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Histochemical and Ultrastructural Study of Clara Cell Granules in the Guinea Pig

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ABSTRACT. Histochemical studies using light and electron microscopes were undertaken to see what chemical components make up the Clara cell granules in guinea pig lung. Light microscopically, it was suggested that they may contain a variable amount of proteins, carbohydrates, and phospholipids. On the other hand, electron microscopic examination with digestive treatment with pepsin as well as chloroform-methanol clearly demonstrated that the main component of the crystalline granules of the guinea pig Clara cells was the proteins. It was, therefore, concluded that Clara cell granules are complex compounds made up mainly of proteins with small amounts of carbohydrates and phospholipids.

Key words : Clara cell — guinea pig — lung — histochemistry — electron microscopy

Clara cells are non-ciliated columnar epithelial cells that are located mainly in the small conducting airways of the lung.[1,2] They are morphologically 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. In addition, in spite of numerous morphologic and cytochemical studies,[3-5] the function of Clara cells still remains to be elucidated. When we started to study the Clara cells of the guinea pig ultrastructurally a few years ago, we found out that they contain unique crystalloid inclusions in addition to electron dense round granules, which were seen to transform into the former on occasions. Histochemical studies on the Clara cell granules have been reported in several species,[4-6] but they have not been well described in the guinea pig. It is usually stated that phospholipids exist in large amounts in these granules.[4,7,8] However, we could not agree with this assertion because such a well-organized crystalline structure is not composed of proteins but of phospholipids alone or as a major component.

In order to clarify that the Clara cell granules of the guinea pig contain proteins as a major component, we have undertaken histochemical studies using both light and electron microscopy. Our results suggest that the main component is, in fact, proteins, possibly with a small amount of PAS-positive materials and phospholipids.

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MATERIALS AND METHODS

Guinea pigs (Hartley) of about 1 kg weight were used for this study. After sacrificing the animals with an intraperitoneal injection of Nembutal, the lungs were removed in toto, and sliced into small pieces for histochemical and ultrastructural examinations. For the identification of phospholipids, the tissues were fixed in a calcium-formalin solution, embedded in gelatin and frozen to be thinly cut. Sections were stained with Baker's acid hematin. In one section, lipids were extracted with a chloroform-methanol solution (2:1) before fixation and the sections were stained in the same manner. For carbohydrates, tissues were fixed in 2.5% glutaraldehyde and embedded in glycol methacrylic acid (GMA). Sections were stained with PAS as well as alcian blue and toluidine blue. For proteins, tissues were also fixed in 2.5% glutaraldehyde, routinely processed and sections were stained with Azan stain, phosphotungstic acid-hematoxylin (PTAH) stain and Mercury bromphenol blue (MBPB) stain. Other staining procedures such as OTA and Klüver-Barerra stains were used, but there was some difficulty in interpreting the results.

