ing effects of deficient ganglioside synthesis during development emphasize their important role in the development of the central nervous system and visceral organs. To avoid confusion with the classical sphingolipidoses, which are caused by deficiencies in catabolic enzymes, we propose the name anabolic sphingolipidosis-type G_{M3} to describe this new disease. It will be interesting to see whether other severe diseases with similar clinical signs will fall into this potentially new class of anabolic sphingolipidoses.

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Polarizing Fucoid Eggs Drive a Calcium Current through Themselves

Abstract. *Calcium ions enter the prospective growth pole of polarizing* Pelvetia *eggs faster than the opposite pole and leave this antipode faster than the growth pole. The resultant calcium current is greatest when first measured at 6 hours after fertilization and decreases as the time of final commitment to growth in a particular direction approaches.*

A unifying feature of the eggs of both plants and animals is the existence of a pattern which foreshadows the organization of the adult. The mechanisms by which these patterns are formed and maintained are not understood. The eggs of the marine brown alga *Fucus* and its close relative *Pelvetia* are convenient for studying this problem since they, unlike almost all other eggs, have no pattern at the time of fertilization. Some hours later they develop an axis, which is manifested by a bulging of the surface about 10 to 12 hours after fertilization. This growing region eventually becomes separated by a cell wall, and the cell thus formed gives rise to the rhizoid or holdfast of the adult plant while the other cell becomes the thallus. The direction in which the axis will form within the egg can be controlled by a variety of external vectors, including unilateral light, which causes the rhizoid to appear on the shaded side.

A clue to the mechanism that leads the fucoid egg from its initial unpolarized state to the grossly polarized embryo came with the discovery that these eggs drive an electrical current through themselves (*1*, *2*). It was suggested that the cytoplasmic field produced by the current might act electrophoretically to move charged entities to the growing region and thus be an essential link in the polarization process. However, the magnitude of the transcytoplasmic electric field depends strongly on which ions are carrying the current (*3*). Ions such as calcium that tend to be strongly bound to cellular components would produce a much larger field than those that are not. We therefore wanted to know if a calcium current was a significant part of the total current through the fucoid egg.

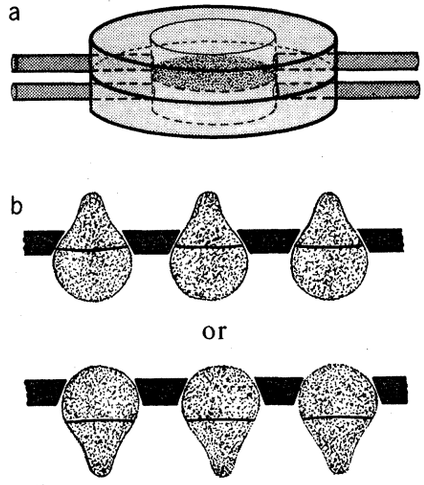

Fig. 1. (a) Chamber of the type used for flux measurements. The nickel screen is positioned as a diaphragm between the upper and lower halves of the chamber. (b) Sketch of a cross-sectional view of two egg-filled screens. The eggs shown here have begun to grow, but the measurements reported in the text were done before growth began so areal corrections were unnecessary.

In order to answer this question, we developed a method for measuring the calcium ion fluxes across either half of the membrane of a developing *Pelvetia* egg. This was accomplished by using a 25-μm-thick nickel screen perforated with many closely spaced, round holes, each 75 μm in diameter (*4*). Each hole of such a screen was filled with an egg 90 μm in diameter (see cover photograph) and the egg-filled screen was then continuously exposed to 1000 foot candles ($\sim$ 11,000 lu/m^2) of unilateral white light. All of the rhizoids later formed on the screen's shaded side, away from the light. The fluxes of $^{45}Ca^{2+}$ into (or out of) the lighted and shaded sides of egg-bearing screens were compared to obtain the desired ratios of calcium fluxes into (or out of) the tentative rhizoid and thallus ends of the eggs.

