

Ammonia and thermotaxis: Further evidence for a central role of ammonia in the directed cell mass movements of *Dictyostelium discoideum*

(slime molds/taxes/kineses/pH)

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ABSTRACT Evidence is presented to support the hypothesis that, in addition to its possible role in mediating chemo- and phototaxis, ammonia (NH₃) is also the key substance responsible for directing thermotaxis of the migrating slugs of *Dictyostelium discoideum*. NH₃ is produced by the cells of the slug and we show that high and low concentrations of NH₃ decrease the speed of the amoebae while intermediate concentrations increase their speed. NH₃ production by amoebae is affected by temperature: the greater the temperature, the more NH₃ is produced. From these facts we speculate that both the positive and the negative thermotaxis found in slugs can be explained by temperature gradients stimulating regional differences in NH₃ production, and depending upon the temperature, the amount of NH₃ will either be in the range that stimulates or inhibits the rate of movement. If this explanation is correct, then minute localized differences in the production of NH₃ and their differential effect on cell speed could account for all the directed movements of the cell masses of these slime molds.

In previous studies we have shown that ammonia (NH₃) plays a key role in orienting cell masses by speeding up the amoebae on one side of a migrating or culminating cell mass, thereby causing a turning by means of the differential forward motion of the internal amoebae. In this way one may account for the orientation of cell masses away from one another (negative chemotaxis) and for positive phototaxis where more light causes an increase in the local production of NH₃ which in turn speeds up the cells in that region (1-4). Here we present evidence that thermotaxis might also be mediated in the same way. During the course of this investigation we examined in detail: (i) the production of NH₃ at various stages of the life cycle of *Dictyostelium discoideum*; (ii) the effect of various concentrations of NH₃ on the rate of movement of isolated amoebae and of migrating slugs; (iii) how the NH₃ produced by an individual slug can affect the speed of its own movement; and (iv) how temperature affects NH₃ production in slugs. All these studies contribute to our understanding of the way NH₃ might play a central role in all the directed morphogenetic movements of the cellular slime molds.

MATERIALS AND METHODS

These experiments were done with *Dictyostelium discoideum* NC-4 grown on *Escherichia coli* B/r.

Life Cycle Experiments. Amoebae were grown on bacteria in Petri plates containing buffered 2% (wt/vol) agar with 1% peptone and 1% dextrose. The NH₃ given off was collected by placing bottoms of plates covered with a lawn of amoebae

upside down over the lid containing a smaller Petri plate (68 × 9 mm deep) with 8 ml of distilled water. The water was changed at 2-hr intervals throughout development. Each experiment involved three plates and was repeated four to nine times (at 23°C under ceiling fluorescent lights). The NH₃ content of the pooled water from each interval was measured with an NH₃ electrode (Orion expandable ion analyzer, EA 940).

Effect of NH₃ on Rate of Movement of Amoebae. Petri plates, each containing a drop of amoebae (from a suspension of 2 × 10⁶ cells per ml) on 2% agar, were inverted over crystallizing dishes (Pyrex, 40 × 80 mm) containing first 10 ml of distilled water and then 5 ml of various concentrations of NH₄Cl combined with 5 ml of 1.0 M NaOH. The movements of the amoebae at 17°C were recorded on a video tape using a Panasonic video camera (WV-1850) with time lapse (AG-6010) feature mounted on a microscope with a 6.3-mm lens. Their speed was calculated from tracings on the screen for 30-min intervals.

Effect of NH₃ on Rate of Movement of Slugs. Sets of three Petri plates, each containing a single slug, were inverted over various concentrations of NH₃-generating solutions (and H₂O controls). The experiments were done for 1 hr at 17°C in the dark, and the distance traversed by the slugs was determined by measuring the track lengths.

Effects of Decreasing Rather Than Increasing the Internal pH of the Cells. We examined the effects of CO₂ on slug speed. The procedure was the same as with NH₃. A gas of 5% CO₂/95% air (from a cylinder) was bubbled through 50 ml of distilled water in a crystallizing dish for 5 min and plates with single slugs were inverted over the dishes. These experiments were also done at 17°C. Parallel experiments on a drop of separate amoebae (2 × 10⁶ cells per ml) were recorded and measured on the video screen.

Thick-Thin Agar Experiments. These experiments were done a number of ways, all giving the same result, but the most successful method was to submerge a small plastic Petri plate lid (50 × 3 mm, Falcon, 1007) right side up in a larger Petri plate (100 × 15 mm) and to pour in 2% agar so the submerged lid was just covered with agar. The rates of the slug migration at 17°C were measured using the time-lapse video camera.

