5. DNA Synthesis in A. formosa

5.1 Introduction

In the previous chapter, it was demonstrated that diel expansion and contraction in the polyps of A. formosa occurs as a result of reorganisation in the tissue layers of the tentacles and the inner body wall. Reorientation of the cells of the inner body wall for a distance of approximately 70μm from the apex of the trabecular tines produced a three- to four-fold longitudinal extension of the tissue envelope around the tine tips. It might therefore be expected that the maximal extension of the envelope is of the order of 210-280μm.

Oliver (1979) reports that growth rates in white tipped branches of A. formosa under aquarium conditions are invariably higher than corresponding rates in the field. Thus, whilst branches of colonies in the field (Nelly Bay) extended 3.7mm per 30 days (i.e. 123μm per day) in July, 1979, similar branches in the aquarium extended 4.6mm per 30 days (i.e. 153μm per day). In either case, however, it is clear that the maximum observed expansion capacity of the tissues of A. formosa over the trabecular tines could not accommodate much more than one day's skeletal growth.

As was reported in section 4.5.3, the tissues of Acropora are anchored in place by the skeleton and cannot move upwards. Furthermore, the tissues do not appear stretched when the polyp retracts shortly before dawn (Section 4.3.3). It would appear, therefore, that in order to accommodate noctural extension, cell division in the branch tips must keep pace with skeletal growth. This section examines the evidence for cell division in the axial polyps of A. formosa.

Three techniques were used to obtain information on patterns of DNA synthesis (the prelude to cell division). The first was the 5-bromodeoxyuridine/Hoechst 33258 technique. Hoechst 33258 is a fluorescent dye which fluoresces more effectively when attached to adenenine-thymine linkages in DNA as compared to DNA in

which the thymine has been replaced by its analogue uracil. Incubation of tissues in 5-bromodeoxyuridine allows substitution of the analogue for thymidine in cells undergoing DNA replication. Subsequent staining of such tissues with Hoechst 33258 therefore produces greatly reduced fluorescence in comparison with that obtained in non-dividing cells (Latt, 1973). Hoechst 33258 alone has been used previously to locate regions of constitutive heterochromatin or DNA replication in metaphase chromosomes of mammalian cells (Hilwig and Gropp, 1972). However, Latt (1973) developed the BrdU/Hoechst 33258 technique as a high resolution fluorometric alternative to autoradiography. It has been used to differentiate human and mouse chromosomes in hybrid cells (Lin, Latt and Davidson, 1974), but, as far as the author is aware, has never been applied to the study of DNA synthesis in coelenterates.

The other techniques used were based on observations of the incorporation of ^{3}H-thymidine into actively replicating DNA using autoradiography or scintillation counting. Autoradiography has previously been applied to the study of patterns of cell proliferation in coelenterates by Campbell (1965, 1967, a,b,c; see also David and Campbell, 1972; and review by Webster, 1971), who used it in studies on freshwater species of Hydra. Similar studies were carried out on a marine hydroid Podocoryne carnea by Braverman (1969, 1971), and Braverman and Schrandt (1969). The only published work describing ^{3}H-thymidine incorporation into corals, however, is that of Cheney (1973, 1974, 1975) who studied A. formosa, A. aspera, Pavona decussata and Pocillopora damicornis. A limited amount of autoradiography was performed on P. damicornis to examine the actual distribution of labelled cells (Cheney, 1973). For the most part, however, these studies used scintillation counting to measure levels of ^{3}H-thymidine in tissue digests following incubation of coral samples in isotope solutions. The level of isotope uptake was taken as a measure of cell proliferation thus providing an index of growth. The effects of a number of different treatments, including long-term maintenance in darkness and altered temperature regimes were investigated (Cheney, 1973, 1974). The stimulation of cell proliferation in tumour-like galls was also examined (Cheney, 1975).

There has, thus far, been no systematic use of the ^{3}H-thymidine techniques in studies of DNA synthesis in corals. The possible influences of diel, tidal or seasonal cycles has not been considered previously, nor have the effects of light versus dark regimes been compared. The relative usefulness of the above techniques was assessed, in this investigation, in a series of experiments designed to answer some of the above questions. DNA synthesis in A. formosa was examined on a diel basis at different times over a two-year period in order to discover whether diel or seasonal rhythms might be associated with cell division. The effect of maintenance in darkness was also assessed.

For the reader's convenience, graphically represented data is incorporated in the text, whilst the raw data are presented in tabular form in Appendix A. Photographic data are located, as usual, at the end of the results section.

5.2 Studies with the 5-bromodeoxyuridine/Hoechst 33258 technique

When unstained sections of branch tips of A. formosa are viewed under ultra-violet light, only the zooxanthellae and nematocysts fluoresce. The former appear red, due to the chlorophyll they contain, the latter blue. If such sections are then stained with Hoechst 33258 and examined in the same way, these colours persist whilst the rest of the coral tissue displays a brilliant green fluorescence. In sections taken from tips exposed to BrdU for one hour at hourly intervals over a 24 hour period prior to fixation and processing, samples taken between 0100h and 1000h (see Figure 67) cannot be distinguished from untreated controls and fluoresce uniformly. However, samples taken between 1100h and 2400h (see Figure 68) show a marked reduction in fluorescence over the tip region of the axial polyp. The tissues of the radial polyps do not exhibit this response; the entire coensarc proximal to the branch tip fluoresces brightly.

Reduction of fluorescence in axial polyps is most obvious in the extreme tip. It reduces gradually down the sides of the polyp and eventually merges with more proximal areas showing full fluorescence (Figure 68). The inner body wall also displays reduced fluorescence for approximately 100μm from the site of the apices of the trabecular tines (Figure 68). Inner body wall tissues deeper within the polyp reverted abruptly to full fluorescence (Figure 68).

The low intensity of the images obtained necessitated long exposure times, hence the rather poor quality of the photographs in Figures 67 and 68, which do not adequately capture the clarity of the original microscope images. Some "flare" was commonly present around the edge of the sections (Figure 68) which may have been due to the presence of microorganisms in the external mucous layer. This could be reduced by using lower concentrations of stain, but only at the expense of clarity of staining. Since this artefact did not interfere with overall interpretation, no preventative measures were taken. The use of the BrdU/Hoechst 33258 technique was not pursued beyond these preliminary experiments.

5.3 Measurement of uptake of ^{3}H-thymidine by scintillation counting

Preliminary experiments using this technique revealed that the level of radioactivity in the branch tips was significantly greater than that associated with either the zooxanthellae or micro-organisms in the incubation medium.

A typical set of results from a 24 hour experiment are displayed graphically in Figure 69. This shows removal of isotope from the incubation medium (Figure 69a) and uptake into the tissue per mg total protein (Figure 69b) on an hourly basis. Although there appears to be a diurnal fluctuation in the amount of isotope removed from the incubation medium by the branch ends (Figure 69a) this is not matched by similar fluctuations in the actual rate of uptake into the tissues (Figure 69 b-d). Uptake rates indicated by this method were, in fact, highly erratic, and no indication of a diel rhythm was ever obtained. Consequently, the use of this method was not pursued further. Possible reasons for the failure to demonstrate clear diel trends of ^{3}H-thymidine uptake are discussed in Section 5.5.

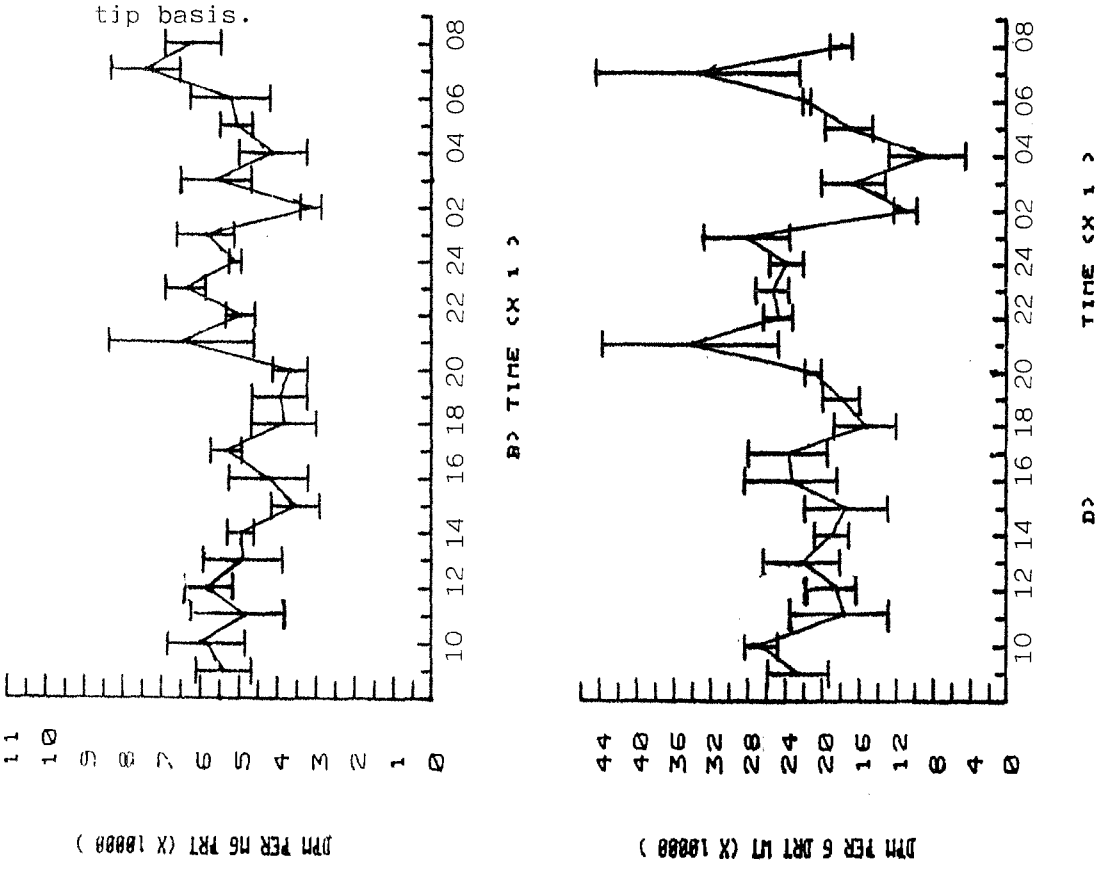
Figure 69(a-d): Uptake of ^{3}H-thymidine into white tipped branch ends of A. formosa (1 hour incubation) at different times over a 24 hour period. Vertical bars represent ± one standard error (n=3).

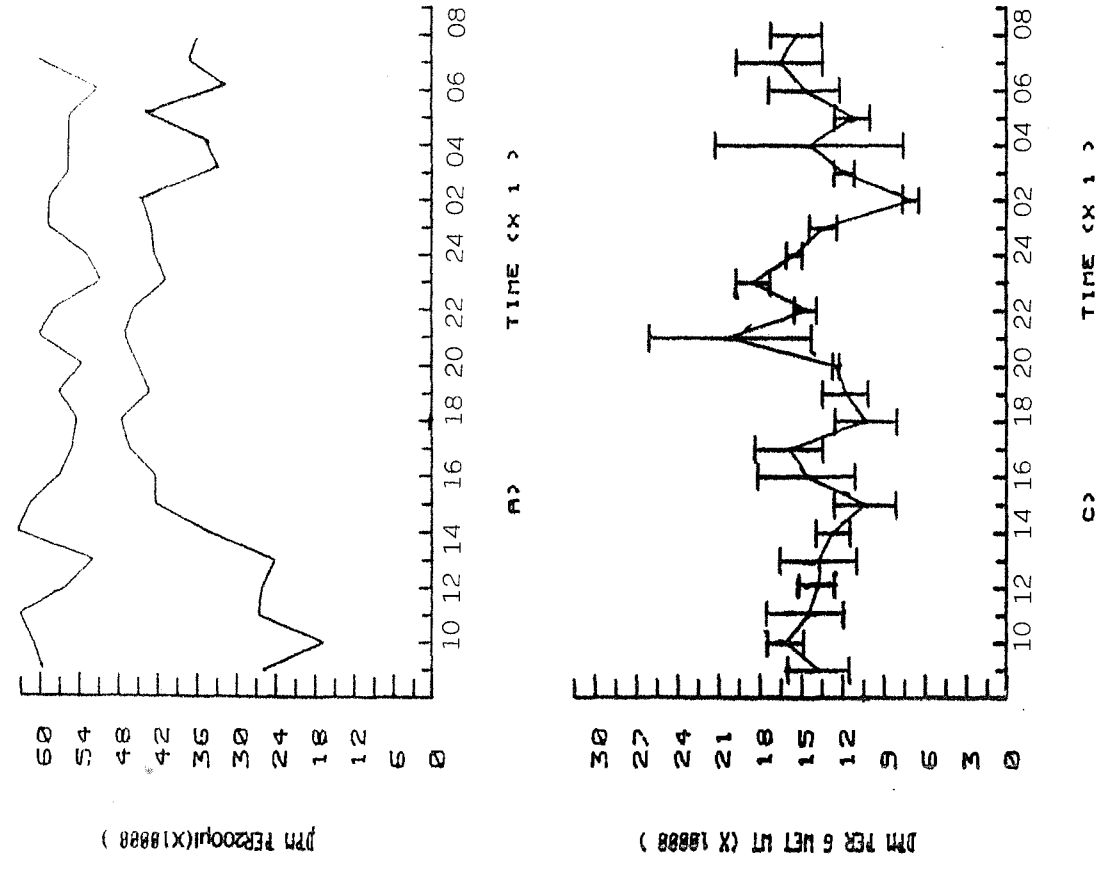
(a) Radioactivity in the pre-(upper curve) and post-(lower curve) incubation medium.

(b) Uptake of isotope into the tips expressed on a protein basis,

(c) Uptake of isotope into the tips expressed on a wet weight of skeleton basis,

(d) Uptake of isotope into the tips expressed on a dry weight of tip basis.





5.4 Measurement of incorporation of ^{3}H-thymidine by autoradiography

Autoradiography experiments yield sections showing dense accumulations of silver grains over cell nuclei (Figure 70). Labelling occurs most frequently in the tip region, but cell layers down the sides of the axial polyp are also labelled. The latter are frequently obscured by mucus precipitation (Figure 71) making routine counting impossible proximal to the extreme tip. Grains occur in both the epidermal and, to a lesser extent, the gastrodermal layer. Calicoblastic cells are also labelled occasionally, but no grains are ever found in association with the zooxanthellae.

The differences in the amount of labelling between the inner epithelial cell layers and the free body walls may not be a true reflection of the relative frequency of DNA replication in these epidermis tissues. It may simply reflect a diffusion gradient of isotope through the body wall which operates when the polyp mouth is closed (Cheney, 1973). Certainly in the present investigation, the mixing procedure used after the addition of isotope to the incubation medium caused both mouth closure and retraction of the tentacles for at least part of the incubation period. Furthermore, it was difficult to count sufficiently large areas of gastrodermal and calicoblastic layers at the tip of the polyp because of the intermittent contact of the skeleton with the body wall. For all these reasons, the analyses described here refer to epidermal counts only and, since it was not possible to distinguish the nuclei of interstitial, glandular and columnar supporting cells after labelling, only total epidermal cell counts are given.

The presence of the silver grains make it impossible to observe whether cells are actually undergoing mitosis at the time of fixation. It is worth noting, however, that examination of many hundreds of routine histological sections revealed only 20 cells which were actually dividing. All occurred in samples taken between sunset and 0200 hours and only two were gastrodermal cells. Mitotic figures, when they were seen, were always located in the outer two thirds of the epidermal cell layer (Figure 72).

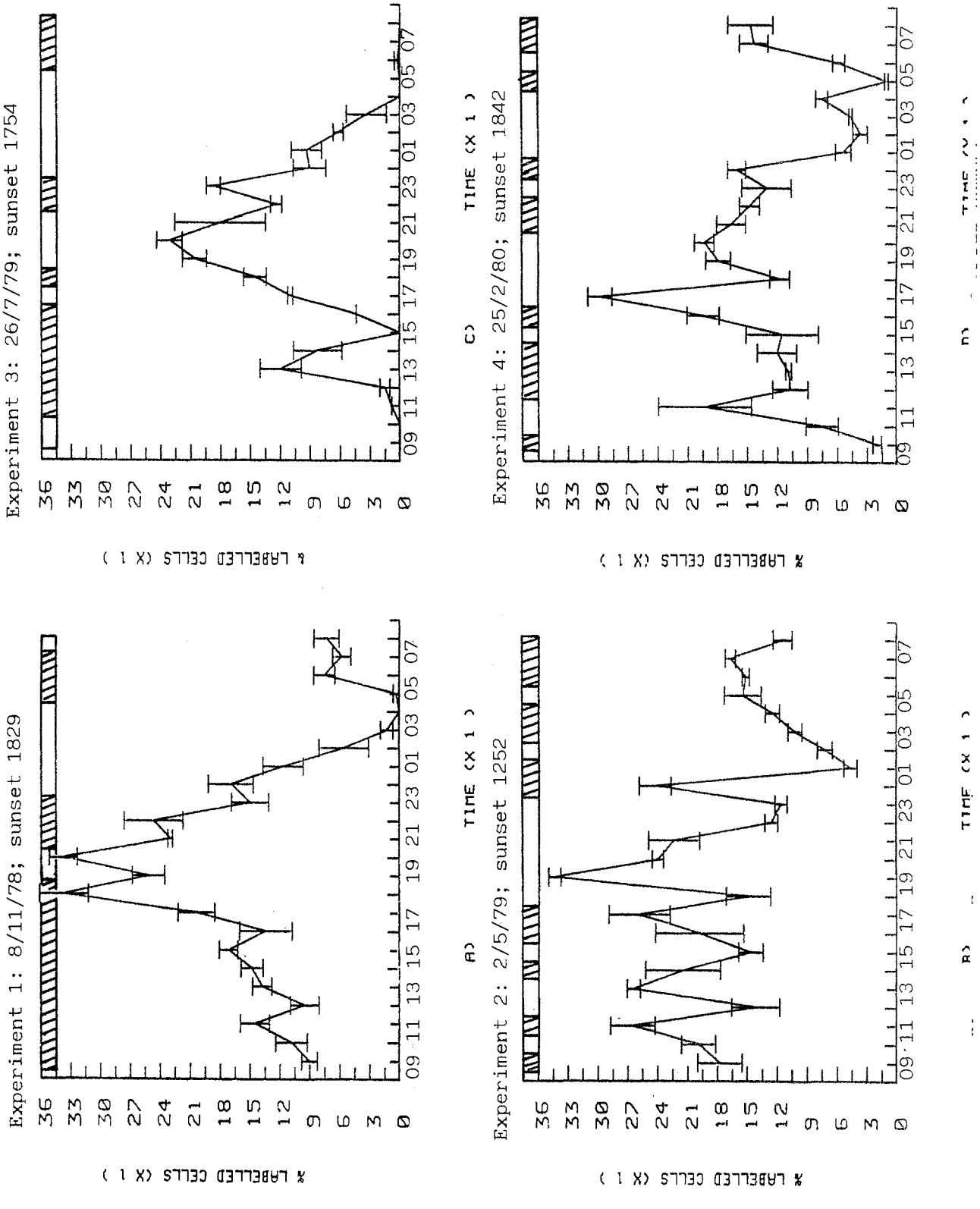
5.4.1 Diel patterns of ^{3}H-thymidine incorporation

Evidence for diel fluctuations in the rate of ^{3}H-thymidine incorporation was sought on four different occasions in experiments (conducted in February, May, July and November) covering a year's growth. The hourly mean % labelled cells (counts corrected for section thickness, Section 2.4.2.2) are recorded in Table I (Appendix A), and plotted against time of incubation in Figure 73 (a-d). It is clear that incorporation of ^{3}H-thymidine (and hence DNA synthesis) is most rapid in the early evening and, with the exception of experiment 2 (Figure 73b), relatively low in the early morning. This trend is most clearly evident in Experiment 3 (Figure 73c), where it appears there is a somewhat lower level of overall incorporation of isotope. The conformational state of the tissues around the trabecular tines of each sample is also recorded in Figure 73 (a-d). As was observed in the diel experiments reported in Section 4.3.3 (Figure 26), there was a general tendency for the polyps to be contracted during the day and expanded at night.

Chi square tests on the above data (Table I, Appendix A) show that the rate of incorporation of ^{3}H-thymidine into tips varies significantly ($p<0.001$) over the 24 hour period during which each experiment was conducted. However, the inter-sample variance is high whilst the within sample variance is very small. The samples were therefore pooled into groups in order to test the premise that the highest level of DNA synthesis occurs around sunset and that the lowest level of DNA synthesis occurs during the early hours of the morning. This premise is based on the observation of Muscatine and Porter (1977) who found that a peak of zooplankton capture in corals accompanies upward migration of the former around sunset. The products of digestion of food so captured might be expected to contribute precursors of DNA synthesis to the metabolism of the polyp. Moreover, the distension of the polyps during the early hours of the morning (Figure 29) could be partially assisted by an increase in volume created by cell division. For the above reasons, it was therefore hypothesised that a high level of DNA synthesis (i.e. ^{3}H-thymidine incorporation) occurs at or soon after sunset, and that a low level of DNA synthesis prevails in the early morning (the presumed time of maximum cell division).

Figure 73(a-d): Autoradiographic measurement of ^{3}H-thymidine incorporation into white tipped branch ends of A. formosa (1 hour incubation) at different times over a 24 hour period at various times of the year. Vertical bars represent ± one standard error (n=3).

KEY: contracted (as in Figure 22) expanded (as in Figure 23)

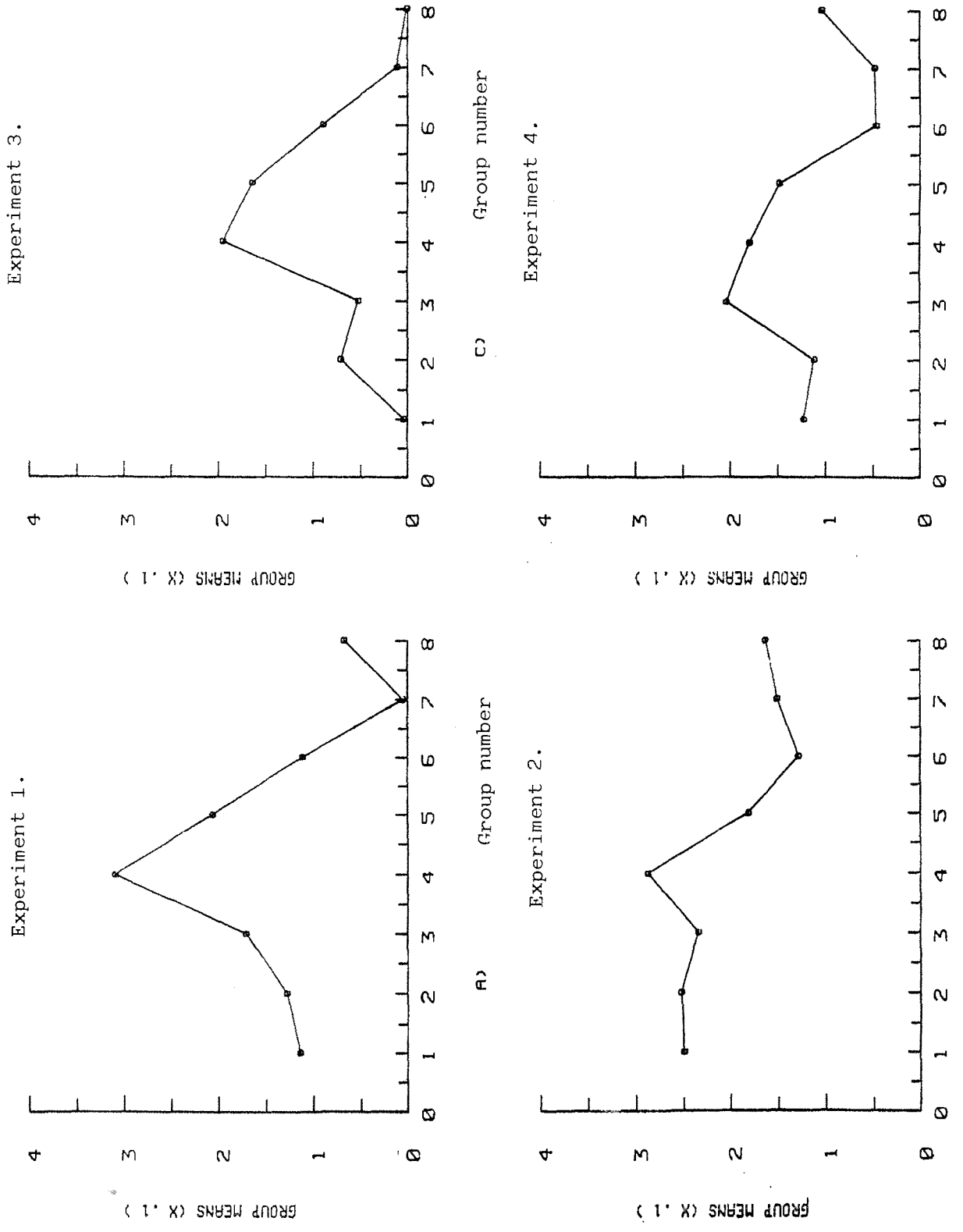


The diel sampling period was therefore divided into eight, three hourly periods (Table II, Appendix A). For every experiment, group 4 includes sunset $\pm$ one hour. Group 7 represents the early morning period which falls nine hours later and excludes the pre-dawn period when Muscatine and Porter (1977) report a second peak of feeding activity in response to the descent of the zooplankton. Examination of isotope uptake in group 4 versus group 7 allows the comparison of relative rates of DNA synthesis at times when feeding activity and polyp expansion respectively are maximal.

Analysis of variance of the grouped data (Table III, Appendix A) shows that there are significant differences between the group means of ^{3}H-thymidine incorporation in Experiments 1, 3 and 4 ($p<0.001$). Experiment 2 ($p>0.05$) is an exception. Comparison of isotope incorporation into tips during the sunset (group 4) and early morning (group 7) periods show that incorporation is consistently greater at sunset in all four experiments (Table II, Appendix A - paired comparisons using t-tests; Experiments 1 and 3 $p<0.001$, Experiments 2 and 4 $p<0.05$). Thus the indication of a diel response curve of ^{3}H-thymidine incorporation obtained from visual inspection of the graphs in Figure 73 and Figure 74 (which plots group mean % of labelled cells against group number) is supported, and the a priori assumption that most DNA synthesis occurs around sunset, with a lull in the early hours of the morning is confirmed.

Analysis of variance (Table IV, Appendix A) reveals no significant seasonal variation between the relative levels of ^{3}H-thymidine incorporation during the early morning and sunset periods respectively ($p>0.05$). However, the between experiments comparison of these values indicates significant ($p<0.05$) seasonal changes in the level of mean incorporation. The within group variance is high, making such interaction effects difficult to detect. Nevertheless, from this it appears that the highest rates of DNA synthesis occur in May and the lowest rates in July. Approximately equal rates of DNA replication are indicated for November and February.

Figure 74 (a-d): Mean percent labelled cells in grouped samples of white tipped branch ends of A. formosa following incubation with ^{3}H-thymidine (for details of arrangement of groups see text and Table II, Appendix A).



5.4.2 Effect of maintenance in light and darkness on diel patterns of ^{3}H-thymidine incorporation

Portions of four A. formosa colonies with a high proportion of white tipped branches were maintained in continuous darkness for three days. After this time, they appeared identical to four control specimens from the same colonies which had been maintained in natural illumination. After 11 days of light deprivation, the situation was considerably changed. One of the specimens had died and the others were heavily bleached and had assumed a pale brown-pink colour.

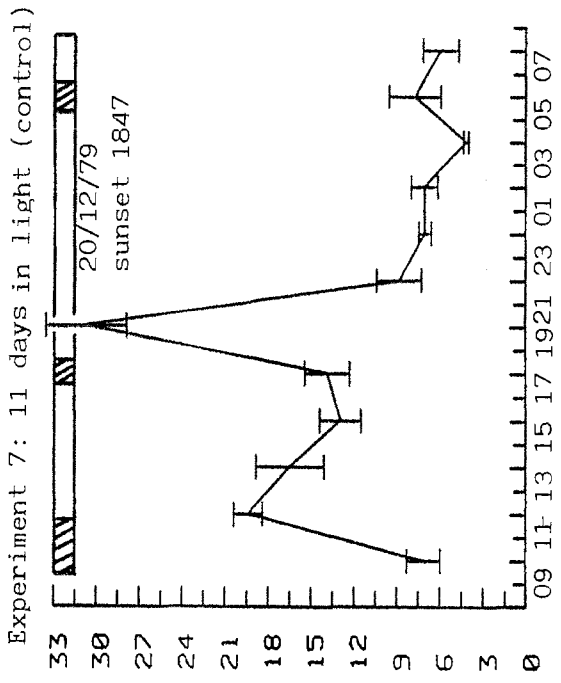
Branch ends were removed for autoradiography from one of the specimens from each group on the third and eleventh days of the experiment. Exposure to isotope occurred at alternate hourly intervals over a 24 hour period, in the same conditions of illumination under which the colony specimens had been previously maintained (i.e. dark incubation for dark maintained tips, and natural illumination for light maintained tips).

