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MORPHOGENESIS

Mechanics of Growth Pulsations as the Basis of Growth and Morphogenesis in Colonial Hydroids

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Abstract—Growth and shaping in colonial hydroids (Hydrozoa, Cnidaria) are realized due to the functioning of special colony elements, growing tips located at the terminuses of branched colony body. Unlike in plants, the growing tips of colonial hydroids are sites of active cell movements related to morphogenesis and lacking proliferation. The activity of hydroid growing tips is expressed as growth pulsations: cyclic repetitions of their apex extensions and retractions. The parameters of growth pulsations are species specific and related to the shape of a forming element. Here, the succession of cell movements and changes in mutual arrangement within the growing tip are described in detail at all pulsation phases. The role of the inner cell layer in the tip activity was demonstrated for the first time. Relationships between the growing tip parameters, length and diameter, and pulsations are discussed. A scheme is proposed for cyclic processes in both epithelial layers. An explanation is provided for the two-step mode of growth pulsations with relative independence of the main phases. It was proposed that successive activities of the tip ecto- and endoderm serve as driving forces provided there is a hard outer skeleton. This scheme makes it possible to explain some patterns of growth and morphogenesis in colonial hydroids, such as gradually increasing growth rate of a new tip and its maximum growth rate, differences in the parameters of growth pulsations between shoot and stolon tips, shoot base inclination towards the stolon tip, etc., and provides a basis for further improvement of the model of morphogenesis in hydroids.

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The phenomenon of morphogenesis or appearance of a new from and structure during development remains one of the largest and so far unsolved problems of current biology. The "rules of morphogenesis," i.e., more or less universal algorithms allowing to deduce forms at each stage of morphogenesis based on the preceding one, are in most cases unknown.

Colonial hydroid polyps possess some advantages as model species for studying and simulating morphogenesis. Their morphology is relatively simple and evolutionarily ancient. The hydroid polyp bud starts its development from a very simple geometrical form, which becomes later monotonously complicated only through changes in local curvature. Given species specific differences, the nascent forms have some deep similarities not only with each other, but also with rudiments of other higher organized animals. Hence, any "morphogenetic rules" revealed through simulation of the development of hydroid polyps can have a more general significance (Beloussov and Grabovsky, 2003).

Colonial hydroid polyps can be represented as a system of branching tubes on whose ends feeding zooids (hydranths) or growing tips are located (Fig. 1). Growing tips are elements of the colony that provide for upbuilding of the colony body and are responsible for morphogenesis of all main elements (Kosevich, 2004). The growth and morphogenesis of colonial hydroid polyps are based on growth pulsations: successive rhythmic extensions and retractions of the shoot and stolon growing tips. Pulsating growth was described for many organisms (Labas *et al.*, 1981; Beloussov *et al.*, 1993) but is most characteristic for hydroid polyps. Growth pulsations in hydroids were described already in the first half of the last century (Saint-Hilaire, 1930; Berrill, 1949) but high emphasis was put on them in the second half of that century (Beloussov, 1961, 1973; Wyttenbach, 1968, 1974; Beloussov *et al.*, 1972). The parameters of growth pulsations were later extensively studied and their main phases were identified (Wyttenbach, 1968), species specificity of the form of growth pulsation was shown (Beloussov, 1961, 1975; Wyttenbach, 1969; Beloussov *et al.*, 1988), and the ratio between the growth pulsation parameters and shape of rudiments was studied (Beloussov, 1961; Beloussov *et al.*, 1984).

One of the main problems, whose solution would significantly facilitate the understanding of growth and morphogenesis in colonial hydroids, is the mechanism of growing tip functioning in the colony. It can be said now that the rhythmic activity of tips is based on periodic proximodistal waves of contractions and relaxations of their ecto- and endodermal cells (Wyttenbach, 1968; Beloussov *et al.*, 1980; Zaraisky *et al.*, 1984). The available data speak in favor of nonmuscle nature

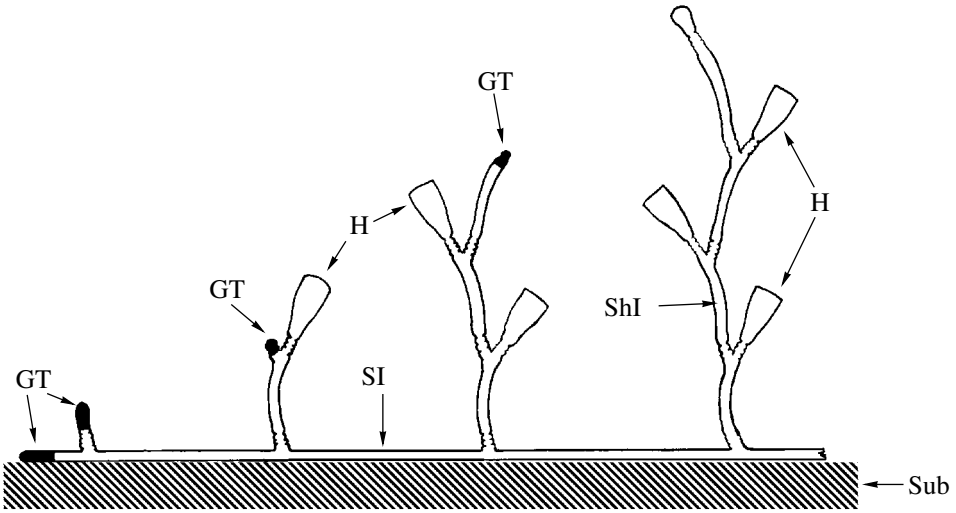

Fig. 1. Schematic diagram of the structure of a hydroid colony. GT, growing tip; H, hydranth; ShI, shoot internode; SI, stolon internode; Sub, substrate.

of cellular movements of the tip (Letunov, 1981; Zaraisky *et al.*, 1984). Based on numerous observations and experiments, Beloussov and his colleagues (Beloussov *et al.*, 1972, 1988, 2000; Beloussov, 1973; Beloussov and Dorfman, 1974; Zaraisky *et al.*, 1984; Labas *et al.*, 1992; Kazakova *et al.*, 1997) determined the cellular (cytophysiological) mechanisms underlying the movements of ectodermal cells of the growing tip, which are responsible for pulsating growth. However, the role of endoderm was not discussed in the proposed scheme of processes occurring in the growing tip during growth pulsation (Zaraisky *et al.*, 1984; Beloussov and Grabovsky), which complicates, in many cases, the explanation of observed morphogenetic processes.

Here, the processes occurring in the growing tip during growth pulsations are described in detail and the main parameters of growth pulsations and their relationships with each other and with characteristics of growing tips are analyzed. Based on the presented data, a scheme is proposed for cyclic processes occurring in both epithelial layers of the growing tip, which allows explanation of some patterns of growth and morphogenesis in hydroid polyps.

