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Acute Exposure to Realistic Acid Fog: Effects on Respiratory Function and Airway Responsiveness in Asthmatics

DIMITRI LEDUC,* SOPHIE FALLY,† PAUL DE VUYST,* ROLAND WOLLAST,† AND JEAN-CLAUDE YERNAULT*

*Chest Department, Erasme University Hospital, Brussels, Belgium; and †Waste Water Treatment and Pollution Department, University of Brussels, Belgium

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Naturally occurring fogs in industrialized cities are contaminated by acidic air pollutants. In Brussels, Belgium, the pH of polluted fogwater may be as low as 3 with osmolarity as low as 30 mOsm. In order to explore short-term respiratory effects of a realistic acid-polluted fog, we collected samples of acid fog in Brussels, Belgium, which is a densely populated and industrialized city, we defined characteristics of this fog and exposed asthmatic volunteers at rest through a face mask to fogs with physical and chemical characteristics similar to those of natural fogs assessed in this urban area. Fogwater was sampled using a screen collector where droplets are collected by inertial impaction and chemical content of fogwater was assessed by measurement of conductivity, pH, visible colorimetry, high pressure liquid chromatography, and atomic absorption spectrophotometry over a period of one year. The fogwater composition was dominated by NH_4^+ and $SO_4^=$ ions. First we evaluated the possible effect of fog acidity alone. For this purpose 14 subjects with asthma were exposed at rest for 1 hr [mass median aerodynamic diameter to a large-particle (MMAD), 9 μm] aerosol with H_2SO_4 concentration of 500 $\mu g/m^3$ (pH 2.5) and osmolarity of 300 mOsm. We did not observe significant change in pulmonary function or bronchial responsiveness to metacholine. In the second part of the work, 10 asthmatic subjects were exposed to acid fog (MMAD, 7 μm) containing sulfate and ammonium ions (major ions recovered in naturally occurring fogs) with pH 3.5 and osmolarity 30 mOsm. Again, pulmonary function and bronchial reactivity were not modified after inhalation of this fog. It was concluded that short-term exposure to acid fog reproducing acidity and hypoosmolarity of natural polluted fogs does not induce bronchoconstriction and does not change bronchial responsiveness in asthmatics.

INTRODUCTION

Water collected from naturally occurring fogs often is found to be contaminated by acidic air pollutants (Waldman *et al.*, 1982; Jacob *et al.*, 1985; Johnson *et al.*, 1987). The pH of polluted fogwater may be as low as 3 (Jacob *et al.*, 1985; Johnson *et al.*, 1987). Acid fogs are formed via heterogeneous phase reaction of sulfur dioxide (SO_2) or nitrogen oxides (NO_X) with atmospheric water droplets. Sulfuric acid (H_2SO_4) and nitric acid (HNO_3) are the most common acid air pollutants.

Sulfuric acid aerosols have been noted to cause significant decrement of pulmonary function in asthmatics by Utell and co-workers (1983) and Koenig and co-workers (1983) after exposure to H_2SO_4 in concentrations of 450 and 100 $\mu g/m^3$, respectively. Exposure to sulfuric acid aerosols has also been associated with increased bronchial responsiveness (Utell *et al.*, 1984).

On the basis of the limited measurements of ionic strengths in liquid fogwater reported to date (Waldman *et al.*, 1982; Jacob *et al.*, 1985; Johnson *et al.*, 1987), we can calculate that fogs usually have an osmolarity lower than 30 mOsm. Inhalation of hypoosmolar aerosols is also a well-established stimulus to bronchoconstriction (Schoeffel *et al.*, 1987; Anderson *et al.*, 1983; Sheppard *et al.*, 1983). Therefore, the effects of hypoosmolarity of fog could theoretically enhance the effects of acidity on the respiratory tract. However, studies addressing the effects of acid aerosols considered aerosols of dry submicrometric particles and may be less relevant in relation to fog exposures: typical fog droplets are at least an order of magnitude larger in diameter and do not have the same pattern of deposition in the respiratory tract, which might change their health risk.

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The aim of this study was to assess the acute effects of a realistic acid fog on respiratory function and bronchial responsiveness. The first step of this multidisciplinary work was the characterization of acid fog in an urban area. In a second step we exposed volunteer asthmatics to a fog as similar as possible to natural acid fog.

METHODS

Brussels Fog Assessment

Site

Samples were collected at eight different sites in the city of Brussels, Belgium, depending on the presence of fog (Fig. 1). The city is located within a dense traffic system; most of the industries and the international airport are located North and Northeast of the downtown area. Brussels is also subject to external anthropogenic sources of pollution due to the proximity (a few hundred miles) of several other large industrial concentrations such as the German Ruhr area, Rotterdam, and Belgian cities (Antwerpen, Ghent, Liège, Charleroi). Brussels is a densely populated (one million inhabitants) and moderately industrialized city.

