Experimentally induced aberrations in the pattern of differentiation in the cellular slime mould *Dictyostelium discoideum*

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SUMMARY

This paper describes a set of perturbatory experiments designed to elucidate aspects of the mechanism by which the normal pattern of differentiation is specified.

Experiments are described which investigate the alterations in development seen in aqueous environments, with changes in humidity and with the introduction of permeable and impermeable barriers.

The following results are reported:

(i) The pattern of differentiation and the various morphologies of fruiting bodies formed when cell masses are placed in or on drops of buffer.

(ii) The alteration of the ratio of cell types formed in aqueous environments in the presence of urethane, mercaptoethanol or EDTA.

(iii) Humidity dependent changes in polarity and the increase of the number of developmental axes with humidity.

(iv) The formation of two developmental axes in cell masses bisected by impermeable barriers and a special case of bisection which induces the whole cell mass to form spores.

(v) The induction of all of any of a cell mass enclosed in a ‘cellulose’ tube to form a tissue ultrastructurally demonstrable to be the same as that of stalk.

These results are discussed in relationship to the work of other authors and the problem of the specification of the patterns of differentiation in the slime moulds. The results are presented in support of a model proposed previously by the author in which the pattern of the two differentiated cell types is inherent in the morphogenetic changes of culmination, and essential requirements of such a model are correlated with these observations.

INTRODUCTION

The cellular slime mould has become a popular experimental system in developmental biology because of its ease of culture and relatively simple pattern of differentiation (Bonner, 1967). The simplicity of synchronous culture and the easily definable morphogenetic sequences have led to the well-established studies of temporal patterns of biochemical changes and attempts have been made to extrapolate, from the knowledge of such sequences, information on the control of morphogenesis and differentiation (Sussman & Sussman, 1969).

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Another currently much used class of experiments designed to elucidate the
control of differentiation is that in which the developing system is perturbed in
some fashion and observation made of the consequent changes in development
(Raper, 1940; Farnsworth, 1973).

There are in the literature several well-established experimental alterations in
the pattern of differentiation of the slime mould. Alteration of physical para-
eters in the environment – for example, light and humidity – can alter the
morphogenetic sequence and the pattern of development (Newell, Telser &
Sussman, 1969). Gerisch (1968) reported that the polarity of a developing cell
mass could be altered by placing it at an air/water interface and showed that
the direction of fruiting body formation and hence the polarity of differenti-
ation was always aligned with respect to the aqueous phase. Gross morphological
changes can be induced by many chemicals which simply block development, by
interfering with RNA or protein synthesis (e.g. Sussman, Loomis, Ashworth &
Sussman, 1967). More subtle alterations of the patterns of differentiation can
be induced by the external application of urethane or mercaptoethanol (Gerisch,
1961). Not only can the relative spatial arrangements of the stalk and spore cells
of the differential cell mass be altered but also the relative numerical proportions
can be altered by temperature (Bonner & Slifkin, 1949), by changes in certain
ionic species like lithium (Maeda, 1970) and by cyclic AMP which alters the
ratio maximally in inducing the formation of stalk cells alone (Bonner, 1970).

This paper describes some more of these perturbatory experiments, all of
which are of a primarily physical nature, and gives the resulting alterations in
the patterns of development seen in aqueous environments, with changes in
humidity, and with the introduction of permeable and impermeable barriers
and tubes.

I have recently proposed (Farnsworth, 1973) a model for culmination in the
slime mould in which the invariance with cell number of the ratio of stalk to
spore cells seen in the fruiting body is a result only of the process of fruiting
body construction. The model proposes that the spatial pattern of the two
differentiated cell types is not specified by a signalling system at any stage of the
life-cycle but is inherent in, and a direct consequence of, the morphogenetic
changes of culmination.

One essential feature of the model is that the differential fate of a cell is de-
dependent only upon its position within the culminate; cells enclosed within the
cellulose stalk sheath are induced to form stalk, those that do not enter the stalk
sheath become spores. Where possible the results of this paper are related to
the predictions of such a model.

