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APOLAR EMBRYOS OF FUCUS RESULTING FROM OSMOTIC AND CHEMICAL TREATMENT¹

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A B S T R A C T

Embryos of the brown alga *Fucus vesiculosus* L. were grown as populations in glass petri dishes in seawater at 15 C in continuous low-intensity unilateral fluorescent illumination for periods up to 2 weeks. A quantitative estimate of increase in nuclear number was made from acetocarmine squash preparations of samples taken at 12-or-24 hr intervals. Over the period of 2-6 days embryos showed a doubling time of about 12-18 hr. Under normal seawater culture conditions each embryo formed a single rhizoid. When grown in seawater supplemented with sugar concentrations above 0.4 M, *Fucus* embryos developed as multicellular spherical embryos lacking rhizoids. In 0.6 M sucrose-seawater, 97% of the embryos were apolar at 2 days; only 37% were apolar at 4 days, many having recovered from the sucrose inhibition. Some embryos remained apolar after growth in 0.6 M sucrose for 2 weeks. Nuclear counts showed that sucrose-seawater markedly inhibited the rate of cell division. Other sugars including D-glucose, D-fructose, D-galactose and the sugar alcohol D-mannitol were also effective. When apolar embryos grown in sucrose-seawater were returned to seawater, embryo growth resumed at the normal seawater rate, judged from nuclear counts. Such embryos formed multiple rhizoids, varying from two to eight rhizoids per embryo, which developed on the embryo quadrant or half away from the unilateral light. Each of the multiple rhizoids originated from a single small cell in the periphery of the multicellular spherical embryo. Thus the rhizoid-forming stimulus apparently had been subdivided among a number of the cells of the apolar embryos. The implications of this finding are discussed. Attempts to produce multiple rhizoids by treatment of embryos with indoleacetic acid or 2,4-dichlorophenoxyacetic acid failed. However, embryos treated with 10^{-4} M or 5×10^{-5} M 2,3,5-triiodobenzoic acid formed 40 and 30% multiple rhizoids, respectively, suggesting that some chemical, perhaps hormonal, mechanism is involved in polarization and rhizoid initiation in *Fucus* embryogenesis.

ASYMMETRICAL DEVELOPMENT of embryos of the brown alga *Fucus* has served as a classical subject for the study of the influence of the physical and chemical environment on the determination of polarity. Factors influencing orientation of polarity were studied in extenso by Whitaker (e.g., 1931, 1936, 1938) and more recently by Nakazawa (1959, 1960, 1962) and by Jaffe (1968) who reviewed this work and the related literature. In nature the large (70-80 μ diam) eggs are released into the seawater where they are fertilized by motile sperm. Each zygote settles and adheres to the substratum by means of a rapidly formed sticky polysaccharide wall. Within about 12 hr at 15 C, depending upon the physical and chemical gradients to which it is exposed, the zygote develops an asymmetrical

protuberance in one-half of the initially spherical cell. By about 18 hr the nucleus undergoes mitosis, forming two nuclei with the spindle oriented in the plane of the protuberance. By 20-24 hr a cross wall forms at right angles to the plane of the protuberance and two quite dissimilar cells result, the pyramidal rhizoid cell and the hemispherical thallus cell. Further divisions of the rhizoid cell lead to a filamentous rhizoid which ultimately forms the holdfast. Divisions of the thallus cell give rise to a pear-shaped multicellular young plant. By the 6th to the 10th day, depending upon illumination and temperature, apical hairs are formed (see Galun and Torrey, 1969); these hairs mark the beginning of the formation of the apical meristem of the vegetative thallus.

The first division is asymmetrical and establishes the fate of the cell progeny. Although recently much interest has centered upon the nature of this differential cell division in ultrastructural terms (Neushul and Liddle, 1968; see also Jaffe, 1968) and in chemical terms (Peterson and Torrey, 1968; Quatrano, 1968), most attention has been paid to the factors controlling the initial polarization of the fertilized egg.

The initiation of the protuberance, and hence the determination of the site of the rhizoid, is

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subject to a variety of environmental factors. The rhizoid forms on the side away from unilateral illumination (Whitaker, 1936; Whitaker and Lowrance, 1936), blue light being most effective and longer wavelengths (580–700 nm) having no effect (Hurd, 1920). Rhizoids form toward the positive pole in an imposed electrical gradient (Lund, 1923), toward the higher concentration of a potassium ion gradient (Bentrup, Sandan, and Jaffe, 1967), on the acidic side of the pH gradient (Whitaker, 1938), on the warmer side in a temperature gradient (Lowrance, 1937), and toward other embryos or a piece of thallus rather than away (the so-called "group effect") (Whitaker, 1931). Jaffe (1966) interpreted these controlling factors in terms of electrical gradients, related perhaps to internal differences of chemical constituents and/or ultrastructural organization, especially in or near the plasma membrane or outer cortex of the cell.

