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BRIEF NOTES

Cells without Cytoplasmic Movements Respond to Cytochalasin[1]

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Cytochalasin B inhibits both the photopolarization and the germination of *Pelvetia* eggs. These are immobile cells without cytoplasmic streaming or other known movements.

In the last five years, low concentrations of cytochalasin B have proved to reversibly inhibit an impressive diversity of developmental phenomena. Wessells *et al.* (1971) have proposed that the drug acts by "disrupting the function of contractile microfilaments"; but strong counter evidence is accumulating. Thus Forer *et al.* (1972) find no effect of the drug upon actin *in vitro*, whereas Palevitz and Hepler (1972) report cytochalasin inhibition of cytokinesis in higher plant cells in which this process does *not* seem to involve contraction and in which microfilaments seem to be absent. Moreover, Bluemink (1971) has carefully studied the drug's inhibition of cytokinesis in amphibian eggs. This seems to be a two-stage process in which the first stage involves filament driven contraction while the second stage (like the whole process in higher plant cells) does *not*. Yet low doses of cytochalasin inhibit the second stage without affecting the first and without disrupting microfilament bundles. Since plasma membrane processes are so prominent in the second stage, Bluemink has argued instead that this membrane is the drug's primary target.

We wish to report that cytochalasin B inhibits photopolarization, germination, and apical hair initiation in developing eggs of the fucoid alga, *Pelvetia fastigiata*. The inhibition of polarization and germination again suggest that the cell surface rather than contractile microfilaments are the drug's first target; both because these cells do not show any of the movements which are supposed to be driven by contractile microfilaments and because polarization and germination seem to be surface-mediated processes.

These plant eggs are encased in a rigid wall and obviously do not show any whole cell movements. Moreover, in contrast to other cytochalasin-sensitive plant cells (Herth *et al.*, 1972), *they do not show cyto-*

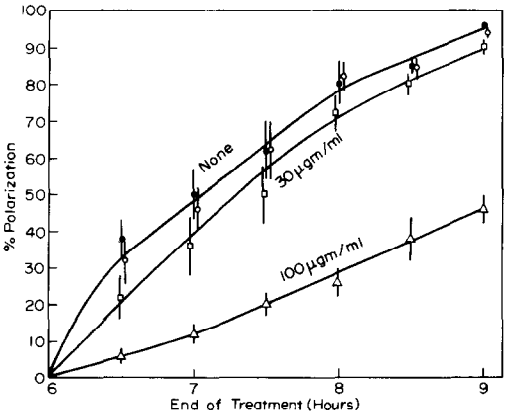

FIG. 1. Inhibition of photopolarization of *Pelvetia* eggs by cytochalasin B. Eggs were treated with the indicated dose of the drug as well as to 100-ft-c of unilateral white light from 6 hr after fertilization until the indicated time. Open symbols represent data from media containing ⅔% dimethyl sulfoxide; closed symbols, media lacking it. All data points are averages of 14 experiments plus or minus the standard deviation.

[1] From D. R. Nelson's undergraduate honor's thesis. Her present address is the Zoology Department, University of Washington, Seattle, Washington 98105. This study was supported by the N.S.F.

TABLE 1

CYTOCHALASIN APPLIED AFTER PHOTOPOLARIZATION DOES NOT DISORIENT *Pelvetia* EGGS[a]

Hour put in dark	Hour CB removed	Control: No CB	30 μg/ml CB	100 μg/ml CB
7	9		42 ± 3	44 ± 4
7	10	48 ± 5	48 ± 4	40 ± 6
7	11		40 ± 5	34 ± 5
7	12		N.G.	N.G.
8	9		70 ± 4	78 ± 5
8	10	72 ± 3	76 ± 6	78 ± 4
8	11		72 ± 5	76 ± 5
8	12		N.G.	N.G.
9	10		90 ± 3	92 ± 1
9	11	92 ± 2	86 ± 4	88 ± 2
9	12		N.G.	N.G.

[a] Eggs were exposed to 100 ft-c of unilateral while light from the time of fertilization until the time shown in column 1. Then cytochalasin was added, and they were placed in the dark. At the time shown in column 2, the drug was removed. The eggs were kept in darkness for an additional day or two, and finally the orientation of the embryos was observed. The numbers in the body of the table indicate the percent of germinated eggs oriented away from the light minus those oriented toward it. NG = none germinated. Each figure is the average of seven experiments in each of which 100 germinated eggs were observed. All media contained ⅔% DMSO. T = 15°C.