For ultrastructural studies, tissues were fixed in 2.5% glutaraldehyde and

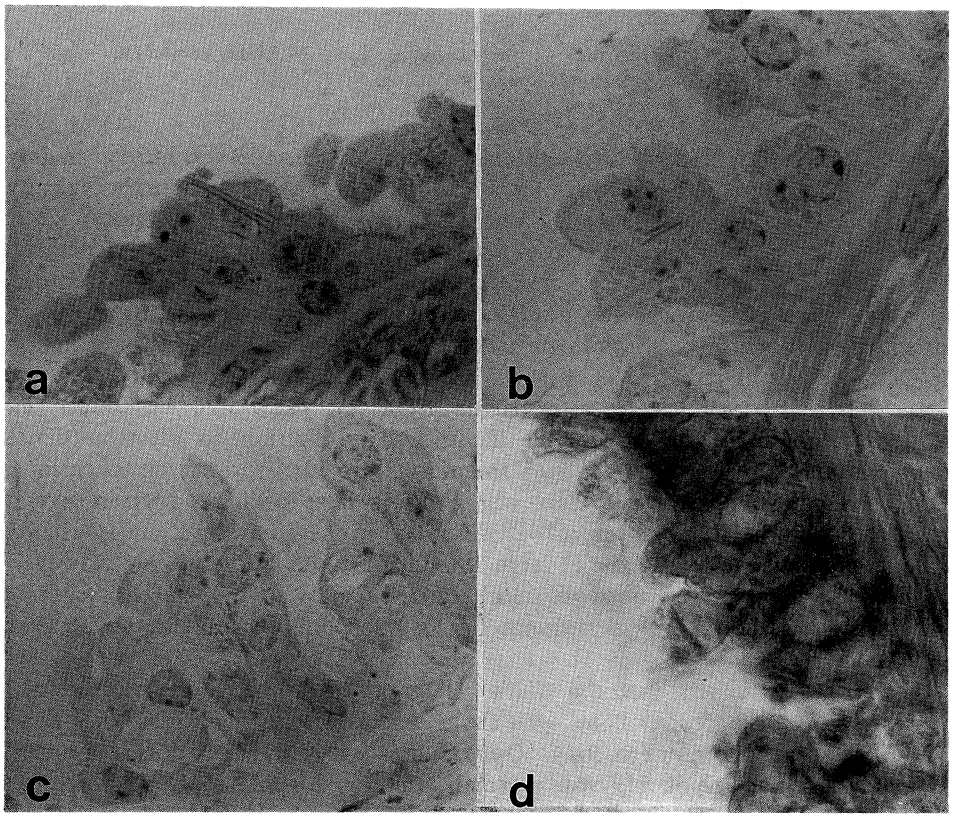

Fig. 1. Needle-like inclusions in the Clara cells. They are positive for MBPB, Azan, PAS, and occasionally Baker's acid hematin. (a. MBPB stain, ×1,000, b. Azan stain, ×1,000, c. Periodic acid-Schiff stain, ×1,000, and d. Baker's acid hematin stain, ×1,300)

then in 1% osmium tetroxide, routinely processed and embedded in Epon 812. For the identification of proteins, thin sections were placed in 5% periodic acid for 20 min and digested by pepsin (0.5%) (Sigma Company) in 0.1 N HCl at 37°C for one hour. For the lipids, tissues were separately fixed in 5% glutaraldehyde, exposed to chloroform-methanol (2:1) for 14 hrs, postfixed in 1% OsO_4 and routinely processed to embed them in Epon 812. Ultrathin sections were examined under a Hitachi HS-9 electron microscope.

RESULTS AND DISCUSSION

Round and oblong crystalloid inclusions were seen in Clara cells of the guinea pig lung light-microscopically (Fig. 1) as well as ultrastructurally (Figs. 2-4). Histochemical results are tabulated in Table 1. Light microscopically, they seem to be composed of proteins, phospholipids and neutral mucopolysaccharides. Granules were stained red for Azan stain. MBPB stains revealed some positivity. Some round and a few oblong crystalloid inclusions were

TABLE 1. Results of histochemical study

Staining Method	Result
MBPB	+
Azan	+
PAS without diastase treatment	+
PAS after diastase digestion	+
Toluidine blue	−
Alcian blue	−
Baker's acid hematin	occasionally +
Baker's acid hematin after chloroform-methanol extraction	all −

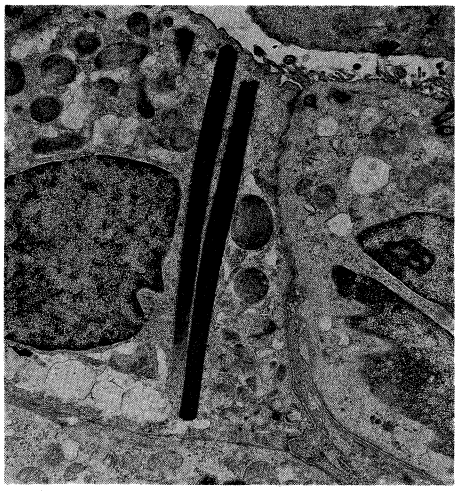

Fig. 2. Electron micrograph of needle-like crystalline inclusions in the Clara cell. Mag., ×6,000

