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The possible role of ammonia in phototaxis of migrating slugs of *Dictyostelium discoideum*

(slime molds/gas orientation/chemotaxis)

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ABSTRACT **Previously we showed that the rising cell masses of cellular slime molds orient away from high concentrations of ammonia gas, presumably by speeding up the cells on one side. Here we show that in the same way NH_3 could also be involved in the highly sensitive phototaxis found in the migrating slugs of *Dictyostelium discoideum*. We have evidence that light increases their speed of migration and their production of NH_3. Since unilateral light is concentrated on the distal side of a cell mass by the "lens effect," this leads to the obvious hypothesis that the light stimulates the local production of NH_3, which, in turn, stimulates the cells in the illuminated region to move faster.**

It has been known for a long time that a gas repels the rising (and migrating) cell masses of the cellular slime molds (1). Recently, we and others (2, 3) have been able to show that the gas involved is NH_3. We also gave evidence that NH_3, in the range of concentrations that orients cell masses, increases the rate of movement of cells in aggregating streams. This combination of facts produced the proposal that NH_3 acts as a repellant by speeding up the cells on the side of a cell mass that has the highest concentration of NH_3, thereby producing orientation away from the gas.

One of the questions that arose was whether or not the strong phototaxis exhibited by cellular slime molds might be mediated by NH_3. It is well known that, as in the mold *Phycomyces*, where it was first discovered by Buder (4), slime mold slugs concentrate or focus the light on the distal side of the cell mass, the so-called "lens effect" (5, 6). As Buder did for *Phycomyces*, Francis (7) concentrated a minute spot of light from above on one side, near the tip (the sensitive region) of the migrating slug of *Dictyostelium discoideum* and showed that the illuminated cells on that side move faster and the cell mass curved away from the spot of light. If light and NH_3 speed up cells within a slug, is it possible that a concentration of light causes a local increase in the production of NH_3, which, in turn, causes the differential speedup of the cells on the distal side? Here we present evidence that supports such a hypothesis.

MATERIALS AND METHODS

The experiments were done with *D. discoideum* NC-4 grown on *Escherichia coli* B/r. To prepare the slugs for measuring the effects of NH_3 on the speed of slugs, amoebae were grown on nutrient agar plates (buffered 1% peptone/1% dextrose/2% agar), washed by centrifugation, and placed in small, concentrated drops on 2%, nonnutrient agar. After the slugs migrated away from the drop, individuals were isolated on a circle of agar with a cork borer and placed in a corresponding hole on a fresh 2% agar Petri dish. This was then inverted over a crystallizing dish (Pyrex 40 × 80 mm) containing 5 ml of 1 mM NH_4Cl and 5 ml of 1.0 M NaOH. The partial pressure produced in the chamber was 0.0065 mmHg. The rates of movement of the slug were measured from video tape taken through a 50-mm lens attached through a microscope to a Panasonic video camera (WV-1850) with time-lapse (AG-6010). The experiments were run at 17°C.

The phototaxis experiments using unilateral light were done in a blackened wooden box (50 × 50 × 50 cm) in which a frosted Plexiglas cylindrical rod (2 cm in diameter) entered vertically down one side of the box. A 15-W incandescent bulb was placed over the rod, but separated from it by a flask containing a 5% solution of $CuSO_4$ to cool the light by filtering out the wavelengths above 650 nm while preserving those important for phototaxis. Covered Pyrex crystallizing dishes (90 × 50 mm) were fitted with a nylon screen rack that came up above the NH_3-generating solution. Right side up on the rack a small, open plastic Petri dish bottom (60 × 15 mm, Falcon no. 1007) was placed that contained 2% nonnutrient agar and a central plug of migrating slugs. The plugs were punched out of a nutrient agar growth plate at the end of the aggregation stage. The crystallizing dishes were placed 10–15 cm from the illuminated bar on shelves within the light-tight box (kept at 17°C).

