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# Regulation of movement speed by intracellular pH during *Dictyostelium discoideum* chemotaxis

(amoeboid movement/random movement/cAMP/signal transduction/pH change)

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**ABSTRACT** **Evidence is presented that the chemoattractant-induced cytoplasmic alkalinization in the cellular slime mold *Dictyostelium discoideum* is essential in regulating locomotion speed during chemotaxis. Intracellular pH was manipulated with weak bases, weak acids, and proton-pump inhibition. Speed of locomotion of individual cells was measured during random and chemotactic movement. We found that (*i*) an increase of cytoplasmic pH increases the speed of randomly moving cells and (*ii*) the chemoattractant-induced rise in intracellular pH is essential for the increase in directed locomotion speed upon chemotactic stimulation. In addition, our experiments support the hypothesis that ammonia plays a key role in the thermo- and phototaxis of migrating slugs by increasing the locomotion speed of individual cells through changes in intracellular pH.**

During chemotaxis of *Dictyostelium discoideum*, amoebae orient in the direction (of the higher concentration) of the chemoattractant gradient and increase locomotion speed (1, 2), as was shown for *Dictyostelium mucoroides* as well (3). Although many intracellular responses to chemotactic stimulation are known, the mechanism of chemotactic movement remains to be clarified (4). Apart from chemotactic stimulation, ammonia addition is also known to increase rate of cell movement (4–6). Because both agents elevate intracellular pH ($pH_i$) of the cells (7–9), it has been argued that increased rate of cell movement during chemotaxis may be mediated by $pH_i$ changes (6, 10, 11).

We present evidence that $pH_i$ is, indeed, involved in regulating locomotion speed during *D. discoideum* chemotaxis. We examined (*i*) the effects of an artificial decrease or increase of $pH_i$ on speed of random movement of individual cells, (*ii*) the effects of an artificial decrease or increase of $pH_i$ on speed of chemotactic movement of individual cells, and (*iii*) $pH_i$ changes upon chemotactic stimulation of cells with artificially decreased $pH_i$. The experiments show directly that locomotion speed during random movement and chemotaxis of *D. discoideum* cells is $pH_i$ sensitive and that the chemoattractant-induced rise in $pH_i$ (7) is necessary for speeding up cell movement. The efficiency of chemotaxis (i.e., directionality) is $pH_i$ independent. In addition, our study provides evidence for the hypothesis that ammonia plays a central role in directed movements of cellular slime molds during pattern formation by influencing $pH_i$ (10, 11).

## MATERIALS AND METHODS

**Cell Culture Conditions.** Experiments were done with *D. discoideum* NC4(H) grown in association with *Klebsiella aerogenes* or *Escherichia coli* 281 on modified SM medium (4.4 g of $KH_2PO_4$, 2.0 g of $Na_2HPO_4$, 1.0 g of $MgSO_4 \cdot 7\ H_2O$, 7.5 g of glucose, 10.0 g of Bacto-peptone (Difco), 1.0 g of yeast extract (Difco), 15 g of agar, 1 liter of $H_2O$) at 22°C. Vegetative cells were harvested with cold 20 mM sodium/potassium phosphate buffer, pH 6.0 (PB), and washed free of bacteria by repeated washes and centrifugation. Starved cells were obtained by incubating cells on nonnutrient agar (1.5% agar in $H_2O$) at a density of $1.5 \times 10^6$ cells per $cm^2$ for ≈4 hr at 22°C, after which cells were shaken in suspension in PB for an additional hour.

**Measurement of Speed of Random Movement and Chemotaxis.** Vegetative or starved cells were deposited on glass coverslips (≈$5 \times 10^4$ cells per $cm^2$) mounted on an open-bottom Teflon culture dish, which was placed on the stage of an inverted microscope (12). The culture dish was filled with 2 ml of saline solution (see below). Cells were observed by phase-contrast microscopy with a 40× objective magnification. Movements of the amoeba at 20°C were recorded on a videotape with a time-lapse video recorder (Panasonic model AG6011A). Tracings of the tracks of individual cells were drawn on copier transparencies from the monitor screen. These tracks were digitized with an *x–y* digitizer and analyzed on a personal computer. Care was taken to ensure that the frequency of position points drawn to form the tracks of individual cells was larger than the turning frequency of the cells.

