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Histology and Histopathology

The histological and the histopathological pattern of conjunctival rhinosporidiosis associated with papillomavirus infection

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Summary. The present study describes for the first time, the clinical, light and electron microscopic findings of two cases of conjunctival rhinosporidiosis. One was with concurrent infection of papillomavirus. Investigations at the ultrastructural level have provided additional information on the development of *Rhinosporidium seeberi* and would suggest that the formation of the wall of this organism is a continuous morphological and biochemical spectrum throughout its cytological maturation. The current observation on the wall formation is probably a modification of the classical pattern as an environmental protection carried out by the fungus against the virus. In contradistinction to the usual histopathological picture of rhinosporidiosis, the case with the viral infection lacked the characteristic marked inflammatory reaction. This finding, together with the relatively short interval of the frequent recurrences of this lesion, have led us to postulate the presence of a localised acquired immune deficiency state. It is possible that this local immune deficiency may be caused by an immunosuppression mechanism. This is probably mediated by papillomavirus and/or due to the weak antigenicity of the host virus-infected cells which contain only copies of viral DNA in an unintegrated form.

Key words: Conjunctiva, Rhinosporidiosis, Papillomavirus

Introduction

Rhinosporidiosis, and papillomavirus infections occur both in man and domestic animals (Sood and Rao, 1967; Robinson and Heath, 1983; Savino and Margo, 1983). Rhinosporidiosis is a fungal infection caused by *Rhinosporidium seeberi* characterised by a granulomatous inflammatory reaction. Infection with papillomavirus, a member of the *Papovaviridae* family, results in cutaneous warts and papillomas of the mucous membranes (Naseman, 1977; Robinson and Heath, 1983; McDonnell et al., 1986). There are only a few reports describing the ultrastructural features of *Rhinosporidium seeberi* in tissue obtained from the conjunctiva (Savino and Margo, 1983). The present study describes for the first time the clinical features, the histopathological details and the ultrastructural findings of simple ocular rhinosporidiosis, in comparison to this condition in the presence of concomitant papillomavirus infection.

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Materials and methods

CASE 1

A 38-year-old Pakistani male, who worked in catering, complained of redness and a foreign body sensation in his right eye, with frequent bleeding during a 6-month period. Examination of the eye revealed an injected pedunculated mass protruding from the inferior conjunctival cul-de-sac measuring 25 × 8 × 5mm. The other eye was normal. The mass was excised and sent for histopathology. Since its removal, the patient has remained well and free from recurrence for a two year follow-up period.

CASE 2

A 35-year-old Indian male nurse, attended our Eye Clinic complaining of a foreign body sensation and redness of this right eye during a three month period. Examination of the eye revealed a dark red polypoid mass protruding from the upper nasal part of the bulbar conjunctiva. The left eye was entirely normal. Apart from small wart-like lesions on the dorsum of his right hand and front of his neck, the patient was in good health. The conjunctival mass was completely excised and sent for histopathology. Follow-up examination four

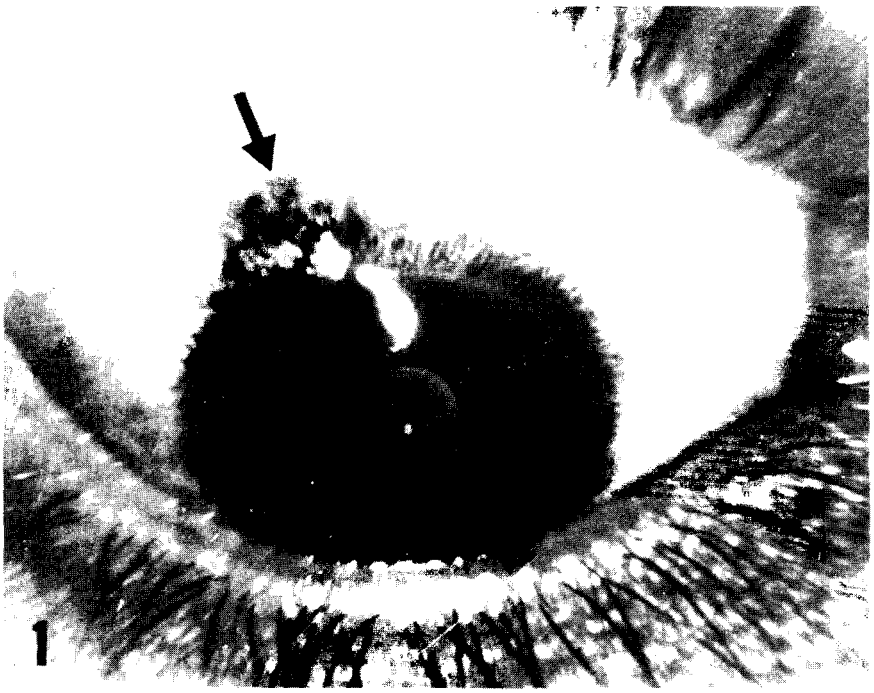


Fig. 1. Clinical photograph of the recurred rhinosporidiosis showing a limbal mass of multicystic nature (arrow).

