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GENETIC ANALYSIS OF DEVELOPMENTAL MECHANISMS IN HYDRA

VI. CELLULAR COMPOSITION OF CHIMERA HYDRA

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SUMMARY

The homeostatic mechanisms that maintain constant cellular ratios in hydra tissue were studied using mutant and chimeric hydra strains.

Mutants having abnormal cellular compositions are isolated through sexual inbreeding of wild hydra, as described in previous papers of this series. Chimeric hydra are produced by making use of a strain (nf-1) which lacks interstitial cells, nerve cells and nematocytes in its tissue. Reintroduction of interstitial cells from other strains (both normal and mutant) into nf-1 leads to creation of chimeric strains having epithelial cell lineages from one strain (nf-1) and interstitial cell lineages from others.

Analyses and comparisons of the cellular compositions of all these strains revealed that the numbers of nerve or interstitial cells in the chimeras were very similar to (statistically significantly correlated with) those in their interstitial cell donors. Since chimeras and their interstitial cell donors share the same interstitial cell lineages, this suggests that interstitial cells or their derivatives (nerves and nematocytes) play major roles in determining the nerve and interstitial cell levels in the hydra tissue. It is suggested that some form of homeostatic feedback mechanisms are probably involved in regulating the levels of these cell types.

INTRODUCTION

Hydra tissue consists of 6 basic types of cells: epitheliomuscular, digestive, gland, interstitial, nerve cells and nematocytes. The first 4 types are mitotic cells while the last 2 are non-dividing cells which arise by differentiation from interstitial cells. In steadily growing hydra, these cell types are present in constant ratios relative to each other (Bode *et al.* 1973; Sugiyama & Fujisawa, 1977*a*).

Hydra strains lacking the last 3 cell types have been isolated in recent years, initially by Campbell (1976) by means of colchicine-treatment, and then by Sugiyama & Fujisawa (1978*a*) by genetic inbreeding. These interstitial cell-deficient strains, which lack interstitial cells and their derivatives (nerve and nematocytes), cannot eat by themselves. However, Campbell (1976) has developed an artificial feeding method by which one can readily and rapidly feed these animals to allow them to grow and reproduce asexually.

Availability of interstitial cell-deficient hydra has opened a way to produce chimeric hydra: when interstitial cells are reintroduced from normal hydra into polyps deficient in these cells, chimeras are produced that consist of epithelial cells of one origin and

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interstitial cells and their derivatives of another (Sugiyama & Fujisawa, 1978*a*, *b*; Marcum & Campbell, 1978*b*). The 2 groups of cells derived from different origins apparently maintain separate lineages in chimeras (Sugiyama & Fujisawa, 1978*b*).

These chimeric strains offer excellent opportunities to examine the roles that different cell types play in hydra development. For example, if a particular character of hydra is primarily determined by epithelial cells, that character in chimeras should resemble that of the epithelial cell donor strains since both share the same epithelial cells. Similarly, if the character is controlled by interstitial cells or their derivatives, the chimera's character should be similar to that of the interstitial cell donor strains.

In previous work we used this strategy to examine various developmental characters, and obtained results which suggested that characters such as growth rate, budding rate, bud developmental rate, tentacle number, polyp size, and regenerative capacity are all controlled primarily by epithelial cells (Sugiyama & Fujisawa, 1978*b*). A similar study has been also carried out by Marcum & Campbell (1978*b*).

In this study, we use the same strategy to examine which cell types control cellular compositions in hydra. As already mentioned, 6 basic types of cells are present in constant ratios relative to each other in steadily growing hydra (Bode *et al.* 1973; Sugiyama & Fujisawa, 1977*a*). When these ratios are artificially altered by chemical means, the animals restore the original ratios gradually (Bode, Flick & Smith, 1976). Changing of culture conditions also alters the cellular ratios, but they eventually reach a new steady state level if animals are continuously kept under the new conditions (Bode, Flick & Bode, 1977). Mutant strains have been isolated that maintain altered cellular compositions (Sugiyama & Fujisawa, 1977*a*). These observations suggest that hydra must have homeostatic device(s) to maintain proper cellular ratios, and that the device becomes defective or altered in mutant strains.

Here we examine which cell types are involved in these homeostatic mechanisms. Chimeric hydra strains were selected that had been produced using interstitial cell donor strains having both normal and altered cellular compositions. Cellular compositions of these chimeras were then determined and compared to those of their epithelial and interstitial cell donor strains. The results to be presented will show that the interstitial cell, nerve cell (and, to a lesser extent, gland cell) compositions in chimeras are similar to those in the interstitial cell donors, suggesting that the levels of these cell types in the hydra tissue are primarily determined by interstitial cells or their derivatives.

MATERIALS AND METHODS

Culture conditions

Self-feeding hydra strains were cultured according to Loomis & Lenhoff (1956) in the modified 'M' solution described by Muscatine & Lenhoff (1965) (1 mM NaCl, 1 mM $CaCl_2$, 0·1 mM KCl, 0·1 mM $MgSO_4$ and 1 mM Tris (hydroxymethyl)aminoethane, adjusted to pH 7·6 with HCl). Freshly hatched *Artemia salinas* nauplii were used as food.

Non-feeding animals were cultured according to Marcum & Campbell (1978*a*). They were cultured in modified M solution containing an antibiotic rifampicin (Boehringer–Mannheim, 50 μg/ml), and fed artificially by forced-feeding.

