EFFECT OF CYTOCHALASIN ON CELL VOLUME AND MORPHOLOGY IN SLICES OF THE DOGFISH SQUALUS ACANTHIAS RECTAL GLAND. John W. Mills and Arnost Kleinzeller, Dept. of Anatomy, Dartmouth Medical School, Hanover, New Hampshire, and Dept. of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania.

Recent evidence indicates that the cytoskeletal components, particularly actin, may play a role in volume regulatory processes. For example, the ouabain resistant component of volume recovery present in rat liver slices swollen at 1°C is completely blocked by treatment of the slices with cytochalasin-B (van Rossum and Russo, J. Membrane Biol. 59:191-209, 1981). In Necturus gallbladder, the regulatory volume decrease that occurs after the initial swelling in hypotonic medium is inhibited by treatment with cytochalasin B (Foskett and Spring, Am. J. Physiol. 248:C27-C36, 1985). In both of these studies a vesicle fusion process, mediated by intact actin filaments, was proposed as part of the mechanism by which ions and water left the cell.

In order to further investigate the role of the cytoskeleton in volume control processes we have begun a detailed examination of the relationship between the state of organization of the cytoskeleton and volume regulation in the dogfish shark rectal gland. This tissue has several characteristics that make it an interesting model system for volume control studies. These are: 1) cell volume does not increase during incubation in ice-cold medium (Kleinzeller and Goldstein, J. Comp. Physiol. B. 154:561-571, 1984); 2) volume regulation in medium made hypotonic by removal of urea involves a relatively slow uptake of K and Cl(ibid); and 3) volume recovery after swelling in high K^+ -Ringer may involve "vesicles" in the cytoplasm (Masur et al., Bull. Mt. Desert Isl. Biol. Lab. 21:48-50, 1981). We report here our initial studies on the effects of the microfilament disrupting agent cytochalasin on volume recovery after swelling in high K-Ringer.

Slices of rectal gland tissue were prepared as previously described (Kleinzeller and Goldstein, J. Comp. Physiol. B. 154:561-571, 1984). After cutting, slices were pooled, lightly blotted and then placed in flasks containing 5ml of either normal shark Ringer's(ibid), with or without cytochalasin, or Ringer's in which all the Na was replaced by an equivalent amount of K, with or without cytochalasin. Each flask also contained 0.2 μCi/ml ^{14}C-PEG. At the end of a ninety minute incubation period a portion of the slices in each flask was removed, blotted and weighed. The remaining slices were lightly blotted and then transferred to flasks containing Na-Ringer's with or without cytochalasin. After a 60 min recovery period the remaining slices were blotted and weighed. Dry weights were obtained after oven drying overnight at 95°C. Na and K was analyzed by flame photometry in extracts obtained by exposure to 0.1N nitric acid. Tissue samples were fixed in shark-Ringer's containing 1% glutaraldehyde. Alternatively some samples were fixed in 5% glutaraldehyde in 0.1M sodium cacodylate (pH 7.4) plus 0.25M sucrose and 0.025% $CaCl_2$ (Ernst et al., J. Membrane Biol. 58:101-114, 1981).

The effect of 100μg/ml cytochalasin-B on steady-state volume and recovery of volume after swelling in K-Ringer for a typical experiment is shown in Table I. In normal Ringer the values for cell water, intracellular water and Na are similar to what has been reported previously (Silva et al., Am. J. Physiol. 233:F298-F306, 1977; Kleinzeller and Goldstein, J. Comp. Physiol. B. 154:561-571, 1984). The value for K is lower than previous reports, but was a consistent finding (mean value for 9 experiments with similar protocols as to that shown in Table I was 106 ± 11mM). Treatment with 100μg/ml cytochalasin-B

had little effect on any of the measured parameters. After swelling in K-Ringer's and re-exposure to Na-Ringer's for 60 min the control tissue showed an intermediate level of recovery of volume and ions. Tissue treated with cytochalasin-B exhibited an apparently more rapid recovery of volume and ions to pre-swelling levels. This same response was also seen in tissue treated as in Table I but exposed to cytochalasin-D at concentrations as low as 10μg/ml. Thus in rectal gland slices recovery of volume after K-induced swelling is not inhibited by treatment with cytochalasins, which presumably disrupted the microfilaments in the cells, and the volume recovery process may even be stimulated by this treatment.

