Chemosensory Behavior in Protozoa

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I. INTRODUCTION

All cells respond to chemicals in their environment. Often these responses are changes in metabolism, membrane electrical properties, surface antigens or growth in reaction to the presence or removal of nutri-

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Table I Protozoan Chemosensory Behavior

Behavior	Organism	Stimulus	Reference
Accumulation	*Dunaliella*	NH_4^+; various amino acids	Sjoblad *et al.* (1978)
	Chlamydomonas	amino acids; fatty acids; inorganic salts	Hirschberg and Rodgers (1978)
	Euglena	Slime trails; O_2	Gunther (1927); Colombetti and Diehn (1978)
	Crypthecodinium cohnii	DOPA, glycine, carbachol; β-dimethylpropiothetin	Hauser *et al.* (1975)
	Astasia	Fatty acid; amino acids; casein; yeast	Mainx (1929)
	Peranema	Tryptone; inorganic acids	Chen (1950)
	Dileptus	Food	Chen (1950)
	Bodo	O_2; meat extract	Fox (1921)
	Trepomonas	Meat extract	Ziegler (1962)
	Polytoma	Meat extract; fatty acid	Ziegler (1962); Links (1955)
	Didinium	Bacterial excretion products	Wessenberg and Antipa (1970); M. Rentz and G. Antipa (personal communication)
	Oxytricha	Bacterial excretion products	G. Gibson and G. Antipa (personal communication)
	Paramecium caudatum	Weak acids; NH_4^+; particulate suspensions	Jennings (1906); Dryl (1973); Nakatani (1968)
	P. tetraurelia	Acetate, lactate, organic anions, NH_4^+	Van Houten (1976, 1978)
	Tetrahymena	Glucose (high levels); amino acids, especially thyroxine	Almagor *et al.* (1977); M. Levandowsky (unpublished results)
	Stentor	pH 6.9–7.7	Pietrowicz-Kosmynka (1971)
	Amoeba	*Tetrahymena* protein	Nohmi and Tawada (1974)
	Physarum	Sugars, amino acids	Kincaid and Mansour (1978a,b)
	Dictyostelium and other acrasins	cAMP folic acid; peptides (?)	Mato and Konijn (1979)

ents, hormones, or neurotransmitters. Cells that move as solitary unicells for at least part of their lives also include changes in motile behavior in their repertoire. Leukocytes migrate to wound areas directed by chemical cues; bacteria accumulate and disperse in response to chemicals; slime mold amebas aggregate by following gradients of attractants; a wide variety of protozoa alter swimming or creeping behavior in response to chemical stimuli. This chapter on chemoreception in protozoa is primarily concerned with chemosensory pathways leading to altered motile behavior rather than altered uptake of nutrients and consequent changes in metabolism.

The chemosensory pathways have a sequence of steps: (1) arrival of the chemical cue at the cell membrane, (2) association of the cue with its receptor (not necessarily at the membrane), (3) transduction of the chemical cue by other, internal signals, (4) eventual translation of these secondary signals into a response, usually a change in the motor activity of the cell, and (5) altered behavior of the cell or group of cells resulting from changed motor activity. We discuss what is known about chemosensory pathways in protozoa roughly according to, but not in order of, the steps in the above stylized pathway. First, we describe external chemical cues and the behaviors protozoa display in response to them. Second, we deal with motor organelles the cells use for the behavioral response and motile mechanisms of the behavior. Finally, we attempt in Section IV to describe the sensory organelles and consider intermediate steps in chemosensory transduction between putative receptors at the membrane of the sensory organelle and the motor response.

Since much of this information has recently been reviewed (Levandowsky and Hauser, 1978; Machemer and de Peyer, 1977) we will, therefore, instead of being exhaustive, attempt to point out common aspects among protozoan chemosensory responses and some parallels with metazoan systems, including the role of calcium and other ions, neurotransmitters, neurohormones, and membrane electrical changes controlling chemoreception and motility.

II. CUES AND BEHAVIORS

Known chemosensory behavior and chemical cues that elicit it are summarized in Table I. Behavior has been divided into four main categories: (1) accumulation, (2) dispersal, (3) host invasion, and (4) settling responses. Accumulation has been further divided into accumulation during mating and other behavior.

A. Accumulation

1. Accumulation during Mating Behavior

Many species of protozoa are known to mate and exchange nuclear material. In the process of mating, cells, usually of complementary mating types, make physical contact, adhere, and fuse. Some species require proper chemical stimuli before the mating process can proceed to adhesion and fusion. These stimuli are either soluble mating type substances that cause cells of complementary mating type to accumulate and to become mating reactive or the stimuli are membrane bound and require contact between the cilia or flagella for complementary cells to become prepared for adhesion and fusion.

In her survey of microbial mating interactions, Crandall (1977) observed that eucaryotic microbes responding to pheromones often can become mating reactive in rich nutritional conditions while cells becoming mating reactive after nutritional deprivation do not respond to soluble pheromones. *Tetrahymena*, as described below, and others may be exceptions and require both nutritional limitation and soluble factors, but, in general ciliates become competent to mate only during nutritional deprivation (Nanney, 1977). For example, in mixtures of two mating types of paramecia that are slightly starved, cells clump together and eventually form pairs that fuse and exchange nuclear material. The signals for clumping are membrane bound and probably are proteins (Metz, 1954). Cell-free filtrates of starved clones do not initiate the mating reaction in the complementary mating type, but preparations of membrane vesicles from cilia of cells of complementary mating type do initiate agglutination of cells (Kitamura and Hiwatashi, 1976, 1980). Isolated cilia from the complementary mating types will agglutinate in the absence of living cells (Takahashi *et al.*, 1974). Thus, in paramecia, the cues for aggregation as a prelude to mating seem to be bound to the surface of the cilia. Potassium in the external medium is necessary to maintain these cues or the ability to respond to them (Takahashi and Hiwatashi, 1974).

These cues can be bypassed with chemical induction of mating, namely exposure to inorganic ions (K, Mg, or Mn), low external calcium, and organic compounds, such as acriflavin (Cronkite, 1976, 1979). After a period of time comparable to the period of agglutination, paramecia cells of the same mating type can pair, fuse and exchange nuclear material. This process circumvents rather than mimics the signals that trigger aggregation and agglutination of cilia prior to pair formation. Therefore, chemical induction of *Paramecium* mating holds little information about

Accumulation for conjugation	*Blepharisma*	Gamone	Honda and Miyake (1975)
	Chlamydomonas	Gamone	Goodenough (1977)
	Oxytricha	Gamone	Esposito *et al.* (1976)
	Euplotes	Gamone	Sonneborn (1978)
Dispersal	*Crypthecodinium cohnii*	Epinephrine; norepinephrine; CO_2	Hauser *et al.* (1975, 1978)
	Stentor	Quinine-HCl; inorganic salts; pH extremes	Pietrowicz-Kosmynka (1972a,b)
	Tetrahymena	Salts; phenylthiocarbamide; strychnine; nicotine; picrate; other hydrophobic compounds	Tanabe *et al.* (1979)
	Paramecium caudatum	Inorganic cations; pH extremes; alcohols; quinine-HCl	Jennings (1906); Dryl (1973)
	P. tetraurelia	pH extremes; OH^-; quinidine-HCl; inorganic cations	Van Houten (1976, 1978)
	Amoeba	Inorganic salts	Jennings (1906)
	Physarum	Inorganic salts; KCN, cycloheximide, sugars (?)	Kincaid and Mansour (1978a,b)
Host invasion	*Entamoeba*	Polysaccharides	Barker *et al.* (1976)
	Trypanosoma cruzi	Vertebrate cells	Dvorak and Howe (1976)
	Leishmania mexicana	Serum; albumin; raffinose; other sugars	R. S. Bray (personal communication)
	Icthyophthirius	Fish slime	Lom and Cerkasovova (1974)
Recognition of surface-bound cues			
Food recognition	*Didinium*	*Paramecium* species	Wessenberg and Antipa (1970); Berger (1979a,b)
Mate recognition	*Tetrahymena*	Membrane protein (?)	Nanney (1977)
	Paramecium	Ciliary membrane protein (?)	Nanney (1977); Sonneborn (1978)
	Chlamydomonas	Flagellar receptor/antigen (?)	Solter and Gibor (1977); Goodenough (1977)

the chemoreception that elicits agglutination of cilia and clumping. Induction does, however, supply the receptor signals for the rest of the mating events. Among these signals is a Ca^{2+} influx (Cronkite, 1976, 1979).

Tetrahymena cells require not only a period of nutritional deprivation, and a period of initiation, but also a period of co-stimulation in the presence of cells of a complementary mating type before cells are competent to adhere, pair and fuse [for a review, see Nanney (1977)]. In general, during initiation the cells prepare for messages to and from complementary mating type cells during the next stage, co-stimulation. However, the presence of complementary mating type cells is not necessary at this time of initiation. Any one of many antibiotics, or changes of incubation medium, disrupt the process of initiation.

During co-stimulation, *Tetrahymena* cells appear not to acknowledge the presence of the complementary mating type, but the opportunity for cell–cell contact is essential for the progression to the next step of pair formation. If a mixture of cells of two mating types is shaken during co-stimulation, they will not become competent to mate. There appears to be a minimum amount of time of contact needed with the complementary cells' surface in order to receive and transduce the membrane-bound signal. Cell-free filtrates will not substitute for the undisturbed cell contact.

Tetrahymena canadensis initiation appears to be unusual in that competence for mating appears to spread from rare initial focal cells to others (Phillips, 1971). Although large aliquots of cells become competent, small aliquots have a chance of not including an initial cell and thus not becoming competent. The spreading factor, isolated from supernatant of starved cultures, is soluble, heat and cold stable, and not mating type specific.

Concentrating starved *Tetrahymena* cells by centrifugation and adding back the supernatant prior to mixing cell types does not disturb and co-stimulation process, whereas concentrating cells and resuspending in buffer apparently removes a soluble factor necessary in co-stimulation (Adair *et al.*, 1978; Wolfe *et al.*, 1979). This factor is neither an agglutinin nor sex attractant (see *Blepharisma* gamones below) because cell-free medium from reactive cell cultures will neither cause homotypic pair formation in the complementary mating type nor substitute for co-stimulation by complementary mating type cells.

Chlamydomonas gametes recognize gametes of the complementary mating type by factors bound to the surface of the gametic flagella (see for review Goodenough, 1977; Solter and Gibor, 1978). Isolated flagella or flagellar membrane vesicles from gametes initiate a mating agglutination of gametes of the complementary mating type, but mixed vesicles or flagella from both mating types will not agglutinate or precipitate without

living cells present (Bergman *et al.*, 1975; McLean *et al.*, 1974; Snell, 1976a,b; see Goodenough, 1977 for a review). Vegetative cells will not aggregate and their flagella will not cause gametic agglutination. The nature of the flagellar substance(s) is still elusive since there is no obvious change in flagellar morphology, gross protein profile, or organization between vegetative cells and gametes or between gametes of the two mating types. Membrane protein, however, is important in the reaction of *C. reinhardii* while both protein and carbohydrate have a role in agglutination of *C. moewusii*, as shown by loss of agglutinability with proteinase and neuraminidase treatment (Weise, 1974). This mating specific reaction of *Chlamydomonas* should not be confused with the acquisition of agglutinability of the tips of gamete flagella by linking with concanavalin A or antibodies.

Chlamydomonas mating-reactive gametes release a substance into the medium that causes cells of complementary mating type to adhere to each other. The gamone activity of *C. moewusii* is actually membrane vesicles complete with mastigonemes that are normally sloughed off the flagella (McLean *et al.*, 1974). Purified vesicles without mastigonemes are active as "gamones" whereas pure mastigonemes are not. The sloughing of vesicles may be incidental and have no real function in the mating reaction itself.

The ciliate *Blepharisma japonicum* of mating type I secretes a soluble substance (gamone I or blepharmone), a glycoprotein (Miyake and Beyer, 1974) that stimulates mating type II to become mating reactive and to produce gamone II (blepharismone) a small molecule related to tryptophan (Honda and Miyake, 1975). Blepharismone, in turn, is a chemoattractant to cells of mating type I, that is, cells of mating type I will accumulate in a drop of buffer with gamone II rather than in a surrounding pool of buffer with no gamone. Once cells are stimulated with gamones, they will adhere and pairs will form between cells of the same or complementary mating type.

Euplotes is a large hypotrichous ciliate genus of cells that characteristically creep by means of cirri, bundles of cilia modified for locomotion. Most species of *Euplotes* require cell–cell contact with the complementary mating type to become mating reactive and eventually pair [see Nanney (1977) for review]. Cells display a recognition of the presence of complementary cells by performing a courtship "dance" while becoming reactive. There are some species of *Euplotes* that do not require cell–cell stimulation for mating, but instead, similar to *Blepharisma*, they produce a soluble substance that causes pairs to form in certain other clones. Once stimulated by filtered media from complementary clones, pairs will form between cells of the same or different clones.

There are multiple mating types of *Oxytricha*. These cells dance, as do *Euplotes* spp., in the presence of cells of another mating type, and this dance can be shortened to immediate pairing in the presence of soluble factor (gamone) from cell-free filtrates of *any* other mating type (Esposito *et al.*, 1976). The gamones do not activate cells that produced them, but once stimulated by a gamone a cell is activated to mate with a cell of any other mating type. There apparently are as many gamones as mating types in this system, although none has been isolated. Nanney (1977) has speculated that unstable gamones are responsible for the chemoattraction that holds the dancing ciliates such as *Oxytricha* together during their courtship dance.

Still other protozoa utilize soluble mating factors. *Tokophyra*, anchored to the substratum, will stretch toward each other to mate, apparently in response to a diffusible signal between them (Sonneborn, 1978). Vorticellids have two forms of cells that fuse, a motile microconjugant and a stationary macroconjugant. The microconjugant recognizes and stops at the macroconjugant rather than at vegetative cells, perhaps recognizing a diffusible factor from the macroconjugant (Grell, 1973). The microconjugant then moves over the surface of the macroconjugant before attaching and fusing. The latter procedure may involve membrane bound chemical cues.

Our discussion of chemical cues that trigger accumulation has ranged from small aromatic compounds to membrane-bound proteins. These varied examples are included because they all involve the sensory transduction of a chemical contact with the cell membrane into signals that change the cell behavior. These contacts most often involve the membranes of cilia or flagella. The role of cilia and flagella in sensory transduction is discussed in Section III.

2. Accumulation Not Related to the Mating Process

Accumulation other than during the mating process generally seems to work for the protozoan cells' advantage by moving them into areas of less unfavorable or optimal conditions for nutrients, osmotic pressure, oxygen pressure, or for slug or plasmodium formation by cellular and acellular slime molds. Several examples given below, however, do not have obvious advantages or functions for the cells. Nonetheless, these examples may provide important information about the mechanisms by which cells talk to each other.

Jennings (1906) recorded the accumulation of paramecia in zones of optimal pH, salt, and temperature over 80 years ago. He assayed chemoaccumulation and dispersal by observing numbers of cells collecting in or outside of a drop of test solution in a thin layer of control solution

on a slide. Dryl (1959, 1973) improved this method by adding grids and counting cells in photographs of the slide, and cateloged more chemicals, including alcohol and ionic repellents. Nakatani (1968) used a capillary method similar to that used by Pfeffer (1884, 1888) for bacterial chemokinesis, in which the number of cells (measured by densitometry) swimming up into a capillary filled with test solution is compared with the number of cells swimming up into a capillary of control solution.

