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Copeman, et al. 1988

GREAT BARRIER REEF MARINE PARK AUTHORITY

Research Report for COTSAC Funded Project

(April 1987 - April 1988)

Project Title: "Studies on the diseases of the Crown of Thorns starfish Acanthaster planci"

Personnel:

Assoc. Prof. D.B. Copeman
Graduate School of Tropical Veterinary Science
James Cook University

Dr J.S. Glazebrook
Research Fellow
Graduate School of Tropical Veterinary Science
James Cook University

Ms K.A. Licastro
Research Assistant

Introduction:

The above project, which started in April 1986, has been run in conjunction with an MST funded project of the title.

i) Disease survey of starfish from the Great Barrier Reef and Suva Reef in Fiji

A total of 120 starfish from 10 different locations have been examined for signs of disease (Table 1). Great Barrier Reef specimens were obtained from Helix, Keeper, Wheeler, John Brewer, Pith, Grubb and Stanley Reefs between April 1985 and November 1987. They included 11 juveniles, 12 sub-adults and 67 adults. All animals from Fiji were juveniles except for 3 adults collected in January 1987. Nine starfish were obviously diseased when taken from aquaria at the University of the South Pacific and James Cook University. Helix, Keeper and Wheeler Reefs were selected as study sites because of their proximity to Townsville and the fact that they were being gradually attacked by large numbers of southward moving starfish. Grubb Reef was the scene of a copper sulphate/slaked lime control program conducted by the Great Barrier Reef Marine Park Authority in July 1986. Diseased starfish were reported on Pith Reef in November 1985. Field sampling decreased in 1987 because of the higher priority given to the identification and culture of a possible disease agent from Fiji.

ii) Clinical disease and pathology

The major line of investigation during 1986 and 1987 was the pathology associated with disease in juvenile and adult starfish from Suva Reef in Fiji. Clinical signs in juveniles ranged from increased transparency of the aboral disc to complete erosion of that area (Fig. 1).

Growth pathological changes indicative of an advanced stage of disease were the absence of a stomach and perforation through the centre of the body. Microscopically, an organism (possibly of the Class Sporozoa, Phylum Protozoa) was associated with degenerative, necrotic changes in the pyloric caecae (Fig. 2). Adult starfish showed signs similar to those recorded in juveniles i.e. erosion of the stomach and ulcerative lesions of the dorsal (aboral) surface. The pyloric caecae of one animal was pitch black and severely atrophied. As well as being normal in appearance, healthy starfish displayed much greater mobility and activity of the tube feet (Fig. 3). Only a scattering of organisms was seen in the pyloric caecae of adult starfish from Suva Reef (Fig. 4). One interpretation of the disease epizootic that occurred in Fiji is that the juvenile starfish were suffering from an acute form of disease and the surviving adults (collected 2 years later) were chronically infected. There is also evidence that the disease agent is quickly transmitted e.g. it took only 2 days for healthy animals to become infected when introduced to the University of the South Pacific aquarium (Zann 1986, pers. com.). Identification of the organism rests with electron microscopy.

Of the 90 starfish examined from the Great Barrier Reef (Table 1), 10% showed some sign of disease. Gross lesions were relatively minor and included prostrate spines, broken skin and an occasional missing arm. Internal changes were seen in the pyloric caecae which were dark brown and watery in consistency. Severe pathological changes were not recorded in this group and compared to their Fijian counterparts, they were in a good state of health. There also appeared to be no focus of infection i.e. diseased starfish were not confined to one particular reef or area. The answer to the question as to whether starfish from Helix, Keeper and Wheeler Reefs are infected with the same organism as Fijian animals (MST Report, 1987) requires further work.

It is important to note that animals such as A. planci have limited means of expressing pathological change because of their low evolutionary position. Degrees of vacuolation and necrosis and a type of phagocytosis seem to be all that the animal is capable of.

Examination of starfish treated with copper sulphate and slaked lime (Grubb Reef, 1986) has produced some interesting results. Copper sulphate is by far the most effective, killing in 2-3 hours, whereas slaked lime can take several days (depending on the dose).

