

## PERIODIC REPEAT UNITS OF EPITHELIAL CELL TONOFILAMENTS

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### INTRODUCTION

Tonofibrils and their component tonofilaments are prominent constituents of various types of epithelial cells. Tonofilaments are of largely undefined length (1), average about 70 Å in diameter, and frequently can be traced into the vicinity of desmosomes, where they may loop through the region of the attachment plaques (2). Ultrastructural examinations have failed to establish consistent evidence of periodicity in tonofibrils, although Brody (3) has shown one example of such a phenomenon.

The current report describes tonofibril periodicity in certain embryonic mouse cells. The cells were noticed first in organ cultures of jaw mesenchyme and submandibular gland epithelium removed from 13-day mouse embryos. Subsequent investigation implied that the epithelial cells containing the fibers were contained originally in traces of oral epithelium associated with the jaw mesenchyme. Since then, periodic fibers have been seen in oral epithelium-jaw mesenchyme cultures, in cultured lower jaw epidermis from 13-day embryos, and in 13- and 16-day embryonic jaw skin that was fixed immediately after dissection.

### MATERIALS AND METHODS

Embryos were obtained from crosses of B/ALB C ♀ with C3H ♂ mice; the morning of vaginal plug discovery was counted as day 0. Dissections and culture procedures duplicated earlier procedures (4), except that the direct recombinations of epithelium and mesenchyme were cultured on the upper surface of a Millipore filter (5) and were held in place with a plasma clot. Cultures were fed on each of 3 days with a mixture of Eagle's basal medium plus 10% horse serum and 10% 9 day chick embryo extract. Oral epithelium for culture was obtained from the lining of the mouth adjacent to the ducts of the salivary glands in 13-day embryos. The lower jaw skin of 13- and 16-day embryos was removed from the area near the mid-ventral line immediately posterior to the symphysis of the mandible.

Cultures were fixed for 3 hr in ice-cold 2½% glutaraldehyde in Sorensen's buffer at pH 7.8. Then they were washed with two 2-hr changes of cold 1% Sorensen's buffer. Osmium tetroxide (1%) in Veronal buffer was used as a postfixative (6) at pH 8.0, and in the presence of sucrose (0.045 g/ml) and calcium chloride (0.0016 g/ml). Postfixation was carried out overnight at 4°C. Dehydration and embedding were as follows: cold 50% EtOH, 5 min; cold 80% EtOH, 10 min; cold 95% EtOH, 15 min; cold 100% EtOH, 20 min (with rise to room temperature); 1% phos-

photungstic acid in 100% EtOH, 30 min (7); 100% EtOH, 20 min; a 1:1 mixture of 100% EtOH and Epon, 1 hr; Epon, 2 hr. The Epon was hardened by heating at 38°C for 24 hr and/or at 60°C for 24 hr.

A Porter-Blum MT-2 microtome was used to obtain thin sections (*circa* 120 m $\mu$ ) that were picked up on grids coated with Formvar and with a film of evaporated carbon. Sections were stained with uranyl acetate (8) and with lead citrate (9) prior to examination using an RCA EMU-3F electron microscope.

## RESULTS

In organ cultures, epithelium typically rounded into cystlike vesicles (Fig. 1). After 3 days *in vitro*, glycogen deposits and keratohyalin granules were prominent in cells at different levels of the epithelium. The fibers described below occurred in cells that also contained glycogen, but did not contain large keratohyalin granules.

As seen in the figures, long stretches of periodicity are visible in many of the tonofilament bundles (Fig. 2). The repeat distance of the dense bands averages 220 Å. Examination of individual tonofilaments by high magnification of the photographs reveals rounded electron-opaque areas regularly distributed down each filament (Fig. 6). Registry of these densities results in the periodicity seen at lower magnification. This registry has been traced across bundles of tonofilaments 0.9  $\mu$  wide (Fig. 4). Note from the figures that all bundles in a given cell do not display the repeat structure. It is not known whether differences in orientation with regard to the plane of section produce this effect, although that seems unlikely. In favorable cases, the periodicity is seen in tonofilaments that extend directly into the attachment plaques of desmosomes at the epithelial cell surface (Fig. 5).

Periodicity was much less prominent in jaw epidermis. Figs. 3 *a* and *b* are representative of the instances in which periodicity with 240 Å repeat distance was seen.

