STUDIES ON THE PHYSIOLOGY OF MOULTING IN THE PENAEID PRAWN, *PENAEUS INDICUS* H. MILNE EDWARDS

THESIS SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
OF THE
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

By
K. K. VIJAYAN, M. Sc.

CENTRE OF ADVANCED STUDIES IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN - 682 031

1988

To My Parents

C E R T I F I C A T E

This is to certify that the thesis entitled ''Studies on the physiology of moulting in the penaeid prawn, Penaeus indicus H.Milne Edwards''is the bonafide record of the research work carried out by Shri K.K.VIJAYAN, under my guidance and supervision in the Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute (CMFRI), and that no part thereof has been presented for the award of any other degree.

Cochin-682031
November,1988

Dr.A.D.DIWAN,Ph.D.,
Associate Professor and
Scientist, CMFRI,
Cochin-31

D E C L A R A T I O N

I hereby declare that this thesis entitled ''Studies on the physiology of moulting in the penaeid prawn, Penaeus indicus H.Milne Edwards''has not previously formed the basis of the award of any degree, diploma, associateship or other similar titles or recognition.

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K.K.VIJAYAN

A C K N O W L E D G E M E N T

It is of great pleasure I express my deepest sense of gratitude to my guide Dr.A.D.Diwan, Associate Professor and Scientist, Central Marine Fisheries Research Institute (CMFRI) for not only suggesting the research topic but also for his invaluable guidance and constant encouragement throughout the study.

I take this opportunity to express my sincere thanks to Dr.E.G.Silas, former Director, CMFRI for encouragement and providng facilities to carry out this study. My thanks are due to Dr.P.S.B.R.James, Director, CMFRI for extending the facilities up to the completion of the thesis.

Iam greatly indebted to Dr. A.Mohandas, Reader, School of Environmental Studies, Cochin University of Science and Techonology for his continued encouragement and generous help in going through the manuscript.

I wish to recall with gratitude all the help and encouragement given by Dr.P.Vedavyasa Rao, Sri.M.S.Muthu, Senior Scientists of CMFRI, and Dr.P.N.K.Nambisan, Professor and Head, Chemical Oceanography, Cochin University of Science and Technology.

My gratitude is due to Dr.M.V.Mohan, Asst.Professor, College of Fisheries, Panangad for generous help in the preparation of drawings.

I express my sincere thanks to Mr.M.Srinath, scientist, CMFRI for his generous help rendered in the statistical analysis.

My special thanks are due to Dr.K.Alagaraja, Senior Scientist, CMFRI, and Sri.K.Narayana Kurup, Scientist for extending the computer facilities.

Thanks are also due to Sri.V.K.Pillai, Scientist, for his help rendered in the mineral analysis, and Sri.M.Nandakumar for his timely help.

My sincere thanks are due to Scientists and other personnels of the Marine Prawn Hatchery Laboratory, Narakkal for providing prawn specimens and other help for the present work.

I wish to record my sincere thanks to my colleague Sri.K.Sunilkumar Mohamed for his unstinted help through out the period of study. My hearty thanks are also due to all my friends, they are many, especially Sri.K.Suresh kumar, Sri.K.K.Joshi, Sri.A.P.Dinesh babu and Sri.Joseph Gilbert for their help at various stages of preparation of this thesis.

The help rendered by Sri.C.Sreekumar, Sri.M.Sudesh, Sri.K.Ramakrishnan, Sri.C.Ravichandran, Sri.M.P.kishor, Sri.Ganesan Iyer, Sri.Thomas Varghese and Sri. M.Karthikeyan in the word processing of this thesis is

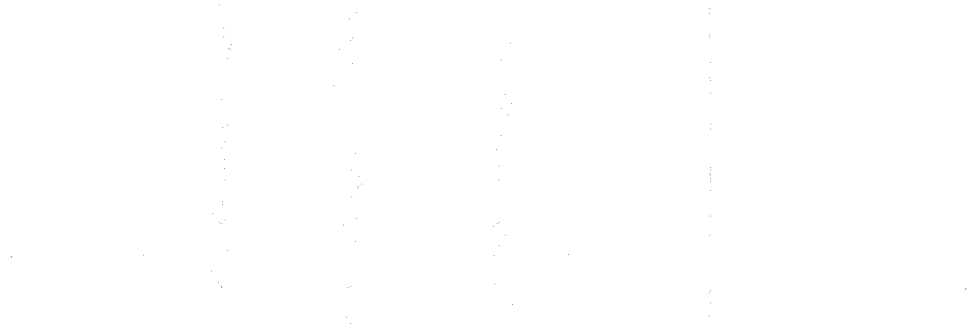
gratefully acknowledged.

Iam also grateful to Mr.P.Raghavan, Photographer, CMFRI for his help in the photomicrography.

I express my gratitude to the Indian Council of Agricultural Research, New Delhi for offering me the Senior Research Fellowship to carry out this work.

Finally, I owe much more than I can to my parents, Smt.K.K.Sarada Amma and Sri.C.Narayanan Nambiar, whose charisma and blessings enabled me to bring out this piece of work.

CONTENTS



P R E F A C E

Penaeid prawns form the most economically significant group in the marine and brackishwater fishery resources of India. This particular group contributes about 62% of the total prawn landings of the country. At present prawns have assumed an important place especially as a commodity supporting an export trade of sizable magnitude. Considerable interest has been shown in the last decade to increase prawn production through various culture practices, mainly due to the high demand for good quality prawns for export coupled with the stagnant and even depleting nature of marine catches.

Available informations suggest that among the 15 species of shrimps and prawns occurring in Indian waters, which are deemed suitable for aquaculture, the Indian white prawn P.indicus is identified as one of the most important commercial species. Considering the increasing importance as an accepted species for prawn culture, P.indicus was selected for the present study. In the life history of prawns, moulting is an important event, which enables the animal to achieve growth. This dynamic physiological event continues through out the life span of the prawn, linking almost all biological activities with

this process. Hence, a good knowledge pertaining to the physiology of moulting is imperative to understand the growth process. This knowledge will be of great use in the scientific prawn farming, so as to achieve high prawn production.

Pioneer workers like Drach in 1939 and Sheer in 1960 initiated the classic studies on crustacean moulting physiology. In subsequent years a great deal of information has been added to this particular field by a number of workers viz. Passano, Aiken, Cooke and Sullivan, Stevenson, Skinner, and Fingerman. In spite of these great contributions, only very limited attention was received to the moulting physiology of natantians especially of penaeids. In this context an effort is made, through the present work, to study some aspects of the moulting physiology of P.indicus.

The main objectives of the present study are:

1. To draw a scheme for the moult cycle classification, and identification of sequential stages of the moulting process

2. To understand the physiology of moulting in relation to following aspects:

a) The structure and function of endocrine systems in

relation to moulting process

b) Studies on the behaviour of important metabolites during the moulting cycle

c) The structure of the cuticle and mapping of some of the important minerals viz. Calcium, Magnesium and Phosphorus and investigation on the mobilization of these minerals during moultig process

d) The effect of some environmental and other factors on moultig process

The thesis embodying the details of the investigation has been organised in five chapters each with an introduction, materials and methods, results, and discussion. Introduction of each chapter highlights the importance of the particular aspects of study covering a review of literature. Under materials and methods the methodology used, and in the results the data obtained are presented. Each chapter is concluded with a discussion followed by a short summary.

The first chapter deals with the characterization and classification of complete moult cycle on the basis of setal development (setogenesis) and cuticle histology. Duration of each moult stage, effect of body size on moult cycle duration, and moulting behaviour are also

investigated on the basis of moult experiments.

The second chapter deals with the structures of neuroendocrine systems and their mechanisms of control on moulting process of the animal. Investigations on the structure and changes of neuroendocrine centres, such as X-organ Sinus Gland complex (Eye), Brain and thoracic ganglia are made using standard staining techniques. Y-organ or the moulting gland and mandibular gland have been found out and their positions were confirmed with histoblogical studies. Alterations in the size and tinctorial affinity of Y-organ cells were noted between the moult stages. Eyestalk ablation and Y-organectomy experiments revealed the production of moult controlling factors in X-organ and Y-organ during the moulting cycle of the prawn.

The third chapter is on the role and involvement of some important metabolites like protein, DNA, RNA, Lipid, glycogen, glucose, glucosamine, chitin, and water content during the moulting cycle of the prawn. Metabolites were estimated in selected tissues such as muscle, hepatopancreas, cuticle, and haemolymph of the animal. Profound changes in the concentration of metabolites were observed during the different stages of moult cycle,

especially between early premoult and postmoult stages.

The fourth chapter is on the distribution and mobilization of some important minerals of the exoskeleton viz. calcium, magnesium and phosphorus in relation to the moult cycle. The bio-concentration of calcium, magnesium and phosphorous was estimated in different tissues such as exoskeleton, muscle, hepatopancreas and haemolymph with changing stages of the moult. Among minerals calcium was found to have a major share and showed significant changes between the moult stages. The mapping of calcium, magnesium and phosphorus in different parts of the exoskeleton was carried out to understand the distribution pattern of these minerals in the exoskeleton.

In the fifth chapter, observations on the effect of some important environmental factors like temperature, salinity, pH, light, and the effect of other factors like starvation and autotomy on moulting cycle of the prawn are included. In the experimental set up, along with the moulting cycle, growth pattern of the prawns was also monitored to find out the effect of environmental factors on growth. Temperature was observed to have prime and direct control over the process of moulting. The moulting cycle of the prawn was severely affected due to starvation

while autotomy has not produced any significant effect on moulting.

A summary of the work, and bibliography are given at the end.

CHAPTER-I MOULTING CYCLE AND ITS CHARACTERISTICS

CHAPTER I

MOULTING CYCLE AND ITS CHARACTERISTICS

1.INTRODUCTION

Crustacean body is ensheathed by a thick outer covering of cuticle called exoskeleton. Due to the presence of the rigid integument, growth process in crustacea can be achieved only through periodic shedding of the cuticular sheath. Thus, shedding of the exoskeleton or moulting forms the most important metabolic event which dominates the life cycles of these specific groups of animals (Highnam and Hill, 1979).

During moult cycle, crustaceans show many cyclic changes including structural, biochemical and physiological resulting in ecdysis (Passano, 1960). Inorder to study the growth and other moult linked process in detail, it is absolutely necessary to know the classification of moulting process by which the division of moult cycle into well defined stages is possible. Drach (1939) was the first to establish the concept of moult cycle in crustaceans as sequences of stages using criteria of readily observable changes in the integument of two brachyurans, viz. Cancer pagurus and Maia squinado. This method was later elaborated by Drach and Tchernigovtzeff

(1967), who compared the moult staging both in lightly and heavily calcified animals. Workers like Travis (1957), Skinner (1962) and Stevenson (1968) developed moult staging schemes based on histological variations of the exoskeleton, while external features of the exoskeleton were used to stage the moult cycle by Bursey and Lane (1971), Nagabhushanam and Vasantha (1971) and Peebles (1977).

Later on many workers have modified the original scheme put forth by Drach (1939), so that the modified scheme becomes applicable to a variety of crustaceans in identification of moult stages (Scheer, 1960; Schafer, 1967; Stevenson, 1972; Aiken, 1973; Freeman and Bartell, 1975; Mills and Lake, 1975; Vanherp and Humbert, 1978, and Vigh and Fingerman, 1985). According to this method, the criterion was to identify the characteristics of new setae that are formed in the tissues of the appendage to replace those lost with the old exoskeleton at the time of ecdysis. It is relatively easy to observe the setal development or setogenesis in crustaceans which have a transparent cuticle. Where atleast one transparent appendage is available for examination, morphological changes of the developing setae (setogenesis) can be observed easily and have been used as a criterion for

moult staging (Aiken, 1980).

Procedure for identifying moult stages of reptantians is well documented but detailed staging of the complete moult cycle in natantians especially among penaeids has not been widely reported. Other than the works of Longmuir (1983) in Penaeus merguiensis and Smith and Dall (1984) in Penaeus esculentus, moult staging studies made by Dall (1965), Schafer (1967), Huner and Colvin (1979), and Pudadera et al. (1984) furnish only limited information. Preliminary works on the moult staging of Penaeus indicus have been reported by Read (1977) and Diwan and Usha (1985).

The method used for moult staging generally involves observations of the degree of hardness of the exoskeleton, and microscopic examination of the transparent edge of the uropods or pleopods, where epidermal withdrawal and development of new setae can be observed (Drach and Tchernigovtzeff, 1967; Yamaoka and Scheer, 1970). However, only a few authors have verified intrepretations of the moult staging criteria using setogenesis with that of histological changes in the cuticle (Travis, 1955a, 1957; Skinner, 1962; Stevenson, 1968, and Smith and Dall, 1984).

Aiken (1973), in the lobster Homarus americanus has observed that the duration of each moult stage in the moult cycle of the animal varied considerably. Earlier investigations on the moult cycle duration with respect to each moult stage of penaeid prawns include the study of Huner and Colvin (1979) in Penaeus californiensis and Penaeus stylirostris, of Smith and Dall (1984) in Penaeus esculentus, and of Pudadera et al. (1984) in Penaeus monodon.

There are still gaps in our knowledge of moulting behaviour of prawns. Basic problems which need further investigations include the time duration for each of the moult stage in the moult cycle, and mechanism of exuviation. The limited information available on the mechanism of exuviation and moulting behaviour of prawns are the works of Jefferies (1964) in Palaemonetes varians, Bursey and Lane (1971) in Penaeus duorarum, Longmuir (1983) in P.merguiensis, and Wassenberg and Hill (1984) in P.esculentus .

In the present work, a detailed study has been carried out to obtain basic data on the moulting cycle of the Indian white prawn Penaeus indicus. The study includes

(1) the staging of moult cycle in greater detail using setogenisis, (2) histology of the integument during the moulting cycle, (3) assesment of stagewise moult cycle duration, (4) relationship between body size versus moult cycle duration and (5) mechanism of exuviation.

2. MATERIALS AND METHODS

Specimen of P.indicus were periodically obtained from the traditional prawn farms of Vypeen island and Marine prawn hatchery laboratory, Central Institute of Brackish Water Aquaculture (CIBA), Narakal, Cochin. The animals were transported to the laboratory in sea water in 25 litre polythelene transportation bags. The prawns were then transferred into a 250 litre rectangular fibreglass tank containing aerated sea water with salinity around 25‰. The size range of the prawns used to establish the moult staging classification varied between 20 mm to 120 mm, i.e. from early juveniles to adults. In the beginning, 20 prawns which are in the premoult condition were held individually in circular plastic cages of 13 cm in height and 20 cm diameter (Fig. 1), to study the changes in the setal morphology occurring immediately after moulting process and thereafter with the advancement of time till the prawns enter into the next successive moult. The cages were floated in a circular perspex tanks of 350 litre

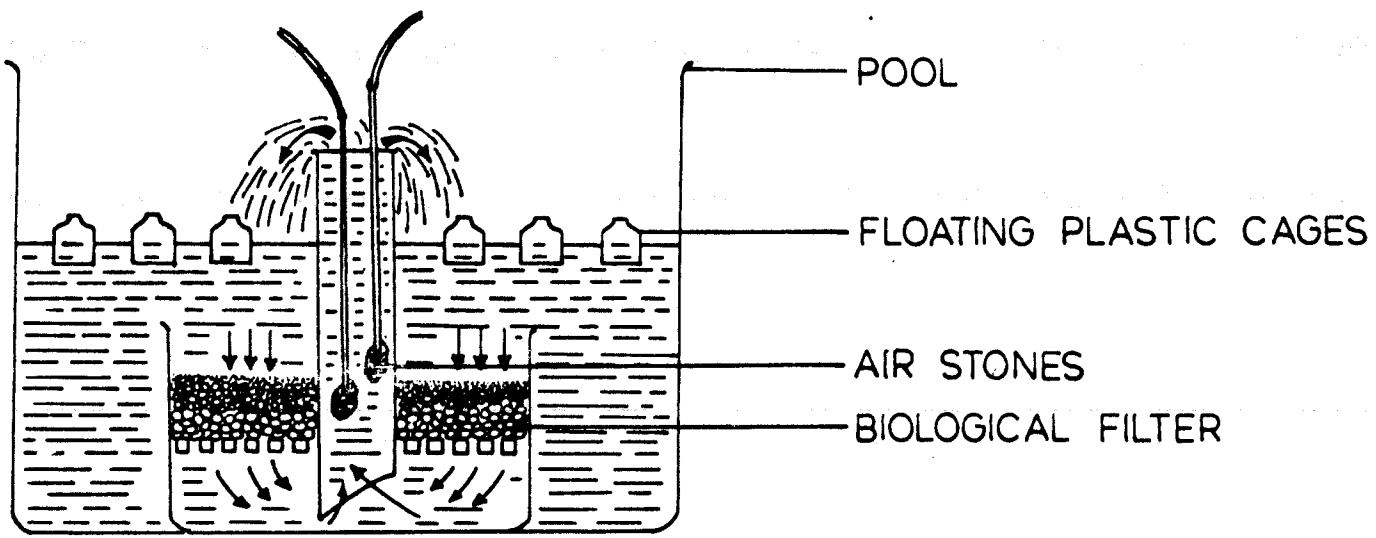


Fig. 2b. Diagramatic representation of animal maintenance system

capacity (Plasty craft corporation, Bombay), fitted with a biological filter (Fig. 2a and 2b) , where the salinity ranged between 20‰ and 25‰ and temperature fluctuated between 29°C and 31°C. All other physical and chemical factors were simulated to those of natural environment. Animals were fed with molluscan and crustacean meat, about 15% of the body weight of the prawns.

Daily observation on the setal development were made in moulted prawns by observing the posterior median part of the inner uropod. Excision of the uropod was not necessary, and the prawn could be held immobile and quiescent while wrapped in a wet paper towel. Moult stages were determined using morphological changes of the setae as a criterion originally proposed by Drach (1939), and further modified by Scheer (1960), and Drach and Tchernigovtzeff (1967). Visual inspection of uropod was carried out using a stereoscopic binocular microscope with either 50X or 100X magnification and transmitted light.

By examining setogenesis daily, approximate time duration for each moult stage was calculated. The total duration of time between two consecutive moults gave the time duration of one moult cycle.

Photomicrographs of setogenesis in different moult stages were taken. The endopodites of the uropods were removed, mounted in filtered sea water on microscope slides, and were photographed using ORWO 125 ASA black and white film in Olympus PM 10AD Binocular Compound Microscope.

Animals used for histological examination of the cuticle to study the integumental changes were killed by severing the cephalothorax. From each prawn, a piece of the cuticle from the lower region of the carapace (Fig. 3) was removed and immediately fixed in Bouin's fixative. Prior to processing and embedding in paraffin wax, the cuticle segments were soaked in solution of 2% nitric acid prepared in 70% alchohol, to decalcify the cuticle. Sections of 7 - 8/u thickness were cut with a rotatory microtome, and stained with haematoxylin and eosin, and Mallory's triple stain.

In order to study the moulting behaviour and mechanism of exuviation, prawns of size 80 to 120 mm in moult stages of D2-3 were selected and kept in separate glass troughs of 10 litre capacity containing aerated sea water. Glass troughs were kept in the dark shadows since the animals have general tendancy to moult in the late

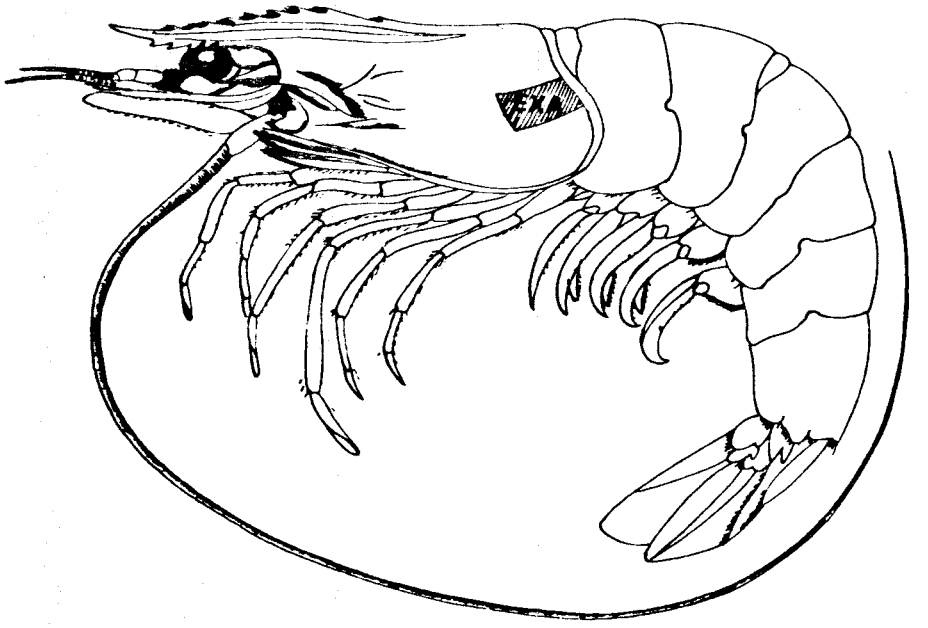


Fig. 3. EXA-Area of exoskeleton sampled for cuticle histology

night or early morning. As and when the animals moulted, the mechanism of exuviation or shedding process of exoskeleton was closely followed and time duration was recorded.

To study the relationship between the body size versus moult cycle duration, observations on the moult cycle were conducted in three different size groups of P.indicus viz. 30 - 40 mm (TL), 60 - 80 mm (TL) and 80 - 120 mm (TL). Each group comprised of 15 experimental animals. Animals were kept under observation till they completed two cycles of moult in the laboratory. Average moult cycle duration of each group was calculated and the results were statistically verified using student 't' test.

3.RESULTS

3.1.Description of moult cycle

The morphological changes associated with setal development of uropod in the prawn P. indicus are found to be a good indicator for identification of the different stages of the moult cycle. On the basis of setogenic events and epidermal retraction observed in the uropods, the moult cycle has been classified intc various well defined stages.

Stage A (early postmoult) Fig:4 and 13

Stage A represented a prolonged state of the actual moult and is the first stage immediately after ecdysis. Soon after the ecdysis, i. e. during stage A, the whole body and the exoskeleton were found very soft and slippery to touch. Granular cytoplasm was continuous through out the setae which filled the setal articulation and setae. The setae were found to be more fragile and delicate. Stage A extended up to a period of 3-7 hrs.

