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EFFECT OF EXPOSURE TO O_3, SO_2 AND NO_2 UPON THE LUNG HISTAMINE CONTENT OF GUINEA PIGS

BY

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ABSTRACT

The lung histamine contents of air-control and O_3-, SO_2-, NO_2- exposed guinea pigs were compared, and the water content of the lungs was estimated in each group. It was noted that the mean histamine content of the lungs of animals exposed to low and high concentrations of ozone was below the mean of the control group. Lung edema was produced by both low and high concentrations of ozone exposure. Massive lung edema was also produced in the lungs of guinea pigs exposed to a high concentration of NO_2, while there was no significant change in the mean histamine content of the lungs. Using an apparatus for perfusion of isolated lungs of guinea pigs, a large amount of histamine was found to be contained in the perfusion out-flow collected during 0 to 90 minutes from O_3-ventilated lungs.

INTRODUCTION

Many investigations have been reported on the toxic effects on men and animals of O_3, SO_2 and NO_2, which are common pollutants in the atmosphere of industry and urban community. It has been well established that exposure to high concentrations of O_3 or NO_2 results in massive lung edema and hemorrhage[1,2]. On the other hand, histamine was mentioned as one of pharmacological agents related to lung edema in a review of Vischer et al.[3] An increased pulmonary capillary permiability is thouhgt to be a primary factor for the pathogenesis of lung edema produced by pharmacological agents. The two most prominent features of local histamine action are smooth muscle contraction and increased capillary permiability. Histamine has long been recognized as one of the participants in the inflammatory process[4,5] and it has been suggested as the initiator of the vascular reaction to tissue injury[6]. There are several observations that the injection of histamine caused pulmonary congestion and edema in guinea pigs[7], and the susceptibility of guinea pigs to histamine increased after

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ozone-exposure[8].

The cause of lung edema following the exposure to O_3 was speculatively attributed by Stokinger[9] to the change in permiability of lung vessels caused by the histamine (or bradykinin) which was liberated from the lung cell under the direct action of O_3 upon the cell. Dixon et al.[10] have shown a release of endogenous histamine of mouse lungs after ozone-exposure. Easton and Murphy[8] reported the mean lung histamine value for guinea pigs sacrificed 90 minutes after the end of ozone-exposure was 20%, although not differed significantly, below the control mean. On the other hand, Troquet and Lecomte[11] concluded that histamine liberation has no great role in O_3 intoxication because no change in lung histamine was found in rats immediately after exposure to 60 to 100 ppm O_3 for 6 hours while it increased four to five-fold 5 days later.

This study was undertaken to throw light on what role histamine might have in lung edema produced by exposure to toxic, edemagenic gases and to know whether the exposure to gases which are known to be injurious (either edemagenic or non-edemagenic) to the lung could cause a change in the lung histamine content.

Guinea pigs were used as experimental animals because of their high lung histamine level (15–35 μg/g)[12]. They were exposed to different concentrations of three kinds of gases, O_3, SO_2 and NO_2. For comparison the effect of injected alpha-naphthyl thiourea (ANTU), which was studied extensively as a mean of producing lung edema[13–15], was also tested. Furthermore, the release of histamine into the perfusate was studied using an apparatus for perfusion of isolated guinea pig lungs.

Materials and Methods

a. Exposure of animals.

Random-bred male guinea pigs (270–370 g) were used. The animals were housed in air-conditioned laboratories and were fed a standard guinea pig diet. Six to eight guinea pigs were exposed to gases in stainless steel whole body exposure chambers ($60\times60\times80$ cm) for 3 hours. Chamber ventilation was approximately three air changes per minute. Ozone was produced by pure oxygen through a dielectric ozone generator and its predetermined volume-flow was diluted with a stream of clean air and then introduced into the gas-exposure chamber so that the concentrations of ozone in the chamber were 1 ppm and 4–8 ppm by volume in air. Metered dilute SO_2 (1.07%) and NO_2 (0.48%) were the source of the exposures; concentrations in the chamber were 10 ppm and 50 ppm, and 10 ppm and 80 ppm for SO_2 and NO_2, respectively. Analyses of concentrations of these gases were made at frequent intervals during exposure by the following

methods. The neutral potassium iodide method of Byers and Saltzman was used for O_3[16], the electroconductivity method of Thomas and Abersold for SO_2[17], and the method of Saltzman for NO_2[18]. Intact guinea pigs were used as the control animal, because air-exposure in the chamber was proven to have no effect on the animals.

