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Chemotaxis by *Pseudomonas aeruginosa*

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Chemotaxis by *Pseudomonas aeruginosa* RM46 has been studied, and conditions required for chemotaxis have been defined, by using the Adler capillary assay technique. Several amino acids, organic acids, and glucose were shown to be attractants of varying effectiveness for this organism. Ethylenediaminetetraacetic acid was absolutely required for chemotaxis, and magnesium was also necessary for a maximum response. Serine taxis was greatest when the chemotaxis medium contained 1.5×10^{-5} M ethylenediaminetetraacetic acid and 0.005 M magnesium chloride. It was not necessary to include methionine in the chemotaxis medium. The strength of the chemotactic responses to glucose and to citrate was dependent on prior growth of the bacteria on glucose and citrate, respectively. Accumulation in response to serine was inhibited by the addition of succinate, citrate, malate, glucose, pyruvate, or methionine to the chemotaxis medium. Inhibition by succinate was not dependent on the concentration of attractant in the capillary. However, the degree to which glucose and citrate inhibited serine taxis was dependent on the carbon source utilized for growth. Further investigation of this inhibition may provide information about the mechanisms of chemotaxis in *P. aeruginosa*.

Motile bacteria are attracted to a wide variety of chemicals, including amino acids and sugars, by a process known as chemotaxis (1, 2, 5). Rapid progress in recent years has been made toward detailing the molecular mechanisms of chemotaxis and sensory transduction in bacteria (5); however, the majority of these studies have dealt with the enteric bacteria *Salmonella typhimurium* (13) and *Escherichia coli* (12) and with the gram-positive bacterium *Bacillus subtilis* (10).

Studies of the behavioral responses of several pseudomonads have been described (3, 4, 11). Moench and Konetzka (11) have reported the chemotaxis of *Pseudomonas aeruginosa* toward nitrogen compounds. No work, however, has yet focused on the mechanisms of chemotaxis in these obligately aerobic bacteria. In this paper we report studies carried out to characterize some of the conditions necessary for chemotaxis by *P. aeruginosa*. In the course of this work, we found that a variety of chemoattractants are able to prevent accumulation in response to a gradient of a second attractant. Further study of this inhibitory effect may aid in understanding the mechanism of the chemotactic response in this organism.

(A prelimary account of this investigation was presented at the annual meeting of the Federation of American Societies for Experimental Biology, 4 to 8 June 1978, Atlanta, Ga. [Fed. Proc. 37:1851, Abstr. no. 3186, 1978].)

MATERIALS AND METHODS

Organism and culture media. The organism used for this study was *P. aeruginosa* strain RM46 (9), a methionine auxotroph of *P. aeruginosa* PAO1. Stock cultures were maintained at 4°C as a dilute suspension in Luria broth (1% NaCl-1% tryptone-0.5% yeast extract, pH 7.0). Fresh stocks were prepared every 4 weeks.

The mineral salts medium (MS) employed for growth of *P. aeruginosa* contained (per liter of distilled water) 7.0 g of K_2HPO_4, 3.0 g of KH_2PO_4, 1.0 g of $(NH_4)_2SO_4$, 0.05 g of $MgSO_4 \cdot 7H_2O$, and 2.5 mg of $FeCl_3 \cdot 6H_2O$ and was supplemented with 5×10^{-4} M L-methionine and either 0.5% D-glucose or an organic acid at 0.4%. Growth of *P. aeruginosa* on a variety of carbon sources yielded cells which were motile and chemotactic. Except where otherwise noted, sodium succinate was used as the carbon source for growth.

The chemotaxis medium (CM) was prepared in glass distilled water and except where otherwise noted consisted of 0.05 M potassium phosphate buffer at pH 7.0 supplemented with 0.005 M $MgCl_2 \cdot 6\ H_2O$ and 0.1 mM disodium ethylenediaminetetraacetic acid (EDTA). For bacterial plate counts, cells were diluted in tryptone broth and plated on tryptone plates containing 1.6% agar.

