DEVELOPMENTAL BIOLOGY 19, 549–565 (1969)

On the Mutual Polarization of Nearby Pairs of Fucaceous Eggs[1]

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Accepted January 22, 1969

INTRODUCTION

The early development of the brown alga *Fucus* is a prototype of the developmental process of *localization*. The recently fertilized egg appears to be a radially symmetrical sphere. A day later it has developed into a pear-shaped embryo, which has cleaved into a rhizoid cell at its pointed end and a thallus cell at the other end. The locus of rhizoid formation may be easily determined by a wide variety of natural vectors such as unilateral light, flow of the seawater medium, and other eggs; and an even wider variety of artificial vectors such as electrical fields, centrifugation, and potassium ion gradients. If shielded from all other effective vectors, the zygotes probably form a rhizoid at the point of sperm entry. Apparently then the localization process here is one which amplifies almost any slight imposed bias into a genetically determined pattern. Analysis of the process then may best focus upon the inner amplification process, which seems likely to have a relatively constant nature, rather than the external vectors which are so exceedingly variable.

With this in mind, it seemed to us to be of particular interest to investigate further the mutual polarizing action of nearby eggs, or so-called group effects. Under some conditions, nearby eggs tend to initiate rhizoids toward each other; under others, away. The former tendency, or positive group effect, is favored by low pH and by high cell concentration; the latter, or negative group effect by high pH and a low cell concentration. In both cases, reflection indicates that whatever factor(s) pass from one egg to another must also act back upon the donor egg.

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Thus these particular external factors must also be parts of the inner amplification system, or at least natural modifiers of it.

Our slight present knowledge of these interactions suggests that they involve emission by the eggs of at least two diffusible and locally effective substances: a stimulator of rhizoid initiation tentatively referred to as rhizin and an inhibitor referred to as antirhizin. It further suggests that rhizin is quite unstable in the extracellular medium while antirhizin is stable, and it proves that rhizin is neither CO_2, O_2, nor H^+ (Jaffe, 1968). It occurred to us that one important further question about the role of these interaction factors might be answered simply by more refined observation of the group effects.

The question may be initially phrased as whether the factor(s) are links in the amplification process or just modifiers of it. The group effects indicate that they are *locally effective*. However, if they are to be links in the amplification process they must also be appropriately *localized*. Thus if a locally effective natural stimulator of rhizoid initiation, rhizin, is somehow localized through the action of each egg near its own tentative rhizoid anlage, it should act to confirm the locus of the anlage, thus ultimately act to further sharpen its own extracellular localization and thus be a true link in the positive feedback loop necessarily characteristic of such amplification. Similarly, antirhizin would be a link in this process if it were naturally localized near the tentative thallus pole of the egg.

However, if these substances were not so localized by the action of each egg—if in particular, these factors were uniformly emitted, destroyed, or absorbed over each egg's surface and thus were uniformly concentrated over this surface (except as disturbed by external factors)—then these factors should be considered modifiers of the amplification process but not links in it.

The refinement in observation of the group effects which could answer this question is this: Observe not just the tendency of pairs of eggs to initiate rhizoids toward (or away) from the other cell, but also toward or away from the other cell's rhizoid anlage. Let us call the former aspect of the "group effect" a *cell effect* and the latter an *anlage effect*. The general nature of the distortion of a random distribution of rhizoid angles producible by a cell effect is obvious enough; that another distinguishable distortion would be produced by an anlage effect is perhaps most easily explained by reference to the simple categorization of nearby pairs of eggs illustrated in Fig. 1.

Populations of cell pairs with more $++$ than $--$ pairs would

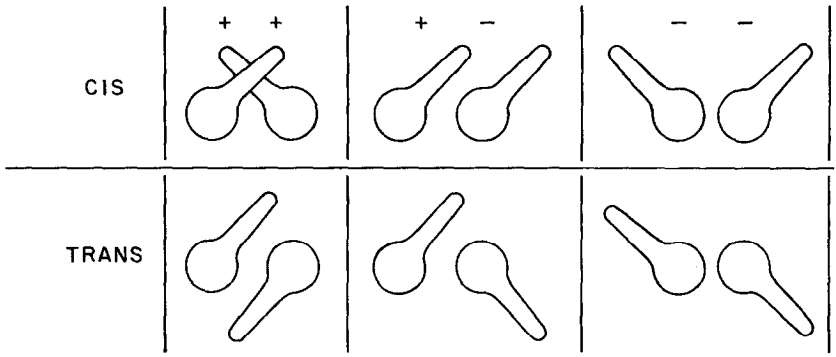


FIG. 1. A method for categorizing nearby pairs of germinated eggs. Plus and minus indicate outgrowths starting toward or away from the other cell. Hence every pair can be categorized as ++, +−, or −− depending upon whether both, one, or neither outgrowth starts toward the neighbor. Every pair can also be categorized as cis or trans depending upon whether the two outgrowths start on the same or opposite side of the line joining their centers. The combination of these criteria serves to divide all pairs into six categories as illustrated.

indicate a positive cell effect. On the other hand, a population with more cis than trans pairs would indicate a positive anlage effect.

