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Effect of Concentration and Cumulative Exposure of Inhaled Sulfuric Acid on Tracheobronchial Particle Clearance in Healthy Humans

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We have previously shown that 1-hr exposures to 0.5 μm sulfuric acid (H_2SO_4) mist at 100 and 1000 μg/m^3 produced transient alterations of bronchial mucociliary clearance of monodispersed 7.6 and 4.2 μm mass median aerodynamic diameter γ-tagged ferric oxide (Fe_2O_3) in healthy nonsmoking humans in a dose-dependent manner. To determine the role, if any, of the length of exposure, 10 healthy volunteers were exposed to 100 μg/m^3 H_2SO_4 for 1 hr and 2 hr on separate occasions, 1 week apart, with measurements of their mucociliary clearance of 5.2 μm Fe_2O_3 particles inhaled both before and after the inhalation of the H_2SO_4. Their rate of bronchial mucociliary clearance was markedly reduced for both Fe_2O_3 aerosols, with slower clearance of the aerosol inhaled after the H_2SO_4 exposure. For the tagged Fe_2O_3 aerosol inhaled after exposure for 2 hr at 100 μg/m^3 H_2SO_4, the tracheobronchial clearance halftime, (T_{50}), tripled from control, and the reduced rate of clearance was still evident 3 hr after the end of exposure. The 1-hr 100 μg/m^3 H_2SO_4 exposure doubled T_{50} from control, and the reduced rate of clearance lasted for about 2 hr after the end of exposure. These results indicate that the effect of doubling the length of exposure was as great or greater than an order of magnitude increase in the concentration of H_2SO_4.

Introduction

Mucociliary clearance is a lung defense mechanism by which inhaled particles that deposit on the conducting airways are removed from the lung. This removal is by a coordinated mucociliary transport system that relies on the beating of the cilia to move the overlying mucus layer carrying the deposited debris.

While the mechanisms by which acid aerosols affect mucociliary clearance are not well understood, it is still important to determine the response of the mucociliary system to these challenges, in particular at concentrations and time periods relevant to environmental and occupational exposures. This paper describes studies on the effect of concentration and length of exposure to inhaled sulfuric acid (H_2SO_4) aerosols on tracheobronchial particle clearance in humans. Studies on the response to acid aerosols on lung clearance in animals has already been discussed at this symposium by R. Schlesinger (*1*).

Our previous results on the effect of H_2SO_4 concentration on mucociliary clearance in humans are shown on Figure 1. Ten healthy volunteers inhaled 0.5 μm H_2SO_4 for 1 hr at concentrations of 100 and 1000 μg/m^3. When the tracer aerosol was 7.6 μm in aerodynamic diameter, the response shown in Figure 1*A* indicates faster clearance at the lower exposure concentration and slower clearance at the higher concentration. In Figure 1*B*, eight healthy volunteers had the same exposures (100 and 1000 μg/m^3 of 0.5 μm H_2SO_4 for 1 hr), but here the tracer aerosol was only 4.2 μm in aerodynamic diameter; the response at the higher concentration is to slow clearance as before, but for 100 μg/m^3, clearance is slower than in the control test. This apparent contradiction has to do with the fact that the mucociliary clearance technique depends on the deposition pattern of the inhaled, tagged aerosols. The larger 7.6 μm particles deposit much more in the large central airways than the 4.2 μm particles, and therefore they will indicate clearance preferentially from a lung region where the effective H_2SO_4 deposited dose is quite small. Thus, it seems that low H_2SO_4 exposure concentrations stimulate mucociliary transport. On the other hand, at high concentrations, the delivered dose is increased, slowing down the mucociliary clearance in

