It is interesting that, even when the mean thickness of the GBM in different conditions are very close, their frequency distribution curves are not always similar in shape and peak. These factors may cause a misleading impression on the thickness of the glomerular basement membrane when a conclusion is made from a small number of measurements. Therefore, it must be stressed that a frequency distribution curve of the thickness of the GBM based on large number of measurements is essential for the evaluation of the thickness.

Conclusion. Several factors responsible for the variation of the thickness of glomerular basement membrane of the rat kidney in various non-pathological circumstances are investigated, and following results are obtained.

(1) The mean thickness and the shape of frequency distribution curve in the glomerular basement membrane vary with condition of the kidney. (2) The expansion and the contraction of the capillary lumens can be one of the important factors responsible for the variation in the thickness in the non-pathological subject. (3) Even when the mean thickness of the glomerular basement membrane in different conditions are close, their frequency distribution curve are not always similar in shape and peak. (4) A compensatory hypertrophy may not produce gross change in width of the glomerular basement membrane in comparison with that of controls. (5) Affected (scarred) glomerular loops in the ischemic kidney show thick irregular basement membrane, whereas unaffected glomerular loops in such kidneys do not show thickening of the basement membrane.

Zusammenfassung. Mehrere Faktoren wurden untersucht, die verantwortlich sind für die Dickenvariabilität der Glomerulisbasalmembran der Rattenniere unter verschiedenen, nicht pathologischen Bedingungen. Durchschnittsdicke und Frequenzverbreitungskurve der Basalmembran ändern sich in Abhängigkeit vom Nierenzustand.

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Effect of 17 β-Estradiol on Early Cleavage Patterns in the Embryo of *Fucus distichus*

At 12 °C early development in *Fucus distichus* L. Powell (= *Fucus gardneri* Silva) is characterized by an oogamous fertilization, with the immediate formation of a fertilization membrane. A gelatinous sheath develops around the zygote over the next 6–8 h, followed by a dramatic polarizing response in the formation of a rhizoidal protuberance within 12 h. The rhizoid is fully formed by 24 h at which time the first cleavage plate is formed at right angles to the polar rhizoidal axis. The resulting cells are now presumed to be different from each other in that the rhizoidal cell gives rise to the holdfast of the mature plant, while the other cell gives rise to the thallus. Various environmental factors directly affect rhizoidal formation in *Fucus*[1]. However, the rhizoidal or polar axis once established preconditions the subsequent 3-dimensional distribution of embryonic mass which is characterized by an orderly displacement of cells. Orderly cell displacement can be viewed as a regulating mechanism for cutting off the regions of intracellular differentiation initiated by the zygote and preconditioned by the polarizing action. These 2 factors then, polarization and a predetermined cell displacement, seemingly are essential conditions for normal development in *Fucus* as in many other organisms.

In *Fucus distichus* it had been observed that young embryos with atypical cleavage patterns during the first 4–6 cell divisions were able to develop into normal embryos within a few weeks[2]. As a consequence, an attempt was made to induce this effect in young embryos in culture through the administration of mitotic poisons; namely, 17 β-estradiol and di-ethyl-stilbestrol, since these hormones were used successfully in inducing atypical cleavages in developing sea urchin embryos and other animal tissues[3]. The objective was to determine whether the heretofore described orderly displacement of cells around the embryonic polar axis was essential to continued embryo growth and survival. Previously, spindles had been rotated in *Fucus* using light as a stimulus. However, the resulting cells were symmetrically divided and the ultimate fate of the embryos was not reported[4].

Uniform cultures of zygotes 1 h old were obtained by following the methods for mass discharge of gametes and timing of fertilization in this monecious alga.

Percentage of population with rhizoids

Hormone concentration in ng/ml	After 24 h	After 4 days	After 5 days
12	40.0	68	98.0
37	34.7	65	97.5
111	24.0	69	97.0
333	17.0	60	91.0
1000	00.0	00	00.0

The relationship of di-ethyl-stilbestrol to the growth rate of young *Fucus* embryos. The growth rate, as evidenced by that percentage of the population which has well-formed rhizoids within a 24 h period, is retarded by an increasing concentration of hormone in a straight-line relationship. By 4 days about 70% of the embryos in all concentrations (except 1000 ng/ml) had polarized and by the 5th day it was close to 100%. Thus, the greater part of an embryo population is affected by a concentration of 333 ng/ml of hormone and growth lags by about 5 days.

