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INTRODUCTION

<u>Acropora formosa</u> Oken 1815, the subject of this study, is a member of a genus which dominates regions of densest coral growth throughout the Great Barrier Reef Province and in most Indo-Pacific reef areas. <u>Acropora</u> are also prevalent in the Atlantic (Goreau and Wells, 1967; Smith, 1971; Gladfelter <u>et al</u>, 1978), and in the Caribbean they often form large monospecific stands (Tunnicliffe, 1981). One of the Carribbean species (<u>A. cervicornis</u>) is closely related to <u>A. formosa</u>. Their distribution is mutually exclusive and, on the basis of similarities in their colony structure, are regarded as geographical analogues (Wallace pers. comm.)

A. formosa belongs to the staghorn group of Acropora sp., which also includes A. cervicornis. Members of this group are characterised by their openly branching coralla (Wallace, 19-79). The distinctive white tipped branches (Bak, 1976) are entirely free of the symbiotic algae (zooxanthellae) which are present throughout the tissues over the rest of the branch surface (see Kawaguti and Nakayama, 1970; Vandermeulen 1972 in Vandermeulen and Muscatine, 1974). Both A. formosa and its presumed geographical analogue, A. cervicornis, are fast growing corals. Growth rates of up to 185mm per year have been reported for A. formosa (Mayer, 1924) and mean growth rates of approximately 100mm per year for A. cervicornis (Shinn, 1966; Lewis et al, 1968; Gladfelter et al, 1978).

Reef forming potential in hermatypic, or reef building corals seems to be linked to the capacity to form colonies which lift the coral tissues above the substratum thus reducing problems with fouling by sedimentation and enhancing food capturing potential (Wells, 1966; Yonge, 1968, 1973). Goreau (1963) has argued that symbiosis with zooxanthellae enables colony, and hence reef, formation by enhancing the overall capacity for calcification to the extent that such hermatypic corals calcify more quickly than the erosional forces of the sea can break them down. The mechanism by which this enhancement is achieved is not completely understood. Reviewers (Muscatine, 1971; Goreau, 1961b; Vandermeulen & Muscatine, 1974; Schmitz and Kremer, 1977; Johnston, 1980) have examined the various models which have been suggested to explain the processes involved. They emphasise that these are not mutually exclusive, nor have they been adequately tested. Moreover, it appears that different mechanisms may operate at different times. Briefly, it is suggested that algal metabolism may influence calcification directly by removing substances which inhibit crystal precipitation or by providing the material for an organic matrix template. Indirectly, the products of algal photosynthesis may provide a stimulus to coral metabolism in general, permitting calcification to proceed at a faster rate, or, more specifically, the zooxanthellae may provide energy for matrix synthesis, or the active transport of calcium and carbonate ions.

In view of this, it is somewhat surprising that some of the fastest rates of calcification ever recorded in hermatypic corals have been reported for the algal-free tips of <u>A. cervicornis</u> (Goreau and Goreau 1959; Goreau, 1961 a and b). In such tips the rates of calcification exceed those of the basal branch regions by a factor of approximately 10. Pearse and Muscatine (1971) were, however, able to resolve this apparent paradox by demonstrating that the products of algal photosynthesis were preferentially translocated to the extreme tip region of such branches. The later work of Taylor (1977), also with <u>A. cervicornis</u>, confirmed this finding, and, in addition, demonstrated the movement of calcium ions towards the ends of white tipped branches.

The rapid rate of growth of <u>A. formosa</u> and <u>A. cervicornis</u>, their comparatively soft skeletons (Kawaguti and Sato, 1968), and the ease with which the sites of maximum calcification can be identified have made these two coral species attractive experimental subjects for a range of investigations. These include investigations of growth (Shinn, 1966; Lewis <u>et al</u>, 1968; Bak, 1976; Gladfelter <u>et al</u>, 1978; Oliver, 1979), translocation (Pearse and Muscatine, 1971; Taylor, 1977; Barnes and Crossland, 1978; Crossland et al, 1980), proximal-distal distribution of zooxanthellae (Kawaguti and Nakayama, 1970; Pearse and Muscatine, 1971), diel rhythms of photosynthesis and calcification (Crossland and Barnes, 1974, 1977; Chalker and Taylor, 1975, 1978; Chalker, 1977), grafting (Collins, 1978), immunology (Cheney, 1975) and regeneration (Kawaguti, 1937a; Connell, 1973; Fichelson, 1973). Despite this extensive interest, however, there is still much about the growth and development of the <u>Acropora</u> (and <u>A. formosa</u>, in particular) that is imperfectly understood.

One area which has yet to be fully investigated concerns the evolutionary origins of the Acropora. At the approximate close of the Paleozoic era, two of the three orders of corals, the Rugosae and the Tabulae became extinct and, concurrently, the Scleractinia emerged; perhaps from the Rugosae (Schindewolf, 1942), but, more probably, independently of any group that has left a recognisable fossil record (Veron, pers. comm.). As far as this record shows (see overleaf, Figure 1 from Wells, 1956), the Scleractinia first emerged as two groups: the sub-order Astrocoeniina, and a complex which gave rise to the remaining four sub-orders encompassing 28 families. The Acroporidae were one of the last major families to emerge, being separable from their astrocoeniid stock only as far back as the upper Cretaceous. Only five genera of the Acroporidae are known, four of which are extant. Of these, Acropora is the most highly speciated genus now in existence (Wallace, 1978).

As far as the writer is aware, there has never been an attempt to look for evidence of the distinct evolutionary origin of the <u>Acropora</u> (as suggested by the above scheme) in the anatomical and histological features of the polyp. This is attempted in the present thesis and evidence is sought from comparison of the histology and histochemistry (of the gland cells in particular) of A. formo's with what is known of other coral species.

As a prelude to the histological investigation, an extensive description of the anatomy and morphology of the polyp of <u>A. form</u>osa and of the skeletal morphology of the corallite is presented.

Figure 1: Evolutionary pattern of the scleractinian suborders and families (reproduced from Wells, 1956, pF363).



These studies form an essential basis for more detailed investigation of aspects of the growth and general behaviour of the colony. Attention is largely directed towards the extreme tip region of the branches. This is justified on the basis of the extreme polarisation of growth shown by <u>A. formosa</u> (Brook, 1893).

The pattern of cell division in the rapidly growing tip region receives special attention. Relatively little is known of cell division in corals nor of the precise control mechanisms which must exist to account for the construction of an elaborate, species specific skeleton (Bourne, 1889; Matthai, 1923; Bryan and Hill, 1941; Wainwright, 1964; Roos, 1967; Vandermeulen and Watabe, 1973). The overall aim of this part of the study is to attempt to relate cell division in the tissues of the tip to calcification and skeletogenesis in <u>A. formosa</u>. The events involved are related to diurnal and seasonal fluctuations in grow-th and to the response of colony growth to environmental changes.

According to Veron (pers. comm.), <u>Acropora</u> may be the most recent of all the Scleractinian genera and can therefore be considered to be near the end-point of Scleractinian evolution. Thus, he suggests that fast growing species, such as <u>A. formosa</u>, may represent the exploitation of the coral-algal symbiosis to the full. The capacity of white tipped branches of <u>A. formosa</u> for rapid growth is therefore further investigated through a detailed comparison of such tips with non-white (brown) tips which are sometimes produced within the colony, and are known (Bak, 1976; Oliver, 1979) to exhibit relatively low rates of branch extension.

Finally, the capacity of <u>A. formosa</u> to repair and regenerate after damage is investigated. These processes do not appear to have been investigated in any detail in the scleractinia, yet Shinn (1966), who worked with <u>A. cervicornis</u>, concluded that branching hermatypic corals are particularly sensitive to adverse environmental conditions, and advocated their use as environmental indicators. Acropora are particularly suitable subjects for such studies since they are known to play an important role in the re-establishment of reefs damaged by catastrophic events (see review Pearson, 1981).

The observations relating to all the above aspects of growth and behaviour of <u>A. formosa</u> are presented, in this thesis, in five separate sections. A more detailed introduction to the various topics appears at the beginning of each section.

2. Materials and methods.

2.1 Collection and maintenance of specimens

Most of the <u>A. formosa</u> specimens collected in this study were taken from a marked site 2m below datum in Nelly Bay, Magnetic Island (Figure 2). Only those colonies which exhibited a short (30-80mm), slender (10-15mm) branched growth form (positively identified as <u>A. formosa</u> by C. Wallace, pers. comm.) and a high proportion of white-tipped branches were selected for use (Figure 3). Portions of these colonies were carefully broken off and transferred to the laboratory in covered, plastic bins (600x500x500mm) which were completely filled with sea water. Some mucus production occurred in the still water (Figure 3), but this stopped soon after the specimens were placed in the running water of the aquarium. A careful note was kept of which colonies the specimens came from.

The aquarium facilities used for maintenance of specimens comprised an open flow cascade system made up of a series of 500mm deep holding tanks. The tanks were screened with Sarlon shade cloth (transmission 29%) to protect the corals from the full intensity of natural sunlight. A 3-day period was allowed for acclimation to the aquarium before branch ends were removed for experimental work.

Some experiments were conducted at the Lizard Island Research Station (14°40'S, 145°27'E). Coral samples were collected from the back reef area of a fringing reef off the southeast side of the island where the depth was approximately three metres at low tide. The <u>A. formosa</u> colonies there had longer interbranch spaces than those collected at Magnetic Island, and the branch tips were blue rather than white (identification verified by C. Wallace, pers. comm.). Specimens were maintained in large, overflowing tanks (1500mm in diameter and 800mm deep) which were connected to an unfiltered sea water supply. Since the transportation distance involved was relatively short, little mucus production was induced. A 24-hour acclimation period was found to be adequate. 2.2 Preparation and examination of skeletal material

Skeletal material was first dried in sunlight. The tissue was removed by soaking in a strong (5-10g/l) calcium hypochlorite solution for 12-24 hours. The skeleton was then rinsed clean under running water, dried, and examined under a stereomicroscope or coated with gold palladium in a Dynavac 300 coating unit and viewed in a ETC Autoscan scanning electron microscope in backscatter emission mode.

2.3 Tissue preparation

2.3.1 Fixation

Preliminary trials showed that the best fixation and response to the staining procedures used was obtained following immersion for 72 hours in 4% sea water formalin buffered with 0.5% sodium glycerophosphate (Steedman, 1976).

Since the study was principally concerned with the extreme tip region of the branches, only the apical 10-15mm of each branch end was processed beyond the stage of fixation. This facilitated subsequent handling and reduced the time required for decalcification.

2.3.2 Decalcification

The tissues were too delicate to withstand the subsequent histological processing once the support of the skeleton had been removed. Specimens were therefore embedded in an agar block prior to decalcification using the method devised by Winsor (pers. comm.). A 25ml syringe (with the nozzle removed) was clamped upright in a rack. The plunger was partially withdrawn and approximately 3ml of molten agar was poured in and allowed to set. The specimen was placed on the top of the agar and oriented so that the branch axis was at right angles to the barrel of the syringe. Molten agar at 30° C was poured in to cover the specimens and allowed to set. The complete agar block was extruded by raising the plunger. The block was then trimmed (Figure 4) to remove excess agar from around the embedded specimen. The base of the specimen was exposed to provide direct contact between the skeleton and the decalcifying fluid, thus allowing the CO_2 generated by the reaction to escape.

Decalcification was carried out using a mixture of formic acid and formalin (Goodins and Stewart's fluid, Culling 1974). In order to make the decalcifying process as gentle as possible, the rate of CO_2 bubble formation was minimised by commencing with a relatively dilute solution of formic acid. Specimens were placed in a 1% formic acid/5% formalin mixture for the first 24 hours. The formic acid content was increased by 1% per day until a final concentration of 5% formic acid/5% formalin was reached. Complete decalcification usually required 5-7 days: the precise end point of the reaction was determined chemically by Arnim's (1935) test, as modified by Drury and Wallington (1976).

2.3.3 Embedding

Decalcified specimens were processed in a Shandon Southern automatic tissue processor using a schedule of two hourly changes. They were dehydrated via an ascending series of alcohols (10, 20, 30, 40, 50, 60, 70 and 90% and 4 changes of absolute alcohol), cleared in toluene (3 changes), and impregnated with "Paraplast" wax (melting point 56.5°C, 3 changes). The tissues were vacuum infiltrated for 30 minutes prior to final embedding in "Paraplast" wax.

2.3.4 Sectioning

A Yamamoto rotary microtome was used to cut 7μ m sections from the blocks. Sections for histochemical studies were cut at 6μ m. Serial longitudinal sections were taken through the mouth region of the axial polyp of each tip. Adhesion of the sections to slides was considerably improved by coating the glass with egg albumen prior to mounting. The slides were then dried overnight in a 56° C oven.

- 2.3.5 Staining
- 2.3.5.1 Histological stains
- The following stains were used for histological sections:-
- (a) Martius yellow brilliant crystal scarlet methyl blue(MSB, Drury and Wallington, 1976).
- (b) Haematoxylin and eosin (Cook, 1974).
- (c) Liisberg's stain (Liisberg, 1961).

The Martius yellow – brilliant crystal scarlet – methyl blue (MSB) technique was selected for routine histological use. This technique clearly distinguished all three cell layers in polyps of <u>A. formosa</u>. The mesoglea stained deep blue and the zooxanthellae bright red. This was of particular importance because observations of conformational changes in cell layers mediated by the mesoglea and changes in the distribution of the zooxanthellae form an integral part of this study.

Muscle fibres stained red, nematocysts magenta. Cell nuclei in the epidermis were deep red to blue purple, and those of the gastrodermis were either red-purple or orange.

Details of the MSB method, as used in the present investigation, are set out below because some modifications to the original method of Drury and Wallington (1976) were evolved during the course of staining large batches of slides.

Modified MSB method

N.B. Townsville water is very soft, and was therefore quite suitable for the washing procedures.

- (1) De-wax and rehydrate sections.
- (2) Stain nuclei with a 1:1 mixture of 1% celestine blue and 4% iron alum for 5 minutes.
- (3) Wash in tap water.
- (4) Stain in Gill's haematoxylin for 2 minutes.
- (5) Wash well in tap water and differentiate in 1% acid alcohol
- (6) Dip three times in Scott's substitute tap water (Drury and Wallington, 1976) to blue nuclei.
- (7) Wash in tap water and rinse in absolute alcohol

- (8) Stain in 0.25% martius yellow in 95% alcohol (modification by Pusey and Edwards, 1978) plus 2% phosphetungstic acid for 2 minutes).
- (9) Wash rapidly in tap water
- (10) Stain in 1% brilliant crystal scarlet in 2% acetic acid for 15 minutes
- (11) Wash well in tap water
- (12) Treat with 1% phosphotungstic acid for 5 minutes to fix and differentiate the red stain.
- (13) Wash in tap water
- (14) Stain in 0.5% methyl blue in 2% acetic acid for 3 minutes
- (15) Rinse in 2 changes 2% acetic acid
- (16) Dehydrate, clear and mount in DPX.

Arginine rich materials, such as erythrocytes or the rhabdites of the nemertine worm <u>Basiodiscus</u> sp., are usually stained bright yellow by the MSB technique (Winsor, pers. comm.). However, the intensity of the colour is lost if either of the solutions containing phosphotungstic acid become exhausted with repeated use. For this reason, control sections of <u>Basiodiscus</u> sp. were routinely included. This problem was impossible to detect in <u>A. formosa</u> because none of the tissue components were coloured yellow in the final preparation. If the phosphotungstic acid solutions became depleted, the zooxanthellae were stained a pale, mottled orange and the rest of the section had an overall red purple tinge due to overstaining by the brilliant crvstal scarlet. Thus it appears that martius yellow has some important pre-staining function in the MSB technique as discussed by Bradbury and Gordon (1977).

Thorough washing between Steps 8 and 10, and 10 and 12, was found to be of crucial importance. Care had to be taken in washing out the Martius yellow because it is highly soluble in water and rapidly leached from the tissues, therefore Step 9 was completed as quickly as possible. The differentiating efficiency of the phosphotungstic acid in Step 12 was quickly reduced if all excess crystal scarlet was not removed in Step 11. Haematoxylin and eosin staining (Cook, 1974) provided excellent general staining but gave poor cellular detail and less colour contrast than MSB and so was not used routinely. It was however used for staining autoradiography slides (section 2.4.1.2) where the only requirement was to be able to distinguish nuclei from cytoplasm.

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Tissues which gave an acidophilic staining reaction with the haematoxylin and eosin methods (i.e. were stained with eosin, an acid dye, thus indicating tissue of a basic character) were stained either by crystal scarlet or by the counterstain, methyl blue. Both of these are acid stains (Lillie, 1977). In contrast, tissues which gave a basophilic staining reaction with haematoxylin and eosin (i.e. were stained by haematoxylin, basic dye, indicating tissue of an acid character) were coloured purple by the MSB technique.

Preliminary tests showed that Liisberg's stain, which Liisberg (1961) devised to differentiate α and β cells in the human pancreas, was particularly effective for demonstrating the position of the zooxanthellae in <u>A. formosa</u>. The algae were coloured deep magenta, as were muscle fibres and some gland cells. The remainder of the section was pale grey. Counterstaining with methyl blue was found to improve contrast for photographic reproduction.

2.3.5.2 Histochemical stains

The contents of the gland cells of <u>A. formosa</u> were investigated by a combination of the following well established histochemical techniques:-

(a) Alcian blue - periodic acid Schiff (after Mowry, 1956 in Cook, 1974).

Initial treatment with alcian blue stains the acid mucins and prevents uptake of PAS. Thus, acid mucins were deep blue whilst neutral mucins, which did not take up PAS, were magenta. Mixed mucins were magenta-purple, purple or purple-black.

(b) Toluidine blue (Lillie and Fullmer, 1976).

Toluidine blue is a thiazine dye which possesses the property of metachromasia (i.e. it has more than one absorbtion spectrum). A shift occurs from the normal monomeric (orthochromatic) form to the polymeric (metachromatic) form when the dye reacts with tissue which contains "chromotropes", i.e. closely apposed (0.5μ m apart) acidic groups. Blue (α) metachromasia denote the orthochromatic form (negative result), purple (β) or red (δ) metachromasia denote the polymeric state (positive result). Thus, sulphated and carboxylated (i.e. acid) mucins display metachromasia whilst neutral mucins do not (Cook, 1977).

Alcohol was found to leach the stain from the cells, thus it was necessary to dehydrate sections with tri-ethyl phosphate (Bancroft, 1967) in order to preserve metachromatic staining.

(c) Voisnet-Fürth reaction (after Serra, 1946; Lillie and Fullmer, 1976)

Voisnet reagent (a fresh solution of 10mls conc. HCl plus 1 drop 2% aqueous formol and 1 drop 0.5% sodium nitrite) reacts with indole compounds to give a purple colour which is specific for tryptophan. It is therefore an indicator of proteinaceous material.

2.4 DNA synthesis

The semi-conservative replication of deoxyribonucleic acid in tissues may be studied by measuring the incorporation of purine or pyrimidine bases, or base analogues, into nuclear material over a period of time. In this study various methods were used to examine DNA synthesis at hourly intervals over a diel (24 hour) period. Two of the methods (fluorescent staining and autoradiography) required histological processing of the tissue as a prelude to measuring the incidence of DNA replication at the cellular level. Scintillation counting was used to examine the overall DNA synthesis and was carried out after digestion of the whole tissue.

2.4.1 5-bromodeoxyuridine incorporation

An hour before the commencement of each incubation period two branch ends approximately 35mm in length were removed from a specimen in the aquarium. These were placed in a wire rack in a glass beaker containing 100ml of filtered sea water $(0.45\mu m)$ Millipore filter membrane) under continuous aeration. Following equilibration for one hour, 1ml of a $10^{-3}M$ solution of 5-bromodeoxyuridine (BrdU) was added to the beaker (final concentration 10^{-5} M). After a one-hour incubation the samples were removed from the incubation medium and dropped into 100ml of 4% sea water formalin containing 0.5% sodium glycerophosphate. No washing was carried out since fixation prevented further incorporation of BrdU and subsequent histological processing removed any excess base analogue. This procedure was repeated at hourly intervals for successive pairs of branch ends over a 24 hour period; thus, as one pair of samples began incubation, the next pair were removed to begin equilibration.

At the end of the experiment, one pair of branch ends was removed and fixed without exposure to BrdU to act as blanks. The extreme ends of all the samples were then sub-sampled to produce tips 10-15mm in length. These were embedded in agar for decalcification and histological processing. Longitudinal serial sections from the centre of the mouth region of each tip were dewaxed and rehydrated. They were stained with a fresh, $1\mu g/ml$ solution of Hoechst 33258 for 15 minutes to differentiate dividing and non-dividing cells (see p113). The sections were mounted in glycerol (DPX was unsuitable because it then quenched flucrescence). Slides were kept cool and in the dark until they were examined (no later than two hours after staining in order to minimise fading). Sections were then examined microscopically for areas of reduced fluorescence (indicating 5-bromodeoxyuridine incorporation and therefore active DNA replication) under transmitted UV light, using a Zeiss photomicroscope 11, equipped with an HBO 200w/4 mercury vapcur lamp, a BG38 excitation filter and barrier filters numbers 44 and 65. Photographs were taken at 400x magnification with high speed (400 ASA) film; exposures required approximately 4 minutes.

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2.4.2 ³H-thymidine incorporation

There were several drawbacks to the BrdU method. The repeated disturbance of the aquarium specimens during sampling interfered with the normal rhythms of expansion and contraction. At night it was necessary to expose the specimens to light in order to remove the branch ends. In addition, the method required handling of the branch ends prior to fixation; consequently, only samples with fully contracted polyps were obtained. Ruptured tissues were unusually common in sections from this experimental technique, suggesting that the samples may have been unduly stressed by the experimental procedure. In order to improve the quality of the material for histological examination, an experimental procedure was devised to minimise handling of branch ends during incubation in ³H-thymidine prior to measuring DNA synthesis by either scintillation counting or autoradiography.

An experimental apparatus (Figure 5) was constructed which held 24 separate incubation chambers under identical conditions. This enabled separate samples to be taken at hourly intervals over a 24 hour period. Each incubation chamber consisted of a 70ml beaker containing a circular perspex disc (10mm thick) with a central stem which protruded 10mm above the rim of the beaker. These perspex racks displaced 10mls of sea water giving a final volume of 60mls in the incubation beakers. The disc was drilled with 3 holes, 10mm in diameter, which accommodated the bases of the severed branch ends and held them upright in the rack.