Morphological effects of the treatments listed in Table I are shown in Figure 1. The amount of swelling caused by K is quite evident in 1c. Even this level of disruption is not irreversible, however, since return to Na-Ringer's results in a complete recovery of normal morphology (Fig. 1d). What is most striking, however, is the marked vesiculation seen in rectal gland after exposure to cytochalasin throughout the swelling and recovery phase (Fig. 1e). The presence of vesicles in cytochalasin treated cells, after near complete volume recovery, was also reported to occur in rat liver slices (van Rossum and Russo, J. Membrane Biol. 59:191-209, 1981).

Our results indicate that disruption of microfilaments does not alter mechanisms of volume maintenance and normal morphology in slices of rectal gland. Volume recovery also can occur, after K-induced swelling, but this is not associated with a return to normal morphology, but rather with a marked vesiculation of the cells. Vesicle formation normally occurs during volume recovery from K swelling and has been implicated as playing a role in the volume recovery process (Masur et al., Bull. Mt. Desert Isl. Biol. Lab 21:48-50, 1981). The fact that the vesicles are retained in cytochalasin treated tissue, but volume recovery is complete, indicates that a mechanism of volume regulation other than vesicle formation and extrusion must be operating, and as proposed by van Rossum and Russo (J. Membrane Biol. 59:191-209, 1981), able to compensate for the loss of the vesicle-extrusion pathway. Since the proportion of tissue water in the extracellular space is higher in tissue allowed to recover in the presence of cytochalasin (Table I), an alternative explanation of the results in Fig. 1 is that the vesicles are actually extracellular, not intracellular. Examination of the tissue at the level of resolution provided by the electron microscope, after exposure of the tissue to electron dense tracers, should provide an answer to this question.

Table I. Effect of cytochalasin-B on cell volume, Na and K in slices of dogfish rectal gland in the steady-state or after recovery from swelling in K-Ringer's. Values are means ± S.D. for four groups of slices for each condition.

	H_2O_e (kg/kg DW)	H_2O_i (kg/kg DW)	Na (meq/kg DW)	K (meq/kg DW)	[Na] (mM)	[K] (mM)
Na-Ringer's (1)	.699	2.720	152	291	56	107
	±.058	±.075	± 7	± 7	± 3	± 2
Na-Ringer's (1) (+100µg/ml cyto B)	.709	2.541	173	296	68	116
	±.083	±.059	±20	±22	± 8	±10
K-Ringer's (1)	.660	3.852	26	923	7	245
	±.086	±.473	± 4	±31	± 1	±15
K → Na (2)	.845	3.010	130	456	43	151
	.075	±.175	±17	±30	± 3	± 5
K → Na (2) (+100µg/ml cyto B)	.997	2.522	150	347	59	137
	±.077	±.091	±18	±11	± 6	± 3

(1) Incubation for 90 min.
(2) Incubation for 90 min in K-Ringer's and then 60 min in Na-Ringer's.
H_2O_e = extracellular tissue water
H_2O_i = intracellular tissue water

Figure 1. Light micrographs of sections of rectal gland exposed to the conditions listed in Table I. X535 a. Control, 90 min incubation in Na-Ringer's. Lumena are patent (L) and overall morphological preservation is good. b. 90 min incubation in Na-Ringer's + 100µg/ml cytochalasin-B. No marked difference between b and a can be seen. c. 90 min in K-Ringer's. Lumena are obliterated, nuclei appear swollen and pale and large clear areas (arrows) can be seen in the cytoplasm. d. 90 min in K-Ringer's and then 60 min in Na-Ringer's. Lumena are again patent and cellular morphology appears similar to a. e. 90 min in K-Ringer's and then 60 min in Na-Ringer's. 100µg/ml cytochalasin-B present for entire time period. Lumena are patent and some cells appear to have returned to normal. However, large vesicles can be seen throughout the rectal gland tubules. Connective tissue cells do not show signs of this vesiculation (arrowhead).

