Stout, J. D., and O. W. Heal. 1967. Protozoology. Pages 149-195 in Soil Biology. Edited by A. Burges and F. Raw. Academic Press. New York.
Thomas, R. 1959. Les Thécamoebiens muscioles et terrecoles: Nations de'ecologie général et comparative. P. V. Soc. Lin. Bordeaux. 97:1-27.
Thompson, R. H. 1959. Algae. Pages 115-170 in W. T. Edmondson, ed., Ward and Whipple's Fresh-water Biology, 2nd ed. Wiley and Sons, New York.
Varga, L. 1935a. Daten zier kenntnis der Protozoenfauna der Waldbodens von Eberswolde (Deutsches Reich). Centralbl. f. Bakt. Jena 93:32-38.
Varga, L. 1935b. Die Protozoa und ihre Vesteilung in Walboden von Tharandt. Centralbl. f. Bakt. Jena 93(2):128-137.

JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE
VOLUME 58, NUMBERS 3 & 4, JULY-OCTOBER 1983

BUD DEVELOPMENT IN *HYDRA:* AUTONOMY vs. POSITIONAL SIGNALS

JAMES A. ADAMS

Tennessee State University
Nashville, TN 37203

ABSTRACT

Bud development in *Hydra* can be divided into three distinct phases, 1) initiation and growth, 2) morphogenesis, and 3) detachment. This study looks at the extent to which these three phases of budding are coupled, the extent to which bud development is autonomous, and the importance of positional value to the budding process.

Morphogenesis of buds (formation of hypostome, tentacles, mouth, hydrant and foot) is found to be autonomous once the bud rudiment is formed thus coupling bud initiation and bud morphogenesis. Complete morphogenesis is observed even when the bud rudiment is displaced from its normal position, or when the rudiment is located on only a small portion (budding region annulus) of the parent body. Detachment of buds on the other hand is severely retarded, and in most cases totally inhibited when the bud does not have access to the area just sub-jacent to the budding region (upper peduncle region). This result is seen when the bud is translocated autoplastically, or when buds develop on budding region annuli from which the peduncle has been removed. Detachment therefore is not coupled to morphogenesis, and appears to be a function of the position of the bud on the parent hydrant.

INTRODUCTION

A common freshwater Cnidarian, *Hydra* has the following body regions in a disto-proximal (oral-aboral) direction: tentacles, hypostome or mouth region, gastric region, budding region (identified by the presence of two or more buds), peduncle, and basal adhesive disk or foot which serves as an organ of attachment to the substratum (fig. 1a.). The body regions of *Hydra* have been subdivided into arbitrarily assigned positions for the purpose of this study (fig. 1b.). H is the most distal position and contains the hypostome and tentacles (head). The gastric region is comprised of positions one, two, and three. Br designates the budding region, while positions four and five constitute the peduncle. F, the most proximal position, represents the foot.

Bud development may be separated into three component developmental processes; 1) initiation and growth of the bud rudiment, 2) morphogenesis (formation of tentacles, hypostome, mouth, and foot), and 3) detachment. It has been demonstrated in colcemid treated hydras (Shostak & Tammariello, 1969; Adams, 1975) that bud initiation and morphogenesis may occur without detachment, leading to supernumerary head formation. Although these results are generally explained in light of the known ability of colcemid to arrest or impede mitosis (Mazi, 1955, Bishoff & Holtzer, 1968) by disrupting microtubular assembly, this disruption could also impact seriously upon cellular movement. The latter thought is intriguing, particularly when viewed in light of the fact that buds move down the body column about the length of their diameter prior to detachment (Shostak & Kankel, 1967). Inhibition of bud detachment by colcemid might well be a result of inhibition of the aforementioned downward-mass-cell movement and thus have very little to do with mitosis per se. The implication therein would be that positional value is involved in bud detachment. Positional value is described as that force which renders cells non-equivalent and thus programs different developmental commitments (Wolpert, 1969; 1978).

This study has several objectives: a) to determine if bud morphogenesis is controlled by the parent body or if the bud rudiment is capable of autonomous morphogenic control, b) to assess the relationship between bud morphogenesis and bud detachment, and c) to look at the role of position in bud detachment.

MATERIALS AND METHODS

Animal Culture

Hydra oligactis and *Hydra littoralis* were mass cultured in 20 cm. diameter Pryex dishes in an incubator at 19±0.2°C. The hydras were fed *Artemia salina* nauplii daily and the medium (artificial pond water, Loomis & Lenhoff, 1956) was changed approximately one hour after feeding to remove dead nauplii. Experimental animals were kept in 15mm × 60mm Petri dishes, one animal per dish, which allowed individual observation to be taken on each animal used in a given experiment.