METHODS

The organism used throughout was the haploid wild-type Dictyostelium
discoideum NC4, which was grown on SM agar in association with Escherichia
coli Bjr at 22 °C.
The staging system used to describe the morphology of the cell masses was as follows:

(8) Aggregation streams disappear.
(9) Surface of cell mass becomes smooth.
(10) Apical tip begins to develop.
(11) Extension of tip begins.
(13) Migration begins.
(15) Migration ends.
(16) Cell mass becomes upright.

These figures correspond approximately to the time in hours that each stage appears after placing washed interphase amoebae on to a non-nutrient agar surface at 22 °C.

Aggregates for aqueous cultures were placed in M/60 phosphate buffer at pH 6-0 for all experiments, and any additional chemicals dissolved in this. The hanging drops were made on 22 mm coverslips which were then sealed on to 'Nucleon' tissue culture chambers, forming watertight transparent incubation chambers.

The cellulose tubes were made by evaporating celloidin wool from mixtures of propanol and acetone, as follows (from Grabar & de Louriero, 1936).

<table>
<thead>
<tr>
<th>Nominal pore diameter (nm)</th>
<th>2000</th>
<th>200</th>
<th>5</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrocellulose (g)</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Propanol (ml)</td>
<td>6.0</td>
<td>3.0</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Acetone (ml)</td>
<td>4.0</td>
<td>7.0</td>
<td>9.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

It was found, however, by examination in the electron microscope that these pore diameters varied by an order of magnitude from batch to batch, dependent upon many factors such as the humidity of the room and the speed of evaporation and that, without elaborate control, sizes were not reproducible. The procedure did, however, always give a set of tubes with graded pore diameters, though their absolute diameters were unknown. It was also noted that the nominally impermeable tubes always had some pores, usually around 5 nm diameter, and these could not be eliminated.

The linear barriers were cut from Melanex type 0, 100 μm gauge (I.C.I. Ltd.), and even though their insertion into the aggregate was controlled by the graduated scale on a micromanipulator, the ease of distortion of the agar and the aggregate meant that any position was only reproducible to within about 10% of the height of the aggregate.

Humidity gradients were set up in a Perspex box with a liquid reservoir at the bottom and a constant stream of air pumped over the surface at a height of 10 cm. A 'Shaw' moisture meter was used to determine the value and stability of the gradient, by placing the sensing probe through holes in the side of the box.
Needles with aggregates on their tips were then fixed to the side of the box at varying heights from the liquid surface.

All experimentally induced differentiation was examined with a binocular microscope, then by high-power phase-contrast and finally by electron microscopy.

For electron microscopy the following schedule was used:

**Fixation.** Karnovsky fixative, 1/8 strength, in 0.05 M cacodylate buffer, pH 7.1, at 5 °C for 60 min.

**Post-osmification.** 1 % OsO₄ in 0.05 M cacodylate, pH 7.4, at 5 °C for 30 min.

**Dehydration.** Ten min each at 5 °C in 50, 70, 90 % ethanol. Ten min each at 5 °C in three changes absolute ethanol.

**Infiltration.** Using Spurr’s (1969) standard resin; 50:50 ethanol:resin for 30 min at 5 °C; 2 changes, 3 h each in resin at 5 °C.

**Embedding.** In standard resin at 60 °C for 12 h.

### RESULTS

#### Development in aqueous environments

**Buffer**

If a cell mass from any stage of development from stage 10 to 16 is placed in a drop of buffer and incubated in the dark at 22 °C in a sealed chamber as a ‘hanging drop culture’, a fruiting body is always formed within a period of 14 h. The morphogenetic sequence is limited to the formation of a tip, the elongation of the cell mass by stalk formation along an axis aligned with the tip, and simultaneous spore formation. The pattern of differentiation appears almost normal. If the drop is large enough to prevent the aggregate reaching the suspending glass surface the cell mass lies at the lowest point of the drop and, in 60 % of the cases observed, a normal fruiting body is formed, the sporophore rising into the air away from the liquid surface (Fig. 1A). In another 20 % of the cases part of the rising spore mass was left on the stalk as the main spore head rose in the air (Fig. 1B) and the remaining 20 % formed normal but multiple fruiting bodies from one aggregate, usually fragmenting into one large and one small one (Fig. 1C).