Most recently a T maze assay has been developed to quantify the *Paramecium* response (Van Houten *et al.*, 1975) (Figure 1). The T maze presents a control and test solution to a population of cells in control solution. Test and control solution differ by only one set of anions or cations, so that attraction or repulsion from a solution can be attributed to one ion species. The number of cells swimming into the test arm divided by the number of cells swimming into either test or control arms (T/T + C) serves as an index of chemokinesis. The T maze has been used to show that *Paramecium tetraurelia* responds to bacterial fermentation and excretion products, and to determine specificity of some responses to these attractants, including the anions acetate, lactate, propionate, and folate, and ammonium ion (Van Houten, 1976, 1978). There appear to be two mechanisms of accumulation by *P. tetraurelia*. Correspondingly attractants fall into two categories. These mechanisms and categories will be discussed in more detail in Section III,E.

Paramecia are food *Didinium* (Figure 2) and the latter ciliates can apparently seek out and accumulate near their food sources by using chemical cues (Wessenberg and Antipa, 1970). The didinia probably indirectly de-

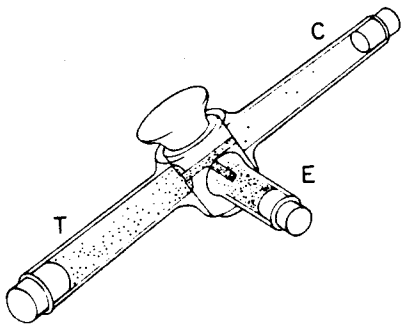

Figure 1. T-Maze assay for chemokinesis of *Paramecium*. Cells in control solution are loaded into entry arm E. Test solution fills T arm. Control solution fills C arm and plug. Stopcock is opened and the cells swim into the plug where they are presented with the test and control solutions. After 30 min the stopcock is closed and the cells in the T, C, and E arms are counted. The number of cells in T divided by the number of cells swimming into either T or C gives an index of chemokinesis. Attraction to the T solution, > 0.5; repulsion from the T solution, < 0.5. (From Van Houten *et al.*, 1975, reprinted with permission.)

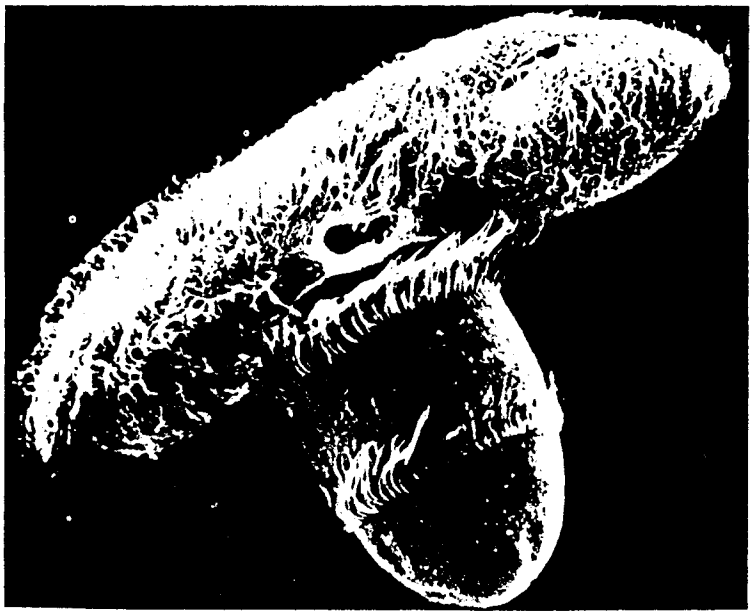

Figure 2. *Didinium* engulfing a *Paramecium*. (Courtesy of G. Antipa, with permission.)

tect populations of paramecia by sensing some substance excreted by the paramecias' food stuff, bacteria. Using the T maze developed for *Paramecium*, Rintz and Antipa were able to demonstrate that didinia are attracted into the arm of the T containing bacterial excretion products in bacteria-modified medium in preference to the arm of the T with medium alone. The activity of the attractant(s) has been traced to a heat stable molecule of between 500 and 1000 daltons (M. Rintz and G. Antipa, personal communication).

Didinia do not detect individual paramecia well if at all by diffusable cues, which is in keeping with attraction of didinia to *Paramecium's* food rather than to populations of paramecia themselves. Individual paramecia occasionally swim close by a didinium without the predator reacting. Didinia can, however, detect individual paramecia from surface chemical cues (Wessenberg and Antipa, 1970). *Didinium* will not ingest every particle that makes contact with its proboscis, but is selective for its food stuff. Contact of the proboscis with prey triggers the ejection of pexicysts, which may serve a sensory function for food recognition, followed by the ejection of toxicysts and ingestion of the prey. The feeding process does not proceed beyond pexicyst ejection if the didinia contact dead paramecia (Wessenberg and Antipa, 1970). Didinia even show preference for feeding on the species of *Paramecium* with which they had previously

been grown (Berger, 1979a). This preference may have a basis in recognition of surface-bound cues and also in size constraints from the didinia's previous growth history (S. Hewett, personal communication).

G. Gibson and G. Antipa (personal communication) observed attraction of *Oxytricha*, similar to attraction of *Didinium*, to bacterial product(s). Unlike *Didinium*, *Oxytricha* feed on bacteria and therefore are attracted to cues directly produced by their food stuff.

The dinoflagellate *Crypthecodinium cohnii* tends to embed in agar gels when grown in biphasic media. This behavior was used in assaying chemoreception by this species (Hauser *et al.*, 1975a). In this assay method, a suspension of cells is layered over both control agar sections and sections with test substances added to the agar. In this situation, *C. cohnii* cells preferentially embedded in agar containing seaweed extracts, as well as L-fucose, dimethyl-β-propiothetin, and betaine, all found in rotting seaweeds, the natural substrate for this saprophytic species (Beam and Himes, 1980). These responses appear adaptive for finding nutrient sources. These organisms, however, also embed in response to low concentrations of dihydroxyphenylalanine (DOPA), glycine, and carbachol, and avoid agar with extremely low concentrations of other neurohormones (Hauser *et al.*, 1975b); we can only speculate about the possible significance of such responses. Using a different method, it was later shown that this species is repelled by a concentration gradient of dissolved carbon dioxide; this response might perhaps serve as a dispersal mechanism in nature.

Spero (1979) studied chemosensory responses of a voracious phagotrophic dinoflagellate, *Gymnodinium fungiforme*, to shrimp extract and amino acids, using capillary methods. The most sensitive responses were to L-serine, L-taurine, and L-glycine, thresholds of 10^{-8} *M* (Table I). Of 13 other dinoflagellate species tested, Spero found chemosensory responses to shrimp extract only in *Crypthecodinium cohnii* and a *Gyrodinium* sp. A response could be induced in *Oxyrrhis marina* by culturing it phagotrophically on living *Dunaliella salina* cells for 72 hr.

It may also be that chemoreception plays a role in the bioconvective patterns often seen in dense cultures of many swimming protozoa (Levandowsky *et al.*, 1975a), although this has not been shown. In very dense old shallow cultures of *Tetrahymena*, with depth much less than the threshold for bioconvection, regular patterns of swimming cells sometimes form that appear to be due to chemosensory behavior (Levandowsky and Hauser, 1978).

Cellular slime mold amebas' chemoreception has been widely studied. *Dictyostelium* and *Polysphondylium* amebas accumulate in the vicinity of folic acid (Pan *et al.*, 1974). During the ameboid stage of the life cycle, the

cells feed on bacteria which probably excrete folic acid. Therefore, movement of amebas toward folic acid should aid in their search for food. When bacteria are depleted, a few amebas of *Dictyostelium* will secrete cyclic AMP (cAMP) in pulses. These pulses of cAMP affect the direction of movement of other amebas and initiate the rhythmic excretion of cAMP from these cells. Hence, cells move inward toward the focal cell and stimulate other cells to follow. Not all slime mold attractants or *acrasins* are cAMP. *Polysphondylium* does not respond to cAMP and its acrasin, probably a small peptide (Wurster *et al.*, 1976) does not affect *Dictyostelium* [see for reviews Mato and Konijn (1979); Konijn (1975); Newell (1977)].

Amebas of the myxomycete *Physarum* differentiate into plasmodia when their food supplies of bacteria run out. The *Physarum* amebas communicate with each other during aggregation and conversion into plasmodia. Cells in the process of differentiation can induce other cells separated from them by a filter to differentiate and form plasmodia (Youngman *et al.*, 1977). The inducer is a diffusible substance, at present uncharacterized. The process of differentiation of myxomycetes into plasmodia is reminiscent of the aggregation of slime mold amebas into slugs and the mating reaction of ciliates that also occurs when food becomes scarce. The plasmodium of *Physarum* is motile and shows oriented movement toward sugars and amino acids (Kincaid and Mansour, 1978a,b) (Figure 3). The sugars need not be metabolizable to be attractants.

There are many reports of other protozoa accumulating near sources of diffusible compounds. Among the euglenids, *Astasia* is attracted to fatty and amino acids (Mainx, 1928); *Peranema* is attracted to casein, yeast, tryptone, and inorganic acids (Chen, 1950); and euglenas follow slime trails of other cells (Gunther, 1927) and accumulate in areas of favorable oxygen concentration (Colombetti and Diehn, 1978). The kinetoplastid *Bodo* aggregates in favorable concentrations of oxygen and meat extract, asparagine, dextrin, and KCl (Fox, 1921). *Trepomonas, Polytoma,* and a *Chlamydomonas* species are attracted to meat extract as well [see Ziegler (1962) for a review]. In each of these last three examples, the organisms are phagotrophic and can feed on meat extract such as peptone medium and their attraction to extract or components of extract would be an advantage in locating their food source. The volvocids *Polytoma* and *Chlamydomonas* are abundant in sewage and both are attracted to fatty acids (Pringsheim, 1921; Pringsheim and Mainx, 1926; Links, 1955; Hirschberg and Rodgers, 1978). *Amoeba* will accumulate in the vicinity of protein extracted from its food organism *Tetrahymena* (Nohmi and Tawada, 1974). The protein was identified by observing the number of amebas that move into a capillary tube with protein relative to a control capillary tube without protein.

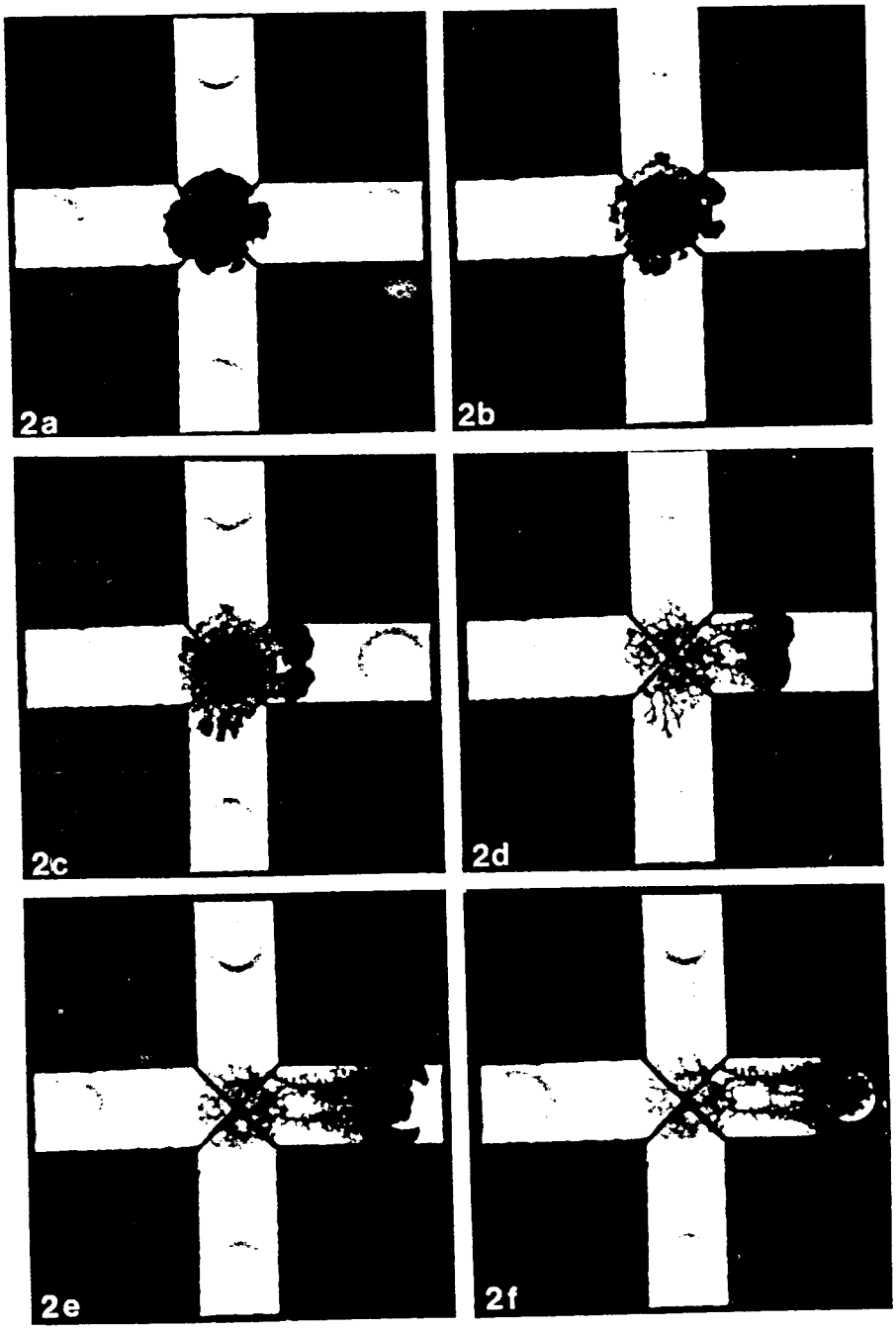

Figure 3. Time course of chemotaxis of *Physarum* toward (3-*O*-methyl)-D-glucose. Panels represent 0.5-hr intervals starting from 2.5 hr. *Physarum* moves toward the pad with attractant and not toward other pads containing only buffer. (Courtesy of Kincaid and Mansour, with permission.)

Chemoaccumulation of the marine green alga *Dunaliella* has been quantified by a capillary technique similar to that used for measuring bacterial chemokinesis (Sjoblad *et al.*, 1978). *Dunaliella* are strongly attracted to capillaries of buffer with ammonium ion, L-tyrosine, L-tryptophan, and L-phenylalanine relative to buffer without these compounds. L-methionine and L-cysteine are weakly attractive while other amino acids, carbohydrates, B vitamins, urea, and nitrate did not elicit accumulation responses. The failure to find all amino acids and NaCl attractive eliminates the possibility that the cells were attracted to the optimal ionic strength for motility, as opposed to sensing specific compounds. Competition experiments described later in Section III,B also support the idea that the cells are detecting specific compounds and are not responding with general increased motility in optimal ionic or osmotic conditions.