Sea water was supplied to each incubation chamber from a central header tank via 24 identical outlets. A constant flow of water to the incubation chambers was controlled by the constant head in the supply tank. The flow rate was arranged so that the water in the beakers was replaced every 15 seconds, giving a flow rate of 17 litres per hour in each chamber. The whole unit stood in a 50mm deep tray which was kept full by the overflow from the beakers and the header tank. This arrangement maintained the contents of the beakers at the same temperature as the sea water flowing through the apparatus. The beakers required only a slight displacement to remove them from the water flow in order to inoculate them with radio-isotope or to fix the coral tissue. This, it was found, could be achieved without causing the withdrawal of expanded polyps. After incubation, the branch ends were fixed by the gradual addition over 4 seconds of a volume of fixative 2-3 times that of the beaker. Fixation by displacement produced the minimal possible disturbance to the samples and was completed sufficiently rapidly to prevent complete withdrawal of fully expanded polyps.

Experimental procedure

After the colony specimens had acclimated in the aquarium for the requisite period (Section 2.1), white-tipped branches of similar diameter were selected for sampling. Their branch ends (30-35mm in length) were removed over a 60mm deep tray of flowing water. This was the only time during the experimental procedure that the samples were exposed to air. Whenever possible, all the branch ends for one experiment were obtained from a single colony. The total number of tips available dictated whether treatments were duplicated or triplicated. Sometimes it was necessary to select and mix randomly branch ends from two separate colonies. Branch ends were placed in the perspex racks of the experimental apparatus and transferred underwater to the incubation beakers, which were then placed under an outlet from the header tank. The branch ends were left undisturbed for 3 hours during which time mucus production ceased and all skeletal debris was carried away by the water flow. Although all samples were fully retracted initially, after this period, the polyps were seen to follow a rhythm of expansion and contraction identical to that of undisturbed specimens in the aquarium.

A beaker was then removed from the water flow at hourly intervals and inoculated with 60μ l of ³H-thymidine (Radiochemical Centre, Amersham, England; specific activity 27Ci/m mol) to give a final activity of 1μ Ci/ml. Mixing was accomplished by gently raising and lowering the perspex rack 10 times, without allowing the ends of the samples to break the surface of the water.

At night, to minimise polyp retraction in response to light, a Cyalume chemical light, which emits green light, was used during these manipulations. Field tests showed that even this subdued light eventually caused polyps to retract. It was therefore used as sparingly as possible to minimise disturbance to the samples.

In experiments where 3 H-thymidine incorporation was measured by scintillation counting, a 200µl aliquot of labelled sea water was removed from each beaker immediately after inoculation with 3 H-thymidine. Following incubation of the tips in the isotope solution for one hour, a second 200µl aliquot was removed. Both the pre- and post-incubation medium samples were placed in 9ml solutions of scintillation fluid (formula - 5g Omnifluor; 250ml Triton X100; 750ml Toluene per litre) with 0.8ml of distilled water for scintillation counting. Comparison of the two sets of counts enabled the rate of disappearance of radioactivity from the medium to be estimated.

The beakers were returned to the water flow to wash for one hour after incubation. Branch ends for scintillation counting were removed from the beaker in turn, allowed to drain for one minute and dropped into a pre-weighed scintillation vial containing 10ml of 1M KOH. The vials were then re-weighed to determine the wet weight of each sample then gently agitated on an automatic shaker for 12 hours to digest the coral tissue.

Branch ends for histology and autoradiography were fixed by the steady addition over four seconds of approximately 150mls of 4% sea water formalin plus 0.5% sodium glycerophosphate. At the end of the experiment, the fixative in all 24 beakers was replaced with fresh fixative. The samples were then left undisturbed for 72 hours before their tips were sub-sampled to obtain 10-15mm tips which were then processed histologically.

2.4.2.1 Scintillation counting

One ml of digest was added to 9ml of scintillation fluid. A further 200μ l was neutralised and assayed for protein by the technique of Lowry <u>et al</u> (1951). The clean skeleton was removed from the vial, washed, dried and weighed.

Radioactivity in the pre- and post-incubation medium samples and the tissue digests was determined with a Packard Tricarb Scintillation Spectrometer Model 3380, interfaced with a PDP 11-70 computer for data acquisition. The counts were normalised against external standard ratios and appropriate quench standards which contained KOH and H_2O . Counts from the tissue digests were expressed as dpm (disintegrations per minute) /mg wet weight of coral, dpm/mg dry weight of skeleton and dpm/mg protein.

2.4.2.2 Autoradiography

When the tips which had been exposed to 3 H-thymidine had been serially sectioned, a slide bearing longitudinal sections from the point nearest the centre of the mouth region was selected from each sample. These slides were dewaxed and dehydrated, then dipped in a 1 in 2 dilution of Ilford K2 Nuclear track emulsion according to the method of Kopriwa and Leblond (1962). The dipped slides were placed in a light-tight box with a packet of silica gel and exposed for 4 weeks at 4°C. After this time, they were developed in Kodak D19 for three minutes, stopped in distilled water for 1 minute, fixed in Hypam Rapid X-ray fixer for 5 minutes, then washed for 30 minutes.

A series of control slides were always included among the test slides. These comprised pairs of alcohol cleaned glass slides which were inserted after every 15 experimental slides. One of these was developed immediately to ensure that the emulsion was not fogged; the other was treated in the same way as the experimental slides. In addition, a slide bearing unlabelled sections and another slide bearing sections from an early autoradiography experiment known to give clear labelling and low background counts were placed at random amongst the experimental slides. After development, the clean glass slides and the unlabelled sections were examined for any significant background to serve as negative controls, whilst the labelled sections were used as positive controls.

Pre-staining of sections with both haematoxylin and eosin and MSB was not successful because the stain washed out during the development procedure. Post-staining with haematoxylin and eosin was preferable to the MSB method since the emulsion was deeply stained by the methyl blue counterstain in the latter method and tended to obscure the section.

For counting of labelled nuclei, a standard eyepiece graticule was designated a "field" diameter. It was found that at 100x magnification, 8 of these usually spanned the end of the tip, but avoided the mouth region and the edges of the section. In routine counting, the number of labelled gastrodermal and epidermal cells in 8 fields were recorded per sample in each of 3 sections taken through the centre of the axial corallite. Although all samples were processed histologically, usually one replicate only was counted. All counts were performed "blind", i.e. the slides were randomly arranged and the sample numbers obscured. The total number of cell nuclei in one field was also recorded for every section.

The β particles emitted from ³H-thymidine are of low energy (E_{max} =18.6kev) giving maximum penetration of 4 μ m in tissue sections. In addition, however, Rogers (1979) reports that efficiency declines drastically with increasing section thickness. On the basis of his information, it was clear that, in this study, only labelled cells within 1 μ m of the section surface would expose the emulsion. Therefore, a factor was applied to the raw counts to calculate the total number of cells which could affect the emulsion. The percentage of labelled cells was in turn derived from this information. Thus the total cell counts were multiplied by 8 (the number of fields examined for labelled cells) then divided by the thickness of the section in microns. (Most sections were cut 7μ m thick, but occasionally 6μ m sections were taken.)

During the course of this investigation a number of problems were encountered in applying the autoradiographic technique to preparations of <u>A. formosa</u>. These included distortion of the pattern of distribution of silver grains caused during drying of the sections (see Rogers, 1979), variability in the quality of the emulsion from one batch to another, and artefacts resulting from the use of xylene stored in metal containers (Rogers, pers. comm.). Only those results not affected by any of the above problems are reported in this thesis.



Figure 2: Map of Cleveland Bay area and position of Nelly Bay collecting site.

Figure 3: Growth form of <u>A. formosa</u> routinely collected from Nelly Bay for experimental work (x 0.75).

The majority of branches terminate in a large white axial polyp (ax), although a few are brown (*). Further growth in one brown tip (arrow) would result in anastomosis with an adjacent branch. Numerous small brown radial polyps (r) project from the sides of the branch. Near the ends of the branches the remainder of the coenosarc (between the radial corallites) is white, but this also becomes brown proximally. Strands of exuded mucus (xmu) emanate from the mouths of some polyps.

Figure 4: Decalcified <u>A. formosa</u> tip embedded in an agar block (x 11.5).

Partially expanded tentacles (te) are present at the mouth of both the axial (ax) and radial (r) polyps. The opaque halo around the specimen is embedding agar (ag).

Figure 5: Experimental apparatus (x 0.2).

24 identical outlets emanate from a central header tank. The flow of sea water from the inlet pipe is regulated to produce a constant, slight overflow. Sediment (x) is precipitated out to the bottom of the tank. The outlets flow into 70ml beakers containing perspex racks which can hold up to three branch tips in the vertical position.

ag - agar; ax - axial polyp; r - radial polyp; te - tentacle; xmu - exuded mucus; asterisk - brown-tipped branch; cross deposited sediment; arrow - obstruction of growth.

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3. The skeleton of A. formosa

3.1 Introduction and terminology

3.1.1 Skeletal morphology of the Scleractinia The adult coral skeleton is almost pure aragonite, the metastable form of $CaCO_3$ (Wainwright, 1963, 1964) and the basic structural unit is a small cone-shaped bundle of these orthorhombic crystals. These units have been variously described as tufts (Jell, 1974), sclerodermites (Vaughan and Wells, 1943; Wells, 1956; Wise and Hay, 1965) and fasiculi (Ogilvie, 1896; Bourne, 1899; Wise, 1969). However, there is now general agreement that the term crystal fibre best describes the crystalline structure and optical properties of these polycrystalline aggregates (Bryan and Hill, 1941; Sorauf, 1970, 1972; Wainwright. 1963, 1964; Vandermeulen and Watabe, 1973). Crystal fibres are always oriented at right angles to the calicoblastic layer when they are laid down, and the point from which they emanate is called the centre of calcification (Ogilvie, 1896; Bryan and Hill, 1941; Sorauf, 1972). Skeletal growth entails the organisation of the crystal fibres into branching rod-like columns, or trabeculae, which form the framework of the skeleton.

The following terms, based on those of Moore <u>et al</u> 1956 are used in this general account of scleractinian skeletal structure:-

basal plate - initial deposition of corallum from which all other skeletal elements arise

calice - open or upper end of corallite

coenosteum – undifferentiated skeleton between corallites (in Acropora – external surface of corallum)

coen - tissue overlying coenosteum

coenenchyme - collective term for coenosteum and coenosarc

coenosteal spine - outwardly projecting spine on coenosteum

corallite - exoskeleton formed by an individual polyp

- corallum exoskeleton formed by a coral colony or solitary polyp
- costa continuation of septum beyond the theca by outward divergence of septal trabeculae

costal spine - outwardly projecting spine on a costa

- crystal fibre aggregation of aragonite crystals into a coneshaped bundle, the basic structural unit of the skeleton
- dentation tooth-like projection on inner (free) edge of septum

dissepiment - horizontal partition across corallite

- entoseptum septum positioned between members of a mesenterial pair, i.e. in the entocoelic spaces (see page43)
- epitheca wall formed around corallite by upward continuation of the basal plate
- exoseptum septum positioned between neighbouring mesenterial pairs, i.e. in the exocoelic spaces (see page 43)
- granulation lateral divergence of crystal fibres from trabeculae to initiate formation of synapticula
- septum radially disposed longitudinal partition of corallite occurring within or between mesenterial pairs
- stereome secondary thickening of coenosteum, mostly by deposition on the synapticulae but also on the costae

synapticula - rod or bar joining opposed faces of adjacent septa synapticulotheca - corallite wall formed from one or more rings of synapticulae theca - wall enclosing polyp column and uniting outer edge of septa (several types)

trabecula - column of crystal fibres

Term introduced in this account:-

trabecular tine - growth point of corallum. Located on the upper surface of a corallite at the junction of the synapticulothecae and septa

The arrangement of the major elements of skeletal structure in an idealised corallite may be shown diagramatically as follows:-



Diagramatic representation of an idealised corallite

The vertical structures, mainly the septa and epitheca, supply the uprights for the framework which supports the tissue. Horizontal elements, such as the dissepiments and synapticulae supply the cross beams which provide internal reinforcement and partitioning, and additional support for the tissue.

In solitary corals the epitheca, an upward continuation of the base plate, encircles the entire corallite, marking the limit of the edge zone (the tissue which hangs over the column wall or theca). In colonial corals, the edge zones become confluent between the polyps to form the coensarc. The skeleton underneath this tissue is the coenosteum, and it and the coensarc are collectively known as the coenchyme. Thus the epitheca persists, if at all, only at the periphery of the colony, and the theca assumes a more important structural role.

Thecae are entirely secondary in origin, and may be derived from various skeletal structures. Most often, there is a general thickening of the edges of the septa to produce a solid wall or septotheca. Dissepimental development may reinforce or entirely replace this to form a paratheca. Perforate or fenestrate synapticulothecae are produced by the combination of many synapticulae to form a wall.

The radially disposed septa are usually in the form of vertical partitions which arise from the basal plate to separate and support the similarly disposed mesenteries (see p. 46) which divide the gastrovascular cavity of the polyp. Trabecular divergence from the septa beyond the theca produces the costae. The mesenteries arise in pairs and, like the septa, follow a hexamerous pattern of development. The first cycle of six septa arise between the members of the first cycle of mesenterial pairs. These regions are known as the entocoelic spaces, therefore these septa are described as entosepta. If the second cycle of mesenteries have formed, the first two septal cycles develop simultaneously and so all 12 are entosepta. If the second cycle of mesenteries has not formed, the next septal cycle develops between the neighbouring pairs of the first cycle of mesenteries in the exocoelic spaces and are described as exosepta. The remaining septal cycles are introduced consecutively, the full complement in the third cycle is 12, 24 in the fourth and so on. However, later stages in development are frequently incomplete.

3.1.2 Previous studies of the skeleton of Acropora

A certain amount is already known about the general characteristics of the skeleton of <u>Acropora</u> from the early work of Duncan (1884), Fowler (1887) and Brook (1893) who made their observations using the light microscope. Wallace (1979) subsequently used the scanning electron microscope, chiefly to examine radial corallites and skeletal elaborations in her taxonomic review of the genus. The simplified skeletal structure displayed by the genus lacks many of the characteristics used in species identification, such as columella, dissepiments and septal structures. There is no epithecal development and the corallite walls are exclusively synapticulothecate.

Secondary deposition, or stereome (Wells, 1956) consolidates the branches proximally (Duncan, 1884), and substantially reduces the porosity of the branches (Spiro, 1974; Gladfelter, 1982). However, rapidly growing branches are characteristically light-ly calcified at the tip (Duncan, 1884; Brook, 1893).

The way in which new corallites always arise below the axial corallite, which dominates each branch end, was first described as "centrifugal" by Ridley (1884 in Brook, 1893), although Brook (1893) proposed the more accurate term, "indeterminate". It contrasts strongly with the mode of growth in other branching forms (e.g. <u>Montipora</u>) where new corallites are added above existing ones ("centrifugally" – Ridley, 1884 in Brook, 1893; "determinately" – Brook, 1893) and in massive forms where all corallites extend simultaneously (Wainwright, 1964; Barnes, 1973). Moreover, <u>Acropora</u>, according to Brook (1893) and Mood-Jones (1907) is capable of both determinate and indeterminate growth within a single colony, since a pad of undifferentiated skeletal growth, which adds corallites at its periphery, initiates growth of the whole colony after settlement. Brook (1893) argued that in <u>Acropora</u> the skeleton between the radial corallites is actually the wall of the axial corallite and that the only true coenosteum occurs at the base of the colony. However, Wallace (1979) points out that a convention has arisen by which the term coenosteum is also applied to the radial corallite walls. This practice is supported by Veron (pers. comm.) who states that secondary deposition in more basal branch regions can completely overlay the original axial corallite wall and may simultaneously submerge the radial corallites. Thus, in <u>Acropora</u>, it appears to be more appropriate to describe the entire external surface of the corallum (i.e. everything outside the corallite boundaries) as coenosteum, and to differentiate within this on the basis of the type and extent of skeletal elaboration.

Another problem related to terminology arises with regard to the extrathecal ridges on the outside of both radial and axial corallites. Brook (1893) stated that they cannot be costae because the number of ridges far exceeds the number of septa expressed in the corallite, and moreover, they are formed before the septa in radial corallite development. This has led to the adoption of alternative terms such as "striations", "rugae" (Brook, 1893) and "pseudocostae" (Vaughan and Wells, 1943) to describe these structures. However, more recent work (Wallace, 1979) stresses the pronounced tendency towards reduced septal development in this genus and Ricart y Mendenez and Friedman (1977) suggest that not all septa are expressed in the calice, but remain immersed in the corallite walls. In the light of this information, the continued use of the term "costae" for <u>Acropora</u> will be evaluated during this study.

With the exception of Collins (1978), who was chiefly concerned with intereactive effects, no other worker has specifically selected the skeleton of <u>A. formosa</u> for morphological study. Nor has any detailed description ever been given of the extreme branch tip region of this genus. Indeed, Fowler's (1887) specimens were branch fragments which lacked axial corallites entirely. The recently published work of Gladfelter (1982; whose findings will be discussed in more detail in Section 4.3.5.2) does include a general description of the tip region of branches of <u>A. cervicornis</u> as part of a study of mineralisation in that species. However, an intimate understanding of the complex interlinkages of the skeleton is necessary for a proper interpretation of the organisation of <u>Acropora</u> polyp tissues. This applies particularly to the evaluation of histological sections, from which the skeleton has, of course, been removed during processing. The branch tip region and, in particular, the axial corallites of <u>A. formosa</u> were therefore examined in detail under the scanning electron microscope. The results are reported here in order to provide the necessary background information for the work which follows. Some observations are also made concerning the response of branch tips to obstruction or reorientation.

3.2 The skeletal morphology of branch tips of A. formosa

3.2.1 General morphology

The branch ends on the upper surface of openly branching colonies of <u>A. formosa</u> (Figure 3) usually terminate in a single, large axial corallite (Figure 6a). Under natural conditions this appears white because the skeleton may be seen through the transparent polyp tissues (Figure 3). The coenosarc proximal to the axial corallite tip gradually assumes a deep, red-brown colour due to the presence of increasing numbers of symbiotic algae whose pigment renders the tissues opaque (Figure 3). Thus the radial corallites are usually brown.

Branch tips in the basal portion of the colony and towards the interior tend to be brown (Figure 3) due to the pigmented zooxanthellae in their polyps which render the tissues opaque. Such tips are known to exhibit little or no longitudinal extension whilst white tips exhibit highly variable growth rates (Bak, 1976; Oliver, 1979). This study is mainly concerned with white tips; however, brown tips are considered in detail in Chapter 6.

Developing radial corallites near the tip of the branch are small and closely aligned to the branch axis (Figure 6b). The more mature radial corallites located proximal to these are larger, and tend to be inclined outwards. Their calices are also displaced outwards, but remain oriented towards the tip (Figure 6b). The combined effect of these arrangements produces a tapering, conically shaped branch end (Figure 3).

There are frequently two cycles of laminar septa bearing pronounced dentations in the central cavity of an axial corallite (Figure 7). Of the six primary septa, one pair is usually slightly more developed than the others, these are the directive septa (Figures 6a,7). In the intact animal they lie between the directive mesenteries (section 4.1). Mature radial corallites have up to two complete septal cycles and the primary direct septa, particularly the outer ones, are much more prominent than the others (Figure 6b). In cross section (Figure 7) it can be seen that the axial corallite wall is made up of a series of highly perforate, concentric cylinders which are interconnected by the radially disposed septa. Each synapticulothecal ring, or cylinder, is formed from a circular pallisade of fine, vertically arranged trabecular columns, and interlinked by simple, horizontally-oriented synapticulae with pores, or fenestrations between them (Figure 6b). The whole structure is extremely porous. Small depressions, or scars, which represent mesogleal attachment sites via desmoidial processes (Wise, 1970) were never observed in <u>A. formosa</u>.

The angle of insertion of the radial corallites dictates that their synapticulothecae are rarely complete (Figure 6b). Their walls contain an average of only 2 - 3 synapticulothecal rings compared with between 4 - 6 in the axial corallites.

Small pointed knobs, or protrusions are present on the upper surface of the axial corallite. They occur at the junctions of the synapticulothecal rings and the upper margins of the septa, and mark the ends of the trabecular columns (Figures 6a and 6b). These features represent the points of the furthest extension of the corallum and are herein named "trabecular tines" (tine = point or prong; Concise Oxford Dictionary of Current English, Fowler and Fowler, 1964).

Near the branch tip the coenosteum is costate (Figure 6b). A direct connection may, on occasion, be observed between septa and their corresponding costae (Figure 6a), justifying the continued useage of this term. Interpretation is frequently difficult since neither the rings nor the septa are continuous and fusion between the septa is common (Figure 6a). There are no costal spines in the distal part of the branch – they begin to appear only at the level of the second circle of radial corallites (Figure 6b). At first, they are restricted to the basal region of the radial corallites, however, they are more widely distributed proximally. A small amount of secondary thickening (stereome) also occurs proximally, which slightly reduces the

porosity of the skeleton compared to the extreme tip (Figure 6b), but this is minimal at this point compared to more basal branch regions (compare Figure 118; Chapter 6).

3.2.2 Formation of Radial corallites

The growth of radial corallites is initiated by the formation of a small pocket of skeleton on the axial corallite wall formed as a result of lateral trabecular divergence from the costae of the coenosteum (Figure 6b). In white-tipped branches this always occurs well away from the corallite margin (c.f. Chapter 6). As the axial corallite extends, the incipient radial corallite grows outwards and the walls develop. Further costal contribution initiates the formation of more synapticulothecal rings (Figure 6b). The third ring is usually at an advanced stage of construction before the first is complete.

The development of the primary septa in the radial corallites may be readily traced in the tip region of a single branch since their order of succession down the branch represents a chronological record of events (Figure 6b). In incipient radial corallites no septal development is seen. The outer directive septum is the first to appear followed by the inner one. Two septa then usually arise together on either side of the outer directive septum. The remaining two septa from the first cycle also arise simultaneously on either side of the inner directive septum. Initially, the septa appear as a series of spines, which project into the calice (Figure 6b). The CaCO₃ deposition associated with subsequent growth usually transforms these into a laminate structure. This occurs near the branch tip in the directive septa, but the other primaries sometimes retain this rudimentary form for some distance down the branch.

3.2.3 Response of branch tips to obstruction or damage <u>A. formosa</u> has the ability to regrow over dead branches by an encrusting mode of growth. A zone of undifferentiated coenenchyme is located at the periphery of the living tissue (Figure 8). The rate at which regrowth occurs is highly variable and may take several months. A similar process seems to occur when the growth of the axial corallite is obstructed, resulting in the

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production of a pad of coenenchyme at the end of the branch over a period of 2-3 weeks. The coenenchyme consists of a mass of undifferentiated coenosteum devoid of corallites at its margin, and an overlying coenosarc which does not contain zooxanthellae (Figure 9). Coenenchyme is also produced, but more rapidly (in 5-7 days), in response to more localised superficial tissue damage (Figure 10).

