

Interaction of *Aspergillus* with Human Respiratory Mucosa

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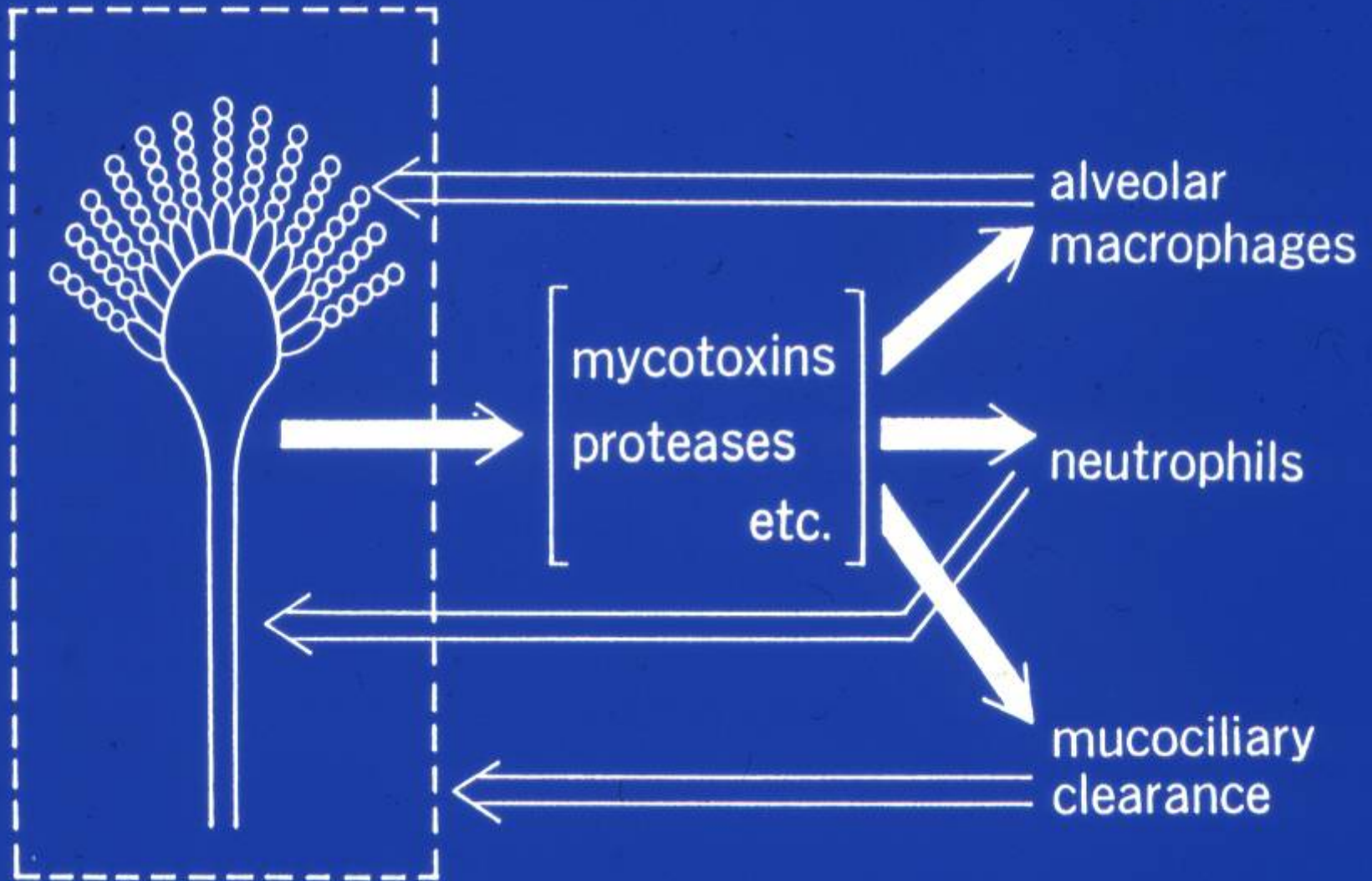
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BACKGROUND

Aspergillus species, most commonly *A.fumigatus* are exogenous fungi and can colonize the airway mucosa in patients with localized underlying bronchopulmonary disorders such as healed tuberculous cavities, bronchiectasis and cystic fibrosis, and occasionally invade the airway mucosa without apparent systemic immunocompromised conditions.

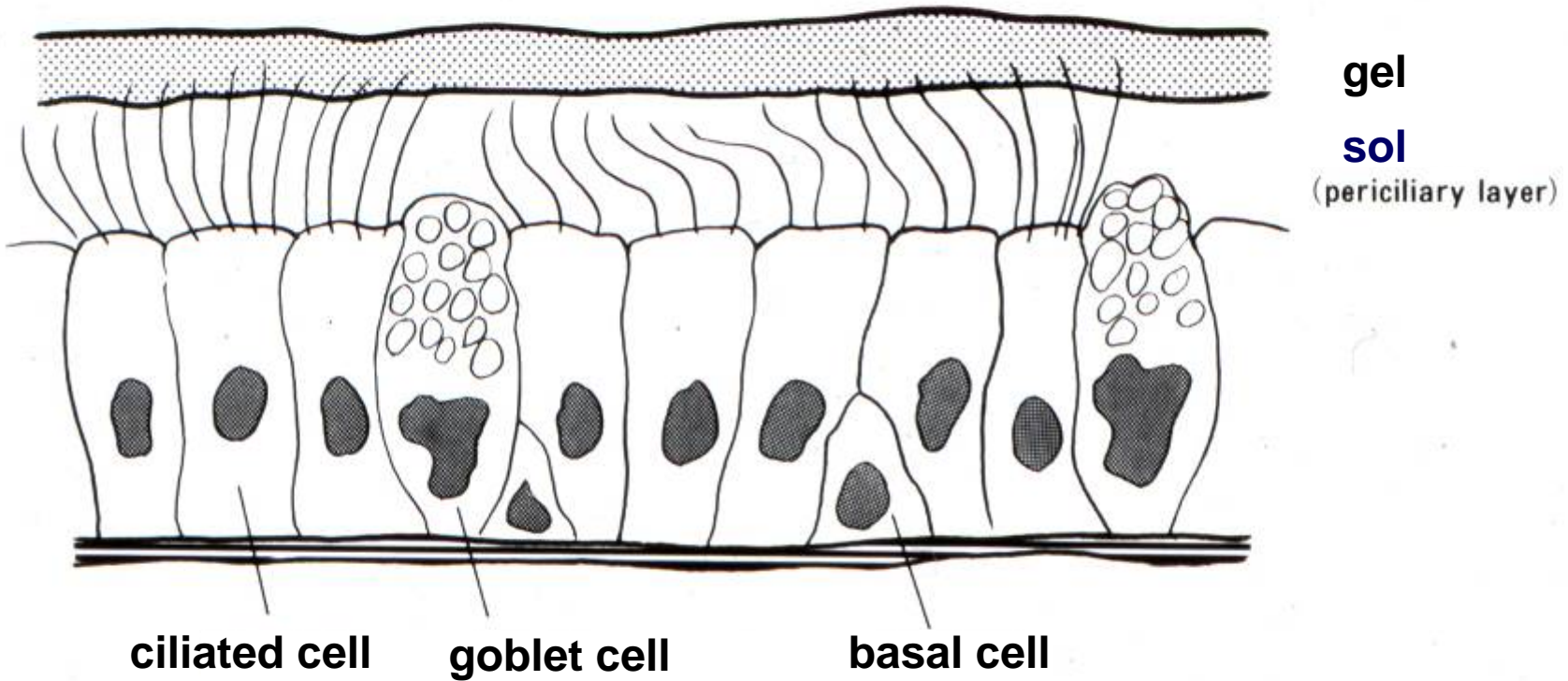
However, the mechanisms of colonization and invasion of airway mucosa, especially the initial step of interaction of *Aspergillus* with airway mucosa after inhalation of conidia through airways are still poorly understood.

[Host-Aspergillus Interaction]



Mucociliary clearance in respiratory tract

larynx ←



PURPOSE

We have morphologically investigated interactions of *A.fumigatus* with human bronchial mucosa in an organ culture model with an air-mucosal interface, in order to elucidate the initial step of invasion of *Aspergillus* conidia (and hyphae) in human bronchial mucosa.

METHODS

- 1) Organ culture model of human bronchial mucosa with an air-mucosal interface was prepared with a modification of a previously reported method.
- 2) *A.fumigatus* conidia were inoculated onto the organ culture tissues, and incubated for up to 24h at 37°C.
- 3) At each timepoint (1, 6, 12, 18 and 24h), adherence and invasion of *A.fumigatus* conidia (and hyphae) in the bronchial epithelium as well as structural changes of the epithelium were investigated by scanning and transmission electron microscopy.



Organ Culture Model of Human Bronchial Mucosa (1)

Organ cultures of human bronchial mucosal tissue were prepared with a modification of a previously reported method

(R. Wilson et al., Am. J. Respir. Crit. Care Med. 153:1130, 1996)

- 1) Bronchial tissues were obtained from proximal bronchi of resected lungs of patients with lung cancer and placed in tissue culture medium MEM containing antibiotics (penicillin, streptomycin and gentamicin).

