Determination in regenerating tissues of *Dugesia dorotocephala*: the influence of nerve cord grafts

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SUMMARY

Lateral fragments which contained no nerve cord were isolated from the postpharyngeal body section of *Dugesia dorotocephala* and fused with nerve cord grafts soon after isolation and at daily intervals through 8 days of regeneration. Fragments fused soon after isolation formed 'headless' regenerates but had normal body proportions. If the lateral cordless fragment was allowed to regenerate for 1 day or longer before fusion with the nerve cord fragment, the head always developed and the body proportions were normal. Therefore, head structures become determined in the lateral fragment within the first 24 h of regeneration; during this time these tissues can also respond to the head-inhibiting influence of the nerve cord. The competence to form particular structures of the postcerebral body regions must emerge after head-forming competence is lost, that is about 24 h after isolation; however, it persists at least through the first 8 days of regeneration. Normal body proportions can be induced by nerve cord grafts throughout the first 8 days of regeneration.

Lateral fragments fused at any time after isolation with another fragment containing no nerve cord developed head structures but failed to differentiate tissues of the postcerebral regions. This confirms that the nerve cord is responsible for inhibition of head structures and induction of differentiation of body regions and normal body proportions.

INTRODUCTION

In a recent study (Sperry, Ansevin & Tittel, 1973), it was demonstrated that in *Dugesia dorotocephala*, tissues other than the main nerve cord have an inherent potential to build head structures only. The nerve cord has an inhibitory as well as an inductive function in regeneration: it inhibits transformation of all available tissues into head structures and induces (directly or indirectly) the differentiation of tissues of the different body regions and the formation of normal body proportions.

Earlier studies (Ansevin, 1969) showed that in the whole postpharyngeal body section (which contains two nerve cords) of the species *Dugesia tigrina*, head structures are determined within several hours after isolation, but the posterior regions of the body are not determined for several days. Also, in prepharyngeal body sections of *Bdellocephala brunnea*, the head becomes determined within several hours after isolation (Teshirogi, 1963).

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In the present investigation we have sought to define the period of time in regeneration during which the inhibitory and inductive actions of the nerve cord are performed. It was assumed that for particular body tissues there is a period of competence of limited duration during which they can be directly or indirectly induced by the nerve cord to form particular body regions and structures, or inhibited from forming some other structures. The goal of this study was to define the periods of specific competences and thus also specify when determinations of particular body structures occur.

The technique of grafting was selected for the present study. Nerve cord was grafted to an isolated postpharyngeal lateral cordless fragment which had been allowed to regenerate for varying periods of time. It was assumed that if, during the 'cordless' period of regeneration, the tissues of the lateral section lose the competence to be induced (or inhibited) to form a specific structure, subsequent grafting of the nerve cord would fail to induce (or inhibit) this particular organ or body part. Conversely, if the grafting of the nerve cord was performed during the stage of this particular competence, induction (or inhibition, as the case might be) of that structure would occur.

METHODS AND MATERIALS

The procedures for maintaining our colony of *Dugesia dorotocephala* and for microsurgical operations were described previously (Sperry et al. 1973).

A lateral cordless fragment was isolated, allowed to regenerate for specified
periods of time, then a fragment containing nerve cord was grafted to the regenerating lateral fragment. The isolation and grafting procedures are shown in Fig. 1. For isolation of the ‘host’ lateral cordless fragment, the entire postpharyngeal section was removed from a whole worm and cut into halves; from each half, the lateral fragment was cut away. The lateral fragments were placed in small culture dishes containing 1-5 ml of the solution of Shapira, Coleman & Castellani (1966) and incubated in the dark at 18 °C for 1–2 h, or for one through eight days. At these daily intervals, the ‘graft’ fragment was prepared from another intact worm: a half-postpharyngeal segment containing a single nerve cord was isolated and the median area was removed.

The grafting procedure, based on that of Brøndsted (1939), is as follows (Fig. 1): From a regenerating ‘host’ lateral fragment a small slice of tissue was cut away from its lateral side in order to create a wounded surface. The cordless fragment and ‘graft’ fragment containing nerve cord were placed on a ‘Schotte table’ (Fig. 2), usually with the ventral surfaces facing upwards, pressed together at the wounded edges, and incubated in the dark at 10 °C for 12–18 h. At this time excess tissue lateral to the nerve cord was removed so that from that moment on the ‘graft’ contained primarily nerve cord. The fused fragments were transferred to a culture dish and allowed to regenerate in the dark at 18 °C. Observations were recorded and medium changed every 2 days for 30–40 days.

