

Im Gefäßinnern (Figur 2, Lu) bildet die Basalmembran kein dichtes Endokard. Dort ist sie zwar an vielen Stellen mehr als doppelt so mächtig wie ihre dem Hämocoel zugewandten Bereiche; die lockere Textur zeigt aber nur an wenigen Stellen Bündel fibrillärer Verdichtungen (Figur 2, Pfeilspitzen). Diese weisen nie ein periodisches Quermuster auf, welches auf Kollagenfasern hindeuten könnte. Da die Basalmembran eine Passage von Stoffen nicht verhindert, ist die beschriebene Gefäßwand porös.

Diese Struktur erlangt vor allem deshalb Bedeutung, weil sie auch Gefäße auszeichnet, die sich zwischen den Lappen endokriner Kopfdrüsen<sup>4</sup> (Figur 1, Kdr) befinden. Über einen engen Hämolympfsinus (Hs) könnten von den Drüsenzellen abgegebene Stoffe bei der diastolischen Erweiterung des Gefäßes leicht in dessen Lumen gelangen, wegen einer Verengung der Poren bei der Systole aber in erster Linie rasch und gerichtet mit dem Häm-

lymphstrom innerhalb des am Ende offenen Gefäßes zu entfernten Körperregionen transportiert werden.

**Summary.** A hitherto unknown structure of the wall of blood vessels in *Scutigera coleoptrata* is described. It is lacking in a continuous adventitia and a dense endocard, and between its muscle fibres there are irregular clefts filled with stroma.

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<sup>4</sup> J. ROSENBERG, *Experientia* 29, 690 (1973).

### Hydroid Stolon Elongation: Evidence of a Distal Locomotory Organ in *Proboscoidactyla flavicirrata*

Stolon elongation in colonial hydroids has frequently been attributed to cellular proliferation in a distal meristematic zone<sup>1</sup>. However, recent studies indicate mitotic activity is sharply reduced or absent in the terminal portions of stolons<sup>2-5</sup>. Nevertheless, there is reason to believe that the specialized terminal stolon section or 'stolon tip' may figure prominently in the elongation process. The tips of elongating stolons 'pulsate', alternately extending and partially retracting on a regular cycle<sup>5-7</sup>. Isolated stolon tips can advance autonomously<sup>5,6</sup>. Experiments described here illustrate the importance of stolon tip activity during stolon elongation in *Proboscoidactyla flavicirrata*.

**Materials and methods.** In nature, *Proboscoidactyla* is a commensal found on the rims of tubes secreted by certain sabellid worms. When flattened pieces of worm tube overgrown with hydroids were held against cover slips and kept in recirculating sea water at 11 to 13°C, new stolons extended from the edges of the tube fragments and across the glass surfaces. Tip pulsations were studied by time-lapse cinematography. For mitotic analysis, whole mounts of the thin transparent stolons were fixed in Fleming's fluid without acetic acid and stained with Heidenhain's iron hematoxylin. Prior to fixation some stolons were exposed to radiation from a Cobalt 60 therapy unit (9950 rad over 15 min). During filming and irradiation experiments the temperature of the 2 mm layer of sea water in the specimen dish was maintained at 12°C by a Cambian electronic cooling unit. A covering of thin plastic film retarded evaporation from the dish.

**Results and discussion.** The stolon tip can be distinguished from the rest of the stolon by its enlarged vacuolated gastrodermal cells and, in most cases, its greater overall girth. No dividing cells were seen in the tips of 16 stolons, although in each of 6 others, there was a single mitotic figure in the tapered transition zone connecting the enlarged tip to the proximal stolon.

