Requirement of ATP in Bacterial Chemotaxis*

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Evidence is presented that chemotaxis requires ATP or a closely related metabolite, in addition to its known requirements of ATP for synthesis of S-adenosylmethionine (AdoMet) and maintenance of the proton motive force. Previous studies demonstrated a loss of tumbling and chemotaxis, and depletion of ATP when hisF auxotrophs of Salmonella typhimurium are starved for histidine (Galloway, R. J., and Taylor, B. L. (1980) J. Bacteriol. 144, 1068-1075). In the present study, intracellular [AdoMet], membrane potential, and [ATP] were measured in a hisF mutant of S. typhimurium. Membrane potential, determined from partitioning of $[^3]H$tetraphenylphosphonium ion between the inside and the outside of the cell, was about -150 mV at pH 7.6, and did not decrease in histidine starvation but was slightly increased. The concentration of AdoMet decreased from 0.4 mM to 0.3 mM during starvation but when cycloleucine, an inhibitor of AdoMet synthetase, was used to decrease [AdoMet] by a similar amount in histidine-fed cells there was little change in tumbling frequency. Intracellular [ATP] was reduced from 4.5 mM to less than 0.2 mM by histidine starvation. About 0.2 mM ATP was necessary for spontaneous tumbling. A similar [ATP] was required for tumbling in arsenate-treated cells. Adenine at concentrations as low as 20 nM caused a transient increase in both tumbling frequency and [ATP] in histidine-starved cells. Thus, out of three parameters tested, only the intracellular [ATP] correlated with changes in tumbling frequency in the histidine-starved cells.

The binding of attractants to chemoreceptors on the surface of a bacterium triggers a behavioral response and simultaneously initiates methylation of a methyl-accepting chemotaxis protein by AdoMet$^1$ (Kort et al., 1979; Springer et al., 1979). The slower methylation process restores prestimulus behavior to the bacterium and is, therefore, the mechanism of behavioral adaptation to the attractant (for a review see Springer et al., 1979; Macnab, 1980; Koshland, 1980; Taylor and Laszlo, 1981). Starvation for methionine depletes the cell of AdoMet and thereby prevents adaptation and spontaneous changes in swimming direction (tumbles) in wild type Escherichia coli and Salmonella typhimurium (Adler and Dahl, 1967; Armstrong, 1972; Aswad and Koshland, 1974, 1975a; Springer et al., 1975).

A requirement for ATP, in addition to methionine, is suggested by two lines of evidence. Arsenate, which depletes the cell of ATP, abolishes chemotaxis by preventing tumbling (Larsen et al., 1974; Aswad and Koshland, 1975a). Starvation for histidine of S. typhimurium hisF strains greatly decreases the intracellular concentration of ATP and also abolishes chemotaxis by preventing tumbling (Galloway and Taylor, 1980). Depletion of ATP could impair chemotaxis by perturbing one of the known reactions or by an unknown mechanism. Because ATP and methionine are substrates for AdoMet synthetase, earlier discussions assumed that the low ATP concentration in arsenate-treated cells caused depletion of AdoMet (Larsen et al., 1974; Aswad and Koshland, 1975a; Springer et al., 1975). Subsequently, it was reported that tumbling in certain mutants of E. coli and S. typhimurium is eliminated by arsenate treatment but not by methionine starvation (Aswad and Koshland, 1975a; Springer et al., 1975; Konodoh, 1980). This suggests that ATP might be required for chemotaxis, in addition to its role in activating methionine.

The possibility that suppression of tumbling is secondary to the effect of arsenate on the proton motive force has not been ruled out. Under appropriate conditions, arsenate can decrease the proton motive force ($\Delta$p$m$) across the inner membrane (Khan and Macnab, 1980a). A minimum proton motive force $\Delta$p$m$ is necessary to maintain the normal probability of tumbling and when $\Delta$p$m$ falls below this value the probability of tumbling in E. coli and S. typhimurium is diminished and may approach zero (Khan and Macnab, 1980a).