Stage B (late postmoult) Fig:5 and 14

Appearance of well developed setal articulation, and the beginning of cuticular node development marked the onset of stage B, i. e. the late postmoult stage. The matrix of the setae was found contracted with the setal lumen so as to fill only the proximal half of the setae. The exoskeleton which at the stage A showed a parchment like consistency became relatively hard in stage B, but it was easily depressible. The late postmoult stage B, lasted 16-22hrs.

Stage C (intermoult) Fig: 6, 15 and 16

In stage C, the most significant observation was that of the presence of fully developed cuticular nodes and setal cones. Distal part of the setae appeared clear and

Fig. 4-12. Schematic representation of setogenesis in P. indicus

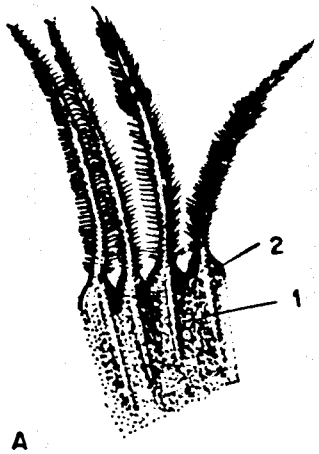


Fig.4
STAGE A

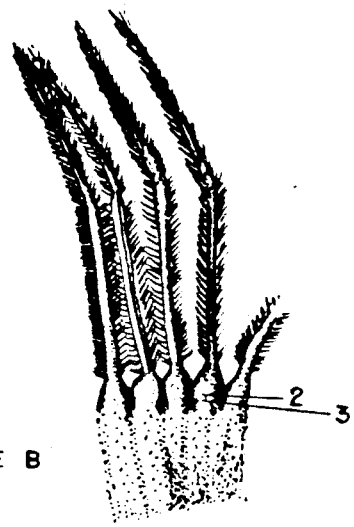


Fig.5
STAGE B

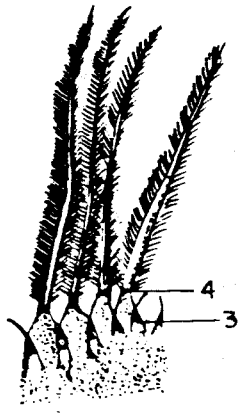


Fig.6
STAGE C

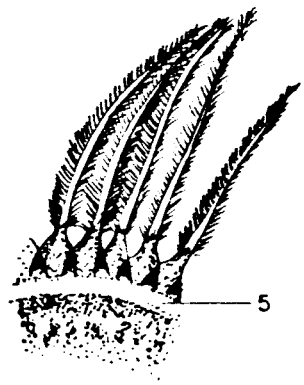


Fig.7
STAGE D_0

Fig 8
STAGE D'_1

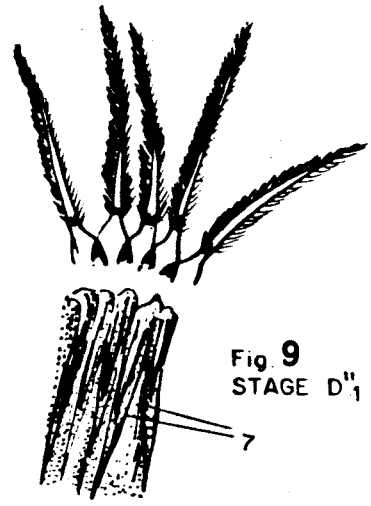


Fig. 9
STAGE D''_1

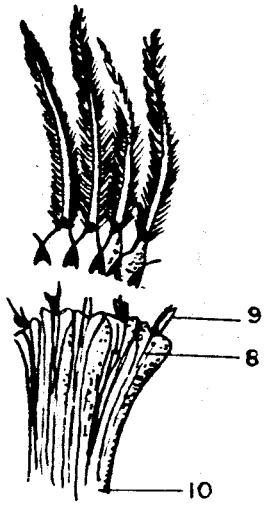


Fig 10
STAGE D'''_1

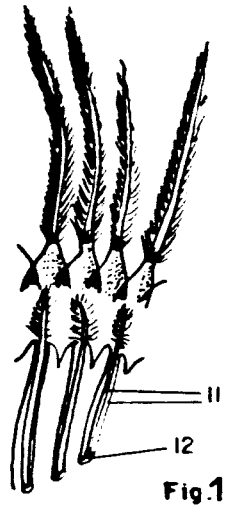


Fig.11
STAGE D_{2-3}

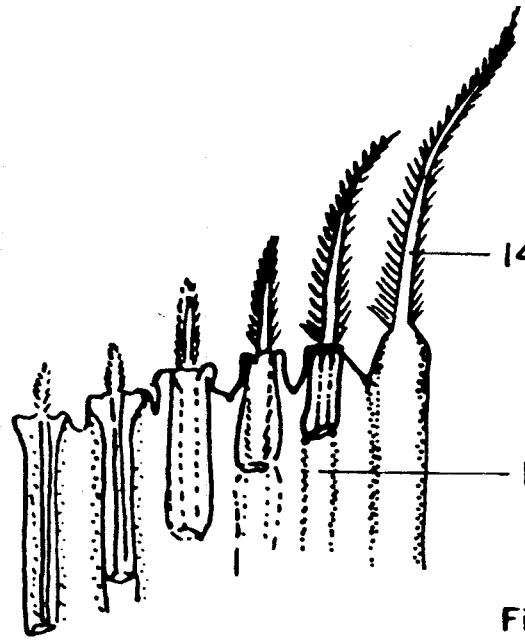


Fig. 12

1. Granular Cytoplasm, 2. Setal articulation, 3. Cuticular nodes,
4. Setal Cones, 5. Amber Coloured Zone, 6. Scalloped Cytoplasm.
7. Setal wall, 8. New Setae, 9. Setal shaft
10. ill defined setal base
11. Tube in tube structure
12. Well defined setal base
13. Setal track
14. New setae

transparent due to the epidermal retraction from the setae. Carapace and rostrum became firm and rigid. Stage C extended for a period of 34-48 hrs.

The onset of premoult period was marked by the separation of cuticle at the base of the setae, and subsequent development of new setae was noticed during this period.

Stage Do (early premoult) Fig: 7 and 17

Appearance of an amber coloured zone at the tip of the uropod, due to the retraction of epidermis from the cuticle between the bases of the setae, was the first indication of premoult as observed in the present study. The epidermal retraction was found to start from the tip and later on extended towards either side of the uropods. This stage lasted for 20-24hrs.

Presence of newly developing setae at the base of old setae in the borders of the uropod was noticed during the stage D1. Stage D1 is divided into substages of D1', D1'', D1''', and D2-3 were determined on the basis of detailed observation on the morphology and extent of the newly developing setae.

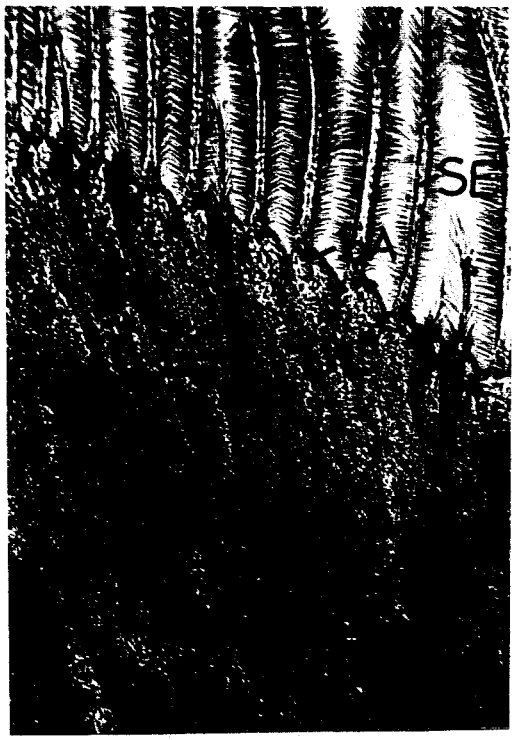
Stage D1' (early premoult) Fig:8 and 18

Fig. 13-22. Photomicrograph of setal development in P. indicus

13. Stage A, GC-granular cytoplasm; SE-Seta; SA-Setal Articulation, X 100 .

14. Stage B, CN-Cuticular Node; SA-Setal Articulation; SL-Setal Lumen, X 100.

15 and 16. Stage C, CN-Cuticular Node; SC-Setal Cone, X 100 and 400X.

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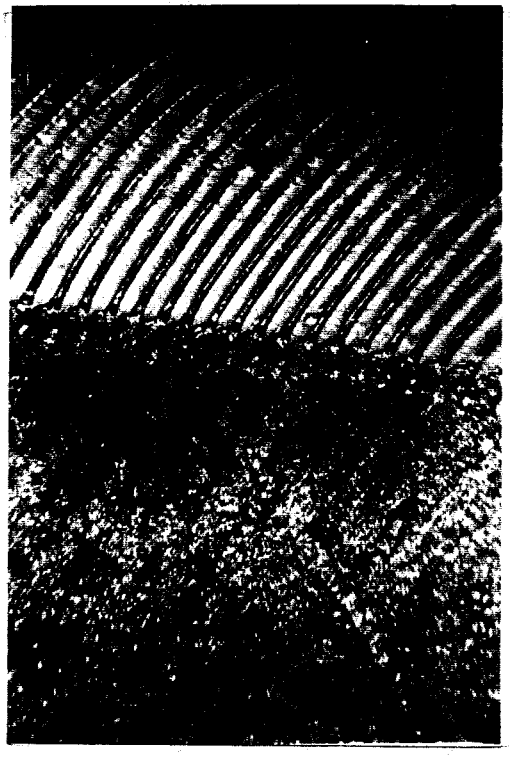


Retraction of the epidermis under the setae increased further in this stage. Condensation of protoplasm was noticed in the region of the formation of new setae. Later, protoplasm invaginated at the site of future setae giving a scalloped appearance. The scalloped apperance of the protoplasm is a striking character of stage D1'. This stage was lengthier with a duration of 48 - 72 hrs.

Stage D1'' (late premoult) Fig: 9 and 19

Relative to stage D1' the setal invagination became more distinct and deepened in stage D1''. Development of the new setal walls was observed while setal shafts had not made their appearance in this stage. D1'' lasted for 24 to 48 hours.

Stage D1''' (late premoult) Fig:10 and 20

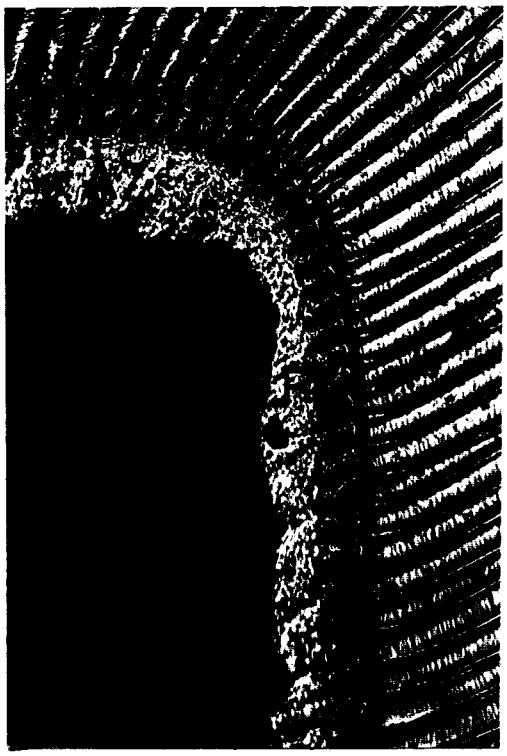
In stage D1''' setal invagination reached its maximum, and the new setae were entirely visible in the matrix. The shafts of the developing setae were visible immediately above the epidermal surface, but the setal shafts were without well developed barbules. Fully developed setae with an ill-defined proximal end, which have a forked appearance, was found to be the main characteristic feature of the stage D1'''. This stage lasted for 20 to 36 hours.

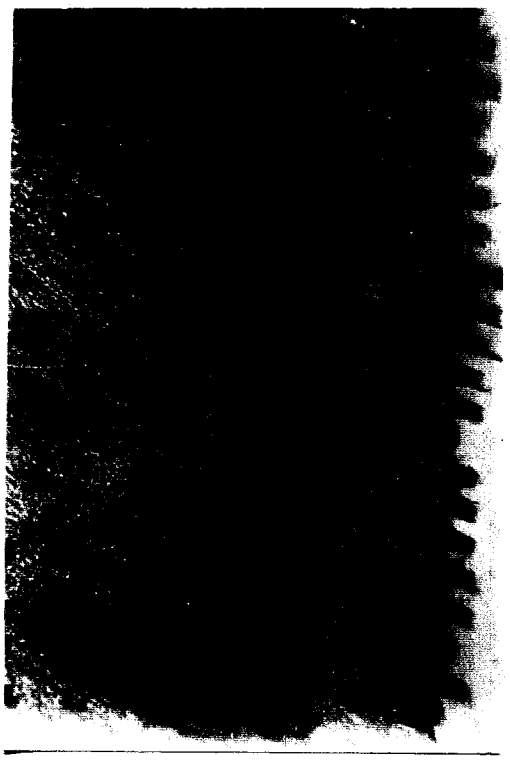
Fig. 17. Stage Do, AZ-Amber coloured zone; RE-Retracted epidermis, X100.

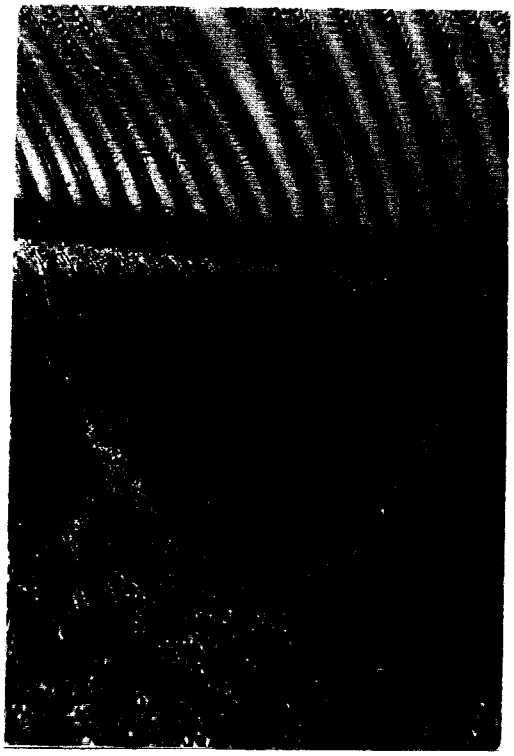
Fig. 18. Stage D1', SP-Scalloped epidermis, X100

Fig. 19. Stage D1'', CP-Condensed protoplasm in the region of the newly developing setae; IN-Invaginated epidermis, X150

Fig. 20. Stage D1''', NS-Newly developed seta with ill defined proximal end; ST-Shafts of the newly developing setae, X150







Stage D2-3 (late premoult) Fig: 11 and 21

Morphological observations have not revealed any notable differences between stages D2 and D3. Therefore, in the present study these two stages were combined and expressed as a single stage. Stage D2-3 varied from the previous stage of D1''' in that, the fully developed new setae had an appearance of 'tube-in-tube' structure with well defined blunt proximal end in place of the bifurcate proximal end. Development of the setae was completed with setal shafts possessing barbules. This is the last and final stage before ecdysis. D2-3 had a shorter duration of 8 to 14 hours.

Stage E (Ecdysis) Fig:12 and 22

Once the animal entered the stage D2-3, the next process observed was the ecdysis, which took place in stage E. This is the process during which the prawn draws out itself from the old exoskeleton and everts the setae of the new exoskeleton. The process of exuviation in P. indicus takes less than 60 seconds.

The moult stages thus identified in the present investigation can be broadly classified into three groups as postmoult, intermoult and premoult. Postmoult was

Fig. 21. Stage D2-3, BP-Blunt proximal end of the new seta; DS-Distal half of the new seta; PS-Proximal half of the new seta ; TT-Tube in tube stage of the newly developing seta , X600.

Fig. 22. Stage E, SE-Setal eversion, X100

comprised of the early postmoult stage A and late postmoult stage B, while the intermoult stage had only one stage, C. Stages Do, D1', D1'', D1''' and D2-3 were categorised under the premoult stage.

3.2.Histological changes observed in the cuticle during moult cycle

Moult staging criteria formulated by setogenesis process in the present study were verified using histological preparations of cuticle collected during different stages of moult cycle. On the basis of cuticular development, postmoult stages of A and B, intermoult stage of C and late premoult stages of D1''' and D2-3 were identified by distinct histological features of the cuticle, but further classification was not possible due to the absence of sharp distinguishable features. Further, the stages identified on the basis of cuticular development confirmed the identity of the stages done on the basis of setal development.

Stage A (early postmoult) Fig: 23a

In the early postmoult stage A, histological studies of the cuticle revealed the presence of only the pre-exuvial layers of epicuticle and exocuticle. Presence of a well-defined epidermal cell layer with elongated

epidermal cells with an average cell height of 40/u was found to be characteristic feature of stage A (Fig.23b). The cuticle was flexible and very soft to touch.

Stage B (late postmoult) Fig:24

Appearance of endocuticular layer marked the onset of stage B. In addition to the fully formed epicuticle and exocuticle, endocuticle made its appearance as a narrow strip under the exocuticular layer. Epidermal cell height was considerably reduced to an average of 17/u

Stage C (intermout) Fig:25

Stage C was found characterised by the presence of all the three fully formed layers of cuticles viz. epicuticle, exocuticle and endocuticle. Epidermal cell layer, during stage C, was not well defined. Cells were of minimum size with an average cell height of 7 /u.

In stages Do and D1' cuticular structure did not show any significant changes from that of stage C.

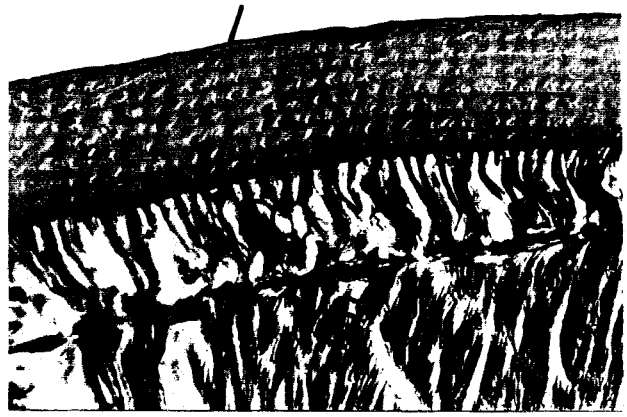
Stages D1''' and D2-3 (late premoult) Fig:26

In the late premoult stages of D1''' and D2-3, development of new cuticle was observed under the old cuticle. Pre-exuvial layers of exocuticle and epicuticle were seen under the old cuticle. In the preparation of

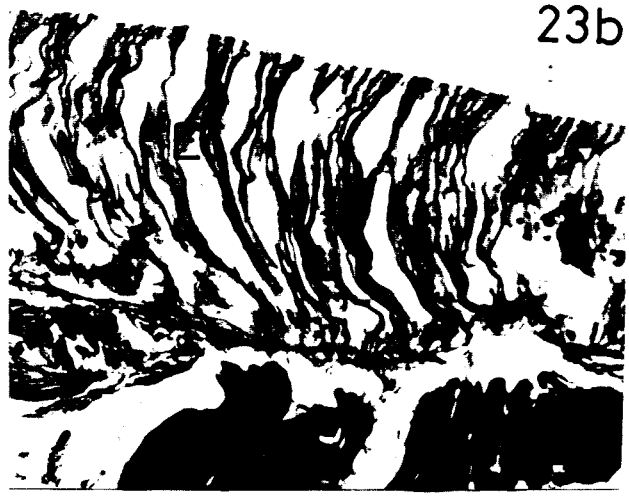
Fig. 23a, 23b, 24 Photomicrographs of the sections of integumental tissue of P. indicus.

23a, Stage A, X200; 23b, Stage A Epidermal layer; 24 Stage B, X400, Mallory's Triple Stain; EC-Epidermal cell layer; EN-Endocuticle; EP-Epicuticle; EX-Exocuticle; MS-Muscle

23a



23b



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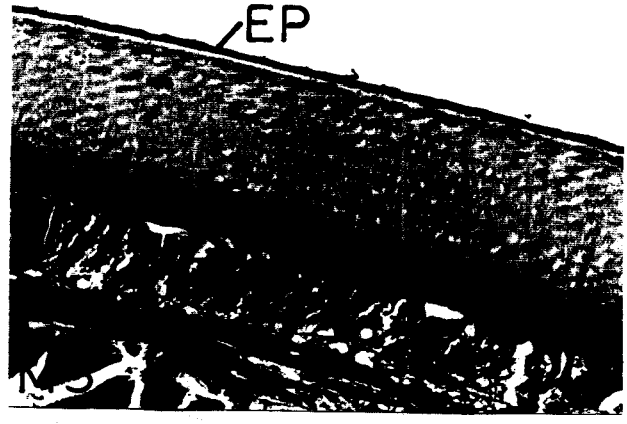
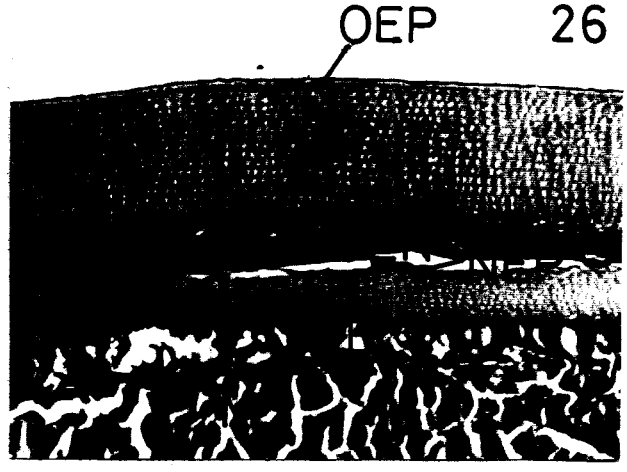


Fig. 25 & 26. Photomicrograph of the sections of integumental tissue of P. indicus.

25, Stage C, X200; 26, Stage D1''' and D2-3 (late premoult), X200. Mallory's Triple Stain.

EC-Epidermal cell layer; EN-Endocuticle; EP-Epicuiticle; EX-Exocuticle; MS-Muscle; NEP-Epicuticle of the newly developing exoskeleton; NEX-Exocuticle of the newly developing exocuticel; OEN-Endocuticle of the old exoskeleton; OEP-Epicuticle of the old exoskeleton; OEX-Exocuticle of the old exoskeleton.