In random movement experiments the speed of locomotion of each cell was calculated by dividing the total track length (*TT*) by time. To get reproducible results drug addition was started after movement of control cells stabilized (2). To avoid changes in cell movement from changes in cell condition (2), we followed cells in each experiment for only ≈8 min.

Starved cells were used for measuring chemotaxis. The tip of a microcapillary (tip diameter, 1–2 μm), filled with bath solution and $2 \times 10^{-4}$ M cAMP, was placed close to the cells. Chemotactic movement toward the capillary was recorded on videotape, and tracks were digitized as described above. Speed of locomotion was obtained by dividing the *TT* by time. In addition, total movement or displacement of each cell in the direction of the chemoattractant gradient (*TG*) was calculated. We used *TG*/*TT* as a measure for chemotactic efficiency (13).

**$pH_i$ Measurements and Manipulation.** The $pH_i$ of individual cells was measured at room temperature as described (8, 14). Briefly, fluorescence intensity at 452- and 493-nm excitation wavelength ($I_{452}$ and $I_{493}$) of individual carboxyfluorescein dibutyrate-loaded cells was measured by using fluorescence

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Abbreviations: $pH_i$, intracellular pH; $pH_e$, extracellular pH; DMO, 5,5-dimethyl-2,4-oxazolidinedione; KPr, potassium propionate; *TT*, total track length of locomoting cells; *TG*, total track length in direction of chemoattractant gradient.

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microscopy. $pH_i$ was calculated from the ratio of fluorescence intensity ($I_{493}/I_{452}$).

$pH_i$ was manipulated by adding the weak acids 5,5-dimethyl-2,4-oxazolidinedione (DMO) and potassium propionate (KPr), the weak bases methylamine and ammonia, or the proton pump-inhibitor sodium azide to the cells. To decrease $pH_i$, cells were bathed in 10 mM sodium/potassium phosphate buffer at pH 6.6 in the presence of DMO (15) or at pH 6.0 in the presence of KPr. $pH_i$ was increased by adding methylamine or $NH_4Cl$ (to add ammonia) to cells bathed in 10 mM sodium/potassium phosphate buffer, pH 7.4 (15). Sodium azide was added to cells bathed in 40 mM NaCl/5 mM KCl/1 mM $CaCl_2$/5 mM Hepes·NaOH, pH 7.0 (16), or in 10 mM sodium/potassium phosphate buffer, pH 6.6. DMO, methylamine, and sodium azide were added to the bath from stock solutions in $H_2O$ of 1 M, 2 M, and 0.1 M, respectively. KPr- and $NH_4Cl$-containing solutions were added by perfusion of the bath with freshly made solutions. Measurements were taken immediately after drug addition; incubation to 20 min, however, yielded the same results.

**Statistics.** Values are expressed as means ± SDs, unless specified otherwise; $n$ equals the number of cells measured. Differences between mean values were tested with the Student's $t$ test.

## RESULTS

**$pH_i$.** $pH_i$ of *D. discoideum* cells can be modified by treating cells with weak acids (such as propionate), weak bases (such as ammonia), and proton pump inhibitors (such as diethylstilbestrol) (8, 9). To examine the effects of $pH_i$ manipulation on cell movement, we used DMO, propionate, and sodium azide for lowering $pH_i$; for elevating $pH_i$, ammonia and methylamine were used. To confirm that the weak acid DMO and the proton pump-inhibitor sodium azide (16) modify $pH_i$ as expected under our experimental conditions, we measured the $pH_i$ of cells in various extracellular solutions used in the cell-movement experiments. Fig. 1 shows that DMO lowers the $pH_i$ in a dose-dependent manner, as has been shown for other weak acids such as proprionate (8, 14). The effect of sodium azide, together with the effects of some other $pH_i$ modifiers, are summarized in Table 1. The extracellular pH ($pH_e$) *per se* does not significantly influence the $pH_i$, as shown in other studies (14, 17). $pH_i$ of methylamine-treated cells was taken from Van Lookeren Campagne *et al.* (15) because our method does not allow measurement of pH values >7.6 due to the dye characteristics. $NH_4Cl$ at 3 mM also can elevate $pH_i$ by at least 0.1 pH unit at the $pH_e$ of 7.4

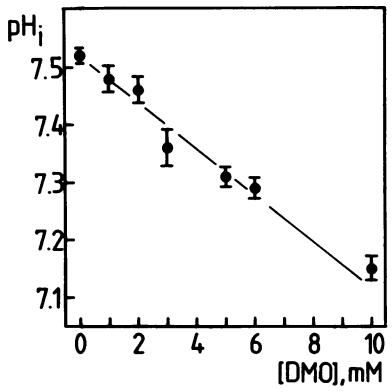


FIG. 1. Mean $pH_i$ of starved *D. discoideum* cells incubated at different DMO concentrations in 10 mM sodium/potassium phosphate buffer, pH 6.6. Error bars represent ±SE. Graph represents 92 single-cell measurements in five independent experiments.

