MORPHOGENESIS IN LOWER PLANTS[1,2]

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The lower plants attract the developmental physiologist with a bizarre variety of systems in which individual, normal, developing cells can be easily and directly observed and experimented upon. For the present purposes, this group includes the slime molds, true fungi, algae, and bryophytes. Only a fraction of the current literature can even be mentioned in the space available here.

Nucleocytoplasmic relations.—Enucleate parts of *Acetabularia,* etc., survive for several months, normally regenerate the complex apical whorls and cap, occasionally regenerate a basal rhizoid [Hämmerling (1)], and carry on a large net synthesis of both proteins and nucleic acids for several weeks [Brachet *et al.* (2)]. However, grafts in which the nucleus comes from one species and the bulk of the cytoplasm from another, while first developing caps of an intermediate character, ultimately are governed by the nucleus. Evidently the cytoplasmic carriers of nuclear information are relatively long lived in the Acetabulareae; no wonder, considering that an adult cell of the order of 10 mm.^{3} probably remains diploid (1). There is evidence of cytoplasmic control of nuclear functions; in polynuclear forms made by grafting, the rate of protein synthesis is unaffected but the volume of the individual nuclei decreases [Werz (3)]. Moreover, when young nuclei are grafted into old cytoplasm they are induced to initiate prematurely the mitoses preliminary to gametogenesis. In weak light, this condition which stimulates mitosis develops without whorl or cap formation [Beth (4)].

The carriers of cap character information produced by the basal nucleus are somehow accumulated in the apical region where caps form (1). A preliminary ultraviolet inactivation study weakly suggests that these carriers are nucleic acids [Six (5)].

Waris (6, 7) and Kallio (8, 9, 10) have reported their studies of desmids of the genus *Micrasterias.* The wild type semicell consists of a centrally located end lobe flanked by two "wings," each of which consists of two side lobes. This structure is cut by two planes of symmetry and is called biradiate. Enucleate forms were obtained by centrifugation at metaphase for the subsequent cytoplasmic division yields an enucleate and a binucleate semicell. The former does not normally survive more than one day but in this period it grows a new semicell whose form, while simplified, has the

[1] The survey of literature pertaining to this review was concluded in August, 1957.

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symmetry of its parent. Thus, an enucleate biradiate wild type semicell with five lobes produces a new semicell with five or three lobes and likewise of biradiate symmetry. An enucleate "defect mutation" semicell—one lacking one wing and, hence, consisting of three lobes cut by one plane of symmetry—produces a new semicell with one or two lobes and likewise of "uniradiate" symmetry [Waris (6)].

Of particular interest is the evidence developed by these workers which suggests that the cytoplasmic determinants of these lobe patterns are immobile primordia which perpetuate some information which is independent of the nucleus. The most suggestive observation is this: the uniradiate defect mutations were observed to arise from the wild type only three times in thirteen years of study [Kallio (9 p. 121)]. Nevertheless, uniradiate semicells sometimes give rise first to a single biradiate semicell which breeds true and subsequently to a series of uniradiate semicells which breed true [Kallio (8, p. 86)].

Moreover, there is graphic evidence that new lobe primordia arise most easily through the splitting of an old primordium rather than *de novo*. Thus, Kallio (8 p. 86) reports that the commonest mode of spontaneous back mutation of a uniradiate form yields, after one or more generations, cells in which an original uniradiate mother semicell is joined to a biradiate semicell bearing two wings which are in a plane perpendicular to that of the mother semicell.

Discovery of a remarkable new species of desmid, *Scottia mira* [Grönblad & Kallio (11)] may extend the possibilities of desmid study to the problem of polarity; for this species is unique among desmids in that each cell consists of two semicells of distinctly different shapes. However, all the observations appear to have been made upon dead specimens, so there is some doubt as to the stability of *Scottia* and its mode of multiplication.

Diploid forms of *Micrasterias,* produced by metaphase centrifugation and other means show the tendency, long established in other forms, to maintain an approximately fixed nucleocytoplasmic volume ratio. In some cases the diploid cells expand chiefly by enlarging their wings; but in others, the wings multiply so as to produce forms with three or four wings of normal size [Kallio (8, 9, 10)].

Bauer (12) has studied the development of a diploid protonema derived by regeneration from a young sporophyte of the moss, *Georgia pellucida.* It passes through the two filamentous phases characteristic of the normal haploid protonema (see p. 364), and except for having the larger cells typical of chromosome doubling it is morphologically indistinguishable from the haploid filaments. However, its subsequent behavior is remarkable. Under proper conditions—these include staling of the medium and the use of 3 per cent agar as opposed to 1 per cent agar—the protonema regularly develops numerous buds which grow directly into sporophytes; the leafy gametophore stage is completely bypassed. While clear-cut control experiments with haploid protonemas are not reported, such vegetative sporophyte development was apparently never observed to occur in haploid

filaments by either Bauer or a number of earlier workers. The vegetative sporophyte only occasionally developed to the point of forming spores, but those spores that did develop were capable of germinating into filaments indistinguishable from normal haploid ones.

These results appear to offer a choice of two interesting conclusions: either a minimum of two chromosome sets is required for sporophyte development in *Georgia,* or protonemas derived by regeneration from the sporophyte retain some subtle cytoplasmic factor which is both essential for sporophyte development and lost during sporogenesis.

Germination.—Forsyth (13) and, more clearly, Allen (14) demonstrated that *Puccinia graminis* uredospores emit a somewhat volatile self-inhibitor of germination. Emission occurs during approximately 5 hr. after floating the spores in water and then ceases (14). The inhibitor is not CO_2. (Forsyth's claim to have identified the inhibitor as trimethylethylene is unconvincing. It is based primarily upon a very crude similarity in the ultraviolet absorption spectra of an alleged inhibitor solution and a trimethylethylene solution.) A self-stimulator of germination emitted by uredospores was recently discovered by Allen (15) and by French *et al.* (16); it inhibits germ tube elongation. This stimulator likewise proves to be volatile and probably has at least two components, one of which has been definitely identified as pelargonaldehyde. However, the capacity of pelargonaldehyde to stimulate germination is also found to a comparable degree in a variety of aldehydes and alcohols of approximately the same molecular size. It is of great interest that pelargonaldehyde likewise induces differentiation of the germ tubes into structures resembling appressoria, infection hyphae, infection pegs, and substomatal vesicles [French *et al.* (16, 17, 18)].

Origin of polarity.—At relatively low concentrations of amoebae of *Dictyostelium discoideum,* aggregation begins with the appearance of small regions in which the amoebae are relatively concentrated, a process of centripetal movement then spreading out from these centers [K. B. Raper (19)]. It would be very difficult to avoid the conclusion that the cells in these primordial centers, called initiators by Sussman (20), are subtly different from the others. Sussman (20) has reviewed a series of studies on the origin of these initiators. He has concluded that at the end of the growth period the fraction of initiator cells is fixed. (The effective fraction is supposed to depend upon the sensitivity of responder cells under the given conditions.) Shaffer (21), on the other hand, concludes that a large fraction of the myxamoebae may act as initiators; the centers of aggregation form around those few which happen to develop this capacity first.

