On the Cellular Composition of *Pelmatohydra robusta* with Special Reference to Its Trend during the Budding Process

By

Syuzo Okazaki

Zoological Institute, Hokkaido University

(With 3 Text-figures and 2 Tables)

The trophic effects of neural factors in the regeneration in some invertebrates such as polychaetes are well known as well as in vertebrates, especially amphibians. As for hydra, however, there still remains a controversy to be settled whether the nerve cells are really involved in morphogenesis. It has been reported that during budding and head- and foot-regeneration nerve cells in hydra increase their number at the site of morphogenesis (Bode *et al.*, 1973). Schaller and his co-workers – in an attempt to isolate morphogenetic substances, or "morphogens", that are produced by nerve cells and affect morphogenesis in normal hydra — claimed the existence of four kinds of morphogens which function in regeneration, *i.e.*, head-activator, foot-activator, head-inhibitor and foot-inhibitor (Schaller and Gierer, 1973; Schaller *et al.*, 1979a). And it was suggested that the head-activator plays an important role in the budding process (Schaller, 1973).

Recently, Campbell (1976) and Sugiyama and Fujisawa (1978) independently produced nerve-free hydras either by means of colchicine treatment or by the isolation of a nerve-free mutant. By performing a series of experiments using such nerve-free hydras, these authors showed that nerve cells are not indispensable in morphogenesis, because the hydras carried out both budding and regeneration. Under these circumstances, it seemed important to re-evaluate possible participation of nerve cells in the budding process in connection with the precise description of the normal morphogenetic process. In the present study, the cellular composition of hydra throughout the course of budding is analyzed in detail and its significance to the budding process is considered.

Materials and Methods

The materials used were *Pelmatohydra robusta*. Animals were cultured, according to the method described by Noda (1972), in glass-distilled water containing 1

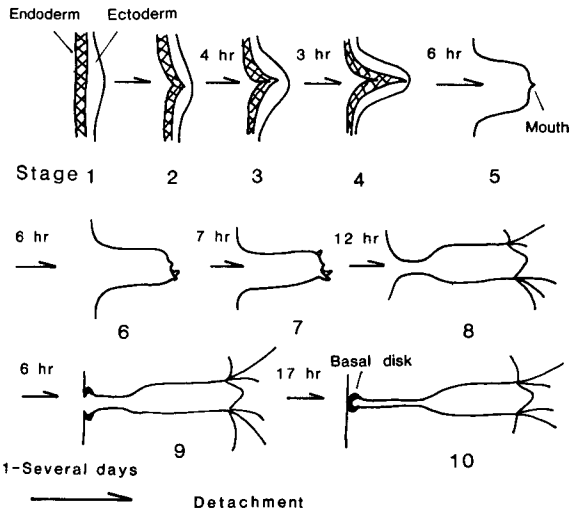

Fig. 1 Developmental stages of *Pelmatohydra robusta* bud. Buds at each stage are shown semidiagrammatically in profile. Time intervals between two consecutive stages are also indicated. The developmental stages were almost comparable — with minor differences — to those revealed by Otto and Campbell (1977) in *Hydra attenuata*. Development of the stage 8 *P. robusta* bud was more advanced than the stage 8 *H. attenuata* bud and the time table differed between the two hydras. A somewhat different sequence of appearance of tentacle rudiments was also seen in the two species. In *P. robusta*, there were usually two initial tentacle rudiments at the position of 4 and 8 o'clock on a dial plate. Additional rudiments appeared first at the position of 12 o'clock, followed by appearances at 6 o'clock and almost immediately at 10 and 2 o'clock, respectively. From the external morphology, the following ten developmental stages are discernible:

Stage 1: A portion of the ectodermal layer becomes thick.

Stage 2: The endoderm with an outward peak protrudes into the ectoderm.

Stage 3: Outward protrusion of the bud continues. The length is, however, less than a half of the parental body column thickness.

Stage 4: Outgrowth of the bud proceeds and its length reaches the thickness of the parental body column. The bud is dome-shaped when the animal contracts.

Stage 5: Opening of the mouth at the tip can be recognized for the first time when animal is fed. The length of the bud is longer than the thickness of parental body column when both parent and its bud are relaxed. The bud is cylinder-shaped when it is at the state of moderate contraction.

Stage 6: The first set of tentacle rudiments appears at the lower surface of the bud.

Stage 7: The length of the first set of tentacle rudiments is longer than their width. Usually, additional tentacle rudiments appear at this stage.

Stage 8: The peduncle is being formed the proximal region becomes somewhat paler in color and narrower in diameter, less than a half of the bud's gastric region. The bud starts feeding independently at this stage.

Stage 9: The basal disk is being formed and the ectoderm becomes translucent at the base of the bud.

