Published Online: 20 May, 1959 | Supp Info: http://doi.org/10.1085/jgp.42.5.959
Downloaded from jgp.rupress.org on July 22, 2018

PARAMETERS OF GASEOUS ION EFFECTS ON THE MAMMALIAN TRACHEA*‡

A. Duration of Effects

B. Minimal Effective Ion Densities

By ALBERT P. KRUEGER and RICHARD F. SMITH

(From the Department of Bacteriology and the Naval Biological Laboratory–Naval Medical Research Unit No. 1, University of California, Berkeley)

(Received for publication, October 17, 1958)

ABSTRACT

A. Duration of Effects

Groups of mice exposed to high densities of unipolar light air ions for 72 hours exhibited persistent alterations in the functional efficiency of their tracheas. These effects lasted at least 4 weeks, and in the case of animals treated with (+) ions included diminished ciliary activity, pale and contracted tracheal mucosa, and enhanced vulnerability to trauma.

Following treatment with (−) ions, animals displayed increased ciliary activity with no other detectable changes. It required at least 60 minutes of exposure to ions to induce such "permanent" functional changes.

B. Minimal Effective Ion Densities

The minimal ion densities producing changes in ciliary activity within an arbitrary period of 30 minutes were determined with extirpated tracheal strips from rabbits and guinea pigs. The threshold value for (−) ions was approximately 2.5×10^3 ions/cm.^{2}/sec. and that for (+) ions was in the range between 1×10^4 and 2.5×10^5 ions/cm.^{2}/sec.

The evidence indicates that ion-induced functional changes in the ciliated epithelium of the pulmonary tree are the results of direct contact of ions with surface cells and do not involve participation of the central nervous system or circulation. So far as ciliary activity is concerned, the number of ions required to produce a change in rate is very small.

Relatively short exposure of animals to high densities of light (+) and (−) air ions (20 minutes) has been shown to affect a number of tracheal

* This research was supported by grants from the Atomic Energy Commission and the Committee on Research of the University of California.

‡ The opinions contained in this report are not to be construed as reflecting the views of the Naval Service.

characteristics, including appearance, ciliary rate, mucus flow, and resistance to mechanical trauma (1). Positive ions in general reduce the functional efficiency of the tissue and the effects persist after the tissue has been removed from the ionized atmosphere. Negative ions increase the efficiency of the surface clearing mechanism but their effects quickly vanish when the ion source is removed.

To determine: (*a*) the duration of these effects when longer periods of air ion exposure are used, and (*b*) the minimal air ion densities required to roduce these effects, the following experiments were performed.

A. Duration of Effects

Methods.—

Young adult male and female albino mice ("Namru" stock) and young adult female guinea pigs were used in these experiments. The mice were exposed to air ions in specially designed masonite cages, 5 x 10 x 7 inches, that included two tritium ion generators in the wire mesh roof, while the metal floor constituted the ground of a rectifying circuit to allow selection of ion charge. The guinea pig cages were similar except that they were 16 x 21 x 9 inches and contained four ion generators in the wire mesh roof. Control cages were identical except for the absence of ion generators. Air ion densities determined with the Beckett probe and Beckman micro-microammeter gave values of *circa* 1×10^7 ions/cm.^{2}/sec. at floor level (2).

Animals were kept in the "ionizing" cages for the prescribed period, then allowed to remain in ordinary room air for varying lengths of time. Finally they were anesthetized with rectally administered sodium pentobarbital, tracheotomized, and the condition of their tracheas (appearance, ciliary rate, and vulnerability to mechanical trauma) studied by techniques already described (3).

Results.—Two groups of six mice were exposed to (+) and (−) air ions respectively for 3 days. At intervals of 1, 2, 3, 4, 5, and 8 days after the conclusion of the ionization period, a mouse was removed from each group for study. Un-ionized control mice were also examined on the 1st and 8th day. The experiment was repeated with animals removed at weekly intervals after the conclusion of the ionization period. Table I summarizes the results of both experiments.

From these data it can be seen that even 4 weeks after the termination of the ionization period, the ion effects on ciliary rate, appearance, and vulnerability to trauma are still present and undiminished.

To obtain an approximate idea of the minimal period of ion-exposure that will produce these long lasting effects, mice were exposed to air ions for brief periods and then examined the following day (Table II).

