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Regulation and Cell Autonomy during Postembryonic Development of *Caenorhabditis elegans*

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The role of cell-cell interaction in the postembryonic development of nongonadal tissues in the nematode *Caenorhabditis elegans* has been explored by selective cell ablation with a laser microbeam. Examples have been found of induction and of regulation in cell lineage and fate. Regulation in which one cell precisely or partially replaces another is seen, but only in certain groups of hypodermal cells which resemble one another closely; cells which are unique are not replaced in this way. The regulation of cell form is more widespread and less restrictive.

INTRODUCTION

The cellular development of the nematode *Caenorhabditis elegans* is now known from hatching to maturity (Sulston and Horvitz, 1977; Sulston *et al.*, 1980; Kimble and Hirsh, 1979). The pattern of divisions and differentiations is constant from animal to animal, and the fate of each terminally differentiated cell is generally correlated with its position in the lineage tree. This might be a consequence of the cell's ancestry or might be due to reproducible interactions with its neighbours so that it is always exposed to the same influences. Selective ablation of cells with a laser microbeam can help to decide between these possibilities, and we have carried out such experiments on the nongonadal tissues of *C. elegans*.

In the past the development of nematodes has been experimentally rather inaccessible, because of the lack of a culture medium for their cells. The egg is surrounded by a rigid shell and the hatched animal by a tough cuticle; extensive perforation of these coverings, in a cutting or grafting operation, causes rapid death. Thus the technique of blastomere isolation, which was employed classically to demonstrate cell autonomy in the early embryogenesis of several invertebrates (reviewed by Davidson, 1976), could not be used in nematodes. Individual blastomeres in nematode eggs could, however, be inactivated by ultraviolet irradiation, and the results were consistent with cell autonomy of development (reviewed by Nigon, 1965). Recently, true blastomere isolation experiments have been carried out in *C. elegans*, and have confirmed the absence of regulation in young embryos of this species (Laufer *et al.*, 1980).

In the absence of any evidence for regulative development, the reproducibility of the cell lineages in nematodes led to the idea that they may be purely mosaic organisms. Here we show that certain cell lineages in *C. elegans* are, in fact, capable of regulation, although the majority appear to be autonomous. We define regulation as the generation of structures from alternative progenitors after the destruction of their normal progenitors.

MATERIALS AND METHODS

The culture and handling of *C. elegans* have been described in the preceding paper and by Brenner (1974).

A laser microbeam system was developed by one of us (J.G.W.) in order to kill individual cells in live animals. The system is

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based on that designed by Berns (1972). A Zeiss universal microscope with Nomarski optics was modified by the addition of a special nose piece containing a semisilvered mirror mounted immediately above the objective. The laser beam was directed in from the side through a 5-cm focal length lens to the mirror and then down through the objective (Plan 100). The lens was set up so that the laser beam was brought into focus at the image plane of the objective and so was also in focus at the specimen plane. An Electro Photonics flashtube-pumped dye laser was used with coumarin 2 dye (Eastman Kodak) which gave pulses of 1-μsec duration with a maximum energy of 250 mJ per pulse at a wavelength of 450 nm. An auxiliary He/Ne gas laser was used in conjunction with a partially reflecting pellicle so that part of its beam would be directed into the cavity of the dye laser. When the incident and emergent beams are parallel they coincide with the beam from the dye laser and so the emergent beam can be used for alignment purposes. A spot size of about 0.8 μm (diameter of central maximum) was obtained with this system. It was found that reasonable depth resolution was obtained with the high (1.25) numerical aperture lens used. It was possible to kill cells which were in the center of an animal with minimal damage to the cells above and below. The use of a laser microbeam to study nematode development has also been described by Samoiloff (1973).

For successful cell ablation the adjustment of microbeam energy is critical, and it is usually more satisfactory to use a series of low energy pulses, during which the condition of the cells can be monitored, rather than a single high energy one. The target cell is not destroyed instantaneously, but often dies in a way reminiscent of programmed cell death. Its refractility increases and then it slowly shrinks. Excessive damage, which may kill neighbouring cells or the whole animal, can be avoided by experience. To ensure that the operations were successful, each ablation was validated after 1–4 hr; by this time it was usually obvious whether the target was dead or had recovered, and the condition of neighbouring cells could be assessed.

Before surgery, nematodes were anaesthetised in 0.5% 1-phenoxy-2-propanol and then mounted on agar containing 0.2% 1-phenoxy-2-propanol, by the quick mount procedure (anaesthetic mount: preceding paper). Before ablation of lateral cells in a young L1, the animal was placed with its dorsal or ventral side uppermost; in this way damage to the intestine was minimized, and bilateral operations were facilitated. On an agar surface free of excess liquid, a young anaesthetised animal adopts this posture when stroked along its length with a hair until it is straight. At ages greater than about 7 hr, this orientation is no longer stable.

Immediately after surgery, animals were placed on individual small NG plates. After 1–4 hr, and subsequently at appropriate times, they were mounted for microscopic observation, and then returned to their plates.

The formaldehyde-induced fluorescence (FIF) procedure for visualizing dopamine was performed as described by Sulston *et al.* (1975).

For polarizing microscopy, the anaesthetic mount was again used. Adult males could be rolled into various positions by manipulation with a hair: ventral, dorsal and ventrolateral views were found most useful. A λ/30 Brace-Köhler rotary mica compensator proved invaluable for analysis of the male sex muscles; we are indebted to Richard McIntosh for suggesting this technique. The objective most used was a Planapo 40, operated at full aperture to maximize vertical resolution.

The lineage nomenclature used in this paper was described by Sulston and Horvitz (1977). After a cell division, each daughter is given its parent's name followed by a letter representing the direction of the division: a, anterior; p, posterior; d, dorsal; v, ventral; l, left; r, right. Thus, Rn.aa is the

anterior daughter of the anterior daughter of Rn. In lineage charts, divisions are anteroposterior (anterior drawn to the left) unless otherwise indicated.

RESULTS

The first sections of the results deal with those areas (ventral and lateral hypodermis, male proctodeum; Fig. 12), in which precise replacement of one cell by another was found. Among the remaining sections, special consideration is given to the mesoderm because no regulation was seen in spite of a striking bilateral asymmetry of certain cell fates in the male.

VENTRAL HYPODERMIS

The ventral hypodermis, in L2 and older animals, comprises cells of the class Pn.p, which are formed in the L1 by division of the ventral cord precursors P1-P12. At hatching, P1-P12 are disposed as six bilaterally symmetrical pairs of hypodermal cells; they subsequently move ventrally to form a single file of cells in the midline, each pair becoming ordered anteroposteriorly at random. They can then be named according to their relative anteroposterior positions (Fig. 12).

P12.p divides once in the L1 of either sex; its posterior daughter dies leaving a unique hypodermal cell P12.pa. The remaining ventral hypodermal cells fuse with the large hypodermal syncytium, except for the groups P3.p-P8.p in the hermaphrodite and P9.p-P11.p in the male (Fig. 1). In the hermaphrodite P5.p, P6.p, and P7.p generate a 22-cell vulva (Fig. 2); P3.p, P4.p, and P8.p divide once only, and their daughters fuse with the large hypodermal syncytium. In the male, P10.p and P11.p add 16 neuronal, supporting, and hypodermal cells to the preanal ganglion (Fig. 3); P9.p fuses with the large hypodermal syncytium, sometimes after dividing once.

Hermaphrodite Vulva

The results of ablations in the vulval lineage are summarized in Table 1.

Regulation is restricted to cells P3.p-P8.p. Even when all the parent cells (P3-P8) are ablated in a newly hatched animal, the remaining ventral hypodermal cells (P1.p, P2.p, P9.p-P11.p) fuse with the large hypodermal syncytium, without division, in the normal way.

After ablation of the gonad, P3.p-P8.p divide once only and all their daughters fuse with the large hypodermal syncytium. The anchor cell, which lies at the midpoint of the gonad, has been shown to be the specific cell required for the induction of the vulva (Kimble, in preparation). Although the gonad and the developing vulva lie close together, they are in relative motion until the end of the vulval divisions, when the gonad becomes attached to the hypodermis. This contrasts with all the other cases of cell-cell interaction reported here, and suggests that a humoral mechanism may be responsible for the induction of the vulva.

The cell, normally P6.p, which will form the central section of the vulva comes to lie directly beneath the anchor, to which its progeny subsequently become attached. The final divisions of this anchor-associated cell are all transverse. In the two experiments in which only P5.p or P6.p were left intact out of the P3.p-P8.p group, the isolated ventral hypodermal cell came to lie beneath the anchor and gave the same division pattern. The division patterns of isolated cells which do not lie beneath the anchor are variable, and do not necessarily correspond to any pattern seen in the wild type.

In two experiments, blast cells which were isolated by ablation of their neighbours divided, but their progeny failed to join the vulva developing under the anchor cell; instead, they formed pseudovulvas (small protrusions) in the adult. Thus division of the blast cells and cooperation of their progeny are separable events. This observation helps in interpreting the development of "multivulva" mutants, in which several pseudovulvas are formed (Horvitz and Sulston, in preparation).

