A QUANTITATIVE STUDY OF CELLULAR RHEOTROPISM

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ABSTRACT Sparsely sowed, hence independent *Botrytis* spores, which are fixed to the wall of a laminar flow chamber, tend to germinate downstream. For a velocity, v_∞, one cell radius from the wall, of 0.1 to 1000 μ/second, this tendency, expressed as a percentage of perfect orientation, approximates $9 \cdot \log (10\ v_\infty)$. Indirect proof is given that this rheotropic response is mediated by the convection across each cell of a diffusible stimulator emitted by it. Analysis of the response indicates that this stimulator has a diffusion constant of the order of 10^{-7} cm^2/second and thus is macromolecular, and a half-life of the order of 10 seconds and thus a radius of action in a stagnant medium of about one cell diameter. The rate of germination falls so slowly with increasing flow rate as to indicate a much lower sensitivity of the rate than of the localization of growth to this stimulator's concentration.

INTRODUCTION

Organic forms may be viewed as the result of a pattern of localized and oriented expansion and—in the case of animals only—contraction. Expansion and contraction in turn may be viewed primarily as outward or inward movements of cellular surfaces, whether these be relatively flexible and reversible in movement as in animal cells or rigid and irreversible in movement as in plant cells. In many cases, *e.g.*, growing nerve axons and fungal hyphae, the regions of rapid expansion are quite restricted and persistent during development. A possible link in the loop(s) which presumably start and maintain such expansion sites would be a diffusible local growth stimulator kept just outside them.

The role of such extracellular substances in locally controlling expansion could be broadly explored by gently forcing the medium to flow and carefully observing the resultant form changes. Indeed, Bonner *et al.* have made effective, if essentially qualitative, use of this notion (1-3), and a number of crude observations of flow effects are also found in the older literature (4-6). One purpose of this study is to broaden the method by applying it in an initial case to essentially isolated cells (previous studies were all on multicellular systems) and to deepen it by a quantitative approach.

Fungal cells are a favorable object for such a study since the frequency and course of hyphal anastomoses has long suggested the significance of extracellular local tip growth stimulators (7). In particular, such observations were made long ago upon hyphae of *Botrytis,* a common imperfect fungus parasitic upon many higher plants (8). There is even a very old and crude report—one in which the term, rheotropism was coined—of growth downstream in young *Botrytis* hyphae and upstream in older ones (4).

To simplify quantitative study, our measurements were restricted to the direction of germination of *Botrytis* spores grown under steady flow. The idea (Fig. 1) was to

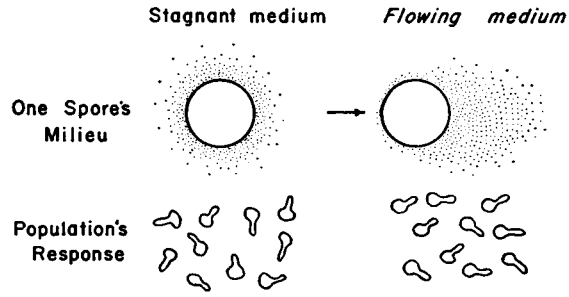

FIGURE 1 Idea of the experiment.

wash stimulator away most effectively from each cell's upstream pole leaving it relatively concentrated to leeward and thus to effect a tendency to grow downstream.

MATERIALS AND METHODS

Source of Botrytis Spores. The strain of *B. cinerea* was that used before (9), and yields ovoid spores averaging about 8 × 11μ. It was maintained by serial culture on 5 mm–deep layers of autoclaved 6.5 per cent Difco Sabouraud dextrose agar in 5 cm pyrex Petri dishes, which were kept at 23°C in dark and sealed 2 liter cans from innoculation until harvesting. Concentrated stock suspensions of spores in inert liquid FC-75[1] were prepared by shaking spores from four 2-week-old cultures into 40 cc of this fluid and straining out cell clumps through a nickel screen perforated by 20μ diameter holes.[2] These were stored at 4°C in the dark. A test showed no fall in germinability after 60 days of such storage, but suspensions more than 3 weeks old were not used in this flow study.

Sowing Spores. Dry spores were fixed to the nickel-reinforced polyethylene roofs of the flow chambers shown schematically in Fig. 2. They were evenly distributed in the desired region(s) and concentration(s) as follows: Such a roof was clamped between a plastic block (electrified by rubbing with filter paper) and a 3mm-thick aluminum stencil. A 2mm-deep aliquot of an appropriate concentration of a suspension of spores in FC-75 was squirted into a channel of the stencil. A minute later the cells had thus been electro-

[1] Fluorochemical 75 made by Minnesota Mining and Manufacturing Company, St. Paul: "Principally isomers of perfluoro cyclic ether $C_8F_{16}O$."

[2] Type 125W made by Perforated Products Company, Brookline, Massachusetts.

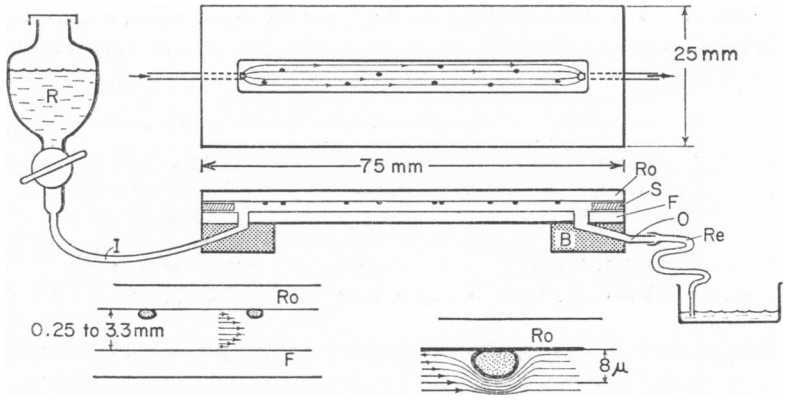

FIGURE 2 Diagrammatic views of the flow chamber. Source and sink of fluid relatively larger than shown. Supports, clamps, connectors, and dark box not shown. R = Two liter reservoir; I = no. 18 black Teflon inlet tube; Ro = Roof of 0.1 mm thick polyethylene reinforced with nickel screen. (This was made by fusing two layers of 50μ-thick polyethylene at 128°C with a nickle screen, 50μ thick containing 60 per cent open area produced by a hexagonal array of 1.3 mm diameter holes. This is the 15 *sp* screen of Perforated Products Incorporated, Brookline, Massachusetts.) S = Sides of Teflon separator; F = Floor made of quartz microslide perforated by 1 mm inlet and outlet holes; 0 = no. 18 Teflon outlet tube; Re = Resistor of long Teflon tubing; B = Support block of type 5052 aluminum.