The Table shows the relationship of varying concentrations of stilbestrol to the early growth rate of *Fucus*. Di-ethyl-stilbestrol has exactly the same effect as 17 β-estradiol except at lower concentrations. The delay in growth closely parallels the increase in concentration up to 1000 ng/ml of the hormone in seawater. At this, the highest concentration level tested, all growth ceases and the embryos do not survive. At 333 ng/ml rhizoid formation is delayed up to 5 days for the greater majority of embryos. At the end of this time, however, most of the embryos have well-formed rhizoids but some of the zygotes remain apolar. Subsequently, these cells undergo the first cleavage rather uniformly. However, the cells formed are markedly unequal in size, the plane of the spindle is quite randomly oriented and a wide variety of 2-celled embryos results (Figure). The second and subsequent cleavages are also disoriented. This effect continues with diminishing consistency for several cell

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[2] E. G. Pollock, unpublished data.
[3] I. Agrell, Nature *173*, 172 (1954).
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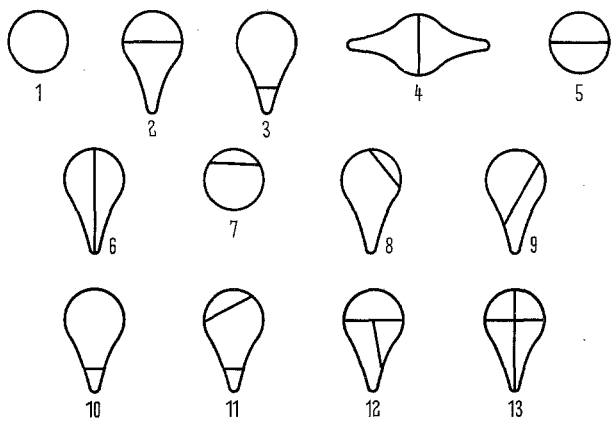
generations so long as the embryos remain in the original hormone solution. Growth, as measured by the increase in cell number per unit time, is slowed by about $^1/_2$ for approximately 10 days. During this period the hormone appears to be used up by the ever-increasing cell population. Within a period of 10–14 days, except for retarded growth rate, the treated embryos cannot be distinguished visually from the controls. If the treated cultures are followed to the first dichotomy of the young thallus, the young plantlets give every indication of continued survival to maturity. By contrast, many animal embryos treated similarly usually give morphological evidence of the hormone treatment.



The effect of 17β-estradiol on early cleavage in *Fucus*. 1, unfertilized egg; 2, normal cleavage at right angles to the polar axis; 3–10, atypical divisions as a result of 333 ng/ml of hormone; 11–13, atypical second division. Spindles are disoriented and division is generally unequal in terms of the size of the resulting daughter cells.

Embryos cultured in solutions of 333 ng/ml of di-ethylstilbestrol in seawater (or 600 ng/ml of 17β-estradiol) where the concentration level was replenished daily for a period of over 2 weeks were capable of continued survival. Cellular division in these materials was still characterized as being unequal and with disoriented spindles throughout the first 10 days of experimental treatment but the overall morphogenesis of these embryos was not noticably different from the normal ones. The growth rate, as measured by the increase in cell number and embryo size, constituted a lag of about 5–6 days. After about 10 days it appeared that the effect on division began to diminish; probably because the hormone was metabolically turned over more quickly by the ever-increasing mass. It should be pointed out that the increase in cell number is logarithmic for the first 40 cells formed, which is a little over 3 days growth in normal embryos. Thereafter, the rate of cell increase drops off and divisions are distributed more or less at random in the developing organism to bring about an expanding 'pear-shaped' morphology.

