tals. Second, the ammonium sulfate used in the suspension medium to maintain the integrity of the crystals and to prevent redissolution of the protein crystallized on drying. We therefore sought a treatment which would impart mechanical strength to the crystals and simultaneously prevent dissolution of the protein in the volatile buffers required for electron microscopy.

Glutaraldehyde has been successfully used as a fixative in the preparation of specimens for electron microscopy (*7*). Quiocho and Richards (*8*) have shown that single crystals of carboxypeptidase A fixed with this reagent have x-ray diffraction patterns very similar to those of the native crystals. These authors have also demonstrated a marked increase in the mechanical stability of crystals prepared for x-ray diffraction studies following treatment with glutaraldehyde.

We therefore fixed the isocitrate dehydrogenase crystals by adding glutaraldehyde to the 60 percent ammonium sulfate suspension of crystals to make the resulting solution 3 percent with respect to this fixative. The suspension was maintained at 4°C for 12 hours. The fixed crystals were recovered by centrifugation at 500*g* for 5 minutes at 4°C, the supernatant was siphoned off, and the crystals were resuspended in 20 m*M* ammonium acetate, *p*H 6.9. This suspension was then centrifuged at 500*g* for 5 minutes at 4°C, the supernatant was removed, and the crystals were again suspended in the ammonium acetate buffer. This procedure was repeated three times. After the final centrifugation, the washed crystals were suspended in 1 ml of the 20 m*M* ammonium acetate buffer.

The treatment of the isocitrate dehydrogenase crystals with this cross-linking agent produced no changes in crystalline morphology that were detectable with the light microscope. The fixed crystals were found to be insoluble in both 20 m*M* ammonium acetate, *p*H 6.9, and distilled water. However, when the ionic strength of the suspension medium was lowered after fixation with glutaraldehyde, the crystals were observed to clump.

The buffered crystal suspension was dispersed on glass cover slips broken to fit scanning specimen stubs and dried at room temperature (23°C). The fixed material was dried with no apparent disruption of the morphology. The cover slips were then mounted on specimen stubs with silver paint, and vapor-coated with carbon followed by gold while they were rotating on a motorized platform. The specimens were viewed with a Cambridge Stereoscan scanning electron microscope at an accelerating voltage of 20 kv.

Examination of the enzyme crystals with the scanning electron microscope provides definitive proof of their octahedral structure. Figure 2A is an electron micrograph of the protein crystals under low magnification. Note that the effective magnifications of the enzyme crystals in Figs. 1B and 2A are approximately the same; yet, the resolution provided by the scanning electron microscope is vastly superior. The amorphous material acting as a cementing substance is assumed to be uncrystallized enzyme which has been cross-linked to the crystals by the glutaraldehyde. Figure 2B is a higher magnification of a small, individual crystal which exemplifies the octahedral morphology postulated from the light microscopic examinations.

This represents the first report of a successful crystallization of isocitrate dehydrogenase, either NAD- or NADP-specific, from any source. Further, to the best of our knowledge, it is the first investigation of the structure of an enzyme crystal by scanning electron microscopy. The techniques described provide a general method for the examination of fragile enzyme crystals with the scanning electron microscope. These crystals, which do not lend themselves to study with either the light microscope or the transmission electron microscope because of their three-dimensional nature, are well suited for observation with the scanning electron microscope. This instrument provides magnification and resolution without loss of the depth of field required for detailed examination of crystals of this nature.