Stolon tips and portions of tips as short as 225 µm (containing fewer than 300 nuclei) continued to progress across the substrate for as much as 55 h after isolation. Although each isolated tip moved as a whole, it gradually lengthened also (Figure 1). No mitotic figures were found in any of 12 isolated stolon tips which had been actively moving just before fixation. Severed and migrating stolon tips of *Proboscoidactyla* pulsed, as has been reported for other hydroid species<sup>5,6</sup>. When 3 stolon tips were filmed before and after isolation, the most obvious behavioral changes were increases in the uniformity of their pulsations; the changes resulting, perhaps, because the isolated tips were no longer affected by asynchronous movements of the hydroplasm generated in other parts of the colony. Like normal stolons, each isolated tip secreted and advanced through a tubular perisarc suggesting that stolon tips may interact with their perisarc to propel themselves. Proximally-directed waves of contraction apparently cause the forward progress of holdfast tips in *Corymorpha*, structures analogous to stolons in many respects<sup>8</sup>.

Irradiated stolons continued to elongate and pulsate as usual. No mitotic figures were found in any part of the 30 stolons fixed and examined 8, 14, or 24 h after irradiation.

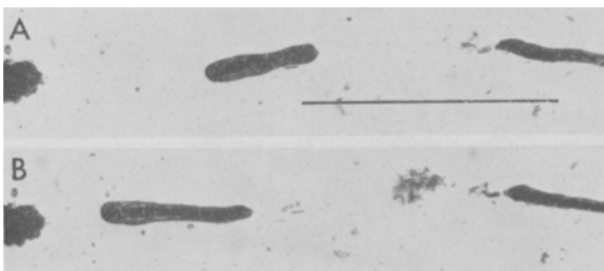


Fig. 1. Photographs of an isolated, migrating stolon tip taken 2 h apart. Scale bar = 5 mm.

<sup>1</sup> N. J. BERRILL, *Growth, Development and Pattern* (W. H. Freeman and Co., San Francisco 1961).

<sup>2</sup> M. BRAVERMANN, *J. Morph.* 135, 131 (1971).

<sup>3</sup> R. D. CAMPBELL, *Biol. Bull. mar. biol. Lab., Woods Hole* 135, 96 (1968).

<sup>4</sup> J. OVERTON, *J. Cell Biol.* 17, 661 (1963).

<sup>5</sup> L. J. HALE, *J. Embryol. exp. Morph.* 12, 517 (1964).

<sup>6</sup> C. R. WYTENBACH, *J. exp. Zool.* 167, 333 (1968).

<sup>7</sup> L. V. BELOUSSOV, L. A. BADENKO, A. L. KATCHURIN and L. F. KURILO, *J. Embryol. exp. Morph.* 27, 317 (1972).

<sup>8</sup> R. D. CAMPBELL, *Biol. Bull. mar. biol. Lab., Woods Hole* 134, 26 (1968).

Buds of hydra also elongated despite total inhibition of mitotic activity by radiation<sup>9</sup>. Hydranth initiation increased in *Podocoryne* colonies subjected to starvation<sup>10</sup>, although mitotic activity would be expected to decrease under these conditions. Localized cellular proliferation is probably not a common morphogenetic mechanism in the hydrozoans.

No elongation was detected in 7 stolons during a 4 h interval between tip ablation and fixation, although the presence of dividing cells was subsequently confirmed. Tipless stolons left undisturbed for 5 h or more usually regenerated motile tip regions and resumed elongation. Isolated sections of stolons sometimes regenerated motile tips at both ends and extended in both directions.

The observations described above suggest that active motility of the stolon tip in *Proboscoidactyla* does not depend upon cellular proliferation and serves a necessary function in stolon elongation. This is not to say that physical stretching is the only way stolons increase in length. Some mitotic activity does occur in the proximal portions of non-irradiated stolons. It has also been suggested that viable cells migrate to growing regions of

hydroid colonies through the gastrovascular cavity<sup>2,5</sup>, although this seems improbable in isolated stolon tips.

Whether cellular proliferation and migration actually figure significantly in the normal elongation of *Proboscoidactyla* stolons cannot be resolved on the basis of the evidence given. However, the stolon tip is clearly identified as a motile organ whose locomotory activity is an essential concomitant of stolon elongation.