## DISCUSSION

Collagen (10) and fibrin (11) fibers both show periodic repeat units when stained in various ways and viewed with the electron microscope. The current observations establish that a similar phenomenon can occur in epithelial cell fibers. We assume that the 220 Å periodic repeat units are associated with tonofilaments because the banded fibers can be traced directly into the attachment plaques of desmosomes, and because the periodic regions often are continuous with aperiodic bundles that in all other respects are typical tonofibrils. It is unknown whether the periodicity results from heavy metal binding to the proteinaceous backbone of the filaments or from binding to other substances associated with the filaments at regular intervals. Matoltsy (12) reported a beaded appearance of filaments that aggregate during drying of purified soluble prekeratin. Whether staggered array of such units could give rise to the observed periodicity is unknown. Obviously, however, some undefined process does result in aggregation of tonofilaments so that lateral registry of the staining moieties is produced.

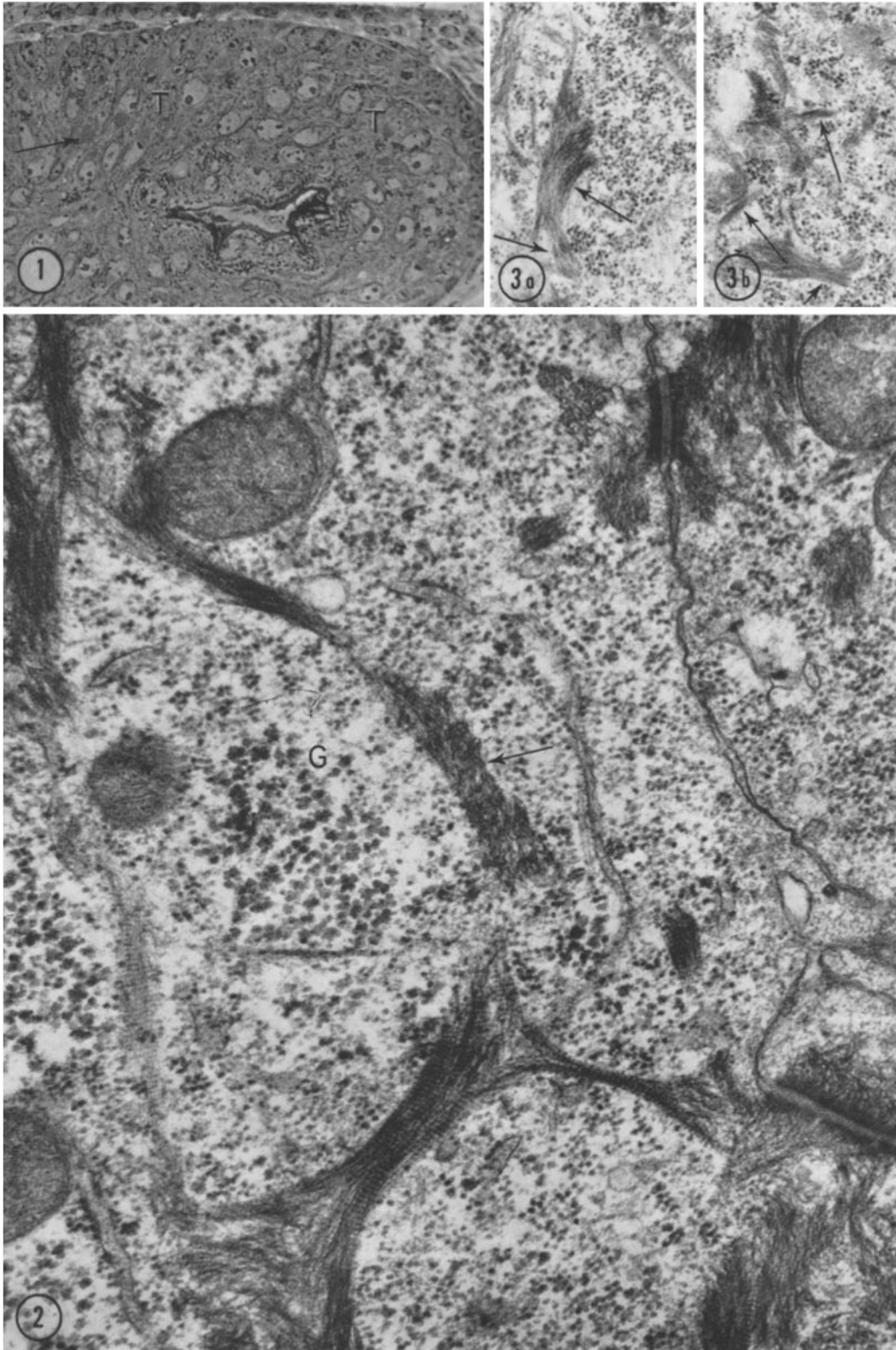
It also is unknown why the periodicity appears most prominently in organ-cultured epithelial cells that also contain glycogen granules. Interestingly, glycogen deposits also were seen in the freshly fixed 16-day embryonic epidermal cells that contained fibrils with similar periodicity. Unpublished experiments by Dr. Kallman indicate