Temperature Gradient Experiments. A temperature gradient was devised by placing a covered 40-W G.E. Showcase light bulb controlled by a variable resistor (Variac) onto an aluminum block (30 × 12 × 2 cm) placed into a cold incubator (12°C). The temperature gradients were measured by two thermocouples. Three Petri plates (100 × 15 mm), each containing a drop of washed amoebae that had been allowed to develop at 17°C in the dark on 2% (non-nutrient) agar, were placed across the aluminum block. The NH₃-generating solution was added to a small Petri dish bottom at the side of the plate (see Fig. 5).

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Effects of Temperature on NH₃ Emission. Amoebae were grown at specified temperatures to the end of the vegetative stage, washed by centrifugation, plated out on 2% agar at a concentration of 4×10^7 cells per plate (100×15 mm), and allowed to develop into slugs in the dark at specified temperatures. Plates of slugs were then inverted over their lids containing smaller Petri dishes (68×9 mm) with 8 ml of distilled water, and three such plates were put into each of the experimental temperatures in the dark for 4 hr. The water aliquots were pooled and tested for NH₃ content by using the NH₃ electrode.

RESULTS

Production of NH₃ During the Course of Development. Cellular slime molds produce NH₃ during the developmental stages (5–10). Here we examined the time course of NH₃ production from the vegetative stage to midculmination, and as can be seen from Fig. 1, there is a steady increase in NH₃ and the multicellular stages give off more NH₃ than the vegetative stage.

Effects of NH₃ on the Rate of Movement. The speed of both individual amoebae and migrating slugs was measured under various partial pressures of NH₃ and then plotted as percent differences from the control without the NH₃. Every effort was made to follow the same amoebae before and after the addition of NH₃. The results from experimental slugs were compared with results from a separate set of control slugs obtained on the same day.

As can be seen in Figs. 2 and 3, the scatter of the data is considerable, yet it is clear that there is a central peak where NH₃ speeds up both cells and slugs, and at NH₃ concentrations above the peak the speed is inhibited. It is interesting to note that the range of NH₃ concentrations where speed is increased is greater for slugs (0.2–0.9 mM) than for amoebae (0.04–0.2 mM). The reason for this is not obvious, although it could simply be that the slime sheath of the slug is less permeable to NH₃ than the membrane of a single cell.

Effects of CO₂ on Movement of Amoebae and Slugs. On the assumption that the increased speed is in some way related

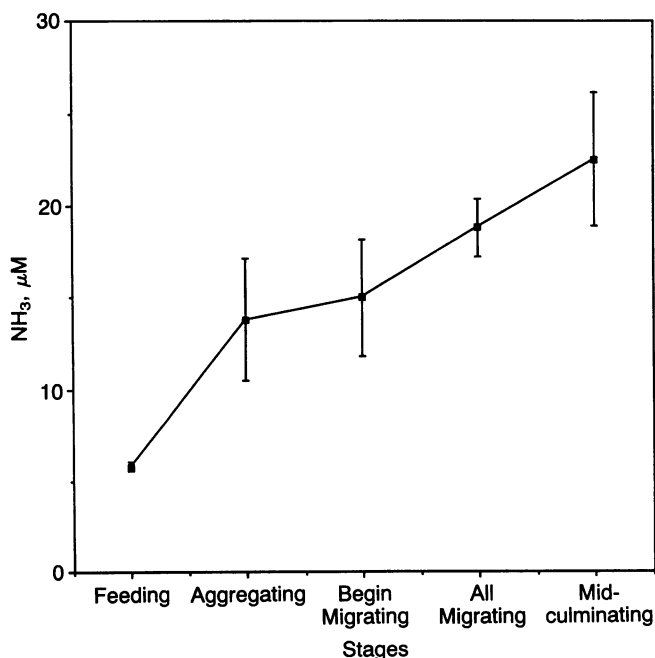


FIG. 1. Amount of NH₃ given off by *D. discoideum* at various stages of development. The points are an average of four to nine experiments, each of which involves three Petri dishes of developing slime molds, and the bars are the SEM for each point. The amount of NH₃ collected (ordinate) is proportional to the rate of NH₃ production per amoeba per unit time.