months after surgery revealed a mass similar to that described earlier, positioned on the upper middle part of the bulbar conjunctiva and not in the previously affected area. The mass was again excised and sent for histopathology. Six months later the patient developed yet another recurrence of a similar lesion which on this occasion appeared at the upper temporal area of the limbus (Fig. 1), and was subsequently excised surgically. At the same time one of the lesions on the dorsum of the hand was also excised and sent for histopathology. A year after the last surgery the patient was seen in the clinic and was free from any apparent lesion in the right eye.

The specimens obtained from the patients were fixed in 10% formalin solution processed and embedded in paraffin wax. Sections were stained with haematoxylin

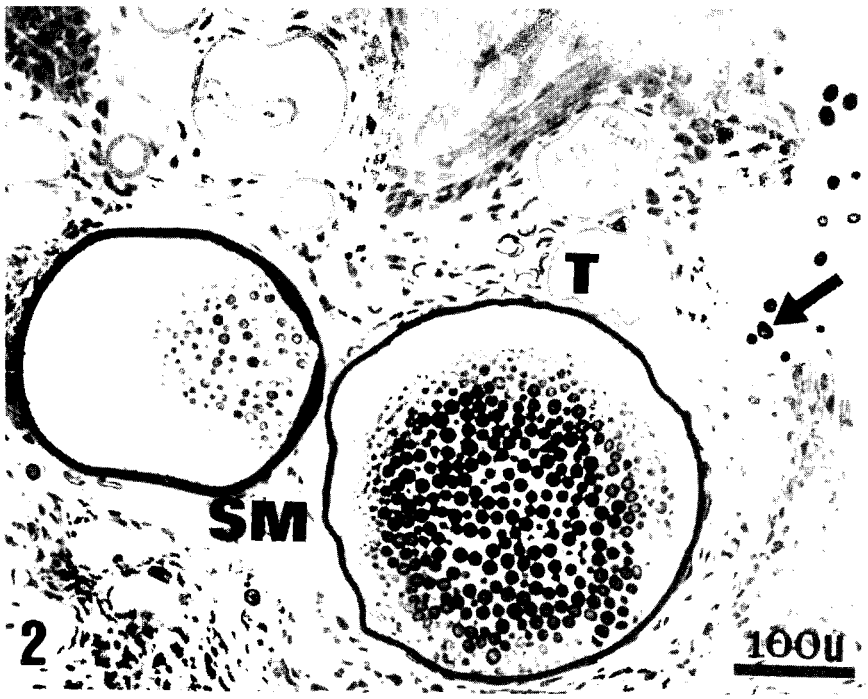


Fig. 2. *Rhinosporidium seeberi* in the conjunctiva at various stages of maturation. Released spores (arrows), trophocytes (T) and mature sporangium (SM). The sporangium is fully packed with immature sporoblast at the periphery and mature ones at the centre. Note the marked differences in the staining pattern of the wall from pale in early trophocytes to dark in mature sporangia. 1-2 μm plastic section stained with toluidine blue. × 167.