Here the data indicate that the effects of a 30 minute exposure to (−) ions are still evident 24 hours later, although a 60 minute exposure is required for the maximal long lasting effect. With (+) ions a difference in the rates of ap-

TABLE I

Condition of Trachea in Mice after a 3 Day Ionization Period

Air ion densities as snout level = 1×10^6 to 1×10^7/cm.^{2}/sec. (probe values).

Time elapsed since ionization	(+) Ionized			(−) Ionized			Un-ionized controls		
	CR	ATS	EVT	CR	ATS	EVT	CR	ATS	EVT
1 hr.	600	Pale and contracted	+	1100	Normal	0	900	Normal	0
1 day	600	" " "	+	1200	"	0	900	"	0
2 days	600	" " "	+	1100	"	0	—	—	—
3 "	600	" " "	+	1150	"	*	—	—	—
4 "	600	" " "	+	1100	"	0	—	—	—
5 "	600	" " "	+	1050	"	0	—	—	—
1 wk.	600	" " "	+	1150	"	0	—	—	—
8 days	600	Normal	+	1150	"	0	900	Normal	0
2 wks.	600	Pale and contracted	+	1150	"	0	—	—	—
3 "	600	" " "	+	1100	"	0	—	—	—
4 "	600	" " "	+	1100	"	0	900	Normal	0

CR, ciliary rate: beats/minute.
ATS, appearance of tracheal surface.
EVT, enhanced vulnerability to trauma.
* Animal died before EVT could be tested.

TABLE II

Condition of Trachea in Mice 24 Hours after Short Ionization Periods

Air ion densities at snout level 1×10^6 to 1×10^7/cm.^{2}/sec. (probe values).

Ionization period	CR	ATS	EVT
	(−) Ions		
0 (control)	850	Normal	0
30 min.	950	"	0
60 "	1050	"	0
	(+) Ions		
0 (control)	850	Normal	0
10 min.	850	"	0
20 "	600	"	0
30 "	Absent	"	0
60 "	600 (Sparse)	Pale and contracted	+
120 "	Absent	" " "	+

CR, ciliary rate, beats/minute.
ATS, appearance of tracheal surface.
EVT, enhanced vulnerability to trauma.

pearance of the three effects is noted. A permanent[1] lowering of the ciliary rate to 600 beats/minute is achieved by a 20 minute exposure to (+) ions, but the enhanced vulnerability to trauma and the pale contracted surface do not become permanent characteristics until after a 60 minute exposure.

This separation of the (+) ion effects was not encountered in the guinea pig, in which a 30 minute exposure is sufficient to make all the (+) ion effects permanent.

DISCUSSION

It appears that once an animal's ciliary rate has been "set" by air ion action at either an abnormally high or an abnormally low level, it will persist at that level indefinitely in ordinary air. The associated surface properties likewise persist.

The discovery that a 20 minute exposure to (+) ions is sufficient to cause a permanent reduction in an animal's ciliary rate is surprising, but not unreasonable. We have observed that when a small area of an extirpated tracheal strip is treated with air ions, this altered rate is transmitted in both caudal and cephalic directions until the entire tissue is beating at the new rate. While caudad transmission is not as rapid as cephalad transmission, it is ultimately just as thorough. Thus it is not necessary for (+) ions to reach the finer ramifications of the respiratory tract in order to exert their effect on all ciliated surfaces; presumably if the ions are able to alter the ciliary rate of merely the upper portion of the trachea, this alteration can be transmitted caudally without further mediation of air ions.

The characteristic pale and contracted appearance of the mucosa and the enhanced vulnerability to trauma resulting from the action of (+) ions require a minimal exposure period of 60 minutes to become permanent.

B. Minimal Effective Ion Densities

In the preceding and in most of our previously reported experiments (1–4), the air ion densities employed have been relatively high, from 1×10^7 to 1×10^9 ions/cm.^{2}/sec. at tissue level in the cases of the extirpated trachea and the tracheotomized animal or at snout level in the case of the intact animal. As might be expected, when the ion density is reduced, the tracheal effects become less marked and the rate at which they occur is retarded.

