Experimental studies on axial polarity in hydra

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SUMMARY

There is evidence which suggests that the polarity of regeneration in hydra is determined by axial gradients of some sort. The mechanisms which may be involved in the establishment and maintenance of the gradients have been investigated by studying the reversal of polarity in graft combinations.

Complete polarity reversal can be effected by a grafted hypostome or by a grafted hypostome and peduncle. Partial polarity reversal can be effected by a graft of a peduncle only. Changes in regional properties associated with polarity changes have been investigated using isolation and transplantation techniques.

The experimental results suggest that the axial gradient behaves as a gradient of a substance. Such a gradient could be produced by either (a) simple diffusion of a substance from a source or (b) unidirectional transport of a substance plus back-diffusion. Some of the experimental results are incompatible with mechanism (a). All the experimental results are compatible with mechanism (b).

Some of the problems raised by the interpretation of the axial gradient in terms of a polarized transport model are briefly discussed.

INTRODUCTION

The nature and organization of developmental field systems is one of the most obscure and challenging problems in developmental biology. Even at the supra-cellular level, our understanding of the control and integration of developmental events within a field is far from complete, and in terms of cellular and molecular processes, it is virtually non-existent.

One of the important problems posed by field phenomena concerns the nature of those vectorial qualities or properties of the field which result in the formation of polarized developmental axes. Hydra provides an ideal system for investigating the vectorial properties of fields since there is only one major developmental axis, the disto-proximal one, and regulation following cutting is rigorously polarized.

There is a considerable body of evidence which suggests that the axial polarity of hydra (and other hydroids) is determined by the direction of slope of axial gradients of some sort (reviewed, Webster, 1970). Insight into the nature of polarity and the mechanisms whereby the axial gradients are established and

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maintained might be expected to come from experiments in which changes or reversals of polarity are induced. In hydra, such changes can be produced in graft combination and many experiments along these lines have been carried out in the past (e.g. Peebles, 1900; King, 1901; Morgan, 1901; Browne, 1909; Goetsch, 1929; Burnett, 1961; Webster, 1966a) but in no case were the changes in polarity analysed in detail. In this paper we present such an analysis.

This work arose as an extension of previous experiments, concerned with the mechanism of transmission of hypostome inhibition, in which it became clear that, under some conditions at least, axial polarity could be very stable (Wilby & Webster, 1970). This observation was in marked contrast to those made earlier (Webster, 1966a, b) which suggested that in certain transplant situations the graded factors responsible for polarity were very labile. These apparently contradictory observations are reconciled in this paper. A further stimulus to the experimental work reported here arose from a reconsideration of the observations of earlier workers on polarity, in particular those of Goetsch (1929), in the light of Lawrence's (1966, 1970) provocative ideas on the nature of polarity in the insect segment.

**MATERIALS AND METHODS**

*Hydra littoralis* were used for all experiments. Details of culture methods, selection of animals, etc., can be found in Webster & Wolpert (1966).

Three types of graft combination were used to study polarity changes:

(a) Proximal hypostome grafts: host animals were prepared by cutting just distal to the youngest bud to remove proximal regions. The hypostome and tentacles from a donor animal were grafted to the proximal end of the host digestive zone using the rod technique previously described (Wilby & Webster, 1970). The hypostome and tentacles of the host animal were removed 3 h after the grafting operation.

(b) Peduncle grafts: host animals were prepared by removing the hypostome and tentacles and, in most cases, the peduncle and basal disc also. Peduncles from donor animals were isolated by cutting just proximal to the oldest bud and were grafted to the distal end of the host digestive zone with opposite polarity.

(c) Hypostome and peduncle grafts: in this case both a hypostome and tentacles and a peduncle were grafted to the isolated digestive zone as described in (a) and (b) above.

All experimental animals were incubated at 26 °C. In most cases regions to be grafted (hypostomes and peduncles) were taken from animals which had been coloured by feeding with *Artemia* nauplii hatched and grown in Evans blue (1:5000 in sea water). After about three meals of the coloured *Artemia* the endoderm of hydra becomes very deeply coloured and the colour persists for more than a week. This method of colouring tissues seems to have no harmful effects on the animals (probably because the dye is concentrated in intracellular
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Vacuoles and they bud and regenerate normally. Grafting experiments with coloured animals produced exactly the same results as with normal, uncoloured hydra.

In the case of the peduncle grafts, some of the graft combinations were left intact and allowed to regenerate. The remainder, and all other graft combinations, were recut after various intervals of time to isolate the host digestive zone whose regeneration behaviour was examined. Recutting was easy to perform since the junction between coloured graft and uncoloured host remained quite sharp for several days. The polarity of the isolated digestive zones was followed using the staining technique described previously (Wilby & Webster, 1970).

