LAMINATED MEMBRANE SURFACE AND OSMIOPHILIC INCLUSIONS IN AVIAN LUNG EPITHELIUM

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ABSTRACT

A study of the fine structure of the avian lung revealed the presence of a unique laminated membrane surface and associated osmiophilic inclusions in epithelial cells of the tertiary bronchi and atria. These structures were not found in the air-capillary epithelium. Each lamination of the membrane surface had the appearance and dimensions of the unit membrane. It is suggested that the laminated membrane surface is associated with the formation of the osmiophilic inclusions and that these inclusions compare with those described in mammalian alveolar epithelium. It is further suggested that the laminated membrane surface is lipoprotein or phospholipid in composition and is responsible for the surface-tension–reducing properties of avian lung extracts.

INTRODUCTION

Early investigations of the anatomy of the avian lung indicated that the blood-air pathway consisted of only a basement membrane and capillary endothelium (11). Later, however, epithelial cells lining the air capillaries were reported in both the pigeon and the chicken (4, 12). Those reports contained conflicting statements as to the presence, in the avian air-capillary epithelial cells, of inclusion bodies corresponding to the inclusion bodies of the alveolar epithelial cells of mammals. The investigators concerned studied only the blood and air capillaries of the lung parenchyma and did not describe the fine structure of the tertiary bronchi or atria.

In mammals, such inclusion bodies have been called mitochondrial transformations, lamellar transformations, lamellar forms, and osmiophilic bodies (8). Klaus *et al.* (7) and Clements (2) suggested that these lamellar inclusion bodies are the source of the "surface-active lining" of the mammalian lung. This surfactant has been demonstrated in mitochondrial extracts from lungs of the mouse, rat, guinea pig, rabbit, cat, dog, and cow. Extracts from lungs of amphibians, reptiles, and birds had less surface activity (7).

The present study describes a laminated membrane surface and associated osmiophilic inclusions in the epithelium of the tertiary bronchi and atria of the avian lung.

MATERIALS AND METHODS

Observations were made on the lungs of two 75-day-old Single-Comb White Leghorn chickens reared under strict isolation and free of all known diseases. The chickens were anesthetized with pentobarbital, the trachea isolated and cannulated, the sternum removed, and the lungs perfused with buffered osmium tetroxide prepared according to the method of Palade (10). Immediately after perfusion the lungs were removed, and small portions of tissue were immersed in fixative solution. The tissues were embedded in methacrylate and were oriented so that a portion of a tertiary bronchus with associated parenchyma would be cut in cross-section. Thin sections were cut on a Porter-Blum microtome with glass knives, post-stained with chromyl chloride according to the method of Bullivant and Hotchin (1), and

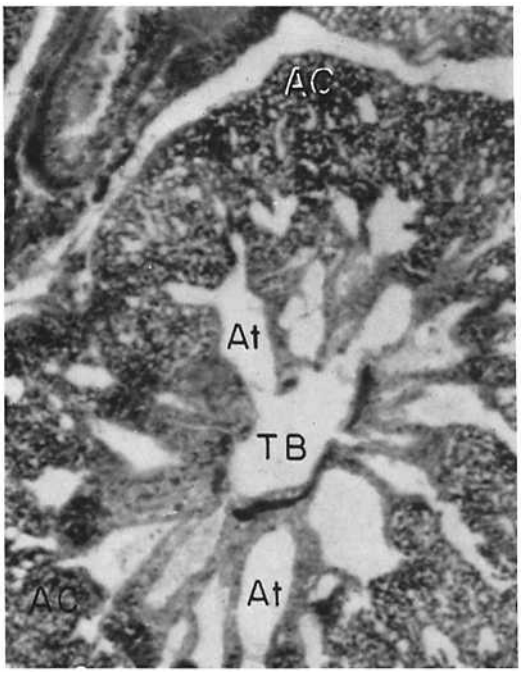


FIGURE 1 A light micrograph of a lobule of the avian lung in cross-section. The lumen of the tertiary bronchus (*TB*), the atrium (*At*), and the air capillary (*AC*) areas are shown. × 700.

examined with an RCA EMU-3E. Sections approximately 1 μ thick were stained by periodic acid–Schiff (PAS), hematoxylin and eosin, and toluidine blue, and were examined by light microscopy.

