

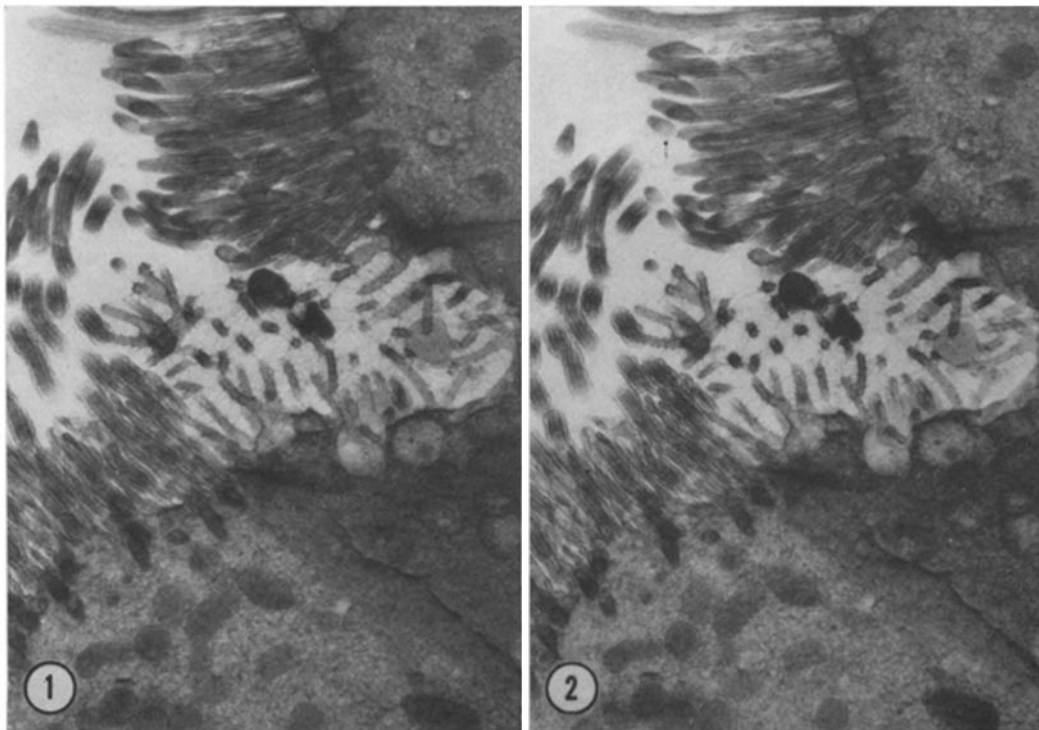
# A STEREOSCOPE OBSERVATION OF TRACHEAL EPITHELIUM OF MOUSE BY MEANS OF THE HIGH VOLTAGE ELECTRON MICROSCOPE

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## INTRODUCTION

The tracheal epithelium of the rodent is a simple columnar ciliated type composed of ciliated cells, goblet cells, nonciliated columnar cells, brush cells, and basal cells. The nonciliated cells are con-

