ORIGINAL REPORT

FIXATION OF LUNG FOR ELECTRON MICROSCOPY

FIXATION OF FRESH FROZEN SECTIONS WITH OSMIUM TETROXIDE

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As the fixation medium penetrates very slowly throughout the lung tissue because of its structural specificity, considerable morphological damages are brought about by the postmortem changes in the tissue. Therefore, in order to retain a satisfactory preservation of fine structure, specific fixation procedures have been employed (1–6). At present, the most reliable procedure for the fixation of the lung is thought to be the intratracheal infusion and subsequent vascular perfusion of the fixation medium (1). However, the intratracheal infusion and vascular perfusion are very difficult to perform in very small animals.

Recent studies have revealed that the satisfactory preservation of fine structure and glucose 6-phosphatase activity of the liver were adequately preserved by fixation of fresh frozen sections with glutaraldehyde (7, 8). With application of this technique, a fixation method for the ultrastructural observation of the lung in cases in which the intratracheal infusion and vascular perfusion were difficult has been developed.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing about 200 g and 3 day old DDD mice were used. Small blocks of lung were removed and frozen immediately on the outer wall of a vessel containing Dry Ice and acetone. Fresh frozen sections (30 μ) were cut in a cryostat at $-17°C$ to $-20°C$ and were placed on slides. The sections were thawed at room temperature, fixed immediately in buffered 1% OsO_4 (9) for 1 hour at 4°C. The sections were dehydrated in graded alcohol, immersed in an Epon-propylene oxide mixture overnight, stripped carefully from the slides with a razor blade and embedded in Epon.

The sections were cut with glass knives on a LKB ultratome, stained with uranyl acetate and lead hydroxide and examined in a JEM-7A electron microscope.

RESULTS AND DISCUSSION

As in Figs. 1 and 2, the alveolar epithelial cells, capillary endothelial cells and alveolar macrophages were observed with the satisfactory preservation of fine structure. The Clara cells and ciliated cells in the bronchiolar epithelium also showed good tissue preservation (Fig. 3). Therefore, the fine structure was not damaged by the freezing and thawing of the fresh tissue in the present experimental conditions. However, the erythrocytes showed a considerable morphological damage in the present study. The membrane integrity of the unfixed erythrocytes might have been destroyed by the freezing and thawing.

There have been a few reports indicating that the preservation of fine structure was hardly damaged by the freezing and thawing of fresh tissue (7) (8) (10). Tice and Barrnett (10) stated that the morphological damage occurred in incubation of fresh frozen sections in reaction medium did not depend on the freezing and thawing but mainly on the incubation procedure itself. In my previous observations on the liver also, the good tissue preservation was found after fixation of fresh frozen sections in glutaraldehyde (7) (8). In accordance with the results of these observations, the present results indicate that the satisfactory preservation of fine structure is also retained in the lung if fresh frozen sections prepared in the adequate conditions are immediately fixed in OsO_4, and the fine structure is not damaged by the freezing and thawing of the fresh lung tissue.

The present method is thought to be useful for the fixation of the lung in very small animals in which the intratracheal infusion and vascular perfusion of fixatives are difficult to perform.

SUMMARY

A fixation technique for the ultrastructural observation of the lung is presented. This technique is useful in very small animals in which the intratracheal infusion and vascular perfusion of fixatives are difficult to perform.

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Fig. 1. Rat lung sectioned at 30 μ in a cryostat, fixed subsequently in buffered 1% OsO_4. Sections stained with uranyl acetate and lead hydroxide. Two type II alveolar epithelial cells (A2) are seen. R: Erythrocyte. ×7500.