After 30 minutes post injection with copper sulphate, degenerative changes can be seen in the muscle and nervous tissue (Fig. 5) at the base of the tube feet. Vaculative changes (non-specific) also begin to appear in the pyloric caecae as well as fragmentation up to an hour after injection (Fig. 6). Nuclei in the gut epithelium become rounded and lose their staining intensity. The coelomic epithelium becomes swollen and separates from the ciliated, squamous epithelium of the pyloric caecae. After 90 minutes severe disintegration of the pyloric caecae and musculature of the tube feet occurred (Figs 7, 8), as well as vacuolative changes in the skin and dermis. The chemical kills the animal by destroying first its form of locomotion, then the gut and finally the integrity of the skin is breached.

In contrast, slaked lime progressively burns a hole through the skin and internal organs and tissues.

iii) Statistical analysis of field cases

The, as yet, unidentified parasite seen in starfish from Fiji and the Great Barrier Reef is being counted (10 fields per tissue per case) to see whether there is any correlation between the presence of these organisms and pathological change. Pathology records indicate that this should be so. Also included in the data set are details of geographical location (reef), time of year (season) age and histological techniques used.

iv) Histology

Although many studies have been conducted on the Crown of Thorns starfish, a detailed description of the normal histology of the animal has not been published. This is essential for the meaningful interpretation of pathological change.

A. planci is advanced in the areas of reproduction and digestion, but has a primitive heart (axial gland) and rudimentary nervous system. Locomotion depends on smooth muscle action alone and the skin is a single layer of epithelium. Structural components and cell types have so far been identified in the skin, cardiac and pyloric stomach, pyloric caecae, gonads and tube feet. Work is continuing on the madriporites, stone canal, otic capsule and axial gland.

Light microscope studies are being augmented with micrographs from the transmission electron microscope.

v) Electron microscopy of material from Fiji

One of the major aims of the trip to Fiji in January 1987 was to obtain "fresh" material from diseased starfish for electron microscopy.

Methods

1. Samples were taken from the skin, pyloric caecae, cardiac and pyloric stomachs of 3 starfish. Tissues adjacent to gross lesions were preferred.

2. Pieces of tissue (2 x 2 mm) were fixed in 2.5% glutaraldehyde for 1.5-2 hours at room temperature, then

3. rinsed in filtered seawater and

4. stored in 50% cacodilate buffer/50% filtered seawater for transport back to Australia.

Results

TEM magnifications of up to x50,000 showed only ovoid, grey, amorphous bodies which could have been mucus secreting (goblet) cells or a type of fat deposit (lipid). Special stains have been applied to try to obtain more information about the nature of these bodies viz. nucleic acid stains and frozen sections for lipids.

The best photographs have been sent to Dr M. Jangoux (an authority on echinoid disease) for his opinion. A recent letter confirmed the presence of sporozoans in acutely infected juveniles from Fiji.

vi) Attempts at cell culture

Four attempts have been made to establish a primary cell culture from the organs and tissues of A. planci (March and November 1986, June and July 1987). The animals had been collected from Keeper, Helix, Wheeler and John Brewer Reefs as part of the University's disease monitoring program on the Great Barrier Reef. All were free of gross (external) lesions and appeared healthy. Three age groups were selected viz. juveniles, sub-adults and adults. Starfish were kept overnight in seawater with adequate aeration.

Methods

1. Prior to dissection of the starfish, the spines were removed, and the skin cleaned with 70% ethanol and rinsed with sterile seawater.

2. Organs and tissues viz. SKIN, AXIAL GLAND and GONAD were removed using aseptic techniques and placed in a sterile petri dish (for explants) or culture medium containing X4 antibiotics with no foetal calf serum (for 1 hour at room temperature) (trypsinisation).

3. Explants were prepared by sectioning material into pieces 1 mm in diameter. Transfer to a culture vessel was carried out in a sterile hood using suction from a sterile Pasteur pipette and teat. They were attached to the scratched bottom surface of the flask and media containing 30% foetal calf serum added.

