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# The interaction of the organizing regions in hydra and its possible relation to the role of the cut end in regeneration

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## SUMMARY

Regeneration in hydra does not necessarily involve cell division, so that the problem of pattern regulation is essentially one of finding the processes by which a given population of cells are assigned appropriate functions in the correct positions. The restoration and nature of the organizers or 'dominant regions' must also be explained.

Lateral transplants of the two different organizer tissues (hypostome and basal disc) into the digestive regions of host animals unexpectedly antagonize one another's inductive ability, suggesting that the heretofore separately studied distal and proximal developmental fields may in fact be interconnected. A model based on this idea, incorporating the extinction results with classical findings, leads to the hypothesis of a role for leakage from the cut surface as a trigger for the regenerative process. This is tested using a ligature technique in which the cut surface is minimized when the hypostome is removed, and the degree of regeneration is found to be significantly lowered, in agreement with prediction.

These results are discussed in relation to 'positional information' and 'special substance' models for pattern formation.

## INTRODUCTION

The regulative capacity of the axial pattern of hydra, the polarity of regeneration, and the ability of elements of the pattern to act like classical embryonic organizers, have been the subjects of intensive study in recent years (reviewed in Webster, 1971) though the phenomena were recognized much earlier (Peebles, 1897; Browne, 1909). Regeneration in hydra does not necessarily involve cell division (Hicklin & Wolpert, 1973) so that the problem of pattern regulation is formally one of discovering the mechanisms by which the population of cells which remain after a portion of the animal is removed are assigned appropriate functional roles in the correct relative positions. A whole functioning organism must result from whatever processes are set in motion. Tied in with the regulation of pattern as a general effect is the restoration of those specific regions, the hypostome and the basal disc (Fig. 1 A), which are unique in their ability to induce partial secondary axes when grafted into host animals.

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Recent experimental work using microsurgical grafting and transplantation techniques has revealed characteristic defect-signalling factors, variously interpreted as organizer inhibitors or activators, apparently distributed as axial gradients with their maxima at the organizing regions. These factors are rapidly changing, in that their levels in a given region can be altered within a few hours. In addition, associated slowly changing competences or thresholds in responsivity to the latter signals have been found, also distributed as gradients along the length of the animal (Webster & Wolpert, 1966; Webster, 1966*a*, *b*; MacWilliams & Kafatos, 1968). Perturbations in the pattern of hydra with various metabolites or metabolic inhibitors (Webster, 1967; Corff & Burnett, 1969; Hicklin, Hornbruch & Wolpert, 1969; Newman, 1973) take on their developmental meanings only in relation to their effects on the phenomenological gradients.

Models accounting for both regeneration and transplantation results have been put forward. Very briefly, those using the concepts of inhibition, activation, and induction have relied on a postulated dynamic equilibrium among cell types, which when disturbed and restored provokes the reformation of organizing or boundary regions as one effect among several (Webster, 1966*b*; Burnett, 1966; Gierer & Meinhardt, 1972). Alternatively, those models using the conceptual framework of positional information have assigned priority to the re-establishment of boundary regions; when this has been accomplished intermediate regions are assigned functions at positions determined in relation to these boundaries (Wolpert, Hicklin & Hornbruch, 1971; Wolpert, 1971). Polar transport has also been suggested as the primary basis of pattern regulation in hydra (Wilby & Webster, 1970), though this has been disputed recently (Gierer *et al.* 1972). Any choice among these possibilities clearly requires detailed study at a variety of levels of organization.

The present work is part of an attempt to further specify the developmental processes in hydra by adducing constraints which narrow the range of possible underlying mechanisms. Evidence is presented that the two organizing regions interfere with one another's abilities to induce secondary structures when transplanted in combination with each other. As previous models have not considered the possibility of organizer–organizer interactions, a new model is proposed, which in turn requires the postulation of a role for the cut end in normal distal and proximal regeneration. This hypothesis is then tested in another experiment whose result is consistent with the model.

## MATERIALS AND METHODS

All experiments were carried out using *Hydra attenuata* grown from a clone supplied by Professor L. Wolpert. Culture methods followed were similar to those used for *Hydra littoralis* by Webster & Wolpert (1966), except for the culturing temperature, which was 17 °C in the present experiments. Animals were fed *Artemia* nauplii three times weekly, and washed after feeding. The medium ('M' solution) was identical to that used for *H. littoralis*.