statically precipitated on the desired surface of the polyethylene. Then the bulk of the now cell-free fluorochemical was removed, the roof freed from the sandwich, and the residual fluid quickly evaporated. Similarly, but without the need for a bounding stencil, spores were precipitated on the bottom of polystyrene Petris used in companion cell interaction studies.

Wetting Spores. Each spore-bearing roof was clamped on to a flow chamber (Fig. 2). To wet the spores and begin their development, each stopcock was opened and growth medium from the reservoir filled its chamber in less than 1 minute. To avoid trapping large bubbles under regions of highly concentrated cells, it was necessary to fill chambers having such areas with medium degassed by boiling and then continue perfusion with the standard aerated medium. Similarly, in the companion cell interaction studies, spores were first wet with degassed medium which was replaced by aerated fluid; here this procedure served to avoid the persistence of minute bubbles between pairs of nearby cells.

The forces acting upon the cells during filling, particularly by the air-water interface at the fluid front, are relatively large. So to help minimize cell movements, a Teflon block electrified by rubbing was placed on *top* of the roof during filling only. The number of cells detached from the roofs during filling (and afterwards) was negligible. However, the cells slid and turned so much during filling as to suggest that residues of this brief but violent process might orient germination. Such ambiguities were minimized by filling different chambers, subsequently run at different flow rates, in an identical way. Such equal filling was attained with groups of chambers covering a one hundredfold rate range by varying only the resistance tubing after the chambers and attaching these components *after* filling the chambers.

Medium. The standard medium, used in most experiments, was a 0.07 per cent Czapek-Dox broth, made up from reagent grade salts dissolved in water redistilled from glass, aerated, and filtered through a type HA millipore filter. Its composition follows:

Ingredient	Per Cent	Molarity
Sucrose	0.06	1.75×10^{-3}
$NaNO_3$	0.006	7.1×10^{-4}
K_2HPO^4	0.002	1.15×10^{-4}
KCl	0.001	1.34×10^{-4}
$MgSO_4 \cdot 7H_2O$	0.001	3.8×10^{-6}
$FeSO_4 \cdot 7H_2O$	2×10^{-5}	7.2×10^{-7}

This standard medium was supplemented with about 0.7 per cent 15,000 CPS Methocel HG[3] to raise its viscosity one hundredfold when so desired; the viscosity was checked with an Ubbelohde viscometer.

To minimize contamination of the medium by the flow apparatus, no grease was used (except for some vaseline applied between the support block and the quartz floor in a few accessory experiments). Thus the only materials in contact with the medium (other than the cells) were glass, quartz, Teflon, polyethylene, and, to a very limited extent, salt water–type aluminum. Both fluid leakage and air entrapment were avoided despite the lack of grease in a rather loose-fitting system by raising the reservoir above the flow chamber so as to maintain a head of about 7 cm of water within it.

Flow Control. All flow chambers (Fig. 2) were 65 mm long internally and the steady flow following filling was always driven by a head of 30 cm water. Other pertinent values are shown in Table I. The flow speeds, v_∞,[4] were those found one cell radius, or 4μ, from the roof and far from any cell. They were calculated with a formula which holds near one of two parallel planes bounding a Newtonian fluid undergoing laminar flow (10).

$$v_\infty = 6B\Delta/wh^2$$

where B = bulk flow rate
Δ = distance from boundary (4μ in this case)
w = chamber width
h = chamber height

(1)

In cases 2*a*, 2*b*, and 4, this formula was confirmed by measuring the velocity of minute carbon particles near the roof. Furthermore, evidence of laminarity in the chamber as a whole and hence, the reliability of equation (1) is provided by calculating Reynolds numbers:

$$\mathrm{Re} = h\bar{v}\rho/\eta$$

where $\bar{v}$ is the average flow speed or B/hw
ρ is the fluid's density
η is the fluid's viscosity

(2)

The highest Reynolds numbers involved (case 4*b*) was 4, far lower than 1000, the

[3] A hydroxypropyl methyl cellulose made by Dow Chemical Co., Midland, Michigan.

[4] *Frequently Used Symbols: a,* radius of cell; *D,* diffusion constant; v_∞, free stream velocity or velocity one cell radius from wall; V_1, per cent orientation $= \Sigma\, p\cos\Theta$; η, viscosity.

TABLE I

FLOW CHAMBER CHARACTERISTICS

Case	v_∞	Viscosity	Bulk rate	Height	Width	Cells/mm²	S_{max}*
	μ/sec.	*cps*	*cc/hr*	*mm*	*mm*		
1	0.1	1	2.4	3.3	15	2	0.15
2	1		24.	3.3	15	4	0.14
2*a*			1.6	1.0	10		
2*b*		100	24.	3.3	15		
3	10	1	16.	1.0	10	8	0.13
3*a*			240.	3.3	15		
4	100		18.	0.50	5		0.06
4*a*			160.	1.0	10		
4*b*		100	18.	0.50	5		
5	1000	1	45.	0.26	5		0.04

* S_{max} is a calculation, using equation (8) of Appendix I, of the highest possible background concentration of any stuff emitted by the cells relative to the concentration it would have at the surface of an isolated cell in this system. It is obtained by considering a cell at the downstream end of the flow chamber, a completely stable substance, and one with the highest plausible diffusion constant, $1 \cdot 10^{-5}$cm²/second.

value known to characterize the transition to turbulence for flow between parallel plates (11).