OEP 26

the approaching moult, the endocuticle of the old exoskeleton partly disintegrated due to the action of the moulting fluid secreted under the old exoskeleton. Hence, during the late premoult stage, the cuticular layers found in P.indicus were the old epicuticle, old exocuticle, a part of the old endocuticle, new epicuticle and the newly developing exocuticle. The epidermal cell layer appeared very distinct and had enlarged considerably, with cell height of 46 to 50 /u.

The changes observed in the epidermal cell size during the different stages of moult cycle were very distinct (Table 1 and Fig.27).

3.3.Moult cycle duration

The experiments conducted on moult cycle behaviour of P.indicus indicated the dominance of premoult period in one complete moult cycle, while the post moult period was of the shortest duration (Table 2 and Fig.28). In the present study it was observed that, the length of the premoult period was the, longest comprising 71% of one moult cycle. Post moult and intermoult occupied the remaining part of the moult cycle, with 18.35% and 10.45%, respectively.

Moult cycle duration for the three size groups of

Table - 1: CHANGES OBSERVED IN THE EPIDERMAL CELL HEIGHT DURING THE DIFFERENT MOULT STAGES OF *P. INDICUS*.

Moult Stage		A	B	C	D_o	D_1'''	D_{2-3}
Height of the epidermal cell (μ)	N	6	6	8	8	8	6
	$\bar{X}$	40	17	7	7	40	46
	SD	± 3	± 4	± 2	± 2	± 3	± 2

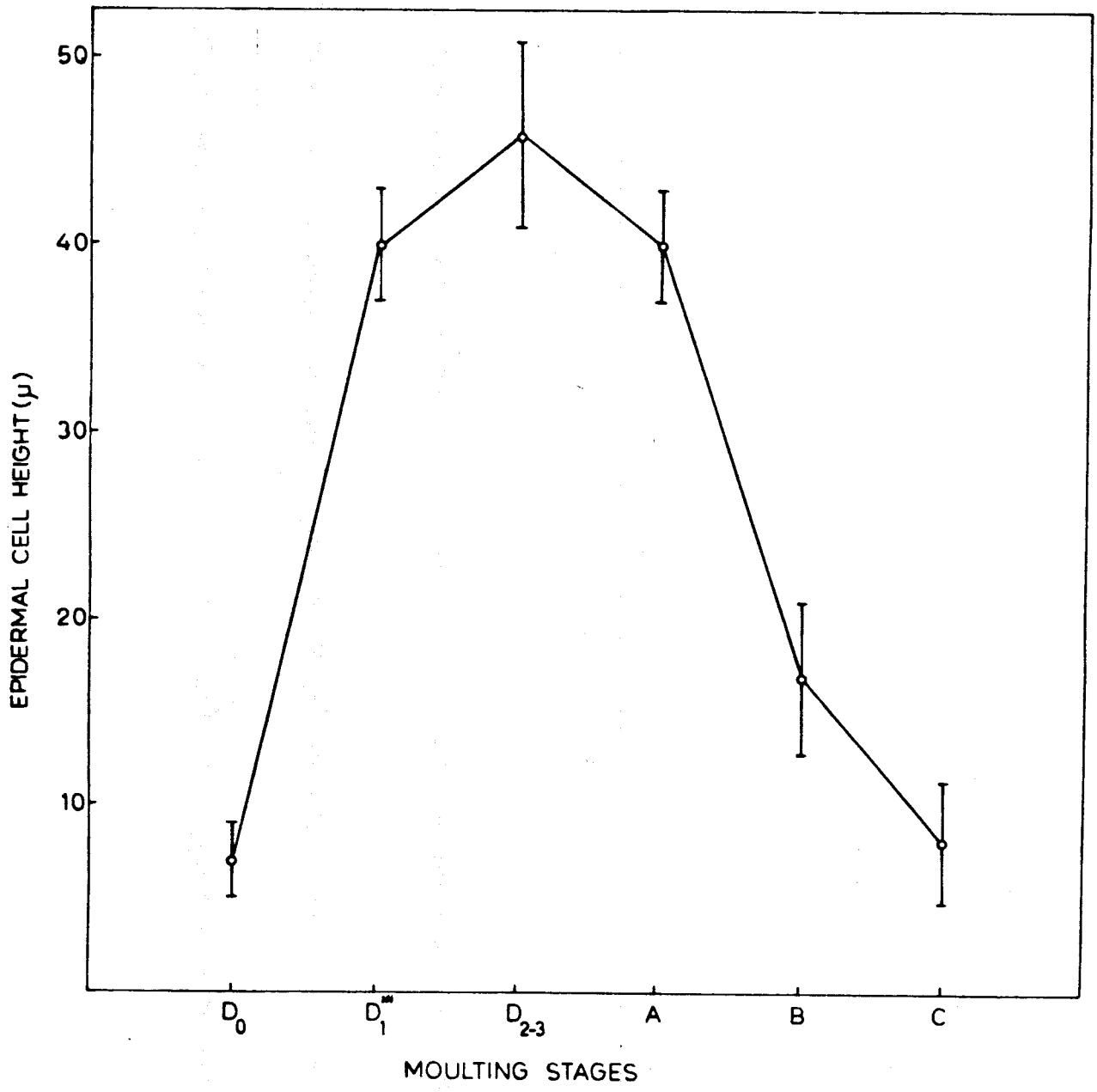


Fig. 27. Changes in the epidermal cell height during different stages of the moult cycle in P. indicus.

Table - 2: CRITERION FOR THE MOULT STAGING IN *P. INDICUS* ON THE BASIS OF SETOGENESIS. DURATION OF STAGES WERE CALCULATED ON A 20 DAYS OBSERVATION.

Moult Stage	Characteristic features	Approximate duration in hrs	Average % duration	
A	Body soft and slippery to touch. Rostrum flexible. Granular protoplasmic matrix continuous in the new setae. Setal cones and cuticular nodes absent.	3 - 7	2.1	Postmoult 10.45%
B	Parchment like integument well developed. Setal articulation and poorly developed cuticular nodes. Setal cones absent.	16 - 22	8.35	
C	Rigid exoskeleton with firm rostrum. Presence of well defined setal cones and cuticular nodes.	36 - 48	18.35	Intermoult 18.35%
Do	Retracted epidermis with an amber coloured zone at the tip of the uropod.	24 - 36	13.05	Premoult 71%
D1'	Setal invagination and scalloped epidermis.	48 - 72	26	
D1''	Protoplasm condenses in the region of the setae. Setal invagination deepened, marking the appearence of new setal walls.	24 - 48	15.2	
D1'''	New setae appears in the uropod matrix with double wall. Setal invagination completed. Shafts visible at the tip of the setae. Proximal part of the setae illdefined.	20 - 36	11.9	
D2-3	Fully developed new setae appears in the matrix as 'tube in tube' structures. Proximal part blunt. Well developed shafts and barbules.	8 - 14	4.75	
E	Rejection of the old cuticle.			

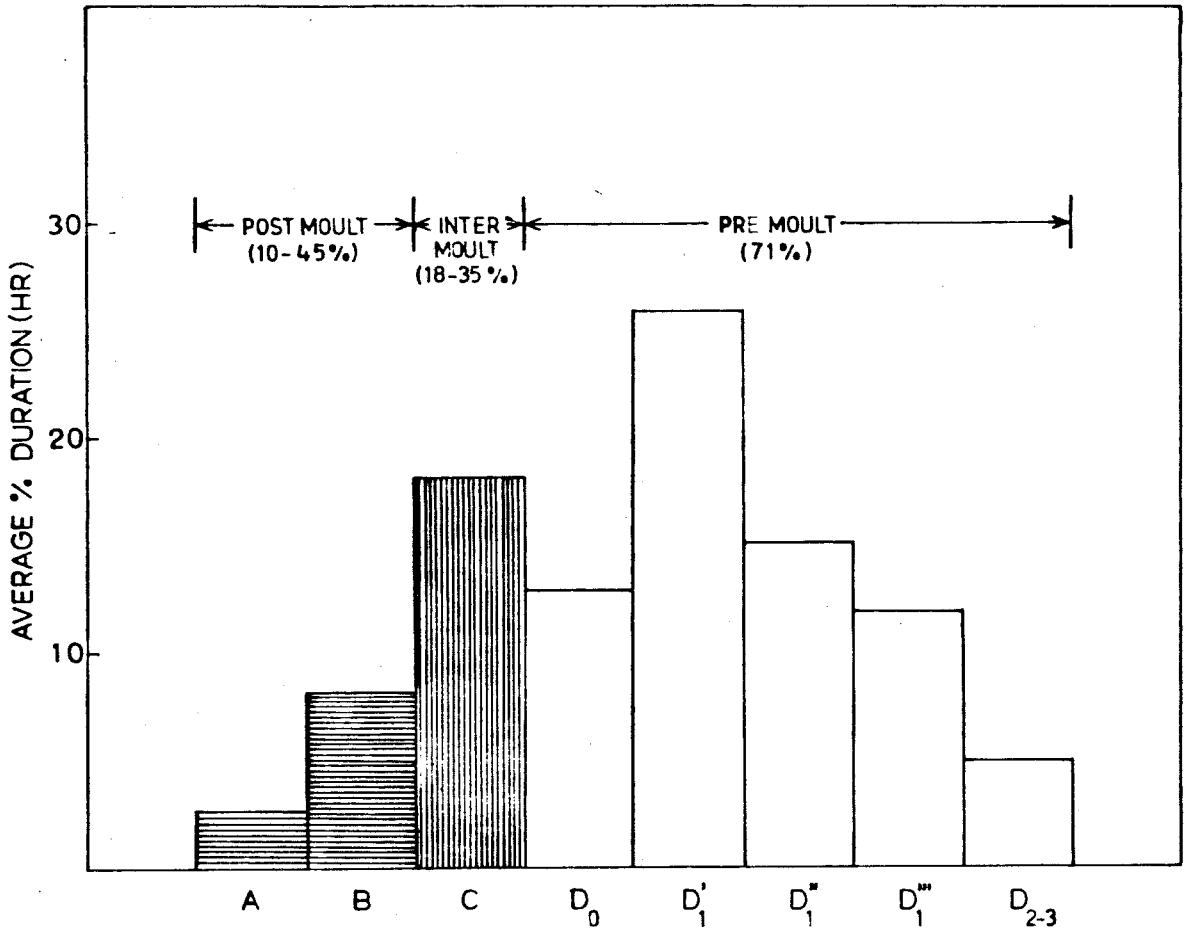


Fig. 28. Average percentage duration of different moult stages in P. indicus.

animals viz. 30-40 mm, 60-80 mm and 80-120 mm in total length, was studied so as to observe relation between the body size or age and duration of moult cycle. Results (Table 3 and Fig.29) showed a significant linear relationship between the size or the age of the animal and the moult cycle duration. The duration of the moult cycle was found to be more with increase in the size of the animal. In juvenile prawns of size 30-40 mm, average moult cycle duration was 96 hours with a premoult period of 76 hours, whereas in young adults of size 60 - 80 mm, average moult cycle duration recorded was 180 hours with an average premoult period of 130 hours. When the animals reached the adult size of 80-120 mm, the average moult cycle duration increased to 240 hours with a premoult period of 165 hours.

Mechanism of exuviation

Of the 38 prawns observed in the laboratory 36 animals (95%) moulted during the night hours (Table 4 and Fig.30). Twenty four animals moulted between 00 to 04 hours of the day. Eight animals moulted in the early hours i.e. between 04 to 07 hours and 4 animals moulted between 22 to 00 hours . Only two animals were observed to moult after 07 hours in the morning.

Table - 3: MOULT CYCLE DURATION FOR DIFFERENT SIZE GROUPS OF *P. INDICUS*.

Size group (mm)	No.	Average premoult duration (hrs) with standard deviation. (hrs)	Average moult cycle duration (hrs) with standard deviation. (hrs)
30 - 40	15	76 ± 14	96 ± 16
60 - 80	15	130 ± 20	180 ± 24
80 - 120	15	165 ± 24	240 ± 48

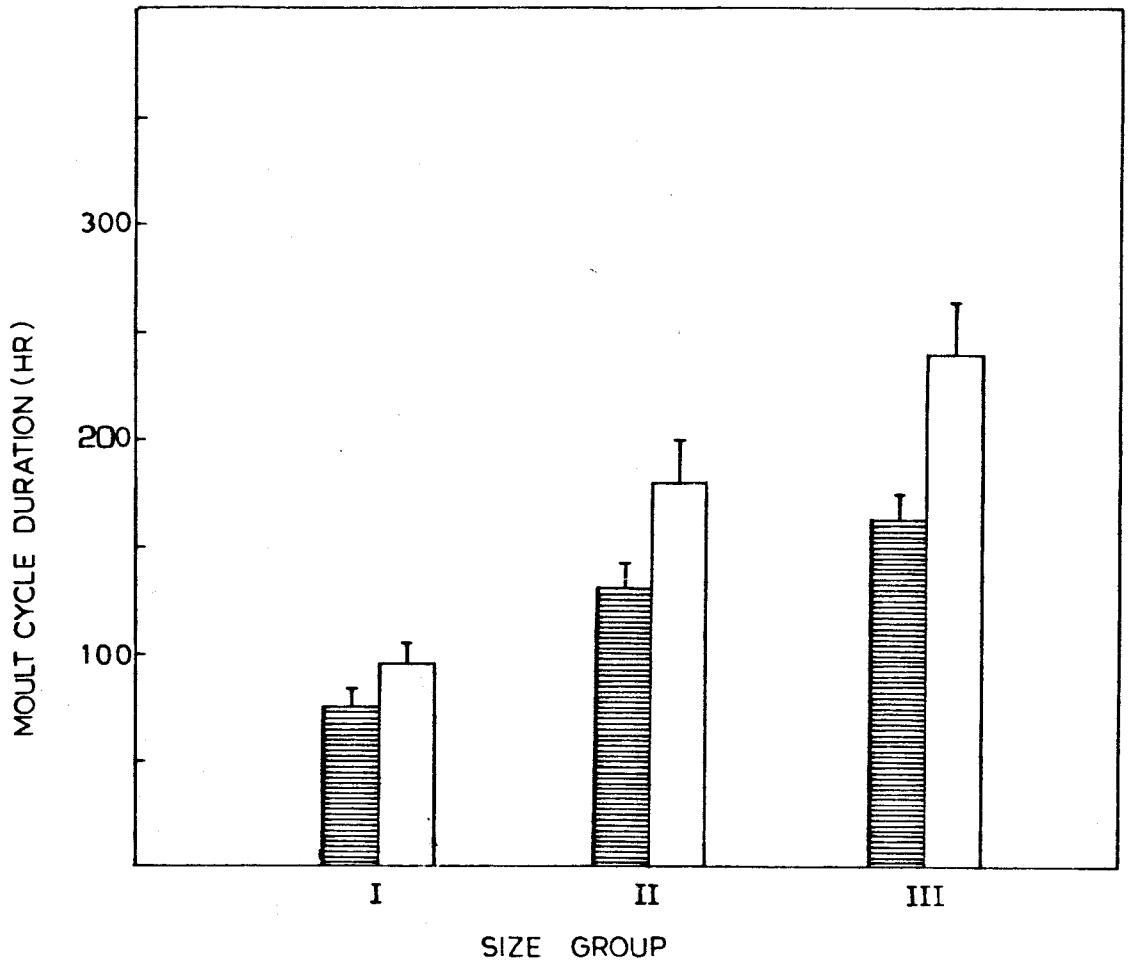


Fig. 29. Average moult cycle duration in different size groups of P. indicus. I, 30-40 mm: II, 60-80 mm: III, 80-120 mm:

Premoult: Total moult cycle:

Table - 4: MOULTING PATTERN OF *P. INDICUS* DURING DIFFERENT HOURS OF THE DAY.

Time of moult (hrs)	No. of prawns moulted	% of prawns moulted
22 - 00	4	10.5
00 - 04	24	63.1
04 - 07	8	21
07 - 11	2	5.2

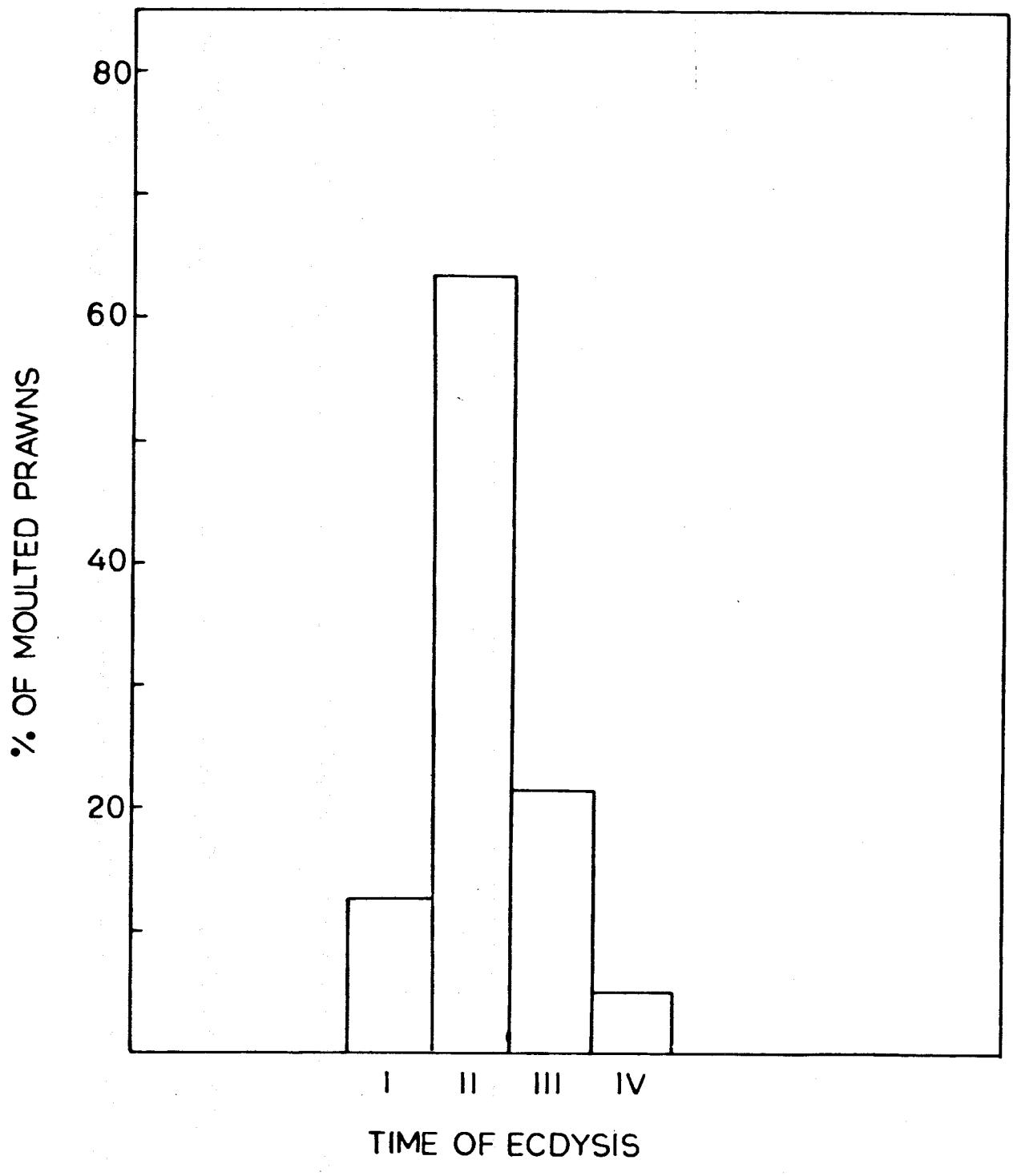


Fig. 30. Frequency of moulting time in P. indicus.

I, 22-00 hrs; II, 00-04 hrs; III, 04-07 hrs; IV, 07-11 hrs.

Prawns in the late premoult stage of D2-3 were generally found to be very active in midnight hours of the day. Prior to ecdysis, prawns were observed to perform several activities like swimming, walking, jumping, flicking, rolling and rotating movements. Rapid propulsion of the animal with the help of pereopods alongwith fluxing of the body convexily at the cephalothorax - abdomen joint was common. Animals stretched the body verticaly with the help of the arch centered on the third abdominal segment. This period of intense locomotion in the late hours of premoult probably helps the animal in the removal of the old exoskeleton from the newly formed one in the process of shedding of the old exoskeleton. It was observed that in the first phase of shedding, carapace was thrown out separately of the cephalothorax. The prawns then flicked violently, lifting the body out of the abdominal and ventral cephalothorax portion of the old exoskeleton. Following the moult, the prawns were found to lay on their side for an average of 5 to 30 minutes before attaining the movements with the aid of pereopods.

4.DISCUSSION

The method of determining moult stages in P.indicus, based on developmental changes in the setae of the uropods

is found in agreement with the studies of other workers who have studied the setogenesis in different crustacean species viz. Leander xiphias and Processa edulis edulis (Scheer 1960), Homarus americanus (Aiken 1973), Astacus leptodactylus (Vanherp and Humbert 1978), and Uca pugilator (Vigh and Fingerman, 1985). The setogenesis process observed in the present study is identical to the setogenesis process observed among other penaeids like P.merguiensis (Longmuir, 1983), P. monodon (Pudadera et al., 1984), and P.esculentus (Smith and Dall 1984). Stages A-B in the present study were characterised by a thin cutcile and a setal lumen which had not completely pinched off to form a setal cone, as noticed in the case of most other decapod crustaceans (Aiken, 1980). Lyle and MacDonald (1983) used granular protoplasm filling setae of the lobster P.marginatus as a major criterion for identifying A and B stages. Vanherp and Humbert (1978) who worked on the setogenesis of the cray fish Astacus leptodactylus also assigned all animals with granular cytoplasm in the setae to stage A. When granular cytoplasm was withdrawn from the distal part of the setae and setal cone began to appear, the moult stage of Astacus leptodactylus (Vanherp and Humbert, 1978) was designated to stage B. Thus, the key features of the postmoult stages

A and B are generally based on the changes in the distribution of internal matrix of the setae and the thickening of setal base. Other investigators like Vigh and Fingerman (1985) in crab, Reaka (1975) in stomatopods and Longmuir (1983) in penaeid have also used granular cytoplasm of the setae to identify the postmoult stages of A and B. Granular cytoplasmic matrix filling the new setae is beleived to help in the evagination of setae at the time of ecdysis and support the setae during the stages of A and B (Smith and Dall, 1984). Contradictory observation was made by Mills and Lake (1975), who have not observed the presence of setal matrix in notable amounts until late postmoult stage B in the crayfish Parastacoides tasmanicus. Workers like Scheer (1960), Peebles (1977), Longmuir (1983) and Smith and Dall (1984) reported the fully developed internal cone and cuticular node as the diagnostic character of stage C, i.e. the intermoult. Similarly in the present investigation the appearance of the internal cones in the setae and development of cuticular node at the setal base marked the end of stage B and onset of stage C in P.indicus. Further division of stage C based on microscopic examination of uropods was not possible.

