Inductive Role of the Nerve Cord in Regeneration of Isolated Postpharyngeal Body Sections of *Dugesia dorotocephala*

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ABSTRACT   Lateral cordless fragments isolated from the postpharyngeal section of *Dugesia dorotocephala* formed a large normal head at a 90° angle to the original antero-posterior polarity; postcerebrally, only a hump of undifferentiated tissue developed. This "head-hump" pattern, and also other types observed in previous studies of lateral fragments, were attributed to the absence of the nerve cord. In order to confirm the inductive role of the nerve cord and to eliminate the possibility that the "head-hump syndrome" was due to the relative proportions of other tissues besides nerve, body fragments of two experimental groups were observed: (1) the five types of fragments which had no nerve cord but had varying proportions of other tissues present formed primarily "head-hump" types of regenerates. (2) Almost all fragments which had varying amounts of nerve cord present but the same proportions of other tissues formed regenerates of normal body proportions. Therefore, the absence of the nerve cord does determine the "head-hump syndrome."

Isolated postpharyngeal half segments containing one nerve cord were allowed to regenerate for varying periods of time before the lateral cordless fragment was isolated. The number of "head-hump" regenerates from lateral fragments isolated after a one-day or longer contact with the nerve cord gradually decreased, and the number of regenerates with incomplete head development or which were more elongated postcerebrally increased. These results indicate that the nerve cord acts gradually to determine the differentiation of specific tissues rather than rapidly to determine the overall body plan.

Diverse functions have been assigned to the central nervous system in regeneration of planaria. Neoblasts appear to be localized along the nerve cords (Pederson, '59; Lender and Gabriel, '60; Brøndsted and Brøndsted, '61; Stephan-Dubois, '61, '65; Kido, '61; Woodruff and Burnett, '65) and some studies have shown that neoblasts migrate along the nerve cords to the wounded area (Lender and Gripon, '62; Kido, '61; Stephan-Dubois, '65). The brain induces eye regeneration via chemical substances (Lender, '50, '51, '52, '55) and, probably indirectly, together with the nerve cords (Kido, '52; Schilt, '72) induces pharynx regeneration (Wolff, Sengel and Sengel, '58; Sengel, '59; Kido, '57). The brain is also responsible for induction of gonads (Vannini, '65). Other studies report that nerve cords exert an inhibitory influence in regeneration (Török and Törö, '62). These functions

may be mediated by neurosecretory substances: neurosecretory cells have been identified in the periphery of the brain and throughout the nerve cords (Lender and Klein, '61; Morita and Best, '65, '66; Best, '67; Oosaki and Ishii, '65; Lender, '64; Lentz, '67), and neurosecretory activity increases during the early phases of regeneration (Lender and Klein, '61).

The nervous system of *Dugesia* consists of cephalic ganglia from which a pair of ventral nerve cords extend posteriorly. The cords are connected by transverse commissures; lateral nerves extend from the cords and branch to form the marginal nerve plexus (Bullock and Horridge, '65).

Our first approach to the study of the

role of the nerve cords in regeneration of the postpharyngeal body section of D. dorotocephala was to observe the morphological and histological differentiation in isolated postpharyngeal lateral fragments which contained no nerve cords. We found that almost all these lateral cordless fragments regenerate in a pattern which we have termed the "head-hump syndrome," characterized by a large normal head and a tall hump of undifferentiated tissue. Beyer and Child ('30) also observed many regenerates of the "head-hump" type developing from lateral fragments of D. dorotocephala from all body levels, but found some regenerates of normal body proportions; a few lacked head structures. The authors reported similar patterns of regeneration for lateral cordless fragments of another species: Planaria maculata (= Dugesia tigrina). Morgan (1898) removed a long lateral fragment from D. tigrina which included portions of the prepharyngeal, pharyngeal, and postpharyngeal regions. Most of these regenerated normal body proportions, but also, a few "head-hump" regenerates were produced. The same author ('03) reported that long lateral fragments from the species D. lugubris always formed heads. More recently, Török ('58) isolated prepharyngeal, pharyngeal, and postpharyngeal lateral cordless fragments of D. lugubris. All formed regenerates of normal body proportions but the frequency of head and eye regeneration showed considerable decline along the antero-posterior axis. In cordless prepharyngeal lateral fragments of Bdellocephala brunnea, Teshirogi and Jin ('64) never observed regeneration of either a head or a tail.

Generally, in all the cases in which the main nerve cords or brain were removed, the resulting patterns of regeneration were attributed to the absence of the central nervous system. However, for the lateral cordless fragments, an alternative possibility had not been explored: the relative amounts of other types of cells and tissues within the regenerate might be the determining factors in differentiation of the regenerating parts. The lateral cordless fragment, isolated from the postpharyngeal section, contains many of the types of tissues also present in a whole transverse postpharyngeal section, but the relative proportions of these tissues differ from those of the whole

section. For example, the lateral region has more of the epidermis with its underlying musculature than does the median region of the postpharyngeal section. The lateral region also contains more gland cells, part of the excretory system and yolk glands; whereas, the median region has more of the digestive system, plus sperm ducts, and parts of the copulatory apparatus. These regions differ in the parts of the peripheral nervous system included. The lateral region has lateral nerves and the peripheral nerve plexus, the median region contains transverse nerves. Thus, lateral fragments differ both quantitatively and qualitatively from the whole postpharyngeal section.

In an effort to get a better understanding of the inductive phenomena in regeneration in planaria, we have attempted to answer this question: Is it the nerve cord or the relative proportions of different cells or tissues that specifies the direction of differentiation in the postpharyngeal body section of Dugesia dorotocephala?

We have also tried to determine whether the nerve cord, if it is indeed responsible for the "head-hump syndrome," acts rapidly to determine the overall plan or regeneration, or gradually to determine the differentiation of specific tissues. This question was based on previous work (Ansevin, '69; Ansevin and Wimberly, '69) which indicated that the future overall body organization is determined in the postpharyngeal section within the first several hours after isolation, but that determination of specific organs occurs at different stages of regeneration.

