

# Effects of Starvation and Neuroactive Drugs on Feeding in *Caenorhabditis elegans*

LEON AVERY AND H. ROBERT HORVITZ

*Howard Hughes Medical Institute, Department of Biology,*

*Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

**ABSTRACT** *Caenorhabditis elegans* concentrates its food, bacteria, by pharyngeal pumping. The rate of pumping is affected by the presence of bacteria. Using a new assay that allows measurement of pumping rate in a population of worms suspended in liquid by measuring their uptake of microscopic iron particles, we have confirmed and quantitated this effect. Furthermore, we demonstrated that starvation stimulates pumping. Worms that had been deprived of bacteria for more than 4 hours pumped in the absence of bacteria under conditions in which well-fed worms did not. Furthermore, starved worms responded to lower amounts of bacteria than did fed worms.

The assay was also useful for measuring effects of drugs on pumping. Of about 30 chemicals screened, 5 had clear effects. The neurotransmitter serotonin and the serotonin uptake inhibitor imipramine stimulated pumping, while the serotonin antagonist gramine inhibits. Imipramine stimulation is greatly decreased in *cat-1* and *cat-4* mutants, which have low levels of serotonin. Muscimol, an agonist for the neurotransmitter GABA, and ivermectin, whose site of action may also be the GABA receptor, both inhibit pumping. Qualitative observations suggested a role for acetylcholine in the regulation of pumping.

Feeding has been extensively studied in *Caenorhabditis elegans*. Worms eat by pumping in bacteria, grinding them up, and passing the debris back to the intestine. The pumping and grinding are done by a neuromuscular organ called the pharynx. Albertson and Thomson ('76) described the anatomy of the pharynx in detail, and briefly described its normal operation. Doncaster ('62) and Seymour et al. ('83) have made more detailed cinematographic observations. The pharynx is a muscular pump that contains its own nervous system of 20 neurons. It is isolated from the rest of the worm by a basal lamina; a pair of extrapharyngeal neurons, the RIPs, form the only anatomical connection between the pharyngeal nervous system and the extrapharyngeal nervous system (Albertson and Thomson, '76; White et al., '86). Pharyngeal pumping is controlled by a variety of factors, including light touch (Chalfie et al., '85), hatching and molting (Singh and Sulston, '78), dauer larva formation (Cassada and Russell, '75), and bacteria (Croll, '78; Horvitz et al., '82).

Croll ('78) looked for an effect of starvation on feeding behavior, but was unable to detect any. He suggested that there is a maximum pumping rate of which the pharynx is capable, and that under the conditions he used, with the worms in a dense bacterial lawn, pumping is maximal for both starved and well-fed worms. This hypothesis

suggests that a difference between starved and well-fed worms might be detectable at lower densities of bacteria. To test this possibility, we have developed a liquid assay for measuring pumping rate in a population of worms, allowing convenient control of the density of bacteria during the assay, and accurate quantitation of the effects. Using this assay we detect a striking increase in pumping rate after four or more hours of starvation.

The assay was also useful for measuring drug effects on feeding. We confirm previous observations of stimulation of pumping by serotonin (Croll, '75; Horvitz et al., '82), and show that imipramine, gramine, muscimol, and ivermectin also affect pumping rate. We also report some qualitative observations suggesting a role for acetylcholine in the control of pharyngeal muscle contraction.

## MATERIALS AND METHODS

### *General methods and strains*

General methods for handling and observing worms are described by Sulston and Hodgkin ('88). Worms were kept at 20°C.

Received March 23, 1989; revision accepted August 9, 1989.

Leon Avery's present address is MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England. Address reprint requests there.

*cha-1(m324) dpy-13 ama-1* worms were obtained as uncoordinated (Unc) larvae from the balanced heterozygote DR697.<sup>1</sup> These are referred to simply as *cha-1* worms. (*dpy-13* and *ama-1* are invisible in an *m324* homozygote and irrelevant to our experiments. *ama-1* confers amanitin resistance, which cannot be seen in worms that do not grow due to the *cha-1* mutation, and young *dpy-13* larvae are indistinguishable from wild-type larvae.) *cha-1(m324) dpy-13 ama-1 unc-22* worms (referred to as *cha-1 unc-22*) were obtained as twitcher (Unc-22) larvae from the balanced heterozygote MT3392. *cha-1(m324)* was isolated by Rogalski and Riddle ('88) in a screen for lethal mutations linked to *ama-1*. *cha-1* encodes choline acetyltransferase (Rand and Russell, '84; Rand, '89) has shown that *m324* maps within the limits of the *cha-1* gene as defined by viable alleles. The recessive mutation *cha-1(m324)* probably reduces or eliminates *cha-1* activity and acetylcholine.