All animals employed in this study had one stage I bud. Budding stages were observed at 24 hrs. intervals in order to determine the rate of bud morphogenesis. The bud staging method of Shostak *et al.* (1968) was used. Stages 1

and 2 represented the bud's initiation and growth phase, stages 3-4 morphogenesis, and stages 5-7 detachment.

Series I - Control of Bud Morphogenesis

In examining the autonomy of bud formation the requirements for axial and circumferential information were tested using *H. littoralis*. To look at the need for axillary information the head was transected at the upper-gastric level and the foot was transected mid-peduncally (fig. 2a). Bud development in these animals was compared to that in untreated controls. In determining the need for circumferential information animals with the upper-gastric and mid-peduncle transections (*ump*-animals) were subsequently transected longitudinally (*empL*-animals). The *empL* transection was performed in such a way as to produce two bi-radial halves, one-half having a bud rudiment and the second bi-radial half being discarded (fig. 2b.). Bud development in these animals was compared to bud development in animals having *ump* transections only (control).

Series II-Control of Bud Detachment

Budding region annuli from *H. littoralis* and grafted *H. oligactis* were employed in this study in an attempt to determine the importance of positional value to bud detachment in hydras. Two groups of grafted *H. oligactis* were used; one group having the gastric regions reversed (g-reversal) and a second group with the gastric and budding regions reversed (gbr-reversal). Grafting was performed by first making the appropriate transections and placing the graft pieces on a strait skewer of human hair. Pre-tied knots of human hair were loosened with watchmaker's forceps and placed on each end of the skewer. The knots were then tightened and slid along the skewer until all cut surfaces were touching (fig. 3a). Grafts were allowed to heal for two and one-half hours followed by removal from the skewer using watchmaker's forceps (fig. 3b). G-reversal animals were used as controls against the gbr-reversal group in assaying the effects of moving the bud away from the subjacent region of the body column on bud morphogenesis and detachment. Budding region annuli were employed in studying effects of removing the body region subjacent to the bud rudiment on bud morphogenesis and detachment. Annuli were prepared by making transections immediately above and immediately below stage 1 buds. Controls were untreated (and unfed) hydras with stage 1 buds. Results from all control and experimentally treated animals were compared using Chi square and a 95% confidence level.

Results

Series I

Table I shows that removing the head and foot from *ump* animals did not have a lasting effect on bud development. Although the rate of bud maturation appeared slower at 24 hrs. in the *ump* group (as evidenced by significantly fewer stage 5 animals) such differences were totally absent by 72 hrs. incubation. All of the buds in the control and *ump* group detached by 96 hrs. of incubation.

The effects of removing the opposite bi-radial half of the parent body from *ump* animals is seen in Table 2. The animals produced by the above mentioned procedure (empL-animals) showed development rates comparable to the control at 24 hrs. At 48 hrs. of incubation the *empL* group displayed more rapid development than did the *ump* control by virtue of the significantly greater frequency of later stages (6 and 7). However, this difference disappeared by 72 hrs. incubation. By 96 hrs. of incubation all animals in both the control and experimental group had achieved 100% bud detachment.

TABLE 1. *Bud stages in* hydra oligactis *following removal of the head and foot of the parent body.*

TREATMENT	TIME HRS	STAGE 1 F , %	STAGE 2 F , %	STAGE 3 F , %	STAGE 4 F , %	STAGE 5 F , %	STAGE 6 F , %	STAGE 7 F , %
UPPER GASTRIC/ MID PEDUNCLE TRANSECTION	24		11 , 27.5	19 , 47.5	2 , 5	8 , 20		
	48				12 , 30	18 , 45	8 , 20	2 , 5.0
N = 40	72						21 , 52.5	19 , 47.5
	96							40 , 100
CONTROL	24		4 , 10	27, 67.5	1 , 2.5	8 , 20		
	48				13 , 32.5	21 , 52.5	6 , 15	
N = 40	72						27 , 67.5	13 , 32.5
	96							40 , 100

F: FREQUENCY

TABLE 2. *Bud stages in* hydra obligactis *following removal of the opposite bi-radial half of the parent body.*

TREATMENT	TIME HRS	STAGE 1 F , %	STAGE 2 F , %	STAGE 3 F , %	STAGE 4 F , %	STAGE 5 F , %	STAGE 6 F , %	STAGE 7 F , %
UPPER GASTRIC/ MID PEDUNCLE/ LONGITUDINAL TRANSECTION	24	9 , 22.5	28 , 70		2 , 5	1 , 2.5		
	48				1 , 2.5	23 , 57.5	13 , 32.5	3 , 7.5
	72						23 , 57.5	17 , 42.5
N = 40	96							40 , 100
CONTROL	24		11 , 27.5	19 , 47.5	2 , 5	8 , 20		
	48				12 , 30	18 , 45	8 , 20	2 , 5.0
N = 40	72						21 , 52.5	19 , 47.5
	96							40 , 100

