Nagoya J. Med. Sci. **49.** 21 ~ 30, 1987

EFFECTS OF ADRENERGIC AND CHOLINERGIC AGENTS AND LEUKOTRIENES ON MUCOCILIARY TRANSPORT FORCE MEASURED BY USING FROG PALATE

KENICHI YAMAKI, YASUNOBU NODA, KENZO TAKAGI, and TATSUO SATAKE

2nd Department of Internal Medicine Nagoya University School of Medicine

ABSTRACT

We have developed a new method to quantify the mucociliary transport force (MCTF). We validated the method by examining the effects of isoproterenol (Iso), norepinephrine (NE), epinephrine (Epi), and acetylcholine (ACh) on MCTF. In the first series of experiments, the effects of leukotrienes (LTs) on MCTF were investigated. In accordance with previous studies, Iso, NE, and ACh induced increases in MCTF, but Epi did not cause any significant effects. In the second series of experiments, LTC_4 induced a significant increase in MCTF and LTD_4 induced a significant decrease in MCTF, although these effects were transient. Neither LTB_4 nor LTE_4 affected MCTF. The effects of LTC_4 and LTD_4 were inhibited by a LT antagonist, FPL55712. These results indicate that we have developed a useful and sensitive method for quantitative determination of the immediate and direct effect of agonists on mucociliary transport, and that selective agonists may be involved in the defense mechanism of the lung to carry mucus plugs or locally produced debris out of the airways.

Key words: mucociliary transport force, isoproterenol, acetylcholine, frog palate

INTRODUCTION

Activity of mucociliary transport has been studied by two different methods: mucociliary transport clearance (MCC) (airway clearance of inhaled particles) and mucociliary transport velocity (MCTV) (the transport rate of markers placed on a mucosa). MCC has been widely used to determine regional clearance of inhaled particles in both animals and human subjects[1,2]; MCTV has been assessed in vitro[3,4] and in vivo[5,6] to determine surface mucus velocity.

The remarkable force of mucociliary transport has been suggested by Hilding, who examined the transport of large mucus plugs occluding the lumen in the trachea[7]. More recently, Stewart examined the weight-carrying capacity of ciliated epithelium of the palate and reported that there was neither decrease nor increase of particle transport at up to a pressure of 20 mg/mm^2 [8]. Although MCTV and MCC have been used conventionally to assess the function of mucociliary transport, it is reasonable to consider that the force of mucociliary transport, rather than the velocity (MCTV) or the clearance (MCC), is an important parameter of mucociliary function in carrying inhaled particles, mucus plugs, or locally produced debris out of airways against gravity.

Accordingly, we developed a new method to determine the mucociliary transport force (MCTF), defined as the tension developed by the mucus flow generated by the ciliated epithelium of a frog palate[9]. Using this method, we can assess MCTF as a more practical index of the activity of mucociliary transport. Moreover, our method has facilitated rapid and direct assessment of effects of pharmacologic agents on mucociliary function, none of which can be examined by the conventional methods.

山木健市，野田康信，高木健三，佐竹辰夫
Received for Publication April 30, 1986

MATERIAL AND METHOD

Bullfrogs were anesthetized with intraperitoneal injection of pentobarbital (25 mg/kg). Each frog was pithed, and its palate was dissected free and stored in Ringer's solution for frogs at 4°C for 48 hr to eliminate possible effects of the anesthetic and pithing. Each palate was pinned onto a silicon rubber bed lining the bottom of an organ bath with the mucosa facing upward. The organ bath (5 ml) was continuously perfused with Ringer's solution (warmed to 25°C) using a peristaltic pump (Tokyo Rikakikai, Tokyo, Japan) at a rate of 2 ml/min. A glass boat, consisting of two thin glass discs (15 mm in diameter) connected by three columns weighing 300 mg, was placed on the surface of a palate. One end of a silk thread was attached to the roof of the boat, and the other was tied horizontally to a force transducer (FD-612-T, Nihonkoden, Tokyo, Japan) (Fig. 1, 2). In order to eliminate the effects of changes in mucus volume retained between the bottom of the glass boat and the ciliated epithelia on MCTF, a loading weight of 2 g was placed on the roof of the glass boat to apply the boat firmly onto the ciliated epithelia, thus keeping the mucus volume between the boat and membrane rather constant. A 2 g weight was considered to be enough to eliminate the effect of the mucus volume between them on MCTF in preliminary studies. Since the surface area of the bottom of the boat was 1.8 cm^2, the pressure applied to the epithelium was calculated 12.2 mg/mm^2.

