ArchJap Chir 47 (6), 643~672, Nov., 1978

原　　著

Studies on Mucous Flow of the Trachea and Scanning Electron Microscopic Studies of Mucous Membrane

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Received for Publication. Sept., 11, 1978

Introduction

Rapid strides in the development of open heart surgical techniques have been translated into excellent operative results in the correction of a variety of congenital cardiac defects in neonates and infants. Disturbances of respiratory function are common after any major operation on the thorax. Additional factors may be important after cardiac surgery, including the presence of pre-existing disease of the lungs, airway or pulmonary, and the effect of cardiopulmonary bypass. Respiratory tracts of infant and neonate are narrow and their secretion of respiratory tract is abundunt. Especially in postoperative period secretion of respiratory tract increases and effort of expectoration decreases, consequently narrowing of obstruction of respiratory tract occurs quickly, which causes complication of the lung. Mucociliary membrane of the respiratory tract always secretes mucous which is transported toward the vocal cord by constant movement of cilia. Disturbance of this mucociliary transport mechanism produced narrowing or obstruction of respiratory tract. Therefore in postoperative respiratory care we must avoid any unfavorable stimulation to mucous membrane of the respiratory tract as much as possible and should maintain physiological action of mucociliary transport mechanism intact. The purpose of this study is to determine the effects of factors which concern mucociliary transport mechanism, i. e., temperature and humidity of inhaled air, total body hypothermia, extracorporeal circulation, intubation by endotracheal tube and suctioning of the respiratory tract.

Materials and methods

Section 1 ; Studies on mucous flow of the trachea

In this experiment the mongrel dogs of both sexes weighing 5 to 12 kg were used. The dogs were anesthetized with 25 mg of sodium pentobarbital (Nembutal®) per kg injected intravenously. Subsequently the trachea of approximately 7 cm length was removed

Key words: Cilia, Mucous flow, Mucous flow rate, Scanning electron microscope, Long term intubation
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just below the cricoid caltilage and the membranous part of the trachea was opened longitudinally, then put into a specially designed plastic chamber for measurement of mucous flow. The temperature and the humidity in the chamber were able to control at any point by electric heater and ultrasonic nebulizer.

Mucous flow was measured by a particle transport technique. As the particle, charcoal powder of approximately 40 μm was placed on the distal portion of the removed trachea and its upward progression was observed through the dissecting microscope. Measurements were made on the time for the particle to move a distance of 1.2 mm and successive reading was obtained every 30 seconds. The readings of the fastest particle transport time in every 5 minutes were expressed as minimal particle transport time and 72 times of the reciprocal of this value was expressed as the maximal mucous flow rate in milimeter per minute. Influences of humidity, total body hypothermia, extracorporeal circulation and intubation of endotracheal tube on mucous flow rate were studied.

Results

1) *Control group*

Mucous flow rate of the trachea of 8 mongrel dogs were measured. The temperature and the humidity in the chamber were maintained at 37°C and 100 per cent throughout the study. The measurements were continued for 60 minutes to assess the time effect on mucous flow rate after removal of the trachea. Maximal mucous flow was maintained relatively unchanged during the first 30 minutes (mean value was 16.78 mm/min.), then decreased gradually as shown in Table 1 and Fig. 1. Measurement of mucous flow in the following studies were completed during the first 30 minutes after removal of the trachea.

2) *Influence of humidity on mucous flow rate*

Influence of humidity on mucous flow rate was studied in six dogs. The humidity in the chamber was changed from 100 per cent to 50-40 per cent while the temperature was maintained constantly at 37°C. Mucous flow rate at 100 per cent humidity was measured

Table 1 (a) Minimal particle transport time of every five minutes after removal of the tracheas measured at 37°C and 100% humidity in control group

No. of dogs	Minimal particle transport time (sec./1.2mm)											
	0~5	~10	~15	~20	~25	~30	~35	~40	~45	~50	~55	~60
1	5.2	5.3	3.1	5.0	3.3	3.1	3.9	3.7	4.2	4.1	4.1	4.2
2	5.7	5.8	5.6	6.0	5.3	5.3	5.7	5.1	5.5	5.7	5.9	5.9
3	4.7	4.7	4.0	3.9	5.0	6.0	13.0	6.5	7.2	10.2	9.6	9.8
4	5.7	5.8	3.7	3.7	3.0	5.4	6.5	7.2	8.7	9.4	9.6	9.7
5	5.0	3.9	3.7	4.2	4.3	4.5	4.1	6.7	5.9	5.2	5.8	5.9
6	3.8	3.0	4.1	3.7	3.9	3.3	3.7	4.9	4.8	5.4	4.7	5.4
7	3.4	2.3	4.3	3.8	2.9	4.0	4.0	3.8	4.9	5.3	5.0	5.4
8	7.0	5.8	3.7	3.7	3.0	5.4	5.5	6.3	7.2	5.4	7.6	8.2
Mean	5.1	4.6	4.0	4.3	3.8	4.6	5.8	5.5	6.6	6.3	5.4	6.9

Table 1 (b) Maximal mucous flow rate after removal of the tracheas measured at 37°C and 100% humidity in control group

No. of dogs	Maximal mucous flow rate (mm/min.)											
	0~5	~10	~15	~20	~25	~30	~35	~40	~45	~50	~55	~60 (min.)
1	13.85	13.58	23.23	14.40	21.82	23.23	18.46	19.46	17.14	17.56	17.56	17.14
2	12.03	12.41	12.86	12.00	13.58	13.58	13.58	14.12	13.09	12.03	12.22	12.22
3	15.32	15.32	18.00	18.46	14.40	12.00	5.54	11.08	10.00	7.06	7.50	7.33
4	12.03	12.41	19.46	19.46	24.00	13.33	11.08	10.00	8.28	7.66	7.50	7.44
5	14.40	18.46	19.46	17.14	16.74	16.00	17.56	10.75	12.20	13.85	12.41	12.20
6	18.75	24.00	17.56	19.46	14.46	21.82	19.46	14.69	15.00	13.33	15.32	13.33
7	21.18	21.10	16.74	18.95	24.83	18.00	18.00	18.95	14.69	13.58	14.40	12.26
8	10.29	12.41	19.46	19.46	24.00	13.33	13.09	11.48	10.00	13.33	9.47	8.78
Mean ±SD	14.83 ±3.62	16.21 ±6.82	18.35 ±2.95	17.41 ±4.22	19.73 ±4.53	16.41 ±4.22	14!60 ±4.73	13.81 ±3.72	12.55 ±3.00	12.30 ±3.41	12.05 ±3.68	11.34 ±3.33

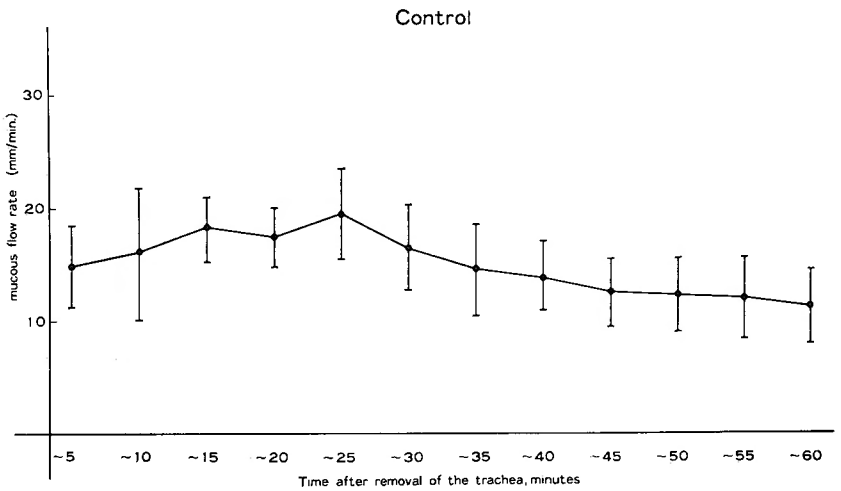

Fig. 1. Maximal mucous flow rate after removal of the tracheas measured at 37°C and 100% humidity in control group

first, then the ultrasonic nebulizer was turned off and measurement of mucous flow was continued during decrease in humidity. When the transport of particles stopped, the nebulizer was turned on again and humidity was increased. The measurements were made until the humidity returned to 100 per cent. Mucous flow decreased parallel with decrease in humidity and in 5 out of 6 dogs it ceased when the humidity reached below 50 per cent. Mucous flow was restored again with increase in humidity of more than 50 per cent and it returned to normal when the humidity reached more than 80 per cent (Fig. 2). Coefficient of correlation between mucous flow and humidity was shown in the figure.

3) *Influence of total body hypothermia on mucous flow rate*

Fifteen dogs were anesthetized by the same method described above. After intubation

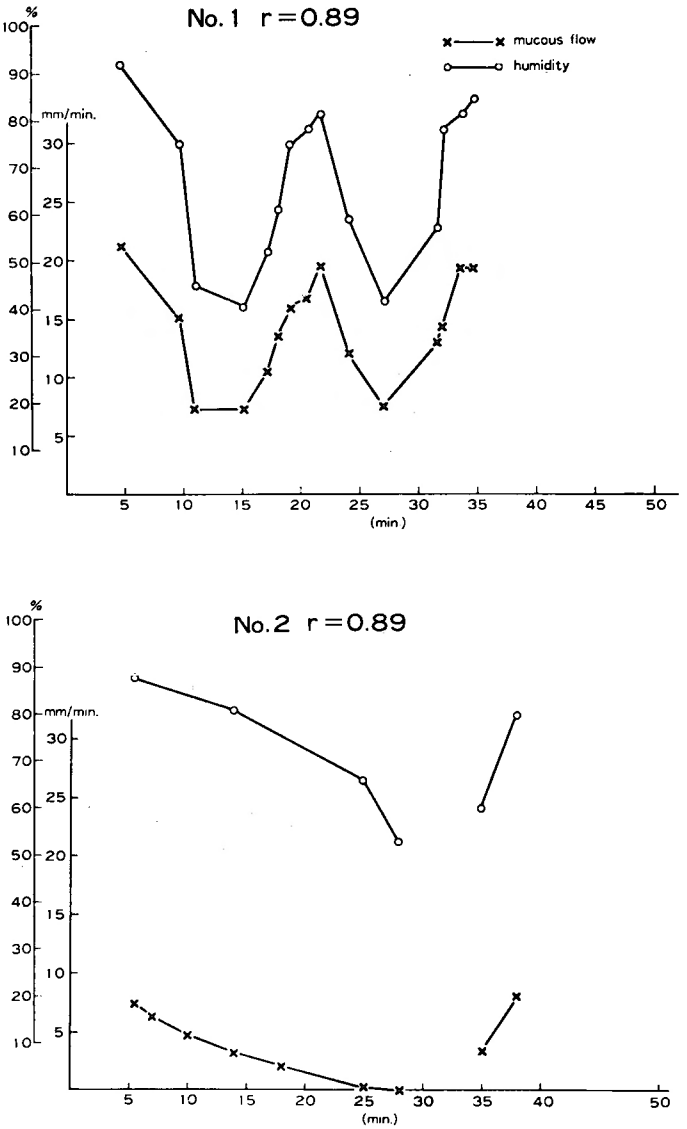

Fig. 2-1 Effect of humidity on mucous flow rate

of endotracheal tube, the dogs were cooled by surface means. During the procedure, respiration was maintained with Harvard Respirator (Respiration Pump Model 614*). The temperature and the humidity of inspired air were 18-24°C and 60-75 per cent. If shivering occurred, Succinylcholine chloride (Succin®) was injected. Tracheas of 5 dogs were removed at the esophageal temperature of 25°C and another 5 dogs at 20°C for measurement of mucous flow rate. Remaining 5 dogs were cooled to 20°C and rewarmed by surface means till 37°C then the trachea was removed for the measurement. Each measurement of mucous