Broken off branch tips produce a zone of coenenchyme within 1-2 weeks of fracture where their undersurface comes into contact with the substrate (Figure 11). The axial corallites of such branch tips slowly lose their physical prominence and new corallites may form on the resealed basal region (Figure 11). Similar tips left undisturbed for 3-4 months on perspex holding racks produce coenenchymal growth which spreads out onto the surrounding substrate. This cement the branch to the substrate and provides a surface from which new branches could presumably be generated.

Generation of new branches was actually observed on the upper surface of broken-off branches. Radial corallites, under these circumstances, developed into incipient axial corallites in response to re-orientation (Figure 12a). Observations over a period of three months (Table 1) showed that the development of those corallites growing directly upwards is favoured over that of the others (Figure 12b). The former remain white and extend with increasing rapidity whilst the latter tend to turn brown and grow more slowly with time and eventually stop growing altogether.

TABLE 1

INITIATION OF NEW BRANCHES IN A

RE-ORIENTED BRANCH TIP OF A. formosa

Branch	L Initial	ength of new b After 1	oranch (mm) After 2	After 3
No.	Condition	month	months	months
1 ³	0.5	1.0	1.6	2.3
2	0.7	1.1	1.2	1.3
3	0.4	1.0	1.4	1.8
4	0.3	0.8	1.0	1.0

¹ See Figure 3.7a

- ² See Figure 3.7b
- ³ Marked * in Figure 3.7a, other branches numbered consecutively with distance away from 1 in Figure 3.7b.
Successive cycles of radial corallites display increasing development with increasing distance down the branch. The first cycle of radial corallites (R_1) are very poorly developed:

ir - outward and lateral trabecular divergence from the costae (arrow) of the axial corallite are in the process of forming a hood-shaped enclosure, the first stage in the development of a radial corallite.

 r_i - has an incomplete synapticulothecal ring (i). The axis of the corallite is closely aligned to that of the branch and its calice abuts directly onto the side of the axial corallite. No septal development is apparent.

In the second circle of radial corallites (R_{II}) the coenosteum at the base of the corallites bears coenosteal spines:

 r_{ii} - the first synapticulothecal ring (i) is two-thirds complete, small spines are present in the position of the outer directive septum, and about one third of a second synapticulothecal ring (ii) is present. The costal contribution to the development of the trabecular columns (tc) of these structures may be traced (see also the adjacent lefthand corallite).

In the third circle of radial corallites (R $_{III}$):

 r_{iii} - the first synapticulothecal ring and approximately half of a second ring (ii) are complete. The corallite axis is inclined further away from that of the axial corallite than in R_I and II. The first cycle of six primary septa is present, the free (inner) margin of the outer directive septum bears dentations (d).

co - coenosteum; d - dentation; dse - directive septum; fe fenestration; ir - incipient radial corallite; r - radial corallite; R - circle of radial corallites; s - synapticula; se septum; sr - synapticulothecal ring; tc - trabecular column; tr - trabecula; tt - trabecular tine; arrow - costa.

- Figure 6: Scanning electron micrograph of a lightly calcified, white tipped A. formosa branch tip.
- (a) End on view of axial corallite (x 25)
- The central cavity contains two cycles of six septa each. The primary septa (se_i) are more prominent than the secondary septa (se_{ii}), and the directive septa of the former (dse) are slightly better developed than the others. There are four concentric synapticulothecal rings (sr_{i-iv}) present within the corallite wall, and these are linked radially by the septal trabeculae (tr). The continuity between the septa and their corresponding costae may on occasion be traced (e.g. dse to cross). Trabecular times (tt) are just visible at the junction of the septa and synapticulothecal rings.

(For enlargement of boxed area see Figure 82).

(b) Oblique view of the same branch (x 29)

The trabecular tines (tt) are more obvious than in (a); they appear as distinct prongs at the junctions of the septa and the synapticulothecal rings. The coenosteum (co) is entirely costate over the end of the branch. Near the tip, the margins of the costae (arrow) are smooth. This condition persists until the level of the second circle of radial corallites (R_{II}). At this point costal spines begin to appear, particularly at the base of the radial corallites. These become more widespread and increasingly well developed with distance down the branch.

The synapticulae (s) between the costae are thin near the branch tip and the fenestrations (fe) are correspondingly large. Deposition of stereome further down the branch slightly thickens the synapticulae, and, to a lesser extent, the costae. This results in some reduction in the size of the fenestrations.

(contd overleaf)



Figure 7: Longitudinal view of the tip region of the skeleton of an <u>A. formosa</u> branch with part of the axial corallite broken away to reveal internal structure (x 25).

Four synapticulothecal rings (i-iv) are present in the wall of the axial corallite. Synapticulae (s) and septal trabeculae (tr) link the rings horizontally and radially respectively. Broken sections of these structures (s' and t') show their three dimensional relationship.

One directive septum (dse), two other primaries (se_i) and two secondary septa (se_{ii}) project into the calice. All are laminar and possess irregular dentations (d) on their margins.

In the foreground, part of the fourth synapticulothecal ring is broken away to show the trabecular columns (tc).

The costae (arrow) of the coenosteum are finely servated near the tip, but further down the branch they become markedly servated due to the presence of costal spines (x).

Figure 8: Recolonisation of dead branches in the basal region of an A. formosa colony $(x \ 0.24)$.

A spreading zone of coenenchyme(co) is present at the edge of the living tissue. The dead skeleton proximal to this is heavily colonised with algae.

co - zone of coenenchyme; d - dentation; dse - directive septum; s - synapticula; s' - broken synpaticula; se - septum; t' broken trabecula; tc - trabecular column; tr - trabecula; roman numerals - number of synapticulothecal rings; cross - costal spine.





- Figure 12: Generation of new axial corallites in a re-oriented branch.
- (a) Initial condition (x 1.3)

The horizontal orientation of this broken off branch end has induced the formation of several incipient axial corallites (ia) on its upper surface. Some of these axial polyps and all of the radial polyps have two complete cycles of tentacles (te), all contain zooxanthellae and all are extended.

(b) After three months (scale = 5cm).

The branch in (a) was rotated 90° for stability. The branch with the axial corallite which has remained white was growing almost directly upwards and and has extended more than any of the others. (Its original position in (a) is marked *.) The remainder initiated the formation of shorter branches which were subsequently infiltrated with zooxanthellae, turned brown and stopped growing (see Table 1).

ax - axial corallite; co - coenenchyme; ia - incipient axial corallite; ir - incipient radial corallite; r - radial corallite; sk - skeleton; te - tentacle; * - original position of longest branch. Figure 9: Pad of coenenchyme developed in response to obstruction after 3 weeks (scale = 5cm).

Undeveloped radial corallites (ir) are present in the pad of coenenchyme(co) at the branch end which was formed against the aquarium wall. The coenosteum is spinulose and there are no zooxanthellae in the coenosarcat the periphery of the pad.

Figure 10: The growth of coenenchymesix days after tissue damage by abrasion (x 7).

Part of the tissue has died back, leaving bare skeleton (sk). A zone of coenenchyme (co) has formed at the periphery of the tissue. There are no zooxanthellae in the overlying coensarc. New radial corallites (ir) are forming close to the edge of the coenosteum.

Figure 11: Coenenchyme growth along the edge of a broken off branch end, $1\frac{1}{2}$ months after fracture (x 2.4).

In the region where the branch end was in contact with the substrate, the tissue has died back, and a zone of undifferentiated coenenchyme(co) has formed around the dead area. The exposed skeleton is heavily colonised with crustose coralline and filamentous algae.

The axial corallite (ax) has turned brown and is no longer any larger than adjacent radial corallites (r). The broken end of the branch has been sealed with tissue which contains zooxanthellae. New radial corallites (ir) are being generated on this surface.

(contd overleaf)











3.3 Consideration of skeletal structure in A. formosa

The skeletal morphology of A. formosa conforms to Duncan's (1884) criteria for fast-growing species. It is lightly calcified, frequently forms radial corallites and shows little skeletal elaboration for some distance down the branch. Thus CaCO₃ deposition appears to be channelled into extension rather than ornamentation near the tip (see also Chapter 6). The presence of distinct growth points specifically located at the junction of the synapticulothecal rings and the septa (the trabecular tines) has not been previously described. However, although no positional details were defined, the photomicrographs in Gladfelter's 1982 paper make it clear that she too has observed trabecular tines on the surface of axial corallites of A. cervicornis. She refers to these structures simply as "axial spines". The author strongly recommends the use of the term "tine" in order to differentiate clearly between these structures and the skeletal elaborations currently described as "spines" (i.e. costal and coenosteal spines).

The absence of attachment scars in the skeleton of <u>A. formosa</u> is entirely consistent with the absence of dissepiments in the calice. The latter are thought to be formed when the polyp periodically detaches its basal region, withdraws up the corallite and secretes a new set of horizontal partitions (Matthai, 1923; Bryan and Hill, 1941; Wells, 1956; Barnes, 1971). Wainwright (1964) suggests that the stimulus for this action is the strain put on the polyp tissue with upward growth of the septa and theca. The extensively ramified structure of the skeleton of <u>A. formosa</u> would clearly make this process impossible without enormous rupture of the tissue. Such anchoring structures are therefore presumably not required in A. formosa.

The formation of radial corallites in <u>A. formosa</u> follows a pattern essentially similar to that described in another, unnamed species of <u>Acropora</u> by Duncan (1884). Thus, <u>A. formosa</u> appears to be highly representative of the genus as a whole and information gained about its manner of growth and regeneration may therefore be extrapolated to the rest of this genus with confidence. This conclusion is supported by the rapidity with which zones of coenenchyme are formed in <u>A. formosa</u> in response to damage or obstruction, and the apparent readiness of radial corallites to change into axial corallites since the <u>Acropora</u> are also well known for their highly developed powers of regeneration (Connell, 1973; Pearson, 1981; Tunnicliffe, 1981). Interestingly, Collins (1978) observed the development of an epithecal rim in his observations of damaged branches in <u>A. formosa</u> colonies. No rim was ever observed in the present study; however, the damage examined was more superficial and did not require extensive skeletal growth.

Kawaquti (1937a) observed a strong tendency for regenerating <u>A. formosa</u> branches to grow upwards following re-orientation, which he suggested was analagous to positive phototropism in plants (Kawaguti, 1944a). Work with <u>A. cervicornis</u> has shown that photosynthesis is essential to calcification (Chalker and Taylor, 1975), and that cycles of algal photosynthesis and calcification are synchronised in order to sustain maximal rates of calcification during daylight (Taylor, 1977; Chalker and Taylor, 1978). Kawaguti's analogy therefore seems to be an apt one. These growth-enhancing factors appear to operate most effectively in branches which point directly upwards towards the light since upwardly-oriented radial corallites are more successful at branch initiation after changing into axial coral-lites.

4. The polyp of A. formosa

4.1 Introduction and terminology

Comparison of general accounts of the structure of coelenterates (Hyman, 1940; Moore, 1956; Bouillon, 1968; Muscatine and Lenhoff, 1974) with more specific studies of the anatomy and histology of corals (Fowler, 1885, 1887; Bourne, 1887a&b; Duerden, 1904a; Matthai, 1923; Hickson, 1924) indicates that the scleractinia have a typical anthozoan structure, and exhibit the tissue composition common to all coelenterates, namely an outer epidermis and an inner gastrodermis separated by the mesoglea. Scleractinian polyps differ from those of the actiniarian sea anemones, the other order in their sub-class, Hexacorallia, only in their ability to secrete a calcium carbonate (CaCO₃) skeleton.

The following general description of the structure of coral polyps is based on the studies mentioned above. It reviews the basic morphology and anatomy of the scleractinia and introduces the specialised terminology associated with this subject. Some of the key terms to be used in this brief review and elsewhere in this thesis are listed below in alphabetical order for ease of reference:-

- canal longitudinal pocket of coelenteron between trabecular columns of skeleton
- canalicula lateral connection between canals through a fenestration in a synapticulothecal ring
- calicoblast epithelial component of calicoblastic epidermis derived from metamorphosis of ectodermal columnar supporting cells during settlement of planulae (Vandermeulen, 1975; Goreau and Hayes, 1977)
- calicoblastic epidermis tissue layer adjacent to and responsible for the secretion of the skeleton

enidoglandular band - densely cellular cap of tissue at the periphery of the mesenterial filaments containing gland cells responsible for digestion

coelenteron - digestive cavity of the polyp

coenosarc - tissue overlying coenosteum

- columnar supporting cell epithelial component of the epidermis
- complete mesentery mesentery which attaches to the stomodeum

desmoidial process - mesogleal attachment points which anchor the polyp to the skeleton

- directive tentacle tentacle located over the outer directive septum
- edge zone fold of body wall extending over the wall of the calice
- epidermis outermost tissue layer, in contact with the environment
- entocoele space between members of a mesenterial pair

exocoele - space between pairs of mesenteries

- gastrodermis tissue layer which lines the coelenteron
- incomplete mesentery mesentery which does not attach to the stomodeum

ingestion – excretion region – part of lateral lobes responsible for particulate ingestion and excretion

inner body wall - tissue which lines the skeleton

lateral lobe - swelling of the gastrodermis just proximal to cnidoglandular band

- mesoglea layer of deformable material which separates the epidermis and gastrodermis
- mesentery radially disposed gastrodermal folds with a mesogleal core bearing either exocoelic (directive mesentery) or entocoelic (non-directive mesentery) longitudinal retractor muscles. All are attached to the column wall and the undersurface of the oral disc.
- mesenterial filament continuation of a mesentery, comprising a non-muscular membranous extension of the mesentery, a pair of lateral lobes and a cnidoglandular band

nematocyst - stinging cell

- nutritive cell major epithelial component of the gastrodermis, responsible for the absorbtion of hydrolysed proteins from digesting prey in the coelenteron
- oral disc oral end of the polyp which bears the tentacles as well as the mouth
- outer body wall tissue which overlies the skeleton to form the exterior of the polyp
- stomodeum oesophagus-like tubular passageway leading from the mouth to the coelenteron
- sulcus --indentation between the cnidoglandular band and the lateral lobes
- tentacle tubular evagination of the oral disc between the members of a pair of mesenteries

The reader is also referred to the diagrammatic representation of the basic anatomical features of the scleractinian polyp overleaf, which illustrates the following description.



Diagrammatic representation of an idealised polyp

Scleractinian polyps are essentially cylindrical with an oral and an aboral end, although this basic form may be extensively modified in colonial types. The lower, aboral end, is described as the inner body wall $^{(1)}$ because it is permanently encased in skeletal material (i.e. the corallite). This is secreted from modified epidermal cells, known as calicoblasts (after Bourne 1887). The calicoblastic layer, and hence the inner body wall, is extensively invaginated to accommodate the vertical skeletal elements. The most prominent of the latter are the septa $^{(2)}$ which arise between the mesenteries (3). The mesenteries themselves are formed by inward folding of the gastrodermis and the mesoglea. The septa and the mesenteries divide the interior of the polyp radially, and have the effect of increasing the area available for absorbtion by the nutritive cells of the gastroder-In contrast, the outer body wall⁽⁴⁾ is relatively mis. uncomplicated. It simply comprises that part of the body wall which lies outside the edge zone of the corallite and is in direct contact with the environment.

The oral end of the polyp bears an oral disc⁽⁵⁾ whose tissues are evaginated over the septa to form the tentacles. These are basically hollow tubes which usually have bulbous ends and rough surfaces, due to the presence of numerous stinging cells or nematocysts. The function of the tentacles is the capture and retention of prey.

A short stomodeum⁽⁷⁾ leads from the mouth and opens into the gut cavity, or coelenteron⁽⁸⁾. Each of the epidermal cells lining the stomodeum bears a long flagellum on its distal surface. These flagellated epidermal cells function in pumping water into the coelenteron. The stomodeum acts as a valve to maintain a positive pressure within the coelenteron which provides support for the animal during expansion. The fluid is, of course, incompressible and thus acts as an antagonist for muscular action. The term "hydrostatic skeleton", suggesting an analogy with the action of muscle against bone in vertebrates, is used to describe this system (see reviews by Chapman, 1958, 1974).

The polyp's axis of lateral symmetry is indicated by the position of two pairs of mesenteries which differ from all other mesenteries. These pairs of mesenteries, known as directive mesenteries, lie diametrically opposite each other across the mouth. Longitudinal retractor muscles $^{(9)}$ form bulges on the faces of all the mesenteries. Their combined action exerts a pull on the underside of the oral disc, and shortens the polyp. The muscles of paired mesenteries usually face each other and project into the endocoelic space enclosed by the pair of mesenteries. However, directive mesenteries are exceptional because they bear their retractor muscles on their outer faces and the muscles project into the exocoelic spaces between adjacent pairs of mesenteries. This makes the directive mesenteries easily recognisable.

Corals have poorly developed musculature compared to the anemones, which probably reflects a reduced need for support in corals because of the presence of their rigid CaCO₃ exoskeleton (Duerden, 1904a; Hyman, 1940). However, ultrastructural studies (Goreau, 1956; Kawaguti, 1964a, 1966; Kawaguti and Yokayama, 1966; Westfall, 1973) have shown that corals are, nevertheless, typically anthozoan in their musculature on two counts. Firstly, the muscle layers are composed of fine sheets of fibrils at the junction of the epitheliomuscular cells and the mesoglea, an arrangement described in actiniarians as muscle fields (Batham and Pantin, 1951). Secondly, with the exception of a thin layer in the epidermis of the tentacles (Hyman, 1940), the longitudinal musculature is entirely restricted to the mesenteries, a characteristic which differentiates the Anthozoa from all other coelenterate groups (Hand, 1966).

Most previous studies of the polyp of <u>A. formosa</u> have been largely concerned with summarising gross anatomical detail for taxonomic purposes (Fowler, 1887; Brook, 1893), or as a prelude to examination at the ultrastructural level (Kawaguti and Sato, 1968; Harrison, 1980). Two studies (Collins, 1978; Gladfelter, 1982) briefly discuss the position of the tissues of Acropora relative to the skeleton. Others restrict themselves to descriptions of the development of particular structures such as the mesenteries (Duerden, 1902a; Atoda, 1951) and gonads (Oliver, 1979). In the present study, a knowledge of the structure of the extreme tip region of <u>A. formosa</u> branches, and in particular, the axial polyp, was considered essential as a basis upon which to interpret observations of the growth and extension of the corallum. None of the studies mentioned above paid particular attention to this region.

As part of this anatomical investigation, a special study was made of the secretory cells of <u>A. formosa</u> in order to evaluate whether the different evolutionary origins suggested for the Acropora by Wells (1956; see p. 3) are reflected in their gland cell composition. With the exception of a preliminary study by Collins (1978), who also examined <u>A. formosa</u>, all previous histochemical investigations of scleractinian secretory cells have been concerned with genera from non-astrocoeniid stock.

Changes which accompany the expansion and contraction of the polyp of A. formosa were investigated from samples taken at different stages of the diel cycle. The sea anemones are the only group of coelenterates in which such changes have been studied in any detail (Batham and Pantin, 1950 a and b, 1951; Chapman, 1949; Robson, 1957; Grimstone et al, 1958; Leghissa, 1965). The findings of Batham and Pantin (1951) may be regarded as representative of all these studies. They report that in Metridium senile, contraction of the polyp is effected by the combined action of the circular muscles of the body wall and the longitudinal muscles of the mesenteries. Their action opens the mouth and shortens the polyp column, thus forcing water out of the coelenteron. Upon relaxation of the muscles, the polyp re-inflates due to reintroduction of water into the body cavity via a ciliated band or bands known as siphonoglyphs. The collapse of the body always produced considerable deformation of the epithelial layers and substantial folding of opposing muscle layers. The latter process was described as "buckling".

There are many reasons why the anemones have been more fully investigated than other coelenterates. The polyps of the former tend to be large, solitary and relatively robust. They are thus easier to work with than other coelenterates which tend to have smaller, fragile polyps and are frequently colonial (G. Chapman, 1974). Corals present additional problems because of their CaCO₃ exoskeleton which creates considerable technical problems by interfering with observation, dissection and histological processing. Moreover, studies by Wells (1932) and Collins (1978) have shown that coral polyps, unlike anemones (see Batham and Pantin, 1951) do not remain expanded when they are anaesthetised. As far as the writer is aware, this study represents the first attempt to describe fully the cellular mechanisms which operate to bring about diel expansion and contraction in the scleractinia. 4.2 The polyp in relation to the skeleton

In a longitudinal section through the stomodeum of an axial corallite of a white tipped branch of <u>A. formosa</u> (Figure 13), the arrangement of the tissues in sectioned polyp material indicates the regions formerly occupied by the skeleton. Such sections reveal that the proportions of skeleton to coelenteron are roughly equal. The three dimensional lattice which comprises the skeleton is entirely covered by the inner body wall, whilst the outer body wall overlies the surface of the corallite.

The position of the trabecular columns and the synapticulae which interlink them to form the synapticulothecal rings can be inferred, as can outlines of the trabecular tines at the ends of the trabecular columns. Similarly, the sites of the trabecular outgrowths which form the perforate septa of the corallite walls and the costae may also be deduced. The coelenteron of each polyp therefore consists of a series of concentrically arranged longitudinal canals, the largest of which is the central cavity. These canals are interlinked through the fenestrations of the synapticulothecae by the canaliculae. The canaliculae also provide continuity between the coelenterons of the axial and radial polyps (Figure 13).

A transverse section through a similar branch at the level of the first circle of radial corallites (Figure 14) shows the intermittent nature of the synapticulothecal linkages. Internal septal development is clearly discontinuous, and it is not possible to trace a direct radial linkage between a septum and a corresponding costa.

At the points where the skeleton meets the polyp surface, there is complete fusion of the inner and outer body walls. At the tip of the corallite, the outer body wall is suspended between the trabecular times (Figure 13). Down the sides of the corallite, it is supported on the edges of the costae (Figure 13 and 14).

4.3 Histology

The polyps of A. formosa may be divided into the following regions: the inner body wall, outer body wall, oral disc, tentacles, stomodeum, mesenteries and mesenterial filaments. The histology of each is examined below. Particular attention is paid to diel changes in the inner and outer body wall (especially where they meet and fuse over projections of the skeleton such as the trabecular tines) and the tentacles. A trilaminar tissue structure of epidermis (either calicoblastic or free), mesoglea and gastrodermis is common to all of the above regions. Each tissue layer contains one particular type of epithelial cell with the exception of the mesoglea which is virtually acellular and consists of a network of fibres and fibrils in a basophilic matrix. The epidermis of the inner body wall is composed almost entirely of squamous, calicoblastic epithelial cells which are spindle-shaped when viewed in section (Figure 15). In contrast, the epidermis of the outer body wall contains tall, thin columnar supporting cells (Figure 16). The gastrodermis throughout the polyp consists largely of nutritive cells (Figures 15 and 17), whose form ranges from cubo-columnar to squamose depending on their location.

