least in part, to a decrease in the density of β-adrenergic receptors. They suggest further that the decline of these receptors may be caused by an inability of aged animals to increase the density of adrenergic receptors in response to reduced activity of the sympathetic nervous system.

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15 February 1978; revised 3 April 1978

Attraction by Repellents: An Error in Sensory Information Processing by Bacterial Mutants

Abstract. *Normal* Escherichia coli *bacteria are repelled by acetate, benzoate, and indole and attracted by α-aminoisobutyrate. We have isolated mutants that are attracted to acetate, benzoate, and indole and may be repelled by α-aminoisobutyrate. These reversed-taxis mutants are defective in a central processing component: a set of methylated proteins known as MCP 1. The mechanism of reversal of taxis is discussed.*

We set out to search for a mutant that is attracted to a chemical which normally repels *Escherichia coli*. From such a reversal of taxis we hoped, ultimately, to learn about the mechanism that a bacterium uses to determine whether it should treat a chemical as an attractant or repellent. We report the discovery and characteristics of such mutants.

The first strains known to exhibit reversed taxis were isolated by selecting for mutants that were attracted into a capillary containing acetate (*1*), which is normally a repellent for *E. coli* (*2*). The chemotactic response to acetate of such a mutant, AW630, and its parent is shown in Fig. 1. The attractant response in the mutant has a threshold of approximately $10^{-3}M$ and a maximum at $1 \times 10^{-1}M$ to $3 \times 10^{-1}M$ acetate. Since repulsion by acetate in the parent shows a similar dependency on concentration (*2*), it appears that the attraction observed in the mutant is not a response to a chemical contaminating the acetate but represents a true reversal of the chemotactic response. Furthermore, since the receptor dissociation constant (*3*) and the specificity (*4*) are the same for acetate attraction in the mutant as for acetate repulsion in the parent, it appears that the same receptor is involved in both processes.

To ascertain whether this reversal of chemotaxis was specific to the acetate receptor or, alternatively, affected the responses also to chemicals detected by other chemoreceptors, we tested various attractants and repellents in the mutant. The chemotactic response to some chemicals was drastically altered, while the response to others was relatively normal. These alterations show a remarkable similarity to the pattern of defects characteristically exhibited by previously isolated *tsr* mutants (*5–8*), although *tsr* strains were not known to be reversed for taxis. However, additional experiments now show that the *tsr* mutants indeed do exhibit such a reversal, and that they and AW630 belong to a single complementation group, the *tsr* cistron (*9*).

Table 1 presents a summary of the responses of a typical *tsr* mutant and its parent in temporal assays (*10, 11*) of chemotactic behavior. Certain chemicals (type 1) show either a reversal or a loss of the response when the mutant is compared to the parent. For example, acetate, propionate, and *n*-butyrate—all detected by the acetate receptor (*2, 11*)—and benzoate and indole—each detected by a separate receptor (*2*)—act as attractants in the mutant, in contrast to their action as repellents in the parent (*2*). This has been confirmed by the capillary assay for acetate (Fig. 1) and for benzoate and indole (*12*). L-Leucine, normally a repellent for *E. coli*, and detected by yet another receptor (*2, 11*), fails to either repel or attract the mutant, as judged by both temporal (Table 1) and capillary (*12*) assays. α-Aminoisobutyrate, a type 1 chemical that is an attractant for the parent, may repel *tsr* mutants; this is indicated by the chemical-in-plug assay (*13*); but results from temporal assays (Table 1) show weak repulsion or, in some experiments, no response. In contrast to type 1 chemicals, the response of *tsr* mutants to type 2 chemicals is relatively normal (Table 1). L-Aspartate, which acts as an attractant in the parental strain, also acts as an attractant for the mutant, and cobalt and nickel ions act as repellents for both parent and mutant.

Since the reversed taxis mutants are all in the *tsr* cistron, the loss of a functional *tsr* product must, in some way, cause the reversal of taxis. It was discovered recently that *tsr* codes for one of the two methyl-accepting chemotaxis proteins (MCP's) (*7, 8*). This protein (actually a set of bands on a sodium dodecyl sulfate gel) is called MCP 1, and it is defective in *tsr* mutants. The other protein (again actually a set), MCP 2, is coded for by the *tar* gene and is defective in *tar*

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mutants (*7, 8*). Behavioral and biochemical data show that *E. coli* has two complementary pathways of information flow from chemoreceptors to flagella, each pathway making use of one type of MCP (*7, 8*). One set of attractants and repellents, called type I (Table 1), act through the Tsr pathway; type 1 attractants increase the level of methylation of MCP 1 while type 1 repellents decrease this level (*7*). Another set of stimuli, called type 2 (Table 1), act through the Tar pathway; in a similar way, type 2 attractants increase the methylation of MCP 2, while type 2 repellents decrease it (*7*).

The *tsr* mutants fail to methylate MCP 1 and are neither repelled by type 1 repellents nor attracted by type 1 attractants, although they show qualitatively normal responses to type 2 stimuli (*7*). Similarly, *tar* mutants fail to methylate MCP 2 and are defective for type 2 stimuli but are qualitatively normal for type 1 stimuli (*7*). It appears that most, if not all, chemotactic information flows through one of these two pathways, since double mutants, which carry both *tsr* and *tar* defects, are generally nonchemotactic (*7*, 8).