OBSERVATIONS

A laminated membrane surface was observed on the epithelial cells lining the tertiary bronchi and atria of normal chicken lungs (Figs. 1 to 3). The laminated surface, which varied greatly in thickness and in number of laminations, was discontinuous and did not line the entire lumen. Thicknesses up to 600 mμ were observed. Breaks and fragments were frequent, indicating fragility. The laminations, which had the dimension and appearance of a unit membrane, consisted of a series of alternating osmiophilic layers approximately 25 A thick separated by electron-transparent layers about 25 A thick (Fig. 4). Separated lamellae were frequently observed to penetrate between two adjacent cells or to extend below the cell surface (Figs. 5, 6). Closely associated with penetration of lamellae into the cells were cytoplasmic vesicles bounded by a membrane (Figs. 6, 7). In thick sections examined by optical microscopy, a PAS-positive, toluidine-blue–negative structure was observed on the free surface of the epithelial cells of the tertiary bronchi and atria.

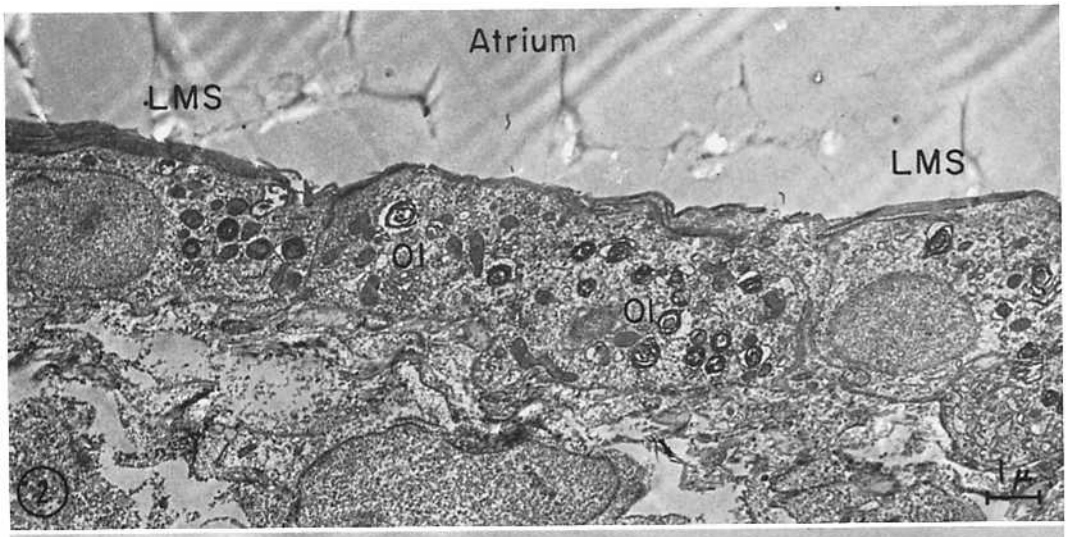
Lamellar osmiophilic inclusions observed in the cytoplasm of epithelial cells lining the tertiary bronchi and atria were distributed throughout the cytoplasm along with well preserved mitochondria (Fig. 3). The osmiophilic inclusions, approximately 0.5 μ in diameter, consisted of spiral or concentric membranes strongly osmiophilic. Atypical inclusions with homogeneous centers of medium electron opacity were observed occasionally (Fig. 8). Inclusions were also found at or near the cell surface in immediate association with the lamellar surface structure, and at these sites much disorganization of the surface was apparent (Figs. 8, 9). Osmiophilic inclusions were never observed in the epithelium of the air capillaries.