#### Pumping iron assays

Densities of bacteria shown in Figure 3 are relative to a saturated L broth culture. *E. coli* strain OP50 was grown to saturation in L broth, harvested by centrifugation, washed once in SB, and resuspended in a volume of SB equal to 1/40 of the volume of the original culture. (SB is 100 mM NaCl, 5 µg/ml cholesterol, 50 mM potassium phosphate buffer [pH 6.0].) This suspension, referred to as 40X OP50, was diluted to the desired density for experiments. The suspension could be kept at 4°C for several months with no diminution in its effects on feeding.

Wild-type worms for pumping iron assays were grown on 10 cm NG or ENG plates spread with NA22, a prototrophic *Escherichia coli* K12 strain. (NG is described by Brenner, '74.) ENG, an enriched variant of NG allowing growth of larger numbers of well-fed worms, contains 0.3% NaCl, 0.5% bactopectone, 0.1% yeast extract, 3% agar, 5 µg/ml cholesterol, 1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, and 25 mM potassium phosphate buffer (pH 6.0). A few N2 L4 hermaphrodites (4–8 for NG plates, 10 for ENG) were placed on a plate. Five days later the plates were checked to see that they still had

abundant bacteria, then the worms were washed off with 4 ml SM. (SM is the liquid growth medium described by Sulston and Brenner, '74.) The worms were centrifuged for a few seconds in a clinical centrifuge, and then sucked up using a 100 µl capillary tube. After two washes in 2.5 ml SM, they were put in 2.5 ml SM, and kept on a roller until use. The suspension contained 500–1,000 worms/ml. For assays on fed worms, 125 µl 40X OP50 was added, and the worms were used within 2 hours. For assays on starved worms, no bacteria were added, and the worms were agitated for 6–12 hours before use. For the experiment shown in Figure 2, worms were prepared as before, except that the time of agitation was varied.

Immediately before use, a 1 ml aliquot of worms was centrifuged in an Eppendorf microfuge, washed in 1 ml SB, and suspended in 1 ml SB. Next, 200 µl of this suspension was added to 400 µl of SB containing 1.5 times the desired concentration of drugs or bacteria, and the mixture was vigorously agitated for 10 minutes. Five microliters of 100 mg/ml iron suspension was added, prepared within 5 minutes of use by adding SB to iron particles (carbonyl iron, Sigma). (For testing the effects of bacteria on starved worms, we used 5 µl of 40 mg/ml iron suspension because otherwise worms took up too much iron for accurate scoring. Controls show that iron uptake is roughly linear with iron density over this range.) Precisely 5 minutes after addition of iron, 20 µl of 100 mM sodium azide was added to stop pumping, followed within a minute by 1 ml of a saturated solution of chloroform in SB to kill the worms. Excess iron was removed by holding a horseshoe magnet to the side of the tube and removing the liquid containing the worms with a Pasteur pipette. Living worms were kept at 20°C at all times.

Tubes were left at 4°C for from 12 hours to several days, then the worm carcasses were pelleted by a brief spin in an Eppendorf microfuge. The carcasses were sucked up in 20–40 µl and spread out on a microscope slide. With the aid of polarized dark-field microscopy, iron particles could be seen as bright reddish points in the dim bluish carcasses. Usually we counted the iron particles in the pharynxes and intestines of each of 100 adult worms, though in some cases we counted as few as 40. Often the iron particles clumped; in these cases we guessed the number of particles in the clump from its size and shape. Because this introduced some subjectivity into the scoring,

<sup>1</sup>The wild-type strain was N2 (Brenner, '74). Mutant strains used were: CB1111 *cat-1(e1111amber) X*, CB1112 *cat-2(e1112) II*, CB1141 *cat-4(e1141) V* (Sulston et al., '75), CB1072 *unc-29(e1072amber) I* (Lewis et al., '80a), DR697 *cha-1(m324) dpy-13(e184sd) ama-1(m118sd) / nT1(IV)*; *+nT1(V)* (Rogalski and Riddle, '88), and MT3392 *cha-1(m324) dpy-13(e184sd) ama-1(m118sd) unc-22(e66) / nT1(IV)*; *+nT1(V)* (which we constructed). *C. elegans* genetic nomenclature is described by Horvitz et al. ('79).

most assays (and all in which bias might conceivably be a problem) were scored blind together with controls. Clumping became a serious problem only when there were at least 10 particles, so worms that had 10 or more particles were recorded as a single class, and treated for averaging purposes as if they had had exactly 10 particles. We use an arrow ( $\rightarrow$ ) in figures to flag assays in which 20% or more of the worms had 10 or more particles, so that the average given is likely to be an underestimate.

