Cell movements, cell division and growth in the hydroid *Clytia johnstoni*

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INTRODUCTION

Although there have been a great many studies on the morphogenesis of hydroids, the emphasis has been largely with regeneration and with grafting experiments, and mostly using the hydras, *Cordylophora* and *Tubularia* as material. Some of these investigations have made reference to cell movements and cell division, but none have considered both these fundamental morphogenetic processes and their interrelationships in a single hydroid. In this paper the occurrence of cell movements and cell division in normal growth and in the regeneration of *Clytia johnstoni* is considered.

The investigation has revealed extensive cell movements, not only in the coenosarc but also passively by the hydroplasm. Cell division is almost exclusively confined to the ectoderm and rarely occurs in the endoderm.

MATERIAL AND METHODS

The animal was cultured in the laboratory on glass plates in running sea-water (Hale, 1957, 1960). All the observations (except those on cell division) were made on the living material kept at 18°C. (± 1). Some observations were supported by time-lapse photography.

Cells were marked by staining them with nile blue sulphate: tiny pieces of cellophane, previously stained with the dye, were left in close proximity to the cells to be marked for 15–30 min. The position of such a marked group of cells was measured immediately after marking and again at intervals.

In some cases movement of cells was observed without staining, and by marking with carbon particles. Cell behaviour was found to be similar with these three methods.

The majority of the cells contain fat vacuoles, the ectoderm numerous small ones, and the endoderm a few large ones. It is undoubtedly because of this fact that nile blue sulphate turned out to be such a satisfactory material to mark the cells in this animal. The stain must diffuse out of the cells extremely slowly; for

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even after 3 or more days, they were still clearly marked, and also there was no evidence that the dye had passed into originally unstained cells.

**General outline of growth**

The animal consists of a stolon of typical coelenterate structure which is normally attached to the substratum. At intervals along the stolon are found hydranths on their stalks; these grow at right angles to the substratum (Text-fig. 1).

![Diagram](image)

**TEXT-FIG. 1.** Diagram to illustrate the morphology of the animal and stages in the growth of a hydranth (from right to left).

![Bar graph](image)

**TEXT-FIG. 2.** Histogram of rates of growth of stolons.
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Growth takes place by an increase in length of the stolon, and, as it grows, new hydranths appear at intervals. The rate of increase in length at $18 \pm 1^\circ C.$ is somewhat variable as shown in Text-fig. 2, which condenses the data on sixty-nine stolons measured for at least 24 hr. The average rate of growth is 3.1 mm./day, the range being from 0.7-5.4. Nearly 80 per cent. grew between 2.0 and 4.3 mm./day.

The tip of the stolon does not grow smoothly. This is shown in Text-fig. 3 in which the position of the tip, relative to a fixed point on the substratum, was measured at equal intervals of time. The pulsing of the tip is directly related to the activity of the contractile region of the stolon just behind its tip. Contraction of this region pushes out the tip, and then as the contraction dies away the tip retracts part of the way. This is repeated at every pulse of the contractile region. Such growth continues without pause if the stolon is cut behind the contractile region (Text-fig. 3).

The leading ectoderm cells of the tip are columnar, in contrast to those in the rest of the stolon which are flattened. These (or at least some of them) secrete
chitin so that as the stolon grows the living coenosarc remains enclosed in a chitinous tube—the perisarc. The newly excreted chitin is soft and sticky and so anchors the stolon to the substratum. The soft chitin slowly hardens.

New hydranths appear at intervals as the stolon grows. A new stalk is first seen a very short distance behind the stolon tip, and always on the side away from the substratum. The stalk increases in length, and its perisarc tube is partly annulated. Eventually the stalk ceases to grow in length, and its end swells up into a bulb, which later becomes cone-shaped with the apex attached to the stalk.

![Text-fig. 4. Histogram of distances between hydranths.](image)

The 'cone' ceases to grow in size, but, inside its chitinous envelope, the hydranth differentiates. When fully formed, the distal end of the perisarc is dissolved away (forming the hydrotheca) and the hydranth stretches out into the sea-water.

During this interval of time (1−2 days) the stolon has continued to grow and usually one or perhaps two more hydranths have started to grow (Text-fig. 1).

