

The Range of Attractant Concentrations
for Bacterial Chemotaxis
and the Threshold and
Size of Response over This Range

Weber law and related phenomena

ROBERT MESIBOV, GEORGE W. ORDAL, and JULIUS ADLER

From the Departments of Biochemistry and Genetics, College of Agricultural and Life Sciences, The University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT Attractant was added to a suspension of bacteria (the background concentration of attractant) and then these bacteria were exposed to a yet higher concentration of attractant in a capillary. Chemotaxis was measured by determining how many bacteria accumulated in the capillary. The response range for chemotaxis lies between the threshold concentration and the saturating concentration. The breadth of this range is different for attractants detected by different chemoreceptors. Attractants detected by the same chemoreceptor can have their response ranges in widely different places. Over the center of the response range (on a logarithmic scale), bacteria give similar sized responses to similar fractional increases of concentration, i.e. they respond to ratios of attractant concentration, but the response peaks at the center of the range. The size of the response is different for attractants detected by different chemoreceptors. For a detectable response, a smaller increase in attractant concentration is needed for attractants detected by some chemoreceptors than for attractants detected by others. Although the data are inadequate, it appears that the Weber law may be observed over a wide range of concentrations for some attractants but not for others. In the Appendix we aim to explain some of these results in terms of the interaction of an attractant with its chemoreceptor according to the law of mass action.

INTRODUCTION

The behavioral repertoire of bacteria includes attraction to light (phototaxis) and attraction to chemicals (chemotaxis) (for a review, see reference 26). As already appreciated by Engelmann and Pfeffer in the 19th century (26), bacterial behavior bears certain similarities to sensory phenomena in higher or-

ganisms. For phototaxis these similarities have been studied and reviewed by Clayton (9, 10). In the case of chemotaxis, we have found that chemo-reception occurs by means of chemoreceptors—specific sensing devices that are located on the surface of the bacterium and that detect attractants in the absence of transport and metabolism of those attractants (1). Each chemoreceptor is believed to contain a protein that specifically interacts with the attractant (13), but beyond this the mechanism of chemoreception in bacteria is unknown.

In the studies reported here, we have explored the following questions:

How broad is the range of concentrations over which bacteria can carry out chemotaxis toward particular attractants? How does the size of the chemotactic response vary within this "response range"? How large a difference in attractant concentration is needed for the bacteria to give a chemotactic response, and is this difference the same throughout the response range?

To answer these questions we used a method for measuring bacterial chemotaxis (2) which consists of placing a capillary tube containing a solution of attractant into a suspension of motile *Escherichia coli* bacteria and then determining the number of bacteria that have accumulated inside the capillary (the response) after a defined period of time. In the experiments reported here we varied not only the concentration of attractant in the capillary, but also the concentration of attractant in the bacterial suspension (the background concentration of attractant).

For these experiments we chose attractants which are not significantly utilized by the *E. coli* strains studied, so that the background concentration of these chemicals would not be reduced by the bacteria during the chemotaxis assay. The attractants chosen were DL- α -methylaspartate, which is detected by the aspartate chemoreceptor (1, 16), and D-galactose, D-fucose (6-deoxy-D-galactose), and L-D-glycerol- β -D-galactoside, which are all detected by the galactose chemoreceptor (1, 13, 14).

In the Appendix we attempt to explain some of the phenomena described here in terms of the interaction of an attractant with its chemoreceptor according to the law of mass action.

MATERIALS AND METHODS

Bacteria

AW405 (3), an *E. coli* K12 strain wild type for chemotaxis, has two detection systems for chemotaxis toward a number of amino acids: the aspartate and serine chemoreceptors (16). Only the aspartate chemoreceptor detects α -methylaspartate (16). In AW518, a serine taxis mutant isolated from AW405 (14), the aspartate chemoreceptor functions normally but the serine chemoreceptor is nonfunctional (14, 16). We used AW518 for studies of α -methylaspartate taxis to avoid possible complications resulting from the presence of the serine chemoreceptor, but subsequent work

has shown that the same results can be obtained with the wild-type AW405. α -Methylaspartate is not significantly oxidized by *E. coli* (16); its uptake has not been measured.

Strain 20SOK⁻, which was used for studies of taxis toward D-galactose, D-fucose, and 1-D-glycerol- β -D-galactoside, is also an *E. coli* K12 derivative and was first described by G. Buttin (7). It is defective in the uptake of D-galactose (1, 7, 19) to the extent of a 99.5% block at 5×10^{-5} M or below (1, 7) owing to the absence of both galactose permease and methylgalactoside permease (19). The methylgalactoside permease is required for the transport of D-fucose and 1-D-glycerol- β -D-galactoside as well (5, 19). In addition to these defects, 20SOK⁻ also lacks galactokinase and β -galactosidase (7) and is therefore unable to metabolize D-galactose and 1-D-glycerol- β -D-galactoside, respectively. D-Fucose is not significantly metabolized by *E. coli* (1 and references cited there).

Chemotaxis Assay

Chemotaxis was measured by the method described previously (2). Bacteria were grown in glycerol-salts medium (2) supplemented with the necessary amino acids. For experiments with strain AW518, the bacterial concentration in the chemotaxis assay was 6×10^7 /ml, while with 20SOK⁻ it was 3×10^7 /ml. The chemotaxis medium contained 10^{-2} M potassium phosphate, pH 7.0, and 10^{-4} M ethylenediaminetetraacetate. For 20SOK⁻, 10^{-3} M $(\text{NH}_4)_2\text{SO}_4$ was added since this was found to stimulate D-galactose taxis about 30% in this strain. Experimental points were obtained in duplicate or triplicate, and means are reported in all cases. The standard deviation for replicate determinations in a single assay is 9% (2).

Chemicals

DL- α -Methylaspartate, D-galactose (substantially glucose free), and D-fucose were obtained from Sigma Chemical Co., St. Louis, Mo. The D-fucose was purified before use (1). 1-D-glycerol- β -D-galactoside was a gift from Dr. Winfried Boos.

RESULTS

Sensitivity Curves

In our first series of experiments, the concentration of attractant in the capillary was always a certain multiple of the background concentration, while the background concentration was varied over a wide range. In this way gradients were set up in which attractant concentrations at the upper and lower limits were always in the same ratio, and we measured the size of the chemotactic response in each of a series of concentration intervals. A plot of the response vs. the geometric mean (square root of the product) of the limiting concentrations is called a sensitivity curve.

A sensitivity curve for α -methylaspartate taxis is shown in Fig. 1. The capillary concentration was always 3.16 times the background concentration (to give points equally spaced on a logarithmic abscissa), and the background

concentration was varied in jumps of 3.16-fold to give nonoverlapping concentration intervals. It can be seen that taxis toward α -methylaspartate occurred between 3×10^{-7} M and about 10^{-1} M with a maximum response at about 10^{-4} M. (Note that for sensitivity curves the geometric mean of the two limiting concentrations, in the capillary and in the bacterial suspension, is plotted on a logarithmic scale on the abscissa.)

