Morphogenetic properties of the parts of the colony of Clytia johnstoni

By L. J. Hale

From the Department of Zoology, University of Edinburgh

SUMMARY

1. The morphogenetic potencies of the various parts of Clytia johnstoni have been ascertained by isolation and by grafting.

2. The various parts of the colony behave on isolation in the following ways:
   (a) Immediate development of a stolon (which in turn may grow hydranths, as in a normal colony). Shown by (i) all parts of the stolon except sometimes the growing tip, (ii) stalk primordia of length 0.5–1.4 mm, (iii) stalks of open hydranths (polarized, the stolon grows out of the cut basal end).
   (b) Immediate differentiation of a polyp. Shown by (i) stalk primordia longer than 1.7 mm and some between 1.4 and 1.7 mm, (ii) bulbs greater than 0.28 mm diameter (i.e. had reached contractile stage), (iii) bulb primordia of all sizes having a ‘stalk’ longer than 0.35 mm; only the larger bulbs became polyps if the ‘stalk’ was shorter than 0.35 mm.
   (c) Immediate incipient differentiation (of a polyp?) was shown by some stalk primordia of length 1.4–1.7 mm long. Partial hydranth differentiation (stomach and buccal region, no tentacles) was shown by an occasional isolated bulb.
   (d) Delayed stolon development. The time delay is variable but not random, with a marked tendency to start after 3–5 days and a less marked tendency after 9 days isolation. Shown by (i) all isolates which differentiated a polyp immediately on isolation, (ii) most bulb primordia less than 0.23 mm diameter and ‘stalks’ shorter than 0.35 mm, (iii) a few bulbs 0.23–0.27 mm in diameter.
   (e) No development at any time. Shown by (i) Stalk primordia less than 0.5 mm long, (ii) bulbs of less than 0.23 mm diameter, (iii) some stolon tips, (iv) most isolated polyps (after regression).

3. There is some evidence that a contractile growth region in an isolate gives it the power of immediate differentiation, in a stalk primordium to produce a stolon and in a bulb to a polyp.

4. The time at which a stalk primordium passes to a bulb primordium appears to correspond to the time when, on isolation, it switches from being stolon producing to being hydranth producing.

5. The peaks of regenerative activity (noted in 2(d) above) are the same as the most likely life-spans of hydranths but it is not known if there is any relation between the two sets of observations.

6. The tips of growing stolons and hydranth stalk primordia have specific but weak inductive powers.

1 Author’s address: Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, U.K.
Hydranths have been favourite material for the study of the differentiation of pattern and form; by far the greatest amount of work has been carried out on *Hydra* and *Tubularia*. The former is attractive on account of the simplicity of its morphology and both animals because of the ease with which experiments may be carried out on them. The recent review of Webster (1971) assesses present knowledge of these two hydroids.

The calyptoblastic hydroids have received very little attention. Morgan (1901a) obtained variable and inconclusive results from isolating stems of *Antennularia*. De Morgan & Drew (1914) described the reconstitution of this animal and Beadle & Booth (1938) of *Obelia* after pressing coenosarc through bolting silk. Nathanson (1955) describes briefly the regeneration process from isolated hydranths of *Campanularia*. Lund (1926 and earlier papers) exposed stem internodes of *Obelia* to an electric current and describes their regeneration and especially the reversal of polarity.

In earlier papers (Hale 1960, 1964, 1973) growth features in *Clytia johnstoni* have been described. It is important to know more about how the morphological pattern of this hydroid is attained and in this paper is described the morphogenetic properties of the various parts of the animal as indicated by regeneration experiments and by grafts of one part to another.

It will be shown that, apart from the extreme growing tip, every part of the stolon is totipotent, and can regenerate all parts of a colony; the tip does not always regenerate a hydranth but possesses weak inductive powers to produce

---

Fig. 1. Regeneration from stolon coenosarc. The original mass of coenosarc was to the right and from it has grown a stolon and this in turn has produced a hydranth primordium. (3 days.)

Figs. 2-4. Regeneration from isolated hydranth stalk primordia of medium length.

Fig. 2. A stolon has grown out from the basal (cut) end (1 day).

Fig. 3. An incipient attempt at hydranth differentiation has occurred at the distal end (1 day).

