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Papers

Ultrastructure of Oral Squamous Cell Carcinoma: A Comparative Analysis of Different Histological Types

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Twenty-five oral carcinomas and five normal oral epithelial specimens were studied using light and electron microscopy. All histological types (well differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma, poorly differentiated squamous cell carcinoma, verrucous carcinoma and spindle cell carcinoma) were seen in the study sample. In addition, 1 case of carcinoma *in situ* was also present. The normal oral epithelium consisted of three keratinising types (gingiva) and two non-keratinising types (buccal mucosa). The ultrastructural features of oral carcinomas showed good correlation with the features seen in light microscopy. The differentiation status of the lesions showed a relationship with cell and nuclear size, tonofilament and keratin content as well as few other cellular abnormalities. It was also observed that the fine details revealed by electron microscopy were often a means of explaining the characteristic histopathological features of oral carcinoma.
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INTRODUCTION

CANCER OF the oral cavity is the most common neoplasm seen in India and accounts for about 25–30% of all cancer cases [1]. Though the oral cavity is easily accessible for clinical examination, malignancy is often missed in its initial stages leading to much morbidity and mortality. Of the various methods available for assessing the malignant status of the oral lesions, electron microscopy has been used as a means of explaining some of the characteristic features observed in conventional light microscopy. Ultrastructure of different oral tumour types have been reported by a number of authors [2–5] and include pathological changes in the basement membrane [6–8], cytoplasmic process [9], intercellular spaces and desmosomes [10]. However, most of the studies carried out so far were restricted to a single clinical type of oral cancer and little effort made to compare the ultrastructural features in different histological types. It thus seemed useful to try and correlate ultrastructural observations with the histological type of cancer. In the present study therefore, ultrastructural features of different histological types of squamous cell carcinoma of the oral cavity were evaluated and compared. These included well differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC), poorly differentiated squamous cell carcinoma (PDSCC), verrucous carcinoma (VC), spindle cell carcinoma (SCC) and carcinoma *in situ* (CIS).

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MATERIALS AND METHODS

25 patients with squamous cell carcinoma of the oral cavity and 5 normal controls were included in the study. Biopsy samples measuring 0.8 × 1.0 cm were taken from the lesions and divided into three portions. The thin middle portion was processed for ultrastructural studies. From normal controls, a small biopsy was taken and divided into two portions. The lateral portions of the cancer biopsies and one portion from the controls were fixed in 10% buffered formalin, routinely processed and sections were stained with haematoxylin–eosin for histopathological examination.

For electron microscopy, tissues were fixed overnight in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in acetone and embedded in epoxy resin, araldite 502. One micron sections were stained with toluidine blue and the ultrathin sections were double stained with uranyl acetate and lead citrate. The observations and documentations were made in a Hitachi H-600 electron microscope.

Table 1. Different types of oral lesion observed

Histological type	Number
Normal keratinising mucosa	3
Normal non-keratinising mucosa	2
Carcinoma *in situ*	1
Well differentiated squamous cell carcinoma	12
Moderately differentiated squamous cell carcinoma	6
Poorly differentiated squamous cell carcinoma	2
Verrucous carcinoma	3
Spindle cell carcinoma	1

RESULTS

Light microscopy

Lesions were histologically classified according to WHO criteria [11]. The different histological types of oral carcinoma seen in the samples studied are shown in Table 1. Semithin (1 μm) sections of various lesions observed are shown in Figs 1–8.

Electron microscopy

For ultrastructural observations the epithelial cells were grouped into three based on their differentiation status, namely, basal type, intermediate type and differentiated type cells. In normal tissue intermediate types are the spinal cells and differentiated type included superficial cells. In carcinomas, basal types (basaloid) are cells seen adjoining the basal lamina. The differentiated types are the cells which have the most squamous differentiating markers (tonofilaments and keratin) while intermediate types are cells with differentiation status between the latter two. Few important sub-cellular structures were selected for evaluation. These include basal lamina, cell shape and size, nuclear features, desmosomes, keratins and tonofilaments. To make comparisons easier, observations in the different type of cells are presented separately in Tables 2–4. Since CIS and SCC have only undifferentiated type cells, ultrastructural features are only shown for the basal type cells (Table 2).

DISCUSSION

A number of roles have been suggested for the epithelial basement membrane in the development and progression of oral carcinogenesis. The term basement membrane was originally coined at light microscopic level, referring to the 1 μm or more thick layer, including the underlying argyrophilic reticular fibres. At the ultrastructural level the term basal lamina (BL) is used to denote the layer which separates the basal cells from the connective tissue [12]. Kobayashi [13] has reported that neoplastic cells invade the stroma irrespective of whether BL is present or not. Smith [14] however, showed that BL restricts neoplastic cells until its continuity is broken and it can no longer retain them. Other studies have also suggested that BL may be the structure that acts as a barrier and determines whether the neoplastic epithelium remains as a carcinoma *in situ* or becomes invasive carcinoma [6, 15]. MacKinney and Singh [8] have postulated a unifying concept for breakdown of the BL and its relationship to oral epithelia undergoing neoplastic transformation. We observed BL in all the lesions except in SCC, where the epithelial–connective tissue junction was indistinct. The BL was thin, discontinuous and fragmented in invasive squamous cell carcinomas while it was continuous and prominent in VC. In CIS, some areas of the BL were multilamellated. Frithiof [6] also observed similar changes which showed the BL to be thin and defective in all the invasive carcinomas studied. Pierce *et al.* [15] suggested that multilamination in BL might be due to the synthesis of additional cellular or extracellular products by the basal cells, as a response to the dysplastic changes occurring in the epithelium. From our present observation, it appears that for tumour invasion, fragmentation or disintegration of BL is essential, though it is not required for carcinogenesis per se.

The presence of poorly developed hemidesmosomes with reduced size and number in all types of lesions except CIS indicates that they may have some role in both carcinogenesis and in tumour invasion. In CIS, we observed relatively high concentrations of intact hemidesmosomes compared with other lesions studied. However, areas with few hemidesmosomes were also evident. Chen and Harwick [2] also found squamous cell carcinomas to have smaller and fewer hemidesmosomes compared to normal tissues. On the contrary Frithiof [7] observed normal hemidesmosomes in the basal cells wherever BL was present.

The absence of basal cell processes in many malignant lesions studied was interesting. This observation is in disagreement with the findings of Shklar [5] and Frithiof [9] who reported basal cell processes to be present in oral leukoplakias, carcinomas and normal mucosa. Shklar [5] observed pseudopodia like cytoplasmic processes in both well differentiated and anaplastic oral epidermoid carcinomas, while Frithiof [9] found cytoplasmic processes extending from the malignant cells through a defective membrane. We have also observed basal cell processes in normal oral mucosa and in leukoplakias [16].

