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Effects of Cytochalasin B upon Microfilaments Involved in Morphogenesis of Salivary Epithelium

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Abstract. Cytochalasin B causes cultured mouse salivary gland epithelium to lose its characteristic shape and to cease undergoing morphogenesis. The drug causes disorganization of the 50 Å microfilaments in epithelial cells that are thought to control cell shape because of contractile properties. Upon removal of cytochalasin, epithelia regain normal shape and resume morphogenesis. Exceptionally large bundles of microfilaments appear in such recovered epithelial cells at points where their contractile activity could account for the changes in cell and tissue shape.

Morphogenesis of many developing organs is a manifestation at the population level of alterations in shape of individual cells. Recent evidence has implicated microtubules[1] and putative contractile microfilaments[2] as the intracellular organelles involved in maintenance or change in cell shape. The latter filaments average 40–50 Å in diameter and apparently insert in the junctional complex regions of epithelial cells (thus differing from the 100 Å filaments that loop through desmosomes). Microfilaments are also found in the microspikes and "fluttering membrane" locomotory organelle of motile cells[3] and of elongating axons,[4] and in the contractile ring responsible for cytokinesis during cell division.[5]

Schroeder[6] has found that the contractile ring filaments of cleaving marine eggs disappear after treatment with cytochalasin B,[7] and that cytokinesis of such eggs ceases. If, as is presumed, the 50 Å filaments of other cell types are similar to the contractile ring filaments, then the drug should produce effects upon such cells. In this paper we describe results of applying cytochalasin B to salivary epithelial cells engaged in morphogenesis.

Methods. Salivary glands from 13-day mouse embryos (BALB/C3H) were cultured on Millipore filter rafts using standard medium[8] and conditions.[9] "Transfilter"[9] cultures had mesoderm above the filter and epithelium clotted below. The latter epithelia, or cytochalasin B-treated ones, were freed of mesoderm with trypsin-pancreatin.[9] Cytochalasin B dissolved in dimethylsulfoxide[7] was added to complete medium at 7 or 10 μg/ml with dimethylsulfoxide at 1%. Control cultures in 1% dimethylsulfoxide medium continue normal morphogenesis. "Recovery" from 18 to 36 hr of cytochalasin B treatment was obtained by washing cultures three times in medium and reincubating in medium. Cultures were fixed in glutaraldehyde and osmium and prepared for electron microscopy as in reference 10.

Results and Discussion. Normal branching morphogenesis continued in all control cultures (Fig. 1*a*). Within 6 to 10 hr after the addition of cytochalasin B, the salivary epithelium began to lose its characteristic shape: the normally

thick, rounded epithelium flattened (Fig. 1*b*); the clefts and bulges, points of morphogenetic activity when the drug was applied, disappeared. To aid visualization, such epithelia were freed of mesoderm with trypsin-pancreatin; when viewed in such a state the epithelium was indeed flat and waferlike as opposed to being of rounded shape. Whole cultures were left in the drug for as long as 72 hr, and no morphogenetic branching took place.

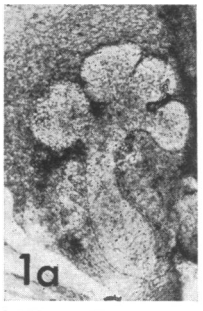
When cytochalasin B was removed from the culture medium, the salivary epithelia rounded up and again formed morphogenetic bulges and branch points (Fig. 1*c*). The initial restorative changes could be detected visually at about 10 hr. With further culturing, such branches showed continuation of salivary-like morphogenesis, indicating that no permanent damage had been done by the treatment.

Epithelia cultured transfilter[9] to salivary mesoderm also lost their normal shape when cytochalasin B was applied, and recovered when the drug was removed. Such recovery took place even if all mesoderm was scraped from the opposite side of the filter. Thus, in the 18 hr after removal of the drug, epithelia rounded up and formed clefts and bulges, on the one hand in the *complete absence* of mesoderm and, on the other, in the absence of direct contact between mesoderm and epithelium. Therefore, restoration in shape of the epithelium must be the result of forces within the epithelial cells themselves.

Electron microscopic observations were made on the columnar salivary epithelial cells. This report centers on the basal ends of such cells, since those regions display striking changes upon the addition and removal of cytochalasin B. Control cells are bounded at their lateral surfaces by regions where 40–50 Å microfilaments approach the cell membrane, appearing to insert into electron-dense material just inside the plasma membrane.

