

SCANNING ELECTRONMICROSCOPIC STUDIES OF *BORDETELLA BRONCHISEPTICA* ON THE RABBIT TRACHEAL MUCOSA

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PLATES VIII–XI

IN previous studies *Bordetella pertussis* was shown to be resistant to the mucociliary clearance mechanism of the rabbit trachea (Matsuyama, 1977). Scanning electron microscopy readily demonstrated *B. pertussis* organisms associated with clusters of the cilia of the epithelial cells. A similar association has been described in chick organ cultures (Iida and Ajiki, 1974, 1975) and hamster organ cultures (Collier, Peterson and Baseman, 1977; Muse, Collier and Baseman, 1977). *B. pertussis* is not a natural pathogen of the rabbit, chick or hamster; on the other hand, *Bordetella bronchiseptica* is a natural respiratory-tract pathogen of the rabbit and the dog (Wilson and Miles, 1975). Bemis, Greisen and Appel (1977) demonstrated experimentally the strong affinity of *B. bronchiseptica* for the canine respiratory tract; they also mentioned similarities between the respiratory infections produced by *B. pertussis* and *B. bronchiseptica*.

In the present study, scanning electron microscopy has been used to observe infections of the rabbit tracheal mucosa with *B. bronchiseptica*, and with *Staphylococcus aureus*—an organism that has no special predilection for the respiratory tract.

MATERIALS AND METHODS

Bacterial strains. *B. bronchiseptica* strain 5376 was supplied by Dr Y. Sato, National Institute of Health, Tokyo, Japan, and was grown either on Bordet-Gengou agar medium containing 20% defibrinated rabbit blood or in a liquid medium containing anion-exchange resin (Ku wajima, Matsui and Kishigami, 1957); it was a phase-1 organism (Nakase, 1957). *S. aureus* strain 209P was obtained from the culture collection of this department, and grown either on Heart Infusion Agar (Nissui, Tokyo) or in Penassay Broth (Difco, Detroit, Michigan, USA).

Tracheal cultures. Embedded rabbit tracheal cultures (ERTC) were prepared as described previously (Matsuyama, 1974). The L-15 Medium used (Grand Island Biological Co., Grand Island, New York, USA) was kept in a humidified environment in a sealed box. Tracheal preparations shown by sterility tests in Thioglycollate Medium (Nissui, Tokyo) to be contaminated with bacteria were discarded. Tracheal preparations were also made from rabbits that possessed serum containing *B. bronchiseptica* agglutinins; serum agglutinin levels were determined with the microtitre equipment described by Manclark (1976).

Point inoculation. Bacterial suspensions were "point inoculated" on to one-day ERTC with a platinum microloop as described by Matsuyama (1974). The infecting bacteria were traced by touching the mucous surface with a platinum coil and "stamping" an agar plate with the coil as described by Matsuyama (1974).

Scanning electron microscopy. Each explant was washed with 2 ml of saline, expelled through a 22-gauge needle at the rate of 0.6–0.8 ml/s to free the ciliated surfaces from mucus. Fixation and observation were as described previously (Matsuyama, 1977).

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RESULTS

B. bronchiseptica infection

Two days after inoculation with *B. bronchiseptica* strain 5376 the infected mucous membrane of the ERTC was observed with a scanning electron microscope. Unlike the uninoculated mucous membrane (fig. 1), the infected mucous membrane was partly denuded of cilia (fig. 2). Tufts of remaining cilia were entangled with large numbers of bacteria (fig. 2). Extrusions of epithelial cells carrying many bacteria among the cilia were frequently observed (fig. 3), but it was difficult to find bacteria in non-ciliated areas.

Sera from some rabbits contained *B. bronchiseptica* agglutinins at titres that did not exceed 128; these agglutinins presumably resulted from natural infection. Explants from rabbits with such agglutinins were infected with *B. bronchiseptica* and observed with a scanning electron microscope. The cilia were entangled with large numbers of bacteria; no differences were recognised between explants from rabbits with agglutinins and those without.

A second phase-1 strain (M1) of *B. bronchiseptica* was examined. This strain, from a guinea-pig, was supplied by Dr Y. Nakase, Kitasato Institute, Tokyo; its behaviour on rabbit tracheal mucosa was similar to that of strain 5376.

