THE EFFECT OF pH ON THE DEVELOPMENT OF ULTRA-CENTRIFUGED FUCUS EGGS

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It has been shown (Whitaker4) that the developmental polarity of fertilized eggs of *Fucus furcatus* is determined by centrifuging at 3000 x g. for 15 or 20 minutes. When the stratified eggs are reared in the dark in normal sea water (pH 7.9–8.0), more than 99% form rhizoids on the centrifugal halves of the eggs, and 93–99% do so within 10° of the centrifugal pole.

Beams3 ultra-centrifuged fertilized eggs of *Fucus serratus* at 150,000 x g. for half an hour and found the polarity to be unaffected by stratification of the visible cell inclusions. It is possible but not probable that the difference lies in the two species of *Fucus*. It also appeared possible that the effects of centrifuging at 3000 x g. might be lost at 150,000 x g. Might some substance or structure be moved at 3000 x g. but be broken down at 150,000 x g.? Thousands of *F. furcatus* eggs were ultra-centrifuged in quartz centrifuge tubes at various forces including 150,000 x g. and 200,000 x g. for various durations, including 5 minutes and half an hour. The eggs were grown in normal sea water (pH 7.8–8.1) in the dark in a constant temperature room at 15°C. in thin cultures to avoid group effect (Whitaker4). Invariably 99% formed rhizoids on the centrifugal halves of the eggs, about 90% or more doing so within 10° of the centrifugal pole. The results are therefore essentially the same at 150,000 x g. and at 3000 x g., except that at the higher force 5 minutes of centrifuging is more than adequate to sharply stratify the eggs and determine polarity.

A number of environmental factors affect the determination of polarity in the *Fucus* egg.2,4 When ultra-centrifuged eggs of *F. furcatus* are illuminated from one side during development, the polarity is affected by the direction of the light as well as by the stratification4 so that in a population rhizoids are observed in all positions with respect to the stratification. The polarity of ultra-centrifuged eggs is also altered by the group effect, if
neighboring eggs are close together, especially if the sea water is somewhat acid. It will be shown below that the response of an isolated egg to its internal stratification depends on the pH of the medium. Beams does not indicate the conditions of his experiments with respect to these factors, and one or a combination of these factors would be adequate to explain why he observed rhizoids in all positions with respect to stratification.

The principal purpose of this communication is to present the results of experiments designed to test the effect of the pH of the external medium on the polarity of centrifuged *Fucus* eggs. A more detailed report will be published elsewhere. Eggs were fertilized and centrifuged in filtered normal sea water. They were centrifuged for 5 minutes at 150,000 x g., beginning 10–20 minutes after fertilization, and were then grown individually in 1 cc. sea water in small individual syracuse dishes in a dark constant temperature room at 15°C. Three hundred and ninety-six eggs were grown in filtered normal sea water, initially at pH 7.9–8.1, and 339 eggs were grown in sea water acidified to pH 5.8–6.1 with McIlvaines' buffer. One hundred per cent of the eggs reared at pH 7.9–8.1 formed rhizoids on the centrifugal halves. Only 10% of the eggs reared at pH 5.8–6.1 formed rhizoids on the centrifugal halves and 90% formed rhizoids instead on the centripetal halves. A considerable number formed rhizoids at the centripetal pole. The developmental response of the egg to its internal stratification is thus reversed with change in external pH. Centrifuged eggs reared at pH 6.0 in mass cultures which were thin so that mutual influences were reduced, but not absent, also statistically showed the reversal, but to a lesser extent.

Only tentative interpretations can be suggested at the present time. It was pointed out earlier that the responses of the *Fucus* egg to a variety of agents could be interpreted on the basis of plant growth hormone (auxin) as one of the links in the reaction chain leading to rhizoid formation. The auxin interpretation is strengthened by the recent work of du Buy and Olson who report having extracted growth substance from *Fucus* eggs. Olson and du Buy also carried out experiments from which they conclude that the rhizoid forms on the side of the egg to which beta-indole acetic acid (hetero-auxin) or its potassium salt is applied in sufficiently high concentration. Auxins are active in the molecular form, and hydrogen ions convert dissociated auxin into molecular auxin. If auxin in the egg is concentrated at the centrifugal end as a result of being adsorbed or attached to larger particles which are moved by centrifuging, the behavior of centrifuged eggs at pH 8.0 can be understood. The reversed response at pH 6.0 could perhaps be explained on this basis by supposing that after acid activation the active auxin at the centrifugal pole is now present in such high concentration as to be inhibitory so that the rhizoid forms at a region of lesser auxin concentration, i.e., more centripetally. Another possibility is that
an amphoteric phenomenon is responsible for the reversal, either through its effect on auxin or through more direct effect on the underlying rhizoid forming processes. Auxin may be unmoved by the centrifuge and amphoteric substances (e.g., protein) concentrated at the centrifugal end may affect either the activity or transport of auxin in a manner which would reverse on either side of the isoelectric point of the amphoteric substances. Both internal pH and electrical gradients might be expected to reverse when the isoelectric point is crossed if amphoteric substances are concentrated at one end of the cell. Experiments are in progress which it is hoped may throw further light on these questions.

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5 As shown earlier in Cystosira with low centrifugal force by Knapp, E., Planta, 14, 731 (1931).

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CHROMOSOME NUMBERS IN NODULES AND ROOTS OF RED CLOVER, COMMON VETCH AND GARDEN PEA

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An observation of 28 chromosomes (4n) on somatic equatorial plates in the meristematic region of the nodule of red clover instead of the 14 chromosomes (2n) found in root tip cells suggested the present investigation. The number of chromosomes characteristic of nodular tissue has heretofore been reported in two instances. Milovidov2 found 42 chromosomes in cells of the cortex of the nodule of Lupinus mutabilis. Lechtova-Trnka3 observed approximately 16 chromosomes in nodular material of both Sophora Moorcroftiana and Robinia viscosa. The exact number was difficult to determine because of the large size and crowded condition of the chromosomes on the equatorial plate.

Nodules and root tips of red clover (Trifolium pratense L.), common vetch (Vicia angustifolia L.) and garden pea (Pisum sativum L.) were used in the present study. They were obtained from field-grown plants and from