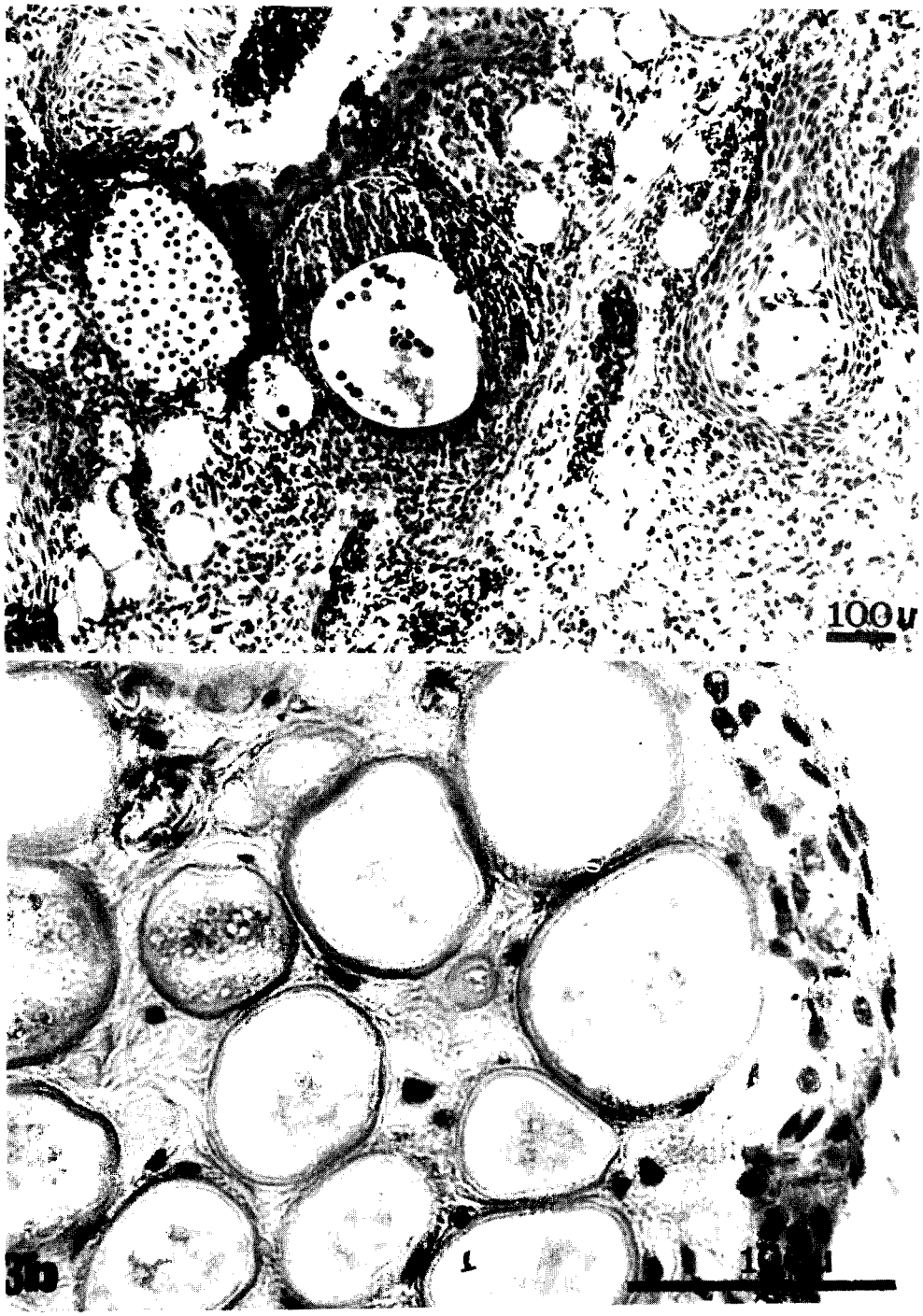
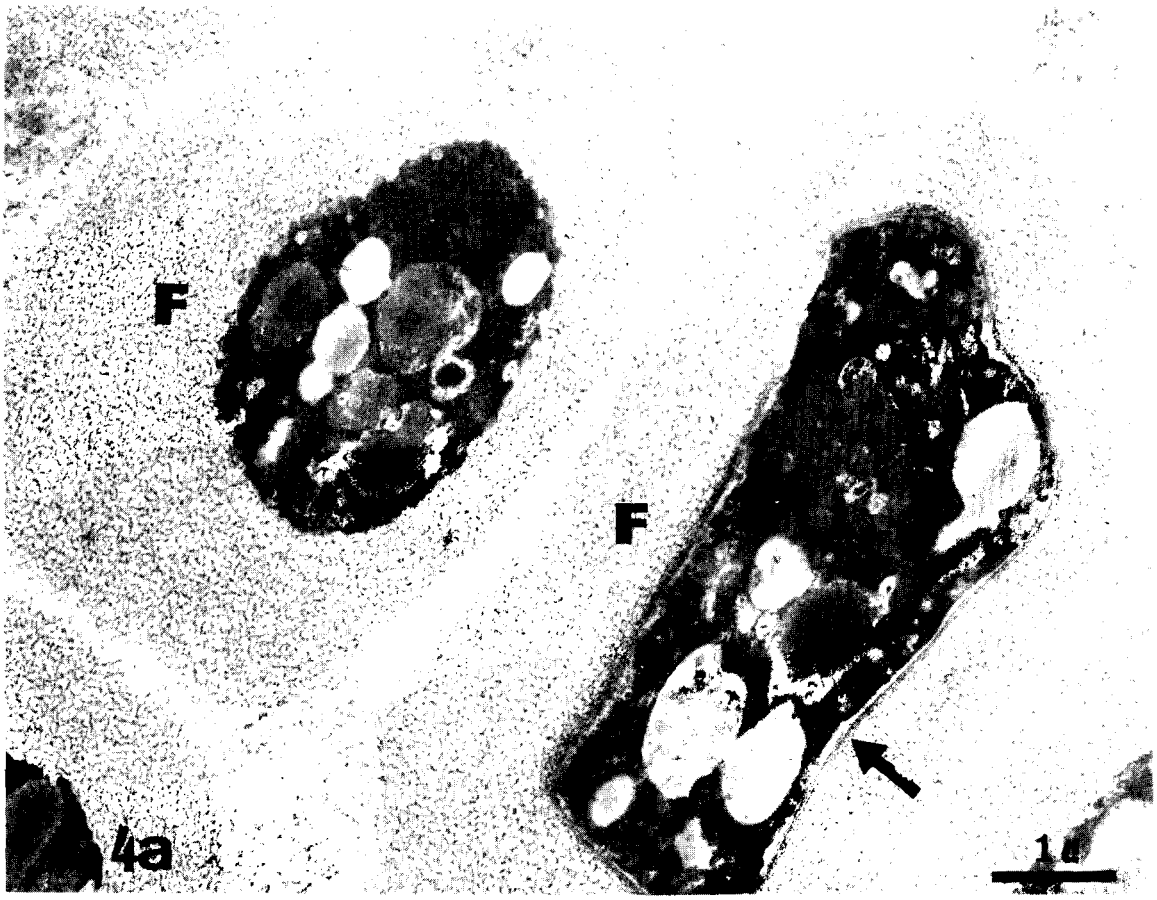


