CALCIUM IN EPITHELIAL CELL CONTRACTION

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ABSTRACT

Epithelial morphogenesis in many organs involves asymmetric microfilament-mediated cellular contractions. Similar contractions, in terms of ultrastructure and cytochalasin B sensitivity, can be induced in the carcinoma cell line C-4II in culture. This line was used to compare total intracellular calcium levels ([Ca]i) in contracted monolayer fragments and in control cultures, and to determine whether epithelial cell contraction depends on influx of extracellular Ca. [Ca]i, defined as Ca not displaceable by lanthanum, was measured by atomic absorption spectrophotometry. Degrees of contraction were determined from shape changes of monolayer fragments. Detachment from the growth surface initiated cellular contractions and caused an immediate increase in [Ca]i, from 1.0 to 4.0–5.0 μg Ca/mg protein in early confluent cultures, and from 0.3 to 1.0–2.0 μg Ca/mg protein in crowded cultures. This increase was followed by a gradual decline in [Ca]i, though Ca levels remained higher than in controls and contraction progressed for 30 min. Contraction was inhibited completely by cold (7°C) and by Ca-free medium, and in a dose-dependent manner by papaverine (2.5×10^{-6} M–2.5×10^{-4} M), lanthanum (1.0×10^{-6} M–1.0×10^{-4} M), and D-600 ($1.0–2.0 \times 10^{-4}$ M). The Ca ionophore A23187 had no effect at 5.0×10^{-6} M and was inhibitory at higher concentrations. The results provide direct evidence for increased [Ca]i in contracting epithelial cells, and suggest that Ca influx is required for such contraction to take place.

During embryogenesis, developing epithelia undergo changes in cell shapes and intercellular organization that are attained through asymmetric coordinated cellular contractions. These morphogenetic processes are mediated by microfilaments positioned in the contracting region of the cells (37), and they result in the folding (e.g., neurulation) or convolution (e.g., gland morphogenesis) of previously flat epithelial sheets (5, 9, 31). There is now widespread evidence indicating that the contractile proteins of nonmuscle cells bear a close resemblance to those of muscle (30, 32, 33) and that, as in muscle, contractile activity is influenced by calcium (1, 17, 38). Indirect evidence suggests that epithelia may require influx of extracellular calcium for contraction to take place: the formation of indentations of the epithelium in salivary gland morphogenesis is reversibly inhibited by Ca-depleted culture medium and by papaverine, a drug affecting Ca flux (1). Amphibian neurulation is also inhibited by papaverine, and this inhibition is counteracted by the Ca ionophore A23187 (27). More direct biochemical analysis of these contractile mechanisms is hindered by the difficulty of obtaining adequate amounts of embryonic tissues and by the dependence of developing epithelia on interactions with mesenchyme and the resulting complexity of the materials under study.

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In the work presented here, epithelial cell contraction was examined in a relatively simple tissue culture system consisting of homogeneous cells that are available in unlimited amounts and capable of contraction in the absence of any heterologous tissues. Over years in culture, cells of the human carcinoma line C-4II have retained epithelial characteristics, including the ability to form basal lamina in vivo and to form apical junctional complexes and hemicysts (domes) in culture (2). If fragments of C-4II monolayers are detached from the growth surface, the suspended cell sheets become convoluted through cell elongation along their apicobasal axis and microfilament-dependent coordinated contractions localized in the cortical cytoplasm subjacent to the basal plasma membrane (3). This process bears a striking resemblance to epithelial morphogenesis in vivo (13) and in vitro (31) in terms of intercellular reorganization, ultrastructural changes, and cytochalasin B sensitivity.

C-4II cells, like smooth-muscle cells and normal epithelial cells, lack the highly developed membranous organelles that sequester and store Ca in striated muscle. It seemed likely therefore that they might require, as does smooth muscle (18, 19), influx of extracellular Ca for contraction to take place. This hypothesis was tested using an adaptation for cell culture[1] of the lanthanum method (35), which has been used extensively to measure Ca levels in muscle. The lanthanum method is based on evidence indicating that extracellular La replaces Ca from the cell coat and outer plasma membrane but does not penetrate cells and that, in the presence of La, Ca flux across membranes is effectively inhibited at low temperatures (11, 16, 19, 23, 36). Therefore, under proper conditions, Ca not replaceable by La is considered to represent total (free + bound) intracellular Ca, and to reflect net changes resulting either from Ca efflux or from uptake of extracellular Ca by cells. With this method, and with agents known to alter Ca exchange in other cell types, direct evidence was obtained indicating that intracellular Ca levels are increased during epithelial cell contraction, and that influx of extracellular Ca is necessary for such contraction to take place.

[1] Lee, H.-C., and N. Auersperg. The use of lanthanum for intracellular calcium measurements in cultured cells. Manuscript submitted for publication.

MATERIALS AND METHODS

Tissue Culture

The cell line C-4II was originally derived from a human squamous cervical carcinoma (2). Stock cultures were maintained in 30-ml plastic tissue-culture flasks in Waymouth's medium MB752/1 with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.), 100 U/ml of penicillin and 100 μg/ml of streptomycin (Microbiological Associates, Walkersville, Md.) at 37°C in humidified 5% CO_2/air. Medium was changed every 2–3 d, and the cells were subcultured every 7–10 d by dissociation in 0.12% trypsin (1:250)/0.025% EGTA in Ca- and Mg-free Hanks' balanced salt solution (Grand Island Biological Co.).

For contraction studies, 1.0–1.8 × 10^6 cells were plated in 60 × 15-mm tissue culture dishes with 2-mm grids on the undersurface (Lux Scientific Corp., Newbury Park, Calif.). Medium was changed on days 2, 4, and 6, and the experiments were performed on day 9 after subculture.

