THE CELLULAR BASIS OF HYDROID MORPHOGENESIS

MAXWELL H. BRAVERMAN[1]

Taos, New Mexico, U.S.A.

With 27 Text-figures

Abstract

Until quite recently, the general framework within which the maintenance of form in the face of continual cell renewal in hydroids has been viewed has been that elaborated by Paul BRIEN, modified with respect to the sites of proliferation by Richard CAMPBELL and the disposition of cells by Stanley SHOSTAK and his students. The growth of hydroids, according to this model, and to the generative scheme described by KUHN, was viewed as similar to the growth of meristematic plants. The form of the animal was believed to arise as a consequence of the location of proliferative regions. Essential to this view of hydroid morphogenesis has been two tenets: (1) that, with the exception of cnidocytes, cells in hydroids move as coherent sheets, and (2) that cells of each of the two layers retain the integrity of their layer of origin—*i.e.* there is no crossing over the mesoglea.

As early as 1930, however, KANAJEW described the movement of vitally stained cells from the epidermis of hydra to the gastrodermis. Subsequently considerable conflicting data has appeared indicating that growth in hydroids is not similar to the meristematic growth of plants, but rather that the sites of cell proliferation are removed in space from the sites of utilization, that cells migrate individually, actively as amoebocytes through the epidermis or passively as epitheliocytes, carried along in the hydrocoel, to their sites of utilization, and that considerable migration across the mesoglea occurs.

A new model of hydroid morphogenesis and morphostasis can now be constructed based upon new information regarding the sites of cell proliferation and cell migration, and accounting for the form of the colony in terms of cellular proclivities such as amoebocytic or epitheliocytic tendencies and cell stickiness, and identifying the decision points of cellular differentiation as a consequence of which colony form is generated. The majority of the data for this model is derived from my studies of the colonial marine hydroid *Podocoryne carnea*. This model will take the form of a flow chart which accounts for the source, distribution and disposition of cellular elements and attempts to account for colony form as a consequence of cellular activities.

Introduction

The colonial marine hydroid, *Podocoryne carnea* filiform and athecate is normally found on hermit crab shells (Fig. 1). Polyps plucked from the shell attach to microscope slides and form colonies there (Fig. 2). *Podocoryne* stolons are totally adherent to the substrate, and are discrete, forming, as they grow and anastomose, a defined, interconnected, network (Fig. 3). Because the stolon network extends in but one

1) Author's address: Ranchos de Taos Pottery, Los Cordovas Route, Box 131, Taos, New Mexico, 87571 U.S.A.

plane a photograph of a colony presents an exhaustive two dimensional map of colony morphology. A series of daily photographs records all gross parameters of colony growth (Fig. 4).

I have obtained such photographs of growing colonies over periods as long as sixty days. My purpose in compiling this record has been to utilize the photographs to determine first the patterns of growth in the apparently random network of stolons and polyps, and then to formulate, in mathematical terms, the kind of biological regulatory dicta which could effect such patterns of growth. These were then used to construct a recursive computer program which would generate a model of the colony in two dimensions. The computer model could thus serve to determine whether any given rule would produce, in interaction with the other rules, the observed colony morphology. Furthermore, the exercise of computer generation could serve to verify or to deny the assumption that the kind of complexity which characterizes developing biological systems can be produced by the recursive application of a small number of regulatory dicta having the form of operational statements.

Our earliest models (BRAVERMAN and SCHRANDT, '66), based to a great extent on assumptions regarding colony growth parameters, were successful to the extent that they showed that a program could be written to serve our purpose (Fig. 5). They further demonstrated that simple growth rules, applied again and again, would generate patterns of biological complexity. Under these circumstances, small changes in the nature of the rules frequently resulted in gross and unpredictable changes in colony form. This observation seems to me to have profound implications regarding the mechanisms of genetics, evolution and development.

The first model was written in gross terms of hydranths, growing stolon tips, branch points and sexual zooids. This seemed appropriate to the view I held, along with most others, at the time, that form in hydroids was a consequence of the location of meristem-like regions of cell proliferation (KUHN, '10; HYMAN, '40; BONNER, '52; BERRILL, '61; BAYER and OWRE, '68). As contra-indicative results from a number of separate laboratories accumulated, it seemed to me that the terms of the model ought to be cellular. Whether this is but a step to understanding biological phenomena at ever finer levels, or whether the cellular fabric is that in which the developmental cloth is stitched, there is no certain knowing. I tend, however, to believe the latter. This is not to deny that the proclivities of cells have their basis in organelle and molecular physiology. They must. Rather, along with Tryggve GUSTAFSON and Lewis WOLPERT ('63), I find the form influencing repertoire of cells to be a highly limited one. The great variety of enzyme permutations of differentiating cells results in but a small number of form generating characteristics. The basis of morphogenesis, in hydroids,

Fig. 1. A colony of *Podocoryne carnea* on a snail shell.
Fig. 2. A young *Podocoryne carnea* colony growing on a microscope slide.
Fig. 3. A 6 week old colony on a microscope slide. N, nutritive zooid; G, generative zooid; S, stolon.
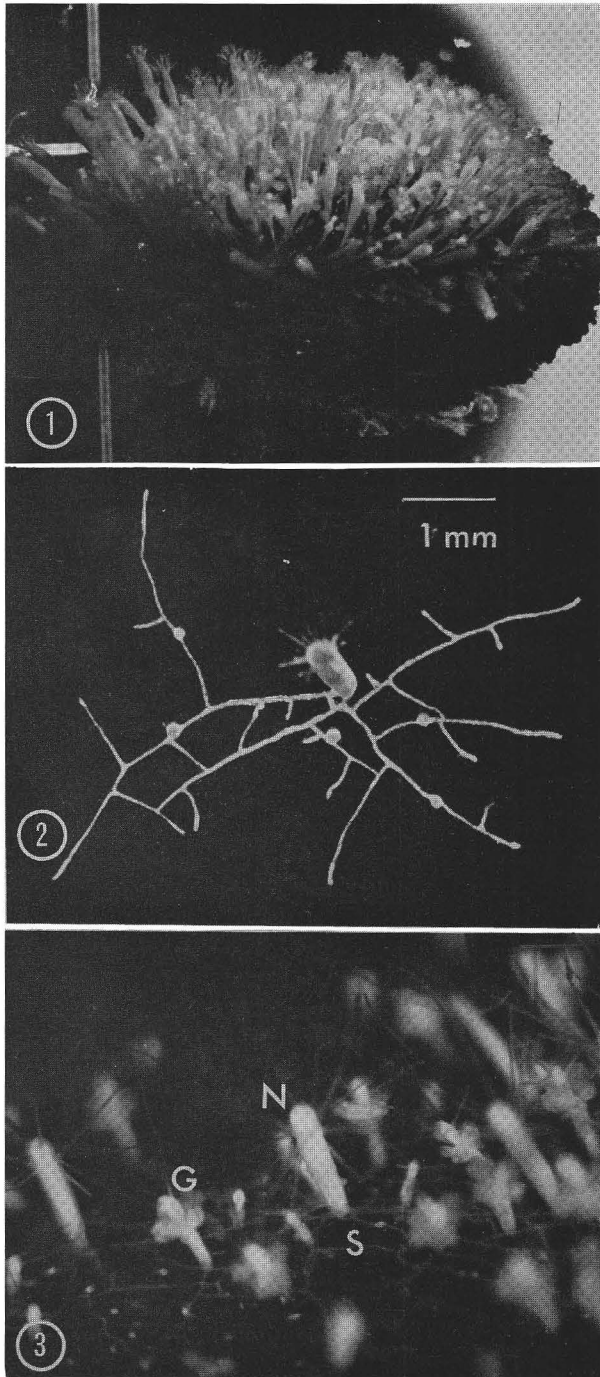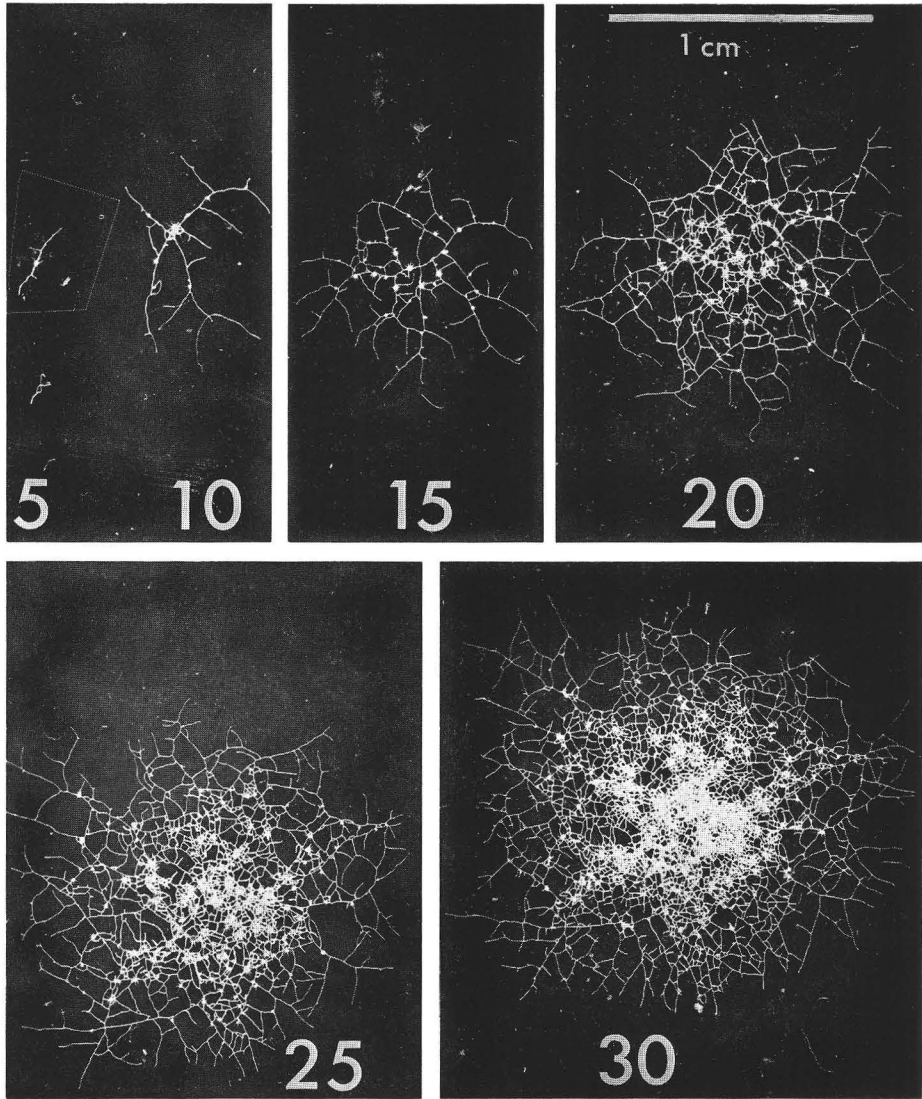
Fig. 4.   A colony of *Podocoryne carnea* growing in artificial sea water at 18 °C over a one month period.   The age of the colony in days is indicated.
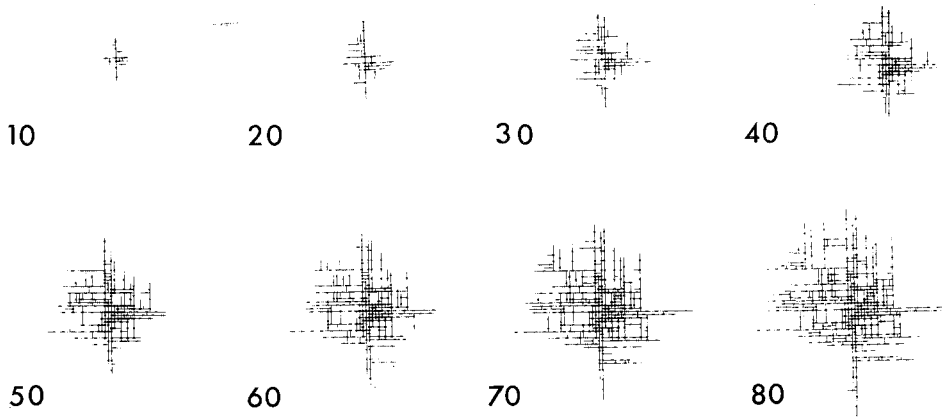
Fig. 5. A computer generated colony of *P. carnea*. The numbers indicate the age of the colony in computer generations.   The computations were done by Robert G. SCHRANDT of The Los Alamos Scientific Laboratory of the U.S. Atomic Energy Commission.

appears, for the larger part, to be cellular.

