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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1. AW574 [S. H. Larsen, J. Adler, J. J. Gargus, R. W. Hogg, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1239 (1974)] was mutagenized with ethylmethane sulfonate [J. S. Parkinson, *J. Bacteriol.* **126**, 758 (1976)] and 3×10^6 bacteria were presented with a capillary containing 0.1*M* sodium acetate, according to the method of J. Adler [*J. Gen. Microbiol.* **74**, 77 (1973)]. Except for individuals swimming randomly, only bacteria that are attracted will go into the capillary. Cells entering the capillary were collected, grown to saturation in tryptone broth, and recycled in this way four times. Ten independent mutants with essentially identical properties were isolated by this procedure.
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3. The apparent dissociation constant for the acetate sensing system, measured according to R. E. Mesibov, G. W. Ordal, J. Adler [*J. Gen. Physiol.* **62**, 203 (1973)], is $10^{-2}M$ in both parent and mutant.
4. Neither the mutant nor the parent responded to these other acetate analogs tested: formate, glycolate, methyl acetate, lactate, and β-hydroxybutyrate. Furthermore, analogs of acetate that *are* repellents for the parent and that inhibit repulsion by acetate in the parent, presumably by competing for the receptor (*2*), also inhibited attraction to acetate in the mutant. Thus acetate, propionate, *n*-butyrate, *n*-valerate, *n*-hexanoate, or *n*-heptanoate (each at $10^{-2}M$ in both the bacterial suspension and the capillary) inhibited attraction of AW630 to acetate ($10^{-1}M$ in the capillary) by more than 90 percent without inhibiting motility.
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12. AW518 was attracted into a capillary containing $10^{-2}M$ indole; there were 240 times more bacteria in such a capillary than in one containing no indole. The threshold for this response was about $10^{-4}M$. For sodium benzoate, accumulation in a capillary containing $10^{-1}M$ was seven times greater than in a capillary without benzoate. The threshold was about $10^{-3}M$. For L-leucine there was no significant attraction between $10^{-7}M$ and $10^{-1}M$. In all cases the parent, AW405, was repelled by the chemical in the capillary.
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.
14. These values were determined as described (*7*).
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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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As previously reported (*9*), 6-mercaptopurine was embryotoxic and reduced litter size, but only at the highest dose (Table 1, group D). Besides this direct lethal effect, 6-mercaptopurine severely impaired the ability of the surviving female offspring to reproduce upon maturity (Table 2). Fewer female offspring were pregnant in groups C ($P < .05$) and D ($P < .01$) than in group A (Table 2). Fewer fetuses (live and dead) were produced by each pregnant mouse in groups C and D than in group A ($P < .01$). The number of live fetuses per pregnant mouse also was less ($P < .01$) in those two groups, and the percentage of the embryos that died and were resorbed in groups C and D was greater ($P < .01$) than that in group A. Ovaries from mice in group D weighed less ($P < .01$) than those from group A. Although all of these reproductive characteristics were affected, offspring of mothers treated with 6-mercaptopurine did not weigh less than offspring of saline-treated mothers at the time of pairing with males.

Table 1. Embryotoxic effects of 6-mercaptopurine in mice.

Group	Dose (mg/kg per day)	*N*	Percentage of		Offspring per pregnant mouse*	
			Mice bred	Bred mice pregnant	Total	Live
A	0	13	69.2	100	10.2 ± 0.8	9.8 ± 0.8
B	0.5	12	100	83.3	9.2 ± 0.8	9.1 ± 0.8
C	1.5	11	90.9	90	8.6 ± 1.0	8.6 ± 1.0
D	3.0	12	58.3	100	5.3 ± 0.6†	3.9 ± 1.0†

*Mean ± standard error of the mean (S.E.M.). †Significantly less than control ($P < .01$).

Table 2. Reproductive characteristics of female offspring of 6-mercaptopurine–treated mice. All female offspring of treated mice were paired with untreated males. To determine if mating occurred, they were examined daily for vaginal plugs; however, vaginal plugs were not found in some mice that were subsequently pregnant. Since the exact day of gestation in these mice was not known, the number of fetuses (total, live, dead, or resorbed) was determined only for those animals in which vaginal plugs were observed. Abbreviation: G, group.

