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Brief Note

Observation of Hexagonal Crystalloid Inclusions in Guinea Pig Clara Cells by Scanning Electron Microscopy

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Key words : Clara cell — crystalloid inclusion — guinea pig — lung — scanning electron microscopy

Clara cells are non-ciliated epithelial cells that are located mainly in small conducting airways of the lung. These columnar cells are ultrastructurally characterized by the presence of a dome-shaped projection of the supranuclear cytoplasm with numerous electron-dense granules and smooth endoplasmic reticulum. There is, however, considerable variability in ultrastructure, distribution, age of maturity and granule content between species. Hexagonal crystalloid inclusions appear to be unique to guinea pigs (Figs. 1, 2). By treating with pepsin, we have previously shown that these crystalloids were composed mainly of proteins. Morphologically, they are membrane-bound, long rod-shaped inclusions; 0.4 μm in diameter at the widest point, and 8.4 μm long on average (Fig. 1). In cross section, the inclusions were regularly hexagonal and show a definite periodicity of alternating dark and light lines (Fig. 2).

In order to better observe the substructure of the inclusions, we tried to examine them with scanning electron microscopy after freeze-fracturing. Guinea

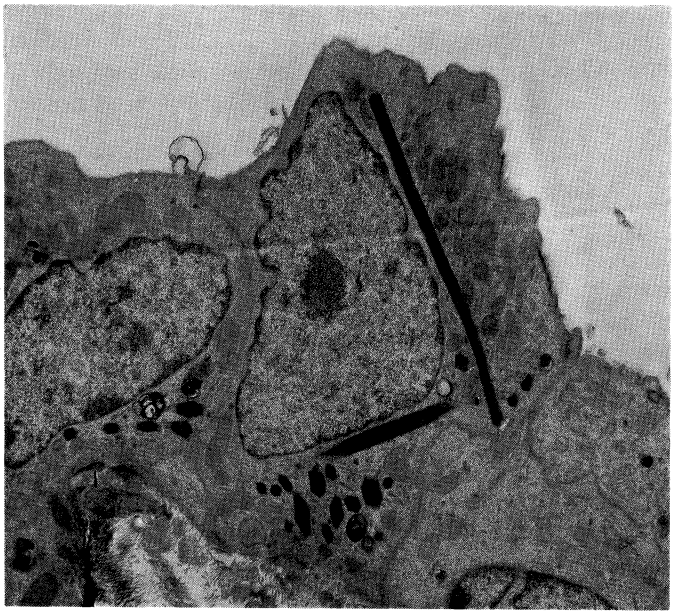

Fig. 1. Clara cells showing long rod-shaped crystalloid inclusions. (TEM, × 4,000)

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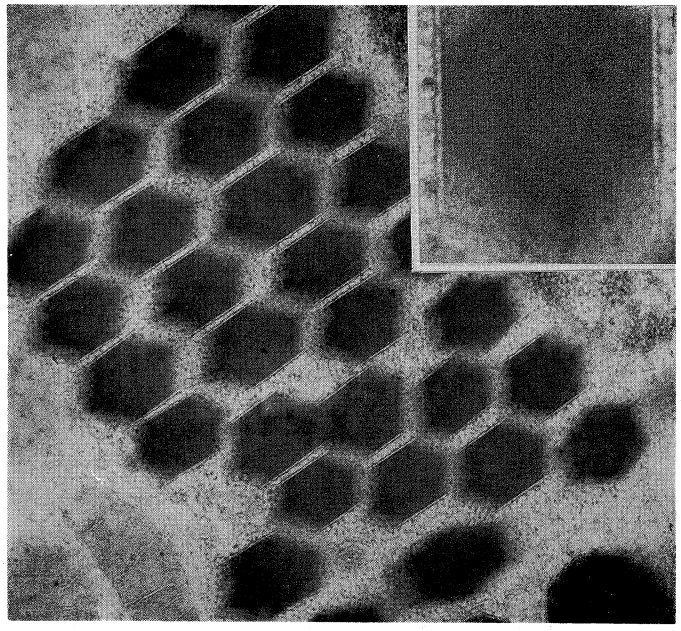

Fig. 2. Cross sections of the crystalloid inclusions, which are hexagonal in shape. (TEM, × 20,000) Inset: Linear periodicity of the crystalloid inclusion. (× 62,000)

pigs (Hartley), weighing 900 g with the age of one year, were utilized for this study. Under anesthesia, the lung was perfused with buffered saline at first and then with 1/4 Karnovsky fixative (0.5% formaldehyde and 0.5% glutaraldehyde in 1/15 M phosphate buffer, pH 7.4) through the right heart. Lung tissues were sliced into 1 × 1 × 4 mm tissue blocks and fixed in the same fixative for one hour. They were postfixed in 1% OsO_4 for 90 min. After a wash with phosphate buffer, they were transferred into 25% and then 50% of dimethyl sulfoxide (DMSO) for 40 min. Practically, we followed the procedure of modified osmium maceration (Aldehyde-osmium-DMSO-osmium) method recommended by Osatake[1] and samples were examined under Hitachi S-570 scanning electron microscope (20 KV). Readers who wish to know the procedure in more detail are recommended to refer to the handout given at Denken Shiryo Gijyutsu Kenkyukai, 1985,[1] and the article written by Tanaka.[2]