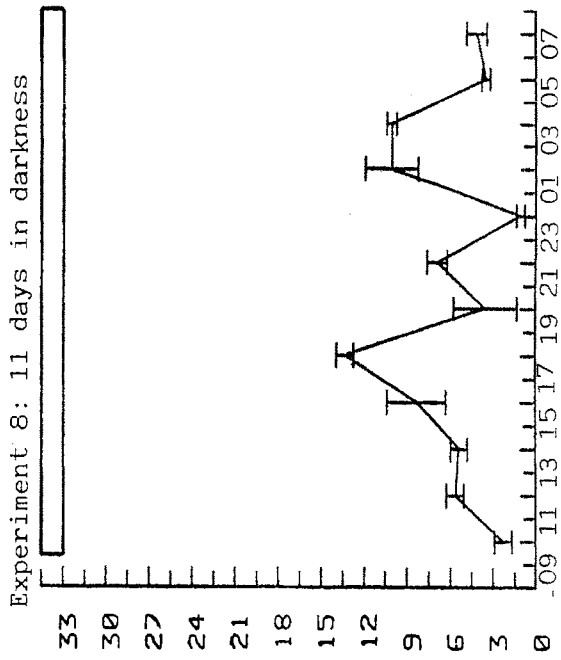
The hourly mean % labelled cells in sections of tips from colonies which had been in continuous darkness for 3 (Experiment 6) and 11 (Experiment 8) days are reported in Table V (Appendix A), along with their appropriate light controls (Experiments 5 and 7 respectively). These data were subject to similar statistical analyses as those applied to the diel incorporation experiments (Experiments 1-4, Section 5.4.1).

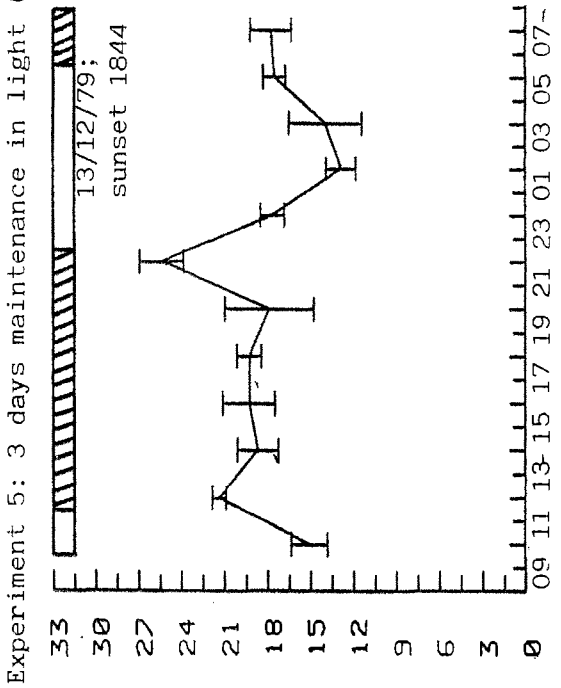
Chi square tests of the counts (Table V, Appendix A) show that the ratio of labelled to unlabelled cells fluctuates markedly over the experimental period in all four experiments ($p<0.001$). Graphs of the mean % labelled cells against time of day (Figure 75 a-d) show that both light controls (Figure 75 a and c) exhibit a pattern of ^{3}H-thymidine incorporation similar to that observed in experiments 1-4 (compare Figure 73 a-d). In contrast, isotope incorporation in dark held and incubated tips follows no such discernible pattern and the overall rate is markedly lower than in the light controls (see Figure 75 b and d).

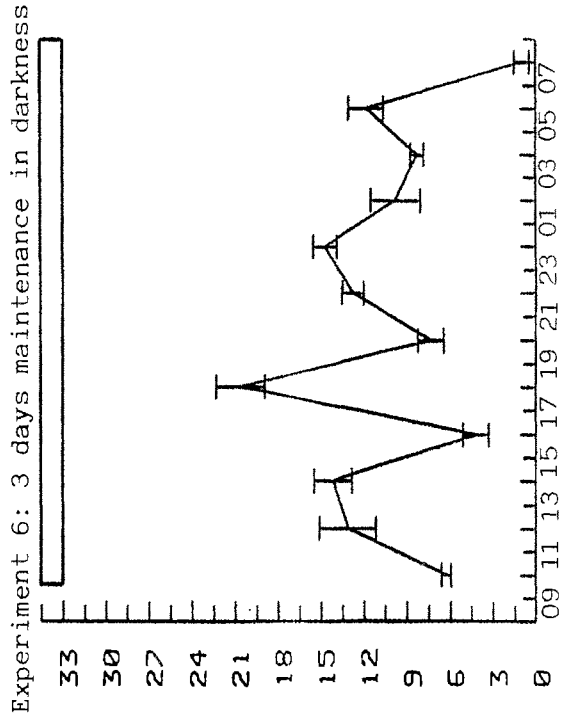
Figure 75(a-d): Autoradiographic measurement of ^{3}H-thymidine incorporation into branch ends of colonies of A. formosa (one hour incubation) at different times over a 24 hour period following maintenance of the colony in continuous darkness. Experiments 5 and 7 (graphs a and c): control tips maintained in natural illumination. Experiments 6 and 8 (graphs b and d): tips deprived of light for 3 and 11 days respectively. Vertical bars represent ± one standard error (n=3).

KEY: contracted (as in Figure 22)  expanded (as in Figure 23)









Interestingly, the diel expansion and contraction behaviour of the dark-held tips, as indicated by the conformation of the tissues around the trabecular tines (Figure 65), is markedly different from that of any other 24 hour experiment. Dark held tips (Experiments 6 and 8) are permanently expanded whereas the light controls (Experiments 5 and 7) display the normal pattern of daytime contraction and noctural expansion, except that the 11 daylight control tips (Experiment 7) tend to be expanded during the afternoon as well as at night (compare Figure 65c and Figure 26). This may have been due to the overcast weather conditions during the time the experiment was conducted (see Section 4.3.5).

Since tips were incubated with isotope for alternate hourly intervals rather than hourly, some adjustments had to be made to the grouping of the data. Each 24 hour period was divided into six four-hourly segments, where tips were exposed to ^{3}H-thymidine during the first and third hours only. The time of sunset $\pm$ 1 hour then falls in group 3, and the early morning period then corresponds to group 5 (Table VI, Appendix A).

The group mean % of labelled cells plotted against group number are shown in Figure 76 (a-d). Analysis of variance of the same data (Table VII, Appendix A) showed no significant differences between groups ($p>0.05$) in incorporation of isotope for any experiment. Moreover, t-tests (Table VI, Appendix A) between the sunset (group 3) and early morning periods (group 5) for each experiment do not reveal any significant differences in ^{3}H-thymidine incorporation ($p>0.05$), with the exception of experiment 7 ($p<0.05$).

Therefore, in these experiments, only number 7, the second light control, provides any statistically significant evidence for diel rhythmicity in ^{3}H-thymidine incorporation. The absence of evidence for this in experiment 5, the first light control, despite a similar pattern of isotope incorporation, may be related to the low level of total incorporation in these experiments as compared with those described in Figure 73 (a-d). In tips which were in continuous darkness, overall incorporation was reduced still further and showed no sign of a diel rhythm.

Figure 76(a-d): Mean percent labelled cells in grouped samples of branch ends of colonies of A. formosa incubated with ^{3}H-thymidine following maintenance in either natural illumination or continuous darkness (for details of arrangement of groups see text and Table VI, Appendix A).

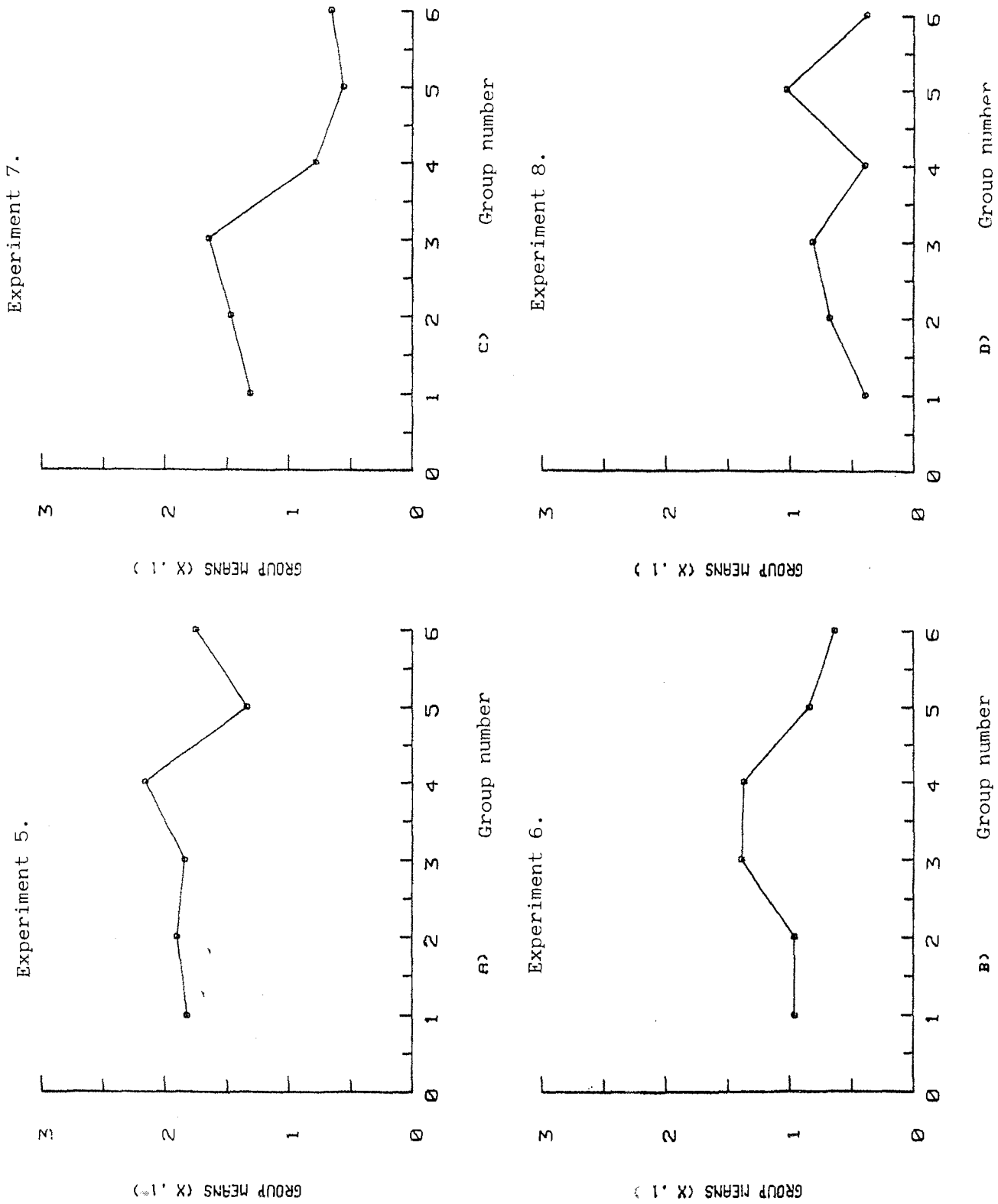


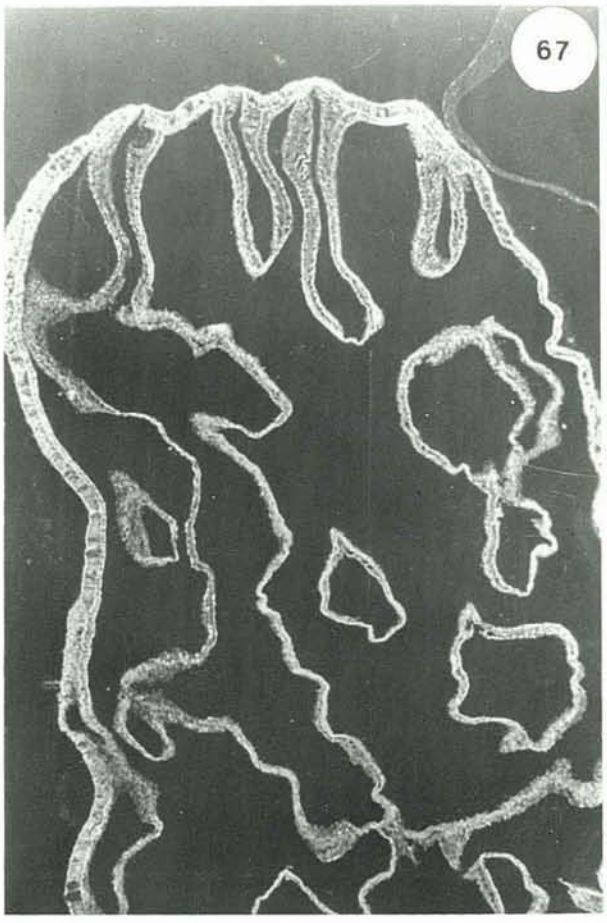
Figure 67: Longitudinal section of a white tipped branch end of A. formosa treated with the BrdU/Hoechst 33258 technique - 4 a.m. sample (x 75)

Half of a longitudinal section through the stomodeum of an axial corallite is shown; the mouth region is to the right. All the tissues display bright fluorescence.

Figure 68: Longitudinal section of a white tipped branch end of A. formosa treated with the BrdU/Hoechst 33258 technique - 4 p.m. sample (x 75).

Histological details are the same as in Figure 67. The outer body wall over the branch tip displays markedly less fluorescence than that in the proximal portion of the branch. Fluorescence in the inner body wall around the trabecular tines is also reduced for a distance of approximately 100μm down the sides of the trabecular tines. Some flare is present around the extreme edge of the sections which is probably due to uptake of the stain by micro-organisms in the superficial mucus layer.

N.B. The tissues of both of these sections exhibit the contracted conformation over the tips of the trabecular tines (c.f. Figure 22). On the basis that the average height of the outer body wall epidermis is 25μm, it is apparent that this conformation affects only a small part of the inner body wall. Roughly 70μm proximal to the extreme tip, the inner body wall displays its usual squamose form (c.f. Figure 15).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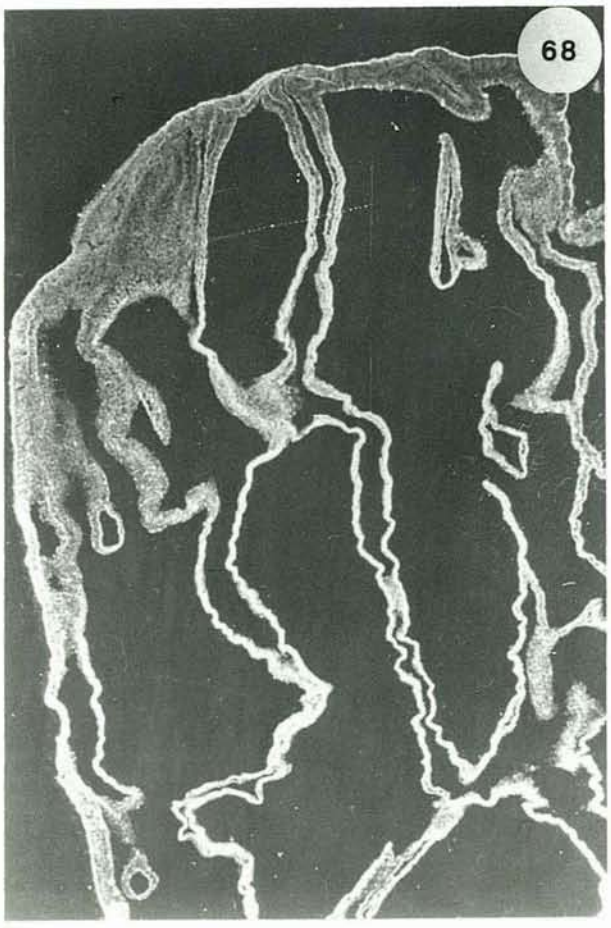
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Figure 70: Autoradiograph of a longitudinal section through the outer body wall over the extreme tip of an axial polyp following exposure of the intact tip to ^{3}H-thymidine. (x 1250).

Dense accumulations of silver grains (sg) are associated with the nuclei of some of the cells of the epidermis (e). The nuclei belonging to the non-staining mucous gland cell (mu_1) cannot be distinguished from those of the more predominant columnar supporting cells. Some of the nuclei of gastrodermal (g) nutritive cells are labelled, but no silver grains are located over the mesoglea.

Figure 71: Masking of silver grains by mucus deposition in an autoradiograph of a longitudinal section of the outer body wall proximal to a branch tip. (x 500).

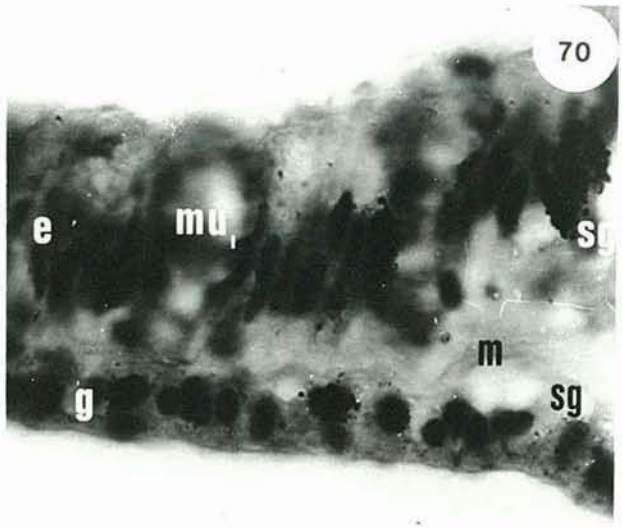
Comparison with Figure 70 shows that far more non-staining mucous gland cells (mu_1) are present in the proximal outer body wall than in the tip region. The contents of these cells have combined with the autoradiographic emulsion and with components of the staining solutions to produce a dense precipitation over the epidermis (e). The precipitate has similar staining properties to the exuded mucus (xmu) making labelled nuclei impossible to count. The mesoglea (m), gastrodermis (g) and coelenteron (ce) are not affected by this precipitation.

Figure 72: Mitotic cells in an axial polyp at the distal edge of the outer body wall epidermis. (x 500).

The epidermis contains 2 dividing cells (dc_1 and dc_2) in the plane of the photograph, a third is out of the focus to the right of dc_2. The first (dc_1) has just divided to produce two daughter cells, whilst the second (dc_2) is in prophase. The third (dc_3) is probably also in prophase.

The section is cut slightly obliquely, therefore the mesoglea (m) appears thicker than usual. The calicoblastic layer (c) does not contain mitotic figures.

ce - coelenteron; dc - dividing cell; e - epidermis; g - gastrodermis; m - mesoglea; mu - mucous gland cell (subscript denotes type); sg - silver grain; xmu - exuded mucus.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mu1
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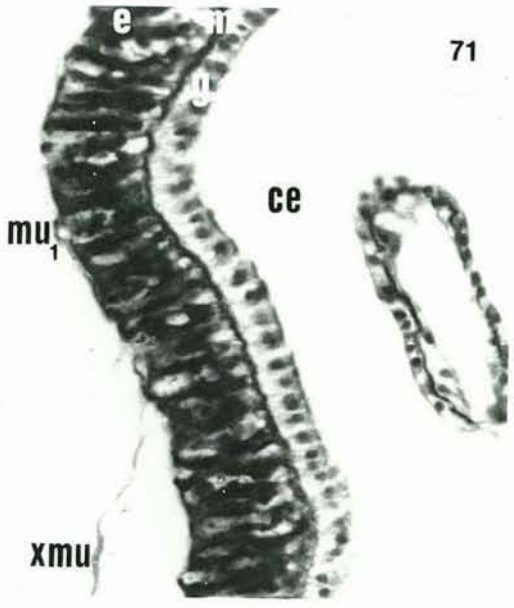
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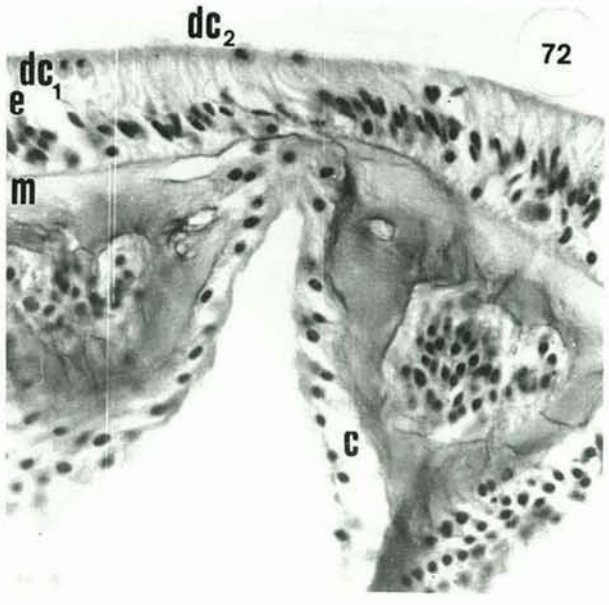
dc2
72
dc1
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5.5 Discussion.

Use of the BrdU/Hoechst 33258 technique allowed the identification of tissue regions where the majority of cells were either in the process of replication, or had recently replicated their DNA. However, the technique was not particularly useful for detailed work because of its essentially qualitative nature. It gave results requiring subjective assessment which could not be satisfactorily recorded or preserved. Nevertheless, the BrdU/-Hoechst 33258 technique clearly identified the extreme tip of the axial polyp as the major site of cell division in A. formosa and suggested that most DNA replication took place during the early afternoon and evening. Furthermore, the region which, with the exception of the tentacles, is most affected by diel expansion and contraction was shown (Section 4.3.3), to correspond closely with that responsible for the generation of new cells. The method also provided evidence that the cells of the epidermis and gastrodermis of the outer body wall replicated their DNA at the same time, justifying the assumption made in the autoradiographic studies that data from the epidermal layer was representative of the outer body wall as a whole.

The conclusion drawn from the BrdU technique results, that cell division in A. formosa is concentrated at branch tips was supported by the scintillation counting measurements of ^{3}H-thymidine uptake along the length of A. formosa and A. aspera branches made by Cheney (1973). These showed a rapid decrease in uptake with increasing distance from the axial corallite. Both findings are consistent with Goreau's (1961a) finding that calcification rates, and hence growth, were greatest at the tips of branches of A. cervicornis.

The extreme polarisation of growth displayed by A. formosa probably contributed largely to the erratic results obtained in this investigation when the scintillation counting technique was used to estimate ^{3}H-thymidine incorporation into tips. Cheney (1973, 1974) obtained meaningful result when he used the scintillation counting technique to examine uptake of ^{3}H-thymidine into branches of Pocillopora damicornis under a variety of

incubation conditions. However, autoradiographic studies of this species (Cheney, 1973) revealed that labelling between calices at the branch tips was uniform, that is, the tissues in adjacent polyps were dividing at the same rate. His data is consistent with what is known of the growth of P. damicornis where it appears that no one polyp at the branch end grows faster than any of the others (Wood Jones, 1907; Wainwright, 1963). Thus apical dominance such as is displayed so strongly by A. formosa does not occur in P. damicornis, and specific regions of cell division are apparently not necessary to accommodate skeletal extension in the latter. In A. formosa, sporadic uptake of ^{3}H-thymidine associated with cell division required to accommodate radial extension in the rest of the branch tip (which may or may not have included the formation of radial corallites) could well have masked any pattern of uptake in the axial corallite as measured in this investigation by scintillation counting.

There are two other potential sources of "noise" in the use of scintillation counting to measure isotope incorporation. The first is that the uptake of ^{3}H-thymidine may occur into pools rather than directly into nuclear material. Cheney (1974) attempted to eliminate this possibility by correlating isotope uptake with DNA concentration. He was unsuccessful, however, because the DNA content of the zooxanthellae, which did not take up ^{3}H-thymidine, could not be differentiated from that of the coral cells. Therefore the effect of such metabolic pools on measurements of this type are unknown at present.

The second potential source of variation concerns the micro-organisms in the mucus coating of the branch tip. These, along with the coral tissues, would have taken up isotope during the incubation period. Since colonies of A. formosa are known to exude copious quantities of mucus even when they are in an unstressed state (Crossland, Barnes and Borowitska, 1980), some of this labelled material would subsequently have been lost to the experimental system during the washing period. This removal could have accounted for some of the discrepancy between data based on the difference between loss of radio-

activity from the medium and uptake into the branch tissues. Autoradiography avoids both of the above problems, since incorporation of ^{3}H-thymidine into cells is measured directly.

The pattern of ^{3}H-thymidine incorporation demonstrated by autoradiography, suggests that there is a strong diel rhythmicity of DNA synthesis in the tip region of A. formosa branches. A peak of isotope incorporation occurs during the early evening feeding period (Muscatine and Porter, 1977), suggesting that products of digestion may contribute to DNA synthesis, whilst a depression of isotope incorporation occurs in the early hours of the morning.

DNA synthesis takes place during interphase and involves three distinct phases which have been well described by Mitchison (1971). Actual incorporation of ^{3}H-thymidine occurs only during the "S", or synthesis phase when replication of DNA takes place. This is preceded by the "G_1" phase and followed by "G_2". All of these phases and mitosis itself are of variable length.

Some clues concerning the length of some of the phases in A. formosa can be obtained from the present results. For example, very few dividing cells were seen during the course of this study (and Collins (1978) reports that he did not observe any in his studies of Acropora). Yet Oliver's (1979) parallel growth studies of A. formosa (see below) showed that similar colonies were actively growing at the time of the experiments described here, indicating that cell division must have been occurring. It would appear therefore that mitosis probably occurs very rapidly in A. formosa.

The distal location of epidermal cells undergoing mitosis as observed here has never been reported previously for any coelenterate. The significance of this positioning in A. formosa is not clear. Perhaps cells migrate to the surface of the cell layer to divide. Since mitotic figures when they were observed appeared only during or just following peak ^{3}H-thymidine incorporation, there is also some indication that epidermal cells

divide very quickly after replicating their DNA. Thus their G_2 phase is probably very short (although the actual number of observations is small). Unfortunately there are no other studies on corals with which these results can be compared.

It is interesting to compare the information on seasonal variations in DNA synthesis in A. formosa tips obtained from this study with the results of a field study of skeletal growth in the same species conducted in Nelly Bay during virtually the same period (Oliver, 1979). Oliver found that maximum extension of branches in A. formosa colonies occurred in mid-April (5.4mm per 30 days), with a minimum in mid-August (2.7mm per 30 days). Experiment 2 of the present investigation was conducted at the beginning of May, immediately after the maximum growth period. By mid-May Oliver found that branch extension rates had decreased to 4.0mm per 30 days, therefore skeletal extension during the period of Experiment 2 was presumably still quite large, somewhere between 5.4 and 4.0mm per 30 days. Significantly, the overall rate of incorporation of ^{3}H-thymidine at this time was considerably higher than in any other experiment. However, the diel pattern of isotope uptake was not as clear as in some of the other experiments, suggesting that the rate of cell division required to keep pace with extension at this time may have resulted in a reduction of diel rhythmicity in DNA synthesis.

In Experiment 3, which took place at the end of July, the converse seems to have occurred. There was less incorporation of ^{3}H-thymidine overall, and the diel rhythm was exaggerated, for the period of reduced nocturnal incorporation persisted well into the following morning. The time at which this relatively low level of DNA synthesis was recorded corresponds to a period close to the yearly growth minimum in August as reported by Oliver, (2.7mm per 30 days, exactly 50% of their extension rates in mid-April).

The absence of labelling associated with zooxanthellae in autoradiographic preparations from this study support Cheney's (1973, 1974) observations with P. damicornis that the algae do

not take up ^{3}H-thymidine. Previous autoradiographic studies with Fungia scutaria (Goreau et al, 1971) have also shown that the symbiotic algae do not incorporate ^{3}H-DL-leucine. One possible explanation is that the isotope molecules cannot penetrate the complex, multi-layered structure of the algal cell walls (see Taylor, 1968, 1973; Kevin et al, 1969; Singh and Mercer, 1976; Muscatine, 1973). Another is that these compounds may not have been metabolically useful to the autotrophic nutrition of the symbiont (see Yonge, 1968).

The use of autoradiography to determine the effect of maintenance in darkness on ^{3}H-thymidine incorporation gave much clearer results than those of Cheney (1974) based on scintillation counting of tissue digests from dark-held branch tips of P. damicornis. Cheney observed low rates of incorporation (on a dry weight basis) in specimens which were totally bleached after 6 weeks in darkness, but could not distinguish whether this was due to tissue loss resulting from a reduction in coral biomass or from the extrusion of zooxanthellae. Direct observation of the ratios of labelled to unlabelled cells by autoradiography as reported in this investigation eliminates this problem.

The results presented here show that maintenance of A. formosa branches in darkness was accompanied by the extrusion of zooxanthellae from the tissues, and disruption of the normal expansion and contraction behaviour of the polyps identical to that reported by Goreau (1959) in similarly treated colonies of Mancinia areolata and Occulina diffusa. In addition, continued darkness reduced the overall rate of DNA synthesis occurring in the branch tips, and was accompanied by a loss of diel rhythmicity in DNA replication. This is consistent with Cheney's (1974) conclusion that the presence of photosynthetically active zooxanthellae is as necessary to sustain high levels of DNA synthesis as it is to maintain maximal rates of calcification.

