Reference: *Biol. Bull.*, **141**: 278–298. (October, 1971)

OBSERVATIONS ON PSEUDOCOLONIAL GROWTH IN HYDRA [1]

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The freshwater hydrozoan polyp *Hydra* (Phylum Cnidaria) has been a favorite tool of developmental biologists for years. It possesses the typical two cell layer hydrozoan body plan in a solitary individual, thereby avoiding the complexity of colonial organization and polymorphism. The great majority of hydrozoan polyps, however, are colonial (Hyman, 1940). Therefore, the question arises, why doesn't hydra also express this type of growth pattern (*i.e.* colonial organization)? What is unique about the hydra which enables it to shed its asexual reproductive products (buds), and thereby remain solitary? Are there any conditions under which hydra does not exhibit a typical solitary growth pattern and assumes a colonial or pseudocolonial existence? If so, what are the characteristics of this transformation?

Previously, Schulz and Lesh (1970) reported that following a rise in temperature, the asexual reproductive products of *Hydra viridis* were not always detached. The result was the generation of pseudocolonial monomorphic hydra. This investigation examines parameters influencing the control and expression of the growth pattern in these pseudocolonial animals. These observations are then compared with existing theories of the control of hydroid growth and polarity in an attempt to determine a basis for colonial *vs.* solitary growth form.

Methods and Materials

H. viridis was mass cultured in eight inch fingerbowls according to the methods of Loomis and Lenhoff (1956), except that demineralized water was substituted for tap water. Cultures were maintained at $22 \pm 1°$ C and were fed daily with *Artemia salina* larvae. The culture solution was changed approximately 1½ hours after each feeding.

Surgical procedures

Routine operations were performed with iridectomy scissors on fully extended, but unanesthetized, 24 hour starved animals. *Regeneration experiments* involved severing the animal completely in the specified region. *Wounding experiments,* alternatively, involved only the removal of a divot of tissue from the specified region.

Grafting experiments employed two distinctly pigmented types of *H. viridis:* untreated *H. viridis* and bleached *H. viridis.* Untreated *H. viridis* was green in color due to the presence of an algal symbiont in the gastrodermal cells. Bleached *H. viridis* had the algae removed from their tissues by treating the animals with glycerine (Whitney, 1907). In this investigation, therefore, four kinds of hydra were available for grafts: green pseudocolonial hydra; bleached pseudocolonial hydra; green normal hydra; and bleached normal hydra.

Grafting was performed in culture solution in a petri dish half-filled with paraffin. Hairs were implanted in the paraffin at right angles to the surface. Host

[1] This work was supported by American Cancer Society Institutional Grant 342-9059 and 9157; and by United States Public Health Service Program-Project Grant PO 1 GM 14891 administered by Dr. Robert D. Allen.

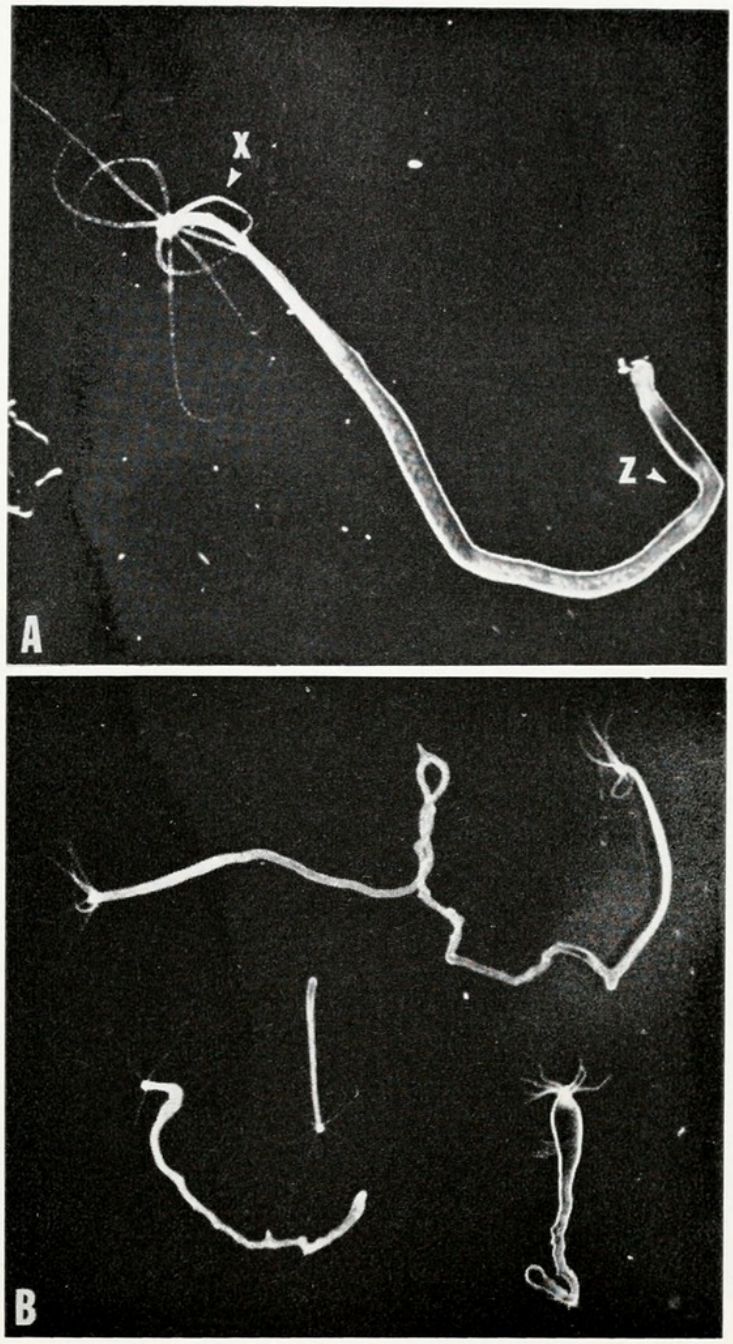


FIGURE 1. Representative monopolar pseudocolonial *H. viridis;* (A.) Single hydra with the regions of wounding indicated by "x" and "z" (see text for description of wounding experiments); (B.) Three monopolar pseudocolonial hydra surrounding a single, relaxed normal *H. viridis,* 4×.

animals were impaled on these hairs through the desired graft position. Tissues to be grafted were then also impaled, so that the cut surfaces of the graft and host tissues were in contact with one another. Graft and host parts were held attached to one another with a hair loop. Grafting was completed within 2–3 hours, after which time the hair loop was released, and the organisms removed from the implanted hair.

Histological studies

Animals were fixed for histological studies in Bouins fixative, dehydrated in ethanol, cleared in toluene, and embedded in paraffin. Serial sections were cut at