Development of multicellular apolar embryos was observed earlier in *Fucus*. Farmer and Williams (1898) described inhibition of rhizoid formation in embryos cultured in seawater at densities higher than normal. Kniep (1907) reported the development of multi-cellular spherical embryos after mechanical destruction of the rhizoid cell. Whitaker (1931) observed occasional apolar embryos developing in the center of a dense population of normal embryos. Sussex (1967) produced apolar embryos in the brown alga *Homosira* by subjecting the fertilized eggs to continuous agitation. The predictable lack of polarization of developing embryos of *Fucus* under specified experimental conditions offers a useful tool in the analysis of the stimuli acting on rhizoid initiation. The work reported below concerned the question of the nature of the rhizoid-initiating stimulus as deduced from experiments with apolar embryos.

MATERIALS AND METHODS—Gamete release and fertilization—The collection of plants, the release of gametes, and fertilization were essentially those described by Peterson and Torrey (1968). Plants of *Fucus vesiculosus* L. were collected from rocks at Sea Wall, Mount Desert Island, Maine, during ebbing and low tides during the summer months. Whole plants were placed in plastic bags, packed on ice in polystyrene storage containers, and carried to the laboratory at Salisbury Cove, Maine. Plants of this dioecious species were separated according to sex by sampling and examining free-hand sections of receptacles of every plant specimen. Whole receptacles were cut from the plants and male and female receptacles stored separately in 10-cm glass petri plates at 4 C in the dark for a minimum of 24 hr but not longer than about 7 days.

To obtain eggs, female receptacles were washed in Millipore-filtered seawater in a funnel to

remove all surface debris and groups of receptacles were floated in fresh, filtered seawater in 10-cm petri dishes placed in a 15 C incubator illuminated from one side by an 8-w cool-white fluorescent lamp. Oogonial release followed by egg release usually occurred within several hours; eggs released more than 12 hr were not used for experiments. Male receptacles were placed moist in 10-cm petri dishes. Usually antheridial release occurred in the refrigerator at 4 C in the dark as manifest by a bright orange exudate on the receptacle surface. Sperm release was effected readily by transferring receptacles covered with such exudate into a small volume of filtered seawater at 4 C. The sperm suspension was kept in a flask on ice until used, usually within a few minutes of suspension.

In order to achieve a clean preparation of eggs free of oogonia or debris, the eggs were filtered through a Nitex nylon filter with a 102 μ pore size (Tobler, Ernst, and Traber, Inc., 71 Murray Street, New York, N. Y. 10007). Sperm suspensions were similarly filtered to remove antheridia. Sperm and egg suspensions were combined in a 125-ml Erlenmeyer flask on ice, were vigorously swirled, and then were allowed to stand on ice for about 1 hr. Although fertilization was achieved very rapidly, a delay before final filtering allowed the fertilized eggs to develop a rigid wall. Filtration and washing of the fertilized egg population at the end of 1 hr on a 35 μ Nitex nylon filter allowed the sperm and unfertilized eggs to pass through the 35 μ pores but caught the rigid zygotes which were then resuspended in fresh, filtered seawater of a selected volume and aliquots were placed in dishes for experiments. Most experiments were carried out in 5-cm Pyrex petri dishes. Small drops of concentrated suspensions of zygotes were pipetted with a Pasteur pipette into 10-ml volumes of the experimental solutions and the dishes immediately incubated at 15 C in the lighted incubator. Each dish was marked with respect to its orientation to the fluorescent lamp. One-sided continuous illumination caused precise orientation of all embryos with the rhizoid growing away from the light. Such oriented embryo development very much facilitated scoring embryonic stages and assured that the asymmetry of rhizoid formation would be quite manifest to the observer. Embryos were observed at regular, usually at 24 or 48 hr, intervals and scored using a dissecting binocular microscope with transmitted light at about 50 \times magnification. In experiments carried more than 3 days, experimental solutions were renewed at least at 3-day intervals. Several series of experiments using seawater with or without sucrose were conducted in the presence of an added antibiotic mixture (25 μ g streptomycin plus 50 units of penicillin per ml). The presence of these antibiotics did not change the observed developmental behavior.

Cytological preparations—Fertilized eggs adhered to glass within a few hours after fertilization and remained attached, especially at the rhizoid end of the embryo, as development continued. This feature served a useful purpose since it allowed petri plates to be emptied and replenished simply by pouring off solutions without loss of the plants. Also unfertilized eggs, sperm and debris were readily washed away. This same feature was used for cytological preparations. Fertilized eggs were pipetted onto albumen-coated 22-mm-square cover slips attached to the bottom of 10-cm petri plates (six per dish). On each cover slip was placed a 20-mm-diam glass van Tieghem ring to restrict the embryos to the cover slip. When the dish was filled with seawater or the experimental solution, all embryos were washed with a common solution. The glass rings could be removed at about 12 hr when the embryos were well affixed to the cover slips. The albumen coat facilitated later squashing procedures and was not essential for adherence of the embryos.