MATERIALS AND METHODS

Materials. The main studies were carried out on colonial hydroids of the family Campanulariidae (suborder Thecaphora): *Gonothyraea loveni* (Allman, 1859), *Laomedea flexuosa* (Hincks, 1861), and *Obelia longissima* (Pallas, 1766). The colonies of these species have the same organization: sympodially branching shoots running from tubular stolons of the hydrorhiza, which creep over the substrate. Shoot and stolon internodes, hydranths (feeding zooids), gonangia (zooids related to sexual reproduction of hydroids), and growing tips are structural elements of the colony (Fig. 1). Soft tissues of the colony (coenosarc) are surrounded by rigid organic skeleton based on sclerotized chitin, perisarc, whose morphological characteristics are used as the main diagnostic features in classification of hydroids of this group. The perisarc of the above species in the area of growing tips is relatively thin, unstained, and transparent, thus allowing observations of the movements of individual cells and cell layers.

Growth and morphogenesis of shoots and stolons proceed at the expense of functioning of special morphogenetic elements of the colony: terminally located growing tips. Morphologically, the growing tip differs from the rest of colony body. Its apical part has a semispherical shape, while ectodermal cells on its sides are in constant contact with the perisarc. The ectodermal cells differ in their shape extended in the apicobasal direction from those in other parts of the colony (Fig. 2) (Hale, 1960, 1963; Wyttenbach, 1968; Zaraisky *et al.*, 1984; Kosevich, 1990). The shape and arrangement of endodermal cells of the growing tip are not that regular and they are characterized by significant vacuolization, unlike those in more proximal regions of the coenosarc (Beloussov *et al.*, 1989) (Fig. 2). Both cell layers are separated by a thin basal membrane, mesoglea. The perisarc surrounding the growing tip tissues is secreted on its apical semispherical surface: here, it remains soft and elastic, while on the tips sides it hardens and is not able of changing its shape (Kossevitch *et al.*, 2001).

Under the constant conditions of colony development, the sizes of stolon growing tip (length and diameter) remain almost unchanged (Kosevich, 1990). The typical growing tip of a rapidly growing stolon in *G. loveni* is 250–350 μm long and has a diameter of 125–130 μm. The length of endodermal tube at phase *C* (see below) is 200–300 μm and its outer diameter is

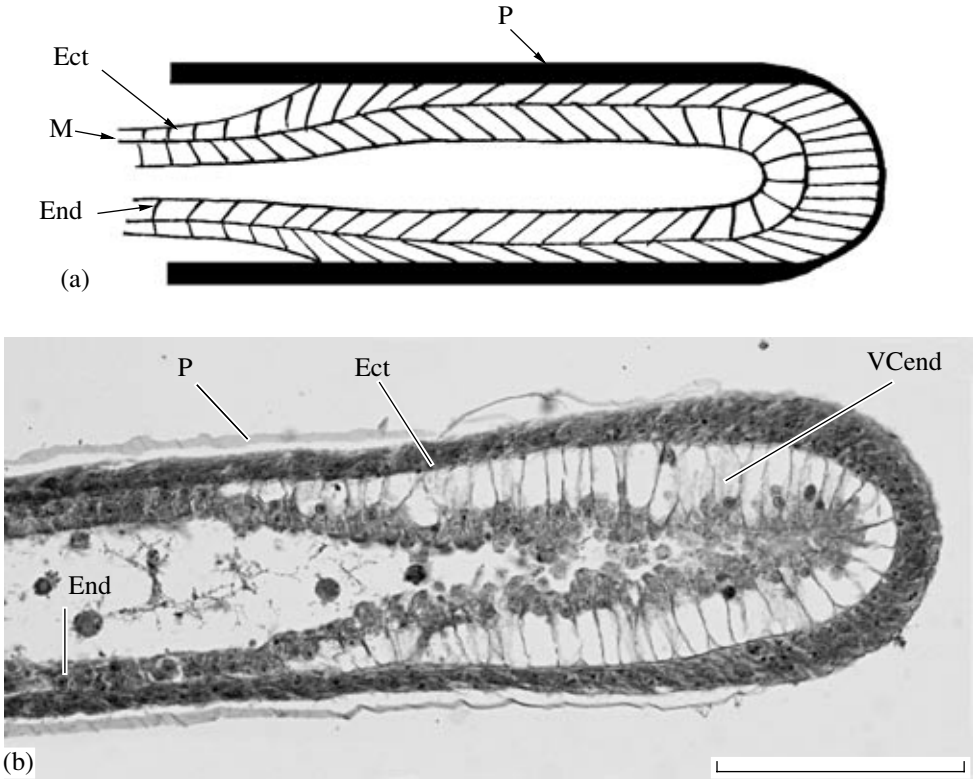

Fig. 2. Structure of the growing tip in a hydroid colony: schematic diagram (a) and microphotograph (b) of a longitudinal section across the middle of stolon growing tip parallel to the substrate. Ect, ectoderm; End, endoderm; M, mesoglea (basal membrane); P, perisarc; VCend, vacuolized cells of growing tip endoderm. Scale: 100 μm.

65–70 μm. The ectoderm on the tip sides at phase *C* is ca. 30 μm thick.

Parameters of growth pulsations. A graphical image of relative position of the apicalmost point of a growing tip (Fig. 3) in time provides a picture of its growth pulsations. The most complicated form of growth pulsations is inherent in the stolon growing tips, while growth pulsations of the shoot growing tips in the species in question are more "smooth" (Wyttenbach *et al.*, 1965; Kosevich, 1990). On the whole, each growth pulsation can be divided into six successive phases (Wyttenbach, 1968) (Fig. 3):

(1) extended and resting at the "peak" level—phase *A*;

(2) retraction of tip—phase *B*;

(3) retracted and resting—phase *C*;

(4) re-extension to near the previous "peak"—phase *D*;

(5) resting at this level—phase *E*;

(6) extension to the level of the next "peak"—phase *F*.

The phases of pulsation are characterized by their **length and amplitude** and **rate** of tip movements (Fig. 3). The main parameters of tip growth pulsations are: **period** (cycle time) (time between successive "peaks") and **growth per cycle (pulsation)**. These two parameters allow a sufficiently precise characterization of the tip growth rate for a long period of time: 12–24 h or more. The third parameter, **range of retraction per cycle (pulsation)**, is the most variable characteristic (Wyttenbach, 1974).

Registration of growth pulsations. Observations of growing tips were carried out under a Biolam microscope (Russia) at ×400. Changes in mutual position of the cells during growth pulsations are more readily distinguished in the stolon growing tips. Short-term registrations of growth pulsations (5–12 successive cycles, total duration of registration ca. 1–1.5 h) were performed by direct observation and registration of the tip apex position through the eye piece with an interval of 12 s. Long-term registrations were performed using a modified microphotometric method (Kosevich *et al.*, 1990).

Growth pulsations were recorded additionally in some tips with an interval of 5 s by three points: apical part of tip—apical part of basal membrane vault—inner surface of endoderm (Fig. 4). This allowed us to follow changes in the thickness of cell layers on the tip apical part during growth pulsation. Directly after registration of growth pulsation of the tip apical part, changes in the outer diameter of the tip endodermal tube were fixed in its middle part (Fig. 5).

