Cell proliferation and morphological patterns in the hydroids *Tubularia* and *Hydractinia*

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**INTRODUCTION**

Morphogenesis has been extensively studied in many hydroids, both during normal growth (Kühn, 1914; Berrill, 1961) and during regeneration (Tardent, 1963). Less is known about the patterns of cell proliferation underlying changes in tissue form. In several cases where cell division has been studied, surprisingly little direct correlation was found between areas of apparent morphological growth and patterns of cell proliferation (Overton, 1963; Crowell, Wyttenbach & Suddith, 1965; Shostak, Patel & Burnett, 1965; Wyttenbach, 1965; Campbell, 1967a, b).

To explore further the relations between tissue growth and body form, I have examined histologically hydroids of two genera, *Tubularia* and *Hydractinia*, each of which has morphological peculiarities. *Tubularia* possesses two whorls of tentacles and one whorl of gonophores, and thus has as many distinct hydranth regions as any hydroid. In the *Hydractinia* colony, four morphologically distinct polyp types are present. In both species, axial as well as radial mitotic patterns were found which can be partly related to the morphological characteristics of the polyps.

**METHODS AND MATERIALS**

Individuals of *Tubularia croeca* were collected from floats in upper Newport Bay, California; those of *T. larynx* from buoys at Arcachon, France. Polyps were fixed for 12 h in the following solutions: 5 ml formalin; 2 ml acetic acid; 0.5 g K$_2$Cr$_2$O$_7$; 25 ml alcohol; 18 ml distilled water; 50 ml sea water.

*Hydractinia echinata* colonies living on shells inhabited by hermit crabs were dredged in Long Island Sound and kept in a running sea-water table for 12–24 h before fixation for 1–4 days in Lavdowsky's fluid (Gurr, 1956).

Polyps were embedded in paraffin and cut in 10 μ thick serial sections perpendicular to their long axes, and stained with Ehrlich's hematoxylin and

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Biebrich scarlet. Individual polyps of both species varied greatly with regard to the abundance of mitotic figures in their tissues. Counts of dividing cells on all sections of a polyp were made on those individuals with the highest mitotic activity. Mitotic cells were mapped out in about forty *Hydractinia* polyps and in sixteen *Tubularia* polyps. The observed patterns are illustrated in this paper by a map of one polyp of each type; these were representative of the general mitotic patterns found in other individuals except where noted. Illustrations above each graph of mitotic cell distribution indicate the relation between axial position and morphology of the individual polyp represented.

The term ‘interstitial cell’ is used here to designate any ectodermal cell other than an epithelial cell. Epithelial cells of the endoderm are termed ‘digestive cells’.

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**RESULTS**

The tubularian hydranth is pear-shaped with a whorl of gonophore stalks inserted between two whorls of tentacles (see Text-fig. 1). The mouth is at the distal tip. The wall of the hydranth and stalk generally consists of an ectoderm and endoderm, separated by a mesolamella, but in some regions this simple bi-layered construction shows elaboration. At the base of the distal tentacle
Cross-sections of *Tubularia croeca* hydranth.

Fig. A. Near oral tip. Scale, 500 μ.
Fig. B. At base of distal tentacles. Scale, 500 μ.
Fig. C. Just above gonophore insertion. Scale, 500 μ.
Fig. D. Through distal tentacle region, showing perradial position of a mitotic digestive cell (arrow). Scale, 20 μ.
Figs. A-C. Cross-sections of *Hydractinia echinata* gastrazooid.

Fig. A. Near oral tip of young polyp. Scale, 50 μ.

Fig. B. At level of primary tentacle insertion, of the polyp shown in Fig. A. Arrows indicate original tentacles, which insert between taeniolae. Scale, 50 μ.

Fig. C. Just below tentacle insertion, showing perradial position of a mitotic digestive cell (arrow). Scale, 50 μ.

Fig. D. Section through tentacular knobs of a gonozooid, showing mitotic digestive cell (arrow). Scale, 50 μ.

Fig. E. Section just above the first gonophore in a gonozooid, showing small digestive cell size and mitotic activity (arrows). Scale, 50 μ.

Fig. F. Section through upper portion of a spiral zooid, showing dividing digestive cell near apex of lumen (arrow). Scale, 50 μ.