This reviewer finds Shaffer's view preferable: Sussman supports his view with the results of a fluctuation test (22). Small numbers of myxamoebae were confined within areas of about 4 mm.^{2} and at cell concentrations otherwise compatible with aggregation. After an interval found to be more than adequate for aggregation in large groups (130 to 500 mm.^{2}), a large fraction of the replicates of this experiment (with small groups) failed to aggregate. However, the conclusion that the nonaggregating

groups lacked initiators is invalidated by a technical mistake; the amoeba were confined by some mysterious property of the agar substratum whic underlies a recently adsorbed droplet of suspended amoebae. This propert was surely no barrier to the diffusion of acrasin, etc; hence, the concen tration of acrasin, etc., in these small groups must have been greatly re duced as compared to cultures on areas of 130 to 500 mm.^{2}. Now in thes latter, a reduction of the cell concentration to about one-half to one-seventl of those used in the small groups completely prevented aggregation, sug gesting that a reduction in the concentration of acrasin, etc., to the degre that must occur in small groups could well block aggregation. Sussma presents a second argument: in large groups, the average number of aggre gations per cell were plotted against cell concentration. The curve shows rather sharp maximum of one aggregate per 2100 cells at the "optimal" density of 200 cells/mm.^{2}. Now, where the small groups had included total of about 1000 cells per group, about two-thirds of the aggregate lacked centers. If this lack really arose from the chance absence of initia tors, then the Poisson equation would yield the fraction of initiators. Th result was one initiator per 2300 cells. It was assumed that at the "optimum" density in large groups the number of aggregates per cell equalled th number of potential initiators per cell. If this were true, then the agree ment between the two numbers would strongly buttress Sussman's con clusion. However, the implications of the above assumption are extremel difficult to swallow: (*a*) The average aggregation size rises rapidly on bot sides of the optimal density. Hence, Sussman's assumption implies that th two mechanisms which rapidly increase the aggregation size at sub- an super-optimal densities must both become virtually inoperative at the opti mal point. (*b*) If wild type cells are mixed with aggregateless mutant one can obtain up to one aggregate per 24 cells, depending upon the mu tant (23). Yet, an ingenious study of wild type and aggregateless mutant separated by thin agar membranes confirms what are in any case the sim plest assumptions (24): (*i*) The initiator cells in the mixture come ex clusively from the wild type component; (*ii*) Aggregateless cells are, i anything, less responsive to acrasin than wild type cells. In the face o these latter facts, the assumption of equal aggregate number and initiato number requires that either (*i*) or (*ii*), or both does not hold when the tw cell types can come into direct contact. In conclusion, it is suggested tha critical evidence may be obtained by repeating the fluctuation test with cell confined by a diffusion barrier.

The early development from carpospores or tetraspores of diverse re algae involves the formation of a cushion of cells lacking any apparen polarity [Fritsch (25 p. 607)]. Jones (26) reports that in *Gracilaria ver rucosa,* a cushion developer, polarity, in the form of apical initials, onl rarely develops except in "rafts" derived from the coalescence of cushion from two or more spores. Is this yet another surface/volume effect?

Various investigators have induced a more or less prolonged apola phase into the development of bryophytes (27 to 30). Thus, Allsopp (30

sowed spores of two species of liverwort on a medium containing 2 per cent glucose. Some of the cultures developed amorphous tissues consisting of largely apolar, undifferentiated, and rapidly growing cells which continued in this state for at least six months. Upon transfer to glucose-free media, normal tissues rapidly developed.

In the normal development of various mosses (31), of *Dictyopteris* (32) and various Fucaceae (33) among the brown algae, and of diverse phycomycete conidiospores (34, 35), *Botrytis* conidiospores (36), *Puccinia* uredospores (37) and basidiospores (36) among the fungi, germination immediately yields a more or less polar structure whose axis can be determined by unilateral light and other gradients applied to the developing spore or zygote. There are many scattered reports of low percentages of bipolar or even multipolar germination in such forms. However, it is not clear whether even approximately normal eggs or spores could so respond. In some cases, the multipolar forms may have arisen from multicellular zygotes or spores arising from the fusion of normal eggs or spores. Thus Whitaker (39) finds that *Fucus vesiculosus* eggs, if raised at excessively high temperatures, often fail to separate from some of their sister eggs in the öogonium; the resultant fusion forms frequently develop in a multipolar manner, though the number of rhizoidal outgrowths initially formed never exceeded the number of constituent cells.

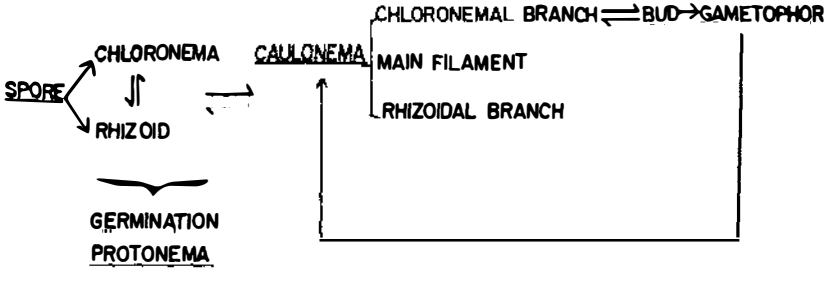
On the basis of these facts, it was possible to entertain the thought that the light or other directive influence in these various cases does not direct a purely epigenetic development of polarity but causes the rotation —to present an extreme alternative—of some single preformed asymmetric and determinative structure such as the nucleus. Indeed this last has been reported to occur within certain green algae zoopores after attachment to a substratum [Kostrum (40)].

Recently, however, it has been found that *Fucus furcatus* zygotes respond to illumination with plane polarized white light by growing out at approximately right angles to the direction of illumination, and with a strong tendency to be in the plane of vibration of the electric vector [Jaffe (41, 42)]. It is clear that every cell is here being stimulated in a symmetric manner, that is, to grow out in both of two opposite directions. In response, up to half the zygotes produce bipolar forms, the rest developing in one direction or its opposite but not both. In this case at least, it seems clear that the polarity arises in some more epigenetic manner than through the directed rotation of some preformed asymmetric structure.

