Studies on pattern regulation in hydra

II. Factors controlling hypostome formation

By GERALD WEBSTER

From the Zoology Department, King's College, University of London

INTRODUCTION

In a previous paper (Webster & Wolpert, 1966a) it was shown that during regulation the time required for the determination of the dominant region, the hypostome, was dependent on the original position of the hypostome-forming region on the linear axis. Hypostome determination occurred more quickly from distal than from proximal regions, suggesting an axial gradient. As Spiegelman (1945) has pointed out, a gradient in time for determination is not sufficient for limited realization to occur in a regulative system (Webster & Wolpert, 1966a). In addition, some mechanism for suppressing potentialities is required. One of the characteristic features of a dominant region is that it inhibits the formation of a new dominant region (Huxley & de Beer, 1934), and earlier work on hydra (Rand, Bovard & Minnich, 1926) has presented some evidence for inhibition of distal regeneration by distal structures. This paper will be concerned with a detailed analysis of the factors controlling hypostome formation.

MATERIAL AND METHODS

Hydra littoralis were used for all experiments. Full experimental details are as given in Webster & Wolpert (1966a).

EXPERIMENTS AND RESULTS

Experiment 1. Hypostome formation from regions transplanted to the digestive zone in the presence and absence of the hypostome

Freshly isolated subhypostomal regions of comparable size to the pieces used in determining the time for hypostome formation (Webster & Wolpert, 1966a) —average volume $1.6 \times 10^{-2} \text{ mm}^3$—were transplanted to the mid-digestive zone of host hydra, and in half the animals the hypostome and tentacles of the host were immediately removed by a cut just proximal to the ring of tentacles (Fig. 1). Freshly isolated pieces from the proximal digestive zone were trans-
planted in a similar manner and the host hypostome and tentacles removed. The behaviour of the transplanted pieces is shown in Table 1. All the host animals regenerated hypostomes and tentacles where these were removed.

The results show very clearly that, in the absence of the host hypostome, transplanted subhypostomal regions give rise to secondary distal axes identical with those produced as a consequence of induction by a determined hypostome—all of type 1 or 3 (Webster & Wolpert, 1966a). If the hypostome and tentacles are present in the host then all the transplanted pieces are absorbed. In the case

![Fig. 1. Schematic representation of Exp. 1. A subhypostomal region is transplanted to the digestive zone of a host hydra in the presence (A) and absence (B) of the host hypostome. In the first case the graft is absorbed, in the second it produces a secondary distal axis.](image)

**Table 1.** Hypostome formation from regions transplanted to the digestive zone in the presence and absence of the host hypostome

<table>
<thead>
<tr>
<th>Source of graft</th>
<th>Host hypostome and tentacles</th>
<th>No. of successful grafts</th>
<th>No. of animals with secondary axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subhypostome</td>
<td>Present</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>20</td>
<td>14 (70%)</td>
</tr>
<tr>
<td>Proximal digestive zone</td>
<td>Absent</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
of the proximal digestive zone, the presence (Webster & Wolpert, 1966a) or absence of host hypostome and tentacles has no effect on the behaviour of the transplanted piece, which is absorbed in both cases.

It is worth noting that the number of tentacles on the primary (regenerated) and secondary (induced) axis often differed, one axis having fewer tentacles than the other.

Discussion of Experiment 1

At first sight the results of this experiment appear to show that a non-hypostomal region is capable of induction. However, a brief consideration of the nature of the experiment will show that this conclusion is not justified. Grafting a subhypostomal region to the digestive zone of a host hydra from which the hypostome and tentacles have been removed produces an animal with two subhypostomal regions. Exps. 1 and 2 of Webster & Wolpert (1966a) indicated that following the removal of the hypostome and tentacles, a new hypostome was formed by the subhypostomal region; if two subhypostomal regions are present in the same animal there is no reason why both should not form hypostomes. This is clearly what has happened in the present experiment. Both subhypostomal regions have formed hypostomes and subsequently induced distal structures, the lateral graft reorientating the tissues of the host (see Exp. 2) to produce a new axis.