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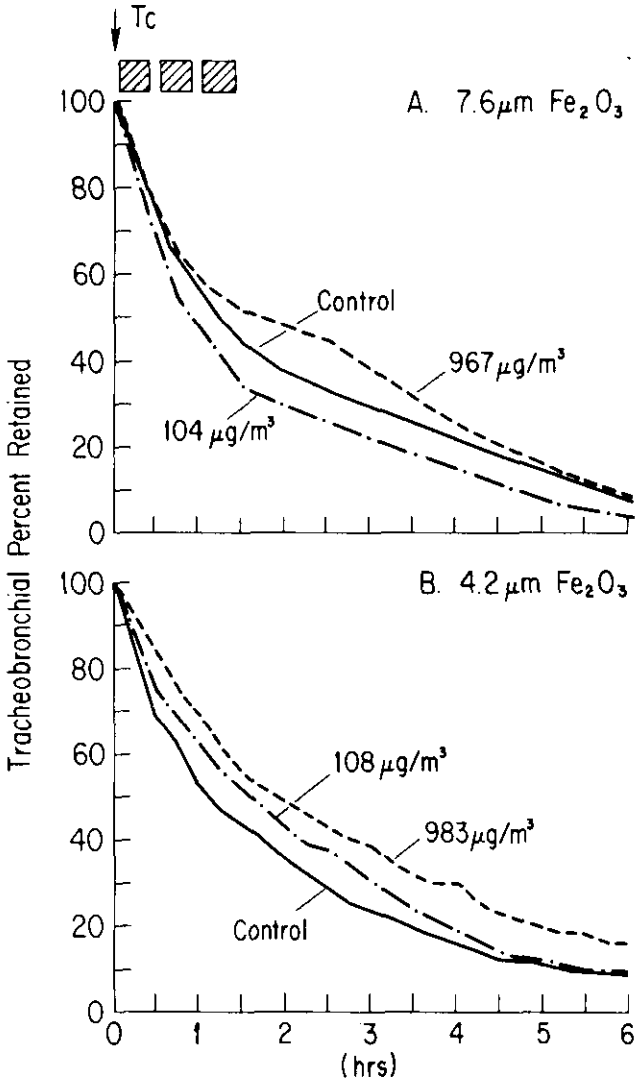

FIGURE 1. Effect of 1-hr H_2SO_4 aerosol exposure to 0 (control), 100, and 1000 μg/m³ concentrations upon tracheobronchial clearance. (*A*) Response of 10 healthy subjects with 7.6 μm MMAD tracer aerosols. (*B*) Response of 8 healthy subjects with 4.2 μm MMAD tracer aerosols.

a dose-dependent manner. Similar responses were obtained from a group of six asymptomatic asthmatics that were exposed for 1 hr to 100, 300, and 1000 μg/m³ H_2SO_4 (*2*). This paper reports the effects of exposure duration of 100 μg/m³ on mucociliary clearance in healthy, nonsmoking adult volunteers.

Methods

Subjects

Ten adult male volunteers participated in the study. Each subject gave informed consent; completed a questionnaire on pulmonary disease, smoking, and work history; and underwent a clinical examination which included chest roentgenogram, electrocardiogram, and blood and urine analyses. All protocols were reviewed and approved by the Board of Research Associates at New York University Medical Center. The physical characteristics of the 10 subjects are displayed in Table 1. There were 8 who had never smoked and 2 exsmokers; all had normal pulmonary values and were asymptomatic at the time of the study.

Sulfuric Acid Aerosols

The H_2SO_4 aerosols were generated and monitored with the same techniques an equipment as in other recent human studies in this laboratory (*3,4*). The aerosol was generated with a Babington-type nebulizer from a dilute (0.1 N) H_2SO_4 solution. Strict control of the rate of addition of the hygroscopic droplets into the airstream and of the relative humidity and temperature were necessary to ensure a constant droplet size.

Airborne H_2SO_4 concentrations were determined by two methods. An in-line Flame Photometric Detector (Model 285, Meloy Laboratories, Springfield, VA) supplied instantaneous direct indications of the sulfur content of the aerosol during exposures and facilitated maintenance of constant exposure levels. An integrated sample was also collected on a glass-fiber filter throughout the entire exposure period. This sample was analyzed by a turbidimetric method. Each subject inhaled the aerosol through a nasal mask while sitting quietly in order to simulate normal human exposure conditions.

The H_2SO_4 droplets had a mass median aerodynamic diameter (MMAD) of 0.5 μm and a geometric standard deviation (σ_g) of 1.9. The electrostatic charge of the aerosol was less than 20 charges per droplet. The air temperature and relative humidity during the 1-hr exposures were 27.1 ± 0.6°C and 47 ± 2% (mean ± SD), respectively. The H_2SO_4 concentrations determined from the filter samples collected during exposure were < 5; 104 ± 21 and 111 ± 22 μg/m³ for the control, 1-hr (short) and 2-hr (long) exposures, respectively, and there were no significant differences in particle size, temperature, or humidity between different exposures.

Table 1. Characteristics of male subjects.

Subject No.	Age	Height, cm	Weight, kg	Race[a]
1	37	193	88	C
2	53	172	66	C
3	32	168	64	C
4	30	170	60	C
5	33	182	73	B
6	36	175	65	C
7	32	186	82	C
8	22	163	51	O
9	22	164	56	O
10	26	177	71	C
Mean	32.3	175	67.6	
SD	9.0	10	11.3	

[a] C, Caucasian; B, black; O, oriental.