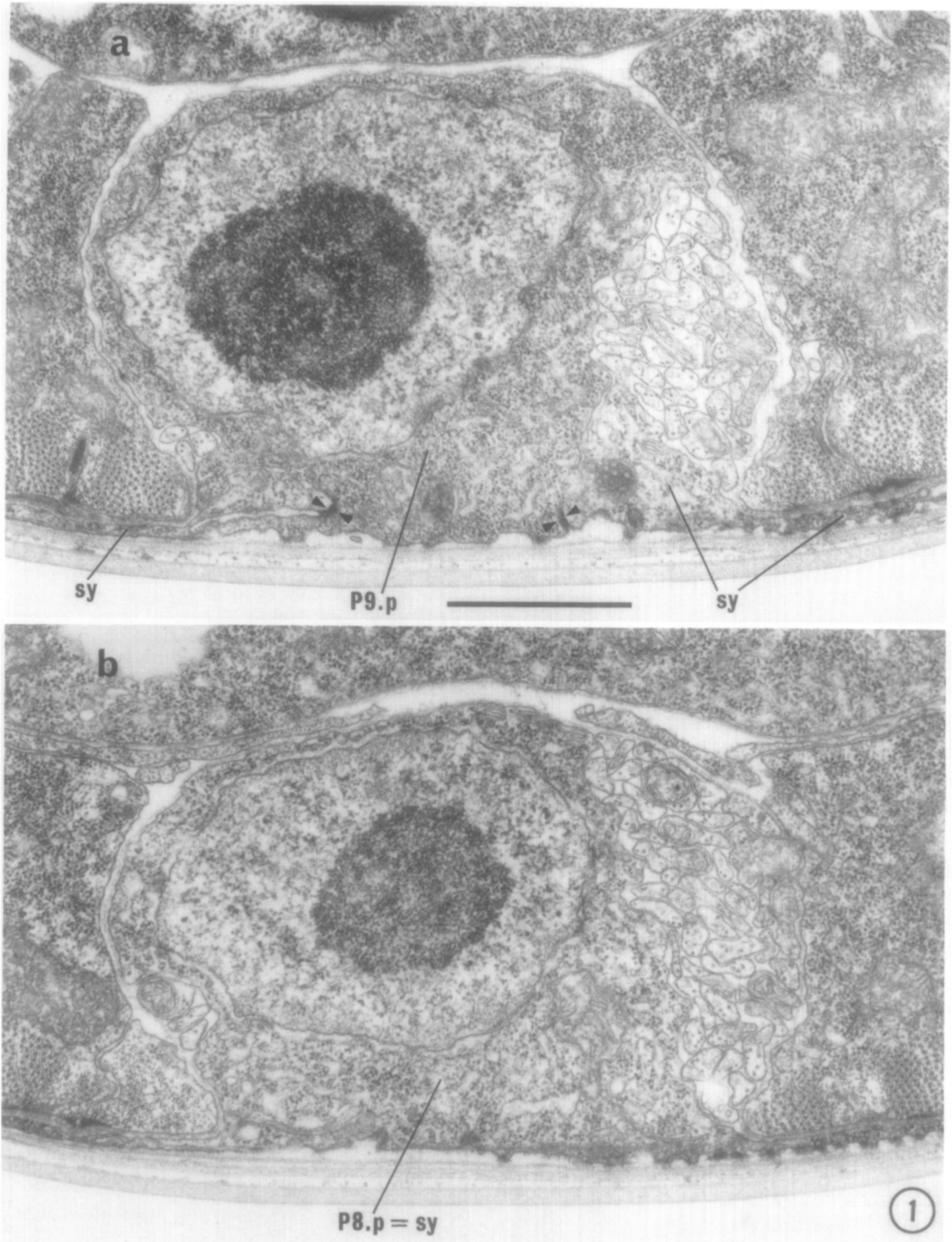


FIG. 1. Male ventral hypodermis, 23 hr; electron micrograph of transverse section; bar = 1 μm. (a) P9.p remains separate from the large hypodermal syncytium (sy); arrowheads point to desmosomes. (b) P8.p has fused with the large hypodermal syncytium.

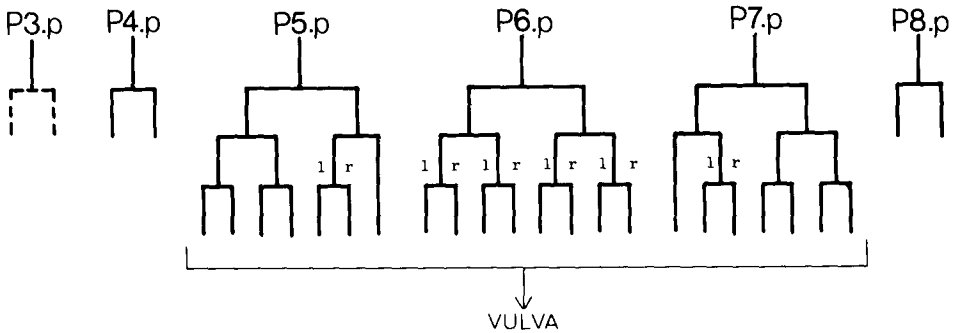


FIG. 2. Lineages of ventral hypodermal cells of the vulval equivalence group in an intact hermaphrodite.

Male Preanal Ganglion

(1) Late cell ablations (L3). When identified cells in the preanal ganglion were ablated during the L3 stage, it appeared from Nomarski observation that the remainder followed their normal fates in terms both of lineage and of morphology. In order to test the extent of this apparent cell autonomy, a systematic series of ablations was carried out, and the presence of the prominent sclerotic hook (Fig. 4) was scored in the resulting adults. The results (Fig. 3) demonstrate that the hook is formed only by cell P10.papp and that this assignment is not altered by cell ablations in the L3. In some cases where adjacent cells were killed, the resulting hook was abnormal and displaced (Fig. 5); however, since there are no other comparable structures, this effect does not cause ambiguity. Thus the hook can be used as one indicator of a normal P10.p-type lineage.

(2) Early cell ablations (L1 and L2). The results of earlier ablations are shown in Table 2 and Fig. 6. Three features were scored: cell lineages, the formation of the hook, and mating behaviour.

In the formation of the most posterior ventral hypodermal cell (P12.pa), P11 can replace P12 provided that the ablation is carried out before the nuclei enter the cord. In the preanal ganglion lineages, P10.p can replace P11.p, and P9.p can replace P10.p or P11.p, but recruitment of other cells has not been seen. This result is consistent with the fusion of P8.p and more anterior cells with the large hypodermal syncytium (Fig. 1). Recruitment is always from anterior to posterior.

Ablation of P11.p in the late L2 does not affect the lineage of P10.p, but does affect

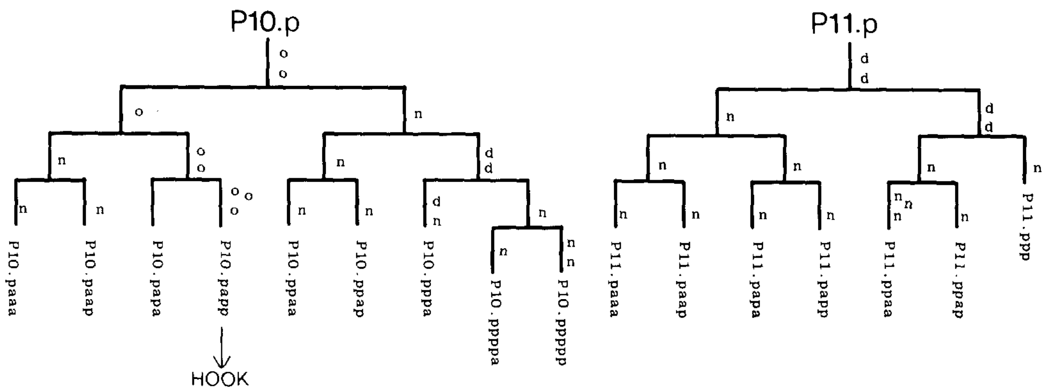


FIG. 3. Preanal ganglion lineages in the L3 male. Each symbol represents an ablation of the designated cell in a separate animal, which was then allowed to mature so that the hook (Figs. 4 and 5) could be scored. n, hook normal; d, hook abnormal, displaced; o, hook absent.