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whorl the gastrodermal core of the tentacles runs along the hydranth mesolamella; the ectoderm forms a single sheath around both the hypostome and the tentacular gastrodermal bases (Plate 1, figs. A, B). In the hypostome the endoderm thickness is not uniform but forms longitudinal ridges (Plate 1, figs. A, B). These ridges, or ‘taeniolae’ (Hamann, 1882), result from some gastrodermal cells being more highly columnar than others. In young specimens these ridges are quite regular and alternate with radii of the distal tentacles. Thus, if the positions of tentacles are termed as ‘radial’ (or ‘perradial’), the taeniolae may be termed as ‘interradial’ in position.

The taeniolae are sharply defined and regular distally, but branch and decrease in size and regularity as they run in a proximal direction. They are not discernible at the level of the gonophore stalks (Plate 1, fig. C).

Below the proximal tentacles an additional tissue layer of gastrodermal origin is interspersed between the ectodermal and endodermal epithelia. It is composed of large vacuolated cells. It is continuous with the tentacular endoderm in T. croeca, but is separated from endoderm in mature T. larynx.

The axial distribution of mitotic figures in one T. croeca hydranth is shown in Text-fig. 1. This pattern is representative of those found in individuals of both species studied. Epithelio-muscular cells show the most broadly distributed mitotic activity. These cells are found dividing in all axial portions of the hydranth. Interstitial-cell mitotic figures are more numerous in lower parts of the hydranth, where these cells are more common. Digestive-cell mitotic activity is greatest in the central regions of the hydranth. No mitotic figures of these cells were found in the most distal hypostomal portions, where the cells are most abundant and densely packed. In all individuals digestive cell mitotic activity was absent or slight above the insertion of the distal tentacles and rose to a maximum between the tentacles and gonophores. Mitotic figures in tentacles occurred only near their bases.

Digestive-cell mitotic figures in taeniolate regions were not randomly disposed.
around the hydranth. They were found predominantly in the grooves between adjacent endodermal ridges; that is, in perradial positions (Plate 1, fig. D).

The radial position of a dividing cell may be quantitated by dividing the number of cells separating it from the perradial direction by the total number of cells in the taeniolate profile. Thus the radial position of a cell in a perradial location would be 0, and that of a cell in an interradial (taeniolate) position would be 0.5.

The radial positions of forty-two mitotic digestive cells are represented in Text-fig. 2a.

The radial positions of mitotic gland cells (Text-fig. 2b) appear to be more random than those of digestive cells.

B. Hydractinia

Four polyp types are present in the Hydractinia colony: gastrozooid (feeding polyp), gonozooid (reproductive polyp), spiral zooid and tentaculozooid (see Müller, 1964).

Histologically, the Hydractinia gastrozooid resembles the distal portion of a Tubularia hydranth. The endoderm exhibits a taeniolate organization which is most regular in the hypostome. Taeniolae become less regular proximally. Radial planes of taeniolae alternate with planes of tentacle insertion. This is especially true in young gastrozooids (Plate 2, figs. A, B), where the radial organization generally has a fourfold symmetry. In older polyps both tentacles and taeniolae increase in numbers; however, tentacles appear more rapidly than new taeniolae, so that some endodermal ridges are along tentacular radii.
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The axial distributions of mitotic figures in a young gastrazooid are shown in Text-fig. 3. Endodermal mitotic figures are absent from the most distal hypostomal region, but are present in other regions. Mitotic epidermal cells occur at

Text-fig. 4. Radial distributions of all dividing endodermal cells (dots) in two young *Hydractinia* gastrozooids. Outlines indicate the configuration of the endoderm at the base of the tentacles.

Text-fig. 5. Axial distribution of mitotic cells in a *Hydractinia* gonozooid. The three arrows indicate positions of mature gonophores not shown on illustration (104 histological sections).

all axial levels but are rare in the distal hypostome. In older gastrozooids there is a greater tendency toward localization of mitotic cells near the tentacle whorl, although dividing cells are still found at all column levels.
The endodermal mitotic cells are localized primarily in or near the radial (tentacular) planes (Text-fig. 4). As in *Tubularia*, this feature is more apparent in digestive cells than in gland cells.