Sampling

Fogwater was sampled using a screen collector (Jacob *et al.*, 1985a). Droplets are collected by inertial impaction on an angled screen made of six layers of 500-μm-diameter polyamide strands. Droplets coalesce rapidly on these strands and flow downward due to gravity and aerodynamically drain into a funnel, which drains the fogwater into a preweighted polyethylene collection bottle. The size cut for 50% collection efficiency, predicted from inertial impaction theory (Friedlander, 1977; Johnson *et al.*, 1987), corresponds to a droplet diameter of 6 μm. The screen collector samples 9.8 m^3 air/min with an efficiency of 90%. A collection rate of approximately 55 ml/h is yielded in a fog with a typical LWC[1] of 0.1 g^{-3}. Prior to sample collection, the collector is rinsed thoroughly with ultrapure water (MQ water). Forty-five fogwater samples corresponding to 18 fog events were taken between 22 October 1991 and 16 February 1993.

[1] Abbreviations used: MMAD, mass median aerodynamic diameter; LWC, liquid water content; FEV_1, forced expiratory volume in 1 s; R_{aw}, airway resistance; SR_{aw}, specific airway resistance; FRC, functional residual capacity; RV, residual volume; ERV, expiratory reserve volume; PD_{20}, cumulative dose of metacholine responsible for a 20% fall of FEV_1; SEM, standard error of the mean; Mch, metacholine; SG_{aw}, airway conductance; VMD, volume median diameter

Analytical Procedures

As soon as possible after collection, samples were filtered through 0.45-μm-pore membrane filters (Sartorius, cellulose nitrate) to prevent as much as possible any changes in the concentrations of the chemical species primarily caused by the solubilization processes of the particulate matter. The conductivity and the pH were measured immediately after sample filtration. The samples were then stored in a refrigerator at 4°C until analyses. NH_4^+ and NO_3^- were analyzed by visible colorimetry on an autoanalyzer. Depending on the concentration range of the sample, Cl^- and SO_4^{2-} were measured by high pressure liquid chromatography or by visible colorimetry. Major cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were determined by atomic absorption spectrophotometry.

Exposure to Acid Fog

Subjects

All the subjects were volunteers and were informed of the risks of the experimental protocol, which was approved by the institutional review board for human studies. Exposed subjects were asthmatics. Asthma was defined by a history of recurrent episodes of wheezing, chest tightness, and reversible airway obstruction (reversibility > 10% of predicted value of FEV_1) and/or airway hyperresponsiveness (PD_{20} < 7.8 μM metacholine).

Theophylline preparations, inhaled β adrenergic agents, or ipratropium were stopped 18 hr before the bronchial responsiveness testing. No subject took oral corticosteroids within the 2 weeks before the test and inhaled corticosteroids were withheld one week before the test. All subjects denied a history of upper or lower respiratory infection within 4 weeks before study. Smokers had to stop smoking 18 hr before the test. Compliance of smoking subjects with the 18-hr cessation interval was difficult to judge objectively. Only two subjects were ruled out because they admitted having smoked during this period. For the rest of the group, compliance was considered relatively good. Perhaps this is due to a relatively moderate level of tobacco consumption in our smoking subjects (mean + SEM: 10.5 ± 2.5 cigarettes per day).

Fog Generation and Exposure

Fog was generated by an air-compressed nebulizer (Hudson Updraft 2, Hendley Medical Supplies, Ltd). The air we used (Oxhydrique Internationale SA) did not contain significant concentrations of atmospheric pollutants (H_2O < 15 ppm, CO < 5 ppm, SO_2 < 0.1 ppm, NO–NO_2 < 1 ppm, NH_3 < 1 ppm, Cl_2