Induction and Quantitation of Contractions

Confluent cultures were rinsed twice with 5 ml of serum-free Waymouth's medium, and the cell sheets were divided into 1 × 2-mm sections with a scalpel, following the grid design on the underside of the dish. The divided monolayers were rinsed with the test solution, and, by use of a plastic wedge, the 1 × 2-mm cell sheets were detached from the substratum in 5 ml of fresh test solution and incubated in suspension for varying time periods. Control cultures with undisturbed cells were incubated for equal periods in the same test solutions. All procedures were carried out at 37°C unless stated otherwise. As described previously (3), detachment from the growth surface causes coordinated contractions limited to the basal parts of the cells. Contraction therefore results in asymmetric shortening and, consequently, in rolling up of the monolayer fragments. If the initial form of the sheets is rectangular, rolling up characteristically occurs along their long axis (Fig. 1).

After incubation, the cells were fixed in 2.5% glutaraldehyde/Millonig's phosphate buffer at 4°C and stored in buffer. Degrees of contraction were expressed as ratios of the lengths over widths of the cell sheets as measured at a magnification of × 4 under a dissecting microscope. Because the initial dimensions of the sheets were 2 × 1 mm, a ratio of ~2.0 indicated that no contraction had taken place. As contraction caused rolling up along the long (2-mm) axis of the sheets, widths became reduced and ratios increased in proportion to contraction. At least 15–30 cell sheets per culture dish were measured.

Ca and Protein Determinations

Each sample of either uncontracted control cells or contracted monolayer fragments consisted of pooled duplicate cultures. Intracellular Ca levels were expressed as ratios of micrograms of Ca to milligrams of protein. The Ca content of cells and test solutions was determined in an Atomsorb model 280 atomic absorption spectrophotometer (Jarrell Ash Div., Fisher Scientific Co., Waltham, Mass.), following removal of cell surface-derived and extracellular Ca by an adaptation of the lanthanum method of epithelial cell culture.[1] Briefly, the cells were rinsed for 30 min at 4°C in 10 mM $LaCl_3$/160 mM Tris-HCl at pH 7.4. The cells were collected by centrifugation, washed in Tris-HCl, and recentrifuged. The supernates were discarded and the cell pellets were dissolved in hot 1:1 (vol/vol) glacial acetic acid:3 M TCA. Protein

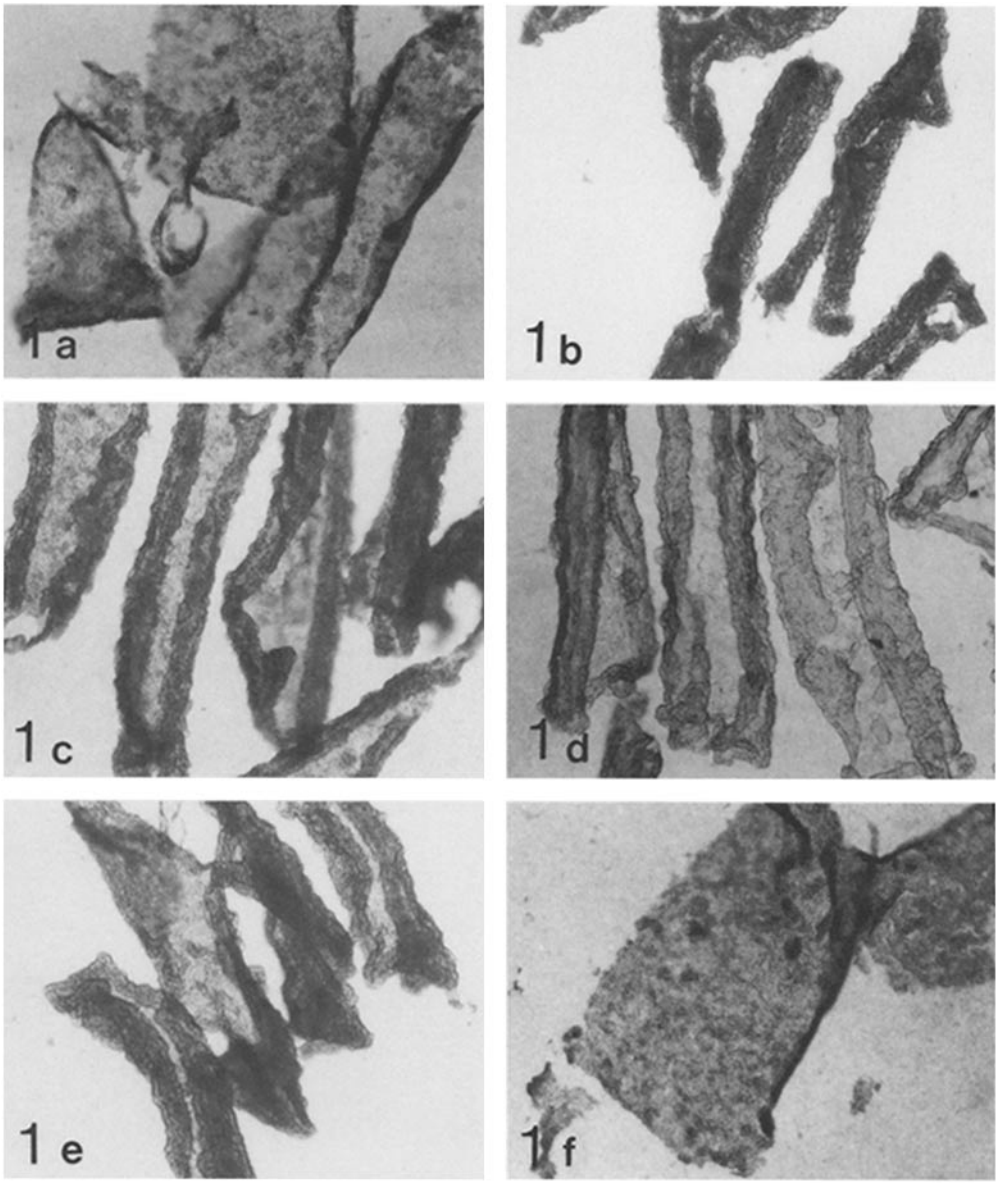

FIGURE 1 2 × 1-mm cell sheets after suspension in different solutions and under different conditions. Unless stated otherwise, 30 min at 37°C. (a) HEPES-BSS; a few minutes after detachment. The length to width ratio is still ~2.0. (*b*) HEPES-BSS; contraction to length to width ratio of 6.80. (*c*) $LaCl_3$, 5.5×10^{-5} M in HEPES-BSS; length to width ratio, 4.00. (*d*) Papaverine, 2.0×10^{-5} M in Waymouth's medium 752/1; length to width ratio, 3.50. (*e*) D-600, 2×10^{-4} M in Hanks' BSS; length to width ratio, 4.5. (*f*) 7°C in HEPES-BSS; length to width ratio, 2.06. Phase optics, × 30.

was aggregated by addition of H_2O and cooling, collected by centrifugation, washed in H_2O, and measured by the method of Lowry et al. (25) using crystalline bovine albumin fraction V (Sigma Chemical Co., St. Louis, Mo.) as the protein standard. The supernates were pooled and used for Ca measurements.