MATERIALS AND METHODS

Planaria of the species Dugesia dorotocephala have been maintained as a large stock colony since 1968 in large preparation dishes containing Hot Springs Ozarka Water (Ozarka-Houston) or tap water filtered through activated carbon to remove chlorine and organics. They are kept in the dark at a constant temperature of 18°C. Each week the worms are fed small pieces of raw calf liver.

Large worms were selected and starved for 7–14 days. Microsurgical operations were performed on worms immobilized by cooling on a petri dish of ice. The cuts were made with extra fine dissecting scalpels,

half spear point blade (Clay-Adams, New York). Immediately after the operation, the segment was transferred to a small culture dish containing 1.5 ml of the solution of Shapira et al. ('66) adjusted to approximately pH 7.6 with Tris buffer, to which 4.0 mg/ml of neomycin sulfate (Upjohn) was added. Cultures were maintained in the dark at 18°C; observations were recorded and medium changed every two days for a period of 30–40 days.

In selected specimens, the nerve cord can be visualized from the ventral surface as a lightly-pigmented streak against the more darkly pigmented areas surrounding it. Histological observation confirmed the "streak" as nerve cord.

I. In order to answer the question whether the amount of nerve or of other tissues determines the pattern of differentiation in the postpharyngeal section, two groups of regenerating fragments were obtained: (a) isolated fragments which contained no nerve cord but contained varying amounts of other tissues, and (b) isolated fragments which contained the same amount of other tissues plus different amounts of nerve cord.

The microsurgical operations are illustrated in figure 1.

(A) *Fragments without nerve cord*

In order to obtain fragments with no nerve cord but with varying amounts of other tissues the following operations were performed:

(1) Lateral fragments containing no nerve cord were cut away from postpharyngeal sections according to the procedure shown in figure 1a: The entire postpharyngeal section was isolated and cut into halves. The lateral fragment was cut away from one half. From the other half, the control piece, a lateral fragment with nerve cord, was isolated. The control piece was then divided into anterior and posterior sections so that each of them would be approximately the same size as the lateral cordless fragment.

(2) Median cordless fragments were isolated from the postpharyngeal sections (fig. 1b). Control pieces were "median fragment + nerve cord," also divided into anterior and posterior sections in order to adjust their size to that of the median piece.

Both lateral and median fragments have no nerve cord but do differ in the types and amounts of other tissues present and in the parts of the peripheral nervous system which they contain.

(3) A segment consisting of lateral and median fragments was isolated according to figure 1f. First a piece containing the nerve cord plus all tissues dorsal and ventral to it was cut out of a whole worm. After the lateral and median fragments had fused (12–24 hours), the section was removed from the worm. This isolate therefore contained tissues of both lateral and median fragments. Controls: unoperated opposite half postpharyngeal segment consisting of lateral and median fragments plus nerve cord.

(4) Another regenerate consisted of two lateral fragments, isolated as shown in figure 1g. Both nerve cords plus the area between the cords were removed from the postpharyngeal section of a whole worm. The two lateral fragments were allowed to fuse and were isolated 12–24 hours later. The resulting segment contained tissues of two lateral fragments. The controls consisted of two fused "lateral fragment + nerve cord" segments, divided into anterior and posterior sections. These were prepared by cutting out the postpharyngeal median area from another worm, allowing the segments to fuse, then isolating the fused segments.

(5) Another isolated segment consisted of the whole lateral half of the postpharyngeal section minus the nerve cord. The nerve cord plus some surrounding tissue was cauterized by means of a laser beam but the other tissues remained intact.

