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Negative Chemotaxis in Cellular Slime Molds

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This study confirms the suggestion of earlier workers that the vegetative amoebae of *Dictyostelium* repel each other while those of *Polysphondylium violaceum* do not. When *Dictyostelium* amoebae were placed in drops on thin and thick agar, the cells moved out faster on the thin agar, presumably because the repellent was more concentrated. This did not occur with *Polysphondylium* amoebae. Also, if 2 drops of cells were placed side by side, or a single drop was placed near an edge, in *Dictyostelium* there were fewer cells emerging between the drops (or near an edge) than on the far side. *Polysphondylium* showed no such difference. However, *Polysphondylium* amoebae were repelled by *Dictyostelium* cells (but not vice versa) when drops of each were placed beside one another. Finally, if *Dictyostelium discoideum* cells were placed in drops over thick and thin agar, but separated from the agar by a dialysis membrane, the cells again spread farther on the thin agar, indicating that the repellent is a dialyzable molecule.

The first evidence for negative chemotaxis came from the work of Samuel (5), who showed in *Dictyostelium discoideum* that vegetative or preaggregation cells tend to repel one another. He placed a concentrated drop of amoebae on non-nutrient agar and put a small cellophane square (covered with a thin layer of agar) near it. When a few of the cells escaping from the concentrated drop moved onto the cellophane, he turned the square 90°. Twenty minutes later, the cells made a right-angle turn and again moved away from the drop of cells. Another way of testing for negative chemotaxis was suggested by Lee Segal (personal communication): if the cells in a drop repel each other, the rate at which they move away should be linear with time, whereas if they leave the drop as a result of random motion, then their speed of outward movement should fall off as the square root of time. Adam (senior thesis, Princeton University, Princeton, N. J., 1973) showed that, indeed, preaggregation amoebae of *D. discoideum* did seem to spread linearly with time, but he also made the interesting observation that in another species, *Polysphondylium violaceum*, the cells move outward in a manner that appeared very roughly equivalent to the square root of time. If these preliminary observations are correct, they would suggest that *D. discoideum* has a repellent and *P. violaceum* does not. The present paper gives evidence that strongly supports such a contention.

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MATERIALS AND METHODS

The cellular slime molds used were: (i) *D. discoideum* NC-4H; (ii) *D. mucoroides* no. 11; *D. purpureum* no. 2; and *P. violaceum* no. 1. They were all grown on *Escherichia coli* B/r.

The slime molds were incubated on nutrient agar (buffered 1% peptone and glucose agar) for approximately 40 h at 21°C in the light. They were then harvested and centrifuged three times for 7 min each at 75 × *g* in 1% (10^{-4} M) standard salt solution (1). The washed amoebae were then suspended in 1% salt solution at a concentration of 10^8 cells per ml. Most of the experiments were run on 2% non-nutrient agar (Difco) in small plastic petri dishes (50 by 12 mm; Falcon no. 1006).

RESULTS

Experiments with deep and shallow agar. The design of experiments with deep and shallow agar followed the principle of those of Twitty and Niu (reviewed by Twitty, 6) for pigment cells in amphibians. Agar was poured into the petri dishes at two depths, 0.5 and 4 mm. Drops of washed amoebae were placed on the surface, and measurements were made of the distance travelled by the outermost cells after 2 and 4 h (see reference 4). *D. discoideum* amoebae moved out much further on the shallow agar than on the deep agar, which is what one would expect if a repellent were responsible for the outward movement of the amoebae (Fig. 1A, Table 1). The same conclusion can be reached for *D. purpureum*. The result is ambiguous for *D. mucoroides* and clearly reversed for *P. violaceum*, in which greater spreading oc-

curs on the deep agar. In this case one might postulate that an attractant plays a part and that since it is more concentrated in the shallow agar, the cells are attracted inward, countering the effect of the outward movement of the cells by random motion. Because the air space was different in the small petri dishes with the two depths of agar, a control with *P. violaceum* was run in which open petri dishes were placed in a moist chamber. The results (for 24 drops) were similar to those described in Table 1; clearly, there is no volatile component affecting the outward movement of the cells.