The premoult stages vary the most, among the assorted

species of crustaceans studied, and have been modified to the greatest extent. Species differences probably account, to a large extent, for the variation observed. Apolysis signals the onset of premoult stage Do in P.indicus as described by Jenkin and Hinton (1966) in other arthropods. Virtually all investigation on crustacean setogenesis had taken the apolysis (Do) as the starting point of premoult development. Subsequent classification of the premoult stages into sub-stages on the basis of neosetal development as done in the present study agrees well with the observation of other workers like Aiken (1973) in Homarus americanus, Freeman and Bartell (1975) in Palaemonets pugio, Vanherp and Bellon Humbert (1978) in Astacus leptodactylus and Smith and Dall (1984) in P.esculentus. After D1''' only one stage is identified in P.indicus, before the actual moult E. Stage D2 and D3 are combined together and counted as a single last stage of the premoult. Further division was not possible due to the lack of noticeable characteristics. Similar type of observations were made by Vanherp and Humbert (1978) in the cray fish Parastacoides tasmanicus. Freeman and Bartell (1975) have not reported any substage beyond D2 while Nagabhushanam and Vasantha (1971) have reported only Do through D3. The fully developed setae in D2-3

stage in P.indicus appeared as a 'tube-in-tube structure' as observed by Stevenson (1968) in the cray fish Orconectes sanborni. This structure which begin its formation in D1'stages probably is similar to Aiken's (1973) 'setal organ'. In the present study D2-3 stage immediately follows stage E, during which time the actual shedding of exoskeleton takes place. Ecdysis in P.indicus is a rapid process which is not sub divided as reported by Longmuir (1983) in Penaeus mergueiensis and Smith and Dall (1984) in Penaeus esculentus. During stage E of the P.indicus all the setae are found to unfold under the cuticle like the 'straightening out of an inverted glove finger'as reported by Vanherp and Bellon-Humbert (1978) in Astacus leptodactylus, and Dexter (1981) in planktonic crustaceans. In the present observation, the new tips of the developing setae never extended into the lumens of the old seatae as described for the prawns, Leander xiphias (Scheer 1960), and cray fish Parastacoides tasmanicus (Mills and Lake, 1975)

The results of the present investigation showed the dominance of the premoult period in the moult cycle of the prawn exhibiting a typical diecdysis moult cycle as described by Knowles and Carlisle (1956) in Leander

serratus. Similar type of findings have been reported by Huner and Colvin (1979), Longmuir (1983) and Pudadera et al. (1984) while working on P.californiensis and P.stylirostris, P.merguiensis, and P.monodon respectively. But the present observation differs from that of Schafer's (1967), where he has reported a very long intermoult period and a very short premoult period for P.duorarum.

In the present study young juvenile prawns moulted faster with short moult cycle duration, while in the adults moult frequency was slow with a lengthy moult cycle. The moult cycle duration which extends depending on the size or the age of the animal indirectly reflects the rate of growth.

The setogenic moult staging in P.indicus was verified on the basis of structural changes of the epidermis and integumentary tissue during the moulting cycle. Histological studies on the integument of P.indicus revealed the general crustacean pattern of cuticular organization, with the exception of a membraneous layer. (Travis, 1960b 1963; Skinner, 1962 and Diwan and Nagabhushanam, 1975). The cuticular changes observed during the moult cycle of P.indicus fall in line with the observation of Dall (1965a) in Metapenaeus sp and Smith

and Dall (1984) in P.esculentus . But Schafer's (1967) report about the presence of membraneous layer in P. duorarum was not observed by any of the penaeid workers so far. Other than the work of Skinner (1962) in Gecarcinus lateralis and Smith and Dall (1984) in P. esculentus, no detailed study is available relating the integumental changes with distinct moult stages. The results of the current study find similarity with the observation of Skinner (1962) and Smith and Dall (1984), who have studied the integumental changes in Gecarcinus lateralis and P.esculentus during the different stages of moult. Drach (1939) and Stevenson (1972) found A2 as the stage in which secretion of post exuvial endocuticle takes place, and they described this stage as the universal criterion for the deposition of principal layer, the endocuticle, where as in P.indicus, beginning of secretion of the post exuvial endocuticle was noted in stage B. Stage C of P.indicus is characterized by the fully developed and hardened cuticle with all layers of epicuticle, exocuticle, and endocuticle. This is true in the case of G.lateralis (Skinner, 1962) and P.esculentus (Smith and Dall, 1984). Further differentiation in the integument of P.indicus was not observed till the animal reached the stage DI'''. This is the stage in which pre-

exuvial layers made their appearance, and by D2-3 the unhardened pre-exuvial layers had developed completely, as observed by Skinner (1962) and Smith and Dall (1984).

The differences observed in the epidermal cells of P.indicus during different moult stages with a maximum cell size in late premoult and early postmoult and with a minimum cell size in intermoult are identical to the observation made in other crustaceans by Travis (1960a), Skinner (1962), Dall (1965a), and Smith and Dall (1984). The large elongated size of the epidermal cell during the late premoult and early postmoult found in the present study is indicative of the higher activity of the cells due to their involvement in the secretion of new cuticle as described by Steveson (1972).

Observation on the moulting behaviour in the present study showed that majority of the prawns (95%) moulted during night hours, of these 63% of prawns moulted between 00 to 04 hrs. Wassenberg and Hill (1984), while working on P.esculentus, also observed that 63% of their experimental animals moulted during the night hours. Available data in Panulirus argus (Lipcus and Herrnkind, 1982), P.Cygnus (Thomas, 1966) and Jasus lalandii (Paterson, 1969) also showed that the crustaceans

generally prefer night hours for moulting. The active movement pattern exhibited by P.indicus during the pre-ecdysal period agrees with the observation made by Travis (1954) in Panulirus argus, Longmuir (1983) in P.Merguiensis and Wassenberg and Hill (1984) in P.esculentus. These authors (Travis, 1954; Longmuir, 1983 and Wasenberg and Hill, 1984) have concluded that the pre-ecdysal movements, no doubt, serve to loosen the old exoskeleton, to enable easy shedding of exuvia. The process of ecdysis in P.indicus was faster which lasted only for 30-50 seconds. In P.merguiensis, ecdysis as reported by Longmuir (1983) lasted 40 seconds while in P.duorarum Bursey and Lane (1971) reported that the time taken for ecdysis was 20-30 miniutes. This disparity could be due to the difference in defining ecdysis. In the act of ecdysis, P.indicus throws out its carapace first, followed by the remaining exoskeleton of abdomen and other appendages as a single unit just similar to the process described for P.esculentus by Wassenberg and Hill (1984).

S U M M A R Y

Characterization and classification of complete moult cycle of Penaeus indicus have been worked out on the

bases of setal development, and epidermal retraction in the uropods (setogenisis). Based on the setal morphology, the moult stages were identified as Postmoult (stages A and B), Intermoult (stage C), and Premoult (stages Do,D1',D1'',D1''' and D2-3). The setogenic moult staging was verified on the basis of structural changes in integumentary tissue, especially the epidermal cells, during the moult cycle. By studying cuticle histology moult stages A and B (postmoult), C (intermoult), and D1''' and D2-3 (late premoult) were identified. Setogenic moult staging used in the present study was found to be a rapid and simple technique for determining the different stages of the moult cycle. Since excision of appendage is not required, this technique is non-destructive and permits repetitive moult staging of an individual.

The average duration of one moult cycle with relative duration of each stage was determined in the adult P.indicus. Premoult occupied the major part of the moult cycle (71%), followed by intermoult (18.35%), and postmoult (10.45%).

During the present work, a linear relationship was observed between the size or age of the prawn and the moult cycle duration. The duration of the moult cycle was

found to be more with increase in size or age of the prawn. Observation on the moulting behaviour showed that majority of the prawns moulted during the late hours of the night, especially between 00 and 04hrs. P.indicus was found to be very active prior to moult, and the whole process of ecdysis was faster which lasted only for 30-50 seconds.

CHAPTER-II STRUCTURE OF NEUROENDOCRINE SYSTEM AND ITS CONTROL ON MOULTING

CHAPTER II

STRUCTURE OF NEUROENDOCRINE SYSTEM AND ITS CONTROL ON MOULTING

1.INTRODUCTION

In crustaceans somatic growth is achieved by the periodic shedding and reformation of the exoskeleton accompanied by the tissue growth. The periodic shedding of exoskeleton is accomplished by moulting, which is the external manifestation of a discontinuous growth process. A great deal of information has been accumulated and reviewed time to time describing the hormonal basis of moulting in crustaceans (Carlisle and Knowles, 1959; Passano, 1960; Highnam and Hill, 1979; Cooke and Sullivan, 1982; Chang, 1985; Quackenbush, 1986, and Fingerman, 1987).

Genuine interest in crustacean endocrinology developed during the period 1928 to 1939, when hormonal control of both moulting and integumentary chromatophores was demonstrated (Perkins, 1928; Kleinholz, 1936; Hanstrom, 1937; Brown and Cunningham, 1939). Since then investigations carried out by several crustacean workers established the classical hypothesis of hormonal control of moulting (Passano, 1960; Aiken, 1978; Cooke and Sullivan, 1982; Skinner, 1985; Fingerman, 1987). According

to this hypothesis the moult cycles in crustaceans are regulated by the interaction of two hormones, the Moult Inhibiting Hormone (MIH) and the Moulting Hormone (MH). The putative moult inhibiting hormone (MIH) in crustaceans is produced in eyestalk neurosecretory cells, designated as X-organ and released from the axonal endings into the sinus gland, a neurohaemal organ of the eyestalk (Aiken, 1978; Andrew et al., 1978; Andrew and Saleuddin, 1979). The moulting hormone is secreted by a discrete pair of endocrine glands situated in the cephalic region of the animal termed as the 'Y-organ'.

In addition to these two specific hormones, possible involvement of a third one, i.e. the Moult Accelerating Hormone (MAH) from the central nervous system, in the moult control has also been reported (Bliss, 1956; Passano, 1960, 1961; Matsumoto, 1962; McWhinne and Mohrherr, 1970a). But the concept of moult control by the antagonistic hormones, MH and MIH remained as the classical hypothesis.

Eyestalk factors have been implicated in the regulation of a large variety of physiological processes. Approximately ten active factors including MIH, pigmentary, ovary inhibiting factors, as well as several

factors that control blood sugar and atleast one that regulate water and salt exchange have been isolated from the eyestalk of various species of crustaceans (Newcomb, 1983; Kleinholz, 1976). Precise bio-chemical information on the nature of the involved active factors and their physiological specificity is, however, still very much limited. It is not known how many individual substances are responsible for the many biological effects of eyestalk extracts (Cooke and Sullivan, 1982). The existence of the Moult-Inhibiting Factor in the eyestalk was demonstrated by various authors using the wellknown technique of eyestalk ablation, which resulted in a precocious moulting, and subsequently injection of eyestalk extract inducing inhibition of moult in the eyestalkless animals (Diwan and Nagabhushanam, 1974; Freeman and Bartell, 1975; Nakatani and Otsu, 1979; Quackenbush and Herrnkind, 1981; Bruce and Chang, 1984; Radhakrishnan and Vijayakumaran, 1984; Webster, 1985; Snyder and Chang, 1986).

Evidence for the 'Y-organ' as the source of moulting hormone (MH) in controlling moult process in Crustacea has been accumulated from a number of different types of observations and experiments. The classical

extirpation and reimplantation experiments of Echalier (1955 and 1959) were the first to provide evidence of the moult controlling function of the Y-glands. Echalier's results have later been confirmed by similar studies, on other brachyura (Passano and Jyssum, 1963 and Carlisle, 1957), in penaeids (Bourguet et al 1977) in isopods (Maissiat, 1970; Charmantier and Trilles, 1977) and in amphipods (Burghause, 1975 and Keller and Willig, 1976). That the moult inhibiting effect of Y-organ removal could be compensated for by the administration of ecdysteroid has later been demonstrated (Blanchet, 1974; Keller and Willig, 1976; Freeman and Bartell, 1976). Recent advanced analytical techniques like Radio Immuno Assay (RIA) and organ culture, made possible direct proof of the ecdysteriod secretory nature of the Y-organ (Chang and O'Connor, 1978; Sumoff and O'Connor, 1982; Jegla et al., 1983; Watson and Spaziani, 1985a, 1985b).

Suspected involvement of other tissues in the moult controlling function with cytological similarity to the Y-organ has been reported by many workers (Byard et al., 1975, Gersch, et al., 1979; Yudin et al., 1980; Borst et al., 1985). These structures were termed as the Mandibular gland or Cephalic gland. Mandibular gland was first classified by Le Roux (1968) in the cephalothorax of

brachyuran decapods. Later reports on the structure and its role as a moult controlling gland were contradictory in nature (Aoto et al 974; Byard, et al., 1975; Hinsch, 1977; Yudin et al., 1980; Borst et al., 1985).

To study the mechanism by which the neuroendocrine and endocrine systems regulate moulting, it is very much essential to know the morphohistology and histochemistry of these systems. Works conducted by earlier workers revealed the complexity in the classification of neurosecretory structures of different Crustacean species (Hisano, 1974; Smith and Naylor, 1972; Vanherp et al., 1977; Bellon-Humbert et al., 1981; Nanda and Ghosh, 1985; Chandy and Kolwalkar, 1985 and Nagabhushanam et al., 1986). Because of the structural complexity of neurosecretory system among the species studied, it becomes necessary to study every species in well-defined conditions, so as to understand the neuroendocrine system of such selected organisms.

Unfortunately, under Crustacea, natantia in general and penaeids in particular has got only little attention with regard to the studies on neurosecretory systems and their related functions. Dall (1965b) in Metapenaeus sp and Nakamura (1974) in Penaeus japonicus studied the

neurosecretory structures, and highlighted its general resemblance to the other group of crustaceans, in its organisation. Among the Indian Penaeids Metapenaeus monoceros (Madhyastha and Ranganekar, 1976), Penaeus monodon (Nanda and Ghosh, 1985), and Parapenaeopsis stylifera (Nagabhushanam et al., 1986) are the few members, which received brief attention, as far as the neurosecretory structures are concerned. But none of these authors has studied these structures in relation to the moult cycle of the prawn. The physiological significance of the neurosecretory systems with regard to the moulting cycle in penaeids still requires elaborate studies.

Studies on the moult regulating endocrine organs in natantia are limited, and in penaeids it is meagre. Basic informations about the Y-organs are available only in Metapenaeus sp (Dall, 1965b), and in Penaeus japonicus (Bourget et al 1977). Detailed studies on the panaeid Y-organs and its moult controlling functions are not available in the literature.

The survey of the literature reveals that there is dearth of basic informations related to the penaeid endocrinology and its control over the key event in crustacean life cycle, viz. moulting. Therefore, in the

present study an effort is made to elucidate the structure and moult linked physiological functions of the endocrine systems of the penaeid prawn, Penaeus indicus.

2. MATERIALS AND METHODS

1. Animals

Specimens of prawn, Penaeus indicus for the present study were collected from the prawn fields in and around Cochin and also from the prawn farm of Marine Prawn Hatchery Laboratory, Narakkal. Throughout the study, healthy adult prawns yet to attain reproductive maturity were selected for avoiding the chance of reproductive interference with the process of moulting.

Live prawns were transported from the field to the laboratory at Cochin in IOL polythene transporting bags (Plastic Craft Corporation, Bombay) filled with water collected from the same area of collection. Transportation bags were properly aerated, with each bag containing 3 to 6 prawns. Animals, after being brought to the laboratory, were maintained individually in floating plastic cages, suspended in the circular plastic pools of 350L capacity with recirculating sea water. Records about the moulting details of each individual animals were maintained.

2. Dissection and fixation of tissues

In order to correlate the histological and histochemical changes occurring in the neuroendocrine centres (Eyestalk, Brain, Thoracic ganglia, Y-organ, and Mandibular organ) in relation to different stages of moulting cycle, live prawns of different moult stages were selected and sacrificed, and different neuroendocrine centres viz. Eyestalk, Brain, Thoracic ganglia, and endocrine organs viz. Y-organ and Mandibular organ were excised out. Tissues were fixed immediately in suitable fixatives. Moult stage identification was done according to the method described in the scheme of moult cycle classification in Chapter-I, using the technique of setal morphology.

For general histological studies, various tissues were fixed in Bouin's fluid, and for histochemical purpose except for lipid and cholesterol, 10% buffered neutral formalin was used as fixative. For lipids, tissues were fixed in formal calcium. Generally, the volume of fixative used was 20 times to that of the tissue. All the tissues fixed were directly transferred to 70% ethanol after 36-48 hours of fixation, and stored in glass tubes

at room temperature for further processing.

3. Processing of tissues

3.1. Decalcification

Cuticular materials of the eyestalk, Y-organ, and Mandibular organ were decalcified using acid decalcification (concentrated nitric acid with 70% ethanol in proportion 2:100 for a period of 2-24 hours).

3.2. Dehydration, clearing, impregnation and blocking

In order to make paraffin blocks for section cutting, dehydration and clearing of tissues were carried-out at the room temperature. From 70% ethanol, tissues were passed through graded series of ethanol. Clearing of tissues was done using methyl benzoate. This reagent ensured the complete clearing of the tissue without over hardening. The tissues from absolute ethanol were transferred to a mixture of methyl benzoate and absolute ethanol in the ratio 1:1. Methyl benzoate was added to ethanol with a dropper so that methyl benzoate formed a layer below the ethanol and the tissues floated at the interphase of the methyl benzoate and ethanol. When the tissues were completely cleared, they sank to the bottom of the container, and became almost transparent. The

tissues were then transferred to benzene for about 2 minutes to remove methyl benzoate and further they were left in a mixture of benzene and paraffin wax in 1:1 ratio at room temperature for over-night for cold impregnation. Before embedding, the tissues were treated with molten paraffin wax (MP 56 °C to 58 °C) for 1hr for hot impregnation. Blocks were made with proper orientation of tissues, and kept with proper code numbers.

4.Sectioning and staining

4.1 Sectioning

Sections for histological and histochemical observations were cut at 6-8 /u thickness using WESWOX MODEL manual rotatory microtome. After deparaffinizing in xylene the sections were hydrated through descending grades of ethanols (100% to 30%) and finally brought to distilled water. Hydrated sections were used for staining with different types of stains depending on the requirements. Histochemical tests for lipid were performed on frozen sections of the respective tissue, which were prepared by fixing the tissue in 10% Baker's formal calcium and then impregnating with 12.5% and 25% gelatin solutions. The blocks were hardened in 5% formaldehyde. Frozen sections of 10 /u thickness were cut in American Opticals Histostat at -20°C, and then stained.

4.2 Staining

For the study of neurosecretory system in the eyestalk, brain, and thoracic ganglia neurosecretory specific staining techniques elaborated by Pearse (1968) were used. Staining techniques used in the present study were Mallory's Triple (Mallory, 1944), Chrome-haematoxylin phloxine (CHP) (Bargman, 1949), and Aldehyde Fuchsin (AF) (Kurup, 1972). The rationale of Chrome-haematoxylin phloxine, Mallory's Triple and Aldehyde Fuchsin staining of the Neurosecretory Material (NSM) is based on the affinity of these stains for the acidic groups, appearing after oxidation of neurosecretory material with oxidising agents such as performic acid and potassium permanganate. The specific oxidation of the NSM involves the formation of the cysteic acid from both cysteine and cystine which are well represented in the neurosecretory material of crustaceans (Lake, 1970). The same oxidation process produces free aldehyde group, to which the basic stains can bind (Gabe, 1953). Y-organ and Mandibular organ were stained with Haematoxylin and Eosin.

Mallory's Triple Stain (Mallory, 1944)

For Mallory's Triple, a primary mordant ($HgCl_2$-acetic acid) was used prior to the fuchsin staining, and then

sections were stained with Mallory's Triple stain.

Chrome - Haematoxylin Phloxine (CHP) (Bargman, 1949)

For CHP hydrated sections were subjected to mordant in Bouin's fluid. After oxidising with Potassium Permanganate and bleaching with oxalic acid, sections were stained with Chrome-haematoxylin in cold (4 °C) followed by phloxine.

Aldehyde Fuchsin (AF) (Kurup, 1972)

In the modified Aldehyde Fuchsin staining, Bouins fluid was used as the preliminary mordant. Then the sections were oxidised in potassium permanganate followed by treatment with Aldehyde Fuchsin stain. Lastly, counter staining was done using Halmi's mixture.

The following histochemical tests were carried out on serial sections of eyestalk, brain, thoracic ganglia, and Y-organ.

Mercuric Bromophenol blue test (For general proteins, Mazia et al., 1953)

Mercuric ions of the bromophenol blue solution react with acidic, sulphydryl, and aromatic residues of the protein to give blue colour.

Ferric-Ferricyanide Test for - SH Groups (Pearse, 1968)

This method depends on the reduction of a fresh solution of potassium ferricyanide in acid solution at pH 2.4 by sulphydryl groups in the tissues. The resulting ferrocyanide combines with ferric ion to give an insoluble prussian blue precipitate. Hydrated slides treated with saturated solution of mercuric chloride served as the control.

Performic Acid Alcian Blue Test For SS Groups (Adam and Sloper, 1956)

Performic acid being an oxidising agent, split the di-sulphide group into sulphonic acid, sulphinic acid, and aldehyde. The sulphonic acid and sulphinic acid combine with the dye and exhibits greenish blue colour. Slides treated directly with alcian blue served as the control.

Methylgreen Pyronin Reaction for Nucleic Acids (Pearse, 1968)

Mixture of basic dyes methylgreen and pyronin stains chromatin (DNA) green, and basiphilic inclusions (RNA) red, at different levels of pH. Sections treated with perchloric acid served as control.

Periodic Acid - Schiff Technique for glycogen and Carbohydrate (Pearse, 1968)

Periodic acid, an oxidant breaks the 'C-C' bonds where these are available as 1, 2 glycols, converts them into dialdehydes but does not oxidise the aldehydes further and these can be localised by Schiff's reagent. Sections treated with diastase for 20 minutes at room temperature served as the control.