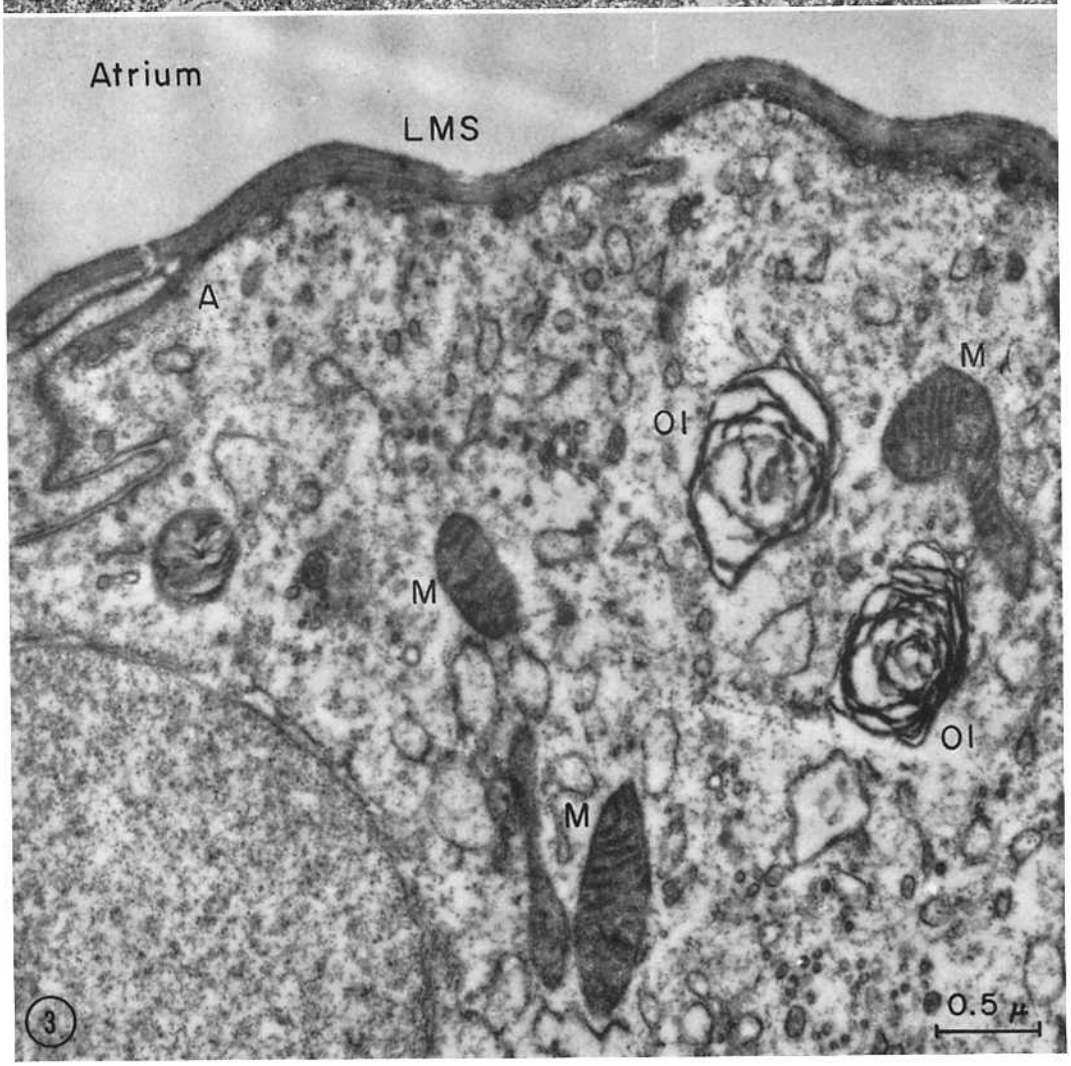
DISCUSSION

Careful investigation of the literature has not revealed description in any cell of a laminated membrane surface similar to the laminated membrane surface structure described in this report. The only similar structure is found in the myelin membranes of nervous tissue, first described by Fernández-Morán (5). The lamellar surface and osmiophilic inclusions were first observed in epithelial cells during preliminary studies of phagocytosis in the avian lung (13). Consequently, additional animals were observed to determine whether these structures were normal cellular constituents or were in some way related to the experimental procedures used to study phagocytosis. Two additional chickens, raised under strict

FIGURE 2 Low magnification electron micrograph showing epithelial cells lining the atrium. Relatively thick laminated membrane surfaces (*LMS*) are seen on the cells with distributed osmiophilic inclusions (*OI*). × 7,000.

