THE EFFECT OF HYDROGEN ION CONCENTRATION UPON THE INDUCTION OF POLARITY IN FUCUS EGGS

II. THE EFFECT OF DIFFUSION GRADIENTS BROUGHT ABOUT BY EGGS IN CAPILLARY TUBES*

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INTRODUCTION

It has been known for a long time (Rosenvinge, Kniep, Hurd, and Whitaker (1-4)) that *Fucus* eggs in the dark tend to form rhizoids in the resultant direction of neighboring eggs. It was shown by Whitaker (4) that unfertilized eggs of another species act as directing agencies, and therefore the effect is non-specific and is not dependent upon growth activity in the directing cells.

More recently (Whitaker (5)) it has been shown that in *F. furcatus*, when eggs are reared in the dark at 14°C in normal sea water at pH 7.8-8.0, aggregations of at least ten or more eggs in close proximity are necessary in order to bring about appreciable mutual control of the polarity. Groups of five eggs or two eggs alone in a dish do not show the effect. These relations suggested that a greater concentration of substances diffusing from the eggs than can be provided by the smaller groups of eggs under conditions of free diffusion is necessary to cause the eggs to respond to each other. Since CO₂, and presumably other acid metabolites are included among the diffusion products, aggregations of various sizes were reared in sea water acidified to pH 6.0. In this medium, all aggregations, including groups of only two eggs alone in a dish, now showed strong mutual inductions of polarity. It was concluded that increased hydrogen ion concentration augments or intensifies the mutual inductive effect (5).

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HYDROGEN IONS AND POLARITY IN FUCUS

Purpose

In the present experiments, single eggs have been reared in the dark within and near one end of a capillary tube so that diffusion products escape more rapidly from one side of the egg than from the other. Also, due to the presence of the tube wall, which limits diffusion, the equilibrium concentration of diffusion products (and also oxygen lack) immediately about the egg is presumably considerably greater than about an egg or a small group of eggs under conditions of unrestrained diffusion. The purpose of the experiments has been to see whether the polarity of an egg under such circumstances might be determined by the gradients resulting from its own metabolism, and to find the effect of initial pH of the medium on the results. Another and original purpose was to test the feasibility of culturing single eggs in capillaries in order to apply gradients of known substances across them, but it is first necessary to know the response of an egg in a capillary to the gradients which the egg itself produces when the medium is initially homogeneous throughout.

Method

Eggs of the hermaphroditic Fucus furcatus f. luxurians were obtained from the same locality and by methods which have been previously described (Whitaker (5-6)). The eggs were in the dark from before fertilization until the end of the experiments except for brief exposure to red light, which does not affect the polarity of Fucus eggs. All work was carried out in a constant temperature room at 15 ± 1°C. No eggs were used which did not appear to be spherical at the start.

The first experiments were carried out in November and December, 1934. A large number of eggs were reared, each in a glass capillary 5 to 100 egg diameters long selected so that the egg fitted with some clearance and was not compressed or elongated.1 The eggs were gently located near one end of the capillary tube, at various distances from the end. The medium was filtered sea water. The results of consecutive experiments were often consistent, but these were interspersed with inconsistent series. While most of the eggs formed rhizoids on the sides of the eggs toward the far ends of the capillaries, it became clear that some variable was not being recognized, and therefore further experiments were postponed at that time.

1 When eggs fit tightly in the capillary and especially if they are considerably elongated, the first cell division does not give rise to a rhizoid cell, but to two more or less equal cells. The first cell plate forms diagonally with respect to the shortened and lengthened diameters of the cell, as if attempting to be about as long as it is in an uncompressed cell.
After the discovery of the striking effect of the pH of the medium on the intensity of the neighbor effect (see Introduction), it appeared quite possible that the response of an egg in a tube to the gradient of its own diffusion products might also be highly sensitive to the pH of the sea water. Subsequent measurements with a glass electrode showed that the pH of sea water collected and filtered as it had been during these earlier experiments ranged over a considerable time from 8.3 to 7.6. More attention to prompt filtering very soon after collecting lessens this range. A new series of experiments was carried out in January, February, and March, 1936, with measurement and artificial control of the pH of the sea water. These new experiments are the basis of the following report.

