HISTOPATHOLOGICAL STUDIES OF SOFT PRAWNS

DISSERTATION SUBMITTED BY
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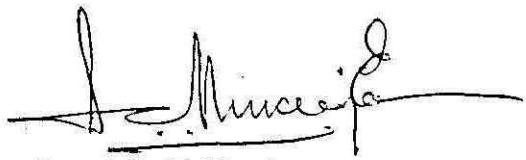
CERTIFICATE

This is to certify that this Dissertation is a bonafide record of the work done by Shri P.R. Remesh, under my supervision and that no part thereof has been presented before for any other degree.



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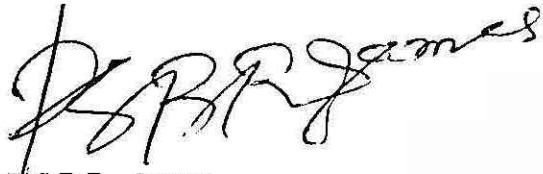

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PREFACE

Unconsciounable utilisation of the renewable marine resources like, marine animals which are of high economic potential, by man, caused a subsequent gradual decrease in the production from feral populations. This rendered development of an alternative to augment the production through aquaculture of these animals in order to meet the ever increasing demand. A tremendous development has been noticed in the field of aquaculture which provided technical achievements for better management of husbandry systems. For aquaculture to be commercially feasible, the economic requirements that maximum number of the individual utilize minimum quantities of space and water, must be realized. This always has been accompanied by the appearance of a variety of debilitating and serious 'disease' problems. The term disease as used here, is defined as "a definite morbid process having a characteristic train of symptoms; it may affect the whole body or any of its part and the etiology, pathology and prognosis may be known or unknown". (Dorland's Medical Dictionary).

Since crustacean losses to disease are one of the single most significant depressants of productivity in the husbandry of these animals, there has been immense progress in the investigation activities of these diseases of crustacean. According to Couch (1978), diseases are second only to predation and periodic physical catastrophies in limiting numbers of crustacean especially penaeid shrimps in nature and second only to nutritional and reproductive requirements in limiting aquacultural success with penaeid shrimps. Though a number of diseases of economically important crustacean

have been described, most of them are of undetermined etiology. Even in cases where etiology is known, the host-agent-environmental interaction is incomplete for many of them. Besides the instinct pathogenicity of the agent, the effect of environmental stressors also play important roles. In such cases, it is very difficult to assess the instigators of the affliction and frequently become apparent only after considerable resource and efforts have been expended in management activities. Same is the case with the "soft-shell syndrome" affecting commercially more important species of penaeids which is a serious threat to the confined population along the culture farms of South India, and is an important limiting factor of the production of penaeid prawns.

Historically, the data pertaining to this syndrome is much inadequate. Although the knowledge of some aspects of the disease has gradually increased, information on the histopathological characterisation of soft prawn syndrome is hardly observed in the literature available. In this perspective, the present study has been taken up to demote the paucity of aquaintance with this critical problem through histological means, to reveal the probable impact of the disease on the normal histological architecture of the animal.

Although, the improvements in general husbandry of penaeids have certainly contributed to lower incidence of shrimp disease presently, the principle means of control is early recognition and subsequent elimination or treatment of infected groups of animals. But in this particular case, no controlling measures have been put forward due to meagre understanding of the etiology and pathogenesis. The only possible alternative which could be adopted to check the economic loss and effort is the early harvest of

the available stock on immediate recognition. In this context; the histopathological examination has to be considred as an essential step to revealing many kinds of important informations including the pathogenesis, that are prerequisites to the development and establishment of reliable and valid diagnostic methods which inturn enables to carry out proper treatment and employ prophylactic measures.

I would like to take this opportunity to express my deep sense of gratitude to Dr. S.C. Mukherjee, under whose scholarly guidance and supervision, this work has been fulfilled. I am thankful to Dr.P.S.B.R.James, Director of Central Marine Fisheries Research Institute, for the facilities provided. I acknowledge my sincere thanks to Dr. P. Vedavyasa Rao for his valuable suggestions. It is my pleasure to thank Mr.A.S.Sahul Hameed for his whole hearted help during the period of my work. Gratitude for the help and advise offered by my friends, especially Dinesan, Ramraj, Muthu Karuppan, Bhaskaran and Gopalakrishnan are also acknowledged with utmost pleasure.

I am grateful to Indian Council of Agriculture Research for providing me with a junior research fellowship.

INTRODUCTION

It is obvious that disease is a ubiquitous phenomenon, and crustacean are no exception to the general rule that all living things are subject to diseases. Though there have been many valuable informations about the crustacean diseases since the earliest part of this century, many of the scientific studies however, been confined to the past two decades, due to the increasing interest in the culture of crustacean that are of direct economic concern. Sindermann in 1970, has given a compiled data of the studies on crustacean diseases since that time. After that, some dedicated works by Bang (1970), Rosen (1970), Sprague (1970, '78), Sindermann (1971a, '71b, '77), Pauley (1975), Lightner (1981), Couch (1983), Overstreet (1983) and others have contributed a great deal to this field of science.

Many diseases of marine invertebrates are inadequately characterised and many others probably, have not even been recognized. The number of diseases and disease mechanisms in crustacean is greater than that known in other groups of marine invertebrates with possible exception of mollusca. Due to the interest and investments in crustacean culture, considerable literature exists concerning diseases of these organisms. Many excellent review articles published by various authors like Lightner (1975, '85) Couch (1978), Egidius (1987) and Sano and Fukuda (1987) have greately contributed to the data available about prawn and shrimp diseases. The valuable studies by Villella (1970), Barkate (1972), Lightner (1973, '78), Barkate et al,.(1974), Paterson and Stewart (1974), Feigenbaum (1975),

Lightner et al.,(1975), Lightner and Redman (1977), Lightner et al.,(1979), Bian and Egusa (1981), Hose et al.,(1984), Sparks et al.,(1985), Lightner and Brock (1987), Strus (1987), Dykova et al,,(1988), Nash et al,. (1988) have comprehensively demarcated different aspects of diseases in crustacean, especially prawns and shrimps. Most of these studies mainly concern with the common etiology, epizootiology and mode of transmission, defense mechanisms and the possible impact and implication of the diseases in these animals.

Extortionate mass mortalities of fishes and crustaceans from natural as well as the culture populations have been reported by different workers like Herrick (1909), Beaven and Truitt (1939), Snieszko and Taylor (1947), Sindermann (1963), Lunz (1968), Lightner (1975, '77a) Delves - Broughton and Poupard (1976), and Couch (1978). Various factors bring-forth inexplicable and catastrophic events, among which, disease is the most important factor (Sindermann, 1970). Since natural populations of many of the economically important crustacean species have become depleted due to irrational exploitation of the available stock, mariculture facilities have been established leading to overcrowding of animals in the culture systems. Lightner (1985) has stated that very often this creates havoc in such populations held in confinement, with higher incidence of disease problems, because of the increased susceptibility to disease than in the natural environment. The classical studies by Overstreet (1973), Aquacop (1974), Johnson (1974) and Delves-Broughton and Poupard (1976) are extremely valuable in this context.

Lightner (1985) expressed the view that the disease outbreaks in feral populations are caused by frank virulent pathogen, whereas facultative pathogens are important causative agents in culture environments. Though diseases are of apparently infectious and non-infectious nature, there is a variety of causative factors such as viruses, bacteria, fungi, protozoan parasites, nutritional, toxic and environmental factors to produce diseases. Majority of the important diseases in prawns are caused by opportunistic organisms that are part of the normal microflora and fauna of prawns.

Different workers have shown that the diseases that occur in prawn populations are consequences of synergestic action of stressors like variations in the temperature of the environment, oxygen level, salinity, hydrogen ion concentration (pH) of water, nutritional factors, chemical pollutants and other toxic substances which influence a great deal in tilting the homoeostatic mechanisms to bring about severe afflictions to the animal health. Studies conducted by Rigdon and Baxter (1970), Venkataramiah (1971a, '71b), Lakshmi et al.,(1978), Akiyama (1982), Doughtie and Rao (1983), Wickins (1984a, '84b), Momoyama and Matsuzato (1987) and Nash et al., (1987) are of great significance in this context.