The fact that a transplanted subhypostomal region forms a hypostome when the host hypostome is absent but is absorbed when it is present indicates that simple cutting is not sufficient to stimulate hypostome formation; this only occurs when the existing hypostome is removed. The corollary of this conclusion is that the presence of a hypostome suppresses or inhibits the formation of another hypostome. The results suggest that in the absence of the hypostome the level of inhibition in the digestive zone falls, so releasing the subhypostomal region from inhibition and permitting it to form a new hypostome.

This experiment has demonstrated that a hypostome situated at the distal end of the animal can inhibit hypostome formation from a subhypostomal region situated at a proximal level. Rose (1957b) has suggested that inhibition always acts in a polarized fashion and the following experiment is designed to test this possibility by seeing whether inhibition can travel in a proximo-distal direction.

Experiment 2. The effect of a transplanted hypostome on the formation of a hypostome from the subhypostomal region

(a) Lateral hypostome grafts to the digestive zone

In this experiment, hypostomes with tentacles and a short piece of axis (to facilitate grafting) were transplanted to the mid-digestive zone of host hydra. Such lateral grafts (Fig. 2A) are rather difficult to accomplish because of the large size of the piece to be grafted; in the first experimental series (10 animals)
both the position of the graft and the length of the axis were somewhat variable. In the second series (10 animals) when some facility had been gained, both the position and length of axis were more uniform throughout the series. Grafts were allowed to heal for 4–5 h, when the host hypostome and tentacles were removed by cutting just proximal to the ring of tentacles.

It will be apparent that this experiment is similar to Exp. 1 except that the relative positions of the hypostome and the subhypostomal regions are reversed.

The number of animals showing regeneration of hypostome and tentacles from the host subhypostomal region is shown in Table 2. Since the results for two series are somewhat different, they are presented separately. It is apparent that a hypostome situated laterally in the digestive zone can, in the majority of cases, prevent the formation of hypostome and tentacles from a subhypostomal region.

In series 1, half the animals regenerated a hypostome and tentacles from the host subhypostomal region and half did not. When the animals were examined 48 h after cutting, it was apparent that the distance between the grafted hypostome and the host subhypostomal region was distinctly shorter in those animals which had failed to regenerate than in those which had succeeded. Measure-
Pattern regulation in hydra. II

ment of this distance in maximally extended animals revealed that the mean distance was nearly twice as great in regenerating as compared with non-regenerating animals (Table 2); however, the difference between these means is not significant ($P > 0.05$) when Student’s ‘$t$’ test is applied.

In the second series only 2 out of 10 animals showed distal regeneration. These animals were measured immediately after cutting and as can be seen from Table 2 the distance between the host subhypostomal region and the grafted hypostome is both shorter than in the first series (possibly as a result of the animals not being as fully extended when measured) and more uniform throughout the series. However, the difference between the mean distance in regenerating and in non-regenerating animals is not significant.

Table 2. The effect of lateral hypostome grafts on the formation of a hypostome from a subhypostomal region

<table>
<thead>
<tr>
<th>Series</th>
<th>No. of animals regenerated</th>
<th>No. of animals not regenerated</th>
<th>Distance, host subhypostome to grafted hypostome*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regenerated animals</td>
<td>Non-regenerated animals</td>
<td></td>
</tr>
<tr>
<td>Series 1 (measured 48 h after cutting)</td>
<td>5</td>
<td>5</td>
<td>1.5 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.0 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.8 -</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>3.26 1.72</td>
</tr>
<tr>
<td>Series 2 (measured immediately after cutting)</td>
<td>2</td>
<td>8</td>
<td>1.4 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.3 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 1.2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>1.35 1.16</td>
</tr>
</tbody>
</table>

* Arbitrary units. † Lost.