F: FREQUENCY

Series II

Detachment in g-reversal animals was 92% complete by 48 hrs. post-grafting (Table 3). Gbr-reversal led to a significantly slower rate of bud detachment at all observation intervals, with more than half (51%) of the buds failing to detach at all (Table 3). Morphogenesis was complete in the buds of both types of grafted animals. Figure 4 shows an animal with a retained bud following gbr-reversal, giving rise to a supernumerary head.

TABLE 3. *Bud retention in* hydra obligactis *following reversal grafting.*

TREATMENT	48H F , %	72H F , %	96H F , %	1 WEEK F , %
GASTRIC REGION REVERSAL				
N = 39	3 , 8	0 , 0	0 , 0	0 , 0
BUDDING REGION AND GASTRIC REGION REVERSAL				
N = 39	33 , 85	25 , 64	20 , 51	20 , 51

F: FREQUENCY

When budding region annuli were employed the rate of detachment was likewise slower than that of the control (Table 4). In this instance 56% of the buds failed to detach and thus formed supernumerary heads (fig. 5). Figure 6 shows that the rate of morphogenesis (rate of attainment of stage four where all body parts were represented) did not differ between untreated control animals and br annuli.

TABLE 4. *Bud stages in* hydra littoralis *following annuli amputation.*

TREATMENT	TIME HRS	STAGE 1 F , %	STAGE 2 F , %	STAGE 3 F , %	STAGE 4 F , %	STAGE 5 F , %	STAGE 6 F , %	STAGE 7 F , %
ANNULI AMPUTATION GROUP	24	1 , 2	25 , 61	15 , 37				
	48		2 , 5	1 , 2	27 , 66	11 , 27		
N = 41	72			1 , 2	18 , 44	6 , 15	16 , 39	
	144			1 , 2	16 , 39	2 , 5	4 , 10	18 , 44
UNTREATED CONTROL GROUP	24		9 , 37	15 , 63				
	48				1 , 4	23 , 96		
N = 24	72						24 , 100	
	144							24 , 100

F: FREQUENCY

Discussion

It is clear from the results of Series I that a bud rudiment possesses the ability to undergo complete morphogenesis and detach from the parent hydrant in the absence of the parent head and foot. Removing the opposite bi-radial half of the parent hydrant is likewise without consequence as far as bud morphogenesis and detachment are concerned. These data suggest that bud morphogenesis and detachment are autonomous and once the bud rudiment has formed no morphogenetic information is required for further development. However, the concept of autonomous bud detachment must be rejected in light of the result of experimental Series II. From the results of the grafting experiments it is seen that when the bud is removed from its normal position (adjacent to region four) bud detachment occurs at a significantly slower rate with more than half of the buds failing to detach at all. Morphogenesis in these bud rudiments is complete with all body regions being formed. Thus, bud morphogenesis is autonomous but detachment of the bud from the parent is not. Furthermore, bud morphogenesis is effectively uncoupled from bud detachment in that morphogenesis may occur without subsequent detachment.

Since buds normally move down the *Hydra's* body column about the length of their diameter prior to detachment buds would actually detach while in region four. Thus this region could contain morphogenetic information necessary for bud detachment. This line of reasoning is further supported by the experiments using br annuli. Region four is removed from the br annuli and the resulting effect is that 56% of the buds fail to detach, giving rise the supernumerary heads. Morphogenesis is complete in the bud rudiments on the annuli however, the parental portions of the annuli may or may not form complete hydrants (fig. 5).

Bud detachment then, is a function of position, or is at least under the controlling influence of bud position. Detachment of buds in the solitary polyp forms of Cnidaria is apparently due to the development of this positional phenomenon. Because of the "position-four phenomenon" budding and 2° hydrant formation (leading to supernumerary heads or colonies) may be distinguished. However the initiation of 2° hydrants and buds are analagous events, both leading to morphogenesis of a new hydrant.

Summary

The first two phases of budding are coupled, with morphogenesis being autonomously controlled by the bud rudiment once a bud is initiated. Morphogenesis of complete hydrants is seen regardless of the buds position, the presence of the parent's head or foot, or the presence of region four. Bud detachment on the other hand, is dependent upon the bud's position. Access of the bud to region four appears to be the key. Since region four may regenerate, it could explain why some buds were able to detach even with translocation of the bud or removal of region four completely. It should be noted that those buds which did ultimately detach under the aforementioned conditions did so at a rate significantly slower than the controls.