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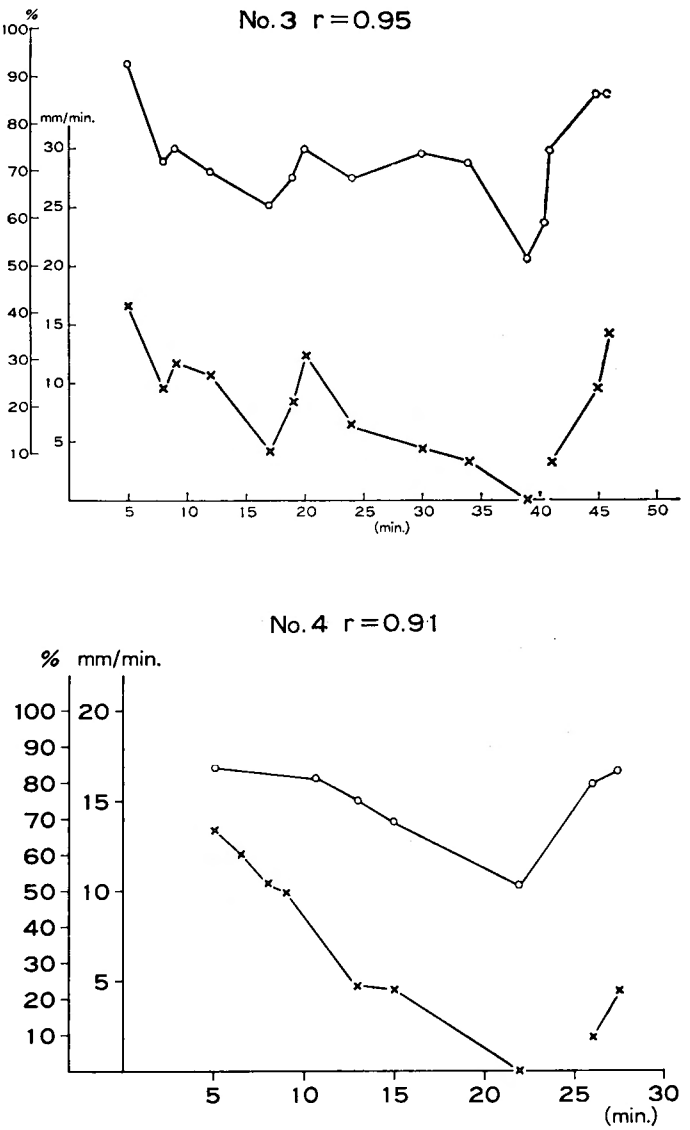

Fig. 2-2 Effect of humidity on mucous flow rate

flow was carried out at the same temperature at which the tracheas were removed and at 100 per cent humidity. Maximal mucous flow rate of the trachea decreased at low body temperature but it was restored when rewarmed to 37°C. Mean value of the maximal mucous flow rate was 12.84 ± 5.48 mm/min. at 25°C of body temperature, 5.88 ± 4.95 mm/min. at 20°C and 18.49 ± 3.86 mm/min. at rewarmed 37°C. (Table 2 and Fig. 3).

4) *Influence of partial and total extracorporeal circulation on mucous flow rate*

Effect of partial and total extracorporeal circulation was studied in 30 dogs. Anesthesia and maintenance of respiration were made by the same method as described above. Central venous

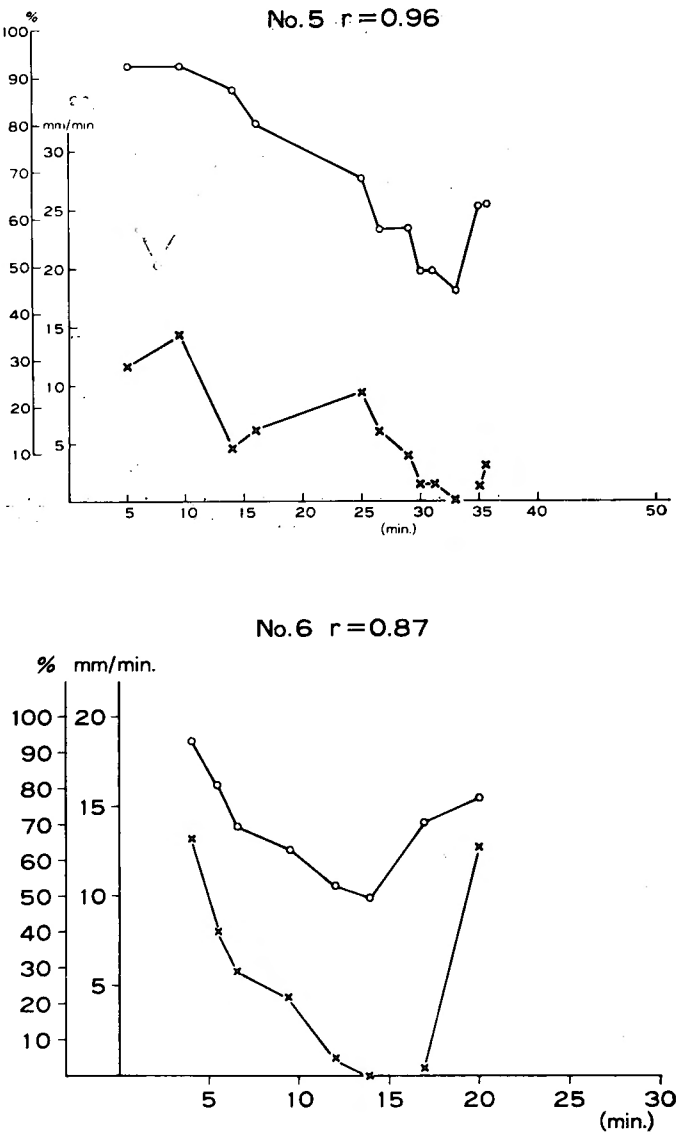

Fig. 2-3 Effect of humidity on mucous flow rate

pressure and arterial pressure were monitored through the saphenous vein and the left femoral artery. The thoracic cavity was opened through the medain sternotomy or by the fourth intercostal incision. The pericardium was opened and a venous cannula was inserted into the right atrium from the right atrial appendage and an arterial cannula into the right femoral artery. These cannulae were connected to pump-oxygenator which consisted of roller type pump (Model 7350**.), hard shell bubble oxygenator (Temptrol Q-130, Bentley Co.) and connecting lines. The apparatus was primed with fresh ACD homolgous blood which was added with

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Table 2 Minimal particle transport time and maximal mucous flow rate of the trachea in total body hypothermia

No. of dogs	Body temp.	Mucous flow sec./1.2mm	Maximal mucous flow rate mm/min.
1	25°C	4.7	15.32
2		3.7	19.46
3		4.3	16.74
4		10.9	6.61
5		11.9	6.05
Mean ±SD		7.1	12.84 ±5.48
6	20°C	4.8	15.00
7		14.6	4.93
8		∞	0
9		13.19	5.46
10		18.0	4.00
Mean ±SD		12.65	5.88 ±4.95
11	Rewarmed 37°C	3.3	21.82
12		5.3	13.58
13		6.0	12.00
14		3.1	23.23
15		3.2	21.82
Mean ±SD		4.2	18.49 ±3.86

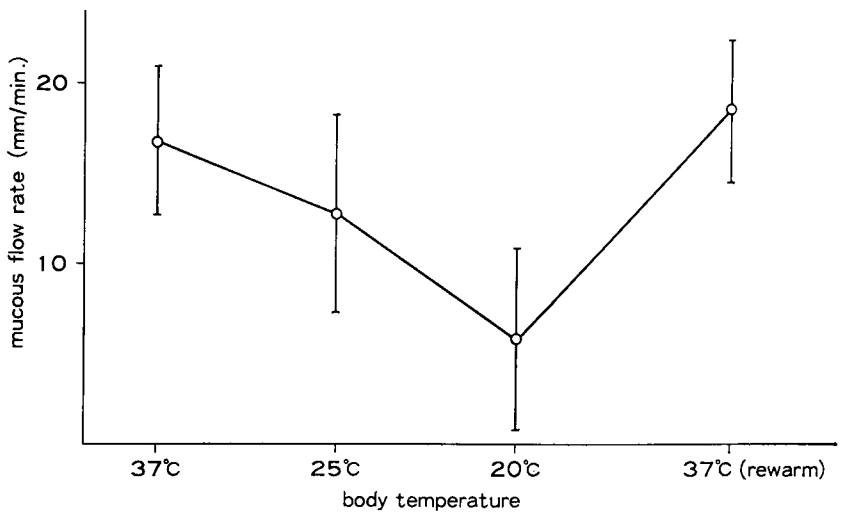

Fig. 3 Effect of total body hypothermia on maximal mucous flow rate

3 ml of heparin and carcium gluconate and 20 ml of 7 per cent sodium bicarbonate. The flow rate of extracorporeal circulation was maintained 50 to 70 ml/kg/min. and mean arterial

pressure was maintained over 50 mmHg during the bypass.

Thirty dogs were devided into two groups; partial cardiopulmonary bypass group and total cardiopulmonary bypass group. In partial cardiopulmonary bypass group, the circulation was maintained by pumpoxygenator and their own hearts and lungs, so that the mechanical respiration was continued with room air (humidity 70 per cent and temperature 25°C) by Harvard Respirator. On the other hand, in total cardiopulmonary bypass group the circulation was maintained by pumpoxygenator only excluding their own circulation by clamping the main pulmonary artery and discontinuing the respiration. After the 30, 60, and 120 minutes' duration of total or partial extracorporeal circulation in every 5 dogs, the trachea was removed and mucous flow was measured in the chamber maintained at 100 per cent humidity and at 37°C. Maximal mucous flow rates of the tracheas after 30, 60, and 120 minutes' partial cardiopulmonary bypass were 13.70±0.95 mm/min., 7.80±0.91 mm/min., and 4.00±2.06 mm/min. respectively (Table 3 and Fig. 4). In total cardiopulmonary bypass group, the mucous flow rates of 30, 60 and 120 minutes' perfusion were 11.73±2.50 mm/min., 12.50±0.62 mm/min. and 13.03±0.71 mm/min. respectively (Table 4 and Fig. 4). Both partial and total cardiopulmonary bypass affected mucous flow, and deliterious effect was progressive in partial bypass group while in total bypass group it was unchanged up to 2 hours' perfusion.

Table 3 Minimal particle transport time and maximal mucous flow rate of the trachea after partial cardiopulmonary bypass

No. of dogs	Perfusion time	Mucous flow sec./1.2mm	Maximal mucous flow rate mm/min.
1	30min.	4.8	15.00
2		5.5	13.09
3		5.7	12.63
4		4.9	14.69
5		5.5	13.09
Mean ±SD			13.70 ±0.954
6	60min.	7.8	9.23
7		11.2	6.43
8		9.6	7.50
9		8.8	8.18
10		9.4	7.66
Mean ±SD			7.80 ±0.71
11	120min.	16.4	4.39
12		∞	0
13		15.0	4.80
14		14.5	4.97
15		12.3	5.85
Mean ±SD			4.00 ±2.06

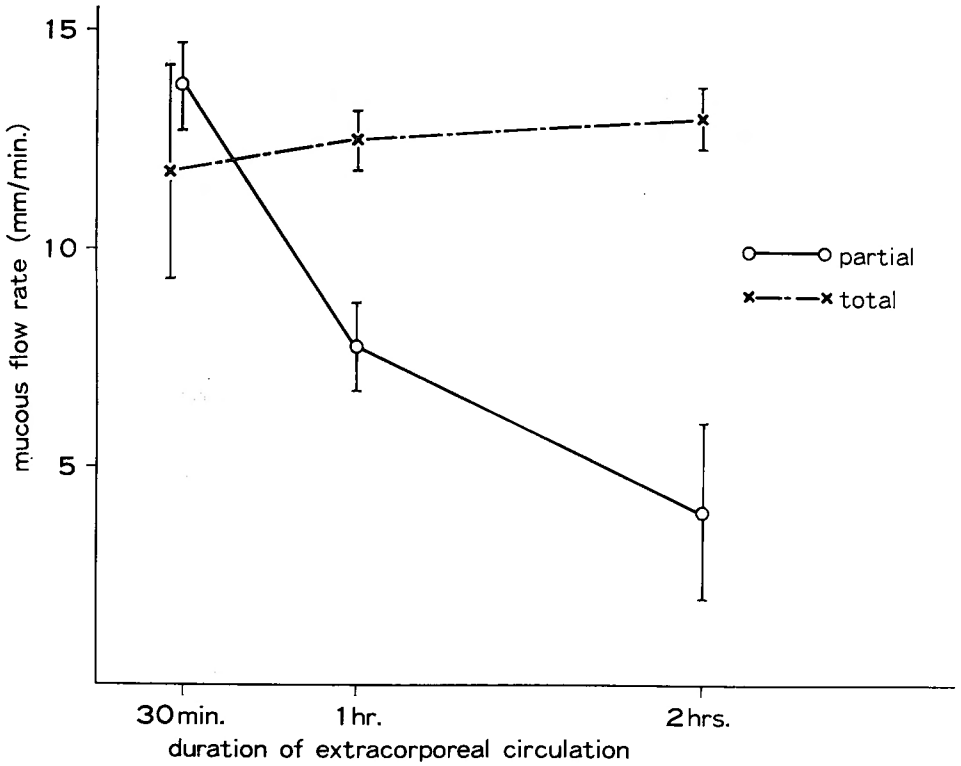

Fig. 4 Effect of partial and total cardiopulmonary bypass maximal on mucous flow rate

5) *Influence of intubation time of endotracheal tube and humidity of inhalated air on mucous flow rate*

Forty five dogs were anesthetized, intubated and their respiration was maintained by Harvard Respirator as described above. Respiration was continued for 3 and 6 hours with inspired air of 30 per cent humidity at 30°C, and 3, 6, 12 and 24 hours with air of 70-80 per cent humidity at 18-24°C and for 6, 12 and 24 hours with inspired air of 100 per cent humidity at 30°C in 5 dogs in each situation.