A number of workers such as Adiyodi (1972), Williams and Lutz (1975), Foster and Howse (1978), Gibson and Barker (1979), Mellon and Stephens (1980), Ravindranath (1980), Rosemark et al.,(1980), Martin and Graves(1985), Al-Mohanna et al.,(1985a, '85b), Al-Mohanna and Nott (1986, '87), Goldenberg et al.,(1986), Benjamin and James (1987), Persson et al.,(1987), Sagrista (1987), Waite and Walker (1988) and Caceci et al.,(1988) have reported normal haematological and histological studies on prawns and other decapods which are of

remarkable importance. Besides these, normal physiological activities in prawns have also been recorded by Travis (1955a, '57), Williams (1960), Dall (1964, '65b, '65d), Waterman (1960), Williams and Lutz (1975), Huner et al., (1979), Cuzon (1980), Fieber (1982), Newman et al., (1982) and Rao et al., (1982), which contribute valuable informations.

Studies on disease of crustacean from India is very meagre and is limited to record of few parasites and their biological considerations. A perusal of literature revealed reports on the diseases of prawns recorded by Mahadevan et al., (1978), Gopalan et al., (1980), Santhakumari and Gopalan (1980), Perumal samy (1982), Rajendran (1982) and Shah et al., (1982). Very recently Soni (1986) has contributed substantial information on diseases of penaeids of both cultured and wild prawns of India, especially on the microsporidiosis.

Among the diseases of cultured prawns of India the "Soft-shell Syndrome" has created a lot of concern among the farmers and thus attracted the attention of fishery scientists. A research project on pathology of 'soft prawns' was envisaged by the Central Marine Fisheries Research Institute (I C A R)in 1982 which has attributed the disease to be due to a multifactorial etiology. The seasonal pattern of occurrence, its discontinuous incidence in a series of ponds having almost similar ecological characteristics and absence of any internal parasites made it difficult to pinpoint a single factor, responsible for bringing forth this syndrome. Rajamani (1982) conducted some biochemical studies on soft prawn and reported an increase in the Non-protein - nitrogen (NPN) content in the soft prawns and assumed that this may be due to the endogenous protein metabolism caused by the

changes in the ecosystems during the extremes of environmental conditions. Soni (1986) has observed some histological changes in the hepatopancreas of prawns having soft-shell syndrome. Barring this no scientific investigations have been carried out on this syndrome in our country.

In Phillipines studies conducted by Baticados et al.,(1986) could produce soft-shelling of about 47 -60% of the prawns in laboratory conditions by an exposure to at least 0.0154 ppm of an organostannous pesticide, for 96 hours. A reversal of the diseased condition was achieved by feeding with 14% mussel meat diet. Similarly Nash et al.,(1988) have reported about 30 - 50% of prawns (Penaeus monodon Fabricus) of the pond reared stock with soft exoskeleton from brackishwater ponds with potentially acid sulphate soils.

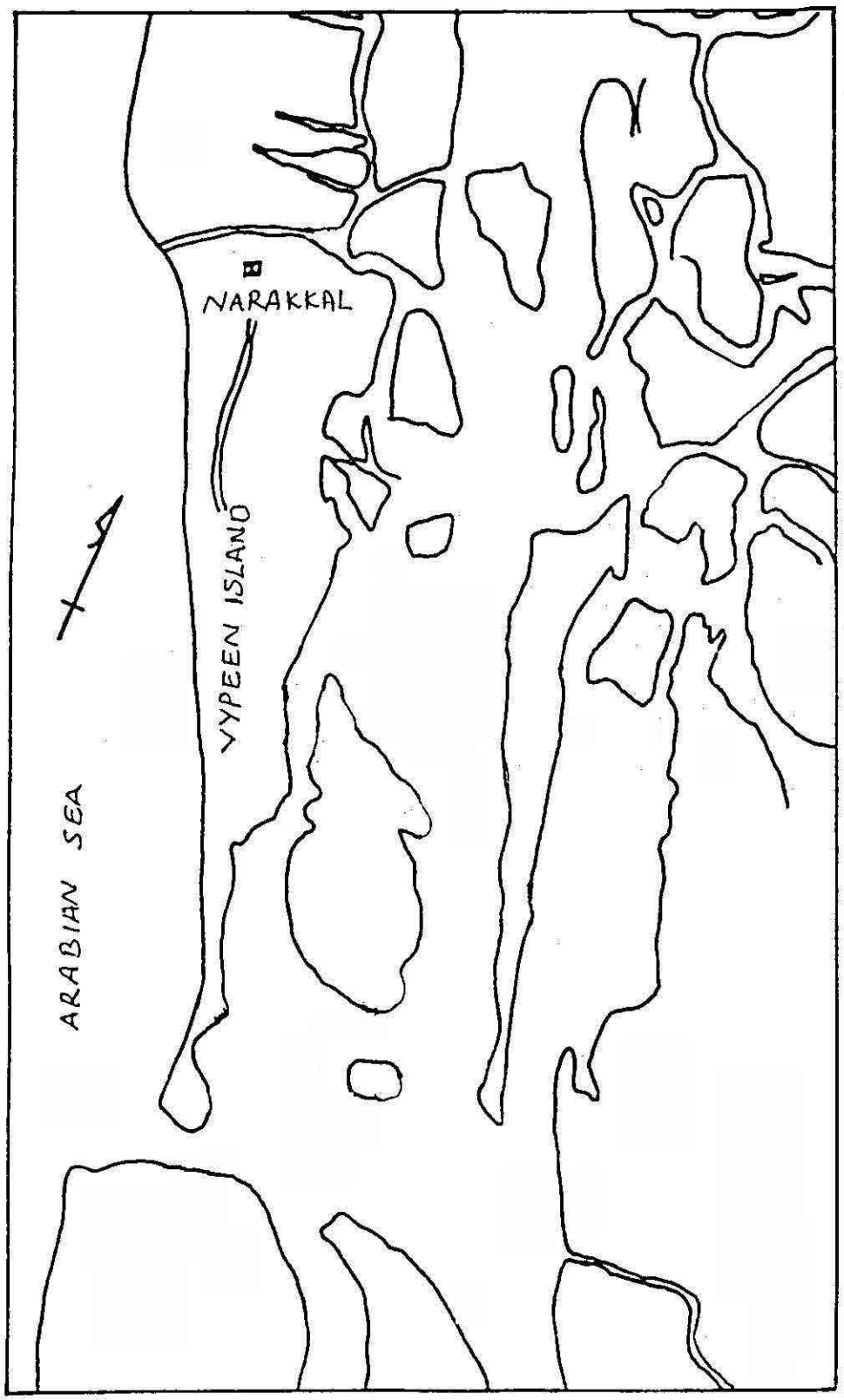
Nash et al.,(1987) while working on Macrobrachium rosenbergii de Man in Thailand, have reported heavy mortality upto the range of 60% per tank in post larvae maintained under intensive conditions in hatchery. Histologically and ultrastructurally severe segmental myofibrillar necrosis unassociated with any infectious agent was reported. The condition has been diagnosed as idiopathic muscle necrosis. Avoidance of overstocking and increased dissolved oxygen in tank water have proved effective in preventing the recurrence of the disease.

Nash et al.,(1988) reported pathological changes in the tiger prawn, P. monodon Fabricius, associated with culture in brackishwater ponds developed from potentially acid sulphate mangroove soils in Johor, Malaysia. A histological and ultrastructural study revealed the accumulation of ferric

hydroxide in the gill lamellae. MacIntosh (1982) reported that 60 percent or more of the fish ponds in the Phillipines and about 90 percent of the mangrove swamps in Malaysia have these characteristics. This is only a part of the approximately five million hectares in Southeast Asia and over 15 million hectares in the tropics which are potentially acid sulphate.

The present study describes clinical and histopathological changes observed in P. indicus and P. monodon cultured in the brackishwater ponds located near Cochin, India.

