OSMOTIC AVOIDANCE DEFECTIVE MUTANTS OF THE NEMATODE *CAENORHABDITIS ELEGANS*

JOSEPH G. CULOTTI AND RICHARD L. RUSSELL

Biological Sciences Department, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

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ABSTRACT

A wild-type strain of the nematode *Caenorhabditis elegans* has been shown to avoid high concentrations of a number of sugars and salts. Individual and population assays for this response were developed and mutants were selected for their inability to avoid high concentrations of fructose or NaCl. Seven nonavoiding mutants representing six complementation groups were isolated and characterized. Genetic studies indicate that the mutants each carry a single recessive mutation responsible for the defective osmotic avoidance behavior. The map locations of the six complementation groups identified by these mutations have been determined. Mutants isolated for their inability to avoid fructose are also unable to avoid NaCl and *vice versa*. The mutants move normally, exhibit normal touch sensitivity, and, like wild type, follow isotherms in a radial thermal gradient. All of the mutants are at least partially defective in the attraction to sodium chloride exhibited by wild type. None of the mutants is temperature sensitive, and all exhibit defective osmotic avoidance behavior as young L1 larvae. Preliminary anatomical studies indicate selective sensory neuron changes in at least one mutant.

THE problems encountered in the development of complex cellular systems in higher organisms are perhaps most acute in the developing nervous system. For example, not only must nerve cells be in some way informed of their position in an array of cells, but they must also establish proper connectivity with other neurons in the array. While much is known about neuronal function from electrophysiological and biochemical studies, information about the rules that govern the development and assembly of neurons into an integrated, functional nervous system is, at the moment, limited (LOPRESTI, MACAGNO and LEVINTHAL 1973; CHANGEUX and DANCHIN 1976). One approach to this problem has been to examine how the instructions contained in the genes are translated into normal wiring and nervous function (BENZER 1971; BRENNER 1974). The soil nematode, *Caenorhabditis elegans*, has the advantage for this purpose that it is both genetically tractable and anatomically simply enough to make a complete identification of a mutationally induced neuronal lesion feasible. In fact, nearly the entire nervous system of the wild-type *C. elegans* has been reconstructed by serial-selection electron microscopy, thereby providing a basis for analyzing behavioral mutants for synaptic defects (WARE *et al.* 1975; WARD *et al.* 1975; ALBERTSON

and THOMPSON 1976; WHITE *et al.* 1976; HALL and RUSSELL, *in* preparation; WHITE, personal communication).

BRENNER and his colleagues have used *C. elegans* to identify the anatomical basis for a number of uncoordinated mutants exhibiting a variety of defects in normal motion (BRENNER 1973), while others have confined their analyses to more specific behaviors such as chemotaxis (DUSENBERY, SHERIDAN and RUSSELL 1975; LEWIS and HODGKIN 1977) and thermotaxis (HEDGECOCK and RUSSELL 1975) with the hope of obtaining lesions confined to a more limited set of neurons. Preliminary results suggest that most chemotaxis- and thermotaxis-defective mutants do turn out to have limited defects in the anterior sensory nervous system, and may be useful for analyzing neural development (RUSSELL, unpublished observations; WARE, personal communication; LEWIS and HODGKIN 1977). This report extends this earlier work by describing the isolation and initial characterization of the first mutants defective in one of the aversive behaviors exhibited by *C. elegans*—avoidance of high osmotic strength conditions. Preliminary anatomical work indicates that some of these mutants also have anterior sensory defects, but ones that affect a different neuronal subset.

MATERIALS AND METHODS

Nematodes: *Caenorhabditis elegans* var. Bristol (strain N2) was originally obtained from S. BRENNER. It was grown monoxenically with *Escherichia coli* strain OP50 as food source (see DUSENBERY, SHERIDAN and RUSSELL 1975) on NGMM-containing petri plates.

Media: NGMM plates and S medium have been described (DUSENBERY, SHERIDAN and RUSSELL 1975). For chemotaxis and osmotic avoidance assays (described below), the tracks of individuals or populations were followed on chemotaxis plates prepared according to WARD (1973). Chemotaxis to NaCl by individuals was assayed by the method of DUSENBERY, SHERIDAN and RUSSELL (1975). Tracking plates for individual thermotaxis assays were prepared according to HEDGECOCK and RUSSELL (1975).

Mutagenesis: Mutagenesis was according to BRENNER (1974) with the following modifications. Wild-type dauerlarvae were purified according to CASSADA and RUSSELL (1975), grown at 20° for 24 hours, and resuspended in S medium containing dextrose and EMS (ethyl methanesulfonate, K&K Laboratories, Plainview, NY), with variations as described in Table 2.