<u>A. formosa</u> polyps possess two types of mucous gland cells (Types 1 and 2) and four types of granular gland cells (Types A-D). Their distribution, morphology and histochemical reactions are discussed in the account of the polyp regions which follow, and summarised in Tables 2 and 3. The distribution of the four types of nematocysts found in <u>A. formosa</u> is also summarised in Table 3. They are categorised with reference to the works of Weill (1934 in Hand, 1961), Hyman (1940), Cutress (1955), Werner (1965) and Mariscal (1974) on the basis of their undischarged appearance. Unless otherwise noted, the staining reactions described are those obtained with the MSB technique (Section 2.3.5.1).

granule diameter (μm)	Cell Size (µm)	MSB ¹	Alcian Blue	-PAS ²	Metachromasia ³	Protein ⁴ (tryptophan)	Conclusions cell contents	Designation
amorphous	interior 5.2x9.0 exterior 7.3x14.4	unstained	+	I	eta violet	1	acid mucin	type 1
	interior 5.2x9.0							
amorphous	exterior 4.7x10.3	deep purple	+	+	α blue	I	mixed mucin	type 2
1.0	3.1x9.4	red (acidophilic)	I	+	8	I	neutral mucin	type A
1.6	5.2x10.4	blue (basophilic)	+	+	ø	l	mixed mucin	type B
0.7	1.4x6.2	purple	I	ł	IJ	+	protein	type C
1.2	2.6x20.8	purple	I	I	ø	+	protein	type D
1 Martiu Methv	s Yellow - Brillian 1 Blue trichome	it Crystal Scarl	et –		³ Toluidine blu	υ		
2 Period	ic Acid Schiff				4 Voisnet-Fürth	reaction		

TABLE 2: Morphology and histochemistry of the glandular cell types in the polyps of A. formosa.

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	outer body wall epidermis	calicoblastic epidermis	tentacle	oral disc	stomodeum	type I mesenterial filament	type II mesenteria filament	outer body wall gastrodermis	inner body wall gastrodermis	
type 1 mucous	+++	-	+	<u>+</u>	+	+	+++	<u>+</u>	++	
type 2 mucous		-	+	+	+	+	-	<u>+</u>	+	
type A granular	+	-	-	-	.	+	++	-	-	
type B granular	-	-	-	-	-	++	+	-	-	
type C granular	-	-	-			++	++	-		
type D granular	-			-	-	+	-		-	
flask- shaped cell	-	-	-	-	. —	<u>+</u>	<u>+</u>			
spirocyst	++	- (++ base)	-	+		, +	-		
microbasic p- mastigophore	+	-	· <u> </u>	-	-	_	+			
microbasic b- mastigophore	+	- (++ tip)	-	-	+	++	-	-	
holotrichous isorhiza	+++	<u>+</u>	<u>+</u>		-		-		+	
zooxanthella	-	-	<u>+</u>			+		++	+	

<u>TABLE 3</u>: Distribution of non-epithelial cell types in the polyps of <u>A. formosa</u> (gland cells described in Table 2).

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+++ = profuse; ++ = moderate numbers; + = scanty; <u>+</u> = occasionally present; - = absent.

4.3.1 Inner body wall

The calicoblastic epidermis of the inner body wall lies immediately adjacent to the skeleton. The spindle-shaped calicoblasts have spherical nuclei in their bulging central region. They are contiguously arranged on the mesoglea to form a thin (1- 2.5μ m) squamous epithelium (Figure 15) which sometimes contains nematocysts (usually holotrichous isorhizae) (Figure 16). The mesoglea itself is extremely thin ($=0.5\mu$ m) in the interior of the polyp. Small ($6.3\times3.5\mu$ m) amoebocytes with fine, granular ($0.3-0.5\mu$ m) acidophilic contents, similar to those sometimes seen in the gastrodermis (Figure 15c), are occasionally present.

The inner body wall gastrodermis is mainly composed of nutritive cells which form a squamous epithelium approximately 5μ m in thickness. Zooxanthellae, which are approximately 7μ m in diameter, form a small but conspicuous component of this layer (Figure 15a). Two types of mucous gland cells are also present in the inner body wall gastrodermis (Tables 2 and 3). They are similar in size ($5.2x9.0\mu$ m) but exhibit different staining reactions. Type 1 mucous gland cells are most abundant, and do not stain at all with MSB (Figure 15a). The others (Type 2) are fewer in number and stain deep purple with MSB (Figure 15b). Both are evenly distributed throughout the polyp interior.

4.3.2 Outer body wall

At the extreme tip of <u>A. formosa</u> branches there are relatively few non-epithelial cells. The outer body wall epidermis consists largely of tall, thin columnar supporting cells which have their oval shaped red-purple nuclei arranged in a distinctive band in the proximal third of the layer (Figure 16). Amongst their basal processes are the spherical, blue-purple nuclei belonging to interstitial cells (Figures 17 and 18). The gastrodermis is separated from the epidermis by a 1.5μ m thick layer of mesoglea, and is composed almost entirely of cubo-columnar nutritive cells roughly 9μ m in height, which do not contain zooxanthellae (see Figure 17).

Proximal to the extreme tip, both the epidermis and the gastrodermis acquire more cell types (Table 3). Nematocysts proper (holotrichous isorhizae, microbasic p- and b-mastigophores) and spirocysts (Figures 17 and 18) occur more frequently in the epidermis. Proximally located cells varying in size from that of an interstitial cell $(4x5\mu m)$ up to that of a holotrichous isorhiza $(6x14\mu m)$ with blue staining (basophilic) cytoplasm also appear (Figures 17 and 19). These probably represent maturation stages in this type of nematocyst. Discharged capsules (Figure 19) are sometimes evident. Small numbers of Type A granular gland cells with acidophilic granules $1\mu m$ in diameter also occur (Figure 19), but the dominant cell type in the proximal epidermis is the large $(7.3 \times 14.4 \mu m)$ non-staining (Type 1) mucous gland cell (Tables 2 and 3). This is difficult to discern, however unless the cell contents are specifically stained (compare Figures 19 and 20). Both purple staining (Type 2) and non-staining (Type 1) mucous gland cells identical to those in the inner body wall gastrodermis occasionally occur in the outer body wall gastrodermis away from the tip (see Figure 20). Zooxanthellae also become increasingly common, and can form an internal lining up to 3 cells thick.

4.3.3 Junction of the inner and outer body walls

Examination of serial sections through the sites of trabecular tines in axial polyps fixed at various times over a diel cycle show that the tissue tends to adopt one of two distinctive conformations around the tip of the tine. In the "contracted" conformation (Figure 22), the tissues appear highly compacted. In the "expanded" state (Figure 23), they are more attenuated. The configuration exhibited by tissues fixed at two hourly intervals over a 24 hour period is summarised for 8 separate diel experiments in Figure 26. From the data, it is clear that the tissues tend to adopt the expanded conformation during the night, and are usually in the contracted conformation during the day. This corresponds to the diel cycle of noctural expansion and daytime contraction exhibited by the whole polyp (Section 4.3.5). The tissue region affected in this way is strictly limited to the extreme tips of the trabecular tines. The inner body wall around the trabecular columns, roughly 70µm proximal to the tip (see Figures 67&68) never changes its conformation.

The main differences between the conformations shown in Figures 22 and 23 are illustrated diagramatically in Figure 27 in order to highlight the changes which occur in the cell layers when the tissues change from the contracted to the expanded state. Reference to that diagram will aid in understanding the following description.

The epidermal columnar supporting cells increase in height from approximately $35\,\mu\text{m}$ in the contracted condition to between 45 and 55 μm in the expanded state. They also become more widely spaced. Their nuclei remain in a band in the proximal third of the layer, but specialised (non-epithelial) cells are shifted distally (compare also Figures 17 and 18).

The mesoglea of the outer body wall changes only slightly between the two conformations, becoming slightly thicker (up to 3µm) in the expanded state. The mesoglea of the inner body wall changes markedly, however. It is always thick and straight in the contracted state, and forms a conspicuous wedge shaped mass at the junction of the inner and outer body walls. This disappears in the expanded state, and mesoglea becomes thin and wavy.

In the contracted state the nutritive cells of both the inner and outer body wall gastrodermis form a uniform, 9μ m thick, cubo-colummnar epithelium. In the expanded conformation, the nutritive cells of the outer body wall show only a slight increase in height and width (up to $12-15\mu$ m). In contrast, those of the inner body wall gastrodermis become cubo-squamose, and form a layer $4-5\mu$ m in thickness. The effect of both these changes is to spread the cells out along the mesoglea. The transition between the inner and outer body wall is abrupt in both conformational states, with little cellular deformation.

The main differences between the contracted and expanded states relate to the calicoblastic layer, where the cells completely reverse their orientation to the mesoglea. In the contracted state there are large intercellular spaces between calicoblasts, and the cells themselves have a tendency to stand perpendicular to the mesoglea. In the expanded state they lie completely flat. The net result is that there are far fewer calicoblasts per unit length of mesoglea in the expanded condition. Since calicoblasts in the tip region are approximately 12.5 μ m long and only 3.7 μ m wide, contracted tissue has potentially 3-4 times as many cells in the same length of mesoglea.

Thus, during expansion of the polyp, it appears that the outer body wall becomes turgid, whilst the inner body wall immediately around the tip of the trabecular times becomes stretched.

The relative position of the skeleton and polyp tissue in the two conformations may be deduced from an examination of damaged sections. In damaged, contracted tissue sections the skeleton appears to have been forced through the outer body wall, causing considerable disruption to the cell layers (Figure 24). Under natural conditions, the skeleton never penetrates the tissue except under conditions of extreme starvation (Yonge and Nicholls, 1931b; Franzisket, 1970) or stimulation (Barnes, 1971; Collins, 1978). Thus it seems that the tissues of contracted polyps are normally closely applied to the skeleton. Consequently they are punctured if the response to fixation is violent.

In contrast, damaged sections in the expanded conformation show no disruption of the cell layers (Figure 25). The tissue appears simply to have torn at the point where the tissues are weakest, at the apex of the site of the trabecular tine. It seems likely that this damage occurred after fixation, perhaps during decalcification, and suggests that there is normally a gap between the skeleton and tissue in such expanded polyps.

4.3.4 Oral disc

The oral disc epidermis (Figure 44) consists almost entirely of columnar supporting cells, reflecting the general tendency (mentioned in section 4.3.2) towards a reduction in the numbers

of specialised cell types towards the branch tip (Table 3). There are a small number of spirocysts, microbasic p-mastigophores and unstained (Type 1) mucous gland cells. Purple staining (Type 2) mucous gland cells are occasionally present and are slightly larger $(5.7 \times 10.3 \mu m)$ than those found in the inner body wall (Table 2). The gastrodermis in this region consists of flattened, cubo-columnar nutritive cells (Figure 45). Zooxanthellae are virtually absent (Table 3).

4.3.5 Tentacles

In full daylight, the tissues of the axial polyps of <u>A. formosa</u> are usually closely applied to the skeleton and the tentacles are completely retracted (Figure 3). The tentacles withdraw into small knobs of tissue situated on the inverted oral disc at the top of the invaginated stomodeum (Figure 30). The tentacles of the radial polyps may sometimes be partially expanded during the day (Figure 28) particularly under conditions of low light intensity (e.g. substantial cloud cover; late afternoon). Full expansion occurs only at night, especially around midnight and the early hours of the morning (Figure 29). During this time, the oral disc, bearing the tentacles, is everted above the calix. The tissue over the surface of the branch end is also inflated and is lifted clear of the corallite surface (Figure 29).

Axial corallites which terminate lightly calcified, fast growing branches have only one cycle of 6 equal sized tentacles (Figure 29), radial polyps have 12 (i.e. 2 cycles). In the latter, the tentacles above the outer directive septum is always considerably longer than any of the others (Figure 28).

The tentacles of axial polyps are generally translucent. Opaque patches (Figure 29) signify areas where nematocysts are concentrated into batteries. Such regions are particularly prevalent towards the tip. The occasional presence of zooxanthellae is indicated macroscopically by brown specs (Figure 28). In radial polyps, however, the tentacles are usually completely brown due to the large number of contained algae (e.g. Figure 12).

Expanded polyps of A. formosa were found to be extremely sensitive to environmental changes, and fixation invariably initiated some degree of contraction before complete fixation could occur. Longitudinal serial sections through a highly expanded oral disc, distal to the stomodeum, (Figures 32 a-d) pass through the bases of the partially retracted tentacles. Such sections show that the mesenteries are attached to the underside of the oral disc (Figure 32 b and c). Mesogleal pleats at this point (see Figure 32d) indicate that the musculature of the mesenteries is contracted (see Batham and Pantin, 1951; Goreau, 1956), thus implicating the mesenteries in the re-invagination of the oral disc. Such sections also show that mesenterial extensions arise from the pair of mesenteries which subtend each tentacle and attach either side of its base (Figure 32a). The attachments appear to be pulled up when the polyp expands and come to lie across the oral disc (Figure 32d), ready to function in the retraction of the tentacles.

The basal regions of the tentacles arise directly from the oral disc and have essentially the same cell structure as that region. The gastrodermis is a flattened, cubo-columnar epithelium, containing few, if any, zooxanthellae. The epidermis chiefly comprises columnar supporting cells, but moderate numbers of both unstained (Type 1) and purple staining (Type 2) mucous gland cells and some spirocysts (Figure 32a) are also present.

Spirocysts are most common in the base of the tentacles. Their numbers decrease along the length of the tentacles as they are gradually replaced by microbasic p-mastigophores (Table 3). In the distal portion of expanded tentacles the mesoglea is very thin and the cells of both the epidermis and gastrodermis are flattened to form a squamous epithelium. The orientation of the nematocysts (Figure 35) indicates that the long axiis of the epidermal cells are oriented parallel to the mesoglea. At the bulbous tentacle tips, the cells resume their normal conformation and batteries of microbasic p-mastigophores are present (Figure 31). The musculature of the tentacles stains red with MSB and the fibres and fibrils are clearly visible against the blue staining cell cytoplasm of the epithelial cells and the mesoglea (figures 33 and 34). The muscle fibrils in the basal processes of the epidermal columnar supporting cells are aggregated into fields of thick, longitudinally oriented fibres which lie between prominent mesogleal folds (Figure 33).

The muscular elements of the gastrodermis of tentacles are similarly arranged, but they are oriented at right angles to the epidermal musculature, forming a circular muscle layer (Figure 34). They are, however, relatively poorly developed, and appear as a diffuse sheet at the base of the gastrodermal cells. Sparse numbers of spherical nuclei (stained red-purple with MSB) are associated with this sheet (Figure 34).

The cellular formations associated with the various stages of tentacle retraction are summarised in Figures 36-38. In the distal part of the tentacle the poorly developed gastrodermal musculature appears unable to resist the action of the longitudinal epidermal muscle fibres and the two cell layers compress together (Figure 36). The alignment of the epidermal cells alters so that they once again become oriented with their long axes perpendicular to the mesoglea. Meanwhile, the gastrodermal nutritive cells resume their cubo-columnar shape.

Contraction of the circular muscle elements reduces the diameter of the tentacle. As a result, the longitudinal muscle fibres are projected into the epidermis on strands of mesoglea, without altering the surface contours of the cell layer. This is the process which Batham and Pantin (1951) described as "buckling". At the same time, the gastrodermal cells are compressed, and the gastrodermis folds into the lumen, creating inwardly projecting undulations (Figure 34). This arrangement is summarised diagramatically in Figure 39a.

At this stage of tentacle retraction, it appears that the gastrodermal musculature is fully compressed, because, upon further contraction of the longitudinal muscles, the circular muscle layer responds by buckling into the gastrodermis (Figures 37 and 38). This action simultaneously deforms the gastrodermal layer and the superficial fibres of the gastrodermal side of the mesoglea and replaces the longitudinal gastrodermal folds with circular ones (Figures 37 and 38). The surface of the epidermis is also thrown into folds which appear to be produced by differential expansion of the stems of the columnar supporting cells (Figures 37 and 38). Thus, the surface of both the interior and the exterior of the tentacle assumes a corrugated appearance (Figure 40). This stage in tentacle retraction is summarised diagramatically in Figure 39b.

Sections through completely retracted tentacles show deep epidermal folds (Figure 41) around the circumference of the knobs of tissue which comprise the tentacles at this stage (see Figure 30). The very high concentrations of epidermal cell nuclei are arranged in thick undulating bands which complement their surface contours. The lumen of the tentacles virtually disappears (Figures 41 and 43) and the fibres of the mesogleal layers align into sheets which are oriented at right angles to each other (Figure 41). Oblique sections through highly retracted tentacles show that the fibres of the mesogleal sheets supply attachment points for the displaced epidermal and gastrodermal cells (Figures 42 and 43).

4.3.6 Stomodeum

The stomodeum of <u>A. formosa</u> is lined by a palisade of tall columnar supporting cells. Their long flagella form a dense brush border which partially occludes the lumen (Figure 48). Non-staining (type 1) and purple staining (type 2) mucous gland cells and spirocysts are sparsely interspersed amongst these cells (Table 3). Thick strands of mucus from the type 2 gland cells are commonly present in the lumen. The gastrodermis around the stomodeum consists entirely of cubo-columnar nutritive cells (Figure 48).

4.3.7 Mesenteries and mesenterial filaments

Serial transverse sections through both radial and axial polyps show that the upper region of the polyps are radially partition-

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ed by 12 mesenteries which are attached to the oral disc (Figure 44). The two directive pairs bear prominent exocoelic pleats, whilst the four non-directive pairs have entocoelic pleats (Figure 44). In radial polyps, the pleats of the outer directive pair of mesenteries (which subtend the enlarged outer directive tentacle) are particularly well developed and contain very fine longitudinal muscle fibrils (Figure 46).

Both members of the two directive pairs of mesenteries are attached to the stomodeum (i.e. they are complete), but one member of each of the four non-directive pairs is incomplete (Figure 45). The overall extent of mesogleal pleating is much reduced at this level compared to that of the oral disc, suggesting that muscular development is decreased around the stomodeum. The incomplete mesenteries are particularly poorly developed, their free edges never bear mesenterial filaments (Figure 46) and they terminate almost immediately below the stomodeum (figure 47).

The cnidoglandular bands of the mesenterial filaments which arise from the complete mesenteries are derived directly from the stomodeum (Figure 47). The mesenteries themselves do not descend far into the coelenteron; the non-muscular mesenterial filaments continue downwards in their place. Only six mesenterial filaments are found in the depth of the polyp (Figure 14), therefore two of the complete mesenteries must be rather short.

The mesenterial filaments in the central cavity of the upper part of the polyp can be readily distinguished from those in the lower part of the central cavity, and in more isolated parts of the coelenteron. The distinction is based on marked differences in the size and inclusions of their lateral lobes and in the composition of their cnidoglandular bands (Figure 52; Table 3). The two types have been designated "I" and "II" respectively.

Both types of mesenterial filament contain different combinations of four types of granular gland cells (types A-D, see Figure 49), which may be readily differentiated by their morphology and by the size and staining reactions of their granules (Table 2; Section 4.3.8.4).

Type A granular gland cells are also found in the outer body wall epidermis (Section 4.3.2). They are conical in shape (approximately 3.1µm wide at their widest point and roughly 19.4µm in length) and contain small $(1\mu m)$ red-staining granules (Figures 49, 52-54). Type B granular gland cells are oval in shape. They are wider and shorter $(5.2 \times 10.4 \mu m)$ than type A (see Figure 56) and are filled with amorphous blue-staining granules approximately 1.6µm in diameter (Figures 53 and 54). The granules of both type C and D granular gland cells (Figure 49) stain red-purple with MSB. Those of type C are only $0.5-0.7\mu$ m in diameter and are irregularly arranged in cells which measure about 1.4 m by 6.2μ m (Figure 57). In contrast, the granules of type D granular gland cells are relatively large $(1.4\mu m)$ and are always arranged in distinct chains, roughly 20.8μ m in length (Figures 49 and 57). Several chains may be contained within a single cell (Figure 56).

A fifth type of gland cell is sparsely distributed throughout the whole length of the mesenterial filaments. These are small $(2.5 \times 4 \mu m)$, highly refractile, flask-shaped cells which do not stain with MSB (Figure 50).

The cnidoglandular bands of the type I mesenterial filaments are derived directly from the epidermis of the stomodeum (Figure 47). Their cellular composition reflects this in that they are chiefly composed of conspicuously flagellated columnar supporting cells (Figure 51). Microbasic p-mastigophores (the only type of nematocyst present, Table 3) and both non-staining (type 1) and purple staining (type 2) mucous gland cells occur in small numbers (see Figure 55). Moderate numbers of blue staining (type B) granular gland cells are present, but there are few of the red staining type A (Figure 53). Similarly, there are moderate numbers of the gland cells with small, red-purple staining granules (type C) but only small numbers of the large granuled, red-purple staining (type D) cells (Figure 57).

The cellular composition of the cnidoglandular bands of type II mesenterial filaments presents a reversal of this picture (Table 3). They are typically paired (Figure 52) and contain few

columnar supporting cells. The dominant cell type is nonstaining (type 1) mucous gland cells, consequently type II mesenterial filaments appear to be highly vacuolate when stained with MSB (compare Figures 53 and 54). Purple staining (type 2) mucous gland cells are entirely absent. The ratio of red staining to blue staining granular gland cells is also reversed, with a marked predominance of type A over type B (Figure 54). Granular gland cells with large, red-purple staining granules (type D) occur in moderate numbers (Figure 56), type C cells with small granules are not present. Type II mesenterial filaments bear both p- and b-microbasic mastigophores, the p-type is by far the most common (Table 3).

Immediately behind the cnidoglandular bands of the mesenterial filaments are the lateral lobes. The two regions are separated by an indentation, or sulcus, linked only by the mesoglea (Figure 52). In the uppermost regions of the type I mesenterial filaments, the lateral lobes are small and compact (Figure 51 and 58). Lower down they become more vacuolate (Figure 60). The lobes may contain broken nematocysts (Figure 58) and zoo-xanthellae (Figure 59). The latter often show signs of degeneration, such as an enlarged accumulation body, an increase in crystalline and vacuolar cytoplasmic inclusions, and even the total loss of internal organisation (Figure 59).

Extrusion of zooxanthellae from the polyp occurs in the distal parts of the lateral lobes of type I mesenterial filament. Ejection takes place near the sulcus (Figure 60). It frequently appears that the algae are still retained within the host cell after extrusion. This region was also observed to be responsible for ingestion (Yonge, 1931) and so, has been referred to as the "ingestion-excretion" region in this study. Clumps of such algae, together with remnants of food and broken nematocysts, are sometimes observed in the coelenteron (Figure 62). They are bound together with mucus to form a bolus which is presumably destined for ejection via the stomodeum.

In complete contrast to the type I mesenterial filaments, the lateral lobes of type II filaments are always extremely reduced and lack inclusions (Figures 52 and 54).