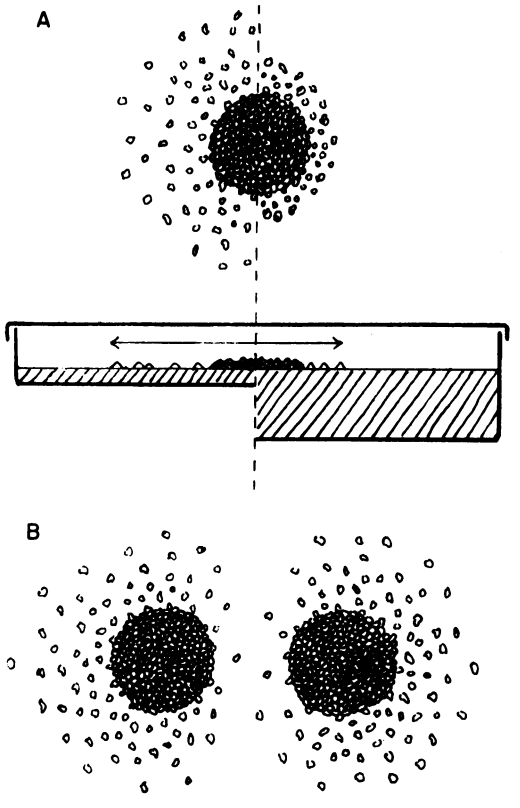

FIG. 1. *Diagrams showing the two basic types of experiments used to demonstrate negative chemotaxis. (A) Thin versus thick-agar experiment seen from surface view (above) and side view (below). Note that the cells spread farther on the thin agar. (B) Two drops of D. discoideum cells side by side on an agar surface. Note that the cell density between the drops is lower than in any other position.*

Experiments with 2 drops. In this sort of experiment, 2 drops were placed close to one another, and after approximately 2 h the number of cells seen through an ocular grid on the dissecting microscope (area, 0.86 mm^2) was counted in two places: on an edge close to the other drop of cells (inside) and on an edge farthest removed from the other drop (outside). If the drops are separated by a range of distances between 1.4 and 1.9 mm, then, as before, the *D. discoideum* amoebae appear to repel one another, whereas the *P. violaceum* amoebae do not (Fig. 1B, Table 2). When the drops are too close (<1 mm apart), then the cells from both drops mingle, making it impossible to record cell density in a meaningful fashion.

This kind of experiment was repeated with one major difference; a drop of *P. violaceum* cells was placed next to a drop of *D. discoideum* cells. It is clear that *P. violaceum* amoebae are effectively repelled by the *D. discoideum* drop but that the *P. violaceum* drop does not repel the *D. discoideum* amoebae (Table 3).

Experiments with cell drops near an agar edge. This is essentially the same experiment as described above, but instead of confronting 1 drop of cells with another, the agar is cut off sharply near the edge of a drop and kept in a moist environment. If the repellent is not vola-

TABLE 2. *Number of cells spread between 2 drops (inside) compared to the number of cells spreading where there are no opposing drops (outside)*[a]

Species	Mean no. of cells/ mm^2 (± SD)		No. of cases	Probability (2-tailed *t* test)
	Outside	Inside		
D. discoideum	106 (±17)	83 (±20)	18	0.001 (3.614)
P. violaceum	98 (±79)	100 (±72)	26	≫0.1 (0.078)

[a] The distance between the drops is in the range of 1.4 to 1.9 mm. SD, Standard deviation.

TABLE 1. *Rates of spreading of cells on shallow and deep agar*[a]

Species	Rate of spreading (mm/h ± SD) in:		No. of cases	Probability (2-tailed *t* test)
	Shallow agar	Deep agar		
D. discoideum	0.42 (±0.19)	0.29 (±0.14)	38	0.001 (4.889)
D. purpureum	0.36 (±0.15)	0.20 (±0.07)	31	0.001 (5.237)
D. mucoroides	0.20 (±0.09)	0.15 (±0.08)	24	0.05 (1.889)
P. violaceum	0.44 (±0.13)	0.54 (±0.14)	36	0.001 (3.259)

[a] These tests were each repeated for a second reading giving similar results except for *D. mucoroides*, which showed identical spreading in both conditions. SD, Standard deviation.