The grafted hypostome and tentacles show a pronounced tendency to ‘grow out’ from the host; that is, the axis elongates. This is especially noticeable in animals which fail to regenerate distally, where it can be clearly seen that this elongation is at the expense of the distal regions of the host which eventually disappear entirely. The result of this outgrowth is that the grafted axis appears to move up the host body. Similar outgrowth occurs in animals which have
regenerated distally, but it is not so pronounced. The impression gained is that the grafted hypostome reorientates the host tissues, and that where two hypostomes are present they ‘compete’ for the available material. It is clear that in view of the morphogenesis which follows grafting, the measurements made at 48 h will only be of significance with regard to the initial (zero time) distance between hypostome and subhypostomal region if all that is involved is reorientation with neither gain nor loss of material. This would appear to be the case, as far as can be determined by visual inspection.

(b) Proximal hypostome grafts to the digestive zone

This experiment was designed to overcome the variation in the position of graft, and in the length of the distal axis attached to the grafted hypostome and tentacles, encountered in the previous experiment.

Hypostomes (with tentacles), isolated by cutting immediately proximal to the ring of tentacles and therefore possessing no distal axis, were grafted to the proximal end of the digestive zone of host animals which had been prepared by cutting just distal to the youngest bud (Fig. 2B). Grafted animals were allowed to heal for 1 h when the host hypostome and tentacles were removed in the usual way. Of 17 animals with this type of graft none showed any signs of distal regeneration. It is clear therefore that a hypostome situated at the proximal end of the digestive zone prevents the formation of a hypostome and tentacles from a subhypostomal region which is situated distal to it.

It is of considerable interest that the majority of experimental animals treated in this way continued to produce buds, but that the new buds formed at the old distal end of the digestive zone, i.e. from the host subhypostomal region or thereabout, and at the maximum possible distance from the grafted hypostome. All the buds grew at the expense of the parents and eventually detached.

As in the previous experiment, the grafted hypostome and tentacles sometimes tended to ‘grow out’ at the expense of the host (in 5 out of 17 animals). In most of these animals (4) a short peduncle with a basal disc formed at the old proximal end of the digestive zone after the outgrowth of the hypostome and tentacles. In some animals where no outgrowth took place (3) a peduncle and basal disc formed at the old distal end of the digestive zone, i.e. there was a reversal of polarity in the host. The remaining animals (10) showed no signs of peduncle and basal-disc formation for as long as 10 days after grafting.

Discussion of Experiment 2

The two experiments demonstrate conclusively that the presence of a proximal hypostome can prevent distal regeneration, presumably by inhibiting hypostome formation from the subhypostomal region.

Exp. 2b is the most convincing with regard to proximo-distal inhibition, since none of the grafted animals regenerated from the distal end. In 2a, on the other hand, some animals were not inhibited; this result may be compared with
Exp. 1, where the hypostome was distal and the subhypostomal region proximal, and all the animals were prevented from forming a secondary axis. The reason for this failure of inhibition is not clear. Superficially, in series 1 it appears to be related to the distance between the grafted hypostome and the host subhypostomal region, but the difference between the mean distance in inhibited and non-inhibited animals is not statistically significant. It will be remembered that these animals were measured 48 h after grafting, but reasons have been given for believing that the distance at this time is probably similar to that at zero time. In series 2, however, measured immediately after grafting, there is no significant difference between inhibited and non-inhibited animals as regards the hypostome–subhypostome distance.

Rand et al. (1926), using a similar experimental situation to that in 2a, concluded that the distance between a laterally grafted hypostome and tentacles and the distal cut surface of the host was important in deciding whether inhibition of distal regeneration would occur or not. Unfortunately, Rand’s experiment is complicated by the fact that in addition to distance the level of the distal cut surface in the host animal also varied although this was not discussed by the authors. This criticism may be equally valid in the present experiment, since, although all animals were cut at the same distal level, the properties of this level with regard to inhibition by a grafted ‘foreign’ hypostome might vary from one animal to another. However, the results of 2b suggest that this is unlikely.

Some evidence for the effect of distance on inhibition comes from work on Tubularia. Tardent (1963) states that the degree of inhibition of distal reconstruction by a hydranth grafted to the proximal end of the stem is a function of the length of the stem. It is apparent that no definite conclusions can be drawn from the present experiment with regard to the importance of distance in hydra. The next experiment (3) was designed to shed further light on this problem.