Population assay for osmotic avoidance: Nematodes growing on NGMM plates were washed twice with water and 100 to 400 worms were transferred in a small drop to the center of a chemotaxis plate into which an annular ring of repellant (1 cm. radius, 0.015 ml of a highly concentrated solute plus the dye Light Green SF Yellowish) had soaked less than five minutes previously. The excess transferred water was absorbed with a Kimwipe, and most of the worms immediately dispersed toward the high concentration ring. After either 15 or 30 minutes (as indicated in the Tables), the plates were exposed to chloroform vapor; worms that were in the annular ring or beyond were scored as nonavoiders and counted as a fraction of the total. Assuming that the solution soaks in immediately and assuming a diffusion coefficient of 2.5×10^{-5} *cm/sec at* 20°, *the peak concentration of a* 5 M *solution applied as described should* vary from approximately 2.6 M to 1.0 M during this time.

Selection: In a modified population assay, approximately 1000 F_2 individuals from each mutagenized line were placed on a chemotaxis plate between two concentric annular rings of either NaCl (4 M) or Fructose (3.6 M). Individuals that moved into or through either ring during the first 30 minutes were picked and cloned. Clones that failed to avoid on retesting by the standard population assay were retained as osmotic nonavoiding mutants.

Individual assay for osmotic avoidance: Individual worms were transferred from an NGMM

growth plate onto a chemotaxis plate, inside a high concentration annular ring (2 cm. radius, 0.03 ml of either 4 M NaCl or 4 M fructose, with dye) deposited less than five minutes previously. After 30 minutes, those individuals whose tracks showed a crossing of the ring were scored as nonavoiders. The dimensions ensure several encounters of the ring during the assay period, yet allow as many as five individuals per plate to be tested without confusion.

Double-blind experiments: Double-blind experiments were performed for each mutant by running individual assays for each of approximately 30 wild-type individuals and 30 mutant individuals (one individual per assay plate). Each worm was allowed to run for 20 to 30 minutes, at which time a picture of the tracking pattern was taken. The pictures were mixed in a random array noted on a master sheet, then scored by a naive observer. The error frequency of each category was determined by reference to the master sheet. Although the numbers of wild-type and mutant individuals were not consciously counted during the scoring, there is undoubtedly some bias introduced by virtue of the observer's knowledge that equal numbers of wild-type and mutant individuals were included in each test; however, the effects of this bias are believed to be minimal.

Crosses and mapping: Crosses were performed by standard procedures (BRENNER 1974; DUSENBERY, SHERIDAN and RUSSELL 1975). The locations of the new mutations on the linkage groups of *C. elegans* were determined by the methods outlined by BRENNER (1974), with the following modifications. Wild-type N2 males were mated to hermaphrodites homozygous for the *dpy* (dumpy phenotype), *lon* (long phenotype), or *unc* (uncoordinated phenotype) marker for which linkage data to the new *osm* (osmotic) mutation was desired. Male progeny from this cross were then mated to the homozygous *osm* mutant hermaphrodites, and approximately 30 to 40 hermaphrodite progeny from this cross were isolated. Their progeny were examined for marker segregants (*i.e.*, *dpy*, *lon*, or *unc*—indicating that the hermaphrodite parent was the trans heterozygote *osm⁻marker⁺/osm⁺marker⁻*); any progeny populations containing these segregants were put through the selection procedure for osmotic nonavoiding mutants and approximately 50 to 200 phenotypically *marker⁺* escapees were picked and cloned. Presumably these individuals were homozygous for the osmotic mutation. They were allowed to self-fertilize, and their progeny were scored for the segregation of the marker phenotype and for their osmotic behavior (as a test of whether the parent was really a homozygous osmotic nonavoiding mutant). For nonlinkage to the *dpy*, *lon*, or *unc* marker, two-thirds of the osmotic nonavoiding clones should segregate the marker; whereas, for complete linkage, none should. The recombination frequency, P, is related to the frequency F of marker segregation by the equation $F = 2P/(1+P)$ which for small P becomes $F = 2P$. The rationale for mapping X-linked mutants was the same as for autosomal mutants, but the procedure for constructing the trans heterozygote (*osm⁻marker⁺/osm⁺marker⁻*) was somewhat different. To do this, *osm* mutant males were crossed to the homozygous marker strain, and nonmutant hermaphrodite progeny (trans heterozygotes) were picked. All subsequent steps were the same as for autosomal mutants.