FIGURE 3 A portion of an epithelial cell showing the laminated membrane surface (*LMS*), two osmiophilic inclusions (*OI*), and several well preserved mitochondria (*M*). At *A* the laminated surface extends between adjacent cells. × 28,000.





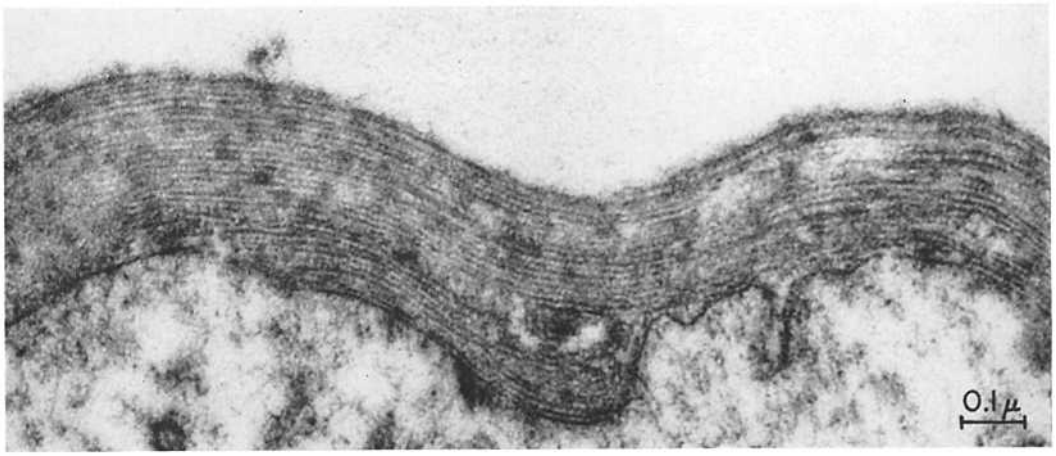


FIGURE 4 High magnification micrograph showing the individual lamellae of the laminated membrane surface. The spacing of the alternating layers is approximately 25 A. The cytoplasmic membrane adjacent to the cytoplasm is shown to be more osmiophilic than the other lamellar membranes. × 90,000.

solation, were examined, and both structures were found in each chicken. Since these chickens were free of all known diseases, it is concluded that these unusual structures are normal constituents of the epithelial cells of the tertiary bronchi and atria.

The sporadic occurrence of the laminated surface membranes would, at first, lead one to conclude that this unusual structure could have been deposited on the surface during perfusion of the lung with fixative solution. However, the continuation of the laminated membrane below the cell surface and between adjacent cells precludes this possibility. Another unusual feature is the membrane-bounded cytoplasmic vesicles (Fig. 7) associated with the penetration of the laminated membrane into the cell, but this cannot be described further at this time. It is concluded that the laminations are external to the cell since the unit membranes immediately bounding the cytoplasm of the cell are more osmiophilic than the other laminations and must represent the cell membrane (Fig. 4).