Transplantation experiments to examine regional properties were carried out along lines similar to those used in previous investigations (Webster & Wolpert, 1966; Webster, 1966a, b).

RESULTS

1. Proximal hypostome grafts

In previous experiments which demonstrated inhibition by a proximal hypostome (Wilby & Webster, 1970) it was observed that within the first 48 h following grafting there was no significant change in the polarity of the inhibited host digestive zone as indicated by the nature of the regeneration at each end. However, if such animals are kept for a longer period of time they do eventually show polarity changes.

The first sign of any change in the host axis occurred after 48 h, when animals which were budding produced new buds from the old distal end of the axis. It is important to note that this shift in the position of the zone of bud initiation occurred before any observable change in regeneration polarity. This observation confirms and extends that of Webster (1966a).

The first morphological indications of alteration in regeneration polarity were observed after about 120 h, when about 75% of the animals produced a peduncle and basal disc at the old distal end. However, it was possible to show that changes in polarity had occurred earlier than this by removing the grafted hypostome and the inhibited distal tip and allowing the animals to regenerate, which they did within 24–48 h of the operation.

The changes in polarity revealed by this experiment followed a consistent pattern in time which is shown in Table 1. The first sign of a polarity change was the appearance of animals with a peduncle and basal disc at each end of the axis (bi-peduncles) and, sometimes but not always, a hypostome and tentacles in the middle of the axis (medial hypostome). It was noted that those animals which produced a peduncle at both ends of the axis, but no hypostome, were actively budding animals and that the buds were initiated in the middle of the axis (these buds always detached). Further changes in polarity occurred in those animals which were kept for a longer period of time; in these cases a complete reversal of polarity occurred and the host digestive zone regenerated a new
hypostome and tentacles from the old proximal end and a new peduncle and basal disc from the old distal end.

From Table 1 it can be seen that although two animals with a peduncle at each end of the axis had appeared 24–48 h after grafting there was no dramatic change in polarity until 72 h, at which time more than half of the animals showed polarity changes. At 96 h the majority of animals showed changes and by 120 h virtually all of the animals showed complete reversal of polarity.

**Table 1. Effect of proximal hypostome grafts on host polarity**

<table>
<thead>
<tr>
<th>Time when graft removed (h)</th>
<th>No. of grafts</th>
<th>Original polarity</th>
<th>Bi-peduncle (no hypostome)</th>
<th>Medial hypostome</th>
<th>Reversed polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>96</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>120</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

It is important to note that although the hypostome and tentacles which developed at the end of the axis were normal, the hypostome which formed in the middle of the axis (a medial hypostome) developed in a characteristic and rather unusual fashion. The first sign of hypostome formation was the production of a lateral outgrowth morphologically identical to an early bud. This continued to elongate exactly like a bud for about 48 h and then produced tentacles at its distal end. At no stage did it detach from the major axis. The development of such a lateral outgrowth (produced by a different experimental method but identical to that described above) is illustrated in Figs. 1–4.

Figs. 1–4. Development of medial hypostome as a result of grafting a hypostome and a peduncle to a digestive zone. The digestive zone was isolated after 24 h in the graft combination.

Fig. 1. Digestive zone 24 h after isolation. The right-hand end is distal.

Fig. 2. Same digestive zone 48 h after isolation, showing early outgrowth in the middle of the axis (arrow). Peduncles and basal discs have regenerated at both ends of the axis.

Fig. 3. Same digestive zone 72 h after isolation. The outgrowth (arrow) has increased in size, evidently at the expense of the major axis.

Fig. 4. Same digestive zone 96 h after isolation. Tentacles have developed at the apex of the outgrowth (arrow).

Fig. 5. Stained peduncle grafted to the distal end of a digestive zone, 48 h after grafting. The arrows indicate the junction between host and graft tissue where an outgrowth is beginning to develop which will eventually produce a secondary axis similar to that shown in Fig. 4. It is composed of both host and graft tissue. A bud is present at the proximal end of the digestive zone.

Fig. 6. Stained peduncle grafted to the distal end of the digestive zone, 96 h after grafting. Arrows indicate host–graft junction. In this case two outgrowths with tentacles have developed some distance away from the junction.
The tips of very early stage outgrowths produced in later experiments were tested for inductive ability by transplantation to intact host animals. All induced the secondary distal axis characteristic of induction by a hypostome and none induced the formation of a bud.