Behavioral assays: Individual assays for chemotaxis and thermotaxis have been described (DUSENBERY, SHERIDAN and RUSSELL 1975; HEDGECOCK and RUSSELL 1975). Wild type *C. elegans* also responds to mechanical stimuli applied to the head or tail (SULSTON, DEW and BRENNER 1975). Sensitivity to mechanical stimuli was tested by tapping the head or tail with a fine human eyelash that had been attached to the end of a toothpick. The wild type response to head tap is very clear—the animal backs up for several wavelengths, then undergoes a deep bend followed by forward motion. A tail tap results in forward motion for a reversing individual or in increased velocity of forward motion for a slowly forward moving individual.

RESULTS

Wild type *C. elegans* is strongly repelled by high concentrations of NaCl. fructose, glucose, sorbitol, sodium acetate, glycerol and ammonium acetate, as shown in Table 1. Because several of these compounds are chemically unrelated, it was reasoned that these responses might all be mediated by a single class of

TABLE 1

Response of wild type to a variety of concentrated chemicals

	Concentration	Fraction nonavoiders (actual numbers)	% Nonavoiders	Time
NaCl	4.0 M	18/382	4.7	30′
Fructose	4.1 M	0/195	0	30′
Sorbitol	4.0 M	5/180	3	30′
Na acetate	4.0 M	0/165	0	15′
NH_4 acetate	4.0 M	10/138	7	15′
Glucose	4.5 M	36/371	10	30′
Glycerol	12.3 M	3/124	2.4	30′
H_2O		188/270	69	30′
H_2O		181/279	65	15′

The population assay was used to monitor the response of wild type (N2) *C. elegans* to a variety of chemicals applied to a standard chemotaxis plate as solutions of concentration indicated in the first column. Each solution also contained 1 mg/ml of the dye Light Green SF Yellowish (MCB) and the pH of each solution was adjusted to approximate neutrality with acetic acid or ammonium hydroxide. The fraction of individuals lying on or outside of each ring (fraction nonavoiders) was scored at times indicated in the right hand column.

receptors, sensitive to high osmotic strength. If so, single mutants defective in response to all these compounds might be obtainable, and if obtained could serve to identify the receptors mediating this avoidance behavior. Mutants were therefore selected for their inability to be repelled by high osmotic strength solutions of either NaCl or fructose.

Mutant selection: Wild-type (strain N2) *C. elegans* hermaphrodites were treated with EMS, divided into 25 independent lines, and grown for mutant expression (see MATERIALS AND METHODS). For 12 lines, selection (see MATERIALS AND METHODS) was for fructose nonavoidance, for five lines it was for NaCl nonavoidance, and for eight lines independent selections were separately performed for both nonavoidance behaviors. Individuals not exhibiting normal avoidance behavior were picked and cloned, and individuals from at least two of the following generations were retested. Seven mutant strains (P801, P802, P808, P811, P813, P816 and P821) were eventually retained for their inability to avoid high concentrations of fructose or NaCl. Results of the selection are summarized in Table 2. Two of the mutants, P813 and P816, were derived from the same line, but as shown below, these strains carried mutations in different genes, indicating that they were of independent origin. The final yield of mutants was four per roughly 20,000 gametes for the fructose selection and three per roughly 20,000 gametes for the NaCl selection, or approximately 0.02%.

The mutants were tested in population assays for their responses to high concentration rings of NaCl or fructose, as shown in Table 3. Although the method has some variability and cannot easily be used to measure response thresholds, it is clear that all of the mutants selected for their inability to avoid fructose are also defective in avoiding NaCl and *vice versa*. This result suggests that a general response to high osmotic strength has been knocked out in all these mutants, as

TABLE 2

Summary of mutant selections

Muta-genesis	EMS	Dextrose	Hrs	No. of lines	Selection	No. individuals	No. picked	No. retained	Mutants
1	0.33%	0.033%	4	5	5-NaCl	13,000	86	1	P821
2	0.33%	0.033%	4	10	10-fructose	10,000	42	1	P811
3	1%	0.1%	6	10	8-NaCl	8,000	50	2	P801, P802
					10-fructose	10,000	99	3	P808, P813, P816

Three mutageneses with EMS yielded 25 mutagenized lines of N2 hermaphrodites. F_2 progeny of the adults used to establish these lines were used to select for NaCl or fructose nonavoiding mutants. Eight of the lines in the third mutagenesis were used to select for both fructose and NaCl nonavoiders.

had been expected. In support, similar response defects for the other compounds avoided by wild type have been observed in all the mutants (data not shown).

Except where specified, all mutant assays were subsequently done using NaCl rings. The tracking patterns for populations of N2 and mutant P813 placed inside high concentration rings of NaCl are shown in Figure 1a.

