This study was undertaken to examine the effects of oxygen exposure on vitamin E deficiency in mice. After 20 hr of exposure to pure oxygen, 5 of 7 mice in the vitamin E deficient group died, but only 1 of 7 in the vitamin E supplemented group died. Pulmonary atelectasis and hemorrhages were the common features in the mice that died. Of the surviving mice, those in the vitamin E deficient group had partial hemorrhages, but those in the control group had no significant pathological changes. A reduction in phospholipid and sulfhydryl groups was observed in the supernatant fractions of the lungs of vitamin E deficient mice. After oxygen exposure further reductions occurred in both groups of mice, but more predominantly in the vitamin E deficient group. In the sediment fraction of the lungs, similar changes were found in the phospholipids in the vitamin E deficient group before and after exposure to oxygen. The sulfhydryl groups in the sediment fractions were decreased significantly in the vitamin E deficient group after oxygen exposure, but they remained unchanged in the control group.

With regard to the care of newborn infants, it is well known that retrolental fibroplasia results from the use of therapeutic oxygen in high concentrations and for a long time, especially in premature infants (1). On the other hand, it is also known that breathing oxygen of high partial pressures causes lung abnormalities, which are ascribed to a toxic effect of oxygen on the lung tissues (2-10). Edema, congestion in the pulmonary interstitium, fibrin membranes formed along the alveolar lining, and hemorrhages in the alveolar space are pulmonary deteriorations resulting from oxygen toxicity. Recently, SHANKLIN and WOLFSON (11) in a retrospective analysis of autopsy materials, suggested the possibility of a direct connection between the use of therapeutic oxygen at any level and pulmonary hemorrhage in newborn infants, especially in premature infants, and that a nearly twofold increase in pulmonary hyaline membrane diseases was also observed in infants receiving oxygen therapy continuously during life.

These changes can be attributed to vitamin E deficiency in the neonatal period,
because vitamin E normally acts as an antioxidant, which effectively protects the biological peroxidation (12–15). With regard to biological peroxidation, most of the literature focuses on aging pathology (16) and lung damage caused by air pollution (17–21). However, it is also important that oxygen may attack the lungs of vitamin E depleted infants in peroxidative chain processes during oxygen therapy. This report presents a study made on the relationship between oxygen poisoning and vitamin E deficiency using mice as test animals.

METHODS

Production of vitamin E deficiency in mice. DDK strain male mice were obtained as weanlings and fed the vitamin E deficient diet shown in Table 1. The animals were kept on this diet for 7 weeks before use. Control mice were fed the same diet supplemented with 1 mg dl-α-tocopheryl acetate per gram of diet. An oxidative hemolytic test with dialuric acid was performed at the end of 7 weeks by a method devised by Rose and Gyorgy (25).

| Ingredient                   | Weight % |
|------------------------------|----------|
| Cornstarch                   | 60       |
| Refined lard                 | 10       |
| Casein (excluding vitamins)  | 18       |
| Dried yeast                  | 8        |
| Salt (mix)\(\textsuperscript{a}\) | 4        |
| Vitamin A                    | 1 I.U. (in 1 g of diet) |

\(\textsuperscript{a}\) NaCl, 1.75; MgSO\(_4\)·Aq, 5.45; NaH\(_2\)PO\(_4\)·Aq, 3.47; K\(_2\)HPO\(_4\), 9.54; Ca(H\(_2\)PO\(_4\))\(_2\)·Aq, 5.40; Citric acid Fe, 1.18; Lactic acid Ca, 13.00.

Animal exposures. The two groups of animals, control and vitamin E deficient, were housed in a single gas chamber, which maintained a 100% oxygen atmosphere. Exposure to oxygen for chemical analyses was 18 hr and that for histological studies was 20 hr.