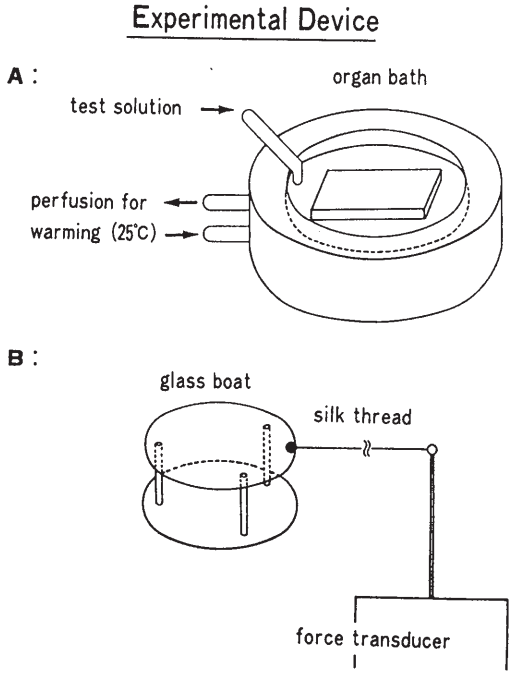

Fig. 1 Experimental device.
The organ bath (5 ml) is filled and perfused with Ringer's solution for frogs and maintained at 25°C (A). The glass boat consists of two thin glass discs with three columns between them. One end of a silk thread is attached to the roof of a glass boat and the other end is tied horizontally to a sensitive force transducer (B).

After positioning the glass boat near the center of the frog palate by adjusting the length of the silk thread, the force developed thereafter was monitored using a sensitive force transducer, amplifier (RD-5, Nihonkoden, Tokyo, Japan), and signal recorder (RECTI-HORIZ-8-K, San-Ei Instrument, Tokyo, Japan). The force developed gradually, reaching a plateau in a few minutes. Ringer's solution was then exchanged for a test solution to examine the effects of drugs on MCTF. One palate preparation was used for only one application of a drug.

In the first series of experiments, the effects of isoproterenol (Iso), norepinephrine (NE), epinephrine (Epi), and acetylcholine (ACh) on MCTF were examined. All drugs were purchased from Sigma Chemical (St. Louis, USA) and dissolved in Ringer's solution for frogs at a concentration of 10^{-5} M. In the second series of experiments, the effects of leukotriene (LT) B_4, LTC_4, LTD_4, and LTE_4 (Merk Frost, Kirkland, Canada) were investigated using the same method. The concentrations of LTs used in this experiment were between 10^{-7} M and 10^{-9} M. Changes in MCTF were followed for 20 min after the application of test solutions or the control Ringer's solution. MCTF values in Tables 1 and 2 were calculated by the tension assessed at the individual time period with the following equation: $\text{Tension}_T/\text{Tension}_0 \times 100$ (T = time period and 0 = introduction of test drug). The values were expressed as the mean ± standard errors.

Statistical analyses were performed by unpaired t-test using the values of tension assessed every 5 min. Differences between the means were considered to be statistically significant when the probability value was less than 0.05.