Growth and preparation of cells. An overnight culture of *P. aeruginosa* was used to inoculate 50 ml of fresh growth medium to an absorbance at 590 nm of 0.05 (1-cm cuvette). This culture was incubated at 35°C with rotary shaking until the absorbance reached 0.25, approximately 3 h, to give an exponentially growing culture. This culture was centrifuged at 12,000 × g for 10 min at 4°C, and the pellet was washed twice with cold (4°C) CM. The final pellet was resuspended

in CM to optical density equivalent to approximately 1.6×10^8 cells per ml. The chemotactic response to serine was found to be proportional to the cell density over a range of 1.6×10^6 to 1.6×10^8 cells per ml.

Chemotaxis assay. The capillary tube assay was performed as described by Adler (2). A small chamber was formed by placing a U-shaped piece of 2-mm glass rod between a microscope slide and cover slip. The chambers were held at 30°C on a slide warmer, and each was filled with 0.4 ml of the above bacterial suspension. The capillaries were filled with an attractant dissolved in CM. Control capillaries contained CM alone and were used as an index of the background motility of the bacteria. The open end of one capillary was inserted into the pool of cells in each chamber.

After 30 min of incubation, the capillaries were removed, the contents were diluted in tryptone broth, and the plate counts were made. Duplicate plates were made for each chamber. The mean accumulation in triplicate assays was expressed in terms of total cells per capillary. To normalize for day-to-day differences in motility, the chemotactic response was also expressed as a ratio of the accumulation in attractant capillaries to that in control capillaries (the relative response). This value showed less variability between different cell preparations than did the absolute accumulations. A relative response of 2.0 or greater was considered to be significant.

The amino acids were all of the L configuation, and glucose was in the D configuration. The organic acids (succinate, malate, and citrate) were used as the sodium salts, adjusted to pH 7.0.

The terms response, threshold, peak, and background are used in this paper as defined by Adler (2).

Taxis inhibition assay. A simple modification of the capillary assay was used to determine the effect of the presence of various substances on the chemotactic response. In this case the test substance was added to CM so that there was no gradient, other than that of the attractant, present at the start of the experiment. Inhibition by the test substance was expressed as the percent decrease in accumulation in capillaries containing attractant when the inhibitor was present as compared with the accumulation when inhibitor was absent.

RESULTS

Serine response. Several amino acids, including aspartate and serine, are strong attractants for *E. coli* (8). By using the capillary assay technique, serine was found to be an attactant for *P. aeruginosa* as well. The concentration-response curve for L-serine (Fig. 1) shows a peak at 10^{-3} M serine and a threshold of approximately 2×10^{-6} M. In experiments on 18 different days, the average response of *P. aeruginosa* to 10^{-3} M serine was 14.8 (± 7.2) $\times 10^4$ cells per capillary, compared with a background accumulation of 0.65 (± 0.33) $\times 10^4$ cells per capillary. This corresponds to a relative response of 22.8. Succinate was a weaker attractant with a threshold of 4×10^{-5} M and a peak relative response of 5.2 (Fig. 1).

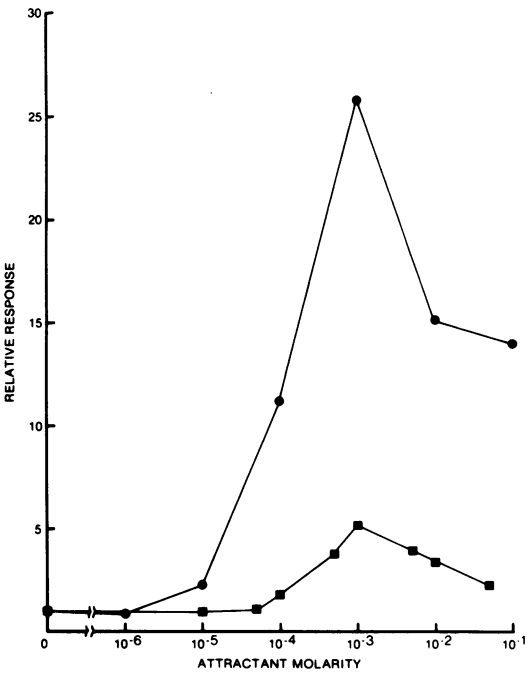

FIG. 1. *Concentration-response curves for chemotaxis of P. aeruginosa toward serine* (●) *and succinate* (■).

