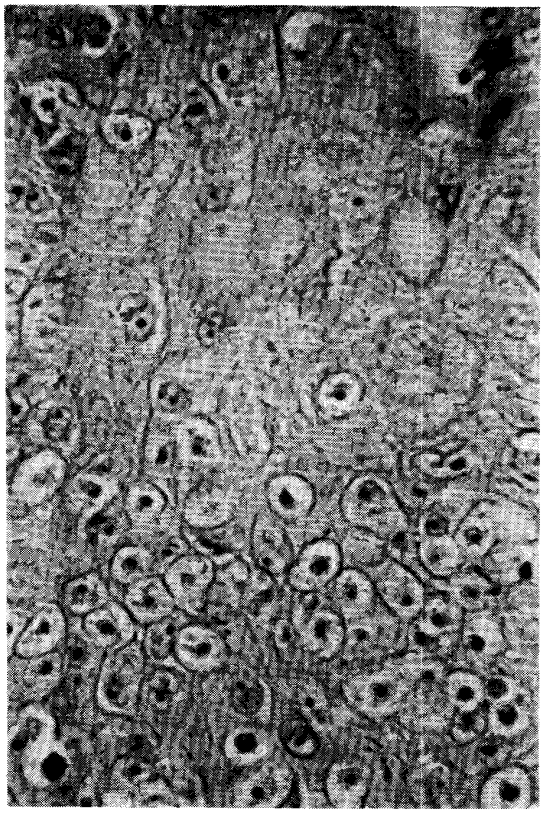

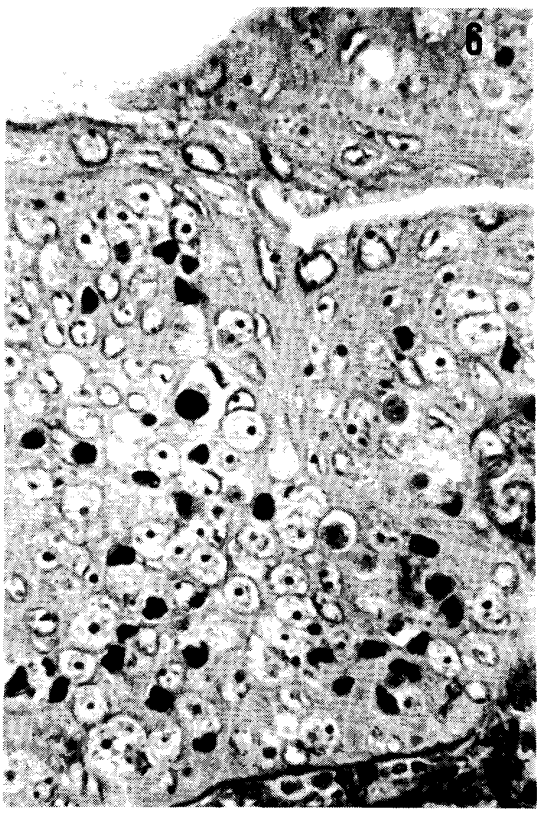
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PLATE Ⅲ

Fig. 7 Epithelial cells in the superficial layer of the epidermis.

Fig. 8 Mucous cells under the superficial layer of the epidermis. Mucous cysts.