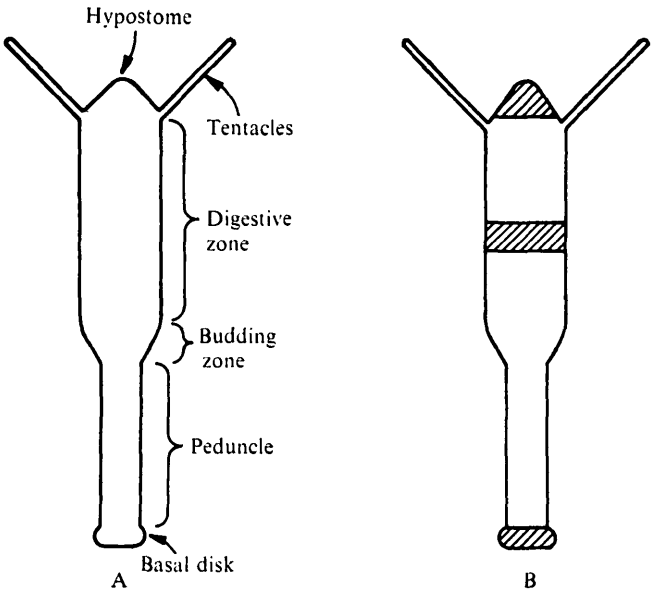


Fig. 1. (A) Diagram of hydra showing principal regions of the axis (adapted from Webster & Wolpert, 1966). (B) Regions used for transplantation experiments.

Actively growing animals, possessing one or two buds, were chosen for experiments approximately 24 h after feeding, and were not fed during the course of an experiment.

*Operative procedures*

(*a*) *Transplantation experiments.* 'Double transplantations' were performed using the lateral grafting technique described by earlier workers (Browne, 1909; Webster & Wolpert, 1966). The technique was adapted in the following manner: Pieces to be transplanted were obtained by isolating rings from the appropriate levels of intact animals (Fig. 1 B) and splitting these up into smaller fragments. These pieces were partially macerated with the cutting needle and inserted either individually or in combination into small incisions made in the mid-digestive zone of a host animal. Because of the difficulty of performing the double transplantation, animals having undergone these grafts were screened 1–2 h after the operation; those animals in which the transplanted tissue had become detached or otherwise lost during this period were discarded.

Experimental animals were kept in an incubator at 26 °C in Petri dishes containing 'M' solution, and were examined every 24 h for three days, after which final tabulations were made.

Inductions of secondary axes were similar to those described by previous workers (Browne, 1909; Webster & Wolpert, 1966). Webster & Wolpert's Types 1, 2 and 3 distal inductions in which a portion of axis was present as well as distal structures, are referred to as 'positive distal inductions' and scored

