Studies on the transmission of hypostome inhibition in hydra

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SUMMARY

The inhibition of hypostome formation in hydra has been investigated using isolation and transplantation techniques.

The transmission of inhibition does not seem to be polarized; a hypostome situated at the proximal end of the digestive zone can inhibit distal regeneration with no change in regeneration polarity. The time required for the proximal hypostome to establish an effective inhibitory 'field' is about 4–7 h. The results confirm that inhibition and polarity are quite separate factors.

The transmission of inhibition by a digestive zone is impaired by treating the region with media containing reduced concentrations of Ca²⁺ and Mg²⁺. This treatment has no effect on the inhibitory activity of the hypostome, nor on the resistance of the tissue to inhibition.

The effect of divalent cations on the transmission of inhibition does not seem to be due to an effect on cell adhesion. There is an effect on general cell permeability but this is not temporally correlated with the effect on transmission.

It is suggested that transmission of inhibition is dependent upon contact-mediated intercellular communication, possibly involving functional coupling between cells.

INTRODUCTION

Previous studies have indicated that the formation of the hypostome in hydra is controlled by an inhibitory mechanism resulting from the action of a present or pre-existing hypostome in conjunction with a threshold mechanism (Webster, 1966a, b).

The present paper is concerned with the mechanism of inhibition and in particular with how inhibitory 'information' is transmitted through the system. In a previous paper (Webster, 1966a) it was shown that a hypostome grafted to the proximal end of a digestive zone could inhibit hypostome regeneration from the distal end, and it was tentatively concluded that the flow of inhibitory 'information' was not one-way or polarized as Rose (1957a) suggested was the case in Tubularia. However, these previous experiments were not entirely satisfactory since it was not shown that the polarity of the digestive zone remained unaltered during the course of the experiment. We have therefore

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returned to this experimental situation and investigated the transmission of inhibition, in relation to polarity, in more detail.

At present the inhibition of hypostome formation can only be discussed in operational terms, and we have no information on events at the cellular level. It is clear that intercellular communication of some kind must be involved and in this paper we report experiments on the effect of divalent cations on the transmission of inhibition which shed some light on the nature of the communication process.

MATERIALS AND METHODS

*Hydra littoralis* were used for all experiments. Details of culture methods and some of the transplantation techniques are given in Webster & Wolpert (1966). In all experiments actively growing animals were used 18–24 h after feeding.

Studies on the inhibition of hypostome formation were made using proximal hypostome grafts. Grafts were prepared as follows: 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 by threading the sections of animals on to fine glass rods and holding the pieces in contact with each other by means of two blocks of silicone rubber. Tissues generally healed together within 1–2 h. The hypostome and tentacles of the host animal were removed at various periods of time after the initial grafting operation.

Graft combinations were incubated at 26 °C and examined for signs of distal regeneration of the host after 24 and 48 h. Animals which showed no sign whatsoever of tentacle regeneration at these times were judged to be completely inhibited; animals which produced a single apical tentacle (comparable to a type 3 axis described in Webster & Wolpert, 1966) were judged to be partially inhibited; animals which produced two or more tentacles in the normal sub-apical position were judged to be not inhibited.

Experiments to determine whether or not there had been changes in the regeneration polarity of completely inhibited animals were performed by removing the grafted hypostome and tentacles after 24 and 48 h. In order to identify the original polarity of the digestive zone, the tissue was lightly stained at either the distal or the proximal end after re-cutting, using an agar block dyed with Nile blue sulphate.

Some experiments were carried out to investigate the effect of divalent cations (Ca$^{2+}$ and Mg$^{2+}$) on the transmission of inhibition from proximal hypostome grafts. Normal hydra medium ('M') contains 0·001 M-Ca$^{2+}$ and 0·0001 M-Mg$^{2+}$. Preliminary experiments showed that the animals would not tolerate the removal of all the Ca$^{2+}$ and Mg$^{2+}$ from the medium and under such conditions disintegrated within a few hours. However, hydra survived and regenerated normally in a medium containing 1 % of the normal concentration of Ca$^{2+}$ and Mg$^{2+}$ (i.e. 0·00001 M-Ca$^{2+}$ and 0·000001 M-Mg$^{2+}$). This medium,
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which we will refer to as Ca-Mg-depleted ‘M’, was made iso-osmotic with normal ‘M’ by the addition of NaCl. Culturing and transplantation operations were performed in Ca-Mg-depleted ‘M’ and the animals subsequently cultured at 26 °C in the same medium unless otherwise stated. Details of treatment and graft combinations used will be given below.