TABLE 1

REGULATION IN THE VENTRAL HYPODERMIS OF THE HERMAPHRODITE[a]

Ablated	No. of animals	No. of descendants from						Egg Laying
		P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	
0	Many	1-2	2	**7**	**8**	**7**	2	+
P6.p	2	1-2	**7**	**8**	—	**7**	2	+
P(4, 5).p	1	2	—	—	**8**	**7**	2	+
P(5, 6).p	1	1	1 + **4**	—	—	**8**	**7**	+
P(5, 7).p	1	2	**8**	—	**8**	—	**8**	+
P(7, 8).p	1	2	2	**7**	**8**	—	—	+
P(5-7).p	1	**8**	**8**	—	—	—	*8*	Slow
P(5-7).p	1	1	**8**	—	—	—	*8*	Slow
P(3, 4, 5, 7, 8).p	1	—	—	—	**8**	—	—	Slow
P(3, 4, 6, 7, 8).p	1	—	—	**8**	—	—	—	−
P(3-8)	3	—	—	—	—	—	—	−
P(1, 2, 9-12)	2	1-2	2	**7**	**8**	**7**	2	+

[a] Ablations performed 10-12 hr after hatching, except for P(1-12) which were ablated in the first hour after hatching. Heavy type: incorporated into vulva; italic type: form pseudovulvas; normal type: fuse with large hypodermal syncytium.

the fates of its progeny: some of them are abnormal in appearance and behaviour, and no hook is formed. It would be reasonable to suppose that debris from the operation blocks the outgrowth of the cell process which forms the hook, were it not for the fact that still later ablations of P11.p (Fig. 3) do allow expression of the hook and yield more normal-looking P10.p progeny. Perhaps in this case determination of lineage precedes determination of cell fate, so that the production of cell P9/10.papp is a necessary but not sufficient condition for the formation of the hook.

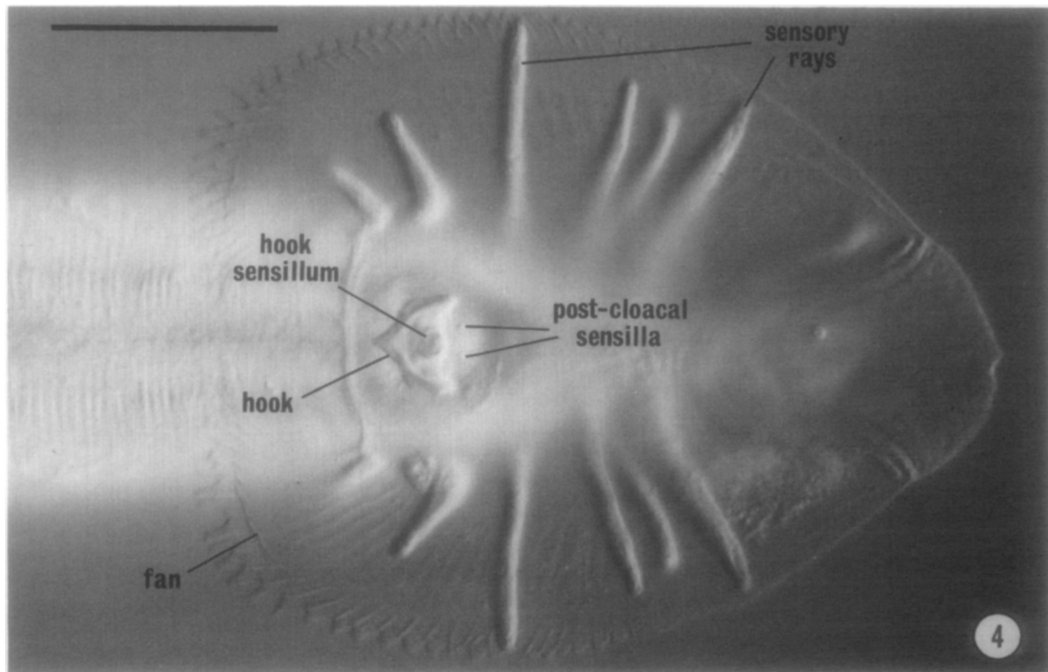


FIG. 4. Ventral view of adult male tail; anaesthetised animal mounted on agar with fan spread against cover slip. Nomarski optics; bar = 20 μm.

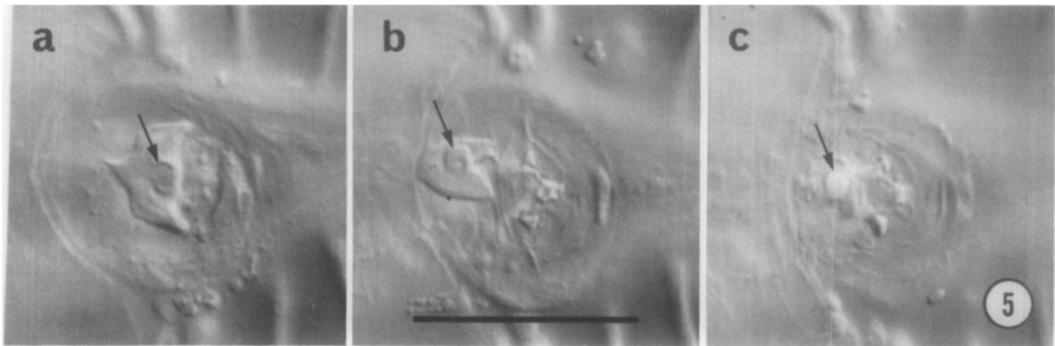


FIG. 5. Effect of cell ablation on the hook. Adult animals mounted as in Fig. 4; Nomarski optics; bar = 20 μm. Arrow points to hook sensillum. (a) Ablation of P10.p in L1: regulation yields normal structure; (b) ablation of P11.ppa: hook displaced; (c) ablation of P10.papp: hook absent.

Ablations of P12.p, B, C, and E showed that these cells are not required for the preanal ganglion lineages to proceed in the normal way.

LATERAL HYPODERMIS

On each side of the newly hatched animal there is a single longitudinal row of seam cells; these are blast cells which give rise to hypodermis and other structures as described below.

Male Sensory Rays

The nine pairs of sensory rays (Fig. 4) are formed by ray precursor cells (Rn) derived from the three most posterior pairs of seam cells (V5, V6, T). Each precursor gives rise to a hypodermal cell (Rn.p) and to a ray cell group comprising two neurons (A and B) and one structural cell (st) (Fig. 7, and preceding paper). In rays 5, 7, and 9, neuron A is dopaminergic.

(1) Late ablations (mid-L3 onwards). A series of ablations in the late L3 and young L4 indicated that the lineages and fates are largely cell autonomous in this period (Sulston and Horvitz, 1977). These experiments have been extended in two ways.

First, in one very young L4 all the Rn.apa cells were destroyed on the left side and all the Rn.aaa cells on the right side (preceding paper). Of the identifiable ray neurons which survived, those on the left side displayed only type A character and those on the right side only type B character. The large amount of debris produced and the accompanying displacement of surviving cells from their normal positions should have given ample opportunity for the reassignment of cell fates, if that had been possible at this stage. Apparently, therefore, the determination of Rn.aaa and Rn.apa cells to the type A and the type B neurons is established early.

Second, the ablation of 98 putative dopaminergic cells or their ancestors was attempted in a total of 29 animals. The resulting adults were examined by the FIF procedure and it was found that in 96 cases the expected cell had disappeared from the FIF pattern. In 2 cases, however, it had not: in both, the target was R5.aaa (the putative dopaminergic cell of ray 5). Probably the cells in question were not killed: in this series of experiments the power of the laser beam was limited in order to avoid damage to neighbouring cells, and because of the rapid development of the animal at this stage validation is difficult. Furthermore, if regulation were truly possible after the death of R5.aaa, it might be expected to occur with still higher frequency after the death of its parent, R5.aa. For that reason 12 R5.aa's were killed in the course of the experiments, but no regulation was observed.

To summarize: in the great majority of experiments, the function of the target cell (but not that of any other cell) was lost.

TABLE 2

REGULATION IN THE VENTRAL HYPODERMIS OF THE MALE[a]

Approx age (hr)	Ablated	No. of animals	P9.p recruitment	P10.p-type lineage	P11.p-type lineage	P12.p-type lineage	Hook	Mating
18–20	P10.p	3	+	c	c	+	+	+
22	P10.p	2	−	−	+	+	−	Slow
12	P11.p	1	+	c	c	+	+	+
12	P11.p	3	−	−	+	+	−	Slow
18	P11.p	1	+	Partial[b]	+	+	+	−
22	P11.p	2	−	+	−	+	−	−
10	P10.p, P11	2	−	−	−	+	−	−
12	P(10, 11).p	1	+	−	+	+	−	Slow
2	P12[c]	3	+	c	c	+	+	+
2	P(9, 10)	1		−	+	+	−	Slow
2	P(11, 12)	1	+	+	+	−	+	−
2	P(9–12)	1		−	−	−	−	−
12	P(9–11).p	1		−	−	+	−	−
12	P12.p	5	−	c	c	−	+	+/−
12	P(4–9).p	1		+	+	+	+	+

[a] Normal lineages of P10.p and P11.p are shown in Fig. 3. In columns 5 and 6: −, lineage absent; c, final cells normal in position and morphology but lineage not followed; +, lineage normal. In column 9: Slow, male was initially unable to locate the hermaphrodite vulva, but yielded progeny after prolonged mating; mating was with four paralysed (E369) hermaphrodites.

[b] P9.pppp did not divide.

[c] The right hand member of the symmetrical pair P11/P12 was ablated before entry into the ventral cord; it is known that P12 usually comes from the right.