The distance between hydranths is somewhat variable. The histogram in Text-fig. 4 summarizes some measurements. The average distance is 4.7 mm. but it can be seen that the great majority are more than 3 mm. apart. Occasionally a stolon will grow for a relatively great distance (up to about 2 cm.) without giving off a hydranth. The reason for this is unknown.

Statistically, the correlation between rate of growth and distance between hydranths (Text-fig. 5) is not quite significant at the 5 per cent. level ($t=1.8$; d.f. = 39; $p = 0.08$). In Text-fig. 5 are three points in circles and another three in
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The former record measurements on stolons which had a relatively high rate of growth coupled with a short distance between hydranths, and the latter cases in which the opposite was found. Each group of three measurements were the only ones made over two particular periods and their apparent lack of conformity might have been the result of some common factor affecting the group. Certainly the exclusion of the measurements on the six stolons results in a very highly significant correlation ($t = 5.3; \text{d.f.} = 33; p < 0.001$). The evidence thus suggests some direct relationship between rate of stolon growth and the distance between the hydranths, but also that other factors are, or can be, involved in the growth of stolon and/or hydranth.

![Text-figure 5](image)

**Text-figure 5.** Relation between rate of growth of stolon and distance between hydranths.

**Results**

1. **Cell movements in the stolon**

The following description of cell movements in the stolon is mainly based on observations of the behaviour of groups of cells marked with nile blue sulphate. A small band of cells (0.2–0.3 mm. wide, both ectoderm and endoderm) was stained and its position measured relative to the growing tip of the stolon and to a fixed point, usually an adjacent hydranth. The same measurements were repeated at intervals until at least 24 hr. later. Thus the amount of growth of the
stolon was determined as well as any movement of the marked cells, together with other relevant observations. One hundred and five experiments were carried out marking cells at various distances from the tip, and from growing or mature hydranths.

Movements of cells were observed in all growing stolons up to about 5–6 mm. from their tips; in many stolons cell movements took place over a longer length and in a few up to about 12 mm. from the tip, but never beyond. The cells moved in the same direction as growth of the stolon. Cells nearest the growing tip moved the fastest, and at increasing distances from the tip the rate of movement decreased.

As there is considerable variation in the rate of growth from one stolon to another (Text-fig. 2) and as this is clearly likely to be reflected in the rate of movement of the cells, the latter was converted to the ratio of cell movement to stolon growth. Thus a ratio of 1·0 indicates that the cells were moving at the same rate as the tip of the stolon. Ratios greater than 1·0 indicate a speed of cellular movement greater than stolon growth, i.e. the cells were moving nearer to the growing tip. A ratio less than 1·0 shows a slower movement than the tip, and an increasing distance between the tip and the marked cells. A figure of 0 means that the cells are stationary. The graphs (Text-fig. 6) summarize the cell movements.

There is considerable variability in the behaviour of cells from one stolon to another; for example, the length of stolon where such movements take place varies from about 6–12 mm. in different stolons. Even allowing for this there still remains considerable variability, the sources of which remain to be found. What is clear is that cell movements in the ectoderm and endoderm always follow these patterns, both individually and relatively. The gradients differ and so do the maxima, although the latter are always greater than 1·0. For purposes of description it is convenient to divide the stolon into four regions.

**Region 1** (about 0–1 mm. from stolon tip). Marked cells move towards the tip (ratio about 1·0–1·1). It is noticeable, also, that the ectoderm cells on the top of the stolon (i.e. away from the substratum) are a little slower moving than similar cells at the sides and bottom of the stolon.

**Region 2** (from about 1 mm. to about 2–3 mm. from the tip). The ectoderm cells are generally moving faster towards the tip (ratios of 1·1–1·5) in contrast to the endoderm cells which move more slowly than the tip (ratios of 1·0–0·6). In the ectoderm the relatively slower rate of movement of cells on the top of the stolon is more marked than in Region 1, so that a band of vitally dyed cells in this region becomes V-shaped after a few hours, the point of the V being directed away from the stolon tip. Both the ectoderm cells, on the top of the stolon, and the endoderm cells tend to become spread out in this region.