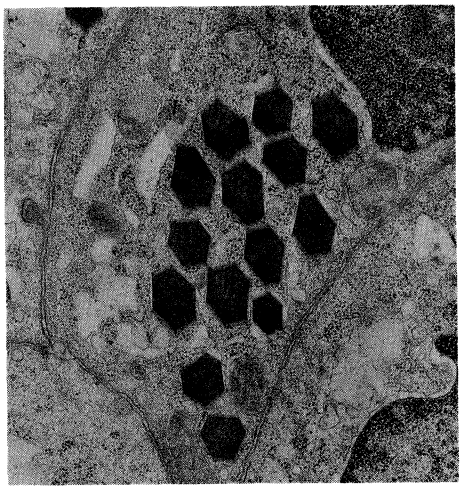

Fig. 3. Cross-sections of the inclusions. They are electron-dense and membrane-bound. Mag., ×11,000

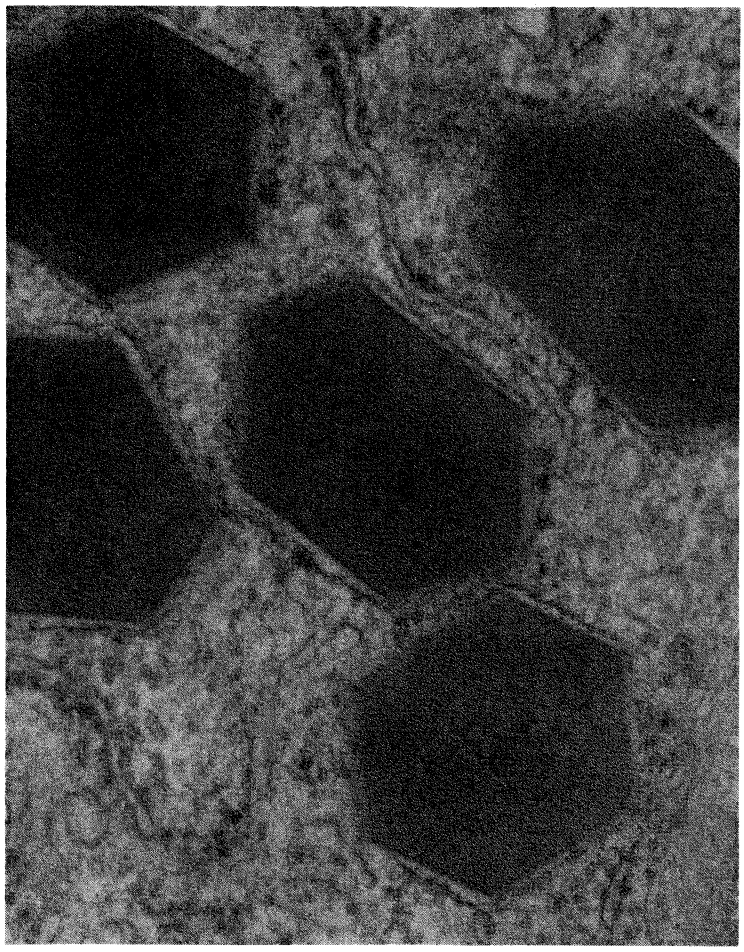

Fig. 4. Higher magnification of cross-sectioned inclusions which exhibit crystalline substructures of dark and clear lines stacked upon each other in parallel fashion. Mag., ×110,000

stained black with Baker's acid hematin, and became negative after chloroform-methanol extraction. PAS stains with and without diastase digestion were both positive in these granules, while toluidine blue and alcian blue stains were consistently negative. The comparison of our results with those of other studies may be difficult because some investigators used different animal species, and because others, although they used the same species, did not describe their staining results well enough for them to be evaluated. It was our feeling, however, that even our procedures did not provide consistent and reliable results. In particular, the procedures for identifying phospholipids were inconsistent and unreliable. For these reasons, we digested the tissue with pepsin, and examined it by electron microscopy, which may provide a better appreciation (Fig. 5). When treated with pepsin, the crystalline substructure of the inclusion bodies disappeared (Fig. 5b). Chloroform-methanol extraction, on the other hand, did not change the crystalloid structures at all (Fig. 5c), although the adjacent areas were widely separated from them by spaces. These results suggested that

