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Negative Chemotaxis in *Escherichia coli*

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Several methods for detecting or measuring negative chemotaxis are described. Using these, we have surveyed a number of chemicals for their ability to repel *Escherichia coli*. Although most of the repellents are harmful compounds, harmfulness is neither necessary nor sufficient to make a compound a repellent. The repellents can be grouped into at least nine classes according to (i) competition experiments, (ii) mutants lacking certain of the negative taxes, and (iii) their chemical structure. The specificity of each class was studied. It is suggested that each class corresponds to a distinct chemoreceptor. Generally, non-chemotactic mutants lack both positive and negative chemotaxis, and L-methionine is required for both kinds of taxis. Repellents at very low concentrations are not attractants, and attractants at very high concentrations are not repellents.

Negative chemotaxis, the movement of organisms away from chemicals, was discovered in bacteria along with positive chemotaxis, movement towards chemicals, nearly a century ago by Engelmann (19) and Pfeffer (41). These and other workers of the late 19th and early 20th century reported taxis away from salts, acids, bases, and alcohol, and from oxygen at high concentrations. In recent years, the only studies of negative chemotaxis in bacteria have been those of Lederberg (30) for phenol taxis in *Salmonella typhimurium*, Clayton (14) for some amino acids and salts in *Rhodospirillum rubrum*, Baracchini and Sherris (11) for oxygen in various bacterial species, Doetsch et al. (18, 45, 48) for acids, bases, salts, and alcohols in *Pseudomonas fluorescens* and other species, Young and Mitchell (53) for hydrocarbons and heavy metals in marine pseudomonads, and Tsang et al. (50) for phenol and repellents, suggested by our present study, in *S. typhimurium*. For a review of the early history see Weibull (51) and Ziegler (54).

We have undertaken a program to study negative chemotaxis in *Escherichia coli* with the aim of learning about its mechanism. The questions we wished to answer are: (i) which chemicals repel *E. coli*; (ii) is there any correlation between harmfulness of a chemical and its ability to repel; (iii) how are the repellents detected; (iv) can mutants be obtained that are defective in negative chemotaxis; (v) what is the relationship between negative and positive chemotaxis; (vi) how do individual bacteria behave when undergoing negative chemotaxis; (vii) how do bacteria make a "decision" when confronted with a "conflict" such as an attractant and repellent presented together?

In the case of positive chemotaxis, we have identified a large number of attractants (6, 34) and shown that chemoreception occurs by means of specific sensing devices, called "chemoreceptors," that are located on or in the bacterial cell envelope and that detect attractants without requiring the metabolism of those attractants (3). The first step in this chemoreception is the binding of an attractant to a recognition component—where identified, a protein specific for the attractant and also involved in transport (6, 26).

In this publication we describe new methods for measuring negative chemotaxis and report a survey of possible repellents. We demonstrate that the detection of repellents is unrelated to the harm they cause. The repellents are classified by competition experiments, the existence of specific mutants, and chemical structure; each class is presumed to be handled by a specific chemoreceptor. An unsuccessful attempt to relate a chemoreceptor to a transport system is reported, and the relationship between positive and negative chemotaxis is explored. The behavior of individual bacteria and the results of "conflict" experiments will be subjects for later publications.

MATERIALS AND METHODS

Chemicals. These were all obtained from commercial sources in analytical grade wherever possible. Chemicals were not purified by us, so the possibility

of contamination by other repellents, attractants, stimulators, or inhibitors must be considered.

Media. Tryptone broth contained 1% tryptone (Difco) and 0.5% NaCl. Glycerol minimal medium contained glycerol (5 g/liter) and the required amino acids L-histidine and L-threonine (0.25 g/liter each), autoclaved separately, and the salts described previously (4).

Chemotaxis medium contained phosphate buffer (pH 7.0; 10^{-2} M) and potassium ethylenediaminetetraacetate (EDTA; 10^{-4} M) (4).

Bacteria. Strain AW574, wild-type for chemotaxis (*his*$^-$, *leu*$^-$, *thr*$^-$, *gal*$^+$) and lacking known suppressor genes, was prepared by S. Larsen and J. S. Parkinson (personal communication) by mating strains AW405 and B7 to eliminate a suppressor gene present in AW405. AW607 is a *leu*$^+$ revertant of AW574; *leu*$^+$ was introduced to avoid any possible problems of L-leucine auxotrophy when dealing with L-leucine taxis. AW607 was used except where otherwise mentioned.

Negative chemotaxis assays: (i) chemical-in-capillary method. To assay positive chemotaxis, a capillary containing a solution of attractant is inserted into a suspension (the "pond") of motile bacteria. The bacteria are attracted to the mouth of the capillary and enter it. The number of bacteria in the capillary is then determined by plating the contents and counting colonies the next day (top curve, Fig. 1). If the chemical is inert or omitted, some bacteria nevertheless enter the capillary owing to random motility of the bacteria (the "background" number; see Fig. 1, zero attractant or repellent). (With a nonmotile strain, only 100 bacteria were found in the capillary at 60 min.) If the chemical is a repellent, fewer than the background number will accumulate in the capillary (bottom curve, Fig. 1).

This method is useful in simultaneously screening for attractants and repellents, but it is limited because repelling effects are often too small to be detected this way. Although inhibition of motility could give the same result as negative chemotaxis, this possibility can be checked by the "motility assay" (middle curve, Fig. 1).

The procedure for the chemical-in-capillary method has been described previously for positive chemotaxis (3, 4, 34). Bacteria were grown in glycerol minimal medium and washed twice with chemotaxis medium at room temperature by the published procedures (4).

(ii) Chemical-in-pond method. The repellent is present in the pond of bacteria, and none is put into the capillary. The bacteria then escape into the capillary for "refuge," and the number accumulated in the capillary is determined as before. Figure 2 is an example of a concentration-response curve, in this case for acetate. Without any repellent, some bacteria enter the capillary by random swimming; with repellent, the accumulation is *larger*, and hence this assay provides a positive result. (The values are never as strikingly above background as for positive chemotaxis in the chemical-in-capillary method.) There is a threshold value for repulsion, near 10^{-4} M in the case of Fig. 2. At high concentrations, loss of motility or viability, or saturation of the chemotactic apparatus,

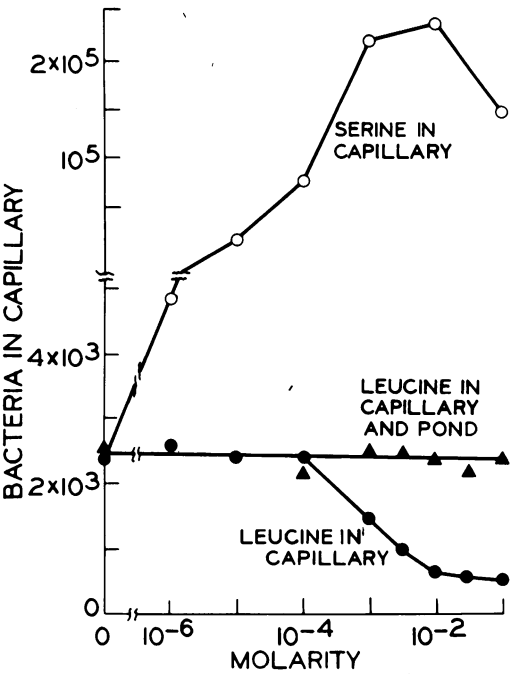

FIG. 1. *Negative L-leucine chemotaxis demonstrated by use of the chemical-in-capillary method. Bottom curve, Capillary contains L-leucine. Middle curve, Capillary and pond contain equal concentration of L-leucine; this is the "motility assay" and in this case shows that L-leucine does not inhibit motility. Top curve, Capillary contains an attractant, L-serine. The experiment was carried out with strain AW405 for 60 min at 30 C. See Materials and Methods.*

leads to a decline, and hence the curve has a peak at 10^{-2} M (Fig. 2). A control experiment with a uniform concentration of repellent in both the pond and the capillary (at various concentrations) shows the effect of the chemical on motility and viability: the "motility assay" (middle curve, Fig. 1). The chemical-in-pond method is useful because it provides positive results and objective data, but it is not as sensitive as the chemical-in-plug method.

The chemical-in-pond method is also useful in studies of repellents that alter agar and thus make the three methods that use agar impractical. An example is shown in the central panel of Fig. 3, which illustrates repulsion by both low and, less effectively, high pH. In a modification, the repellent (pH 4.5 or 8.5) is again placed in the pond, but various concentrations of repellent are placed in the capillary (outside panels, Fig. 3).