Cell Movements

Morphogenesis occurs continually in colonial hydroids as stolons extend, apparently without limit.   In *Podocoryne,* colony growth consists of two parameters: stolon growth and new hydranth formation.   These two parameters are mediated by three variables: stolon extension, stolon branching, and new hydranth formation (Fig. 6).   In *Podocoryne* not only do new colony regions continually form, old ones are
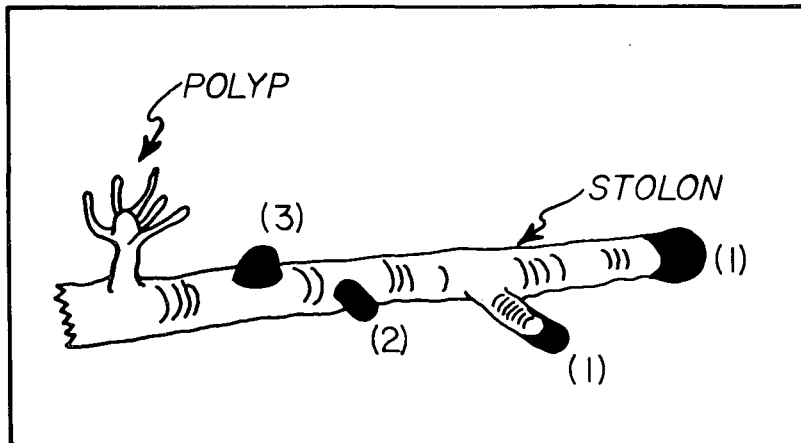

Fig. 6. *P. carnea* grows by increasing stolon length and adding new hydranths.   These two parameters are mediated by three variables: (1) stolon extension, (2) stolon branching, (3) new polyp formation.

also maintained. To account satisfactorily for the role of cells in colony morphogenesis we shall have to account for cell proliferation, cell movement, the final disposition of cells, and finally, gain some idea of the priorities and proclivities governing cell disposition. But cells are disposed not only to provide for new colony growth, they also move to replace expendable cellular elements such as nematocysts, and others which cycle through the growing regions.

I. FROM POLYP ECTODERM TO POLYP GASTRODERM: Cell proliferation in hydra has been described by BRIEN (BRIEN and RENIERS-DECOEN, '49; BRIEN, '51) to occur in a discrete growth region below the tentacles, and to effect a movement of cells from that region up into the tentacles and down the column of the hydra to the peduncle. CAMPBELL ('65 a,b; '67 a,b,c,d) subsequently argued that proliferation is not limited to a discrete region, but distributed through the body column. He, along with SHOSTAK (SHOSTAK and KANKEL, '67, SHOSTAK, '68), emphasized that the majority of downward moving cells move into the budding polyps (Fig. 7).

TRIPP   CAMPBELL   CAMPBELL
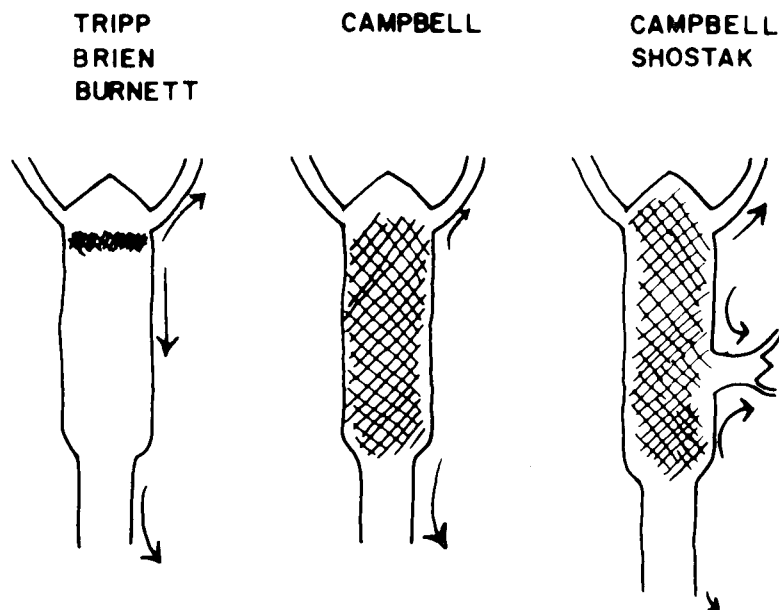BRIEN   SHOSTAK
BURNETT

Fig. 7. Ideas of how hydra grows and maintains itself have expanded the original hypothesized site of proliferation to include almost the entire column of the hydra, and have emphasized detrainment of cells into buds rather than off the foot.

This pattern of cell movement is not a general one among the hydrozoa, but rather is unique to hydra. Probably the well known morphogenetic movements in hydra represent an adaptation to the solitary hydroid's mode of budding. Even so, emphasis on the proximal movement of sheets of cells in hydra ignores an important cell movement which does appear to be ubiquitous among the hydrazoa. This is the movement

of cells from the epidermis to the gastrodermis.

When hydroids are lightly stained in any of a number of vital dyes, then removed, the dye is localized primarily to the epidermis. Subsequently the dye disappears from the epidermis and is seen in the gastrodermis of the animal. This description has been reported by KANAJEW ('30) for *Pelmatohydra oligactis,* by SHOSTAK (personal communication) using *Hydra viridis,* by HALE ('64) for *Clytia johnstoni,* and by BRAVERMAN ('69) for *Podocoryne carnea.*

ROSE and BURNETT ('68 a,b; '70 a,b) have devoted a series of papers to the nature and origin of the mucous and zymogen glands of hydra and other hydroids. They contend that the initial source of these cells is basophilic cells of the polyp epidermis. The basophilic cells then migrate individually across the mesoglea into the gastrodermis, there to differentiate as gland cells. Their most convincing evidence consists of radioautographs showing radioactive gland cells in *Cordylophora* recombinants of labeled epidermis and unlabeled gastrodermis.

There is a strong correlation between the pattern of vital dye localization and the pattern of thymidine incorporation in polyps exposed to these cell markers. In *Podocoryne* vital dyes mark the epidermis, most heavily in the mid polyp region, the cut proximal end of the hydranth and the cells lying in the furrows of the hypostomal gastrodermis. Thymidine is incorporated, during a one hour pulse, by cells of identical regions (Fig. 12). In polyps on colonies the mid body epidermis is labeled, as are cells occupying the furrows between gastrodermal ridges. When polyps are plucked from the colonies, then put into radioactive thymidine, cells of the cut surface incorporate label for 4 to 12 hours after (BRAVERMAN '69). This correlation suggests that the two markers identify the same population of cells, and that therefore at least some of the cells which migrate across the mesoglea first divide.

II. *FROM POLYP GASTRODERM TO HYDROPLASM AND THENCE TO OTHER PARTS OF THE COLONY:* The best indication of the subsequent disposition of those cells that were marked with vital dyes in the epidermis and then moved into the gastrodermis comes from the experiments of HALE ('64), using *Clytia johnstoni,* and of BRAVERMAN ('69) with *Podocoryne carnea. Clytia* is a calyptoblast in which polyps regularly regress and are replaced. *Podocoryne* is a gymnoblast in which once formed, polyps endure for the life of the colony. Movement of cells out of *Clytia's* polyps occurs *only* when those polyps regress. Movement from *Podocoryne's* polyps occurs continually. With this exception passive migration of cells originating in the polyp gastroderm is identical in the two species.

Vitally stained polyp gastroderm cells move to four locations in growing colonies. *A. From the Proximal Gastrodermis to the Distal Gastrodermis.* In grafts of *Podocoryne* polyps consisting of dyed bottoms and undyed tops, the dye, initially in the proximal epidermis, finally locates in the distal gastrodermis. In the reverse graft, dye slowly disappears from the distal epiderm. In neither case is there any indication of distal or proximal movement of dyed cells in the epidermis (BRAVERMAN '69, Fig. 8).

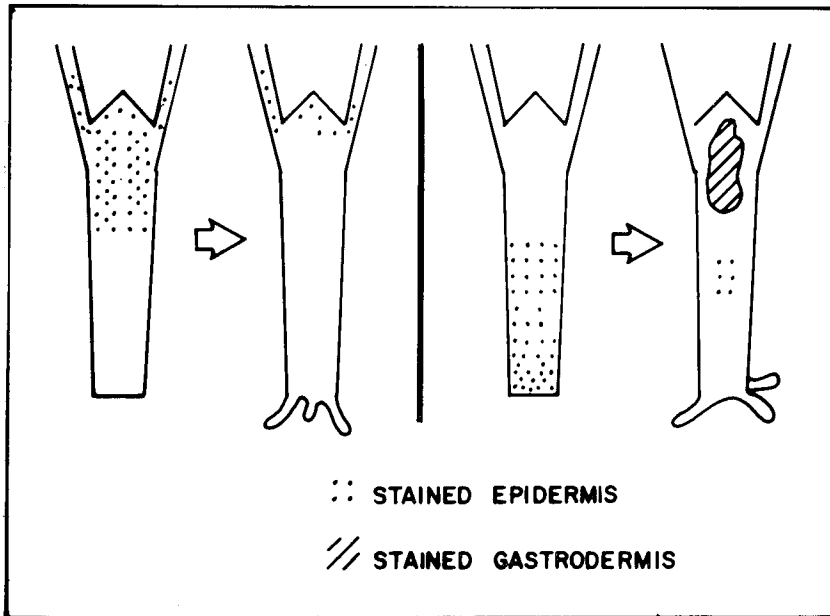: : STAINED EPIDERMIS

// STAINED GASTRODERMIS

Fig. 8.   Vital dye grafts of *Podocoryne carnea* indicate no proximal movement of the dye.   Dye
moves from the epidermis to the gastrodermis, and distally in the gastrodermis (after
BRAVERMAN, '69).

FED

5 - 17   5 - 19   5 - 24   5 - 27   6 - 1

STARVED

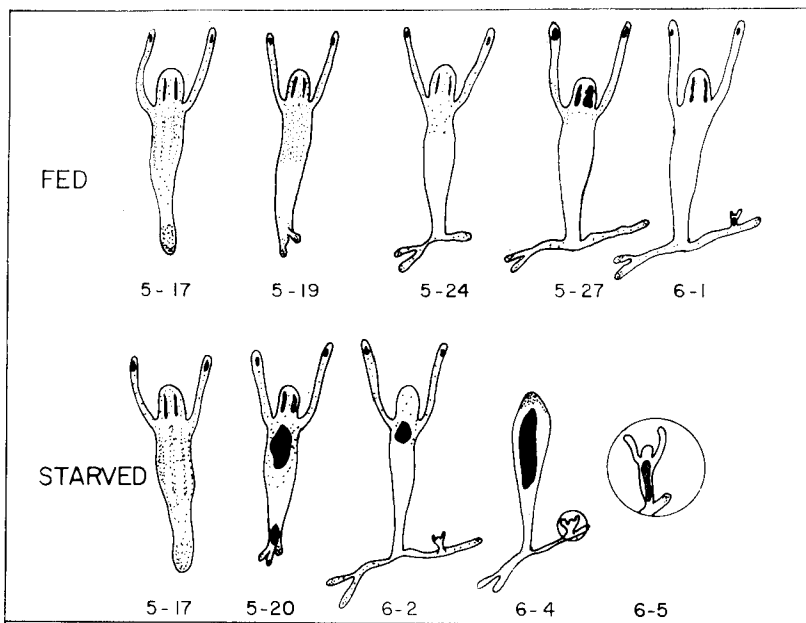5 - 17   5 - 20   6 - 2   6 - 4   6 - 5

Fig. 9.   New hydranths, forming on colonies initiated with dyed polyps, contain dye in their
gastrodermal cells.   This is more noticeable in starved colonies, but occurs in fed ones as
well (from BRAVERMAN, '69).

This distad movement of gastrodermal cells has been described by STEINBERG ('54, '55) in *Tubularia* stems as one of the first steps to occur during hydranth regeneration. Four to eight hours after decapitating a hydranth there is a distinct shifting of endodermal cells in a distal direction within the hydrocaulus. It is this distad movement, no doubt, that is responsible for the gastroderm denudation that occurs in repeatedly regenerating *Tubularia* stems (TARDENT, '63).