All of the 25,000 holes in each screen were filled with eggs by a simple procedure. The screen was placed as a 25-mm-wide diaphragm in a Plexiglas chamber filled with seawater (Fig. 1a). The holes are funnel-shaped, and each screen was placed with its funnels inverted, that is, with its wide apertures downward (Fig. 1b). A suspension of recently fertilized *Pelvetia* eggs, obtained as described elsewhere (*5*), was injected into the lower part of the chamber and thus forced upward through the screen. After every hole was plugged with an egg, the eggs were

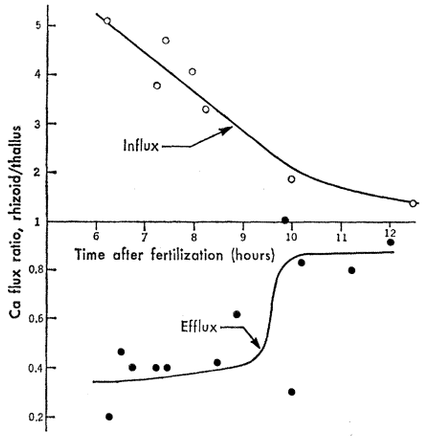

Fig. 2. Calcium influx and efflux ratios in developing *Pelvetia* eggs. Each point is the result of two simultaneous flux measurements on half-eggs made by using a pair of screens as shown in Fig. 1b.

kept in place by maintaining a slight upward flow of seawater through the screen. By 5 or 6 hours after fertilization the eggs had secreted a glue that stuck them very tightly in the holes, so further flow became unnecessary (indeed, impossible) and the screen was ready for flux measurements.

To determine the asymmetry of calcium influx during a given stage, seawater containing $^{45}Ca^{2+}$ was passed through the upper part of two chambers, and thus applied to the narrow-aperture sides of two egg-bearing screens. However, one of these was illuminated from below throughout the experiment, so in it the tracer was applied to the shaded, hence tentative rhizoid ends of the eggs; while the other was illuminated from above, so the tracer was applied to the tentative thallus ends. The two chambers compared differed only in their illumination direction, so any difference in $^{45}Ca^{2+}$ uptake must have resulted from this difference in illumination. As labeled seawater was passed through the upper, narrow-aperture compartments, unlabeled seawater was passed through the lower compartments to wash away any tracer that diffused through the screen (*6*). The concentration of tracer that appeared in this wash was always less than 1 percent of that in the labeled side at the flow rates used (about 5 ml/min). After 30 minutes the $^{45}Ca^{2+}$ was washed out, and the egg-bearing screens were removed from the chambers and washed for 15 minutes in order to remove the tracer from the cell walls. They were then dried and counted in a gas-flow planchet counter. These exposure and washing times were chosen with consideration of exchange times for cytoplasmic and cell-wall calcium in whole eggs (*7*).

The asymmetry of calcium efflux was determined in a similar way. At various stages, we forced unlabeled seawater past two oppositely illuminated egg-bearing screens that had been evenly preloaded with $^{45}Ca^{2+}$ (*6*). We collected and counted the effluents (*8*) from the upper, narrow-aperture faces of both screens and thus the $^{45}Ca^{2+}$ emitted (during 30 minutes) by the tentative rhizoid ends of one population of eggs and the tentative thallus ends of another. Each screen was preloaded by at least 3 to 4 hours exposure to labeled seawater from both sides; each was washed for 15 minutes while transferring it to a fresh chamber for effluent collection.