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FIGURE 1 Cross-section of oral epithelium that has rounded into a cyst within the jaw mesenchyme during the 72 hr *in vitro*. The outermost epithelial cells are proliferative. Keratohyalin granules are visible in the cytoplasmic regions of the cells near the central lumen, and large masses of glycogen (arrow) are found in some intermediate level cells. Scattered cells at the level indicated by *T* contain the tonofibrils with the repeat structure.  $\times 400$ .

FIGURE 2 Cytoplasmic regions of intermediate-level cells of oral epithelium in which periodicity of the fibrillar system is seen. Tonofibril bundles containing periodic regions lead toward typical aperiodic clusters of tonofilaments (arrow) in the center. Glycogen granules (*G*) are located nearby.  $\times 50,500$ .

FIGURE 3 *a, b* Suggestions of periodicity in tonofilaments (arrows) in freshly fixed jaw skin from 16-day mouse embryos. Although not so frequent or prominent as in cultured cells, the repeat distance is nearly the same, 240 Å.  $\times 21,000$ .



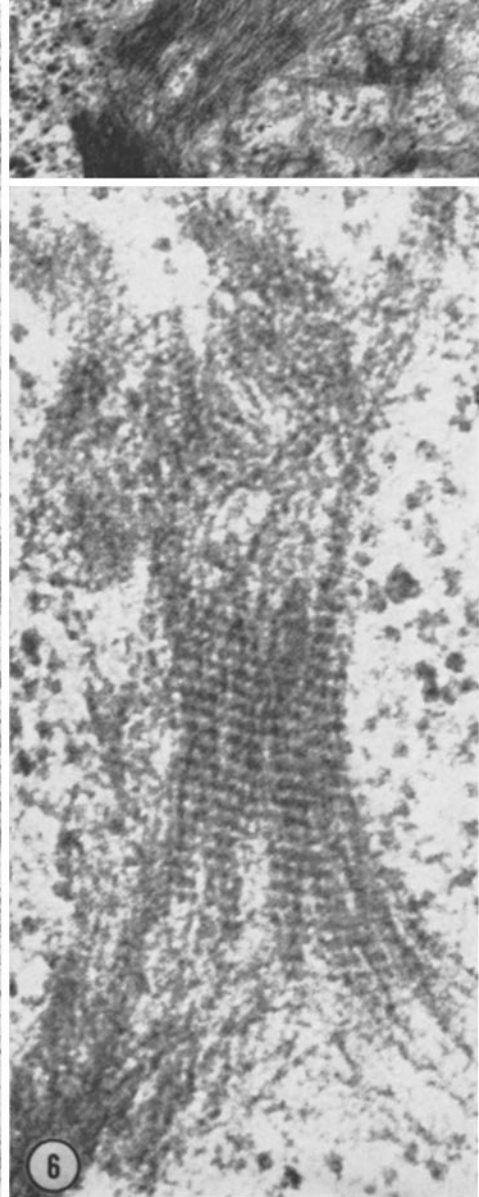
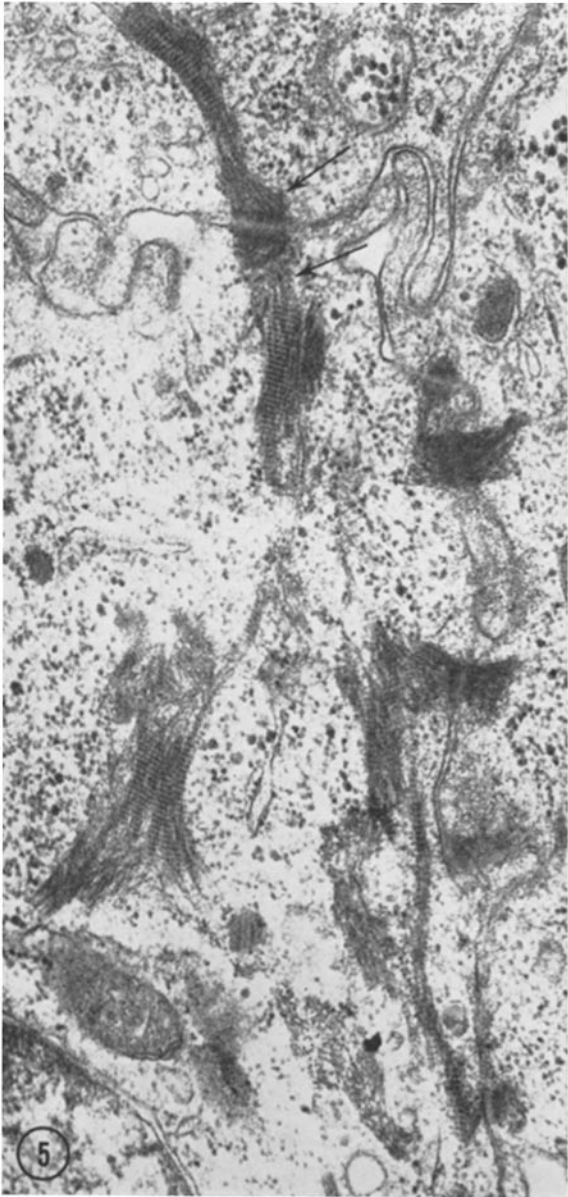