B. *To the Gastroderm of Newly Forming Polyps and to the Distal Gastroderm of Sexual Zooids.* If *Podocoryne* hydranths are removed from a colony, lightly stained in Nile Blue Sulfate for 24 hours, then placed on slides, they will attach there and initiate new stolon and hydranth growth. As the dye disappears from the epidermis, it can be seen simultaneously to appear in the gastrodermis of newly forming polyps (Fig. 9, BRAVERMAN '69).

When entire three month old colonies of *Podocoryne carnea* are lightly stained in Nile Blue Sulfate, the dye initially resides, as it does in single polyps, in the epidermis. Young polyps are the most heavily dyed. The largest polyps in the colony take up but little dye, gastrozooids take up none at all. After three weeks of culture in undyed
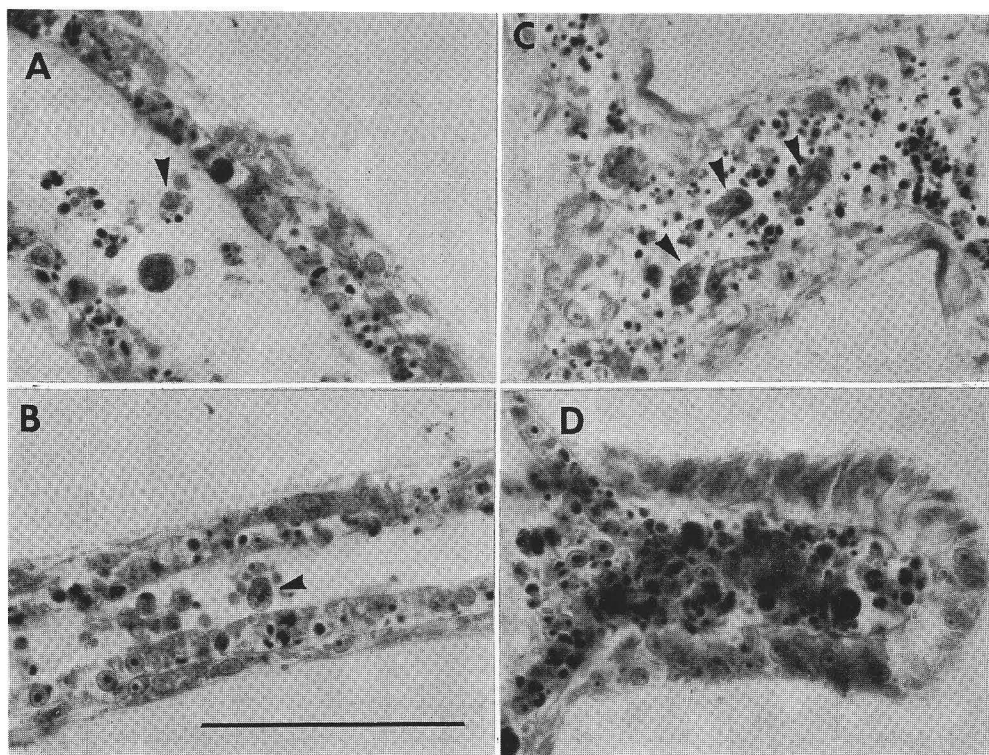

Fig. 10. The gastrodermis of *P. carnea* is a highly labile tissue. (A and B), gastrodermal cells containing nutriment float in the hydroplasm of the stolon. (Arrows indicate the nuclei of these "epitheliocytes.") (C) Zymogen-like cells (arrows) form in the stolon. These are being carried in the hydroplasm. (D) At times the gastrodermis of the stolon appears to be a fluid tissue. This is a newly formed stolon. The bar is 50 μ in length.

sea water, little dye remains in the epidermis. Considerable dye is resident in the proximal gastrodermis of young polyps, old polyps and gastrozooids.

C. To the Stolon. GOLDIZEN ('66) describes that when vitally stained hydranths of *P. carnea* are placed on a microscope slide bearing a young colony of the same clone, they attach to the slide, and stolons which form anastomose with those of the colony already established. Two days later palely stained cells can be observed scattered throughout the stolon gastrodermis of the host.

D. To the Stolon Tips. L.J. HALE ('64) carried out extensive staining experiments in which a short length of stolon, or a hydranth, on a small piece of colony, was dyed. He describes that dyed gastrodermal cells were transported to all regions of the stolon (as well as to the other colony regions described above), but especially to the region 1–5 mm behind the growing tip.
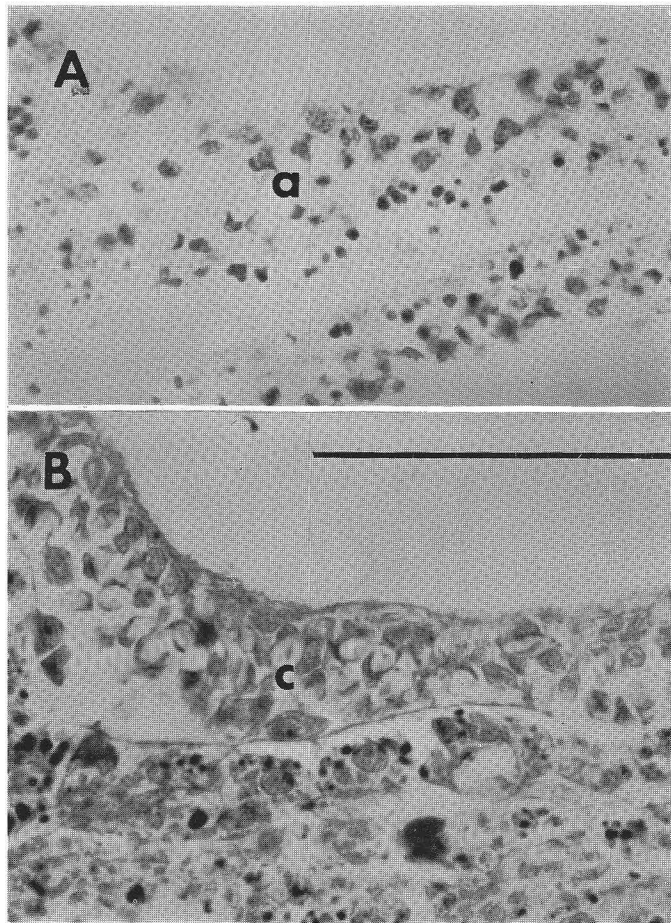

Fig. 11. (A.) Amoebocytes, a, and (B) cnidocytes, c, in proliferation regions of the stolon of *P. carnea*. The bar is 50 μ long.

E. Special Considerations. The passive movement of gastroderm cells through the hydroplasm was observed by GOLDIZEN (*ibid.*) in time lapse films of growing stolons. He describes intact cells floating along in the hydroplasm. These cells adhered to the gastroderm wall of the stolon, displaced adjacent cells and adhered to the mesoglea. HALE (*ibid.*) makes virtually the same observation of cells floating in *Clytia's* hydroplasm.

I have seen, in histological preparations, numerous examples of cells floating in the hydroplasm of *Podocoryne*. In fact, in many colonies the gastroderm of the stolon appears to be a fluid tissue consisting of rounded nucleated cells floating among the contents of the hydroplasm (Fig. 10). In the majority of the colonies, the gastroderm was a discrete, regular tissue of cuboidal cells, clearly defined and firmly adherent to the mesoglea. The liquid tissue rarely encompassed all the gastroderm of a colony. There is little chance that *Artemia* nuclei, which are frequently found in the hydroplasm from the third hour after feeding on, can be mistaken for nuclei of the hydroid, for the shrimp nuclei are intensely basophilic.

Dyed epidermal cells were never observed to migrate directly to other regions of the colony, without first passing through the gastroderm. Apparently, the cells incorporating dye move on a one-way street from epidermis to gastrodermis and thence to the hydroplasm for distribution to the other regions of the colony.

III. FROM STOLON EPIDERMIS TO HYDRANTHS: In older regions of the stolons of *Podocoryne carnea* the epidermis is expanded beyond the single layer found in newly formed stolons. These expanded regions consist of cells with small nuclei, frequently bearing numerous chromatic figures, nematocytes in various stages of development, and other cells, amoeboid in shape (Fig. 11). The regions in which nematocysts develop have been called cnidogenic regions (BOUILLON, '68) and have long been known (AGASSIZ, 1862). It is, of course, not possible, looking at an a-moeboid cell, to establish that it is a unique cell type, and not a cell on its way to becoming an cnidocyte. It is only the occurrence of these amoeboid cells in the upper half of the polyp epidermis, and the rare specimens caught in histological preparations half way between epidermis and gastrodermis, that indicates that under some circumstances amoebocytes retain their amoeboid shape until they reach their ultimate tissue destination. The rapid migration of thymidine incorporating cells into the polyp, further indicates that many of the amoebocytes seen in amoebogenic regions of the stolon are not in the process of differentiating as nematocytes.

Hydranths removed from cultures of *Podocoryne carnea* that had been incubated for one hour in tritiated thymidine, contain few labeled gastroderm cells, except those associated with the hypostomal gland cell region. Labeled cells in the epidermis are, for the most part, confined to the mid-region of the polyp. This initial distribution of radioactivity indicates which cells were in the process of incorporating thymidine into acid stable polynucleotide during the pulse. The distribution of radioactivity in hydranths removed from the same colony twenty-four hours later shows where the
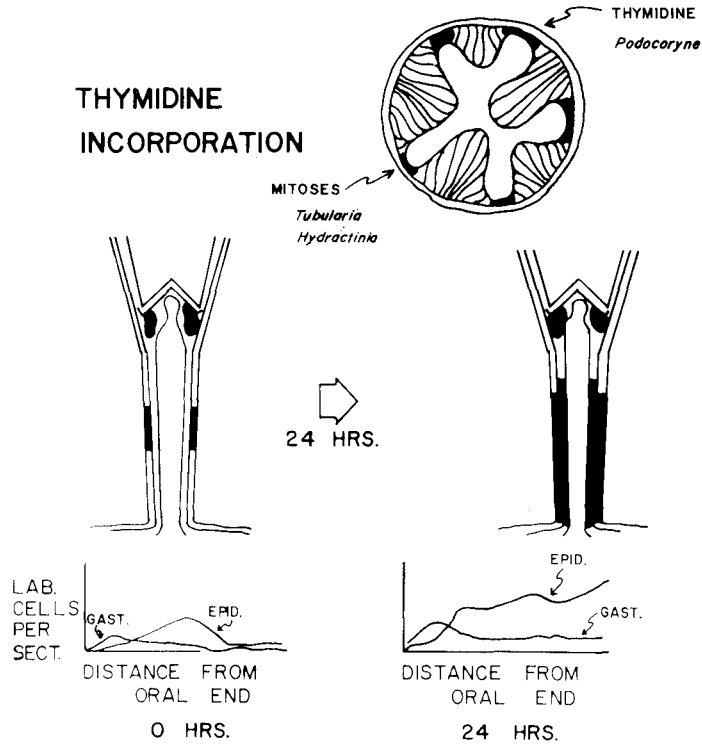
Fig. 12. Thymidine is incorporated in the mid-body epidermis and in the proliferating cells of the hypostomal mucous gland region. With the exception of that circumscribed region, the only cells of the hydranth to incoporate thymidine are those of the epidermis, mainly in the mid-body region. Twenty-four hours after a one hour pulse, both gastrodermal cells and epidermal cells of the proximal polyp are radioactive.

cells which took up the lable, originally, now reside. The distribution of label after twenty-four hours indicates considerable cell migration (Fig. 12; see BRAVERMAN '69 for experimental details). Whereas initially the only region of the polyp below the hypostome to bear labeled cells was the epidermis of the mid-region, in the twenty-four hour sample the entire proximal polyp, both epidermis and gastrodermis, was heavily populated with radioactive cells. Virtually no cells of the proximal endoderm were initially labeled, after twenty-four hours about 12% of the cells were. This increase in labeled cells can only be accounted for by positing that labeled cells migrate up into the polyp from the stolon.

Conceivably, some of the increase in labeled cells might be due to cell division. Such increase would then be proportional to the original labeling. Surely this is the case in the gastroderm of the upper third of the polyp (BRAVERMAN '68, describes cell movements in this circumscribed region). The increase in proximal gastroderm and epiderm labeling is, however, not proportional to the original label, and must therefore

reflect the movement to the region of cells which were resident elsewhere during the time of the pulse. The only possible source for such cells is the stolon.

