ELECTRON MICROSCOPY OF ORAL CELLS IN VITRO

I. Annulate Lamellae Observed in Strain KB Cells

M. KUMEGAWA, M. CATTONI, and G. G. ROSE. From the Department of Medicine (Periodontics), The University of Texas Dental Branch, and the Department of Biology, The University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77025

INTRODUCTION

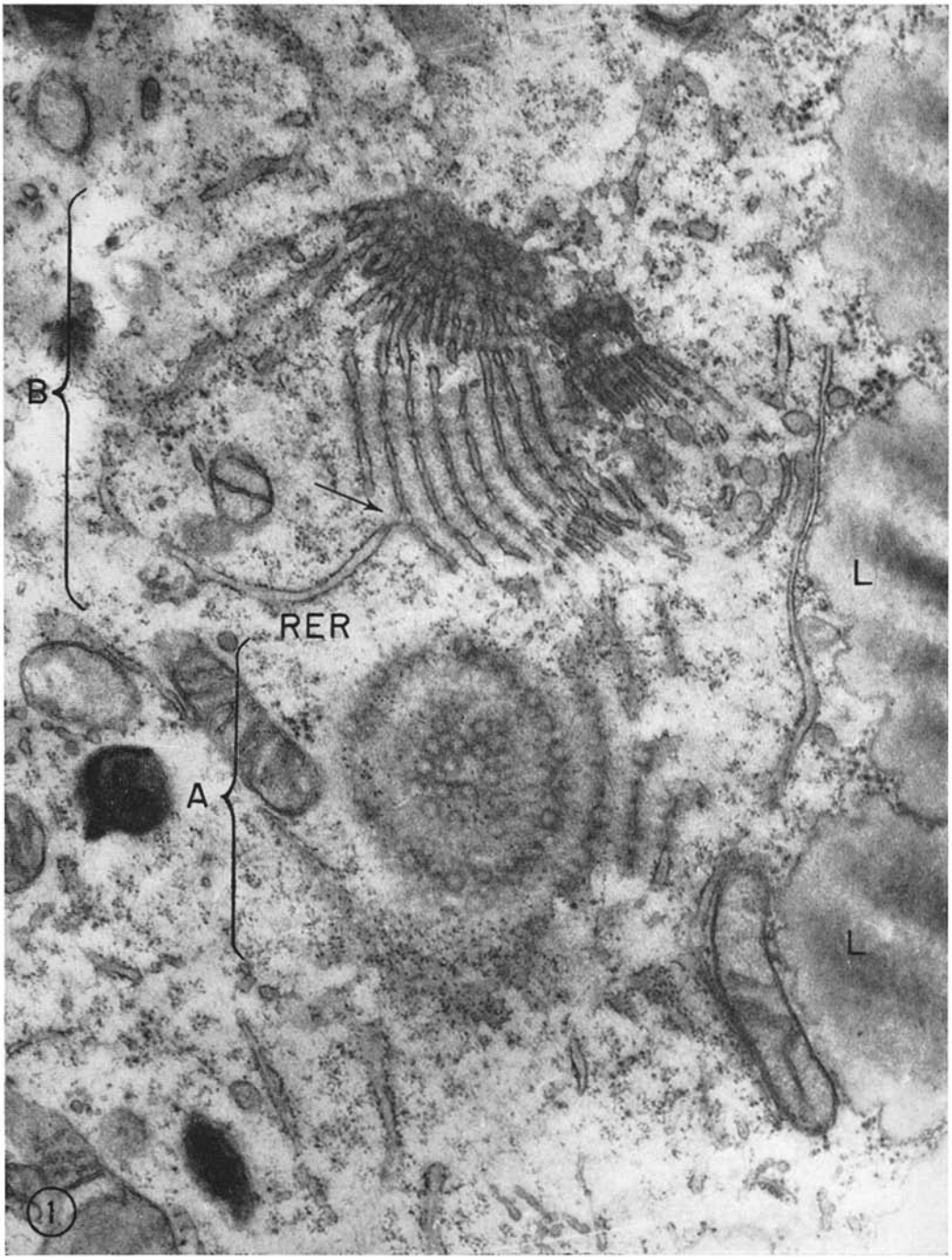
The term "annulate lamellae" was first used by Swift (22) to describe pairs of cytoplasmic membranes arranged in parallel stacks and containing periodically spaced annuli. Generally, annulate lamellae have been observed in the cytoplasm of lower organisms (7–9, 11, 14, 22). However, they also have been found in the cytoplasm of the adrenal cortex of fetal rats (19), the liver and pancreatic acinar cells of rats (6), the myocardium of chick embryos (10), and various kinds of animal tumor cells (1, 4, 5, 21). Intranuclear annulate lamellae are rare, thus far having been reported to occur in a few cells (7, 9, 11). These elements have not been reported to occur in many human cells. Our survey of the literature revealed descriptions of annulate lamellae in human cells in only three instances: Epstein (3) observed them in strain HeLa cells, Zamboni et al. (23) found them in the pronuclear stage of the ovum, and Nagano (12) identified them in Sertoli cells of the male gonad. Although they have been found in a wide variety of species, little is known of their origin and function (8, 9, 11).