Fig. 4. The partially differentiated hydranths have regressed and stolons grow at the basal end (2 days).

Figs. 5-9. Regeneration from isolated bulbs of hydranth primordia.

Fig. 5. A small bulb; these fail to regenerate.

Fig. 6. A larger bulb from which a stolon has grown from the base (2 days).

Fig. 7. A larger bulb sometimes partially differentiates; this one produced a buccal region and stomach but no tentacles (1 day).

Fig. 8. A larger bulb which differentiated completely but did not open (1 day).

Fig. 9. Most regenerated polyps regress and die within a few days of isolation. Sometimes (as in this one) a stolon grows from the base (3 days).

Fig. 10. Regeneration from an isolated open polyp. Most regress and die within a few days but a few grow a stolon from the cut end (2 days).
Morphogenetic properties of Clytia
a stolon branch. Isolated hydranth primordia regenerate in different ways depending upon their size; the tip of a stalk-like primordium has weak inductive properties.

MATERIALS AND METHODS

The animal was cultured as described in earlier papers (Hale, 1973). Further details are described under each experiment.

RESULTS

(a) Stolon

(i) Tip

The tip region in the normal animal has a characteristic columnar ectoderm with vacuolated endoderm cells inside. The ectoderm cells secrete chitin and, as the stolon grows, the perisarc tube is laid down by it.

When the tip (ca. 0.5 mm) is isolated, being left on its substratum, it firstly grows as a stolon (Fig. 1). The direction of growth can be either way, i.e. continuing the same way or in the reverse way, or both ways, or change direction. The rate of growth increases to a maximum between 1 and 2 days after isolation and then decreases so that growth ceases normally at 4–5 days. During this time the regenerate becomes 2–3 times as long and much thinner than the original stolon.

Many but not all of these regenerating pieces develop a hydranth; out of 52 such experiments 27 grew a hydranth. The hydranth is small but is otherwise normal in the features of its differentiation and final structure.

The reason why some isolated tips produce a hydranth and others do not is obscure. It might be expected that if, at the time of isolation, the tip is likely to bud off a hydranth branch, i.e. it is 3+ mm from the nearest existing hydranth, it would often do so. These experiments show no evidence of this; the capability of isolated tips to produce a hydranth appears not to be related to its distance from the nearest node at the time of isolation.

An alternative experiment is to press the coenosarc out of an isolated tip and allow it to regenerate. The cell mass forms a roughly spherical hollow ball and secretes a chitinous envelope. Fifteen such experiments were undertaken and in seven of them a stolon grew out and then the stolon produced a hydranth. In one the stolon grew very slowly and failed to produce a hydranth. In the other seven no growth took place. Once again the production or failure of hydranth growth could not be related to any other feature.

A further experiment was made to elucidate the morphogenetic function of the stolon tip; this was to graft it on to the side of a stolon some distance away from the growing region. Of 18 successful grafts three initiated a branch stolon and the remainder were absorbed. In some experiments the graft was prestained with Nile blue sulphate. When a branch grew the tip of the branch was stained
blue showing that the grafted cells had formed the growing point of the stolon. Where the graft was absorbed by the host the coloured cells became scattered along the endoderm of the host stolon.

To summarize, stolon tips have a weak property of initiating new stolons. When isolated, stolon tips often grow as small stolons which later produce hydranths; in a significant number no growth takes place.

(ii) Other regions of stolon

Lengths of stolon approximating to an internode (ca. 4 mm) were isolated and allowed to grow. This they did (in all the 12 cases) by firstly producing a stolon tip at each end and growing in both directions; later one end usually ceased to grow. In this way the stolon was drawn out into a long thin structure. In all cases a hydranth grew in the ‘normal’ way behind the growing stolon tip.

In the second experiment the coenosarc was pressed out of the perisarc tube and transferred to another plate. The mass rounded-up somewhat covered itself with a chitinous envelope and within 24 h one (10 cases) or two (3 cases) stolons grew out and these in turn produced hydranths. In five regenerations an additional hydranth grew straight from the reconstitution mass, perhaps indicating that the original stolon might have possessed secondary hydranth primordia.