Removal of the host hypostome presumably results in a fall in the level of inhibition (see Exp. 1) which is counteracted by the effect of the grafted hypostome. The latter must make its presence felt at the distal end before hypostome determination can occur. In 2a inhibition due to the grafted hypostome must have reached an adequate level within 8·5–9·5 h after grafting (4–5 h delay before removal of host hypostome + 4·5 h for hypostome determination in 50 % of the host animals (Webster & Wolpert, 1966a); in 2b within 5–6 h (1 h delay + 4·5 h for hypostome determination). These figures indicate that any explanation of the different results of 2a and 2b must involve factors other than (or in addition to) the time required for the grafted hypostome to establish inhibition.

**Experiment 3. Hypostome formation from regions transplanted to the basal disc**

The procedure in these experiments was exactly the same as that in Exp. 1. Freshly isolated subhypostomal regions were transplanted to the basal disc (split longitudinally) of the host hydra and in half the animals the hypostome and
tentacles were removed. Pieces from the proximal digestive zone were transplanted in a similar manner but the host animals were left intact.

The formation of secondary axes by the transplanted pieces is recorded in Table 3; all the induced axes were of type 2 (Webster & Wolpert, 1966a).

It is evident that a subhypostomal region when transplanted to the basal disc can form a hypostome and induce a secondary axis irrespective of whether the host hypostome is present or absent; the difference in the number of secondary axes in the two cases is not significant when the $\chi^2$ test is applied. The majority of proximal digestive zone pieces, however, do not induce secondary axes in the presence of the host hypostome, but form either a basal disc or are absorbed, as are the non-inducing subhypostomal regions.

**Table 3. Hypostome formation from regions transplanted to the basal disc**

<table>
<thead>
<tr>
<th>Source of graft</th>
<th>Host hypostome and tentacles</th>
<th>No. of successful grafts</th>
<th>No. of animals with secondary distal axes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subhypostome</td>
<td>Present</td>
<td>20</td>
<td>13 (65%)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>20</td>
<td>18 (90%)</td>
</tr>
<tr>
<td>Proximal digestive zone</td>
<td>Present</td>
<td>20</td>
<td>1 (5%)</td>
</tr>
</tbody>
</table>

It is noteworthy that the secondary axes which are produced by the transplanted subhypostomal regions do not appear until some time after the host animals have regenerated hypostomes and tentacles; 48–72 h for the formation of secondary axes compared with 18–20 h for distal regeneration. That this is partly due to a slower response to inductive stimuli on the part of the basal disc is suggested by the results of a few experiments in which hypostome fragments were transplanted to these two regions; induced axes were formed more quickly by the digestive zone (within 24 h) than by the basal disc (approximately 48 h).

In striking contrast to the behaviour of subhypostomal regions transplanted to the digestive zone (in the absence of the hypostome) where tentacles are produced before any considerable outgrowth of the axis occurs, in the present experiments outgrowth of an axis usually preceded tentacle formation. The sequence of events in the development of a secondary axis from a subhypostomal region in the basal disc is thus very similar to that in the development of a bud. However, none of the induced axes separated from the hosts, though observed for periods of up to 14 days.

**Discussion of Experiment 3**

The formation of secondary axes from the transplanted regions in this experiment is interpreted in exactly the same way as in Exp. 1, i.e. the grafts first form hypostomes and subsequently induce distal structures.

In contrast to the results obtained in Exp. 1 where the behaviour of sub-
hypostomal regions transplanted to the digestive zone was dependent upon whether the host hypostome was present or absent, in the present experiment subhypostomal regions in the basal disc formed hypostomes irrespective of the presence or absence of the host hypostome. The proximal digestive zone, on the other hand, behaved very much as in exp. 1 of Webster & Wolpert (1966a) and as in Exp. 1 above, except that one case of hypostome formation was observed in the presence of a pre-existing hypostome as compared with no cases where this region was transplanted to the digestive zone.