We estimated the sources and sizes of errors in pumping iron assays by analysis of variance on 84 control assays of starved worms, done over a period of 10 months. Assays done on the same day on worms from the same population varied by about 21% (i.e., the standard error of  $\ln(p)$  where  $p$  is the result of an assay, was 0.21). We therefore considered drug effects of two-fold or more compared to a concurrent control likely to be significant, and retested them. Assays done on different days varied by 45%. We traced part of this between-days variance to the effects of old iron. Assay results dropped gradually over 6 months that we used iron from the same jar, but returned to the initial high level when we used iron from a freshly opened jar.

### *Chemicals*

Ivermectin (22,23-dihydro avermectin) was a gift from Merck, Sharp, and Dohme, Inc. All other chemicals were from Sigma. The hydrogen tartrate salt of nicotine was used, and the creatine sulfate complex of serotonin.

### *Single worm assays of imipramine stimulation of pumping*

An individual L4 hermaphrodite was placed on a 6 cm NG plate seeded with OP50. One day later the adult was transferred without bacteria to an NG plate. After 5 minutes to allow the worm to adapt, pumps were counted during 5 successive 1 minute periods. Often the counts were interrupted by the worm crawling to the edge of the plate. When this happened, we put the worm back in the center, then waited 1 minute before resuming counts. The median of the 5 counts was taken as the worm's basal pumping rate. The worm was put back on its seeded plate for at least 10 minutes, then tested on an NG plate containing 20  $\mu\text{g/ml}$  imipramine. In a few cases we did the imipramine test first, followed by the control; this had no obvious effect on the results. Paired control and imipramine plates were made at the same

time from melted NG agar and a concentrated imipramine stock less than 48 hours before use, and each pair was used for a mutant and a wild-type control. Wilcoxon's signed rank test (Sokal and Rohlf, '81) was used to test imipramine stimulation for a given strain for significance, and to test for significant differences between mutant and paired wild-type controls. Assays were done at room temperature, which varied from 20 to 25°C. We have since discovered that temperature has a strong effect on pumping, worms pumping faster at higher temperatures. The need for paired assays could probably be eliminated by doing the assays at constant temperature.

## RESULTS AND DISCUSSION

### *Effects of bacteria and starvation on pumping*

By counting pharyngeal muscle contractions in individual worms, Croll ('78) and Horvitz et al. ('82) showed that bacteria stimulate pumping. Croll ('78) looked for but was unable to detect any effect of starvation on pumping. To explore in detail the effects of bacteria and starvation on pumping, we developed a method for simultaneously measuring pumping in a large number of worms. Worms were suspended in liquid with 5  $\mu\text{m}$  iron particles and any drugs or bacteria to be tested, and the particles eaten by each of about 100 worms were counted. Figure 1 shows histograms obtained using well-fed or starved worms in the presence or absence of bacteria. The average number of iron particles per worm was used to quantitate feeding. These measurements confirmed the stimulation of pumping by bacteria, and demonstrated a previously undetected stimulation of pumping by starvation. Both of these effects make sense: pumping is more useful when food is present, and the need for food is greater when starved. Figure 2 shows how pumping in the absence of bacteria depends on time of starvation.

Starved worms responded to much lower amounts of bacteria than were necessary to elicit a response in well-fed worms (Fig. 3). Since the assays were finished 15 min after the addition of bacteria, this stimulation was rapid; indeed, direct observation of worms on plates showed that they responded within a minute. (See also Croll, '78.) (The assays on starved worms in Figure 3 were done at a lower iron concentration than normal, to prevent saturation at these high pumping rates. The vertical scales of the two curves in