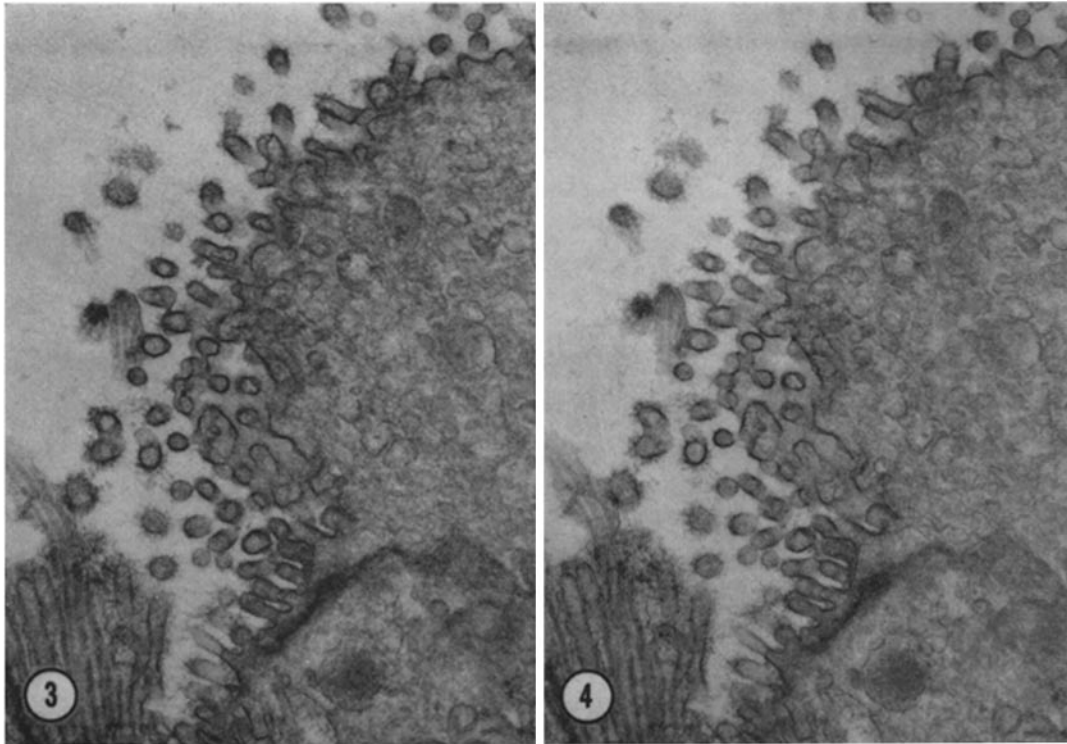
cells have been described in detail (Rhodin and Dalhamn, 1956; Rhodin, 1966; Leeson, 1961; Cireli, 1966; Steinman, 1968), the three-dimensional construction of the tracheal epithelium has never been observed at the fine structural level.



FIGURES 1, 2 These two pictures are a stereo pair showing the apical surfaces of two ciliated cells and a nonciliated secretory cell. The surface of the ciliated cell is studded with many cilia and microvilli. Many microvilli which branch irregularly can be seen on the surface of the nonciliated cell. The thin threads of surface coating material are seen to be webbed among the microvilli. A secretory granule is seen among the microvilli. The surface of the nonciliated cell exhibits a spherical indentation and small protrusion which is caused by a secretory granule beneath it. Thickness of the section,  $1 \mu$ ; accelerating voltage, 800 kv.  $\times 8500$ .

sidered to represent goblet cells in different stages of mucous granule formation (Rhodin and Dalhamn, 1956; Cireli, 1966). Though the fine structure and embryonal development of these

For an understanding of the three-dimensional organization of the biological fine structure, it has been necessary to employ reconstruction by means of serial sections. Even in this case, the results are



FIGURES 3, 4 These pictures are a stereo pair showing the luminal surface of a brush cell. The surface is seen to be very complicated, exhibiting many short microvilli and intervillar grooves. The surface of the microvilli is covered by a surface coat which projects from the surface as fuzzy threads. Thickness of the section,  $1 \mu$ ; accelerating voltage, 800 kv.  $\times 13,000$ .