Respiratory Mechanics

The possible effects of H_2SO_4 aerosol upon respiratory mechanics were investigated by having each subject perform a series of respiratory function tests prior to exposure, within 15 min after the cessation of exposure, and again 3 hr later. Subjects performed the tests while wearing a nose clip in a sitting position, and all parameters were standardized to body temperature, pressure, and water saturation (BTPS). A series of maximum expiratory flow-volume maneuvers yielded forced expiratory volume at 1 sec (FEV_1), forced vital capacity (FVC), mid-maximal expiratory flow rate (MMEF), and peak expiratory flow rate (PEFR). These measurements were performed with a low-resistance (< 0.2 cm H_2O/L/sec) wedge spirometer (Model 170, Med-Sciences Electronics, Inc., St. Louis, MO), a waveform recorder (Model 1015, Biomation, Inc., Cupertino, CA), and plotted with an X-Y-T chart recorder (Model 2000, Houston Instruments, Austin, TX). Values were expressed as a mean of three tracings in which the FVC was within 95% of the best effort. Thoracic gas volume (V_{tg}), airway resistance (R_{aw}), and airway conductance G_{aw} (the inverse of R_{aw}) were determined by body plethysmography. Tests were conducted with a 600-L stainless-steel body plethysmograph and associated electronics (Model 2000, Cardio-Pulmonary Instruments Corp., Houston, TX). Measurements were made approximately 3 min after each subject entered the plethysmograph to allow for thermal equilibrium. Values were expressed as the means of five panting maneuvers after subtracting either the measurements shutter assembly dead space (35 mL) or the equipment resistance (0.28 cm H_2O/L/sec).

Single-breath nitrogen washout tests were performed with a nitrogen analyzer (Model 1410, W. Collins, Inc., Braintree, MA). Volumes were measured by the integration of the expiratory airflow as measured by a heated pneumotachograph (No. 3, A. Fleisch, Switzerland). The slope of the alveolar plateau of phase III ($\Delta\%N_2$/L), closing volume (CV), and the ratio of closing volume to vital capacity (CV/VC) were determined.

γ-Tagged Ferric Acid Aerosols

The possible effect of H_2SO_4 dose on mucociliary clearance was studied by monitoring two inhaled monodispersed γ-tagged aerosols. Immediately before the acid exposure, the subjects inhaled, via a mouthpiece, a monodispersed Fe_2O_3 aerosol for about 2 min. This aerosol, tagged with the γ-emitter gold-198 (^{198}Au, E_γ = 410 keV, $t_{1/2}$ = 2.7 days) was generated with a spinning disc generator. Immediately after the acid exposure, the second aerosol, tagged with technetium-99m (^{99m}Tc, E_γ = 144 keV, $t_{1/2}$ = 6 hr) and generated as the first one, was inhaled. All inhalations were at respiratory rate, 14.1 ± 0.2 bpm; tidal volume, 1.09 ± 0.31 L; peak inspiratory flow, 69.4 ± 11.2 Lpm: average inspiratory flow, 40.8 ± 7.9 Lpm: inspiratory time, 1.70 ± 0.2 sec. The diameter of the tagged aerosols were chosen to produce a ratio of about 70% tracheobronchial, 30% alveolar initial deposition. This was determined, for each subject, on a test run in which their baseline mucociliary clearance was measured using simultaneously two aerosols of about 3.5 μm and 6.0 μm aerodynamic diameter tagged with ^{198}Au and ^{99m}Tc, respectively. From this run, the subject's optimum particle aerodynamic diameter was determined; the group mean was d_A = 5.2 μm, with a range of 4.0 to 5.8 μm.

Measurement of Mucociliary Clearance

Lung clearance was determined from serial measurements of right lung retention, using two large NaI (Tl) collimated scintillation detectors aligned over the upper right lung field and connected to a preamplifier-amplifier-single channel analyzer-interface-Apple II microcomputer. The single channel analyzers energy discrimination windows were set for the ^{99m}Tc and the ^{198}Au peaks. The Apple II was programmed to accept the counts, correct them for background and decay, and to display the corrected retention curves in real-time on the screen. All γ-retention measurements were taken inside a low-background chamber.

