species established that these exocrine structures were the source of the pyrazines.
5. An LKB-9000 combined gas chromatograph and mass spectrometer equipped with a 10 percent SP-1000 on Supelcoport 80-100 column was used. Secretions were also examined on a column containing 1 percent OV-17 on the same support. Both columns were programmed from 70°C at 8°C per minute. We thank H. M. Fales and W. Comstock of the National Heart and Lung Institute for the use of this instrument.
6. Program developed by S. R. Heller of the Division of Computer Research Technology, NIH. See S. R. Heller, *Anal. Chem.* **44**, 1951 (1972).
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Role of Relative Humidity in the Synergistic Effect of a Sulfur Dioxide–Aerosol Mixture on the Lung

Abstract. *Experimental evidence concerning the physicochemical and biological factors involved in the potentiation of the irritant property of sulfur dioxide in combination with an aerosol is reported. Relative humidity is an important variable for those aerosols capable of absorbing water at relative humidities below 95 percent.*

During many of the episodes of urban air pollution associated with increased rates of illness and death, elevated levels of sulfur dioxide and suspended particulates, low ambient temperature, and high relative humidity (RH) have been recorded (*1*). Still, the individual pollutants in these episodes did not approach levels required to impair the function or structure of the lungs in laboratory tests. To account for this paradox, it has been suggested that urban pollutants interact with each other giving rise to more potent products, usually in the form of aerosols. To date, only Amdur has shown convincingly an intensified or synergistic effect of gas-aerosol mixtures on pulmonary function (*2*). Her studies were done on guinea pigs; subsequent attempts to duplicate the results in man (*3*) and cats (*4*) have been unsuccessful. We now report an experiment testing the hypothesis that an elevated RH, by enhancing the interaction between SO_2 and certain aerosols (*5*), is important in determining whether gas-aerosol synergism occurs (*6*).

A modification of the method of Amdur and Mead (*7*) was used to measure pulmonary flow resistance (R_L) in guinea pigs (average weight, 334 ± 31 g). The animals were anesthetized with intramuscular injection of ketamine (100 mg/kg), to permit insertion of the pleural catheter. The measurements were made in a pressure plethysmograph, with the animals under light sedation (*8*) with sodium pentobarbital (10 mg/kg). Sulfur dioxide (1.1 ± 0.1 ppm), a polydispersed sodium chloride (NaCl) aerosol (900 to 1000 $\mu g/m^3$), and water vapor were mixed with filtered air to provide a flow of 10 liter/min, and a residence time in the reaction chamber of about 8 to 10 minutes. A polydisperse aerosol was produced by flowing filtered air through a fritted glass sparger submerged in a 5 percent NaCl solution. The generator and the size distribution of the aerosol have been studied by Pueschel *et al.* (*9*) and more recently by Covert *et al.* (*10*). The size distribution is essentially the same as that of urban air, as described by Junge (*11*) and Butcher and Charlson (*12*). The peak particle count occurs at 0.1 μm and because of settling in the reaction chamber, no particles greater than 2 μm reach the guinea pig; these observations were confirmed by electron photomicrographs.

There were six modes of exposure: (1 and 2) SO_2 at low (< 40 percent) and high (> 80 percent) RH; (3 and 4) NaCl at low and high RH; (5 and 6) SO_2 + NaCl at low and high RH. The temperature throughout the reaction chamber was 22°C. As the guinea pig breathes, the inspired gases and aerosols are rapidly brought to or near body temperature and humidity by the upper airways.

After a control period, each animal was exposed to one mode for 1 hour, allowed to recover on ambient, filtered air for 1 hour, and exposed again to another mode for a second hour. The sequence of modes was random. The latter half (30 minutes) of the recovery period served as the control for the second exposure. Thirty-six animals were studied to provide 72 exposures: 12 for each mode, half being first and half second exposures.

The average change in R_L during the period of exposure, relative to the average R_L during the control period preceding exposure, is shown (Fig. 1a) as a percentage. The control values of R_L were variable (average, 0.566 ± 0.32 cm-H_2O ml^{-1} sec^{-1}). The tendency for R_L to decrease during exposure in four of the modes is unexplained. This same tendency has been seen in control animals breathing filtered air for 5 hours. The R_L (average value for the entire exposure) increased significantly only in mode 6 when SO_2 plus NaCl were administered at high RH. We also divided the 1-hour exposure into 15-minute periods and obtained average values for these four periods (Fig. 1b). The increase in R_L, although pulsatile in character, was present throughout the exposure and tended to increase with time.

The concentration of gaseous SO_2 decreased significantly in mode 6. A decrease of approximately 10 percent was observed in mode 5 (SO_2 plus NaCl at low RH) which was probably due to adsorption of the gas on the dry aerosol.