The focused output beam of a two joule ruby laser system operating at a wavelength of 6943 Å was used to cauterize the nerve cord. A whole worm was immobilized on a petri dish of ice. The worm and dish were covered with a piece of cheese cloth and placed in a vertical position on the platform of a micromanipulator. Convenient alignment of the ruby laser and focusing system (a Leitz research microscope) with respect to the specimen was accomplished with a He-Ne laser beam and a beam steering reflector mounted in a precision mirror mount. A series of about ten pulses was delivered along the nerve cord at 30 second intervals. The energy required to produce cauterization was about 0.5 joule. Each pulse resulted in the formation
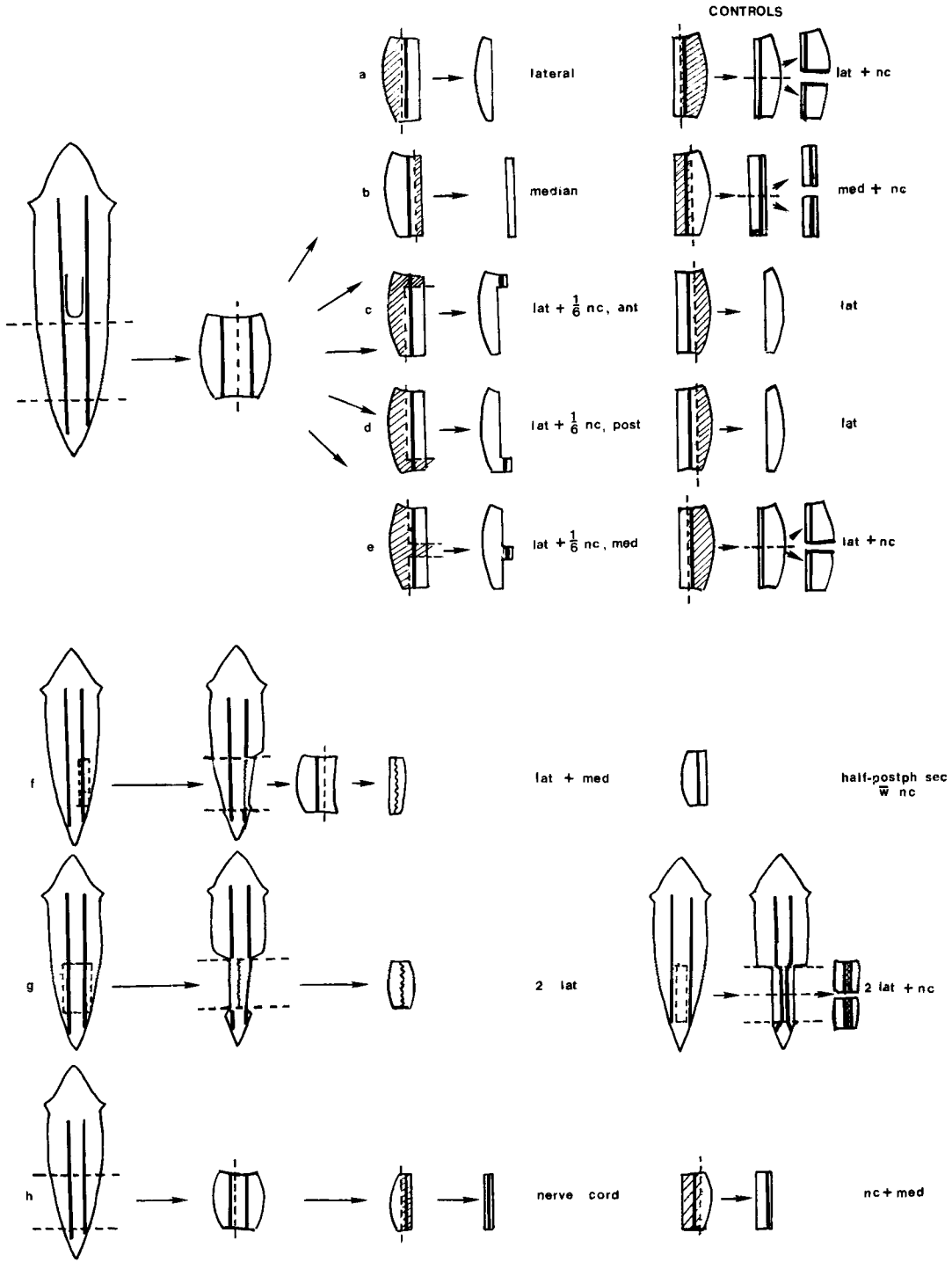
Fig. 1　Microsurgical operations. The control is shown to the right of each experimental segment.

of a light spot with a diameter of about 250 μ with an available power density of 2.3 $\times$ 10^6 W/cm^2 for a 350 μ sec laser pulse. Within two hours after this operation, the postpharyngeal section was removed from the worm. About 12 hours later the section was divided in half; the uninjured lateral half consisting of intact nerve cord, lateral, and median pieces served as a control.

(B) Fragments containing nerve cord

In order to obtain fragments which had different amounts of nerve cord but the same amounts of other tissues, the operations shown in figure 1c–e were performed. Lateral pieces plus a small section of the nerve cord left either anteriorly, posteriorly, or medially were isolated. The nerve cord extended along approximately one-sixth the length of the isolated lateral fragment. Controls for "lateral + one-sixth nerve cord, anterior" and "lateral + one-sixth nerve cord, posterior" were lateral fragments containing no nerve cord. For the "lateral + one-sixth nerve cord, median" group, the controls were lateral pieces plus the entire nerve cord.

In addition, a body fragment containing primarily nerve cord was isolated according to the procedure shown in figure 1h. These narrow fragments consisted of the nerve cord plus parts of the digestive and reproductive system and the epidermal layers immediately dorsal and ventral to the nerve cord. The control consisted of nerve cord + median fragment.

In a separate series of experiments, lateral cordless fragments from body regions other than postpharyngeal were obtained. Lateral fragments were cut away from five levels of the body: two fragments from the prepharyngeal region (anterior and posterior sections), one fragment from the pharyngeal region, and two fragments from the postpharyngeal region (anterior and posterior sections).

II. In order to answer the question concerning gradual or rapid induction by the nerve cord, lateral fragments were cut away after exposure to the nerve cord for 1, 2, 3 . . . 8 days. The procedure shown in figure 1a was followed except that the postpharyngeal half sections were allowed to regenerate for varying periods from one through eight days before the lateral cord-

less fragment and control "lateral + nerve cord" fragments were isolated.

For all cases, except that in which two fused lateral fragments were isolated, the control was prepared from the other half of the same postpharyngeal section.

For histological observations, specimens were fixed in Bouin's fixative for three days and embedded in paraffin. Serial sections of 7 μ were prepared and stained with Mallory-Heidenhain Stain, the rapid one-step method (Cason, '50). This stain allowed us to visualize the nerve cords and transverse and lateral branches but the fine marginal nerve plexus could not be detected.

RESULTS

The following types of regenerates were observed in the experiments:

(1) "Head-hump syndrome." This was the predominant type of regenerate from fragments which had no nerve cord. These regenerates consist primarily of a very large but otherwise completely well-developed head. The postcerebral portion of the body consists only of a hump of undifferentiated tissue. The steps in the formation of one such "head-hump" regenerate are shown in figure 2. (a) By two days the fragment has healed completely and the posterior end has folded over and fused with the median part of the fragment. In these fragments total or partial fusion of the anterior and posterior ends may occur to produce a rounded regenerate. (b) By four to six days a blastema has formed at the midsection of the fragment or at the point where the ends have fused. This head blastema forms at a 90° angle with respect to the normal antero-posterior polarity of the worm. (c) The head blastema continues to enlarge and the postcerebral area remains rounded (6 days). (d) By eight to ten days one eye has formed. (e) By 10–12 days the second eye appears as well as other head structures such as auricles. The post-cerebral region has formed a tall hump. (f) A fully-formed "head-hump" regenerate displays an oversized head and postcerebral tall hump (20 days).