During all observations, the colonies were kept in a chamber with running sea water at a constant temperature. Registrations were performed at approximately the same time with reference to feeding of hydroids.

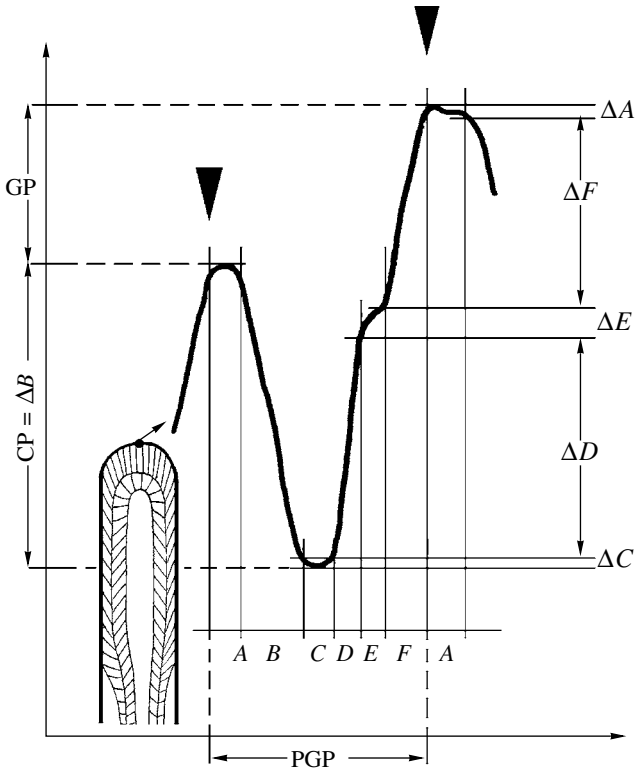

Fig. 3. Graph of growing tip pulsations. Here and in figs 4 and 5: abscissa—time; ordinate;—relative position of tip apex. *A*, *B*, *C*, *D*, *E*, and *F* –phases of growth pulsation and their duration; Δ*A*, Δ*B*, Δ*C*, Δ*D*, Δ*E*, and Δ*F*–amplitudes of tip apex movements at the corresponding pulsation phases; GP, growth per pulsation; PGP, period of growth pulsation; RP, retraction per pulsation; (▼) moments of pulsation "peak."

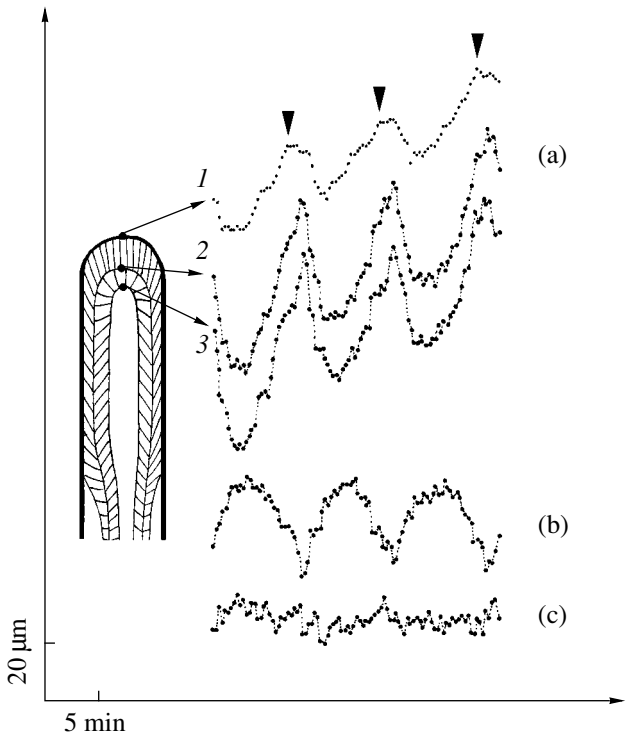

Fig. 4. Registration of growth pulsation of a growing tip by three points: (*1*) tip apex, (*2*) apical part of basal membrane vault, (*3*) inner surface of endoderm of the tip apical part. (a) graphs of registration of successive pulsations; (b, c) changes in thickness of ectodermal (b) and endodermal (c) layers on the tip apical part (calculated from the data of graph a) (▼) moments of pulsation "peaks."

Histological studies. For histology, the colonies of hydroids were fixed by Bouin mixture and embedded in paraffin using the standard method. Sections, 5–8 μm thick, were stained by haematoxylin after Caracci.

RESULTS

The published data (Beloussov *et al.*, 1972; Donaldson, 1973; Kosevich, 1991a; Beloussov, 1988) suggest that the growing tip of colonial hydroids is capable of autonomous functioning, i.e. its activity is determined above all by the processes occurring in the tip. Hence, the structure and changes taking place in the tip during growth pulsation should be related to the pattern of its functioning.

Description of processes occurring in the growing tip during growth pulsation (Fig. 6). We will begin with **phase *A***—arrest at the "peak" level. Cell movements are practically absent. Ectodermal cells on the tip sides in its proximal part are oriented perpendicularly to the perisarc. Closer to the apical part, the inner ends of ectodermal cells are located more distally than their outer ends: the cells are "thrown back" (term from Beloussov and Dorfman, 1974). In the apical part, the cells are bent. The endodermal tube has the minimal outer diameter and is somewhat narrowed in the distal direction. Its vault is practically cone-shaped. In the tip proximal part, characteristic widening of the endodermal tube is observed (Figs. 5, 6a, and 6e).

Phase *B*—phase of retraction. The following changes are observed in cell layers. The widening of the endodermal tube spreads in the proximodistal direction and is particularly pronounced in the vault region. In the proximal part, the endodermal tube widening observed at the preceding phase disappears and its outer diameter becomes the same all along the tube. Thereafter, the basal membrane with the attached cell bases retracts in the proximal direction, which leads to altered orientation of the cells of both layers. In the apical part, the ectodermal cells are elongated and thinned. At the end of this phase, the outer ends of ectodermal cells are rapidly displaced along the tip sides in the distal direction (Fig. 6b).

