Microbial Flora of the Larynx, Trachea, and Large Intestine of the Rat after Long-term Inhalation of 100 Per Cent Oxygen

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Effects of long-term inhalation of 100 per cent oxygen on the microbial flora of the rat larynx, trachea, and large intestine were studied. Rats were kept 14 days in an atmosphere of 100 per cent oxygen after being conditioned to high oxygen concentrations by exposure to three cycles of 100 per cent oxygen (two days) alternating with 40 per cent oxygen (two days). Controls were kept under similar conditions in normal atmosphere. Rats were sacrificed, and at necropsy laryngo-tracheal swabs and fecal material from the large intestine were obtained and cultured for bacteria and fungi.

*Streptobacillus moniliformis,* the predominant microorganism in the upper tracheal of controls, was not isolated from the oxygen-treated rats. Alpha-hemolytic streptococcus and *Staphylococcus albus* were present in control rats, but were found less frequently in rats exposed to oxygen. *Pseudomonas* and *Proteus,* infrequently isolated from controls, were predominant and sometimes the only microorganisms isolated from oxygen-treated rats. The data indicate that prolonged exposure of the rat to 100 per cent oxygen shifts the microbial flora in the upper respiratory tract from mainly gram-positive to mainly gram-negative bacteria. In contrast, there was no significant difference between the microbial flora in large intestines of control and oxygen-treated rats. The possibility that similar changes may occur in man should be considered when prolonged oxygen therapy is contemplated. (Key words: Oxygen, respiratory flora; Infection, respiratory tract.)

PROLONGED EXPOSURE to high concentrations of oxygen is now frequently a part of inhalation therapy of critically ill patients. The development of the chronic phase of oxygen toxicity, with attendant pulmonary honey-combing and interstitial scarring, is one of the main complications of prolonged exposure to 100 per cent oxygen. Impairment of the phagocytic activity of alveolar macrophages is another. This, in turn, may facilitate microbial proliferation. Enhancement of viral infections in mice following prolonged exposure to high concentrations of oxygen has also been reported. On the other hand, hyperbaric oxygen is used to treat patients infected with anaerobic organisms, such as clostridia, and has also been shown to be bacteriostatic or even bactericidal to aerobic organisms in vivo and in vitro. Since various microorganisms have access to the upper respiratory tract, it is important to determine whether inhalation of high concentrations of oxygen could significantly increase or decrease the numbers and types of potentially pathogenic organisms. The present investigation was designed to study this in experimental animals. While previous studies in vivo and in vitro have tested the effects of relatively brief exposures (a few hours to several days) to high oxygen concentrations, the present report deals with the effects of a more prolonged exposure to 100 per cent oxygen.
TABLE 1. Microorganisms Isolated from Tracheas of Rats after Prolonged Exposure to 100 Per Cent Oxygen*

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Oxygen Treated</th>
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<tbody>
<tr>
<td></td>
<td>Experiment I</td>
<td>Experiment II</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>3+ 4+ 4+ 3+ 2+ 3+</td>
<td>3+ 4+ 4+ 2+ 2+ 2+</td>
</tr>
<tr>
<td><em>streptococci</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>+ 2+ 2+</td>
<td>+ 2+ 3+ 2+ 2+ 3+</td>
</tr>
<tr>
<td><em>albus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>+ 2+ 3+ 2+</td>
<td>+ 2+ 3+ 2+ 3+ 2+</td>
</tr>
<tr>
<td><em>coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>+ 2+ 2+ 2+ 2+</td>
<td>+ 2+ 2+ 2+ 2+</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>spp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em></td>
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<tr>
<td><em>spp.</em></td>
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* The designations 1+ to 4+ represent growth of the microorganisms on one to four quadrants of the culture plates, respectively, using the four-quadrant streaking system.

**Methods**

Male, CFE, Sprague-Dawley rats (275–350 g) were randomized into experimental and control groups. The experimental group consisted of 48 animals and the control, ten. The experiment was performed in two sessions because a) space in the animal tanks was limited, and b) we wished to double-check the normal flora in control animals by ordering animals from the supplier at two separate times. The same number (half) of the experimental as well as the control rats were processed during each session (Experiments I and II in table 1). The room and tank temperatures were the same during the two experiments, 75–78 F.

The experimental animals were subjected to a 12-day regimen in order to prolong survival in 100 per cent oxygen. The regimen consisted of the following sequence of oxygen concentrations: two days in 100 per cent, two days in 40 per cent, two days in 100 per cent, two days in 40 per cent, two days in 100 per cent, two days in 40 per cent. After this regimen, rats were kept in 100 per cent oxygen for 14 days.

Thirty-eight of the experimental animals died from oxygen toxicity before the 14-day period in 100 per cent oxygen was completed, but ten survived and were sacrificed on the fourteenth day by cervical dislocation. Controls were concurrently kept in room air and sacrificed at the same time as the experimental animals. The rats were weighed at the beginning of the experiment, every week thereafter, and at necropsy.