More recently, Arai (1981) reported that a small amount of phosphate can restore tumbling to arsenate-treated E. coli without greatly increasing the concentration of ATP. Furthermore, an uncA mutant has normal tumbling frequency at ATP concentrations that are lower than those in arsenate-treated wild type cells. Arai concluded that a phosphorylated compound other than ATP is required for chemotaxis. There is, therefore, considerable uncertainty about the mechanism of the arsenate effect on chemotaxis.

Histidine starvation of hisF strains of S. typhimurium is particularly useful in probing the role of ATP in chemotaxis because it induces adenine starvation in contradistinction to arsenate treatment which induces phosphate starvation. Adenine and ATP are depleted in histidine starvation as a consequence of the loss of feedback inhibition of phosphoribosyltransferase, the first enzyme in the histidine biosynthetic pathway (Shedlovsky and Magasanik, 1962a, 1962b). In minimal medium containing glucose, hisF strains cease tumbling about 2 h after they are transferred to histidine-free medium, but vigorous motility continues for more than 24 h (Galloway and Taylor, 1980). Tumbling is rapidly restored in histidine-starved cells by addition of adenine. Swimming speed and adaptation to serine and aspartate are normal in the histidine-starved bacteria, suggesting that the tumbling defect is not

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$^1$ The abbreviations used are: AdoMet, $S$-adenosyl-$L$-methionine; $\Delta$V, membrane potential; $\Delta$p$m$, proton motive force; TPP$, tetraphenylphosphonium bromide; TPRMP$, triphenylmethylphosphonium bromide.
the result of lower ΔΨm or AdoMet depletion. In this study, we measured the AdoMet level and ΔΨ in histidine-starved cells and demonstrated that they are adequate to support tumbling. Close correlations between the ATP level and tumbling frequency under various conditions indicated a requirement for ATP or a closely related metabolite in chemotaxis. A preliminary account of this work has been presented (Shioi and Galloway, 1981).

**EXPERIMENTAL PROCEDURES**

**Materials**

L-[methyl-3H]Metionine (20 Ci/mmole) and S-adenosyl-L-[methyl-3H]Metionine (6.6 Ci/mmole) ([3H]AdoMet) were obtained from ICN Chemical and Radioisotope Division, [methyl-3H]Triphosphonperte (1[H]TPMP)] (3.59 Ci/mole) was obtained from New England Nuclear. [phenyl-3H]Tetraphenylphosphonium bromide ([3H]TPP)) (5.6 Ci/mole) was purchased from Nuclear Research Center, Negev, Israel. Cyclooxygen (1-amino-1-carboxylic acid), AdoMet, and SP-Sephadex C-25 were obtained from Sigma. Sodium tetraphenylboron and triphenylphosphonium bromide were obtained from K and K Laboratories, Plainview, NY. Tetraphenylphosphonium bromide was obtained from Aldrich. ATP Monitoring Reagent was purchased from LKB-Wallace.

**Bacterial Strains**

*S. typhimurium* ST171 (hisF thyA cheA) is a constantly tumbling mutant derived from *S. typhimurium* ST23 (hisF thyA) which is wild type for chemotaxis (Aswad and Koshland, 1975b). *S. typhimurium* SL3730 (galK galU str) is known to be permeable to the fluorescent dyes, 8-anilino-1-naphthalene sulfonate and dis-C2-(S) (Laszlo and Taylor, 1981).

**Growth and Starvation of Cells**

Cells were grown at 30 °C in Vogel and Bonner medium E (Vogel and Bonner, 1956), fortified with the auxotrophic requirements of the strain and glucose (7 mg/ml) as the carbon source. Overnight cultures were diluted 1:10 into growth medium and the cells were harvested during the exponential phase of growth (OD540 = 0.6 to 0.8) by centrifugation at 6000 × g for 10 min. For histidine starvation, the pellets were washed twice in starvation medium (histidine-free growth medium), resuspended in a volume of starvation medium equivalent to the initial culture volume, and incubated at 30 °C in a shaker incubator. The timing of starvation similarly was commenced at the final resuspension in starvation medium.

