* A detailed report of the data and anatomical verification of the lesions will appear elsewhere.

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DO FUCUS EGGS INTERACT THROUGH A CO_2-pH GRADIENT?

BY LIONEL JAFFE*

HOPKINS MARINE STATION, PACIFIC GROVE, CALIFORNIA

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In 1889 Rosenvinge[1] reported that the direction of the main morphological axis, or polarity, of the eggs of the Fucaceae could be determined by neighboring eggs. Developing in darkness, they were found to germinate proximally, i.e., to develop their rhizoids or basal poles toward their neighbors. This so-called "positive group effect" has been well confirmed,[2, 3, 4] though a tendency toward proximal germination between the smallest of groups, a pair of eggs, was found to occur only below pH 7.6.[5, 6]

An understanding of this mutual orientation would bear upon two broader problems: the origin of polarity and the mechanisms of morphogenetic interaction between cells. Rosenvinge[1] suggested an oxygen gradient to be responsible for the positive group effect; Olsen and du Buy,[7] an auxin gradient; Whitaker,[8] a CO_2-pH gradient. Whitaker supported his proposal by demonstrating that when *Fucus* eggs are exposed to a CO_2-pH gradient in darkness, where they respire, they do, in fact, germinate toward the high CO_2, high H^+ pole (except at very low pH's, on the border line of growth inhibition).[9] Moreover, so many other facts fit neatly into this picture that Whitaker's hypothesis has won wide acceptance: In a gradient of white light, rhizoids develop from the darker side; in a thermal gradient, from the warmer side; in a gradient of dinitrophenol (averaging 10^{-5} *M* at threshold) or of potassium indole acetate (averaging 10^{-3} *M* at threshold), from the side of higher concentration.[8, 10]

However, the positive group effect has been reported to appear in illuminated, and hence presumably photosynthesizing, cultures.[1, 2, 3] Although this suggests oriented development in a direction opposite that expected on the basis of Whitaker's hypothesis, the results are ambiguous. First, it cannot be inferred from the published data that the illuminated eggs maintained net photosynthesis. Second, the observed effects may in part have been due to mutual shading of the eggs, since in a gradient of white light rhizoids develop from the darker sides. For these reasons the author has conducted further experiments on the germination of *Fucus* eggs, illuminated under conditions not subject to the above criticism.

Light gradients were minimized by illuminating the cultures directly from above and, by means of mirrors, also from below, so that very little light struck the eggs at angles low enough to cause shadowing. Furthermore, red instead of white light was used, because red light has been reported not to orient *Fucus* eggs even when unilaterally applied.[3, 11] This claim has been verified in experiments with eggs grown under conditions identical with those used to study the mutual orientation of illuminated pairs, except that the light was directed unilaterally instead of from above and below. The results showed that 57 per cent of 222 eggs had germinated from their darker hemispheres and 43 per cent from their lighter ones.

The red light used in all experiments was obtained by filtering light issuing from a tungsten filament lamp through $^1/_2$ meter of water and an F-29 Kodak Wratten filter reported to eliminate radiation of wave lengths shorter than 600 mμ; its intensity was *ca.* 800 foot-candles.

The experiments were conducted with zygotes of *F. furcatus*, obtained as described by Whitaker,[12] and were carried out in an 11° ± $^1/_2$° C. constant-temperature room or water bath.

1. A suspension of recently fertilized *Fucus* eggs in filtered sea water was placed in a cylindrical culture chamber. After settling, the eggs nearly covered the bottom with a single layer of cells. The chamber was closed with a cover slip so as to exclude gas bubbles, sealed with paraffin oil, and illuminated with red light.

In this culture gas bubbles accumulated; after 21 and 33 hours their estimated volumes were 5 and 9 μl., respectively. No gas bubbles were observed in a control experiment with sea water without eggs and sterilized with $HgCl_2$. The culture was then left in darkness for 36 hours; during this period the gas volume fell to 1 μl.

Examination of the culture after 33 hours of illumination showed that practically 100 per cent of the eggs bordering the few clear spaces had germinated away from these spaces and toward their neighbors. If the gas production is attributed to O_2 evolution by photosynthesis, this simple experiment shows that the positive group effect is not mediated by a CO_2 (or an O_2) gradient.

2. This was corroborated by an experiment with more widely spaced eggs. That net photosynthesis was maintained here was demonstrated in a parallel experiment using Warburg manometers.

Three thousand recently fertilized eggs were distributed on the bottom of a Carrel flask (5 cm. in diameter) containing 8 cc. of sea water buffered at pH 6.0 with 0.016 *M* phosphate, plus 2 cc. of natural sea water added just before the flask was sealed so as to provide sufficient CO_2 for prolonged photosynthesis (5×10^{-4} *M* $CO_2 + HCO_3^-$). The flask was illuminated with red light until after germination was completed. Then the culture was carefully scanned for semi-isolated pairs of eggs (those less than $1^1/_2$ egg diameters apart whose separation from the nearest third egg was at least 3 times their distance apart). Of 78 eggs observed in such illuminated pairs, 82 per cent had germinated proximally, the rest distally. Two control cultures developed in darkness. In one, bearing an initially identical medium, 68 per cent of 84 eggs germinated proximally. In another dark control, not supplemented with natural sea water, 77 per cent of 88 eggs germinated proximally. Thus, if anything, the illuminated, photosynthesizing culture showed a more marked positive group effect than the dark, respiring controls.