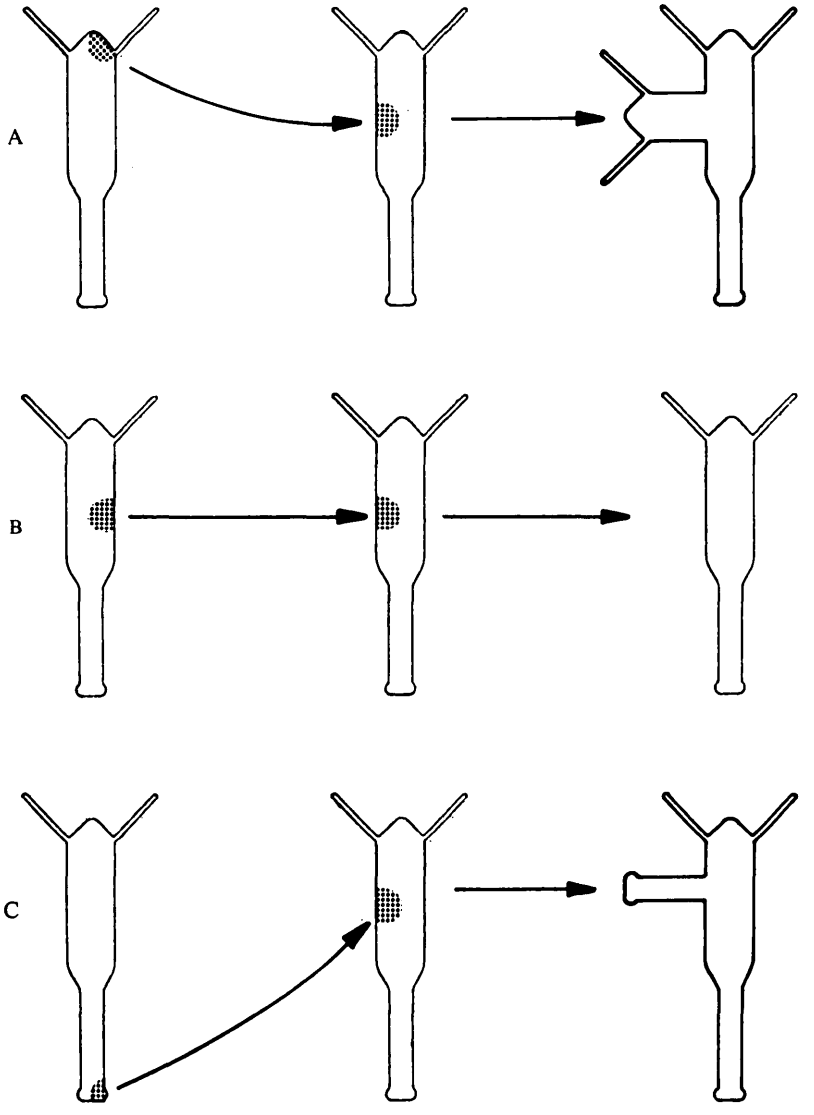


Fig. 2. Diagram illustrating some main results obtained from 'single' lateral grafts. (A) A piece of head will induce a secondary distal axis when grafted into the mid-digestive zone of an intact hydra. (B) A similarly grafted piece from the digestive zone will be absorbed. (C) A similarly grafted piece of basal disc will induce a secondary proximal axis.

together in the final tabulations; the corresponding proximal inductions are similarly scored. Inductions of a single tentacle (Webster & Wolpert's Type 4) or of a sticky patch with no secondary axes are referred to as 'weak distal or proximal inductions' where they are present at 72 h, and scored accordingly. The most common results for 'single transplantations' as reported in the literature and confirmed in preliminary experiments, are given in Fig. 2.

Table 1. *Inductions resulting from double transplantations of tissue from various sources*

| Sources of graft | No. of successful grafts | No. and type of positive inductions | No. and type of weak inductions | No. failing to induce |
|---|---|---|---|---|
| Hypostome–basal disc | 14 | 2 (D) | 4 (D)+3 (P) = 7 | 5 |
| Hypostome–digestive region | 9 | 8 (D) | 1 (D) | 0 |
| Digestive region–basal disc | 11 | 11 (P) | 0 | 0 |

D = distal, P = proximal.

(*b*) *Ligature experiments.* Animals were tied just below the hypostome with a blond human hair pulled just until it began to stretch, incubated at 26 °C, and examined every 24 h.

## EXPERIMENTS AND RESULTS

*Experiment* 1. *The interaction of the organizing regions.* Tissue from three sources – hypostome cone (head), digestive region, and basal disc (foot) (Fig. 1 B) – were excised from donor animals and partly macerated with a tungsten needle. Pieces corresponding in size to about half a hypostome cone were grafted within 15 min in pairs into a small incision made in the mid-digestive zone of a host animal in the following combinations: hypostome–basal disc, hypostome–digestive region, digestive region–basal disc. Care was taken that both pieces of tissue were in contact with each other, partly buried in the incision and partly protruding from the host's surface. Grafts were counted as successful if this situation still obtained 1–2 h later when some healing had taken place.

Results are summarized in Table 1.

The tabulated results show an unexpected mutual extinction of inducing ability in the combined hypostome–basal disc transplantations. The results for the other sets of double grafts are consistent with expectations based on earlier results (see Fig. 2). In these cases, digestive region, not having inducing ability of its own, appears to enter more or less inertly into the inductive process (Fig. 3).

The pattern of extinction in the mixed organizer grafts is also of interest. The proportion and distribution of those that succeeded in inducing (43 % distal, 21 % proximal, of which 77 % were otherwise rare weak inductions) along with the proportion which failed to induce (36%) point to the existence of a characteristic 'extinction mixture', though exact proportions are hard to ascertain. Since an attempt was made to keep all grafted pieces of tissue the same size (by visual comparison), it might appear that the results indicate a greater intrinsic 'inductive strength' for the hypostome than for the basal disc; both cases of positive induction for hypostome–basal disc combination were of the distal type, as were four out of seven weak inductions. However, the sample size is too