If the drop is small enough to allow the aggregate to reach both the glass surface and the surface of the drop, the aggregate becomes firmly attached to the glass and a fruiting body is formed, the lower part of which is beneath the liquid. In such situations only 2 % of the cases observed formed normal single fruiting bodies (Fig. 1D), whilst about 20 % formed two or three fruiting bodies from one aggregate (Fig. 1E). In the majority (80 %) of the cases an apparently normal fruiting body with the spore mass trapped at the air/water interface, and the stalk extending beyond, was formed (Fig. 1F). About half of these cases had, on closer examination, bifurcate stalks hidden beneath the spore mass, which either rejoined (Fig. 1G) or remained separate to give a Y-shaped fruiting body (Fig. 1H).
Pattern formation in Dictyostelium

Fig. 1. Alternative fruiting body morphologies following culmination in hanging-drop culture.

Urethane

If the buffer in which the cell mass is suspended is supplemented with urethane the pattern of differentiation seen is markedly altered. Table 1 shows the cumulative results of the effects of various concentrations of urethane on different stages of development. Concentrations below 0.05 M do not affect morphogenesis or differentiation as compared with hanging-drop controls, and concentrations of 0.15 M or over kill all the cells within 3 h. However, at a concentration of 0.1 M abnormal morphogenesis and patterns of differentiation are seen and this pattern appears to depend on the stage the cell mass has reached when the exposure to urethane begins. As demonstrated in Fig. 2 true stalk cells, defined by having thickened angular cell walls with no visible cytoplasm (Fig. 4B) and which show fluorescence when treated with Calcofluor white as described by Harrington & Raper (1968), are always formed, but their relative proportions appear age-dependent, a stage 9 or 10 aggregate forming much more stalk than
Table 1. *Effect of urethane on the pattern of differentiation*

<table>
<thead>
<tr>
<th>Stage of initial chemical treatment</th>
<th>Concentration of urethane (M)</th>
<th>Resultant cell population as mean % of total</th>
<th>Typical morphology</th>
<th>No. of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After 12 h 'Stalk'</td>
<td>'Spore'</td>
<td>Dead</td>
</tr>
<tr>
<td>8–16</td>
<td>0·05 or less</td>
<td>30</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0·1</td>
<td>30</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>0·1</td>
<td>45</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>10</td>
<td>0·1</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>0·1</td>
<td>15</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>12</td>
<td>0·1</td>
<td>15</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>13</td>
<td>0·1</td>
<td>20</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td>14</td>
<td>0·1</td>
<td>20</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>0·1</td>
<td>20</td>
<td>45</td>
<td>35</td>
</tr>
</tbody>
</table>

Note: The typical morphology refers to the shape of the cell population after urethane treatment.
Pattern formation in Dictyostelium

Fig. 2. Changes with the age of the cell mass of the pattern of differentiation induced by 0.1 M urethane. □, Spore; ■, stalk.

Fig. 3. Alternative morphologies following the insertion of a linear barrier. (A) Side view of barrier insertion. (B) Barrier only just into aggregate, the culminating sporophore avoids the barrier. (C) Barrier two-thirds of the way into aggregate gives a pile of spores. (D) Barrier bisecting aggregate gives two new axes and two fruiting bodies.
any others. Another notable feature is that the stalk cells are always formed in
discrete regions, usually only one, or more rarely two, contiguous groups of cells,
all displaying the stalk properties after 12 h incubation and not altering after
this period (see Fig. 5 A, B).