As mentioned earlier, *Pelvetia* eggs in the light begin to germinate about 10 to 12 hours after fertilization, and their axes of polarity are then irrevocably fixed. Since the eggs become well enough stuck for experimentation by 6 hours after fertilization, it is on this time period—6 to 12 hours—that we focused our attention. It happens that this largely spans the time period during which the eggs are maximally sensitive to light as an agent which can orient the axes (*2*). The upper curve in Fig. 2 shows the ratio of the amount of calcium that entered the tentative rhizoidal (shaded) poles of the eggs to the amount that entered the tentative thallus (lighted) poles. At 6 hours, five times as much entered the prospective rhizoid as the prospective thallus, and this ratio decreased as the time of commitment was approached, although it remained greater than 1.

The lower curve in Fig. 2 represents similar measurements of the efflux ratio. While the exact time course is not certain, it is clear that initially three times as much calcium left the prospective thallus as left the prospective rhizoid, and this ratio also approached but did not quite reach 1 by 12 hours.

Taken together, these two curves establish that there is a substantial calcium current through the polarizing *Pelvetia* egg and that this current diminishes as the period of photosensitivity passes and germination begins. In these experiments, the eggs were typically 50 percent germinated by 11 hours after fertilization. The polarity axis seems to become irreversibly determined 1 or 2 hours before visible germination in *Pelvetia* (*2*). In absolute terms, the measured calcium current was 2 pa per egg at 6 hours and 0.4 pa at 10 hours. Comparison with electrical measurements of the total current in *Pelvetia* (*2*) suggests that at 6 hours calcium ions may carry nearly all of the current but by 10 hours they carry only a small fraction of the total. While we do not know the identity of the current-carriers at the later stages, our preliminary results indicate that sodium ions, chloride ions, and calcium ions may all be involved (*9*).

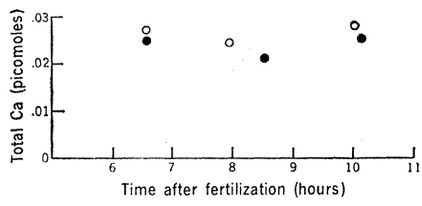

Fig. 3. Each point here was generated by adding the flux data from a pair of screens. The points shown are from one representative influx experiment (open circles) and one representative efflux experiment (closed circles).

We know that the inward movement of calcium is energetically downhill (*7*) so the influx is presumed to be passive while the efflux must be by way of a pump. Thus the calcium current seems to be produced by both a concentration (or activation) of leaks at one end of the cell membrane and a concentration (or activation) of pumps at the other end. Furthermore, by adding instead of dividing the flux data, as in Fig. 3, we learned that both the total calcium influx and the total calcium efflux remain sensibly constant over the period studied. Hence the mechanism which redistributes the surface concentrations or activities of both calcium pumps and calcium leaks appears to conserve their total surface concentrations or activities. In view of this, it is tempting to make the simplest of interpretations, namely that the total number of leak sites and the total number of pump sites stay fixed within the membrane and that they are simply redistributed within this membrane to produce the current.

Calcium currents have been found in at least one other developing system. It has been shown that the isolated chick chorioallantoic membrane produces a calcium current of about 1 $\mu a/cm^2$ under short-circuited conditions (*10*). This compares with 0.03 $\mu a/cm^2$ in the *Pelvetia* egg at 6 hours after fertilization if one assumes a uniform current density in the interior of the cell. Incidentally, this chick epithelium is formally quite similar to our

egg-bearing screens. Of course, the function of the calcium transport is very different. The chorioallantoic epithelium is involved in moving calcium from the shell to the embryo. Thus, its function lies in producing changes external to the epithelial cells themselves.

What is the role of the calcium current in the polarizing *Pelvetia* egg? We believe that it is the creation of an intracellular gradient of free calcium ions, with the higher concentration being at the leaking end. We are encouraged in this view by the finding (*11*) that there is an unequal distribution of calcium along the axis of the slug of the cellular slime mold *Dictyostelium discoideum*, with more calcium found in the anterior prestalk region. It was also shown that an increased level of extracellular calcium induced stalk formation and inhibited spore formation, which suggests that calcium does control the developmental fate of these cells.