**Observation of Motility**

A small drop (10 μl) of bacterial suspension was placed on a microscope slide and the bacteria were observed at a magnification of ×100 through a Leitz Dikablu trinocular microscope with dark-field optics and an objective lens (L32) with a long working distance. The tumbling frequency of the bacteria was measured by the photographic procedure of Spudich and Koshland (1975). Measurement of swimming velocity has been described previously (Galloway and Taylor, 1980).

**AdoMet Assay**

**Isotope-labeling Method—Aliquots (7 ml) taken from a starved or unstarved culture were labeled for 20 min with 250 μCi L-[3H]Metionine in the presence of 200 μg/ml of chloramphenicol. Six milliliters of labeled culture was removed and quickly added to the cell suspension which had been preincubated with L-[3H]Metionine for 10 min in the presence of 200 μg/ml of chloramphenicol. After incubation for 30 min, cell motility was analyzed and the cell suspension was treated with cold trichloroacetic acid solution and assayed for AdoMet.

**EDTA Treatment**

The permeability of the intact cell to TPP⁺ varied from 1 day to another. The cells were consistently permeable after treatment with EDTA by the procedure of Leive (1965) or Szmelcman and Adler (1976), but these treatments partly reversed the behavioral change observed in histidine-starved cells. By simplifying the procedure as described below, the smooth motility in histidine-starved cells was successfully preserved. Cells were harvested by centrifugation at 6000 × g for 8 min and the pellet was resuspended in 0.1 M Tris-HCl, 10 mM EDTA (pH 8.0) to one-fourth of the initial volume. After incubation for 1 min at 30 °C, the suspension was diluted 1:10 into motility medium (pH 7.1) which consisted of 10 mM potassium phosphate, 0.1 mM EDTA, and 20 mM potassium lactate. The pH of the final suspension was 7.6 and the cell concentration was about 1.4 × 10⁹ cells/ml. The permeability to TPP⁺ of *S. typhimurium* treated by this simplified procedure and resuspended in motility medium was similar to the permeability of cells treated by the complex procedure (Szmelcman and Adler, 1976) and of the permeable *S. typhimurium* mutant SL3730. The cells remained permeable for more than 30 min after treatment. *S. typhimurium* treated by the simplified procedure was not permeable to the ions immediately after treatment. Therefore, treated cells could not be transferred into growth medium or starvation medium for measurement of membrane potential.

**Measurement of Membrane Potential**

TPP⁺ uptake was determined by the method of Shioi et al. (1980) with the following modification. Permeable *S. typhimurium* suspended in motility medium (0.25 to 0.75 ml) was incubated with 10 μCi [1H]TPP⁺ at 30 °C with vigorous shaking. The optimal concentration of cells had an optical density of 600 nm of 2 (1.4 × 10⁹ cells/ml). At higher densities, the cells became anaerobic. Aliquots (50 μl) removed after 3, 4, and 5 min were diluted with 2 ml of the motility medium, filtered on Millipore filters (Millipore, pore size 0.45 μm), and washed with 2 ml of the motility medium. Between 20% and 40% of the total TPP⁺ was retained on the filters. Nonspecific binding of the radioactivity to the cells was small and was corrected for after treating the cells with 2% toluene. The uptake of TPP⁺ reached a maximum after 3 min and gradually decreased after 5 min. Membrane potential (ΔΨ) was calculated from the partitioning of [3H]TPP⁺ between the inside and outside of the cells using the Nernst equation. The uptake of TPM⁺ in the presence of sodium tetrathylboron gave similar ΔΨ values but equilibrated more slowly.

**ATP Assay**

Aliquots of the trichloroacetic acid extract were diluted more than 10-fold with 0.1 M Tris-HCl, 2 mM EDTA buffer (pH 7.75) or were extracted with ether three times. A purified luciferase ATP Monitoring Reagent (LKB-Wallace) was used to determine ATP because it

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CA with a Beckman LS5700 liquid scintillation counter. AdoMet appeared in a single peak eluted by the step pH gradient but was not present in these fractions if the trichloroacetic acid extract was heated in boiling water. In unstarved cells, about 0.1% of the radioactivity in t-[3H]Metionine added to the external medium appeared in AdoMet isolated from the bacteria. The recovery of AdoMet was 77 ± 8%. The intracellular concentration of AdoMet was calculated on the assumption that 1 A unit at 600 nm corresponds to 0.88 μl of cell volume/ml.