For cytological preparations the cover slip was simply picked up from the culture dish with stainless-steel forceps and transferred to a small screw-cap bottle containing a small volume of 10% formalin in seawater at room temperature. The embryos could be stored indefinitely in this fixing solution but were held at least 24 hr before further processing.

Detailed quantitative information about the course of cellular events during embryogenesis necessitated our development of a method of accurate nuclear or cell counting, based on a large sample size. We developed the following squash technique modified from procedures described by Roberts (1966). Embryos were carried through all the procedures adhering to the cover slip. Checks were made after each step to see that treatment had not removed the embryos.

After 24-hr fixation in 10% formalin-seawater, the embryos were given at least three changes of tap water during one hour. The embryos were transferred to 6% Na_2CO_3 solution in distilled water and warmed gently on a hot plate; boiling was avoided. Higher heat treatment could be tolerated by the young embryos than by the older embryos which macerated very readily (Fig. 12). After 15 min in Na_2CO_3 solution, the embryos were given at least three tap water rinses during at least 15 min.

The cover slip was then placed embryo-side down on a clean glass slide and the cover slip gently pressed to squash the embryos in a drop of tap water. The amount of squashing required depended upon the age of the embryos and the effectiveness of the carbonate maceration. After squashing, drops of acetocarmine solution (2 g carmine in 100 ml 45% acetic acid) were allowed to seep under the cover slip reaching all the embryos. Then the slide was heated gently

over an alcohol lamp flame for a few seconds, avoiding boiling, and allowed to stand. The preparation was then gently resquashed with filter paper taking up the excess stain. In some preparations this procedure gave good nuclear staining with relatively light cytoplasmic staining. The nuclear stain could be intensified with a drop of very dilute iron tartrate solution which, however, acts progressively with time to produce overstaining. Most cell walls stained poorly but could be discerned by their exclusion of stain. The rhizoid cell and its adherent polysaccharide stained dark red. Also in apical-hair stage embryos an internal skeleton-like wall component took up the carmine stain. Nuclear counts were usually made on temporary mounts. However, in some cases, air-dried slides were made permanent simply by popping off the cover slip, drying, and mounting in Permount.

RESULTS—*Normal development of Fucus embryos in seawater at 15 C*—In Fig. 1 is presented in terms of the number of nuclei per embryo the time course for normal development of *Fucus* embryos grown in seawater at 15 C in continuous low-intensity unilateral white light. In Fig. 2-7 are illustrated squash preparations of embryos

