The Effect of Various Conditions on Tracheal Mucociliary Transport in Dogs[1] (36570)

YASUO SAKAKURA AND DONALD F. PROCTOR
(Introduced by F. B. Bang)

Departments of Environmental Medicine and of Pathobiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205

In spite of increasing attention to the role of mucociliary transport in defence against the atmospheric environment, and a large number of investigations into various factors which may impair this mechanism many questions remain insufficiently answered. Cigarette smoking, one of the most common chemical contaminants among human population, is known to depress the rate of mucus transport in mammalian mucociliary systems (1, 2). Various airborne contaminants may affect mucociliary physiology and may slow down mucus transport (3, 4). Delay in elimination of potentially pathogenic inhaled particles may result in accumulation of such particles in the bronchial tree. Thus mucociliary malfunction could be related to such common diseases as pulmonary emphysema, chronic bronchitis, and laryngeal and pulmonary cancer. Knowledge of factors which affect mucociliary clearance continues to assemble from a variety of invertebrate and vertebrate systems (5–7). At the same time, increasing urban industrialization, mobility and intermixture of human virus populations and sudden changes of environment constantly impose new burdens on the human respiratory mucous membranes.

In this study we present a series of investigations of the effect of several extrinsic and one intrinsic factor on mucociliary transport rates in the dog trachea. We chose the dog because of its long habituation to human-related environment, and the close similarity of its tracheal physiology to that of human.

Materials and Methods. The methods of monitoring mucociliary flow rates were developed in this laboratory and have been reported in detail (8–10).

Observations were made on 34 mongrel dogs of both sexes ranging from 9 to 16 kg in weight. The dogs were anesthetized with 20 mg of sodium pentobarbital/kg of body weight injected intravenously 30 min after injections of 0.3 ml/kg of Innoval[2] intramuscularly. The aim had been to maintain a relatively constant level of anesthetic depth throughout the measurement.

This mixture of anesthetic drugs was found to impair mucociliary clearance less than barbiturate anesthesia alone. A 3-cm vertical incision was made through the skin and the anterior part of the trachea was exposed by blunt dissection in order to avoid disturbance of tracheal blood vessels and nerves. Using a heated needle, a small hole was made in the cartilage of the 16th to 21st tracheal ring, depending on the size of the dog. A plastic catheter (1 mm o.d. diam) was inserted into the lumen of the trachea through this hole to place the tagged particle on the mucous membrane of the posterior tracheal wall. No attempt was made to carry out these studies under aseptic conditions.

The particles used in this study were anion exchange resins in the form of small (less than 0.5 mm diam) spherical beads (Dowex 1-x8 20–50 mesh).[3] The resin beads were labeled with the ^{99}Tc pertechnetate (TcO_4^{2-}) ion, by soaking in a solution of technetium-99m. Each particle possessed approximately

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[2] Innoval (1 ml) = 0.05 mg fantanyl citrate + 2.5 mg droperidol. McNeil Laboratory, Inc., Fort Washington, PA 19034.

[3] Cal-Biochem, Los Angeles, CA 90054.