We have carried out some simple experiments in an attempt to determine whether the Ca-Mg-depleted ‘M’ has any effect on the general permeability of hydra and, therefore, whether the failure of inhibition which is observed under these conditions could be due to leakage of some inhibitory substance into the medium. Hydra were cultured at a density of five animals/ml in normal ‘M’ and in Ca-Mg-depleted ‘M’. After 24 h the supernatant medium was removed, filtered through a Millipore filter and its ultraviolet absorption spectrum recorded on a Cary 15 recording spectrophotometer. The same animals were cultured for a second period of 24 h in fresh medium and the procedure repeated.

RESULTS

Inhibition of hypostome regeneration by a proximal hypostome graft

Proximal hypostome grafts were made as described above and graft and host allowed to heal together for periods of 0, ½, 1, 2 and 3 h when the host hypostome and tentacles were removed. Graft combinations were kept and examined for signs of distal regeneration: results are shown in Table 1. It should be noted that distal regeneration from this level of the axis in normal ungrafted animals always occurs within 18-24 h of hypostome removal.

Table 1. Effect of a proximal hypostome graft on distal regeneration

<table>
<thead>
<tr>
<th>Time after grafting when host hypostome removed (h)</th>
<th>Distal end of host 24 h after grafting</th>
<th>No. not inhibited</th>
<th>No. partially inhibited</th>
<th>No. completely inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
<td>15</td>
<td>3</td>
<td>2 (10 %)</td>
</tr>
<tr>
<td>½*</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>6</td>
<td>1</td>
<td>6 (46 %)</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>7</td>
<td>5</td>
<td>8 (40 %)</td>
</tr>
<tr>
<td>3</td>
<td>182</td>
<td>58</td>
<td>0</td>
<td>124 (68 %)</td>
</tr>
</tbody>
</table>

* After ½ h grafts were not completely healed and removal of host hypostomes probably disturbed the graft-host relationship in some cases. This may account for the absence of any inhibition as compared with animals at 0 h.

It can be seen from Table 1 that a proximal hypostome can inhibit distal regeneration in experimental animals after 24 h—thus confirming the previous observations—and that the degree of inhibition, as judged by the number of animals affected, is dependent upon how long the graft has been in contact with the host before the host hypostome is removed. Contact for 0–½ h is insufficient
to establish inhibition but after contact for 1–3 h maximum inhibition is obtained. This indicates that a hypostome situated at the proximal end of the digestive zone can establish inhibition at the distal end relatively quickly.

It should be noted that at 48 h some proximal hypostome grafts begin to separate from the hosts. After 72 h some of the animals which were inhibited at 24 h have now regenerated and by 120 h only about 35 % (40 out of 117) of the animals remain completely inhibited.

At 24 and 48 h some of the animals which were completely inhibited were cut to remove the grafted hypostome and the tentacles and also the inhibited distal tip as a control for the cut at the proximal end. One end of the axis was stained with Nile blue sulphate so that its polarity was known, and the pieces were allowed to regenerate. All the animals (except two) regenerated with their original polarity (Table 2): the hypostome developed at the original distal end and the peduncle at the original proximal end. This result clearly shows that a hypostome situated at the proximal end of an axis can inhibit hypostome formation at the distal end without producing any other detectable change in this region or in the proximal region, i.e. without altering the regeneration polarity of the axis.

Table 2. 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>Reversed polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The effect of divalent cations on the transmission of inhibition

We have examined the effects of divalent cations on the transmission of inhibitory ‘information’ in hydra in the hope of gaining some insight into the mechanisms involved. The experimental situation used was that described above: a hypostome grafted to the proximal end of the digestive zone.

Graft combinations of the following type were made (‘untreated’ means that the tissues came from animals grown in normal ‘M’; ‘pretreated’ means that the tissues came from animals cultured for 24 h in Ca–Mg-depleted ‘M’):

(a) Untreated hypostome grafted to pretreated digestive zone.
(b) Pretreated hypostome grafted to pretreated digestive zone.
(c) Pretreated hypostome grafted to untreated digestive zone.
(d) Untreated hypostome grafted to untreated digestive zone.
(e) Untreated hypostome grafted to untreated digestive zone, cultured in ‘M’ with normal Ca²⁺ and Mg²⁺.