The transition from type I to type II mesenterial filaments is a gradual one. The structural differences illustrated here represent points in a continuum of change during which the cnidoglandular bands of type I mesenterial filaments acquire more glandular cell types as they descend into the polyp. Their lateral lobes become larger and have increasing numbers of inclusions (compare Figures 58 and 60). In contrast, the cnidoglandular bands of type II mesenterial filaments have progressively fewer gland cell types away from the mouth until type 1 mucous gland cells come to predominate. At the same time, the lateral lobes regress and eventually almost disappear (compare Figures 52 and 56). The convoluted nature of the filaments make it difficult to trace the transition from type I to type II mesenterial filaments. Thus, it is not possible to state whether the pairing of the type II mesenterial filaments originates from a merging of two type I filaments or the splitting of one.

The presence of type II mesenterial filaments throughout the coelenteron suggests that they are highly mobile. This is further indicated by Figure 66 which shows a series of sections through either a group of type II mesenterial filaments, or one convoluted one, projecting through a perforation in the outer body wall.

The mesoglea of both types of mesenterial filament is fibrillar in the lateral lobes where it occasionally contains cells (Figure 51). It becomes a single layer in the membranous part of the filament where it forms a core covered on either side by a layer of extremely flattened gastrodermal cells. Zooxanthellae are occasionally present in the membranous portion of type I mesenterial filaments, forming a conspicuous feature of this structure (Figure 61).

The intra-mesogleal development of the gonads on type I mesenterial filaments is also a very obvious process. In monthly samples taken from Nelly Bay, ova were first observed in April. Increasingly mature stages of development are then observed (compare Figures 63 and 65), until October when the largest eggs are found. Zooxanthellae are not associated with the ova at any stage. Testes are present for only a short period from September to October, and they quickly mature in this time (see Figure 64). They may appear on an unoccupied mesenterial filament, or develop below a maturing ovum (Figure 63). Zooxanthellae are commonly found amongst the developing loculi of immature testes, but are not present in the mature testis (compare Figures 64a and b). Planulae are never seen inside the polyp, but after October there is no more reproductive material produced until the cycle begins again in April.

4.4 Histochemistry

Unstained (type 1) mucous gland cells are distributed throughout the polyp, with the exception of the calicoblastic epidermis (Table 3). Their size varies widely $(5.2 \times 9.0 \mu \text{m} \text{ to } 7.3 \times 14.4 \mu \text{m})$, but they are considered to be the same cell type because of the uniformity of their histochemical reactions (see Table 2). Their cell contents stain heavily with Alcian blue but not with PAS (Figure 20). They are highly metachromatic (Figure 21), and can therefore be presumed to manufacture acid mucins.

Purple staining (type 2) mucous gland cells are more restricted in their distribution. A few are found in the gastrodermis of both the inner and outer body walls where they are $5.2 \times 9.0 \mu$ m in size. The majority are larger ($4.7 \times 8.3 \mu$ m) and are concentrated in the epidermis of the mouth region and the upper portion of the mesenterial filaments (Table 3). The cellular products of purple staining (type 2) mucous gland cells is non-metachromatic and stains red-purple with Alcian blue-PAS (Figures 20 and 55) indicating the presence of a mixture of mucins which are predominantly neutral in character.

Type A and type B granular gland cells of the mesenterial filaments, and, in the former case, free body wall (Table 2), also produce mucins. The acidophilic granules of Type A granular gland cells are PAS positive (Figures 55 and 56) and non-metachromatic, implying the presence of neutral mucins. The basophilic granules of type B granular gland cells are also non-metachromatic, but stain with both Alcian blue and PAS (Figure 56) suggesting the presence of mixed mucins. The purple-staining granules of the type C and D granular gland cells of the mesenterial filaments do not stain with Alcian blue-PAS (Figure 56) which indicates that they do not contain mucins. This is confirmed by the positive result with the Vois-net-Fürth reaction (Table 2) which indicates the presence of tryptophan and thence protein material. These findings suggest that Types C and D granular gland cells may be involved in enzyme synthesis. This conclusion is supported by the colouration of these glands with Liisberg's stain (a stain designed to differentiate α and β cells in the hangerhans islets of the human pancreas), which does not stain any of the other gland cell types.
Figure 13: Longitudinal section through a white tipped branch end of A. formosa (x38).

The site of the skeleton has been stippled to show the relationship between the axial corallite (ax) and its polyp. Two radial polyps (r) are also sectioned obliquely. The tissue outlines the positions of four trabecular columns (tc) belonging to the synapticulothecal rings which make up the corallite wall. Each column terminates in a trabecular time (tt).

The outline of the septal trabeculae (tr) which link the synapticulothecal rings radially are seen in longitudinal section, as are the outward trabecular divergences which produce the costae (arrow). The sites of the synapticulae (s) which interlink the trabecular columns are seen in transverse section only and would project out of the plane of the page.

The coelenteron (ce) consists of a series of longitudinal canals (ca) running parallel to the trabecular columns. Continuity between these canals and the central cavity and the coelenterons of the radial polyps is effected by small canals or canaliculae (*).

The tentacles (te) of the axial polyp are retracted onto the oral disc (o). The stomodeum is not well represented, and continuity of tentacles and oral disc with the mesenterial filaments (mf) in the depth of the central cavity (cc) has been disrupted during preparation of the section (refer instead Figure 30).

ax - axial corallite; ca - canal in coelenteron; cc - central cavity; ce - coelenteron; mf - mesenterial filament; o - oral disc; r - radial corallite; s - synapticula; sk - skeleton; tc trabecular column; te - tentacle; tr - septal trabecula; tt - trabecular tine; arrow - costa; * - canalicula; stippling position of skeleton



Figure 14: Transverse section of a branch tip of <u>A. formosa</u> at the level of the second circle of radial corallites (x29).

As in Figure 13, the site of the skeleton is stippled. The central cavity (cc) of the axial polyp and those (cr) of the first circle of radial polyps (R_1) are situated deep within the specimen. Around the periphery of the branch the second circle of radial polyps are sectioned at several levels. All are cut obliquely due to their angle of insertion to the branch. Polyp 1 (1) is cut almost at the junction of the stomodeum (st) and the oral disc (o); polyp 2 (2) is sectioned through the oral disc near its tip; polyps 3 (3) and 4 (4) have been sectioned deep in the central cavity; they contain six mesenterial filaments (small numbers). Costal development (arrow) is most noticeably pronounced near the base of the polyps. Polyp 5 (5) has been cut almost longitudinally, and the section passes obliquely through the outer directive septum (dse).

cc - central cavity; cr - central cavity of radial polyp; dse directive septum; o - oral disc; R - circle of radial corallites; st - stomodeum; small numbers - mesenterial filaments; large numbers - radial polyps discussed in text; arrow - costa; stippling - position of skeleton.



Figure 18: The outer body wall at night (x1250)

The mesoglea (m) in this section is aligned with that of the daytime section in Figure 17 to allow the relative expansion of the tissue layers to be observed. The cells of both the epidermis (e) and gastrodermis (g) are substantially taller and the mesoglea is markedly thicker. The oval nuclei of the columnar supporting cells (cs) are still aligned in a band in the proximal third of the layer. A microbasic b-mastigophore (b) is embedded amongst them. The increased distance between the nuclear band and the mesoglea allows the spherical nuclei of the interstitial cells (i) to be readily distinguished. Zooxan-thellae (z) are present in the gastrodermis.

a - amoebocyte; b - microbasic b-mastigophore; c - calicoblastic layer; ce - coelenteron; cs - columnar supporting cell; f
flagellum; g - gastrodermis; h - holotrichous isorhiza;
i - interstitial cell; in - incipient nematocyst or cnidoblast;
m - mesoglea; mu - mucous gland cell (subscript denotes type);
p - microbasic p-mastigophore; sp - spirocyst; tc - trabecular column; tr - septal trabecula; z - zooxanthella.

Figure 15: Portions of the inner body wall (x1250).

The calicoblastic epidermis (c) is extremely thin. The calicoblasts form a squamous epithelium, with the spindle-shaped cells joined end to end. The nutritive cells of the gastrodermis (g) are also flattened and some contain a zooxanthella (z). Non-staining (mu₁) and purple-staining (mu₂) mucous gland cells, and an amoebocyte (a) containing small (1 m) acidophilic granules also present. A thin band of mesoglea (m) separates the two epithelial layers.

Figure 16: Nematocysts in the calicoblastic epidermis around a trabecular column near a branch tip (x500)

The calicoblastic layer (c) contains holotrichous isorhizae (h). The shape of the tissue indicates the site of a trabecular column (tc) and a septal trabecula (tr). The free body wall epidermis (e) consists almost entirely of columnar supporting cells.

Figure 17: The outer body wall during daylight (x1250)

The outer body wall comprises two epithelial layers – the epidermis (e), and the gastrodermis (g). These are separated by the acellular mesoglea (m). The nuclei of the flagellated (f) columnar supporting cells (cs) are aligned in a band in the proximal third of the layer. Interstitial cells (i) are located between their basal processes. A variety of nematocysts are present; spirocysts (sp), microbasic p-mastigophores (p) and holotrichous isorhizae (h). The cnidoblasts (in) in the proximal region of this layer are differentiating interstitial cells which appear to be producing holotrichous isorhizae. Unstained mucous gland cells (mu₁) are present but they are difficult to discern (c.f. Figure 19). Lining the coelenteron (ce) are the cubo-columnar nutritive cells of the gastrodermis (g).



Figure 19: MSB stain of the outer body wall (x1250)

The cytoplasm of the columnar supporting cells of the epidermis (e) stains pale grey, their oval nuclei are red-purple to purple. The outline of unstained mucous gland cells (mu₁) may be discerned. In the distal portion of the epidermis there are two acidophilic (type A) granular gland cells. Mature holotrichous isorhiza (h) stain pink, whilst developing cnidoblasts (in) are intensely acidophilic. Transparent blue capsules the same size as the former are probably discharged holotrichous isorhizae (xh). The spherical nuclei of the interstitial cells (i) are purple, their cytoplasm is grey. The mesoglea (m) stains bright blue and is very prominent. The nuclei of the gastrodermal cells (g) stain a variety of colours from red, red-purple to purple. Zooxanthellae (z) are mottled, some granular cell contents are acidophilic, the pyrenoid is pink.

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Figure 20: Alcian blue-PAS stain of the inner and outer body walls (x500)

The contents of type 1 mucous gland cells (mu_1) are alcian blue positive. Type 2 mucous gland cells (mu_2) in the inner body wall are red-purple, indicating a mixed reaction. The majority of the rest of the tissues including a zooxanthella (z) are PAS positive.

Figure 21: Toluidine blue stain of outer body wall (x500)

The contents of the type 1 mucous gland cells (mu_1) show β metachromasia, the rest of the tissues are orthochromatic.

e - epidermis; f - flagellum; g - gastrodermis; gr - granular gland cell (subscript denotes type); h - holotrichous isorhiza; i - interstitial cell; in - incipient nematocyst; m - mucous gland cell (subscript denotes type); xh - discharged holotrichous isorhiza; xmu - exuded mucus; z - zooxanthella.



Figure 23 (contd)

There is an abrupt transition from the cubo-columnar nutritive cells of the outer body wall gastrodermis to the cubo-squamous cells of the interior. The cells in both regions contain zooxanthellae (z). The cells of the calicoblastic layer (c) form a squamous epithelium. Their long axes are oriented parallel to the inner body wall mesoglea, and they are in contact only at their extreme tips.

Figure 24: Rupture of tissue in the contracted state (x500)

Internal pressure from the trabecular tine appears to have forced the calicoblastic layer through the mesoglea, pushing aside the cells of the free body wall epidermis.

Figure 25: Rupture of tissue in the expanded state (x500)

No evidence of internal pressure is present; the tissue appears to have torn at the apex of the tissue cavity which held the tine without disruption of any of the layers.

b - microbasic b-mastigophore; c - calicoblastic layer; cs columnar supporting cell: e - epidermis; g - gastrodermis; i - interstitial cell; m - mesoglea; mu - mucous gland cell (subscript denotes type); sp - spirocyst; tt - trabecular tine; z - zooxanthellae; * - wedge-shaped mesoglea at the point of fusion of the inner and outer body walls; arrow - intercellular space.

Figure 22: Tissue in contracted state around the site of a trabecular tine (x500)

The outer body wall is compact and the columnar supporting cells (cs) are closely apposed to each other. The majority of the oval nuclei are located in the proximal third of the layer. The mesoglea (m) is continuous over the top of the site of the trabecular tine (tt), and is thick and straight down its sides. At either side of the original position of the apex of the tine the mesoglea of the inner body wall fuses with that of the outer body wall forming a characteristic wedge-shape (*). The cells of the gastrodermis (g) are cubo-columnar in form throughout and do not contain zooxanthellae.

The spindle-shaped calicoblastic cells (c) are arranged with their longest axes parallel and line the cavity which held the trabecular tine (tt). At the apex of the cavity they are oriented at 90° to the mesoglea; this angle reduces to approximately 60° further down the tine. The calicoblasts appear to be in close contact at the distal portion of the layer, but there are intercellular spaces proximally (arrow).

Figure 23: Tissue in the expanded state around the site of a trabecular time (x500)

The tall epidermal layer (e) is less compact than in Figure 22 due to the expanded condition of the cells and the presence of larger intercellular spaces. The nuclei of the columnar supporting cells (cs) are arranged in a diffuse band in the proximal third of the layer. Two types of nematocyst (spirocysts (sp) and a microbasic b-mastigophore (b)) and an unstained mucous gland cell (mu₁) are present in the distal portion. Spherical nuclei belonging to interstitial cells (i) are located next to the mesoglea. The mesoglea itself fuses without thickening at the junction of the inner and outer body walls and is thin and wavy down the sides of the site of the time.







Figure 26: Conformation of tissues over the trabecular tines of white tipped branch ends of <u>A. formosa</u> fixed at 2 hourly intervals over a period of 24 hours.

1.	23/5/78	5.	15/10/80
2.	20/12/79	6.	15/10/80
3.	2/7/80	7.	25/10/80
4.	23/7/80	8.	5/1/81



- Figure 27: Diagramatic representation of the conformations of tissue found over the trabecular tines (see text for full explanation)
- (a) contracted
- (b) expanded

c - calicoblastic layer; e - epidermis; m - mesoglea; mu mucous gland cell; sp - spirocyst; tt - trabecular tine.



Figure 31: Longitudinal section through a tentacle tip (x1250)

The long axes of the columnar supporting cells (cs) are at right angles to the mesoglea (m) at the end of the tentacle but are angled more obliquely proximally. The gastrodermal cells (g) also become more flattened away from the tip. Several microbasic p-mastigophores (p) are grouped together to form a battery. The lumen (l) of the tentacle is an extension of the coelenteron.

ca - gastrodermal canal; cc - central cavity of axial polyps; cs - columnar supporting cells; dt - directive tentacle; g gastrodermis; ir - incipient radial corallite; l - lumen; m mesoglea; mf - mesenterial filament; o - oral disc; p - microbasic p-mastigophore; r - radial corallite; st - stomodeum; te tentacle; arrow - costa; * - superimposed tentacles; x transition from straight to convoluted cnidoglandular band. Figure 28: Tip region of a white tipped branch end of <u>A. form</u>osa in late afternoon (5.00pm) (x14)

The coenosarc over the tip of the axial corallite (ax) is free of zooxanthellae and is closely applied to the skeleton. A few zooxanthellae can be seen associated with a newly forming radial corallite (ir). Radial polyps (r) further down the tip are partially extended, each has twelve tentacles. The tentacle located over the directive septum, the directive tentacle (dt), is substantially longer than the others. The white striations down the outside of the radial corallites indicate the position of the costae (arrow) because the tissue is stretched over the coenosteum and contains no zooxanthellae. The gastrodermal canals (ca) between the costae contain algae.

Figure 29: Tip region of a white tipped branch end of <u>A.</u> formosa in early morning (1.30am) (x19)

The polyp tissue is less closely applied to the skeleton at night than during the day (c.f. Figure 28). The oral disc (o) is completely everted and bears one cycle of six tentacles (te) of equal length (one is superimposed over the other at *). The opaque regions probably indicate the presence of concentrations of stinging cells, particularly at the tip where they are grouped into batteries (see Figure 31).

Figure 30: Dissection of decalcified tip of <u>A. formosa</u> with retracted tentacles (x12)

The tentacles (te) are retracted into compact knobs of tissue on the oral disc (o), below them is the stomodeum (st). The continuity of the mesenterial filaments (mf) from the base of the tentacles, through the stomodeum and into the central cavity (cc) may be traced. Near the tip the cnidoglandular bands of the mesenterial filaments are almost straight. Further down the branch they are highly convoluted (x). These two regions are probably type 1 and type II mesenterial filaments respectively. (contd overleaf)







Figure 32: Longitudinal sections through an expanded oral disc and tentacles

(a) (x 79) The section passes through the edge of the oral disc, the bases of 5 tentacles (1-5) and 2 trabecular tines (tt). The columnar supporting cells of the epidermis (e) are greatly distended throughout these regions, and the tissue around the trabecular tines are in the expanded conformation. Mesenterial extensions from the mesenteries which subtend the centre tentacle (3) extend upwards and are attached to its base (*), well above the level of the oral disc. The mesenteries themselves continue down into the central cavity (cc). There is substantial folding in the gastrodermal and epidermal epithelia at the base of the tentacle, indicating buckling in both muscle layers. Large numbers of unstained (mu₁) and purple stained (mu₂) mucous gland cells are present in the epidermis.

(b) (x 79) Section through the edge of the oral disc $(54\mu m \text{ from } (a))$.

The mesenterial extensions appear to be stretched over the oral disc. Note the contracted appearance of the mesenteries on the underside of the oral disc (x) (see also Figure 44).

(c) (x 79) Section through the back wall of the centre tentacle $(30\mu m \text{ from (b)})$.

The mesenteries now appear discontinuous (c.f. adjacent tentacles in (a) between 4 and 5).

(d) (x200) Enlargement from (b) of point of contact of mesenterial extensions with oral disc

The mesenterial extensions appear to be discrete from the oral disc (arrow). The mesoglea of the mesentery is extensively pleated immediately below the oral disc (x), indicating that the muscle fibrils are contracted.

cc - central cavity; e - epidermis; g - gastrodermis; me mesentery; mu - mucous gland cell (subscript denotes type); tt
- trabecular tine; 1-5 - tentacle number; arrow - mesenterial
attachment stretched over oral disc; x - mesogleal pleating.



Figure 33: Transverse section through the basal region of a slightly retracted tentacle (x1250)

The acidophilic staining of the musculature at the base of the epidermal cells (e) contrasts strongly with the basophilic mesoglea (m). The longitudinal epidermal musculature takes the form of thick fibres (fi_L) interspersed between mesogleal folds. The circular muscles (fi_C) of the gastrodermis (g) are far less well developed. Nuclei (mn) are present at intervals along this circular muscle layer which may belong to myofibrils.

Figure 34: Transverse section through the mid-region of a moderately retracted tentacle (x500)

The contraction of the circular muscle fibrils (fi_C) in the gastrodermis has caused folding of the surface of the layer, resulting in partial occlusion of the lumen (1). At the same time, the longitudinal epidermal muscle fibres (fi_L) have buckled into the epidermis as strands of mesoglea. Spirocysts (sp) and microbasic p-mastigophores (p) are present in considerable numbers in the epidermis.

e - epidermis; fi_C - circular muscle fibrils; fi_L - longitudinal muscle fibres; g - gastrodermis; l - lumen; m - mesoglea; mn - myofibril nucleus; p - microbasic p-mastigophore; sp spirocyst.



Figure 38 (contd)

of the epidermis (e). The surface of the mesoglea (m) adjacent to the epidermis is fibrillar instead of smooth, in order to accommodate the basal processes of the highly compressed columnar supporting cells. The nuclear band of this cell layer is wider than usual and the surface folds are more pronounced than in Figure 37.

e – epidermis; g – gastrodermis; l – lumen; m – mesoglea; p – microbasic p-mastigophore; arrow – epidermal cells resuming vertical orientation to the mesoglea. The nuclei of the columnar supporting cells of the epidermis indicate that the long axiis of these cells are aligned parallel to the mesoglea (m). A microbasic p-mastigophore (p) is similarly oriented. The cells of the gastrodermis (g) form a squamous epithelium.

Figure 36: Longitudinal section of tentacle undergoing initial stages of retraction (x500)

Epidermal cells (e) are beginning to resume their orientation perpendicular to the mesoglea (at arrows).

Figure 37: Longitudinal section of buckling in the circular musculature of the gastrodermis of a retracting tentacle (x500)

The nuclei of the cells in the epidermis (e) are packed closely together. Folds are beginning to appear on the surface of the layer to accommodate the compression caused by the contraction of the epidermal muscle fibres. This shortening has also forced the circular muscle sheet to buckle at right angles to the direction of contraction. The superficial fibres of the mesoglea (m) and the gastrodermal cells (g) accompany this deformation, producing tree-like extensions into the gastrodermis and gastrodermal folds into the lumen (1) of the tentacle.

Figure 38: Longitudinal section of an advanced state of buckling in a retracting tentacle (x500)

The gastrodermal (g) corrugations in the lumen (l) of the tentacle are much more pronounced than in Figure 37. This is due to increased buckling of the circular muscle layer in response to further contraction of the longitudinal muscle fibbres



Figure 39: Diagramatic representation of buckling in a retracting tentacle of A. formosa.

(a) Epidermal buckling

The surface of the gastrodermis (g) is thrown into longitudinal folds which project into the lumen (1) as a result of compression by the gastrodermal circular muscle fibrils (fi_C) . At the same time, the epidermal musculature (fi_L) is buckled into the epidermis (e) by the deformation of the epidermal surface of the mesoglea (m). Individual muscle fibres are forced outwards on strands of mesoglea without altering the surface contours of the epidermal layer.



Figure 39: (contd)

(b) Gastrodermal buckling

Contraction of the longitudinal epidermal muscle fibres (fi_L) shortens the tentacle and forms transverse folds in the superficial epidermis (e). The longitudinal folds of the gastrodermis (g) in (a) are replaced by circular corrugations as the gastrodermal layer is forced to buckle into the lumen (l) of the tentacle under the influence of the better developed longitudinal muscle fibres. The circular muscle layer $(fi_C)carries$ the overlying gastrodermis with it and also the superficial fibres of the underlying mesoglea (m). These form the core of the resultant invaginations.

e - epidermis; fi_C - circular muscle fibril; fi_L - longitudinal muscle fibre; g - gastrodermis; l - lumen; m - mesoglea.



Figure 43: Oblique transverse section through a highly retracted tentacle (x200)

The lumen (1) of the tentacle has almost disappeared due to the extent of gastrodermal folding. The gastrodermis (g) itself appears syncitial. The "warp and weft" arrangement of the mesoglea (m) is seen edge on (c.f. Figure 41). The sheets of mesoglea are oriented at right angles, their fibres appear to provide attachment points for the attenuated basal processes of the compacted epithelial cells (e).

e - epidermis; fi_L - longitudinal muscle fibre; g - gastrodermis; l - lumen; m - mesoglea; mu - mucous gland cell (subscript denotes type); o - oral disc; sp - spirocyst; st stomodeum; * - point of transition from epidermal to gastrodermal buckling; arrow - orientation of sheets of mesogleal fibres.