Stage 10: The bud remains attached to the parent, but no longer has a common gastric cavity with it.

mM NaCl, 1 mM $CaCl_2$, 0.1 mM $MgSO_4$, 0.1 mM KCl and 1 mM Tris (hydroxymethyl) aminomethane, the pH being adjusted at 7.3–7.6 by HCl. The animals were maintained at about 20°C and fed once every day with newly-hatched *Artemia* nauplii. The medium was changed twice a day, about 30 min and 6 to 8 hr after feeding.

Fully-grown animals, with about 9 mm body column length in the state of moderate contraction, produced 1.0–1.5 buds every day, and the developmental stage of a bud was determined from its external morphology as shown in Fig. 1. A newly formed hydra started the first budding 1 to 3 days after detaching from its parent hydra.

The cell types of macerated hydras were essentially identified according to the method devised by David (1973). In brief, five to ten dissected tissues were dissociated in 100–500 μl of a maceration solution consisting of glycerin: acetic acid: water (1: 1: 13); the resulting cell suspension was fixed by adding one or two drops of 20% formalin. The cell suspension, spread on gelatine-coated slide glass and dried, was examined under a phase contrast microscope. A total of at least 2,000 cells was counted per specimen. The absolute number of cells was obtained by determining the cell concentration with a Neubauer hemocytometer (0.1 mm depth). For technical reasons, in order to determine their cellular composition, the early buds were examined as a whole, whereas the older, and therefore larger, buds were investigated in their several regions. To examine the cellular composition of a mature hydra, several regions along the body axis were cut: the tentacles, the hypostomal region including the tentacle base, the upper gastric region, the lower gastric region, the budding region excluding buds, the peduncle and basal disk. The upper and lower gastric regions were obtained by transversely cutting the gastric region in half with a scalpel. In this paper, an animal which had been detached from the parent hydra at least 10 days previously and bore at least 4 buds is designated as a "mature" hydra. The maceration of hydra tissues was performed about 24 hr after the last feeding to avoid possible contamination by *Artemia* cells.

Results

The composition of cell types, the relative cell densities$\pm$S.D. (%) of each cell type and the total number of cells in various regions of the growing bud and mature hydra are shown in Tables 1 and 2. The young hydra bearing a single bud at the early stages produces a second bud on the opposite side of the budding region. Therefore, after removing the pre-existing very young bud from the parent hydra, the remaining part of the budding region was regarded as being at pre-budding stage, or stage "O" (see the scheme in Table 1). The changes of relative density of each cell type during growth of the bud are represented graphically in Fig. 2 (a to g). It is apparent that the cellular composition of early buds prior to stage 4 is almost identical to the budding region at stage "O". The first detectable changes in cellular composition was the increase in number of the nerve cells in the stage 4 bud

Table 1. Cellular composition of developing bud

Stage & Region	Cell No.		E.P. + Digestive	Battery	Big I-cell	Little I-cell	Nerves	Nemato-blasts	Nemato-cytes	Mucous	Zymogen
"0"		%	16.0 ± 2.30	—	16.5 ± 1.88	27.0 ± 1.35	2.0 ± 0.72	23.0 ± 1.82	8.5 ± 1.41	2.0 ± 1.06	3.5 ± 0.55
	13,000	#	2,090	—	2,150	3,519	260	2,990	1,110	460	460
2		%	15.5 ± 0.36	—	16.5 ± 1.96	29.5 ± 1.63	1.5 ± 0.08	23.0 ± 2.49	9.5 ± 1.56	1.5 ± 0.18	3.0 ± 0.57
	7,800	#	1,210	—	1,290	2,300	120	1,790	740	120	230
3		%	15.0 ± 0.81	—	18.0 ± 2.05	30.0 ± 0.57	2.3 ± 0.13	22.0 ± 2.62	8.0 ± 0.21	1.5 ± 0.66	3.0 ± 0.86
	7,500	#	1,130	—	1,350	2,250	170	1,650	600	110	230
4		%	14.0 ± 4.13	—	15.0 ± 1.66	29.5 ± 3.68	5.0 ± 1.04	21.5 ± 2.12	10.0 ± 1.18	2.0 ± 1.59	3.0 ± 1.14
	9,500	#	1,330	—	1,430	2,800	480	2,040	950	190	290
5 Tip	8,500	%	16.5 ± 2.85	—	13.0 ± 1.80	34.0 ± 2.06	5.0 ± 0.28	17.0 ± 1.17	12.0 ± 2.17	1.0 ± 0.26	2.0 ± 0.04
		#	1,410	—	1,110	2,890	430	1,450	1,020	90	170
Base	8,500	%	16.0 ± 1.56	—	15.0 ± 1.65	29.0 ± 1.38	1.5 ± 0.04	23.0 ± 1.45	11.0 ± 1.57	1.5 ± 0.20	3.0 ± 0.63
		#	1,360	—	1,280	2,470	130	1,960	940	130	260
Total	17,000	#	2,770	—	2,390	5,360	560	3,410	1,960	220	430
		%	16.3	—	14.1	31.5	3.3	20.1	11.5	1.3	2.5
6 Tip	9,000	%	19.0 ± 1.80	—	11.0 ± 2.64	25.5 ± 3.38	7.8 ± 1.94	20.0 ± 2.65	11.5 ± 1.75	3.0 ± 0.92	3.0 ± 0.49
		#	1,720	—	990	2,300	700	1,800	1,040	270	270
Base	9,000	%	13.0 ± 1.86	—	17.0 ± 1.06	28.0 ± 2.56	3.0 ± 0.85	28.0 ± 0.32	8.0 ± 2.00	1.0 ± 0.38	2.0 ± 0.26
		#	1,180	—	1,530	2,520	270	2,520	720	90	180
Total	18,000	#	2,900	—	2,520	4,820	970	4,320	1,760	360	450
		%	16.1	—	14.0	26.7	5.4	24.0	9.8	2.0	2.5