Graft combinations were kept and examined for signs of distal regeneration after 24 h: results are shown in Table 3.
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It can be seen that pretreatment of the host-digestive zone with Ca–Mg-depleted ‘M’ reduces the inhibitory effect of the proximal hypostome: compare (a) and (b) with controls (e). The difference between the results of (a) and (b) and those of (e) is significant ($P < 0.01$) using the $\chi^2$ test. On the other hand, pre-treatment of the hypostome with Ca–Mg-depleted ‘M’ has no significant effect on the inhibitory properties of this region: compare (c) with (d) or (e). It would therefore seem that the reduction in the degree of inhibition at the distal end of the host axis is brought about by some change in the properties of the host caused by Ca$^{2+}$ and Mg$^{2+}$ depletion. This is borne out by comparing (a) or (b) with (c) in Table 3; these results are significantly different by the $\chi^2$ test ($P < 0.01$).

Table 3. Effect of treatment with Ca–Mg-depleted ‘M’ on inhibition by proximal hypostome grafts

<table>
<thead>
<tr>
<th>Graft combination</th>
<th>No. of grafts</th>
<th>No. of hosts inhibited after 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Untreated hypostome + pretreated host</td>
<td>10</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>(b) Pretreated hypostome + pretreated host</td>
<td>13</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>(c) Pretreated hypostome + untreated host</td>
<td>6</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>(d) Untreated hypostome + untreated host (cultured in Ca–Mg-depleted ‘M’)</td>
<td>13</td>
<td>10 (77%)</td>
</tr>
<tr>
<td>(e) Untreated hypostome + untreated host (cultured in ‘M’)</td>
<td>30</td>
<td>18 (60%)</td>
</tr>
</tbody>
</table>

These changes in the host digestive zone are observed most clearly using a 24 h pretreatment with Ca–Mg-depleted ‘M’ and observing the animals 24 h after grafting. If those animals which have regenerated at this time (those in which inhibition has failed) are cut to remove the regenerated hypostome and tentacles and then replaced in Ca–Mg-depleted ‘M’, the majority (ca. 80%) do not show any sign of regeneration 24 h later, i.e. the proximal hypostome is now able to exert an inhibitory effect. This suggests that after 48–72 h in Ca–Mg-depleted ‘M’ the digestive zone has regained its normal properties. This, we feel, is most probably due to the animals concentrating Ca$^{2+}$ and Mg$^{2+}$ from the medium. Two further observations confirm that the change in properties is a transitory phenomenon. In case (d) Table 3, neither graft nor host was pretreated but the graft combination was cultured in Ca–Mg-depleted ‘M’. After 24 h ten animals were inhibited, as recorded in Table 3, but by 48 h seven of these animals had regenerated, i.e. after 48 h in the presence of low Ca$^{2+}$ and Mg$^{2+}$ the inhibition present at 24 h had disappeared. A further experiment (not recorded in Table 3) involved pretreating the digestive zones of host animals for 48 h and then grafting an untreated hypostome; in this case virtually all the animals (11 out of 12) did not regenerate: the digestive zone had completely normal properties. We presume that in this case, as in the first experi-
ment, loss of normal properties occurred during the first 24 h and these were restored during the second 24 h.

We emphasize these results in order to make it clear that loss of normal properties with respect to inhibition as a result of Ca$^{2+}$ and Mg$^{2+}$ depletion is a transient event, and that any demonstration of a requirement for divalent cations in the inhibitory process requires very careful timing of experiments.

The change in the properties of the host axis revealed by this experiment could be either (i) an increase in the resistance of the host subhypostomal region to inhibitory influence, i.e. an elevation of threshold for inhibition, or (ii) a reduction in the ability of the axis to transmit inhibitory 'information'.