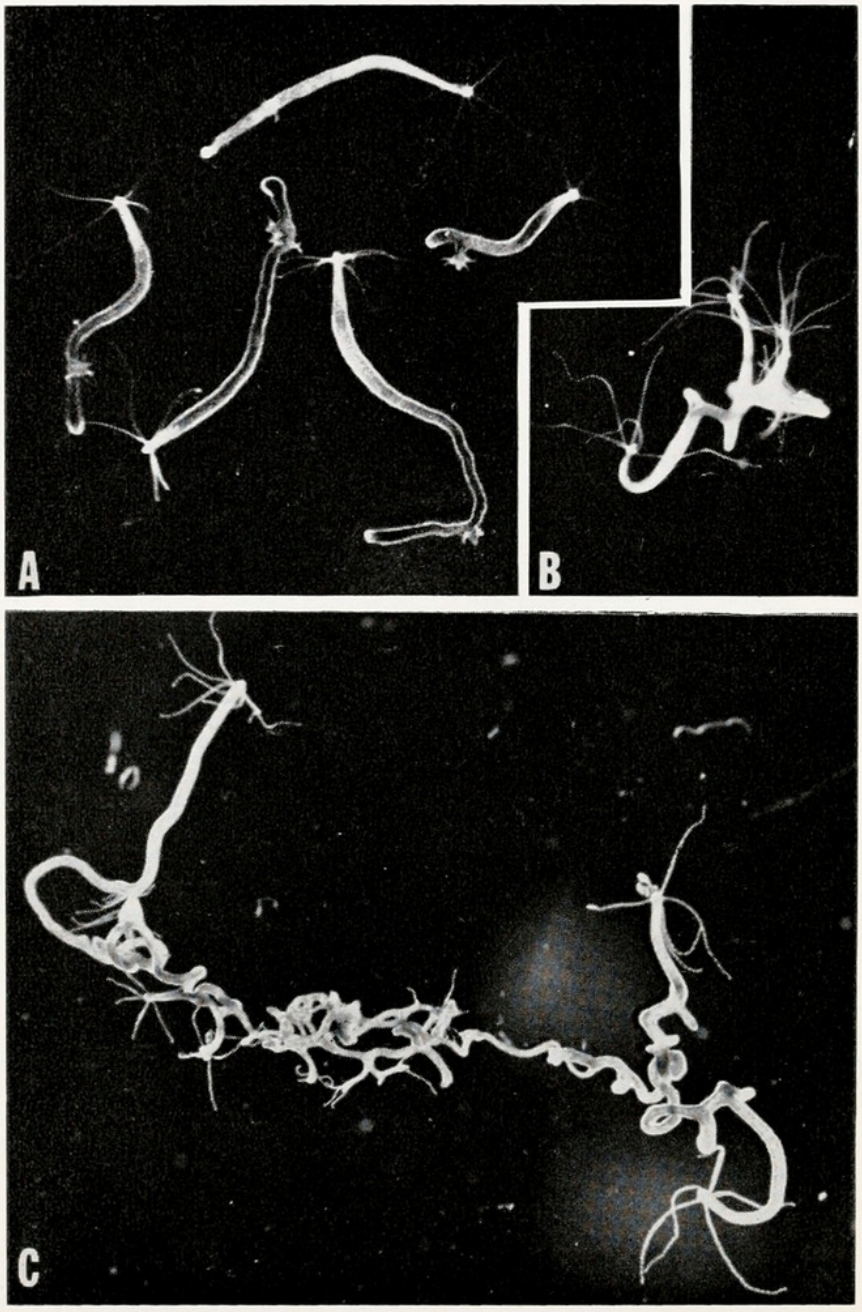


FIGURE 2. Representative pseudocolonial hydra after developing supernumerary hypostomes and/or basal discs; (A.) Initial stages in the development of supernumerary hypostomes and tentacles, 4×; (B.) Intermediate development of supernumerary structures, 3×; (C.) Typical pseudocolonial form, 2×.

5 μ and stained in 0.1% aqueous toluidine blue at pH 8. All histological observations reported were based on sections of a minimum of 10 animals for each experimental situation.

RESULTS

Occurrence of pseudocolonial hydra

A culture of *H. viridis* was inadvertently maintained at 25 ± 2° C for over one month. After this period animals possessing a unique morphology began to appear in the culture (Schulz and Lesh, 1970). These organisms were intensely green in color, and were 2–5 times the length of normal *H. viridis* (Fig. 1). In time

these elongated animals also lost the typical apical-basal polarity seen in the hydra. Rather than possessing a single hypostome distally and a basal disc proximally, these hydra developed hypostomes and/or basal discs all along the body column (Fig. 2). Furthermore, the supernumerary hypostomes developed either singly or in clusters. For these reasons these modified hydra have been termed "pseudocolonial" hydra. They will be referred to as such in this report.

Pseudocolonial animals removed from the 25° C culture and returned to normal culturing temperatures (22 ± 1° C) retained their peculiar morphology for as long as one year. (N.B. cultures were not kept longer than one year.) Animals cultured at 20 ± 1° C however, gradually returned to a normal morphology after several months. A constant supply of pseudocolonial forms was maintained during the duration of the investigation by allowing one culture to remain at 25° C. All experiments described in this report, however, were performed at normal culturing temperatures (22 ± 1° C), as detailed in the Methods and Materials section.

Histological organization of pseudocolonial hydra

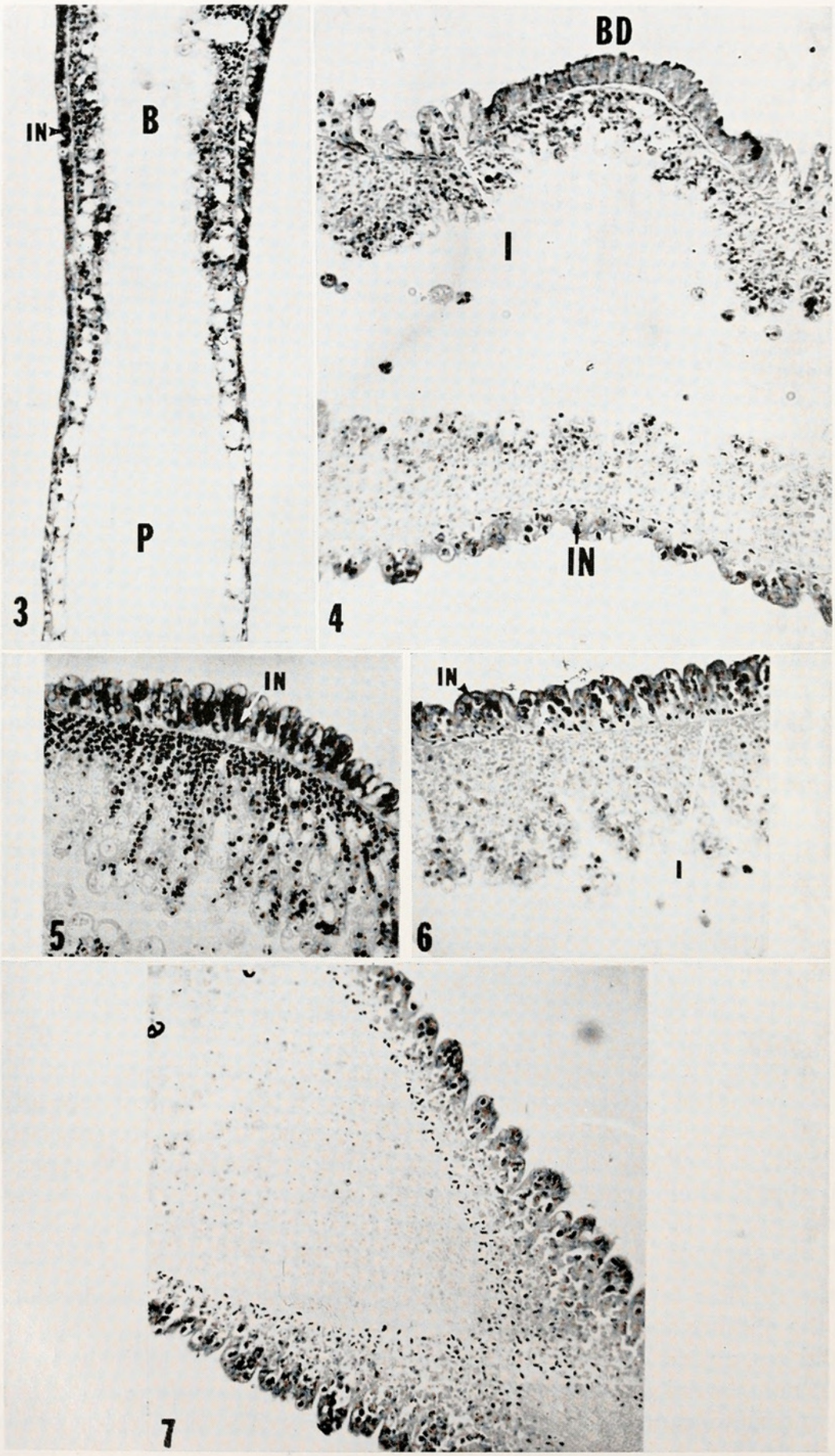
Histological examination of the pseudocolonial hydra revealed marked differences in both the body organization and distribution of cell types from that normally observed in hydra. Normally, proceeding proximally from the hypostome, the body column of the hydra is organized into a "growth" region, gastric region, and budding region. Each of these areas is histologically and histochemically distinct (Burnett, 1959). The budding zone is followed by a peduncle or stalk region, which immediately precedes the proximal basal disc. The peduncle region is characterized by the presence of a vacuolated gastrodermis, almost devoid of both food reserves and symbiotic algae. The epidermis is also characteristically thin and virtually lacks the small basophilic interstitial cells, so prevalent in the regions distal to the peduncle (Fig. 3). Two interstitial cell derivatives, cnidobasts and cnidocytes, are also normally absent from the peduncle. Therefore, the peduncle represents a region of decreased cellular activity. Quite possibly, cells are degenerating here prior to their being sloughed in the region of the basal disc.