Making and Preparing the Capillary Tubes.—Clean new pyrex tubing was drawn out to form capillaries which were selected for internal diameter as measured by ocular micrometer. By means of a Taylor micromanipulator, pieces of suitable bore were cut quite squarely into measured lengths between a diamond splint and the edge of a razor blade. The capillary lengths used were 400, 700, and 1400 or 1500 microns. As the eggs vary in diameter from about 65 to 90 microns, averaging about 75, these lengths correspond approximately, on the average, to 5.5, 10, and 20 egg diameters. Large numbers of capillaries were cut, and by selecting eggs and capillaries of appropriate diameters, any degree of clearance between the egg and the capillary could be obtained. Similar capillaries were also made from clear fused quartz (silica).

To reduce dangers of contamination of various sorts, capillaries were never used a second time. The new tubes were placed one each in a small Syracuse dish (Bureau of Plant Industry model) in 1 cc. sea water. A fine glass pipette which was held in the hand and operated by a rubber tube to the mouth was placed against one end of the short capillary on the bottom of the dish and a large volume of medium was sucked through to flush it. After being flushed and then soaked overnight (usually 24 hours) in sea water, each capillary was transferred through three dishes of the medium to be used in the experiment and was copiously flushed in each to remove any solutes from the glass walls of the capillary and to equilibrate with the experimental medium.

Placing the Eggs.—Eggs were fertilized in normal sea water and then between 30 and 90 minutes after fertilization (during which time the cell wall progressively becomes more adhesive) a single fertilized egg was placed in each small Syracuse dish of experimental medium containing a prepared capillary. Eight of these dishes had already been placed in a levelled Petri dish on the stage of a microscope. The Petri dish also contained sea water and served as a moist chamber. Several microscopes, each bearing such a levelled Petri dish, were mounted on a heavy concrete table in the constant temperature room as it was found that either vibration or slight slope of the capillaries would cause the eggs to move along the capillaries or to roll out. The Petri dish was not moved from the stage of the microscope after the eggs were placed.

Under the compound microscope an egg was washed and placed near one end of the capillary and medium was gently sucked through the capillary from the
other end by means of the mouth pipette. This carried the egg into the capillary without abuse or distortion and the distance from the end of the capillary at which the egg came to rest could be accurately regulated. If the egg came too far into the capillary it was rolled back by gently blowing medium into the opposite end of the tube. The stickiness of the egg at this time reduced the tendency to move. By about 2 hours after fertilization the sticky wall has hardened and attaches the egg to the glass. After an egg was placed in each of the eight capillaries in a moist chamber, the dishes containing the capillaries were rotated so that the capillaries lay in different positions with respect to the compass as a control against any unknown directional influence in the environment. The compass direction of the capillary had no effect on the result. The results were observed and either drawn or photographed about 24 hours after fertilization when the rhizoid protuberance was well developed and the spore had usually developed to the two cell stage.

The Experimental Medium.—The medium inside and outside the capillary was always the same at the start of the experiment. Eggs were reared in normal sea water of measured pH, ranging in these experiments from 8.09 to 7.85 (see Table I). In the same experiment, eggs were also reared in samples of the same sea water which had been artificially made more acid or more basic. In some cases the sea water was acidified by adding from 3 to 5 volumes to the hundred of acid McIlvaine's secondary sodium phosphate-citric acid buffer. In most cases concentrated HCL instead was added to the sea water in such small amount as to have no appreciable effect on the total osmotic pressure. Sea water was artificially made more basic by adding from 1 to 5 volumes to the hundred of a basic isosmotic mixture of Na2CO3 and NaHCO3.

