BI418-AQUACULTURE

PRACTICAL PROJECT

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AN INVESTIGATION INTO THE BIOLOGY AND SPAWNING MATURITY OF ANADARA SPP. (BIVALVIA : ARCIDAE) IN FIJI.

ABSTRACT : The anatomy and histology of the Fijian Anadara mollusc, are described. Its structure is similar to other lamellibranchs of the family Arcidae. The gender determination of live specimens was deemed impossible, due to the inaccessibility of the gonads. Peculiar white patches were found on the mantle of all specimens, suggesting a reaction to disease or pollution which warrants further investigation.

INTRODUCTION :

The bivalves of the genus Anadara are a marine group, and belong to the family Arcidae, subfamily Anadarinae.They are an important source of protein in many tropical, subtropical and warm temperate areas. In Fiji, the most common species is A. antiquata (Lin.), followed by the second most-common species A. subcrenata (Lischke) and lastly A. inequivalvis (Brugiere).

They are epibenthic bivalves, showing some primitive morphological features (eg. external chevron ligament, straight taxodont hinge, unfused mantle margins, and byssus). The family is also peculiar in possessing the red blood pigment haemoglobin. The fossil record shows that the first few species appeared in the Oligocene, but by the Miocene many, widely distributed Anadara spp were present. Today, there are about 60 living species, mostly tropical and sub-tropical. They inhabit inter-tidal mudflats and sub-tidal areas, seaward of mangrove forests.

Nothing much is known about recruitment of Anadara spp. in Fiji, but Maybin (1989) showed that apparently some part of the population is mature throughout the year, with a definite peak in maturity in December (the hot and rainy season).

It has been suggested (Fiji MAF, 1982) that Anadara spp could be a potential organism for aquaculture in the Fiji Islands. In this respect, a knowledge of the spawning periodicity, species composition and distribution and growth rates is essential in order to assess the viability of the mariculture of these molluscs. The subject of this present investigation is to throw some light on the reproductive biology and population structure of Anadara in Fiji, as well as to find a way, if possible, of determining the sex of live animals with minimum damage, which would be of obvious advantage if mariculture of these organisms is envisaged. Also, the nature of certain peculiar patches that have recently appeared in certain Anadara populations is looked into, especially so as to determine any possible disease and/or pathological reactions in present bivalve populations.

MATERIALS AND METHODS :

At the outset of this project, it was proposed to make an aim of identifying the sex of live Anadara specimens with a novel approach, which would consist of of attempting to drill a hole of suitable size at the right location through the shell of the animal, so as to enable extraction of gonadial samples via an hypodermic needle for further investigation of its content and maturity stage.

For this purpose, it was envisaged to procure a suitable drill (eg. an electronic printed-circuit board drill, in default of a much more expensive dentist's drill) and to carry out dissections on a few specimens so as to determine the exact location of the gonads in respect to the shell. It is impossible to determine the gender of these molluscs from external characteristics, as sexual dimorphism is only apparent in the content and color of the gonads, being pink for ovarian tissue and whitish for testes (Maybin, 1989).

About 50 Anadara antiquata specimens were bought from the Suva market, and kept in an aquarium supplied with mudflat silt. Preliminary investigations into the general morphology of the molluscs were carried out, and dissections revealed that the internal anatomy of Anadara closely ressembles that of other lamellibranchs of the family Arcidae. Results and drawings of the dissections are to be found in Appendix A.

These dissections revealed that the gonads were far less accessible than previously thought, being intimately associated with the foot and digestive gland, and located dorsally and closely apposed to the shell hinge. Hence, it was deemed not practically feasible to sample gonadial tissue via drilled holes, since there was too much risk of damaging adjacent vital organs in the process. Consequently, this phase of the investigation was abandoned, and the project took a different course.

During the initial dissections, it was noticed that nearly all of the specimens investigated had peculiar white patches on the mantle edges, particularly at the posterior end. These superficially looked like calcareous incrustations of some kind, and were apparently not present in Anadara populations looked at a few years ago (Maybin, personal com.). The mysterious aspect of this phenomenon induced some research into the nature and possible origin of these patches, as well as any implications for the potential culture and consumption of these molluscs.

The third phase of this investigation, which ran concurrently with the second, consisted in carrying out histological analysis of the gonads, in order to determine the reproductive maturity of the animals.

The method used was to fix selected gonad tissue into

Bouin's fluid for a set time, followed by progressive dehydration in graded ethanols. Xylene clearing followed, after which the tissues were embedded in wax. Sections 11μm thick were obtained of the blocks using a rotary microtome, and the resulting slides were stained using eosin and haemotoxylin. A detailed fixing and staining schedule is to be found in Appendix B.

During the course of this investigation it was also decided to try a different staining technique, in order to highlight any stellate cells and associated granules in the tissues, as the above dyes do not stain these features (Sullivan, 1960). The distribution of stellate cells follows that of whitish mottling on the mantle of A. trapezia (Sullivan, 1960) and it was considered worthwhile to investigate whether this was also the case in A. antiquata.

To this effect, waxed tissue sections of the mantle and gonad were brought to water and stained with Gomori's aldehyde-fuchsin dye for elastic fibres (Drury et al, 1967). However, the desired effect was not obtained, and the slides remained blank. Possible reasons for the failure of this exercise are given in the discussion. A detailed account of the staining schedule is to be found in Appendix C. Photomicrographs of relevant slides were obtained using an Olympus photo-microscope.

RESULTS :

I. Mantle-tissue investigation :

A drawing of a section of so-called "patchy" mantle is shown in Fig.1, as well as the accompanying photomicrographs. Portions where the patches were present appear darker-staining, due to the presence of numerous, small cells. Teased-out "patch" material, viewed through a phase-contrast microscope, revealed what looked like long "strands" of non-descript material, with numerous black "granules" embedded in it. It could not be ascertained whether these were part of the mantle epithelium or foreign bodies, but their structure was evidently more complex than initially thought.

Anadara antiquata specimens collected from the mudflats opposite the Suva Pony Club turned out to also possess the peculiar white patches, albeit to a much lesser extent. Here the mantle was soft and smooth, in contrast to the Market specimens, where the incrustations were profuse on the posterior mantle edges, ctenidial suspensory arches and adjacent tissue, imparting a tough and apparently "calcareous" texture to these organs. However, the fact that these incrustations survived long periods of decalcification in Bouin's fluid suggest that they are not entirely calcareous in nature.