The dried air of 30 per cent humidity at 30°C (10 per cent biohumidity) was obtained by passing the air through $CaCl_2$ packed in the plastic chamber maintained at 30°C with heater coil (Fig. 5).

The wet inspired air of 100 per cent humidity at 30°C (70 per cent biohumididity) was obtained by passing the room air tbrough the hot water as shown in Fig. 6. As the inspired air of 70-80 per cent humidity at 18-24°C (40-50 per cent biohumidity) used room air itself. Mucous flow rate was measured within the chamber maintained at 37°C and at the same biohumidity level in each study. Maximal mucous flow rate of 10 per cent biohumidity group was 5.63 ± 0.58 mm/min. after 3 hours' exposure and O mm/min. after 6 hours' exposure (Table 5 and Fig. 7). Maximal mucous flow rate of 40-50 per cent biohumidity group was 14.00 ± 2.15 mm/min. after 3 hours' exposure, 13.56 ± 2.48 mm/min. after 6 hours'

Table 4 Minimal particle transport time and maximal mucous flow rate after total cardiopulmonary bypass

No. of dogs	Perfusion time	Mucous flow sec./1.2mm	Maximal mucous flow rate mm/min.
1	30min.	5.5	13.09
2		5.1	14.12
3		10.3	6.99
4		5.6	12.86
5		6.2	11.61
Mean ±SD			11.73 ±2.50
6	60min.	5.7	12.63
7		5.4	13.33
8		5.5	13.09
9		6.3	11.43
10		6.0	12.00
Mean ±SD			12.50 ±0.62
11	120min.	5.6	12.86
12		6.0	12.00
13		5.1	14.12
14		5.5	13.09
15		5.5	13.09
Mean ±SD			13.03 ±0.71

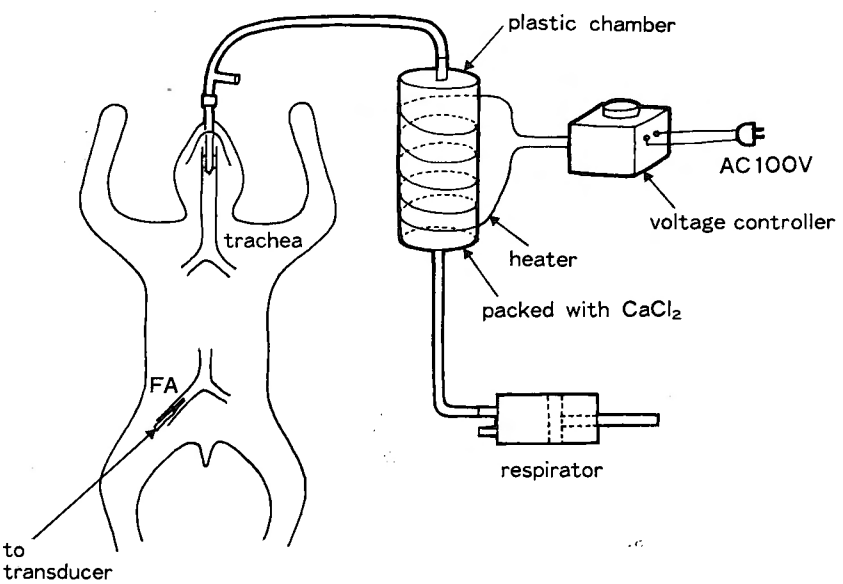

Fig. 5 Influence of inhalated air on mucous flow rate (at 10 percent biohumidity)

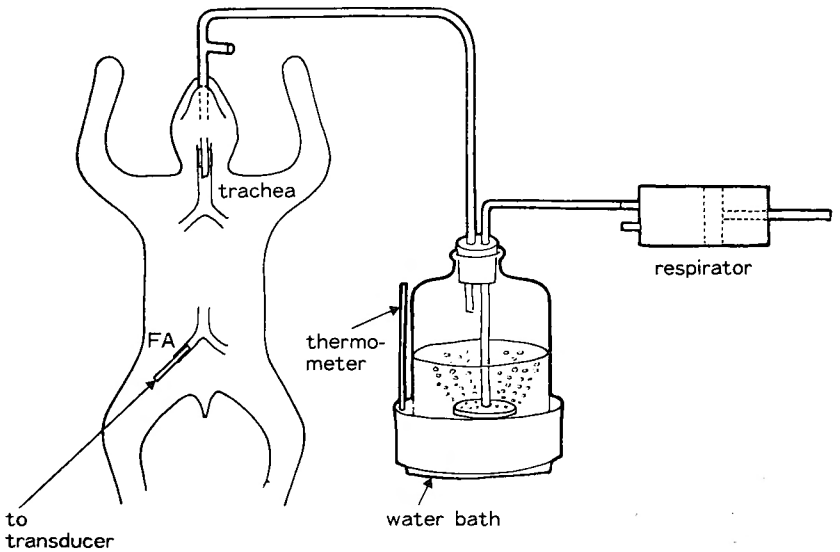

Fig. 6 Influence of inhalated air on mucous flow rate (at 70 percent biohumidity)

Table 5 Minimal particle transport time and maximal mucous flow rate when intubated with 10 per cent biohumidity

No. of dogs	Intubation time	Mucous flow sec./1.2mm	Mucous flow rate mm/min.
1	3 hrs.	12.5	5.76
2		11.5	6.29
3		15.0	4.80
4		13.5	5.33
5		12.0	6.00
Mean ±SD			5.63 ±0.58
6	6ths.	∞	0
7		∞	0
8		∞	0
9		∞	0
10		∞	0
Mean ±SD			0

exposure, 7.16±0.33 mm/min. after 12 hours' exposure and 5.39±0.23 mm/min. after 24 hours' exposure (Table 6 and Fig. 7). Maximal mucous flow rate of 70 per cent biohumidity group was 14.41±2.10 mm/min. after 6 hours' exposure, 13.11±1.79mm/min. after 12 hours' exposure and 14.55±1.99mm/min. after 24 hours' exposure (Table 7 and Fig. 7). In dogs intubated with an endotracheal tube with cuff the mucous was dammed by the cuff and accumulated. On this accumulated portion of the trachea the mucous flow stopped completely.

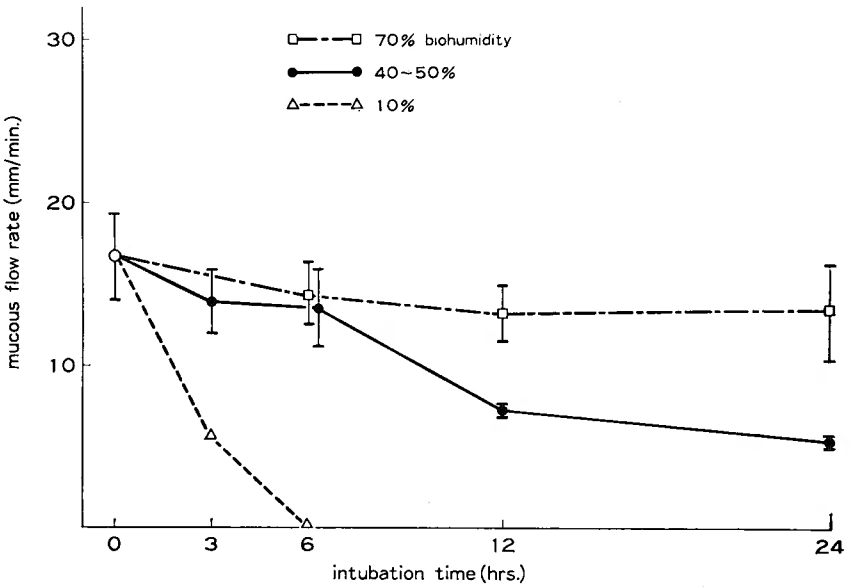

Fig. 7 Effect of intubation time and biohumidity of inhaled air on mucous flow rate

Section 2; Scanning electron microscopic studies of mucous membrane.

Materials and methods

Changes of the mucous membrane of the trachea affected by several factors were studies by scanning electron microscope (MINISEM, Hitachi Akashi Co. Ltd.) and Field Emission Scanning Electron Microscope (HSF-20 HITACHI Co. Ltd.). The trachea of mongrel dogs under anesthesia with 25 mg per kg of sodium pentobarbital was removed and sliced 3 mm by 3 mm in size. Mucous was washed out with physiolosic saline and the specimens were prefixed in 2 per cent glutaraldehyde for one hour at 4°C. Then the specimens were washed in 0.1M phosphate buffer (pH 7.4) and postfixed with 1 per cent osmic acid (OsO_4) for two hours at 4°C. After post-fixation the specimens were dehydrated by passage through graded solution of ethanol of from 50 per cent to absolute. Then amyl acetate was substitued for the ethanol. Then the specimens were dried by critical point method using carbon dioxide. Prepared specimens were mounted on the stubs, and coated with gold in the vacuum evaporator (HITACHI HSU-5GB) or iron coater (GIKO IB-30) and were examined by scanning electron microscope.

Results

1) *Normal mucous membrane*

Scanning electron micrograph of the normal mucous membrane of the trchea revealed ciliated cells, non-ciliated cells and mucous producing cells. Cilias were protruded straight,

Table 6 Manimal particle transport time and maximal mucous flow rate when intubated with 40~50 per cent biohumidity

No. of dogs	Intubation time	Mucous flow sec./1.2mm	Mucous flow rate mm/min.
1	3hrs.	5.4	13.33
2		4.9	14.69
3		6.4	11.25
4		4.2	17.14
5		5.3	13.58
Mean ±SD			14.00 ±2.15
6	6hrs.	6.5	11.08
7		5.7	12.63
8		4.1	17.56
9		5.1	14.12
10		5.8	12.41
Mean ±SD			13.56 ±2.48
11	12hrs.	10.6	6.79
12		9.5	7.58
13		10.5	6.86
14		10.0	7.20
15		9.8	7.35
Mean ±SD			7.16 ±0.33
16	24hrs.	13.9	5.18
17		13.3	5.41
18		12.7	5.67
19		14.0	5.14
20		12.9	5.57
Mean ±SD			5.39 ±0.23

smoothly curved from the ciliated cells, and arranged regularly in harmony. The surface of cilia was smooth and the tip of it was tapered. Hemispherical shaped mucous producing cells were seen between these ciliated cells and several microvilli, and discharged mucous granules were seen on their surface (Fig. 8, 9, 10).