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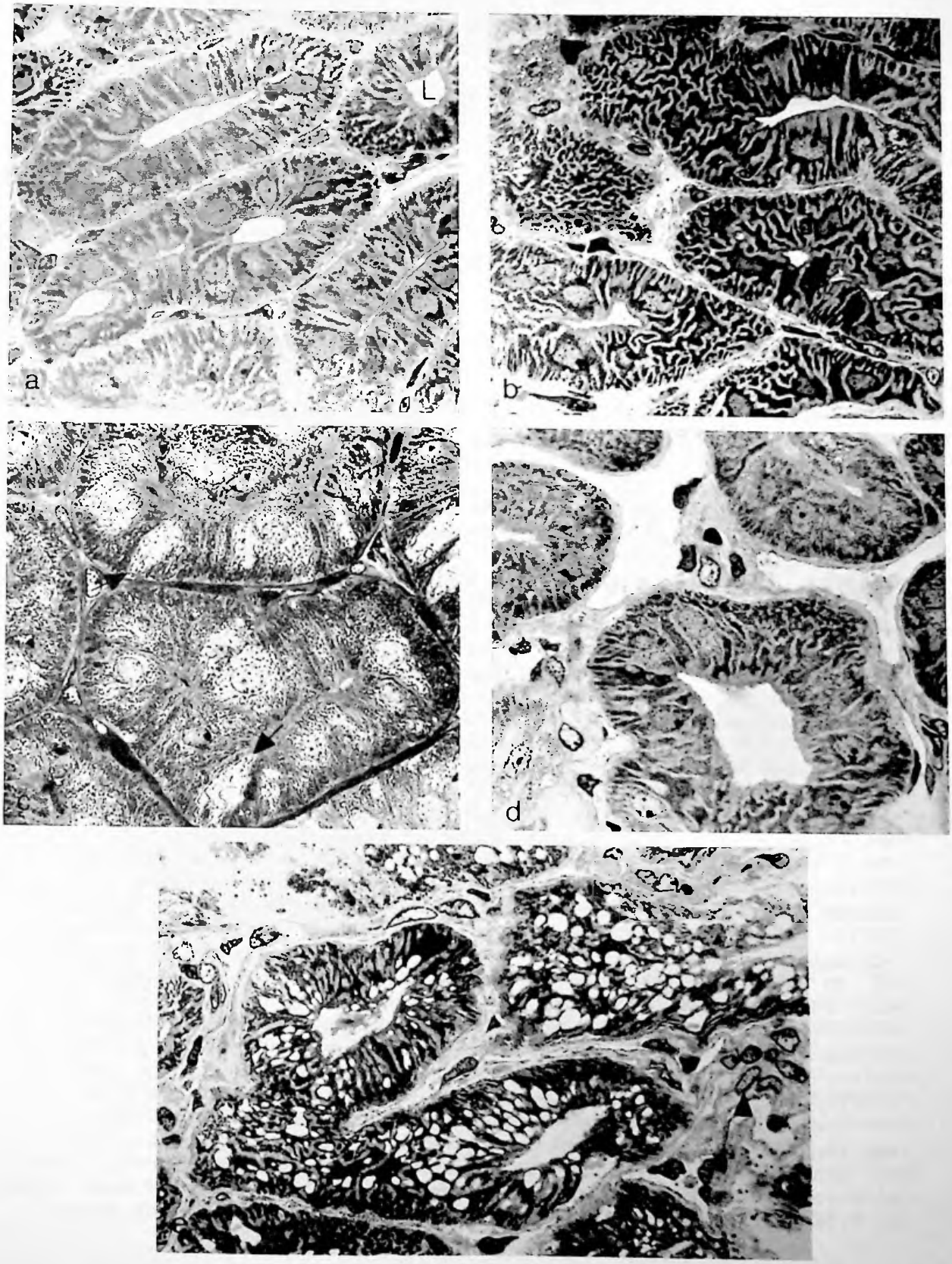
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