Large masses of granular and filamentous material are seen near the lateral surfaces at each end of the cells treated with cytochalasin B (Fig. 2). The filaments are 40–50 Å in diameter but, unlike those seen in untreated cells, occur only in short lengths and are randomly oriented with respect to each other. These masses are seen in the vicinity of the junctional complexes at the apical end and near the regions where filaments inserted at the basal end of the cells. Such masses are never seen in a control or a recovered cell. In some preparations, very little of the electron-dense material was seen associated with the inner side of the plasma membrane at points where filaments normally insert. This observation raises the possibility that cytochalasin B may affect the attachment of filaments to the cell membrane in addition to the integrity of filaments in organized bundles. In treated cells, microtubules and 100 Å filaments appear normal in number and distribution.[11]

Epithelia fixed after recovery from cytochalasin B provide remarkable views of organized filament bundles. As seen in Figure 1*c*, the clefts of such epithelia are unusually broad and deep. In every such cleft examined are found extraordinarily thick bundles of 40 to 50 Å filaments, running from one side of a cell to the other (Fig. 3). Single thin sections show such bundles in cell after cell, giving the impression of a continuous band of filaments at the base of the cleft. It is interesting that highly convoluted cell surfaces are often seen immediately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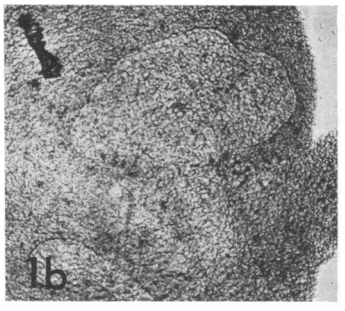
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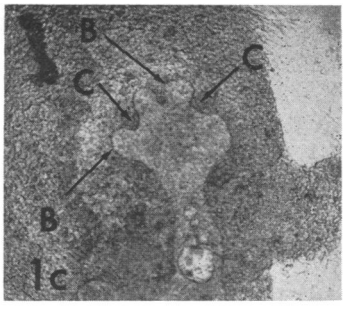
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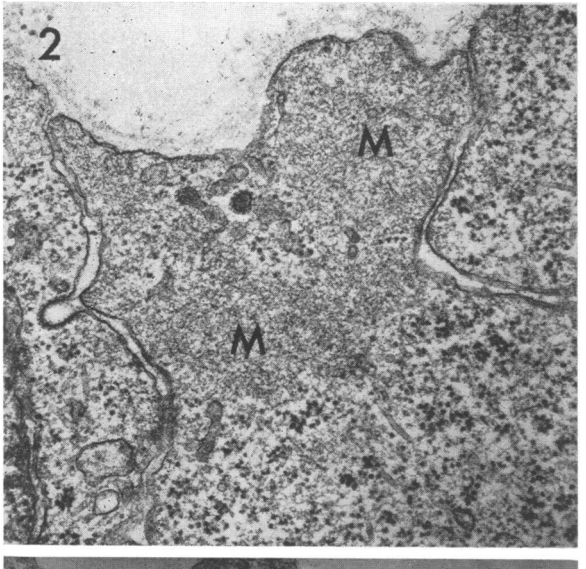
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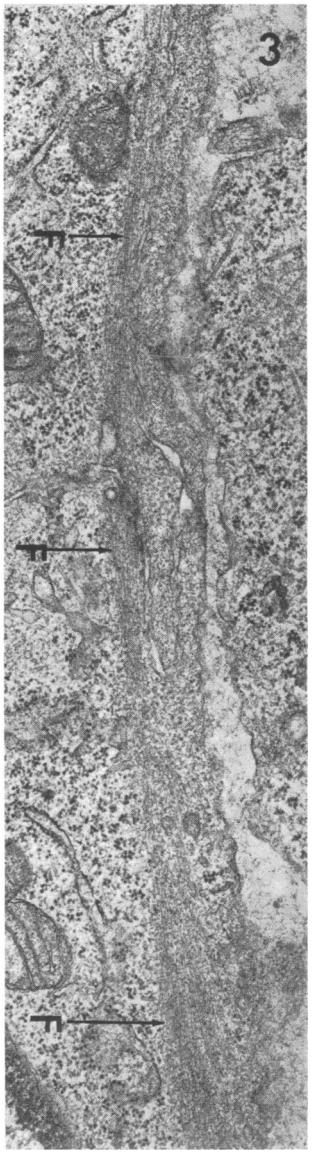
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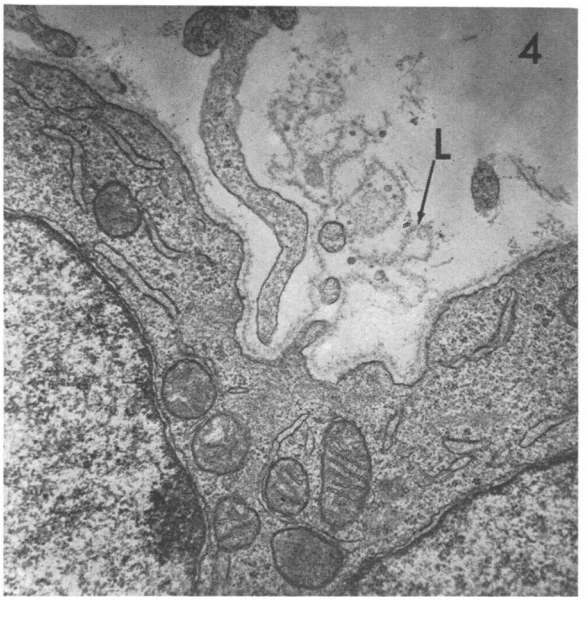
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L

