Published November 1, 1960

The Biological Mechanisms of Air Ion Action

II. *Negative air ion effects on the concentration and metabolism of 5-hydroxytryptamine in the mammalian respiratory tract*

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ABSTRACT Negative air ions are shown to decrease 5-hydroxytryptamine concentrations in extirpated strips of rabbit trachea and in the respiratory tracts of living mice. An initial exposure of guinea pigs to (−) air ions causes a transient rise in urinary 5-hydroxyindoleacetic acid excretion which is not observed upon subsequent exposures.

These findings are compatible with the hypothesis advanced earlier that (−) air ion effects depend on the ability of (−) ions to accelerate enzymatic oxidation of 5-hydroxytryptamine.

INTRODUCTION

Recently we advanced the hypothesis that the functional changes induced by exposure of the mammalian trachea to positive air ions are mediated by the release of free 5-hydroxytryptamine (5 HT, serotonin), while negative air ion effects are due to the ability of negative ions to accelerate the enzymatic oxidation of 5-HT (1). We pointed out that the experimental observations offered in support of this hypothesis, while suggestive, were not conclusive and stated our intention to seek definitive evidence by direct analysis of 5-HT levels in exposed tissues.

This research was supported by (1) a contract with the United States Air Force (A.F. 49 (638)-669) monitored by the Air Force Office of Scientific Research of the Air Research and Development Command, (2) a grant from the National Institutes of Health, and (3) a grant from the Committee on Research of the University of California.

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

Received for publication, May 18, 1960.

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Published November 1, 1960

The present paper reviews the data derived from analyses of whole mouse lungs and rabbit tracheal strips for 5-HT and of guinea pig urine for output of the specific metabolite 5-hydroxyindoleacetic acid during exposure to air ions. The results are compatible with the hypothesis.

MATERIALS AND METHODS

Animals used in these studies include male and female "Namru" stock mice (20 to 35 gm.), male and female New Zealand rabbits (7 to 10 pounds), and male and female guinea pigs (300 to 500 gm.).

Living mice and guinea pigs were exposed to negative air ions by means of special ionizing cages (2). Sections of rabbit trachea were exposed to negative air ions at room temperature in humid air chambers already described (3).

5-hydroxytryptamine was assayed in mouse and rabbit tissues by means of the spectrophotofluorometric method of Udenfriend, Weissbach, and Clark (4). Levels of 5-hydroxyindoleacetic acid were determined by the colorimetric method of Udenfriend, Titus, and Weissbach (5) as modified by Dalgliesh (6). "Sigma" preparations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were used as standards.

Individual procedures followed in each of the three sets of experiments are given below.

A. *5-HT Levels in the Mouse Respiratory Tract*

After being kept in control or ionizing cages for the desired period, mice were sacrificed with rectally administered nembutal. The respiratory tract of each animal was carefully removed from just below the larynx, washed, and trimmed, then immersed in liquid nitrogen. The frozen specimen was pulverized with a mortar and pestle and extracted with 0.1 N HCl.

Mice used in these experiments ranged in age from 3 to 8 weeks. However, the mice in any single experiment were always the same age; comparisons between control and test values were only made on mice of the same age, sex, and ancestry.

B. *5-HT Levels in the Extirpated Rabbit Trachea*

The tracheas of normal rabbits varied so widely from one another in 5-HT content that it was impossible to make comparisons between ionized and control animals, as was done with mice. For this reason the following "double control" technique was employed.

Each rabbit was sacrificed with a blow on the head or with intraperitoneally administered nembutal. The trachea was removed from just below the larynx to the point of bifurcation. After being washed and carefully trimmed of adventitious fat and connective tissue, the trachea was divided into three sections of equal weight (Fig. 1). Sections A and C were used as controls. Section B was exposed to negative air ions.

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In each control animal, the 5-HT levels of the three sections were almost always the same. Occasionally the level in section C was somewhat lower than the levels in sections A and B, presumably due to the increased ratio of cartilage to soft tissue in the lower part of the trachea. No cases were ever encountered among the controls in which section B was lower than sections A or C.

In order to exaggerate the differences between the control and ionized tissues, a higher level of tissue 5-HT was provided in some experiments by injecting 10 mg. 5-HT (1 ml. of a 1 per cent solution) into the animal at the moment when respiration ceased; under these conditions the heart continued to beat for several minutes after the injection.