Animals were all cultured in a constant temperature room maintained at 18 ± 1 °C.

Strains

All strains used have been described previously (Sugiyama & Fujisawa, 1978*a*, *b*), and they are listed in Table 1. Strain sf-1 is a sexually inbred clonal line (F_6) which appears normal in every respect except one; in stock culture of this strain one can occasionally find polyps lacking interstitial cells and being unable to feed by themselves. We call these polyps non-feeders, or nf-1, and their self-feeding parental strain sf-1. Nf-1 is maintained by the forced-feeding method of Marcum & Campbell (1978*a*) described above. Their tissue consists of epithelial cells only, and lacks interstitial cells, nerve cells, and nematocytes. Nf-1 polyps are used as the epithelial cell host (recipient of interstitial cells) in chimera production.

Table 1. *List of chimeric strains used (all epithelial cells were derived from strains nf-1)*

Chimera		Interstitial cell donor		
Code	Designation	Designation	Property	Reference**
1	Chim-1	105	Wild type	(1) (2) (4)
3	Chim-3	Reg-16	Regeneration-deficient	(1) (3) (4)
4	Chim-4	Nem-3	Nematocyst (holotrichous isorhiza)-deficient	(1) (4)
6	Chim-6	Nf-17	Food ingestion-deficient	(4)
8	Chim-8	L2*	Wild type	(4)
10	Chim-10	L4*	Wild type	(4)
12	Chim-12	SSE	Normal, produced by sexual crosses	(4)
17	Chim-17	Maxi-1	Large size	(1) (4)

* Original parental strains from which sf-1 was derived by sexual inbreeding.

** (1) Sugiyama & Fujisawa (1977*a*); (2) Sugiyama & Fujisawa (1978*a*); (3) Sugiyama & Fujisawa (1977*b*); (4) Sugiyama & Fujisawa (1978*b*).

Chimeric hydra

Chimeric hydra strains were produced by the procedure described previously (Sugiyama & Fujisawa, 1978*a*, *b*), and it is schematically shown in Fig. 1.

Cellular composition

Cell type identification and counting was done following the maceration procedure described by David (1973). Small polyps bearing no buds (recently detached from parents) or bearing a small protrusion (indicating the initiation of the first bud) were selected from daily fed cultures 1 day after the last feeding. The hypostomal region (including hypostome and tentacles) was removed just proximal to the base of the tentacles, and the remainder of the body was macerated in a solution containing glycerol:glacial acetic acid:water (1:1:13) at room temperature. The resulting cell suspension was spread on a microscope slide to dry, and cells were counted with phase-contrast optics at a magnification of 400 times.

Statistical methods

Correlation coefficients and regression coefficients were calculated and their significance tested according to the standard procedure as described in text books such as that by Snedegar (1956).

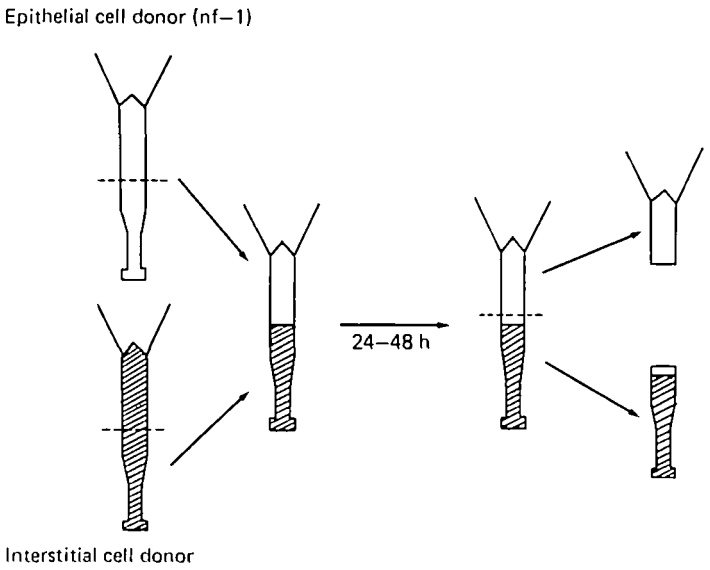

Fig. 1. Scheme of temporary grafting for chimera production. During grafting, interstitial cells and nematocytes (but no epithelial cells) migrate from the interstitial cell donor tissue (cross-hatched) into the epithelial cell host (nf-1 strain) tissue. Hence, the upper-half polyp which is obtained when the graft is separated again contains epithelial cells derived from nf-1 origin and interstitial cells and their derivatives from the interstitial cell donor origin. (For detail see Sugiyama & Fujisawa, 1978*a*, *b*).

RESULTS

The scheme employed for producing chimeric hydra is shown in Fig. 1. The epithelial cell host, strain nf-1, is an interstitial cell-deficient strain that lacks interstitial cells and their derivatives (nerve and nematocytes). When an upper-half polyp of nf-1 and a lower-half polyp of a wild type (strain 105) were grafted together for 24–48 h, interstitial cells and nematocytes migrated in massive numbers from the lower to the upper half. Epithelial cells, however, did not migrate under the same conditions. Upon graft separation, therefore, an upper-half polyp was obtained that contained epithelial cells derived from strain sf-1 (the source of strain nf-1) and interstitial cells and their derivatives from strain 105. The chimeric clone derived from this polyp was named chim-1.