Phase *C*—resting after retraction—moment of "downfall (trough)." During this phase, cell movements are practically not registered. As a result of pre-

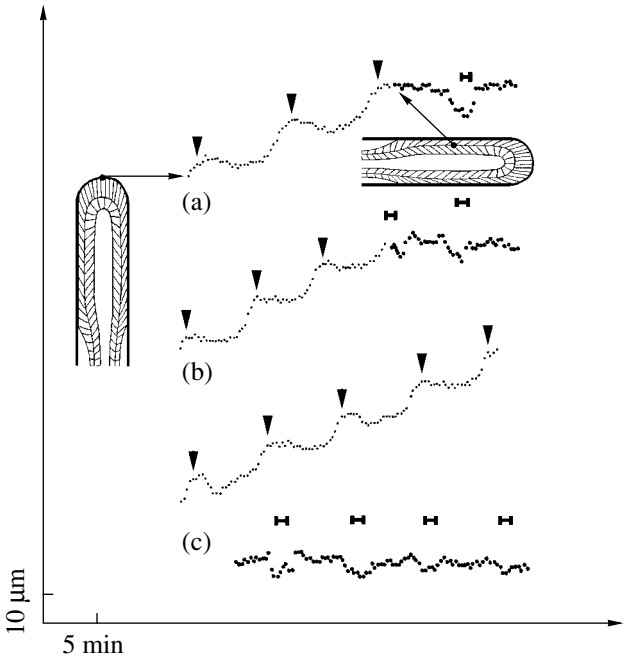

Fig. 5. Registration of different variants (a–c) of changes in the endodermal tube diameter in the tip middle part during growth pulsation. (···) results of registration of growth pulsations on the growing tip apex; (•••) subsequent registration of changes in the endodermal tube diameter; (→) corresponding sites of registration; (▼) registered moments of pulsation "peaks"; (⊢) calculated moments of "peaks" of subsequent pulsations.

vious movements, cell layers are oriented in the following way (Figs. 6c and 6f). Ectodermal cells on the tip sides are inclined with their outer ends in the distal direction. In the apical part, they are narrow and strongly extended (Fig. 4b). The ectoderm thickness on the tip sides is minimal. The endodermal tube has the maximum outer and inner diameters (Fig. 5). Its vault is practically semispherical. Endodermal cells on the tip sides are inclined with their inner ends in the distal direction. Perisarc is often shriveled on the apical tip surface.

Phase *D*—re-extension to the level of the previous "peak." In the beginning of this phase, the basal membrane with the attached cell ends begins to move in the distal direction. Movement begins in the proximal tip part and spreads in the distal direction. In a tip ca. 300 μm long, the basal membrane moves all along within approximately 3 s after the beginning of movement in the proximal part. The orientation of ectodermal cells undergoes changes. Their ends attached to the basal membrane move in the distal direction, while at the site of contact with the perisarc, they remain immobile. The ectoderm thickness in the apical tip part remains practically unchanged throughout this phase (Fig. 4b). Endodermal cells practically do not change their orientation during movement of the cell layer with the basal membrane. The endoderm movement is noticeable in the coenosarc regions closest to the growing tip, at least within the nearest 200 μm.

Phase *E*—resting at a level of the previous "peak." The main differences in the tip structure from that at phase *C*—long axes of ectodermal cells located on tip sides, which are practically perpendicular to the perisarc, and somewhat thinner ectoderm in the apical tip part. Endodermal cells may have a smaller angle of inclination of the lateral walls with reference to the basal membrane (Figs. 6d and 6g).

Phase *F*—extension of tip to the level of a new "peak." The phase is characterized by widening of the endodermal tube in the tip proximal part and constriction in other parts of the tip. Constriction of the endodermal tube spreads in the proximodistal direction in a wave-like mode. The endodermal tube vault becomes con-shaped and is "intercalated" in the ectoderm. Ectodermal cells on the tip sides in its distal part are "thrown back." In the apical part, ectodermal cells are compressed along the longitudinal tip axis and bend laterally (Fig. 6a).

Registration of changes in the **endodermal tube** outer diameter in the middle part of the stolon growing tip during growth cycle has shown that the minimal diameter corresponds to the moment of peak, while the maximum diameter, to the moment of tip compression (Fig. 5). The diameter varied within 5–7 μm, on average (up to 10 μm).

The data on registration of growth pulsations by three points make it possible to isolate on graphs of **basal membrane movements** in the apical part two, sometimes three, sections upon transition from "downfall" to next "peak." These sections differ by the rate of basal membrane movement and may correspond to different phases or processes that determine the tip movements. The basal membrane movements during transition of the growing tip from "peak" to next "downfall" are faster, but monotonous, "smooth" and their rates are practically the same (Fig. 4a).

The **ectoderm thickness** in the growing tip apical part of undergoes similar changes. Their amplitude is 30 μm, on average. During transition from a "peak" to a "downfall" the ectoderm thickness increases rapidly and uniformly. On the contrary, during transition from "downfall" to the next "peak," its thickness decreases in two distinct stages. These stages agree quite well with pulsation phases (Fig. 4b). In the end of movements to both "peak" and "downfall," the ectoderm thickness undergoes insignificant changes. One gets an impression that there is a minimal and maximal length of the ectodermal layer.

Analysis of parameters of tip growth pulsations. The proposed division of pulsations into six phases (Wyttenbach, 1968) proved to be sufficiently precise, detailed, and convenient and was used in our studies. During analysis of growth pulsation, the main attention was paid to interdependence of individual phases and

