Sulfur Dioxide (SO_2) Exposure and Recovery Effects on Mice[1] (36253)

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Numerous studies have described the distressing symptoms on the upper respiratory passages due to sulfur dioxide exposure (1, 2) and effects on eyes (3), lungs (4, 5), and respiratory disturbances (6). There are data on SO_2 effects on blood constituents (7) and some evidence that animals recover quickly from SO_2 inhalation of sublethal levels (8).

However, the time responses of an animal to adjust physiologically to continuous SO_2 exposure and the response times of recovery following exposure have not been reported for mice.

A longevity study on rats (9) showed a decreased life span due to various levels of SO_2 from 1 to 32 ppm.[3] However, no study has reported the time responses of the animals to continuous SO_2 exposure and the response time in recovery following the exposure. Therefore, the objectives of this study were to gain a better understanding of the prolonged effects of atmospheric SO_2 on the gross physiological responses; nasal and pulmonary tissue changes in mice; and the ability to recover from, or compensate for, such exposures.

Mature male mice (200 days old) of the Charles River strain were divided randomly into two groups: a control and gas exposed group each having 12 animals. Each of the groups was divided into two cages of six animals before being placed in exposure chambers. Water and Rockland Rat and Mouse Diet were available at all times to the mice. Measurement of group feed and water intake and body weights were made daily. Oxygen consumption and individual daily feed and water intake and body weights were measured 33 days after initial exposure and similarly 32 days after exposure ceased. Oxygen consumption was measured with an 8-chamber Regnault-Reiset volumetric apparatus (10). Histological studies of the upper respiratory tract and lung tissues were performed on mice sacrificed immediately following these measurement periods. Ambient temperature of the chambers fluctuated around a room mean temperature of approximately 20° (range of 16 to 27°). The chambers were opened for approximately 5 min once daily for cleaning and feeding.

The mice were housed in three-mesh stainless steel screen cages (24 cm deep and 12 cm high) which permitted maximal exposure to the air–SO_2 mixture and allowed observations of animals. The chambers were modified refrigerators with the freezer units and coils removed; and the back panels were replaced with a Plexiglas pane. Air to each chamber inlet was filtered by two 25 cm × 25 cm glass wool filters, monitored by a flow meter and drawn into the top of the chamber via the tangential feedbox by a squirrel-cage exhaust blower attached to the floor. The air flow, monitored by an air-flow meter, was exchanged in each chamber once every 2 min.

SO_2 was introduced to a tangential mixbox and mixed by the tangential feed system (Hinners, personal communication). This assembly was attached near the ceiling of each chamber. The SO_2 concentrations in the unit were monitored near the air exhaust duct and maintained with an electroconductivity analyzer (Davis model 70A1-01 Standard portable SO_2 monitor, Newark, NJ). This in-

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[3] ppm concentrations signify ppm on a volume basis.

TABLE I. Feed and Water Intake and Body Weights of Control and SO_2 Exposed Mice for a 7-Day Period During SO_2 Exposure and Postexposure.

Period	Group	N	Feed intake (g/day)	Water intake (ml/day)	Body wt (g)
Exposure, 4–11 days[a]	Control	12	7.3[d]	6.9[d]	45.7[d]
	SO_2	12	2.7	2.8	36.8
Postexposure, 5–12 days[b]	Control	6	4.9	4.6	44.1[c]
	SO_2	3	6.5[d]	12.6	37.5

[a] Mean value for days 4–11 exposure.
[b] Mean value for days 5–12 postexposure.
[c] $p < .05$.
[d] $p < .01$.

strument automatically corrected for CO_2 levels.

For the initial study, animals were exposed to 40 ppm SO_2 in the air. To monitor the high range and dependably detect a mixture of 40 ppm of SO_2 in the air at 70–80% of recorded scale, the high range span pot was adjusted. The SO_2 was infused at a rate sufficient to produce a mixture of 40 ppm from SO_2 cylinders (commercial grade 99.9% SO_2, 35 psig) procured from Matheson Company, Inc.[4] Our monitor (analyzer), calibrated as described, was used for the final determination of the SO_2 rate of flow. A small Gilmont flowmeter (range 0.2–90 ml of air/min) was used for visual observation and adjustment of the flow of SO_2.

Table I shows the average feed and water intake and body weight values based on group measurements of mice on continuous exposure to 40 ppm for 4–11 days and subsequently at 5–12 days postexposure. Within a few days of SO_2 exposure, a marked decline occurred in body weight and feed and water consumption. The mean feed and body weight values for the SO_2 exposed groups were significantly lower during the 4–11-day period of exposure. The body weight values for the SO_2 exposed groups were also significantly lower than the control group, when values from 4–11 days were pooled for the 7-day period. As shown in Fig. 1, these differences in feed and water intake diminished on continued SO_2 exposure, although the body weights remained markedly lower than the controls. To provide greater statistical reliability, at 33 days of exposure the mice were placed in individual cage partitions to measure the feed and water daily on an individual basis for a 3-day period (Table II). Individual values on all SO_2 exposed mice for this pooled 3-day period showed a significant difference in body weight; although the feed and water intake had gradually recovered to that of control animals. However, the oxygen consumption of the SO_2 animals was significantly lower (Table II) at this 33–35-day period of exposure.

These exposed mice appeared generally in poor condition with dull, dry, and colorless eyes compared to the controls. Breathing was irregular, with evidence of respiratory sneezing, rattling, and nasal "sniffling." The hair was extremely ruffled; and tails of the animals seemed dry and inflexible. The bodies of the animals assumed a "humped" position much of the time. These general symptoms persisted throughout the exposure period, with one animal dying at 11 days and two at 36 days of exposure. Following the above measurements, 6 control and 6 experimental animals were sacrificed for histological observations at 35 days of exposure. Several abnormalities were present in the upper respiratory tract. Examination of the epithelium tissue revealed the presence of many leukocytes, mucus, and some cellular debris. Stratified squamous epithelium was found in the tract tube 3–4 layers thick and poorly organized. The turbinate bones were thin and

[4] Matheson Scientific Co., 3160 Terrace Street, Kansas City, MO 64111.