(2) Early ablations (young L1). It can be seen from experiments 3, 4, and 7–9 in Table 3 that substantial, though not perfect, regulation is possible after destruction of some of the ray-producing seam cells (Fig. 8). Lineages, ray morphology, and the

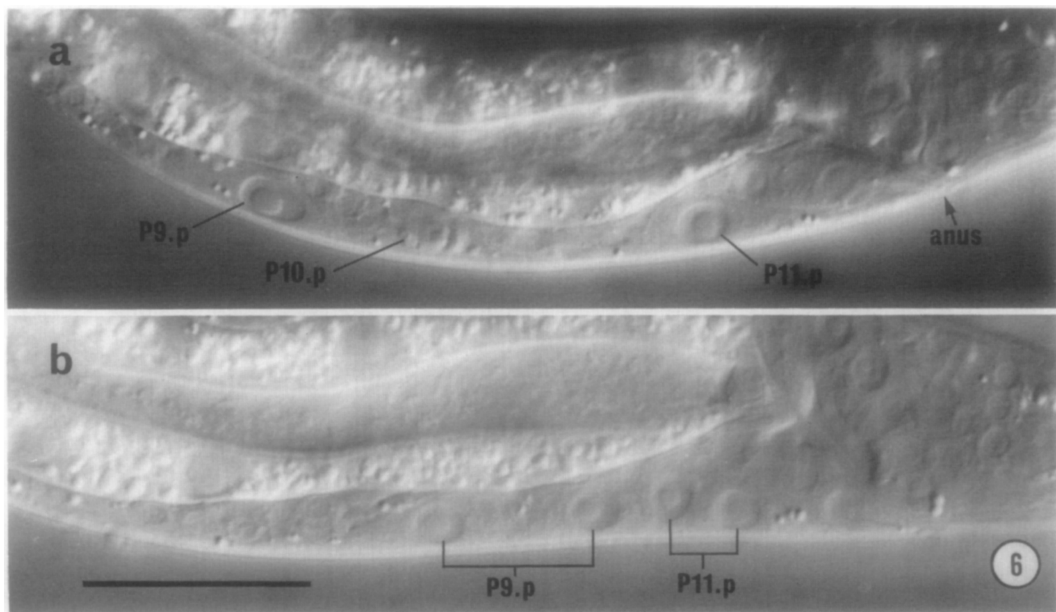


FIG. 6. Regulation in the ventral hypodermis; P10.p ablated at about 20 hr. Nomarski optics; bar = 20 μm. (a) 7 hr after ablation; (b) 12 hr after ablation.

FIG. 7. Lineages of lateral hypodermal cells in the wild-type male and in some experimental animals (see Table 3). The timing of divisions in the experimental animals is not known accurately. DN, dopaminergic neuron; rcg, ray cell group; s, sister cells which appear alike; se, hypodermal seam; sy, large hypodermal syncytium.

synthesis of dopamine are all subject to regulation. The recruitment of cells is always from anterior to posterior. Only one of the resulting adults yielded progeny when mated with paralysed hermaphrodites, but this low success rate is probably due in part to the loss of hypodermal cells causing distortion of the bursa.

In all cases, descendants of one or more V cells were recruited to the ray area, in the sense that they migrated posteriorly and went through additional rounds of division. V5 was able to replace V6 completely, V4 replaced V6 partially, and in one animal (10) V3.pppp approached the cloaca at L3 lethargus and divided to yield two seam cells and two syncytial nuclei. Thus, no distinct borderline between recruitable and nonrecruitable V cells was detected. After ablation of T, however, no V cell moved into its position and the posterior three rays were not produced; the only replacement of a T function occurred in experiment 1 when an extra dopamine cell was formed (see below). Equally, T never changed its lineage to resemble that of V6. It is not clear whether this division between T and V cells is inherent or due to mechanical constraints.

In experiments 1, 3, 4, and 7, dopamine

TABLE 3

REGULATION IN THE LATERAL HYPODERMIS OF THE MALE

(a) Source of Postdeirid and Sensory Ray Cells on One Side of a Normal Animal (Intact Male)

Derived from	Postdeirid cell group[a]	Ray cell groups	Rays	Dopaminergic ray cells
T	0	3	3	2
V6	0	5	5	1
V5	1	1	1	0
Total	1	9	9	3

(b) Results of Early Ablations at Age 0–4 hr (Experimental Males)

Experiment[b]	Ablated	Postdeirid cell group[a]	No. of ray cell groups from: V4	V5	V6	T	No. of rays[c]	No. of dopaminergic ray cells
{ 1	T	+		1	5		3	2
{ 2	T	+		1	5		3	1
3	V6, T	−		5			5	1
4	V6, T	−		4			2	1
{ 5	V(5, 6), T	−	0				0	0
{ 6	V(5, 6), T	−	1				1	0
7	V(4, 6), T	−		5			3	1
{ 8	V(5, 6)	−	2			3	5	
{ 9	V(5, 6)	−	4			3	7	
10	V(4–6)	−				3	3	2
11	V(3–5)	−			5	3	8	
12	V(2–5)	−			4	3	7	
13	V(2–4)	−		1^e	5	3	8	
14	V(1–4)	−		3^e	5	3	8	
15	V(1–4)	−		$1 + 1^e$	5	3	8	
16	V(1–4)	−		$1 + 1^{er}$	5	3	8	
17	V(1–3)	+		1^e	5	3	8	

[a] The postdeirid cell group includes one dopaminergic neuron.

[b] Bracketed pairs are in single individuals.

[c] Ray cell groups do not necessarily form rays. The cell group is of more significance than the production of a ray as such; even in the wild type, it is not uncommon for a ray to be missing even though the appropriate cells have been formed. e, ectopic ray cell group (anterior to normal region); r, reversed lineage (see Fig. 9).

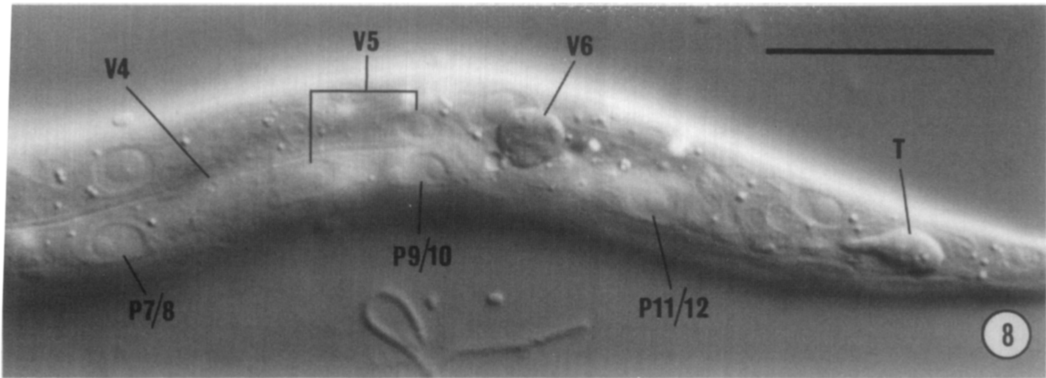


FIG. 8. Left lateral hypodermis 10 hr after ablation of V4, V6, and T (Expt 7, Table 3). V4 has disappeared; V5 has divided; V6 and T are refractile blobs. Nomarski optics; bar = 20 μm.

was synthesized by cells which would not normally do so. In 3, 4, and 7 the new dopaminergic cell was derived from V5. Since the latter failed to make the postdeirid (see below), it produced in all only one dopaminergic cell, as usual; nevertheless, although the exact identity of the new cell is not known, it was formed from V5.ppp rather than from V5.paa as is the postdeirid. Experiment 1 is more striking in that a total of three dopaminergic cells (one postdeirid and two ray neurons) were derived from V5 and V6, which normally produce only two.

The lineages shown in Fig. 7 demonstrate that recruitment of the L1 seam cells to ray formation is not an all-or-none decision restricted to the possibilities seen in the wild type, and suggest that some regulation may occur at later stages as well. This was confirmed by the ablation of V6.pa and V6.pp in a young L2, which led to the production of two rays, instead of the usual one, from V5.ppp. Ablations at slightly later times, in the mid-L2, have not led to any regulation of cell lineages.

(3) Ectopic ray cell groups. When a sufficiently large gap is created in the seam, some of the remaining cells yield more hypodermal daughters than usual and the behaviour of the ray cell progenitors may be modified. The most striking change is in V5, which can give rise both to additional hypodermal cells and to more than one ray cell group; some of the additional ray cell groups so formed lie well anterior to the cloaca and are termed ectopic (Table 3, experiments 13–17; Fig. 9).

Although ectopic ray cell groups did not give rise to rays visible by light microscopy, electron microscopical reconstruction of two animals (15, 16) revealed that the cell group had formed a typical ray tip embedded in the cuticle. Such rays would not be expected to elongate, because the necessary hypodermal withdrawal does not take place in this region (see preceding paper). These experiments demonstrate two important points about sensory ray production: first that the total number of ray cell groups can exceed the normal number, and second that the position of the blast cells at the time of ray cell group formation is not decisive in the control of their lineages.