In most of these cases of accumulation there is some association between the food source of the organism and the compounds that elicit the accumulation response. Often the attractant compounds are excretion products of the food organisms or are breakdown products of components of the food. Most often the attractants are diffusible substances, but there are cases of cell surface cues that filter feeders or suctorians use to differentiate food and nonfood particles (see below). The role of attraction of the dinoflagellate *Crypthecodinium* to neurohormones and related compounds may also eventually be shown to be related to feeding, but at present its function, if any, is not known.

Phagotrophic ciliates can be divided into two groups by feeding behavior: gulpers and swirlers (Machemer and de Peyer, 1977). Didinia are gulpers and apparently detect populations of prey by diffusible cues and individual prey by surface-bound cues (see discussion in Section II,A). *Paramecium* and *Stentor* are among the filter feeders that swirl food particles into a vacuole by the beating of cilia in the oral groove. Not all particles are whirled indiscriminately into the vacuole. Rather, nonfood particles, such as carmine are sorted out (Jennings, 1906; Tartar, 1961). If chemical cues are involved, the cues that affect this behavior are probably on the surface rather than diffusible. Though not an attraction or accumulation, this feeding behavior is included here because most of the accumulation behavior described above arises from food cues.

B. Dispersal

Dispersal, strictly speaking, is inseparable from accumulation, since each example of accumulation necessarily includes dispersal from a relatively "unattractive" solution. Dispersal will, however, be treated separately from the accumulation responses; in particular swimming up a gra-

dient of attractant may not always have an equivalent mechanism to swimming down a gradient of repellent (see Berg, 1975, for a bacterial example).

Crypthecodinium accumulates in areas containing DOPA but is repelled from areas of epinephrine and norepinephrine at exceedingly low concentrations (Hauser *et al.*, 1975b). The functions of these behavioral responses, benefits to the cell, or even possible interference with normal cell function are not known. The level of epinephrine detected by the cells is small (perhaps, on the basis of crude estimates, as low as one molecule per cell) and argue for a specific function in the cell rather than a behavioral response as an artifact caused by an overwhelming amount of the compound. The intriguing problem of the roles of neurohormones in the cells is discussed in Section IV.

Paramecia disperse away from solutions of high cation concentration, pH extremes, quinine, quinidine, and alcohols (Jennings, 1906; Dryl, 1973; Van Houten, 1976, 1978). Dispersal behavior may function to remove cells from toxic conditions (Dryl, 1961a), but toxicity alone is not sufficient for repulsion: the cells must change their swimming behavior and not merely become sick in the solutions in order to be repelled (Jennings, 1906; Van Houten, 1976).

Like paramecia, amebas will move away from areas of high salt (Jennings, 1906). However, motility, and hence mechanisms of dispersal of paramecia and amebas, are different and are discussed in Section III.

The ciliate *Stentor* can be sessile or free swimming. The free-swimming cells are repelled from areas of quinine or high salt solution (Pietrowicz-Kosmynka, 1971, 1972a,b). After being adapted in high concentrations of potassium, the cells will no longer respond to repellent stimuli (Pietrowicz-Kosmynka, 1972a). This adaptation is reversible and cells again disperse from repellents after one or more hours in low potassium solutions. Adaptation to high potassium concentrations is more accurately called desensitization, making the cell unresponsive to most stimuli, and should not be confused with the adaptation that is a common feature of steady state behavior discussed in Section IV.

Both sessile and free swimming *Stentor* cells react to mechanical stimuli by contracting (Wood, 1975; Huang and Mazia, 1975). After repeated stimulation, the sessile cells may detach and swim off (Jennings, 1906). The cells then settle in a new area. Sessile *Stentor* has no known similar escape response to chemical cues.

Myxomycete plasmodia show negative chemotaxis to inorganic salts (Coman, 1940; Kincaid and Mansour, 1978a), potassium cyanide, and cycloheximide (Durham and Ridgway, 1976). There are conflicting reports about the repellent properties of the sugars sucrose, ribose, and fructose

(Hato *et al.*, 1976; Durham and Ridgway, 1976; Kincaid and Mansour, 1978a,b). The differences in results may be due to different assay conditions. In the chemotaxis assay of Kincaid and Mansour (1978b), the plasmodia are weakly attracted to these sugars and this attraction has no relation to the ability of the sugar to support growth of *Physarum*. Except in the possible cases of repulsion by sugars, repellents appear to be signaling unfavorable conditions to the plasmodium.

It is not clear whether most protozoan dispersal behavior has a function in the wild or is a laboratory phenomenon. Vegetative amebas of the cellular slime mold are repelled from each other by a diffusible substance (Keating and Bonner, 1977). Most reported cues eliciting dispersal are conditions such as high salinity, extremes in pH, unfavorable ionic or osmotic strength, and conditions unfavorable for the growth of food organisms.

As noted above, the dinoflagellate *C. cohnii* swims down concentration gradients of carbon dioxide. The "aerotaxis" observed in wet mounts of preparations of this species, in which dynamic aggregates of cells form around bubbles or at the edge of the coverslip, proved to be due not to an attraction to oxygen but rather to an avoidance of carbon dioxide, produced presumably in the organisms' metabolism (Hauser *et al.*, 1978).

C. Host Invasion

Protozoa can live as parasites in hosts from mosquitoes to man. There is evidence that these protozoa use chemical cues to facilitate the invasion of a proper host. The ciliate *Icthyophthirius* parasatizes fish and is attracted to agar impregnated with fish slime (abstract, Lom and Cerkasovova, 1974). The advantage of this attraction is obvious.

Entamoeba, the cause of human dysentery, is attracted to carbohydrates (Barker *et al.*, 1976), which, perhaps, could serve as cues for the successful invasion of host tissues.

Trypanosoma cruzi, the cause of Chagas' disease, is attracted to cultured vertebrate cells (Dvorak and Howe, 1976). The trypanosomes preferentially invade heart tissue of the host and the mastigotes appear to reflect this preference in stronger attraction to muscle over Hela cells in culture. Robert Bray (personal communication) has adapted the Boyden chamber assay technique used with leukocyte chemotaxis to the study of another kinetoplastid parasite, *Leishmania mexicana*. Two fractions from fetal calf serum proved attractive to culture forms (corresponding to the insect stage of the parasite); preliminary fractionations indicate the presence of at least two active principles, one of which is probably albumin. Several sugars were also attractive, in particular the trisaccharide

raffinose, at very high concentrations (corresponding perhaps to very high concentrations seen in the insect crop at times).

D. Settling Responses

Some protozoa have sessile stages in their life cycle. Settling and development of the sessile form are likely to involve recognition of chemical cues for the proper substrate. Langlois (1975) reported settling of the marine ciliate *Vorticella marina* on particular algae. The preferences among algae can be paralleled with algal excretion products affecting the choice of substrates of the ciliates and survival in the laboratory. The relationship between choice of substrate and survival is not completely clear, however, and this will be an interesting area of future study. Other flagellates and ciliates such as *Stentor* settle, but the means of recognizing a suitable settling spot are not known.

II. Motor Organelles and Mechanisms of Motile Behavior

Discussion of unicellular chemosensory responses in modern terms requires careful attention to the details of motility. Although several aspects of protozoan locomotion are treated in detail in other chapters of this treatise, it will be helpful for what follows to review the subject briefly here, with particular attention to aspects of locomotory morphology and physiology that are intimately involved in chemosensory responses.

Although protozoa display a rich variety of locomotory behavior in response to chemicals, the underlying motility can be divided into essentially four types: (1) ciliary/flagellar movement; (2) ameboid movement; (3) contraction and change of body shape; (4) gliding mediated by no apparent organelle.

A. Cilia and Flagella

The structural components of flagella and cilia are better known than the molecular and physical mechanisms within the cilia and flagella that produce the bending movement of the organelles [for reviews see Warner and Satir (1974); Satir (1976); Linck (1976); Dute and Kung (1978)]. It is generally accepted that neighboring microtubule doublets slide relative to each other and that this sliding is accompanied by the ATP-mediated breaking of dynein arm links to neighboring doublets (Satir, 1976; Warner and Satir, 1974). The outer nine doublets are connected to the inner pair of tubules by radial spokes. Since these spokes are perpendicular to the

inner tubules, they may be the structures that resist the sliding, converting the sliding of the outer doublets into a bend of the entire cilium (Warner and Satir, 1974; Warner, 1977). *Chlamydomonas* mutants lacking radial spokes are paralyzed, adding support to this argument (Witman *et al.*, 1978; Luck *et al.*, 1977).

The central tubules of *Opalina* are in a fixed orientation to the direction of bend of the cilium (Tamm and Horridge, 1970). This requires that the central tubules twist or rotate to remain normal to the bend. There is recent evidence that in *Paramecium* the central tubules do indeed twist during the ciliary beat (Omoto and Kung, 1979). The central doublet rotates a full 360° per beat and by its rotation may coordinate the pairs of outer tubules that either by sliding or some other mechanism cause the cilium to bend. It is not yet known whether this twisting is active or passive or whether the twisting causes or is caused by the bending that moves the cilium.

In many flagellate groups flagella range in number from one to four, varying in position on the cell. Anterior flagella are used to pull the cell forward in a breaststroke fashion as with the two flagella used by *Chlamydomonas*, in a whipstroke as with the single flagellum used by *Peranema*, in a sinusoidal wave leading the cell as used by the chrysomonads *Ochromonas* and *Chromulina* and certain trypanosomatids, or a backward-pointing spiral as in *Euglena* (see Jahn and Bovee, 1967; Jahn and Votta, 1972; and Figure 4). Trypanosomes have a flagellum attached to the cell body for part or all of the length of the cell by an undulating membrane. Trichomonads likewise have a flagellum attached

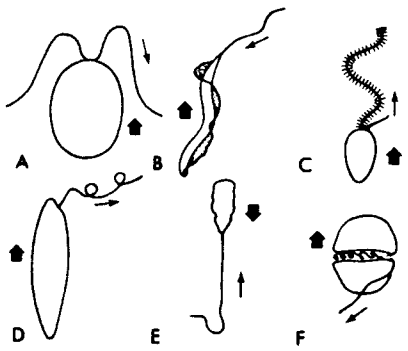

Figure 4. Varieties of wave forms in flagellar motility. Thin arrow indicates the direction of flagellar wave movement; fat arrow indicates the direction of cell movement. (A) *Chlamydomonas*, distally directed, planar wave; (B) *Trypanosoma*, basally directed, planar wave; (C) *Ochromonas*, distally directed, planar-pulling wave; (D) *Euglena*, distally directed helical wave; (E) *Peranema*, basally directed helical wave; (F) *Cryptthecodinium*, circumferential, helical wave. [See Jahn and Bovee (1967) and Grell (1973).]

to an undulating membrane in addition to four anterior flagella. Flagella extending beyond the posterior can push the cell forward as does the planar wave of the trailing flagellum of *Crypthecodinium* (Figure 4). Helical waves are also seen in the transverse flagellum of this dinoflagellate (Figure 4). The green alga *Platymonas* moves by a breaststroke beating of two anterior flagella. The flagella sit in a depression that limits the amount of force generated by the beating flagella. At the base of the flagella is a pair of rhizoplasts, myofibril-like striated structures capable of contraction and in a position to modify the form of the flagellar beat by altering the shape of the depression as they contract (Salisbury and Floyd, 1978). Algae not having flagella in a depression do not have the rhizoplast; the rhizoplast may be involved in overcoming the physical constraints on the power stroke of flagella in depressions like those of *Platymonas*.

Ciliates display an amazing assortment of arrangements of cilia that are used to propel the cell and to move food particles into a gullet or oral apparatus. Somatic (nonoral) cilia generally beat in an oarlike manner. During the power stroke the cilium is rigid and moves toward the posterior in one plane; during the recovery stroke the cilium is more bent and moves back toward the anterior almost circumscribing a semicircle in other planes. Unlike some flagella that continually undulate, cilia require a recovery stroke to complete the beat cycle.

There are three basic arrangements of cilia. *Opalina* and euciliates such as *Paramecium*, *Tetrahymena*, *Stentor*, and *Spirostomum* have cilia covering most of the body and may have oral cilia arranged in special membranelles. Hypotrichous ciliates, such as *Euplotes* and *Stylonichia*, swim as other ciliates, but also have the ability to walk or creep along the substratum by tufts of fused ventral cilia called cirri. Peritrichous ciliates are stalked and bell shaped with cilia arranged into membranelles coordinately beating to force food particles into a funnel-like peristome. Peritrichs are capable of rapid, but limited movement by contraction of the stalk that attaches them to the substratum, as discussed in Section III,B, and like *Vorticella* also have a free-swimming form with an additional posterior ring of cilia for movement.

A picture of membrane electrical control of ciliary activity has emerged from the electrophysiological study of several very different ciliates. Despite the different body shapes and arrangements of cilia in *Paramecium*, *Opalina*, *Euplotes*, and *Styloychia*, the control of cilia is basically the same (Naitoh and Eckert, 1974; Eckert and Naitoh, 1972; Epstein and Eckert, 1973; de Peyer and Machemer, 1977, 1978a). The frequency and angle of ciliary beating that determine speed of swimming are functions of membrane potential (more accurately, a change of membrane potential away from the resting potential) and calcium conductance (Machemer and Eckert, 1975;

Eckert *et al.*, 1976). A small change in the hyperpolarizing direction will increase the frequency and angle of ciliary beating to increase speed; a small depolarization will decrease frequency and change angle to decrease speed (Machemer, 1974; Eckert and Machemer, 1975). Speed can be altered in *Paramecium* by a mutation that increases resting membrane potential through altered resting potassium permeability (Satow and Kung, 1976). Cells will spontaneously change direction by transiently backing up and then moving forward again in a new, random direction [the avoiding reaction described by Jennings (1906)]. The transient reversal of cilia that causes the transient backward swimming corresponds to a calcium action potential. Calcium carries the active current and increased internal calcium causes the cirri or cilia to reverse (Eckert, 1972; Oertel *et al.*, 1977; de Peyer and Machemer, 1978a,b; see Figure 5). For example, in *Paramecium*, calcium above 10^{-6} *M* will cause reversed beating of the cilia, as demonstrated in detergent-treated cells (Naitoh and Kaneko, 1972, 1973). Magnesium and ATP allow these essentially dead leaky cells to swim forward; addition of calcium above 10^{-6} *M* will cause them to swim backward. Both speed and direction of detergent treated cells are dependent on calcium concentration, and, hence, upon calcium concentration within the cilium (Naitoh and Kaneko, 1972).