The above discussion concerns the origin of apicobasal polarity. Study of the origin of dorsiventral polarity, using liverwort gemmae, began long ago; to my knowledge, Mirbel's 1835 paper on this subject (43) is the earliest study of the origin of polarity in any form. Since the polarity of the developing gemma is readily determined by various external influences, these gemmae have been recently considered as being comparable in their organization to *Fucus* eggs, moss spores, etc. However, the careful work of Fitting (44), his student Halbsguth (45, 46) and "grand-student"

Kohlenbach (47) clearly show this interpretation to be misleading. Th external directive agents are not acting to establish dorsiventral polarit *de novo*. Rather they act to favor one or the other of two competing meri stems which have a preformed tendency to lay down lobes with opposit dorsiventral polarities: A gemma is a disc-shaped structure with two op positely placed notches in its edge. These notches are the site of the tw apical meristems whose activity primarily constitutes germination. Sinc each notch behaves independently, the further discussion will refer to single notch and its associated tissue. Both flat outer layers of the gemm exhibit a preformed, visible and fixed ventral differentiation. Seen in micro scopic section, each notch usually contains what can be more or less clearl seen as two apical meristems, each nearer one of the flat faces (46). Unde illumination directed at one flat face, it appears that the meristem neare to the light is relatively inhibited; the farther one becomes dominant an lays down a thallus lobe with a ventrally differentiated face extending th layer already present in the gemma and therefore lying away from th light, and a dorsally differentiated layer lying toward the light. But, unde illumination directed equally at both flat faces, so-called isolateral form sometimes develop. That is, both meristems develop to an equal degree, s a form with two ventrally differentiated layers lying toward the light de velops. Dorsally differentiated tissue develops when the two meristems be gin to diverge so as to lay down lobes diverging from that plane of sym metry which is parallel to the flat faces. This dorsal tissue develops t some extent internally, proximal to the crotch, and subsequently at the in side faces exposed by the splitting growth (44).

Origin of buds in mosses.—On the basis of the older work, togethe with more recent papers, particularly those of Wallner (48), Heitz (31 49), Meyer (50), Sironval (51, 52), Fitting (53), Bopp (54 to 57), an Allsopp & Mitra (58), the following tentative scheme can be drawn:



An important feature of this scheme is that the protonemal stage whicl intervenes between spore and bud consists of two distinct substages: firs what I will refer to as a germination protonema, and secondly the caulo nema (48, 51, 56). The buds in most species form only upon the proxima cells of the chloronemal branches of the caulonema (58).

Although quantitative studies are lacking, the literature allows the following qualitative characterizations of the germination protonema: (*a*) The chloronema and rhizoid are extremes of a continuum of forms (51, 53). (*b*) A chloronema-generating filament apex can be converted to one generating a rhizoid and visa versa by changes in pH and salt concentration (53); clear evidence that mature cells can be so transformed is lacking. (*c*) Intergrades are somewhat less stable than more extreme forms of the chloronema-rhizoid continuum (50, 51, 53). Thus, (i) a spore commonly sprouts both a distinctly chloronemal and a distinctly rhizoidal filament (49, 50); (ii) Apex conversion [see (*b*)] may not occur until a few days after the causal environmental change and then occurs quite suddenly (53 pp. 654-55). (*d*) Point (c–i) also indicates that under many common conditions, either extreme form alone is in turn less stable than both together. This conclusion is buttressed by Fitting's claim that conversion from chloronemal to rhizoidal generation occurs relatively readily in germlings lacking any rhizoidal filaments (53 pp. 654–55).

The caulonema is generated by a transformed chloronemal apex. This transformation to a caulonema is favored by sunlight as opposed to light from tungsten filament bulbs, by the presence of growing *Penicillium* sp. hyphae, and by material from a degenerate chloronema [Sironval (51)]. The reverse transformation, from caulonema to chloronema, can be induced by isolating caulonemal sections from the culture in which they had developed. This holds for cells newly generated by the apical cell and probably also for cells formed before the isolation [Bopp (56)].

Bud formation is greatly stimulated by various 6-(substituted) aminopurines in concentrations of as little as 10^{-8} *M* [Gorton *et al.* (59)]. Which transformation is so hastened in the chloronema to bud sequence is uncertain. Bud formation is greatly inhibited and buds are induced to revert to the filamentous stage by 10^{-4} *M* indoleacetic acid [Bopp (57)].

Origin of reproductive structures, heterogenous chemical control.— Raper (60) has carefully reviewed the older literature. Hustede (61) has found some striking effects of β-indole derivatives on the development of reproductive structures in two green algae. As little as 10^{-8} *M* indoleacetic acid stimulates zoospore formation in *Stigeoclonium*. As little as 10^{-9} *M* indoleacetic acid or tryptophan likewise stimulates zoosporangial formation but inhibits gametangial formation in *Vaucheria*, while as little as 10^{-7} *M* indole, indolealdehyde, or indolecarboxylic acid has the opposite effect, favoring gametangia and opposing zoosporangia. Moreover, these latter, short-side-chain compounds tend to favor öogonia as opposed to antheridia.

Cantino has published on the development of *Blastocladiella*, a water mold metabolically notable for producing no net CO_2 at all (62 p. 333). He has found that if the bicarbonate concentration of the medium lies in a concentration range (.004 *M* to .01 *M*) slightly below that which completely

inhibits growth (.05 *M*), the zoospores are induced to develop into thick-walled resistant sporangia (R.S.) instead of into thin-walled sporangia (63). He believes that the bicarbonate acts by speeding certain carboxylation reactions. Most probably, however, the bicarbonate acts by lowering the extracellular or intracellular pH, or both. (*a*) In the experiment cited above, the medium was so poorly buffered that the 2½ fold range of R.S.-inducing CHO_3^- concentrations is associated with a twofold range of extracellular hydrogen ion concentrations. (*b*) Addition of bicarbonate to medium B, which is buffered with .013 *M* phosphate failed to induce R.S. formation (62 p. 333). (*c*) "Gaseous CO_2 at concentrations permitting growth never induced more than 50 per cent (average *ca.* 5 to 25 per cent) of a population . . . to form resistant sporangia" (62 p. 350). (*d*) "Extreme reduction of the atmospheric CO_2 concentration often induced the formation of large quantities (50 to 85 per cent) of R.S. plants" (62 p. 333).

Origin of reproductive structures, autogenous chemical control.—Gussewa (64) has demonstrated that zoospore production is set off when the respiration of the green alga, *Oedogonium* has raised the extracellular concentration of CO_2 to a level equivalent to a few per cent in the gas phase. He showed that this is not an extracellular pH effect but pointed out that the CO_2 may well operate through an intracellular pH drop.

Studies of the hormonal initiation (and control) of sexual reproduction in the fungi have been carefully covered in recent reviews by J. R. Raper (60, 65). Since the time of these reviews, the important paper of Plempel (66) on *Mucor* has appeared. He has clearly demonstrated that sexual interaction in *M. mucedo* involves at least four stable substances: (*a*) the plus progamone; the filtrate from the isolated plus mycelium contains this agent as shown by stimulation of the minus mycelium to liberate a second substance; (*b*) the minus gamone; thi sagent induces the otherwise untreated plus mycelium to develop zygophores; (*c*) the complementary minus programone; (*d*) the complementary plus gamone.