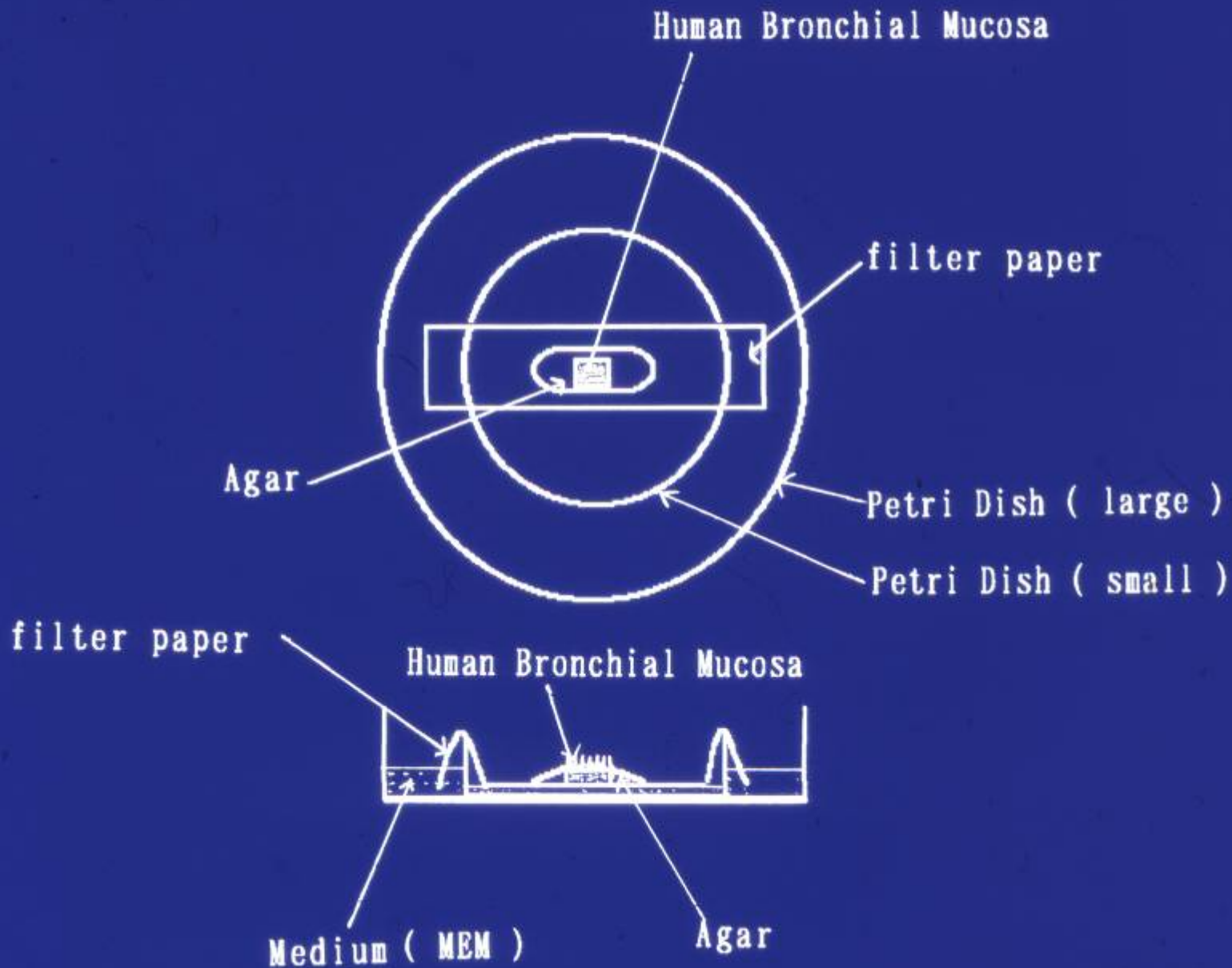
Organ Culture Model of Human Bronchial Mucosa (2)

- 2) Each tissue was checked by light microscopy, and a tissue with a smooth surface and actively beating cilia was selected.
- 3) The tissue was then dissected into 6mm×6mm squares with a thickness of 2-3mm, immersed in MEM with the antibiotics for at least 4h to eradicate bacteria, and then placed in antibiotic-free MEM for 1h.
- 4) To prepare the organ culture system, a 3.5 cm-diameter Petri dish was placed within a 6 cm-diameter Petri dish aseptically. MEM without antibiotics was added into the outer large Petri dish. A strip of sterile filter paper was immersed in MEM and then laid across the inner Petri dish, with its ends immersed in MEM in the outer large Petri dish.

Organ Culture Model of Human Bronchial Mucosa (3)

- 5) A single square of bronchial mucosal tissue was placed with its ciliated surface upwards onto the filter paper strip in the inner Petri dish.
- 6) Semi-molten 1% agar at 40°C was carefully pipetted around the edges of the tissue in order to seal all the cut edges.
- 7) The organ culture tissues were then incubated for at least 1h in a humidified atmosphere containing 5%CO₂ at 37°C before experiments.

Organ Culture System of Human Bronchial Mucosa





Inoculation and Incubation of *A.fumigatus*

- 1) A clinical isolate of *A.fumigatus* from a patient with invasive pulmonary aspergillosis was grown on PDA slants at 37°C for five days.
- 2) Conidia were harvested in distilled water containing 0.1% Tween 80, washed and resuspended in MEM at an appropriate concentration (1×10^7 /ml)
- 3) Two organ cultures were prepared for each experiment. One organ culture was inoculated with 30µl of MEM containing *A.fumigatus* conidia, and another was inoculated with 30µl of MEM without conidia (control).
- 4) The organ cultures were incubated for 1, 6, 12, 18 or 24h in a humidified atmosphere containing 5% CO₂ at 37°C.

Light Microscopical Assessment of Bronchial Epithelium

- 1) At each timepoint, the organ culture tissues were removed from the filter paper, and placed in Petri dishes containing MEM.
- 2) The bronchial epithelium with beating cilia along the edge of the tissue was viewed directly by light microscopy($\times 400$ magnification), and the epithelial integrity was assessed by the investigators who were unaware of the origin of the organ culture tissues.
- 3) Ciliary beat frequency (CBF) was measured by a photometric technique, and was calculated as the mean of 10 separate areas of beating cilia.
- 4) The mean CBF value of infected organ culture tissue was compared with that of uninfected organ culture tissue (control) at each timepoint.

Electron Microscopical Examinations

- 1) At each timepoint, after light microscopical assessment, each organ culture tissue was divided into two pieces : one for scanning electron microscopy (SEM), the other for transmission electron microscopy (TEM).
- 2) The organ culture tissues for SEM and TEM were processed by conventional procedures. Specimens were coded so that the observers were unaware of their origin including incubation time, and were examined by SEM and TEM.
- 3) Electron microscopical studies were performed from the viewpoints of respiratory epithelial damage and interaction between *A.fumigatus* conidia (or hyphae) and bronchial ciliated epithelium.

RESULTS



Light Microscopical Assessment & CBF Measurement

- 1) At 1 to 12h, no significant difference in CBF (infected vs control)
- 2) At 18, 24h, CBF of infected tissue was significantly lower (vs control)
- 3) Epithelial disruption occurred at 12h, the degree of disruption gradually increased throughout the 24h-experiment in *Aspergillus* infected tissues; (undulation → extrusion → detachment of epithelial cells).
- 4) In uninfected organ culture tissues (control), no epithelial disruption throughout the 24h-experiment.

SEM Findings (1)