Electrical and ionic control of motility in flagellates is not as well established as in ciliates. They are more difficult to impale with electrodes for standard electrical measurements because of their size, and, in some cases, their hard coverings. Nichols and Rikmenspoel managed to impale *Chlamydomonas* and *Euglena* and determined that injection of Mg^{2+} increases frequency of flagellar beating while injection of Ca^{2+} decreases frequency (Nichols and Rikmenspoel, 1977, 1978). Hyams and Borisy (1978) eliminated injection problems by isolating the *Chlamydomonas* flagellar apparatus that retains the ability to beat the flagella independent of the cell body. In the presence of more than 10^{-6} *M* Ca^{2+} the flagellar beat frequency changes and flagellar beating form changes to that charac-

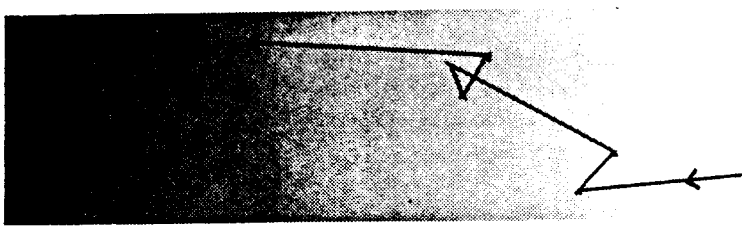

Figure 5. Schematic representation of a cell swimming up a gradient of attractant. The cell decreases the frequency of turning as it detects increasing attractant concentration and will be attracted by klinokinesis. Note increased mean free path in swimming up gradient.

teristic of backward swimming. If the apparatus is made leaky with detergent, the flagella reverse in 10^{-7} *M* Ca^{2+}. This is similar to the dependence on concentrations greater than 10^{-6} *M* external calcium for the living cells to reverse flagellar beat in response to a light stimulus (Schmidt and Eckert, 1976). It can be construed from these studies that steadily increasing internal Ca^{2+} first decreases beat frequency and eventually reverses the beat direction, in much the same way as in *Paramecium* (Naitoh and Kaneko, 1973). The role of Mg^{2+} in ciliate and flagellate beating, however, is not yet clear. Frequency and direction can be modulated in the absence of external magnesium (see Eckert *et al.*, 1976 and Eckert and Machemer, 1975, for discussions). Mg^{2+} possibly plays an opposing role to Ca^{2+} in the intact cell with decreasing amounts enhancing beat reversal (Nakoka and Toyotama, 1979). (See Section IV,D, for further discussion of calcium in chemoreception.)

B. Ameboid Movement

Cells lacking cilia or flagella sometimes locomote by organelles called lamellipodia or pseudopodia, sheet-like extensions of the cytoplasm. The lamellipodia, appropriately referred to as ruffles, slowly wave in the medium and make temporary attachments to the substrate. When several ruffles have attached, the cell becomes spread and stretched. One or two of the temporary ruffles' attachments retain contact with the substrate and the rest collapse back into the cell. The cell then pulls in its rear and moves into its new leading edge by waves of contraction and moves forward [for reviews, see Komnick *et al.* (1973); Revel *et al.* (1978); Lazarides and Revel (1979); Hitchcock (1977); Clarke and Spudich (1977); Allen and Allen (1978)].

Ameboid protozoa have many variations of this basic movement. The lamellipodia can take on different characteristic shapes and hence different names (Grell, 1973). Essentially, these organelles allow the cell to creep in ameboid fashion along the substrate. Cells moving in this fashion include free and parasitic amebas, cellular slime molds, myxomycete plasmodia and amebas, sporozoan parasitic ookinetes, and some members of Rhizomastigida that have both flagella and pseudopodia (Jahn and Bovee, 1967).

The molecular mechanism of movement by ruffling is still a puzzle but at least several pieces of the puzzle have been identified (Larazides and Revel, 1979; Hitchcock, 1977; Clarke and Spudich, 1977; Hellewell and Taylor, 1979). Filaments of actin and myosin and associated proteins are present in motile cells and the filaments change structure with the degree of motile activity of the cells. In nonmotile cells, actin filaments are or-

ganized in bundles, some of which attach to cell membranes at sites of cell adhesion to substrate and very few of which are found in ruffled membranes. Actively motile cells' actin is organized into a meshwork particularly in the ruffles and in association with the cell membrane. The meshwork may be comparable to actin gels in cytoplasmic extracts which contract in the presence of myosin and calcium (Allen and Allen, 1978). This suggests that cell motility is achieved by the reversible contraction of the actin meshwork in the presence of myosin and calcium. How contraction accounts for the mechanisms of ameboid movement by producing endoplasmic streaming, fountain zone contraction, or membrane receptor capping is still a matter of dispute [for discussion, see Komnick *et al.* (1973); Stossel (1978); Allen and Allen (1978); Hellewell and Taylor (1979)].

Much of the work establishing this model of nonmuscle cell contraction and movement has been performed on protozoa such as *Physarum* (Jacobson *et al.*, 1976; Hatano and Owaribe, 1976), *Amoeba* (Taylor, 1976), and *Acanthamoeba* (Pollard *et al.*, 1976; Gordon *et al.*, 1976), on the cellular slime mold *Dictyostelium* (Cooke *et al.*, 1976), and on tissue culture cells (Lazarides and Revel, 1979). A picture of nonmuscle motility is emerging with components common to cells from protozoa to platelets (Nachmias and Asch, 1976). (See Sections IV,C–E for discussion of roles of membrane electrical properties and calcium in ameboid movement.)

C. Change of Body Shape as Limited Movement

The previous section dealt with cells that constantly change body shape by putting out ruffles and pseudopodia during locomotion. Many other protozoa have well defined body shape that can be altered in a specific way by contraction or extension in response to stimuli. Contraction is a much more limited movement than free swimming or ameboid creeping, but is included in a discussion on motility because it is a characteristic protozoan motor behavioral response to external stimuli, including chemicals.

For hypotrichous and peritrichous ciliates, contraction is similar to an avoiding reaction, a means of quickly removing most of itself from the area of a stimulus. This behavior has particularly obvious value to those peritrichs that contract into a protective hard lorica.

Heterotrichous ciliates, such as *Spirostomum* and *Stentor*, are capable of both swimming and contraction (Huang and Mazia, 1975). *Spirostomum*, a long ciliated cell that swims by beating of its body cilia, is able to shrink to less than half its length by the contraction of myonemes, struc-

tures resembling muscle filaments. *Stentor* swims freely or takes on a sessile form that uses its cilia not to swim but to collect food. In the sessile or free form *Stentor* is able to contract into a spherical shape, also by means of myoneme filaments.

Peritrichous ciliates, such as *Vorticella*, have a free-swimming form and a sessile stalked form. Within the stalk, is a structure called a spasmoneme, biochemically quite different from a myoneme, that can contract into a coiled or zig-zag shape to shorten the cell stalk and, hence, the overall length of the cell (Amos, 1975). *Zoothamnium* has a colony of individuals connected to a single branched spasmoneme system. Contraction occurs simultaneously throughout the system (Amos, 1975).

Heterotrich and peritrich contraction are similar in that they are rapid, extreme, and mediated by identified contractile fibers. The ciliate groups differ in the arrangements of fibers: in peritrichs, the fibers are restricted to the stalk, a contractile organelle with a dense filament and an elastic sheath; in the heterotrichs, the fibers are in bundles localized in the cell cortex and associated with ribbons of microtubules that are probably responsible for reextension of the cell after contraction (Huang and Mazia, 1975).

Both spasmoneme and myoneme contraction are dependent on calcium and not on ATP. Both systems may use the electromotive force generated by a calcium gradient for the energy for contraction. In neither type of fiber is the molecular basis of contraction understood. The spasmoneme proteins have been isolated and actin and tubulin can be no more than minor components (Amos, 1975). Therefore, the mechanism of contraction will undoubtedly be different in many respects from muscle contraction.

D. Gliding

There are motile protozoa with no apparent motor organelle (Grell, 1973; Jahn and Bovee, 1967). It remains to be determined how sporozoan ookinetes make helical movements or how gregarines and eugregarines glide. However, ultrastructural studies of gliding and nongliding eugregarines establish the correspondence of the ability to glide with the presence of undulating pellicular folds (Vavra and Small, 1969). Eugregarines unable to glide have folds that are fused at the tip or completely missing while gliding eugregarines have pellicular folds that are free to undulate. Gregarine gamonts glide and waves moving from front to back across the cell surface may be the rhythmic motion that produces their gregarine gliding movement (Grell, 1973).

E. Behavioral Mechanisms

As stated before, protozoa can respond behaviorally to chemicals by accumulation, dispersal or settling. All these responses require movement of the motor organelles just described, but it is not immediately apparent how movement of flagella or other organelles cause the final behavior response that we observe. Unfortunately, motor mechanisms of protozoa have received little attention as compared to the elegant and extensive studies of motile bacteria [for review, see Berg (1975a,b); Koshland *et al.* (1976)]. What we can describe here as possible behavioral mechanisms for protozoa come in large part from studies of small metazoa and bacteria. Analogies with larger and smaller organisms must be made with caution, however, because of scaling peculiarities of the hydrodynamic regime of protozoan swimming (Holwill, 1977). All but the very tiniest of swimming metazoa have Reynolds number (essentially, the ratio of inertial to viscous forces) much greater than one—that is, inertial considerations dominate the mechanical problem and viscosity is relatively insignificant. On the other hand, it is the reverse for bacteria, which have Reynolds number much less than one and experience mainly viscous forces. For swimming protozoa, the Reynolds number is typically in the range of 0.01–1.0, so that while viscous forces are dominant inertial effects may be important in some cases (see Chapter 2 by Roberts).

Most behavioral responses to chemicals can be considered either a *chemotaxis* or a *chemokinesis*. Chemotaxis refers to an *oriented* movement—the cell orients and moves toward or away from the chemical source—whereas chemokinesis denotes an unoriented change of movement by (unoriented) cells. Kineses require modulation of some motor response as the cells move in a stimulus gradient, in order to cause eventual accumulation or dispersal. For organisms such as *Paramecium*, with a modified random walk type of swimming, the motor response can be in the frequency of random turns (klinokinesis) or the speed of movement (orthokinesis) (see Table II), in a variety of ways. Perhaps the best studied example of klinokinesis is bacterial "chemotaxis" (Adler, 1976a,b). Bacteria, such as *E. coli*, *Bacillus subtilis*, and *Salmonella typhimurium*, swim by means of rotating flagella. While swimming, the cells will occasionally stop, tumble in place, and resume swimming in a new direction. This is the "twiddle" or tumble behavior and it occurs spontaneously at a ground rate. Chemical stimuli modify this ground rate of twiddling: attractants temporarily suppress twiddling and repellents temporarily increase the rate of twiddling. These responses result in a longer mean free path for cells swimming up a gradient of attractant and a shorter mean free path for cells swimming up a gradient of repellent (Figure 5). With fewer turns to

Table II Classic Mechanisms of Accumulation and Dispersal[a]

Classic mechanism	Motor response for attraction	Motor response for repulsion
Klinokinesis	Decreased frequency of change of direction when swimming up a gradient	Increased frequency of change of direction if swimming up a gradient
Orthokinesis	Decreased speed (regardless of orientation)	Increased speed (regardless of orientation)
Taxis	Orientation and movement toward stimulus	Orientation and movement away from stimulus

[a] Based on Frankel and Gunn (1961).

interrupt their swimming, cells accumulate near attractants and with many turns to interrupt their paths, cells disperse from repellents. Speed of swimming does not enter into this mechanism.

A second classic mechanism of kinesis is orthokinesis, modulation of speed of movement regardless of orientation (Table II). Cells moving slowly can accumulate in a region as though in a traffic jam, whereas cells moving quickly and smoothly disperse, as do cars on the open road (Fraenkel and Gunn, 1961). Paramecia are capable of both kinds of klinokinesis and orthokinesis, as will be discussed below. Further discussion of the various kinds of kineses and their theoretical properties are found in Chapter 6 by Lapidus and Levandowsky.

1. *Kineses of Ciliates*

Paramecia have long been studied for their accumulation and dispersal behavior in chemicals. The cells accomplish these behaviors by modifying their two main components of swimming behavior: the avoiding reaction that is the mechanism to change swimming direction (Figure 6) and the speed of swimming that is determined by frequency and angle of ciliary beating. Since the late 1800s, it has been observed that cells swimming toward attractants decrease the frequency of avoiding reaction (F_{AR}) and increase F_{AR} when swimming toward repellents (Jennings, 1906). Hence, it was assumed that cells swim in longer mean free paths when going toward attractants and eventually accumulate in attractants; and that cells swim in short mean free paths when swimming toward repellents and eventually disperse from the repellents (Dryl, 1973; Jennings, 1906) (Figure 7). This mechanism of attraction and repulsion for *Paramecium caudatum* is, at first glance, similar to the klinokinesis of bacteria, i.e., dependent on frequency of change of direction (F_{AR}). However, both F_{AR} and the speed of swimming are functions of membrane potential. Predicting from the

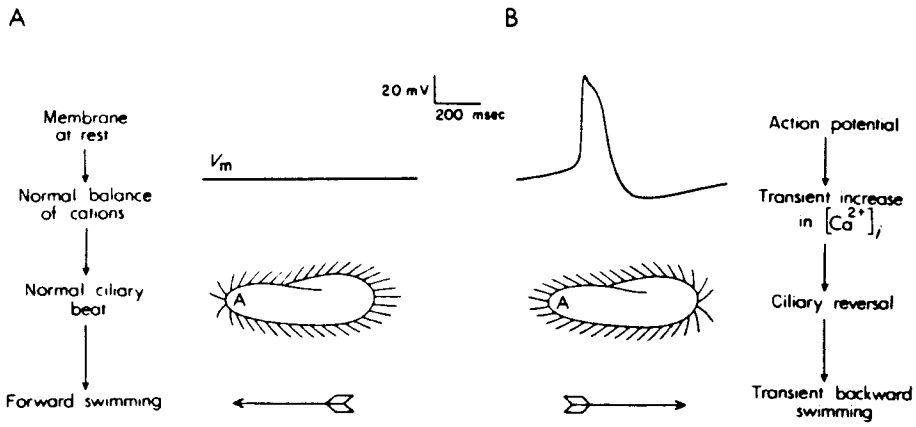

Figure 6. Relationship of membrane potential and locomotor behavior of *Paramecium*. (A) Cell at rest; tracing represents membrane potential of approximately −25 mV. (B) Cell stimulated with barium. Note relation of action potential with reversed beating of cilia. [From Kung *et al.* (1975) with permission. Copyright 1975 by the American Association for the Advancement of Science.]

known electrical control of ciliary movement (Eckert, 1972), an attractant stimulus that decreases the frequency of avoiding reaction would also tend to increase speed of swimming by changing the frequency and angle of ciliary beating. It was observed by Dryl (1961b) and later by Nakatani (1970) that *P. caudatum* does indeed swim faster in attractants that decrease the F_{AR}. (We will return to the role of speed in chemokinesis.)