Large numbers of labeled cells are found in the cnidogenic and amoebocytic regions of the stolon after a one hour tritiated thymidine pulse. For the most part, the labeled cells are adjacent to concentrations of the smaller cnidocytes and amoebocytes, and resemble most characteristic cells of the stolon. Thirty-five hours after such a pulse considerably more cnidocytes and amoebocytes are labeled (Fig. 13). The large
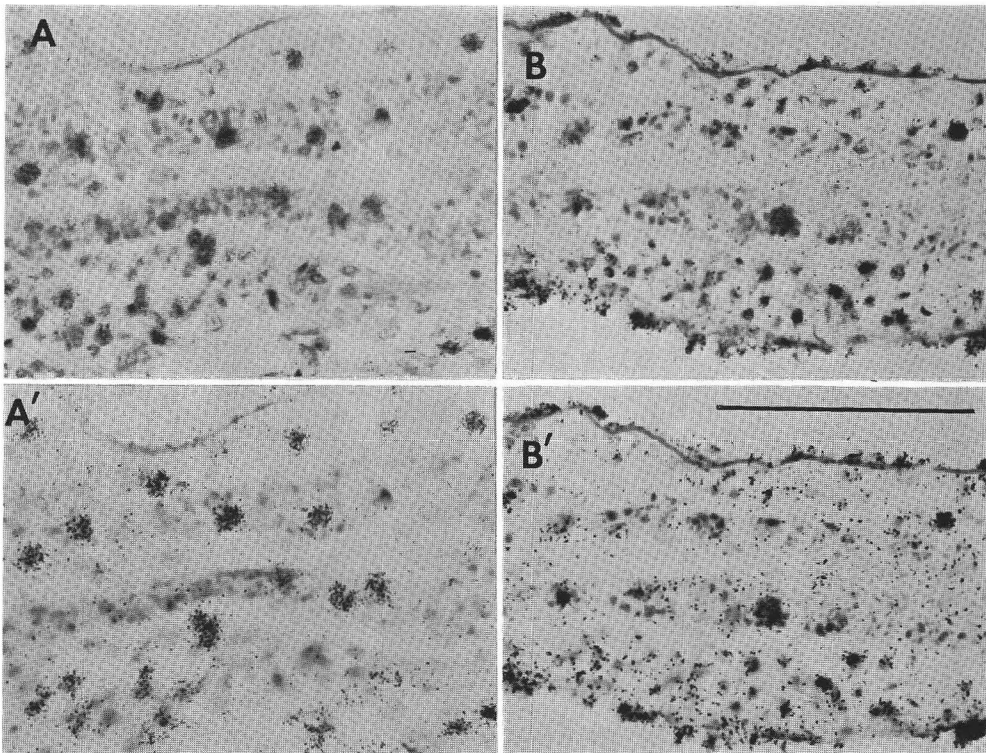

Fig. 13. Stolon proliferating regions (amoebogenic or cnidogenic) (A and A') immediately after a one hour pulse, and (B and B') 35 hours after a one hour pulse, with tritiated thymidine. In A and B the camera was focused on the tissue section; in A' and B' on the film emulsion. Only large cells are labeled just after the pulse, but 35 hours later many small nuclei are radioactive. This shows that the amoebocytes and cnidocytes are not a self-reproducing population, but rather derive from the characterstic epidermal cells of the stolon. The bar is 50 μ long.

numbers of labeled cells in these epidermal proliferation centers indicates that these could be the sources for the labeled cells which migrate into the polyp. Unfortunately, the polyp epidermis is so crowded with small cells, that a direct identification of which cell type migrates into the epidermis is impossible. If the labeling pulse is sufficiently concentrated, and the exposure interval sufficiently long to provide unequivocal labeling, then the grains mask the morphology of the tiny cell over which they lie.

The distribution of label in large nuclei immediately after a labeling pulse, and in small ones thirty-five hours later, indicates that amoebocytes and cnidocytes are not a self-reproducing population, but rather arise from characteristic cells of the stolon epidermis.

HALE ('64) has described that no mitotic figures can be found in the gastroderm of either stolon or polyp of *Clytia*. Nor have I been able to find more than a few mitotic figures in the gastrodermis of *Podocoryne carnea,* with the exception of the
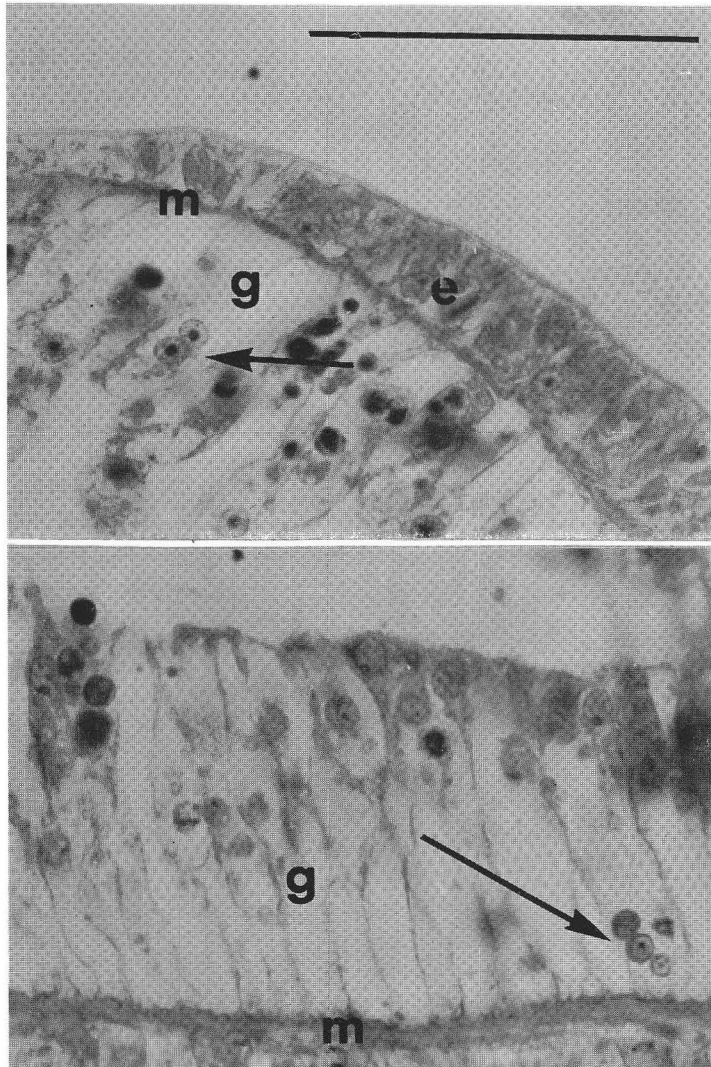

Fig. 14. Polynuclear cells (arrows) in the proximal gastroderm of *Podocoryne* hydranths. These colonies were starved for four days, fed once, then starved for three days more. Mesoglea, m; gastroderm, g; epiderm, e. The bar is 50 μ long.

circumscribed proliferation region of the hypostome glands. Thymidine is, however, incorporated by the gastrodermal cells of the stolon. This in itself is a puzzling situation. How can cells be continually incorporating thymidine into polynucleotide, but never undergo division? The entire question of cell division in hydroids is puzzling. *Podocoryne* and *Clytia,* at least, have very little Feulgen demonstrable DNA. Even in the epidermis of *Podocoryne* very few division figures can be recognized. Furthermore, multiple nucleated cells are frequently seen in the gastroderm of *Podocoryne* (Fig. 14). Earlier investigators speculated that amitotic division occurred in the hydrozoa (cf. BRAVERMAN, '71b). That is unlikely, but the problem remains.

Since thymidine incorporation does occur in the stolon, stolon gastrodermal cells could be responsible for the increase in labeled cells of the polyp gastrodermis. Gastrodermal cells, after synthesizing DNA, might round up, and thus lose their connection to the stolon mesoglea to float in the hydroplasm. It is possible that these cells might then insinuate themselves among the proximal polyp gastroderm, but it is unlikely, for cell movement in the polyp gastroderm is from proximal to distal, suggesting that the distal region is stickier than the proximal, and dye-labeled cells moving into polyps tend to become established in the distal, not the proximal region. It is more likely that the source of labeled cells in the polyp epidermis and gastrodermis is the amoebocytic cells which migrate up into the polyp from the stolon, some of which move across the mesoglea into the gastroderm.

That similar cells migrate in the epidermis of other species has previously been reported by TARDENT and MORGENTHALER ('61) and by LENHOFF ('59). The latter investigator demonstrated the distal migration in hydra of ^{14}C labeled cnidocytes. BURNETT *et al.* ('68) have described how in tissue cultures of *Tubularia* cells, first, endoderm cells migrate out from the inoculum, then cells they describe as interstitial cells follow. In *Cordylophora,* similar cells move across the mesoglea and differentiate as gland cells (ROSE and BURNETT '70b). Indeed, these migrating amoebocytes appear to bear many of the properties previously claimed for interstitial cells, with the major exception that they are not a self-sustaining population, but arise from epidermal cells of the stolon.

IV. OUT OF STOLON GASTRODERMIS: The gastrodermis of the stolon of *Podocoryne carnea* varies in its structure. At times it can be seen to be a discrete, regular, tissue bearing regularly shaped gastrodermal cells which entirely fill the mesogleal space; at other times the tissue appears virtually fluid: cells are not adherent to the mesoglea but rounded up, packed tightly in some areas of the stolon, absent from others (Fig. 10). This apparent fluidity is consistent with the picture of gastroderm fluidity described by STEINBERG to occur during *Tubularia* regeneration (STEINBERG '54, '55) and by MOOKERJEE and BHATTACHERJEE ('66) to occur during hydra regeneration. BURNETT (personal communication) has also described gastrodermal cells of the hydra polyp breaking loose and floating about in the gastrovascular cavity.

It seems that under circumstances, at present not clearly understood, but certainly

during starvation and during polyp regression, but also under more normal circumstances, cells of the gastroderm lose their adhesiveness, float free from the mesoglea, moving to wherever the currents and the pulsations of the hydroplasm carry them. In those hydroids undergoing regression and replacement of their polyps (probably calyptoblasts in general, CROWELL, '53) gastroderm release occurs solely during polyp resorption.   In species in which polyps are not regularly resorbed (probably gymnoblasts in general) release of gastroderm cells occurs during normal colony growth. In both, gastroderm cells seem to be continuously attaching and releasing from the stolons.

   V. SUMMARY OF CELL MOVEMENTS: Two major types of cell movements are found in the hydrozoa.   Epidermal cells migrate as amoebocytes and cnidocytes from their proliferation sources in the stolon and in the mid-regions of polyps, into polyps and thence across the mesoglea into the gastroderm or up into the tentacles.   Gastrodermal cell movements are passive and involve a loss of adhesivity of gastrodermal cells to the underlying mesoglea and release to the hydroplasm.   There gastrodermal cells are carried about, passively propelled by currents created by hydrostatic pressures of polyps filling and emptying, by the peristaltic contractions demonstrated by stolon tip expansion and contraction, and by the flagella of the cells of the gastroderm.

   At this time it is not at all clear whether the sole proliferating tissue in hydrozoan colonies is the epidermis (with the exception of the circumscribed proliferating region of the hypostomal gastroderm), or whether the thymidine incorporating cells of the stolon gastroderm function as a cell source, despite the lack of noticeable mitotic figures in this tissue.   A clear picture of cell movements and cell proliferation is dependent upon the resolution of this paradoxical situation regarding gastroderm cell proliferation.

Patterns and Priorities of Colony Growth

   I. NUTRITION: The developmental repertoire of a growing colony consists of stolon extension, stolon branching and new polyp initiation.   Nutrition is equitably distributed among colony elements.   The starved halves of colonies, from which hydranths are removed to effect starvation, continue to grow stolon (the parameter grossly most sensitive to starvation) at the same rate as the halves which are capable of eating (BRAVERMAN, '71a).   Virtually identical experiments were reported by CROWELL ('57) with identical results.   The mixing of nutriment in the stolon network, and/or the indirect influence of nutriment on growth, seems to be complete and equitable.   The proximity of stolon regions to nutriment sources, the feeding polyps, thus plays no role in morphogenesis.

   II. TEMPORAL CONSTRAINTS: There are, however, temporal constraints on branching and hydranth formation.   Hydranths form on stolons of 2, 3 and 4

Table 1. The distribution of hydranths and branch points with respect to "random" distribution.

