closely associated with the enzyme due to similar physical-chemical properties. Two peaks of reduced and oxidized cytochrome were found by gradient elution. Total purification of the specific enzyme activity was about 100-fold over the original supernatant.

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Effects of Gaseous Ions on Tracheal Ciliary Rate.* (24061)

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Recent experiments have shown that (+) and (−) air ions have marked effects on ciliary rate and other properties of the mammalian trachea, both *in vitro*(3) and *in vivo* (4). It seems reasonable to conclude that these effects stem from physiologically significant alterations in state of gases present in ambient atmosphere and that deliberately produced changes in composition of this atmosphere would be reflected in the response of the tracheal tissue. The following experiments were performed to test this assumption.

Methods. Anesthetized rabbits were tracheotomized and placed in humid-air ionizing chamber according to technic already described(4). Gases used were warmed to 25-28°C, bubbled through water, and blown continuously into the chamber. Since earlier work(2) had shown that presence of smog or cigarette smoke in ambient atmosphere altered the action of air ions on bacterial suspensions, cigarette smoke was employed in some of these experiments. It was produced in mechanical device operating under negative pressure, and then treated in the same way as gases. For N_2 and CO_2 experiments it was necessary to sacrifice the rabbits with an overdose of nembutal; experiments with O_2 and cigarette smoke permitted exposure of living as well as sacrificed animals. As in previous work(1-4): (a) ions were generated by β radiation from sealed tritium foils, a rectifying circuit allowing selection of (+) or (−) ions, (b) the same stroboscopic method was employed to determine rate of ciliary beat (accuracy ± 50 beats/min.), and (c) atmospheric temperature in exposure chamber varied from 25-28°C on different days, while relative humidity remained close to 90%.

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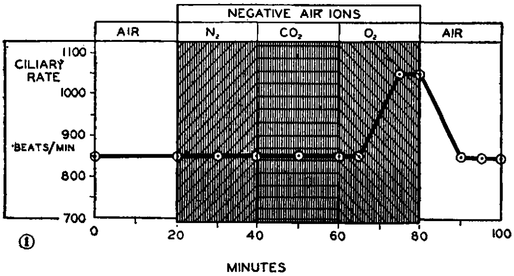

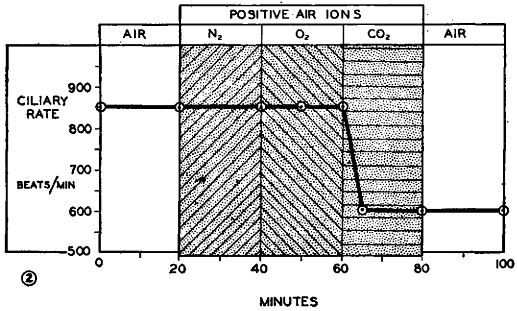

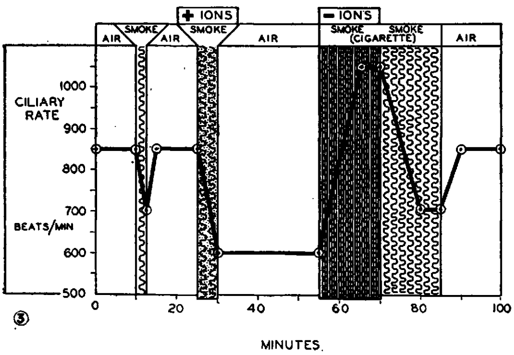

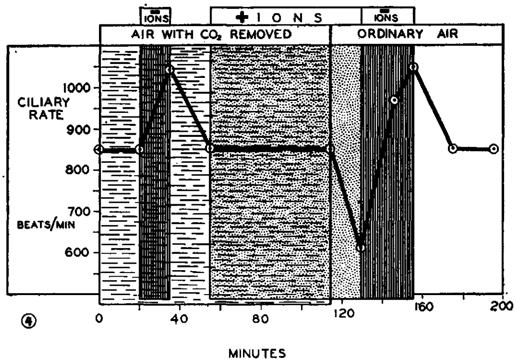

FIG. 1. Action of negative ions on tracheal ciliary rate in N_2, CO_2, and O_2.
FIG. 2. Action of positive ions on tracheal ciliary rate in N_2, O_2, and CO_2.
FIG. 3. Action on tracheal ciliary rate of cigarette smoke, cigarette smoke and positive ions. cigarette smoke and negative ions.
FIG. 4. Action of positive ions in air with and without CO_2. (Negative ions were administered before and after the experiment to establish that the tissue was normally reactive.)