The progressive change in properties of the inhibited distal end which are revealed by allowing this end to regenerate can also be shown by transplantation experiments. The procedure used was that previously devised by Webster (1966a) in which the distal tip is transplanted into a host from which the hypostome and tentacles have been removed. If this region possesses normal subhypostomal properties (i.e. if it is unchanged), it will induce the formation of a secondary axis in the host; if it does not possess these properties but possesses those of more proximal regions, it will be assimilated or induce the formation of a peduncle and/or basal disc.

Distal tips, taken 24–48 h after grafting on the proximal hypostome, induced secondary distal structures in 60% of the cases, the remainder being assimilated (twenty-four grafts); transplants made at 72 h were all assimilated (eight grafts); at 96 h and 120 h 70% of the grafts were assimilated and 30% produced a peduncle and/or basal disc (thirteen grafts).

This experiment shows very clearly the progressive change in properties of the inhibited region from typical, distal, subhypostomal properties to those characteristic of more proximal regions and finally to those characteristic of the most proximal regions, the peduncle and basal disc. The sequence of changes is the same as that revealed by the regeneration experiments. It is important to note that no detectable changes occur in the inhibited tip until 48–72 h after grafting on the proximal hypostome.

2. Peduncle grafts

The formation in the previous experiments of a hypostome in the middle of the axis recalls experiments performed by Goetsch (1929), in which he obtained a similar shift in the position of hypostome formation in a combination consisting of a peduncle grafted with opposite polarity on to the distal end of a host axis. These observations suggested to us that the changes in polarity might be explicable in terms of Lawrence's (1966) 'sand model' and we therefore thought it desirable to repeat and extend Goetsch’s experiments.

Peduncle grafts were prepared as described in the Methods section but in some cases the host peduncle was left in place. Some of the graft combinations were left intact and others were recut after various intervals of time in order to remove the grafted peduncle.

In those graft combinations which were left intact, the new hypostome was produced in exactly the same manner as in the experiment with a proximal hypostome graft in which a medial hypostome was produced; i.e. an initial lateral outgrowth comparable to a bud followed by the development of tentacles.

In all cases this lateral outgrowth, which seems to be the first sign of new
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Hypostome formation, appeared after a somewhat greater time than that normally required for regeneration from a subhypostomal region which is about 24 h. In intact combinations the outgrowth did not appear in the majority of animals until 48 h after grafting and tentacles did not appear until about 72 h. Thus the whole process of producing distal structures took considerably longer in the graft combination.

Table 2. Effect of peduncle grafts on host polarity (graft combination left intact)

<table>
<thead>
<tr>
<th>Nature of host</th>
<th>No. of grafts</th>
<th>No. animals with hypostome at junction*</th>
<th>No. animals with medial hypostome</th>
<th>No. animals with no hypostome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host with own peduncle</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Host minus own peduncle</td>
<td>58</td>
<td>34</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

* Fifty % of these outgrowths contained equal proportions of host and graft tissue, the rest had less graft tissue.

Table 3. Regeneration of host axes and peduncles after isolation from graft combination

<table>
<thead>
<tr>
<th>Time in graft combination before isolation (h)</th>
<th>No. of pieces isolated</th>
<th>No. of pieces regenerating</th>
<th>Nature of regeneration at 48 h</th>
<th>Bi-peduncle (no hypostome)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>At 24 h</td>
<td>At 48 h</td>
<td>Normal</td>
</tr>
<tr>
<td>Isolated host axes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (freshly isolated)</td>
<td>18</td>
<td>15 (83 %)</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>7 (70 %)</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>4-5</td>
<td>16</td>
<td>6 (37 %)</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>4 (36 %)</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>7 (70 %)</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>48</td>
<td>8</td>
<td>6 (75 %)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Isolated peduncles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>18</td>
<td>0</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>4-5</td>
<td>16</td>
<td>0</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>4 (40 %)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>48</td>
<td>8</td>
<td>6 (75 %)</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

In the intact, uncut combination the majority of outgrowths appeared at the junction between graft and host (see Table 2) and, since the peduncle had been stained, it could be seen that the lateral outgrowth was often made up of both
host and graft tissue in about equal proportions (Fig. 5). In some cases, the lateral outgrowth did not arise from the junction but from a position one-third to half-way down the host axis (Fig. 6). These results confirm Goetsch's (1929) observations that a shift in the site of hypostome formation can occur in this graft combination.

Although in the majority of cases in which the graft combination was left intact there was no pronounced shift in the site of hypostome formation, the experiments in which the combination was recut and the regeneration of the isolated peduncle and host axis examined showed that there was interaction between the graft and host, with a resultant change in the properties of both regions.