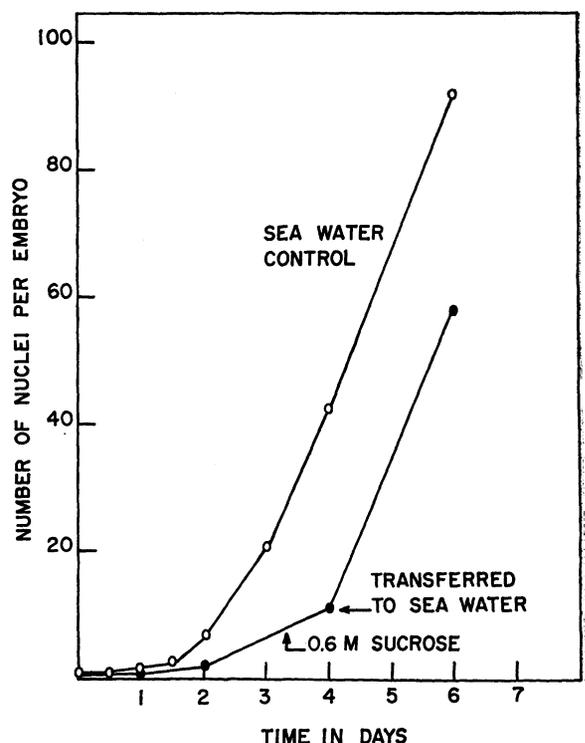
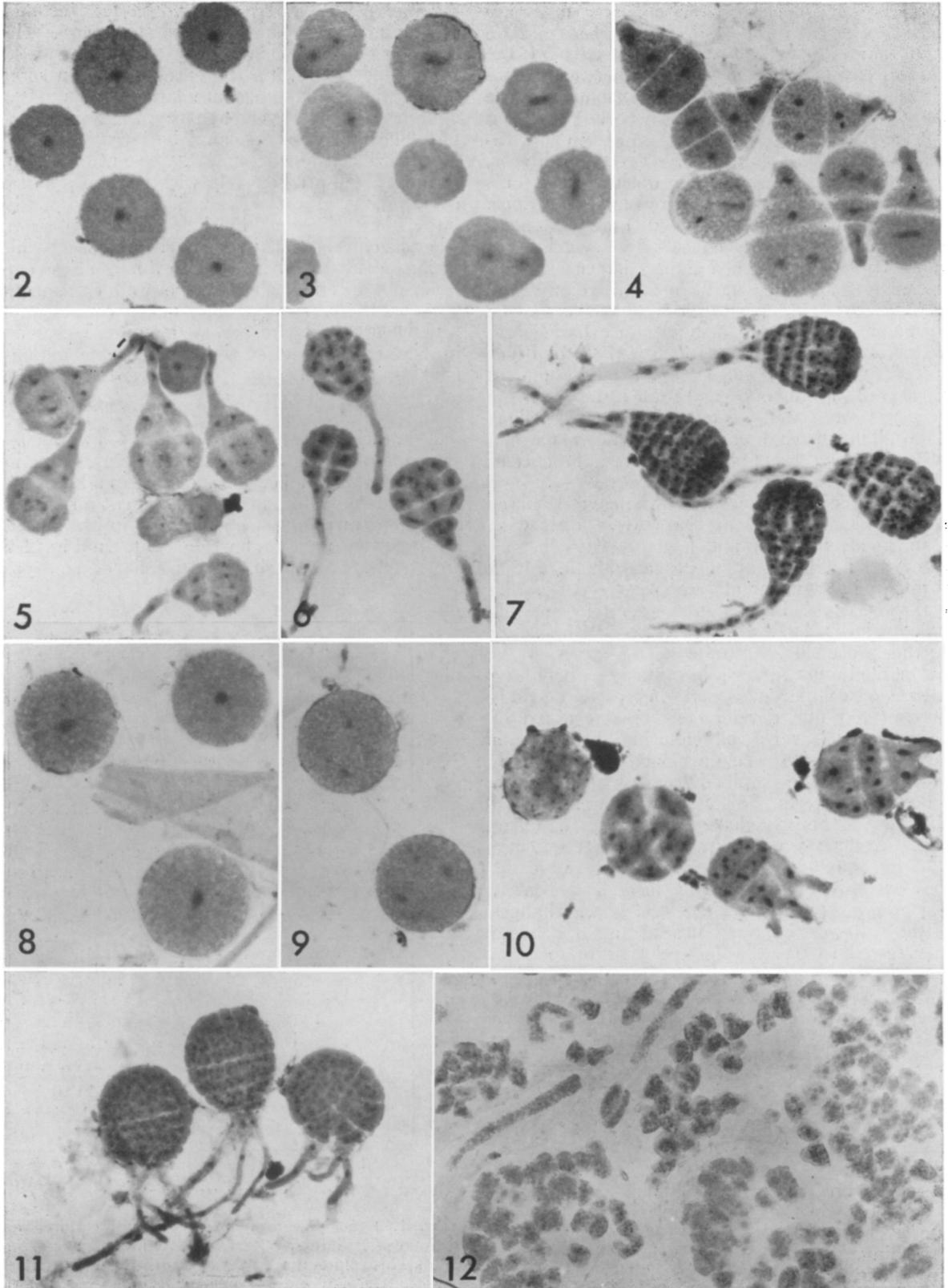


Fig. 1. Development of *Fucus* embryos cultured in continuous low-intensity light at 15 C in seawater or in 0.6 M sucrose plus seawater for 4 days then transferred to seawater. Development is expressed as number of nuclei per embryo and each point is based on the mean of 25-30 embryos. Fertilization occurred at day 0.



showing some of these stages. Note that orientation of the embryos was disturbed in these preparations by the squash procedure. Polarization of the embryo developed between 12 and 20 hr after fertilization (Fig. 2, 3) and by 24 hr mitosis and cell division had occurred. By 48 hr a mean value of 6.5 nuclei per embryo was observed. In some series accelerated rates were observed; for example, in one set a mean value for nuclear number of 3.9 at 36 hr and 8.5 at 48 hr was recorded (Fig. 4, 5). With a cell doubling time of approximately 12–18 hr between day 2 and day 6, mean nuclear number increased to 10.6 at 60 hr, 20.7 at 72 hr, and 42.1 at 96 hr (Fig. 6, 7). The rhizoid cell which initially represented almost one-half of the dividing zygote, divided again parallel to the initial plane of division (Fig. 4); these cells elongated markedly and divided again to form a single long rhizoid made up of six to eight large elongate cells almost devoid of chloroplasts and quite poor in cellular organelles (Fig. 7). These rhizoid cells produced a thick outer layer of sticky polysaccharide which cements the embryo to its substratum.

The thallus cell of the two-celled embryo proceeded to cleave into many small cells as the thallus body increased slowly in size (cf. Fig. 2, 6). The pattern of cell divisions was not precise. No multinucleate cells were observed during this study, and the nuclear counts represent cell numbers. By the 6th to the 7th day, the first sign of apical hair formation was the development of a small papilla on the thallus body approximately 180° from the rhizoid end of the embryo. This hair developed by intercalary growth from internal cells and usually more than one hair developed (Galun and Torrey, 1969). Apical hair formation was preceded by internal differentiation of cells (Nienburg, 1931). Such internal differentiation was not apparent at 4 days (Fig. 7).

