

## Scanning Electron Microscopic Studies on Intubation Damage of Tracheal Mucous Membrane<sup>\*</sup>

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### ABSTRACT

The effects of tracheal tube of cuff on dog tracheal mucosa were studied by scanning electron microscopy. Additionally, specimens of human tracheal epithelium were investigated. The specimens obtained from total laryngotomy cases under endotracheal intubation anesthesia for 3 to 6 hours. In injured areas of dog and human tracheal mucosa normal ciliary arrangement was disturbed and the cilia became shortened or had disappeared. The epithelial cells were also irregular in shape and arrangement. The scattered cells were also seen.

### INTRODUCTION

The use of endotracheal intubation during surgery and artificial ventilation in critical patients has become most acceptable. However, as usage increased, many complications have been reported<sup>1, 7-10, 12, 16, 12, 23)</sup>. Many studies have been reported concerning the damages caused by intubation, but in many of them the observation of the tracheal damage was done by fiberoscopy, light microscopy<sup>16, 21)</sup> and transmission electron microscopy (TEM)<sup>23)</sup>. There are only a few reports of tracheal damage observed by scanning electron microscope (SEM)<sup>19, 23)</sup>.

The general conclusion from the investigation of the tracheal damage is that an increase of cuff pressure or intubation time leads to progressive extension of mucosal damage. But many publications have described the damage caused by the pressure of cuffs or else caused by tubes are that larger than the diameter of the trachea. However, there are not many SEM studies on tracheal damage by intubation anesthesia. Therefore this report concentrates on tracheal damage influenced by cuffs under intubation.

### MATERIALS AND METHODS

Ten dogs were used. Two of the dogs were

used for the study of the normal morphology of the tracheal mucosa, and eight for the study of the effects of endotracheal intubation.

Specimens of human tracheal epithelium were also observed. The specimens were obtained from total laryngotomy cases which had been done under the endotracheal intubation anesthesia for 6 hours.

The structure of the tracheal mucosa was examined by scanning electron microscope. The specimens for SEM were fixed in buffered 2.5% glutaraldehyde and then Tannin-Osmium conductive staining was done. Before being dried by critical point technique, they were dehydrated in graded ethanol series and stored in pure isoamylacetate. After coating with gold in a "sputter", the specimens were examined in a JSM-T200 and FT-15 microscope.

The dogs were anesthetized by an intravenous injection of 2% sodium pentobarbital but they were able to breathe spontaneously throughout the experiment. An endotracheal tube with a prestretched large cuff (Portex) was placed in the cervical portion of the trachea, the cuff was inflated and the tube was left for 1 hour to 24 hours. Then the intracuff pressure was measured. The intracuff pressure was kept at 40 mm H<sub>2</sub>O constantly.

After this procedure, the tracheal epithelium with cartilage was taken.

<sup>\*</sup> 佐々木尚：挿管による気管粘膜障害の走査電顕的研究

## RESULTS

The normal tracheal epithelium:

The normal tracheal wall of a dog was found to consist of a mucous membrane and series of horse-shoe shaped cartilage of the same appearance at those in human trachea. The surface layer of the mucosa is built up of a ciliated cell containing goblet cells.

In scanning electron micrograph, long and numerous cilia of ciliated cells covered a large part of the tracheal surface (Fig. 1). The ciliated cells contained more than 200 cilia per cell. These ciliated cells possessed microvilli, also goblet cells had short microvilli, they were usually obscured from view. But the apical surface of goblet cells in active stage could readily be distinguished from ciliated cells. The goblet cells had released their content and globular substances could be seen between the ciliated cell. In the inactive stage of the goblet cells the goblet cell surfaces were covered with short microvilli.

The tracheal mucosa of dogs after intubation for one hour:

The ciliary arrangements of the tracheal mucous membrane were almost relatively unaltered after one hour intubation. But the globular substances secreted from the goblet cells were distinguished in the cuff site (Fig. 2). The secretion seems to be caused by irritation of cuff pressure.

The tracheal mucosa of dogs after intubation for 3 hours:

The tracheal mucous membrane damage caused by the endotracheal tube cuff began to increase. The ciliary arrangement was disturbed and shorted cilia could be seen. The tips of cilia in some parts were fused. But the change was not so remarkable (Fig. 3).