The procedure for the chemical-in-pond method is the same as that for the chemical-in-capillary method (4), except that the test chemical is placed in the bacterial suspension instead of in the capillary. Bacteria were grown and washed as for the first method. The standard deviation for replicate assays was 13%.

(iii) Chemical-in-plug method. In a petri plate, bacteria sufficiently concentrated to give visible tur-

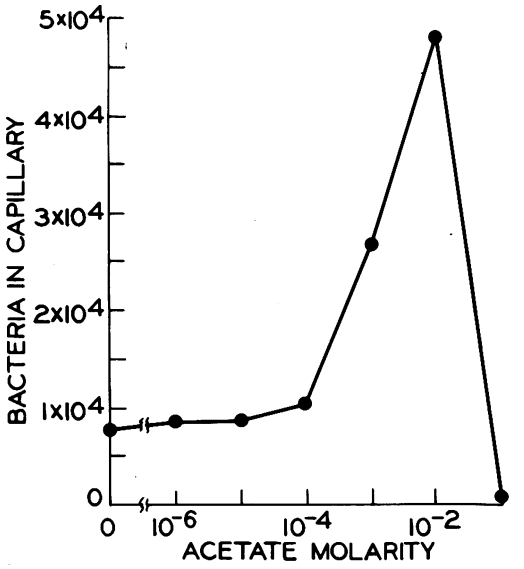

FIG. 2. *Negative chemotaxis assay of acetate repulsion by use of the chemical-in-pond method. The experiment was carried out with strain AW607 for 60 min at 30 C. See Materials and Methods.*

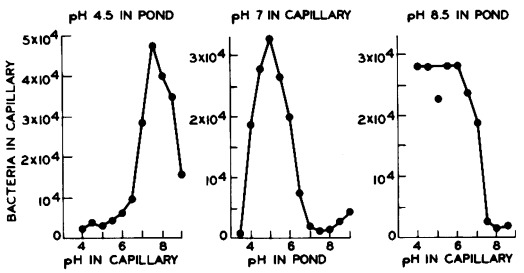

FIG. 3. *Assay of chemotaxis away from low and high pH by use of the chemical-in-pond method. Phosphate buffer was used throughout, but similar patterns were obtained with other buffers. The shapes of the pH gradients were not determined. The experiment was carried out with strain AW405 for 60 min at 30 C. In the central panel the decline below pH 5 is due to inhibition of motility. See Materials and Methods.*

bidity are suspended in agar that is soft enough (0.3%) to allow motility. A plug of hard agar (2%) containing repellent repels bacteria, creating a clear area around the plug within 30 min. Beyond is a ring of bacteria, individuals that used to be in what is now the clearing. Figure 4 shows this at various concentrations of repellent, in this case acetate. The distance from the plug to the ring provides the basis for a concentration response curve (Fig. 5). The concentration gradient (L-leucine) established in this method is shown in Fig. 6. This is the most useful method because it is the most sensitive one, providing lowest thresholds, and it is also technically the simplest to handle; in addition, mutants can be isolated from the clear zone.

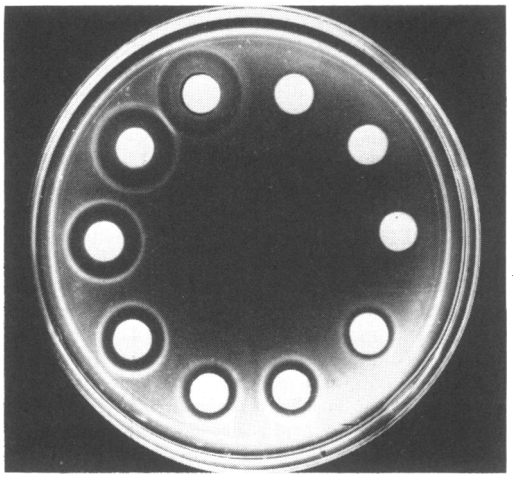

FIG. 4. *Negative chemotaxis assay of acetate repulsion by use of the chemical-in-plug method. The plugs contained dilutions of acetate starting with none at about 1 o'clock and increasing from 3 × 10^{-4} to 3 M clockwise (same concentrations as shown in Fig. 5). The assay was allowed to develop 30 min at room temperature (27 C), and then the plate was photographed. Bacteria (AW607) were initially distributed at equal cell density throughout the plate (including the center), but this is not clear in the figure due to an artifact in photography resulting from uneven lighting. See Materials and Methods.*

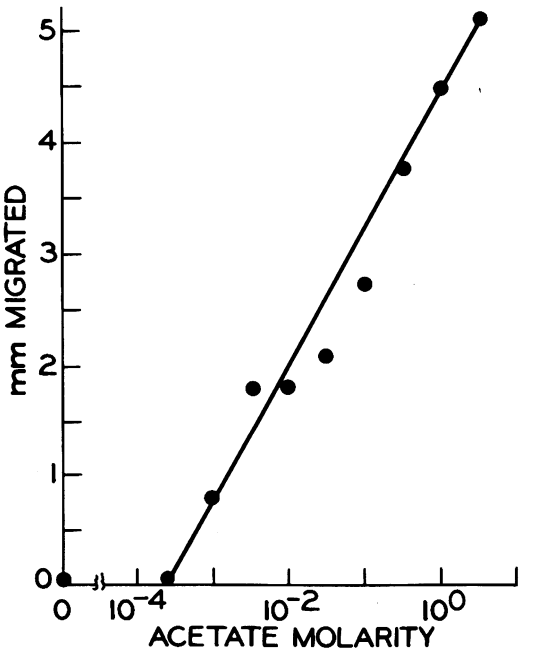

FIG. 5. *Concentration response curve for acetate by use of the chemical-in-plug method. Measurements come from Fig. 4 and are converted to the real response size in the petri dish; Fig. 4 is smaller than real size. The intersection with the abscissa is considered the threshold concentration.*

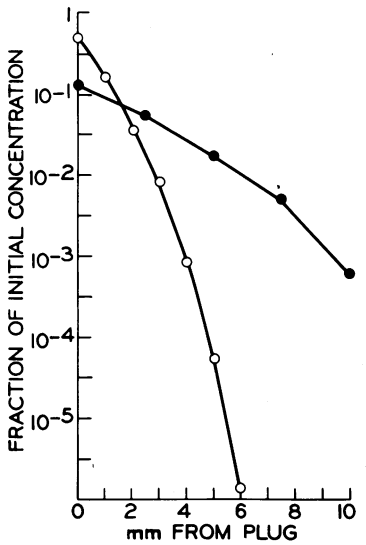

FIG. 6. *Shape of gradient at 20 min. The curve with open circles was calculated for 20 min from the diffusion equation adapted to two-dimensionl diffusion from a plug source (15). The diffusion coefficient of* L*-leucine was taken as 7.26 × 10⁻⁶ per cm² per s (32) at 25 C. The curve with closed circles is an experimental result with 10⁻¹ M* L*-[¹⁴C]leucine in the presence of 5 × 10⁷ motile bacteria per ml for 20 min; initial temperature, 40 C, final temperature, 27 C. Radioactivity was measured in samples picked up by 1-μliter capillaries at various distances from the plug. The discrepancy between the theoretical and experimental curves may result from (i) disturbing the gradient by inserting the plug and removing samples, (ii) convection caused by the change in temperature during the experiment, (iii) change in the diffusion coefficient as the temperature changed, and (iv) each sample representing an average of surrounding fluid.*

This method also allows identification of attractants: in this case the bacteria accumulate adjacent to the plug of agar and leave a clear region that they vacated away from the plug.

To prepare the agar plug containing the test chemical, 4% agar in glass-distilled water was autoclaved and allowed to come to 70 C in a 70 C bath. Phosphate buffer (pH 7.0) and EDTA were added to 10^{-2} and 10^{-4} M, respectively, and then this was mixed with an equal volume of the test chemical at 70 C, at twice the required concentration in chemotaxis medium. (In some cases at the highest concentration tested, the chemical was added to the agar as a suspension because of solubility limitations.) An 8-ml volume of the resulting liquid agar solution was poured into a plastic petri dish (50 by 12 mm) and allowed to solidify at room temperature overnight. Plugs of this agar containing the chemical were cut out with a sharpened, stainless-steel cutter of 8 mm diameter (made from no. 304 stainless steel tubing, Steel Sales Co., Chicago, Ill.); a cork borer also works.