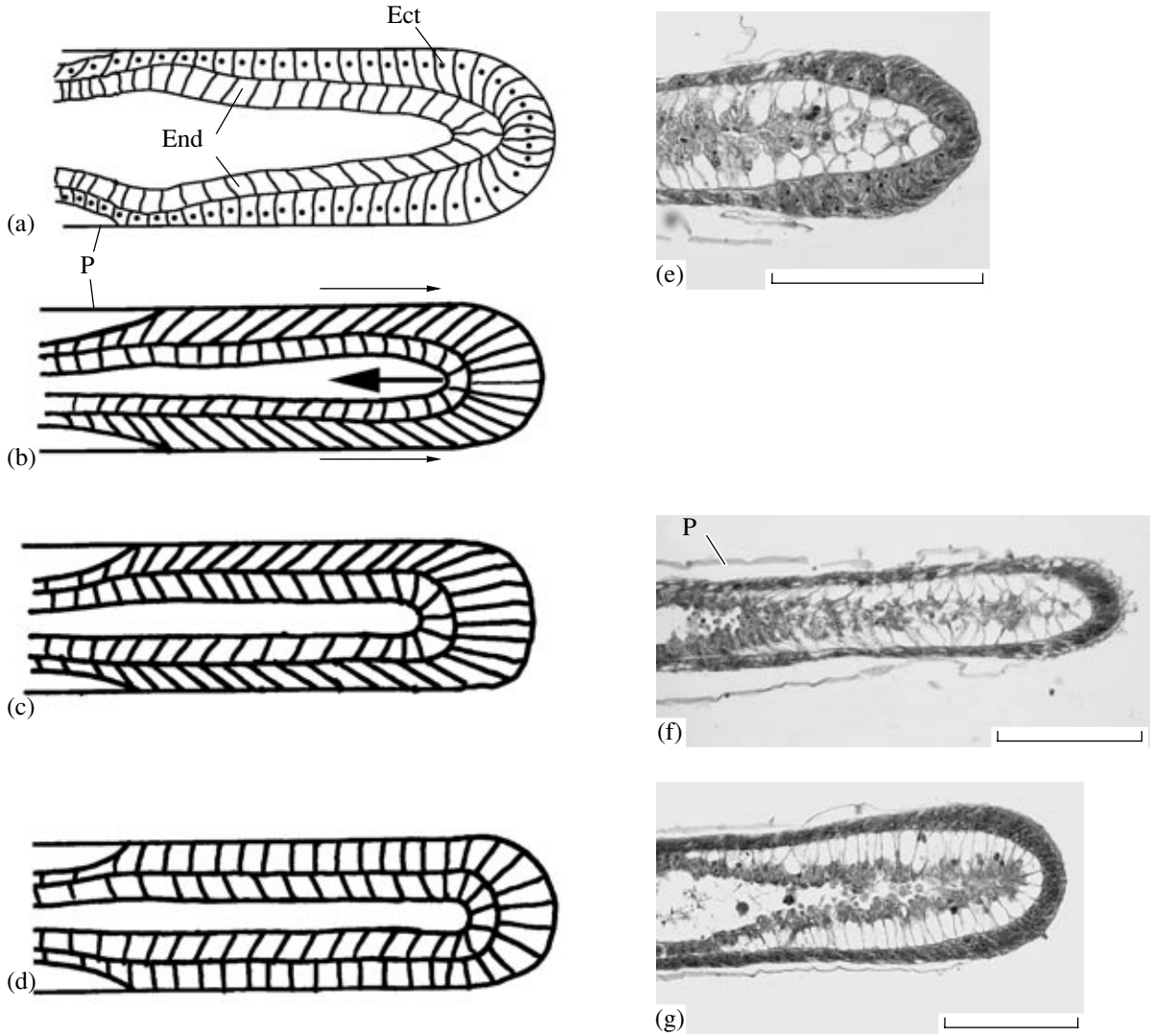

Fig. 6. Observed changes in the tip structure during growth pulsation. (a–d) phases of growth pulsation (scheme): (a) "peak," the greatest tip extension; (b) retraction; (c) resting in retracted state (phase *C*); (d) arrest at the level of the preceding "peak" (phase *E*). (e–g) microphotographs of sections: (e) apical part of tip at phase *A*; (f) growing tip at phase *C*; (g) the same at the level of the preceding "peak" Scale: 100 μm. Directions of movement: (⟵) tip apical vault; (⟶) distal ends of tip ectodermal cells. For other designations see Fig. 2.

variations of their parameters. This in view, correlation analysis of the relative duration of pulsation phases and amplitude of tip movements in continuation of these phases was carried out.

All phases were expressed most fully in growth pulsations of the stolon tips. However, under constant external conditions, growth pulsations remain rather uniform and are characterized by constant parameters (Crowell and Wyttenbach, 1957; Wyttenbach, 1968; Wyttenbach *et al.*, 1973; Kosevich, 1990). Therefore, analysis was performed for 22 registrations of 151 growth pulsations of a stolon tip in a colony of *Gonothyraea loveni*, which developed at a dosed diet for 52 days. At such a diet, the colony obtained a *fixed* amount of food daily irrespective of its size. Therefore, the diet could be considered excessive at first, which led to a gradual deceleration of its growth as a whole and stolon tips in particular (Burykin, 1979; Kosevich, 1991a). Each registration included a record of 5 to 11 successive growth pulsations of the tip. Based on these records, parameters of each growth pulsation was calculated and the data were averaged for each registration. The resulting mean values were used for correlation analysis. Since registration was performed at different temperatures (15 to 20°C), **relative duration of pulsation phases** (relative to the period of a given pulsation) was used for calculations.

The main coefficients of correlation between the duration of growth pulsation phases (*A, B, C, D, E*, and *F*) and amplitude of movements of the growing tip dur-

ing the corresponding phases (ΔB, ΔD, and ΔF) in a colony of *G. loveni* (Fig. 3) are as follows:

	B	*C*	*D*	*E*	*F*	ΔB	ΔD	$\Delta D + \Delta F$
A	−0.40				−0.53			
B		−0.09			0.40			
C			−0.35					
D	0.24			−0.43				
E					−0.46			
F			−0.01					
ΔB	0.70							0.93
ΔD			0.57			0.60		
ΔF					0.64	0.74	0.09	
D + *F*	0.46							0.37.

Analysis revealed a negative correlation between the relative duration of successive growth pulsation phases, thus confirming the **constancy of the period** of growth pulsation and its genetic determination (Wyttenbach, 1969, 1974): the period of pulsation remains practically unchanged, while the duration of individual phases could vary. Note practically full absence of any correlation between phases *B* and *C*: the coefficient of correlation r_{BC} equals 0.09. Hence, it can be proposed that pulsation is "broken" between phases *B* and *C*, i.e. the cycle of tip growth activity **starts from phase *C* and ends by phase *B***.

Analysis has shown a sufficiently high positive correlation between the durations of "active" phases (*B*, *D*, and *F*) and amplitude of movements of the tip (ΔB, ΔD, and ΔF) at these phases, i.e. the amplitude of movements depends on the duration of phases. Zaraisky *et al.* (1984) also indicate to constant rates of cell movements and relate this constancy to the rate of processes underlying the cell mechanisms of tip movements.

Correlation between amplitudes of tip movements at different phases confirms the independence of its growth per pulsation from other parameters of growth pulsations. The amplitude of tip movements at phase *F* does not depend on that at phase *D* but affects the value of retraction ΔB: the bigger the extension of tip at phase *F*, the longer distance it covers at the phase of retraction. The amplitude of tip movements at phase *D* increases with ΔB: the stronger the tip is retracted, by a longer distance it should re-extend to return to a level of the previous peak.

The coefficient of correlation between growth per pulsation and growth rate per 24 h equals 0.87 (significant for 0.1% level of significance).

DISCUSSION

Although this study is mostly descriptive, the data obtained allow us to apply several interesting considerations to specific features of the growth pulsations, the phenomenon underlying morphogenetic processes in hydroid polyps.

The following conclusions can be drawn from the data of correlation analysis of growth pulsation phases. Growth pulsation begins with the phase of resting after a maximum retraction (phase *C*) and ends by a tip retraction ((phase *B*). The duration of each subsequent phase is inversely dependent on that of the preceding phase, which determines the constancy of the period of growth pulsations at a given temperature. The rate of tip movement at phases *B*, *D*, and *F* is constant, thus determining the stability of growth pulsation parameters at the constant conditions: the relative duration of "active" phases varies much less than that of "resting" phases: *A*, *C*, and *E*. This confirms indirectly once more that the active tip movements are inherent in the tip. Independence of the amplitude of tip movements at phase *F* (extension from the level of the previous "peak" to that of a new "peak") from the preceding phases, which are more vulnerable to the inner colony factors, confirms even more the relative autonomy of the tip growth (Beloussov *et al.*, 1972; Donaldson, 1973; Kosevich, 1991a; Beloussov, 1998). The absence, practically full, of correlation between total duration of phases *D* and *F* and total amplitude of tip movements at these phases [$((\Delta D + \Delta F) - r_{(D+F)/(\Delta D+\Delta F)}) = 0.37$] suggests that these growth pulsation phases are determined by different, practically unrelated processes in the tip.