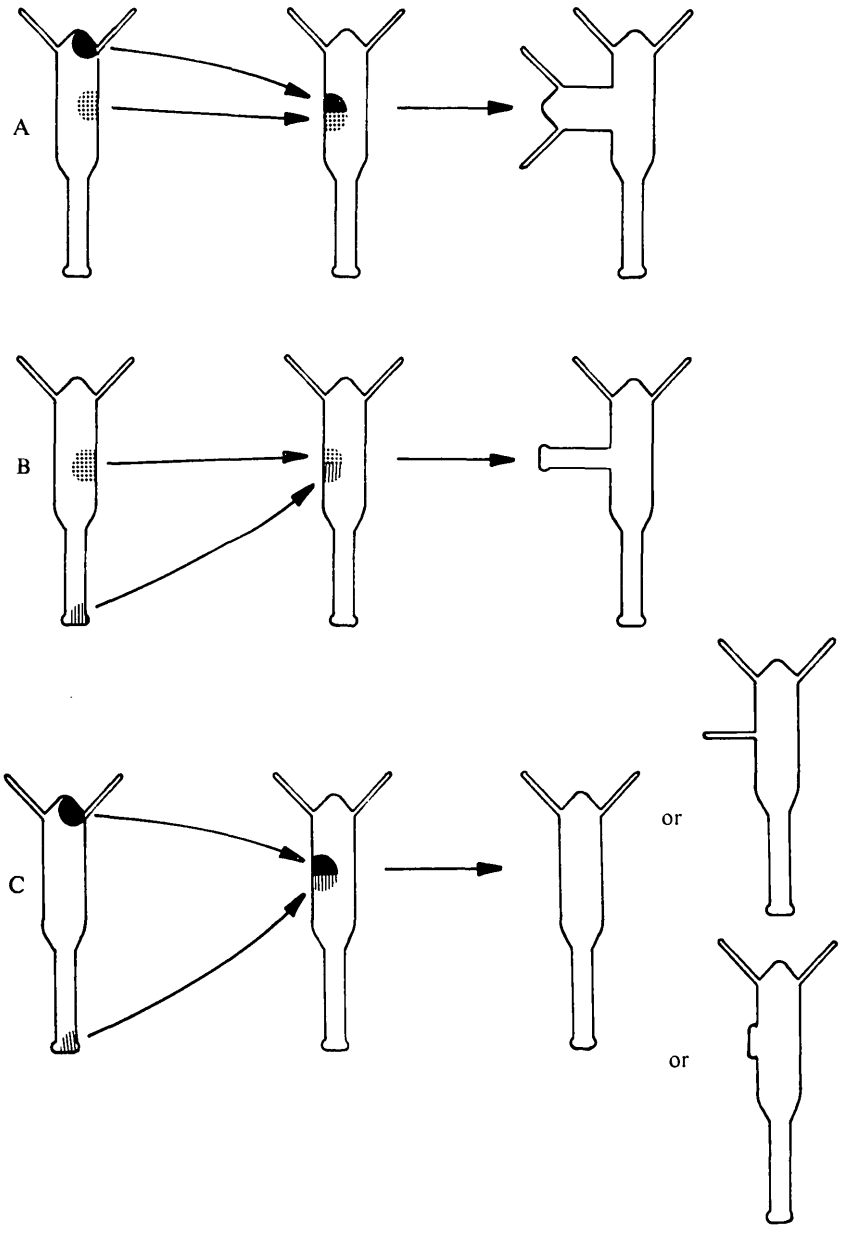


Fig. 3. Diagram illustrating main results obtained from 'double' lateral grafts (Expt. 1). (A) Hypostome–digestive zone combination grafted laterally to host's mid-digestive region gives distal induction. (B) Basal disc–digestive zone combination gives proximal induction. (C) Hypostome–basal disc combination gives weak distal induction, no induction or weak proximal induction.

small to warrant this conclusion. Furthermore, the constitution of the basal disc is such that samples of it are likely to contain a larger proportion of dead material than an equivalent amount of hypostome tissue (Lentz, 1966).

*Discussion of results of Experiment* 1. Though the formation of distal and proximal organizing regions in hydra have been investigated separately (Webster & Wolpert, 1966; Webster, 1966*a*, *b*; MacWilliams & Kafatos, 1968) the formal results in each case have been similar in that a slowly varying 'threshold' (Webster, 1966*a*; MacWilliams, Kafatos & Bossert, 1970), or 'positional value' (Wolpert, 1971) which is graded along the length of the animal has been postulated for each subsystem, along with a much more rapidly varying and also graded 'inhibitor' (Webster & Wolpert, 1966; MacWilliams & Kafatos, 1968), 'signal' (Wolpert, 1971), or 'activator' (Gierer & Meinhardt, 1972). The results of Expt. 1 indicate a constraining relationship between the two subsystems and allow us to make a set of inferences about the nature of the processes involved:

(1) Whether or not the effect on hypostome and basal disc tissue of being combined in a double graft is ultimately to alter their corresponding slowly varying or 'threshold' factors ($S_D$ and $S_P$, distal and proximal respectively) it is clear that the initial failure to induce must be due either to an interaction between the rapidly varying factors ($r_D$ and $r_P$) or a cross reaction between opposite slowly varying and rapidly varying factors ($S_D \rightarrow r_P$; $S_P \rightarrow r_D$). The slowly varying factors themselves are presumed not to be directly communicable by diffusion between bits of tissue (Wolpert, 1971).

(2) If we make the plausible inference that the result of the tissue–tissue interaction is to 'proximalize' the distal bit, and 'distalize' the proximal bit (that is, transform them to intermediate values of the $r$'s and/or $S$'s which render them incapable of induction) a schematic model can be outlined which integrates the previously studied distal and proximal subsystems of hydra in a way consistent with the results of Expt. 1 (Fig. 4A).