The tracheal mucosa after intubation for 6 hours:

The tracheal damage caused by the endotracheal tube was remarkable. The ciliary arrangements of tracheal mucosa was disturbed (Fig. 4). The cilia were shortened and non ciliated cells could be observed easily. The surface of the non ciliated cells was not greatly damaged. However, the non ciliated cells became loose in some area and some ciliated

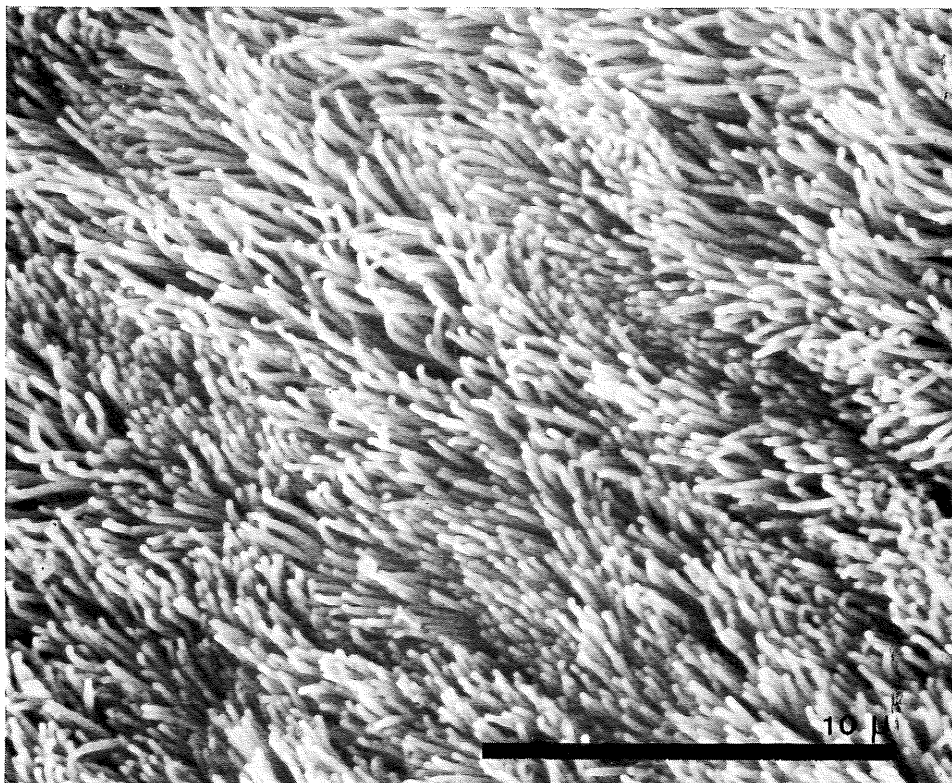
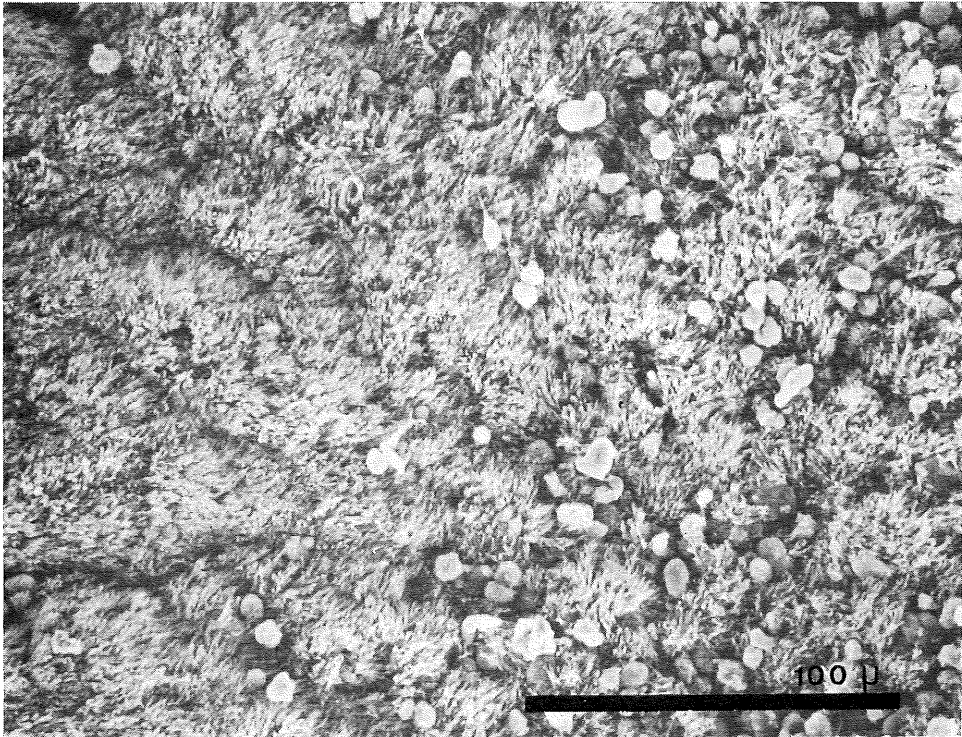
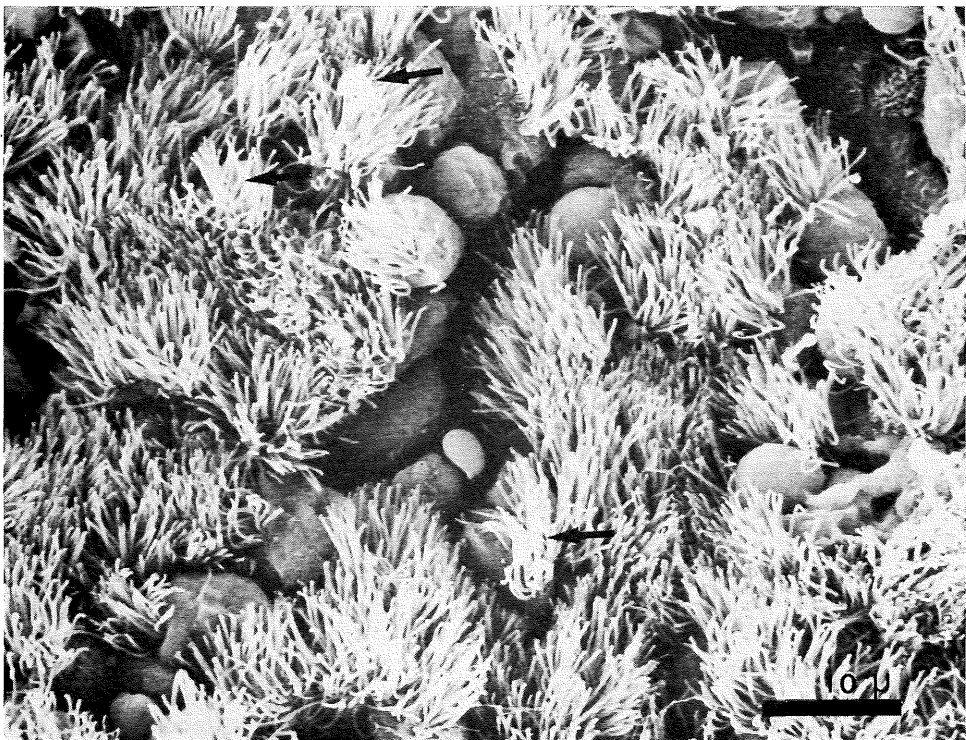