The present study also brings forth the existence of a possible direct relationship between cell size and its grade of differentiation; cell size being directly proportional to its degree of differentiation. WDSCC had the largest cell size compared with other grades of differentiated carcinomas. This may possibly be due to the overproduction of differentiating materials such as keratin and tonofilaments. In the case of nuclei, the nuclear size was inversely proportional to the degree of differentiation. A larger nuclear volume was found in poorly differentiated cells. The low content of heterochroma-

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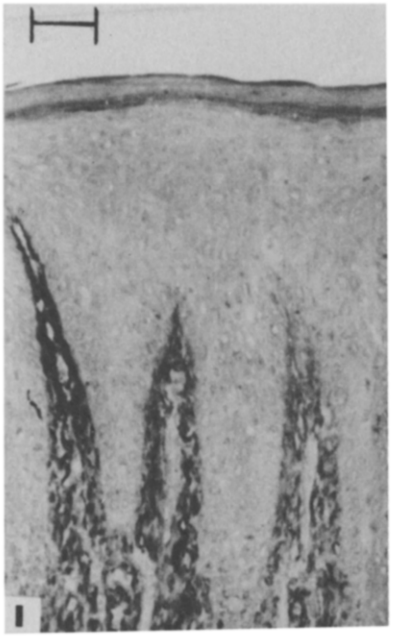
Fig. 1. Normal keratinising mucosa. Toluidine blue stained 1 μ thick resin embedded sections (Bar = 50 μm).
Fig. 2. Normal non-keratinising mucosa. For staining information see Fig. 1.
Fig. 3. Carcinoma *in situ*. For staining information see Fig. 1.
Fig. 4. Well differentiated squamous cell carcinoma. For staining information see Fig. 1.
Fig. 5. Moderately differentiated squamous cell carcinoma. For staining information see Fig. 1.
Fig. 6. Poorly differentiated squamous cell carcinoma. For staining information see Fig. 1.
Fig. 7. Verrucous carcinoma. For staining information see Fig. 1.
Fig. 8. Spindle cell carcinoma. For staining information see Fig. 1.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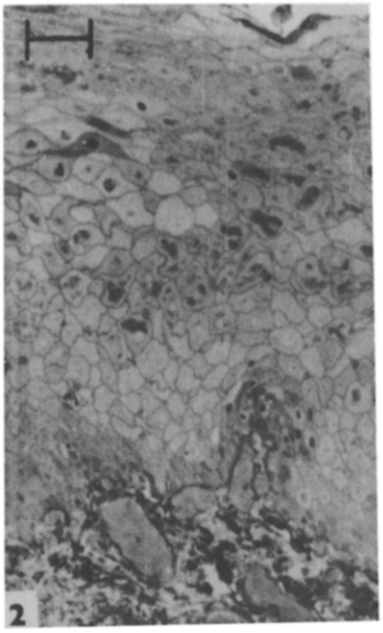
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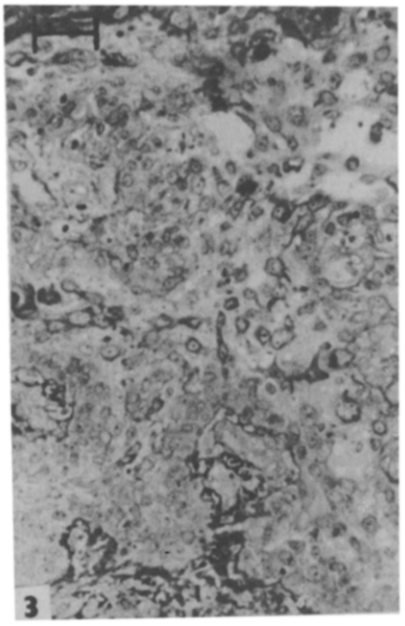
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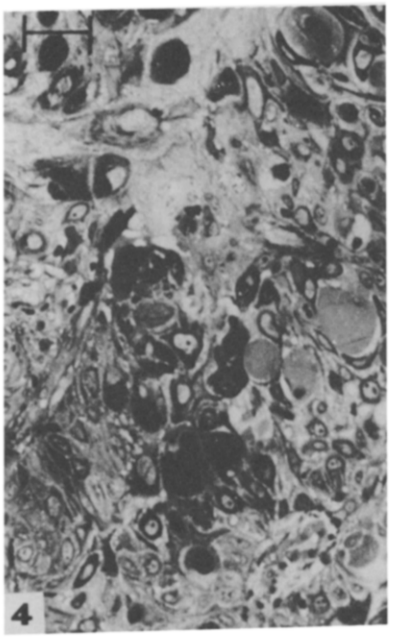
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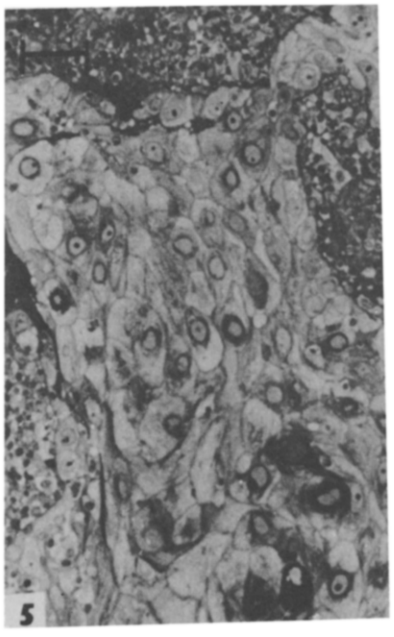
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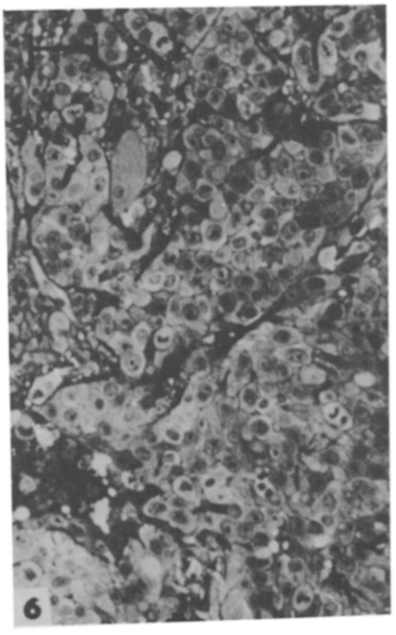
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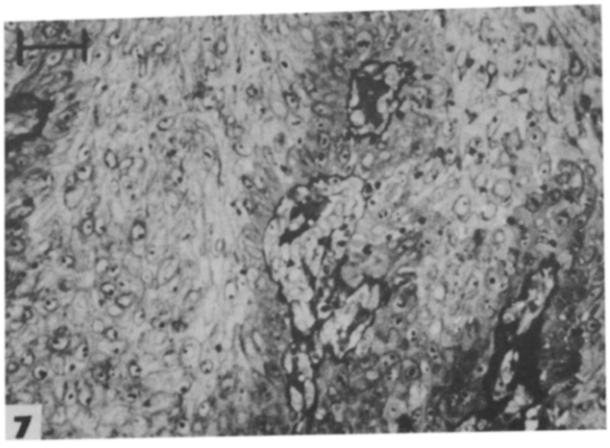
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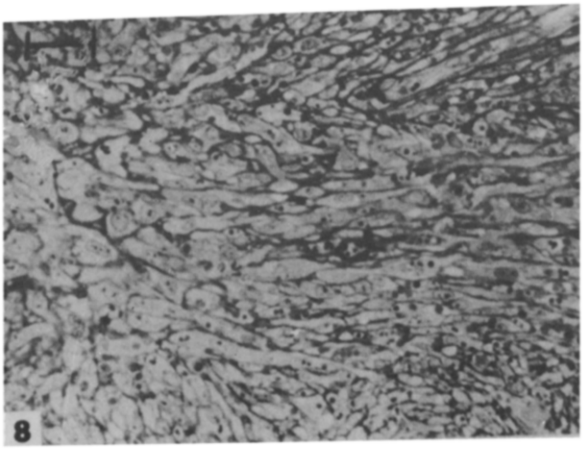
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Table 2. Comparison between ultrastructural features of basal cell type of different oral lesions

