PULMONARY GRANULAR PNEUMOCYTES

Loss of Mitochondrial Granules during Hyperoxia

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INTRODUCTION

Hyperoxia is known to injure tissues and cells (1). In a series of papers the quantitative influence of hyperoxia on some aspects of lung ultrastructure was elucidated (2–4). These studies revealed that hyperoxia resulted in rapid destruction of the pulmonary capillaries, a thickening of the air-blood barrier, and, eventually, a repopulation of the alveolar epithelium by granular pneumocytes at the expense of the type I alveolar cell.

While studying the interrelation between protein synthesis by the lung and the ultrastructure of the pulmonary granular pneumocyte under hyperoxic conditions (5, 6), we noted that the mitochondria of granular pneumocytes from O_2-exposed rats seemed to contain fewer granules than those from rats exposed to compressed air. This led to the present study in which we used stereological techniques to examine the influence of in vivo hyperoxia on the dense granule content of mitochondria of rat lung granular pneumocytes.

MATERIALS AND METHODS

Animals

Dublin Sprague-Dawley derived male rats (Flow Research Animals, Inc., Dublin, Va.) were exposed to either compressed air or greater than 98% oxygen at 1 atm for 48, 72, or 96 h (5).

Preparation of Tissue

Rats were anesthetized with sodium pentobarbital, 30 mg/kg intraperitoneally, and sacrificed by exsanguination. Thin slices were cut from the lower lobe of the right lung. These were diced into cubes of about 1 mm^3, fixed for 1 h in cold cacodylate-buffered 1% osmium tetroxide, and then dehydrated and embedded (7). The blocks were sectioned on an ultramicrotome with a diamond knife, and sections with a silver interference color were picked up on 200-mesh copper grids. They were stained with uranyl magnesium acetate and lead citrate (8) and examined with an AEI-6B electron microscope (AEI Scientific Apparatus Inc., Elmsford, N. Y.).

Sampling Procedures

We made 40 tissue cubes from the right lower lobe of the lung of each rat. Five of these blocks were sectioned at 1 μm for light microscopy and at 600–900 Å for electron microscopy. For rats examined after 48- or 72-h exposures, two electron micrographs of granular pneumocytes were taken from each of these five blocks. Thus, from each rat we used five blocks and made 10 micrographs. We examined 4 micrographs from each block of each animal exposed for 96 h; therefore, in this group 20 micrographs were examined from each rat. The micrographs of granular pneumocytes were taken randomly. Granular pneumocytes were identified as alveolar wall cells having lamellar inclusion bodies and microvilli (9). Mitochondrial granules were identified as dense intramitochondrial bodies (Fig. 1). In our control rats (72-h exposure to compressed air) these had a diameter of 360 ± 8.5 Å (mean ± SEM, 100 dense granules measured).

Stereologic Procedure

Measurements were made at a magnification of × 25,000 on 8 × 10-inch enlarged prints of the micrographs. A square grid of white lines was superimposed on each print by lengths of 7-mil wire placed on a frame at 1-inch intervals. Calibration checks were made on the electron microscope weekly and on the enlarging equipment before each use. The micrographs from all rats were coded, placed in random order, and measurements made in that order without knowledge of which group of animals the photographs represented. Lineal analysis was used to determine the mitochondrial area (10, 11). The number of dense granules counted in the mitochondria were related to the area of mitochondrial profiles.

RESULTS

After 48 h of hyperoxia there is a marked decrease in the number of mitochondrial granules in the rats exposed to hyperoxia. After 72 h of hyperoxia we were unable to detect mitochondrial granules in the O_2-exposed rats (Table I and Fig. 2). In this part of the study the rats exposed to hyperoxia had an initial weight of 283 ± 36 g (mean ±

TABLE I

Influence of Hyperoxia on Mitochondrial Granules

Exposure	Atmosphere	Mitochondrial granules per μm^2 mitochondrial profile	Mitochondrial granules per mitochondrion
h			
48	Air (5)	1.5 ± 0.3	1.5 ± 0.3
	O_2 (5)	0.1 ± 0.0	0.1 ± 0.0
		$P < 0.001$	$P < 0.05$
72	Air (3)	2.1 ± 0.1	2.7 ± 0.7
	O_2 (3)	0	0
		$P < 0.005$	$P < 0.05$
96	Air (5)	2.1 ± 0.3	1.4 ± 0.1
	O_2 (5)	0.1 ± 0.1	0.1 ± 0.1
		$P < 0.001$	$P < 0.05$

Values in parentheses indicate numbers of rats. Mean ± SEM are given.

