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Aerotaxis in *Salmonella typhimurium*: Role of Electron Transport

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Sensory transduction in aerotaxis required electron transport, in contrast to chemotaxis, which is independent of electron transport. Assays for aerotaxis were developed by employing spatial and temporal oxygen gradients imposed independently of respiration. By varying the step increase in oxygen concentration in the temporal assay, the dose-response relationship was obtained for aerotaxis in *Salmonella typhimurium*. A half-maximal response at 0.4 μM oxygen and inhibition by 5 mM KCN suggested that the "receptor" for aerotaxis is cytochrome *o*. The response was independent of adenosine triphosphate formation via oxidative phosphorylation but did correlate with changes in membrane potential monitored with the fluorescent cyanine dye diS-C_3-(5). Nitrate and fumarate, which are alternative electron acceptors for the respiratory chain in *S. typhimurium*, inhibited aerotaxis when nitrate reductase and fumarate reductase were induced. These results support the hypothesis that taxis to oxygen, nitrate, and fumarate is mediated by the electron transport system and by changes in the proton motive force. Aerotaxis was normal in *Escherichia coli* mutants that were defective in the *tsr*, *tar*, or *trg* genes; in *S. typhimurium*, oxygen did not stimulate methylation of the products of these genes. A *cheC* mutant which shows an inverse response to chemoattractants also gave an inverse response to oxygen. Therefore, aerotaxis is transduced by a distinct and unidentified signalling protein but is focused into the common chemosensory pathway before the step involving the *cheC* product. When *S. typhimurium* became anaerobic, the decreased proton motive force from glycolysis supported slow swimming but not tumbling, indicating that a minimum proton motive force was required for tumbling. The bacteria rapidly adapted to the anaerobic condition and resumed tumbling after about 3 min. The adaptation period was much shorter when the bacteria had been previously grown anaerobically.

Bacterial migration in response to oxygen gradients was demonstrated as early as 1881. Engelmann (13) observed microscopic accumulation of bacteria about air bubbles, at the edges of a cover glass, and around photosynthetically active plant cells. On the basis of the macroscopic bands that formed beneath a cover glass, Beijerinck defined three categories of aerotactic response: aerobic bacteria formed a band adjacent to the air interface, anaerobes moved as far as possible from the interface, and spirillar bacteria (microaerophiles) congregated a short distance from the interface (6). More recently, Baracchini and Sherris (5) surveyed 24 species of highly motile bacteria and confirmed that aerobic and facultative anaerobic species showed positive aerotaxis, whereas three anaerobic species of *Clostridia* showed negative aerotaxis. Evidently, bacteria seek an oxygen concentration that is optimal for their metabolic "life style," and oxygen may function as an attractant or a repellent, depending on the species studied. The dual properties of oxygen as a chemoeffector, together with evidence for a novel sensory transduction mechanism, have stimulated an investigation of the mechanism of aerotaxis in our laboratory. In the studies described in this paper, aerotaxis was investigated in *Salmonella typhimurium* and *Escherichia coli*, which are attracted to oxygen.

Aerotaxis and chemotaxis to amino acids and sugars are functionally equivalent (1, 2). In an isotropic medium, *E. coli* and *S. typhimurium* consume oxygen and carbon sources in their environment, thereby creating gradients. The bacteria then move preferentially toward regions of a more concentrated oxygen or carbon source, depending on which substance is limiting. Considerable progress has been made in understanding the mechanism of the chemotactic response in *S. typhimurium* and *E. coli* (18, 25, 28, 46; B. L. Taylor and D. J. Laszlo, *In* D. Norris (ed.), *The Perception of Behavioral Chemicals*, in press). Swimming bacteria change direction

(tumble) about once per second and, as a result, move in a three-dimensional, random walk pattern (7). When the bacteria detect an increase in attractant concentration or a decrease in repellent concentration they temporarily suppress tumbling so that they continue to swim in the favorable direction (29). The molecular events associated with the regulation of tumbling include formation of a chemoeffector-receptor complex and interaction of the complex with a membrane bound signalling protein which ultimately affects the probability of tumbling. Methylation of the signalling protein mediates adaptation to attractant stimuli (22, 43, 46, 47, 49). At least 12 gene products are required for signal transduction and processing (11, 42, 44, 55). Neither the electron transport system nor changes in the proton motive force are required for chemotaxis to sugars and amino acids (35, 37, 51; M. Snyder, J. Stock, and D. E. Koshland, Jr., personal communication).

In contrast to the extensive studies of chemotaxis, the mechanism of aerotaxis is relatively unexplored; even the identity of the oxygen receptor has not been clearly established. Clayton (9) observed that cyanide inhibits aerotaxis in *Rhodospirillum rubrum* and suggested that both electron transport and the rate of oxidation of the carbon source are important in aerotaxis. Cytochrome *c* oxidase has been proposed as the receptor for aerotaxis in *Euglena* sp. (38). Recently, Taylor et al. (51) confirmed that cyanide also inhibits aerotaxis in *S. typhimurium* and proposed that aerotaxis is a specific example of a more general phenomenon that they named electron acceptor taxis. *S. typhimurium* is attracted by a variety of alternative electron acceptors for the respiratory chain, including nitrate, fumarate and trimethylamine oxide. Electron acceptor taxis to nitrate and fumarate requires the appropriate electron transport pathway and is abolished by specific mutations in the pathway. As a result of these studies, it was hypothesized that electron transport mediated changes in the proton motive force are a common factor in electron acceptor taxis (51). This paper presents direct evidence for the role of electron transport and the proton motive force in aerotaxis in *S. typhimurium*.

(Preliminary reports of this work have been presented elsewhere [B. L. Taylor and D. J. Laszlo, Fed. Proc. **39**:2103, 1980; D. J. Laszlo and B. L. Taylor, Clin Res. **28**:9A, 1980.].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains of *S. typhimurium* and *E. coli* used in this study are described in Table 1. Cells were grown at 30°C in Vogel-Bonner citrate medium (53), pH 7.0, supplemented with glycerol (1.0% [vol/vol]) or glucose (0.6% [wt/vol]) and the auxotrophic requirements of the strain. Early log-phase cells (optical density at 600 nm = 0.2 to 0.25) were washed and suspended in Vogel-Bonner medium with either glucose or glycerol and with appropriate supplements.