All these data suggest the integrity of growth pulsation and its integrity and significant autonomy as a physiological act. The data obtained confirm a significant constancy of its main parameters, period and growth per pulsation, against the background of possible oscillations and variability of individual pulsation phases (Wyttenbach, 1968, 1969, 1974; Wyttenbach *et al.*, 1974).

On the basis of these observations and published data, the following hypothetical scheme of cyclic processes in the tip is proposed, which helps in explaining some specific features of growth and structure of colonial hydroids.

Hypothetical Model of Cyclic Processes in the Tip Growth

It is more advisable to begin the description of processes occurring in the growing tip during growth pulsation from the established moment of pulsation start: **phase *C*, maximum tip retraction**. At this moment, the ectodermal cells on the tip sides are inclined with their distal ends to the tip apical part. In the apicalmost part, they are narrow and markedly extended; the thickness of ectodermal layer is minimal. The outer diameter and inner lumen are maximal. The endodermal cells are inclined with their inner ends to the tip apical part (Fig. 7a).

At **phase *D***, **transition from the level of maximum retraction to that of previous "peak,"** proximodistal movement of the basal membrane and of the ends of cells of both layers attached to it takes place. The move-

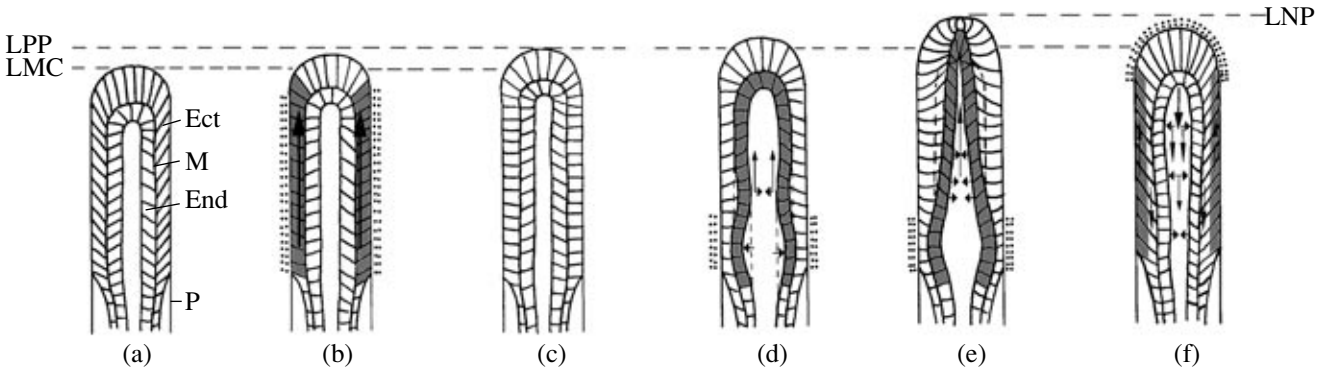

Fig. 7. Scheme of successive processes occurring in the tip. (a) resting in retracted state, phase *C*; (b) re-extension to the level of the previous "peak"; phase *D*; (c) resting at the level of the previous "peak," phase *E*; (d) beginning of extension to the level of a next "peak," phase *F*; (e) resting at the level of a next "peak"—maximum extension, phase *A*; (f) retraction of the tip after the next "peak," phase *B*; LMR, level of maximum retraction of the tip; LNP, level of next "peak"; LPP, level of the previous "peak." For other designations see Fig. 2. (::::) sites of "support" for active movements, (■) layers responsible for tip active movements at a given phase; (→) directions of layer movements (arrow length corresponds to shift value); (– –) contour of endodermal at the preceding phase.

ment starts at the tip proximal part and spreads distally. The endodermal cells located slightly more proximally to the tip also move. Reorientation of ectodermal cells tending to locate perpendicularly to the perisarc can serve as a **driving force** of basal membrane and endoderm movement at this phase. This reorientation spreads as a proximodistal process (Beloussov, 1998). This distally directed movement can be supported by contacts between the apical walls of ectodermal cells and perisarc, as well as by mechanical friction of plasmalemma and perisarc all along the tip as a result of a certain increase in the diameter of the ectodermal layer due to increased volumes of component cells (Beloussov and Dorfman, 1974; Beloussov *et al.*, 1980, 1984; Zaraisky *et al.*, 1984) (Fig. 7b).

As a result of these movements, the endodermal cells practically "normalize" their position with reference to the perisarc. They also some what decrease the angle of their inclination to the basal membrane due to a slight mutual displacement as a result of a decrease in the endodermal tube diameter under the pressure of a somewhat thickened ectoderm. Ultimately, the basal membrane and the entire endodermal tube of the tip are drawn in the distal direction. The apical part protrudes to a level of the preceding peak. The ectoderm thickness in the apical part remains practically unchanged, because the young perisarc compressed during the preceding proximal movement of the tip relaxes, not interfering with the cell movement (Beloussov, 1975; Kossevitch *et al.*, 2001). At the end of phase *D*, the movement of cell layers is slowed down and the tip apical part is arrested approximately at a level of the preceding "peak," **phase *D*** (Fig. 7c). Further movement of the tip at the expense of reorientation of the ectodermal cells is **impossible.**

The endodermal tube elongation is a driving force of the tip protrusion to the level of a new "peak" at **phase *F***. The phase begins with the endodermal tube widening in the tip proximal part, which produces a **foothold** for subsequent tip extension. This widening is followed by a decrease in the endodermal tube diameter in the proximodistal direction all along the tip, distally to the site of widening (Fig. 7d). One gets an impression that a wave of compression spreads along the endodermal tube in the growing tip. Meanwhile, the thickness of endodermal layer remains practically unchanged.

On assumption of the constancy of volumes of the endodermal cells, the decrease in the endodermal tube diameter leads to its elongation. The endodermal cells are enlarged along the tip axis, in parallel to the basal membrane and extend this latter. Since the endodermal tube is closed as a vault at the distal end, the inner ends of endodermal cells are displaced in the distal direction to a lesser extent than their bases. As a result, the endodermal cells are inclined with their inner ends from the tip apical part. Enlargement of the endodermal cells along the basal membrane and on the vault in the distal part of the endodermal tube leads to an increase in the vault surface and makes it to bend stronger and protrude additionally in the distal direction (Fig. 7e).

At the expense of the above described processes, the tip ectoderm cells together with the basal membrane are drawn, by their bases, to the apical part. In the process, the more distally is the ectodermal cell located, the greater is distance over which its base is drawn. As a result, the distalmost ectodermal cells located on the tip sides are "thrown back" in the distal direction. The ectodermal cells on the apical part pressured by a protruding endodermal tube are compressed and somewhat bent to the sides (Beloussov, 1998). However, devoid of the possibility of strong compression and bending because of the cell layer continuity and cell contacts, the ectodermal cells are pushed out in the distal direction and extend a newly formed perisarc (Kossevitch

et al., 2001). The tip extends to the level of a new "peak." The cell layers do not move for a certain time: **phase *A*** (Fig. 7e).