Light requirements for development.—In general, low intensity developmental light requirements ($< 10^5$ ergs/mm.^{2}) are satisfied only by the blue part of the visible spectrum. Thus, blue light is required for fruit body maturation in *Coprinus lagopus* [Borriss (67); Bünning *et al.* (68)] at a dose of $\geq 10^2$ ergs/mm.^{2} [Stiefel (69)], and to speed sporophore initiation in *C. lagopus* at $\geq 10^{-1}$ ergs/mm.^{2} [Madelin (70)]; for sex organ initiation and development in *Pyronema confluens* (71), at $\geq 10^4$ ergs/mm.^{2} [Kerl (72)]; for the initiation of long conidiophores in *Aspergillus gigantens* at $\geq 10^4$ ergs/mm.^{2} [Gardner (73)]; for apothecium formation in *Ascophanus carneus* [Stoll (74)]; for trophocyst formation in *Pilobolus Kleinii* [Page (75)]; for öogonial formation in *Laminaria* [Harries (76)]; for rhizoid formation in *Spirogyra fluviatilis* [Weihe (77)]. Moreover, there is evidence that short wavelengths are more effective than long ones in induc-

ing the localization of conidiophores and conidia in those hyphal tips of *Fusarium discolor* which are young during light exposure; $\geq 10^2$ ergs/mm.^{2} are needed [Bisby (78)]. The alleged effect of red light in favoring sporangiophore formation in *Choanephora cucurbitarum* [Christenberry (79)] was probably due to the temperature of the illuminated plant which was 10°C. higher than the dark control; high temperature is found to favor sporangiophore formation [Barnett & Lilly (80)].

The only clear exception to the blue light rule is the initiation of fruit body formation in the acellular slime mold, *Didymium eunigripes* [Straub (81); Lieth (82)]. According to Lieth (82), the light requirement for fruiting can be met not only by $\geq 10^4$ ergs/mm.^{2} blue light but also by $\geq 10^5$ ergs/mm.^{2} red light; moreover, $\geq 10^4$ ergs/mm.^{2} of green light markedly inhibits the initiation of fruiting by a simultaneous dose of red light. Radiation lying between 350 and 390 mμ. is likewise reported to stimulate fruting (81).

It would not be surprising if these low intensity blue morphogenetic effects ultimately prove to be mediated by the same mysterious photoreceptor responsible for phototropism.

Borriss (67) made the remarkable discovery that mechanical stimulation will substitute for blue light in allowing fruit body maturation in *Coprinus*. Moreover, the same dark reaction seems to limit the effectiveness of both forms of stimulation [Stiefel (69)].

In various cases it has been observed that these blue light effects are restricted to illuminated regions of the plant and are not translocated (70, 71, 78, 83).

Origin of reproductive structures, other physical factors.—Hawker's (84) review can be consulted here. More recently, Sproston & Pease (85) have made the first observation of a thermoperiodic response in a cryptogam (formation of apothecial initials in *Sclerotium*). In a careful study, Plunkett (86) has established that the initiation of sporophore rudiments as well as the initiation of caps in *Polyporus* is markedly speeded by high transpiration rates brought about by dry air flow. Now there is evidence in the higher fungi (as well as the phycomycetes) that mycelial protoplasm is induced to flow to a region of low external water activity [Buller (87 pp. 85, 96, 123)]. Hence, Plunkett's paper suggests an experimental attack upon the often observed relation between protoplasmic accumulation and the initiation of primordia.

Neoplasms.—Thomas *et al.* (88) have shown that rose-comb disease of mushrooms bears notable resemblances to animal cancer. The disease which is induced with particular effectiveness by diesel oil fumes is characterized by large, typically cerebriform outgrowths from the cap. These tumors may greatly outgrow the normal sporophore tissues; they often lack any gill tissue differentiation; and they are made up of cells which have an ab-

normally large number of nuclei and abnormally high basophilia, and exhibit "endomitosis, multipolar spindles, chromosome aggregation, polyploidy, aneuploidy, and micronuclei."

Persistence of differentiation.—Recent work continues to provide evidence of the relative stability of polarity even in simple systems: (*a*) An individual myxamoeba of *Dictyostelium* when confronted with a suddenly reversed gradient of acrasin usually "makes a U turn without ever losing its elongate shape"; occasionally it balls up, at the same time sending out a pseudopod toward the acrasin [Bonner (89)]. (*b*) Myxamoebae stuck together in fabricated loops as well as in the previously observed naturally occurring ones [Shaffer (90)] stream in a constant direction, that is either clockwise or counterclockwise. Bonner (89) argues—I believe correctly—that such behavior cannot be explained by an extracellular chemical gradient. For if, at any one time, cells in one part of the loop are exposed to a clockwise gradient, then others at this same time must necessarily be exposed to a counterclockwise one. Moreover, there appears to be inadequate reason to postulate waves constructed of successive zones of (i) acrasin gradient stimulation, (ii) response via chemotaxis and acrasin secretion, (iii) refractory state, . . . etc., passing around the rings —a possibility implied in Shaffer's discussions. (*c*) Hyphae of *Saprolegnia* when exposed to a suddenly reversed gradient of a mixture of amino acids toward which they respond chemotropically, subsequently grow in a hairpin turn, although they also react in part by developing new laterals far in back of the tip (35). (*d*) In the absence of strong external counter gradients, isolated cells of *Cladophora* [see(91)], *Enteromorpha* (92), *Chara* and *Nitella* (93), and *Griffithsia* [see (91)] show at least a strong tendency to regenerate new shoots and rhizoids with unchanged polarity.

On the other hand, in the case of various cellular, filamentous forms with apical meristems, environmental changes can convert the shoot-generating apical cell to a rhizoid-generating one or visa versa. The older work of this sort on *Cladophora, Sphaceleria,* and various Florideae is reviewed by Bloch (91). An impressive example of this phenomenon is furnished by Fitting's study of certain moss protonemas (53). A chloronema transferred to media relatively lacking in salts or relatively alkaline began generating a rhizoidal filament at its tip within a few days, while a rhizoidal filament transferred to relatively acid media soon began generating a chloronemal filament at its tip.

Similarly, in various large coenocytes, *Bryopsis, Dasycladus* and *Caulerpa* as well as similar large multinucleate isolated internodal cells of *Griffithsia* (91), *Chara* and *Nitella* (93), the application of reversed gradients or other agents can reverse the polarity.

A number of workers have succeeded in forcing more or less differentiated gametangial cells to sprout vegetative hyphae. Kerl (72) induced such effects by surgically isolating gametangia of *Pyronema* from the rest

of the mycelium. All cells of all stages in gametangial development frequently sprout vegetatively upon isolation except for the mature ascogonial cell; i.e., the as yet undelimited primordia, the recently delimited gametangia, the mature antheridia, trichogynes, and ascogonial stalk cells all do so. The mature ascogonial cells, however, which may be likened to eggs, respond to isolation with numerous small outgrowths which suggest abortive ascogenous hyphae rather than vegetative ones—behavior resembling parthenogenesis.