Results of these experiments are shown in Table 3. Let us first consider the changes which occur in the subhypostomal region of the host axis. After 2 h in contact with the peduncle the rate of regeneration of the subhypostomal region did not appear to have changed; however, the regenerated structures were often abnormal and a high proportion of animals produced a single apical tentacle. This suggests some sort of inhibition of distal regeneration since such structures are often produced in the presence of inhibitory chemicals (see Webster & Wolpert, 1966). After 4½ h of contact the majority of host axes showed delayed regeneration, taking about 48 h to produce distal structures (as compared with the normal time of 18–24 h); a number of these were of the single tentacle form. Some animals did not produce any distal structures but produced peduncles and basal discs at the distal end. After 6 h of contact the majority of animals again showed delayed tentacle regeneration and some animals produced peduncles and basal discs at the distal end. In one of the latter cases a medial hypostome was also produced.

Thus after 6 h of contact with a peduncle the properties of the subhypostomal region of the host axis have been changed quite dramatically, the rate of regeneration has altered, and in some cases this region has quite clearly acquired properties characteristic of much more proximal regions.

However, when host axes which have been in contact with a peduncle for 24 or 48 h were examined the changes in properties appeared much less striking. After 24 h of contact few of the animals showed delayed regeneration; no single tentacles were produced but in one case a peduncle was regenerated at the distal end. After 48 h of contact the results were much the same, but no regeneration of distal peduncles was observed.

These results indicate that in the graft combination the properties of the subhypostomal region are at first rapidly altered, but that after 24–48 h the region seems to have regained its normal properties.

The initial, rapid changes in the properties of the subhypostomal region were confirmed by transplantation experiments (see Table 4). After 4½ h in combination the subhypostomal regions were transplanted into host animals with or without hypostomes and tentacles. In the hosts without hypostomes, 80 % of the
subhypostomal regions were assimilated, i.e. behaved as though they had lost their characteristic properties and acquired those of more proximal regions; 50% behaved as peduncles or basal discs and induced proximal axes. The majority of normal subhypostomes induce distal axes in this situation.

In the hosts with hypostomes no distal axes were induced and 40% of the grafted regions again induced proximal axes. After 41/2 h of regeneration the majority (ca. 70%) of normal subhypostomal regions induce distal axes in this situation since a hypostome has been determined (see Webster & Wolpert, 1966).

Thus the transplantation experiments indicate that hypostome determination in the subhypostomal region was inhibited, and furthermore, that the properties of this region had been altered and properties characteristic of more proximal regions acquired.

Let us now consider the changes which occur at the distal end of the peduncle in the graft combination (Table 3). After 41/2 h the properties of the peduncle appeared to be unchanged; after isolation it regenerated in the normal time (about 36–48 h) and the distal structures produced were completely normal. After 24 h in the combination, however, about 40% of the isolated peduncles regenerated in a considerably shorter period of time than normal (about 24 h) and again the regenerates were normal. After 48 h in combination the majority of peduncles exhibited this reduction in the time required for regeneration and produced distal structures within 24 h.

Thus in the peduncle there is no rapid change in properties comparable to that which occurs in the subhypostomal region and the change which does occur, the increase in rate of regeneration, takes a substantial period of time.

It is most important to note that after a period in graft combination of between 24 and 48 h both the subhypostomal region and the peduncle are, when isolated, capable of regenerating distal structures in the same period of time, i.e. about 24 h. It will be remembered that it was about 48 h after preparation that the intact graft combination began to produce the lateral outgrowth which we believe to be the first sign of hypostome formation (and which, on transplantation into an intact host, always induces a secondary distal axis) and that, in many cases, this outgrowth appeared at the junction between graft and host and appeared to be composed equally of graft and host tissues. This fact is consistent with the observation that the rates of regeneration of graft and host become virtually identical after a period in graft combination. Transplantation of the host subhypostomal region as before into animals with or without hypostomes and tentacles confirms that, after 24 h in the graft combination, many of these regions have reacquired their original properties (see Table 4). About 50% of the grafts induced distal axes in hosts without hypostomes, none induced proximal axes. Ten% induced distal axes in intact hosts, indicating hypostome determination in a few cases.
3. Hypostome and peduncle grafts

The previous experiments have shown that the hypostome is capable of completely reversing the regeneration polarity of an axis but that the reversal takes a considerable period of time; the peduncle, on the other hand, although incapable of bringing about complete reversal of polarity, can effect partial reversal and can produce rapid, though temporary, changes in regional properties. It was therefore of interest to examine the effect of the two regions when present at the same time in a graft combination.