Serial sections of these regenerating fragments were examined at different periods during the process of regeneration. In the early stages of regeneration — two to four days — a number of lateral nerves
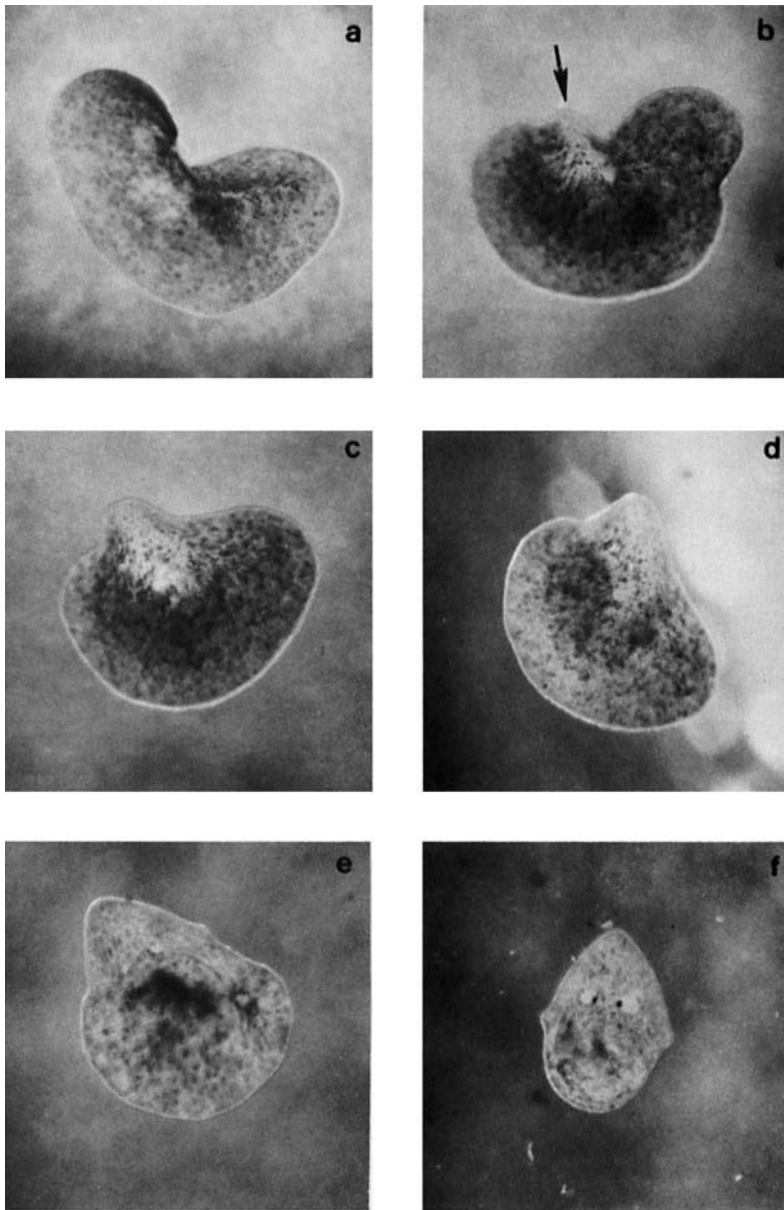
Fig. 2   Steps in the formation of a "head-hump" regenerate from a postpharyngeal lateral cordless fragment. (× 25.) (a) Two days. Anterior end at upper left. (b) Four days. Blastema indicated by the arrow. (c) Six days. (d) Eight days. (e) Twelve days. (f) Twenty days.

are evident. By four to six days the blastema has formed as a bulge of undifferentiated cells in the median area of the fragment directly anterior to the lateral nerves. Within the blastema a mass of nerve tissue appears — this will form the cerebral ganglia. During the 8–12 day period the number of lateral branches decreases to about three, the blastema enlarges, and the mass of the cerebral ganglia tissue increases. Connections are established between the cerebral ganglia and the lateral branches.

(2) *Head-hump, with two heads* (fig. 3). These regenerates form in the same manner as the "head-hump syndrome," except that two head blastemas form in the median area or near the extremities of the fragment. In some cases these anterior blastemas develop two complete heads. In other instances the two blastemas partially fuse in the median region; within each blastema two eyes form but only one auricle develops. In all cases, the postcerebral area remains as a hump of undifferentiated tissue.

(3) *Incomplete head, + hump.* This type is similar to the "head-hump" type except that head development is incomplete. "Incomplete" head development is characterized by the lack of one or both auricles and a delay of four or more days in appearance of eyes. The postcerebral region consists of a hump of undifferentiated tissue.

(4) *Head + head-like* (fig. 4). In this type two head-like blastemas formed near the extremities of the fragment; one became a normal head. The other blastema remained distinctly independent for about 25 days, but in most cases did not form auricles or eyes. The postcerebral region remained rounded and hump-like.

(5) *Head, + elongated postcerebral region* (fig. 5). These regenerates formed an enlarged head identical to the "head-hump" type, but the postcerebral region was elongated and formed only a small hump; no tail developed.

(6) *Normal* (fig. 7). These formed head structures of normal size and were normal in body proportions; a tail developed.

(7) *No head* (fig. 8). These regenerates formed normal body proportions with a tail. However, no head regenerated. A tiny anterior blastema formed but the anterior end remained rounded and no head structures appeared. They displayed normal antero-posterior polarity as they moved in one direction only.

The groups entitled "head-hump, with two heads," "incomplete head + hump," and "head + head-like" are modifications of the "head-hump" type — all have head structures but lack posterior structures. The groups entitled "normal" and "no head" are of normal body proportions, with or without a head. The type "head, + elongated postcerebrally" is intermediate; it

has an oversized head characteristic of the "head-hump" groups, but is more elongated although body proportions are not completely normal.