Furthermore, the flow around each cell is shown to be locally laminar by calculating the Reynolds number for a submerged sphere (11):

$$\mathrm{Re} = 2a\rho v_\infty/\eta \tag{3}$$

Approximating v_∞ by the flow rate one cell radius from the wall, one obtains a range of Reynolds numbers of $1 \cdot 10^{-7}$ to $1 \cdot 10^{-4}$ for our experiments. These are safely below the critical figure of 0.1.

Temperature and Light Control. All experiments were run in a room thermostated at 23°C. Observations of germination rates in the "dark" were done with red light found in separate tests not to effect this process. "Blue light" was obtained by filtering the radiation from a 100 watt tungsten bulb through 10 cm of 5 per cent $CuSO_4$.

Determining Growth Orientation. Previously described methods for measuring and characterizing the orientation of the cells' outgrowth, (9, 12) were modified only by a device to speed recording of the measured angles. A ring of fixed electrical contacts, each nearly 10° wide, and connected to a separate electromechanical counter was placed under the goniometer eye piece. Associated circuitry was such that after the operator had rotated the eye piece to align its reticle with a particular germ tube, pressing a pedal recorded one count in the correct 10° interval. In this way, the time required to measure and record about a hundred angles and thus characterize the orientation of one population is reduced from about an hour to 15 minutes. Calculation of the statistical parameters used to characterize these distributions were greatly speeded by the Johnson Foundation's Control Data 160-A computer.[5]

[5] This facility is supported by Public Health Grant FR 15.

In all cases we counted only cells separated by a gap of more than one long spore axis from any other cell.

THE RHEOTROPIC PHENOMENON

Dry and sparsely sowed—hence essentially isolated—cells were fixed to the roofs of straight flow chambers (See Fig. 2, Table I, and Appendix I). The chambers were filled with a simple sugar-salt solution thus initiating development. From the time

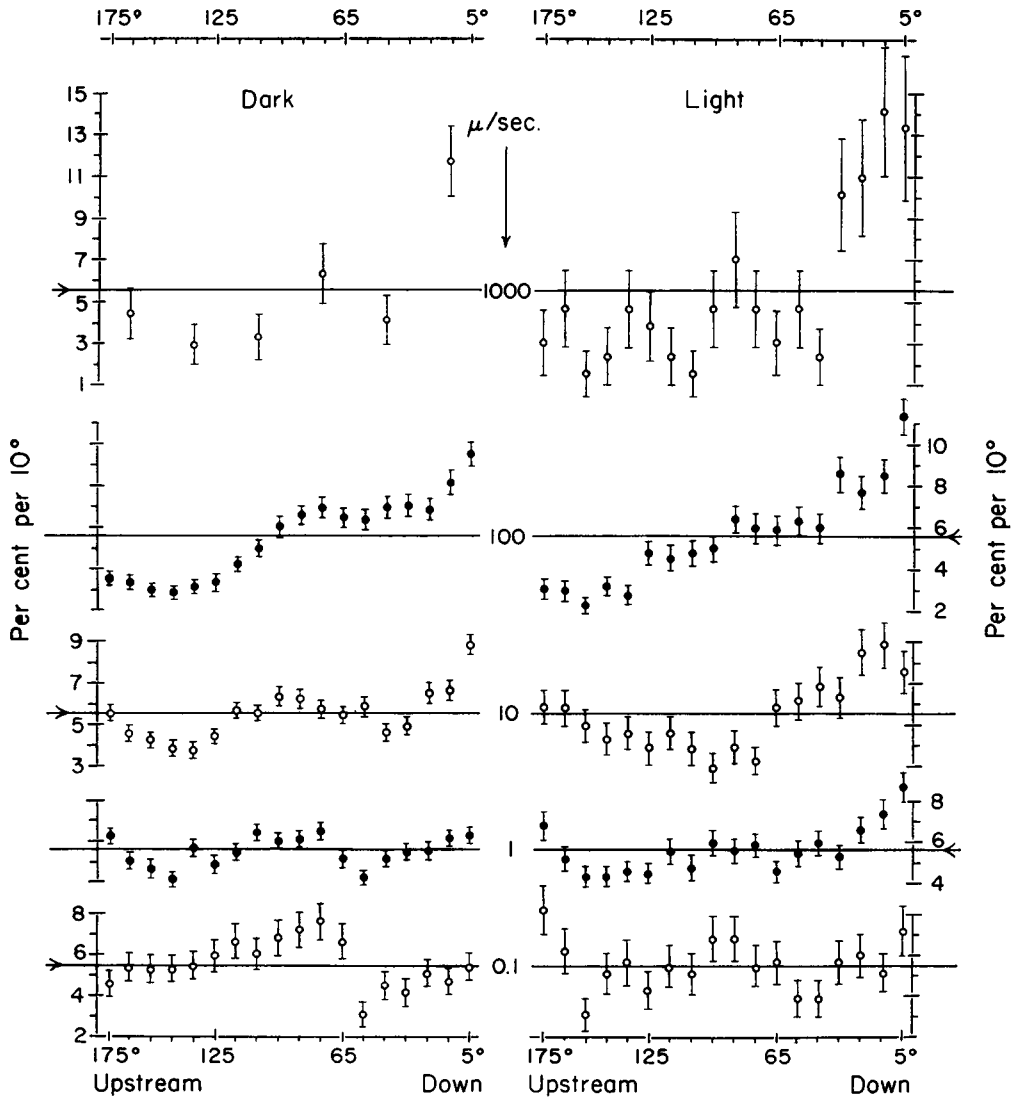

FIGURE 3 Pooled outgrowth distributions in response to flow.

the cells were thus wet until the time at least 90 per cent of them had formed one outgrowth, steady laminar flow of this medium was maintained through each chamber. Then the cells were killed with ultraviolet light and the distributions of their outgrowth angles determined.