Observation of motility and measurement of speed. Bacteria were observed at a magnification of ×500 or ×800 by using a Leitz Dialux trinocular microscope with dark-field optics and an objective lens with a long working distance (UMK50 or L32). The microscope was fitted with interchangeable photographic (Nikkormat ELW) and video equipment (camera, Sanyo model VC3300X; recorder, Sanyo model VTC7100; monitor, Hitachi model VM-172).

The photographic analysis of motility was similar to the procedure of Spudich and Koshland (48). A 2-s exposure was made of bacteria with 5-Hz stroboscopic illumination. The fraction of smoothly swimming bacteria was determined as the ratio of tracks without abrupt change in direction to the total number of tracks.

The video analysis of motility was made during replay in slow motion. The path of a bacterium during a timed interval was marked on the monitor screen, and the speed was calculated. Tumbling frequency was determined as the reciprocal of the mean time interval between consecutive tumbles by a bacterium.

For observation of the light response, bacteria were illuminated with a high-intensity mercury arc lamp (Osram HBO100w/2) as described previously (50, 51). For normal observation, a long-pass orange filter (Oriel 5150, 50% transmission, 530 nm) was inserted in the light path to protect bacteria from intense blue light (30). The effect of a pulse of blue light on cell motility was observed when the filter was briefly removed from the light path.

Aerotaxis assays. Both the spatial and temporal aerotaxis assays utilized a Lucite microchamber (3.8 by 8.0 by 0.4 cm) with a cover glass top (2.4 by 6.0 cm, no. 1 thickness) and glass bottom (3.8 by 7.5 by 0.1 cm); one end of the chamber was removable. Gas was introduced via Teflon tubing (type FEP) which extended into the closed end of the microchamber. The gas stream from the perforated end of the tubing was diffused by a baffle of polyurethane foam. The microchamber was placed on the stage of a Leitz Dialux microscope equipped with a Leitz 606 dark-field condenser. A drop of culture was placed near one end of a microscope slide which was then inserted into the chamber. Observation of the bacteria through the top of the chamber was possible with a long-working-distance objective.

For the spatial assay, bacteria grown in Vogel-Bonner medium with glycerol were washed and suspended in Vogel-Bonner medium with glucose (optical density at 600 nm = 0.10). A 3-ml amount of cell suspension was placed in a test tube (15 by 1.8 cm) and sealed with a stopper perforated by a gas inlet, an outlet, and a length of needle stock (24 gauge) that reached the bottom of the tube and was bent at a right angle at the upper surface of the stopper. The cells were gassed with nitrogen for 30 min. By briefly closing the gas outlet, a drop of the culture was injected between a slide and cover glass spaced by capillary fragments

TABLE 1. *Bacterial strains*

Strain	Relevant genotype[a]	Source or reference
S. typhimurium		
ST1	Wild type (selected for serine chemotaxis from LT2)	3
ST23	ST1 *hisF8786 thyA1981*	3
ST171	ST23 *cheZ221*	3
ST832	*trg*	D. E. Koshland, Jr.
ST352	*unc*	T. D. Ingolia and D. E. Koshland, Jr.
ST134	ST23 *cheC84*	43
ST55	ST23 *cheW226*	55
ST162	ST23 *cheR212*	55
ST108	ST23 *cheS58*	47
ST176	ST23 *cheY62*	55
SL2516	SL4213 *cheV107*	10
SL3730	*galK galU463 str*	M. Snyder
BT10	ST23 *cheZ221 met*E862::Tn*10*	14
BT11	ST23 *cheB111*::Tn*10*	14
E. coli		
RP477	F′ *thi thr*(Am) *leu his* Δ(*gal-att, eda, rpsL*)	39
RP437 (*tar*)	RP437 *eda*$^+$ *tar-539*	J. S. Parkinson
RP4790	RP477 *thr*$^+$ *tsr-14*	40
AW702	*trg thr leu his thi*	24

[a] The gene designations shown are the nomenclature adopted recently by workers in chemotaxis. For *S. typhimurium che* genes, the original *P*, *Q*, *T*, *U*, and *Y* have been replaced by *A*, *Y*, *Z*, *C*, and *B*, respectively (14).

within the nitrogen-filled microchamber. A capillary tube (1-μl Drummond Microcap) was filled with either oxygen or nitrogen and sealed at one end. The open end was inserted into the drop, and the behavior of the cells around the end was observed. KCN, when used, was added anaerobically to the test tube to a final concentration of 5 mM.

For the temporal assay the microchamber was connected to a gas proportioner (Fig. 1). A flow meter (Matheson 1472) with a mixing chamber was fitted with stainless steel inlet tubes connected to cylinders of prepurified nitrogen and oxygen or 1% oxygen in nitrogen (Matheson). In some of the early experiments, argon was used in place of nitrogen. A three-way valve permitted selection between two ranges of oxygen flow rates. An additional system provided humidified, oxygen-free nitrogen; gas from a low-pressure regulator was directed through a pair of gas washing bottles (Kimax) connected with butyl rubber tubing (Fisher Scientific). One of the bottles contained a solution of 0.15 M pyrogallol in 10 M KOH to remove residual oxygen, and the other bottle held distilled water. A four-way valve permitted selection between scrubbed nitrogen and the oxygen mixture. A Beckman E2 oxygen analyzer was used to calibrate the proportioner. Direct measurements were obtained over the range of 24 to 0.1% oxygen by mixing pure oxygen and nitrogen. More dilute oxygen mixtures (0.002 to 0.24%) were formed by mixing 1% oxygen in nitrogen with nitrogen.