Using the same procedure, we have produced a number of chimeric strains in previous work (Sugiyama & Fujisawa, 1978*b*). Strain nf-1 derived from strain sf-1 was used as the epithelial cell host in all cases, while various strains having diverse types of characters were used as the interstitial cell donors.

From these chimeras, 8 strains were selected for this study and they are listed in Table 1 together with their interstitial cell donors. Table 2 shows the cellular composition of these strains.

Table 2. *Cellular compositions*

Strain	No. of specimens examined	Total no. of cells counted	Cellular composition (± standard deviation)						
			Epithelio-muscular	Digestive	Large interstitial	Small interstitial	Nematocyte (+blast)	Nerve	Gland
105	14	20276	15·4 ± 2·4	16·2 ± 2·2	13·6 ± 2·6	16·2 ± 2·5	29·9 ± 3·3	2·3 ± 0·6	6·6 ± 1·2
Chim-1	5	5094	14·4 ± 1·5	15·5 ± 2·1	15·2 ± 1·6	11·7 ± 2·9	34·5 ± 4·4	2·4 ± 0·4	6·3 ± 1·7
Reg-16	15	17812	19·6 ± 6·6	21·3 ± 5·3	10·1 ± 2·7	13·5 ± 3·8	27·2 ± 8·2	1·5 ± 0·6	6·8 ± 1·6
Chim-3	5	5028	13·5 ± 1·5	14·8 ± 2·0	13·0 ± 2·2	8·6 ± 1·9	43·4 ± 4·9	1·2 ± 0·6	5·5 ± 1·7
Nem-3	13	13523	29·8 ± 3·9	29·5 ± 3·5	5·5 ± 2·2	7·5 ± 3·2	16·9 ± 6·6	3·0 ± 0·9	7·9 ± 2·6
Chim-4	10	10238	15·6 ± 2·3	15·9 ± 2·9	10·1 ± 1·7	9·4 ± 3·6	42·1 ± 5·0	2·3 ± 0·6	4·7 ± 1·4
Nf-17	5	6113	13·7 ± 1·5	13·8 ± 1·0	14·8 ± 1·5	14·1 ± 2·1	38·2 ± 2·6	1·8 ± 0·5	3·6 ± 0·6
Chim-6	5	6100	16·1 ± 2·7	15·2 ± 2·6	13·4 ± 1·0	14·3 ± 2·9	36·2 ± 2·5	1·6 ± 0·6	3·2 ± 0·9
L2	10	10178	14·9 ± 2·1	16·6 ± 1·2	11·7 ± 1·1	10·9 ± 3·1	39·9 ± 2·6	1·2 ± 0·4	4·7 ± 0·9
Chim-8	10	10296	14·8 ± 2·7	16·8 ± 2·2	11·9 ± 2·5	10·5 ± 4·1	40·3 ± 3·6	1·5 ± 0·5	4·6 ± 0·8
L4	5	5090	16·6 ± 0·5	18·0 ± 0·5	11·6 ± 1·9	6·9 ± 1·5	37·9 ± 2·8	1·8 ± 0·5	7·2 ± 1·1
Chim-10	5	5070	17·8 ± 1·6	17·8 ± 1·2	12·4 ± 1·8	9·5 ± 1·8	34·8 ± 1·2	1·5 ± 0·5	6·1 ± 0·9
SSE	10	10202	15·7 ± 2·8	17·0 ± 1·6	13·6 ± 2·8	9·4 ± 3·9	36·8 ± 3·7	1·3 ± 0·6	6·1 ± 1·4
Chim-12	10	10322	15·0 ± 2·6	17·2 ± 1·4	12·0 ± 2·5	11·2 ± 2·4	37·8 ± 3·9	1·0 ± 0·4	5·8 ± 1·1
Maxi	5	5818	17·5 ± 1·4	17·3 ± 2·2	10·3 ± 2·3	11·9 ± 4·7	32·9 ± 1·9	2·3 ± 0·8	7·8 ± 1·3
Chim-17	5	5082	19·5 ± 2·5	20·0 ± 2·7	14·7 ± 3·8	6·0 ± 1·6	32·6 ± 3·9	1·5 ± 0·6	5·8 ± 0·9
Sf-1	9	8980	19·6 ± 3·2	23·2 ± 3·2	8·4 ± 2·7	12·0 ± 2·7	28·8 ± 4·1	2·0 ± 0·6	6·1 ± 1·2

Cellular composition of chimeras and their interstitial cell donors

Fig. 2 shows the statistical relationship between the nerve cell content of the chimeras and that of their interstitial cell donors. The data for this and following figures are all taken from Table 2. Each point in the figure shows the average nerve cell content of an interstitial cell donor on the abscissa and that of the corresponding chimera on the ordinate. Numerals next to points show the code numbers of the chimeric strains