Table 4. Effect of depletion of Ca$^{2+}$ and Mg$^{2+}$ ions on the threshold for inhibition of the subhypostomal region

<table>
<thead>
<tr>
<th>Subhypostomal region transplanted into</th>
<th>No. of transplants</th>
<th>Assimilated</th>
<th>Induced hypostome and tentacles</th>
<th>Induced peduncle and basal disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact host</td>
<td>12</td>
<td>11</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Host minus hypostome and tentacles</td>
<td>13</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

We have attempted to distinguish between these alternative explanations by examining the effect of Ca$^{2+}$ and Mg$^{2+}$ depletion on the transplantation behaviour of subhypostomal regions. Intact hydra were cultured in Ca–Mg-depleted 'M' for 24 h and subhypostomal tissue from these animals transplanted into the digestive zone of intact hosts or hosts from which the hypostome and tentacles had been removed. Hosts were cultured in Ca–Mg-depleted 'M'. Results are shown in Table 4: tissues transplanted into intact hosts were assimilated in all cases but one. This indicated that the resistance of the treated region to inhibition has not been increased since untreated subhypostomal regions normally behave in this way (Webster & Wolpert, 1966). In the case of transplantation to hosts minus hypostome and tentacles about 50 % of the transplants induced secondary distal structures and the remainder were either assimilated or induced secondary proximal structures. Untreated subhypostomal regions transplanted in a similar way induce secondary distal structures in about 70 % of the cases (Webster, 1966a). These results therefore confirm that there has been no increase in resistance to inhibition and suggest that resistance may in fact be lowered by depletion of Ca$^{2+}$ and Mg$^{2+}$ ions.

These observations indicate that the effect of Ca$^{2+}$ and Mg$^{2+}$ depletion on the inhibitory influence of a proximal hypostome is not caused by a change in the resistance of the host axis to inhibition. It therefore seems reasonable to conclude that the effect is on the ability of the host axis to transmit inhibition.
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The results of the experiments carried out to determine whether Ca–Mg-depleted ‘M’ has an effect on the general permeability of hydra are shown in Table 5. It is clear that during the first 24 h hydra in Ca–Mg-depleted ‘M’ leak very much more u.v.-absorbing material into the medium than do control hydra cultured in normal ‘M’. This is presumably due to a general increase in cell permeability in the presence of reduced concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) ions. However, in the second period of 24 h the leakage from animals cultured in Ca–Mg-depleted ‘M’ is identical to that from control animals and is, in fact, reduced compared with that occurring during the first 24 h period. There is therefore no correlation between leakage of u.v.-absorbing materials and ability to transmit inhibition, since, it will be remembered, animals transmit normally during the first 24 h in Ca–Mg-depleted ‘M’ but transmission is impaired in the second 24 h period.

Table 5. Ultraviolet absorption of supernatant from animals cultured for 24 and 48 h in normal ‘M’ and Ca\(^{2+}\)-Mg\(^{2+}\)-depleted ‘M’

<table>
<thead>
<tr>
<th>Animals cultured in</th>
<th>Supernatant from 1st 24 h in culture. Optical density at 260 m(\mu)</th>
<th>Supernatant from 1st 24 h in culture. Optical density at 280 m(\mu)</th>
<th>Supernatant from 2nd 24 h in culture. Optical density at 260 m(\mu)</th>
<th>Supernatant from 2nd 24 h in culture. Optical density at 280 m(\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ‘M’</td>
<td>0.040</td>
<td>0.030</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Ca–Mg-depleted ‘M’</td>
<td>0.33</td>
<td>0.25</td>
<td>0.15</td>
<td>0.13</td>
</tr>
</tbody>
</table>

DISCUSSION

The results obtained with the proximal hypostome grafts confirm and extend those obtained previously (Webster, 1966a) and indicate that inhibition of hypostome formation can occur in a proximo-distal direction as well as in the normal disto-proximal direction.

We have observed a certain amount of variability in the present experiments in the degree of inhibition obtained with proximal hypostome grafts compared with those previously reported, and the proportion of animals inhibited at 24 h —about 70%—was also lower than in previous experiments when all the animals were inhibited. However, in the present experiments we have used many more animals than previously, and we are confident that the results obtained reflect the true nature of the situation. This variability may in part account for the results of Hicklin, Hornbruch & Wolpert (1969), who performed an apparently identical experiment but failed to obtain distal inhibition in any of their animals. These authors do not discuss the discrepancy between their results and those reported previously by us (Webster, 1966a), nor do they give details of the number of animals employed. Their failure to obtain distal inhibition is presumably due to some difference in experimental technique.