**Résumé.** Les portions distales des stolons du *Proboscoidactyla flavicirrata* sont des organes moteurs dont les activités jouent un rôle essentiel dans l'élongation du stolon.

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<sup>9</sup> S. G. CLARKSON and L. WOLPERT, *Nature*, Lond. 214, 780 (1967).

<sup>10</sup> M. BRAVERMANN, *Biol. Bull. mar. biol. Lab., Woods Hole*, 139, 404 (1970).

### Micronuclear Mitosis in the Life Cycle of a Plurimicronucleate Strain of *Euplotes crassus*<sup>1</sup>

In *Euplotes crassus*, a ciliate marine species, it has been found<sup>2</sup> that starvation may bring about a physiological reorganization of pellicular structures as well as a mitotic division of the single micronucleus, not accompanied by cytokinesis. By successive reorganization processes, some micronuclei may accumulate in a common cytoplasm. However, only 1 micronucleus divides during the vegetative reproduction which ensues after reorganization, while the supernumerary ones are sorted out in daughter cells without resorption. In this way unimicronucleate cells are again formed as asexual reproduction goes on.

A search has been made to find out whether any one of the nuclei present in the cytoplasm can enter mitosis in the life cycle of the ciliate, or if the nuclear products of the same nucleus always take part in the division process. It must be noted that all nuclei, no matter how many, are derived through mitosis from the same mother-nucleus and that all of them should be in G<sub>2</sub> phase as DNA duplication stage begins at late telophase in *Euplotes*<sup>2,3</sup>.

The routine techniques used are reported in detail elsewhere<sup>2</sup>. Only those strictly necessary will be described here. As material, strain 17 of *E. crassus*, Pisa collection, has been used extensively. Lines of this strain consists of uni- and plurimicronucleate cells.

**Results.** First of all, it was ascertained by spectrophotometric analysis that the amount of Feulgen positive material of different micronuclei in resting stage inside a cell, and of different cells, averaged around the same arbitrary units (Figure 1), confirming that all nuclei are

in G<sub>2</sub> phase. This analysis was carried out using the apparatus described by BENEDETTI and VIOLA MAGNI<sup>4</sup>.

Cells of a plurimicronucleate line were allowed to divide 2 or 3 times in culture fluid with 3H-thymidine

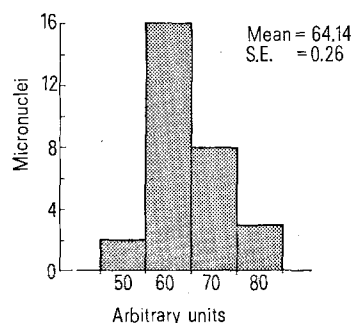


Fig. 1. Content of micronuclear Feulgen positive material expressed in arbitrary units (abscissa); in ordinate, number of micronuclei. The data were derived from 14 cells.

<sup>1</sup> Supported by a grant from Consiglio Nazionale delle Ricerche (C.N.R.).

<sup>2</sup> P. LUPORINI and P. BRACCHI, *Monitore zool. ital.*, in press (1973).

<sup>3</sup> D. M. PRESCOTT, R. F. KIMBALL and R. F. CARRIER, *J. cell Biol.* 13, 175 (1962).

<sup>4</sup> P. A. BENEDETTI and M. P. VIOLA-MAGNI, *J. scient. Instrum.* 43, 141 (1966).

Life cycle phases	No. specimens examined	Unimicronucleate specimens	Plurimicronucleate specimens	Specimens with 1 labelled micronuclei	Specimens with 2 labelled micronuclei
Asexual reproduction	577	406	171	577	—
Conjugation	215	155	60	215 <sup>a</sup>	—
Reorganization	176	59	75	134	42
1st Post-reorganization fission	438	223	191	414	24

Micronuclear behaviour in the plurimicronucleate strain 17 in different life cycle phases. Strain 17 includes uni- and plurimicronucleate cells. Other explanations in the text. <sup>a</sup> In conjugation, the labelled micronucleus is that undergoing the first pregamic fission.