Histological features	Normal keratinising epithelium (Fig. 9)	Normal non-keratinising epithelium (Fig. 12)	Carcinoma *in situ* (Figs 15–17)	WDSCC (Fig. 18)	MDSCC (Fig. 21)	PDSCC (Fig. 24)	Verrucous carcinoma (Fig. 27)	Spindle cell carcinoma (Figs 27–30)
Basal lamina	Dense and continous	Dense and continous	Dense continous and multilamellated in certain areas	Thin and discontinous	Thin and discontinous	Thin and fragmented	Continous and slightly thickened	Indistinct
Hemidesmosomes	Plenty	Plenty	Plenty and reduced in certain areas	Less and poorly formed	Less and poorly formed	Few and poorly formed	Less and poorly formed	—
Basal cell shape and size	Cuboidal and uniform	Cuboidal and uniform	Oval or round and size almost normal	Pleomorphic and enlarged	Pleomorphic and enlarged	Pleomorphic and enlarged	Oval and size almost normal	Spindle or oval and large
Nucleus	Round and monomorphic	Round and monomorphic	Round enlarged and slightly pleomorphic	Enlarged, bizzare, and pleomorphic	Enlarged, oval and slightly pleomorphic	Enlarged, oval and highly pleomorphic	Mostly oval and enlarged	Oval, highly enlarged and pleomorphic
Chromatin nature	Granular and with marginal chromatin condensation	Granular and with a thin marginal condensation	Homogeneous granular with trace of heterochromatin	Prominent marginal condensation and presence of perichromatin granules	No marginal condensation, perichromatin granules were present	No chromatin condensation plenty of perichromatin and interchromatin granules	Fine granulated chromatin with few aggregates	Granular chromatin with plenty of perichromatin and interchromatin granules
Nucleolus	Round and seen in most cells	Round and vesicular	Moderately developed and more than one per nuclei	Present in many cells	More developed and reticular	Well developed more than one in each nuclei	Well developed marginated nucleoli	Marked development and more than one per nuclei
Keratin and tonofilaments	Loosely arranged tonofilaments and keratin granules	Trace amount of tonofilaments and granules	Sparse distribution of tonofilament and keratin granules	Plenty of tonofilaments and keratin granules	Mild amount of tonofilaments and keratin granules	Trace amount	Moderate amount	Almost absent and few tonofilaments
Desmosomes	Many and intact	Many and intact	Few and poorly formed	Few and poorly formed	Moderate amount	Rare and poorly formed	Less in number	Absent and seen as small dense zones in the cell membrane
Spongiosis	Trace	Trace	Moderate	Moderate	Mild	Severe	Mild	Severe

Table 3. Comparison of ultrastructural features in intermediate type cells of different oral lesions

Histological features	Normal keratinising epithelium (Fig. 10)	Normal non-keratinising epithelium (Fig. 13)	WDSCC (Fig. 19)	MDSCC (Fig. 22)	PDSCC (Fig. 25)	Verrucous carcinoma (Fig. 28)
Nucleo-cytoplasmic index	Low	Very low	Low	High	Very high	Low
Nucleus	Oval	Small round	Large round	Oval and enlarged	Large, round and more than one per cell	Round
Chromatin nature	Sparse amount of chromatin with few condensed areas	Fine granular and without any heterochromatin	Coarse granular and with condensed areas	Dense and granular	Granulated without any hetrochromatin	Less amount of chromatin
Nucleolus	Small and rarely present	Conspicuous nucleoli	Rare and small	Well developed nucleoli in plenty of cells	Well developed and more than one per nucleus	Only present in few cells
Keratin and tonofilaments	Plenty of loosely scattered slender bundles	Trace	Increased significantly, tonofilaments grouped into bundles	Comparatively less than WDSCC	Trace	Plenty and scatterly distributed
Desmosomes	Numerous and intact	Plenty and intact	Less and poorly formed	Plenty of desmosomes and poorly formed	Almost absent	Less and poorly formed
Spongiosis	Trace	Absent	Mild	Moderate	Trace	Trace

Table 4. Ultrastructural features of differentiated type cells of different oral lesions

Histological features	Normal keratinising epithelium (Fig. 11)	Normal non-keratinising epithelium (Fig. 14)	WDSCC (Fig. 20)	MDSCC (Fig. 23)	PDSCC (Fig. 26)	Verrucous carcinoma (Fig. 29)
Size and shape	Flat, elongated	Swollen spindle shaped	Very large with a very low nucleo-cytoplasmic index	Large cell but smaller than WDSCC and with low nucleo-cytoplastic index	Large but comparatively high nucleo-cytoplasmic index	Mostly elongated and flattened
Nucleus	Condensed nuclei present in few cells	A small nucleus in most of cells	Small, showing karyorhexsis	Larger than that of WDSCC	Very large, round	Small dense nucleus
Chromatin nature	Sparse amount of granular chromatin	Finely granulated chromatin without heterochromatin	Sparse amount of granular chromatin	Sparse amount of chromatin	Plenty of dense chromatin	Sparse granular chromatin
Nucleolus	Absent in most of cells	Absent in most of the cells	Small dense nucleolus	Small reticular nucleolus	Well developed reticular nucleoli	Small nucleolus rarely present
Tonofilaments and keratin	Cytoplasm almost filled with bundles of tonofilaments and granular keratin	Tonofilaments were trace and cytoplasm contain plenty of keratin granules	Whole cytoplasm filled with bundles of tonofilaments and keratohyaline aggregates	Plenty of swirled bundles of tonofilaments seen in one side of the cells, focal and plenty of keratohyaline materials	Moderate amount of tonofilaments in thin bundles and granules of keratin	Plenty of fine keratin granules and scattered arranged tonofilaments
Desmosomes	Plenty and intact	Plenty and intact	Few and poorly formed desmosomes	Few and poorly formed	Few and poorly formed	Moderate number

tin and nuclear bodies, presence of interchromatin and perichromatin granules in the malignant cells, well developed nucleoli, abundance of well developed cell organelles and marked increase of polyribosomes indicate the hyperactive status of these cells [5].