Fig 1. STUDY AREA



MATERIALS AND METHODS

The present study was conducted during the period of May, 1988 to August 1988. It included the collection of penaeid prawns of species Penaeus indicus and Penaeus monodon, having softness of the exoskeleton and muscles, from the culture ponds for carrying out clinicopathological, gross and histopathological examination. The affected specimens were collected from the perennial prawn culture ponds in and around Cochin, especially from Narakkal area in Vypeen island. This particular island is located parallely west to the main land of Cochin surrounded on three sides by the Cochin backwater system and on the western side by the Arabian sea. It has a length of 25km. with an area of 69.63 Km^2, and has extensive marshy low lands, interconnecting tidal canals and paddy fields suitable for seasonal and perennial prawn culture systems (Fig. 1).

Collection of Specimen:

The test specimens were collected by operating cast net from private and government owned ponds where the occurrence of "Soft syndrome" in prawns was reported during monsoon season. The general informations about the stocking, feeding schedule and composition of feed given, previous occurrence of the disease and current state of the culture system etc. were gathered either by enquiry or by direct observation. The analysis of the hydrological parameters like water temperature (T), hydrogen ion concentration (pH), salinity (S) and

dissolved oxygen (DO) were carried out from time to time by the following methods.

An ordinary immersible mercuric thermometer graded upto 50°C (accuracy of 0.1°C) was used to measure the water temperature of the pond. The hydrogen ion concentration was measured using a portable pH meter (Biochem make) at the collection site itself. For determining the salinity, water samples were collected in stopperred reagent bottles, and for determining the DO content water samples were collected in 125 ml. glass bottles without agitating the water and stopperred without entangling air bubbles, after fixing immediately with Winkler's solution. Later, in the laboratory the salinity was determined using argentometric method (Strickland and Parson, 1968) and DO content by Winkler's method (Strick-land and Parson, 1968)

Pathological investigations:

The pathological investigations included observation and recording of clinical signs, behavioural changes and gross lesions in spontaneous cases of affected animals. The grading was done as G1 & G2 according to the shell quality. The total length was expressed as the distance from the tip of rostrum to the tip of telson and all the animals were weighed using a monopan balance (Yamato). The sex of the animals was determined by identifying the secondary sexual characteristics.

The live specimens of apparently normal and diseased ones were separately transported to the laboratory for detail studies, in the water

collected in plastic bins of 50 litres capacity from the same pond, at a density of 1 No/2 litres with occasional agitation of the water. The specimens were then examined in the laboratory within 48 hours of collection. A number of ten diseased and same number of apparently normal animals were collected each time for laboratory study.

Haematological studies:

(i) Total hemocytic count (THC):-

The haemolymph was drawn directly from the heart using a 26 guage hypodermic needle pre-treated by flushing with an anticoagulant (3% tri-sodium citrate) attached to a 1 ml glass syringe. From the collected haemolymph 0.05 ml was drawn into a WBC pipette and diluted with WBC diluting fluid. (Analytical solution, SD's Lab-Chem industry) and agitated. A drop from the pipette was used to charge the Neubauer - counting chamber and the hemocytes were counted under low power objective in light microscope. The number of cells were expressed as cells per cubic milli-metre.

(ii) Haemolymph glucose determination:-

A part of the haemolymph was used to determine the glucose content. It was done using a glucometer (Model : Ames) at a particular time of the day (between 11.00 hrs - 12.00 hrs) in order to avoid diurnal variation of haemolymph glucose concentration.

Gross and histopathological studies:

The gross changes if any, were examined in the hepatopancreas, gut, gill, body muscles and exoskeleton of the diseased as well as the normal

animals. Small tissue pieces were collected in 10% phosphate buffered formalin (pH 7.0). Dead field specimens were immediately fixed at the collection site by (a) injecting the fixative below different parts of the exoskeleton using a hypodermic syringe, (b) by longitudinally cutting the dorsal aspect of the exoskeleton to enhance penetration of the fixative or (c) collecting tissue samples by dissecting out the organs or fixing the whole animal in sufficient quantity of fixative. The fixative was changed after 24 hours with fresh fixative.

Tissues were removed from the fixative after proper fixation, washed in running tap water for 6-8 hours before processing them. The embedding, sectioning and staining were accomplished using routine histological methods. Paraffin wax (BDH, 56-58°C melting point) was used for making blocks. The sections were cut at 4-8 μ thickness in a manual rotary microtome (Weswox Optik Model MT-1090 A) and stained by Hematoxylin-Eosin method. Stained sections were examined under a binocular research microscope (Olympus). Photomicrographs were taken wherever necessary.

RESULTS

The present study was conducted on two species of penaeid prawns ie. Penaeus indicus and Penaeus monodon, collected from private and Government owned farms of Vypeen island during the period of May, 1988 to August, 1988 when there was the occurrence of "Soft Syndrome".

Survey data:

The incidence of "Soft Syndrome" this year, was first noticed in the farms in the first week of May, when few prawns showed affection revealing clinical signs. A steep increase in the incidence was noticed after three to five days. Early harvest was done in such ponds to avoid the loss as there was rise in the occurrence of "softness" in the animals leading to high morbidity and mortality. The yield was 20 to 25% of the stocked population on harvesting, from these ponds.

P. indicus of mostly 91 to 132mm length and P. monodon of 112 to 141 mm length of both sexes were affected and were collected from the culture ponds having high incidence of mortality. During the period of study, the pond salinity ranged from 20..3 ppt to 2.2 ppt due to continuous dilution by monsoon rains. The temperature varied from 32.2° to 34.7°C. The pH range was in between 7.8 to 8.0 and dissolved oxygen content was 3.73 to 4.21 ml/l.

Clinical signs and gross pathology:

The affected animals were often dull and sluggish in their movements. The defense reflexes were minimum as evidenced during their capture. The exoskeleton were thin, soft and palpable with cuticular lesions distributed on the tergum in extreme cases. The muscle tissues were fragile and moderate to markedly soft to touch. The gut which could be seen through the semitransparent exoskeleton showed wavy nature especially in the portion of first three abdominal segments. The gut was full, with food particles mainly blue green algae, diatoms and detritus and appeared yellow to orange yellow in colour at the anterior region. In the case of P. indicus the body was light greenish in colour and general distribution of some dark greenish spots in the abdominal musculature was noticed. This was not vivid due to the species specific pigmentation of the body in P. monodon. The hepatopancreas of the affected prawns were comparatively smaller than their normal counterparts of the same size group which appeared loose.

During the initial stages it was very difficult to differentiate the 'soft prawns' from the early post moulted prawns. Clinically, both of them showed soft exoskeleton. But, with progression of the disease, the intestine of the affected animals appeared enlarged and wavy, particularly at the anterior part of the abdomen. Such prawns exhibited sluggish movement and progressive emaciation.

Haematological findings:

(i) Haemolymph glucose estimation:

25 animals examined under G1 grade (extremely soft) showed a Haemolymph glucose level of 12.75 ± 2.82 mg/100 ml. with a range of

8-17mg/100 ml

31 animals examined under G2 grade (Moderately soft) showed a haemolymph glucose level of 23.0 ± 3.15 mg/100 ml. with a range of 14-27 mg/100 ml.

22 numbers of apparently normal animals examined, showed, a haemolymph glucose level of 27°0 ± 5.07 mg/100 ml with a range of 22-41 mg/100 ml.

The haemolymph glucose content is shown in Table No.1.

(ii) Total Hemocyte Count (THC):-

The total hemocyte count of 25 animals examined under G1 grade was 13,910 ± 5.153 cells/mm^3 with a range of 8050-24750 cells/mm^3.

The total hemocyte count of 31 animals examined under G2 grade was 10,308 ± 2,403 cells/mm^3 with a range of 7,500 - 17,750 cells/mm^3.

The total hemocyte count of 22 apparently normal animals (N) examined was 9,864 ± 3,609 cells/mm^3 with a range of 4,250 - 16,950 cells/mm^3.

The total hemocyte count is shown in Table No.2.

Histopathology:

Histopathological changes were noticed in the exoskeleton, muscle, gill, hepatopancreas heart and gut.