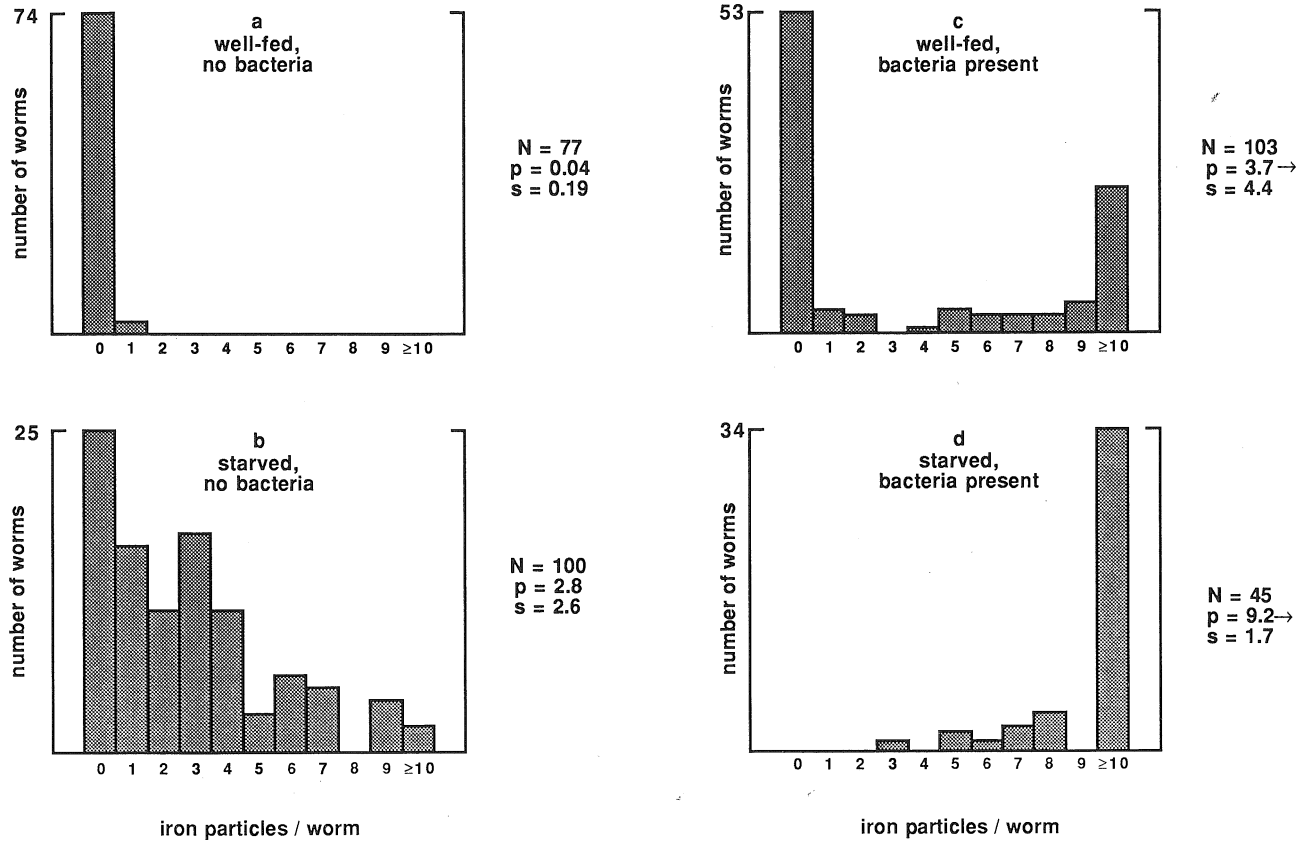


Fig. 1. Pumping rate histograms. Pumping was measured using the pumping iron assay described in Materials and Methods, and the number of iron particles in each of about 100 worms counted.  $N$  is the total number of worms counted,  $p$  the average number of particles/worm (treating all worms in the  $\geq 10$  class as if they had exactly 10 particles; see Materials and Methods), and  $s$  is the sample standard deviation. **a:** Well-fed worms in the absence of bacteria. Well-fed worms were given abundant food until a few minutes before the assay. There was little or no pumping. **b:** Starved worms in the

absence of bacteria. Starved worms were suspended in liquid without food for more than 6 hours. The distribution of pumping rates was broad, but unimodal. **c:** Well-fed worms in the presence of bacteria. The worms were divided into two populations on the basis of pumping rate. Because many worms were in the  $\geq 10$  class, the average 3.7 underestimates pumping rate. This is indicated by the following  $\rightarrow$ . **d:** Starved worms in the presence of bacteria. All worms pumped rapidly. As in **c**, the average 9.2 probably underestimates pumping rate.

Figure 3 have been adjusted so that the curves are directly comparable.) Furthermore, starved worms responded to bacteria with uniformly rapid pumping (Fig. 1d), but only half the well-fed worms pumped rapidly (Fig. 1c).

The response of worms to bacteria was neither a simple chemical response nor a simple response to particles. Pumping was not significantly stimulated by bacterial culture supernatant, by soluble material from bacteria extracts, or by  $1 \mu\text{m}$  latex beads (data not shown). That the regulation of pumping by bacteria is complex is not surprising, since there are many chemosensory and mechanosensory neurons whose connectivity suggests they might regulate feeding in response to bacteria (Albertson and Thomson, '76; White et al., '86). Iron uptake was roughly linear with iron

density at the levels used here (data not shown), suggesting iron particles had little or no effect on pumping rate.

#### *Drugs that affect pumping*

We used the pumping iron assay to quantitate the effects of about 30 drugs on pumping. Five had clear effects: serotonin, imipramine, and gramine, which are known to have effects on serotonergic transmission, and muscimol and ivermectin, which may affect GABAergic transmission. In addition, although side effects prevented meaningful quantitation, we made some qualitative observations suggesting that drugs that influence cholinergic transmission may affect pumping.