**Spectrophotometric Method**—The entire 200 ml of starved or unstarved culture was centrifuged at 6,000 × g for 10 min. The pellet was resuspended in 7 ml of starvation or growth medium, and the optical density at 900 nm was determined. Six milliliters of the cell suspension was mixed with 4 ml of the ice-cold 20% (w/v) trichloroacetic acid and [3H]AdoMet was added to quantitate the recovery. AdoMet was extracted and chromatographed by the procedure used for labeled AdoMet and the concentration was determined from absorbance at 257 nm.

**Alteration of Intracellular AdoMet Level**

Cyclooxygen, an inhibitor of AdoMet synthetase, was ground finely in a mortar and added directly to the cell suspension which had been preincubated with L-[3H]Metionine for 10 min in the presence of 200 μg/ml of chloramphenicol. After incubation for 30 min, cell motility was analyzed and the cell suspension was treated with cold trichloroacetic acid solution and assayed for AdoMet.

**A preliminary account of this work has been presented (Shioi and Galloway, 1981).**
produced a relatively constant luminescence. Luminescence was counted for 0.1 min with a Beckman LS7500 liquid scintillation system with the coincidence circuit off (Lundin and Thore, 1975). The minimal detectable ATP concentration was $10^{-11}$ M. The assay was performed in duplicate and corrected for an extracellular ATP concentration of about 15 nM.

RESULTS

Behavior in Histidine Starvation—*S. typhimurium* ST171 has auxotrophic requirements for histidine and thymine, and has a constantly tumbling phenotype in which the bacterium performs an erratic tumbling motion in the unstimulated state. Various treatments induce translational movement termed "smooth swimming." The motility was quantitated photographically using a 1-s exposure of bacteria illuminated with a 4.7 Hz stroboscopic light source (Macnab and Koshland, 1972; Spudich and Koshland, 1975). Each swimming bacterium was represented on the photographic negative by a sequence of five images. The bacterium was scored as "tumbling" if the images were superimposed or indicated an acute change in direction, or "smooth" if the images formed a straight, or gently curving line. Smooth tracks were essentially absent from photographs of a normal motility of ST171. When ST171 was starved for histidine in minimal medium containing glucose and thymine, the total population of cells changed to smooth swimming over a short time interval, about 2 h after washing (Fig. 1). On the other hand, thymine starvation in the growth medium did not change the behavior at all (data not shown).

Some requirements for suppression of tumbling by starvation were investigated (Fig. 1). There was little change in tumbling frequency when ST171 was starved for histidine in chemotaxis buffer (10 mM potassium phosphate, pH 7.1, 0.1 mM EDTA, 7 mg/ml of glucose). The addition of Mg$^{2+}$ to the chemotaxis buffer slightly accelerated the decrease in tumbling frequency during starvation and the addition of NH$_4^+$ caused a 50% decrease in tumbling. The simultaneous addition of Mg$^{2+}$ and NH$_4^+$ resulted in an even greater effect on tumbling in histidine starvation. It is likely that a nitrogen source stimulated protein synthesis and thereby facilitated depletion of the intracellular histidine pool. In the following experiments, bacteria were starved for histidine in medium E which includes both NH$_4^+$ and Mg$^{2+}$.