FIG. 1.—(*a*) Control salivary gland in culture, 24 hr. (*b*) Salivary gland in cytochalasin, 24 hr. (*c*) The same gland shown in Fig. 1*b*, but after cytochalasin removal and 24-hr reincubation. This recovered gland has both rounded up and formed deep wide clefts (*C*) during the recovery period. *B* = bulge. ×170.

FIG. 2.—Cytochalasin-induced masses (*M*) of granular and filamentous material at the basal end of salivary epithelial cells. Organized bundles of filaments would normally be seen in these regions. ×31,000.

FIG. 3.—A montage of the basal ends of cells at the base of a cleft in a recovered gland (as in Fig. 1*c*). Organized filament bundles (*F*) have reappeared. ×22,500.

FIG. 4.—Convoluted epithelial cell surfaces and "separated" basal lamina (*L*) in the cleft of a recovered culture. These phenomena are never seen on the bulges or sides of recovered epithelia. Bundles of organized filaments (as in Fig. 3) are seen in other sections through this cell. ×16,000.

over the band (Fig. 4). At points, the overlying basal lamina is separated from the epithelial cell surface and thrown into tortuous folds. The convolutions of plasma membrane might result if the underlying filamentous network had contracted to generate the cleft, thus reducing the cross-sectional area of the cells in the base of the clefts. Furthermore, separation of the basal lamina might result if its surface area cannot be reduced rapidly enough to adjust to the decreasing cell surface area caused by such a contraction.

In "recovered" epithelial cells located at the tips or on the sides of the epithelial bulges, the drug-induced masses of disorganized filaments have disappeared, and typical organized aggregates of filaments are seen associated with the junctional complexes. The basal ends of such cells are smooth and free of convolutions, and the basal lamina is smooth and closely associated with the plasma membrane in all regions. Thus, at these locations in the epithelium, there is no hint of the unusual reductions in cell cross-sectional area that were discerned in the clefts. The reconstituted filament systems of cells at the tips and the sides of bulges may well be functioning, however, in the restoration of the "rounded" epithelial shape.

These observations imply that, in large part, epithelial shape is controlled by microfilament systems in the epithelial cells.[10] The experiments reinforce the idea that such filaments are contractile, since the other known effects of cytochalasin B—upon cytokinesis,[6] cell motility,[7] gland formation in oviduct,[12] and growth cone function in elongating axons[4]—are best explained by inhibition of contractile filaments.* Epithelia that have recovered from cytochalasin B by forming extraordinarily wide clefts provide a strong correlation between the presence of thick bundles of filaments and this "morphogenetic movement." From the point of view of normal organ morphogenesis, the important question becomes: what controls the distribution and the contractile function of such filaments in time? Since specific salivary gland mesoderm[13] must be near the epithelium if any epithelial morphogenesis is to occur, the role of that tissue in filament organization and function deserves attention.

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* *Note added in proof:* The possibility that cytochalasin acts by inhibiting protein synthesis has been eliminated by recent experiments showing that ^{14}C-amino acid incorporation into hot trichloroaceticacid-insoluble material in control, dimethylsulfoxide-treated and cytochalasin-treated glands is identical at 23 and 46 hr of incubation. Furthermore, incorporation into glands that were cytochalasin treated for 23 hr and then "recovered" for 23 hr is the same as that into glands that were in cytochalasin for 46 hr.

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[11] A further effect of cytochalasin B is upon the distribution of mitotic cells in the epithelium: in controls, mitosis always occurs at the luminal surface; in cytochalasin B-treated ones, mitosis frequently takes place at the basal surface with the cells often protruding into mesenchymal spaces (see also[12]). The drug apparently inhibits the processes responsible for the premitotic movement in normal tissue.
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