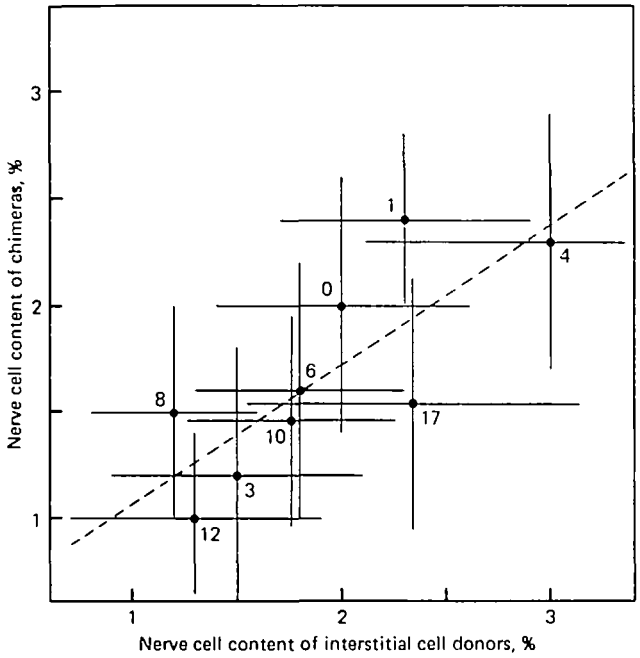

Fig. 2. Correlation between the nerve cell content of the chimeras and that of their interstitial cell donors. Each point shows the average nerve cell content of an interstitial cell donor strain (abscissa) and that of the corresponding chimera (ordinate). Bars show standard deviations (horizontal bars for interstitial cell donors and vertical bars for the chimeras). Numerals next to the points show the code numbers of the chimera strains (see Table 1). The numeral 0 represents the nerve cell content of the epithelial cell donor (strain sf-1), using the same value both on ordinate and abscissa for this strain. The dotted line shows the regression of the chimera's nerve cell content on that of the interstitial cell donors. r (correlation coefficient) = 0·783 ($P < 0{\cdot}02$); b (regression coefficient) = 0·655 (this value is statistically not significantly different from unity: $t = 1{\cdot}751$, $P > 0{\cdot}1$).

involved (Table 1). Thus, the point with numeral 1 shows that chim-1 contains an average of 2·4% nerve cells (± standard deviation of 0·4%) while the content of the same cell type in its interstitial cell donor, strain 105, is 2·3% (±0·6%). The figure also includes the nerve cell content of the epithelial cell donor, strain sf-1. Attempts to produce a 'homochimera' using strain sf-1 as the interstitial cell donor and nf-1 as epithelial cell host have all failed (Sugiyama & Fujisawa, 1978*b*). For reference purpose, however, the nerve cell content of strain sf-1 is also included (indicated by numeral 0), plotting the same value (2·0 ± 0·6%) on both the abscissa and ordinate.

The figure shows that nerve cell content varies widely between different interstitial cell donors, ranging from the lowest of about 1·2% to the highest of about 3%, and that chimeras also show a similar range of variation. An important point to be noted here is that the nerve cell content of each chimeric strain is generally very similar to that of its interstitial cell donor strain, but not necessarily to its epithelial cell donor strain (indicated by numeral 0).

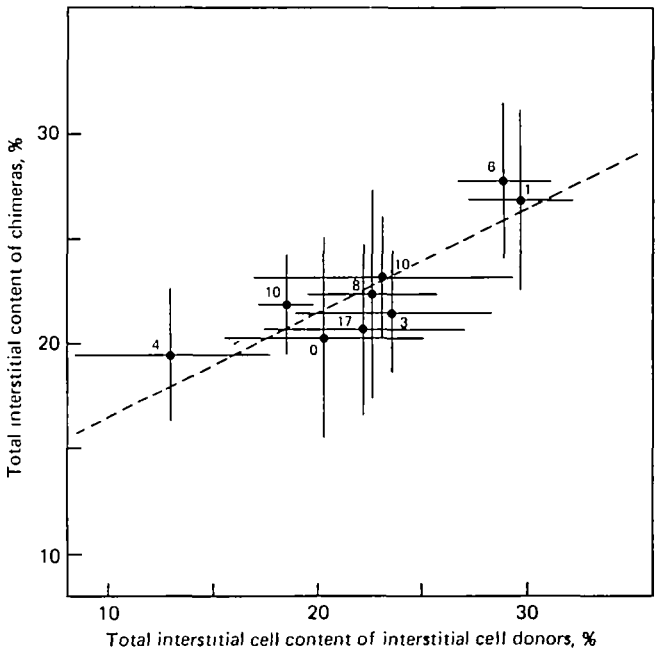

Fig. 3. Correlation between the total interstitial cell content of the chimera and that of their interstitial cell donors. Total (large + small) interstitial cell contents of the chimeras and their interstitial cell donors are shown in the same way as explained in the legend of Fig. 2 for nerve cells. $r = 0{\cdot}872$ ($P < 0{\cdot}01$), $b = 0{\cdot}494$ (this value is statistically significantly different from zero ($t = 4{\cdot}709$, $P < 0{\cdot}01$) and from unity ($t = 4{\cdot}833$, $P < 0{\cdot}01$)).

When statistically analysed, the correlation between the average nerve cell content of chimeras and that of their interstitial cell donors is highly significant ($r = 0{\cdot}783$, $P < 0{\cdot}02$). The regression of the former on the latter content is shown by the dotted line in the figure. Its slope (regression coefficient $b = 0{\cdot}655$) is not significantly different from unity when statistically analysed by the t-test ($t = 1{\cdot}751$, $P > 0{\cdot}1$) (see Discussion).

In hydra tissue, nerve cells are present scattered between epithelial cells. Thus, one way to express nerve cell content is by the ratio of nerve to epithelial cells. When expressed this way, however, correlation between the chimeras and the interstitial cell donors is much lower ($r = 0{\cdot}454$, $P > 0{\cdot}1$) than when expressed by percentage of total cells.