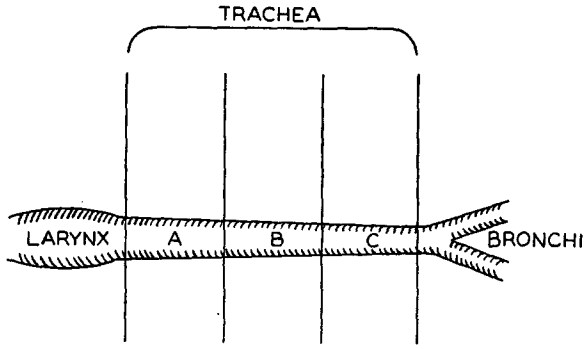

FIGURE 1. Method of sectioning and labeling the rabbit trachea for 5-hydroxytryptamine assay.

C. *5-HIAA Levels in Guinea Pig Urine*

Guinea pigs were held in specially designed ionizing metabolism cages; their filtered urine was collected daily, extracted, and assayed. Animals had continuous access to water, standard diet pellets, and chard. Daily observations of each animal's appearance and behavior were made throughout the experiment.

EXPERIMENTAL RESULTS

A. *Effect of Negative Air Ions on the 5-HT Content of the Mouse Respiratory Tract*

5-hydroxytryptamine levels in the respiratory tracts of young mice of the same age and history show remarkably little variation. This makes mice especially suited to a study of agents suspected of altering 5-HT concentrations.

The results of two typical experiments are shown in Fig. 2. Exposure to negative air ions invariably caused a drop in the 5-HT content of the mouse respiratory tract. The extent of the drop varied considerably, but in each

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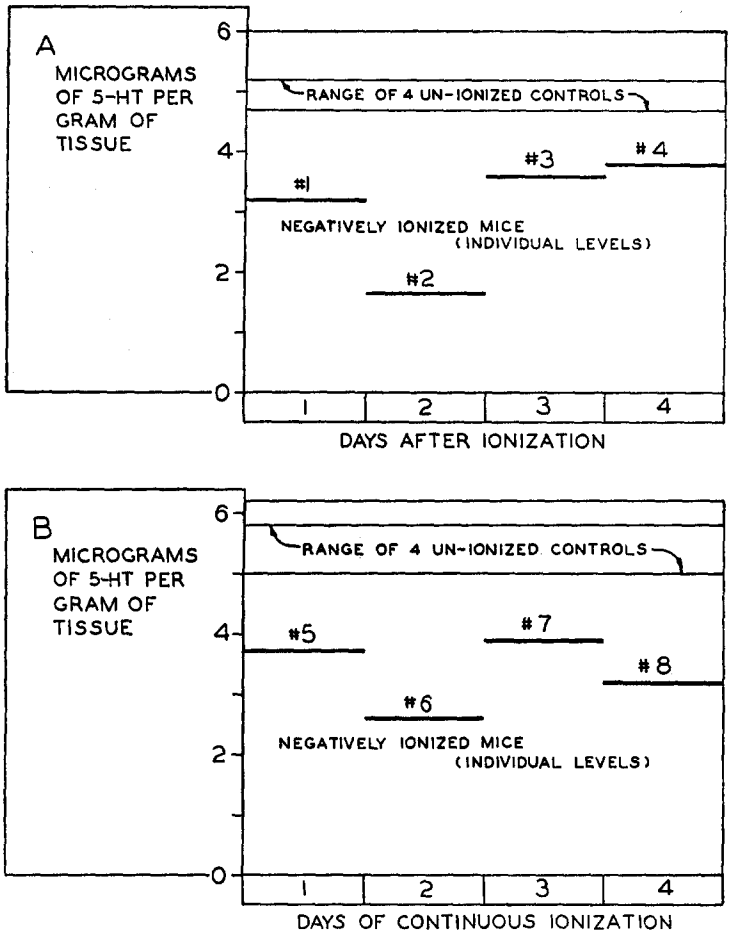

FIGURE 2. Reduced level of 5-hydroxytryptamine in the respiratory tract of ionized mice (eight control and eight ionized mice).

A. Following 14 hours' exposure to negative air ions (6 week old mice).

B. During continuous exposure to negative air ions (7 week old mice).

TABLE I

STATISTICAL VALUES FOR THE EXPERIMENTS SHOWN IN FIG. 2

	Mean	Difference	Standard deviation	Standard error of means	Standard error of difference (68 per cent)	Standard error of difference (99.5 per cent)
A.						
Control (4)*	5.0	1.90	0.19	0.09	0.42	1.26
Test (4)	3.1		0.82	0.41		
B.						
Control (4)	5.4	2.10	0.30	0.15	0.32	0.96
Test (4)	3.3		0.50	0.25		

* No. of animals used.