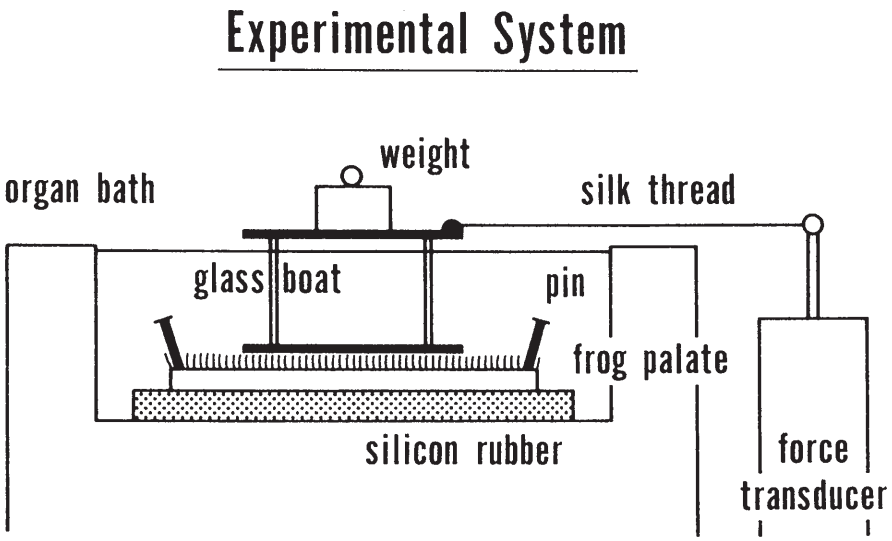

Fig. 2 Experimental system.
The determination is performed on a frog palate. When the glass boat is placed in the center of a mucosa, the force, as the tension between the glass boat and the force transducer, develops soon due to mucociliary transport. In order to eliminate the possible effect of the changes in the mucus between the bottom of the glass boat and the ciliated epithelium on MCTF, a loading weight of 2 g is placed on the roof of the glass boat to apply the glass boat tightly to the mucosa.

RESULTS

Tension created by mucociliary transport developed rapidly after the glass boat was placed on the center of a frog palate (Fig. 3, 5), and reached a plateau at about 1.1 g. Table 1 and Fig. 3 and 4 show the effects of NE, Epi, Iso, and ACh on MCTF. Fig. 3 shows a typical trace of MCTF for the control (top), and the traces to demonstrate the effects of 10^{-5} M Iso (middle) and 10^{-5} M ACh (bottom) on MCTF. Iso, NE, and ACh induced rapid increases in MCTF, whereas Epi did not cause any significant change in MCTF throughout the 20 min observation period. The changes in MCTF were calculated with the following equation: $(MCTF_T - MCTF_B)/MCTF_B \times 100$ (T = test drug and B = base line). Iso, NE, and ACh increased MCTF with percent increases of 19.2 ± 2.7%, 10.0 ± 2.6%, and 16.2 ± 3.1% at 20 min after the drug application, respectively (n = 10).

Table 1. Effects of adrenergic and cholinergic agents on MCTF.

	5	10	15	20 (min)
Epinephrine 10^{-5}M (n=10)	98.9±0.9	97.9±1.7	96.4±2.0	94.8±2.3
Norepinephrine 10^{-5}M (n=10)	99.8±1.5	100.8±1.8^{+}	101.3±2.1^{++}	100.4±2.4^{++}
Isoproterenol 10^{-5}M (n=10)	105.1±1.4^{++}	108.4±1.7^{++}	108.4±2.1^{++}	108.7±2.3^{++}
Acetylcholine 10^{-5}M (n=10)	106.7±1.7^{++}	109.1±2.1^{++}	107.4±2.5^{++}	106.0±2.7^{++}
Control (n=10)	98.4±0.6	96.1±0.5	93.3±1.0	91.2±1.4

+ : P<0.05, ++ : P<0.005 mean ± SEM

The values are calculated by the tension assessed at individual time period. NE, Iso, and ACh induced significant increases in MCTF, but Epi did not affect MCTF.

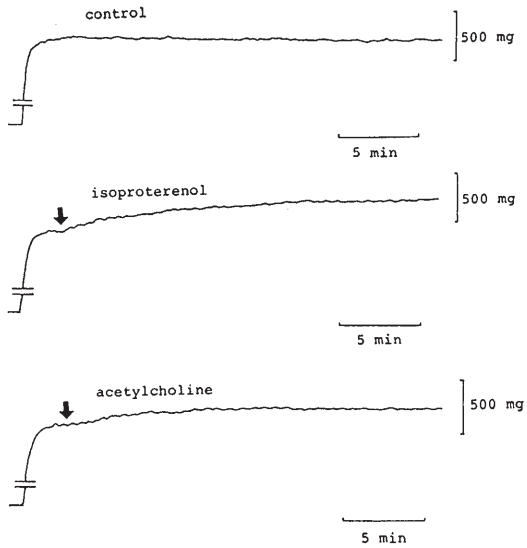

Fig. 3 Trace of MCTF of isoproterenol and acetylcholine.
Typical traces of MCTF for control (top), 10^{-5} M Iso (middle) and 10^{-5} M ACh (bottom) are shown.