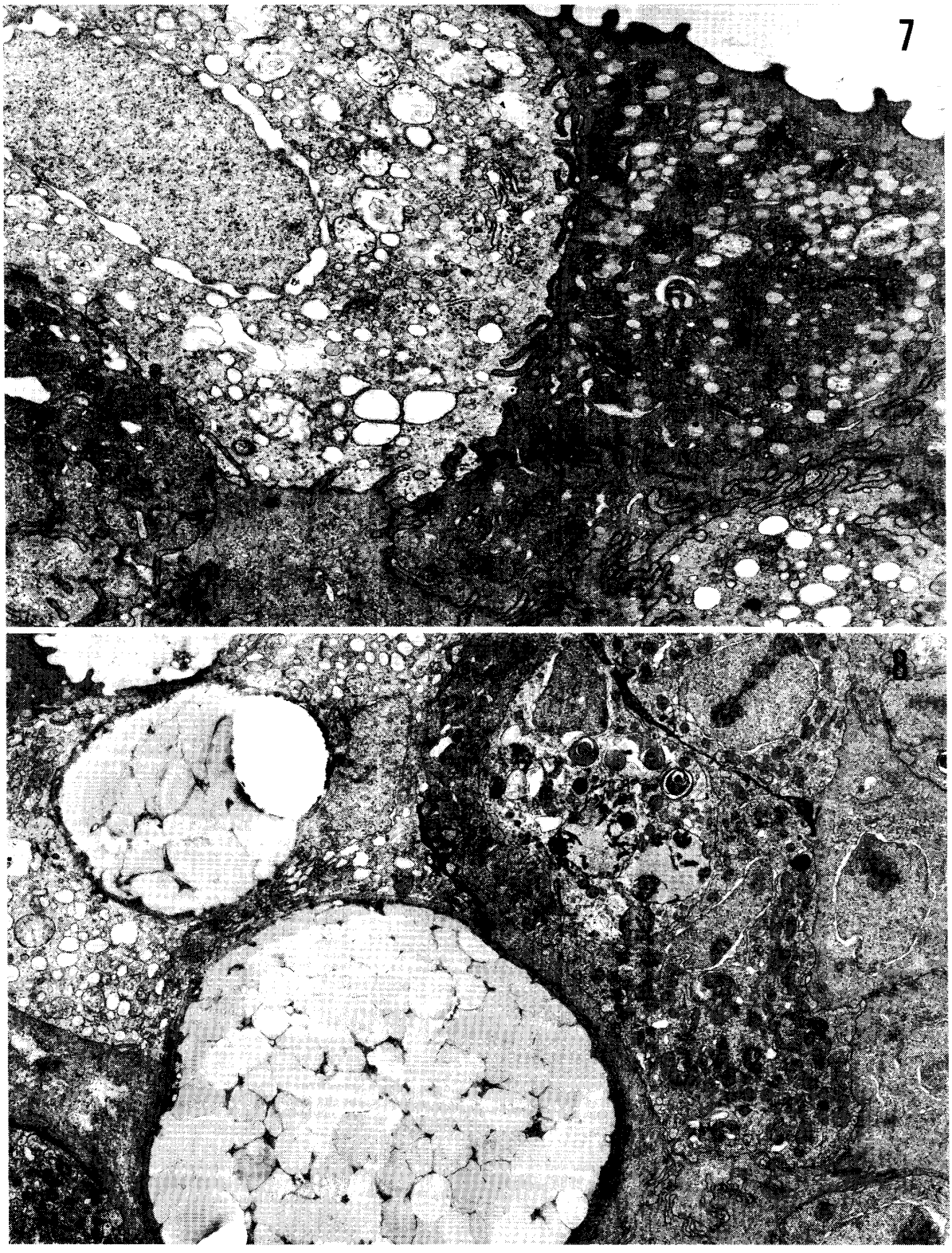
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PLATE IV

Fig. 9 In the intermediate and basal cell layers of the epidermis. Numerous X-cells (↑) are seen.

Fig. 10 The basal cell layer of the epidermis.

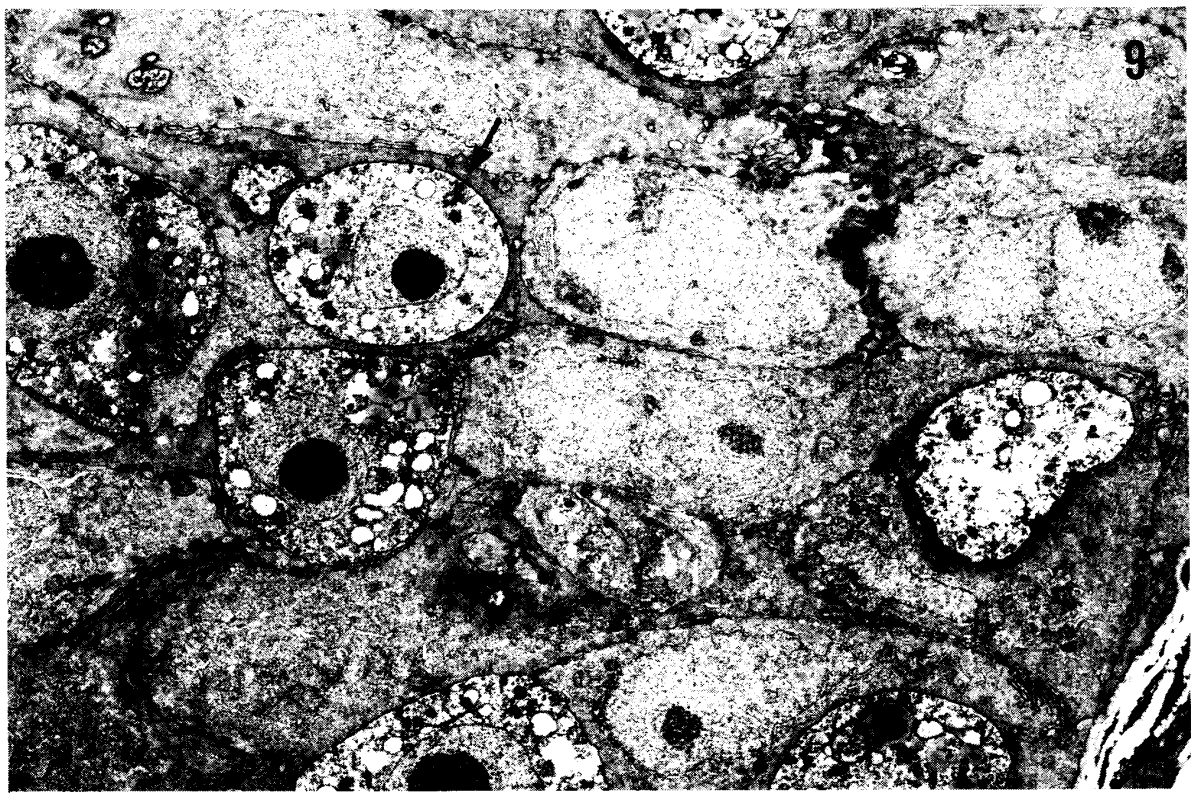
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PLATE V