Both light control Experiments (Experiments 5 and 7) might have been expected to exhibit a clearer diel rhythm in ^{3}H-thymidine uptake. However, although the pattern of incorporation in

Experiment 5 followed the expected pattern, the differences in rates of incorporation were not significant. The second light control experiment (Experiment 7) did exhibit a distinct diel rhythm, even though the overall level of uptake was lower than in Experiment 5. This suggests that there was something unusual about the incubation conditions during Experiment 5. The most likely explanation is that the overcast weather conditions during the execution of this experiment influenced the pattern of isotope incorporation.

In terms of colony growth as a whole, the evidence from this study suggests that the overall rate of DNA synthesis in each branch tip is related to seasonal changes in skeletal extension rates. The diel pattern of ^{3}H-thymidine incorporation observed here for axial polyps suggests that cell division occurs at night, a time when the polyps are fully expanded and the tissues are lifted away from the growth points of the corallite, allowing uninterrupted growth when extension rates are maximal (see Section 4.5.3.2). The size of the tissue envelope around the extending trabecular tines therefore increases at night, allowing the new skeletal accretion to be accommodated when the polyp retracts just before dawn. Thus, it appears that cell division in A. formosa is synchronised with polyp behaviour and skeletal growth, on both an annual and a daily basis. This synchrony ensures that tissue growth keeps pace with skeletal growth whilst, at the same time, minimising cellular constraints on skeletal extension.

6. Comparison of white and brown branch tips of A. formosa

6.1 Introduction

Macroscopic examination of colonies of A. formosa (section 3.2.1)reveals that branch tips may be white or brown depending upon whether or not they contain zooxanthellae. Measurement of rates of longitudinal extension in branch tips of A. palmata (Bak, 1976) and A. formosa (Oliver, 1979) has shown that white tips (defined as those tips which are markedly paler than the rest of the branch) undergo variable growth rates, whilst brown tips exhibit little or no longitudinal growth. Oliver (1979) examined the skeletons of brown tips of A. formosa by light microscopy and found that they are subject to increasing consolidation with age which he summarised as follows:-

(i) increased density of the skeleton caused by thickening of skeletal elements,

(ii) progressive "rounding off" of the profile of the apical calice caused by coenosteal growth,

(iii)encroachment of the lateral calices around the apical calice, together with reduction in size of the apical aperture.

Bak (1976) and Oliver (1979) concluded that browning and its associated depression of skeletal growth rates was a spontaneous, reversible process, but neither reported on the timing of the changes, nor on how the conversion from one state to the other was brought about.

Changes in the algal distribution of polyps of Lobophyllia hemplichii (Kawaguti, 1954) and Montastrea cavernosa (Lasker, 1979) have been correlated with alterations in normal patterns of expansion and contraction. However, the behavioural changes associated with browning in A. formosa have not thus far been examined, nor has the nature of calcium carbonate accretion which causes skeletal consolidation in brown tipped branches been determined. These questions are investigated in this section which also includes a detailed comparison of white and brown tips and their intermediaries. The investigation is based on morphological and histological examination of the coral tissues and scanning electron microscopy of the skeleton.

6.2 Results of examination of white and brown tips

6.2.1 Position of white and brown tips and behaviour of polyps

White tipped branches occur most frequently on the upper surface of A. formosa colonies. They are found almost without exception on branch ends which are growing directly towards the light. In contrast, brown tips tend to become more common towards the base of the colony, particularly in laterally oriented branches and in the interior of the colony (Figure 3).

The appearance and behaviour of polyps in white tipped branches of A. formosa has been described in detail in Section 4.3.5.1. Full expansion in such tips occurs only at night provided that they are healthy and actively growing. In brown tips (Figure 85), the tentacles of both radial and axial polyps contain zooxanthellae and are commonly well extended both during the day and the night. Brown axial polyps always have two complete cycles of six tentacles (Figure 85).

6.2.2 Distribution of zooxanthellae in white and brown tips

In white tipped A. formosa branches (Figure 77), the terminal portion of the axial polyp contains virtually no zooxanthellae. The tissue is transparent, and the axial corallite is clearly visible beneath it. The coenosarc is also transparent for some distance down the branch, and the coenosteum can be seen through it (Figure 77).

Macroscopically, the zooxanthellae appear as brown specs when they occur in low concentrations. In the distal tip region, they first appear in the intercostal spaces of newly formed radial polyps (Figure 77). Further down the branch, as radial corallites gradually become more mature, the presence of increasing numbers of algae renders the whole coenosarc progressively more opaque. Eventually, the coenosarc becomes a uniform red-brown colour, except where the tissue is supported on skeletal structures such as costae and trabecular tines. These points of contact exclude zooxanthellae, and so remain white. The lines over the costae (Figure 77) are straight, and show no evidence of skeletal elaboration.

Longitudinal sections from white tips treated with Liisberg's stain (Figure 78) confirm that the coenosarc of the axial polyp is virtually devoid of zooxanthellae for some distance down the branch. They also show that there are very few algae in the gastrodermis of the inner body wall.

In brown branch tips (Figure 79), the tissues, as can be judged from their colour, are uniformly well endowed with zooxanthellae, except where they rest on the skeleton. The pattern of colouration over the costae reflects a more complex underlying skeletal structure than that found in white tips (compare Figures 77 and 79). Longitudinal sections through the axial polyp of brown tipped branches stained with Liisberg's stain (Figure 80) show that zooxanthellae are present throughout the gastrodermis of both the inner and outer body walls.

The axial corallites of branches with brown tips are much more rounded than those with white tips. The distance between the extreme tip and the first circle of radial corallites is also markedly reduced (compare Figures 77 and 79). The radial corallites themselves all appear to be fully developed (Figure 79); there is no graduation of maturity with distance down the branch as observed in Section 3.2.2.

6.2.3 Skeletal differences between white and brown tips

Examination of skeletons from white and brown tips shows substantial differences in their structures which are consistent with the macroscopic observations reported in Section 6.2.2. In white tips (Figure 6) the skeleton is lightly calcified and growth points are conspicuous on the surface of the axial corallite, even at low magnification. At high magnification (Figure 82), the ends of the crystal fibres which make up the trabecular tines are clearly visible. The skeletal linkages in such tips appear to be fragile, and pairs of granulations, which signal the formation of new synapticulae (Wells, 1956), are frequently observed (Figure 82).

The skeleton of brown tips (Figure 81) is a much more heavily calcified structure. Substantial consolidation of growth is apparent between the synapticulothecal rings, the growth points appear blunted, and the synapticulae are much thicker. The appearance of the surface of the skeleton in brown tips is also quite different (compare Figures 82 and 83). It is much smoother; and although some granulations are present, there are no signs of the formation of new synapticulae. Examination of a broken trabecular tine (Figure 84) reveals that the entire structure is, in fact, overlaid with secondary deposition. As would be expected from Gladfelter's (1982) description of skeletal accretion in A. cervicornis (Section 4.5.4.2), the centre of the tine appears unstructured, and highly organised crystal fibres radiate outwards from it. However, around the exterior of the tine, the surface irregularities have been smoothed out by the deposition of a thin layer of stereome.

There are considerable differences between the position and degree of development of the first circle of radial corallites in the two types of tip. In white tips (Figure 6b), the first layer of radial corallites arises some distance down the branch, and exhibits only rudimentary development. Overall, the coenosteum shows little increase in skeletal elaboration proximally. In brown tips (Figure 81b), however, the first layer of radial corallites is frequently located almost at the tip of the branch and their corallite walls usually contain at least two synapticulothecal rings. Some coenosteal elaboration is present between the members of the second circle of radial corallites in white tips (Figure 81b), but in brown tips the entire coenosteum, up to and including the extreme tip of the axial corallite, can become heavily ornamented (Figure 79).

6.3 Intermediate patterns of distribution of zooxanthellae

The white and brown tips described above represent two extremes. Between them is a range of intermediate conditions. The conversion of a white tip to a brown tip is usually preceded by the development of a second cycle of tentacles in the axial corallite. All 12 tentacles then become brown due to an increase in the number of zooxanthellae. This is accompanied by an increasing tendency for the axial polyp, along with the radial polyps, to expand during the day as well as at night (Figure 86). Then follows a gradual infiltration of zooxanthellae into the distal coenosarc (Figure 85).

Various stages in the sequence of events described above are most commonly found in samples collected during the period July to August when the proportion of brown to white tips is at its highest. A shift towards a predominance of brown tips also occurs in colonies which have been maintained in the aquarium in excess of 4-6 weeks.

The gradual infiltration of zooxanthellae into the distal coenosarc sometimes comes to a halt and may even be reversed. More often, it continues until the branch tips come to assume the same colour as the rest of the branch. The structure of the axial corallites in such tips gradually become less porous as they undergo internal consolidation as described in Section 6.2.3. Their physical prominence is also reduced by continued growth in adjacent radial corallites. The time taken for these processes to occur ranges from 6-10 weeks.

6.4 Induction of browning

Conversion of white to brown tips can be artificially induced by maintaining severed tips (25mm-30mm long) in racks in an aquarium for 2 weeks. Characteristic morphological changes were observed in a group of 10-15 tips so treated, which were taken from colonies of A. formosa collected at Lizard Island. Expansion and contraction rhythms were not monitored and no attempt was made to quantify these changes since the size of the sample was too small to compensate adequately for the inherent variability of skeletal form in this species (see Wallace, 1979). These qualitative results are nevertheless of some value since they substantiate the observations reported above.

The blue pigmentation characteristic of A. formosa colonies from Lizard Island (Section 2.1) rapidly disappears from tips within about 5 days of transfer to the aquarium, but there is no accompanying loss of zooxanthellae. No extrusion or loss of brown colouration occurs, in fact the tips appear more brown, presumably due to the removal of the masking effect of the pigment. The portion of the coenosarc of the axial polyp which is free of algae reduces rapidly from approximately 15mm upon collection (compared to a maximum of 10mm in colonies from around Magnetic Island) to 75mm in 1 week and after about 2 weeks extends for only 25mm from the tip. Within the time available for observation, none of the tips under observation actually became completely brown.

The most marked changes in such tips occur in the skeleton, particularly with regard to the form and arrangement of the radial corallites. In freshly collected branch tips, the skeleton is extremely fragile and susceptible to damage; the radial corallites near the tip are poorly developed and widely spaced. Over the course of two weeks, the skeleton appears to consolidate. The radial corallites become larger and the intercorallite distance is markedly reduced (Figure 87).

Figure 80: Longitudinal section through the axial corallite of a brown tipped branch of A. formosa stained with Liisberg's stain (x20)

The location of the section and histochemical details are as in Figure 78. The ratio of skeleton (sk) to coelenteron (ce) is much higher than in Figure 78. The section is slightly crushed at x. Zooxanthellae (z) form a monolayer in the gastrodermis of the outer and inner body wall. They are not associated with the oral disc region (o), but some algae are present in the basal portion of the contracted right hand tentacle (te).

ax - axial corallite; ce - coelenteron; co - coenosteum; g - gastrodermis; o - oral disc; r - radial corallite; R - circle of radial corallites; sk - position of skeleton; te - tentacles; z - zooxanthellae; arrow - costa; x - damaged portion of section.

Figure 77: White tipped branch of A. formosa fixed with buffered sea water formalin (x12)

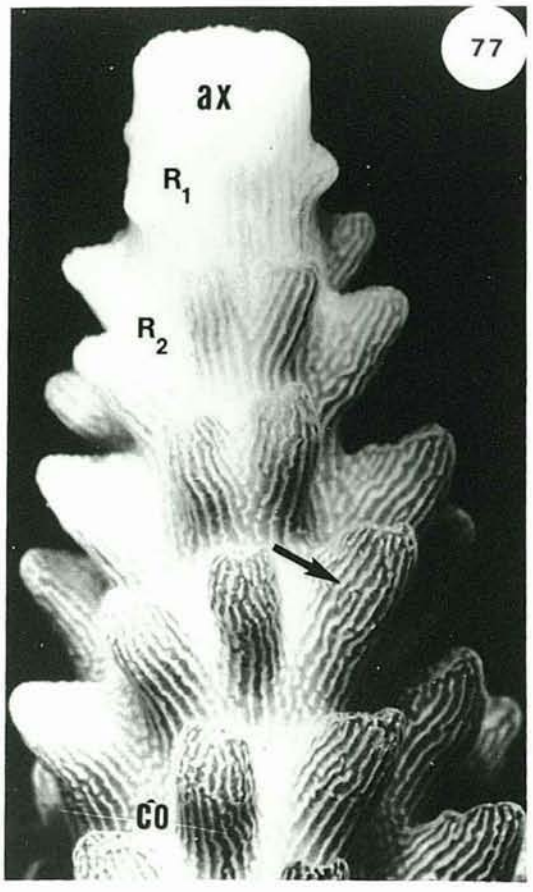
There are no zooxanthellae in the coenosarc of the extreme tip of the axial corallite or the first circle of radial corallites (R_1). Algae begin to appear in the polyps of the second circle of radial corallites (R_2) and the intercorallite region proximal to this is thoroughly infiltrated. All the radial corallites are prominently nariform. The pattern produced in the tissue by the underlying skeleton shows that the coenosteum (co) is prominently costate (arrow), becoming slightly spinulose between the corallites at the base of the tip.

Figure 78: Longitudinal section through the axial corallite of a white tipped branch of A. formosa stained with Liisberg's stain (x31)

The section is taken through the edge of the stomodeum (st), and retracted tentacles (te) are obliquely sectioned on the invaginated oral disc (o). The proportion of skeleton (sk) to coelenteron (ce) is extremely low throughout. The zooxanthellae (z) appear as round black dots in the gastrodermis (g). There are virtually no zooxanthellae in the gastrodermis of the outer body wall above the level of the top of the oral disc, and few throughout the inner body wall gastrodermis. Those on the right hand side of the section are probably associated with the radial corallite (r) (c.f. Atoda, 1951).

Figure 79: Brown tipped branch of A. formosa fixed with buffered sea water formalin (x10)

The coenosarc of the branch tip is entirely infiltrated with zooxanthellae. The outline of the underlying coenosteum suggests that the costae (arrows) of the radial corallites are highly elaborate whilst the intercorallite region is densely spinulose. The axial corallite (ax) seems blunted (c.f. Figure 77), and the radial corallites are rounded. The latter all appear equally well developed and are present right up to the rim of the axial corallite, diminishing its physical prominence.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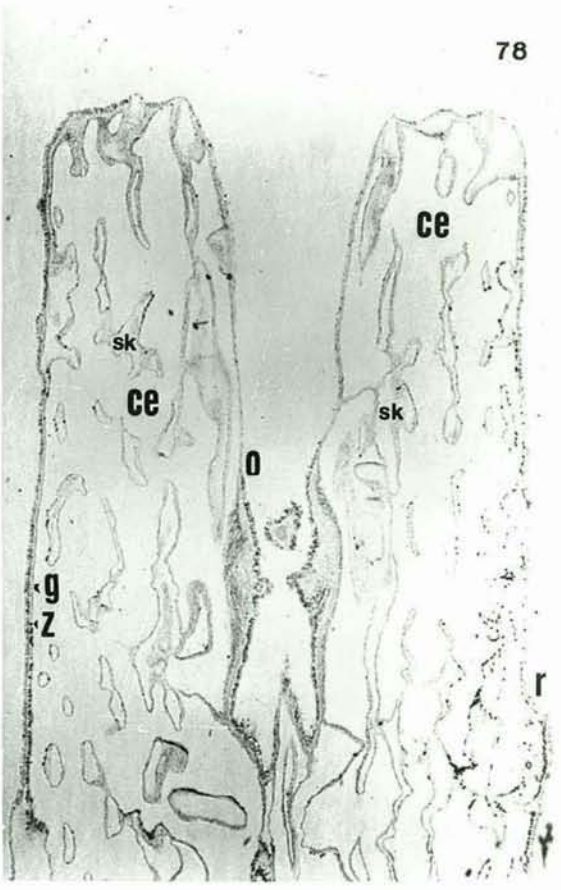
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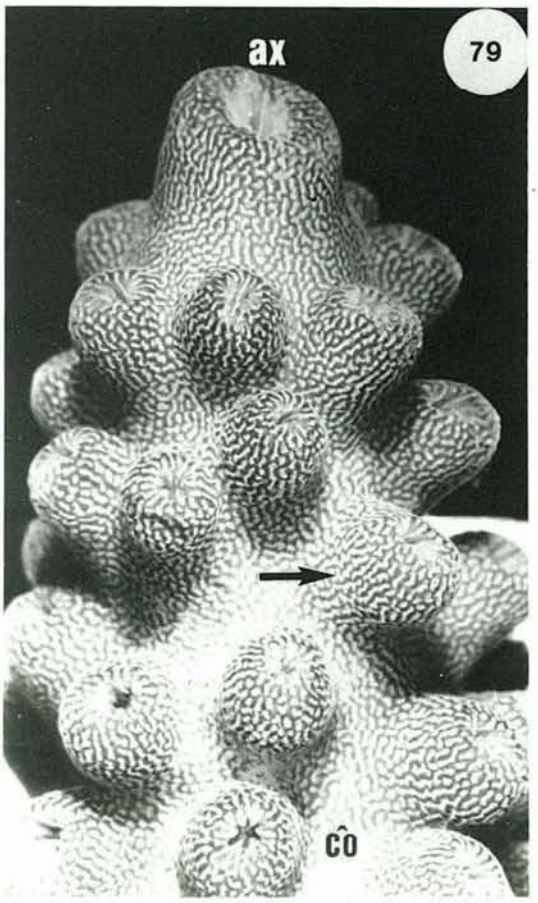
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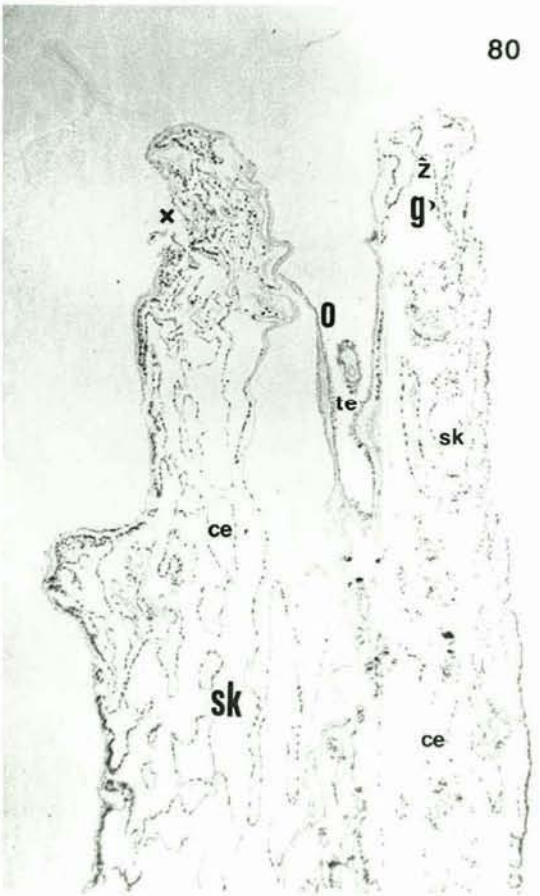
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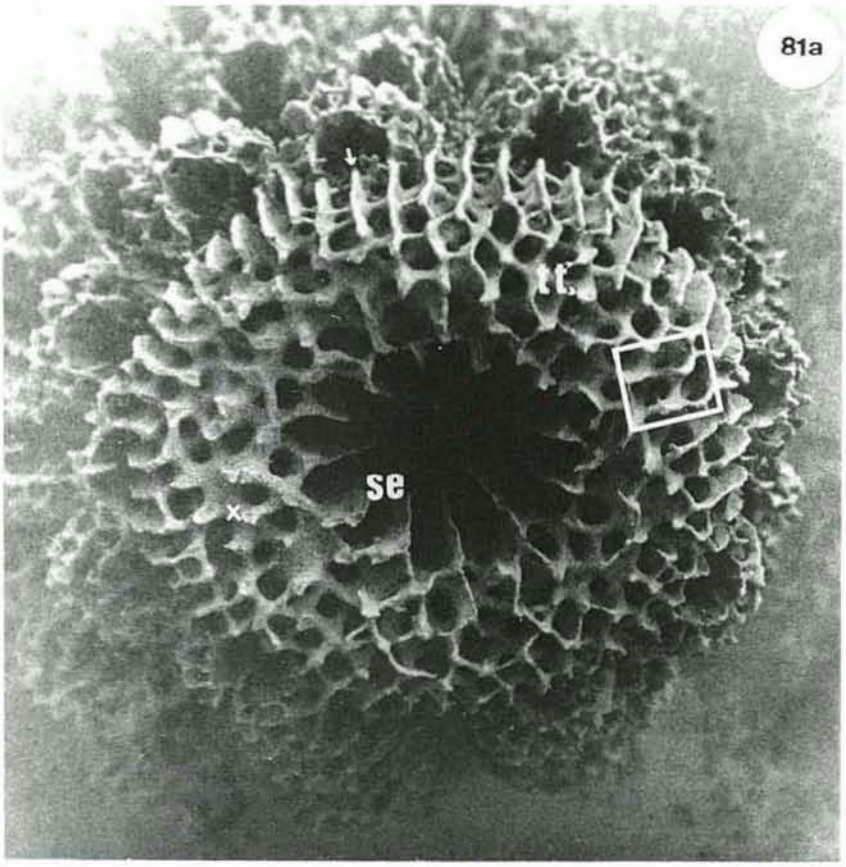
Figure 81: Skeleton of a branch tip of A. formosa recently infiltrated by zooxanthellae (for comparison with Figure 6)

(a) end on view (x30)
The structure of the axial corallite is not as porous as that of a white tip (Figure 6) and substantial thickening is present between the synapticulothecal rings. The septa (se) and the costae (arrow) have smooth margins but are heavily calcified. The trabecular tines (tt) are blunt in appearance, with flattened rather than pointed ends. Some were accidentally broken on the left hand side of the tip, the one marked "x" is enlarged in Figure 84.
(For enlargement of the boxed area see Figure 83)

(b) Oblique view (x25)
The whole structure is more heavily calcified than in Figure 6; the synapticulae (s) are well formed and the fenestrations (fe) are relatively small. Radial corallites (ir) are forming right up to the rim of the axial corallite. The first circle of radial corallites (R_1) have a well developed outer directive septum and two developing synapticulothecal rings. Members of the second circle of radial corallites (R_2) have at least four primary septa and 3 synapticulothecal rings. Costal spines begin to occur on costae (arrow) just below the first circle of radial corallites (R_1).

fe - fenestration; ir - incipient radial corallite; R - circle of radial corallite; s - synapticula; se - septum; tt - trabecular tine; arrow - costa; x - broken trabecular tine


81a
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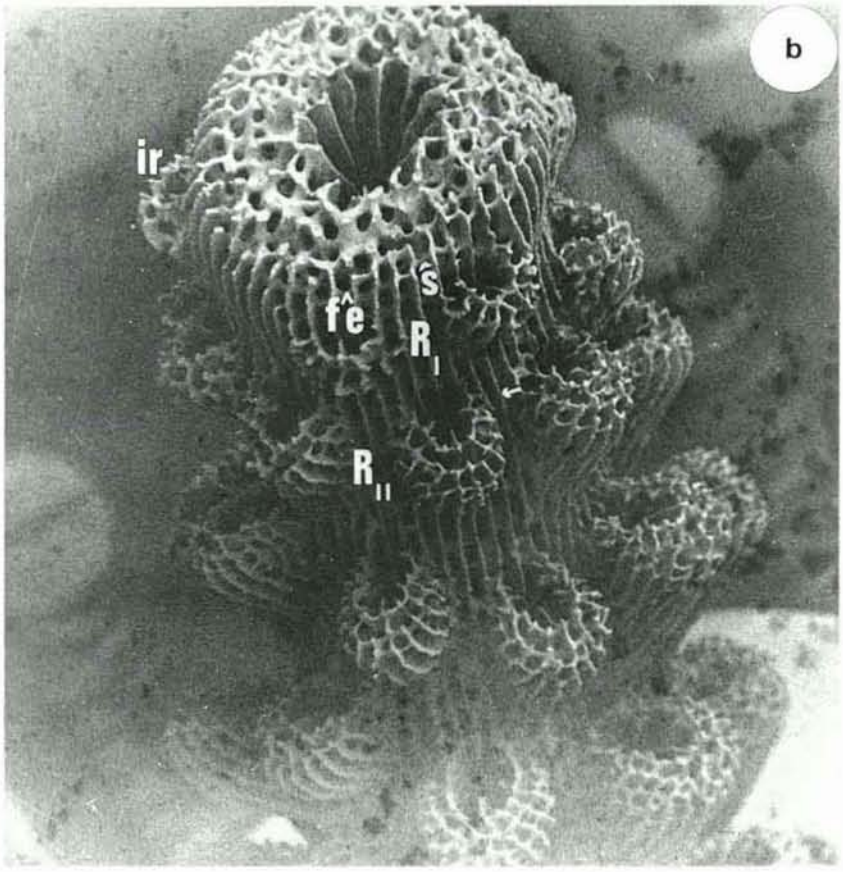
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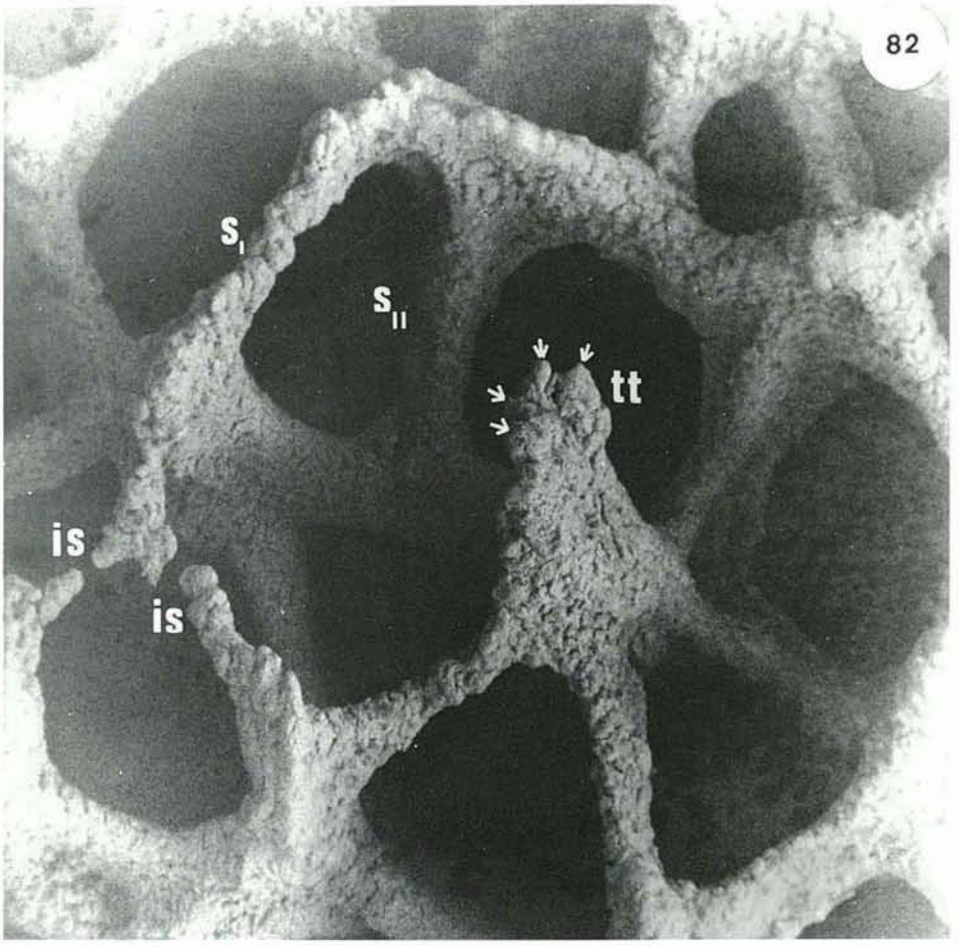
Figure 82: Detail of a trabecular tine (from Figure 6a) in white tip (x270)

The ends of the crystal fibres (arrows) which form this trabecular tine (tt) may be discerned. The comparative ages of the synapticulae may be gauged from their positions relative to the skeletal surface. Newly formed synapticulae (s) appear very fragile and retain the irregular appearance of those still in the process of formation (is). Those further down (S_{II}) are thicker and smoother. Overall, however, the skeletal surface is highly irregular compared to Figure 83.