**Region 3** (from about 2–3 mm. to about 4–6 mm. from the tip). Useful measurements of groups of stained cells were difficult in this region, since in both cell layers the marked cells became spread out with indistinct limits.

**Region 4** (from about 4–6 mm. to about 6–12 mm. from the tip). In this region,
at increasing distance from the tip, the cells move more and more slowly and eventually stop. The ectoderm cells still tend to be more active (ratios 0.9–0) than the endoderm (ratios 0.6–0).

Similar results for ectoderm cell behaviour were obtained by marking with carbon particles. Using a micromanipulator and microsyringe a suspension of carbon particles (a drop of Indian ink diluted with sea-water) was injected through a hole made in the perisarc. Some of the particles adhered to the ectoderm cells and their movement was observed and measured over 1 hr. with the high-power microscope (×10 objective and ×10 eye-piece). Confirmation of the behaviour of endoderm cells was made by direct observation. This is possible as their fat vacuoles have a distinct orange colouration (from an orange pigment in the Artemia nauplii given as food).

A diagrammatic illustration of these cell movements is shown in Text-fig. 6. Beneath the graphs are three figures which represent the growing ends of stolons; the centre figure shows the pre-growth conditions and the upper and lower ones the post-growth conditions. If cells are marked at equal intervals along the first 6–12 mm. of a stolon (centre figure), a day later they will have moved to the
positions shown in the upper (ectoderm) and lower (endoderm) figures. The tendency for ectoderm cells to accumulate just a little behind the growing end of a stolon is clearly shown; it is in this position that new hydranths first begin to grow. Further back from the tip a decrease in cell density is shown; it will be seen later that additional cells are added to this region.

2. Cell movements in stolon and hydranth formation

Early on in these experiments it was noted that some marked stolon cells subsequently appeared in a growing hydranth stalk and later still in the hydranth itself. Marked cells in the initial 0·3 mm., whether ectoderm or endoderm, never appeared in the hydranth. These cells stayed at the extreme tip of the stolon. Behind this extreme tip, marked ectoderm cells moved into a hydranth stalk if one were growing up in their pathway and were moving nearer to the tip (i.e. up to about 6–12 mm. from the tip). Thus, in their forward movement, if the cells came to a growing hydranth (which is, of course, fixed), some of the cells would move into it. The cells which move in are those on the ‘top’ of the stolon (away from the substratum) and not those at the sides or bottom of the stolon; the latter pass it by in their forward progression.

The region of the stalk in which these cells appear depends upon their distance from the tip of the stolon at the time they migrate into the stalk; the further away the cells are from the stolon tip, the further away are they from the tip of the growing hydranth.

The rate of movement of cells into the hydranth stalk slows down and eventually ceases at the same time as the hydranth itself grows and differentiates. The slowing down of this migration process corresponds to the slowing down, and then the cessation of movement of the stolon ectoderm cells at increasing distance from the stolon tip.

A few endoderm cells migrate into a hydranth stalk during its initiation just behind the tip of the stolon, but not thereafter. The endoderm of a growing stalk, and of a differentiating hydranth, has a derivation different from that of the ectoderm, cells being transported there in the hydroplasm (see 4 below).

3. Cell movements in growing hydranth

Marked stolon cells could frequently be traced right through into the fully formed hydranth, but further observations were made by marking a band of cells at varying distances from the tips of growing hydranth stalks of various lengths, and following their movements (thirty experiments).

As a stalk grew in length, the marked cells maintained their positions relative to the tip, growth taking place by the addition of cells at the base. This distal movement of cells continued as the tip of the stalk swelled into a bulb, but at a decreasing rate. Once the hydranth begins to differentiate, the ectoderm cells
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cease to move. The endoderm cells continue to move for a little longer, but soon become stationary. During the swelling up of the bulb at the top of the stalk, the cells continued to maintain their relative positions so that the cells forming the various regions of the hydranth could be traced back to a particular region of the growing stalk. The cells in the stalk are arranged in the order in which they appear in the hydranth; at the tip of the stalk they are destined to form the buccal region, and then, at increasing distances from the tip, they are destined to form the tentacles, the tentacular ring (base of tentacle) and stomach. Table 1 summarizes these results.

