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Effect of SO_2 on the Pathogenesis of Viral Upper Respiratory Infection in Mice (39725)

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Introduction. Epidemiologic studies have consistently shown an association between respiratory infection and air pollution. Most of these studies have been concerned with effects on the mucous membranes of the tracheobronchial tree (1-5). Among the many airborne contaminants to which the Yokkaichi industrial area of Japan was heavily exposed from about 1960-1966, SO_2 was the major pollutant, with levels ranging from 0.2 to 2.5 ppm (6), and during those years the incidence of respiratory diseases was significantly increased over that of nonpolluted areas. In 1968, after rigorous control measures, the range was lowered to 0.071-1.0 ppm. Nonetheless, Ohyana and Miyoshi found in 1972 that the incidence of rhinitis and tonsillitis in school children in Yokkaichi was still significantly higher than that of children in nonpolluted areas (7). Previous reports of increased respiratory disease had been concerned with industrial workers in the United States and in England after exposure to SO_2 levels as high as 55 and 36 ppm, respectively (8).

There have been several histopathologic studies of lower respiratory mucosae in experimental animals after exposure to various levels of SO_2 (9-16) and three studies of experimental exposure of nasal mucous membranes to high (10-36 ppm) concentrations of SO_2 (17-19), but the effects of *continued* exposure of nasal tissues to *low levels* of SO_2 have not been reported. Since the inhaled gas initially impinges on nasal tissues and may interact with other factors to enhance respiratory tract infections, it seemed important to follow the pathogenesis of intranasal virus infection in association with low levels of exposure. The present study was designed to determine the effects of constant exposure of nasal mucous membranes of mice to low levels of SO_2 on the pathogenesis of influenza virus infection in these animals.

Materials and methods. Animals. Male mice of the Japanese DS strain, derived from the commercial dds strain maintained by the Abulahi Laboratory, Shionogi Co., Shiga prefecture, Japan, were used throughout the study. They were all 4-5 weeks old and weighed 15-20 g when first exposed to SO_2.

Virus. The mouse-adapted PR_8 strain of influenza A virus was serially passed six times in unanesthetized mice by intranasal inoculation. After each passage, macerated lung tissues were diluted tenfold in normal saline and used as the successive inocula. Lung tissues from the final passage were inoculated into the allantoic fluid of 12-day-old chick embryos. Allantoic fluid was harvested 24 hr after inoculation and was stored at $-70°$ until used. The virus had a hemagglutination titer of approximately 2024.

Exposure condition. The SO_2 exposure apparatus is shown in Fig. 1. During exposure, approximately 30 mice were housed in 3290-cm^3 stainless steel wire cages within the vinyl chloride exposure chamber, which had a volume of 6880 cm^3. Sulfur dioxide was generated by a standard gas generator (Kimoto SCCl, 3780), utilizing a Teflon permeation tube containing liquified SO_2. The gas was mixed with filtered air and introduced into the exposure chamber at a rate of 9 liter/min. Sulfur dioxide was maintained at a concentration between 0.03 and 0.1 ppm throughout the exposure period. The SO_2 concentration was determined daily by the *p*-rosaniline method (20). Food and water were provided *ad libitum*.

Experimental procedure. Mice were divided into three experimental groups of five

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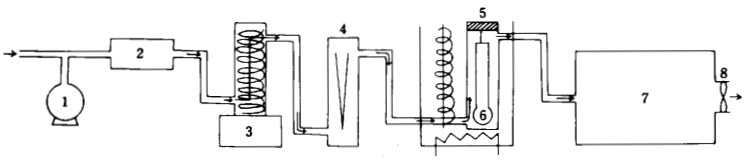

FIG. 1. Diagram of SO_2 exposure apparatus: (1) air compressor; (2) air filter containing activated charcoal and silica gel; (3) refrigerator; (4) flow meter; (5) water bath, 25°; (6) SO_2 permeation tube; (7) exposure chamber; (8) exhaust fan.

mice each: (i) controls which were exposed to neither virus nor SO_2; (ii) those receiving virus alone; and (iii) those exposed to SO_2 and then inoculated with virus. Following 4 weeks of exposure to SO_2, the mice in group 3 were inoculated intranasally with 0.02–0.03 ml of undiluted virus suspension; they were not anaesthetized. The mice were killed at 3, 6, and 12 hr, and 1, 2, 3, 5, 7, 14, and 21 days after inoculation. Mice in the two control groups were killed at the same time intervals. Body weights of mice in each experimental group were recorded through the experiment.