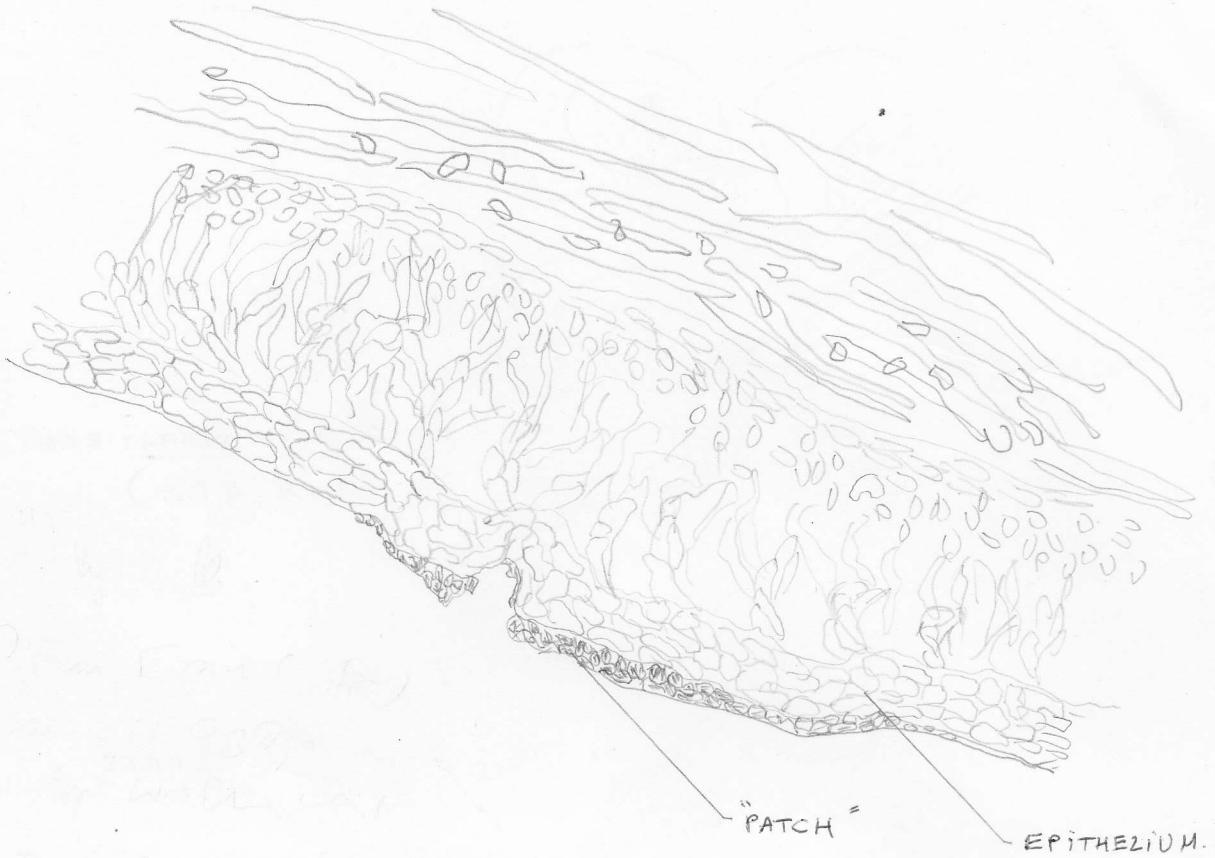

Fig. 1. ANADARA ANTIQUATA = "PATCHY" MANTLE
(× 400)

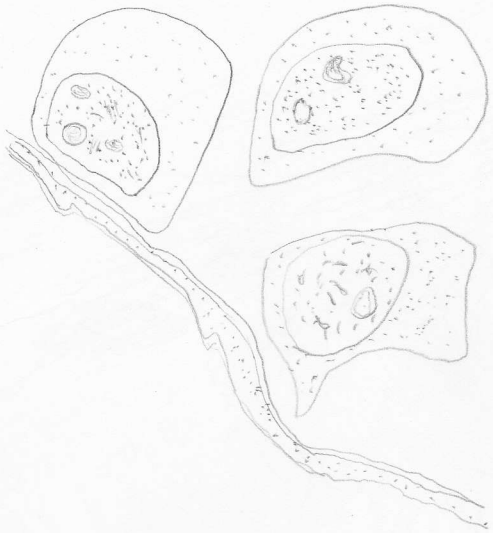

Fig. 2. A. ANTIQUATA : EGGS
(X 400)

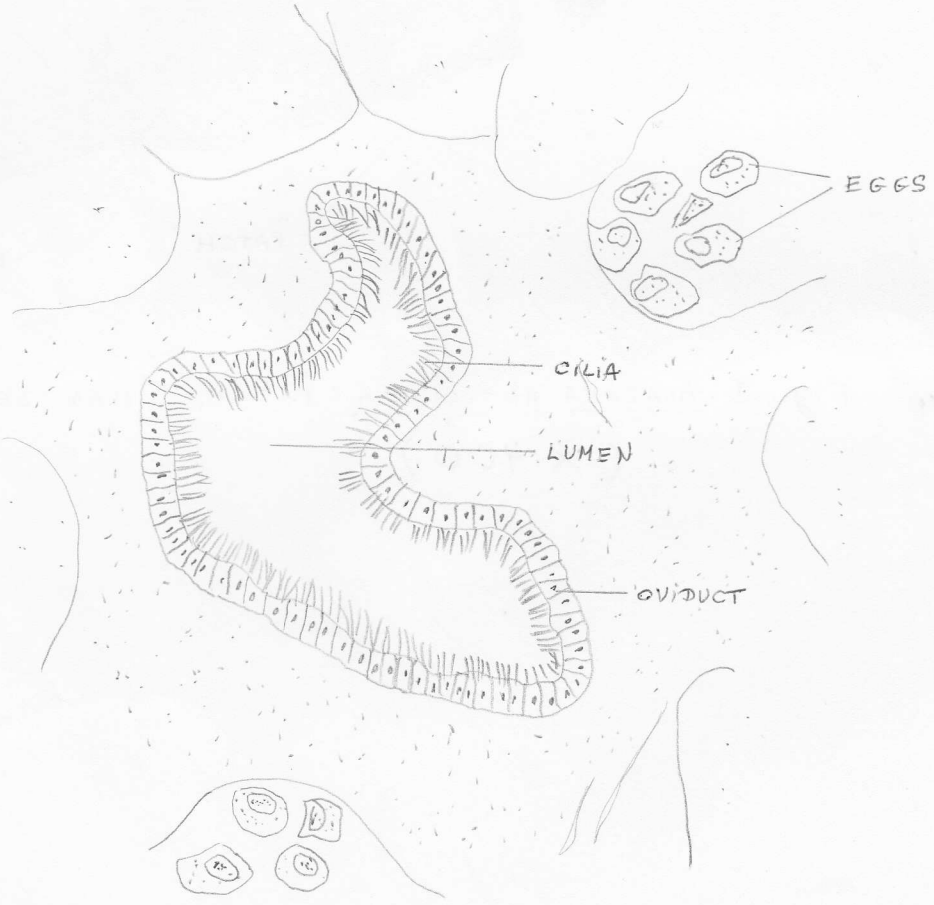

Fig. 3. A. ANTIQUATA : OVIDUCT AND EGGS
(X 100)

II. Gonad Histology :

A number of acceptable slides were obtained of both ovarian and testicular tissue, and these were looked-at under the microscope. Drawings of these can be found in Figs. 2 and 3, and the accompanying photomicrographs.