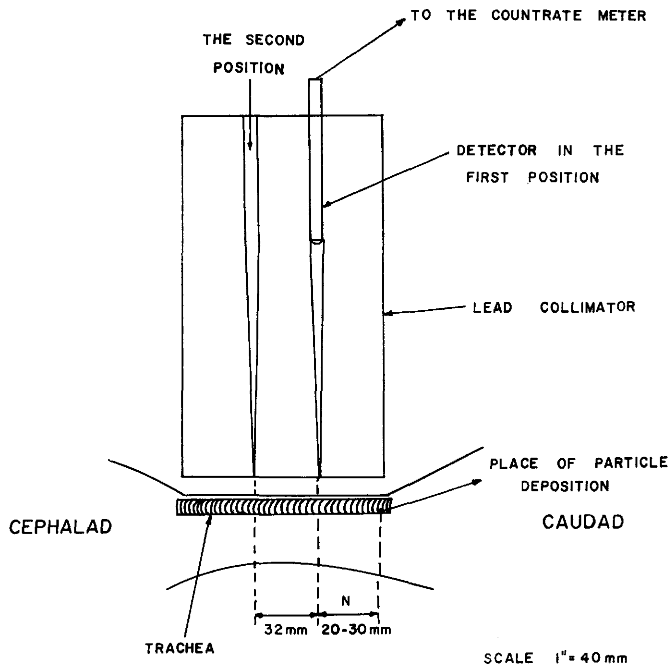

FIG. 1. Diagram showing a relationship between a point of deposition of a particle in the trachea and a lead collimator.

50 μCi of activity. The particles were stained with bromophenol blue[4] for easy visualization.

The dogs were placed ventral side up in a two-section plastic chamber. The head section was separated from the neck and body section by an insulated rubber collar and maintained at constant temperature and relative humidity, at any desired level.

A single lead collimator with two holes was placed over the upper part of the neck in line with the trachea. A small scintillation detector was first put over the proximal hole of the collimator (the first position) and later shifted 3.2 cm to the distal one (the second position) as soon as the particle passed under the first position. The distance between the first position and the site where the particle was initially placed varied from 2 to 3 cm according to the size of dog (Fig. 1). The catheter and incision were closed during the measurements. As the radioactive particle passed in sequence under these two positions, the activity was picked up by the detector first at the proximal and later at the distal position. The signal from the detector was fed through a count rate meter to the recorder. The rate of movement of the particles was calculated from the record and expressed as millimeters per minute.

All dogs were initially exposed to ambient air at 24°, 78% RH (18 mm Hg of water vapor pressure). Ambient air in the head section was then changed to the test condition. The temperature and relative humidity in the head section were recorded during the measurement with an electric thermometer and hygrometer.[5] Rectal temperature and respiratory rate were also recorded. A typical recording is demonstrated in Fig. 2.

Pilocarpine was administered intravenously 1 mg/kg at the condition of 24°, 78% RH after control measurements of flow rate.

In the study of cigarette smoke, a thin vinyl tube 2 mm in diameter was inserted into the hypopharynx orally. Nonfiltered commercial cigarettes were used. A 15-ml

[4] Matheson Company, Inc., Cincinnati, OH.

[5] Electric hygrometer indicator, Hygrodynamics, Inc., Silver Spring, MD. Stated by manufacturer to be accurate to ±2% relative humidity with sensitivity of 0.15% relative humidity.

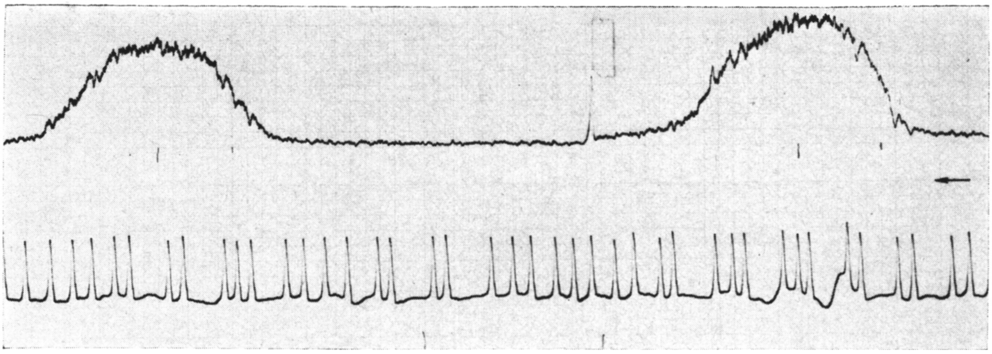

FIG. 2. Read from right to left (this record represents 6 min). Record of detected radioactivity, above, showing passage of first and second detectors (3.5 min from appearance at first to appearance of second). Below, record of respiratory frequency. This is a typical "normal" record and shows a flow rate of 9.1 mm/min. Increasing radioactivity as particle passes by detector is indicated by an upward deflection of the upper trace.

puff of smoke was insufflated manually with a syringe through the tube at the beginning of the every fourth inspiration in 9-puff runs, and every second inspiration in 12- and 15-puff runs, respectively. After completion of one measurement in the cigarette smoke study, the next run was started after 30 min to avoid an accumulation effect.