Chemicals

Ionophore A23187 (a gift from Dr. R. Hamill, Eli Lilly International Corp., Indianapolis, Ind.) was used from a stock solution of 5 mg/ml 95% ethanol, D-600 (Kroll AG, Ludwigshafen/RL, W. Germany) from stock solutions of 10 and 20 mg/

ml dimethyl sulfoxide (DMSO), and papaverine-HCl (Sigma Chemical Co.) from a stock solution of 1.0 mg/ml H_2O. All stock solutions were stored frozen. Controls for all cultures treated with the above-mentioned drugs contained comparable amounts of solvents.

Most experiments were carried out in Hanks' balanced salt solution (Hanks' BSS). For experiments in which effects of $LaCl_3$ or of high Ca concentrations on cells were examined, a carbonate- and phosphate-free, HEPES-buffered, balanced salt solution (HEPES-BSS; HEPES from J. T. Baker Chemical Company, Phillipsburg, N. J.) was prepared (Table I).

RESULTS

Changes in Intracellular Ca Levels with Contraction

The 1 × 2-mm cell sheets started contracting within minutes upon detachment from the growth surface and began to roll up along their long axis. The degree of contraction increased gradually over 30 min, after which time the cells had formed tubular structures and contraction proceeded more slowly (Fig. 1 *a* and *b*). Cells in mitosis and cells that appeared dead or severely damaged on the basis of morphology and staining characteristics did not appear to contribute to the process of rolling up (Fig. 2). Immediately after detachment from the culture dish (Figs. 3 and 4), the basal cytoplasm contained a cortical filament network and particulate organelles. Intercellular contact was maintained by overlapping cell edges, as in undetached cultures (2). There was no evidence of damage to the basal plasma membranes. After

TABLE I

Composition of Balanced Salt Solutions

Components	Hanks' BSS	Mg-, Ca-free Hanks' BSS	HEPES-buffered phosphate-, carbonate-free BSS
	mM	*mM*	*mM*
NaCl	136.89	136.89	129.86
KCl	5.37	5.37	4.55
$CaCl_2$	1.26	—	1.20
$MgSO_4$	0.406	—	0.201
$MgCl_2$	0.492	—	0.492
Na_2HPO_4	0.423	0.423	—
KH_2PO_4	0.441	0.441	—
Glucose	5.55	5.55	6.11
$NaHCO_3$	4.17	4.17	—
HEPES	—	—	10.5
Phenol red	200 mg/ liter	200 mg/ liter	50 mg/ liter

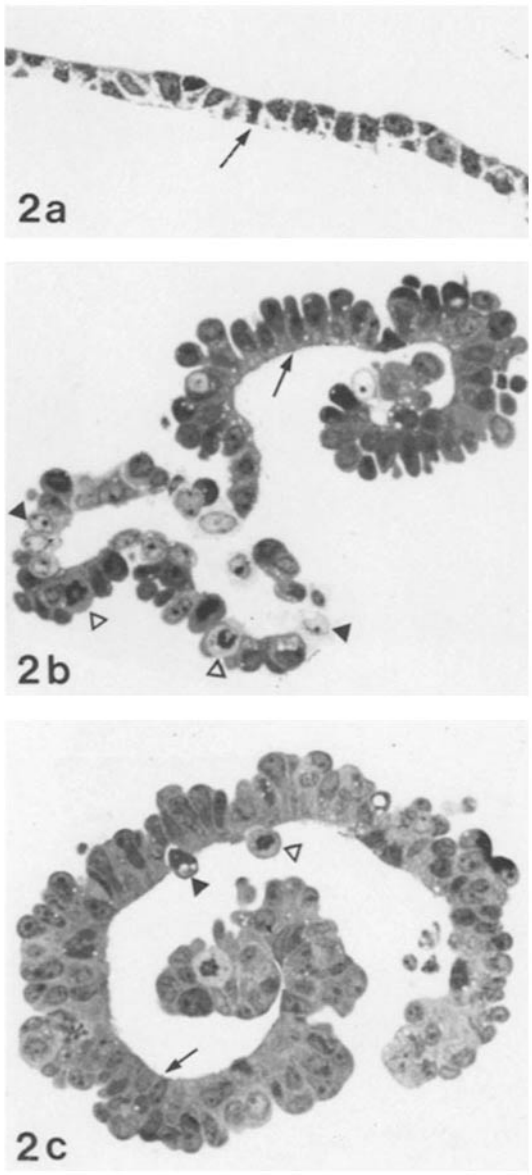

FIGURE 2 Cross sections through monolayer fragments of C-4II cells, detached from the culture dish, suspended in Hanks' BSS and fixed (*a*) immediately, (*b*) 10 min after detachment, and (*c*) 1 h after detachment. Arrows point to the basal surface, i.e., to the side of the cell sheets that was attached to the substratum. The sheets roll up as the cells elongate along their apicobasal axis, and become closely apposed and contracted in the basal region. Dead or damaged cells (▲) and mitotic cells (△) do not seem to take part in this process. Epon 812 embedding, toluidine blue. × 440.