In the pseudocolonial hydra, however, there was normally no cytological evidence for the existence of a peduncle. Basal discs developed at varying points along the body columns (see Figs. 2B and C), with no indication of the vacuolation and degeneration characteristic of a peduncle. Interstitial cells and interstitial cell derivatives also persisted in abundance in the areas immediately adjacent to these basal discs. Figure 4 shows numerous isorhizas cnidocytes next to a basal disc. The disc itself appears normal histologically, possessing the typical intense mucus border and elongate epitheliomuscular cells.

Cellular morphology

At the cellular level the only significant changes in the pseudocolonial form were in the number and distribution of interstitial cell derivatives. Interstitial cells *per se* did not appear any more abundant in pseudocolonial individuals. No specific cell counts were made, however, a superficial comparison of Figures 5 and 6 indicates no significant differences would be found. The occurrence of one interstitial cell derivative, however, is markedly increased.

Specifically, increased numbers of isorhizas cnidocytes were noted. This type



FIGURES 3–7.

of nematocyst is most often concentrated in the hypostome and tentacle regions of hydra (Lesh, 1970). In *H. viridis* they are nearly absent from the body column (Fig. 5). This distribution contrasts markedly with that seen in the column of the pseudocolonial *H. viridis* (Fig. 6). In the modified form virtually a layer of this type of cnidocyte is found just external to the mesoglea. The layered order of these cnidocytes is most apparent in Figure 7 which shows an oblique section through the column of a pseudocolonial hydra.

Growth pattern of pseudocolonial hydra

Ten individual pseudocolonial *H. viridis* were isolated in the "elongate" stage (*i.e.* prior to the development of any branches or supernumerary hypostomes or basal discs) (see Fig. 1). This "elongate" stage has been designated the monopolar pseudocolonial condition, and will subsequently be referred to as such. The growth pattern exhibited by these animals was recorded daily for 5–6 weeks. Accurate positioning of developing supernumerary structures was obtained by diagramming the organisms on graph paper as they appeared on a similar piece of paper affixed to the microscope stage. Two representative patterns are shown in Figure 8A. A representative growth pattern of a typical monomorphic, colonial, hydroid *Cordylophora* is presented also for comparison (Fig. 8B).

These growth patterns readily revealed two differences between the pattern of pseudocolonial *H. viridis* and that of *Cordylophora.* First, the pseudocolonial hydra separated portions of the "colony" at various times during its growth. The *Cordylophora* colony, alternatively, remained intact throughout the 5–6 week observation period. This observation is consistent with Fulton's previous growth pattern studies on *Cordylophora* (Fulton, 1961, 1963). In some instances (see Fig. 8A) the units separated from the hydra pseudocolonies were normal buds (Schulz and Lesh, 1970). Alternatively, the separation occurred at random (*i.e.* unpredictable) positions along the "colony." The latter event resulted in the formation of 2–3 smaller "colonies," often considerably unequal in size.

Superficially, the pattern exhibited by the pseudocolonial hydra recalls both the heterocyte-derived mutant reported by Lenhoff (1965) and the stolonizing mutant of Haynes, Burnett and Deutschman (1964). Both of these mutants often assumed multipolar forms. The occurrence of normal asexual reproduction, however, separates the present growth modification from Lenhoff's non-budding strain. The

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FIGURE 3. Longitudinal section of normal *H. viridis* comparing the budding zone (B) with the peduncle (P). The budding zone (B) possesses an epidermis containing numerous interstitial cells (IN) and gastrodermal cells packed with food reserves and symbiotic algae. Within the peduncle (P), the epidermal layer is thin and contains no interstitial cells. The cells of the gastrodermis are correspondingly thin and vacuolated, 160 ×.

FIGURE 4. Supernumerary basal disc (B) on a pseudocolonial hydra. Isorhizas cnidocytes (I) and interstitial cells (IN) are present in the areas immediately adjacent to the disc. A peduncle region, however, is absent, 160 ×.

FIGURE 5. Gastric region of a normal *H. viridis* showing numerous interstitial cells (IN), but an absence of isorhizas cnidocytes, 160 ×.

FIGURE 6. Gastric region of a pseudocolonial *H. viridis* illustrating a comparable number of interstitial cells (IN) to that observed in Figure 5. The added presence of layer of isorhizas cnidocytes (I), just external to the mesoglea is also evident, 160 ×.

FIGURE 7. Oblique section through a pseudocolonial animal to more directly demonstrate the layered distribution of the isorhizas cnidocytes in this form, 160 ×.

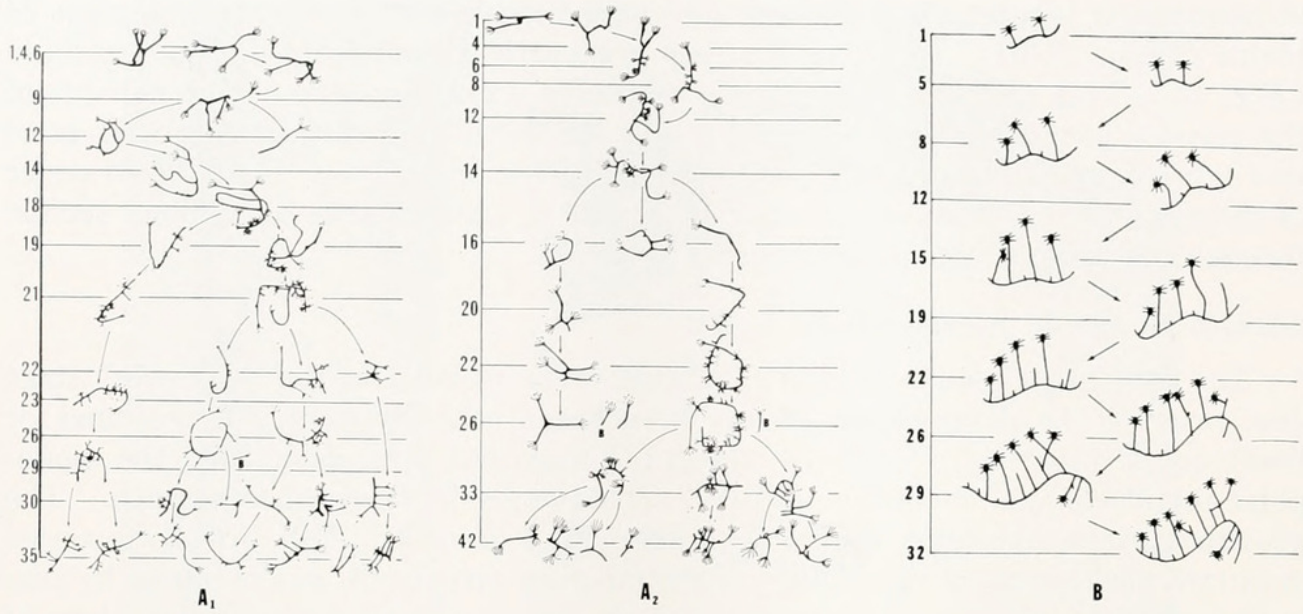

FIGURE 8. Growth patterns of two pseudocolonial hydras (A_1, A_2) and a typical colonial hydroid *Cordylophora* (B). In each of the patterns the ordinate represents days.

further observation that no part of the "colony" lacking a hypostome was ever detached from the parent mass distinguishes this aberrancy from the stolonizing growth modification described by Haynes, Burnett and Deutschman, 1964.