b. Intraperitoneal injection of alpha-naphthyl thiourea (ANTU).

The solution of ANTU was prepared containing 10 mg/ml in propylene glycol, and the animals were injected intraperitoneally with 100 mg/kg, then sacrificed three hours later.

c. Measurement of lung water content.

After 3 hours exposure to the gases, the animals were sacrificed by decapitation. The lungs were removed and dissected just above the tracheal bifurcation, washed with Ringer's solution and any excess fluid on the surface was removed gently with a filter paper. After weighing, the right lung was dried at 110°C for 24 hours, and weighed again. The weight of edematous lungs is known to increase due to not only water but protein accumulating in the lung[19,20]. However, for the sake of simplicity, the difference in weight between fresh and dried lung was assumed in this study to represent the water content of the fresh lung, and the dried lung weight was assumed to represent the lung parenchyma weight. The normal range of the lung water content in this study was 79.3±0.2% and independent of body weight. Thus, lungs with a water content significantly above this range can be presumed to be edematous.

d. Estimation of histamine.

The estimation of the histamine content of the lungs, and of the perfusate in the perfusion experiments was performed by the method described by Shore et al.[12] with a slight modification. Fluorescence readings were converted to histamine content by reference to a curve for readings of histamine standards containing 5–15 μg of histamine base in 4 ml of 0.1 N HCl, put through the same procedure as the tissue. Throughout this procedure, the recoveries of authentic histamine added to tissue homogenates ranged from 90 to 100%. On the other hand, the fluorescence reading of authentic histamine treated by the procedure of Shore et al. was constantly 60% of the reading of same amount of histamine being directly put into 4 ml of 0.1 N HCl and not carried throughout the procedure. Because histamine may be diluted in the edematous lungs by the accumulated fluid, the histamine content per gram of fresh lung weight is thought not to give an appropriate measure for comparing the quantitative change of histamine in the lung. Therefore, histamine contents of the lungs were compared on the basis of per gram of lung parenchyma (μg histamine per g fresh lung tissue/% parenchyma$\times$1/100), of the total lung histamine content

(μg histamine per g fresh lung tissue $\times$ fresh lung wt.), and of total histamine content per 100 g body weight.

e. Perfusion of isolated lungs of guinea pigs.

A separate group of animals was used in this experiment. Guinea pigs were anesthetized by intraperitoneal pentobarbital (30 mg/kg), and heparinized (2 mg/kg) intravenously. The chest was opened and the trachea cut. After being freed from surrounding tissue, the trachea, lung and heart were excised en bloc, then after removal of the pericardium, a vinyl cannula filled with perfusion fluid was inserted into the main pulmonary artery through the right ventricle. The left ventricle was opened and a vinyl cannula was inserted. The trachea was cannulated and the lung was gently inflated through this cannula, and Ringer's solution was sprayed on the surface of the lung. The trachea, lung, heart and pulmonary vessels were transferred to a humidified, constant-temperature chamber shown in Fig. 1. The trachea cannula was connected through a cover plate to a Harvard respirator with which the lung was ventilated with air or 8–12 ppm ozone; tidal volume was 20 ml and respiration rate was 25 per minute. A perfusion pump was connected to the perfusion cannula. The perfusion fluid was a mixture of 100 ml Tyrode's solution containing clinical polyvinylpyrrolidone (6% solution) and 4 ml of blood plasma of the donor guinea pig. For perfusion, the volume in-flow was kept constant at the rate of 0.8 ml per minute. The out-flow from left ventricle was collected in separate tubes 30, 60 and 90 minutes after the start of perfusion and the histamine content was estimated as soon as possible. Also the histamine content remaining in the lungs which were used in the operation described above was estimated.