4. Trypsinisation - After immersion in basal medium for an hour, tissues were macerated using sterile scalpel blades and resuspended in ATU (active ingredient trypsin, a pig pancreatic enzyme). The cell/tissue suspension was agitated for varying periods of time using a sterile magnetic stirrer (at room temperature and 4°C). Centrifugation at 2000 rpm for 5-10 minutes followed and the resultant pellet was washed in basal medium before seeding the culture vessel. As is normal practice for 1° cell cultures, 30% foetal calf serum was incorporated into the medium.

5. 27°C was chosen as a suitable incubation temperature, being intermediate between maximum and minimum sea temperatures.

6. Newly established cultures were examined daily under the inverted microscope and kept for at least a month.

7. A change in pH from alkaline (7.3-7.4) to acid indicated active cell metabolism and the medium was changed. Three media viz. TL125, T199 and TMEM which were modifications of mammalian media (taurine added) were used.

The skin was difficult to remove aseptically, particularly when it was separated (by dissection) from the underlying network of fibrous tissue (dermis). The same applied to the axial gland, which lies adjacent to the stone canal below the madreporite.

Common contaminants included marine fungi and yeasts (Rhodoturula spp) and when these were overcome by increasing the concentration of fungizone, a protozoan (possibly of the Family Thraustochytrium) became dominant. Tissue separation techniques have so far failed to eradicate this organism.

Other difficulties included poor adherence of tissue expaltns from Crown of Thorns to the bottom of the culture vessel.

Summary and Conclusion:

Fibroblastic cell lines from echinoids have been successfully established overseas (Quiot and Rammou 1972). It was encouraging to observe outgrowths from skin explants (Medium T199).

The protozoan presently contaminating primary cell cultures could prove to be a major obstacle to the establishment of a cell line. Protozoa of the Phylum Labyrinthomorpha; Order Labrinthulida are described in Levine et al (1980) as being saprobic and parasitic on algae in marine and estuarine waters. To be absolutely sure of their identity, a unique cell-surface organelle (sagenogenetosome) needs to be found under the electron microscope. A Chief Investigator (J.S. Glazebrook) plans to visit the Virginia Institute of Marine Science in the United States in September this year to discuss with Dr L. Ellis ways of overcoming the same problem. The same organism has been frustrating overseas attempts to establish a coral cell line.

Forthcoming Publications:

a) Zann, L., Glazebrook, J.S. and Brodie, J. (in preparation). A disease epizootic in juvenile Crown of Thorns in Fiji.

b) Glazebrook, J.S., Owens, L. and Zann, L. (in preparation). Pathology associated with the chemical control of Crown of Thorns on the Great Barrier Reef.

c) Glazebrook, J.S., Campbell, R.S.F. and others. Crown of Thorns Starfish: An Atlas of Histology.

TABLE 1 Crown of Thorns starfish examined for signs of disease.

ORIGIN	GREAT BARRIER REEF						SUVA RE FIJI
	HELIX	KEEPER	WHEELER	JOHN BREWER	PITH	GRUBB	STANLEY	
Date of Collection	April 86 Aug. 86 July 87	April 85 June 85 Feb. 86 Aug. 86 June 87	April 86 Aug. 86 Oct. 86 June 87	Nov. 86 June 87	Feb. 86	July 86	Aug. 86	April 86 Jan. 87
No./age	1 J 3 SA 14 A	3 J 9 SA 20 A	1 J 19 A	1 J 7 A	3 A	3 A	5 J	18 J 3 A

J = juvenile
SA = sub adult
A = adult

One adult was also examined from the School of Biological Sciences aquarium at James Cook University in March 1984 and 8 juveniles from the University of the South Pacific aquarium in November 1984.