Some dogs were studied on 2 to 5 occasions at 1-week intervals. After the dogs were sacrificed by injection of excess sodium pentobarbital intravenously, the trachea was removed between tracheal rings 12 and 17. Tissues were fixed in 10% formalin and embedded in paraffin. Sectioned preparations were stained with H&E and AB-PAS for histopathological examination.

Results. A total of 535 measurements of mucociliary transportation rate were attempted in 63 experiments on 34 dogs. Measurements were successfully completed in 31.1% of the attempts. In the 63 experiments, 20 showed no particle movement. The trachea of most of these dogs were histologi-

TABLE I. Mucociliary Transport Rates (mm/min) Under Control Condition; 24°, 78% RH (wvp 18 mm Hg).[a]

Expt. no.	Mean	No. of measurements	Expt. no.	Mean	No. of measurements
4	14.0	2	46	4.0	4
19	14.6	3	48	11.4	2
20	6.4	3	50	11.4	2
21	12.0	2	52	18.1	2
23	1.2	3	53	14.4	2
25	8.4	3	54	11.2	2
26	3.0	3	55	24.4	6
32	9.3	3	56	11.5	4
33	4.0	4	57	16.7	5
34	15.6	3	58	0.7	3
35	2.4	3	59	12.7	5
36	21.0	2	60	35.1	3
37	6.9	3	62	7.0	3
38	13.0	3	63	4.3	4
42	5.6	7	65	10.3	3
44	2.9	3	66	3.9	3

[a] Mean mucociliary transport rate: 10.5 mm/min (SD 7.39), $N = 32$.

TABLE II. Daily Change in Mucociliary Transport Rate (mm/min) Under Control Condition; 24°, 78% RH (wvp 18 mm Hg).

						Range (times)	Means	SD
Expt. no.	31	32	34	36				
Dog 23	(12)[a]	9.4	15.6	21.0		2.2	15.3	5.80
Expt. no.	33	35	37					
Dog 24	4.0	2.3	6.9			3.0	4.4	2.33
Expt. no.	38	39	41	44				
Dog 25	13.0	2.9[b]	(3)[a]	2.9		4.5	6.3	2.60
Expt. no.	45	46	50	51	53			
Dog 28	3.6[b]	4.0	11.4	(5)[a]	14.4	3.6	8.4	5.39
Expt. no.	52	54	55					
Dog 30	18.1	11.2	24.4			2.2	17.9	2.56

[a] On these occasions measurements were not successful. Numbers in parentheses indicate number of measurements attempted.

[b] On these occasions only one measurement was successful so the data are not listed in Table I.

cally normal, but others had such pathological changes as squamous metaplasia, chronic inflammation, and possible dehydration.

Mucociliary transport rates were measured under four environmental conditions: (a) 24°, 78% RH (wvp 18 mm Hg) (control); (b) 24°, 100% RH (wvp 22 mm Hg); (c) 24°, 21% RH (wvp 6 mm Hg); (d) 7.2°, 32% RH (wvp 3 mm Hg). All studies were conducted under nasal breathing conditions.

In the control condition, mucociliary transport rates ranged from 0.7 to 35.1 mm/min, on the basis of measurements from 103 particles in 32 experiments on 19 dogs; the mean was 10.5 mm/min (SD 7.39). As shown in Table I, the transit rates among different dogs varied widely: 9.4% were below 3 mm/min and 3.1% were above 30 mm/min. Table II shows the daily change of the transit rates in 5 dogs measured 3 or more times at weekly intervals.

