REGENERATION IN THE GORGONIAN *PLEXAURA FLEXUOSA* (CNIDARIA, OCTOCORALLIA)

by

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ABSTRACT

Regenerative capability of *Plexaura flexuosa* is investigated in experiments which simulate the injuries caused by predation of the ovulid snail *Cyphoma gibbosum*: partial or total removal of the coenenchyme from the axial skeleton.

Regenerative growth of the coenenchyme is primarily induced by the possibility of linear extension of the axial epithelium; settlement of fleshy algae or e.g. *Millepora* (fire-coral) on the axial skeleton inhibits the recovery of the coenenchyme. Coenenchyme growth is accompanied by evagination of the polyp cavity wall and by increased mesoglea production. The cellular basis for regeneration lies in the migration capability and morphogenetic plasticity of at least two cell types of the coenenchyme epidermis: interstitial cells and "transitional" cells.

Repeated removal of re-grown coenenchyme causes a decrease of the polyp density followed by the formation of new polyps. These are formed by an extra-tentacular budding mechanism which has its primordia in the epidermis of the coenenchyme.

INTRODUCTION

The ovulid snail *Cyphoma gibbosum* Linnaeus, 1758, is a common predator on shallow-water octocorals. It browses on the surface of gorgonians, sometimes stripping branches completely of their coenenchyme. Kinzie (1970) estimated the rate of feeding by *Cyphoma* and suggested that, under normal circumstances, the rate of predation by *Cyphoma* is less than the rate of regeneration by the gorgonian. Heavy predation resulting from "subsocial" behaviour of the snails (Kinzie, 1970) and repeated grazing by *Cyphoma* on the same branches, may cause the destruction of complete colonies.

Cary (1914) investigated the regenerative abilities of some gorgonians by checking the recovery of colonies that had been subjected to various injuries. He examined histological sections of regenerating branches of *Antillogorgia* (as *Gorgonia*) *acerosa* (Pallas, 1766), but apart from his work the information on regenerative processes in octocorals is extremely scanty. The present study deals with the regenerative capacity of *Plexaura flexuosa* Lamouroux, 1821, following simulated predation by *Cyphoma* and places particular emphasis on the underlying morphogenetic events during regeneration.

MATERIAL AND METHODS

During the period March through December, 1975, a series of regeneration experiments was carried out with *Plexaura flexuosa* (Cnidaria, Octocorallia), a common gorgonian on the shallow reaf platform of the Southwest coast of Curaçao, using SCUBA diving. Only the terminal branches of colonies measuring about 1 m in height and occurring at a depth of 4 to 6 m were used in the experiments (fig. 1).

Predation by *Cyphoma* was simulated by stripping branches of their coenenchyme. Fifteen branches belonging to one colony were subjected to the following treatments (3 branches per treatment): stripped subapically on one side and on both sides (fig. 2a and b), and stripped apically on one side and on both sides (fig. 2c and d). The coenenchyme was removed over a distance of 4 cm, leaving the axial skeleton bare, except for one series of three branches where the innermost layer of spicules, the axial sheath, and the axial epithelium were left intact (fig. 2e). The branches were checked at regular time intervals and the rate of regeneration was determined.

For the purpose of histological examination of the regenerating material 32 branches belonging to one colony were stripped of their coenenchyme, 16 according to diagram b and 16 according to diagram d of fig. 2. Four regenerating branches of each type were sampled at 3, 7, 14, and 28 days after stripping. Four normal branches of the same colony

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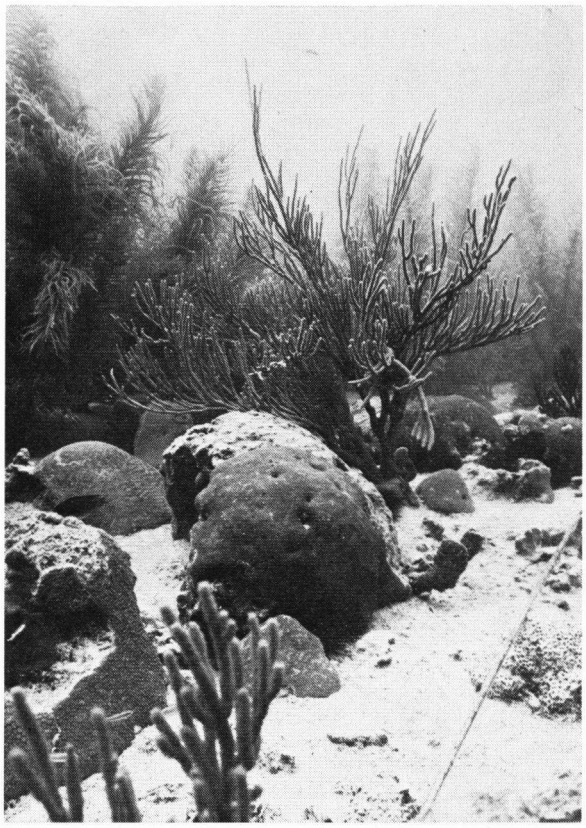

Fig. 1. Colony of *Plexaura flexuosa* on the shallow reef platform of Curaçao.