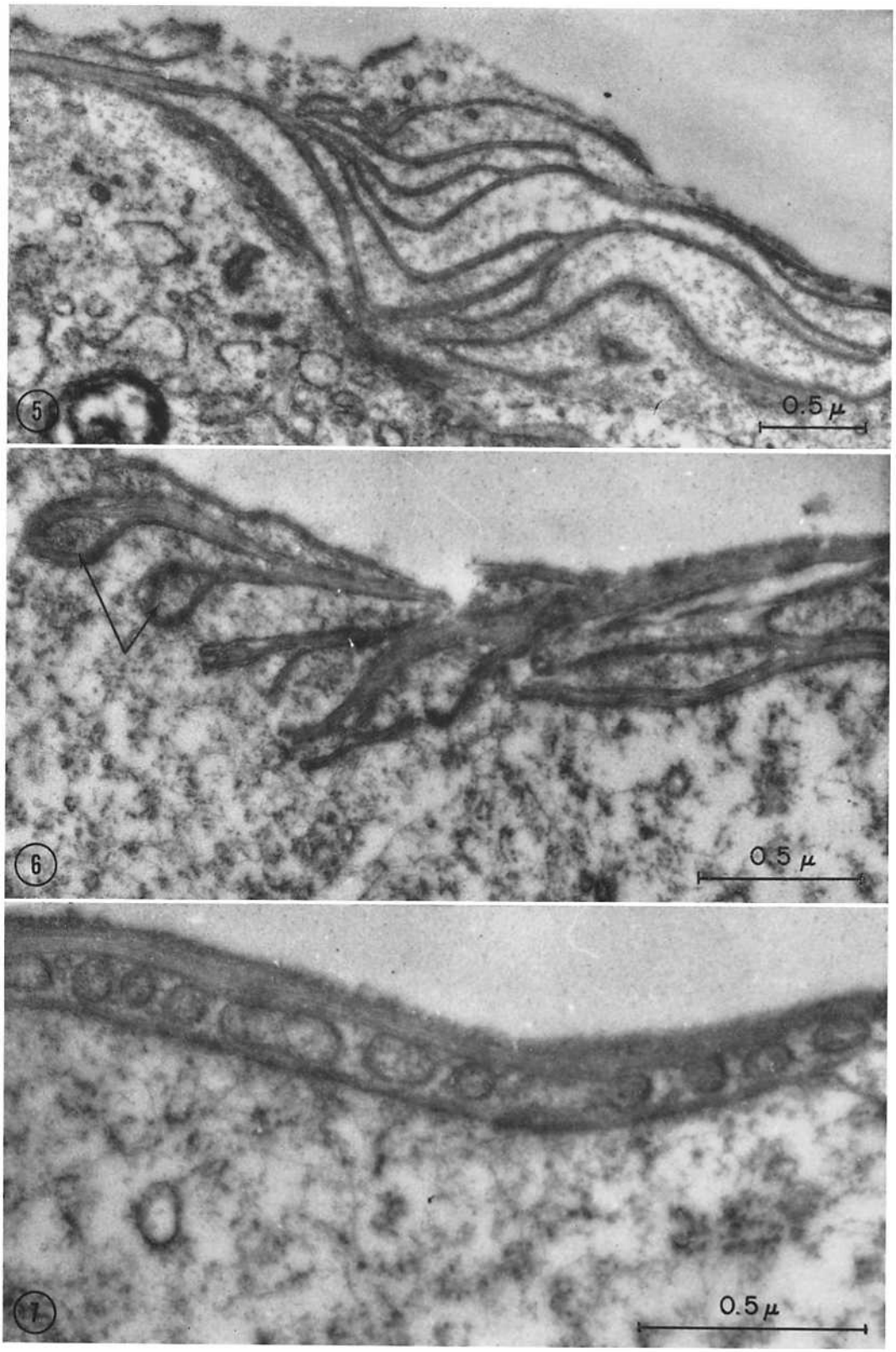
It is suggested that the laminated membrane surface is intimately associated with the formation of the osmiophilic inclusions. This suggestion is based on the observation that in each case in which an osmiophilic inclusion was observed at the surface of the cell the laminated surface structure was present and in many cases was continuous with the membrane structure of the inclusion (Figs. 8, 9). Osmiophilic inclusions were never seen at the surface if the laminated structure was absent.

Although osmiophilic inclusions have previously been reported in the lungs of mammals (7, 8, 11), they have not been observed in previous studies of the avian lung (4, 12). This exclusion is, no doubt, due to the restricted location of these bodies in the avian lung, since previous investigations of the avian lung centered on the blood-air pathway and no reports exist of the ultrastructure of the tertiary bronchus and atrium. In DeGroodt's (4) report of the blood-air pathway of the chicken, "band-like formations" in the epithelium were visible in his Fig. 2 and mentioned in the caption.