Using the T mazes to measure accumulation and dispersal of *P. tetraurelia* and also measuring F_{AR} and speed in test and control solution, Van Houten (1976, 1978) demonstrated two mechanisms of attraction and repulsion. As noted by Jennings and Dryl for *P. caudatum*, attractants that decrease the frequency of avoiding reaction also increase speed while repellents that increase F_{AR} also decrease swimming speed of *P. tetraurelia* (Van Houten, 1978). The attractants and repellents classified as type I require that the cell be able to modulate F_{AR}. "Pawn" mutants that cannot reverse their cilia and can swim only forward (Kung, 1971) are unable to be attracted or repelled by these compounds (Van Houten, 1978). Another class of attractants and repellents have qualitatively similar behavior changes, but cause completely opposite accumulation and dispersal results (type II). For example, repellents of type II depress F_{AR} and increase speed, similar to attractants of type I. However, repellents II decrease F_{AR} almost to zero and increase speed so that the mean free path is very long. The cells are repelled by an orthokinetic mechanism (Table II), that is, by the relatively fast smooth swimming, and pawns are also

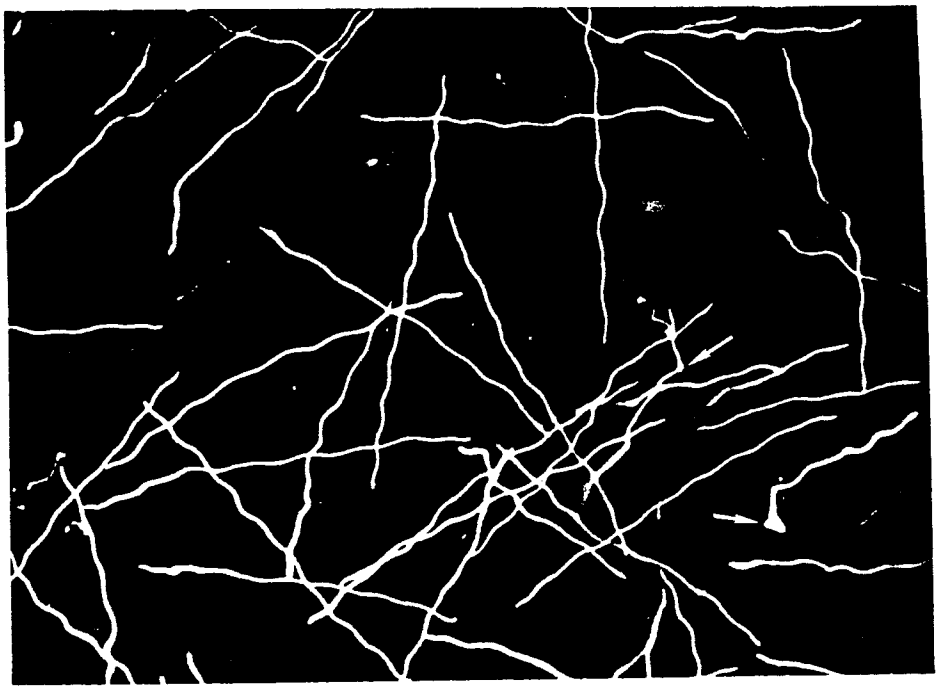

Figure 7. Long exposure (4 sec) photograph of *Paramecium tetraurelia* swimming in attractant potassium acetate (KOAc). Attraction by KOAc is associated with an increased mean free path (decreased frequency of avoiding reaction and increased swimming speed). Kinks in the tracks indicate avoiding reactions of the cells (arrows).

repelled by this mechanism (Van Houten, 1978). The use of other behavioral mutants that do not normally reverse their cilia and perform avoiding reactions and manipulation of normal cells has confirmed that there is a mechanism of accumulation and dispersal not dependent on the avoiding reaction (Van Houten, 1976, 1978).

A unifying explanation for all this apparently complex behavior at the subcellular level comes from the established electrical control of ciliary beating of *Paramecium* (Van Houten, 1978) (Figure 8). A hyperpolarizing stimulus will decrease F_{AR} and increase speed, responses consistent with attractants of type I. A large hyperpolarization decreases F_{AR} to near zero and increases speed more, responses appearing qualitatively similar to the slight hyperpolarization but resulting in repulsion type II, probably because of the high speed and resultant extremely long mean free path. Conversely, repellents type I depolarize the membrane slightly, increasing F_{AR} and decreasing velocity. Attractants type II depolarize the membrane more and greatly increase F_{AR} and decrease velocity, causing cells

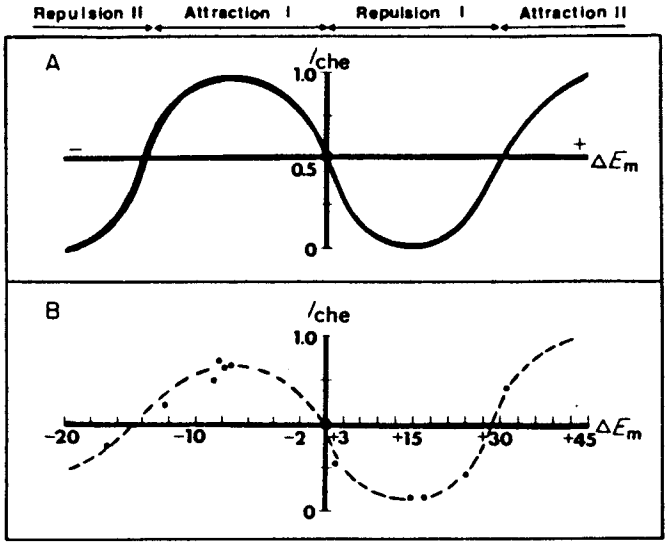

Figure 8. (A) Model for membrane potential control of chemokinesis behavior in *Paramecium tetraurelia*. (B) Fit of experimental data of membrane potential and indices of chemokinesis with model. (See Figure 1 for definition of indices.) $I_{che} > 0.5$, attraction; $I_{ch} < 0.5$, repulsion. As chemical stimuli change membrane potential (E_m) relative to control, animals will be attracted or repelled, depending on the magnitude and direction of the E_m change. [From Van Houten (1979) with permission. Copyright 1979 by the American Association for the Advancement of Science.]

to move little and, hence, to accumulate. All four classes of chemical stimuli have been identified and electrical measurements have been made to verify the predictions of the hypothesis (Figure 8). Several mutants defective in behavior have been used to test the hypothesis and many more mutants specificially defective in chemokinesis are now available to perturb and probe the chemosensory transduction pathway of *Paramecium* (Van Houten, 1978; J. Van Houten, M. Di Nallo, and E. Martel unpublished results).

It is not sufficient to leave *Paramecium* chemokinesis with a qualitative description of individual behaviors. Causality between the F_{AR} and speed of swimming and the observed accumulation or dispersal of populations must be established. However, classical mechanisms of kineses (Table II) cannot be directly applied to *Paramecium* behavior because klino- and orthokinesis are not neatly separable in paramecia. For example, an attractant type I will decrease F_{AR}, which is consistent with attraction by klinokinesis, but will also increase speed which will act to cause repulsion by orthokinesis. Therefore, orthokinesis and klinokinesis appear to be at odds in paramecia with speed changes tending to cause dispersal and F_{AR}

changes tending to cause attraction. Because decreased F_{AR} and increased speed do in fact accompany attraction I, either the klinokinetic mechanism dominates or the two motor changes both contribute to an increased mean free path which, in turn, leads to accumulation of cells by a previously undescribed mechanism. Rohlf and Davenport (1969) devised a two-dimensional computer simulation of a model of kinesis and obtained more efficient accumulation of cells with decreased F_{AR} and increased speed than with decreased F_{AR} alone. (Adaptation is also required, as discussed in Section IV,F). These results although not done with a T maze geometry, seem to imply that the two motor responses reinforce rather than oppose each other for attraction I to give population behavior consistent with klinokinesis. (For repulsion II, the same qualitative changes of individual behavior must interact to give population behavior consistent with orthokinesis!) It will be important to establish causality between the modulation of F_{AR} and speed and the accumulation and dispersal responses, and to understand the interaction of F_{AR} and speed. Mathematical modeling and simulation may provide helpful clues (see Lapidus and Levandowsky, Chapter 6), as will the continued use of mutants with specific defects in components of motor behavior.

Doughty and Dodd (1978; Doughty, 1979) modified the *Paramecium* T maze assay with a small stopcock bore of 1 mm that retards the cells from leaving an arm of the T once they have entered. The T maze (compare Section II,A, Figure 1) in this altered configuration may perhaps be measuring a different aspect of motility, not steady state accumulation or dispersal by a kinesis. For example, T arms with solutions that induce fast or smooth swimming initially could have many cells enter and then be "trapped" for a time. However, there are two mechanisms of attraction and repulsion of paramecia that can be detected by the Van Houten apparatus: there are repellents *and* attractants that, to different degrees, induce fast, smooth swimming, and it is not clear whether the small stopcock bore would allow differentiation between these (poorly understood) mechanisms. Doughty noted increased velocity of cells in acetylcholine and nicotine and speculated that these compounds cause accumulation in the modified T maze by positive orthokinesis (increased velocity with increasing concentration) (Table II). Positive orthokinesis *alone* should result in repulsion from these solutions (Table II), but cells may accumulate transiently in the acetylcholine test arm, being trapped, for a time, in the arms of the T that they first enter because of the relatively small size of the stopcock opening. Kineses causing attraction or repulsion after the populations in the T maze or other assay have come to a steady state are probably using somewhat different mechanisms (Van Houten, 1976; Fraenkel and Gunn, 1961). Thus, because of the differences of geometry

in the two kinds of stopcock experiments, it may not be possible to compare the experiments of Doughty and Van Houten in a simple way. Different behaviors are probably being seen. Some other important differences in the two sets of experiments are noted below.

The chemosensory behavior of the smaller ciliate *Tetrahymena*, a favorite tool of biochemists because it is easy to culture axenically in defined media, has been neglected. Recently, however, several groups reported preliminary results with this organism (Almagor *et al.*, 1977; Tanabe *et al.*, 1979; Lapidus and Levandowsky, 1980). The Japanese group (see also Ueda *et al.*, 1975) gave an abbreviated account of a new assay method in which cells are in both control and test solutions at the onset, and these are gently added, simultaneously, at opposite sides of a rectangular plankton counting chamber until the chamber is filled. After a period, the relative abundances of cells are observed on the sides where test and control solutions were added, and this is used as a measure of chemosensory attraction and repulsion. In principle this seems a very useful addition to the experimental repertoire for behavioral studies; Levandowsky (unpublished results) experienced difficulty with the method because of mixing of the two solutions on contact, due to strong capillary forces acting when the two menisci come into contact. The other two groups (Almagor *et al.*, 1977; A. Ron, personal communication; Lapidus and Levandowsky, 1980) used capillary techniques. Lapidus and Levandowsky used flattened capillaries with a rectangular cross-section. The latter workers found that cells swam much slower in solutions with attractant substances, such as casein hydrolysate, suggesting that a classical orthokinetic effect may be the dominant factor in these studies. As to the turning frequency, so important in understanding *Paramecium*, the situation is less clearly defined for *Tetrahymena*. The basic swimming behavior does consist of straight (actually, tightly spiral) "runs" interspersed with turns; however, the turns are of at least two sorts. Some are smooth, with a continuous change of direction, whereas others are sharp, discontinuous changes of motion, resembling more the classical avoidance reaction of *Paramecium*. It seems clear that to understand in detail the results obtained by various assay methods with this genus much careful descriptive work with basic swimming behavior will be needed.

2. *Chemotaxis Mechanisms of Ameboid Cells*

A fair amount is known by now about mechanisms of random movement of the amebas *A. proteus*, *Chaos*, *Acanthamoeba*, and of *Physarum* plasmodium (see Section III,C), but little is known about the alteration of these mechanisms to cause oriented movements of chemotaxis.

Motile nonmuscle cells typically ruffle and one of the ruffling mem-

branes becomes the new leading edge of the cell (Revel *et al.*, 1978). Presumably chemoattractants and repellents help determine the new leading ruffle and, hence, the direction of movement. The cell then moves into its new leading edge by means of waves of contraction and the cell moves forward.

Although much larger than amebas, myxomycete plasmodia move in a related manner by the above ameboid pattern. Because of large size of the plasmodia, they are convenient as a model motile system (Hatano and Owaribe, 1976; Jacobson *et al.*, 1976). *Physarum* plasmodium cytoplasm streams rhythmically back and forth through a network of channels. Rhythmic contraction (probably of actin and myosin) causes the streaming and hence the waves that move over the surface of the plasmodium and cause slow random movement of the whole organism. Chemical stimuli alter this to a directed movement.

Durham and Ridgway (1976; Ridgway and Durham, 1976) established that waves of contraction which are the basis of plasmodial motility move opposite the organism's direction of movement, and that the waves increase in frequency in attractants and decrease in frequency in repellents. They propose that if there were a gradient of attractant across the plasmodium, mechanical waves would move away fastest from the area high in attractant concentration, in turn moving the membrane and the plasmodium toward the attractant source. This would be a relatively simple explanation of the mechanism of chemotaxis of *Physarum* toward attractants. Conversely, repellents decrease the frequency of waves. Plasmodia must be repelled by preventing leading edges from forming on the side of the plasmodium nearest the repellents. Any contractions initiating will then be on the edges of the plasmodium away from the repellents and the macrocell will move anywhere but toward the repellent.

Free internal calcium increases in concentration in areas initiating contraction (Ridgway and Durham, 1976). Attractants and repellents may perhaps control the frequency and sites of initiation of contraction waves by controlling the permeability of the plasma membrane to calcium. Changes in calcium permeability would be expected to change some membrane electrical properties, which have, indeed, been observed during exposure to attractants and repellents (see Sections IV,C, D). Detailed membrane electrical studies will undoubtedly provide clues to the mechanisms of chemosensory transduction in plasmodia.

Though *Physarum* plasmodia appear to orient and move directly toward some stimuli, it is not completely obvious that crawling cells will always respond with a true taxis toward or away from chemicals. Information from leukocytes and cellular slime mold ameba chemotaxis indicates that kinesis may also play a role in the mechanism of protozoan ameboid

chemotaxis. For example, attractants not only cause the formation of pseudopodia and ruffles on the side of the cell closest to the source of attractant but uniform concentrations of attractants also increase speed of leukocyte motility and alter frequency with which the cell makes turns (Zigmond, 1978a). Therefore, even in what clearly appears to be chemotaxis, kineses may play a role in the behavior. Cellular slime molds move about as individual amebas while feeding on bacteria and for 8–12 hr after the bacteria are depleted. During this time the cells are attracted to folic acid. At the end of this time, aggregation begins and the cells move inward toward a focal cell in response to pulses of cAMP to eventually form a slug [for a review, see Mato and Konijn (1979)]. Ameboid *Dictyostelium discoideum* preaggregative cells have a characteristic speed and persistence time [time between spontaneous changes of direction (Potel and Mackay, 1979)]. Some mutants unable to aggregate in response to cAMP have abnormal preaggregative speeds and/or persistence times, indicating that normal random motility as well as orientation is associated with normal "chemotaxis" of aggregation. Aggregating amebas increase speed of movement in response to cAMP as well as orient in and move up gradients of the acrasin (Bonner *et al.*, 1966; Bonner, 1970), that is, the amebas show both chemokinesis and chemotaxis.

Many assays purporting to measure chemotaxis are actually measuring chemokinesis which is a general motility response of the cell to attractant [see Konijn (1975) for a review of assays]. Similar problems of distinguishing taxis from kinesis have been directly addressed in leukocyte chemotaxis assays (Zigmond, 1978b). At present it is almost impossible to sort out the relative contributions and interactions of kinesis and taxis to attraction and dispersal of ameboid cells, and thus, the mechanisms of attraction and repulsion of amebas are not fully understood at this time. We emphasize here the dangers of inferring mechanisms of population behavior from population assays without observing behavior of individual cells or organisms. The use of mutants selected for specific defects in random motility or in oriented movements will help in this analysis.