Loss of intercellular bridges or desmosomes has been

reported to be a feature of carcinomatous change [2–6]. Our studies are in agreement with earlier reports, since we observed only few poorly defined desmosomes in the different types of lesions studied. However, MDSCC lesions were an exception where areas with moderate number of intact desmosomes were observed. Loss of desmosomes may be a reason for the widening of intercellular spaces and ruffling of cell boundaries. Intercellular junctions are vital in maintaining the level of cell differentiation and overall metabolic activity.

To conclude, comparison of the ultrastructure of different

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Fig. 9. Electron micrograph of normal keratinising oral nucosa. Basal region showing continuous basal lamina (Bl), cuboidal cells with large nucleus and cytoplasm containing loosely arranged tonofilaments (T) (Bar = 3 μm).

Fig. 10. Electron micrograph of normal keratinising oral mucosa. Intermediate cells showing small nucleus and cytoplasm containing plenty of tonofilaments (T). Mild intercellular spaces (I) are also present (Bar = 3 μm).

Fig. 11. Electron micrograph of normal keratinising oral mucosa. Differentiated cells showing pyknotic nuclei (N) and cytoplasm with abundant tonofilaments and keratin (Bar = 3 μm).

Fig. 12. Electron micrograph of normal non-keratinising oral nucosa. Basal region showing continuous basal lamina (Bl) and basal cells with large nuclei. Lamina propria (L) also seen (Bar = 3 μm).

Fig. 13. Electron micrograph of normal non-keratinising oral mucosa. Intermediate cells showing small nuclei with cytoplasm containing few tonofilaments. Plenty of desmosomes are also seen (Bar = 3 μm).

Fig. 14. Electron micrograph of normal non-keratinising oral mucosa. Differentiated cells with very low nucleo-cytoplasmic index and very small nuclei (N) (Bar = 3 μm).

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Fig. 15. Electron micrograph of carcinoma *in situ*. Basal region showing continuous basal lamina (Bl) and basal cells with large nuclei, numerous tonofilaments (T) and few desmosomes (arrow) (Bar = 3 μm).

Fig. 16. Electron micrograph of carcinoma *in situ*. Basal region showing reduplicated basal lamina (Bl) demarcating epithelium (E) from lamina propria (L) (Bar = 3 μm).

Fig. 17. Electron micrograph of carcinoma *in situ*. Cells with large irregular nuclei (N) and granular cytoplasm with plenty of cell organelles (Bar = 3 μm).

Fig. 18. Electron micrograph of well differentiated oral squamous cell carcinoma. Basal region showing thin basal lamina (Bl) which is also absent in one area (arrow). Basal cells are irregular with bizarre nuclei (N). Intercellular space (I) are prominent. Few poorly formed desmosomes (arrow head) are also seen (Bar = 3 μm).

Fig. 19. Electron micrograph of well differentiated oral squamous cell carcinoma. Intermediate cells with high nucleo-cytoplasmic index, cytoplasm contains numerous tonofilaments and keratohyalin material. Intercellular spaces (I) are prominent (Bar = 3 μm).

Fig. 20. Electron micrograph of well differentiated oral squamous cell carcinoma. Differentiated cells with small nuclei (N) and cytoplasm containing full of keratohyalin material and tonofilament bundles (T) (Bar = 3 μm).

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Fig. 21. Electron micrograph of moderately differentiated oral squamous cell carcinoma. Basal region showing continuous basal lamina (Bl) and basal cells with plenty of cell organelles (Bar = 3 μm).

Fig. 22. Electron micrograph of moderately differentiated oral squamous cell carcinoma. Intermediate cells with high nucleo-cytoplasmic index, cells contain large nucleus and well developed nucleolus (Bar = 3 μm).

Fig. 23. Electron micrograph of moderately differentiated oral squamous cell carcinoma. Differentiated cell with small nucleus (N) and cytoplasm containing large amount of tonofilaments concentrated in one side of the cell (Bar = 3 μm).

Fig. 24. Electron micrograph of poorly differentiated oral squamous cell carcinoma. Basal region with fragments of basal lamina (arrow); basal cells contain large nuclei with prominent multiple nucleoli. Intercellular spaces (I) are prominent (Bar = 3 μm).

Fig. 25. Electron micrograph of poorly differentiated oral squamous cell carcinoma. Intermediate cells—a binucleated cell with prominent nucleoli and cytoplasm devoid of tonofilaments (Bar = 3 μm).

Fig. 26. Electron micrograph of poorly differentiated oral squamous cell carcinoma. Differentiated cell showing enlarged nucleus (N) with prominent nucleolus (n) and mild amount of tonofilament (T) in the cytoplasm (Bar = 3 μm).

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Fig. 27. Electron micrograph of verrucous oral carcinoma. Basal region showing slightly thickened basal lamina (Bl) with basal cells having uniform size and large nuclei. Inflammatory cells (Ic) are also present (Bar = 3 μm).

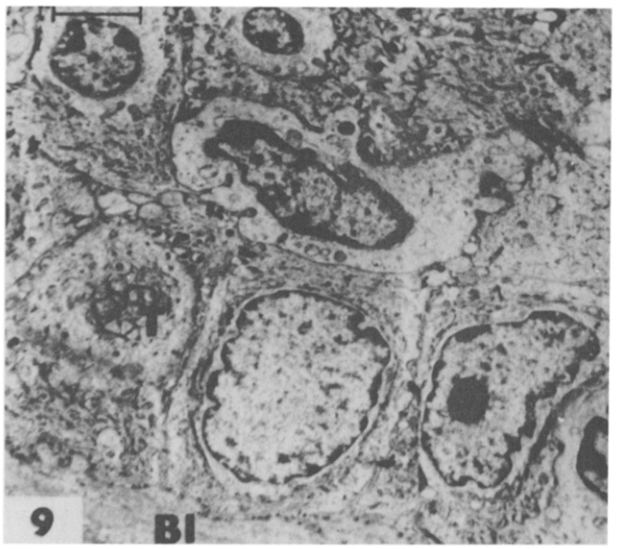
Fig. 28. Electron micrograph of verrucous oral carcinoma. Intermediate cells containing large nuclei with prominent nucleoli and mild amounts of tonofilaments seen in the cell periphery (Bar = 3 μm).

Fig. 29. Electron micrograph of verrucous oral carcinoma. Differentiated cells with small nuclei and haphazard arrangement of tonofilaments (Bar = 3 μm).

Fig. 30. Electron micrograph of spindle cell oral carcinoma. Degenerating spindle cells (C) having electron dense nuclei and clear cytoplasm. Dense stroma (S) is also seen in between the cells (Bar = 3 μm).

Fig. 31. Electron micrograph of spindle cell oral carcinoma. Poorly differentiated spindle cell containing large irregular nucleus and cytoplasm with plenty of organelles (Bar = 3 μm).