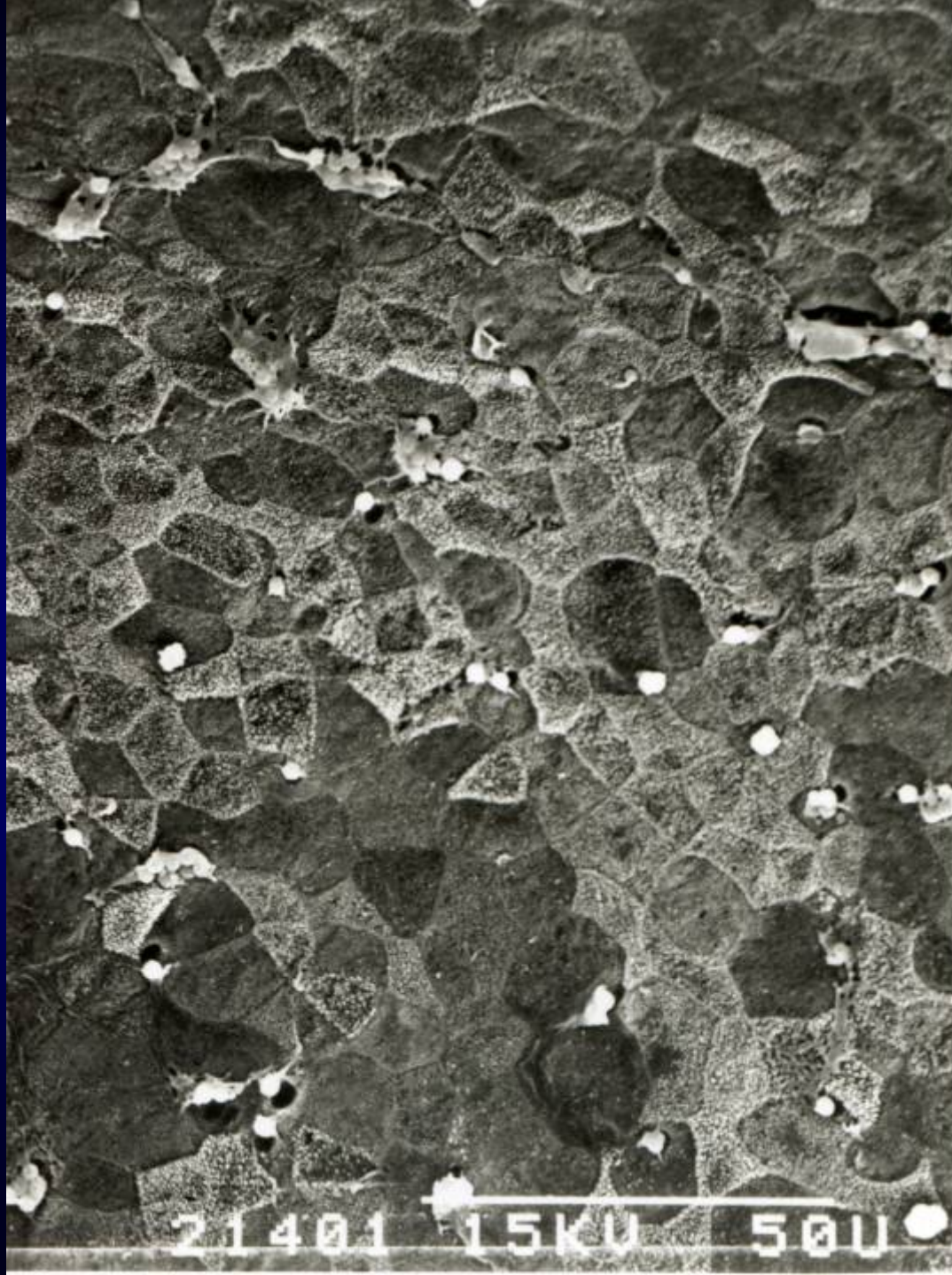
- 1) At 1 and 6h, Conidia predominantly adhered to damaged epithelial cells and mucus, with a smaller amount of conidia adhered to unciliated (not damaged) cells and ciliated cells.
- 2) At 6h, conidia were observed in indentations like craters on the surface of the damaged cells and unciliated cells.
- 3) At 6h, about one third of conidia attached to the mucosa germinated.
- 4) At 12h, mild epithelial damage (undulation of the epithelial surface and loss of cilia) occurred in a restricted area, most conidia became germinated conidia or hyphae.

SEM Findings (2)

- 5) At 18h, SEM revealed disorganization of cilia, extrusion and detachment of ciliated epithelial cells, separation of intercellular junctions between epithelial cells, and marked hyphal growth in some areas of mucosal surface. Hyphae were seen in the gaps formed between epithelial cells, and a part of hyphae seemed to directly enter the epithelial cells.
- 6) At 24h, epithelial damage and hyphal growth became more remarkable,
- 7) In uninfected organ culture tissues (control), SEM revealed almost no epithelial damage throughout the 24h-experiment.