Sudan Black B Test for Lipids (Pearse, 1968)

Being an azo dye and due to the slightly basic nature of amino groups, it combines with acidic groups of compound lipids such as phospholipids and produces black or dark blue colour. Lipids from the control slides were removed using chloroform-methanol extraction.

Test for Cholesterol (Windaus, 1910)

Digitonin forms crystals with free sterols and these can be examined under polarised light, where the needle of rosette is characteristic of cholesterol.

5. Cell Measurement of NSC

The size of the organs and cells was measured using a monocular Olympus microscope fitted with a calibrated ocularmeter (Erma, Japan).

6. Quantitative study of neurosecretory cells

Quantitative study on the distribution of

neurosecretory cells in eyestalk, brain and thoracic ganglia was carried out, during postmoult, intermoult, and premoult. For each stage, number of active and passive neurosecretory cells present in the selected median sections of eyestalk, brain, and thoracic ganglia were counted. For each stage, cell counts from five animals were made and the percentage average of the cells were recorded.

$$\% \text{ of active/passive cells} = \frac{\text{No. of cells in active/passive phase}}{\text{Total no. of cells counted}} \times 100$$

7. Photomicrography

The photomicrographs of the histological sections were taken on Universal Research Microscope (Vanvox model PM 10 AD) using black and white film (ORWO 100 ASA).

8. Electron microscopic studies

Inorder to study the nature of neurosecretory vesicles of neurosecretory cells, the tissue containing the medulla terminalis area of the eyestalk was fixed in 4% ice cold (4 °C) gluteraldehyde solution prepared in Millonig's phosphate buffer at pH 7.2 for 12 hours. Then the used fixative was replaced with fresh ones and stored at 4 °C. Fixed tissues were washed several times in cold (4 °C) Millonig's phosphate buffer (7.2 pH) followed by post

fixation using osmium tetraoxide for about two hours at 4 °C. Specimens were then dehydrated in ascending series of ethanol concentrations. After keeping for 30 minutes (15 minutes, 2 changes) in 100% propylene oxide, the tissues were embedded in araldite. Silver to grey ultrathin sections were cut with LKB Ultratome. After post staining with uranyl acetate (Watson, 1968) and lead citrate (Reynolds, 1963), stained grids were examined in a JOEL JEM 100 CX electron microscope at 80 KV.

9. Eyestalk removal and Eyestalk extract administration experiments

To elucidate the relative effect of the removal of eyestalk X-organ complex over the moulting process of prawns, eyestalk ablation (unilateral and bilateral eyestalk ablation) and eyestalk extract injection experiments were conducted. P.indicus of size 60-80 mm in total length, in the intermoult stages were used. Eighty prawns were selected and were divided into five experimental groups viz. A to E, each group consisted of 16 prawns. All prawns of group A were subjected to unilateral eyestalk surgery. In group B, both eyestalks were removed. Group C comprised intact prawns, kept as the control for group A and B. Group D composed prawns from

which both eyestalks were removed followed by injection of eyestalk extract. In group E, bilateral eyestalk surgery was performed followed by the administration of physiological saline. The prawns of group E served as the control for group D. Removal of the eyestalks were carried out with the help of an Electrocautery apparatus (Fig. 1). Eyestalk removal was done at the narrow proximal end in the region of articulating membrane. Eyestalk extract for the injection was prepared by homogenizing fresh eyestalks in double distilled water using a glass tissue homogenizer. The homogenite was centrifuged for five minutes at 2000 rpm in a clinical centrifuge. After centrifugation, supernatent was removed and used for injecting into prawns. Each prawn received 0.2 ml of eyestalk extract. The concentration of the material injected was equivalent to two eyestalks (2 eyestalks/ 0.2ml). The extract administered was intramuscular at the anterior region of the ventral abdomen of the prawn. Single injection was given to each prawn during the course of the experimental period.

Experimental prawns were maintained individually in floating plastic cages as described in chapter I. Prawns were fed with molluscan and crustacean meat, about 15% of

the body weight of the prawns. Moult stage development of each prawn in the experimental and control groups was observed daily and records of moulting details of individual prawns was kept. Prawns were kept under observation till the animal completed one moult cycle in the experimental system. The effect of eyestalk ablation and eyestalk extract injection on the moult cycle of the prawns were assessed by recording the premoult duration of experimental prawns.

10. Y-Organectomy experiments

Experiments on Y-organ removal were performed in reproductively immature adult prawns of intermoult stage (80-120 mm in total length) to study the role of Y-organ on moult cycle. In total 41, prawns were selected for the experiment. Y-organ removal was done in 24 prawns. The remaining seventeen prawns were kept and treated as controls, (shamoperation) in which small piece of muscle tissue to the size of Y-organ was removed from the nearby Y-organ region of each individual prawn. For the successful removal of Y-organ from the live prawns, a 'gill irrigator' (Fig. 2) was designed and used in the experiment. In the gill irrigator system continuous flow of sea water (salinity, 25$\pm$ 2.5‰; temperature 30 $\pm$ 1 °C, and

pH 8 ± 0.2) was maintained through a pair of capillaries which helped to wash the gills of the prawns continuously while operation. Gill irrigator system helped to keep the prawn alive during the course of surgery. Bilateral removal of Y-organ was accomplished with the help of sharp scalpel, pointed scissors and forceps. The wounds were later sealed immediately by electrocauterization. Both the experimental and control prawns were kept individually in floating plastic cages as described in chapter-I. During the experimental period prawns were fed with molluscan and crustacean meat, about 15% of the body weight. Moult stage developments of each prawn in the experimental and control groups were observed daily and records of individual moulting details were kept. Prawns were kept under constant observation till each animal completed atleast one moult cycle during the experimental period.

11. Statistical Analysis

Results obtained in the present observation were tested using student 't' test (Snadocor and Cochran, 1970) to verify the significance between the experiments.

3. RESULTS

1. Structure of Neuroendocrine Systems

Investigation on serial sections of eyestalk (Optic

ganglia), brain (Cerebral ganglia), and thoracic ganglia using histology and histochemistry has revealed the structure and pattern of neurosecretory system of the penaeid prawn, P.indicus. Basically the general organisation and structure of neurosecretory system of the P.indicus resemble to those described for other crustaceans, although species specific variations do exist.

Neurosecretory cells of varying size and shapes were found distributed over the specific areas of the ganglionic nerve tissue of eyestalk, brain, and thoracic ganglia. In the present study the neurosecretory cells have been classified into five different types on the basis of the size, shape and staining characteristics (Fig. 3-7). Neurosecretory cells (NSC) in general were observed as monopolar with a clear single nucleus and rich in cytoplasm. Table 1 shows the size distribution of different neurosecretory cell types.

1.1 Cell Types

Giant Neurosecretory Cell (G type NSC) Fig. 3a,3b

This is the largest neurosecretory cell identified among the five types of cells present in the eyestalk, brain, and thoracic ganglia. The giant neurosecretory

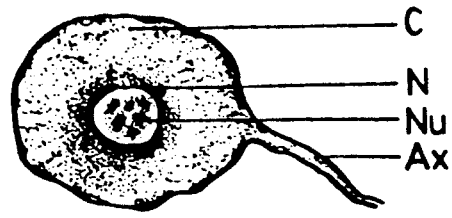
Table - 1: NEUROSECRETORY CELL TYPES IN *P. INDICUS*
CLASSIFICATION ON THE BASIS OF CELL SIZE

Cell type		Cell diameter (μ)	Nucleus diameter (μ)
Giant neuron - GN	N	25	25
	$\bar{X}$	75	15
	SD	$\pm$12	$\pm$4
Large oval cell - A	N	35	35
	$\bar{X}$	40	12
	SD	$\pm$17	$\pm$4
Small oval cell - B	N	35	35
	$\bar{X}$	23	10
	SD	$\pm$ 4	$\pm$4
Club shaped cell - C	N	35	35
	$\bar{X}$	15	6
	SD	$\pm$ 4	$\pm$3
Small round cell - D	N	35	35
	$\bar{X}$	9	7
	SD	$\pm$ 4	$\pm$2

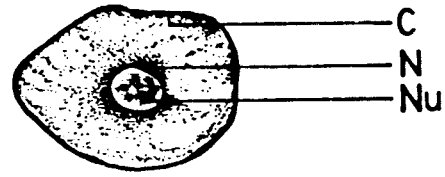
Fig. 3-7. Diagramatic representation of neurosecretory cell types of P. indicus.

3. Type G cell; 4. Type A cell; 5. Type B cell; 6. Type C cell; 7. Type D cell; AX-axon; C-cytoplasm; N-nucleus; NU-nucleolus

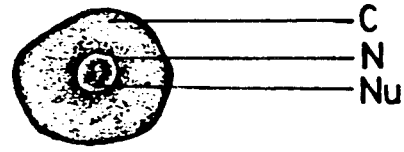
3 TYPE 'G' CELL



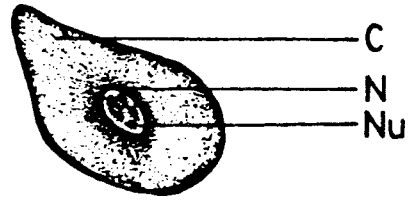
4 TYPE 'A' CELL



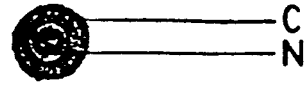
5 TYPE 'B' CELL



6 TYPE 'C' CELL



7 TYPE 'D' CELL



cells were detected only in the brain and thoracic ganglia of the prawn, and were not found in the optic ganglia. The cell bodies observed were large and oval in outline with a cell diameter ranging from 63 to 87 /u (average 75 /u). Generally the cells were seen with an axon and uniformly distributed rich cytoplasm. The large single nucleus (11 to 19 /u diameter) had 1 or 2 nucleoli. Chromatin material of the nucleus was found scattered in the nucleoplasm.

Large Oval Cell (A type NSC) Fig. 4a, 4b

Type A cells were large oval cells, and found widely distributed in the eyestalk, brain, and thoracic ganglia. The cells were found predominantly present in the thoracic ganglia. The size of the cells generally varied from 23 to 57/u with an evenly distributed cytoplasm. Spherical or oval nucleus had a diameter of 8 to 16/u. Nucleoli were not clearly seen except for some diffused spots.

Small Oval Cells (B type NSC) Fig.5a. 5b

Type B cells identified in the present study were small and oval in shape, and found well distributed in the eyestalk, brain and thoracic ganglia. The cell diameter ranged between 19 to 27/u with a proximally or centrally placed single nucleus of 6 to 14/u in diameter.

Fig. 3a. G type neurosecretory cell of thoracic ganglion in the active phase of neurosecretion, X400, Aldehyde Fuchsin.

Fig. 3b. G type neurosecretory cell of thoracic ganglia in the passive phase of neurosecretion, X400, Aldehyde Fuchsin.

Fig. 4a. A type neurosecretory cell from the eyestalk in the passive phase of neurosecretion, X400, Aldehyde Fuchsin.

Fig. 4b. A type neurosecretory cell from the brain in the active phase of neurosecretion, X400, Aldehyde Fuchsin.

Fig. 4c. A type neurosecretory cell from the thoracic ganglion in the active phase of neurosecretion, X400, Aldehyde Fuchsin.

Fig. 4d. A type neurosecretory cell from the eyestalk in the active phase of neurosecretion, X400, Aldehyde Fuchsin.

CP-Cytoplasm; GC-Glial cell; N-Nucleus; NU-Nucleolus; V-Vacuole; VC-Vacuolated cytoplasm.

Club Shape Cell (C type NSC) Fig. 6a, 6b

These were club or conical shaped cells found in the eyestalk, brain and thoracic ganglia. The size of the cell varied from 11 to 19/u in diameter with a prominent nucleus of 3 to 9/u diameter. These cells showed limited distribution when compared to the distribution pattern of B type NSC.

Small Round Cells (D type NSC) Fig. 7

Type D cells were small round cells and the smallest of the all the five type NSC observed, with a cell diameter ranging between 5 to 13/u. Nucleus is large and prominent (5 to 9/u), while the cytoplasm was scanty and cell margins ill-defined. The cell bodies were generally found in groups in the eyestalk, brain, and thoracic ganglia.

Staining of the neurosecretory cells with selected neurosecretory specific stains, viz. MTP, CHP and AF showed the presence of neurosecretory materials or granules in the cytoplasm of all NSC types except D type cells. The D type cells showed little affinity towards the neurosecretory stains.

Different neurosecretory cells when stained with AF,

Fig. 5a. B type neurosecretory cell from brain in the passive phase of neurosecretion, X400, Aldehyde Fuchsin.

Fig. 5b. B type neurosecretory cell from the eyestalk in the active phase of neurosecretion, X400, Aldehyde Fuchsin.

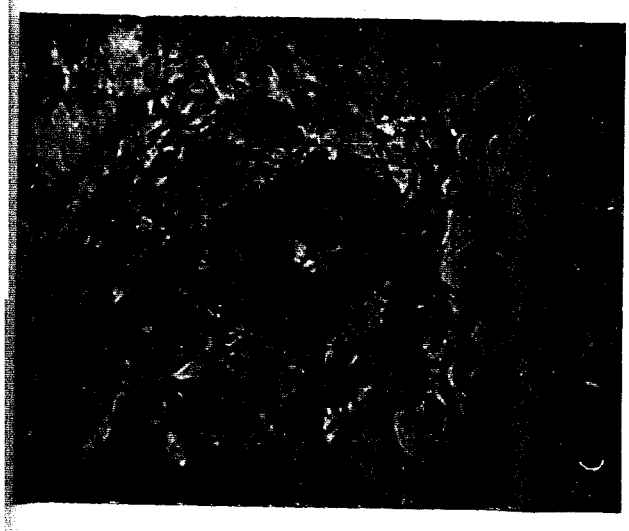
Fig. 5c. B type neurosecretory cell from the eyestalk in the active phase of neurosecretion, X400, Aldehyde Fuchsin.

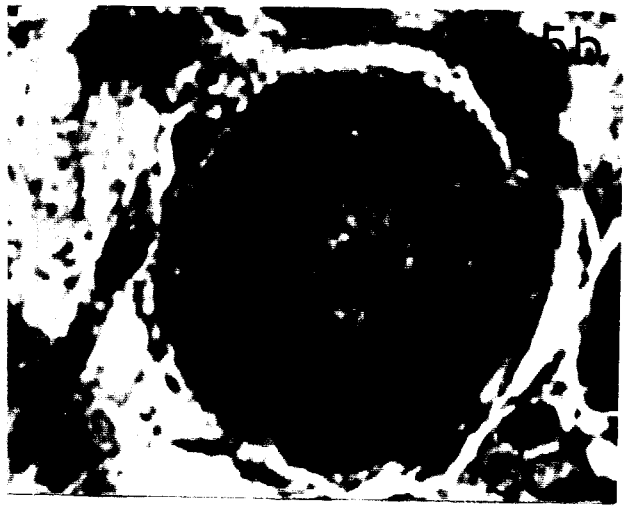
Fig. 6a. C type neurosecretory cell from the eyestalk in the active phase of neurosecretion, X1000, Aldehyde Fuchsin.

Fig. 6b. C type neurosecretory cell from the brain in the passive phase of neurosecretion, X400, Aldehyde Fuchsin.

Fig. 7a. D type neurosecretory cell from the eyestalk, X400, Aldehyde Fuchsin.

AX-Axon; CP-Cytoplasm; D-D-type cell; GC-Glial cell; N-Nucleus; NU-Nucleolus; V-Vacuole; VC-Vacuolated - Cytoplasm




5b


V


V

CHP and MTP, the cell cytoplasm stained in different shades of purple colour. Nucleoplasm of the cell, with AF gave a pale yellow colour, where as it showed light shades of blue colour with CHP and brownish red with MTP. With AF, nucleoli of the neurosecretory cells stained in different shades of violet while with CHP stain nucleoli appeared purple. In the case of MTP, nucleoli of the G type cells showed red colour, but nuclei of all other cells showed blue colour. The reactions of the 5 types of the NSC to the three histological staining technique are given in Table 2.

1.2.Histochemical Observations of NSC

To reveal the chemical nature of neurosecretory material of NSC, various histochemical tests were performed and the results indicated that the NSC material is predominantly a protein with small amounts of carbohydrates, and little lipids.

Bromophenol blue test for general protein gave a very strong reaction with neurosecretory cells and sinus gland showing the presence of high protein contents. All neurosecretory cells except type D were rich in cystine and cysteine. Strong positive nature of NSC except the D cells to AF, CHP and MTP indicated the presence of

Table - 2: REACTIONS OF NEUROSECRETORY CELLS OF *P. INDICUS* TO DIFFERENT STAINING TECHNIQUES

NSC	Staining technique	Cytoplasm/ Neurosecretory material	Nucleoplasm	Nucleoli
Giant neuron 'G'	PAF	Deep purple	Pale Yellow	Violet
	CHP	Greyish Purple	Bluish grey	Red
	MTP	Deep Purple	Brownish red	Red
Large oval Cell 'A'	PAF	Reddish Purple	Pale Yellow	Violet
	CHP	Purple	Pale Blue	Purple
	MTP	Reddish Purple	Brownish red	Reddish blue
Small oval Cell 'B'	PAF	Brownish Purple	Pale Yellow	Pale Violet
	CHP	Violet	Light blue	Red
	MTP	Deep Purple	Brownish red	Blue
Club shaped Cell 'C'	PAF	Violet	Pale Yellow	Purple
	CHP	Purple	Pale blue	Brown
	MTP	Bluish Purple	Yellowish red	Blue
Small round Cell 'D'	PAF	Pale Yellow	Pale Yellow	Pale Violet
	CHP	Majenta	Pale blue	Purple
	MTP	Pale Purple	Pale Yellow	Blue

proteinaceous secretion of cystine and cysteine. This was again indicated by the reaction of Ferric-Ferricyanide test and performic acid alcian blue test. Periodic schiff reaction demonstrated the presence of carbohydrates, and a number of related substances. Control sections subjected for diastase digestion showed that the PAS positivity of NSC is largely due to the presence of glycogen. Sudan Black B test for lipid gave a very feeble reaction with NSC. The results of the reaction of different staining material in the NSC were observed for the secretory material only. The results of the histochemical tests are given in the Table 3a, b, c and d.

1.3.Histology of the eyestalk neuroendocrine complex

Histological studies, using light microscope, revealed the structure of neuroendocrine complex of the eyestalk (Fig. 8 and 9). The central axis of the eyestalk was found to consist of an optic ganglia enclosed by a thin connective tissue sheath. Based on the distinct morphological features the optic ganglion was further divided into three different medullae. On the basis of earlier literature the three medullae have been named as Medulla Terminalis (MT), Medulla Interna (MI), and Medulla Externa (ME), and terminal portion above the ME i.e. the Lamina Ganglionaris (LG). The lamina ganglionaris has been

Table 3 a. HISTOCHEMICAL REACTION OF NEUROSECRETORYCELLS OF EYESTALK IN *P. INDICUS*
A. POSTMOULT (ACTIVE PHASE)

Test applied	Type A NSC	Type B NSC	Type C NSC	Sinus Gland	Reference
Aldehyde fuchsin (AF)	+++	+++	++	+++	Kurup (1972)
Chrome Haematoxylin Phloxine (CHP)	++	++	++	++	Bargman (1941)
Mallory's Triple Stain (MTP)	++	++	+	++	Mallory (1944)
Mercuric Bromophenol Blue test (MBT)	+++	+++	++	+++	Mazia *et al.* (1953)
Ferric-Ferricyanide test (FFT)	++	++	+	++	Pearse (1968)
Mercaptide	-	-	-	-	
Performic acid Alcian Blue Test (PFAB)	++	++	+	+++	Adam & Sloper (1956)
Alcian blue alone	-	-	-	+	
Periodic Acid Schiff(PAS)	++	++	+	++	Pearse (1968)
Diastase digestion	+	+	±	+	
Sudan Black B Test (SBT)	++	+	+	++	Pearse (1968)
Chloroform methanol	-	-	-	-	

Table 3 b. HISTOCHEMICAL REACTION OF NEUROSECRETORYCELLS OF EYESTALK IN *P. INDICUS*

B. PREMOULT (PASSIVE PHASE)

Test applied	Type A NSC	Type B NSC	Type C NSC	Sinus gland	Reference
Aldehyde fuchsin (AF)	++	+	+	+	Kurup (1972)
Chrome Haematoxylin Phloxine (CHP)	+	+	+	+	Bargman (1941)
Mallory's Triple Stain (MTS)	++	+	+	+	Mallory (1944)
Mercuric Bromophenol Blue test (MBT)	+	+	+	+	Mazia *et al.* (1953)
Ferric-Ferricyanide test (FFT)	+	+	+	+	Pearse (1968)
Mercaptide	-	-	-	-	
Performic acid Alcian Blue Test (PFAB)	±	±	±	+	Adam & Sloper (1956)
Alcian blue alone	-	-	-	-	
Periodic Acid Schiff (PAS)	++	+	+	++	Pearse (1968)
Diastase digestion	-	-	-	±	
Sudan Black B Test (SBT)	+	+	+	+	Pearse (1968)
Chloroform methanol	-	-	-	-	

Table 3 c. HISTOCHEMICAL REACTION OF NEUROSECRETORYCELLS OF BRAIN AND THORACIC GANGIA IN *P. INDICUS*: A PREMOULT (ACTIVE PHASE)

Test applied	Type G NSC	Type A NSC	Type B NSC	Type C NSC	Reference
Aldehyde fuchsir (AF)	+++	+++	++	++	Kurup (1972)
Chrome Haematoxylin Phloxine (CHP)	+++	++	++	++	Bargman (1941)
Mallory's Triple Stain (MTP)	+++	++	++	++	Mallory (1944)
Mercuric Bromophenol Blue test (MBT)	+++	++	+++	+	Mazia *et al.* (1953)
Ferric-Ferricyanide test (FFT)	+	++	++	+	Pearse (1968)
Mercaptide	±	±	+	±	
Performic acid Alcian Blue Test (PFAB)	++	++	+	+	Adam & Sloper (1956)
Alcian blue alone	±	±	-	-	
Periodic Acid Schiff (PAS)	++	++	+	++	Pearse (1968)
Diastase digestion	±	±	±	+	
Sudan Black B Test (SBT)	±	+	+	±	Pearse (1968)
Chloroform methanol	-	-	-	-	