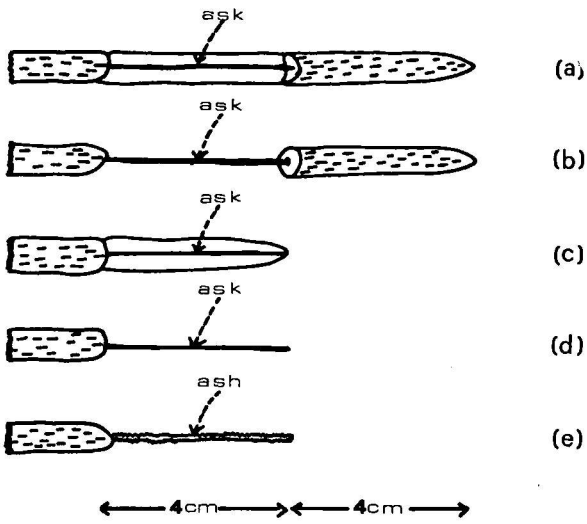

Fig. 2. Stripping of the coenenchyme to simulate predation by *Cyphoma*. (ask — axial skeleton, ash — axial sheath).

served as controls. The branches were cut off the parent colony, brought to the laboratory in sea water and fixed in Heidenhain's Susa fixative. They were left in ample amounts of fixative for 48 hours to ensure a thorough decalcification of the coenenchyme. At 12 hours intervals fresh fixative was added. Previous experience indicated that a weak decalcifying agent was needed when the tissues were being fixed; the site of the embedded spicules in the mesoglea is maintained and partly filled with residual matter. After fixation the branches were transferred to 70% ethanol. They were dehydrated through a graded series of ethanol, cleared in xylene and infiltrated in Paraplast (M.P. 56°-57°C). Sections of 5 μm were prepared, both parallel and transverse to the axial skeleton.

Sections were stained with Harris Hematoxylin (McClung Jones, 1950) counterstained in eosin Y and differentiated in 95% ethyl alcohol solution saturated with Orange G (Paulete-Vanrell, 1967). The differentiation in the Orange G alcoholic solution gives a light orange-brown color to the mesoglea of the outer coenenchyme and a yellow-brown color to the cytoplasm of the globular granular cells of the epidermis, enabling one to distinguish the latter cells in the mesoglea cell cords from the granular gastrodermal cells of the polyp cavity wall and of the solenia network. Methyl Green-Pyronin staining technique was employed to determine the presence of type I-cells (after Lender & Gabriel, 1960).

Microscopy was done with a Wild M 20 microscope and photomicrographs were taken with a Wild M Ka 1 camera attachment using Panatomic X film.

To investigate the regenerative capability of the coenenchyme and the fate of the polyps when a colony is subjected to repeated injury, 12 branches of one colony were treated as follows: the coenenchyme was removed from 4 to 8 cm and from 12 to 16 cm below the branch tip (fig. 3). The branches were re-stripped twice at 14 days intervals and samples were collected for histology at both one and two weeks after each stripping. Of an additional colony 12 branches underwent the same treatment but the branches were re-stripped three times at intervals of 9 to 11 days and three branches were collected 9 to 11 days after each stripping. The length and diameter of the regenerating isolated coenenchyme were measured and the total number of polyps on this coenenchyme was determined. Nineteen untreated branches of the same latter colony served as controls; the total number of polyps on 4 cm of coenenchyme length (the part 8 to 12 cm below the branch tip) was determined.

ACKNOWLEDGEMENTS

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RESULTS

In situ observations of regeneration

The branches that were subjected to simulated predation by *Cyphoma* show a remarkable similarity in their recovery according to observations in situ.

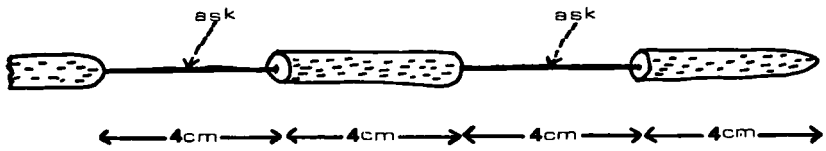


Fig. 3. Stripping of the coenenchyme to investigate regeneration of isolated coenenchyme tissue. (Abbreviations as in fig. 2).

Already three days after the stripping healing of the cut surfaces is taking place. Seven days after the stripping the linear coenenchyme growth averages about 0.9 cm in the apically and sub-apically stripped branches (fig. 2d, e and b). Comparable measurements could not be taken where the coenenchyme was removed on one side of the branch only (fig. 2a and c), because regeneration in those branches is the result of both linear extension of the coenenchyme and an inward folding of the edges of the cut surface. Fourteen days after the stripping the coenenchyme has completely covered the barren areas of all branches with the exception of those branches with the axial sheath left intact. The regeneration of the latter clearly lags behind, as only about 50% of the stripped surface has been re-covered by the coenenchyme. Only after about 8 weeks the stripped areas are completely covered, but the upper 1.5 cm of the branches still shows clearly the bluish-purple spicules of the axial sheath and is devoid of polyps. All other branches seem to have completely regenerated within 4 weeks after stripping. The only visible traces of the injury are a slight thickening of the tissue or a faint bluish color — due to spicules of the axial sheath — at the encounter site of the coenenchyme.