WILLIAM F. BURKE
JAMES R. SWAFFORD
HENRY C. REEVES
Department of Botany and Microbiology, Arizona State University, Tempe 85281

References and Notes

1. G. D. Greville, in *Citric Acid Cycle*, J. M. Lowenstein, Ed. (Dekker, New York, 1969), p. 25.
2. J. M. Lowenstein, in *Metabolic Pathways*, D. M. Greenberg, Ed. (Academic Press, New York, 1967), vol. 1, p. 146; G. W. E. Plaut, in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myerbach, Eds. (Academic Press, New York, 1963), vol. 7, p. 105; T. E. Rogland, T. Kawaski, J. M. Lowenstein, *J. Bacteriol.* **91**, 236 (1966).
3. R. F. Chen, D. Brown, G. W. E. Plaut, *Biochemistry* **3**, 552 (1964).
4. H. C. Reeves, G. O. Daumy, C. C. Lin, M. Houston, *Biochim. Biophys. Acta* **258**, 27 (1972).
5. W. B. Jakoby, *Anal. Biochem.* **26**, 295 (1968).
6. S. Inoue, J. Hardy, G. H. Counsineau, A. K. Bal, *J. Ultrastruct. Res.* **29**, 350 (1969).
7. D. D. Sabatini, K. Bensch, R. J. Barrnett, *J. Cell Biol.* **17**, 19 (1963).
8. F. A. Quiocho and F. M. Richards, *Proc. Nat. Acad. Sci. U.S.A.* **52**, 833 (1964).
9. We are indebted to Peter B. Mee of Advanced Systems and Technology, Honeywell Information Systems Inc., and John J. Burke for their technical assistance. Supported by NSF grant GB-1588.

27 March 1973

Common Mechanism for Repellents and Attractants in Bacterial Chemotaxis

Abstract. *The migrational response of* Salmonella typhimurium *away from compounds such as phenol, indole, acetic acid, and leucine occurs because the bacteria tumble less frequently while* descending *gradients of repellents. This contrasts with their response of tumbling less frequently while* ascending *gradients of attractants. The results of competition experiments suggest that repellents, like attractants, operate through specific receptors, and the algebraic additivity experiments indicate that repellents and attractants utilize a common memory mechanism for taxis.*

The migration of bacteria toward attractants and away from repellents was observed in the last century by Pfeffer and Engelmann (*1*). Since that time the response to attractants has been documented extensively by Adler and co-workers (*2, 3*) and more recently by Berg and Brown (*4*) and in our laboratory (*5, 6*). However, except for the finding by Lederberg (*7*) of negative phenol taxis and the *p*H and inorganic ion studies by Doetsch and co-workers (*8*), repellents have received little attention. The recent development of new techniques (*5, 6*) has facilitated the study of repellents as well as attractants. Moreover, the finding that attractant gradient sensing employs specific receptors (*2, 9, 10*) and a memory mechanism

(*6*) made such studies of theoretical interest, particularly in view of the suggestion (*8*) that repellents may operate differently. Studies with a variety of possible repellents (*11*) were therefore initiated to document their effects and to establish their relationship to attractants.

The studies were carried out with *Salmonella typhimurium* LT2 (selected for motility on soft agar) grown aerobically at 30°C in Vogel-Bonner citrate medium (*12*), with 1 percent (by volume) glycerol as an additional carbon source. Concentrated repellent solutions were adjusted to the *p*H of the buffer (7.0) prior to dilution. The compounds were studied in two ways. The first method utilized the temporal gradient apparatus (*6*) whereby bacteria are subjected to sudden concentration changes from one uniform spatial environment to another. Their subsequent motility responses, observed microscopically, are therefore the result of temporal gradient sensing. Because of the steepness of the gradient, this method is capable of detecting very weak repellents. The second method utilized the population migration apparatus (*5*) which measures, by light scattering, the movement of a population swimming in a spatial gradient. The concentration changes sensed by the bacteria in this case are generated by their movement through the gradient. By using a step gradient in this apparatus, the response to all but the weakest repellents could be measured. An exponential gradient which decreases by a factor of 2 over several millimeters, however, evoked a measurable response only with the strongest repellents.

The responses in the temporal gradient apparatus are summarized in the first column of Table 1 and

Table 1. Properties of repellents to *Salmonella typhimurium*.