Histopathologic examination. After removing the lower jaw, skinned heads were fixed in 10% formalin, then decalcified in 8% trichloroacetate. Tissues were dehydrated in graded ethyl alcohol and embedded in paraffin. Transverse sections were made across the nasal cavity through the anterior ridge of the orbits, and 6–8-μm sections were stained with H&E and PAS. PAS-positive cells were counted in the respiratory epithelia of the nasal septal mucosa. All mice in all groups were evaluated histologically, and all slides were read blind.

Titration of influenza antibody in serum. Venous blood was taken from the eye. The sera were separated and inactivated at 56° for 30 min. Sera from each group of five mice were pooled, and HI antibody was titrated for each group at each time interval after inoculation. Four hemagglutinating doses of viral antigen were used in these assays.

Results. Body weight. In the virus control group, the mice lost approximately 10% of their body weight from the prechallenge level during the first 7 days after inoculation, then subsequently showed normal weight gains. The group exposed to SO_2 also lost 10% of their body weight by the 7th day after the initial exposure, and thereafter gained weight at normal rates. In the SO_2 plus virus group, the mice lost 5% of their body weight during the first 2 days after virus inoculation, and thereafter showed the same daily increments as did the virus controls.

Development of serum antibody titers. In the virus control group, HI antibody developed on Day 10 postinoculation and reached maximum levels on Day 14. In the SO_2 plus virus group, these levels reached 1024 on Day 6 and rose to 4096 by Day 8 after inoculation (Fig. 2). Thus, the antibody response was significantly more rapid; at 7 days the HI titer was 500 times greater in the SO_2-exposed group. There is no known statistical test that can be applied to these geometric mean titers.

Histopathology. At 3 hr after virus inoculation, the virus control and SO_2 plus virus groups showed no histopathologic differences.

By 12 hr, there were scattered discrete changes in the middle portion of the nasal septum in the virus control group: loss of cilia (arrow), vacuolization of the epithelial columnar cells, and inflammatory cell infiltration in the subepithelial layer (Fig. 3). At the same time the SO_2 plus virus group showed extensive loss of cilia, inflammatory cell infiltration in the mucosa, and congestion of the subepithelial capillaries (Fig. 4).

At 48 to 72 hr after inoculation, both groups showed focal desquamation of respiratory epithelia in the nasomaxillary turbinates and nasal septum, with frequent fibrinopurulent exudation into the nasal cavity. In the SO_2 plus virus group, the changes were much more extensive in both extent and severity, with complete loss of cilia, areas of flattened respiratory epithelium, many islands of desquamated respiratory epithelium, and great masses of fibrinopurulent exudate in the nasal cavity lumen. The

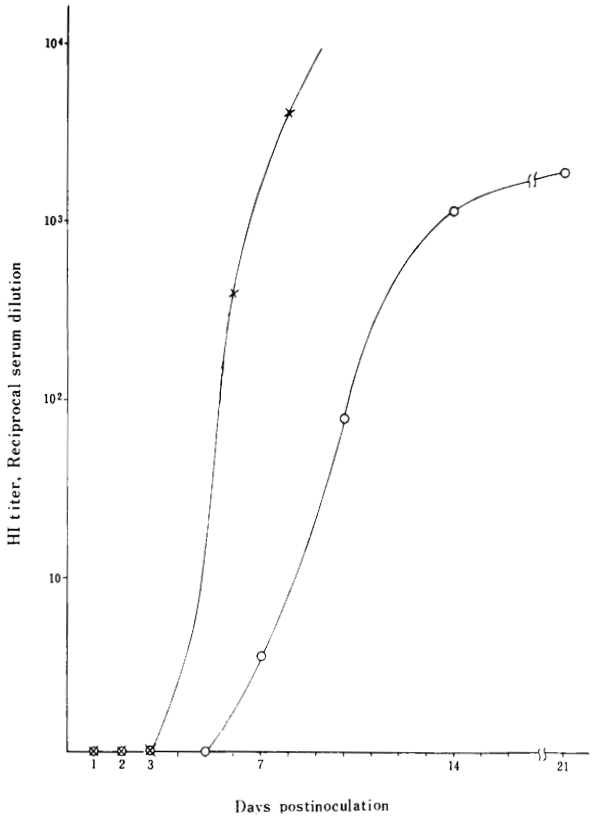

FIG. 2. Changes in HI antibody titer after intranasal inoculation of influenza virus. ×——×, SO_2 plus virus; ○——○, virus only.

olfactory mucosa was also inflamed.