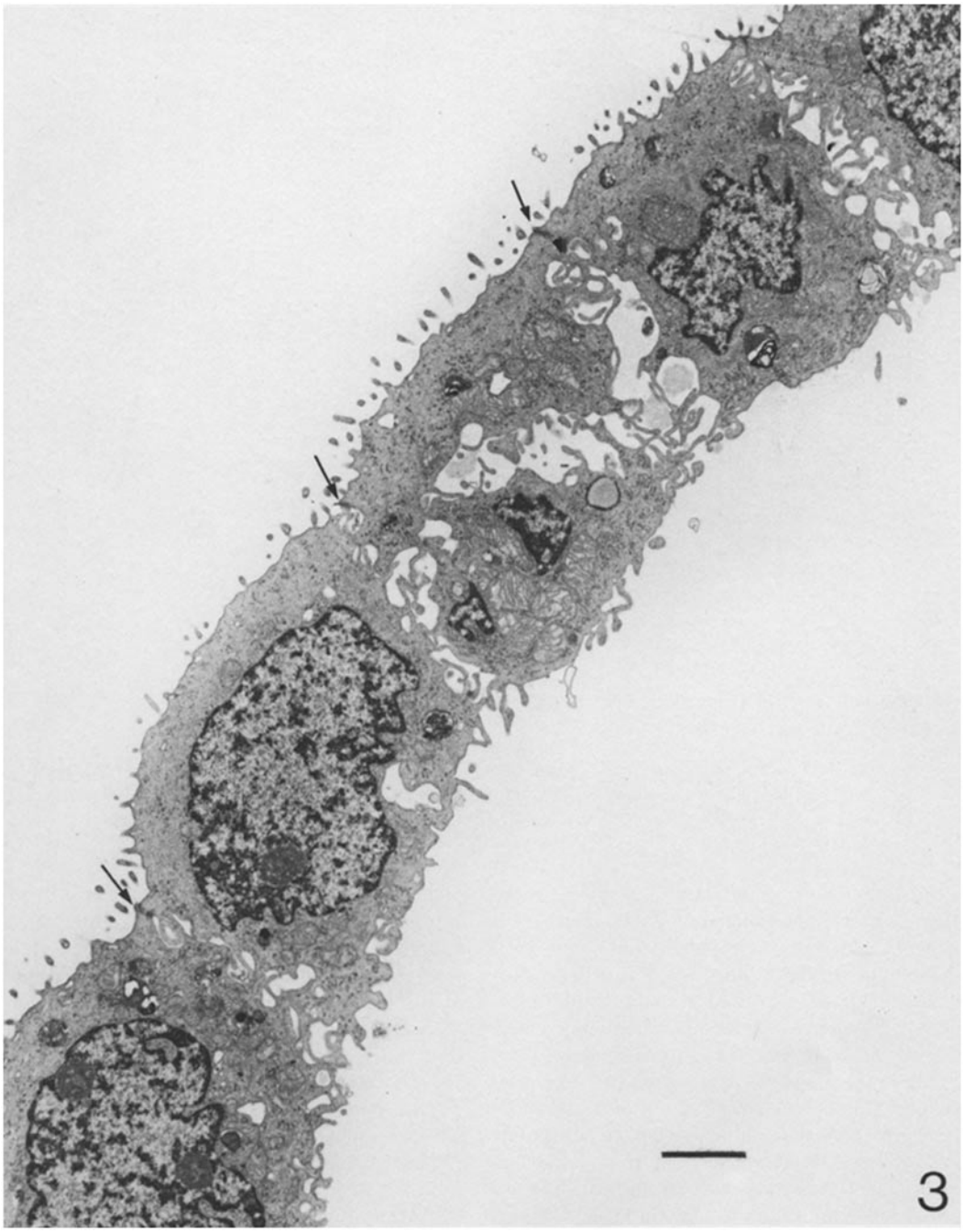

FIGURE 3 Electron micrograph of section through cell sheet fixed immediately after suspension (see Fig. 2*a*). At the apical surfaces, the cells form microvilli and are joined by junctional complexes (arrows). Basally, intercellular contact is maintained by nonspecialized overlapping cell edges. There is no evidence of cell damage at the basal plasma membrane, and there is no filament condensation in the basal cytoplasm. Bar, 2.0 μm. × 6,100.