Total of 120 animals

TABLE 2 Summarizes the results obtained so far. (cell culture)

ORIGIN	AGE	ORGAN/ TISSUE	MEDIUM USED	OBSERVATIONS
Helix Reef March 1986	JUV.	SKIN	TL15	Explants - no growth up to 3 weeks then contamination Trypsinized cultures - small, rounded cells which failed to attach.
John Brewer Reef November 1986	JUV. ADULT	SKIN	TL15	as above
Helix and Wheeler Reefs June - July 1987	ADULT	SKIN AXIAL GLAND GONAD	TL15 TMEM T199	Explants - fibroblastic outgrowths from skin/T199 culture; others - no growth or contaminated. Trypsinized cultures - contaminated with protozoan (F. Thraustochytrium)

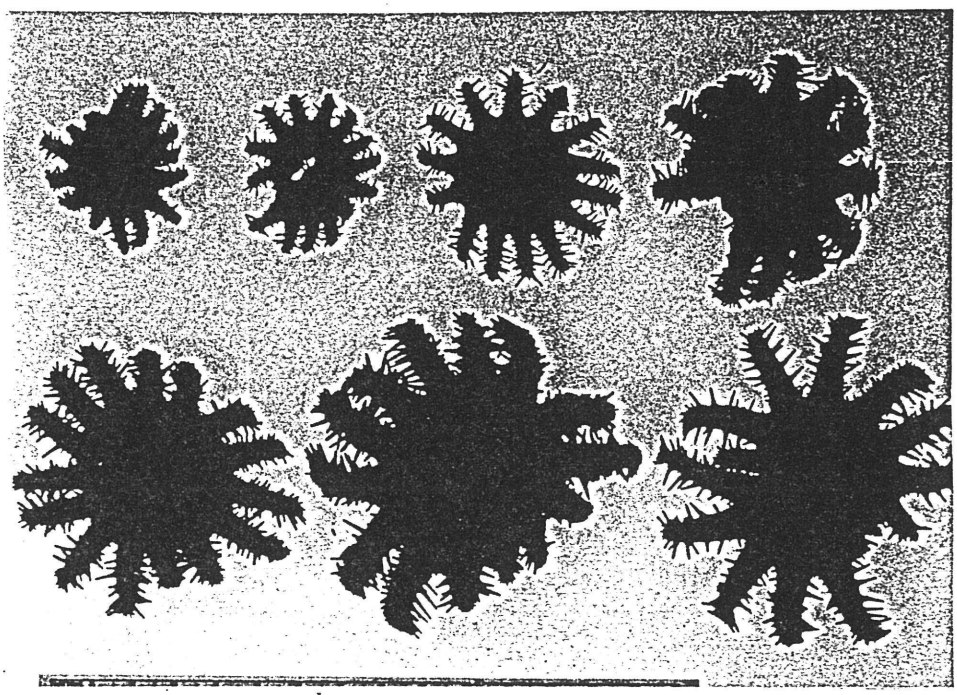

Fig. 1 Juvenile starfish from Suva Reef, Fiji showing perforation of the central disc area and missing arms. November, 1984. Collector: L. Zann.