SD) for 48-h O_2, 283 ± 36 g for 48-h compressed air, 243 ± 12 g for 72-h O_2, and 250 ± 15 g for 72-h compressed air-exposed rats. Rats of this size rarely live beyond 72 h when exposed to this oxygen concentration. Smaller rats, about 100 g, survive beyond this period of time and seem to recover from some of the initial ill effects of hyperoxia. To examine rats during this later period, we exposed smaller rats, 112 ± 4 g for O_2 and 107 ± 1 g for compressed air, to >98% O_2 or compressed air for 96 h. We found that the number of dense granules was still markedly decreased (Table 1).

DISCUSSION

Rosenbaum, Wittner, and Lenger reported mitochondrial changes in pulmonary granular pneumocytes of rats exposed to 100% oxygen (12). They found that the cristae lost their usual perpendicular arrangement to the mitochondrial inner membrane or were completely lost and that a spotty increase in matrix density occurred.

The present study has shown a loss of mitochondrial granules in the granular pneumocytes of rats exposed to oxygen (>98%). The function of mitochondrial granules is unclear but it has been suggested that they are related to cation accumulation within mitochondria (13). If this is the case, the loss of mitochondrial granules suggests the loss of intramitochondrial cations in granular pneumocytes.

Fisher et al. (14) have made the interesting observation that Ca^{++} activates α-glycerophosphate oxidation by lung mitochondria. α-Glycerophosphate plays an important role in the synthesis of surface-active phospholipid in the lung (15, 16) which is in turn necessary for normal lung function. If our observations on mitochondrial dense granules reflects a loss of mitochondrial cations, including Ca^{++}, this could result in alterations in the intracellular concentration of α-glycerophosphate and alterations in the synthesis of phospholipid in the lung.

