which the patients had normal cholesterol levels. It might be postulated that Vit. A either inhibits synthesis of cholesterol in cases of over-synthesis, or causes mobilization and excretion of cholesterol when blood levels become excessively high.

These results are in direct disagreement with work done by Wendt(6) and Lasch(7). Their reported increase in serum cholesterol after Vit. A administration may be explained on the basis of the interference of Vit. A with cholesterol determination as pointed out by Kinley and Krause(10). Since Lasch and Wendt were using a reagent containing ferric chloride which was used both for cholesterol determinations and Vit. A determinations, their apparent elevations were possibly due to a color produced by both cholesterol and Vit. A. The same type of elevation was seen in this laboratory until the Schoenheimer-Sperry method was adopted for separation of Vit. A from the serum before carrying out cholesterol determinations.

Summary. Oral administration of 100,000 I. U. of Vit. A acetate for 4 to 6 months significantly reduced the elevated serum cholesterol levels in atherosclerotic patients but had no effect on individuals with normal cholesterol levels.

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Further Studies of Gaseous Ion Action on Trachea.*† (25246)

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During the past 3 years, it has been demonstrated that light air ions markedly affect the functional efficiency of the mammalian trachea(1,2). In general, (+)ions depress ciliary activity, contract the tracheal wall and induce a state of vasoconstriction and hyperreactivity to mechanical trauma. (−)ions accellerate the rate of ciliary beat, reverse the (+)ion-induced contraction of the wall, and do not alter normal vascularity or the response to trauma. Experiments performed to determine which atmospheric components in ionized form account for the observed functional changes implicated negatively charged oxygen and positively charged carbon dioxide respectively as the specific mediators for acceleration and inhibition of ciliary activity (3). However, these observations apply to only one physiological effect, ciliary activity. Because experiments on other effects, such as enhanced vulnerability to trauma, require exposure of the trachea *in situ* to nitrogen and carbon dioxide for long periods of time, a procedure not compatible with life, a method was devised which permitted the animal to respire through a separate airway while response of the trachea to treatment with various gases was observed.

Methods. 1) Operative technic: Albino rabbits weighing 10-12 lb were anesthetized

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† The opinions expressed herein are not necessarily those of the Air Force or Navy.

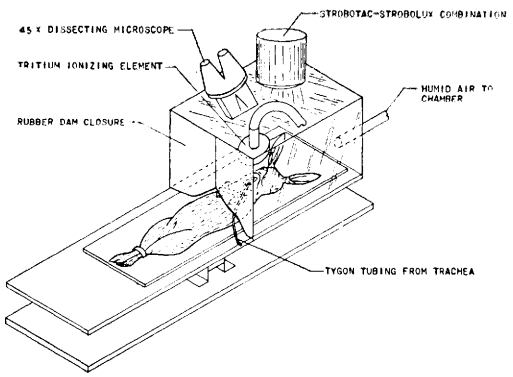

FIG. 1. Experimental arrangement for exposing animal trachea to various gases.

with sodium nembutal administered intravenously. The thyroid gland, cricoid cartilage and first 3 to 6 tracheal cartilages were exposed through a midline incision extending from the hyoid bone to the manubrium sterni. So far as possible, adjacent tissues of the larynx and trachea were not disturbed. Next, a longitudinal incision was made into the cricothyroid ligament, care being taken not to penetrate the underlying mucosa. Through this incision the mucous membrane was bluntly dissected from the overlying structures for a distance of one centimeter under the thyroid cartilage cephalad and for 1-2 cm under the cricoid and first 4-6 tracheal cartilages caudad. The exposed cartilages were cut in the midline, the cut ends gently retracted by ligatures, and all bleeders clamped and tied. A perforation barely large enough to admit one blade of a small mosquito forceps was made through the laryngeal mucosa, the blade inserted and the mucosa crushed in the midline for the length of the proposed opening into the trachea. The final incision was made, producing an aperture 1 $\times$ 2 cm extending from the lower part of the larynx into the upper part of the trachea. The width could be adjusted by gentle traction on the ligatures tied to the severed ends of the cartileges. To prepare a separate airway, the trachea was intubated as close as possible to the manubrium sterni. The surgical technique was essentially that employed in making the aperture for observation, and as soon as the opening was ready, a snugly fitting polyethylene tube was inserted, connecting the lungs with normal room air (Fig. 1). Two advantages are secured through the laryngeal approach to the trachea; (1) exposure of the large preformed cavity facilitates illumination of the adjacent tracheal field; (2) hemorrhage is minimized and oozing is controlled by the fact that the direction of blood flow is away from the tracheal field and toward the oral cavity. 2. *Humid air chamber.* The head and thorax of the surgically-prepared rabbit was placed inside a glass and plastic chamber so constructed that the mucosa of the trachea could be observed through a microscope during exposure to unionized or ionized gases. To maintain a high humidity the gases were bubbled through water at 60°C before entering the chamber. The temperature within the chamber remained at 25°-26°C. 3. *Gaseous ion source.* Small gaseous ions were generated within the chamber by β radiation from H^3 contained in sealed foils mounted 5-6 cm above the tissue surface. An electrostatic field of low intensity was used to allow selective removal of ions of either charge, leaving an atmosphere containing unipolar ions(4). In some experiments the ground of the rectifying circuit was placed in contact with the trachea just caudal to the viewing aperture, while in others no ground was employed; no difference was noted in the experimental results obtained. Between 0.5 and 1.0 $\times$ 10^9 ions of either charge impinged on each cm^2 of exposed tissue(4).