Similar analyses were carried out on other cell types, and Fig. 3 shows the result

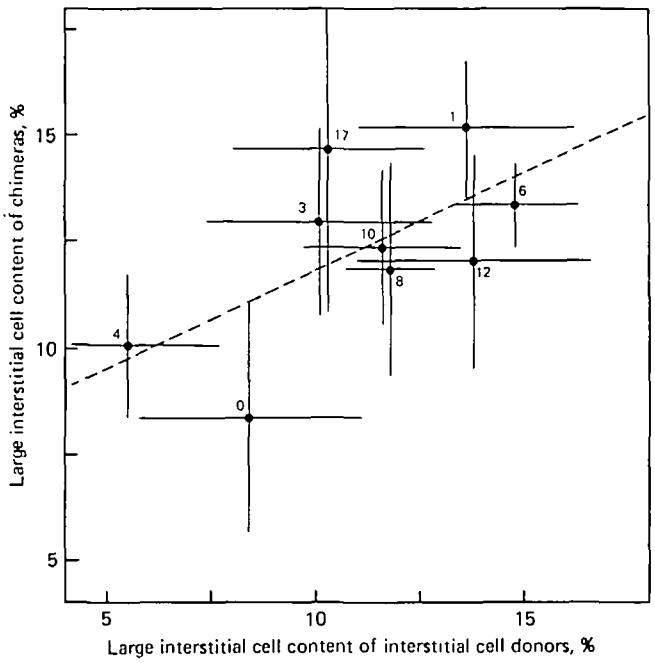

Fig. 4. Correlation between the large interstitial cell content of the chimeras and that of their interstitial cell donors. $r=0{\cdot}622$ ($P < 0{\cdot}1$), $b=0{\cdot}457$ ($t=2{\cdot}101$, $P < 0{\cdot}1$).

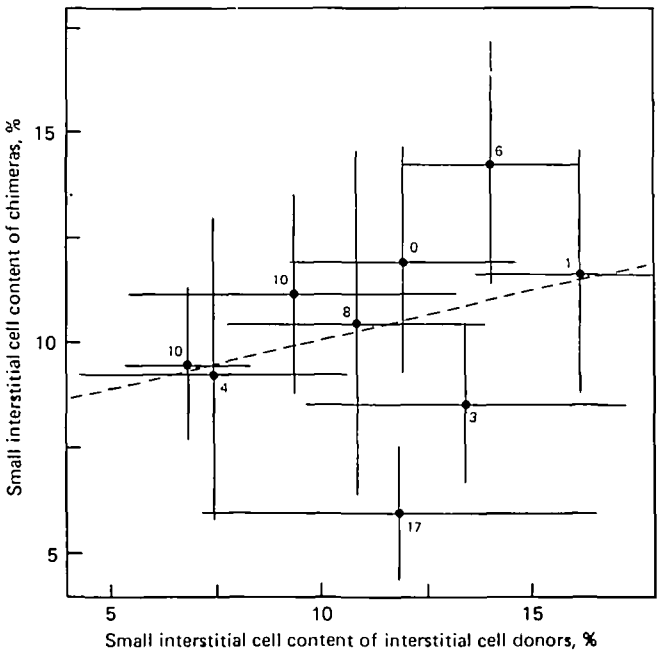

Fig. 5. Correlation between the small interstitial cell content of the chimeras and that of their interstitial cell donors. $r=0{\cdot}312$ ($P > 0{\cdot}1$), $b=0{\cdot}240$ ($t=0{\cdot}770$, $P > 0{\cdot}1$).

obtained for the total (large + small) interstitial cells (expressed by percentage of total cells). Here, the correlation between the chimeras and their interstitial cell donors is statistically significant ($r = 0{\cdot}872$, $P < 0{\cdot}01$), but the regression coefficient ($b = 0{\cdot}494$)

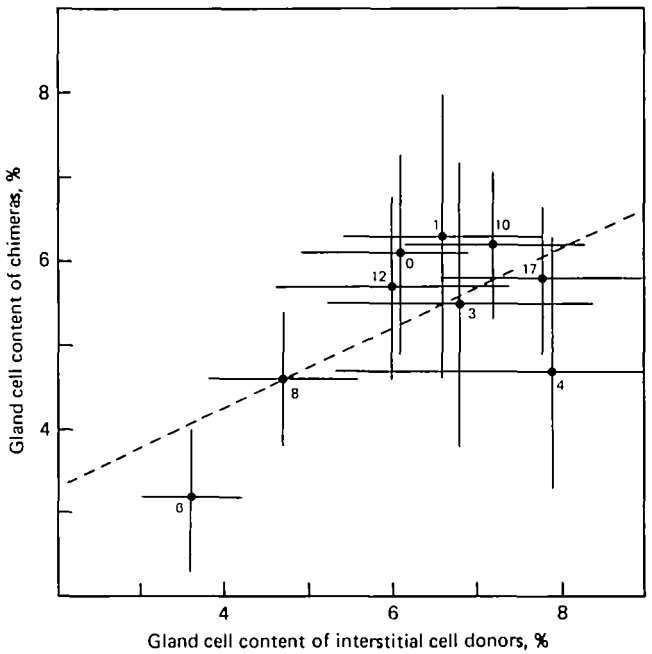

Fig. 6. Correlation between the gland cell content of the chimeras and that of their interstitial cell donors. $r = 0{\cdot}657$ ($P = 0{\cdot}05$). $b = 0{\cdot}468$ ($t = 2{\cdot}305$, $P < 0{\cdot}1$).