Table 4. Transplantation of subhypostomal regions from ungrafted animals and host subhypostomal region from graft combinations ('Peduncle-only' grafts)

<table>
<thead>
<tr>
<th>Time after removal of hypostome, or in graft combination, when transplanted (h)</th>
<th>No. of pieces transplanted</th>
<th>Intact host Type of inductive response:</th>
<th>Host minus hypostome and tentacles Type of inductive response:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Secondary distal axis</td>
<td>Graft absorbed</td>
</tr>
<tr>
<td>Control subhypostomes from ungrafted animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4.5</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>4.5</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Subhypostomes from grafted animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>14</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>4.5</td>
<td>14</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>24</td>
<td>21</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Hypostome and peduncle grafts were prepared as described in the Methods section. In some cases both grafted regions were left in place for varying periods of time and then removed simultaneously. In other cases either the hypostome or the peduncle was removed after an interval, and the other region left in place for a further period of time before it too was removed. In both sets of experiments the regeneration behaviour of the isolated digestive zones was observed. One end of this region was stained in order that polarity could be followed. Results are shown in Table 5.

The isolated axes showed three types of regeneration: (a) normal regenerate with original polarity; (b) a medial hypostome with a peduncle at each end of the axis; (c) a normal regenerate with reversed polarity. In other words, exactly
the same forms as were observed in the experiments using hypostomes alone as grafts.

The normal regenerates, whether of original or reversed polarity, were produced within about 24 h of cutting. Medial hypostomes took somewhat longer to develop, the initial outgrowths not appearing in the majority of cases until 48 h after isolation of the axis.

Let us consider first the cases in which both grafted regions were removed simultaneously. From Table 5 it is apparent that the axial polarity of the digestive zone was very rapidly altered. After only 4 $\frac{1}{2}$ h in the graft combination, regenerates with medial hypostomes were produced; after 24 h half of the animals had medial hypostomes and two showed complete reversal of polarity; after 48 h there were two animals with the original polarity and the majority had completely reversed.

**Table 5. The effects of hypostome and peduncle grafts on host polarity**

<table>
<thead>
<tr>
<th>Treatment of graft combination</th>
<th>Host regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of hypostome removal (h)</td>
<td>Time of peduncle removal (h)</td>
</tr>
<tr>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>24</td>
<td>4.5</td>
</tr>
<tr>
<td>4.5</td>
<td>24</td>
</tr>
<tr>
<td>48</td>
<td>24</td>
</tr>
<tr>
<td>24</td>
<td>48</td>
</tr>
</tbody>
</table>

It is clear that this graft combination can produce polarity changes very much more rapidly than can a graft of a hypostome alone, which it will be remembered takes about 72 h to produce medial hypostomes and 96–120 h to produce complete reversal.

In view of these observations it seemed of interest to try to determine what were the relative contributions of the two grafted regions to the rapid reversal of polarity. The procedure used was to remove the two regions at different times (see Table 5).

In the first case the peduncle was removed at 4 $\frac{1}{2}$ h and the hypostome at 24 h. Comparing these animals with those in which both components were removed at the same time, 4 $\frac{1}{2}$ h, the proportion of animals showing polarity changes was not altered, i.e. the rapid reversal of polarity, if initiated, could not be continued by the hypostome alone. In the second case the hypostome was removed at 4 $\frac{1}{2}$ h and the peduncles at 24 h. Although the proportion of animals showing polarity changes did increase, this increase is not statistically significant (by $\chi^2$ test). It
would seem therefore that during the first 24 h both regions are necessary to bring about rapid changes in polarity.

Similar experiments were carried out in which the peduncle was removed at 24 h and the hypostome at 48 h and vice versa. Again, the hypostome alone had no statistically significant effect on polarity changes. The peduncle alone, however, although having no significant effect on the proportion of animals showing complete reversal, did significantly ($P < 0.1$ by a one-tailed $\chi^2$ test) increase the number of animals showing partial reversal, i.e. those with a medial hypostome.

It would seem therefore that in most cases rapid and complete reversal of polarity requires the continuous presence of both hypostome and peduncle. There is some evidence to indicate that in those animals in which reversal takes place relatively slowly, a partial reversal can be brought about by the peduncle alone.

We have also examined the effects on the distal end of the peduncle of this graft combination. It will be remembered that, in the experiments in which the graft consisted of a peduncle alone, the properties of the peduncle, as judged by regeneration rate, were altered after 24–48 h in the combination. We examined peduncles after 48 h in the hypostome and peduncle graft combination and found no alteration in the rate of regeneration. Eight peduncles were examined, all took the normal 48 h to regenerate distal structures after removal from the combination, as compared with the 24 h observed in the previous experiment.

**DISCUSSION**

Let us first briefly summarize the significant observations on polarity reversal in the digestive zone.

