ORIGINAL REPORT

THE ULTRASTRUCTURE OF THE CLARA CELL IN THE BRONCHIOLAR EPITHELIUM

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INTRODUCTION

Two types of cells, ciliated and non-ciliated bronchiolar epithelial cells, lining the terminal bronchioles were first described by KÖLLIKER[17] in 1881. In 1937 CLARA[7] confirmed this finding and the non-ciliated bronchiolar epithelial cell is now often referred to as the Clara cell.

The ultrastructure of the Clara cell was described by KARRER[13], KISCH[15], GMELICH et al.[12] and NIDEN et al.[20,21] Most of them believe that this cell has a secretory function.

The introduction of the scanning electron microscope into biological research revolutionised the possibilities of studying the surface structures of biological specimens. The majority of biological investigations have been on the surface of hard tissues[6,9,22,23], although a few other studies have been reported.[3~5,10,24]

The present investigation is a study of the ultrastructure of the Clara cell with the scanning and the conventional electron microscope.

MATERIALS AND METHODS

Studies with the scanning electron microscope

Lungs of the normal adult mouse (DDD strain) and guinea pig were used in the present study. The tissue must be completely dried for the scanning electron microscopy. The author made use of three different methods of drying tissue: (a) freeze drying; (b) freeze drying of fixed tissue; (c) dehydration of fixed tissue in graded series of acetone solutions.

For method (a) the lung was frozen by the liquid nitrogen gas under general anesthesia then dried at −40°C. The fixative used for method (b) and (c) was 2% glutaraldehyde solution buffered with phosphate buffer solution or 10% formalin. As BARBER et al.[4] mentioned, the fixing method used seemed to be comparatively

unimportant since all these methods gave adequate preparation of materials for scanning electron microscopic study. A sufficient cleaning of the surface is possible by washing with a 0.9% NaCl solution containing 0.1% Trypsin.

The cut surfaces of lungs were coated with gold (ca. 200Å in thickness) in the rotary vacuum evaporator, and examined under the scanning electron microscope (JSM-2).

Studies with the conventional electron microscope

The lungs were fixed by intratracheal instillation of ice-cold 2% glutaraldehyde buffered at pH 7.4 with phosphate. Small pieces of the fixed lung tissue were postfixed in ice-cold 1% osmium tetroxide buffered at pH 7.4 with phosphate, washed in the buffer solution, dehydrated in graded series of ethanol, and embedded in Epon 812. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome, stained with lead hydroxide, and examined with an JEM-7 electron microscope.

OBSERVATIONS

The surface of small bronchus

The surface of a small bronchus of the guinea pig was characterized by regularly arranged ridges parallel to the longitudinal axis of the bronchus (Fig. 1). The surface of the trachea and the large bronchus is lined with the large number of ciliated cells. One of the most obvious things that was observed in the small bronchus of the guinea pig was the comparative increasing of non-ciliated cells. In the higher magnification, the individual cilia could clearly be seen on the surface of ciliated cells. The large number of microvilli were well seen on the surface of the non-ciliated cell which seemed to correspond to the goblet cell (Fig. 2).

The surface of the terminal bronchiolus

The surface of the terminal bronchioles of the mouse and the guinea pig was observed to be covered with the uneven mucous membrane in lower magnification (Fig. 3). In the higher magnification, the large number of papillary protrusions were seen on the surface of the terminal bronchiolus (Figs. 4 and 5). Comparative examinations of stereoscopic pictures and conventional micrographs of the sections suggested that protrusions corresponded to the Clara cells (Fig. 6).

Ultrastructures of the Clara cell

The Clara cells had club-shaped protoplasmic processes projecting into the lumen, but the ciliated cells did not project beyond the luminal surface of the terminal bronchiolus (Fig. 7). The cytoplasm of the Clara cell was rich in mitochondria and agranular, tubular or vesicular endoplasmic reticulum. Agranular endoplasmic reticula of lamellar form were also observed; they tend to encircle the mitochondria in multilayers. Granular endoplasmic reticula were poorly developed. Modified mitochondria with few to absent cristae were prominent throughout the cytoplasm without any apparent polarity (Fig. 8).

Osmiophilic lamellar inclusions could be occasionally found in the cytoplasm of the Clara cell (Fig. 9). The problem whether these inclusions are identical with those

which are found in the B-type alveolar epithelial cell or not remains to be solved.

Agranular endoplasmic reticula collected at the apex of the cell, and were extruded from the cell into the bronchiolar lumen (Fig. 10). This finding indicated that the Clara cell might have an apocrine secretory function.

DISCUSSION

The ultrastructure of the Clara cell was studied by a few workers.[13,15,20,21] They described an abundance of mitochondria and endoplasmic reticula, and GMELICH et al.[12] described "cells with relatively short and plump pseudopods on the luminal surface" that contained "small electron-translucent vacuoles" in their apices. The stereo ultrastructure of the Clara cell has not been previously reported in the literature.

Most workers believe that the Clara cell has a secretory function. CLARA[7] was certain that the material produced by this cell was not of a mucoid nature. VON HAYEK[25], however, maintained that the secretion of this cell is "a kind of apocrine secretion which produces the eosinophilic mucus". NIDEN et al.[20,21] and AZZOPARDI et al.[1] attributed the synthesis of lipoprotein to the Clara cell and recognized it as an important source of the surfactant of the pulmonary alveoli. However, there is no proof that the agranular endoplasmic reticulum extruded into the bronchiolar lumen is the source of the alveolar surfactant.