(3) In the proposed model $m_1$ and $m_2$ are assumed to be diffusible head and foot activators respectively, each exercising its function when its absolute concentration in a given section of tissue exceeds appropriate values ($T_1$ and $T_2$). $E_1$ is the distributed source of $m_1$ and sink of $m_2$, while $E_2$ is the distributed source of $m_2$ and sink of $m_1$. Lastly, $m_1$ and $m_2$ cause the formation of $E_2$ and $E_1$ respectively, when they fall below appropriate values ($T_3$ and $T_4$). The *production* and *destruction* expressions for $m_1$ and $m_2$ are assumed to be linear or quasi-linear in $E_1$, $E_2$, $m_1$, and $m_2$, whereas the processes of head and foot determination and induction of $E_1$ and $E_2$ will clearly depend in a non-linear way on $m_1$ and $m_2$, in so far as they are threshold phenomena. The separate distal and proximal slowly and rapidly varying factors $S_D$, $S_P$, $r_D$, $r_P$, considered above are now subsumed into the two vectors ($E_1$, $E_2$) and ($m_1$, $m_2$) which take on their values as functions of position along the hydra's axis. The polarity of any piece of tissue is defined by the slopes of its ($E_1$, $E_2$) distribution.

(4) The results of Experiment 1 are accounted for in the following way: hypostome tissue with a high value of $E_1$, a low value of $E_2$, a suprathreshold ($T_1$) value of $m_1$ and a low value of $m_2$, is brought into proximity with basal disc tissue with a high value of $E_2$, a low value of $E_1$, a suprathreshold ($T_2$) value of

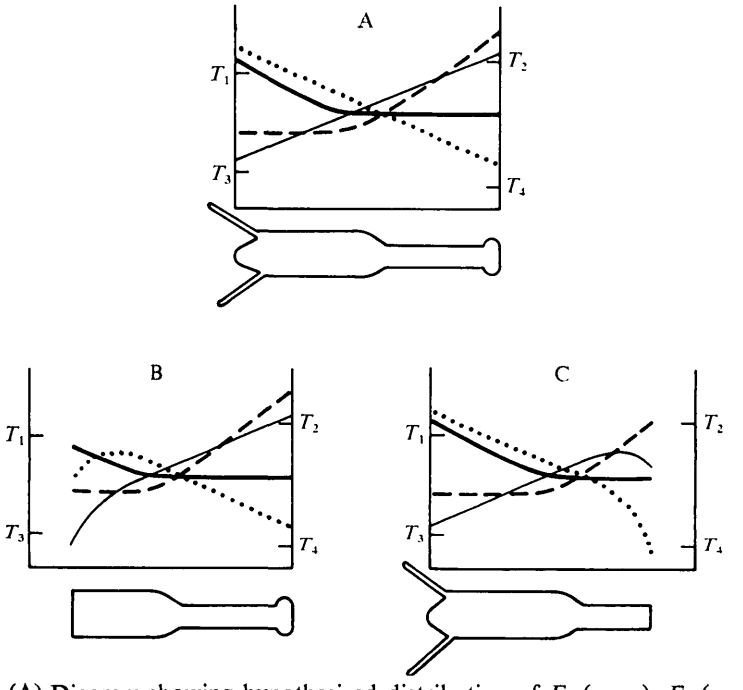


Fig. 4. (A) Diagram showing hypothesized distribution of $E_1$ (——), $E_2$ (– – – –), $m_1$ (. . . . .) and $m_2$ (——) in an intact hydra (see text). (B) Removal of hypostome causes distal leak of $m_1$ and $m_2$, which has the effect of depressing $m_2$ below threshold $T_3$, a level not attained in the intact animal. (C) Removal of basal disc causes proximal leak of $m_1$ and $m_2$, depressing $m_1$ below $T_4$.

$m_2$ and a low value of $m_1$, in a double transplantation into the mid-digestive zone of a host animal. The value of $m_1$ in the distal piece is depressed below $T_1$ by the high level of its sink in the adjacent proximal piece. Similarly, $m_2$ in the proximal piece is lowered below $T_2$, and neither tissue is capable of inducing the appropriate structures.

(5) The simple model presented here can account for many of the classical results on hydra pattern regulation – for example, those involving the induction of secondary distal and proximal structures (Browne, 1909) as well as certain effects previously interpreted in terms of inhibition (Webster & Wolpert, 1966; Webster, 1966*a*, *b*). This being the case, it is of interest to pursue the model in its present minimal form somewhat further. For a satisfactory account of regeneration a subsidiary, testable hypothesis must be made concerning processes taking place at the cut distal or proximal end. The following experiment, therefore, is strongly suggested by the form of the model, and depending on its outcome, can provide circumstantial evidence for or against models of this form.