Complete reversal of axial polarity can be brought about by a proximal hypostome graft but this reversal occurs very slowly; a graft combination consisting of a proximal hypostome and a distal peduncle is able to reverse polarity much faster. A peduncle alone can under no circumstances bring about a complete reversal of polarity but can sometimes effect a partial reversal. Polarity reversal in all cases seems to follow the same pattern: the site of hypostome formation first shifting from the distal end of the axis to the middle of the axis, and then shifting to the opposite, former proximal, end. These changes in the site of hypostome formation are invariably accompanied by the formation of a peduncle and basal disc at the old distal end; in some cases the peduncle and basal disc appear in this position and thus indicate a change in polarity even though there is no apparent formation of a hypostome. The reversal of regeneration polarity is associated with a temporal sequence of changes in the transplantation properties of affected regions.

Throughout this discussion it will be assumed that the site of hypostome formation occurs at, and indicates the presence of, the high point of a gradient.
or gradients, and the site of peduncle and basal disc formation similarly indicates the low point. In other words, regeneration polarity is determined by the direction of slope of an axial gradient of some sort. Evidence in support of this assumption is presented elsewhere (Webster, 1970).

We believe that the experiments reported in this paper provide information on two major problems raised by the idea of axial gradients: (a) the nature of the axial gradient; (b) the mechanism involved in the establishment and maintenance of the gradient.

(a) The nature of the axial gradient

The experiment in which a peduncle was grafted to the distal end of the digestive zone resulted in the formation of a medial hypostome in a significant number of animals. This observation indicates that the graft combination has resulted in the high point of the gradient being displaced from the distal (subhypostomal) end of the host some way along the host axis, since as noted above the assumption is that a hypostome forms at the high point of the gradient. Such a movement of the gradient peak could occur if the gradient was of some substance which could diffuse within the tissues. In this graft combination, regions with different concentrations of gradient substance are opposed and a discontinuity in the gradient is initially present at the graft-host junction; substance would therefore tend to diffuse from high to low concentration, i.e. from host to graft. The situation can be visualized (Fig. 7) in terms of Lawrence's (1966) 'sand model' developed to account for polarity changes in the insect segment. It is clear that in the graft combination in which both a hypostome and peduncle were present, the large number of animals with medial hypostomes produced after 24 h can be explained in exactly the same way; in fact, the statistical analysis of the results suggests that it is the presence of the peduncle which is primarily responsible for their formation.

In the peduncle-only grafts the results of isolating the host and graft after a period in combination and examining the changes in regeneration rate and transplantation properties are completely consistent with this interpretation. As shown in Fig. 7, the gradient level in the host subhypostomal region will fall considerably as a result of 'flow' of substance into the graft and the experiment revealed that such changes did occur, the host subhypostomal region acquiring properties characteristic of more proximal regions (i.e. lower gradient levels). Fig. 7 indicates that the initial changes in gradient level in the grafted peduncle are relatively small and, in fact, none could be detected experimentally.

If this interpretation of the experimental results is correct, it indicates that diffusion of gradient substance can occur relatively quickly since, in the peduncle-only grafts, changes could be detected (by both regeneration and transplantation tests) within 4½ h. In a previous experiment in which a subhypostomal region was transplanted into the digestive zone of an intact host, it was shown that the region quickly lost its characteristic properties (Webster, 1966b). This result can
also be explained in terms of the ‘sand model’ since the experiment involves placing a region with a high concentration of gradient substance into a region containing a lower concentration. Diffusion or ‘flow’ will occur from the transplanted subhypostomal region into the host and the gradient level in the former will be reduced. This reduction in gradient level accounts for the loss of

Fig. 7. Diagrammatic representation in terms of Lawrence's ‘sand model’ of the changes in the gradient which occur when a peduncle is grafted to the distal end of the digestive zone. The gradients are drawn as linear for simplicity; the long arrows in the diagrams of the graft components indicate both the regeneration polarity and the direction of transport of the gradient substance.

(A) Initial form of the gradients immediately after grafting. ‘Flow’ of a substance will occur from the high level in the host to the low level in the grafted peduncle (curved arrow).

(B) Form of the gradients after ‘flow’. The high point of the gradient is now in the middle of the host axis. The gradient level at the distal end of the host axis has fallen considerably while that in the distal end of the grafted peduncle has risen slightly. This situation is unstable unless changes in the direction of transport occur (see text).