Table. 3. *Correlation of cellular content between chimeras and their interstitial cell donors*

Cell type	Correlation coefficient	Significance level
Epitheliomuscular	0·027	> 0·1
Digestive	0·160	> 0·1
Interstitial		
Total	0·872	< 0·01
Large	0·622	< 0·1
Small	0·312	> 0·1
Nematocyte (+blast)	−0·249	> 0·1
Nerve	0·783	< 0·02
Gland	0·657	≒ 0·05

is lower than in the previous case. Unlike nerve cells, this relationship is not significantly changed when the content is expressed by the ratio to epithelial cells instead of by percentage of total cells ($r = 0{\cdot}776$, $P < 0{\cdot}02$; $b = 0{\cdot}477$).

Interstitial cells can be classified into 2 subtypes: large interstitial cells and small interstitial cells (David, 1973). Relationships between the contents of these subtypes in the chimeras and in their interstitial cell donors are shown in Figs. 4 and 5. It is

clear that correlations are much less significant for both large interstitial cells (Fig. 4) and small interstitial cells (Fig. 5) than for the total (large + small) interstitial cells (Fig. 3).

Fig. 6 shows the correlation between the gland cell percentages of the chimeras and their interstitial cell donors. Here, the correlation is marginally significant ($r = 0{\cdot}657$, $P \doteqdot 0{\cdot}05$).

Table 3 summarizes the correlations between the chimeras and their interstitial cell donors for all the cell types analysed. Significant correlations exist for interstitial, nerve and gland cells, but not for epitheliomuscular cells, digestive cells, or the nematocytes (including nematoblasts) which are present in the body column.

DISCUSSION

Hydra tissue consists of 6 basic types of cells, some of which are mitotic cells while others are not. Epitheliomuscular and digestive cells are mitotic cells, the cell cycle time of which is roughly equal to the doubling time of animals (about 3 days). This suggests that these cells maintain their population through self-proliferation (David & Campbell, 1972). Interstitial cells are also mitotic cells, but these cells, in addition to maintaining their own population by division, also differentiate into non-mitotic nerve cells and nematocytes. The average cell cycle time of interstitial cells (less than 1 day) is much shorter than the doubling time of the animals (Campbell & David, 1974). Gland cells are also mitotic, but they may also arise by differentiation from other types (Campbell, 1974; Sugiyama & Fujisawa, 1978*a*).

In spite of these diverse and interconnected patterns of proliferation and differentiation, the 6 types of cells are maintained in nearly constant ratios relative to each other in steadily growing hydra (Bode *et al.* 1973). This suggests that hydra must have homeostatic mechanisms to maintain proper cellular ratios in its tissue.

To study the nature of these mechanisms, Bode and his colleagues used various means to perturb the homeostasis and then examined the responses of animals (Bode *et al.* 1976, 1977). In the present study, we used hydra strains whose cellular compositions are genetically 'perturbed'. Wild type hydra strains originally collected from ponds generally show very similar cellular compositions (Sugiyama & Fujisawa, 1977*a*). However, some wild type strains and many sexually produced laboratory strains have cellular compositions that differ markedly from the majority of wild type strains (Table 2).

In the present study we used these strains as the donors of interstitial cells to produce chimeric strains, and then compared the cellular compositions of the resultant chimeras to those of their interstitial and epithelial donor strains. If the primary determinant of the content of a given cell type is located in epithelial cells, the abundance of this cell in chimeras should be similar to that of the epithelial cell donor since both share the same epithelial cells. Similarly, if the determinant is in interstitial cells or their derivatives, the cell's abundance in chimeras should be similar to that of the interstitial cell donors.

In general, the experimental results have shown that the cellular compositions of

chimeras resemble those of their interstitial cell donors much more closely than those of their epithelial donor strain. This suggests that interstitial cell lineages play predominant roles in determining the cellular compositions in the hydra tissue. The detail of this is discussed below for individual cell types.

Interstitial cells

The contents of total (large + small) interstitial cells in the chimeras are generally very similar to those in their interstitial cell donors, as indicated by the good statistical correlation between the 2 contents ($r = 0{\cdot}872$, $P < 0{\cdot}01$; Fig. 3). This suggests that interstitial cell content is primarily determined by interstitial cells or their derivatives (nerve cells and nematocytes).

This observation is consistent with the suggestion made by Bode *et al.* (1976) that interstitial cells can 'measure' and regulate their own density within the tissue by some form of a negative-feedback mechanism. According to them, interstitial cells send out feedback signals, presumably humoral in nature, into the tissue at a constant rate. At low interstitial cell density, the signal in the tissue is weak, permitting interstitial cells to divide to increase their density. As the interstitial cell density becomes higher, the feedback signal becomes correspondingly stronger, preventing further build-up of the interstitial cell population.

Our chimeras support this idea that interstitial cells regulate their own densities. Interstitial cell contents vary between strains since the feedback mechanism regulating their levels varies between strains. Interstitial cell contents are similar between chimeras and their interstitial cell donors because the homeostatic mechanism is located in the interstitial cells themselves.

There are, however, 2 aspects of the chimeras that can not be directly explained by this model. Bode *et al.* (1976) proposed their model to explain the homeostasis of large interstitial cells. In the present study, we found a good correlation in the contents of total (large + small) interstitial cells between the chimeras and their interstitial cell donors. However, the contents of large interstitial cells alone or of small interstitial cells alone show much less-significant correlations. This suggests that the regulatory mechanism acts on the level of total interstitial cells rather than of large or of small interstitial cells individually.