Mutant responses: When placed inside a high concentration ring of NaCl, wild-type individuals are at first attracted to the NaCl (Figure 1b) but as they move up the concentration gradient, they are eventually repelled by some high threshold concentration. At this point the worm stops and usually moves backward by reversing the direction of wave propagation, much as in response to a tap on the snout. This is most often followed by a deep bend and subsequent forward motion away from the ring. Sometimes, however, an individual will

TABLE 3

Mutant responses to high concentrations of fructose and NaCl

	NaCl or fructose isolation	4 M NaCl		4.1 M fructose	
		Fraction nonavoiders	% Nonavoiders	Fraction nonavoiders	% Nonavoiders
N2		16/263	6	0/195	0
P801	N	218/292	75	47/139	34
P802	N	176/274	64	89/196	45
P808	F	301/356	85	87/232	38
P811	F	188/293	64	118/154	77
P813	F	186/218	85	158/171	92
P816	F	101/149	68	121/184	65
P821	N	149/222	67	48/177	27

The population assay was used to monitor the response of wild type and mutants to a ring of 4.0 M NaCl or 4.1 M fructose applied to a standard chemotaxis plate as described in MATERIALS AND METHODS. The fraction of individuals lying on or outside of each ring (fraction nonavoiders) was scored after 30 minutes. The difference in the fraction nonavoiders between NaCl and fructose for each of the mutants is not believed to be significant; similar variation (most probably due to small differences in overall tendency of the worms to disperse from the application site) is seen in repeat experiments involving the same mutant.