Regeneration of isolated contractile regions followed the same pattern as non-contractile regions. Thus the stolon possesses all the necessary growth equipment to produce more stolon and also hydranths.
In an earlier work (Hale, 1973) it was shown that different parts of the internode have different potentialities to produce hydranths and to produce stolons. An internode (undivided by secondary hydranths) was therefore cut into lengths of about 0.75 mm, making usually five or sometimes six pieces. Each piece, in its perisarc, was transferred to another dish to regenerate; they were separately attached to the glass plate in marked positions with a tiny smear of Vaseline and kept in running sea water. Stolons grew in the first day and firmly anchored them to the glass. They were observed for 20 days.

A pair of internodes was used from each of six stolons; the internode between the first two open hydranths nearest a growing tip and the internode next but one further from the tip. Fifty-five pieces were thus successfully grown and in 50 of them a hydranth stalk primordium grew on the stolon; all but nine of the latter differentiated a hydranth. Details of the time of initiation of hydranth stalk primordia are recorded in Table 1.

First, it is obvious that all regions of an internode can, and most did, produce a hydranth. Two of the pieces of stolon which failed to produce a hydranth primordium were adjacent to a hydranth in the parent stolon and three pieces were in the middle of the parent internode.

Secondly, the times taken to initiate hydranths by pieces of stolon from the middle of internodes was compared with the times taken by stolon pieces from the ends of internodes (near to nodes) but there was no significant difference (t = 0.28, d.f. = 27, 0.8 > P > 0.7).

Thirdly, 'parent' stolons differ from each other in the times taken for hydranths to be initiated.

Fourthly, the times taken to initiate hydranths show a predominant peak at 4–5 days and a small peak at 9 days. Most require 4–5 days to initiate a hydranth stalk. Then there is another smaller peak at about 9 days.

To summarize, this experiment demonstrated that any part of the internode could produce a hydranth. The reason for the preponderance of secondary hydranths arising in the central part of the internode and branch stolons at its ends must be sought elsewhere.

(b) Hydranth

A hydranth starts its development as a branch, nearly always from a stolon in Clytia. The branch is much thinner than a stolon, grows nearly perpendicular to the substratum and is normally partly annulated. After 1–2 days growth the branch (called here a 'stalk primordium') begins to swell at its tip (now called a 'bulb primordium'). At first the swelling is spherical, then pear-shaped with the stalk of the pear towards the future hydranth stalk, then cone-shaped. The coenosarc is hollow and, up to the beginning of the pear-shaped stage, closely adherent to the perisarc which it is secreting. The close adherence of coenosarc and perisarc continues at the distal end where growth continues for a time. During this time the region of the coenosarc which is further back detaches
Morphogenetic properties of Clytia

from the perisarc as it becomes contractile; this part becomes the stomach of
the hydranth. The more distal coenosarc differentiates by the growth of ten-
tacles and buccal region. Lastly the perisarc at the end of the cone is dissolved
away and the hydranth opens. The whole hydranth normally takes 2–3 days to
grow and differentiate.

The morphogenetic potencies of the various parts have been determined; these parts are (i) the stalk primordium, (ii) the bulb (from a bulb primordium),
(iii) the entire bulb primordium (i.e. bulb + ‘stalk’), (iv) isolated open hydranths,
(v) isolated stalks of open hydranths.

(i) The stalk primordium

The stalk primordia were isolated by cutting the stolon on either side of the
base and detaching them from the glass plate, the short piece of stolon remain-
ing on each was then cut off. The primordia were placed in small pits in a perspex
plate which was then placed in running oxygenated sea water. Observations
were made over a period of 20 days on 32 stalks ranging in initial length from
0.3 to 2.1 mm.

The subsequent regenerative behaviour was basically either (a) no develop-
ment or (b) to produce a stolon at the cut (basal) end, or (c) to produce, or to
attempt to produce, a hydranth at the original growing (distal) end.

Five failed to differentiate, all less than 0.5 mm in length. Flagella activity
and movement of particles in the coelenteron and its general appearance
indicated livingness. During 20 days the only change was a very gradual de-
crease in size obviously due to its using up material to support its life. Hargitt
(1915) noted that reconstitution masses from a number of hydroid species
lived without differentiation for ‘some weeks’.