To obtain sensitivity curves for the sugars, it was necessary to go to 100-fold concentration differences between the capillary and the bacterial suspension because the responses to 3-fold or even 30-fold differences were too close to the blank values (the accumulations in the absence of attractant) to be accurately measured. As can be seen in Fig. 2, taxis occurred between 10^{-9} M and 3×10^{-5} M for galactose, between 10^{-7} M and 10^{-4} M for glycerol galactoside, and between 10^{-5} M and 10^{-2} M for fucose. The maximum responses to the three sugars occurred at the following concentrations:

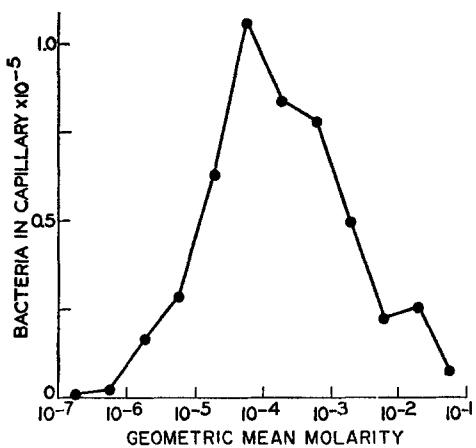


FIGURE 1

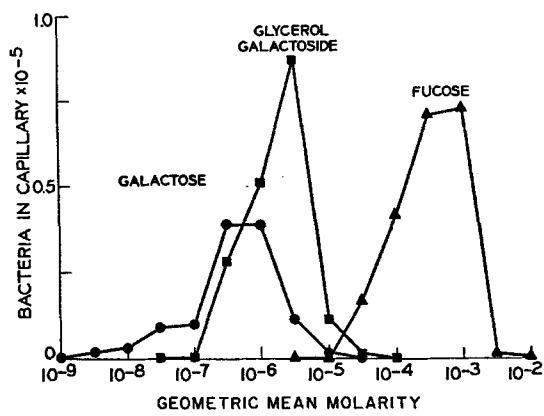


FIGURE 2

FIGURE 1. Sensitivity curve for taxis towards α -methylaspartate. The concentration of attractant in the capillary was 3.16 times higher than the concentration of attractant in the bacterial suspension; the geometric mean (square root of the product) of these two limiting concentrations is plotted on the abscissa. The number of bacteria (*E. coli* strain AW518) accumulating in the capillary after 1 h at 30°C is plotted on the ordinate. The number of bacteria accumulating in the absence of attractant (4,700) has been subtracted from each point.

FIGURE 2. Sensitivity curves for taxis toward galactose (\bullet — \bullet), glycerol galactoside (\blacksquare — \blacksquare), and fucose (\blacktriangle — \blacktriangle). The concentration of attractant in the capillary was 100 times the concentration in the bacterial suspension; the geometric mean (square root of the product) of these two limiting concentrations is plotted on the abscissa. The number of bacteria (*E. coli* strain 20SOK $^{-}$) accumulating in the capillary after 1 h at 30°C is plotted on the ordinate. The number of bacteria accumulating in the absence of attractant (8,000 for the experiment with galactose, 16,200 for that with glycerol galactoside, and 17,400 for that with fucose) has been subtracted from each point.

galactose between 3×10^{-7} and 10^{-6} M, glycerol galactoside 3×10^{-6} M, and fucose between 3×10^{-4} and 10^{-3} M. (All values on the abscissa are geometric means of the limiting concentrations in the capillary and in the bacterial suspension.)

Concentration-Response Curves

In our second series of experiments, the background concentration was held constant while the concentration of attractant in the capillary was varied over a wide range (all values being greater than the background concentration). A plot of response vs. logarithm of the capillary concentration is called a concentration-response curve. Concentration-response curves were determined at a variety of background concentrations for each attractant.

Concentration-response curves for taxis toward α -methylaspartate at five different background concentrations are shown in Fig. 3.

We define "threshold concentration" as that concentration of attractant in the capillary which gives a just detectable increase over the blank value. "Just detectable" is taken to mean two standard deviations above the mean blank value. 13 determinations of the blank values on 5 different days (for the strain and conditions used in Fig. 3) ranged between 3,500 and 7,520 bacteria; the mean was 4,700 and the SD was 1,000. The threshold for α -methylaspartate was 5×10^{-7} M when no α -methylaspartate was initially present in the bacterial suspension (curve A in Fig. 3). With 10^{-5} , 10^{-4} , 10^{-3} , or 10^{-2} M α -methylaspartate in the suspension, the threshold shifted to 0.9×10^{-6} , 1.0×10^{-4} , 1.7×10^{-3} , and 1.3×10^{-2} M, respectively (curves B, C, D, and E in Fig. 3). Considering the error of the assay, we regard these four values as experimentally indistinguishable from slightly above 1.0 times the background concentration.

We define "saturating concentration" as the highest background concentration at which bacteria fail to give a just-detectable increase over the blank value to yet higher concentrations in the capillary. From Fig. 3 saturation can be seen to be just above 10^{-2} M (curve E). At background concentrations of 0, 10^{-5} , or 10^{-4} M, the slopes of the rising portions of the curves (A, B, and C, respectively) were similar, while the slope was reduced at a background concentration of 10^{-3} M (curve D) and reduced further at 10^{-2} M (curve E).

Sensitivity curves can also be used to estimate thresholds (when there is insignificant attractant in the background) and saturating concentrations. For α -methylaspartate (Fig. 1) these values would be about 3×10^{-7} M and about 10^{-1} M, respectively, in good agreement with those obtained from concentration-response curves.

Concentration-response curves for galactose taxis at four different background concentrations are shown in Fig. 4.

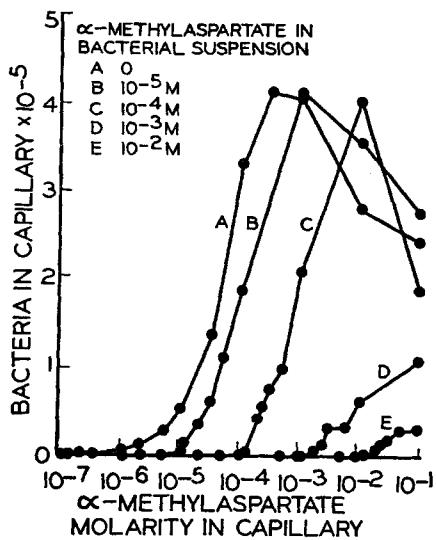


FIGURE 3

FIGURE 3. Concentration-response curves for taxis toward α -methylaspartate by *E. coli* strain AW518. The concentration of attractant in the capillary is plotted logarithmically on the abscissa. The number of bacteria accumulating in the capillary after 45 min at 30°C is plotted on the ordinate. Each capillary was placed in a bacterial suspension containing 0, 10^{-5} , 10^{-4} , 10^{-3} , or 10^{-2} M α -methylaspartate (curves A, B, C, D, or E, respectively). Data from several experiments have been combined in this figure. The number of bacteria accumulating in the absence of attractant (4,500) has been subtracted from each point.