Series I. The results on the amount of nerve vs. amount of other tissues are summarized in table 1

A. *Isolated fragments containing no nerve cord but varying amounts of other tissues*

(1) *Lateral fragments.* Thirty-three of the 36 cordless lateral fragments isolated formed regenerates of the "head-hump" type. One of these regenerated two heads. The other three of this group formed regenerates which had an enlarged head of the "head-hump" type but were elongated postcerebrally.

The control segments for the lateral fragments consisted of lateral fragments plus nerve cord, divided into anterior and posterior pieces. In all cases (63) these formed normally proportioned worms. Thirty-three of these were completely normal but 30 did not regenerate head structures ("no head" type).

(2) *Median fragments.* Mortality in this group approached 100%. Many parameters — type and amount of mediam, pH, type of buffer, temperature, sterility, time of removal from segment — were varied, but no improvement in viability was obtained. Only one median fragment remained alive for sufficient time to observe regeneration. It regenerated as a "head-hump" type with two heads. The control — median + nerve cord fragment — developed as a normal worm.

(3) *Lateral + median fragments, fused.* Eleven of the 16 fragments regenerated as "head-hump" types. Included in the "head-hump" regenerates were three cases in which two heads developed. One of the 16 fragments regenerated as "head + head-like." Two formed a large head but were elongated postcerebrally, and two were completely normal.

The controls for this group were entire lateral halves of the postpharyngeal section which contained one nerve cord. All controls were of normal body proportions; 12 were completely normal and four were of the "no head" type.

(4) *Half-postpharyngeal section minus*
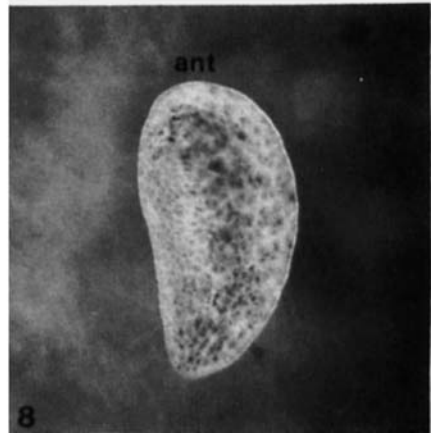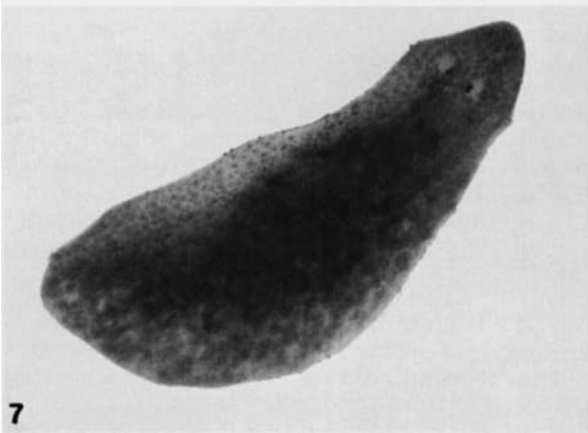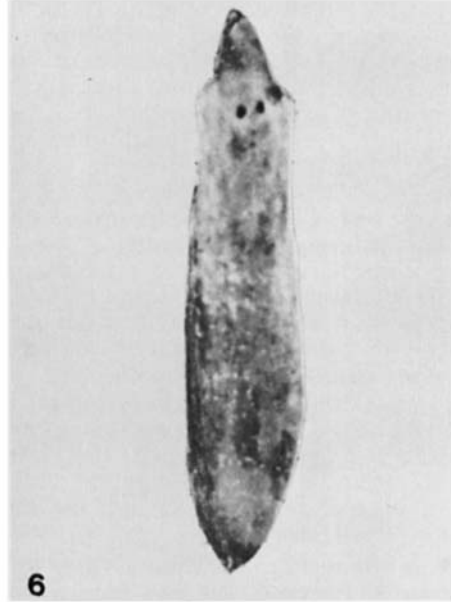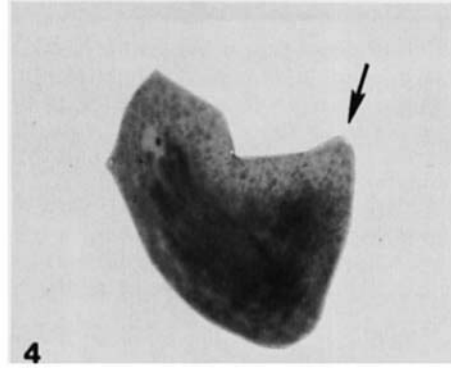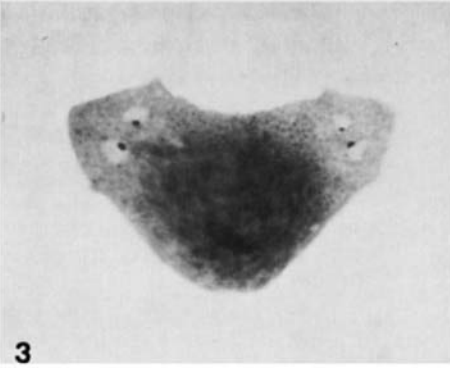
nerve cord. In these fragments an area which included primarily nerve cord was cauterized by the laser beam. Twenty of the 53 fragments regenerated as "head-hump." Nine of these had two heads. Eight regenerates had a large head but were elongated postcerebrally. An additional twenty regenerated completely normal worms and five were normally proportioned but had no head.

The controls consisted of the entire half of the postpharyngeal section which had one nerve cord. All 78 controls were normally proportioned regenerates; 69 were completely normal but nine did not regenerate head structures.