Many of the longer primordia produced a stolon at the cut end (Fig. 2). Nine of these did so without showing signs of any immediate differentiation
of the hydranth. They were between 0.5 and 1.4 mm long. The stolon began to
grow within 1 or 2 days of isolation and continued to do so usually up to the
4th (4), 5th (4) or 6th (1) day. Three of these stolons produced a hydranth stalk
primordium, in two at the moment the stolon ceased growing and in another
after an interval of 11 days. In one of these a small polyp grew on the end of the
stalk but in the others the stalk ceased to grow without further differentiation.

Seven of the longer primordia in the 1.4–1.7 mm range attempted to differ-
entiate (a hydranth?) at the growing tip end of the stalk (Fig. 3). This was
demonstrated by a small amount of growth in length, usually a swelling at the
end and a thickening of the terminal ectoderm. The morphology of these
incipient growths was different from that seen in a growing stalk tip or stolon
tip, recognizable hydranth parts were less certainly identifiable. These changes
took place during the first day of isolation but during the second day a stolon
grew out of the other cut end, and the incipient growth regressed (Fig. 4).
Table 2. Frequency of incipient or complete hydranth differentiation according to length of isolated hydranth stalk primordium

<table>
<thead>
<tr>
<th>Length of isolated stalk primordium (mm)</th>
<th>1·40--1·64</th>
<th>1·65--2·13</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incipient hydranth</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Complete hydranth</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Totals</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

Probability of association (Fisher's 'exact' test) = 0·05.

Four of the regenerates went on to produce a hydranth stalk primordium but these did not differentiate into hydranths.

Eleven of the longer primordia rapidly differentiated a hydranth as if they were still attached to the parent stolon, taking the normal 1–2 days to do so. Seven of them opened but four did not. After 2–3 days the hydranths regressed and all but three of the isolated pieces died quickly, six days after their initial isolation. Of the three that did not follow this fate, two produced a stolon at the cut end and were still alive after 20 days; the third died at 16 days.

It will be seen that the behaviour of the isolated primordia falls into four categories according to their length at isolation. In the first, up to 0·5 mm, no differentiation takes place. In the second, a stolon is typically produced from the cut end of stalks of length 0·5–1·4 mm. Stalks longer than 1·4 mm all make a successful or unsuccessful attempt at differentiating into a hydranth within the first 1–2 days of isolation. Those that made an initial attempt at hydranth formation and failed all went on to behave like those in the second category and produced a stolon from the cut end. The isolated primordia which only made an incipient attempt at hydranth differentiation were generally the shorter ones, as is shown by a contingency analysis (Table 2). Thus the third category are primordia of lengths between 1·4 and 1·7 mm, the majority of which form incipient hydranths in the first day and then as these regress they behave like the primordia in category two. The fourth category comprise primordia longer than 1·7 mm, but a few shorter ones (down to 1·4 mm) also behave in the same way; they differentiate into a hydranth as in the intact animal.

The tips of isolated stalk primordia may be grafted on to a stolon. In ten such experiments with tips between 0·25 and 0·40 mm long five regressed leaving an empty perisarc tube, four grew and produced a normal hydranth with normal length stalk and one which failed to make organic attachment ‘grew’ slowly leaving behind as a ‘trail’ an empty perisarc tube.
(ii) The bulb

Twenty-four experiments were carried out in which a series of growing bulbs were isolated. They ranged from very young stages of 0.15 mm diameter up to larger ones of approximately 0.3 mm diameter, and then another nine where the bulb was elongating from 0.3 to 0.6 mm with relatively little increase in diameter.

The isolated pieces behaved differently according to whether they had (or had not) differentiated far enough for the coenosarc to be separating from the hydrotheca; this stage corresponds to the onset of pulsations in the hydranth. The first 15 in order of size (up to a length of 0.3 mm and diameter 0.25 mm) had neither coenosarc separation nor pulsations at the time of isolation. Eight of them failed to differentiate any structure (Fig. 5); the smaller ones succumbed after 6 or 7 days, but the larger ones lived up to 15 days. Four others grew a stolon (Fig. 6) (after 3, 8, 9 and 10 days) but only one (the largest) produced a hydranth stalk; three of the four were relatively large but one was a smaller primordium. Two others immediately elongated very considerably, as in normal polyp differentiation, one partially differentiated showing buccal and stomach regions only (Fig. 7), and the other differentiated completely (Fig. 8); the partial differentiations were a little different from those occurring in regenerating stalk primordia in that more clearly recognizable hydranth structures were formed.