After the addition of the HCl, or the Na2CO3-NaHCO3 mixture, the sea water was vigorously aerated by means of a large sintered glass nozzle which produced tremendous clouds of minute air bubbles in order to re-equilibrate with atmospheric CO2. The acid or base was added until the desired pH was attained, with aeration after each addition, until successive measurements with a glass electrode gave approximately constant pH readings. The pH values obtained in this way are shown in column 6 of Table I, and have been designated "pH at end of adjustment period." Experiments 1 to 3 inclusive were started in this medium soon afterwards, but in Experiments 4 to 10 inclusive, after adjustment of the pH, the medium stood for 12 to 40 hours (usually 18 to 24 hours), before the beginning of an experiment. The measured pH at the beginning of an experiment, when compared with that at the end of the adjustment period, gave further measure of equilibration and the stability of the pH. In Experiments 5, 9, and 10 the sea water to which HCl had been added was aerated overnight through sintered glass after the adjustment period, and in Experiment 10 the sea water to which Na2CO3-NaHCO3 was added was treated in similar fashion. The data in Table I show that in the other cases in Experiments 4 to 10 inclusive, in which no final aeration took place after the period of adjustment, the pH is in general about equally constant.

At about 24 hours after the start of the experiments the pH of the experimental
medium in the small dishes was measured. The changes which had occurred during the progress of the experiments are shown in column 8 of Table I.

RESULTS

In more than 600 cases studied, the rhizoid protuberance formed either on the side of the egg toward the far end of the capillary, into which it extended, or else it formed on the opposite side of the egg i.e., on the side toward the near end of the capillary, in which case it extended toward or out of this end of the capillary. Photomicrographs in Fig. 1 show eggs in each category located at several different distances from the end of the capillary. Table I gives the conditions of the experiments and the number of eggs in each experiment. The percentage of eggs in the different media of each experiment which formed the rhizoid protuberance on the side of the egg toward the far end of the capillary i.e., on the side of the egg from which diffusible substances from the egg could diffuse away least readily, is given in column 10 of Table I.

These percentages bear a very definite relation to the initial pH of the medium (i.e., the pH at the start of the experiments, as shown in Table I, column 7), which remained essentially unaltered outside the capillary during the course of the experiments. The percentages are plotted against initial pH in Fig. 3, which shows that in acid sea water a very high percentage of the rhizoid protuberances form toward the far end of the capillary. Near pH 8.0, however, the percentage drops rapidly to 50, which represents a condition of random direction, and at higher pH values up to 8.5 or 8.6, the percentage drops down to less than 25. In other words, in basic sea water most of the rhizoid protuberances form on the opposite side of the egg, toward the near end of the capillary. As the sea water is made more basic, the percentage of rhizoid protuberances forming toward the near end of the tube does not approach 100, however, as in the reverse phenomenon in acid sea water, but attains only 75 or 80 at the maximum.

In order to find out if this percentage would more nearly approach 100 under still more basic conditions, 59 eggs were tested at pH 9.1–9.2. A complication comes in, however, since above about pH 8.6 precipitation of certain salts from the sea water takes place and it is not known what effect this may have on the development of polarity.
FIG. 1. Photomicrographs of typical examples of *Fucus* eggs growing at several
distances from the ends in capillaries 1.4 to 1.5 mm. long. (A) Rhizoids on sides
of eggs toward far ends of capillaries (typical result in acid sea water). The
initial pH in the cases shown (from top to bottom) was 7.4, 6.4, 7.9. (B) Rhizoids
toward near ends of capillaries. Initial pH (from top to bottom) was 8.4, 8.6, 8.4. See text.