The formation of spores is more complicated, though again apparently age-
dependent. After 12 h incubation many spore-like cells are visible, these being
smaller than the amoebae, condensed, oval to elliptical, with a smooth outline
and little substructure visible; they do not, however, take on the refractility
characteristic of the spores under phase-contrast (see Fig. 5 A). After a further
6–12 h incubation almost all of these cells have disappeared and the number of
dead cells, characterized by the spherical swollen appearance, with a coarsely
granulated cytoplasm and a central vacuole occupying at least two-thirds of the
cell volume which often contains bacteria showing Brownian movement,
increased correspondingly (Fig. 5 B). In all cases of treatment with 0.1 M urethane
in aqueous environments a significant proportion of the cells are killed and a
normal fruiting body is never formed, though the differentiated cell mass is not
always amorphous as indicated by the sketches in Table 1.
The use of either mercaptoethanol or EDTA in the hanging-drop cultures again showed disruption of morphogenesis and differentiation, but failed to reveal any evidence of age dependence of the effect. The minimal effective concentration of EDTA was $2.5 \times 10^{-2} \text{M}$, and after 12 h incubation in such a medium all stages had differentiated into $35 \pm 5\%$ stalk and $55 \pm 7\%$ spore, with the other $10\%$ of the cells being dead. These are average deviations from 15 replicates of each of 7 different stages. In such cultures the stalk cells again were closely spatially associated, and the spores appeared to remain unaltered in number with time after the first 12 h.

Similarly with mercapoethanol the cells became, within 12 h, $40 \pm 5\%$ stalk and $45\%$ spores, the spore proportion dropping to $5\%$ after 25 h, leaving $55\%$ of the cell population dead; the results being the same whatever stage was initially used.
Table 2. Effect of humidity on the number of axes developed by a cell mass supported on a needle

<table>
<thead>
<tr>
<th>Humidity (% R.H.) at 20 °C</th>
<th>% fruiting bodies</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Double</td>
<td>Multiple</td>
<td>Amorphous</td>
</tr>
<tr>
<td>Low 94</td>
<td>55</td>
<td>23</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Medium 96</td>
<td>25</td>
<td>38</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>High 99</td>
<td>4</td>
<td>26</td>
<td>30</td>
<td>40</td>
</tr>
</tbody>
</table>

Means of 120 determinations. Standard error, ± 4 %.

Humidity-dependent changes in the polarity of differentiation

Gerisch (1968) showed that the polarity of the subsequent development of a mechanically agglomerated cell mass could be controlled by placing it at an air/water interface, the culmination always being away from the aqueous phase, and when it was placed in water in the interstices of a net, at an air/water/air interface, two developmental axes were induced, one to each side. This work prompted investigation of such a procedure on naturally aggregated amoebae. Cell masses from stages 8 to 16 were each placed in phosphate buffer + 200 μg/ml streptomycin in the apertures of 200- or 400-mesh nylon nets. These were then incubated vertically in the dark in an atmosphere of saturated water vapour, at 22 °C for 24 h. It was found that whatever age the initial stage the same result was attained: 24 % of all samples gave one fruiting body, 50 % gave two fruiting bodies, 24 % gave three or four fruiting bodies and 2 % showed no differentiation (see Fig. 5 C). As an extension of the idea of providing alternative air/water interfaces, aggregates were also placed at the tips of needles, the moist surface round the aggregate providing an almost continuous air/liquid interface round the whole cell mass. The pattern of differentiation seen following incubation of aggregates on needles was found to be not age-dependent but directly related to the humidity of the incubation atmosphere. Apparently normal morphogenesis of culmination occurs and fruiting bodies are often formed but, as summarized in Table 2, it is apparent that at low humidities, in the majority of cases only one or two tips and consequent developmental axes develop but, as the humidity is increased, the tendency to form many developmental axes is increased (Fig. 5 D). Under all incubation conditions, a proportion of the cell masses, which increases with increasing humidity, shows terminal differentiation but abnormal morphogenesis; the cell mass differentiates into regions of stalk (35 % of the cell mass) and spores (65 %) but the aggregate has no characteristic gross morphology (Fig. 5 E) though the normal yellow pigmentation of the spore head develops after 36 h, colouring the whole cell mass.
Pattern formation in Dictyostelium