In addition to delayed growth and unequal cleavages, many embryos in culture were characterized by the formation of 3 unequal cells at the time when normally 2 cells are formed. This would indicate that multipolar spindles are formed; an observation consistent with those for echinoderm embryos exposed to estradiol. Due to an unequal distribution of chromosomes it appeared that some of these cells died. Nevertheless, the general form of the embryo was maintained by the remaining cells which soon filled in the gaps. Estradiol is a mitotic poison and apparently acts upon the mitotic spindle in a way similar to colchicine[5]. When the embryos are removed from its influence, moreover, normal division is resumed.

Developing embryos were grown in 2 μc/ml solutions of C^{14}-17β-estradiol in seawater for 6 h time periods and for the first 24 h of growth. These embryos were collected and the number counted for each sample. The samples were then extracted with methanol and chloroform and the extracts and the residue mass were counted on a Nuclear Supplies Liquid Scintillation Counter. The results were the same for all samples in this experiment: 80% of the label was in the estradiol fraction. These results indicate that estradiol is metabolized readily into a more stable and possibly inactive compound within the alga and, that the effect on mitosis is probably initiated by estradiol directly rather than some intermediate.

Autoradiographs of these materials using the Kodak NTB-2 liquid emulsion procedure revealed that the label is fairly equally distributed throughout the nucleus and cytoplasm of the cells, though somewhat less in the nucleus. Also, the label did not appear to be associated with any particular cell organelle when observed at the light microscope level. These observations indicate that the hormone is probably more closely associated with cytoplasmic fractions rather than with nuclear ones, and this seems likely in view of its action on the spindle. Any discrete association between the hormone and DNA, as suggested by many other studies on the action of estradiol in other tissues, is not readily apparent from these data[6].

Since *Fucus* displays a strong polar response and a 2-cell differentiation early in embryogeny, researchers have readily acceeded to the supposition that the fate of any cell in the young embryo can be said to be predetermined by (1) intracellular differentiation of the zygote due to polarization and (2) orderly cleavage patterns. Such orderliness in early embryogeny along with a 2-cell differentiation would suggest a rigidity in the embryonic plan whereby excessive deviation from it would be detrimental to survival.

Although it is too early to draw definite conclusions concerning these observations, it is possible to put forward some modified considerations governing early growth in *Fucus*. (1) As long as general polarity has established a differential separation of molecules within the zygote, it would appear that the manner by which the mass is cleaved up is not prerequisite to normal growth. (2) The displacement of anyone cell to a particular locus within the embryo body is usually determined by orderly cell lineage, but its fate or ultimate function is not predetermined by these events; and these young cells appear not to be differentiated in terms of any criteria other than position within the embryonic mass. It seems clear that the position a cell occupies within the embryo may come about through a diversity of pathways. (3) It may be argued that polarization is the primary critical event in *Fucus* development and the normal cleavage pattern observed in early growth is not more than 'happenstance' regularity which truly is not a precondition for normal morphogenesis. The early 2-cell differentiation following polarization would lead one to generalize the opposite. In fact, the differences are *regional* – and this point suggests a re-evaluation of the significance of the 2-cell differentiation versus asymmetric mitosis in terms of the overall plan of embryogenesis in *Fucus*.

These suggestions may be supported by some additional considerations. Developmental botanists have ascribed to the classical laws of embryogeny which seek to standardize the conditions of uniform cell displacement[7]. These

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[6] T. HAMILTON, Science *161*, 649 (1968).
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formulations stem from many observations on higher plant embryos where, for the most part, cleavage patterns are quite regular. Notable exceptions have been recorded, however, for embryos of *Gossypium* and *Capsella* and one cannot say with certainty that orderly cell displacement is a requirement for normal growth in these organisms[8]. Furthermore, notwithstanding the dramatic 2-cell differentiation in *Fucus*, its histogenesis is quite simple and 'indefinite' as compared with more advanced systems. The second apparent differentiation in *Fucus* comes about when the embryo is comprised of approximately 100 cells; i.e., the formation of a peripheral cell layer which is likened to a dermatogen. Therefore, up to this point in growth the function of cleavage would be merely to 'cut up' the existing, generally polarized mass, and the pattern of doing so need not to be uniform. In this respect the young *Fucus* embryo is like an early phase of echinoderm embryos. It is of interest to consider, however, that the overall form of the treated embryos of *Fucus* is maintained in spite of rotated spindles and unequal cleaving. This fact leads one to emphasize the regional nature of polarization in the embryo, and to assign a less critical role to orderly cleavage patterns, cell displacement and early differentiation. If this holds true it should be possible to reverse the initial events in early embryogeny (up to a point) and still obtain normal embryos e.g., multicellular, apolar embryos which later polarize. Further, in view of these considerations, one might ask, 'how different really are the first-formed 2-cells of the embryo?'