The purpose of this report dealing with strain KB cells cultivated in roller tubes is to describe the presence of well-developed annulate lamellae in increasing numbers as compared to those found in KB cells under culture conditions less favorable for prolific growth. Many of these organelles had a special arrangement of their lamellae, appearing bell- or dome-shaped. This feature is shown in three micrographs of serial sections and a diagram derived from them and other illustrations.

MATERIALS AND METHODS

The KB cell strain was established as an in vitro strain from a human epidermoid carcinoma by Eagle (2) in 1955. The cultures used in this report were obtained in roller tubes from a commercial source (Microbiological Associates, Inc., Bethesda, Md.). In this laboratory, the KB strain has been maintained in roller tubes; the nutrient medium has consisted of equal parts of Medium 109 and Medium V-614 (Difco Laboratories, Detroit, Mich.) supplemented with calf serum (20%, Microbiological Associates, Inc.), whole egg ultrafiltrate (5%, Microbiological Associates, Inc.), phenol red (0.001%), and penicillin G (1,000 units/ml).

After 8 and 30 days of growth in roller tubes, the KB cells were harvested. All specimens were fixed in phosphate-buffered osmium tetroxide (1%) for 1 hr at 4°C or phosphate-buffered glutaraldehyde (3%) for 1 hr before fixation in the osmium tetroxide. Subsequent to their rapid dehydration in a series of cold alcohols and treatment with propylene oxide, the specimens were embedded in Araldite. After dehydration by the 95% alcohol, the KB cells were removed from the roller tubes with a rubber policeman, and were centrifuged into pellets. The embedded specimens were sectioned on a Porter-Blum microtome with a glass knife, and were stained with lead citrate. Examinations and micrographs were made with an RCA EMU-3C electron microscope.