The ultrastructure of the osmiophilic lamellar bodies that are rarely found in the Clara cell resembles to that of the B-type alveolar epithelial cell. It is generally accepted that the osmiophilic lamellar inclusions of the B-type alveolar epithelial cell (=type II alveolar cell, granular pneumocyte, alveolar wall cell, large alveolar cell) are the source of the alveolar surfactant.[2,14,16] However, the problem whether both inclusions are identical with each other or not remains to be solved.

According to the author's observation, the Clara cells are not found in all kinds of vertebrata but in mouse, guinea pig and some other mammalia. It seems to show that the Clara cell is not an important source of the alveolar surfactant.

SUMMARY

The terminal bronchiolar epithelium of the mouse and the guinea pig were examined with the scanning electron microscope and the conventional electron microscope. The ciliated cells and the Clara cells were well preserved and it is possible to discern individual cell with the scanning electron microscope. The apical part of the cytoplasm containing the agranular endoplasmic reticulum was occasionally seen to be extruded into the bronchiolar lumen. This finding indicated that the Clara cell has an apocrine secretory function. Osmiophilic lamellar inclusions are rarely found in this cell. However, there is no proof that they are the important source of the pulmonary surfactant.

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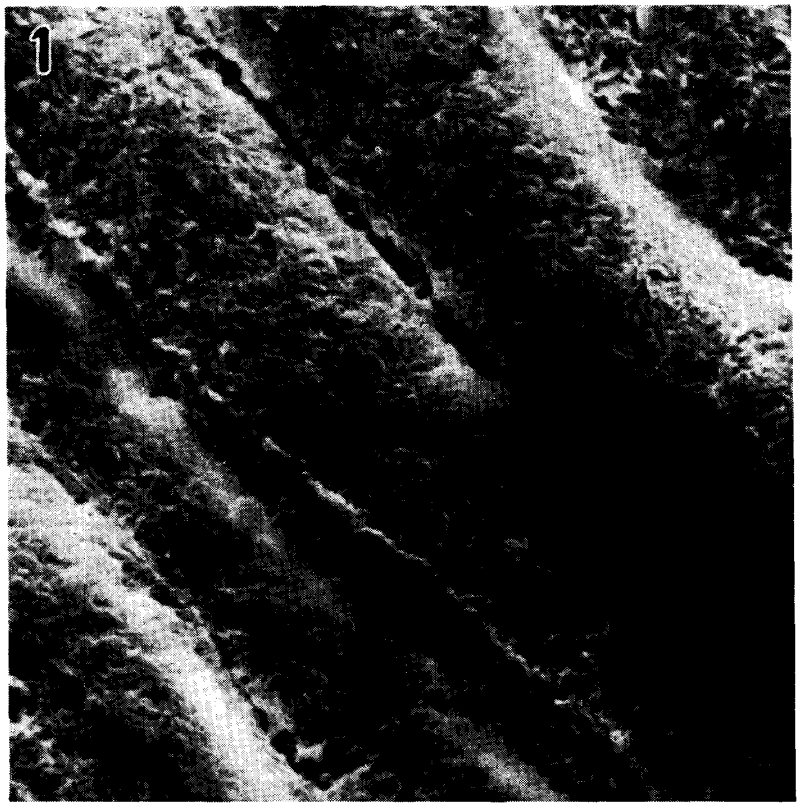
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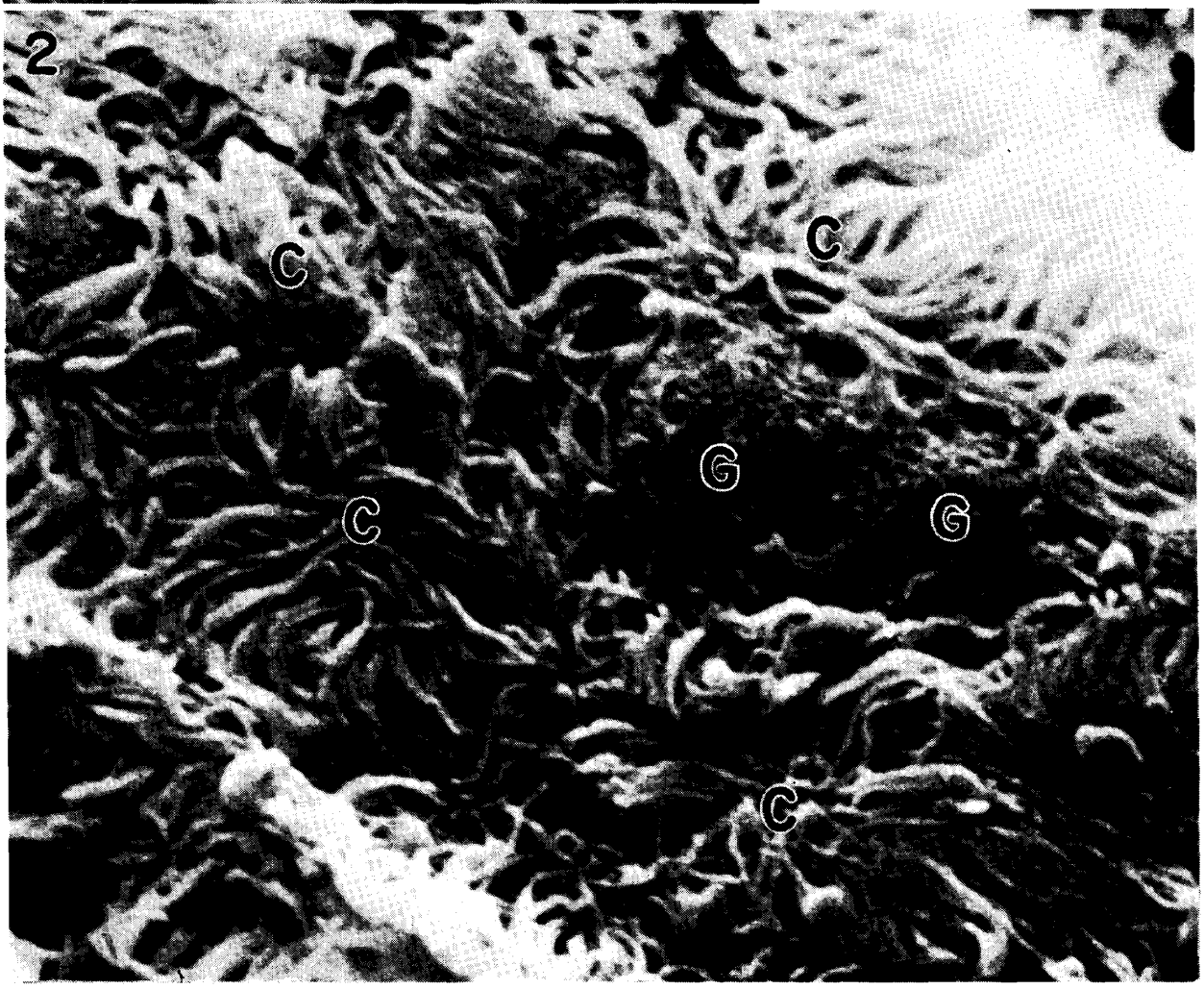