Phase of retraction after "peak" **(*B*)** begins with the increase of the endodermal tube diameter in the proximodistal direction. Then follows the endodermal tube vault compression, which accelerates the tip retraction. All this induces drawing of the basal membrane from the tip apical part together with the bases of cell of both layers. It may well be that elasticity of the extended basal membrane and of the not yet hardened perisarc plays an active role in this process (Fig. 7f) (Beloussov, 1998).

At the final stages of retraction, the endodermal tube proximal part is compressed, as a result of which the last **foothold** is lost. At this moment, the most remarkable event of the retraction phase is observed: fast (1 μm/s), almost synchronous sliding of distoapical ends of ectodermal cells along the perisarc inner surface in the distal direction. It was initially proposed that this takes place at the expense of contraction of the ectoderm-covering plasmalemma extended during the tip extension, which causes "sliding" of the outer ends of ectodermal cells in the distal direction (Beloussov and Dorfman, 1974). However, it was shown later that this "sliding" is an active, rather than passive process: it is related to the formation of distally oriented ectodermal cell processes along the perisarc leading to the fixation of cells at a new, more distal level (Labas *et al.*, 1992; Beloussov, 1998). At the same time, this "sliding" is to a certain extent enhanced by a decrease in cross diameters of the ectodermal cells on the tip apical part due to their longitudinal extension by the retracting endoderm: tight contacts between the cells (Overton, 1963; Makarenkova, 1989) induce "drawing" of the entire endodermal layer along the tip longitudinal axis.

The above considered "sliding of ectodermal cells provides for the movement of the tip cells along the perisarc in the distal direction and thereby fixes the tip increase per pulsation. In addition, the tip retraction is less than its protrusion. This is enhanced by that a newly formed and partially hardened perisarc fixes to a certain extent the new increase and does not let the tip to retract to the previous level. It is more difficult to compress a thin and elastic perisarc than to extend it (Beloussov, 1998). At the end of the retraction phase, the orientation of the cells of both layers returns to the initial state, **phase *C***; so-called moment of "downfall" occurs.

The pattern of endodermal tube movements in a growing tip during growth pulsation creates an impression of proximodistally oriented peristaltic wave of compression–relaxation running along, whose length is approximately twice that of the endodermal tube. This agrees to a certain extent to the suggestion of a chemical nature of the signal, which determines the tip movements (Zaraisky *et al.*, 1984).

Consequences from the Model

The above considered model of movements of the cell layers in the growing tip makes it possible to explain the presence of discrete phases in growth pulsation. This concerns above all the tip movement from the level of maximum retraction (phase of "downfall") to the level of a new "peak." This extension of the tip apical part cannot be explained by the ectoderm activity alone, as was repeatedly done by Beloussov and his colleagues (Beloussov, 1973, 1998; Beloussov *et al.*, 1980; Zaraisky *et al.*, 1984; Labas *et al.*, 1992; Beloussov and Grabovsky, 2003). Although those authors indicate that such a simplification is rather crude simplification, they believe that in the first approximation both layers are involved and behave in growth pulsations in a similar way. At the same time, it follows from the data presented that the activities of ectoderm and endoderm have some significant distinctions.

The expression and independence of some "passive" phases (phases of "resting" at the level of previous "peak" and "resting" at the level of a new "peak" and in a retracted state) from the processes inside the colony (direction of hydroplasmic currents) have already been mentioned (Fulton, 1962; Wyttenbach, 1968, 1973, 1974) for a number of colonial hydroids. The proposed scheme explains these phenomena by switching from one driving force to another at the growth pulsation phases in question.

The outer skeleton, perisarc, plays an important role in the proposed model. Only its presence and rigidity on the growing tip sides allow the tip tissues to move in the distal direction. The site of formation of a new perisarc and the rate of its hardening also play significant roles. If we assume relative constancy of growth pulsation parameters, such as period and growth per pulsation, changes in the relative rate of hardening of a new perisarc may determine to significant extent successive changes in the shape of the tip and rudiment as a whole. If the boundary between the already hard and still elastic (newly formed) perisarc remains at the same relative distance from the tip apex, at the interface of semispherical apex and lateral parallel sides of the tip, tubular structures with a constant diameter are formed (Fig. 8, *1*). Relative deceleration of the perisarc hardening means a shift of the boundary between still elastic and already hard perisarc proximally from the tip apical part, which allows the tip widening at the moment of its extension. Hence, the formed structure will be characterized by successive increase in its diameter (Fig. 8, *2*). Acceleration of the new perisarc relative hardening means a distal shift of the boundary "elastic–hard perisarc" and, as a consequence, gradual decrease in the diameter of the tip and rudiment (Fig. 8, *3*) (Kossevitch *et al.*, 2001). Thus, the proposed model makes it possible to complement the earlier explanation of changes in the rudiment shape in colonial hydroids by the form of growth pulsations and shift of the highest apicobasal

activity in a growing tip (Beloussov, 1975; Beloussov *et al.*, 1984).

The proposed model allows also: (1) calculation of a maximum tip growth per cycle and maximum amplitude of tip movements on the basis of empirical data; (2) explanation of reaching a maximum growth per pulsation of a new stolon tip within several days after its appearance; (3) explanation of distinctions of growth pulsation parameters of stolon and shoot tips, and (4) explanation of the shoot first internode inclination towards the stolon growing tip.

Calculation of maximum amplitude of tip movements. According to the presented model, the maximum amplitude of tip movements during pulsation consists of the following components: (1) tip movements as a result of reorientation of the ectodermal cells on the tip sides and (2) "pushing out" of the apical part by an elongating endodermal tube. The movement determined by reorientation of the ectodermal cells equals a product of the ectodermal layer thickness by cotangents of the initial cell inclination to the perisarc at phase *C*. Extension of the apical part due to the endodermal tube elongation equals a product of the endodermal tube length at phase *C* by the ratio of its maximal outer diameter to the minimal one, except the initial length. The amplitude of changes in the ectoderm thickness in the apical part during growth pulsation should be deducted from the resulting values.

Let us consider a specific example. The thickness of ectodermal layer on the stolon tip sides equals 30 μm and inclination of cells to the perisarc amounts to 30° (maximum inclination equals 30°– 40°—Beloussov and Dorfman, 1974), while cotangents is 1.73. The endodermal tube length at phase *C* equals 200 μm and its maximal and minimal diameters amount to 65 and 60 μm, respectively. The amplitude of changes in the ectoderm thickness in the apical part during growth pulsation is 30 μm. After all calculations, we obtain a value of 38.6 μm, which agrees quite well with the empirical data on growth pulsations of the stolon tips (Wyttenbach, 1969, 1974; Wyttenbach *et al.*, 1973). The calculations based on the model confirm the earlier data that the maximum increase of the tip per pulsation is determined by the endodermal tube elongation and, hence, depends on the tip length, but does not practically depend on its diameter (Kosevich, 1990). In addition it can be seen from the proposed model that there is a limit of increase per pulsation and, correspondingly, of the tip growth rate, which was earlier proposed on the basis of indirect data (Kosevich, 1991a).