The other nine larger bulbs all immediately elongated: one differentiating to the stomach and buccal region only and the other eight completely, although only two of the latter opened. The partly differentiated polyp soon regressed but a stolon grew out of the cut end and this in turn grew a hydranth stalk primordium. The eight fully differentiated polyps also regressed and five had died by 7 days; a sixth lived 11 days and a seventh 18 days; none of these produced any further structure, but the eighth produced a stolon (Fig. 9).

Thus almost all of the young bulbs up to the onset of contractility fail to differentiate a polyp on isolation; after this stage polyp differentiation takes place.

When young, precontractile bulbs are grafted into the stolon, some grow and differentiate into sessile hydranths and others regress leaving an empty perisarc bulb (five each in ten experiments). This result is similar to that of grafting stalk tips. Older primordia always differentiate fully, as expected from the results of the isolation experiments.

(iii) The bulb-primordium

(a) Variable bulb size. In this experiment 20 developing bulb primordia were isolated, having the stalk part trimmed to ca. 0.3 mm length; two of the bulbs were in the earlier contractile stages but the remainder were a series of younger, non-contractile bulbs.
Eleven smaller pieces of tissue, those having a bulb of up to 0.2 mm in diameter, did nothing in the first 2 days of isolation. Five of the nine longer primordia all immediately (1–2 days) differentiated partly and four wholly into a hydranth, the latter including the two isolated in an early contractile stage.

Of the 11 younger ones 4 produced a stolon 4–9 days after isolation, 3 in the ‘normal’ way from the cut end of the stalk but 1 directly out of the bulb; 3 (including the latter) developed a hydranth stalk and 2 of these (the smaller primordium and this aberrant one) a small polyp. Two more primordia produced a hydranth stalk directly from the primordia after 10 and 12 days quiescence. Of the other 5, 3 died after 4 days and 2 after 9 days without differentiating.

The nine longer primordia, which partly or wholly differentiated into hydranths, regressed during the 3rd and 4th day. Of the five partial differentiations four died in 4–6 days without further change but the other produced a stolon (following by a hydranth stalk) from the cut end of the stalk. Of the four which fully differentiated, one soon died and the other three produced stolons and hydranth stalks, two of them hydranths as well.

Thus the presence of ‘stalk’ on the bulbs enables them to differentiate normally and directly into hydranths from an earlier developmental stage than without the ‘stalk’, though a stalk primordium of this length or a bulb of this diameter, on their own, would fail to do so.

(b) Variable ‘stalk’ length. Hydranth primordia were isolated with variable lengths of stalk in four groups, the diameter of these pre-contractile bulbs being respectively 0.14–0.15 mm, 0.17–0.18 mm, 0.20–0.21 mm and 0.22–0.23 mm. The subsequent changes in the four groups were not distinguishable and the results are described as a single group of 30 primordia with lengths of attached stalk varying from 0 to 1.66 mm.
Morphogenetic properties of Clytia

In the group of ten primordia with 'stalk' lengths less than 0.34 mm only one differentiated into a hydranth (and this, curiously, had a very short 'stalk' - 0.14 mm) and the other nine failed to do so. All but one produced a stolon after 3-4 days but only one of these produced a hydranth stalk primordium.

Of the 20 primordia isolated with 'stalks' 0.35 mm and upwards all but one (stalk 0.5 mm) differentiated in 1-2 days into a hydranth. Seven opened but the remaining 12 failed to do so. There were no partial differentiations. After regression, ten of them grew a stolon from the cut end of the 'stalk' and five of these went on to grow a hydranth stalk, but none of the latter produced a hydranth. The other nine failed to produce any further structure but six failed to survive beyond 6 days.

Thus to summarize, young bulb primordia (before the contractile stage) will develop into a complete hydranth provided they have more than about 0.35 mm of 'stalk' attached. Without 'stalks' such primordia do not proceed directly to hydranth differentiation. Stalk primordia of this length fail to differentiate at all.