Two additional features of these experiments are worth mentioning, although at present there is insufficient data to justify further comment. First, V5 and V6 sometimes give rise to a group of small cells, in a manner which suggests that a ray lineage has been attempted but has failed. An example is shown in Fig. 7 (Expt. 4).

Second, following the more extensive ablations, polarity reversals sometimes occur in the lineages. An anterior daughter may become a seam cell while its posterior sister joins the hypodermal syncytium: normally, such an orientation is seen only at

the ends of the animal. In experiment 16 (Fig. 9) the anterior-posterior reversal of an entire ray lineage was seen.

Hermaphrodite Lateral Hypodermis

As in the male, the creation of a sufficiently large gap in the seam of a hermaphrodite can cause the remaining cells to proliferate more than usual. The response is limited: no more than one extra hypodermal cell per juvenile blast cell has been seen. Usually it is due to some of the anterior daughters, which normally fuse with the hypodermal syncytium, persisting as seam cells and subsequently dividing again.

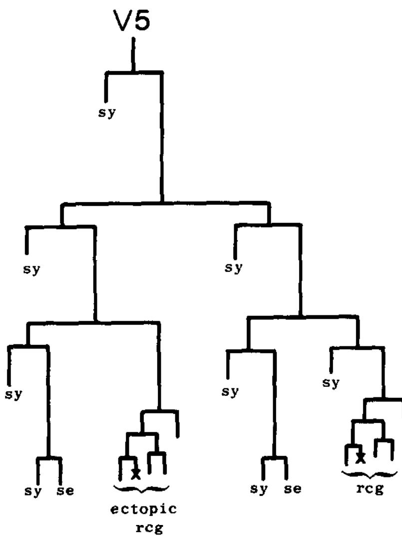
Postdeirid

The postdeirid is a sensillum embedded in the lateral cuticle in the posterior half of the body of both sexes; its single sensory neuron is dopaminergic. Underlying the sensillum is the postdeirid cell group, comprising the cell bodies of the postdeirid neuron, its socket cell, its sheath cell, and an accompanying neuron. Their origin is shown in Fig. 7.

Like the sensory ray lineages, the postdeirid lineage is cell autonomous after late ablations (Sulston and Horvitz, 1977). The results of early ablations are shown in Table 3b and Table 4. The postdeirid lineage is all-or-none in that either V5.pa gives rise to the entire lineage, or it divides to give one syncytial cell (anterior) and one seam cell (posterior); no intermediate cases have been seen. No cell other than V5 gives rise to the postdeirid. Production of the postdeirid is not strongly position dependent: after ablation of T, it was formed more posteriorly than usual, and after ablation of V4 it was formed more anteriorly. Conversely, after ablation of V (3, 4, 6), V5 failed to form the postdeirid although it did not move appreciably.

A plausible explanation for these observations is that V5, and no other cell, has the potential to form the postdeirid, yet if it loses contact with either of its neighbours in the L1 it behaves as an ordinary seam cell. Nomarski observations are consistent with this hypothesis, but it has not been tested by electron micrographic reconstruction.

The inability of other V cells to replace V5 indicates that position can only be of importance in very young animals if at all. Possibly V5 is programmed by its special ancestry, which differs from that of the



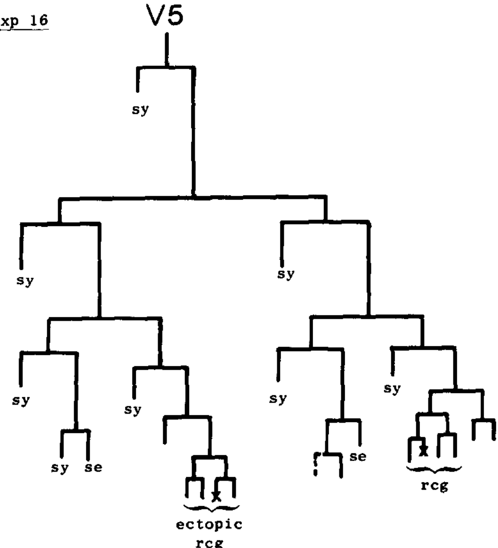


FIG. 9. Lineages of V5 in denuded seams (see Table 3); formation of ectopic ray cell groups. Note the inversion of the ectopic ray lineage in Expt 16.

TABLE 4

EFFECT OF ABLATIONS IN THE LATERAL HYPODERMIS ON THE PRODUCTION OF THE POSTDEIRID[a]

Ablated	Post-deirid cell group	No. of ablations	
		⚥	♂
QV5	–	3	
V5	–	4	6
V4	+	2	
V6	–	4	
T	+		4
V(3, 4, 6)	–	1	1
V(2–4)	–	1	1

[a] Except for QV5, which was ablated about 6 hr before hatching, the cells were ablated in newly hatched animals.

other V cells. V5 is the posterior daughter of a cell (QV5) which divides in the lateral hypodermis one hour before hatching (Fig. 7); the anterior daughter is Q1/2. The other V cells do not go through an equivalent division and so cannot be strictly homologous.

MALE PROCTODEUM

In the lineage of blast cell B, which generates many of the cells in the male proctodeum (cloaca and copulatory apparatus), there are four pairs of cells that have a choice of fates in normal development. The members of each pair are made by bilaterally symmetrical sublineages of B, and subsequently move to the midline (Fig. 12, and preceding paper). There are two fates open to each pair of cells, the first being followed by one cell and the second by the other. In the pairs B.alaa/B.araa and B.alpp/B.arpp the homologues become ordered anteroposteriorly and subsequently differ in lineage; after ordering they are renamed Bα/Bβ and Bγ/Bδ (Fig. 12). In the pairs B.alapaav/B.arapaav and Bγ.ald/Bγ.ard one homologue lives and the other dies. After removal of one member of a pair, usually achieved by ablation of its parent, the remaining member may invariably follow one fate; if so, that fate is said to be primary for that pair.

After ablation of B.ala or B.ara, B.araa or B.alaa, respectively, gave a normal Bα lineage; there were no Bβ derivatives. Furthermore, if the putative Bα (the more anterior cell) was ablated when its commitment was visible but before it reached the midline (23–24 hr), then the putative Bβ gave a Bα lineage. Therefore the Bα fate is primary.

After ablation of B.alapaa or B.arapaa, B.arapaav or B.alapaav, respectively, did not die but formed part of the vas deferens (the usual function of the surviving cell). Therefore, hypodermal function is probably primary for this pair.

In the dorsal proctodeum, the results were not decisive. The commitment of Bγ and Bδ seems to occur as soon as they are born. After ablation of one parent (B.alp or B.arp), either Bγ or Bδ could be formed; it seemed that the relative position of the dead cell was important in determining the outcome. In one animal the survivor (B.arpp) lay neither anterior nor posterior to the dead cell but spanned the entire gap between the normal neighbours of Bγ and Bδ; it gave an intermediate type of lineage, consisting of a single anterior-posterior division. After ablation of one grandparent (B.al or B.ar), B.arpp or B.alpp, respectively, again spanned the entire Bγ/Bδ region and again gave an intermediate type of lineage. It is possible, then, that neither the Bγ nor the Bδ fate is primary, and that contact of a single cell with both anterior and posterior neighbours leads to confusion.

The consequence of ablating Bγ.al or Bγ.ar was also unpredictable; Bγ.ard or Bγ.ald, respectively, sometimes survived and sometimes died.

The apparent difference between the dorsal and ventral regions of the proctodeum may be due partly to the way in which it develops. All the cells originate from B, which lies dorsal to the rectum, and a close contact between left and right sides persists at this point; the ventral bilateral homologues contact one another only later in development. This means that in dorsal

ablations there is more risk of damaging the contralateral cell as well as the target cell, and that cytoplasmic processes may establish the anteroposterior ordering of dorsal cells at an early stage.

MALE MESODERM

Experiments on the mesoderm were carried out in the male rather than the hermaphrodite, because of its greater complexity and the asymmetry of certain cell fates.

The single juvenile mesoblast (M) divides in the L1 to yield body muscles and three sex mesoblasts (SM1, SM2, SM3) on each side. In the L3, the sex mesoblasts divide to yield male specific muscles and a single coelomocyte.

The ablation experiments were carried out with two questions in mind. First, are the sex mesoblasts SM1 and SM2, which lie close together dorsally, interchangeable? Second, what is the basis for the difference in cell assignments between the left and right sides of the animal? Whereas the lineages of the sex mesoblasts are identical on the two sides of the animal, the fates of five of their progeny are not (Fig. 10). Since the five cells involved, which are derived from SM1 and SM2, initially lie close together in a row (preceding paper, Fig. 27), a possible explanation might be a system of hierarchical determination in which the fate of each cell depends upon the fate of its neighbour.

The male specific muscle cells can be recognized by the characteristic positions of their nuclei in the late L4, and most of them can be identified in the adult by the position and orientation of their birefringent myofilaments (Fig. 11).

(1) Late ablations (L3). SM2.pp was killed on the left in two animals and on the right in five animals; SM2.pa was killed on the left in one animal and on the right in one animal. The cells were killed as soon as possible after their birth. In every case the fates of the remaining cells were normal. When the left SM2.pa was killed, the left SM2.pp moved to the midline as usual but was unable to move anteriorly; nevertheless, it differentiated into a coelomocyte in the normal way.