Gradual increase of a new stolon tip per pulsation. The proposed model and calculations suggest that the increase per pulsation is directly related to the growing tip elongation. A newly appeared stolon growing tip is much smaller than that of the "established" (at the age of four to six days with constant growth rate under favorable conditions) stolon (Wyttenbach, 1968). The growing tip reaches its final size within several days:

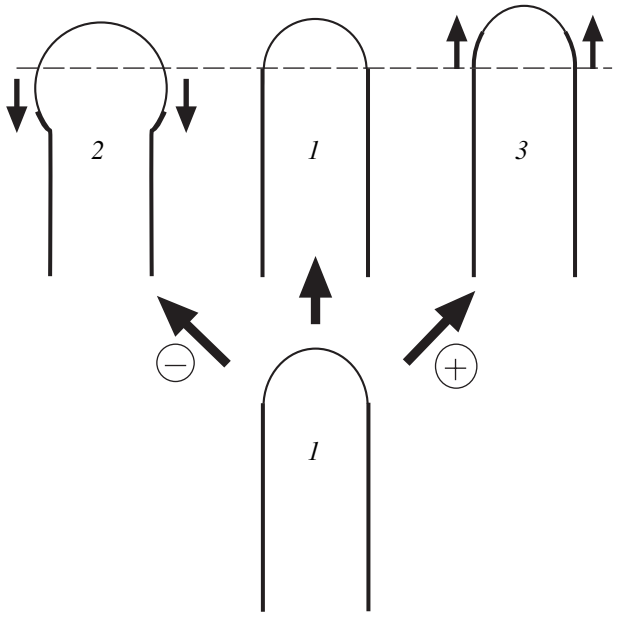

Fig. 8. Scheme of changes in the growing tip shape with alterations of relative rates of its growth and hardening of a newly formed perisarc. *1*, initial state: boundary of perisarc hardening remains at the level of transition of tip apex spherical surface into its lateral surfaces; (*2*) deceleration of perisarc hardening with respect to the tip increase; (*3*) acceleration of perisarc hardening with respect to the tip increase. Perisarc: (——) already hardened, (——) newly formed elastic. (— —) relative level of perisarc hardening at the initial state; (⟶) shift of the boundary of perisarc hardening with reference to the tip apex; (⊕) and (⊖) relative acceleration or deceleration of the perisarc hardening, respectively.

four to six. Mainly its length increases (Kosevich, 1990), and, correspondingly, the growth per pulsation.

Distinctions of growth pulsation parameters of stolon and shoot tips. The shoot growing tips in most representatives of the Campanulariidae are smaller that the stolon tips: their diameter is less and, what is more important, they are shorter (Wyttenbach *et al.*, 1965; Kosevich and Marfenin, 1986; Kosevich, 1990, 1991a). Based on the proposed model, one can explain the main distinctions of growth pulsations of the shoot and stolon tips. A lesser increase per pulsation is related to a lesser relative and absolute elongation of the endodermal tube in the shoot tips. A shorter period of growth pulsations in the shoot tips appears to be related to the time of spreading of the waves of reorientation of the ectodermal cells and narrowing of the endodermal tube: the shorter the distance over which a wave spreads, the faster is one process completed (one phase of pulsation) and begins another. And the rates of spreading of these waves appear to be similar in the stolon and shoot tips, like the mechanisms underlying growth pulsations.

It may well be that smaller shoot tips in species of the Campanulariidae are related to their short life span: 24 h, on average (Marfenin and Kosevich, 1984;

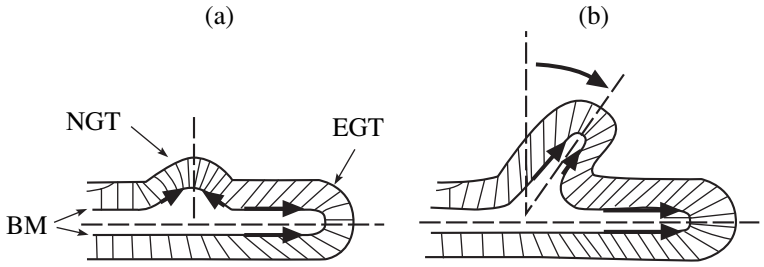

Fig. 9. Inclination of a new growing tip towards the existing tip: (a) initial moment, appearance of a new growing tip; (b) a late stage, change in the direction of growth of the new tip (only one cell layer is shown). BM, basal membrane; EGT, existing growing tip; NGT, new growing tip. (— —) direction of growth of tips; (⟶) direction of basal membrane shift during growth pulsations; the arrow length is proportional to the shift value.

Kosevich and Marfenin, 1986; Kosevich, 1990, 1991a), i.e. the tip does not succeed to "full-grow." This is confirmed to a certain extent by the fact that growth pulsations of the tips of *O. longissima* "giant" shoots, approaching the stolon tips by their size, are very similar in both form and parameters to growth pulsations of the stolon tips (Kosevich, 1991b).

Inclination of the shoot first internode towards the stolon growing tip. The first internode of the shoot in most colonial hydroids of the Campanulariidae and some species of other families of thecate hydroids is inclined at the base towards the stolon growing tip (Marfenin and Kosevich, 1984; Kosevich and Marfenin, 1986). This is related to the fact that the growing tip of the next shoot is laid down in the direct vicinity of the stolon tip (Kosevich, 1990, 1999). Actually, they can be considered as interacting rudiments. During growth pulsations at the initial stages of shoot development upon extension of the shoot tip, the basal membrane on its side facing the stolon tip moves in the direction of the shoot growing tip over a shorter distance than on the opposite side due to its shorter length and lesser involvement in movements. As a result, according to Beloussov (1965), the shoot growing tip is inclined towards the stolon tip at the initial stages of its growth (Fig. 9). The interaction of closely located pulsating rudiments (growing tips) is enhanced by that, according to the proposed scheme, an active role in extension to the level of a new "peak" is played by endoderm. The difference in the freedom of movement or extension of basal membrane (mesoglea) on different tip sides leads to enhanced deviation of direction of the tip apex growth from the initial direction (perpendicular to the longitudinal axis of a mother structure) towards the closes active growing tip.

The appearance of secondary peaks or absence of stolon tip compression during two or three successive pulsations at the moment of appearance of the next shoot growing tip are related to such an interaction of two closely located tips (Kosevich, 1990).

The above said is confirmed to a certain extent by the absence of inclination of the first internode of a shoot forming from a settled planula. The growing tip of the *O. longissima* first shoot developing from an attached frustula is also much less inclined towards the stolon growing tip, since it appears approximately in the middle of frustula: hence, the basal membrane state is the same from both ends (Berrill, 1948; Makarenkova *et al.*, 1985).

Based on the proposed scheme of growing tip functioning and patterns of interaction of neighboring rudiments (Beloussov, 1965), one can propose that the degree of separation of hydrothecae from the stem axis or shoot branch in colonial hydroids depends on the time of appearance of a hydranth-forming growing tip. To test this suggestion, it is necessary to study in detail the processes of morphogenesis in different representatives of this group of colonial invertebrates.

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