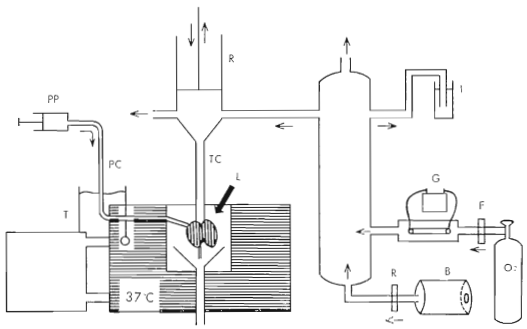

Fig. 1. Apparatus for perfusion of guinea pig isolated lungs. L: lung, PC: perfusion cannula, PP: perfusion pump, R: respirator, TC: trachea cannula, T: thermometer, F: Flow-metor, G: O_3 generator, B: blower, I: impinger.

Results

Table 1 shows that the average lung water contents of animals exposed to 4–8 ppm ozone and 80 ppm NO_2 were considerably higher than that of control guinea pigs ($P<0.01$), while the animals exposed to the lower concentration of O_3 and 10 ppm NO_2 had slightly increased water content (O_3: $P<0.01$, NO_2: $P<0.05$). When compared to control animals, the average total lung histamine content of guinea pigs exposed to O_3, both 1 ppm and 4–8 ppm, was significantly reduced ($P<0.05$), but that of guinea pigs exposed to NO_2 was within normal range. Also on the basis of either the content per gram of lung parenchyma or per 100 g of body weight, it is evident that the average histamine content of animals exposed to O_3 significantly reduced (1 ppm: $P<0.05$, 4–8 ppm: $P<0.01$), but not in the case of animals exposed to NO_2 (Fig. 2).

The reduction of the lung histamine content observed in animals exposed to O_3 might mean the release of endogenous histamine from the lung cells. This gives rise to question of where the released histamine goes. It could be possible that the endogenous histamine liberated from the cells by exposure to O_3 enters the circulation. The experiment of the lung

Table 1. Lung Water and Histamine Content of Guinea Pigs Exposed to O_3, SO_2, NO_2 for 3 Hours, and Injected α-Naphthyl Thiourea

Groups		Lung Water Content (%)	Total Lung Histamine Content (μg)	Lung Histamine Content μg/g Parenchyma	Lung Histamine Content μg/100g Body wt
Control (n=13)		79.3±0.2	57.4± 4.2	114.0± 8.4	16.9±1.5
O_3	1 ppm (n=12)	81.0±0.2**	42.8± 3.8*	78.6± 2.9*	12.2±1.1*
	4–8 ppm (n=13)	86.2±0.5**	41.2± 5.5*	78.4± 8.8**	12.3±1.4**
SO_2	10 ppm (n= 6)	79.8±0.2	74.4±18.3	135.2±26.0	21.6±4.9
	50 ppm (n= 6)	79.4±0.4	57.3± 8.4	112.3±18.7	16.5±2.7
NO_2	10 ppm (n= 5)	80.4±0.3*	70.6±12.3	148.2± 9.0	21.0±2.2
	80 ppm (n= 7)	83.9±0.8**	60.6±13.8	111.9±26.4	17.0±4.0
Injected α-Naphthyl Tiourea. 100mg/kg (n=8)		79.6±0.2	62.3± 3.3	105.1± 5.1	15.4±0.74

Figures are mean values±standard error. *: $P<0.05$ **: $P<0.01$

perfusion evidently showed that a larger amount of histamine was contained in the perfusion outflow collected during 0 to 90 minutes from O_3-ventilated lungs rather than that collected from air-ventilated lungs (Table 2). The combine value of the total histamine content in the per-

