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ULTRASTRUCTURE OF THE CAPSULAR EPITHELIUM OF RADICULAR CYSTS

by

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INTRODUCTION

The radicular cyst develops from an apical granuloma either by epithelial proliferation and subsequent lining of a cavity within the granuloma (*Hammer, 1934, Kronfeld, 1955*) or by a breakdown of a central part of an epithelial nest located in the granuloma (*Hill, 1930*).

The expansive growth of the cyst is dependent upon the osmotic pressure within the cystic fluid (*Tratman, 1939, Stokke, 1956*); this pressure has been measured by *Toller (1948)* and *Stokke (1956)*. The osmotic pressure is considered to be primarily due to inflammation, which results in decomposition of desquamated epithelial cells, with a subsequent rise in the molarity of organic components (*Stokke, 1956*).

The light microscopic features of the radicular cyst are familiar. The object of the study reported in this paper was to analyze the fine structural characteristics of the capsular epithelium.

MATERIAL AND METHODS

The material comprised twelve radicular and residual cysts which were studied by electron microscopy (Table 1).

Immediately after careful removal of the cystic capsule from the bony cavity a small piece about 2 mm wide was immersed

Table 1
Presentation of material

Cyst no.	Sex & age of patient		Type of cyst:			Appearance of epithelium				
			Position	Radicular Residual	Inflam- mation ^b	Light microscope ^c			Electron microscope	
					0	1	2			
1	F	37	Maxilla	Ra			x	found		
2	M	44	»	Re			x	»		
3 ^a	F	33	Mandible	Ra			x	»		
4 ^a	F	33	»	Re	x		x	not found		
5	M	39	»	Ra			x	found		
6	F	56	Maxilla	Re	x		x	»		
7	M	40	Mandible	Re			x	»		
8	M	67	»	Re		x	x	not found		
9	M	70	»	Re		x	x	found		
10	M	65	»	Re		x	x	»		
11	F	42	Maxilla	Ra			x	»		
12	M	58	Mandible	Re	x		x	»		
Total:	Male: 7		Maxilla: 4	Ra: 4	3	5	4	1	4	7
	Female: 5		Mandib.: 8	Re: 8						

^a Cysts nos. 3 and 4 were from one patient.

^b 0: no inflammation, 1: moderate or mild inflammation, 2: severe inflammation.

^c 0: not present, 1: local, 2: covering the cystic cavity.

in cold, one per cent buffered osmium tetroxide solution (*Rhodin*, 1954) and kept at 5° C for 2—3 hours. After rinsing and dehydration the specimen was embedded in Epon or Vestopal. Thin transverse sections of the epithelial capsule were cut with an Ultratome (LKB), collected on Formvar-coated copper grids, stained in 1.5 per cent uranyl acetate or lead citrate solution and examined by electron microscopy (Siemens Elmiskop I). The rest of the cystic capsule was fixed in 10 per cent formaldehyde and submitted to routine histologic examination.

RESULTS

In two capsules no epithelium could be found in the tissue prepared for electron microscopy. In the other 10 specimens the capsules were lined with a stratified squamous epithelium, which was only 3—10 cells in height (Fig. 1).