The degree of any rheotropic effect should depend upon the flow rates near the cells, *i.e.*, within a few cell diameters of the roof. Here the flow rate rises linearly with distance (starting from zero at the boundary of course); we characterize the flow pattern in any chamber by the velocity, $v\infty$, one cell radius or 4μ from the roof, and far from any cell. The effects of velocities, $v\infty$, of 0.1, 1, 10, 100, and 1000 μ/second were tested both upon cells in chambers kept in darkness and ones lit from above with 50 μwatt/cm^2 of blue light.

In Fig. 3 we have pooled and graphed all of the resultant distributions. Accessory data appears in Table II. For each condition, per cent of all outgrowths per 10° interval is plotted against direction, 0° being downstream and 180° upstream. The horizontal reference lines represent uniform distributions; these would have 5.6 per cent of their outgrowths within every 10° interval. The vertical bars indicate standard errors.

The most obvious deviation from randomness in these distributions is an accumulation downstream which increases with flow rate, first becoming evident with dark-grown cells at 10 μ/second and with light-grown ones at 1 μ/second. In order to characterize the degree of this downstream tendency (or so called negative rheotropic effect) in each distribution, we calculate an appropriate parameter, essentially the average cosine, that we call the unipolar orientation, V_1, and express as a per cent:

$$V_1 = \Sigma p \cos \theta \tag{4}$$

where p is the per cent of outgrowths in the angular interval centered on θ. These orientation values appear in Fig. 4 and Table II.

TABLE II
THE RHEOTROPIC PHENOMENON

	Flow rate v_∞	No. of runs	No. of chambers	No. of angles	Orientation V_1
	μ/sec.				*per cent*
Dark	0.1	2	5	847	-2.6 ± 2.4
	1	9	25	3490	2.2 ± 1.2
	10	5	15	2900	9.6 ± 1.3
	100	5	16	2858	23.4 ± 1.3
	1000	2	2	88	25.1 ± 7.4
Light	0.1	1	2	487	-0.2 ± 3.2
	1	5	10	1433	8.0 ± 1.8
	10	1	3	642	12.7 ± 2.8
	100	3	7	1243	26.5 ± 1.9
	1000	2	2	127	34.4 ± 6.0

Note that for a population all growing exactly downstream, $V_1 = +100$ per cent; upstream, $V_1 = -100$ per cent; while for a uniform distribution, $V_1 = 0$ per cent. The standard deviations recorded are values theoretically characteristic of a so called circular normal distribution containing the measured number of angles accumulated to the degree, V_1 (12).

For both dark- and light-grown cells statistically significant orientation downstream first appears at a flow rate, v_∞ of 1 μ/second, rises at about 10 per cent per decade until 100 μ/second and probably continues to rise at a slower rate until 1000 μ/second. However, at all speeds tested, the dark-grown cells are a bit less oriented; the dark response curve would coincide with the light one if it were moved about one half log unit or 3-fold toward lower flow rates. It is our tentative judgment that the light curve is the more reliable one, the dark cells' orientation being disturbed by their greater tendency to originate toward the roof.

For the purpose of succinct description, as in our abstract, it may be noted that the light curve can be approximated as:

$$V_1 = 9 \cdot \log (10 v_\infty) \tag{5}$$

A roofward tendency would indirectly reduce flow sensitivity in two ways: first, the most effective level of the fluid would be closer to the roof and hence slower. Secondly, there might be more movement of the cells by the mechanical reaction to germ tubes growing against the roof. We have two evidences for such a tendency: first unilateral light of the sort used is known to elicit germination slightly away from, in fact at about 115° from, the light (9). Secondly, we made some measure-

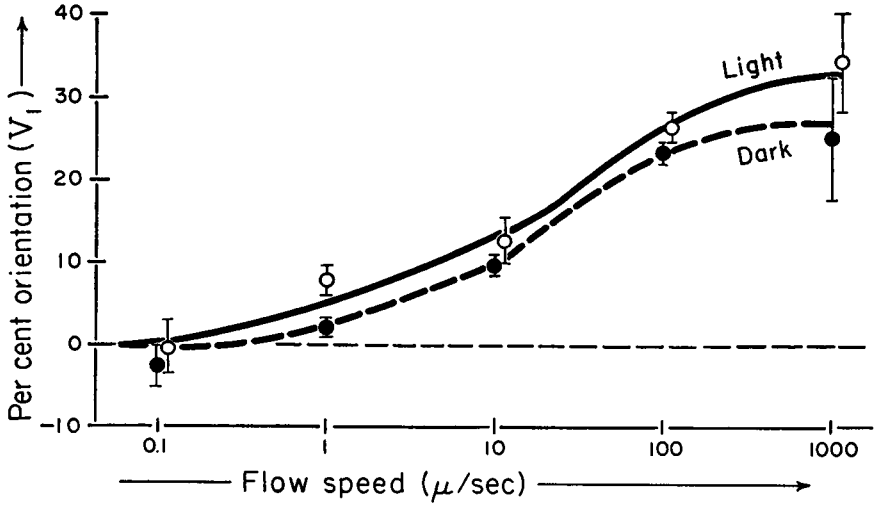

FIGURE 4 The rheotropic phenomenon. Tendency to germinate downstream *versus* flow rate, v_∞, one cell radius from the wall.

ments, shown in Table III, of the distances from the roof of the germ tube origins of light- and dark-grown cells. These were made after the germ tubes were several spore diameters long, and the reaction to outgrowths starting very near the roof would soon tend to push their origins away. Hence the true tendency of the dark-grown cells to grow toward the roof must be even greater than measured.