The bacteria were grown in tryptone broth to an absorbancy (590 nm) of 0.6 to 0.8 (an absorbancy of 1.0 is equivalent to 6×10^8 bacteria per ml), 5 ml of this culture was washed once in chemotaxis medium by centrifugation at room temperature, and then the bacteria were suspended at a final absorbancy of 0.06 to 0.1 in 20 ml of 0.3% agar containing chemotaxis medium at 50 C in a 50 C bath. This cell-agar suspension was immediately poured into a plastic petri dish (100 by 15 mm). After 5 min for minimizing convection due to cooling, the agar plug containing the test chemical was planted, with toothpicks, into the cell agar suspension. It is easier to plant the plug before pouring the cell agar suspension, but the resulting ring is irregular owing to disturbance of the gradient by the pouring. This was allowed to solidify and develop for 30 min at room temperature. The plate was then photographed, and the distance from the edge of the plug to the ring of bacteria was measured on the photograph with a ×7 measurement magnifier (Bausch and Lomb, Inc., Rochester, N.Y.). The measurements were plotted (Fig. 5), and the threshold of the response was determined as described there. For 0.1 M L-leucine in 13 separate experiments the size of the ring averaged 1.7 mm and the standard deviation was 11.5%. Threshold determinations for L-leucine in 7 separate experiments ranged from 6×10^{-5} to 5×10^{-4} with an average of 2×10^{-4} M.

The plates were kept at room temperature, since the size of the ring at 20 min was nearly the same at 25 and 30 C; at 35 C, the optimum, the ring was about 25% larger, whereas at 40 C chemotaxis was practically abolished. The size of the ring increased only slowly after 20 min, and by 60 min it had nearly reached a maximum.

Even plugs without repellent caused a slight (<1 mm) clearing with accumulation of bacteria on the plug, but never a ring of bacteria at the edge of the clearing away from the plug. This is clearly an artifact, since it was shown by generally nonchemotactic mutants. (It is evident in Figure 9 in the case of the amino acid mutant.)

For competition experiments, the competing chemical was added to the agar plug and to the cell agar suspension at the same final concentration.

(iv) Chemical-in-plate method. In this method, repellent is contained in hard agar throughout a petri plate, and a soft agar suspension of bacteria, concentrated enought to see, is placed in a well. A 20-ml volume of 3% agar containing chemotaxis medium and the test chemical was poured into a plastic petri dish (100 by 15 mm) and allowed to stand overnight at room temperature. Then as many as 40 holes, 8 mm in diameter, were cut out with a cutter. Bacteria were grown and washed as for the chemical-in-plug method, and each hole was just filled (about 0.15 ml) with the strain of bacteria to be tested at an absorbancy at 590 nm of 0.06 to 0.1 in 0.2% agar. The plate was then kept at room temperature. The bacteria escape visibly from the repellent within 30 min (Fig. 7).

This method is especially suited for testing a large number of colonies of bacterial cultures (up to 40 per plate), as in screening for mutants.

(v) Test tube method. A 2-ml volume of 2% agar containing chemotaxis medium and the test chemical was pipetted into each plastic tube (12 by 75 mm)

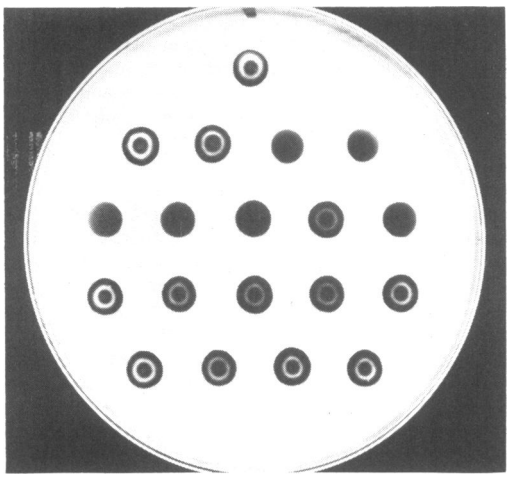

FIG. 7. *Negative chemotaxis for* L-*leucine by use of the chemical-in-plate method. The plate contained* 10^{-1} *M* L-*leucine, and various strains of E. coli, including taxis mutants of various sorts, were placed in each of the holes. Holes with rings indicate a response to the* L-*leucine gradient; those without indicate no response. The assay was allowed to develop for 30 min at room temperature (27 C), and then the plate was photographed. (The holes all contain bacteria at a visible turbidity, but this does not always show up due to low fidelity in photography.) See Materials and Methods.*

supplied with cap, and allowed to solidify at room temperature overnight. Then 2 ml of bacteria, grown and washed as for the chemical-in-plug method, at room temperature in chemotaxis medium at an optical density (590 nm) of 0.06 to 0.1 (turbid enough to see), was carefully layered onto each tube. A clear area developed above the agar within 30 min (Fig. 8). This method is suitable for surveying chemicals and for isolating mutants from the clear zone.

Isolation of negative chemotaxis mutants. Bacteria were first mutagenized according to the following procedure (J.S. Parkinson, personal communication). After growth in tryptone broth to an absorbancy (590 nm) of 1, 1.5 ml of the bacteria was sedimented and then suspended, at the same density, in the salts of the growth medium (4) containing 0.3 M ethyl methane sulfonate. This was kept at room temperature for 1 h and then sedimented. Finally, a small (<0.005 ml) volume of the pellet was grown in tryptone broth overnight.

To obtain mutants by the chemical-in-plug method, a sterile 1-μliter micropipette was used to pick up bacteria that remained in the middle of the clear zone around the plug containing the specific repellent. These bacteria were grown in tryptone broth. Usually the procedure had to be repeated 5 to 10 times to reach a point where a clearing no longer formed. To eliminate generally non-chemotactic mutants and nonmotile mutants, a positive chemotaxis step was then interposed. Bacteria from the clearing were grown in tryptone broth and spotted at the center of a tryptone soft agar plate, which results in swarming due to chemotaxis towards L-serine and L-aspartate in the tryptone (2). The origin of inoculation, containing the undesired mutants, was removed and discarded. Then the chemical-in-plug method was repeated 5 to 10 times until a clearing no longer formed for the specific chemical but did form for other repellents. The mixture around the plug containing the specific chemical was then cloned, and individual clones were tested for chemotaxis by the chemical-in-plate method. This procedure was used to isolate the

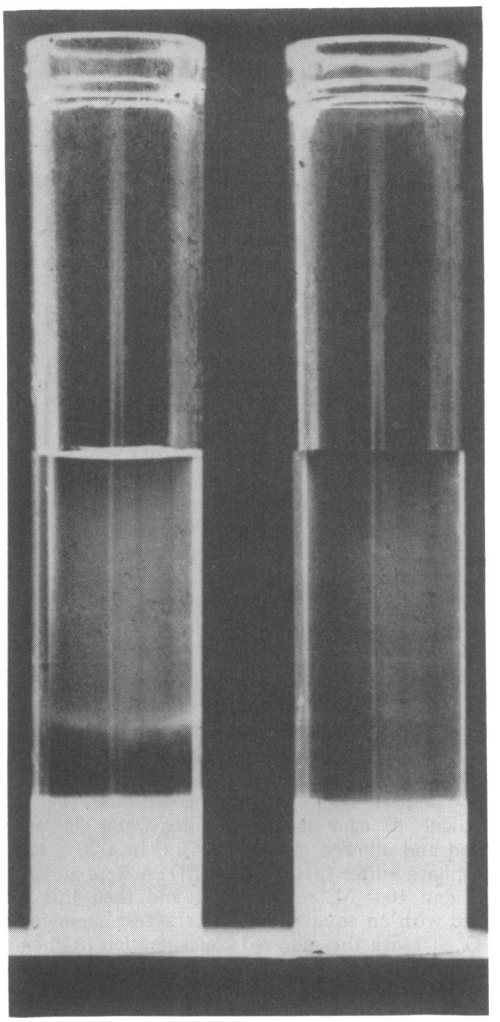

FIG. 8. *The test tube method for demonstrating negative chemotaxis. The tube at the left contained 0.1 M acetate in the agar at the bottom of the tube; note clear region above the agar. The tube at the right contained no repellent. The experiment was carried out with strain AW607 at room temperature (27 C) for 30 min and then the tubes were photographed. See Materials and Methods.*

specific, negative chemotaxis mutants described below.

When the test tube method was used for isolating mutants, a sterile melting point tube, held closed on top by a finger, was lowered into the clear region atop the agar layer but below the band of bacteria repelled by the chemical, and then fluid containing the bacteria was picked up by capillary action. These bacteria were grown up and the procedure was repeated 10 to 15 times. Otherwise the procedure is similar to that just described.