Fig. 1. The normal tracheal mucosa of a dog



**Fig. 2.** The tracheal mucosa of a dog intubated for one hour. The globular substances secreted from the goblet cells are seen.



**Fig. 3.** The tracheal mucosa of a dog after intubation for 3 hours. The tips of cilia were fused (arrows).

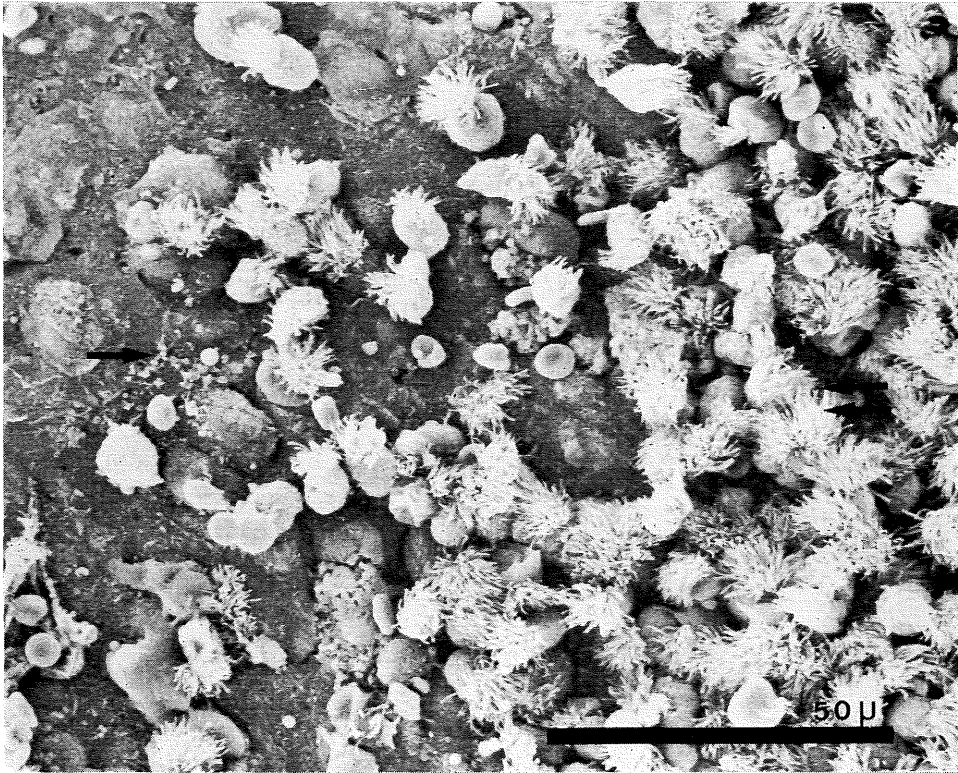


Fig. 4. The tracheal epithelium of a dog after intubation for 6 hours. The borderline between the cuffed area and the uncuffed area is clearly distinguished (—→ cuffed area, ⇐— uncuffed area).

cells had disappeared from the mucous membrane. The borderline between the cuffed area and the uncuffed area could be clearly distinguished.