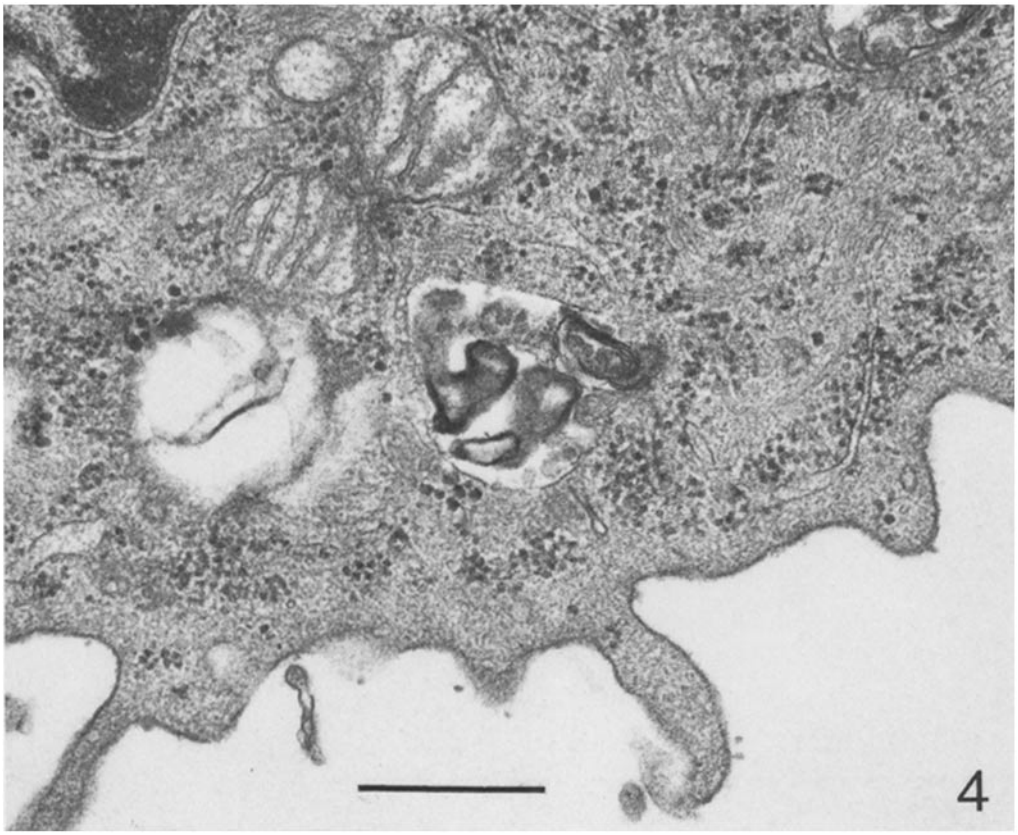

FIGURE 4 Electron micrograph of a preparation similar to that in Fig. 3, showing details of the basal region of one cell. The plasma membrane is intact, and the cytoplasm contains a meshwork of cortical filaments as well as particulate organelles. Bar, 0.5 μm. × 48,000.

suspension for 1 h, the basal cortical filaments had formed prominent bands that displaced other organelles and were associated laterally with intermediate intercellular junctions. The cells had become elongated, narrowed at the base, and more closely adherent to one another than immediately after detachment from the substratum (Figs. 5 and 6) (3). Fig. 7 shows the relationship between intracellular Ca content and duration of contractions in Hanks' BSS, with data pooled from 14 separate experiments. Detachment from the plastic was associated consistently with an immediate severalfold increase in intracellular Ca levels. This increase was followed by a gradual decline though, in every experiment, contracted cells after 30 min still contained significantly more Ca than the controls. In controls and after contraction, intracellular Ca levels in cells from crowded cultures (>4.0 mg protein per sample) were lower than those from less crowded cultures, in agreement with other observations.[1] Contractions comparable in degree to those in Hanks' BSS (1.26 mM Ca) and similarly associated with increased intracellular Ca levels also occurred if the cell sheets were suspended in Waymouth's medium (0.82 mM Ca) or in HEPES-buffered, carbonate- and phosphate-free Hanks' BSS (1.2 mM Ca) (Table II).

The possibility was considered that the higher Ca levels in contracted cell sheets resulted from the cells being arranged into more compact multicellular structures after contraction, and some of the extracellular Ca therefore being less accessible to La displacement. This is unlikely, however, because (*a*) the difference in Ca levels between controls and contracted cells was greatest 1 min after detachment from substrata, i.e., before visible morphologic changes and rearrangements within the cell sheets; (*b*) a significant difference between

controls and contracted cells persisted if the cell sheets were broken up into small clumps and single cells at the onset of La-Tris-HCl treatment; (*c*) the high levels of Ca in contracted sheets stabilized after 30 min in spite of continued La-Tris-HCl treatment.[1]

Effects on Contraction of Divalent Cation Depletion and Agents That Alter Ca Flux

Evidence presented above indicates that C-4II cells take up extracellular Ca when detached from the growth surface, and that intracellular Ca levels remain elevated during contraction. The following series of experiments was undertaken to determine whether the Ca taken up was actually required for contraction to take place.

DIVALENT CATION DEPLETION: Contraction of the cell sheets was completely inhibited in Ca-, Mg-free BSS, and Ca-free BSS but not in Mg-free BSS (Table III). In Hanks' BSS with Ca concentrations of 0.32 mM and above, contraction proceeded at a normal rate, but the rate was reduced if the Ca concentration was lowered to 0.13 mM (Table III). When incubated for 40 min in Ca-, Mg-free BSS, or Ca-free BSS, cells released an average of 1.43 and 1.60 μg Ca/mg protein, respectively, whereas no Ca release was detected in complete or Mg-free Hanks' BSS. In Ca-, Mg-free BSS, cell sheets gradually disintegrated; the cells became rounded and, if left in this solution for >3 h, many cells died as indicated by lack of dye exclusion. This apparent inability of the cells to maintain physiological intracellular Ca levels in the absence of extracellular Ca agrees with suggestions based on observations in cultured kidney cells (6) and 3T3 cells (34). The effects of divalent cation depletion were in keeping with an extracellular Ca requirement for C-4II cell contraction. However, it was not possible to distinguish the effects of Ca lack on contractility from deleterious effects on intercellular adhesion that interfered