Figure 40: Longitudinal section through part of a partially retracted tentacle (x76)

The oral disc (o) is re-invaginated inside the calice. In the distal part of the tentacle both epithelial layers are cubo-columnar (as in Figure 36). In the mid-region theỳ are more compressed, the epidermis (e) is columnar and slightly corrugated whilst the gastrodermis (g) is cubo-columnar (this state of retraction corresponds to that in Figure 39a, but this cannot be discerned in longitudinal section). Proximally (analagous to Figure 39b), the amplitude of the epidermal corrugations increases abruptly (*); simultaneously, the gastrodermal muscle layer buckles into the lumen (l) to form "trees" like those in Figure 38.

Figure 41: Longitudinal section of a completely retracted tentacle (x200)

The tentacle is retracted into a knob of tissue like those in Figure 30. Non-staining and purple-staining mucous gland cells $(mu_1 \text{ and } mu_2 \text{ respectively})$ and spirocysts (sp) are present in the deeply folded epidermis (e). The gastrodermis (g) is apparently syncitial. The epidermal muscle fibres (fi_L) , cut in both longitudinal and transverse section, are pronounced, presumably due to their highly contracted state. The fibres of the mesoglea (m) are parallely arranged in sheets oriented at right angles to each other.

Figure 42: Oblique longitudinal section through a highly retracted tentacle (x200)

This section passes close to the edge of the stomodeum (st). The tentacle is almost completely retracted onto the invaginated oral disc (o). Proximally, the section cuts through the mesoglea (m), just below the bases of the epidermal cells (e). Distally, some nuclei are present where it partially intersects the gastrodermis. The fibres of the mesoglea are arranged in parallel in sheets of differing orientation (arrows).



Figure 47: Transverse section of a radial polyp at the base of the stomodeum (x79)

The section passes slightly obliquely through the base of the stomodeum (st) at the point where the cnidoglandular bands (cb) of the complete mesenteries separate from the stomodeal tissue. The proximal incomplete mesenteries (im) have almost terminated.

ca - canal of coelenteron; cb - cnidoglandular band; cm - complete mesentery; dse - site of directive septum; dt - directive tentacle; en - entocoelic mesogleal pleats; ex - exocoelic mesogleal pleats; fi_L - longitudinal muscle fibre; f - flagellum; im - incomplete mesentery; sr - synapticulothecal rings; st - stomodeum; arrow - costa; 1-12 - numbers of mesenteries.

Figure 44: Transverse section of a radial polyp at the level of the oral disc (x79)

In radial polyps the mouth opening is displaced distally. This section is so near the tip of the polyp that the linkages of the synapticulothecal ring (sr) appear incomplete proximally. The tissue of the oral disc is deeply invaginated over the position of the outer directive septum (dse) to form the directive tentacle (dt). The oral disc epidermis contains moderate numbers of unstained mucous gland cells (mu₁) and occasional purple staining mucous gland cells (mu₂). All 12 mesenteries (1-12) are attached at this point, and show differing degrees of mesogleal pleating. The mesenteries on either side of the directive septum have prominent exocoelic pleats (ex), the non-directive pairs have small entocoelic pleats (en) which reduce gradually in size proximally. Arrows mark the position of costae produced by outward trabecular divergence from the synapticulotheca (see also Figure 45).

Figure 45: Transverse section of a radial polyp at the level of the stomodeum (x79)

There are 8 complete mesenteries (cm) which are attached to the stomodeum (st) and 4 incomplete mesenteries (im). The columnar supporting cells of the epidermis of the stomodeum bear conspicuous flagellae (f). The position of the pair of prominent directive septa (dse) between 2 pairs of complete mesenteries is indicated. The site of one complete (sr_1) and one incomplete (sr_2) synapticulothecal rings can also be deduced. The gastrodermal canals (ca) lie between them.

Figure 46: Transverse section of a complete and an incomplete mesentery (x500)

The incomplete mesentery (im) bears no mesenterial filament and no obvious musculature. In contrast, the adjacent directive mesentery has prominent exocoelic pleats (ex) in the mesoglea. Longitudinal muscle fibres (fi_L) are sectioned transversely near to the point where the mesentery joins the stomodeum (st).



Figure 51 (contd)

The position of the skeleton (sk) is marked, it is lined with a squamous epithelium of spindle-shaped calicoblastic cells (c).

c - calicoblastic layer; cb - cnidoglandular band; cs - columnar supporting cells; f - flagellum; fl - flask-shaped gland cell; g - gastrodermis; gr - granular gland cells (subscript denotes type); ll - lateral lobes; m - mesoglea; me - mesentery; mem membranous part of mesenterial filament; mu - mucous gland cell (subscript denotes type); sk - skeleton; st - stomodeum; su - sulcus; z - zooxanthellae; I - type I mesenterial filament.
Figure 48: Transverse section through part of the stomodeum of a radial polyp (x500)

The epidermis of the stomodeum (st) contains both non-staining and purple-staining mucous gland cells (mu₁ and mu₂ respectively). The flagella (f) on the columnar supporting cells (cs) are very long. The mesenteries (me) enclose pockets of coelenteron (ce), they are made up of a strand of mesoglea (m) sandwiched between two layers of gastrodermis (g). All are continuous with corresponding layers in the stomodeum.

Figure 49: Longitudinal section through the cnidoglandular band of a type I mesenterial filament (x1650)

The contents of four types of granular gland cells (gr_{A-D}) may be distinguished. A large differentiating nematocyst (in) is also present, its cell contents are amorphous, but a distinct barb may be observed distally.

Figure 50: Longitudinal section through the cnidoglandular band of a type l mesenterial filament (x1250)

The free edge of the cnidoglandular band contains a non-staining (mu₁) mucous gland cell and two refractile, flask-shaped cells (fl).

Figure 51: Transverse section through a type 1 mesenterial filament in the upper part of a polyp (x640)

The mesenterial filament arises from the inner body wall where the squamous gastrodermal cells (g) occasionally contain zooxanthellae (z). The mesoglea provides a wedge-shaped point of attachment then forms the thin central core of the membranous part of the filament (mem). It becomes fibrillar in the compact lateral lobes (ll) and then passes across the sulcus (su) to form a T-junction in the cnidoglandular band (cb), providing a basement membrane for the conspicuously flagellated (f) columnar supporting cells. Cell nuclei (arrow) are present in the mesoglea, perhaps indicating the presence of wandering cells (amoebocytes).

(contd overleaf)



cb - cnidoglandular band; ce - coelenteron; fl - flask-shaped cell; gr - granular gland cell (subscript denotes type); im incomplete mesenteries; in - incipient nematocyst; ll - lateral lobe; mu - mucous gland cell (subscript denotes type); sk skeleton; z - zooxanthellae; I - type I mesenterial filament; II - type II mesenterial filament The relative positions of the coelenteron (ce) and skeleton (sk) are marked. An incomplete mesentery (im) projects into the coelenteron. The cnidoglandular bands of the type II filaments (II) appear more vacuolate in structure than the type I (I) due to the presence of unstained mucous gland cells (mu_1) (c.f. Figure 56). The type II filaments contain more acidophilic (gr_A) than basophilic (gr_B) granular gland cells. (A flask-shaped gland cell (fl) is also present.) The ratio is reversed in the type I filaments. The lateral lobes (ll) of the type II filaments are markedly reduced compared to those of type I.

Figure 53: Longitudinal section of a type I mesenterial filament (x500)

The cnidoglandular band (cb) has moderate numbers of purplestaining mucous gland cells (mu₂) as well as a complement of acidophilic (gr_A) and basophilic (gr_B) granular gland cells. Basophilic gland cells predominate. The elongate cell with basophilic cytoplasm and a thick, acidophilic barb-like structure is an immature nematocyst (in) similar to that in Figure 49. Zooxanthellae (z) are present in the lateral lobes (11).

Figure 54: Longitudinal section of a type II mesenterial filament (x500)

Compared to type I mesenterial filaments (Figure 53), there appear to be relatively few cells in the cnidoglandular band (cb) and lateral lobes (ll) of type II mesenterial filaments. However, the intercellular spaces are packed with non-staining mucous glands (mu₁) (see Figure 56). The ratio of acidophilic (gr_A) to basophilic (gr_B) granular gland cells is the reverse of that in type I filaments (c.f. Figure 53), the acidophilic type predominate.

(contd overleaf)







Figure 55: Alcian blue-PAS stain of a type I mesenterial filament (x1250)

The granules of the type A granular gland cells (gr_A) are intensely PAS positive. The contents of the type 1 mucous gland cells (mu_1) are Alcian blue positive, whilst those of the type 2 mucous gland cells (mu_2) give a red-purple colouration, indicating a mixed staining reaction.

Figure 56: Alcian blue-PAS stain of a type II mesenterial filament (x1250)

The granules of the type A granular gland cells (gr_A) give a strong PAS positive reaction whilst the contents of the type 1 mucous gland cells (mu_1) are strongly Alcian blue positive and form the bulk of the cnidoglandular band. Type B granular gland cells (gr_B) stain with both Alcian blue and PAS but those of type D (gr_D) do not stain with either.

Figure 57: Liisberg's stain of a type I mesenterial filament (x1250)

The granules of type C (gr_C) and D (gr_D) granular gland cells are stained red by this method, as is the barb of the microbasic p-mastigophore (p). The type A granular gland cells (gr_A) are unstained.

gr - granular gland cell (subscript denotes type); mu - mucous gland cell (subscript denotes type); p - microbasic p-mastigophore.



Figure 58: Spirocyst in a lateral lobe of a type I mesenterial filament (x500)

A spirocyst (sp) is present in a lateral lobe (ll) of this type I mesenterial filament

Figure 59: Zooxanthellae in an oblique section of a lateral lobe of a type I mesenterial filament (x1250)

Many zooxanthellae are present and each is contained in a vacuole (v). Some of the algae appear entirely normal (z); internal structures such as the pyrenoid are clearly visible. One algal cell is in the process of dividing (dz); the opposing faces of the two daughter cells are characteristically flattened. Other algae appear pycnotic, with crenellated cell walls and amorphous cell content (xz).

Figure 60: Zooxanthellae in the process of extrusion from the ingestion-excretion region of the lateral lobes of a type I mesenterial filament (x500)

The zooxanthellae (z) in the lateral lobes (ll) show various stages of internal disorganisation. The actual site of extrusion (arrow) discharges into the sulcus (su) which separates the lateral lobes and the cnidoglandular band (cb) (the latter is obliquely sectioned). The extruding zooxanthella appears to be intracellular since the nucleus (*) of the coral cell which contains it is clearly visible.

Figure 61: Zooxanthellae on the membranous portion of a type I mesenterial filament (x500)

Two zooxanthellae (z) are enclosed in vacuoles in the gastrodermis of the membranous part of the mesenterial filament (mem).

cb - cnidoglandular band; dz - dividing zooxanthellae; ll lateral lobe; mem - membranous part of mesenterial filament; sp - spirocyst; su - sulcus; v - vacuole; xz - degenerate zooxanthella; z - zooxanthella; arrow - ingestion-excretion zone; * - nucleus of coral cell.



gv – germinal vesicle; h – holotrichous isorhiza; l – lumen; m – mesoglea; n – nucleolus; oo – ooplasm; ov – ovum; sp – spirocyst; spe – sperm; ta – sperm tails; ts – testis; xmu – exuded mucus; xsp – broken spirocyst; xz – degenerate zooxanthella; z – zooxanthella; I – type I mesenterial filament. Figure 62: Bolus of material for extrusion in coelenteron of an axial polyp (x640)

Embedded in the mucus (xmu) are zooxanthellae in a variety of conditions, ranging from normal (z) to degenerate (xz); intact spirocysts (sp), broken spirocysts (xsp), and holotrichous isorhizae (h).

Figure 63: Longitudinal section through an hermaphrodytic radial polyp taken in mid-October (x79)

There are two mature ova (ov) with granular ooplasm (oo) and prominent germinal vesicles (gv). The section passes through a well developed nucleolus (n) in the uppermost ovum. In contrast the testes (ts) are immature, the loculi are difficult to discern, no central cavities are present (c.f. Figure 64a).

Figure 64: Comparison of mature and immature testes (x500)

(a) Mature. The mesoglea (m) of the mesenterial filament is continuous around the testis and also separates the loculi. The sperm (spe) are well developed with densely staining basophilic heads, their tails (ta) project into the cavity (1) at the centre of each loculus.

(b) Immature. The loculi are poorly defined and the sperm are less well developed than in (a). There are zooxanthellae (z) present within the testis.

Figure 65: Developing oocyte on a type I mesenterial filament (x500)

An ovum is embedded in the mesoglea (m) of a type l mesenterial filament (I), the germinal vesicle (gv) is situated in the amorphous ooplasm (oo) and contains a densely stained nucleolus (n).

(contd overleaf)



Figure 66(a-d): Serial sections through type II mesenterial filaments extruded through the outer body wall (x200)

Part of one or more paired type II mesenterial filaments (II) have been extruded through a rupture (ru) in the outer body wall. The relative positions of the coelenteron (ce) and the skeleton (sk) are indicated.

ce - coelenteron; ru - rupture; sk - skeleton; 11 - type 11 mesenterial filament.



4.5 Discussion

4.5.1 Anatomy

As might have been expected from the structure of the skeleton (Section 3.2.1), the coelenteron of A. formosa polyps is composed of a highly porous arrangement of cross-linked, concentrically arranged longitudinal canals. According to workers such as Hyman (1940) and Hand (1966), the invaginations of the inner body wall which accommodate the septa and produce the mesenteries in coral polyps substantially increase the total surface area available for absorbtion. The large surface area presented by the interconnected canals in Acropora could perhaps be said to compensate for the fact that the septa are almost completely immersed in the corallite walls, and for the relatively poorly developed mesenteries which terminate only a short distance below the stomodeum. The axial and radial corallites are essentially similar in structure, however, the former is enormously elongated and in the latter the axis of symmetry of the polyp is displaced outward from the branch.

Unfortunately it was not possible, in the present investigation, to confirm Fowler's (1887) claim that some mesenteries were longer than others, but two of the eight mesenterial filaments which continue down the central cavity in place of the complete mesenteries do seem to terminate abruptly. It was, however, possible to confirm the reports of Hyman (1940) and Hand (1966) that the development of the mesenteries in <u>Acropora</u> is arrested in the Edwardsian stage (i.e. there are eight complete and four incomplete mesenteries). Duerden's (1902a) contention that the four incomplete mesenteries never developed mesenterial filaments was also supported.

The mesenteries of <u>A. formosa</u> are attached to the underside of the oral disc, and so conform to the normal condition described by Wells (1956) in his general account of the structure of the scleractinian polyp. Nevertheless, <u>A. formosa</u> polyps are unusual in that their musculature is better developed at the level of the oral disc than at the stomodeum. Previous descriptions of large polyped, fleshy corals such as <u>Balanophyllia regia</u> (Kinchington, 1980), or anemones like <u>Metridium senile</u> (Batham and Pantin, 1951), show the longitudinal retractor muscles of the mesenteries to be best developed below the stomodeum. However, in <u>A. formosa</u>, the concentration of the musculature above the stomodeum is consistent with the limited movement shown by the polyps, where full expansion involves virtually only the oral disc and tentacles. Invagination of the oral disc is presumably effected by the musculature around the mouth rather than by retraction along the length of the column as required by larger polyps (Batham and Pantin, 1951).

The mesenterial extensions observed attached high up in the base of the tentacles of <u>A. formosa</u> are unlike anything previously reported in other anatomical studies of coelenterates. It is proposed that their presence is also related to the limited movement of the polyp in this species.

In the cate corals, an inward movement of the body wall commonly accompanies tentacle retraction (Duerden, 1904a; Barnes, 1971). In <u>A. formosa</u> however, the complex structure of the synapticulotheca would prevent such a movement. It therefore seems likely that the mesenterial extensions assist in the retraction of the tentacles after the oral disc has been drawn inside the calice.

As far as the author is aware, this is the first time that the anatomical continuity between the cnidoglandular bands of the mesenterial filaments and the stomodeum has been demonstrated histologically in the scleractinia. This transition zone between the exterior and the interior of the polyp has been thoroughly investigated in Hydra (Wood, 1979) where it is said to be marked by a distinct groove and the presence of transition cells which are unlike gastrodermal or epidermal cells. This was not the case in A. formosa. The similarity between the cellular structure in the epidermis of the stomodeum and the upper cnidoglandular bands of type I mesenterial filaments in A. formosa supports the contention of Wilson (1888) and Matthai (1923) that in corals the latter tissue layer is ectodermal in origin. It is therefore contrary to the suggestion of Duerden (1902b) that the cnidoglandular bands are derived from the gastrodermis.

Although the internal pressure of the hydrostatic skeleton of coelenterates is small (Batham and Pantin, (1950a) recorded that pressures of 2-7mm in water were normal for the coelenteron of <u>Metridium senile</u>), it is essential for functional efficiency that they are maintained consistently within the polyp. Usually the stomodeum (Barrington, 1979) and the mesenteries (McFarlane, 1978) act as internal valves to minimise leakage if the mouth of the polyp is opened. In <u>A. formosa</u> the convoluted gastrovascular cavity and the mesenterial extensions in the tentacles probably provide an additional restriction to water loss.

4.5.2 Tissue Structure

4.5.2.1 Epithelial cells

The epidermal cells of <u>A. formosa</u> are similar to those described for various coral species by Goreau (1956) and Duerden (1902b) except that in <u>A. formosa</u> a distinct nuclear band is often located in the proximal third of the layer rather than the centre. The spindle-shaped calicoblastic cells of the inner body wall, and the cubo-columnar nutritive cells of the outer body wall gastrodermis which are flattened into a squamous form in the inner body wall also resemble previous description from other corals by Duerden (1902a), Hyman (1940) and Goreau (1956).

Flagellae were difficult to distinguish except as an indistinct layer on the cell surfaces, but were definitely present. This is consistent with ultrastructural studies of <u>Astrangia danae</u>, <u>Mussa angulosa</u> (Goreau and Philpott, 1956), <u>Balanophyllia regia</u> (Lyons, 1973 a and b) and <u>Pocillopora damicornis</u> (Vandermeulen, 1975). The flagellae were longest on the columnar supporting cells of the stomodeum, presumably indicating an efficient mechanism for pumping water into the coelenteron to create and maintain positive hydrostatic pressure (see Chapman, 1949; Batham and Pantin, 1950a). The columnar supporting cells of the cnidoglandular bands of the type I mesenterial filaments also bore conspicuous flagellae. These may be involved in water circulation in the coelenteron, and also in the movement of the filaments themselves (Stephenson, 1920). Although light microscopy is scarcely adequate to reveal the distribution of the epithelial musculature, muscle fibres, which comprise amalgamations of finer muscle fibrils (Barnes, 1968), were nevertheless seen in the epidermis and gastrodermis of highly retracted tentacles, and in the uppermost portion of the mesenteries. The presence of muscle fibrils at the site of the longitudinal retractor muscle along the length of the mesenteries, could be inferred from the permanent mesogleal folds like those described in the mesenteries of both anemones (Batham and Pantin, 1951; Grimstone et al, 1958) and other corals (Duerden, 1902b; Goreau, 1956). Such folds are known to increase the area available for muscle attachment (Chapman, G., 1974).

The intimate association of cell nuclei with the gastrodermal musculature in the tentacles strongly suggests that the contractile power of this layer is supplemented by the action of myofibrils (musculo-epithelial cells with a reduced epithelial component, see D. M. Chapman, 1974). These cells have been described in anemones (Hyman, 1940) and in the retractor muscles of cubomedusae polyps (D. M. Chapman, 1974), but there have been no previous reports of their presence in corals.

4.5.2.2 Interstitial cells

The small basophilic cells observed amongst the basal processes of the epidermal columnar supporting cells of <u>A. formosa</u> resemble the interstitial cells of hydrozoans (Davis, 1969; review D. M. Chapman, 1974), and confirms Harrison's (1980) report of interstitial cells in this species. Interstitial cells appear to be rare in the scleractinia. Apart from Harrison's observations, they have only been reported previously in species of <u>Fungia</u> (Bourne, 1887a) and <u>Porites</u> (Franzisket, 1970), and in <u>As-</u> trangia danae (Szmant-Froelich et al, 1980).

Although it is now recognised (see review Bouillon, 1968) that interstitial cells do not have the capacity for totipotency suggested in the early literature, they can nevertheless differentiate into a variety of cell types. In other coelenterates, where they have been better studied, they have been observed to generate gonads (Jennison, 1979), neurosecretory cells (Davis, 1969), and nematocysts (Westfall, 1966). The relatively large number of interstitial cells in the epidermis at the tip of <u>A. formosa</u> branches and the paucity of non-epithelial cell types (Table 3) suggests that the full complement of cell types may be in the process of being generated in this region.

4.5.2.3 Nematocysts

According to previous descriptions (Hyman, 1940; Hand, 1966; Mariscal, 1974) the cnidom of the scleractinia comprises spirocysts, microbasic p- and b-mastigophores and holotrichous isorhizae. The nematocysts found in <u>A. formosa</u> in this study are therefore highly characteristic of this group of coelenterates. Harrison (1980) reported the presence of a fifth type, which he tentatively identified as atrichous isorhizae. These were not observed during this study, suggesting that they may be restricted to more basal branch regions in their distribution. There is a strong resemblance between the cells with intensely acidophilic contents observed from time to time in the proximal outer body wall epidermis in this study and Harrison's (1980) description of cnidoblasts in the early stage of differentiation. This resemblance supports the assumption made here that these cells were incipient nematocysts.

Details of the distribution of nematocysts in the terminal portion of axial polyps of <u>A. formosa</u> (Table 4) have not been previously described. According to Mariscal (in Mariscal, 1974) each nematocyst type has a different function and may act by a combination of one or more mechanisms. These include penetration of the prey by the barb, entanglement with the thread and production of toxic substances. Thus, the distribution of all four nematocyst types throughout the outer body wall of <u>A. formosa</u> indicates a capacity for a variety of offensive and defensive strategies. A shift in nematocyst type from spirocysts in the basal region of the tentacles to microbasic p-mastigophores at the tip has also been reported in the ahermatype <u>Hoplangia durotrix</u> (van Präet, 1978). Presumably the batteries of heavily barbed microbasic p-mastigophores act in defense or prey capture whilst the spirocysts, which function by entanglement (Mariscal, 1971), subdue the prey by restraint as it is introduced into the mouth. The possible significance of the nematocyst composition of the cnidoglandular bands of the mesenterial filaments is considered in section 4.5.3.