The tracheal mucosa after intubation for 12 hours:

The cuffed area changed the ciliary arrangement remarkably. The cell junctions separated from each other. Some cells separated from the mucous membrane (Fig. 5, 6). Therefore, the high cuff pressure influenced the mucous membrane, disturbing the blood supply to the tracheal wall.

The tracheal mucosa after intubation for 24 hours:

In the cuffed area the mucous membrane showed remarkable deterioration. In some areas basal cells were seen like bamboo shoots popping out (Fig. 7.) Some ciliated cells bodies with cilia were isolated on the mucous membrane and the basal membrane was seen in some areas. Fibrine nets could be seen on the

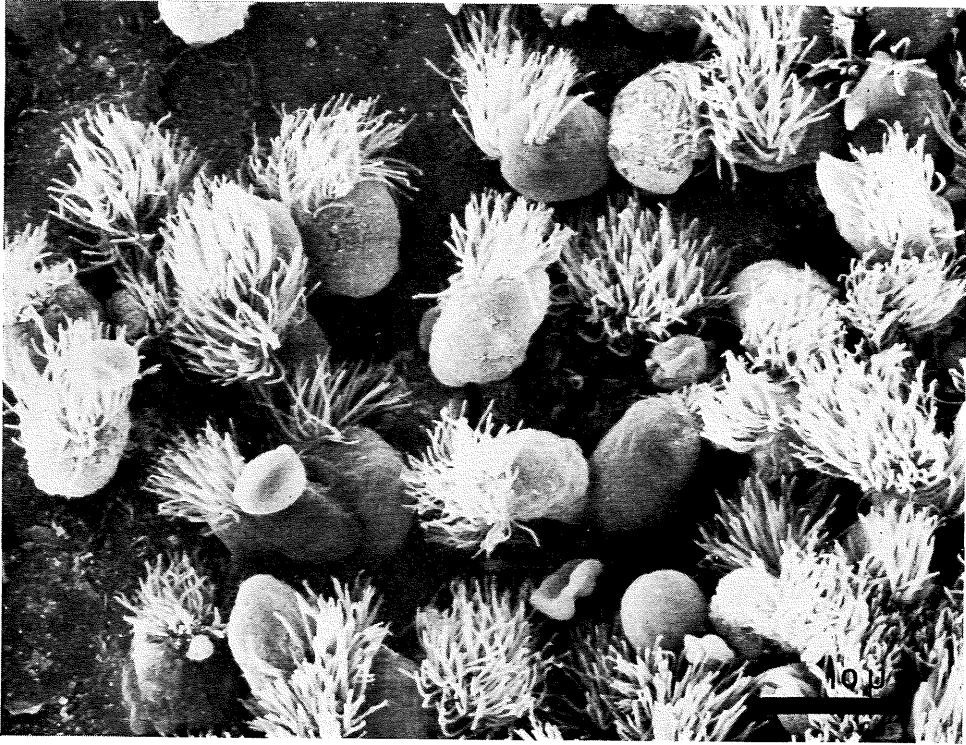
deteriorated parts of some specimens (Fig. 8).

In human materials taken from intubation anesthesia (3-6 hours):

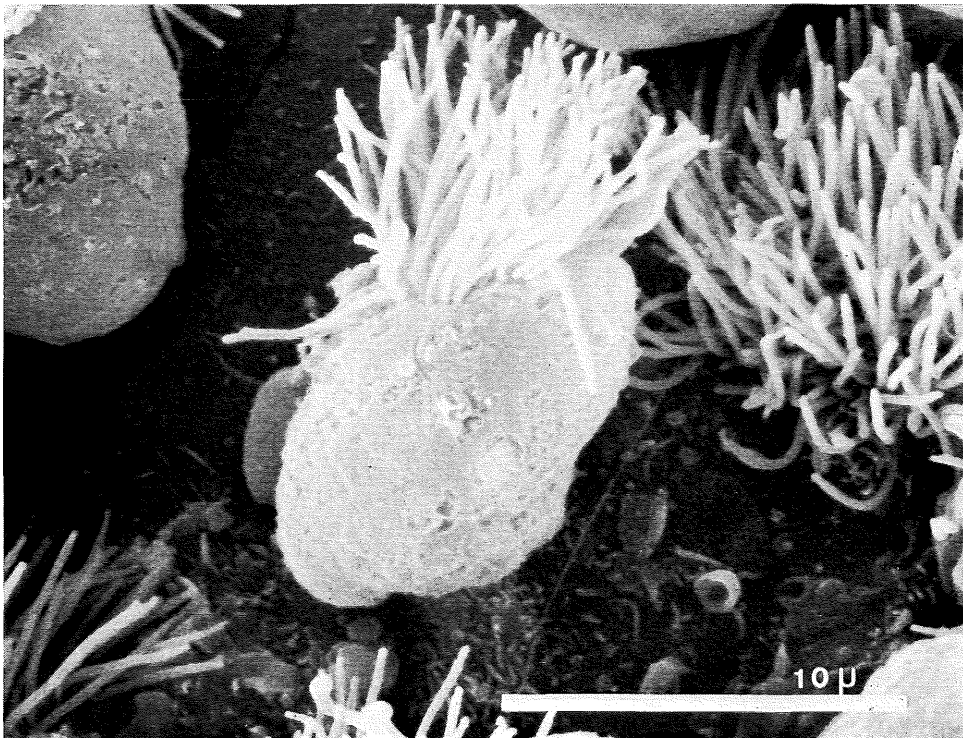
Specimens of human tracheal mucosa were taken from 4 cases of total laryngectomy under general anesthesia. After 3 hours anesthesia small specimens were taken from the cuffed area. The ciliary arrangement of the tracheal mucosa did not change so remarkably (Fig. 9). The globular substances which were secreted from the goblet cells could be seen here and there. But the human mucous membranes were not as beautiful as those in the dogs; the non ciliated cells were numbered more than that in the dogs.