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experiment the difference between control and test samples was greater than could be accounted for by chance. Statistical values for the experiments shown in Fig. 2 are given in Table I.

A 12 hour exposure was sufficient to give a maximal decrease in 5-HT. Longer exposure did not bring about a correspondingly greater decrease in 5-HT. The reduced 5-HT levels persisted long after exposure to negative air ions had ceased.

TABLE II

EFFECTS OF NEGATIVE AIR IONS ON THE 5-HYDROXYTRYPTAMINE CONTENT OF THE EXTIRPATED RABBIT TRACHEA

Rabbit no.	Length of time section B exposed to (−) air ions	u 5-HT/gm. in each trachea triplicate (see Fig. 1) A (Control)	B (Test)	C (Control)
	I. Untreated series			
1	0.0 (control)	1.8	1.8	1.8
2	0.0 (control)	2.5	2.5	2.3
3	0.5 hrs.	1.5	1.2	1.5
4	0.5 hrs.	2.8	1.8	2.8
5	1.0 hrs.	1.9	1.7	1.9
6	1.0 hrs.	1.7	1.3	1.7
7	2.0 hrs.	1.9	1.3	1.9
8	3.0 hrs.	1.4	1.8*	1.4
	II. Pretreated series‡			
9	10 min.	2.9	1.8	2.9
10	30 min.	3.9	2.6	3.9
11	30 min.	1.5	3.8*	1.2
12	60 min.	2.2	4.7*	2.1

* These values were subsequently shown to be due in large part to 5-HIAA (see text).
‡ Each animal received 10 to 20 mg. 5-HT intravenously before sacrifice.

B. *Effect of Negative Air Ions on the 5-HT Content of the Extirpated Rabbit Trachea*

Using the three tracheal sections, we found small but reproducible decreases in the 5-HT content of the negatively ionized section (Table II, *I*). This decrease occurred after exposure periods from 20 minutes to 2 hours. With longer exposure periods, the 5-HT content of the ionized sample appeared to increase. When these samples were made alkaline, however, their fluorescence increased; the fluorescence of 5-HT under these conditions decreases. Upon further study it was found that these samples showed the characteristic optical properties of 5-hydroxy-indoleacetic acid (4).

In the living animal, 5-HIAA is removed by the blood stream as fast as it is formed from 5-HT. Under the conditions of our experiment, however, 5-HIAA slowly accumulated. The extraction method used in this assay was designed

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for fresh tissues in which 5-HIAA is normally absent. Since 5-HIAA fluoresces to a lesser extent than 5-HT at the acid pH used in the assay, it is necessary