The presence of nematocysts in the calicoblastic layer as observed in this study has also been reported for other corals. Bourne (1899) found nematocysts in a degenerate condition in this layer in <u>Fungia</u>. Kinchington (1980) observed "differentiating nematocysts" in <u>Balanophyllia regia</u>, and van Praët (1977) identified healthy-looking mastigophores in <u>Hoplangia durotrix</u> and noted the additional presence of small cells exhibiting different staining characteristic from the calicoblastic cells. The author concurs with Kinchington that the presence of nematocysts probably indicates the occurrence of interstitial cells in the calicoblastic layer, and suggests that they are represented in <u>Hoplangia durotrix</u> by the unusually staining cells described by van Praët.

4.5.2.4 Amoebocytes

In 1931, Yonge and Nicholls (1931a) reported the presence of "carrier", or "wandering" cells throughout the tissues of several species of coral. Like the cells observed in the present investigation in the mesoglea and gastrodermis of <u>A. formosa</u>, these cells were filled with small granules. Yonge and Nicholls found that wandering cells were most numerous in ahermatypic species. However, their numbers markedly increased in hermatypic species, which had extruded their zooxanthellae. Such extrusion was said to occur by migration along the membranous part of the mesenterial filaments.

Yonge (1973) described the zooxanthellae as "automatic agents of excretion" on the basis of observations (Yonge and Nicholls, 1931a) that they could utilise products of protein breakdown such as ammonium and phosphates. Consequently, he postulated that wandering cells had two alternative functions; either they acted as carrier cells for the zooxanthellae, or, in corals deprived of the cleansing function of the symbiotic algae, he suggested that they gathered up metabolic waste products for excretion.

Despite infrequent but consistent reports in the literature concerning cellular inclusions in the mesoglea of the sceractinia (Duerden, 1902a, 1904; Matthai, 1923; Kawaguti and Yokayama, 1966) it is often stated that the mesoglea of corals is acellular (e.g. Goreau, 1956; Chapman, G., 1974). Apart from Kawaguti (1964c) who claims that zooxanthellae are extracellular, it is also generally assumed (e.g. Goreau, 1961b) that the zooxanthellae are contained within modified nutritive cells, but this has not been confirmed ultrastructurally (Muscatine, 1973).

Histological observations from this study support the observations of Yonge and Nicholls (1931a). The mesoglea of A. formosa does sometimes contain cells, and, improbable as it may seem, zooxanthellae appear to migrate along the membranous part of type I mesenterial filaments in the manner first described by Yonge in 1931. In addition, however, it was observed that the cells which contain the zooxanthellae are sometimes extruded along with the algae, suggesting that extrusion of zooxanthellae may present a greater metabolic drain on the host than previously supposed. A significant proportion of the extruded zooxanthellae in this and other studies of symbiotic coelenterates (Taylor, 1969a, 1973a; Goreau et al, 1970; Reimer, 1971; Trench 1974; Steele, 1975, 1976, 1977) appeared perfectly healthy, and all of them may not be lost to the coral. If the ejected coral cells and their intracellular algae were capable of reinserting themselves into the gastrodermis, extrusion may also be a mechanism by which the algae are redistributed in the polyp. For example, the algae-free coensarc just proximal to the growing tip may acquire its algal symbionts in this way.

In Harrison's (1980) recent ultrastructural study of <u>A. formosa</u> zooxanthellae are described as being contained within gastrodermal nutritive cells. However, close examination of his micrographs reveals that whilst the cytoplasm of the nutritive cells contains many inclusions, and their nuclei consistently have an electron-opaque band around their periphery, those that contain zooxanthellae have noticeably fewer cytoplasmic inclusions, and their nuclei are electron lucent at their edges. It is therefore possible that these cells may be of a completely different type and not merely modified nutritive cells.

The acidophilic granule-containing cells observed in the mesoglea and gastrodermis of A. formosa are almost certainly identical with the wandering cells described by Yonge and Nicholls (1931a). They have been described as amoebocytes in this study because of their superficial resemblance to the amoebocytes of anthozoa (c.f. Bouillon, 1968) and also because of their histochemical similarity to the ameobocytes of the scyphozoa (c.f. D.M. Chapman, 1974). The limited resolution of the light microscope makes it difficult to compare accurately observations made here with the published ultrastructural descriptions of amoebocytes, such as those of Grimstone et al (1958) in Metridium senile, and Young (1974) and D.M. Chapman (1974) in Calliactis parasitica. However, their structural similarity to what Kinchington (1980) called "amoeboid cells" in Carophyllia smithi strongly suggests that amoebocytes do occur indeed in the scleractinia.

Amoebocytes are known to be concerned with wound healing and tissue repair in both the Scyphomedusae (Metchnikoff, 1892, in Robson, 1957) and the Actiniaria (Young, 1974). These processes are investigated in <u>A. formosa</u> in Chapter 7 where the in-volvement of the amoebocytes is examined in detail.

4.5.2.5 Gonads

The structure of developing gonads in <u>A. formosa</u> is essentially similar to that described for the few other coral species where reproductive material has been examined in detail. These comprise studies of <u>Favia doryensis</u> (Marshall and Stephenson, 1933), <u>Stylophora pistillata</u> (Rinkevitch and Loya, 1979) and <u>Astrangia danae</u> (Szmant-Froelich <u>et al</u>, 1980). Therefore, the proposed evolutionary difference between astrocoeniid and nonastrocoeniid corals (Wells, 1956, see p. 3) does not appear to be reflected in any differences in gonad formation.

The descriptions of mature gonads given here confirm the findings of Oliver (1979) and Harrison (1980) with <u>A. formosa</u> but contribute more information about the early stages of development. Rinkevitch and Loya (1979) and Fowler (1888) observed that <u>Pocillopora damicornis</u> produces gonads which project into the coelenteron on stalks. The former suggest that this is a characteristic of branching or small-polyped species, and report unpublished data that one species of <u>Acropora (A. hemprichii)</u> conforms to this pattern. In this investigation however, it has been shown that the developing gonads of <u>A. formosa</u> are always located on the mesenteries.

Goreau (1956) reported that zooxanthellae were associated with developing ova in Porites furcata. In Stylophora pistillata, Rinkevitch and Loya (1979) observed that zooxanthellae were present in the gastrodermis around developing ova and testes; algae were also found in the gastrodermis and mesenterial filaments of the planulae. In the present work, zooxanthellae were present only in the early stages of development of the testes of A. formosa. Since no brooding of fertilised eggs was ever observed, it would appear that reproductive material is shed into the water for external fertilisation and that it is free of algal symbionts when it is released. Re-infection may occur from the zooxanthellae which are periodically released from adult coral colonies (Marshall, 1972; Goreau et al, 1970), or from mobile free living stages such as those which arise in axenic laboratory cultures of G. microadriaticum (Kawaguti, 1944b; Zahl and McLaughlin, 1957; McLaughlin and Zahl, 1959; Freudenthal, 1962; Kinzie, 1974; Steele, 1977; Schoenberg and Trench, 1980b).

The monthly sampling programme carried out during 1980 for this study showed that colonies of <u>A. formosa</u> in Nelly Bay produced eggs in the period between April and October, and testes in October. Release of reproductive material occurred in October-November, coinciding with an annual increase in water temperature (Kenney, 1974). It must be noted, however, that in this investigation histological observations were confined to the extreme tip region, a region known to be a less reliable source of reproductive material than the mid-branch region (Harrigan, 1972). The evidence cannot therefore be regarded as conclusive. However, in a recent study, Oliver (1979) has used a more sensitive technique involving microscopic examination of portions of decalcified branches. He reports a similar pattern of development and release, but was able to detect earlier stages in development. His observations show that gonad development in the more proximal regions of the branches began 4 months sooner than at the tips.

Available evidence therefore suggests that <u>A. formosa</u> sheds eggs and sperm into the water around October to undergo external fertilisation. Stimson (1978) describes this mode of reproduction as being typical of deep water species rather than those of the reef flat which tend to brood their planulae. This is consistent with Wallace's (1979) observations that the preferred habitat of A. formosa is the reef slope.

4.5.2.6 Gland cells

Table 4 summarises the results of all other histochemical studies of the gland cells of corals, and notes any apparent similarities between those descriptions and the information obtained for A. formosa in this study.

Goreau (1956) reported the presence of many highly refractile, eosinophilic, weakly PAS positive "hyaline" glands in the lower third of the cnidoglandular bands of the corals which he studied. He offered no explanation of their possible function. The refractile flask-shaped cells observed in the present investigation do not resemble the cells described by Goreau. They are much smaller and are only sparsely distributed throughout the cnidoglandular bands. None were seen in Alcian blue-PAS preparations and their function is completely unknown.

Of the five histochemically distinct types of mucous gland cell described in the scleractinia (Table 4), two occur in <u>A. formosa</u>. These cells produce β metachromatic acid mucins and

		1			- 11	00-									
	Comparison with this study		n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.		n.c.	n.c.	n.c.	n.c.	'pe
	lryptophan	J													nd ty
	blue a a blue a blue a blue blue blue blue blue blue blue blue		8	8	β	Ş	β	β	1		β	β	a	ø	y secor
	A 97uzł	,									~ .	_			nant]
	PAS		I	I	+	I	+	+	weak		e +	e) +	I	+	edomi
	Staining reaction AB-PAS														above, pre
	AB		+	+	I	+	I	I			<i>ر</i> .,	I	ç.	ç.	ង ខ្ល
	Nature of cell product		acid mucin	acid mucin	neutral mucin	acid mucin	neutral mucin	neutral mucin	not known		muco-protein	muco-or glyco- protein	not stated	not stated	
	gland cell type		mucous	mucous	granular	mucous	granular	granular	hyaline ⁽²⁾		granular	mucous	granular	mùcous	
• 101 1030	Region of polyp		epidermis - body wall	- oral disc	(1) - stomodeum	gastrodermis		cnidoglandular band	σ		ectodermis - planula				epidermis - tentacle
- IN TA CONTRACT	Author and species studied	GOREAU (1956)	Astreoides calicularis	Dendrogyra cylindricus Dinloria clivosa	Diploria strigosa	Isophyllia multiflora	<u>Isophyllia rigida</u> Isonhvllia sinuosa	Mancinia areolata	Montastrea annularis Porites furcata Tubastrea tenuilamellos	TYONS (1973b)	Balanophyllia	regia			

Table 4: Summary of the major results of histochemical studies of gland cells in corals compared with the writer's findings with A. formosa.

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(contd overleaf)

Author and species studied	Region of polyp	gland cell type	Nature of cell product	Stainin reaction AB AB-PAS	PAS CP	Tryptophan Tryptophan Toluidine blue a s f f f f f f f f f f f f f f f f f f	Comparison with this study
VAN PRAËT (1977)			n de la constante de la constan				
Hoplangia	epidermis - body wall	mucous	acid mucin	+	+	÷	n.c.
durotrix		granular	$1 \mu m$ eosinophili	c granules		·	gr_{A}
	- oral disc	mucous	acid mucin	+	+	+	n.c.
	- stomodeum	mucous	acid mucin	+	+	+	n.c.
	gastrodermis	mucous	acid mucin	+	+	+	n.c.
		granular	protein	$1-2\mu m$ electi	ron opaqı	le granules +	gr_{D}
		granular	protein	0.2µm elecù	ron opagı	le granules +	gr _C
	cnidoglandular band	mucous	acid mucin	+	+	+	n.c.
		granular	protein	$1-2\mu m$ electi	ron opaqı	le granules +	$gr_{\rm D}$
		granular	$1-3\mu$ m moderatel	y electron o	oaque gra	nules	gr B
		granular	0.7-1 μ m slightl	y electron o	oaque gra	nules	gr _A
						(cont	l overleaf)

Table 4 (contd)

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species studied	Region of polyp	grand cell type	product	AB	reaction AB-PAS	PAS	A sauza S saibiuloT a suibiuloT a suid a suid	tudy this	
LINS (1978)									
formosa	epidermis – body wall	mucous	wk acid mucin	+	-/+	I	I	un ₁	
	- stomodeum	mucous	acid mucin	+	-/+	I	1	- ^L nu	
		granular	mixed mucin	i	purple	+	+	- ² mu	TTO
	gastrodermis	"active sites"	mixed mucin	+	+/wk	weak	I	mu2	
	cnidoglandular bands	mucous	acid mucin	+	-/+	ł	I	nu_1	
		granular	mixed mucin	I	purple	+	+	mu ₂	

n.c. - no comparison; mu - mucous gland cell (type denoted by subscript); gr - granular gland cell (type denoted by subscript)

(1) except hermatypic corals which also have granular gland cells in the body wall epidermis (2) intensely eosinophilic

(3) diastase resistant

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Table 4: (contd)

non-metachromatic neutral mucins, which were found in association with the type 1 and type 2 mucous gland cells respectively. Goreau (1956) additionally found δ metachromatic acid mucins and β metachromatic neutral mucins in the corals which he examined. Van Praët (1977) identified the contents of the mucous gland cells of <u>Hoplangia durotrix</u> as acid mucins, but the staining reaction which he recorded (Table 4) indicate the production of a fifth type of mucus. This comprised a mixture of

mucins which were predominantly acid in character.

A variety of functions have been proposed for mucus production in corals. These include feeding and cleansing, the retardation of epizooic growth (see Muscatine, 1973), and the removal of surface bacterial growth (Ducklow and Mitchell, 1979). Mucus may also provide a buffer to some forms of physical damage (Benson <u>et al</u>, 1978). <u>Acropora</u> species are well known to produce copious quantities of mucus following damage or exposure to air (Coles and Strathman, 1973; Benson and Muscatine, 1974). The ubiquitous distribution of mucous gland cells reported in this study is consistent with the above observation, and also with the finding of Crossland <u>et al</u> (1980) that in <u>A. acuminata</u> (= <u>formosa</u>, Veron pers. comm.) up to 40% of the net carbon fixation is dissipated as exuded mucus.

Electron microscopic studies of scleractinian mucous gland cells (Lyons, 1973b; van Praët, 1977; Kinchington, 1980) support the suggestion made in this study that cells in the outer body wall of the polyp are the same as those in the interior. It follows therefore that the structures which Collins (1978) described as "active sites" in the inner body wall of <u>A. formosa</u> (Table 4) are probably identical with those described here as purple staining (type 2) mucous gland cells. Collins did not fully describe what he called granular gland cells in his study of <u>A. formosa</u>, but their distribution pattern and histochemical reactions (Table 4) correspond to the type 2 mucous gland cells described in this study.

In the hermatypic corals which he studied, Goreau (1956) consistently observed an abrupt transition between acid mucin producing mucous gland cells in the epidermis to neutral mucin producing granular gland cells in the stomodeum and cnidoglandular bands. In contrast, acid mucin (type 1) producing and neutral mucin producing (type 2) mucous gland cells in <u>A. formosa</u> were not mutually exclusive in their distribution at the stomodeum. There was a distinct shift towards a predominance of type 2 mucous gland cells in the stomodeum and cnidoglandular bands of the type I mesenterial filaments, but this tendency was then reversed in the type II mesenterial filaments.

Harrison (1980) described five types of granular gland cells in A. formosa on the basis of the size of their granules and their staining reactions with Gomori trichrome. However, he did not include descriptions or illustrations of the glands themselves. Two of these granule types were identical to those in the type A granular gland cells of this study and were found in the same locations, namely the outer body wall and the cnidoglandular bands of the mesenterial filaments. One type stained red and the other blue. Harrison (1980) examined tissue more proximal to the tip region, and therefore older than that examined here, suggesting that red granules may mature into blue granules. Two other granule types recognised by Harrison (1980) stained orange with Gomori trichrome stain and correspond to the contents of type C and D granular gland cells described in the present study. The final type of granule described by Harrison corresponds to those found in type B granular gland cells, and, as in this study, they stained blue.

The incomplete descriptions of Lyons (1973b) do not readily permit comparisons with this study. However, none of the cells she describes (Table 4), with the possible exception of the mucous gland cells in the outer body wall, corresponds to any cell type found here. In contrast, there is a strong correlation between the physical appearance of the granular gland cells described in <u>Hoplangia durotrix</u> by van Praët (1977) and the granular gland cell composition of <u>A. formosa</u> (Table 4). The two which resemble types C and D in <u>A. formosa</u> were examined histochemically by van Praët. They too were found to contain tryptophan. Despite the finding that there are more granular gland cells in <u>A. formosa</u> than were indicated by Collins' (1964) preliminary study, at least one non-astrocoeniid coral (<u>Hoplangia durotrix</u>) appears to have a similar gland cell composition. Therefore the difference between the evolutionary origins of <u>A. formosa</u> and other corals which have been studied is not reflected in the nature of its secretory products.

4.5.3 Diel expansion and contraction rhythms

The work of Duerden (1904a), Kawaguti (1954), Porter (1974) and Lasker (1977) on numerous scleractinian species indicates that the behaviour pattern of night expansion and daytime contraction described here for the axial polyps of <u>A. formosa</u> is typical of the majority of reef building corals. A tendency for partial daytime expansion in radial polyps has also been noted previously in <u>Acropora</u> by Stephenson (in Yonge, 1940) and Lewis and Price (1975).

Detailed histological examination of the axial polyps of <u>A. for-mosa</u> revealed that it is not only the oral disc and tentacles that move during expansion. The inner and outer body walls over the extreme tip also alter their positions. The outer body wall becomes distended at night, irrespective of whether it is located over the coelenteron or the skeleton. At the same time the conformation of the tissues of the inner body wall immediately around the trabecular tines is also altered, allowing the outer body wall to be lifted clear of the skeleton, thus creating a space above the tines. Only a limited amount of the suggestion made in Chapter 3 (p.40) that the bulk of the polyp is anchored in place by the internal cross-linking of the skeleton.

The correlation was not absolute between the occurrence of the expanded and contracted tissue conformations with night and day respectively. In coelenterates, however, a variety of external influences may disrupt diel patterns of polyp expansion. These include mechanical disturbances (Horridge, 1957), abnormal alterations in light level (Lasker, 1979), atypical abundance of food (Goreau, 1956) and changes in the level of state indicated by tissue conformation during the course of these experiments.

4.5.3.1 <u>Cellular mechanisms of expansion and contraction</u>(a) Outer body wall

If the outer body wall of the polyp was merely inflated in response to the increase in hydrostatic pressure which produces polyp expansion, it might be expected that the tissues would become stretched and thus thinner overall. In fact, the reverse occurs and the cells of all three external tissue layers become thicker when the polyp expands and the tissues adopt the expanded conformation. The work of Robson (1957) with Metridium senile showed that the epithelial layers of coelenterates contain fluid in intercellular spaces. According to Robson the mechanical properties of this sub-epithelial fluid "enable the epithelium to function as a whole without excessive strain" in response to muscular deformation. In addition, her micro-injection experiments showed that an increase in the volume of the sub-epithelial fluid produced localised distension of the tissues. It seems reasonable to conclude, therefore, that the conformational changes observed here in the outer body wall of A. formosa are due to an overall increase in volume of the sub-epithelial fluid in the tissues at night when the polyp is expanded.

The increase in hydrostatic pressure which accompanies expansion (Batham and Pantin, 1950a) presumably squeezes the inner body wall proximal to the tip against the skeleton. It is possible, therefore, that the sub-epithelial fluid in the outer body wall gastrodermis becomes supplemented by that displaced from the inner body wall. The volume of the sub-epithelial fluid of the epidermis could be increased either by the movement of water, under pressure, from the coelenteron, or by absorption from the exterior. The mesoglea of coelenterates is known to be able to absorb large quantities of water (Barrington, 1979), thus, the increase in thickness of the mesoglea which accompanies epithelial distension may be accomplished by one of the methods suggested for the epithelial layers. Interestingly, the distension of the columnar supporting cells of the epidermis does not appear to displace the nuclear band from its position in the proximal third of the layer. This suggests that the distal portion of the cells extends at least as much as the proximal portion. This is interesting because only the basal portion of epithelial cells have previously been reported to be affected by compression (see Robson, 1957; D.M. Chapman, 1974).

(b) Inner body wall

It was not possible, with light microscopy, to demonstrate any muscular involvement in the processes of expansion and contraction around the trabecular tines. However, electron microscopic studies of the inner and outer body wall of A. formosa (Kawaguti and Sato, 1968; Harrison, 1980) have revealed the presence of very fine muscle fibrils in the gastrodermis adjacent to the mesoglea. These appeared to be located in the expanded basal processes of the nutritive cells. Harrison also noted the complete absence of any muscular material in the columnar supporting cells of the outer body wall. In the light of this information it appears likely that the expansion and contraction of the coensarc is under the control of а gastrodermal slow (non-nervous) conducting system like that observed to act in the coensarc of the brain coral Meandrina meandrites (McFarlane, 1978). These systems appear to act by muscular inhibition rather than by direct stimulation (McFarlane and Lawn, 1972; McFarlane, 1974). Their action therefore relaxes the gastrodermal musculature and reduces muscle tone. Expansion of the coensarc is then induced by positive pressure in the coelenteron.

The nutritive cells of the gastrodermis of <u>Acropora</u> are known to have large intercellular spaces between their basal processes (Kawaguti and Sato, 1968). Passive deformation of these spaces by an increase in hydrostatic pressure in the coelenteron could readily account for the observed change from cubo-columnar to cubo-squamose. This supports the suggestion made previously (Section 4.5.3.1a) that the inner body wall gastrodermis is a source of sub-epithelial fluid. The wedge of mesoglea at the junction of the inner and outer body wall bears a superficial resemblance to the compressed mesoglea described by D.M. Chapman (1974) in the base of retracting tentacles in the scyphozoan Aurelia aurita. This structure is thought to act as a tensile spring which is released when the tissue expands. If this occurred in A. formosa, it might be expected to assist the tissue around the tine to adopt the extended state. However, G. Chapman (1974) points out that the coelenteron is hydraulically unimportant in the medusae, and stresses that the scyphozoa are the only group of coelenterates in which the mesoglea clearly acts as an antagonist to the musculature. He adds that compression of actiniarian mesoglea produces little restorative power because this simply deforms the fibres, rather than bending them to generate tensile strength. The appearance and disappearance of the wedge-shaped mass of mesoglea in the tissue around the trabecular tines of A. formosa must therefore be interpreted as a passive process, caused by a contraction of the gastrodermal musculature and positive pressure in the hydrostatic skeleton respectively.

It is important to note that, except in cases of obvious rupture, the mesoglea is always continuous over the tips of the trabecular tines. Thus, in <u>A. formosa</u>, the epidermal cells do not move inwards to become calicoblastic cells as Barnes (1971, 1972) described in the edge zone of <u>Mancinia areolata</u>. Kawaguti and Sato (1968) state that the cells of the calicobastic layer in <u>A. formosa</u> are concentrated over the spines (= trabecular tines) in a multi-layered arrangement. Comparison of their longitudinal sections with serial sections through the same region obtained in this study shows that they obtained a misleading impression by sectioning obliquely, effectively cutting a transverse section of the calicoblastic layer down the side of the tine. Sections obtained in this study show that the calicoblastic layer is never more than one cell thick.