The ovarian tissue contained a large number of oocytes, and was very similar to the ovarian tissue of mature Anadara granosa specimens investigated by Broom (1983). The testicular tissue contained many spermatozoa, apparently at various meiotic stages. In addition, some ciliated stomach epithelium and a number of concave erythrocytes are visible. It was found that a variation of the treatment shown in Appendix B gave better-contrasted slides; this consisted in a brief immersion (4-5 sec) in Eosin following blueing in lithium carbonate, and prior to immersion in haemotoxylin. These slides are labelled "var".

DISCUSSION :

The peculiar aspect of the "patches" on the mantle is their apparent recent emergence, and this suggests that they are not an innate physical trait. The fact that these patches are more prominent on the posterior, light-exposed part of the mantle has some parallels with observations made by Sullivan (1960) on A. trapezia, where "peculiar translucent granules" were found in the connective tissue of the mantle border, the ctenidial arches, etc., being more concentrated on the posterior end of the animal. Their composition was not determined, and they were believed to be associated with the processes of the "stellate cells", peculiar branched cells peculiar to the molluscan phylum, and which remain colourless with haemotoxylin stain.

Sullivan (1960) also states that in fresh specimens, a whitish mottling of the mantle border is found, whose distribution corresponds to that of the translucent granules in the tissues. Atkins (1936, in Sullivan, 1960) considered that these patches are present on tissues that are exposed to light.

Whether the latter observations are relevant to the white patches observed in present Anadara populations is not certain, however it may be significant that their distribution is also in areas of the mantle most likely to be exposed to light. The actual significance of this observation is yet unknown.

The presence of numerous, small and dark-staining cells in "patchy" areas of the mantle epithelium are reminescent to some extent of "reaction tissue" found in other organisms, and hence brings up the question of whether these features are the result of disease, pathogens or irritant agents. If these were indeed pathological in origin, it would certainly be of an epidemic proportion, considering the fact that virtually 100% of the samples had profuse distributions of patches on them. Also,

the restriction to areas likely to be reached by light is not entirely explained by a pathological cause. A further alternative might be that they are caused by pollution of some sort, which triggers this peculiar (defensive?) reaction from the molluscs.

Sullivan (1960) states that in A. trapezia, the distribution of white patches on the mantle is concurent to the distribution of stellate cells and peculiar translucent granules in the mantle epithelium. The latter remain colourless with eosin and haemotoxylin, and stain red with Azan and purple with Gomori's aldehyde-fuchsin. However staining the A. antiquata tissues with this stain proved fruitless, and a possible reason could be that the basic fuchsin employed was not pure or not of the kind especially prepared for use with this and Schiff's reagent (Drury et al, 1967). Also, the fixation technique employed may have been inadequate (excessive decalcification in Bouin's) for this kind of material to survive. Had this effort proved successful, it would certainly have thrown some light into the nature of these white patches, and whether they are associated with the stellate cells.

Hence, it is proposed that further investigation is required into the nature of these patches, particularly to ascertain whether they are associated with the "granules" of stellate cells. To achieve this, sections of mantle tissues would need to be stained with Azan or Gomori's aldehyde-fuchsin, as recommended by Sullivan (1960), but using the appropriate basic fuchsin. If the latter association proves true, then perhaps research into the actual function of these granules and stellate cells could throw some light into the nature of these white patches.

In addition, a survey could be carried out of Anadara spp. in various sites around Fiji, to establish the occurence and distribution of these patches. An investigation into the level and types of any pollutants in the water and mollusc tissues could also be undertaken, so as to determine any possible correlation between pollution levels and patch occurence.

II. Gonad sections :

The close ressemblance of the gonad tissue to that of A. granosa (Broom, 1983) indicates that the reproductive pattern of both species is similar. It seems that the specimen in question was fairly mature, which was not immediately evident since the gonad was not particularly large. It was found upon dissection that gonad growth proceeds a fair bit into the foot and around the digestive gland, which makes it less obvious externally, and also complicates any attempt to sample the tissue on live specimens, as mentioned earlier.

Out of about 8 specimens dissected, only two were males, suggesting that the male to female ratio in this

population may not be 1:1, as reported for A. granosa (Broom, 1983). This fact should add further impetus for research into sex-determination of these molluscs, as the latter is essential if breeding of cockles in an aquaculture context is envisaged.

No attempt was made to induce spawning in the A. antiquata specimens, although the latter has been tried previously without success, mainly due to failure of indentifying the sex of the brood-stock (Maybin, pers. comm.). Broom (1985) recommends artificial induction of spawning in Anadara spp. by thermal shock techniques, or immersion of ripe females in a sperm-seawater suspension.

CONCLUSIONS AND FURTHER RESEARCH :

From this investigation, it is clear that the determination of the gender of live A. antiquata specimens is very difficult, due to the absence of external sexual dimorphism and the relative inaccessibility of the gonads. Since the sex-determination of the brood stock in an aquaculture venture is of crucial importance, further research into the sex-determination of these molluscs is warranted. The possible effects of disease or pollution, especially with respect to the peculiar white patches on the mantle of present Anadara population, needs looking into.