Exoskeleton:

Microscopically, the upper most layer of the exoskeleton ie., the epicuticle revealed marked thinning with erosions and in some places the continuity was broken giving it a desquamated appearance (Fig. 2). The subepicuticular pigmented layer or the exocuticle composed of chitin and calcium showed thinning and uneven thickness in some places. The calcified endocuticle showed remarkable microscopical alteration. In most of the extreme cases, the calcified layer was not at all present (Fig. 3) leaving behind the exocuticle alone. Whereever present, the calcified endocuticular layer stained deep pink with haematoxylin and eosin exhibiting a decalcified nature. In some cases the membranous layer was markedly thickened showing hyperplastic changes. Extensive separation of the epidermal layer from the uncalcified layer was also noticed (Fig. 4). Besides, this epidermal layer showed degenerative changes in many areas. The sub-epidermal layer showed extensive vacuolation to give a reticulate appearance (Fig. 5) with mild to moderate focal aggregation of hemocytes. Scattered, pleomorphic 'reserve cells' resembling mononuclear cells were found as in the normal. In places where gross lesions in the exoskeleton was noticed (G1), infiltration of hemocytes was evident in the underlying subepidermal layer. The tonofibrils which traverse the epidermal layer showed focal detachment from the uncalcified layer of the endocuticle (Fig. 6 & 7). exhibiting hyperplastic and hyperchromatic characteristic.

Muscle:

Stricking changes were however seen in the muscle tissues underlying the exoskeleton.

Histomorphological changes consisted of myofibrillar necrosis and degeneration of varying degree, extend throughout the striated musculature of the body without any apparent site of predilliction. The muscle fibres displayed a variety of morphological changes characteristic of progressive myofibrillar degeneration and necrotic myopathy. Evidence of any bacterial, protozoan or parasitic agent could not be demonstrated. In some focal areas, muscle fibres or a group of muscle bundles showed Zenker's necrosis with ground glass appearance or hyalinization (Fig. 8). fragmentation, granular degeneration, vacuolation, hemocytic infiltration (Fig. 9) and mineralization (Fig. 10 and 11).

Focal to multifocal areas revealing hyalinisation, swelling, and loss of cross striations were often associated simultaneously with fragmentation of the muscle fibres (Fig. 12). Areas of myofibrillar disorganisation along with hyperchromatic cytoplasm (Fig. 13) and pyknotic nuclei were evident. In some cases severe sarcomere atrophy and oedema of muscle bundles were also noticed. (Fig. 14). Extensive myofibril necrosis with destruction of parenchyma were displayed by vacuolar changes with 'moth-eaten' appearance (Fig. 15). Some cases were noticed with the appearance of large vesicular myonuclei with prominent and marginated heterochromatin granules. Interstingly, sarcolemmal nuclear proliferation with slight to moderate haemocyte infiltration was also noticed multifocal or focal aggregation of haemocytes was evident in many areas (Fig. 16 & 17). Under higher magnification infiltrating hemocytes showed phagocytic activities and encapsulation of necrotic muscle tissues containing pyknotic and karyorrhectic nuclei (Fig. 18). In early stages

of degeneration, the muscle fibres either fuse together and/or showed detachment from the myoseptum. Replacement of necrotic tissue by connective tissue was evident (Fig. 12). Focal areas of dystrophic calcification at the site of necrotic muscle tissue was evident at few places (Fig.10 & 11). The calcification was later confirmed by special staining method of Von Kossa and Alizarin red S stains.

Gill:

In most of the cases the epidermal covering around the lamellar sinuses showed degeneration. Marked atelectatic changes showing collapse of the anterior sinus with emphysema or distension of the outer lamellar sinuses were usual findings in affected gills (Fig. 19). Gill epithelium in the lamella exhibited flattened appearance due to atrophy or necrotic changes. Inflammatory cell reaction was scanty. The brancial septum separating the branchial canal into afferent and efferent canals appeared thickened with scattered branchial cells (Fig. 20). The intracellular connective tissue was abundantly present in association with oedema and scattered haemocytes at the branchial septum.

Hepatopancreas:

The absorptive cells (F- cells) showed hyperchromatization and derangement in the architecture. The epithelial cells of hepatopancreatic tubule were completely disintergrated and denuded leaving the basal lamina alone in extreme cases. The covering elastic tissue and basement membrane were rendered thin and tense (Fig. 21). Fusion of the basal luminae of the ruptured, disintegrated tubules was evident in some cases.

Occasionally tubular cells exhibited vacuolation and hyperchromatic characters (Fig. 22 & 23). Few vacuolar cells in these areas showed round to oval basophilic cytoplasmic inclusions.

Heart:

Heart muscle showed myocardial degeneration with pyknotic and karyorrhectic nuclei in the muscle bands. Other changes were characterised by hyalinization and vacuolation associated with moderate hemocytic infiltration. Pericardium showed marked thickening due to hyperplastic changes and hemocytic infiltration.

Gut:

Moderate to marked histopathological changes were seen in foregut, midgut and hindgut. Necrosis of the cuticular lining was evident almost throughout the entire length of the gut (Fig. 24). The mucosal epithelium was darkly stained and had sloughed off appearance at some places. Other changes included focal areas of necrosis in the tunica muscularis and submucosa (Fig. 25), hemocytic infiltration in the connective tissue of submucosa and occasional presence of nematodes in the lumen (Fig. 26). Serosal layer often showed invagination and lumen contained debris.

Filtering apparatus:

The chitinuous plates were markedly damaged in most cases with loss of chitogenous epithelium. Setal secreting cells showed loss of nuclei (Fig. 27). Connective tissues were abundantly present.

TABLE No. 1

TABLE SHOWING THE HAEMOLYMPH GLUCOSE CONTENT(in mg/100 ml)

Grade	Number of animals examined	$\bar{X}$	SD	Range
G1	25	12.75	± 2.82	8 - 17
G2	31	23.0	± 3.15	14 - 27
N	22	27.0	± 5.07	22 - 41

G1 - Extremely soft

G2 - Moderately soft

N - Apparently normal

TABLE No. 2

TABLE SHOWING THE TOTAL HEMOCYTE COUNT (THC) (Cells/mm^3)

Grade	*Number of* Animals examined	$\overline{X}$	SD	Range
G1	25	13,910	± 5,153	8,050 - 24,750
G2	31	10,308	± 2,403	7,500 - 17,750
N	22	9,864	± 3,609	4,250 - 16,950

G1 - Extremely sott

G2 - Moderately soft

N - Apparently normal.

Fig. 2. Photomicrograph of exoskeleton showing marked thinning of the cuticle. Note the sloughing (arrow) and the gap between cuticle and muscle tissue H&E X30

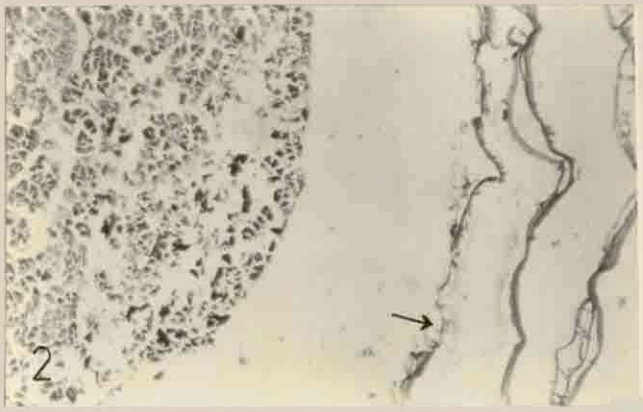
Fig. 3. Note the loss of calcified endocuticle and disorganised muscle bundles. H&E X30

Fig. 4. Note the separation of subepidermal layer from the epidermal layer (arrow). Wide gap between exoskeleton and muscle layer is also evident H&E X30

Fig. 5. Photomicrograph showing vacuolation in the subepidermal layer H&E X30

Fig. 6. Note the tonofibrils (T) and it's focal detachment (arrow) from the uncalcified layer of endocuticle,and the abnormal thickening of the pigmented layer. The lightly stained materials between the tonofibrils and muscle (M) are hemocoele and few scattered hemocytes. H&E X30