4. Movement of cells in hydroplasm

The hydroplasm (i.e. the fluid in the coelenteron) contains cells. It has been found that during the ebb and flow of the hydroplasm these cells, derived from non-growing and regressing parts of an animal, preferentially adhere to the growing regions. The experiments demonstrating this phenomenon were carried out either by initially staining a short length of stolon or a hydranth. In the latter case no transfer of cells occurs unless the hydranth regresses. Most of the experiments were carried out on a piece of colony (c. 15–20 mm. of stolon with its hydranths) kept for a few days in an observation cell in running sea-water.

The results of one experiment (of twelve carried out) are illustrated in Text-fig. 7.

Similar results were obtained in the other experiments, including those in which the cells in a short length of the stolon were marked with vital dye. These results may be summarized as follows:

A. The cells of a regressing hydranth are transported by the hydroplasm to other parts of the animal, and used again.

B. Cells of the stolon are used in a similar way. Cells from a regressing hydranth are frequently temporarily 'stored' in the stolon.

C. Cells transported by the hydroplasm may stick on in the following regions:

(a) Stolon. (i) Scattered everywhere. (ii) More densely about 1–5 mm. behind the tip of a growing stolon. If a stolon ceases to grow, cells are transported from it to other growth regions.

(b) Hydranth. (i) Growing (or regenerating) stalk. (ii) Growing (or regenerating) hydranth. (iii) Into stomach of differentiating hydranth, and newly opened hydranth, but not later.

In these experiments the cells which were transported by the hydroplasm were certainly of endodermal origin, and later became part of the endoderm elsewhere in the animal. Where a regressing hydranth was marked, ectoderm cells were also marked, but no trace of such cells were found later in the ectoderm elsewhere in the animal. It is not impossible that such cells, if scattered throughout the animal, would be difficult to detect; but though they might have been so scattered, no
**TABLE 1**

*Origin of parts of hydranth: presumptive regions of growing stalk*

The overlap of the regions is largely a result of variability between growing stalks. The final length of most stalks is between 2 and 4 mm.

<table>
<thead>
<tr>
<th>Part of stalk: distance from tip (mm.)</th>
<th>0·0–0·2</th>
<th>0·15–0·30</th>
<th>0·25–0·40</th>
<th>0·30–0·50</th>
<th>0·4–1·2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part of hydranth formed from this length of stalk</td>
<td>Buccal region</td>
<td>Distal ends</td>
<td>Middle regions</td>
<td>Bases</td>
<td>Distal end</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

>l-2 >
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Vitally stained hydranth
Cut end of stolon
Growing hydranth

Day 0

Hydranth regressing

Day 1

Cut end of stolon regenerated and growing

Day 2

Stolon ceased growing

Day 3

Stolon now a growing hydranth
Hydranth regressed

Day 4

Hydranth beginning to regenerate?

Day 5

5 mm

Lengths of stolon and hydranth stalks; other dimensions exaggerated

TEXT-FIG. 7. Transfer of cells by the hydroplasm, illustrated by drawings from an experiment. Dots represent marked cells.
trace of them was ever found. They were certainly not concentrated at any point, as, for example, in the growing regions.

Where a length of stolon was marked, the ectoderm cells behaved like normal ectoderm cells in this position; many of the marked endoderm cells moved to other regions in the colony as already described, and behaved no differently from endoderm cells derived from a regressing hydranth.

Many cases of the actual attachment of a marked cell at a new site were seen. For some time after their attachment they were moved by the flagella which are active during the movement of the hydrolasm, but they slowly became incorporated in the endoderm.

![Diagram of cell movements during stolon regeneration](image)

**Text-fig. 8. Regeneration of a stolon tip, movement of ectoderm cells.**

5. **Cell movements during stolon regeneration**

If the growing end of a stolon is removed it will regenerate. Fourteen such experiments were carried out in which a variable length of the first few millimetres of a stolon was removed, and marked cells at different distances from the cut were observed. Measurements were made as before to ascertain cell movements and the renewal of stolon growth.