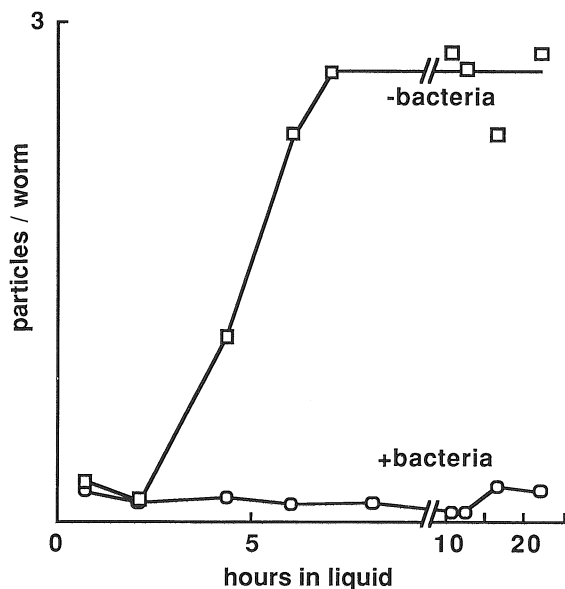


Fig. 2. Effects of starvation on pumping. At time zero worms were harvested from a plate that had a thick bacterial lawn, washed free of bacteria, and suspended in SM. Bacteria were added to the control worms ( $\circ$ ) to a density twice that of a saturated L broth culture; the experimental worms ( $\square$ ) were left without bacteria. The worms were agitated at 20°C for the time shown, then washed again (so that both experimental and control worms were assayed without bacteria) and assayed for pumping iron as described in Materials and Methods.

### Serotonin

We have confirmed the finding of Croll ('75) and Horvitz et al. ('82) that serotonin stimulates pumping (Fig. 4a). Furthermore, high concentrations of the serotonin antagonist gramine (Evans and Shea, '78) inhibited pumping (Fig. 4b), and at low concentrations the serotonin uptake inhibitor imipramine (Briley, '75; Desai and Horvitz, '89) stimulated pumping (Fig. 4c).

Although small, the imipramine stimulation of pumping was reproducible (8/8 experiments). Moreover, it could be reliably seen in single worms (Table 1); 20  $\mu$ g/ml imipramine stimulated pumping in 29/30 wild-type worms tested. (The exceptional worm moved sluggishly on the imipramine plate and pumped only 4 times in 5 minutes. It may have been injured when it was transferred to the plate.) Since one effect of imipramine is to enhance the effect of endogenous serotonin (Briley, '75; Desai and Horvitz, '89), we used mutations in the genes *cat-1*, *cat-2*, and *cat-4* to test whether imipramine stimulation was correlated with endogenous serotonin levels. Table 1 shows that imipramine stimulation of pumping was



Fig. 3. Effects of bacteria on pumping. Pumping was measured with bacteria present at the indicated densities during the assay. The density of bacteria is relative to a saturated L broth culture. The concentration of iron in the assays on well-fed worms ( $\circ$ ) was 0.83 mg/ml, that in the assays on starved worms ( $\square$ ) 0.33 mg/ml. The vertical scales have been adjusted so that the plotted curves can be compared directly. Arrows on some points on the fed curve indicate that more than 20% of the worms in the assay had  $\geq 10$  particles in them, so that the pumping rate plotted is probably an underestimate.

greatly decreased in *cat-1* and *cat-4* mutants, which have low levels of serotonin and dopamine, but not in *cat-2* mutants, which have very little dopamine but roughly normal levels of serotonin (Sulston et al., '75; Horvitz et al., '82; Desai et al., '88). Thus imipramine stimulation correlated with serotonin levels. In the absence of imipramine, *cat-2* worms were superficially normal. *cat-1* and *cat-4* worms were slightly small and pale, *cat-4* more so than *cat-1*, suggesting that in the presence of bacteria they ate less than wild-type or *cat-2* worms. *cat-4* worms also pumped significantly less than wild-type worms in the absence of bacteria and imipramine (Table 1). One possible explanation is that endogenous serotonin may be necessary for normal pumping.

Although low concentrations of imipramine stimulated pumping, high concentrations were inhibitory. This may be a general anesthetic effect. Morgan and Cascorbi ('85) have shown that several general anesthetics paralyze *C. elegans* and that, as in higher animals (Meyer, '37), the potency of an anesthetic is related to its hydrophobicity. Imipramine and all other hydrophobic drugs we tried caused paralysis as well as cessa-