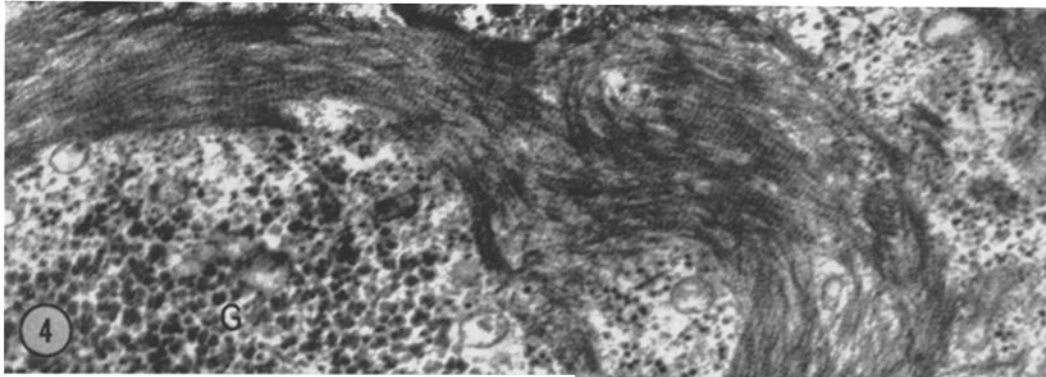


FIGURE 4 A wide bundle of tonofilaments in which the periodic densities can be traced for unusually long distances. Glycogen (G).  $\times 50,300$ .

FIGURE 5 A case in which periodicity is associated with tonofilaments that can be traced directly (arrows) into the attachment plaques of a desmosome.  $\times 31,500$ .

FIGURE 6 A portion of Fig. 5. Note the suggestion of individual densities spaced at regular intervals along individual tonofilaments; lateral registry results in the periodicity seen at lower magnification.  $\times 102,000$ .

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that the cultured cells are healthy as defined by uridine- $^3\text{H}$  and leucine- $^3\text{H}$  incorporation. Further analysis of the periodicity will depend upon finding reproducible conditions that evoke large scale filament alignment.

The observations reported in this brief paper were made by Dr. Frances Kallman prior to her death in March 1966. The manuscript has been prepared by Dr. Wessells with the gracious assistance of Mrs. Jean Evans.

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