Therefore, although the pseudocolonial hydra was able to retain its asexual reproductive products temporarily, it still maintained the capacity for separation. In this respect it differs from the majority of colonial Cnidarians (Hyman, 1940).

The second difference exhibited in the growth pattern of pseudocolonial organisms was an absence of the spatial regularity in the development of individuals witnessed in the *Cordylophora* colony (see also Fulton, 1961, 1963). This irregularity was most apparent in the formation of hypostomes. Regeneration experiments by many workers have indicated that, with the exception of the peduncle and basal disc, the entire body column of a hydra normally possesses the capacity for hypostome differentiation. Despite this potential, hypostome formation is normally restricted to the distal end of the animal and to the budding region, an area nearly 60% of the length of the body column removed from the existing oral hypostome.

Pseudocolonial hydra developed hypostomes either singly or in clusters at nearly *any* point along the body column. The proximity of another hypostome appeared to have no effect on the subsequent development of additional hypostomal regions. This type of growth is markedly different from that typically exhibited in the hydra. Figure 9 compares hypostome positions along the column in 24 randomly selected, budding pseudocolonial and normal *H. viridis*. Each hydra selected was measured linearly and the position of each hypostome marked on graph paper. The hydra were then divided linearly along the body column into 20 equal segments and the segments containing hypostomes recorded. As all of the hydra possessed hypostomes in segment one, these are not indicated in the figure. In normal *H. viridis* hypostomes were concentrated within sections 12–16 (the budding region). The same analysis performed on pseudocolonial hydra, however, generated the open circle plot in Figure 9. With the exception of section two, hypostomes developed at any point along the body column of a pseudocolonial hydra. In normal *H. viridis*

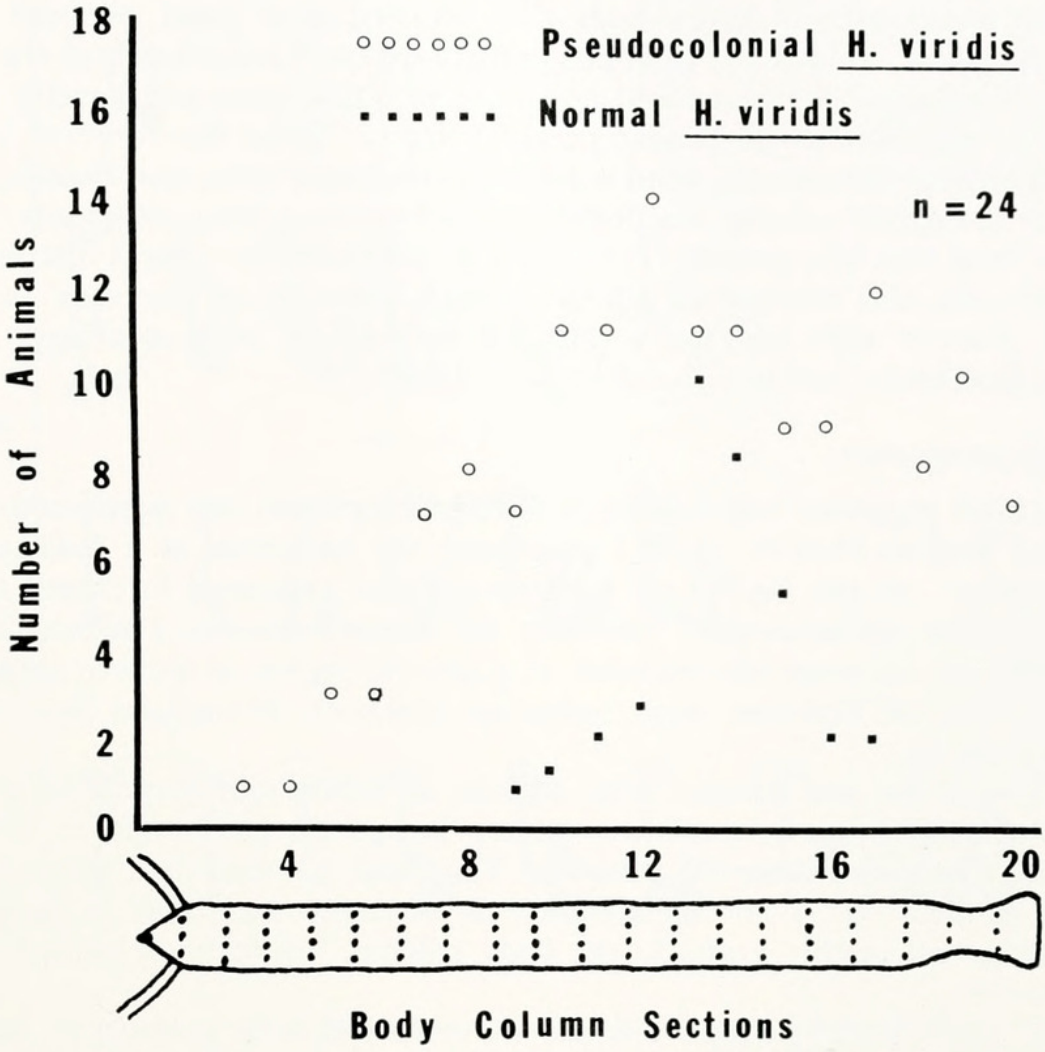

FIGURE 9. Distribution of hypostome formation in normal *H. viridis,* compared with pseudocolonial *H. viridis.*

hypostomes never occurred distal to section nine, nor proximal to section 17. The height of the distributions shown in Figure 9 is irrelevant. Pseudocolonial hydras, by possessing greater numbers of hypostomes, also result in an increased number of animals in each category. The significant point is the distribution of the hypostomes, not the absolute number of these structures formed.

From these observations, therefore, it is also evident that the normal factors operating to control hypostome development in hydra are either not operating or are greatly modified in the pseudocolonial hydra.

Bud development

An additional parameter of the growth pattern of any hydra is the production and detachment of true buds. As both normal and pseudocolonial hydra release buds (Schulz and Lesh, 1970), the similarities and the differences between the processes exhibited in both organisms must be examined.

Buds are normally initiated along the column at a point approximately 60% of the way down the body column. They then move through the budding and peduncular regions, forming a peduncle and basal disc, and are shed in the proximal portion of the adult peduncle (Baird and Burnett, 1967).

In the pseudocolonial hydra buds also initiated at a point removed from a parent hypostome. However, since the parent possessed no peduncle in the vicinity of the budding zone, the bud could not move into that zone and develop its own peduncle in association with similar parental tissue. Buds did, however, progress proximally along the column, form a peduncle and basal disc, and detach. When shed from the parent column the pseudocolonial bud was morphologically indistinguishable from that of a normal *H. viridis*. It possessed the typical distribution of interstitial cells and interstitial cell derivatives characteristically seen in normal animals. Twenty such buds were collected on each of three occasions. All became pseudocolonial within two weeks after being shed.

Basal disc movements

It has been suggested that regions of differential growth may occur in the hydra. Brien and Reniers-Docoen (1949) postulated the existence of a subhypostomal growth region. In this region cell proliferation was presumed to exceed that evidenced elsewhere in the animal. Recently, the autoradiographic evidence of Campbell (1965) has disputed the existence of a growth region or growth center. He has shown that cell divisions occur somewhat uniformly throughout the column of the individual.

One method to test whether any regions of differential growth exist in the pseudocolonial hydra is to mark the column in specific places and to follow the markers as they move along the column. The basal disc acts as a natural marker for such studies. It is not metabolized, is normally incapable of autonomous development, and readily grafts to the body column, providing reliable placement of the marker.

Eleven green pseudocolonial animals were marked with a bleached basal disc grafted in the growth region (or at the top of the gastric region). It made no difference if normal or pseudocolonial basal discs were used as grafts. Five additional animals received two grafted basal discs placed immediately adjacent to one another along the column, also in the growth region. The movement of these grafted basal discs was followed for a three week period. Three representative animals are shown in Figure 10. These diagrams were constructed with graph paper in the manner described in a previous section.