TABLE 3. *Number of cells spread between (inside) a drop of D. discoideum and a drop of P. violaceum (1.4 to 1.9 mm apart) compared to the number of cells that spread where there are no opposing drops*

Species	Mean no. of cells/mm² (± SD)[a]		No. of cases	Probability (2-tailed *t* test)
	Outside	Inside		
D. discoideum	78 (±16)	85 (±12)	11	>0.1 (1.247)
P. violaceum	91 (±25)	37 (±19)	11	0.001 (5.678)

[a] SD, Standard deviation.

TABLE 4. *Number of cells spread from a drop near an agar edge (edge side) compared to the number of cells that spread on the side where there is extended agar (outside)*

Species	Mean no. of cells/mm² (± SD)		No. of cases	Probability (2-tailed *t* test)
	Outside	Edge side		
D. discoideum	63 (±13)	46 (±9)	12	0.001 (3.740)
P. violaceum	220 (±28)	229 (±37)	6	≫0.1 (0.459)

[a] The distances between the drop and the edge range from 1.4 to 1.9 mm. SD, Standard deviation.

tile, as previously described experiments indicated, then it should accumulate in greater concentration near an edge than on the opposite side, where there is a long stretch of agar for continued diffusion. Here, over a wide range of distances (1.4 to 2.5 mm) between the drop and the edge, it is clear that in *D. discoideum* there are fewer cells on the edge side (hence a greater concentration of repellent), whereas there is no difference between sides for *P. violaceum* (hence no repellent) (Table 4).

Experiments with a dialysis membrane. To see if the repellent will pass through a dialysis membrane, the experiment with shallow and deep agar was repeated, but in this case the agar was covered with a dialysis membrane (which had been washed by boiling for 10 min in 10^{-3} M sodium ethylenediaminetetraacetate). To keep the membrane flat, it was placed in a small double-ring frame resembling a miniature embroidery hoop. The depths of the agar were 0.3 mm (shallow) and 11 mm (deep) in plastic petri dishes (100 by 15 mm; Falcon no. 1001).

The results are similar to the previous shallow-deep experiments (Table 1): the amoebae of *D. discoideum* spread much farther on the shallow dishes, 0.37 (±0.06) mm/h, than on the deep dishes, 0.27 (±0.07) mm/h, in 14 and 16 cases tested (P = 0.001; 3.653 by two-tailed t test). Therefore, the repellent is a small molecule that can pass through a dialysis membrane. We are presently attempting to devise a bioassay so that further characterization of the repellent will be possible.

DISCUSSION

It is clear from these experiments that the vegetative amoebae of *D. discoideum* (and *D. purpureum*) produce a dialyzable repellent that causes the amoebae to spread away from one another. The situation for *D. mucoroides* is marginal, but *P. violaceum* does not produce the repellent, although it is sensitive to that produced by *D. discoideum*.

It is unlikely that any of the differences shown here are due to differences in rates of locomotion of cells, but rather, they are due to differences in orientation. This was shown by Bonner et al. (3) and Adam (senior thesis), for the rates of movement of individual amoebae are not significantly affected by gradients of acrasins; they only affect cell orientation.

Another basic difference between *Dictyostelium* and *Polysphondylium* is that the former uses cyclic adenosine 3′,5′-monophosphate for its acrasin, whereas the latter does not. During the vegetative stages, it is known that the *Dictyostelium* amoebae produce a small amount of extracellular cyclic adenosine 3′,5′-monophosphate and have some sensitivity to its gradients at that stage (2). Therefore, the repellent must overcome or counteract any tendency for the vegetative cells to clump; and it can only be when the cyclic adenosine 3′,5′-monophosphate production and the sensitivity to it are each increased dramatically that the repellent can be counteracted and aggregation can occur. On the other hand, in *Polysphondylium* we have presented evidence that the attractant is operating at all stages; it is simply not strong enough to cause genuine aggregation at the vegetative stage.

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