In humans, the amount of cilia in ciliated cells was slightly less than those of dogs. This may be caused by aging and smoking and 6 hours intubation anesthesia. The cilia arrangement of tracheal mucosa was disturbed. The cell junction became loose and the epithelial cells became irregular in shape and arrangement

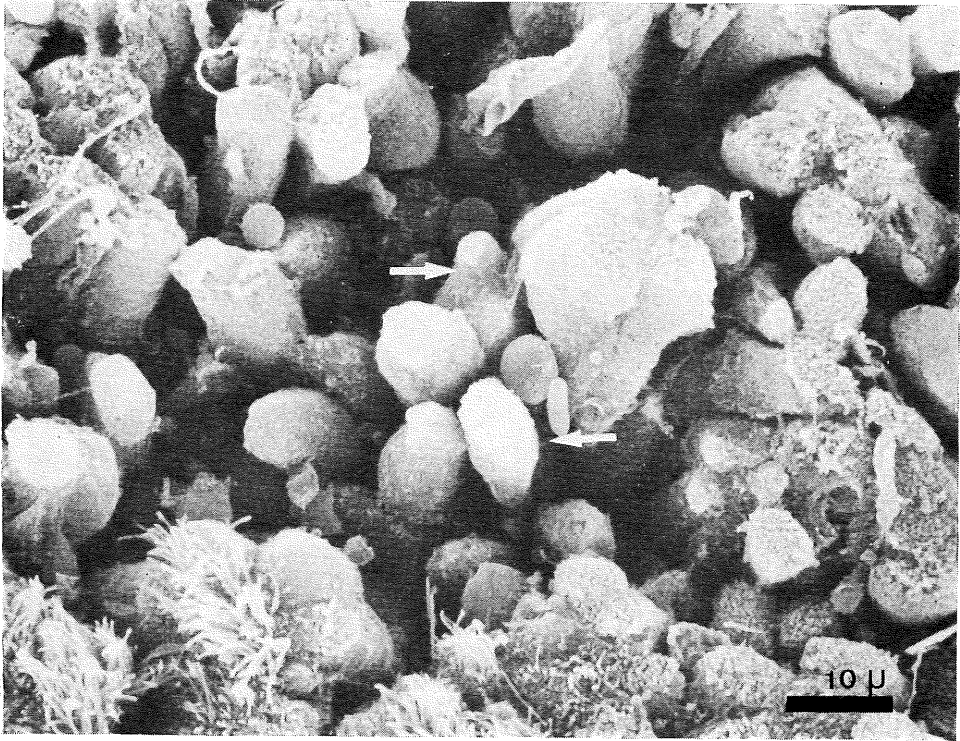




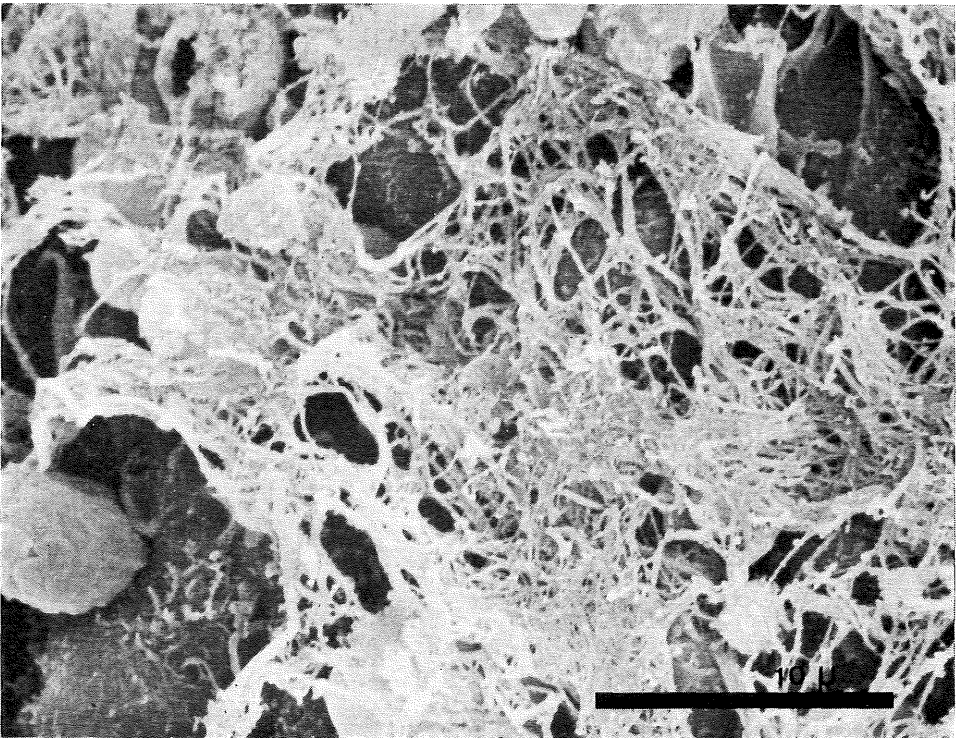
**Fig. 5.** The tracheal mucosa of a dog intubated for 12 hours. The cells are separated from each other.