To see if light affects the amount of NH_3 given off by migrating slugs, the slugs were prepared by washing the amoebae off plates before aggregation (as described above) and the cells in the centrifuge pellet were diluted to 4×10^7 cells per ml. One milliliter of such a suspension was spread on a 2% nonnutrient agar Petri plate (100 × 15 mm), allowing the excess liquid to evaporate. Slugs developed within 16 hr in the dark at 17°C. For each experiment, eight Petri plates with the slugs were placed upside down and a small Petri dish bottom containing 8 ml of distilled water was inserted inside each Petri dish so that its edges were flush against the agar ceiling containing the migrating slugs. Four dishes were then put in the dark box and four were put in a box with a slit illuminated by unilateral light from a fiber optic lamp, all at 17°C. The water was then changed and the plates were put under the reciprocal conditions for another 2-hr period. To measure the NH_3 that had accumulated, the four dishes of water from each condition were pooled and tested for NH_3 by using an Orion NH_3 electrode and an Orion expandable ion analyzer, model EA 940. The method of collecting and measuring the NH_3 is that of Ira Feit, who kindly gave us advice and the benefit of his experience.

RESULTS

Effect of NH_3 on Slug Speed. At first we had great difficulty with these experiments for we used plates with many slugs on them and there was no difference in their speed before and after adding NH_3. We thought that this might be because all of the slugs on the plate were giving off NH_3 that was saturating the environment before any further NH_3 was added. If only one or two slugs were placed in the dish, then

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we were able to obtain differences in the speed before and after the addition of the NH_3-generating solution. In 16 experiments where each slug was measured for 40 min, the mean speed (±SEM) over water was 1.31 ± 0.02 mm/hr (n = 16) and for the same slugs after the addition of NH_3 it was 1.45 ± 0.02 mm/hr (n = 16). By using a paired t test (one-tailed), the difference is significant at the level of $P < 0.05$.

Slug Movement in the Absence of NH_3. We also performed some experiments in which the NH_3 was removed following the enzymatic method used by Schindler and Sussman (8). When a slug was placed on a Nuclepore filter (pore size = 5 μm) over a filter pad soaked with a mixture of 25 mM ketoglutarate/25 mM NADH/60 activity units of glutamine dehydrogenase/20 mM Na/K phosphate buffer, pH 7.3, in a small, well-closed Petri dish (50 × 9 mm; Falcon no. 1006), all forward movement of the slug ceased. This was not the case when any one of the three components of the mixture was tested separately in buffer, indicating that the key factor in preventing locomotion was probably the absence of NH_3.

Orientation to Unilateral Light in an Atmosphere of NH_3. One way to test if NH_3 might be involved in phototaxis by locally speeding up cells in the slug is to saturate the atmosphere with a nontoxic level of NH_3 around slugs that are illuminated from one side to see if the excess NH_3 cancels out the normal phototaxic response. This experiment works remarkably well and has been repeated many times. As can be seen in Fig. 1, if two plates of slugs in identical containers with the same source of unilateral light are compared, the slugs over water show normal phototaxis, whereas the slugs over NH_3 show little or no response to the light.

Another way in which the absence of phototaxis in an atmosphere of NH_3 can be shown is by having slugs crawling on the surface of agar in a Petri dish inverted over a crystallizing dish (80 × 40 mm) containing an NH_3-generating solution (again involving a range of partial pressures from 0.0065 to 0.026 mmHg of NH_3). The slugs are not affected by lateral light or light from above (as they are in controls), which makes them hug the agar. Instead, the normally inconsequential effect of gravity takes over and a very high percentage of the slugs points straight downward, often continuing to migrate so that they become suspended on a thread of slime sheath, ultimately fruiting in midair (Fig. 2). If the plates are right side up in an atmosphere of NH_3, no such effect is seen. Nor do they orient in the fashion shown in Fig. 2 if they are inverted over water in the dark.

Effect of Light on the Emission of NH_3. Again we tried numerous ways to do this experiment and finally adopted a method devised by Ira Feit (personal communication), where nonnutrient agar plates containing a heavy concentration of washed amoebae are inverted over an open Petri dish containing distilled water when the cells have reached the migration stage. The water is collected after a 2-hr interval and measured for NH_3 with the NH_3 electrode. Such plates were put in a box with a slit and given unidirectional light for 2 hr and then placed in the dark in a light tight box for 2 hr, or the sequence was reversed. In 20 such experiments the mean NH_3 concentration was 15 μM in the light and 11 μM in the dark. Since the measurements were made in millivolts, the means ± SEM are −29.8 ± 3.7 mV in the light and −22.2 ± 3.5 mV in the dark. In a paired t test (one-tailed) the difference is significant at the level of $P < 0.005$. It is unfortunate that it is not possible, because of the difficulty in obtaining accurate NH_3 measurements at such low concentrations, to do a dose–response curve to see how much the amount of NH_3 given off by the slugs varies with light intensity.