Establishment of accurate threshold levels is handicapped by two factors: (*a*) the difficulty in obtaining accurate ion density measurements at the lower levels, and (*b*) variation in individual susceptibility to air ion action, which becomes more in evidence as the air ion density is reduced. However, it is

[1] We describe these effects as "permanent," since they continue unchanged for periods of up to 4 weeks and apparently can only be reversed by artificially administered doses of air ions of the opposite charge.

possible to establish a range of values below which air ion effects are seldom observed and above which air ion effects are almost always observed.

Because of the ease with which ciliary rates can be determined we have conducted all threshold experiments with ciliary activity as the sole criterion of gaseous ion action.

Methods.—

Young adult female rabbits and guinea pigs were used in these experiments. They were sacrificed with intraperitoneal injections of sodium pentobarbital, their tracheas removed and placed in a high humidity "ionizing" chamber according to a technique already described (3).

Ion densities were reduced either by increasing the distance of the ion source from the tissue, or by reducing the voltage in the rectifying circuit by means of two variable transformers ("powerstat") in series. Both methods gave substantially the same results.

The determination of minimal dosage levels for (+) and (−) light ions presented certain problems of mensuration. It is common practice to measure air ion concentrations by collecting the ions on a series of thoroughly insulated polarizing plates arranged in a duct. Air is drawn past the plates with a small blower at a velocity of *ca.* 75 cm./sec. and the current resulting from the deposition of ions is measured with a micro-microammeter. This method provides a means of determining the absolute ion density for each polarity, but was not applicable to our experiments because of its relatively huge volume demand and the small dimensions of the exposure chamber.

A preferable method involves the Beckett target probe. It is placed at the same distance from the ion source as the tissue surface and ions migrating in the field of the rectifying circuit impinge upon it. The current produced is measured with a Beckman ultrohmeter and is converted into ions/cm.^{2}/sec. by substitution in the equation $N = I/qA$ in which

I = current measured
$q = 1.6 \times 10^{-1}$: coulombs
A = area of probe surface

So far as experiments with the extirpated trachea are concerned, the probe values have more significance than would numbers of ions/cm.^{3} as determined by the ion collector. The former depend upon impingement of ions on a given surface area of probe and their physiological activity is exerted upon a corresponding area of tisuse. This is not the case for ions/cm.^{3}; these ions are picked up by the collecting plates during the measuring process, but only an unknown fraction of them could be expected to impinge on the tissue surface.

Probe measurements are accurate for high densities of ions, but at lower densities the ultrohmeter readings are not steady. However, by averaging the scale readings and by referring to the extrapolated theoretical curves for distance and voltage variation it was possible to obtain reasonably accurate values.