RESULTS

Demonstration and measurement of negative chemotaxis. Five methods were developed for studying negative chemotaxis; typical results are shown in Fig. 1 (bottom curve) for the chemical-in-capillary method, in Fig. 2 and 3 for the chemical-in-pond method, in Fig. 4 and 5 for the chemical-in-plug method, in Fig. 7 for the chemical-in-plate method, and in Fig. 8 for the test tube method.

Survey of repellents. Using these methods, we tested a number of chemicals for repulsion. Table 1 lists the chemicals that were found to be repellents and those that were found to be inert or very weakly repellent. Attractants have been listed elsewhere (6, 34).

For those repellents studied, negative chemotaxis appears to be constitutive, neither inducible nor repressible. The same size response to six repellents (2×10^{-1} M acetate, 3×10^{-1} M iso-butanol, 10^{-1} M L-leucine, 6×10^{-3} M indole, 10^{-1} M benzoate, and 10^{-1} M salicylate) was obtained with bacteria grown in minimal glycerol medium with or without each of the repellents (3×10^{-2} M acetate, 10^{-1} M ethanol, 10^{-2} M L-leucine, 3×10^{-4} M indole, 10^{-3} M benzoate, or 10^{-4} M salicylate) or in tryptone broth.

Is there any relation between repulsion and harmfulness? The mechanism for detection of repellents appears unrelated to deleterious effects of repellents. This is parallel to our demonstration that the mechanism for detection of attractants is unrelated to beneficial effects (such as adenosine triphosphate production) of attractants (3).

Table 2 shows the ability of each repellent to kill, to inhibit growth, and to inhibit motility, along with its threshold for chemotaxis. It may be seen most of the repellents are harmful chemicals, but not all of them are (at least by the criteria tested), for example, L-tryptophan, L-phenylalanine, and L-leucine. (See, however, reference 23, which shows that L-leucine can prolong the lag period that occurs when *E. coli* is shifted from complex to defined media.) Even leucine auxotrophs, which would benefit from attraction to L-leucine, are repelled by it (data not shown). Thus chemicals do not have to be harmful to be repellents. Even the harmful chemicals bring about repulsion at concentrations that are too low to produce harm.

As already noted by Pfeffer (41), some harmful chemicals are not repellents (Table 1), for example, the antibiotics penicillin and streptomycin, the toxic (47) L-leucine-containing dipeptides, and some toxic (7) metal ions such as Cu^{2+}. Thus, harmfulness is not sufficient to make a chemical a repellent.

A chemical can be a repellent in one strain but not in another, yet the harm done in the two strains is the same. Lederberg reported that phenol repels *Salmonella* but not *E. coli* (30), a result that we have confirmed. Yet the growth of both is completely inhibited by 10^{-2} M (and uninhibited at 10^{-3} M). Indeed, phenol at high concentrations is an attractant for *E. coli* (threshold 3×10^{-4} M, peak response of 300,000 bacteria per h at 10^{-1} M). A generally non-chemotactic mutant failed to respond, so this is in fact positive chemotaxis. A mutant (see below) that is no longer repelled by indole is just as much harmed as the parent (data not shown). Likewise, a mutant (see below) that is not repelled by low pH remains just as sensitive to it as its parent, and a mutant (see below) that is only poorly repelled by salicylate is harmed by it as much as its parent (data not shown).

Some mutants are resistant to the harm caused by a repellent, yet they are still repelled. For example, valine-resistant mutants (50% inhibition of growth at 5×10^{-2} M) are repelled by valine with an unaltered threshold (2×10^{-4} M). *E. coli* W, an independent isolate of this species (16), is naturally resistant to L-valine (29), yet is repelled by it with the same threshold as *E. coli* K-12.

Although harm done could not be the basis for detection of repellents, the function of negative chemotaxis appears to be to allow the bacteria to escape from harmful or crowded environments; thus, negative chemotaxis must have survival value for bacteria. Most of the repellents are indeed harmful (Table 2); harm has been documented previously for short-chain fatty acids (20, 46, 52), ethanol (36), indole (J. A. Boezi, Ph.D. thesis University of Illinois, Urbana, 1961; R. D. DeMoss, personal communication), L-valine (28), L-isoleucine (23), L-leucine (23), Co^{2+} (1, 7, 39), and Ni^{2+} (1, 7). Some of the repellents are antimicrobial food preservatives (20).

The following repellents are excretory products of *E. coli* and may thus be indicators of a

TABLE 1. *Repellents and nonrepellents for E. coli*[a]

Class of compound	Repellents	Threshold[b] (M)	Weak or nonrepellents	Highest concn tested (M)
1. Fatty acids and analogues:				
Straight chain	Acetate	3×10^{-4}	Formate	1
	Propionate	2×10^{-4}	Palmitate	3×10^{-2}
	n-Butyrate	10^{-4}	Stearate	3×10^{-2}
	n-Valerate (*n*-pentanoate)	3×10^{-4}		
	n-Caproate (*n*-hexanoate)	10^{-4}		
	n-Heptanoate	6×10^{-3}		
	n-Caprylate (*n*-octanoate)	3×10^{-2}		
	n-Caprate (*n*-decanoate)	3×10^{-2}		
Branched chain	Iso-butyrate	3×10^{-4}		
	Iso-valerate	10^{-4}		
Unsaturated	Crotonate (2-butenoate)	3×10^{-4}		
	Sorbate (2,4-hexadienoate)	6×10^{-4}		
Hydroxy acids	L-2-Hydroxy-iso-caproate	10^{-2}	DL-Lactate	10^{-1}
			DL-3-Hydroxy-*n*-butyrate	10^{-1}
			4-Hydroxy-*n*-butyrate	10^{-1}
			2-Hydroxy-iso-butyrate	10^{-1}
Mercapto acids	2-Mercaptoacetate (thioglycolate)	5×10^{-2}		
Keto acids	2-Keto-iso-caproate	10^{-2}		
Some amino acids			L-2-Amino-*n*-butyrate	10^{-1}
			4-Amino-*n*-butyrate	10^{-1}
			6-Amino-*n*-caproate	10^{-1}
			8-Amino-*n*-caprylate	10^{-1}
			DL-2-Amino-*n*-caprylate	10^{-2}
Aromatic acids	Benzoate	10^{-4}	*p*-Hydroxy benzoate	3×10^{-2}
	Phenylacetate	10^{-3}	*p*-Phenylbenzoate	3×10^{-2}
			β-Naphthylacetate	3×10^{-2}
Dicarboxylic acids[c]			Oxalate	10^{-1}
			Malonate	3×10^{-2}
			Adipate	10^{-1}
			Sebacate	10^{-1}
Thioacids	Thioacetate	2×10^{-3}		
Amides	Acetamide	5×10^{-2}	*n*-Valeramide	10^{-2}
	Iso-valeramide	2×10^{-3}		
Esters			Methyl acetate	3×10^{-1}
			Ethyl acetate	3×10^{-1}
			Amyl acetate	10^{-1}
			Methyl propionate	3×10^{-1}
2. Aliphatic alcohols:				
Straight chain	Methanol	10^{-1}[d]		
	Ethanol	10^{-3}		
	n-Propanol	4×10^{-3}		
	n-Butanol	10^{-3}[d]		
Branched chain	Iso-propanol	6×10^{-4}		
	Iso-butanol	10^{-3}		
	Iso-amyl alcohol	7×10^{-3}		
Amino alcohols			DL-2-Amino-1-propanol	1
			2-Amino-2-methyl-1-propanol	1
Dialcohols	Ethylene glycol	10^{-3}		
Aldehydes			Formaldehyde	1[d]
			Acetaldehyde	3×10^{-1}[d]
Mercaptans	Mercaptoethanol	10^{-3}		
	2-Propanethiol	3×10^{-3}		
3. Amino acids and analogues:				
L-α-Amino acids[g]	L-Leucine	10^{-4}[e]	L-Arginine	10^{-1}
	L-Isoleucine	1.5×10^{-4}[e]	L-Lysine	10^{-1}
	L-Valine	2.4×10^{-4}[e]	L-Tyrosine	10^{-3}[f]
	L-Tryptophan	10^{-3}[e]	L-α-Amino-*n*-butyrate	10^{-1}
	L-Phenylalanine	3.5×10^{-3}[e]		
	L-Glutamine	3×10^{-3}[e]		
	L-Histidine	5×10^{-3}[e]		
	L-Norleucine (α-amino-*n*-hexanoate)	3×10^{-4}[e]		
	L-Norvaline (α-amino-*n*-pentanoate)	2×10^{-3}[e]		