As Bonner & Dodd (1962) demonstrated, there is a gaseous hormonal system controlling the orientation of the developmental axes in the slime mould, whereby the attitude of a culminating cell mass is influenced by its immediate physical environment such that the distance from the final position of the spore head to any solid substrate is maximal on all sides. Experiments using impermeable external sheet barriers have been carried out to determine whether a similar direct physical influence can induce changes in the pattern of differentiation, but it was found that wherever external barriers were placed round developing cell masses the tip moved to, or was reformed at, the nearest available unobstructed position, and culmination occurred normally, in the direction dictated by the tip. However, it was found that the placing of impermeable barriers within the cell mass did alter the subsequent pattern of differentiation. If a barrier was placed up to half-way (± an estimated 10%) into a cell mass (see Fig. 3A) the tip moved or was reformed and morphogenesis continued normally, the barrier being physically ejected, or if it was firmly fixed into the agar, simply avoided (Fig. 3B). If the cell mass was completely bisected by the barrier, two tips and two new developmental axes, giving two normal fruiting bodies, were seen irrespective of the prior developmental state of the cell mass (Fig. 3D). It was found, however, that a special case could be induced with the barrier between the two extremes described above. If the barrier was pushed an estimated two-thirds of the way into the cell mass (again the actual distance of penetration being 60% of the total height of the cell mass, ±10% due to the error in the techniques used) the tip was destroyed but did not re-form, the whole cell mass differentiating into a pile of spores (Fig. 3C). This occurred in 80% of the cases which did not form one or more normal fruiting bodies; the other 20% of these formed spores and discrete regions of stalk in a ratio of 2:1, and again appeared age-independent, but was most easily performed on stages 9–11.

Development with cylindrical barriers

It was found whilst experimenting with permeable cellulose barriers that the mechanical process of fruiting could, in part, be mimicked. The first step seen during culmination is the formation of a cellulose tube beneath the tip, delineating the region where stalk formation is initiated (Raper & Fennel, 1952). This was simulated by inserting tubes of cellulose nitrate into preculminating cell masses, whereupon it was found that all the cells included in such tubes differentiated into stalk cells, even if a whole cell mass was pushed into one, providing that the tip was destroyed in the process. In this context stalk cells were identified as cells showing at least a twofold increase in diameter and the characteristic angular thickening of the cell walls (Fig. 6), and also giving fluorescence when treated with 'Calcofluor T' as described by Harrington & Raper (1968) as typical of cellulose. This distinguishes the cells from dead ones which show
Fig. 6. Electron micrographs of (A) normal stalk, (B) stalk induced by enclosure in cellulose tube. A, undifferentiated amoebae outside the stalk sheath but enclosed by slime sheath; S, slime sheath; C, cellulose stalk sheath; W, angular cellulose walls of stalk cells.
only a slight volume increase and a distinct spherical central vacuole having no thickened cellulose walls with angular corners. If the tip was left mechanically undisturbed when the cell mass was put into a tube, so that the aggregate was merely surrounded by the tube, only in contact with it at the base on the agar surface, almost normal terminal morphogenesis occurred; a fruiting body being formed in the lumen of the tube, along its axis. ‘Cellulose’ tubes with varying permeabilities with a range of average pore diameters from 0 to 400 nm were prepared as described by Grabar & de Louriero (1936). All these tubes caused enclosed amoebae to differentiate into stalk, irrespective of pore diameter, though examination by electron microscopy of the supposedly impermeable membrane showed it to have pores of 5 nm diameter in it. Naturally occurring carbohydrate tubes such as the hairs from nettle leaves and the spines from the rear legs of cockroaches failed to show induction of stalk cells, as did glass capillaries and glass capillaries lined with ‘cellulose’. Direct induction of stalk formation by the same ‘cellulose’ in forms other than in tubes was found to be impossible. Simple contact with sheets of ‘cellulose’ or the implantation in the cell mass of sheets or rods of ‘cellulose’ or of stalks from other fruiting bodies all failed to induce stalk cell formation.