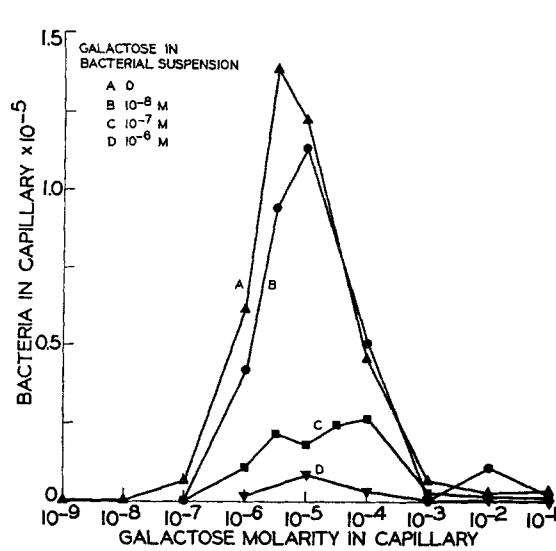


FIGURE 4

FIGURE 4. Concentration-response curves for taxis toward galactose by *E. coli* strain 20SOK-. The concentration of attractant in the capillary is plotted logarithmically on the abscissa. The number of bacteria accumulating in the capillary after 1 h at 30°C is plotted on the ordinate. Each capillary was placed in a bacterial suspension containing 0, 10^{-8} , 10^{-7} , or 10^{-6} M D -galactose (curves A, B, C, or D, respectively). The number of bacteria accumulating in the absence of attractant (10,300) has been subtracted from each point. A, ▲—▲; B, ●—●; C, ■—■; D, ▽—▽.

To determine the threshold concentrations, a just-detectable increase over the blank value was again taken to be 2 SD above the mean blank value. 20 determinations of the blank value in one experiment (for the strain and conditions used in Fig. 4) ranged from 4,500 to 12,300 bacteria; the mean was 8,800 and the SD was 1,800. The threshold for galactose was 4×10^{-8} M when no galactose was present in the bacterial suspension (mean of eight experiments including curve A in Fig. 4). With 10^{-8} or 10^{-7} M galactose in the suspension, the threshold shifted to 1.2×10^{-7} and 3×10^{-7} M, respectively (curves B and C in Fig. 4).

From the sensitivity curve (Fig. 2), threshold (for a background concentration that can be regarded as insignificant) can be estimated to be about 10^{-8}

M (capillary concentration) for galactose, in fair agreement with the 4×10^{-8} M above.

At a background concentration of 10^{-6} M (curve D of Fig. 4), the response to galactose was almost eliminated, in agreement with the absence of a response at 3×10^{-6} M background concentration in the sensitivity curve (Fig. 2). The saturating concentration for galactose is thus about 3×10^{-6} M.

For glycerol galactoside and fucose, based on sensitivity curves (Fig. 2), thresholds can be estimated at 10^{-6} and 10^{-4} M, respectively, and saturation at background concentrations of 10^{-6} M and 10^{-3} M, respectively.

Concentration-response curves for glycerol galactoside and fucose with no attractant in the background are shown for comparison with galactose in Fig. 5. The threshold for glycerol galactoside is at 4×10^{-7} M, and for fucose it is at 7×10^{-6} M (mean of three experiments), in rough agreement with those reported above from sensitivity curves. Concentration-response curves show a peak and, at higher concentrations, a decline to the blank value. The peak concentrations for galactose, glycerol galactoside, and fucose (Fig. 5) occurred at 3×10^{-6} M (5×10^{-6} M for mean of four experiments), 2×10^{-5} , and 10^{-2} M (5×10^{-3} M for mean of three experiments). The peak and declining

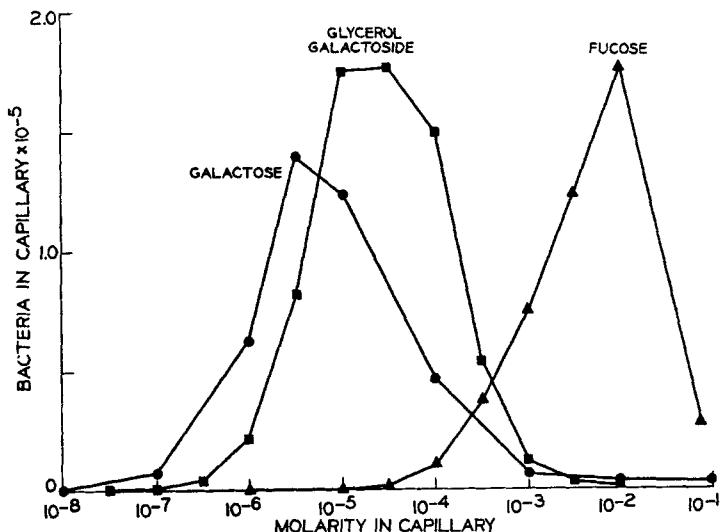


FIGURE 5. Concentration-response curves for taxis toward galactose (●—●), glycerol galactoside (■—■), and fucose (▲—▲) by *E. coli* strain 20SOK⁻. The concentration of attractant in the capillary is plotted logarithmically on the abscissa. The number of bacteria accumulating in the capillary after 1 h at 30°C is plotted on the ordinate. The curve for galactose is repeated from Fig. 4. The three curves were obtained on separate days, but the results for glycerol galactoside and fucose were normalized to that of galactose by including 10^{-6} M galactose in the experiments. The number of bacteria accumulating in the absence of attractant (10,300, 25,000, and 22,000, respectively) has been subtracted from each point.

portions of the concentration-response curve are regarded as being due to saturation of the chemoreceptor; this is explained in more detail in the Appendix.

The similarity between the sensitivity curves (Fig. 2) and the concentration-response curves (Fig. 5) for the sugars results from the fact that it was necessary to use 100-fold intervals for the sensitivity curves, and the entire response range for the sugars is only about that large (see Discussion).

DISCUSSION

The Response Range for Chemotaxis

A response range for chemotaxis may be defined as the range of concentrations bounded by the threshold concentration and the saturating concentration; thus gradients (of sufficient steepness) which include concentrations of attractant greater than threshold or less than saturation can elicit a chemo-tactic response. The response range for taxis toward α -methylaspartate extends from a threshold concentration of $3-5 \times 10^{-7}$ M to a saturating concentration of about 10^{-1} M (Figs. 1 and 3). For galactose taxis the response range extends from a threshold of $1-4 \times 10^{-8}$ M to saturation at about 3×10^{-6} M (Figs. 2 and 4); for glycerol galactoside from $4-10 \times 10^{-7}$ M (Figs. 2 and 5) to 10^{-5} M (Fig. 2); and for fucose from $7-10 \times 10^{-5}$ M (Figs. 2 and 5) to 10^{-3} M (Fig. 2). It is clear that the breadth of the response range is different for attractants detected by different chemoreceptors: for the amino acid it is relatively broad—five to six decades, while for the three sugars detected by the galactose chemoreceptor it appears about equally narrow—between one and two decades. A further conclusion is that the location of the response range on the concentration scale (the abscissa) can vary, even among attractants detected by a single chemoreceptor—for example galactose, glycerol galactoside, and fucose (see Figs. 2 and 5).

An interpretation of the response range, threshold, and saturation in terms of binding between chemoreceptor and attractant is considered in the Appendix.

The Size of the Chemo-tactic Response

Fig. 1 shows that for a given relative increase in α -methylaspartate concentration (3.16-fold) the size of the response was greatest at around 10^{-4} M. However, although the sensitivity curve shows a maximum, the response does not fall off sharply on either side of the peak. Thus a 1,000-fold variation in the concentration of α -methylaspartate in the capillary (10^{-5} to 10^{-2} M) is accompanied by less than a fivefold variation in the size of the response (Fig. 1). For serine taxis in *Salmonella*, Dahlquist et al. (11), using preformed exponential gradients, also showed that bacteria give similar sized responses to

similar fractional increases of concentration over a broad range, and that these responses peak at a certain concentration (see their Fig. 8).