Table - 3 d. HISTOCHEMICAL REACTION OF NEUROSECRETORYCELLS OF BRAIN AND THORACIC IN *P. INDICUS* : B POSTMOULT (PASSIVE PHASE)

Test applied	Type G NSC	Type A NSC	Type B NSC	Type C NSC	Reference
Aldehyde fuchsin (AF)	+	+	+	+	Kurup (1972)
Chrome Haematoxylin Phloxine (CHP)	++	+	+	+	Bargman (1941)
Mallory's Triple Stain (MTP)	+	+	+	+	Mallory (1944)
Mercuric Bromophenol Blue test (MBT)	+	+	±	+	Mazia *et al.* (1953)
Ferric Ferricyanide test (FFT)	+	+	±	±	Pearse (1968)
Mercaptide	+	±	±	-	
Performic acid Alcian Blue test (PFAB)	+	+	+	±	Adam & Sloper (1956)
Alcian blue alone	-	+	±	±	
Periodic Acid Schiff (PAS)	+	++	+	±	Pearse (1968)
Diastase digestion	±	+	±	±	
Sudan Black B Test (SBT)	+	+	+	±	Pearse (1968)
Chloroform methanol	-	-	-	-	

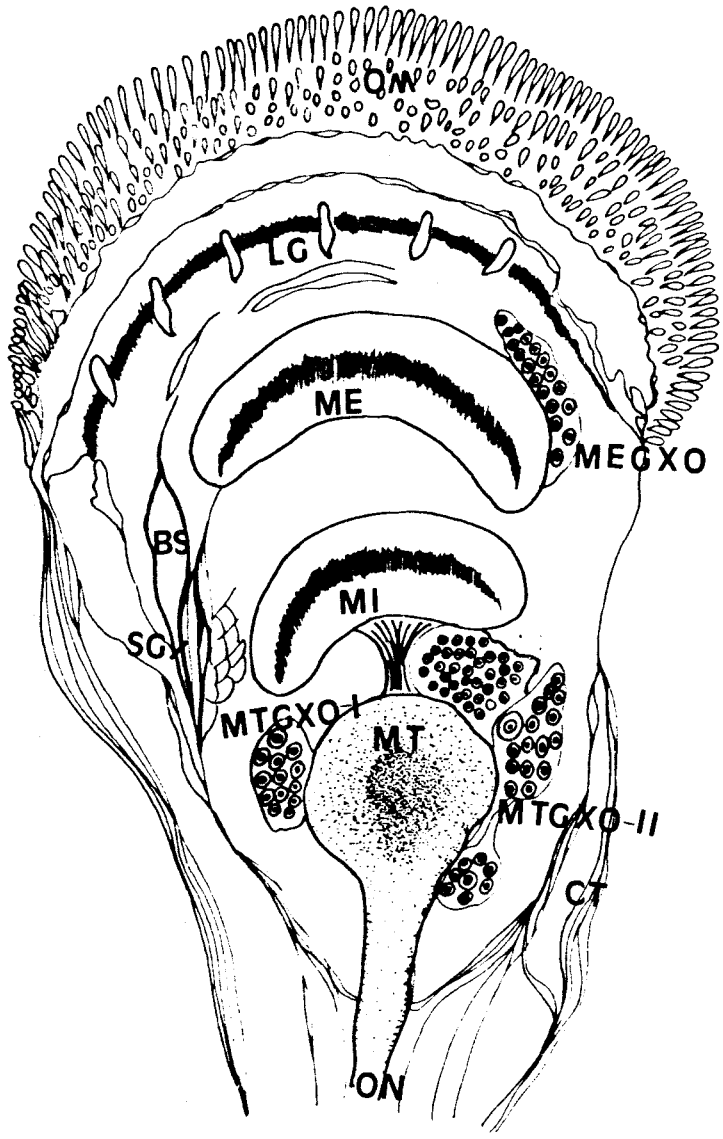
detected as the outermost lobe lying just below the ommatidia. This lobe was found connected to the first lobe of the three medullae, the medulla externa, with nerve bundles. Followed by the medulla externa, the other two lobes detected were medulla interna and medulla terminalis. Among the three medullae observed, the largest and the most prominent was the medulla terminalis, situated in the most proximal region of the central axis of the eyestalk. Medulla terminalis was found connected with the brain by an optical nerve tract.

Neurosecretory cells were found distributed on the peripheral areas of the optic lobes in localised regions. The neurosecretory cell groups, which were specifically stainable with AF, CHP and MTP were found as distinct group over the peripheral regions of medulla terminalis and medulla externa.

In accordance with the nomenclature of earlier literature distinct neurosecretory cell groups associated with the optic peduncle ganglia in P.indicus were given the terms as the Medulla Terminalis Ganglionic X-organs (MTGXO-I, MTGXO-II) and Medulla Externa Ganglionic X-organ (MEGXO). X-organs were observed to be comprised of different type of neurosecretory cells with typical

Fig. 8 and 9. Longitudanal section of eyestalk (8 - Diagramatic representation and 9 - reconstructed photomicrograph).
BS-Blood sinus; CT-Connective tissue; LG-Lamina ganglionaris; ME-Medulla externa; MI-Medulla interna; MT-Medulla terminalis; MTGXO I-Medulla terminalis ganglionic X-organ I; MTGXO II-Medulla terminalis ganglionic X-organ II; MEGXO-Medulla externa ganglionic X-organ; OM-Ommatidia; ON-Optic nerve; SG-Sinus gland.

Fig.8




S
TGXO
TGXO
ON

secretory characteristics. The MTGXO-I and MTGXO-II situated in the medulla terminalis were found to contain different types of NSC viz. A, B, C and D (Fig.10-16). Cell type C were very few in numbers while other NSC types were predominantly noticed in the ventral and lateral regions of the medulla terminalis. An isolated group of NSC was detected on the surface of the medulla externa. This NSC group detected dorsolaterally at the rostral side of the eyestalk on the surface of the medulla externa has been termed as the Medulla Externa Ganglionic X-organ (MEGXO) (Fig. 17).

Groups of small fuchsinophilic cells were observed distally around the medulla externa and similar cells were also seen in association with the ventral part of the medulla interna. Generally it was not possible to see any cytoplasm associated with these cells and they were considered in the present investigation as neural cells (Fig.23).

The principal neurohaemal organ detected in the eyestalk complex in the present observation has been termed as Sinus gland in line with the studies of earlier crustacean workers. The gland was found to occupy a position between the medulla interna and medulla

Fig. 10. Medullaterminalis ganglionic X-organ I of the eyestalk, X200, Chrome-Haematoxylin Phloxine.

Fig. 11 Medulla terminalis ganglionic X-organ I of the eyestalk, X200, Aldehyde Fuchsin.

Fig. 12 and 13 Medulla terminalis ganglionic X-organ II of the eyestalk X100, Mallory's Triple Stain.

Fig. 14 and 15 Medulla terminalis ganglionic X-organ I of the eyestalk, X100, 200, Aldehyde Fuchsin.

AT-Axonal tract; AX-Axon; BS-Blood sinus; CS-Connective tissue; MT-Medulla terminalis; MTGXO-Medulla terminalis ganlionic X-organ.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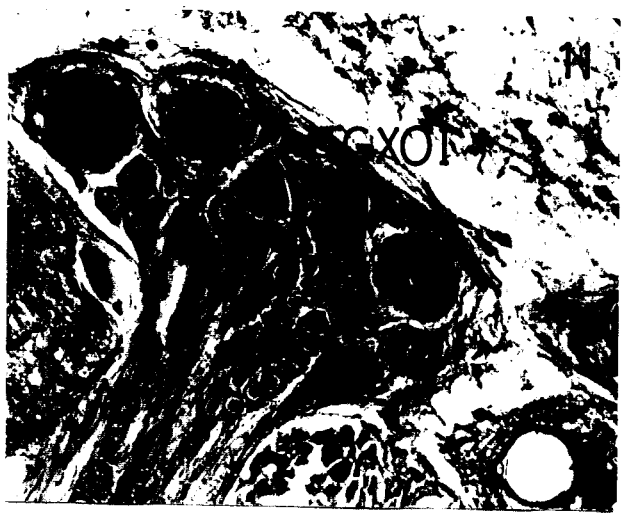
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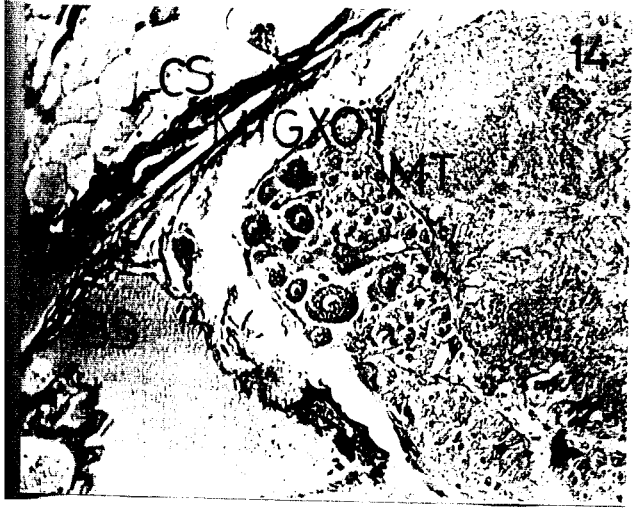
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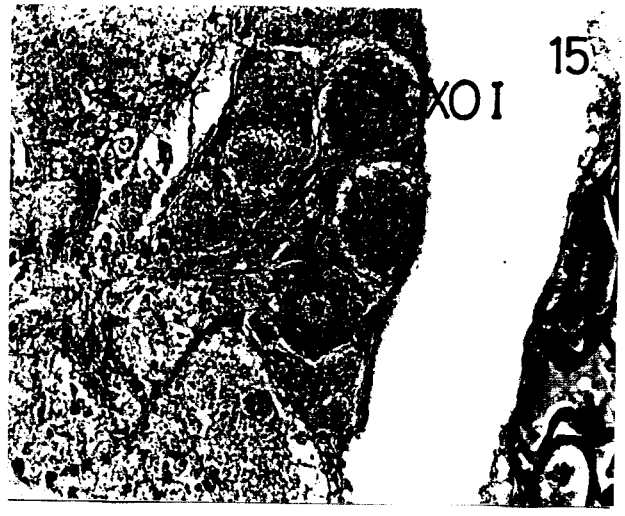
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XO I

terminalis in a latero-longitudinal axis of the eyestalk. Sinus gland as observed in the present study has a rough triangle shape, one of whose apices was found tapering off in a proximal direction to touch the medulla terminalis (Fig.8 and 9). The central part of the gland has been found to occupy a blood sinus. The gland was found separated from the external blood sinus by a thin membrane which took dark blue colour with MTP and purple colour with AF. The organ has copious circulation and joins with the major blood sinus of the eyestalk (Fig. 18 and 19). Axonal tracts composed of axonal fibres were found to connect the sinus gland and X-organs of the eyestalk (Fig. 11, 20, 21 and 22). Careful examination of the tract revealed the presence of phloxinophilic secretory materials along the tract. Sinus gland showed strong positive reaction towards all the three neurosecretory specific stains (MTP, AF and CHP) used in the present study.

1.4. NSC of Brain and Thoracic Ganglia

Sections of brain and thoracic ganglia revealed that neurosecretory cells were distributed mainly over the peripherals of neuropile area on both dorsal and ventral sides. Serial sections showed that these cells were

Fig. 16 and 17 — Medulla terminalis ganglionic X-organ II of the eyestalk, X200, Chrome-Haematoxylin Phloxine

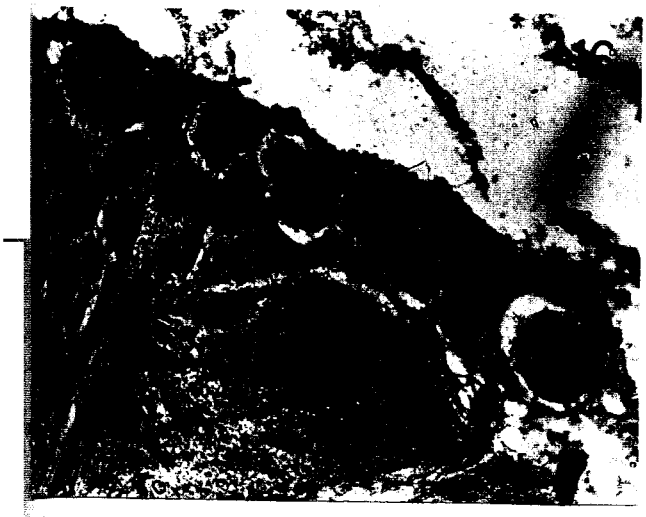
Fig. 18 — Sinus gland, X200, Aldehyde Fuchsin.

Fig. 19 — Large blood sinus associated with the sinus gland, X200, Aldehyde Fuchsin.

Fig. 20 — Axonal tract connecting the Medulla terminalis ganglionic X-organ I and sinus gland, X200, Aldehyde Fuchsin.

Fig. 21 — Axonal tract connecting the Medulla externa ganglionic X-organ and sinus gland, X200, Chrome-Haematoxylin Phloxine.

AT-Axonal tract; BS-Blood sinus; CT-Connective tissue; ME-Medulla externa; MTGXO-Medulla terminalis ganglionic X-organ; NP-Neuropile; SG-Sinus gland; XO-X-organ




SG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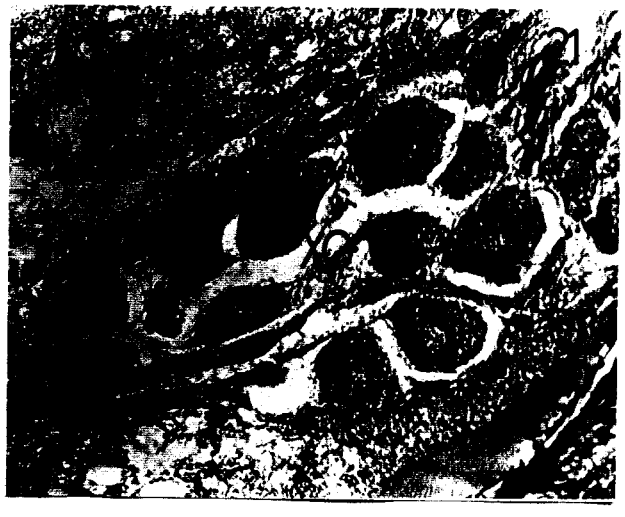
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organised as well defined groups in three important regions viz. anterior, median, and posterior. (Fig. 24A, 24B, 24C, and 25 to 27). Dorsal part of the brain and ventral plane of the thoracic ganglia were found to be notably richer in large neurosecretory cells of G and A types (Fig. 28 to 31). The most prominent area for the localization of neurosecretory cells on the dorsal side of the brain and ventral side of the thoracic ganglia was the central group (Fig. 28 to 32). When compared to the brain, the thoracic ganglia was found to contain larger number of G and A cells (Fig. 29). The somata of the neurosecretory cells was found to be insulated by glial cells that separate the neurosecretory cells from one another. (Fig. 3b and 5b).

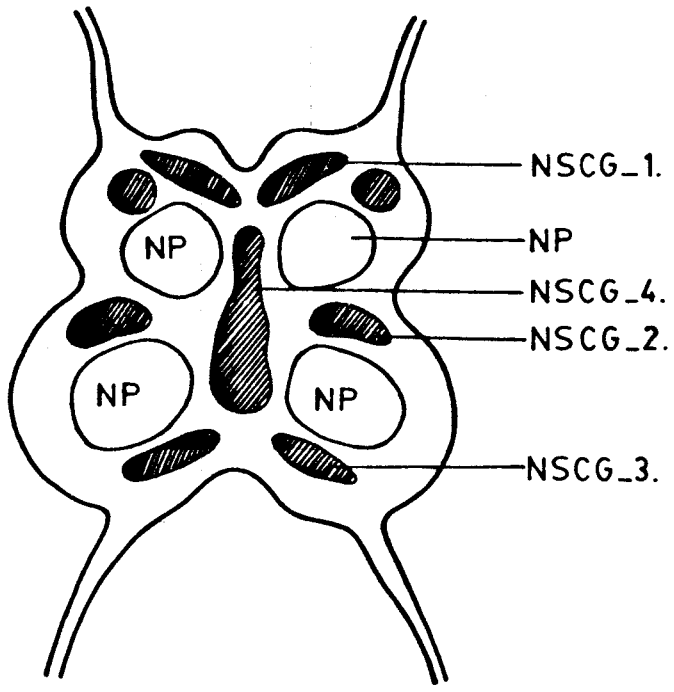
1.5. Ultra structure of Neurosecretory Granules

Electron microscopy of the eyestalk neurosecretory cells showed neurosecretory vesicles of 120 to 130 nm diameter. Ultrastructrally these granules appeared as a hallowed dense cored vesicles of spherical nature (Fig. 33). The neurosecretory vesicles were found to be surrounded by a thin protective membrane.

2 Histology of Y-organ

The location and the structure of the Y-organ were

BRAIN DORSAL VIEW



BRAIN VENTRAL VIEW

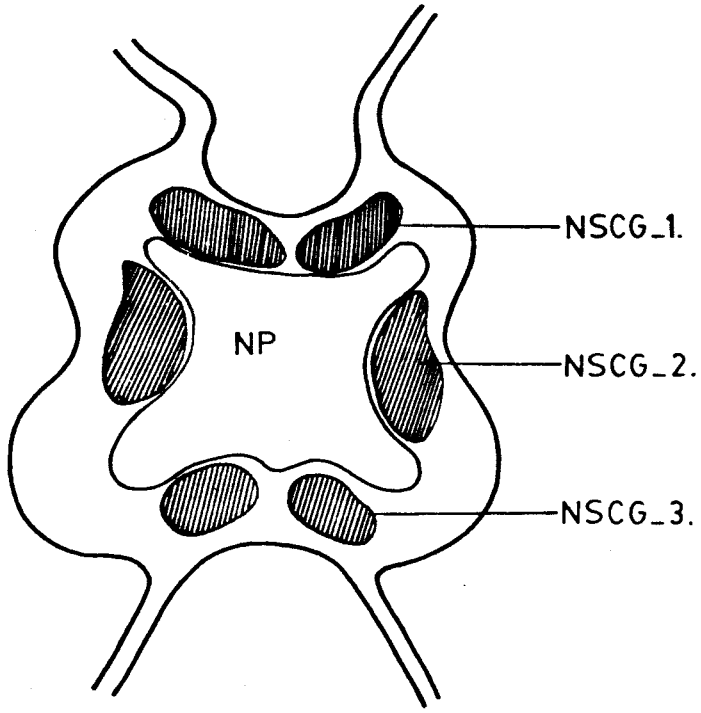
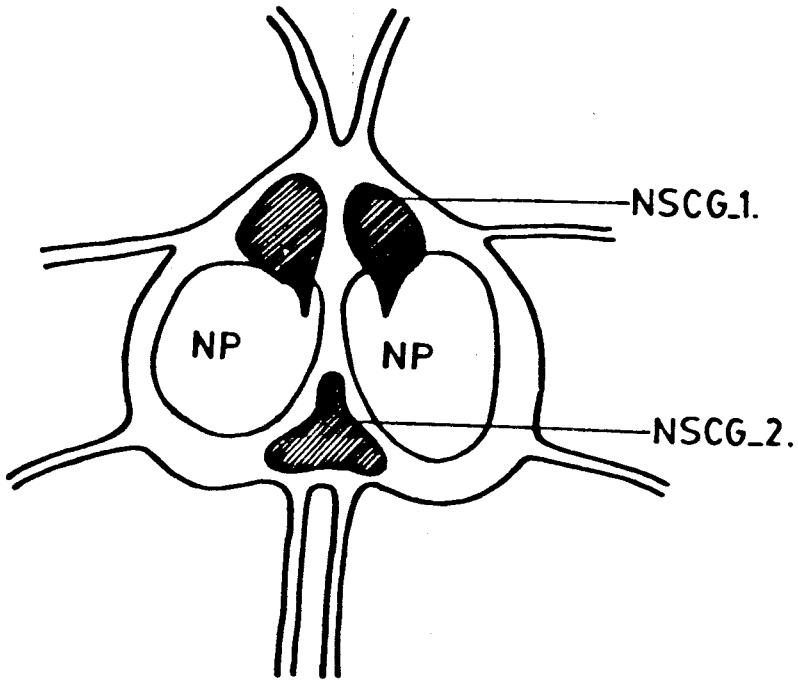


Fig. 24A. Diagramatic representation of the longitudanal section of the brain, NP-Neuropile; NSCG-Neurosecretory cell groups.

THORACIC GANGLIA:

DORSAL VIEW



VENTRAL VIEW

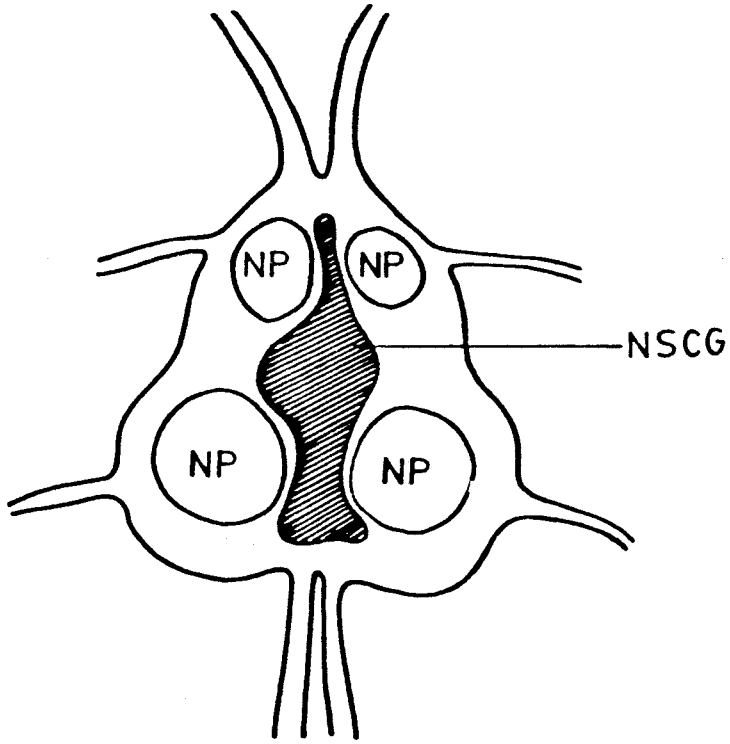


Fig. 24B. Diagramatic representation of the longitudanal section of thoracic ganglia, NP-Neuropiole; NSCG-Neurosecretory cell groups.

ig. 22 Axonal tract connecting medulla terminalis ganglionic X-organ II and sinus gland, X200, Aldehyde Fuchsin.