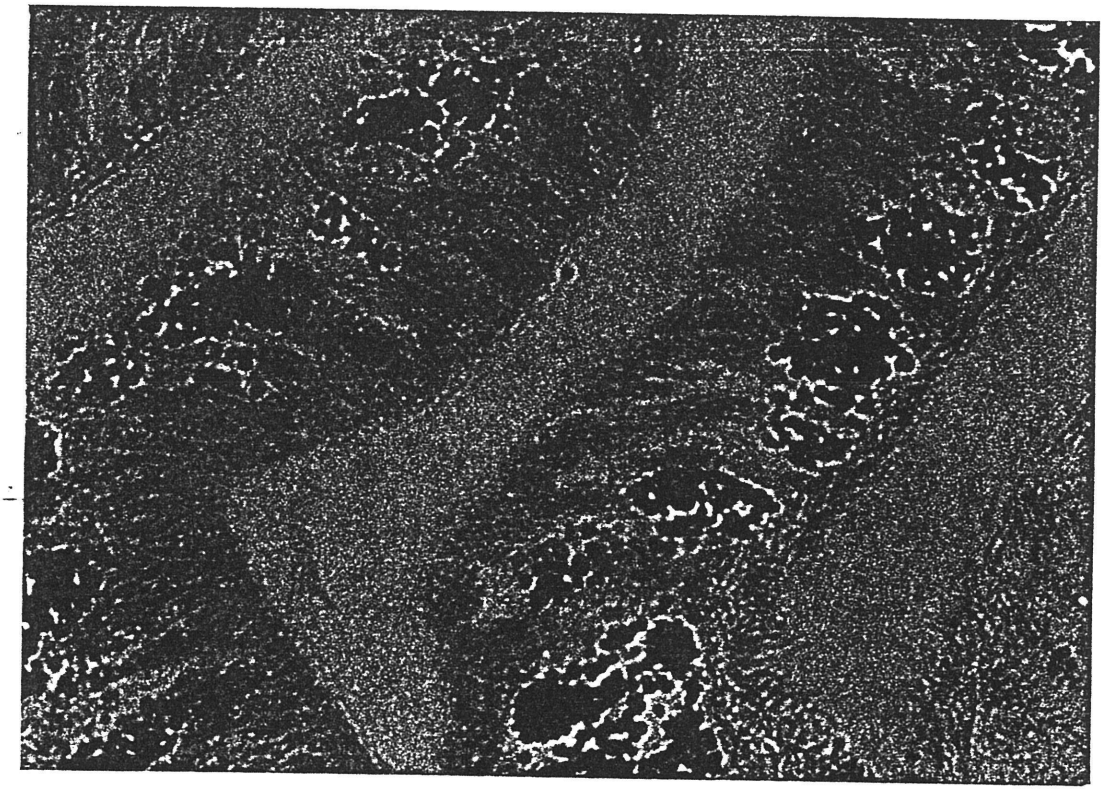

Fig. 2 Intracellular parasites (possibly sporozoans) in the pyloric caecae of juvenile A. planci from Fiji.

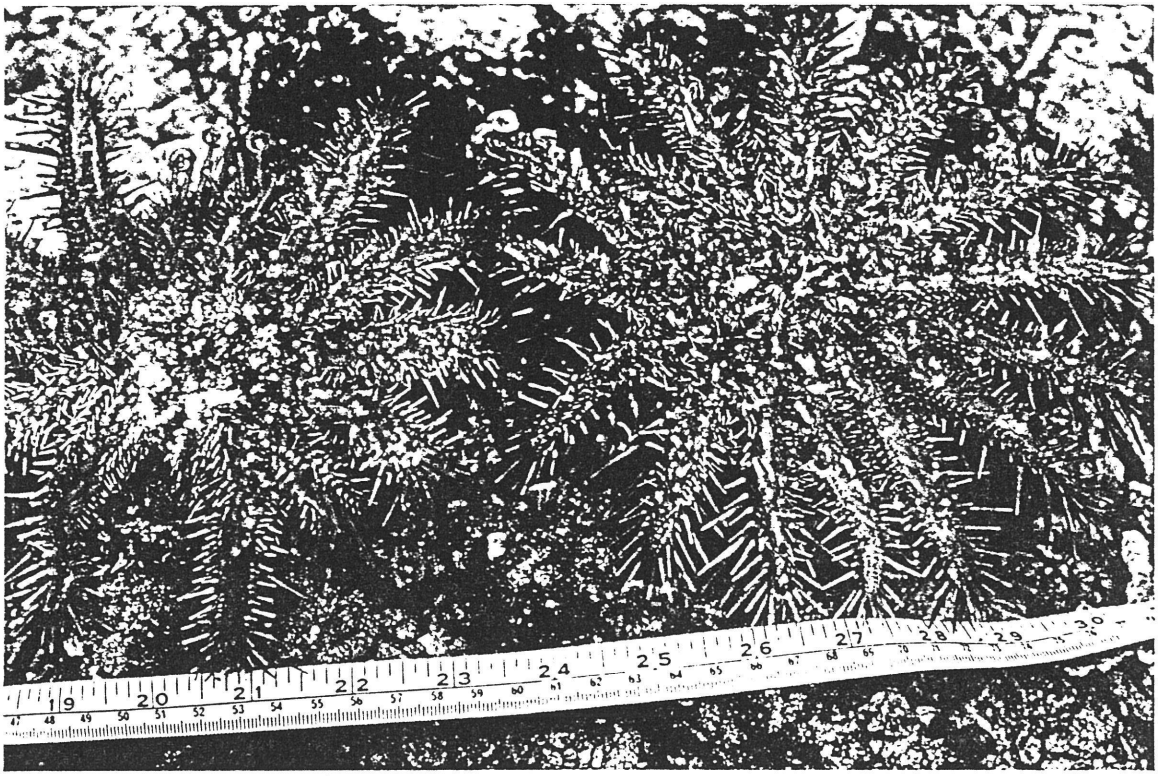

Fig. 3 Diseased and healthy starfish on Suva Reef in January, 1987.
NOTE: severe erosion of the cardiac stomach and reduced mobility of the tube feet in the diseased animal.