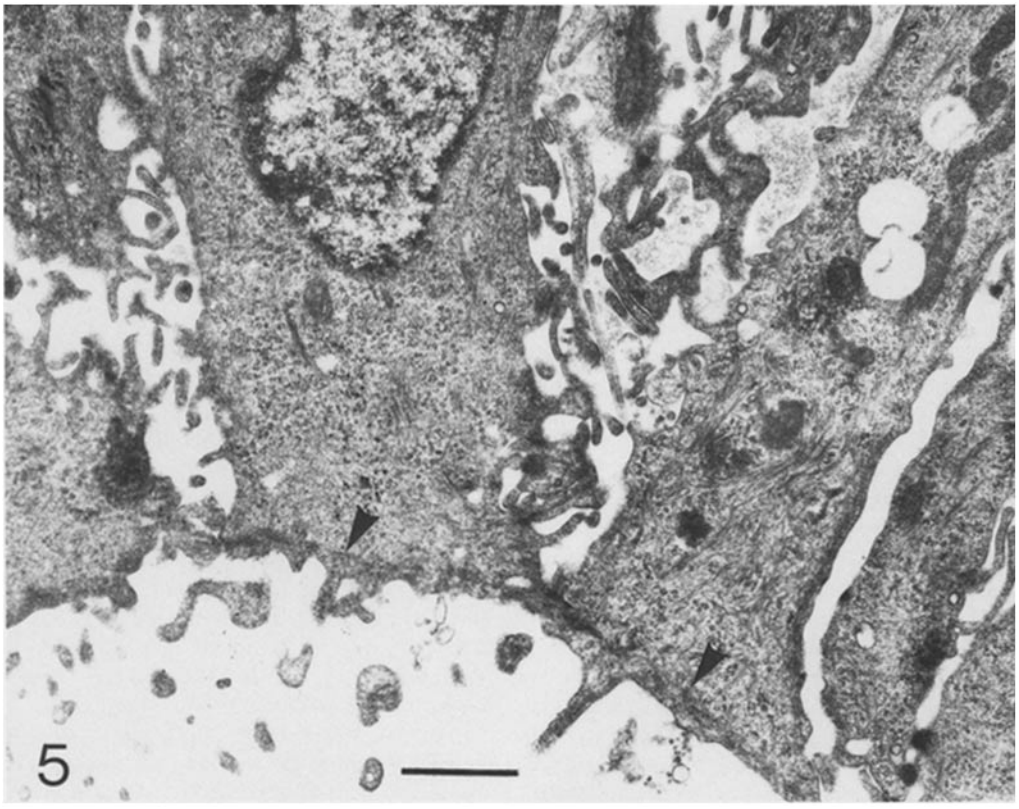

FIGURE 5 Electron micrograph of section through the basal portion of a cell sheet fixed 1 h after suspension (see Fig. 2*c*). The cell diameters parallel to the base are shortened; the plasma membrane is folded, and adjacent to it there is a dense filament band (arrowheads). Bar, 1.0 μm. × 15,000.

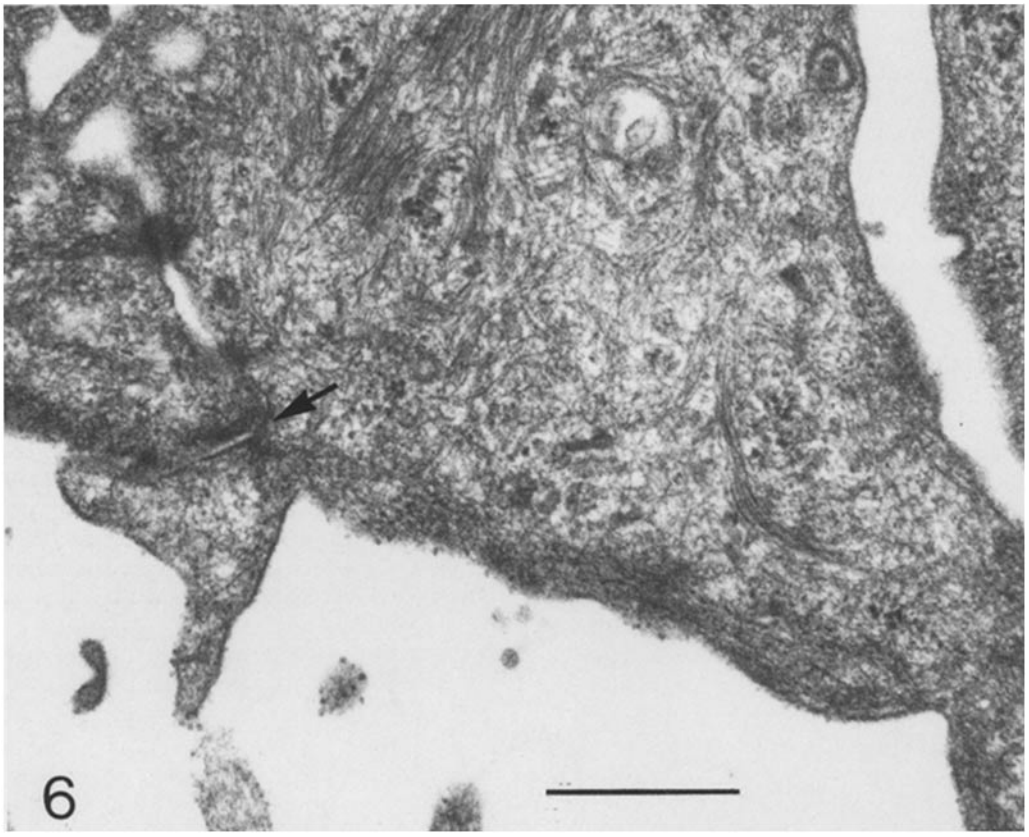

FIGURE 6 Electron micrograph of a preparation similar to that in Fig. 5, showing details of the basal region of one cell. The region near the plasma membrane is taken up by a filament layer to the exclusion of other organelles. Note the intermediate junction in the left lateral cell wall, continuous with the filament band (arrow). Bar, 0.5 μm. × 49,500.

with the rolling up of the cell sheets. To examine the possible extracellular Ca requirement for contraction more specifically, we suspended cell sheets in solutions containing agents that block Ca flux (papaverine, lanthanum, D-600) or facilitate Ca flux (ionophore A23187).

PAPAVERINE: In Waymouth's medium, papaverine inhibited contraction completely at 2.5 × 10^{-5} M and above, partially at 2.5 × 10^{-6} M, and had no effect at 2.5 × 10^{-7} M (Table III, Fig. 1). The inhibitory effect of papaverine was reversed by transfer of the cells to papaverine-free medium.

LOW CONCENTRATIONS OF $LaCl_3$: Effects on cellular contraction of low concentrations of $LaCl_3$ in HEPES-buffered, carbonate- and phosphate-free BSS are shown in Table III and Fig. 1. Contraction was inhibited to increasing degrees by concentrations of 10^{-5} M $LaCl_3$ and above, and inconsistently by 10^{-6} M $LaCl_3$.

D-600: The effect of D-600 on contraction was examined in Hanks' BSS with varying concentrations of Ca, as well as in Waymouth's medium. In standard Hanks' BSS (1.26 mM Ca), contraction was partially inhibited by 2 × 10^{-4} M but not by 1 × 10^{-4} M D-600. In Waymouth's medium (0.82 mM Ca) and in Hanks' BSS with Ca concentrations reduced to 0.31 and 0.13 mM, contraction was inhibited by 1 × 10^{-4} M and 2 × 10^{-4} M but not by 5 × 10^{-5} M D-600 (Table III, Fig. 1).