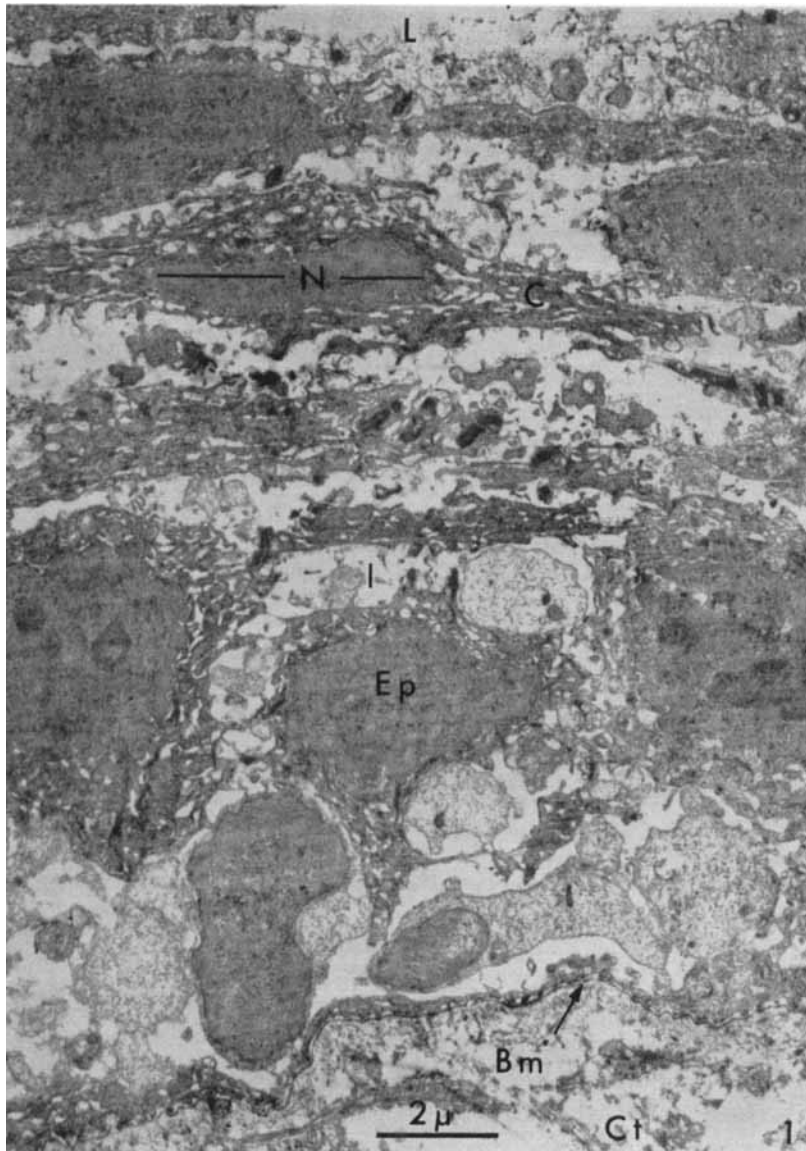


Fig. 1. Epithelial capsule from cyst no. 1. Between the epithelium and connective tissue (Ct); is the basement membrane (Bm). The epithelial cells (Ep) are separated by a large intercellular space (I). Large highly electron dense nuclei (N). The cytoplasm (C) is split up by numerous less dense channels and vacuoles. Flattened cells close to the cystic lumen (L). $\times 8,000$.