Unfortunately, under the experimental conditions used to cauterize the nerve cord with the laser beam, it was necessary to destroy some neighboring tissues in addition to the nerve cord. The reasons for this were the following: (1) the only marker visible was the lightly pigmented "streak" which was mainly but not totally nerve cord. (2) Even in a cold-immobilized worm localized contractions could occur which would vary the position of the nerve cord. (3) The energy from the laser varies as much as 15% from pulse to pulse. (4) An attempt was made to move the worm an equal distance between each pulse but in some cases more or less overlapping occurred.

In order to determine if cauterization was an effective means to destroy the nerve cord completely, whole postpharyngeal sections in which one side was cauterized were fixed immediately after the operation and the operated side was compared to the half containing the intact nerve cord. In two-thirds of the 14 segments fixed immediately, one nerve cord was completely cauterized, but in one-third of the segments,

a small amount of nerve cord remained on the cauterized side of the segment.

(5) *Two lateral fragments, fused.* Nine of the 18 segments regenerated as the "head + head-like" type. Four of the regenerates were "head-hump" and four others were incomplete in head development but retained the hump. One displayed an enlarged head but was elongated postcerebrally.

The controls were "two lateral + nerve cord fragments fused" in which the median area was removed. They regenerated normally proportioned worms in almost all cases. Nine of the 14 were completely normal and four were of the "no head" type. One control regenerate showed the "head-hump" pattern with two heads.

B. *These results on isolated fragments containing varying amounts of nerve cord but the same amount of other tissues are also included in table 1*

Lateral fragments were isolated with a small portion of nerve cord (about one-sixth) remaining in either the anterior, posterior, or median position. Almost all of these regenerated as normally proportioned worms; most of these had normal head structures, but a few did not form a head.

(1) *Lateral + one/sixth nerve cord, anterior.* Eight of the nine cases were of normal body proportions; six were completely normal and two had no head structures. One regenerate was incomplete in head development, with a hump.

The controls were lateral fragments with no nerve cord. Eight of the nine regenerates displayed the "head-hump syndrome." One was incomplete in head structures, with the hump.

(2) *Lateral + one-sixth nerve cord, posterior.* Twelve of the 13 regenerates were completely normal. One regenerate showed head structures typical of the "head-hump" type but was elongated postcerebrally.

The controls for this group were also lateral cordless fragments. Ten of the 13 were of the "head-hump" type. Two were incomplete in head development with the hump and one was entirely normal.

(3) *Lateral + one-sixth nerve cord, median.* Nineteen of the 20 regenerates were of normal body proportions, but four had

Fig. 3 A "head-hump" regenerate, with two heads, 33 days after isolation. (× 25.)

Fig. 4 Regenerate of the "head + head-like" type, 14 days after isolation. (× 25.) Arrow indicates "head-like" blastema.

Fig. 5 Regenerate of the type "head, + elongated postcerebral region," 14 days after isolation. (× 25.)

Fig. 6 Type of regenerate which has normal body proportions but abnormal head development, 72 days after isolation. (× 42.)

Fig. 7 Typical normal regenerate, 20 days after isolation. (× 25.)

Fig. 8 A "no head" regenerate, 33 days after isolation. (× 25.) ant: anterior end.

TABLE 1

Regeneration of body fragments in Series I: Amount of nerve vs. amount of other tissues

Experimental

Type of fragment	Total number	Types of regenerates						
		Head-hump	Head-hump 2 heads	Inc. head + hump	Head + head-like	Head + elong. post	Normal	No head
lateral	36	33 (92%)	1			3 (8%)		
median	1	1	1					
lat + med	16	11 (69%)	3		1 (6%)	2 (12.5%)		
half-postph sec − nc	53	20 (38%)	9		8 (15%)		20 (38%)	5 (9%)
2 lat	18	4 (22%)	2	4 (22%)	9 (50%)	1 (6%)		2 (11%)
lat + 1/6 nc, ant	9			1 (11%)			6 (67%)	2 (22%)
lat + 1/6 nc, post	13					1 (8%)	12 (92%)	
lat + 1/6 nc, med	20					1 (5%)	15 (75%)	4 (20%)
nerve cord	15						1 (7%)	14 (93%)

Controls

Controls: type of fragment	Total number	Types of regenerates						
		Head hump	Head hump 2 heads	Inc. head + hump	Head + head-like	Head + elong. post	Normal	No head
lat + nc	63						33 (52%)	30 (48%)
med + nc	1						1	
half-postph sec − nc	16						12 (75%)	4 (25%)
half-postph sec − nc	78						69 (90%)	9 (10%)
2 lat + nc	14	1 (7%)	1				9 (64%)	4 (29%)
lat	9	8 (89%)		1 (11%)				
lat	13	10 (77%)	2	2 (15%)			1 (8%)	
lat + nc	34						16 (47%)	18 (53%)
nc + med	11						7 (64%)	4 (36%)

no head structures. One regenerate had an oversized head but was more elongated postcerebrally than the "head-hump" specimens.

For this group controls consisted of lateral fragments plus the entire nerve cord. All regenerated normal body proportions, but 18 of the 34 regenerates did not form head structures.

C. Isolated nerve cords.

Fragments containing primarily nerve cord with a minimum amount of the surrounding tissues were isolated. All of the 15 regenerates formed normally proportioned worms but 14 of them did not form head structures (table 1).

The controls, nerve cord + median fragment, also regenerated as normally proportioned worms but only 4 of the 11 cases had no head structures; the other specimens were completely normal.

D. Most of the lateral cordless fragments from body regions other than postpharyngeal also regenerated as "head-hump." Lateral fragments were removed from five levels of the body: two fragments from the prepharyngeal region (anterior and posterior sections), one from the pharyngeal region, and two fragments from the postpharyngeal region (anterior and posterior sections). The number of head-hump regenerates from each level is as follows: (A) prepharyngeal, anterior — 6/10 (B) prepharyngeal, posterior — 11/12 (C) pharyngeal — 11/12 (D) postpharyngeal, anterior — 10/10 (E) postpharyngeal, posterior — 9/11. The remaining regenerates were of the type "incomplete head, + hump."