Fig. 7. Higher magnification of Fig. 6.
Note the uneven thinning of the calcified endocuticular layer (C) and corresponding detachment of tonofibrils (T). Hemocytes (H) are distinctly visible H&E X85.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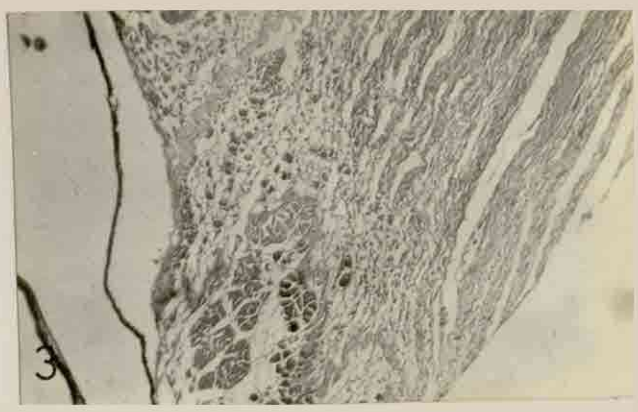
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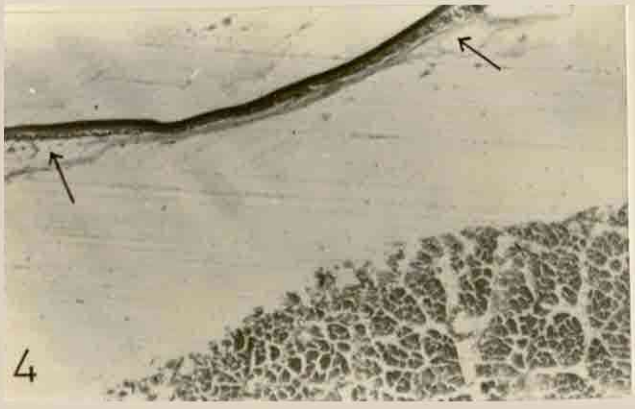
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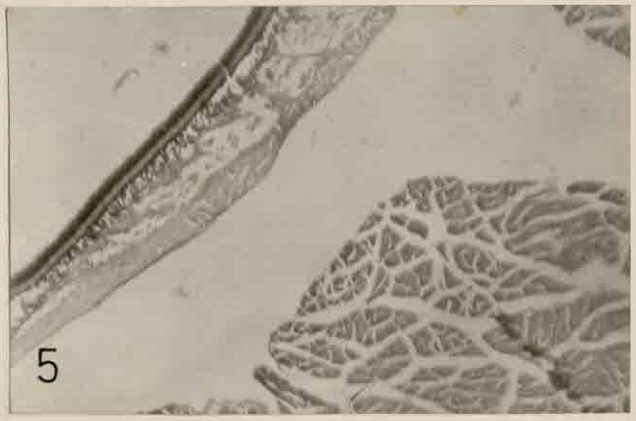
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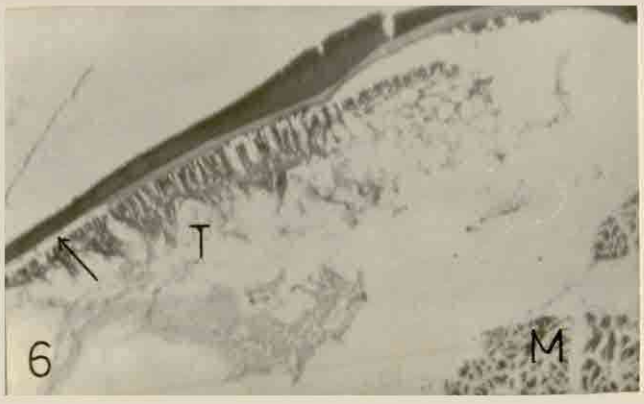
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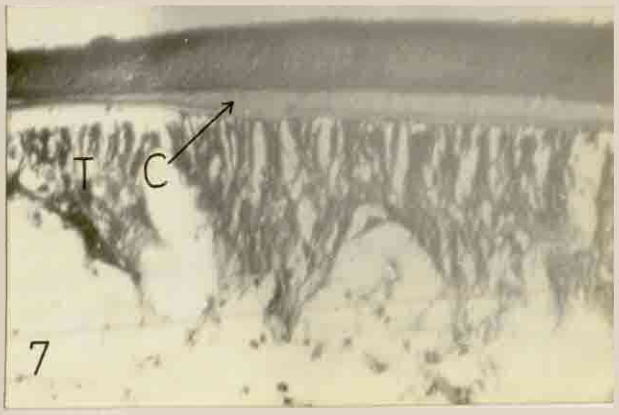
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Fig. 8. Note the loss of striation and ground-glass appearance of muscle tissue, showing Zenker's necrosis. Dark stained area is a necrotic muscle bundle with hemocytic infiltration. H&E X85.

Fig. 9. Photomicrograph showing extensive muscular necrosis with hemocyte infiltration. Note the fragmentation and separation of muscle fibres H&E X85.

Fig. 10. Necrotic muscle fibres with loss of striation and loss of myonuclei. Darkly stained area (arrow) showing dystrophic calcification. H&E X30

Fig. 11. Higher magnification of Fig. 10. showing calcification. H&E X85.

Fig. 12. Note the hyalinization and fragmentation of the muscle fibres. Oedema and focal aggregation of hemocytes are also noticed (arrow). H&E X85.

Fig. 13. Photomicrograph showing myofibrillar disorganisation. Note hyperchromatic muscle fibres and multifocal hemocyte infiltration H&E X30


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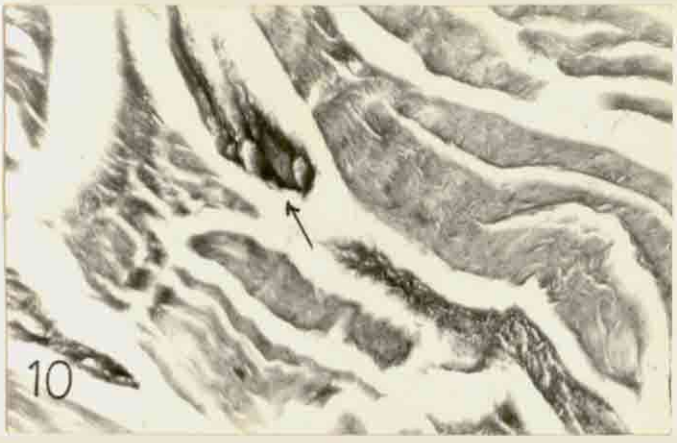
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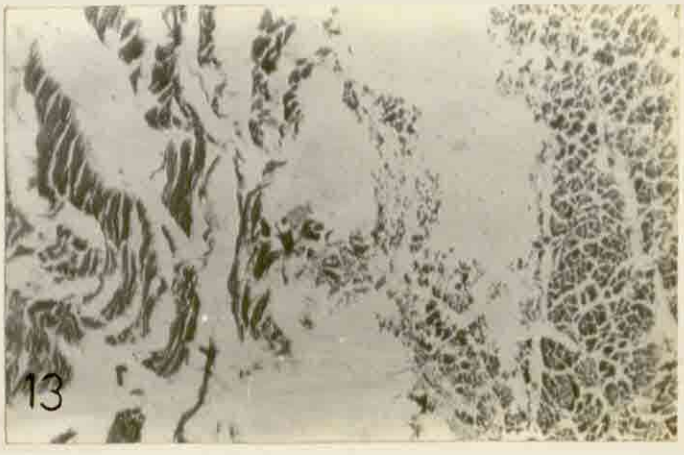
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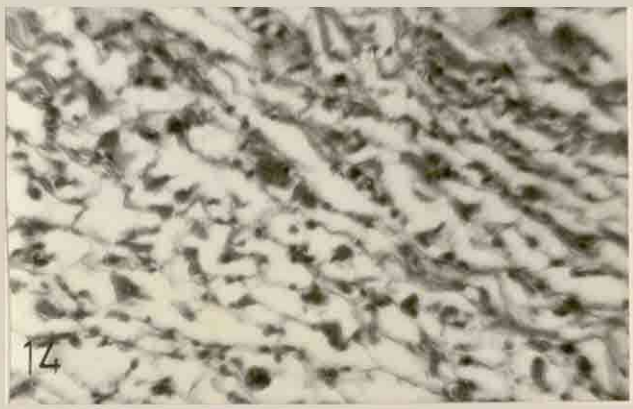
Fig. 14. Muscle tissue showing severe sarcomere atrophy and oedema with pyknotic myonuclei adhering to sarcomere. H&E X400.

Fig.15. Extensive myofibrillar destruction displaying "moth-eaten" appearance. Few aggregation of cellular debris are seen scattered H&E X85.

Fig.16 Note the focal aggregation of hemocytes in necrotic myofibres H&E X85.