Item	Taxis threshold* (M)	Motility impairment threshold (M)	Response in spatial gradient†	
			Concentration step (M)	Transfer velocity (μm sec^{-1})
		Class A‡		
Acetate	3×10^{-4}	7.5×10^{-2}	$10^{-3} \longleftrightarrow 10^{-4}$	1.1
Propionate	10^{-4}	7.5×10^{-2}	$10^{-3} \longleftrightarrow 10^{-4}$	0.7
Butyrate	3×10^{-4}	7.5×10^{-2}	$10^{-3} \longleftrightarrow 10^{-4}$	0.9
		Class B		
Leucine	3×10^{-4}	None§	$10^{-3} \longleftrightarrow 10^{-4}$	0.6
Isoleucine	3×10^{-4}	None§	$10^{-3} \longleftrightarrow 10^{-4}$	0.2
Valine	3×10^{-4}	None§	$10^{-3} \longleftrightarrow 10^{-4}$	< 0.1
		Class C		
Indole	10^{-5}	3×10^{-4}	$3 \times 10^{-5} \longleftrightarrow 3 \times 10^{-6}$	1.5
Phenol	3×10^{-5}	3×10^{-3}	$10^{-4} \longleftrightarrow 10^{-5}$	1.2
Tryptophan	3×10^{-3}	None§	$10^{-2} \longleftrightarrow 0$	?||
Tyrosine	10^{-3}	None§	$10^{-3} \longleftrightarrow 0$	0¶

* The lowest concentration which, when reduced by a factor of 4 in the temporal gradient apparatus, produced a smooth response. † Response in the population migration apparatus to the indicated step change in concentration. For definition of transfer velocity, see text. ‡ Classes based on competitive inhibition experiments (see text). The following inhibitor/repellent combinations were tested with inhibitor concentration chosen just below the motility impairment threshold and the repellent concentration chosen to give a submaximal response. After each inhibitor/repellent combination, the observed inhibition of transfer velocity is given: propionate/acetate, 100 percent; butyrate/acetate, 100 percent; acetate/propionate, 100 percent; valine/leucine, 100 percent; leucine/isoleucine, 100 percent; isoleucine/leucine, 100 percent; phenol/indole, 50 percent; indole/phenol, 100 percent; phenol/acetate, 0; propionate/leucine, 20 percent; phenol/leucine, 20 percent; leucine/propionate, 0; idole/acetate, 0. § No impairment observed, up to solubility limit ($10^{-2}M$, branched-chain amino acids and tryptophan; $2.5 \times 10^{-3}M$, tyrosine). || No response was noted when the concentration interval was $10^{-3} \longleftrightarrow 0M$. It was questionable whether the slight response at $10^{-2} \longleftrightarrow 0M$ was due to tryptophan or trace impurities. ¶ Tyrosine gave no response to the largest step change that could be used (solubility limitation).