Several points became apparent in examining these movements. First, (1) the basal discs always moved proximally down the column. None moved distally into the hypostome or tentacle region. Also, (2) the total distance moved varied from one animal to another. Third, (3) grafted basal discs moved normally along the column until they reached a point at least the distance of the proximal surface of a normal hydra. At this juncture one of two events occurred. Either a peduncle formed and the pseudocolony split, with the grafted basal disc serving as a basal disc for the newly isolated individual (Figs. 10A and B). Alternatively, if a hypostome developed between the original distal hypostome and the grafted basal disc, no peduncle developed, no separation occurred, and the basal disc continued its movement along the pseudocolony (Figs. 10A and C). The animal shown in Figure 10C was followed for six weeks. At the end of that period the grafted basal disc was still attached to the pseudocolony.

An important point can be made from these observations. In order for separa-

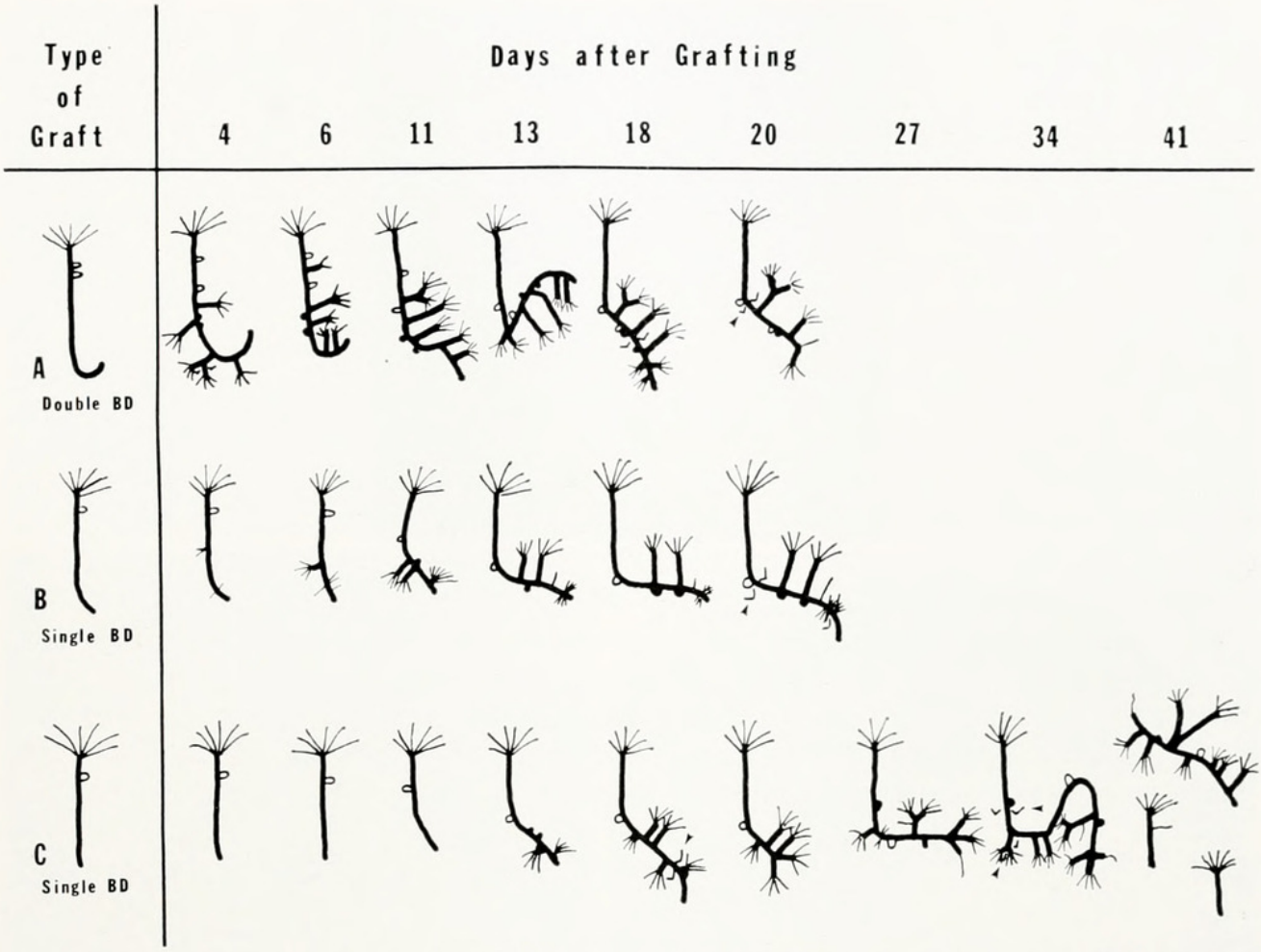

FIGURE 10. Comparison of the movement of grafted basal discs in pseudocolonial hydras. Note that (1) the basal disc always moves proximally down the column; (2) the total distance moved varies from one animal to another; and (3) the grafted basal discs move normally along the column until they reach a point at least the distance of the proximal surface of a normal hydra. Only after this point may separation occur or not occur, depending upon the relative positions of hypostome formation.

tion to occur in a pseudocolony a peduncle, which is normally not evident in a pseudocolony, and basal disc are required. The basal disc, however, need not be elaborated by the pseudocolony. Any basal disc of the appropriate species may serve as a potential separation point.

Regenerative capacity

One of the most intriguing aspects of hydra regeneration is that despite the labile capacity for hypostome and basal disc formation within the column, the animal rigorously maintains its disto-proximal polarity during the regeneration process (Burnett, 1961). Colonial hydroids, on the other hand, do not so readily adhere to their existing polarity. Either extremely short or extremely long stolon pieces often will exhibit a bipolar regeneration, forming hydranths at both cut surfaces (Tardent, 1963).

Furthermore, one unique characteristic of Lenhoff's (1965) heterocyte mutant hydra was that upon bisection both cut portions invariably developed hypostomes. Basal discs apparently failed to regenerate in these forms.