Acknowledgments

The author wishes to thank Solomon Pollard, Gwendolyn Rees and Eva Williams for technical assistance in conducting this study.

This investigation was supported in part by NIH Grant No. 5 SO6 RR 08092-08.

References

Adams, J. A. 1975. Developmental aspects of the cell cycle in *Hydra viridis:* The effects of colcemid, colchicine, FU, and FUdR. Unpublished. Ph. D. Thesis, University of Pittsburgh, Pittsburgh.

Bishoff, R. and H. Holtzer. 1968. The effects of mitotic inhibition on myogenesis *in vitro*. J. Cell Biol. *36:* 111-127.

Loomis, W. F. and H. M. Lenhoff. 1956. Growth and sexual differentiation in *Hydra* in mass culture. J. Expl. Zool. *132:* 555-568.

Mazi, D. 1955. The organization of the mitotic apparatus. Symp. Soc. Expl. Biol. *9:* 335-357.

Shostak, S. and D. R. Kankel. 1967. Morphogenetic movements during budding in *Hydra*. Dev. Biol. *15:* 451-463.

Shostak, S., J. W. Bisbee, C. Ashkin, and R. V. Tammariello. 1968. Budding in *Hydra viridis*. J. Expl. Zool. *109:* 423-430.

Shostak, S. and R. V. Tammariello. 1969. Supernumerary heads in *Hydra viridis*. Nat. Cancer Instit. Monogr. (USA). *31:* 739-750.

Wolpert, L. 1969. Positional information and the spatial pattern of cellular differentiation. J. Theoret. Biol. *25:* 1-47.

Wolpert, L. 1978. Pattern formation in biological development. Sci. Am. *239:* 124 (October).

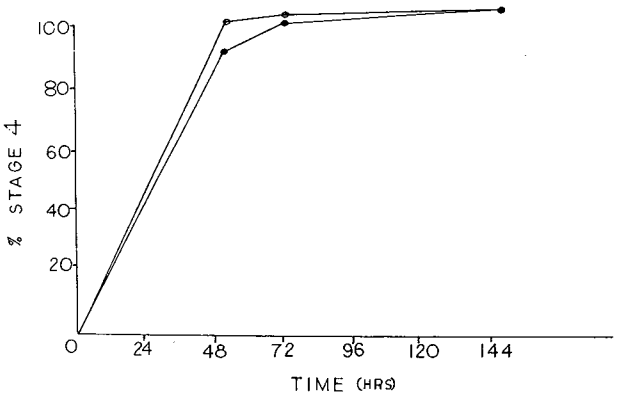
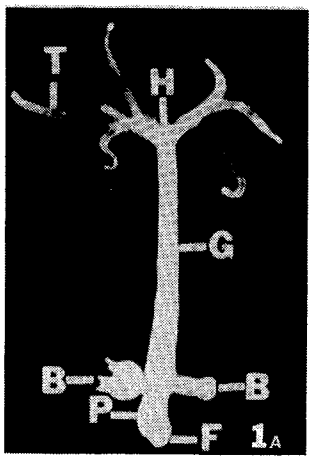
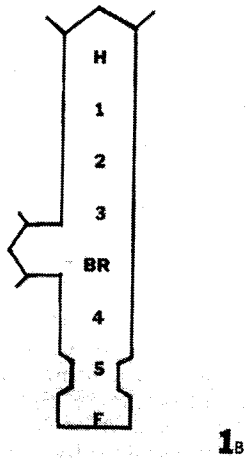
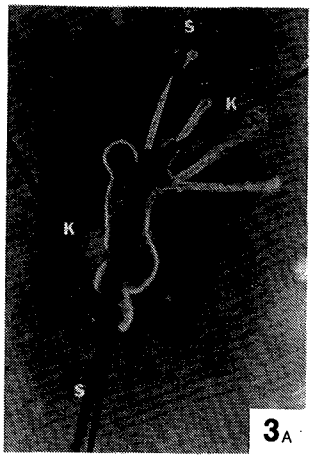
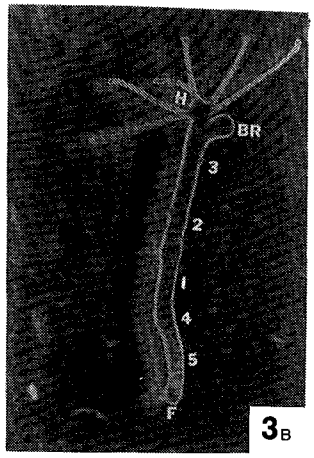