IONOPHORE A23187: Contraction of C-4II cells in Waymouth's medium over periods up to 60 min was not affected by 5 × 10^{-6} M A23187 (Table III). At concentrations of 2 × 10^{-5} M and above, contraction was increasingly inhibited, possibly as a result of toxicity.

Effects of Cold on Contractility of C-4II Cells

Incubation of detached cell sheets in Hanks' BSS at 4°–7°C for periods ranging from 15 to 120 min completely and reversibly inhibited contraction. However, once contracted at 37°C, cell sheets remained contracted for up to 48 h upon transfer to 7°C (Table III, Fig. 1).

DISCUSSION

The appearance of organized microfilaments in locations where contractions produce changes in cell shape led originally to the suggestion that these cellular components are the contractile elements of nonmuscular cells (13). Numerous correlations of contraction-inhibitory effects of cytochalasin B with the disappearance of microfilaments in nonmuscle cells greatly strengthened this hypothesis (17, 31, 37). The suggestion that the mechanism of C-4II contraction is comparable to that of cells in developing epithelia is based on the ultrastructural changes involved as well as on the sensitivity of the process to cytochalasin B (3). In the present study, additional evidence was obtained supporting the validity of C-4II cells as a model for epithelial cell contraction. The lack of contraction at 7°C is in keeping with the concept that the rolling up of C-4II monolayers represents an energy-requiring contractile process as distinct from elastic recoil upon release from the growth surface. As in developing epithelia, C-4II contraction was inhibited by the smooth muscle relaxant papaverine, at doses comparable to or below those inhibitory to cardiac and smooth-muscle contraction (14, 18), salivary gland morphogenesis (1), and amphibian neurulation (27). Though papaverine alters Ca flux, its mode of action is complex, and many of its effects may be attributed to increased cAMP levels resulting from phosphodiesterase inhibition. However, there is some evidence that, in salivary gland morphogenesis, increased levels of cAMP are not the primary means by which papaverine inhibits epithelial contraction (1). The dependence of rolling up of C-4II cell sheets on the presence of extracellular Ca is also comparable to the results obtained in embryonic systems, though it cannot be taken as direct evidence for a Ca requirement in contraction, because Ca is needed to maintain intercellular adhesion and the integrity of the cell sheets.

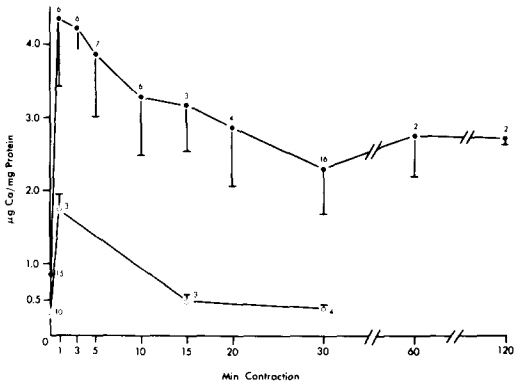

FIGURE 7 Effect of different times of contraction in Hanks' BSS on intracellular Ca levels. ●, Samples with <4.0 mg protein; ○, samples with >4.0 mg protein. Means and SD. Numbers indicate number of samples.

TABLE II

Effect of Contraction on Intracellular Ca Content of C-4II Cells*

Sample	Intracellular Ca (ratio of Ca to protein)
	μg/mg
Waymouth's medium	
Controls	0.32 ± 0.06
Contracted	2.10 ± 0.96
HEPES-BSS	
Controls	0.49 ± 0.25
Contracted	0.73 ± 0.34

Means ± SD of four samples per determination.

* In Waymouth's medium 752/1 (0.82 mM Ca) and in carbonate- and phosphate-free HEPES-buffered Hanks' BBS (1.2 mM Ca).

In their response to detachment from substrata, C-4II cells differ from many types of cultured cells that respond by rounding up, forming microvilli over their entire surface, and converting the microfilament bundles present in stress fibers into a filamentous meshwork that is most prominent near the nucleus and microvilli (15). In contrast, the contraction of C-4II cells resembles the differentiated function of a multicellular organized unit. It would be interesting to know whether this morphogenetic capacity of C-4II cells is dependent on their ability to form an organized epithelium with apicobasal polarity: as C-4II cultures become crowded, the cells change from a flat to a cuboidal or columnar shape; at the same time, the intercellular and basal spaces form a compartment separated from the culture medium by apical junctional complexes (2) and therefore likely to be of

TABLE III

*Effects of Divalent Cation Depletion, Agents Affecting Ca Flux, and Cold on the Contractility of C-411 Cells**