Table 1. (Continued to next page)

Stage & Region		Cell No.		E.P. + Digestive	Battery	Big I-cell	Little I-cell	Nerves	Nemato-blasts	Nemato-cytes	Mucous	Zymogen
7	Tip		%	16.5 ± 0.48	0.5 ± 0.01	10.5 ± 0.60	29.0 ± 2.13	8.5 ± 1.43	16.0 ± 1.62	13.5 ± 0.84	2.5 ± 0.24	3.0 ± 0.47
		8,500	#	1,400	40	890	2,470	720	1,360	1,150	210	260
	Base		%	14.5 ± 3.30	—	16.0 ± 0.73	34.0 ± 2.16	2.0 ± 0.16	21.0 ± 2.61	8.5 ± 1.75	1.0 ± 0.52	2.2 ± 0.09
		12,000	#	1,740	—	1,920	4,080	240	2,520	1,020	120	260
	Total	20,500	#	3,140	40	2,810	6,550	960	3,880	2,170	330	520
			%	15.3	0.2	13.7	32.0	4.7	18.9	10.6	1.6	2.5
8	Head		%	11.0 ± 0.35	3.0 ± 0.15	5.0 ± 0.20	16.5 ± 4.6	13.0 ± 2.00	14.0 ± 1.90	34.0 ± 2.15	3.0 ± 0.10	1.5 ± 0.10
		4,200	#	460	130	210	690	550	590	1,430	130	60
	Upper Body Column		%	13.5 ± 1.31	—	16.0 ± 1.53	33.0 ± 3.83	2.5 ± 0.46	23.0 ± 2.28	8.0 ± 1.05	1.5 ± 0.36	2.5 ± 0.40
		11,000	#	1,490	—	1,760	3,630	280	2,530	850	170	280
	Lower Body Column		%	24.0 ± 2.37	—	12.5 ± 1.15	24.0 ± 2.44	4.0 ± 0.92	20.0 ± 2.75	11.0 ± 2.20	1.5 ± 0.61	2.5 ± 0.44
		10,000	#	2,400	—	1,250	2,400	400	2,000	1,100	150	250
	Total	25,200	#	4,350	130	3,220	6,720	1,230	5,120	3,380	450	590
			%	17.3	0.5	12.8	26.7	4.9	20.3	13.4	1.8	2.3
9	Tentacles		%	2.5 ± 0.83	6.0 ± 0.55	—	0.3 ± 0.20	3.0 ± 0.28	—	87.0 ± 1.13	—	—
		3,800	#	100	230	—	10	110	—	3,310	—	—
	Hypostomal Region		%	22.5 ± 1.28	1.0 ± 0.12	3.0 ± 0.49	9.0 ± 2.05	15.0 ± 0.71	10.5 ± 3.31	28.0 ± 3.89	9.5 ± 3.17	2.0 ± 0.33
		3,100	#	700	30	90	280	470	330	870	290	60
	Gastric Region		%	14.5 ± 1.14	—	16.0 ± 1.41	28.0 ± 5.13	2.0 ± 0.30	24.0 ± 6.01	11.0 ± 2.16	1.5 ± 0.63	3.0 ± 0.21
		22,500	#	3,270	—	3,600	6,300	450	5,400	2,480	340	680
	Foot		%	48.5 ± 7.77	—	5.5 ± 2.12	12.0 ± 3.34	12.0 ± 2.03	8.0 ± 1.48	12.0 ± 4.37	1.0 ± 0.16	1.0 ± 0.30
		3,500	#	1,700	—	190	420	420	280	420	40	40
	Total	32,900	#	5,770	260	3,880	7,010	1,450	6,010	7,080	670	780
			%	17.5	0.8	11.8	21.3	4.4	18.3	21.5	2.0	2.4

Table 1. (Continued to next page)