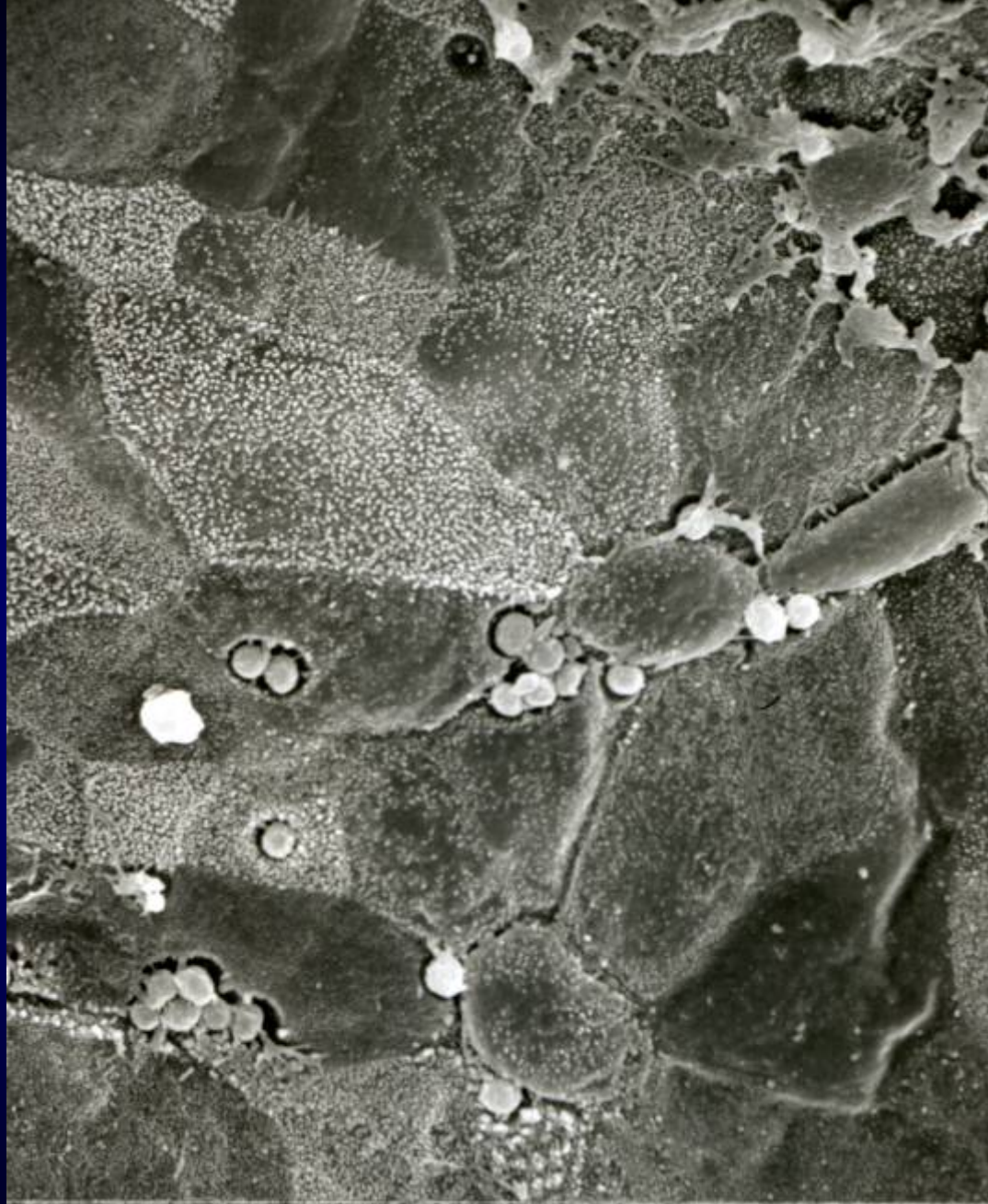






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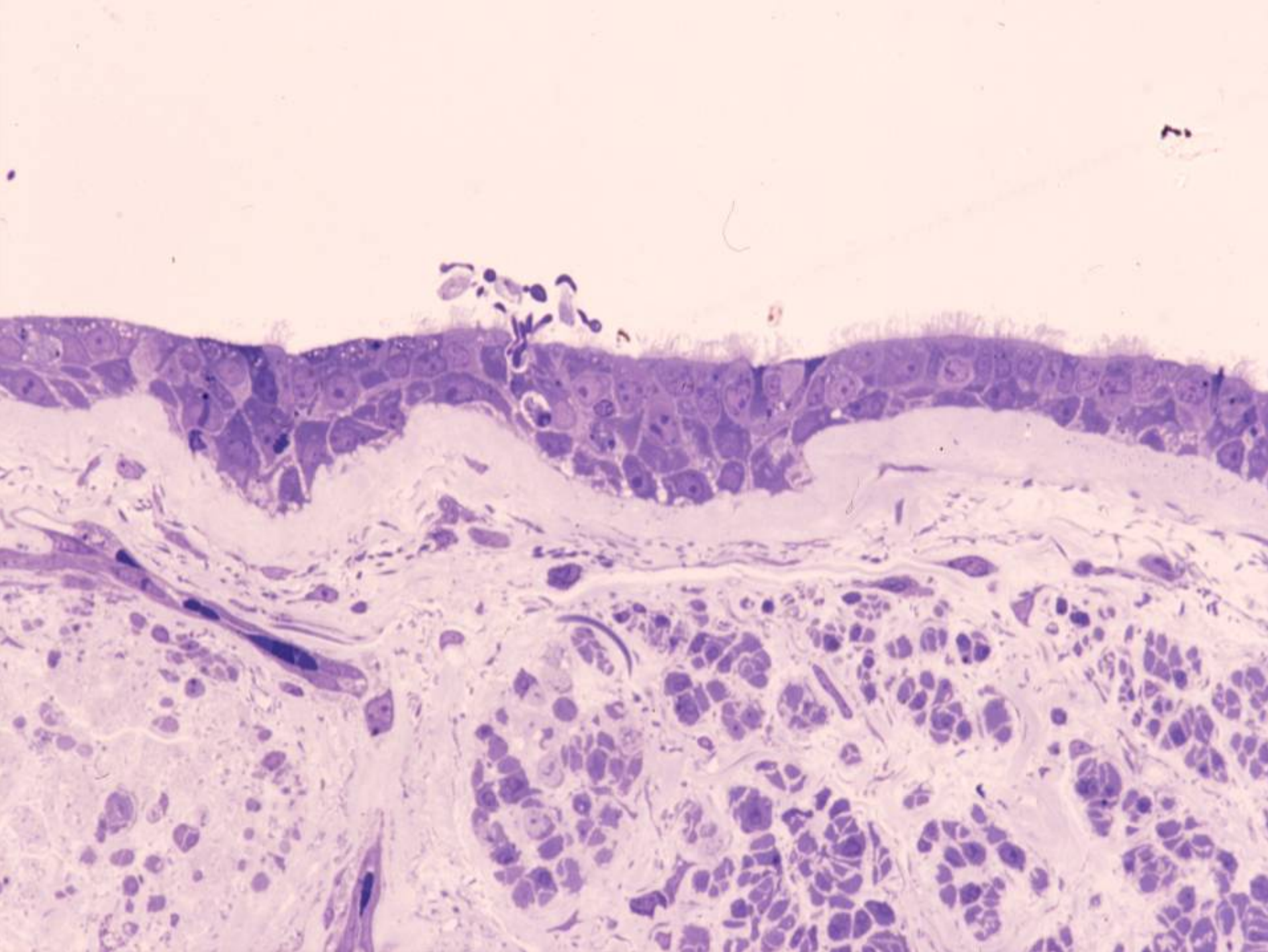
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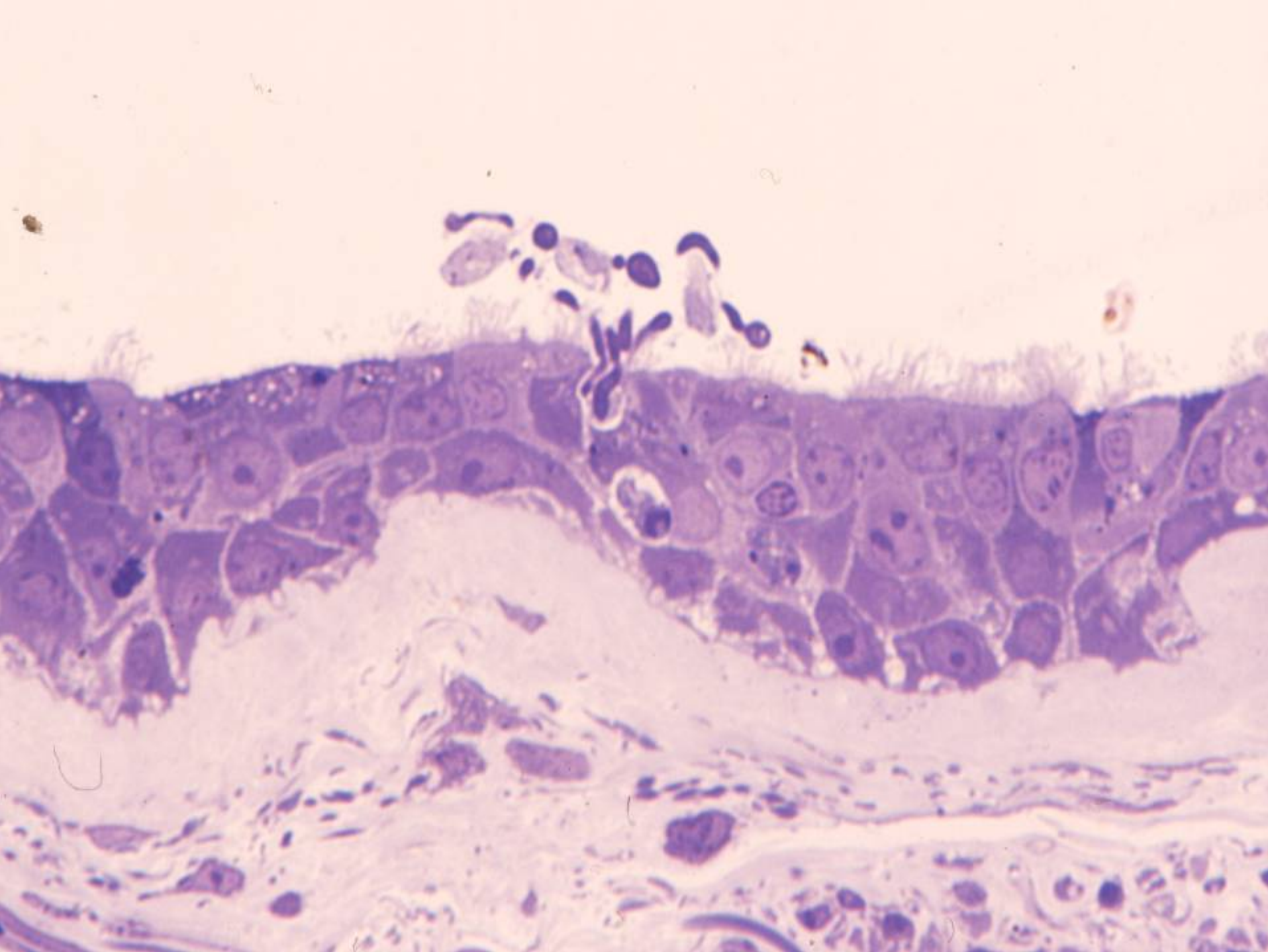
TEM Findings (1)

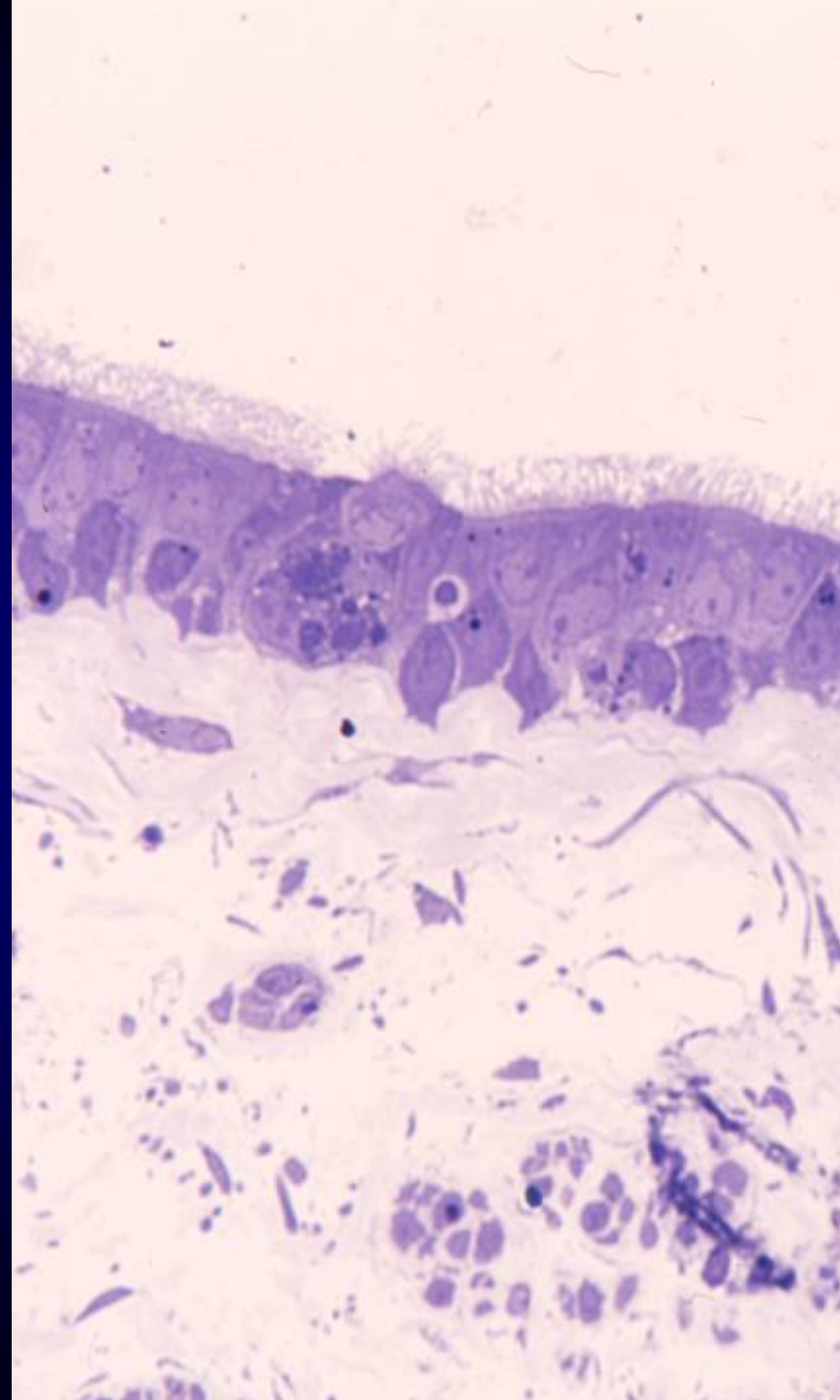
- 1) In the infected organ culture tissues, almost normal ultrastructure of bronchial epithelium was revealed at 1 and 6h.
- 2) At 12 and 18h, epithelial damage including loss of cilia, extrusion and detachment of epithelial cells were demonstrated.
- 3) At 24h, more remarkable epithelial disintegration was demonstrated in most mucosal surface area.
- 4) In the uninfected organ culture (control), almost normal ultrastructure of the epithelium was revealed throughout the 24h-experiment