Regenerative growth of coenenchyme

The main morphogenetic events during the regeneration as observed from examination of the sections proved to be of the same nature in the apical regeneration and in the subapical regeneration. Therefore we will limit the discussion of the results to the regeneration of the subapical parts.

Three days after the denudation of the axial skeleton we observed that the "proximal" and "distal" regions facing the bare axis had grown an average length of 1 to 1.5 mm, respectively. Both growing regions assume a cone-shaped form, the cones themselves measuring 4 to 6 mm in length. The bases of the cones are continuous with the cylindrical coenenchyme. Therefore we will call the coenenchyme cone together with the adjoining cylindrical coenenchyme the "regenerating front". The cone-shaped form of the regenerating front is maintained as long as 12 days after the start of the experiments. The cones represent the healed site of the wounds caused during stripping of the coenenchyme. Depending on the place where the cut was made polyps were observed to occur almost up to the apex of the regenerating front. Often they were damaged by the denudation of

Fig. 4. Photomicrograph of epidermis of normal coenenchyme. 1000 ×.

Figs. 5, 6. Photomicrographs of group of interstitial cells in the inner coenenchyme. 1000 ×.

Fig. 7. Photomicrograph of general view of the outer coenenchyme and of a polyp sectioned at the level of the distal part of the siphonoglyph. 40 ×

Fig. 8. Photomicrograph of general view of the outer coenenchyme and of a polyp showing the anatomical changes in the polyp cavity wall during regeneration. 40 ×.

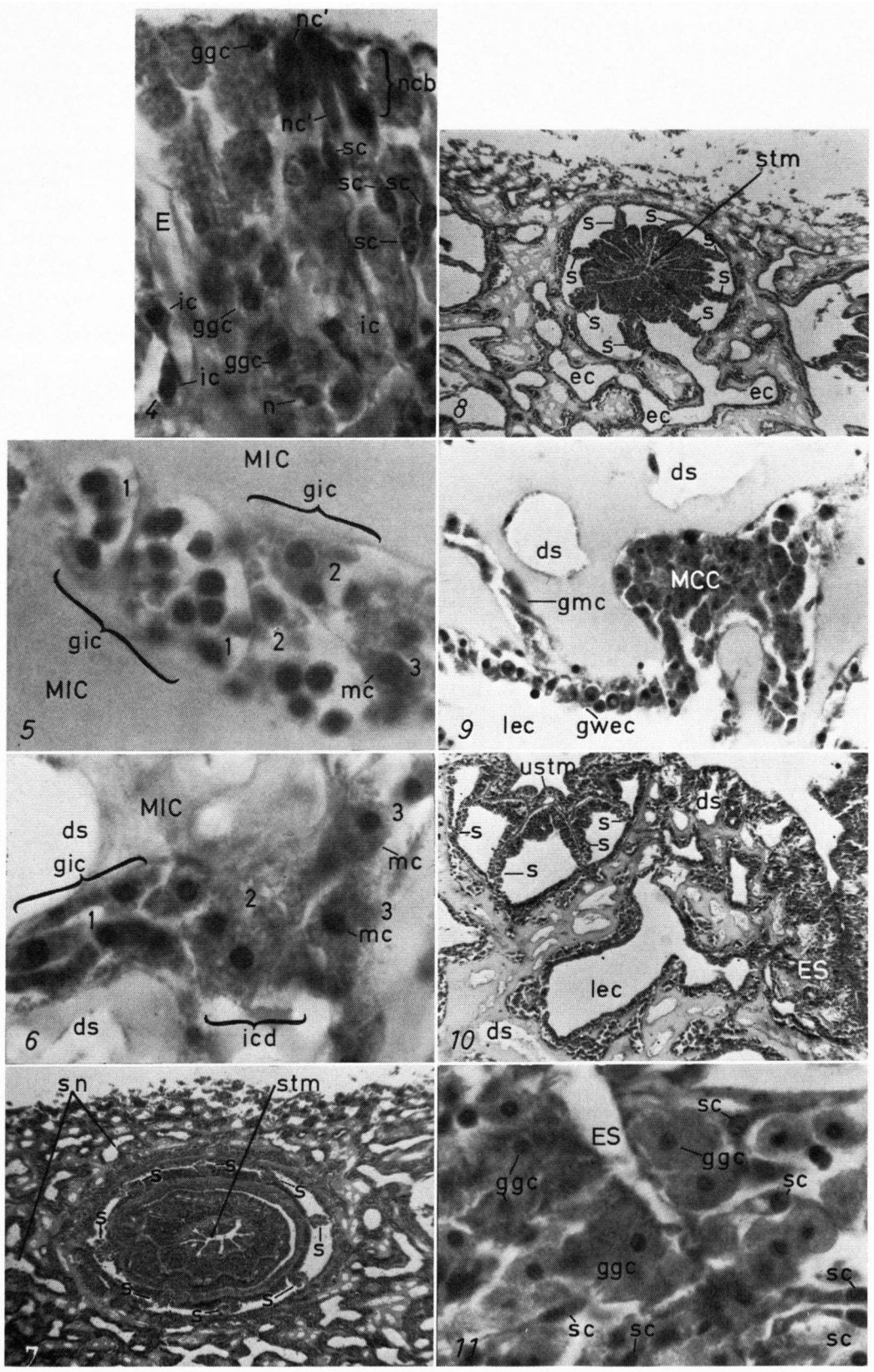
Fig. 9. Photomicrograph of outer coenenchyme showing the close contact between the evaginated chamber wall and a mesoglea cell cord. 400 ×.