Stolon age in days	Hydranths			Branch Points		
	Observed	Predicted	Obs. Pred.	Observed	Predicted	Obs. Pred.
1	2.2	13.1	0.2	26.6	89.7	0.3
2	14.1	12.3	1.1	168.9	68.0	2.5
3	19.0	9.2	2.1	66.3	49.0	1.3
4	13.8	6.7	2.1	19.4	33.8	0.6
5	4.6	5.0	0.9	6.6	22.3	0.3
6	1.6	3.6	0.4	2.9	14.6	0.2
7	0.2	2.4	0.1	2.4	8.9	0.3
8	0.0	1.3	—	0.0	4.4	—

days of age. Two times as many form on 3 and 4 day old stolon and about as many form on 2 day old stolon as random distribution would predict (Table 1). Branches tend to form on somewhat younger stolons. Two and a half times the randomly predicted number on two day old stolon, 1.3 times the predicted number on three day old stolon. Cnidogenic and amoebogenic regions tend, at least in younger parts of the colony, to be located at branch points.

III. FEEDBACK REGULATION: Hydranth formation is further constrained as a feedback function, apparently related to the number of hydranths present on a colony. In those colony halves from which polyps were removed, new hydranths formed at about two and a half times the rate they did in the control halves (BRAVER-
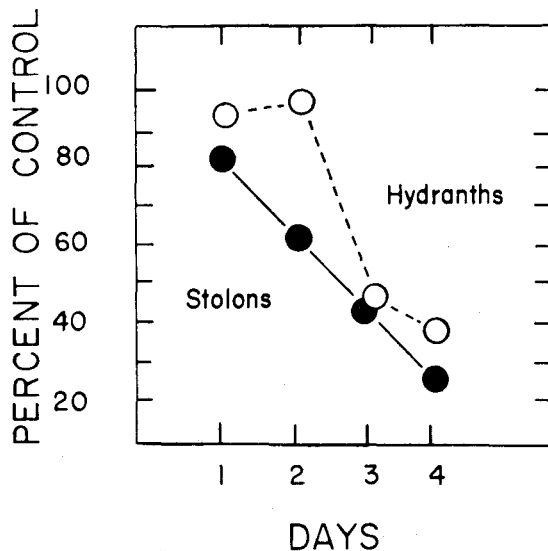

Fig. 15. The rate of stolon growth (●) falls off linearly and immediately in starved colonies of *P. carnea*. Hydranths (○) remain at the control level for two days.

MAN, '71a). Thus there is some kind of control which regulates the number and
location of new hydranth formation. Since the control applies to where hydranths
form, it is unlikely that materials moving in the hydroplasm exert this control, as they,
if food distribution be any criterion, are ubiquitously distributed.

   IV. PRIORITIES: Analysis of starving colonies demonstrated interesting
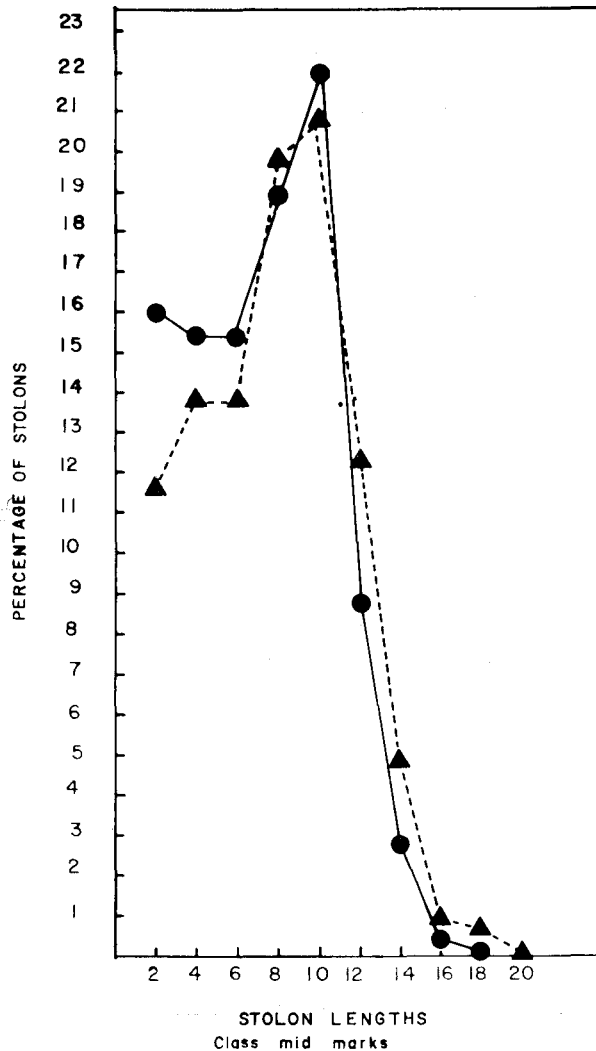

Fig. 16. Frequency distribution of the lengths individual stolons grow in
   colonies starved for four days (●) and in control colonies (▲) fed
   daily. Measurements were taken on each of four days and summed.
   n fed=1148; n starved=698. The rate of growth of already existing
   stolons does not differ from the growth of stolons in control, fed,
   colonies.

priorities among the three growth parameters. When colonies are starved stolon growth falls off linearly and immediately; hydranth formation lags two days in demonstrating the effects of starvation (Fig. 15). Although the total stolon length of the stolons forming in starved colonies is reduced, the growth of individual stolons is not. Frequency distribution analysis (Fig. 16) of the lengths individual stolons grow in starved colonies, and in fed controls, shows that those stolons which do grow, in starved colonies, grow the same amount as those growing in fed controls. The difference in total stolon length grown reflects the difference in branching. In starved colonies branching is severely curtailed. The absolute number of new branches is reduced, as well as is the number of new branches relative to the amount of new stolon growth. Those individual stolons already in existence continue to grow at the same rate as their counterparts in fed controls. This is dramatically demonstrated by photographs of colonies that were starved for seven days beginning on the 20th day of culture, compared to controls that were fed during the entire time. The peripheral area subtended by the stolons of the starved colonies is equal to that of the fed colonies, but
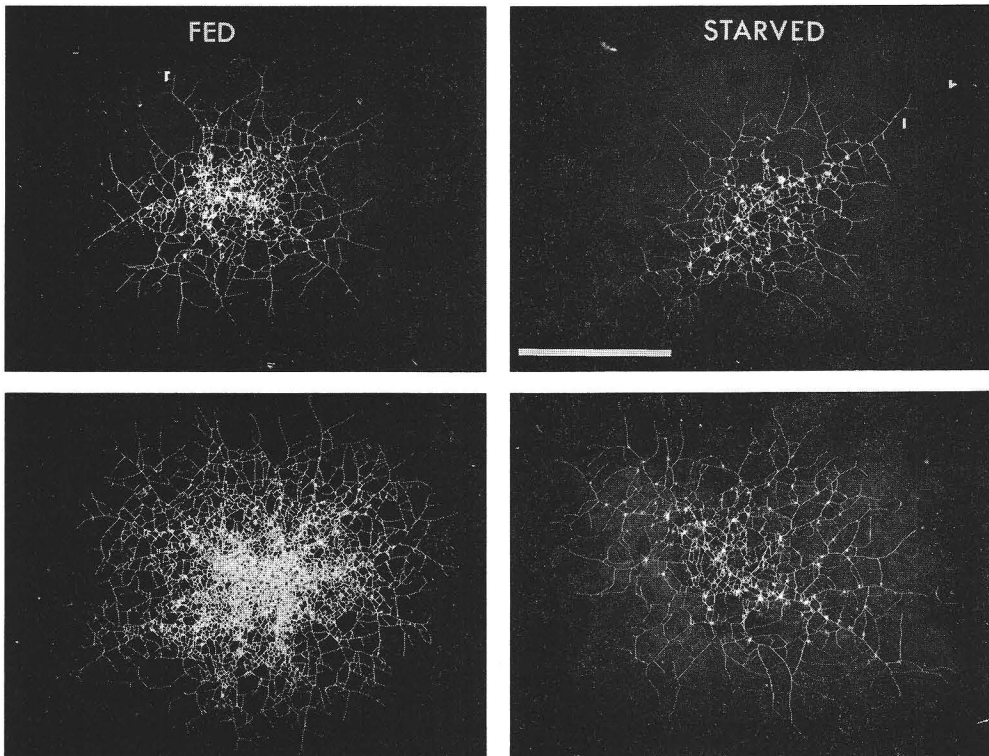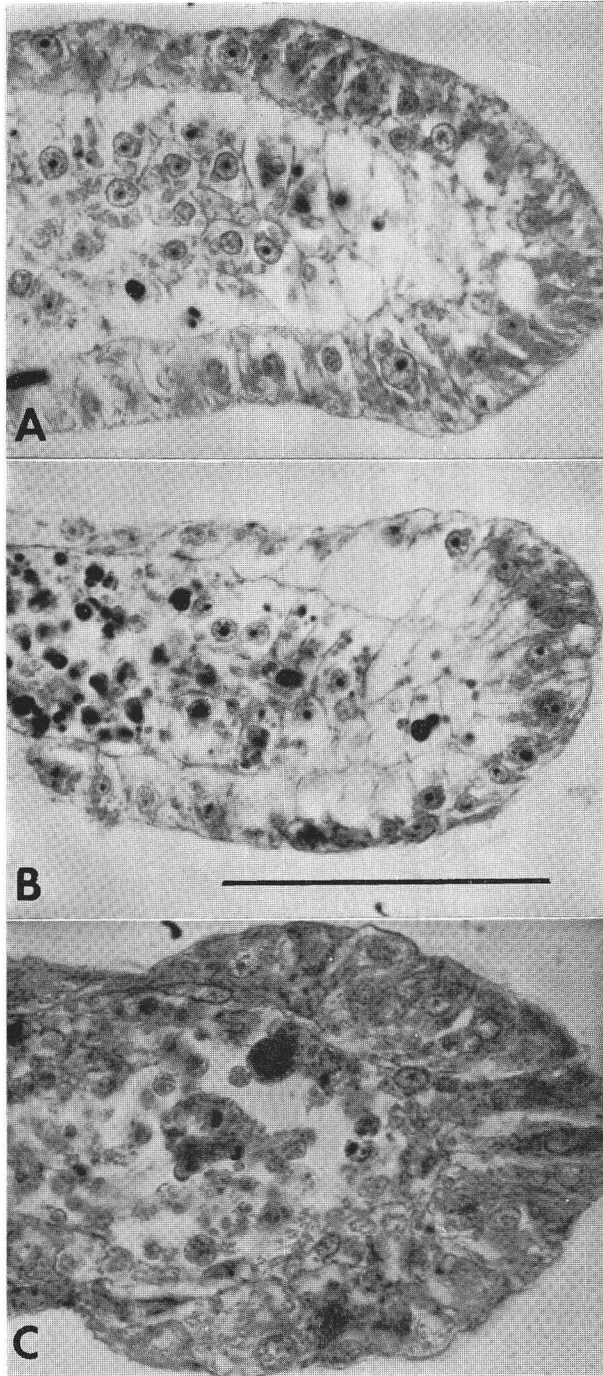

Fig. 17. Colonies fed and starved for one week, beginning on their twentieth day. The colonies are depicted on day 20 and day 27. The stolons of the starved colony extend as far peripherally as do those of the fed colony, but branching in the starved colony has been severely repressed. The bar is one centimeter long.

the stolon density is considerably less (Fig. 17).

Although the new branches/new stolon length were reduced in the starved colonies to about 1/3 the number of those in the fed, the number of new hydranths/new stolon length was the same in starved and in fed colonies. From this data a morphogenetic priority can be constructed. Stolon extension will continue despite starvation and new hydranth formation will occur on stolons of the appropriate age, in proportion to the length of new stolon formed. Branch formation, however, will be curtailed in response to limited nutrition.

Startlingly similar results regarding growth priorities were reported by Sears CROWELL ('57) on the basis of experiments similar to, but considerably more extensive and more elegantly controlled than, these. CROWELL observed, "There is good correspondence between the total quantity of the stolonic system and the quantity of food," but he showed that branching to form new uprights is severely retarded under starvation conditions. This is true, he reports, in both the calyptoblast *Campanularia flexulosa* and the *Cordylophora* sp. CROWELL's analysis of the growth priorities, taking into account an earlier description by BERRILL ('50) is lucid: "it seems to be a clear rule that locations where proliferation is occurring have precedence over zones of prospective growth." This was written before CROWELL's vital dye studies led him to question that growth regions are proliferation regions (CROWELL, WYTTENBACH and SUDDITH, '65).