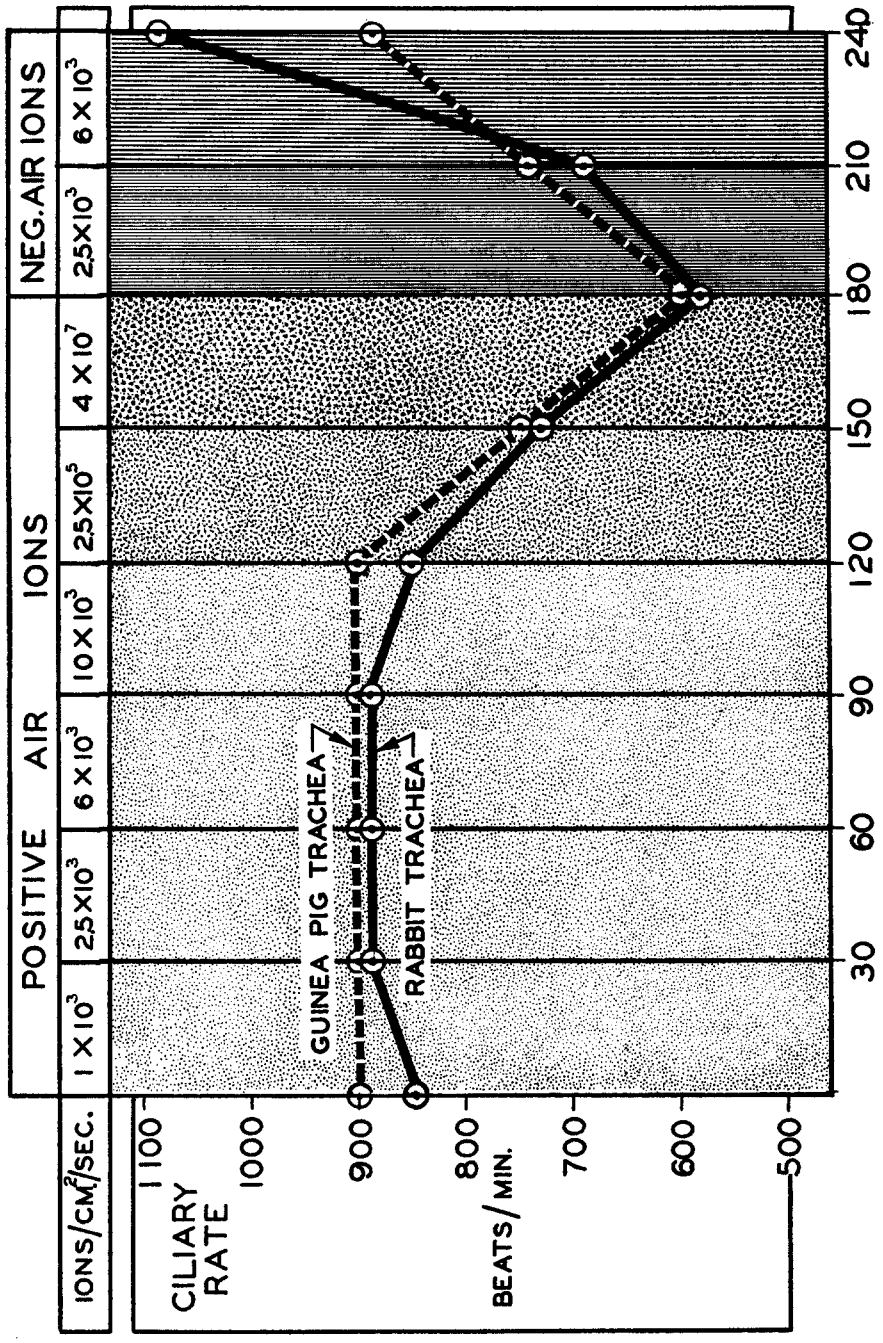

FIG. 1. Determination of minimal ion densities affecting ciliary rates of extirpated guinea pig and rabbit tracheas.
(−) ion threshold = 2.5×10^3 ions/cm.²/sec.
(+) ion threshold = *ca.* 2.5×10^5 ions/cm.²/sec.