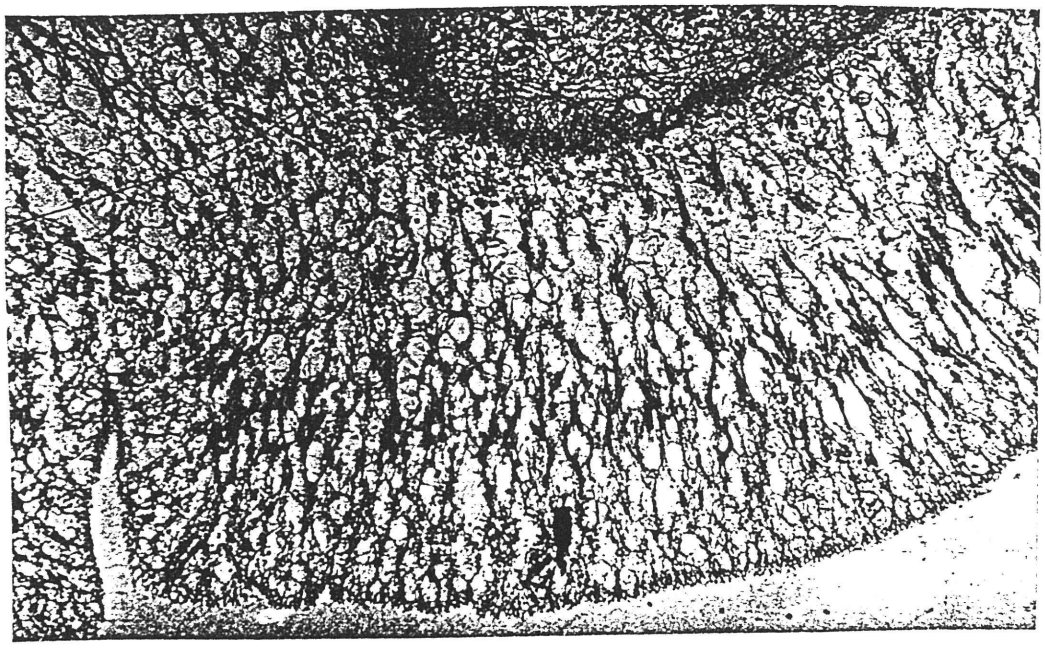

Fig. 4 Low grade (chronic) infection of parasites in the pyloric caecae of an adult starfish from Suva Reef. January, 1987.