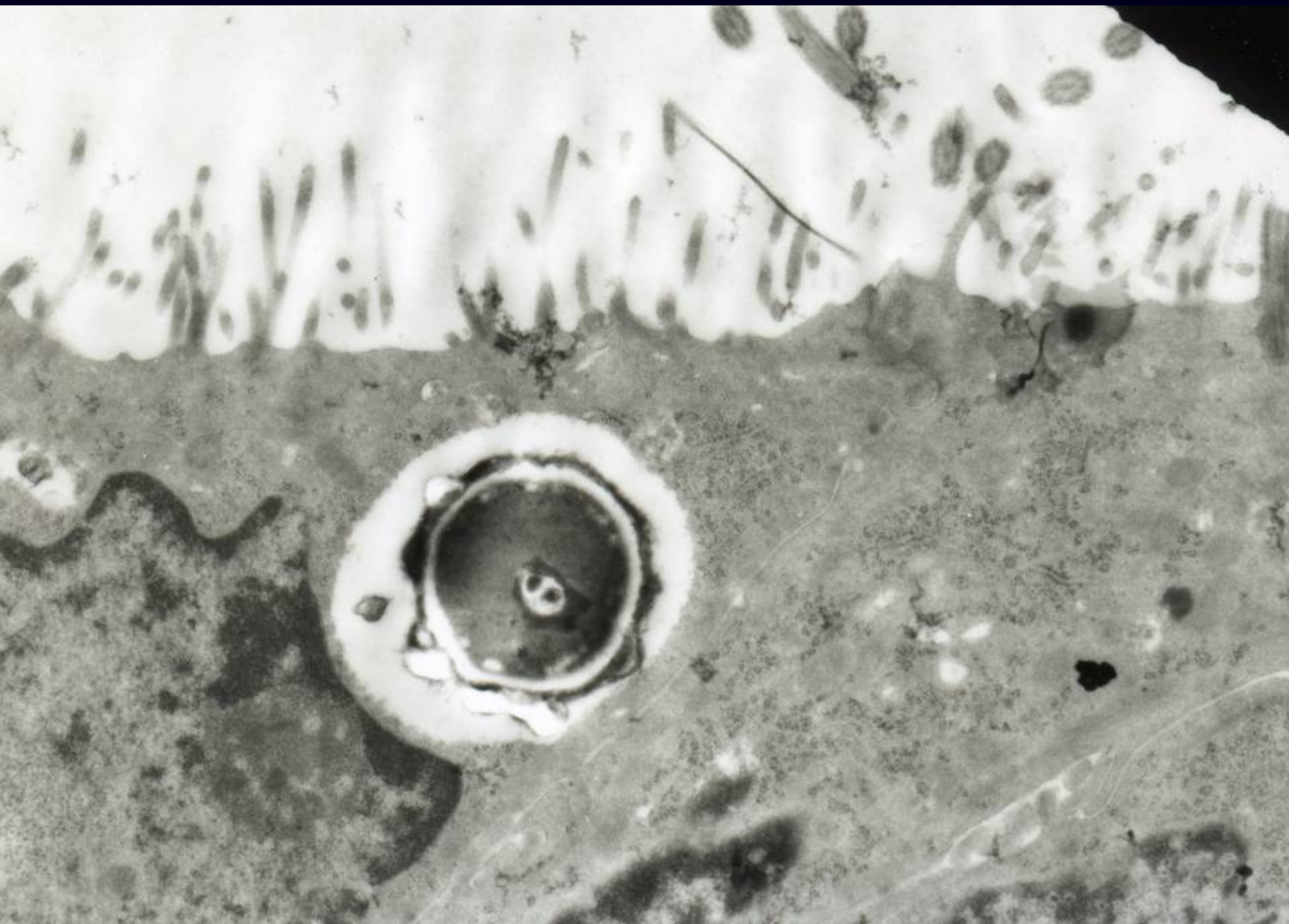
TEM Findings (2)

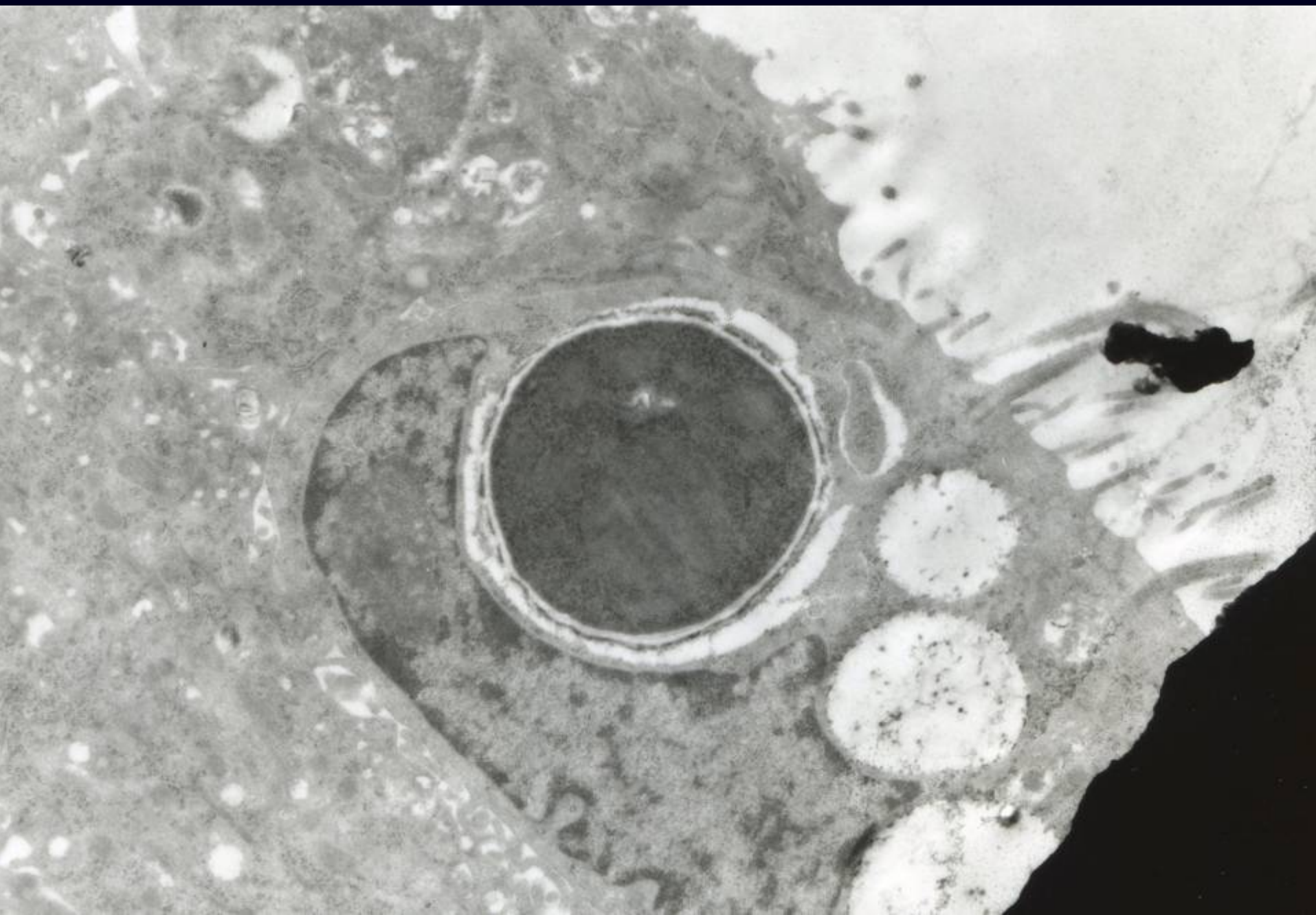
- 5) At 1h, conidia were observed closely associated with the mucosal surface, predominantly adhering to damaged epithelial cells and mucus, and occasionally adhering to cilia.
- 6) At 6h, intracellular conidia were occasionally observed in both ciliated and unciliated epithelial cells. Most intracellular conidia were not enclosed in membrane-bound vacuoles, and appeared to be free within the cytoplasm of the epithelial cells. Intercellular conidia were also seen within the epithelium associated with dilatations of intercellular space.
- 7) At 18 and 24h, *Aspergillus* hyphae were seen directly penetrating ciliated cells, and some hyphae were seen in the intercellular space.