Bistis has studied *coitus interruptus* in *Ascobolus* (65, 94). If the oidium, a sort of male cell which induces ascogonial development, is removed before the ascogonium has matured, the latter sometimes sprouts vegetatively from its tip. However, the differentiated cells of the mature reproductive structure (except for the stalk cells which "are indistinguishable morphologically from vegetative hyphal cells") do not so respond. Denffer & Hustede (95) induced young antheridial primordia of *Vaucheria* to continue their elongation in the form of vegetative filaments rather than maturing sexually, by treatment with as little as 10^{-9} *M* indoleacetic acid. Mature gametangia, however, do not respond to the IAA (see p. 365).

Raper caused young, undelimited öogonial primordia of a variety of homothallic species of the Saprolegniaceae either to drain their contents back into the parent hypha or sprout antheridial hyphae by treating them with crude concentrates of hormone A from *Achlya*. Once delimited, however, the öogonia no longer respond (96). Bopp (56) has succeeded in causing *Funaria* buds to revert to a filamentous form via 10^{-4} *M* indoleacetic acid, and *Funaria* caulonemal filaments to revert to the chloronemal form via isolation. Sandan (97) reports a remarkable transformation of a mature rhizoidal cell of *Nitella* into an internodal one as a result of isolation from the rest of the plant. This metamorphosis began about two months after isolation and yielded an internodel cell of about one-eighth normal diameter with a linear instead of a spiral white line and streaming pattern. It should be noted that, with the exception of the reports of Sandan and of Bopp, the various transformations reviewed here do not represent so much the metamorphosis of an entire mature cell from one differentiated state to another as a change in the cell type generated by an obvious meristem or at most the sprouting of a changed cell type from a restricted region of a partially differentiated cell.

Scherr & Weaver have reviewed the extensive literature on the dimorphism phenomenon in yeasts (98). A reading of this review as well as the discussion in *Henrici's* handbook (99), indicates that the transformation from the yeast phase toward the mycelial phase involves a failure to carry "cell division" to the point of cell separation, a lengthening of individual cells, and a tendency to restrict budding to the anterior ends of cells attached in chains [see (99), Fig. 116; see also (98), Plate II, Fig. 4, Plate V, Fig. 4, Plate VI, Fig. 1].

Evidently, the last two features may be considered as a change in cell polarity, but it is not clear from the published data whether these changes involve only cells formed in a new environment, or whether cells already formed are likewise changed. Moreover, the polarity changes are not quite as extreme as superficial examination suggests for Barton (100) carefully observed *Saccharomyces cerevisiae* and found that successive buds showed a strong tendency to be antipodal.

Intracellular loci and directions of wall extension.—According to Mitchison (101), about 90 per cent of cells of the fission yeast, *Schizosaccharomyces Pombé* elongate by growth restricted to one end. Moreover, studies of various isolated fungal hyphae have clearly indicated a similar restriction of growth in *Peziza* (102), in the stage I sporangiophore of *Phycomyces* (103), and in *Rhizopus* (34). Now, the shape changes in the budding of yeasts are incompatible with such tip growth (104). It may, therefore, be guessed that both the cell lengthening and the apical localization of budding which characterize the passage of dimorphic yeasts toward the pseudomycelial or mycelial phase are manifestations of an increasing restriction of growth to the apical pole of each cell.

Green (105, 106) has carefully studied the growth of the lateral wall of the enlarging internodal cell of *Nitella*. At least as averaged over short intervals of time, wall extension goes on at an equal rate at all points. Extension in the axial and circumferential directions are allometrically related, that is if C is the cell's circumference and H its length, then $dC/C = K\, dH/H$. K is twice as high at 36°C. as at 22°C.

Correlates of local growth.—The wall in expanding tips of the juvenile *Phycomyces* sporangiophore is much thinner than elsewhere (107). It may be, then, that here and in other cases of hyphal growth, the resulting higher tension in the wall's tip is in some manner responsible for the concentration of cytoplasm. Direct evidence that high wall tension can stimulate fibril synthesis is reported by Green (108). In *Nitella,* reduction of cell turgor, and hence wall tension, with polyethylene glycol solutions greatly inhibits cellulose synthesis.

Wilson's observations with visible light of the striations and scattering patterns on the walls of the largest cells of both *Valonia* and the related *Dictyosphaeria* (109, 110) when combined with the earlier x-ray work of Preston & Astbury (111, 112) and the electron microscope work of Steward & Mühlethaler (113, 114) yield a consistent and convincing picture: the cellulose fibrils in these cells lie in three systems of orientation, two spiral and one approximately meridional, which converge on their developmental poles.

One might imagine that the growth response of *Fucus* zygotes to polarized light is effected by the direct photochemical creation of an oriented molecular array which defines two developmental poles. However, the wave-length dependence and other details indicate that the phenomenon

is simply a variant of the directive influence of ordinary light on these zygotes, and more broadly of the general phenomenon of phototropism; specifically, it is suggested that the photoreceptor molecules tend to lie periclinally so that those molecules at the site of future rhizoid development are so oriented as to lie effectually in the dark [Jaffe (42)].

Lund (115) has measured the extracellular potential gradients on the surface of the filamentous green alga, *Pithophora* when the plant was suspended in moist air. The filament is septate with growth largely confined to the apex of the thallus. Within each cell cytoplasm including the chloroplasts accumlates apically and when branching occurs it originates from apices of intercalary cells. The potential distribution showed three main features: (*a*) Potential maxima (regions where the potential was most positive) were found at or slightly in back of the apex of each cell; (*b*) In moving basally from the apical cell, the height of successive maxima fell; (*c*) Potential maxima were also found near the cytoplasmic condensations associated with the laying down of a cross-wall during an intercalary division. These last two features of the distribution represent a correlation between positive extracellular potential and the site of wall growth. It would be of considerable interest to know if wall growth in *Pithophora* is concentrated at the apical end of each cell, as the distribution of cytoplasm and of branching suggests. For if it were, the positiveness of extracellular potential would be even more closely correlated with the rate of underlying wall growth, a conclusion on the cellular level which would conform to the correlation at a grosser level exhibited elsewhere (116 to 119).