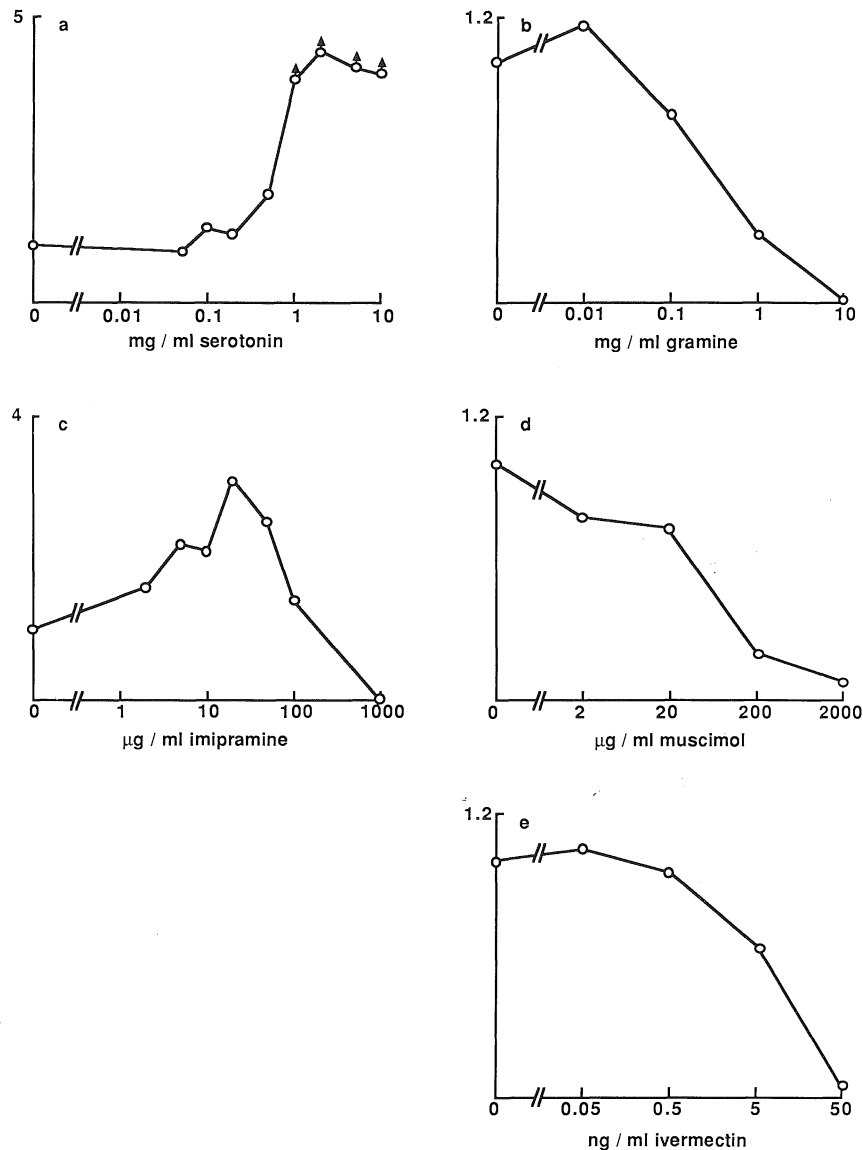


Fig. 4. Effects of drugs on pumping. Starved worms, prepared as described in Materials and Methods, were assayed for pumping iron in varying concentrations of serotonin (a), gramine (b), imipramine (c), muscimol (d), or ivermectin (e). (Ivermectin assays contained 1% dimethyl sulfoxide.) The

plots have been normalized so that the ordinate at zero drug concentration is 1. The arrows on points in a indicate that more than 20% of the worms in these assays had  $\geq 10$  iron particles, so that the indicated pumping rate is probably an underestimate.

tion of pumping, and their potency was inversely related to their water solubility (data not shown).

#### GABA

The GABA agonist muscimol inhibited pumping (Fig. 4d). Ivermectin, which may affect GABAergic transmission (Kass et al., '80; Drexler and Sieghart, '84), was an extremely potent inhibitor of pumping (Fig. 4e).

#### Acetylcholine

We used a mutation in the choline acetyltransferase gene, *cha-1(m324)* (Rand and Russell, '84; see also Materials and Methods), to test whether acetylcholine was necessary for normal pumping. *cha-1* worms hatch and can be recognized as small, extremely uncoordinated larvae, which never grow. When we examined *cha-1* worms by Nomarski microscopy (these worms are too small

TABLE 1. Imipramine stimulation of pumping in single worms<sup>1</sup>

Genotype	N	Imipramine ( $\mu\text{g/ml}$ )		Stimulation
		0	20	
+	30	33 $\pm$ 5	111 $\pm$ 7	78 $\pm$ 6
<i>cat-1</i>	10	25 $\pm$ 6	36 $\pm$ 8**	11 $\pm$ 5**
<i>cat-2</i>	10	26 $\pm$ 5	82 $\pm$ 12**	56 $\pm$ 10*
<i>cat-4</i>	10	10 $\pm$ 4**	24 $\pm$ 8**	14 $\pm$ 6**