2) *Influence of humidity on mucous membrane*

The shape, surface and arrangement of cilias which were exposed to inhaled air of 10 per cent biohumidity for 3 hours were almost the same as that of the normal cilia except for swelling of the tips (Fig. 11,12). The mucous membrane exposed to inhaled air of 10 per cent biohumidity for 6 hours were shown in Fig. 13 and 14. Cilias are bended and intertwined each other and were twined round by the thread of mucous as though a small insect was caught by the cobweb, in spite of washing-out of mucous from the surface of the mucous membrane in the process of fixation with phosphate buffer. Mucous granules between cilia

Table 7 Minimal particle transport time and maximal mucous flow rate when intubated with 70 per cent biohumidity

No. of dogs	Intubation time	Mucous flow sec./1.2mm	Mucous flow rate mm/min.
1	6hrs.	6.0	12.00
2		5.4	13.33
3		4.2	17.14
4		5.3	13.58
5		4.5	16.00
Mean ±SD			14.41 ±2.10
6	12hrs.	5.0	14.40
7		6.5	11.08
8		6.4	11.25
9		5.1	14.12
10		4.9	14.70
Mean ±SD			13.11 ±1.79
11	24hrs.	5.9	12.20
12		4.2	17.14
13		5.2	13.85
14		4.5	16.00
15		5.3	13.58
Mean ±SD			14.55 ±1.99

and small lumps on the surface of cilias were observed, and the tip of some cilias were irregularly swollen. Whereas, the mucous membrane which was exposed to inhaled air of 70 per cent biohumidity up to 24 hours showed only slight changes. Cilias exposed for 6 hours, were almost normal in their shape, surface and arrangement except for the swelling of the tips (Fig. 15, 16). When exposed for 12 hours, cilias showed some stiffness, swelling of the tips and a few lumps on the surface. However, intertwining of cilias, mucous granules between cilias and the thread of mucous were not observed (Fig. 17, 18). When exposed for 24 hours, a few thread of mucous, a few lumps on the furface and swelling of the tips of cilias and some stiffness of cilias were observed. However the swelling of the tips were not irregular, and intertwining of cilias and mucous granules between cilias were not observed (Fig. 19).

3) *Infuence of endotracheal tube on mucous membrane*

Intubation of endotracheal tube lasting for 12 and 24 hours showed marked damage to mucous membrane. When the surface of mucous membrane was pressed by the tip of endotracheal tube, it produced a clear boundary line (Fig. 20). The structure of cilia was not seen on the surface of mucous membrane which was pressed by the tip of endotracheal tube in both 12 and 24 hours' intubation. A trace of probable cilias was found on the mucous membrane in the case of 12 hours' intubation (Fig. 21). The structure of cilia was

destroyed completely on the surface of mucous membrane which was pressed by the endotracheal tube for 24 hours and some erythrocytes were seen in this area (Fig. 22).

4) *Influence of suction on mucous membrane*

Suctioning test was performed on the mucous membrane of the excised trachea using three different types of suction catheter; Nelaton rubber tube (No. 7), Argyle MAR 2502-14 and Argyle Aero-Flo Tip (Fig. 23). Each suction was performed for 10 seconds with 200 mmHg negative pressure. The procedure was repeated 5 times at the same spot and each sucked area was offered to scanning electron microscopic study.

Sucttion with Nelation rubber tube showed falling-off and deformity of cilias and hemorrhage (Fig. 24). On the other hand, suction with Argyle MAR 2502-14 and Argyle Aero-Flo Tip showed no remarkable changes (Fig. 25, 26).

Discussion

The mucous membrane of the conducting air way is composed of mature ciliated cells, mucous producing cells and non-ciliated cells[7)11)17)18)19)] In the normal tracheal mucosa there are about five ciliated cells for each mucous producing cell[25)]. Each ciliated cell has 200 cilias[12)]. The stalk extending from the body of cell is about 0.2μm in diameter and 5μm to 15μm long[11)]. Cilias produce metachronal wave[11)] which moves mucous. The producing cell is called goblet cell. Secretary granules are formed in the golgi region. Golgi membrane and vacuoles gradually develop into premucous granules. Gradually the mucous granules become densely packed and fill the upper part of the cell, which become expanded giving rose to the goblet shape[21)]. The mucous producing cell discharges the abundunt mucous granules by breaking the cell membrane[15)]. (Fig.12). Mucous flow in the respiratory tract was first described in 1830 by SHARPY. According to LUCAS[12)] the mucous blanket consists of two layers; watery layer and mucinous layer. In watery layer the cilias stand and beat, and mucinous layer, which float on the top of the watery layer, makes contact only with the tip of cilia. DALHAMN[6)] described in the trachea of rat the depth of mucous is about 5 micron. When the depth of mucous increased, mucociliary system could not perform the normal function. The blanket of mucous in the most part of the respiratory tract serves to entangle dust particles and bacteria[26)]. So the mucous works for prevention from infection. The mucous stream flows continuously from the bronchiolar region to the trachea[9)10)] and transports the dust particle and sputum.

In the the trachea of rats, DALHAMN[6)] found a ciliary frequency of 1317 beats per minutes or 22 beats per second with mucous flow of 13.5 mm/min.. SADE et al[24)]. reported that the velocity of mucous transport was 12 to 30 mm/min. in frog and toad plates, and ASMUNDSSON[2)] reported that the tracheal mucous flow rates were 5 to 19 mm/min. (mean 12.6 mm/min.) in mongrel dogs. In this experiment the tracheal mucous flow rates in 8 mongrel dogs of control group were 5.54 to 24.83 mm/min. (mean 16.78 mm/min.). ASMUNDSSON et al. used a jet nebulizer powered by compressed air as a humidifier. However, the water particle of the jet nebulizer is large and wet mucous blanket and cilias which may disrupt or stop

mucociliary transport.

In this study, ultrasonic nebulizer was used as humidifier which produced the particle of less than 5-6μm. Difference of velocity of mucous flow between ADMUDSSON's data and this experiment seems to be due to the size of water particle. Mucous flow and ciliary beat are largely affected by environmental humidity[3)14)]. The effect of drying on the respiratory mucosa of rabbits was discribed in detail by PROETZ (1933). He stated that as the membrane dried the ciliary activity decreased and finally ceased. Drying for about 15 minutes produced irreversible damage to the ciliary cells and the ciliary activity was not recommenced by the moistening with physiologic saline or Ringer's solution. He added that the only natural enemy known to the cilia in their line of function is excessive drying. In the removed trachea when drying was prolonged for more than 10 minutes to 15 minutes, ciliary beating was not observed and transport was not restored by moistening the surface of the mucous membrane.

DALHAM reported that at 30 per cent relative humidity ciliary movement ceased only 3 to 5 minutes after the trachea was opened, at 50 per cent ciliary movement ceased after 8 to 10 minutes, and at 70 per cent there was no discernible reduction of the ciliary activity when the rats had lain in the chamber for 60 minutes. The velocity of mucous flow slowed with the decrease of humidity, and when the humidity became less than 50 per cent, the transport of particle stopped in 5 of 6 dogs in this study whereas ciliary beat was still maintained. Shortly after ciliary beat also stopped. If the humidity increased whithin a few minutes, ciliary beat was restored at first and then the transport of the particle started again and accerelated to the level of normal. Ciliary movement and mucous flow were reversible after short-time exposure to low humidity, and mucous flow rate depended on environmenntal humidity. DAHLAMN reported that the mucous flow rate and ciliary beat of the rat at 31.2°C of body temperature were reduced to one half normal values. No other reports concerning low body temperature and mucous flow of the trachea were seen in the litarature.

Clinical application of deep hypothermia of around 20°C has been employed recently in open heart surgery in infants. However, the mucous flow at low body temperature below 30°C was not known. In this study, mucous flow rate was reduced to three fourth of normal at 25°C, one third at 20°C. These reduction recovered completely after rewarmed to 37°C. Low metabolic rate at hypothermia may cause the reduction of ciliary beat and mucous flow rate. Effect of extracorporeal circulation on the mucous flow has not been reported previously. It is imporfant to clarify this problem with the respect of postoperative respiratory care. This study revealed that both partial and total extracorporeal circulation reduced mucous flow rate to 80 per cent of normal value in 30 minutes. In total perfusion, this slightly reduced mucous flow rate was maintained unchanged for 2 hours. In partial perfusion, on the other hand, mucous flow rate was reduced progressively and became 23 per cent of normal flow rate after 2 hours' perfusion. The reduction during cardiopulmonary bypass may be due to inadequate peripheral circulation which may cause hypoxia of the mucous membrane.

Marked reduction in mucous flow rate with partial perfusion was attributed to low humidity of the trachea caused by mechanical respiration with air of 40 to 50 per cent biohumidity during extracorporeal circulation in this study. With total extracorporeal circulation, on the other hand, respiration was completely stopped during the perfusion, so that trachea may be highly humidified by perspiration of respiratory tract.

A relative humudity at any temperature which is converted into the humidity at 37°C is called a biohumidity. So that relative humidity of 30 per cent, 50-70 per cent and 100 per cent is equal to biohumidity of 10 per cent, 40-50 per cent and 70 per cent, respectively. When the controlled respiration with inhaled air of 10 per cent biohumidity continued for three hours, the mucous flow rate became 25.8 per cent of normal, and when continued for 6 hours, it stopped completely. The mucous flow rate decreased gradually when ventilated with 40-50 per cent biohumidity and showed 42.7 per cent of normal value after 12 hours, and 32.1 per cent after 24 hours. Meanwhile, when ventilated with 70 per cent biohumidity the mucous flow rate was well maintained and showed 80 per cent of normal value even after 24 hours.

As shown in these results, dried air such as that with 10 per cent biohumidity gave marked damage to the mucous membrane within six hours. Air of 40 to 50 per cent biohumidity also gave unfaborable effect on the mucous membrane within 24 hours. On the other hand, suitable moisture such as 70 per cent biohumidity scarcely gave influence on the mucous membrane. There are few reports on morphorogical study of normal mucous membrane of the trachea and the bronchus through scanning election microscope[20)21)18)11)]. Scanning electron microscopic study demonstrates clear and fine morphological structure. Mucous which covered mucous membrane must be washed out before fixation for this study. The mucous membrane consists of ciliated cell and nonciliated cell (mucous producing cell). Ciliated cells are five times of nonciliated cell in number. There are about two hundred cilias per one ciliated cell. Between cilias there are some microprotuberance called microvilli. In normal ciliated cell cilia protrudes straight and the tip of it becomes narrow gradually and the surface of it is smooth. When the mucous membrane was dried up by exposing to dried air for 15 minutes mucous on the surface was dried, hardened and sticked fast to cilia. Consequently cilia could not produce metachronal wave and mucous transportation. When the mucous memrane was exposed to inhaled air of 10 per cent biohumidity for three hours, mucous flow rate became one forth of normal rate although the shape of cilia was almost normal. When exposed to same air for six hours, cilia was bended and intertwined each other and mucous flow stopped completely, and the thread of mucous was twined round cilia in spite of washing-out of mucous before fixation. That is, at first dried air affected only mucous which protected cilia and cells, and when exposed to dried air for long time, cilia and mucous producing cell were affected i. e., cilia was bended and mucous granules could not melt into mucous due to deficiency of mucous.

Consequently, mucous flow could not occur. On the contrary, when proper moisture such as 70 per cent biohumidity was given to the mucous membrane for 24 hours, cilia

didn't bend or intertwine and hardening of mucous was not observed. Trauma, for example pressure by cuff[4)5)13)] or the tip of endotracheal tube[1)] and suction[1)] gave marked damage to the tracheal mucosa[13)]. Among them the pressure to mucous membrane by the tip of endotracheal tube was the worst. The structure of cilia and mucous producing cell was not seen on the mucosa pressed by the tip of endotracheal tube. In these areas mucous flow was not observed. Therefore, when intubated for long time the mucous membrane was destroied and the mucous was dammed up, and consequently it caused narrowing or obstruction of the tracheal tract. Moreover the function of prevention from infection was eliminated, and it easily provoked infection of respiratory tract. FLEMMING[8)] reported that in only 2 out of 128 patients who received mechanical ventilatory support for an average 9 days after combat injury tracheotomy cultures were persistently negative. In 53 patients pulmonary sepsis occurred and caused death in 21. Thus the most common complication of long term intubation and long term mechanical ventilatory support is respiratory tract infection. When iutubation was made, tracheal secretion increased so that the tracheal tract should be aspirated directly through the endotracheal tube. It was also pointed out that if the suction catheter was adhered to the mucosa and was pulled directly, the aspiration gave damage to the mucous membrane. PULM[20)] and DUNNING reported that noninterrupted vacuum during tracheal broncheal suction might lead to severe mucosal damage. They also pointed out that if the suction catheter adhered to the mucosa and pulled directly away from it such technic was tantamount of a crude biopsy. SACKNER[22)23)] et al investigated the pathogenesis and prevention of mucosal hemorrhage and erosion in the tracheobroncheal tree asociated with suctioning. When vacuum was applied to the catheter and its eyes came into close proximity to the broncheal mucosa, the mucosa was elevated and invaginated into side or end hole or both depending on the configulation of the catheter tip. These areas became hemorrhagic immediately after cessation of vacuum. But Argyle Aero-Flo Tip should minimize damage to the tracheobroncheal mucosa compared with a standard catheter ,thereby minimizing bacterial colonization and disturbance in mucociliary transport in patients who required suctioning of the tracheobronchial tree. In this experiment the mucous membrane which was aspirated by three types catheters was examined through scanning electron microscope. The Nelaton catheter gave maximal damage to the mucous membrane and Argyle Aero-flo Tip gave minimal damage. To prevent respiratory tract infection, mucous membrane which protects from infection should be gurded from a trauma such as suctioning and pressure by cuff or tip of the endotracheal tube.