Fig. 3. (a) Case No. 1. A paraffin section of conjunctival rhinosporidiosis with massive chronic inflammatory cell infiltration, H&E. × 100
(b) Case No. 2. Marked infestation of the conjunctival stroma with *Rhinosporidium seeberi* fungi. In contrast to case No. 1, note the absence of inflammatory reaction, H&E. × 380

and eosin (H&E), Periodic Acid Schiff's reaction (PAS), and alcian blue. For semi-thin sections and transmission electron microscopy the specimens were fixed in a solution containing 3% glutaraldehyde, 1% formaldehyde, and 0.1M phosphate buffer. The tissue was post-fixed in buffered 1% osmium tetroxide, dehydrated in graded ethanol, and embedded in epoxyresin. Plastic sections, 1-2 μm thick, were stained with toluidine blue, and examined under a light microscope. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and were examined under a Jeol 1200 EX transmission electron microscope. For scanning electron microscopy the osmicated tissue was dehydrated in acetone, dried by the critical point technique and coated with a thin (200 Å) layer of gold and examined under a Jeol JSM 840 scanning electron microscope.



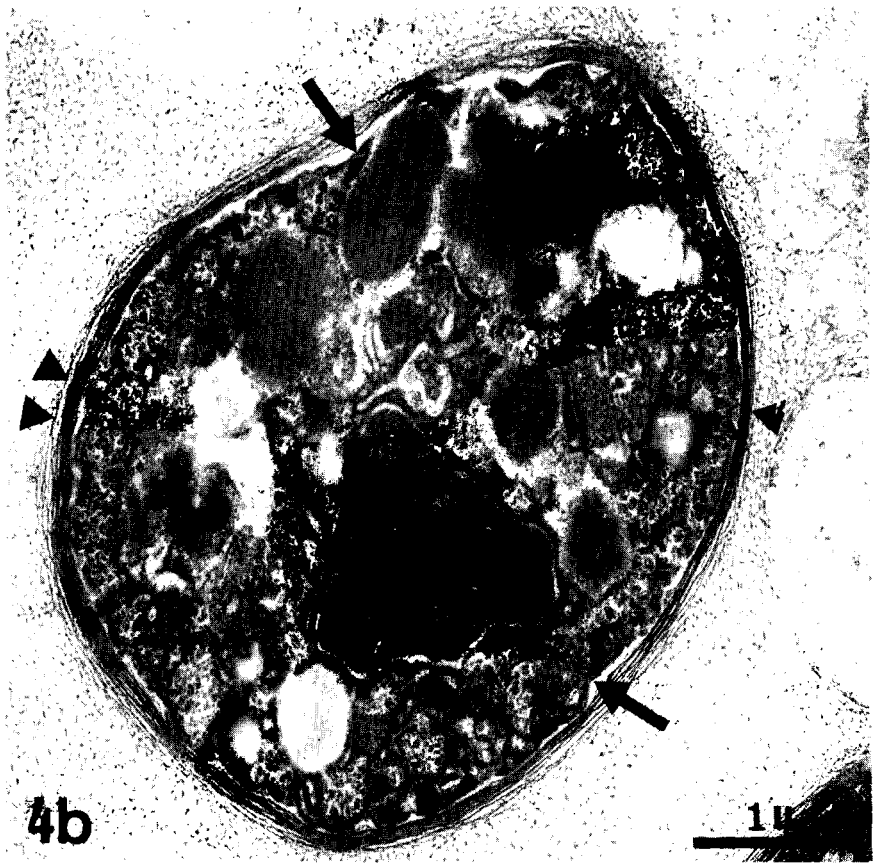


Fig. 4. (a) Electron micrograph showing developing sporoblast. The wall consisted of single and wide filamentous layer (F). The condensation of the inner aspect of this wall forms membranous structure (arrows). Lead citrate and uranyl acetate. × 16,640
(b) Electron micrograph of a later stage of sporoblast. The wall consists of well developed multilaminated membranous inner layer (arrowheads). The irregular plasma membrane (arrows) fuses in certain places with the innermost lamina of the membranous layer. Lead citrate and uranyl acetate. × 20,000

Results

Light microscopy

Examination of sections obtained from the conjunctival lesions of both cases revealed organisms characteristic of *Rhinosporidium seeberi* at various stages of development from trophocytes to mature sporangia together with the released sporoblasts. Sections stained with H&E, PAS, alcian blue and toluidine blue showed that the wall of the organism stained differently at different stages of maturation. In the early stage of development, the wall stained faintly (acidophilic) while in later stage, the wall stained darkly (basophilic), (Fig. 2).

Case No. 1

The conjunctival stroma also showed diffuse infiltration of lymphocytes, monocytes, plasma cells, polymorphonuclear cells, and multinuclear giant cells (Fig. 3a).