Fig. 11 Cellular infiltration in the dermis.

Fig. 12 X-cells in the epidermis.

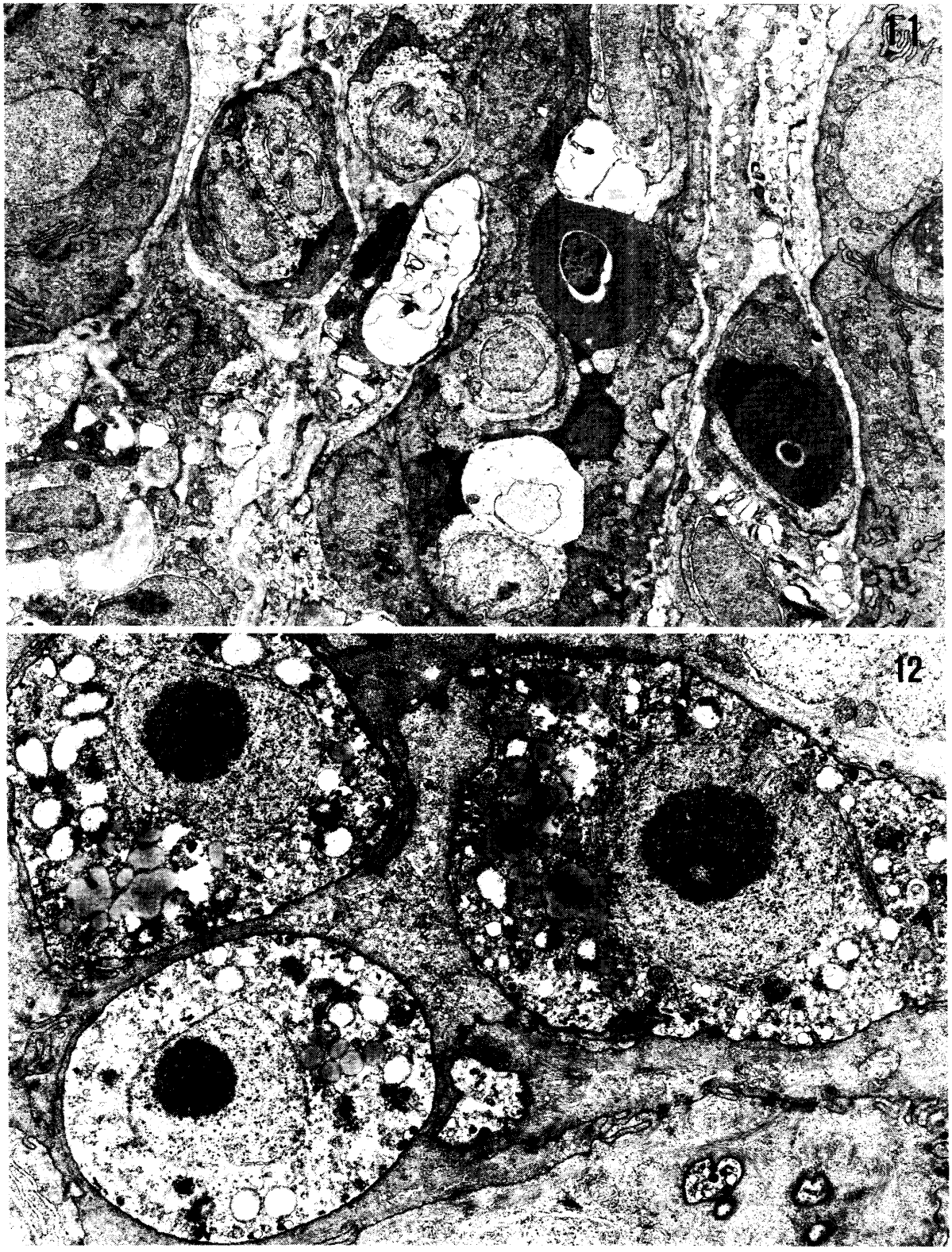
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PLATE VI