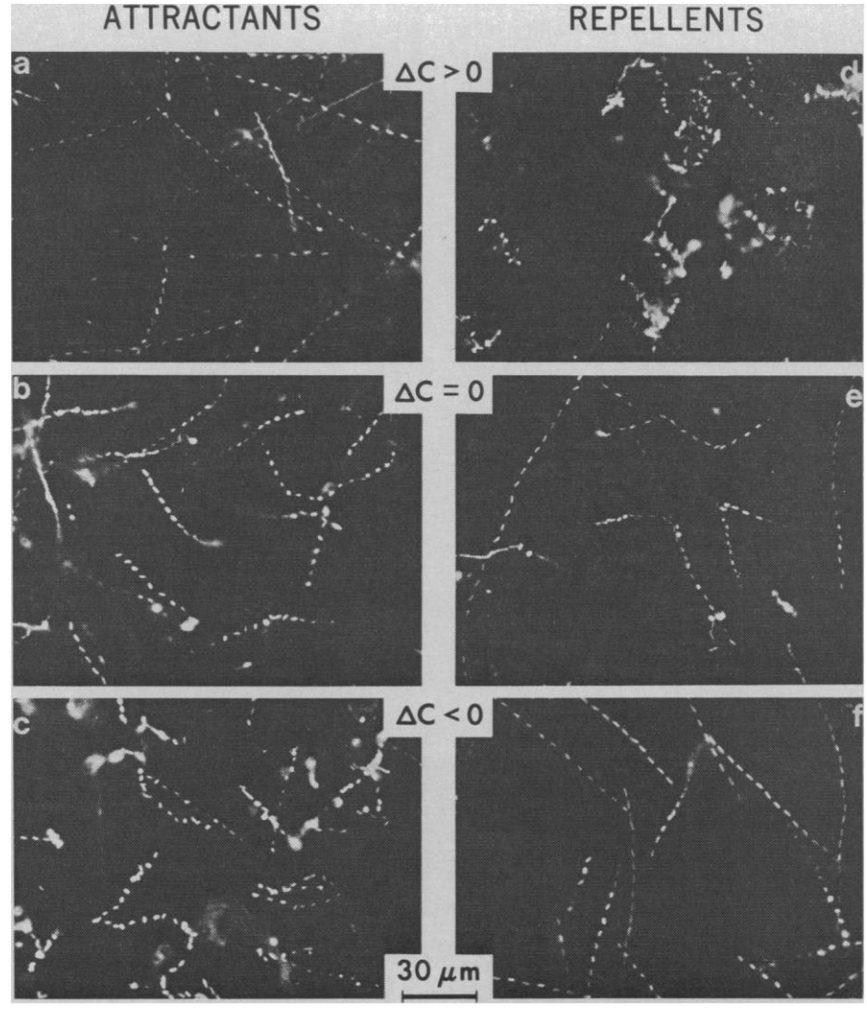

Fig. 1. Motility tracks of *Salmonella* in the interval 2 to 7 seconds after subjection to temporal gradients of attractants and repellents. Photographs taken in dark-field with stroboscopic illumination operating at 5 pulses per second. (a, b, and c) Attractants. (a) Serine increase from 0 to $7.5 \times 10^{-4}M$; (b) no concentration change (control); (c) serine decrease from 10^{-3} to $2.4 \times 10^{-4}M$. (d, e, and f) Repellents. (d) Phenol increase from zero to $7.5 \times 10^{-4}M$; (e) no concentration change (control); (f) phenol decrease from 3×10^{-4} to $7.5 \times 10^{-5}M$. The smooth motility response to "favorable" gradients (a and f) and the tumbling response to "unfavorable" gradients (c and d) eventually give way to the normal motility pattern (b and e).

are illustrated by motility tracks in Fig. 1. When, for example, bacteria were subjected to a *decrease* in phenol concentration from 3×10^{-4} to $7.5 \times 10^{-5}M$, the normal motility pattern, consisting of roughly straight line runs over moderate distances interrupted by turns or tumbles (Fig. 1, b and e), was initially replaced by one in which bacteria swam for long distances without tumbling (Fig. 1f). The tumbling gradually returned so that after about 18 seconds, 50 percent of the population was judged to be swimming normally. Conversely, a sudden *increase* in phenol concentration from 0 to $7.5 \times 10^{-4}M$ caused the bacteria initially to swim erratically with frequent tumbles (Fig. 1d) and then to relax back to a normal pattern after about 10 seconds. Thus repellents, like attractants, utilize a rudimentary memory device involving a comparison of past and present concentrations. These responses are just the opposite of those observed with an attractant, where a sudden increase in concentration causes suppression of tumbling and a sudden decrease increases the frequency of tumbling (*6*). The inverse relationships of a strong attractant (serine) and a strong repellent (phenol) can be seen in Fig. 1. The duration of the smooth response to a given decrease in concentration of repellent is greater than that of the tumbling response to the corresponding increase. This asymmetry has been noted for attractants (*4*, *6*).