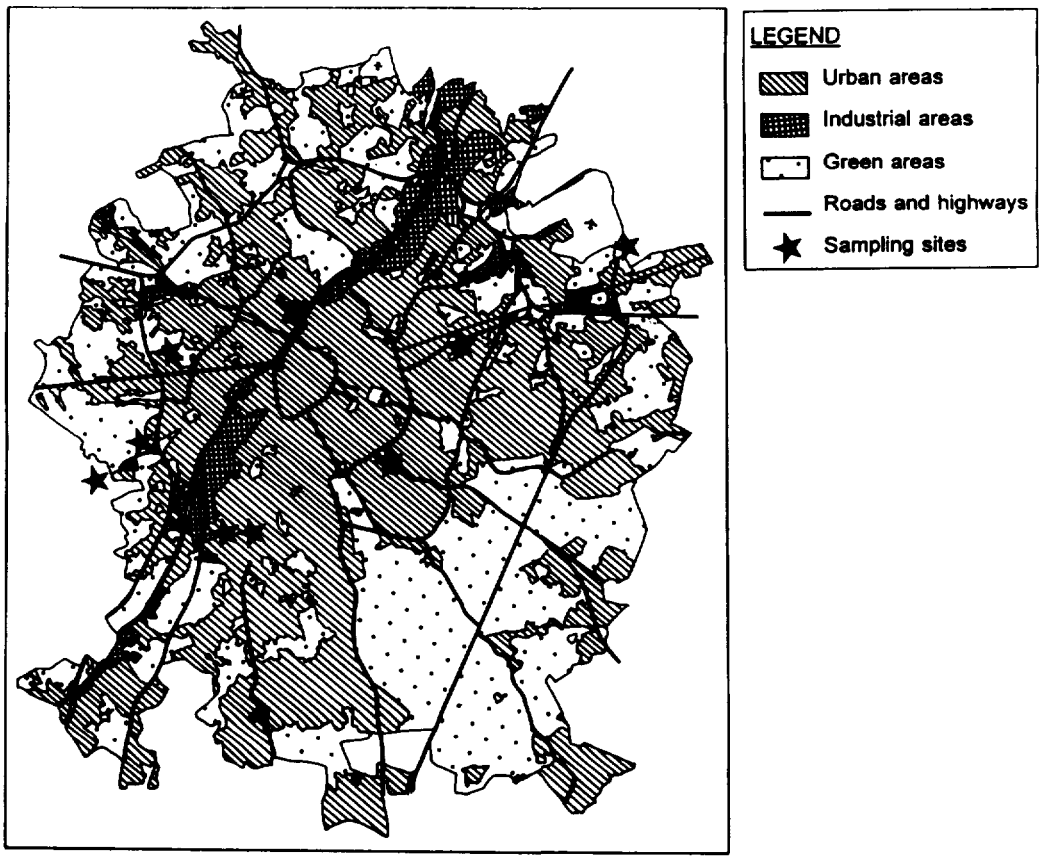

FIG. 1. Map of survey area (Brussels, Belgium).

< 0.1 ppm). Subjects were exposed to acid fog through a face mask (covering mouth and nose). Granulometry was controlled by an optical aerodynamic particle counter (Polytec-Waldbrown, RFA) under conditions of temperature (~18°C) and relative humidity (~75%) identical to exposure conditions. The physical and chemical characteristics of the fog are summarized in Table 1. In the first step, H_2SO_4 was suspended in NaCl solution to produce an isoosmolar acid fog with H_2SO_4 concentration 500 $\mu g/m^3$. In this step we investigated only the effects of acidity in fog. In the second step, the hypoosmolar and acid reconstituting conditions of the fog were very similar to those of naturally occurring fog. Both exposure conditions corresponded to that of very dense fog (LWC $\geq$ 0.5 g/m^3).

Measurement of Pulmonary Function

Subjects performed pulmonary function studies in an integrated-flow, pressure-corrected body plethysmograph (Leith and Mead, 1974). Airway resistance (R_{aw}) was measured during panting as described by Leith and Mead (1974) and Dubois and co-workers (1956). Airway resistance was corrected for volume and expressed as specific airway resistance (SR_{aw}). Before measurements of SR_{aw}, a 30-s period of normal breathing was monitored on oscilloscope to avoid reflex changes in bronchial tone induced by rapid changes in lung volume. Then, beginning at the end of a normal tidal breath, the subject panted against a closed shutter for measurement of functional residual capacity (FRC). To calculate the residual volume (RV), the subject expired maximally and expiratory reserve volume (ERV) was subtracted from FRC. After this, the subject inspired maximally and TLC was calculated by adding RV and inspiratory forced vital capacity. Finally, the subject expired forcefully to RV and the forced expiratory volume in 1 sec (FEV_1) was measured. A minimum of three maneuvers were performed and we used acceptability and reproducibility criteria recommended by the American Thoracic Society (1987) to select the measurement we recorded.

Assessment of Bronchial Responsiveness

Bronchial responsiveness was assessed pharmacologically using a classic Mch challenge (Sterk *et al.*, 1993). Metacholine was delivered by a MEFAR MB dosimeter (calibrated output: 14 μl per puff; droplets size, polydisperse aerosol from 0.5 to 5 μm).

For each metacholine dose, subjects inhaled five times to their TLC. FEV_1 was measured 30 and 90 sec following each five inhalations and the best value was chosen. The metacholine concentrations were progressively increased (0.125–0.250–0.5–1–2–5–10–20–25 mg/ml). The FEV_1 measured after inhalation of diluent (NaCl 0.9%) was taken as the pretest value and the test was stopped when FEV_1 had decreased at least 20%. For each subject a dose–response curve was established where the percentage fall in FEV_1 was plotted against the cumulative dose of metacholine on a log scale (maximal cumulative dose of metacholine, 22.5 μM). PD_{20} values were measured on these curves.

Variability of pulmonary function measurements is not a specific characteristic of smokers but is found in all the asthmatic subjects. It is related to level of bronchial hyperresponsiveness. Bronchial responsiveness assessed by PD_{20} at baseline was not significantly higher in smokers than in nonsmokers in our study (PD_{20} at baseline: mean in smokers = 1.84 μM Mch and in nonsmokers = 1.6 μM McH). To reduce effects of variability of pulmonary function in time we used minimal delay between assessment of baseline situation and exposure situation in the experimental protocol. Also, to minimize effects of variability of pulmonary function between subjects, we compared paired differences and used nonparametric statistical methods to analyze results.

Experimental Protocol

On the first day, respiratory function and bronchial responsiveness were assessed for each subject. The next day, respiratory function was reassessed and these measure was considered as baseline respiratory function.

Prior to exposure, subjects gargled grapefruit juice to reduce levels of oral ammonia that might neutralize inhaled acid. Subjects were then exposed to acid fog for 1 hr at rest. Exposure time included three periods of 5 min of voluntary hyperventilation alternating with tidal breathing periods. One-half hour after exposure, respiratory function and bronchial responsiveness tests were performed at the same hour in the day.

Statistical Analysis

The paired differences of FEV_1, SR_{aw}, and PD_{20} were each noted not to be normally distributed, and therefore nonparametric statistical methods were used throughout the analysis. The FEV_1, SR_{aw}, and PD_{20} were expressed as means ± SEM (standard error of the mean). Paired differences for values of FEV_1, SR_{aw}, and PD_{20} before and after fog exposure were compared using the Wilcoxon matched-pairs signed-rank test. A $P < 0.05$ was considered significant.