*Experiment* 2. *The role of the cut end in regeneration.* The model of the preceding section is required to explain normal distal and proximal regeneration and does so by the synthetic effect stimulated by sub $T_3$ and $T_4$ levels of the rapidly changing factors $m_1$ and $m_2$. Simple removal of head or foot tissues alone is not

Table 2. *Degree of regeneration in tied and cut hydra*

| Method of head removal | 48 h | 72 h |
|---|---|---|
| Tied (total no. = 24) | 15 (F)+5 (S)+4 (N) | 17 (F)+5 (S)+2 (N) |
| Cut (total no. = 25) | 23 (F)+2 (S) | 23 (F)+2 (S) |
| Tied (total no. = 15) | 7 (F)+4 (S)+4 (N) | 9 (F)+3 (S)+3 (N) |
| Cut (total no. = 15) | 14 (F)+1 (S) | 14 (F)+1 (S) |

F, full regeneration; S, single tentacle regeneration; N, no regeneration.

Table 3. *Increase in number of tentacles during regeneration in tied and cut hydra*

| Method of head removal and time elapsed | No. of animals with $N$ tentacles | | | | | | Mean no. tentacles |
|---|---|---|---|---|---|---|---|
| | $N = 0$ | 1 | 2 | 3 | 4 | 5 | |
| Tied (24 h) | 4 | 14 | 1 | 1 | 0 | 0 | 0·95 |
| Cut (24 h) | 1 | 5 | 7 | 6 | 1 | 0 | 2·05 |
| Tied (48 h) | 2 | 5 | 1 | 4 | 6 | 2 | 2·65 |
| Cut (48 h) | 0 | 2 | 1 | 1 | 12 | 4 | 3·75 |
| Tied (72 h) | 1 | 5 | 0 | 2 | 6 | 6 | 3·25 |
| Cut (72 h) | 0 | 2 | 0 | 0 | 8 | 10 | 4·20 |

Total: 20 animals cut, 20 tied.

sufficient to create these subthreshold levels; for this it is necessary to invoke leakage of $m_1$ and $m_2$ from the open surfaces which are created by the excision of these tissues. Both factors presumably leak from both ends; however, only at the distal end will $m_2$ fall below threshold, and similarly for $m_1$ at the proximal end (Fig. 4B, C). The purpose of the present experiment was to determine the effect on regeneration of minimizing the area of the cut surface. Only distal end regeneration was looked at, as the characteristic structures (tentacles, hypostome cone) are easier to recognize and quantify than is the basal disc.

Hydra were tied tightly with a human hair just proximal to the ring of tentacles and placed in 'M' at 26 °C. Control animals were decapitated with a razor blade and incubated under the same conditions. The ligated animals were screened over the next few hours to determine that the hypostome ultimately detached: only those animals for which this occurred between 1 and 4 h after tying were retained. In these cases the distal-most surface of the animal appeared smooth at 20× under the dissecting microscope.

Results of two separate experiments in which animals were manipulated as described and observed at 24 h intervals are given in Table 2.

For the purposes of Table 2, 'full regeneration' is defined as the presence of two or more tentacles (always accompanied by a hypostome) at the time of observation, while 'single tentacle regeneration' is indicated where one tentacle

was grown from the center of the distal surface in lieu of a mouth opening, hypostome cone, and normal ring of tentacles.

The tabulated results indicate a qualitative diminution in regenerative ability where the animal was ligated rather than cut. In particular, at 72 h after head removal, by which time the pattern had almost invariably stabilized, a small proportion of the tied animals had always failed to regenerate, a situation never found in the controls. Furthermore, the proportion of single tentacle regenerates was significantly greater in the tied animals (21 % *vs.* 8 %). When failures to regenerate are combined with single tentacle regenerates, and tied and cut animals are compared, the percentages are 33 % *vs.* 8 %.

However, a better quantitative estimate of the diminution of regenerative ability in the ligated animals can be obtained (Table 3) by following the time course of tentacle formation during regeneration (Schaller, 1973).

The differences in the mean number of tentacles are highly significant ($P < 0{\cdot}0005$) at 24 h and still significant ($P < 0{\cdot}05$) at 48 and 72 h when Student's $t$ test is applied.

*Discussion of results of Experiment* 2. The hypothesis of a role for leakage from the cut end as the evoker of regeneration is not bound to the model presented in the last section, but arises from any description which postulates a *distributed* production of the diffusible factors which signal a pattern defect and makes use of the quasilinear assumption of the previous section. (However, see Gierer & Meinhardt (1973) for a *non-linear* distributed source model which does not require leakage.) Where highly localized sources of 'inhibitor' (Webster & Wolpert, 1966; Wolpert *et al.* 1971) or 'positional signal' (Wolpert, 1971) are postulated, the level of this factor drops below a critical threshold simply by virtue of removal of source tissue (hypostome, basal disc) from the system. In quasilinear distributed source models such as that presented here, axial production of the signalling factor continues after the removal of any portion of tissue, suggesting that the open surface itself may play a role in depressing (or otherwise altering) its value.