The distributions plotted in Fig. 3 may also show weak peaks cross-stream at about 90° and perhaps upstream at 0°. Since these minor peaks seem uncorrelated with flow rate, we consider them to be artifacts. The cross-stream artifact is probably a joint consequence of the following two facts: (*a*) The cell movements which

TABLE III

OUTGROWTH ORIGINS WITH RESPECT TO ROOF

Distance from roof (in cell radii)	0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
No. of outgrowths in dark:	1	2	5	4	1	4	4	0	0	0
No. of outgrowths in light:	0	0	0	2	7	6	2	3	0	1

occur during wetting showed a weak tendency to leave the ovoid spores with their long axes parallel to the flow direction. (Measurements on 804 cells in 8 chambers yielded a distribution showing 9 ± 3 per cent of such orientation). (*b*) About two thirds of the cells are found to germinate more nearly across than along their own long axes.

PROOF OF CONVECTIVE MECHANISM

A combination of evidence proves that the observed downstream growth is effected by convection of a stimulator across each cell. We first present three positive confirmations and then arguments gainst five alternative explanations:

1. *Growth of neighboring cells toward each other.*[6] Plainly, the supposed stimulator might well manifest itself in the centripetal growth of nearby cells. As Table IV shows, they do show such a tendency:

Spores were sown at 50 cells/mm^2 on the bottoms of polystyrene Petris and cultured under 5 mm of unstirred medium. Some were grown in darkness and some under white light coming from above; this included about 100 μwatt/cm^2 of energy in the blue and thus was probably very similar in its effects to that used in the main studies on flow. After germination each dish was scanned for all pairs of cells which were *not* in actual contact, separated by a gap of less than one short cell

[6] This particular phase of the work was executed by Dr. Erica Kronfeld while holding a Public Health Service traineeship in developmental biology.

TABLE IV

GROWTH OF NEARBY CELLS TOWARD EACH OTHER

	Runs	Dishes	Cells	Scored plus
				per cent
Dark	7	18	766	74.7 ± 1.6
Light	6	15	708	76.1 ± 1.6

diameter (*i.e.*, $< \sim 8\mu$) but at least three cell diameters from any third cell. Each outgrowth in such a semi-isolated pair was scored plus or minus if it began more nearly toward or away from the vicinal cell respectively. In both dark- and light-grown cultures about 75 per cent of the outgrowths were marked plus, that is began relatively centripetally.

2. *Interference by upstream cells.* It should be possible to swamp out the supposed convective gradient of stimulator across a cell with brackground material coming from other cells upstream, provided only that these latter are sufficiently close and numerous. Hence such an upstream source should greatly reduce or eliminate the rheotropic effect. Table V summarizes evidence that this does indeed happen:

Cells were distributed in flow chambers in three regions:

A. An upstream, control area, 18 mm long and bearing cells so sparsely sowed (8 cells/mm^2) as to be essentially independent.

B. A midstream, source region, 10 mm long and containing cells so concentrated

TABLE V

EFFECT OF CONCENTRATED UPSTREAM CELLS ON RHEOTROPISM

v_∞	Region:	*A*, upstream	*B*, midstream	*C*, downstream
100μ/sec	Dark No. chambers	4	3	6
	No. cells	426	680	901
	Orientation (*per cent*)	17 ± 3	8 ± 3	18 ± 2
	Light No. chambers	4	3	6
	No. cells	456	653	869
	Orientation	24 ± 3	7 ± 3	27 ± 2
1000μ/sec	Dark No. chambers	2	3	3
	No. cells	88	616	419
	Orientation (*per cent*)	25 ± 7	12 ± 3	32 ± 3
	Light No. chambers	2	3	3
	No. cells	127	627	515
	Orientation (*per cent*)	34 ± 6	15 ± 3	35 ± 3

(1300 cells/mm^2) as to raise high the ratio, S of the background concentration of a stable substance to that maintained at its own surface by each cell, both in the rear of B and in:

C. A downstream, test region extending from 1 to 19 mm beyond the border of B and again containing relatively dispersed cells (15 cells/mm^2). (We got minimum S values of 7 and of 2 to 5 for the observed parts of B and C respectively by assuming D to be 10^{-7} cm^2/second in equation (9), Appendix I.)

In both dark- and light-grown cultures, in the region centered about a distance 4 to 12 mm beyond B's border, there is *not* any depression of rheotropism; if anything there is actually a small enhancement relative to the control area. Within the rear part of B, itself, however—and this is the point—orientation is reduced to about 40 per cent of its normal value.

These results, then, again appear to support the convective mechanism but obviously indicate that the supposed stimulator must be very unstable.

3. *Correct dependence on velocity.* In the companion paper (13), a model is analyzed to yield the steady-state concentration gradient across a spherical source of a diffusible stuff as a function of the free stream velocity, or, to state the truth more fully, of the so called Peclet number:

$$\begin{aligned} \mathrm{Pe} &= a \cdot v_\infty / D \\ \text{where } a &= \text{sphere's radius} \\ v_\infty &= \text{free stream velocity} \\ D &= \text{stuff's diffusion constant} \end{aligned} \qquad (6)$$

One obvious simplification is that the model sphere is fixed in the stream by imagination rather than attachment to a wall. Nevertheless, it is our judgment that the model is applicable if, as the dual use of v_∞ implies, the velocity one radius from the wall is taken as the free stream velocity. Comparison of the rheotropic curve (Fig. 4) with the model gradient curve (Fig. 5 of this paper or Fig. 4 in the companion paper) shows an obvious if rough similarity. The gradient curve rises at about 8 per cent per decade for two decades and starts to level off, reaching 22 per cent after three decades; the rheotropic curve (of light-grown cells) rises at about 10 per cent per decade for two to three decades and then seems to start levelling off, reaching 34 per cent after a total of three to four decades.