RESULTS

Brussels Fog Assessment

The fogwater composition, shown in Table 1 and Fig. 2, is dominated by NH_4^+ and SO_4^{2-} and to a lesser extent by NO_3^- and Cl^-. Earlier fog studies in urban environments (Waldman *et al.*, 1982; Jacob *et al.*, 1985; Johnson *et al.*, 1987) have reported similar patterns. The frequency distributions show maxima for the following classes of concentrations: 1.5 to 2 mEq/liter for NH_4^+, 0.5 to 1 mEq/liter for SO_4^{2-} and Cl^-, and 0.0 to 0.5 mEq/liter for NO_3^-. The mean cations/anions balance is equal to 1.22, which means that all major species have been accounted for. However, the maximum value (1.63) suggests that anions are missing in some samples. This anion deficiency ("Deficit" in Fig. 2), probably corresponds to highly soluble organic species such as formaldehyde, acetic acid, and formic acid, as reported elsewhere (Jacob *et al.*, 1983; Munger *et al.*, 1983, 1990). The composition of fogwaters collected at different times and places is found to be highly variable because it depends on a great number of chemical, physical, and meteorological factors such as temperature in-

TABLE 1
Physical and Chemical Characteristics of Reconstituted Fogs

	MMAD[a] (μm)	LWC (g/m^3)	Osm (mOsm)	pH	SO_4 (mEq/liter)	NH_4 (mEq/liter)
Naturally occurring fog						
Means ± SEM	6.6 ± 2.1	0.04 ± 0.011	9.053 ± 1.65	4.34 ± 0.12	2.53 ± 0.51	3.75 ± 0.56
Limit values	0.5–10	0.01–0.5	2.3–25	3.4–5.5	0.7–7.1	1.2–8.9
First artificial fog	9 ± 0.5	2	300	2.5	5	—
Second artificial fog	7 ± 0.5	0.5	30	3.5	15	12

[a] MMAD, mass median aerodynamic diameter (means ± SEM and limit values observed for naturally occurring fogs; means ± SEM for artificial fogs); LWC, liquid water content; Osm, osmolarity.

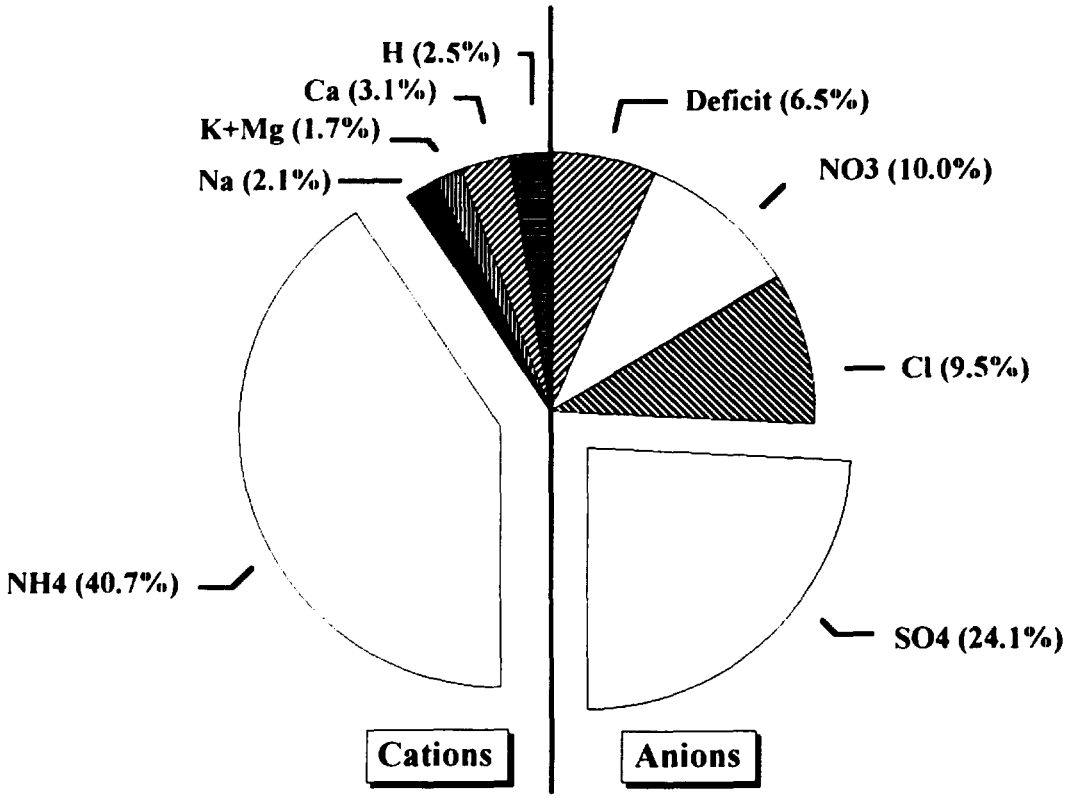

FIG. 2. Average composition (%) of fogwater in Brussels, Belgium.

version, adjacent sources of a specific pollutant, and liquid water content.