Fig. 23 Neural cells of eyestalk, X200, Aldehyde Fuchsin.

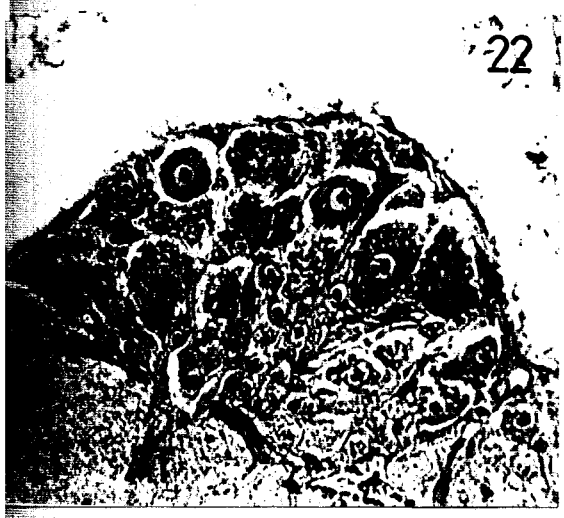
Fig. 24c Groups of neurosecretory cells in the anterioventral part of the brain, X200, Aldehyde Fuchsin.

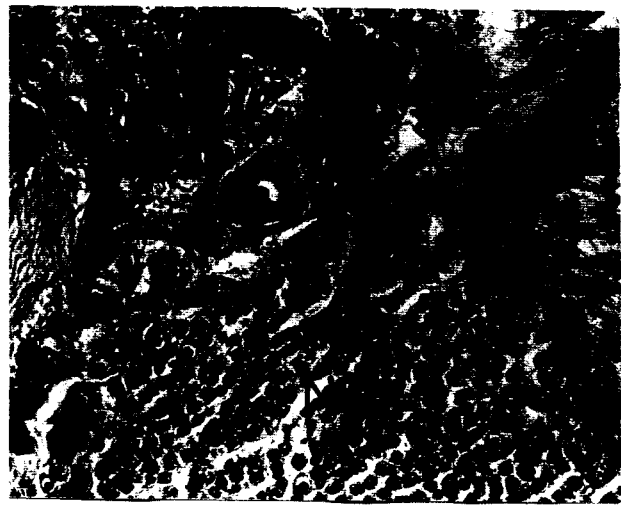
Fig. 25 Groups of neurosecretory cells in the ventral median part of the brain, X100, Mallory's Triple Stain.

Fig. 26 Groups of neurosecretory cells in the ventral median part of the thoracic ganglia, X100, Aldehyde Fuchsin.

Fig. 27 Groups of neurosecretory cells in the posteriodorsal part of the thoracic ganglia in the active phase of neurosecretion, X200, Aldehyde Fuchsin.

AT-Axonal tract; MT-Medulla terminalis; NP-Neuropile; NS-Neural cells; NSCG-Neurosecretory cell groups. XO- X Organ.





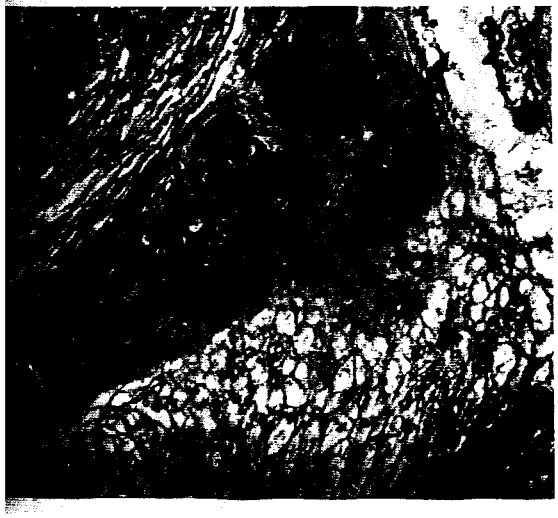


Fig. 28 Groups of neurosecretory cells in the dorsomedian central part of the brain in the passive phase of neurosecretion, X100, Aldehyde Fuchsin.

Fig. 29 Groups of neurosecretory cells in the ventromedian central part of the thoracic ganglia in passive phase of neurosecretion, X100, Aldehyde Fuchsin.

Fig. 30 Groups of neurosecretory cells in the ventromedian central part of the thoracic ganglia in passive phase of neurosecretion, X100, Mallory's Triple Stain

NSCG-Neuro secretory cell groups.

Fig. 31 Groups of neurosecretory cells in the ventromedian central part of the thoracic ganglia in active phase of neurosecretion, note the exocytosis in A type cells, X100, Aldehyde Fuchsin.

Fig. 32 Groups of neurosecretory cells in the dorsomedian central part of the brain in the active phase of neurosecretion, X100, Chrome-Haematoxylin Phlixine.

Fig. 33 Electron micrograph of neurosecretory vesicle from the eyestalk X-organ complex, X27000.

EX-Exocytosis; NSCG-Neuro secretory cell groups;

NSV-Neuro secretory veside.

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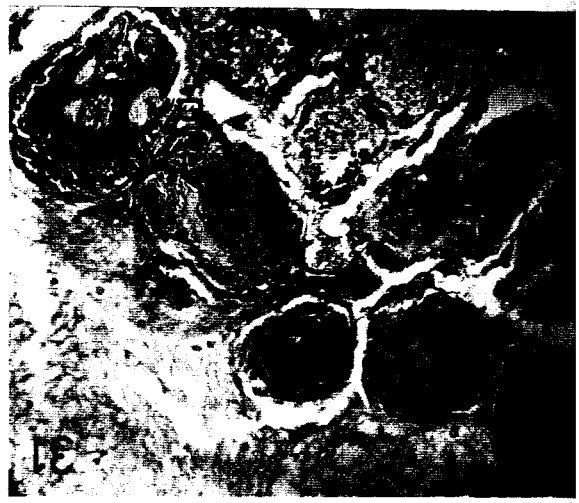
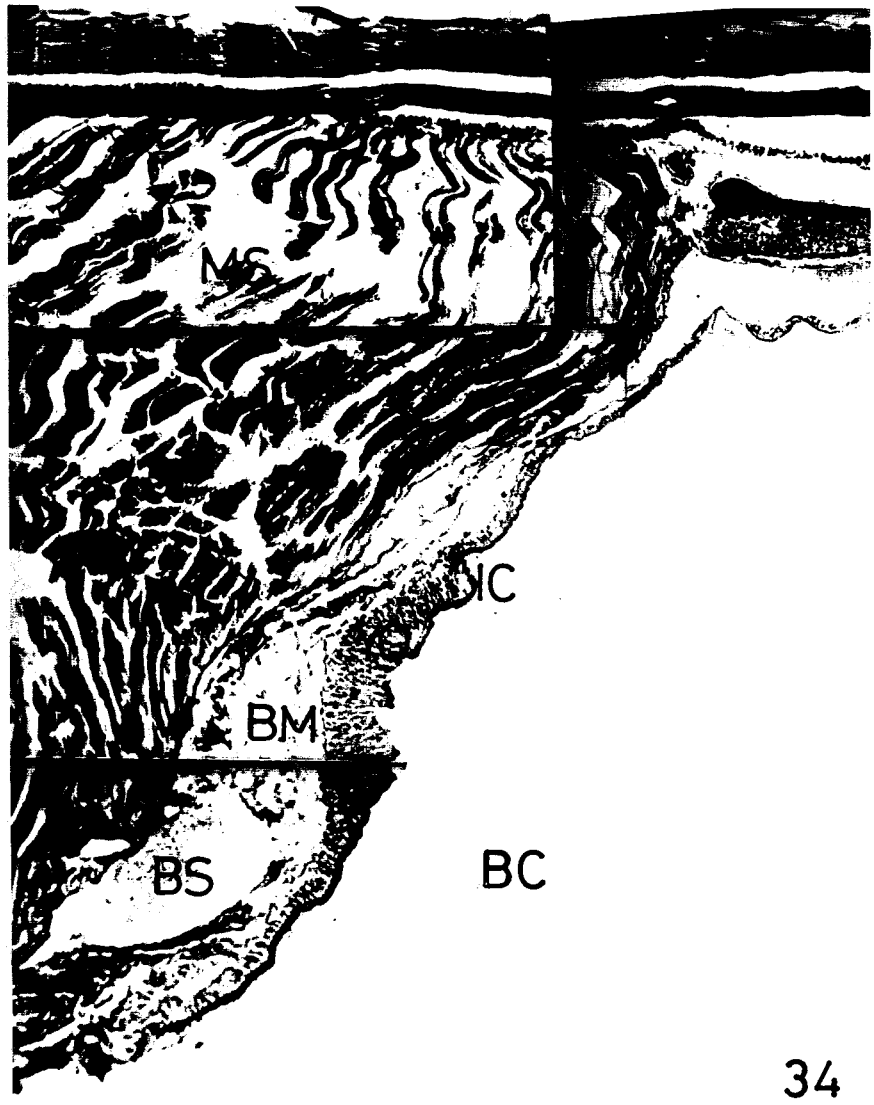


found for the first time in the P.indicus. The Y-organ was seen as a pair of narrow strip of glandular tissue at the junction of prebranchial and branchial chambers (Fig. 34 to 37). The organ appeared as transluscent in live specimens. The anterior portion of the gland lay adjacent to the mandibular muscles on an epidermal shelf and projected inwards from the innerwall of the branchiostegite. Posteriorly, the gland was found tapering and was adjacent to the lateral end of the posterior dorsoventral muscle. (Fig. 39A and B). In an adult animal, the size of the Y-organ varied from 1 - 2 mm in length, and 0.3 mm in width. The cuticular layer of the branchiostegite surrounding the Y-organ was seen to be characterised by extensive infoldings (Fig. 36 and 37). The innercuticle of this region was considerably thick, measuring on an average 6/u. The organ was separated on the inside from the surrounding blood sinus, muscle and connective tissue by a thin basal membrane. Minute capillaries and blood spaces were seen adjacent to the gland. No direct supply of nerve to Y-gland was evident in the tissue.

In the histological picture of the Y-organ, it was observed that the cells were closely packed and stained intensely with haematoxylin indicating its basophilic

Fig. 34. Reconstructed photomicrograph of Y-organ, BM-Basal membrane; BS-Blood sinus; BC-Branchial chamber; YO-Y-organ; IC-Inner cuticle; MS-Muscle; OC-Outer cuticle.


MS
IC
BM
BS
BC

Figs. 35, 36 and 37. Y-organ, X200, Haematoxylin and Eosin.

Figs. 38a and 38b. Y-organ cells, X400, Haematoxylin and Eosin.

Fig. 41 Cells of mandibular organ, X400, Haematoxylin and Eosin.

CYO-Cells of Y-organ; CMO-Cells of Mandibular organ; IC-Inner cuticle; YO-Y-organ.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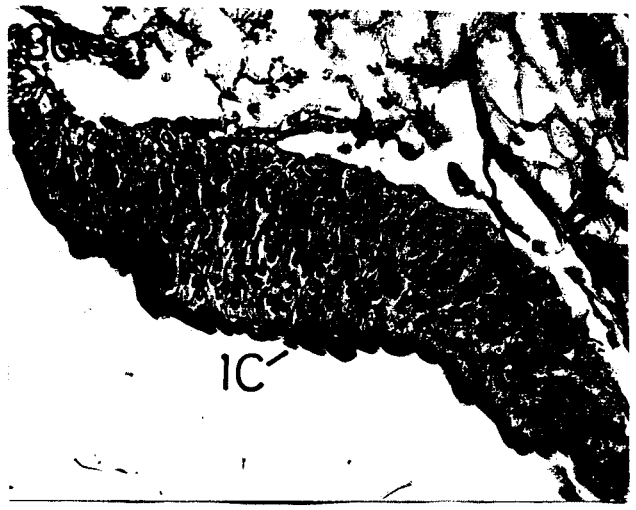
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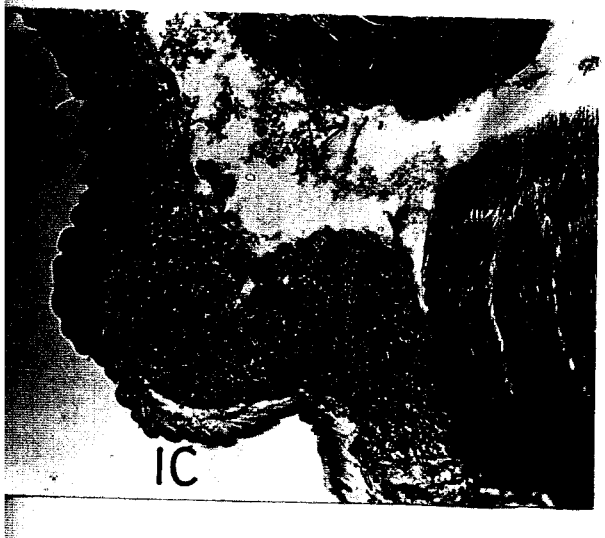
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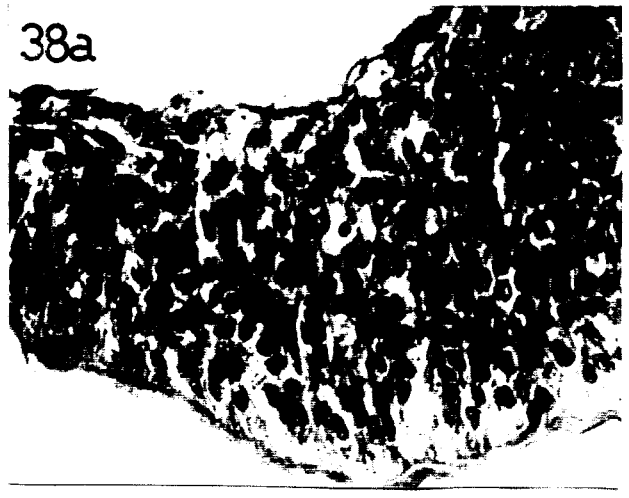
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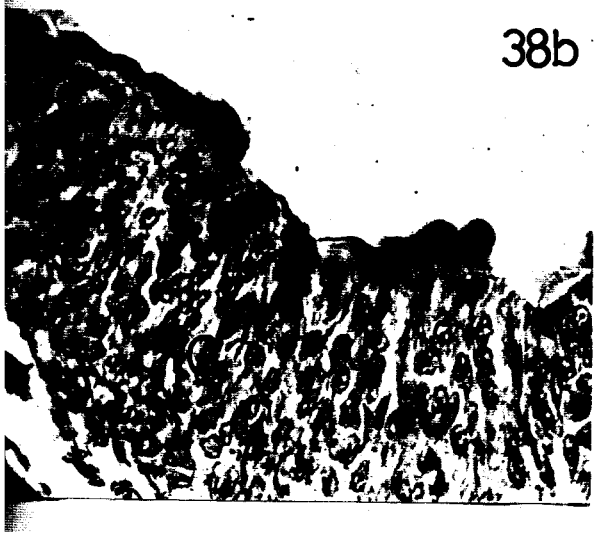
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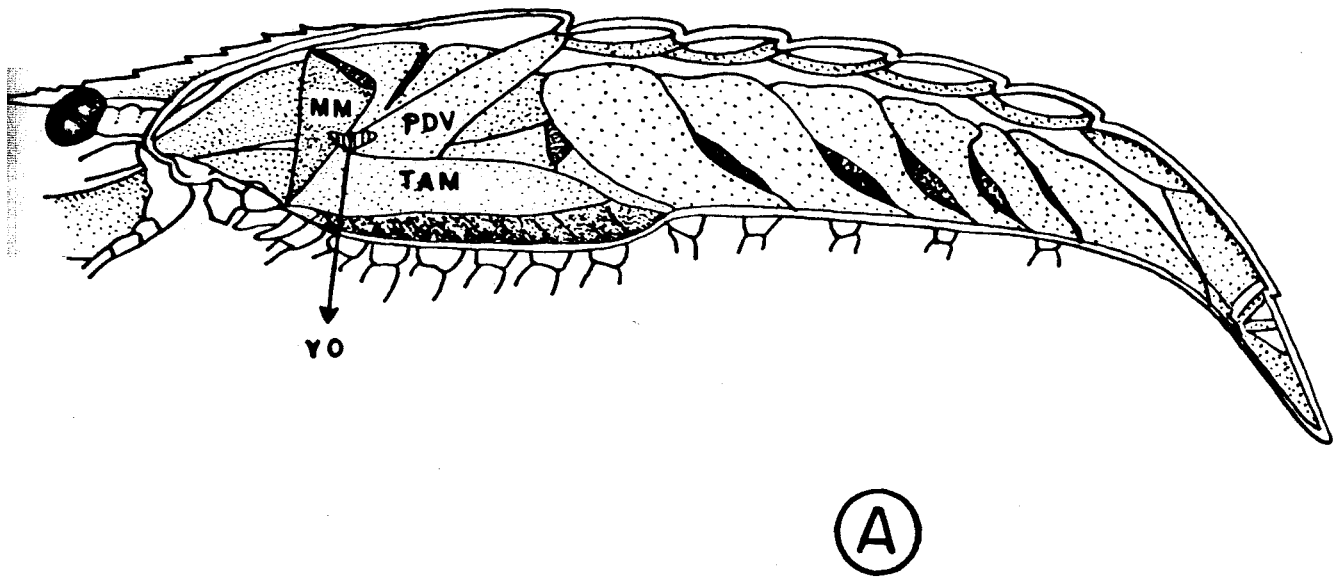
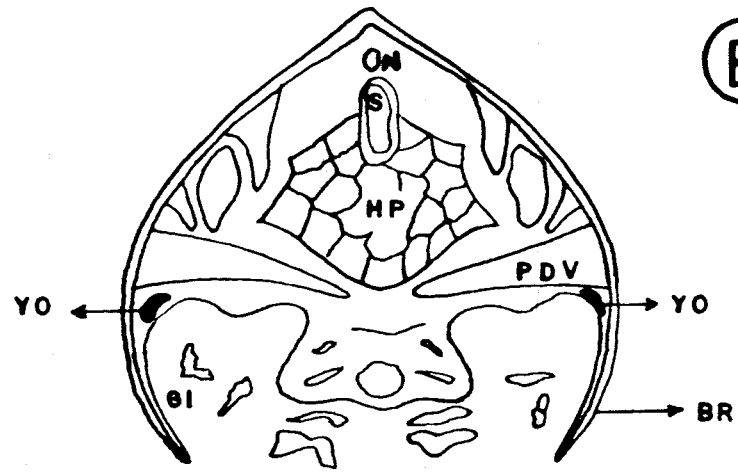


Fig.39 Schematic representation of the Y-organ in Penaeus indicus.

Not to scale. IA - Lateral view of the position of the Y-organ in relation to the adjoining muscles. MM - Mandibular Muscle, PVD - Posterior dorsoventral muscle, TAM-Thoracicoabdominal Muscle and YO-Y-organ. IB - Transverse section of the anterior cephalothorax through the level of Y-organ. S - Stomach, HP - Hepatopancreas, PDV - Posterior dorsoventral Muscle, YO-Y-organ, GI-Gills, N - Nerve cord, and BR - Branchiostegite.



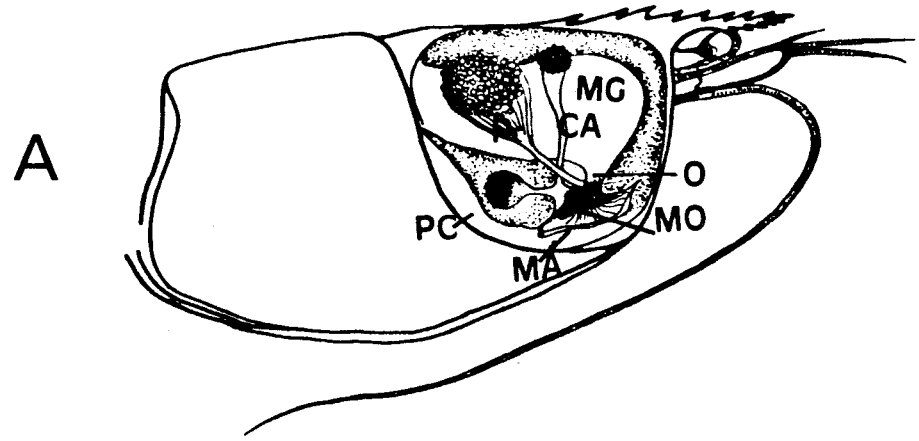
nature. The whole gland was lobulated, each lobe having 7 to 12 cells (Fig. 36). The gland cells have rare cytoplasm and cytoplasmic limits were hardly discernible with light microscopy (Fig. 38a, b). The nuclei were oval to spherical in shape and measured about 3 to 5 /u in diameter. Nuclei were centrally or peripherally placed and chromatin granules were also seen. The cells were generally positive to protein with the nuclear wall showing intense staining with mercuric bromophenol blue. The PAS test employed gave a negative reaction thereby indicating the absence of any carbohydrates. Sudan Black B and Digitonin test employed did not show any positive reactions. Methyl green pyronin test gave RNA positive reaction for the cytoplasm. The results of the histochemical tests are given in Table 4.