(C) The stable situation if no changes in the direction of transport occur. The gradient peak has moved back to the host-graft junction which is the point where the two polarized transport systems meet ‘head to head’.
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characteristic properties, and the time taken for this to occur (in 50% of the animals) is 4–5 h, i.e. identical to the time taken for changes to occur in the peduncle-only grafts. Thus two experimental results can be interpreted in terms of a gradient of substance, and both suggest that this substance can diffuse relatively quickly.

(b) The establishment and maintenance of the gradient

The experimental results reported in this paper give some insight into the sort of mechanism which may be involved in producing and maintaining the axial gradient. There are only two simple mechanisms for generating stable gradients of a substance (Webster, 1970):

(i) A simple diffusion mechanism involving continuous production of a substance by a source and continuous removal by a ‘sink’. Either the sink or the source must be localized at one end of the axis.

(ii) A mechanism involving polarized (unidirectional) transport of a substance coupled with back diffusion (Lawrence, 1966).

There is no doubt that many of our results can be explained in terms of a simple diffusion model in which the hypostome acts as a source. Some results, however, are very difficult to account for in these terms.

1. In the hypostome-only grafts regeneration polarity, and therefore the axial gradient, is stable for at least 48 h, possibly somewhat longer. This stability is very difficult to account for in terms of a simple diffusion model since, as noted above, other experiments indicate that gradient substance can diffuse relatively quickly (within 4–5 h). We would, therefore, expect changes to occur at the high end of the gradient (that is the old distal end of the host) as a result of diffusion down the concentration gradient within a few hours at the most, and would not expect the gradient level to remain stable for 48 h. This intuitive reasoning is supported by theoretical calculations by O. K. Wilby which will be published elsewhere.

2. In the hypostome-only grafts changes in regional properties (and therefore gradient level) occur first, in some animals at least, at the end of the axis opposite to the grafted hypostome, i.e. at the point farthest removed from the region which might reasonably be supposed to act as a source. Furthermore, regions adjacent to the supposed source retain their characteristic properties for a long period of time (48–72 h).

3. In the hypostome-only grafts some animals develop a hypostome, and therefore a gradient peak, in the middle of the axis. This situation seems impossible to explain in terms of a simple diffusion model. If the whole animal, apart from the hypostome, acts as a sink a medial peak obviously cannot be formed; if the sink is localized in the peduncle and/or the basal disc, a medial peak could be generated if these regions were present, but the experimental results show that the fall in gradient level at the distal end of the host occurs before these regions can be detected.
(4) In the peduncle-only grafts there are initial rapid changes in the properties of the host subhypostomal region which we have explained in terms of the 'sand model'. Further studies of this graft combination (when left intact) using transplantation techniques show that these changes are transient and that the host subhypostomal region regains its characteristic properties within 24–48 h. At the same time, the distal end of the peduncle also shows changes in gradient properties and becomes identical to the subhypostomal region. The transient nature of the changes is also made clear by comparing the number of graft combinations which show evidence of interaction between graft and host (with a resultant change in the gradient properties of the host) with the number which finally produce a medial hypostome (i.e. retain a displaced gradient peak). The regeneration data indicate that 55% of the combinations have interacted after 4½–6 h. The transplantation experiments (into decapitated hosts), which provide a more reliable demonstration of changes, indicate that 80% of the combinations have interacted after the same time. However, only about 30% of the combinations eventually produce a medial hypostome, the remainder producing a hypostome at or near the graft–host junction.

Fig. 8. Effect of reversing the polarity of transport on a gradient of a substance produced by a polarized transport–back diffusion mechanism. The solid arrow indicates the original direction of transport, the solid curve (0) the initial, stable, gradient. The dashed arrow indicates the reversed direction of transport and the dashed curves the gradient profiles at different intervals of time after reversal of the direction of transport. After a time interval of twelve units, the gradient peak is approximately in the middle of the axis (based on unpublished calculations of O. K. Wilby).
We suggest that these observations indicate that the gradient peak, which is initially displaced along the axis as a result of 'flow' of gradient substance from host to graft, is slowly moved back to the graft-host junction. Such a change is most difficult to account for in terms of a simple diffusion model. It may be noted that Hicklin, Hornbruch & Wolpert (1969) have reported similar transient changes in gradient properties following treatment with dithiothreitol.

Although the above results are not easily explicable in terms of a simple diffusion model of gradient formation, they are explicable in terms of a polarized transport model, and such a scheme is consistent with all the experimental results presented in this paper. In terms of this model, a reversal of regeneration polarity is caused by a reversal of the direction of transport of gradient substance. We will briefly summarize how such a model can be used to account for our results; a more detailed consideration will be given elsewhere (Webster, 1970).

