A Preliminary Report

Effect of a Combination of SO_2 and H_2SO_4 On Guinea Pigs

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RESEARCH of the past 4 years has shown that low concentrations of either sulfuric acid mist alone (*1*) or sulfur dioxide alone (*2*) cause a reflex production of shallow, rapid respiration in normal humans. Since both these substances can, and as a matter of fact often do, occur together, the obvious next step was to test the effect of a combination of the two. This was best done first on animals in which lung pathology as well as respiratory effects could be studied. This paper reports the results of these very brief preliminary studies on guinea pigs.

In the experiments using humans, the concentrations of sulfur dioxide or sulfuric acid mist varied in order of magnitude from those occurring in the atmosphere during such disasters as the London fog to those considered as safe levels for routine exposures in industry. In the case of sulfur dioxide, the average concentration for the day at the height of the London fog was 1.3 p.p.m. (see *Public Health Reports*, May 1953, p. 474). This is about 10 times the amount usually found in the London atmosphere and about one-tenth of the maximum allowable concentration for industry (10 p.p.m.).

Dr. Amdur, research associate in the department of industrial hygiene, School of Public Health of Harvard University, who has been engaged in a series of studies on the effect on man and animals of sulfur dioxide and of sulfuric acid mist, presented this preliminary report on the effect of a combination of the two on guinea pigs, at the meeting in Boston of the American Association for the Advancement of Science, December 29, 1953.

In the case of sulfuric acid mist, atmospheric concentrations of 0.25–0.4 mg. per cu.m. have been reported and the industrial level is 1 mg. per cu.m.

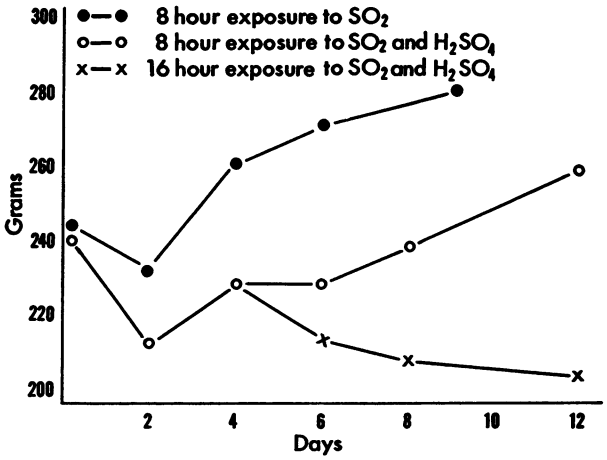
In the experiments using guinea pigs, concentrations much higher than these are used since the guinea pig is considerably more resistant to these substances than are humans. Levels 8 to 9 times the allowable industrial concentrations were used in the work reported here. These levels were chosen so that neither substance present alone would cause appreciable damage to the animals for the period of the exposure used.

Experimental Procedure

The apparatus was similar to that used in previous work on sulfuric acid mist alone (*1*, *3*). The mist was generated by warming concentrated sulfuric acid in a three-necked flask. Sulfur dioxide from a tank was metered into one neck of the flask while compressed air entering the second neck swept the vapors out of the flask through the third neck which was connected to the main air stream entering a 2-cu. ft. exposure chamber, made of corrosion-resistant materials (A).

For sampling the air in the chamber for sulfuric acid mist and sulfur dioxide, a membrane filter (B) and a sintered glass bubbler were connected in series. The sulfuric acid mist particles, which had a mean diameter of 1 micron, were caught by the filter. This filter was then washed with water and the conductivity of the resulting solution was measured. Concentration was calculated from a curve prepared from

Figure 1. Growth of guinea pigs following exposure to 89 p.p.m. of sulfur dioxide alone and to 89 p.p.m. of sulfur dioxide with the addition of 8 mg. per cu. m. of sulfuric acid mist.



standard sulfuric acid solutions. A sensitive fuchsin-formaldehyde colorimetric reagent (*4*) failed to indicate the presence of sulfur dioxide on the aerosol as collected. The sulfur dioxide, which passed the membrane filter (B), was trapped in the hydrogen peroxide-sulfuric acid reagent of Thomas and Abersold (*5*) and determined by conductivity. On several samples, the sulfur dioxide was determined by the iodine method. This checked the concentration found by conductivity showing that no appreciable quantity of the sulfur gas was present as sulfur trioxide.

In these experiments, 8 guinea pigs were exposed for 8 hours to a combination of 8 mg. per cu.m. of sulfuric acid mist and 89 p.p.m. of sulfur dioxide. Two of these animals were re-exposed 4 days later to the same concentrations for another 8 hours. Control data on the effects of exposure to sulfuric acid mist alone were readily available since in the course of previous work done in the same laboratory (*3*) a total of 68 guinea pigs from the same source had been exposed for 8 hours to 8 mg. sulfuric acid mist per cu. m. and 30 animals had been exposed to this concentration for 72 hours.