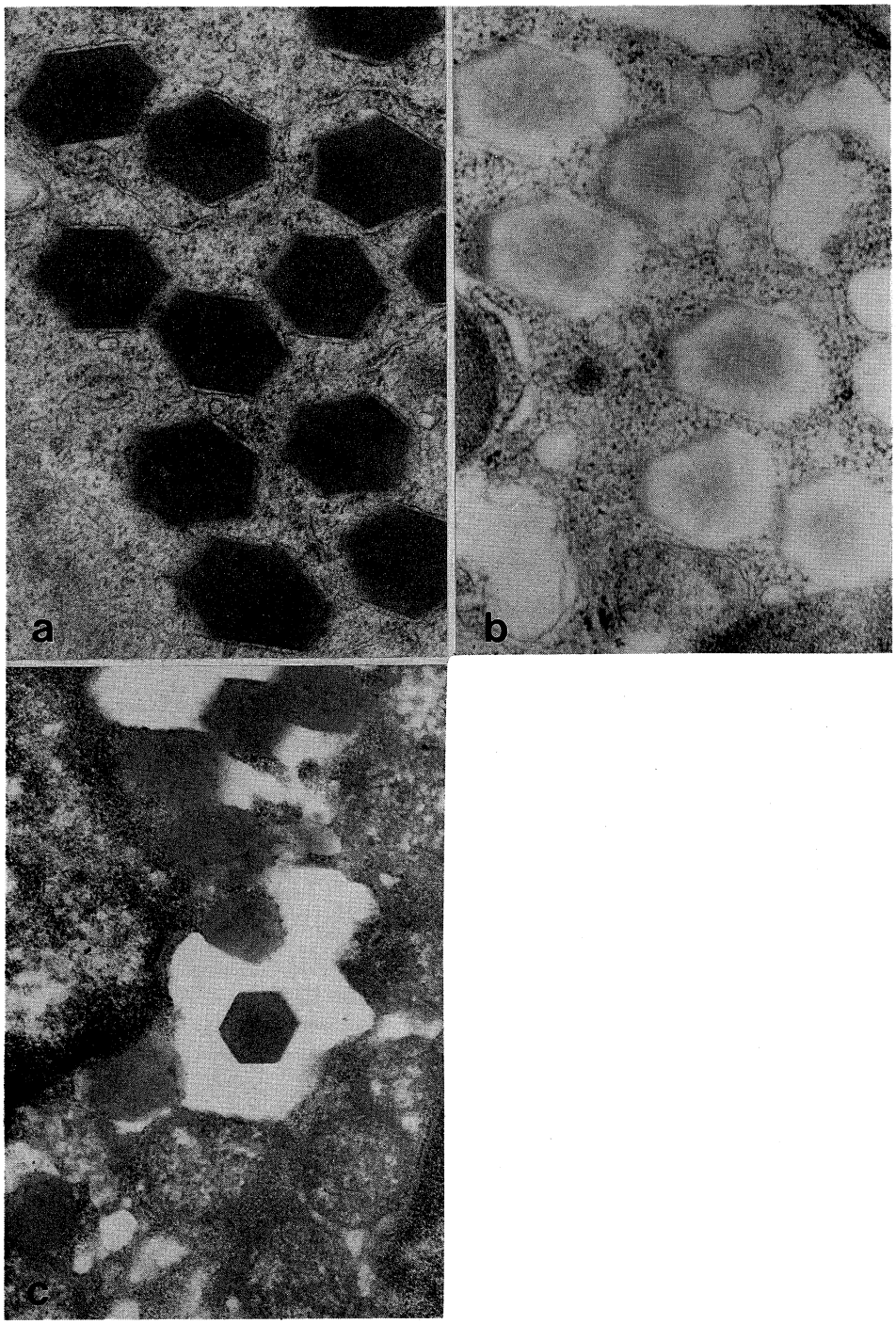

Fig. 5. Crystalline structures treated with pepsin and chloroform-methanol. a. Those without any digestive treatment for comparison. Mag., ×45,000 b. Those digested with pepsin. Note that crystalline structures are fading away. Mag., ×45,000 c. Those treated with chloroform-methanol. Crystalline structures remain intact, but areas adjacent to these structures widen. Mag., ×30,000

at least the crystalloid structures were composed mainly of protein substances. In view of the positive result in light microscopical and histochemical studies, however, we think that a small amount of phospholipids and polysaccharides somehow exist within these inclusions. As there seem to be considerable variations in the morphology among species, there may be some variations in their chemical composition. This assumption would explain the different results among investigators.[4–8] Further studies are, of course, required to determine whether Clara cells of different species pursue the same or similar functions.

Acknowledgment

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