Over a range of initial concentrations, a sudden fourfold decrease in concentration causes a significant decrease in tumbling. The lowest (threshold) initial concentration for which such a response is observed is shown for a number of compounds in the first column of Table 1. The threshold concentration that produces damage to the motility of the organism is much higher, as shown in the second column. The concentrations required to produce significant repellent reaction (10^{-5} to $10^{-3}M$) are appreciably higher than the corresponding concentrations for attractants, many of which are effective at $10^{-6}M$ (*2*, *3*).

To test the compounds under conditions of a spatial gradient the population migration apparatus was used with a step gradient (Fig. 2a). Under these conditions, the bacteria migrated from the higher to the lower concentration, producing after some time a distribution as shown in Fig. 2c. The equivalent response to an attractant is also given (Fig. 2b). A "transfer velocity" was then calculated from the rate of increase of the area of the bacterial peak appearing on the low concentration side of the repellent gradient (Table 1, column 3). The stimulus provided by the temporal gradient apparatus is much more extreme than that derived from the bacterial motility through spatial gradients; tryptophan and tyrosine, which elicited short responses even at high concentrations in the temporal gradients, failed to elicit responses in the spatial gradient. The relative order in the two types of gradient, however, remains the same. Thus acetate, propionate, and butyrate give roughly equal responses, whereas indole and phenol are much stronger than tryptophan and tyrosine. When a shallower spatial gradient was used, that is, one that decreased by a factor of *e* in 6.7 mm, only indole and phenol gave marked responses. The migration velocity of the population, defined previously (*5*), was 1.9 μm sec^{-1} for indole and 1.5 μm sec^{-1} for phenol, at plateau concentrations of $10^{-4}M$ and $10^{-3}M$, respectively. By comparison, the migration velocity was 2.8 μm sec^{-1} when a similar gradient of serine (an excellent attractant) was used.

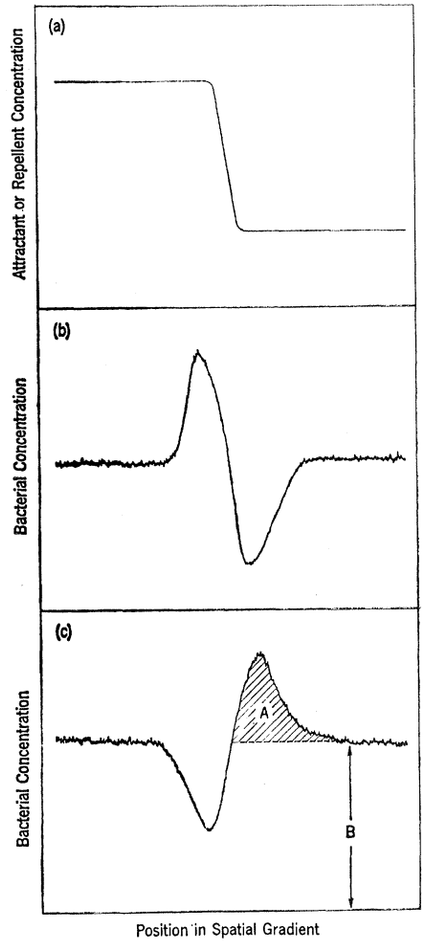

Fig. 2. Response of *Salmonella* to spatial gradients of chemicals in the population migration apparatus. (a) Form of the gradient (a step function). (b) Response to an attractant, serine, $10^{-3} \leftrightarrow 10^{-4}M$, after 8 minutes. (c) Response to a repellent, phenol, $10^{-4} \leftrightarrow 10^{-5}M$, after 8 minutes. Transfer velocities were calculated from the rate of increase of the hatched area A, divided by the mean bacterial concentration B.