This similarity both supports the convective mechanism and suggests that *per cent growth orientation approximately equals per cent gradient evoking it.* Two arguments in turn buttress this latter inference and thus further support the convective mechanism:

First, it is notable that in the *only* cases in which cell orientation as a function of gradient have been measured—the orientation of *Botrytis* and *Osmunda* spores by light gradients (9)—per cent orientation does in fact prove to about equal per cent evoking gradient. Secondly, the 75 per cent centripetal fraction reported under point 2 for cells an average of one radius apart is about what this relation would

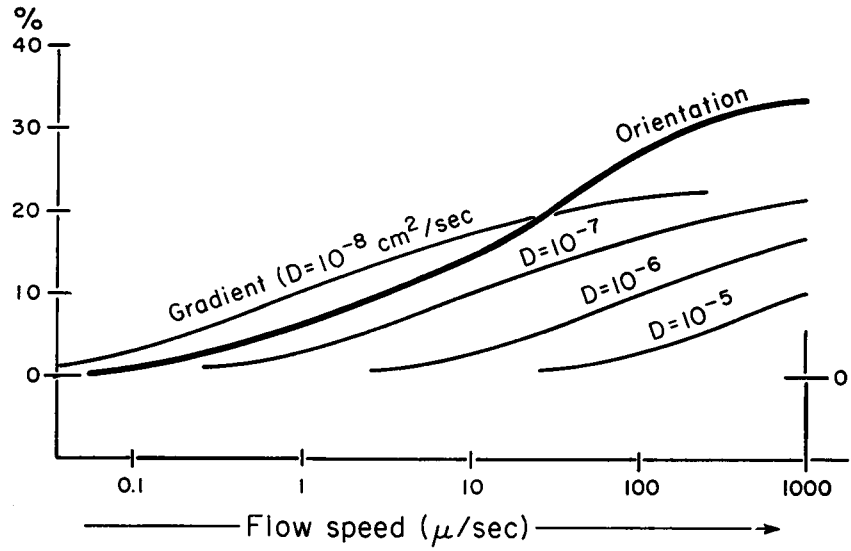

FIGURE 5 Comparison of the rheotropic curve (for light-grown cells) with the model gradient curves, the latter being drown for various assumed diffusion constants.

predict, at least using the very crude analysis so far available (see Appendix II). The force of this last point may perhaps be gauged from our guess that if the centripetal fraction had proven to be < 60 per cent or > 90 per cent, then the assumption that orientation equals gradient would be seriously shaken.

4. *Not a pressure effect.* Flow establishes a pressure gradient across each cell, pressure decreasing steadily from front to rear. One might easily imagine that this gradient somehow orients growth by deforming the ungerminated spores, or directly orients the outgrowths by swinging them downstream. However, we have examined these hypotheses and found them wanting.

A dimensional analysis shows that all surface forces in such a laminar flow system (whether pressures or shearing forces) are proportional to both velocity and viscosity (Appendix III). Hence both hypotheses are inconsistent with the logarithmic dependence of orientation on velocity. Also both are inconsistent with data (reported in Table VI) which show orientation to be unaffected by a 100-fold rise in viscosity; for on their bases such a rise should be equivalent to a 100-fold rise in velocity. [The viscogen, a 0.7 per cent methocel, was unlikely to hinder *diffusion;* for 0.5 per cent agar, which is likewise a dilute, long straight chain polysaccharide, hardly slows even a haemocyanin with a D of 1×10^{-7} cm²/second (14)].

The deformation hypothesis is rendered further unlikely by calculations which show the walls to sustain flow pressure differences (from front to rear) which are a very small fraction of the total pressure, of osmotic origin, which they sustain.

TABLE VI

VISCOSITY INDEPENDENCE OF RHEOTROPISM

v_∞	1 centipoise	100 centipoise
(μ/sec.)		
1	2,−3,−5,−2,−3,0 average −2 ± 1	−3,−6,0,−6,4,1 average −2 ± 2
100	10,8,21 average 13 ± 4	10,13,8,14,8 average 12 ± 2

Each value in the body of the table is a per cent orientation, V_1, obtained from about 200 outgrowth angles measured in one flow chamber. Data from two experimental runs are pooled.

The pressure difference, maintained by slow flow across a sphere (15) is given by:

$$\Delta P = 3\eta v_\infty / a \tag{7}$$

For v_∞ equal to 10 μ/second, a speed which evokes substantial orientation, this formula yields a pressure *difference* of only $6 \cdot 10^{-8}$ atmospheres. This is a minute fraction of the 10 to 100 atmospheres of osmotic pressure typical of the cells of fungi, like *B. cinerea* which are parasites of high plants (16).

The hypothesis that the outgrowths are swung downstream is further undermined by some direct observations reported in Table VII. While the germlings do undergo substantial rotations, the degree of orientation of populations of outgrowths is scarcely influenced. For this reason and because comparable rotations occur in the absence of flow, we have concluded that these rotations are adjustments to growth pressures against the roof, rather than to flow.

5. *Not a molecular orientation effect.* Calculations show that only im-

TABLE VII

ROTATIONAL INDEPENDENCE OF ORIENTATION

Experiment no.	v_∞	No. cells counted	Average rotation	Orientation	
				uncorrected	corrected
	μ/sec.			*per cent*	*per cent*
1	0	83	18°	—	—
2	0	96	10°	—	—
3	0	96	14°	—	—
4	100	62	3°	—	—
5	100	46	44°	16	17
6	100	87	47°	9	7
7	1000	38	13°	51	41

Rotation of each germling counted was determined by measuring the angle between the axes of its ovoid spore case and of flow, both just after wetting and at the experiment's end. The average rotations listed are of the absolute values of these rotations. The corrected orientations listed were obtained by subtracting the rotational angle of each germling from its outgrowth angle and then recomputing V_1 for the thus corrected population of outgrowth angles.

plausibly long molecules could be much oriented by the rheotropically effective velocities:

It is easily shown that near a sphere under Stokes flow (10), the maximum velocity gradient occurs at the equator and is given by

$$\beta \max = 1.5v_\infty/a \tag{8}$$

About 10 per cent rheotropic orientation occurs at v_∞ equal to 10 μ/second. Here, then βmax is only 3 second^{-1}. Now the angular distribution function for molecules slightly oriented by shear is given by Boeder in reference (17).

$$f \propto (1 + \beta \cdot \sin 2\theta/4\Theta) \tag{9}$$

where f is the molecular fraction lying at the angle Θ to flow

β is the velocity gradient

Θ is the rotary diffusion constant

Hence the molecular fraction at the 45° maximum will exceed that at the 135° minimum by only 2 per cent when $\beta/4\Theta$ equals 0.01. For β equal to 3 seconds^{-1}, even this minute degree of orientation, then would require a molecule with a rotary diffusion constant of 75 seconds^{-1} or one about 0.5μ long.