It should be noted that I am not asserting that an open surface is necessary for all secondary head and foot formation in hydra. According to the model, the formation of these structures is strictly dependent on the concentrations of $m_1$ and $m_2$ respectively exceeding certain threshold values $T_1$ and $T_2$ in a given piece of tissue. Regardless of the mechanism that brings about the suprathreshold value of one of these variables, the appropriate structure is expected to form precisely where that value is attained. The model is therefore entirely consistent with experiments which demonstrate hypostome or basal disc determination in unwounded tissue (see Webster, 1971, for such examples). Leakage from cut surfaces does not by itself create these suprathreshold values of the inducing agents; rather, it is postulated to set off a chain of events whose final result is the exceeding of the threshold by one of the $m$'s and the consequent induction of hypostome or basal disc.

Experiment 2 makes use of the fact that the surface area available for the escape of a diffusible signal is ideally reduced to a small hole at the center of the distal end of the animal when the head detaches from the partially healed ligature constriction. The relative rates of healing and the escape of signal presumably determine whether a single tentacle or nothing at all will regenerate from this hole.

The following observations on hydra which eventually regenerated after being ligated may help explain how a leakage mechanism might be involved even after the open end has ostensibly healed:

The smooth, healed distal ends of these animals would swell over the period of about one day to several times their original size, apparently under internal pressure from the digestive cavity. If the hydra had a well-developed bud, the swelling process would not occur until after the bud had detached. For the next day or so, tentacles would then form, distributed randomly over the swollen distal surface; the swelling would decrease, and the tentacles would regulate into a ring around the newly formed hypostome cone.

On the basis of the hypothesized role of leakage from an open surface, I would interpret this sequence of events in the following manner:

Non-budding, decapitated hydra with completely healed distal surfaces are topologically distinct from intact animals, in that in the former the ectoderm forms an unbroken surface, effectively isolating the stomach from the external medium. Any internal–external pressure differentials cannot equalize in the normal way and lead to swelling if the inner pressure exceeds the outer. This pressure leads to local breaks in the swollen surface, allowing leakage to occur and tentacles to form at these sites. The eventual appearance of an oral opening (perhaps by a similar mechanism) puts an end to this process and possibly influences the arrangement of the tentacles.

A possible criticism of the assay used in Experiment 2 is that *morphogenesis* (i.e. hypostome and tentacle formation) is looked at, whereas *determination* (i.e. ability to induce a hypostome and tentacles in an intact host) is clearly the key event under investigation. An argument might be that the former is interfered with by the ligature, while determination occurs just as quickly in the tied as in the cut animals. I think that this is unlikely to be true for the following reasons: (1) The ligature ceased to act directly on the animal at a maximum of 4 h after it was applied. The average delay in regeneration, however, was of the order of days. (2) The unbroken surface left behind by the ligature is unlikely to be a deterrent to morphogenesis if the appropriate inductive stimuli are present. This is shown by both the formation of buds, and by experiments of Wilby & Webster (1970) in which full hypostomes and the normal number of tentacles form from the unbroken surface under appropriate conditions. (3) In the marine hydroid *Tubularia* ligation is also found to inhibit distal regenerative ability (Rose & Rose, 1941). This effect was overcome in ligated distal ends whose normal electronegativity was restored by application of an electric field (Rose, 1970),

indicating that in this hydroid at least, a tied surface is no deterrent to morphogenesis if the physiological conditions associated with determination are present.

## GENERAL DISCUSSION

The axial pattern of hydra appears to be organized into two distinct developmental fields, each having its own 'dominant region' (Huxley & DeBeer, 1934). The results of the first experiment presented here indicate that these dominant regions are mutually antagonistic in their roles as organizers of secondary axes, and suggest the possibility of interconnections in the fields themselves and in the processes they govern. It is worthwhile systematizing what is known of the dynamics and constraints of the pattern variables in the simplest reasonable manner, both as a starting point for further investigations at the level of pattern, and as a means for suggesting possible molecular mechanisms for the phenomena.

The model presented along with the results of Expt 1 is not proposed as an 'explanation' for the pattern-forming process in hydra, but as a formal representation of certain classical results on the distal and proximal fields in which the abstract components are integrated in a way consistent with the extinction phenomenon. Its usefulness is as a logical summary of previous experiments and a suggestor of new ones; as such it is one tool in the investigation.

Part of the economy of this particular model is the use it makes of continuously distributed sources and sinks of hypothetical head and foot activating substances. A strong advantage of this proposal is the resultant ease with which experimental concepts such as 'threshold' and 'competence' are thereby interpreted. The analogy with Lawrence, Crick & Munro's (1972) model for the pattern of the epidermis in the insect *Rhodnius* should also be noted. Here a given tissue's ($E_1$, $E_2$) value plays the role of a 'remembered' variable which provides the dynamic conditions for the homeostatic equilibration of ($m_1$, $m_2$) around a characteristic mean (Newman, 1972).