Fig. 10. Photomicrograph of area bordering the epidermis scar showing modification of the microscopic anatomy of one polyp. 40 ×.

Fig. 11. Photomicrograph of the epidermis scar. Note that the scar is a deep invagination of epidermis epithelial zones after the encounter of two regenerating fronts. 1000 ×.

All: Hematoxylin-Eosin-Orange G staining.

Abbreviations: ds = decalcified spicule; E = epidermis; ec = evaginated chamber; ES = epidermis scar; ggc = globular granular cell; gic = group of interstitial cells; gmc = group of mesoglea cells; gwec = gastrodermis wall of evaginated chamber; ic = interstitial cell; icd = interstitial cell differentiation; lec = lumen of evaginated chamber; mc = mesoglea cell; MCC = mesoglea cell cord; MIC = mesoglea of inner coenenchyme; n = nematoblast; nc' = nematoblast capsule; ncb = nematoblast cell in battery; s = septum; sc = supporting cell; sn = solenia network; stm = stomodaeum; ustm = unfolded stomodaeum; 1, 2 and 3 = the series of events leading to mesoglea cell production.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the axial skeleton and just a few remnants were observed.

In preparations of the normal epidermis of the coenenchyme we observed a three-layered feature. The distal layer, the epithelial zone, is made up of long cylindric supporting cells (20 to 30 μm) with a plate-like external surface. The opposite side is a long cylinder that gradually narrows and branches at its proximal end. In few cases these cytoplasm extensions reach the underlying mesoglea. The nucleus is ovoid, rather voluminous when compared with other cells (4 to 7 μm in diameter) and with dense chromatin granules. Among the supporting cells are scattered globular cells, with small compact nuclei and granular cytoplasm lightly stained with eosin and sharply stained with Orange G turning to a yellow-brown color. Nematocyst cells bearing capsules sharply stained with eosin in batteries of six to ten (fig. 4). The intermediate layer is made up of thin branched cytoplasm cells with voluminous nuclei that resemble the nuclei of supporting cells, bearing a small nucleolus. These cells represent a transitional cell type between interstitial cells and supporting cells. They originate from dividing interstitial cells of the subepithelial zone. They loose the nucleolus when differentiating into supporting cells and are non-dividing. Therefore we will call them "transitional cells". The proximal layer, the subepithelial zone, is composed of globular granular cells like the ones scattered among the supporting cells in the epithelial zone, nematoblasts and interstitial cells. The nematoblasts have small reniform nuclei while the interstitial cells have massive nuclei varying from 3 to 6 μm in diameter and acidophilic cytoplasm.

The wound is healed by the migration of the epidermis cells of the coenenchyme. The epidermis migration structurally changes toward the anterior limits of the regenerating fronts. Over the posterior half of the regenerating front the epidermis stays structurally intact, except for the reduction of its thickness — from about 60 μm to about 30 μm — and more cohesive attachment of cell elements. The supporting cells migrate as well as the other cells, the latter more likely capable of amoeboid movements. Nevertheless there is an increase of the number of globular granular cells and interstitial cells in the subepithelial zone in relation to the number of supporting cells. One may think the former cells are capable of faster migration and can be responsible for a draggling effect upon the normally fixed supporting cells.

The transitional cells and the interstitial cells show a unique migration behaviour in moving through the mesoglea. The transitional cells are usually migrating into the mesoglea of the outer coenenchyme. They are found separately, associated to the outer solenia network, or in groups. In the first case they insinuate themselves among the entoderm cells which line the small solenia and which are crowded with zooxanthellae. In the second case they form groups in the mesoglea just beneath the subepithelial zone of the epidermis. These groups of cells grow exclusively by draggling of additional transitional cells and they form small spheric chambers. The new chambers fuse with the small solenia whenever they occur close to them.

The interstitial cells migrate alone or with the globular granular cells in the mesoglea cell cords; in the normal coenenchyme the interstitial cells and the globular granular cells of the epidermis are sunk in the mesoglea and arranged in masses or cords. The independent kinetic behaviour of interstitial cells differs from the migration behaviour of transitional cells; the former wander as deep as the mesoglea of the inner coenenchyme in greater numbers than the transitional cells and they form groups of as many as 15 cells. After the formation of groups we observed two mechanisms of differentiation, both having the production of mesoglea cells as the ultimate result. One pattern is the direct differentiation of the interstitial cells into mesoglea cells by the decrease of the nucleus-cytoplasmic ratio, by granulation of the cytoplasm and by individualization through the production of a feeble basophilic matrix (fig. 5). Another pattern takes place in two stages. First the cells form a network by means of a delicate cytoplasmic expansion. Small spaces are left between the meshes of cytoplasm network while the enclosing mesoglea looses its homogeneity. Again a basophilic matrix is laid in the spaces pushing the cells apart. Individualization is followed by differentiation (fig. 6). Both patterns suggest an

efficient mesoglea producing mechanism. It is rather difficult to evaluate the bulk increase of the mesoglea if one considers that the cell migration has been accompanied by a general increase of the intercellular matrix. Chapman (1966) reported similar difficulties in quantifying the mesoglea production of the developing ephyra larvae of the jelly-fish *Aurelia*.

