Lung 5-Hydroxytryptamine and Ozone Induced Pulmonary Edema in Rats.* (26572)

RALPH G. SKILLEN, CLINTON H. THIENES, JOSEPH CANGELOSI AND LORRENE STRAIN
Institute of Medical Research, Huntington Memorial Hospital, Pasadena, Calif.

Pulmonary edema and hemorrhage are well unkown results of exposure of animals to toxic doses of ozone(1-5). Experiments on dogs by Kabins *et al.*(6) suggest that 5-hydroxytryptamine (5-HT) may have a role in formation of lung edema. Since 5-HT is present in the lungs of rats in appreciable amounts(7,8), this investigation was undertaken to study the effects of ozone induced pulmonary edema on the lung 5-HT content.

Materials and methods. Male albino Wistar rats (325-375 g) were placed in wire cages 20 × 28 × 20 cm high set upon a hardware cloth shelf within an exposure chamber, constructed of galvanized sheet steel, 76 × 76 × 79 cm high. Air, passed through a Barnebey-Cheney Model 8CF air filter was admitted at the top, directed throughout the chamber by baffles, and pumped from the chamber at each of the bottom corners. Air was changed once every minute. Ozone, produced from tank oxygen in a 12,000 volt discharge ozonizer of the type employed by Quilligan *et al.* (9), was mixed with the filtered air before entering the chamber. Air samples were taken from a point just above the animals every 30 minutes during an experiment, and ozone content was determined by the neutral buffered 1% potassium iodide method of Byers and Saltzman(10), liberated iodine being measured photometrically at 352 mμ in a Beckman DU Spectrophotometer. Ozone concentration was maintained at 6 parts per million (p.p.m.) by volume, and exposure time was 4 hours in all experiments. Control animals were handled in the same manner except that addition of ozone to the chamber was omitted. Each group consisted of 11 animals. After exposure, these animals were anesthetized with pentobarbital sodium, and the lungs were removed, weighed, and assayed for 5-HT spectrophotofluorometrically in an Aminco-Bowman Spectrophotofluorometer by the method of Bogdanski *et al.*(11). The lungs of additional control and ozone-treated groups of rats, 11 animals each, were assayed for monoamine oxidase (MAO) activity by the method of Sjoerdsma *et al.*(12) using the colorimetric procedure of Udenfriend *et al.* (13). Protein contents of all lungs in this study were measured by the method of Lowry *et al.*(14). Blood samples were taken by cardiac puncture from 2 more groups of 11 rats each, exposed as before, one group to ozone and the other to air alone; these samples were obtained immediately after exposure and assayed for 5-HT by the method described by Udenfriend *et al.*(15). In the last hour of the 4-hour exposure, a few deaths occurred among those animals subjected to ozone. These animals were removed from the chamber immediately, the assay made, and the results included in the data for that experiment except when blood 5-HT was to be determined. In this case it was necessary to expose 3 additional rats to obtain blood from living animals.

Results. Rats exposed to 6 p.p.m. ozone by volume for 4 hours showed severe pulmonary edema and hemorrhage. The effects of this exposure on lung weight, lung protein, and lung 5-HT content are summarized in Table I.

Since there was an approximately 3-fold increase in lung weights and a 2-fold increase in lung protein contents of the ozone-exposed rats, consideration of the results obtained with these animals on a "per gram of tissue" or a "per gram of lung protein" basis is misleading. Therefore, results have been related to body weight in Table I. The findings show that 4 hours exposure to 6 p.p.m. ozone resulted in significant increases in lung weight, total protein in the lung, and total lung 5-HT.

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TABLE I. Mean Lung Weight, Protein, and 5-HT of Normal Male Rats Exposed for 4 Hours to 6 p.p.m. Ozone and Air Alone.

Group	g lung/100 g body wt	g lung protein/100 g body wt	μg lung 5-HT/100 g body wt
Control	.56 ± .09	.09 ± .02	.56 ± .07
Ozone	1.50 ± .30*	.19 ± .06*	1.33 ± .24*

* $P < .001$.

Although no definite explanation for the 5-HT increase can be given, the increased lung weight and protein content are considered to be the results of the edema and hemorrhage. Lung MAO activities of rats receiving a 4-hour exposure to 6 p.p.m. were compared to those of rats exposed to air alone. These data are summarized in Table II.