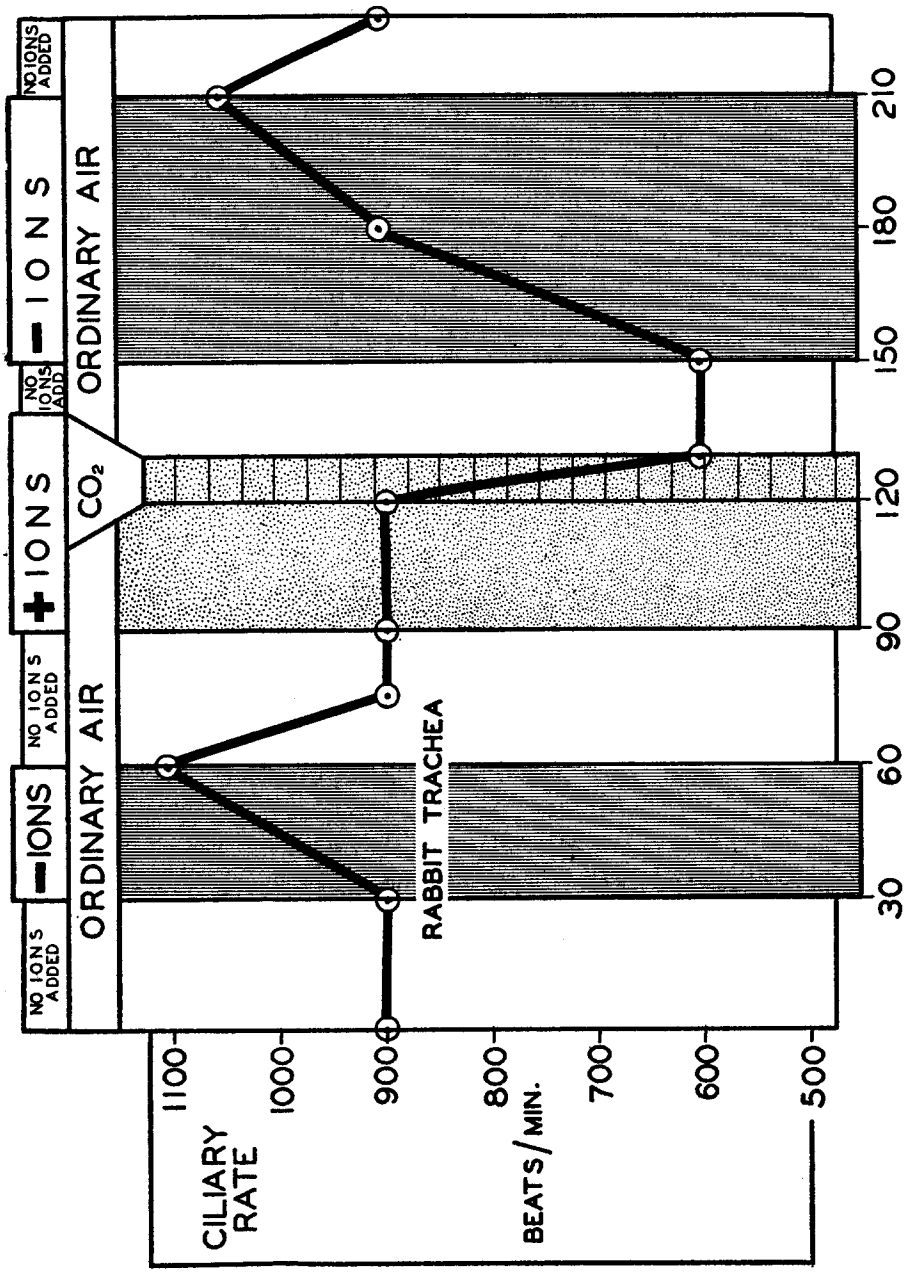

FIG. 2. Effects on ciliary rate of extirpated rabbit trachea of (−) and (+) air ions at density of 1×10^3/cm.^{3}/sec.
1. (−) ions accelerate rate; rate returns to normal when ion source is removed.
2. (+) ions do not affect rate; ionized CO_2 inhibits rate and effect persists when ion source is removed; inhibition is reversed by (−) ions.

Results.—When an exposure period of 30 minutes was used, the threshold range for producing a definite effect on the ciliary rate of most animals occurred between 1×10^3 and 1×10^5 ions/cm.^{2}/sec. A few sensitive individuals were affected by air ion concentrations below 1×10^3, while a few resistant individuals required concentrations above 1×10^5.

In any given individual, the (−) ion effects occurred at ion densities below which (+) ions had ceased to have any effect. Tracheal tissue displaying clear cut (−) ion effects at 2.5×10^3 did not show (+) ion effects until the density reached 1×10^4 or even 2.5×10^5 (Fig. 1).

In one experiment additional CO_2 was supplied to a chamber in which the (+) ion density of 1×10^3/cm.^{2}/sec. had failed to alter the tracheal ciliary rate. As can be seen from Fig. 2, the positively ionized CO_2 produced an immediate fall in the ciliary rate even at this previously ineffective density. Un-ionized CO_2 is known to have no effect on the tracheal ciliary rate (4).

DISCUSSION

On the basis of earlier work we concluded that negatively charged oxygen ions and positively charged CO_2 ions are the components of ionized air responsible for effects on the ciliary rate. These conclusions were based on exclusion rather than on positive identification. The available data on the production of gaseous ions were derived by physicists from rigorously controlled and relatively simple systems. The production of ionic types under the complex conditions existing in our experimental chamber has not been investigated and the exact ionic pattern is not assured, although Tüxen's experiments provide a basis for speculation (5).