Results. When administered in unmodified humid air, (–) air ions increase ciliary rate by about 200 beats/min (reaching maximum effect within 10-20 minutes), while (+) air ions lower ciliary rate by about 300 beats/min or abolish ciliary activity altogether (reaching maximum effect within 15-20 minutes). These effects are seen both in tracheas of living animals and in extirpated tracheal strips(3,4). However, when commercially-produced gases or cigarette smoke were substituted for air, a number of deviations from the usual pattern were observed:

1. *Negative ions in gases and cigarette smoke.* (–) ions had no effect whatever on tracheal ciliary rate in N_2 or CO_2 (Fig. 1). In O_2 (Fig. 1) or in cigarette smoke (Fig. 3) (–) ions raised the ciliary rate to the same extent and at same rate as in ordinary air.

2. *Positive ions in gases and cigarette smoke* . (+) ions had no effect whatever on tracheal ciliary rate in N_2 or O_2 (Fig. 2). In CO_2 (Fig. 2) or in cigarette smoke (Fig. 3) (+) ions lowered ciliary rate more rapidly (within 2-5 minutes) than in ordinary air (within 15-20 minutes). When ordinary air was passed through a CO_2-absorber before reaching exposure chamber, (+) ions had no effect on ciliary rate; when CO_2-absorber was removed, (+) ions produced usual ciliary-rate depression at usual rate (Fig. 4).

3. *Gases and cigarette smoke alone.* N_2, O_2, or CO_2 by themselves had no effect on ciliary rate within the 20-minute exposure employed. Cigarette smoke alone, however, promptly decreased ciliary rate by about 200 beats/min. The decrease persisted only so long as cigarette smoke remained in the chamber. When the smoke was flushed out with fresh air, the ciliary rate returned at once to its initial level (Fig. 3).

These experiments make it clear that (–) ion effects on tracheal cilia can take place only when more than a certain minimum of O_2 is present. Evidently these effects proceed at maximal rate in ordinary air, and are not accelerated by 100% O_2, or diminished by additional components of cigarette smoke.

Analogously, (+) ions require more than a certain minimum of CO_2 to exert their effects. However, increases in CO_2 concentration above that of ordinary air do increase the rate at which (+) ions produce their effects.†

The agent in cigarette smoke that causes reversible lowering of ciliary rate is not known. Whatever it may be, its action is effectively neutralized by (−) ions, which raise the ciliary rate just as well in a heavy atmosphere of cigarette smoke as they do in fresh air.

On the other hand, the reversible drop in ciliary rate induced by cigarette smoke alone is quickly lowered still more and made permanent by (+) ions; "permanent" in the sense that the low rate will continue indefinitely after smoke and (+) ions have been removed, although it can still be reversed by (−) ions. The extent of the (+) ion lowering in cigarette smoke is the same as in ordinary air; the rate of lowering is 3-10 times faster in cigarette smoke. Whether this more rapid rate of action of (+) ions in smoke is due to increased CO_2 concentration alone, or involves other components of cigarette smoke as well, remains to be established.

† In ordinary air, rate of (+) ion action is significantly higher *in vivo* than *in vitro*. (+) ion action mediated through additional CO_2 from expired air of living animal is evidently sufficient to cause this difference.

In experiments performed in humid air about 1 x 10^9 ions/cm^2/sec, as measured with the Beckett probe and Keithley electrometer, impinged on tissue surface. Ion density measurements did not vary greatly from this figure when N_2, O_2, or CO_2 were substituted for air, but this does not signify that equivalent numbers of ions necessarily were produced from the various gases, for the ambient atmospheres were far from pure. Among sources of pollution were 1) air seepage into the plastic chamber, 2) impurities in the commercially-made gases which were bled from metal tanks, 3) water vapor purposely introduced to keep the tissue moist. Nevertheless, these gases did not seem to introduce any complicating factors, presumably because the physiologically active components (O_2 and CO_2) were present in quantities below their threshold levels.

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Infectious Canine Hepatitis Virus Studies with Special Reference to Passage of Raccoon Tissue Cultures. (24062)

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Previous reports have described growth of infectious canine hepatitis (ICH) virus in tissue cultures derived from dog kidney(1,2,3). More recently, it has been shown that ICH virus grows in tissue cultures of ferret and pig kidney origin(4). Green *et al.* reported transmission of fox encephalitis virus infection to 1 of 14 raccoons, *Procyon lotor*, by intracerebral injection, while raccoons given virus by intraocular route were routinely infected(5), indicating that certain cells within the ocular tissues and central nervous system of these animals support growth of virus. Recently it was shown that there are no sero-

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