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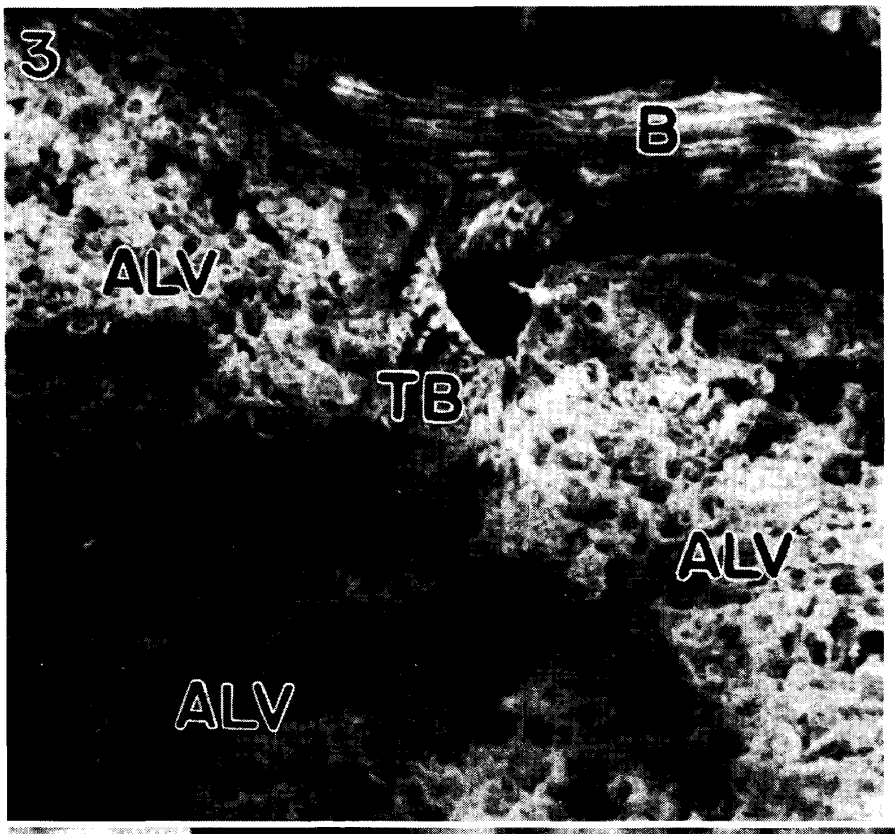
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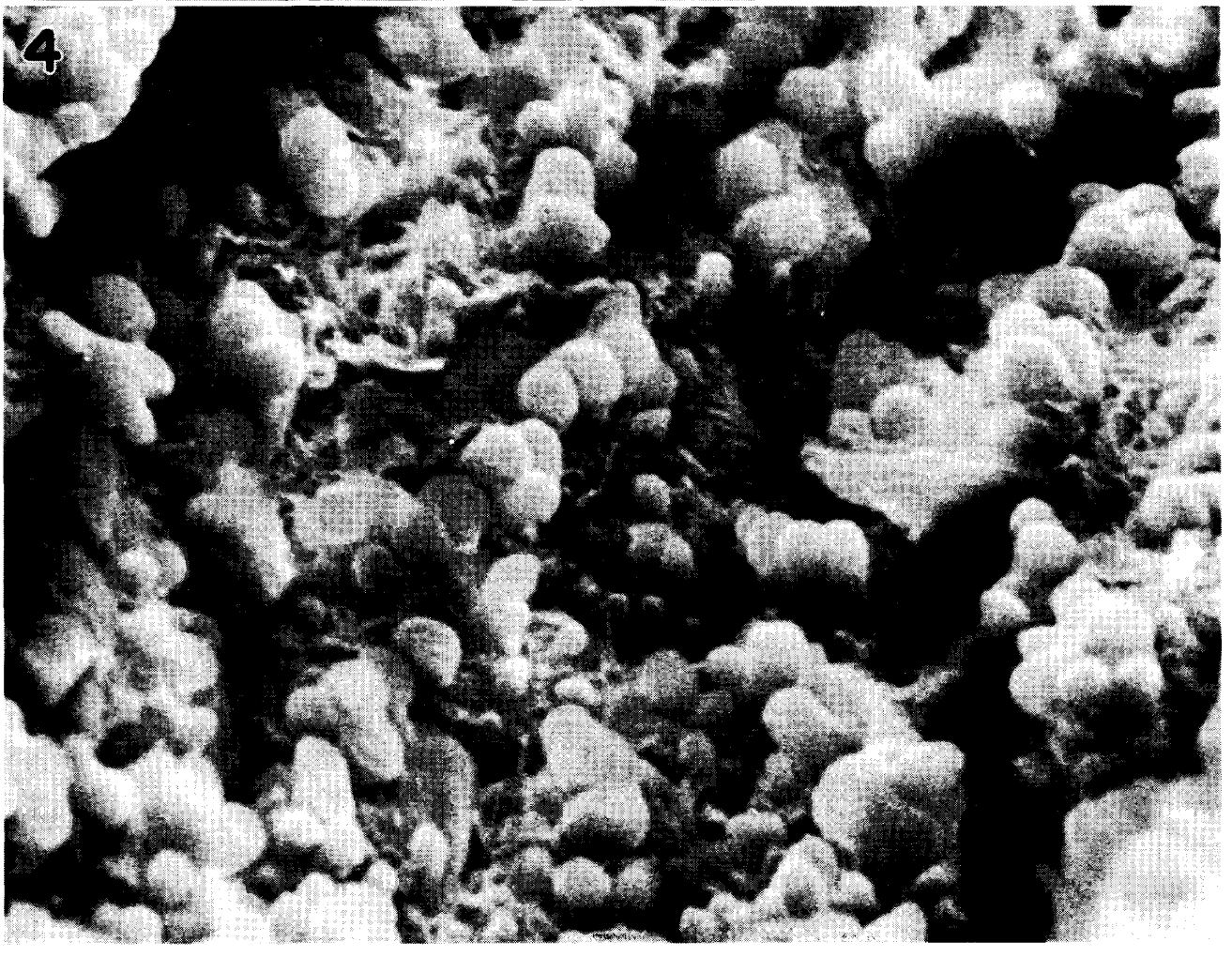
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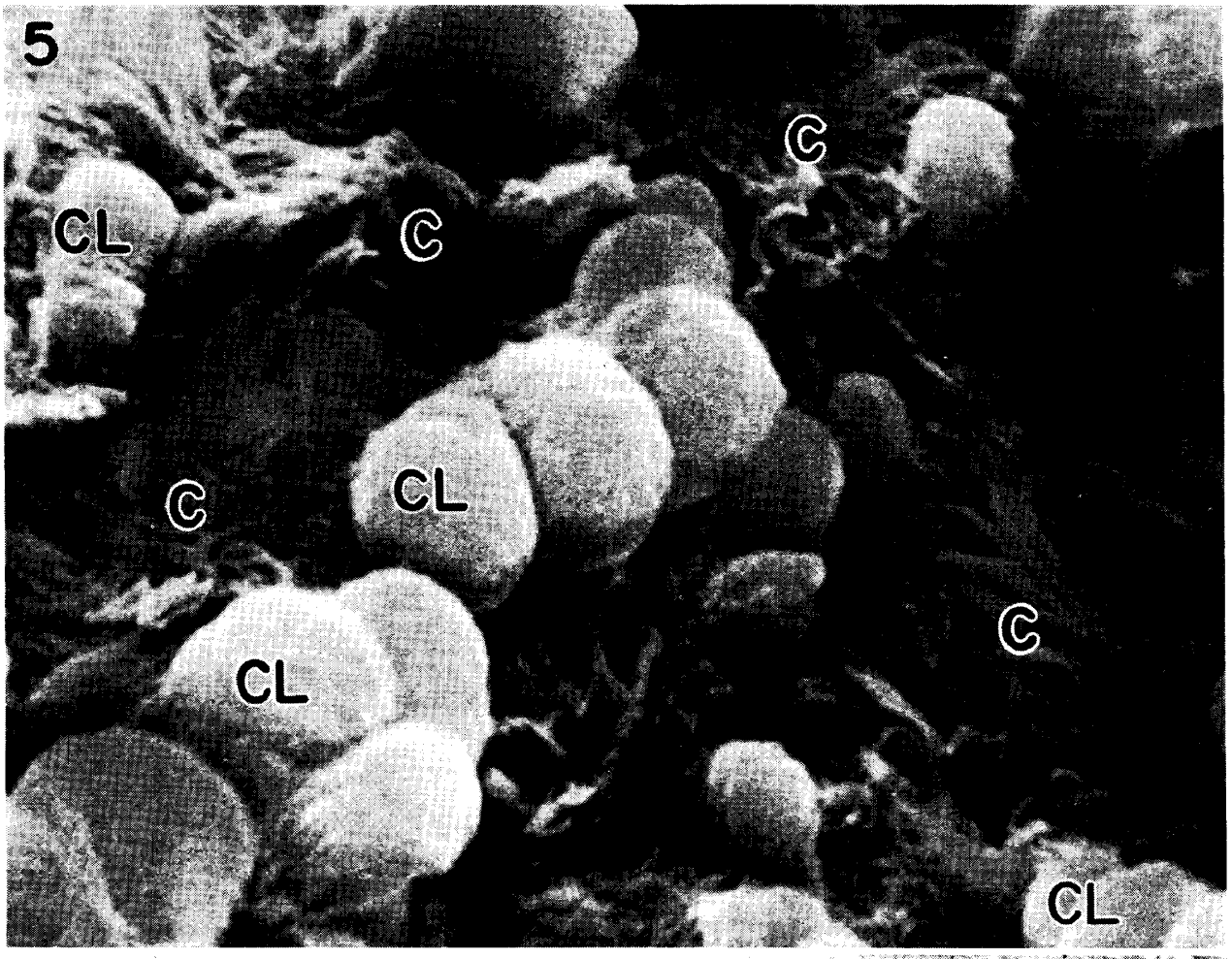
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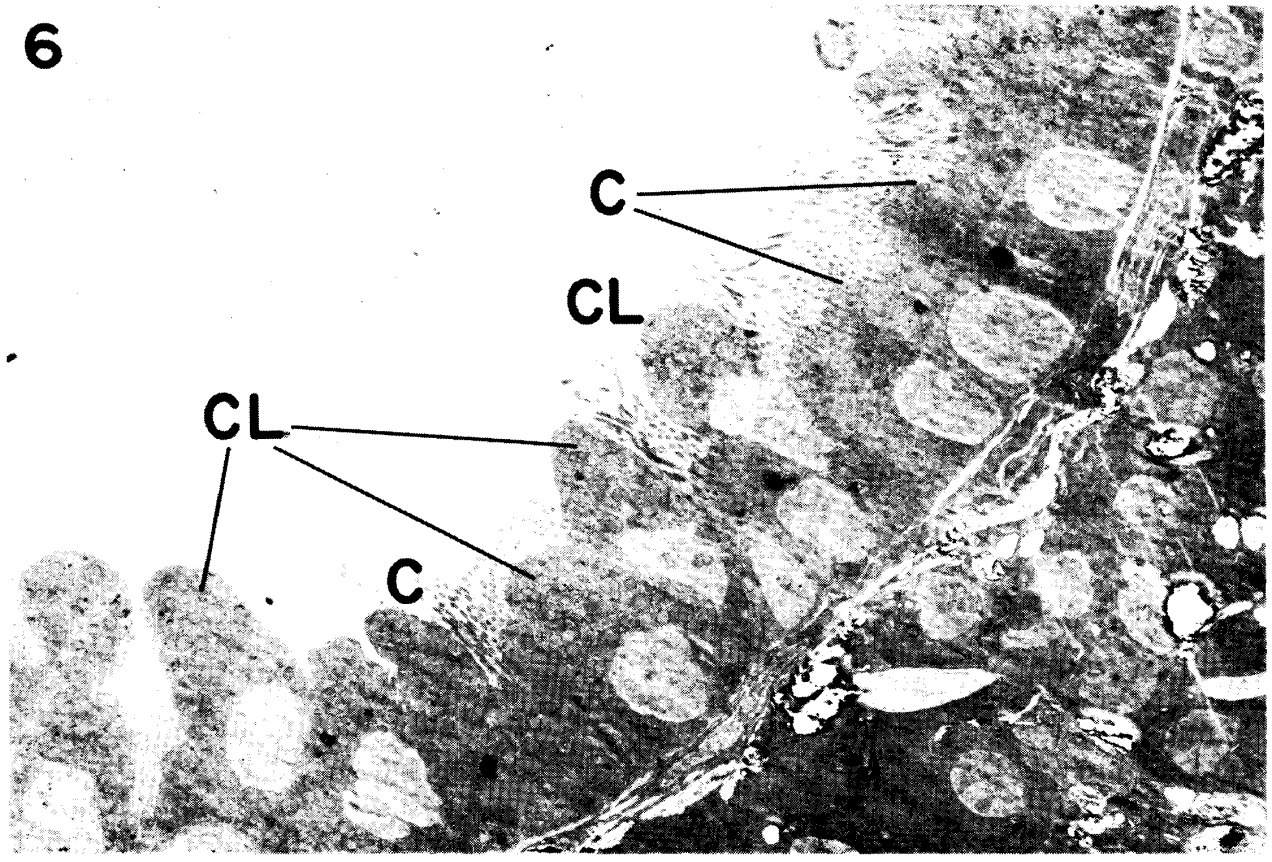