<sup>1</sup>A well-fed worm was placed on a plate with no bacteria and no imipramine, and on one with 20  $\mu\text{g/ml}$  imipramine dissolved in the agar, and the pharyngeal pumps in 5 successive 1-minute intervals were counted for each plate. The median of the 5 counts is the pumping rate for that worm. N is the number of worms so tested. The 0  $\mu\text{g/ml}$  and 20  $\mu\text{g/ml}$  imipramine columns give the average of the medians. Each mutant worm was paired with a wild-type worm tested on the same plates within 30 minutes. The results of the control tests are pooled in the first row. The stimulation for a given worm is the difference between the 0  $\mu\text{g/ml}$  imipramine and 20  $\mu\text{g/ml}$  imipramine values. All numbers are mean  $\pm$  SEM. The stimulation in *cat-4* was statistically significant at the 5% level, suggesting *cat-4* may not completely eliminate serotonin. The stimulation in *cat-1* worms was not quite statistically significant.

\*Significantly different from +,  $0.01 < P < 0.05$  (Wilcoxon's signed rank test).

\*\*Significantly different from +,  $0.001 < P < 0.01$  (Wilcoxon's signed rank test).

to eat iron particles), we found that they pumped only rarely (less than once a minute), even in the presence of 5 mg/ml serotonin. We sometimes saw coordinated twitches, in which all the terminal bulb muscles twitched at once, followed by a slight peristaltic twitch of the isthmus muscles. (See Albertson and Thomson, '76, for pharynx anatomy.) We interpret this movement as a very brief pump.

*cha-1* worms pumped more frequently in the presence of the nicotinic acetylcholine agonist nicotine, and the pumps were more often complete, rather than twitches. (In the presence of 5 mg/ml serotonin 0/4 *cha-1* worms pumped. 0/9 pumped in 5 mg/ml serotonin and 10  $\mu\text{M}$  nicotine; 1/10 in 5 mg/ml serotonin and 100  $\mu\text{M}$  nicotine; and 9/14 in 5 mg/ml serotonin and 1 mM nicotine. Each worm was observed for only a few seconds, and coordinated twitches were not counted.) The muscarinic agonist oxotremorine (1  $\mu\text{l/ml}$  or 10  $\mu\text{l/ml}$ ) did not obviously increase the frequency of pumping in *cha-1* worms, but the rare pumps that did occur became long-lasting. These observations suggested to us that acetylcholine acting at nicotinic receptors might promote pharyngeal contraction, while acetylcholine acting at muscarinic receptors might inhibit relaxation. The effects of cholinergic drugs on pharyngeal muscle in wild-type worms were consistent with this model.

In wild-type worms nicotine stimulated pharyngeal contraction, in some cases leading to tetanus: sustained contractions lasting more than 30 seconds. (Natural pumps are always less than 1 second long.) The muscarinic agonists pilocarpine (50 mM) and oxotremorine (1  $\mu\text{l/ml}$  or 10  $\mu\text{l/ml}$ ) both lengthened pharyngeal contractions: in some wild-type animals pumping continued, but the contractions lasted longer, while in others the pharynx went into tetanus for up to several minutes. In the muscarinic antagonist scopolamine (10 mM or 50 mM) pumps were generally briefer than normal; in extreme cases we saw only coordinated twitches.

If a nicotinic receptor is involved in the control of pumping, it is pharmacologically and genetically distinct from the well-characterized levamisole receptor present on body muscle (Lewis et al., '87b). Levamisole did not cause pharyngeal tetanus or rescue pumping in *cha-1* worms. This failure was not due to a toxic effect of levamisole, because levamisole did not block the effects of nicotine. *unc-29* worms, which lack detectable levamisole receptors (Lewis et al., '87a), pumped normally, and the effect of nicotine on the *unc-29* pharynx was identical to that in wild-type, even though the *unc-29* mutation blocks the effect of nicotine on body muscle (Lewis et al., '80a).

#### ACKNOWLEDGMENTS

This work was supported by research grants GM24663 and GM24943 from the U.S. Public Health Service. H.R.H. is an investigator of the Howard Hughes Medical Institute. L.A. was supported by a postdoctoral fellowship from the Damon Runyon-Walter Winchell Cancer Fund. We thank Theresa Rogalski for strains, and Jim Rand for communicating unpublished results. We also thank Marty Chalfie for noticing that ivermectin inhibits pumping.

#### LITERATURE CITED

- Albertson, D.G., and J.N. Thomson (1976) The pharynx of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. Ser. B, 275:299-325.
- Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. Genetics, 77:71-94.
- Briley, M. (1975) Imipramine binding: Its relationship with serotonin uptake and depression. In: Neuropharmacology of Serotonin. R. Green, ed. Oxford University Press, New York, pp. 50-70.
- Cassada, R.C., and R.L. Russell (1975) The dauer larva, a postembryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol., 46:326-342.
- Chalfie, M., J.E. Sulston, J.G. White, E. Southgate, J.N.