These experiments suggest that the concept of hierarchical determination is incorrect. However, because the mesoblasts are both large and mobile, there is a possibility that debris from the dead cells may restrict the regulatory behaviour of the remaining ones.

(2) Early ablations (L2). In order to interpret the results of these experiments (Table 5) it was necessary to score all the muscles on both sides of the animal, because sometimes cells migrated across the

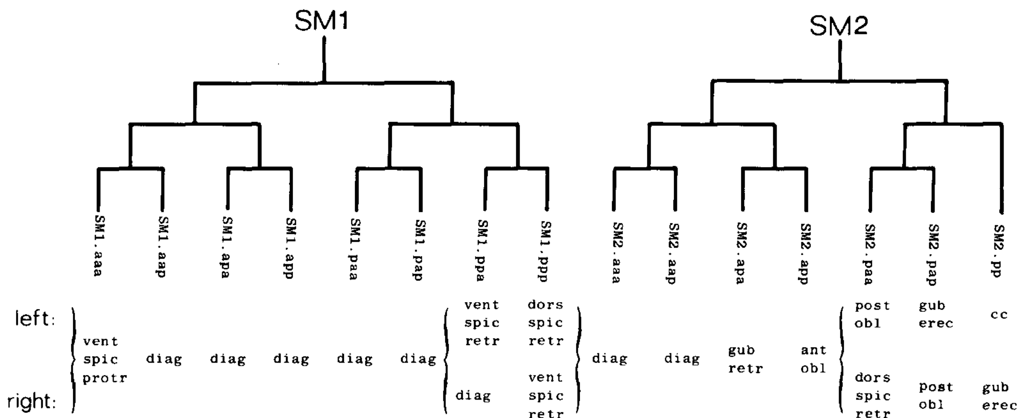


FIG. 10. Lineages of the dorsal sex mesoblasts, showing side-specific fates of their progeny. ant, anterior; post, posterior; dors, dorsal; vent, ventral; cc, coelomocyte; diag, diagonal; erec, erector; gub, gubernacular; obl, oblique; protr, protractor; retr, retractor; spic, spicule.

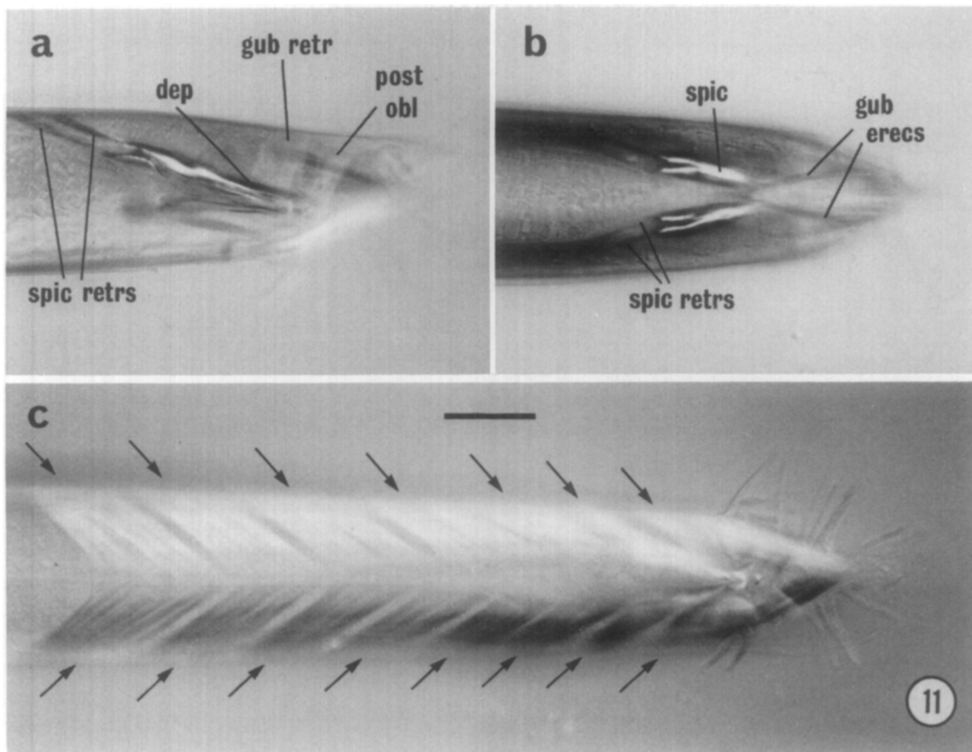


FIG. 11. Birefringence of male tail; anaesthetised animal mounted on agar; crossed polarisers, with λ/30 Brace-Köhler compensator set for maximum transmission, and Planapo 40 objective. The compensator causes muscles running in one direction to appear darker, and those running in the perpendicular direction to appear lighter, than the background. The strongly birefringent spicules always appear bright. dep, depressor (reorganised); other abbreviations as in Fig. 10. (a) Ventrolateral; (b) dorsal; (c) ventral, diagonal muscles arrowed.

midline. It is interesting that the transferred cells appeared to retain their commitments.

It is immediately apparent that the ablation of one mesoblast had little effect upon the fates of the others. This was true even when SM1 or SM2 was ablated at 16 hr, only 1 hr after its birth (since these two cells are sisters, this is the earliest time at which the experiment is possible). Furthermore, the surviving cells behaved normally not only with regard to their ultimate fates but also at intermediate times. Unless the precise time at which the cells approach the cloaca is decisive, it is difficult to avoid the conclusion that there is an intrinsic difference between SM1 and SM2.

A more detailed inspection of Table 5 rules out an extensive fate hierarchy among the cells whose assignments vary from left to right. After ablation of SM1, SM2 produces its normal complement of muscles and fails to produce more than one spicule retractor muscle; after ablation of SM2, SM1 fails to produce the coelomocyte, the gubernacular erector, and the posterior oblique muscle. The cells behave as though they are limited to the variation seen between left and right. If the basis for this limited variation is a posterior to anterior hierarchy, which would be consistent with the results of SM1 ablation, then SM1 in the absence of SM2 should produce two spicule retractors; on the contrary, it invariably produces only one. However, presumptive spicule retractors are often seen to develop into diagonal muscles (e.g., after ablation of the unrelated mesoblast SM3);

perhaps the general disturbance following ablation of SM2 causes a switch in the same way.

The dorsal coelomocyte (cc) is a unique cell which is normally formed from the left SM2. It migrates anteriorly in the midline and eventually moves to the right. On one occasion an intact animal was seen to produce two dorsal coelomocytes; one was the left SM2.pp and the other came from the right, probably being the right SM2.pp. This observation suggests that both SM2.pp's have the potential to be coelomocytes. However, the coelomocyte was not formed after ablation of the left SM2 (six animals) although it was still formed after ablation of the right SM2 (three animals); this result is consistent with the later ablations described above.

OTHER TISSUES

When the blast cells B, C, E, F, K, or M were ablated no lineage regulation was seen. All these cells are unique in morphology (with the exception of K, which has a contralateral homologue) and fate. There was also no lineage regulation when endodermal cells were ablated.

SEARCH FOR AN ORGANISER IN THE MALE TAIL

As described above, the gonad is necessary for the development of the hermaphrodite vulva; this process involves the movement of several ventral hypodermal cells towards the centre of the gonad. The movement of various groups of male cells (lateral hypodermal, ventral hypodermal, mesodermal, gonadal) towards the tail suggested that another such organising centre might be located there. Furthermore, the general cell movements involved in the final maturation of the male might also be controlled by a particular tissue. Therefore, as many cells as possible were ablated in an effort to abolish the recruitment of the blast cells and the shaping of the tail. In different animals, the gonad, all the tail neurons, the tail hypodermal cells and many of the rectal cells (including B, C, E, F, K, and K′ in various combinations) were eliminated. Some of the animals died, but in those that survived the characteristic movements and

TABLE 5

ABLATION OF THE SEX MESOBLASTS[a]

Side	Ablation		SM1 derivatives			SM2 derivatives							SM3 derivatives	Other	Contralateral abnormality
	Age (hr)	Cell	vent spic protr	diag	spic retr	diag	gub retr	ant obl	spic retr	post obl	gub erec	cc			
L		—	1	5	2	2	1	1	0	1	1	1	6		
R		—	1	6	1	2	1	1	1	1	1	0	6		
{L	16	SM1	0	0	0	2	1	1	0	1	1	1	6		
{R	16	SM1	0	0	0	2	1	1	1	1	1	0	6		
L	~21	SM1	0	0	0	2	1	1	0	1	1	1	6	1 spic retr	−1 spic retr
R	~21	SM1	0	0	0	2	1	1	1	1	1	0	6	2 diag	−2 diag
R	~21	SM1	0	0	0	2	1	1	1	1	1	0	6	2 diag	−2 diag
{L	16	SM2	1	6[b]	1	0	0	0	0	0	0	0	6		
{R	16	SM2	1	6	1	0	0	0	0	0	0	0	6		
{L	~21	SM2	1	6	1	0	0	0	0	0	0	0	6		
{R	~21	SM2	1	6	1	0	0	0	0	0	0	0	6		
R	24	SM2	1	6	1	0	0	0	0	0	0	0	6	{1 gub retr, 1 ant obl}	− {1 gub retr, 1 ant obl}
L	~21	SM3	1	5	2	2	1	1	0	1	1	1	0		
R	~21	SM3	1	6	1	2	1	1	0	1	1	0	0		+1 diag

[a] The lineages were not followed, but cell types were determined by Nomarski observation of the late L4 and polarised light microscopy of the adult (see text). The results are matched as nearly as possible to the normal patterns (shown in the top two lines); some cells (e.g., diagonals) may be derived either from SM1 or from SM2, but most (e.g., spicule protractors, coelomocyte) are normally derived from one particular mesoblast. Abbreviations: ant, anterior; post, posterior; vent, ventral; cc, coelomocyte; diag, diagonal; erec, erector; gub, gubernacular; obl, oblique; protr, protractor; retr, retractor; spic, spicule.