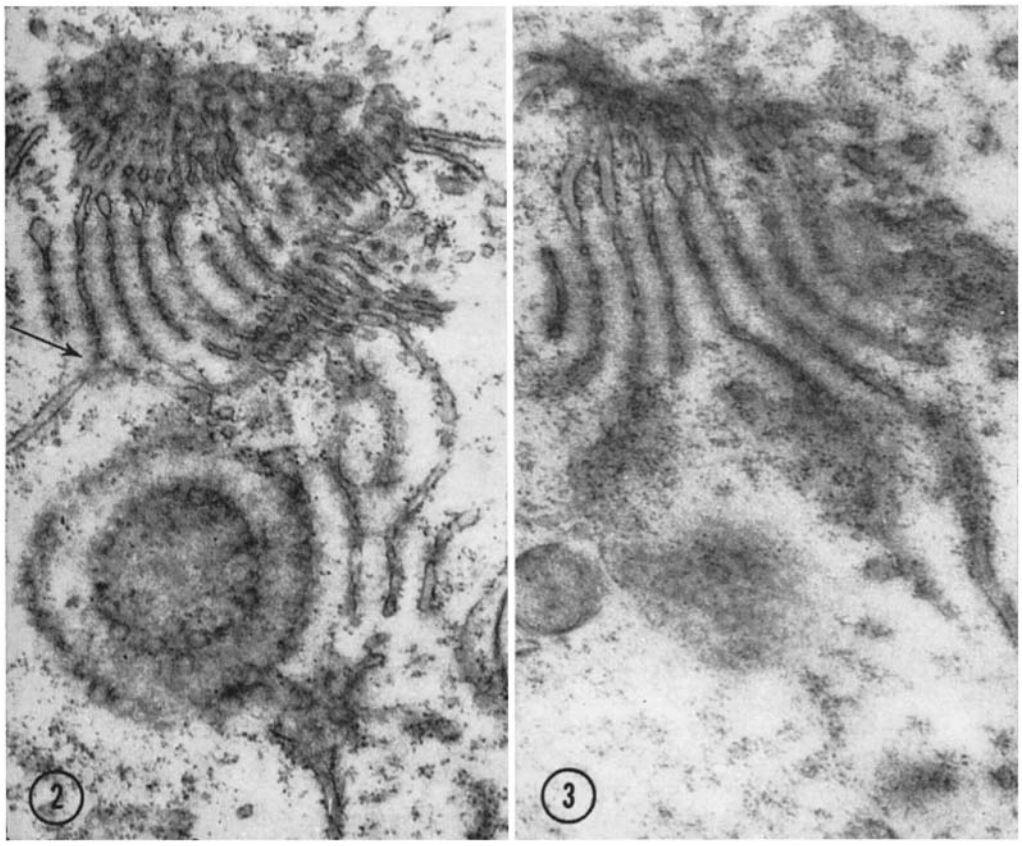
FIGURES 1–3 Serial sections through a bell-shaped stack of annulate lamellae in KB cells grown in roller tubes. The radial section (stack *B*) shows a periodicity of the lamellae, and the transverse section (stack *A*) shows the concentric arrangement of the pores. In the lower section through stack *A* (Fig. 1), the number of rings of pores is increased over that of the upper profile levels (Figs. 2 and 3). In the outer lamellae, the pores were sectioned progressively more obliquely. Communication with the rough-surfaced endoplasmic reticulum (*RER*) is noted in Figs. 1 and 2 (arrows). × 39,000.

RESULTS

Cytoplasmic annulate lamellae were observed in many KB cells cultivated in roller tubes. The lamellae were well developed and characteristically were arranged in stacks of 2–20 layers. Individual lamellae often appeared to expand into spherical or elongated vesicles and to be continuous with the rough-surfaced endoplasmic reticulum (Figs. 1 and 2, arrows). Ribosomes were sometimes observed along the outer surfaces of the lamellae as well as in the cytoplasm between the lamellae.

Structural details of the annulate lamellae in KB cells were not different from those described for annulate lamellae in other cells, but special arrangements of the lamellae often were encountered. Two stacks of lamellae (*A* and *B*) at right angles and adjacent to each other are shown in serial sections (Figs. 1–3). The three levels of stack *A* suggest that the lamellae might be roughly bell-shaped, for when the sections were cut nearly transversely a circular symmetry of pores was revealed. The number of rings increased from Fig. 3 to Fig. 1 as the sections were cut deeper into the *A* portion of the organelle, the pores of the outer ring being sectioned progressively more obliquely. In the profile of stack *B* the symmetry at the top of the dome was not so perfect, although the bell-shaped design is apparent. A three-dimensional schema was derived from these three sections, from other micrographs not shown in the report and from studies of other researchers (14, 19, 21); the schema is shown in Fig. 4. It should be remembered that this schema is only a diagrammatical interpretation of the bell-shaped arrangement of the lamellae.

When the lamellae were sectioned grazingly, small circular profiles or annuli were observed which were similar to the pores in the nuclear envelope. In cases in which the lamellae were



FIGURES 2 and 3 See legend under Fig. 1.