Depending upon the position of the subject relative to the detectors, the viewed field included either the whole head or approximately 90% of the right lung. The detectors were first aligned over the subject's head for a measurement of the initial amount of aerosol deposited in the upper respiratory tract (URT). The subject was then repositioned so that the detectors viewed the right lung. After the first measurement, used to determine the initial amount of tagged aerosol deposited in the lung, the subject breathed from an air stream containing either the H_2SO_4 aerosol at the preset concentration or the distilled water control aerosol. Serial measurements of lung retention were made during the next 5 hr. The same procedure to determine URT deposition and initial lung deposition was done after the H_2SO_4 exposure for the second tagged aerosol. All subjects returned for an additional retention measurement the following morning.

Tracheobronchial retention curves were constructed in which the initial tracheobronchial retention value was 100% and the 24-hr thoracic retention value was 0%. As previously, the 24-hr thoracic retention was considered an indicator of the deposition in the nonciliated pulmonary or alveolar region (ALV). For each run, we obtained two tracheobronchial clearance curves, one starting immediately before the H_2SO_4 inhalation and continuing for about 5 hr, and another with initial time at the end of the acid exposure. From each clearance curve we obtained the following characteristic tracheobronchial clearance times: T_{50}, the time to complete 50% of tracheobronchial clearance; and MRT, the mean residence time for particles on the tracheobronchial airways.

Protocol

The experimental protocol followed a similar sequence to our previous studies on the effect of H_2SO_4 on healthy and asymptomatic asthmatics subjects (*3,4*), with the addition of a second tagged aerosol inhalation following the end of the period of inhalation of the H_2SO_4. A baseline respiratory function test preceded a 2-min ^{198}Au-Fe_2O_3 test aerosol inhalation during each run day, followed by 3-min head and initial lung retention measurement. This was immediately followed by three 20-min inhalations for the short (1-hr) exposure, or six 20-min inhalations for the long (2-hr) exposure, either a distilled water sham aerosol, for the control runs, or 100 $\mu g/m^3$ of 0.5 μm diameter H_2SO_4. Between the three or six inhalation periods, 3-min lung retention values for the ^{198}Au-aerosol were recorded and, at the completion of the H_2SO_4 exposure, the second tagged aerosol, ^{99m}Tc-Fe_2O_3, was inhaled for 2 min and head and initial ^{99m}Tc chest counts were recorded. Thereafter, simultaneous measurements of retention were performed for both ^{198}Au and ^{99m}Tc-aerosols. After accruing 1 hr of retention data, the second set of respiratory function tests was obtained. Another 30 min of retention measurements preceded a 30-min lunch period, followed by more retention measurements to complete the 5-hr period from the initial ^{198}Au-aerosol inhalation. Before the end of the day, the last set of pulmonary function tests were performed.

Results

Respiratory Functions

The respiratory mechanics of the 10 subjects were not significantly affected by the exposure to H_2SO_4 at a concentration of 100 $\mu g/m^3$ for neither the short 1-hr exposure nor for the long 2-hr exposure.

Regional Deposition of Tagged Aerosols

Regional shifts in the deposition of the tagged aerosol can create artifacts in the thoracic retention curves, and parameters such as size distribution of the aerosol and breathing pattern during inhalation must be controlled within runs. The measurements of regional deposition for the group (i.e., initial upper respiratory tract, URT; tracheobronchial retention, TB; 24-hr thoracic retention as an indicator of the deposition in the nonciliated pulmonary or alveolar region (ALV), are presented in Table 2. There were no significant differences between the values for the control and for the H_2SO_4 exposure runs neither when the tagged aerosol was inhaled before the H_2SO_4 nor when it was inhaled after acid exposure. If there was any variation in regional deposition, it was not large enough to alter the apparent rate of clearance of the Fe_2O_3 aerosol.

Bronchial Mucociliary Clearance

Table 3 shows that the alterations in bronchial clearance after inhalation of H_2SO_4 were statistically significant. For 1-hr and 2-hr exposures at 100 $\mu g/m^3$ H_2SO_4, the average clearance halftimes, T_{50}, were significantly greater than those following the sham exposure, indicating a delay in mucociliary clearance. The effect of H_2SO_4 on clearance was greater when the tagged aerosol was inhaled after the acid dose exposure. A similar result is indicated by the effects on mean residence time, MRT. The group mean MRT values increased after 1 hr of H_2SO_4 exposure, and increased even more after 2 hr of exposure.