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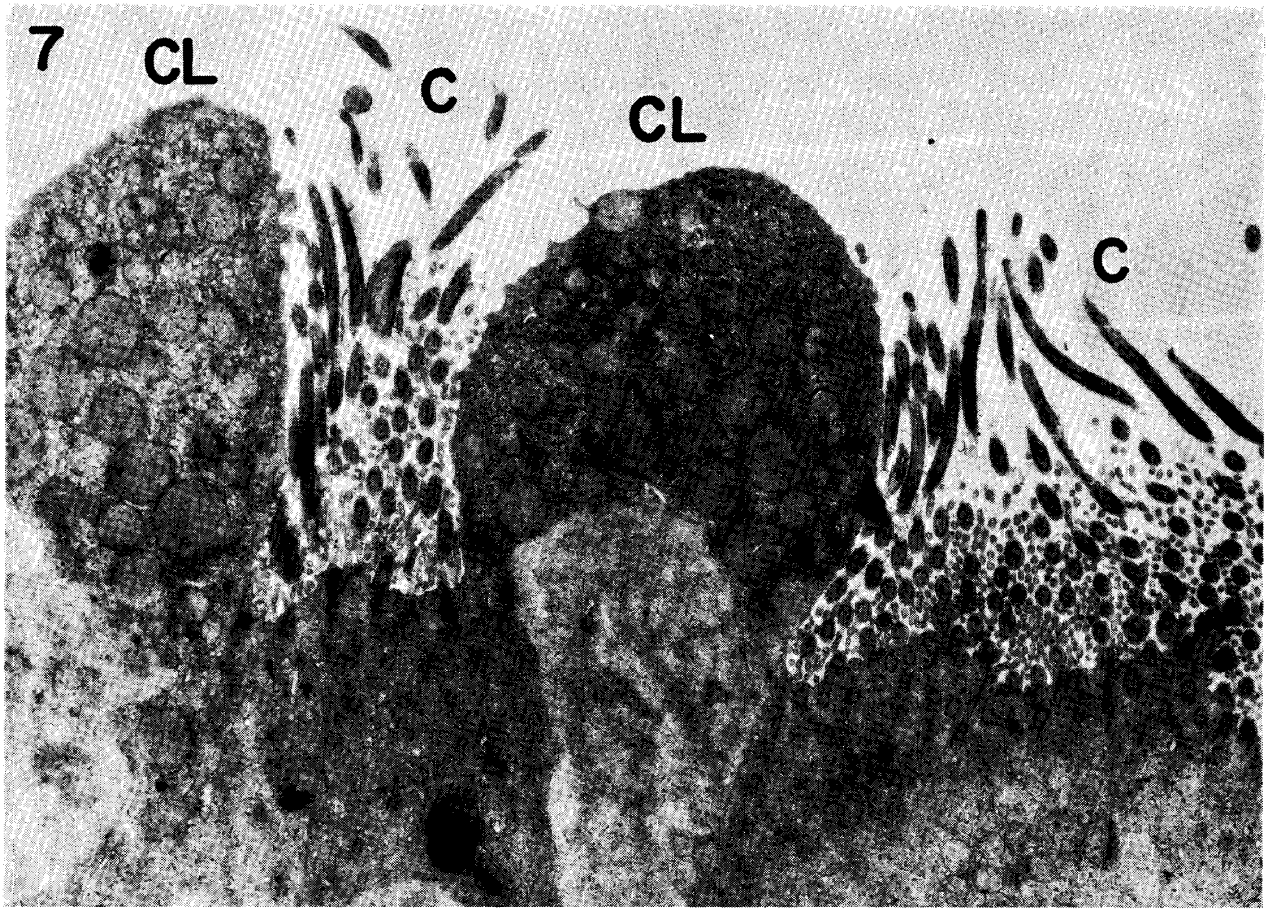
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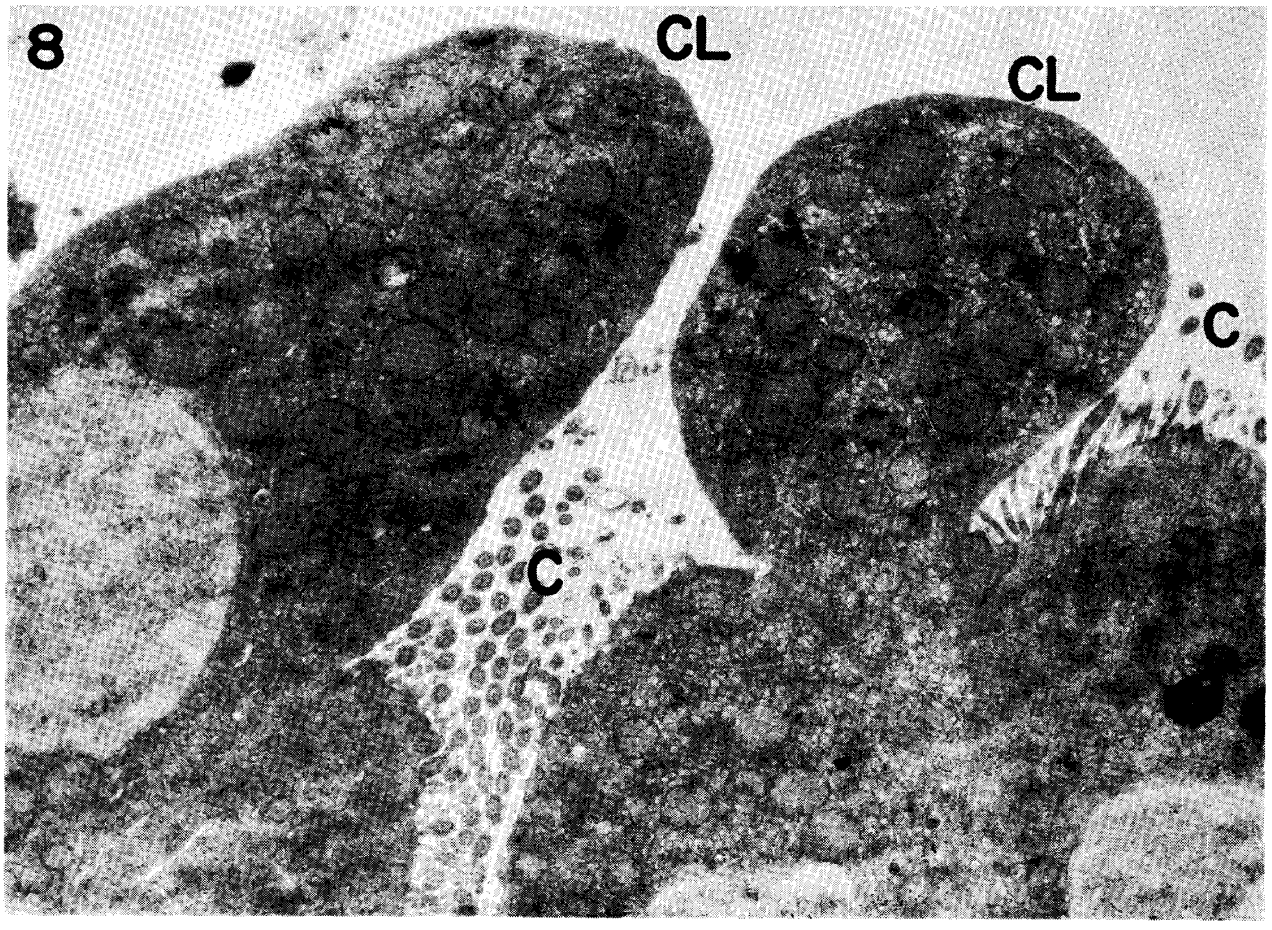
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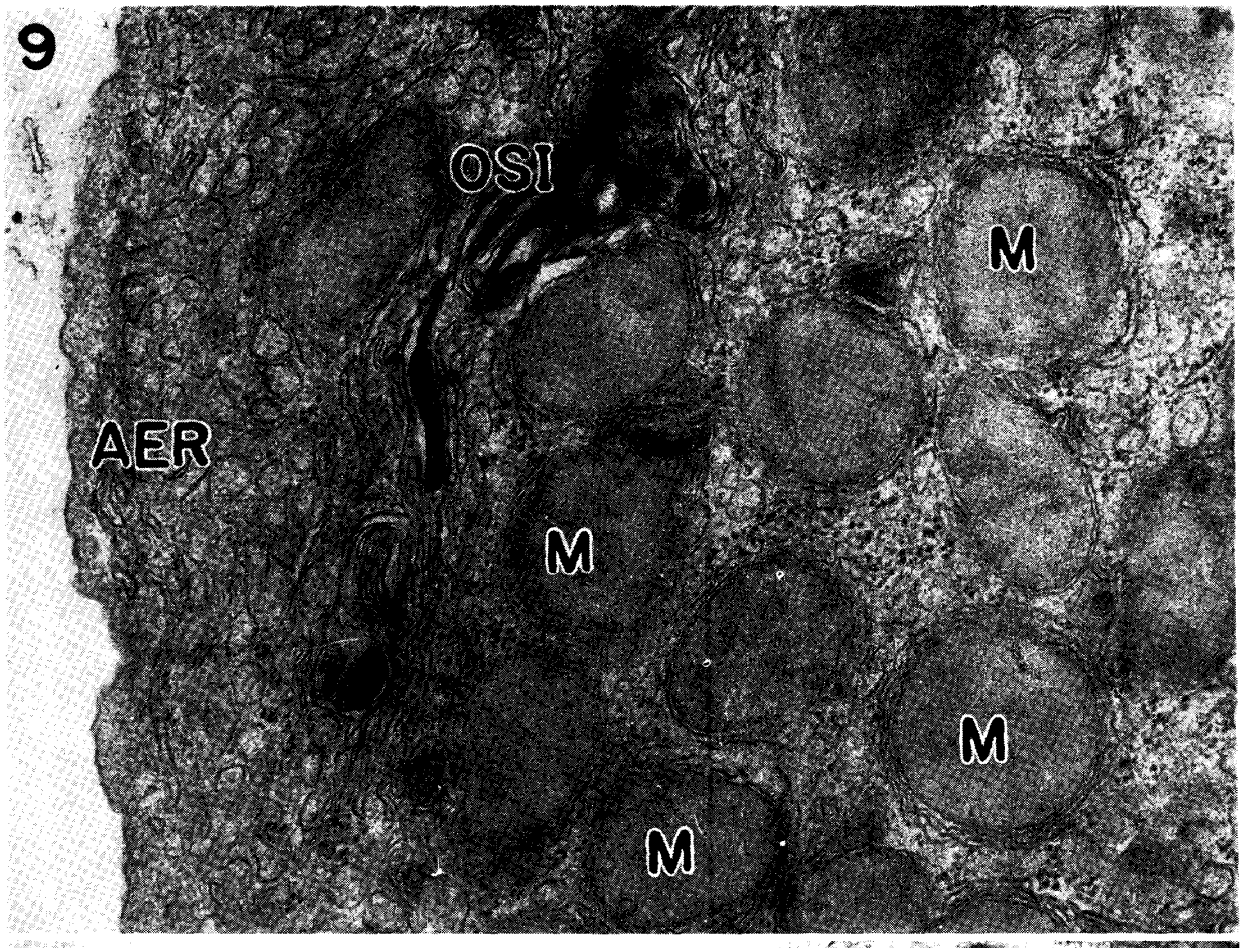
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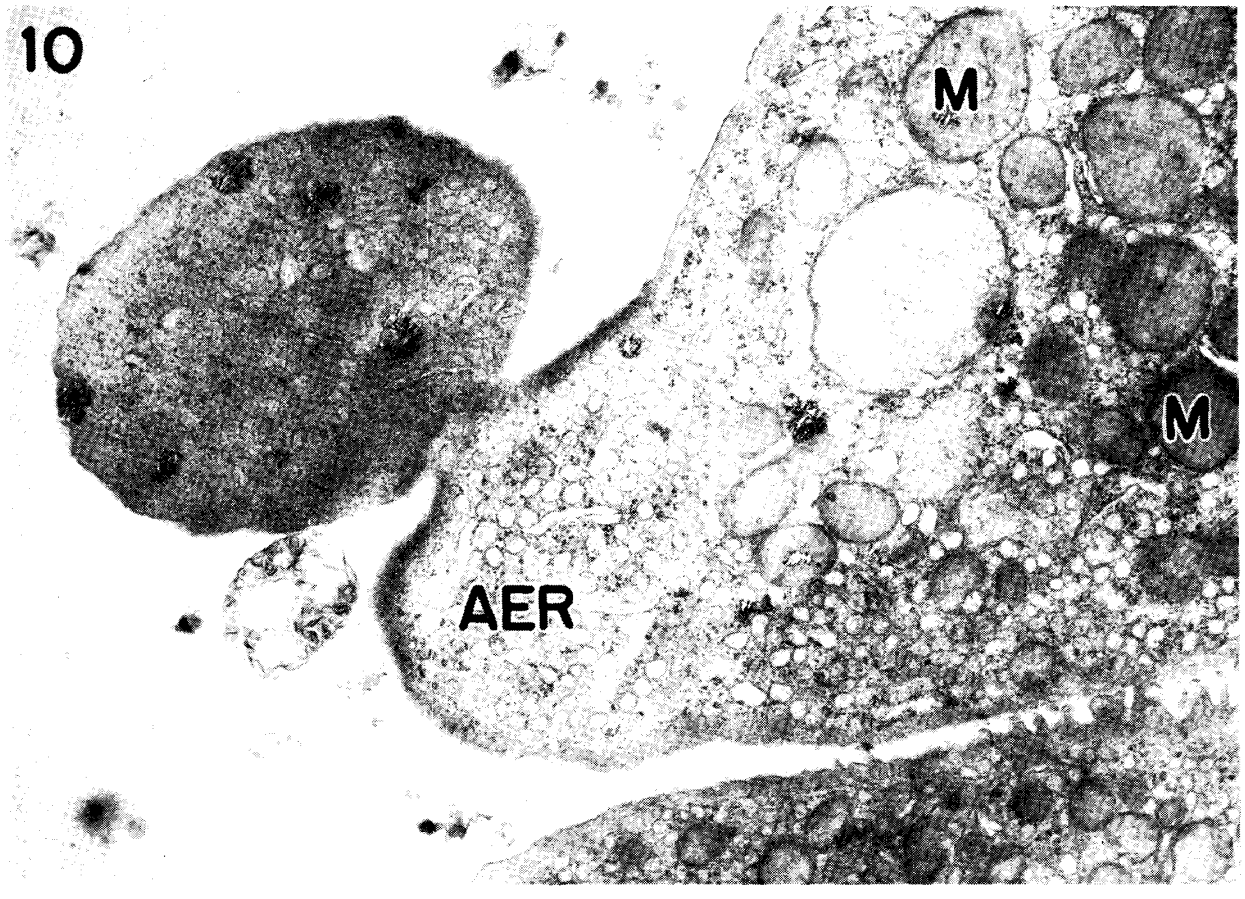
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Explanation of figures