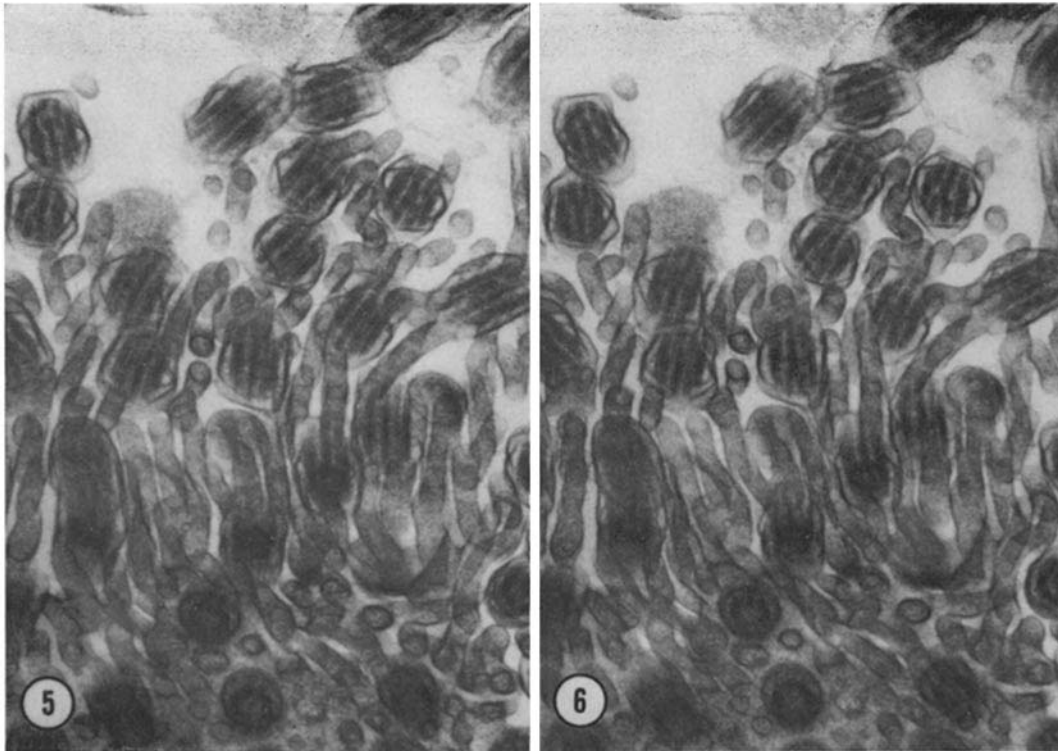
not always reliable because of the uneven thickness of the sections and the distortions of the sections caused by the compression at sectioning. Electron microscope stereoscopy has been tried (Kelly, 1966; Gray and Willis, 1968), but the stereo effects were not so remarkable because of the thinness of the sections used in these studies. Electron microscope stereoscopy of thick sections is strongly required for a better understanding of the biological ultrastructure.

The penetrating power of electrons is known to increase remarkably at higher accelerating voltage (Dupouy and Perrier, 1962; Kobayashi et al., 1964; Ueda and Nonoyama, 1967), and it already has been found that a  $0.5 \mu$  section can be observed at 500 kv (Hama and Porter, 1969). Consequently, stereoscopy is expected to be effective with the use of high accelerating voltage and thick sections. The present paper deals with the stereoscope observations of the fine structure of the tracheal

epithelium with the high voltage electron microscope.

#### MATERIALS AND METHODS

The specimens used were tracheas of mice. Tissues were dissected from anesthetized mice and put into 2% osmium tetroxide buffered with *s*-collidine at pH 7.4. After 20 min the specimens were transferred to 3% glutaraldehyde adjusted to pH 7.4 with the same buffer, and they remained in this fixative for 1 hr. The specimens were washed briefly with distilled water, then refixed in 1% osmium tetroxide for 1 hr. The specimens were stained en bloc with 2% uranyl acetate for 40 min, dehydrated, and embedded in Epon 812.  $1 \mu$  sections were prepared and were double stained with alcoholic uranyl acetate for 20 min and with lead citrate for 20 min, and then were examined under the Hitachi Hu 1000 electron microscope operated at 800 kv. The stereophotographs were taken by tilting the stages  $16^\circ$  between the two exposures.



FIGURES 5, 6 This stereo pair represents enlarged pictures of the surface of the ciliated cell. The cilium is surrounded by a series of microvilli. Notice that the outline of the cilium is clearly seen only at the upper and under surfaces of the section, whereas the central and peripheral filaments are clearly seen from the surface to the bottom of the section. The basal bodies are seen under the surface of the cell. Thickness of the section,  $1 \mu$ ; accelerating voltage, 800 kv.  $\times 28,000$ .

