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Maturation of the fetal lung II. Effect of hyperoxia on phosphatidic acid phosphatase, pyruvate kinase and superoxide dismutase activity in the newborn rat lung

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Oxygen-induced lung toxicity was studied in newborn rats. Three-day-old rats were exposed to 85—90% oxygen or air for 48 h. The activity of pulmonary antioxidant defenses, as measured by the activity of superoxide dismutase (SOD), showed a progressive increase in activity. The activity of phosphatidic acid phosphatase (PAPase) showed a statistically significant decrease in the oxygen-poisoned lung. In the activity of pulmonary pyruvate kinase no significant change occurred. After two days of oxygen exposure (FiO_2 085—0.9) marked histological changes appeared including an increased number of interstitial cells, atelectasis, pulmonary oedema, diminution of lamellar content, loose lamellar bodies and increasing tubular myelin in the alveolar space.

In a previous study of the lungs of fetal and newborn rats [12] a change has been noted in the activity of PAPase, an enzyme which has an important role in the synthesis of surfactant in the fetal lung. The present paper reports on a study of the influence of postnatal hyperoxia on PAPase and PK activities together with the activity of SOD and the picture of the oxygen-poisoned lung.

MATERIAL AND METHODS

Three-day-old Wistar R/A rats (Laboratory Animal Breeding Centre, Gödöllő) were used in the experiments. They received standard rat chow and tap water ad libitum. The rats were exposed to hyperoxia (FiO_1 0.85—09) for 48 h in a modified infant incubator at 23—25 °C with the chamber atmosphere continuously circulated and filtered. The oxygen concentration was controlled every two hours. Control animals were maintained in room air under similar conditions. Neonatal rat pups from four litters of the same age were randomly assigned to either a group exposed to hyperoxia or a control group exposed to air. A foster mother was then provided for each mixed litter. The littermates of the newborn rats exposed either to oxygen or air were anaesthetized with ether and exsanguinated. The lungs were excised and washed well in cold physiological saline to remove as much intravascular blood as possible. Three to four rat lungs were homogenized with 1:10 isotonic NaCl in tissue grinder homogenizer (Thomas, USA) for 20 s and sonicated for 30 s (Sonic 1510, Braun, Melsungen GFR). The values reported are the mean ±S. D. of six determinations.

The activity of phosphatidic acid phosphohydrolase or phosphatidate phospha-

TABLE I

Effect of hyperoxia on PAPase, PK and SOD activity in the lungs of 5-day-old rats. Three-day-old animals were exposed to hyperoxia for 48 h (FiO_2 0.85—0.9) (mean ±S.D.)

	PAPase		PK	SOD
	nmol/g tissue/min	nmol/mg protein/min	mU/mg protein	U/g tissue
Control n=6	358.2±33.6	14.38 ±0.68	302.73±38.03	202.58 ±11.78
Hyperoxia n=6	263* ±19.87	11.51*±1.14	286.52±19.83	287.96*±13.96

* $p < 0.001$

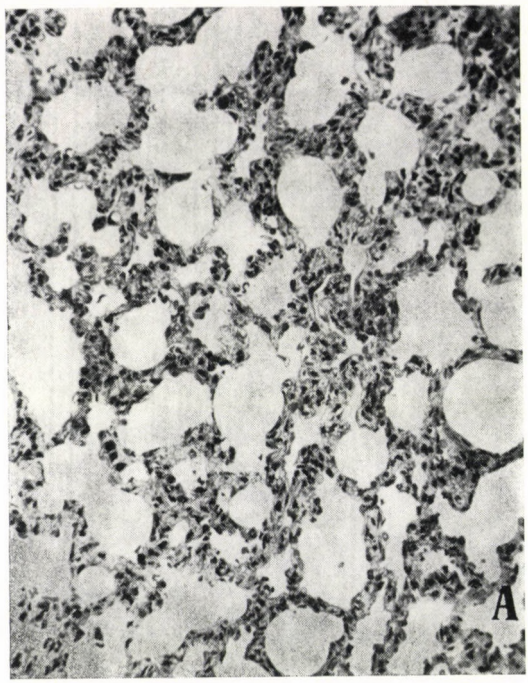
tase, EC 3.1.3.4. (PAPase) in the lung homogenate was estimated by the method of Coleman and Hübscher [3] modified by us [13]. Results were expressed as nmol/g/tissue/s or nmol/mg protein/s. The activity of superoxide dismutase EC 1.15.11 (SOD) and pyruvate phosphotransferase, EC 2.7.1.40 (PKATP) was assayed after centrifugation at 12 000 g in the supernatant of the whole lung homogenate at 4°C for 30 min. The activity of PK was determined by the method of Guttman and Bernt [8] and the results were expressed as mU/mg of protein determined as described by Lowry et al [14]. SOD activity was measured by the adrenochrome method of Matkovics [15]. The essence of this method is that adrenaline is spontaneously transformed to adrenochrome in the presence of air at pH 10.2. This spontaneous oxidation of adrenaline is inhibited to an extent depending on the amount of SOD. One unit of superoxide dismutase activity was defined as the amount of enzyme which caused a 50% inhibition of adrenaline oxidation. Measurements were made at 25°C and 480 nm in a Specord M 40 spectrophotometer, results were expressed as U/g tissue. Statistical evaluation of the data was performed by Student's *t* test.