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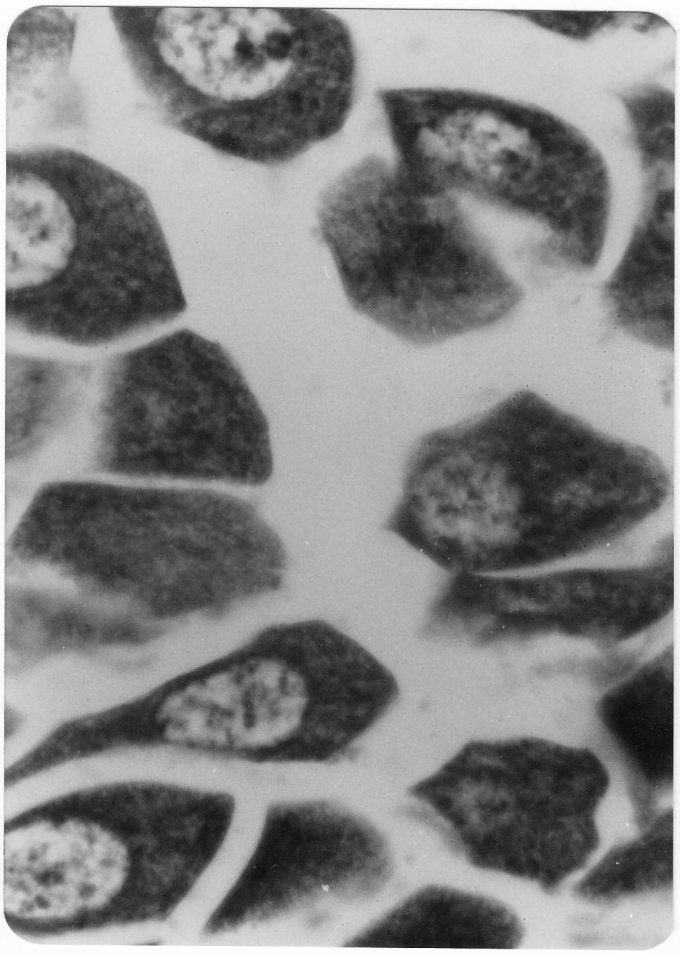
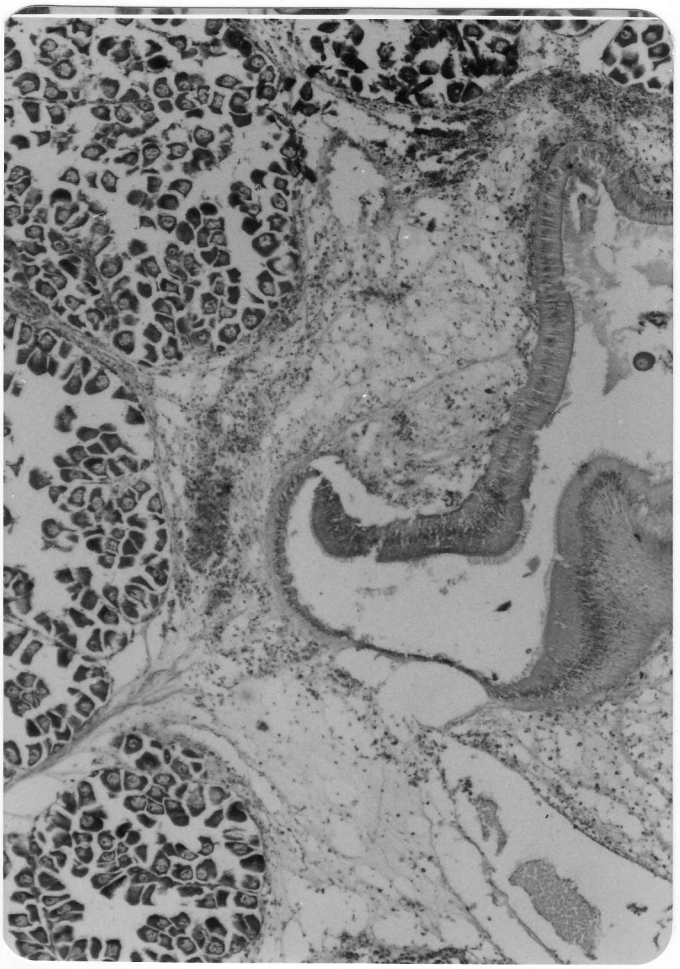
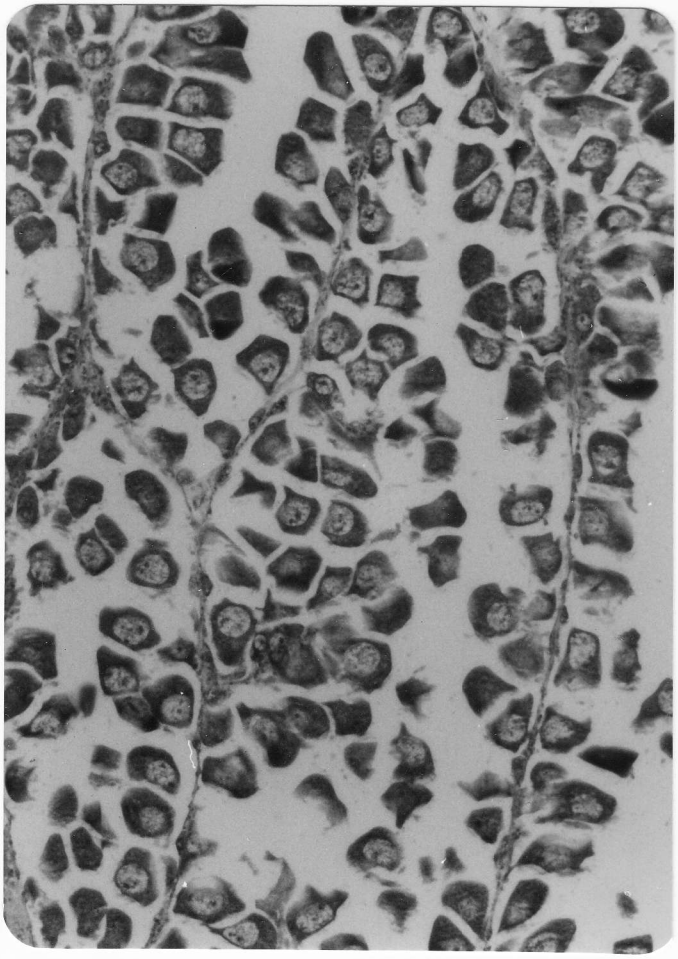
SULLIVAN, G.E. (1960). Functional morphology, micro-anatomy, and histology of the "Sydney Cockle", Anadara trapezia (Deshayes). Aust. j. zool., 9 : 219-256.



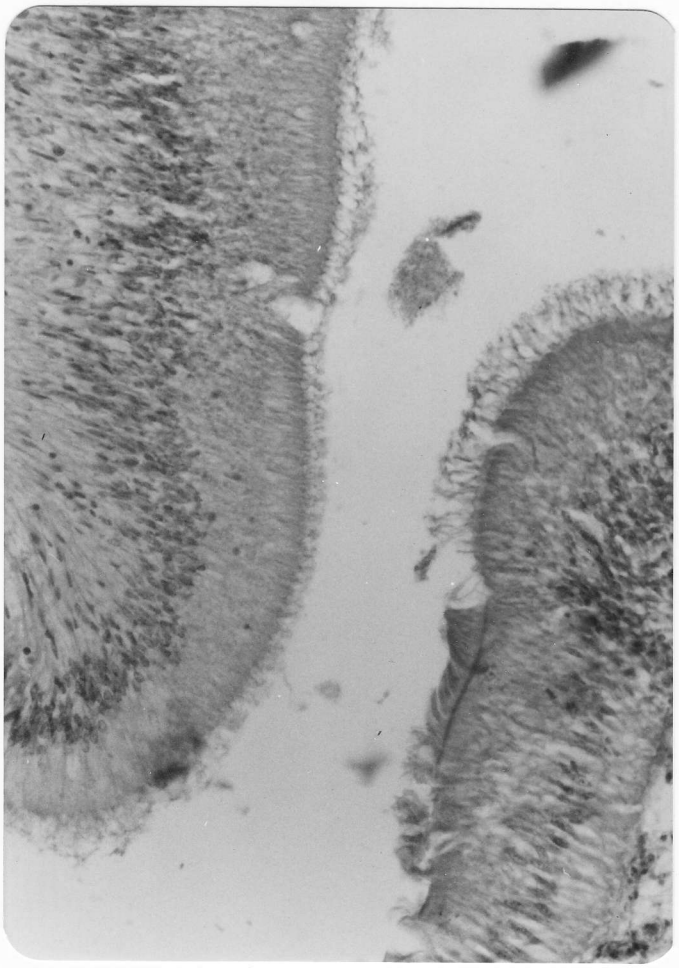
I. CLOSE-UP VIEW OF EGGS (×400).