Working with air and pure gases, Tüxen identified O^-, O_2^-, NO_2^-, NO_3^-, OH^-, and H^-, but found no N^-, N_2^-, He^-, Ne^-, or A^-. In our test chamber containing air at an R.H. of *ca.* 90 per cent, we might expect to encounter during negative ionization OH^-, H^-, O^-, and O_2^-, but no N^- or N_2^-. When the air in the test chamber was replaced with oxygen, the same physiological results were obtained; when the air was replaced with nitrogen, no effects were obtained. Since water vapor was present in all three experiments, H^- and OH^- ions can be ruled out as the physiological mediators, leaving O^- and O_2^- as the active agents. The same type of experiment and reasoning led to the conclusion that CO_2^+ is the physiological mediator during positive ionization.

While O^- and CO_2^+ are physiological opposites in their influence on ciliary rate, it appears that their effectiveness, ion for ion, is of the same order of magnitude. The minimal effective dose for negatively ionized air is approximately 1×10^3/cm.^{2}/sec. for 30 minutes. The minimal effective dose for positively ionized air is several times this value. However, this difference may be more apparent than real and could be due to the fact that air normally contains only 0.05 per cent CO_2 as compared with 21 per cent O_2. Oxygen

ions would represent a major part of the 1×10^3/cm.^{2}/sec. negative threshold, while CO_2 ions would be a much smaller percentage of the 1×10^4 to 2.5×10^5 ions/cm.^{2}/sec. positive threshold. This hypothesis is supported by the experimental observation that an ion density of 1×10^3 (+) ions/cm.^{2}/sec. in room air (0.05 per cent CO_2) produced no change in ciliary rate, while a prompt drop in rate occurred when a large volume of CO_2 was added to the atmosphere with ion density remaining constant (Fig. 2).

In the course of determining threshold values for light air ions of either charge, many observations were made of the time required for various ion densities to induce measured changes in the rate of ciliary activity. When these data were analyzed for a (time) $\times$ (concentration) constant it was clear that no such relationship obtained; even when the greatest possible allowance was made for the limitations of the strobotachometric method of evaluating ciliary beat there was no evidence that the total number of gaseous ions impinging on the tissue surface either determined the degree of change in ciliary rate or constituted a valid expression of threshold when the end point employed was the minimal detectable effect on ciliary rate. Further, the change in rate was not proportional to ion density.

It is apparent that whatever mechanism underlies the change of ciliary rate induced by gaseous ions, the essential condition is direct contact of ions with the surface cells. To begin with, central nervous system action can be excluded, for the phenomenon occurs in the isolated tracheal strip as well as in the living tracheotomized and living intact animal (1). For the same reason absorption and distribution of ions by the blood stream are not involved. This places the locus of action in the epithelium and very likely in the most superficial cells, since gaseous ions have low velocities and accordingly very little power to penetrate.

Just what transpires after contact of the ion with the cell surface is difficult to say. Essentially nothing is known about the permeability of pseudostratified ciliated columnar epithelium to gaseous ions and little more is known about the functioning of the cellular elements thought to be responsible for the control of ciliary activity. The consensus seems to be that:

1. Each cilium consists of a cylinder of nine equidistant straight fibers arranged around two somewhat thinner central fibers. The peripheral fibers may be composed of two filaments, the proximal ends of which join in a basal plate more or less closely applied to a well defined basal body, the kinetosome.

2. Associated structures, *e.g.* mitochondria, rootlets, and various cytoplasmic fibers, are not involved in the production of ciliary motion.

3. The central fibers may be specialized for conduction, while the peripheral fibers are contractile (6). Contractile impulses could originate at a given point in the basal body, circulate around it, and produce localized contractions in the outer fibers.

4. The cycle of contraction and relaxation may depend upon a reaction between the contractile protein of the fiber and some energy-rich molecule.

5. In mammals no specific structural elements connect the kinetosomes of adjacent cells (7); consequently there exists no recognizable apparatus for conduction of regulatory impulses.

We do know, however, from our own experiments and from those of others (8) that changes in the rate of ciliary beat can be transmitted from one small area along the surface for moderate distances.