This decrease in gas phase SO_2 was greater than expected from calculated uptake of gas on the aerosol, both in the dry and droplet form. Some wall loss of SO_2 is expected at high RH and also some loss of SO_2 on the filter in the SO_2 sampler line. This difference between the calculated and observed uptake of the gas by the droplet is not critical to the interpretation of the results. The *p*H of the droplets in mode 6, measured with a *p*H meter (Instrumentation Laboratory, Inc., model 245) was 3.2 ± 0.5. The reduction in *p*H could be due only to absorption of SO_2 by the aerosol (a droplet at high RH) (*10*). No decrease in SO_2 concentration was

observed or expected for the other modes. No sulfuric acid mist was detected by a method (*10*) in which the sulfuric acid is reacted with ammonia (NH_3) to form ammonium sulfate, which is then identified by its deliquescent point. The instrument is described fully (*10*). A second qualitative check for the presence of H_2SO_4 was made by a mass spectrometer method (*13*). The results of this test were negative for sulfates and positive for SO_2 and bisulfite.

One of our associates (*14*) has estimated the *p*H of an aqueous aerosol in contact with SO_2 at 1 ppm at 20°C. If there is virtually no NH_3, and if CO_2 is normal for ambient air (325 ppm), the aerosol will have a *p*H of 3.8, a bisulfite ion concentration of $1.5 \times 10^{-4}M$, and a sulfite concentration of $6.3 \times 10^{-8}M$ if there is no H_2SO_4 formation. If, however, there is a trace (1 ppb) of NH_3 in equilibrium with the system, the *p*H will then be 4.9, bisulfite concentration will be $1.5 \times 10^{-3}M$, and sulfite concentration will be $7 \times 10^{-6}M$.

Once the RH is sufficiently elevated, the hydration of the particles and their subsequent uptake of SO_2 proceed rapidly. Presumably, this process could occur within the upper airways where RH rises rapidly. However, the uptake of SO_2 by the upper airways is also rapid (*15*). (Guinea pigs are obligatory nose breathers; the nose is a highly efficient scrubber for SO_2.) The latter competing mechanism is likely to prevail unless the aerosol concentration is large, since the nasal passages provide a large, moist surface which will immediately begin to absorb SO_2 while the particles are still attracting water vapor.

The results of this experiment are in agreement with the hypotheses we have proposed and provide a realistic basis for gas-aerosol synergism. To our knowledge, this is the first demonstration of such synergism in which the physicochemical basis for the biological effect is shown experimentally.

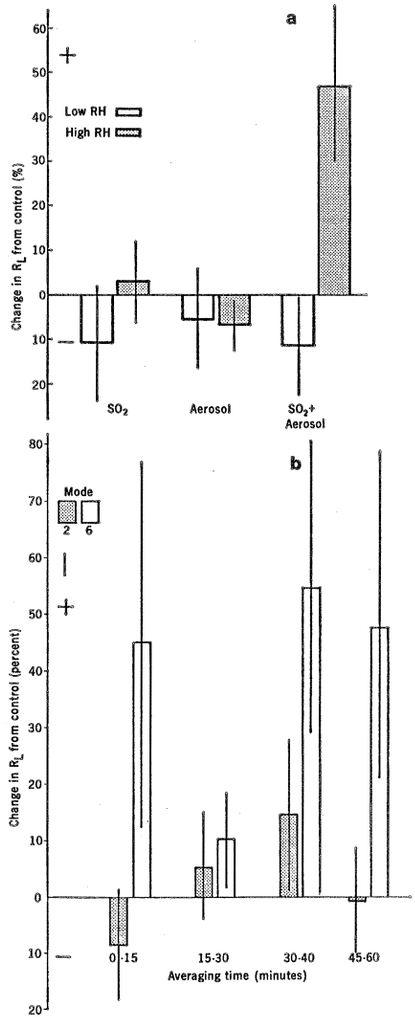

Fig. 1. (a) Values of R_L from 12 exposures were averaged (cm-H_2O ml^{-1} sec^{-1}; means ± S.E.) for each of the six exposure modes (SO_2, 1 ppm; in combination with NaCl aerosol, 1 mg/m^3). The results of the first and second exposures were not significantly different and were therefore combined. R_L was measured at approximately 4-minute intervals. The increase in R_L for mode 6 exceeds the other changes by the following: compared to mode 2, $P < .05$; compared to modes 1, 3, 4, and 5, $P < .01$. (b) The exposure (1 hour) was divided into four 15-minute periods, and an average value for R_L was obtained for each period. Only mode 2 (SO_2 at high RH) is compared to mode 6 since the change in R_L in the other four modes was less than in mode 2. The single highest peak R_L in any mode other than mode 6 occurred in mode 2 (average, 30 to 45 minutes).

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