TABLE 1—*Continued*

Class of compound	Repellents	Threshold[b] (M)	Weak or nonrepellents	Highest concn tested (M)
	DL-α-Amino-*n*-octanoate	10^{-2}[e]		
	p-Amino-DL-phenylalanine	10^{-4}[e]		
D-α-Amino acids	D-Leucine	10^{-4}[e]		
	D-Phenylalanine	3×10^{-3}[c]		
	D-α-Aminophenylacetate	7×10^{-4}[e]		
Amino acids other than α			ω-Amino-*n*-hexanoate	10^{-1}
			ε-Amino-*n*-octanoate	10^{-1}
Amino acid amides			L-Leucine amide	10^{-1}
			L-Tryptophan amide	10^{-1}
Amino acid esters			L-Leucine ethyl ester	10^{-1}
			L-Phenylalanine methyl ester	10^{-1}
Decarboxylated			Tryptamine	1.5×10^{-3}
			DL-Amphetamine (DL-α-Methylphenylalanine)	10^{-1}[d]
N-acyl amino acids	*N*-benzoyl-DL-leucine	3×10^{-2}[e]	*N*-acetyl-D-leucine	10^{-1}
			N-acetyl-L-phenylalanine	10^{-1}
N-alkyl amino acids	*N*-methyl-DL-leucine	3×10^{-2}[e]		
α-Keto acids	α-Keto-iso-caproate	10^{-2}		
α-Hydroxy acids	L-α-Hydroxy-iso-caproate	10^{-2}		
Dipeptides			Glycyl-L-leucine	10^{-1}
			L-Leucyl-glycine	10^{-1}
			L-Leucyl-L-leucine	10^{-1}
4. Indole and analogues:				
	Indole	10^{-6}[h,i]		
Position 2	2-Methylindole	3×10^{-5}[h]	Indole-2-carboxylate	10^{-1}
Position 3	Skatole (3-methylindole)	10^{-6}[j]	Indole-3-acetate	3×10^{-3}
	Tryptophol	10^{-4}[k]	Indole-3-pyruvate	3×10^{-3}
	Tryptophan	10^{-3}[k]	Tryptamine	2×10^{-3}
			Gramine [3-(dimethylaminomethyl)indole]	2×10^{-3}
Position 5	5-Methylindole	10^{-6}[h]		
	5-Hydroxyindole	2×10^{-5}[j]		
	Indole-5-carboxylate	10^{-4}[h]		
Position 6			6-Aminoindole	3×10^{-3}
Position 7	7-Methylindole	10^{-6}[h]		
Altered rings			7-Azaindole	2×10^{-3}
			Adenine	3×10^{-3}
5. Aromatic compounds:				
A. Benzoate and analogues:				
	Benzoate	10^{-4}		
Esters	Methylbenzoate	10^{-4}		
	Ethylbenzoate	10^{-2}		
Amides			Hippuric acid (*N*-benzoylglycine)	10^{-1}
Alcohol-replacing carboxyl	Benzyl alcohol	6×10^{-4}	Phenol	3×10^{-1}[d]
Ortho substituents	Salicylate (*O*-hydroxybenzoate)	10^{-4}[l]		
	Thiosalicylate (*O*-thiobenzoate)	3×10^{-4}		
	Anthranilic acid (*O*-aminobenzoate)	10^{-3}		
	Phthalate (*O*-carboxylbenzoate)	10^{-2}		
Meta substituents	*m*-Chlorobenzoate	3×10^{-4}		
	m-Nitrobenzoate	3×10^{-3}		
	Saccharin (*O*-benzoic sulfimide, sodium salt)	3×10^{-2}		
Para substituents	*p*-Aminobenzoate	3×10^{-3}	*p*-Phenylbenzoate	3×10^{-2}
	p-Hydroxybenzoate	10^{-2}	Folic acid	3×10^{-2}
	p-Toluic acid (*p*-methylbenzoate)	2×10^{-2}		
Heterocyclic	Nalidixic acid	10^{-3}		
B. Salicylate and analogues:				
	Salicylate	10^{-4}[l]		

TABLE 1—*Continued*

Class of compound	Repellents	Threshold[b] (M)	Weak or nonrepellents	Highest concn tested (M)
	Thiosalicylate	3×10^{-5}		
Esters of carboxyl	Methylsalicylate	1.5×10^{-5}		
Amides of carboxyl			Salicylanalide	3×10^{-2}
Esters of alcohol	Aspirin (acetylsalicylate)	10^{-3}		
Alcohol-replacing carboxyl			Saligenin (*O*-hydroxybenzyl alcohol)	10^{-1}
Meta-substituents			Gentisic acid (*m*-hydroxysalicylate)	10^{-1}
Para-substituents	*p*-Aminosalicylate	3×10^{-4}		
6,7. H^+ and OH^-				
	Low pH	3×10^{-7} M H^+[d]		
	High pH	3×10^{-7} M OH^-[d]		
8. Sulfide	Na_2S	3×10^{-3}[d]		
Mercaptans	2-Propanethiol	3×10^{-3}		
9. Other inorganic ions	$CoSO_4$	2×10^{-4}	$MgSO_4$	10^{-1}
	$NiSO_4$	2×10^{-5}	$CrCl_3$	10^{-4}[d]
			$CuCl_2$	10^{-5}[d]
			$NiCl_2$	10^{-5}[d]
			$NaHCO_3$	10^{-1}[o]
			$NaPO_4$ (pH 7)	10^{-1}[o]
			Na_2SO_4	10^{-1}[o]
10. Additional nonrepellents:				
Sugars and analogues			D-Arabinose	10^{-1}[m]
			L-Fucose	10^{-1}[m]
			D-Galactose	10^{-1}[n]
			Lactose	10^{-1}[n]
			D-Ribitol	10^{-1}[m]
			Sucrose	10^{-1}[m]
Antibiotics			Penicillin	10^{-1}
			Streptomycin	10^{-1}
Substances bitter to man			Caffeine	10^{-2}
			Quinine	10^{-1}
Detergents			Sodium dodecyl sulfate	10^{-1}
			Triton X-100	0.03%
Miscellaneous			Cyclic adenosine 3',5'-monophosphate	10^{-2}

[a] Assays were by the chemical-in-plug method, except for those marked for footnote *d*, which were by chemical-in-pond method. For H^+ and OH^-, the thresholds are averages of experiments like those in the central panel of Fig. 3 and like those in the side panels where the pH in the pond is systematically varied until a response is no longer obtained.

[b] Thresholds were determined as shown in Fig. 2, or Fig. 1 when marked footnote *d*. Thresholds determined in separate experiments differ by a factor of three or less. For certain cases where related chemicals are compared, we list thresholds obtained from a single experiment. Thresholds are actually lower than indicated, since the bacteria respond away from the plug or capillary.

[c] Succinate, fumarate, maleate, and malate are weak attractants detected by the aspartate chemoreceptor (34). They were nevertheless tested as repellents up to 10^{-1} M, but no repulsion was detected.

[d] Assay was carried out by repellent-in-pond method for various technical reasons. This method gives thresholds about 10- to 50-fold higher than chemical-in-plug method.

[e] These compounds are not repellents in the mutant lacking hydrophobic amino acid taxis; remaining compounds tested under "Amino acid analogues" were equally effective in this mutant and its parent, AW607.

[f] Near limit of solubility (2.5×10^{-3} M). Negative chemotaxis with a threshold of 10^{-6} M has been reported for *Salmonella* (49).

[g] L-α-Amino acids that are commonly found in proteins but not listed are known to be attractants (34). They were nevertheless tested for repulsion up to 10^{-1} M, but showed none. This includes L-proline, with cells grown in the absence of L-proline where it is not an attractant (34).

[h] Not detected in mutant lacking indole taxis.

[i] Threshold averaged for many experiments is 7×10^{-6} M. Here we report the threshold found in the experiment where indole and its analogues were compared.

[j] Threshold increased 10-fold in mutant lacking indole taxis.

[k] Equally effective in mutant lacking indole taxis and its parent AW607.

[l] Much reduced in salicylate taxis mutant (Table 4). All the other aromatic compounds in this table gave normal responses in this mutant.

[m] Non-attractants for *E. coli* (6).

[n] Attractants for *E. coli* (6).

[o] Sodium salts tested because K^+ is weakly attractive (unpublished data); potassium salts were also not repellents.