Sensitivity curves for the sugars also showed maxima (Fig. 2) with the peak response occurring at a different concentration in each case. In the case of the sugars, however, the response falls off more sharply on either side of the peak than in the case of the amino acid.

It is interesting that for different chemoreceptors the height of the sensitivity curve is different: compare Figs. 1 and 2 and take into account that different sized concentration intervals were used, or compare Fig. 2 with Fig. 7 B of the Appendix for equal-sized concentration intervals. Also the maximum response in the concentration-response curve is different for the amino acid and for the sugars: compare Fig. 5 and curve A of Fig. 3.

Sensitivity curves, concentration-response curves, and maximum response size are interpreted in the Appendix.

Smallest Increase in Concentration of Attractant Necessary for a Response

Pfeffer, who studied chemotaxis microscopically using a set-up similar to ours, reported (18, 26) in 1888 that bacteria (*Bacterium termo*) suspended in 0.01% meat extract were attracted to 0.05% meat extract, but were not attracted to weaker solutions. When 0.1% meat extract was present in the suspension, the threshold for chemotaxis was raised to 0.5%; with 1% meat extract in the suspension, the threshold was 5%. Pfeffer pointed out that bacterial chemotaxis thus appears to obey the Weber law, according to which the least perceptible change in the intensity of a stimulus (e.g., the concentration of meat extract) is a constant multiple of the stimulus intensity already present. Pfeffer thus found that over a 100-fold concentration range the Weber law constant was 5; i.e., a fivefold increase in stimulus intensity was required to elicit a detectable chemotactic response. Weber law behavior has also been observed in phototaxis by certain photosynthetic bacteria (8, 10, 20, 22). In these instances the Weber law constant was about 0.05.

Does chemotaxis in *E. coli* obey the Weber law? As reported, thresholds for α -methylaspartate taxis were found to be 0.9×10^{-5} , 1.0×10^{-4} , 1.7×10^{-3} , and 1.3×10^{-2} M when the background concentrations were 10^{-6} , 10^{-4} , 10^{-3} , and 10^{-2} M, respectively (Fig. 3). Assuming no systematic variation in these relative increases of concentration needed for a detectable response, these data are consistent with Weber law behavior with a Weber law constant of 1.2 (average of 0.9, 1.0, 1.7, and 1.3). However, this finding is inconclusive due to the error of the assay. (Since the putative Weber law constant of 1.2 is close to 1.0, it is also possible that threshold at a particular background concentration equals that concentration *plus* a constant rather than *times* a constant. For example, the above thresholds might really be 1.2×10^{-5} , 1.02×10^{-4} ,

1.002×10^{-3} , and 1.0002×10^{-2} M at background concentrations of 10^{-5} , 10^{-4} , 10^{-3} , and 10^{-2} M, respectively.)

A 12-fold increase in attractant concentration in the capillary was needed for a just-detectable response to galactose at a background concentration of 10^{-8} M, while a threefold increase sufficed at a background concentration of 10^{-7} M (Fig. 4). Thus the Weber law does not appear to be obeyed in the case of galactose taxis.

The range of concentrations over which Weber law behavior might be expected is discussed in the Appendix.

In brief, the results presented in this paper demonstrate that bacteria can detect remarkably small changes in attractant concentration under certain conditions. Further, there is no single, fixed threshold for chemotaxis; bacteria can sense increases over the background concentration over a wide range of background concentrations. Thus bacteria that find themselves in a gradient of attractant can migrate from the lowest detectable concentration to the concentration that saturates the cell's chemotactic machinery.

Similarities to Sensory Physiology in Higher Organisms

Finally, we wish to point out a few similarities between results reported here for bacterial chemotaxis and data on sensory phenomena in higher organisms.

(a) The concentration-response curves for chemotaxis in bacteria are S-shaped up to the peak concentration (Fig. 5 and curve A of Fig. 3). Similarly in higher organisms a plot of response size vs. the logarithm of stimulus strength gives S-shaped curves extending from threshold to saturation. An example from chemoreception is the response of the male *Bombyx* moth to the female sex-attractant, bombykol, whether measured behaviorally (21) or electrophysiologically (electroantennogram, receptor potential, or frequency of nerve impulse) (4). Furthermore, the stimulus-response curves for various bombykol isomers are of the same shape as the bombykol curve, but are shifted to higher concentrations (4). Also, the response range is broader for some insect chemoreceptors than others (4).

(b) Fechner predicted (see references 23, 24) that a constant *relative* increase in stimulus intensity produces a constant *absolute* increase in the size of the sensation (often extrapolated to response), i.e., that organisms respond to ratios of stimulus intensity. For bacterial chemotaxis this has been found, roughly, to be the case (see the middle portion of Fig. 1, and also data of Dahlquist et al. [11]), but only over a narrow range of attractant concentration. In animals, too, the Fechner relation often holds, approximately, over a part of the response range. Stevens (23, 24), however, has emphasized that in animal physiology the Fechner relation is not applicable over the entire response range. He has pointed out that were the relation to apply, the curve of

response size vs. logarithm of stimulus strength should be linear in place of the upturned or S-shaped curves ordinarily obtained.

(c) The Weber law may be valid in certain cases of bacterial chemotaxis (see Fig. 3 and Pfeffer, references 18, 26). This law has frequently been found applicable in sensory reception in higher organisms (25), but only over a limited part of the response range. The part where it applies, as well as the Weber law constant, differ widely for various sensory processes. For examples, see Tamiya (25) and references cited there, and—for taste—see Pfaffmann et al. (17).

(d) As in the case of bacterial chemotaxis, so for sensory reception in higher organisms there is no single, fixed threshold for a stimulus. Instead, organisms adapt to a background stimulus, and are then able to respond to a stronger stimulus, until the background intensity is so high that the receptor becomes saturated. For examples of adaptation in animals, see references 6, 12, 17, and 27. Recently, Macnab and Koshland (15) have shown that a sudden change in attractant concentration elicits a change in tumbling frequency of bacteria (the behavioral correlate of chemotaxis); this change persists for some seconds or minutes, then tumbling returns to the normal frequency, i.e., the bacteria adapt to the new concentration.

It is not known whether these similarities between bacterial chemotaxis and sensory phenomena in higher organisms are accidental, or reflect some fundamental, universal laws or mechanisms of sensory reception.