The response to a temporal gradient of oxygen was determined by placing 2 to 10 μl of culture on a microscope slide in the microchamber and ventilating

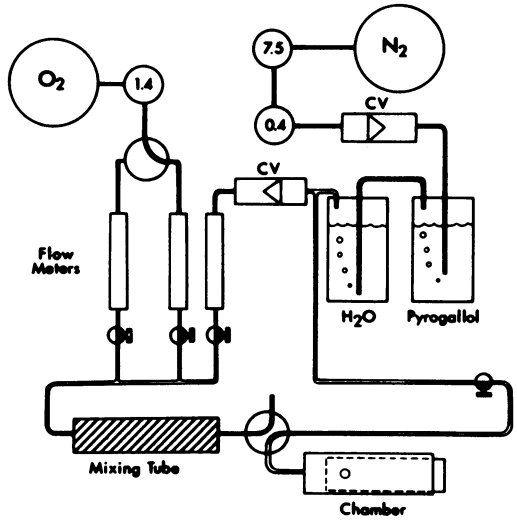

FIG. 1. *Gas flow diagram for oxygen temporal gradient apparatus. The cylinder designated O_2 contained either oxygen or 1% oxygen in nitrogen. In some earlier experiments, argon was substituted for nitrogen. The four-way valve between the mixing tube and chamber was used to switch the ventilating gas from nitrogen to an oxygen plus nitrogen mixture. Gas regulators are shown as small circles, with the selected pressure (in kilograms per square centimeter) indicated within the circle. cv, One-way check valve.*

the chamber with nitrogen for 4 min. Oxygen was readmitted to the chamber as a mixture with nitrogen or as air that rapidly diffused into the chamber when the nitrogen flow was stopped. The behavior of the bacteria was observed and videotaped for analysis. For the dose-response studies, 2 μl of culture was spread on the slide to a diameter of 6 mm. The culture was equilibrated with nitrogen for only 1 min (to minimize evaporative loss) before exposure to the desired oxygen concentration.

Fluorometric measurement of membrane potential. A 1-μl amount of an ethanol solution (1 mM) of the cyanine dye diS-C_3-(5) (45) or 2 μl of 8-anilino-1-naphthalene sulfonate (5 mM) was added to 3 ml of cell suspension (optical density at 600 nm = 0.3) in a fluorometer cuvette. The cells were stirred continuously by a magnetic bar, and a stream of air or nitrogen was directed into the capped cuvette. Fluorescence was measured in a Spex Fluorolog spectrofluorometer with multialkali photodetector, with excitation and emission wavelengths of 622 and 670 nm, respectively, for diS-C_3-(5) and 375 and 472 nm, respectively, for 8-anilino-1-naphthalene sulfonate. The band-pass was 10 nm. Simultaneous measurement of oxygen concentration was made with a chemical microsensor (Transidyne General) connected to a needle oxygen electrode (Transidyne General no. 760) and a silver-silver chloride reference electrode positioned in opposite corners of the fluorometer cuvettes so that they were out of the light path. Additions were made with a Hamilton syringe through the gas outlet pore in the cuvette cap. At the end of the experiment, 1 drop of culture from the cuvette was examined under the microscope to confirm that the bacteria were motile and had not been killed by the photodynamic action of the dye (36, 50).

RESULTS

Spatial assay for aerotaxis. The aerotaxis assays used by most previous investigators depended upon bacterial oxygen consumption to generate the gradients to which the bacteria responded (2, 5, 6). This type of assay is clearly unsatisfactory for investigating the role of electron transport in aerotaxis. For example, cyanide would inhibit respiration-mediated formation of the gradient whether or not respiration is required for aerotaxis. We have developed alternative assays which can be used in the presence of respiratory inhibitors. An artificially generated spatial gradient of oxygen was used for qualitative measurements, and a temporal assay was used to quantitate the aerotactic response to precisely determined oxygen concentrations.

For the spatial assay, a dilute culture of *S. typhimurium* was made anaerobic by bubbling with nitrogen, and 1 drop was then placed in the nitrogen-ventilated microchamber (see above). An oxygen-filled capillary was inserted into the drop, and the distribution of bacteria was observed under the microscope. The results obtained with the wild-type strain ST1 are shown in Fig. 2. The bacteria formed a distinct cluster at the mouth of the capillary tube within 5 min (Fig. 2A). The cluster persisted for up to 2 h. High magnification (×800) revealed that cells within the cluster swam at normal speed (30 μm/s), whereas cells at the periphery of the drop moved at approximately one-half this speed. The presence of aspartate (1 mM) or serine (1 mM) did not prevent cluster formation. No cluster formed around the mouth of a control capillary filled with nitrogen gas (Fig. 2B) or at the mouth of an oxygen-filled capillary when the medium contained 5 mM KCN (Fig. 2C). To ensure that the KCN-inhibited cells were anaerobic, the culture was gassed with nitrogen for 30 min before the assay.

Temporal assay for aerotaxis. The sensing mechanism for chemotaxis involves comparison over time of changes in effector concentration rather than comparison of simultaneous measurements made at different points in space (29). As a result, the bacteria respond to a step increase in attractant concentration by suppressing tumbling and swimming smoothly for an interval before relaxing to a normal (random) motility pattern. The time required for 50% of the bacteria to resume random motility is a valid measure of the chemotactic response (48).

An analogous temporal assay for aerotaxis was designed by using the apparatus shown in Fig. 1. A small drop of bacteria was placed in a microchamber on the microscope stage. The bacteria rapidly became anaerobic when the chamber was ventilated with purified nitrogen or argon. After the bacteria adapted to the anaerobic conditions they were exposed to oxygen by ventilating the chamber with an oxygen-nitrogen (or oxygen-argon) mixture. The composition of the mixture was controlled by a gas proportioner and could be varied to give an equilibrium concentration of dissolved oxygen in the medium between 0.03 and 300 μM.

When anaerobic *S. typhimurium* ST1 in glucose medium was exposed to oxygen, the bacteria suppressed tumbling and swam smoothly for about 15 s before resuming random motility (Fig. 3). The transition from anaerobic to aerobic conditions was accompanied by changes in velocity and tumbling frequency. As previously observed in the spatial assay, the mean swimming speed of anaerobic *S. typhimurium* in medium with glucose was 19 μm/s. Anaerobiosis affected the population uniformly; the distribution of speeds about the mean was normal and was not skewed by a disproportionate number of sluggish bacteria. When aerated, strain ST1 organisms rapidly accelerated with a slight