TABLE 2. *Effect of repellents on viability, growth rate, and motility of E. coli*[a]

Repellent	Viability[b]	Growth rate[c]	Motility[d]	Threshold[e]
Acetate	>1	10^{-1}	9×10^{-2}	3×10^{-4}
Sorbate	2×10^{-1}	3×10^{-2}	2×10^{-1}	6×10^{-4}
Ethanol	1.5	5×10^{-1}	>1	10^{-3}
L-Leucine	$>10^{-1}$	$>10^{-1}$	$>10^{-1}$	10^{-4}
L-Isoleucine	$>10^{-1}$	4×10^{-2}	$>10^{-1}$[f]	1.5×10^{-4}
L-Valine	$>10^{-1}$	4×10^{-4}	$>10^{-1}$[f]	2.4×10^{-4}
L-Tryptophan	$>5 \times 10^{-2}$	$>5 \times 10^{-2}$	$>5 \times 10^{-2}$	10^{-3}
L-Phenylalanine	$>10^{-1}$	$>10^{-1}$	$>10^{-1}$	3.5×10^{-3}
Indole	5×10^{-3}	10^{-3}	3×10^{-3}	10^{-6}
Benzoate	6×10^{-1}	2×10^{-2}	6×10^{-2}	10^{-4}
Salicylate	2×10^{-1}	6×10^{-4}	3×10^{-2}	10^{-4}
Acid	pH 4.5	pH 4.5	pH 4	pH 6.5
Base	pH 9.6	pH 8.5	pH 8.5	pH 7.5

[a] *E. coli* wild-type for chemotaxis, AW607, was used, except that (only for historical reasons) for ethanol, acid, and base the related wild-type W405 was used. > Indicates that there was no effect at this highest concentration tested, except that for footnote *f* 10^{-1} M L-isoleucine inhibited motility 30% and 10^{-1} M L-valine inhibited motility 20%. All acids were tested at pH 7.

[b] Concentration (molar) required for 50% loss in 1 h. Viability 1 h after addition of various concentrations of each repellent in chemotaxis medium was measured by plating cells and counting colonies the next day. The bacteria had previously been grown in minimal glycerol medium and washed in chemotaxis medium (see Materials and Methods).

[c] Concentration (molar) required for 50% inhibition. Growth rate in minimal glycerol medium containing various concentrations of each repellent was measured individually at an optical density at 590 nm. The bacteria had previously been grown in that medium.

[d] Concentration (molar) required for 50% inhibition. Motility in chemotaxis medium containing various concentrations of each repellent was measured by the motility assay (see text). For indole and salicylate, the results were confirmed by microscope observations. For sorbate, motility was studied only with a microscope.

[e] From Table 1.

crowded environment: acetate (49), ethanol (49), indole (25), and $H_2S(Na_2S)$ (17). By use of the chemical-in-plug method, it can be shown that a highly concentrated *E. coli* suspension (2×10^9/ml), boiled or not, in the plug repels the same (and other) species of bacteria in the plate to the extent of a 1-mm clearing. Even a mutant, AW518, insensitive to most of the repellents studied (see Table 4), was repelled. The responsible repellents have not been identified.

One class of repellents that may have been missed in this survey is harmful compounds whose threshold for chemotaxis lies too close to the threshold for toxicity.

Repellent classes based on competition experiments. Using the chemical-in-plug method, we placed one repellent, A, at 50- to 200-fold above its threshold in the plug and a second repellent, B, at as close to saturating concentration as possible (considering solubility limits and harm to motility) everywhere in the plate including the plug. If the two repellents use the same chemoreceptor, the response to A should be inhibited; if they do not, the response should not be affected. (Inhibition could result from a loss of motility or of chemotaxis, but this was always checked by including a plug of unrelated repellent.)

The results, available for certain of the repellents and summarized in Table 3, show that these repellents can be classified into at least five classes: short-chain fatty acids, aliphatic alcohols, some amino acids, indole and some of its relatives, and aromatic compounds. Benzoate may be a relatively weakly detected member of both the fatty acid class and the aromatic class, based on its ability to inhibit fatty acid taxis.

Repellent classes based on mutants. Chemotaxis mutants are of three, fully motile types: (i) specific, which lack one chemoreceptor activity only (6, 27); (ii) general, which lack all chemotaxis (10); and (iii) multiply defective, a new, in-between class which has several chemoreceptor activities defective due to a single mutation in a step beyond the chemoreceptors (R. W. Reader, G. W. Ordal, W-W. Tso, J. S. Parkinson, J. Adler, manuscript in preparation). Some mutants that were previously thought to be specific (34) are in fact multiply defective mutants; hence, for assigning chemi-

TABLE 3. *Competition experiments for negative chemotaxis in E. coli*[a]

Class	Repellent	Remaining response with competitor listed[b] (percentage of response without competitor)			
		Acetate	L-Leucine	Indole	Benzoate
Fatty acids	Acetate	5	95	60	35
	Propionate	20			
	n-Butyrate	35			
	Crotonate	15			
	Sorbate	10		90	15
Aliphatic alcohols	Iso-butanol	100			
Hydrophobic amino acids	L-Leucine	95	0	55	100
	L-Isoleucine		0		
	L-Valine		0		
	L-Tryptophan		0	50	
	L-Phenylalanine		0		
	L-Glutamine		0		
	L-Histidine		0		
Indole and analogues	Indole	85	100	0	80
Aromatic compounds	Benzoate	95	100	75	15
	Salicylate	75		80	45

[a] Bacteria wild-type for chemotaxis, AW607, were grown in tryptone broth and washed in chemotaxis medium (see Materials and Methods). Competition was studied by the chemical-in-plug method, with repellent in the plug only and competitor in both plug and soft agar at equal concentration. At 30 min, the clear region around the plug was measured in this case and in a control without competitor present, as described in the legend to Fig. 4. Then by use of a concentration response curve for each repellent (for example Fig. 5), these measured values were converted into effective concentration of repellent.

[b] Competitors were used at the highest concentration possible in an attempt to saturate that chemoreceptor; this concentration was dictated either by the limit of solubility (for L-leucine) or by the need to avoid inhibition of motility (Table 2). Competitor and repellents were at the following concentrations: acetate, (1.5×10^{-1} M) and repellents (200-fold above threshold), L-leucine (0.16 M) and repellents (50-fold above threshold), indole (3×10^{-4} M) and repellents (50-fold above threshold), benzoate (4×10^{-2} M) and repellents (200-fold above threshold). Competition by salicylate was difficult to establish reliably, since it inhibits motility at a concentration that is too close to its taxis threshold. In "self-competition" experiments, for example acetate competing with acetate, the response was usually not totally eliminated because the plug always contained more repellent than the plate, and the chemoreceptor was usually not saturated. Empty space indicates experiment not carried out.

cals to a class, mutants must be used with caution because they might be of this type. Even specific mutants can be misleading, since the mutation may cause an altered specificity rather than loss of activity to all members of a class. Other criteria such as competition data need to be considered together with data from mutants.

Negative chemotaxis mutants were isolated by picking from the clearing of a chemical-in-plug plate or from the clearing above the repellent-containing agar in a test tube (see above for details of the mutant isolation).

Two mutants, AW608 and AW616, fail to show any repulsion by L-leucine (Fig. 9), L-isoleucine, L-valine, L-tryptophan, L-phenylalanine, L-glutamine, and L-histidine, but chemotaxis is normal for other repellents (Fig. 9 and Table 4) and for all the attractants tested except L-serine, which has a 30-fold higher than normal threshold. These mutants thus support the suggestion from competition experiments that these amino acids belong to one class. AW608 was studied further and found to lack chemotaxis away from those other amino acids and analogues shown to be repellents in Table 1 (footnote *e*), and thus these all belong to the same class of repellents.

Two other mutants, AW609 and AW617, fail to show any repulsion by indole (Table 4). AW609 was studied further and found to show little or reduced repulsion by indole analogues (Table 1, footnotes *h* and *j*), except for tryptophan, which elicits a normal response (Table

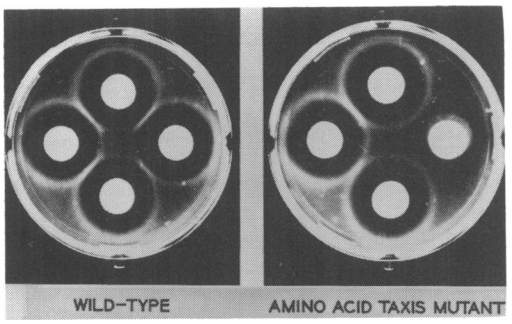

FIG. 9. *Parental bacteria (AW607) and a hydrophobic amino acid taxis mutant (AW608) studied by chemical-in-plug method with four of the more extensively studied repellents. The four plugs contained salicylate (10^{-1} M), acetate (10^{-1} M), indole (3×10^{-3} M), and L-leucine (10^{-1} M), starting from the bottom and going clockwise. The taxis mutant (right) shows taxis to salicylate, acetate, and indole, but not to L-leucine (apparent small response is artifactual; see Materials and Methods) nor (not shown) to any of the other repelling amino acids. Assay was done as described in Materials and Methods except that it lasted 1 h and 50-cm petri dishes were used.*

4). Chemotaxis is normal for other repellents (Table 4) and for all attractants tried.