II. OVARY + OVIDUCT (×40)

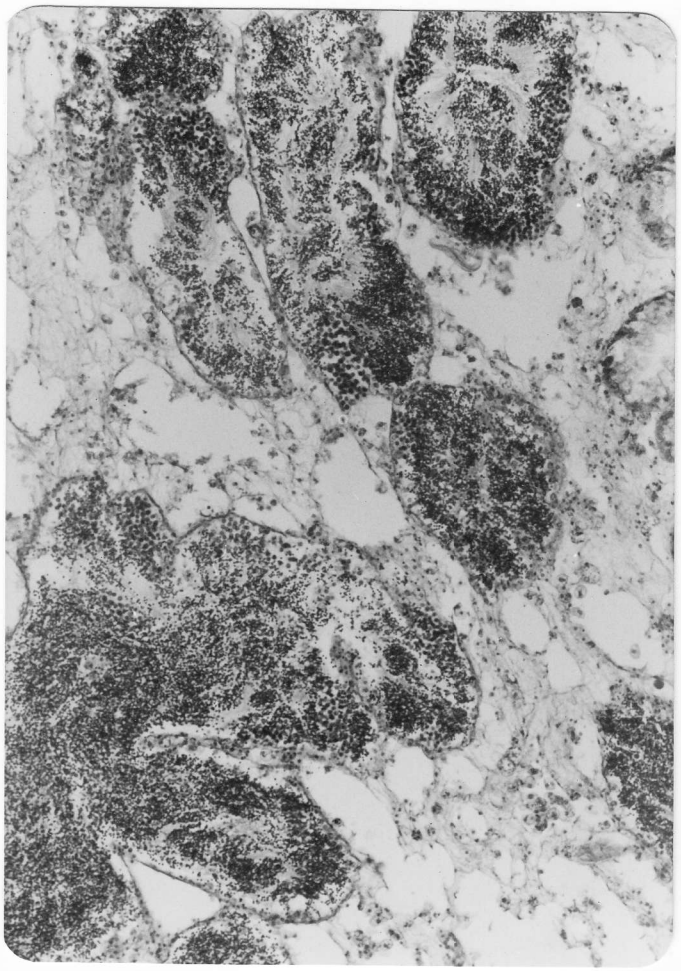


III. EGGS (×100)

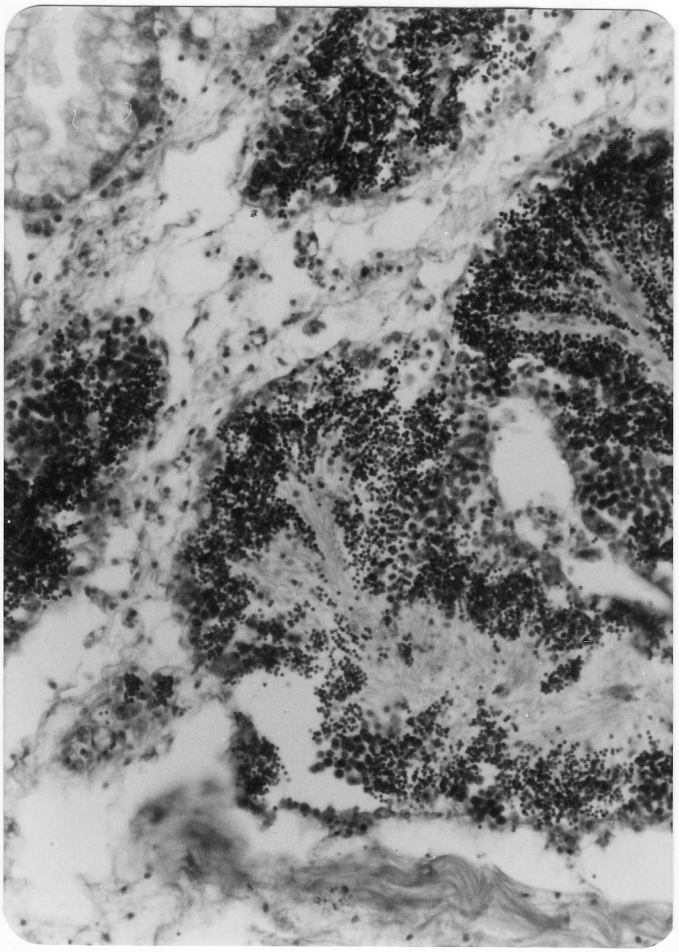


IV. CILIATED EPITHELIUM : OVIDUCT (×400)

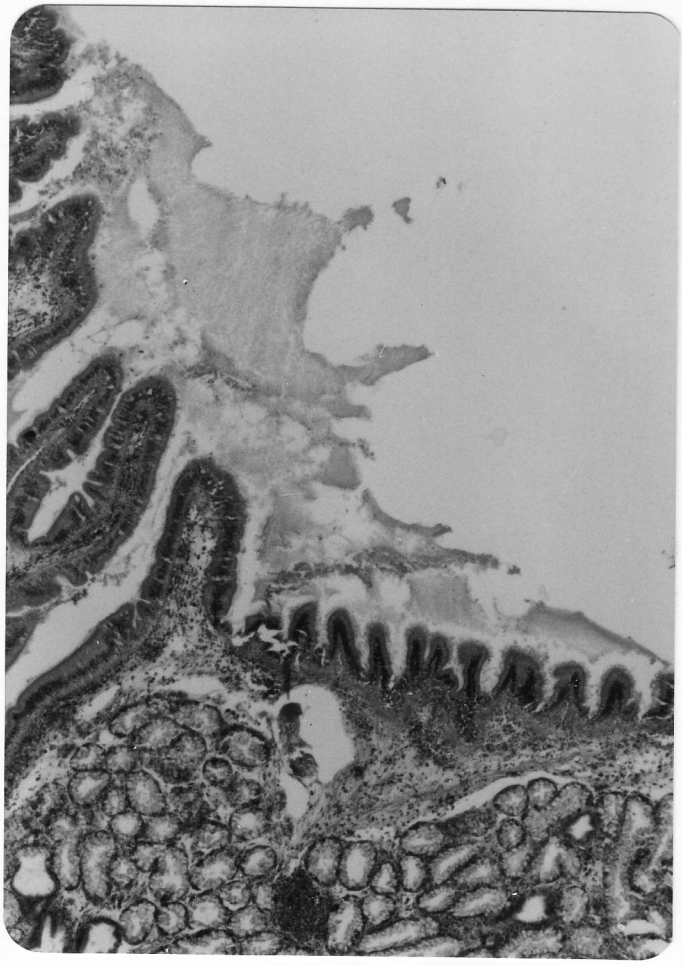
A. ANTIQUATA : PLATES