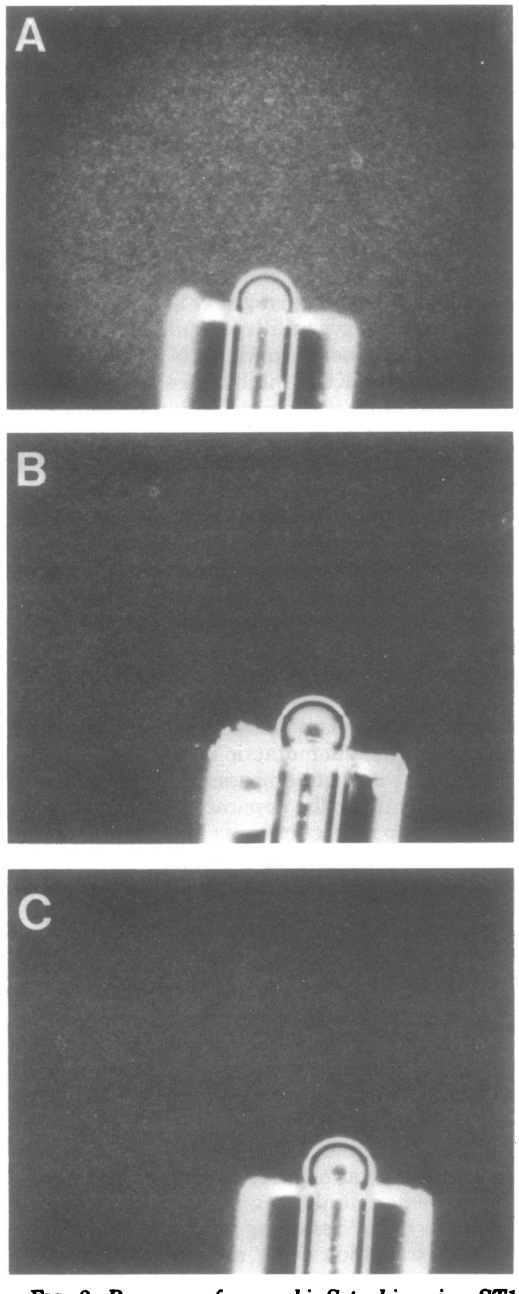

FIG. 2. *Response of anaerobic S. typhimurium ST1 to an oxygen spatial gradient in the presence and absence of KCN. The preparation was photographed with dark-field illumination 5 min after insertion (described in the text) of a gas-filled capillary into anaerobic S. typhimurium ST1 in Vogel-Bonner medium with 0.6% (wt/vol) glucose. (A) Capillary contained oxygen. (B) Capillary contained nitrogen. (C) Capillary contained oxygen, and the medium contained 5 mM KCN.*

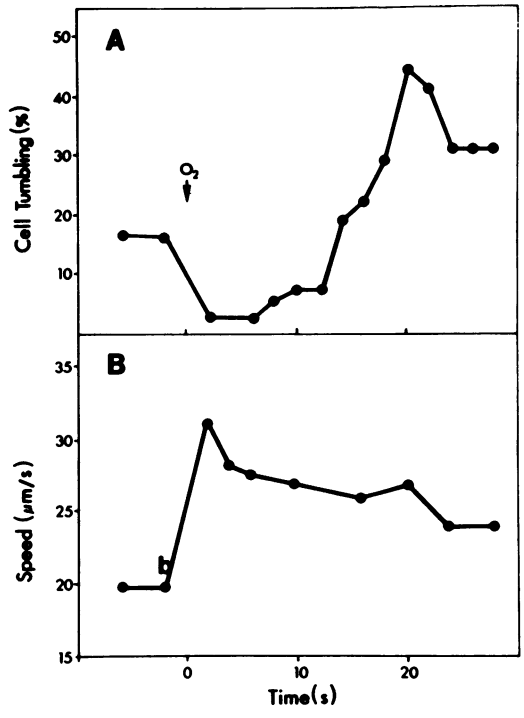

FIG. 3. *Effect of a temporal gradient of 0 to 270 μM oxygen on the motility pattern of S. typhimurium ST1. Strain ST1 in Vogel-Bonner medium with glucose was placed in a microchamber and made anaerobic by ventilation with argon gas. After 4 min of preincubation, 21% oxygen in argon was introduced as the ventilating gas at zero time. The motility of strain ST1 was photographed with a stroboscopic lamp; the tumbling frequency (A) and speed (B) of the bacteria were measured as described in the text.*

overshoot before steady-state swimming speeds were approached. The smooth response to oxygen was also followed by an overshoot in which the tumbling frequency was increased over the normal aerobic tumbling frequency of this organism. The speed overshoot and the tumbling overshoot were not simultaneous.

The random motility of anaerobic *S. typhimurium* ST1 at zero time in Fig. 3 was observed only after the bacteria had been anaerobic for 2 to 3 min. The time course of change in motility during the temporal aerotaxis assay is seen more clearly in Fig. 4, which shows a longer time interval. At the commencement of anaerobiosis, the cells began to swim smoothly. This was not the result of a favorable stimulus caused by the loss of oxygen. On the contrary, when an aerobic culture of *S. typhimurium* ST1 was flushed with nitrogen, the cells first entered a tumbling phase, then slowed down and swam smoothly. Al-

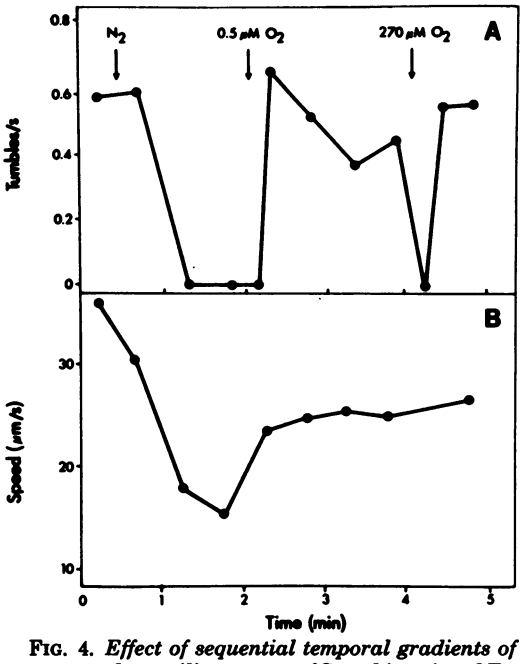

FIG. 4. *Effect of sequential temporal gradients of oxygen on the motility pattern of S. typhimurium ST1. The experimental procedure was similar to that described in the legend to Fig. 3 except that the ventilating gas was N_2 with two step increases in oxygen concentration to give the dissolved oxygen concentrations indicated. The response of the bacteria was videotaped, and the tumbling frequency (A) and speed (B) were determined as described in the text. The data points shown are the means of measurements made of 5 to 10 bacteria.*

though the tumbling phase was consistently observed in the microscope, it was not evident in Fig. 4 because the response was brief (approximately 3 s) compared with the time scale in the figure. This led us to consider whether the tumbling response might be interrupted by a loss of energy required for tumbling.