Suspending medium	Additive or variable	Contraction‡		
		Length to width ratio§ (mean ± SD)	Control‖	*P*¶
			%	
Hanks' BSS	None	5.77 ± 1.51	100	—
	Mg-free	5.45 ± 1.45	92	>0.05
	Ca-free	2.34 ± 0.82	9	<0.001
	Mg-, Ca-free	2.09 ± 0.49	2	<0.001
Waymouth's medium 752/1	None	5.61 ± 1.35	100	—
	Papaverine, 2.5×10^{-7} M	5.72 ± 1.20	103	>0.05
	Papaverine, 2.5×10^{-6} M	4.55 ± 1.22	71	<0.05
	Papaverine, 2.5×10^{-5} M	2.36 ± 0.51	10	<0.001
	Papaverine, 1.3×10^{-4} M	2.11 ± 0.39	3	<0.001
HEPES-BSS	None	6.65 ± 1.45	100	—
	$LaCl_3$, 10^{-6} M	5.84 ± 1.34	83	<0.05
	LaCl, 10^{-5} M	5.22 ± 1.55	69	<0.005
	LaCl, 5×10^{-5} M	3.58 ± 1.76	34	<0.001
	LaCl, 10^{-4} M	2.61 ± 0.86	13	<0.001
Hanks' BSS (1.26 mM Ca)	0.5% DMSO**	5.52 ± 0.78	100	—
	D-600, 5×10^{-5} M	5.51 ± 1.02	100	>0.05
	D-600, 1×10^{-4} M	5.24 ± 1.19	92	>0.05
	D-600, 2×10^{-4} M	4.41 ± 1.36	64	<0.01
Hanks' BSS (0.32 mM Ca)	0.5% DMSO**	6.24 ± 1.13	100	—
	D-600, 5×10^{-5} M	6.25 ± 1.15	100	>0.05
	D-600, 1×10^{-4} M	5.49 ± 1.47	82	<0.05
Hanks' BSS (0.13 mM Ca)	0.5% DMSO**	4.5 ± 1.18	100	—
	D-600, 1×10^{-4} M	3.72 ± 1.38	68	<0.05
	D-600, 2×10^{-4} M	3.60 ± 1.70	63	<0.05
Waymouth's medium 752/1	0.5% DMSO**	6.20 ± 1.29	100	—
	D-600, 1×10^{-4} M	4.87 ± 1.39	68	<0.01
Waymouth's medium 752/1	0.5% Ethanol**	5.13 ± 1.49	100	—
	A23187, 5×10^{-6} M	5.06 ± 1.30	97	>0.05
HEPES-BSS	None	6.57 ± 1.04	100	—
	7°C (≤90 min)	2.02 ± 0.56	0.4	<0.001
	7°C (60 min) followed by 37°C (30 min)	6.09 ± 1.55	89	>0.05
	37°C (30 min) followed by 7°C (24 h)	6.32 ± 1.10	94	>0.05

* All experiments were carried out at 37°C unless stated otherwise.

‡ Contraction over 30 min after detachment from the plastic of 2 × 1-mm monolayer sheets.

§ Ratios of the length over width of 20–40 cell sheets per sample measured at the end of the contraction period.

‖ Percent calculated from actual ratios minus 2 (because a ratio of 2.0 equals zero contraction; see Materials and Methods for detail). Controls, cultures with no additives or variables.

¶ Student's *t* test, control vs. experimental.

** Solvent control.

an ionic composition different from that of the medium, as demonstrated for other cultured cells of similar morphology (12).

The mechanism of induction of C-4II contraction, i.e., detachment from the growth surface, does not show any obvious resemblance to developmental or physiologic processes. As suggested previously (3), microfilaments in the basal cytoplasm of adherent monolayer cultures probably exert an intracellular tensile force that interacts with extracellular adhesive forces in the maintenance of colonial organization. Therefore, contraction could result from a sudden imbalance resulting from persisting tensile forces in the basal cytoplasm after detachment from the substratum. The contractions might also, perhaps, be related to local changes in physical properties of the cytoplasm upon the sudden release from tension, analogous to contractions that occur in cells upon decompression (33). It is interesting that cells of amphibian neural plate, which normally constrict apically to form the neural tube, can be induced to constrict basally instead through separation from underlying tissues (8, 9). There are also less direct analogies between the induction of C-4II cell contraction and normal morphogenetic processes. In gland development, an undisturbed association with underlying basal lamina is necessary for the development (5) and maintenance (4) of epithelial morphology. The localized epithelial contractions that result in lobulation and branching of the glands are closely associated, both chronologically and spatially, with changes in the chemical composition and macromolecular organization of structures immediately underlying the basal surfaces of the contracting cells.

Localized accumulations of cytofilaments, thought to have a contractile function, also appear in response to cell wounding (20). Therefore, the possibility cannot be ruled out that contractions in the basal cytoplasm of C-4II cells were induced by mechanical damage, caused when the cells were scraped off the dishes. However, it is unlikely that damage alone can account for the observed response. Careful ultrastructural examination of cells immediately after detachment from the growth surface failed to show any damage to basal plasma membranes. Furthermore, the filament bands that formed during contraction extended uninterrupted along the length of the basal cell surfaces, tended to be located internal to plasma membrane folds, and were associated with intermediate junctions on the lateral cell walls, thus mimicking the "purse-string" effect in contracting embryonic epithelia. Filament accumulations in response to cell damage alone would be expected to be more randomly distributed and focal, and probably not associated with the lateral cell walls.

Whether the influx of Ca upon detachment from the culture dish triggers contraction in C-4II cells or whether it is a secondary event is not known, but Ca influx does seem to be required for contraction to take place. The results with Ca-deficient BSS and papaverine in support of this conclusion have already been commented on. The requirement for extracellular Ca was investigated further by two agents that affect Ca flux more specifically than does papaverine, and lack the deleterious effects on cell adhesion of Ca depletion. D-600, a derivative of Verapamil (21), drastically and specifically reduces Ca influx into cells and has been shown to interfere with the contraction of smooth and cardiac muscle (22, 28) as well as with Ca-dependent secretory activity of pancreatic β cells (26) and brain slices (29). In the present study, C-4II contraction was significantly inhibited by D-600. Lanthanum, at concentrations ranging from 10^{-6} M to 10^{-4} M, blocks Ca flux into cells and thereby interferes with Ca-dependent functions including contraction of smooth muscle (36) and cultured myoblasts (10), the chemotactic migratory response of leukocytes (7), and locomotion of cultured glial cells (24). C-4II cell contraction was increasingly inhibited by similar concentrations of lanthanum in a dose-dependent manner.

No enhancing effect of A23187 on C-4II cell contraction was demonstrated. This result is not too surprising in view of the large and rapid increase in intracellular Ca induced by detachment from the substratum alone and the complex effects of A23187 on cellular Ca in other cultured cells (6). No enhancement by A23187 of contraction beyond control levels has been reported in any other epithelial tissues, though A23187 does counter the inhibitory effect of papaverine on amphibian neurulation (27).

In conclusion, this study shows by a direct method that intracellular Ca levels are increased during contraction in at least one type of epithelial cell and provides evidence that influx of extracellular Ca is required for such contraction to take place. In view of the many similarities in structure and in responses to external agents and conditions between the C-4II line and developing epithelia, it seems reasonable to assume that the observations

reported here may be relevant to epithelial cell contraction in other systems.

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