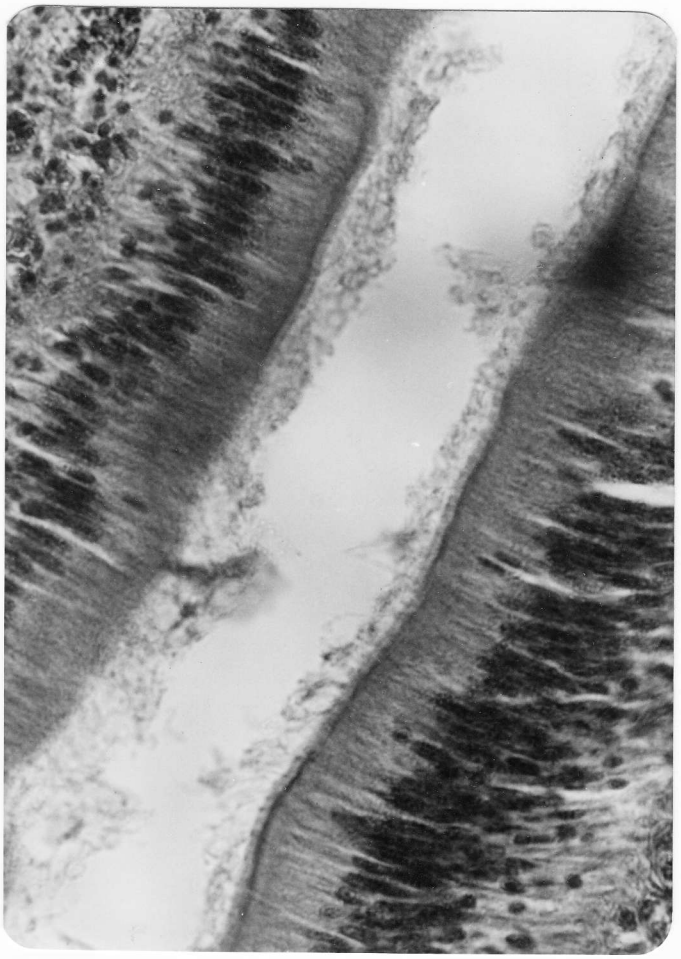
V. TESTICULAR TISSUE (X100)



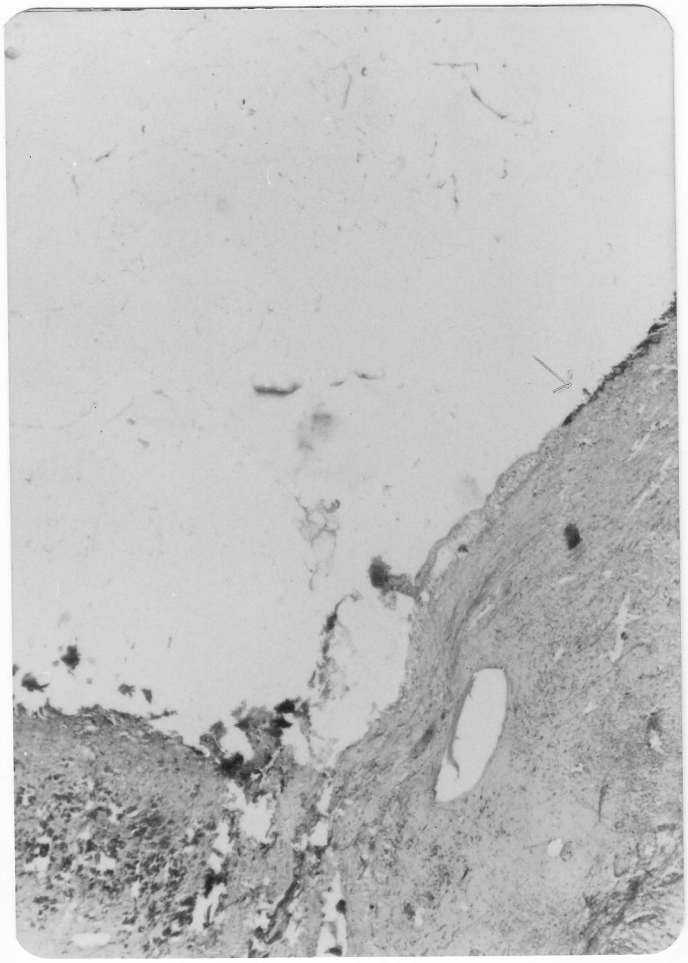
VI. SPERMATOZOA (X 200)



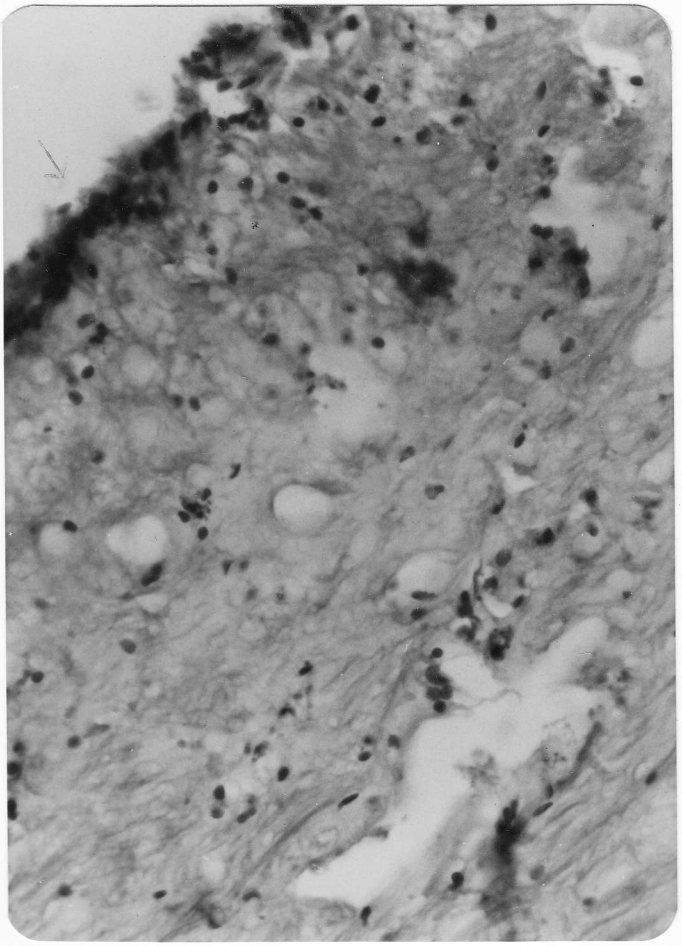
VII. DIGESTIVE GLAND + STOMACH LUMEN. (X40)