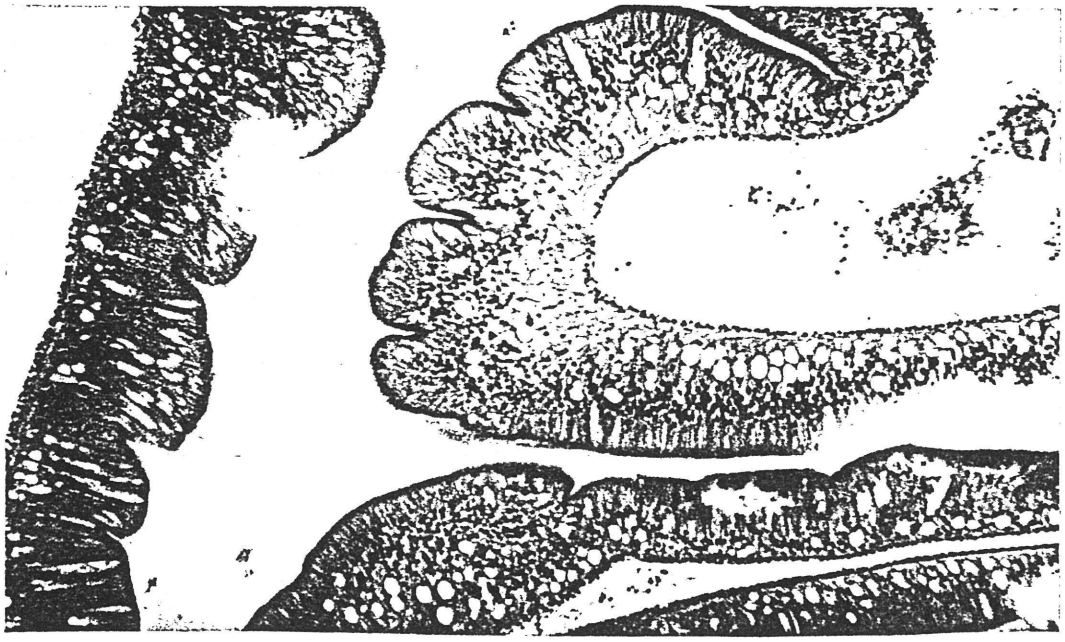

Fig. 5 Non-specific vacuolation of the pyloric caecae. 30 minutes post-injection

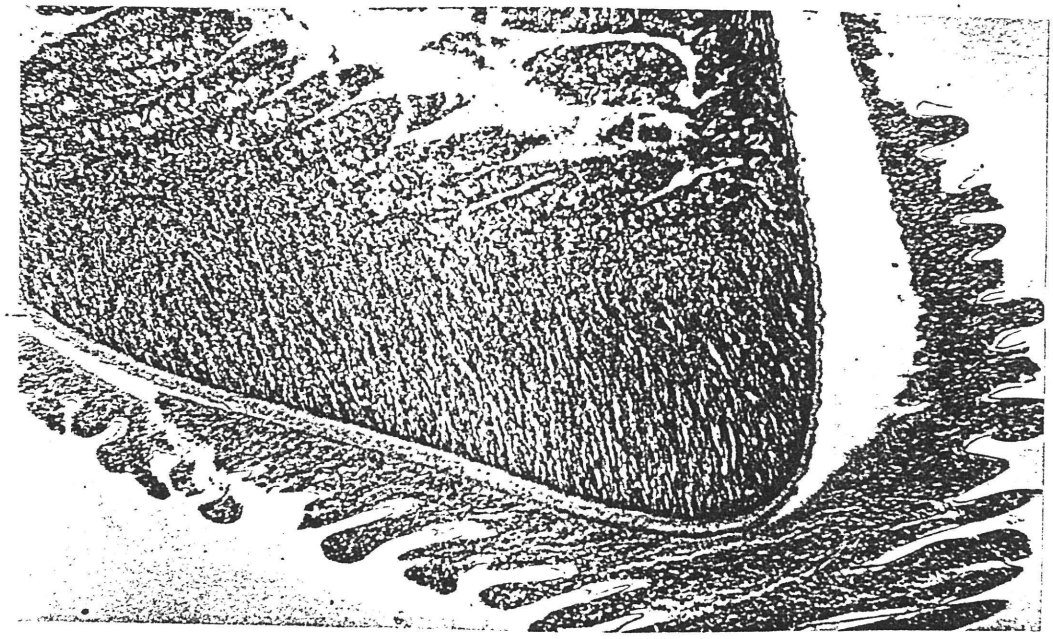

Fig. 6 Vacuolation of the tube feet musculature (internally). 30 minutes. P.I.