Series II. Rapid vs. gradual induction by nerve cord

Lateral cordless fragments were removed from regenerating postpharyngeal half-segments at daily intervals through eight days. The types of regenerates observed have been described earlier in this section. The "head + head-like" type was not encountered among the regenerates of this series, but one additional type was found. This regenerate has normal body proportions but is incomplete in head structures; a projection with two eyes at the base formed in place of the head (fig. 6). The results in this series of experiments are summarized in table 2.

There is a decrease in the number of "head-hump" regenerates in the fragments which were in contact with the nerve cord for one or more days. Between "0 day" (lateral fragments isolated immediately) and "one day," the percent of "head-hump" regenerates drops from 92% to 63%. In the "two day through eight day" groups the per cent of "head-hump" regenerates is similar, ranging from 35% to 47%, with the exception of "four day" isolates which showed 65% "head-hump" regenerates.

A greater variation in the types of regenerates was observed in lateral cordless fragments isolated after one day or longer contact with the nerve cord. One or two cases of each of the types of regenerates were found in the "one day" group even though the predominant type was "head-hump." In the "two day through eight day" groups a frequent type (found in 20 –41% of the cases) was the "incomplete head, + hump." In the "2-, 3-, 6-, 7-, 8-day" groups, some regenerates, ranging from 12% to 30%, were of the "head, but elongated postcerebral region" type; no regenerates of this type were found in the "four day" and "five day" groups.

Regenerates of normal body proportions (normal head, incomplete head, or no head) were more frequent from the lateral fragments in contact with the nerve cord for one or more days. Lateral fragments isolated immediately ("zero day") did not form any normally proportioned regenerates. The "two-day" groups also did not form regenerates of normal body proportions. In the other groups, the percent of normally proportioned regenerates is as follows: "3-day, 7-day" — 5–6%; "4-, 5-, 6-, 8-day" — 12–18%; "1-day" — 26%.

The controls consisted of "lateral plus nerve cord" fragments, divided into anterior and posterior portions, so that each control segment was approximately the same size as the lateral fragment. These were also cut away from regenerating half postpharyngeal sections at daily intervals through eight days. All of the controls in each group formed normally proportioned worms, most of which displayed normal head formation. In the fragments isolated immediately ("zero day"), 48% had no head, but in the "1- 8-day" groups, only 3–19% had no head structures. A few of

TABLE 2

Regeneration of body fragments in Series II: Rapid vs. gradual induction by the nerve cord

Number of days lateral fragment in contact with nerve cord	Total number	Types of regenerates							Controls: days on which "lat + nc" fragment isolated	Total number	Types of regenerates						
		Head-hump	Head-hump 2 heads	Inc. head + hump	Head + elong. post	Normal propor with proj.	Normal propor no head	Normal			Head-hump	Head hump 2 heads	Inc. head + hump	Head + elong. post	Normal propor with proj	Normal propor No head	Normal
0 day	36	33 (92%)	1		3 (8%)				0 day	63						30 (48%)	33 (52%)
1 day	19	12 (63%)		1 (5.3%)	1 (5.3%)	2 (10.5%)	2 (10.5%)	1 (5.3%)	1 day	27						2 (7%)	25 (93%)
2 day	18	8 (44%)		7 (39%)	3 (17%)				2 day	27						4 (15%)	23 (85%)
3 day	20	9 (45%)		4 (20%)	6 (30%)		1 (5%)		3 day	31						1 (3%)	30 (97%)
4 day	17	11 (65%)		4 (24%)		1 (6%)		1 (6%)	4 day	22						1 (5%)	21 (95%)
5 day	17	8 (47%)	1	6 (35%)			1 (6%)	2 (12%)	5 day	27						3 (11%)	24 (89%)
6 day	17	6 (35%)		6 (35%)	3 (18%)	2 (12%)			6 day	32						5 (16%)	27 (84%)
7 day	18	8 (44%)		5 (28%)	4 (22%)		1 (5.5%)		7 day	29						3 (10%)	26 (90%)
8 day	17	6 (35%)	1	7 (41%)	2 (12%)	2 (12%)			8 day	31						6 (19%)	25 (81%)

the normally proportioned worms had two normal heads (6% of the "6 day"; 11% of "5 day").

DISCUSSION

The results show that over 90% of the lateral fragments with no nerve cord regenerate in a pattern which we have termed the "head-hump syndrome." These regenerates form a very large, otherwise normal head, but are deficient in postcerebral structures; instead of an elongated body only a hump of undifferentiated tissue forms. The head is oriented at a 90° angle to the original polarity of the worm. In contrast, the control fragments which contain a nerve cord regenerate normally proportioned worms. About half of the controls form normal head structures but the other half fail to form a head.

Earlier workers who observed the "head-hump" phenomenon associated this type of regeneration with the absence of the main nerve cord. Beyer and Child ('30) concluded that the lateral nerves of the fragments played a role in the localization of the head in the central-median region (rather than at the anterior end), but that the nervous system was simply an expression of existing physiological gradients. Morgan (1898, '03) suggested that the appearance of head structures in the central-median region of a long fragment of *D. tigrina* and *D. lugubris* was due to the absence of the antero-posterior polarizing influence of the main nerve cord. Other investigators who found an absence (Teshirogi and Jin, '64, *B. brunnea*) or decrease in frequency (Török, '58, *D. lugubris*) of head formation in the regeneration of lateral cordless fragments also attributed their results to an insufficient amount of nerve present. In order to confirm this inductive role of the main nerve cord, other possible factors which might have been responsible for the observed results had to be eliminated. This task was undertaken in the present study. We considered the possibility that the "head-hump syndrome" could be due to the relative amounts of other types of tissues within the regenerate rather than to the low amount of nerve present (only lateral branches and peripheral plexus as compared to the main nerve cord). Therefore, we obtained several types of regenerates which lacked a nerve cord but had varying proportions of other types of tissues. Also we obtained regenerates which had varying amounts of nerve present but the same proportions of other tissues.