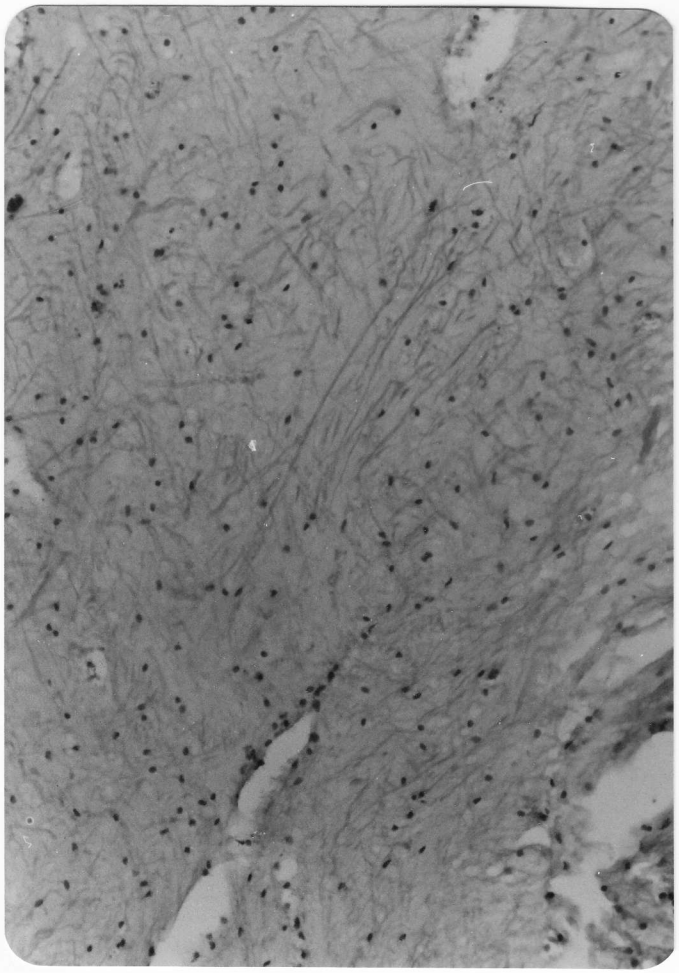
VIII. CILIATED EPITHELIUM : STOMACH. (X 400)



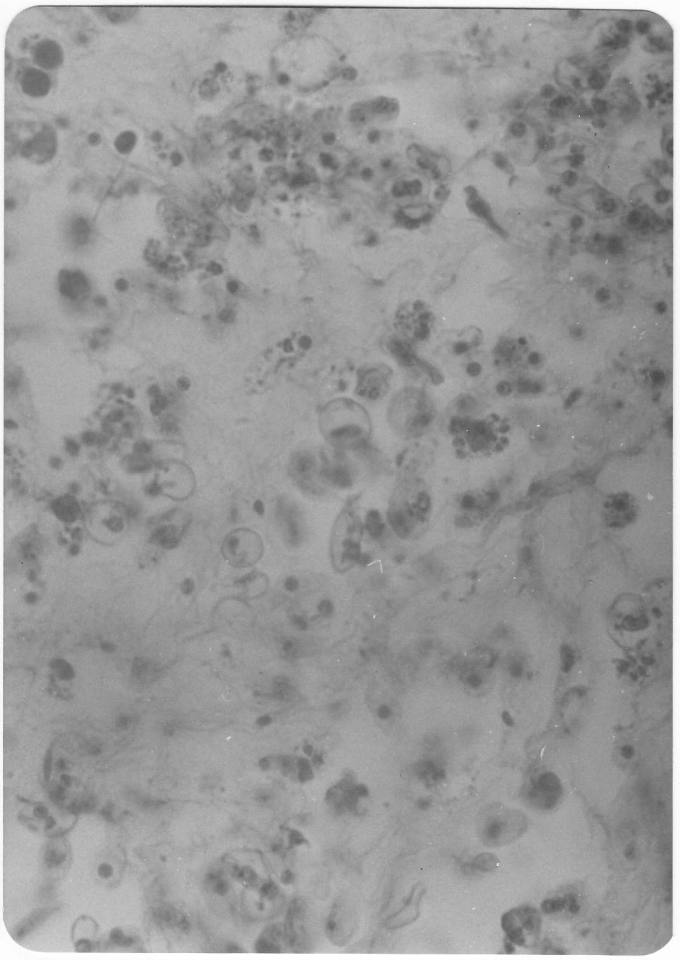
IX. MANTLE BORDER + "PATCH" (X 40)