G	*N*	Weight at pairing* (g)	Percentage of mice pregnant		Fetuses per pregnant mouse*		Dead or resorbed fetuses (%)	Ovarian weight* (mg)
			Total	With observed vaginal plugs	Total	Live		
A	33	26.9 ± 0.4	100	78.8	13.0 ± 0.4	11.3 ± 0.4	12.8	8.6 ± 0.4
B	46	28.9 ± 0.3	95.7	81.8	12.8 ± 0.3	10.5 ± 0.4	17.8	8.7 ± 0.3
C	33	26.2 ± 0.5	78.8†	88.5	10.6 ± 0.5‡	8.0 ± 0.6‡	25§	7.3 ± 0.6
D	9	27.0 ± 1.2	22.2‡	100	6.5 ± 3.5‡	3.5 ± 1.5‡	46.2§	2.2 ± 0.6‡

*Mean ± S.E.M. †Significantly less than control ($P < .05$). ‡Significantly less than control ($P < .01$). §Significantly greater than control ($P < .01$).

Histological examination of ovaries from the female offspring of mothers treated with 6-mercaptopurine revealed that there were few oocytes and ovarian follicles. Many ovaries from mice in groups C and D were completely devoid of follicles (Fig. 1). Apparently 6-mercaptopurine reduced the number of oocytes that arose mitotically from a pool of less than 100 primordial germ cells in the fetus (*10*). This probably accounted for the small litters in groups C and D. The additional high incidence of fetal mortality in these two groups suggests that the oocytes that were present contained genetic damage that was lethal to the fetus after fertilization. This damage was probably acquired while the offspring were developing in the uterus of their mothers during treatment. It is unlikely that such damage would be eliminated or repaired as has been reported in somatic cells because synthesis of DNA stops when the germ cells are arrested in prophase of the first meiotic division in utero, and no new DNA is

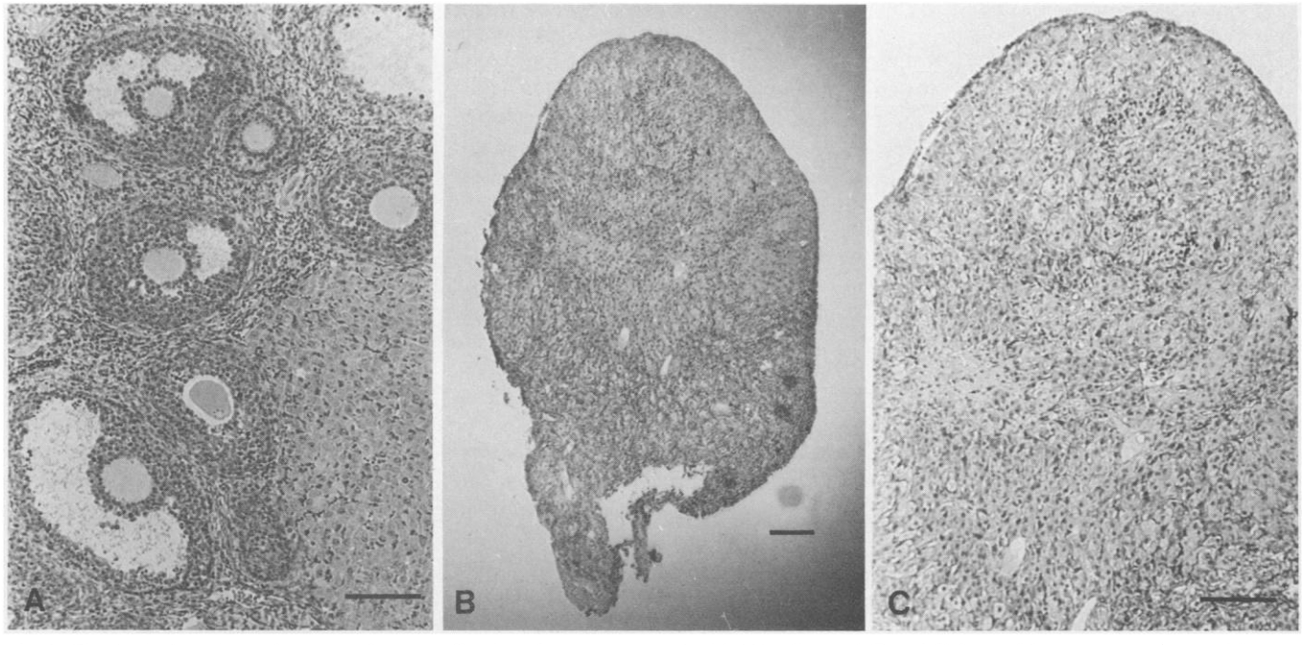

Fig. 1. Histological sections of an ovary from an offspring of a mouse treated with (A) saline and (B and C) 3 mg of 6-mercaptopurine per kilogram of body weight, daily. Abundant oocytes and follicles are present in (A) whereas in (B) and (C) they are absent. Scale bars = 0.1 mm.

synthesized until after fertilization (*11*).