The formation of apolar embryos in sucrose-seawater—Early in our studies it was observed that *Fucus* embryos cultured in small-volume dishes (e.g., Bureau of Plant Industry dishes which hold 1 ml) unpredictably produced multicellular spherical embryos completely lacking rhizoids. A search of the literature suggested that osmotic conditions influence embryogenesis and led us to test the effect of increasing the osmotic pressure of the seawater on embryo development. These preliminary experiments with concentrated

TABLE 1. *Effect of seawater supplementation with sugars or sugar alcohols of different concentrations on polarization of Fucus embryos*

Sugar concentration in seawater ^a	Number of embryos counted		% polarized	
	48 hr ^b	96 hr	48 hr	96 hr
0 sucrose	130	124	100	100
0.45 M "	100	125	51	86
0.5 M "	136	132	32	80
0.55 M "	135	157	24	84
0.6 M "	123	116	3	63
0.65 M "	133	153	7	35
0.4 M D-mannitol	153	154	44	77
0.5 M "	144	118	5	23
0.6 M "	102	134	1	11
0.6 M D-galactose	—	216	—	75
0.6 M D-glucose	—	162	—	68
0.6 M D-fructose	—	239	—	88

^a Embryos were cultured in 10 ml of medium in 5-cm glass petri dishes at 15 C in continuous unilateral white fluorescent light.

^b Embryos were transferred to fresh solutions of the same constitution at 48 hr.

seawater, with increased NaCl content, and with sugar additions to seawater were done in collaboration with Margaret McCully, whose help on the early experiments with sucrose is acknowledged.

In Table 1 are presented in summary form the results of experiments showing the effects of sugar concentration in seawater on embryo polarization. Embryos were transferred to the experimental conditions 1 hr after fertilization and were cultured in 10 ml of medium in 5-cm glass petri plates at 15 C in continuous low-intensity unilateral white fluorescent light. Random counts of two dishes from each treatment were made at 48 and 96 hr of continuous exposure to the treatment solutions after fertilization.

In the seawater controls all embryos showed polarized development at 48 hr and continued to develop thereafter in a polarized fashion as already described. In all the sugar solutions tested at 0.4 M or higher some embryos developed which were apolar. In 0.6 M sucrose in seawater 97% of the embryos showed apolar development at 48 hr (Fig. 13). In 0.6 M mannitol only 1% of the embryos were polarized. Thus, these high

Fig. 2–12. Embryos of *Fucus* cultured in continuous light at 15 C for various periods of time after fertilization, fixed in 10% formalin-seawater, treated with sodium carbonate solution, squashed and stained with acetocarmine. All $\times 180$ except Fig. 12, $\times 245$.—Fig. 2. Embryos in seawater 12 hr.—Fig. 3. Embryos in seawater 24 hr.—Fig. 4. Embryos in seawater 36 hr.—Fig. 5. Embryos in seawater 48 hr.—Fig. 6. Embryos in seawater 72 hr.—Fig. 7. Embryos in seawater 96 hr. Note single rhizoids.—Fig. 8. Embryos in 0.6 M sucrose-seawater 36 hr.—Fig. 9. Embryos in 0.6 M sucrose-seawater 48 hr.—Fig. 10. Embryos in 0.6 M sucrose-seawater 4 days; then transferred to seawater 2 days. Note multiple rhizoids.—Fig. 11. Embryos in 0.6 M sucrose-seawater 4 days; then transferred to seawater 4 days.—Fig. 12. *Fucus* embryos in seawater 4 days, then fixed and squashed, showing the maceration possible with the sodium carbonate treatment.