With these considerations in mind then, we measured the mutual orientation angles developed by large populations of nearby pairs of fucaceous eggs, and characterized these distributions by parameters indicative of a cell effect on the one hand and of an anlage effect on the other. These parameters as a function of the distance between the cell pairs and of certain changes in the medium selected to elicit different degrees of positive or negative cell effect are the chief data presented in this paper. They will be seen to yield rather clearly interpretable results.

Some results from a second, related study are also reported here. In this we similarly measured the cell effect between pairs of eggs developing while the medium was forced through the substratum (and thus between them) at various known rates. The object of this cross-flow study was to obtain an indication of the extracellular mobility of the interaction factors. For we reasoned that sufficiently rapid cross flow should interrupt communication between the pairs by carrying away the interaction factors before they could diffuse from cell to cell. The more mobile the factor (i.e., the higher its diffusion constant), the faster the cross flow that should be needed to interrupt the interaction.

However, it will be seen that these cross-flow data show unexpected complexities which limit their present interpretability.

MATERIALS AND METHODS

Obtaining and washing zygotes. Ripe fronds of *Fucus furcatus* and of *Pelvetia fastigiata* were collected for us by Mrs. A. Phillips of the Hopkins Marine Station, Pacific Grove, California. The moist fronds were air-mailed to us in ice-cold hermetically sealed canning jars. Upon receipt they were stored in loosely capped jars at about 3°C. Healthy gametes were usually obtainable from the fronds from about 1 to 3 weeks after collection. Before this they are unlikely to shed enough gametes when stimulated; afterward they are likely to shed gametes that fail to fertilize. To stimulate shedding, some fronds were transferred to a room kept at 15°C where all subsequent operations were carried out. The fronds were cleaned by rubbing them between one's fingers under seawater and were then transferred to fresh seawater. This usually sufficed to induced shedding by the *Fucus* fronds within about a half hour; *Pelvetia* fronds were induced to shed by prolonged illumination followed by a dark shock (Jaffe, 1954). Each ripe frond sheds both egg and sperm capsules in these species. Dissolution of the egg capsules and fertilization occur within about a half hour after shedding. Fertilization may fail unless the gametes are aerated, as by shedding into relatively shallow seawater (say, 5 mm deep) which is occasionally swirled during the process. Under these circumstances, we find that motile sperm are usually a sufficient indicator that nearly 100% of the eggs will be fertilized and will develop.

A critical variable in the subsequent interactions of the zygotes proved to be whether or not they were vigorously washed. Zygotes were so washed as follows: First, oversize materials were removed by straining them through an appropriate Nitex[4] cloth (one with 85-μ holes for *Fucus:* with 110-μ holes for *Pelvetia*). They then were held upon a 1 cm diameter 35-μ Nitex cloth while at least 20 ml of fresh natural seawater was run through the filter at a rate of at least several millimeters per second.

Media. The basic medium was natural seawater obtained from the Marine Biological Laboratories, Woods Hole, Massachusetts; it was usually stored for several months before use. In some cases this medium was modified by whatever materials passed into it during shedding,

[4] Tobler, Ernst & Traber Inc., 71 Murray St., New York, New York 10007.

capsule dissolution, and fertilization; 100 ml of *shedding water* contained material emitted by 3–10 fronds and 50–100 thousand eggs. In any one experiment, the pH of the shedding water differed by no more than 0.1 pH unit from that of the unmodified seawater. Both had pH's of 7.6 ± 0.1 in all experiments. When so desired, both were sometimes acidified to pH 6.1 ± 0.2 with 0.01 *M* phosphate.

Cell distribution. A sufficient number of sufficiently isolated pairs of eggs generally resulted from the random sowing, at 1–2 hours after fertilization, of 1500–3000 eggs in each of a number of 42-mm petri dishes. A pair was considered to be sufficiently isolated if the gap between the nearest third cell and either cell in the pair was at least twice that between the pair, or four cell diameters, whichever was larger. This method gave enough pairs separated by gaps of up to four egg diameters. However, to obtain enough pairs 4–9 diameters apart, a screen technique was resorted to: We used two grades of thin electroformed nickel screens each containing a hexagonal array of conical holes; they had hole diameters of 1.0 and 1.5 mm and center-to-center distances of 1.7 and 2.5 mm, respectively. Each screen was held about one hole diameter above the bottom of a 42-mm petri dish, with the wider part of the hole upward, and immersed in a 3-mm deep layer of medium containing about 0.4% of Dow Chemical's Type 90 HG, 15,000 centipoise Methocel to raise its viscosity 20-fold. An equal amount of such medium containing eggs was then layered on top. When it held two eggs per hole, subsequent settling of the eggs through the screen yielded many more sufficiently isolated pairs, 4–9 diameters apart, than did random sowing. (As is later demonstrated in Fig. 3a, pair interaction proved to be independent of this Methocel.)