The effect of relative humidity at constant temperature is shown in Table III. The overall difference between control and 100% RH was not significant, but in two of the four individual experiments there was an in-

TABLE III. Effect of Change of Relative Humidity on Mucociliary Transport Rate (mm/min).

Expt. no.	24°, 78% RH (wvp 18 mm Hg)	No.[a]	24°, 100% RH (wvp 22 mm Hg)	No.[a]	24°, 21% RH (wvp 6 mm Hg)	No.[a]
32	9.3	3			8.5	3
33	4.0	4			1.6	3
34	15.6	3			11.4	3
35	2.4	3			2.7	4
Mean	7.8	$N = 4$			6.1	$N = 4$
21	12.0	2	19.6[b]	3	20.3	3
23	1.2	3	3.3[b]	3	4.7[bc]	4
25	8.4	3	7.4	3	12.1	2
26	3.0	3	1.9	2	3.2[bc]	4
Mean	6.2	$N = 4$	8.1	$N = 4$	10.1	$N = 4$

[a] No. = number of measurements.

[b] Statistically significant, compared with the base line value.

[c] Statistically significant, compared with the value measured at 24°, 100% RH (wvp 22 mm Hg).

TABLE IV. Effect of Pilocarpine on Mucociliary Transport Rate (mm/min) Under Control Condition; 24°, 78% RH (wvp 18 mm Hg).

Expt. no.	Control	No.[a]	Pilocarpine[b]	No.[a]
53	14.4	2	37.9[c]	7
58	0.7	3	13.2[c]	3
59	12.7	5	33.0[c]	3
60	35.1	3	34.9	4
Mean	15.5	$N = 4$	29.8	$N = 4$

[a] No. = number of measurements.

[b] Pilocarpine was injected intravenously at a concentration of 1 mg/kg.

[c] Significantly different from the control.

creased rate at the higher humidity. The difference between control and 21% RH was not significant, but the mean transit rate at 21% RH increased after subsequent exposure to 100% RH for 2 hr.

The change in both temperature and relative humidity was studied at control conditions and 7.2°, 32% RH (wvp 3 mm Hg). No significant change in transit rate was found.

In some dogs intratracheal air temperature was measured by inserting a thermometer through the same hole previously used for particle deposition. After 2 hr of exposure to 7.2°, 32% RH, intratracheal air temperature varied between 34.5 and 35.5°, and body temperature was between 34.5–36.0°. The temperature fluctuated from 0.4 ± 0.2° between inspiration and expiration.

Pilocarpine increased the mucociliary transport rate (Table IV). The difference in the rate was highly significant in 3 of 4 occasions. The range of increase of transit rate varied from nil to 14.5 times. This effect of pilocarpine continued during 3 hr of observation. The accelerating effect on mucociliary activity and respiration appeared immediately on injection.

In the study of the effect of cigarette smoke (Table V), mucus transport rates were measured in 6 experiments on 5 dogs at control environmental conditions. After base line measurements each dog was exposed to cigarette smoke over a total time span varying from 2 to 4 min according to their respiration rates.

Nine puffs of nonfiltered cigarette smoke showed inconsistent effects on mucociliary activity; 2 of 4 occasions showed increased transit rate, while one showed a decrease and one showed complete arrest of mucociliary function for the observation period of 40 min. Twelve puffs had a consistent inhibitory effect on mucociliary activity. In each case the difference between base line and exposure line was significant. In one dog exposed to 15 puffs, 3 particles did not move for the period of 40 min observed. An injection of the standard dose of pilocarpine did not reactivate mucociliary function which had been arrested after 9 or 15 puffs. The effect of

TABLE V. Effect of Cigarette Smoke on Mucociliary Transport Rate (mm/min) Under Control Condition; 24°, 78% RH (wvp 18 mm Hg).