In this preliminary study, the preservation of the ultrastructure was not perfect but as shown in Figs. 3, 4 and 5, crystalloid bodies were satisfactorily observable. Other organelles of the Clara cells were also seen (Fig. 3). In this technique, crystalloid bodies were composed of aggregates of round granular materials, each measuring approximately 30 nm in diameter. Even in longitudinal sections (Fig. 4), they appeared granular and linear structures were not present. In areas where the substructures were rather clearly seen, granular particles were aligned in parallel, staggering each other (Fig. 5). Limiting membranes outlined the inclusions on occasion. Although transmission electron microscopy (TEM) disclosed linear substructures, the hexagonal inclusions were revealed with this technique to be condensed aggregates of round particles in so-called crystalloid organized arrangement which were embedded within the limiting

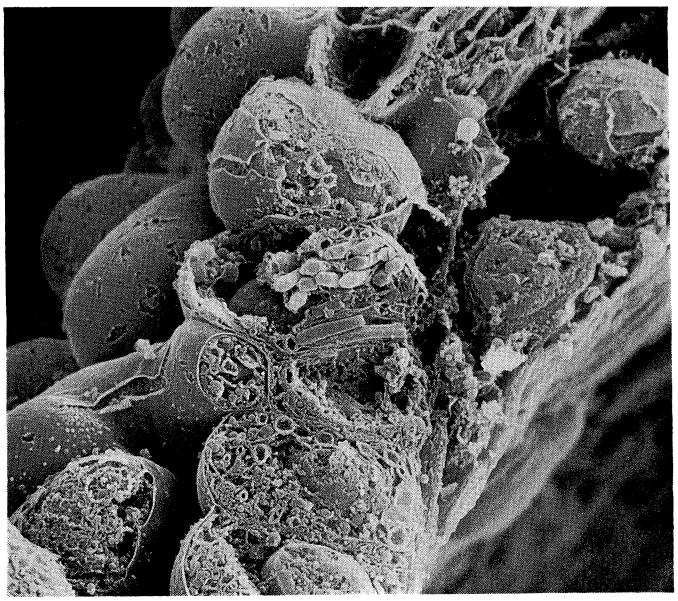

Fig. 3. Scanning electron micrograph showing Clara cells with crystalloid inclusions. (× 1,800)

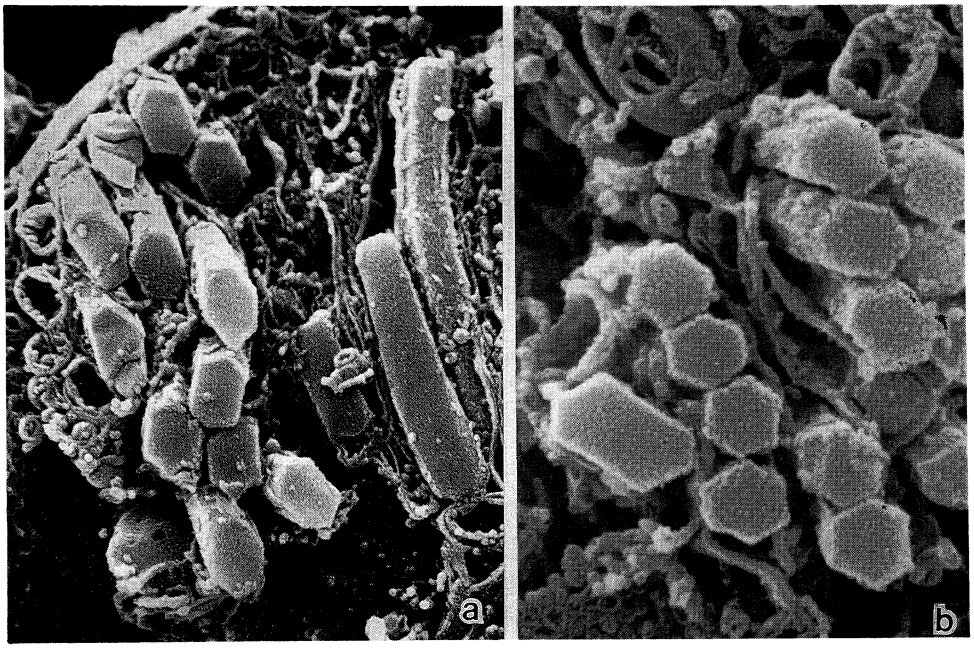

Fig. 4. a. Crystalloid inclusions cut transversely and longitudinally. Note that they are surrounded by the membranous structure in places. (SEM, × 10,000)
b. A close-up view of fractured surface of the crystalloid inclusions. Note that the surfaces are granular. (SEM, × 19,000)

membrane. Parallel arrangement of the particles may have imparted a linear appearance in TEM.

Scanning electron microscopy associated with a method of fracturing cells enabled the observation of tridimensional subcellular structures. However, this technology still bears some limitations and problems to be solved. For the better appreciation, therefore, techniques in many aspects should be improved. With such improvement and through extensive studies on crystalloid inclusions in guinea pig Clara cells, our findings may be confirmed.

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REFERENCES

1) Osatake, H.: Chugoku-chiku Denken Shiryo Gijyutsu Kenkyukai, Tottori, Japan 1985 (in Japanese)
2) Tanaka, K. and Mitsushima, A.: A preparation method for observing intracellular structures by scanning electron microscopy. J. Microsc. **133** : 213-222, 1984