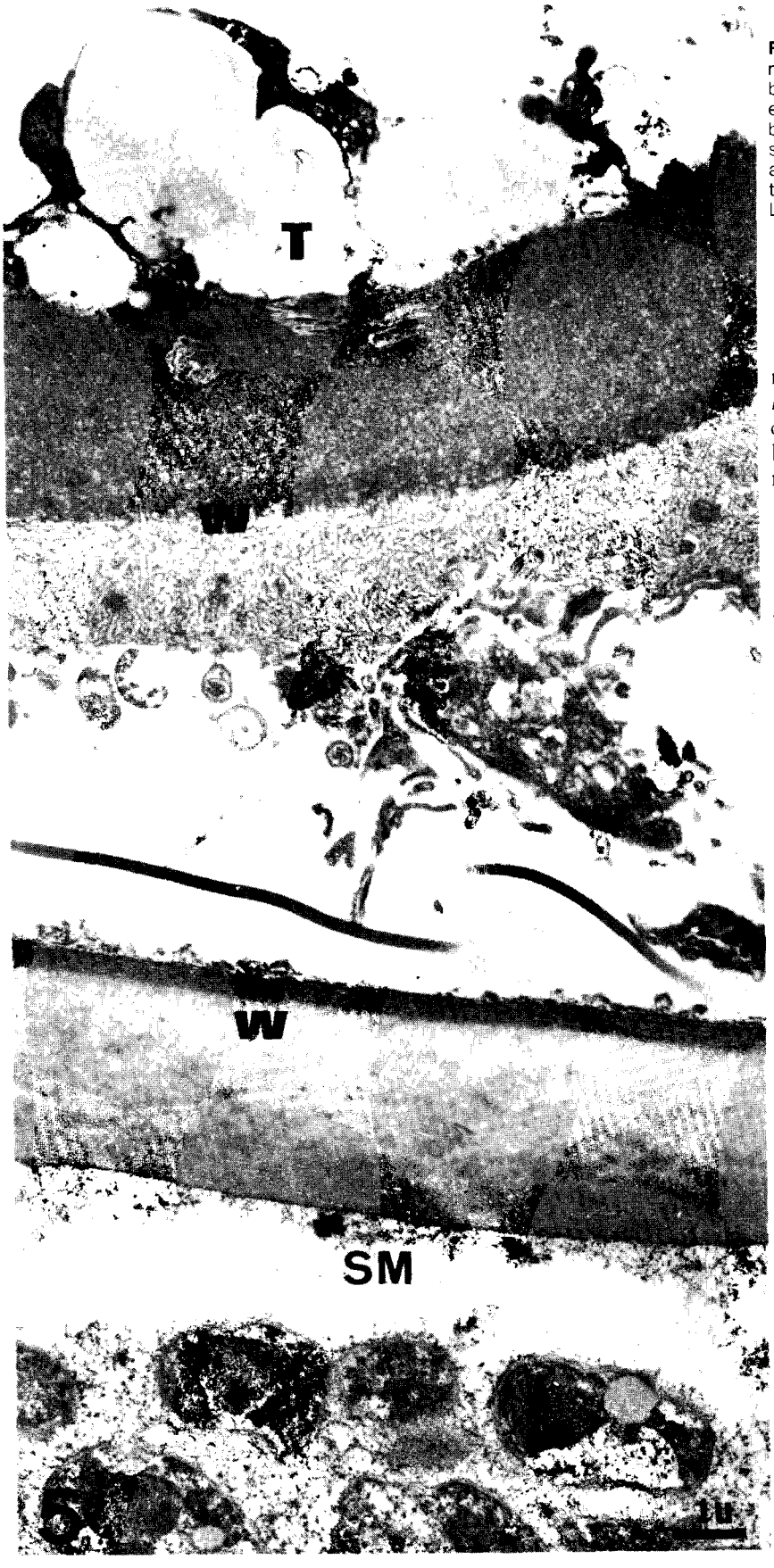


Fig. 5. Electron micrograph of trophocyte (T), and mature sporangium (SM). Note the differences between the structure of the wall (W) of the two elements. In trophocytes the inner zone of wall became wide with an electron-dense compact fibrillar structure. In mature sporangium the wall consists of an amorphous with moderate electron density. Note the decrease or absence of the filamentous zone. Lead citrate and uranyl acetate. × 11,750

Case No. 2

The conjunctival lesion exhibited a massive infestation of the stroma with *Rhinosporidium seeberi,* and showed occasional lymphocytic infiltration. The lesion lacked the marked inflammatory reaction observed in case No. 1, (Fig. 3b).

Electron Microscopy

The ultrastructural features of *Rhinosporidium seeberi* in cases No. 1 and No. 2 were similar. The structure of the wall of the sporoblast varied according to the stage of maturation. In the early stage the wall was not well defined and consisted of a single wide layer of fine filaments appearing as a lattice-work of microfibrillae. The innermost part of the microfibrillar layer condensed to form a membrane-like structure which became multilaminated by the time of maturation (Figs. 4a, b). The