For light microscopy of the lungs the thoracic cavity was opened, the trachea cannulated and 10% formalin was instilled at a perfusion pressure of 25—30 cm water. Specimens obtained from various areas were embedded in paraffin and stained with haematoxylin and eosin. For electron microscopic studies specimens of the lungs were excised and fixed in ice-cold 3% glutaraldehyde in Millonig buffer pH 7.4, postfixed in 1% phosphate buffered osmium tetroxide and embedded in Durcupan (Fluka). Ultrathin sections were cut by Reichert OmU3 ultramicrotome and stained with uranyl acetate and lead citrate before examination with a Tesla BS 500 electron microscope.

RESULTS

The oxygen-induced biochemical changes are shown in Table I.

Exposure to 85—90% oxygen for 48 h resulted in a statistically significant decrease in pulmonary PAPase activity in the lung of three-day-old rats (263 ±19.87 nmol/g tissue/min or 11.51 ±1.13 nmol/mg protein/min). In PK activity no significant change occurred following exposure to hyperoxia. Forty-eight hours after the start of oxygen exposure, SOD activity in the neonatal rat lung increased to 287.96 ±13.95 U/g tissue, and in



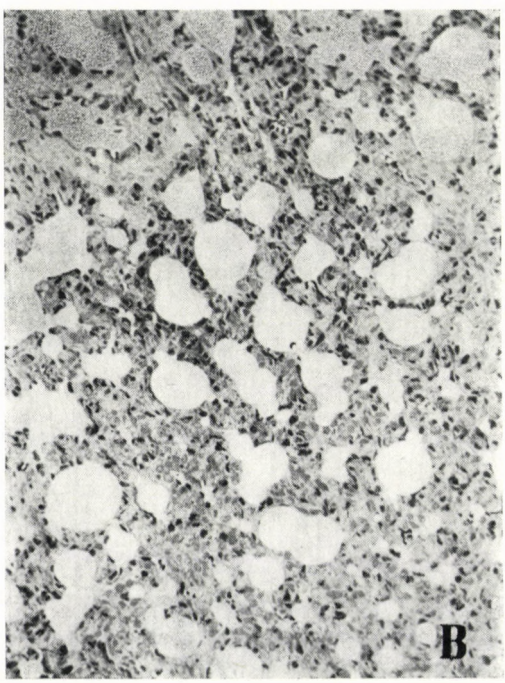


Fig. 1. Light micrograph of air and oxygen-exposed rat lungs. Three-day-old rats were exposed to either air or 85—90% oxygen for 48 h. A: exposed to air (×250), B: exposed to oxygen (×250)

the controls to 202.58 ± 11.77 U/g tissue. The increase was significant statistically ($p < 0.001$).

To assess the extent of oxygen-induced lung injury and its effect on pulmonary maturation, lungs from