Stage & Region	Cell No.		E.P. + Digestive	Battry	Big I-cell	Little I-cell	Nerves	Nemato-blasts	Nemato-cytes	Mucous	Zymogen
Newly-dropped		%	2.0 ± 0.42	6.0 ± 0.30	—	—	2.5 ± 0.20	—	89.5 ± 0.65	—	—
Tentacles	7,700	#	150	460	—	—	190	—	6,900	—	—
		%	29.0 ± 1.30	0.5 ± 0.25	2.0 ± 0.65	2.0 ± 0.79	22.0 ± 6.41	0.5 ± 0.21	31.0 ± 3.39	12.5 ± 3.12	0.2 ± 0.23
Hypostomal Region	4,300	#	1,250	20	90	90	950	20	1,330	540	10
		%	16.5 ± 2.34	—	18.5 ± 1.05	26.0 ± 0.49	2.5 ± 0.30	22.0 ± 1.90	10.0 ± 2.49	1.5 ± 0.59	3.0 ± 0.30
Upper Gastric Region	18,000	#	2,970	—	3,330	4,680	450	3,960	1,800	270	540
		%	18.0 ± 2.42	—	17.5 ± 1.05	26.5 ± 4.15	2.0 ± 0.47	22.0 ± 2.51	10.0 ± 1.77	1.5 ± 0.84	2.5 ± 0.57
Lower Gastric Region	10,000	#	1,800	—	1,750	2,650	200	2,200	1,000	150	250
		%	45.5 ± 6.78	—	4.5 ± 1.55	10.0 ± 4.82	10.0 ± 2.17	9.5 ± 3.15	17.0 ± 1.22	2.5 ± 0.44	1.0 ± 0.42
Peduncle	2,500	#	1,140	—	110	250	250	240	430	60	30
		%	69.5 ± 2.17	—	—	0.4 ± 0.37	21.0 ± 1.67	—	8.0 ± 0.89	1.0 ± 0.10	—
Basal Disk	1,600	#	1,110	—	—	10	340	—	130	20	—
Total	44,100	#	8,420	480	5,280	7,680	2,380	6,420	11,590	1,040	830
		%	19.1	1.1	12.0	17.4	5.4	14.6	26.3	2.4	1.9

Table 1. For examination, the developing buds were divided into several parts shown schematically in the left column. The relative density was expressed as a ratio of the number of a particular cell type to the total number of all cell types existing in each region.

Table 2. Cellular composition along body axis of a mature hydra

Region	Cell No./ Region		Epithelio-muscular	Battery	Digestive	Big I-cell	Little I-cell	Nerves	Nemato-blasts	Nemato-cytes	Mucous	Zymogen
Tentacles	36,000	%	–	4.6 ± 0	0.7 ± 0.20	–	–	2.8 ± 1.20	–	91.7 ± 1.45	–	–
		#	–	1,660	250	–	–	1,010	–	33,000	–	–
Hypostomal	9,500	%	8.3 ± 2.39	1.1 ± 0.54	12.5 ± 0.74	1.4 ± 0.28	3.6 ± 0.24	18.6 ± 1.93	1.2 ± 0.16	30.4 ± 1.32	22.0 ± 2.49	0.1 ± 0.08
		#	790	100	1,190	130	340	1,770	110	2,890	2,090	10
Upper Gastric	43,000	%	8.0 ± 1.23	–	9.2 ± 1.28	17.5 ± 1.66	28.5 ± 2.20	1.6 ± 0.49	15.9 ± 1.93	8.9 ± 1.97	6.5 ± 1.39	4.3 ± 0.80
		#	3,440	–	3,960	7,530	12,260	690	6,840	3,830	2,800	1,850
Lower Gastric	44,000	%	8.0 ± 0.52	–	11.0 ± 0.74	16.9 ± 0.61	27.5 ± 1.13	1.2 ± 0.38	18.4 ± 4.39	9.4 ± 2.29	3.7 ± 1.35	3.4 ± 1.39
		#	3,520	–	4,840	7,440	12,100	530	8,100	4,140	1,630	1,500
Budding	26,000	%	8.2 ± 0.54	–	11.0 ± 0.49	15.9 ± 2.29	25.7 ± 1.11	1.9 ± 0.36	19.7 ± 4.59	11.5 ± 1.99	3.0 ± 1.02	2.4 ± 0.33
		#	2,130	–	2,860	4,130	6,680	490	5,120	2,990	780	620
Peduncle	7,100	%	36.3 ± 2.50	–	14.8 ± 3.00	3.1 ± 0.15	8.7 ± 1.60	9.4 ± 0.30	7.2 ± 2.25	15.9 ± 2.25	3.8 ± 0.25	0.3 ± 0.10
		#	2,580	–	1,050	220	620	670	510	1,130	270	20
Basal Disk	2,200	%	55.6 ± 1.36	–	9.3 ± 0.40	–	1.0 ± 0	18.8 ± 1.95	–	13.9 ± 2.95	1.3 ± 0.10	–
		#	1,220	–	200	–	20	410	–	310	30	–
Total	167,800	#	13,680	1,760	14,350	19,450	32,020	5,570	20,680	48,290	7,600	4,000
		%	8.2	1.0	8.6	11.6	19.1	3.3	12.3	28.8	4.5	2.4