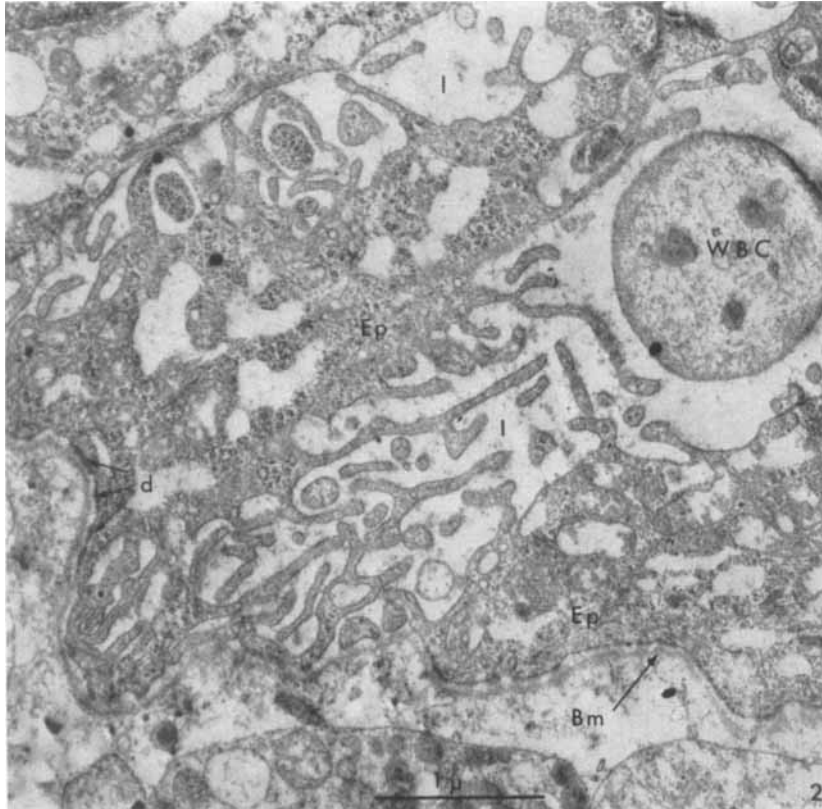


Fig. 2. Epithelial capsule from cyst no. 5. Long cytoplasmic processes of the epithelial cells (Ep) project into the intercellular space (I). White blood corpuscles (WBC) within the epithelium. Close to the basement membrane (Bm) the half-desmosomes (d) are seen as thickenings of the plasma membrane. $\times 22,000$.

All 10 capsules displayed structures which are known to be present in non-keratinizing epithelium of the oral cavity (*Sognnaes, 1958, Themann, 1958, Fasske, 1959, Albright, 1960, Zelickson, 1962*). However, the over-all pattern displayed considerable differences between the specimens as regards the spatial relation between the epithelial cells, the quantities of ribosomes, mitochondria, tonofilaments and desmosomes (compare figs. 1—4). In several capsules the intercellular space was strikingly large and numerous slender cytoplasmic processes were found (Figs. 1, 2). In these specimens there were only a few desmosomes.

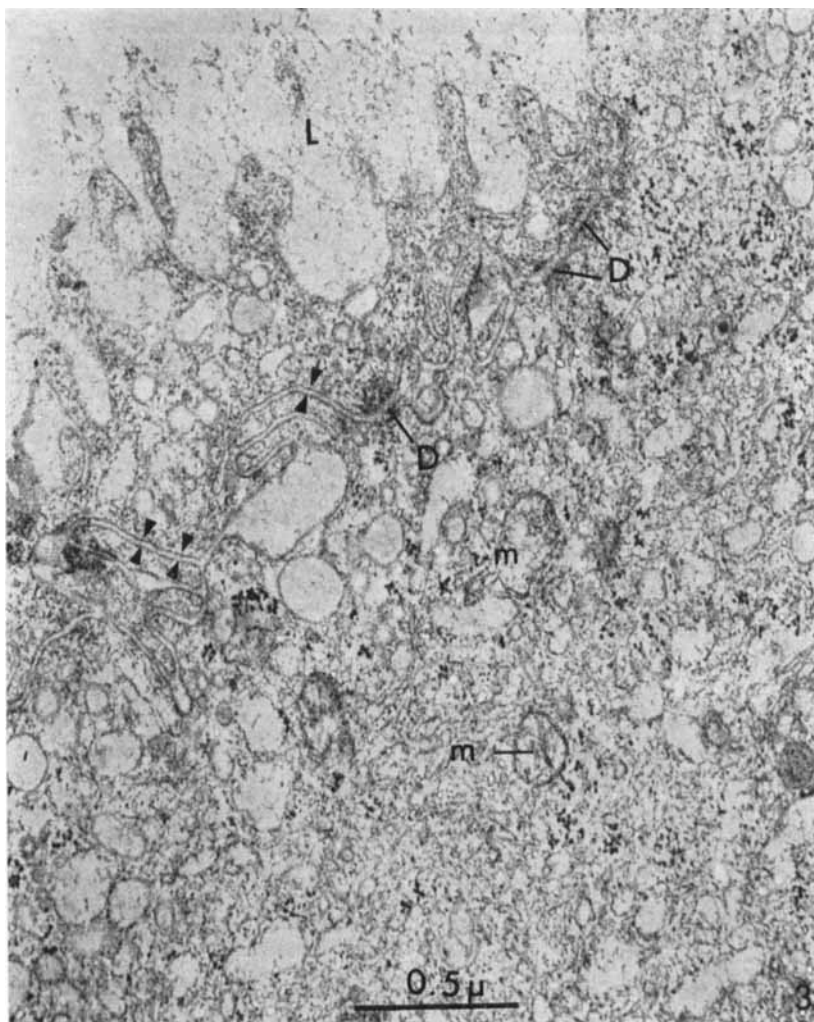


Fig. 3. Epithelial capsule from cyst no. 3. Cells in the superficial part of the epithelium are seen close together and the spaces between them are of constant width (arrow-heads). These areas of proximity are easily distinguished from the desmosomes (D). Mitochondria (m) can be identified even in superficial cells. L: Lumen. $\times 44,000$.