Fig.17. Note the degree of affection and the infiltrating cells in the process of phagositosing the necrotic tissue H&E X85.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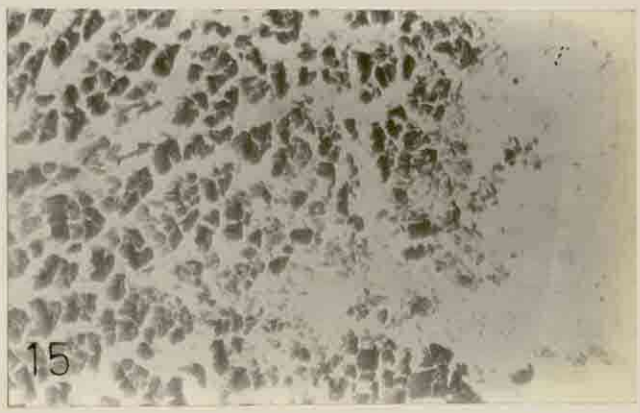
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Fig. 18. Hemocytic aggregation in a necrotic focus with phagocytosing necrotic tissue. Note the nuclei of hemocytes which are variable in shape. H&E X320.

Fig.19. Note the absence of lamellar cuticle, shrunken epithelial cell and collapsed sinuses. Moderate hemocyte accumulation is apparent throughout the gill H&E X85.

Fig. 20. Focal agtregation of hemocytes in the gill lamellae (arrow) and branchial septum H&E X85.

Fig. 21. Photomicrograph of hepatopancreatic tubule showing marked disintegration of tubular cells leaving empty space within the basal connective tissue H&E X30.

Fig. 22. Cross section through hepatopancreatic tubule displaying distented lumen (L), hyperchromatic and degenerating tubular cells fat cells (F) and connective tissue (C.T) H&E X85.

Fig. 23. Longitudinal section through hepatopancreatic tubules. Note the distented lumen (L), flattened hyperchromatic cells and vacuolations H&E X 85.


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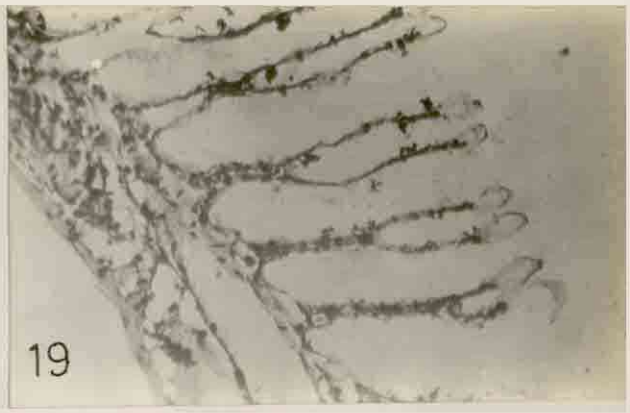
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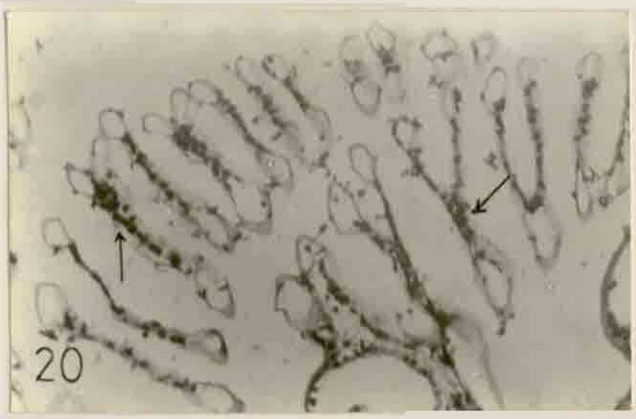
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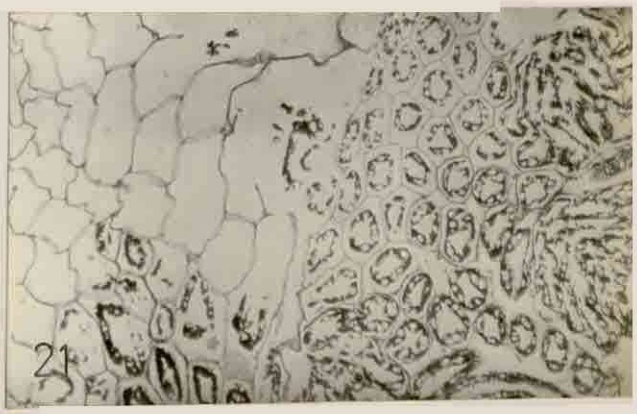
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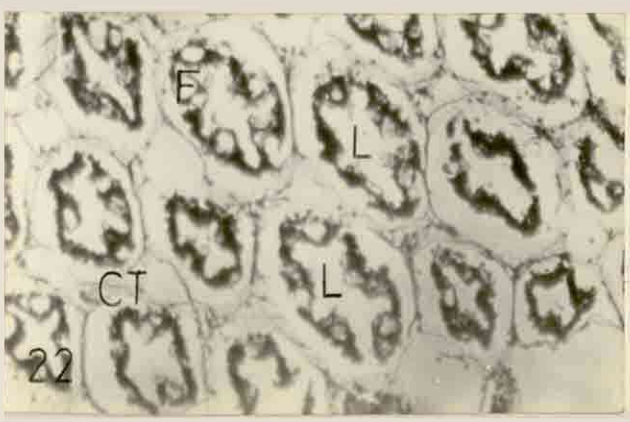

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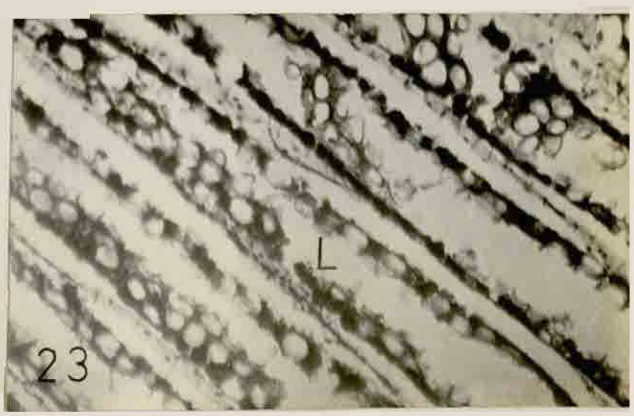

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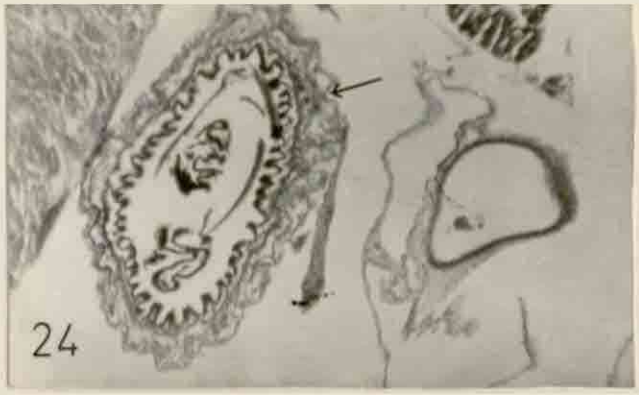
Fig. 24. Photomicrograph of foregut exhibiting necrosis of cuticular lining. Marked invagination of outer layer (arrow) and part of filter chamber (F) on the right H&E X85.

Fig.25. Midgut showing necrosis of tunica muscularis,(arrow) and submucosa. Note the invagination of serosa H&E X85.

Fig. 26. Lumen of the gut showing cross section of hematodes and cuticular debris. Note apparently normal hepato-pancreatic tubules (top left) and Multivacuolated tubular cell (arrow) H&E X85.