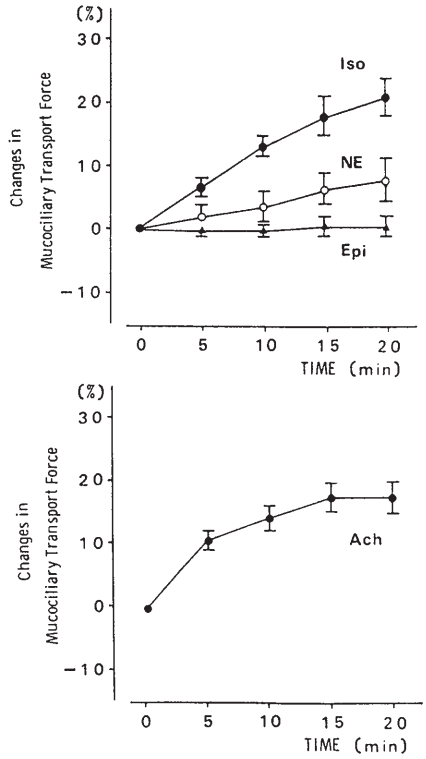

Fig. 4 Effects of adrenergic and cholinergic agents on MCTF.
The changes in MCTF of 10^{-5} M Iso, 10^{-5} M NE, 10^{-5} M Epi, and 10^{-5} M ACh are shown, The data were expressed as the mean ± standard errors. Iso, NE, and ACh induced significant increases in MCTF ($p < 0.005$), but Epi did not affect MCTF.

Table 2, Fig. 5, and Fig. 6 show the effect of LTs on the MCTF of frog palates. Figure 5 shows a typical trace of MCTF for the controls (top), and the traces to demonstrate the effects of 10^{-7}M LTC_4 (middle) and 10^{-7}M LTD_4 (bottom) on MCTF. LTC_4 induced a significant increase in MCTF at 5 and 10 min after introduction of the drug. LTC_4 (10^{-7}M) increased MCTF with percent increases of 6.1 ± 2.4%, 6.6 ± 3.2%, and 7.0 ± 4.1% at 5, 10, and 15 min after the drug application (n = 5), respectively while LTD_4 induced a significant decrease in MCTF at 5 and 10 min after the application. LTD_4 (10^{-7}M) decreased MCTF with percent decreases of 6.3 ± 1.7% and 3.5 ± 1.1% at 5 and 10 min after the application (n = 5), respectively. Decreases in MCTF induced by LTD_4 were also observed at concentrations of 10^{-8}M and 10^{-9}M. The effects of these drugs were transient and diminished at 20 min after the application. In contrast to these results, neither LTB_4 nor LTE_4 caused any significant effect on MCTF even at our highest concentration (10^{-7}M).

To investigate the action of a LT antagonist against the changes of MCTF induced by LTC_4 or LTD_4, the frog palates were perfused with 10^{-8}M FPL55712 dissolved in the Ringer solution together with 10^{-7}M LTC_4 or LTD_4. FPL55712 inhibited both the increase in MCTF induced by LTD_4 and the decrease in MCTF by LTD_4.

Table 2. Effects of leukotrienes on MCTF.

	5	10	15	20 (min)
LTC_4 10^{-7}M (n = 5)	104.4 + 2.3†	102.4 + 3.0†	99.8 + 3.8†	98.1 + 4.4
10^{-8}M (n = 5)	99.3 + 0.8	96.6 + 1.1	93.5 + 1.5	90.6 + 2.1
10^{-9}M (n = 5)	97.8 + 1.4	95.0 + 1.9	91.7 + 2.0	89.0 + 2.0
LTD_4 10^{-7}M (n = 5)	92.2 + 1.8†	92.7 + 1.1†	90.0 + 2.1	87.4 + 1.8
10^{-8}M (n = 5)	93.1 + 1.6†	91.0 + 1.6†	89.1 + 2.1	86.7 + 1.8
10^{-9}M (n = 5)	96.6 + 0.9	92.6 + 2.0†	89.6 + 4.7	87.5 + 1.7
LTB_4 10^{-7}M (n = 5)	98.7 + 0.6	97.9 + 1.3	94.6 + 2.2	92.5 + 2.2
LTE_4 10^{-7}M (n = 5)	97.1 + 1.0	95.4 + 1.0	92.4 + 1.0	90.1 + 1.9
Control (n = 10)	98.4 + 0.6	96.1 + 0.5	93.3 + 1.0	91.2 + 1.4