F. Detection of Temporal or Spatial Gradients—Characteristics of the Chemoreceptors

Protozoa may sample chemical cues from their environment in a temporal or spatial manner. Bacteria, for example, appear to sample their environment with time. Cells physically transferred to a new but uniform concentration of attractant or repellent will react to this change because they apparently compare their information before and after the transfer and react accordingly. Other types of cells that require a physical gradient

of attractant or repellent will not react to a new but uniform environment. Leukocytes will react to uniform concentrations of attractant with increased random motility but seem to sample a physical gradient of attractant across the length of the cell (Zigmond, 1978a). Stationary cells put out ruffles on the side of the cell nearest the highest concentration of attractant and begin immediately to move up the gradient of attractant. If the cell had to sample the environment with time, cells would move out randomly and then gradually become oriented up the gradient. The type of detection of chemicals, temporal or spatial, is not determined for most protozoa. Amebas and cells displaying ameboid chemotaxis may be capable of sensing a gradient across the length of the cell. Other cells, such as paramecia that display kineses and swim many lengths of the cell per second, could sample the environment temporally. Responses of paramecia on transfer to uniform pools of attractants and repellents indicate that they are indeed capable of temporal sensing (Van Houten, 1978; Dryl, 1973). The type of sensing mechanism, temporal or spatial, may have implications for the arrangement of the chemoreceptors on protozoa (see Section IV,B). As Zigmond (1978a) observes for leukocytes, temporal sensing only requires that the cell have one receptor or detector of the chemical and a memory for the cell to respond appropriately, while it seems clear that cells spatially sensing chemical gradients would need more than one receptor to make simultaneous camparisons of attractant or repellent concentration of different sides of the cell.

IV. SENSORY ORGANELLES, MEMBRANES, ELECTROGENESIS, CALCIUM, ADAPTATION, HORMONES, AND NEUROCHEMICALS

A chemosensory pathway in a model protozoan is outlined in Figure 9. We have described chemical stimuli (Figure 9A), final observable behavior (accumulation, dispersal, and so on), in response to the stimuli (Figure 9C), the motor organelles that are responsible for the behavior (Figure 9B), and the mechanism by which movement of these organelles results in the observed behavior. We have addressed mainly the motor effector end of the chemosensory transduction pathways. Other than describing the nature of chemical cues, we have not discussed the receptor end of the pathways. This omission unfortunately reflects the dearth of information regarding the nature of the sensory receptors, and transduction of chemical information into electrical or other forms that, in turn, could affect the cell's motor organelles and behavior. Nevertheless, we can offer some general suggestions regarding sensory organelles and receptors, and the roles of membranes, membrane potentials, calcium,

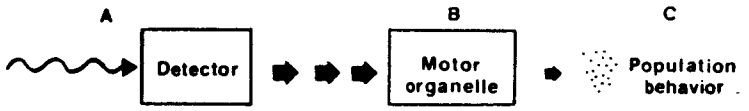

Figure 9. Flow of information along a chemosensory transduction pathway. A chemical cue (A) is detected by the cell (perhaps at the cell membrane) and this chemical information is transduced into other signals, possibly electrical or ionic, that affect the motor organelles (B) and eventually the behavior of a population of cells (C).

and possibly hormones and neurochemicals in protozoan chemoreception and transduction.

A. Sensory Organelles

Plasma membranes are the first possible sites of contact between chemical stimuli and the cell. In general it is not known whether any of the cells' specialized membrane-bound organelles serve as sensory organelles to receive and transduce chemical signals from the environment. Cilia, flagella, and filopodia are membrane bound and intimately involved in the chemosensory pathway as the cell's effector organelles for movement, and these same structures are candidates for sensory organelles as well.

Although cilia, or at least arrays of microtubules resembling axonemes, seem nearly always to be part of sensory (including chemosensory) organs of metazoa (Barber, 1974), their function as sensory organelles has been demonstrated only for mechanoreception of hair cells (Hudspeth and Jacobs, 1979; Hudspeth and Corey, 1977; Moran *et al.*, 1977), and for mating signal transduction of *Chlamydomonas* (Solter and Gibor, 1976). Atema (1975) elaborated this theory (see below). While cilia or flagella are likely to be sensory organelles, by analogy with mechanosensory organelles, they are perhaps unlikely to be *exclusive* sites of chemosensory receptors in protozoa. For example, deciliated paramecia lose the regenerative potential from a tap on the anterior but retain the depolarizing and hyperpolarizing receptor potentials in response to anterior and posterior mechanical stimulation (Ogura and Takahashi, 1976; Ogura and Machemer, 1980; see Section IV,C, for detailed discussion). Likewise, preliminary electrical recordings from deciliated cells in attractants and repellents are near normal, indicating that chemoreceptors, like mechanoreceptors, are not exclusively on the *Paramecium* cilia (J. Van Houten, unpublished observations). Additional support for nonciliary sensory organelles comes from the flagellate *Euglena*, which appears to use a cytochrome, possibly internal, as

chemoreceptor to detect the attractant oxygen (Miller and Diehn, 1978).

Filopodia are fine processes put out by the ruffling membrane of motile eucaryotic cells. The filopodia are believed (but not proven) to serve a sensory function in leukocytes and tissue culture cells in recognizing substratum and neighboring cells (Revel *et al.*, 1978; Zigmond, 1978a). The tactile and/or chemical cues sensed by the leukocyte filopodia would be transduced into the motor response of the cell—that is, the organization of appropriate subcellular fibers in the ruffle or elsewhere in the cell that cause oriented movement or immobilization.

Protozoa that move in ameboid fashion also put out ephemeral extensions (ruffles, lamellipodia, lobopodia) that guide the cell flow and creeping movements over the substratum. These extensions could have sensory as well as locomotor functions. *Dictyostelium*, *Physarum*, or *Amoeba* may sample the chemical environment with their slowly waving extensions and the information they gather will determine whether lamellipodia are retracted or remain attached to substrate to direct the cell's movement (see Section IV,B, for a discussion of the role of microtubules in oriented movement).

B. Receptors

Chemical cues are likely to affect the cell by first binding to specific sites on or in the cell. If binding to these sites is necessary for the subsequent response of the cell, then the binding sites are considered receptors and part of the chemosensory pathway. Receptors may be a specific membrane protein or glycoprotein population, phospholipids, or internal membrane components. Even though little is known about the location or composition of possible chemoreceptors in protozoa, some indirect information can be gathered about their sensitivity and specificity for chemical cues.

Specificity of a putative receptor necessary for chemosensory response can be expected to show parallels with the specificities of chemosensory behaviors. Specificity of chemoresponses can be described by assaying the behavior first in response to a stimulus and then in response to the stimulus with a saturating amount of a second stimulus present. For example, attraction of *Dunaliella* is assayed by the accumulation of cells in capillaries with attractant compared to the accumulation of cells in capillaries of buffer without the attractant compound (Sjoblad, *et al.*, 1978). A second attractant at 10 times higher concentration was added to all solutions and the accumulation of cells in the optimal concentration of the first attractant was again assayed. If the uniform presence of the

second attractant inhibits the accumulation in the first attractant, the two attractants are thought to be sensed by the same systems (possibly through the same receptors). If there is no inhibition, the two attractants are considered to be sensed by two systems. In this assay, evidence was found in *Dunaliella* for one NH_4^+ receptor and one receptor that detects all three of the best attractant amino acids (tryptophan, tyrosine, and phenylalanine) and NH_4^+ (Sjoblad *et al.*, 1978).

In the T maze assay, more paramecia accumulate in the arm of the T with potassium acetate than in the arm with KCl (Van Houten, *et al.*, 1975). Cells still accumulate preferentially in potassium acetate even when a saturating amount of a second attractant (potassium lactate) is included in all the arms of the T maze (Van Houten, 1976). The second attractant does not interfere with the sensing of the first attractant; therefore, it is possible that the two attractants are sensed by two separate receptors with different specificities. By this criterion, a minimum of three systems for detecting attractants exist in *P. tetraurelia* (Van Houten, 1976). Parallels of these behavioral studies have been made (1) with attractant binding and uptake and inhibition by a second attractant, and (2) with electrical responses of the cells to one or combinations of attractants. Specific responses argue for sensory transduction mechanisms that are specific and following separate pathways at least for early sensory events. Receptors can provide such specific mechanisms. Mutants of *Paramecium* that are normal in response to all repellents and attractants but one provide additional evidence for specific receptors for chemical cues (J. Van Houten, M. Di Nallo, and M. Wohlford, unpublished results).

Chlamydomonas gametes sense the presence of a gamete of complementary mating type when their flagella come in contact. Roseman (1970) proposed that membrane glycosyltransferases can serve as chemoreceptors for recognition and other sensory transduction processes by forming enzyme-substrate complexes with acceptors on other cells. Gametes may recognize mates, therefore, by binding to the other's flagella by glycosyltransferase–carbohydrate–acceptor complexes and subsequent transduction of this signal. McLean and Bosmann (1975) found support for this hypothesis in (1) the higher glycosyltransferase activity of gametes of *C. moewusii* relative to vegetative cells, (2) the stimulation of flagellar membrane vesicle glycosyltransferase activity upon mixing vesicles from complementary mating type gametes, and (3) the lack of stimulation of glycosyltransferase activity upon mixing vesicles from vegetative cells. Incompatible with Roseman's hypothesis are Goodenough's observations (1977) that nucleoside and nucleotide sugars do not inhibit or reverse *C. reinhardi* mating agglutination.

C. Role of Plasma Membrane and Electrogenesis in Chemoreception

Whatever the nature of the sensory organelles, the chemical cues must be transduced into signals that direct the movement of the cell. Control of protozoan movement is best understood for ciliary motion and for contraction. Ciliary movement is under electrical control at the cell membrane (see Section III,A). Likewise, contraction has electrical correlates. *Stentor* and *Zoothamnium* will contract in response to mechanical and chemical stimuli, and the contraction response is associated with a membrane depolarization (Wood, 1975; Moreton and Amos, 1979). Therefore, it is very likely that chemical signals are *generally* transduced into electrical signals that, in turn, affect protozoan behavior. This likelihood is increased with the realization that membrane potential changes appear to be associated with every chemosensory response system examined so far: *Physarum* chemotaxis (Hato *et al.*, 1976), bacterial chemokinesis (Szmelcman and Adler, 1976), leukocyte chemotaxis (Gallin and Gallin, 1977); metazoan invertebrate and vertebrate gustatory and olfactory chemoreception (Vinnikov, 1974; Dethier, 1971; Price and Desimone, 1977; Wenzel, 1973; Hansen, 1978); *Paramecium* chemokinesis (van Houten, 1979); and *Tetrahymena* (Tanabe *et al.*, 1979).

An analogy with mechanoreceptor systems will be helpful in discussion of electrical control. Ciliates that swim or creep characteristically back up upon bumping their anterior, but move forward fast after their posterior has been tapped (Machemer and de Peyer, 1977; Jennings, 1906). As can be predicted from the swimming direction and speed of the cell, these responses to mechanoreception correspond to an action potential and a hyperpolarization respectively, whether cilia are arranged over the body as in *Paramecium* or in specialized cirri for locomotion as in *Euplotes* (Machemer and de Peyer, 1977). Mechanoreceptors can be mapped along the cell by local stimulation while recording membrane potential from the cell (Eckert *et al.*, 1972; de Peyer and Machemer, 1978; Ogura and Machemer, 1980). Characteristically the extreme anterior responds to mechanical stimuli with the largest depolarization, the most posterior with the largest hyperpolarization (de Peyer and Machemer, 1978a). From deciliation and other experiments, it appears that, although the calcium gates required for the calcium action potential are associated with the ciliary membrane, the structures necessary for the mechanoreception depolarizing and hyperpolarizing receptor potentials are in the plasma membrane of both *Paramecium* and *Stylonychia* (Ogura and Takahashi, 1976; Dunlap, 1977; de Peyer and Machemer, 1978a,b; Ogura and Machemer, 1980).

In mechanoreception, then, the stimulus is transduced into an electrical message that affects the cells' motor apparatus. The behavioral response can be simple—a transient change in direction or frequency of ciliary beat is sufficient to cause an avoiding reaction or transient fast forward swimming of an individual cell. Responses to *chemical* stimuli, however, can be complex, requiring a series of steps. For example, in chemokinesis of *Paramecium*, the *frequency* of ciliary reversals (F_{AR}'s) and *frequency* and *angle* of forward beating cilia—hence the *speed*—are both changed to cause eventual accumulation or dispersal of a population of cells in a way not yet completely understood. In chemotaxis of the leukocytes and *Dictyostelium*, the cell's motor apparatus becomes oriented and the cell often moves faster in and toward attractants (Eckert *et al.*, 1977; Malech *et al.*, 1977; Zigmond, 1978a; Bonner, 1970). Therefore, it is of interest to know the electrical correlates of the more complex response to chemoattractants and repellents. These may be signals to the motor organelles. Electrical changes should be predictable for the ciliates at least, if simple changes in F_{AR} and speed that are under known electrical control are indeed bases of and sufficient for the mechanisms of accumulation and dispersal from chemicals, as they appear to be (Van Houten, 1979; see Section III,A).

Van Houten (1978) provided evidence for two types of attractants and repellents for *Paramecium tetraurelia*, each associated with characteristic changes of F_{AR}, speed, and membrane potential relative to controls. Membrane potential changes are predictable from the swimming behavior of the cells displayed in attractants and repellents (see Sections II and III,E). Even though the mechanisms by which the components of swimming cause accumulation and dispersal of populations are not yet understood, we can predict that the control of the complex behavior of chemokinesis in *Paramecium* probably reduces to the electrical control of these components (F_{AR} and speed) (Van Houten, 1979). All other ciliates and flagellates (de Peyer and Machemer, 1978a; Nichols and Rikmenspoel, 1977, 1978; Hyams and Borisy, 1978) that have been examined appear to be under a similar electrical control of ciliary/flagellar beating and, hence, components of locomotor behaviors (see Section III,A). Likewise, the chemokinesis behavior of other ciliates and flagellates will probably reduce to the transduction of chemical information into the electrical information that controls the ciliary or flagellar beating, while individual differences between genera may perhaps arise in the types of cues to which cells respond and in the means by which the chemical cues alter the membrane potential or other membrane electrical properties.

Food-deprived didinia respond to contact of proboscis with prey with a depolarization and subsequent hyperpolarization (Hara and Asai, 1980).

The depolarization is a mechanoreceptor response as it can be elicited by contact with an inert object. The hyperpolarization is a result of nutrition-dependent sensory transduction of surface-bound food cue(s) since a recently fed didinium will respond to contact with prey with a depolarization alone.