Table 1. $pH_i$ of cells treated with weak base, weak acid, and proton pump inhibitor

| $pH_e$ | Addition* | $pH_i$ ($n$) |
|---|---|---|
| 6.0 | None | 7.50 ± 0.05 (60) |
| 6.0 | 2 mM KPr† | 7.34 ± 0.09 (45) |
| 6.6 | None | 7.53 ± 0.06 (38) |
| 6.6 | 5 mM DMO† | 7.33 ± 0.05 (35) |
| 6.6 | 100 μM sodium azide† | 7.28 ± 0.08 (59) |
| 7.0 | None | 7.53 ± 0.05 (26) |
| 7.0 | 100 μM sodium azide† | 7.28 ± 0.03 (64) |
| 7.4 | None | 7.55 ± 0.09 (40) |
| 7.4 | 2.5 mM methylamine | 7.97‡ |

$n$, no. of cells measured.
*Composition of solutions is as described in *Materials and Methods*.
†Difference between $pH_i$ of treated and control cells is significant at 99.5% confidence limit.
‡Value was obtained from ref. 15.

(9). We conclude that DMO, KPr, and azide decrease $pH_i$, whereas methylamine and ammonia increase $pH_i$.

**$pH_i$ and Random Movement.** The effect of $pH_i$ manipulation on random cell movement was investigated by measuring the speed of locomotion during random cell movement with different DMO and methylamine concentrations (Fig. 2). DMO has little effect on the random-movement speed at concentrations of 5 mM and below; higher DMO concentrations decrease the speed. KPr at 2 mM, which induces a $pH_i$ decrease similar to 5 mM DMO (≈0.2 pH unit, Table 1), also has not much effect on random-movement speed (Table 2). Methylamine, on the other hand, increases locomotion speed in a dose-dependent manner (Fig. 2), as does ammonia (Table 2). Sodium azide, which decreases $pH_i$ by ≈0.2 pH unit (Table 1), has little effect on speed of locomotion for both extracellular conditions ($pH_e$ = 7.0 and 6.6), as shown in Table 2.

**$pH_i$ and Chemotaxis.** The speed of locomotion of starved amoebae during chemotaxis toward a microcapillary filled with cAMP was measured under conditions that alter $pH_i$. Fig. 2 shows the effects of DMO and methylamine on the speed of directed locomotion. Methylamine, at concentrations <5 mM, has little effect on locomotion speed during chemotaxis, whereas it depresses chemotactic locomotion at 10 mM. Addition of 5 mM $NH_4Cl$ increases chemotactic locomotion speed slightly (Table 2). DMO strongly decreases the speed of locomotion during chemotaxis, even at low concentrations. KPr has the same effect as DMO and decreases the chemotactic-locomotion speed considerably (Table 2). On the other hand, we find, quite unexpectedly, that sodium azide, which like DMO and KPr decreases basal $pH_i$ (Table 1), has little effect on chemotactic-locomotion speed (Table 2).

The fact that DMO, KPr, and sodium azide lower $pH_i$, but that only DMO and KPr decrease locomotion speed during chemotaxis may seem contradictory. This apparent contradiction was resolved by measuring $pH_i$ in sodium azide and DMO-treated cells during cAMP stimulation. Chemotactic stimulation itself has been shown to increase $pH_i$ by ≈0.15 pH unit (7). With DMO, however, the cAMP-induced alkalinization is weak, as shown in Fig. 3 [mean $pH_i$ before stimulation, 7.33 ± 0.08 ($n$ = 7); maximum $pH_i$ at $t$ = 90 s after stimulation with $10^{-6}$ M cAMP, 7.38 ± 0.05 ($n$ = 7)]. In sodium azide-treated cells, however, a large rise in $pH_i$ upon cAMP stimulation is still seen [mean $pH_i$ before stimulation, 7.28 ± 0.07 ($n$ = 6); maximum $pH_i$ at $t$ = 90 s after stimulation with $10^{-6}$ M cAMP, 7.44 ± 0.06 ($n$ = 6) at $pH_e$ 7.0, mean $pH_i$ before stimulation, 7.28 ± 0.07 ($n$ = 11), and maximum $pH_i$ after stimulation with $10^{-6}$ M cAMP, 7.40 ± 0.07 ($n$ = 11), at $pH_e$ 6.6; differences between stimulated and unstimulated $pH_i$ were significant at 99.5% confidence limit].