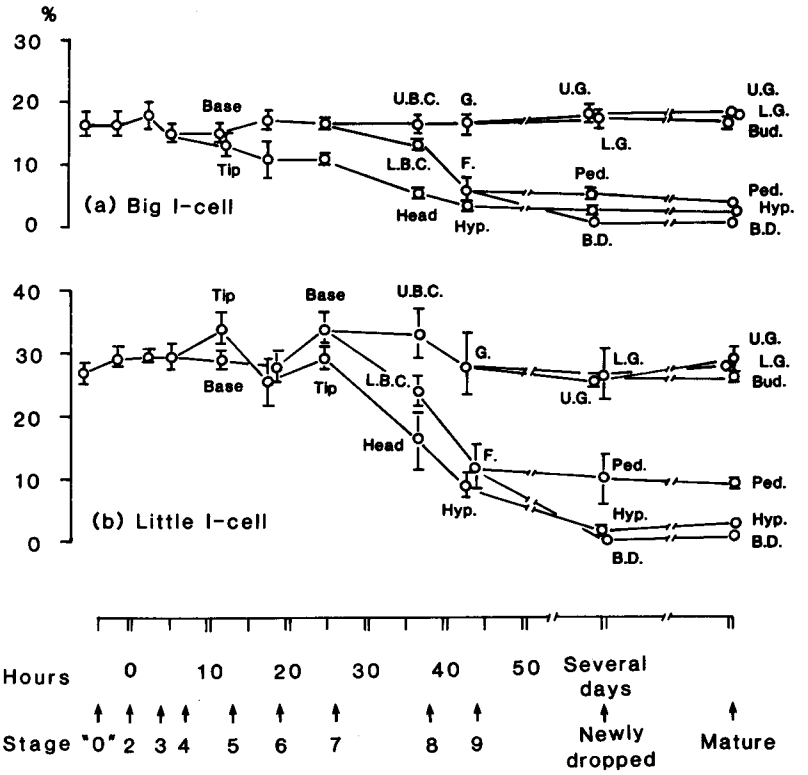

Fig. 2. (a to g). Change in relative density of a particular cell type of developing bud (data from Table 1).

Ordinate: percent of the number of each cell type to the total cell number of all cell types existing in each region.

(a) Big I-cell, (b) little I-cell, (c) nerve cell, (d) nematoblast, (e) nematocyte, (f) mucous cell and (g) zymogen cell.

B.D., basal disk; Bud., budding region; F., foot; G., gastric region; Hyp., hypostomal region; L.B.C., lower body column; L.G., lower gastric region; U.B.C., upper body column; U.G., upper gastric region; Ped., peduncle; T., tentacles.

just prior to the opening of the mouth. After this stage, the relative number of nerve cells continued to increase at the bud tip (presumptive head) and the maximum relative density was attained by the newly-dropped hydra (Fig. 2c). At the tip of the stage 5 bud, the nematoblasts started to decrease gradually in relative number until the time of detachment (Fig. 2d). At the time of differentiation of the tentacles (st. 6) and thereafter, the nematocytes continued to increase in relative number at the tip of the bud until the time of detachment (Fig. 2e). There were more mucous cells at the tip than the base of the stage 6 bud and they were

found clearly accumulated in the tip of older bud at stage 9 (Fig. 2f). Decrease in the relative number of big I-cells started in the tip of the bud from stage 5 or 6 (Fig. 2a), followed by a similar decrease of little I-cells between stage 6 and 7 (Fig. 2b). The decrease of both kinds of I-cells lasted until the time of detachment. In the base region of the stage 8 bud, nerve and nematocytes began to increase their relative densities (Figs. 2c and 2e), while big I-cells and little I-cells began to decrease from the same stage (Figs. 2a and 2b). The nematoblasts decreased their relative density in older buds (at st. 8 and 9, Fig. 2d). No change occurred in the density of the zymogen cells before stage 8, but, after this stage, both head and foot regions had smaller populations of this cell (Fig. 2g). The time when these changes occurred in the cellular composition of the bud was apparently well correlated with the time of differentiation of head or hydranth (hypostome and tentacles) (from st. 5 to 6) and that of foot (peduncle and/or badal disk) (from st. 8 to 9).