	Control		9 puffs		12 puffs		15 puffs	
Expt. no.	Mean	No.[a]	Mean	No.[a]	Mean	No.[a]	Mean	No.[a]
42	5.6	7	9.0[b]	3				
46	4.0	4	5.0[b]	2				
55	24.4	6			14.8[b]	5		
56	11.5	4			3.3[b]	3	0	3
57	16.7	5	0	3				
62	7.0	3	5.1[b]	3	4.9[b]	3		
Mean								
9 puffs	5.3	$N = 4$	4.8	$N = 4$				
12 puffs	14.3	$N = 3$			7.7	$N = 3$		
15 puffs	11.5	$N = 1$					0	$N = 1$

[a] No. = number of measurements.

[b] Significantly different from the control.

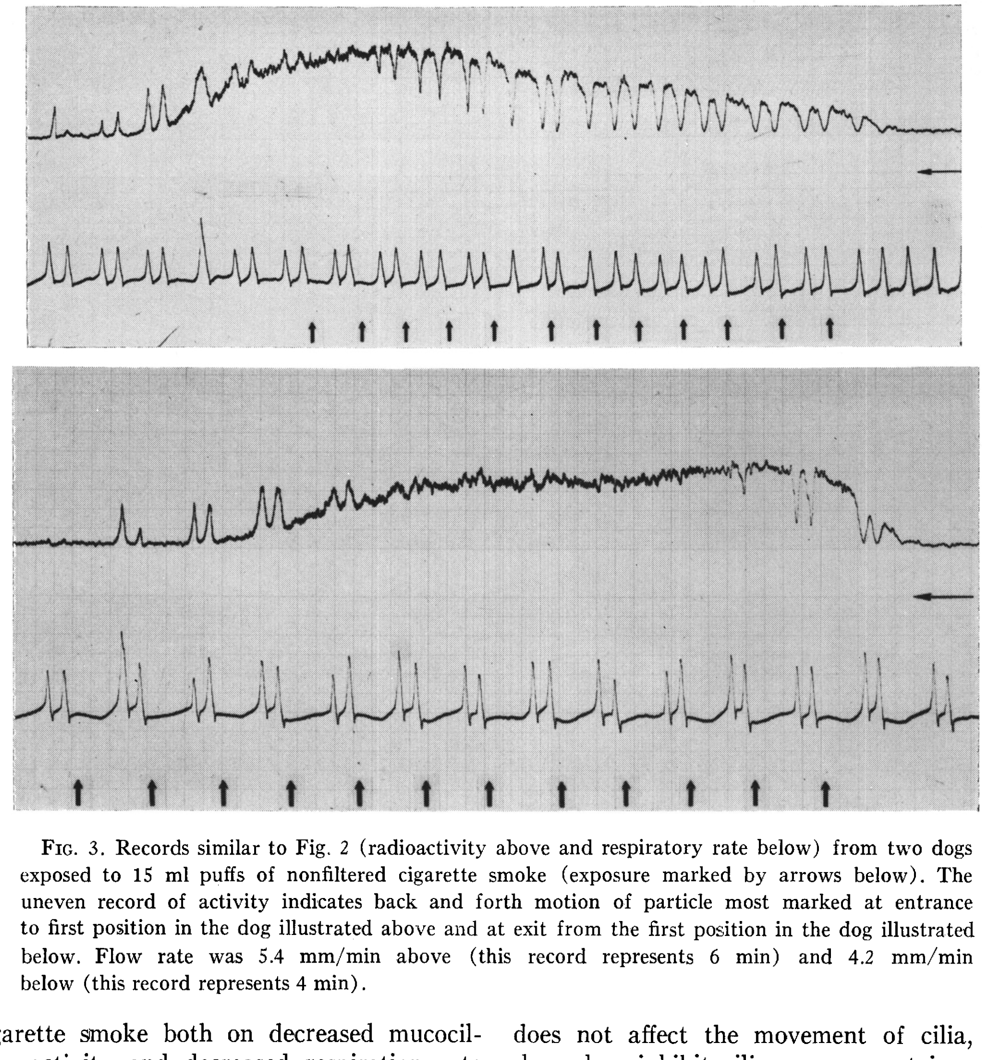

FIG. 3. Records similar to Fig. 2 (radioactivity above and respiratory rate below) from two dogs exposed to 15 ml puffs of nonfiltered cigarette smoke (exposure marked by arrows below). The uneven record of activity indicates back and forth motion of particle most marked at entrance to first position in the dog illustrated above and at exit from the first position in the dog illustrated below. Flow rate was 5.4 mm/min above (this record represents 6 min) and 4.2 mm/min below (this record represents 4 min).

cigarette smoke both on decreased mucociliary activity and decreased respiration rate and amplitude appeared within 1 min.

Graphs showing alterations of mucociliary activty at the first position induced by cigarette smoke are shown in Fig. 3. Figure 4 shows a possible reversal of the direction of mucus flow, which was never observed in an experiment without cigarette smoke.