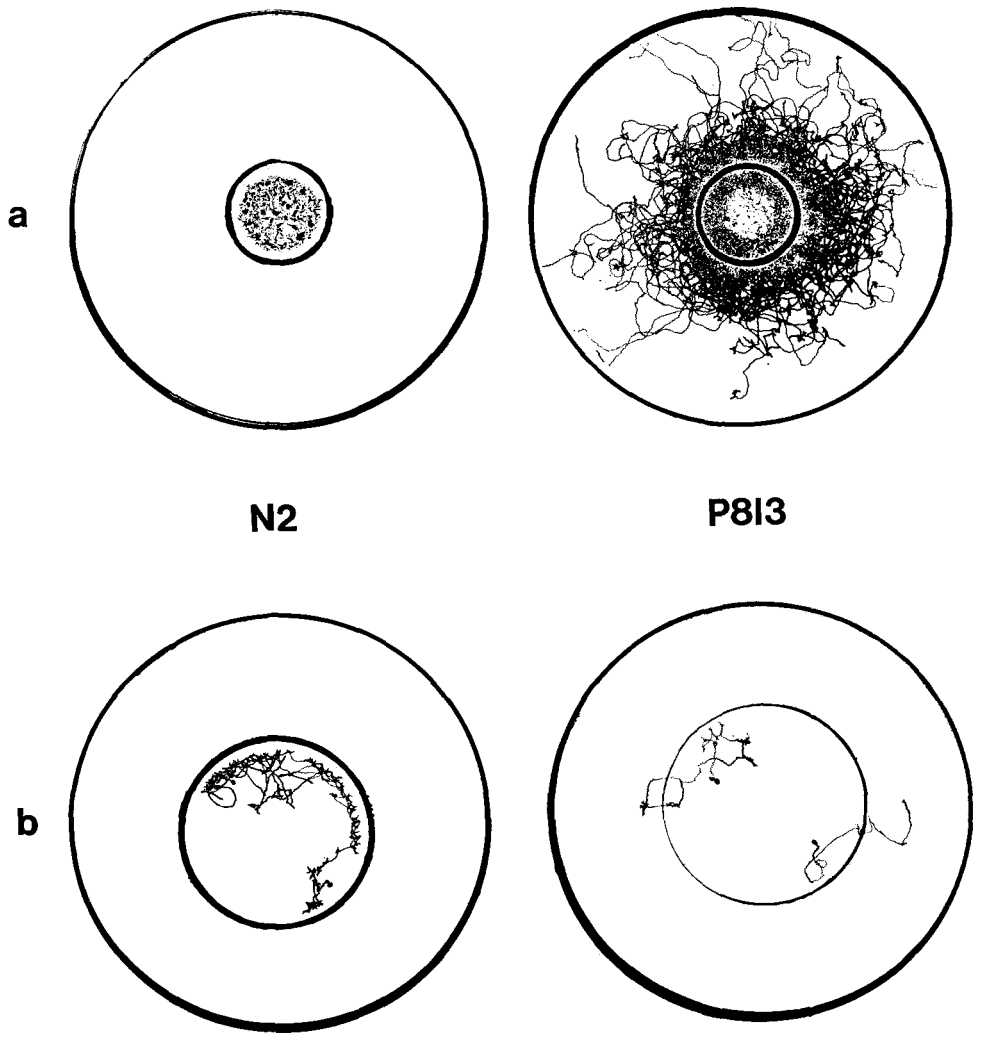

FIGURE 1.—(a) Population response of strain N2 (wild type) and strain P813 (osmotic nonavoiding mutant) to a high concentration ring of 4 M NaCl. The NaCl (0.015 ml of a 4 M solution) was applied to a standard chemotaxis plate (WARD 1973) in a ring of radius 1 cm and allowed to soak in. Populations of N2 (193 individuals) or P813 (238 individuals) growing in NGMM plates were washed with water, placed down in the center of the ring, and allowed to disperse. These pictures of the tracking patterns were taken ten min later; no N2 and 179 P813 individuals had escaped. (b) Individual responses of strain N2 (wild type) and strain P813 (osmotic nonavoiding mutant) to a high concentration ring of NaCl. The NaCl (0.03 ml of a 4 M solution) was applied to a standard chemotaxis plate in a ring of radius 2 cm and allowed to soak in. From populations growing on NGMM plates, two individuals of strain N2 were picked and placed on plate A, and two individuals of strain P813 were placed on plate B. These pictures of the tracking patterns were taken 20 min later. One P813 individual escaped on its second approach to the ring, while the other required four approaches.

forego the immediate reversal in wave propagation by simply bending away from the ring and subsequently moving forward.

In general, the mutants are not noticeably attracted to the salt ring, but when the ring is approached by chance, any one of three basic behavior patterns may be unpredictably observed: (1) the individual may move through the ring, seemingly without hesitation, (2) it may stop and search with its head before moving through the ring, or (3) it may stop and reverse, or stop and turn away from the ring like the wild type. The wild-type response is infrequent, but when it does occur for a particular mutant individual, it is most often followed by one of the other two responses on the next chance approach to the salt ring. The net result is that the individual is able to escape the ring within two or three chance approaches, as shown in Figure 1b.

Individual tracking patterns of wild-type and mutant individuals were tested for distinguishability in double-blind experiments (see MATERIALS AND METHODS). As Table 4 shows, mutant and wild-type tracking patterns are easily distinguished; the least reliably scored mutant is P821 at approximately 93% accuracy —more than sufficient for most genetic experiments.

Nature of the mutations: Hermaphrodites of each mutant strain were separately crossed to wild-type males. From the phenotypes of the resulting male progeny and hermaphrodite progeny (see BRENNER 1974), the defects of four of the mutants (P801, P802, P811 and P821) could be ascribed to autosomal recessive mutations, while those of the other three (P808, P813 and P816) could be ascribed to *X*-linked recessive mutations. For each mutant, heterozygous hermaphrodite progeny of the cross were allowed to produce progeny by self-fertilization; in all cases, very nearly one-fourth of the (approximately 100) progeny tested were osmotically nonavoiding, indicating that each mutant most probably harbors a single mutation (or less likely, two closely linked mutations) responsible for the osmotic nonavoidance phenotype. In keeping with accepted nomen-

TABLE 4

Results of double blind experiments

	Fraction of individuals identified as wild type	
	Mutant strain	Wild type
P801	0/26	30/31
P802	1/30	29/30
P808	1/28	29/30
P811	1/31	27/27
P813	0/28	27/29
P816	1/30	29/30
P821	2/30	27/28

Tracking patterns for individuals of the osmotic nonavoiding mutants and for simultaneously tested wild-type individuals were scored by the double-blind method described in MATERIALS AND METHODS. The fractions are given as actual numbers of individuals scored.

TABLE 5

Complementation properties of mutants

Male parent	*dpy* hermaphrodite parent						
	P801	P802	P811	P821	P808	P816	P813
P801	3/23(—)	24/24(+)	18/20(+)	24/24(+)			
P802	24/24(+)	3/24(—)	24/24(+)	22/24(+)			
P811	16/16(+)	N.D.	4/39(—)	N.D.			
P821	N.D.	15/15(+)	24/24(+)	3/31(—)			
P808					N.D.	0/9(—)	10/10(+)
P816					0/5(—)	N.D.	10/10(+)
P813					10/10(+)	10/10(+)	N.D.
	Autosomal				X-linked		

Mutant males were derived as described in MATERIALS AND METHODS and crossed with double mutants of each osmotic mutation with a dumpy marker. The fraction indicated is the fraction of nondumpy cross progeny exhibiting wild-type behavior. Each fraction presented is in the actual number of individuals scored. N.D. implies the cross was not done.

clature, these mutations are labelled according to the strain of origin (p801 for strain P801, etc.).