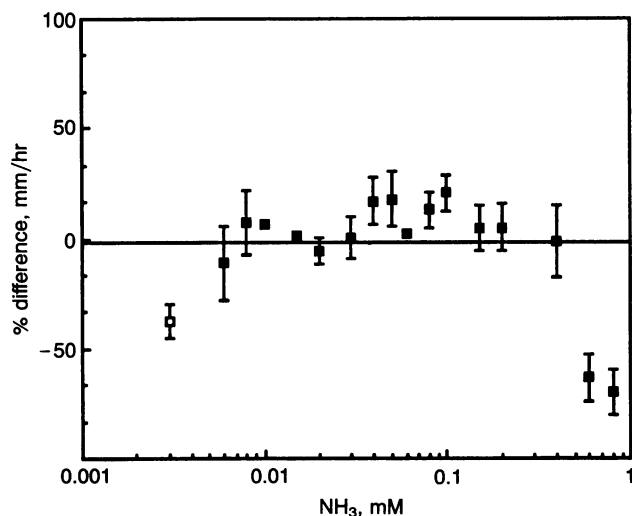


FIG. 2. Percent differences of the rates of movement of vegetative amoebae under various concentrations of NH₃ before and after the addition of the NH₃. The solid squares are the average of three or four experiments, each involving 6–10 amoebae, which were measured for 30 min before and after the addition of NH₃. The percent difference between the control and the experimental is shown for various concentrations of NH₃. The bars show the SEM for each point. [To convert to partial pressure divide the concentration (mM) by 190 to give the partial pressure (mmHg).] A similar experiment was done 12 times with 5% CO₂/95% air (open square).

to the NH₃ causing an increase in pH inside the cells, we tested the speed of amoebae and slugs in an atmosphere of 5% CO₂/95% air, which should have the opposite effect. As can be seen in Figs. 2 and 3, CO₂, which would be expected to lower the pH of the cells, generally causes a reduction in the rate of movement.

Slug Speed on Agar Thickness. Another way to see the effects of NH₃ on the rate of movement in slugs is to have them migrate toward a light from thick agar (2.3–3.3 mm deep) to shallow agar (0.6–0.9 mm deep). The speed is significantly faster over the thin agar. [The mean speed for eight slugs was 1.51 ± 0.13 mm/hr (SEM) and for the same slugs 1.19 ± 0.08 mm/hr (SEM) over thick agar. $P < 0.05$.] This is well illustrated in the best case of several runs where

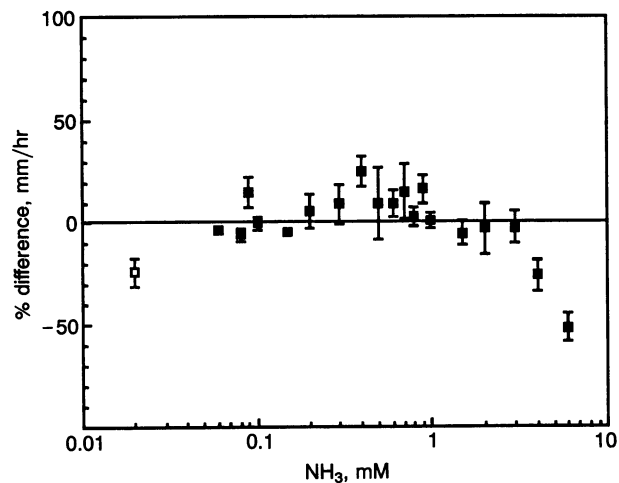


FIG. 3. Percent differences of the speed of migrating slugs over various concentrations of NH₃ versus the controls where no NH₃ was added. Each solid square is the average of three or four experiments (\pm SEM). On each day of an experiment, sets of three Petri plates, each containing one slug, were subjected to various concentrations of NH₃ and each of these sets was compared to one set of three control plates with no added NH₃. A similar experiment was done nine times with 5% CO₂/95% air (open square).

we have a continuous record of a single slug that went from thick to thin agar (Fig. 4A). Clearly by the time at least half of the slug is over the thin agar the speed increases.

It is obvious we cannot prove that this effect is due to NH_3 and not some other diffusible substance. NH_3 is, however, the most likely candidate for a very interesting reason. It was difficult to lead the slug over the thin agar with a weak directional illumination. For each success there were many failures, where, as soon as the slug tip reached the edge of the thin agar over the submerged plastic platform, the slug would shy away by turning (Fig. 4B). Since a local high concentration of NH_3 has been observed to cause such repulsion of cell masses (2, 3), one can reasonably postulate that the NH_3 diffusing into the thin agar reaches a higher local concentration than in the thick and, consequently, repels.