If these potentials are like all other actively maintained biolectric potentials whose nature is at all understood, they will originate in the cell membrane. Hence the region just below the membrane which in turn underlies an extracellular locus of relaively high (i.e., positive) potential will be the site of a relatively negative potential as compared to other portions of the cytoplasm within this cell and should attract inclusions having a positive electrophoretic potential and repel those with a negative one. The available evidence, though sparse, consistently indicates that nuclei have a positive electrophoretic potential [Churney (120)], while chloroplasts have a negative one (121, 122). Put together, then, these facts suggest an electrophoretic explanation for the long established tendency of nuclei to migrate toward sites of wall growth in plant cells [Haberlandt (123)], and the sometimes associated displacement of chloroplasts away from these sites, as in normal (123) or regenerative (124) growth of *Vaucheria.*

Buller's (87) studies of hyphal fusion in a variety of Ascomycetes and Basidiomycetes are of fundamental interest. They strongly suggest that one of the links in the mechanism which originates and maintains tip growth is emission by the tip of an unstable substance(s) which in turn

acts back on the emitting tip to speed its growth. In a tip-to-tip fusion, two hyphal tips grow close enough by chance (usually within about 10 to 15 μ). They then grow directly or almost directly toward each other, make contact and fuse. In peg-to-peg fusions, two older hyphae lying no more than 25μ apart mutually induce each other to form simultaneously peg pairs lying opposite each other which then grow toward each other, touch and fuse.

Whitaker put forward the plausible suggestion that the positive group effect in *Fucus*, i.e., the tendency of the eggs to germinate toward their neighbors, was simply mediated by a CO_2-pH gradient (33). However, a closer examination indicates that such a gradient does not in fact play a significant role in producing this interaction [Jaffe (125)].

Stadler (34) has studied a negative group effect in *Rhizopus*, a marked tendency of the conidiospores to germinate away from each other which is referred to as the staling reaction. He clearly confirmed the earlier demonstration of Clark that this reaction is due to a negative chemotropic response. He further shows that the grown mycelium produces an unstable substance which markedly inhibits spore germination (it could hardly have a half life more than about 30 min.), and makes the plausible assumption that this latter substance is the same as the one responsible for the group effect.

Heitz (49) reports that *Funaria* spores developing in diffuse white light tend to develop chloronemas away from their neighbors. However, since the chloronemas tend to grow toward unilateral white light, it is uncertain whether or not this negative group effect is simply a consequence of mutual shading.

Fischer & Werner's work (35) indicates that the tendency of hyphae of *Saprolegnia ferax* to grow away from each other is due, at least in part, to a positive chemotropic influence of amino acids in the medium. The chemotropic response is manifested both by rapid turning of the direction of growth of the hyphae and by the initiation of new laterals from the hyphal wall facing a source of amino acids. The remarkable discovery was made that the response cannot be elicited by any one amino acid; the simplest combination of acids giving more than a very weak response was leucine, plus glutamic acid, plus cysteine; various combinations of five or six amino acids gave somewhat greater responses; no one amino acid was essential to the response. In one experiment, swarmers germinating on otherwise nutrient-free agar were exposed to a gradient. From the data given, it can be estimated that in the threshold region the amino acid concentration lay between 10^{-5} and 10^{-4} *M* but the setup precludes any estimate of the minimal effective gradient.

Fischer & Werner hypothesize that the amino acids act to reduce the extensibility of the wall. However, in view of the fact, documented above, that in all cases investigated hyphae not bearing a terminal spore mass

extend through tip growth, and considering that the acids act to initiate new laterals toward the high amino acid side, there is hardly any doubt that in fact the amino acids act to increase the walls' extensibility.

Since antheridial hyphae of various members of the Saprolegniales were likewise attracted to an amino acid mixture, it has been suggested (126) that Raper's substance C (which attracts *Achlya* antheridia to the öogonia) consists of such a mixture. Whatever its nature, the action of substance C is remarkable with regard to the enormous distance, several thousand μ, from the öogonial source through which the 10μ wide antheridia can respond chemotropically (65). The so-called zygotropism of *Mucor,* etc., is likewise reported to appear at distances apart from the zygophores as great as about 2000 μ (66, 127, 128). But to my knowledge, there is no evidence that the initial long distance phase of this "attraction" does not in fact represent a self repulsion (via negative chemotropism) of the zygophores of the plus and also of the minus strain. Indeed, Banbury (127) presents some evidence that suggests, but unfortunately fails to prove, his point. Two other established cases of sexually significant positive chemotropism are the attraction of the trichogynes of *Ascobolus* [Bistis (94)] and of *Bombardia* [Zickler (129)] to the "male" cells; the maximal distances of attraction for *Ascobolus* are said to be about 20 μ [Raper (65)] and for *Bombardia,* judging from Zickler's photographs, about 50μ.

The work of Bopp (130, 131) extends the evidence of many predecessors that the moss calyptra quite generally exports a growth hormone to the developing sporophyte; for excision of the calyptra results in a more or less marked shortening and apical thickening of the developing sporophyte.

Bonner *et al.* (132), in a refinement of various older observations, report that the stipe of the commercial mushroom elongates primarily through the extension of axially oriented cells in a short region beneath the cap. The experiments of Urayama (133) furnish some evidence that the gills of this plant export a growth stimulator to these cells. Thus, if the gills are all cut off, agar blocks placed on the cut undersides of the cap and an excised gill placed upon one of these blocks, then the stipe bends away from this latter block. As much as 10^{-2} *M* indoleacetic acid was a completely ineffective substitute for the gill.

This last finding is in conformity with the emerging pattern of auxin action: it can only effect the elongation of cells with cellulose-bearing walls. Basidiomycetes have chitinous, not cellulosic walls [Brian (134)].) Thus Davidson (135) made the striking observation that as little as 10^{-11} *M* indoleacetic acid markedly increased the length of the middle and inner walls of the green alga *Rhizoclonium hieroglyphicum* while not effecting any elongation of the outer wall; it is indirectly indicated by Smith (136) that the inner wall is cellulosic, the middle wall made of pectin, and the outer one chitinous.

Likewise, indoleacetic acid effects marked elongation of *Fucus* germlings at down to 10^{-8} *M* [Davidson (137)]; of *Nitella* plants regenerating from isolated internodal cells at down to 10^{-6} *M* [Sandan *et al.* (138)]; of apical segments of the green alga *Codium* at 10^{-5} *M* [Williams (139)]. According to the best available evidence, all these forms have cellulose in their walls. [For *Fucus,* see (25 p. 24), (140), (141); for *Nitella* and *Codium,* see (142).]

On the other hand, when indoleacetic acid is applied to a wide variety of fungi whose walls are not constructed of cellulose, no effects are observed except in concentrations $\geq 10^{-5}$ *M* (134, 143, 144).

Brian (134) has studied the effects of griseofulvin on 51 fungi including representatives of every major group. All 38 of the chitin-walled fungi showed distinct morphological responses to as little as 3×10^{-7} to 3×10^{-5} *M* solutions depending upon the species. On the other hand, all of the 12 fungi without chitinous walls showed no response to 6×10^{-5} *M* solutions. This latter group included 10 öomycetes and two yeasts.