A striking feature of a few of the capsules was the great number of mitochondria within the basal part of the epithelium (Fig. 4). They were often elongated and exhibited a distinct system of transverse cristae. The mitochondria decreased in number to-

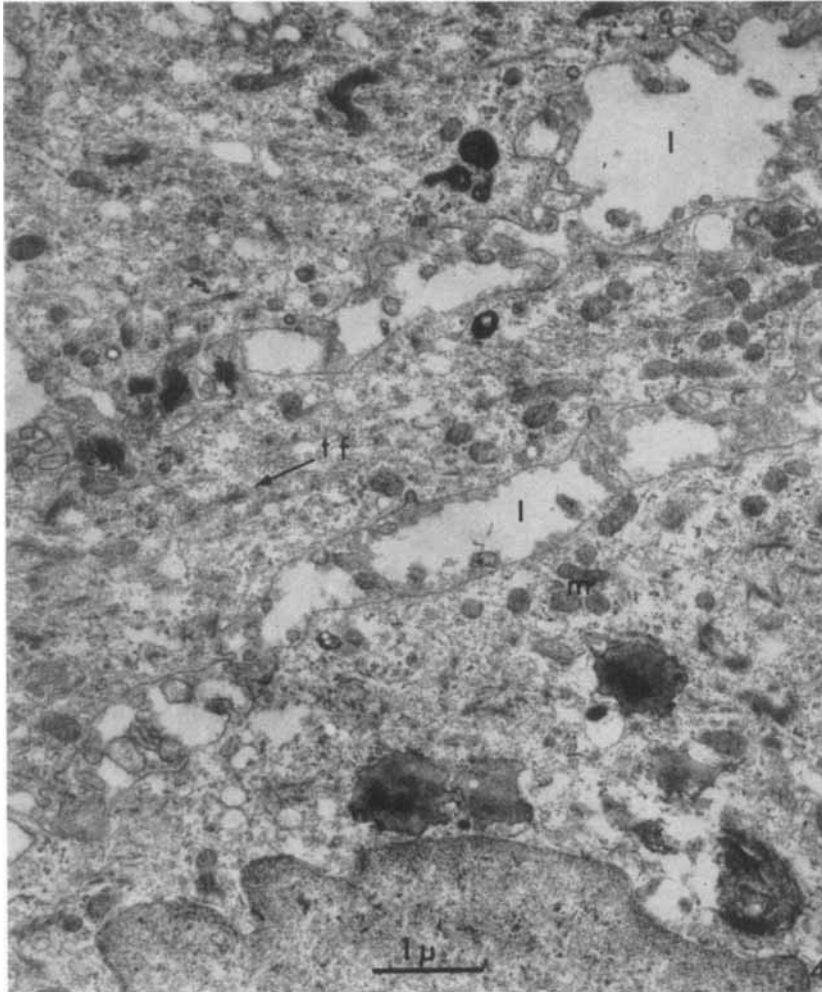


Fig. 4. Cyst no. 6. Central part of epithelium. Tonofilaments (tf) and mitochondria (m) are seen in the cytoplasm. I: Intercellular space. $\times 15,000$.

wards the surface of the epithelium but could still be identified in the superficial cells (Fig. 5). Ribosomes, numerous in most specimens and often ranged along α -cytomembranes, occurred throughout the epithelium. Tonofilaments were present in all specimens but varied in number from case to case.