Fig. 27. Section of filtering apparatus showint damaged chtinous plate (C) and median ridge (M), Setal secreting cells (S), Setae of filter (F) and connective tissue (CT) H&E X85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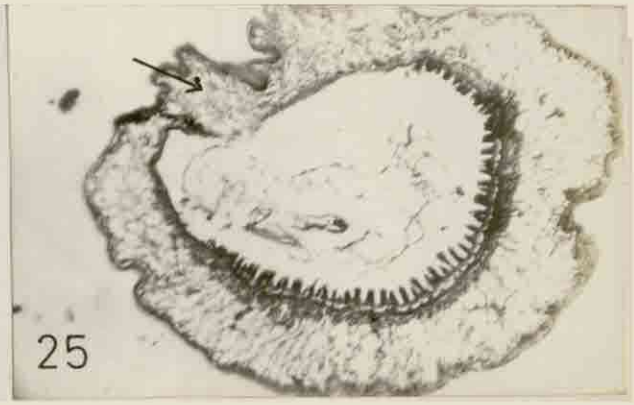
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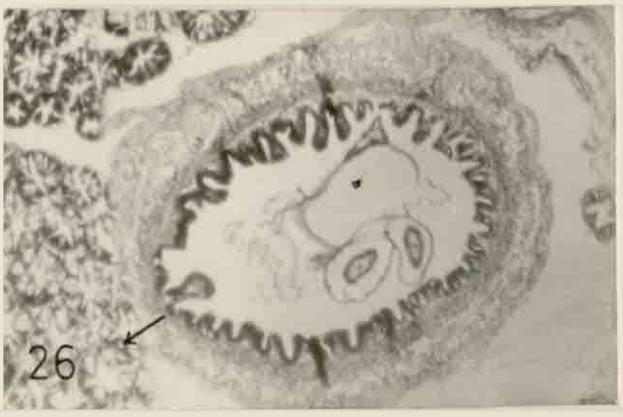
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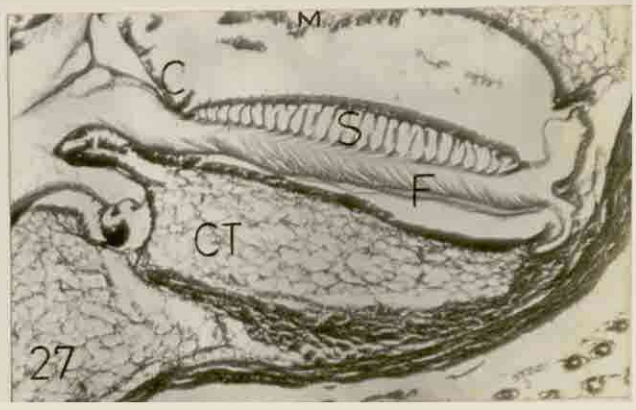
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DISCUSSION

A considerable amount of loss is incurred every year due to the "soft-shell syndrome" which sweep through the stocked populations of penaeid prawns in the grow-out systems, along the southern part of India causing immense loss in the production. In recent years prawn production in brackishwater ponds in Philippines (Baticados et al., 1986) and mangrove soils in Malaysia (Nash et al., 1988) has been adversely affected by a similar chronic soft shelling syndrome. Multi-disciplinary approaches in the field of pathobiology, ecology, physiology, biochemistry and nutrition have already been made earlier to understand the causative factor(s), inducement and manifestation of this syndrome in penaeids. However, histopathological studies of various organs of soft prawns are inadequate. The microscopical changes recorded in the present study may help in elucidating pathogenesis of 'Soft prawn', to a considerable extent.

The notable features in this condition are the thinning of the exoskeleton and tenderness of the body muscles rendering the animal to feel soft. Similar softness is also seen in prawns immediately after moulting whose cuticle gets hardened within few hours, whereas the cuticle of the soft prawns remain soft and thin. Subsequently this condition leads to poor growth resulting greater morbidity and mortality. As selective stocking is being practised by the farmers either with P. indicus or P. monodon, the pathological conditions were observed mainly in these two species and occassionally in Metapenaeus dobsoni.

Published reports on epidemiological studies (Anon. 1987) revealed that the syndrome usually make its appearance during the period of March to September every year in the tide-fed brackishwater farms or pump-fed farms. As the present study was confined to the period of May-August, the detail picture of the occurrence of the phenomenon through a Calendar year could not be presented. However, there is very little doubt about the seasonal occurrence of the syndrome which was first recorded in the first week of May, after monsoon rains, this year (1988) and continued upto the middle of August during which period the environmental parameters showed a wide fluctuation in salinity, temperature and oxygen content.

Report presented under the research project on the "Pathobiology of soft prawns" (Anon. 1987) pointed out that soft-shell syndrome occurred in widely fluctuating ecological conditions, with temperature ranging from 26.2° - 39.0°C, salinity from 2.4 - 33.67‰, pH from 6.06 to 9.33 and DO from 0.45 to 9.35 ml/l. In the present study too, it has been observed that the condition appeared when the salinity and oxygen level of water were very low whereas temperature was very high. The sudden change in the ecological condition might have acted as a triggering factor to induce the softness in the prawns. Momoyama and Matsuzato (1987) have reported a similar condition in Kuruma Shrimp cultered in Japan, producing muscle necrosis which has been attributed to environmental or physical stress due to high temperature, low dissolved oxygen and over-crowding. But these workers failed to find any relation between the occurrence of this disease and water quality.

A statistical analysis by Boyd (1982) showed that unfavourable pond conditions of high soil pH, low water phosphate and low organic matter content when occur together, result into soft shelling among prawns in the ponds. The organic matter content of the soil serve as a measure of pond fertility. It is either directly used by the prawns as food or these may decompose and release inorganic nutrients which inturn enhance the growth of phytoplankton. Boyd (1982) was of the opinion that low organic matter content in the soil may reduce the available food for prawns. He also expressed that the significantly higher incidence of soft-shelling during the summer months (March-June) could be related to the relative difficulty in the process of water exchange during dry season.

The gross anatomical changes noticed in the present study was rather interesting and may help in the identification process of the affected animals. Besides softness of the exoskeleton, the animals exhibited pale musculature with ground glass appearance. The underlying hollow space beneath the cuticle invariably contained bubble like materials which may be considered as a frequent observation. The wavy appearance of the gut - especially, in the anterior third of the body in extreme conditions often containing grayish black undigested food materials is of diagnostic importance. Similar observations were also made by other workers (Soni, 1986; Anon. 1987) who, of course could not attribute any reason for this. It can be opined that this wavy feature may be due to intestinal dysfunction.

Results showed that, there is a considerable increase in total hemocyte count in haemolymph in comparison to the normal ones which indicated the body's response to the triggering effects. Although similar observations, were made by Rabin (1965); Sindermann (1971a), Couch (1978),

Gunnarsson & Lackie (1985) and Persson and Soderhall (1987), there is a possible exception of Gaffkemia where the hemocyte number declined (Stewart and Rabin, 1970) due to the disease. The increase in the granular hemocytes indicated the role of these cells in acute inflammatory conditions. Interestingly, a decline in the haemolymph glucose level may be caused by an energy debt in the tissues due to improper assimilation of carbohydrate and the ultimate effect of stress. A similar hypoglycemic condition has been noticed in terminal growth (TG) prawns (Brock, 1983), in Macrobrachium. sp.

However, calcium levels in the exoskeleton, muscles and haemolympl was also studied earlier which indicated difference in the calcium levels in the haemolymph, muscle and exoskeleton in different seasons, rendering to imbalance in the absorption and transportation of calcium (Anon, 1987).

Histopathological studies of various organs indicated some complex phenomenon interrelated to each other. The thinning of the epicuticle and endocuticle or the decalcified nature of the endocuticle observed in this study require further elucidation. Nevertheless, these changes may be attributed to a sort of 'leaching' of the cuticular calcium or a kind of resorption by the body. But, it is well understood that the cuticular resorption is mainly aimed at the conservation of organic constituents. It is reported that, about only 5% of the cuticular calcium is resc:bed by Carcinus during the premoult phase (Lafor, 1948).

It is appropriate to discuss the possible impact of microbial population on this disease problem. Pillai (1982) through transmission experiments and field observations stated that, vibriosis caused by Vibrio anguillarum in P. indicus produces white patches on abdomen and reddish discolouration of the rostrum. In acute cases the prawns become emaciated with softening of muscle tissue and thinning of the cuticle. Kurien (1982), Muthu et al.,(1982) and Nandakumar (1982), reported the occurrence of this phenomenon in cultured prawns. While Muthu et al., (1982) observed that this was the most common disease in P. indicus, Kurien (1982) was of the opinion that, the thinning of the exoskeleton might be due to the metabolic changes owing to the changes in the environment. Attempts were made for isolation of pathogenic bacteria in the present study which proved futile. Besides, necrotic body tissue failed to demonstrate the presence of any bacterial pathogen histopathologically. The studies carried out by Baticados et al., (1986) could isolate chitinoclastic bacteria like Vibrio and Aeromonas from the lesions of soft shelled prawns, but experimental transmission attempts were unsuccessful. Few workers (Hood and Meyers, 1974) believed that chitinoclastic bacteria are normal part of the microflora of penaeids and presence of these organisms in the cuticular lesions may be of secondary infection.