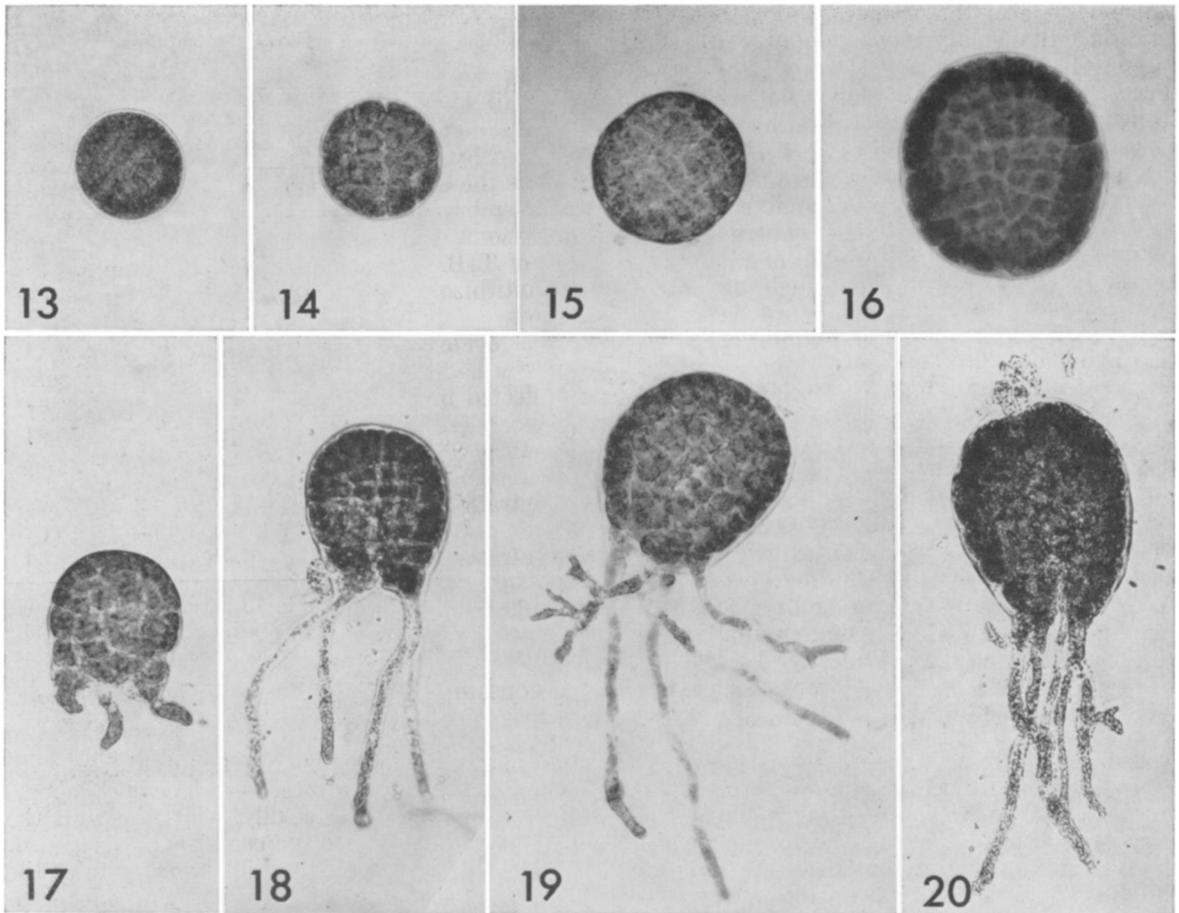


Fig. 13-20. Whole mounts of embryos of *Fucus* fixed in 10% formalin-seawater after culture for various periods of time in 0.6 M sucrose-seawater. All $\times 200$.—Fig. 13. Embryo in 0.6 M sucrose-seawater for 2 days.—Fig. 14. Embryo in 0.6 M sucrose-seawater for 4 days.—Fig. 15. Embryo in 0.6 M sucrose-seawater for 6 days.—Fig. 16. Embryo in 0.6 M sucrose-seawater for 10 days.—Fig. 17. Embryo in 0.6 M sucrose-seawater for 4 days; then transferred to seawater for 2 days.—Fig. 18. Embryo in 0.6 M sucrose-seawater for 4 days; then transferred to seawater for 4 days.—Fig. 19. Embryo in 0.6 M sucrose-seawater for 4 days; then transferred to seawater for 6 days.—Fig. 20. Embryo treated as in Fig. 19. Note beginning of apical hair formation.

concentrations of sugars added to the seawater dramatically changed the developmental pattern, almost completely suppressing rhizoid formation.

With time, an increasing proportion of the population recovered from the osmotic inhibition of rhizoid formation. Thus, in 0.6 M sucrose in seawater only 37% of the embryos were still apolar after 4 days. These embryos were enlarged, multicellular spheres (Fig. 14). If maintained in high-sucrose seawater a proportion of the embryos continued to grow as spherical embryos, showing progressive increase in cell number and in total volume but showing no polarization and no rhizoid initiation (Fig. 15, 16). Similar responses were observed in treatments of embryos with other sugars at the same concentration. In the high concentration of mannitol, what were apparently toxic effects interfered with embryo development; hence this reagent was not useful for further experiments.

Since mannitol is metabolized by *Fucus* (Bidwell and Ghosh, 1962), its uptake at these high concentrations may have "swamped" normal metabolic processes, causing inhibition and toxicity.

For subsequent experiments, sucrose at 0.6 M in seawater was selected. At this concentration sucrose delays cell division. In Fig. 1 is shown the time course of an experiment in which embryos were cultured in 0.6 M sucrose in seawater for 4 days and then were transferred to fresh seawater alone. During the first 36 hr in sucrose-seawater no cell division was seen (Fig. 8). Mitosis occurred at about 48 hr (Fig. 9). By 4 days the mean nuclear number was 11.2 as compared to 42 for seawater controls.

Release of the sucrose-induced inhibition by transfer of the embryos to seawater led to a rapid restoration of the normal rate of cell division as is apparent in the curve in Fig. 1.