The equivalent proportion between NH_4^+ and SO_4^{2-} is found to be remarkably constant for most fog events. NH_4^+ concentration plotted versus SO_4^{2-} concentration shows a very good correlation, with the exception of the highest concentrations of NH_4^+. The slope of the linear regression (without the data corresponding to $[NH_4^+] > 5$ mEq/liter) is 1.9. Such a feature was also described by Sigg *et al.* (1985) and Behra *et al.* (1989).

Thus, SO_4^{2-} is partially neutralized by NH_4^+ in most samples. The NH_4^+ cation, acting as the main acid-neutralizing component, originates from gaseous NH_3. Intensive cattle breeding and agricultural activities provide important sources of NH_3. We can summarize by saying that Brussels fogwater can be largely assigned to a solution of ammonium salts such as $(NH_4)_2SO_4$ and NH_4HSO_4. These salts are primarily present in the aerosol phase and act as condensation nuclei for the growth of fog droplets, where they are subsequently partially dissolved.

Exposure to Acid Fog

Effects of Sulfuric Acid Fog

Subjects characteristics, lung function testing, and bronchial responsiveness challenge tests are summarized in Table 2 and Fig. 3. The means ± SEM were as follows: FEV_1 (% of predicted value) before exposure: 79 ± 4.9% and after exposure: 76 ± 5.6% (NS; $P = 0.14$); SR_{aw} (L × cm H_2O/L/s) before exposure: 8.19 ± 1.7 and after exposure: 8.83 ± 2.5 (NS; $P = 0.87$); PD_{20} (μM Mch) before exposure: 1.267 ± 0.5 and after exposure: 1.041 ± 2.4 (NS; $P = 0.36$).

Differences observed in FEV_1 and SR_{aw} before and after fog exposure were not significant. Only 8 of the 14 subjects demonstrated an increase of SR_{aw} after fog exposure. Moreover, changes were very small: increased SR_{aw} exceeded 10 L × cm H_2O/L/s (considered as normal range) in only two cases and the mean change of SR_{aw} was 4%. Significant change of bronchial responsiveness after H_2SO_4 fog exposure was not observed ($P = 0.36$). When an increase of bronchial responsiveness was observed, this change remained very tenuous. The mean change of bronchial reactivity was −18% of PD_{20} in the sense of an increase of bronchial responsiveness.

Effects of Ammonium Sulfate Fog

Subjects characteristics, lung function testing, and bronchial responsiveness assessment are sum-

TABLE 2

Individual Characteristics and Responses to H_2SO_4 Fog Exposure

Subject	Sex	FEV_1 (% of predicted value)		FVC (% of predicted value)		SR_{aw} (L x cmH_2O/L/s)		PD_{20} (μM Mch)		Medications	Smoker	Reversibility[a] (% of predicted value)
		Before	After	Before	After	Before	After	Before	After			
1	M	61	69	86	100	9.2	6.8	0.25	1.88	Ad,[b] IC, SC	–	26.1
2	M	76	64	112	100	8.5	10.3	0.34	0.274	Ao, IC, SC	+	15.2
3	F	112	118	133	133	1.9	2.8	0.46	0.104	Ad, IC, SC	–	ND
4	M	98	98	115	124	3.4	4.7	0.06	0.189	Ad, IC, SC	+	ND
5	F	90	85	100	96	3.8	4.7	4.65	7.15	—	–	ND
6	M	65	61	96	94	14.1	11.8	1.04	0.51	Ao, IC	+	25.8
7	M	76	78	96	93	10.4	10.3	6.52	1.45	Ao	+	13.1
8	M	66	62	75	75	6.2	5.9	0.608	0.51	Ao, T, IC	–	30.1
9	M	64	55	77	68	9.9	6.7	0.33	0.08	Ad, T	+	14.7
10	M	44	38	76	69	28	40	0.63	0.15	Ao	–	35.3
11	M	90	85	119	118	8.1	8.6	0.29	0.18	Ao	–	ND
12	F	84	67	96	80	3	3.3	0.81	0.28	Ad, IC	–	15.3
13	F	77	79	95	88	4.7	2.8	0.59	0.13	Ao	–	18
14	F	103	105	113	115	3.5	5	1.17	1.69	—	+	ND
	Mean:	79	76	99	96	8.19	8.8	1.26	1.041			
	±SEM:	±4.9	±5.6	±4.7	±5.4	±1.8	±2.5	±0.5	±0.4			

[a] Reversibility of airway obstruction was assessed only in subjects presenting with obstructive ventilatory impairment at baseline.
[b] Abbreviations used: A, β adrenergic agonist (d = daily; o = occasionally); IC, inhaled corticosteroids; T, theophyllin; SC, systemic corticosteroids (during exacerbations); ND, not done.

marized in Table 3 and Fig. 4. The means ± SEM were as follows: FEV_1 (% of predicted value) before exposure: 76 ± 7% and after exposure: 73 ± 6% (NS; $P = 0.15$); SR_{aw} (L x cm H_2O/L/s) before exposure: 6.93 ± 2.12 and after exposure: 7.33 ± 1.81 (NS; $P = 0.2$); PD_{20} (μM Mch) before exposure: 1.68 ± 0.71 and after exposure: 1.71 ± 0.61 (NS; $P = 0.8$). We did not observe significant change in FEV_1 and SR_{aw} before and after exposure to ammonium sulfate fog. In 7 of the 10 subjects, SR_{aw} was increased but this increase was relatively small (mean increase = 36.4%) and in only 2 subjects, values of SR_{aw}