Our work demonstrates that type 1 repellents not only fail to repel *tsr* mutants, but in many cases they act as attractants for these strains. In a like manner, type 1 attractants may act as repellents for these mutants. We have looked for, but so far have failed to find, any evidence that chemotaxis toward type 2 stimuli is reversed in the *tar* mutants.

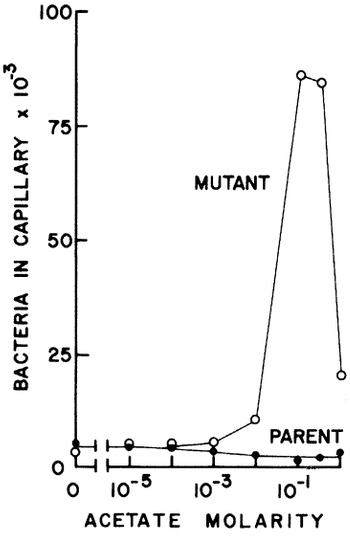

Fig. 1. Comparison of acetate taxis of the mutant, AW630 (open circles), and its parent, AW574 (closed circles). Capillaries containing sodium acetate (*p*H 7.0) at various concentrations were inserted into chambers containing a suspension of bacteria, with each capillary in a separate chamber (*1*). After 60 minutes at 30°C, the number of bacteria in each capillary was measured (*1*). Cells had been grown in glycerol minimal medium (*1*) and assayed at 7×10^7 per milliliter. Decreasing accumulation with increasing concentration, characteristic of a repellent response (*2*), is evident for the parent; for results from other methods that show more strikingly the repulsion of the parent by acetate, see (*2*). Increasing accumulation with increasing concentration (to a peak value) characteristic of an attractant response (*1*), is evident for the mutant. Data represent mean values for three assays of both mutant and parent.

Since *tsr* mutants cannot methylate MCP 1, information specifying the reversed response must either pass through the Tar pathway, and therefore MCP 2, or through a still unidentified third pathway. To investigate the former possibility, we studied the effects of type 1 stimuli on the methylation of MCP 2 in a *tsr* mutant (AW518). We found a 36 ± 8 percent ($n = 5$) increase in methylation when the repellent sodium benzoate (30 m*M*) was added and a 33 ± 10 percent ($n = 5$) decrease in methylation when the attractant α-aminoisobutyrate (50 m*M*) was added (*14*). These changes in methylation, which are opposite to those normally found for attractants and repellents (*7*), suggest that the reversed responses are mediated by the Tar pathway.

How does elimination of the Tsr pathway lead to the reversal of responses to type 1 stimuli? Since information which results in these reversed responses is apparently carried by the Tar pathway, two different models can be proposed.

1) First, it may be that in wild-type strains type 1 stimuli utilize both the Tsr and Tar pathways. Thus, in addition to having the previously reported large effects on MCP 1 (*7*), these stimuli may have small effects of opposite polarity on MCP 2 that have not been detected because of incomplete separation of the methylated proteins on sodium dodecyl sulfate gels (*7*). In *tsr* mutants only the MCP 2 effects remain.

2) Alternatively, type 1 stimuli may have no effect on MCP 2 in wild-type *E. coli*, but in some way the lack of MCP 1 function in the *tsr* mutants may lead to abnormal processing of these stimuli by MCP 2. Once complete separation of MCP 1 and 2 has been accomplished, it will be possible to distinguish definitively between these two models.

Attractant and repellent responses to type 1 stimuli are greater in *tar* mutants than in the wild type (*7*); this suggests that removal of MCP 2 has relieved the cells of an inhibitory effect on type 1 responses. This increased responsiveness is a necessary consequence of the first alternative but not the second.

If the first model is correct, what role might this play in the behavior of the organism? (i) The phenomenon may simply be a consequence of the particular mechanisms that have evolved to control the methylation of MCP 1 and MCP 2, and thus have no behavioral significance. (ii)

Table 1. Temporal assays of chemotaxis in a reversed taxis mutant and its parent. For temporal assays (*10, 11*) chemical and bacterial suspensions were mixed on a slide at room temperature (25° to 26°C), and the tumbling frequency was immediately observed microscopically. An attractant response is indicated by suppression of tumbling ("smooth" swimming) while a repellent response is indicated by an increased frequency of tumbling ("tumbly" swimming). The number of experiments in each case is shown in parentheses. The parental strain was AW405 (*5*), while the reversed taxis strain was the *tsr* mutant AW518 (*5*). Acids were used as the sodium salts.