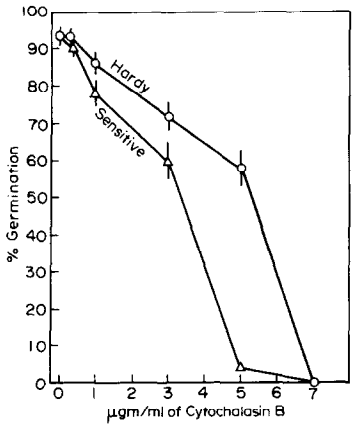

FIG. 2. Cytochalasin B inhibits the germination of *Pelvetia* eggs. Cytochalasin was added at 5.5 hr after fertilization and left in the cultures until germination was observed at 50 hr. Normally eggs germinate between 8 and 14 hr. The drug-resistance of the eggs showed some variability from batch to batch which was correlated with the season. The curve marked "hardy" averages data from the five most resistant batches, all of which grew in the winter; "sensitive" averages the six most sensitive ones, all of which grew in the spring or fall. All media contained ⅔% DMSO. All cultures grew at 15°C and were exposed to 100 ft-c of white light throughout the experiment.

plasmic streaming; or at least they do not stream during their first day of development, a period which includes both their polarization and germination. The lack of streaming is shown by the presistence of the stratification produced by centrifugation (Lowrance and Whitaker, 1940) as well as by direct observation.

Evidence that photopolarization is surface mediated comes from studies of its dependence upon the polarization of the light (the developmental axis forms in the plane of the E vector), studies of polarized plasmolysis, and studies of the transcellular current (Jaffe, 1968). One indication that germination is surface mediated is that it is driven by an increase in osmotic pressure in turn produced by a surface-mediated uptake of potassium chloride (Allen *et al.*, 1972).

Figure 1 summarizes our evidence that 30–100 μg/ml of cytochalasin B inhibits the photopolarization of *Pelvetia* eggs. These cells were cultured in natural sea water at 15°C in the dark until 6 hr after fertilization, then unilaterally illuminated

TABLE 2
CYTOCHALASIN INHIBITS APICAL HAIR FORMATION IN *Pelvetia* EMBRYOS[a]

μg/ml of CB	Apical hairs		
	Normal	Retarded	Absent
0	11	0	0
1	7	4	0
3	0	3	8
5	0	0	11

[a] Embryos were observed 14 days after fertilization. Apical hairs normally form at about 10 days under these conditions. Numbers in the table indicate numbers of dishes in which the apical hairs were normal (i.e., had formed a dense network throughout the dish) were absent, or obviously retarded. The medium was changed every 3 days in all dishes since cytochalasin solutions were found to lose half their effectiveness in inhibiting germination in this time. All media contained ⅔% DMSO; all cultures grew under 100 ft-c of white light at 15°C.

while in cytochalasin sea water or in control media for various periods and finally returned to natural sea water in the dark to germinate. Later the directions of germination were observed, and the degree of photopolarization was expressed as the percent germinating away from the previous light (to any degree) minus the percent towards it. It seems clear that 30–100 μg/ml of cytochalasin can markedly inhibit photopolarization. In other experiments, summarized in Table 1, cytochalasin was applied after illumination. Orientation was scarcely reduced. So the apparent inhibition of photopolarization is real; concentrations of cytochalasin up to 100 μg/ml neighter *de*polarize nor disorient the eggs.

Figure 2 summarizes our evidence that 1–5 μg/ml of cytochalasin inhibits the germination of *Pelvetia* eggs. That is, it blocks the local growth that generates a rhizoid. It evidently requires about a 30 times lower concentration of cytochalasin to block germination than to block polarization. We have also made qualitative observations indicating that the block to germination can be reversed by removal of the drug.

Finally, Table 2 summarizes our evidence that 1–5 μg/ml also inhibits apical hair formation.

As a large, isolated, unvacuolated marine cell, the fucaceous egg is relatively amenable to investigations of membrane physiology. Perhaps, then, it will prove to be a favorable material for studying the mechanism of cytochalasin action.

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