man, at least, the plasma protein bindings of drugs in the fetus and neonate has been reported to be less than in the adult (*9*). Such data are not available for the rat but the plasma concentration of albumin [the main drug-binding component of plasma (*1, 2*)] is less in the fetal rat than in the adult (*10*), and there is a relation, although not a proportional one, between binding and albumin concentration (*1, 5*).

An indication of the potential hazard to the fetus and newborn from this type of interaction comes from the report in 1956 of the production of fatal kernicterus in several premature infants who were given sulfisoxazole as part of a prophylactic antibacterial regimen (*11*). This highly bound sulfonamide displaced sufficient bilirubin from binding to plasma protein to cause a toxic concentration to be reached in the brain of susceptible infants. Although this did not happen in utero, the data in Table 1, A and B, attest to the possibility of such an occurrence, particularly in light of the deficient binding in the fetus and neonate previously noted (see above). In addition, we had reported earlier that displacing agents are more effective in the presence of a deficient binding (*5*). It is also conceivable that the displacement of potentially hazardous, highly bound drugs in the mother may play some role in the production of birth defects and in the relatively high fetal mortality rate in this country. Recently, Bleyer *et al.* (*12*) published a list of the drugs commonly ingested by mothers during pregnancy. Some of these drugs are highly bound to plasma protein and a number have been shown experimentally to have a deleterious effect on the fetus (*13*). In light of our limited knowledge and experience with drug interactions in man, the decision to administer drugs to a pregnant patient for treatment of a minor complaint should be weighed against the potential hazard to the fetus.

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2 March 1973

Nematode Morphogenesis: Localization of Controlling Regions by Laser Microbeam Surgery

Abstract. *Laser microbeam studies reveal that postembryonic development of the free-living nematode* Panagrellus silusiae *is under the control of specific regions. Growth is regulated by the hindgut, and ecdysis by nerve cell bodies situated anterior to the nerve ring, and gonad development is under the control of the nerve ring. This latter event is presumably neuronally mediated, while the other events are under hormonal control.*

In the free-living nematode *Panagrellus silusiae* the major events of postembryonic development are growth, cuticle formation, ecdysis, gonad development, and the development of sexual behavior (*1*). Coordinate control of these events has been postulated. Such control could be either hormonal or nervous. However, as nematodes are eutelic, with little or no capacity for regeneration, with a high internal hydrostatic pressure, few experimental studies have been carried out to determine the possible sources of coordination of postembryonic development. Ligature and microirradiation studies of larger animal parasitic species have demonstrated that the general region of the nerve ring controls the events of exsheathment (*2*). Laser microbeam irradiation has been used to determine the receptor site for mating attraction (*3*). This technique is most suited to the nematode system, as a high-energy flux is delivered to the tissue in a localized region without disruption of the body wall and the resultant loss of internal pressure.

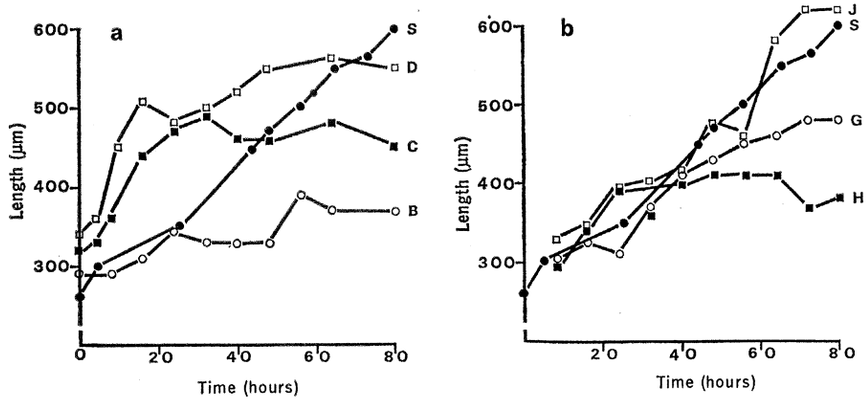

Fig. 1. Growth of individual nematodes after laser microbeam irradiation with a 7-μm spot (260-joule input). (a) Growth after irradiation in anterior region; (b) effects of irradiation in posterior region. Key to the regions indicated: *B*, cell bodies of the nerve ring; *C*, nerve ring; *D*, pharynx; *G*, gonad primordium; *H*, hindgut; *J*, extreme posterior region of the nematode; *S*, sham (laser spot delivered to medium near nematode).