There were no Odland bodies (*Odland*, 1960), which are char-

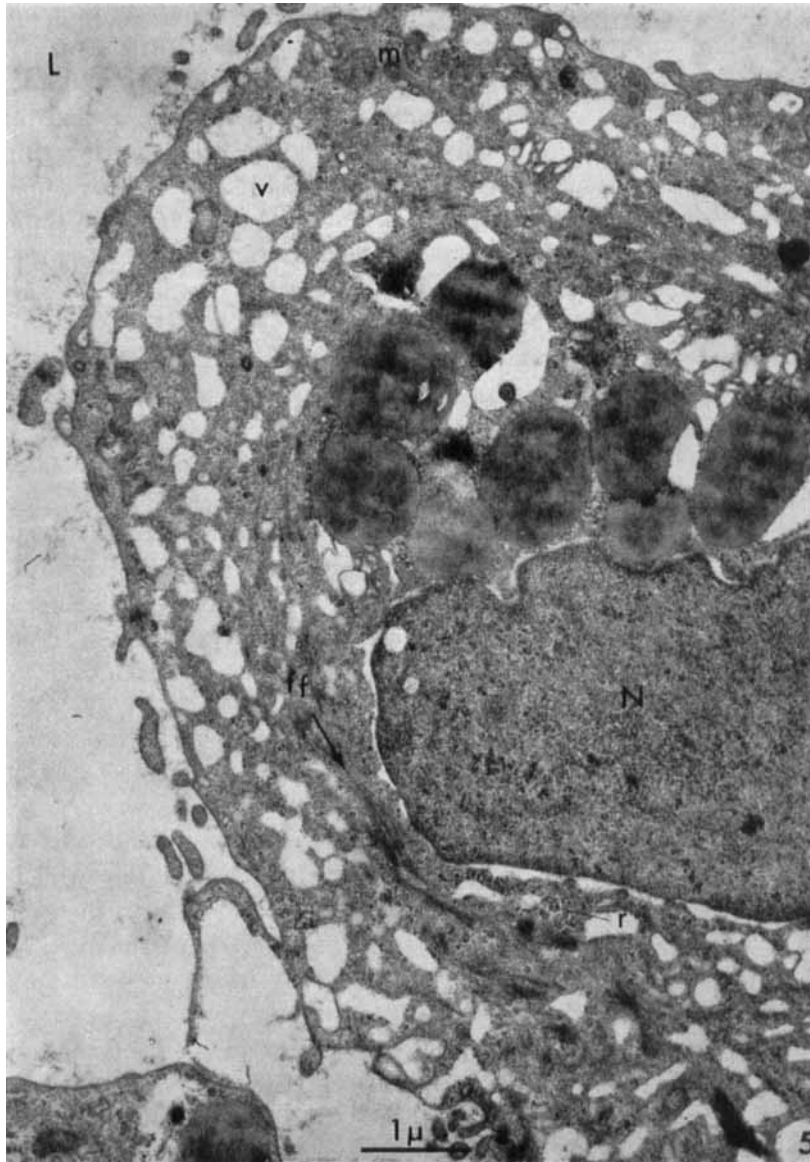


Fig. 5. Cyst no. 6. Part of superficial cell with nucleus (N), tonofilaments (tf), mitochondria (m) and ribosomes (r). v: Vacuole, L: lumen. $\times 15,000$.

acteristic of keratinizing oral epithelium (*Frithiof & Wersäll, 1965*) and no keratohyaline substance.

Partly decomposed fragments of epithelial cells and white blood corpuscles were occasionally observed on the surface of the epithelium, White blood corpuscles were often seen within the connective tissue and the epithelium (Fig. 2).

In all specimens the epithelium was separated from the connective tissue by a distinct basement membrane. There were numerous half-desmosomes, visualized as dense areas of the plasma membrane facing the basement membrane (Fig. 2).

In one case the cystic lumen was separated from the epithelium by a thin, well-defined membrane under which flattened erythrocytes were found.

In spite of the presence of all the organelles known from studies on normal non-keratinizing human oral epithelium (*Zellickson, 1962*), the capsular epithelium differs strikingly in appearance from that of the oral mucosa. The most outstanding characteristics of the capsular epithelium in the present material are the intercellular relationship described above, the loose structure of the cytoplasm (Figs. 1, 2, 5) and a large number of intact organelles in superficial cells.

DISCUSSION

Tratman (1939) proposed that the capsule as a whole constitutes a semipermeable membrane such as would be appropriate for the production and maintenance of the osmotic pressure necessary to account for the expansive properties of the cyst. No specific structure within the capsule that might be associated with this function has been found, however.