Complementation and mapping: As Table 5 shows, complementation experiments, done in all possible pairwise combinations, revealed that only mutations p808 and p816 are in the same complementation group. These mutations define the gene *osm-1*, whereas p801, p802, p821, p813 and p811 define genes *osm-2*, *osm-3*, *osm-4*, *osm-5* and *osm-6*, respectively.

TABLE 6

Mapping of osmotic avoidance mutations

Osmotic mutant	Gene name	Linkage group	Markers used	F	$P=\frac{F}{2-F}\times 100$	95% Confidence limits
P801	(*osm-2*)	I	dpy-5	8/123	3.4	1.7–6.8
			unc-13	1/107	0.5	0.1–2.3
P802	(*osm-3*)	IV	dpy-13	1/141	0.4	0.1–1.7
			unc-24	8/75	5.6	2.8–11.7
P808	(*osm-1*)	X	dpy-7	28/83	21.2	14.1–29.3
			lon-2	44/99	28.6	20.9–37.2
			unc-3	52/434	6.4	6.2–6.6
P811	(*osm-6*)	V	dpy-11	10/141	3.6	2.0–7.0
			unc-51	33/83	24.8	17.3–33.8
P813	(*osm-5*)	X	dpy-7	21/110	10.5	6.2–15.2
			lon-2	7/95	3.8	1.9–8.2
P821	(*osm-4*)	IV	dpy-13	5/40	7.1	2.8–17.0
			unc-24	2/132	0.8	0.2–2.4

F is the fraction of osmotic defectives (in actual numbers) that segregated the marker phenotype (see MATERIALS AND METHODS). P is the map distance calculated from F as indicated. The right hand column indicates the 95% confidence limits for each P, calculated according to MILLER and FREUND (1965).

The approximate locations of the *osm* genes on the chromosome map of *C. elegans* were determined by the methods outlined in MATERIALS AND METHODS. The linkage data are shown in Table 6 and the results are summarized in Figure 2. Only one three-factor cross has been carried out, that to establish the location of *osm-1*. This was done by crossing a *lon-2* male (long phenotype) to a *osm-1 unc-3* double-mutant hermaphrodite (uncoordinated and osmotic nonavoiding phenotypes). Wild-type hermaphrodite cross progeny were picked and allowed to self-fertilize. Sixty-seven osmotic avoidance defective progeny exhibiting normal motion were selected and again allowed to self-fertilize. Sixty-five of the osmotic avoidance defectives segregated both long and uncoordinated phenotypes, whereas two segregated only the long phenotype. This result places *osm-1* to the right of *unc-3* and extends the right end of the *X*-linkage group by approximately 6 map units.

Developmental studies: The osmotic responses of wild-type and mutant populations of newly hatched L1 juveniles were monitored in the population assay. The ability of wild-type individuals to avoid high salt concentrations is present from the time of hatching, and therefore does not depend on neurons generated