The results of Exp. 1 were interpreted as suggesting that in the absence of the hypostome the level of inhibition in the digestive zone falls, so releasing the subhypostomal region from inhibition and permitting it to form a hypostome. If this view of the events leading to hypostome formation is correct, it is clear from the results of the present experiment that in the intact animal, the level of inhibition in the basal disc is lower than in the digestive zone, since a subhypostomal region forms a hypostome when transplanted there in the presence of a pre-existing hypostome. Whether, when the hypostome is removed, the level falls as it does in the digestive zone is difficult to say; the difference in number of secondary axes produced in the presence and absence of the hypostome is not statistically significant.

It is apparent that the possibility of inhibition being affected by distance, suggested by the results of Exp. 2, is confirmed by the results of this experiment. The level or effectiveness of inhibition declines as the distance from the hypostome increases, being high distally and low proximally.

It is apparent that the subhypostomal region and the proximal digestive zone behave very differently when transplanted to the basal disc of intact animals ($P < 0.001$ by $\chi^2$ test for difference in numbers of secondary axes produced). This difference in behaviour under the same, constant, inhibiting conditions implies that the two regions have different thresholds for inhibition. The threshold of the subhypostomal region (producing 65% hypostomes) is higher than that of the proximal digestive zone (5% hypostomes), which in turn is higher than that of the basal disc (produces no hypostomes). In other words the threshold for inhibition, like the level of inhibition, declines as the distance from the hypostome increases. As with the time for hypostome determination, it seems permissible to postulate the existence of axial gradients in properties which give rise to a gradient in threshold for inhibition by a hypostome and to a gradient in the level of inhibition.

**GENERAL DISCUSSION**

The main conclusions to be drawn from the above experiments are that an existing hypostome inhibits the formation of a new hypostome. The level or effectiveness of inhibition declines as the distance from the hypostome increases, being high distally and low proximally.
The results with regard to inhibition confirm those of Rand et al. (1926). The most important new concept is that inhibition is a threshold phenomenon; regions differ in the amount or level of 'inhibitor' required to prevent hypostome formation. Distal regions have higher thresholds than proximal regions. The fact that the level of inhibition falls when the hypostome is removed and, as will be shown in the next paper, rises when the new hypostome is formed, suggests that inhibitor (if it is a substance) is continually produced by the hypostome and continually lost, inactivated or destroyed by the rest of the system. Presumably in the intact animal the rate of production is equal to the rate of loss, inactivation or destruction and an equilibrium is attained with a constant level of inhibition. The observation that the level of inhibition decreases with distance from the hypostome is in accord with this view. The dynamic control of hypostome formation provided by such a system, is in fact, a necessary feature of a regulative system, since, if a missing part is to be replaced, some mechanism must inform the rest of the system of its absence (Webster & Wolpert, 1966b).

It is important to realize that hypostome formation occurs as a result of a
release from inhibition. This mode of control implies that it is a spontaneous and autonomous process. These properties should be contrasted with those of regions whose differentiation is dependent on inductive influences.

A schematic representation of the hypothetical relationships between level of inhibition and threshold in the intact animal is depicted in Fig. 3. The 'curves' are drawn linear and parallel for simplicity and the slope is, of course, arbitrary. The fact that the proximal digestive zone produced one case of hypostome formation has been taken to mean that the level of inhibition in the basal disc is at approximately the threshold level for this region. This is perhaps a rather tentative conclusion in view of the small number of experiments performed, but it constitutes the only available information on the relative values of inhibitory level and threshold for any region and for this reason has been used in constructing the diagram. Even if this conclusion is inaccurate, it is clear that the level of inhibition must always be well above the threshold, otherwise transplantation of any distal region (high threshold) to a slightly more proximal position (lower level of inhibition) would result in release from inhibition and hypostome formation. This has not been observed.