The polyps present in the posterior half of the regenerating front underwent modifications of their microscopic anatomy. In sections transverse to the skeleton plane we observed that the distal limit of the polyp cavity wall is slightly projecting from the coenenchyme, as a result of the rapid migration of epidermis elements. In the distal regions the polyp cavity wall is also evaginated into the coenenchyme forming a system of branched chambers. The solenia network originates from proximal regions of the polyp cavity wall, at the level of the mesenteries. In sections parallel to the plane of the axial skeleton the normal polyp cavity wall is devoid of lateral expansions to the level of the distal part of the siphonoglyph (polyps in contracted condition).

The evaginated chambers differ from the solenia network in two aspects. They have a larger lumen (compare fig. 7 with fig. 8) and they are lined with a simple layer of entodermal cells of the multilayered polyp cavity wall lacking the abundance of zooxanthellae of the solenia.

Over the anterior half of the regenerating front the epidermis is replaced by an ectodermal layer solely made up of transitional cells, globular granular cells, interstitial cells and nematoblasts. The transitional cells are arranged in the distal part. They seem to colonize a cytoplasmic condensation originating from globular granular cells. Scattered among them are interstitial cells and nematoblasts. The proximal part is composed of globular granular cells with enlarged nuclei, interstitial cells and nematoblasts. Residual matter of decalcified spicules is found between the latter ectodermal elements, indicating that the mesoglea must have been broken down. Tardent (1962) had also observed that mesoglea was broken down for the distal shift of hydrocaulus entoderm during regeneration of the hydroid, *Tubularia larynx*.

At the apex of the regenerating front the ectoderm invaginates as a tube, giving rise to the axial epithelium (fig. 12). The interstitial cells form clusters in the most proximal part of the invaginated arch, being continuous with the germinal layer of the axial epithelium (fig. 12). The destiny of the globular granular cells and of the interstitial cells is uncertain. Methyl Green-Pyronin technique failed to reveal the extent of redifferentiation of such elements. We think that the redifferentiation of the ectoderm to axis epithelium cells is accomplished through a mesenchymal ground-phase. The globular granular cells seem to fuse together. The remaining ectoderm cells colonize the fixed part and differentiate.

Seven days after the denudation of the axial skeleton the regenerating fronts have grown on the average about 1 cm. The basic mechanisms observed during the previous phase are well established now. The independent migration of interstitial cells and transitional cells is still restricted to the regenerating front. On the other hand the evagination of the polyp cavity wall is increased. Polyps as far as 1.5 cm from the regenerating front have their polyp cavity walls evaginated into the coenenchyme. The growth of the evaginated chambers is increased by the fact that they are coming in close contact with the mesoglea cell cords (fig. 9). The globular granular cells of the latter undergo metaplasia assuming characteristics of entodermal cells. So they contribute to the growth of the evaginated chambers; they can directly line the evaginated chambers or the mesoglea cell cords can become hollow extensions of the evaginated chambers. We were able to estimate the added growth by the cavitation of the mesoglea cell cords since the latter lack zooxanthellae. We figured an average size increase of about 1/5 of the lumen of the evaginated chambers. We believe the increase to be proportional to the size and depth of the mesoglea cell cords.

The two regenerating fronts encounter fourteen days after the denudation of the skeleton. The regions in which the two fronts meet is swollen and marked by a ring-like epidermis scar. After the encounter the epidermis of the coenenchyme still migrates, which results in a great supply of ectodermal elements into this area. The encounter is followed by the rapid fusion of the ectodermal