Although the cellular composition of newly-detached hydra was almost similar

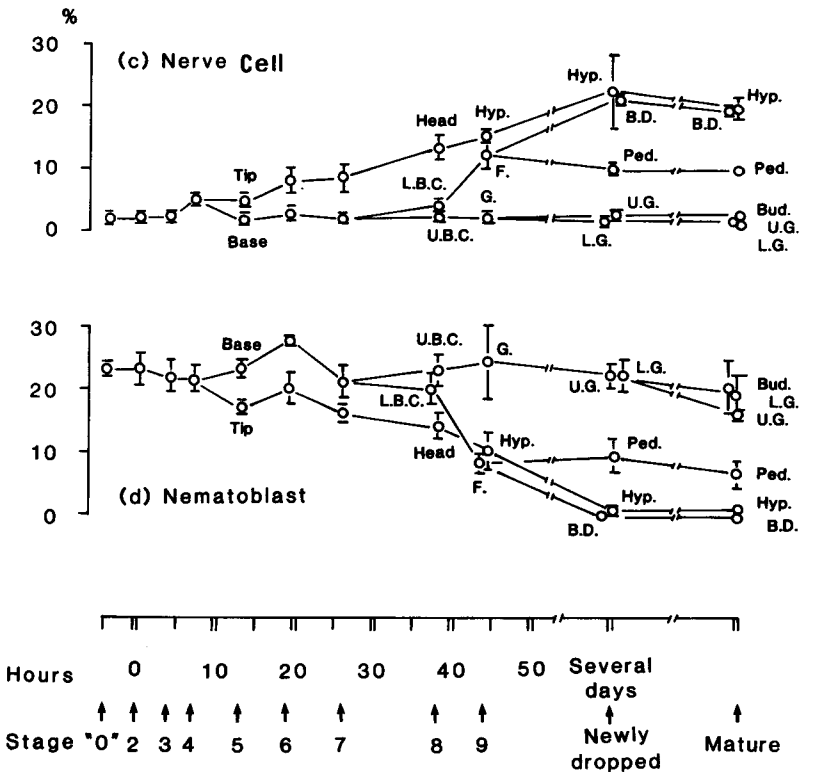

Fig. 2 (c), (d). (For explanation see page 415)

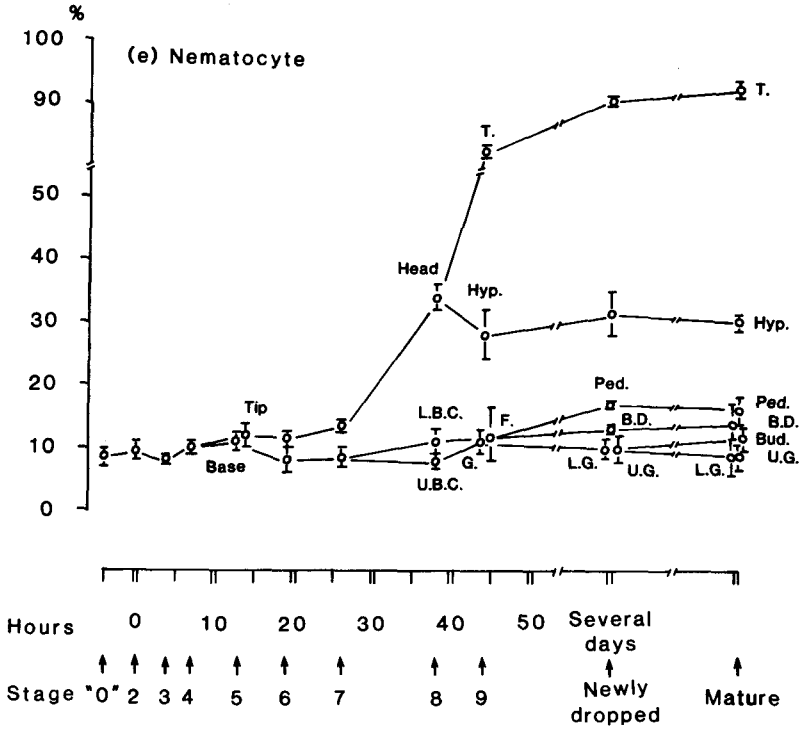

Fig. 2 (e). (For explanation see page 415)

to that of mature ones, the relative density of nerve cells was slightly larger in the former at each corresponding region (Tables 1 and 2): The percentage of nerve cells to the total cell number in the whole hydra was about 5.4% for newly-detached hydra and 3.3% for mature hydra. On the other hand, there were more mucous cells in mature hydra (4.5%) than in newly-dropped hydra (2.4%). In a mature hydra, it was clear that each morphologically specialized region had the characteristic cellular composition (Table 2). It seemed significant that cellular composition of the gastric region, both upper and lower, was almost identical to that of the budding region minus buds (Table 2).

It was estimated that a young hydra, at the point when its first bud is being formed, consisted of about 53,000 cells in its body column excluding tentacles and the bud, whereas a mature hydra has about 132,000 cells in the body column.