REFERENCES

1. ADLER, J. 1969. Chemoreceptors in bacteria. *Science (Wash. D. C.)*. **166**:1588.
2. ADLER, J. 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. *J. Gen. Microbiol.* **74**:77.
3. ARMSTRONG, J. B., J. ADLER, and M. M. DAHL. 1967. Nonchemotactic mutants of *Escherichia coli*. *J. Bacteriol.* **93**:390.
4. BOECKE, J., K. E. KAISLING, and D. SCHNEIDER. 1965. Insect olfactory receptors. *Cold Spring Harbor Symp. Quant. Biol.* **30**:263.
5. BOOS, W. 1969. The galactose binding protein and its relationship to the β -methylgalactoside permease from *Escherichia coli*. *Eur. J. Biochem.* **10**:66.
6. BOYNTON, R. M., and D. N. WHITTEN. 1970. Visual adaptation in monkey cones. Recordings of late receptor potentials. *Science (Wash. D. C.)*. **170**:1423.
7. BUTTIN, G. 1963. Mécanismes régulateurs dans la biosynthèse des enzymes du métabolisme du galactose chez *Escherichia coli* K12. I. La biosynthèse induite de la galactokinase et l'induction simultanée de la séquence enzymatique. *J. Mol. Biol.* **7**:164.
8. CLAYTON, R. K. 1953. Studies in the phototaxis of *Rhodospirillum rubrum*. I. Action spectrum, growth in green light, and Weber law adherence. *Arch. Mikrobiol.* **19**:107.
9. CLAYTON, R. K. 1953. Studies in the phototaxis of *Rhodospirillum rubrum*. III. Quantitative relations between stimulus and response. *Arch. Mikrobiol.* **19**:141.
10. CLAYTON, R. K. 1959. Phototaxis of purple bacteria. In *Encyclopedia of Plant Physiology*. W. Ruhland, editor, Springer-Verlag KG., Berlin. **17**(1):371.
11. DAHLQUIST, F. W., P. LOVELY, and D. E. KOSHLAND, JR. 1972. Quantitative analysis of bacterial migration in chemotaxis. *Nat. New Biol.* **236**:120.

12. DOWLING, J. E., and H. RIPPS. 1971. S-potentials in the skate retina. Intracellular recordings during light and dark adaptation. *J. Gen. Physiol.* **58**:163.
13. HAZELBAUER, G. L., and J. ADLER. 1971. Role of the galactose binding protein in chemotaxis of *Escherichia coli* toward galactose. *Nat. New Biol.* **230**:101.
14. HAZELBAUER, G. L., R. E. MESIBOV, and J. ADLER. 1969. *Escherichia coli* mutants defective in chemotaxis toward specific chemicals. *Proc. Natl. Acad. Sci. U.S.A.* **64**:1300.
15. MACNAB, R. M., and D. E. KOSHLAND, JR. 1972. The gradient-sensing mechanism in bacterial chemotaxis. *Proc. Natl. Acad. Sci. U.S.A.* **69**:2509.
16. MESIBOV, R., and J. ADLER. Chemotaxis toward amino acids in *Escherichia coli*. *J. Bacteriol.* **112**:315.
17. PFAFFMANN, C., L. M. BARTOSHUK, and D. H. McBURNEY. 1971. Taste biophysics. In *Handbook of Sensory Physiology*. L. M. Beidler, editor. Springer-Verlag New York Inc., New York. **4(2)**:75.
18. PFEFFER, W. 1888. Über chemotaktische Bewegungen von Bakterien, Flagellaten, und Volvocineen. Untersuchungen aus dem Botanischen Institut in Tübingen. **2**:582.
19. ROTMAN, B., A. K. GANESAN, and R. GUZMAN. 1968. Transport systems for galactose and galactosides in *Escherichia coli*. II. Substrate and inducer specificity. *J. Mol. Biol.* **36**:247.
20. SCHLEGEL, H.-G. 1956. Vergleichende Untersuchungen über die Lichtempfindlichkeit einiger Purpurbakterien. *Arch. Protistenkd.* **101**:69.
21. SCHNEIDER, D. 1963. Electrophysiological investigation of insect olfaction. In *Olfaction and Taste*. Y. Zotterman, editor. The Macmillan Company, New York. **1**:85.
22. SCHRAMMECK, J. 1934. Untersuchung über die Phototaxis der Purpurbakterien. *Beitr. Biol. Pflanz.* **22**:315.
23. STEVENS, S. S. 1970. Neural events and the psychophysical law. *Science (Wash. D. C.)*. **170**:1043.
24. STEVENS, S. S. 1971. Sensory power functions and neural events. In *Handbook of Sensory Physiology*. W. R. Loewenstein, editor. Springer-Verlag New York Inc., New York. **1**:226.
25. TAMIYA, H. 1952. A new interpretation of the Weber law and the Weiss law. Some additions to our knowledge of comparative physiology of irritability. *Cytologia (Tokyo)*. **17**:243.
26. WEIBULL, C. 1960. Movement. In *The Bacteria, A Treatise on Structure and Function*. I. C. Gunsalus and Y. Stanier, editors. Academic Press Inc., New York. **1**:153.
27. WERBLIN, F. S. 1971. Adaptation in a vertebrate retina: intracellular recording in *Necturus*. *J. Neurophysiol.* **34**:228.

APPENDIX

GEORGE W. ORDAL and JULIUS ADLER

The purpose of this Appendix is to report an attempt to understand the phenomena described in the text in terms of the law of mass action as applied to interaction between a chemoreceptor and its substrate. Previous (and in certain ways similar) efforts to understand chemoreception in terms of this law have already been made by Beidler (2 A-4 A) and Kaissling (7 A). For combination of a protein (the recognition component or binding protein of a chemoreceptor) with its substrate (the attractant) according to the equilibrium $P + A \rightleftharpoons PA$, the law of mass action gives the well-known equation

$$K_D = \frac{[P][A]}{[PA]}, \quad (1)$$

where K_D is the dissociation constant of the protein attractant complex; A , the attract-

ant; P , the free-binding protein; and PA , the complex between binding protein and attractant. Rearrangement of Eq. 1 gives:

$$\text{Fraction of protein in complex} = \frac{[PA]}{[P_t]} = \frac{[A]}{K_D + [A]}, \quad (2)$$

where P_t is the total binding protein, and is equal to the sum of $P + PA$.

Eq. 2 can be plotted as shown in Fig. 6 A, and its first derivative as shown in Fig. 6 B. According to our theory, concentration-response curves are similar to Fig. 6 A, and sensitivity curves are approximations to Fig. 6 B. (Note that the intervals are vanishingly small for Fig. 6 A but 3.16- or 100-fold for the experimental sensitivity curves.)

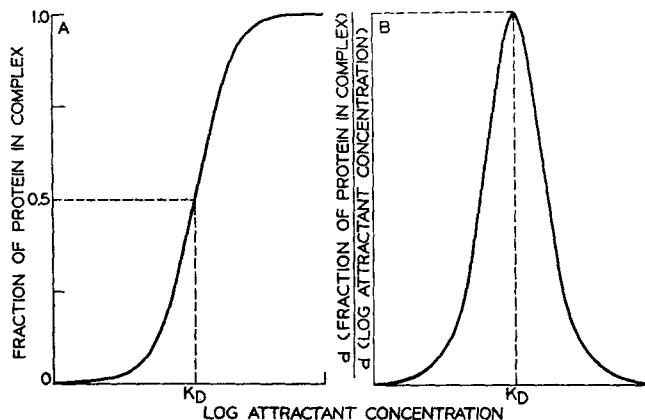


FIGURE 6 A. A plot of Eq. 2. The concentration of attractant that results in complexing of half the binding protein is the dissociation constant, K_D .

FIGURE 6 B. A plot of the first derivative of Eq. 2. The concentration of attractant that gives the maximum in the curve is the K_D .

To apply Eq. 2 to our chemotaxis experiments, three assumptions will be made:

(a) Each chemoreceptor has a binding protein that forms reversible complexes with the attractants detected by that chemoreceptor, according to Eq. 1 above. For the galactose chemoreceptor this recognition component has been identified (13) as the galactose-binding protein (5, 1 A, 6 A). A binding protein has not yet been identified for the aspartate chemoreceptor.