The Nature of Growth Regions

I. THE STOLON TIP: Growing stolon tips are characterized by a cap of epidermal tissue, elongated in the direction of stolon growth. These cells bear what appear to be vacuoles proximally. The vacuoles are adjacent to what appear to be similar vacuoles at the distal border of gastrodermal cells. The nuclei of the tip epidermis characteristically bear proximally directed adherent chromatic appendages. Perhaps these are associated with their perisarc depositing function. The structure of the tip gastroderm can be either well or poorly defined (Fig. 18).

By means of time lapse cinematography GOLDIZEN demonstrated ('66) a pulsating pattern of advancement of the tip of *Podocoryne* stolons similar to that shown for *Clytia* (HALE, '64) and for *Campanularia* (WYTTENBACH, '64). There appears to be some correlation between the expansion and contraction of the tip, the degree of fluidity of the gastroderm, and the distribution of a bolus of cells and free nuclei which is frequently seen, during the contraction phase, some distance behind the tip. Although it is difficult to be certain that the effect is not an artifact of tangential section, it also appears that during contraction, the epidermis contains an increased number of the

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Fig. 18. The gastrodermis of the stolon tip varies from a discrete, regular tissue (A) to a poorly defined, virtually liquid tissue (C). This change in tissue fluidity may be related to stolon extension and the pulsating expansion and contraction of the tip. The bar is 50 μ long.

aforementioned vacuolar spaces (Fig. 18).

The pattern of movement of vital dyes in the stolon relative to the movement of the elongating stolon tip (HALE, '64; OVERTON, '63; CROWELL, WYTTENBACH and SUDDITH, '65) indicated that contrary to earlier expectations, stolon extension was not effected by terminal proliferation in a meristem-like region. The indirect evidence of dye movements has subsequently been directly verified. Little thymidine incorporation occurs at the stolon tip. The per cent of labeled cells increases more or less regularly for the first 0.5 mm from the tip. Incorporation is approximately equally divided between epidermal and gastrodermal cells (BRAVERMAN, '71b). Thirty-five hours after a one hour labeling pulse with tritiated thymidine, however, a considerable number of labeled cells can be seen in both epidermis and gastrodermis of the tip (Fig. 19). The gastrodermal cells no doubt arrive there carried by the stolon currents. It is not equally clear how labeled epidermal cells can be present at the tip unless (a) tip cells are regularly sloughed, or (b) gastroderm cells move into the tip epidermis.

II. NEW BRANCHES: The sequence of events characterizing branch formation begins with the reorientation of epidermal cells perpendicularly to the mesoglea. The cells continue to elongate. Their nuclei develop characteristic proximally directed tabs. The perisarc is dissolved and, shortly thereafter, gastrodermal cells reorient with respect to the new tip. By histo-morphological criteria, branch formation appears to be an event initiated by the epidermis. No increased incidence of thymidine incorporation is present in the cells which reorient to form new branches, thus direct proliferation is no more responsible for new branch formation than it is for stolon extension (Fig. 20).

Stolon anastomosis is, interestingly, accomplished via the same mechanism as branch formation. As a stolon tip approaches another stolon, the cell morphology characteristic of the epidermis of new branching is induced in the recipient stolon. This morphogenetic induction at a distance, across intervening sea water, is, to my knowledge, a unique phenomenon (Fig. 21). It has previously been described to occur in anastomosing stolons of *Hydractinia echinata* (MÜLLER, '64). Thus, apparently, is the perisarc broached.

GOLDIZEN ('66) has described that advancing tips which approximate empty stolons of old portions of the colony do not invade the empty stolons, but move over them, corroborating the observation that perisarc dissolution is accomplished by the recipient stolon. Subsequently the two epidermal layers fuse, and, by a mechanism unknown to me, the fused epidermis layers part and the gastrodermis becomes confluent.

III. NEW POLYPS: Polyp formation in *Podocoryne,* unlike those hydroids with terminal polyps, requires that a new "growth" region be initiated along with polyp differentiation. I previously (BRAVERMAN, '71b) described this new region as being identical in origin to that of stolon outgrowth (branching). In that description I was mistaken, and probably misled by aberrant stolon tips growing upward from the
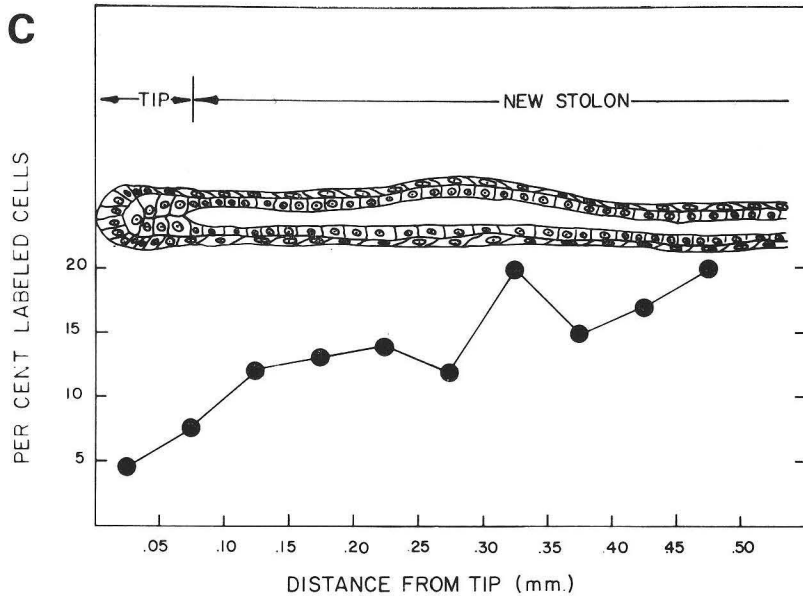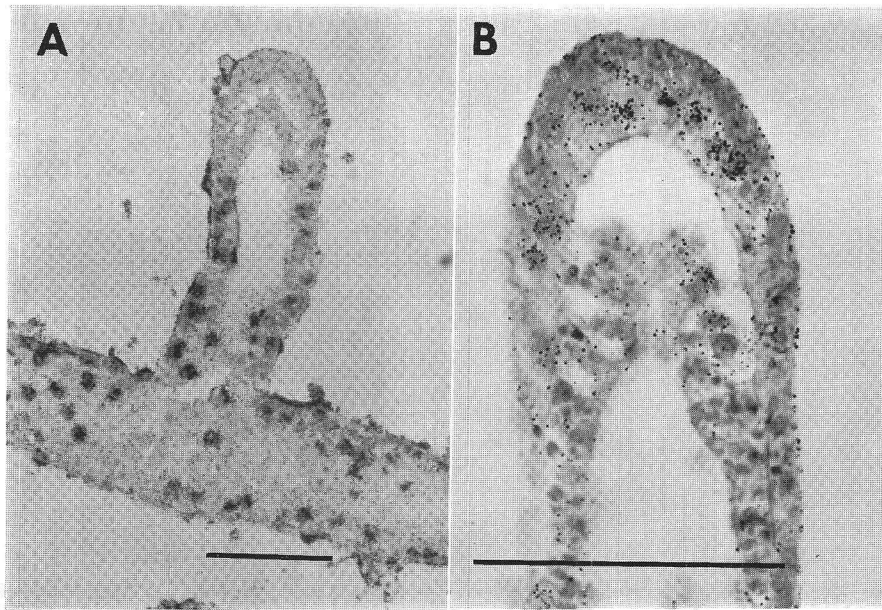
Fig. 19. The distribution of radioactive nuclei, in the stolon tip, immediately after (A and C) and 35 hours after (B) a one hour incubation in tritiated thymdine. Although tip nuclei do not incorporate thymidine, labeled nuclei are present at the tip thirty-five hours later. A possible explanation is that epitheliocytes float through the hydroplasm, adhere to the tip and insinuate themselves among the cells of both the epidermis and the gastrodermis. The bar is 50 μ long.
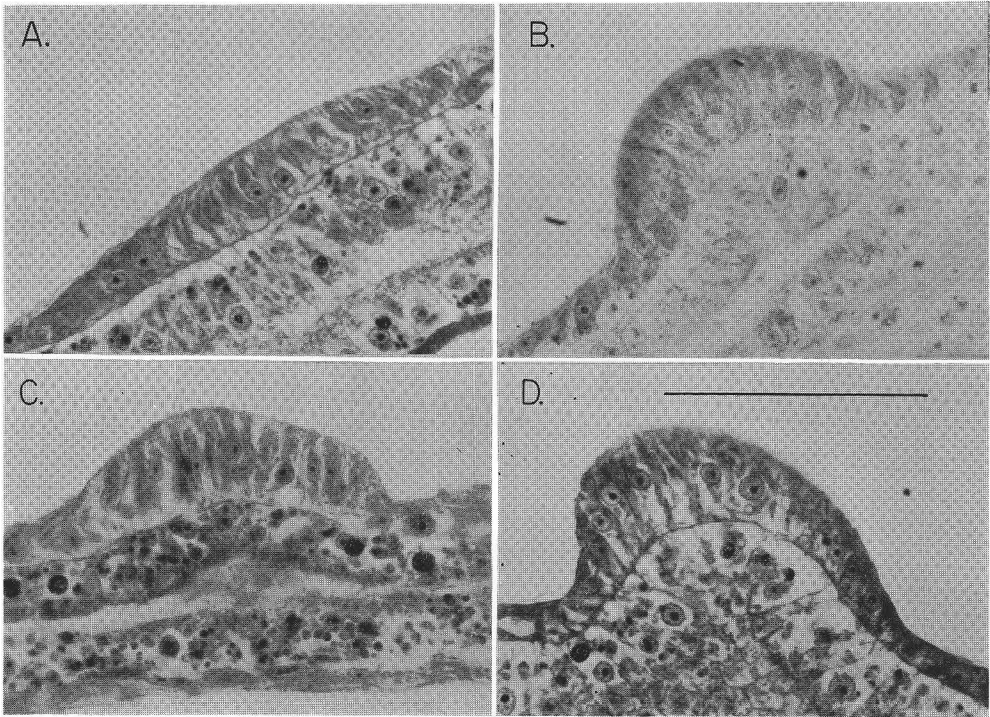
Fig. 20. Branch point genesis. The initial morphological event consists of elongation of epidermal cells in the direction of the new stolon. The bar is 50 μ long.

surface of the substrate. Although it remains difficult, unequivocally, to identify the earliest stages of hydranth outgrowth, the first recognizable form appears to be sphere shaped and without perisarc (Fig. 22). BERRILL ('61) described that not only are what appears to be vacuolated cells characteristic of the advancing terminal stolon, but also these vacuolated cells are present in terminal hydranths developing, in *Obelia, Campanularia,* and many related genera, at the ends of stolons. These vacuole-like structures seem to be ever present in hydroid regions of tissue extension. They may well play some critical role in accommodating the cellular accretion which must occur there.

A Model for the Cellular Basis of Morphogenesis
and Morphostasis in Hydroids

Two important aspects of the cellular mechanisms relevant to the growth and maintenance of hydroid colonies remain yet unknown. (1) Gastrodermal cells incorporate thymidine but do not demonstrate mitosis. Do these cells divide? If so, how? Alternatively, is the epidermis the only proliferation source in the colony?
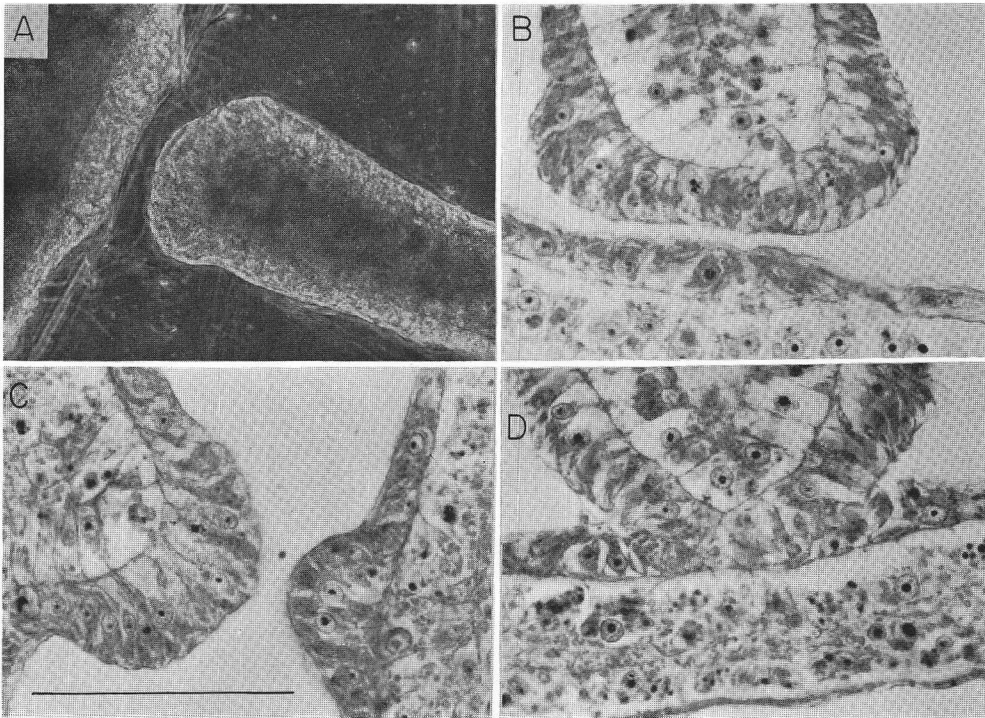
Fig. 21. Stolon anastomosis. Perisarc dissolution in the recipient stolon is effected by branch formation induced in that stolon by the anastomosing one. (A) Phase contrast photomicrograph of living stolons. (B,C, and D) Stages in anastomosis. The bar is 50 μ long.