Composition of CM. Due to the known toxic effects of EDTA for *P. aeruginosa* (16), it was important to determine whether the presence of EDTA was necessary for chemotaxis by this organism as it is for *E. coli* (2). Therefore, experiments were performed to determine the effect of varying the EDTA and magnesium composition of CM on the response to serine. The results are shown in Fig. 2. When the EDTA concentration was varied in the presence of 0.005 M magnesium chloride (Fig. 2A), a sharp dependence of serine taxis on EDTA was found. The response in the absence of EDTA was only 9% of the response occurring in the presence of 1.5×10^{-5} M EDTA. The response to serine, however, decreased steadily when EDTA was added in excess of 1.5×10^{-5} M. Motility was also sensitive to the EDTA level; the background accumulation was found to increase with increasing EDTA up to 7×10^{-4} M.

Figure 2B shows the effect on serine taxis and motility of varying the magnesium concentration in the presence of 1.5×10^{-5} M EDTA. A significant response to serine was detected in the absence of magnesium, but the accumulation was increased more than threefold by the inclusion of 0.005 M magnesium chloride in CM. In a separate experiment, increasing the magnesium level to 0.015 M did not improve the chem-

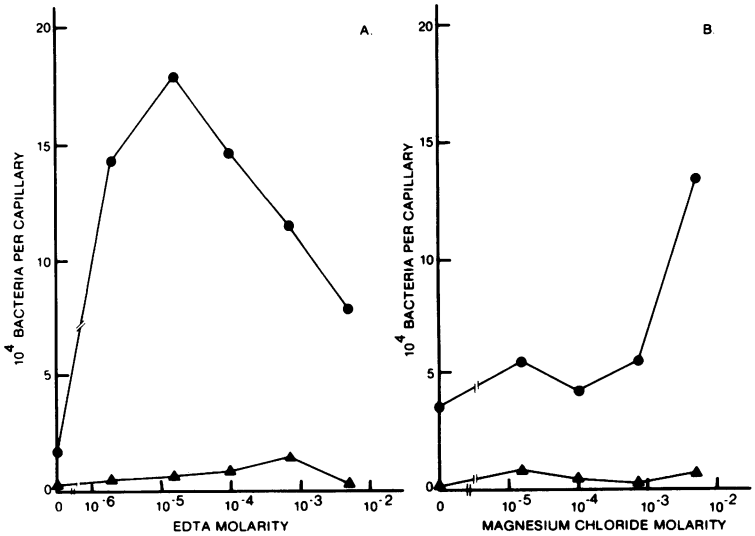

FIG. 2. *Effect of EDTA and $MgCl_2$ on motility and chemotaxis toward serine. P. aeruginosa was grown as usual, and then washed and resuspended in CM consisting of 0.05 M phosphate buffer supplemented with either (A) 0.005 M $MgCl_2$ and varying concentrations of EDTA, or (B) 1.5 × 10^{-5} M EDTA and varying concentrations of $MgCl_2$. Capillaries contained either 10^{-3} M serine in CM (●) or CM alone (▲).*

otactic response significantly. On the basis of these results, a CM containing 1.5 × 10^{-5} M EDTA and 5.0 × 10^{-3} M $MgCl_2$ was adopted for further work with *P. aeruginosa*. No loss of viability was detected after incubation of bacteria in this medium at 30°C for 1 h, which was sufficient time to complete the chemotaxis assay.