Recording data. Recording of the outgrowth angles α and β (Fig. 2) and of the gap between each pair was speeded by a specially designed electromechanical device, the "SNARC".[5] Using it, the operator observed each pair with a microscope equipped with an image-splitting eyepiece, made three adjustments of the apparent positions of the split images each followed by pressing a footswitch, and thus recorded the encoded data on paper tape. The first adjustment superimposed one egg's "second" image upon the other's "first"; the second and third successively caused each egg's two images to lie in its outgrowth's direction. The taped data were then analyzed with the use of a Johnson Foundation computer.[6]

[5] SNARC was designed, built, and programmed by Mr. David M. Director.

[6] This facility is supported by Public Health Grant FR15.

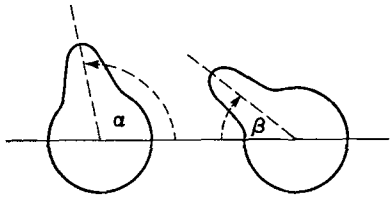


FIG. 2. Angles measured in analysis of cells' interaction.

Analysis of data. Consider Fig. 2 showing a pair of embryos. The parameter used to indicate the degree of the cell effect was simply the average cosine of all the outgrowth angles α and β in the population of n pairs considered. That is:

$$V = 1/2n \sum_{i=1}^{i=n} (\cos \alpha_i + \cos \beta_i)$$

The parameter used to indicate the degree of the anlage effect was

$$L = 1/2 \, (L_{\pm 45} + L_{\pm 135})$$

where

$$L_{\pm 45} = 1/n \sum_{i=1}^{i=n} \cos (\alpha_i - \beta_i)$$

for all those pairs in which α and/or β lies in the sector $0 \pm 45°$, while $L_{\pm 135}$ is the same calculated over all pairs in which α and/or β lies in the sector $180 \pm 45°$.

We concocted L in an effort to attain a measure of the degree of *correlation* between two identical distributions of angles; thus a parameter which would have the value $+1$, if $\alpha_i = \beta_i$ for all pairs; -1, if $\alpha_i = \beta_i + 180°$ for all pairs; zero if the two distributions were *uncorrelated.*

Uncorrelated means that all the subdistributions of β in those pairs for which α has any fixed value are identical. [See Jaffe (1968, Fig. 11) for an illustration of the concept of angular correlation.] It is obvious that L has the values $+1$ and -1 in the cases of perfect correlation and anticorrelation. Moreover, we verified that L is zero for a number of simple uncorrelated distributions. For example, we easily showed L to be zero for a population of uncorrelated pairs in which the fraction of outgrowths at $+45°$ and also at $-45°$ approach one half, at $+135°$ and also at $-135°$ *approach* zero, and at all other angles *is* zero.

We feel that these examples are a sufficient indicator of the reliability

of L in the present application. However, for future studies it would appear better to use a more rigorously tested angular correlation coefficient, R, which has recently been derived for this use by Dr. H. Rubin of Purdue's mathematics department:

$$R = \frac{C\Delta - [(C\alpha)\,(C\beta) + (S\alpha)(C\beta)]}{\sqrt{[1 - (C\alpha)^2 - (S\alpha)^2][1 - (C\beta)^2) - (S\beta)^2]}}$$

where

$$C\alpha = 1/N \sum \cos \alpha \qquad S\alpha = 1/N \sum \sin \alpha$$

$$C\beta = 1/N \sum \cos \beta \qquad S\beta = 1/N \sum \sin \beta$$

$$C\Delta = 1/N \sum \cos (\alpha - \beta)$$

Cross-flow. For this study cells were held upon a Nitex[4] screen woven of 45 μ diameter Nylon threads which frames 35-μ diameter holes. Flow was started before the eggs settled and continued until practically all had germinated. The desired flow rates were effected by a 50- to 100-cm head pushing fluid through resistance elements consisting of Teflon tubes about 1 meter long and of 0.5- to 1.5-mm bore.