Fig. 32. Electron micrograph of spindle cell oral carcinoma. Poorly differentiated cell containing large nucleus (N) and well developed nucleolus (n). Adjacent cell membranes have electron dense desmosome like areas (arrows) (Bar = 3 μm).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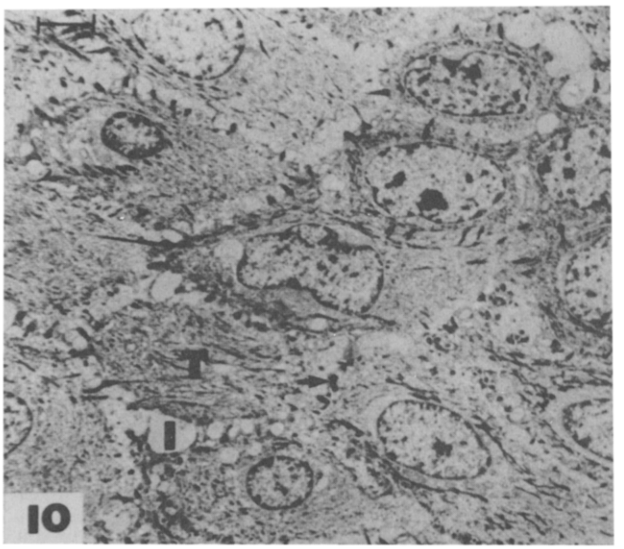
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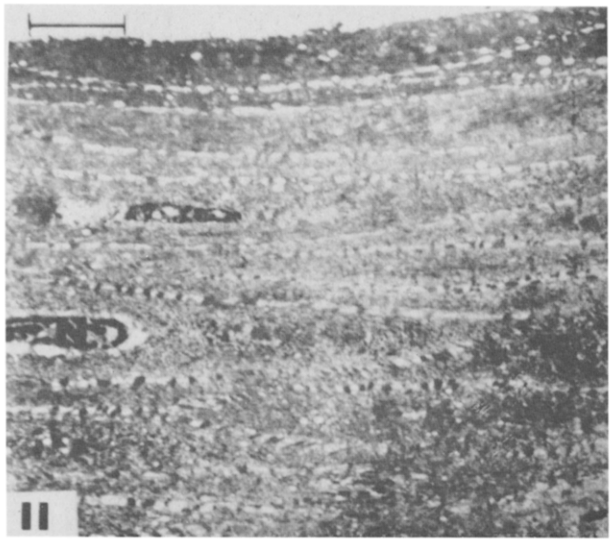
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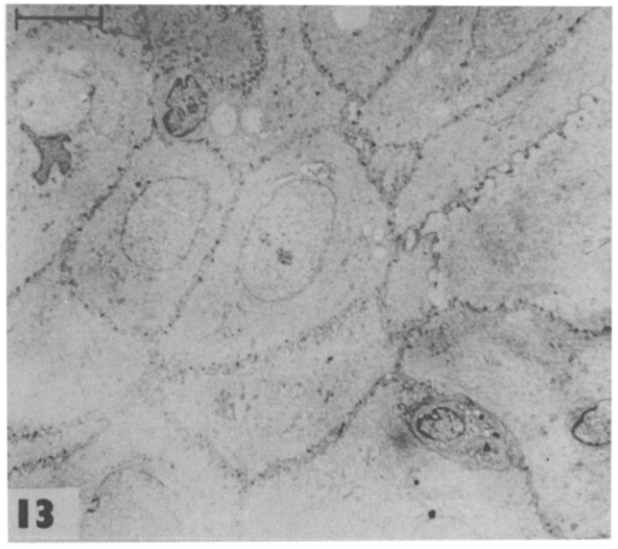
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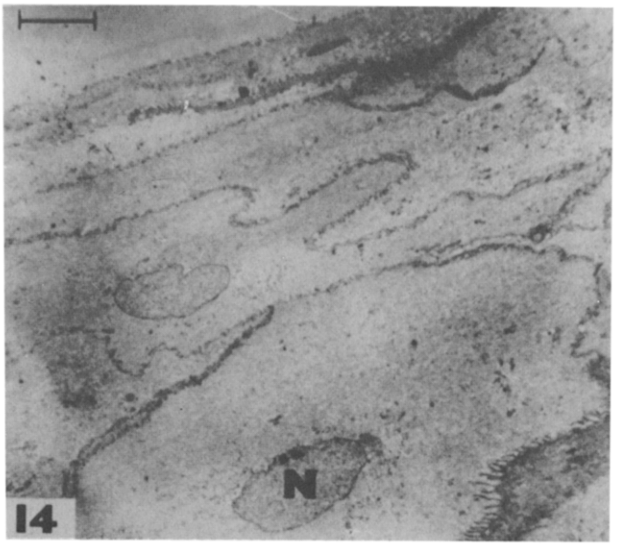
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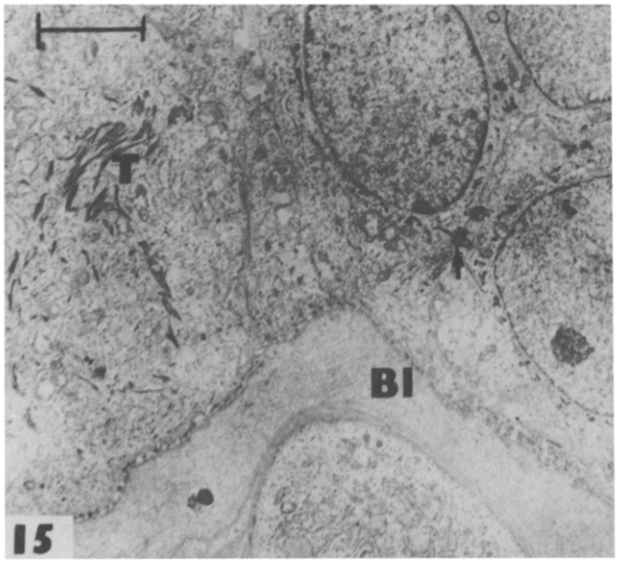
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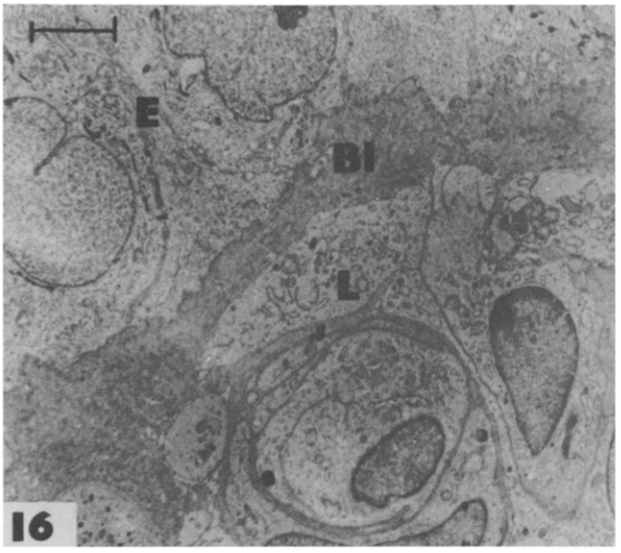
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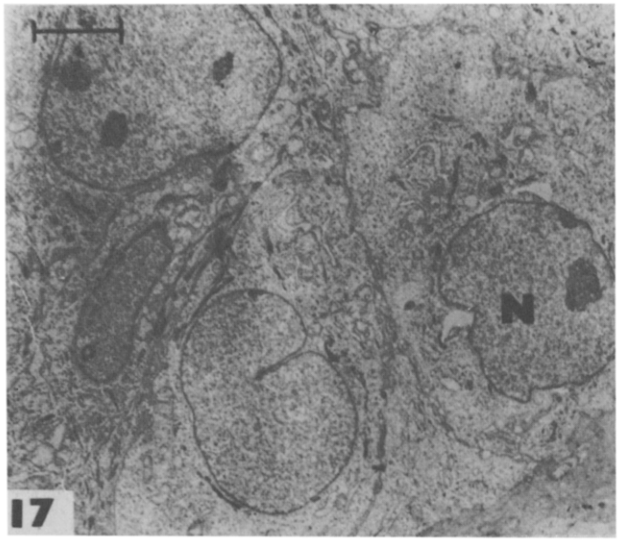
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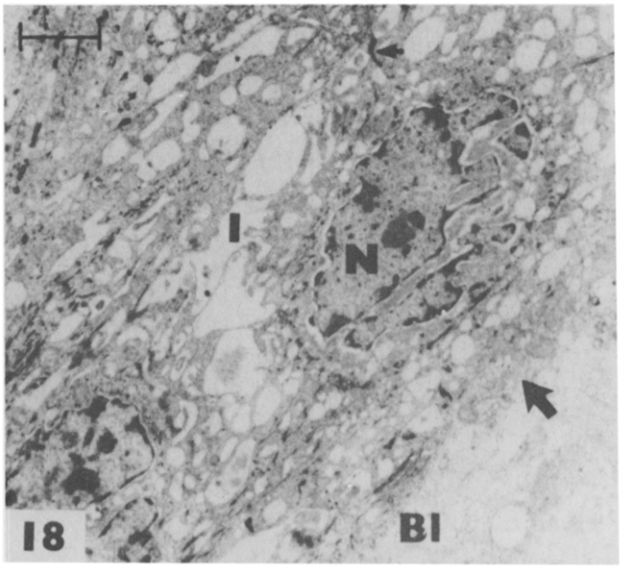
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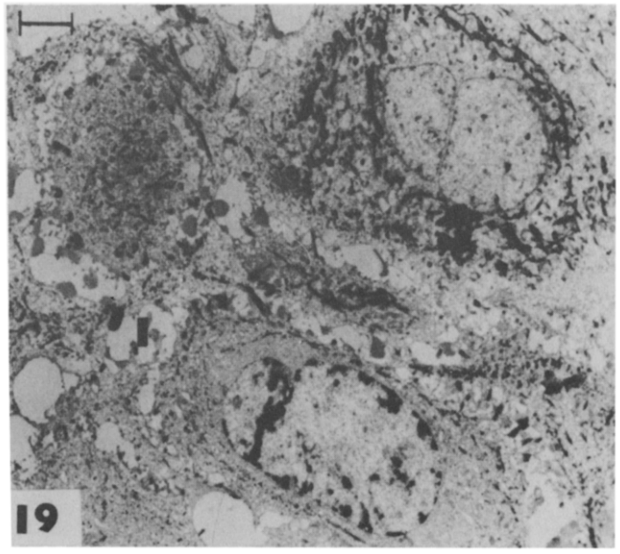
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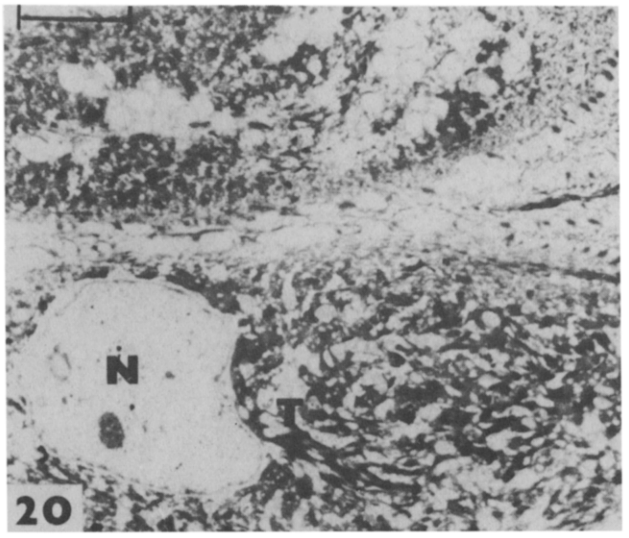
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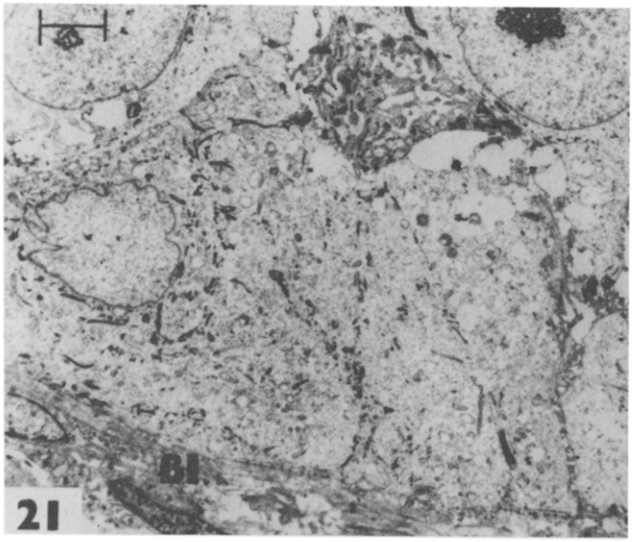