The *P. aeruginosa* strain (a methionine auxotroph) used for these studies gave a strong response to serine without the addition of methionine to CM. This is in contrast to *E. coli* which was nonchemotactic in the absence of methionine (2). In an attempt to deplete any intracellular methionine pools and to decrease the levels of amino acid carried through the wash procedure, an overnight culture of bacteria was inoculated into MS containing 5 × 10^{-6} M methionine. When growth of the culture ceased after 3 h due to methionine limitation, the culture was washed and resuspended as usual. A strong response to serine was detected, and the magnitude of the response was not significantly different from that of a parallel culture grown on a nonlimiting concentration of methionine. Similarly strong chemotactic responses were obtained without addition of methionine to CM when other strains were tested, including the prototrophic parent *P. aeruginosa* PAO1 and two auxotrophs carrying lesions in methionine biosynthesis different from that in RM46.

Rate of accumulation. Figure 3 illustrates the rate of accumulation of bacteria in capillaries containing either 10^{-3} M serine, 0.5% yeast extract, or CM alone. The number of bacteria in capillaries containing serine, after a lag of about 5 min, showed a linear increase until peaking at 30 min of incubation. Bacteria appeared to accumulate in yeast extract capillaries without lag,

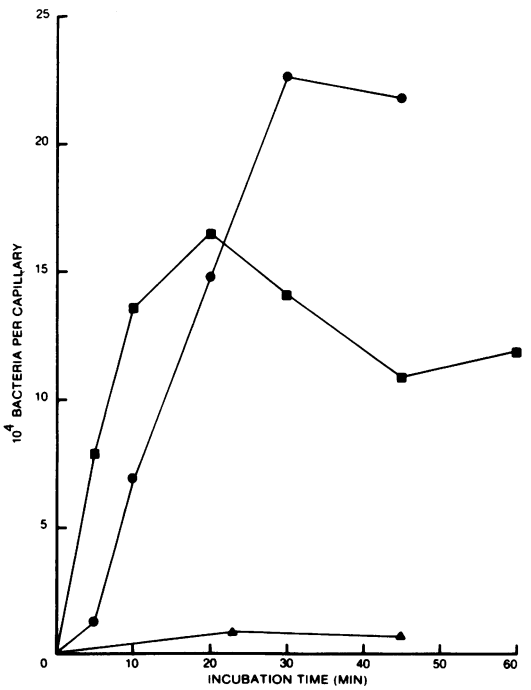

FIG. 3. *Rate of accumulation of P. aeruginosa in capillaries containing either 10^{-3} M serine (●), 0.5% yeast extract (■), or CM alone (▲).*

and the accumulations reached a peak at 20 min. The background accumulation in capillaries containing only chemotaxis medium reached 0.84 $\times 10^4$ cells per capillary after 23 min and was not significantly different at 45 min. Based on the serine taxis data, a standard incubation time of 30 min was adopted.

Other attractants. *P. aeruginosa* was tested for chemotactic responses toward a limited group of chemicals which included amino acids, organic acids, and glucose. The concentration-response curves for several of these substances are shown in Fig. 4. The response of this organism to succinate and serine has been described above (Fig. 1). Strong responses were obtained toward glutamate and α-ketoglutarate (peak relative responses were 23.7 and 15.5, respectively). No response toward α-methylaspartate was detected over a concentration range of 10^{-5} to 10^{-1} M.

Positive chemotactic responses were obtained toward the sodium salts of organic acids including succinate (Fig. 1), citrate (Fig. 5A), pyruvate, and malate (data not shown). Sodium ion acts as an attractant for some bacteria (6). However, when NaCl was tested as an attractant for *P. aeruginosa*, no response was detected in the concentration range of 10^{-4} to 10^{-2} M. This suggests that chemotaxis toward the organic acids is due to detection of the acid itself and is not a response to the sodium ion alone.