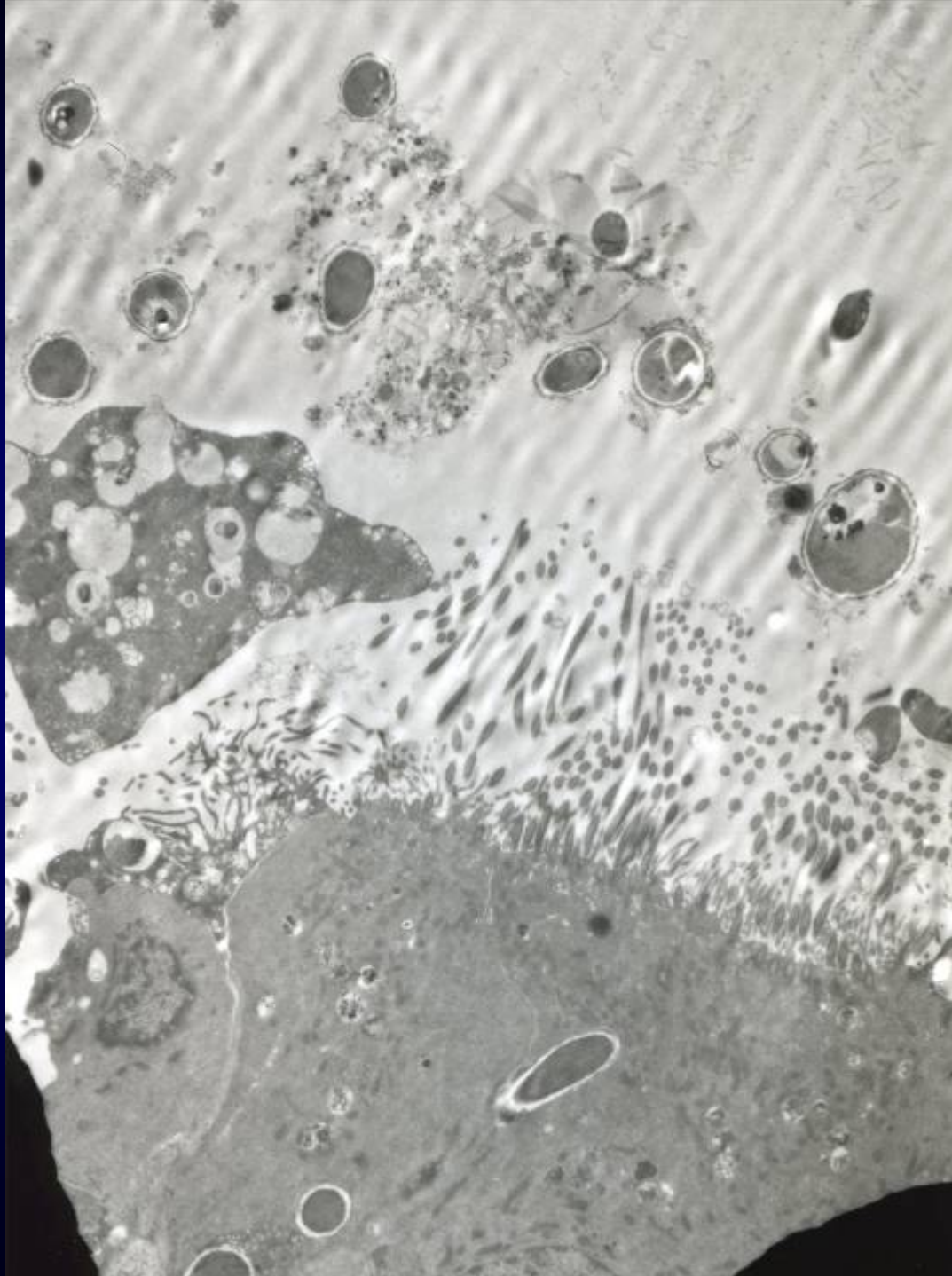


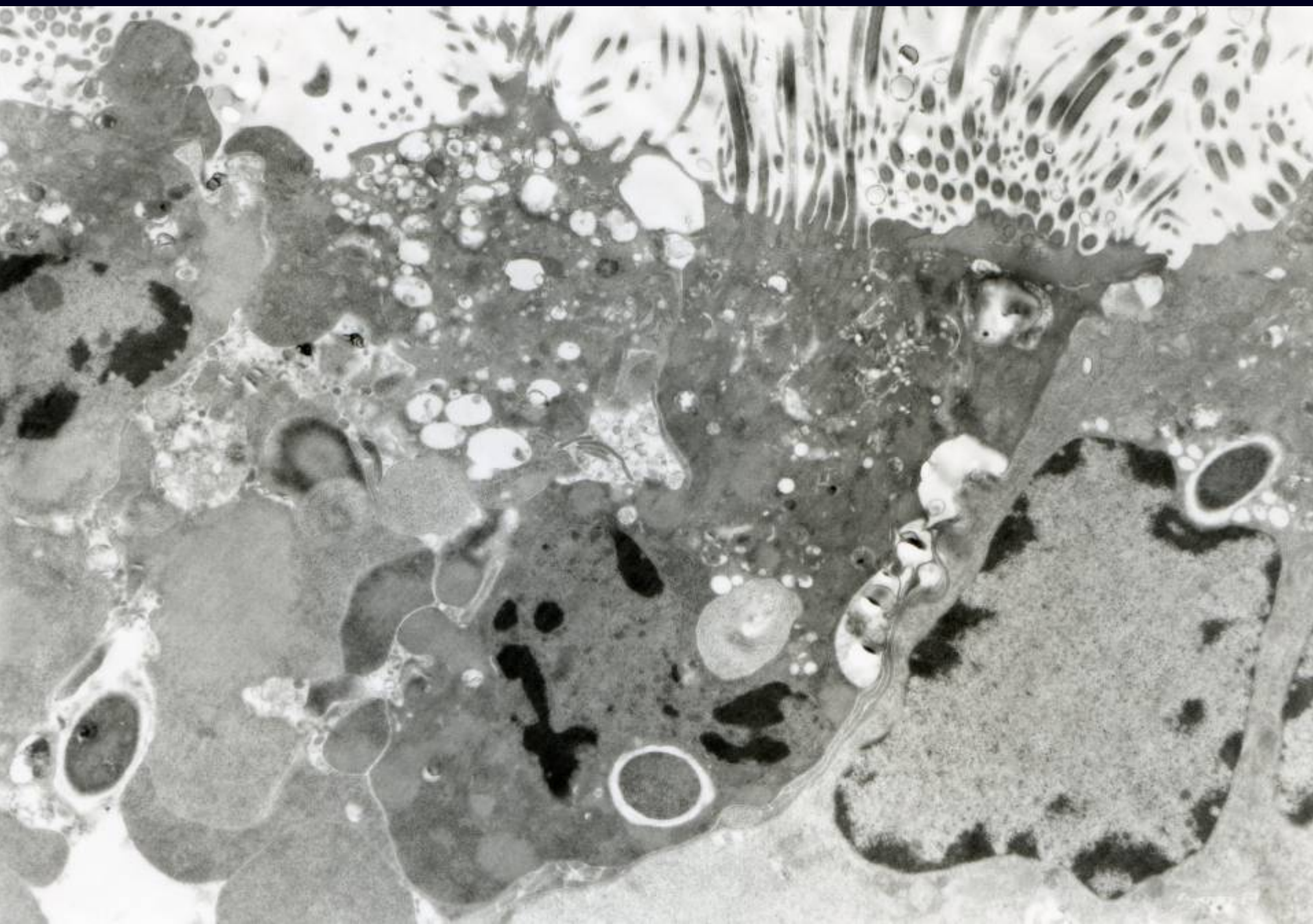


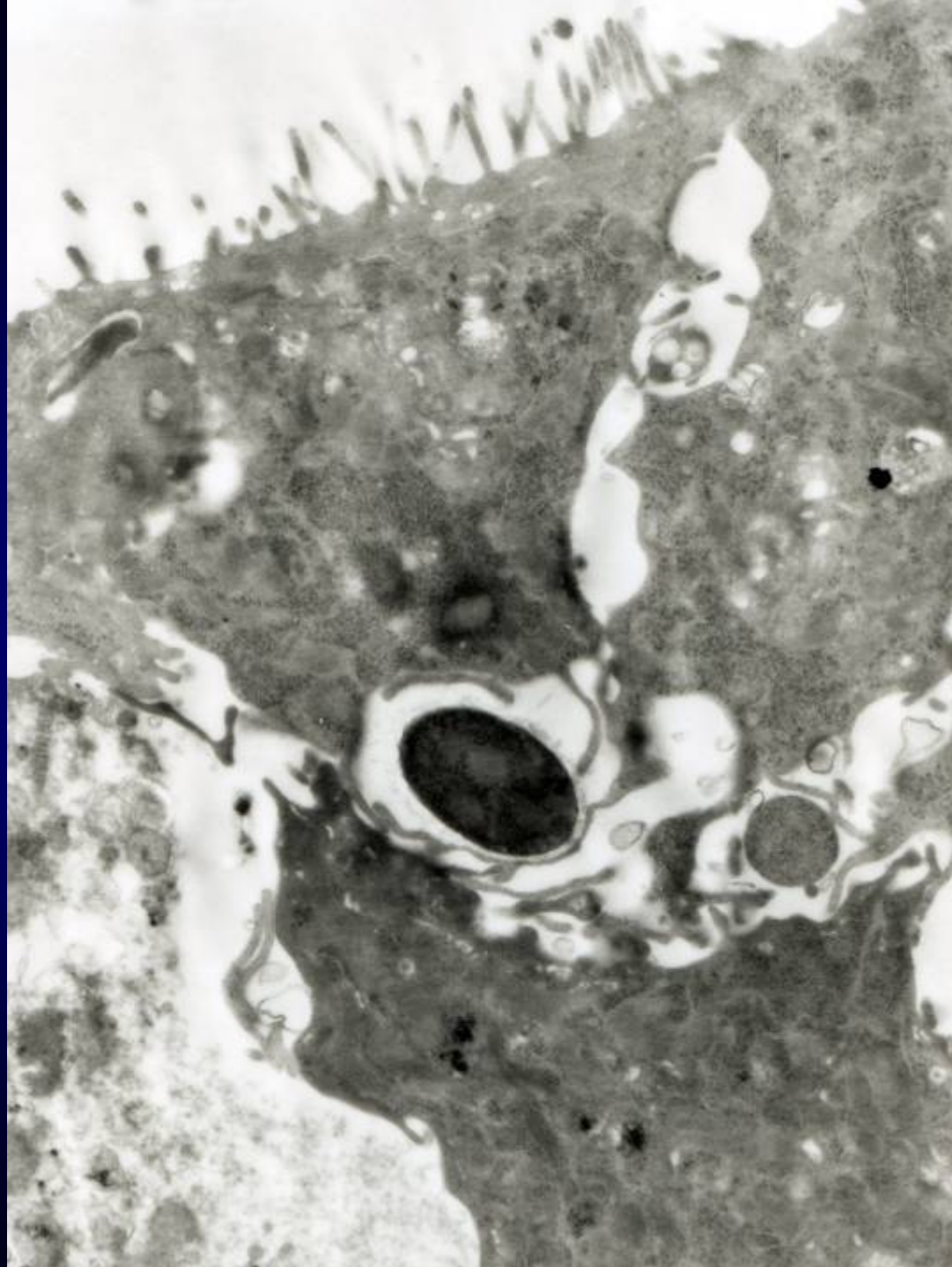


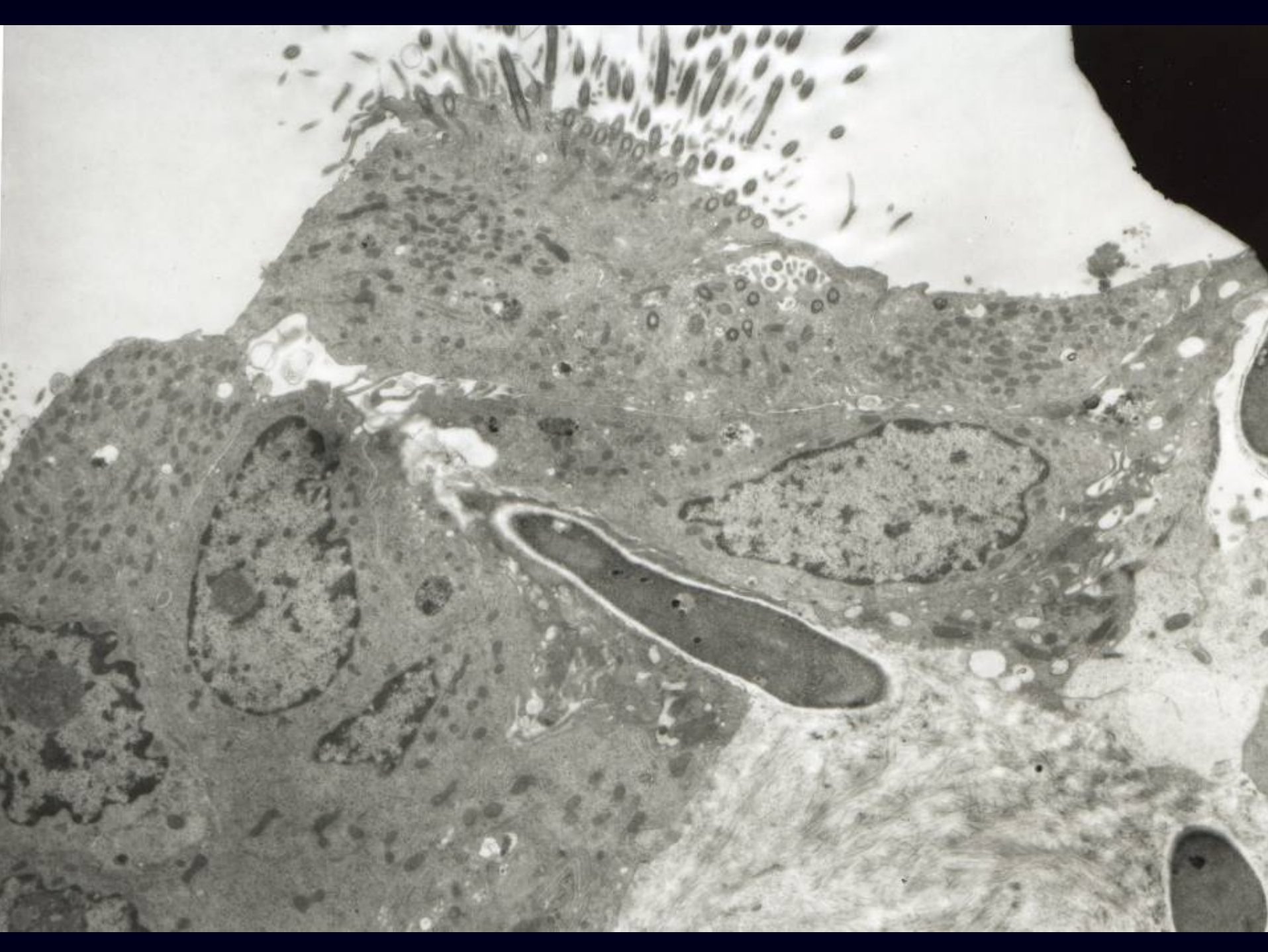


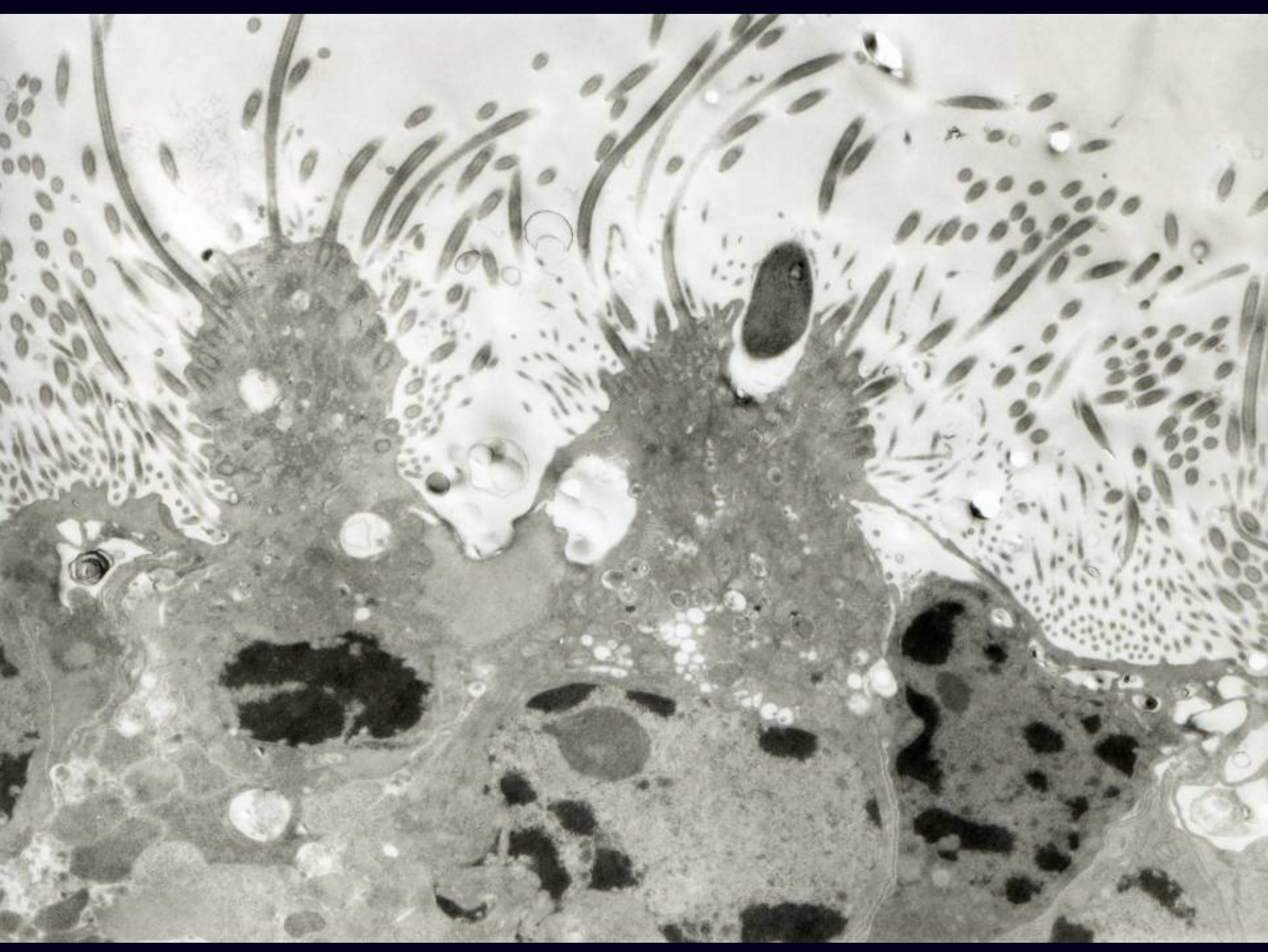


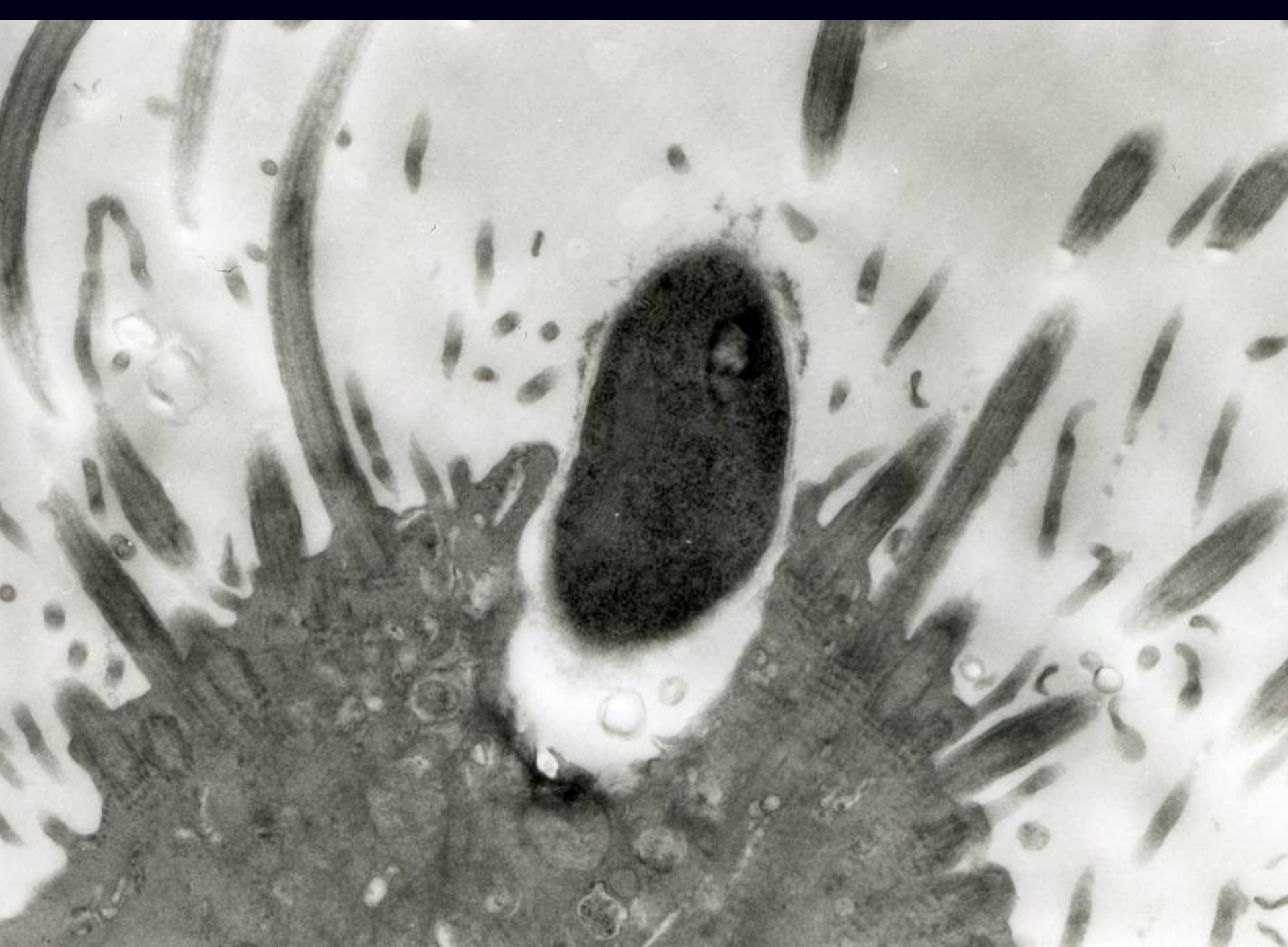


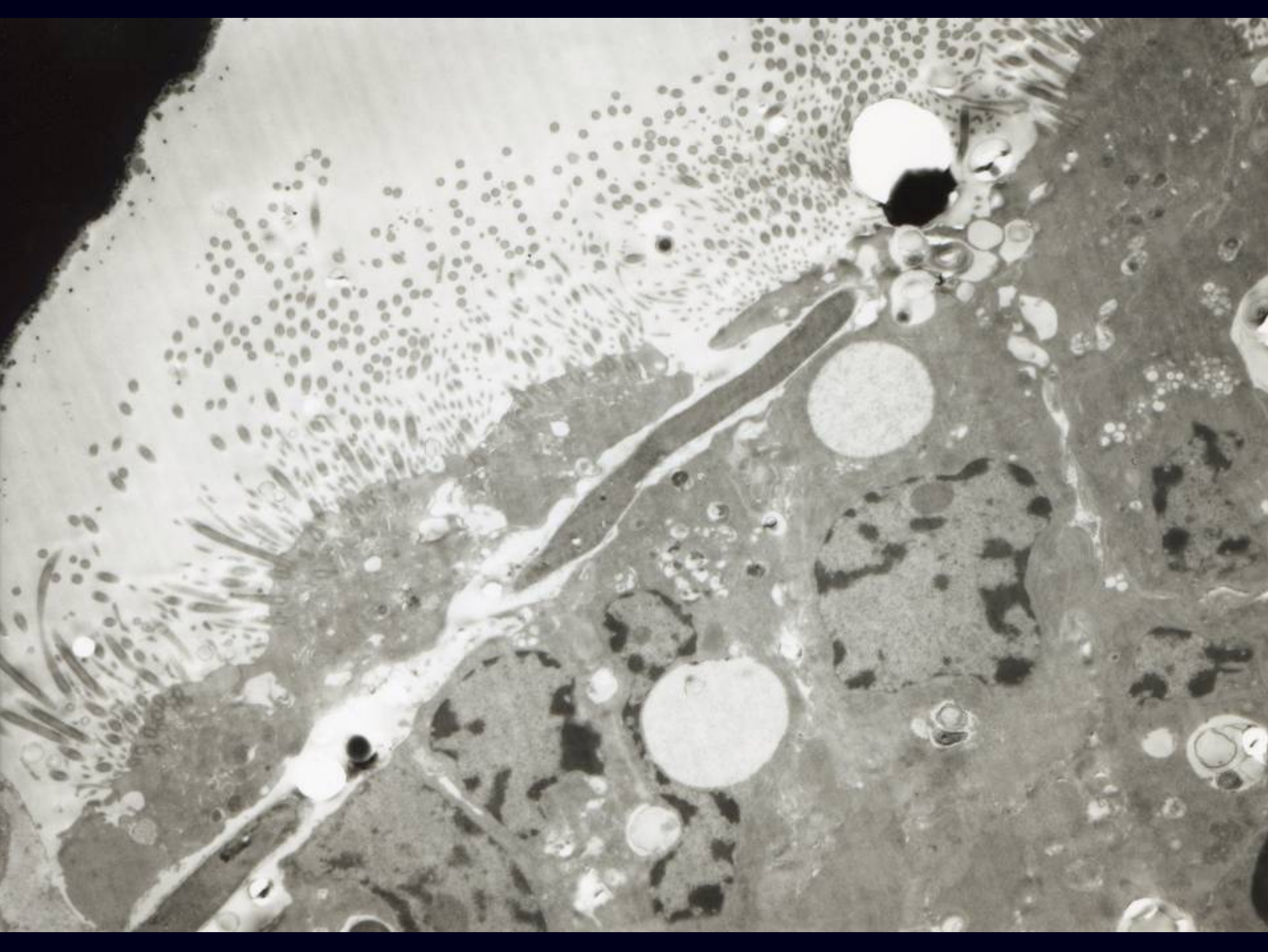












CONCLUSION and DISCUSSION (1)

- 1) We demonstrated serial morphological changes of both human bronchial epithelium and *A.fumigatus* conidia (and hyphae) using an organ culture model of human bronchial tissue with an air-mucosal interface.
- 2) *A.fumigatus* caused damage to human respiratory mucosa associated with slowing of ciliary beat frequency.
- 3) A part of *A.fumigatus* conidia were internalized within ciliated and non-ciliated epithelial cells, and hyphae penetrated through both intercellular and intracellular space of the bronchial epithelium.

CONCLUSION and DISCUSSION (2)

4) These findings suggest that there might be at least three pathways by which *Aspergillus* invades the bronchial mucosa : (1) penetration of germinated conidia (or hyphae) through the intercellular space of ciliated epithelium, (2) direct penetration of hyphae through the epithelial cells, and (3) internalization of conidia within epithelial cells (although the fate of the internalized conidia is unknown).