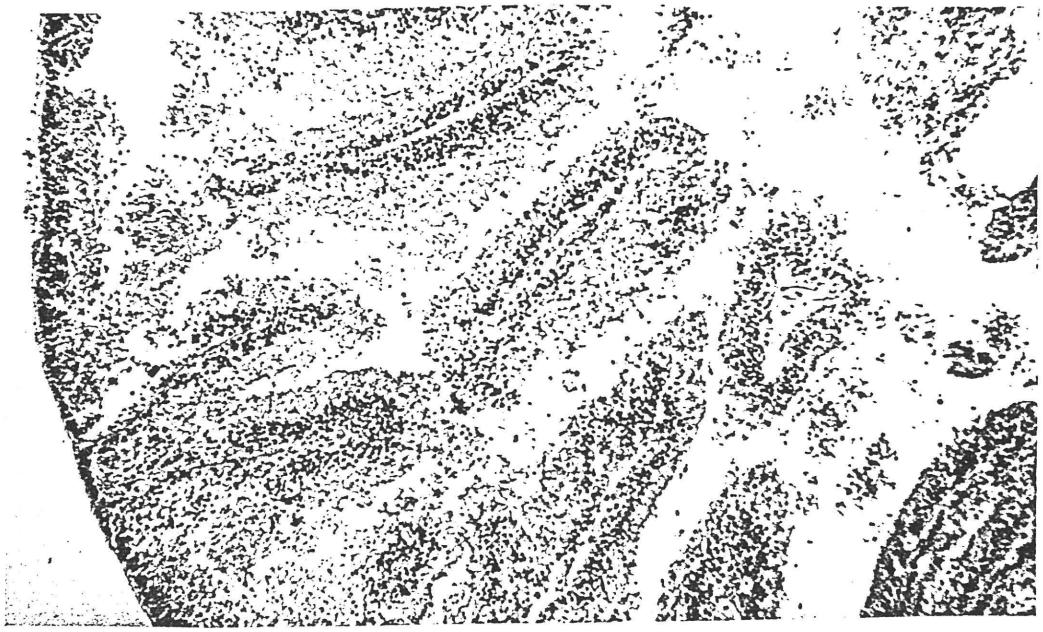

Fig. 7 Disintegration of the pyloric caecae and shedding of the coelomic epithelium. 90 minutes P.I.

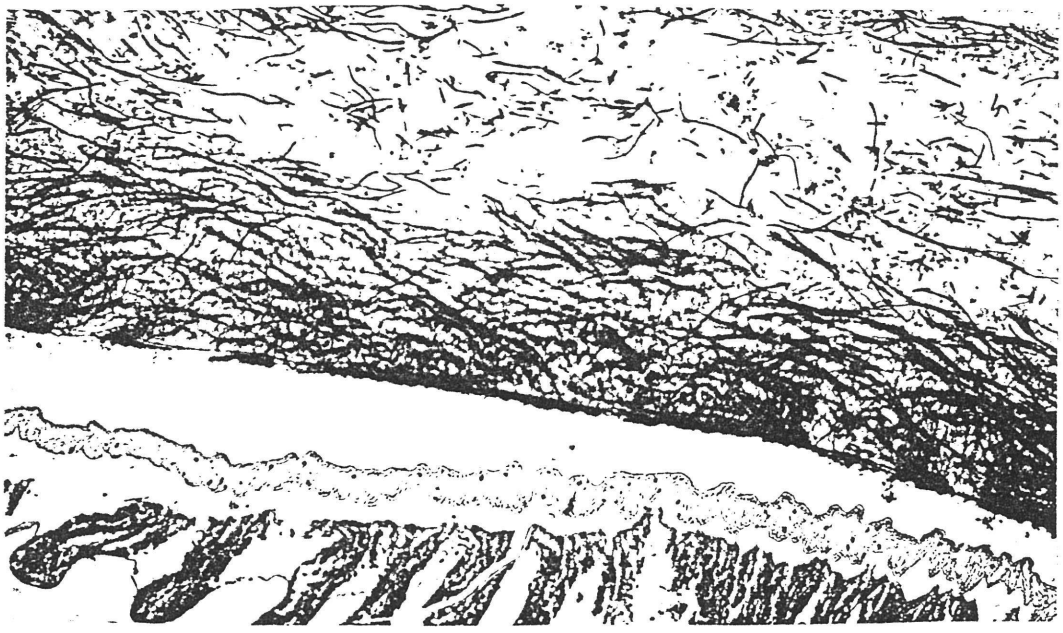

Fig. 8 Severe degeneration of the tube feet musculature with separation of individual bundles and fibres. 90 minutes P.I.