Another mutant, AW610, shows reduced repulsion by salicylate (Table 4) while showing normal taxis away from the other (including other aromatic) repellents (Table 1, footnote *l*, and Table 4). Chemotaxis toward all attractants tried is normal. This mutant is thus regarded as defective in the activity of a surprisingly highly specific "salicylate chemoreceptor"; alternatively an "aromatic chemoreceptor" may have been so altered as to affect only salicylate taxis in this mutant.

We have tried, but not succeeded, to isolate a mutant missing fatty acid or benzoate taxis.

The previously reported serine taxis mutant AW518 (27, 34) is clearly a multiply defective mutant since it is defective not only in serine taxis, but in addition it shows reduced repulsion by fatty acids, hydrophobic amino acids, indole, benzoate, and low pH, but it still responds to aliphatic alcohols, salicylate, high pH, Co^{2+}, and Ni^{2+} (Table 4). This suggests that benzoate and salicylate are detected by different mechanisms, and the same appears to be true for low and high pH.

Specificity of each class of repellents: (i) fatty acid class. The fatty acid (Table 1) class includes straight-chain, branched-chain, and unsaturated fatty acids. Short-chain fatty acids are most active, from C2 to C6, with declining activity up to C10; C1 (formate) and acids much longer than C10 are not active. Inactivity of the long-chain fatty acids could be due to their relative insolubility in water. Phenyl carboxylic acids are repellents.

Hydrophobicity of the side chain is important: addition of a hydroxyl, amino, sulfhydryl, keto, or a second carboxyl group greatly decreases the activity. The carboxyl group is crucial: mercaptoacids, amides, and esters have much reduced activity. The detergents tested (Table 1) were not found to be repellents.

Due to the lack of a fatty acid taxis mutant, assignment of all the fatty acids and analogues to a single class must rest on competition experiments (Table 3) and structural similarity.

(ii) Aliphatic alcohols class. The aliphatic alcohols class (Table 1) includes short-chain, straight or branched alcohols, C2 to C4 being most active. Thiols are active. The alcohols do not belong to the fatty acid class, based on competition experiments (Table 3) and the mutant AW518 (Table 4). They also do not belong to the class of phenolic compounds that includes salicylate since the salicylate taxis mutant is unaltered for alcohol taxis (Table 4), nor are alcohols detected by the same mechanisms as hydroxyl ions, since in a competition experiment 10^{-1} M iso-butanol did not inhibit hydroxyl ion taxis (data not shown).

(iii) Hydrophobic amino acid class. Based on the competition experiments (Table 3) and studies with the amino acid negative taxis mutant (Table 4 and footnote *e* in Table 1), the hydrophobic amino acid class includes a large variety of amino acids that have been established (37) as having relatively hydrophobic side chains. The D-isomers are about as active as the L-isomers. Basic amino acids are not detected; neither are acidic amino acids and other hydrophilic amino acids, most of which are attractants (34).

The carboxyl group is essential since amides, esters, and decarboxylated derivatives are inactive. The amino group is required: there is loss of activity when it is acylated or alkylated, or replaced by a keto or hydroxyl group. Dipeptides are inactive.

It is noteworthy that repulsion by tryptophan is not due to production of the repellent indole, since a tryptophanase mutant, T_sA (13), is repelled normally by tryptophan, giving the normal threshold of 10^{-3} M.

(iv) Indole class. Indole and a number of analogues are active, for example the various methylated derivatives such as skatole (Table 1). On the other hand, addition of amino or carboxyl groups or alteration of the ring structure destroys the activity.

TABLE 4. *Mutants in negative chemotaxis*

Class	Repellent	Threshold[a] (m)					
		Wild-type AW607	Hydrophobic amino acid taxis mutant AW608	Indole taxis mutant AW609	Salicylate taxis mutant AW610	Serine taxis mutant AW518	Generally non-chemotactic mutant[b]
Fatty acids	Acetate	3×10^{-4}	3×10^{-4}	7×10^{-4}	8×10^{-4}	$>10^{-1}$	$>10^{-1}$
	Propionate	2×10^{-4}			4×10^{-4}		
	n-Butyrate	10^{-4}			10^{-4}		
	Iso-butyrate	3×10^{-4}			2×10^{-4}		
	Sorbate	6×10^{-4}				$>10^{-1}$	
Aliphatic alcohols	Ethanol	10^{-3}			10^{-3}	10^{-3}	$>10^{-1}$
	Iso-butanol	10^{-3}	10^{-2}		10^{-3}	10^{-3}	
Hydrophobic amino acids	L-Leucine	10^{-4}	$>10^{-1}$	10^{-4}	2×10^{-4}	$>10^{-1}$	$>10^{-1}$
	L-Isoleucine	1.5×10^{-4}	$>10^{-1}$				$>10^{-1}$
	L-Valine	2.4×10^{-4}	$>10^{-1}$				
	L-Tryptophan	10^{-3}	$>10^{-1}$	10^{-3}	10^{-3}	$>10^{-1}$	
	L-Phenylalanine	3.5×10^{-3}	$>10^{-1}$				
	L-Glutamine	3×10^{-3}	$>10^{-1}$				
	L-Histidine	5×10^{-3}	$>10^{-1}$				
	See others in Table 1, footnote *e*						
Indole and analogues	Indole	7×10^{-6}	8×10^{-6}	$>5 \times 10^{-3}$[c]	1.5×10^{-5}	$>5 \times 10^{-3}$[c]	$>5 \times 10^{-3}$[c]
	See others in Table 1, footnotes *h*, *j*, and *k*						
Aromatic compounds	Benzoate	3×10^{-4}			3×10^{-4}	$>10^{-1}$	$>10^{-1}$
	Salicylate	3×10^{-4}	4×10^{-4}	6×10^{-4}	3×10^{-2}	2×10^{-3}	$>10^{-1}$
	See others in Table 1, footnote *l*						
H^+		3×10^{-7}	3×10^{-7}			$>10^{-4}$[d]	$>10^{-4}$[d]
OH^-		3×10^{-7}	3×10^{-7}			3×10^{-7}	$>10^{-5}$[d]
Metallic cation	$CoSO_4$	2×10^{-4}	2×10^{-4}			2×10^{-4}	
	$NiSO_4$	2×10^{-5}	2×10^{-5}			2×10^{-5}	

[a] Chemical-in-plug method used except for H^+ and OH^-, where chemical-in-pond method was used. Thresholds determined in different experiments differ by a factor of three or less. The thresholds reported in this table were obtained by comparing each chemical in the wild type and mutant in the same experiment. Empty space indicates experiment not carried out.

[b] In nearly all cases a *cheA*, *cheB*, and *cheC* (9) representative (M593, M590, and M497, respectively) was tested and gave identical results.

[c] Highest concentration allowed by solubility.

[d] Highest concentration that can be used without severely inhibiting motility.

The most active compounds fail to repel, or repel less well than normal, the indole taxis mutant. Repulsion by L-tryptophan is normal in this mutant, but is lost in the amino acid taxis mutant (Table 4). That, competition experiments (Table 3) and the result that L-tryptophan did not at all compete with indole taxis (data not shown) make it apparent that tryptophan does not belong to the indole class.

(v) Aromatic class. Salicylate and benzoate and many of their analogues are repellents for *E. coli* (Table 1); however, only salicylate taxis is defective in the salicylate mutant (Table 4). Thus, either salicylate belongs to a separate class or else a more general "aromatic chemoreceptor" has been so altered by mutation that only salicylate is affected. Consistent with the idea of a separate chemoreceptor for salicylate is the fact that salicylate taxis but not benzoate taxis survives in AW518 (Table 4). Aromatic acids could belong to the fatty acid class, but this is not the case for the repelling aromatic esters.

(vi and vii) Low and high pH. Strain AW518 (27, 34) was found to lack taxis away from low pH, along with many other negative taxes

(Table 4), but it retained taxis away from high pH. Although this mutant is multiply defective, the above fact suggests that hydrogen ions are detected by a different mechanism than the one for hydroxyl ions.

(viii) Sulfide. Sodium sulfide (pH 7.0) repels *E. coli*, and therefore presumably hydrogen sulfide would do the same. Mercaptans are also repellents (Table 1). This taxis has not been studied further.