(b) Loss or gain of attractant from the binding protein is the crucial element in initiating a chemotactic event. (The bacteria swim in straight lines, interrupted by spontaneous twiddling or tumbling, which is followed again by straight lines in new, randomly chosen directions. The chemotactic event consists of an inhibition of the twiddling or tumbling whenever the bacterium happens to swim towards the source of attractant [15, 5 A], and also an increased frequency of the twiddling or tumbling if the bacterium happens to swim away from the attractant [15]. The mechanisms by which loss or gain of attractant might bring about a chemotactic event are unknown.)

(c) The size of the chemotactic response (i.e., the number of bacteria accumulating in the capillary in the chemotaxis assay) is proportional to the difference in the fraction of complexed binding proteins at the attractant concentration initially present in the bacterial suspension and that in the capillary.

(Of course bacteria do not sense that entire concentration difference at any one time since their dimensions are small compared to the distance over which the gradient extends, and numerous chemotactic events are needed to bring a bacterium into the capillary. Probably the number of chemotactic events is proportional to the difference in fraction of binding protein complexed at the two concentrations, and the number of bacteria accumulating in the capillary is proportional to the number of chemotactic events. For convenience, we ignore the intermediate happenings and consider only the final state—the number of bacteria that have accumulated in the capillary.)

This difference in fraction of protein complexed is

$$\Delta \frac{[PA]}{[P_t]} = \frac{[A_2]}{K_D + [A_2]} - \frac{[A_1]}{K_D + [A_1]}, \quad (3)$$

where A_1 is attractant initially present in the bacterial suspension and A_2 that in the capillary.

Sensitivity Curves

Eq. 3 may be used to deduce a theoretical sensitivity curve, for example the one obtained experimentally for α -methylaspartate (Fig. 1). The peak of a sensitivity curve should occur at the K_D , since $\Delta[PA]/[P_t]$ reaches a maximum when the interval includes the K_D . The K_D used in calculating the theoretical sensitivity curve was determined as follows. First the K_D was estimated by finding (by eye) the center of symmetry of the experimental curve (Fig. 1). Then the theoretical sensitivity curve was calculated employing Eq. 3 for 3.16-fold concentration intervals (used in Fig. 1). Then by comparison of the theoretical with the experimental curves, the K_D was reestimated to reduce disagreement. After such successive approximations, a K_D of 1.3×10^{-4} M was found. Finally, a theoretical sensitivity curve was again calculated using Eq. 3, and its vertical scale was adjusted to give a good fit with the experimental curve. The result is shown in Fig. 7 A.

The reasonable agreement between the experimental and theoretical curves of Fig. 7 A makes it plausible that a single species of binding protein, having a K_D in the neighborhood of 1.3×10^{-4} M, participates in taxis toward α -methylaspartate. If there were several species, each having a different dissociation constant, there should be several peaks in the sensitivity curve, unless the different dissociation constants were close to each other. Discrepancies between the predicted and observed sensitivity curves may be due in part to error in the assay, or inexactness of assumption (c).

Using Eq. 3 and the procedure described above, we have calculated theoretical sensitivity curves for galactose, glycerol galactoside, and fucose and have superimposed them on the experimentally obtained curves of Fig. 2. The results are shown

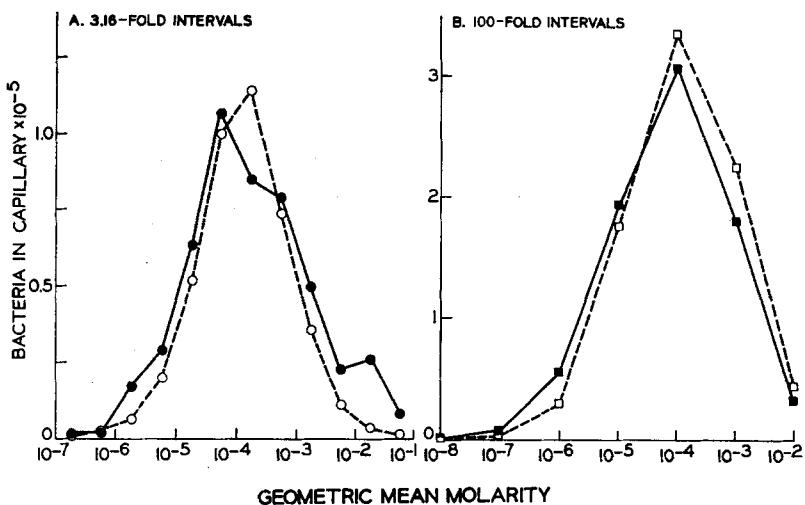


FIGURE 7. Theoretical sensitivity curves have been fitted to the experimental curves for α -methylaspartate. Abscissa and ordinate as in Fig. 1. Fig. 7 A is for 3.16-fold concentration intervals. ●—●, experimental curve for α -methylaspartate taken from Fig. 1. ○—○, theoretical curve; the plotted values were calculated from Eq. 3, see text. Fig. 7 B is for 100-fold concentration intervals. ■—■, experimental curve; except for the difference in concentration interval, the experimental procedure was the same as used for Fig. 1. □—□, theoretical curve, as above.

in Fig. 8. The experimental curves are much narrower than the theoretical curves. It will be recalled that for the sugars it was necessary to go to 100-fold concentration intervals, while threefold intervals sufficed for the amino acid. These discrepancies are not due to the use of 100-fold concentration intervals per se, since no such discrepancies occurred with α -methylaspartate when 100-fold intervals were used (Fig. 7 B). Rather, the discrepancies may be explained in the following way.

It is inherent in assumption *c* (above) that no matter how low or high the concentration of attractant in the gradient, or no matter how shallow the gradient, the accumulation of bacteria in the capillary is still proportional to the change in fraction of binding protein complexed at the two extremes of concentration. It was thus assumed that all portions of the gradient are effective in recruiting bacteria. This assumption appears to be wrong for the sugars. For gradients outside the response range (either for low concentrations near the edge of the bacterial suspension or for high concentrations near the capillary mouth) and for shallow gradients, bacteria carry out taxis less efficiently than predicted. Hence bacteria are recruited only from a narrow part of the gradient. For smaller than 100-fold concentration intervals (30-fold or less) the gradients are too shallow to be effective.

The reason for this low efficiency of taxis outside the response range or in shallow gradients may be that there exists a minimal change in fraction of binding protein that has to change state within a certain minimal period of time in order to be effective. In other words, if the fraction of binding protein changing occupancy over a certain distance within a certain period of time is too small, then the chance

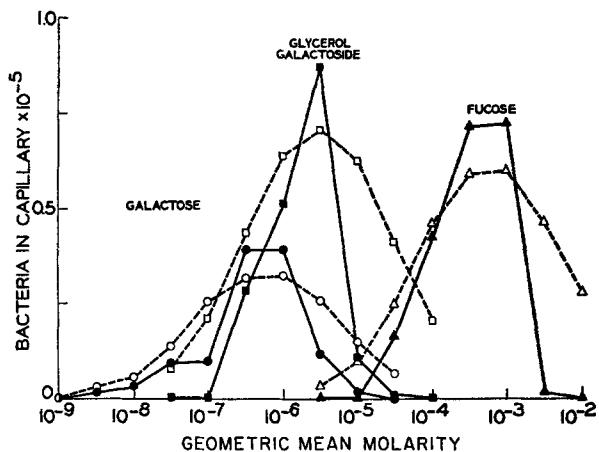


FIGURE 8. Theoretical sensitivity curves (dashed lines) have been fitted to the experimental curves (solid lines) for galactose, glycerol galactoside, and fucose. Experimental data for galactose (\bullet — \bullet), glycerol galactoside (\blacksquare — \blacksquare), and fucose (\blacktriangle — \blacktriangle) were taken from Fig. 2. Theoretical values for galactose (\circ — \circ), glycerol galactoside (\square — \square), and fucose (Δ — Δ) were calculated from Eq. 3; see text. 100-fold concentration intervals were used. Abscissa and ordinate as in Fig. 2.

for a chemotactic event is less than expected by the theory. For further discussion see the section on Threshold below.