Finally, these observations on the relationship of polarity, cell cleavage and histogenesis suggest a profound flexibility in the early embryogeny of *Fucus*. This is noteworthy in view of the fact that mature forms of this species are characterized by a similar degree of morphological variation even within a short range of the intertidal zone[9-11].

Zusammenfassung. 17β-Östradiol verändert die Lage der Spindelachse und verursacht eine abnormale Mitose in jungen Embryonen von *Fucus distichus*. In der Folge zeigen die wachsenden Embryonen Furchungsmuster, welche keine geordnete Abfolge der Teilungen mehr zeigen. Trotzdem können die Embryonen eine örtliche Polarisierung aufrecht erhalten und überleben.

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[11] Sincere appreciation is extended to Professor RALPH HATHAWAY of the University of Utah for help and encouragement in initiating this work.

Catecholamines in the Avian Carotid Body

The presence of catecholamines in cells, which was formerly detected only by their chromaffin reaction, has recently come to be studied by fluorescence histochemical methods of ERÄNKO, FALCK and HILLARP[1]. This modern method has made it increasingly evident that the mammalian carotid body, long believed to be a chemoreceptor, represents a kind of endocrine gland consisting of cells which secrete catecholamines[2]. However, the avian carotid body does not seem to have been observed by either fluorescence or electron microscopy. The carotid body in some birds is known to be characteristically enclosed by the parathyroid, like the relation of the medulla to the cortex in the mammalian adrenal, to form the so-called parathyroid-carotid body complex[3] and, if studied by modern methodologies, seems to provide a key to the elucidation of the function of this organ.

Material and methods. Adult love-birds, *Uroloncha domestica,* were used in this investigation. The carotid body was found bilaterally in the thoracic inlet, beside the common carotid artery just beyond the origin of the subclavian artery.

For the examination of catecholamines in the carotid body, the histochemical method described by DAHLSTRÖM and FUXE[1] was applied. Freeze-dried tissues were treated with gaseous formaldehyde and embedded in paraffin. Sections were examined under an Olympus microscope 'Photomax' with a HB 100A high pressure mercury lamp (Ushio), an activation filter U (maximum transmission, 365 nm), a dark field condenser for immersion oil and a barrier filter FY3 (transmission, 410 nm).

For electron microscopy, the brachiocephalic artery and its branches were perfused, via a polyethylene tube, with 2.5% glutaraldehyde in phosphate buffer at pH 7.1. The carotid body, together with surrounding tissues, was further fixed in glutaraldehyde followed by 1.3% osmium tetroxide. After dehydration with ethanol, the tissues were embedded in Luft's Epon. Thin sections were cut with a Porter-Blum microtome and stained with uranylacetate and lead hydroxide. A Hitachi HS 7s electron microscope was used for observation.

Thicker (2μ) serial sections of Epon embedded materials were stained with toluidine blue and were studied light microscopically and partially reconstructed using photomicrographic methods (x c. 400). Light microscopic chromaffin reaction was examined with bichromate fixed specimens.

Results and discussion. In the fluorescence microscopy, the cytoplasm of the glomus cell of the carotid body showed green fluorescence which was interpreted as indicating the presence of catecholamines rather than indolalkylamines[1]. This interpretation was supported by the electron microscopic observation that cored vesicles, resembling the catecholamine-containing granules of the adrenal medullary cells, occurred in the cytoplasm of the glomus cells. A series of electron micrographs were obtained which seemed to indicate different stages of the

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