Figure 83: Detail of trabecular tines (from Figure 81a) in a brown tip (x270)

The entire skeletal surface, including the apices of the trabecular tines (tt), has a smooth appearance. One granulation (gr) is present but there is no corresponding outgrowth from an adjacent synapticula. The same gradation of strengthening and consolidation as observed in Figure 82 is present in the synapticulae (S_I vs S_{II}) but the finest ones on the surface are as smooth as those found in the depths of the skeleton. Thickening below the corallite surface considerably reduces the gaps between the skeletal elements.

gr - granulation; is - incipient synapticula; s - synapticula; tt - trabecular tine; arrow - crystal fibre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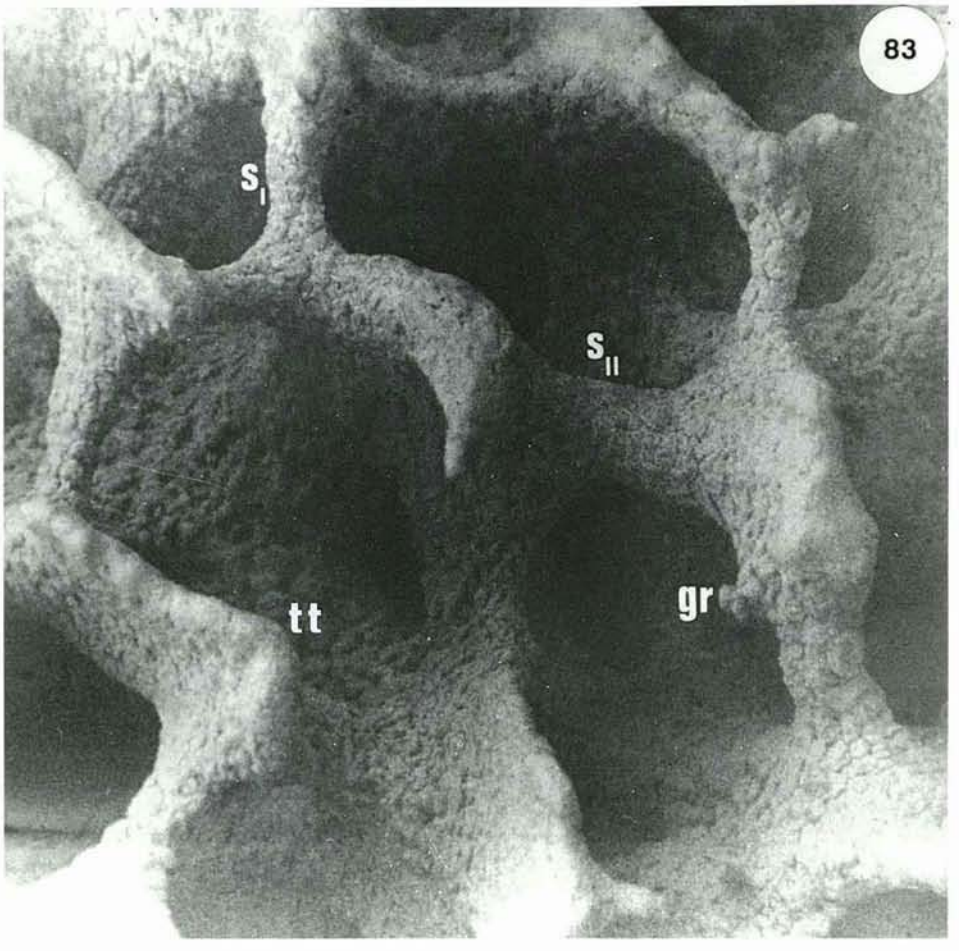
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s_I
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Figure 84: Detail of a broken trabecular tine (from Figure 81a) showing stereome deposition (x360)

The smooth appearance of the skeletal surface of Figure 81a is shown to be composed of large flakes of stereome deposition (sd), a thin layer (arrow) overlies the outer edge of the crystal fibres (cf) which form the bulk of the trabecular tine (tt).

Figure 85: Brown tipped branches of A. formosa showing daytime expansion (x3.7)

The entire coenosarc and the tentacles (te) of both the axial (ax) and radial (r) polyps are infiltrated with zooxanthellae. All are partially expanded. The axial corallites appear rounded and less prominent than that in Figure 77.

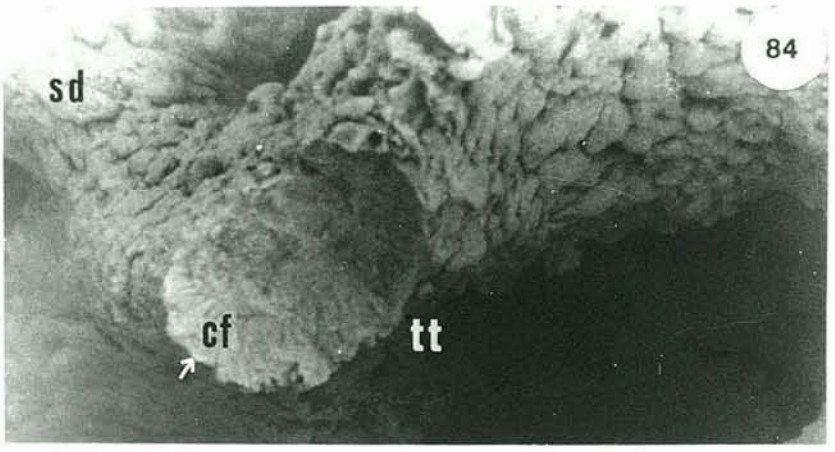
Figure 86: White axial polyp of A. formosa with brown tentacles showing daytime expansion (x1.3)

The white axial polyp of the axial corallite (ax) at the branch tip has 2 cycles of zooxanthellae containing tentacles (te) which are fully expanded.

Figure 87: Skeletal compression in white tipped branches of A. formosa after prolonged maintenance in an aquarium (x3)

These tips were all taken from the uppermost branches of the same colony and placed in perspex racks in the aquarium. They were sampled immediately (1), after one week (2) and after two weeks (3) respectively. There appears to have been a general consolidation of skeletal structure with time, accompanied by a reduction in intercorallite distance and an increase in the proximity of radial (r) corallites to the extreme tip of the branch.

ax - axial corallite; cf - crystal fibre; r - radial corallite; sd - stereome deposition; te - tentacle; tt - trabecular tine; arrow - layer of stereome.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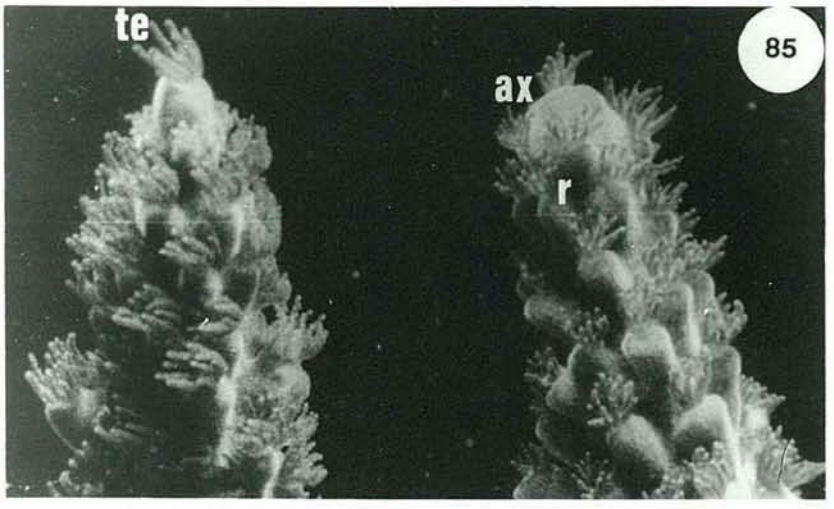
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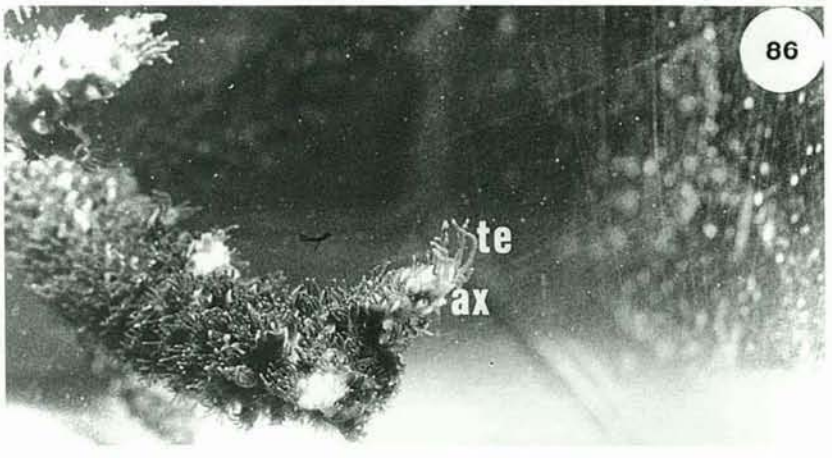
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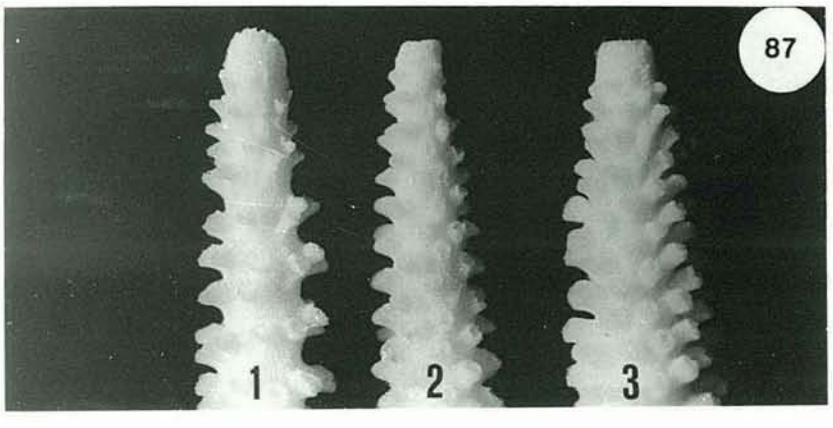
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6.5 Discussion

The foregoing results suggest that as long as a white tipped branch of A. formosa is actively growing, zooxanthellae remain virtually absent from the end of the branch, the axial polyp never develops more than one cycle of tentacles and calcification in the axial corallite is largely devoted to the longitudinal extension of the branch, with little structural consolidation. Under certain conditions, which another study (Oliver, 1979) has shown correspond to the cessation of longitudinal extension, the axial polyp develops a second ring of tentacles, and the end of the branch becomes infiltrated with zooxanthellae. Calcification then becomes channelled into skeletal elaboration and the formation of stereome, a type of deposition which has been shown (Spiro, 1974; Gladfelter, 1982) to be associated exclusively with infilling of basal branch regions which are no longer actively growing. At the same time, the axial polyp undergoes a marked change in behaviour. Instead of exhibiting a distinct diel rhythmicity of expansion and contraction, it becomes virtually permanently extended.

The net result of these alterations is that, upon cessation of longitudinal growth, the axial polyp and its corallite undergo a sequence of events comparable with the gradual maturation of radial polyps and their corallites with increasing distance from the tip of the branch (Section 3.2.2). Although this study has concentrated on the initial stages of this process, there was clear evidence that, as the process continues, the axial polyp ultimately becomes indistinguishable from adjacent radial polyps.

As Barnes (1973) has pointed out, since the polyp secretes the skeleton, and the skeleton in turn supports the tissues, cell division and calcification cannot take place independently. In Section 5.4 it was demonstrated that cell division rates do decrease in response to reduced skeletal growth rates. Thus in brown tipped branch ends of Acropora, where longitudinal extension is zero (Bak, 1976; Oliver, 1979), it would seem likely that cell division has also virtually ceased.

If the factors which reduce rates of coral cell division do not affect reproduction in the symbiotic algae, one possible explanation of browning could be the overgrowth of these non-dividing cells by the zooxanthellae. However, numerous studies of symbiosis in coelenterates suggests that this is unlikely to be the case; the animal host appears to maintain control of its algal partners at all times. For example, in cross infection experiments in anemones, heterologous strains of algae undergo both morphological (Pardy, 1976) and physiological (Schoenberg and Trench, 1980 a and c) changes under the influence of a new host. There is also ample evidence that coelenterates routinely prune their algal population (Taylor, 1969a, 1973a; Goreau et al, 1970; de Reimer, 1971; Yonge, 1973; Trench, 1974; Steele, 1977; Muscatine and Pool, 1979).

Under adverse conditions, algae are either bleached or extruded depending upon the severity of the stress (Yonge and Nicholls, 1931 a and b; Kawaguti, 1973b; Goreau, 1964; Kinsman, 1964; Marshall, 1972; Jokiel and Coles, 1974, 1977; Coles and Jokiel, 1978; Jaap, 1979). Algal rejection was never observed in colonies which exhibited browning; the degree of pigmentation simply increased. This suggests that the condition of browning is not induced by some kind of acute stress. The almost permanent expansion of polyps in brown tipped branches supports this view because corals under stress tend to contract (Abe, 1938; Lewis, 1971)

Since in the browning of tips the axial polyp adopts the appearance of a radial polyp, it is interesting to speculate as to whether it also assumes the functional role of the latter in the production of algal photosynthate to enhance calcification (see Pearse and Muscatine, 1971, Taylor, 1977). If this does occur, then the material so produced must be capable of being deployed to stimulate growth in white tips since it would be of no immediate use to the non-growing, brown tipped branch responsible for its manufacture. Cheney (1975) provided indirect evidence for the occurrence of inter-branch translocation of algal photosynthate when he observed that the development of

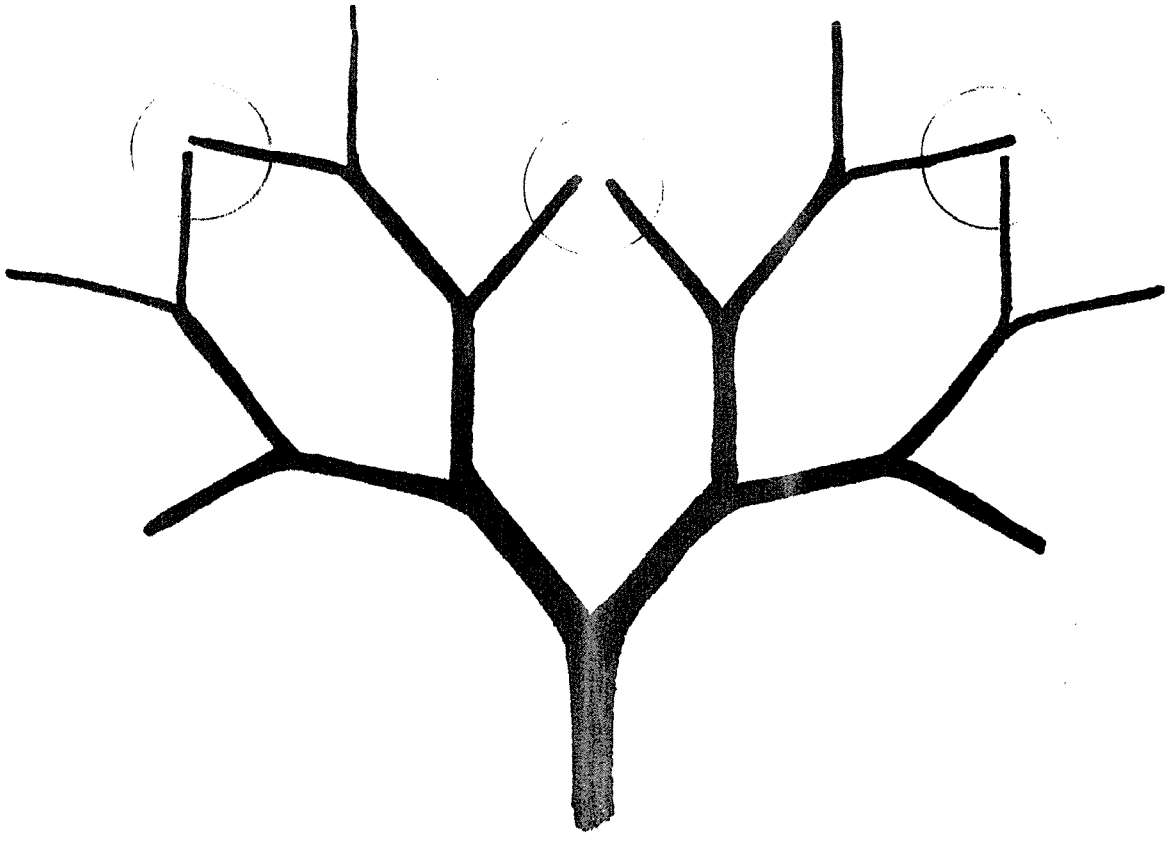
tumour-like growths in A. formosa colonies appeared to occur at the expense of longitudinal extension in adjacent white tipped branches.

Porter (1976) separated the scleractinia into two groups on the basis of their surface to volume ratio in relation to polyp size. He concluded that species with small polyps, like Acropora, relied relatively little upon zooplankton capture to satisfy their nutritional needs and depend heavily upon photosynthesis. The studies of Gladfelter (1975) and Sebens and de Reimer (1977) with several species of anemones, and Fricke and Vareski (1982) with the hard coral Plerogyra sinuosa showed that polyp structures with dense populations of algae (such as modified tentacles, auxillary structures of the column wall and pseudo-tentacles) always responded positively to light with respect to their orientation and by expansion. This maximised the exposure of zooxanthellae to light by increasing the total surface area presented for irradiation. Thus the virtually permanent expansion of the polyps of brown tipped branches of A. formosa suggest that algal infiltration is accompanied by an increase in dependence upon photosynthesis, rendering prey capture even less important in the overall nutrition of the colony. This conclusion is further supported by the work of Lasker (1976) with Montastrea cavernosa who observed that tentacles which contain zooxanthellae are not as sensitive to touch stimuli, or as efficient at prey capture as tentacles which do not contain symbiotic algae.

The reasons why vigorously growing white tipped branches become brown are clearly very complex. They may be related to the control of the pattern of growth in an extensively branching system, where theoretical consideration of colony growth (see below) indicates that inference between extending branches is inevitable. The work of Lang (1971, 1973) and Richardson et al (1979) on coral aggresion in the Faviina clearly demonstrates that coral polyps can sense the presence of other polyps. Moreover, anastomosis between branches in A. formosa colonies is known to be rare (Collins, 1978; Oliver, 1979). Thus the ability to terminate longitudinal extension in certain branches

may be an important part of the overall mechanism controlling morphology of the colony. This hypothesis is consistent with Oliver's (1979) observation that in healthy, rapidly growing colonies of A. formosa, the majority of brown tips are in the interior of the colony where there is also a tendency for all branches to grow more slowly.

Diagramatic representation of growth in a branching colony



This diagram assumes a two-dimensional growth pattern with bifurcate, 60° branching at regular intervals. The situation is more complex in colonies of A. formosa which are, of course, three-dimensional. They display trifurcate branching at roughly 60° at irregular intervals.

The above explanation could not, however, account for the fact that occasionally branches on the exterior of the colony, particularly towards the base, may also become infiltrated with algae. These changes are unlikely to be caused by a reduction of calcification rates associated with shading (Goreau and Goreau, 1959). Illumination on the reef is not unidirectional and

incident light is often diffuse (Glynn and Stewart, 1973; Kinzie, 1973). Therefore, shading is not a constant feature of any situation of the open reef, particularly for an openly branching species like A. formosa.

The causes of browning, although probably varied, are clearly part of the mechanism governing the rate and optimal directions of branch growth with respect to the colony as a whole. The net result of such a control mechanism appears to ensure that the metabolic resources of the colony are channelled into producing upward growth. This is consistent with the observations described earlier in this thesis (Section 3.2.3) concerning the pattern of growth emanating from the radial corallites of re-oriented, white tipped branches.

The fact that the ratio of brown to white tips appears to be subject to change on a seasonal basis is consistent with the preliminary conclusion of Bak (1976) and Oliver (1979) that the infiltration of algae into branch tips was a reversible process. The period July to August, when the highest proportion of brown tips were found, corresponds to a yearly growth minimum (Oliver, 1979) when DNA synthesis is also depressed (Section 5.4.2). From this it may be concluded that under a particular set of environmental conditions, the colony can only sustain growth in a limited number of tips. This number will decline if conditions become less favourable and vice versa.

Although colonies of A. formosa are known to be able to survive for long periods in the aquarium (up to 18 months, Barnes and Crossland, 1980), it was observed during this study that maintenance of colonies for prolonged periods in the aquarium resulted in a gradual increase in the number of brown tips per colony. This alteration was presumably associated with an overall reduction in growth rate in response to the change in environmental conditions. Certainly isolated tips, showing skeletal compression, apparently resulting from deprivation of nutritional input from the rest of the colony, also undergo changes typical of the first stages of browning, but at a greatly accelerated rate.

7. Repair and regeneration in A. formosa

7.1 Introduction

Repair and regeneration have been extensively investigated in some coelenterate groups. Work on these processes in hydra began with Trembley (1744, in Lenhoff and Loomis, 1961). Since then the capacity of these animals to regenerate their hypostome and tentacles (Rowley, 1902; Ham and Eakin, 1958; Clarkson, 1969; Spangenberg and Eakin, 1962; Diehl and Burnett, 1965; Park, et al, 1967, 1970), and their ability to reform from single layer explants by an elaborate process of de- differentiation and re-differentiation (Haynes and Burnett, 1963; Davis, et al, 1966; Polteva and Berisova, 1974) has been examined in considerable detail. The latter capability has also been observed in other hydrozoans, for example, Cordylophora lacustris (Zwilling, 1963), Coryne pusilla (Aizu, 1968a) and Coryne lovenii (Polteva and Berisova, 1974); and in the scyphozoan Aurelia aurita (Steinberg, 1963; Aizu, 1968b). Superficial wound repair has been described in the actiniarian Calliactis parasitica (Young, 1974; Young 1971, and Chapman and Young (unpub.) in Chapman, G., 1974), whilst regeneration following varying degrees of traumatic amputation has been observed in several other species of anemones including Metridium senile (Polteva, 1970, 1972) and Aiptasia diaphana (Singer and Palmer, 1969; Singer, 1971).

Although scleractinian corals are well known for their ability to repair damage to colonies (Stephenson and Stephenson, 1933; Kawakami, 1941; Stephenson, et al, 1958; Bosch, 1967; Connell, 1973; Pearson, 1981), no previous studies have examined the processes involved on a histological or ultrastructural basis. Thus the few existing reports on tissue repair in corals (Fichelson, 1973; Bak, et al, 1977; Bak and Steward-van Es, 1980), have been largely based on macroscopic observations. Therefore, any information gathered about repair and regeneration in corals must, in the first instance, be related to studies of the other coelenterate groups mentioned above.

In his extensive review of cnidarian histology, D.M. Chapman (1974) reports that whilst Hydrozoa have basophil interstitial cells, the Scyphozoa have amoebocytes which are not especially basophil. Both cell types have been shown to participate in repair and regeneration of the animal concerned. Interstitial cells are reputed to account for the superior regenerative capacity of Hydrozoans as compared with Anthozoans (Young, 1974). They are said to be able to differentiate into a variety of different replacement cells (Lentz, 1965; Davis, 1969). In addition, however, somatic epithelial cells are also known to divide (Steinberg, 1963; Diehl and Burnett, 1965; David and Campbell, 1971).

The role of amoebocytes is less clear. They are rarely observed to divide (D.M. Chapman, 1974). They appear to be responsible for resynthesis of the mesoglea and are also incorporated into the reforming epidermis in the anemone Calliactis parasitica (Young, 1974; Chapman and Young (unpub.) in G. Chapman, 1974). In other species, amoebocytes are either weakly phagocytic, as in the scyphozoan Aurelia aurita (Prazdnikov and Mikhalova, 1962), or strongly phagocytic as in the anemone Metridium senile during regeneration (Polteva, 1970). This is a property never shown by interstitial cells (D.M. Chapman, 1974). There is some suggestion (Chapman and Young (unpub.) in G. Chapman, 1974) that mesogleal resynthesis and phagocytosis may be carried out by two different types of mesogleal cell, or, alternatively, by different phases of the same cell type.

The situation is not, however, as clear cut as D.M. Chapman (1974) suggests, since some anemones are known to have interstitial cells (Westfall, 1966; Singer, 1971). In Aiptasia diaphana, for example, it appears that amoebocytes play no part in the resynthesis of the mesoglea (Singer, 1974) and new cells for regeneration are largely provided by differentiation and division of the interstitial cells (Singer, 1971). The histological studies reported in Section 4.3 show that A. formosa, like Aiptasia diaphana, has both interstitial cells and amoebocytes.

It was considered relevant, therefore, to determine the role of these cells in repair and regeneration in A. formosa and thus gain an insight into repair mechanisms in corals generally.

In corals the repair process requires resealing of the tissue, formation of the polyp, and regrowth of the skeleton. The regenerative power of A. cervicornis is so prodigious that Tunnicliffe (1981) concluded that it is a more important method of substrate colonisation than sexual reproduction. However, her descriptions of regeneration from fragments of branches in A. cervicornis (and also those of Shinn, 1972) and in A. formosa (Collins, 1978) have thus far been restricted to gross macroscopic observations. The same applies to studies of regeneration of single branches in other Acropora by Wood Jones (1907) and Kawaguti (1937a).

In this investigation branch tips of A. formosa were deliberately severed in order to study response to damage in more detail. Cellular processes involved in wound repair were examined histologically until the branch end was sealed over. Regeneration of branch ends was then monitored until the branch had completely regrown. At various stages of regrowth both the tissue and the skeleton were examined microscopically.

7.2 Repair

7.2.1 Experimental procedures

Preliminary experiments showed that branch ends were completely resealed 48 hours after severance. All experiments to examine the cellular processes involved in repair were therefore planned within this time scale.

24 branch ends approximately 50mm in length were removed from a large, blue-tipped colony of A. formosa. The extreme tip of each of the apical polyps was removed and the remainder of the branch was cut into two roughly equal portions. Two cut surfaces, one 5mm from the branch end and in the white, zooxanthellae-free region of the tip, and the other well into the brown region of the branch (approximately 25mm from the end) were thus available for study. The pair of pieces from each individual branch end were put into separate incubation beakers of the experimental apparatus (described in Section 2.4.2). One set of pieces were fixed at once, the remainder were placed under the water outlets in the experimental apparatus.

For the first hour of the experiment the contents of a beaker were fixed in buffered sea water formalin every 15 minutes, for the next hour every half hour, for the next 5 hours every hour, for the next 15 hours every alternate hour and for the remaining 16 hours every 3 hours. Thus samples were fixed at 0, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, $1\frac{1}{2}$, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19, 21, 23, 27, 31, 34 and 38 hours after the initial cutting. At the end of the experiment sub-samples were taken from about 1cm from the damaged surface of each branch portion and prepared for histological examination in the usual way.

7.2.2 General observations

Examination of serial sections taken from the above specimens showed that the broken surface of a branch severed 25mm from the tip is sealed within 38 hours by a layer of tissue which is virtually indistinguishable from the normal outer body wall in the tip region of an axial corallite (Figure 110). The sequence of events which result in wound repair in branches cut 25mm

from the tip are summarised in Table 5 along with an assessment of the approximate timing of these events at a position mid-way between the wound edge and the centre of the branch. There is considerable variability between samples with respect to the initiation and duration of each stage but it appears that they are initiated consecutively and then proceed concurrently. Branches cut 50mm from the end repair themselves in an identical manner, but lag behind the 25mm severed tips by about 2-4 hours.

Immediately after the tip is severed, the canals of the coelenteron and the trabecular columns of the skeleton are completely exposed to the environment. There is an abrupt transition from the jagged, cut surface and the undamaged tissue around the edge of the wound (Figure 88). The canals seal over quite quickly (after about 13 hours) (Figure 89), but the re-establishment of the tissue over the exposed skeleton (Figure 90) takes longer and the branch end is not completely sealed until at least 17 hours after cutting and the process may take up to 21 hours.

There is an abrupt transition between the extent of tissue repair on either side of the trabecular columns of the synapticulothecal rings. The tissue between each ring tends to exhibit the same state of regeneration, with the most advanced stages of repair being found near the edges of the wound (Figure 97).

In the early stages of repair there is evidence to suggest that the gastrodermis supplies cells to the reforming epidermis. Thus, zooxanthellae are sometimes seen in the epithelial cells of this layer and loose zooxanthellae which appear to have been recently extruded are often seen next to seemingly intact epidermal cells (Figure 91). However, the epidermis appears to "reciprocate" at a later date by supplying cells to the gastrodermis since nematocysts are quite frequently observed in the gastrodermis of samples taken from 17 hours onwards (Figure 92) and may be present even after the tissue over the branch end has been completely repaired.

Table 5: Time course of the repair process in branches of *A. formosa* severed 25mm from the tip

Hours	Stage of Repair
0-0.25	Extrusion of mesenterial filaments into broken surface
0.75-13	Mucus on wound surface covering extruded mesenterial filaments
5-6	Mitosis of epidermal cells Gastrodermal cells sealing canals of coelenteron
11-15	Maximum numbers of mesenterial filaments extruded
13-17	Canals sealed. Gastrodermis is cubo-columnar, epidermis, when present, forms squamous epithelium. No mesoglea.
17-21	Entire wound surface sealed by gastrodermal and epidermal layers.
19-23	Nematocysts appear (horizontally-oriented) in epidermis
21-27	Mucous gland cells appear in epidermis and mesoglea starts to form
23-27	Epidermal epithelium becomes increasingly cubo-columnar, particularly over gastrodermis
27-31	Small acidophilic granules appear in epithelial layers and at the site of the regenerating mesoglea
27-34	Cells containing vacuoles filled with acidophilic granules evident in reformed mesoglea. Concentration of differentiating cnidoblasts at wound edge
27-38	Calicoblastic layer reformed. Epidermal epithelial cells become columnar, nematocysts and mucous gland cells vertical to new mesoglea, tissue slightly disorganised over skeletal spines
48	New outer body wall established.