Proton motive force in anaerobic and aerobic cells. The energy for the flagellar motors in bacteria is supplied by the proton motive force across the plasma membrane (20, 26, 32–34, 52). At energy levels that are less than saturating for the motors, the swimming speed is proportional to the proton motive force. Changes in swimming speed are therefore an indication of change in the proton motive force. Anaerobic bacteria were immotile in glycerol medium but swam slowly in glucose medium, indicating that the proton motive force of *S. typhimurium* in glucose medium was intermediate between the normal aerobic value and the value near zero expected in glycerol medium. This was confirmed by using fluorescence of 8-anilino-1-naphthalene sulfonate, which is inversely related to membrane potential (4, 54). *S. typhimurium* SL3730 was used, since it is a motile strain that is permeable to the fluorescent dyes 8-anilino-1-naphthalene sulfonate and diS-C_3-(5) (M. Snyder, J. Stock, and D. E. Koshland, Jr., personal communication). When this strain became anaerobic in glucose medium, there was an increase in fluorescence corresponding to a decrease in potential (Fig. 5). The addition of the uncoupler carbonyl cyanide-*m*-chlorophenyl hydrazone further decreased membrane potential and abolished motility. Thus, the membrane potential in anaerobic *S. typhimurium* is intermediate between the values for aerobic and depolarized cells.

The observation that the slowly swimming *S. typhimurium* organisms in anaerobic glucose medium do not tumble suggested that there was a minimum proton motive force required for tumbling. Tumbling is resumed after 3 to 5 min

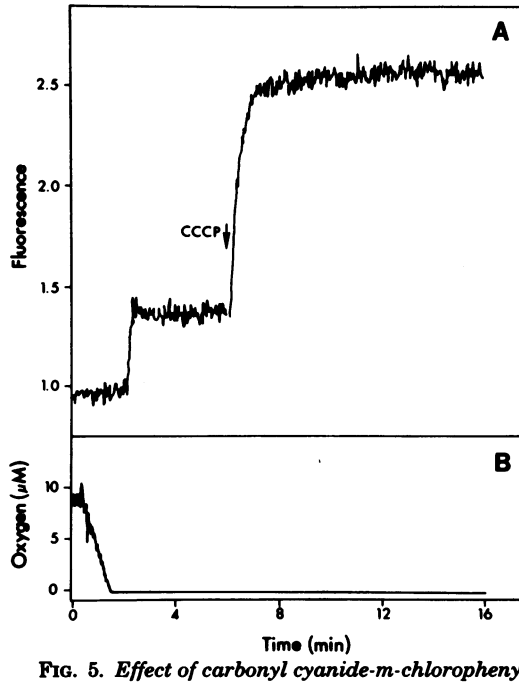

FIG. 5. *Effect of carbonyl cyanide-m-chlorophenyl hydrazone (CCCP) addition on fluorescence of 8-anilino-1-naphthalene sulfonate in the presence of anaerobic S. typhimurium SL3730. The bacteria were prepared in Vogel-Bonner medium with glucose as described in the text except that EDTA (0.1 mM) was added to the medium in which the cells were suspended. The bacteria became anaerobic after the air space in the cuvette was flushed with nitrogen; fluorescence and oxygen concentration were measured simultaneously. CCCP (1 μM) was added through the gas outlet with a Hamilton syringe. Fluorescence intensity here and in Fig. 8 is given in arbitrary units: the base-line fluorescence in Vogel-Bonner medium was given the value 1.*

but there is no reproducible change in fluorescence during this time. Either the ΔpH component of the proton motive force increases or the bacteria adapt to the lower proton motive force.

The aerotactic response of smooth swimming did not result from a partial depolarization of the membrane. The bacteria experienced two sequential step increases in oxygen concentration (Fig. 4). The first oxygen level (0.5 μM) was sufficient to support normal random motility yet the bacteria responded to a further increase in oxygen concentration by suppressing tumbling and swimming smoothly for a brief interval.

Dose-response relationship. In a series of temporal assays *S. typhimurium* ST1 in glucose medium was kept anaerobic for 1 min and then exposed to a predetermined concentration of oxygen, and the response (smooth swimming) of the bacteria was videotaped and timed (Fig. 6). The half-maximal aerotactic response was elicited by 0.4 μM oxygen, which is similar to the K_m (0.2 μM) for cytochrome *o* in *E. coli* (41). The accuracy of the dose-response plot depended in part on a rapid equilibration of oxygen gas with the medium in which the bacteria were suspended. We have calculated that by using a 2-μl drop of culture spread to 6 mm in diameter, the dissolved oxygen concentration would reach 95% of the equilibrium value within 1 s. It is assumed that the profile of an increase in oxygen concentration is not important and that bacteria respond to the change in oxygen bound rather than the time rate of change in oxygen bound. This assumption is supported by the characteristics of the chemotactic response to ribose by *S. typhimurium* (48).

No evidence was found for oxygen receptors with a dissociation constant of greater than 10^{-6} M. The response time for 300 μM oxygen was similar to that for 2 μM oxygen. When the spatial assay for aerotaxis was performed in the presence of 2.5 μM oxygen in the bacterial medium, there was no accumulation at the mouth of the oxygen-filled capillary. No evidence was found in the temporal or spatial assay that *S. typhimurium* is repelled by high concentrations of oxygen.

Role of electron transport. Oxidative phosphorylation is not essential for aerotaxis. *S. typhimurium* ST352, which is deficient in the energy transducing ATPase (T. Ingolia and D. E. Koshland, Jr., personal communication), had a normal aerotactic response. Taylor et al. (51) proposed that electron transport-induced changes in the proton motive force were the signal that regulated tumbling frequency in aerotaxis. The correlation of changes in oxygen concentration with membrane potential and swimming pattern provide direct support for this hypothesis (Fig. 7). Fluorescence of the cyanine dye diS-C_3-(5) was used to monitor membrane potential (35, 37, 54), and a needle oxygen electrode in the fluorometer cuvette permitted simultaneous measurement of potential and oxygen concentrations. At the same time, 1 drop of the culture was monitored in the microchamber used for the temporal assay of aerotaxis. Oxygen concentrations in the microchamber were altered in parallel to changes in the fluorometer cuvette. Two anaerobic-aerobic cycles are shown in Fig. 7. Membrane potential (measured as negative fluorescence) decreased with anaerobiosis and increased with aerobiosis as predicted by the hypothesis.