Summary

The mucociliary transport mechanism was studied from the functional and morphological aspects. The following results were obtained.

1. The inhaled air of low biohumidity gave damage to the mucous membrane of the respiratory tract within 6 hours. On the other hand, suitable biohumidity maintained the mucous membrane of the respiratory tract intact.

2. Total body hypothermia decreased the function of mucociliary transport mechanism but it is reversible.
3. The partial extracorporeal circulation reduced the function of mucociliary transport mechanism more than the total extracorporeal circulation.
4. The pressure of the the tip of the endotracheal tube produced greater damage to the mucociliary membrane and the pressure of the cuff of the endotracheal tube gave slight damage to the mucociliary membrane.
5. None of the dogs in whom suction was performed with the Argyle Aero-Flo tip suction catheter showed gross abnormality. All dogs in whom suction was performed with Nelaton rubber tube showed gross changes on the mucociliary membrane.

From these results, any unfavorable stimulation gave some damages to mucous membrane of the respiratory tract. Especialy in postoperative respiratory care we must protect mucous membrane of the respiratory tract from any unphysiological stimulation.

Acknowlegement

I express deep gratitude to Prof. YORINORI HIKASA for his kind advice and supervision. I am greatly indebted to Chief Surgeon Ryusuke MURAOKA of Shizuoka Children's Hospital for his valuable guidance and helpful discussion. The technical assistance of Mr. M. FUJIOKA made these study possible.

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和文抄録

気道線毛上皮に関する機能的及び形態学的研究

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新生児乳児の心臓手術後呼吸管理は，近年症例が増えると共に重要な課題となりつつある。気道が単に酸素や空気の流入路として，作用するのみでなく気道に流入した異物除去分泌物の除去，その他極めて重要な働きを有している．実際に気道分泌物による気道狭窄や閉塞が，種々の肺合併症の原因となる事が多い．気道の粘膜線毛上皮が，気管内挿管，体外循環，低体温，吸気の湿度，吸引によっていかなる影響を受けるかを検討した。

150余頭の雑種成犬を用いて，著者が設計した温度及び湿度が自由に調整できるプラスチック製の箱の中に，ネンブタール麻酔下の犬に種々の因子を加え気管を摘出し，機能的には，気管膜様部を切開展開し，直径40μmの炭粉を置き，粘液異物運搬速度を計測し，形態学的には，粘膜線毛上皮を，グルタールアルデヒド，オスミウム酸を用いる二重固定を行なった後，脱水及び臨界点乾燥を行ない，金蒸着を行ない，走査電子顕微鏡により観察を行ない，次の様な結果及び結論を得た．

1) 低湿度の吸気は，6時間以内に気道粘膜に損傷を与える．一方適度の湿度では，気道粘膜には損傷を与えない．

2) 低体温は粘液異物運搬能や線毛運動の機能は低下するが，これは可逆的である．

3) 部分体外循環は，完全体外循環よりも粘液異物運搬能に影響を及ぼす．

4) 気管挿管におけるチューブの先端が粘膜にあたる部分は，線毛上皮の損傷は甚だしく，又チューブのカフが圧迫する部分は，少なからず線毛上皮に損傷を与える．

5) アーガイルエアフローチップによる気管内吸引では，ほとんど線毛上皮に影響を及ぼさないが，ネラトンチューブによる吸引では線毛上皮の剝離が見られた．

以上の結果より気道粘膜線毛上皮の生理的粘液運搬能は，気道の乾燥，長期挿管，気管チューブの圧迫，吸引等により障害される．特に術後の呼吸管理においては，気道粘膜には必要以上な非生理的な刺激を与えない様気をつけなければならない．

Fig. 8 Normal mucous membrane of the dog. (×1000)
Fig. 9 Normal mucous membrane of the dog. Cilia and mucous producing cell. (×4000)
Fig. 10 Mucous producing cell. (×5000)
Fig. 11 Cilias exposed to inhaled air of 10 per cent biohumidity for 3 hours. (×5000)
Fig. 12 Cilias exporsed to inhaled air of 10 per cent biohumidity for 3 hours. Swelling of the tips is seen. (×10000)
Fig. 13 Cilias exposed to inhaled air of 10 per cent biohumidity for 6 hours. Cilias are twined round by the thread of mucous. (×10000)
Fig. 14 Cilias exporsed to inhaled air of 10 per cent biohumidity for 6 hours. Mucous granules and small lumps on the surface of cilias are observed. (×10000)
Fig. 15 Cilias exposed to inhaled air of 70 percent biohumidity for 6 hours. (×10000)
Fig. 16 Cilias exposed to inhaled air 70 per cent biohumidity for 6 hours. Swelling of the tips is seen. (×20000)
Fig. 17 Cilias exposed to inhaled air of 70 per cent biohumidity for 12 hours. (×8000)
Fig. 18 Cilias exposed to inhaled air of 70 per cent biohumidity for 12 hours. Swelling of the tips is seen. (×20000)
Fig. 19 Cilias exporsed to inhaled air of 70 per cent biohumidity for 24 hours. A few thread of mucous, a few lumps on the surface and swelling of the tips of cilias are observed. (×10000)
Fig. 20 The surface of mucous membrane pressed by the tip of endotracheal tube for 12 hours. A clear boundary line is seen. (×1000)
Fig. 21 The surface of mucous membrane pressed by the tip of endotracheal tube for 12 hours. (×5000)
Fig. 22 The surface of mucous membrane pressed by the tip of endotratheal tube for 24 hours. The structure of cilia is destroyed completely. (×5000)
Fig. 23 Suction tubes. Argyle Aero-Flo tip, Argyle MAR 2502-14 and Nelaton rubber tube. (left to right).
Fig. 24 The surface of mucous membrane sucked by Nelaton rubber tube. (×5000)
Fig. 25 The surface of mucous membrane sucked by Argyle MAR 2502-14. (×5000)
Fig. 26 The surface of mucous membrane sucked by Argyly Aero-Flo tip. (×5000)

Fig. 8

Fig. 9.

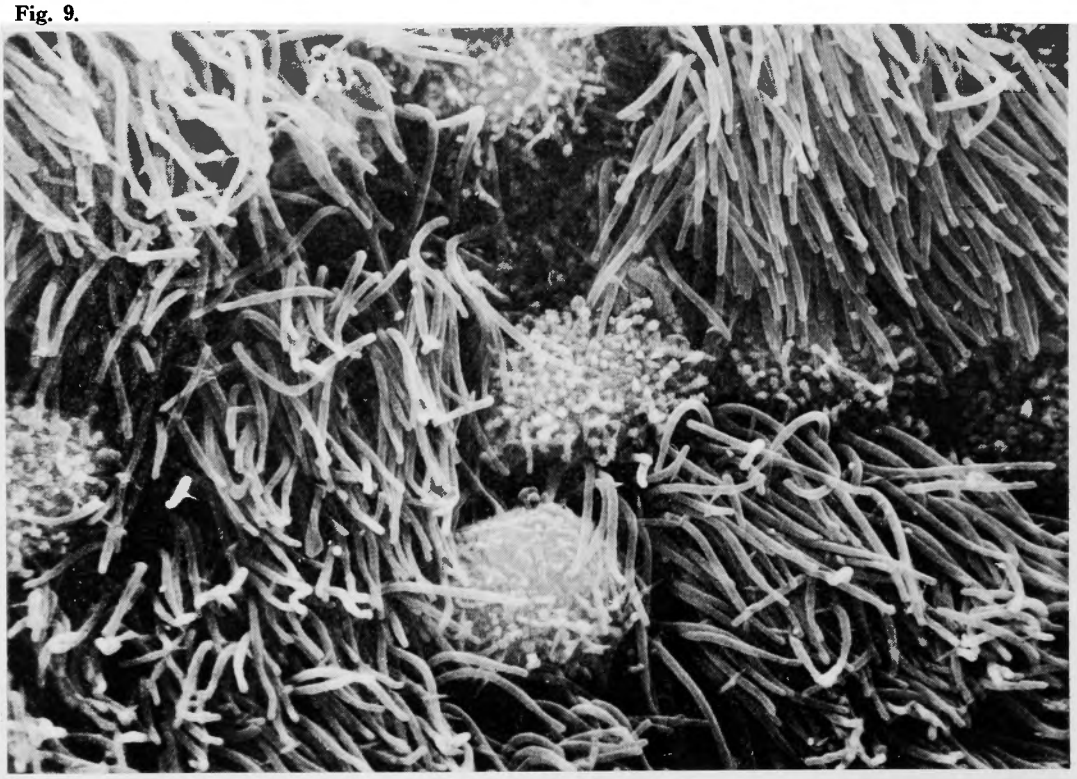


Fig. 10.

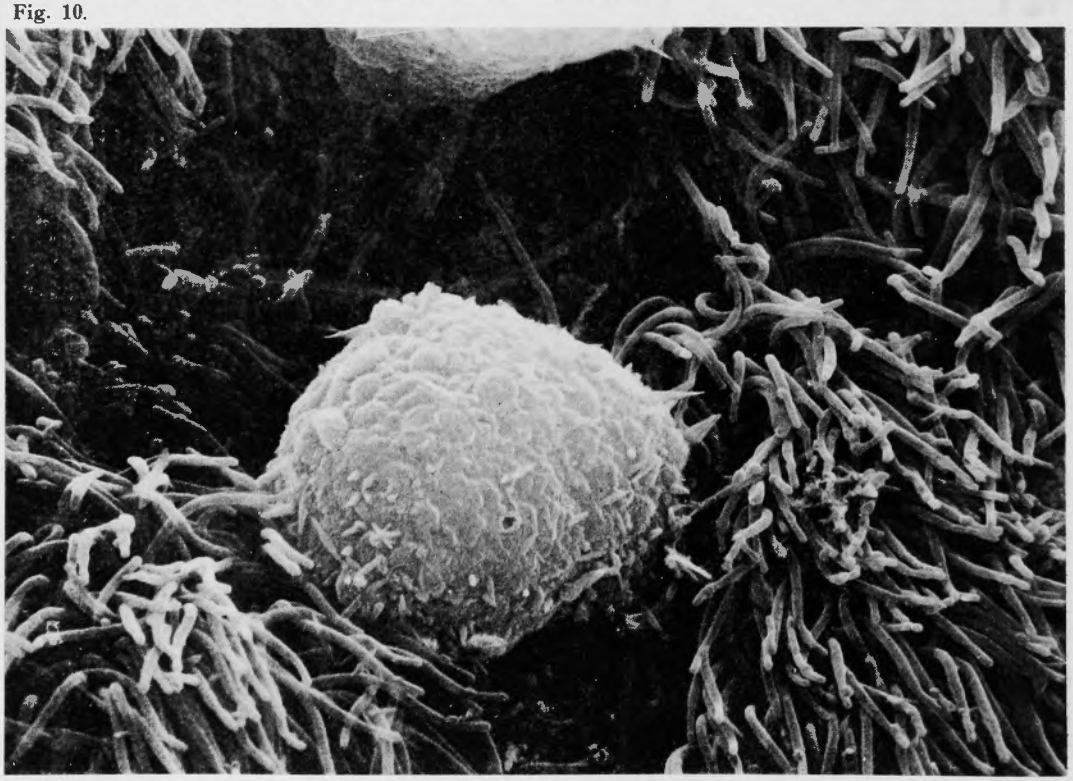


Fig. 11.