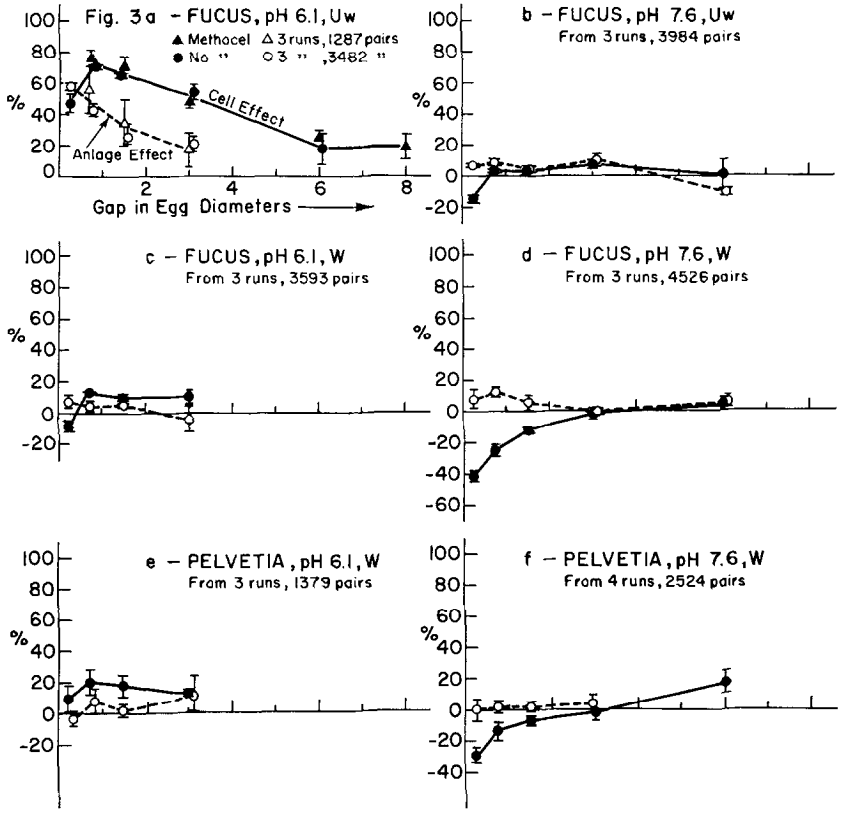
RESULTS AND ANALYSIS

The chief data collected are displayed in Figs. 3 and 4. In each of the ten graphs, the degree of mutual polarization of populations of pairs of *Fucus* or *Pelvetia* eggs is plotted against the size of the gap between them. Two aspects of this polarization are so plotted: the cell effect, and the anlage effect indicative of a tendency of the rhizoids to originate toward (or away) from the other cell or rhizoid anlage, respectively. If this initiation tends toward the other cell or anlage, the polarization is described as positive; if away, negative. The conditions varied as is indicated on each graph and further explained in the legend.

The main question considered in this study is answered by considering the anlage effects under those conditions proving to give the strongest positive and negative cell effects. We shall consider these first.

In brief, relatively large positive anlage effects were shown where nearby eggs showed a large positive cell effect but *not* where they showed a comparably large negative one. Most notable are the consistently large positive anlage effects shown by unwashed, relatively close *Fucus* eggs at pH 6 (Fig. 3a). Substantial positive anlage effects were also shown by *Fucus* eggs grown under those other conditions used which also elicited a large positive cell effect (Fig. 4a). In sharp contrast to

this were the relatively weak anlage effects which accompanied a large negative cell effect (Fig. 3d). Thus, in comparing Figs. 3a and 3d, note that among the closest eggs (those 0 to $\frac{1}{2}$ egg diameters apart), while the cell effects were 47 and -42%, the anlage effects were $+58$ and $+8 \pm 5\%$, respectively.



FIGS. 3 and 4. The mutual polarization of pairs of fucaceous eggs. Measures of the tendency of the rhizoids to originate toward (plus value) or away from (negative value) the other cell ("cell effect" O——O) or rhizoid anlage ("anlage effect" O- - -O) are plotted against the gap in egg diameters between the pairs. *Uw*, means unwashed eggs; *W*, washed eggs; *S*, eggs were washed and then returned to shedding water; 6.1 and 7.6, pH of the medium. For figures 4c–d, the curves marked "2μ/sec" or "13μ/sec" show the cell effects between eggs lying on a screen perfused at the indicated rate; control eggs lay on such a screen without flow. Points with an abscissa of $\frac{1}{4}$ egg diameter describe the interaction of eggs 0 to $\frac{1}{2}$ egg diameters apart; $\frac{3}{4}$, indicates $\frac{1}{2}$ to 1; $1\frac{1}{2}$, 1 to 2; 3, 2 to 4; 6, 4 to 8; 8, 7 to 9. Errors are standard deviations of values from the indicated number of replicate runs; the total number of pairs measured is also shown.

