

Polarity reversal in hydra by oligomycin

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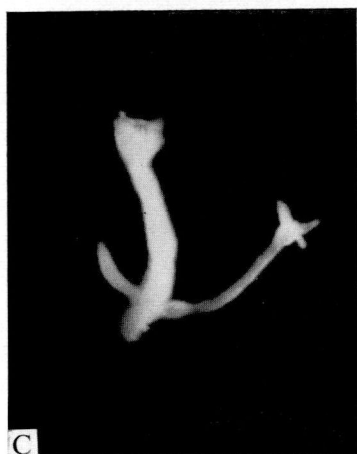
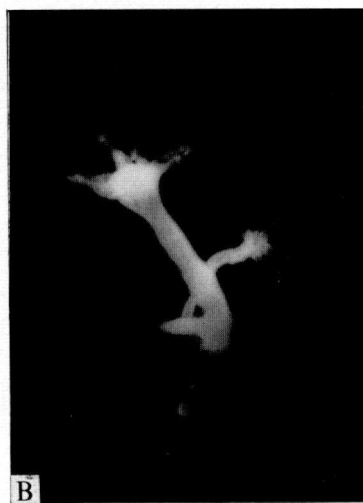
SUMMARY

Intact hydra treated for 24 h with oligomycin gradually lose their head structures and the distal ends form feet. Grafting experiments show that the distal ends of treated animals induce proximal structures.

INTRODUCTION

We have attempted to account for regeneration and pattern regulation in hydra in terms of positional information and have put forward a model involving the interaction between two gradients (Hicklin, Hornbruch, Wolpert & Clarke, 1973; Wolpert, Hornbruch & Clarke, 1974). It would be highly desirable if some way of investigating the biochemical basis of such gradients were available, the main obstacle to a direct approach at present being the lack of a suitable assay. A more oblique approach is to treat hydra with various agents in order to interfere with regeneration or regulation, in the hope that if agents with specific effects at the biochemical level produce specific developmental effects, some insight into the underlying processes may be obtained. Particular care has to be taken with such studies to ensure that the effect is specific to the agent and not just a non-specific trauma. The claim, for example, by Lesh & Burnett (1966) to have isolated a polarizing substance should be treated with caution, as Müller & Spindler (1971) have been unable to repeat their results and suggest the effects they obtained were due to a component of nematocyst toxin which causes the formation of supernumerary tentacles. Several agents, such as colchicine and low temperature (Corff & Burnett, 1969, 1970) and dithiothreitol (Hicklin, Hornbruch & Wolpert, 1969) can, however, cause regenerating hydra to form feet in place of heads at their distal ends. While it was tempting to implicate microtubules from such results (Wolpert, Hicklin & Hornbruch, 1971) we knew that quite unrelated compounds such as actinomycin D could cause regenerating distal ends when grafted to the mid-gastric region to induce proximal structures (Clarkson, 1969). We therefore investigated the stability of intact and regenerating hydra against insult by a variety of chemical agents (Wolpert *et al.* 1974) and found that varied and apparently

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unrelated compounds, such as butanol and 8-chloroxanthene, could cause foot regeneration at the head end of *Hydra littoralis*. This almost never occurred with *H. attenuata*, with which several compounds such as concanavalin A and diamide could lead to extra tentacles forming, which never happened with *H. littoralis*. It is important to note that all the agents were active at concentrations close to the lethal concentration. The single most dramatic result occurred with the inhibitor of oxidative phosphorylation, oligomycin, when applied to *H. littoralis* which converted intact head ends to feet. We have therefore studied it in more detail.

MATERIALS AND METHODS

H. littoralis were used and cultured in 'M' as in previous experiments (Hicklin *et al.* 1973). All experiments were carried out at 26 °C. The oligomycin was obtained from Sigma Chemicals and comprised 15 % oligomycin A and 85 % oligomycin B.

RESULTS

Polarity reversal of intact animals

Hydra were incubated in 10 µg/ml of oligomycin for 24 h in batches of 10–15 animals in 10 ml. After 24 h the animals were washed thoroughly in several changes of 'M' and kept in 'M' at 26 °C. The medium was changed every 24 h for 5–8 days during which time the animals were not fed. With this treatment, 80 % of the animals are found to have feet at their head end and 20 % of the animals die.