One way in which a calcium gradient might act to polarize these eggs is by producing an electric field across the cytoplasm. The magnitude of the gradient—and hence the field—depends on the concentration of fixed calcium-binding sites and mobile calcium-binding molecules. We have shown elsewhere (*9*), using estimates from other systems for these quantities, that a calcium current of 0.03 $\mu a/cm^2$ could produce a cytoplasmic field across a *Pelvetia* egg of 0.1 volt/cm or more. Such fields are quite large enough to segregate cytoplasmic components (*3*).

One can envision other ways in which a cell might make use of a calcium gradient to bring about polarization. The control of contractility by calcium ion is well established, and extends even to primitive systems; Taylor *et al.* (*12*) have shown that cytoplasmic contractility of amoebas may be regulated by the level of free calcium ion. Another effect of calcium has been demonstrated by Weisenberg (*13*). Working with rat brain tubulin, he found that a free calcium concentration of $6 \times 10^{-6} M$ blocked the repolymerization of microtubules, and he concluded that "calcium appears to be a logical candidate as a regulator of microtubule polymerization *in vivo*."

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Spontaneous Regression of Friend Virus Induced Leukemia: Coinfection with Regressing and Conventional Strains of Virus

Abstract. *Mixtures of Friend virus (CFV) and the regressing strain of Friend virus (RFV) induce leukemia which regresses. The dominance of the regressing phenotype is solely a function of a threshold dose of RFV. The minimum amount of RFV which induced regression of CFV leukemia is below the titer for induction of Friend disease, but does correlate with the titer of lymphocytic leukemia (helper) activity in these stocks.*

The systematic study of spontaneous cancer regression, some 200 cases of which have been documented for the human disease, has been stimulated by the recent availability of suitable animal models. Friend virus leukemia is a progressive neoplastic disease characterized by massive splenomegaly leading inevitably to splenic rupture and death (*1*).

We have reported the isolation and characterization of a virus strain capable of inducing a disease initially indistinguishable from that induced by Friend or Rauscher leukemia virus (*2*). The disease induced by this agent differs from that initiated by conventional leukemia viruses in that it appears to be self-limiting. The characteristic splenic response, histologically indistinguishable from conventional Friend virus leukemia, does not lead to death; rather, in a significant proportion of leukemic mice, the massive proliferation of splenic cells is reversed, and the organ returns to normal architecture and mass. The virus synthesis which accompanies the disease similarly subsides.

In studies carried out shortly after the initial isolation of RFV, coinfection with both the conventional Friend virus (CFV) and the regressing Friend virus strain (RFV) did not demonstrate a marked influence of RFV on CFV induced leukemia (*3*). Subsequent sug-

Table 1. Spontaneous regression of leukemia induced by regressing and conventional strains of Friend leukemia virus.

Virus inoculation			Incidence		
CFV	RFV	Ratio	Leukemia (No. leukemic/ No. inoculated)	Regression (No. regressed/ No. leukemic)	Percent
100*	—		11/11	0/11	0
10	—		9/10	1/ 9	11
1	—		5/10	0/ 5	0
0.1	—		1/10	0/ 1	0
—	776		10/10	2/10	20
—	78		10/10	2/10	20
—	7.8		7/10	7/ 7	100
—	0.8		6/10	6/ 6	100
25	388	0.06	9/10	2/ 9	22
25	39	0.64	10/10	9/10	90
25	3.9	6.41	10/10	6/10	60
25	0.4	64.1	9/10	4/ 9	44
2.5	388	.006	10/10	3/10	30
2.5	39	.06	9/10	4/ 9	44
2.5	3.9	.64	9/10	6/ 9	67
2.5	0.4	6.4	7/ 9	1/ 7	14

* Leukemic dose, 50 percent: (that dose cf virus which will induce leukemia in 50 percent of inoculated weanling Swiss/ICR mice within 21 days).