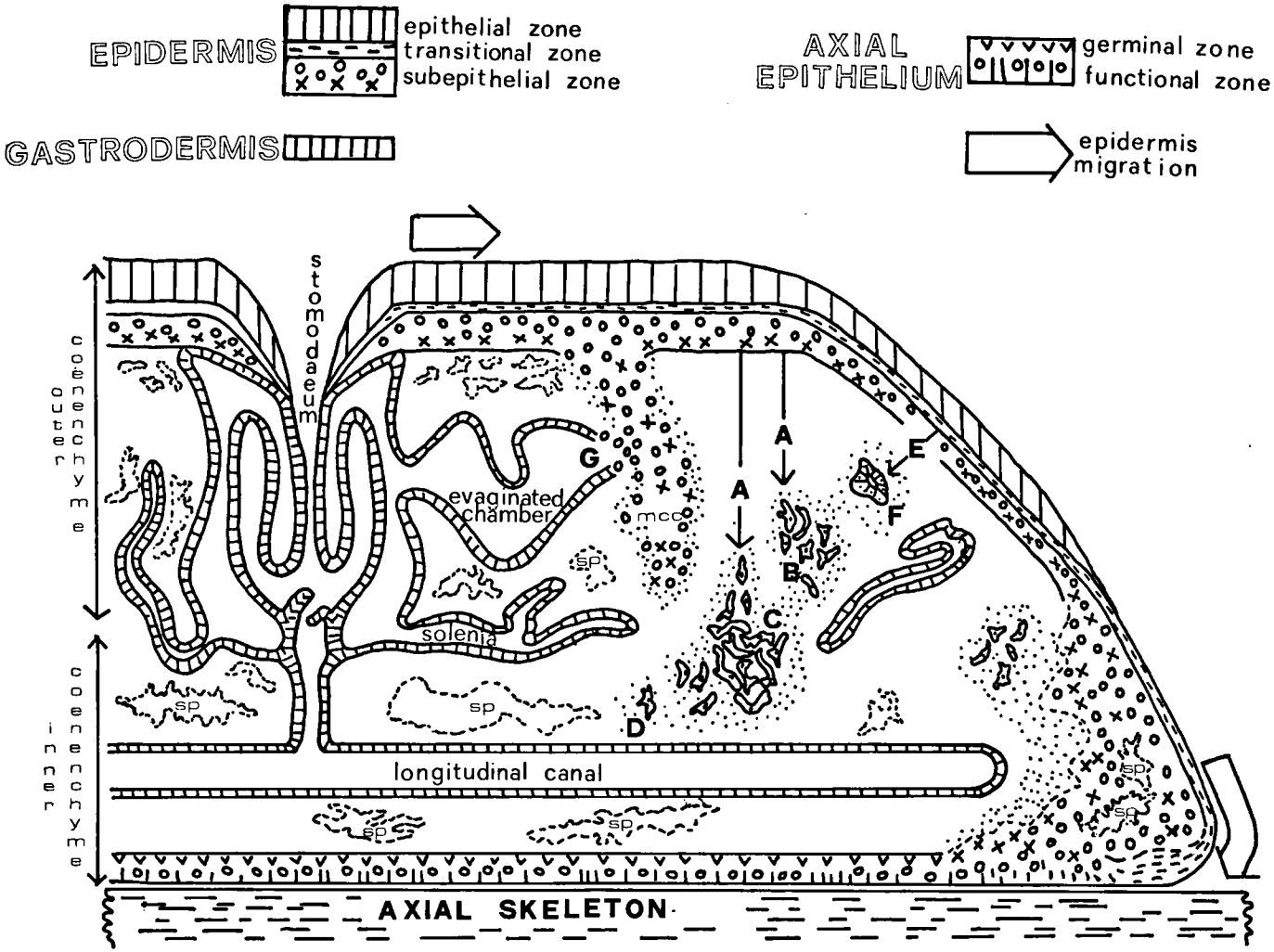

Fig. 12. Diagram of a longitudinal section through ½ of a regenerating front and its adjoining coenenchyme. A — interstitial cells from the epidermis subepithelial zone migrate through the mesoglea; B — cells form groups and differentiate; C — cells form a net system with small spaces; D — cells are pushed apart by the increase of basophilic matrix produced within the spaces; E — transitional cells wander through the mesoglea of the outer coenenchyme; F — groups of transitional cells form spheric chambers which can connect with the outer solenia network; G — evaginated polyp cavity wall reaches a mesoglea cell cord, globular granular cells of mesoglea cell cord can insert the wall of the new gastrodermal chambers.
sp = spicule; mcc = mesoglea cell cord.

layers next to the axis. The transitional cells and the interstitial cells must rapidly differentiate into mesoglea cells or undergo cytolysis since they are absent at this level. We are inclined to adopt the first hypothesis since narrow strands of basophilic mesoglea are observed to be highly populated with cells. The axial epithelium is the first tissue to regain its normal structure. Indications of its fast linear growth are still found in its multilayered germinal zone — around 5 layers of germinal cells.

The scar in the outer coenenchyme is a deep invagination of the epidermis (fig. 10). The epidermis epithelial zones of the encountering fronts maintain their organization for a long extent of the invagination (fig. 11). On the other hand the remaining epidermis cells migrate in all directions undergoing multiple redifferentiation. The polyps bordering the epidermis scar underwent almost complete modification of their anatomy. The column unfolds with the stretching of the polyp cavity during evagination of its wall. Nevertheless the septa remained unharmed (fig. 10).

The first linkage of the solenia network occurs between the evaginated chambers. The transitional cells of the epidermis differentiate into gastroderm supporting cells contributing to the connections between solenia. Thick mesoglea strands are reformed after the establishment of an integrated solenia system. The bimodal re-differentiation of interstitial cells into mesoglea cells accompanied with the production of intercellular matrix ensures a rapid replacement of the mesoglea.

Fourteen days after the encounter of the regenerating fronts only the strongly basophilic nature of the mesoglea in the outer coenenchyme testifies to the site in which the regenerative processes occurred. A swollen condition of the coenenchyme is no longer observed.

Polyp formation

Histological examination of regenerating branches that were subjected to repeated stripping indicate that the morphogenetic processes as described above for the growth of the coenenchyme continue to occur. The solenia network of the isolated coenenchyme appears to be gradually replaced by mesoglea layers highly populated with cell elements, resulting in a striking decrease in width of the coenenchyme. The proximal wall of the polyp cavity of the pre-existing polyps is almost in contact with the axial epithelium, and longitudinal canals are observed in regions corresponding to the outer coenenchyme of normal conditions.