3. In the parallel manometric experiment, four test vessels plus two thermobarometers were so rigged as to allow determinations of both oxygen and carbon dioxide exchange, in darkness as well as in red light. Each vessel contained in its main compartment 2 ml. of artificial sea water, buffered at pH 6.0 with 0.015 *M* phosphate; the four test vessels were each supplied with 17 mm.^{3} of eggs. Two of the test vessels were illuminated with red light, while two were kept in darkness. One illuminated test vessel, one "dark" vessel, and one thermobarometer each contained in its center well and appendices 1 ml. of a CO_2 buffer to maintain a constant pressure of 0.3 per cent CO_2 in the gas phase (0.321 *M* $KHCO_3$ plus 0.154 *M* Na_2CO_3).[13] Here the pressure changes were due exclusively to oxygen production or utilization. Carbon dioxide assimilation or production were computed from simultaneously measured pressure changes in the companion vessels without CO_2 buffer, equilibrated with a gas phase composed of air plus 0.3 per cent CO_2; any decrease in the rate of photosynthesis resulting from CO_2 depletion was minimized by flushing the illuminated vessel at approximately 3-hour intervals with this gas mixture. The experiment was conducted in a water bath at 11° C.; photosynthesis was measured over the period from $2^1/_2$ to 23 hours after fertilization; nearly all the eggs had germinated. Measurements in the dark were continued for another 13 hours, at which time germination had here been practically completed.

The results show that the rate of photosynthesis, not corrected for respiration, remained constant at 0.8 μl gas/mm^3 eggs × hr, double the rate of respiration, which was constant at 0.4 μl gas/mm^3 eggs × hr. Both the assimilatory and respiratory quotients were within 5 per cent of unity.

In so far as they are comparable, these results harmonize with those of Whitaker,[14] who found that at 18° C. fertilized *F. vesiculosus* eggs produced more than 2 μl O_2/mm^3 eggs × hr in white light at an intensity of 100,000 foot-candles and consumed 1.0 μl O_2/mm^3 eggs × hr in darkness.

These experiments convincingly demonstrate that in red light photosynthesis exceeded respiration, and calculations readily provide assurance that, in the stationary cultures used to study the group effect, diffusion brought enough CO_2 to the eggs to maintain this condition without the 24-hour period needed to complete germination. Hence it can be safely inferred that the positive group effect persisted in red light, despite the fact that the CO_2-pH gradient was opposite that of the dark cultures.

4. Further evidence against the hypothesis that a CO_2-pH gradient is responsible for proximal germination is furnished by the failure of wide variations in buffer capacity to influence the group effect. Since orientation is not an all-or-none phenomenon, and its manifestation seems to depend on the steepness of a gradient, it should be expected that, the greater the latter, the more pronounced the degree of orientation.

The author has examined shadowgraphs of cultures of eggs, in sea water buffered at pH 6.0 with 0.016 and 0.002 *M* phosphate, respectively, after development in darkness. In each experiment 3,000 eggs were distributed over the bottom of Petri dishes 5 cm. in diameter. The shadowgraphs were analyzed so as to allow direct comparison with the results of Whitaker's[5] similar exeriments on dark-grown *F. furcatus* eggs in sea water buffered at pH 6.0 either with 0.008 *M* phosphate-citrate or with bicarbonate; in the latter case the medium was in atmospheric equilibrium and hence contained 0.000024 *M* CO_2 plus HCO_3^-. The concentration near a pair of eggs must have been slightly greater due to respiration; it can be estimated as 0.00003 *M*.

Mutual orientation was determined for egg pairs separated from each other by $^1/_2$–$1^1/_2$ egg diameters, and more than 7 egg diameters from the nearest third egg. The extent of orientation has been listed in Table 1; it is expressed under two categories, viz., general orientation, covering the cases of rhizoids developing proximally, 0°–90° toward their neighbors, and strong orientation, for rhizoid development in the direction of neighboring cells within 10° of the line joining the egg centers.

TABLE 1

MUTUAL ORIENTATIONS OF PAIRS OF EGGS REARED IN SEA WATER AT pH 6.0 IN VARIOUS BUFFERS

			PER CENT OF ALL RHIZOIDS		
OBSERVER	BUFFER	TOTAL NO. OF EGGS	Distal 90°–180°	Proximal 0°–90°	Proximal 0°–10°
W	3×10^{-5} *M* carbonate	104	12	88	26
J	2×10^{-3} *M* phosphate	74	11	89	23
W	8×10^{-3} *M* phosphate + citrate	97	8	92	33
J	1.6×10^{-2} *M* phosphate	48	11	89	29

It is clear that the results of Whitaker's experiments and mine are fully comparable; over the 500-fold range of buffer concentrations involved, no difference is observed. Since the first pK of carbonic acid and the second of phosphoric acid in sea water are both about 6.1, the range of buffer concentrations also covers an equal range of buffer capacities. And, because the steepnesses of the H^+ ion gradients established are inversely proportional to the buffer capacities,[15] it may therefore be concluded that the identical orientations cannot be attributed to these gradients.

Summary.—The tendency of *Fucus* eggs to develop their basal poles toward each other persists despite the replacement of respiratory CO_2 emission by photosynthetic CO_2 uptake and is not influenced by a 500-fold change in buffer capacity. Hence this interaction is not mediated by gradients of H^+, CO_2, or O_2.

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