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An Epithelial-Like Cell Line in Continuous Culture from Normal Adult Human Gingiva.* (31032)

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There have been several reports of the continuous cultivation *in vitro* of adult human epithelial-like cells obtained from abnormal tissue(1-4). Continuous cultivation of epithelial-like cells derived from normal adult human tissue is comparatively uncommon(5, 6).

This report deals with the isolation and growth in continuous culture of an epithelial-like cell line derived from an area of clinically normal adult human attached gingiva.

Source of tissue. The tissue was obtained from a 19-year-old white female in apparent good health. The attached gingiva, labial mucosa, tongue, cheeks and oropharyngeal regions were clinically normal.

Using mandibular block anesthesia (lidocaine HCl 2% with epinephrine, 1:100,000) with no local infiltration into the biopsy areas a strip of attached gingiva measuring 2 × 6 mm was removed in the mandibular anterior region (Fig. 1). At the same time a small fragment of labial mucosa (2 × 3 mm) was also excised.

Material and method. The tissue was washed in Hanks' salt solution containing 0.05% $NaHCO_3$ to remove adherent blood and saliva.

The gingival specimen was cut into 3 sections and the labial specimen was cut in half (Fig. 2). The fragments were placed in separate sterile 60 mm petri dishes with the cut surface against the glass. The fragments were held in position with a small strip of gelatin film(7) until outgrowth occurred at which time the gelatin film was removed. The medium consisted of Eagle's basal solution supplemented with 15% calf serum.† The Eagle's solution was modified by the addition of inositol(8) and the use of Hanks' salt solution containing 0.05% $NaHCO_3$. Penicillin, streptomycin and amphotericin B were added to the medium to give final concentrations of 100 units, 100 μg and 4 μg, per ml, respectively. Two ml of medium were placed in each petri dish. The culture dishes were then incubated at 36-37°C in an atmosphere of approximately 5% CO_2 in air. Cultures were examined twice weekly when the medium changes were made.

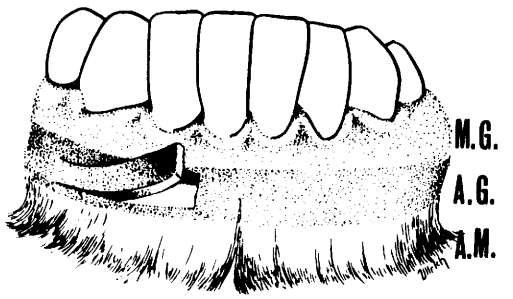

FIG. 1. Diagram showing area where gingival specimen was obtained. M.G., marginal gingiva; A.G., attached gingiva; A. M., alveolar mucosa.

Results. Epithelial-like outgrowth was noted from 2 gingival and one labial explant by the third week. One section of labial mucosa showed only fibroblast-like outgrowth and one fragment of gingiva did not grow. During the fourth week a few small separate colonies of compact epithelial-like cells were noted in the 4 plates showing growth. These

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colonies rapidly increased in size and number. The cells composing the colonies showed no contact inhibition and appeared to pile up with the central area becoming granular and necrotic.