Thus the sucrose treatment had no residual deleterious effects. The most striking effect of sucrose treatment was that when normal cell division rates were resumed, the apolar embryos showed polarization and multiple rhizoid initiation on the side of the embryo away from light. After 2 days in seawater two or more rhizoids were evident, each developing from a separate peripheral cell of the multicellular sphere (Fig. 10, 17). Usually, these rhizoids arose from adjacent or neighboring cells, within one quadrant of the embryo. As many as seven or eight separate rhizoids were observed in some embryos arising from seven or eight separate peripheral cells of the spherical embryo (Fig. 11, 19, 20). Thus the stimulus to rhizoid initiation, which in the unicellular zygote is normally distributed to one of the two cells formed at the first division, was distributed during the period of sucrose treatment among several or many cells of the embryo. Only later was rhizoid initiation expressed. Multiple rhizoids originated and developed simultaneously, forming a complex embryo (Fig. 11, 17-20). Hair formation followed approximately its normal time course and typically occurred at the normal site 180° removed from the position of the rhizoids (Fig. 20).

Attempts to initiate multipolar embryos by hormone treatments—Davidson (1950) reported that embryos of *Fucus* cultured in seawater containing different concentrations of auxins such as indoleacetic acid, indolebutyric acid or naphthalene acetic acid ranging from 10^{-6} to 10^{-10} M, were stimulated to form many rhizoids on each embryo. No mention was made of an apolar stage in this development; however, only one observation was made which was at the end of the experiment 40 days after fertilization. As auxins were invoked by Davidson as probably controlling rhizoid initiation, we were interested to test these observations.

Fertilized *Fucus* eggs were transferred after filtration and washing into solutions of auxins prepared in seawater in 5-cm petri plates. Several hundred embryos per dish were set up in triplicate, each dish containing 10 ml of medium. Tests were made of 2,4-dichlorophenoxyacetic acid (2,4-D) at 10^{-5} and 10^{-6} M; of indole-3-acetic acid (IAA) at 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} and 10^{-9} M, and 2,3,5-triiodobenzoic acid (TIBA) at 0.1, 0.5, 1, 5, 10, 50 mg/liter (2×10^{-7} M to 10^{-4} M). Embryos were grown in continuous unilateral light at 15 C. In the experiment with IAA, a duplicate set was run in total darkness at 15 C.

In the experiments with 2,4-D and IAA the embryos all formed single rhizoids and were developmentally indistinguishable from the seawater controls. There was no indication over a period of 12 days that auxin treatment had influenced the number of rhizoids formed.

In the highest TIBA concentrations (10^{-4} and 5×10^{-5} M in seawater) embryos with multiple rhizoids were formed in good numbers, i.e., about 40 and 30%, respectively, after 6 days (Table 2). These numbers were lower than those produced by the sucrose treatment, but in other respects the embryos were comparable. At 2 days apolar embryos were observed; by 6 days up to five rhizoids per embryo were observed in some cases of TIBA treatment, although the majority of multirhizoid embryos formed two or three rhizoids.

It is evident that TIBA at a concentration of 10^{-4} M had an inhibitory effect on cell division as reflected in the average number of nuclei per embryo. Here again the effect was less marked than that caused by 0.6 M sucrose in seawater. The striking fact is that with relatively low concentrations of TIBA (10^{-4} M) we were able to reproduce a response elicited by very high concentrations (0.6 M) of another substance which presumably acts by osmotic means rather than by influencing events at metabolic or hormonal levels.

DISCUSSION—Normal development of *Fucus* embryos proceeds with remarkable precision. Under controlled environmental conditions, even 6-day-old embryos show considerable synchrony in their changing morphology and anatomy. Up

TABLE 2. The effect of 2,3,5-triiodobenzoic acid (TIBA) on increase in average nuclear number per embryo and the initiation of multiple rhizoids in *Fucus* embryos

Treatment ^a	Hr after fertilization			
	24	48	96 ^b	144
<i>Average number of nuclei per embryo^c</i>				
Seawater control	1.7	6.6	33.8	92.5
0.6 M sucrose in seawater	1.0	1.9	11.2	58.1
TIBA at 10^{-4} M in seawater	1.0	3.6	18.8	70.6
<i>Percent of total number of embryos with multiple rhizoids^d</i>				
Seawater control	—	—	4%	4%
0.6 M sucrose in seawater	—	—	60%	62%
			(apolar)	
TIBA at 10^{-4} M in seawater	—	—	38%	42%
TIBA at $5\frac{1}{2} \times 10^{-5}$ M in seawater	—	—	18%	29%

^a Embryos were cultured at 15 C in continuous unilateral white fluorescent light.

^b All embryos were transferred after 96 hr to fresh filtered seawater.

^c Nuclear counts are based on 25 embryos for each treatment time.