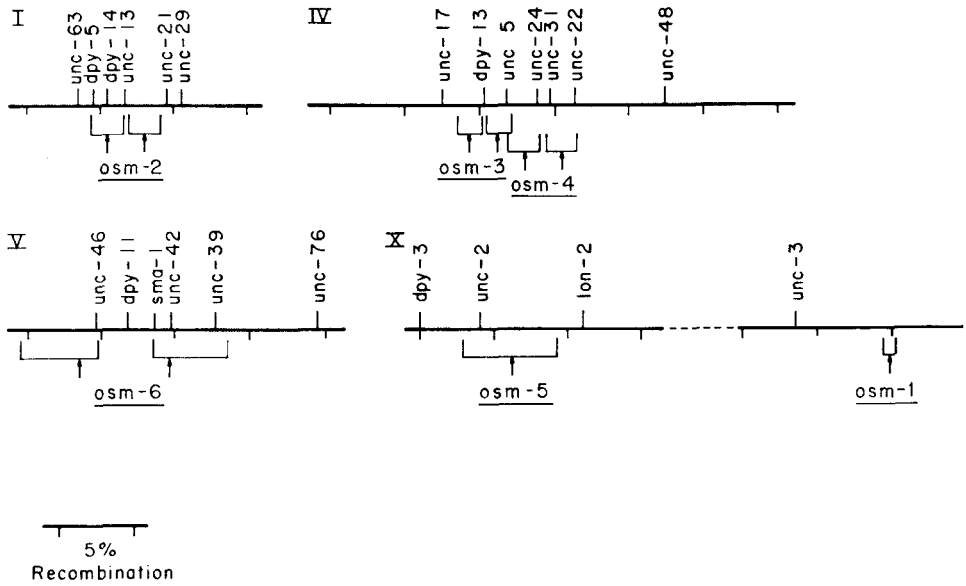

FIGURE 2.—Map locations of osmotic avoidance mutations. Map distances from two marker mutations on the same chromosome were determined for each of the six osmotic avoidance complementation groups (data shown in Table 6). Probable map locations relative to the closer marker are indicated by arrows (with 95% confidence intervals indicated by brackets). For *osm-2*, *osm-3*, *osm-4*, and *osm-6*, linkage data for the more distant marker were insufficient to distinguish between the two possible map locations (to the left or right of the closer marker), thereby necessitating the use of two arrows to indicate these possibilities. For *osm-5*, linkage data for the more distant marker (*dpy-7*) were sufficient for an accurate determination of its relative map location. A three-factor cross was done to determine the relative map location of *osm-1*.

by post-embryonic cell divisions (SULSTON 1976; SULSTON and HORVITZ 1977). Moreover, newly hatched individuals of all the mutants are unable to avoid high salt concentrations, suggesting that none of the mutants is post-embryonically degenerative in nature. Wild-type individuals also respond normally following several generations of growth at 16° or 25°, whereas the mutants all respond abnormally following growth at either temperature. Therefore, none of the mutants is temperature-sensitive for development of avoidance behavior.

Populations of mutant hermaphrodites, started with three synchronously laid eggs, were allowed to develop undisturbed for about two generations, and the size distribution of the resulting population was measured on an electronic nematode counter (BYERLY, CASSADA and RUSSELL 1975). This size distribution was fitted by computer to a similarly obtained size distribution for wild-type *C. elegans* by varying scale factors for developmental rate, size, egg-laying rate, and developmental spread, as described previously (BYERLY, SCHERER and RUSSELL 1976). The results are shown in Table 7. The χ^2 values for the mutant parameters indicate that there are some differences from the wild-type developmental pattern for all of the mutants. With the exception of P816, the near wild-type values for the mutant parameters suggest that these differences are not of the major quantitative sort that would be expected if the mutants possessed major developmental defects. P816, however, has a significantly lower EGF (egg yield per individual) than wild type and may possess some developmental defect that lowers fertility. With the possible exception of mutant P816, therefore, the osmotic avoidance defects are not due to broadly acting mutations with extensive pleiotropic effects on development.

Behavioral spectrum of mutants: Approximately ten individuals from each osmotic nonavoiding mutant clone were assayed for other known *C. elegans*

TABLE 7

Mutant developmental parameters

	DRF	SIF	EGF	SPF	χ^2
N2	0.95	0.98	1.25	2.12	44.7
P801	0.88	1.02	1.22	1.44	56.0
P808	0.88	1.00	1.06	2.06	73.6
P811	0.93	0.98	1.00	1.88	86.7
P813	0.94	0.96	0.94	2.29	55.6
P816	0.94	0.98	0.73	1.66	112.3
P821	0.92	0.94	0.91	2.20	66.8

Using the method described by BYERLY, SCHERER and RUSSELL (1976), populations (two to four for each mutant) were started with three synchronously laid eggs and allowed to develop for about two generations (166.3 to 177.7 hr) at 20° thereafter. The resulting population size distribution was measured with an electronic nematode counter (BYERLY, CASSADA and RUSSELL 1975) and then computer-fitted to variations of a normative wild-type distribution generated by independently varying factors for developmental rate (DRF), size (SIF), egg yield per individual (EGF), and intrinsic temporal spread (SPF). The values giving the best fit are recorded; for the normative distribution, the factors would all be 1.00. The goodness-of-fit is indicated by the χ^2 value at the right; different N2 (wild type) populations typically give χ^2 values from 25 to 50. Development of P802 was not monitored.

behaviors. In general, all of the mutants moved normally and showed normal sensitivity to light head and tail tapping with a human eyelash. Additionally, individuals from each mutant strain were able to track isothermally as indicated by circular tracking patterns in radial thermal gradients of the type described by HEDGECOCK and RUSSELL (1975). However, when tested in individual tracking assays for chemotactic attraction to NaCl (WARD 1973; DUSENBERY, SHERIDAN and RUSSELL 1975), none of the mutants appeared to be attracted nearly as well as wild type. Although some individuals of every mutant showed attraction to NaCl, this attraction was slight, and other individuals of the same mutant type showed no apparent attraction. Mutant P802 is the best mutant in this respect—its tracking pattern most closely resembles the wild-type pattern.