By 5–14 days, the virus control group showed a low columnar respiratory epithelium with few cilia, and intensive desquamation of the olfactory epithelium. In the SO_2 plus virus group, there was beginning repair of the septal and maxillary turbinate epithelia. The subepithelial layer was only loosely infiltrated by small numbers of inflammatory cells, and the olfactory epithelium was partially replaced by ciliated cells.

By 2 weeks postinoculation, the nasal mucosae were essentially restored in both groups. The sequence of pathological changes is summarized in Fig. 5. Histopathological changes peaked on Day 7 after inoculation in the virus controls and 2 days postinoculation in the SO_2 plus virus group.

Number of goblet cells. After 4 weeks of exposure to SO_2, the number of goblet cells in the respiratory epithelium of the nasal septum was found to be six times greater than in nonexposed mice. In the virus control mice, the number of goblet cells increased progressively after inoculation throughout the experiment. In mice exposed to both virus and SO_2, goblet cells increased for 3 days postinoculation and then rapidly decreased (Fig. 6).

Discussion. There is considerable literature linking increased frequency and severity of lower respiratory disease with exposure to high levels of SO_2. To select clinical examples from three communities: French *et al.* (1) found that excessive rates of acute respiratory disease were clearly linked to heavy SO_2 pollution; Buechley *et al.* (21) found mortality rates greater than expected when SO_2 levels were above 500 $\mu g/m^3$; and Yoshida *et al.* (6) reported that prevalence of bronchial asthma was directly proportional to concentrations of SO_2.

The nose has been curiously neglected in clinical and experimental studies of SO_2 ⇋

virus interactions, even though it is known that nasal mucous membranes absorb most inhaled SO_2 (22) and that both irritant gases and virus infections initiate inflammatory and immune responses in the nose. Matsumura's (23) concept that under certain conditions SO_2 may have an adjuvant effect on antibody production seems reinforced by the present experiments in which HI responses were more rapid and reached higher titers when virus was introduced after long-term exposure to SO_2.

The histopathologic changes induced by *virus alone* in the nasal mucosae of mice in the present experiments were similar to those described after intranasal inoculation of influenza virus in unanesthetized ferrets (24) and mice (25), and of NDV inoculation in chickens (26).

The increased numbers of goblet cells produced in nasal tissues by *SO_2 alone* were similar to responses described after long-

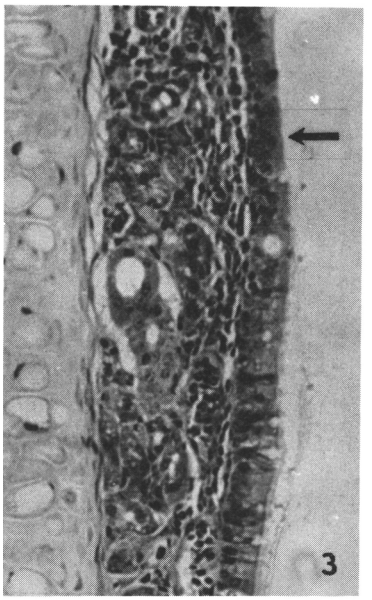

FIG. 3. Epithelium of midseptum 12 hr postinoculation, virus only, showing moderate loss of cilia (arrow) and mild inflammatory response.

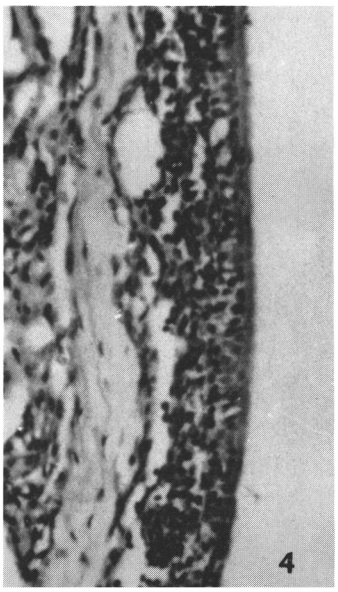

FIG. 4. Epithelium of inferior turbinate 12 hr postinoculation after SO_2 exposure showing epithelial cell metaplasia and intensive inflammatory response.