The coenosarc seals itself immediately after it is cut. In the following 6–12 hr. the cut end becomes reorganized to become a normal growing stolon. The ectoderm cells migrate towards the cut in a manner similar to their movement towards a normal stolon tip; those at the side of the stolon move faster than those on the top (Text-fig. 8). Thus, ectoderm cells accumulate at the tip; there forming the columnar arrangement characteristic of a growing tip. Endoderm cells do not move during this period. If any or all of the contractile region is cut away, a new
one develops in the normal position. As these processes are completed the stolon slowly resumes its normal growth.

6. **Cell movements in the regeneration of an isolated length of stolon**

In these experiments a short length of stolon was stained with nile blue sulphate. The growing end of the stolon was cut off and the stolon was also cut some distance from the other side of the stained region, as shown in Text-fig. 9.

![Text-fig. 9. Regeneration in an isolated piece of colony. The small and large dots represent marked ectoderm and endoderm cells respectively.](image)

In the four experiments carried out, both cut ends regenerated a new tip and began to grow. In three, the cut end nearest the original tip regenerated a little earlier than the other end, and it grew a little faster; these differences were not marked.

The behaviour of the marked ectoderm cells was just the same as in the experiments where only one cut was made. The marked cells, which were nearer to the
cut further away from the original tip, behaved as if this were the only regenerating end. Many of the marked endoderm cells were carried by the hydropasm and became attached throughout the length of the stolon, with particular concentrations just back from each growing tip. Thus stained ectoderm cells and stained endoderm cells were found at the end remote from the original tip, but stained endoderm only at the other growing region.

TEXT-FIG. 10. Fate of cells in a hydranth stalk. The diagram shows the position of marked cells (dotted) in the stalk of a normally growing (to the left) and regenerating (to the right) hydranth stalk.

7. Regeneration of hydranth stalks

In these experiments a similar procedure was carried out on growing hydranth stalks as on the stolons. A short length of the stalk was marked with the vital dye and the end of the stalk cut off at various distances from the marked region. Nine experiments were carried out.

Regeneration follows and is of a pattern similar to the regeneration in a stolon; the tip was reformed from adjacent cells and growth renewed. A stalk of normal length was formed and then a normal hydranth differentiated. Marked cells formed that part of the hydranth as expected from their position in the growing
stalk relative to the cut end, and not relative to their position before cutting. Text-fig. 10 illustrates two such experiments.

8. Movement of cells in regressing hydranth

Hydranths occasionally regress. When this happens, a hydranth retracts into the base of the hydrotheca, the tentacles shorten, becoming mere knobs and the buccal region and stomach form a rounded mass of cells. The structure slowly diminishes in size and eventually disappears, leaving a normal looking stalk but an empty hydrotheca. The fate of these cells has been described above.

The stalks of regressed hydranths often regenerate. The stalk begins to grow again, increasing in length until it has grown above the original hydrotheca. Its end then swells and differentiates into a new hydranth. This whole process, regression followed by regeneration, may be repeated a number of times so that, eventually, the hydranth has a very long stalk with three or four cups of previous hydranths decorating it.

By marking cells in the stalk of a regressing hydranth (six experiments), it can be shown that the stalk cells remain in the stalk and do not become detached to be carried away in the hydroplasm as do the cells of the hydranth. Moreover, the stalk cells do not change position, marked cells remain in the same place.

When the stalk begins to regenerate to produce a new hydranth the cells in it behave as they would in a newly formed stalk. Their positions in the stalk relative to the tip again determine the part of the hydranth they will form.

9. Cell division

This was investigated on material fixed in Bouin's fluid, stained in bulk in Ehrlich's haematoxylin and sectioned longitudinally. The incidence of mitoses was determined with a 1:12 oil immersion objective. The Feulgen method of colouring the chromosomes was found to be unsatisfactory, presumably owing to a small amount of DNA in them.

The incidence of mitoses was studied in thirteen stolons, in four of them for over 15 mm., from a growing tip (Text-fig. 11). In addition, five growing hydranth stalks, six developing hydranths and six open hydranths and their stalks were scrutinized. Due to branching, it is rare for any part of a stolon to be further than about 15 mm. from a growing tip, but six pieces of stolon at such distances were investigated.

Mitoses were found as follows:

(a) The number in a stolon was very variable; in five none were found, and the other eight had a variable number up to a maximum frequency of forty in 1·5 mm. of stolon.