Chemotaxis of cells with ameboid movement also has electrical correlates. Prior to pseudopod formation leukocytes undergo a slight depolarization followed by a more prolonged hypolarization when exposed to chemoattractants (Gallin *et al.*, 1978). The depolarization reflects a Ca^{+2} and possibly a Na^+ influx. The increased internal calcium could directly depolarize and then indirectly hyperpolarize the cell by triggering a K^+ efflux (Meech, 1974; Gallin *et al.*, 1978). The changes of internal calcium could also affect the polymerization of microtubules or control actin-myosin microfilament systems, both necessary for oriented movement (see Sections III,B, IV,D, and IV,E). The putative influx of sodium could help to account for the depolarization and the observed increase in the membrane Na^+–K^+ ATPase activity (Becker *et al.*, 1978).

There are some differences between the myxomycete plasmodium and leukocyte membrane electrical responses to chemical stimuli. Plasmodial membrane potentials, measured by direct electrical recording and more often by indirect means (EPG or electroplasmagram), change to more depolarized (positive) values with increasing amounts of attractants *and* repellents (Hato *et al.*, 1976; Ueda *et al.*, 1975). The depolarizations are gradual and are superimposed on an oscillation of the EPG that has the same period as contraction and variation in free intracellular Ca^{+2} (Ridgway and Durham, 1976). [Attractants increase the frequency of contractions and repellents decrease the frequency (Durham and Ridgway, 1976).] (see Section III,B).

The correspondence between the relatively rapid electrical changes of leukocyte attractants and the potential changes during chemotaxis of plasmodia is not yet understood. The plasmodial potential changes appear to reflect changes necessary for altered motility in an adaptation to chemical stimuli in general, whereas, the leukocyte response may reflect the initial responses of the cell attempting to orient in the gradient of attractant rather than a general change characteristic of chemosensory transduction or adaptation.

Braatz-Schade (1978) found that some agents that inhibit motility of *Ameba proteus* also cause changes in cell shape and membrane potential. The effects of these agents, which include Mn^{2+}, La^{3+}, verapamil, and other calcium antagonists, are reversible by external Ca^{2+}. Nuccitelli *et al.* (1977) demonstrated both steady and pulsed currents through *Chaos* cells, the steady current entering the tail region and pulses entering pseudopods.

Ion substitution experiments indicate that external calcium ions carry at least part of the current. These observations are consistent with changes in membrane potential and intracellular calcium distribution in leukocyte and *Physarum* during motility and chemotaxis discussed in Sections IV,C,D, and with *Chaos* calcium distributions discussed in Section IV,D.

D. Role of Calcium in Movement and Chemoreception

Protozoa respond to stimuli, including chemical stimuli, with a limited number of movements (avoiding reaction, fast forward swimming, oriented movement and contraction) and calcium appears to have a fundamental role in most of them. Whether a cell moves by means of two or thousands of cilia, speed and the transient change of beating direction that causes the change of swimming direction are dependent on calcium (see Eckert, 1972; Naitoh and Eckert, 1968; Eckert *et al.*, 1976; Schmidt and Eckert, 1976; Hyams and Borisy, 1978; Holwill and McGregor, 1975). Glycerol or detergent treatments make ciliary apparatuses permeable to external compounds in *Paramecium*, *Chlamydomonas*, and *Crithidia* (Naitoh and Kaneko, 1973; Holwill and McGregor, 1975; Hyams and Borisy, 1978). These permeable cells and ciliary apparatuses beat as for *in vivo* forward swimming when supplied with ATP and magnesium and reverse the beat to the backward swimming mode with the addition of sufficient calcium. (See Sections III,A for further discussion.)

The role of calcium in control of protozoan cilia is consistent with a general control of all types of ciliary motion. Increasing intracellular calcium causes beating metazoan gill cilia to arrest (the counterpart to the protozoan ciliary reversal) and intracellular calcium controls the frequency of metazoan ciliary beating (Walter and Satir, 1978; Eckert and Murakami, 1972; Murakami and Takahashi, 1975; Satir *et al.*, 1976; Satir, 1975). Calcium does not arrest or reverse cilia by inhibiting sliding of outer doublets (Walter and Satir, 1979). Instead, calcium might affect the timing of rotation of the central tubules that, in turn, may control the direction of the power stroke of the ciliary beating (Omoto and Kung, personal communication).

Intracellular calcium concentrations affect the contraction and organization of contractile filaments responsible for nonmuscle cell ameboid movements (Lazarides and Revel, 1979). Chemoattractants and repellents probably alter the frequency of waves of contraction of *Physarum* plasmodium by affecting local free calcium concentration that, in turn, affects the contraction of actin and myosin filaments responsible for movement (Durham and Ridgway, 1976). In particular, Ridgway and Durham (1976)

have shown free calcium concentration increases in contracting regions of the plasmodium.

Spasmonemes and myonemes of *Spirostomum*, *Stentor*, and vorticellids contract with increasing calcium concentration, independent of ATP concentration or availability (Huang and Mazia, 1975; Amos, 1975) (see Section II,C). Contraction of the rhizoplast at the base of the flagella of the alga *Platymonas* is also dependent upon calcium (Salisbury and Floyd, 1978). If we extrapolate from the ionic control of *Chlamydomonas* and *Euglena* flagella, local calcium concentrations would appear to control flagellar beat frequency, beat direction, and the contraction cycle in the rhizoplast that facilitates the flagellar beating in *Platymonas* (see Section II,A).

Protozoan chemoreception systems are at least indirectly dependent on calcium because their motile apparatuses are affected by calcium. There are several examples of specific calcium concentration requirements for protozoan chemoreception, as opposed to motility in general: the repulsion of *Crypthecodinium* by carbon dioxide (Hauser *et al.*, 1978); the chemokineses of *Paramecium tetraurelia* (Van Houten, 1976, 1978) and *P. caudatum* (Dryl and Kurdybacha, 1978); and the chemokinesis of *Stentor* (Pietrowica-Kosmynka, 1972b). A role for calcium in *Blepharisma* mating attraction is only hinted at by the fact that Gamone II binds Ca^{2+} (Kubota *et al.*, 1973) and therefore could possibly function as attractant and mating substance by altering surface charge, sequestering surface calcium, or transporting calcium by acting as an ionophore (Crandell, 1977).

The best documentation of the involvement of calcium in chemoreception independent of its involvement in random motility comes not from protozoan but from leukocyte chemotaxis. Many changes in leukocyte calcium content and distribution have been reported (see below). Most of the changes, however, are measured in whole cell preparations in the presence and absence of uniform amounts of attractant instead of in gradients. These changes are difficult to correlate with the sequence of local morphological changes within the cell that result in oriented motility and chemotaxis. It appears clear, however, that calcium serves several chemotaxis functions separated in time and in space in the cells and we will attempt to summarize these changes below.

Chemoattractants stimulate two rapid calcium changes. One is a rapid calcium influx dependent on the presence of extracellular calcium (Boucek and Snyderman, 1976). The other is a rapid transient release of calcium from membranes, presumably into the cell, independent of external calcium (Naccache *et al.*, 1979ab). The independent calcium release may be an initial response of attractant binding to receptor, while

the early calcium-dependent influx may correspond to observed initial membrane potential changes that precede pseudopod formation in response to attractants (Gallin and Gallin, 1977; Gallin *et al.*, 1978). The membrane first depolarizes and then hyperpolarizes when attractant is locally perfused onto the cell. The potential changes are thought to be partly due to a calcium influx, that, in turn, stimulates a postassium efflux (Meech, 1974; Gallin *et al.*, 1975). The cell next responds by decreasing calcium influx and reducing free calcium in the cell by shifting calcium from cytoplasmic to granular membrane fractions (Gallin and Rosenthal, 1974).

It appears from these whole cell measurements that initial responses of leukocytes to chemoattractant include a transient increase in free calcium, followed by a lowering of free calcium. These whole cell changes must be related to local changes within the attracted cell. Gallin and Rosenthal (1974) hypothesize that randomly migrating cells are in a random microtubule polymerization–depolymerization state (see also Bandman *et al.*, 1974). In a chemotactic gradient, a decrease in cytoplasmic (free) calcium nearest the stimulus would favor polymerization of tubulin, orient the cell, and bias its movements toward the attractant. Indeed, divalent cations (probably calcium) are deposited on the cytoplasmic side of the plasma membranes of pseudopods on the leading edge of a responding leukocyte (Cramer and Gallin, 1979). These deposits may represent the cells' mechanism for sequestering calcium and lowering free calcium concentration.

Recent studies of *Chaos carolinensis* provide more information about the local changes of calcium during ameboid locomotion. There are differences in distribution of free calcium (assayed by aequorin fluorescence) between the tail to leading end of the large *Chaos* amoeba (D. L. Taylor, personal communication). Free calcium remains consistently high in the tail area, probably causing contraction of the actin–myosin complexes and perhaps subsequently forcing endoplasm to stream toward the leading edge of the cell. At the leading edge, free calcium transiently increases in concentration, perhaps causing the contraction and sloughing off of actin containing sheets. Peeling off of actin sheets from the plasma membrane of the pseudopod might weaken the membrane and facilitate the movement of endoplasm forward into the pseudopod, pushing forward the cell's leading edge and producing oriented movement. There is no complete agreement about the roles of contraction-produced cytoplasmic streaming of endoplasm in locomotion in leukocytes and giant amebas and hence the role of the free calcium in the tail [for discussion, see Stossel (1978); Komnick *et al.* (1973); Allen and Allen (1978)].

The transient increases in free calcium in the leading edge of *Chaos* are dependent on external calcium and are reminiscent of the transient changes of calcium in leukocytes putting out pseudopodia in response to attractants. Unlike leukocytes, *Chaos* has few microtubules and therefore, cannot polymerize microtubules to produce orientation (D. L. Taylor, personal communication). Instead in *Chaos*, orientation may be produced by the weakening of the pseudopod membranes to allow forward movement.

It is important to note that cellular pH also changes during locomotion of *Chaos* (D. L. Taylor, personal communication), and that Hellewell and Taylor (1979) demonstrated a calcium and pH-dependent contraction of actin and myosin containing extracts of *Dictyostelium*. Since *in vivo* free calcium and pH changes appear inseparable, it seems impossible at present to determine the cause and effect relationships between changes in calcium and pH and subcellular events necessary for movement. Also keep in mind that other regulatory mechanisms, such as myosin phosphorylation, have not been discussed here.

Physarum plasmodia increase frequency of waves of contraction in attractants (Durham and Ridgway, 1976) and contraction is associated with a transient increase in free calcium (Ridgway and Durham, 1976) as postulated for contraction that produces crawling of leukocytes. Like leukocytes and *Chaos*, plasmodia must transiently (but cyclically) increase free calcium on the side nearest the attractant. The local calcium increases preferentially initiate waves of contraction from that side to produce movement toward the attractant. There may also be undescribed local decreases in free calcium near the plasmodium leading edge to promote microtubule polymerization, pseudopod formation, and oriented movement, or perhaps there are fundamental differences between leukocytes, *Chaos*, and *Physarum* in the mechanism of movement or orientation that require different roles for calcium in chemotaxis.

Harold (1977), McMahon (1974), and Rasmussen (1977) have predicted that cells use a limited number of small molecules such as calcium ions or cyclic nucleotides to act as second messengers that modulate the physiology of the cell in response to external cues. In protozoan chemoreception, calcium can play at least two roles, even within the same protozoan. Changes of calcium currents or conductance are involved in the transduction of external cues into electrical signals, and calcium appears to be the messenger commonly used as a signal to the motor organelles to alter their function (for cilia or flagella to alter beat form or frequency; for actin–myosin mesh to contract, and for lamellipodia to form, oriented by the stimulus). The choice of calcium may be dictated by the nature of attractants or repellents or may be dictated by the fact that

polymers involved in movement (microtubules, actin, myosin) all polymerize or contract (move) as a function of calcium concentration.

E. Role of Microtubules in Chemoreception

Nonbeating cilia are found in almost all types of metazoan sensory organs (Barber, 1974). The microtubules of these cilia may themselves function as the sensory organelles, or may be necessary in some other way for proper structure of such organelles.

Functional axonemal microtubules have of course a direct and obvious role in the motor behavior of ciliates and flagellates, and interference with the axonemes with antitubulin drugs will disrupt motility and, hence, chemoreception behavior. However, the antitubulins vinblastin and vincristine interrupt dinoflagellate dispersal from some repellents at concentrations that do not affect motility or positive (attraction) responses (Levandowsky *et al.*, 1975b), suggesting that microtubules may have a role in the transduction of chemical information as well as in the motion of the effector organelles.

Chemosensory response by motile nonmuscle cells, but not ability to move, is disrupted by the antitubulin colchicine. [See Dustin (1978) for a review of role of microtubules in orientation and microfilaments in movement of ameboid cells.] Intact microtubules are not essential for movement of leukocytes (Malech *et al.*, 1977), slime mold amebas (Eckert *et al.*, 1977) or fibroblasts (Gail and Boone, 1971). Leukocytes and amebas treated with colchicine have difficulty not with movement but with oriented movement, making more and larger turns (Bandman *et al.*, 1974; Cappuccinelli and Ashworth, 1976). Eventually these cells can accumulate up a gradient of attractant, but cannot successfully migrate chemotactically through filters (Zigmond, 1978a). Likewise, aggregation by chemotaxis of *Dictyostelium* but not motility is inhibited by colchicine and several other antitubulins (Cappuccinelli and Ashworth, 1976; Eckert *et al.*, 1977). It is not clear whether microtubules in nonmuscle cells are functioning in cell shape and oriented motility alone or in sensory transduction during the chemosensory response as well. In interpreting the results of experiments with antitubulins, however, other actions of these drugs should be kept in mind. Thus, colchicine has effects on several transport systems and on cell shape in the absence of microtubules (Beebe *et al.*, 1979), and vinblastine has been shown to inhibit the activity of brain cyclic AMP phosphodiesterase (Watanabe *et al.*, 1979).

Intracellular nonaxonemal microtubules are known to have a function in transmembrane flow of information in lymphocytes. Microtubules may influence restriction of lateral movement of membrane receptors of

lymphocytes in response to lectin crosslinking lymphocyte membrane receptors (McClain and Edelman, 1978). Microtubular structures are envisioned as receiving or transmitting signals along their length by the propagation of conformation changes of the tubulin molecules (Atema, 1975). *Chlamydomonas* gametes first adhere at the flagella and then proceed with the mating process. It has been suggested that the chemosensory signal of contact with the complementary mating type travels down the flagellum to the gamete by way of the flagellar membrane or axoneme, and that some mutants unable to carry out the mating process may be defective in signal production, reception or propagation (Goodenough, 1977; Solter and Gibor, 1977, 1978). It would not be surprising if microtubules played a role other than as a motor organelle structure in protozoan chemoreception. Early protozoologists suggested chemosensory functions for various cilia that seemed to have little direct role in locomotion (reviewed by Hyman, 1940).

F. Adaptation

Bacteria accumulate in or disperse from chemicals by a chemokinesis mechanism (see Section III,E). In response to being transferred to a uniform concentration of attractant, the cells transiently suppress spontaneous changes of swimming direction (twiddles, comparable to the avoiding reaction of ciliates). When their environment is no longer changing, the cells' frequency of twiddling returns to a basal level. This adaptation to the attractant is accompanied by the methylation of membrane proteins (Goy *et al.*, 1978; Springer *et al.*, 1979; Goy and Springer, 1978). Conversely, in response to being transferred to repellent solutions, bacteria increase the frequency of twiddling and eventually the frequency returns to basal level. This adaptation to repellent is accompanied by the removal of methyl groups from sets of membrane proteins. Adaptation (Zigmond and Sullivan, 1979) and methylation of a membrane protein also occurs during the chemotaxis of leukocytes and may prove to be common to many chemoreception systems (O'Dea *et al.*, 1978; Pike *et al.*, 1978).