To test whether repellent classes exist, competition experiments were performed in which a high uniform concentration of a compound was tested for its ability to inhibit the response to a spatial step gradient of a second compound in the population migration apparatus. (The classification in Table 1 is based on these experiments.) Thus, a tenfold excess of a compound from one class was capable of eliminating the bacterial response to a gradient of other compounds of the same class; for example, acetate eliminates the response to propionate. On the other hand, a compound from one class caused little or no inhibition of the response to the compounds in other classes; for example, phenol has little effect on the response to acetate. The existence of classes and the chemical similarity of members of a class suggest that receptors exist for repellents as well as attractants, and that the degree of specificity of these receptors is similar to that of attractant receptors and binding proteins involved in transport.

In order to examine the interaction between compounds, additivity experiments were carried out in the temporal gradient apparatus. The duration of a response of one type (either "smooth" or "tumbling") to a given gradient stimulus could be progressively reduced by a simultaneous opposing stimulus of increasing strength, and eventually replaced by a response of the opposite type. For example, the smooth response to a particular decrease in phenol concentration could be reduced by superposing either a decrease in serine concentration or an increase in acetate concentration. If the second stimulus was large enough, a tumbling response resulted. This is consistent with the idea of algebraically additive stimuli into a common mechanism.

It is reasonable to ask why certain compounds are attractants and others

are repellents. All of the attractants discovered so far are compounds such as amino acids and sugars capable of sustaining rapid cell growth, while repellents can all be associated with some deleterious effect. Phenol and indole are toxic at high concentrations, and are excretion products of tyrosine phenol lyase and tryptophanase reactions of organisms in soil and fecal matter (*13*). The inhibition of isoleucine biosynthesis by valine and hence inhibition of cell growth is well known (*14*). Acetate and other carboxylic acids are excretion products of fermentative metabolism and may be warning signals of crowded conditions. It was interesting that attractants act at considerably lower thresholds than repellents, but that the magnitudes of the responses above threshold are similar. These facts are in keeping with the respective significance of attractants and repellents to the organism. If highly toxic repellents are found, their taxis thresholds should be correspondingly low.

The relationship between chemotaxis and transport is still not clear. Chemotaxis toward an attractant is, however, a futile event if the compound is not subsequently transported and utilized, whereas chemotaxis away from a harmful repellent is a successful event in itself. Substances such as phenol and indole cannot be utilized as carbon sources by *Salmonella* (*15*), suggesting that the taxis binding protein may function solely as a sensory receptor devoid of any function in transport.

Our conclusions from this initial survey of repellents are the following. First, the response of bacteria to gradients of repellents is essentially the inverse of the response to attractants. In each case the favorable direction, whether a positive gradient of attractant or a negative gradient of repellent, causes decreased tumbling and hence a prolongation of travel in that direction. Second, competition experiments support the notion of discrete chemoreceptors with restricted specificity, as has already been found for attractants (*2, 9, 10*). Third, the additive nature of attractants and repellents suggests they may operate through a common mechanism. As has been discussed for attractants (*6*) this involves a biochemical "memory" which allows a past environment to be compared with a present one. Assuming that the initial event in chemotaxis is the binding of the chemical to a receptor protein, it is possible that the attractant-receptor complex of an attractant system is equivalent to the uncombined receptor of a repellent system and vice versa, but that subsequent elements of the two systems are identical and may in fact be shared with other tactic stimuli such as *p*H, oxygen, and (for photosynthetic organisms) light.

NORA TSANG
ROBERT MACNAB
D. E. KOSHLAND, JR.
Department of Biochemistry, University of California, Berkeley 94720