Table 3 summarizes these results.

(iv) Isolated open polyp

The polyps regressed after 1-3 days except for one which remained open for 6 days. Fifteen of the 21 observed soon died (within 6 days of isolation) without further differentiation. The other six developed a stolon from the cut end of the hydranth (Fig. 10) and the latter then regressed; the stolon later produced a hydranth stalk primordium and all but one went on to develop a hydranth. The six were still alive at 20 days.

(v) Isolated stalks from open hydranths

Stalks ranging in length from 1.5 to 3.3 mm long (i.e. some came from regenerated hydranths) were isolated by removing the polyp and either leaving them attached to the culture plate after cutting away the parent stolon or transferring them to pits in a perspex plate, the latter being kept, as usual, in running sea water.

In all 21 cases the coenosarc slowly retreated down the tube and one or two stolons grew out of the base. In five of these a hydranth was then formed on the stolon. The tissues behaved like small pieces of stolon.

In a further eight stalks the contents were separately pressed out on to a glass plate. Initially an irregular mass was formed which secreted a chitinous covering. Within a day a stolon grew out and this in turn (in six cases) produced a hydranth. Again the stolon regeneration pattern was followed.
DISCUSSION

(a) Morphogenetic properties of stolon

Except for the first 0.5 mm at the growing tip any part of the stolon has a capability of producing all the parts of a colony. An explant does this by first organizing one or more growing stolon branches and then these in their turn often bud off hydranths in the normal way. The cells in a stolon are thus capable of becoming organized into hydranth structures as well as stolon.

In one respect this result was surprising as earlier observations (Hale, 1973) have shown that branches of the stolon (to form hydranths or branch stolons) do not arise at random. The experiments recorded here show no differences between different regions of the stolon in their ability to differentiate hydranths or stolons when isolated. Some other explanation must be found to account for this observation.

(b) Morphogenetic properties of hydranth

A quite different state of affairs is found after isolation of various parts of a growing or 'adult' hydranth. A more meaningful interpretation follows from the results of earlier work (Hale, 1964) on cell movements and cell division in the animal. It will be recalled that the growth of the primordium takes place mostly by the migration of cells from the stolon, partly as a coherent ‘sheet’ and partly by the adherence of cells floating in the hydroplasm to the contractile growth regions. Cell division is absent except at the time and place where the tentacles are being formed. A further point to be noted is that the cells in a stalk primordium are arranged in the order in which they later occur in the polyp: this differentiation is reversible in the stalk primordium.

(i) Stalk primordium

Taking first the results of growing the stalk primordia in isolation it is seen that they go through three main phases. Young (i.e. short) ones up to about 0.5 mm long do not differentiate. Longer primordia, between about 0.5 and 1.4 mm differentiate by growth of a stolon from the proximal (basal) cut end. Primordia longer than 1.7 mm always immediately differentiate fully into a hydranth. Primordia between 1.4 and 1.7 mm may either make an incipient attempt at hydranth differentiation or a successful hydranth differentiation. Nathanson (1955) made some similar experiments on Campanularia but with no quantitative details. Morgan (1901a) and Peebles (1902) also did some preliminary regenerations with Antennularia and with Pennaria and Eudendrium respectively.

The difference between the first and second groups in Clytia is significant. The short (0.5 mm) growing stalks lack some factor, or factors which completely prevents any kind of differentiation. A slightly longer growing stalk possesses
Morphogenetic properties of Clytia

Fig. 11. Morphological structures developed from isolated hydranth stalk primordia of various lengths. The hydranth stalk primordium passes through successive stages of regeneration according to its length. Explanation of the three parts of the primordia is given in the text.

full equipment to differentiate into any structure, first by differentiating a stolon and then this in turn produces hydranths. This longer stalk behaves just like an isolated length of stolon except that the structure is polarized and the stolon always grows out of the cut, basal end. Thus one might deduce that the basal end of such a stalk possesses stolon forming characteristics not shown by the disal end; this is in contrast to a length of stolon both ends of which develop stolon-forming characteristics.