In most of the cystic capsules of the present material the epithelium has a comparatively loose structure with a large intercellular space, which appears to be continuous from the lumen of the cyst to the basement membrane. To judge from the electron microscopic structure the epithelium would seem unable to maintain the required pressure difference. However, the lack of structure in the intercellular space does not necessarily mean that it contains only tissue fluid. It has been reported that substances containing polysaccharides are also present (*Wislocki*

et al., 1951). In some of the cysts closely packed cells were observed superficially in the epithelium, the intercellular space being reduced to a narrow gap of approximately constant width (Fig. 3). It was impossible to ascertain whether these areas were part of a continuous zone of adherence delimiting the cystic cavity, or whether they constituted only a limited area, as is the case of the desmosomes (*Odland*, 1958). The significance of the intercellular space as an osmoregulatory barrier is therefore difficult to judge.

In other parts of the organism, for example the kidneys and the capillary wall, the basement membrane, which is probably synthesized by the epithelium (*Pierce et al.*, 1964, *Mukerjee et al.*, 1965), has important functions (*Pease*, 1958). Combined electrophysiological and electron microscopical studies on frog skin (*Ottoson et al.*, 1953) have shown that a resting potential is maintained across the basement membrane. It is therefore probable that the semipermeable properties necessary for the expansive growth of the cyst are possessed by the basement membrane.

The condition of the cystic epithelium is dependent on the degree of inflammation (*Forsberg & Hägglund*, 1959, *Molyneux*, 1964, *Shear*, 1964). Mild, chronic inflammation may stimulate the proliferation of the epithelium, whereas more severe inflammation might lead to degeneration, ulceration and disintegration of the epithelium. In the absence of inflammation the epithelium is thin and regular, with smooth surfaces towards the lumen and the connective tissue. The factors governing the appearance of the epithelium as seen under the light microscope might also be reflected on the ultrastructural level, the different degrees of inflammation accounting for the structural variations found within the epithelial capsules in this small series. A classification of a larger material into expansive or static cysts would provide a firmer basis for the morphological interpretation.

SUMMARY

In an electron microscopic study of squamous epithelium from the capsule of radicular cysts, wide structural variations between specimens were found. These variations are probably ascribable to differences in the degree of inflammation within the cystic capsule.

10 capsules of the 12 cysts studied displayed a non-keratinized, stratified squamous epithelium which, however, was easily distinguishable from normal oral mucosa.

The expansive growth of the cyst is known to be due to the hypertonicity of the cystic fluid. The basement membrane probably acts as a semipermeable membrane.

Avenues of approach for further studies are suggested.

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RÉSUMÉ

ULTRASTRUCTURE DE L'ÉPITHÉLIUM CAPSULAIRE DU KYSTE RADICULE-DENTAIRE

Au cours d'une étude au microscope électronique de l'épithélium pavimenteux provenant de la capsule de kystes radiculo-dentaires, de grandes différences de structure ont été trouvées entre les spécimens. Ces variations peuvent probablement être attribuées à des différences dans le degré d'inflammation existant à l'intérieur de la capsule kystique.

Les 10 capsules considérées présentaient toutes un épithélium pavimenteux stratifié non kératinisé, qui était cependant facile à distinguer de la muqueuse buccale normale.

On sait que la croissance expansive du kyste est due à l'hypertonie du liquide kystique. La membrane basale agit probablement comme une membrane semi-perméable.

L'auteur suggère des voies d'approche pour la réalisation d'études ultérieures.

ZUSAMMENFASSUNG

ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN DES EPITHELS DES ZYSTENBALGES

Grosse ultrastrukturelle Variationen zwischen verschiedenen Präparaten wurden festgestellt. Man nimmt an, dass diese Variationen im gewissen Verhältnis zum Grad der Inflammation im Balge stehen.

Sämtliche 10 Fälle zeigten ein nichtkeratinisiertes mehrschichtiges Plattenepithel mit deutlichen ultrastrukturellen Abweichungen von normaler Mundschleimhaut.

Das expansive Wachstum der Zyste ist von einer Druckdifferenz osmotischer Natur abhängig. Die bemerkenswerte lockere Struktur des Balgenepithels mit grossen intercellulären Räumen scheint nicht eine solche Druckdifferenz aufrecht zu erhalten. Die für die Basalmembran bekannten Eigenschaften haben wahrscheinlich Bedeutung für die Aufrechterhaltung des Überdrucks im Lumen der Zyste.