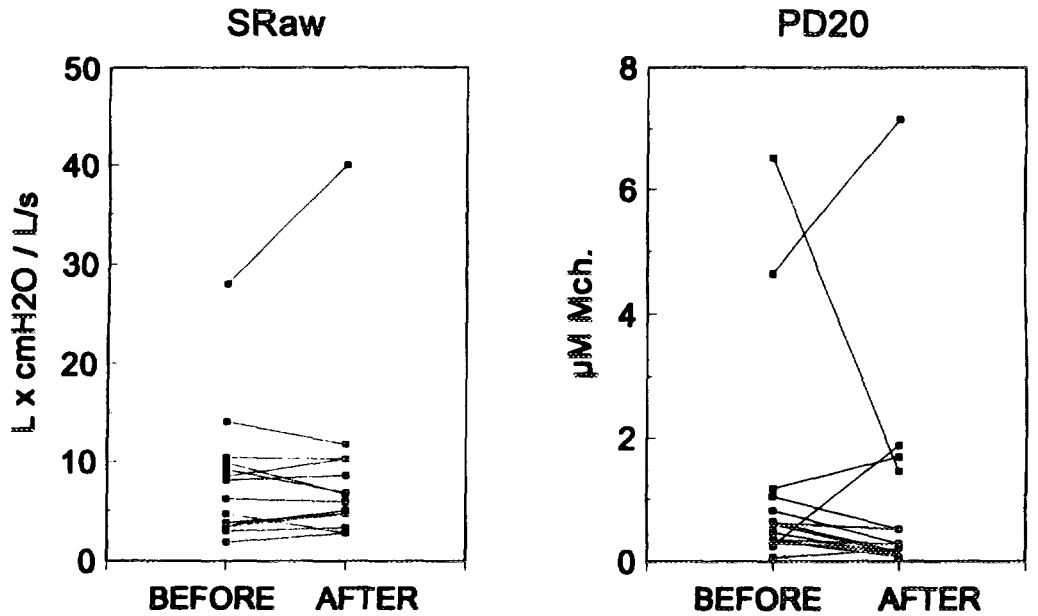

FIG. 3. Effects of H_2SO_4 fog exposure on airway resistance (SR_{aw}) and bronchial responsiveness (PD_{20}).

TABLE 3

Individual Characteristics and Responses to Ammonium Sulfate Fog Exposure

Subject	Sex	FEV_1 (% of predicted value)		FVC (% of predicted value)		SR_{aw} (L × cmH_2O/L/s)		PD_{20} (μM Mch)		Medications	Smoker	Reversibility[a] (% of predicted value)
		Before	After	Before	After	Before	After	Before	After			
1	F	106	103	116	118	2.1	1.3	1.147	1.44	—	–	ND
2	M	55	57	74	73	6.1	7.9	0.47	0.39	Ao,[b] IC	+	31.2
3	F	61	53	80	77	13.6	16.7	0.93	3.32	—	–	13.4
4	F	100	88	106	102	2.5	2.6	0.76	1.12	—	–	ND
5	F	79	83	84	89	1.9	3.2	1.19	0.54	Ad, IC, SC	–	12.3
6	M	62	55	97	93	9.4	12.9	0.45	0.24	Ad, IC, SC	–	23.4
7	F	104	92	123	114	5	7	0.08	0.01	Ao	+	ND
8	M	42	50	73	72	22.7	15.5	0.86	1.56	Ad, IC	+	21
9	F	86	90	96	96	4	3.1	7.6	6.5	—	+	20.1
10	M	70	61	62	59	2	3.1	3.31	2.01	Ao, IC	–	17.5
	Mean:	76.5	73.2	91.1	89.3	6.9	7.3	1.68	1.71			
	±SEM:	±7	±6.2	±6.2	±6.04	±2.1	±1.8	±0.7	±0.6			

[a] Reversibility of airway obstruction was assessed only in subjects presenting with obstructive ventilatory impairment at baseline.
[b] Abbreviations used: A, β adrenergic agonist (d = daily; o = occasionally); IC, inhaled corticosteroids; T, theophyllin; SC, systemic corticosteroids (during exacerbations); ND, not done.

reached 10 L × cm H_2O/L/s (considered as normal range).

Sulfate ammonium exposure did not significantly change bronchial responsiveness (P = 0.87). In all subjects, changes in bronchial responsiveness were small and the mean change of PD_{20} from preexposure baseline was +2% in the sense of a decrease of bronchial responsiveness.

The statistical power of Wilcoxon matched-pairs signed-rank test in these studies (assessing importance of β, the type II error) (Freiman *et al.*, 1978) was as follows:

concerning exposure to H_2SO_4 fog: 10.36% for FEV_1, 7.44% for SR_{aw}, and 9.06% for PD_{20},

concerning exposure to ammonium sulfate fog: 9.52% for FEV_1, 6.59% for SR_{aw}, and 5.37% for PD_{20}.