Five types of fragments from the postpharyngeal section were obtained which did not have a nerve cord but had varying amounts of other tissues. Most of these formed "head-hump" regenerates, or regenerates with slight modifications of the "head-hump syndrome." Regenerates which had varying amounts of nerve cord present but the same proportions of other tissues almost all regenerated normally proportioned worms. In addition, fragments containing primarily nerve cord also regenerated normally proportioned worms which, however, lacked head structures in most cases. Therefore, we can conclude that it is indeed the absence of the nerve cord which determines the "head-hump syndrome."

The large number of normally proportioned regenerates from the group "half postpharyngeal section minus nerve cord" were probably the result of incomplete destruction of the nerve cord by the laser beam. As we have shown, fragments with even a small section of the nerve cord remaining regenerate normal body proportions. In addition, the section with the destroyed nerve cord was not isolated from the entire worm immediately but was allowed to heal. During this time nerve cord from other areas may have influenced this section before it was isolated from the worm.

The second question of our study was, "Does the nerve cord act rapidly in the postpharyngeal section to determine the overall plan of regeneration, or does it act gradually over a period of time to determine the differentiation of specific tissues?" In order to determine if the nerve cord acts rapidly or gradually, one half of the postpharyngeal section was allowed to regenerate for one through eight days before the lateral cordless fragment was isolated from the section. By eight days the regeneration of the postpharyngeal lateral half section is quite extensive. An earlier study in this laboratory (Sperry and Ansevin, unpublished) showed that by eight days half of the cerebral ganglia had formed and one eye had developed on the side of the original nerve cord. The postpharyn-

geal lateral half always regenerates as a normally proportioned worm with normal head structures. The lateral fragments isolated from the postpharyngeal half section at daily intervals through eight days showed a decrease in the number of "head-hump" regenerates. A marked decrease in the "head-hump" regenerates occurs between "zero day" and "one day." The tendency to form an oversized head still remained in the lateral fragments in contact with the nerve cord for one or more days, but many regenerated incomplete heads or were more elongated in the postcerebral region. Only a few regenerates of normal body proportions were found in lateral fragments isolated after one day.

The results indicate that the determination of the postpharyngeal half segment in the direction of normal proportions — under the influence of the nerve cord — does begin within the first 24 hours of regeneration. This is shown by the decrease in the number of "head-hump" regenerates in lateral fragments isolated after one day or longer contact with the nerve cord. However, some "head-hump" regenerates formed in all cases, even from lateral fragments isolated as late as eight days. Also, among the lateral fragments isolated after one day there was a greater variety of regeneration patterns, ranging from "head-hump," to "incomplete head + hump," and "large head but elongated postcerebrally," to normally proportioned regenerates. Therefore, determination by the nerve cord is gradual rather than rapid.

Based on the results of these various experiments one might attribute several functions to the nerve cord in the regeneration of the postpharyngeal body section of *D. dorotocephala*:

(1) Specification of the antero-posterior polarity of the worm.

    (a) A fragment which contains the nerve cord develops an anterior end which usually forms a head and a posterior end which forms a tail.

    (b) Even a small portion of the nerve cord can specify the development of normal antero-posterior polarity; lateral fragments with a small part of the nerve cord remaining develop normally proportioned worms.

    (c) The lateral branches of the nervous system can also specify polarity; in the isolated lateral fragment, the head always develops at the ends of the lateral branches.

(2) Specification and maintenance of the development of various tissues along the length of the regenerate.

    (a) In the pieces containing nerve cord information is released at different levels along the body to specify the development of different tissue types in different proportions. For example, more digestive tissues form in the anterior region than in the posterior region. The nerve cord may act indirectly in this function but our results show that pieces with nerve cord do form different tissue types at different levels of the body. Even a small portion of the main nerve cord (regardless of its position along the antero-posterior axis of the lateral fragment) allows the isolate to express its original polarity and to develop differentiated tissues of the various body regions, thus forming normal body proportions.

    (b) This specification occurs gradually in the process of regeneration. In lateral cordless fragments isolated from the postpharyngeal section after one or more days there is a decrease in the number of "head-hump" regenerates and an increase in the number with incomplete head or more elongated postcerebral regions.

    (c) In contrast, the lateral branches cannot specify the development of different types of tissues. In the "head-hump" regenerates only undifferentiated tissue forms in the postcerebral region. These "head-hump" regenerates eventually elongate (after about 30 days) and form differentiated tissue but only after the main nerve cords have regenerated.

(3) Inhibition of the formation of head structures.

It seems that the potential to form head structures is constantly present in most areas of the body. Not only is it present in the postpharyngeal area but isolated lateral cordless fragments from other levels of the body (pharyngeal and prepharyngeal) also reveal it in the "head-hump" type of regeneration.

    (a) Isolated cordless fragments always regenerate head structures. The lateral and median areas of the postpharyngeal section do not normally form heads since they are in contact with the nerve cords of the sec-

tion. However, when these areas are isolated from the inhibiting influence of the nerve cord, head structures form.

(b) Isolated fragments containing primarily nerve cord with some of the surrounding tissue did not form heads in over 90% of the cases. However, the normal antero-posterior polarity and normal proportions of body tissues were maintained.

(c) Some of the control regenerates with a nerve cord formed normal body proportions but did not form head structures. These formed only a tiny head blastema. It might be hypothesized that a certain critical number of undifferentiated cells within the blastema are necessary for head formation to be initiated. In the "no-head" regenerates, the number does not reach this threshold value — presumably because the nerve cord inhibits these cells before they can become established in the head blastema.

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