FIG. 2. Photomicrographs of typical *Fucus* eggs reared in sea water of different
pH. The eggs shown in A, B, and C, have all been fertilized within 15 minutes of
26 hours (15°C., dark). The pH of the sea water in the three cases was respectively 7.0, 7.9, and 9.1. The eggs in C show the typical altered type of rhizoid,
with the narrow base, which forms at high pH. See text.
Fig. 3. Graph showing percentage of eggs which formed rhizoids on the sides toward the far ends of the tubes (where diffusion is most impaired) when the pH of the medium initially (i.e., when the eggs were placed in the tubes at the beginning of the experiments) was as shown. The curves are freely drawn. In the more basic range (curves intermittent) special considerations make the results less certain (see text). The response of the eggs to their own diffusion gradients is seen to be somewhat less pronounced in the shorter tubes, as might be expected. The significant data upon which each point is based are shown in Table I.
TABLE I

Conditions of the experiments, and results upon which Fig. 3 is based. The columns, left to right, show (1) number of the experiment; (2) capillary tube lengths to nearest 100 μ (tubes are pyrex in Experiments 1 to 8 inclusive, and quartz in Experiments 9 and 10); (3) clearance between egg in tube and tube wall (range and average); (4) distance from near end of tube to nearest edge of egg (range and average); (5) composition of medium, which was either unaltered sea water, or sea water to which acid or base was added (see text); (6) pH of medium at end of adjustment period (see text); (7) pH at start of experiment, when eggs were placed in tubes; (8) change in pH of medium surrounding tubes as measured 24 hours later; (9) number of eggs, each in a separate tube and dish; (10) per cent of these which formed rhizoids on sides toward far ends of tubes. See text.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Tube length in μ</th>
<th>Clearance between egg and tube in μ</th>
<th>Distance: egg to near end of tube in μ</th>
<th>Medium</th>
<th>pH at end of adjustment period</th>
<th>pH at start of experiment</th>
<th>pH change in 24 hr in medium</th>
<th>No. of eggs</th>
<th>Rhizoids toward far end of tube</th>
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<td>1</td>
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<td>750-241</td>
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<td>7.85</td>
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<td>400-111</td>
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in the egg. There is a marked difference in the type of the rhizoid protuberance which forms at such high pH (Fig. 2). It is decidedly narrower at the base and its extension is retarded. There is no indication whether this is a result of the high pH as such or whether it is due to the loss of precipitated salts from the sea water. At this high pH the eggs in the capillaries show a reduction of the tendency for rhizoid protuberances to form toward the near end. The percentage drifts back toward 50, or randomness of the results. The number of experiments is less in the extreme basic range, and the special circumstances of precipitation and altered developmental type combine to make this apparent reduction in the control of polarity difficult to interpret at the present time. The results here must be regarded as merely exploratory and tentative. However, it does appear relatively safe to conclude that as the pH is increased from 8.5 to 9.2 the percentage of the protuberances forming toward the far end of the capillary does not approach 0. The effect of the base in controlling the response of the egg does not become almost absolute, as in the case of the acid.

In addition to the experiments recorded in Table I and Fig. 3, 50 eggs were reared in four experiments which were similar to the others except that the glass capillaries were only 400 microns long. This is only slightly over 5 egg diameters, and eggs which were 1 egg diameter or more in from the end of the capillary were therefore fairly close to the center of the capillary. In consequence, the difference in diffusion from the two sides of the egg was less than in the case of longer capillaries. The results were very similar to those obtained in the longer capillaries, but as might be expected, the determinative effects on the eggs were somewhat less marked. In Table I and Fig. 3 it may also be seen that the response of the eggs in acid sea water to the gradients in the capillaries was not quite as strong in capillaries 700 microns long as in capillaries 1400 or 1500 microns long.

DISCUSSION

When a single Fucus egg develops in a gradient of its own diffusion products in a medium which is initially homogeneous, the developmental response of the egg to the gradient appears to be greatly affected by the hydrogen ion concentration of the medium. It has
already been shown (Whitaker (5)) that when two eggs develop near each other, the rhizoid protuberances develop on the side toward the neighbor if the sea water is acidified, while in normal sea water they do not. The present results are quite in agreement with this observation, and a reasonable interpretation is that upon sensitization with a slight amount of acid the rhizoid protuberance develops on the side of the egg subjected to the greatest concentration of diffusion products of the egg itself for the same reason that it develops on the side toward a neighboring egg.