S. aureus infection

Two days after inoculation with *S. aureus*, the area of the mucous membrane showing bacterial growth by the "stamping method" was observed with a scanning electron microscope. Partial disappearance of cilia and extrusion of epithelial cells were easily recognised. However, it was extremely difficult to find the infecting bacteria on these damaged areas. Most of the remaining cilia were free from bacteria (fig. 4). *S. aureus* seemed to have no intimate contact with tufts of cilia of the extruding epithelial cells (fig. 5).

DISCUSSION

Infection of ERTC with *B. bronchiseptica*, like infection with *B. pertussis* (Matsuyama, 1977), resulted in intimate interactions between bacteria and ciliated epithelial cells. On the mucous membrane infected with *B. bronchiseptica* most of the remaining cilia were entangled with large numbers of bacteria. This was in clear contrast to the mucous membrane infected with *S. aureus*. It was extremely difficult to find *S. aureus* on the mucous membrane; even when infecting bacteria were seen, there were no intimate interactions between them and the mucous membrane. Most of the *S. aureus* cells were probably lost in the washing process that preceded the scanning electron microscopy; light microscopy showed that unwashed explants carried many *S. aureus* cells (Matsuyama, 1974).

Although the precise mechanism for the interaction between *B. bronchiseptica* and the beating cilia has not yet been elucidated, the phenomenon may be important in relation to pathogenesis. *B. bronchiseptica* infection usually started from the point-inoculated site on the ERTC, as in *B. pertussis* infection (Matsuyama, 1977). It seems possible that the interaction between cilia and organisms assists *B. bronchiseptica* in persisting in the respiratory tract. Recently, delayed clearance of *B. bronchiseptica* from the infected canine trachea was shown by a study of aerosol infection. *B. bronchiseptica* required 14 weeks to be cleared from the trachea of an infected dog; in contrast, *S. aureus* and other organisms were cleared in 1-3 days (Bemis *et al.*, 1977).

Organ cultures in liquid medium have been used to study specific interactions between micro-organisms and epithelial cells; such studies have been confined to infections with viruses, mycoplasmas, and a few species of bacteria lacking the ability to grow in the medium itself. ERTC and the point-inoculation method have made possible the study of infections of the mucous membrane with several species of bacteria.

B. BRONCHISEPTICA ON THE TRACHEAL MUCOSA

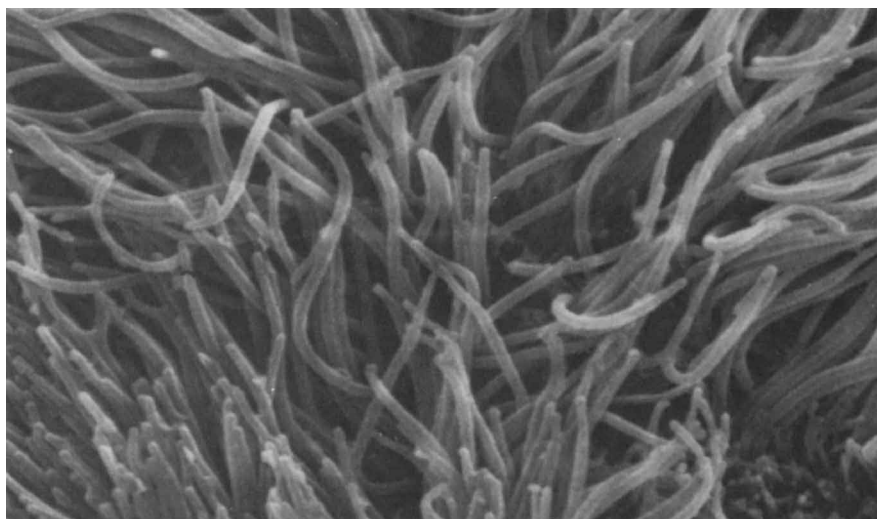


FIG. 1.—Mucous membrane of an uninfected rabbit tracheal explant. Scanning electron micrograph (SEM). $\times 9300$.