At the end of the oligomycin treatment a significant change in the appearance and behaviour of the animals was noted (Fig. 1 B). The foot no longer adheres to the substratum and the peduncle appears shorter, bulging out balloon-like over the foot. Most long tentacle buds detach during the 24 h period of the treatment but small buds are stopped in their development showing the same elongation of the gastric region as the parent. The gastric region of the animals is very elongated, the spontaneous movements of the animals are much reduced and appear slower than in untreated hydra. The reaction to touch is very much slower and the subsequent contraction is like a slow-motion replay, starting at the area of contact and spreading distally slightly more rapidly than proximally. The relaxation period is also much increased in time compared to the normal behaviour of the animal. The dome of the hypostome is clearly recognizable. The tentacles have shortened drastically (remining one of daisy petals). They

FIGURE 1

Changes in a hydra treated with oligomycin for 24 h. (A) normal hydra; (B) hydra after treatment; note tentacle regression and swelling of the body; (C) 24 h after treatment; (D) 48 h after treatment; the tentacles have disappeared from the original head end (arrow); (E) 96 h after treatment; the foot-like head end is indicated by an arrow.

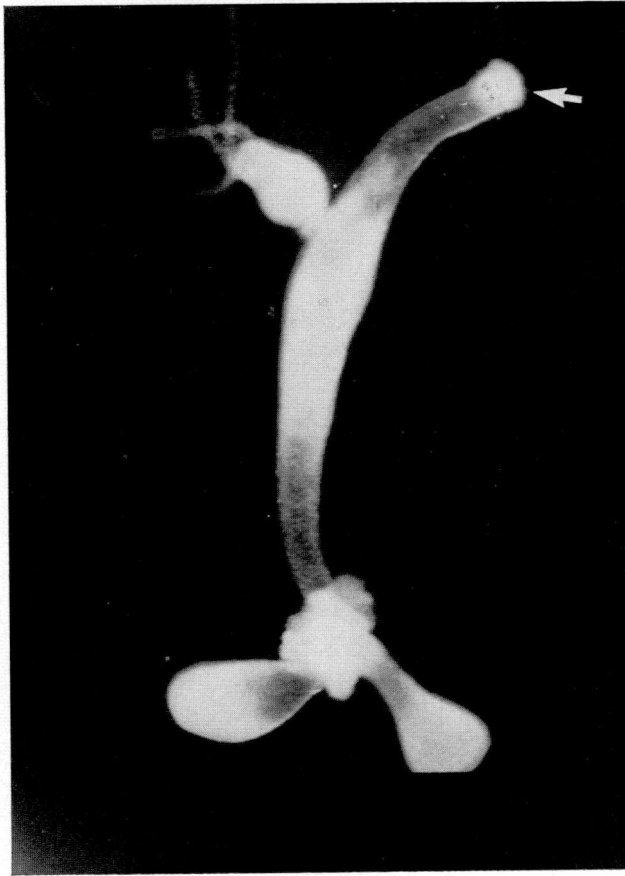


Fig. 2. A treated hydra after 8 days. The arrow indicates the foot at the original head end. Two old buds are still attached near the foot end.

are very narrow at the base, widening to a hollow sphere with or without a nipple at their most distal end. Both foot and tentacles appear pear-shaped pointing outward.

Twenty-four hours later the peduncle is further reduced in length so that the buds emerge almost at the proximal end of the axis (Fig. 1 C). Buds are not growing out and no more new buds have been initiated. The gastric region is still very elongated, and there is hardly any spontaneous movement. The response to touch is, however, almost normal, the contraction following immediately after the contact although the movement itself is still slow. The dome of the hypostome is no longer clearly identifiable and the tentacles have altogether disappeared. Most animals show a broadened ring of loosely connected tissue at their distal end, where dead and dying cells are sloughed off.

After 48 h the animals are beginning to recover. The foot does not yet attach to the bottom of the dish but the peduncle is elongating and becoming narrower. New buds are initiating. The gastric region is no longer excessively elongated,

Table 1. Behaviour of head ends of animals treated with oligomycin for 24 h and then grafted laterally into intact animals

Concn. oligomycin	Time after treatment (h)	No. of animals	Absorbed	Heads	Feet
2.5 $\mu\text{g/ml}$	0	12	—	12 (100 %)	
	24	10	—	10 (100 %)	
	48	10	1 (10 %)	8 (80 %)	1 (10 %)
5 $\mu\text{g/ml}$	0	19	8 (42 %)	8 (42 %)	3 (16 %)
	24	22	11 (50 %)	2 (10 %)	9 (40 %)
	48	10	2 (20 %)		8 (80 %)
10 $\mu\text{g/ml}$	0	30	18 (60 %)	10 (33 %)	2 (6 %)
	24	20	15 (75 %)	—	5 (25 %)
	48	29	8 (27 %)	—	21 (73 %)
	72	10	1 (10 %)	—	9 (90 %)
15 $\mu\text{g/ml}$	0	19	19 (100 %)	—	
	24	10	9 (90 %)	—	1 (10 %)
20 $\mu\text{g/ml}$	0	30	24 (80 %)	—	6 (20 %)

in fact some of the animals have regained their normal proportions. Which is to say, through the loss of a great many cells the animals are smaller than untreated animals starved for the same length of time. Several animals show a narrowing of the distal end. There is no sign of head-like structures reappearing.