Table 2. Random and chemotactic speed of locomotion and chemotactic efficiency (*TG/TT*) before and after addition of different $pH_i$-influencing chemicals

| | Control cells, μm/s (*n*) | Treated cells, μm/s (*n*) |
|---|---|---|
| Random movement | | |
| 2 mM KPr | 0.061 ± 0.007 (258) | 0.066 ± 0.031 (123) |
| 5 mM DMO | 0.070 ± 0.015 (74) | 0.063 ± 0.012 (116) |
| 100 μM sodium azide* | 0.050 ± 0.012 (85) | 0.045 ± 0.011 (128) |
| 100 μM sodium azide† | 0.049 ± 0.008 (170) | 0.047 ± 0.008 (241) |
| 2.5 mM methylamine | 0.060 ± 0.011 (388) | 0.077 ± 0.012 (345)‡ |
| 2.5 mM $NH_4Cl$ | 0.064 ± 0.009 (248) | 0.074 ± 0.010 (274)‡ |
| Chemotactic movement | | |
| 2 mM KPr | 0.188 ± 0.026 (195) | 0.131 ± 0.012 (425)‡ |
| 5 mM DMO | 0.156 ± 0.019 (286) | 0.098 ± 0.016 (373)‡ |
| 100 μM sodium azide* | 0.095 ± 0.019 (270) | 0.091 ± 0.017 (323) |
| 100 μM sodium azide† | 0.125 ± 0.021 (241) | 0.128 ± 0.019 (308) |
| 2.5 mM methylamine | 0.140 ± 0.031 (275) | 0.144 ± 0.035 (226) |
| 5.0 mM $NH_4Cl$ | 0.100 ± 0.013 (235) | 0.117 ± 0.019 (241)‡ |
| *TG/TT* | | |
| 2 mM KPr | 0.84 ± 0.06 (195) | 0.84 ± 0.05 (425) |
| 5 mM DMO | 0.89 ± 0.11 (286) | 0.81 ± 0.14 (373) |
| 100 μM sodium azide* | 0.80 ± 0.06 (270) | 0.82 ± 0.07 (323) |
| 100 μM sodium azide† | 0.78 ± 0.12 (241) | 0.81 ± 0.07 (308) |
| 2.5 mM methylamine | 0.78 ± 0.09 (275) | 0.81 ± 0.05 (226) |
| 5.0 mM $NH_4Cl$ | 0.76 ± 0.08 (235) | 0.87 ± 0.07 (241)‡ |

Extracellular solutions were as described in *Materials and Methods*. *n*, number of cells measured.
*$pH_e$ was 7.0.
†$pH_e$ was 6.6.
‡Difference between control and treated cells was significant with 99.95% confidence limit.

This difference in $pH_i$ response upon chemotactic stimulation is most likely due to a strong buffering capacity of the many DMO ions in the cytoplasm of DMO-treated cells: Simple calculations indicate that proton pump activity of untreated cells increases by ≈40% upon chemotactic stimulation (from data in ref. 7). Increase in proton pump activity upon stimulation in sodium azide-treated cells is calculated at ≈45% from data obtained in the present study. In DMO-treated cells, however, decrease in cytoplasmic proton concentration from increased proton pump activity upon chemotactic stimulation should be much smaller than that expected from the 40–45% increment in pump activity. Buffering capacity of the cytoplasm of *D. discoideum* cells has been estimated at 10–40 milliequivalents of $H^+$ per pH unit per liter (18, 19). The additional buffering capacity of cytoplasm from 5 mM DMO at a $pH_e$ of 6.6 is calculated at ≈40 milliequivalents of $H^+$ per pH unit per liter ($\delta$[DMO]/$\delta$pH at constant DMO concentration; see p. 391 of ref. 28). Then the $pH_i$ of DMO-treated cells, initially 7.33, would only become ≈7.38 after chemotactic stimulation, about the same as actually observed (see above).