Chemotaxis and thermotaxis mutants previously isolated in this laboratory (DUSENBERY, SHERIDAN and RUSSELL 1975; HEDGECOCK and RUSSELL 1975) were assayed in the population assay for their osmotic avoidance behavior. All of the mutants responded approximately like wild type.

DISCUSSION

The results described above show that *Caenorhabditis elegans* avoids high concentrations of at least two chemically unrelated groups of compounds, sugars and salts. They also show that this response depends on the products of at least six genes, identified by seven "osmotic avoidance" mutants, which map in widely separated positions. These gene products are also required for normal chemotactic attraction to NaCl, but not for responses to thermal and mechanical stimuli, nor for normal movement or development.

The fact that mutants isolated for their inability to avoid high concentrations of fructose are also unable to avoid high NaCl and *vice versa* suggests that a single response—probably an osmotic one—is involved. However, we cannot rule out the possibility that there really are two distinct receptors for the two kinds of chemicals and that the mutants have affected only the elements commonly required for the two responses. No evidence exists to refute this argument, and it is difficult if not impossible to test by competitive saturation experiments of the kind used in studying chemotaxis (WARD 1973). Additional mutants in new genes might be of some help, particularly if selection were made directly for the loss of one response and the retention of the other. Additional genes for these responses could almost certainly be found, given the fact that only two of the original seven mutants fail to complement.

The fact that all seven osmotic nonavoiding mutants show normal movement and thermotaxis demonstrates that at least some of their sensory capacities and most probably their entire motor control program are intact. Why mutants defective in reversal were not obtained is unknown. Perhaps a search for more osmotic avoidance mutants would turn up mutants defective in the motor program associated with the avoidance response.

The observation that all of the osmotic avoidance mutants fail to be attracted properly to NaCl demonstrates that chemotaxis to NaCl and osmotic avoidance

of NaCl (or fructose) share some common gene functions. (Apparently, these functions are not required for thermotaxis). The osmotic avoidance behavior therefore serves as a means of further subdividing the gene functions required for chemotaxis. It will be interesting to determine the complete array of chemotactic responses exhibited by the osmotic nonavoiding mutants (see DUSENBERY 1976).

The map positions of the osmotic avoidance mutants suggest some interesting functional possibilities. The *osm-1* complementation group, for example, is closely linked to a gene required for dauerlarvae formation (*daf-6*, D. RIDDLE, personal communication). Since there are suggestions that P808 and P816 might be unable to form SDS resistant dauerlarvae (R. L. RUSSELL, unpublished observations), it is possible either that these two mutant strains represent additional alleles of the *daf-6* gene or that they identify a nearby gene of related function. Similarly, P801 maps close to the *tax-1* complementation group (DUSENBERY, SHERIDAN and RUSSELL 1975; R. L. RUSSELL, unpublished results) with whose mutants it shows functional similarities; however, in this case two separate genes are indicated by complementation tests. The significance of such linked, functionally related genes remains to be seen.

From a purely utilitarian point of view, the osmotic avoidance mutations should provide useful markers for chromosome mapping studies since they are located on four of the six *C. elegans* linkage groups and they are compatible with many other mutant phenotypes. The *X*-linked mutations should be especially useful for *X*-chromosome mapping studies since they exhibit a nonmorphological phenotype that does not render hemizygous males incapable of mating, as do many other *X*-linked marker mutations with morphological or behavioral aberrencies. Along these lines, it is interesting to note that the *osm-1* complementation group is located on the extreme right end of the *X* chromosome and is covered by the duplication strain Dp(*X*;*V*)1 isolated by HERMAN, ALBERTSON and BRENNER (1976) (R. HERMAN and J. CULOTTI, unpublished results). Finally, the fact that rare osmotic defective homozygotes can be directly selected makes these mutants especially useful for three-factor crosses in which the production of homozygotes by recombination occurs infrequently.

Anatomical studies with the osmotic avoidance mutants, to determine the structural basis for the mutant defects, are currently underway. Preliminary serial section EM results obtained by R. WARE (personal communication) suggest that mutant P811 has defects affecting the sensory endings of the anteriorly located cephalic neurons (WARD *et al.* 1975; also called LSM neurons by WARE *et al.* 1975). At least two other mutants, P808 and P813, appear to have an altered distribution of the presumed neurotransmitter (dopamine) normally found in these neurons (SULTON 1976; J. CULOTTI, unpublished results). Whether these are indeed the neurons mediating the osmotic avoidance response must await the analysis of further mutants and the execution of selective cell-ablation experiments.

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