Fig. 13 In the left, small particles (↑) in the nucleus of the X-cell. In the right, X-cell has fat droplet and tangential section shows many nuclear pores.

Fig. 14 X-cell, numerous nuclear pores and microtubuli (↑) are seen in the tangential section of the nucleus.

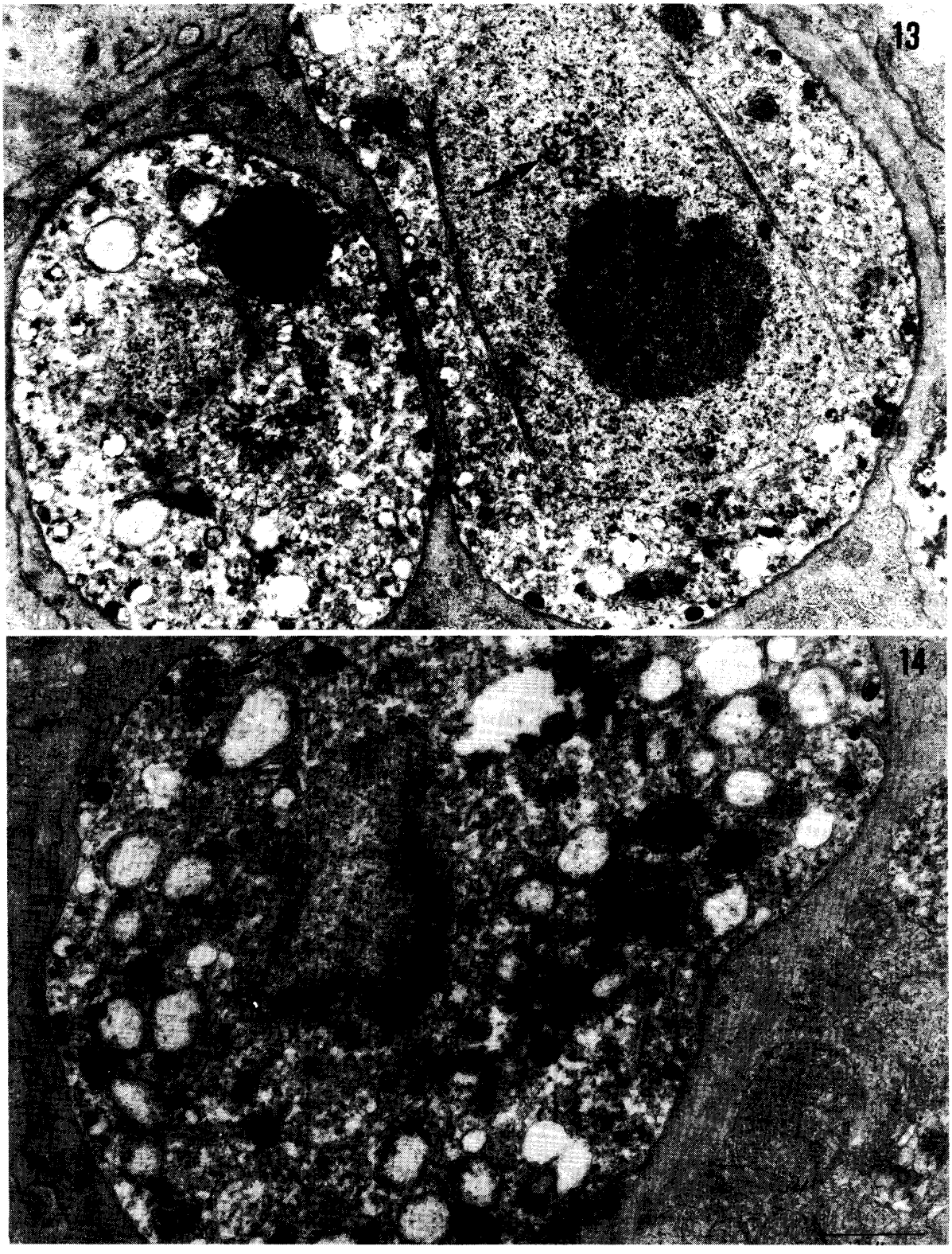
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PLATE VII