In the case of the egg in a capillary, a lesser acidification of the medium suffices to sensitize the egg, so much less that sea water as collected is often sufficiently acid without alteration. This is not surprising, because a consideration of diffusion areas shows that an egg in the end of a capillary is subjected to a greater concentration of diffusion products of the egg (including CO₂) than are two eggs in a dish under conditions of unrestricted diffusion. The developing Fucus egg has a relatively high respiratory rate in the dark. It is of the order of 5 times that of a fertilized sea urchin egg (Whitaker (7-8)). The single egg in the capillary therefore undoubtedly increases acidity about itself very considerably, particularly on the side with more restricted diffusion.

The egg near one end in the capillary is developing in a gradient of CO₂ and H ions as well as of other diffusion products of the egg, and of O₂ tension. The present experiments do not indicate what the substance or substances are which determine the polarity of the egg. But since a gradient of H ions in slightly acid sea water causes the rhizoid to form on the more acid side (Whitaker (9)), this gradient in the capillary may be the one which is effective. If so, it is not now possible to say whether such an effect is specifically due to a gradient of CO₂ or whether it is due to a gradient of H ions, since the two go together in a carbonate buffer system such as sea water.

The failure of the region of highest concentration of diffusion products to induce the formation of a rhizoid protuberance when the medium is more basic is comparable to the failure of two eggs in a dish to form rhizoids toward each other when the medium is not acidified. The actual reversal above pH 8.0, with more than half of the rhizoids forming on the side with the least concentration of diffusible products.
can be given only as an observation. Extended discussion of this phenomenon would be premature at the present time. It might be noted, however, that although the relation was not stressed in a previous paper, the data in Table I (Whitaker (5)) show that two eggs alone in a dish in the most basic medium used (pH 7.8–8.0) showed a statistical tendency to form rhizoids on the sides of the eggs away from the neighbor.

The variable results obtained in capillaries in 1934 (see page 58) can be explained adequately by the variations of pH of the different samples of sea water as it was collected at that time (see Fig. 3).

In the case of the single egg in the capillary, responding to its own diffusion gradient, in contrast to the aggregations of two or more eggs affecting each other, there appears to be no possibility that the effect is mitogenetic.

**Errors and Alternative Interpretations.**—So far in the discussion the view has been favored that the polarity of the egg is determined, depending on the hydrogen ion concentration of the medium, by a gradient of products diffusing from the egg. Two or three other possibilities must be considered. In the first place, substances coming into solution from the pyrex capillaries would be present in a concentration gradient across the egg. It was with this in mind that only new, clean, soaked pyrex was used and that capillaries of pure fused silica (quartz) were substituted in Experiments 9 and 10, with essentially similar results. If there is any such effect from glass, or an effective differential adsorption from the medium, it also takes place in quartz. It does not appear to the authors very probable that the principal results are due to such factors.

Since the eggs in the dark consume oxygen at a relatively high rate (Whitaker (7–8)) a gradient of oxygen tension must be present across the egg in the capillary, in the opposite direction to the gradient of products diffusing from the egg. A gradient of oxygen tension has never been established across *Fucus* eggs to test its effect. Winkler (10) found that an oxygen gradient across the eggs of *Cystosira*, another member of the Fucaceae, had no effect on the polarity in that form. It has already been noted that a gradient of hydrogen ions and CO₂ does determine the polarity of the *Fucus* egg, at least in slightly acidified sea water, and this, together with the pronounced
sensitivity of the phenomenon to pH and the general physiological effectiveness of hydrogen ions and CO₂, suggests the greater importance of these substances in the present instance. The rôle of the oxygen gradient has not been determined, however, and it may be an important part of the picture. In any event, it is not supposed that only one substance or its reactions determines polarity. It is much more probable that a number of substances are involved, any one of which might become limiting. The participation of some type of growth substance inside the cell, if not diffusing from it, appears quite probable on general grounds.