Above about 1-4 mm length the growing stalks develop a greater tendency (immediately after isolation) to differentiate first into a stalked hydranth until those over 1-7 mm long always do so. In any one instance the differentiation is either an initial stolon (in some shorter ones) or hydranth production; no case was observed of both or neither occurring. Thus a growing ‘stalk’ goes through a situation where it passes rapidly from a primordium with the potentiality of differentiating into a stolon on isolation to a primordium having the potentiality of differentiating partially or fully into a hydranth on isolation.

A similar distinction can probably be made between those growing stalks in the 1-4–1-7 mm category which only partially differentiated into hydranths, the shorter ones being more likely to differentiate partially in contrast to the longer ones being more likely to differentiate fully. An explanation for these observa-
tions on the stalk-like primordium follows from the indication that a fully
grown stalk has three linearly arranged regions (see Fig. 11). The first is at the
growing end (A) and is about 0.5 mm long; a second is adjacent to it (B) and
about (1.4 - 0.5) = 0.9 mm long and the last (C) of variable length, adjacent to
the stolon region. A may well correspond to that part of the primordium giving
rise to the buccal region and B to the stomach region. Some cells at the junction
of A and B must be primordial tentacle cells which, by rapid mitosis later
give rise to these structures. If these suggestions are right then C will give rise
to the stalk coenosarc.

A on its own does not differentiate into any structure; it remains alive but
lacks the means both of growth and morphological differentiation.

When B joins A (in normal growth) the joint structure suddenly (even with
a small amount of B) acquires the means of growth and differentiation. The
pattern is to produce first a stolon then later, from this, a hydranth differentiates.
Does B (which later in normal growth might become the buccal region of a
hydranth) possess some essential stolon-characterizing material? (One wonders
whether there is any significance in the fact that the buccal region of the hydranth
and the stolon growth regions are the main contractile regions of the animal.)
Another possible explanation is that once B joins A there is sufficient material
for differentiation.

More difficult to interpret is how the addition of C to (A + B) changes the
short-term morphogenetic capacity of the whole to a hydranth. In the longer
term, after the hydranth has regressed, (A + B + C) seems to have the same
morphogenetic potency as (A + B) without C.

(ii) Stalk and bulb primordia

A stalk primordium 0.5 mm long contains about the same volume of coeno-
sarc as a 0.2 mm diameter bulb from a bulb primordium. Neither primordium
grows or differentiates after isolation and it is likely that the cells of such a bulb
are those which previously were in the stalk. Similarly, the volume of coenosarc
in a stalk primordium of 1.5 mm long is about the same as that in a bulb 0.3 mm
in diameter; almost up to these sizes rapid (i.e. 1- to 2-day) hydranth differentia-
tion does not take place when such pieces are isolated. Larger primordia
and longer stalks do rapidly differentiate into complete hydranths or incomplete
hydranths. There are some differences in detail between the two sets of observa-
tions indicating some changes from the stalk-like primordium to the bulb
primordium; nevertheless the correspondence in differentiation potencies is
considerable.

After isolation a bulb primordium develops further than a similar sized bulb
without a stalk; in the former case much of the material in the stalk moves into
the bulb as it does in normal development. Further, the appearance of the bulb
in a primordium corresponds with a marked change in its development
potentialities. A stalk primordium 0.3 mm long does not differentiate into any
Morphogenetic properties of Clytia

structure, but a bulb primordium of the same length with a very small bulb produces a stolon after 3–4 days. A similar result is obtained with a stalk primordium of length 0.4 mm, but here a very small bulb converts a primordium from being unable to differentiate to being able to transform directly into a hydranth in 1–2 days. Therefore some change occurs the moment a bulb starts to form which confers on a primordium an ability to differentiate into either a stolon or a hydranth; just before this occurs, no structural differentiation takes place. This moment is a point of full determination. Barth (1944) found it to be very late in the Tubularia hydranth. Further investigation of such changes would clearly be of interest.

(iii) Contractile regions

The youngest stalk primordia are not contractile although they grow from the adjacent stolon which is; later the stolonic contractility spreads into the lower end of the primordium. This fact may be connected with the lack of growth of the youngest stalk primordia when isolated and with the proximal stolonic growth characteristic of somewhat older primordia when isolated. It is also pertinent to note that in normal growth this contractile part of the stalk primordium becomes the stomach of the polyp, another contractile region.