Discussion. Measurements of mucociliary activity in this study were successful in only 39.1% of the attempts. This could be due to the difference in susceptibility of individual dogs to anesthetic, abnormal mucosa in the trachea of some dogs, possible flaws in the experimental model, or to a combination of these factors.

Although Rivera (11) says that Nembutal does not affect the movement of cilia, the drug does inhibit ciliary movement in mammalian mucociliated organ cultures *in vitro* (12). The inhibitory effect of the dosage of Nembutal recommended for dog anesthesia (13) was observed in this study. In order to decrease the dose of Nembutal, Innoval was administered in a concentration of 0.3 ml/kg intramuscularly 30 min before injection of Nembutal. In this case anesthesia was satisfactory with Nembutal at a concentration of 20 mg/kg. Nevertheless, we observed that there was marked individual susceptibility to this dose, and there was a variation of the anesthesic depth in many cases. In some dogs particles failed to move not only at the deep stage of anesthesia but also at the very light stage.

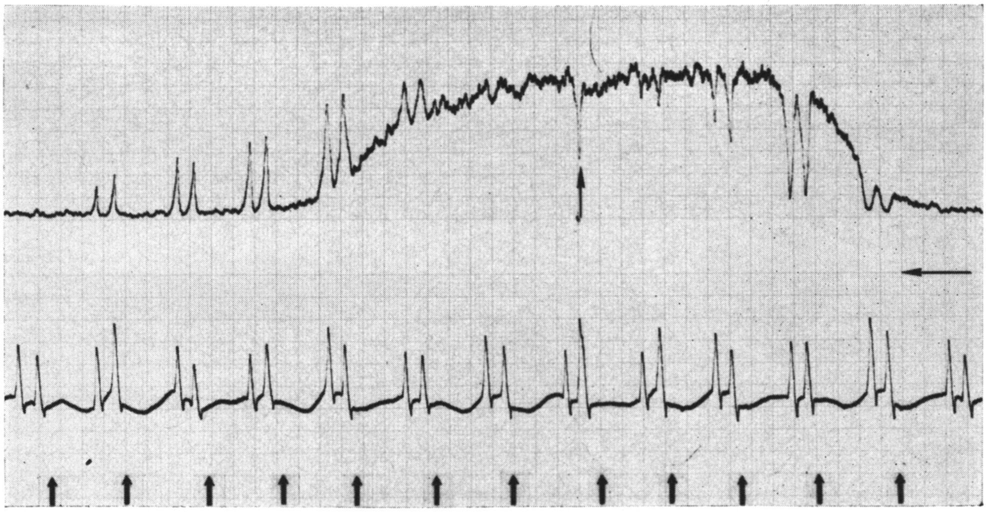

FIG. 4. Record as in Fig. 3 showing even more marked movement of particle in and out of first position. Large arrow indicates possible reversal of motion. Small arrows indicate an exposure to 12 successive 15-ml puff of nonfiltered cigarette smoke. The particle moved at a rate of a 5.6 mm/min (this record represents 4 min).