7.2.3 Histological observations

Within 15 minutes of severance, the broken surface has become covered by both type I and II mesenterial filaments (Figure 89), although they do not reach their maximum concentration until 11 to 15 hours after cutting. They remain close to the surface even after the end has become completely sealed (Figure 90).

A conspicuous layer of mucus becomes established over the entire wound surface within an hour of cutting (Figure 94). This layer also envelops the extruded mesenterial filaments (Figure 95) and persists until the branch end is sealed. The mucous gland cells in the cnidoglandular bands of the mesenterial filaments may be responsible, in part, for the secretion of this mucus layer. However it seems to be substantially derived from the normal coating of the outer body wall, because, in common with the latter, the mucus on the wound surface contains large numbers of bacteria and diatoms. Unlike the body wall coating, however, the wound mucus also contains zooxanthellae and cell fragments. Considerable quantities of this material appear to be sloughed off the branch end (Figure 96). Accumulations of cellular debris, bound together by mucus, occur in the crevices of the broken surface (Figure 94).

Dividing nuclei are rarely seen in sections of A. formosa and no more than twenty were seen during the examination of hundreds of slides taken over many diurnal cycles. Four of these mitotic figures were observed in regenerating tissue in a sample taken 5 hours after cutting; two of them are shown in Figure 72.

The pockets of ruptured coelenteron appear to become sealed off initially by the gastrodermal layer only. Epidermal cells appear later. The gastrodermal cells on the wound surface originate from the cut edges of the inner body wall gastrodermis (Figure 98). These cells rapidly assume a cubo-columnar form, even though the cell layer from which they originate is a squamous epithelium. By about 15 hours after wounding the new body wall gastrodermis is only half as thick as usual, but contains about the usual number of nuclei per unit length of tissue (Figure 100 c.f. Figure 17).

Replacement of the epidermis does not begin until 2-4 hours after reformation of the gastrodermis. The renewing epidermal layer retains a flattened squamous form only 2-3μm thick (compared to the normal 20-25μm for at least 23 hours after severance of the tip. New epidermal cells are extremely attenuated, possessing long basal processes. They do not originate from the calicoblastic layer lining the site of the broken skeleton, but from the undamaged epidermis at the periphery of the branch. It is these extended epithelial cells which initially bridge the gap across the severed trabecular columns. A sinuous sheet of contiguous cells emanates from the side nearest the wound edge until it is long enough to attach to the far side (Figure 103). The movement of specialised epidermal cells into the repairing epidermis then begins as the epithelial sheet moves on and the calicoblastic layer reforms (Figures 104, 109). Fully differentiated horizontally-oriented nematocysts appear in the epidermis from 19 hours onwards (Figure 101), and after 23 hours distorted mucous gland cells are also present (Figure 102).

Some nematocysts appear to be generated in the tissue immediately adjacent to the wound edge since large numbers of differentiating cnidoblasts are observed there (Figure 111). Only fully differentiated nematocysts and mature mucus cells are ever seen in the regenerating region. Neither MSB nor Massons trichrome stains give any evidence for the presence of mesoglea between the epidermis and the gastrodermis during the first 21 hours after wounding (see Figure 100). This layer eventually reappears, after about 27 hours, between the epidermal and calicoblastic epithelia, following reformation of the latter.

At the same time that the mesoglea begins to reappear, small (0.1-0.8μm) acidophilic granules can be seen throughout the repairing tissue, particularly at the site of the reforming mesoglea and in the epidermis (Figure 106). By 34 hours after severance the granules tend to be concentrated in the vacuoles of what appear to be mesogleal cells (Figure 107). This material appears to be identical to that which accumulates in the lateral lobes of type I mesenterial filaments (Figure 108).

The epidermal layer gradually thickens, and the orientation of the specialised cells tends to conform to that of the epithelial cells. A cubo-columnar epithelium is present between 23 and 27 hours after cutting (Figure 101), but virtually all epidermal cells become vertically oriented with respect to the regenerated mesoglea by about 27 to 38 hours following wounding (Figure 102). During this final stage, the gastrodermis exhibits its normal thickness and some zooxanthellae may be present.

By 38 hours after wounding, some cellular disorganisation is still evident in the epidermis, particularly over the new skeletal spines (Figure 110). This is most obvious in relation to the nuclei of the columnar supporting cells which are not arranged in their usual discrete band (compare Figure 17). Nevertheless, the repair process is almost complete by this time and the new body wall is virtually morphologically identical to the outer body wall in the rest of the tip region.

7.3 Regeneration

7.3.1 Experimental procedures

Regeneration experiments were carried out in the aquarium on small colonies of A. formosa, bearing approximately 20 white tips. No more than 5 white tips per colony were severed, in an attempt to avoid excessive shock to the colony which might have inhibited its recovery. The branches were cut approximately 40mm from the tip. Regeneration was observed at weekly intervals until the branches had either completely regrown, or until it was obvious that complete recovery was not going to occur and that a static situation of partial regrowth had been reached. Sample pairs of branch ends representing various stages of regeneration were taken at various times. One branch was fixed in buffered sea water formalin (Section 2.3.1), photographed, and prepared for histological examination. The skeleton of the accompanying branch was prepared for examination under the scanning electron microscope (see section 2.2). Response to colonisation of the cut surfaces by filamentous algae was also investigated.

7.3.2 External appearance of regenerating branch ends

The jagged nature of the surface of a severed branch gradually diminishes following the resealing of the branch end with tissue. After 18 days the surface of the coenenchyme has become rounded and the fractured edges of the corallites blunted (Figure 112a). Regrowth is initiated by elongation of the axial corallite. This is followed by the development of radial corallites down the sides of the new growth as it increases in length and breadth (Figures 113a and 114a). These processes continue until the region of new growth appears continuous with the original branch and the distribution of both radial corallites and zooxanthellae are indistinguishable from that of an undamaged branch tip (Figure 115).

The positions of the severed corallites are delineated (soon after resealing) (Figure 112a) by the distribution of the zooxanthellae which gradually infiltrate the intervening coenosarc. At first there are few zooxanthellae in the new portion of the

branch (Figure 113a), but their gradual appearance first in the radial corallites and then in the rest of the coenosarc is indicated by the accumulation of brown pigmentation.

The distribution of zooxanthellae in the outer body wall gastrodermis clearly outlines the position of the underlying skeleton, and indicates that the regenerated coenosteum is costate (Figure 114a). The coenosarc at the tip of the axial corallite invariably remains white in branches which regenerate successfully (Figure 115). Infiltration of this region by zooxanthellae is a reliable indication that regeneration has been arrested at that particular stage of regeneration. This is a frequent occurrence when the water in the aquarium is exceptionally turbid, for example, after monsoonal rains (between January and April).

Table 6 shows the rate of extension of the axial corallites, and of the development of radial corallites during regeneration. The experiment was carried out during September-December, the period of optimum conditions for regeneration when successful regeneration of severed branch ends occurred most frequently.

Under aquarium conditions, complete regeneration (from branches 6-8mm in diameter) is achieved after approximately 100 days. Field observations indicate that regeneration occurs more rapidly in the natural environment, probably 60-80 days, but the processes involved appear to be exactly the same.

Table 6: Rate of extension of the axial corallite and development of radial corallites in a regenerating branch tip of A. formosa

Days after severance	No. of Samples measured	Average No. of regenerated radial corallites	Average No. of regenerating radial corallites[1]	Average Extension of axial corallite (mm)
18	4	0	0	0
26	3	5	5	0
34	2	25	8	9
46	3	43	7	15
84	2	68	8	24

[1] First synapticulothecal ring complete

7.3.3 Histological observations

Sections through regenerating tips show a marked difference in the ratio of coelenteron to skeleton between the original branch and the regenerating portion (Figures 113b, 114b). The new portion is lightly calcified in contrast to the original portion. In the new growth, type II mesenterial filaments do not at first penetrate the distal regions of the coelenteron (Figure 113b). They do not appear until an advanced state of regeneration has been reached (Figure 114b).

At the point of transition between old and new growth, there is an abrupt reduction in the number of zooxanthellae present in the outer body wall gastrodermis. In the former the algae are 2-3 deep, whilst in the latter the layer is never more than one cell thick. As in undamaged branches (Figure 78) the algae become increasingly sparsely distributed towards the tip. Similarly, the number of specialised cells is also reduced in the tissue over the extreme tip of the axial corallite.

7.3.4 Microscopic appearance of the skeleton

Low power scanning electron micrographs of branches which have become resealed but have not yet commenced regenerating show that the smooth macroscopic appearance of the skeleton (Figure 116a) is not due simply to the rounding off of calcium carbonate splinters. It is the result of the formation and extensive elaboration of numerous spines on the coenosteum accompanied by the deposition of substantial amounts of stereome (Figure 116b). This spinulose coenosteum is identical with that found on the exterior of the undamaged basal portion of the branch (Figure 116a). The broken edges of the axial corallite are not ornamented in this way (Figure 116a) and, as the corallite regenerates, it develops the costate coenosteum (Figure 117), previously hinted at by the observed pattern of distribution of the zooxanthellae (see Section 7.3.2). The costae have smooth margins near the tip and only develop spines proximally (Figure 117). However, the extent of development of the spines is more marked than that normally seen in white tips (compare Figures 117 and 6b).

The regenerating radial corallites, although extremely well developed, tend to be less uniformly oriented than in undamaged branches. Even those near the branch tip have at least two synapticulothecae, a complete primary cycle of septa and sometimes a partially developed secondary cycle as well (Figures 117, 118). Apart from the unusually well-developed costal elaboration in close proximity to the tip, the axial corallite appears normally developed with two complete septal cycles and 2-3 synapticulothecae (Figure 118).

7.4 Response to colonisation of severed branches by filamentous algae

Severed branches frequently become infected with filamentous algae which interfere with the repair process. This is particularly common in the mid-year period (June-July). Sometimes, however, the coral is able to combat the infection. This involves the formation at the periphery of the branch of a rounded rim of coenenchyme which has a zooxanthellae-free coenosarc (Figure 119). This first stage takes about a week, then a zone of coenosteum encroaches over the surface of the infecting algae during the ensuing 2-6 weeks (Figure 120). The entire branch end thus becomes sealed off, trapping the algae underneath (Figure 121). New corallites later develop on this site. Zooxanthellae do not infiltrate the overlying coenosarc until the incipient radial corallites appear.

Figure 88: Longitudinal section from near the centre of a severed branch of A. formosa immediately after removal of the tip. (x 170).

There is no continuity of the tissue over the trabecular columns (tr) of the skeleton or the canals of the coelenteron (ca). The free body wall is undamaged (u) right up to the edge of the wound (x).

In this and all the subsequent figures, the position of the former branch end is towards the upper edge of the photograph and the cut surface is oriented horizontally.

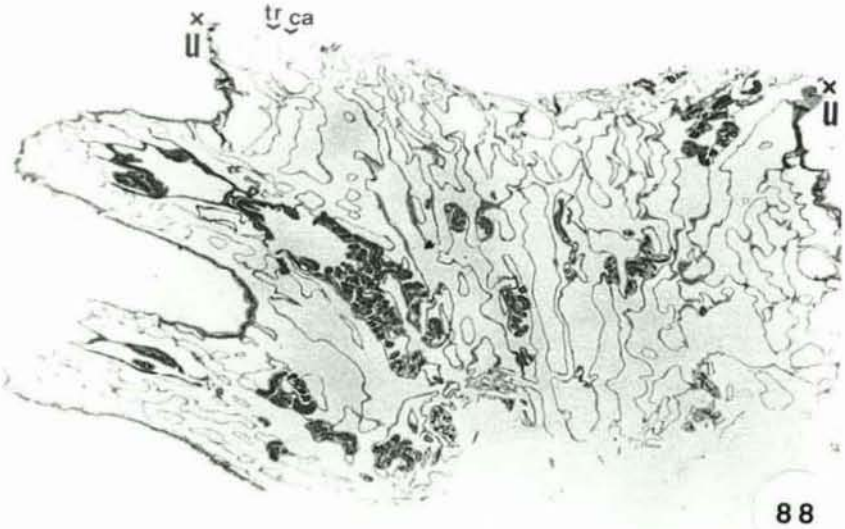
Figure 89: Longitudinal section from near the centre of a severed branch of A. formosa , 3 hours after removal of the tip. (x 230).

Some canals are sealed off (arrows) but there is still considerable disruption of the tissues. Shreds of tissue (*) are attached to the edge of the wound (x), and the trabecular columns (tr) are still completely exposed. The coelenteron is not sealed in the centre of the branch and type II mesenterial filaments (II) are extruded over the central portion of the broken surface. Undamaged body wall (u) is present at the right hand side of the section.

Figure 90: Longitudinal section from near the centre of a severed branch of A. formosa, 31 hours after removal of the tip. (x 130).

A thin layer of tissue covers the entire wound surface, type I mesenterial filaments (I) are accumulated just below the resealed surface.

ca-canal of gastrodermis; tr-site of trabecular column; u-undamaged tissue; I-type I mesenterial filament; II-type II mesenterial filament; x-edge of wound; arrow-sealed gastrodermal canals; *-shred of tissue.



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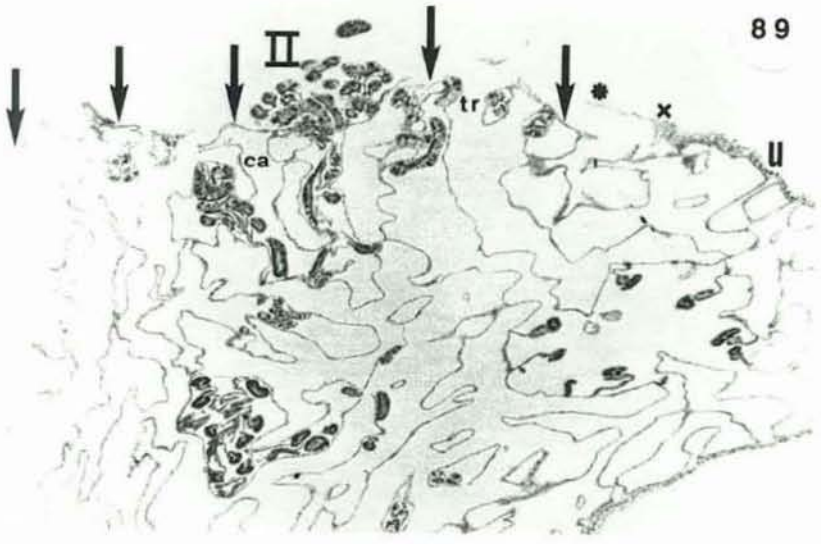

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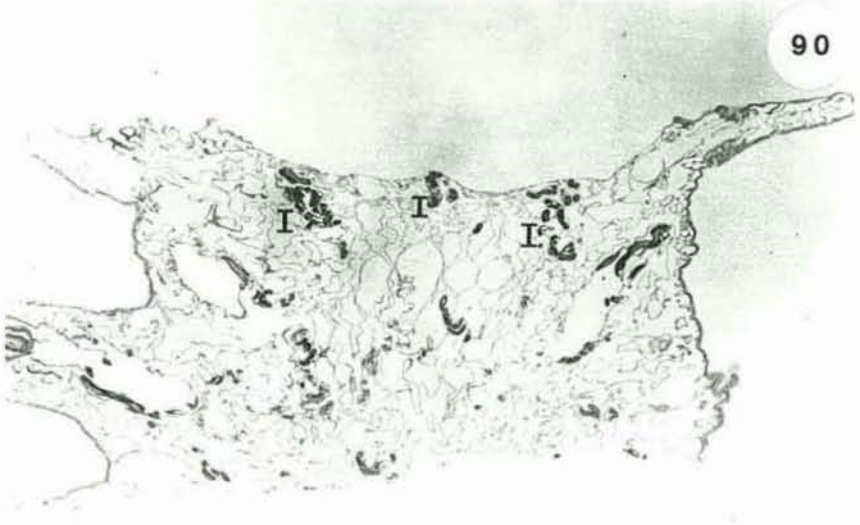

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Figure 91: Gastrodermal cells in repairing epidermis 13 hours after removal of the tip (x 1600).

The epidermal layer (e) is in the form of a cubo-columnar epithelium. Two of the cells contain a zooxanthella (z). Other algae cells nearby appear to have been recently extruded.

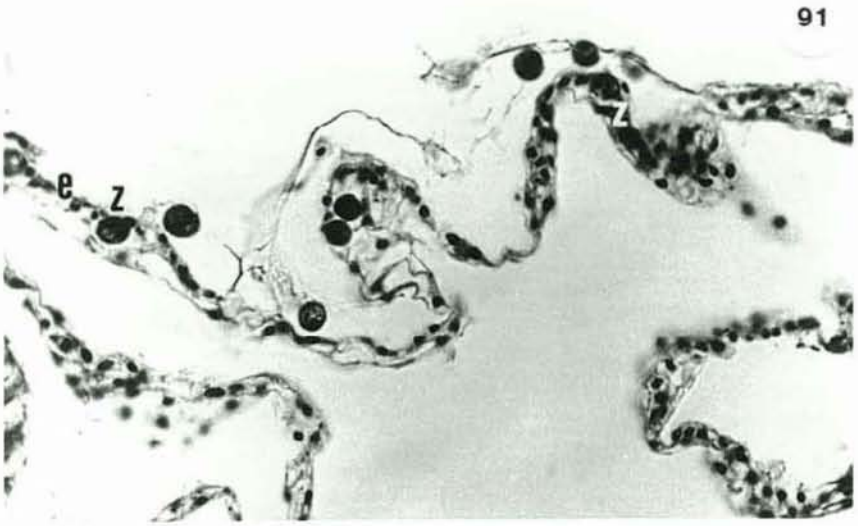
Figure 92: Nematocysts in repairing gastrodermis 15 hours after removal of the tip (x 1250).

The gastrodermis (g) contains a holotrichous isorhiza (h) which partly obscures the nuclei of two underlying gastrodermal cells. The epidermal cells (e) are extremely attenuated, they are contiguously arranged in a thin squamous epithelium. There is no evidence of a mesogleal layer.

Figure 93: Nematocysts in repaired gastrodermis 38 hours after removal of the tip (x 200).

The section is poorly preserved and the tissue has been stretched over the reformed trabecular tine(tt). The epidermis (e) and gastrodermis (g) are fully repaired. The epidermis contains spirocysts (sp) in the distal part of the layer and holotrichous isorhizae (h) proximally. The gastrodermis also contains holotrichous isorhizae which are oriented perpendicularly to the mesoglea (m) in the outer body wall, and parallel to it alongside the tine.

e-epidermis; g-gastrodermis; h-holotrichous isorhiza; m-mesoglea; sp-spirocyst; tt-trabecular tine; z-zooxanthella.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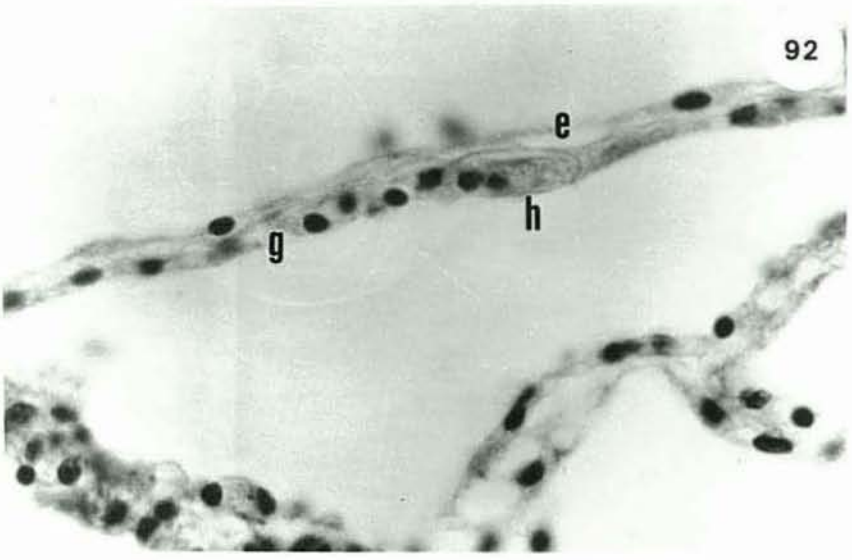
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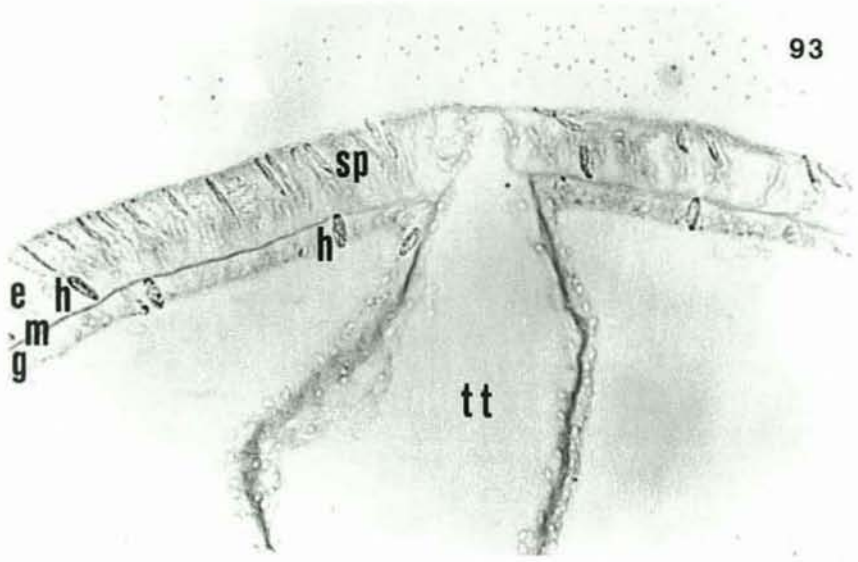
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Figure 94: Mucus secretion over a severed branch end 13 hours after removal of the tip (x 500).

A conspicuous layer of mucus (xmu) seals the end of the branch, it lies across a sealed portion of the coelenteron (ce) and an unsealed site of a trabecular column (tc). Cellular debris bound by mucus is present in the crevices of the broken surface (arrow).

Figure 95: Extrusion of type II mesenterial filaments from a severed branch end 11 hours after removal of the tip (x 500).

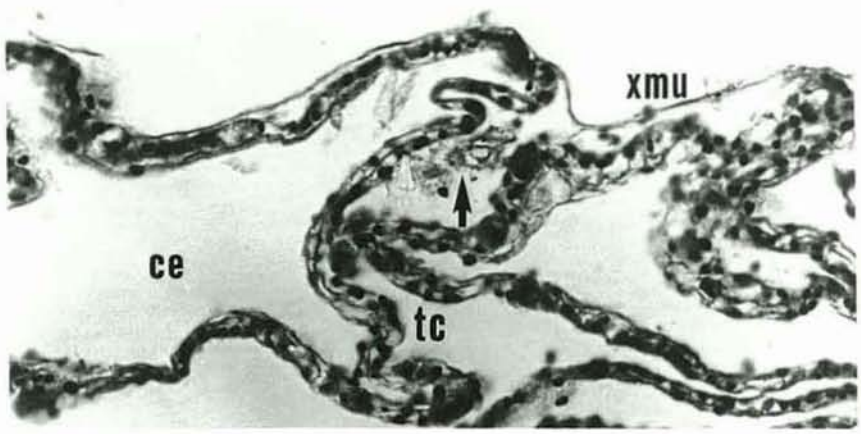
Extruded type II mesenterial filaments (II) on the surface of the branch are bounded by a layer of mucus (xmu). Types A (gr_A) and B (gr_B) granular gland cells are present in their cnidoglandular bands.

Figure 96: Detail of mucus exuded from a branch end 6 hours after removal of the tip (x 200).

Mucus (xmu) exuded from the broken branch end is laden with bacteria, zooxanthellae and other algae, including diatoms. The coelenteron (ce) is sealed but the site of a trabecular column (tc) is still open to the exterior. A second column and a septal trabecula (tr) are sectioned obliquely.

ce-coelenteron; gr-granular gland cell (subscript gives type); tc-trabecular column; tr-septal trabecula; xmu-exuded mucus; II-type II mesenterial filament; arrow-cellular debris.

94



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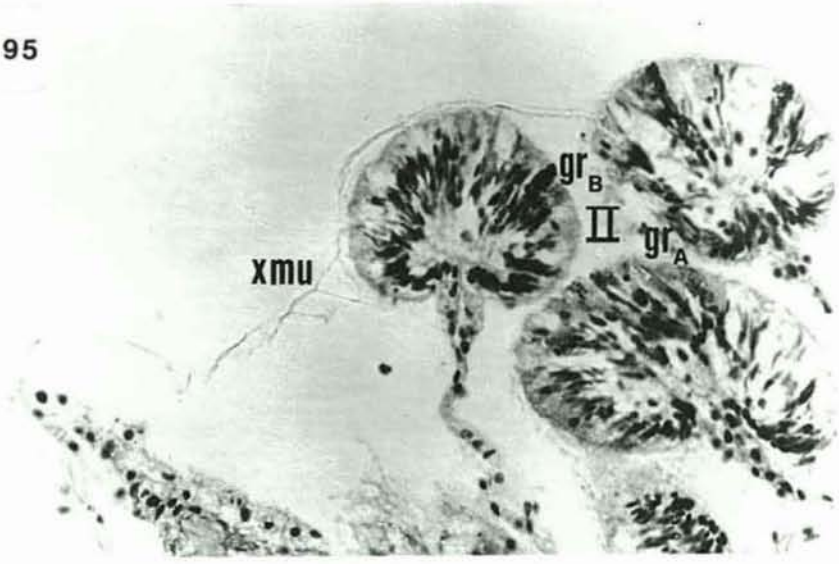

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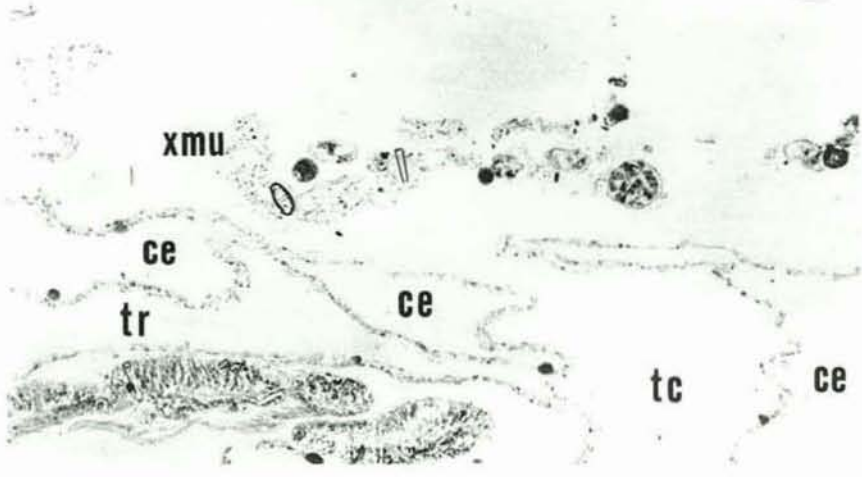


Figure 97: Different stages in the progress of repair between the trabecular columns of successive synapticulothecal rings of A. formosa 27 hours after removal of the tip (x 79).

The tissue on the right of the section is undamaged (u) and is situated at the edge of the wound (x). The section passes through the sites of the trabecular columns of two synapticulothecal rings (sr_1 and sr_2). The tissue between the rings is well repaired, the gastrodermis (g) appears normal and the epidermal cells (e) are almost re-oriented with their long axiis perpendicular to the mesoglea (m). The innermost tissue (left side) is less well repaired, the gastrodermis is cubo-columnar, but the epidermis is squamous in form.

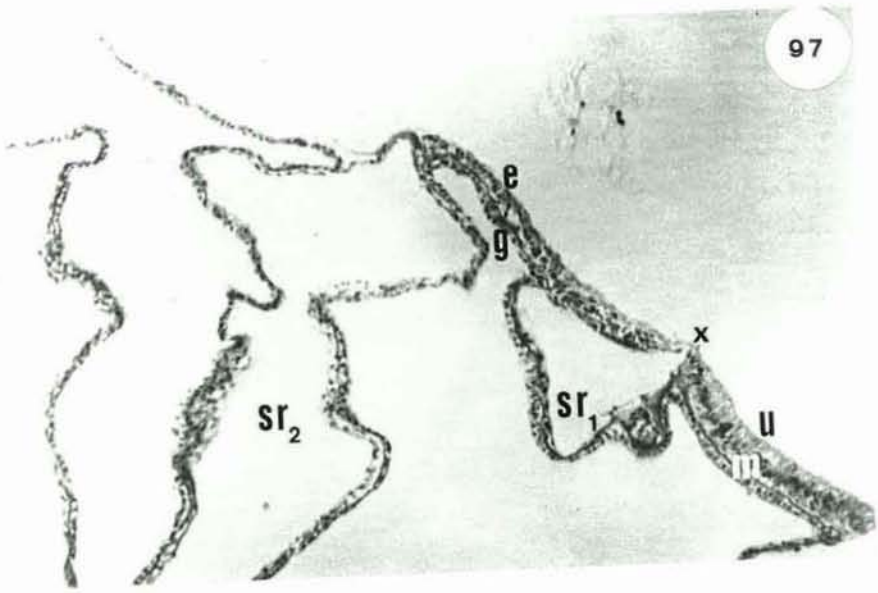
Figure 98: Gastrodermal cells sealing the coelenteron 13 hours after removal of the tip (x 1250).