The axial distributions of mitotic figures in gonozooids and spiral zooids are shown in Text-figs. 5 and 6. Both polyp types have mitotic figures associated with the abortive tentacular knobs (Plate 2, fig. D). Below this 'hydranth region' the gonozooid shows an intense localization of dividing cells at the base of the constricted part of the column (Text-fig. 5; Plate 2, fig. E), at which point gonophores are originated. Below this region, mitotic figures are found sporadically along the column mainly in the ectoderm near the bases of developing and mature gonophores. Mitotic activity is also distributed along the length of the spiral zooid in both ectoderm and endoderm. Mitotic figures appeared to be axially clustered in all spiral zooids examined.

The endoderm in the gonozooid and spiral zooid does not show the typical taeniolate organization as found in the gastrozooid hypostome. The gastric lumen of the entire spiral zooid and of the gonozooid above the constriction is frequently, however, spindle-shaped or polygonal in cross-section (Plate 2, fig. F). This may reflect a radial organization of the endoderm basically similar to the taeniolate organization, but modified by the cells being highly vacuolate and therefore sparse. Mitotic figures along the spiral zooid and above the constricted region in the gonozooid are generally, but not always, at an apex of the lumen as seen in cross-section (Plate 2, fig. F).

In all three polyp types, ectodermal mitotic figures are not localized with
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respect to the gastrodermal taeniolae. In the spiral zooids, however, mitotic figures were found largely on the outer side of the coiled polyp, the side which also shows the greatest development of mesogloea and ectodermal muscular processes.

DISCUSSION

The mitotic patterns of Tubularia and Hydractinia show broad resemblances to one another, in that mitotic figures are present in most parts of the polyp except for the distal tentacle regions and in the distal hypostomal endoderm. In this respect they are qualitatively similar to mitotic patterns reported in hydra (Campbell, 1967a, b).

The most noteworthy feature of the mitotic patterns described is the radial localization of mitotic figures in the taeniolate hydranth regions. This radial organization is coincident with the polyp’s radial symmetry as manifested by the gastrodermal structure and the placement of tentacles. Braverman (personal communication) found that the nuclear incorporation of $^3$H-thymidine was similarly localized in the endodermal furrows in Podocoryne.

The radial aspects of hydroid morphology have not been adequately studied. In Hamann’s (1882) neglected article the taeniolate structure provided the basis of a hydrozoan classification system. In the early literature the taeniolate organization was often described (see Kühn, 1914). In the more recent literature, there is a tendency to consider taeniolae as endodermal folds, such as are found in most highly extensible tissues. However, in Hydra, for example, the gastric portion of the column undergoes much greater extension during feeding than does the hypostomal region; the mouth does not generally open wide during ingestion, and when the hydra is not feeding the gastric region frequently is more constricted than the hypostomal area. Yet the taeniolate structure in hydra is quite restricted to the hypostomal area, and thus is not correlated with tissue distension.

A second point suggesting that the taeniolate organization is not a superficial one is the phyletic breadth of its occurrence. It is present in Siphonophora, as was pointed out by Hamann (1882). It is also present in the young stages of scyphozooan and actinian development, and the taeniolate structures in these forms appear to provide the architectural primordium of the later radial elaborations in these groups. It occurs in almost all hydrozoans. Thus the taeniolate organization is exceedingly general among coelenterates, although it is not present in the group now known as thecate hydroids.

The radial organization of polyps should probably be considered as fundamental as the axial organization. This is particularly true because radial symmetry is one of the primary characteristics binding members of the phylum Coelenterata in a group. An example of the developmental interactions and importance of different aspects of radial organization is apparent during Tubularia regeneration (Campbell & Campbell, 1967).
The correspondence between morphology and growth patterns, illustrated in the polymorphic forms of *Hydractinia*, emphasizes the possibility that some patterned growth may be the cause of morphological specialization. Spiral zooids, for example, are peculiar in being very long as well as in coiling away from the nearest colony edge which is maintained stationary by irritation from a hermit-crab's movements. It is possible that this irritation of the colony edge promotes tissue growth on adjacent sides of nearby polyps, thus leading to the exaggeration and asymmetry of elongation of the spiral zooid. Epidermal mitotic figures were found mainly on this side of the polyps, as predicted by this hypothesis.