Discussion

Bronchial mucociliary clearance was altered by 100 $\mu g/m^3$ H_2SO_4 exposure in a group of 10 healthy subjects in a dose-dependent manner, similar to that established in this laboratory for H_2SO_4 concentrations of 100, 300, and 1000 $\mu g/m^3$ for 1-hr, (*3,4*). There was a transient delay in mucociliary clearance as defined by higher values of T_{50}, increasing with longer exposure times. The differences were statistically different from control ($p < 0.05$) for both the 1-hr and 2-hr exposures. Figure 2 shows the mean clearance for the group, for each exposure, and for the control run.

These results are analogous to our previous results (Fig. 1), where the subjects inhaled H_2SO_4 for 1-hr at 100 and 1000 $\mu g/m^3$. The magnitude of the effects on T_{50} and MRT are summarized in Table 4. For the 1-hr 100 $\mu g/m^3$ H_2SO_4 exposure, we obtained a similar increase in T_{50} for the eight healthy subjects that inhaled a 4.2 μm tagged aerosol before the H_2SO_4. The increase in T_{50} after 2 hr of 100 $\mu g/m^3$ H_2SO_4 exposure is quite close to our previous 1 hr 1000 $\mu g/m^3$ H_2SO_4. These results show that increasing the time of exposure

Table 2. Reproducibility of regional deposition.

Tag	Exposure	% Regional deposition (± SD)		
		TB[a]	Alveolar	Upper respiratory
^{198}Au (before H_2SO_4)	Control	53 ± 21	26 ± 12	21 ± 13
	1 hr	54 ± 25	23 ± 14	23 ± 15
	2 hr	55 ± 22	27 ± 14	18 ± 14
^{99m}Tc (after H_2SO_4)	Control	50 ± 20	31 ± 17	19 ± 16
	1 hr	53 ± 23	23 ± 10	24 ± 13
	2 hr	53 ± 25	24 ± 10	23 ± 15

[a]TB, tracheobronchial retention.

Table 3. Effects of 100 μg/m³ inhalation exposures to submicrometer H_2SO_4 on tracheobronchial clearance half times for tagged Fe_2O_3 particles in healthy subjects.

	Clearance half time before H_2SO_4, min			Clearance half time after H_2SO_4, min		
Subject	Control	1 hr	2 hr	Control	1 hr	2 hr
1	40	60	67	39	93	115
2	20	36	60	14	50	55
3	12	30	47	15	61	65
4	9	8	12	13	63	70
5	64	82	95	66	95	125
6	75	93	143	78	140	210
7	75	124	170	95	160	230
8	60	70	78	55	94	110
9	34	53	67	36	66	78
10	15	30	47	9	14	44
Mean (SD)	42 (26)	59 (26)*	79 (45)*	42 (30)	84 (43)*	110 (64)*

* Statistically significant ($p < 0.005$).

has an effect similar to an increase in the concentration of H_2SO_4. The increase in MRT with H_2SO_4 exposure is less dramatic than on T_{50}; these values should be considered lower bounds to ΔMRT because of the way they were calculated. In order to avoid arbitrary extrapolations, we defined MRT for the period of time for which we have actual data. By inspecting the clearance curves for control and exposure runs, we can see that increasing the integration period will only increase the ΔMRT values.

We have previously shown in this laboratory in donkeys that there is a time period, of the order of 30 min, for the effect of an acute H_2SO_4 exposure to appear as an alteration of mucociliary retention curves (5). When comparing the retention curves of inhaled tagged aerosols before and after the same H_2SO_4 exposure (Fig. 3), it becomes noticeable that both curves become parallel to each other after about 40 min, indicating the same rate of clearance of particles from the lung.

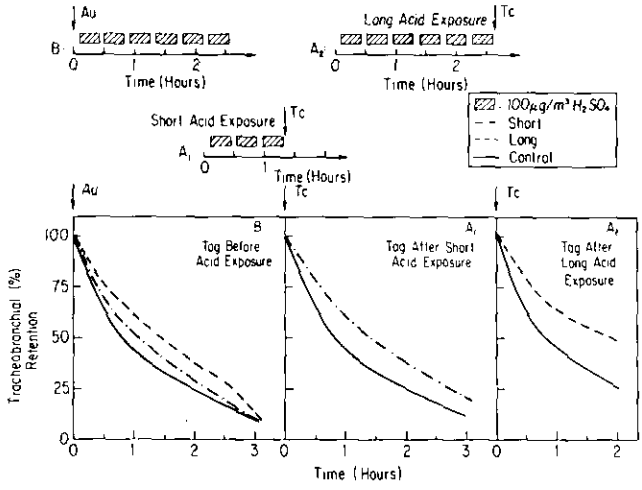

FIGURE 2. Effects of 1-hr and 2-hr inhalations of 100 μg/m³ H_2SO_4 mist on bronchial mucociliary clearance of a tagged aerosol inhaled (*A*) after acid exposure; and (*B*) before exposure.