6. *Not convection of an inhibitor used up by the cells.* It might be supposed that the growth medium, despite its preparation from water redistilled in glass and low percentages of a few reagent grade salts, inadvertently contains effective traces of a locally acting inhibitor, and further supposed that this reaches the cells' lee poles at a diminished rate because it is used up at their front poles *en route*. This notion seems precluded by a crude dimensional argument which indicates that the supposed inhibitor would have to have a diffusion constant so low as to be macromolecular.

Such orientation should rapidly disappear below a certain flow rate because inhibitor would reach the lee pole by diffusion up or cross-stream. On dimensional grounds this threshold speed should be about D/a. The rheotropic curve indicates that this critical speed would be about 1μ/second. Hence D would be about 5μ × 1μ/second or 5 × 10^{-8} cm^2/second. Crude as this argument is, it should nevertheless suffice to preclude the action of the small inhibitors, *e.g.*, heavy metals, which are at all likely to be in the medium.

7. *Not a wetting effect.* Finally, logic compels us to consider the possibility that the rheotropic effect is caused by the very brief but violent process of initially wetting the cells and filling the flow chamber, rather than the subsequent steady flow. If this were true, then the apparent dependence of rheotropism upon steady flow velocity (Fig. 4) would really arise from some correlated variation in the wetting process. Suffice it to state that inspection of an appropriate graph of our data reveals no such correlation.

This notion is also inconsistent with the following experiment. Eight chambers

were perfused at a v_∞ of 100μ/second as usual. Thirteen hours after wetting, when 5 per cent of the cells had germinated, the flow in three test chambers was stopped but here as in the five controls (in which perfusion continued) the cells were not killed until 5 hours later, when >80 per cent of the cells had germinated. The control orientations were 23, 20, 19, 28, and 17 per cent for an average of 21 ± 4 per cent; the test ones, 2, 8, and, 9 per cent for an average of 6 ± 4 per cent. Apparently, most orientation occurs in the last quarter of the pregermination period, let alone the 1 minute wetting interval.

PROPERTIES OF THE STIMULATOR

Mobility. The model developed in the companion paper yields the gradient maintained by flow as a function of its rate for any assumed diffusion constant. These gradient curves are compared to the rheotropic curve in Fig. 5. It is argued, under point 3 above, that per cent growth orientation approximates the per cent gradient evoking it; though considerations such as the somewhat greater steepness of the rheotropic curve suggest that orientation may exceed evoking gradient a bit. Hence 10^{-7}cm^2/second is considered to be the most probable order of magnitude of D. This corresponds to a molecular weight of 10^5 to 10^8 (17).

Stability. The 40 per cent depression of orientation seen amidst very concentrated cells (point 2 above) is inferred to arise from a proportional rise in stimulator background concentration, C_B, effected by upstream cells. Thus:

$$\frac{\Delta C/(C_B + C_{SA})}{\Delta C/C_{SA}} \cong 0.4 \quad \text{or} \quad C_B/C_{SA} \cong 1.5 \tag{10}$$

where ΔC is the absolute concentration gradient across a cell and
C_{SA} is the average concentration at the surface of the isolated cell.

Then using equation (9) of Appendix I, the observed values of 1.3×10^5 cells/cm^2 for n and 3×10^{-3} cm for g, the average gap between the test cell and the first upstream source cells, one can calculate the length, $\bar{x}$ of the contributing zone of upstream cells. One thus gets values of 0.25 mm and 0.4 mm in the 100 and 1000μ/second flow rate studies, respectively; stimulator from more remote cells presumably decays *en route*. In such a flow system, one can estimate the message time t_m or average time taken by a molecule in moving from one cell to another a distance, l, downstream as:

$$t_m = 5\sqrt[3]{a^2 l^2 / D v_\infty^2} \tag{11}$$

If l is taken as $(0.5\ \bar{x} + g)$, then the half-life, $\bar{t}$ should approximate t_m. Using $D = 10^{-7}$cm^2/second, then, one obtains half-life values of 8 and 3 seconds, respectively from the low and high flow rate studies.

Radius of Action. We define the radius of action, $\bar{R}$ of the stimulator in a stagnant medium as:

$$\bar{R} = \sqrt{Dt} \tag{12}$$

Thus this biologically important value is a joint consequence of mobility and stability. Using $\bar{t} = 5$ seconds and $D = 10^{-7}\text{cm}^2/\text{second}$, one obtains 7μ or about one cell diameter for R; a plausible value for a cell growth localizer.

Finally, note that if one substitutes equation (11) in (12), it is seen that R has really been obtained by the relation:

$$R = 2.2\sqrt[3]{alD/v_\infty} \tag{13}$$

Hence it is only weakly effected by the error in D; a ten fold error in estimating D leads to only a two fold error in $\bar{R}$.

GERMINATION RATES

Our observations on germination rates under different flow rates are summarized in Table VIII. There is only a small—perhaps 10 per cent—decrease in germination rate as the flow rate increases from 0.1 to 100μ/second.

TABLE VIII

TIME FOR HALF OF CELLS TO GERMINATE

Flow rate (μ/sec.)	0.1	1	10	100
Experiment	*hrs.*	*hrs.*	*hrs.*	*hrs.*
1	10.0 ± 0.3	9.8 ± 0.1		
2	12.4 ± 0.6	13.6 ± 0.2	13.8 ± 0.3	
3		26.3 ± 0.5	26.0 ± 0.5	27.8 ± 0.7
4		8.2 ± 0.3	8.6 ± 0.3	9.1 ± 0.1
		Ratios of 0.98, 1.10		Ratios of 1.05, 1.10

Figures given are average of four chambers in each case. In any one experiment, spore batch, chamber dimensions, and flow rate were the same in all chambers.