Next, if we consider in detail the situation obtaining in a restricted area of ciliated epithelium during exposure to gaseous ions, it becomes evident that small numbers of ions bring about the change in ciliary rate. Measurement of the tracheal cells of the rat and guinea pig with the eyepiece micrometer indicates a surface area of *ca.* 80 μ^2/cell. In one cm.^{2} there are approximately 1.3×10^6 cells, and a significant change in ciliary beat in these cells follows exposure to 1×10^3 (−) ions/cm.^{2}/sec. for 30 minutes. Assuming the lowest degree of ionic efficiency, *i.e.* that all the ions reaching the surface during this period are required to establish the change in rate, there would be a total of 1.8×10^6 ions distributed over a surface containing 1.3×10^6 cells. If the distribution occurs according to the Poisson equation, the majority of the cells will receive at least one ion and those receiving more than one will receive at most a few.

Since each cell bears approximately 100 cilia projecting from the free surface into the tracheal lumen, it is conceivable that a portion of the air ions never reach the cell surface proper, but instead impinge on the cilia. This would further reduce the number of ions available for inducing a physiological response in the cell itself, unless of course it is assumed that direct action on the cilia occurs. Such a possibility cannot be excluded, for it is conceivable that air ions might act directly on some component of an intraciliary enzyme system.

In addition to the suggestive evidence that the change in rate of ciliary beat follows from air ion action on some component of the ciliated epithelial cell, it is logical to conclude that other known effects depend on reactions at different sites. For example, changes in the nature and volume of mucus present on the epithelial surface have been observed (1) and the inference follows that they are induced by air ions reaching the secretory cells.

Further, we have reported the induction of a state described as enhanced vulnerability to trauma (EVT) when the living trachea is exposed to (+) air ions. In this condition the slightest touch with a probe produces in the tracheal wall a red ecchymotic disc or web of tangled capillaries which tends to persist and is surrounded by a zone of progressive ischemia. Our earlier papers also include a description of contracture of the posterior tracheal wall (1, 3) presumably due to the action of smooth muscle located in the posterior wall and attached to the outer surface of the tracheal cartilages.

Both these effects involve tissue elements located beneath the epithelium and one is forced to the conclusion that gaseous ions serve as the stimuli which activate them, although it is not clear whether the ions actually reach the smooth muscle cells and the capillary walls or initiate their effect indirectly, perhaps by triggering surface components from which the stimuli are transmitted. The latter mechanism may seem to be an unlikely possibility, but the production of effects remote from the site of air ion application is known to occur. For example, Nielsen and Harper have reported the reduction of succinic dehydrogenase activity in the adrenals of rats exposed to (+) air ions (9), and Worden has demonstrated an increase in the CO_2 combining power of plasma of hamsters treated with (−) air ions (10).

Our experiments establish in general terms the cellular sites involved in air ion action on the trachea, but do not pin-point the ultimate enzymatic substrate. Recently (11) we reported evidence suggesting that this may be the iron prophyrin constituents of the cells and additional experiments have served to confirm our earlier report. A detailed account of this work will appear elsewhere.

BIBLIOGRAPHY

1. Krueger, A. P., and Smith, R. F., *J. Gen. Physiol.*, 1958, **42,** 69.
2. Krueger, A. P., Hicks, W. W., and Beckett, J. C., *J. Franklin Inst.*, 1958, **266,** 9.
3. Krueger, A. P., and Smith, R. F., *Proc. Soc. Exper. Biol. and Med.*, 1957, **96,** 807.
4. Krueger, A. P., and Smith, R. F., *Proc. Soc. Exp. Biol. and Med.*, 1958, **98,** 412.
5. Tüxen, O., *Z. Physik*, 1936, **103,** 463.
6. Bradfield, J. R. G., Fibrous proteins and their biological significance, Symposia of the Society for Experimental Biology, Cambridge, Cambridge University Press, 1955, 306.
7. Fawcett, D. W., and Porter, K. R., *J. Morphol.*, 1954, **94,** 221.
8. Gray, J., Ciliary Movement, London, Cambridge University Press, 1928, 120.
9. Nielsen, C. B., and Harper, H. A., *Proc. Soc. Exp. Biol. and Med.*, 1954, **86,** 753.
10. Worden, J. L., *Fed. Proc.*, 1954, **13,** 168.
11. Krueger, A. P., and Smith, R. F., The Physiological Significance of Positive and Negative Ionization of the Atmosphere, presented at the First International Symposium on Space and Submarine Medicine, United States Navy Submarine Base, New London, Connecticut, September, 1958.