1 Note added in proof. Hicklin, Hornbruch & Wolpert inform us that they are now able to obtain inhibition by a proximal hypostome using the procedure described in this paper.
The most important extension to the original observations on inhibition by a proximal hypostome is the demonstration that this can occur without any change in the regeneration polarity of the host axis. Since this polarity is, at present, the only sort of polarity definitely known to exist in the system, these observations remove the element of doubt present in the previous experiments that the grafted hypostome might have repolarized the system. That such repolarization does occur is indicated by further experiments reported in another paper (Wilby & Webster, 1970), but this does not take place until some considerable time after inhibition is established at the distal end of the host axis.

As noted above, in the present experiments only 70% of the subhypostomal regions were inhibited at 24 h. This may be compared to the situation in which a subhypostomal region is transplanted to the proximal digestive zone of an intact host (i.e. a situation in which inhibition must act in a normal disto-proximal direction), in which all the transplants were inhibited (Webster, 1966a; Webster & Wolpert, 1966). This difference in the effectiveness of hypostome inhibition in the two experimental situations might indicate that inhibition moves less readily in a proximo-distal direction than it does in a disto-proximal direction. It is clear, however, that there is no absolute restriction on proximo-distal transmission of inhibition and the different results may well be due to the fact that, in the case of the proximal hypostome grafts, the transplanted hypostome has to establish communication with the host and establish a new inhibitory field, whereas in the case of the subhypostomal transplant the region in question is subjected to extant and stable inhibitory influence.

We therefore conclude that there is no reason to assume the existence in hydra of a polarized or one-way flow of inhibitory 'information' such as has been postulated by Rose (1957a, b) in Tubularia.

These observations also indicate that a region can be prevented from developing into a hypostome and yet remain unchanged in its 'potential' for hypostome formation, and that those properties which determine potential for hypostome formation, referred to in previous papers as 'time-threshold' properties (Webster 1966a, b) are relatively stable. This observation is compelling evidence in favour of the assumption, previously made but until now unproven, that the inhibitory situation in a region can change relatively quickly but that the 'time-threshold' properties of a region are retained for a longer period of time. The results also, of course, confirm that regulation involves an interaction between at least two factors, the 'potential' for hypostome formation and the inhibition of hypostome formation.

The results of the experiments on the time course of the establishment of inhibition indicate that inhibitory 'information' is transmitted relatively quickly from the proximal hypostome to the distal end of the host axis. The results showed that a delay of 1–3 h was required in order to obtain significant inhibition of distal regeneration in the host. Taking into account the fact that about 4 h is required for hypostome determination from the distal end of the host axis
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(Webster & Wolpert, 1966) it is evident that the proximal hypostome must establish a threshold level of inhibition at the distal end at some time between 4 and 7 h after it is placed in contact with the proximal end.

It seems probable that this time is made up of the separate times required for at least two different processes to occur: (a) the time required for the grafted hypostome to establish functional contact with the host tissue, i.e. the ability to interact and communicate with it; (b) the time required for the transmission of inhibition.

The results obtained by treating hydra with Ca–Mg-depleted ‘M’ provide further information on the nature of the tissue interactions and allow some speculation about the mechanism whereby inhibitory ‘information’ is transmitted.

Our results demonstrate that a reduction in the Ca$^{2+}$ and Mg$^{2+}$ content of the medium significantly impairs the inhibitory effect of a proximal hypostome graft, and that this result is brought about by some change in the properties of the host axis, since the ability of the hypostome to inhibit is unaffected. The resistance of the subhypostome region of the host axis to inhibitory influences (i.e. its threshold) is not increased by depletion of Ca$^{2+}$ and Mg$^{2+}$ ions and may even be lowered and it therefore seems probable that the change in the host axis is a change in its ability to transmit inhibition. The results cannot be accounted for in terms of an effect on the establishment of functional contact between grafts and host since the combination of untreated hypostome plus pretreated host behaves very differently from the combination of pretreated hypostome plus untreated host.

The fact that the transmission of inhibition by the digestive zone can be experimentally impaired in this way indicates very clearly that transmission occurs through the cells of the body wall of the animal and not through the enteron. This confirms previous, unpublished, experiments by G. Webster which suggested that inhibition does not occur by the free diffusion of some substance, but that intimate contact between the interacting tissues is required. For example, hypostomes placed within the enteron of host animals do not inhibit distal regeneration; also, isolated hypostomes cultured with decapitated animals in microdrops of medium do not inhibit the regeneration of the latter.