Studies of this type can identify mutagens and determine the risk associated with exposure to known mutagens. Exposure of the fetus to low doses of mutagens may not cause immediate, obvious effects such as morbidity or birth defects, but can have severe consequences when the otherwise normal female offspring reach puberty.

Caution must be exercised when data gathered from animal experiments are extrapolated to the situation in humans. However, many children have been born to mothers treated with azathioprine (*2, 12*), and these children should be observed carefully to determine whether their reproductive function has been adversely affected by the drug.

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Peptide Formation in the Prebiotic Era: Thermal Condensation of Glycine in Fluctuating Clay Environments

Abstract. *As geologically relevant models of prebiotic environments, systems consisting of clay, water, and amino acids were subjected to cyclic variations in temperature and water content. Fluctuations of both variables produced longer oligopeptides in higher yields than were produced by temperature fluctuations alone. The results suggest that fluctuating environments provided a favorable geological setting in which the rate and extent of chemical evolution would have been determined by the number and frequency of cycles.*

Recently we proposed (*1*) that environments where both water content and temperature fluctuate diurnally and seasonally presented the most favorable and geologically relevant settings for condensation reactions in the prebiotic era. Simulation of the main characteristics of such a system—that is, variable water content and temperature, high solute concentration during a dehydration process, the presence of a catalytically active solid surface, and the opportunity for redistribution of the organic molecular species during a hydration process—led to condensation of glycine to oligopeptides. For purposes of comparison, systems that were only subjected to temperature fluctuations were also examined (*2*).

In a typical experiment, 1 ml of 23 m*M* glycine was added to the clay mineral in the Na^+ form (kaolinite, 60 mg; bentonite, 20 mg). After hydration of the clay, the suspension was treated as follows for wetting-drying and temperature fluctuations (WDTF): (i) dehydration at 60°C for 1 to 2 days; (ii) heating at 94°C for 2 to 3 days; (iii) rehydration with 1 ml of water, and (iv) repetition of steps (i) to (iii) for *n* number of cycles. Suspensions tested for temperature fluctuations (TF) with dehydration were treated as in step (i) above, with subsequent intermittent heating at 94°C and cooling to 25°C; these TF treatments were performed in parallel with the wetting-drying series. Products were extracted from the clay with water and 1*N* NH_4OH (*3*). Identification of glycine oligopeptides was based primarily on their elution times as determined with an automatic amino acid analyzer (*4*).

In the absence of clay, trace amounts of diglycine formed occasionally after lengthy heating. The presence of clays, however, consistently brought about the synthesis of peptides, and diglycine was readily detected after 1 week at 94°C.

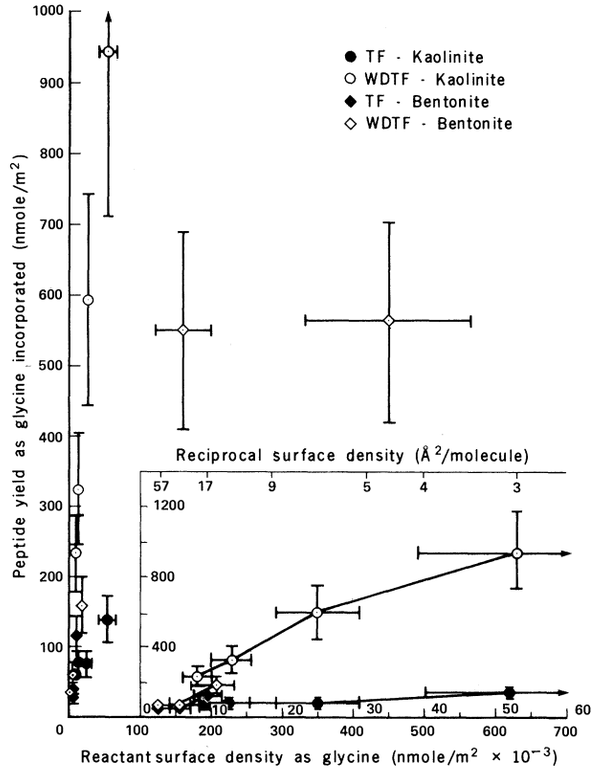

Fig. 1. Effect of initial glycine surface density on the total yield of peptide. In the inset the lower left-hand corner of the figure is redrawn for clarity. Values for the reciprocal surface density are provided on the upper scale of the inset for various reactant surface densities. Error bars represent the ± 25 percent uncertainty in the surface area estimates; replicate analyses from Table 1 typically exhibit much smaller uncertainties.

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