(2) How does extension at the stolon tips occur? Where do the epidermal and gastro-dermal cell components for each of these come from?

In order to construct a model based on the source, movement and distribution of cells in growing colonies I shall make the following assumptions with regard to the two problematical aspects of cell proliferation and colony growth.

(1) Gastrodermal cells of the stolon can give rise to other gastrodermal cells (no assumption of method is necessary). This applies only to gastrodermal cells of the stolon; little thymidine incorporation is demonstrated by gastrodermal cells of the polyp stalk.

(2) Stolon tip extension results from division of cells distributed among the adjacent stolon epidermis and from the adhesion to the growing region of epitheliocytes floating in the hydroplasm. I shall further assume that under conditions of starvation, and perhaps under other conditions presently unknown, the contribution from adjacent epidermis is minimal and that the majority of cellular material for growth is obtained from epitheliocytes.

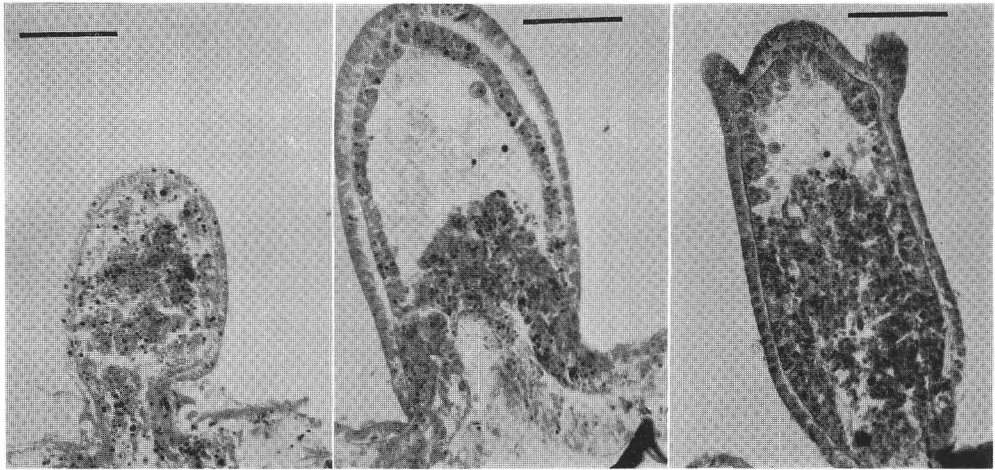*I. STOLON EXTENSION AND BRANCHING:* Stolon extension in the epidermis

Fig. 22. Three stages in the development of a new polyp. All are characterized by a bolus of cellular and nutritive material. The bar is 50 μ long.

of *Podocoryne carnea* proceeds partially, or wholly, by virtue of cell division occurring between the stolon tip and the closest stolon branch. The extension of tip gastrodermis is accomplished by means of epitheliocytes which float in the hydroplasm, accumulate at the stolon tip and interdigitate among those already there, during the expansion phase of tip pulsation. Presumably advancement of the two layers is balanced by the extent of epitheliocyte contribution to the epidermis. In a normally fed colony a proliferative balance exists between the rate at which the tip moves forward
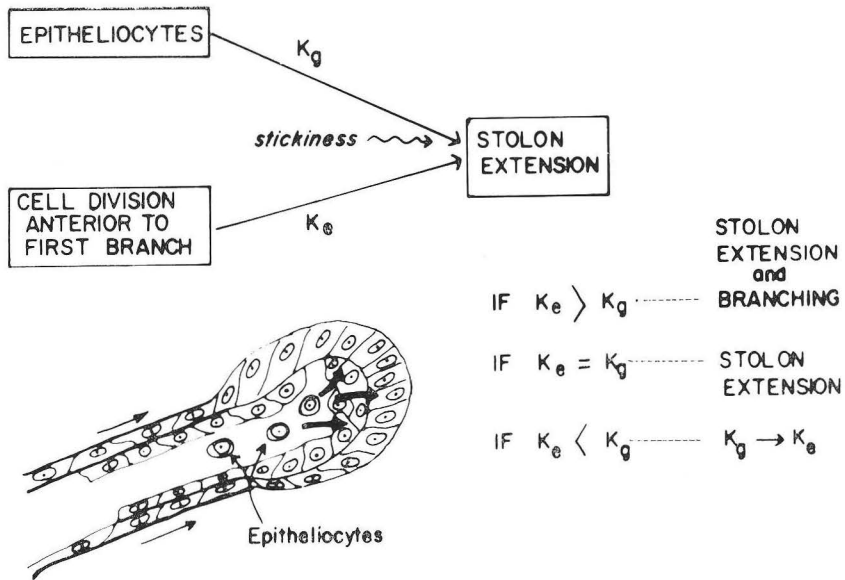

Fig. 23. The cellular basis of stolon extension in *Podocoryne carnea*.

and the rate of proliferation in adjacent stolon ectoderm. If the latter outruns the former, the number of cells in the space defined by mesoglea and perisarc laterally, and the tip and a branch antero-posteriorly, increases. This increase in cell volume within a contained space results in increased pressure which stimulates epidermal cells to reorient in the characteristic palisaded form of new stolon branches, and stolon outgrowth occurs at that point (Fig. 23).

II. HYDRANTH FORMATION: Although some of the constraints on hydranth origin are known, viz.: (1) that hydranths form preferentially on stolon of two and three days of age, and (2) that some feedback control regulates the distribution of new hydranths, there is no evidence relating to the cellular basis of either of these constraints. That is, it does not now seem that hydranth formation is a consequence of the modes of proliferation and cell movement which characterize stolon extension and stolon branching.

A simple mechanism for hydranth genesis, and one that is consistent with what is know of cell movements during *Tubularia* regeneration, is the following. Inhibition of gastrodermal stickiness is effected by some cellular constituent of finite lifetime which has its origin in hydranths, and moves through the cells of the adjacent stolon. When new stolons form, sufficiently far from existing hydranths, and when the stolon tissues reach an age which renders them competent, gastrodermal cells, or their underlying mesoglea become sticky, thus attracting a concentration of gastroderm cells. Since "stickiness" is a relative measure of the adhesive qualities of both involved elements, an increase in the stickiness of the epitheliocytes, which could occur during starvation, could also increase the probability of hydranth formation, thus accounting for the initial increase in hydranth number recorded under some circumstances of starvation (FULTON, '62; BRAVERMAN, '71a). Attractive as this theory is, I have found no histological evidence to corroborate it, not, perhaps, because it does not occur, but rather because small accumulations of gastroderm in stolon, still bearing perisarc, would be indistinguishable from the usually observed distribution of gastroderm which appears very irregular. Therefore this aspect of colony morphogenesis, hydranth formation, will be left out of the model.

III. EPIDERMAL REGIONS OF LOCALIZED PROLIFERATION: Radioautographs show that between fifteen and twenty per cent of the epidermal cells of the stolon are synthesizing DNA during a one hour period. In newly formed stolon, distally to the first branch, this proliferation no doubt contributes to stolon extension. Proximal to the first branch cnidogenic and amoebogenic regions are formed. Presumably, the constant level of epidermal proliferation is channeled into these compartments in these older regions of the colony. The specific stimulus to this developmental rechanneling is presently unknown. Epidermal cells divide, and dividing become smaller. The daughter cells probably divide again. These cells become either amoebocytes or cnidocytes. In either case, at some stage in their differentiation they migrate from the proliferation region. Their movement in the stolon itself is most

likely at random, but a constraint is probably imposed which favors distal migration into and through the polyp.   In the polyp, the cnidocytes migrate to the tentacles, the amoebocytes, at each level, make a decision as to whether to cross the mesoglea or move upward.   This decision is apparently influenced by the numbers of gastrodermal digestive cells and gastrodermal gland cells at each level (A. BURNETT, personal communication with respect to Zymogen glands).   How this influence is mediated is presently unknown.

Proliferation regions, similar to those of the stolon, are present in the mid-polyp region.   How the rates of production of these two sources of epidermal cells are related and regulated is not known.

Adjacent epidermal cells are continously recruited into the proliferation regions of the stolon, and probably into that of the polyp, too (Fig. 24).
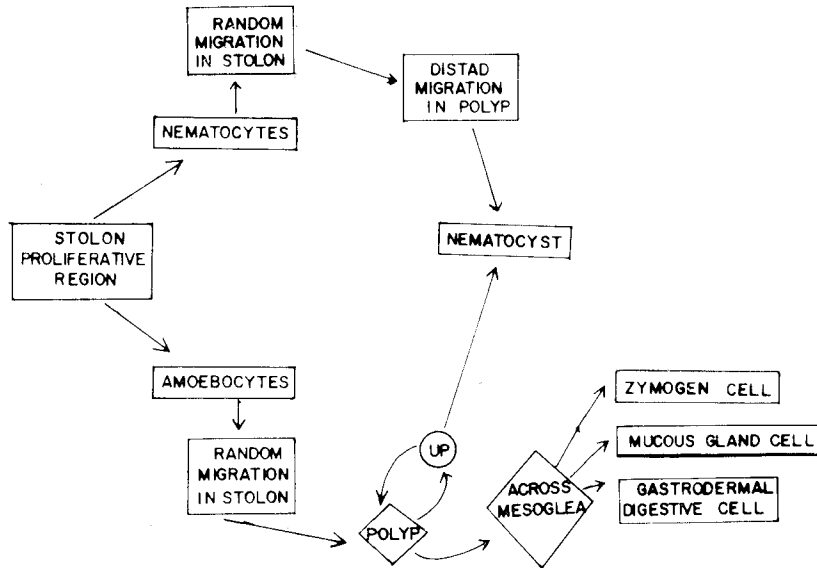

Fig. 24.   Proliferation and migration of amoebocytes and cnidocytes in *P. carnea*.

IV.   GASTRODERMAL DIGESTIVE CELLS: A. Of the Polyp.   The factors regulating the duration of residence of gastrodermal digestive cells in a polyp are presently unknown.   On one hand, thymidine incorporation does not occur in the polyp, but does occur in the gastrodermal cells of the stolon, so that it is likely that digestive cells ingesting nutriment lose their attachment to their polyp site to migrate to stolon regions and to initiate DNA synthesis.   On the other hand, the polyps of starved colonies are divested of gastrodermal digestive cells of the polyp.   The continued growth of stolons in starved colonies suggests that even during starvation epitheliocytes are available for stolon extension.   Thus it appears that gastrodermal cell release is effected at a constant rate, insensitive to nutritive conditions.

As with amoebocytes and cnidocytes, some force stimulates gastrodermal cells to move distally in polyps. This could be effected by reducing adhesive forces proximally relatively to those in the distal polyp gastroderm. Conceivably changes in adhesivity, and the distal attraction of amoebocytes and cnidocytes, might reflect the differences in electrical potential which ROSE ('63 a,b) has recently suggested play an important role in defining the circumstances under which regneration can occur in *Tubularia.*

Whatever the regulative mechanism, digestive cells lose their contact with the mesoglea and move into the hydroplasm as cnidocytes. There, they are carried passively until they come into contact with a region of the mesoglea that is sufficiently sticky. Such sticky regions exist, apparently, at the stolon tip, in newly forming hydranths, in the distal portion of all hydranths, and in some regions of the stolon.