A. A gradient produced by polarized transport of substance in a proximo-distal direction plus back diffusion would be very stable. The gradient could be linear or non-linear, depending upon the characteristics of the transport process. A reversal of the direction of transport in a system in which the gradient was non-linear will produce changes in the gradient (Fig. 8) such that (a) the gradient level at the distal end of the axis will fall but there will be little change at the proximal end; (b) at a certain time after reversal the gradient peak will be more or less in the middle of the axis (hence a medial hypostome could be produced); (c) after a further period of time the slope of the gradient will be completely reversed and hence regeneration polarity will be reversed. All the changes are consistent with the experimentally observed changes in regeneration and transplantation properties.

B. Transient changes in gradient level as observed in the peduncle-only grafts are explicable; as can be seen in Fig. 7, the junction between graft and host is the point at which two polarized transport systems meet 'head-to-head'. The gradient peak, initially displaced as a result of 'flow', will be unstable in its new position and will tend to move back to the junction which is the stable position. This is obvious by intuition, but is also supported by the unpublished calculations of O. K. Wilby. Such movement is consistent with the restoration of the characteristic properties of the subhypostome, since the movement of the peak back to the junction will result in an elevation of the gradient level at this point.

Several problems are raised by an interpretation in terms of a polarized transport model. First, we may ask what is the cause of the reversal in direction of transport of the substance? It is clear from the combination in which a hypostome only was used as a graft that this region can completely reverse regeneration polarity, and therefore in these terms cause a reversal in the direction of transport. The peduncle also seems to play some role in bringing about a reversal since, in a combination consisting of hypostome and peduncle, complete reversal of regeneration polarity occurred a good deal faster than one
in which the hypostome only was used, i.e. 24–48 h compared with 72–96 h. It is not clear whether this observation indicates that the peduncle has ‘special’ properties comparable to those of the hypostome. A second problem is to account for the production of stable medial hypostomes in the peduncle-only grafts since we have argued that the displaced gradient peak is unstable and will tend to move back to the junction. In order to produce a medial hypostome, the displaced peak must be stabilized in its new position. There are at least two ways in which this could occur: (a) if a hypostome is determined relatively quickly at the peak it could change the polarity of transport of the host axis and thereby stabilize itself; (b) the direction of transport of the substance could be changed in relation to the new gradient established as a result of ‘flow’ (Fig. 7), in other words, the substance is always transported against the concentration gradient. Such a change would also stabilize the displaced peak.

Finally, there is the problem of how polarized transport of the substance is brought about. One mechanism, similar to that suggested by Lawrence (1966), would involve polarized cells all orientated in the same direction and capable of (net) unidirectional active transport. With regard to the mechanism of repolarization, it is worth bearing in mind that the hypostome seems to establish a gradient in level of inhibition (Webster, 1966a); such a gradient could provide a vector which could reorientate and repolarize cells and thereby bring about an eventual reversal of regeneration polarity.

The model for polarity which we have suggested must be regarded as no more than a working hypothesis. It is, admittedly, somewhat speculative, but it is also consistent with all of the experimental observations and seems to have considerable heuristic value. It can also account for many features of regulation in hydra (Webster, 1970).

Note added in proof. Since this paper was submitted for publication we have carried out more experiments on polarity reversal by proximal hypostome grafts. Using digestive zones from starved, non-budding, animals 53 grafts produced 5 examples of medial hypostomes. The rarity of such hypostomes suggests that the conditions necessary for their formation are transient.

RÉSUMÉ

Etudes expérimentaux sur la polarité axiale chez l’Hydre

Certains faits suggèrent que la polarité de la régénération chez l’Hydre est due à des gradients axiaux. Le mécanisme qui peut être impliqué dans l’établissement et le maintien des gradients a été étudié en analysant les effets de l’inversion de la polarité dans des combinaisons de greffes.

Une inversion totale de la polarité peut être réalisée par une greffe d’hypostome ou par celle d’un hypostome et d’un pédoncule. Une inversion partielle peut être réalisée par une greffe d’un seul pédoncule. Les changements des propriétés locales associées à des altérations de la polarité ont été étudiées par des techniques d’isolement et de transplantation.

Les résultats expérimentaux suggèrent que les gradients axiaux se comportent comme des gradients de substances. Un tel gradient peut être le fruit (a) d’une simple diffusion de substance à partir d’une source ou (b) d’un transport d’une substance dans une direction déterminée suivi d’une diffusion rétrograde. Certains des résultats expérimentaux sont
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incompatibles avec le mécanisme (a). Tous les résultats expérimentaux sont compatibles avec le mécanisme (b).

Certains problèmes soulevés par l'interprétation des gradients axiaux, en prenant comme modèle un transport polarisé, sont brièvement discutés.

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REFERENCES


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