Here are reported the effects on the subsequent development of the organism of irradiation of second-stage juveniles with a 7-μm laser spot. The experimental procedure involved removal of second-stage juveniles from mass cultures of *P. silusiae* strain C grown xenically on Czapek Dox agar, immobilizing the juveniles in 0.2 percent propylene phenoxitol, laser irradiation in one region, and transfer of the juveniles for a 7-day growth period in depression slides containing Hieb and Rothstein's (*4*) medium inoculated with *Escherichia coli*, after which the animals were killed, mounted in lactophenol, and examined under Nomarski interference microscopy for total body length, persistent laser damage, sex, and gonad development. For purposes of laser irradiation the nematodes were divided into ten morphological regions: region A containing the anterior sense organs, region B containing the bulk of the cell bodies of the nerve cells, region C containing the nerve ring, region D containing the pharynx, region E containing the excretory cell, region F containing primarily the middle of the intestine, region G containing the four cells of the gonad primordium, region H containing the hindgut, region I containing the anus, and region J consisting of the posteriormost portion of the animal. Sham experiments were performed in which the laser hit within 10 μm of the anesthetized animal. Initial experiments were performed with 260-joule power output to the laser (a Hadron Biolaser, coupled to a Wild M40 inverted microscope). The growth of the irradiated animals was followed by video recording at 8-hour intervals (Fig. 1). Later studies were performed with a laser input of 130 joules (Table 1). This latter energy input permitted the majority of treated animals to reach adulthood. In the latter series of experiments, 30 second-stage juveniles were treated in each region.

In those experiments in which a higher-energy laser pulse was delivered, growth was inhibited most markedly in animals treated in the B and H regions (Fig. 1). Animals treated in B were observed to have tatters of cuticle present along their surface, especially at the anterior end, implying that damage to the B region blocks ecdysis, but not cuticle formation. No evidence of molting was detected in animals irradiated in H, and presumably these animals did not form a new cuticle. Irradiation in C, D, and G resulted in a partial inhibition of growth (Fig. 1), while normal growth was observed in animals irradiated in A, E, F, and J. These results indicate that the anterior end, foregut, and posterior end of the nematode play no role in regulating the growth of the animal. However, it does appear from this evidence that the hindgut and nerve ring play an important role in regulating growth of the animal.

Table 1. The effects of low-energy laser microbeam irradiation in subsequent growth, survival, and gonad development. Each treatment was performed on 30 second-stage juveniles.

Region irradiated	Dead	Adults	Normal adults	Males: females (ratio)
A	6	22	18	11 : 11
B	7*	18†	14*	7 : 11
C	8†	19†	3‡	10 : 9
D	6	22	17*	12 : 10
E	4	25	18†	11 : 14
F	2	26	18†	7 : 19*
G	7*	20*	16*	8 : 12
H	9†	19†	16	9 : 10
I	7*	23	20	14 : 9
J	7*	19†	14†	8 : 11
Sham	2	26	25	15 : 11

* Significantly different from the sham with $P < .10$. † Significantly different from the sham with $P < .05$. ‡ Significantly different from the sham with $P < .001$.

Following low-energy laser irradiation significant decreases in survival and growth to the adult stage occurred in animals irradiated in the B, C, G, H, I, and J regions (Table 1). In all cases the length of adults obtained from treated animals was not significantly different from that attained by sham-treated animals. Larvae treated in the C region grew to adult size in 63 percent of the cases, but only 16 percent of these had normal adult gonads. The remainder had incomplete gonads similar to those resulting from inhibition of DNA synthesis. It appears that the C region is the site for regulation of the development of the reproductive system. Animals irradiated in the regions between the nerve ring and the gonad primordium all produced a significant number of adults with abnormal gonads, which suggests a physical connection between the nerve ring and gonad. Such a connection is in all probability neuronal. Damage to the G region containing the gonad primordium did not produce abnormalities in gonad development. As it is unlikely that the germinal primordium escaped damage it is possible that the cells in this tissue are capable of regeneration or regulation. Irradiation of the F region, just anterior to the germinal primordium, produced a significant increase in the proportion of adult females. This difference cannot be accounted for by mortality. During gonad development the male gonad primordium grows in an anterior direction while the female gonad primordium grows in a posterior direction. The increased proportion of females can be explained by assuming that development of the male gonad requires a stimulus from a localization of material just anterior to the gonad. Failure of the primordium to receive this signal promotes development of a female reproductive system. Supporting this hypothesis is the observation of a significant increase in the number of females with abnormal gonads following irradiation in the F region. If these females are considered to be genetic males with abnormal gonad development, a sex ratio of 1 : 1 is obtained.

Animals irradiated in the J region showed normal growth for several days, but with significant numbers dying or developing abnormally after 7 days. This effect is probably not indicative of a controlling region but rather reflects the fact that the 7-μm spot was larger than the diameter of the region, producing lesions in the body wall and disrupting the hydrostatic skeleton.

The results reported here demonstrate the complex nature of the control of postembryonic development in nematodes, with separate control over growth, gonad development, and molting. The role of the hindgut in promoting growth was unexpected, although numerous reports of a "feeding stimulus" for development have been made. Continuing laser microbeam studies should reveal the precise cellular sites for the control of postembryonic development.

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