The responses of *Botrytis alii* hyphae were studied most carefully. The lowest effective concentration, 3×10^{-7} *M,* causes "a marked spiral wave of the hyphae without noticeable reduction in the growth rate"; 3×10^{-6} *M* causes "excessive branching and distortion of the hyphae"; 3×10^{-5} *M* "causes the production of highly stunted and gnarled germ-tubes, usually with spatulate extremities," yet even 3×10^{-4} *M* neither blocks germination of the conidia nor causes any detectable change in O_2 uptake. Brian presents cogent arguments for the view that the griseofulvin acts by somehow producing a diffuse increase in wall plasticity. Thus the griseofulvin syndrome resembles that produced in root hairs by calcium deficiency, an effect which is apparently mediated by a reduced deposition of calcium pectate, the hairs' chief skeletal element [Cormack (145)]. Banbury (143) reports that 3×10^{-4} *M* griseofulvin in lanolin produces comparable reactions when locally applied to the *Phycomyces* sporangiophore. When applied unilaterally to the sub-sporangial growth zone in stage IV, the cell bends away from the griseofulvin, presumably because of greater wall growth there. It also tended to bend to the right as viewed from the griseofulvin and to continue growth in a clockwise helix as viewed from above. Similarly, application of the griseofulvin paste to the tips of sporangiophores before sporangium formation again yielded such clockwise spiraling. It should be noted that this griseofulvin-induced "spiraling" both here and in Brian's study is "helicogenic"—it produces a permanent helical form—and is geometrically distinct from the normal "spiraling" of the *Phycomyces* sporangiophore, a process which consists of a rotation of the growing wall about the longitudinal axis so as to lay down a straight sporangiophore [Castle (146)]. The griseofulvin-induced helicogenic spiraling appears to be a cogent argument in support of Frey-Wyssling's suggestion (147) that normal "spiral growth" of *Phycomyces* arises from a helically proceeding

path of localized wall growth. A large increase in the amount of local growth per unit path length in the postulated process would generate a visible and permanent helix instead of an apparently straight structure [See Castle's criticism (146)].

Protoplasmic movement.—Kühn (148) has clearly reviewed Steinecke's (149) important study of protoplasmic movements during the reversal of polarity in *Bryopsis*. In brief, there is apparently a movement of an apically situated "plasma" (as indicated by vital dyes) as well as apically aggregated chloroplasts to the former basal region and a concomitant movement of a "basal plasma" (again as indicated by vital dyes) to the former apex.

Köhler (150) has confirmed and extended older observations of the extreme tendency of the protoplasm in the cells of the filamentous green alga *Chaetomorpha* to flow basally. Swarmers germinating at an air-water interface develop an elongate tube instead of a basal holdfast; in each cell of an old filament transferred to a fresh medium the protoplasm shows a striking basal accumulation before dividing transversely. If an old filament is ripped off the substratum, it does not regenerate a holdfast but protoplasm from successively more apical cells forces its way to the basal tip, ballons out and dies.

Buller (87) has studied and reviewed protoplasmic streaming in the hyphal fungi. In general, flow is observed toward growing tips and also toward regions of relatively high external osmotic pressure. Buller believes that the regions evacuated by the flowing protoplasm are filled up largely by synthesis of new protoplasm on rich media and by vacuole growth on poor ones. Vacuoles are said to move with the stream only at relatively high streaming rates. It is necessary to explain the discrepancy between stream rates which are commonly of the order of 1000 μ/min., and tip growth rates which are of the order of 10 μ/min. Buller's report suggests that at least part of the difference lies in the fact that the rapid streaming is only observed in a few main hyphae; the total cross section of the growing tips may well greatly exceed that of the transport hypha which feeds them. Moreover, in the hyphae of the phycomycetes, though not in those of the higher fungi, a counterflow is clearly seen in the periphery of the hyphae.

The migration of the pseudoplasmodium of the remarkable new species *Dictyostelium polycephalum* [Raper (151)] provides at least a superficial transition from the intracellular protoplasmic movements considered above to the amoeboid movements to be considered next. Both myxamoeboid movements (151, 152) and hyphal tip growth (35, 102) commonly proceed at rates of the order of 10 μ/min.; the thickness of the *D. polycephalum* pseudoplasmodium (about 50 μ), is comparable to many hyphae; the slime sheath is at least superficially comparable to the hyphal wall.

It is in order to comment upon the intensively investigated phenomenon of aggregation in the cellular slime molds, a subject recently reviewed by

K. B. Raper (153). Except where counter indicated, this discussion refers to *D. discoideum*. In 1953, Raper reported that the process spreads out from collecting centers (19); in 1940 he reported that at low amoebal densities, amoebae reach the collecting centers from relatively distant points by means of conspicuous streams, rather than directly (154). In 1947, Bonner demonstrated that the initial stage in the aggregative movement was produced by positive chemotaxis (155). From these simple facts, it could have, but was not, deduced that a chemotactic chain mechanism is at work. That is, the chemotactic agent, acrasin, induces its own secretion, with a chain of such events starting at the primordial collecting centers. In 1953, Shaffer reported a critically important experiment which, among other things, made the above conclusion much more obvious, if not more compelling (156). Acrasin-sensitive amoebae were sandwiched between a block of agar and a supporting slide. The wash from centers was applied to the meniscus surrounding the block. Within 5 to 10 min., the amoebae began to move directly toward the nearest edge of the block and soon thereafter to form streams indicative of induced acrasin secretion (152, 156). Moreover, Shaffer pointed out that this chain mechanism is not only present, but that it increases the sizes of the aggregates far beyond the possibility of the direct mechanism (152).

The agar block experiments also demonstrated that acrasin in the wash from newly forming centers had a half life of the order of minutes. Obviously the more unstable a substance is, the more rapidly its concentration will decline on a trip from its source. Hence, Shaffer argues, an unstable chemo-orienting agent will be less likely to leave cells in the position of the ass between the hay stacks. Moreover, Shaffer presents a qualitative argument to show that as long as a source produces the chemotactic agent rapidly enough to maintain a concentration in the Weber-Fechner range, a cell can be lured to it over an indefinitely extended range by an unstable substance but not by a stable one (152). Additional conviction and clarity are lent by a mathematical argument. Routine solution of the diffusion equation for the case of a spherical source steadily emitting a substance suffering first order decay into an infinite medium yields the following simple result:

$$\frac{\Delta C}{C} = a\left(\frac{1}{r} + \sqrt{\frac{.7}{DT}}\right)$$

Where ΔC is the concentration difference across the test cell of diameter, a, of a substance with diffusion constant, D, and half life T, and at a distance r from the center of the source. (This expression never falls below $a\sqrt{.7/DT}$ which is 4 per cent where $a = 10\mu$, T = 100 seconds, $D = 5 \times 10^{-6}$ cm.^{2}/sec.)