(c) Peaks of regenerative activity

In the results, attention has been drawn to the time delays between isolation of a part of the animal and the onset of differentiation. It has been seen that differentiation, either of a stolon or a hydranth, tends to happen at three particular times. The first peak of activity is ‘immediate’, i.e. within 1–2 days of isolation. These are cases where, clearly, the whole mechanism for stolon or hydranth differentiation is already present.

A second peak has been shown to occur at about 4–5 days after isolation and possibly a third is found at about 9 days. The reason for this pattern of differentiation activity is obscure. Is there some cycle of events leading to possible differentiations every 4–5 days? In the intact animal hydranth branches tend to be initiated every 1–2 days but this is more likely to be connected with rate of stolon growth and, presumably, the distance of stolon tip from the previously differentiated hydranth. One event in the normal animal which also demonstrates two 4- to 5-day cycles is the length of life of hydranths (Hale, 1973). Are all the phenomena correlated or are they a manifestation of an intrinsic clock?

(d) Possible inductive properties of stolon tips and tips of hydranth stalk primordia

Experiments on grafting stolon tips and the tip of stalk-like primordia indicate that they may have weak inductive properties. The fact that many grafts of these structures regress indicates that the stolon may not always be in a condition to react to this growth and differentiation stimulus. Further
experiments are desirable to add to knowledge of the conditions under which growth occurs or fails to occur.

Since the tip of a stalk primordium goes on to give rise to the buccal region of the hydranth it has some similarities, morphologically and embryologically to the oral cone in hydra and the gymnoblasts.

(e) **Incomplete regeneration**

In many regenerations a stolon first grows and this may or may not be followed by the appearance of a hydranth stalk and this in time may or may not differentiate a hydranth. Morgan (1901b, 1902, 1903) obtained incomplete regenerations from small pieces of tissue in Tubularia. A shortage of cells seems to be the most likely explanation of incomplete regenerations in Clytia but no experiments have been done to test this.

(f) **Regenerative ability**

The observations have shown that certain isolates live but fail to differentiate into any structure. They are all small pieces of coenosarc and their behaviour might be explained as being due to there being insufficient cellular material to create either of the two basic structures, stolon or hydranth. Mere smallness may not be the reason, or only reason, as a similar small piece of stolon coenosarc, other than from the tip, can successfully differentiate, if only partially.

Another possible factor is that development of an isolate requires the presence of more than one cell type and that the non-developing isolates lack one or more cell types. It is to be noted that the non-differentiating isolates are from the growing ends of either stolon or hydranth and, being structures specialized for chitin production, they may not possess every cell type necessary for differentiation of a whole stolon or hydranth. In the case of the hydranth stalk primordium the addition of even a small amount of coenosarc B (see (b)(i) above) converts an inactive tip isolate A to a differentiating one and A and B have been shown to be distinguishable in their morphological potentialities (Hale, 1964).

This idea gains further support from a second feature of the results of these experiments. When differentiation of isolates takes place the immediate result is the production of either a stolon or a hydranth. Is it possible that the direction of differentiation is governed by the cell types present? Some support to this suggestion comes from the observations of isolated older hydranth stalk primordia; when the third (basal) region, C, is added to the two distal regions A and B the structure changes from stolon producing to hydranth producing.

In the development of the hydranth the term ‘cell type’ must almost certainly refer to partially differentiated cells. In the case of isolated pieces of stolon the experiments do not make it clear whether differentiation is due to the presence and organization of such partially differentiated cells or of totally differentiated cells, or of both. Whether the ‘adult’ cells of Clytia are able
Morphogenetic properties of Clytia

to take part in such differentiations is not clear although there may be a pointer from other experiments described here. It has been noted that most open, i.e. differentiated, polyps die a few days after isolation. Their cells seem to be incapable of any form of reconstitution on their own. Yet it is known that, in the presence of its stalk, the cells are re-used in further differentiations. Perhaps therefore there is one, or more than one cell type, which is essential for both kinds of structural differentiation in this animal.

To summarize, while there is likely to be a minimum number of cells which can create a stolon or hydranth there is evidence that a process of determination is also involved before structural organization can take place.

REFERENCES


(Received 21 August 1972, revised 14 June 1973)