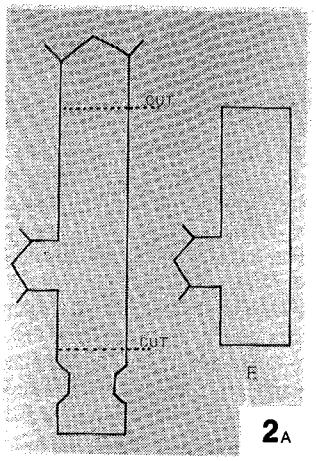
FIGURE 6. *The rate of attainment of stage four (completion of morphogenesis) is shown for stage 1 buds on untreated control hydras (0) and br annuli (●).*

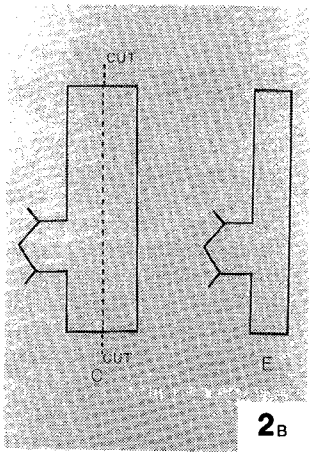


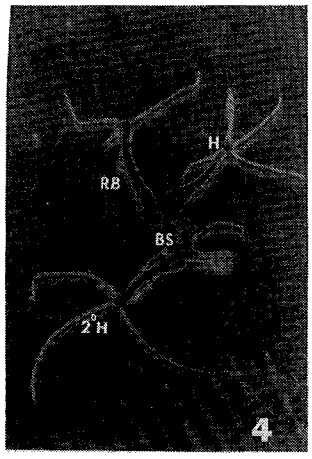


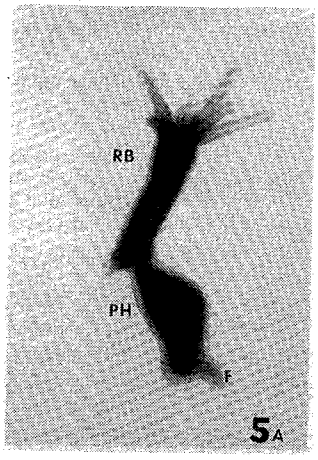












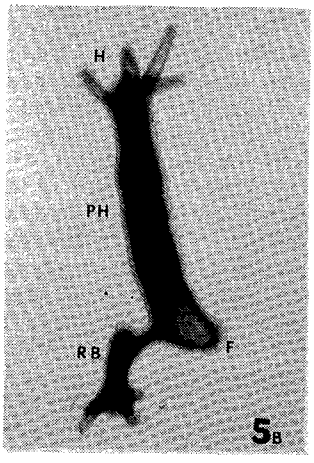


Figure Legends

FIGURE 1. Hydra's *morphology is shown in 1a. In a disto-proximal (oral-aboral) direction are the hypostome (H), tentacles (T), gastric region (G), budding region (B), peduncle (P), and foot or basal adhesive disk (F). The positions assigned to the morphological regions of the* Hydra *are illistrated in 1b.*

FIGURE 2. *Experimental Series I employs untreated control animals (C) and experimental animals (E) with the head removed mid-gastrically and the foot removed mid-peduncally (ump-animals) in testing for the bud's dependence upon information from the terminal structures during development (fig. 2a). When examining the need for circumferential information (fig. 2b) by a developing bud the controls are ump-animals (C) and the experimental group (E) are ump-animals with the opposite biradial half removed (empL-animals).*

FIGURE 3. *The animal above (fig. 3a) has been transected at the positions 1-H border and the positions Br-4 border followed by reversal of the gastric region and budding region (gbr-reversal) and threading of the graft pieces on a skewer (S) of human hair. Two knots (K) of human hair have been placed on either end of the graft pieces to insure apposition during healing. A healed gbr-reversal-grafted hydra is shown below (fig. 3b).*

FIGURE 4. *This gbr-reversal grafted animal shows a 2° head (2° H), a retained bud (RB), the terminal head of the original animal (H), and the body stalk (BS) of the original animal.*

FIGURE 5. *This figure shows animals with retained buds following br annulus isolation. A br annulus with a retained bud (RB) but having failed to regenerate a terminal head is shown on the left (6 days post-grafting) (fig. 4a). The animal on the right (fig. 4b) is an example of a br annulus with a retained bud (RB) as well as regeneration of the terminal head (H) and foot (F) on the parent hydrant (6 days post-grafting).*