As early as seven days after the second stripping we found that new polyps were being formed. They are formed by an extra-tentacular budding mechanism. In some areas the coenenchyme epidermis thickens and is closely associated with an outer solenium. The epidermis invaginates into the coenenchyme and its cells intrude into the associated solenium. Entoderm ridges are formed around the solenium plugged with epidermis cells. The oral aperture and the pharynx are formed when the "plug" of epidermis cells bursts at the coenenchyme surface. At the same time the definitive septa are formed by delamination of the entoderm ridges by thin layers of mesoglea.

The sites of polyp budding do not present any remarkable cell population. The thickening is uniformly developed with all types of epidermis cells but supporting cells of the epithelial zone; the thickening occurs in a distal-proximal gradient. Later stages of polyp development are characterized by the differentiation of epidermis cells into ciliated siphonoglyph cells.

The results of the polyps countings and measurements on repeatedly stripped branches are summarized in table I. The interval between stripping and sampling was determined such as to prevent the isolated coenenchyme from joining the neighbouring coenenchyme. The rate of linear extension of the coenenchyme decreases after each successive stripping as does the width of the branches. The formation of new polyps, as observed by microscopy, is not shown in the table since it was impossible to count the new polyps with any reliability. They appear as small whitish bumps in the coenenchyme and were very difficult to distinguish from accumulations of spicule debris. Therefore the pre-existing polyps, that is those of which a mouth opening was clearly visible, were selectively counted. The numbers are presented in such a way that the number of polyps counted on the isolated coenenchyme at the moment of sampling (the observed number of polyps) can easily be com-

pared with the number of polyps that was present on the 4 cm of coenenchyme length at the time of the preceding stripping. The latter, of course, is a figure calculated from the observed number of polyps of each preceding sample, or, in the case of the first sample, from the number of polyps in the controls. We have called this figure the expected number of polyps. The correspondence between the observed and the expected number of polyps supports our assumption that the existing polyps do not take part in any way in the regeneration process.

DISCUSSION

The cone-shaped form of the regenerating front, in which the apex is an anterior ectodermal layer close to the axial skeleton, suggests a mechanism primarily induced by the linear extension of the axial epithelium. The linear extension of the axial epithelium determines the growth of the coenenchyme. A smooth and clean substratum is a basic requirement for the effectiveness of the overall process. The coenenchyme is expected to grow whenever this condition is satisfied. Kinzie (1970) and Van 't Hof (in prep.), when fixing colonies of gorgonians in plastic (PVC) tubes, observed that the coenenchyme grew over the tubes. We found that the coenenchyme growth is inhibited when fleshy algae had settled on the axial skeleton and the coenenchyme growth decreases when the axial sheath had been left during stripping. The stretching of the coenenchyme during growth causes the evagination of the polyp cavity wall. To some extent the evagination of the polyp cavity wall represents an attempt to compensate for the slow rate of growth of the solenia network. Moreover we observed that even the mesoglea cell cords form cavities during this phase. The hollow architecture of the coenenchyme is a transitory condition since mesoglea production is enforced by the increased number of mesoglea cells and the hollow condition of the coenenchyme is gradually replaced by new strands of mesoglea.

The cellular basis for the growth of the coenenchyme lies in the migration capability and morphogenetic plasticity of at least two cell types of the epidermis: the interstitial cells and the transitional cells. Therefore one may think we are observing an epigenetic mechanism associated with a morphallactic mechanism: the interstitial cells give rise to mesoglea cells and to the cells of the axial epithelium germinal zone while the transitional cells give rise to gastrodermis cells and axial epithelium supporting cells. Nevertheless we have to consider that the transitional cells are an early stage of differentiation of interstitial cells into supporting cells of the epidermis epithelial zone.

The interstitial cells should represent a proliferous cell type during regeneration since they are differentiating into a large number of new cell elements. We did not observe the increase of mitotic activity of interstitial cells. Therefore we are forced to support the hypothesis that the interstitial cells are not a self-sustaining population and must also arise through de-differentiation of somatic cells. In this case the interstitial cells themselves are morphallactic in origin excluding the epigenetic transformation system. We think that the decrease of coenenchyme growth, as it was observed after repeated stripping, can be related to an exhaustion of necessary cell supply for regeneration.

Considering the remaining cell types of the epidermis we bring forward the suggestion that the supporting cells are irreversibly fixed elements. They did not undergo transformation at the moment of encounter between the regenerating fronts, leaving the encounter site marked with a deep scar-like epidermis evagination. Nematoblasts are also unlikely to de-differentiate. Nematoblasts were observed scattered among elements of the mesoglea cell cords during the last phases of regeneration following repeated stripping of the coenenchyme. The globular granular cells are more likely capable of dedifferentiation. They seem to fuse together in the apex of the regenerating front ectodermal layer creating a mesenchyme-like phase colonized with transitional cells and interstitial cells. We failed to follow the destiny of this ground-phase employing Methyl-Green Pyronin technique since the nuclei of the transitional cells and of the globular granular cells looked alike: voluminous with dispersed chromatin and devoid of a nucleolus. Moreover the granular cytoplasm phase was peculiarly positive to methyl-green, a fact not yet understood

considering the acidophilic nature of such granules. The destiny of the globular granular cells remains intriguing since no cell type provided with a granular cytoplasm was ever observed in the axial epithelium.