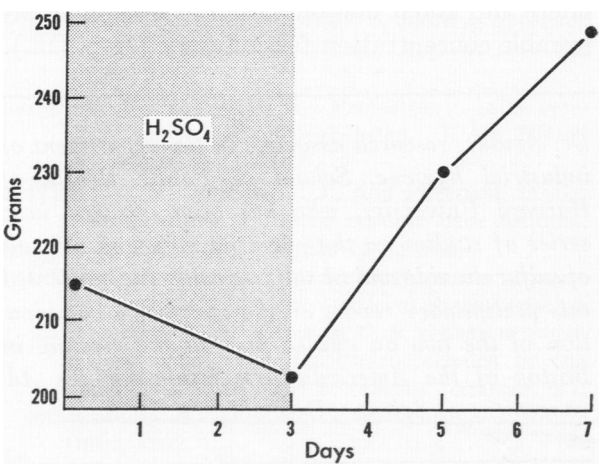
Due to various limiting factors, only 4 guinea pigs were exposed to 89 p.p.m. of sulfur dioxide uncontaminated by sulfuric acid mist. The response of the guinea pig to this gas has been, however, well documented by Weedon, Hartzell, and Setterstrom from the Boyce Thompson Institute (*6*). The response of the 4 animals exposed was as expected from the data reported.

Results

As criteria of damage, growth curves, respiratory response, and lung pathology were used. These combined criteria show that the effect of the combination was much more marked than the effect of either sulfuric acid mist or sulfur dioxide alone at the same concentrations.

Figure 1 shows the growth curves for animals exposed to sulfur dioxide alone and to the combination. In all cases the weight had fallen off the day following exposure. In the case of sulfur dioxide alone, growth was quickly resumed. When both sulfuric acid mist and sulfur dioxide were present, growth was slow to resume. The two animals that had been re-exposed ceased to grow entirely during the period of observation following re-exposure. The effect of the combination on growth is definitely greater than the effect of sulfuric acid mist alone, since in previous experiments even the survivors of LD_{50} concentrations (18 mg. per cu. m., a dose lethal to 50 percent of the animals exposed) or of 72-hour exposures to 8 mg. per cu. m. resumed growth following exposure. Figure 2 shows the growth curve of 12 animals exposed to 8 mg. sulfuric acid mist per cu. m.

Figure 2. Growth of guinea pigs following exposure to 8 mg. per cu. m. of sulfuric acid mist alone for 72 hours.



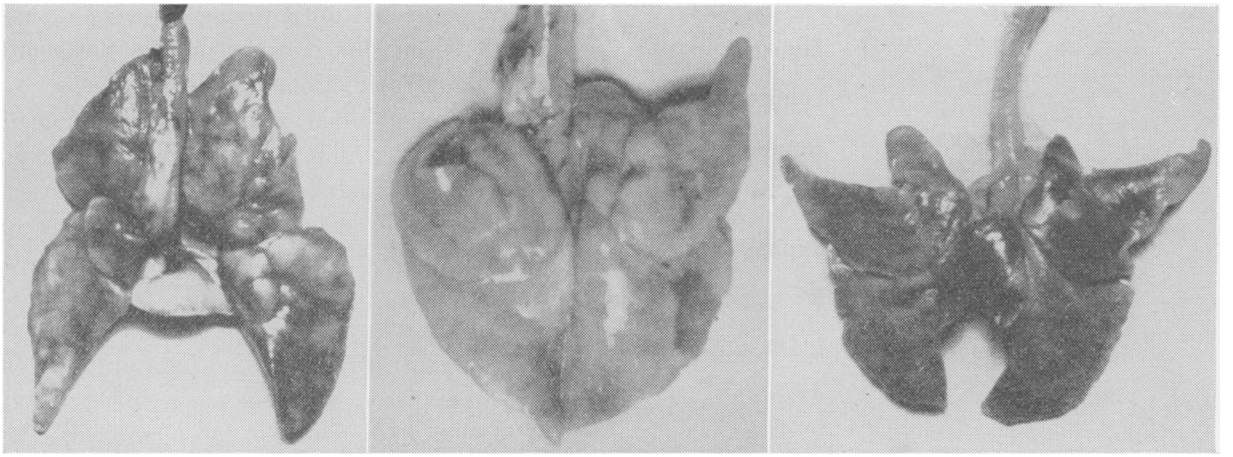


Figure 3. Lungs of three guinea pigs exposed for 8 hours to 89 p.p.m. of sulfur dioxide.

for 72 hours. The weight lost during exposure was quickly regained.

The lung pathology produced by the combination was much more extensive than that resulting from the same exposure to sulfuric acid mist alone or sulfur dioxide alone at the same dose levels. The lungs of animals exposed for 8 hours to 8 mg. per cu. m. of sulfuric acid mist alone (*3*) showed some damage with occasional areas of consolidation mostly limited to the hilar regions. Lungs of animals exposed to 89 p.p.m. of sulfur dioxide alone showed some damage, including pinpoint hemorrhages and occasional small areas of consolidation. Figure 3 shows the lungs of three of these animals. In neither case, however, was the lung damage severe.