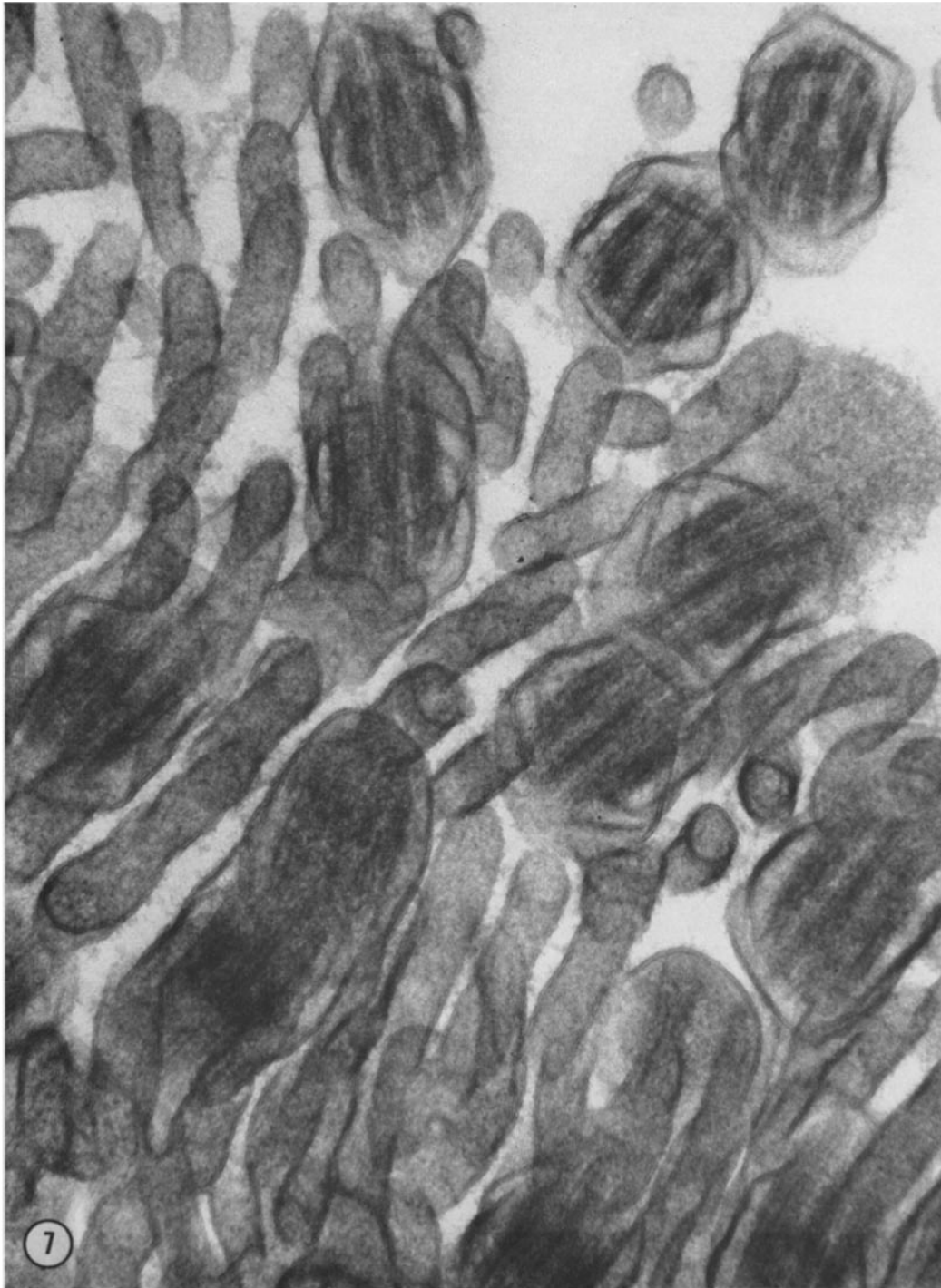
## RESULTS

In the electron micrograph of the tracheal epithelium of the mouse, ciliated cells, goblet cells, nonciliated columnar cells, and brush cells can easily be distinguished. In the present paper the apical surface of the ciliated cells, nonciliated columnar cells, and brush cells will be described. The apical cytoplasm of the nonciliated cell is occupied by a number of secretory granules about  $0.5 \mu$  in diameter (Figs. 1, 2). Some of the granules are situated near the apical end of the cell, and a few of them push up the surface membrane toward the lumen. On the surface of the nonciliated cell there are spherical indentations  $500 \text{ m}\mu$  in diameter that are probably the result of the release of secretory material from the surface. Further, many short microvilli that stud the surface of the nonciliated cell are found to have branches. Therefore, the surface of the non-

ciliated cell is very irregular and complex. Threads of surface-coating material are seen to be webbed among the surfaces of the adjacent microvilli at various heights like wire entanglements. Granules having the same density and diameter as the secretory granules in the apical cytoplasm are seen among the microvilli. On the surface of the cilia of an adjacent ciliated cell the surface coat is not evident.