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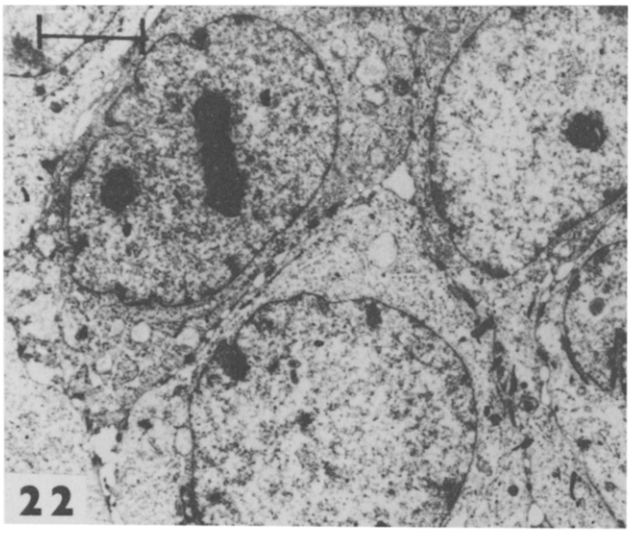
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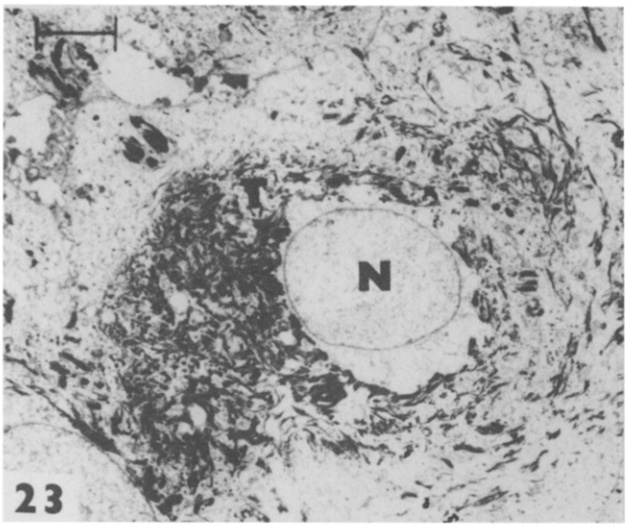