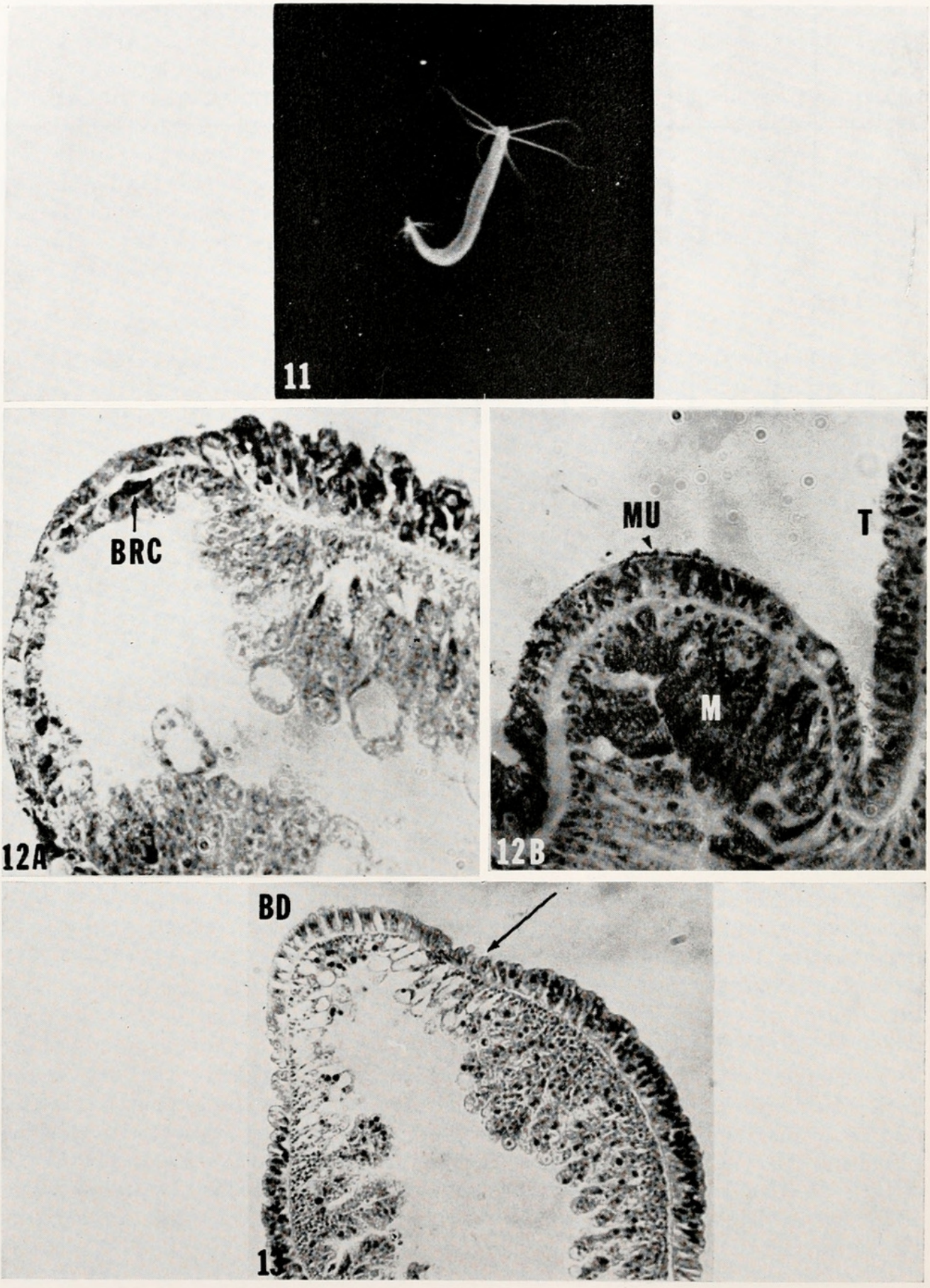

FIGURE 11. Typical bipolar regenerate which develops if a monopolar pseudocolonial hydra is severed through the lower third of its body column (see Table II for further explanation of the type of cut), 4 ×.

TABLE I

Regeneration following bisection of pseudocolonial and normal H. viridis. *Abbreviations are: Dps = distal half of pseudocolonial* H. viridis, *Dn = distal half of normal* H. viridis, *Pps = proximal half of pseudocolonial* H. viridis, *Pn = proximal half of norma* H. viridis.

Type of cut	Piece	Number of pieces	Structure regenerated at cut surface	
			Hypostome	Basal disc
Bisection of psuedocolonial and normal *H. viridis*.	Dps	15	2	13
	Dn	15	0	15
	Pps	15	14	1
	Pn	15	15	0

Bisection of the pseudocolonial hydra normally resulted in a polarized regeneration (Table I). Occasionally, however, the pseudocolonial animal failed to maintain its existing polarity and developed a bipolarity instead. Of 15 distal halves, 13 regenerated basal discs at the cut surface, corresponding to the existing polarity of the organism. Two, however, developed hypostomes, giving rise to a bipolar regenerate. Likewise 14 of 15 proximal pieces regenerated hypostomes distally. One, however, formed a basal disc.

This result led to the question of what would occur if a pseudocolonial hydra were allowed to achieve its elongated monopolar state (prior to any development of supernumerary hypostomes and/or basal discs) and were then severed through the extended lower third of the animal. This extended region, as shown in Table II, lies below the peduncle and basal disc regions of a normal hydra. Therefore, it is also further displaced from the distal hypostome than any part of a normal animal. When this type of cut was made, the distal pieces regenerated exclusively hypostomes at the cut surface (Fig. 11). Thirty hydra were excised, 30 regenerated hypostomes. This result is directly opposed to the regeneration observed above when the animal was bissected normally; and with the result obtained if one severs a normal *H. viridis* within the lower third of the body column (Table II).

Histological investigations confirmed the results observed grossly (Fig. 12). Within 12 hours after excision of the pseudocolonial hydra very little organized mesoglea was apparent and large numbers of gastrodermal basal reserve cells had collected in the wound area. Few interstitial cells were present (Fig. 12A). By 24 hours, tentacles were forming and increased numbers of interstitial cells were found. Mucous cells lined the gastrodermis and isorhizas cnidocytes were plenti-

FIGURE 12. Histological sections of regeneration occurring at the cut surface of monopolar pseudocolonial hydra severed through the lower third of the body column; (A.) Twelve hours of regeneration; Note the accumulation of basal reserve cells (BRC) in the cut region; (B.) Twenty-four hours of regeneration. Tentacles (T) have formed and mucous cells (M), characteristic of hypostome formation, line the gastrodermis. The typical external mucous border of the hypostome (MU), is also evident, 160 ×.

FIGURE 13. Histological section of regeneration occurring at the cut surface of normal *H. viridis* severed through the lower third of the body column. After 24 hours regeneration typical basal disc cells (BD) are present, 160 ×.

TABLE II

Regeneration following two types of cuts in the proximal (lower) third of pseudocolonial and normal H. viridis. *Abbreviations are: Dps = distal ⅔ of pseudocolonial* H. viridis, *Dn = distal ⅔ of normal* H. viridis, *Pps = piece of tissue isolated from the proximal (lower) third of pseudocolonial* H. viridis, *Pn = piece of tissue isolated from the proximal (lower) third of normal* H. viridis.

Type of cut	Piece	Number of pieces	Structure regenerated at cut surface	
Pseudocolonial and normal *H. viridis* severed in two places. One cut was made ⅔ of the distance from the hypostome to the basal disc. The second cut was ~3 mm above the basal disc.	Dps	30	Hypostome	
	Dn	27	Basal disc	
			Structure regenerated at cut surface	
			Distal surface	Proximal surface
	Pps	11	Hypostome	Basal disc
		2	Hypostome	Hypostome
		2	Basal disc	Basal disc
	Pn	15	Hypostome	Basal disc

ful. These events are characteristic of hypostome morphogenesis (Burnett, 1959). The mucous border seen in the hypostome region is also present (Fig. 12B).

Normal *H. viridis* severed in a similar manner showed only vacuolated, structureless gastric region cells after 12 hours regeneration. Few interstitial cells, cnidoblasts or food reserves were present in the wound region. By 24 hours typical basal disc cells were found and no interstitial cells were present. In fact, a distinct line (see arrow in Fig. 13) existed below which interstitial cells occurred only rarely (Fig. 13).

Proximal pieces whose origin was entirely within the extended portion of the pseudocolonial animal regenerated diverse structures at their cut surfaces (Table II). Eleven of 15 regenerated a hypostome at the distal cut surface and a basal disc proximally; conforming to the normal polarity of the organism. Two formed hypostomes at each cut end and two formed simultaneous basal discs at the cut surfaces.

These results support earlier conclusions regarding the possible modifications of hypostome control in a pseudocolonial hydra. Obviously, any inhibitory control emanating from the hypostomal region is active only over a portion of the entire body column of the pseudocolonial animal.

Effects of wounding

The possibility of a unique role for the hypostome in the control and expression of form in the pseudocolonial hydra led to the question of what would result if, rather than severing the animal, one only wounded it in a desired region. Two regions were selected for wounding (see Fig. 1A), an area slightly subjacent to the existing distal hypostome (arrow "x" Fig. 1A) and a region in the extended portion (lower third) of the column (arrow "z" Fig. 1A). These regions were

selected for several reasons. The area subjacent to the existing hypostome should be influenced by the presence of a hypostome in its proximity. Normal *H. viridis* wounded in this region simply healed the wound and formed no supernumerary structures at the wound site. Nine of 15 pseudocolonial hydra wounded in this region, however, formed basal discs at the wound site. Only 6 healed normally.

A wound in the lower third of the normal animal also was normally healed with no stimulation of regeneration of supernumerary structures. In the pseudocolonial hydra, however, this area is considerably further from the distal hypostome than in a normal animal. Also, the regeneration experiments indicate that this region may not be under any inhibitory influence from the existing distal hypostome. As predicted, 14 of 15 pseudocolonial organisms developed supernumerary hypostomes when wounded in this region.