There was a relatively high incidence of histopathological changes in the trachea of dogs used in this study. For instance, squamous metaplasia of the tracheal epithelium was found in 25% of dogs examined. No particle movement was observed in the trachea which showed squamous metaplasia. However, in many dogs histopathology of the trachea was not correlated with particle transport rate. For instance, the slowest particle transport (0.7 mm/min) in this study was observed in the trachea with normal morphology, while relatively high particle transport rates (11.5 and 12.7 mm/min) were observed in the trachea which showed chronic inflammation.

Since each dog was placed ventral side up, the membranous portion of the trachea often sagged to one side of the tracheal wall forming a fold in the membrane. We were unable to control the formation of this fold. Particles placed on the edge of such a fold did not move. Asmundsson and Kilburn (14) reported that mucus moved either in a clockwise or counterclockwise helical path up the trachea of dogs, and that if a particle reached a fold in the membrane, its progress ceased. In our experiments, particles were necessarily positioned blindly.

Mucociliary transport rates in the trachea of dogs averaged 10.5 mm/min (SD 7.39) in this study. Our normal average flow rates are the same as those obtained in *in vitro* studies by Asmundsson and Kilburn (14), 10.0 mm/min, and slower than those obtained by Hilding (15), and Kensler and Battista (7), also *in vitro*, 14–15 mm/min and 36.1 mm/min, respectively. Hach (16) reported that particles placed in bronchioles traveled to the trachea at a rate of 4.6 mm/min *in anesthetized dogs*. In experiments with the extirpated trachea of dogs Lommel (17) found the "optimal" speed of mucus flow to be 19.8 to 24 mm/min, whereas Von Gebhart (18) reported a maximum flow rate of 12 mm/min. These moderate differences in mucociliary transport rate could be due to the factors discussed above, including differences in method. Anesthesia and extirpation could both cause significant deviation from normal rates.

A series of successive weekly observations of mucus flow rates in the same human subjects has been made only by Bang, Mukherjee and Bang (19), who studied nasal mucociliary rates and reported that, in many individuals, they were sufficiently constant to be used as their own controls. Since one of our dogs showed a 4.5-fold difference in the course of weekly testing, it is suggested that in using stray animals of unknown age, disease history, or state of nutrition, the control rate of each animal should be measured each time before subjecting the animal to an experimental condition. The mean SD on the runs on individual dogs was 2.63 mm/min ranging from 0.19 to 7.16 mm/min.

While Archard (20) has stated that most observers seem to favor humidity levels around 50% RH and a temperature between 65 and 75°F, we selected 24° and 78% RH as the basic environmental condition in order to make the cold and/or dry air change more definitive, since Dalhamn (21) and Ewert (22) reported that dry air was detrimental to mucociliary activity.

As might have been expected from the studies reported by Perwitzschky (23) and Ingelstedt (24, 25), during nasal breathing neither high nor low relative humidity showed any definite effect on mucociliary activity in the trachea of dogs. In 2 of 4 of our experiments in which the dogs were exposed to 24°, 100% RH (wvp 18 mm Hg) rates were significantly faster than base line rates, while all 4 experiments at 24°, 21% RH (wvp 6 mm Hg) failed to show a significant difference from control conditions. Differences in rates between the control and cold dry environments (7.2°, 32% RH and 3 mm Hg wvp) were not significant.

Baetjer (9) studied mucociliary clearance rates in the chicken trachea using a similar method, and reported that clearance rate of droplets (saline solution of Na-^{131}I) injected into the trachea of living chicks was faster when chicks inhaled air at high temperature than when they inhaled cold air. This difference in reaction to temperature may be due to differences in adaptation in chicks and dogs. The difference between body and intratracheal temperature is 5 times greater in chicks than in dogs. All facts indicate the excellent capacity of the canine nose to condition inhaled air. Andersen, Lundqvist and Proctor (26) observed no change in human nasal mucus flow rates during a stay of 8 hr at 70% RH 23°, and no significant difference between the mucus flow rates at 70, 50, 30, and 10% RH.