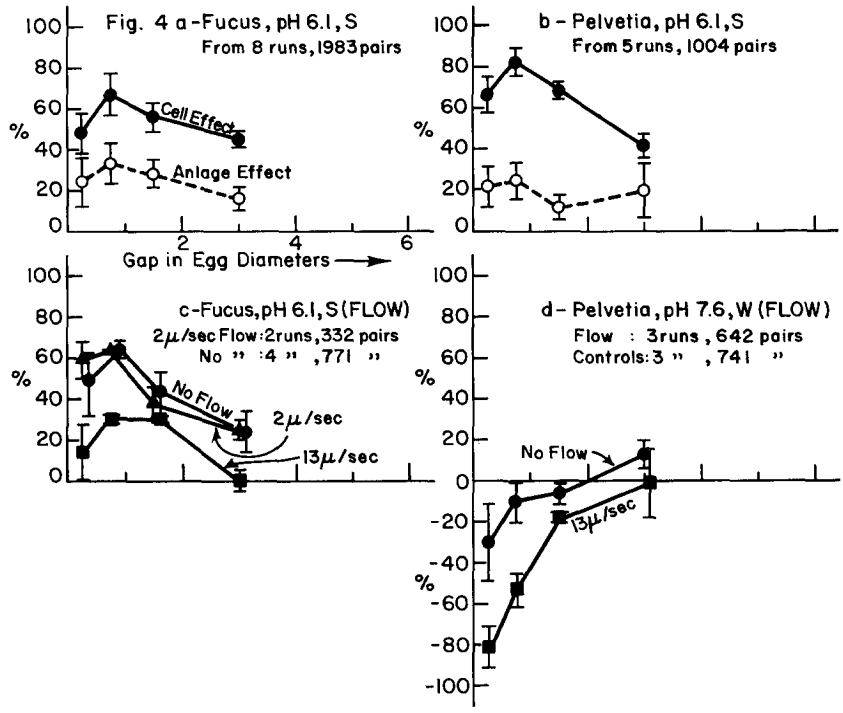


FIG. 4.

A comparison of Figs. 4b and 3f shows similar though less clear results with *Pelvetia* eggs.

Cell Effects

Figure 3 shows that slight acidity and a failure to wash the eggs both favor the positive cell effect whereas slight alkalinity and washing of the eggs markedly favor the negative cell effect. Thus, the strongest positive cell effect is found with unwashed eggs at pH 6.1 (Fig. 3a), and the strongest negative one with washed eggs at pH 7.6 (Figs. 3d, 3f).[7] A comparison of the graphs on the left and right sides of Fig. 3, i.e., a and b, c and d, e and f, isolates and demonstrates the strong pH effect; a comparison of those on the top and middle rows of Fig. 3, i.e., a and c, b and d, isolates and demonstrates the strong washing effect. However,

[7] One experiment was run with washed *Fucus* eggs in seawater buffered at pH 8.4 with 0.05 *M* Tris. We found a negative cell effect, but one of somewhat lesser degree than those found at pH 7.6.

it makes relatively little difference whether the eggs are those of *Fucus* or *Pelvetia* (cf. 3c and 3e, 3d and 3f, 4a and 4b); whether the eggs are unwashed *or* washed and then have shedding water readded (cf. 3a and 4a); and whether or not the medium's viscosity is raised 20-fold with 0.4% Methocel (see Fig. 3a).

It is notable that the positive cell effect falls much more gradually with the distance between the egg pairs than does the negative one. Thus the strong positive effect exhibited in Fig. 3a falls to one-half of its peak value when the intercellular gap reaches about 4 egg diameters; while the strong negative effects shown in Figs. 3d and 3f fall to one-half maximum at about 1 egg diameter.

Indeed, the shapes of all these cell effect curves seem to show the sum effect of a positive influence which falls steadily but slowly with distance and a negative one which falls steadily but much more steeply; only the proportions of these components appear to vary with the conditions. Thus when the positive effect is quite dominant, as in Figs. 3a (and also 4a and b), some negative influence seems yet to be seen in the reduced cell effect in the nearest distance class (of 0 to $\frac{1}{2}$ cell diameters as compared with the next class of $\frac{1}{2}$ to 1 cell diameters). When the two influences seem nearly to balance (as in Figs. 3b and 3c), the cell effect changes sign, shifting from weakly negative to weakly positive between these same distance classes. When the negative effect is quite dominant, as in Figs. 3d and 3f, the positive influence seems yet to be seen in a crossover to a weak positive effect in the longest distance class of 4 to 8 cell diameters.