X. CLOSE-UP VIEW: PATCH. (X 400).



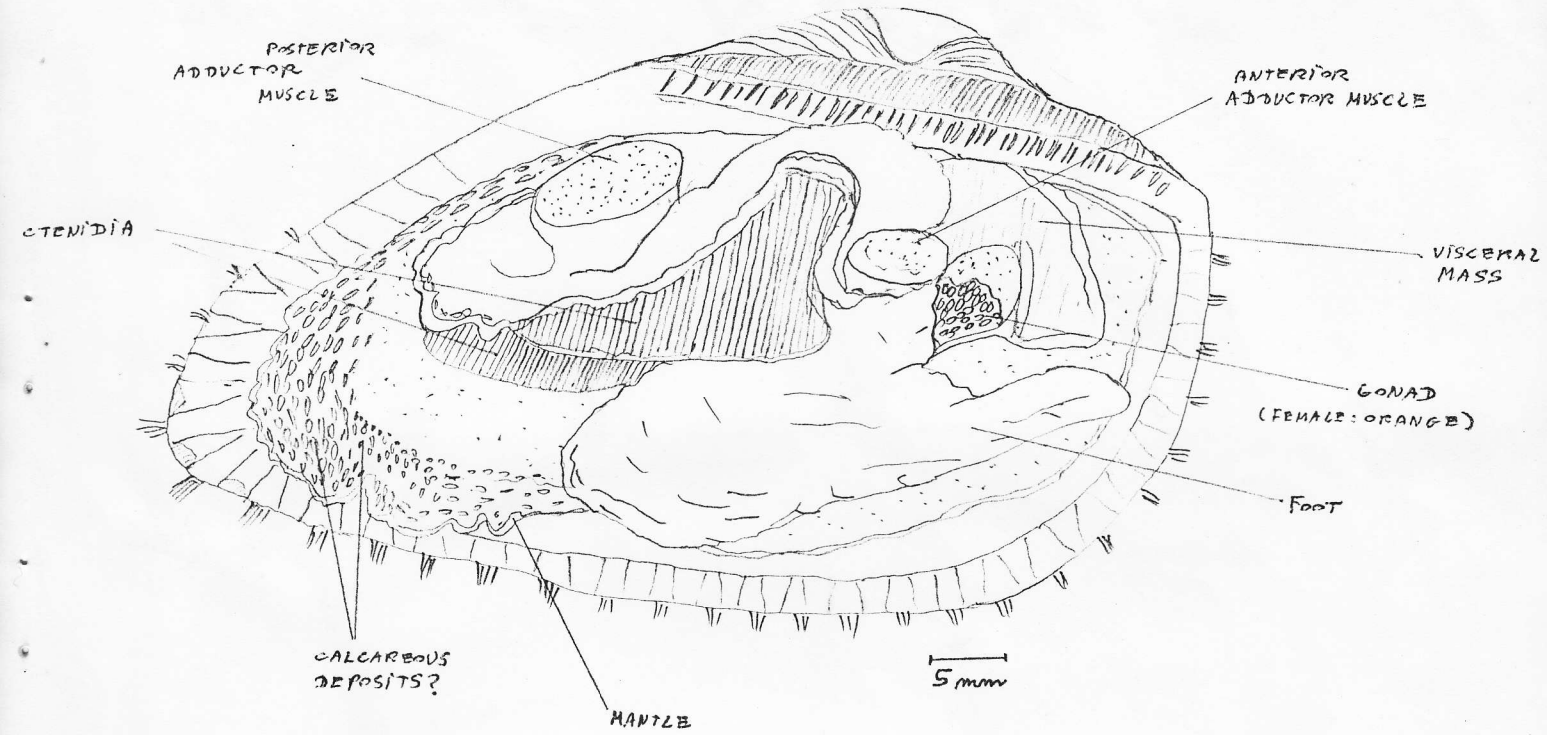
XI. CONNECTIVE TISSUE OF MANTLE. (X 400)



XII. RED BLOOD CELLS (GREEN FILTER, X 400).

APPENDIX A

Anadara antiquata : General Anatomy



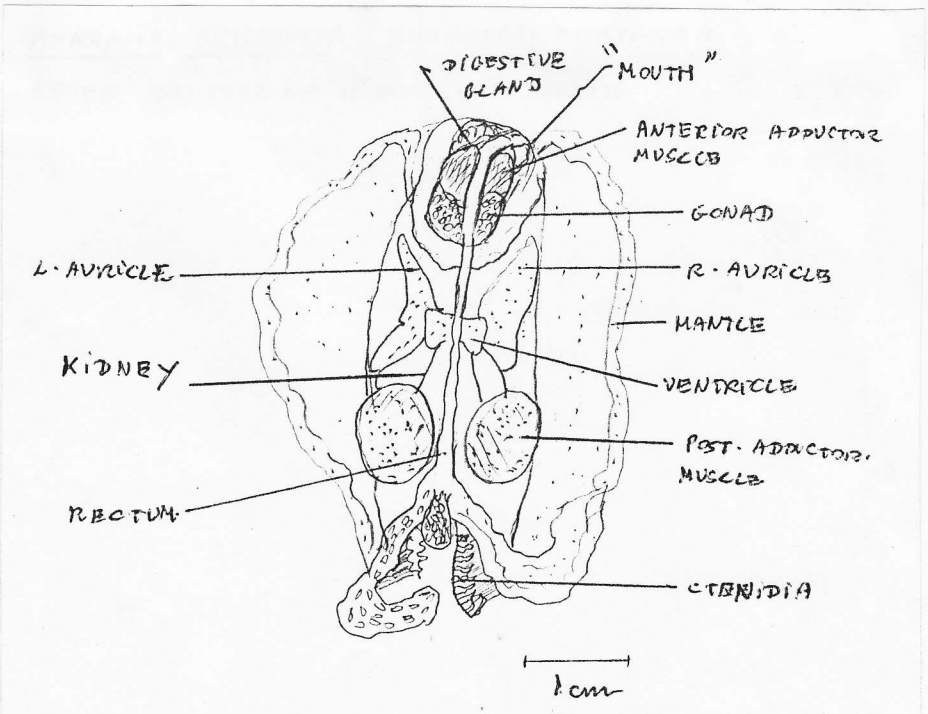


Fig 3. *Anadara antiquata*:
DORSAL VIEW OF VISCERAL MASS.

APPENDIX B

Fixing and staining schedule for the specimens :

I. Tissue Fixation :

SOLUTION	TIME IMMERSED
Bouin's Fluid	48h
10% ethanol	12h
20% ''	''
50% ''	''
75% ''	''
95% ''	4h
ABS ''	8h
Xylene	12h

II. Waxing :

The tissues were immersed in paraffin wax, and placed in a vacuum oven at 65C for 12h to remove the xylene.

After this liquid wax was poured into plastic moulds, and the tissues were placed in these.

Once set, the wax blocks were labelled and attached to wooden chucks for placement onto the microtome stage.

Sections were cut at 11μm, and floated on a warm water bath. Albumen-coated glass slides were used to pick up selected sections.

III. Staining :

SOLUTION	TIME IMMERSED
Xylene (removes wax)	2 min
ABS	1 ''
95% ethanol	''
75% ''	''
45% ''	''
20% ''	''
Distilled water	indefinite
Lithium Carbonate (removes picric acid)	dip
tap water wash	
Haemotoxylin (Chr.)	1 min
tap water wash	
Lithium Carbonate ("blueing")	dip
tap water wash	
Eosin	30 sec

The slides were then dehydrated in graded ethanols, cleared in xylene, and mounted with Canada Balsam.

APPENDIX C

Gomori's Aldehyde Fuchsin stain for elastic fibres :

I. Preparation :

Basic fuchsin (Schiff-compatible)	0.5g
70% ethanol	100ml
Conc. Hydrochloric acid	1ml
Paraldehyde	1ml

II. Method :

The dye is dissolved into the alcohol, and the acid and paraldehyde added. The mixture is left at room temperature for 12-72 h, or until a deep purple colouration is assumed.

III. Technique :

The sections are taken to water, and treated as per the following schedule :

SOLUTION	TIME IMMERSED
Lugol's iodine	10 min
tap water wash	
2.5% sodium thiosulphate	2 min
tap water wash	
70% ethanol wash	
Aldehyde fuchsin	10 min
95% ethanol wash	

The slides can be counterstained as desired.
Mounting should be done using a synthetic resin medium.