REFERENCES

- Albright, J. T.*, 1960: Electron Microscope Studies of Keratinization as Observed in Gingiva and Cheek Mucosa. *Ann. N. Y. Acad. Sci.* 88: 351.
- Fasske, E.*, 1959: Über das Dechepithel der menschlichen Mundschleimhaut. *Z. Zellforsch.* 49: 515.
- Forsberg, A. & G. Hägglund*, 1959: Den radikulära tandcystans genes och fysikaliska expansion. *Svensk tandläk.-T.* 52: 223.
- Frithiof, L. & J. Wersäll*, 1965: A Highly Ordered Structure in Keratinizing Human Oral Epithelium. *J. Ultrastr. Res.* 12: 371.
- Hammer, H.*, 1934: Untersuchungen über die Pathogenese der radikulären Züsten. *Dtsch. Zahn-, Mund- u. Kieferheilk.* 1: 319.
- Hill, T. J.*, 1930: The Epithelium in Dental Granulomata. *J. dent. Res.* 10: 323.
- Kronfeld, R.*, 1955: *Histopathology of Teeth and Their Surrounding Structures.* Kimpton, London, 4. ed. Editor: Boyle, P. E.
- Molyneux, G. S.*, 1964: Observations on the Structure and Growth of Periodontal and Residual Cysts. *Oral Surg.* 17: 756.
- Mukerjee, H., J. S. Ram & G. B. Pierce*, 1965: Basement Membranes V Chemical Composition of Neoplastic Basement Membrane Mucoprotein. *Amer. J. Path.* 46: 49.
- Odland, G. F.*, 1958: The Fine Structure of the Interrelationship of Cells in the Human Epidermis. *J. biophys. biochem. Cytol.* 4: 529.
- 1960: A Submicroscopic Granular Component in Human Epidermis. *J. invest. Derm.* 34: 11.
- Ottoson, D., F. Sjöstrand, S. Stenström & G. Svaetichin*, 1953: Microelectrode Studies on the E. M. F. of Frog Skin Related to Electron Microscopy of the Dermo-Epidermal Junction. *Acta physiol. scand.* 29: 611.
- Fease, D.*, 1960: The Basement Membrane: Substratum of Histological Order and Complexity. IV Int. Kongr. für Elektr. Mikrosk., Berlin 1958. Springer Verlag, Berlin P. 139.

- Pierce, G. B., et al.*, 1964: Basement Membranes IV Epithelial Origin and Immunologic Cross Reactions. *Amer. J. Path.* 45: 929.
- Rhodin, J.*, 1954: Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney. Thesis, Stockholm.
- Shear, M.*, 1964: Inflammation in Dental Cysts. *Oral Surg.* 17: 756.
- Sognaes, R. F.*, 1958: Electron Microscopy of the Epithelial Lining of the Human Oral Mucosa. *Oral Surg.* 11: 662.
- Stokke, T.*, 1956: Osmotic Pressure in Odontogenic Cysts. *Acta odont. scand.* 14: 65.
- Themann, H.*, 1958: Elektronenmikroskopische Untersuchungen der Normalen und der pathologisch veränderten Mundschleimhaut. *Fortschr. Kiefer- u. Gesichtschir.* 4: 390.
- Toller, P. S.*, 1948: Experimental Investigation into Factors concerning the Growth of Cysts of the Jaws. *Proc. roy. Soc. Med.* 41: 681.
- Tratman, E. K.*, 1939: Diffusion as a Factor in the Increase in Size of Dental and Dentigerous Cysts. *Brit. dent. J.* 66: 515.
- Wislocki, G. B., D. W. Fawcett & E. W. Dempsey*, 1951: Staining of Stratified Squamous Epithelium of Mucous Membranes and Skin of Man and Monkey by the Periodic Acid-Schiff Method. *Anat. Rec.* 110: 359.
- Witzell, J.*, 1896: Über Zahnwurzelzysten, deren Entstehung, Ursache und Behandlung. *Dtsch. Msehr. Zahnheilk.* 14: 369.
- Zelickson, A. S.*, 1962: An Electron Microscope Study of Normal Human Non-Keratinizing Oral Mucosa. *J. invest. Derm.* 38: 99.