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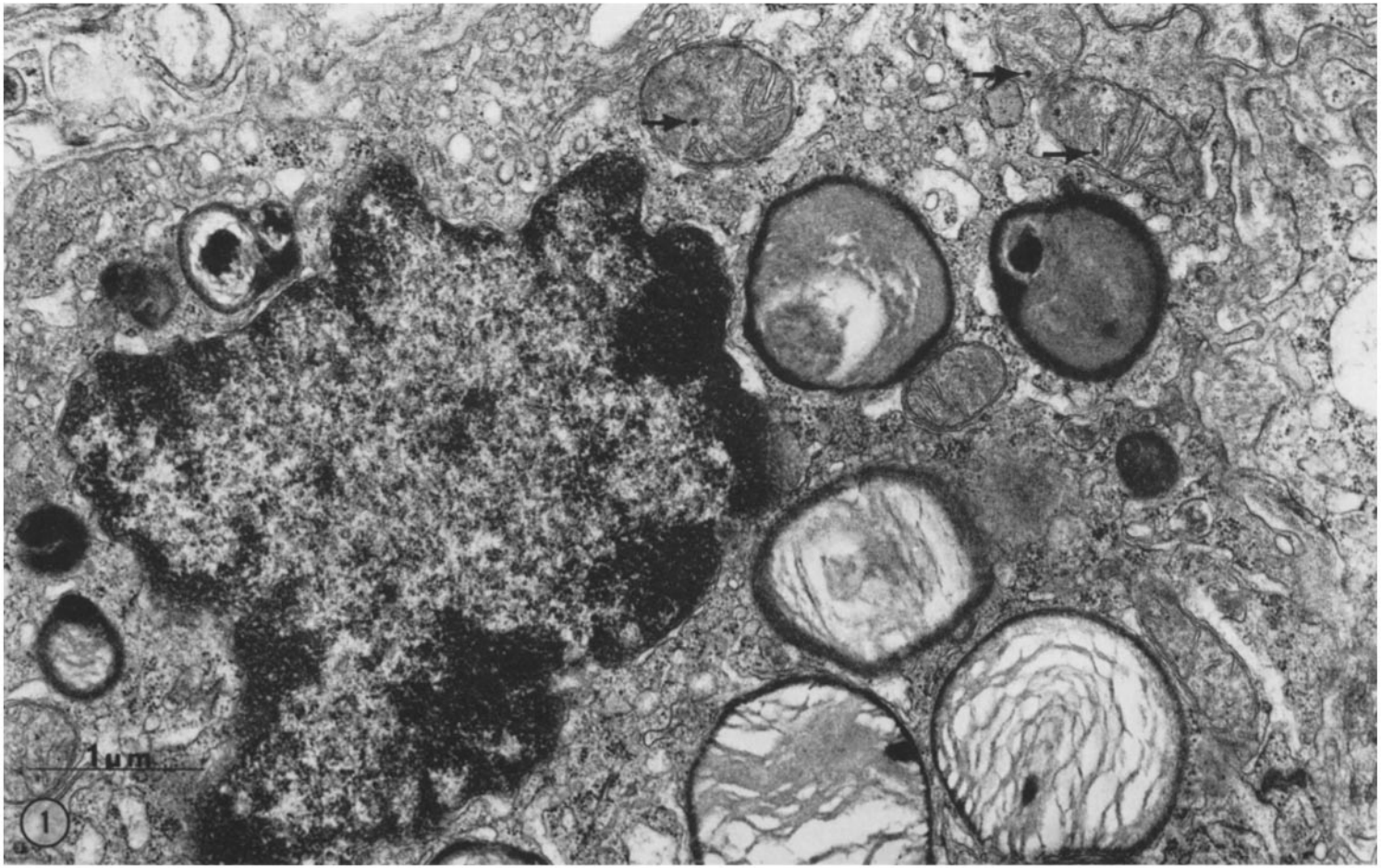
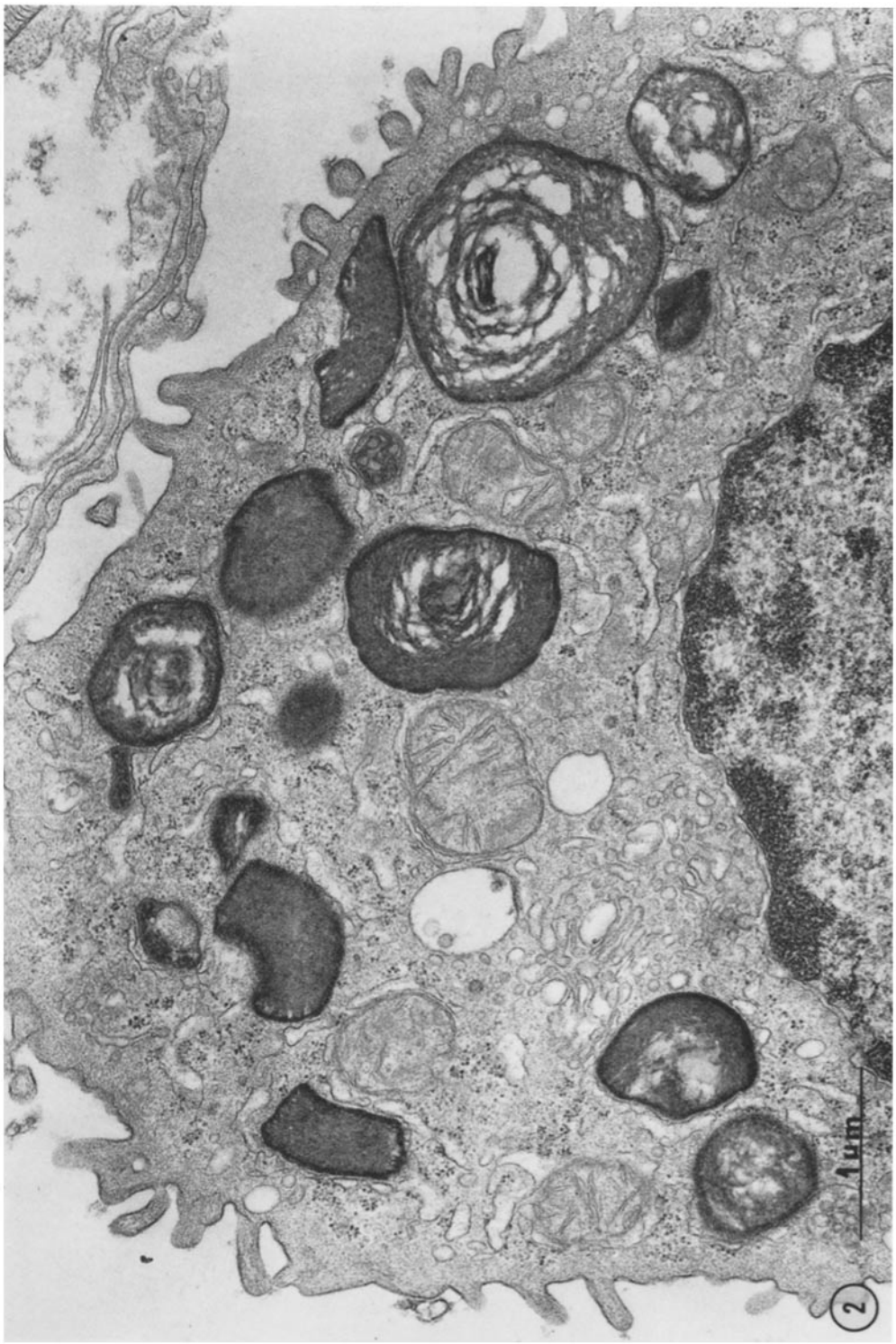


FIGURES 1 and 2 Electron micrographs of pulmonary granular pneumocytes. Fig. 1 is from a compressed air-exposed rat with arrows showing mitochondrial granules. Fig. 2 is from a 72-h O_2-exposed rat showing absence of mitochondrial granules. Both figures, × 25,000.



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