[b] Three normal, three abnormal morphology.

divisions of the hypodermis and mesoderm were always seen.

These results do not rule out the existence of a general male tail organiser; they may mean simply that it is difficult to inactivate the organiser without killing the animal, or that the proper combination of cells was not eliminated. They do, however, suggest an alternative possibility: that in their initial movements and cell divisions the tissues are directed only by their own internal programme and perhaps by an anteroposterior polarity.

The experiments did reveal another, more limited, example of organisation in the male. After ablation of E, the vas deferens fails to connect to the proctodeum and consequently the adult is sterile. Although derivatives of E are not used structurally, they do serve two important functions: the first is the engulfing and killing of a specific gonadal cell, and the second (which may be a consequence of the first), is to stimulate the attachment of the proctodeal cells K.a and K′ to the vas deferens (see preceding paper).

FORM REGULATION

After adoption of their discrete fates, which are expressed by movements and final positions as well as morphology, cells can show plasticity of form. Examples which are of importance to the organism are the ability of intestinal and hypodermal cells to grow, without division, to replace lost neighbours. An extreme case of repair was seen when five of the eight cells which normally form the posterior half of the intestine were destroyed in a young L1; the remaining three elongated and shaped themselves appropriately to regenerate a functional intestine.

In the mesoderm some of the cells behave as though they are competing for attachment sites. When both spicule retractors on one side are lost, a spicule protractor sometimes grows anteriorly and attaches to the body wall. In these cases it is not obvious that form regulation is useful to the organism, but they do indicate another factor controlling differentiation of muscle cells.

DISCUSSION

SUMMARY

We have examined the extent of cell–cell interaction in the postembryonic development of nongonadal tissues of the nematode *C. elegans*. Cells were ablated with a laser microbeam, and the consequences were assessed mainly by light microscopy. Much of the development appeared to be cell autonomous by this criterion, but three types of cell interaction could be distinguished. With the exception of the induction of the vulva, all appear to involve direct contact between cells.

(1) Induction. The development of a structure is dependent upon the presence of cells which do not themselves contribute to that structure. Two cases were found: the induction of the vulva by the gonad and the organisation of the vas deferens at the cloaca. In neither case is there any homology between the inducing and induced cells. Another type of interaction, related to induction, is that between a cell programmed to die and its killer (see preceding paper).

(2) Lineage regulation. The lineage of one cell is changed in such a way as to compensate for the loss of another. This can involve simple replacement in which the regulating cell switches from its normal lineage to that of the lost cell or a less well-defined proliferation in which extra cells are generated by a lineage not seen in the intact animal. In no case, however, is regulation complete: even when a functional adult is produced, it contains fewer cells than normal. The groups of cells which can regulate in this way are depicted in Fig. 12. The members of each group show substantial homology with one another. Conversely, there is no lineage regulation between cells which lack homology.

(3) Functional regulation. The function of one cell is changed in such a way as to

compensate for the loss of another. Simple replacement, in which the nature of one cell changes completely to that of another, is seen only in two pairs of bilateral homologues in the male tail. Form regulation, in which a cell grows in such a way as to combine its own normal function with that of the lost cell, is more widespread and is seen in the ectoderm, the mesoderm, and the endoderm.

CELL AUTONOMY

When the behaviour of a cell is not affected by the ablation of any of its neigh-

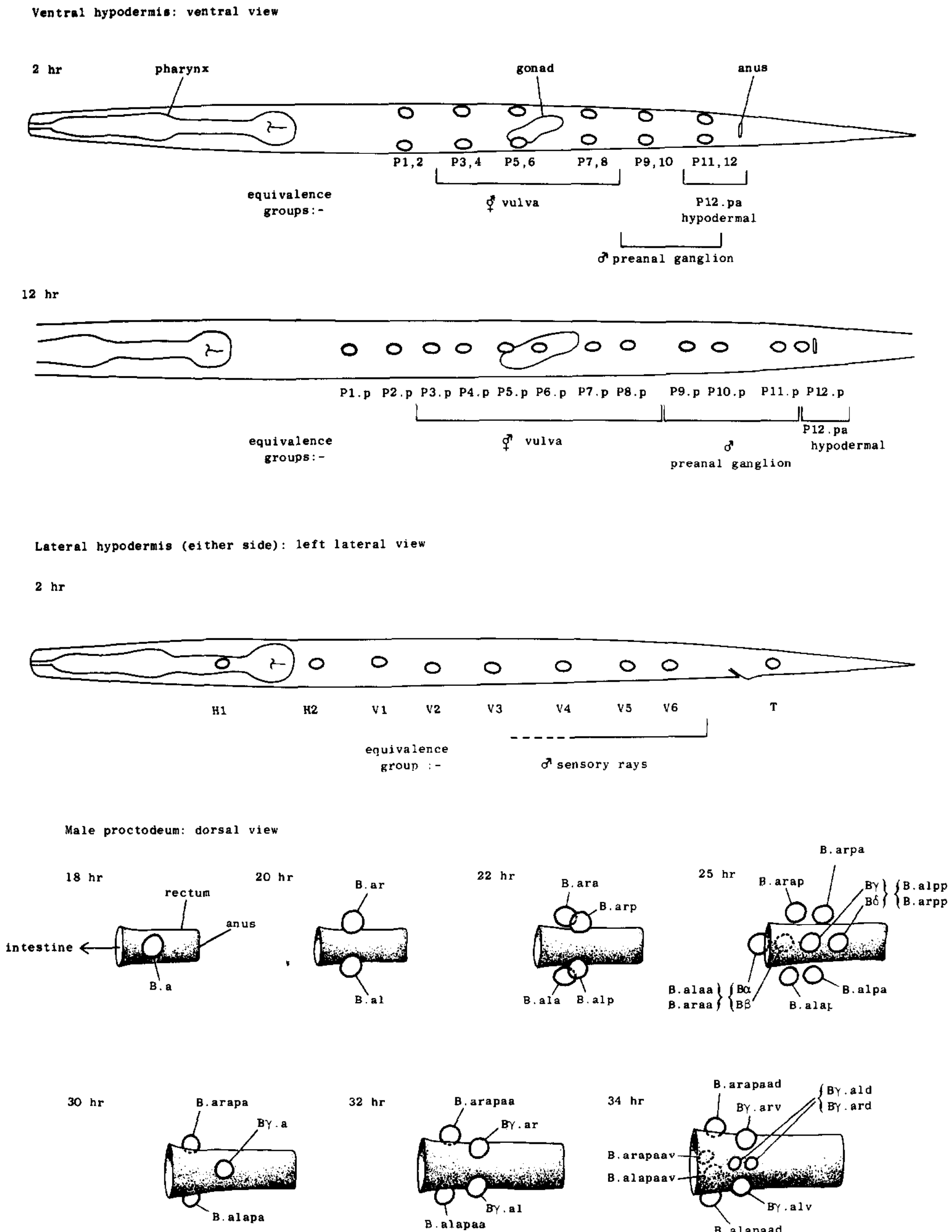


FIG. 12. Schematic drawings showing positions of blast cells which form equivalence groups.

bours it is reasonable to conclude that it is behaving autonomously. Autonomous behaviour of cells at late stages is also suggested by their extensive and apparently independent movements in many areas. It must be remembered though, that these observations principally concern nuclear movements, and that the cytoplasms of quite distant cells may be in contact. In the absence of grafting experiments (which cannot as yet be performed on nematodes) the proposal of cell autonomy is necessarily a negative conclusion, and to that extent lacks conviction. There are three important ways in which ablation experiments which imply autonomy may be misleading.

First, parts of the target cell may survive for a few hours, and continue to signal in the normal way to neighbouring cells. By definition, however, the nucleus of the target cell is not seen again and the cell does not divide; thus dynamic exchange of information between surviving pieces and other cells is unlikely.