Fig. 12

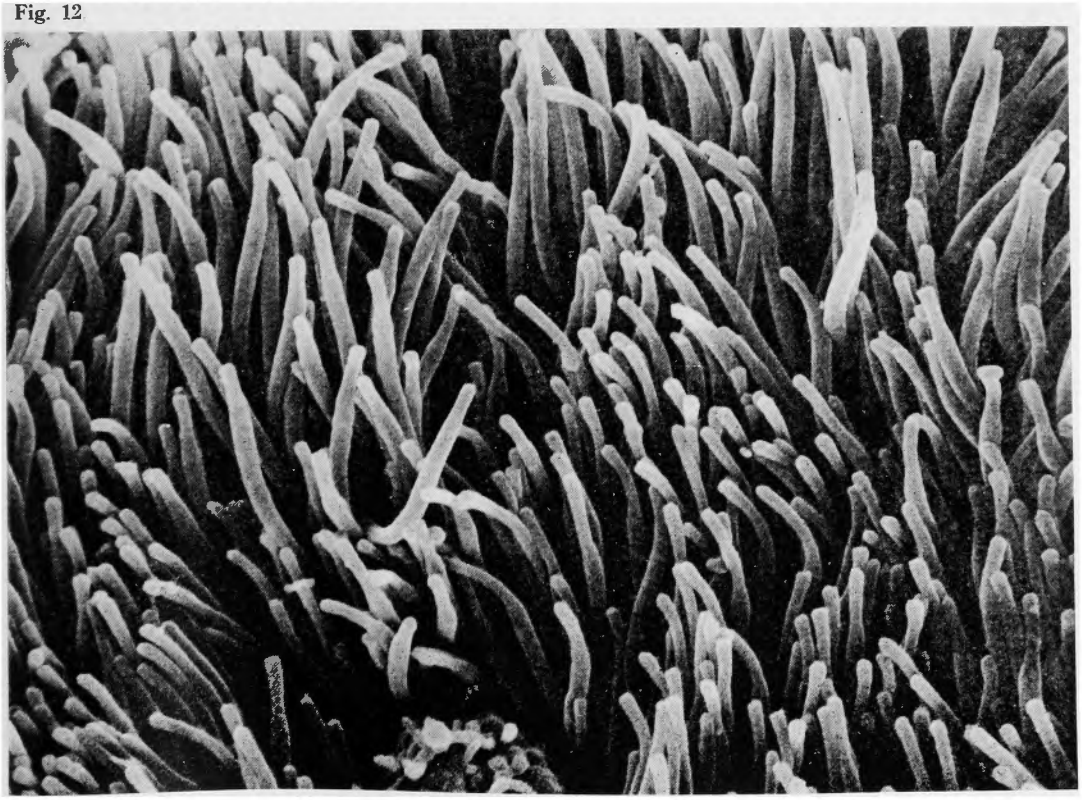
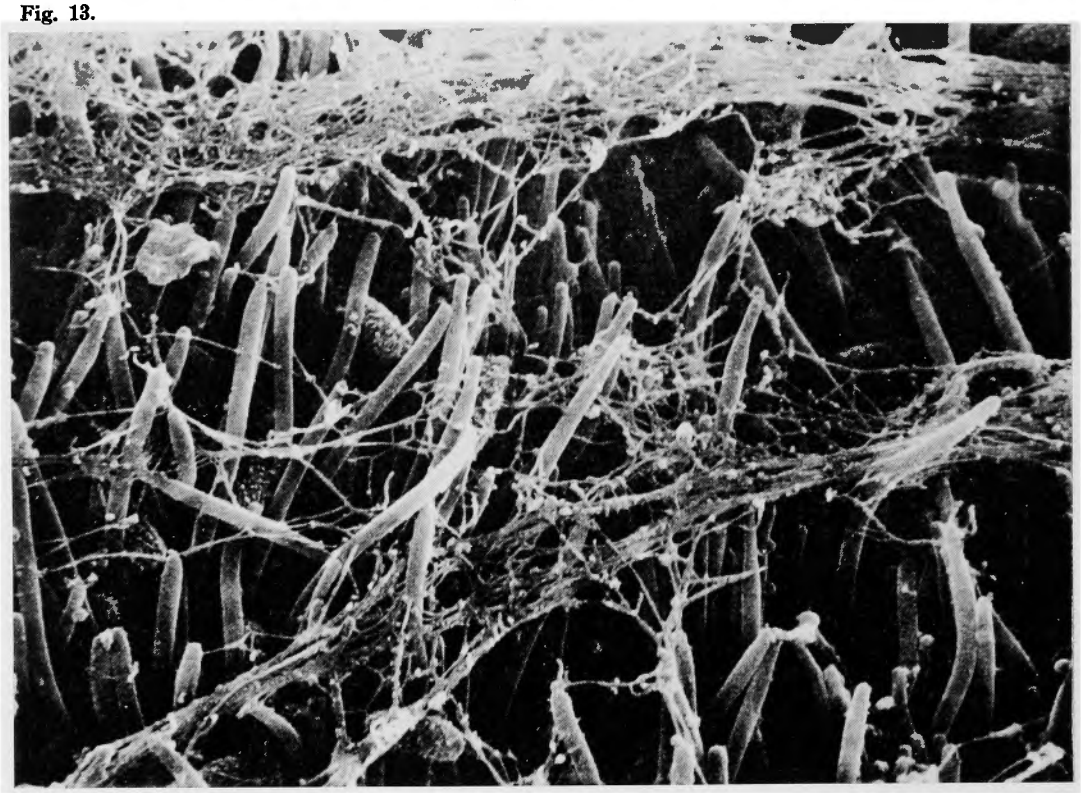


Fig. 13.



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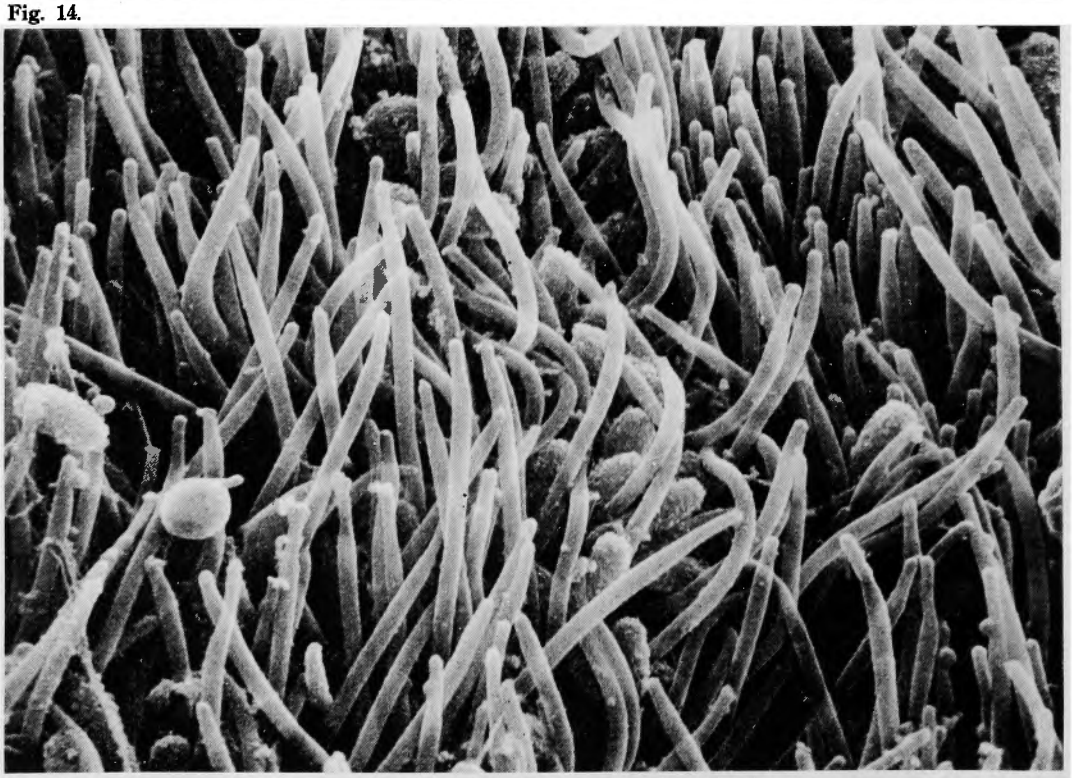

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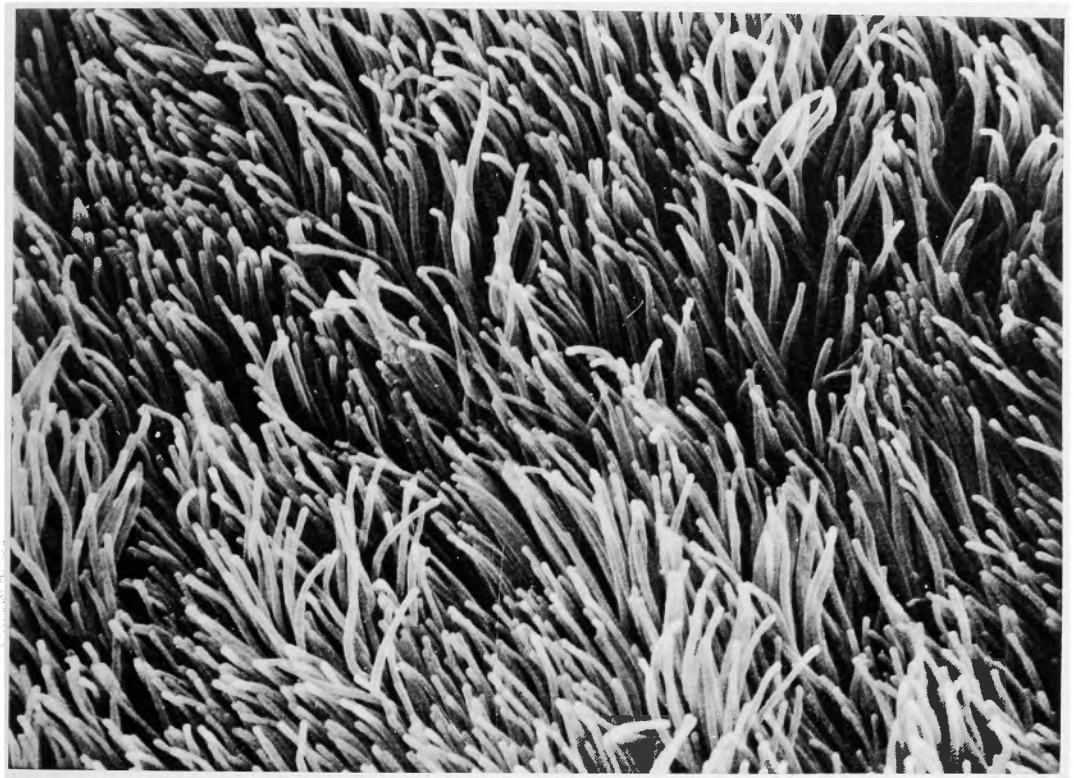

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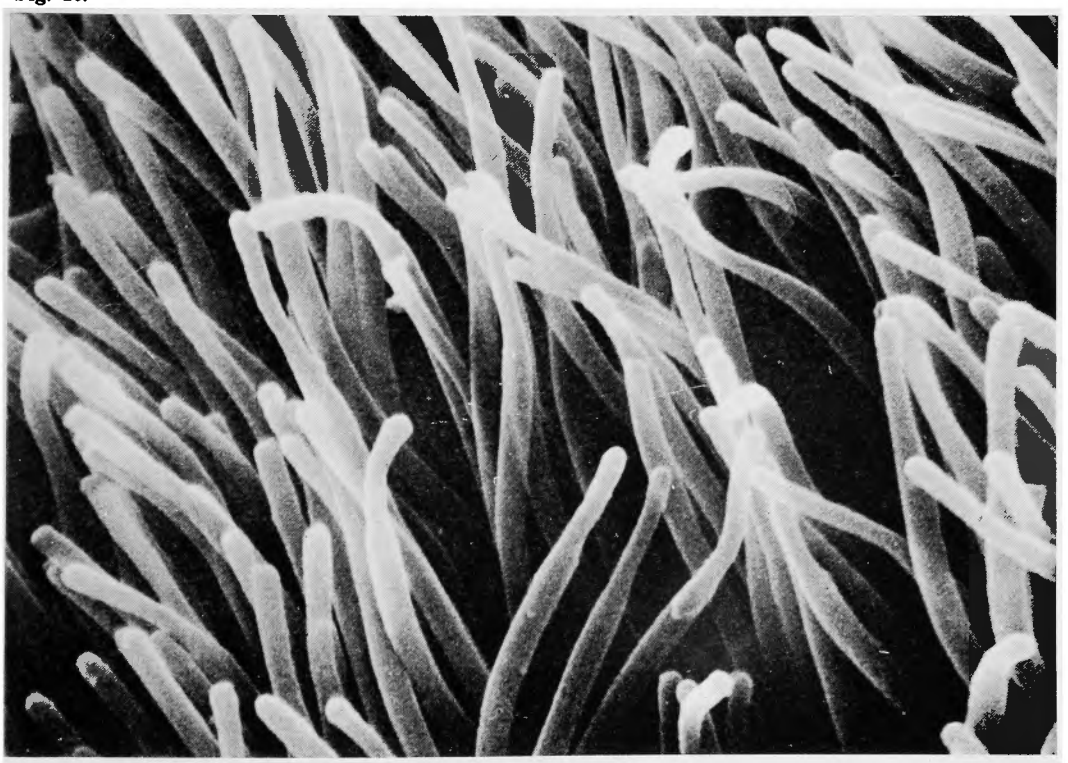