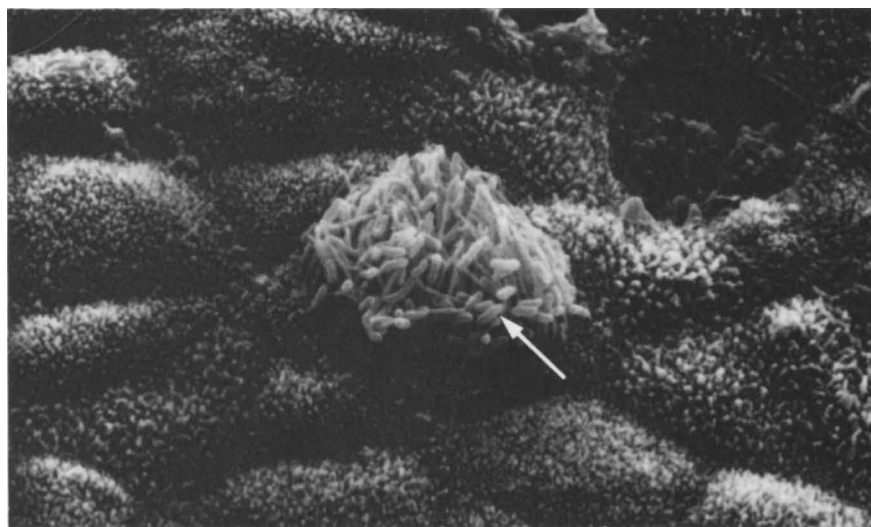


FIG. 2.—Mucous membrane of a rabbit tracheal explant, 2 days after inoculation with *Bordetella bronchiseptica*. Cilia are entangled with infecting bacteria (arrow). SEM. $\times 3300$.

B. BRONCHISEPTICA ON THE TRACHEAL MUCOSA

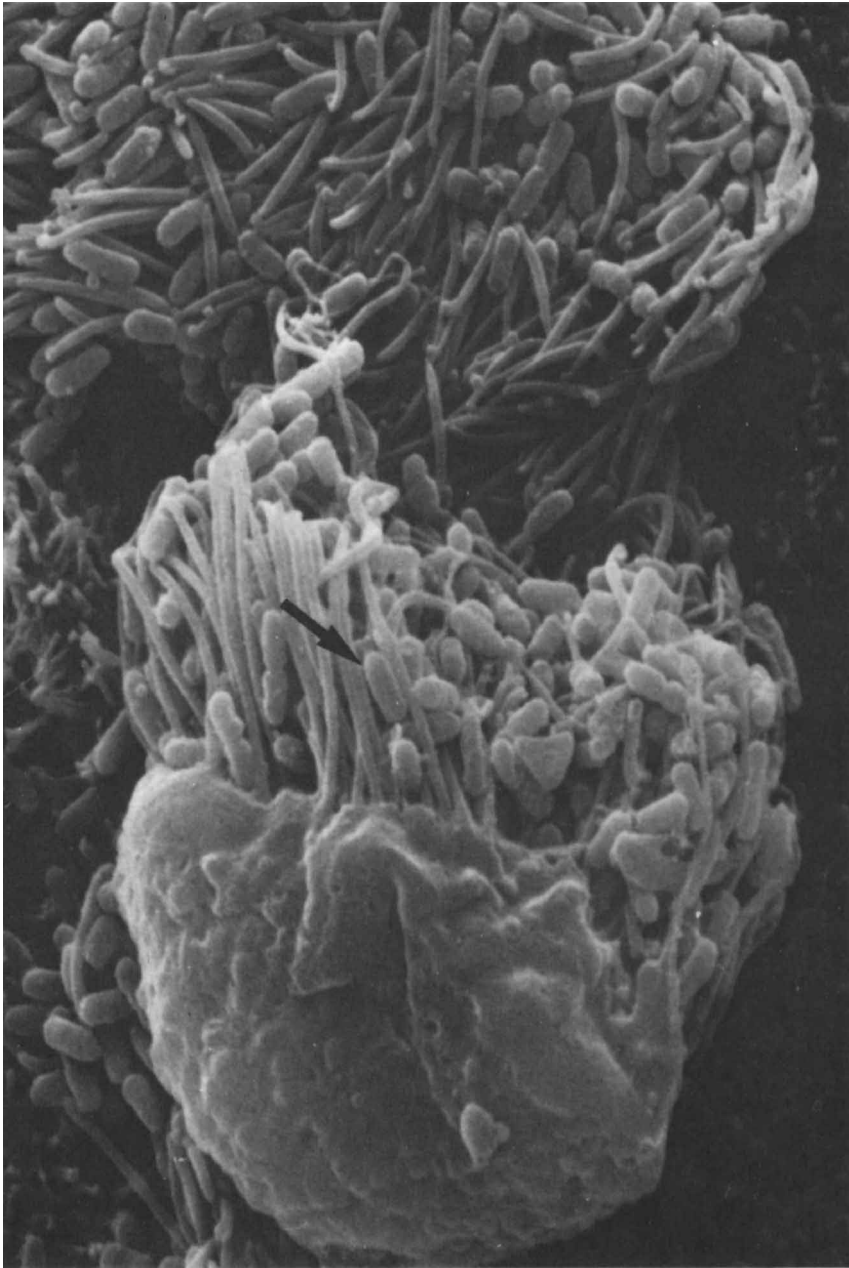


FIG. 3.—An extruding ciliated epithelial cell and infecting bacteria (arrow), 2 days after inoculation with *B. bronchiseptica*. SEM. $\times 9900$.