Second, the adjacent cells may continue to develop normally because of their position, with respect either to distant landmarks or to their remaining neighbours. This possibility can be investigated by causing more extensive damage, so that a surviving cell is shifted from its normal position and isolated as far as possible from its normal interactions. Experiments of this kind have been performed in the terminal development of the postdeirid and the sensory rays; in these areas, at least, the cells do seem to behave autonomously by this criterion.

Third, when cells are ablated late in development, there may be insufficient time for the survivors to establish and respond to new contacts.

EQUIVALENCE GROUPS

The ability of one cell to replace another is an indication that the two cells are equivalent in developmental potential. We refer to groups of such cells as equivalence groups (Kimble *et al.*, 1979).

The members of an equivalence group resemble one another closely in structure and function, and in most cases they are of similar origin as far as this is known. Following ablation of unique cells, replacement does not occur though form regulation may be seen. On the other hand, similar cells are not necessarily equivalent (cf. Lewis and Wolpert, 1976).

Equivalence Groups in Intact Animals

Some equivalence groups were known previously, as a result of the analysis of particular cell lineages in several individuals. Each of these groups comprises a bilaterally symmetrical pair of cells which move to, and meet in, the midline; subsequently, one cell (sometimes from the left and sometimes from the right) follows one fate while the other follows a second fate. It is possible either that the pairs are never truly symmetrical and that the members are differentially committed before centralization, or alternatively that the cells, having reached the midline, compete with each other for one of the fates. In certain cases the second hypothesis has been confirmed by cell ablation; this has the added advantage of revealing which, if either, fate is primary (i.e., is invariably followed by the surviving cell).

The groups which show natural ambiguity in this way are the following (see Fig. 12):

(1) The six pairs of ventral cord precursors P1–P12 become ordered anteroposteriorly in the ventral midline. Certain neurons derived from P1 differ from the homologous neurons derived from P2; the behaviour of the ventral hypodermal cells derived from each of the pairs P3/4, P5/6, P7/8, P9/10, and P11/12 differ in either the hermaphrodite or the male or both. Cell ablations have shown that the P1 fate is primary in the first pair (White, unpublished results) and that the P12 fate is primary in the last. The behaviour of the ventral hypodermal cells is governed by the equivalence groups which they join. All

twelve precursors may be potentially equivalent but we have not succeeded in testing this idea by causing one of them to move into the region normally occupied by an adjacent pair.

(2) The pair of cells $B\alpha/B\beta$ is formed by bilaterally symmetrical lineages and so is the pair $B\gamma/B\delta$; the members of each pair differ in subsequent lineage. The fate of $B\alpha$ is primary.

(3) The cell pairs B.alapaav/B.arapaav and Bγ.ald/Bγ.ard are also formed by bilaterally symmetrical lineages. One member of each pair dies and the other takes on a structural role; the structural role is primary in B.alapaav/B.arapaav.

Another equivalence group showing natural ambiguity was found by Kimble and Hirsh (1979) in the development of the gonad. Its members are not bilaterally symmetrical but are radially symmetrical about the twofold rotation axis of the gonad.

Equivalence Groups in Regulation

Other equivalence groups were revealed by the cell ablation experiments described in this paper. They lie in the ventral and lateral hypodermis, and comprise cells which are arranged anteroposteriorly. Within each group, the cells are recruited in a definite hierarchy (presumably on account of their arrangement), and so no ambiguity is seen in the lineages of intact animals.

(1) Ventral hypodermis. In the hermaphrodite, the ventral hypodermal cells P(3–8).p form an equivalence group for generation of the vulva. In intact animals P(5–7).p give rise to the vulva (Fig. 2); after ablation of one or more of these cells, recruitment of their neighbours towards the centre of the group is possible. At least for the central cells, the P6.p fate is primary. P3.p is only occasionally recruited, perhaps because it fuses prematurely with the hypodermal syncytium (see below).

In the male, P(9–11).p form an equivalence group for late development in the preanal ganglion. In intact animals P10.p and P11.p are used; after cell ablation recruitment is from anterior to posterior. The P11.p fate is primary.

With the exception of P12.pa and those involved in the ventral equivalence groups, the ventral hypodermal cells fuse with the large hypodermal syncytium by the L2 stage (Fig. 1). This fusion programme appears to be laid down before hatching, because ablation of some of the precursor pairs at this time does not affect the fusions of the hypodermal daughters of the remainder. This may be the mechanism by which both boundaries of the vulval equivalence group and the anterior boundary of the preanal equivalence group are defined. The posterior boundary of the preanal group falls between P11.p and P12.p, whose parents are themselves equivalent until entry into the ventral cord.

In embryogenesis, therefore, developmental boundaries seem to be set up between some of the pairs of ventral precursors (Fig. 12). The boundaries at P2/P3 and at P8/P9 are revealed also by the fates of Pn.aap cells. In the hermaphrodite, these cells die outside the group P(3–8) but become Class C ventral motor neurons inside it (White *et al.*, 1976); in the male our information is incomplete but there is a discontinuity in terms of lineage at P2/P3 and in terms of fate at P8/P9 (preceding paper).

(2) Lateral hypodermis. In the male the lateral hypodermis on each side contains an equivalence group for sensory ray production. The group seems to have a posterior boundary between V6 and T, but the anterior boundary is less well defined. In intact animals, V6 gives rise to five rays and V5 to one; after cell ablations, V4, V5, and V6 can be recruited posteriorly and can generate replacement rays. V3 also responds to some extent, although it has not been seen to generate ray cells; since V4 is itself less active in ray formation than V6, we cannot say whether V3 is potentially

capable of generating rays or whether its response is part of the general hypodermal proliferation (see below). It may be that all V cells are potentially alike, but that when the lateral hypodermis is greatly denuded, the generation of additional hypodermal cells conflicts with the formation of ray cells. Alternatively, or in addition, the more anterior V cells may be incapable of producing rays because of their initial position. To a first approximation, the V6 function is primary.

The posterior boundary of the sensory ray group may arise from mechanical restraint by the rectum, which lies between V6 and T. T, which generates three rays, is morphologically similar to V6 though its lineage in the hermaphrodite (Sulston and Horvitz, 1977) is very different. Stronger confirmation of a developmental boundary at this point comes from a mutant isolated by Hodgkin (1974); in this mutant (e1239) the V cells are virtually unable to generate rays, while T behaves normally.

In both sexes, some of the hypodermal blast cells (seam cells) respond to a sufficient reduction in their numbers by a limited and variable proliferation. Also, sensory ray formation seems to show an intermediate type of regulation in which either complete (V5→V6) or partial (V4→V6) replacement can occur, and in which a proliferative response is possible (the production of ectopic rays by V5). Provisionally, then, we shall say that the lateral hypodermis contains equivalence groups for sensory ray production in the male, and for hypodermal proliferation in both sexes, but that the properties of these groups are not as well defined as those of the ventral hypodermis.

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REFERENCES

BERNS, M. W. (1972). Partial cell irradiation with a tunable organic dye laser. *Nature (London)* **240,** 483–485.

BRENNER, S. (1974). The genetics of *Caenorhabditis elegans. Genetics* **77,** 71–94.

DAVIDSON, E. H. (1976). "Gene Activity in Early Development," 2nd ed; p. 245 ff., Academic Press, New York.

HODGKIN, J. A. (1974). "Genetic and Anatomical Aspects of the *Caenorhabditis elegans* Male," Ph.D. thesis, University of Cambridge, England.

KIMBLE, J., and HIRSH, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans. Develop. Biol.* **70,** 396–417.

KIMBLE, J., SULSTON, J., and WHITE, J. (1979). *In* "Cell Lineage, Stem Cells and Cell Determination," INSERM Symposium No. 10 (N. le Douarin, ed.), pp. 59–68, Elsevier.

LAUFER, J. S., BAZZICALUPO, P., and WOOD, W. B. (1980). Segregation of developmental potential in early embryos of *Caenorhabditis elegans. Cell* **19,** 569–577.

LEWIS, J. H., and WOLPERT, L. (1976). The principle of non-equivalence in development. *J. Theor. Biol.* **62,** 479–490.

NIGON, V. (1965). *In* "Traité de Zoologie" (P.-P. Grassé, ed.), Vol. 4, pp. 249–256. Masson, Paris.

SAMOILOFF, M. R. (1973). Nematode morphogenesis: localization of controlling regions by laser microbeam surgery. *Science* **180,** 976–977.

SULSTON, J. E., ALBERTSON, D. G. and THOMSON, J. N. (1980). The *Caenorhabditis elegans* Male: Postembryonic Development of Nongonadal Structures. *Develop. Biol.* **78,** 542–576.

SULSTON, J., DEW, M., and BRENNER, S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans. J. Comp. Neurol.* **163,** 215–226.

SULSTON, J. E., and HORVITZ, H. R. (1977). Postembryonic cell lineages of the nematode *Caenorhabditis elegans. Develop. Biol.* **56,** 110–156.

WHITE, J. G., SOUTHGATE, E., THOMSON, J. N., and BRENNER, S. (1976). The structure of the ventral nerve cord of *Caenorhabditis elegans. Phil. Trans. Roy. Soc. London B.* **275,** 327–348.