DISCUSSION

We have given evidence to support the idea that not only do gradients of NH_3 orient cell masses of slime molds but also that this phenomenon could be involved in positive phototaxis: the light is concentrated on the distal side by the lens effect, more NH_3 is presumably generated in the illuminated region, and this increase stimulates the cells in that region to move more rapidly, thereby causing orientation toward light. The evidence that we have to support such a hypothesis is that (*i*) NH_3 causes cells in the slug to move more rapidly and a removal of NH_3 causes them to stop moving, (*ii*) light increases the amount of NH_3 given off by the cells, and (*iii*) an excess of added NH_3 inhibits phototaxis but not migration.

Such a hypothesis fits in well with the experiments of Kitami (9), who showed that slugs of *D. discoideum* migrating against centrifugal forces moved more rapidly toward light than in the dark. As he points out, this is in keeping with the earlier observations of Poff and Loomis (10), who showed that slugs moving phototactically increase their rate of

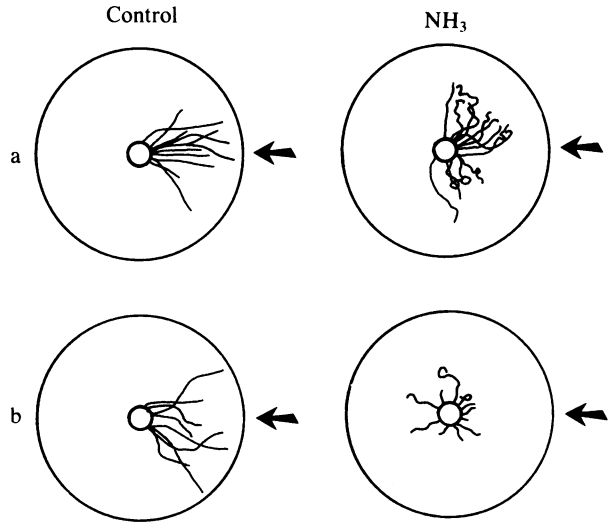

FIG. 1. Effect of NH_3 on phototaxis. Two experiments showing slime tracks of slugs that have migrated out from a central plug of agar in a Petri dish. The arrows show the direction of the light source. Note that in an atmosphere of NH_3, the slugs become disoriented and fail to move toward the light. This is partially true in *a*, where the partial pressure of the NH_3 is 0.005 mmHg, and completely so in *b*, where the partial pressure is 0.008 mmHg.

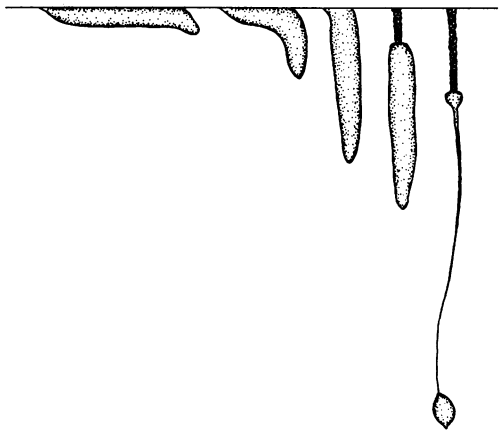

FIG. 2. Semidiagrammatic drawing in a time sequence showing (from left to right) that if slugs are migrating on the ceiling of a dish containing NH_3 vapor, gravity appears to take over and the slugs point straight downward. Note the migration continues off the surface of the agar so that the fruiting body is suspended on a thread of slime sheath. (Also note the curious shape of the basal disc under these circumstances.)

movement with the intensity of illumination. As one can see from their data, a 10× increase in illumination produces a 1.3× increase in speed. However, it should be pointed out that Smith *et al.* (11) find no difference in speed under similar conditions. Also a number of workers have tried to see if general, undirected illumination affects the rate of speed, but no difference could be observed between light and dark (5, 12).

One other fact in the literature fits in with the NH_3-speed hypothesis, although this point is far more speculative and fanciful. It has been known for a long time that larger slugs move more rapidly than smaller ones (13–15). Could this be because a greater number of cells will produce a larger concentration of NH_3? This would also account for the well-known fact that very small slugs do not migrate at all but fruit immediately. [This also fits in with Schindler and Sussman's (8) evidence that NH_3 favors migration over fruiting.]

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