<table>
<thead>
<tr>
<th>Source of graft</th>
<th>Transplanted to</th>
<th>Host hypostome</th>
<th>Level of inhibition in relation to threshold</th>
<th>Behaviour of graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subhypostome</td>
<td>Digestive zone</td>
<td>Present Above</td>
<td>Absorbed</td>
<td>Hypostome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absent Below</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subhypostome</td>
<td>Basal disc</td>
<td>Present Below</td>
<td>Hypostome</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absent Below</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal digestive zone</td>
<td>Digestive zone</td>
<td>Present Above</td>
<td>Absorbed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absent ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal digestive zone</td>
<td>Basal disc</td>
<td>Present Above/below</td>
<td>Absorbed/hypostome</td>
<td></td>
</tr>
</tbody>
</table>

It will already be clear from the above discussion that the interaction between threshold and level of inhibition is believed to control hypostome formation. If the level of inhibition is above the threshold of a region then the formation of a hypostome will be prevented. Conversely, if a region is released from inhibition as a result of a fall in the level of inhibition below the threshold, then a hypostome will be formed. As a simple illustration of how these concepts can be used to explain the behaviour of a region, the results of the transplantation experiments performed so far are summarized in Table 4 and interpreted in terms of inhibition levels and thresholds.

The table is self-explanatory with the exception of the case of the proximal digestive zone transplanted to the digestive zone in the absence of the host.
hypostome, where the relationship between inhibitor level and threshold is queried. With the information available it is not possible to decide whether the proximal digestive zone is released from inhibition when the level of inhibition falls in the digestive zone and then inhibited when the new hypostome forms (from the subhypostomal region) or whether the level of inhibition always remains above the threshold for this region. This raises the whole question of what may be called the dynamics of regulation, which is the subject of the next paper in this series.

It will be apparent that throughout the above discussion it has been assumed that the level of inhibition in a transplanted piece becomes adjusted relatively quickly to that characteristic of its new position. However, the piece is assumed to retain its characteristic threshold properties for a somewhat longer period of time. There is no evidence to support these assumptions, but they are necessary for any satisfactory explanation of the transplantation experiments. The stability of threshold is the object of experiments to be reported in the next paper in this series.

It is now possible to consider the regulation of a hydra into a distal hypostomal region and a proximal non-hypostomal region in terms of the concepts of inhibition and threshold and time for hypostome determination.

Consider the case where the hypostome only is removed from a hydra. For simplicity, it will be assumed that the gradients in threshold and level of inhibition are linear and parallel (Fig. 3). When the hypostome is removed, the level of inhibition falls, and we will assume that it falls below all thresholds throughout the system. All regions begin forming hypostome, but hypostome determination occurs more quickly in distal than in proximal regions. The new hypostome at the distal end produces inhibitor either as it is forming or immediately after it has become determined and it is resistant to inhibition. When inhibition is restored to a level above all thresholds, hypostome formation will cease, and the original pattern is restored.

In the above explanation, time for hypostome determination is considered to be an independent factor and it is in fact the gradient in time that determines the polarity of the system. However, it appears that there is also a gradient in threshold and it is possible that it is this gradient that could determine the polarity. Moreover, it can provide an explanation for the gradient in time.

As pointed out in the previous paper (Webster & Wolpert, 1966a), one possibility for the origin of the observed differences in time for hypostome determination is that all regions start from the same point, develop at the same rate, but differ in the time at which development is initiated. The interaction between level of inhibition and threshold could provide a basis for such a mechanism. If we postulate that the gradients in level of inhibition and threshold differ in slope such that regions differ in the extent to which the inhibitor level is above the threshold, then, when the hypostome is removed, and assuming that the level of inhibition falls at the same rate throughout the animal, different
regions will be released from inhibition at different times (Fig. 4). This is a very attractive possibility because the time difference is regarded as something arising from the relative values of the other two factors, not as an independent factor. Unfortunately there is no evidence to support this suggestion, but use of it is made later in a speculative model for proportionate regulation.

The above interpretations of changes in level of inhibition after removal of the hypostome should only be regarded as giving a general indication of how these may operate. A detailed explanation requires knowledge of the dynamics of the system. We require details of the changes in level of inhibition, possibly of threshold, and also of time for hypostome formation after removal of the hypostome. This will be considered in the next paper. It is also evident that the gradients in level of inhibition and threshold could provide an explanation for many of the numerous grafting experiments that have been carried out on hydra. This will be deferred until the dynamics have been investigated.
Relation to work on other hydroids