A single layer of gastrodermal cells (g), some of which contain a zooxanthella (z), seals off a pocket of coelenteron (ce). A mucous layer (xmu) persists across the healing surface.

Figure 99: Epidermal cells from the wound edge migrating across the wound surface 13 hours after removal of the tip (x 1250).

Cells from the cubo-columnar epidermal epithelium (e) at the edge of the wound transform into squamous epithelial cells to migrate across the resealing surface in the direction of the arrow.

ce-coelenteron; e-epidermis; g-gastrodermis; m-mesoglea; sr-synapticulothecal ring; u-undamaged tissue; xmu-exuded mucus; z-zooxanthella; arrow-direction of migration of epidermal cells; x-wound edge.



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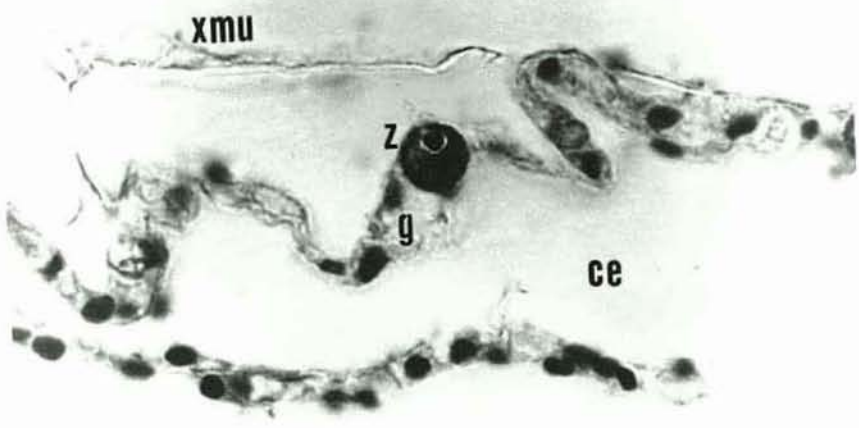

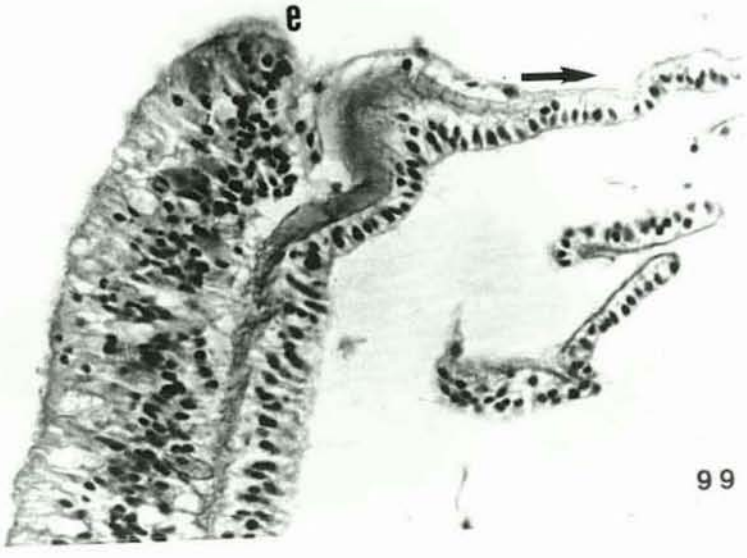

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Figure 100: State of repair of the tissues over the coelenteron 21 hours after removal of the tip (x 1250)

The gastrodermal cells (g) are cubo-columnar in form. The nuclei occur at approximately the same density as in normal gastrodermis but they almost fill the smaller sized cells. The epidermal layer (e) is complete and forms a flattened epithelium with few nuclei per unit length of tissue. No mesogleal layer is evident between the two epithelia.

Figure 101: State of repair of the tissue over the coelenteron 27 hours after removal of the tip (x 1250)

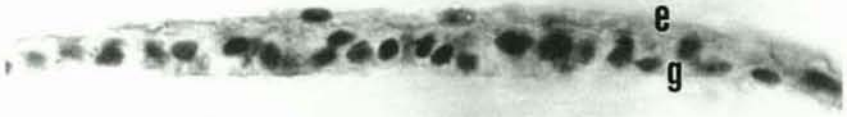
Both the epidermal (e) and gastrodermal (g) cells are cubo-columnar in form and the presence of the mesoglea (m) is indicated by a faint line. A holotrichous isorhiza (h) lies in the epidermis with its longitudinal axis parallel to the mesoglea.

Figure 102: State of repair of the tissues over the coelenteron 34 hours after removal of the tip (x 1250)

The epidermal cells (e) are midway between cubo-columnar and columnar in form. The gastrodermis is in a similar state to that shown in Figure 100 Two holotrichous isorhizae (h), oriented obliquely to the mesoglea (m), and distorted unstained (type 1) mucous gland cells (mu_1) are present in the epidermis.

e-epidermis; g-gastrodermis; h-holotrichous isorhiza; m-mesoglea; mu-mucous gland cell (subscript denotes type).

100



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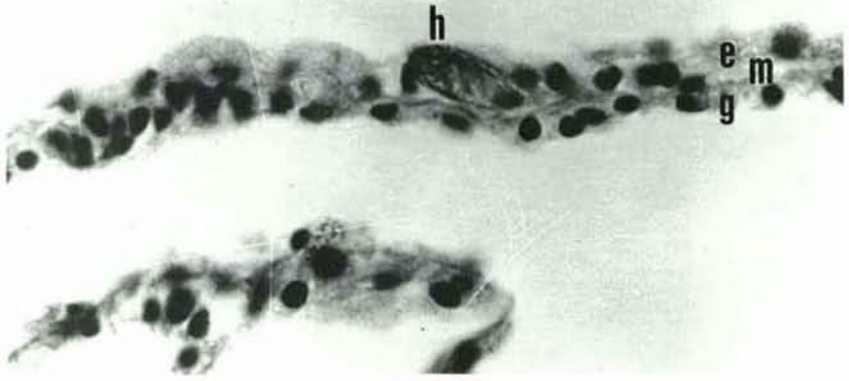

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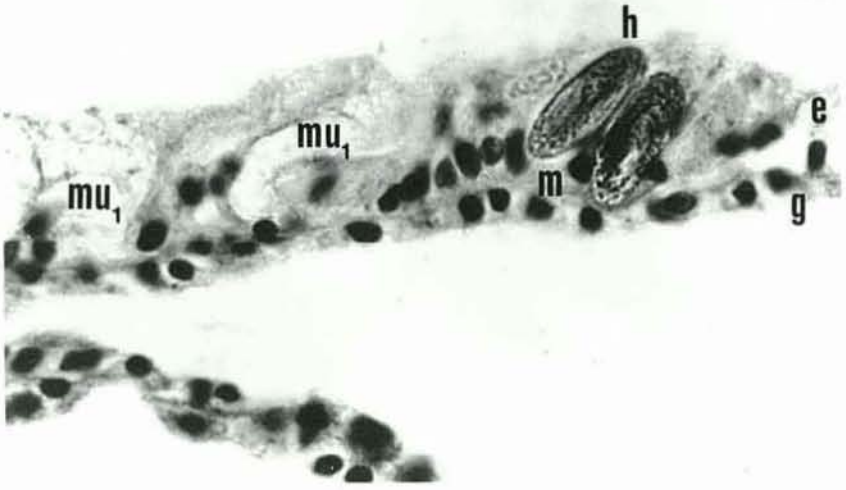


Figure 103: State of repair of the tissues over the site of a trabecular column 27 hours after removal of the tip (x 1250)

The gastrodermis (g) on either side of the site of the severed trabecular column (tc) forms a well developed cubocolumnar epithelium. In contrast, the adjacent calicoblastic layer (c) is torn and disrupted. Epidermal cells (e) are stretched over the gap formerly occupied by the trabecular column, their direction of migration is indicated by the arrow. The cells are greatly attenuated and are in contact only at their extreme ends.

Figure 104: State of repair of the tissues over the site of a trabecular column 31 hours after removal of the tip (x 1250)

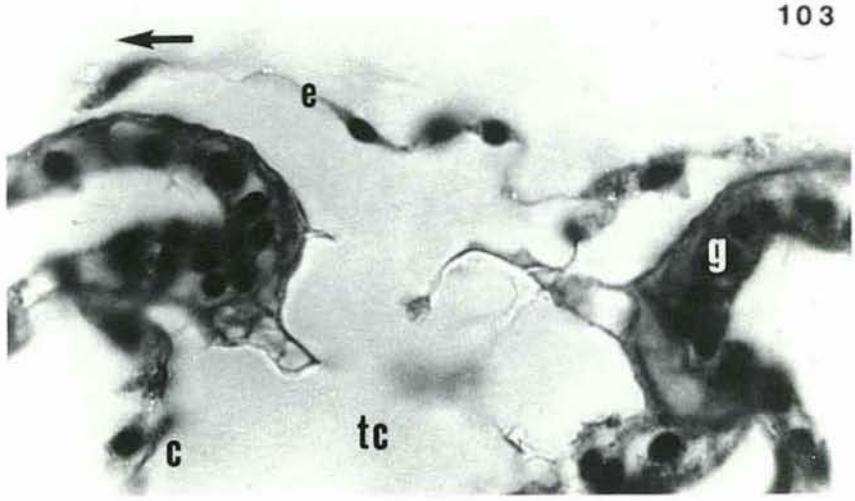
Both the epidermis (e) and the calicoblastic layer (c) form a thin, squamous epithelium over the site of the skeleton. No mesoglea is evident between the two layers.

Figure 105: State of repair of the tissues over the site of a trabecular column 34 hours after removal of the tip (x 500)

Since the tissue is reformed, the trabecular column is presumably in the process of regenerating, thus creating a new trabecular tine. The site of the skeleton is therefore labelled accordingly (tt). The calicoblastic layer (c) is completely regenerated and a new mesoglea (m) is just visible. There is a difference in the organisation of the epidermis (e) on either side of the tine (c.f. Figure 97). On the right hand side it is beginning to assume a cubo-columnar arrangement, but on the left hand side, which is nearer the centre of the branch, it is still squamous in form and contains a horizontally-oriented holotrichous isorhiza (h).

c-calicoblastic layer; e-epidermis; h-holotrichous isorhiza; m-mesoglea; tc-trabecular column; tt-trabecular tine; arrow direction of migration.

103



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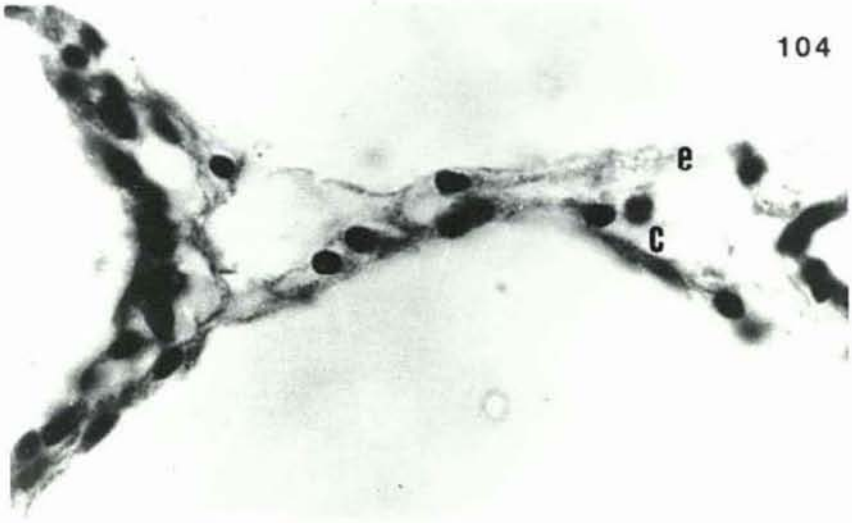

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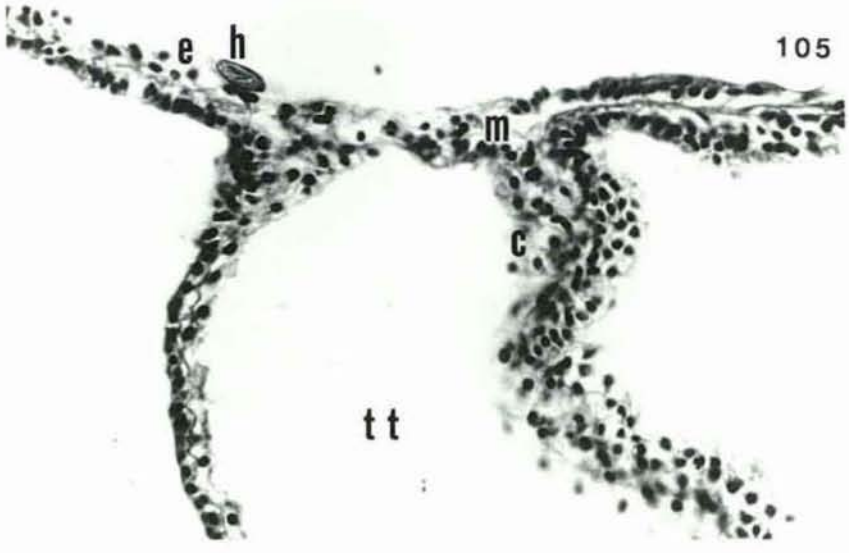


Figure 106: Acidophilic granules and granule-containing vacuoles in repairing tissues 27 hours after removal of the tip (x 1250).

Groups of small (0.2-0.7μm) acidophilic granules (arrows) are present throughout the tissues. Some are localised near the presumed site of the reforming mesoglea (m).

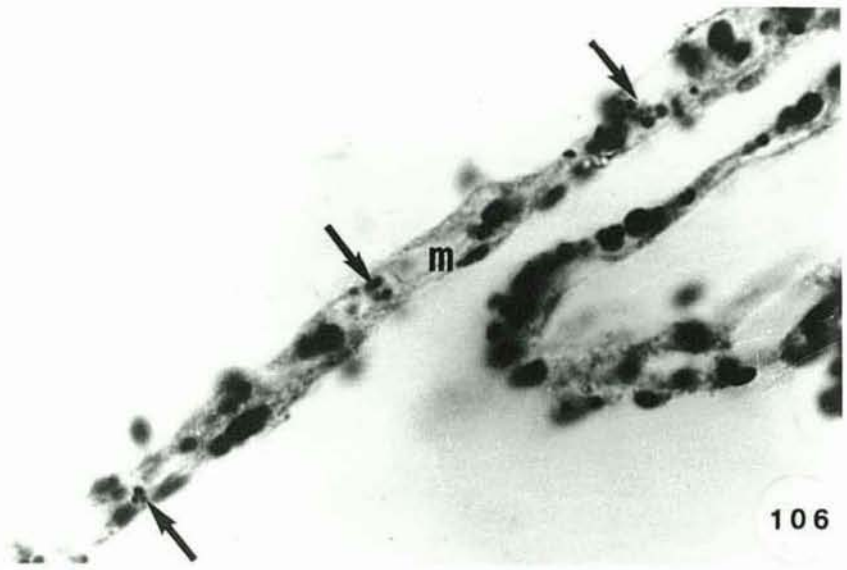
Figure 107: A mesogleal amoebocyte in repairing tissue 31 hours after removal of the tip (x 1250).

An amoebocyte (a) is present in the mesoglea (m). It is filled with granules similar to those shown in Figure 106. Such granules are absent from the rest of the tissue. The epidermis (e) is not well repaired and forms a squamous epithelium, although the gastrodermis (g) has assumed its usual cubo-columnar shape.

Figure 108: Lateral lobes of a type I mesenterial filament near the repairing surface 31 hours after removal of the tip (x 1250).

Small acidophilic granules (small arrows) resembling those in Figures 106 and 107 are present in the absorption-excretion region (ae) of the lateral lobes (11) of a type I mesenterial filament.

a-amoebocyte; ae-absorption-excretion region of lateral lobes; e-epidermis; g-gastrodermis; ll-lateral lobes of mesenterial filament; m-mesoglea; small arrow-acidophilic granule; large arrow-group of acidophilic granules.



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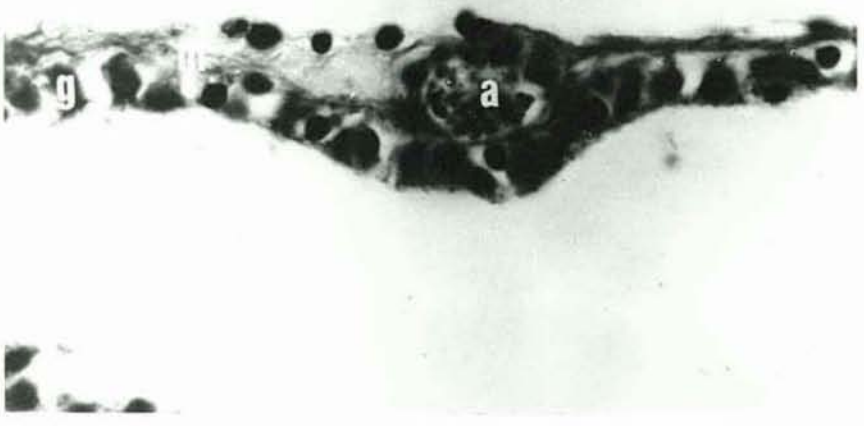


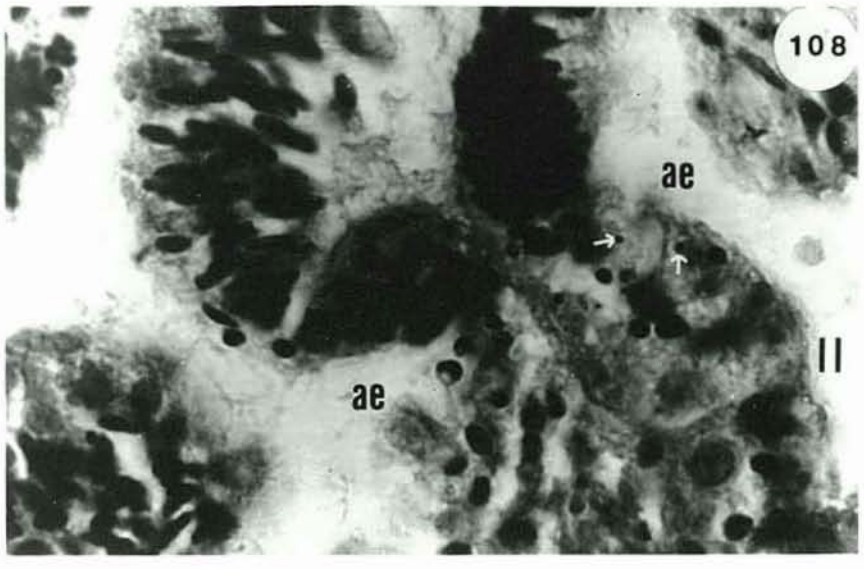


Figure 109: State of repair of outer body wall 34 hours after removal of the tip (x 500)

The epidermis (e) of the outer body wall is columnar on either side of the site of the trabecular tine (tt). Interstitial cells (i) are present amongst the basal processes of the columnar supporting cells. The area over the tine tip is rather disorganised. The calicoblastic layer (c) in this region is in the process of reforming, and the mesoglea (m) is very diffuse. The position is shown of a septal trabeculum (tr) which diverges out from the trabecular column (tc).

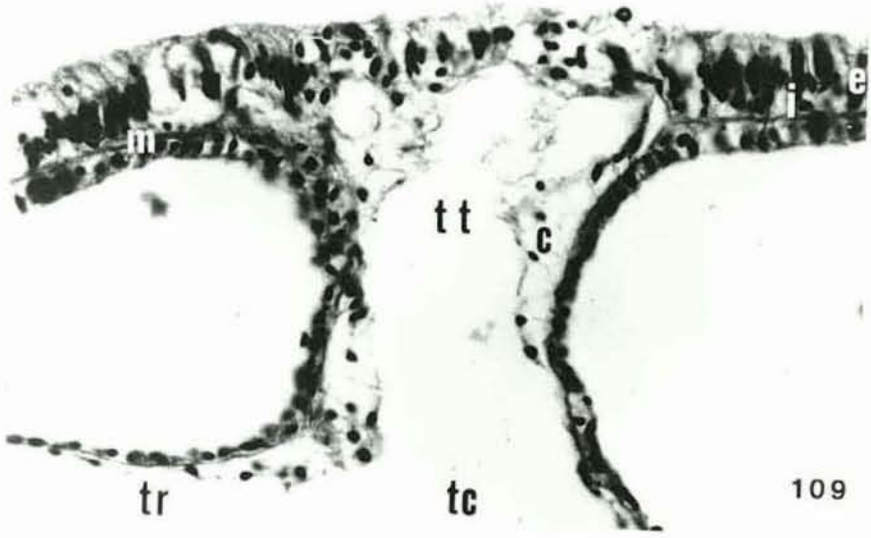
Figure 110: State of repair of the outer body wall 38 hours after removal of the tip (x 500)

The epidermis (e) is almost normal in appearance, although the nuclear band of the columnar supporting cells is slightly more diffuse than usual. More interstitial cells (i) are present than in Figure 109. The calicoblastic layer (c) and the mesoglea (m) are completely regenerated over the site of the trabecular tine (tt). The positions of two septal trabeculae (tr_1 and tr_2) are also shown.

Figure 111: Differentiating interstitial cells in the pre-wound epidermis 31 hours after removal of the tip (x 500)

A mixture of mature holotrichous isorhizae (h) and developing cnidoblasts (in) are present amongst large numbers of undifferentiated interstitial cells (i) in the undamaged epidermis (u) in the pre-wound region of the branch. Two mature-appearing holotrichous isorhizae are in the process of migration across the wound surface and have adopted an orientation horizontal to the mesoglea (m) to do so.

c-calicoblastic layer; e-epidermis; h-holotrichous isorhiza; i-interstitial cell; in-incipient nematocyst; m-mesoglea; tc-trabecular column; tr-septal trabeculum; tt-trabecular tine; u-undamaged tissue.



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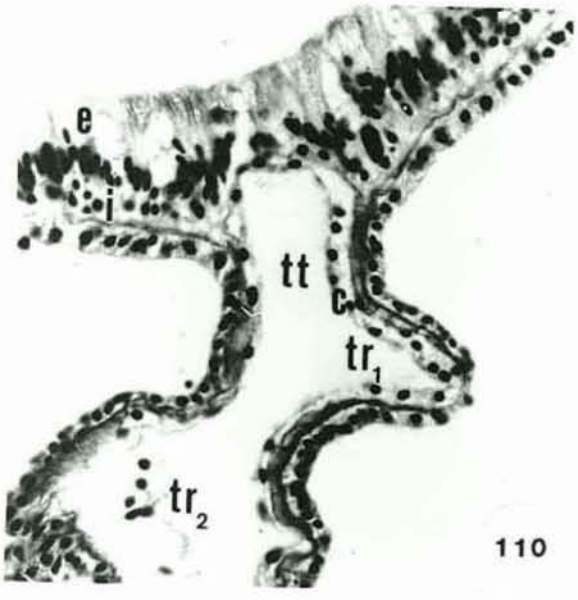

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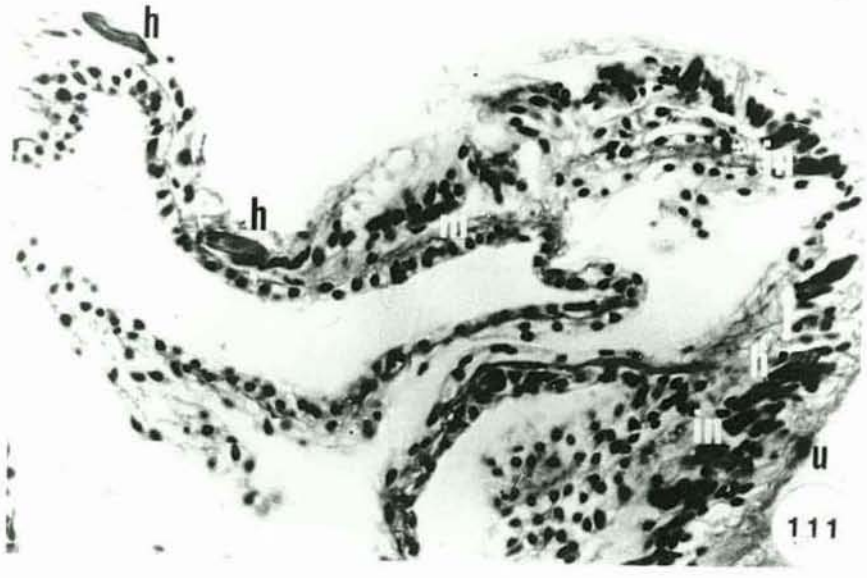


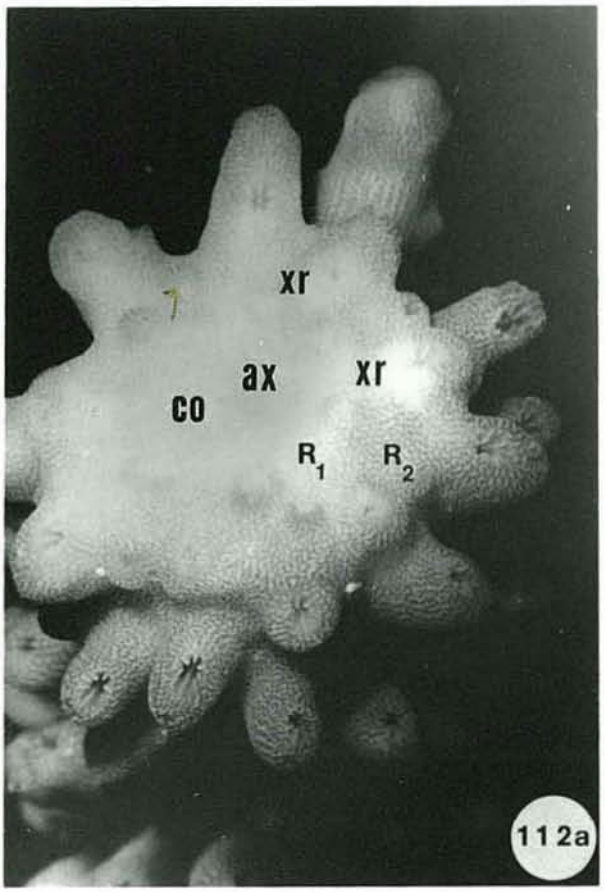
111

Figure 112: Broken branch end of A. formosa after 18 days regeneration

(a) Macroscopic view (x6.3)
Zooxanthellae are absent from the tissue of the coenenchyme around the axial corallite (site marked ax, contrast does not allow the axial corallite to be clearly discerned) and the innermost circle of radial corallites (R_1). The second ring of radial corallites (R_2) contains damaged (xr) and undamaged corallites. The undamaged corallites around the periphery of the branch contain zooxanthellae throughout their tissues, whilst the damaged corallites have few zooxanthellae associated with their broken calices.