For controls a lateral fragment containing no nerve cord was grafted to a regenerating lateral cordless fragment (Fig. 1). The figure shows that control and experimental fragments were isolated from the same worm; in practice, however, in most cases separate worms were used for the control series.

In these experiments, a ‘Schotte table’ (Brøndsted, 1939; Fig. 2) was prepared by stretching a piece of muslin cloth across a plastic ring which was 2·3 cm in diameter and 0·4 cm in height; a second ring of slightly smaller diameter interlocked with the first ring to hold the cloth taut across the ‘table’. The ‘Schotte table’ was placed in a small Petri dish and sufficient saline solution was added so that the cloth was well moistened. Maintenance of the fragments on ‘Schotte tables’ and at low temperatures for the first hours after the operation were necessary for the fragments to remain immobilized long enough for fusion to take place.
RESULTS

A representative graft of the host lateral cordless fragment and the nerve cord fragment is shown in Fig. 3 at two days after fusion. The anterior and posterior ends of the host lateral fragment had fused prior to grafting to produce a rounded regenerate.

Altogether 63 successful grafts were obtained in the experimental series and 14 in the control group. Efforts were made to obtain a significant number of successful grafts at 1–2 h after isolation ('0 day') and at '1 day' after isolation; thereafter, emphasis was placed on the even days of regeneration.

The following results are shown in Table 1.

(A) In all cases in which the lateral cordless fragment was fused with the nerve cord fragment within 24 h after isolation ('0 day'), 'headless' regenerates developed (types 1–3, Table 1; Fig. 4). Neither the host cordless fragment nor the nerve cord fragment differentiated head structures, but normal body proportions were established.

Figures 3–8

Types of regenerates formed as a result of a nerve cord fragment grafted to a lateral cordless fragment (scale line = 0.4 mm).

Fig. 3. Representative graft of a nerve cord fragment to a host cordless fragment, shown 2 days after grafting. × 25.

Fig. 4. A 'headless' regenerate (type 1), 68 days after grafting. The nerve cord fragment was grafted to the cordless fragment at '0 days' after isolation. (Fixed specimen, × 50.)

Fig. 5. Regenerate of normal body proportions with normal head structures (type 4), 14 days after grafting. The cordless fragment has formed the anterior regions and the nerve cord graft has developed tissues of the posterior region. ×25.

Fig. 6. Regenerate of normal body proportions with atypical head structures (type 5), 56 days after grafting. × 50.

Fig. 7. A 'doublet' type regenerate (type 6), 40 days after grafting. Individual on the left developed from the nerve cord graft fragment. × 25.

Fig. 8. Regenerate of normal body proportions with atypical head structures (type 11), 40 days after grafting. Tissues on the right half of the body developed from the cordless fragment and tissues on the left half are of nerve cord graft origin. × 25.

Figures 9–11

Types of regenerates formed as a result of a cordless fragment grafted to a regenerating lateral cordless fragment.

Fig. 9. Regenerate in which host and graft fragments have developed as 'head-hump' regenerates of opposing antero-posterior polarity (type 14), 29 days after grafting. ×25.

Fig. 10. 'A head-hump' regenerate (type 16), 14 days after grafting. ×25.

Fig. 11. A 'head-hump' regenerate having one large but otherwise normal head ('H') and two incomplete heads (arrows), type 18, 16 days after grafting. ×25.
Table 1. *Types of regenerates formed when a nerve cord fragment or a cordless fragment was grafted to a cordless lateral fragment at various stages of regeneration*

Each block represents one regenerate. Shaded regions represent tissues which developed from the nerve cord graft.

<table>
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<th>Number of days lateral fragment had regenerated when graft attached</th>
<th>Types of regenerates</th>
<th>Nerve cord graft</th>
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Nerve cord grafts in regenerating Dugesia

(B) When the lateral cordless fragment was allowed to regenerate for one day or longer before fusion with a nerve cord fragment, the resulting regenerates formed head structures and normal body proportions (types 4-13, Table 1).

(1) The most common type of regenerate, found in all groups except '3 day', was one in which the cordless fragment formed the anterior region while the graft gave rise to the posterior region (types 4 and 5, Table 1; Figs. 5 and 6). Some of these regenerates formed atypical head structures: three eyes appeared but only one auricle developed (type 5, Table 1, Fig. 6).