If we nevertheless assume that the peak response in the sensitivity curves for the sugars occurs at the K_D , then the K_D 's for galactose, glycerol galactoside, and fucose are 6×10^{-7} , 3×10^{-6} , and 6×10^{-4} M, respectively (Fig. 8). (In the case of galactose and fucose, the peak of the experimental curves was assumed to lie midway between the highest points, which were experimentally indistinguishable.)

How do the dissociation constants deduced for α -methylaspartate and for galactose and its analogs compare with known values? The dissociation constant for a recognition component- α -methylaspartate complex has not yet been experimentally determined, since a binding protein has not yet been identified for this chemo-receptor. The galactose-binding protein, the recognition component of the galactose chemoreceptor, is known to have two dissociation constants for galactose, 10^{-7} M and 10^{-5} M (6 A). The former value is in reasonable agreement with 6×10^{-7} M, deduced above as the K_D for galactose in chemotaxis. The dissociation constant(s) for the complex between glycerol galactoside and the galactose-binding protein is not accurately known, but it is probably 10-fold higher than for galactose, since a fluorescence increase resulting from complex formation between protein and sugar occurs half maximally at 10^{-6} M for galactose and at 10^{-5} M for glycerol galactoside (6 A). Our result would be in agreement with this. The dissociation constant for the complex between fucose and the galactose-binding protein has not been directly determined, but it is clear from fluorescence studies that the value has to be in the order of 10^{-4} M (personal communication from Dr. Winfried Boos). This would also be in agreement with our result.

Concentration-Response Curves

One can employ Eq. 3 and the same assumptions used before to predict the shape of concentration-response curves at any background concentration and to predict K_D 's from these curves.

Fig. 6 A shows a theoretical concentration-response curve when no attractant is present initially in the bacterial suspension. The rising portion of the experimental curves (Fig. 5 and curve A of Fig. 3) resembles the theoretical curve (Fig. 6 A). However, the theoretical curve reaches a plateau, whereas the experimental curves form peaks. A likely reason for this difference involves saturation of the chemoreceptors: at high concentrations of attractant, chemoreceptors of the bacteria just outside of the mouth of the capillary are beginning to be saturated with the attractant that has diffused there, and the bacteria are hence less able to detect the yet higher concentrations in the capillary. (*Outside* the capillary a cloud of bacteria still forms.) The falling portion of the curve is thus regarded as resulting from approaching the saturation of the chemoreceptors.

Whenever the peak concentration occurs not too far from the saturating concentration (which is the case for the sugars), one can deduce the K_D from a concentration-response curve as that concentration that gives 50% of the maximum response when no attractant is present initially in the bacterial suspension. (Substitution of $A_1 = 0$ and $A_2 = K_D$ into Eq. 3 gives $\Delta[PA]/[P_i] = 0.5$. See also Fig. 6 A.) Accordingly, experiments including those shown in Fig. 5 and curve A of Fig. 3, give the following values for K_D 's: for α -methylaspartate, 7×10^{-6} M (mean of three experiments); for galactose 10^{-6} M (mean of four experiments); for glycerol galactoside 3×10^{-6} M; and for fucose 6×10^{-4} M (mean of three experiments). Comparison with values based on sensitivity curves (see above) indicates reasonable agreement.

As stated, Beidler developed a theory of taste-receptor stimulation that is similar to ours (2 A-4 A) and from concentration-response curves (electrophysiological responses of rat taste nerve to stimulation of tongue with varying concentrations of salts) he has also predicted equilibrium constants.

Threshold

In terms of the present theory, a certain minimum change in fraction of binding protein complexed must occur in order for a response to be detectable, and that occurs at the threshold concentration.

What is the change in fraction of binding protein complexed at threshold? We reported in the text that the threshold for galactose is 4×10^{-8} M, 1.2×10^{-7} M, or 3×10^{-7} M, when the background concentration is zero, 10^{-8} M, or 10^{-7} M, respectively (from Fig. 4). If the K_D is 6×10^{-7} M, then, respectively, a 6, 15, and 19% change in fraction of the binding protein complexed must occur to initiate a detectable response. For glycerol galactoside and fucose we reported thresholds of 4×10^{-7} M and 7×10^{-6} M, respectively, when the background concentration is zero (from Fig. 5). If the K_D 's are 3×10^{-6} M and 6×10^{-4} M, then, respectively,

a 12 and 10 % change in fraction of the binding protein complexed needs to occur to initiate a detectable response. These five values fall into the range 6–19 % (average 12%); error of the assay may account for this variation. Thus it appears that a detectable response (in our assay) will not occur unless there is at least a 12 % change in the fraction of the galactose-binding protein complexed, and there is no apparent difference among galactose, glycerol galactoside, and fucose in this regard. Since there are about 50,000 galactose-binding proteins per cell (W. Boos, personal communication), about 6,000 per cell would have to change occupancy by attractant molecules in order to produce a detectable response. (It is not possible to state how many proteins need to change occupancy for a single chemotactic event, since many such events must have to occur for a bacterium to end up in the capillary.)

In the case of α -methylaspartate, we reported the threshold to be 5×10^{-7} M when the background concentration is zero (from Fig. 3). If the K_D is 1.3×10^{-4} M, then only 0.4 % of the α -methylaspartate-binding protein needs to change occupancy in order to initiate a detectable response (in our assay). At background concentrations of 10^{-5} , 10^{-4} , 10^{-3} , and 10^{-2} M, the thresholds should then be 1.06×10^{-5} , 1.02×10^{-4} , 1.04×10^{-3} , and 1.43×10^{-2} M. The experimental results were 0.9×10^{-5} , 1.0×10^{-4} , 1.7×10^{-3} , and 1.3×10^{-2} M (from Fig. 3). Error of the assay may account for these discrepancies. The similarity of the experimental and corresponding theoretical numbers supports the conclusion that there has to be at least a 0.4 % change in fraction of this binding protein occupied for a detectable response to occur. Since the binding protein for α -methylaspartate has not yet been identified, the number of molecules per cell corresponding to 0.4 % is unknown.

Thus the minimal effective change in fraction of binding protein occupied—the “responsiveness”—is very different for the two chemoreceptors: 12 % for the sugars and 0.4 % for the amino acid. As a result, the response range for the sugars is much narrower than for the amino acid. For the sugars cells are recruited only from a small part of the gradient, the part that is close to the K_D and sufficiently steep (see discussion under Sensitivity Curves above), while for the amino acid cells can be recruited from a larger part of the gradient.