Cell Effects under Cross-Flow

Under conditions eliciting a strong positive cell effect, *Fucus* eggs grown upon a Nitex filter while a steady cross-flow of 2 μ/sec passed through this support showed the *same* tendency to initiate rhizoids toward each other as did control cells grown upon the same support without cross-flow (Fig. 4c).[8] Thus it appears that rhizin can diffuse across a gap, L, of at least 2 *Fucus* egg diameters, d, despite a cross-flow velocity, v, of about 2 μ/sec. Thus:

$$L \geq 3d$$

where $d = 1$ *Fucus* egg diameter

The time, T, available for this diffusion is given by:

[8] Preliminary studies with lower flow rates showed these also to be ineffective.

$$T \leq d/v$$

Hence

$$D \geq L^2/T = 9dv = 1 \times 10^{-5} \text{ cm}^2/\text{sec}$$

Thus this finding is evidence that rhizin is a comparatively small molecule. However, the responses at cross-flows of 13 μ/sec (Fig. 4c and d) are *not* interpretable on the basis of whether or not cross-flow interrupted communication; for at these speeds cross-flow had the largest effect when the cells were *closest*, favoring a negative cell effect both in cases where the medium favored a positive cell effect (Fig. 4c) and where it favored a negative one (Fig. 4d). We can offer no precise alternate interpretation of these latter data at this time, noting only that at these high speeds, *individual* cells show strong rheotropic responses (Bentrup and Jaffe, 1968), and that the flow patterns past these cell pairs are not known. Hence at these speeds the cells may well have communicated hydrodynamically, i.e., through their effects upon the flow which in turn effected the other cell, rather than solely through diffusion.

DISCUSSION

1. The chief question which we tried to answer was whether extracellular rhizin and/or antirhizin are links in the amplification process which irreversibly localizes the rhizoid initial in the fucaceous egg and thus polarizes it *or* whether they are just modifiers of this process. Our data showing a marked tendency for relatively nearby egg pairs to initiate rhizoids toward the neighbor's rhizoid initial (i.e., the positive anlage effect), under conditions and only under conditions which also elicit a strong tendency to initiate rhizoids toward the other cell—these data are clear evidence that extracellular rhizin but not antirhizin is somehow localized near the very pole whose initiation it favors. Thus it is clear evidence that rhizin is a link in the localization process while antihizin is only a modifier of it.

2. Some confirmation of these conclusions is provided by an analysis of those of our data which show the cell effect as a function of intercellular distance:

First, let us point out that if extracellular rhizin were a link in the amplification process, then its secretion might be expected to begin during this process. However, it seems very probable that a *Fucus* egg population's asynchrony is so great compared to the time required for polarization within any one egg that the two eggs in any independent

pair are likely to polarize during nonoverlapping period.[9] Hence the faster egg in most such independent pairs would secrete rhizin during a period sufficient to polarize an egg and before the other began. Furthermore the rhizin diffusion constant of about 10^{-5} cm^2/sec (yielded by our cross-flow data) indicates that the time taken for rhizin to diffuse even 4 egg diameters (or 0.03 cm) is only about 100 seconds, a time which is certainly very short compared with the 3 hours it takes half of a *Fucus* egg population to begin germinating (Whitaker, 1936; Jaffe, 1968). Finally, then, if put together, these considerations indicate that *if rhizin were an amplification link, then in most nearby pairs, rhizin from the faster developing cell should reach the slower one before it began to secrete this stuff.*

Now let us show that an analysis of the data showing cell effects as a function of distance offers strong support for just this requirement for a (largely) one-way cell effect. Suppose the opposite, i.e., suppose the interacting pairs to be perfectly synchronous in their development. In this case the fractional gradient resulting from either cell's action upon the other would be reduced ("drowned out") by the secretion of stuff by the other, and an analysis of the steady-state diffusion pattern about two identical spherical sources of some stable stuff gives an upper limit to the fractional gradient they can impose upon each other (Appendix). If the interaction occurs before the concentrations have risen to steady-state levels, or if the stuff decays en route between cells, the gradients will be yet lower because both these conditions should reduce the concentration provided by the "donor" cell more than they reduce those provided by the "receptor" cell.

Now, the available quantitative studies upon the polarization of cells by light or chemical gradients support the empirical rule that *percent polarization of a population approximates the percent gradient evoking it.* This is true for the polarization of *Osmunda* and of *Botrytis* spores by light gradients (Jaffe and Etzold, 1962), of the polarization of *Fucus*

[9] Except under special circumstances, such as illumination by polarized light, eggs initiate two rhizoid poles so rarely as to indicate that the germination, i.e., initiation of one rhizoid pole, by an egg marks the practical completion of its polarization; on the other hand, the decline in a population's photoreversibility marks the beginning of its irreversible polarization. A comparison then of germination and photoreversibility curves shown by one population indicates that the process of irreversible polarization within each cell takes less, probably much less, than 2 hours, and the germination curve itself indicates that even within any 2-hour period only about 20% of the cells complete their polarization (Jaffe, 1968, Fig. 7).