DISCUSSION

In the light of the results of Gerisch (1968) it is not surprising that cell masses, when placed in hanging drops, produce fruiting bodies, but it is interesting to note that all stages of morphogenesis when placed in such a situation proceed straight to culmination with the same time course. This demonstrates unequivocally that the migrating grex stage is not essential for the specification of the normal pattern of differentiation, and gives further evidence against the notion that internal cellular rearrangement occurs at this time in order to give pre-patterns for differentiation (Bonner, 1959; Farnsworth & Wolpert, 1971). A similar conclusion can be drawn from the induction of two developmental axes from one grex when it is placed in a net at an air/water/air interface. The only limitation to the formation of normal fruiting bodies in hanging drops appears to be that part of the aggregate must be outside the water, and it is at this point that the tip is formed and the developmental axis initiated. This would seem to suggest that, either the site of tip formation is dependent upon an external inhomogeneity which is not present under water, or that immersion in water interferes with some local internal inhomogeneity or signal at the tip site. It is interesting that culmination can occur with all of the cell mass except the very tip submerged, as evidenced by stalks being formed within the liquids such that they touch the suspending glass surface and hold spore heads outside the drop.

The aberrations in pattern seen in fruiting bodies formed in hanging drops appear to be simply mechanical; the spore head becomes trapped by the physical
forces at the meniscus whilst the stalk rises out into the air. It may, however, be that the tip region is so much wetter than usual that the normal internal inhibitory system which prevents more than one tip being formed fails, allowing double tips to form and hence double stalks (Farnsworth, 1973).

The alterations of the patterns of differentiation by the three chemical treatments used, agree in general with the findings of Gerisch (1961, 1968). The preferential age-dependent alterations of the pattern by urethane in such systems would appear to be a direct chemical effect, and it seems explicable when one considers that the sequence of biochemical differentiations is rigidly ordered in time and co-ordinated with the stages of cytodifferentiation. Whilst it is extremely difficult to deduce or even guess which of the events is being specifically altered by the urethane, the result does imply, as one would quite reasonably intuitively suppose, that the accumulation of a sufficient number of biochemical events is a prerequisite of cytodifferentiation, and that this competence arises with age, and furthermore that the prerequisite biochemical changes necessary for spore differentiation are possibly larger in number or occur later than do those necessary for stalk formation. This finding of the differentiation of cell types being related directly to a temporal sequence of events is predicted by my model and it is certainly consistent with the model that the initiation of spore differentiation occurs later, or more slowly than, that of stalk formation. As the undifferentiated cell mass rises up the stalk as it is formed, there must be a source of undifferentiated cells which can enter the cellulose stalk sheath and become incorporated into more stalk. This requires that at least some of the stalk is formed before spore formation is irreversibly initiated.

The failure of EDTA and mercaptoethanol to show age-dependent variations in their effect on differentiation adds a further complication which it appears can only be resolved by answers to very specific biochemical questions, as to the nature of the actual process which is being affected.

The fall in ‘spore’ numbers between 12 and 24 h after treatment with urethane and mercaptoethanol has two likely explanations; either the cells are not fully differentiated, as histologically appears to be the case, or the spores are forming and then germinating and dying. This latter explanation would also explain why the spore number does not fall in the EDTA-treated cells, where the EDTA is inhibiting germination and so preventing the cell from damage. A similar argument would explain previous reports that mercaptoethanol preferentially induces differentiation into stalk cells (Gerisch, 1961), since if it did not inhibit germination the liberated amoebae could be killed by osmotic damage, leaving only stalk cells.

Further evidence that the migratory stage is not essential for normal pattern formation is given by the experiments where any developmental stage can produce, directly, a normal fruiting body when placed on a needle. The experiments clearly show that, as the humidity increases, the tendency to form more than one tip increases, and each tip gives one fruiting body. The extreme case of this
Pattern formation in Dictyostelium is that no tip at all is formed and hence no fruiting body; effectively the cell mass is tip all over its surface and no axis is formed, but differentiation still occurs. This result implies that the determination of the site of tip formation is the sole deciding factor in the specification of the developmental axes (Farnsworth, 1973) and that the site of tip formation is influenced by humidity. Speculation as to possible mechanisms for this provides several alternatives, the most likely of which appear to be those involving drying of the slime coat of the cell mass, with its concomitant alterations in surface tension or mechanical pressure, or those in which there is an aqueous disruption of some internal or external chemical inhomogeneity or gradient.