The efficiency of chemotaxis (*TG/TT*) was not affected by treatment of the cells with DMO, KPr, sodium azide, methylamine, or ammonia (Fig. 4 and Table 2).

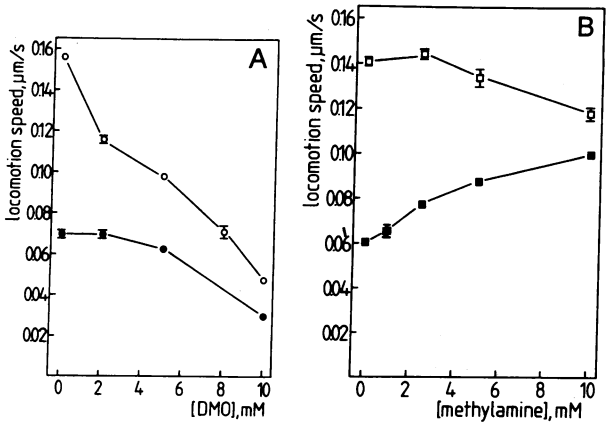


FIG. 2. Mean speed of locomotion during random movement (●, ■) and chemotaxis (○, □). Error bars represent ± SE (no bar means SE <0.02 μm/s). (*A*) Effects of different DMO concentrations on a total of 448 (random movement) and 1356 (chemotaxis) cells in at least three independent experiments. (*B*) Effects of different methylamine concentrations on a total of 1371 (random movement) and 785 (chemotaxis) cells in at least three independent experiments.

## DISCUSSION

These experiments show that speed of locomotion of individual *D. discoideum* cells is $pH_i$ dependent. This $pH_i$ dependence is present during both random movement and chemotaxis. Evidence is presented that the cAMP-induced alkalinization in *D. discoideum* (7) is essential in speeding up the cells during their chemotactic response: (*i*) Decrease in $pH_i$ to ≈7.3 with 5 mM DMO (Fig. 1) has little effect on random-movement speed (Fig. 2*A*), and a similar result was found with the weak acid KPr (Table 2). DMO and KPr inhibit (i.e., buffer) the cAMP-induced alkalinization (Fig. 3; ref. 15) and decrease locomotion speed during chemotaxis (Fig. 2*A*, Table 2). The effects of DMO and KPr are probably not due

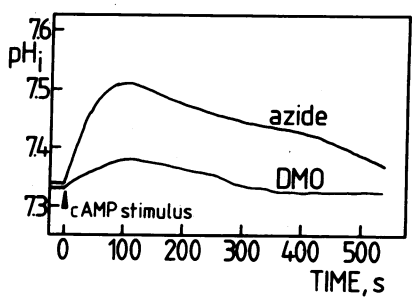


FIG. 3. $pH_i$ after stimulation of individual cells with $10^{-6}$ M cAMP in the presence of 5 mM DMO or 100 μM sodium azide. Cells were stimulated at $t = 0$ by perfusion of the measuring chamber (8) with bath solution containing cAMP. Two typical responses are shown.

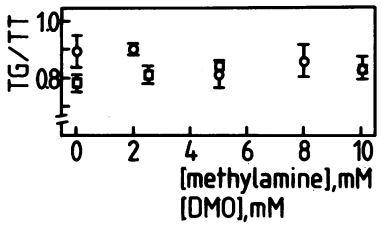


FIG. 4. Mean efficiency of chemotaxis, *TG/TT* (13), for different DMO concentrations (○) and methylamine concentrations (□). *TG/TT* value of 1 means complete chemotaxis; a value of 0 means random movement. Graph represents measurements in 1356 (DMO) and 785 (methylamine) cells in at least three independent experiments; error bars represent ± SE.