The responsiveness of *P. aeruginosa* to at least two attractants was found to depend on the carbon source utilized for growth (Fig. 5). Figure 5A illustrates the response to citrate of bacteria grown on MS with citrate and on MS with glucose. Whereas glucose-grown cells exhibited a small, but significant, response to 10^{-2}

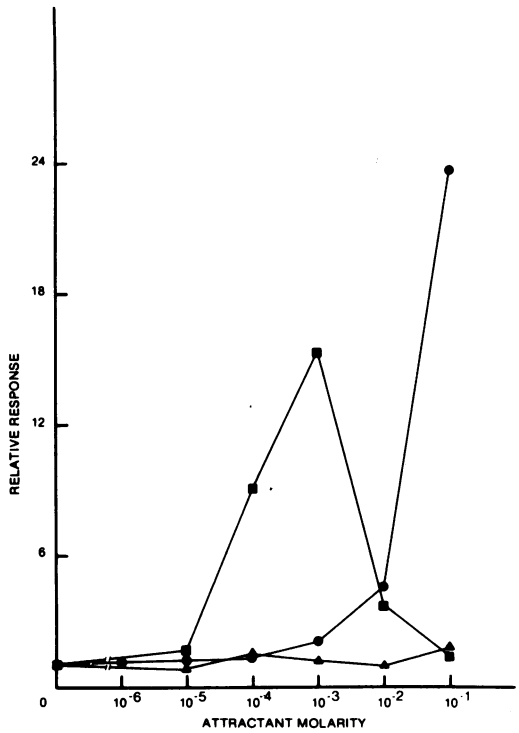

FIG. 4. *Concentration-response curves for chemotaxis of P. aeruginosa toward glutamate (●), α-ketoglutarate (■), and α-methylaspartate (▲).*

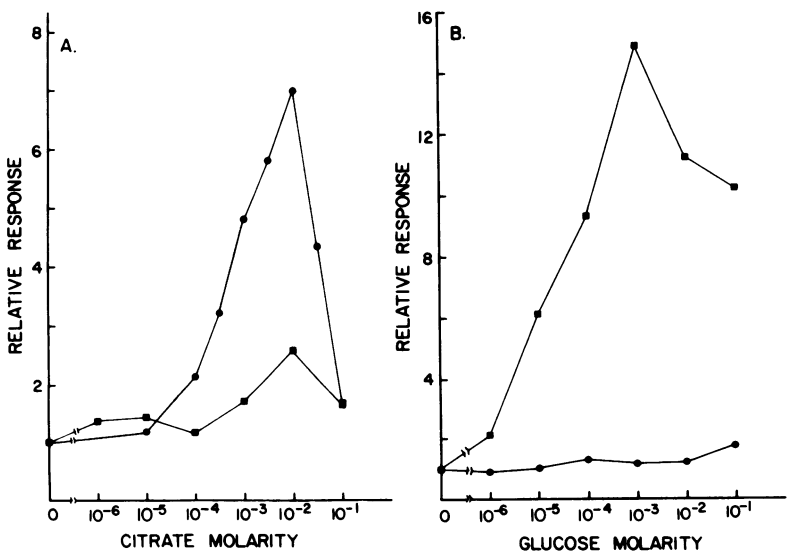

FIG. 5. *Concentration-response curves for chemotaxis of P. aeruginosa toward glucose and citrate. Bacteria were grown in either MS with citrate (●) or MS with glucose (■). Capillaries were filled with (A) CM containing citrate at the indicated concentrations or with (B) CM plus glucose at the indicated concentrations.*

M citrate, the response range of citrate taxis was extended down to 10^{-4} M citrate, and the peak response increased to 7.0 in citrate-grown cells.

In the case of chemotaxis toward glucose (Fig. 5B), no response was seen at all in citrate-grown cells, whereas glucose-grown cells exhibited a threshold of approximately 10^{-6} M glucose and a peak relative response of 10.5 at 10^{-3} M glucose. No significant response to 3×10^{-2} M glucose was detected in cells grown on succinate or on malate (data not shown). Unlike glucose, the attractants serine, succinate, and malate (at 3×10^{-2} M) elicited a strong chemotactic response from *P. aeruginosa* regardless of whether the bacteria were grown on MS supplemented with succinate, citrate, malate, or glucose (data not shown).