4. *Determination of ciliary rate.* A strobotactomer (Strobotac-Strobolume combination) was used to determine the rate of ciliary beating as described earlier(1,2).

Results. Observations were made on a total of 8 rabbits breathing normal room air through a separate airway, while the tracheal mucosa was exposed to various gases, ionized and un-ionized. In each case, rate of ciliary beat was determined after a 20 minute period of exposure to a given gas and the vulnerability to trauma was tested by lightly touching the mucosal surface with a blunt probe. Normally this causes no more than a fleeting hyperemia but under some circumstances the vessels may dilate and form a prominent web or a definite ecchymosis appears. Either of the latter conditions is described as enhanced vulnerability to trauma (EVT)(2,5).

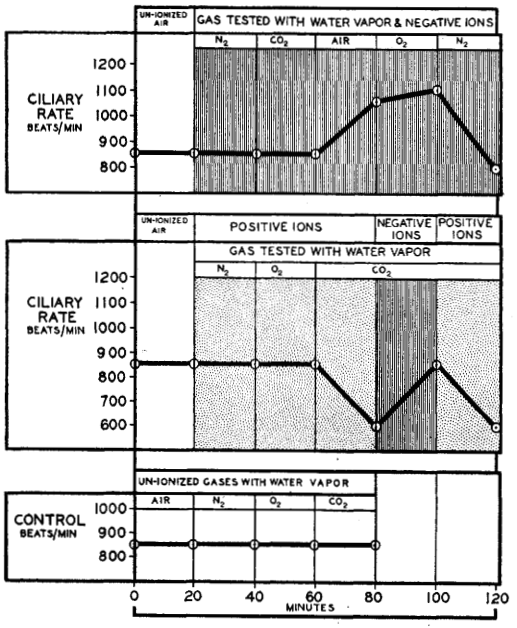

FIG. 2. Effects of ionized and un-ionized gases on ciliary activity in trachea of living rabbit.

Un-ionized N_2, O_2 and CO_2 had no effect on ciliary activity. Under conditions permitting formation of (+)ions, typical inhibition of the ciliary beat occurred in an atmosphere of CO_2 but not in N_2 or O_2. When the rectifying circuit was reversed so that only (−) ions could be generated, atmospheres of N_2 and CO_2 were without effect, while O_2 and ordinary air accelerated the rate of beat (Fig. 2).

N_2, O_2 and air in the un-ionized state or under conditions favoring (−)ion formation failed to induce EVT. This also was true for CO_2 as a rule, but occasionally a barely detectable EVT was produced by untreated CO_2 bled directly from a tank. Probably this resulted from an ionized fraction of the CO_2 produced by natural forces; extremely small numbers of positively charged CO_2 ions will induce a state of EVT(5). With the rectifying circuit reversed so that only (+)ions would be generated N_2 and O_2 were without effect, but CO_2 or air containing CO_2 permitted the prompt formation of a marked EVT.

These findings corroborate and extend our earlier experiments conducted with excised tracheal strips and living tracheotomized rabbits.

It is clear that positively charged CO_2 is the agent responsible not only for reduction of ciliary activity but also for the following effects: (1) Contracture of the trachael smooth muscle; (2) Pronounced mucosal ischemia; (3) Exaggerated lability of the mucosal vessels (enhanced vulnerability to trauma).

Summary. The trachea of the living rabbit was observed during exposure to un-ionized and ionized N_2, O_2 and CO_2 using a separate airway for respiration. Positively charged CO_2 was found to be responsible for: reduction of ciliary activity, contracture of smooth muscle, ischemia and enhanced vulnerability to trauma.

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Emergence of Hepatitis Virus in Mice Injected with Ascites Tumor. (25247)

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An ascites tumor that originally arose in Princeton mice during transfer of *Eperythrozoon coccoides* was reported from this laboratory in 1956(1). After a long series of passages with ascitic fluid, carrying the tumor cells, lesions that were similar to those produced by the virus of murine hepatitis, MHV (Pr) (2), were observed in the livers of the