The present knowledge of the cellular basis of regeneration in coelenterates has been particularly focused on some members of the Hydrozoa and recent research has shown that regeneration can occur without the presence of interstitial cells (for references see Tardent, 1965). Burnett (1966) presented the hypothesis that two basic classes of cells should be taken in account among coelenterates: fixed epithelial cells and free cells of the interstitial type. Transformation can occur among each cell type and both must be present for regeneration. Moreover Summers & Haynes (1969) added embryological evidence to Burnett's hypothesis studying the development of the hydroid *Pennaria tiarella*. They showed that, in the planula, cells are segregated into an embryonic endodermal central mass (interstitial cells) and into two outer epithelia (ectoderm and endoblast). The interstitial cells migrate into the ectoderm only when the larva transforms into polyp. Braverman (1973) followed Burnett's idea limiting the hydroid cell repertoire to "epitheliocyte", "cnidocyte" and "amoebocyte". The three cell types can be fixed elements as well as motile elements. Ectodermal elements are supposed to move actively while endodermal elements are supposed to move passively. He suggests that interstitial cells are no more than an amoebocytic cell type occurring in the epidermis.

We believe Braverman's approach to the cell repertoire of hydroids is consistently true for *Plexaura flexuosa* considering the morphallactic origin of interstitial cells and their kinetic behaviour. Moreover we verified that free cell movements are restricted to cells originating from the epidermis. So far the gastrodermis cells of the solenia network do not seem to play any important rôle in regeneration of *P. flexuosa*. We must bear in mind that the primordia of new polyps are a product of epidermis cells. Therefore the coenenchyme epidermis is the tissue layer which provides the cellular basis for morphogenesis in *P. flexuosa*.

Our histological analysis did not indicate that any cell element of the polyps participates in the regeneration mechanism. Observations on the number of polyps of the regenerated coenenchyme after repeated stripping support this finding, even though some discrepancies occur between the observed and the expected number of polyps (table I). To explain these discrepancies we must take into account that the number of polyps present on the regenerating surface of the isolated coenen-

TABLE I

Coenenchyme growth and average number of polyps on isolated coenenchyme after repeated stripping.

Description of sample (no. of branches in parentheses)	Average length of the isolated coenenchyme (cm)	Average diameter of regenerated branch (cm)	Observed no. of polyps (average no. of polyps on total regenerated surface)	Expected no. of polyps (average no. of polyps on 4 cm of coenenchyme length at the time of the preceding stripping)
Regenerating branch 11 days after 1st stripping (3)	7.5	0.33	353	372 [1]
Regenerating branch 9 days after 2nd stripping (3)	5.8	0.28	173	188
Regenerating branch 10 days after 3rd stripping (3)	5.3	0.28	124	119
Regenerating branch 11 days after 4th stripping (2)	5.1	0.25	71	93

[1] This figure represents the number of polyps counted on 4 cm of coenenchyme of the controls (mean of 19 branches).

chyme at any time depends on the number of polyps present on the 4 cm length of coenenchyme left in between the two stripped areas. This implies that, after the first stripping, the number will vary with the variation in the number of polyps of the controls; during regeneration after repeated stripping the number of polyps on the regenerated surface depends also on the coenenchyme growth following the preceding stripping. The average total number of polyps per 4 cm of coenenchyme length of the controls is 372.1 $\pm$ 33.4 (min. 287, max. 416) of 19 branches. This variation may influence the average total number of polyps after each stripping, considering the small sample size in each step of our experiment (3 branches).

In our observations the polyp represents not only the individual of the system, but also the component which acquired the terminal condition (functional maturity through the absence of cell metaplasia capabilities; Zwilling, 1963). The coenenchyme represents the colony component through which the individuals are integrated and is also the component which, by its morphogenetic plasticity, enables the maintenance of the polyp terminal condition.

Whether or not successful regeneration will occur after injury or predation depends not only on the type of damage and the extent, but also on the possibility to replace tissues rapidly and effectively. Therefore the regenerative capability of a species has a distinct ecological importance. Our simulated predation experiments with *Plexaura flexuosa* show that this gorgonian is able to restore quickly a variety of injuries. Partial removal of the coenenchyme requires much more time for recovery than complete removal, for reasons explained above. On the other hand, complete removal of the coenenchyme, leaving the axial skeleton bare, provides an ideal opportunity for fleshy algae and for planulae of e.g. the fire-coral, *Millepora*, to settle. This competition inhibits regrowth of the coenenchyme over the damaged areas and may eventually result in a complete destruction of the colony. Our experiments indicated that the settlement of algae on the axial skeleton is directly related to the time during which the substratum is "available": settlement by algae was only observed after repeated stripping of the coenenchyme when the axial skeleton remained bare for more than two weeks. Observations on natural predation by *Cyphoma* support the findings of our regeneration experiment involving repeated stripping. Colonies were found in which *Cyphoma* were actively feeding on some branches, while other branches in the same colony showed a markedly decreased width of the coenenchyme, the axial skeleton denuded of the coenenchyme, and in still others the axial skeleton is covered with fleshy algae. We conclude that predation by *Cyphoma* in itself does not normally cause mortality in gorgonians, but that colonies are destroyed due to competition following intensive repeated predation by *Cyphoma*.

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