B. Of the Stolon. Gastrodermal digestive cells of the stolon carry out polynucleotide synthesis, presumably synthesizing DNA. Presumably some form of proliferation occurs, despite repeated failure by a number of investigators to identify mitotic figures in cells of the stolon gastrodermis (HARGITT, '03; BILLARD, '04; HALE, '64). Cells are released from the stolon gastrodermis, under circumstances presently not understood, into the hydroplasm, where they join the epitheliocyte population (Fig. 25).
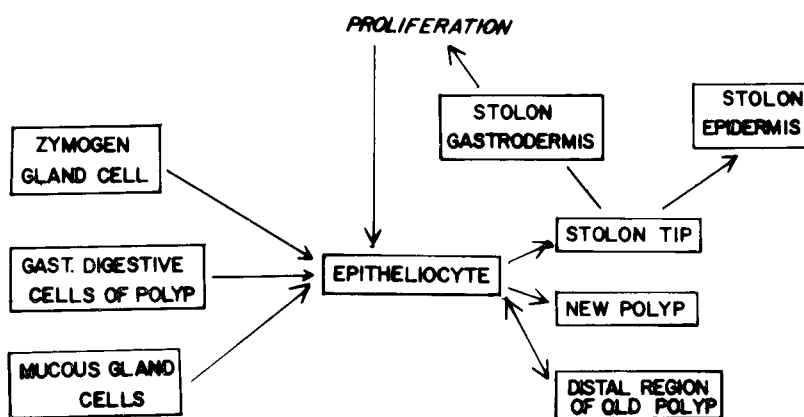

Fig. 25.   Epitheliocyte origin and migration in *P. carnea.*

V. ON INTERSTITIAL CELLS: In his book, The Germplasm (1892), August WEISSMAN spelled out his interpretation of the ROUX-WEISSMAN hypothesis that cell division could distribute the hereditary material equally according either to quantitative or qualitative standards.   He hypothesized, "Ontogeny depends on a gradual process of disintegration in the development of each individual ... Finally, if we neglect possible complications, only one kind of determinant remains in each cell, viz. that which has to control that particular cell or group of cells." (quoted in WILSON, '11).

If cells of the adult phenotype contain but a small fraction of the hereditary determiners, then it is necessary that a special group of cells be set apart as germ cells. WEISSMAN's ideas about the location and behavior of germ cells derive primarily from his study of hydroids, indeed, from his study of *Podocoryne carnea*. How WEISSMAN's theorizing distorted his observations is reviewed by BERRILL and LIU ('48). Similarly, the high degree of regeneration of which hydroids are capable was inconsistent with the severely restricted genetic potency of adult cells, unless one hypothesized that along with the germ line a second group of cells, embryonic in that they retained qualitatively complete nuclei, was set aside. These were the interstitial cells which ostensibly provided totipotent nuclei for regeneration. Even in 1911 WILSON identified the "quasi-metaphysical character which almost places it outside the sphere of legitimate scientific hypothesis" of WEISSMAN's theorizing (WILSON, '11, p. 407). He cites, in contradiction, the general observation that "in ordinary mitosis, the division of the chromatin is carried out with the most exact equality," and cites also numerous experiments in which complete embryos result from isolated blastomeres.

Experiments with hydroids by NORMANDIN ('60), ZWILLING ('63), HAYNES and BURNETT ('63), and by DIEHL ('68), leave no doubt that interstitial cells are not requisite to regeneration of whole animals. A remnant of the Weissmanian view that only I-cells are totipotent resides nevertheless in the belief that interstitial cells are a self-sustaining population, and that if they are selectively destroyed, they cannot be replaced from other cell types (BRIEN and RENIERS-DECOEN, '49; DIEHL and BURNETT, '64). Both experiments upon which this conclusion rests depend on an agent presumed to be selectively effective against interstitial cells: in one case radiation, in the other nitrogen mustard. But that assumption, in fact, assumes the conclusion, inasmuch as both are effective against *any* dividing cells. Thus not only I-cells but also any other cells which might divide to give rise to I-cells would be affected.

It has been necessary to consider the nature of the interstitial cell, and the controversy surrounding it, because in many respects the amoebocytic cells of *Podocoryne* resemble what has been described as interstitial cells. They are migratory, differentiate into a number of cell types, and reside in the epidermis. Amoebocytic cells of *Podocoryne* do not, however, resemble the nests of I-cells which are present in *Hydra* (Fig. 26). Nevertheless it seems likely that the role of I-cells and the role of amoebocytes is similar. Neither uniquely totipotent, nor a self-reproducing population, they are the proliferative and migratory phase of epidermal cells of the hydrozoan.

VI. SUMMARY AND CONCLUSIONS: Many assumptions have been utilized to construct this model. Naturally, a model constructed rigorously would be preferable. Nevertheless, the construction of an explicit model from inadequate data has the virtue of identifying clearly what is known, making assumptions explicit, and emphasizing what is unknown. Attention is thus focused on areas requiring further investigation (Fig. 27).

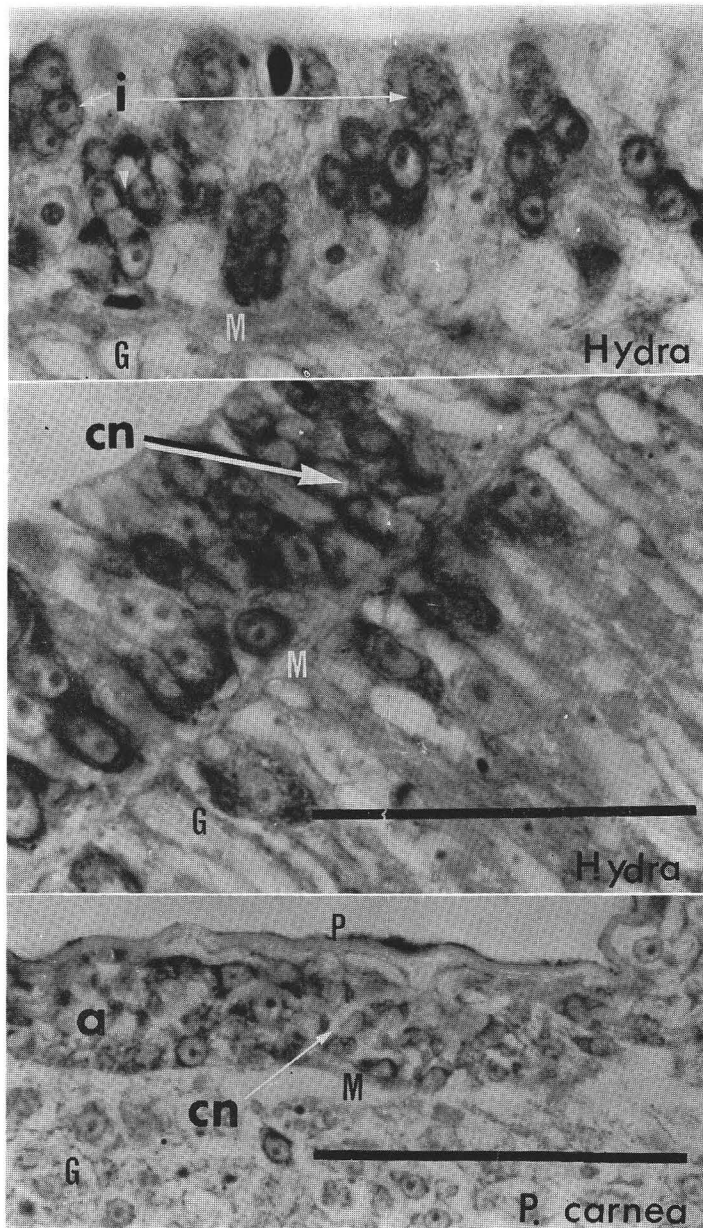In retrospect it seems that the perspective upon which this model depends is the

Fig. 26. "Interstitial cells" (i), top, and cnidocytes (cn), middle, in *Hydra*. Bottom, cnidocytes (cn) and amoebocytes (a) in *P. carnea*. I-cells are ovoid with a single nucleolus; amoebocytes are amoeboid and contain much dispersed chromatic material. Both preparations are stained with Toluidine blue. *Hydra* cells are much larger than those of *P. carnea*. The bar is 50 μ long.
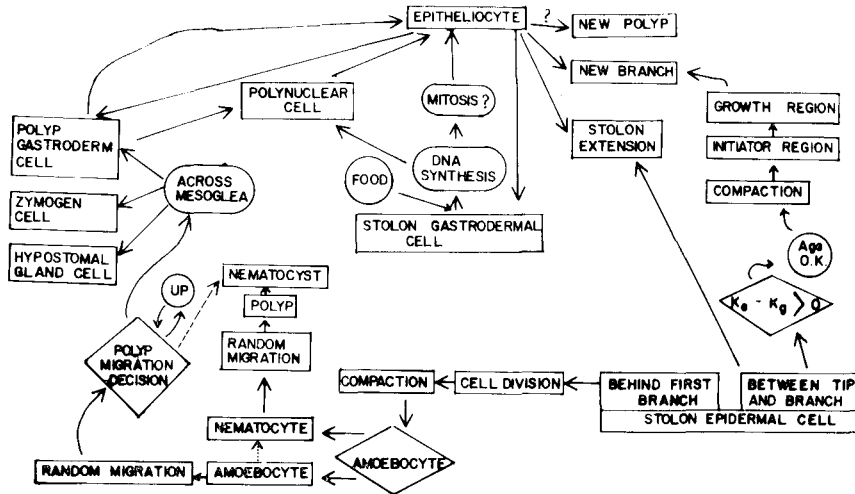
Fig. 27. The cellular basis of morphogenesis in *Podocoryne carnea*.

insight that the factors determining growth in the Hydrozoa are quite different from those determining form. The idea that form is a consequence of the location of proliferative regions is an attractive one for many reasons and one slow to die. Although data suggesting other bases of morphogenesis were produced simultaneously in a number of laboratories, I believe S. CLARKSON and L. WOLPERT ('67) were the first to make explicit the general implications for hydroid morphogenesis of the dependence of bud genesis on the initiation of proliferative regions. They, of course, concurred with a number of other investigators in negating this dependence.

The repertoire of cellular alternatives which account for morphogenesis and morphostasis in *Podocoryne* is extremely limited: cells can be epitheliocyte or amoebocyte; they can remain in place or they can be motile; they can make or dissolve perisarc. Certainly there is a broad spectrum of synthetic activities of which hydroid cells are capable, but these are not, for the larger part, activities which directly influence colony form.

Not all form generating activities have been considered in the model. The hydranth has been taken as a given, although I have attempted to show how cell proclivities can account for the steady state of the polyp. The origin of sexual persons has been ignored. The model is, by no means, exhaustive. My intent has been to demonstrate that colony morphogenesis and morphostasis is the consequence of a small number of variables in cell proclivities, that the building blocks out of which colony form is constructed are few.

To contend that the basis of morphogenesis is cellular seems to be a truism. It is, of course, true that animal architecture is constructed of cellular units. It is also a truism to add that any theory which purports to account for animal form must eventually be relevant to cellular characteristics. That the alternate expression of

cells that influence shape are few is not, however, a truism. I corroborate, in this study, the conclusions of GUSTAFSON and WOLPERT ('63) who identified many of the same cellular proclivities: adhesion or the lack of it, tendency to form pseudopods, as responsible for the morphogenesis of the sea urchin pluteus. It begins to appear that the complexity of animal form, in its phylogenetic diversity, is structured of a small number of cellular proclivities reflecting primitive cellular characteristics. The biochemical variety underlying these alternatives of cellular behavior may be diverse, but the repertoire of cell states which influence form is highly limited.

Acknowledgments

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DISCUSSION

MILLARD: I have two questions to ask, but first I would like to be quite clear that by hydroplasm is meant coelenteron.

BRAVERMAN: Yes.

MILLARD: 1) There are two species known (one *Eudendrium* and one *Hydractinia*) in which the manubrium is completely blocked by cells. Does Dr. BRAVERMAN think this is a transitory phase and of no specific value?
2) How do the cells moving through the hydroplasm avoid digestion?

BRAVERMAN: 1) Yes, transitory. Of value? I don't make value judgements regarding my material.
2) How do cells of our stomach avoid digestion?

WERNER: With respect to the paradox on that there have not been mitotic figures: did you preserve material over a 24 hour turn? Sometimes there are mitotic figures only in short time.

BRAVERMAN: Yes, but I found no period of increased mitosis.