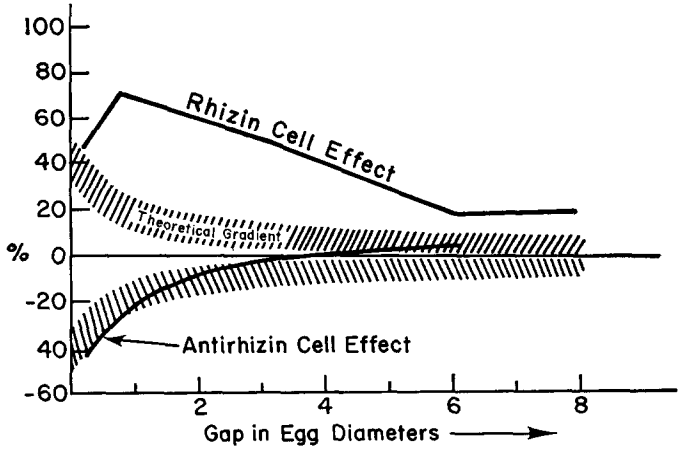


FIG. 5. A comparison of the theoretical steady-state gradients imposed upon each other by identical spherical sources with the mutual polarizations of *Fucus* eggs which are mediated primarily by rhizin (from Fig. 3a) and primarily by antirhizin (from Fig. 3d). The calculated limiting values for the theoretical gradients (from Eqs. 5 and 7, Appendix) bound the gray bands. This same band is drawn both positively and negatively to allow comparison with the polarization produced by rhizin and antirhizin, respectively.

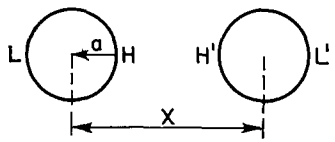


FIG. 6. Diagram of spheres whose centers are x apart.

eggs by hydrogen ion gradients (Bentrup *et al.*, 1967), and of the polarization by the gradients in turn evoked by slow flow past both *Botrytis* spores (Müller and Jaffe, 1965) and *Fucus* eggs (Bentrup and Jaffe, 1968). Therefore, while this empirical rule is not yet intelligible it is nevertheless sufficiently documented to predict that the mutual polarization of cell pairs should approximate the gradients they impose upon each other. If then, synchronous cells interact through steady-state gradients of a stable stuff, their degree of mutual polarization should approximate the gradients calculated in the Appendix and graphed in Fig. 5; if synchronous cells interact through gradients of an unstable stuff and/or through pre-steady-state gradients, then the degree of mutual polarization should be *less* than these theoretical gradients; in

no case should synchronous cells impose a mutual polarization larger than these gradients. Also in Fig. 5 we plot, for comparison, both the mutual polarization which is in fact mediated primarily through rhizin (the cell effect in Fig. 3a) and that mediated primarily through antirhizin (the cell effect in Fig. 3d).

It is striking to see that while antirhizin interaction approximates the theoretical curve, rhizin interaction is far larger, particularly at long distances. What may this mean? It clearly suggests that *antirhizin's* production is synchronous, that it does not decay en route between cells, and that interaction occurs through antirhizin gradients in the steady state; i.e., it suggests the simplest possible circumstances. Moreover, this is consistent with previous evidence of antirhizin's relative stability (Jaffe, 1968), and it may also be considered further support for the rule that polarization approximates evoking gradient.

However, with regard to *rhizin*, which is the chief concern here, a very different analysis is required. We seem forced to abandon the trial supposition of synchrony; it *cannot* explain the large polarizations observed. On the other hand, these large polarizations *are* explicable if cell pairs are sufficiently asynchronous in beginning rhizin production. Indeed, if the slower cell responds fast enough to a low enough absolute concentration of rhizin, then the effective fractional gradient across it, one produced by the edge of the rhizin gradient spreading from the faster cell, could be as large as desired as far away as desired. Thus, in principle, sufficient asynchrony makes possible up to 100% polarization of one member of every pair at any separation, and thus an average polarization of up to 50% for the population of pairs at any separation. Hence asynchrony can explain 50% or lower polarization at 3 or more egg diameter gaps (Fig. 3a); to explain the yet higher polarization of cells separated by less than two cell diameters, it is only necessary to add the assumption of some "back-talk," i.e., that at these short distances, the slower cell acts back to some degree upon the faster.