AdoMet Level in Histidine Starvation—To determine whether smooth swimming was caused by AdoMet depletion, the intracellular concentration of AdoMet was monitored during the course of histidine starvation. In the isotope-labeling procedure, L-$[^3]$H]methionine added to the medium was rapidly transported into the cell and incorporated into $[^3]$H] AdoMet. Due to a rapid turnover of AdoMet, it was necessary to add at least 0.25 mM methionine to the medium in order to achieve a steady state level of intracellular $[^3]$H]AdoMet (data not shown). The steady state AdoMet concentration of unstarved *S. typhimurium* ST171 was a function of the extracellular methionine concentration, and was 0.4 mM in the presence of 0.25 mM methionine and 1.4 mM in the presence of 5 mM methionine. Using the spectrophotometric procedure, the intracellular AdoMet concentration was found to be 0.32 mM in methionine-free medium.
When ST171 was starved for histidine, there was a 30% decrease in the intracellular concentration of AdoMet that was concomitant with the change of behavior (Fig. 2). The observed decline in the AdoMet level was not an artifact of the isotope-labeling method and was confirmed using the spectrophotometric assay for AdoMet (data not shown).

In order to evaluate the significance of a 30% decrease in AdoMet, the intracellular concentration of AdoMet in unstarved S. typhimurium ST171 was decreased by addition of cycloleucine, an inhibitor of AdoMet synthetase (Lombardini et al., 1970). A logarithmic increase in the concentration of cycloleucine, over the range 10 mM to 400 mM, caused a linear decrease in the concentration of AdoMet (Fig. 3). The unstimulated behavior of ST171 was affected by reduction of AdoMet to low concentrations but a 30% decrease in AdoMet level had little effect on the tumbling frequency, even though the same decrease in AdoMet doubled the response times to L-serine and L-aspartate. Therefore, the 30% decrease of AdoMet level cannot explain the drastic decrease in tumbling frequency in histidine starvation.

Confirmation of this conclusion was found in two additional experiments. Tumbling was restored to histidine-starved cells within a few seconds of the addition of 10 μM adenine, but more than 15 s was required for restoration of the AdoMet level (Fig. 4A). In the presence of 100 mM cycloleucine, adenine restored tumbling quickly, but did not increase the AdoMet level (Fig. 4B).

The Proton Motive Force in Histidine Starvation—Whenever the probability of tumbling in bacteria is diminished as the result of a decrease in ΔψH+, the swimming speed is also diminished (Khan and Macnab, 1980b; Laszlo and Taylor, 1981). The swimming speed of S. typhimurium ST171 was found to be constant during histidine starvation although the tumbling frequency changed drastically (Fig. 5A). This suggested that ΔψH+ remained above the saturating level for swimming velocity and for tumbling.

The membrane potential of intact ST171, determined from the distribution of [3H]TPP+ between the intracellular space and the extracellular medium, was about -150 mV and was slightly increased, rather than decreased, by histidine starvation (Fig. 5B). Because the membrane potential (Δψ) is the only significant component of the proton motive force at an external pH of 7.6 (Zilberstein et al., 1979; Khan and Macnab, 1980b; Felle et al., 1980; Slonczewski et al., 1981), the results indicated that ΔψH+ of the cells was not diminished by histidine starvation.

Correlation of ATP Concentration and Tumbling Frequency—In contrast to Δψ and the concentration of AdoMet, the intracellular concentration of ATP was greatly reduced in histidine starvation (Fig. 6A). The ATP level began to decline during cell washing and was 10% of normal after 1 h of histidine starvation. When the ATP level decreased to less than 0.3 mM (5% of normal), there was a steep drop in tumbling frequency, and very low concentrations of ATP correlated with complete suppression of tumbling.

The addition of 1 μM adenine to histidine-starved cells restored tumbling within 3 s with a gradual increase in ATP concentration in the cells (Fig. 6B). Even 0.1 μM adenine induced a transient recovery of tumbling. Adenine uptake also increased the concentration of ATP, and, at the lowest concentration of adenine, the time course of the change in ATP corresponded to the transient change in tumbling frequency. Although the change in ATP level induced by 0.1 μM adenine was small, it was nevertheless significant as is evident from a similar experiment where the concentration of ATP was plotted on an expanded scale (Fig. 7).
In Fig. 7, the concentration of adenine added to histidine-starved ST171 varied from 10 nM to 100 nM; and at each concentration, there was a correlation between the percentage of tumbling cells and the concentration of ATP. The increase in intracellular ATP concentration from 0.15 mM to 0.17 mM in Fig. 7 is equivalent to depletion of 35% of the 20 mM extracellular adenine. In the previous figures, cells were scored as tumbling if they tumbled once or more during a 1-s exposure used to record motility. This measure of total tumbling cells included constantly tumbling cells and the random cells that tumbled during the exposure. In Fig. 7, the total tumbling cells and the constantly tumbling cells are shown separately, and both estimates of tumbling frequency correlate with the ATP level.