Seventy-two hours after the termination of the treatment most of the animals are sticking to the dish with the original foot. The peduncle is translucent and elongated. Buds which had been stopped in their development during the treatment and recovery period are beginning to show tentacles and new buds are initiating distal to them. At the same time, new buds are initiated just proximal of the original head. The head region has turned narrow and translucent like a peduncle (Fig. 1E). Later, the animals have bipolar feet and the gastric region is divided into two budding centres (Fig. 2).

With a higher concentration of oligomycin, 20 $\mu\text{g/ml}$, an 8 h treatment gave 100 % normal animals but 15 h treatment gave 70 % reversal.

Grafting experiments

A more sensitive assay than observing polarity reversal in intact animals is to use a standard grafting technique. As shown by Browne (1909), a piece of the head end grafted into the gastric region of another animal induces a new distal axis. We have extensively used this as an assay for head-end determination (Webster & Wolpert, 1966) and have also shown that proximal regions, such as the peduncle, will induce a proximal axis (Hicklin & Wolpert, 1973). We therefore assayed the properties of the distal end by lateral grafting in order to follow changes with time and the effect of concentration. The results are shown in Table 1.



Fig. 3. A gastric region treated for 24 h after 6 days, showing feet at both ends and two buds formed in the central region.

At low concentrations of oligomycin ($2.5 \mu\text{g/ml}$) nearly 100 % of the grafts behave like a control series giving head-end inductions. With increasing concentration the number of head-end inductions immediately following treatment falls markedly, and at $15 \mu\text{g/ml}$ 100 % are absorbed, neither heads nor feet being induced. However, with time after treatment the situation changes and an increasing number of foot inductions occur. This is particularly clear with the $10 \mu\text{g/ml}$ series. At concentration of $15 \mu\text{g/ml}$ and above most animals die 24 h after the end of treatment. In most cases where the graft induced feet, the tentacles of the treated animal had fully regressed and it was not clear whether a hypostome was still present or not.

Regeneration of treated animals

The ability of treated animals to regenerate both distal and proximal structures was investigated. The animals were treated with oligomycin for 24 h and then cut at the mid-gastric region; that is, into H12 and 34B56F. Distal halves

quite often gave animals with reversed polarity – that is, with feet at each end; some animals failed to regenerate at all; other animals appeared to be normal but we do not know from which end the head came. Some proximal halves failed to regenerate and some appeared to regenerate heads; however, these may represent animals in which incipient buds take over at the distal end and do not detach.

Animals treated for 24 h with 10 $\mu\text{g}/\text{ml}$ then had their heads removed and were allowed to regenerate for 24 h. Lateral grafting of the distal tip gave 40 % absorbed (out of 10 animals) and 60 % feet inductions. If the animals were left 24 h before removing the head end 90 % foot inductions were obtained.

Some experiments were carried out by treating isolated gastric regions. Most animals died but on occasion animals with feet at each end and buds in the middle formed (Fig. 3).

DISCUSSION

Reversal of polarity can be brought about by a variety of agents. The special feature of oligomycin is that it is the first compound to exert its effect on intact animals; in the other cases where, for example, colchicine (Corff & Burnett, 1969) or dithiothreitol (Hicklin *et al.*, 1969) was used, it was necessary for the head end to be cut off. Like these other compounds, oligomycin was effective at concentrations close to the lethal concentration and there is quite a lot of cell death. Many of the cells of the head end are killed by the treatment and it may be region 1 that forms the foot end and not the hypostome. In this case the effect of oligomycin is much more like that of other agents reversing polarity, its particular effect being to chemically remove the head. This latter could be related to both increased permeability of the cells at the head end to oligomycin and their greater sensitivity to it, compared to other regions. Surprisingly, buds, even with tentacles, are much less affected by the treatment, as was found with our experiments with dithiothreitol.

Though the effect of oligomycin is very dramatic, at this stage we do not understand what mechanism is involved in this, and other cases, where feet form at the head end. One may be tempted to think that the head end is a region of high metabolic activity which is differentially affected, with respect to the foot end, by a variety of inhibitors. One may even be tempted to implicate mitochondria since these are thought to be the sites where oligomycin acts, and Gustafson (1965) has, for example, drawn attention to the possible role of mitochondria in relation to gradients in sea-urchin activity. However, one must be very cautious with such extrapolations: for example, rotenone, which is also an inhibitor of respiration and which brings about foot formation at the head end of regenerating hydra (Wolpert *et al.*, 1974), has been recently found to break down microtubules (Brinkley, Barham, Barranco & Fuller, 1974). We also need to know more about the metabolic patterns in hydra; our recent work does in fact suggest that the pentose phosphate shunt is more active at the foot end

than at the head end and that oligomycin has a significant effect on eliminating gradients of enzymes (Baquer, McLean, Hornbruch & Wolpert, 1974).

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