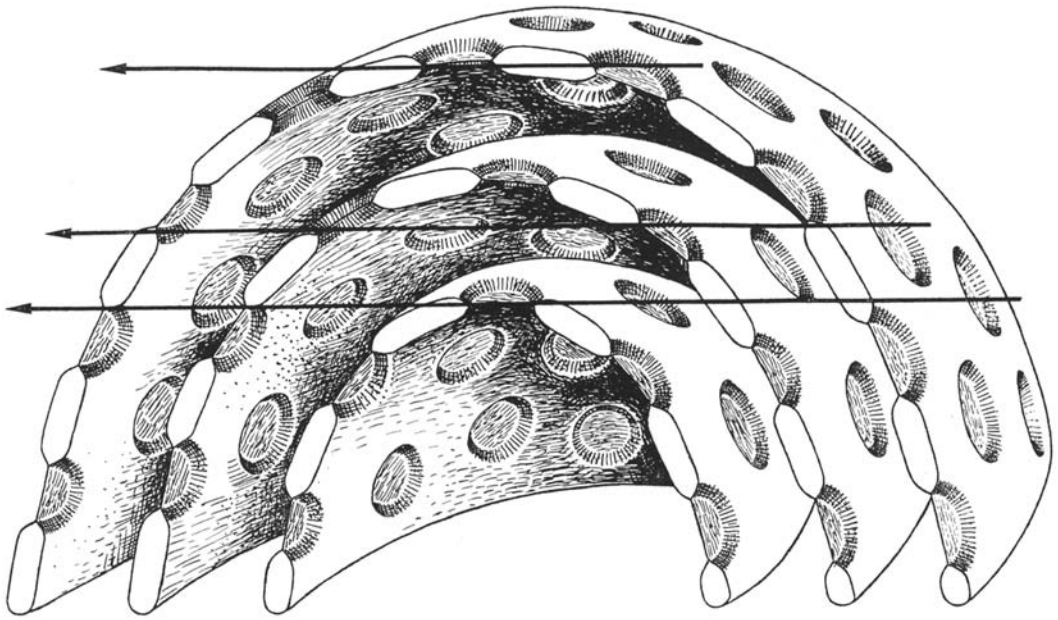


FIGURE 4 Diagram of a bell-shaped stack of annulate lamellae constructed from stack *A* in the series of three micrographs in Figs. 1–3. The arrow-tipped lines coincide with the level of stack *A* in the three micrographs: Fig. 1, lower line; Fig. 2, middle line; Fig. 3, upper line.

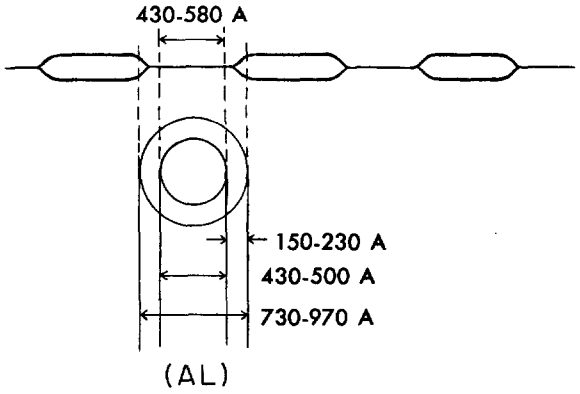


FIGURE 5 Schematic diagram showing the dimensions of the pores and membrane spacings of the annulate lamellae (*AL*) in KB cells.

sectioned normally so as to display the annuli in side view, dense, diaphragm-like membranes appeared to traverse the annular regions. The pore sizes and membrane spacings are shown in the diagram in Fig. 5.

DISCUSSION

Porter (13) stated that annulate lamellae were most prominent in actively growing and multiplying cellular populations. The KB strain, derived from a human epidermoid carcinoma, is an excellent example of such a population. Merkow and Leighton (10) added to this basic concept the effect of environmental alterations, as they had found an increase in the numbers of annulate lamellae in the myocardium of chick embryos incubated at abnormally low temperatures.

In our study, the well-developed annulate lamellae were observed in many cells of the rapidly growing cultures in roller tubes. Although these organelles were found also in the KB cells cultured under other conditions, such as the serum-free environments under dialysis membranes (15–18) and the serum-restricted environments of perforated cellophane used according to Sandstrom's method (20), their frequency and size were decreased. The special arrangements of the lamellae in bell-shaped forms were not detected in the KB

cells cultured under these membrane-restricted conditions which reduced their multiplication propensity.

Despite the production of well-developed annulate lamellae in the KB cells by the roller tube culture method, new insights into their origin were not obtained. Similarly, except for the demonstration of their continuity with the rough-surfaced endoplasmic reticulum, their function remains an enigma. Present studies are centered about studying the effects of physical and biological factors of the microenvironment on the size, shape, and numbers of annulate lamellae and other organoids and organelles specifically as they relate to the growth and population differentiation of this cell line.