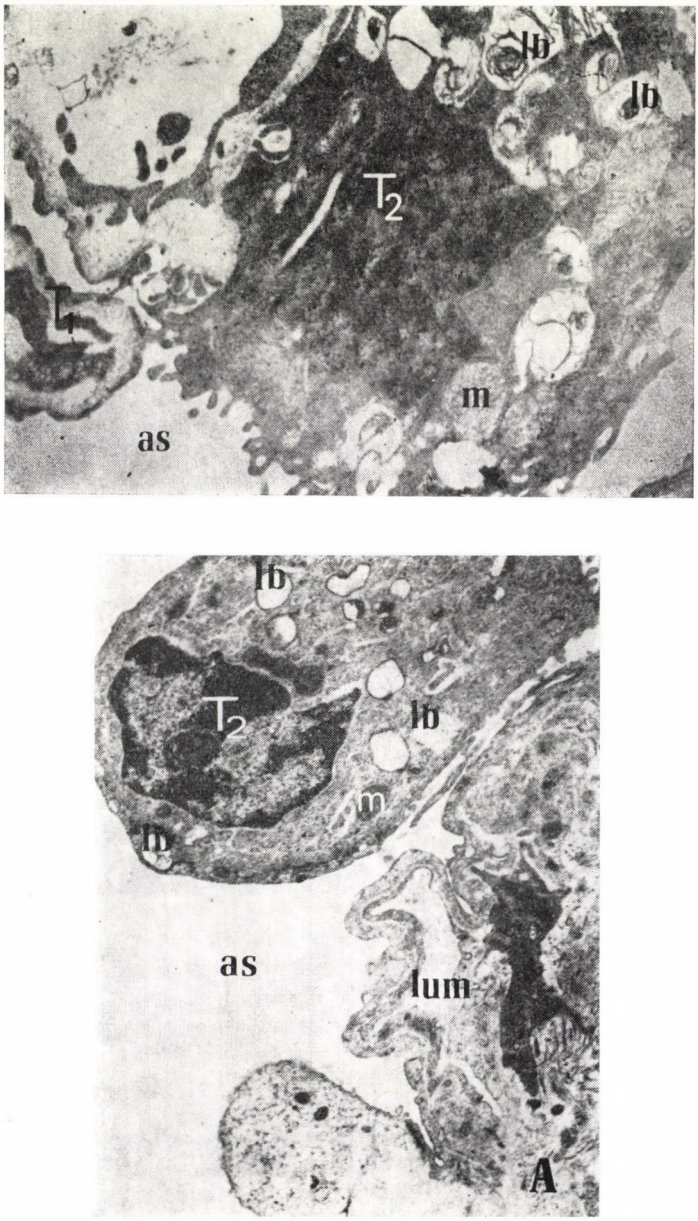
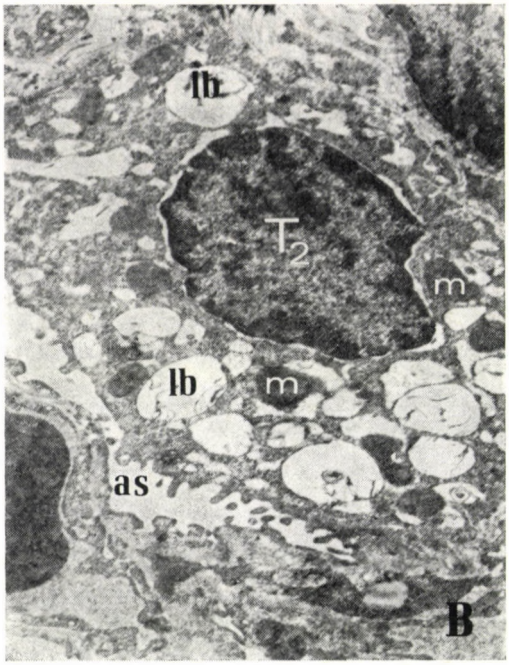


FIG. 2. Electron micrograph of lungs of control air-exposed 5 day-old rats ×12 000. T_1: type I epithelial cell, T_2: type II epithelial cell, lum: capillary lumen, as: alveolar space, l b: lamellar body, m: mitochondrium



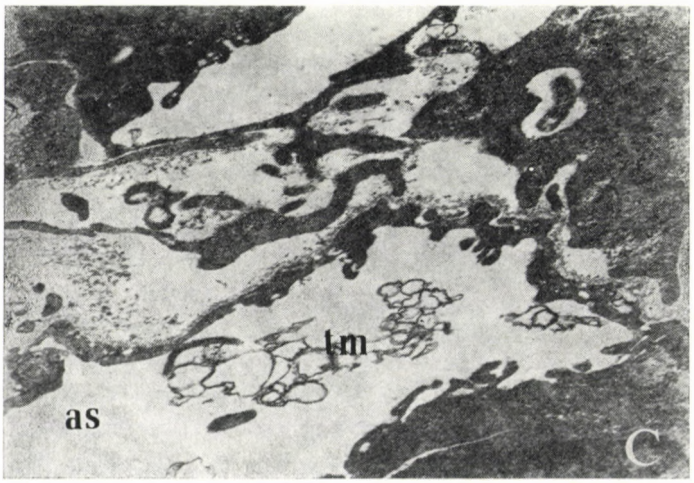


FIG. 3. Electron micrographs of lungs of five-day-old rats which had been exposed to 85—90% oxygen for 48 h. At the end of exposure, the animals were sacrificed and their lungs were fixed and stained as described in Materials and Methods. A: In type II epithelial cell the lamellar contents are diminished ×8000. B: loose lamellar bodies in type II epithelial cell. ×10 000. C: Tubular myelin (tm) from type II cell, 14 000

the various groups were examined by light and electron microscopy. Exposure to FiO_2 0.85—0.9 for 48 h produced marked changes: increased numbers of interstitial cells that led to an augmentation of tissue area (Fig. 1). Electron microscopy revealed focal areas of interstitial and perivascular oedema, type II cells appeared to have loose lamellar bodies, the lamellar contents diminished, tubular myelin released from type II cells appeared in the alveolar space (Figs 2, 3).