Discussion

The cellular composition along the body axis in *Pelmatohydra robusta* is essentially identical to that reported on *Hydra attenuata* (Bode *et al.*, 1973), except for some

minor points. For instance, in *P. rubusta*, the relative density of the little I-cells is higher than that of the big I-cells, whereas in *H. attenuata*, the relative density of little I-cells is the same as that of big I-cells. Population of most cell types, except for the epithelial cells, started to change at various developmental stages of the bud (Fig. 3). Thus, it is evident that in terms of the cellular composition the budding process can be divided into two major phases. During the first phase (phase I: st. 1 to 3), the parental wall protrudes and elongates perpendicularly to the body axis. Phase I, however, is characterized in that no changes in cellular composition are detected. No accumulation of any special types of cells, even nerves, could be detected during this phase. In the next phase (phase II: from st. 4 to detachment of young hydra), the ratios of several types of cells to the total number of cells start to change correspondent with morphogenetic events such as the formation of the head (mouth and tentacles) and foot (peduncle and/or basal disk). Prior to the appearance of the mouth, the nerve cells first begin to increase their ratios and thereafter nematoblasts, big I-cells, nematocytes, mucous cells and little I-cells start to decrease or increase in their relative densities

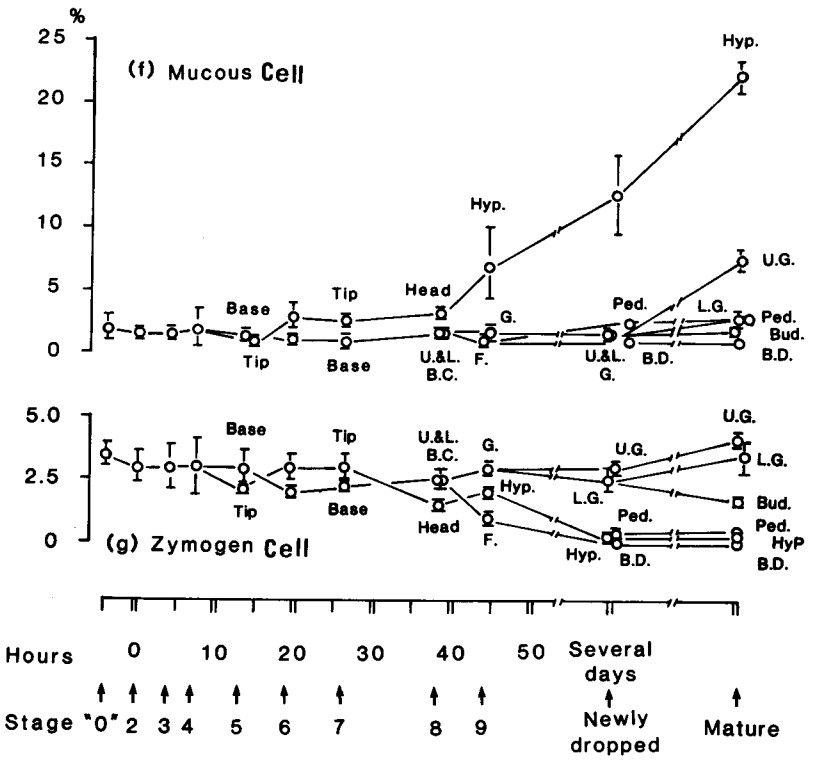

Fig. 2 (f), (g). (For explanation see page 415)

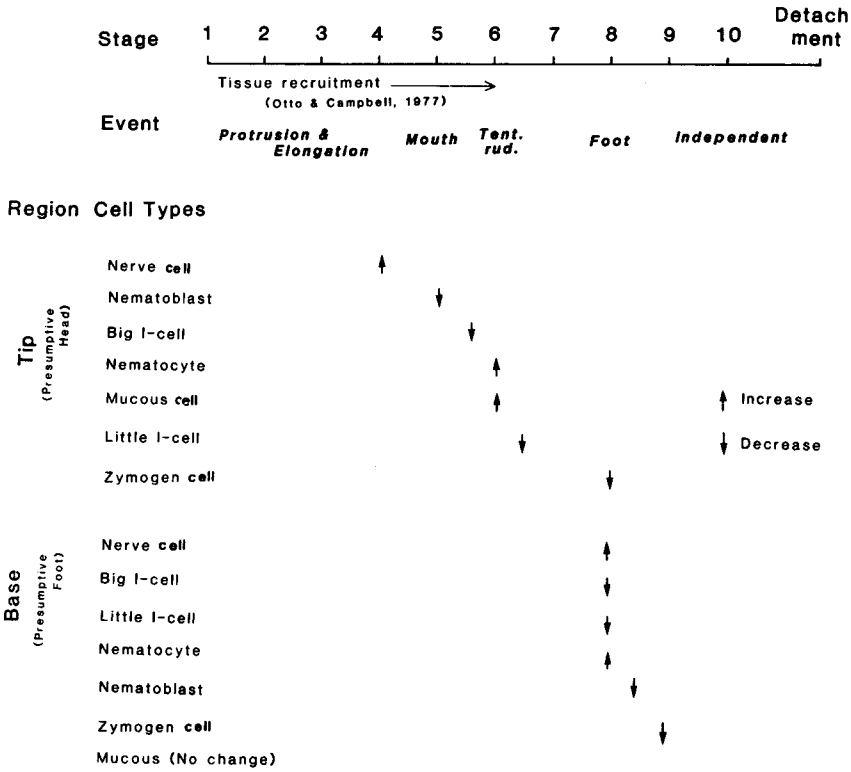

Fig. 3. The developmental stages at which a particular cell type started to change its relative density in the tip (presumptive head) and the base (presumptive foot) of the bud.
↑: increase in relative density of cells
↓: decrease in relative density of cells