The results of the experiments in which impermeable plastic barriers were inserted in cell masses demonstrates some of the properties of the system by which the slime mould specifies its developmental fields. The results show that two new developmental axes can be formed, and an existing one negated by bisecting the cell masses at any stage, the temporal aspects of this phenomenon having been reported elsewhere (Farnsworth, 1973). This implies that the tip, as suggested by the work of Raper (1940), is the organizing region which dominates the cell mass by some communicatory system which is disrupted by impermeable barriers. Experiments with permeable barriers (if materials of graded porosity could be found which had mechanical properties suitable for the physical process of insertion into cell masses) could provide useful information about the nature of the signalling system.

The most interesting result from such barrier experiments, where the whole cell mass differentiates into spores, suggests some further properties of the system which specifies pattern. It would seem that, if the barrier separates the cell mass enough to disturb the tip and the origin of the initial developmental axis, but not enough to allow two new independent axes to be formed, no new tips and no axes are made, but differentiation then proceeds, no stalk is formed and the whole mass becomes spores. This again lends much support to the proposed model, the barrier disturbing the old tip, but not enough to induce the formation of a new one. The absent tip cannot induce or direct the formation of the stalk sheath and so cells cannot enter it. There is thus no location within the culminate which has the conditions for inducing stalk formation, so, as differentiation proceeds, the whole cell mass becomes spores.

The differentiation, into stalk, of all of any cell mass which is enclosed inside a cellulose nitrate tube suggests a mechanism by which stalk is normally formed. As proposed earlier (Farnsworth, 1973), this observation firmly supports the hypothesis that cells are only induced to form stalk if they are enclosed in the cellulose sheath which initially delineates the stalk forming region (Raper & Fennel, 1952). This relatively simple property could account for a large part of the normal pattern of differentiation, and obviates the need for any dynamic signalling system for tissue proportioning, the imposition of pre-pattern and the determination of cell types prior to culmination. A simple sequence of depend-
encies can lead to the induction of the correct proportion of stalk given that only cells which are enclosed in the stalk sheath are induced to form stalk. Assuming the rate at which cells enter this sheath is proportional to its diameter, and the diameter of the sheath is governed by the diameter of the tip of the culminate and finally that the diameter of the tip is proportional to the diameter of the cell mass (related possibly by a simple parameter like surface tension), then a fixed proportion of any size cell mass will form stalk. (For a simple arithmetic demonstration of this see Farnsworth, 1973.)

The mechanism by which enclosure in a cellulose tube induces stalk formation is not clear. However, it is easily shown that it is not simply a mechanical process, enclosure in tubes of other materials does not have the same effect. Furthermore the phenomenon is not simply one of direct chemical induction by contact with cellulose. Rods and sheets of cellulose nitrate or the stalks from other slime moulds all failed to give the same effect when introduced into the cell masses.

The formation of stalk cells within the cellulose tube appears to be neither directly physical (e.g. pressure) nor directly chemical, though it has been shown that some compounds by direct action can induce stalk formation. The most likely explanation appears to involve both physical and chemical factors. If the sheath were to act as a 'filter', keeping, by selective binding, inclusion or exclusion, some molecule or even ion (see Maeda, 1970) within or from its lumen, the resultant inhomogeneity could then affect the choice of the cells to form stalk. Though the work reported here has failed to show, as one would expect were this mechanism true, any direct correlation between stalk induction and the pore size of the cellulose nitrate tube used to induce it, it may be that the discriminating effect occurs over a very narrow range of, or at a very small, pore size.

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REFERENCES


Pattern formation in Dictyostelium


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