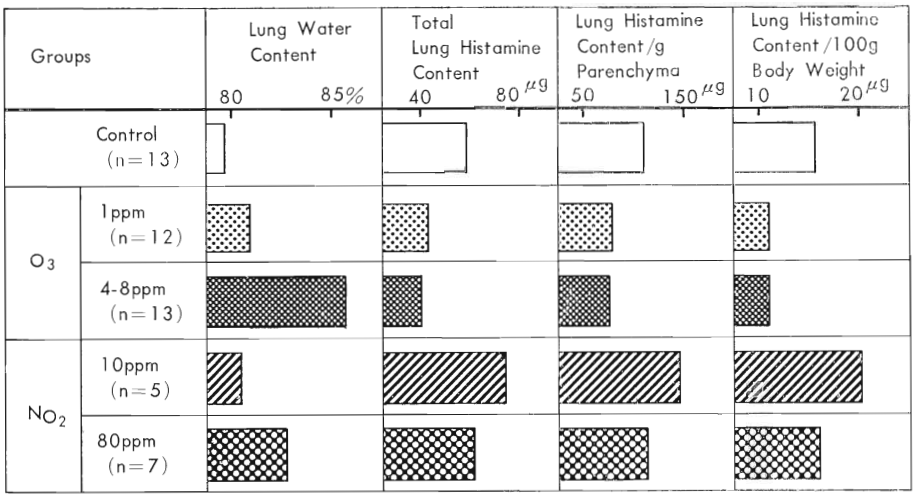

Fig. 2. Development of lung edema and lung histamine content of guinea pigs exposed to O_3 and NO_2 for 3 hours.

Table 2. Histamine Contents in Outflow Perfusion Fluid and Isolated Lungs Ventilated with Air and 8–12 ppm Ozone

Groups	Experiment No.	Histamine Release in Perfusion Fluid (μg) Min. 0–30	30–60	60–90	Total	Residual Histamine Content of Perfused Lung (μg)	Lung Weight of Perfused Lung (g)	% Histamine Content Perfusion Fluid	Perfused Lung
Air Ventilation	1	0	0	0	0	74.3	3.3	0	100
	2	0	0	1.3	1.3	41.0	4.0	3.1	96.9
	3	4.7	0	1.1	5.8	42.9	3.9	11.9	88.1
	4	6.1	0	0	6.1	31.0	3.2	16.5	83.5
	5	1.3	0.9	2.2	4.4	57.0	3.8	7.2	92.8
	Mean	2.4	0.2	1.0	3.5	49.2	3.6	7.7	92.3
O_3 Ventilation	6	1.4	1.4	0	2.8	35.9	3.9	7.2	92.8
	7	7.2	8.5	2.8	18.5	31.7	3.8	36.9	63.1
	8	5.4	3.5	1.8	10.7	20.5	5.7	34.3	65.7
	9	3.6	1.8	7.5	12.9	51.6	6.5	20.0	80.0
	10	2.2	3.6	2.0	7.8	45.0	4.0	14.8	85.2
	Mean	4.0	3.6*	2.8	10.5*	38.9	4.8	22.6*	77.4*

*: $P<0.05$

fusion out-flow plus the residual histamine content of the perfused lung was assumed to be the original histamine content which was contained in the lung before perfusion, and the percentages of each fraction were calculated to the original histamine content of the lungs. The amount of histamine in the out-flow was to average 22.6% of the original histamine content of the lungs ventilated with O_3 (Fig. 3), while it was only 7.7% of the original histamine content of the lungs ventilated with air ($P<0.05$). There was a slight difference in the weight between the lungs ventilated with air and ozone (air: 3.6 g, O_3: 4.8 g), but it did not attain statistical significance.