After anesthesia with ketamine chloride, the animals were sacrificed, and their lungs were removed en bloc from the chest. The lungs were kept in 10% formalin for light microscopy or stored at -50°C for chemical analysis. The lungs were divided into two fractions as follows. The lungs have a specific lining material, the alveolar surfactant, containing phospholipids, carbohydrates, and proteins. To separate the lung tissue components from the surfactant materials, the lungs were homogenized and centrifuged at 3,000 rpm. The supernatant fractions consisted mainly of the pulmonary surfactant, and the remaining sediment fractions contained the other tissue components, including all kinds of biological membranes.
**Chemical analysis**

a) Total phospholipids, the fractions of the lung were extracted in 20 volumes of chloroform: methanol (2:1). The extracts were filtered through a glass filter G-3 and evaporated to dryness under nitrogen in vacuo. The evaporated residues were reextracted with chloroform: methanol (9:1). This filtration and evaporation process was repeated twice. The last evaporated residues were weighed out as the total lipids. Phospholipids were determined by a modification of the method of Bartlett (22).

b) Total sulphydryl groups, the total sulphydryl groups were determined using Ellman's method modified by Sedlack and Lindsay (23).

(c) Protein, protein concentration was measured using the method of Lowry et al. (24).

**Histological examination.** The block of the lungs kept in formalin solution was dehydrated in increasing concentrations of alcohol and embedded in paraffin by the usual procedure. The histological slices were stained with hematoxylin-eosin and prepared for light microscopy.

**RESULTS**

After the animals had been fed the vitamin E deficient diet for 7 weeks, their erythrocytes were greatly vulnerable to dialuric acid. No susceptibility was observed in erythrocytes of the vitamin E supplemented animals, as shown in

| Group                              | No. | % Hemolysis with dialuric acid |
|------------------------------------|-----|-------------------------------|
| Control                            | 1   | 7.2                           |
| (supplemented with vitamin E)      | 2   | 2.5                           |
|                                   | 3   | 0                             |
|                                   | 4   | 2.0                           |
|                                   | 5   | 5.0                           |
| Vitamin E deficient                | 1   | 98.6                          |
|                                   | 2   | 100.0                         |
|                                   | 3   | 100.0                         |
|                                   | 4   | 90.4                          |
|                                   | 5   | 89.9                          |

| Groups                              | No. | Died | Pulmonary bleeding |
|-------------------------------------|-----|------|--------------------|
| Control                             | 7   | 1 (14.3%) | 1 (14.3%)         |
| Vitamin E deficiency                | 7   | 5 (71.5%) | 7 (100%)          |
Fig. 1. Atelectasis, edema, and hemorrhage in the lungs of mice that died after exposure to 100% oxygen for 20 hr: (A) Vitamin E deficient; (B) control (Hematoxylin and eosin stain, ×200).
After 20 hr of exposure to 100% oxygen, 5 of 7 vitamin E depleted mice died, but only 1 of 7 vitamin E supplemented animals died (Table 3). Pulmonary atelectasis and hemorrhages were visually evident in the lungs of the dead mice of both groups (Fig. 1). Both of the surviving vitamin E deficient mice had par-
tial hemorrhages, possibly initiated in the pulmonary interstitium, as shown in Fig. 2. No remarkable histological changes were found in the 6 surviving control group mice.

After 18 hr of exposure to oxygen for chemical analysis, no animals in either group died. However, vitamin E depletion and additional oxygen exposure produced significant changes in the phospholipid contents of the lungs. In the supernatant fractions of the lungs, vitamin E deficiency produced a decrease in the total phospholipids. With exposure to pure oxygen for 18 hr, much greater decreases of the phospholipids were observed in the control than in the vitamin E deficient mice, as shown in Fig. 3. These changes in the phospholipids corresponded to those in the total lipids in the supernatant fractions of the lungs. In the sediment fractions, containing the lung tissue components including all kinds of membranes, similar changes were observed in the total phospholipids based on wet weight of the tissues. The decrease in the total phospholipids, however, might be more specific in the sediment than in the supernatant fractions, because it did not correspond to changes in the total lipids, as shown in Fig. 4.