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REFERENCES

1. CHAMBERS, V. C., and R. S. WEISER. 1964. An electron microscope study of Sarcoma I in a homologous host. I. The cells of the growing tumor. *Cancer Res.* **24**:693.
2. EAGLE, H. 1955. Propagation in a fluid medium of a human epidermoid carcinoma, strain KB. *Proc. Soc. Exptl. Biol. Med.* **89**:362.
3. EPSTEIN, M. A. 1961. Some unusual features of fine structure observed in HeLa cells. *J. Biophys. Biochem. Cytol.* **10**:153.
4. HOSHINO, M. 1963. Submicroscopic characteristics of four strain of Yoshida ascites hepatoma of rats. A comparative study. *Cancer Res.* **23**:209.
5. HRUBAN, Z., H. SWIFT, and M. RECHEIGL, JR. 1965. Fine structure of transplantable hepatomas of the rat. *J. Natl. Cancer Inst.* **35**:459.
6. HRUBAN, Z., H. SWIFT, and A. SLESERS. 1965. Effect of azaserine on the fine structure of the liver and pancreatic acinar cells. *Cancer Res.* **25**:708.
7. KESSEL, R. G. 1964. Intranuclear annulate lamellae in oocytes of the tunicate, *Styela partita*. *Z. Zellforsch. Mikroskop. Anat.* **63**:37.
8. KESSEL, R. G. 1964. Electron microscope studies on oocytes of an Echinoderm, *Thyone briareus*, with special reference to the origin and structure of the annulate lamellae. *J. Ultrastruct. Res.* **10**:498.
9. KESSEL, R. G. 1965. Intranuclear and cytoplasmic annulate lamellae in tunicate oocytes. *J. Cell Biol.* **24**:471.
10. MERKOW, L., and J. LEIGHTON. 1966. Increased numbers of annulate lamellae in myocardium of chick embryos incubated at abnormal temperatures. *J. Cell Biol.* **28**:127.
11. MERRIAM, R. W. 1959. The origin and fate of annulate lamellae in maturing sand dollar eggs. *J. Biophys. Biochem. Cytol.* **5**:117.
12. NAGANO, T. 1966. Fine structure of the sertoli cells in the human testis. Proceedings of the Sixth International Congress on Electron Microscopy, Kyoto, 1966. R. Uyeda, editor. Maruzen Co., Ltd., Tokyo.
13. PORTER, K. R. 1961. The ground substance; observations from electron microscopy. 1961. *In* The Cell. J. Brachet and A. E. Mirsky, editors. Academic Press Inc., New York. 652.
14. REBHUN, L. I. 1956. Electron microscopy of basophilic of some invertebrate oocytes. II. Fine structure of the yolk nuclei. *J. Biophys. Biochem. Cytol.* **2**:159.
15. ROSE, G. G., C. M. POMERAT, T. O. SHINDLER, and J. B. TRUNNEL. 1958. A cellophane-strip technique for culturing tissue in multipurpose culture chambers. *J. Biophys. Biochem. Cytol.* **4**:761.
16. ROSE, G. G., M. CATTONI, and C. M. POMERAT. 1963. Observations on oral tumors *in vitro*. 1. Zeiosis versus the nucleus and endoplasmic reticulum in strain KB. *J. Dent. Res.* **42**:38.
17. ROSE, G. G., M. CATTONI, and C. M. POMERAT. 1964. Observations on oral tumors in vitro. 2. Karyobiosis in strain KB cells. *J. Dent. Res.* **43**:580.
18. ROSE, G. G. 1966. Observations on oral tumors in vitro. 3. Morphological properties, longevity of nonvital intranuclear inclusions. *J. Natl. Cancer Inst.* **36**:747.
19. ROSS, M. H. 1962. Annulate lamellae in the adrenal cortex of the fetal rat. *J. Ultrastruct. Res.* **7**:373.
20. SANDSTROM, B. 1965. Studies on cells liver tissue cultivated *in vitro*. I. Influence of the culture method on cell morphology and growth pattern. *Exptl. Cell Res.* **37**:552.
21. SCHULZ, V. H. 1957. Elektronenmikroskopishe Untersuchungen eines Mammarkarzinoms der Ratte. *Oncologia.* **10**:307.
22. SWIFT, H. 1956. The fine structure of annulate lamellae. *J. Biophys. Biochem. Cytol.* **2** (Suppl. 2): 415.
23. ZAMBONI, L., D. R. MISHELL, JR., J. H. BELL, and M. BACA. 1966. Fine structure of the human ovum in the pronuclear stage. *J. Cell Biol.* **30**:579.