Role of the hypostome

As evident from the preceding results, a physiological uniqueness of the pseudocolonial hydra appears to be the role of its hypostome in the control of growth and form. Normally, the hypostome is considered the "dominant" part of a hydra from a developmental stand point. It is the source of inductive material that is responsible for the gradient in interstitial cell differentiation along the column (Lesh, 1969, 1970). Furthermore, its presence presumably results in an inhibition in the development of similar structures nearby (Rand, Bovard and Minnich, 1926; Webster and Wolpert, 1966; Webster, 1966).

To examine the role of the hypostome in the control of growth and form in the pseudocolonial hydra, three types of grafting experiments were performed. In all cases grafts were between green and bleached animals so that the graft could be easily distinguished and followed. In the first experiment (1) the hypostome and growth region were exchanged between pseudocolonial and normal *H. viridis*. Second (2), pseudocolonial or normal hypostomes were grafted to the body column of the opposite hydroid type in a typical E. Browne Harvey induction graft (Browne, 1909). Finally (3), the gastric and upper budding regions were excised from pseudocolonial animals and the organisms were then reassembled without the gastric region. Following healing of the graft, a cut was made "x"-days after the graft through the "extended portion" of the animal (*i.e.* the original lower third of the body column). The previous regeneration experiments indicated this extended region normally regenerates a hypostome when severed.

Graft #1: Exchange of hypostome and growth regions. Hypostome and growth regions were exchanged between pseudocolonial and normal hydra to test the capacity of each hypostome to direct its own growth pattern. Ten grafts were performed in each case. In pseudocolonial hydra receiving a normal hypostome (N/PS) no morphological changes from the pseudocolonial form were observed over a 48-day observation period. The animals remained pseudocolonial. Normal hydra receiving a pseudocolonial hypostome (PS/N), however, would not "accept" this hypostome. The hypostome of the first bud that developed invariably became the hypostome of the organism. The pseudocolonial hypostome simply remained attached to the body of the organism and was in time sloughed from the base. These individuals never exhibited a pseudocolonial form nor did any of their buds.

Graft #2: Induction. Normal hypostomes grafted to the body of a pseudo-

colonial animal (N → PS), (retaining its own hypostome), induced a secondary axis of growth in 16 of 16 grafts. Pseudocolonial hypostomes grafted to the body column of normal hydra (PS → N), however, successfully induced a secondary axis in only 6 of 13 experimental organisms. Furthermore, none of these newly induced outgrowths ever exhibited the pseudocolonial morphology. In the remaining 7 grafts no induction occurred. The grafted pseudocolonial hypostome simply remained affixed to the body of the normal hydra.

Graft #3: Elimination of gastric and upper budding regions. Pseudocolonial hydra that had been reassembled with the gastric and upper budding regions eliminated were naturally smaller than normal pseudocolonial hydra. Consequently the original hypostome was in much closer proximity to the base of the animal. When these animals were severed one day after grafting 23 of 27 animals developed basal discs at the cut surface, while four formed hypostomes. If the graft remained in place for two days before the cut was made, only half of the regenerates (13 of 27) formed basal discs at the amputation site. The remainder developed hypostomes. Allowing the graft to remain longer than two days resulted in still greater proportions of the animals developing hypostomes at the cut surface. The inhibitory effect of the hypostome, therefore, is an immediate one, and appears to be maintained only over a limited growth period. Within 48 hours the situation has changed and hypostome formation is no longer so severely restricted. The animal appears to be reverting to its original pseudocolonial condition in which hypostome formation is inhibited only over a limited portion of the body column.

Discussion

When all of the parameters separating the pseudocolonial hydra from the typical solitary form are considered, the most pronounced and definitive characteristic is a morphological one, the existence of a peduncle region immediately distal to each and every basal disc in the solitary organism. This region, defined by its low metabolic activity, virtual absence of interstitial cells and cnidocytes, and its inability to autonomously regenerate, is also characteristically absent in colonial hydroids. Thus the question arises, is there, overtly, a morphological basis for the occurrence of the pseudocolonial hydra? Does the capacity to form a peduncle possibly separate colonial from solitary cnidarians?

The unique growth effects caused by the presence of a peduncle are most apparent in the growth patterns exhibited by the pseudocolonial hydra. Here hypostomes and basal discs form all along the body column. Separation, however, occurs only if a peduncle develops immediately adjacent to a basal disc. This is most evident in individuals detached from the pseudocolonial form as buds.

A further indication of the role of the peduncle in separation is seen in the experiments using basal discs as markers to detect differential growth along the body column. Here, too, if a peduncle develops, the pseudocolony separates at that point (Fig. 10). If no peduncle forms, no detachment occurs. Interestingly, peduncles form only in those regions where basal discs occur. However, the presence of a basal disc does not always result in peduncle formation.

Of all regions within the hydra, the peduncle has been literally overlooked in both theoretical and experimental considerations of the control of growth and form

in the organism. This oversight is not without justification. An isolated peduncle is normally incapable of independent existence. Furthermore, it exerts no apparent inductive growth influence on other regions of the animal (Browne, 1909). Its existence, therefore, has normally been explained as a result of the activity of one of the two presumed organization centers within the animal; the hypostome and/or the basal disc.

Hypostome as an organization center

Clearly, hypostome function is modified in the pseudocolonial hydra. The evidence indicates further that the modification is a suppression or lessening of the rigid hypostomal control normally witnessed in hydra. This conclusion is appropriate whether one is considering a hypostome's inductive (stimulatory) or its inhibitory mechanism of control.

Several observations support the conclusion that pseudocolonial hypostomes possess reduced inductive activity. For example, although the pseudocolonial hypostome is capable of maintaining its own morphological conformation, it neither induces nor remains the dominant hypostome in a normal *H. viridis*. Grafting experiments showed that $< 50\%$ of the pseudocolonial hypostomes were able to induce a secondary axis of growth in normal *H. viridis*. In grafts where a pseudocolonial hypostome was exchanged for the normal hypostome in a normal hydra, the pseudocolonial hypostome graft was not "accepted." The hypostome of the first bud of the normal animal assumed hypostome control of the organism. Normal hypostomes, alternatively, induced their morphological pattern in a pseudocolonial hydra 100% of the time. The latter observation also confirms that the cells of the pseudocolonial form have not lost the ability to respond to inductive materials.

Two additional consequences of hypostome inductive activity in the pseudocolonial hydra that require consideration are (1) the increased number and distribution of isorhizas cnidocytes; and (2) the continuous occurrence of interstitial cells along the body column of the animal. Normally isorhizas are confined to regions of high inductive activity and are thus restricted to the distal areas of *H. viridis* (Lesh, 1970). Interstitial cells are usually prevalent in the gastric and budding regions, but are drastically reduced in number proximal to the budding region, and are virtually absent in the lower third of the body column (*i.e.* peduncle and basal disc regions).

Both of these modified distributions are consistent with the hypothesis that the pseudocolonial hydra possesses a more uniform distribution of inductive influence throughout the body of the organism than one normally finds in hydra. A relatively consistent level of inductive capacity would result in the entire animal possessing nearly maximal concentrations of this activity. The fact that this maximum might be somewhat lower than that found in solitary animals would be irrelevant. In such a situation, events characteristic of high levels of inductive activity would persist. Among these events are the differentiation of isorhizas cnidocytes and a high concentration of interstitial cells (Lesh and Burnett, 1966; Lesh, 1969, 1970). Events associated with decreased levels of inductive activity would not occur. The formation of a peduncle represents one such event (Burnett, 1966).

The presence of hypostomes in virtually all of the 20 segments of a so-divided pseudocolonial hydra attest to a decrease in the inhibitory powers of the pseudocolonial hypostome. Furthermore, regeneration experiments indicate that this inhibition may not simply be reduced, but may also operate over only a limited portion of the body column. The regeneration of hypostomal structures at the proximal cut surface when a monopolar pseudocolonial hydra was severed within the proximal (lower) third of the body column is consistent with this conclusion. Likewise, the formation of supernumerary hypostomes when a pseudocolonial hydra is wounded in the proximal third of the body column also offers support.