Evidence for a diffusible head-activating substance which is very specific and active at extremely low concentrations has been presented recently (Schaller, 1973). This substance has been shown to be largely structure-bound in membranous particles that could presumably act as its sources under certain conditions (Schaller & Gierer, 1973). These particles are graded distoproximally along the animal's axis. While there is nothing to directly link these factors with the microsurgically studied rapidly and slowly changing developmental variables (Webster & Wolpert, 1966; Webster, 1966*a*, *b*; MacWilliams *et al.* 1970; Wilby & Webster, 1970; Wolpert *et al.* 1971) their correspondence seems reasonable in the context of the present model. Gierer & Meinhardt (1972) also draw on this evidence in formulating their distributed source model for the distal field of hydra. However, their use of inhibitory variables and non-linear kinetics makes their treatment not directly comparable with the one presented here.

The 'leak hypothesis' which arises from the model appears to be confirmed in

some form by the results of Expt 2. It need not be assumed that the interrelationship of the developmental variables is accurately portrayed in Fig. 4 to conclude that there is a strong case that the *escape* of a diffusible substance, rather than the excision of its localized source (Wolpert, 1971), is responsible for signalling the defect. In this regard it is noteworthy that Schaller & Gierer (1973) report a measurable drop in head activating activity 4–8 h after the onset of head regeneration, the substance being released from the regenerating surface into the surrounding medium. This is precisely the period during which determination takes place (Webster & Wolpert, 1966). In our scheme this drop would be predicted for $m_1$ (head activator) even though the smaller drop in the nearer-to-threshold $m_2$ would be the actual signal for determination at the distal end. At this point the existence of a diffusible foot-activating agent and its sources is entirely hypothetical, and while the techniques of Schaller & Gierer (Schaller, 1973; Schaller & Gierer, 1973) could certainly be used to look for them, the main difficulty would be the lack of a good quantitative assay for basal disc development.

The model under discussion obviously provides only a partial description of the developmental processes in hydra. Thus, while experiments concerning bud formation can certainly be formulated in this context, the model is essentially incomplete with respect to that question. A more serious problem, since it directly concerns axial regeneration, is the rigorous maintenance of polarity which is found experimentally but is not a property of the model for small pieces which do not include the 'cross-over point' of the gradients (see Fig. 4A). For these cases the model would predict mirror-image regeneration, which is not found in hydra. Some form of inhibitory effect (Webster, 1966*a*; Wolpert *et al.* 1971; Gierer & Meinhardt, 1972) is therefore likely to be necessary in addition to the postulated factors in a more complete version of the model.

Even under the constraints set by the results of Expts 1 and 2, many models, employing a variety of conceptual frameworks, could be put forward to account for the relevant observations on the pattern formation of hydra. Therefore, it would be instructive to conclude with an example of a fairly decisive point of disagreement between 'special substance' models (using concepts such as 'head activating agent') such as the one presented here and that of Gierer & Meinhardt (1972) and 'positional information' models for hydra as they have thus far been formulated (Wolpert *et al.* 1971; Wolpert, 1971).

Compare the following two situations:

(1) A slowly varying factor, 'positional value', governs differentiation in hydra in the sense that a certain set of values of this variable will be interpreted by the genome of a pluripotent cell as 'hypostome', a second set as 'digestive region', etc. Characteristic substances (inhibitors, signals) may be produced by these cells as secondary consequences of say, head differentiation, but the determining aspect in the evocation of any tissue type is the set of positional values that correspond to it.

(2) No single factor corresponds to a positional value. Tissue-specific activating substances, possibly produced everywhere, induce the appropriate differentiations when their concentrations attain critical values. Since these determinative factors are rapidly changing relative to their source and sink concentrations, their concentrations are biased by the latter but can change independently of them under certain conditions. A 'coordinate system' and its 'interpretation' are not easily separable.

The first case corresponds to existing models for hydra pattern formation in the positional information framework (Wolpert, 1969, 1971; Wolpert *et al.* 1971), while the second corresponds to the present model and that of Gierer & Meinhardt (1972).

A positional information model requires that the whole array of tissue types in a given developmental field be evoked by varying levels of a single factor. Where only a simple yes/no choice is available in the field in response to a given signal, the model should be considered as being of the induction type.

The model presented here contains no one variable which in different quantities is permissive for more than two *qualitatively different* tissue types (e.g. hypostome and not-hypostome). But unless such a factor can be demonstrated experimentally it must be concluded that the axial pattern of hydra is a specific, complex interaction between special inductive factors and quantitatively varying competences in a manner not easily schematized by separating a 'map' from its interpretation.

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