Fig. 15 Aggregate of the fine hollowed particles in the nucleus of X-cell.

Fig. 16 Aggregate of particles with double membranes in the cytoplasm of X-cell.

Fig. 17 Enlargement of Fig. 16. Particles in the cytoplasm.

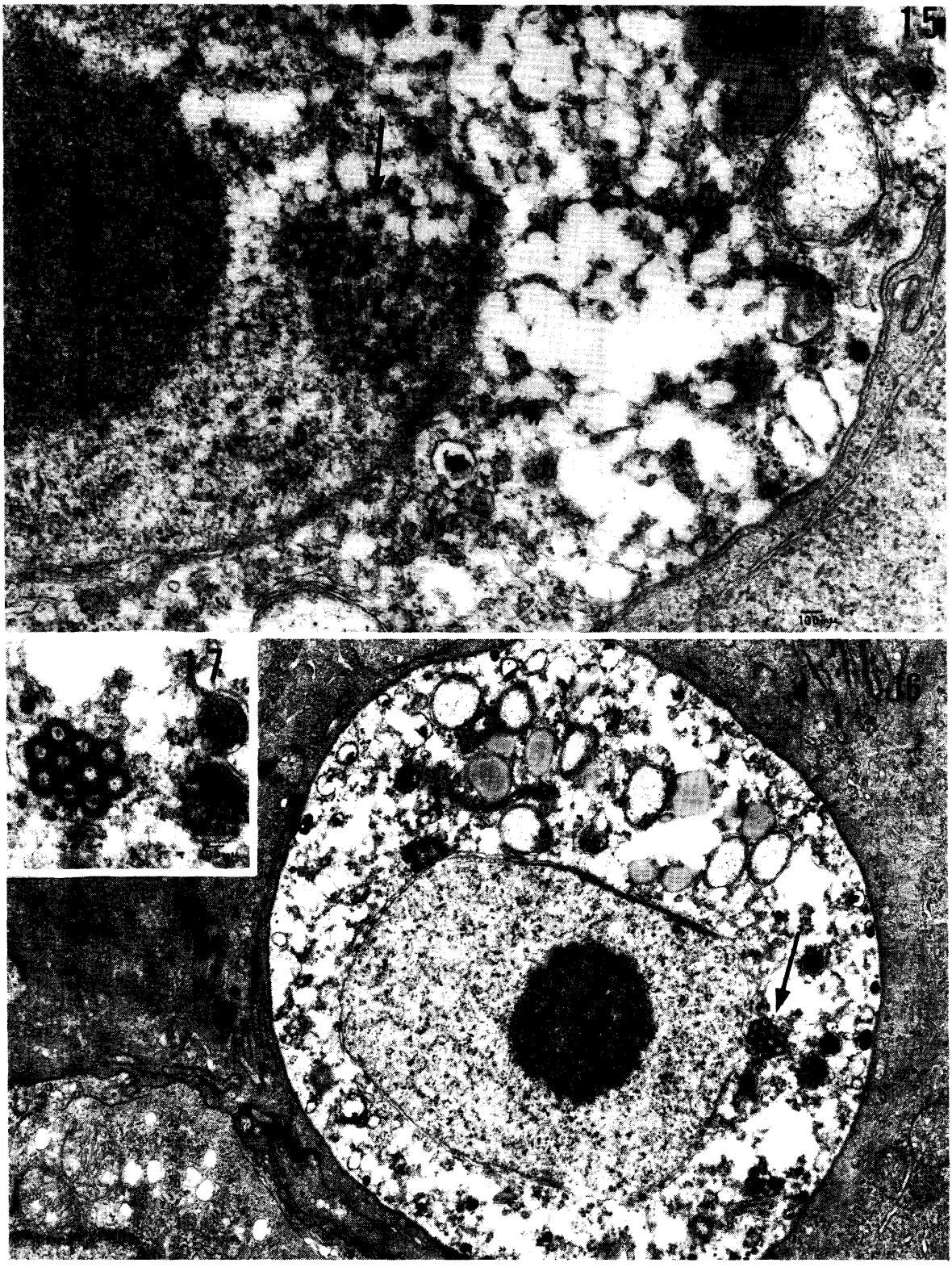
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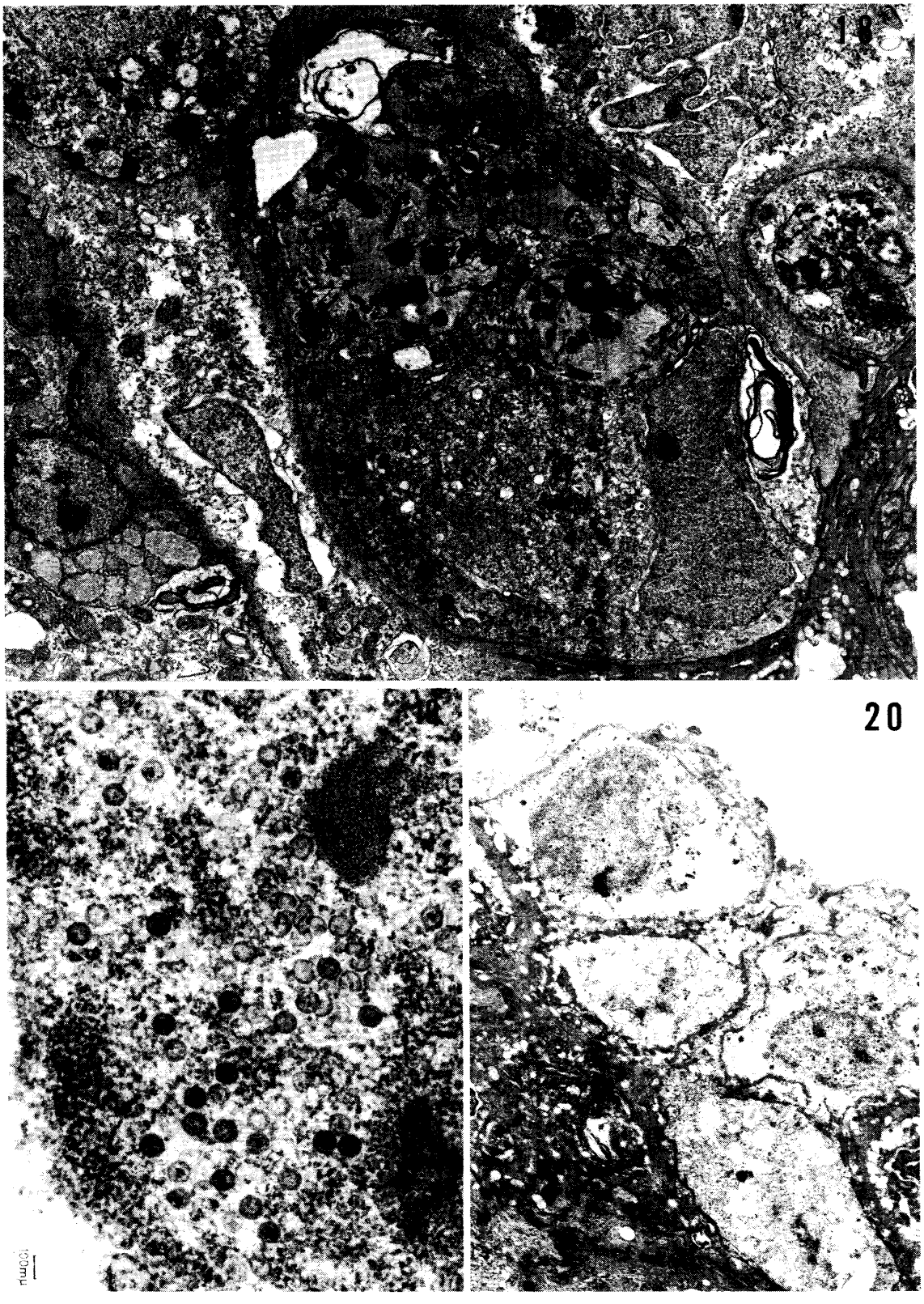
PLATE VIII

Fig. 18 Macrophge.

Fig. 19 Virus-like particles in the nucleus of the epithelial cells of the epidermis.

Fig. 20 Degenerative changes of the epithelial cells of the epidermis and they contain numerous virus-like particles in their nuclei.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