(b) Mitoses were never seen in the first 0·3 mm. of a growing stolon.
(c) In the eight stolons where mitoses were seen, they were not randomly distributed; this is seen in the histograms in Text-fig. 11, and is supported by test (in A: \(\chi^2 = 55.1, \text{d.f.} = 14, p < 0.001\); and in B: \(\chi^2 = 22.1, \text{d.f.} = 11, p \approx 0.02\)). The groups of mitoses appear to have no relation to the incidence of hydranths, developing or fully grown.

\[
\begin{array}{c}
\text{Distance from stolon tip—mm} \\
\text{TEXT-FIG. 11. Distribution of mitoses along two stolons (mitoses in hydranths and stalks not included).}
\end{array}
\]

(d) During the development of a hydranth, mitoses were rare, except at one stage and in one position. A great many were seen during the period of development of the hydranth when the tentacles and buccal cavity were differentiating; the cells concerned were located at the junction of these two structures.

(e) Practically all the mitoses were in ectoderm cells; only very rarely was one ever seen in the endoderm.

DISCUSSION

1. Cell division

The investigation has shown that cell division is virtually confined to the ectoderm cells and, apart from one period during the genesis of the hydranth, it is almost entirely confined to the cells of the stolon. Any of the stolonic ectoderm
cells may divide except those in the first 0.3 mm. or so from a growing tip; there is thus no evidence of a terminal meristematic region.

Berrill (1949a) states that in *Obelia*, in a growing stolon, 'cell proliferation has been found only at the extreme tip of both epidermis and endodermis'. He also states that a similar state of affairs exists in the growing hydranth stalk, and then, as the end of the stalk swells, these mitoses disappear. Later (Berrill adds) there is a slow division of the endoderm cells in the distal rim of the disc of the differentiating hydranth. Chapman (1937) also thought there were terminal proliferating regions in which the endoderm was prominent.

*Obelia* and *Clytia* are very closely related genera and it would be expected that the disposition of cell divisions in them might have been similar. As it is, Berrill's and Chapman's accounts could hardly be more different than mine; confirmation is clearly necessary.

The stolonic ectoderm cells which divide in *Clytia* are flattened, epithelial-like cells without any other obvious specialization. Those found in the contractile region do not possess contractile fibrils (Hale, 1960), and are thus not highly specialized; they certainly divide. More highly specialized cells are found in the stolon tip (chitin secretion) and the differentiated hydranth (muscle tails, etc.); mitoses have not been seen in these cells.

It is often assumed that hydroids possess 'interstitial cells' (I-cells) whose main function is to provide new cells for growth, repair, etc. These persistent embryonic cells, well known in the hydras and in *Cordylophora*, are not found in *Clytia*. Neither were they seen by Berrill (1949a, 1949b) in *Obelia*, *Bougainvillea* and *Aselomaris*. In the hydras, McConnell (1932, 1933a, 1933b) found that both ectodermal and endodermal cells divide; not only do the interstitial cells divide but also the more specialized ectodermal musculo-epithelial cells and endodermal secretory cells. Mitoses are not localized in regenerating hydras according to Rowley (1902). After X-radiation, which is reputed to destroy interstitial cells selectively, limited regeneration still occurred in the hydras (Brien & Reniers-Decoen, 1955) and in *Pennaria* (Puckett, 1936). Thus even in hydroids which possess interstitial cells, other, presumably more specialized, cells are also involved in producing new cells.

An interesting feature of the distribution of mitoses in the stolon of *Clytia* is that they are not randomly distributed. Not only are they grouped at intervals down a stolon, but the numbers vary from stolon to stolon and may be absent altogether. The regions of higher mitotic activity do not correspond to growing hydranths. A possible explanation is that waves of cell division, initiated near the tip, pass down the stolon from time to time. Further data are required about this.