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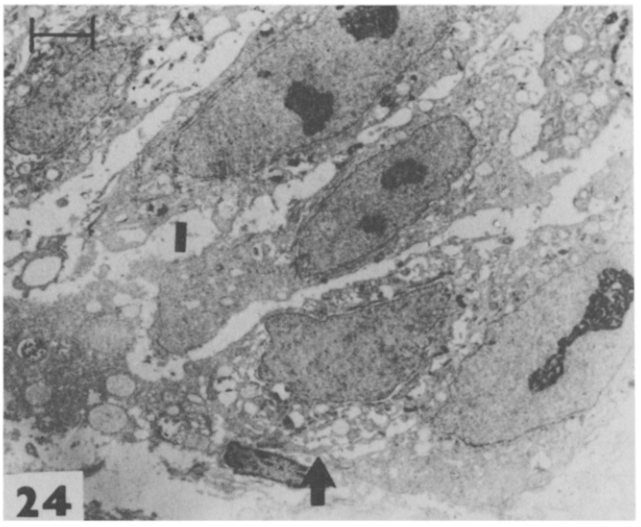

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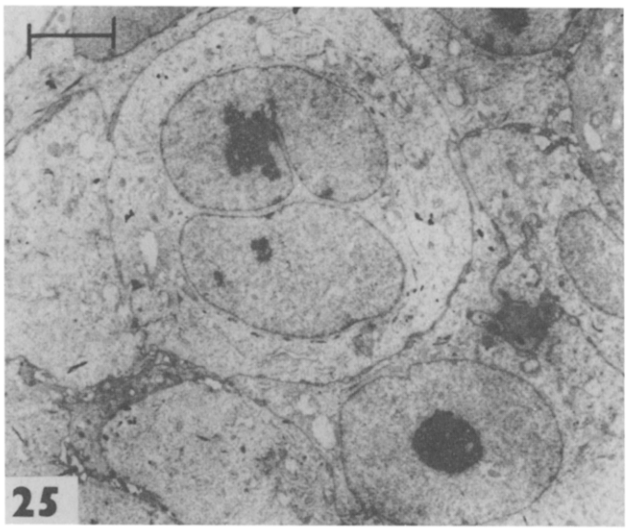
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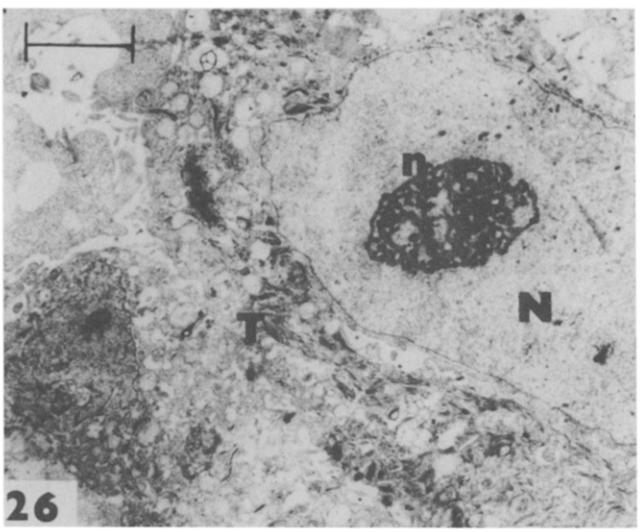