In other hydroids, particularly Tubularia, the inhibitory aspects of dominance and inhibition of hydranth reconstitution have been the subjects of much experimental work (Barth, 1938; Child, 1941; Rose & Rose, 1941; Steinberg, 1954, 1955; Tardent, 1960, 1963). A supposed inhibitory interaction during the individuation of the parts of the hydranth has been investigated by Rose (1955, 1957a, 1963). The wealth of data available indicates that the situation in Tubularia and other morphologically elaborate hydroids is probably complex and reference will only be made to those results which are immediately relevant. The most important points are: (1) the hydranth in Tubularia can inhibit the reconstitution of other hydranths; (2) the inhibition declines in effectiveness with increasing distance from the hydranth; (3) the time for hydranth formation increases disto-proximally. These ideas have lead to attempts to explain the maintenance and alterations in polarity in terms of rate of regeneration and inhibition. In general terms, these concepts are similar to those proposed for hydra above. The main difference, however, is the introduction of the concept of threshold with its connotation of all-or-none inhibition. It should be noted, for example, that work of Tubularia has usually dealt with inhibition in terms of its effect on rate of regeneration (Tardent, 1963).

The present experiments demonstrate that in the absence of the hypostome the subhypostomal region will form a new hypostome irrespective of whether this region is situated at the distal end of the animal or not. In contrast, the proximal digestive zone, whether in situ or transplanted elsewhere in the digestive zone, does not form a hypostome, irrespective of whether a hypostome is present or absent, as long as more distal regions are present. This is presumably true of all regions proximal to the subhypostomal region.

Very similar results were obtained by Child (1932, 1941) working on Corymorpha. Lateral grafts of distal regions of the stem, in the absence of the host hydranth, produced secondary axes in a large number of cases but pieces from more proximal levels were less effective. Child interpreted his results in terms of his theory of axial gradients and dominance; a region of high ‘activity’ from a high distal level of gradient being able to dominate and organize lower gradient levels. The difference between host and graft was assumed at the time of interaction to be purely quantitative. In view of the arguments put forward (Webster & Wolpert, 1966a) that the primary step in distal reconstitution in all hydroids is the formation of a distal organizer (hypostome) it is felt that Child’s results can be explained in exactly the same terms as those used for the results with hydra, i.e. the transformation of the graft into a hypostome followed by the induction of distal structures and a secondary axis. The fact that quite a large proportion of the grafts from proximal levels produced secondary axes in the presence of more distal regions, as compared with hydra where all the proximal pieces are absorbed (inhibited), is probably related to the fact that in
Corymorpha organization is much more labile and subject to environmental influence than in hydra (bipolar reconstitution is common—Child, 1941). An organizer can, under certain conditions, form from a region which is not the most distal, e.g. a simple incision in the stem made at a distal level will produce a secondary axis in many cases, provided that the hydranth has been removed (Child, 1932).

The results of transplanting distal and proximal regions of the stem of Corymorpha to various levels of the axis in the presence and absence of the host hydranth led Child (1932) to conclude that the lower the level of implantation, the greater the effect of a graft of given origin with regard to the production of a secondary axis. This is clear evidence of a decline in the effectiveness or level of inhibition along the axis. Child’s results also suggest that the distal and proximal regions of the axis have different thresholds; at any given level of implantation a graft from a distal region was more effective than one from a proximal in causing the formation of a secondary axis. The situation in Corymorpha therefore appears to be very similar to that in hydra, and encourages the belief that the concepts developed for hydra may have a general significance.

The problem of the bud

In view of the suggestion that a hypostome will only form when the level of inhibition is below the threshold, one is forced to consider the formation of a new hypostome during budding. It is a question of great interest whether there is any similarity between the processes involved in the formation of a hypostome during budding and the formation of a hypostome during regulation. There is good evidence that as soon as a bud appears its tip possesses organizing properties. When transplanted it will induce a bud (Li & Yao, 1945), that is, it induces the formation of a complete miniature hydra which detaches. This behaviour should be contrasted with the behaviour of a hypostomal graft in which only tentacles and a short distal axis are induced. Typical hypostomal properties are acquired by the tip of the bud later, at about the time the bud forms its own tentacles. Another difference between the early bud organizer and the hypostome is the distance over which inhibition of hypostome formation can occur. As was shown in Exp. 2, a hypostome can inhibit in a proximo-distal direction over a distance equal to that between the budding zone and the hypostome. A bud, however, cannot inhibit over such a distance, although there is some evidence that a new bud may be able to inhibit distal regeneration from the digestive zone over a much smaller distance (Lenhoff, 1957). There are thus important differences between an early bud and a hypostome; in its early stages a bud may only possess the ability to reorient the host material to produce a new axis and only later develops typical hypostomal properties.