(b) Longitudinal section through the axial polyp (x12.6)
The edges of the axial polyp (ax) and a radial polyp (r) are slightly raised above the wound surface (between crosses). The whole surface is covered with a layer of tissue.

ax-axial polyp/corallite; co-coenenchyme; r-radial polyp/corallite; R-circle of radial corallites; xr-broken radial corallite; x-edge of wound.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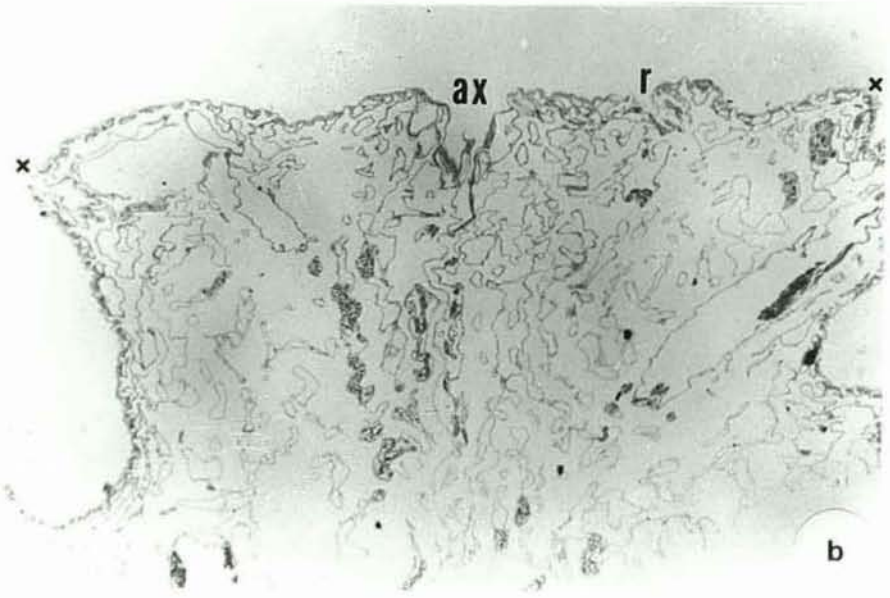
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Figure 113: Broken branch end of A. formosa after 26 days regeneration

(a) Macroscopic view (x 6.3)

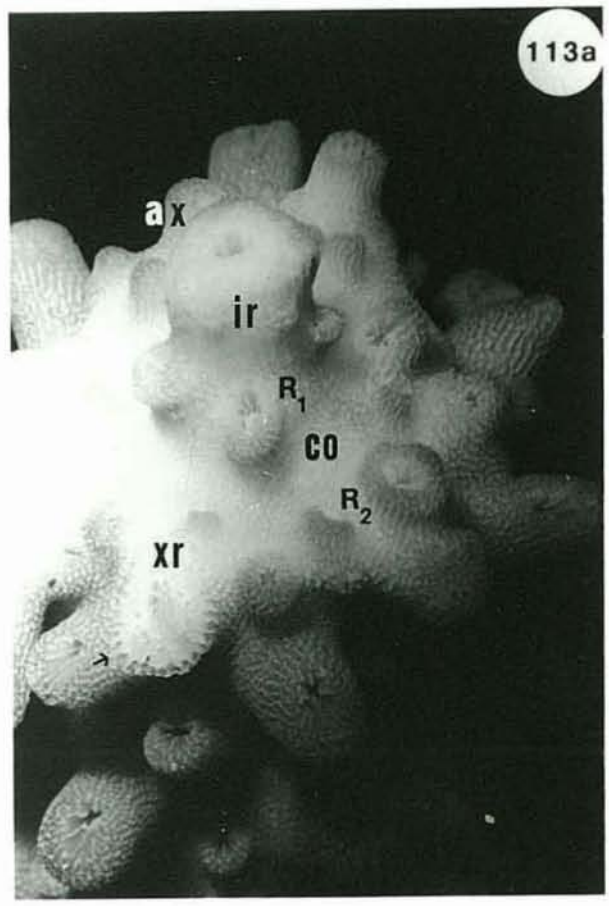
The regenerating axial corallite (ax) is 4mm long. Zooxanthellae have spread into the tissue of the coenenchyme (co) which covers the original broken surface, and they are present in the first circle of radial corallites (R_1). A new circle of radial corallites is beginning to form near the tip. The tissue over these incipient radial corallites (ir) and around the axial corallite does not contain algae. The orientation of the second circle of radial corallites (R_2) is markedly disrupted.

In the foreground a radial corallite (xr) has been broken transversely after fixation, the tissue can be seen to be supported on the edges of the costae (arrow).

(b) Oblique longitudinal section through the axial polyp (x 11)

The area formerly occupied by the skeleton (sk) shows that the lower part of the branch is relatively heavily calcified with only small pockets of coelenteron (ce) present. The line of fracture may be discerned from the abrupt transition to a more lightly calcified structure in the regenerated portion of the branch (between arrows). Above the fracture line the proportion of coelenteron area to skeleton area increases to approximately 1:1. It is also clear that the whole branch end has extended not just the central portion of the branch.

ax-axial polyp/corallite; ce-coelenteron; co-coenenchyme; ir-incipient radial corallite; r-radial polyp/corallite; R-circle of radial corallites; sk-skeleton; xr-damaged radial corallite; small arrow-costae; large arrow-line of fracture.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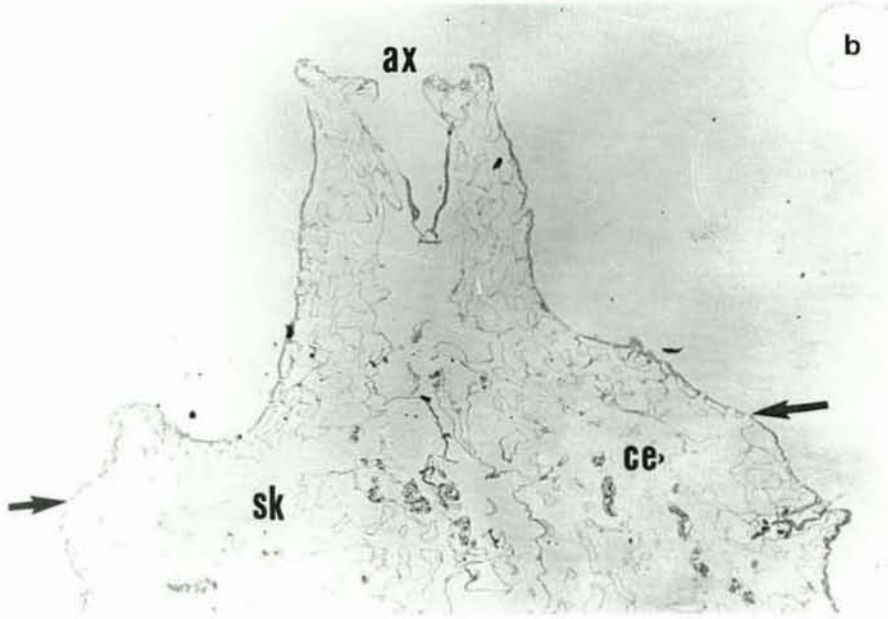
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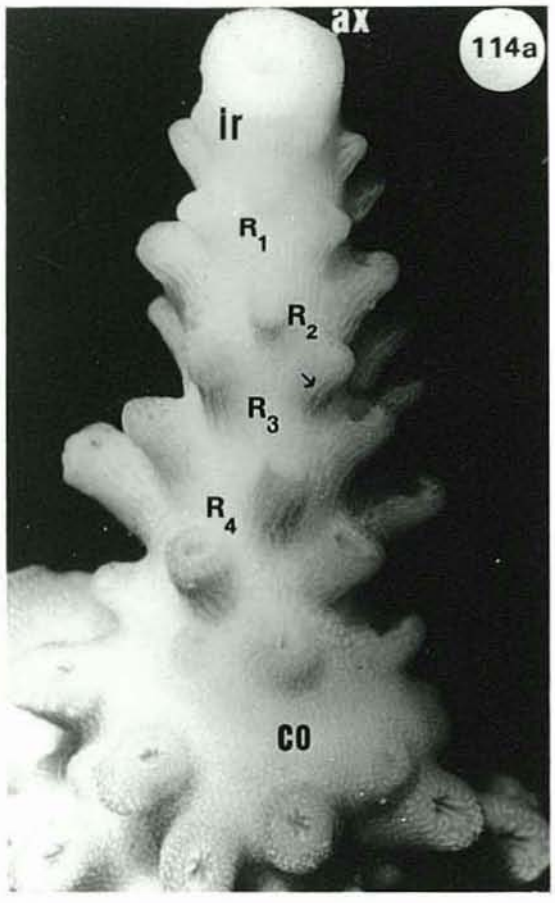
Figure 114: Broken branch end of A. formosa after 46 days regeneration

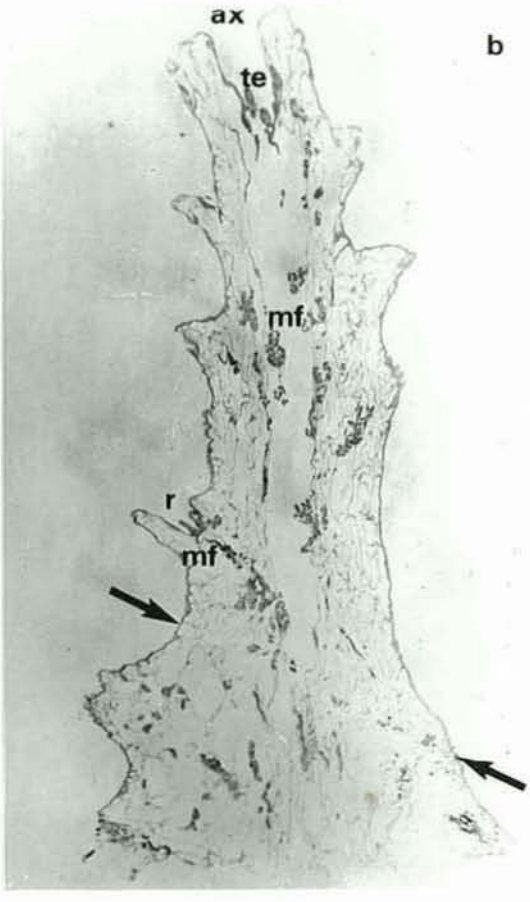
(a) Macroscopic view (x 6.3)
The coenosteum over the original broken surface is highly elaborate and the coenosarc is completely infiltrated with zooxanthellae. In the regenerated portion (approx 120mm in length) the coenosteum is costate (arrow). Zooxanthellae are present throughout the tissue up to the level of the fourth circle of radial corallites (R_4). Between circles R_2 and R_4 the algae are concentrated in the radial polyps. There are virtually no zooxanthellae present at or above the first circle of radial corallites (R_1), which makes the formation of incipient radial corallites (ir) difficult to discern from the photograph except in profile.

(b) Longitudinal section through the axial polyp (x 5.6)
The line of fracture (between arrows) is indicated (as in Figure 113b) by a transition from a heavily calcified structure in the original part of the branch, to lightly calcified in the new growth. In the latter, the tentacles (te) and mesenterial filaments (mf) have regenerated in both the axial (ax) and the radial (r) corallites (The apparent curvature of the new portion is an artefact produced during sectioning).

Figure 115: Broken branch end of A. formosa after 84 days regeneration (scale bar; = 50mm)
The regenerated portion is 24mm long. There is only a slight indentation and a small amount of disorganisation amongst the radial corallites to indicate where the break (x) occurred. The coenosarc at the extreme tip is free of zooxanthellae; however, they are present elsewhere. There is a continuous proximal gradation of colour from light to dark brown along the branch.

ax-axial corallite; co-coenenchyme; mf-mesenterial filament; r-radial corallite; R-circle of radial corallites; te-tentacle; small arrow-costa; large arrow-line of fracture; x-point of breakage.


ax
114a
ir
R1
R2
R3
R4
co


ax
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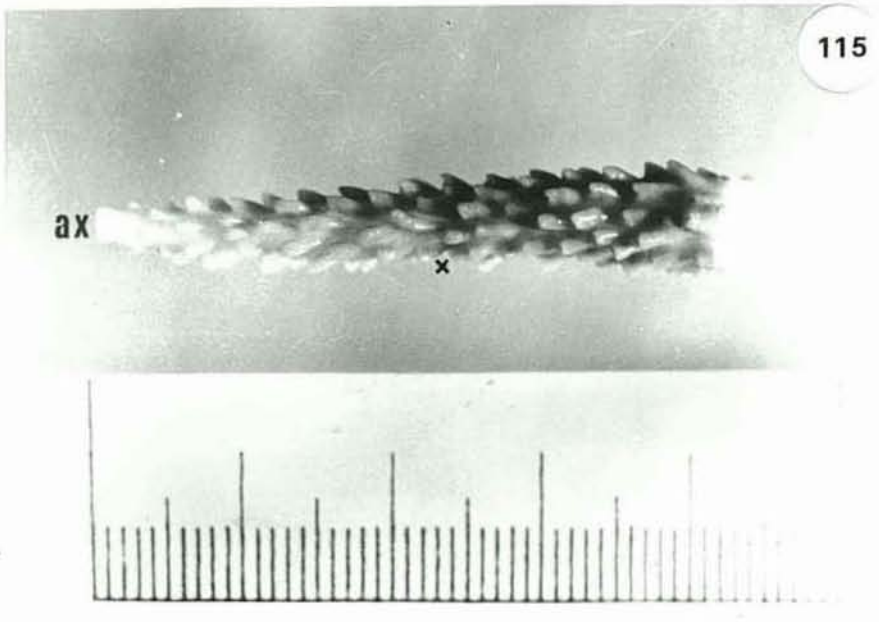
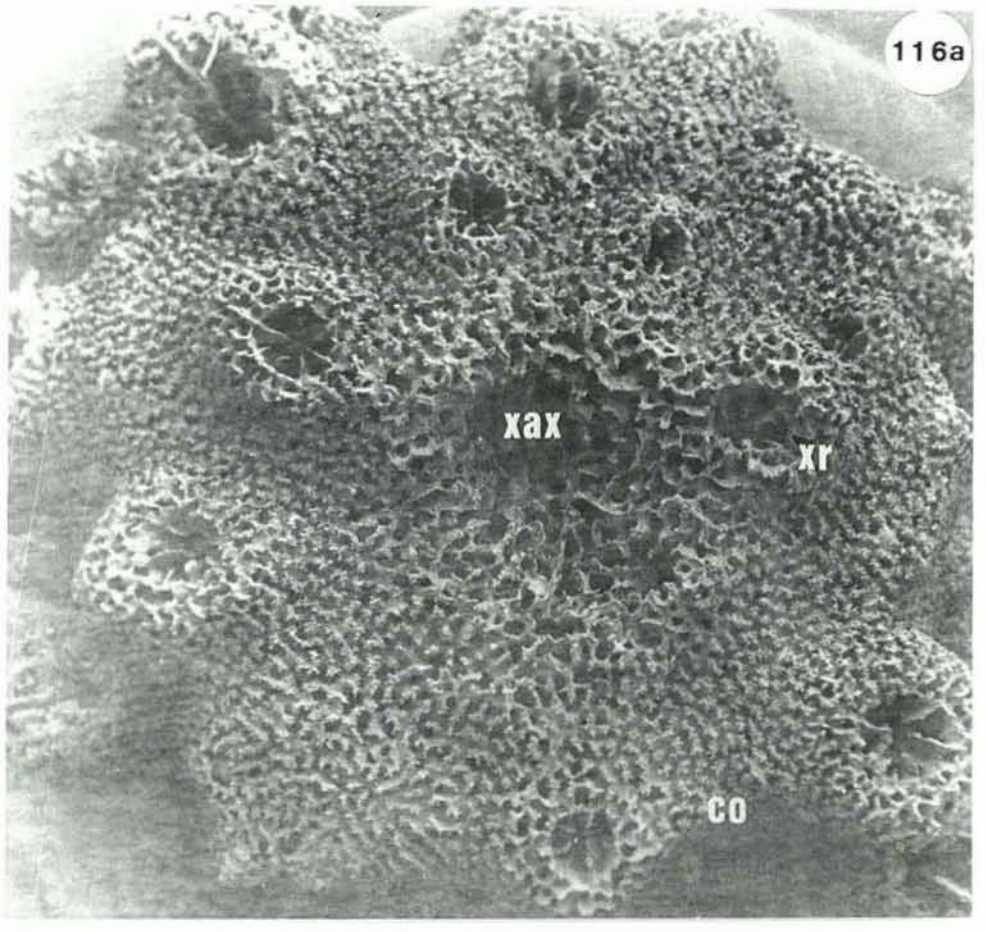

115
ax
x

Figure 116: Skeleton of a branch of A. formosa after 8 days regeneration

(a) low power (x25)
The edges of the severed axial (xax) and radial (xr) corallites are rounded. The surrounding coenosteum (co) is densely spinulose and there has been some stereome deposition between the spines. The spines are less obviously elaborate towards the centre of the branch.

(b) high power (x 270)
The axial corallite is located off the photograph to the right. The complexity of the elaborations on the coenosteal spines increases with distance from the axial corallite. Stereome (sd) overlays all skeletal linkages.

co-coenosteum; sd-stereome deposition; xax-broken axial corallite; xr-broken radial corallite.


116a
xax
xr
co

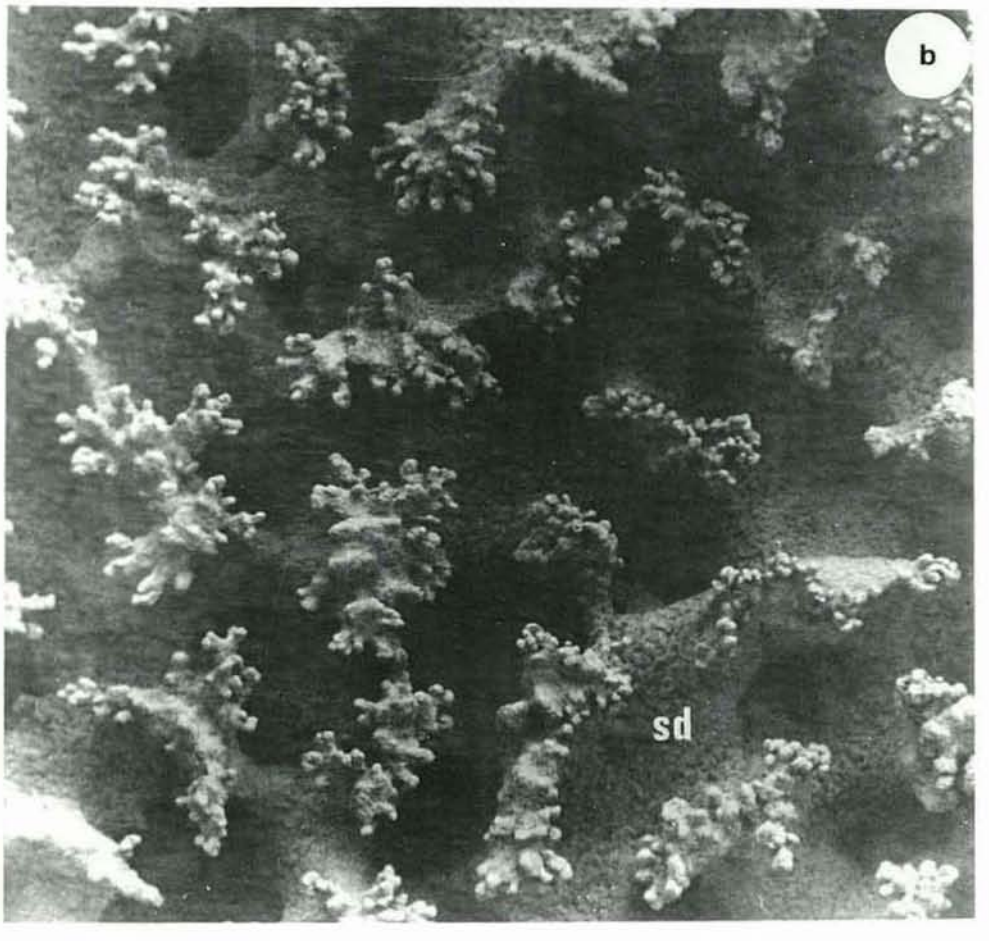

b
sd

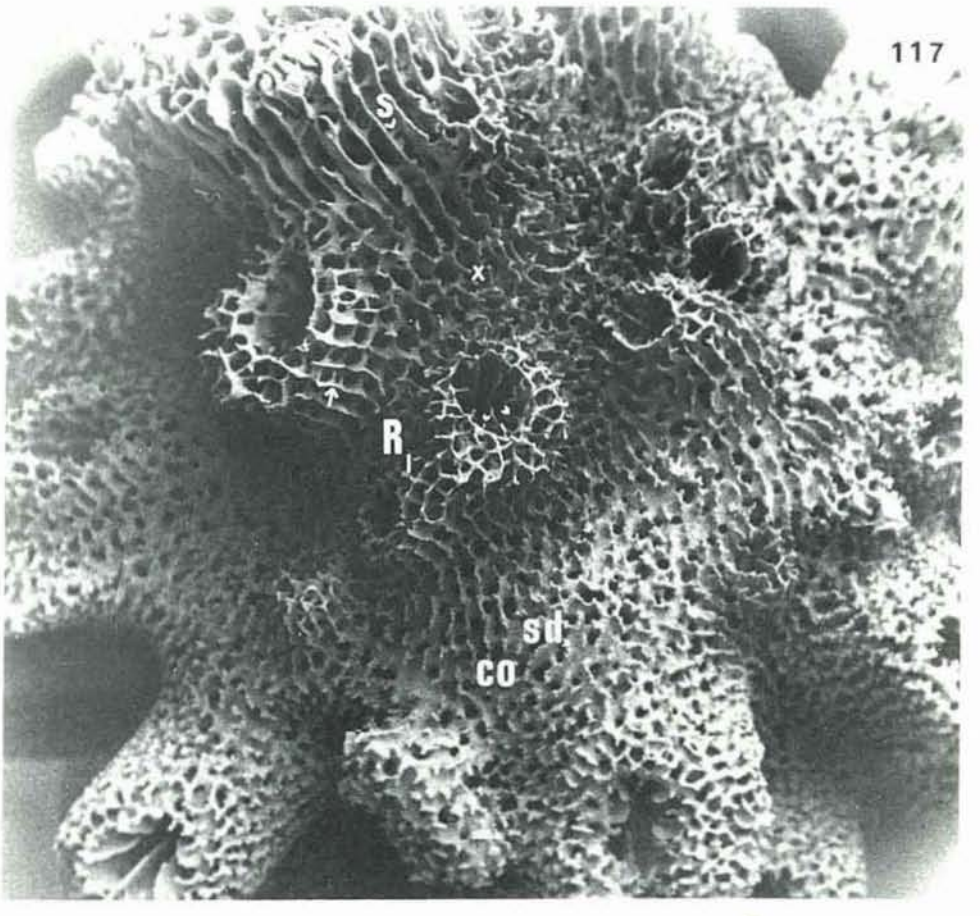
Figure 117: Skeleton of a branch end of A. formosa after 26 days regeneration (x 25)

As in Figure 116a, sterome (sd) has been deposited between the spines on the coenosteum (co) over the original broken surface. The coenosteum of the regenerated portion is costate (arrow). Near the tip the margins of the costae are smooth. Costal spines (x) are present at and below the level of the first cycle of radial corallites (R_1). Each corallite has 3 synapticulothecal rings in various stages of development. The radial corallite in the centre of the photograph has a complete cycle of primary septa and the first pair of a secondary cycle are present on either side of the outer directive septum (pointers). The synapticulae (s) of the tip region are thin and widely spaced, creating large fenestrations.

Figure 118: Skeleton of a branch end of A. formosa after 46 days regeneration (x 25)

The axial corallite is fully developed at the tip with 2 complete cycles of septa in the calice and 3 synapticulothecal rings in the wall. The costae (arrow) and the synapticulae (s) are more heavily calcified than in Figure 117 and the fenestrations are accordingly reduced in size. There are 2 radial corallites (ir) developing approximately 2mm from the tip. The corallites of the first circle of radial corallites (R_I) have 2 incomplete synapticulothecal rings and a complete cycle of primary septa. Those in the second circle (R_{II}) have 3 synapticulothecal rings (one of which is complete). The corallite to the right of the "co" label has a complete cycle of primary septa and a fully developed secondary cycle, only three of the latter are visible (pointers). The coenosteum between the first and second circle of radial corallites is costate. The margins of the costae appear serrated because of the presence of large numbers of costal spines. Below the second circle of radial corallites the coenosteum is spinulose and overlaid with stereome (sd). The third layer of radial corallites (R_{III}) is embedded in this.

co-coenosteum; ir-incipient radial corallite; R-circle of radial corallites; s-synapticuale; sd-stereome deposition; arrow-costa; pointer-secondary septum; x-costal spine.


117
s
x
R
sd
co

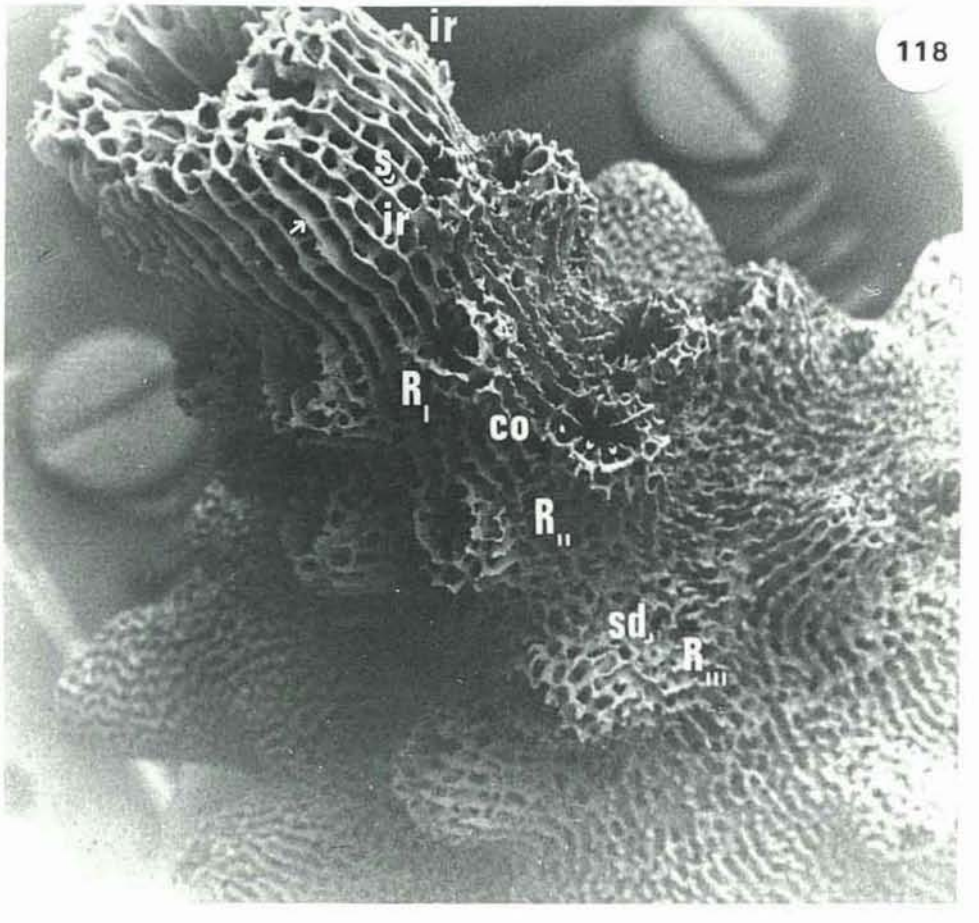

118
ir
s
ir
R
co
R
sd
R

Figure 119: Broken branch end of A. formosa 7 days after colonisation by filamentous algae (x 6.3)

A rim of coenenchyme (co) has formed at the periphery of the branch, adjacent to the infecting algae (al). There are no zooxanthellae present in the overlying coenosarc.

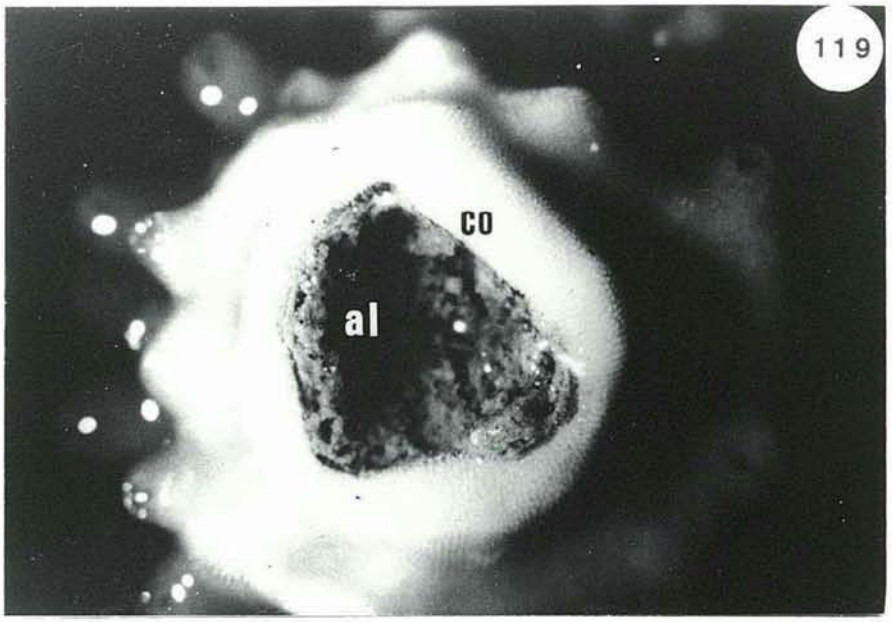
Figure 120: Broken branch end of A. formosa 23 days after colonisation by filamentous algae (x 6.3).