(2) 'Doublet' type regenerates were found among the '1-4 day' group (types 6-13, Table 1). A few of these were bipolar: the host and graft fragments developed anterior and posterior regions of opposing polarity (types 6 and 7, Table 1; Fig. 7). Some regenerates formed two anterior regions but had one tail (types 8 and 9, Table 1). Others fused to form a single head and tail in which the nerve cord graft developed tissues of one half of the body and the cordless fragment formed tissues of the other half (types 10 and 11, Table 1; Fig. 8). Some of these also developed atypical head structures (type 11, Fig. 8).

(3) Only in the '1 day' group a few regenerates were formed in which the cordless fragment was almost completely absorbed by the nerve cord fragment (type 12, Table 1) or the nerve cord graft almost fully incorporated into the cordless fragment (type 13).

(C) The control series gave uniform, distinct results. Lateral cordless fragments which at any time after isolation were fused with a fragment which had no nerve cord formed 'head-hump' regenerates: an oversized but otherwise normal head regenerated, but normal body proportions failed to develop and the post-cerebral region formed only a 'hump' of undifferentiated tissues (types 14-18, Table 1). A few cases appeared in the control '1 day' group where host and graft lateral fragments each formed 'head-hump' regenerates but of opposing polarity (type 14, Table 1; Fig. 9) and in a few regenerates the host fragment developed as 'head-hump' but the graft formed only a 'hump' (type 15, Table 1). More often the fragments fused to develop a single 'head-hump' regenerate (types 16 and 17, Table 1; Fig. 10), a type found in all control groups. Head structures were incomplete in some cases: only one eye and one auricle formed (type 17). A few regenerates developed multiple head structures, resembling 'Janus-head' forms, with a single 'hump' (type 18, Table 1; Fig. 11). A regenerate of this type had one oversized but otherwise normal head ('H', Fig. 11) and two atypical heads, each of which had two eyes but lacked auricles (arrows, Fig. 11).

DISCUSSION

The present study shows that induction of normal body proportions in Dugesia dorotocephala can occur and is effective at any time through eight days of regeneration, since nerve cord grafts fused with the lateral pieces as late as after eight days of regeneration still prevented the 'head-hump syndrome'. Head differentiation could be inhibited only during the first 24 h of regeneration.
of a lateral postpharyngeal fragment. This we consider to be evidence that the competence of tissues to become determined as head structures lasts for less than the first 24 h of regeneration and that head determination is always accomplished by 24 h or sooner. Thus, the 'non-head' competence (to form other organs of the body) must emerge close to the end of the first 24 h. However, it persists for at least the next 7 days since determination of specific organs and body parts can be induced by nerve cord grafts in lateral pieces that were regenerating in isolation for as long as eight days.

These results confirm the observations of Ansevin (1969) and Teshirogi (1963) which showed that head structures were determined in isolated body sections within several hours after isolation, but that more posterior regions were determined later in regeneration. The results also further confirm the gradual nature of determination of body tissues under the influence of the nerve cord (Sperry et al. 1973).

The present study has also further confirmed that the inhibition of head structures and the determination of the body tissues is specifically a function of the nerve cord. A fragment containing only branch nerves when fused with a cordless fragment failed to inhibit head formation even if it was grafted soon after isolation of the host fragment. In some cases where multiple head structures developed, head formation appeared to be stimulated under the influence of the cordless graft. Also, branch nerves did not induce development of tissues of more posterior regions: postcerebral regions remained hump-like.

No attempt was made in the present study to control the antero-posterior orientation of the nerve cord graft with respect to that of the host cordless fragment. By chance one would expect a 50:50 relationship between the number of homopolar and heteropolar grafts and thus regenerates. However, only 4 out of 63 regenerates in the experimental series were bipolar (types 6 and 7) and these occurred only in the '2–4 day' groups. In all other cases the form of the regenerate indicated that the polarity of the nerve cord graft coincided with the polarity of the host cordless fragment. The present results suggest that the polarity of the final regenerate may be under the control of the differentiating tissues of the host lateral cordless fragment and that the polarity becomes determined within the first four days after isolation. However, other possible explanations such as sampling error or greater survival of homopolar grafts have not been eliminated. The question of establishment of polarity should be pursued further before definite conclusions can be drawn.

The nature of the processes by which planarian tissues become determined to form particular structures is unknown. The earlier and present findings suggest that the information coded and released in each cell of the body (other than nerve) is to form head structures. Unless this message is altered in another direction the information is expressed as head structures. The nerve cord plays a decisive role in altering the head-forming potential and inducing the development of other body tissues.
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The authors express sincere thanks to Mrs Elga Lewis for diligent care of the worm colony. This work was submitted in partial fulfillment of the requirements for the Ph.D. degree (P.J.S.).

REFERENCES


(Received 23 April 1974)