Discussion

Exposure to elevated oxygen concentrations is a commonly used therapy of many conditions including the disturbances of adaptation, hypoxia and the respiratory distress syndrome of the newborn. There are several potential mechanisms which may play a role in the oxygen effect. The toxic influence of oxygen on the lung varies depending upon the concentration and duration of oxygen exposure. In addition, individual vulnerability by oxygen may vary depending upon the stage of maturation of the lung at the time of oxygen exposure [10, 15].

The lung toxicity of oxygen is complex; it may appear in acute or chronic conditions and vary according to species [20, 23]. It is widely believed that the damage caused in the lungs by oxygen is due essentially to the initiation of lipid peroxidation which by generating highly reactive free radicals destroys the cell membranes and impairs biochemical functions [1, 6]. These free radical formations do not, however, proceed uncontrolled as there are certain mechanisms protecting against oxygen toxicity. One mechanism involves the enzyme SOD which scavenges superoxide (O_2^-) and plays an important role in the detoxication of oxygen [11, 17, 23].

Several investigators have found that neonatal rats have an ability to increase specifically and rapidly the activity of pulmonary SOD [9, 20]. According to our data the significant increase of SCD activity points to hyperoxia in the lung cells, and on this basis we could determine the activities of two additional lung enzymes, PAPase and PK.

The phospholipids appear in the last stage of gestation in the lung tissue and amount until delivery. These changes coincide with a marked increase in the concentration of desaturated PC, depending on different enzymes. Among these, PAPase has a significant role. The activity in normal newborn animals has been studied by several authors [12, 16, 19, 21], but we have not found any data concerning PAPase activity in the oxygen-poisoned lung. Gilder et al [7] reported that the incorporation into PC of labelled choline and palmitate was inhibited in the lung slices prepared from rabbits exposed to 100% oxygen at 3 atm for 3 h. The enzymes which transfer fatty acids to lyso-PC were also depressed in the microsomes of the oxygenated lung. In the oxygen-poisoned lung

the surfactant activity of the alveolar lining is generally decreased. This may be due to a direct oxidation or inactivation of the pulmonary surfactant, or to alveolar destruction and diminished synthesis of surfactant lipids [1].

Our experiments showed that exposure to 85—90% oxygen for 48 h resulted in a 27% decrease in PAPase, activity in the lung of newborn rats and the decrease of surfactant synthesis might be ascribed to the diminished PAPase activity.

We also studied the activity of PK which has an outstanding role in glycolysis. In our experiments, there was no significant change in the activity of pulmonary PK following exposure to hyperoxia. Simon et al. [20] observed that exposure of cell monolayers to 95% O_2 for 96 h did not cause any specific alteration in energy metabolism. Thus, neither the key mitochondrial enzyme cytochrome oxidase nor the two rate-limiting glycolytic enzymes pyruvate kinase and phosphofructokinase had been affected.

Morphological studies have shown that atelectasis and oedema were typical features of the oxygen-poisoned lung [2, 18, 23]. Hyperoxia brought about a slowing of lung maturation and a reduction in the number and surface area of the alveoli. It also had a direct toxic action on the cells of the lung resulting in destruction of the capillary endothelium and alveolar epithelium [23].

The amount of total phospholipid in the endobronchial washing fluid was comparatively low before lung oedema had developed but it increased sharply after its appearance during oxygen poisoning. The causes of the increase in alveolar phospholipids may be that the oedema fluid seeping across the alveolar wall would wash off the lining layer, the types I and II alveolar cells may be damaged by oxygen [4, 5, 22] and there may be leakage of plasma through the damaged alveolar capillary membranes. In spite of the increase in alveolar phospholipids, surfactant activity seems to be decreased in the oxygen-poisoned lung, due perhaps to increased amounts of surface active inhibitors such as plasma in the alveolar space [1]. This would then manifest itself after only two days of oxygen exposure with histological changes such as an increase in the number of interstitial cells, atelectasis, pulmonary oedema, diminution of lamellar contents, loose lamellar bodies and an increase of tubular myelin in the alveoli.

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