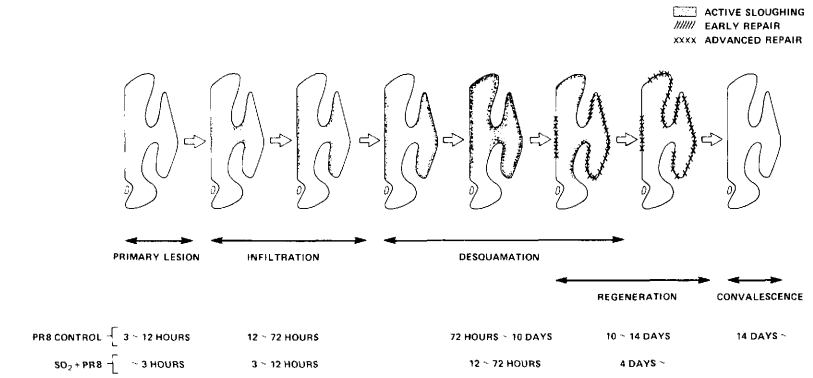

FIG. 5. Diagrammatic summary of differential sequence of pathological changes after virus inoculation with and without SO_2 exposure. Stippling represents epithelial damage; hatched lines and crosses represent early and later repair; dash lines indicate regenerated mucosae.

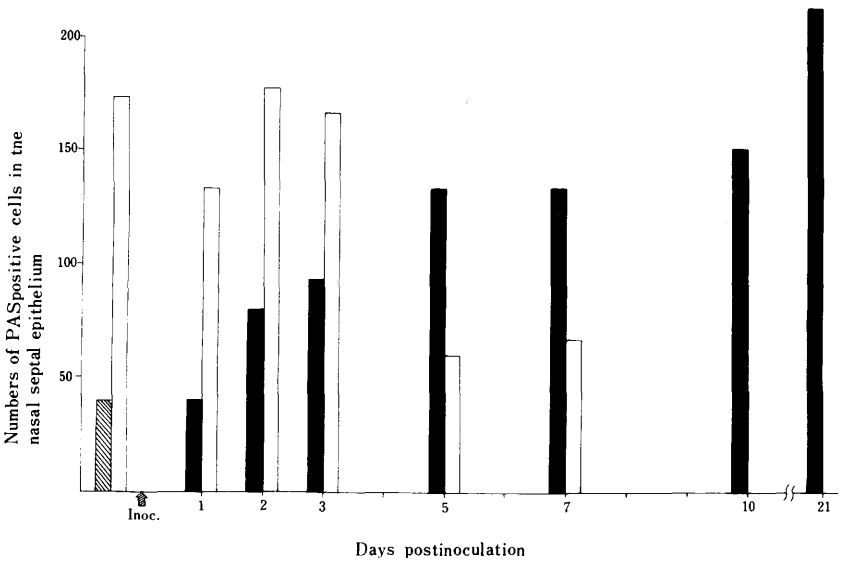

FIG. 6. Changes in the numbers of goblet cells in septal mucous membrane over time. Hatched bar indicates control, no virus or SO_2; black bar indicates virus alone, no SO_2; white bar indicates SO_2 plus virus.

term exposure of tracheal epithelia to low levels of SO_2 (27) and after intermittent exposure of bronchial epithelia to heavy concentrations of SO_2 (14).

The histopathology produced in the nose by *combined SO_2 and virus* in the present experiments suggests that progressive alteration of nasal mucosae by SO_2 allowed very rapid proliferation of virus in these tissues. In nature, this breakdown in the normal defense mechanisms in the nose would leave the lower tract tissues vulnerable to effects of gases, viruses, or combinations of the two.

Clearly a systematic experimental study of interactions between chronic air pollution and respiratory virus infections in nasal tissues, and subsequent effects on tracheobronchial tissues, has not yet been achieved. Especially lacking are data on the differential effects of pollutants on altered production of secretory antibody, interferon, and lysozyme.

Summary. The mouse-adapted strain of PR_8 influenza virus was inoculated intranasally into unanesthetized DS mice which had been continually exposed to 0.03–0.1 ppm of SO_2 for 4 weeks. Histopathological changes in nasal tissues showed that inflammatory response was more rapid and more severe, and regeneration was initiated sooner, in these mice than in virus-inoculated controls. The HI titer also developed more rapidly and reached higher levels in mice exposed to SO_2 plus virus than in mice receiving virus alone, and antibody to the virus appeared earlier in the exposed mice.

Animals which were exposed to SO_2 and were not given the virus showed a sixfold increase in the number of goblet cells in nasal respiratory epithelial cells, though the olfactory epithelial cells were apparently unaffected.

The results suggest that increased severity of influenza infection after continued exposure to low levels of SO_2 resulted from a progressive alteration of the nasal mucous membranes by SO_2 which then allowed rapid destructive proliferation of subsequently introduced virus. This destruction of nasal tissues would eliminate a major defensive barrier against lower tract disease.

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