Adaptation to chemical stimuli plays an important role in protozoan chemoreception, and methylation was recently found to be involved in transduction in *Dictyostelium* (J. Mato, personal communication). Jennings (1906) observed that paramecia respond and then adapt to changes in chemicals in their environment. When physically transferred to a new but uniform solution, the cells first respond to the new environment by changing F_{AR}; then, gradually, the cells return to basal levels of turning. In general, after transfer from control to attractant or repellent

solution, the turning frequency and speed of *P. tetraurelia* change, but eventually return to near basal levels (of cells transferred from control to control solutions) (J. Van Houten, 1976, unpublished results). This adaptation of behavior has membrane electrical correlates. Upon transfer to an attractant or repellent solution, the membrane potential changes, hence changing the frequency of ciliary beating and the frequency of depolarizations reaching threshold for action potentials (Van Houten, 1979) (see Section III,E). Machemer (1976) observed that *P. caudatum* ciliary beating frequency eventually returns to a basal level after a new resting potential has been established in a new solution and that the threshold for action potentials changes to a new level relative to the new resting membrane potential. This accommodation of membrane properties essential for chemokinesis can be observed in the *immediate* response of *Paramecium* to a change in solution. For example, paramecia hyperpolarize in one type of attractant (such as acetate ion) (Van Houten, 1979) (see Section IV,B). In a uniform concentration of attractant, the membrane potential remains more negative and the membrane accommodates, that is, the threshold for action potentials shifts in the hyperpolarizing direction to a new level relative to the new resting potential and the resistance returns to a basal level (Machemer, 1976). The new lower action potential threshold allows the cell to react to a small amount of control solution (relative repellent) by depolarizing slightly and triggering an action potential (hence an avoiding reaction), turning the cell and increasing its chances of reentering the attractant area (Figure 10). If the membrane had not accommodated and the behavior had not adapted, the cell would have to swim far into the relative repellent to depolarize sufficiently to trigger an action potential and a change of swimming direction. The farther into the repellent the cell swims the more likely the cell will remain trapped there by frequent AR.

Adaptation of swimming behavior is an essential aspect for simulation and mathematical models of mechanisms of attraction and repulsion (Table II). Rohlf and Davenport (1969) and Fraenkel and Gunn (1961) have discussed the importance of adaptation in kineses (see further discussion by Lapidus and Levandowsky in Chapter 6). Adaptation (desensitization) is also a characteristic of chemoreceptors in general (Mukherjee *et al.*, 1975; Archer *et al.*, 1973; Katz and Thesleff, 1957; Sugiyama *et al.*, 1976) including chemoattractant receptors in leukocytes (O'Flaherty *et al.*, 1979). The cross adaptation of the cell behavior potentially could be used to provide information about characteristics of receptors involved in chemoreception of protozoa. This adaptation should not be confused with the extreme long-lasting desensitization of paramecia to chemical stimuli resulting from prolonged exposure to high concentrations of some cations, such as potassium (see Section IV,G).

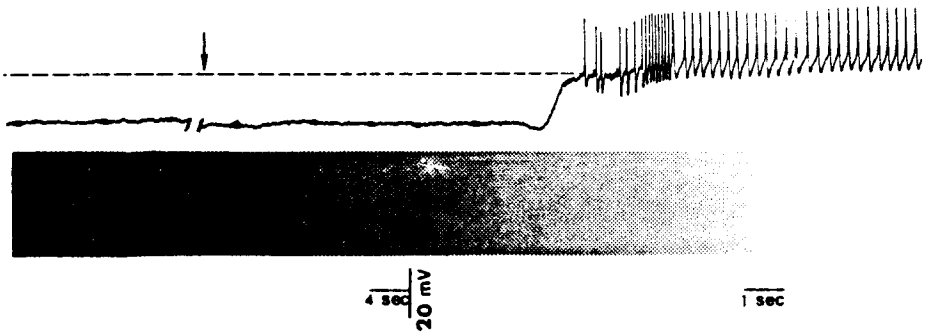

Figure 10. Membrane potential recording from *Paramecium tetraurelia* bathed in a relative attractant (5 m*M* KCl in buffer) and repellent (2.5 m*M* $BaCl_2$ in buffer) (J. Van Houten, unpublished data). To simulate a paramecium swimming from an area of attractant (type I) toward a repellent (type I) (i.e., down the gradient of attractant) the bath solution flowing into the chamber was changed from 5 m*M* KCl to 2.5 m*M* $BaCl_2$ at the arrow. Within 38 sec, before the entire bath solution had reached 2.5 m*M* $BaCl_2$, the cell depolarized and gave action potentials. Since each action potential corresponds to an avoiding reaction, the free-swimming cell in a gradient of decreasing KCl and increasing $BaCl_2$ would experience avoiding reactions long before it swims far up the gradient of repellent. These avoiding reactions would facilitate the cell's swimming back into the relative attractant (KCl). The cell had been in KCl for 6 min prior to the change of bath solution and, therefore, was at least partially adapted to KCl. The quick response to $BaCl_2$ was probably facilitated by this adaptation, which would include a shift of the action potential threshold toward the adapted KCl resting membrane potential (Machemer and de Peyer, 1977).

G. Role of Hormones and Neurochemicals in Chemoreception

Protozoan behavior has obvious parallels with nerve function and muscle movement. Neurotransmitters mediate these functions in metazoans, but the possible role of neurochemicals in chemoreception of protozoa is puzzling at present. Protozoa have the enzyme pathways to produce epinephrine and norepinephrine (Janakedevi *et al.*, 1966a), serotonin (Janakadevi *et al.*, 1966b), and possibly acetylcholine (Bayer and Wense, 1936; Bulbring *et al.*, 1949). The presence of an acetylcholinesterase is in dispute (Seaman and Houlihan, 1951; Seaman, 1955; Tibbs, 1960; Aaronson and Bensky, 1963; Torres de Castro and Couceiro, 1955). These neurohormones may perhaps function as intracellular modulators of metabolism of glycogen and phagocytosis rather than in intercellular communication akin to neurotransmitters, as suggested by Blum (1967) and others. Some recent observations, however, suggest that neurohormones might also have important functions as external chemical stimuli for communication or for modifying protozoan behavior.

The dinoflagellate *Crypthecodinium cohnii* is repelled by minute amounts of epinephrine and norepinephrine (Hauser *et al.*, 1975b). It is interesting that sensitivity of phototaxis in another dinoflagellate is affected antagonistically by acetylcholine (Forward, 1977).

Contraction behavior of *Stentor* can be modified by curare binding to receptors, that, unlike metazoan curare binding sites, do not have high affinity for acetylcholine (Wood, 1975), but the significance of this for sensory physiology is not clear.

Csaba and co-workers located binding sites for thyroxine and insulin on *Tetrahymena* cellular membranes as well as in food vacuoles (Csaba *et al.*, 1977). The fact that these hormones affect the rate of phagocytosis and glucose metabolism, the presence of binding sites for hormones, and the effects of externally applied hormones on the cells suggest that the cells could extract information from these compounds in their environment.

Acetylcholine was an essential component of an "artificial prey" that elicited a feeding reaction in suctorians (Hull, 1961), and may play a role in the aggregation of *Polysphondylium* amebas (Clark, 1977). Cholinesterase, eserine, the insecticide 2,2 dichlorovinyl dimethyl phosphate and acetylcholine increase the number of aggregation centers. Atropine has the opposite effects. The mechanism for interference with the aggregation process is not known.

Doughty and Dodd (1978; Doughty, 1979) have reopened the investigation of the effects of neurochemicals on *Paramecium* behavior. They found that paramecia increase motility in sodium buffer with acetylcholine, d-tubocurare, nicotine, and gallamine, and decrease motility in sodium buffer with β-ionine, relative to sodium buffer alone. Preincubated cells also respond to ionic strength changes along since cells swim into sodium buffer with added KCl or NaCl relative to sodium buffer alone (Doughty and Dodd, 1978). The dose response curves for these experiments are very unusual and the results appear very different from those obtained by Dryl (1973) and Jennings (1906), who concluded that KCl and NaCl decrease motility relative to buffer without the salts. The differences may be attributable to the assay or the incubation.

Comparisons of their results with other behavioral work are somewhat difficult to make because of: (1) their use of a modified T maze that by trapping cells appears to measure motility rather than steady-state accumulation and dispersal (see Section III,E,1); and (2) the incubation of cells in variable high amounts of sodium (6–8 m*M*) buffer up to 24 hr before use. This latter procedure starves the cells and alters their avoiding reaction response to potassium [cells giving one prolonged backward

swimming episode and recovery instead of repeated avoiding reactions (Dryl, 1973; Jennings, 1906; Hansma, 1979; Doughty and Dodd, 1978)]. The procedure of incubating cells in sodium buffer was intended to adapt the cells to the control solution prior to the T maze test so that cells would not be reacting to the test solution in the T maze while also in the process of adapting to the control solution (Doughty, 1979). Prolonged exposure of the cells to this control solution might have significant effects on sensitivity to some stimuli (Dryl, 1959); Hansma 1979). More generally, the effects of starvation on both motility and sensitivity need study. For example, is there anything in *Paramecium* which is comparable to the striking morphological and behavioral changes in starved *Tetrahymena* (Nelsen and De Bault, 1978)?

There may be several types of physiological "adaptation" in responses to the chemical environment, depending on the time scale involved. In the studies by Van Houten, cells "adapt" to uniform chemical environments (as in the ends of the arms of the T) on a time scale of minutes and rapidly "de-adapt" to quickly respond to changing chemical environments (as near the stopcock plug) (Figure 1). Repeated adaptations of this sort to solutions during this assay may be required for a steady-state accumulation or dispersal by klinokinesis (Rohlf and Davenport, 1969; Fraenkel and Gunn, 1961; Lapidus and Levandowsky, 1980) and allows this kind of chemoaccumulation and dispersal to be measured. As noted earlier, the "trapping" effect used by Doughty and Dodd may be measuring a different phenomenon, not without interest in its own right, after cells have undergone a much longer and probably very different type of adaptation.

The meaning of responses to neurochemicals and hormones is difficu't to assess. While it appears that some protozoa respond to some neurohormones applied externally, it is not clear at all whether these compounds play a normal role either in intracellular communication and motile behavior or in intracellular regulation of metabolism or motility. It is also conceivable that the same neurohormone can directly affect both motility and metabolism as serotonin does in parasitic flatworms (Mansour, 1979).

Perhaps the most convincing demonstrations of roles for neurohormones in protozoa are the studies in *Tetrahymena* of epinephrine and norepinephrine that affect carbohydrate metabolism by acting through a glucose and cyclic nucleotide sensitive system to repress expression of the galactokinase gene (Roberts and Morse, 1978; Nandini-Kishore and Thompson, 1979) and through a glucose-sensitive system to increase glycogen content and growth rate (Blum *et al.*, 1966; Blum, 1967;

Lowry and Gorde, 1972). However, no complete pathway for the synthesis and regulation of epinephrine and norepinephrine has yet been described for protozoa. In general the uncertainty about the natural presence or the control and role of neurohormones in cells makes it difficult to decide whether cells changing behavior in solutions of neurohormones are doing so in response to a natural behavioral modulator or to a change in carbohydrate metabolism (and relative levels of ATP or cAMP) or to compounds that interfere with normal functioning and cause changes that are artifactual but, perhaps, informative all the same. However, the dispersal response of the dinoflagellate *Crythecodinium* to minute amounts of epinephrine and norepinephrine suggests a possibility of a very specific and sensitive detection of catecholamines by these cells and a natural role for norepinephrine in their swimming behavior, and perhaps in the behavior of other protozoa as well. A study of the behavior of protozoan mutants defective in neurohormone metabolism would be exceedingly useful.

V. SUMMARY

Protozoa respond to a large variety of chemical cues. When the cues are produced by food in the environment, the cells often respond by aggregating near the food (see Section II,A); when they are produced by other protozoa, the response is often contact or preparation for mating. In some cases cells are repelled by chemicals produced by cells of the same species (Section II,A,2), presumably a dispersal mechanism. Suboptimal pH, salt, ionic strength, or oxygen tension are often cues to disperse, escape, or contract (Section II,D). Intriguing possibilities of microbial interaction or communication are hinted at in the relatively few examples of responses to nonfood, in particular neurochemical, cues (Sections II,A, II,B, and IV,G).

After almost a century of observation of protozoan behavior, there are many important but neglected aspects of protozoan chemosensory pathways (Figure 9). With the exception of work with slime molds, few attempts have been made to identify sensory organelles or putative receptors of chemical stimuli or to describe the specificities of receptors (see Sections IV,A,B). Controls of the movement of ciliary, flagellar, or contractile apparatuses (Figure 9B) are much better understood (see Sections III,A–D) than the mechanisms by which the cells transduce chemical signals into signals affecting the motor organelles. While the studies of electrical bases of ciliary motility have surged ahead,

quantitative descriptions of the actual movements of the cells as a result of changes in ciliary movement are almost nonexistent. A qualitative description of individual behavior is not always sufficient to predict population behavior (see Section IV,E); quantitative data are essential for simulation and mathematical modelling of the latter (Figure 9C). Simulation and modeling are, in turn, important techniques for assigning causal relationships between behavior of individuals and behavior of populations. Mutants defective in individual components of behavior are of tremendous help in evaluating the roles of individual cells' movements in population behavior, but cannot eliminate the need to describe the behavior mathematically.

No entire chemosensory pathway is currently understood from receptor to effector. Protozoa present chemoreception model systems that could conceivably be completely dissected because of the following aspects:

1. Cells respond to stimuli, including light, touch, temperature, and chemical stimuli with changes in motile behavior.
2. These behavioral responses can be studied on the level of the individual or population.
3. Responses of an individual can be reduced to a few clear cut components of motility.
4. Since many species are large enough for standard electrical intracellular recording, these components have characterized electrical bases. For ciliates in particular, it is possible to monitor the membrane electrical properties indirectly by observing swimming behavior.
5. The genetics and mutagenesis of many protozoa have been carefully investigated (Sonneborn, 1974). Genetic dissection has been particularly useful in studying *Paramecium* ciliary movement (Kung *et al.*, 1975; Takahashi and Naitoh, 1978) and chemokinesis (Van Houten, 1977, 1978) and, more recently, *Tetrahymena* swimming behavior (M. Takahashi, personal communication).
6. Pure clones of cells can be cultured and some species can be grown at sufficiently high density to provide large quantities of material for biochemical study. This is particularly important if putative membrane bound receptors molecules are to be identified and characterized.

Even though there is not a synapse in sight, excitable protozoan unicells have provided information relevant to metazoan neuronal systems (Eisenstein, 1975; Bentley, 1976; Ward, 1977). Likewise, information relevant to the study of metazoan chemoreception can be gathered in the process of studying how protozoa detect and respond to their chemical environment, and, perhaps, talk to each other.

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