The stimulus which sets off the isolated burst of mitotic activity in the developing hydranth is also a mystery. Presumably, insufficient cells migrate into the hydranth from the stolon to complete its construction, and the deficit is made up in this way.
Since mitoses are virtually confined to the ectoderm in Clytia, this layer must produce all the new cells for growth, both for the ectoderm and endoderm. Zwilling (1963) has recently shown that isolated Cordylophora ectoderm is capable of producing a complete hydroid, with endoderm. Gilchrist (1937) obtained the same result with the scyphistoma larva of Aurelia (confirmed by Steinberg, 1963). In these cases it would be interesting to know whether the endoderm is produced by the ectoderm during normal growth. Normandin (1960) reports the reverse situation in Hydra—that the endoderm will reconstitute a new ectoderm. In contrast, Beadle & Booth (1938) failed to obtain regeneration with either isolated ectoderm or endoderm in Cordylophora and Obelia. Although some confusion remains, there is good evidence that at least in some hydroids the two cell layers can be, and in Clytia must be, derived from the progeny of cells in one layer.

There appears to be no difficulty in cells passing from one layer to the other through the mesogloea; according to Roudabush (1933) it happens in Hydra when it is turned inside out.

2. Transportation of cells by the hydroplasm

The experiments described here show that during the ebb and flow of the hydroplasm (Hale, 1960) cells are transferred from regressing hydranths and non-growing regions of the stolon to the growing parts of the animal. The success of this method of cell transfer must depend not only upon the movement of the hydroplasm, but also upon changes in the mutual adhesiveness of the cells. They must lose their stickiness in order to be released into the hydroplasm, but must be mutually sticky with cells in the growing regions.

Hydranth regression is a well-known phenomenon. Thacher (1903), Huxley & De Beer (1923) and Child (1923) all record both cells and cell debris in the hydroplasm during this process. Although the hydroplasm is liable to contain cell debris from food materials, these authors probably believed that it was derived from the death of the hydranth cells. The observations on Clytia do not entirely resolve this point, but the greater amount of solid material in the hydroplasm in animals kept without food for 24 hr. or more, and therefore presumably devoid of food materials, consists of normal cells, as seen in the living animal and in fixed and stained sections. It seems unlikely, therefore, that there is much death of hydranth cells during their regression.

Probably all the cells transported in the hydroplasm will become endoderm cells. Some ectoderm cells enter the hydroplasm as a result of hydranth regression, but they will almost certainly become endoderm cells.

The passive carriage of cells by the transportation system is obviously of fundamental importance in the morphogenesis of this animal. Lefèvre (1898) and Deviney (1934), in studies on the colonial ascidian Perophora, observed that un-specialized undifferentiated cells in the blood-stream contributed to the formation
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of buds. Weiss & Andres (1952) injected dissociated potential pigment cells from embryos of coloured chicks into the blood-stream of embryos of unpigmented varieties (and reciprocal experiments). If these cells reached a location with appropriate conditions, they proliferated and colonized the region.

How far this method of cell transportation is important in morphogenetic processes in other animals remains to be seen. It could not occur in the early embryogenesis of animals before a transportation system had been formed; but it could then operate as Weiss & Andres' experiments show. It might be important in the regeneration of lost parts and in wound healing; it could be valuable as a more rapid means of supplying cells than is offered by 'normal' cell progression or local growth and division of cells.

3. Cell movements of coenosarc

The more usual kind of cell movement which is seen in Clytia is the forward movement of the coenosarc of about the first centimetre or so of the stolon and in a growing hydranth. In the stolon the considerable movement of ectoderm cells towards the tip should result in an increasing collection of cells at the tip, but this does not occur. Some of these cells might be used up in replacing the chitin secreting cells; the considerable secretory activity of these cells might indicate a short life.

It is certain that many of these cells take part in the growth of a hydranth. Possibly the initiation of a hydranth is due to a concentration of ectoderm cells just back from the tip (see Text-fig. 6) and then, as shown above, the ectoderm cells continue to migrate up the stalk, contributing to its formation and the formation of the hydranth. Only a few of the endoderm cells behave in this way, forming the core of the tip of the stalk only; this correlates with the comparatively small accumulation of endoderm cells in the stolon. In a study of Tubularia Steinberg (1955) has related the regeneration of the hydranth with cell movements.

A noticeable feature of the movement of the ectoderm cells is that they move slightly faster at the sides of the stolon; this mainly occurs about 1–2 mm. away from the tip and happens whether the cells become involved in hydranth stalk growth or not. In the regeneration of a stolon, it was shown that the same phenomenon occurred and that the cells at the side of the stolon were more particularly concerned with the reformation of the columnar, chitin secreting tip. This suggests that the different behaviour of the cells on the top and at the side of this part of the stolon might be related to the formation of hydranths and maintenance of the stolon tip respectively.