plasma membrane fused with the innermost layer of the membranous structure forming the limiting membrane (Fig. 4b). In the trophocyte this inner multilaminated membranous structure blended together to become a wide, compact, electron-dense fibrillar material. The outer filamentous zone of the wall decreased in thickness and the microfibrillae became coarse in comparison to the earlier stages (Fig. 5). In mature sporangia the inner part of the wall became amorphous and exhibited moderate electron density, whilst the outer filamentous zone became extremely narrow or absent in some places (Fig. 5). The sporangia contained sporoblasts at various stages of maturation. At the periphery, they were immature and closely packed, but towards the centre sporoblasts were mature and isolated with well defined cellular organelles (Figs. 2, 4, 5). The ruptured sporangia released spores into the conjunctival stroma and the life cycle of the organism continued (Figs. 2, 6).

In case No. 2, the stroma surrounding the fungi contained cells with vacuolated cytoplasm. The nuclei and the cytoplasm of

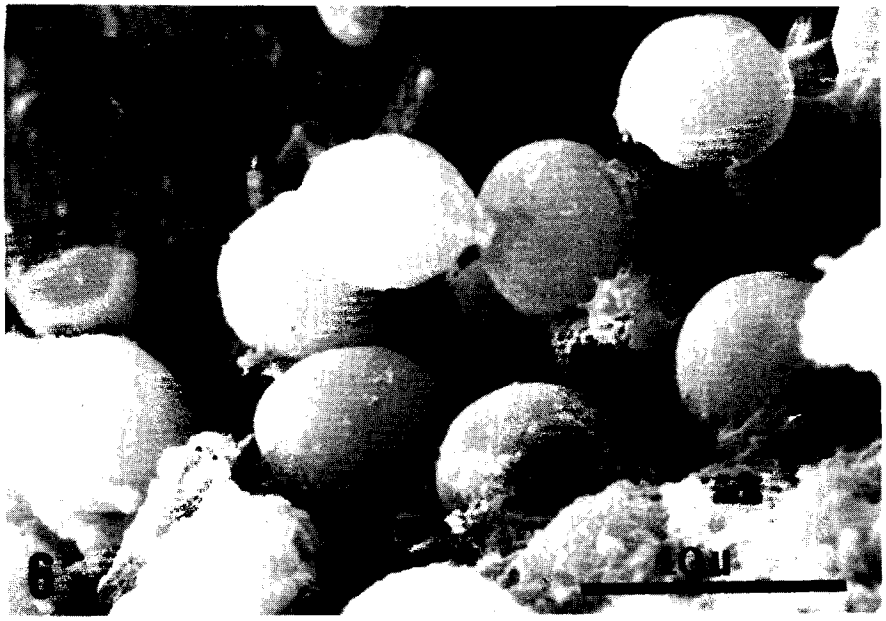


Fig. 6. Scanning micrograph of case No. 2 showing released spores. Note the scarceness of inflammatory cells. × 3,524