† : $P < 0.05$ mean ± SEM

The values are calculated by the tension assessed at individual time period. LTC_4 induced a significant increase, and LTD_4 induced a significant decrease in MCTF. Neither LTB_4 nor LTE_4 affected MCTF.

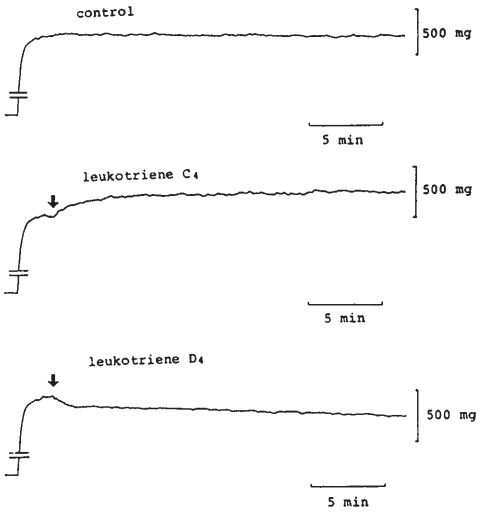

Fig. 5 Trace of MCTF of LTC_4 and LTD_4.
Typical traces of MCTF for control (top), 10^{-7}M LTC_4 (middle), and 10^{-7}M LTD_4 (bottom) are shown.

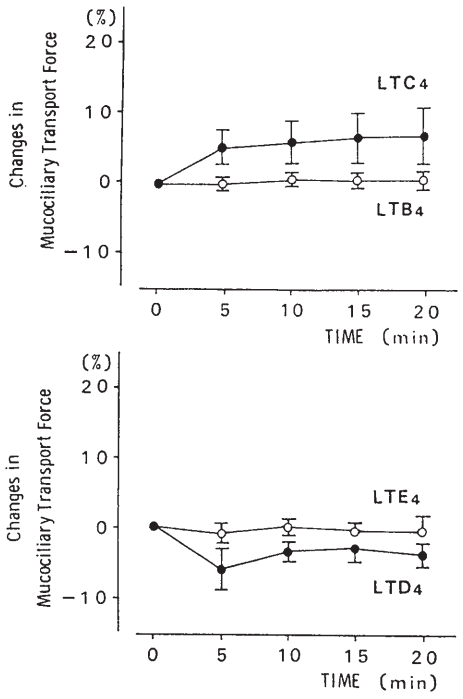

Fig. 6 Effects of leukotrienes on MCTF.
The changes in MCTF of 10^{-7}M LTB_4, 10^{-7}M LTC_4, 10^{-7}M LTD_4, and 10^{-7}M LTE_4 are shown. The data were expressed as the mean ± standard errors. LTC_4 induced an increase in MCTF, and LTD_4 induced a decrease in MCTF ($p < 0.05$). Neither LTB_4 nor LTE_4 affected MCTF.

DISCUSSION

The clearance of inhaled particles, mucus plugs, or locally produced debris from the tracheobronchial tree by the action of mucociliary transport is an important pulmonary defense mechanism against the development of various diseases. Conventional methods for the determination of the activity of mucociliary transport have their methodological limitations. For example, assessment of MCC is possible only in vivo using radioactive particles, and the clearance patterns thus obtained depend not only on mucociliary transport but also on the sites of particle deposition. Although the determination of MCTV can be performed both in vivo and in vitro, MCTV is known to vary considerably with the sites of measurement. Moreover, the in vivo determinations of MCC and MCTV are invariably influenced by various factors, such as changes in air flow, mucus volume, and the caliber of airway. These factors might make the changes in MCC and MCTV induced by some agonists in the former studies much greater than those of MCTF that we observed in this study. Moreover, MCTV, which determines the horizontal distance of the particle transported, can not be a reliable index in the assessment of the activity of mucociliary transport to expel the particles or mucus plugs upward out of the airways against gravity. Neither MCTV nor MCC has been able to provide information on the immediate and direct effects of the agents on mucociliary transport. Therefore, we have introduced a new method, by which the activity of mucociliary transport can be assessed in a prompt and direct manner.