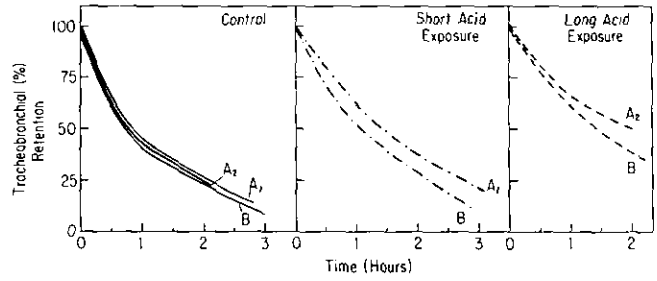

FIGURE 3. Comparisons of mucociliary clearance of tagged aerosols that were deposited before H_2SO_4 exposure with those inhaled immediately after 1-hr and 2-hr exposures at 100 μg/m³ H_2SO_4.

Table 4. Changes in characteristic tracheobronchial clearance mean times associated with inhalations of submicrometer H_2SO_4 by healthy humans.

	ΔT_{50},%				ΔMRT,%			
	1 hr			2 hr	1 hr			2 hr
Study	100 μg/m³	300 μg/m³	1000 μg/m³	100 μg/m³	100 μg/m³	300 μg/m³	1000 μg/m³	100 μg/m³
Tag after H_2SO_4 (n = 10) $4.0 < D_a < 5.8$ μm	100	—	—	162	21	—	—	33
Tag before H_2SO_4 (n = 10) 4.0 μm $< D_a <$ 5.8 μm	44	—	—	93	8	—	—	12
Tag before H_2SO_4[a] (n = 8) D_a = 4.2 μm	38	33	78	—	16	23	19	—

[a] From Leikauf et al. (4).

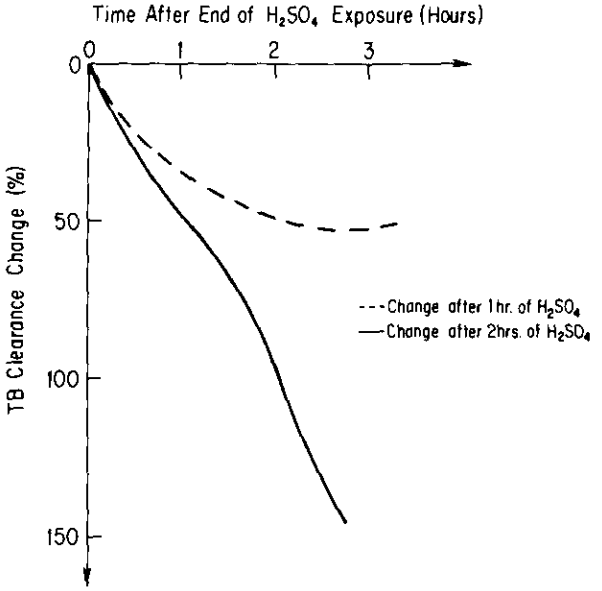

FIGURE 4. Changes in bronchial clearance after 1-hr and 2-hr H_2SO_4 exposure relative to control as a function of time after end of exposure.

The magnitude of an effect can be characterized by the amount of change produced and the duration of this alteration for a given end point. In the present study, changes are defined by the variations in the rate of clearance of tagged particles from the lung. Figure 4 shows the percentage change (from control) of clearance halftime of aerosols inhaled after 1-hr and 2-hr exposures to 100 $\mu g/m^3$ H_2SO_4, as a function of time after the end of the H_2SO_4 exposure. The two exposures yield very different rates of clearance on the same time scale. After the 1-hr H_2SO_4 exposure, tracheobronchial clearance slows down for about 2.5 hr and, even though the amount cleared is only at this time about 50% of that in control runs, the rate of clearance has reached the level of that in control runs. For the 3 hr following the 2-hr H_2SO_4 exposure mucociliary clearance is slowed to ~ 50%/hr of that in the control test, and there is no evidence of it leveling off.

In conclusion, the duration of an acute H_2SO_4 exposure to 100 $\mu g/m^3$, affects mucociliary clearance not only in magnitude but also in the persistence of the changes.

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