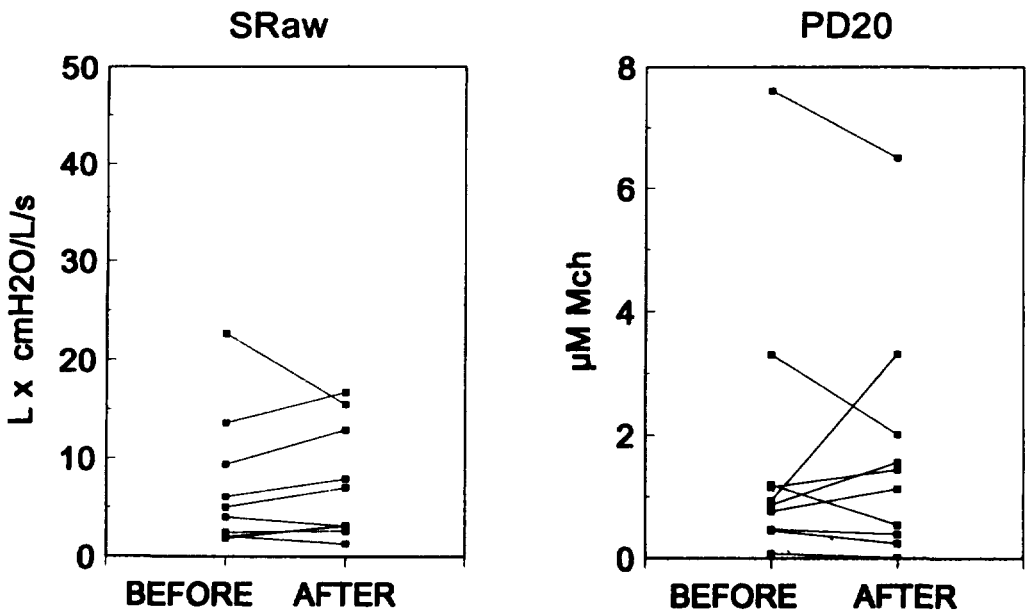

FIG. 4. Effects of ammonium sulfate fog exposure on airway resistance (SR_{aw}) and bronchial responsiveness (PD_{20}).

DISCUSSION

In this study, exposure of asthmatics at rest to a realistic acid fog did not cause significant change in respiratory function or bronchial responsiveness. The challenge with H_2SO_4 fog was designed to isolate the causal role of acidity among the effects of acid fogs. To rule out a possible combined effect of hypoosmolarity, fog solution was made isoosmolar. The pH of solution was extreme but realistic. LWC of this fog was chosen very high to increase sensibility of the experiment. Lack of bronchoconstrictor effect and lack of bronchial reactivity change observed after exposure to H_2SO_4 fog in this first part suggest that, after short-term exposure, acidity of natural fogs does not influence respiratory function and bronchial responsiveness in asthmatics.

The second part of the work was designed to assess global respiratory effects of fog with physical (granulometry) and chemical characteristics (pH and titratable acidity, major ions components, osmolarity) similar to those of fogs measured in an urban area such as Brussels, Belgium. The negative results of the second part, again, suggest that naturally occurring fogs do not exert acute respiratory effects in asthmatics.

In our population sample, it was not possible to detect discriminating factors among subjects who worsened their bronchoconstriction or bronchial hyperresponsiveness and those who improved it or remained unchanged after exposure. Comparing severity of asthma (assessed by usual medication requirements), smoking habits, level of obstructive ventilatory impairment at baseline, and level of bronchial responsiveness at baseline in these subgroups of subjects, we did not find significant differences ($P > 0.05$ using nonparametric Wilcoxon test). This suggests that individual changes of SR_{aw} or PD_{20} likely are rather caused by random variations around the baseline than by the existence of reactive subgroups in the asthmatic population.

Our subjects, being ages 26 to 70 years old and having relatively moderate medication requirements, might not have been representative of the asthmatic population, but are likely comparable to asthmatics in previous studies. Because we studied subjects for a short period in this study, we cannot rule out the possibility that we missed delayed effects or effects of acid mist possibly occurring after a more prolonged exposure. Our experimental protocol did not include exercise during exposure (only three periods of hyperventilation). Exposure at rest could be a less sensitive method of assessing acid fog effects, but likely corresponds to more realistic exposure conditions for most of the asthmatic population. Although the two protocols reported here have limited statistical power, it is noteworthy that individual changes in SR_{aw} and PD_{20} are small, contrasting with situations observed with well-known bronchoconstrictors (histamine, SO_2, distilled water aerosols) or "bronchial responsiveness increaser" agents (SO_2, viral infections), where SR_{aw} and PD_{20} characteristically are modified by several hundred percentages in asthmatics.

Previous controlled exposure studies of the effects of H_2SO_4 involving subjects with asthma have generated conflicting data. Utell and co-workers demonstrated significant decreases in airway conductance (SG_{aw}) after exposing asthmatic subjects to aerosols (MMAD 0.7 μm) containing 450 $\mu g/m^3$ of H_2SO_4 for 16 min through a mouthpiece (1983). Koenig and co-workers exposed 10 adolescent asthmatics to 100 $\mu g/m^3$ of H_2SO_4 and NaCl aerosol (MMAD 0.6 μm) through a mouthpiece and found a significant FEV_1 decrease immediately after a 10-min exercise period (1983). Utell also demonstrated that exposure to aerosol containing 450 $\mu g/m^3$ H_2SO_4 increased bronchial reactivity to carbachol in asthmatics (1984).