An interesting delay was also observed in the agar block experiment. "A single addition of a concentrated acrasin solution (free of acrasinase)

produced dramatic orientation within 2-3 minutes" (152). It seems almost impossible to account for this lag on the basis of diffusion times. The dialyzable molecules of acrasin are very unlikely to have a diffusion constant, D, lower than 2×10^{-6} cm.²/sec. (157 pp. 13–14). For points close enough to the edge of the agar block and short enough times, the solution for the diffusion of a solute from an infinitely long column of solution into a similar column of solvent should be an adequate approximation. Using this model, one finds where $D = 2 \times 10^{-6}$ cm.²/sec., that a point 0.1 mm. from the block's edge will reach one-tenth maximal concentration in 26 sec. It seems a fairly safe conclusion, then, even from this preliminary report that there is normally a lag of at least a minute and probably more between chemotactic stimulus and response.

Now, Raper has found that *D. polycephalum* during aggregation sometimes exhibits conspicuous concentric zones of more and less concentrated amoebae; the wavelength being about .08 mm. [(151) Plate 2, Fig. 6]. It may be suggested that a stimulus-response lag plays a critical role in producing this phenomenon, as well as the "ripples . . . a few amoeba-lengths between their crests" reported by Shaffer (21) to appear in a film by Arndt of *D. mucoroides*. Each sparse zone would represent a region left behind by the group of stimulated amoeba proximal to it in the lag period before the stimulus was relayed from the proximal group to the next most distal group. The speed of chemotactic movement in the agar block test, which was of the order of 10 μ/min. or one amoeba length per minute is consistent with this interpretation (152).

Using the agar block experiment as a bioassay, both Shaffer (152, 158) and Sussman *et al.* (159) have presented strong evidence that the instability of acrasin is due to an enzyme. By various methods they inactivated and removed the putative enzyme and obtained stable crude concentrates of acrasin. Sussman, moreover, has presented preliminary evidence suggesting that acrasin consists of at least two components.

Bonner grafted the anterior section of a migrating pseudoplasmodium, labeled with a vital dye, on to the rear end of another intact pseudoplasmodium (160). The grafted front piece was then seen to migrate faster than the host so as to reach its front end within a few hours. Bonner puts forward the interesting suggestion that a process of velocity selection plays a part in determining the ultimate cell fate. In support of this, Bonner presents some evidence that even under normal circumstances, some of the individual cells move at rates different from the pseudoplasmodium as a whole. In assessing the significance of this process, one may note that of the 10 (?) members of the Acraseae carefully studied, only two, *D. discoideum* and *D. polycephalum* [Raper (151)], have a migratory phase, and even in *D. discoideum,* growth under relatively high temperatures or other slightly adverse conditions eliminates the migratory phase [Bonner (161); Raper (154)]. On the other hand, a process of velocity selection may well

play a general, if limited, role during aggregation. Thus, Shaffer (90) reports that nonaggregated myxamoebae show wide and persistent individual variations in their rates of turning in the absence of an acrasin gradient, and in their time of becoming reactive to acrasin gradients. Moreover, in aggregation streams, individual cells are sometimes seen to slip past each other.

Raper & Fennel (162) have published a study of "stalk formation in *Dictyostelium*," primarily *D. discoideum*. It includes a meticulous description of the process, an analysis of the sorophore sheath, and an analysis of the physical forces involved in the upward growth of the sorophore. It is argued that the two principal forces responsible for this process are "the swelling of stalk cells entrapped in the elongating sorophore sheath" and the surface tension of the sorogen, while a second and progressively diminishing force results from the coordinated pseudopodial movements of the myxamoebae that comprise the sorogen." Considering that the stalk cells swell to about five times their initial volume after deposition within the sorophore sheath, there can be no question that their swelling contributes a substantial fraction of the elongation of the cells after entrapment; however, that swelling and surface tension play a major, or indeed any, role in forcing cells into the sheath is open to serious doubt: (*a*) The only initial ascension rates published (those for fruiting bodies with relatively large sori) are those of Bonner *et al.* (163) and these vary from .8 to 1.3 mm./hr. These figures are little lower than the maximum rates of pseudoplasmodial migration found by Bonner *et al.* (164), namely 2 mm./hr.; the pseudoplasmodium, of course, is propelled solely by "pseudopodial movements." (*b*) It is true, as Raper & Fennel emphasize, that a diminishing fraction of the sorogen cells retain their power of amoeboid movement as ascension proceeds; it is likewise true that the rate of ascension diminishes during culmination (163). Moreover, it is notable that these cells which retain the power of locomotion always remain in a mass touching the sorophore sheath, which thus provides the necessary substratum for their traction. (c) It is true that the upper cells of the sorogen are oriented transversely to their axis of motion; but this is likewise true of the analogously situated anterior cells of the migrating pseudoplasmodium (161). (*d*) Recently Raper (153) has described the remarkable new species, *Acytostelium leptosomum*. In this form, the stalk is acellular

Other polar gradients.—Heitz (49) reports that *Funaria* spores germinating under relatively dim white light sprout chloronemas first and rhizoids later or not at all. If, however, 6×10^{-11} *M* indoleacetic acid (or sometimes less) is added to the medium, then the spores sprout a rhizoid and no chloronema. This plainly suggests a significant role for auxin in determining the spore's polarity.

Various experiments suggest roles of indoleacetic acid in the establishment or maintenance of both the apicobasal and dorsiventral polarity o

the liverworts: extracted *Lunularia* tips yield an auxin as determined by the *Avena* curvature test, which a chromotographic study suggests consists solely of indoleacetic acid [La Rue & Narayanaswami (165)]. 10^{-5} *M* indoleacetic acid applied in an agar block to the cut apical tip of a *Lunularia* thallus whose apex was removed inhibits both apical regeneration and the germination of gemmae in the brood cups (165). As little as 6×10^{-9} *M* indoleacetic acid allows the dark germination of gemmae, which, because they developed on thalli in relatively little light, would not otherwise have germinated [Fitting (166)]. As little as 6×10^{-7} *M* indoleacetic acid weakens the directive influence of various external agents on the development of dorsiventral polarity in the gemmae of *Marchantia* [Kohlenbach (47)]. (See p. 364 of this review.)

Bonner (160) has vitally dyed vegetative amoebae of *D. discoideum* with neutral red, etc. With the otherwise undefined "beginning of differentiation" in the pseudoplasmodium, "the anterior region of presumptive stalk remains dark, while the posterior presumptive spore region in a matter of 10 to 15 min. bleaches considerably. The division line between these two regions is invariably clear and sharp." If one makes a "transection just at the division line between the bleached posterior section and the dark anterior section, then soon afterward the posterior end will again acquire a dark tip, and the anterior section will begin to blanch at its posterior end." It appears difficult to avoid the conclusion that the blanching and darkening represent processes of reduction and reoxidation.

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