FIGURE 5 A micrograph showing branching of the laminated membrane surface. × 33,000.

FIGURE 6 Membrane-bounded cytoplasmic inclusions (arrows) surrounded by extensions of the laminated membranes that extend below the cell surface. × 51,000.

FIGURE 7 A row of membrane-bounded cytoplasmic particles between two layers of laminated membranes. × 71,000.



However, these structures were not described in his text. DeGroodt's micrograph may have been of a portion of the lobule bordering the atrium.

The osmiophilic inclusions described in the present report appear identical to those reported in mammalian alveolar epithelium cells (7, 8, 11). They are not, however, similar to the cytoplasmic inclusions found in the epithelium of the toad lung by Schultz (11), who considered these inclusions to be similar to the osmiophilic bodies or transformed mitochondria found in the alveolar epithelium of mammals. The relationship of the inclusions with medium-dense centers (Fig. 7) to the typical osmiophilic inclusions is uncertain.

Klaus *et al.* (7) related the surface activity found in mitochondrial fractions from lung tissue to the presence of the osmiophilic inclusions Although Miller and Bondurant (9) found that extracts prepared from chicken and frog lungs had less surface activity than extracts from mammalian lungs, it can be predicted that extracts of the tertiary bronchi and atria would have more surface activity than extracts from the whole lung.

When mammalian lung extracts were fractionated, the surface-active material was in the lipoprotein fraction (6). This activity persisted when the protein was removed and only phospholipid remained (3). Pure phospholipids, including lyolecithin, sphingomyelin, and dipalmitoyl lecithin, produce area-tension curves similar to those for mammalian lung extracts (6). The staining reactions of the laminated surface membrane indicate that it is a phospholipid or a lipoprotein and is probably the source of the surface-tension–reducing material of the avian lung.

Many additional observations must be made before the nature and function of laminated membrane surface and the osmiophilic inclusions of the chicken lung are fully elucidated.

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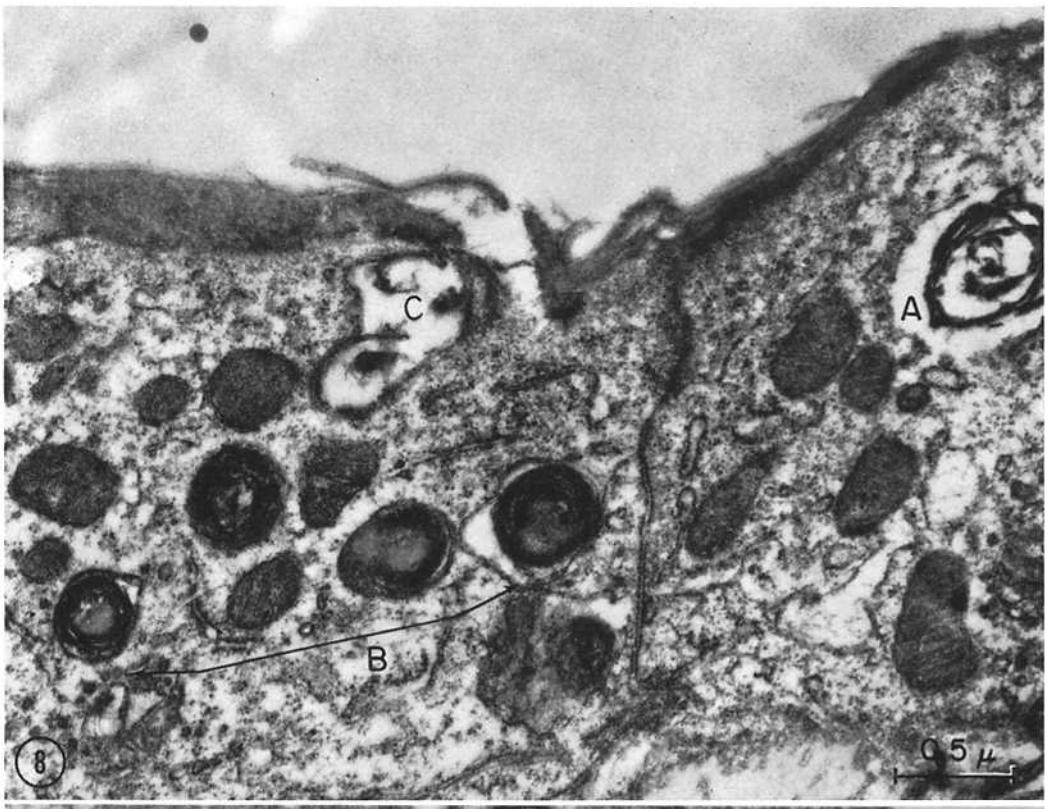
FIGURE 8 Several different forms of osmiophilic inclusions. A single inclusion at (*A*) shows the usual osmiophilic structure, whereas along area *B* several inclusions with medium-dense centers are observed. At *C* an inclusion is seen in contact with the laminated membrane surface. × 31,000.