during the course of the head formation (Fig. 3: Region, Tip). As differentiation of the foot proceeds, all types of cells, but not the mucous cells, start to increase or decrease their relative densities (Fig. 3: Region, Base). It is, therefore, assumed that the change in composition of these cells is closely related to morphogenetic processes during the growth of the bud.

The mechanism of budding in hydra is still under debate. Bode *et al.* (1973) reported that the nerve cells of *H. attenuata* increased their number at the site of morphogenesis, and Schaller *et al.* (1979a) claimed that the nerve cells in the same hydra produce four morphogens which stimulate or inhibit the morphogenesis such as head and foot regeneration and budding in normal animals. In the present study, however, it should be emphasized that the gastric region in newly-detached hydra and the budding region at stage "0" and the very young bud prior to stage

4 have almost identical cellular composition. It was found that number of the nerve cells increased only after a lapse of a certain time following the initiation of bud formation.

As for the ability of nerve-free hydras to perform morphogenesis (Campbell, 1976; Sugiyama and Fujisawa, 1978), Schaller *et al.* (1973) interpreted that the epithelial cells in nerve-depleted hydra have taken over the morphogen-producing activity of the nerve cells in such cases. In the present study, no abrupt increase in the number of the nerve cells was seen in the bud at early stages of development. The role of the nerve cells as the site of morphogen-production remains questionable if we assume that these morphogens do exist in hydra and that the amount of the released morphogens corresponds to the number of the nerve cells present in the budding region. In *P. robusta*, the cellular composition of the growing bud changed at the time of the formation of each organ, and no appreciable change was seen prior to the bud formation.

Summary

The budding process in *Pelmatohydra robusta* was examined in terms of its cellular composition. The process can be divided into two phases, I and II. In phase I, a portion of body wall of the parent hydra protrudes outward and it elongates without any detectable changes in cellular composition. Phase II is the period of organogenesis accompanying changes in cellular composition, in which an increase of the nerve cells was detected for the first time in the tip of the bud. Changes in the number of other cell types took place after this. It is suggested that the change of cellular composition may be caused by organogenesis in budding process.

Acknowledgements

The author wishes to express his sincere appreciation to Prof. Tomoji Aoto for his invaluable advice throughout the course of this study and for improvement of the manuscript. Thanks are also due to Dr. K. Noda, Tokyo Metropolitan Institute of Gerontology, for providing him with the present materials.

References

Bode, H., S. Berking, C. N. David, A. Gierer, H. Schaller and E. Trenkner 1973. Quantitative analysis of cell types during growth and morphogenesis in *Hydra*. Wilhelm Roux's Archives **171**: 269–285.

Campbell, R. D. 1976. Elimination of hydra interstitial and nerve cells by means of colchicine. J. Cell Sci. **21**: 1–13.

David, C. N. 1973. A quantitative method for maceration of hydra tissue. Wilhelm Roux's Archives **171**: 259–268.

Noda, K. 1972. Inhibitory effects of X-rays on the budding of hydra and the reformation of buds by the transplantation of the intact cell mass. Jour. Fac. Sci. Univ. Tokyo, sec. IV **12**: 429–438.

Otto, J. J. and R. D. Campbell 1977. Budding in *Hydra attenuata*: Bud stages and fate map. J. Exp. Zool. **200**: 417–428.

Schaller, H. C. 1973. Isolation and characterization of a low-molecular weight substance activating head and bud formation in *Hydra*. J. Embryol. exp. Morph. **29**: 27–38.

———, and A. Gierer 1973. Distribution of head-activating substance in *Hydra* and its localization in membranous particles in nerve cells. *Ibid.* **29**: 39–52.

———, T. Schmidt and C. J. P. Grimmelikhuijzen 1979a. Separation and specificity of action of four morphogens from *Hydra*. Wilhelm Roux's Archives **186**: 139–149.

———, C. J. P. Grimmelikhuijzen, T. Schmidt and H. Bode 1979b. Morphogenetic substances in nerve-depleted *Hydra*. *Ibid.* **187**: 323–328.

Sugiyama, T. and T. Fujisawa 1978. Genetic analysis of developmental mechanisms in hydra. II. Isolation and characterization of an interstitial cell-deficient strain. J. Cell Sci. **29**: 35–52.