NH_3 and Thermotaxis. The first way we examined the question of whether NH_3 was involved in thermotaxis was to repeat the kind of experiment we had done for phototaxis (4), that is, to put slugs on a temperature gradient and flood the atmosphere with NH_3 at various concentrations. This was done for both positive and negative thermotaxis, the latter having been demonstrated by Whitaker and Poff (11). They showed that thermotaxis is positive if the gradient is above the growth and development temperature and negative if it is below. In both cases added NH_3 disoriented the slugs, although the effect is less striking in negative chemotaxis, probably because our temperature gradient was suboptimal (Fig. 5). Note that as the concentration of NH_3 increased, the length of the tracks of the slugs decreased, clearly indicating an inhibition of speed by the NH_3 . This would be expected for a NH_3 concentration of 6 mM as can be seen in Fig. 3.

Effect of Temperature on NH_3 Production. Next we examined the effect of temperature on the emission of NH_3 by migrating slugs, and, as can be seen from Fig. 6, the higher the temperature, the more NH_3 was produced. Furthermore, the emission was affected by the temperature at which the amoebae developed: those cultures that developed at higher temperatures gave off more NH_3 at any one temperature than those that developed at a cooler temperature (Fig. 6).

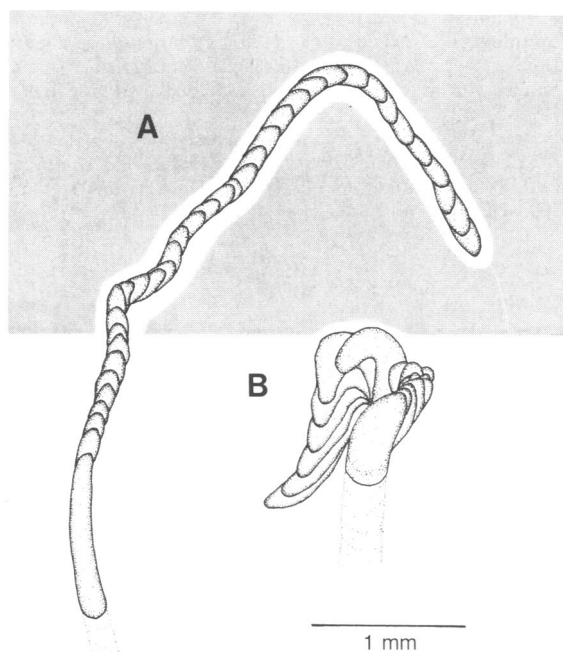


FIG. 4. (A) Tracings from a video screen taken at 10-min intervals of a migrating slug going from thick (white background) to thin (gray background) agar. Note that once the slug is over the thin agar it moves more rapidly. (B) Similar tracings of a slug that has turned upon reaching the edge of the thin agar.

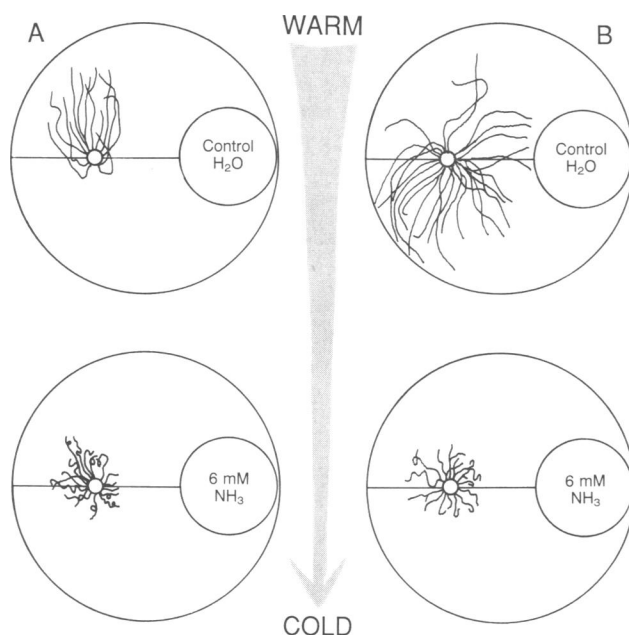


FIG. 5. Effect of adding NH_3 on the orientation of slugs in a heat gradient. (A) Positive thermotaxis of slugs in a temperature gradient above the temperature at which development occurred. The control (Upper) shows strong orientation toward the warmer side, but the orientation is inhibited by 6 mM NH_3 (Lower). (B) Negative thermotaxis in a temperature gradient below the development temperature. The slugs in the control show greater orientation toward the colder side than do those of the experimental plate in an atmosphere of NH_3 .