The increase in ATP concentration and tumbling frequency induced by adenine was not affected by the presence of 100 mM cycloleucine (data not shown), although the increase in AdoMet concentration was completely abolished. Guanine restored tumbling poorly and cyclic AMP and cyclic GMP included constantly tumbling cells and the random cells that tumbled during the exposure.

**Fig. 6.** Comparison of the intracellular concentration of ATP and tumbling frequency of *S. typhimurium* ST171 after (A) L-histidine depletion and (B) addition of adenine to L-histidine-starved cells. At intervals, motility was photographed (see Fig. 1). 0.6 ml of the culture was removed and added to 0.4 ml of 20% trichloroacetic acid, 5 mM EDTA. After incubation for 20 min at 0°C, ATP was measured as described under “Experimental Procedures.” A, the procedure for histidine starvation is described in Fig. 1. (C) ATP concentration, (D) percentage of cells that tumbled. B, after starvation for 4 h, adenine was added (t = 0) at the indicated concentrations. Open symbols, ATP concentrations; closed symbols, tumbling cell fractions. Adenine concentration: (C) 0.1 μM, (D) 1 μM, (E, F) 10 μM.

**Fig. 7.** Change in intracellular ATP and tumbling frequency in S. *typhimurium* ST171 after addition of low concentrations of adenine. After 4 h of L-histidine starvation, various concentrations of adenine were added to the culture. ATP concentrations (C) were assayed as described in Fig. 6. Behavior was assayed as described in Fig. 1. A tumbling cell (●) was defined as one that appeared as a blurred spot on the photographic negative (see “Experimental Procedures” for details). A tumbling cell (△) had one or more abrupt changes of direction on the negative. Adenine concentration: (A) 10 nM, (B) 20 nM, (C) 50 nM, (D) 100 nM.

**Fig. 8.** Intracellular ATP concentration (●) and tumbling frequency (•) in arsenate-treated *S. typhimurium* ST171 before and after the addition of sodium phosphate. ST171 cells, grown as described in Fig. 1, were washed and resuspended in medium (pH 7.0) containing 10 mM sodium arsenate, 0.1 mM EDTA, and 10 mM sodium lactate (Arai, 1981). The cells were incubated at 30°C for 130 min before 1 mM sodium phosphate (pH 7.0) was added to the cell suspension. Motility and ATP analyses were performed as described in Fig. 6.
that observed in histidine-starved cells (Figs. 6 and 7), and it is likely that the concentration of ATP regulates tumbling frequency in arsenate-treated cells.

**Preservation of Tumbling in Histidine-starved Bacteria—**
Several variables counteract the suppression of tumbling in histidine starvation. These include addition of chloramphenicol, simultaneous starvation for methionine and histidine, and the carbon source (Galloway and Taylor, 1980). Chloramphenicol inhibition and methionine starvation were assumed to decrease utilization of L-histidine and ATP by stopping protein synthesis. If tumbling suppression is the result of ATP depletion, conditions that prevent or reverse the loss of tumbling should increase the concentration of ATP in histidine-starved cells. This was found to be true for two conditions that we investigated. When histidine starvation of *S. typhimurium* ST171 was carried out in succinate medium rather than glucose medium, the loss of tumbling was delayed by approximately 3 h (Fig. 9). Compared with cells in glucose medium (Fig. 2), there was a slower decline in ATP level in ST171 in succinate medium and this accounted for the delayed loss of tumbling.

Chloramphenicol (200 µg/ml) restored tumbling in histidine-starved ST171 and there was a concomitant increase in the ATP level (Fig. 10).