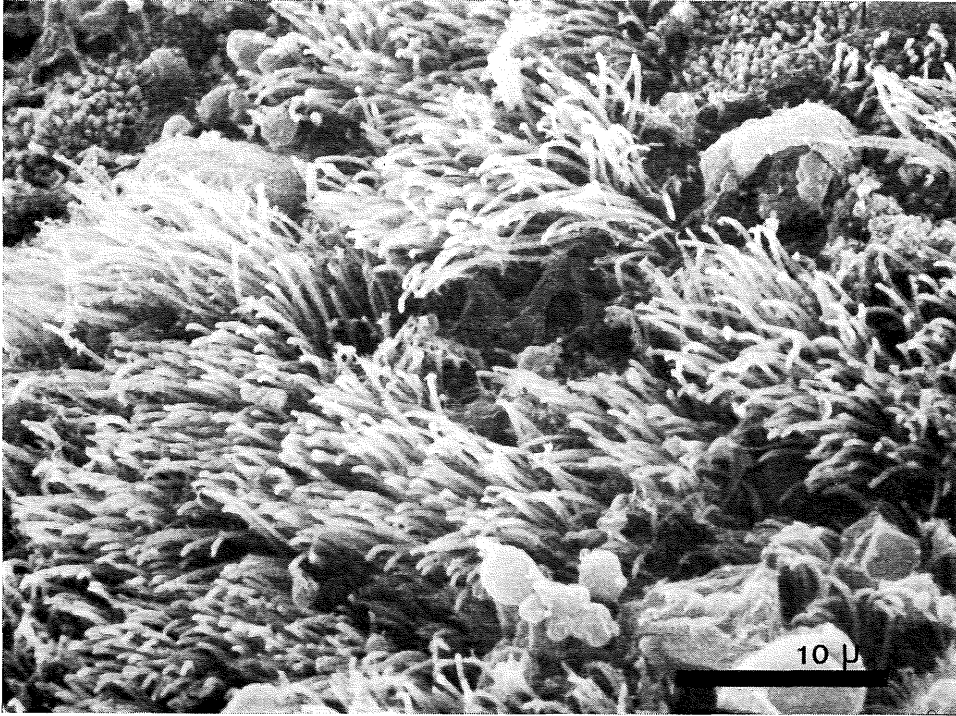
**Fig. 6.** The tracheal mucosa of a dog intubated for 12 hours. The scattered ciliated cells are seen.



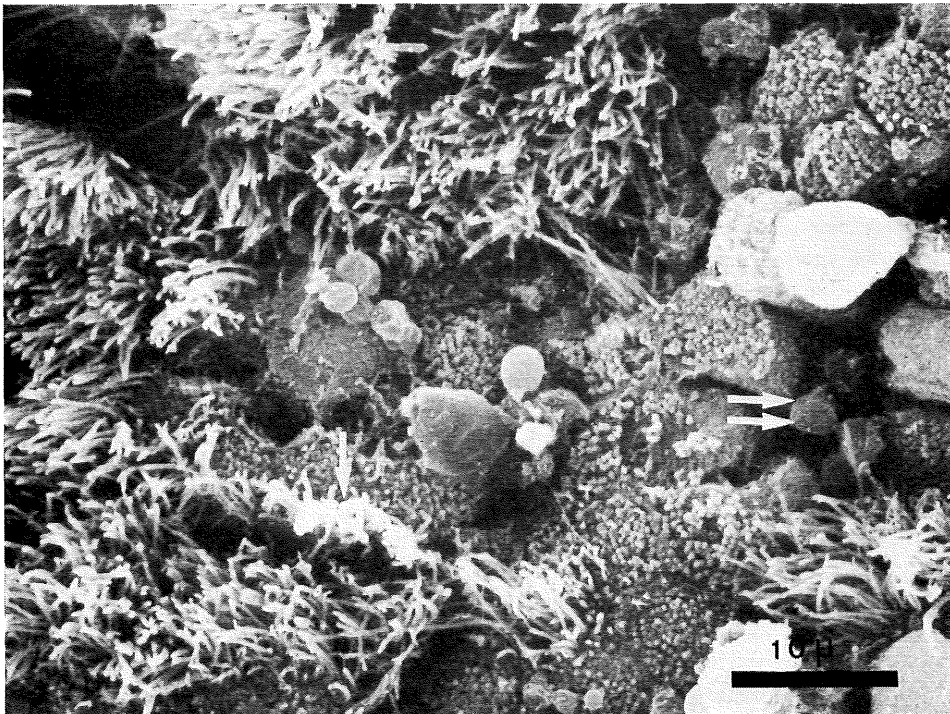
**Fig. 7.** The tracheal mucosa of a dog after intubation for 24 hours. The basal cells are seen.