References and Notes

1. T. W. Engelmann, *Pflügers Arch. Ges. Physiol.* **30**, 95 (1883); W. Pfeffer, *Untersuch. Bot. Inst. Tübingen* **2**, 582 (1888).
2. J. Adler, *Science* **166**, 1588 (1969).
3. R. Mesibov and J. Adler, *J. Bacteriol.* **112**, 315 (1972).
4. H. C. Berg and D. A. Brown, *Nature* **239**, 500 (1972).
5. F. W. Dahlquist, P. Lovely, D. E. Koshland, Jr., *Nature New Biol.* **236**, 120 (1972).
6. R. M. Macnab and D. E. Koshland, Jr., *Proc. Nat. Acad. Sci. U.S.A.* **69**, 2509 (1972).
7. J. Lederberg, *Genetics* **41**, 845 (1956).
8. J. L. Smith and R. N. Doetsch, *J. Gen. Microbiol.* **55**, 379 (1969); R. N. Doetsch and W. F. K. Seymour, *Life Sci.* **9**, 1029 (1970).
9. G. L. Hazelbauer and J. Adler, *Nature New Biol.* **230**, 101 (1971).
10. R. Aksamit and D. E. Koshland, Jr., *Biochem. Biophys. Res. Commun.* **48**, 1348 (1972).
11. We thank J. Adler for the suggestion of compounds to study as repellents, based on work of W.-W. Tso and J. Adler (in preparation).
12. H. J. Vogel and D. M. Bonner, *J. Biol. Chem.* **218**, 97 (1956).
13. H. A. Barker, in *The Bacteria*, I. C. Gunsalus and R. Y. Stanier, Eds. (Academic Press, New York, 1967), vol. 2, p. 179.
14. H. E. Umbarger and M. Freundlich, *Biochem. Biophys. Res. Commun.* **18**, 889 (1965).
15. D. Gutnick, J. M. Calvo, T. Klopotowski, B. N. Ames, *J. Bacteriol.* **100**, 215 (1969).
16. Supported in part by NIH grant 8M-GM-09765.

12 March 1973

Induction and Ecological Significance of Gigantism in the Rotifer Asplanchna sieboldi

Abstract. *Dietary α-tocopherol and cannibalism together induce the giant, campanulate morphotype. Campanulates, unlike the two smaller female morphotypes, do not respond to α-tocopherol by forming male-producing offspring. Campanulate production probably is very significant ecologically, allowing a rapid, adaptive response to increased prey size and a rapid reproductive rate uninterrupted by sexuality and consequent dormancy.*

Asplanchna sieboldi is a large, planktonic, usually predatory, ovoviviparous rotifer that can reproduce both sexually and parthenogenetically (*1*) and which may exhibit extensive nongenetic polymorphism in female body size and shape (*2–7*). The mode of reproduction depends on whether mictic or only amictic females are present. Amictic females usually predominate and always produce diploid females parthenogenetically. The morphologically similar, mictic females produce haploid eggs which develop into males parthenogenetically or into thick-walled resting eggs if fertilized. The phenotypic plasticity of females is extraordinary and involves three basic morphotypes with intermediates: (i) a relatively small, saccate type; (ii) an intermediate-sized, humped or cruciform type (*8*); and (iii) a very large, campanulate type (Figs. 1 and 2). Each transformation from one morphotype to another occurs between two or more generations, the phenotype of a given female being fixed shortly before birth (*9*).

Dietary α-tocopherol (vitamin E) induces amictic saccates to produce cruciform and some mictic offspring (*6, 10*). Resting eggs always develop into amictic saccates (*3, 4*), and this type of female continues to reproduce itself as long as the diet contains subthreshold levels of tocopherol. The factors necessary for the induction of campanulates have not been determined, although their presence is associated with the previous existence of cruciforms and with cannibalism or the ingestion of large prey (*4, 5, 7*). In this study the effects of α-tocopherol and cannibalism on the induction and mode of reproduction of campanulates are examined.

Cultures of two clones of *A. sieboldi* were kept at 25°C in the dark and fed a diet of either *Paramecium aurelia* (*11*) or conspecifics. Stock and experimental cultures were transferred daily. Generation times of the rotifers under these conditions were about 30 hours. When the rotifers became adults bearing late-stage embryos, they were killed and fixed in 30 percent and then 70 percent ethanol; they were measured from a dorsoventral aspect with an ocular micrometer in a Wild M-5 stereomicroscope at 37 or 50 magnifications (*12*). The standard errors of the