It is well known that Ca$^{2+}$ and Mg$^{2+}$ ions have profound effects upon the adhesive properties of cells (Curtis, 1967) and changes in adhesion between cells might be expected to impair contact-dependent communication. However, under our experimental conditions there is no evidence to indicate that the adhesive properties of the cells are altered since there is no sign of cell disaggregation and tissues from treated animals can be grafted together as readily as can those from untreated ones.

At present, the nature and cause of the inhibitory effect in hydra is unknown. The simplest hypothesis is that inhibition is caused by some substance. Although there is no satisfactory evidence for such a substance in hydra (Webster, 1970),
there is some evidence (not entirely satisfactory) for the presence of such substances in *Tubularia* (Rose, 1967). If such a substance is present in hydra, the depletion of Ca$^{2+}$ and Mg$^{2+}$ ions might interfere with inhibition by altering the general permeability (see Davson, 1959) of the animals so that the substance leaked away into the medium, with the result that a threshold concentration could not be established.

Our observations on animals placed in Ca-Mg-depleted 'M' indicate that leakage of ultraviolet absorbing materials is increased under these conditions. These observations, however, are difficult to reconcile with those on the transmission of inhibition. During the first 24 h in Ca-Mg-depleted 'M', hydra leak more material into the medium than do control animals in normal medium but, it will be remembered, are able to transmit inhibition normally. During the second 24 h in Ca-Mg-depleted 'M' leakage also occurs, though to a lesser extent, but these animals have a significantly reduced ability to transmit inhibition. There is thus no temporal correlation between the general leakiness of the hydra and their ability to transmit inhibition, and we do not believe that this hypothesis can account satisfactorily for our results.

Recently, Loewenstein and his colleagues (Loewenstein 1966, 1967) have shown that intercellular communication involving functional coupling between cells is profoundly influenced by divalent cation concentration. The results reported in this paper make it tempting to speculate that the transmission of inhibition in hydra may be dependent upon functional coupling between cells. At present there is no direct evidence for functional coupling between hydra cells. The presence of functional coupling seems a reasonable possibility, however, since the layers of the body wall of hydra are typical epithelia closely comparable to the epithelial systems in which functional coupling has been demonstrated. More significantly, hydra cells are bound together by septate desmosomes (Wood, 1959), which are a characteristic feature of many of the epithelial systems in which functional coupling occurs and have been considered as a possible site for coupling (Loewenstein, 1966; Bullivant & Loewenstein, 1968). It may be noted that the time required for the establishment of inhibition in hydra (4-7 h) is consistent with the idea that the establishment of functional contact between graft and host and the transmission of inhibition are dependent upon the establishment of functional coupling between cells. In other systems in which coupling has been studied in detail, intercellular communication is established within about 1-4 h of cell contact and substances diffuse very rapidly within coupled cell systems (Loewenstein, 1966, 1967; Loewenstein & Penn, 1967).

The development of our tentative hypothesis on the mechanism of transmission of inhibition must await electrophysiological studies on hydra comparable to those carried out on other epithelial systems.
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RÉSUMÉ
Etudes sur la transmission de l'inhibition de l'hypostome chez l'Hydre

L'inhibition de la formation de l'hypostome a été étudiée en utilisant des techniques d'isolements et de transplantations.

La transmission de l'inhibition ne semble pas polarisée; un hypostome situé à l'extrémité proximale de la zone digestive peut inhiber une régénération distale sans aucune altération de la polarité de régénération. Le temps requis pour qu'un hypostome établisse un 'champ' effectif d'inhibition est de l'ordre de 4 à 7 h. Ces résultats confirment que l'inhibition et la polarité sont des facteurs complètement séparés.

La transmission de l'inhibition par une zone digestive est affaiblie en traitant cette région par des milieux à contenance réduite de Ca²⁺ et de Mg²⁺. Ce traitement n'affecte pas l'activité inhibitrice de l'hypostome, ni la résistance du tissu à l'inhibition.

L'effet de cations divalents sur la transmission de l'inhibition ne semble pas être due à un effet sur l'adhésion cellulaire. Il existe une action sur la perméabilité cellulaire en général mais elle ne présente pas de corrélation chronologique avec l'effet sur la transmission.

Il est suggéré que la transmission de l'inhibition dépend de communications intercellulaires résultant de contacts qui, éventuellement, peuvent impliquer des couplages fonctionnels entre les cellules.

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REFERENCES


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