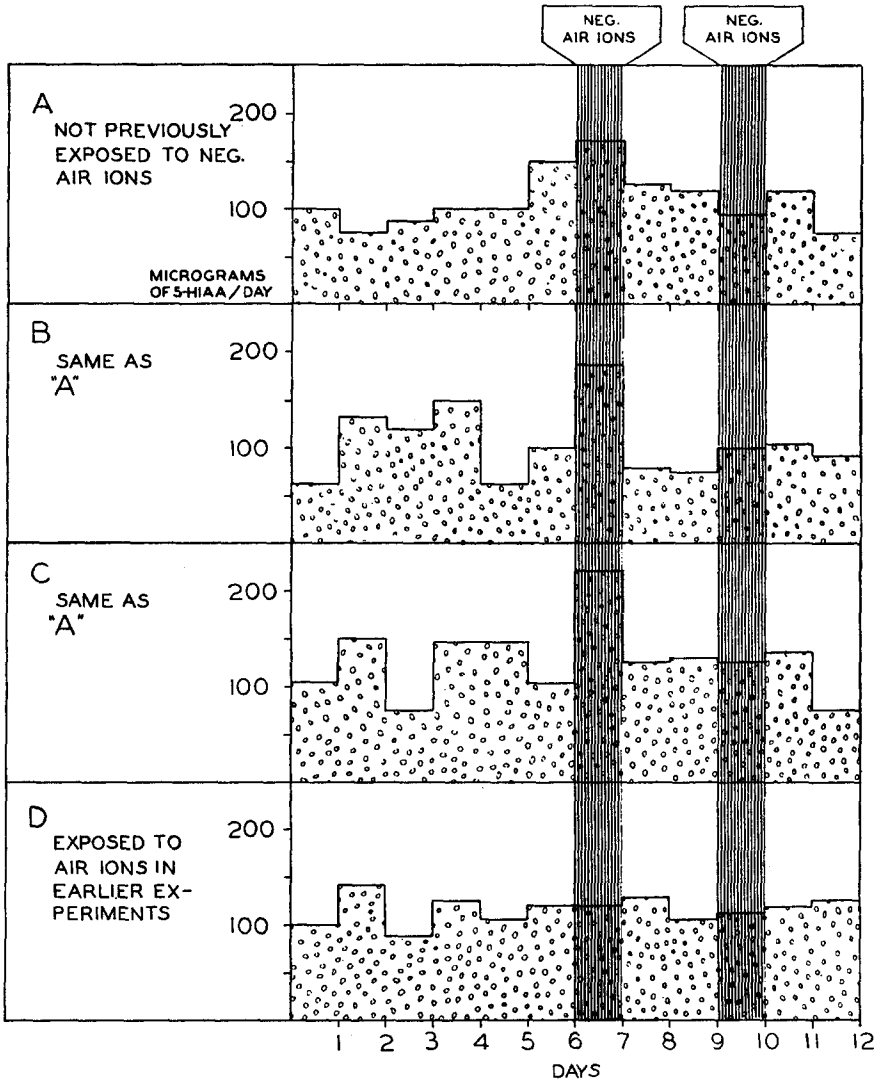

FIGURE 3. Increased excretion of 5-hydroxyindoleacetic acid from initial exposure to negative air ions but not in successive exposures (four guinea pigs, A, B, C, and D).

to assume that some bound 5-HT in the tissue was released and contributed to the 5-HIAA accumulation.

The (−) ion-induced sequence of events was accelerated when 5-HT was given to the animal intravenously just before death. In the ionized section of the trachea, the decline in 5-HT occurred within 10 to 30 minutes, while the accumulation of 5-HIAA became apparent within an hour (Table II, *II*).

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C. *Effect of Negative Air Ions on the 5-HIAA Excretion of Guinea Pigs*

When a guinea pig which had never previousy been exposed to unipolar air ions was allowed to breathe negative air ions for 24 hours, the level of 5-HIAA in its urine invariably rose. However, subsequent exposures to (−) air ions were completely without effect (Fig. 3).

Even after being kept in ordinary atmospheres for several months, the once ionized guinea pig remained unresponsive. Positive air ions were just as effective as negative air ions in bringing about this state of unresponsiveness.

DISCUSSION

The data presented here agree with our earlier hypothesis: that (+) and (−) air ion effects are mediated by the higher or lower 5-HT concentrations they produce in the exposed tissue (1). The one apparent departure from the hypothesis—the high values obtained with prolonged exposures of extirpated rabbit trachea to (−) ions in part B—turned out, upon closer study, to be an additional proof that (−) ions accelerate the oxidation of 5-HT.

Parts A and C of these experiments are in accord with one of the most puzzling of our earlier observations: the persistence of air ion effects in living animals for weeks after the cessation of ionization. For example, we had found that the enhanced ciliary rate induced in mice by (−) ions could still be detected after a month in ordinary air (2).

Part A of the present study shows that the ion-induced 5-HT decrease in the mouse respiratory tract likewise persists long after exposure to (−) ions has ceased. In part C, the increased excretion of 5-HIAA by guinea pigs occurred only on the very first exposure to (−) ions and could not be duplicated on successive exposures, presumably because the first exposure had already brought about a maximal depletion. These findings may be related to the subjective experience of human beings who often report that they "feel better" during a first exposure to (−) air ions, but find subsequent exposures to be without effect.

The question naturally arises as to how significant a reduction in the 5-HT content of the respiratory tract may be in various species. It would appear to be highly significant in mice; Fink (7) and Fox, Einbinder, and Nelson (8) have shown that anaphylaxis in mice is mediated chiefly through 5-HT and can be blocked by 5-HT antagonists. In the guinea pig and man, however, 5-HT appears to play a very minor role in comparison to histamine. Sparrow and Wilhelm (9), for example, report that histamine is 500 times more potent than 5-HT in causing changes in vascular permeability in the guinea pig. Brocklehurst (10) and others have been unable to implicate 5-HT in any human sensitization reactions.

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The question then can be answered only in part. There is at present no reason to anticipate that air ion–induced changes in the 5-HT of the respiratory tract in man or in the guinea pig would effectively change the pattern of pulmonary hypersensitivity. This does not mean, however, that all air ion effects on this state are precluded. So far as we know, no reliable data are as yet available on alterations in histamine release and metabolism brought about by exposure to (−) ions.

We wish to express our sincere thanks to Dr. H. A. Barker for allowing us to use his Aminco-Bowman spectrophotofluorometer and for giving us laboratory space while our own laboratory was being remodeled.

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