**Discussion**

In bacterial chemotaxis, migrational behavior is accomplished by modulating the frequency of tumbling in the swimming cells. As a result, bacteria that are deficient in tumbling are also deficient in chemotaxis. In *S. typhimurium* and *E. coli*, a minimum concentration (0.2 mM) of intracellular ATP was required to maintain spontaneous tumbling and chemotaxis. A direct relationship between the ATP concentration and tumbling frequency is indicated by the following evidence.

1. In cells that were starved for histidine in minimal medium containing glucose, cessation of tumbling occurred after 2 h and was concurrent with depletion of ATP (Fig. 6).
2. In succinate medium, the ATP level declined more slowly in histidine starvation and suppression of tumbling was delayed until ATP decreased to about 0.3 mM (Fig. 9).
3. Low concentrations of adenine rapidly and specifically restored tumbling in histidine-starved bacteria. The increase in tumbling after addition of 20 nM to 100 nM adenine was transient and paralleled a transient increase in ATP (Fig. 7).
4. The addition of chloramphenicol to histidine-starved cells restored tumbling and the recovery of normal behavior was coincident with an increase in ATP level (Fig. 10).
5. The relationship between ATP and tumbling frequency was qualitatively similar in histidine starvation and in arsenate treatment (compare Figs. 6 and 8). That is, depletion of adenine and depletion of phosphorylated compounds have similar effects on behavior. This strongly suggests that a phosphorylated adenylate compound is required for tumbling. In adenine-depleted bacteria, factors which increased the energy charge (e.g. inhibition of protein synthesis by chloramphenicol) also aided recovery of tumbling. We conclude that the essential adenylate compound is ATP, although it is possible that a metabolite derived from ATP is the active agent.

The ATP requirement in chemotaxis is independent of the requirement for AdoMet and δ-lys. In histidine starvation, the level of AdoMet decreased by 30%, but on the basis of Fig. 3, this would account for a 3% decrease in tumbling frequency, not the 90% decrease in tumbling that was observed (Fig. 2). Furthermore, recovery of tumbling after addition of adenine was independent of change in AdoMet concentration (Fig. 4). Histidine starvation did not alter the swimming speed of *S. typhimurium* although it increased membrane potential slightly (Fig. 5). An increase in potential would not cause prolonged smooth swimming.

The elimination of AdoMet or membrane potential as the basis for the loss of tumbling in adenine-depleted cells, implies that ATP has a novel, unidentified role in chemotaxis. This laboratory recently demonstrated that a methylation-independent sensory adaptation mechanism is involved in aero- and in chemotaxis to phosphotransferase substrates (Niwan and Taylor, 1982). A methylation-independent adaptation to repellents was reported by Stock et al. (1981). It is possible that ATP is required in one of these adaptation mechanisms and histidine starvation will be a useful probe to explore this possibility.

The ATPase-deficient *E. coli* AN120 (uncA) continues to tumble in arsenate medium until the intracellular ATP concentration decreases to extremely low levels (0.03 nmol/mg of protein or 0.02 mM) (Arai, 1981). This suggests that the ATP threshold for tumbling is lower in AN120 than in unc + strains. The minimum concentration of ATP required for chemotaxis might also be lowered by adaptation. In histidine starvation, we consistently observed that the ATP level required to support a given tumbling frequency was slightly lower after *S. typhimurium* adapted to depletion of ATP. For example, compare the ATP levels in the depletion and recovery phases
in Fig. 8. Although these differences were small they were observed in all our experiments.

The relationship between AdoMet concentration and spontaneous tumbling frequency suggests that the steady state tumbling frequency is also regulated by the absolute level of methylation of the methyl-accepting chemotaxis proteins. If cycloleucine, which is not a chemoeffector, was added to *Salmonella typhimurium* ST171 that had previously adapted to attractants in the medium, the spontaneous tumbling frequency in ST171 was only slightly affected by small changes in the phenotype of strains which are defective in methylation (Springer et al., 1979; Koshland, 1981; Parkinson, 1977).

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