Inhibition of chemotaxis. Because motility and chemotaxis are known to be energy-requiring processes (7), a potential energy source was added to CM in an attempt to stimulate the chemotactic response. The effect of succinate on the response to serine is shown in Fig. 6. Compared with the response in the absence of succinate, the response to serine was significantly stimulated by the addition of 10^{-5} M succinate to CM. Higher concentrations of succinate caused inhibition, rather than stimulation, of serine taxis. The effective inhibition range of succinate concentration was the same regardless of the serine concentration in the capillary. Succinate at greater than 10^{-5} M reduced the accumulation of bacteria in response to serine, and 10^{-3} M succinate exerted approximately 90% inhibition. Succinate did not appear to inhibit motility because use of CM containing 10^{-4} M or greater succinate caused a three- to fourfold increase in background counts compared with assays performed in the absence of succinate.

Not only succinate, but also malate, citrate, and glucose inhibited the response of *P. aeruginosa* to serine (Table 1). Similarly, pyruvate and methionine inhibited serine taxis by 80 and 94%, respectively. When succinate and methionine were tested as attractants, the addition of 10^{-1} M serine to CM caused a 67% inhibition of the succinate response and 95% inhibition of the methionine response.

Table 1 shows the effect of the carbon source utilized for growth of *P. aeruginosa* on the degree of inhibition of serine taxis exerted by four different inhibitors. Both succinate and malate caused approximately 90% inhibition of serine taxis regardless of the carbon source used for growth. Citrate, however, exerted this high level of inhibition only if the bacteria had utilized citrate for growth. Similarly, glucose exerted strong inhibition of serine taxis only in the case of glucose-grown cells.

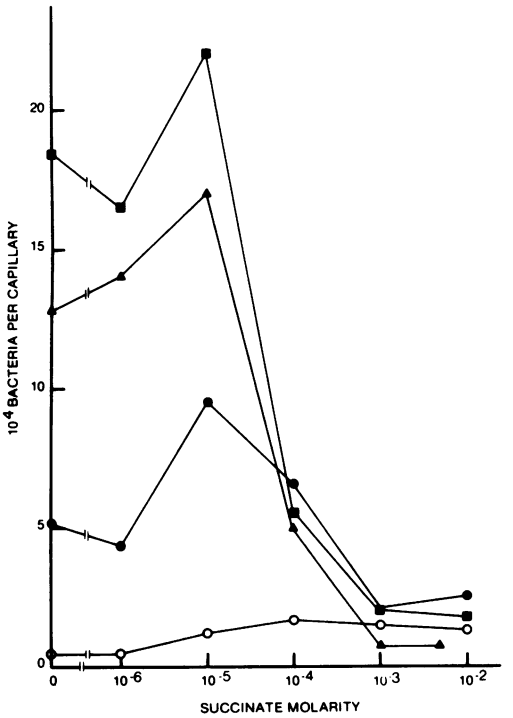

FIG. 6. *Inhibition by succinate of chemotaxis by P. aeruginosa toward serine. The chemotaxis medium (CM) was supplemented with succinate at the indicated concentrations. The capillaries contained either 10^{-1} M serine in CM (▲), 10^{-3} M serine in CM (■), 10^{-4} M serine in CM (●), or CM alone (○).*

TABLE 1. *Inhibition of serine[a] taxis by glucose and organic acids*

Carbon source used for growth	Inhibition (%) exerted by:[b]			
	Succinate	Malate	Citrate	Glucose
Succinate	92	93	43	40
Malate	91	94	56	69
Citrate	88	94	93	54
Glucose	87	92	64	93

[a] Capillaries contained 0.1 M serine.
[b] CM contained the respective inhibitors at 3×10^{-2} M.