In Figs. 3 and 4 the surface of the brush cell is studded with short microvilli that are relatively few in number. The nonmicrovillous surface between microvilli displays irregularly branching intervillar grooves, the surface of which is covered by filamentous coating material. On the surface of the microvilli are seen many short projections of coating material.

In addition to having long cilia, the ciliated cell, has short microvilli that irregularly branch in



**FIGURE 7** This picture shows at higher magnification a part of the field shown in the previous stereo pair (Figs. 5, 6). The trilaminar unit membrane structure is clearly resolved on the surface of the microvilli. Thickness of the section,  $1\ \mu$ ; accelerating voltage, 800 kv.  $\times 85,000$ .

many places (Figs. 5, 6). A cilium is surrounded by series of microvilli. In some cases where the staining is insufficient, the surface plasma membrane around the cross-sectional profiles of the cilia is clearly seen at both the upper- and the under-surfaces of the section but is vaguely defined between the two surfaces except where there are folds, whereas the central and peripheral filaments are stained deeply throughout the thickness of the section. On the other hand, the microvilli are rather sharply outlined through their whole length in the section. In general, the outer membrane of the microvilli is more sharply defined than that of the cilia, probably because of the presence of the thicker outer coat on the surface of the microvilli. A three-layered unit membrane structure is resolved on the surface of the microvilli and the cilia (Fig. 7), which indicates the fairly good resolution achieved even in very thick sections.

#### DISCUSSION

The penetrating power of electrons at 800 kv has proved to be sufficient for observing sections 1  $\mu$  thick. The resolution achieved is reasonably high so that the unit membrane structure can be recognized on the surface of the cilia and the microvilli. Thus high resolution stereoscopy of thick tissue sections could be performed by using the high voltage electron microscope.

The complexity of the surface structure of the nonciliated secretory cells and of the brush cells of the trachea was first recognizable by using stereoscopy. Also, the observation of the entire architecture of the microvilli and the webbing surface coat material could be first achieved by employment of stereoscopy of the thick sections.

From the observations mentioned above, it is supposed that the mucous material is secreted as a single granule from the apical surface of the nonciliated cells and that the material keeps its granular form for some time after release, a spherical surface indentation being left behind. It is likely that the surface coat which exists as a thick layer on the surface of the nonciliated cell is different from the mucous material secreted from the same cell; it is considered to be closely related to the cell surface forming the microenvironment (Fawcett, 1965; Ito, 1965).

Judging from the fact that the microvilli and the inner filamentous structures of the cilia are intensively stained throughout the entire 1  $\mu$  section, even in the case in which the stain is in-

sufficient, it is evident that the stain did penetrate the entire thickness of the section. However, the affinities to the stain of the materials constituting the cellular elements must be different. The surface membrane of the cilia is not so strongly stained as the surface of the microvilli or the ciliary filaments; the plasma membrane of the cilia is clearly seen only where it lies in the viewing axis, and it is almost transparent where it runs at an angle to the viewing axis. In addition, the cell membrane seems to stain more strongly at the surface of the section. The above mentioned phenomena, that the outlines of the cilia are clearly seen at the upper and under surfaces of the section and that frequently they are vaguely defined between the two surfaces, probably can be explained in this way. The employment of an embedding material which is more permeable to the stain, the development of an effective block staining method, or the use of a more stable stain for a longer staining time is required to overcome the staining problem when thick sections are used for high voltage electron microscopy.

#### SUMMARY

The surface of the tracheal epithelium of the mice was observed stereoscopically by using the 1  $\mu$  section with high voltage electron microscopy. A three-dimensional arrangement of the cilia and the microvilli on the ciliated cell and the complexity of the surface of the nonciliated secretory cell and brush cell including branched microvilli were observed. The surface coat material was found to cover the nonmicrovillous grooves and to web the adjacent microvilli as wire entanglements. Secretory material was considered to be released as a single granule, a spherical indentation being left behind. The problem of staining thick sections is discussed.

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