Many workers (Bourne, 1887b, 1899; Wilson, 1888; Ogilvie, 1896; Goreau, 1956) have observed that the calicoblastic layer is normally cubo-squamose in form, but noted that a columnar epithelium is frequently associated with skeletal growth points. However, the capacity of this arrangement to create space for growth of the skeleton as observed in this study has not been previously recognised.

Although the ability of the spindle-shaped calicoblastic cells to slide against each other and completely reorient themselves relative to the mesoglea has not previously been proven, it has been suggested to occur in the lateral margin of settling Balanophyllia regia larvae (Kinchington, 1980). Epithelial cells in coelenterates are commonly joined by desmosomal contacts at their distal surfaces (D.M.Chapman, 1974), and Harrison's (1980) electron microscopic study showed that A. formosa is no exception. Johnston's (1980) superficial transverse sections through the calicoblastic layer of P. damicornis showed that such contacts are continuous around the distal perimeter of the cells. Since coelenterates can readily break and reform desmosomal contacts (Overton, 1963; Bibb and Campbell, 1973; Campbell, 1974), there would appear to be two possible ways by which the calicoblastic cells could adopt the changes in configuration reported in this study. These are represented diagramatically below:-

(a) Breaking of desmosomal contacts at the distal ends of the cells



The cells re-orientate along the mesoglea and desmosomes reform between the original distal end of one cell and the former proximal end of the next.

(b) Breaking of desmosomes and reformation halfway down the cells



The original lateral axis is converted into a longitudinal axis aligned parallel to the mesoglea.

The illustration above represents a very simplified concept of the possible mechanisms of cellular movement in the calicoblastic layer. Electron microscopic studies of calicoblasts in <u>A. formosa</u> (Kawaguti and Sato, 1968) and <u>Pocillopora damicornis</u> (Vandermeulen, 1975; Johnston, 1977, 1980) confirm the existence of large intercellular spaces adjacent to the mesoglea, but emphasise the extreme interdigitation of the cells which makes interpretation at high magnification difficult.

(c) The tentacles

Retraction of the tentacles of <u>A. formosa</u> is a continuous process which on the basis of histological evidence presented here can be divided into three distinct phases:-

- Conversion of the squamose epithelial layers of the fully extended tentacles to a form resembling that found in the outer body wall.
- 2. Contraction of the circular muscle layer which buckles the longitudinal muscle layer outwards and throws the gastrodermis into longitudinal folds
- 3. Further contraction of the longitudinal muscle layer which buckles the circular musculature muscle layer inwards, deforming the gastrodermis into transverse corrugations and compressing the epidermis to give similar, outwardly projecting folds.

The light microscope can resolve the fibres only in the epithelial muscle fields of the tentacles where they are highly contracted. However, it seems reasonable to assume that the observed conversion from a squamose epithelium to a cubo-columnar epithelium in the gastrodermis, and a columnar epithelium in the epidermis during the first stages of retraction is due to contraction of the longitudinal muscle fibres of the epidermis. Morphological changes of this type are known to occur readily in other coelenterates. Thus, describing a similar process in the tentacles of <u>Aurelia aurita</u>, D.M. Chapman (1970) attributed the dimensions of the tentacular elements entirely to the degree of shortening.

The sub-epithelial fluids described by Robson (1957), see Section 4.5.3.1a) presumably transmit the alterations in hydro-

static pressure which create the changes in the epithelial cell layers associated with the initial stages of contraction. The subsequent increase in height of first the gastrodermis, and then the epidermis, in response to increasing muscular compression (stages 2 and 3 respectively), can also be attributed to the action of the compressed sub-epithelial fluid on the basal processes of the cells. The detailed work of Robson (1957) describes how this squeezes the upper portion of the cells away from the mesoglea.

During their ultrastructural study of Metridium senile, Grimstone et al (1958) postulated that desmosomal contacts at the distal surface of the epithelial cells (similar to those observed in A. formosa by Mahanskii et al, 1977; Harrison, 1980) permitted the retention of surface features such as the complex flagellar apparatus during deformation of the cell layers. However, they made no mention of how non-epithelial cell types responded to re-orientation. Presumably, mucous gland cells are capable of substantial deformation since their cell contents are amorphous. In contrast, nematocysts have a tough, double-membraned capsule (Mariscal, 1974) which might have been expected to resist deformation. However, Westfall (1970) demonstrated that these cells have a hinge-like process in their basal region to allow them to conform to a change in orientation from vertical to horizontal as required. Observations of the behaviour of nematocysts in contracting tentacles in this study are consistent with the possession of such processes.

Electron microscopic studies (Goreau, 1956; Kawaguti and Yokayama, 1966) have shown that the mesoglea of corals contains both branched and unbranched fibrils. In other coelenterates, such fibrils are known to become aggregated into fibres under muscular compression to create a highly deformable three dimensional lattice (Chapman, 1953, 1959; Alexander, 1964). G. Chapman (1974) concluded that since this is a passive process in the anthozoa, the role of the mesoglea is predominantly that of a cell substratum which permits great changes in shape. The organisation of the mesogleal fibres in this way has been variously described as "warp and weft" in Calliactis parasitica
(Chapman, 1953), and "warp and woof" in <u>Metridium senile</u> (Grimstone <u>et al</u>, 1958). It is most pronounced at the surface of the mesoglea since this is closest to the muscle layers and therefore subject to most compression. The result is a flexible surface for muscle attachment which Batham and Pantin (1951) and Chapman (1953) suggest can readily accommodate to buckling actions. The parallel alignment of the mesogleal fibres observed here in retracted tentacles of <u>A. formosa</u>, and their subsequent organisation into sheets of differing orientations is identical to that described from anemones. In addition, Kawaguti and Yokayama (1966) observed that the mesoglea of <u>Dendrophyllia cribrosa</u> appeared densest at its boundaries with the epithelial layers. These observations strongly suggest that a mechanism similar to that described by Batham and Pantin (1951) also operates in corals.

Robson (1957) defined "buckling" as a process by which the fibres of a muscle field accommodate when they are displaced by the contraction of a reciprocal muscle. The behaviour of both the longitudinal and circular muscle layers in retracting tentacles of <u>A. formosa</u> clearly justifies the continued use of the term. Transverse sections of tentacles of <u>A. formosa</u> show buckling in the epidermal musculature which is exactly analagous to that reported in the tentacles of the anemones <u>Metridium senile</u> (Batham and Pantin, 1951) and <u>Actinia equina</u> (Leghissa, 1965). Batham and Pantin (1951) also observed superficial buckling of the gastrodermal muscle layer in longitudinal sections of <u>Metridium senile</u> tentacles but report tree-like invaginations like those observed here only in the body wall.

The only previous description of folding of the epidermal layer at right angles to the direction of contraction of the longitudinal muscle fibres was supplied by Robson (1957) and relates to the tentacles of <u>Metridium senile</u>. She referred to the effect as "fine wrinkling", and stated that it only occurred under conditions of maximal contraction, attributing it to the relatively small amounts of sub-epithelial fluid in these structures compared to the outer body wall. In <u>A. formosa</u>, the compression of the entire tentacle into a small knob of tissue clearly conforms to the condition of extreme contraction and resulted in the production of deep epidermal folds.

4.5.3.2 Functional significance of expansion and contraction According to Lewis and Price (1975) the genus <u>Acropora</u> belongs to a group of corals which normally expand fully only at night. This group obtains food by a combination of tentacle capture and entanglement by mucus filaments. Since the latter process does not require the polyp to be expanded, this combination of feeding strategies appears to allow continuous food capture over a diel period.

Zooplankton are most abundant in reef waters at night (Glynn, 1973; Porter et al, 1977), therefore the advantages of nocturnal expansion of the tentacles for food capture is immediately obvious. In contrast, the advantages of nocturnal expansion of the coensarc is not so clear. McFarlane (1978) showed that expansion of this tissue is not necessarily a consequence of the expansion of the oral disc and tentacles since the two slow systems which control expansion in these regions act independently. In the brain coral Meandrina meandrites, for example the coensarc is commonly expanded during the day when the oral disc is retracted. This arrangement probably exposes the contained zooxanthellae to maximal illumination (see Drew, 1976; McFarlane, 1978). Thus it seems very likely that the observed tendencies for the coensarc at the tips of the axial corallite in A. formosa to be expanded at night, and its close application to the skeleton during the day also has some definite physiological significance.

Time-lapse photography on branch tips of <u>A. formosa</u> by Barnes and Crossland (1980) has shown that skeletal extension at night consistently' exceeds that occurring during the day. Based on previous observations of growth in the dissepiments of <u>Isophyllia sinuosa</u> (Barnes, 1970, 1972), they suggest that the pattern of deposition at night (when, according to Chalker (1976) and Taylor (1968), calcification rates are low) is oriented towards extension. Deposition resulting from the substantially higher daytime calcification rates (Kawaguti and Sato, 1968; Goreau, 1959; Goreau and Goreau, 1959; Vandermeulen <u>et al</u>, 1972), although volumetrically greater, is, according to Barnes and Crossland, geared towards consolidation of nocturnal growth. This suggests that there are two types of deposition with different control mechanisms operating during the day and night. Gladfelter's (1982) study of $CaCO_3$ accretion in the axial corallites of <u>A. cervicornis</u> supports this view.

Gladfelter (1982) observed two distinct phases of accretion and her diagramatic representation of the sequence of crystal deposition and growth in an extending axial spine (= trabecular tine) is reproduced below.

Diagramatic sequence of crystal deposition and growth on an extending spine in an axial corallite of A. cervicornis (drawings reproduced from Gladfelter, 1982)



- A. Deposition of fusiform crystals (1.5-3 μ m in length) on extreme tip of trabecular tine.
- B. Fusiform crystals act as foci for clusters $(3-6\mu m \text{ in diamet-er})$ of randomly oriented crystals.
- C. Proximal to the tip, groups of needles projecting from the clusters in direction of calicoblastic epidermis extend to form tufts $5-6\mu m$ wide.
- D. Several extended tufts combine to form fasiculi (= crystal fibres) 15µm in width.

The first phase of skeletal accretion (steps A and B) involves extension of the trabecular tines by deposition of randomly oriented fusiform crystals which serve as growth foci for "numerous needle-like crystals projecting in many directions from the fusiform crystal surface". Gladfelter suggested that the accretion which occurs during the first phase resembles the precipitation that takes place in a cavity containing a super-saturated solution of CaCO₂. This was based on previous reports from other coral species (Bryan and Hill, 1941; Jell, 1974) which pointed out the similarity between the observed patterns of crystal accretion and the purely physio-chemical process of inorganic spherulite formation. Also relevant were the findings of Wind and Wise (1976) that nucleation and growth of crystals in the mantle fluid of a marine archeogastropod occurs by an apparently random process of precipitation. She therefore tentatively suggested that the tissue would be lifted away from the skeleton when this type of growth occurred. She stresses that "the mechanism of formation of the putative cavity can only be postulated at this time", stating that it "could involve tissue growth, muscular action or increased fluid pressure".

The second phase of accretion (steps C and D), according to Gladfelter, begins just proximal to the tip. It not only increases the diameter of the delicate first phase accretion to the same width as the rest of the branch, but also produces thickening throughout the length of the corallite. Because of interference during competitive crystal growth (first described by Barnes, 1970), accretion occurs only on those crystals from step B which are oriented perpendicular to the calicoblastic epithelium (step C). This produced bundles of parallely oriented crystals which then group together to form "fasiculi" (= crystal fibres) in step D.

Gladfelter acknowledges that the organisation of the crystal during second phase accretion in <u>A. cervicornis</u> is the same as that found in many other corals (e.g. Ogilvie, 1896; Wells, 1956; Wise, 1969, 1972), when it has sometimes been suggested (Wainwright, 1963, 1964; Sorauf, 1972) that an organic matrix template may be involved in directing precipitation. Gladfelter further points out that second phase deposition is highly reminiscent of that described by Johnston (1980) in <u>Pocillopora dam-</u> icornis in a recent study which proved the existence of a highly organic matrix for the first time. However, she concluded "how a matrix may be involved in <u>A. cervicornis</u> has not yet been ascertained".

Histological evidence from the present investigation confirms that Gladfelter's "putative cavity" is a reality in <u>A. formosa</u>. It is formed almost exclusively at night by a combination of muscular relaxation and an increase in hydrostatic pressure. Since <u>A. cervicornis</u> follows the same expansion and contraction rhythm as that described here for <u>A. formosa</u> (see Lewis and Price, 1965), it is safe to conclude that during darkness the tissues of the axial polyps in both <u>A. formosa</u> and <u>A. cervicornis</u> are indeed lifted away from the growth points of the corallites, i.e. the trabecular tines. It follows, therefore, that nocturnal extension in the trabecular tines of <u>A. formosa</u> can be presumed to occur as in <u>A. cervicornis</u>, by the precipitation of fusiform crystals and micritic needles.

The use of Johnston's (1977) fixative on A. formosa (Harrison, 1980) resulted in the preservation of a delicate skeletal organic matrix similar to that described by Johnston in Pocillopora damicornis (Johnston, 1980). Radio-isotope work (Muscatine and Cernichiari, 1969; Pearse and Muscatine, 1971; Young et al, 1971; Schmitz and Kremer, 1977) has shown that products of algal photosynthesis are involved in the manufacture of organic material in coral skeletons. Since the high rates of daytime calcification in the tips of A. cervicornis branches are known to result from preferential translocation of algal photosynthate towards the branch end (Pearse and Muscatine, 1971; Taylor, 1977), it seems likely that a matrix similar to that observed in A. formosa is also present in A. cervicornis. There is every reason to conclude, therefore, that daytime calcification in both species produces crystal fibres proximal to the tip whose precipitation is enhanced and oriented by a structured organic matrix.

The diel pattern of accretion proposed here for the trabecular tines of A. cervicornis and A. formosa, based on the observations of Gladfelter (1982), does not correspond to that observed in the only other diel study of skeletal growth to date by Barnes (1970, 1971). In the dissepiments of Isophyllia sinuosa Barnes found that new crystals and the subsequent random growth which formed the basis for the crystal fibres occurred only during the day. In contrast to what has been observed for A. formosa, nocturnal growth in Isophyllia sinuosa took the form of highly ordered growth resulting in the extension of existing crystals in a thin primary layer. From his observations, Barnes argued that high daytime calcification rates were necessary to induce spontaneous precipitation of crystals. The model presented here for A. formosa would clearly not apply to Isophyllia sinuosa and points to fundamental differences between the two species. Barnes observed that the end result of daytime growth in Isophyllia sinuosa was the insertion of large highly organised crystal fibres over the whole growth surface, including the leading edge of the primary layer, whilst Gladfelter found that such growth (her second phase accretion) only took place proximal to the tip.

It is important to note that the formation of dissepiments in species which produce them occur only periodically (Wells, 1956) when the polyp base is uplifted (see p. 40). In contrast, the sustained growth of the corallum in <u>A. formosa</u> relies upon continuous extension of the trabecular tines. It would be therefore expected that the latter may have a higher "priority" in the metabolic processes of the colony. In addition, the position of the dissepiment at the base of the polyp means that the overlying tissues are not influenced by diel rhythms of expansion and contraction (Wainwright, 1964). Thus the type of deposition produced by the uplifted calicoblastic epithelium as described for A. formosa is not seen in dissepiments.

Goreau (1959) found that coral calcification continues, albeit at a low level, in the absence of zooxanthellae and in darkness. He concluded from this that algal photosynthesis was not essential to the process of calcification but merely enhanced its basic rate. It seems highly probable that the diel pattern of deposition observed by Barnes in the dissepiments of <u>lsophyl-</u><u>lia sinuosa</u> is the physical expression of this effect. In contrast the pattern which is suggested here for the trabecular times of <u>A. formosa</u> and <u>A. cervicornis</u> would enable continuous growth which combines maximal extension with adequate structural consolidation.

4.5.4 Structure and function in the mesenterial filaments Yonge (1930b) made a thorough examination of the process of digestion in fungiid corals. Although the methods used were crude by modern standards (Lenhoff, 1968; Muscatine, 1973) there has been no suggestion that their general conclusions were incorrect. In contrast to the anemones where initial stages of digestion require an alkaline pH (Hyman, 1940), the immediate effect of food entering the coelenteron of the corals was reported as a drop in pH. These more acid conditions, it was suggested, provided the optimum conditions for the action of a powerful proteolytic enzyme which was secreted from the gland cells of the mesenterial filaments. Yonge (1931) reports that most of the ingestion of the resultant smaller particles took place via the ingestion excretion zones in the lateral lobes of the mesenterial filaments. Others (Hyman, 1940; Meglitsch, 1972) have suggested that the gastrodermis as a whole is more likely to be responsible for the absorbtion of initial products of digestion. Peptidases then complete the hydrolysis into amino acids intracellularly. Progressive digestion of this type is known to be typical of coelenterates and the preliminary proteolytic stages always tend to occur in the upper part of the polyp (Yonge, 1937; Meglitsch, 1972).

The structure of the cnidoglandular bands of type I mesenterial filaments in <u>A. formosa</u> is consistent with the above pattern of events. They bear heavily barbed, possibly poisonous (Mariscal, 1974) microbasic p-mastigophores and numerous mucous gland cells. Thus, they appear well equipped to complete the subjugation of prey when it is introduced into the coelenteron and to apply the mucus coating, which appears to be an integral part of the coelenterate digestive process (Krijgsman and

Talbot, 1953). They also have large numbers of granular gland cells which are predominantly basophilic in character, implying that their cell contents are acidic. The nature of the secretions from these cells would therefore be consistent with the drop in pH suggested by Yonge (1930b) to be required for optimal functioning of the proteolytic enzymes. Two of these gland cell, granular gland cell types C and D, appear to be involved in the production of enzymes (possibly proteases). The remaining two (types A and B) are primarily involved in the synthesis of neutral mucins which, according to Phillips <u>et al</u> (in Lenhoff, 1968), have been implicated in the complexing of proteins prior to hydrolysis.

The extremely reduced lateral lobes of the type II mesenterial filaments of <u>A. formosa</u> suggests that their absorbtive ability is poor. Their tendency to be distributed in the depth of the polyp and in the gastrodermal canals outside the central cavity gives further support to the suggestion that they are not directly involved in the digestive process described above. The majority of the gland cells in the cnidoglandular bands of type II mesenterial filaments give an acidophilic staining reaction and it is possible that their alkaline secretions may have some buffering effect in the more remote regions of the coelenteron, thus restricting proteolysis to the central cavity.

Type II mesenterial filaments could be involved in the mixing of gut contents to promote more efficient absorbtion, a role suggested for mesenterial filaments in general by Hand (1966). However, the strong ciliary currents which have been observed in the gastrodermal canals of <u>A. cervicornis</u> (Gladfelter, in prep. in Gladfelter, 1982) suggest that, in <u>Acropora</u>, the flagellated gastrodermis is capable of maintaining an efficient circulation within the narrow confines of the convoluted coelenteron. Some other function must therefore be assigned to the type II mesenterial filaments.

It has been reported by Collins (1978) that the presence of food on the outer surface of an <u>A. formosa</u> colony induces spontaneous extrusion of mesenterial filaments through the coensarc. Histological examination of extruded filaments during this study has shown that it is the type II filaments which are involved. It appears, therefore, that their function may be extra coelenteric food capture and digestion.

Viewed in this context, the rather incongruous presence of both type p- and b- microbasic mastigophores deep in the polyp may be readily understood if it is assumed that they are in fact reserved for use when the filaments are outside the body. (They may also have a protective role when filaments are extruded in response to tissue damage by pollutants such as DDT and oil (Lewis, 1971). The presence of substantial numbers of type D granular gland cells suggests a considerable capacity for the digestion of proteinaceous material. Equally importantly, the large numbers of mucous gland cells would enable the projection of the copious quantities of mucous which have been observed to be associated with extra-coelenteric digestion (Matthai, 1918; Goreau, 1956). Observations on anemones have shown that such mucous coatings ensure continuous close contact of the filaments with the prey and prevent dilution of the digestive enzymes by the surrounding water (Nichol, 1959).

The extremely reduced state of the lateral lobes of the type II filaments presumably enhances their flexibility but as mentioned above (p. 127) must also reduce their capacity for absorbtion. However, Harrison (1980) has shown that A. formosa is typical of other scleractinia such as Mussa angulosa, Astrangia danae (Goreau and Philpott, 1956) and Balanophyllia regia (Lyons, 1973 a and b) in that it has microvilli on the columnar supporting cells of the free epidermis. These structures are thought to increase the surface area available for absorbtion in 1956; Goreau et al, 1971) and corals (Goreau, anemones 1960) which carry out extra-coelenteric digestion. (Batham, They may well perform the same role in A. formosa.

Thus, the structures described here as type II mesenterial filaments appear to fulfill a role in feeding and defence in <u>A. formosa</u> which is analagous to that performed by acontial filaments in the acontiarian anemones (Stephenson, 1920). Several workers (Fowler, 1885; Bourne, 1887a; Collins, 1978) have used the term "acontia" to describe extruded mesenterial filaments of corals. In addition, Harrison (1980) used the same term in his study of <u>A. formosa</u> to describe regions of the mesenterial filaments which are histologically identical to the type II filaments of this study. The author believes the use of this term to be inaccurate and misleading. Extrusion of mesenterial filaments is a common phenomenon in corals (Yonge, 1930a; Goreau, 1956), but Duerden (1904a) emphasised that it is only "comparable with" not identical to the emission of acontia.

Acontia are specific taxonomic features used in the identification of anemones and they are structurally different from the mesenterial filaments of corals. Many, though not all, anemones bear ciliated tracts on either side of the cnidoglandular band in the pharyngeal region, giving the band a trilobate appearance. Below the pharynx of such species the ciliated tracts disappear and the mesenterial filaments take on the appearance common to all anemones. These structures are indistinguishable from the mesenterial filaments of corals (see illus. Hyman, 1940, p. 572; Meglitsch, 1972). Acontia are threadlike continuations of the cnidoglandular bands of such filaments (Hyman, 1940) attached only at their distal ends. They may be readily differentiated histologically from mesenterial filaments by the presence of longitudinal muscle fibres along the mesogleal core, and the presence of many large nematocysts which are typically larger than those found on the upper cnidoglandular band (Stephenson (1920).

Histological evidence presented in this investigation suggests that some structural changes do occur proximally in the cnidoglandular bands of <u>A. formosa</u> (from type I to type II), but they are not so pronounced as the transition from cnidoglandular bands to acontia in anemones. No muscular development was observed in the cnidoglandular bands of type II mesenterial filaments and, most importantly, it is clear that these filaments are not free, but are still attached to the membranous part of the structure which accompanies them when they are extruded through the body wall. Thus, there is no evidence that true acontia ever arise from the mesenterial filaments of A. formosa.