Assuming D to be $10^{-7}\text{cm}^2/\text{second}$, the Peclet number rises from 0.04 to 40 over this range. According to the model then, a 60 per cent fall in the average surface concentration of the stimulator (13) effects only a *10 per cent* fall in rate while a 16 per cent increase in concentration gradient effects a *27 per cent* orientation rise. Evidently the localization of growth is far more sensitive to the concentration of stimulator than is its rate. Such a relation is usually found with light, and we would suggest a general explanation. The level of adaptation to both light and to the tip growth stimulator is relatively unlocalized.

APPENDIX I

RELATIVE BACKGROUND CONCENTRATION IN A STRAIGHT FLOW SYSTEM

Consider a laminar perfusion system with a linear velocity profile and cells on the wall.

Consider a stable substance produced at the same rate by all the cells.

Consider the average concentration of this stuff at the surface of one test cell.

Let us find the swamping ratio, S, of the part of this material arising from all other cells in the system (the background concentration, C_B) to that part arising from the test cell (C_{BA}).

Let x be the coordinate in the flow direction with flow going toward higher x.

Case I. Cells uniformly distributed from $x = o$ to $x \geq \bar{x}$. Test cells at $x = \bar{x}$.

Let β be the velocity gradient.

Let q be the flux of stuff from cells on the wall.

Then C is given by the analogous Levecque solution for heat transfer from a uniformly heated wall (18).

$$C_B = 4.6q\sqrt[3]{\bar{x}\beta/D^2} \tag{1}$$

Let n be the cell concentration.

Let K be the rate of emission of the stuff per cell.

Obviously, $q = Kn$ and

$$C_B = 4.6Kn\sqrt[3]{\bar{x}\beta/D^2} \tag{2}$$

The concentration at the surface of an isolated spherical source in an infinite stagnant medium can easily be shown to be given by:

$$C_{SO} = K/4\pi aD \tag{3}$$

The companion paper (13) computes the ratio $\bar{C}_{SA}$ of the average surface concentration under Stokes flow to that in a stagnant medium. Then the average concentration at the surface of a sphere on the wall of a semi-infinite medium with a linear velocity profile should be given approximately by:

$$C_S = 2\bar{C}_{SA}C_{SO} = \bar{C}_{SA}K/2\pi aD \tag{4}$$

Hence using (2) and (4), one gets the desired ratio:

$$S = C_B/C_S = 29naC_{SA}{}^{-1}\sqrt[3]{\bar{x}D/\beta} \tag{5}$$

The Peclet number is defined in this system as:

$$\mathrm{Pe} = av_\infty/D \tag{6}$$

Hence:

$$S = 29na^{5/3}\bar{C}_{SA}{}^{-1}\mathrm{Pe}^{-1/3}\bar{x}^{1/3} \tag{7}$$

Let $\alpha = 29\ C_{SA}{}^{-1}Pe^{-1/3}$. *Hence:*

$$S = \alpha a^{5/3}\bar{x}^{1/3}n \tag{8}$$

where α is a function of Pe only. For convenience, Table IX lists α values, calculated from the C_{SA} values given in (13).

TABLE IX

α VALUES TO COMPUTE S VALUES

Pe: small	0.5	1	3	10	100	1000	∞
α: $29\mathrm{Pe}^{-1/3}$	40	36	30	25	21	19	18

Case II. Cell concentration, n is high from $x = o$ to $x = \bar{x}$; relatively low from $x = \bar{x}$ to $x = \bar{x} + g$ where test cell lies.

A minor variation of the above reasoning leads to:

$$S = \alpha a^{5/3}[(\bar{x} + g)^{1/3} - g^{1/3}]n \tag{9}$$

APPENDIX II

CRUDE ANALYSIS OF CHEMOTROPIC INTERACTION OF CELL PAIRS

Consider a point at a distance r from a spherical source (or cell) of unit radius. The concentration at r in the steady-state is:

$$C(r) = K/4\pi r D \tag{1}$$

Where K is the rate of emission of a diffusible stuff.

Normalize the system by letting $K = 4\pi D$. Then:

$$C(r) = 1/r \tag{2}$$

Consider two such cells separated by a small gap g.

Let Co be the concentration on the most distal part of one cell's surface; Ci, the most proximal.

In the steady-state, (using 2):

$$\begin{aligned} &Co \cong 1 \\ &Ci \cong 1 + 1/(1 + g) \\ &V_1 = \text{orientation} \cong \text{gradient} \cong (Ci - Co)/Ci = 1/(2 + g) \end{aligned} \tag{3}$$

Suppose that the orientation of the outgrowth is scored only as + or − depending upon whether an outgrowth is more nearly toward or away from the neighbor respectively.

$$\therefore\ V = f_+ - f_-$$

Where f_+ and f_- are the fractions of outgrowth, scored + and − respectively.

Since, $f_+ = (1 - f_-)$,

$$\begin{aligned} V_1 &= 2f_+ - 1 \\ f_+ &= (3 + g)/2(2 + g) \end{aligned} \tag{4}$$

Thus for g equal to one cell radius, approximately 67 per cent of the outgrowths would be expected to form proximally.

APPENDIX III

DIMENSIONAL ANALYSIS OF SURFACE FORCES EXERTED BY STEADY VISCOUS FLOW

On a submerged body

Let P = A surface force per unit area, *i.e.*, either a pressure or a shearing force.

Let l_1, l_2 $\cdots$ *etc.* be various characteristic dimensions of the body.
Obviously at any point on the body:

$$P \equiv (\eta)^b (v_\infty)^c f(l_1, l_2 \cdots)$$

$$\eta \equiv ML^{-1}T^{-1}; \qquad v_\infty \equiv LT^{-1}; \qquad l_n \equiv L; \qquad P \equiv ML^{-1}T^{-2}$$

$$\therefore b = 1 \quad \text{because of mass term}$$

$$\therefore c = 1 \quad \text{because of time term}$$

$$\therefore P \propto \eta v_\infty \tag{1}$$

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