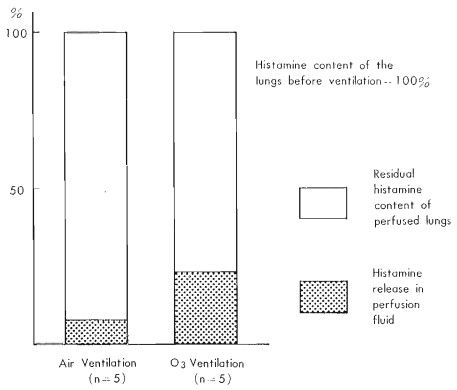

Fig. 3. Average histamine content in perfusion outflow fluid and perfused lungs of guinea pigs.

SO_2-exposure, both in low and high concentrations of the gas, had no effect on the water and histamine content of the lungs of the animals. Guinea pigs administrated ANTU did not show any change in both the lung water and lung histamine contents.

Discussion

A current theoretical concept concerning the biochemical mechanisms of ozone toxicity is that ozone reacts with cellular sulfhydryl groups, causing direct damage to the proteins requiring this chemical groups and toxic effects are then propagated further by free radicals formed during the interaction of ozone with sulfhydyl groups. These free radicals would change the cell permeability and cause histamine liberation or bradykinin formation. The liberated histamine might increase the permeability of lung vessels and cause lung edema[9].

It is supported that the physiological effects of endogenous histamine occur only when certain stimuli cause its transfer to the extracellular space,

because intracellular histamine is physiologically inert. Such stimuli may be chemical, enzymatic, or physical in nature[21].

The present study showed evidently that the development of lung edema advanced in parallel with reduction of the lung histamine content of the guinea pigs exposed to O_3, and the perfusion experiment strongly suggested that the reduction of lung histamine content was due to its increased liberation into the pulmonary circulation. Therefore, the present evidences concerning with the effect of O_3 appear to support the concept described above.

Prior to the experiment of NO_2, the author expected the change of lung histamine content similar to that of O_3 exposure, because it has been known that the way in which NO_2 physiologically acts closely parallels that of O_3. For instance, both O_3 and NO_2 are deep-lung irritants, and protection against acute lethal effects by sulfur-containing compounds is about equally good for both substances[22]. Against my expectation, lung edema developed in the guinea pigs exposed to high concentration of NO_2 without reduction of the lung histamine content, which suggested the different mechanisms involved in toxic edema reaction of NO_2 other than the mediation of endogenous histamine of the lungs.

Although the details of those mechanisms were not revealed in this study, the most probable cause is a direct action of NO_2 upon the lung vessels. However, the secondary responses also should be considered to know the pathway of action of NO_2. The reason why O_3 and NO_2, which are both edemagenic, have different effects upon the lung histamine content should be further investigated.

Besides the similar actions of O_3 and NO_2, there are several evidences indicating somewhat different modes of toxicological and physiological action. The bronchiolitis fibrosa obliterans is developed by exposure to NO_2, but not to O_3[23], and the effect of *in vitro* exposure to NO_2 on lung surface tension properties is qualitatively opposite to that of O_3[24]. Chemically, NO_2 is known to be more soluble in water than O_3[23], which might partially determine the penetration rate into the respiratory passages. Moreover many compounds were reported which could cause the liberation of histamine from the lungs with or without production of lung edema[25]. Therefore, it seems likely that a certain chemical agent can cause lung edema without having direct relation to the histamine release.

Alpha-naphthyl thiourea was tested here as one of pharmacological agents producing lung edema in guinea pigs. Large amounts of ANTU were given by intraperitoneal injection. The attempt to produce lung edema failed, as this rodenticide is a relatively specific poison for Norway rats[15], therefore it might not be toxic enough to produce the lung edema in guinea pigs. The guinea pigs exposed to SO_2 did not show any

change in the lung water content and histamine content possibly because SO_2 differs markedly from O_3 and NO_2 in that its acutely irritant effects are confined to the upper respiratory tract.

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