Specimens for microbial examinations were collected by introducing sterile swabs through an incision in the upper trachea and by collecting material from the large intestine after opening the abdominal cavity. Material on one tracheal swab was cultured on sheep blood agar (BA), colistin and nalidixic acid (CN) agar, chocolate agar, and Mac Conkey agar (MAC) plates. A second swab was streaked onto Sabouraud dextrose agar (SDA) and mycobiotic agar (MA) plates for isolation of fungi. A third swab was used for the preparation of Gram-stained smears. Fecal specimens from the large intestine were cultured on BA, CN, MAC, and Hektoen agar plates, in a Gram-negative broth tube, and on SDA and MA agar plates. The bacterial cultures were incubated at 36 C in an atmosphere of 10 per cent CO₂–90 per cent air. In addition, duplicate BA and CN agar plate cultures were incubated anaerobically at 36 C in a GasPak jar (Baltimore Biological Laboratory, Cockeysville, Maryland). The method for producing anaerobiosis is described elsewhere. Fungus cultures were incubated at 30 C in atmospheric air for four weeks. The culture plates of materials from both controls and oxygen-treated animals were examined by a code system, using a blind technique.
Results

The results of isolation of bacteria and fungi from the upper tracheas of oxygen-treated and untreated rats are summarized in Table 1. Experiments I and II were performed at different times using equal numbers of animals under similar experimental conditions. *Streptococcus moniliformis*, the etiologic agent of rat-bite fever in the United States, was the predominant microorganism in the upper tracheas of control rats, but was not isolated from the tracheas of rats treated with oxygen. Two other bacteria, alpha-hemolytic streptococci and *Staphylococcus albus*, present in control rats, were absent or less frequently isolated from the oxygen-treated animals. In contrast, *Pseudomonas* and *Proteus* species, less frequently isolated from control rats, had become the predominant microorganisms in the experimental animals.

The intestinal tracts, especially the colons, of the experimental animals were usually considerably dilated by feces and gas. However, there was no qualitative difference between the microbial flora of the large intestines of control and oxygen-treated rats. *Escherichia coli*, *Proteus*, *Pseudomonas*, *Clostridium* spp., *Diptheroids*, *Bacteroides*, and *Enterococcus* were present in the feces in both experimental and control rats.

The experimental animals lost, on the average, 14 per cent of their starting weights, while the controls gained about 22 per cent during the same period. Weight losses ranged from 6 to 29 per cent.

Gross and microscopic examination of the tracheas, major bronchi, and large intestines did not reveal any significant differences between experimental and control rats. Only one experimental animal had small patches of squamous metaplasia in several of the bronchial sections. None was observed in the controls. The gross and microscopic changes seen in the lungs of rats with chronic oxygen toxicity varied in severity. Such changes have been described.1,2,4

Discussion

The data presented demonstrate that prolonged inhalation of 100 per cent oxygen altered the microbial flora of respiratory tracts but not the flora of gastrointestinal tracts of rats. In contrast, several previous studies in vitro and in vivo failed to show any significant effect on aerobic organisms of 100 per cent oxygen at one atmosphere.8,9 At first glance, it may appear that these results are contradictory to our findings. However, in the present investigation, long-term exposure to oxygen was used, while the previous investigators relied on short-term, one- to three-day exposures. The techniques for prolonging the survival of rats in 100 per cent oxygen were developed recently1-4 and were not available to earlier investigators studying microbial changes.

The alpha-hemolytic streptococci isolated from the tracheas of the control animals were not present in four of the five experimental animals of Experiment I (Table 1). The persistence of the streptococci in the tracheas of the animals from the second experimental group despite the prolonged exposure to 100 per cent oxygen was the main difference between the two experiments. This could be accounted for by possible strain differences in sensitivity to high concentrations of oxygen. Such variations have been reported by others.6

Persistent contamination or even growth of gram-negative organisms in the tubing and other components of the respiratory care equipment used for long-term administration of high oxygen concentrations has been encountered (unpublished data).

At present it is not clear whether the alterations of the tracheal flora resulted from a direct action of oxygen on the microorganisms or some other factor(s), such as inhibition of pulmonary macrophages. The rate of microbial clearance from the respiratory system has been attributed to the activity of macrophages, and varies from organism to organism.12 The effects of high oxygen concentrations on pulmonary clearance mechanisms have been reported.5 The possibility that the observed changes are a premortem effect seen in critically ill animals must be considered also. However, the latter is unlikely because the experimental animals from which cultures were obtained were still quite active at the time of sacrifice, i.e., they were not moribund. Their weight losses had not reached the extreme proportions shown by other survivors of long-term administration of 100 per cent oxygen.4
which lost as much as 50 per cent of their starting weights before succumbing to chronic oxygen toxicity. Since in-citro studies have shown sensitivity of microbial cultures to high oxygen tensions, it appears more likely that the changes observed in this study were the result of a direct action of oxygen on the microorganisms. For example, the growth of *Staphylococcus aureus* and that of *Achromobacter P6* were inhibited and the uptake of leucine by *Pseudomonas saccharophila* was decreased by high concentrations of oxygen.

The inhibition of growth of *Pseudomonas in vitro* by oxygen was reversed by amino-acid supplements, which led another investigator to speculate that high oxygen tension interferes with the transport of nutrient and thus inhibits protein synthesis and growth. Since the strains of microorganisms isolated in the present study were different from those described by the above-mentioned investigators, it is not certain whether high oxygen concentrations would have had the same effect on them in vivo.

While this study showed that long-term inhalation of 100 per cent oxygen changed the laryngotracheal flora, it also showed that these changes will not necessarily lead to infection of the lungs or upper respiratory tract. Although the rats were sick and their lungs showed the typical morphologic changes of chronic oxygen toxicity, no histologic evidence of bacterial infection was observed either in the lungs or in the upper respiratory tract. Rats are known to be rather resistant to bacterial infection. This may account for the failure of the organisms comprising the normal flora to lead to infection. However, in clinical practice susceptibility to infection by normal flora may be considerably greater. Therefore, alterations of bacterial flora induced in the upper respiratory tract of animals by high concentrations of oxygen have potential clinical significance, since the widespread use of oxygen therapy in hospitals may result in similar changes in man.

**References**