these cells exhibited dense particles, hexagonal in shape, arranged in crystalloid clusters, which measured about 40-52 nm in diameter. The morphology and the measurement of these dense particles were identical to those of papillomavirus (Figs. 7a,b).

Discussion

Rhinosporidiosis is a fungus infection predominantly affecting the mucosal membrane of the nasal passages. However, there have been a few reports describing the disease in the conjunctiva (Satyanarayana, 1960; Sood and Rao, 1967; Neumayr, 1977; Prevost et al., 1980; Savino and Margo, 1983). This disease is endemic in India, Sri Lanka, East Africa and parts of America (Vonhaccke and Mugliston, 1982). In the Arabian Gulf region, rhinosporidiosis has been reported in Iran, Bahrain and recently in Kuwait (Firouz-Abadi et al., 1971; Alhilli, 1985; Matusik et al., 1986). The present cases and those reported elsewhere in the region were all male expatriate workers from the Indian subcontinent.

In the present study the electron microscopic observation of different phases of the life cycle has provided additional knowledge of the development of the wall of the organism. It has been reported that the early developed sporoblast was surrounded by a lattice-work of microfibrillae with no obvious cell wall, while the fully formed cell wall consisted of a distinct limiting membrane and a thick fibrillar envelope (Kannan-Kutty and Teh, 1974; Naghasfar et al., 1986). On the contrary, our study showed clearly that the innermost layer of the microfibrillar envelope of the early stage had condensed together «probably due to expansion of the sporoblasts» (Kannan-Kutty and Teh, 1974) to form a membrane-like structure, which became multilaminated by the time of maturation.

In the trophocyte this multilaminated membranous structure subsequently fused together to form a single electron dense layer of a compact fibrillar material. The fibrillar structure of this layer changed gradually to become a morphously homogeneous material in the mature sporangium. The outer filamentous layer became extremely thin or absent at this stage. The current observation on the wall formation is probably a modification in the maturation stages occurring as an environmental protection carried out by the fungus against the presence of the virus. The ultrastructural findings of the present study coincided with the histochemical results which indicated that the chemical composition of the wall changed with cytological maturation i.e. acidophilic in the early stages and basophilic in the later stages. Thus the development of the wall of *Rhinosporidium seeberi* constitutes a continuous morphological and biochemical developmental spectrum throughout the stages of maturation; wide and filamentous initially and narrow and homogeneous at the end with various degrees of condensation in between.

The route of infection with *Rhinosporidium seeberi* was most probably exogeneous in the present cases because both patients were free of any other lesion due to this organism. However, the papilloma virus in the second case had probably reached the conjunctiva by contamination from the warts present on the patient's skin. Indeed, this latter virus usually infects the conjunctiva secondarily via lesions in the eyelid and elsewhere (Naseman, 1977; Robinson and Heath, 1983; Naghasfar et al., 1986). Electron microscopic examination of both the lesion on the conjunctiva and that on the skin demonstrated the presence of morphologically identical viruses, which supported the conclusion that the conjunctival infection was secondary to the skin lesion.

Histopathological studies on *Rhinosporidium seeberi* infections reported by other workers as well as that of the first case in the present study showed a diffuse inflammatory cell infiltration of the stroma about these organisms in the form of a chronic inflammatory reaction (Sood and Rao, 1967; Alhilli, 1985; Matusik et al., 1986). The second case associated with papillomavirus infection showed very few inflammatory cells. An additional, interesting observation in this latter case was the massive infestation of the conjunctival stroma by the fungus at different stages of maturation. It has been reported that viral infection can result in a decreased immune response through the selective activation of human suppressor T-cells (Reinherz et al., 1980). Immunosuppression by papillomavirus is not certain, but it is tempting to postulate that the absence of an inflammatory reaction in