The experiments and observations on the regeneration of a stolon or a hydranth show that the cells differentiate according to their position in the sequence of cells starting from the tip. It is not known how this pattern is impressed on the cells. A similar situation is found in the 'slug' of the cellular slime mould Dictyostelium discoideum (Bonner et al., 1955; Bonner, 1963).
SUMMARY

1. In *Clytia johnstoni*, mitosis is almost exclusively a function of the ectoderm cells; only very rarely does an endoderm cell divide. Thus new endoderm cells must be produced by the ectoderm cells.

2. Cell divisions may occur anywhere in the stolonic ectoderm except for approximately the first 0.3 mm. from a growing tip. During the development of a hydranth mitoses are rare except for a local wave of ectodermal cell division contributing to the formation of the tentacles and buccal region.

3. Growth only takes place in the first 6–12 mm. of a stolon. The ectoderm cells in the first 2–3 mm. move towards the tip; those on the ‘top’ of a stolon (i.e. away from the substratum) are concerned in the initiation of hydranths and those at the sides and bottom with stolon growth. In the remainder of this region ectoderm cells on the top of the stolon migrate into and form the ectoderm of the hydranths.

4. The hydroplasm contains cells, mostly endoderm, which are derived from non-growing regions of the stolon and from regressing parts of the animal. During the ebb and flow of the hydroplasm, such cells are transported to, and adhere to the growing regions of stolons and to growing hydranths. The endoderm of hydranths is almost entirely supplied by this means.

5. Cells differentiate into the various regions of the hydranth and stolon according to their positions relative to the growing tip.

RÉSUMÉ

*Mouvements cellulaires, division cellulaire et croissance chez l’Hydraire Clytia johnstoni*

1. On décrit des recherches sur le rôle des mouvements cellulaires et de la division cellulaire dans la croissance de l’Hydraire colonial *Clytia johnstoni*. On a mis les mouvements cellulaires en évidence par marquage au sulfate de bleu de Nil et observation pendant la croissance normale et la régénération. La fréquence des mitoses a été déduite de l’examen des coupes histologiques. Ces caractères et d’autres qui s’y rapportent révèlent le mode de croissance suivant.

2. La région du stolon contractile rythmiquement provoque l’elongation des premiers millimètres (6–12) du stolon.

3. Dans cette région de croissance, les cellules ectodermiques s’accroissent par division. Des cellules endodermiques s’y ajoutent aussi, provenant de cellules en suspension dans l’hydroplasme; comme cet hydroplasme est animé de mouvements de flux et de reflux, les cellules adhèrent à lui de préférence.

4. Les cellules ectodermiques se divisent aussi dans les régions du stolon qui ne croissent pas et leur descendance doit peupler l’endoderme car une cellule endodermique ne se divise que très rarement.

5. L’endoderme des régions du stolon qui ne croissent pas forme une réserve
Cell movement, cell division and growth in Clytia

de cellules. Les cellules en suspension dans l'hydroplasme dérivent partiellement de lui et partiellement des régions en régression de la colonie.

6. Les cellules ectodermiques se déplacent vers l'extrémité dans les 2 ou 3 premiers millimètres d'un stolon en croissance et tendent à s'y accumuler, ébauchant probablement de nouveaux hydranthes. D'autres cellules ectodermiques migrent dans l'hydranthe en croissance à partir de la région de croissance du stolon.

7. Les cellules ectodermiques du 'dessus' du stolon (c'est-à-dire éloignées du substratum) sont particulièrement liées à la formation et à la croissance des hydranthes, et celles ces 'côtés' et du 'bas' à la croissance du stolon.

8. Une vague locale de divisions cellulaires ectodermiques contribue à la formation des tentacules et de la région buccale d'un hydranthe.

9. L'endoderme d'un hydranthe dérive presque entièrement de cellules transportées dans l'hydroplasme.

10. Les cellules se différencient dans les différentes régions de l'hydranthe et du stolon selon leur distance à partir de l'extrémité sécrétant la chitine.

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