The function of mucociliary transport is known to be influenced not only by the ciliary motility but also by the volume and the chemical and physical properties of mucus. Since we constantly perfused the organ bath and used a 2 g weight on the glass boat, the effect of changes in mucus volume on MCTF can be considered negligible. Thanks to this, we preliminarily validated good reproducibility of the experiments and the precise determination of MCTF, which is considered to be produced by the flow of mucus generated by ciliary activity. Advantages of measuring MCTF as an index of the activity of mucociliary transport are its abilities to (1) determine the over-all function of mucociliary transport generated by mucus flow and ciliary motility, (2) continuously monitor the direct effects of drugs without being influenced by the changes in air flow and bronchial constriction, etc., (3) apply drugs to mucociliary transport in homogeneous distributions and conditions, and (4) assess the activity of mucociliary transport as an area rather than as a line, as in the case of MCTV.

Effects of adrenergic and cholinergic agents on mucociliary transport have been studied extensively by assessing the changes in frequency of ciliary beat[10,11], mucus secretion[12,13] rates of mucociliary transport[14,15], and clearance of inhaled particles[16,17]. These studies have shown that administration of adrenergic agents stimulates the activity of mucociliary of adrenergic agents stimulates the activity of mucociliary transport by increasing the ciliary beat frequency and the mucus secretion. In accordance with these studies, we found that a beta-adrenergic agent, Iso or NE, and a cholinergic agent, ACh, induce a prompt and significant increase in MCTF. Iso induced the same degree of increase in MCTF as ACh, and induced a more significant increase than NE at a concentration of 10^{-5} M. However, Epi induced only a slight increase in MCTF which was not considered to be significant. Although the responses achieved by these drugs in our method were not as remarkable as those reported in MCC and MCTV studies, this is probably due to the fact that the responses in MCC and MCTV studies were augmented by various factors such as the changes in air flow and the airway caliber. Changes in the mucociliary transport function per se could be found in this method.

Effects of LTs have been studied and reported as follows: LTC_4 increased the ciliary beat frequency in vitro[18]; LTD_4 induced the secretion of mucus[19,20]; and FPL55712, a specific antagonist of LTs, increased MCTV in the in vivo experiment using a dog[21]. In our experiment, in which the effect of mucus volume had been virtually eliminated, LTC_4 increased MCTF but LTD_4 decreased

MCTF soon after the application. This finding is worth noting because both of these agents are known to be potent constrictors of smooth muscles of the bronchi[22] and because a decrease in MCC or MCTV has been reported in patients with asthma[23,24]. Since LTD_4 induced greater change, a decrease, in MCTF than LTC_4 did at lower concentrations, LTD_4 would seem to play a more important role than LTC_4 in the maintenance or the development of physiological or pathogenic conditions.

In this study, we confirmed that the determination of MCTF by the use of our new method is valid for the assessment of the activity of mucociliary transport, and found that not only Iso, NE, and ACh, but also LTC_4 and LTD_4 alter the magnitude of MCTF. The effects of Iso, NE, and ACh were more significant that those of LTC_4 and LTD_4. Therefore, it is implicated that LTs partly contribute to the maintenance or the determination of physiological function of mucociliary transport.