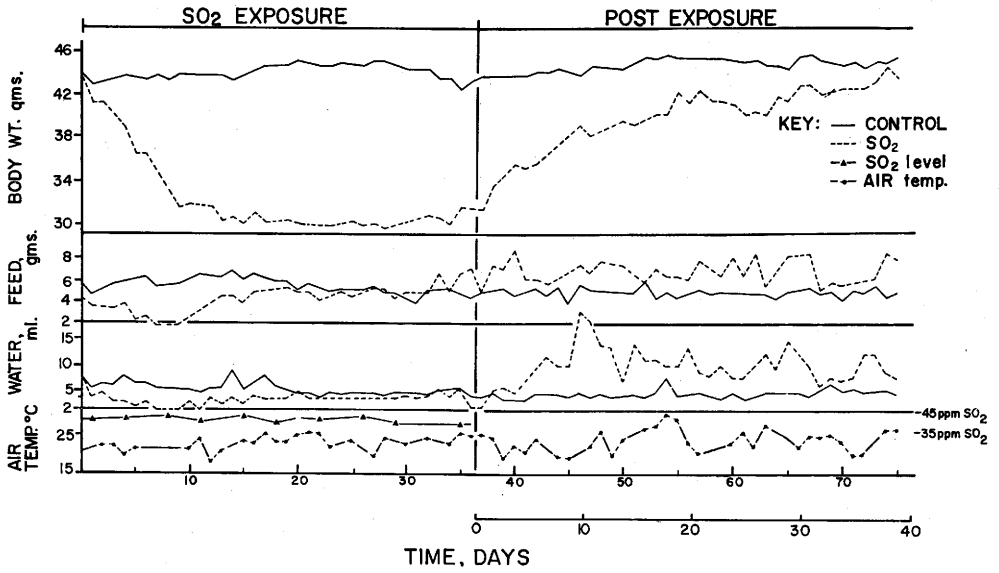

FIG. 1. Time–response curves of various functions to SO_2 exposure and postexposure.

disorganized; and chronic inflammation had resulted in some dissolution of the turbinate bones. However, no gross changes were found in the lungs.

Since it seemed that the body weight and other values had attained a stable level of acclimation, we terminated the SO_2 exposure to observe any postexposure changes (Fig. 1). Within a few days following cessation of SO_2 exposure, feed and H_2O intake (Tables I and II) had overcompensated. Water intake was considerably higher, although the variability was too great to be significant. Body weights were attained at a level insignificantly different than controls at 32 days postexposure (Table II). After 32 days postexposure (Fig. 1), all values, including O_2 consumption, were similar to control levels. The feed values were still somewhat higher and significant at the .05 level.

TABLE II. Average Individual Feed and Water Intake and Body Weights of Control and SO_2 Exposed Mice.

Period	Group	Feed intake (g/day)	H_2O intake (ml/day)	Body wt (g)	O_2 consumption (ml/hr)
Exposure, 33–35 days[a]	Control	5.12	6.10	43.96[e]	169.5[e]
		(36)[c]	(36)	(36)	(12)
	SO_2	5.18	5.46	33.20	96.3
		(33)	(33)	(33)	(11)
Postexposure, 32–34 days[b]	Control	4.45[d]	5.56	44.72	127.8
		(18)	(18)	(18)	(12)
	SO_2	5.40	7.22	42.06	119.0
		(9)	(9)	(9)	(9)

[a] Average daily values of individual animals for days 33, 34, and 35 exposure.
[b] Average daily values of individual animals for days 32, 33, and 34 postexposure.
[c] Number.
[d] $p < .05$.
[e] $p < .01$.

A second group of mice was sacrificed 34 days after exposure ceased. There were no gross or microscopic effects on the upper respiratory tract, as was observed during the exposure period. The treated and the control animals appeared very similar. The trachea and lungs of both groups were also normal except for the presence of traces of chronic murine pneumonia. Although the number of animals for histology were limited, it seemed that the 40-day period after exposure to SO_2 was sufficient to repair the damage to the nasal sinuses.

The conclusion, after about 20 days of SO_2 exposure it appears that the mice reassumed a near-control level of feed intake, although the body weights and O_2 consumption were subnormal, indicating a decreased metabolic activity of the SO_2 exposed individuals. We propose to do further metabolic and endocrine studies to clarify mechanisms involved in this physiological adjustment to SO_2 exposure. The most amazing aspect of this study was the rapid recovery in body weight and other measured variables after cessation of SO_2 exposure. The upper respiratory tracts of the mice even appeared normal at 35 days postexposure. With the extremely high level (40 ppm) of SO_2, it is surprising to see such rapid recoveries to such an irritating and unpleasant atmosphere as SO_2. As stated earlier, several animals which were exposed died, compared to no losses of control animals. Furthermore, residual effects may be revealed in longevity studies.

Summary. Continuous exposure of mature, male mice to 40 ppm atmospheric SO_2 depressed feed and water intake, body weight, and O_2 consumption; and caused upper respiratory damages. After approximately 20 days of exposure, feed and water intake returned to control values, although body weight and metabolism remained low. Immediately following cessation of SO_2 exposure, the animals began to recover body weight and overcompensated in feed and H_2O intake. Oxygen consumption was normal at 32–34 days postexposure; and limited histological evidence showed no apparent damaging effects of exposure on the surviving mice.

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