The hypothesis further required that the tumbling frequency be regulated by changes in membrane potential. In *Bacillus subtilis*, uncoupler- or ionophore-mediated depolarization causes a tumbling (repellent) response, and hyperpolarization causes smooth swimming (35, 37). Responses to change in the proton motive force were less dramatic in *S. typhimurium* and were consequently harder to detect. Nevertheless, there was a brief tumbling response (ca. 2 s) to the addition of carbonyl cyanide-*m*-chlorophenyl hydrazone or KCN and a smooth response after membrane hyperpolarization by valinomycin.

Competition between oxygen and other electron acceptors. The electron transport systems of *S. typhimurium* and *E. coli* are branched pathways that can utilize oxygen or alternative terminal electron acceptors (15). The most studied alternate electron acceptors are nitrate and fumarate, and the terminal components of the electron transport systems that interact with these acceptors are nitrate reduc-

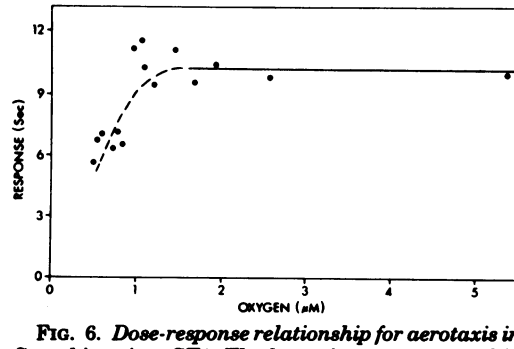

FIG. 6. *Dose-response relationship for aerotaxis in S. typhimurium ST1. The bacteria were prepared in Vogel-Bonner medium with glucose, and aerotaxis was measured by the temporal assay as described in the text. Timing of the response commenced when the bacteria accelerated after exposure to oxygen and ended when 50% of the bacteria had resumed random swimming.*

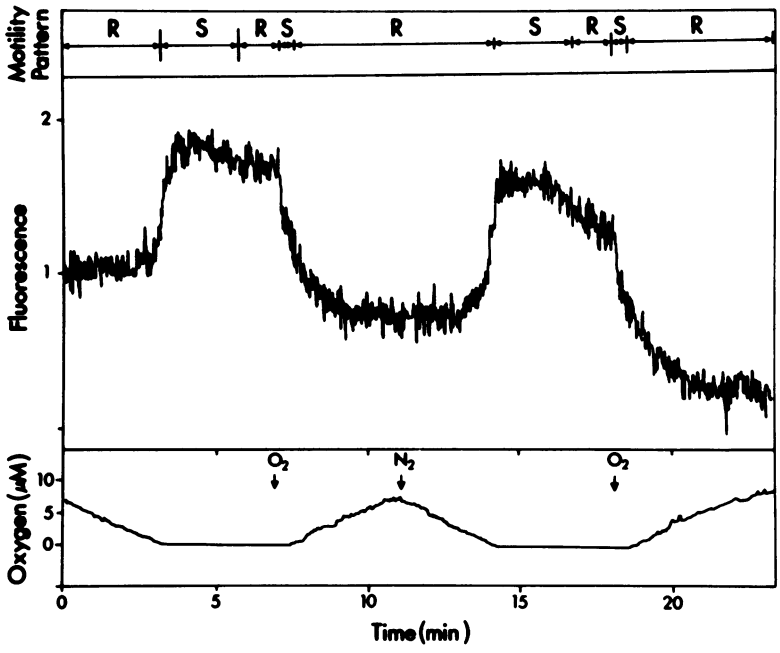

FIG. 7. *Effect of increasing and decreasing temporal oxygen gradients on the fluorescence of dis-C_3-(5) and on motility of S. typhimurium SL3730. The bacteria were prepared as described in the text, and fluorescence and motility were simultaneously examined in the fluorometer and in the microchamber, respectively. The oxygen concentration in the fluorometer cuvette was altered by flushing the air space at the top of the cuvette with either nitrogen or air. The composition of the gas ventilating the microchamber was regulated manually to correspond to the reading of the oxygen electrode in the fluorometer cuvette. Two anaerobic-aerobic cycles are shown. R, Random swimming pattern; S, smooth swimming pattern.*

tase and fumarate reductase, respectively. Both nitrate and fumarate are attractants for *S. typhimurium* and *E. coli*, and in each case the receptor is the terminal reductase (51). If taxis to electron acceptors is mediated by electron transport and the proton motive force, aerotaxis should be competitive with taxis to nitrate and fumarate. This was demonstrated (Table 2). The aerotactic response was significantly reduced in the presence of nitrate or fumarate ($P < 0.001$). Inhibition by nitrate plus fumarate was similar to inhibition by either electron acceptor used separately. Induction of the terminal reductase was required for competition (data not shown).

Signalling protein for aerotaxis. The known signalling proteins for chemotaxis are the products of the *tsr*, *tar*, and *trg* genes (23, 24, 46). Mutations in these genes in *E. coli* and in *trg* in *S. typhimurium* did not affect aerotaxis (Table 3). This indicated that aerotaxis was not focused through one of the known signalling proteins. The response to intense blue light (30, 50), which is apparently mediated by the proton motive force (51), was also found to be normal in *tsr*, *tar*, and *trg* mutants of *E. coli*. The normal aerotactic response in these mutants was verified by the temporal and spatial assays, and the RP477 (*che*$^+$) response was not inhibited by serine (1 mM) or aspartate (1 mM) (31).

TABLE 2. *Effect of nitrate and fumarate on aerotaxis in S. typhimurium ST1*[a]

Additions to medium		Response to oxygen gradient (s)[b]
Carbon source	Electron acceptor	
Glycerol	None	16.0 ± 1.9 (11)
	Nitrate	10.9 ± 1.2 (6)
	Fumarate	10.8 ± 1.1 (5)
	Nitrate and fumarate	10.3 ± 1.8 (6)
Glucose	None	14.7 ± 1.5 (7)

[a] *S. typhimurium* ST1 was grown anaerobically overnight in Vogel-Bonner medium with glycerol and nitrate (30 mM) or fumarate (30 mM). The cells were washed in aerated Vogel-Bonner medium containing the indicated carbon source and electron acceptors. Aerotaxis was measured by the temporal assay.