Another complex aspect of our work is the effects of epithelial cells on the interstitial cell content. Although total interstitial cell content in the chimeras and in their interstitial cell donors are significantly correlated ($r = 0{\cdot}872$, $P < 0{\cdot}01$), the regression coefficient of the former on the latter ($b = 0{\cdot}494$) is intermediate between 1 and 0. Theoretically, this coefficient (slope of dotted line in Fig. 3) should be nearly unity if the interstitial cell content is determined solely by interstitial cells or their derivatives, and nearly zero if determined by epithelial cells alone. Such clear-cut examples exist in the case of other characters. For example, the regression coefficient is close to (statistically not significantly different from) unity in the case of nerve cells (see below) and nearly zero in the case of developmental characters such as population growth rate (see fig. 3 in Sugiyama & Fujisawa, 1978*b*). The fact that the coefficient is about half way between these 2 theoretical values suggests that epithelial cells also play some

role in determining the level of interstitials. Epithelial cells may influence the interstitial cell level indirectly by affecting the passage of feedback information through the tissue. It is also conceivable that epithelial cells are more directly involved in the regulatory mechanism itself in some way.

Nerve cells

Nerve cells represent the most clear-cut pattern in the present analysis. Their contents in the chimeras and in the interstitial cell donors show a strong correlation ($r=0{\cdot}783$, $P < 0{\cdot}02$). The regression coefficient ($b=0{\cdot}655$, the slope of dotted line in Fig. 2) is somewhat lower than the theoretical value of 1. This difference, however, is not significant when statistically analysed by the t-test ($t=1{\cdot}751$, $P > 0{\cdot}1$). This strongly suggests that the level of nerve cells in hydra tissue is primarily determined by interstitial cells or their derivatives. The influence of epithelial cells, if there is any, appears to be less significant than in the case of interstitial cells.

The present experiments do not show which cell type of the interstitial cell lineage is directly responsible for determining the nerve cell content. However, the simplest explanation would be that nerve cells themselves regulate their own level in the tissue by some form of feedback mechanism. Nerve cells cannot divide, and they all arise by differentiation from interstitial cells. Nerve cell density could be regulated if this differentiation process were controlled by feedback information sent from nerve cells themselves.

There are, however, 2 important aspects which should be mentioned here concerning this mechanism. The first is that, although correlation between the chimeras and the interstitial cell donors is very high when the nerve cell content is expressed as a percentage of total cells ($r=0{\cdot}783$, $P < 0{\cdot}02$), it becomes much lower when the nerve cell content is expressed as the ratio to epithelial cells ($r=0{\cdot}454$. $P > 0{\cdot}1$). (This is not true for gland cells or interstitial cells.) This difference may be revealing the nature of the mechanism regulating the nerve cell level. If the mechanism is designed to maintain a constant number of nerve cells per tissue mass, this should be more closely reflected in nerve-to-epithelial cell ratio than in the percentage of nerve cells since epithelial cells (which are much larger than other cell types) provide the majority of the tissue mass. That this is not the case appears to suggest that the mechanism operates more on the basis of cell numbers than on the basis of cell density per tissue mass. That is, the mechanism maintains a constant ratio between the number of nerve cells and the number of total cells in the polyp. We believe such a mechanism is conceivable if the feedback information sent out by nerve cells, whatever its nature may be, is diluted or inactivated nearly equally by other cell types regardless of their sizes.

The second important aspect requiring consideration is the regional distribution of nerve cells. It is known that nerve cells are more numerous in developmentally more active regions (head, budding, foot region) than in other regions (Bode *et al.* 1973). Thus, the average nerve cell content of a strain may be influenced if the relative proportions of developmentally more-active to less-active tissues show significant variation between strains. However, this is apparently not an important factor in the

present case for the following 2 reasons. First, we have previously shown that little difference exists between the developmental characters (budding rate, size, regenerative capacity, etc.) of various chimeric strains. However, nerve cell contents do show significant differences between these strains (Table 2), suggesting that these differences are unrelated to developmental activities. Secondly, we directly examined correlations between nerve cell contents and developmental characters (budding rate, bud developmental rate, size, tentacle numbers) of various wild type and mutant strains. No significant correlations, however, were present (Sugiyama & Fujisawa, in preparation).

Gland cells

Gland cell levels in the chimeras and in the interstitial cell donors show a marginally significant correlation ($r = 0{\cdot}657$; $P \doteqdot 0{\cdot}05$), and the regression coefficient ($b = 0{\cdot}549$) is intermediate between 0 and 1 (Fig. 6). This suggests that interstitial cells or their derivatives play major roles, while epithelial cells play secondary roles, in determining the level of gland cells in the tissue. However, the evidence for this is not as strong as in the cases for nerve or interstitial cells, and analyses of a much larger number of strains will be needed to settle this issue. At present, it may be nevertheless interesting to speculate on how the gland cell level can be regulated by interstitial cells or their derivatives. Two possibilities can be considered depending on the origin of gland cells in the chimeras. The first is that gland cells in the chimeras are derived from the epithelial cell host (strain sf-1) and their levels are determined by interstitial cells or nerve cells. This mechanism, if true, involves a second cell type to regulate the level of gland cells, while in the cases already discussed (interstitial cells and nerve cells), each cell type probably regulates its own density. The second possibility is that gland cells in the chimeras are largely the differentiation products of the reintroduced interstitial cells and they determine their own density as in the cases of nerve cells or interstitial cells.