REFERENCES

1) Yeates, D.B., Larkridge, C.G., Marrinelli, A., *et al.* Unsedated animal models of pulmonary mucociliary transport. *Biorheology,* **21**, 273–283, 1984.
2) Foster, W.M., Langenback, E.G., and Bergofsky, E.H. Respiratory drugs influence lung mucociliary clearance in central and peripheral ciliated airways. *Chest,* **80**, 877–880, 1981.
3) Purchelle, E. and Zahm, J.M. Influence of rheological properties of human bronchial secretions on ciliary beat frequency. *Biorheology,* **21**, 265–272, 1984.
4) Hill J.R. The influence of drugs on ciliary activity. *J. Physiol.,* **139**, 157–166, 1957.
5) Santa Cruz, R., Landa, J., Hirsch, J., *et al.* Tracheal mucous velocity in normal man and patients with obstructive lung disease: effects of terbutaline. *Am. J. Respir. Dis.,* **109**, 458–463, 1974.
6) Wanner, A., Zarzecki, S. Hirsch, J., *et al.* Tracheal mucous transport in experimental canine asthma. *J. Appl. Physiol.,* **39**, 950–957, 1975.
7) Hilding, A.C. and Minn, D. The role of ciliary action production of pulmonary atelectasis, vaccuum in the paranasal sinuses, and in otitis media. *An. Otol. Laryngol.,* **52**, 816–833, 1943.
8) Stewart, W.C. Weight-carrying capacity and excitability of excised ciliated epithelium. *Am. J. Physiol.,* **152**, 1–5, 1948.
9) Yamaki, K., Satake, T., Yamakawa, I., *et al.* A new method to evaluate the ciliary transport function by utilizing the organ bath. *Panminerva Medica,* **28**, 145–147, 1986.
10) Verdugo, P., Johnson, N.T., and Tan, P.Y. β-adrenergic stimulation of respiratory ciliary activity. *J. Appl. Physiol.,* **48**, 868–871, 1980.
11) Van As, A. The role of selective β_2-adrenoceptor stimulants in the control of ciliary activity. *Respiration,* **31**, 146–151, 1974.
12) Leikoauf, G.D., Ueki, I.F., and Nadel, J.A. Autonomic regulation of viscoelasticity of cat tracheal gland secretions. *J. Appl. Physiol.,* **56**, 426–430, 1984.
13) Sturgess, J. and Reid, L. An organ culture study of the effect of drugs on the secretory activity of the human bronchial submucosal gland. *Clinical Science,* **43**, 533–543, 1972.
14) Kordic, P., Bülbring, E., and Burn, J.H. Ciliary movement and acetylcholine. *Brit. J. Pharmachol.,* **7**, 67–79, 1952.
15) Yamada, S. and Maruyama, I. Effects of various autonomic drugs on ciliary movement on a palatine mucosa of a frog. *Pharmacometricus,* **22**, 587–596, 1981.
16) Foster, W.M., Bergofsky, E.H., Bohning, D.E., *et al.* Effect of adrenergic agents and their mode of action on mucociliary clearance in man. *J. Appl. Physiol.,* **41**, 146–152, 1976.
17) Camner, P., Strandberg, K., and Philipson, K. Increased mucociliary transport by adrenergic stimulation. *Arch. Environ. Health.,* **31**, 79–82, 1976.
18) Wanner, A., Maurer, D., Abraham, W.M., *et al.* Effects of chemical mediators of anaphylaxis on ciliary function. *J. Allergy Clin. Immunol.,* **72**, 663–667, 1983.
19) Johnson, H.G. and McNee, M.L. Secretogogue responses of leukotriene C_4, D_4: comparison of potency in canine trachea in vivo. *Prostaglandins,* **25**, 237–243, 1983.
20) Marom, Z.V.I., Shelhamer, J.H., Bach, M.K., *et al.* Slow-reacting substances, Leukotrienes C_4 and D_4, increase the release of mucus from human airways in vitro. *Am. Rev. Respir. Dis.,* **126**, 449–451, 1982.

21) Ahmed, T., Greenblatt, D.W., Birch, S., *et al.* Abnormal mucociliary transport in allergic patients with antigen-induced bronchospasm: role of slow reacting substance of anaphylaxis. *Am. Rev. Respir. Dis.*, **124**, 110–114, 1981.
22) Stjernschantz, J. The leukotrienes. *Med. Biol.*, **62**, 215–230, 1984.
23) Mezey, R.J., Cohn, M.A., Fernandez, R.J., *et al.* Mucociliary transport in allergic patients with antigen-induced bronchospasm. *Am. Rev. Repir. Dis.*, **118**, 677–684, 1978.
24) Foster, W.M., Langenback, E.G., and Berkofsky, E.H. Lung mucociliary function in man: interdependence of bronchial and tracheal mucus transport velocities with lung clearance in bronchial asthma and healthy subjects. *An. Occup. Hyg.*, **26**, 227–244, 1982.