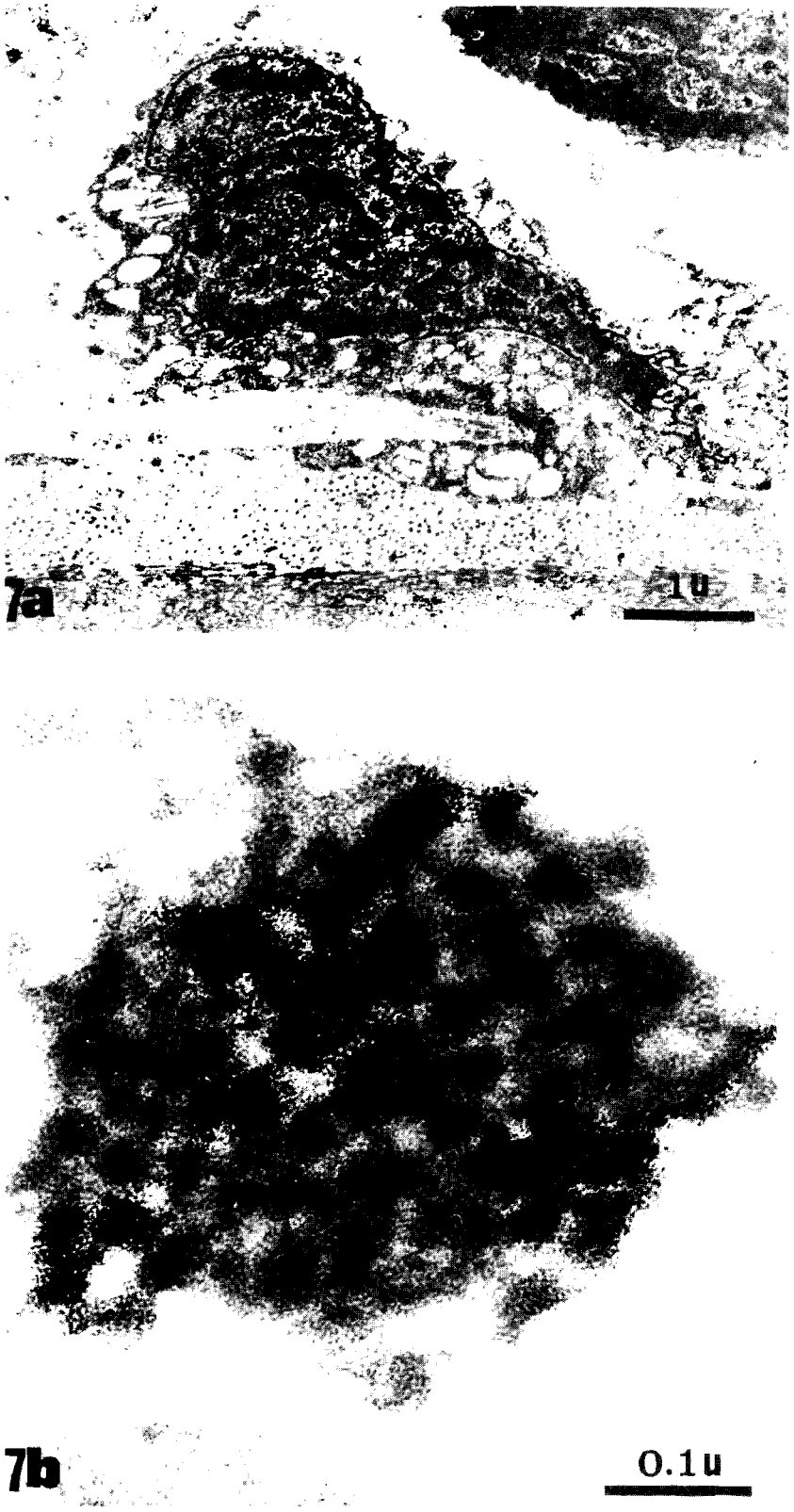


Fig. 7. (a) Electron micrograph of case No. 2 showing a cell abutting at a trophocyte wall. The cell exhibits vacuolate cytoplasm and multiple nuclear inclusions. Lead citrate and uranyl acetate. × 18,200 **(b)** Higher magnification of the nuclear inclusion, showing dense particles of typical papillomavirus. Lead citrate and uranyl acetate. × 207,500

the second case with concurrent viral infection might possibly be due to a localised immune deficiency state caused by some immunosuppression mechanism involving this virus. It is equally possible that the absence of an inflammatory reaction in this case might be due to the fact that, in viral papillomas, the majority of virus-infected host cells are in a non-productive state i.e. containing only multiple copies of viral DNA in an unintegrated form (Shah, 1985). The antigenicity of these cells would therefore be weak an incapable of stimulating the immune system to induce inflammation (Shah, 1985). Similarly, the wide-spread *Rhinosporidium seeberi* infection and the relatively early and frequent recurrences after two complete excisions could be interpreted on the same basis of a weak immune response to this infection prior to and following surgical intervention.

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