[b] The times shown for the smooth swimming response are the mean ± standard deviation, with the number of determinations shown within parentheses.

Aerotaxis was also examined by introducing a band of bacteria beneath a glycerol gradient in chemotaxis buffer. The bacteria consumed the oxygen in their environment and migrated in response to the resultant oxygen gradient (1, 2).

TABLE 3. *Response to a temporal gradient of oxygen by mutants of S. typhimurium and E. coli*[a]

Strain	Chemotaxis mutation	Unstimulated response	Response to oxygen decrease	Response to oxygen increase
S. typhimurium				
ST1	None	Random	Tumbling	Smooth
ST832	*trg*	Random	Tumbling	Smooth
ST134	*cheC*	Random	Smooth	Tumbling
ST55	*cheW*	Smooth	None	None
ST162	*cheR*	Smooth	None	None
ST108	*cheS*	Smooth	None	None
ST176	*cheY*	Smooth	None	None
SL2516	*cheV*	Smooth	None	None
BT10	*cheZ*	Tumbling	None	Smooth[b]
BT11	*cheB*	Tumbling	None	None[b]
E. coli				
RP477	None	Random	Tumbling	Smooth
RP437 (*tar*)	*tar*	Random	Tumbling	Smooth
RP4790	*tsr*	Random	Tumbling	Smooth
AW702	*trg*	Random	Tumbling	Smooth

[a] Strains were grown in nutrient broth and examined for aerotaxis by the temporal assay procedure.
[b] Observed only after preincubation in anaerobic medium for 1 h or longer.

The rate of migration was similar for *E. coli* RP477 (*che*$^+$), RP437 (*tar*) and RP4790 (*tsr*), provided that the strains were all highly motile (Fig. 8). Strains that were not highly motile were slower in moving up the column. RP437 (*tar*) was the strain most susceptible to poor motility and was initially thought to be defective in aerotaxis. Subsequent selection of RP437 (*tar*) for improved motility on tryptone semisoft agar gave a highly motile strain whose migration was consistently similar to the results shown in Fig. 8. The selected strain was confirmed to be *tar*.

Convergence of pathways for aerotaxis and chemotaxis. Although electron acceptor taxis is not focused through one of the known chemotaxis signalling proteins, it is evident (Table 3) that the pathways eventually converge into a common tumble-regulating system. This is most clearly illustrated by *S. typhimurium* ST134 (*cheC*). The *cheC* gene product is a component of the flagellar basal body and is apparently at the interface between the sensing system and the motor (21, 43). *S. typhimurium* ST134 has an extreme clockwise bias, resulting in inverse responses to chemoeffectors; that is, repellents cause smooth swimming, and attractants cause tumbling. The response by this strain to a temporal gradient to oxygen is also the inverse of the response by *che*$^+$ strains; decreased oxygen causes smooth swimming, and increased oxygen causes tumbling (Table 3). In the spatial assay, *S. typhimurium* ST134 was repelled by oxygen.

Strains with *che* mutations other than *cheC* did not show a response to oxygen in the routine temporal assay except that *S. typhimurium* BT10 responded positively to an increase in oxygen concentration after being incubated anaerobically for 1 h or longer (Table 3). The oxygen response of *S. typhimurium* ST1 was lengthened by aerobic growth in Vogel-Bonner medium with glucose and by anaerobic incubation or growth in the same medium.

DISCUSSION

The temporal assay that was developed for use in these studies has made it possible to determine the dose-response relationship for aerotaxis in *S. typhimurium*. This is the first such determination for a microorganism, and the method should be useful for studying the oxygen response by other organisms. For organisms with a longer response time, it should be possible to obtain a precise measurement of the half-maximal response from a double-reciprocal plot of the data. We estimated the half-maximal response (approximately 0.4 μM) for *S. typhimurium* directly from the dose-response plot.

Certain cytochrome oxidases have K_m values similar to the half-maximal response for aerotaxis in *S. typhimurium*. We conclude from this and from the inhibition of aerotaxis by KCN (Fig. 2) that the oxygen receptor for aerotaxis is a terminal oxidase. The known cytochrome oxidases (*d* and a_1) in *S. typhimurium* (12) are not good candidates for the aerotaxis receptor. In *E. coli*, cytochrome *d* (K_m = 0.02 μM) and cytochrome a_1 are found in stationary-phase cells (8), whereas our studies utilized early exponential-phase cells. Furthermore, during the dose-response investigations, it was evident from the relationship between oxygen concentration and

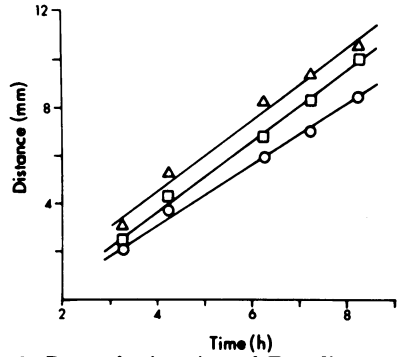

FIG. 8. *Rate of migration of E. coli mutants in response to a self-generated oxygen gradient. A 1 to 3% glycerol gradient in 10 mM potassium phosphate, pH 7.0, 0.1 mM EDTA, and 0.3 mM $(NH_4)_2SO_4$ was preformed in glass tubes (7.5 by 1.0 cm). E. coli RP477 (che$^+$)* (○), *RP437 (tar)* (□), *and RP4790 (tsr)* (△) *were grown at 30°C in Vogel-Bonner glycerol medium with required supplements until the optical density at 600 nm reached 0.45 to 0.50. The cells were washed twice and suspended in phosphate-EDTA-$(NH_4)_2SO_4$ with 5% glycerol. A 0.1-ml portion of the washed bacteria was introduced beneath the glycerol gradient; the surface of the gradient was gassed with nitrogen, and the tubes were stoppered and sealed with Lubriseal. The bacteria consumed the oxygen in their environment and migrated as a band in response to the resulting oxygen gradient. The distance that each mutant had migrated from the bottom of the tube was measured at the times indicated.*

swimming speed that the principal cytochrome oxidase determining the energy production rate in early exponential-phase *S. typhimurium* had a K_m similar to the half-maximal response for aerotaxis. We have determined that the oxidase in *S. typhimurium* used in these studies was cytochrome *o* (B. Fandrich and B. L. Taylor, unpublished data) which is the principal cytochrome oxidase in *E. coli* and has a K_m of 0.2 μM (41). The dose-response relationship suggests that cytochrome *o* is also the receptor for aerotaxis. The terminal oxidase appears to be the receptor for aerotaxis in *Euglena* sp. (38). The evidence that the aerotaxis receptor is cytochrome *o* is important in view of the finding by MacGregor and Bishop (27) that cytochromes do not function as oxygen sensors in the regulation of nitrate reductase biosynthesis. It is possible that cytochrome *d* is also a receptor for aerotaxis but this would not have been detected in our studies.