**Fig. 8.** The tracheal mucosa of a dog after intubation for 24 hours. The fibrine nets covered on basal cells.



**Fig. 9.** The human tracheal epithelium intubated for 3 hours. The ciliary arrangement of the tracheal mucosa did not change so remarkably.



**Fig. 10.** The human tracheal epithelium intubated for 6 hours. The tips of cilia were fused (→) and the cell junction of the non ciliated cells became loose(⇔).

(Fig. 10). These cells seem to be falling off, while the remaining cilia became shortened or bundled.

## DISCUSSION

The fine structures of the tracheal epithelium have been extensively observed by several authors, both by TEM<sup>18,23,26)</sup> and by SEM<sup>2,3,11)</sup>.

Rhodin distinguished at least three different cell types in the tracheal epithelium. Ciliated cells and goblet cells from the upper portion of the epithelial lamina and basal cells from an irregular layer at the basement membrane. In a scanning electron microscopic study by using freeze cracking method the three types of tracheal epithelial cells were recognized by Harada and Sasaki<sup>14,15)</sup>.

Normal tracheal epithelium of dogs were covered with cilia of ciliated cells and non ciliated cells with microvilli. The non ciliated cells were usually concealed by the cilia. And all ciliary beats leaned towards the larynx. Non ciliated cells seemed to be goblet cells. The goblet cells have three stages, active stage (secretion stage), intermediate stage and inactive stage<sup>14,15)</sup>.

Numerous studies have been reported concerning damage caused by intubation, but in many of the cases the small high-pressure cuff has been used. Analysis of the cuff problem was made by many research groups. It was found that the large cuff was able to "seal" at a much lower pressure than the small cuff<sup>4-6)</sup>. Several reports demonstrated that the number tracheal injuries caused by a large cuff are lesser than those caused by a small cuff. This suggested the use of a prestretched large cuff in this study. Cooper and Grillo reported a case in which 8 dogs which were intubated with large cuffs<sup>7)</sup>. In one dog there was a slight discoloration of the tracheal mucosa after 13 days, while the rest showed non microscopic damage. At light microscopy, only mild inflammatory changes were seen.

Magovern et al.<sup>22)</sup> reported that dogs intubated for 3-30 days with a large cuff had no microscopic damage. After 30 days of intubation the light microscopy revealed a well preserved mucosa with flattened epithelium. They also reported 3 patients intubated for 44, 55 and 200 days respectively. These patients showed no mucosal damage in endoscopic examination,

Klainer et al.<sup>19)</sup> reported scanning electron microscopic studies of cuff induced damage. They intubated dogs with large cuffs and found a diffused ciliary denudation at cuff site. However, the same areas revealed almost complete destruction of the tracheal mucosa under light microscopy.

Nordin<sup>23)</sup> reported a study on rabbits by SEM and TEM. He stated the degree of damage was directly related to the cuff-to-tracheal wall pressure and if cuff-to-tracheal wall pressure is above 50 mmHg, it often produced wide spread areas of destroyed epithelium.

In this study the borderline between the cuffed and the uncuffed area was clearly observed. In the uncuffed area the tracheal surface of dogs was covered with cilia and the ciliary arrangement was leading to the larynx. They seemed to be normal.

At the cuff level, the decrease of cilia was remarkable and shortened cilia were observed. The non ciliated cells with microvilli were also observed. These non ciliated cells with microvilli are usually concealed by the long cilia of the ciliated cells. On other areas the remaining cilia became shortened or irregular in shape. The prestretched large cuff caused the damage to the tracheal mucosa of dogs.

In the specimens of human tracheal epithelium taken from a total laryngectomy case, the cell junction became loose and the epithelial cells were irregular in shape and arrangement.

Although there are some reports of large cuffs causing less damage than the smaller ones, we must not ignore the tracheal damage reported in this study. Further experimental study is necessary.