Pilocarpine has been found to increase particle transport rate in mucociliated epithelia when administered locally or systemically in the trachea of cats (27), the chick nose (28), the frog pharynx (29, 30), and the sea slug (31). But this drug has no effect on the motility of cilia *per se* (28, 32). In our experiments, the base line transport rates were accelerated as much as 2.6 times by pilocarpine on 2 occasions, and on another by as much as 19 times. On one occasion when the base line was 35.1 mm/min, the fastest rate in all experiments, the rate was not affected. This supports the general concept that the mucociliary transport rate depends more on the quality and quantity of mucus than on the ciliary "base rate." The present study indicates that the maximum particle transport rate that can be induced in the dog trachea may be around 33–38 mm/min.

The exposure of cigarette smoke was comparable to the situation in the human smoker. The dog inhaled fresh smoke in a volume of 15-ml puffs which is proportional to their minute volume of 3.1 liters/min compared to a 35-ml puff proportional to a minute volume of 6.4 liters/min for a man (33).

Using a variety of different materials and methods, the cilia-toxic effect of cigarette smoke has been extensively studied (34–37), and results have been in general agreement. Cigarette smoke has produced inhibitory effects on ciliary beating, on mucus flow and on transport of particles. However, contrary findings cannot be ignored. Bair and Dilley (38) studied the effect of cigarette smoke on pulmonary clearance in dogs and reported that prolonged heavy daily smoking did not alter the deposition of inhaled 0.10-μ particles nor the capability of the respiratory tract to remove them. Among authors who reported the stimulatory effect of cigarette smoke on ciliary epithelium, Albert *et al.* (39) studied smoking and clearance of inhaled particles (0.7–3.1 μ) from the bronchi. They observed that there was some acceleration of lower lung clearance, and stated that mucus output may be an important determinant of the rate of clearance since cigarette smoke is thought to increase mucus production but not alter the rate of ciliary beat.

In the present study, there was a large individual variation in response to 9 puffs of cigarette smoke: on 2 of 4 occasions the particle transport rates increased, on one occasion the rate decreased, and on one occasion the particle failed to move. While the rate was inhibited after 12 puffs in each of 11 experiments, the inhibition varied from 28.7 to 70%. Since the rate of transport was decelerated or halted, and since movement of the blanket was not restored by pilocarpine injection, it may be that ciliary activity was in fact inhibited by cigarette smoke as Carson, Goldhamer and Carpenter (1) have suggested. The possible ciliary reversal observed in this study at the first position during exposure to cigarette smoke suggested that cigarette smoke disturbs the coordination of ciliary movement. Under the conditions of these experiments, smoking only a few puffs seemed enough to produce a harmful effect on mucociliary activity in the dog trachea. The difference in the response to 9 puffs and 12 puffs may be due to the difference in the time interval between the exposures.

A major disadvantage in the study method is that transport rates must be measured under narcosis. The method's great advantage is the high degree of accuracy in measurement of transport rates.

Summary. Tracheal mucociliary transport rate in dogs was measured by a radioactive particle transport technique. The transport rates averaged 10.5 mm/min in a condition of 24°, 78% RH (wvp 18 mm Hg). Neither change in relative humidity (100% RH, wvp 22 mm Hg and 21% RH, wvp 6 mm Hg) at the same temperature (24°), nor in both temperature and relative humidity (7.2°, 32% RH wvp 3 mm Hg) showed significant difference in mucociliary transport rate. These results indicate the excellent capacity of the canine nose to condition inhaled air. Pilocarpine stimulated the mucociliary transport rate. Nine puffs of nonfiltered smoke showed inconsistent effects on transport rates, while 12 puffs had a consistent inhibitory effect on mucociliary activity. It is suggested that cigarette smoke disturbs both ciliary beating and ciliary coordination in the trachea of the dogs.

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