Nevertheless, not all of the controlled exposure studies of the effects of H_2SO_4 aerosols in subjects with asthma demonstrated significant changes in pulmonary function and bronchial responsiveness. In the study of Sackner and co-workers there was no significant change in FEV_1 or total respiratory resistance in five adults with asthma exposed at rest to H_2SO_4 aerosols (MMAD 0.1 μm) at 10, 100, and 1000 $\mu g/m^3$ for 10 min through a mouthpiece system (Sackner *et al.*, 1978). Linn and co-workers exposed asthmatics to H_2SO_4 aerosols (MMAD 0.6 μm) at 122, 242, and 410 $\mu g/m^3$ and did not find significant change in respiratory function, symptoms score, or bronchial reactivity to breathing cold dry air (1986).

Studies with controlled exposure to acid fogs are less numerous than those with smaller droplets size aerosols. Avol and co-workers exposed normal and asthmatic adult subjects to fogs (MMAD 10 μm; LWC 0.1 g/m^3) containing 0, 500, 1000, and 2000 $\mu g/m^3$ H_2SO_4 in an exposure chamber for 1 hr: there was a statistically significant increase in respiratory symptoms, but there was no significant change in pulmonary function or bronchial responsiveness (Avol *et al.*, 1988). Although some authors concluded that acid fog inhalation exposure is associated with increased mucociliary clearance (Laube *et al.*, 1993), Aris and co-workers exposed asthmatic subjects to H_2SO_4 fogs (volume median diameter VMD 6 μm and LWC 1.8 or 0.5 g/m^3) without demonstrating

effects on pulmonary function or on respiratory symptoms (1991). In a recent study, Morrow *et al.* exposed asthmatic subjects to near ambient levels of sulfuric acid aerosols (100 $\mu g/m^3$ H_2SO_4) and did not observe significant change of lung function (1994).

In our study, exposure conditions combined several bronchial stimuli including hypoosmolarity (Schoeffel *et al.*, 1981; Anderson *et al.*, 1983; Sheppard *et al.*, 1983), acidity (Utell *et al.*, 1983; Koenig *et al.*, 1983), and even titratable acidity (Fine *et al.*, 1987). Despite association of all these potential bronchial effectors, exposure to this fog did not produce significant change in pulmonary function or bronchial responsiveness.

One hypothesis is that the lack of effect is, at least partly, linked to the physical properties of natural acid fog. In order to integrate our data with previous studies, it is possible that at the same pH values and H_2SO_4 concentrations, acid fogs consisting of large droplets (>7 μm) may be less toxic for the respiratory tract than aerosols of smaller droplets ($\leq$ 1 μm). Such findings have already been reported in animal exposure studies (Amdur *et al.*, 1978) and suggested in some human exposure studies (Aris *et al.*, 1991; Balmes *et al.*, 1988). The specific mechanisms underlying acid-induced pulmonary functional changes are not known but, acting as irritant agents, acid aerosols likely exert respiratory effects by causing epithelial damage leading to inflammation and mediators release (Barnes and Lundberg, 1991) and by stimulating sensory receptors: the rapidly adapting irritant receptors and the C-fiber receptors (Coleridge and Coleridge, 1986; Sant'Ambrogio, 1987).

Since these receptors are mainly concentrated in subglottic large airways (in particular for the rapidly adapting irritant receptors), we can speculate that the preferential target site for acid aerosols to induce functional changes is likely located in the tracheobronchial region. Because of hygroscopic characteristics, deposition of fog in the respiratory tract is difficult to assess. Growth of hygroscopic particles during transit in humid respiratory airways makes extrapolation of data concerning dry aerosols deposition difficult (Morrow, 1986). However, isotopical studies demonstrated that, using monodisperse aerosol with MMAD = 10 μm, deposition in the tracheobronchial region did not exceed 25% of the total amount inhaled, the major part depositing in oropharynx (Bowes *et al.*, 1989). Perhaps discrepancies of deposition sites between coarse (>7 μm) and fine ($\leq$1 μm) aerosols could explain the different respiratory effects previously described. In this regard it is interesting to note that Hackney and coworkers, comparing difference of effects between fine (MMAD = 0.9 μm) and coarse (MMAD = 10 μm) acid aerosols in asthmatics, observed that fine aerosols were more likely to change pulmonary function, while coarse aerosols were more likely to increase respiratory symptoms (Hackney *et al.*, 1989). They suggested that this pattern might be explained by assuming that symptoms are caused primarily by large acid droplets deposited in upper airways and that disturbances of pulmonary mechanics in asthmatics are caused primarily by smaller acid droplets deposited more distally in the bronchial tree.

Moreover, fine aerosols usually have a lower LWC than coarse aerosols: for the same concentration of H_2SO_4 in air, acid pollutants and H^+ ions are thus more concentrated in droplets of fine aerosols than of coarse aerosols, where they are diluted. These very concentrated particles could be more effective on irritant receptors in stimulating bronchoconstriction.

In conclusion, short-term exposure to realistic acid fog does not cause significant change in respiratory function or bronchial responsiveness in asthmatics at rest. The understanding of the discrepancies of effects between acid fogs and finer acid aerosols requires further investigations, addressing hygroscopic aerosol deposition in the respiratory tract and mechanisms of respiratory toxicity of acid aerosols.

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