- Thomson, and S. Brenner (1985) The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *J. Neurosci.*, 5:956-964.
- Croll, N.A. (1975) Indolealkylamines in the coordination of nematode behavioral activities. *Can. J. Zool.*, 53:894-903.
- Croll, N.A. (1978) Integrated behavior in the feeding phase of *Caenorhabditis elegans* (Nematoda). *J. Zool. (Lond.)*, 184:507-517.
- Desai, C., G. Garriga, S.L. McIntire, and H.R. Horvitz (1988) A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. *Nature*, 336:638-646.
- Desai, C., and H.R. Horvitz (1989) *C. elegans* mutants defective in the functioning of the motor neurons responsible for egg laying. *Genetics*, 121:713-721.
- Doncaster, C.C. (1962) Nematode feeding mechanisms. I. Observations on *Rhabditis* and *Pelodera*. *Nematologica*, 8:313-320.
- Drexler, G., and W. Sieghart (1984) Properties of a high-affinity binding site for [<sup>3</sup>H]avermectin B<sub>1a</sub>. *Eur. J. Pharmacol.*, 99:269-277.
- Evans, P., and M. Shea (1978) The identification of an octopaminergic neuron and the modulation of a myogenic rhythm in the locust. *J. Exp. Biol.*, 73:235-260.
- Horvitz, H.R., S. Brenner, J. Hodgkin, and R.K. Herman (1979) A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. *Mol. Gen. Genet.*, 175:129-133.
- Horvitz, H.R., M. Chalfie, C. Trent, J.E. Sulston, and P.D. Evans (1982) Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science*, 216:1012-1014.
- Kass, I.S., C.C. Wang, J.P. Walrond, and A.O.W. Stretton (1980) Avermectin B<sub>1a</sub>, a paralyzing anthelmintic that affects interneurons and inhibitory motoneurons in *Ascaris*. *Proc. Natl. Acad. Sci. USA*, 77:6211-6215.
- Lewis, J.A., C.-H. Wu, H. Berg, and J.H. Levine (1980a) The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. *Genetics*, 95:905-928.
- Lewis, J.A., J.S. Elmer, J. Skimming, S. McLafferty, J. Fleming, and T. McGee (1987a) Cholinergic receptor mutants of the nematode *Caenorhabditis elegans*. *J. Neurosci.*, 7:3059-3071.
- Lewis, J.A., J.T. Fleming, S. McLafferty, H. Murphy, and C. Wu (1987b) The levamisole receptor, a cholinergic receptor of the nematode *Caenorhabditis elegans*. *Mol. Pharmacol.*, 31:185-193.
- Meyer, K.H. (1937) Contribution to the theory of narcosis. *Trans. Faraday Soc.*, 33:1062-1068.
- Morgan, P.G., and H.F. Cascorbi (1985) Effect of anesthetics and a convulsant on normal and mutant *Caenorhabditis elegans*. *Anesthesiology*, 62:738-744.
- Rand, J.B., and R.L. Russell (1984) Choline acetyltransferase-deficient mutants of the nematode *Caenorhabditis elegans*. *Genetics*, 106:227-248.
- Rand, J.B. (1989) Genetic analysis of the *cha-1/unc-17* gene complex in *Caenorhabditis*. *Genetics*, 122:73-81.
- Rogalski, T.M., and D.L. Riddle (1988) A *Caenorhabditis elegans* RNA polymerase II gene, *ama-1 IV*, and nearby essential genes. *Genetics*, 118:61-74.
- Seymour, M.K., K.A. Wright, and C.C. Doncaster (1983) The action of the anterior feeding apparatus of *Caenorhabditis elegans* (Nematoda: Rhabditida). *J. Zool. (Lond.)*, 201:527-539.
- Singh, R.N., and J.E. Sulston (1978) Some observations on moulting in *Caenorhabditis elegans*. *Nematologica*, 24:63-71.
- Sokal, R.R., and F.J. Rohlf (1981) *Biometry*, 2nd ed. W.H. Freeman and Co., San Francisco.
- Sulston, J.E., and S. Brenner (1974) The DNA of *Caenorhabditis elegans*. *Genetics*, 77:95-104.
- Sulston, J.E., M. Dew, and S. Brenner (1975) Dopaminergic neurons in the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.*, 163:215-226.
- Sulston, J.E., and J. Hodgkin (1988) *Methods*. In: *The Nematode Caenorhabditis elegans*. W. Wood, ed. Cold Spring Harbor Press, Cold Spring Harbor, N.Y., pp. 587-606.
- White, J.G., E. Southgate, J.N. Thomson, and S. Brenner (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. Ser. B*, 314:1-340.