TABLE 2: Effect of Mucosal [K] on J_{Na}^{ms} and J_{Cl}^{ms}

Mucosal [K]	<0.05 mM	0.1 mM	0.6 mM	1.1 mM	Ouabain + Papaverine
J_{Na}^{ms} (μEq/cm^2·h)	4.05	4.18	4.35	4.24	0.58
(n = 7)	±0.41	±0.36	±0.39	±0.32	±0.11
J_{Cl}^{ms} (μEq/cm^2·h)	3.61	3.80	3.77	3.85	1.71
(n = 6)	±0.47	±0.49	±0.51	±0.66	±0.18

Furosemide has been shown to inhibit NaCl entry in several types of epithelia and Renfro has previously reported an inhibitory effect on the flounder bladder using a concentration of 1 mM. The effect of this concentration of furosemide did not uniformly produce inhibition of J_{Na}^{ms} and J_{Cl}^{ms}. J_{Na}^{ms} was reduced only in bladders where control values were greater than 3 μEq/cm^{2}h. In bladders where inhibition occurred, there was only a partial inhibition of net flux. The effect on J_{Cl}^{ms} was similar but there tended to be some inhibition even at low (<3 μEq/cm^{2}h) rates. When the effect of furosemide was examined on J_{Na}^{ms} and J_{Cl}^{ms} measured simultaneously, J_{Cl}^{ms} was inhibited to a greater extent than was J_{Na}^{ms}. The data are displayed in Table 3. The reduction in J_{Na}^{ms} was 0.70 ± 0.30 while the reduction in J_{Cl}^{ms} was 1.33 ± 0.38. Thus the reduction in J_{Cl}^{ms} was almost twice the reduction in J_{Na}^{ms} ($p < 0.01$).

TABLE 3: Effect of 1 mM Furosemide on J_{Na}^{ms} and J_{Cl}^{ms} (n=12)

	Control	Furosemide	Ouabain + Papaverine
J_{Na}^{ms} (μEq/cm^2·h)	3.52	2.82[a]	0.63[c]
	±0.56	±0.30	±0.06
J_{Cl}^{ms} (μEq/cm^2·h)	4.49	3.16[b]	1.38[c]
	±0.70	±0.34	0.08

[a] $p < 0.05$ compared to control; [b] $p < 0.005$ compared to control; [c] $p < 0.001$ compared to furosemide.

These data, when considered together, provide evidence that Na and Cl absorption is a coupled process which occurs by an electroneutral mechanism. Evidently it does not occur via a parallel exchange system nor does it need mucosal K^+ to operate optimally. Although furosemide does not inhibit net Na and Cl absorption completely, it does reduce the absorptive flux when control values are high. The net rates of Na and Cl absorption are not different but furosemide reduces J_{Cl}^{ms} to a significantly greater degree than it does J_{Na}^{ms}. Although the changes in unidirectional flux were differentially reduced by furosemide, there was no evidence of any change in the electrical properties of the epithelium. Thus the most likely explanation for this differential effect is an effect on both the neutral NaCl transport system and a parallel effect on a Cl-Cl exchange system. This work was supported by a grant from the National Institutes of Health AM 25231. We appreciate the advice and assistance of David Andrew and David Dawson.

FURROWING ACTIVITY IN ENDOPLASMIC FRAGMENTS OF THE SAND DOLLAR EGG

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The physical mechanism which accomplishes cytokinesis is localized anew in each cell cycle in the equatorial surface as a consequence of interaction between the surface and the mitotic apparatus. In order to learn more of the organizational factors involved in the interaction, the original egg surface was drastically disrupted and the consequent

effects on cytokinesis were observed. The jelly, membranes and hyaline layer of fertilized E. parma eggs were removed by repeated washings with 1 M glycine beginning 1 min after fertilization. At various times thereafter, extensive portions of the surface were rapidly disrupted by contacting them with an expanding drop of mineral or silicone oil which was expelled from the nozzle of a micropipette. The immediate result was an explosive destruction of part of the surface, followed by massive outflow of endoplasm. Within seconds of the beginning of the outflow, new membrane rapidly appeared on the naked endoplasm. The new membrane connected with the persisting part of the original surface so that most of the egg contents were then covered by a combination of original and new surface. By mechanical pressure the mitotic apparatus and variable amounts of the surrounding endoplasm were coaxed into the region bounded by the new membrane, which subsequently protruded and often became completely separated from the remainder of the egg. As the volume of the protrusion increased, the thickness of the original surface increased as the area it covered decreased. Original and new surfaces became easily distinguishable because the original surface appeared to be covered with projecting, enmeshed linear structures and to be underlain by a cortical zone from which granular components were nearly absent. No granular free area or linear structures were associated with new surface.

In nucleated endoplasmic fragments, mitotic cycling continued and, at its maximum, the appearance of the mitotic apparatus was typically radiate. In the presence of a well-developed mitotic apparatus, the surface of the endoplasmic fragment developed typical furrows, the number and permanence of which were correlated with the number of asters and their size. Although failure of furrowing in endoplasmic fragments was not rare, the fact that failure also occurred in eggs in which the endoplasmic mass had previously rejoined the portion covered by the original surface suggests that the difficulties arose from disorganization rather than absence of an essential component. In cases where the endoplasm rejoined the portion covered by the original surface, the original and the new surface remained contiguous and discrete on the surface of the nearly spherical mass. These observations indicate that cleavage furrow establishment does not require a surface with a stable, long-standing organization. This investigation was supported by NSF Grant PCM7902624.

POLAR LOBE FORMATION AND CYTOKINESIS IN FERTILIZED EGGS OF ILYANASSA OBSOLETA AND MYTILUS EDULIS

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Fertilized eggs of the marine mud snail, Ilyanassa obsoleta (Nassarius obsoletus), and of the blue, edible mussel, Mytilus edulis, form a constriction slightly below the equator that tightens and then relaxes several times before and during early cleavage. This shape change is an excellent mimic of cytokinesis. Our purpose has been to determine what accompanying changes occur in intracellular ionic activities and in the cytoskeletal proteins.

Electrodes. We attempted to construct Ca^{2+}-selective microelectrodes following the methods of Tsien and Rink (1981, J. Neurosci. Meth. 4:73) and of Lanter et al (1982. Anal. Chim. Acta 135:51). The calcium-selective neutral carrier, ETH 1001, was kindly donated by Professor W. Simon (Swiss Federal Institute of Technology, Zurich). Standard calibration solutions contained 0.125 M K^+ and free Ca^{2+} concentrations of 10^{-3}-10^{-8} M (Tsien and Rink, 1981). Silane treatments of microelectrodes with tri-n-butylchlorosilane in gaseous form or mixed with 1-chloronaphthalene were unsuccessful. However, when N-trimethylsilyldiethylamine was used (5%, v/v, in 1-chloronaphthalene), a few electrodes displayed excellent response to Ca^{2+}, even detecting a difference between pCa∞ and pCa 8, even after such electrodes were stored in air overnight. Two single barrel electrodes (tip diameter, 2 μm, O. D.) and two double barrel electrodes (overall tip diameter, 3 μm, O.D.; one barrel filled with 3 M KCl and used for determining membrane potential) calibrated satisfactorily, but insufficient numbers of Ilyanassa eggs were punctured to yield data on the intracellular pCa. Repeated attempts to produce more such single or double-barrel electrodes were unsuccessful.

Thiophosphorylation. Our previous experiments had suggested that polar lobe-like constrictions could be induced experimentally in Ilyanassa eggs when cAMP levels are raised by microiontophoretic injection of cAMP or by inhibiting