Ozone exposure resulted in a loss of MAO activity per g of lung. However, this appears to be a dilution effect which can be attributed to edema and hemorrhage since no significant difference exists between the 2 groups when MAO activity is considered in relation to quantity of lung per 100 g body weight. No significant difference was found in whole blood 5-HT levels. The group exposed to air alone had a value of 0.31 ± 0.02 μg/ml, while the ozone-exposed group had a value of 0.32 ± 0.02 μg/ml. A total of 36 rats were exposed to 6 p.p.m. ozone. In this group there occurred 5 deaths (14%) near the end of the 4-hour exposure period.

Discussion. Associated with ozone induced pulmonary edema is an increased lung 5-HT content. The source of this increase and the problem of whether it is cause or effect of pulmonary edema are open to question. Several possible explanations for this increased 5-HT can be suggested. One possibility is that the lung of the rat under certain conditions is able to trap and store 5-HT released from other tissues and that ozone exposure in some way induces such a release. If actually operative to greater or lesser degrees in the lung and possibly other tissues as well, such a trapping mechanism might be a partial explanation of the variable sensitivity of different tissues to the 5-HT releasing property of reserpine encountered by Parratt and West (7). However, the increase in 5-HT in the edematous lung may be simply a trapping of fluid containing this amine. The changes in 5-HT content and weight of the lungs are proportionally similar, 138 and 168% respectively; therefore, the 5-HT concentration of this fluid would necessarily approach that of the lung. Since this is approximately 2.5 to 3 times the concentration found in whole blood and since essentially all blood 5-HT normally is found in the platelets and none in the plasma(16), this seems unlikely. Retention of platelets in the lungs of ozone-exposed rats would be a more reasonable explanation although failure to demonstrate a change in blood 5-HT of these animals constitutes an objection to this. Another possible cause of the increased lung 5-HT following ozone exposure may be the decreased MAO activity per g of lung. This is considered to be the result of dilution by edema and hemorrhage since no such significant change is found when evaluation is in terms of activity per lung per 100 g body weight, this latter measurement indicating destruction rather than dilution of activity. If this dilution of MAO is the sole reason for the increase of lung 5-HT, then pulmonary edema must be considered the cause rather than its effect. Still another possible answer may be that ozone exposure results in an increased activity of 5-hydroxytryptophan decarboxylase, the enzyme responsible for the decarboxylation of 5-hydroxytryptophan to 5-HT. This possibility as well as those previously mentioned are now under investigation.

TABLE II. Mean MAO Activity as Measured by 5-HT Utilization in Lungs of Normal Male Rats Exposed for 4 Hours to 6 p.p.m. Ozone and Air Alone.

Group	Moles × 10^{-5} consumed/hr	
	Per g lung	Per lung/100 g body wt
Control	1.03 ± .21	.56 ± .14
Ozone	.33 ± .20*	.46 ± .24

* $P < .001$.

Summary. Rats exposed to 6 p.p.m. ozone by volume for 4 hours developed severe pulmonary edema and hemorrhage with significant increases in lung weight, lung protein content, and lung 5-HT when related to body weight. Ozone exposure was found to decrease MAO activity; this was an effect of dilution by edema rather than destruction of the enzyme. Ozone exposure did not alter blood 5-HT content. Possible mechanisms involved in the lung 5-HT increase after exposure to ozone were discussed briefly.

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Passage of Thiopental and Barbital Into Ocular and Cerebrospinal Fluids of Dog.* (26573)

PETER G. DAYTON, LEONARD BRAND, DOLORES TALLER AND LESTER C. MARK

New York Univ. Research Service, Goldwater Memorial Hospital and Dept. of Anesthesiology, Columbia Presbyterian Medical Center, N. Y. City

The passage of barbital and thiopental into ocular and cerebrospinal fluids was investigated in order to extend previous studies of the passage of barbiturates into various compartments such as brain, cerebrospinal fluid, fat and muscle(1-3). The comparison between ocular and spinal fluid was made because embryological and physiological similarities exist between the 2 fluids(4).

Methods and materials. Barbital was determined by the method of Giotti and Maynert(5). Thiopental was measured by the method of Brodie *et al.*(1). When concentrations were low, after extraction of drug by the organic solvent, the drug was extracted into 1 or 2 ml of aqueous phase and measured either in micro or semi-micro cuvettes. The methods gave negligible blank values and recoveries averaged 90-100%.

Mongrel, adult female dogs weighing 7-20 kg were used for this study. Barbital sodium (100 mg/kg) as a 10% neutral solution or thiopental sodium (50 mg/kg) as an alkaline (pH 10.5) 2.5% solution was injected intravenously within 5 seconds. Artificial respiration was given to the thiopental-treated dogs

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