Fig. 1. Surface view of a small bronchus of guinea pig. Some ridges of the mucuous membrane are arranged parallel to the longitudinal axis of the bronchus. (x 1,400)

Fig. 2. A portion of Fig. 1 at higher magnification. Individual cilia of the ciliated cell (C) and microvilli of the goblet cell (G) can clearly be seen. (x 8,400)

Fig. 3. Low-power micrograph of a small bronchus (B), at terminal bronchiolus (TB) and alveolar area (ALV) of guinea pig. (x 150)

Fig. 4. Surface view of a terminal bronchiolus of guinea pig. The large number of papillary protrusions are seen on the mucous membrane. Some ciliated cells are also seen. (x 2,500)

Fig. 5. A portion of Fig. 4 at higher magnification. The Clara cells (CL) are very well shown. Note also the ciliated cells (C) are scattered among them. No microvilli are seen on the surface of the Clara cell. (x 7,500)

Fig. 6. Cross section of the terminal bronchiolus of mouse. The Clara cells (CL) had club-shaped protoplasmic processes projecting into the lumen, but the ciliated cells (C) did not project beyond the luminal surface. (x 2,000)

Fig. 7. High-power micrograph of the Clara cells (CL) and the ciliated cells (C). (x 8,000)

Fig. 8. Clara cells (CL) and ciliated cells (C). (x 8,000)

Fig. 9. The cytoplasm of the Clara cell. Modified mitochondria (M) with few cristae are lamellarly encircled by agranular endoplasmic reticula. Agranular endoplasmic reticula of vesicular or tubular form (AER) are abundantly found in apical area of the cell. An osmiophilic inclusion body (OSI) can be seen. (x 30,000)

Fig. 10. Extrusion of an apical part of cytoplasm of the Clara cell. (x 15,000)