B. BRONCHISEPTICA ON THE TRACHEAL MUCOSA

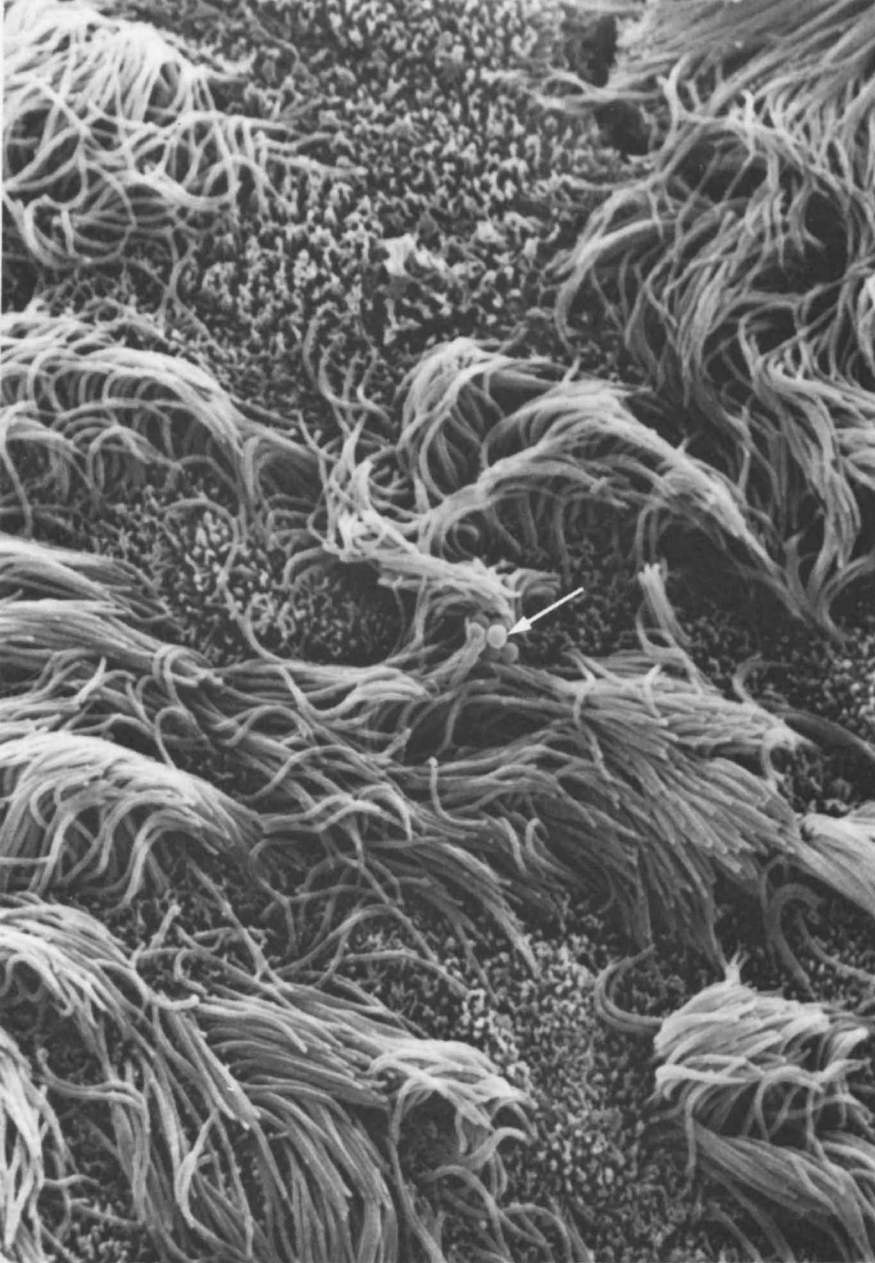


FIG. 4.—Mucous membrane of rabbit tracheal explant, 2 days after inoculation with *Staphylococcus aureus*. Most of the cilia are free from bacteria (arrow). SEM. $\times 4600$.