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## REFERENCES

1. **Andrews, M.** 1971. The incidence and pathogenesis of tracheal injury following tracheostomy with cuffed tube and assisted ventilation. Analysis of a 3-year prospective study. *Brit. J. Surg.* 749-755.
2. **Barber, V. C. and Boyde, A.** 1968. Scanning electron microscopic studies of cilia. *Z. Zellforsch.* 84 : 269-284.
3. **Breipohl, W., Herberhold, C. and Kerschek, R.** 1975. Die Oberflächemorphologie des Trachealepithels münlicher Albinoratten. *Arch. Oto. Rhino. Laryng.* 211 : 267-281.
4. **Carroll, R. G., Hedden, M. and Safar, P.** 1969. Intratracheal cuffs: Performance characteristics. *Anesthesiology* 31 : 275-281.
5. **Ching, N. P., Ayres, S. M., Paegle, R. P., Linden, J. M. and Nealon, T. F.** 1971. The contribution of cuff volume and pressure in tracheostomy tube damage. *J. Thorac Cardiovasc Surg.* 62 : 402-410.
6. **Ching, N. P., Atres, S. M., Spina, R. C. and Nealon, T. F.** 1974. Endotracheal damage during continuous ventilatory support. *Ann Surg* 179 : 123-127.
7. **Cooper, J. D. and Grillo, H. C.** 1969. Experimental production and prevention of injury due to cuffed tracheal tubes. *Surg Gynec. Obstet* 129 : 1235-1241.
8. **Cooper, J. P. and Grillo, H. C.** 1969. The evolution of tracheal injury due to ventilatory assistance through cuffed tubes. *Ann Surg.* 169 : 334-348
9. **Donnelly, W. H.** 1969. Histopathology of endotracheal intubation. *Arch. Path.* 88 : 511-520.
10. **Dunn, C. R., Dunn, D. L. and Moser, K. M.** 1974. Determinants of tracheal injury by cuffed tracheostomy tubes. *Chest* 65 : 128-135.
11. **Greenwood, M. F. and Holland, P.** 1972. The mammalian respiratory tract surface. A scanning electron microscopic study. *Laboratory Investigation* 27 : 296-304.
12. **Geffin, B., Grillo, H. C., Cooper, J. D. and Pontoppidan, H.** 1971. Stenosis following tracheostomy for respiratory care. *JAMA* 216 : 1894-1988.
13. **Grillo, H. C., Cooper, J. D., Geffin, B., and Pontoppidan, H.** 1971. A low-pressure cuff for tracheostomy tubes to minimize tracheal injury. A comparative clinical trial. *J. Thorac Cardiovasc Surg.* 62 : 898-907.
14. **Harada, Y. and Sasaki, H.** 1976. Scanning electron microscopy of tracheal epithelium. Freeze cracking method. *A. N. L.* 3 : 93-100.
15. **Harada, Y., Sasaki, H. and Gaafar, H.** 1977. The effect of an expectorant on the tracheal mucosa. *ORL* 39 : 113-121.
16. **Hedden, M., Ersoz, C. J. and Safar, P.** 1969. Tracheoesophageal fistulas following prolonged artificial ventilation via cuffed tracheostomy tubes. *Anesthesiology* 31 : 281-289.
17. **Hilding, A. C. and Hilding, J. A.** 1952. Tolerance of the respiratory mucous membrane to trauma: surgical swabs and intratracheal tubes. *Ann Otol.* 71 : 455-479.
18. **Hilding, A. C.** 1971. Laryngotracheal damage during intratracheal anaesthesia. *Ann Otol.* 80 : 565-580.
19. **Klainer, A. S., Turndorf, H., Wu, W-H., Mawal, H. and Allender, P.** 1975. Surface alterations due to endotracheal intubation. *Am. J. Med.* 58 : 674-681
20. **Knorádová, V.** 1966. The ultrastructure of the tracheal epithelium in rabbit. *Folia Morphol.* 14 : 210-219.
21. **Lindholm, C-E.** 1969. Prolonged endotracheal intubation. *Acta Anaesthesiol Scand. Suppl.* 33.
22. **Magovern, G. J., Shively, J. G., Fecht, D. and Thevoz, F.** 1972. The clinical and experimental evaluation of a controlled-pressure intratracheal cuff. *J. Thorac Cardiovasc Surg.* 64 : 747-758.
23. **Nordin, U.** 1977. The trachea and cuff-induced tracheal injury. *Acta Otolaryngol Suppl.* 345.
24. **Pearson, F. G., Goldberg, M. and Silva, A. J.** 1968. A prospective study of tracheal injury complicating tracheostomy with a cuffed tube. *Ann Otol.* 77 : 867-882.
25. **Rhodin, J. and Dalhamn, T.** 1956. Electron microscopy of the tracheal ciliated mucosa in rat. *Z. Zellforsch.* 44 : 345-412.
26. **Rhodin, J.** 1956. Ultrastructure of the tracheal ciliated mucosa in rat and man. *Ann Otol. Rhinol. Laryngol.* 68 : 964-974