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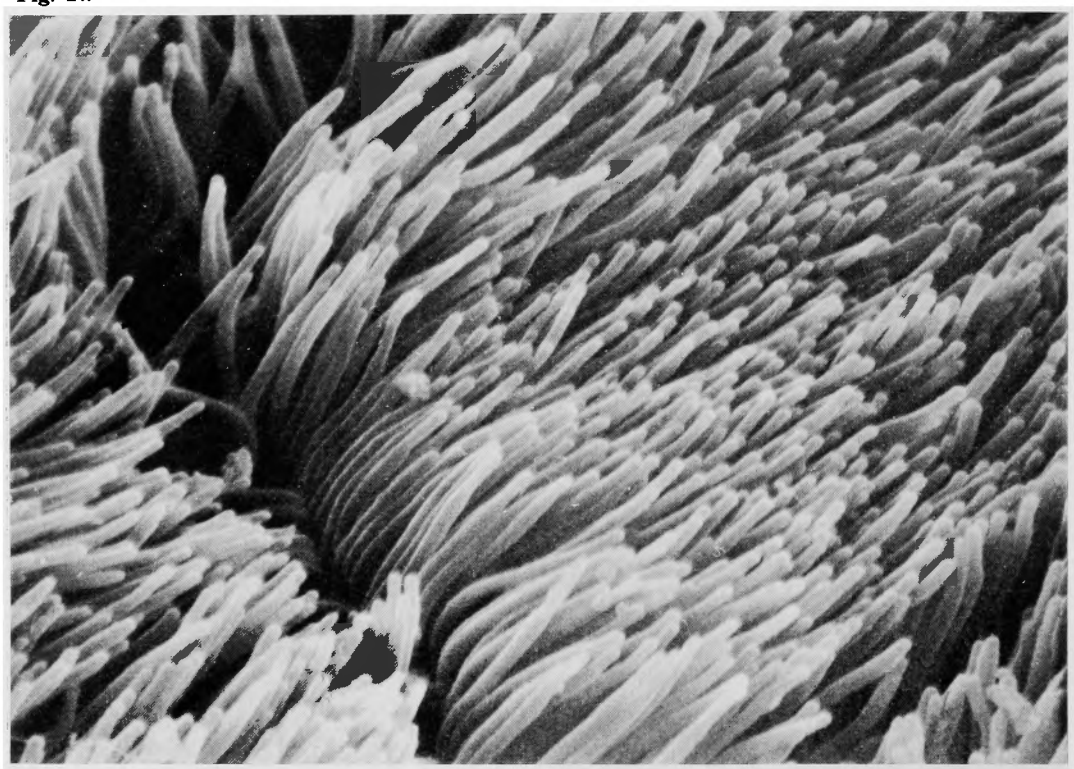
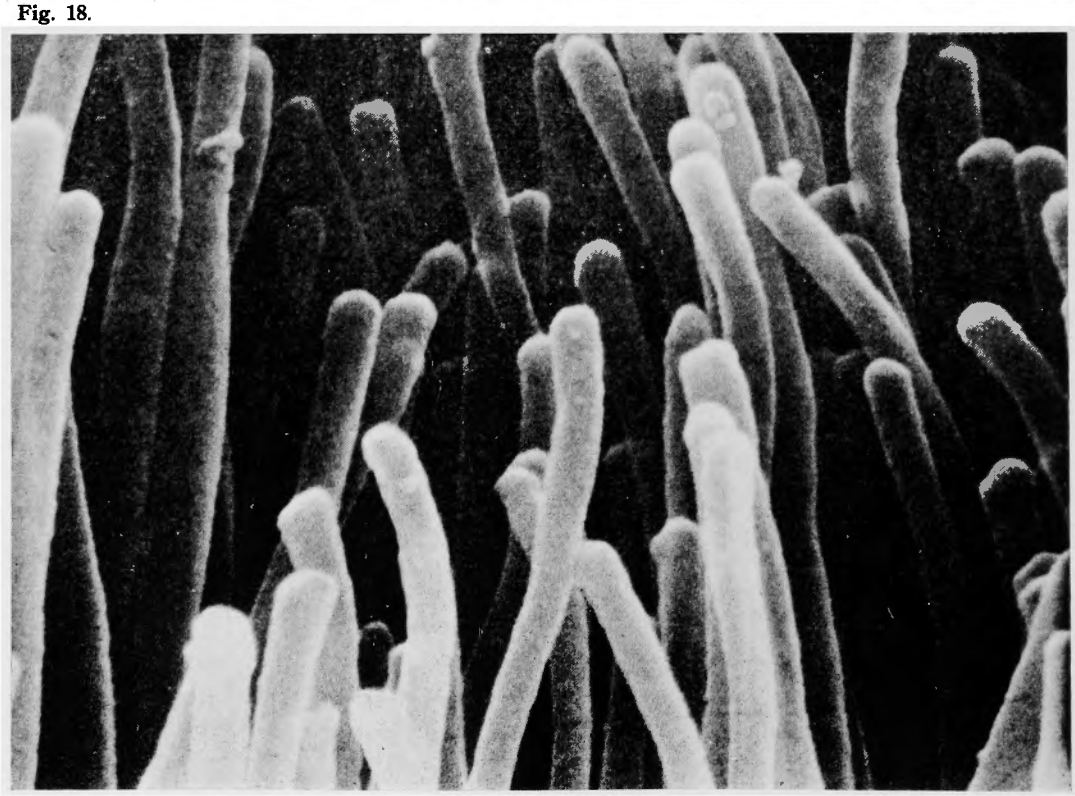


Fig. 18.



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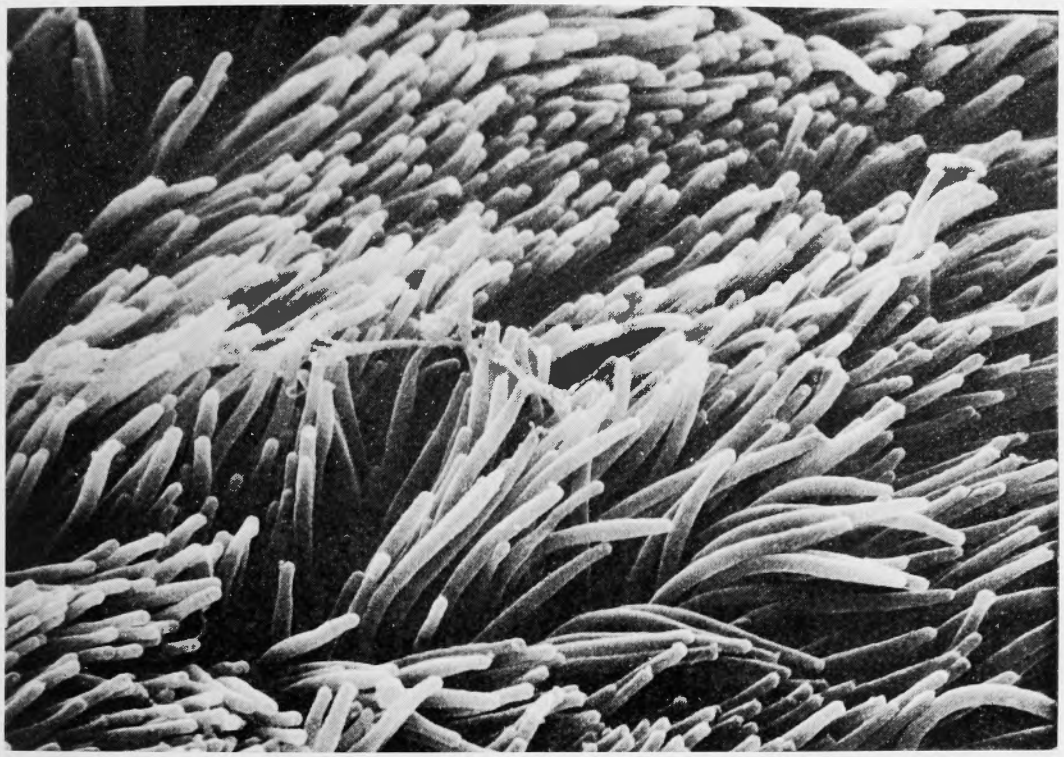

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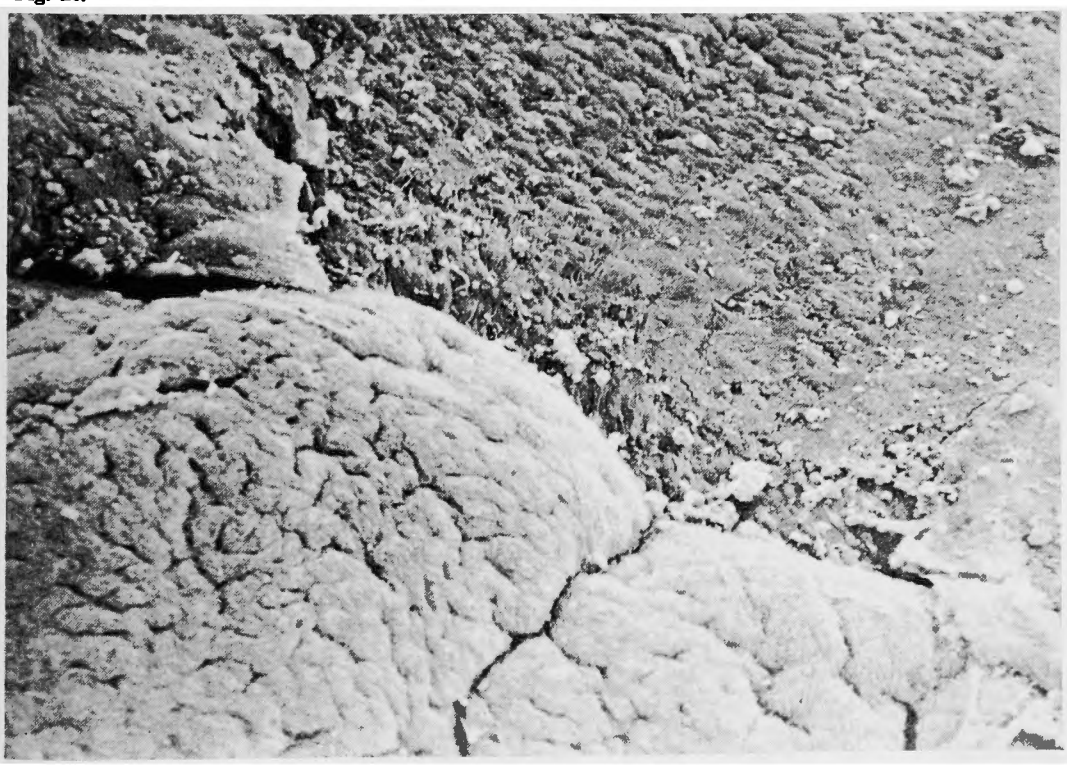
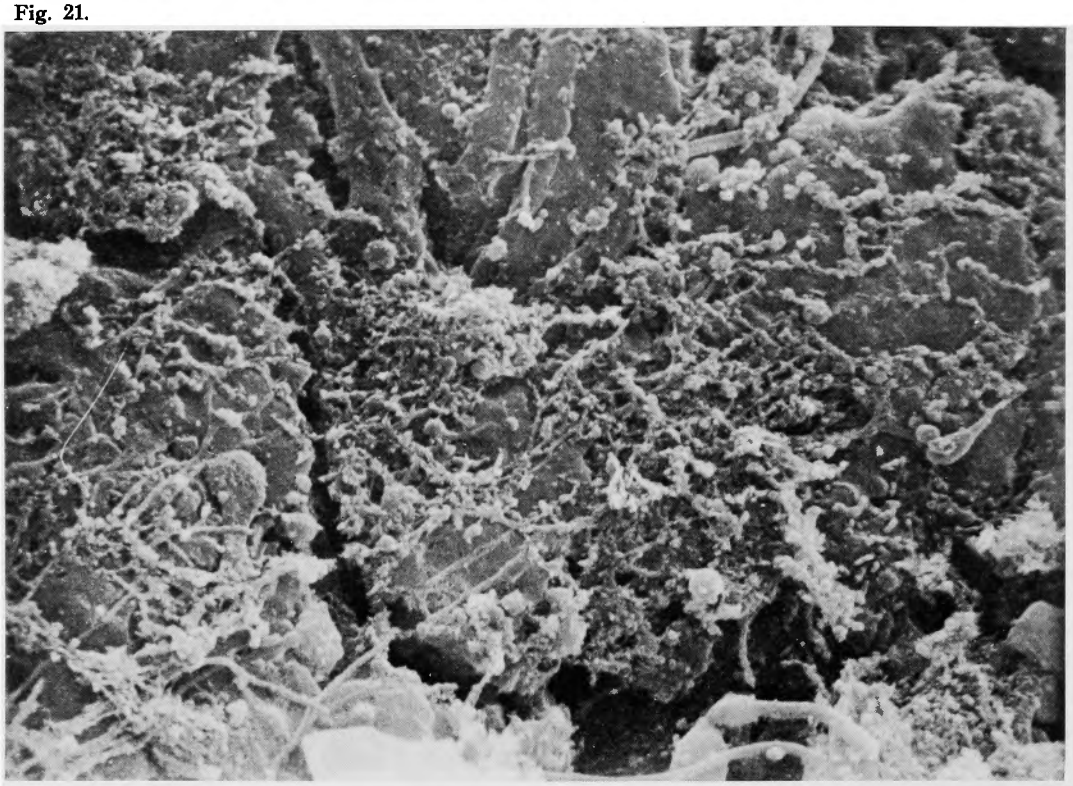