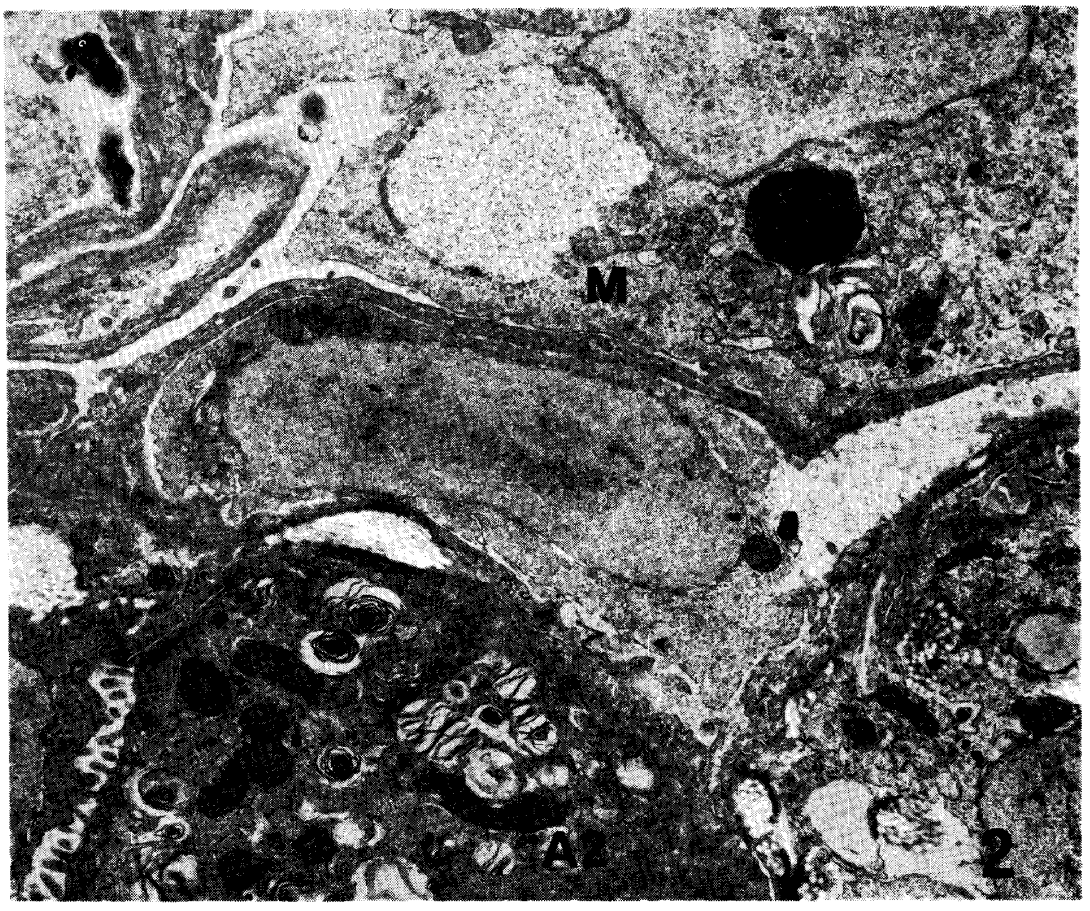

Fig. 2. Rat lung sectioned and fixed as in Fig. 1. A type II alveolar epithelial cell (A2) and alveolar macrophage (M) are seen. ×10000.

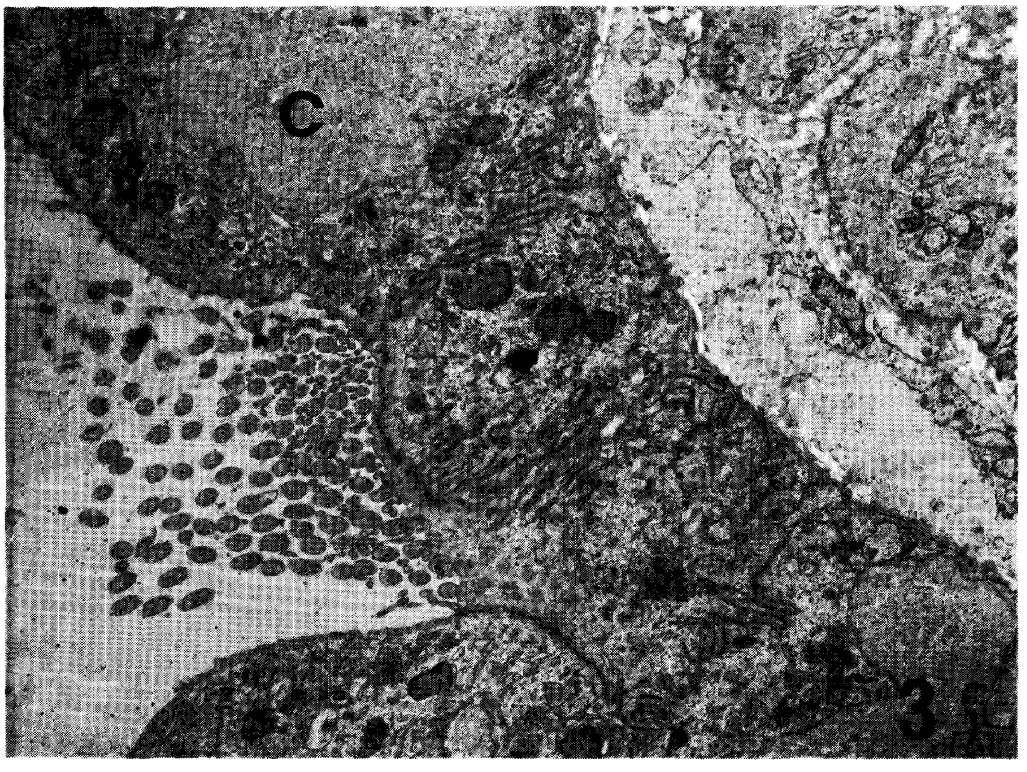

Fig. 3. 3 day old mouse lung sectioned and fixed as in Fig. 1. The Clara cells (C) and ciliated cell in the bronchiolar epithelium are observed. ×7500.