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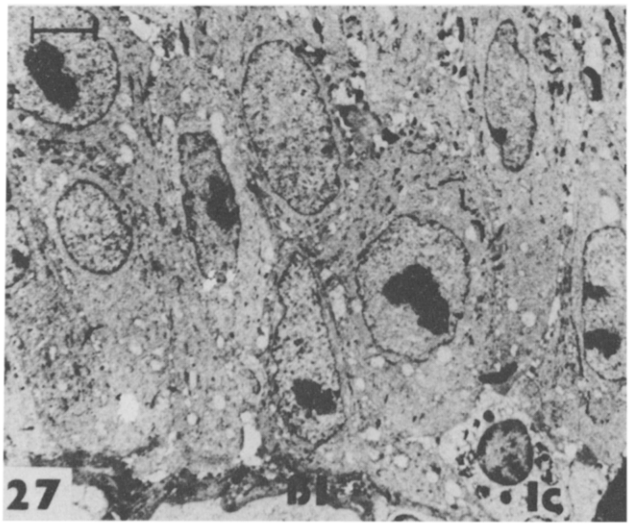
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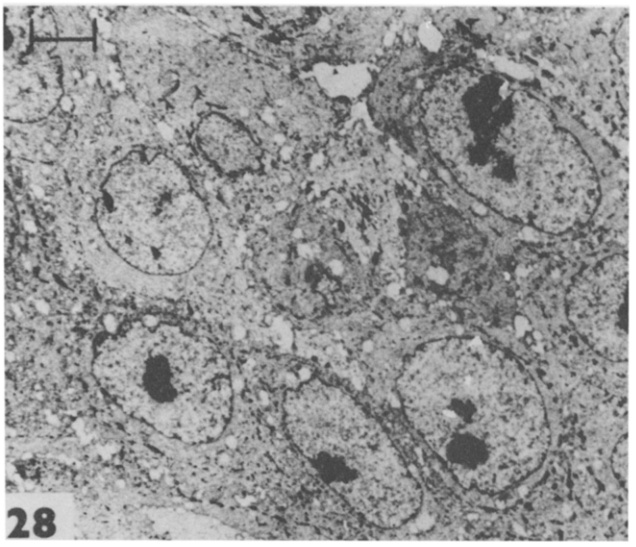
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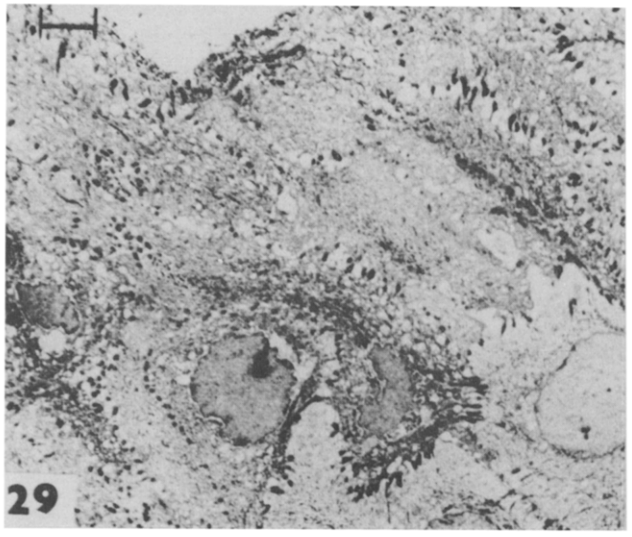
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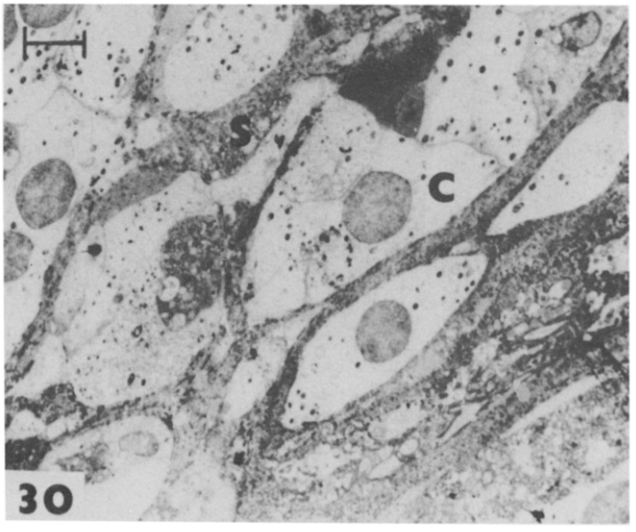
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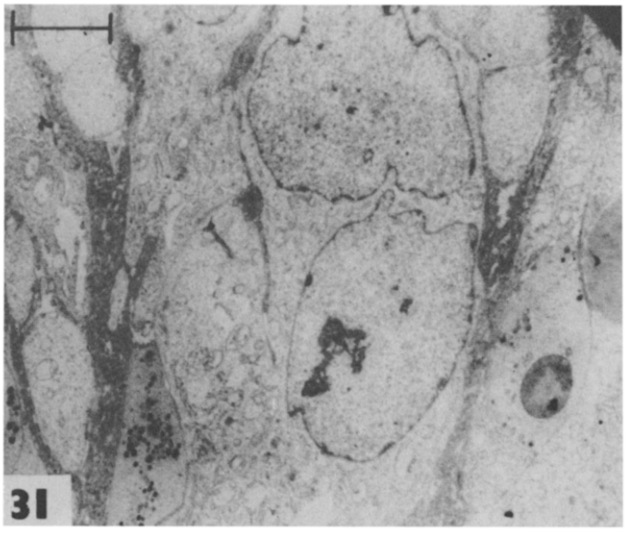
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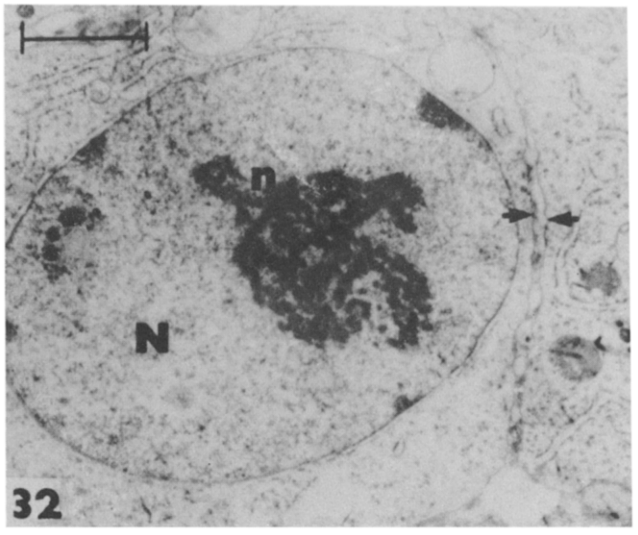
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types of oral squamous cell carcinoma revealed a close similarity to exist between various features of the different malignant cells. In addition, each type of cell was also found to show its own characteristic morphological features. Elucidating these conspicuous changes can help in understanding routine histopathological observations and its correlation to the process of carcinogenesis.

1. Jayant K, Notani PN. Epidemiology of oral cancer. In Rao RS, Desai PB, eds. *Oral Cancer*. India, Professional Publication Division, Tata Memorial Hospital, 1991, 1–17.
2. Chen SY, Harwick RD. Ultrastructure of oral squamous cell carcinoma. *Oral Surg* 1977, **44**, 744–753.
3. Someren A, Karciogly Z, Clairmount AA, Ga A. Polyploid spindle cell carcinoma (pleomorphic carcinoma). *Oral Surg* 1976, **42**, 474–489.
4. Prioleau PG, Santa Cruz DJ, Meyer JS, Baner WC. Verrucous carcinoma: a light and electron microscopic, autoradiographic and immuno-fluorescence study. *Cancer* 1980, **45**, 2849–2857.
5. Shklar G. Ultrastructural pathology of oral cancer. In Shklar G, ed. *Oral Cancer*. Philadelphia, WB Saunders Co, 1984, 20–30.
6. Frithiof L. Ultrastructure of the basement membrane in normal and hyperplastic human oral epithelium compared with that in the preinvasive and invasive carcinoma. *Acta Pathol Micro Biol Scand* 1969, **200**, 1–63.
7. Frithiof L. Electromicroscopic observations on structures related to the epithelial basement membrane in squamous cell carcinoma. *Acta Otolaryngol* (Stockh) 1972, **73**, 325–334.
8. McKinney Jr RV, Singh BB. Basement membrane changes under neoplastic oral mucous membrane. *Oral Surg* 1977, **44**, 875–888.
9. Frithiof L. Cytoplasmic processes in normal and in malignant oral epithelium. *Acta Otolaryngol* (Stockh) 1972, **74**, 212–229.
10. Luzardo MJ, Castejon OJ. Epithelial function in the stratum spinosum of the human oral mucosa. *Parodontologia* 1969, **23**, 177–182.
11. Wahi PN, Cohen B, Luthra UK, Torloni H. Histological typing of oral and oropharyngeal tumors. International Histological Classification of Tumours No. 4, World Health Organisation, Geneva, 1971.
12. Ghadially FN. *Diagnostic Electron Microscopy of Tumours*. 1st ed. London, Butterworth Heineman, 1980.
13. Kobayashi T. Dermo-epidermal junction in invasive squamous cell carcinoma. *Acta Dermatol Venerol* 1969, **49**, 445–457.
14. Smith CJ. The epithelial tissue junction in the pathogenesis of human and experimental oral cancer. In Tarin D, ed. *Tissue Interactions in Carcinogenesis*. New York, Academic Press 1972, 191–225.
15. Pierce GB, Nakane PK. Basement membranes: synthesis and deposition in response to cellular injury. *Lab Invest* 1969, **21**, 27–41.
16. Kannan S, Balaram P, Pillai MR, *et al.* Ultrastructural variations and assessment of malignant transformation risk in oral leukoplakia. *Pathol Res Pract* 1993 (in press).

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