The experimental technique used in this investigation would not reveal whether the formation of a hypostome in regenerating animals involves a sequence of events similar to that involved in the formation of a hypostome.
during bud development. The early stages of hypostome formation (‘bud-type organizer’ determination) would not be detected by this method when grafts were made to the digestive zone, since all host animals were actively budding. However, many grafts were made to the basal disc, and in these cases there was never any sign of the graft inducing the development of a bud. This is by no means conclusive evidence, but it suggests that the formation of a hypostome does not involve a ‘bud-type organizer’ stage.

**SUMMARY**

1. The factors controlling hypostome formation in hydra have been investigated using transplantation techniques. Two factors have been demonstrated, (a) inhibition of hypostome formation and (b) threshold for inhibition.
2. The presence of a hypostome inhibits the formation of another hypostome. This confirms the results of other workers.
3. Inhibition can act in either a disto-proximal or a proximo-distal direction.
4. The level of inhibition is lower in proximal than in distal regions, i.e. the level or effectiveness of inhibition declines as the distance from the hypostome increases. An axial gradient in level of inhibition is postulated.
5. The difference in behaviour of regions from different axial positions when transplanted to the basal disc, i.e. when subjected to identical inhibiting conditions, indicated that regions have different thresholds for inhibition. Distal regions have higher thresholds than proximal regions; an axial gradient in threshold is postulated. The idea of a threshold for inhibition is a new concept as far as regulation in hydroids is concerned.
6. The interaction between threshold and level of inhibition is believed to control hypostome formation. Polarized regulation of hydra into a distal hypostome and a proximal non-hypostomal region is discussed in terms of these concepts.
7. The results are discussed in relation to those obtained by other workers on other hydroids. Re-interpretation of these results suggests that inhibition and threshold are controlling factors in the regulation of other hydroids.

**RÉSUMÉ**

*Études sur la régulation chez l’hydre. II. Facteurs contrôlant la formation de l’hypostome*

1. Les facteurs contrôlant la formation de l’hypostome chez l’hydre ont été étudiés au moyen de techniques de transplantation. Deux facteurs ont été démontrés, (a) inhibition de la formation de l’hypostome et (b) seuil de l’inhibition.
2. La présence d’un hypostome inhibe la formation d’un autre hypostome. Ceci confirme les résultats d’autres auteurs.

4. La force de l'inhibition est plus faible dans les régions proximales que dans les régions distales, c'est-à-dire que l'effet inhibiteur diminue au fur et à mesure que la distance à partir de l'hypostome augmente. L'existence d'un gradient axial des inhibitions est considérée.

5. La différence de comportement des fragments provenant de différentes régions axiales et greffés sur le disque basal, c'est-à-dire soumis à des conditions inhibitrices identiques, indique que ces régions ont des seuils différents d'inhibition. Les régions distales ont des seuils plus élevés que les régions proximales; l'idée d'un gradient axial de seuil est proposée. L'idée d'un seuil d'inhibition est un nouveau concept dans les cas de la régulation chez les hydroïdes.

6. L'interaction entre seuil et force d'inhibition est considérée comme contrôlant la formation de l'hypostome. La régulation polarisée de l'hydre en un hypostome distal et une région proximale non-hypostomale est discutée à la lumière des ces idées.

7. Les résultats sont discutés en relation avec ceux obtenus par d'autres auteurs sur d'autres hydroïdes. Une ré-interprétation de ces résultats suggère qu'inhibition et seuil sont des facteurs contrôlant la régulation d'autres hydroïdes.

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REFERENCES


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