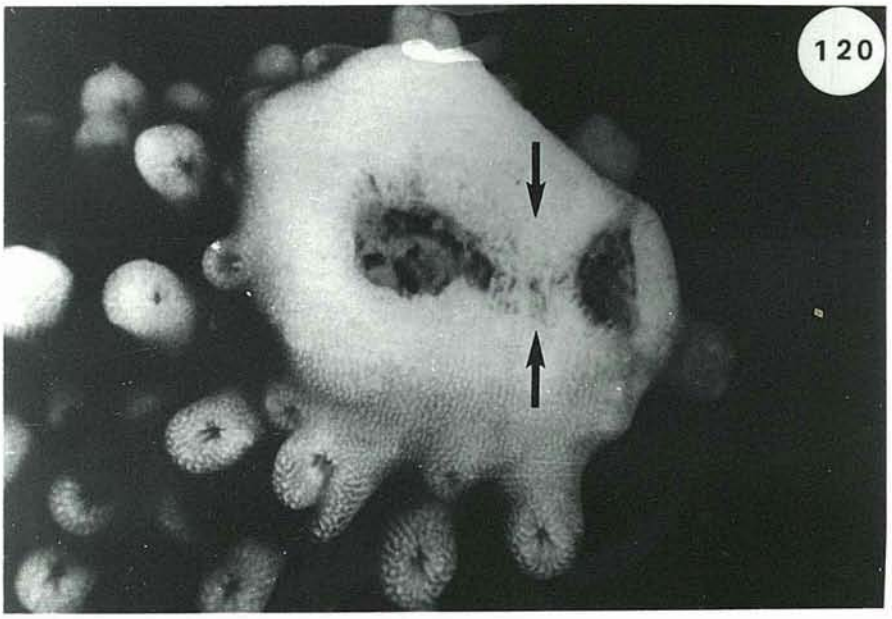
Undifferentiated coenenchyme growth is extending over the algal surface, sealing it off (between arrows)

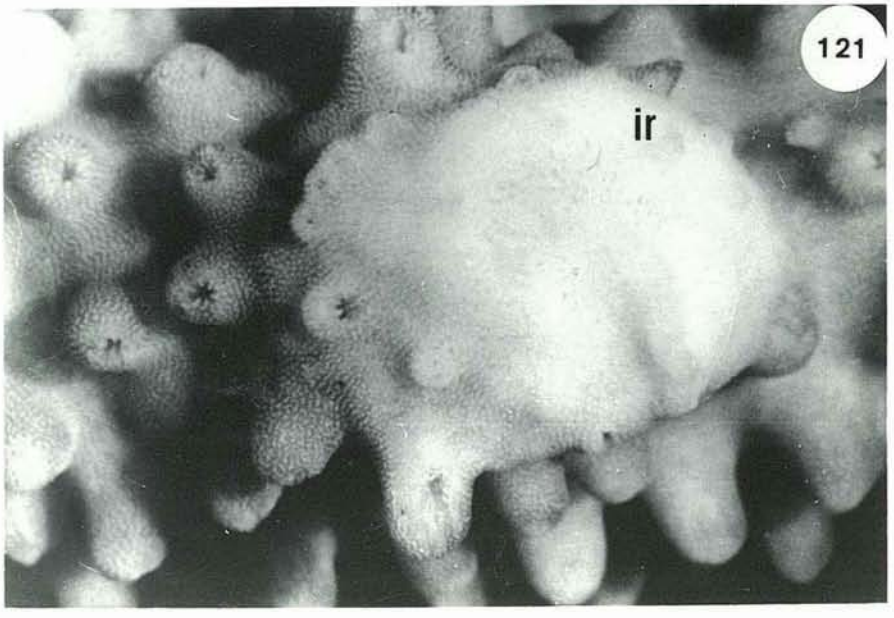
Figure 121: Broken branch end of A. formosa 40 days after colonisation by filamentous algae (x 6.3)

The whole branch end is covered with a layer of coenosteum which has trapped the colonising algae underneath. The small protrusions on the surface are early stages in the regeneration of new radial corallites (ir).

al-filamentous algae; co-coenenchyme ir-incipient radial coral-lites; arrows-direction of coenenchymal growth.


119
co
al


120


121
ir

7.5 Discussion

7.5.1 Cell division and differentiation during repair

Two independent reports exist of a brief burst of mitotic division in hydra following severance of the hypostome. The first, 45 minutes after cutting (Ham and Eakin, 1958) was not confirmed by the later work of Clarkson (1969). The second, 5 hours after cutting (Rowley, 1902), appears similar to that observed in this study. However, Rowley concluded that no reliable inferences could be drawn from this occurrence since as in the experiments reported here it was observed in only one specimen.

Subsequent work has shown that cell division is not an essential prerequisite for repair in coelenterates. For example, in both hydra (Clarkson, 1969; Park, et al, 1967) and anemones (Singer, 1971; Young, 1974) no increase in cell proliferation was observed in response to wounding until at least 24 hours had elapsed. In hydra the hypostome and tentacles were completely regenerated in that time, and subsequent cell division over the entire animal replenished the stock of cells (Spangenberg and Eakin, 1962). The anemone Aiptasia diaphana required 24 hours to reseal itself, and there was no regeneration of the polyp during the first 24 hours after cutting. Cellular material for the regeneration of the oral disc and tentacles which followed was mainly provided by the division of interstitial cells (Singer, 1971). Complete regeneration took a further 7-10 days (Singer and Palmer, 1969).

From the above studies it appears that in both hydra, and in anemones which have interstitial cells, tissue repair is effected entirely by the migration of epithelial cells and differentiation of interstitial cells, whereas regeneration generally requires cell division. Cheney (1974) observed a 48 hour delay before cell division occurred in the scleractinian coral Pocillopora damicornis in response to damage caused by handling. On this basis, it can be assumed that the single mitotic figure observed in this part of the present study may be atypical and that repair in A. formosa probably follows the same pattern as that previously described for the Hydrozoa and Actiniaria.

7.5.2 Exchange of cells between layers during repair

The contribution of gastrodermal cells to the reforming epidermis as reported here has previously been reported for both anemones (Singer, 1971) and hydra (Haynes and Burnett, 1963). Electron microscopic studies of epithelial explants in hydra (Haynes and Burnett, 1963; Davis, et al, 1966) have described subsequent transformation of gastrodermal nutritive cells to epidermal epitheliomuscular cells. The process involved the removal of food vacuoles, the evolution of mucus droplets and, of course, the loss of algal symbionts. The observation in this study of epithelial cells located in the epidermis which appear to have recently extruded zooxanthellae strongly suggests that a similar process occurs during early stages of repair in A. formosa.

The significance of the presence of nematocysts in the gastrodermis of A. formosa at a later stage of repair is more difficult to evaluate. From studies of hydrozoans (Tardent, 1954; Burnett, 1959; Slautterback and Fawcett, 1959; Slautterback, 1961) and anemones (Westfall, 1966), interstitial cells are well known to be the source of cnidoblasts. However, such interstitial cells have been shown to be derived from a variety of other cells, including gastrodermal epithelial cells (Haynes and Burnett 1963; Davis, et al, 1966) and both epidermal (Burnett, et al, 1966) or gastrodermal (Davis, 1970; Lesh, 1970) mucous gland cells. Thus, the regeneration of the full gastrodermal cell complement has several potential alternative routes, by virtue of the extraordinary plasticity of coelenterate cells which, in addition to allowing the direct conversion of one cell type into another, as described above, also permits dedifferentiation from one cell type into an interstitial cell then redifferentiation into another, entirely different type of cell.

Based on his observation that there was no increase in the number of ^{3}H-thymidine labelled gastrodermal cells in wounded specimens of the anemone Aiptasia diaphana, Singer (1971) suggested that the repairing gastrodermis is supplemented by the movement of cells from the epidermis to the gastrodermis

through the mesoglea. Singer's evidence came from observations of epidermal explants from hydroids where the epidermis has been shown to be capable of reforming the mesoglea and the gastrodermis (Zwilling, 1963; Hale, 1964; Lowell and Burnett, 1969; Polteva and Borisova, 1974) even after the interstitial cells have been removed (Normandin, 1960).

In A. formosa branches undergoing repair, any movement of epidermal cells, other than nematocysts, into the gastrodermis would be impossible to detect using light microscopy. At the stage at which this transfer might be expected to occur, all epithelial cells are cubo-columnar and therefore morphologically similar to normal nutritive cells. They would therefore remain undetected if they differentiated into gastrodermal epithelial cells. The more obvious transformation into gastrodermal mucous gland cells, which was observed in hydra (Lowell and Burnett, 1969) was not seen in the present investigation. However, this is not surprising since it would only occur infrequently, for there are characteristically few mucous gland cells in the tip region of A. formosa (Section 4.3.2).

The presence of nematocysts in the gastrodermis of A. formosa, where they clearly cannot be functional, may be the result of erroneous differentiation of interstitial cells, perhaps because of damage to the control mechanism after wounding. In that event, the cells would probably be resorbed in the same manner as discharged capsules (Blanquet and Lenhoff, 1966). Alternatively, these nematocysts may be able to dedifferentiate, and so represent a potential source of gastrodermal glands or epithelial cells after re-differentiation. Only an electron microscopic study could distinguish between these alternatives since it is only on the basis of the sub-microscopic changes preceding these events that they can be detected (see Davis, et al, 1966).

7.5.3 Contribution of different tissue layers and cell types to repair

(a) Gastrodermis

The gastrodermis has long been known to be responsible for the initial sealing of the coelenteron in wounded hydra (Rand,

1900; Mattes, 1925). The results presented here suggest that the same is true for A. formosa. Gastrodermal cells have previously been shown to be capable of limited independent migration in both anemones (Singer and Palmer, 1969) and hydra (Tardent, 1962). Papenfuss (1934) and Bibb and Campbell (1973) report that during sealing, attachment of the cells is initiated by the intermeshing of temporary superficial cell processes. The rapid formation of desmosomes then stabilises the contact. Since desmosomes have been observed between undamaged gastrodermal cells in A. formosa (Harrison, 1980), it is probable that a similar mechanism operates to reseal the branch end in the coral.

(b) Epidermis

The majority of cells in the repairing epidermis of A. formosa appear to arise from the migration of epithelial cells from the edge of the wound. This is consistent with observations of wound healing in mammals, where epithelial cells from the periphery of the wound migrate across the wound surface until they meet and fuse with other cells, thus sealing the broken surface (Johnson and McMinn, 1960; Krawczyk, 1971). This certainly occurs in both anemones (Young, 1974) and hydroids (Stevens, 1903; Spangenberg and Eakin, 1962). Bibb and Campbell (1973) used electron microscopy to show the formation of desmosomes upon initial contact of the extended processes of migrating squamous epidermal cells and the formation of more desmosomes when the area of surface contact increased as the cells resumed their columnar form.

The presence of horizontally-oriented, fully differentiated nematocysts and flattened, apparently mature mucous gland cells in the repairing epidermis of A. formosa indicates that a similar epidermal migration is involved. The whole cell layer seems to be in motion carrying the specialised cells along passively in the migrating epithelium. Such motility of the epithelial layers has been described as a normal occurrence in the column wall of growing hydra (Campbell, 1967b).

The accumulation of differentiating nematocysts in the pre-wound region at a later stage (from 27 hours after wounding) is reminiscent of that observed by Spangenberg and Eakin (1962) in the pre-tentacle area of regenerating hydra, 16 hours after cutting. Campbell (1967c) found that newly differentiated nematocysts in hydra are capable of independent movement. Migration from the pre-wound region is presumably important in ensuring that the tissue that forms over the branch end has its full complement of nematocysts. Since nematocysts are well known to be derived from interstitial cells (Slautterback and Fawcett, 1959; Slautterback, 1961), this observation lends support to the suggestion made in Section 4.5.2.2 that the interstitial cells commonly found in the tip region of normally growing A. formosa may represent a pool of potential specialised cells.

(c) Mesoglea

Chiakulas (1952) has shown that epithelial migration is often prevented in vertebrates if the basement membrane fails to regenerate. This study, and those with various hydroids (Spangenberg and Eakin, 1962; Zwilling, 1963; Bibb and Campbell, 1973) show that this is clearly not the case for all coelenterates. Sealing in A. formosa is effected first by the gastrodermis and epidermis with the mesoglea being reformed later.

Singer and Palmer (1969) and Singer (1971) provide no information on this point from their study of the anemone Aiptasia diaphana because they did not examine samples taken sooner than 24 hours after cutting. By then the mesoglea, though thinner than normal, is intact, and wound closure is complete. The work of Young (1974) suggests that mesogleal reformation is essential for epidermal migration in the anemone Calliactis parasitica, but her results are not directly comparable to these studies because she restricted her study to superficial wounds and did not cut through the mesoglea at any stage.

Studies on retardation of regeneration in hydra (Spangenberg and Eakin, 1961) suggest that the mesoglea constitutes a

barrier which somehow substantially reduces the incidence of differentiation of interstitial cells. Spangenberg (1961) points out that differentiation does not cease entirely because some differentiation is observed in both regenerating and non-regenerating animals. Nevertheless, Spangenberg and Eakin (1961) report that interstitial cells differentiate more frequently if the mesoglea is absent, and that abnormal development (such as the formation of extra tentacles) results if mesogleal function is suppressed. Conversely, they found that if the mesoglea reforms too soon in the repair process, the regeneration process is prevented. Burnett and Hausman (1969) later went so far as to suggest that it is the mesoglea which is responsible for the control of morphogenesis in hydra. In this context, it is interesting to note that no zooxanthellaecontaining cells are ever seen in the epidermis of A. formosa after the reformation of the mesoglea. Moreover, the only obvious demonstration of migration from the epidermis to the gastrodermis (the nematocysts) was observed after the mesoglea was resynthesised. This may mean that the reappearance of the mesoglea in repairing A. formosa terminates gastrodermal to epidermal movement and signals the beginning of cellular migration from the epidermis to the gastrodermis.

Amoebocytes in anemones may adopt two different roles in response to wounding. In Calliactis parasitica they resynthesise the mesoglea and become incorporated into the reforming epidermis (Young, 1974; Young and Chapman (unpub) in G. Chapman, 1974), whereas in Metridium senile they become phagocytic (Polteva, 1970). The amoebocytes of A. formosa bear a striking resemblance to Young's (1974) description of "granular phase" mesogleal cells in C. parasitica. However, they are so few in number, it is unlikely that they could successfully fulfill the same function. Instead, the observed similarity between the cell contents of A. formosa's mesogleal cells and the material which accumulates in the ingestion-excretion region of the lateral lobes of the mesogleal filaments strongly suggests that the former are in fact phagocytic.

Perhaps, then, amoebocytes are responsible for the collection of cell debris from the wound region and its subsequent transportation to the lateral lobes for extrusion. This would be consistent with previous reports of the presence in corals of "wandering cells" (Young and Nicholls, 1931a), which were presumed to be involved in excretion. The occasional observation of similar cells in the inner body wall region of normally growing branches of A. formosa (Section 4.3.1) implies that such scavenging activity is a normal occurrence. Damage to the branch probably stimulates the migration of such cells to the wound surface to assist in the repair process by removing cell debris.

(d) Calicoblastic layer

In anemones (Singer and Palmer, 1969) and hydra (Spangenberg and Eakin, 1962) gastrodermal cells have been observed to migrate out from the cut surface of the septa, join up and reseal the coelenteron. In corals, however, there are gaps in the tissue at the sites of the trabecular columns of the skeleton. It is probably indicative of their advanced state of differentiation that during repair the calicoblastic cells seem incapable of rejoining before the epidermal layer is re-established over the top of the trabecular columns and the mesoglea is re-synthesised. The calicoblastic layer was never seen to contribute cells to the reforming epidermis, nor does the reverse appear to occur. This suggests, firstly, that the differentiation of epidermal cells to calicoblasts is irreversible, and secondly that it does not occur in response to wounding but only at a particular place and time, for example upon settlement of the coral planulae (Vandermeulen, 1975; Goreau and Hayes, 1977; Kinchington, 1980) or as observed in the edge zone of massive coral such as Mancinia areolata (Barnes, 1972). Further, the regeneration of this layer must therefore depend on division of the existing cells, with or without the differentiation of interstitial cells whose presence in the layer is indicated by the occasional presence of nematocysts (Section 4.3.1). A full explanation of how the calicoblastic layer repairs itself must await further investigations at the ultrastructural level.

(e) Zooxanthellae

The rate at which epidermal migration from the wound edge bridges the gaps over the skeleton dictates the speed at which the branch end repairs itself. It is therefore interesting that branches severed 25mm from the tip consistently repair themselves more quickly than those only 5mm away from the end of the branch. The skeleton is always thicker further down the branch (Spiro, 1974; Gladfelter, 1982) and might have been expected to create more of a barrier to the migrating tissue.

The 50mm point of severance is well into the brown portion of the branch. Algal photosynthate from these more proximally located regions is normally translocated distally in order to sustain rapid growth in the tip (Pearse and Muscatine, 1971). However, the removal of the tip presumably halts this process as a result of the collapse of the hydrostatic skeleton upon which this transport system depends. This condition presumably persists until the branch end is resealed and ciliary currents (observed by Gladfelter, in prep., in Gladfelter, 1982) can be re-established in the coelenteron. Until this occurs, it seems likely that repair processes in the brown portion of severed branches would be stimulated by localised accumulation and utilisation of photosynthetic products from the algae. This, then, would explain why these more proximal regions are able to repair themselves more rapidly than the near-tip regions after severance of the branch. The latter are cut off from their normal supply of photosynthesis products and, because they lack zooxanthellae, they have no immediate alternative source.

Despite their observation that symbiotic algae in decapitated Aiptasia diaphana become markedly concentrated in the region from which the tentacles regrow, Singer and Palmer (1969) concluded that zooxanthellae played no direct part in repair and regeneration because regeneration occurred at the same rate in animals having both heavy and light algal populations. It may be relevant, however, that these experiments were maintained in darkness which would minimise the effect of any contribution from algal photosynthesis. It was noted, however, that even under these conditions, the decapitated animals

tended to retain their symbionts more strongly than the control animals, pointing to a possible indirect contribution to the repair process by the algae.

Conclusive evidence for the potentially beneficial influence of algal metabolism with regard to repair has been obtained from corals. Cheney (1974), for example, found that a reduction of the algal population depressed normal rates of cell division in Pocillopora damicornis and Pavona decussata. In addition, Franzisket (1970) demonstrated an absolute requirement for zooxanthellae in the regeneration of atrophied coral tissue in severely starved specimens of Porites sp. Thus it is probable that the more rapid repair rates of proximal portions of A. formosa branches is indeed attributable to the presence of algae in the adjacent tissue.

7.5.4 Regeneration of severed branches

Wood-Jones (1907, 1910) was the first to ascribe the properties of "perpetual growth and perpetual youth" to the "dominant apical zooid" of Acropora branches. Both he and Kawaguti (1937) found that, within certain limits of branch width and distance from the tip, the axial corallite will reestablish itself, after the branch has been severed, leaving no trace of damage.

The rate of repair of A. formosa corresponds closely with that of the "haimei type" of Acropora described by Kawaguti (1937). His specimens regenerated an average of 17mm and 42 radial corallites in 45 days, compared with an average of 15mm and 43 radial corallites in 46 days observed in A. formosa in the present investigation.

Previous reports by Gravier (1915) indicate that although deep water ahermatypes such as Stephanotrochus nobilis Moseley and Deltocyathus andamanicus Alcock are able to recement their skeletons when broken, they are permanently scarred and thereafter display considerable septal disorganisation. Thus, the ability of A. formosa to completely regrow after severance of branches, leaving virtually no trace of damage, suggests

that hermatypic corals have greater powers of regeneration than ahermatypes.

This capacity is presumably directly related to the stimulative effect of algal photosynthesis upon calcification (Vandermeulen et al, 1972). Once the tissues have been repaired it therefore seems likely that translocation of products of photosynthesis (as observed by Pearse and Muscatine, 1971, and Taylor, 1977) occurs during regeneration of A. formosa as well as in normal branch extension.

The regenerating radial corallites emerge virtually complete from the side of the regrowing axial corallite and the coensteum acquires complex elaborations typical of a mature branch with unusual speed. This is consistent with the observations of Loya (1976) who found that damaged branches of Stylophora pistillata grew twice as fast as undamaged controls in the first two months of regeneration so that colonial symmetry was rapidly restored. In addition to restoring the branch diameter, this enhanced deposition presumably eventually occludes the interior of the reforming tip to match that of the original severed branch. These observations suggest that regenerating tips which remain white may be subject to stimulation of growth due to translocation of products of algal photosynthesis at a rate in excess of that normally experienced by undamaged white tipped branches (c.f. Section 6.5).

Only branch ends where the coenosarc remains free of zooxanthellae in the initial stages of regeneration are able to regrow successfully. The failure of branch ends which become infiltrated with algae to regrow supports the conclusion reached in Section 6.5 that calcification rates under tissues which contain symbiotic algae are characteristically low.

7.5.5 Response to colonisation of damaged surfaces by filamentous algae

Algal colonisation of damaged surfaces was observed to be a regular occurrence during repair of tissue lesions in the hermatypic corals Agaricia agaricites and Porites astreoides

(Bak and Steward-van Es, 1980). These two species were found to exhibit completely different types of regrowth. The former created a dome-shaped roof of new skeleton over the original lesion (Bak, et al, 1977). In contrast, the latter formed a regenerating lip behind which new polyps were formed; the regenerating coenenchyme remained completely in touch with the damaged surface and simply displaced the algal contaminants (Bak, and Steward-van Es, 1980). Kawaguti (1937) described an identical process to that of Porites astreoides in the regeneration of severed branch ends in another un-named species of Porites, and reported that the same process occured in Styllophora, Pocillopora and Millepora.

Earlier in this investigation (Section 3.2.3), A. formosa has been shown to be capable of generating a zone of coenenchyme similar to that described for P. astreoides. However, in response to colonisation by filamentous algae, A. formosa appears to adopt a different strategy which has the effect of overgrowing and smothering the algae. In this it is more like Agaricia agaritites. This conclusion is supported by the work of Fichelson (1973) who studied the succession of organisms on dead portions of colonies in several species of branching corals (including some species of Acropora). In particular, he examined Stylophora pistillata which in previous work (Kawaguti, 1937, see above) had been shown to respond to damage in the same way as A. formosa, by forming a zone of closely applied coenenchyme to recolonise the skeleton. In all cases where algal infestation occurred, Fichelson found that recolonisation of the skeleton was effected by growing over the top of the colonising algae. Thus both A. formosa and S. pistillata appear to change their regenerative strategy in response to colonisation of the cut surface by filamentous algae.

SUMMARY AND CONCLUSIONS

1. The structure of the axial corallite of the characteristically white tipped branches of A. formosa, the manner of formation of radial corallites and alterations in skeletal growth in response to obstruction and re-orientation are all typical of the Acropora, suggesting that this species is highly characteristic of the genus as a whole.

2. The growth points of the corallum, the trabecular tines, are located at the end of the trabecular columns, at the junction of the synapticulothecal rings and the upper margins of the septa.

3. The skeleton bears no attachment scars, suggesting that the polyp is held in place by the extensive cross-linking of the skeletal elements and not by desmoidial processes.

4. The structure of the coelenteron of the polyp of A. formosa is highly convoluted to accommodate the ramifications of the skeleton. In addition, the musculature is unusual in that it is predominantly located above rather than below the stomodeum, and the mesenteries bear special extensions. These modifications, which function in tentacle retraction, appear to compensate for the relative immobility of the polyp compared to polyps of other corals which have a simpler skeletal structure.

5. During the day the coral tissue is usually closely applied to the skeleton. At night the polyps expand, apparently as a result of the relaxation of the gastrodermal muscles of the inner body wall and an increase in hydrostatic pressure in the coelenteron. The outer body wall is lifted away from the surface of the corallite, and the cells of the inner body wall are reorganised to produce a cavity above the tips of the trabecular tines.

6. It is concluded that extension of the trabecular tines occurs at night by a special type of $CaCO_3$ precipitation into the space created above the tip of the tine during noctural

expansion of the tissue. Consolidation of the skeleton proximal to the tip occurs during the day by way of a second type of precipitation which is probably directed by an organic matrix secreted by the polyp and contoured by the contracted tissues.

7. Histochemical characterisation of the secretory products of the gland cells of the polyp gave an indication of their probable functional roles. The distribution of particular types of gland cell is consistent with what is known of the secretions and digestive processes of coelenterates.

8. Two types of mesenterial filaments are recognised. The glandular structure of the cnidoglandular bands of the upper part of the polyp suggests that they are chiefly concerned with the digestion of prey which is introduced into the central cavity by the tentacles. The convoluted regions of the mesenterial filaments elsewhere in the polyp appear to be capable of extracoelenteric digestion.

9. Amoebocytes occur in the gastrodermis and mesoglea of the polyp, whilst interstitial cells are consistently present in the epidermis. Both cell types are known to be involved in repair and regrowth in different coelenterate species, but they are not usually found together. Their joint occurrence in A. formosa may be related to the marked capacity of this species to recover from tissue damage.

10. The anatomy, histology and reproductive biology of A. formosa, as described here, is not consistent with the suggestion that its ancestral group, the Astrocoeniina, has a separate and distinct origin from that of the other scleractinian sub-orders and families.

11. The use of the 5-bromodeoxyuridine/Hoechst 33258 fluorescent staining technique yielded qualitative evidence that the highest levels of DNA replication (and hence, cell division) occurs at the extreme tip of the branch. Maximum DNA replication takes place between 11.00h and midnight, with little or none taking place during the morning period 01.00h to 10.00h.

12. Attempts to measure the rate of ^{3}H-thymidine incorporation by scintillation counting of tissue digests yielded erratic results due, it is thought, to masking of ^{3}H-thymidine uptake into the axial corallite by additional but sporadic uptake into the coenosarc proximal to the tip. The sporadic nature of the latter is probably related to the intermittent formation of radial corallites.

13. Autoradiography of sections from branch tips incubated with ^{3}H-thymidine over a diel cycle, confirmed, in a quantitative way, the pattern of diel cell division indicated by the BrdU/Hoechst 33258 technique. A maximum level of ^{3}H-thymidine incorporation around sunset and a minimum in the early hours of the morning is interpreted in relation to what is known of the pattern of prey capture and feeding by the polyps.

14. The night-time peak of cell division corresponds with the state of maximum extension of the polyp and is clearly important as a follow-up to the cellular re-orientation mechanism involved in the creation of space above the extending trabecular tine.

15. Although the diel rhythmicity in ^{3}H-thymidine incorporation persists throughout the year, there is some seasonal variation in the amount of the base incorporated. The lowest rates correspond to periods of reduced skeletal growth, and the highest, most erratic rates occur when branches are extending most rapidly. These observations confirm that cell division is depressed when skeletal accretion is reduced, and is most frequent at times of rapid skeletal accretion.

16. Maintenance of colonies of A. formosa in darkness results in extrusion of zooxanthellae from the coral tissue. After 11 days in darkness, the diel rhythm of ^{3}H-thymidine incorporation into the tip region is lost and the polyps, instead of their usual pattern of expansion and contraction, are permanently expanded. Clearly the reduced rate of skeletal growth known to occur in darkness (or under reduced illumination) is accompanied by a reduction in the rate of cell division.

17. Infiltration of zooxanthellae into a white tip (browning) appears to convert the axial polyp, both physically and functionally, into the equivalent of a radial polyp. It is proposed that browning is associated with the rationalisation of the metabolic resources of the colony. Thus, under conditions close to optimal for growth, it is only the branches in the interior and basal portions of the colony which display browning. The resultant reduced rate of growth ensures that they do not grow into other branches. In sub-optimal conditions, however, increasing numbers of brown tips appear.

18. Re-orientation of a branch of a colony may result in conversion of radial polyps into functional axial polyps which initiate new branch growth. Under such conditions upward regrowth is favoured and browning seems to be induced in the control process. It is suggested that this process is dependent upon the translocation of products of algal photosynthesis from brown tipped branches into the axial polyps of adjacent white tipped branches. Input of these products to the algae free axial polyps, over and above those supplied by the radial polyps of their own branches, may allow growth in the remaining white tipped branches to be sustained at the expense of longitudinal extension in the brown tipped branches.

19. Histological examination of tissue repair in A. formosa shows strong similarities to the repair processes of both hydra and anemones. The gastrodermis initiates the re-sealing of the tissues and contributes extensively to the reformation of the epidermis until the mesoglea is re-synthesised. Once this is accomplished, there may be some movement of non-epithelial cell types from the epidermis to the gastrodermis.

20. Amoebocytes accumulate in the site of the reforming mesoglea but appear to play no direct part in its reformation. It is suggested that they collect up cellular and skeletal debris, and transport it to the lateral lobes of the mesenterial filaments for extrusion.

21. The calicoblastic layer of the inner body wall is the last cell layer to be repaired. There was no clear evidence from the present studies as to whether these highly differentiated cells are able to divide or whether new calicoblasts arise from the differentiation of the interstitial cells which are present in this layer.

22. The presence of zooxanthellae in the outer body wall immediately adjacent to the damaged tissues enhances the rate at which they are able to repair themselves. This is presumed to be due to the local diffusion of products of algal photosynthesis which are no longer available for translocation because of the disruption of circulation in the coelenteron.

23. Observations of regeneration in severed branch tips reveals that regrowth of the axial and radial corallites and the acquisition of skeletal elaborations of their coenosteum, occur with unusual speed. This indicates a degree of channelling of the resources of the colony into the regenerating area. The zooxanthellae undoubtedly assist in this process via the translocation of photosynthate from basal branch regions to stimulate calcification. Failure of a branch to regenerate is invariably accompanied by infiltration by zooxanthellae, an observation consistent with the presumed link between browning and the control of colony growth.

24. Colonisation of severed branch ends by filamentous algae results in an altered pattern of regeneration.