DISCUSSION

This study has demonstrated that *P. aeruginosa* RM46 is able to respond chemotactically to glucose as well as to amino acids and organic acids. Furthermore, the glucose response of this organism was found to depend on prior growth on glucose. The lack of a response to glucose when *P. aeruginosa* was grown on succinate, citrate, or malate is consistent with the induction by glucose of a glucose-binding protein, described by Stinson et al. (14, 15), which functions

as the chemoreceptor. The observation of inducibility of glucose taxis could explain the absence of glucose taxis of *P. aeruginosa* reported by Moench and Konetzka (11), because their growth medium utilized succinate as the carbon source.

Like glucose taxis, chemotaxis toward citrate appears to be inducible. In contrast, initial studies (data not shown) of succinate and malate taxis by bacteria grown on several carbon sources indicate that the succinate and malate responses are not inducible. This may suggest that the inducible dicarboxylic acid-binding protein described by Stinson et al. (14) is not the succinate and malate chemoreceptor. Further studies are required to explore the nature of these receptors.

The presence of EDTA in CM was found to be essential for chemotaxis in *P. aeruginosa*. This result was similar to the studies of *E. coli* (2) which indicate that heavy metals inhibit motility and chemotaxis. The EDTA requirement is of particular interest because of the known sensitivity of *P. aeruginosa* to this substance (16). Decreased chemotactic responses observed in the presence of 10^{-4} M or greater EDTA probably reflect toxicity of the chelating agent. The inclusion of magnesium in CM may provide some protection to the bacteria against removal of essential Mg^{2+} from the cell envelope.

No addition of methionine to CM was necessary for chemotaxis by the methionine auxotroph. Attempts to lower intracellular methionine pools by growth in a limited methionine medium had no effect on chemotaxis. Thus, no methionine requirement was demonstrated for *P. aeruginosa*. If the methionine requirement is to be explored in this organism, it will be necessary to impose more stringent conditions for methionine depletion.

The results of taxis inhibition studies indicate that chemotaxis toward serine was inhibited by a variety of other attractants—succinate, citrate, malate, methionine, glucose, and pyruvate. Similarly, serine could inhibit the response to succinate and methionine. The succinate inhibition of serine taxis showed no dependence on the serine concentration used as attractant and occurred over a range of succinate concentrations that corresponds to the rising portion of the chemotactic response range for succinate (10^{-5} to 10^{-3} M, Fig. 1).

The extent of inhibition of serine taxis by four attractants—succinate, malate, citrate, and glucose—appears to correlate with the ability of the inhibitor to elicit a chemotactic response from *P. aeruginosa*. Succinate and malate, which elicited strong positive responses from *P. aeruginosa* regardless of growth substrate, also exerted maximum inhibition of serine taxis regardless of growth substrate. Citrate and glucose exerted maximum inhibition only when the bacteria were grown under conditions which led to induction of citrate and glucose taxis, respectively.

It appears unlikely that inhibition results from increasing ionic strength or other such chemical effect because this could not explain the growth substrate dependence. Furthermore, motility was stimulated by succinate at levels which abolished the chemotactic response. This suggests that inhibition was not due to decreased motility of the bacteria.

In the taxis inhibition assay, inhibition would be expected to result if the two test substances were detected by the same chemoreceptor. However, considering the variety of attractants that inhibited serine taxis, it seems unlikely that all could be detected by the same receptor. Thus, other mechanisms must be hypothesized to explain inhibition.

The work of Springer et al. (12) has shown that in *E. coli* various attractants are detected by two different, but complementary, pathways of information processing. The organization of these pathways appears to be such that two attractants, detected by different receptors, can feed information through the same pathway with the result that the presence of one attractant can prevent the accumulation of bacteria in response to a gradient of the second attractant. Such an inhibition of the chemotactic response was initially thought to be the result of receptor site competition between attractants (8). The inhibition of chemotaxis described here for *P. aeruginosa* could be explained by hypothesizing that the signals from all the attractants tested feed through the same information-processing system. From these studies we have no evidence for multiple systems as described for *E. coli* (12). To further explore the organization of information processing in this organism, it will be necessary to carry out genetic studies of chemotaxis-defective mutants.

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