At present, however, the origin of gland cells in the chimeras is uncertain. Gland cells can divide mitotically (Campbell, 1974) and they may maintain their population by self-proliferation. However, gland cell levels are very low (or absent) in interstitial cell-deficient strains (Marcum & Campbell, 1978*a*; Sugiyama & Fujisawa, 1978*a*), but return to normal levels when interstitial cells are reintroduced (Table 2). This suggests that, although gland cells can divide mitotically, their long-term maintenance may require input of new gland cells by differentiation from interstitial cells.

General discussion

Bode *et al.* (1976) originally proposed that the interstitial cell level in hydra tissue is homeostatically maintained by some form of a negative-feedback mechanism. Our chimera data support this view. In addition our data further suggest that the level of nerve cells (and perhaps the level of gland cells) in the tissue is also regulated by similar mechanisms. However, it is not known whether the mechanisms regulating the levels of these cell types are mutually related to, or independent of, each other; no indication has been obtained that suggests the existence of a master homeostatic mechanism that monitors and controls the levels of all these cell types.

The nature of feedback information used in the homeostasis cannot be deduced from the present experiment; it may or may not be humoral. The limitation inherent in the statistical approach used here must also be mentioned. The similarity of cellular compositions between chimeras and interstitial cell donors could be due to statistical coincidence. Thus, the conclusions drawn from this study need to be verified by independent and more direct approaches.

Within these limitations, however, the present study strongly suggests that interstitial cells or their derivatives control the levels of interstitial cells and nerve cells (and probably also of gland cells). Epithelial cell content in the hydra tissue varies between strains, but there is evidence to suggest that this variation is the indirect result of the variations of the content of interstitial cells and their derivatives (Sugiyama & Fujisawa, in preparation). Thus, overall cellular composition of hydra is primarily determined by interstitial cells and their derivatives. This is in a sharp contrast to developmental or morphological characters that are primarily determined by epithelial cells (Sugiyama & Fujisawa, 1978*b*; Marcum & Campbell, 1978*b*).

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REFERENCES

BODE, H., BERKING, S., DAVID, C. N., GIERER, A., SCHALLER, H. & TRENKNER, H. (1973). Quantitative analysis of cell types during growth and morphogenesis in hydra. *Wilhelm Roux Arch. EntwMech. Org.* **171**, 269–285.

BODE, H. R., FLICK, K. M. & BODE, P. M. (1977). Constraints on the relative sizes of the cell populations in *Hydra attenuata*. *J. Cell Sci.* **24**, 31–50.

BODE, H. R., FLICK, K. M. & SMITH, G. S. (1976). Regulation of interstitial cell differentiation in *Hydra attenuata*. I. Homeostatic control of interstitial cell population size. *J. Cell Sci.* **20**, 29–46.

CAMPBELL, R. D. (1974). Cell movement in hydra. *Am. Zool.* **14**, 523–535.

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

CAMPBELL, R. D. & DAVID, C. N. (1974). Cell cycle kinetics and development of *Hydra attenuata*. II. Interstitial cells. *J. Cell Sci.* **16**, 349–358.

DAVID, C. N. (1973). A quantitative method for maceration of hydra tissue. *Wilhelm Roux Arch. EntwMech. Org.* **171**, 259–268.

DAVID, C. N. & CAMPBELL, R. D. (1972). Cell cycle kinetics and development of *Hydra attenuata*. I. Epithelial cells. *J. Cell Sci.* **11**, 557–568.

LOOMIS, W. F. & LENHOFF, H. M. (1956). Growth and sexual differentiation of hydra in mass culture. *J. exp. Zool.* **132**, 555–574.

MARCUM, B. A. & CAMPBELL, R. D. (1978*a*). Development of hydra lacking nerve and interstitial cells. *J. Cell Sci.* **29**, 17–33.

MARCUM, B. A. & CAMPBELL, R. D. (1978*b*). Developmental roles of epithelial and interstitial cell lineages in hydra: Analysis of chimeras. *J. Cell Sci.* **32**, 233–247.

MUSCATINE, L. & LENHOFF, H. M. (1965). Symbiosis of hydra and algae. I. Effects of some environmental cations on growth of symbiotic and aposymbiotic hydra. *Biol. Bull. mar. biol. Lab., Woods Hole* **128**, 415–424.

SNEDEGAR, G. W. (1956). *Statistical Methods*, 5th edn. Iowa State College Press.

SUGIYAMA, T. & FUJISAWA, T. (1977*a*). Genetic analysis of developmental mechanisms in hydra. I. Sexual reproduction of *Hydra magnipapillata* and isolation of mutants. *Development, Growth and Differentiation* **19**, 187–200.

SUGIYAMA, T. & FUJISAWA, T. (1977*b*). Genetic analysis of developmental mechanisms in hydra. III. Characterization of a regeneration deficient strain. *J. Embryol. exp. Morph.* **42**, 65–77.

SUGIYAMA, T. & FUJISAWA, T. (1978*a*). Genetic analysis of developmental mechanisms in hydra. II. Isolation and characterization of an interstitial cell-deficient strain. *J. Cell Sci.* **29**, 35–52.

SUGIYAMA, T. & FUJISAWA, T. (1978*b*). Genetic analysis of developmental mechanisms in hydra. V. Cell lineage and development of chimera hydra. *J. Cell Sci.* **32**, 215–232.

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