(ix) Metallic cation class. Of various metallic cations tested, only Co^{2+} and Ni^{2+} were found to be repellents. It is possible that both belong to one class since they are both group VIII transition elements and are both detected by strain AW518. Competition experiments have not been carried out because motility is inhibited by low concentrations of these ions (7). Mg^{2+}, which shares a transport system with Co^{2+} (38), is not a repellent.

Negative chemotaxis and transport. Possibly the detection of repellents involves transport systems for the repellents, as is the case for various attractants (6, 26), but we have not been able to establish this.

E. coli contains a shockable binding protein for L-leucine, L-isoleucine, and L-valine (42), another one specific for L-leucine (22), and another one more or less specific for L-isoleucine (21); all three are strongly repressed by L-leucine in the growth medium (28, 40). Since chemotaxis away from the amino acids is not at all repressed by L-leucine (see above), it is most unlikely that these binding proteins play any role in chemotaxis. Oxender's group has isolated a mutant, EO 0323, that is unable to use these binding proteins for transport, although the binding activities are still present presumably due to lack of a subsequent component in the permease (43), and another mutant, EO 0321, that has lost the L-leucine-specific binding activity and L-leucine specific transport and has reduced levels of the activity of the protein that binds L-leucine, L-isoleucine, and L-valine (43). These mutants are normally repelled by the hydrophobic amino acids, giving the same thresholds as the wild type. Transport mediated by these shockable binding proteins and the L-leucine-specific binding activity do not appear, therefore, to be required for this negative chemotaxis.

E. coli has at least one additional transport system for L-leucine, L-isoleucine, and L-valine that is membrane-bound and does not use shockable binding proteins (31). The amino acid taxis mutant AW608 is normal in L-leucine transport over the range from 10^{-8} to 10^{-4} M, which includes this transport system (data not shown; confirmed by D.L. Oxender, personal communication). In addition, L-tryptophan and L-phenylalanine are not substrates for this system, and mutants presumed to be missing this system, such as MI183a (24) and MI238 (M. Iaccarino, personal communication), show normal taxis away from the hydrophobic amino acids (data not shown).

Thus, chemoreception of the hydrophobic amino acids does not employ the transport systems we have studied so far. Perhaps yet another transport system operative at very high substrate concentrations is involved. We have not studied the possible role of transport systems for the other repellents.

Relation between negative and positive chemotaxis. Generally non-chemotactic mutants, previously shown to be fully motile but lacking all positive chemotaxes (10), also lack all negative chemotaxes (Table 4). No mutants were found that lack all negative chemotaxes but have all the positive ones. Thus the pathways for analyzing sensory data on repellents and attractants do not appear to be totally separate, but rather they converge in a final common pathway. Positive chemotaxis, though not motility, requires or is stimulated by L-methionine in strains unable to synthesize it (4, 5). This has now been shown to be the case also for negative chemotaxis: without L-methionine, a methionine auxotroph responded to a plug of 0.1 M acetate with a clearing of 1.4 mm, whereas with 10^{-7} M or higher concentrations of L-methionine the clearing was 3.0 mm. The role of L-methionine is presumably in the common pathway, but details of the mechanism of action are still unknown.

Thresholds for repellents are usually 100- to 10,000-fold higher (Table 1) than for attractants (6, 34). This seems reasonable from point of view of providing a selective advantage to the bacteria, since the repellents studied are harmful only at high concentrations, whereas the attractants are utilized even at the low concentrations.

Are repellents at very low concentrations attractants, and are attractants at very high concentrations repellents? Apparently not. L-Leucine and acetate, repellents with thresholds near 10^{-4} M, were tested for attraction between 10^{-4} and 10^{-9} M, but none was found. The attractants L-aspartate, L-serine, and D-galactose did not repel at 10^{-1} M. In the case of D-galactose this study included a mutant ($20SOK^-$) whose threshold is 10^{-8} M and whose positive taxis response is saturated at 10^{-5} M (35).

Osmotaxis, a repulsion by high osmolarity, is reported in the older literature (44). Indeed, we have indications that a wide variety of chemi-

cals at concentrations above 10^{-1} M can repel *E. coli*, but this has not been investigated further.

It is noteworthy that the organic repellents have both a hydrophobic and a hydrophilic moiety. (The detergents tested, however, are not repellents [Table 1].) In contrast, attractants so far identified (6, 34) are more hydrophilic.

DISCUSSION

Chemoreceptors for negative chemotaxis. The mechanism of chemosensing for negative chemotaxis can perhaps best be explained in terms of "chemoreceptors," as was done for positive chemotaxis (3). A chemoreceptor is a sensing device that signals a change in concentration of repellent or attractant; its mechanism does not involve "sensing" of harm or benefit, but rather interaction of a specific recognition component with a specific chemical or a group of closely related chemicals. The recognition component has been identified as a protein in some chemoreceptors for positive chemotaxis (6, 8, 26), but for negative chemotaxis we have no information about its chemistry. The idea of a generalized sensitivity or a nonspecific detection mechanism (4, 5, 48) is rejected. The evidence supporting this point of view in the case of negative chemotaxis follows.

(i) There is no correlation between repulsion and harmfulness, and thus harm caused cannot be the cause of repulsion. Two strains can be equally harmed by a chemical, yet only one is repelled; this can be explained by the hypothesis that one strain has a chemoreceptor for that chemical whereas the other strain lacks it. Those harmful chemicals which are repellents are sensed at a concentration below that required to produce detectable harm, and this can be explained by the existence of chemoreceptors with thresholds for detection below the level of repellent that causes harm (Table 2).

(ii) Repellents can be classified on the basis of competition experiments. Each class would correspond to a chemoreceptor with its specific recognition component.

(iii) Repellents can be classified by mutants lacking taxis toward one or a group of closely related chemicals. Each such mutant would be missing one chemoreceptor or the ability to pass information from that receptor to the flagella.

At least nine chemoreceptors for repellents have, by these criteria, been identified, and their specificity has been described. It should be emphasized that the evidence is not equally good for each. They are: a short-chain fatty acid chemoreceptor, an aliphatic alcohol chemoreceptor, a hydrophobic amino acid chemoreceptor, an indole chemoreceptor, a chemoreceptor for aromatic compounds (with probably a distinct chemoreceptor for salicylate), separate chemoreceptors for high and for low pH (alternatively, there may not be chemoreceptors for H^+ and OH^-, and the chemotactic response may result from an effect of H^+ or OH^- on some compound of the chemotaxis machinery; in the mutant AW518 this component may be altered), a sulfide chemoreceptor, and a metallic cation chemoreceptor.

No doubt additional repellents and corresponding chemoreceptors remain to be discovered.

We agree only in part with the three chemoreceptor classes defined by the competition experiments of Tsang et al. for *Salmonella typhimurium* (50).

Behavior of individual bacteria. Bacteria in the absence of a gradient swim for a while (a "run"), then they tumble (a "twiddle" or series of twiddles), then they again run in a new random direction (12, 33). Berg and Brown (12) and Macnab and Koshland (33) have discovered that in a gradient of attractant bacteria tumble, or twiddle, less frequently if they happen to swim toward the source of attractant and (of lesser significance) more frequently if they swim away; in this way they migrate toward attractants. Tsang et al., studying *Salmonella typhimurium*, showed that the opposite is true for repellents: bacteria swimming up the gradient have an increase in the frequency of tumbling, whereas those swimming down gradient have a decrease; and in this way the bacteria escape repellents (50). Thus, attractants and repellents affect the tumbling frequency in opposite ways. Tsang et al. showed that the inhibition of tumbling caused by addition of attractant could be counteracted by addition of repellent and thereby argued that repellents and attractants use a common mechanism (50).

We have confirmed these results and have shown that the specific negative taxis mutants fail to tumble when presented with the respective repellents, for example L-leucine or indole. We will report on this and related matters in a separate communication (G. W. Ordal, R. W. Reader, E. N. Kort, W-W. Tso, S. H. Larsen, and J. Adler, manuscript in preparation). We will also report experiments in which bacteria are forced to "choose" whether or not to enter a capillary containing both attractant and repellent (J. Adler and W-W. Tso, manuscript in preparation).

Apparently one chemoreceptor can signal for either repulsion or attraction, but not both, no matter how high or low the concentration of the chemical. The signal leading to attraction must in some way act differently and oppositely from the signal leading to repulsion, but the nature of these signals is unknown. It is curious that phenol, a harmful chemical, repels *Salmonella* but attracts *E. coli*, as if a mutational difference between the strains had led to a switch in the polarity of signals.

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