Competition among nitrate, fumarate, and oxygen (Table 2) suggests that there is a common component in the signalling pathways for taxis to electron acceptors. The receptors (nitrate reductase, fumarate reductase, and cytochrome *o*) could interact directly with a common signalling protein; however, the evidence suggests that the common component is a product of the electron transport system. 2-Heptyl-4-hydroxyquinoline-*N*-oxide, which blocks electron transport at a site other than the terminal oxidase, inhibits aerotaxis in *B. cereus* (W. W. Goral and B. L. Taylor, Clin. Res. 27:5A, 1979). If electron acceptor taxis is mediated by the electron transport system, the receptors for this type of taxis are not receptors in the same sense used for other chemoattractants because the classical chemoreceptor interacts directly with the chemotaxis sensing system.

The role of the electron transport system in aerotaxis was previously assumed to be in ATP synthesis (9). However, this possibility is excluded by the observed normal aerotaxis in *S. typhimurium* ST352 which is deficient in the energy-transducing ATPase (unpublished data). The following evidence is consistent with electron transport-mediated changes in the proton motive force as the parameter that regulates the behavioral response in aerotaxis: (i) the correlation between membrane potential and oxygen concentration (Fig. 8); (ii) the response (smooth swimming) to valinomycin-induced hyperpolarization of the proton motive force; and (iii) in the competition studies (Table 2) the magnitude of the increase in swimming speed after the bacteria became aerobic appeared to correlate with the response time. The increase in swimming speed reflects a change in the proton motive force (19). If the proton motive force is the first common component for electron acceptor taxis, we assume that there is a signalling protein responsive to changes in the proton motive force. One of the surprising findings was the short response of *S. typhimurium* to a temporal oxygen gradient. This made it difficult to determine the quantitative relationship between response time and change in the proton motive force.

Smooth swimming observed after *S. typhimurium* became anaerobic is the result of a decrease in the proton motive force, which, in turn, decreases the swimming speed. Tumbling was prevented in aerated *S. typhimurium* when the membrane potential was lowered by addition of valinomycin or arsenate (unpublished data). This relationship has been quantitated by Khan and Macnab (19), who found that the clockwise-counterclockwise bias of the flagella motor is an exponential function of the speed of rotation. Whenever the proton motive force is decreased, the motor becomes more counterclockwise biased, thereby reducing the tumbling frequency. Smooth swimming in response to localized conditions that decrease the proton motive force might disperse the bacteria and enhance

their survival (19). However, such a response would be disadvantageous to facultative anaerobes in generalized anaerobiosis because it would preclude chemotaxis to fermentable energy sources. It is therefore significant that *S. typhimurium* adapted to anaerobiosis within 2 to 3 min and resumed random swimming. This adaptation, which is independent of protein synthesis, might result from a metabolic shift that increases the anaerobic proton motive force, although the possibility that the tumble regulator adapts to a decreased proton motive force cannot be excluded at this time.

It is clear from both spatial and temporal assays that aerotaxis is normal in *E. coli* mutants that are defective in the *tsr*, *tar*, or *trg* gene (Table 3; Fig. 8). Thus, sensory transduction in aerotaxis does not involve the known signalling proteins, which are the products of these genes (23, 46). The observation that oxygen did not stimulate methylation of these gene products in *S. typhimurium* supports a similar conclusion for that species. The response to blue light is also independent of these signalling proteins. Sugars that are attractants and that are transported by the phosphotransferase system are focused through a distinct signalling protein (17, 23). In view of this, it seems that there are at least five signalling proteins (Fig. 9). We would assume that effectors such as light (16, 51), which also modulate the proton motive force, would be focused through the signalling protein for aerotaxis. The results obtained with the *cheC* mutant of *S. typhimurium* ST134 indicate that the point of convergence for the chemotaxis and aerotaxis pathways is either before or at the switch in the flagellar basal body. It will be of considerable interest to discover how changes in the proton motive force are transduced into a chemotactic signal that impinges on the common response system.

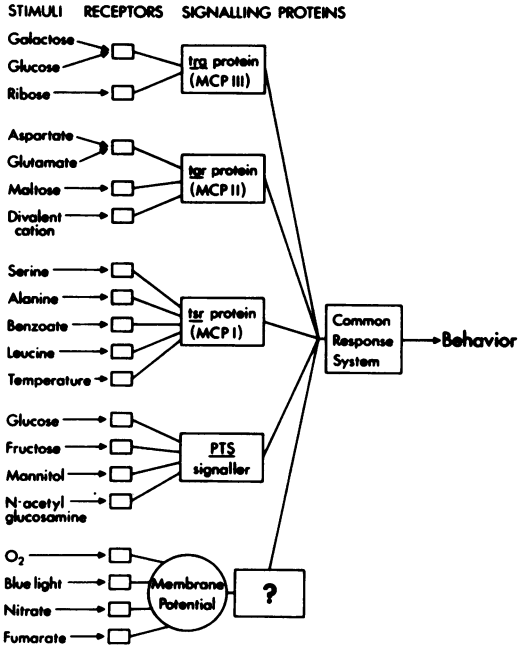

FIG. 9. *Diagram showing a minimum of five signalling proteins that are involved in focusing chemotaxis signals into the common response system. Evidence for the membrane potential signalling protein is found in the text, and evidence for the trg, tar, tsr, and phosphotransferase system (PTS) signalling proteins has been reported previously (17, 23, 24, 46). MCP, Methyl-accepting chemotaxis protein.*

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