The limited nature of the inhibitory effect is further documented by two additional observations. A wound made directly below the distal hypostome results in the development of a supernumerary basal disc, rather than a hypostome. This result is indicative of some degree of inhibition in regions close to an existing hypostome. Also, when the gastric and budding regions are removed from a pseudocolonial hydra, and the hypostome is placed in direct contact with the proximal third of the body column, an inhibition to hypostome formation (as measured by regeneration experiments) is immediately established in this part of the animal. Within 48 hours this inhibition is vastly reduced. The hypostome has now presumably grown further from the most proximal body regions, and thus cannot exert as great an inhibitory effect.

Interpreting these observations regarding hypostomal influences along the body column of the pseudocolonial hydra, the following situations could occur.

(1). The combined effect of a uniform distribution of inductive activities and the innately low level of hypostomal inhibition observed throughout the pseudocolonial hydra may not permit the elaboration of a normal budding zone. Some balance between the factors stimulating (inductive) and those inhibiting hypostome formation must exist to permit bud development. For at present unexplained reasons this balance may not occur in some hydra.

(2.) If no normal bud forms, no region of intense hypostomal inhibition is created along the body column of these hydra.

(3.) In the absence of asexual reproduction, the animal would simply continue to grow. The material and cells normally employed to formulate a bud would not elaborate additional body column. No dilution of interstitial cells to bud formation would occur and these cells would be available throughout the column.

(4.) The combined availability of interstitial cells and the lack of hypostomal inhibition would make the structuring of a peduncle impossible. No separation would occur in such an organism.

(5.) As a consequence of a uniform distribution of inductive substances and the presumed gradient distribution of inhibitory materials, hypostomes could occasionally develop along the column. The presence of a supernumerary hypostome could potentiate two developments. (i) The presence of a secondary hypostome could result in the achievement of the required balance between inhibition and stimulation to generate the formation of a peduncle and basal disc. In these cases separation will occur. (ii) Alternatively, the availability of large numbers of interstitial cells plus the position of the secondary hypostome relative to existing

hypostomal structures might result in no alteration of conditions in the body column. Here, the newly formed hypostome would simply remain and grow. In the latter situation one could still expect some manifestations of an increased level of inhibition due to hypostome formation. This increase should be most apparent distal and lateral to the developing hypostome. The inhibitory influence would probably not be in a proximal direction, as here the influence of the existing body column would be the greatest. Encouragingly tentacles form on all secondary hypostomes, and isolated tentacles do occasionally develop in areas near supernumerary hypostomes (see Fig. 8).

The end result of these activities would be the generation of a pseudocolonial organism.

Basal disc as an organization center

Mac Williams and Kafotos (1968) have presented evidence that the basal disc can also act as an inhibitor of its own differentiation. Through grafting experiments they found that the presence of a basal disc, in the vicinity of a proximal wound site, inhibited the regeneration of that structure at the cut surface. Therefore, the possibility exist that the basal disc could control peduncular formation, and consequently, the development of the pseudocolonial condition.

It is quite apparent from the growth patterns and the activity of grafted basal discs that the presence of a basal disc is essential for separation in the pseudocolonial form. The animal, however, has no noticeable difficulty elaborating these structures. They develop singly or in groups all along the body column. Furthermore, the presence of a basal disc does not insure separation will occur.

These facts, together with observations that during normal asexual development a peduncle forms before a basal disc, lead one to conclude that although the presence of a basal disc is important for its own differentiation, it is probably not the controlling agent for a pseudocolonial or colonial existence.

Organization centers in colonial hydroids

Before any ideas about the generation of a pseudocolonial condition can be accepted, however, they must be compared with the growth patterns exhibited in colonial hydroids. Theories exploring mechanisms for the control of growth and form in colonial hydroids have been suggested for many years. Berrill (1961) contains an excellent review of much of the early literature on hydroid polarity. The principal question arising here, however, is this: Could an explanation of hypostomal function, similar to that which has been proposed to explain the occurrence of the pseudocolonial hydra, also at least partially account for the growth pattern seen in *e.g. Cordylophora?*

Initially one must determine what portion of a colonial organism corresponds developmentally to the distal hypostome of a pseudocolonial individual. Two choices present themselves: the hypostome of an individual hydranth or the growing tip of a stolon. Several facts point to the stolon tip as a probable analogue. First, the stolon tip could be considered the "growth center" of a colony. It grows away from the existing colony (Fulton, 1963). Likewise, the hypostome of a pseudocolonial form, by developing an increased column length also displaces itself

further from the remainder of the animal. The growth pattern shown in Figure 8 also supports this conjecture, as secondary branches develop first in the proximal region of a pseudocolony and progress distally. This patterning corresponds directly to the growth pattern described for *Cordylophora* (Fulton, 1963).

At the cellular level, the organization and distribution of cell types is also consistent with the hypothesis of analogous roles for the stolon tip and pseudocolonial hypostome. Moving proximally from the pseudocolonial hypostome, one finds a uniform occurrence of interstitial cells and their derived cell types. Only in the buds of pseudocolonial animals is the typical hydra gradient in the distribution of these cells exhibited. The stolon of a colonial hydroid also contains abundant and stable levels of interstitial cells and of their derived cell types. In the uprights and hydranths, however, gradients in cell distribution become evident (Diehl, 1969).

Unfortunately, in a detailed analysis of inductive areas in *Cordylophora lacustris* Moore (1952) was unable to achieve induction with any tissues other than those possessing hydranth differentiation. However, she was able to correlate the capacity for induction with the availability of interstitial cells in the host tissue. Although stolon tips failed to induce secondary outgrowths in her system, considerable technical difficulty was experienced in these grafts. The grafted stolon tip invariably secreted perisarc around itself and separated from the remainder of the tissue mass. This separation could have prevented the necessary tissue interactions and/or temporal requirements requisite to an inductive event. Consequently, the inductive capacity of stolon tips should be reinvestigated.

Therefore, it is postulated that the presence of a level distribution of hypostomal inductive influences throughout the body column of a Cnidarian will ultimately result in the continued availability of interstitial cells throughout the column. Together these facts can prohibit the formation of a peduncle region. This morphological aberrancy restricts the capacity for separation in these organisms and could hence result in the generation of a pseudocolonial or colonial growth pattern.

The ecological reasons why this strain of hydra exhibits a pseudocolonial form when subjected to a condition of environmental stress (increased temperature) remains unknown at this time. Colonial patterns of organization are typically limited to animals of comparatively simple body organization and that reproduce asexually (Barrington, 1967). Conceivably, the possibility to increase the density of individuals in a population for purposes of feeding and sexual reproduction under stress conditions could provide a basis for this modification.

The technical assistance of Mrs. Mary Esther Haggerty is gratefully acknowledged. The author's appreciation is also extended to Mr. Ryland Loos who prepared the final pseudocolonial growth pattern diagrams.

Summary

1. Following a rise in culturing temperatures the reported strain of *H. viridis* grows in length and does not always detach its asexual reproductive products (buds). This aberrancy ultimately leads to the development of a pseudocolonial organization in these animals. Once elaborated, the pseudocolonial condition remains stable at normal culturing temperatures.

2. Analyses of several growth parameters in the pseudocolonial animals reveal that the normal role of the hypostome in the control of growth and form in hydra is modified in pseudocolonial individuals.

3. The principal morphological modification resulting from the altered hypostomal control is the absence of a peduncle region in the pseudocolonial hydra. Normal solitary hydra develop this histologically and histochemically distinct region immediately distal to the basal disc. Colonial hydroids, alternatively, possess no comparable region.

4. Comparing possible mechanisms for the control of growth and form in solitary, pseudocolonial, and colonial hydroids, the observations reported lead to the suggestion that there could be a morphological basis for colonial organization in the Cnidaria (*i.e.* the presence or absence of the capacity for peduncle formation).

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