When acid is added to sea water, CO₂ is produced, and when base is added, CO₂ is absorbed from the air. Until equilibration with the atmosphere has taken place, CO₂ will continue to be given to or taken from the air for a long time, with a resulting drift of the pH of the sea water. If acidified sea water contained CO₂ considerably in excess of atmospheric tension at the start of an experiment in a capillary, further diffusion of excess CO₂ out into the air would proceed more rapidly from the medium outside the capillary than from the medium in the capillary, from which diffusion is partly restricted. This would result in a gradient of CO₂ across the egg, and would correlate with a rising pH of the medium outside the capillary. Conversely, alkalinized sea water not in equilibrium with atmospheric CO₂ would produce a gradient of CO₂ across the egg in the opposite direction. Since such artificial gradients would be in the right direction to explain the results, their magnitude must be considered. The best measure is the drift of the pH of the medium exposed to air.

A comparison of columns 7 and 6 in Table I shows that in 15 of the 18 measurements in Experiments 4 to 10 inclusive, in which the media stood for 13 to 40 hours after adjustment of the pH and before the beginning of an experiment, the change in pH as measured was not more than 0.02 unit. In two cases it was 0.13 unit. All changes which took place were slightly in the acid direction, as might be expected from the action of microorganisms. If acidified sea water had been giving off CO₂, it would have become more basic. Column 8 shows the changes in pH of the media surrounding the capillaries in 24 hours after the start of the experiments. Since the place of rhizoid origin is determined in about the first half of this time, only
about half of the changes shown in column 8 took place during the significant period. Column 8 shows that the changes in pH are very slight in most of the experiments, and ordinarily the drift of the pH of the media to which acid or base had been added was no greater than that of the normal sea water. In all of these, the slight drifts are predominantly in the acid direction, even in the acidified sea water. The pH values were measured to 0.01 unit, which did not exceed the limit of the glass electrode. Such highly refined measurement is not regarded as fully significant, but the values are recorded as measured because the significance at least approaches such limits.

In view of the comparative constancy of the pH of the media in most of the experiments, it appears very probable that artificial CO2 gradients resulting from delayed equilibrations with the atmosphere after addition of acid or base must have been small compared with the gradients of catabolic CO2 established by these rapidly respiring eggs.

SUMMARY

1. When a Fucus egg develops near one end in a close fitting capillary tube of pyrex glass or silica (quartz), diffusion of substances passing to and from the egg is more impeded on the side of the egg toward the far end of the tube.

2. The egg therefore develops in a gradient of its own diffusion products, and of oxygen tension.

3. More than 600 eggs have been reared, each near one end in a capillary, in sea water at various regulated and measured pH values.

4. When the medium, which is initially homogeneous inside and outside the capillary, is initially at pH 6.5 to 7.6, nearly all of the eggs develop rhizoid protuberances on the sides of the eggs toward the far ends of the capillaries. This is on the sides of the eggs where the concentration of substances diffusing from the eggs is greatest.

5. The polarity and developmental pattern of the egg is thus determined either by a concentration gradient of products diffusing from it, or by a gradient of oxygen tension. The former interpretation is favored.

6. This is regarded as an extension of earlier observations that rhizoid protuberances form on the sides of two neighboring eggs in the direction of the neighbor if the sea water is acidified.
7. It appears hardly possible that a mitogenetic effect could be responsible for the response of an egg to its own diffusion gradients.

8. When the medium is made more basic, the percentage of the eggs which form rhizoid protuberances toward the far end of the tube decreases to about 20 or 25 per cent between pH 8.1 and 8.6. The response of the egg to the gradients which it produces is thus statistically reversed. The determination of the polarity of the eggs by the diffusion gradients does not become as complete in alkalinized as in acidified sea water.

9. When the pH of the sea water is elevated to 9.1 or 9.2, salts precipitate out. The type of development is altered and the control of the diffusion gradients over the polarity of the eggs decreases.

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