Several of the separate colonies were iso-

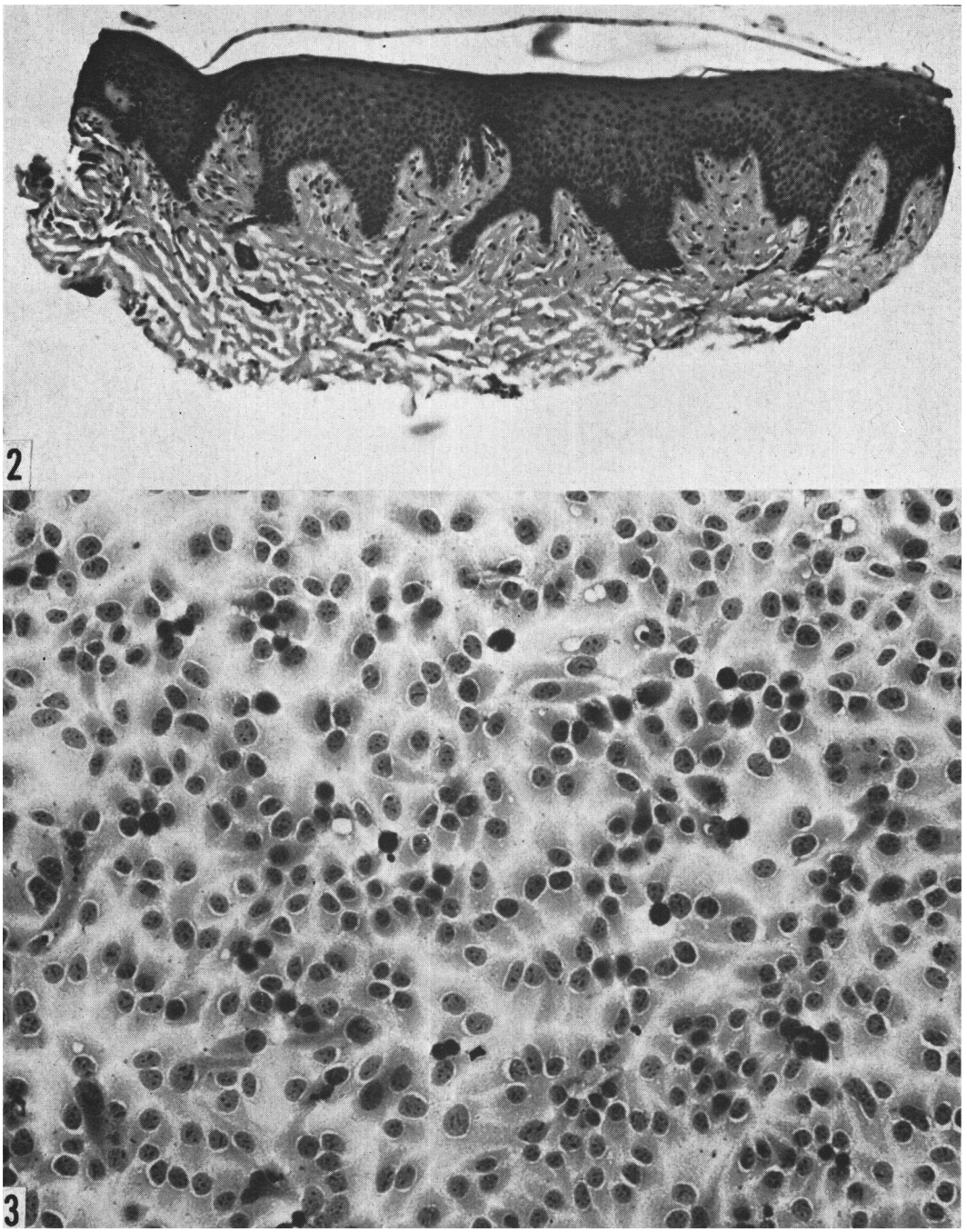

FIG. 2. Histologic section of attached gingiva obtained from same area as cultivated tissue showing normal appearance of tissue. Hematoxylin and Eosin. orig. mag. ×100.

FIG. 3. Six-day culture of gingival cells in 31st passage. Note numerous mitotic figures. Hematoxylin and Eosin. orig. mag. ×200.

lated and washed with saline A and removed from the glass with 0.1% trypsin (Difco "1:250") in saline A. Medium containing 15% calf serum was added to the trypsinized cells to stop the action of the trypsin. They were spun at 900-1000 rpm for 10 minutes. The cells were resuspended in medium, placed in loosely stoppered Yerganian tubes and incubated at 36-37°C in an atmosphere of 5% CO_2 in air. The cultures proliferated rapidly and were composed of compact polygonal cells. Daily fluid changes were made and the cultures were subcultured on the seventh day as growth was heavy. Thereafter, subcultures were made weekly with medium changes 3 times per week. The line is currently in the 41st passage.

The cells are polygonal with a single nucleus containing 2-5 chromocentral masses (Fig. 3). The cytoplasm is usually clear but occasional granular cells are seen.

Cells in the 16th passage were inoculated into cheek pouches of 22 animals of 3 hamster species (Syrian, Chinese and Armenian). Inoculations varied from 5×10^5–5×10^6 trypsinized cells. In addition, 10 of the animals received 2.5 mg of cortisone subcutaneously at time of inoculation with 4 additional injections during the 11-week period of observation. There was no evidence of tumor formation.

The growth rate in an atmosphere of 5% CO_2 is relatively rapid with an approximate generation time of 24 hours during the logarithmic phase. The cells also grow readily in tightly stoppered vessels without addition of CO_2. Plating efficiency in 60 mm petri dishes seeded with 100 cells is approximately 80-90%.

Preliminary chromosome analysis indicates a rather stable complement in the triploid range (68-70). A detailed karyotypic analysis will be reported elsewhere.

Discussion. The establishment of a permanent epithelial-like cell line from clinically normal adult human tissue is relatively uncommon. Fibroblast-like strains initiated from this type of tissue have the diploid chromosome number and a relatively short *in vitro* life span(9). The chromosome complement of peripheral lymphocytes of the individual from whom this line was derived was normal. Two subsequent attempts to obtain independent epithelial-like colonies capable of serial passages from similar biopsies of this person were not successful although outgrowth from the fragments occurred. Over 300 cultures from similar specimens of more than 100 individuals were initiated over a 2-year period. Fibroblast-like cells were the predominant or sole cell type in all cultures maintained over 4 months.

The possibility that the epithelial cell line reported here may have arisen as a contaminant from other already established cell lines is ruled out by the fact that established cell lines are not cultured in this laboratory. Similarly, cellular extracts were never used. It is conceivable that a virus present in the oral cavity caused a transformation of some cells to an abnormal chromosome number and thus permitted its continuous cultivation. The susceptibility of these cells to a number of viruses is now under study.

Summary. An epithelial-like cell line has been established from an area of clinically normal adult human attached gingiva. It has a generative time of approximately 24 hours in logarithmic phase and a plating efficiency of about 80-90%.

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