to cell damage because 5 mM DMO and 2 mM KPr have little effect on randomly moving cells, whereas DMO and KPr strongly inhibit chemotactic locomotion. Furthermore, the effects of DMO and KPr on locomotion as well as $pH_i$ are reversible, and chemotactic efficiency is unchanged (Fig. 4; Table 2). Cells treated with DMO and KPr look healthy and develop normally (15, 20). (*ii*) On the other hand, sodium azide, at the concentration that decreases $pH_i$ similarly to that of 5 mM DMO and 2 mM KPr, has no effect on random- or chemotactic-locomotion speed at either $pH_e$ 7.0 or 6.6 (Table 2; ref. 16). The cAMP-induced rise in $pH_i$ is also intact in sodium azide-treated cells (Fig. 3). From this result we conclude that the increase in locomotion speed during chemotaxis closely correlates with the cAMP-induced alkalinization of the cytoplasm. (*iii*) Increasing $pH_i$ with methylamine and ammonia increases locomotion speed during random movement (Fig. 2*B*; Table 2). However, chemotactic-locomotion speed was not further increased when $pH_i$ was increased with methylamine (Fig. 2*B*), and ammonia only slightly increased chemotactic-locomotion speed (Table 2). This result can be explained by the fact that the raised $pH_i$ in methylamine-treated cells cannot be further increased by cAMP stimulation (i.e., methylamine buffers this response, as do DMO and KPr; ref. 15) and by a leveling off of the (still hypothetical) velocity vs. $pH_i$ curve.

Our data show that $pH_i$ is strongly involved in regulating locomotion speed. However, $pH_i$ and locomotion speed do not exhibit one-to-one correspondence. For instance, the chemotactic-locomotion speed of DMO- and KPr-treated cells is greater than the random-movement speed of untreated cells (Fig. 2*A*; Table 2); however, the $pH_i$ of the former cells is much lower, and the cAMP-induced rise in $pH_i$ is suppressed (Figs. 1 and 3; Table 1). Also methylamine and ammonia are unable to speed up randomly moving cells to the same level as chemotactically moving cells (Fig. 2*B*; Table 2). Measurements in sodium azide-treated cells show that a relative rise in $pH_i$ is sufficient for a normal chemotactic response (Fig. 3; Table 2). These facts may indicate an additional speed-regulating mechanism. However, we should remember that the speed of randomly moving cells can easily be underestimated because direction of movement changes frequently and, more importantly, the different stage of development can cause different "basal" speeds of locomotion during random and chemotactic movement. We conclude that a decrease in resting $pH_i$ to 0.2 pH unit has little effect on locomotion speed (Figs. 1 and 2*A*) but that inhibition of the chemoattractant-induced rise in $pH_i$ impairs speed of locomotion during chemotaxis. Furthermore, we found that the chemoattractant-induced increase in locomotion speed can be mimicked in randomly moving cells by an artificial $pH_i$ increase.

Changes of $pH_i$, increase as well as decrease, do not change efficiency of chemotaxis (*TG/TT*) (Fig. 4; Table 2). This fact strongly suggests that the orientation of the cells in a chemoattractant gradient is regulated independently of $pH_i$ and speed of locomotion. In other words, locomotion and orientation are regulated independently.

Chemotactic migration of human neutrophils has been shown also to depend on $pH_i$ (21). Although the mechanism of $pH_i$ regulation differs between animal cells, such as neutrophils, and nonanimal cells, such as *Dictyostelium*, chemotactic activation of a $Na^+/H^+$ exchanger in animal cells has an effect identical to that caused by the chemotactic activation of electrogenic proton pump in nonanimal cells (9, 21). $pH_i$ might act via changing structure, activity, and interactions of contractile elements in the cell (for review, see ref. 21). pH-dependent actin-binding proteins that regulate actomyosin and filament cross linking are present in *D. discoideum* (22–24). The actin-binding protein hisactophilin is thought to respond in its actin-binding activity to changes in $pH_i$ upon chemotactic stimulation (25).

Our measurements support the hypothesis of Bonner *et al.* (5, 6, 10) that ammonia plays a central role in directed cell mass movements. The weak base ammonia, produced by the cells, will increase $pH_i$, which increases cell locomotion speed. In this way ammonia production and evaporation in the aggregates of *D. discoideum* cells might regulate phototaxis and thermotaxis of migrating slugs by changing locomotion speeds of individual cells. Bonner *et al.* (5, 10) reported that ammonia speeds up movement of cells in aggregation streams, although various effects on isolated cells occur. This result may appear to contradict the absence of effect in this study of another weak base, methylamine, on the speed of chemotactically moving cells (Fig. 2*B*). However, in aggregation streams, in contrast to our experiments, cells move in an oscillating chemoattractant field, and this situation is also the case with movement of slug cells (26, 27). In these cells, $pH_i$ will also change in response to the oscillating cAMP level, whereas adding the weak base will prevent any decrease in $pH_i$ during the low cAMP phase. This absence of $pH_i$ decrease may increase the overall locomotion speed.

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