The sulfhydryl group contents in the lung tissues were also affected by vitamin E depletion as well as by additional oxygen exposure. The level of the sulfhydryl groups was determined on the basis of tissue protein. In the vitamin E deficient, unexposed group, a decrease in the sulfhydryl groups was observed in the supernatant fractions, but no remarkable changes occurred in the sediment ones. After exposure to 100% oxygen for 18 hr, the sulfhydryl groups in the supernatant fractions were markedly decreased in the vitamin E supplemented and depleted groups. The rate of sulfhydryl decrease was slightly greater in the latter group, as expected. With regard to the sediment fractions, there were no changes in the vitamin E

![Fig. 3. Effect of oxygen exposure on total phospholipids in the supernatant fractions of lungs. Each column represents the average and standard error of 6 mice.](image)
supplemented group after exposure, but a significant decrease of the sulfhydryl contents occurred in the depleted group exposed to oxygen (Fig. 5).

In summary, the phospholipid and sulfhydryl groups, which are required for the maintenance of membrane integrity, were decreased significantly in the lungs of vitamin E deficient mice after exposure to oxygen.

Fig. 5. Effect of oxygen exposure on sulfhydryl groups in the lungs. Each column represents the average and standard error of 6 mice.
DISCUSSION

There are a number of recent reviews compiling investigations concerning oxygen poisoning over the past twenty years (26–28). Studies involving plants and animals indicate that all living organisms can be harmed by high pressures of oxygen. From the pulmonary lesions resulting from intense oxygen exposure in several species of animals and from autopsy materials in man, the general features of the lung injury seem to be similar in men, dogs, rats, and monkeys (8, 10, 29). The exposure to 100% oxygen at one atmosphere induces predominant lesions including the capillary endothelium and the alveolar type I cells. As these cells disrupt, the pulmonary interstitium becomes edematous and the fibrin membrane may be formed along the alveolar lining. This is followed by a diffuse parenchymal lung lesion associated with congestion, edema, bleeding, and fibrosis (9, 30, 31). Our histological findings of hemorrhage and atelectasis in the lungs after oxygen exposure is fundamentally in agreement with the views cited in these reviews. In additional, the evidence of higher mortality in the vitamin E depleted mice after exposure may be important for the following reasons:

The mechanism involved in cellular injury of the lung by oxygen is still not completely understood. It is clear, however, that oxygen accelerates the rate of oxidation of oxidizable substances, in which lipids containing polyunsaturated fatty acids as well as proteins including the sulfhydryl groups may be involved. Furthermore, there is also evidence that oxygen in high concentrations promotes the formation of free radicals by reacting with unsaturated lipids; the free radicals once formed would occasionally cause random, uncontrolled reactions typical of antioxidative chains (16). During exposure to a high concentration of oxygen, the radical chain reactions would start and could produce serious disturbances in cell chemistry. Numerous enzymes and coenzymes are inactivated by oxygen, especially those in which the sulfhydryl groups are essential (27). In additional, all the various membranes of the cells are critical sites for oxidative damage, since the membranes contain a high proportion of unsaturated lipids associated with proteins including sulfhydryl groups. The above provides the reasons for pulmonary damage resulting in edema, congestion, and/or hemorrhage after exposure to high concentrations of oxygen.

On the other hand, $\alpha$-tocopherol(vitamin E) is known to prevent lipid peroxidation by its antioxidant action, from the findings in rancidity chemistry (12–15). The study by Tappel and Horwitt strongly supported such an antioxidant action of vitamin E in vivo, which may also play a role in the prevention of excessive peroxidation in biological tissues. In many studies, a direct connection has been demonstrated between vitamin E deficiency and the extent of lipid peroxidation. For example, the progress of vitamin E deficiency is characterized by increased erythrocyte hydrogen peroxide hemolysis, which is probably due to oxidation of red cell membrane components (32–34). Hydrogen peroxide is a strong oxidant.
Similar hemolysis has been observed after exposure of red cells to oxygen flow, as reported previously (35).

If the lungs of vitamin E deficient animals are exposed to pure oxygen, damage similar to that observed in the red cell membrane might occur on the lung membranes, and the damage would be increased with vitamin E deficiency. This theory is supported by our finding that a remarkable decrease in phospholipids and sulfhydryls was evident in the lungs of vitamin E depleted mice after oxygen exposure. Furthermore, the decrease in these components in the sediment fractions of the lungs may reflect damage in the membranes of the lung tissues. This is in agreement with the histological suggestion that an interstitial hemorrhage was initiated in the alveolar hemorrhages.

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