3. In previous studies of the group effect, the significance of washing the eggs was not recognized. In the three quantitative studies available, the eggs were washed primarily by settling and other gentle processes in which the flow of fluid past the egg probably did not exceed about 10^{-2} cm/sec, and the total dilution of the original shedding water probably did not exceed about a thousandfold. The eggs were washed in the present study with speeds of perhaps 0.3 cm/sec, thus speeds at least 30-fold greater than those used before; moreover, the original shedding water was diluted in the present study to a degree, that, while

TABLE 1

COMPARISON OF THE CELL EFFECT IN PRESENT AND OLDER STUDIES[a]

	pH 6.1		pH 7.6	
	Unwashed	Washed	Unwashed	Washed
Present study	65	5	0	−25
Older studies	80[b]		−5[c]	

[a] To make the results relatively comparable, the responses in the present study are an average of those of cells 0–2 cell diameters apart; moreover the older results, which were prevously presented as p = percent of rhizoids initiated between 0 and 90° of the neighbor are here converted to $2(p\text{-}50\%)$

[b] Jaffe (1955) and Whitaker (1937).

[c] Whitaker and Lowrance (1940).

hard to estimate, was certainly far greater than that used before. It is of some interest then to compare quantitatively the older results with the present ones, and this is done in Table 1. It is seen that the relatively gentle and incomplete washing done before was approximately equal in its consequence for the cell effect to that of no washing at all.

SUMMARY

We have measured the mutual polarization of populations of pairs of fucaceous eggs as a function of their distance apart. We did this in different circumstances, of which the medium's pH and washing of the eggs proved most consequential.

Under circumstances in which each egg tends to initiate its rhizoid pole (or germinate) toward the other, it also tends to initiate it toward the other's rhizoid initial; however, when it tends to germinate away from the other, its germination direction is independent of the other's. We infer that *rhizin* (an extracellular locally effective stimulator of rhizoid initiation) is concentrated near the developing rhizoid initial and is thus a link in a regenerative process that irreversibly polarizes the egg; however, *antirhizin* (an extracellular locally effective inhibitor of rhizoid initiation) is inferred to be uniformly emitted by each egg and thus only modifies this process.

When eggs interact through antirhizin, their mutual polarization approximates the fractional gradients calculated to be imposed upon each other by synchronous spherical sources of a stable stuff in the steady state; so antirhizin interaction is inferred to have this simple character. However, when eggs interact through rhizin, their polariza-

tion greatly exceeds such gradients; we infer asynchrony, i.e., that the faster cell in each pair sends rhizin to the other, slower one before this latter starts to emit it. These inferences, in turn, are believed to confirm rhizin as a link in the polarization process, and antirhizin as only a modifier of it. A flow of the medium through the substratum and thus between pairs interacting via rhizin fails to modify their interaction at speeds of up to 2 μ/sec; it is inferred that rhizin has a diffusion constant of the order of 10^{-5} cm^2/sec and thus is a relatively small molecule.

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APPENDIX

A calculation of the limiting values for the size of the fractional gradients of some stable stuff which are imposed by two spherical sources upon each other in the steady state.

1. Consider one sphere of radius a steadily and uniformly emitting some stuff which diffuses into an infinite medium.

Let r = the distance from its center

Let C = the stuff's concentration outside of the source.

The diffusion equations are easily solved to show that in the steady state:

$$C = K/r \tag{1}$$

where K is a constant.

2. Now consider two such spheres whose centers are X apart (Fig. 6). Let C_H be the (high) steady state concentrations at points H and H'; C_L the (low) ones at L and L'.

Let G = the fractional gradient across each sphere. Then, by definition:

$$G = \frac{C_H - C_L}{C_H} \tag{2}$$

3. It is clear that to some degree each sphere will impede the diffusion of stuff from the other. A solution taking this into exact account will not be attempted here. Rather, by very simple means, limits to the solution will be obtained:

Case I. Assume that each sphere does not impede diffusion of stuff emitted by the other at all. This assumption plainly yields a lower limit for G. In this case, using Eq. (1), one gets:

$$C_H = K/a + K/X - a \tag{3}$$

$$C_L = K/a + K/X + a \tag{4}$$

Putting these values into Eq. (2), yields:

$$G_{\min} = 2a^2/X(X + a) \tag{5}$$

4. *Case II.* Assume that each sphere completely blocks diffusion of material from the other source to this one's distal side, so that:

$$C_L = K/a \tag{6}$$

while the proximal concentration, C_H, remains as in Case I. This assumption would appear to yield an upper limit for G.

Substituting Eqs. (3) and (6) into Eq. (2) then, yields:

$$G_{\max} = a/X \tag{7}$$