Fig. 21.



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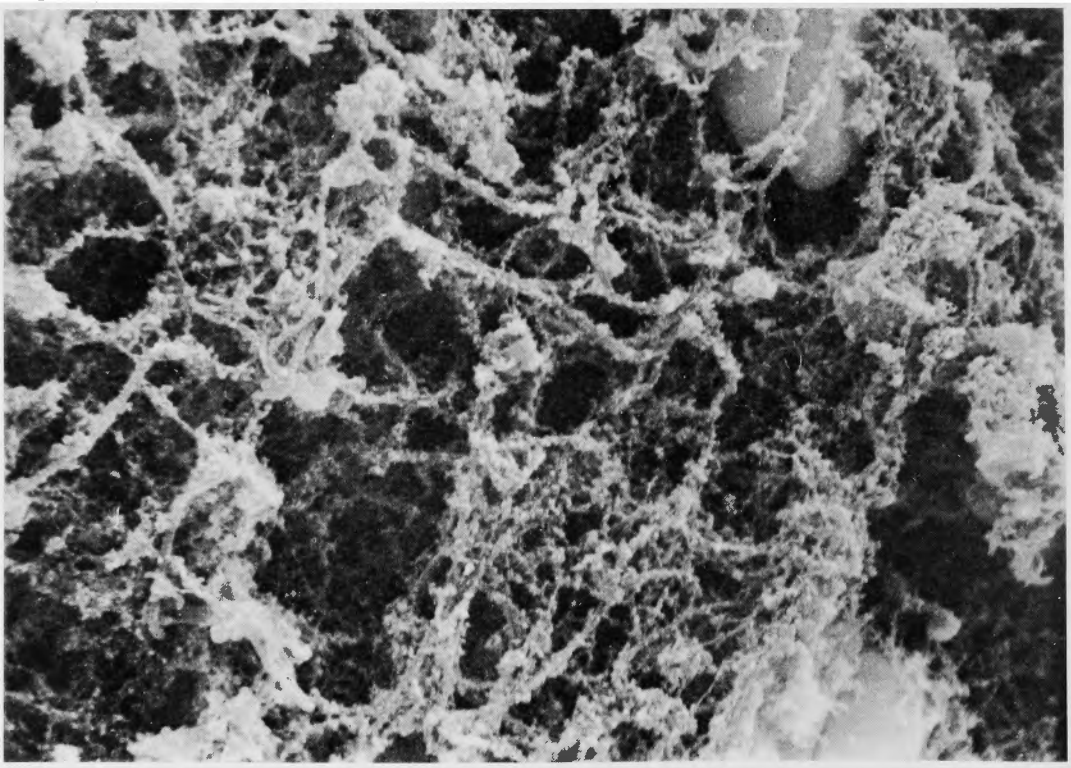
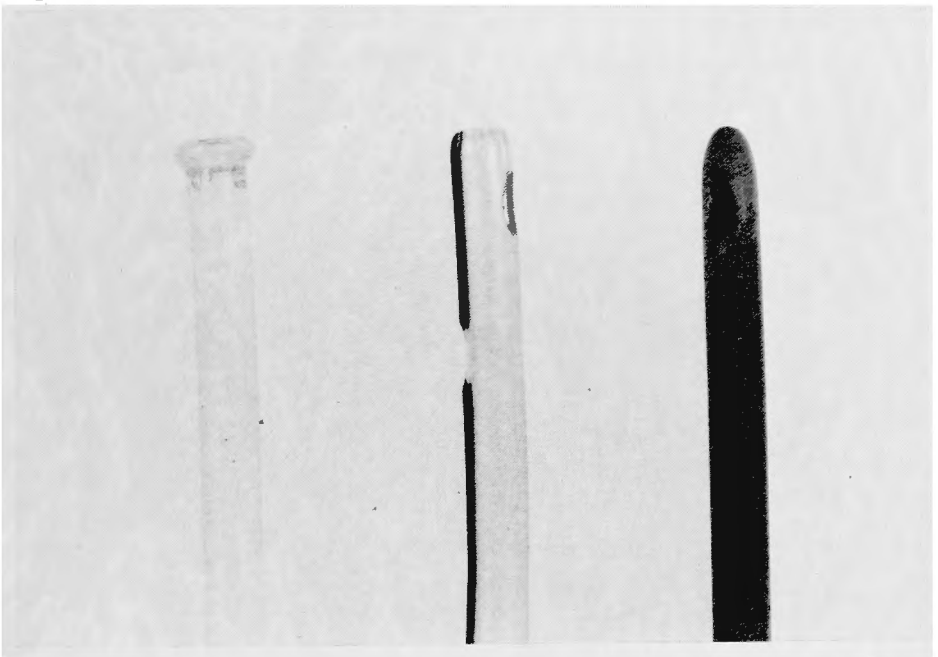


Fig. 23.



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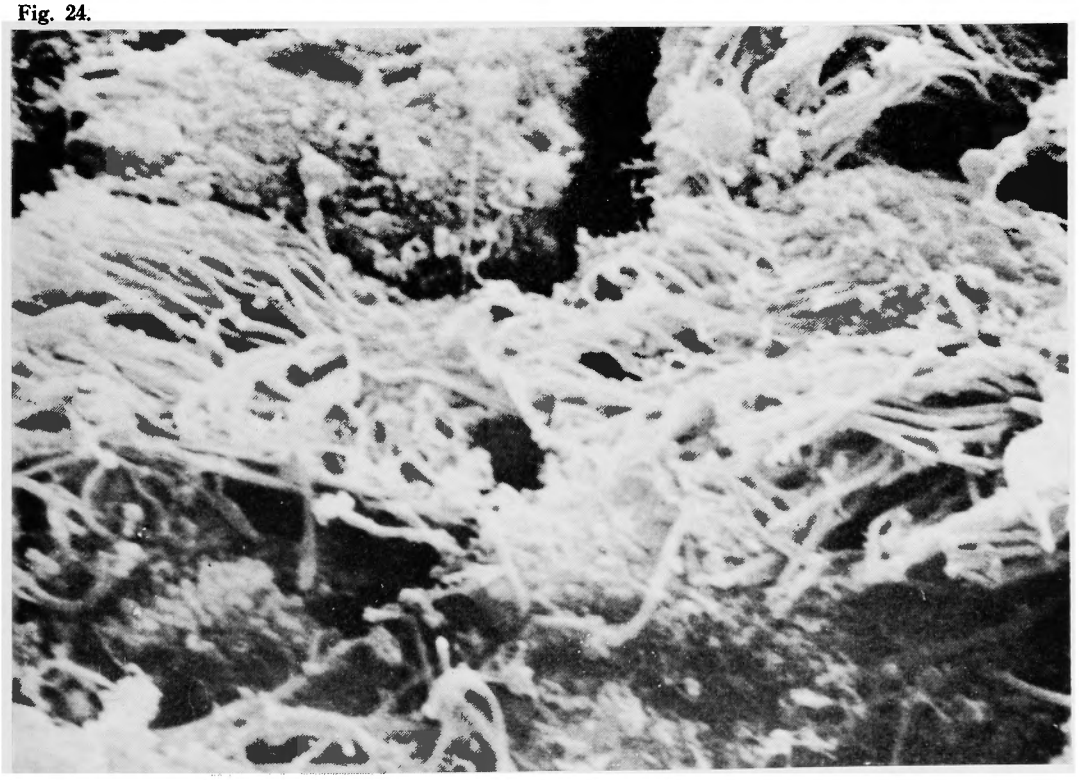

Fig. 25.

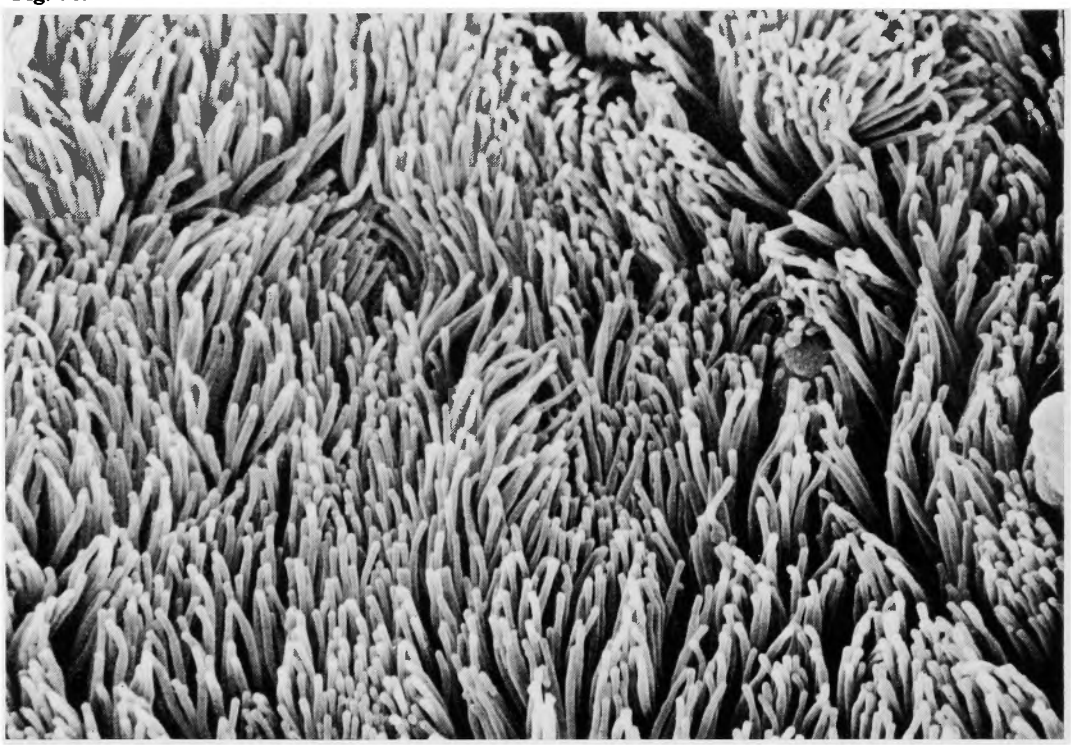

Fig. 26.