Alternatively, morphogenetic and proliferation patterns could be controlled in common by other processes. The wealth of experimental data on the growth of hydroids emphasizes the developmental plasticity of their specialized tissues, but no critical test of the necessity of cell division for morphogenesis has been made.

Another interesting aspect of the mitotic patterns presented here concerns the homology of hydranth parts in different species of hydroids. In the *Tubularia* hydranth the following major axial regions may be distinguished proceeding from the oral pole: hypostome, distal tentacle whorl, gonophore (reproductive) region, and proximal tentacle whorl. Many hydroid polyps are lacking in some of these regions, but the deleted parts are almost always the proximal ones. In the *Hydractinia* gonozooid, proximal tentacles are lacking; in the gastrozooid, proximal tentacles and reproductive organs are missing. Structures are rarely absent other than in this proximal–distal order. For example, there is no polyp lacking a reproductive region but possessing proximal tentacles. The existing series of polyp forms fits with the idea (see Rose, 1957) that development begins at the distal pole and proceeds proximally, if it is supposed that different polyp types represent different proximal extents to which development has continued. This would indicate developmental homology between similar axial regions of many hydroids (for example, the tentacles of *Hydra*, which are between hypostome and reproductive regions, the tentacles of *Hydractinia* gastrozooid and gonozooid, and the distal tentacles of *Tubularia*).

The mitotic patterns reported here support this interpretation of comparative hydroid structure. In all polyps studied here (and in hydra) mitotic activity is found mainly in the region between the distal tentacles and reproductive structures. In all polyps, the endodermal mitotic activity is radially organized in the hypostome and the vicinity of the distal tentacles, but not in the vicinity of the reproductive structures. The distal hypostomal endoderm is devoid of mitotic activity. There is thus considerable similarity in the mitotic patterns of the different polyp types.
SUMMARY
1. Mitotic figure distributions in the hydroids Tubularia and Hydractinia are related to both the axial and radial morphological organizations of the polyps. Cells divide in all regions of the body except at some extremities, but the abundance of dividing cells varies along the polyp axis. For example, in Hydractinia gonozooids mitotic figures are concentrated in the constricted part of the column, and in the Tubularia hydranth they are most abundant just above the whorl of gonophores.

2. The polyps of both species of hydroids have a similar radial organization. In the distal hydranth portions this is manifested both in the positions of tentacles and in the positions of longitudinal gastrodermal ridges called taeniolae. Taeniolae generally are spaced between tentacles.

3. In Tubularia and in Hydractinia gastrozooids, mitotic digestive cells are radially localized between taeniolae, that is, along the tentacular radii. The taeniolate organization appears to be a fundamental one in view of its wide phyletic distribution and its relation to other morphological and mitotic patterns.

4. The similarity in mitotic patterns among different polyps is consistent with wide homologies existing between the structures of diverse hydroid types.

RÉSUMÉ
1. Les distributions de figures mitotiques chez les hydroides Tubularia et Hydractinia sont en relation avec l'organisation morphologique axiale et radiale des polypes. Les divisions cellulaires sont réparties sur l'ensemble du corps de l'animal, sauf à certaines extrémités, mais leur abondance varie le long de l'axe du polype. Par exemple, dans les gonozooides d'Hydractinia les figures mitotiques sont groupées à la constriction de la colonne, tandis que chez l'hydranthe de Tubularia elles dominent juste au dessus de l'anneau des gonophores.

2. Les polypes de ces deux espèces d'hydroïdes ont une organisation similaire. Dans les régions distales de l'hydranthe, cela se manifeste par la position des tentacules et des crêtes longitudinales du gastroderme appelées 'taeniolae'. Les 'taeniolae' alternent avec les tentacules.

3. Chez Tubularia et dans les gastrozooides d'Hydractinia, les cellules digestives en division sont réparties radialement entre les 'taeniolae', soit le long des rayons tentaculaires. L'organisation des 'taeniolae' apparaît être fondamentale par sa vaste distribution phylétique, par sa relation aux répartitions mitotiques et à d'autres modes d'organisation morphologique.

4. La similarité des distributions mitotiques parmi les différents polypes est compatible avec la présence d'homologies existant entre les structures des divers types d'hydroïdes.
REFERENCES


(Manuscript received 19 December 1966)