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Mortality and Histopathology of Germ-Free Rats and Mice Exposed To 100% Oxygen.* (31158)

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Many workers have noted similarities in the histology of lungs from animals exposed to hyperoxia and from those suffering from various pulmonary diseases(1-5). Rats and mice are known to be chronically afflicted with murine pneumonia and related diseases which can cause thickening of the alveolar wall, atelectasis, edema, lymphoid invasion, etc., much the same as in oxygen toxicity(6). In fact, the oxygen toxicity syndrome has been referred to as oxygen pneumonitis(7). The extent to which such chronic infections contribute to animal mortality in oxygen toxicity or at least to its pulmonary histopathological aspects have never been fully determined, although attempts have been made to reduce the problem by use of Caesarian derived animals(8) and to define the nature of the infection by culture of the lungs of afflicted animals(1-5,9).

Although one might expect pulmonary disease to heighten susceptibility to O_2 toxicity, our general impression from work with rats is that those with chronic respiratory conditions tended to live longer. Our preliminary attempts to culture lungs of rats which died of oxygen poisoning resulted in finding certain entero-bacteria which might be called opportunists that migrate to areas of least resistance in the body. Others, however, have found no bacteria in the lungs of guinea pigs and rats exposed to oxygen for varying periods of time(1-5,9). It seemed to us that an alternate and perhaps more direct method of delineating the role of chronic infection in oxygen toxicity would be to study the response of germ-free animals to hyperoxia.

Materials and methods. Two germ-free experiments have been accomplished involving a total of 8 rats and 14 mice breathing 99 ± 1% oxygen at one atmosphere (OAP). Germ-free Sprague-Dawley rats, about 200 g in weight, and germ-free adult white mice were obtained from the germ-free colonies bred and maintained by Laboratory Animal Facilities of Ohio State University. For exposure to OAP, they were transferred directly *via* locks into a peracetic-acid-sterilized poly-

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TABLE I. Mortality in Germ-Free Rodents Exposed to 100% Oxygen at One Atmosphere.

	No.	Time of death (hr) Avg	SE	C*	Range
Mice					
Germ-free	14	97.7	2.6	10.0	91-115
Conventional	44	146.2	7.8	35.6	96-384
Rats					
Germ-free	8	81.4	3.7	12.8	73- 91
Conventional	23†	88.4	3.9	21.2	70-120†

* Coefficient of variation.
† 3 rats still alive at 120 hr.

vinyl isolator similar to those described by Trexler(11).

A continuous, adjustable flow of 100% oxygen was passed from a pressurized tank through spun glass sterile filter media into the germ-free isolator in place of the usual supply of room air from a compressor. By adjusting O_2 flow, nitrogen, monitored on a Med-Science Nitrolyzer, was kept below 2% and CO_2, analyzed by a Kitagawa gas detector, below 0.5%. Gas temperature in the isolator ranged between 70-74°F and relative humidity 60-70%. Controls of similar, but conventionally raised animals were exposed to essentially identical O_2, temp and relative humidity conditions in an isolator which was not germ-free and in which the gas recycled(10). Time of death was recorded and lungs were saved for histological examination.

Results and discussion. As shown in Table I, germ-free mice died on the average a significant 48 hours (33%) sooner and within a much narrower time range than conventional mice. The 3-4-fold larger coefficient of variation for the control mice clearly reflects the greater dispersion of their mortality data. A similar pattern of shorter average survival time and narrower time spread of mortality is observed in the results for germ-free compared to conventional rats, but the differences are of smaller magnitude because some of the control runs were terminated before all animals were dead. Within each species first deaths occurred at about the same time for both germ-free and conventional animals, and the wide range in mortality in the conventional groups was due to much prolonged survival in a few animals.

Lungs removed from the germ-free animals were more uniform in gross appearance than control lungs. Histologically, however, they showed somewhat the same picture of atelectasis, perivascular edema and inflammatory cell infiltration in the alveoli as did the lungs from conventional animals. Since it is assumed that the germ-free animals had no pulmonary disease, it follows that the inflammatory cell infiltration seen in O_2 toxicity is independent of infectious processes. The alveolar wall from lungs of germ-free animals also appeared thinner than those with chronic infection. This would agree with the work of Smith *et al*(4) who suggested that the thicker alveolar walls observed in young rats may have played a role in their increased resistance to oxygen toxicity.

The overall picture presented by these tests is one of greater and more uniform susceptibility to O_2 toxicity in germ-free rodents. Implied here is that the chronic pulmonary infection present in the conventional rats and mice leads to changes in the lung structure which confer some increased resistance to high concentrations of oxygen. Because of their more uniform response, germ-free animals may serve as a useful test system in studying the mechanism of O_2 toxicity.

Summary. Germ-free rats and mice tended to die sooner and within a narrower time span than conventional animals on exposure to 100% O_2, suggesting that chronic respiratory conditions may increase resistance to O_2 toxicity. Lung pathology of germ-free animals dying in O_2 appeared much like that of chronic respiratory infection, indicating that inflammatory cell infiltration of the alveolar wall can occur in animals presumed free of disease.

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Physiological Thymidine Reutilization in Rat Bone Marrow.* (31159)

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The method of studying cell turnover in whole organs or tissues with the aid of a label for DNA requires a tracer which is metabolically available for a short time only and which is not reutilized following catabolism of DNA. This requirement must be met also when cell proliferation is examined over periods of several cycles.

Labeled thymidine, because of its specificity as DNA precursor and because of its rapid incorporation by cells, is widely used for turnover studies(1). However, the advantages of labeled thymidine as tracer may be offset by the fact that it is at least partially reused when DNA is catabolized(2-9). Since DNA as a carrier of genetic information in chromosomes is retained throughout the life of the cell under normal conditions (exclusive of possible repair processes), the reutilization of labeled thymidine can only begin after death of those cells that had incorporated the tracer during DNA synthesis.

A DNA catabolite, such as thymidine, its nucleotides, or even nucleotide chains, may be reutilized by direct intercellular exchange, or transported *via* the blood or lymph circulating between cells in different organs either in a free form or bound to cells acting as carriers. For example in the bone marrow, direct intercellular exchange of DNA catabolites may occur, since the most mature nucleated precursors of red cells extrude their nuclei in the process of becoming transformed into reticulocytes. These nuclei are at least partially phagocytized in the marrow(10), where catabolites of DNA consequently may become available for reutilization without mediation of the circulation. Whatever the mechanism of reutilization may be, the passage of DNA catabolites from one cell to another requires a more quantitative evaluation.

While the phenomenon of reutilization of thymidine—or its nucleotides—has been established in principle, the magnitude of this reutilization pathway and the type of material reutilized has remained largely uncertain mainly because of the lack of a proper tracer as reference for measuring true DNA renewal with exclusion of reutilization. This problem may now find a solution by using as a reference tracer the thymidine analogue labeled 5-iodo-2′-deoxyuridine (IDU), in parallel with labeled thymidine. IDU labeled with I^{131} has been used(11,12) to specifically label DNA in cells, and it was recognized that data on DNA renewal obtained with I^{131}-DU were not in agreement with results obtained with tritiated thymidine, the discrepancy being due to poor reutilization of the former in relation to the latter(2,7,9,13). The first results using IDU as reference tracer in bone marrow cells and interpreted in terms of thymidine reutilization were published previously(7,14). They are now extended and further discussed. It appears that, in rat bone marrow, 35% of the liberated thymidine, or its nucleotides, is physiologically re-

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