^d Percents forming multiple rhizoids are based on random counts of > 200 embryos for each treatment time.

until 4 days, nuclear counts in any given population show only slight variability. Thus, embryonic development in *Fucus* is an excellent system for quantitative studies. The embryo also has a plasticity in its developmental pattern which allows experimental manipulation.

Inhibition of protuberance formation and subsequent rhizoid formation represent a dramatic upset in the normal well-ordered developmental program. The spherical embryos which form are reminiscent of globular-stage embryos in some dicotyledonous plants. Osmotic inhibition of rhizoid formation is non-specific, since effective inhibition can be achieved with a number of different osmotic agents. Even seawater concentrated above its usual osmotic pressure prevents rhizoid formation. Sucrose appears to be particularly useful for experimental work since it has no deleterious aftereffects. Tulecke (1957) reported that high sucrose concentrations caused *Ginkgo* male gametophytes to develop as spherical structures. A similar effect of sucrose treatment was reported by Valanne (1966) in apolar embryo production in mosses. These effects of sucrose are in some respects reminiscent of the effects of high sucrose concentration on isolated embryo cultures of *Capsella* described by Raghavan and Torrey (1963) in which osmotic conditions determined whether normal or abnormal embryonic development occurred.

Nakazawa (1959) has postulated the following sequence of events leading to rhizoid initiation: a localized area of increased surface energy develops in the cortical layer of the cell as the result of an external environmental stimulus such as unilateral light; an accumulation occurs locally of a surface-active agent and potential membrane component such as lecithin; this results in a localized enhancement of membrane permeability, a lowered surface tension, and then a localized bulging leading to rhizoid formation.

Protuberance formation probably results from a localized softening of the cell wall of the zygote and a distention caused by internal pressures. Presumably concentrated solutions outside the cell prevent protuberance formation either by preventing the softening process, i.e., inhibition of a chemical process, or by preventing the expression of the internal pressure, i.e., by a physical process. In the experiments reported here, both osmotic inhibition by concentrated sugar-seawater solutions and chemical inhibition by TIBA treatment resulted in apolar embryos. In the former case cell division was markedly inhibited; in the latter case only a slight delay in the rate of cell division was noted. It would seem that inhibition of cell division did not play a critical role in producing apolar embryos.

In all these experiments only a portion of the population responded to the treatment. With sucrose treatments a good proportion of the embryos recovered from initial inhibition. Pre-

sumably sucrose was absorbed and as the internal and external osmotic pressure became more nearly equalized, the localized softening became apparent through normal rhizoid formation. The difference in susceptibility of embryos to sucrose inhibition of embryo development is not understood. At present we have no information about the fate of the sucrose provided in the external solution.

The most striking and potentially most informative observation in these experiments is the fact that sucrose-inhibited apolar embryos formed multiple rhizoids when returned to seawater. Rhizoid initiation occurred among a number of peripheral cells of these multicellular spherical embryos so that several rhizoids developed. This response means that the information on how to form rhizoid cells had been distributed to a whole series of cells in a portion of the multicellular embryo. This response is no longer the definitive unequal or asymmetrical cell division determining cell fates which one observes in the first division in normal *Fucus* embryogenesis. Rather, several to many cells on one side of the multicellular embryo are informed by the environmental stimulus of unilateral illumination to divide in such a way that each will form a rhizoid. Thus the rhizoid-stimulus has become divided among a number of cells.

In accounting for the polarization mechanism which results in rhizoid initiation, one must now take into account this phenomenon that the rhizoid-initiation stimulus can be subdivided and expressed in a number of cells of the multicellular embryo. Careful analyses of the timing of multiple rhizoid orientation and initiation in sucrose-inhibited embryos may make it possible to clarify the nature of these rhizoid-forming differences.

That auxin of the IAA-type is the chemical basis for rhizoid initiation seems unlikely from the experiments described here. No multirhizoid embryos were formed in any of the experiments with exogenously supplied IAA or 2,4-D. One can surmise that Davidson (1950) probably observed multirhizoid formation in *Fucus* embryos arising from osmotic effects of evaporating seawater solutions in his small-volume, long-term experiments.

Yet one can elicit multirhizoid formation by treatment with TIBA at near-hormonal levels; these concentrations are equivalent to those effective in many higher plant experiments. How TIBA acts in *Fucus* is not known. It is not reasonable to interpret these results in terms of blocking auxin transport between cells, as has been shown in higher plants (Keitt and Baker, 1966). However, TIBA has been reported to act directly on individual cells in some cases as, for example, on the growth of root hairs (Gorter, 1949), where the mechanism remains unknown. Perhaps the TIBA is acting in *Fucus* as a sulf-

hydriyl-inhibiting agent (Leopold and Price, 1956). That one can effect the formation of multiple rhizoids by such chemical treatment encourages one to believe that a direct attack on the nature of the chemical stimulus initiating rhizoids in *Fucus* embryos should be possible.

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