This difference in minimal effective change in fraction of binding protein occupied explains why it was necessary to go to steeper (100-fold) concentration intervals for the sugar sensitivity curves, while shallower (threefold) intervals sufficed for the amino acid. It also explains why the height of the sensitivity curves (compare Figs. 7 B and 1) and the height of the concentration-response curves (compare Fig. 5 and curve A of Fig. 3) are less for the sugars than for the amino acid.

What is the molecular basis for the difference in minimal effective change in fraction of binding protein occupied? One possibility is that the α -methylaspartate-binding protein might somehow be coupled more effectively to the response mechanism. Alternatively, there might be a larger number of α -methylaspartate-binding proteins per cell than galactose-binding proteins. Thus if there were 1.5×10^6 of the former, as compared to 5×10^4 of the latter, the 30-fold difference might be explained. Indeed, when the number of galactose-binding proteins per cell was increased 6.5-fold by induction, i.e. by growth in the presence of galactose, the response to fucose increased from 3×10^4 to 25×10^4 bacteria accumulated in 1 h at the peak of a

concentration-response curve, which occurred at the same concentration (10^{-2} M) in both cases (unpublished data).

Saturation

According to the present theory, saturation should occur at that concentration where practically all of the binding protein is already occupied. In the case of galactose, saturation occurs at about 3×10^{-6} M (Figs. 2 and 4) where 83% of the binding protein is occupied if the K_D is 6×10^{-7} M. If a 12% change in the fraction of the binding protein occupied is needed for a detectable response and 6×10^{-7} M is the K_D for galactose, we would have expected the saturating concentration to have been 4×10^{-6} M (the concentration at which 88% of the binding protein is occupied). In the case of α -methylaspartate, saturation occurs at about 10^{-1} M (Fig. 1), where 99.9% of the binding protein is occupied if the dissociation constant is 1.3×10^{-4} M. If a 0.4% change in fraction of the binding protein occupied is needed for a detectable response, then we would have expected saturation to have occurred at about 3×10^{-2} M (the concentration at which 99.6% of the binding protein is occupied). Thus the observed values of saturating concentrations are in broad agreement with the predicted ones.

Weber Law

From Eq. 3 it is possible to calculate for background concentrations throughout the response range how much more attractant needs to be in the capillary to get a detectable response. For galactose we will assume that a 12% change in binding protein occupied is necessary for a detectable response, and for α -methylaspartate a 0.4% change, the values obtained above. The results are shown in Fig. 9.

It can be seen from Fig. 9 that adherence to the Weber law can be expected only over a range of concentrations centered at the dissociation constant. For a relatively unresponsive chemoreceptor (curve A, galactose) this range will be narrower than for a responsive one (curve B, α -methylaspartate). The experimental results (Figs. 3 and 4) are in fair agreement with these expectations. In the case of galactose at a background concentration of 10^{-8} M (about $\frac{1}{60}$ of the deduced K_D) a higher fold increase (12-fold) was required in the capillary for a detectable response than at a background concentration of 10^{-7} M (threefold increase). The values predicted from Fig. 9 are 10-fold and 2-fold, respectively. In the case of α -methylaspartate, where the K_D has been deduced to be 1.3×10^{-4} M, for backgrounds between 10^{-6} and 10^{-2} M the increases needed in the capillary, 1.0- to 1.7-fold, were found to be experimentally indistinguishable. These values are in agreement with the prediction from Fig. 9.

It is generally recognized that the Weber law is valid only for intermediate stimulus intensities; it fails with quite weak and very large stimuli (17, 20; 25 and references cited there).

Conclusion

We have been able to explain certain features of bacterial chemotaxis by means of the equation that defines a dissociation constant for the complex between the attract-

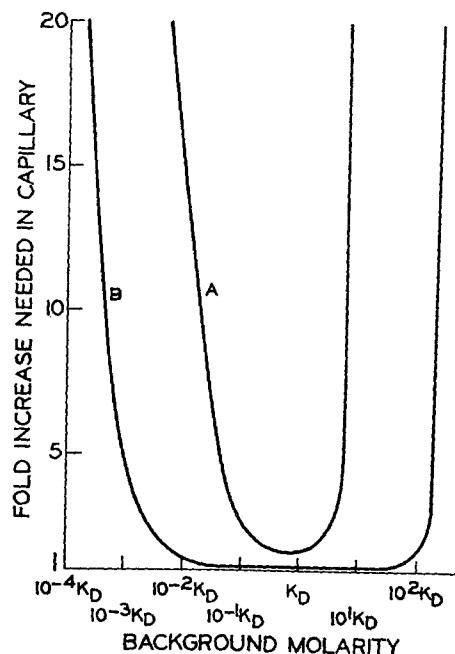


FIGURE 9. Theoretical Weber law curves. For various concentrations of attractant in the bacterial suspension (abscissa) we have plotted the fold increase in attractant concentration needed in the capillary to get a detectable response (ordinate). For curve A, a detectable response occurs when there is a 12% change in fraction of the binding protein complexed (as for galactose taxis); for curve B it is a change of 0.4% (as for α -methylaspartate taxis). The curves were calculated from Eq. 3, substituting 0.12 or 0.004 for the left-hand side.

ant and the component of a chemoreceptor that recognizes the attractant. We have used this theory to deduce sensitivity curves and concentration-response curves, and, in part, these agree reasonably well with the experimental results. The theory can thus be used to predict from these curves the dissociation constant for any attractant, and from the shape of the sensitivity curve, to tell whether one or more species of recognition component is present. Further, the theory predicts the range of concentrations over which the Weber law may be obeyed. However, the theory reveals nothing concerning the *mechanism* of chemoreception.

We thank Margaret M. Dahl for having carried out most of the experiments reported here. This investigation was supported by U. S. Public Health Service Grant AI-08746 from the National Institute of Allergy and Infectious Diseases.

R. E. Mesibov held a U. S. Public Health Service Predoctoral Fellowship from the National Institute of General Medical Sciences. G. W. Ordal held an American Cancer Society Postdoctoral Fellowship, and is now a U. S. Public Health Service Postdoctoral Fellow.

Received for publication 28 November 1972.

REFERENCES FOR APPENDIX

- 1 A. ANRAKU, Y. 1968. Transport of sugars and amino acids. I. Purification and specificity of the galactose- and leucine-binding proteins. *J. Biol. Chem.* **243**:3114.
- 2 A. BEIDLER, L. M. 1954. A theory of taste stimulation. *J. Gen. Physiol.* **38**(2):133.
- 3 A. BEIDLER, L. M. 1962. Taste receptor stimulation. *Progr. Biophys. Biophys. Chem.* **12**:107.
- 4 A. BEIDLER, L. M. 1971. Taste receptor stimulation with salts and acids. In *Handbook of Sensory Physiology*. L. M. Beidler, editor. Springer-Verlag New York Inc., New York. **4**(2):200.
- 5 A. BERG, H. C., and D. A. BROWN. 1972. Chemotaxis in *Escherichia coli*: Analysis by three-dimensional tracking. *Nature (Lond.)*. **239**:500.
- 6 A. BOOS, W., A. S. GORDON, R. E. HALL, and H. D. PRICE. 1972. Transport properties of the galactose-binding protein of *Escherichia coli*. Substrate-induced conformational changes. *J. Biol. Chem.* **247**:917.
- 7 A. KAISSLING, K. E. 1969. Kinetics of olfactory receptor potentials. In *Olfaction and Taste*. C. Pfaffman, editor. The Rockefeller University Press, New York. **2**:52.