Extensive morphological alterations were demonstrated histopathologically in the present study which in many respects are similar to the reports of earlier workers. Rigdon and Baxter (1970) were the first workers to observe development of white or opaque abdominal musculature in spontaneous muscle necrosis of prawns and described the histological

condition as "degenerated foci of striated muscle", in brown shrimp. Lakshmi et al., (1978) reported similar disease problem which had been attributed to variety of unfavourable environmental conditions during or immediately following a hyper-activity. Similarly Akiyama (1982), Momoyama and Matsuzato (1986), and Nash et al., (1987) have described the condition as white muscle disease, idiopathic myopathy or idiopathic muscle necrosis (IMN). However, no reference have been made regarding the soft exoskeleton in these cases. Besides the above mentioned workers, Venkataramiah (1971a, 1971b), Sindermann (1977), Brock (1983) and Lightner (1983) have considered the muscle necrosis as a result of predisposing environmental stressors including extreme and sudden fluctuations in salinity, temperature, DO levels, hyperactivity, over-crowding and physical handling. The present findings in muscle tissue also corroborated with the observations made by above workers and may be attributed to the predisposing environmental stressors.

Lactic acid is believed to be the major cause of postactivity acidosis in crustaceans (Phillips et al., 1977; Mc Mahon et al., 1978). Maximum lactic acid and minimum pH levels may occur 1-2 hrs following stress due to low body temperature (McDonald et al., 1979), their open circulatory system or combined effect of both (Spotts & Lutz, 1981). During this period, there may be an increased susceptibility to the effect of further stress conditions such as low DO content or infection (Spotts and Lutz, 1981).

Nash et al., (1988) observed softening in about 40 - 50% of the stocked population of P. monodon cultured in brackish water ponds of potentially acid sulphate soil and this may be attributed to reduced

pH due to rapid loss of alkalinity which causes precipitation of calcium salt making it nonavailable to the animals. Studies conducted by Wickins (1984) could demonstrate the weight loss of carapace in P. monodon due to reduced pH, though the calcium levels remained constant in the exoskeleton. In the present study, denudation of calcified endocuticular layer was noted which may be due to resorption or improper mineralization of the exoskeleton. The factors influencing mineralization other than temperature and physiology, include bicarbonate (Greenaway, 1974), external calcium level (Cripps and Nakamura, 1979) and pH (Malley, 1980). However, Lahti (1988) found that the mineralization of the exoskeleton in Astacus did not get much affected in calcium deficient water.

According to the pond surveys made by Baticados et al.,(1986), the occurrence of soft shelling could be predicted with 98% accuracy under poor soil and water conditions in the ponds especially with low amount of phosphate. They suggested that this may be another reason for making calcium nonavailable to the animal along with reduced pH.

Although no clinical chemistry parameters have been taken up simultaneously with the histopathological study in this study, it is considered that stress induced hyperactivity leading to rapid development of muscle hypoxia and accumulation of lactic acid during anaerobic glycolysis were the most likely reasons in the pathogenesis of muscle necrosis. The increase in the non-protein nitrogen (NPN) reported by Rajamani (1982), may be due to the protein degradation in the muscle tissues during the necrotic changes.

In the case of muscle diseases, nutritional myopathy associated with vitamin E and trace element selenium have been reported to be the cause of muscular dystrophy in domestic animals (Hulland, 1985), and fish (Roberts, 1986; Collins & Rice, 1986 and Richards, 1986), but has not been described in prawns. Accumulation of free radicals causes peroxidation of membrane lipids and damage to protein molecules leading to cellular injury beneath the cuticle and muscle. Estimation of selenium in the soil, in the affected ponds may be appropriate.

Another important factor causing soft shelling is aquatic pollutants. Among which the pesticides used in the ponds for control of weed animals, have been implicated as chitin synthesis inhibitors (Corbett, 1974; Dale, 1975). Laboratory studies by Baticados et al.,(1986) could produce about 47-60% of soft shelling in P. monodon by a 96 hrs exposure to an organo-stannous pesticide. The effective interference of the chemical pollutants is largely controlled by the environmental conditions, species differences and also the physiology of the animal.

Sis et al., (1980) reported changes in the gut of penaeid shrimp as a result of environmental stressors. Some of the histological changes reported by them and Lee et al., (1985) like necrosis of the lining cuticle, focal necrosis and general hemocytic infiltration were similar to those observed in the present study. Although hemocytosis was mainly observed in the submucosa, eosinophilia was not much pronounced.

Histopathology of the organs examined in this study suggest that certain histologic changes may be useful as early warning indicators of stress. Inflammatory reaction in the gut and hepatopancreas are notable examples of stress indicators. Similar views have also been offered by Lee et al., (1985). They have also opined that the hepatopancreas has important metabolic functions and is an organ responsible to chemical injuries involving organic and inorganic poisons, pesticides and biotoxins. They added that although the shell is in direct contact with the environment, the highly protective epicuticle diminishes its value under extreme environmental stress conditions.

In these perspective, the present findings could provide a basic information about the impact of this syndrome on animal which may subsequently lead to establishment of proper controlling and preventive measures through better management systems. In order to find out the exact cause of the syndrome, and to understand the problem precisely, further studies like histochemical studies, mechanism of soft shelling by exposure to pesticides used in prawn culture, it's mode of action, role played by pH, the calcium - phosphorus - carbonate relationships in the ionic regulation of prawns and the selenium content of the soil have to be taken up.

SUMMARY

1. Penaeid prawns of two species ie., Penaeus indicus and Penaeus monodon showing soft-shell syndrome wre obtained from Matsyafed farm and private farms of Vypeen Island, Cochin, for carrying out the clinicopathological and histopathological studies. The study was conducted during the period of May-August '88 when there was the occurrence of "soft prawns" in these culture fields.

2. Haematological studies included Total Hemocyte Count (THC) and estimation of haemolymph glucose level. The haemalogical examination revealed the increase in the number of circulating hemocytes (hemocytosis) and the decrease in the haemolymph glucose content (hypoglycemia) conditions in the soft prawns. The comparative value with the control group of animals have been presented in (Table 1 & 2).

3. The histopathological changes were observed in the exoskeleton, muscle, gill, hepatopancreas, heart and gut.

4. The exoskeleton exhibited marked thinning or decalcified nature mainly in the calcified layer of endocuticle. Focal detachment of tonofibrils from the uncalcified layer was also a regular finding.

5. Degeneration of the epidermal layer and vacuolation of the subepidermal connective tissue layer was also noticed in soft prawns.

6. Remarkable changes were noticed in the muscle tissue. Progressive degenerative changes were evident in the striated musculature. Focal to multifocal necrotic areas with occasional hemocytic infiltration without presence of any bacterial or protozoan agent were invariably present throughout the musculature in extremely soft animals. The necrotic changes were characterized by early degenerative change, loss of striations, fragmentations of myofibres, severe sarcomere atrophy with pykno tic and karyorrhetic nuclei, distintergration, hyalinization and mineralization in the necrotic foci. Focal areas of Zenker's type of necrosis were also evident.

7. Gill alterations were characterized by flattening of brachial epithelial cells and distension of the outer lamellar sinuses and thickening of branchial septum with focal aggregation of hemocytes.

8, Histopathologically, the hepatopancreas showed degenerated tubular epithelial cells which showed hyperchromatization with basophilic cytoplasmic inclusions in some cells and extensive vacuolation.

9. The heart exhibited myocardial degeneration with pyknotic and karyorrhectic nuclei.

10. Necrosis of the cuticular lining, with sloughing in some places, hemocytic infiltration in the submucosal layer and invagination of the serosal layer were the pathomorphological alterations observed in the gut. Marked damage in the chitinous plates was noticed in the filtering organ.

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