B. BRONCHISEPTICA ON THE TRACHEAL MUCOSA

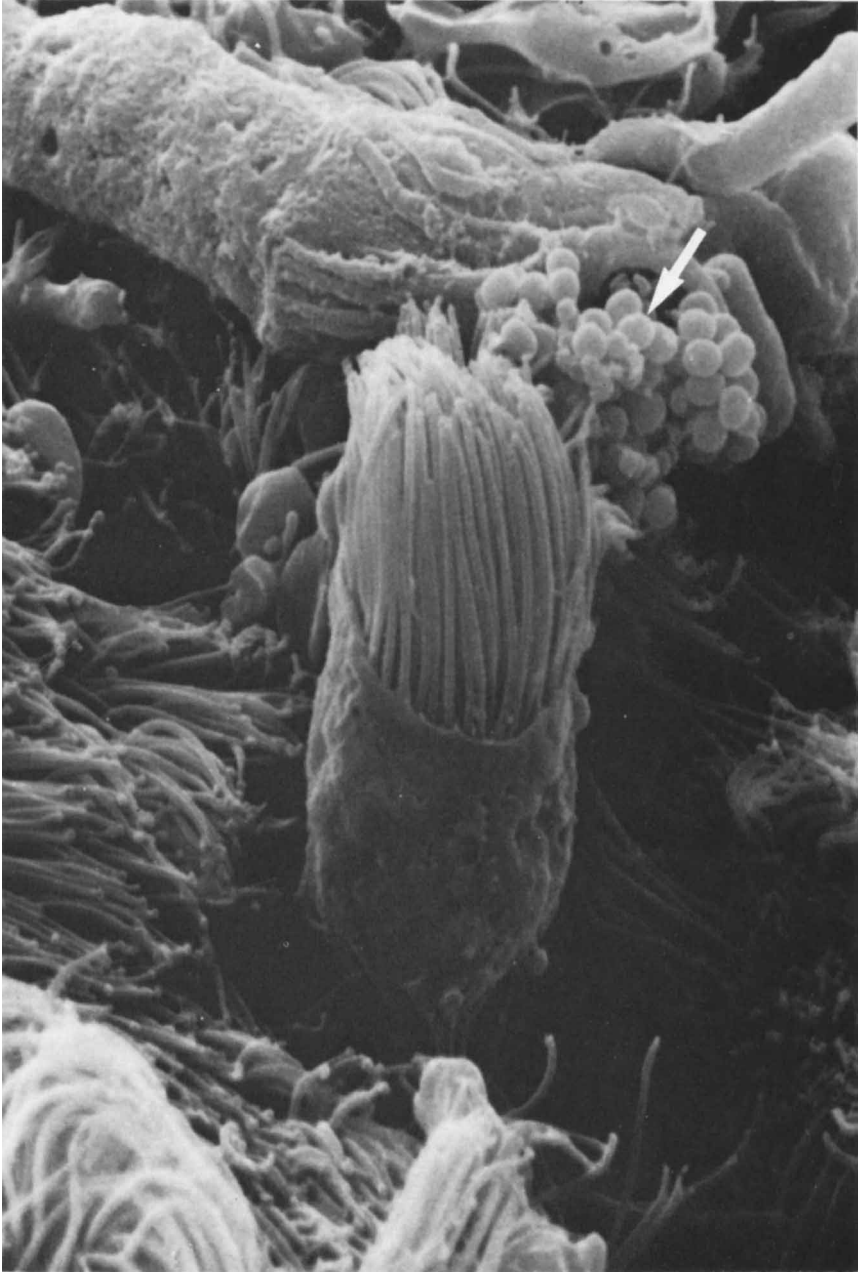


FIG. 5.—An extruding ciliated epithelial cell and infecting bacteria (arrow), 2 days after inoculation with *S. aureus*. SEM. $\times 8300$.

SUMMARY

Rabbit tracheal mucous membrane cultivated on solid agar medium was infected with either *Bordetella bronchiseptica* or *Staphylococcus aureus*. Scanning electron microscopy revealed that large numbers of *B. bronchiseptica* cells became entangled with the cilia of the tracheal epithelial cells. In contrast, *S. aureus* cells showed no such intimate interaction with the cilia.

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