DISCUSSION

The simplest model for all these results assumes that NH_3 directly controls the rates of movements of the amoebae inside a slug; the amoebae are all moving in the same direction toward the anterior tip of the slug due to the internal

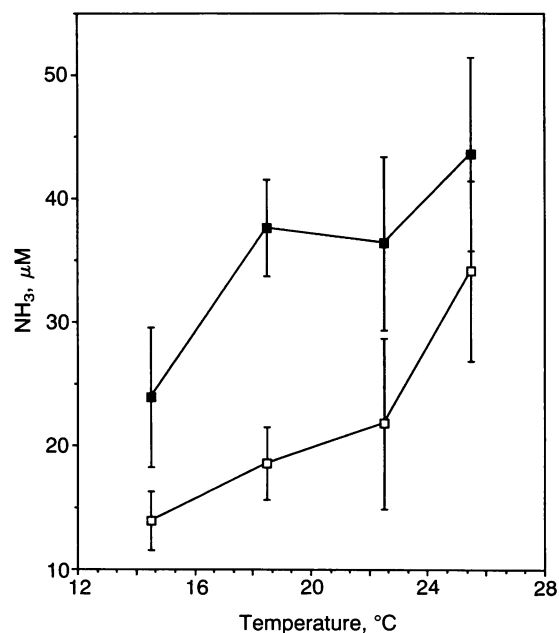


FIG. 6. Effect of temperature on NH_3 production of migrating slugs. Note that although NH_3 production consistently increases with temperature, the effect is greatly influenced by the temperature at which the slugs develop. Each point is the mean of four to nine experiments, each consisting of three plates of slugs, and the bars are the SEM for each point. Developmental temperatures: ■, 22–23°C; □, 18–19°C.

antero-posterior pulse gradient of cAMP (12–14). If one removes all the NH_3 produced by the slug using an enzymatic method, then all movement stops (4). What we have done here is compare the rate of movement of isolated slugs with and without added NH_3 ; in other words, we are comparing slugs with two levels of NH_3 and then we find that some addition of NH_3 causes an increased speed, while the addition of even more NH_3 results in an inhibition of the rate of cell movement (Figs. 2 and 3). This means that in the earlier work on repulsion of cell masses (2, 15) the concentration of NH_3 that repelled slugs was in the optimal range for increased cell speed. The same is true for the effect of directional light that is concentrated on the far side of the slug by the “lens effect”; again the light must be concentrated enough to cause the cells to produce the optimum amount of NH_3 for fast cell movement.

In the present study of thymotaxis there is the further interesting complication that, besides the long-established positive thymotaxis, Whitaker and Poff (11) showed that positive thymotaxis occurs only in temperature gradients spanning temperatures higher than the temperature at which the amoebae developed. Further, if the slugs are subjected to gradients spanning temperatures less than the development temperature, they are negatively thymotactic and move away from the warmer side. (See controls in Fig. 5.)

The model can easily encompass these facts for we have shown that the higher the temperature the more NH_3 is produced and that the speed of movement of the amoebae is NH_3 dependent: low NH_3 stimulates the rate of cell movement (over a restricted range of concentrations) and high NH_3 inhibits the rate of cell movement. For negative thymotaxis, where the overall production of NH_3 is relatively low, we postulate that the amount of NH_3 on the warmer side of the slug is within the NH_3 concentration range that stimulates cell movement, and for this reason the slug orients away from heat. For temperature gradients above the developmental temperature, the overall production of NH_3 has been pushed up so high we postulate that the NH_3 concentration on the warmer side of the slug is in the inhibitory range and, therefore, the amoebae on the far side move faster and orient the slug toward the heat.

This model raises some interesting questions. First, how does NH_3 stimulate or inhibit cell locomotion? Perhaps locomotion is affected through pH (an idea supported by our CO_2 results), because increased intracellular pH results in the increased activity of contractile proteins and cell motility [for a review, see Simchowicz and Cragoe (16)]. Furthermore, an

increase in pH stimulates chemotactic movement in leukocytes (16).

Second, how is the production of NH_3 within the slugs controlled? We do not know whether there is a specific deamination reaction that responds to small changes in temperature and light. Perhaps the most puzzling aspect of the tactic responses in these slime mold slugs is that differences in temperature as small as 4–5/10,000 of a °C across the tip of a slug are sufficient to cause orientation (17, 18). This means that the response system must be exquisitely sensitive to minute differences in temperature and light and that these differences are somehow amplified, through the aegis of NH_3 , into sizeable differences in cell speed.

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