Figure 4 shows the lungs of three animals exposed to the combination of sulfuric acid mist and sulfur dioxide for 8 hours. The lungs in all cases showed large areas of complete consolidation and hepatization involving entire lobes. Figure 5 shows the lung of one of the animals that was re-exposed for a second 8-hour period 4 days after the initial exposure. In this case hemorrhage and consolidation are widespread throughout the whole lung. The general ill health of these animals was very likely related to the presence of this extensive lung damage.

When guinea pigs are exposed to 8 mg. per cu. m. of sulfuric acid mist alone, there are no noticeable respiratory effects. This is true for exposures up to 72 hours. Grossly, the animals breathe and behave in a normal manner. At 89 p.p.m. of sulfur dioxide, the animals appear restless and annoyed when the gas is first turned on, but this passes in 5 to 10 minutes and they appear to become accustomed to it. Weedon and co-workers (*6*) report that at 150 p.p.m. of

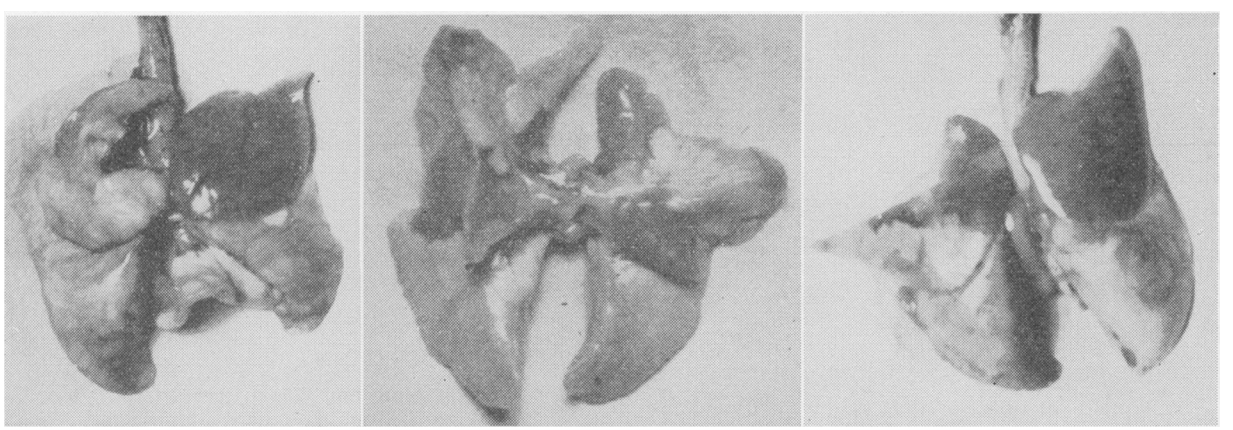


Figure 4. Lungs of three guinea pigs exposed for 8 hours to 89 p.p.m. of sulfur dioxide and 8 mg. per cu. m. of sulfuric acid mist.

Figure 5. Lung of guinea pig after 16-hour exposure to 8 mg. per cu. m. H_2SO_4 and 89 p.p.m. SO_2

sulfur dioxide severe respiratory distress does not set in until the animals have been exposed for 48 hours, and that even at 296 p.p.m. evidence of dyspnea only became apparent during the second day of exposure.

When these two substances are given together at the concentrations used, signs of respiratory distress become apparent within the first hour of exposure and become progressively worse as the exposure continues. Frothy fluid appears in the nose and mouth and dyspnea is very pronounced. Even more surprising is the fact that this labored breathing continues for 24 to 48 hours after the animals have been removed from the exposure chamber. At concentrations of sulfuric acid mist alone capable of killing 50 percent or more of the animals in 8 hours, those that survived were breathing normally within 2 hours after the exposure ended except in a few rare instances.

Discussion

None of the animals in these experiments died spontaneously either during exposure or in the short period of observation following exposure. Both the depressed growth curves and the severe lung pathology, however, indicated that considerable damage had occurred. Both these factors were markedly aggravated by the repeated exposure.

If a quantity of sulfuric acid mist equivalent to 89 p.p.m. of sulfur dioxide were given, guinea pigs would be dead within an hour or less of laryngeal spasm. With three times this concentration of sulfur dioxide alone, however, breathing is not affected until the second day of exposure. From this it appears that the small amount of sulfuric acid mist, which is ineffective alone, when combined with a concentration of sulfur dioxide, which is also ineffective alone, produces a very marked respiratory response.

Since only one concentration of sulfuric acid mist and sulfur dioxide was used in these experiments, the results must be regarded as purely preliminary. Further studies using different concentration combinations are needed before an explanation of the nature of the joint toxic action of these two compounds can be postulated.

Summary

It has been shown that for 8 mg. per cu. m. of sulfuric acid mist and 89 p.p.m. of sulfur dioxide given in combination to guinea pigs the effect on growth, lung pathology, and respiration are much more marked than would be predicted from the use of either alone.

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EQUIPMENT REFERENCES

(A) Lucite. Rohm and Haas, Philadelphia.

(B) Filter, Millipore. Type HA, plain, 47 mm. discs in packages of 100. Lovell Chemical Co., Watertown, Mass.