FIGURE 9 An osmiophilic inclusion at the cell surface with its membranes apparently laminated membrane surface. × 37,000.



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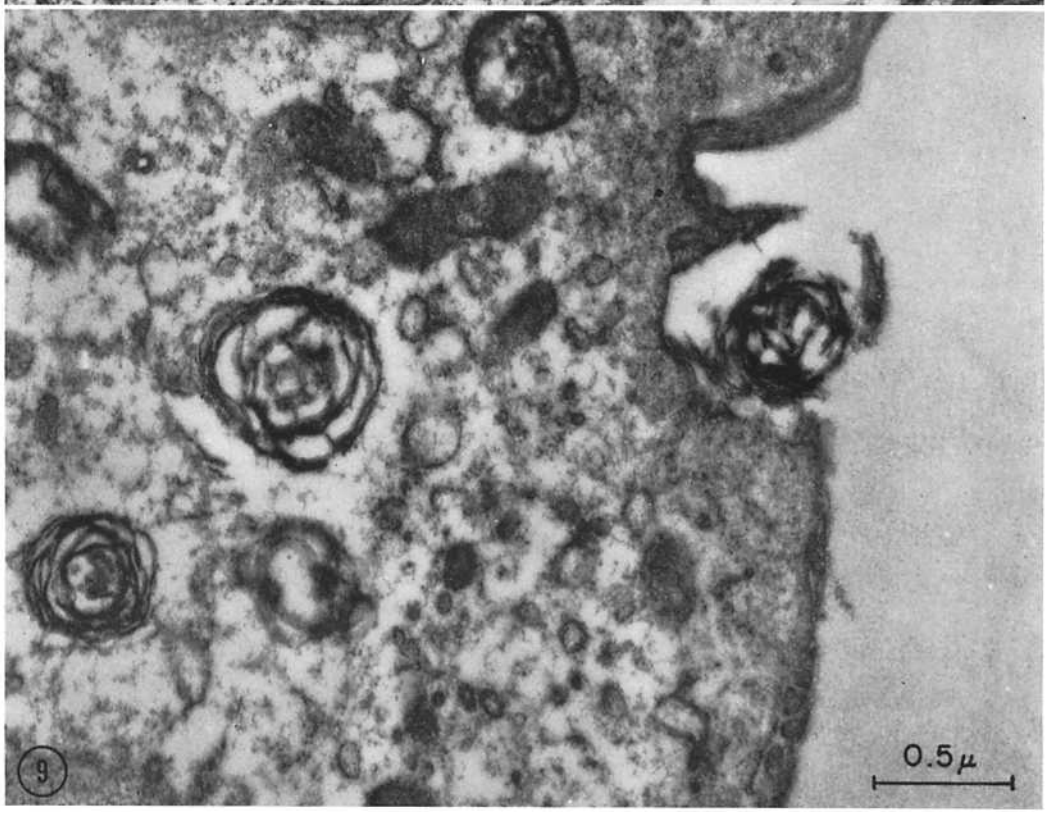

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