Chemical	By parent responses		By mutant responses	
	Type	Length (sec)	Type	Length (sec)
		Type 1 repellents		
Acetate, 17 m*M*	Tumbly	16 ± 1 (2)	Smooth	15 ± 3 (2)
Propionate, 17 m*M*	Tumbly	15 ± 3 (3)	Smooth	19 ± 3 (2)
n-Butyrate, 17 m*M*	Tumbly	15 ± 2 (3)	Smooth	16 ± 2 (2)
Benzoate, 33 m*M*	Tumbly	33 ± 3 (2)	Smooth	45 ± 5 (2)
Indole, 0.33 m*M*	Tumbly	30 ± 5 (6)	Smooth	35 ± 5 (2)
L-Leucine, 33 m*M*	Tumbly	14 ± 1 (4)	No response	(2)
		Type 2 repellents		
Co(II), 0.5 m*M**	Tumbly	25 (1)	Tumbly	50 (1)
Ni(II), 0.5 m*M*†	Tumbly	30 ± 2 (4)	Tumbly	46 ± 3 (4)
		Type 1 attractant		
α-Aminoisobutyrate, 3 m*M*	Smooth	95 ± 5 (2)	Tumbly	‡
		Type 2 attractant		
L-Aspartate, 5 μ*M*	Smooth	130 ± 3 (2)	Smooth	170 ± 3 (2)

*Considered as $CoSO_4$; †Considered as $NiSO_4$. ‡See text.

Or, if the bacteria can modulate the dominance of the Tsr (MCP 1) or Tar (MCP 2) pathways, a particular chemical may be an attractant in some situations and a repellent in others. This ability to alter the pattern of response could be of advantage to the cell. For example, acetate, which is an excretory product of *E. coli* (*2*), may signal a crowded, suboptimal environment, and hence normally act as a repellent for the bacteria. However, if no other carbon and energy source is available, acetate can be used (*15*), and in this situation the organism may profit by responding to it as an attractant rather than as a repellent.

While we now know the identity of the biochemical species (MCP) involved in the reversal of taxis in the *tsr* mutants, we do not yet know what determines whether binding to a given receptor leads to methylation or demethylation of MCP 1 and MCP 2. Elucidation of this point is essential for understanding why some compounds are attractants and others are repellents and for determining how information is processed in the chemotactic response.

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13. In the chemical-in-plug assay (*2*) the α-aminoisobutyrate is contained in a plug of agar that is immersed into a bacterial suspension in agar of such a low concentration that motility is possible. Repulsion is indicated by a vacant region around the plug and, around the vacant region, a ring of bacteria, those that fled the repellent in the now vacant region. In the case of AW518 repulsion was obvious when the plug contained 0.3*M* α-aminoisobutyrate; 0.1*M* gave a weak response, but 0.01*M* failed to elicit any response. For the parent, AW405, repulsion was not detected at any of these concentrations.
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10 January 1978; revised 20 March 1978

6-Mercaptopurine Treatment of Pregnant Mice: Effects on Second and Third Generations

Abstract. *The immunosuppressive drug 6-mercaptopurine is embryotoxic in mice. Of the surviving female offspring of mice treated with low doses of 6-mercaptopurine during pregnancy, despite normal body weight and general appearance, many were either sterile or, if they became pregnant, had smaller litters and more dead fetuses as compared to offspring of mothers that had not received the drug.*

Women of childbearing age are frequently exposed to chemicals capable of causing genetic damage to the fetus. Exposure can be intentional, as during treatment with certain drugs, or unintentional, as from pollutants in the environment. Severe genetic damage may lead to fetal death or birth defects, but, if the genetic damage is less severe, obvious effects may not be observed.

The immunosuppressive drug azathioprine [6-(1-methyl-4-nitro-5-imidazolyl) mercaptopurine] induces gaps, fragments, deletions, and breaks in chromosomes of treated patients (*1, 2*). Abnormal chromosomes have also been observed in somatic cells from children of mothers treated with low doses of azathioprine during pregnancy (*2*). Azathioprine and its metabolite 6-mercaptopurine (*3*) induce genetic damage by inhibiting synthesis of DNA (*4*) and by being converted to thioguanine, which is incorporated as a nucleotide analog into DNA (*5*).

Both azathioprine and 6-mercaptopurine cause genetic damage in germ cells as well as in somatic cells (*6*). Chromosomal damage in somatic cells of the human fetus induced by treatment of pregnant women with azathioprine disappears by 20 months after birth (*2*). However, genetic damage in the germ cells of the female fetus may remain since these cells divide mitotically early in development, enter meiosis before birth, and remain in prophase of the first meiotic division until just before ovulation (*7*). Therefore, the consequences of such damage would not be manifested until the female offspring of treated mothers reach puberty and attempt to reproduce (*2*), a full generation after exposure to the drug.

Female CD1/CR mice received treatments with either physiological saline or one of three doses of 6-mercaptopurine. The *p*H of all solutions for injection was adjusted between 8 and 9. Between 0.1 and 0.2 ml of injection solutions were administered subcutaneously every day, beginning 3 days before pairing with untreated males for breeding through day 18 after mating. Females were paired with males for a maximum of 5 days. The live and dead offspring of the treated mice were counted after birth. At 70 days of age, all surviving female offspring were paired with normal males for 12 days (approximately three estrous cycles). On day 18 after mating, these females were killed and the number and viability of the fetuses were determined. Binomial data were analyzed by the χ^2 test. Continuous data were subjected to one-way analysis of variance, and treatment means were compared to control means by Dunnett's test (*8*).

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