3 Mandibular Organ

Mandibular organ has been found and located using histologic techniques. Morphological difficulty was there to locate the gland due to the camouflage with the surrounding tissues. The mandibular organ was found and detected near the posterior ventral base of the adductor muscle of each mandible (Fig.40 A and B). Histological preparations showed that the gland is composed of oval

Table - 4: HISTOCHEMICAL REACTIONS OF Y-ORGAN IN *P. INDICUS*

Test applied	Y - Organ Cells					Reference
	Cytoplasmic matrix	Nucleus	Nuclear wall	Nucleolus	Inner cuticle	
Mercuric Bromophenol Blue	+	++	+++	++	+	Mazia *et al* (1953)
Periodic acid Schiff (PAS)	-	-	-	-	++	Pearse (1968)
Diastase digestion	-	-	-	-	-	
Sudan Black B	-	-	-	-	+	Pearse (1968)
Chloroform methanol	-	-	-	-	-	
Cholesterol (Digitonin)	-	-	-	-	-	Windaus (1910)
Methyl green pyronin	++(Pink)	+(Pink)	-	+(Pink)	-	
Hot perchloric acid	-	-	-	-	-	



CA-Cephalic apodeme; CNS-Central nervous system; MA-Mandible; MG-Anterior chamber of foregut; MO-Mandibular organ; O-Oesophagus; P-Posterior adductor of mandible; PC-Pre branchial chamber

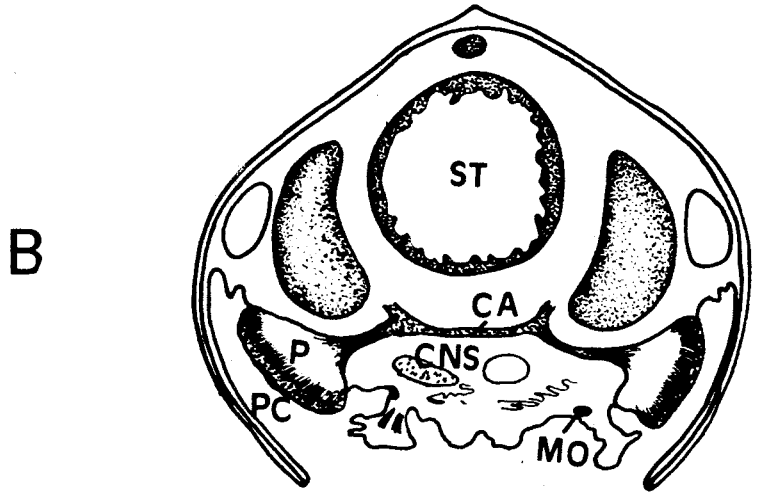


Fig. 40. Diagramatic representation of Mandibular Organ
A : Side view B : Cross section

shaped glandular cells. The diameter of the cell ranged from 3 to 6/u. Very little cytoplasm was noticed in the cells. The gland was not lobulated and the cells were fairly loosely packed (Fig. 41). In the present study significant structural changes were not seen in the mandibular gland in relation to different stages of moult cycle. Mandibular organ removal was not successful due to the death of the operated animals.

4 Neuroendocrine control of moulting

4.1. Secretory characteristics of neurosecretory cells in relation to moulting cycle.

Observations on the neurosecretory cells during the different stages of the moult cycle of eyestalk, brain, and thoracic ganglia showed cyclic changes in secretion of NSC material. These changes have been distinguished on the basis of staining intensity of the neurosecretory materials in the cytoplasm and a gradual vacuolization of the same. Except the type D-cell all the neurosecretory cells exhibited the secretory cycle. Each cell has been assumed to pass through different phases of the secretory cycle culminating in the release of neurosecretory material either in to the haemolymph or to the sinus gland, through axons. The secretory cycle of the cells

has been divided into three different phases depending on the appearance of stainable secretory granules in cytoplasm and on the appearance of the vacuoles (Fig. 42 a, b and c).

Phase I (Passive) Fig. 3b, 4a, 25 and 30

This phase has been considered as the passive phase of the neurosecretory cells. In the passive stage the cytoplasm generally filled the perikarya of the cell which gave a moderate reaction with NSC specific stains. Chromatin material was found evenly distributed in the well defined nucleus.

Phase II (Active) Fig. 3a, 4b and 5b

This phase has been identified as the active phase of NS-cells because of the appearance of deeply staining neurosecretory material in the cytoplasm. Cytoplasm was coarse with intermingled small vacuoles. In the majority of the cell, chromatin material showed a condensing tendency. The staining activity was maximum at this phase of the cell.

Phase III (Active) Fig.4c, 4d and 5c.

This phase has been the final stage or the advanced stage of the activity of the cell. The cytoplasm occupied

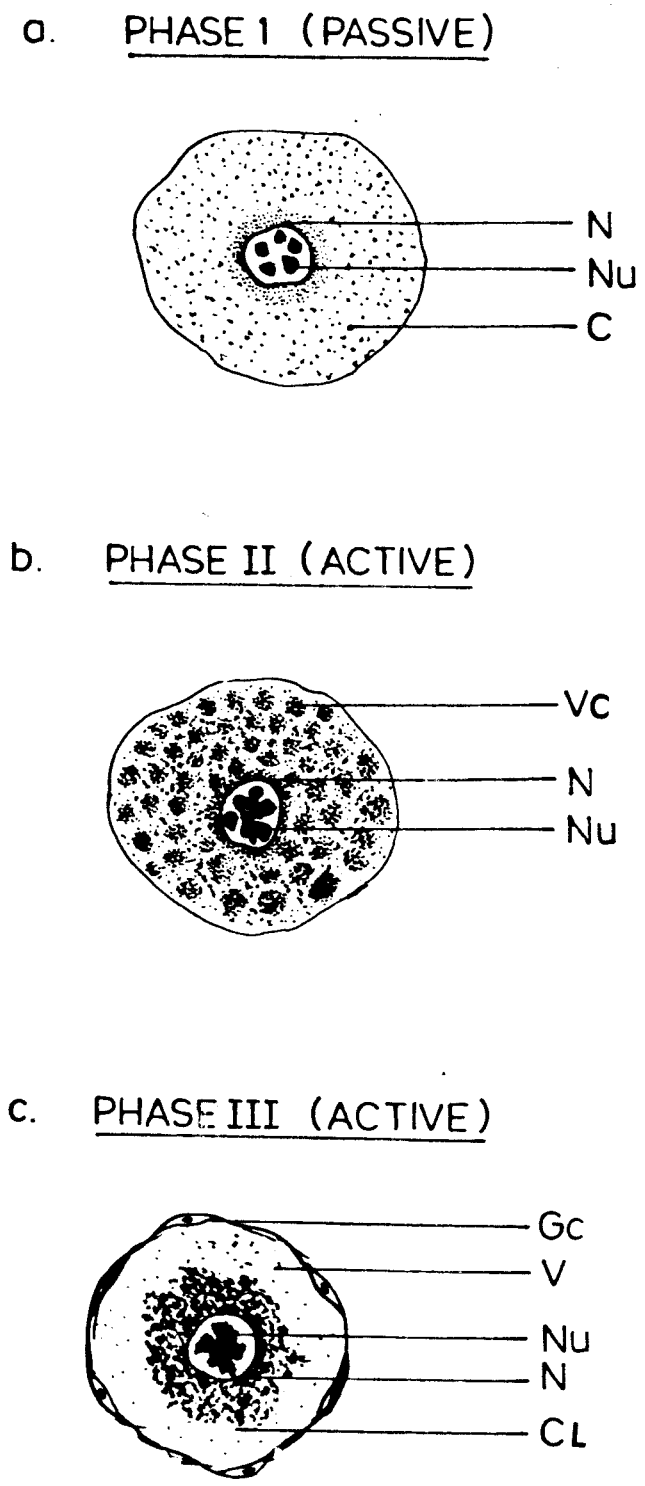


Fig. 42. Diagramatic representation of neurosecretory cycle.

C-Cytoplasm; CL-Cytoplasmic layer around the nucleus; GC-Glial cell; N-Nucleus; NU-Nucleolus; V-vacuole; VC-Vacuolated cytoplasm

by large vacuole and a cytoplasmic layer was observed around the nucleus. The central layer of cytoplasmic material was well stained with neurosecretory stains.

Neurosecretory cells in different phases of activity were detected in all the three stages of moult viz. postmoult, intermoult and premoult. Secretory activity of A, B and C cells in the eyestalk and G, A and B cells of brain were observed with postmoult, intermoult and premoult stages. Cells in the secretory phase II and Phase III were grouped into one and counted as active cells. Investigation revealed that quantitative occurrence of NSC in active and passive phase varied significantly from postmoult and intermoult to premoult. Between the postmoult to intermoult stage no significant variation was observed between the quantitative distribution of active and passive NSC ($P>0.01$) (Table 5 and 6,Fig .43)

In the postmoult and intermoult stages the percentages of active cells recorded in the eyestalk were 67.61 ± 6.94, and 69.61 ± 6.69, respectively, while the percentage of passive cells were only 32.38 ± 6.9 and 30.34 ± 6.73, respectively. The count showed an opposite trend during the premoult period. During premoult the

Table - 5: CHANGES IN THE EYESTALK NEUROSECRETORY CELL ACTIVITY ACCORDING TO THE MOULT CYCLE IN *P. INDICUS*

Moult Stage		% Active Cells	% Passive Cells
Postmoult	N	12	12
	$\overline{X}$	67.61	32.38
	SD	±6.94	±6.91
Intermoult	N	12	12
	$\overline{X}$	69.61	30.34
	SD	±6.69	±6.73
Premoult	N	12	12
	$\overline{X}$	35.41	65.33
	SD	±4.2	±4.1

Table - 6: CHANGES IN THE BRAIN AND THORACIC GANGLIA NEUROSECRETORY CELL ACTIVITY ACCORDING TO THE MOULT CYCLE IN *P. INDICUS*

Moult Stage		% Active cells	% Passive cells
Postmoult	N	12	12
	$\bar{X}$	33.91	66.08
	SD	±4.21	±4.21
Intermoult	N	12	12
	$\bar{X}$	34.29	65.70
	SD	±4.55	±4.56
Premoult	N	12	12
	$\bar{X}$	66.03	33.96
	SD	±4.26	±4.27

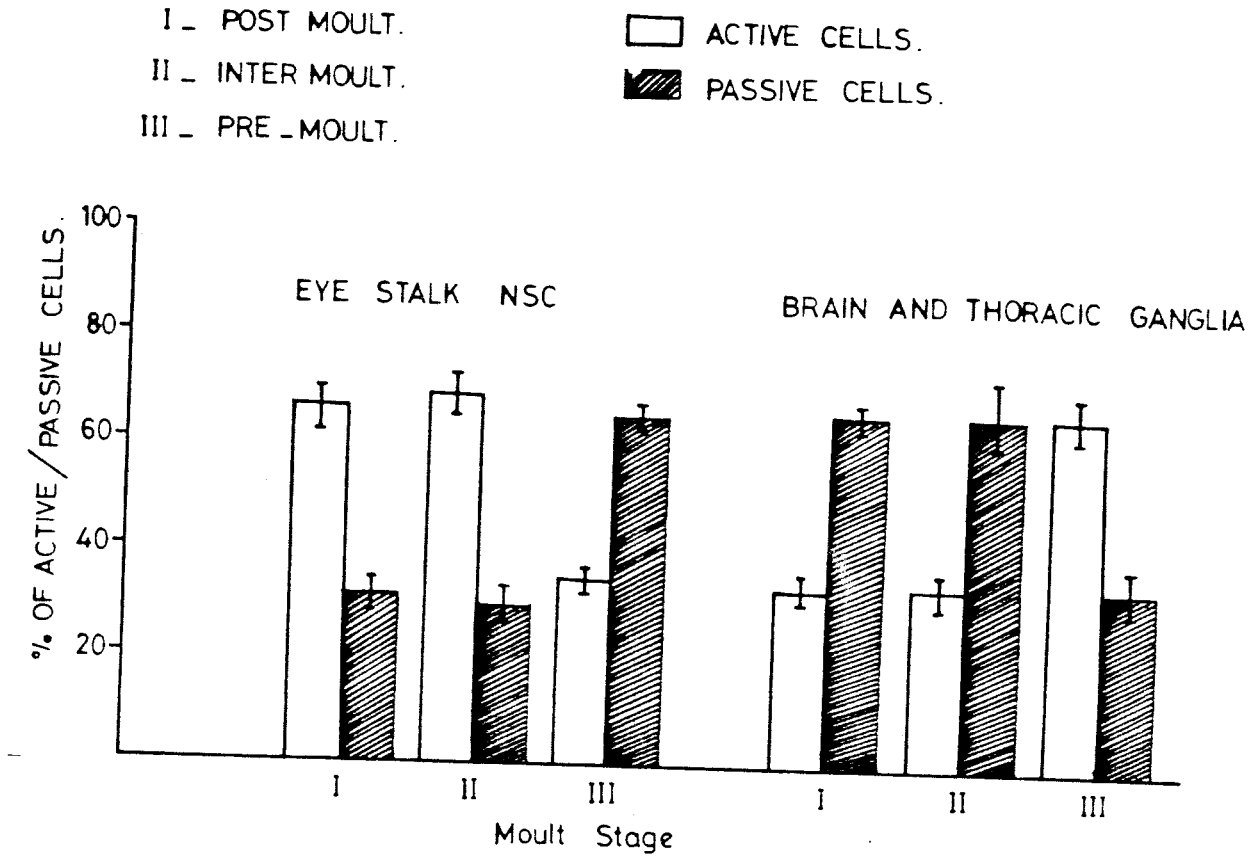


Fig. 43. Percentage variation of active and passive neurosecretory cells of eyestalk and brain in P. indicus during postmoult, intermnoult, and premoult.

percentage of passive cells was higher (65.33± 4.1) than the active cells (35.41± 4.2). The quantitative difference between the percentage of active cells in postmoult and intermoult was not significant, while percentage variations of active cells in postmoult and intermoult to the premoult were statistically significant ($P<0.01$) (Table 5). In brain and thoracic ganglia, percentage of active cells recorded in the premoult stage of the animal was 66.03± 4.26. At the same time during postmoult and intermoult the percentages of active cells were considerably low i.e. 33.91±4.21 and 34.29±4.55, respectively, when compared to the percentages of passive cells (66.08±4.21 and 65.7±4.56). The percentage differences of active cells of premoult to intermoult and postmoult were statistically significant ($P<0.01$) (Table 6). Histochemical observation also revealed increased secretory activity in the neurosecretory cells during the active phase than the passive phase. It was interesting to note that the histochemical test for protein showed increased affinity towards the active NSC than to the NSC of the passive phase. Both cysteine and cystine groups were abundantly present in the active NSC. Carbohydrates also showed more affinity towards the active cells. Sinus gland of the intermoult and postmoult prawns gave intense

positive reaction towards the neurosecretory specific stains, while in the premoult stage the reaction was weak. (Table 3a, b, c and d).

4.2. Eyestalk ablation and Eyestalk extract injection experiments on moulting.

These experiments were conducted to elucidate the role of eyestalk X-organ complex on the moult cycle of the prawns. The results are summarized in Table 7 and Fig.44.

Unilateral eyestalk ablation:

Unilateral eyestalk ablation did not show any precocious moulting among the experimental animals. The average premoult period in such animals was 120±20hrs, which was the same duration of premoult recorded for the intact control prawns. The duration of premoult period recorded in the experimental and control group has not showed any significant difference ($P>0.01$).

Bilateral eyestalk ablation:

Bilateral eyestalk ablation resulted in significant reduction in the moult cycle duration. Precocious moulting occurred in the bilaterally eyestalk ablated animals with a significantly shortened premoult period of 80±12 hrs ($P<0.01$). At the same time in the control group of

Table - 7: EYESTALK REMOVAL AND EYESTALK EXTRACT INJECTION EXPERIMENTS IN *P. INDICUS*.

Experiment		Average Premoult period in hrs. ± SE	Student's 't' test
A. Unilateral eyestalk ablation	N	16	
	$\bar{X}$	120	P > 0.01
	SD	±20	
B. Bilateral eyestalk ablation	N	16	
	$\bar{X}$	86	P < 0.01
	SD	±12	
C. Intact controls	N	16	
	$\bar{X}$	120	
	SD	±20	
D. Bilaterally eyestalk ablated with eyestalk extract injection	N	16	
	$\bar{X}$	130	P < 0.01
	SD	±13	
E. Bilaterally eyestalk ablated with saline injection	N	16	
	$\bar{X}$	80	P< 0.01
	SD	±12	

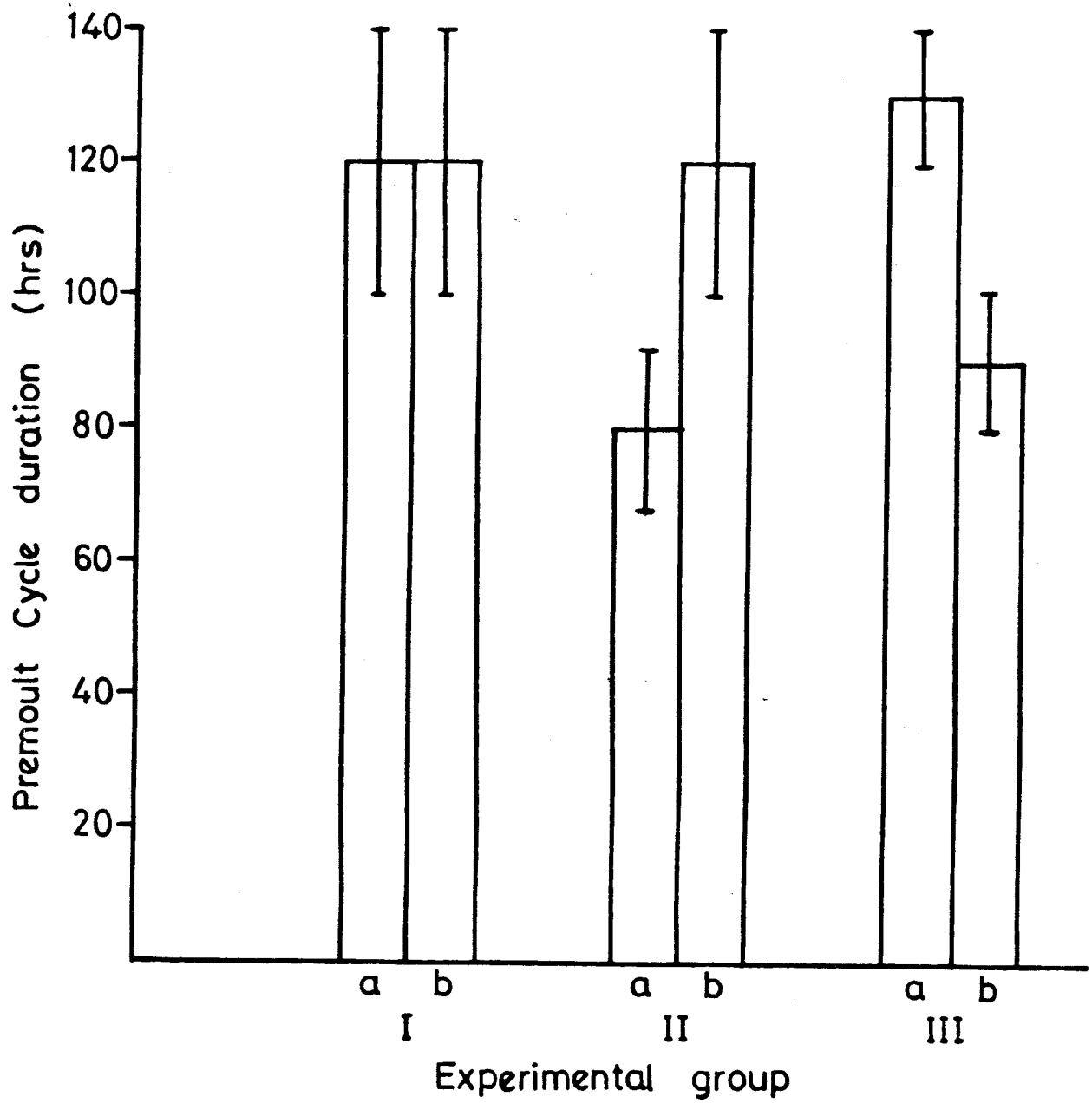


Fig. 44. Effect of eyestalk ablation on the premoult duration of P. indicus. I-Unilaterally eyestalk ablated prawns, II-Bilaterally eyestalk ablated prawns, III-Bilaterally eyestalk ablated and eyestalk extract injected prawns.
a : Experimental animals, b : Intact controls.

animals with both eyes intact, the time taken for the completion of premoult period was 120±20 hrs.

Eyestalk extract injection:

In the experimental group of bilaterally eyestalk ablated with eyestalk extract injected animals, the premoult period was found extended considerably for a period of 130±13hrs, while in bilaterally eyestalk ablated with saline water injected controls, the premoult period was completed with a significantly short period of 80±12hrs ($P<0.01$).

4.3. Morphohistology of Y-organ cells during the moulting cycle

Histological and histochemical observations of the Y-organ were made in relation to various stages of moult cycles to see if any correlation exists between the Y-organ and moulting process. Observation showed cyclic changes in size and staining properties of the Y-organ cells in relation to moult cycle. The premoult Y-organ cells were comparatively larger in size (8 to 9 /u), when compared to the postmoult and intermoult Y-organ cells (4 to 7 /u). This volume change in Y-organ cells was due to the increase in the cytoplasm of the cell. Cell cytoplasm of the Y-organ cells was found to be minimum during the

intermoult stage (C) of the prawn (Fig. 37), while cytoplasm was observed to increase in volume from early premoult stage (Do) to attain the maximum in late premoult stage D1'''and D2-3 (Fig. 38 a and b). The amount of cytoplasm was found depleted again in postmoult (Fig. 35). RNA content of the Y-organ cells showed variation during the different stages of the moult cycle. Maximum staining intensity was noticed among the premoult cells, while in the postmoult and intermoult, RNA detected was minimum. Y-organ cells deeply stained with haematoxylin in premoult stages, where as during the intermoult and postmoult stages, affinity of the Y-organ cells towards haematoxylin was poor (Table 4).

4.4. Y-organectomy experiment

Out of 24 animals in which Y-organ removal was carried out, 19 survived during the experimental period and virtually all the 19 prawns failed in performing the successful moult. Out of 19 prawns, seven animals showed an extraordinary extended intermoult period (148±48 hrs). These animals died without entering into the premoult. Four animals entered the premoult stage and proceeded towards moulting. But all of them failed in completing the ecdysis successfully and died during their effort in exuviation. Rest of the eight animals remained in pre-

Table - 8: Y-ORGANECTOMY EXPERIMENT IN *P. INDICUS*

Observations	
No. of animals used for Y-organ removal	- 24
Animals survived after operation	- 19
Animals died in intermoult	- 7
Animals died in premoult period	- 8
Animals died during moulting	- 4
No. of sham operated controls	- 17
No. of controls moulted	- 17

moult for a very long time (384±48 hrs.) and all of them died without moulting. At the same time all the 17 prawns in the control group completed the normal moult cycle successfully with a moult cycle period of 216±48 hrs (Table 8).

4. DISCUSSION

Despite the large information on the neurosecretory system in crustaceans, classification of NSC has been incomplete and even the identification and localization of cell types and cell groups varied considerably between the species. In the present study five different types of neurosecretory cells have been identified in *P.indicus*, mainly on the basis of shape and size of the cells. Except the G-type NSC, which is the largest among the five, all other four types of NSC were detected in the Optic Ganglia, while the G cells along with the other four types of neurosecretory cells were abundantly present in the thoracic and cerebral ganglia.

Attempts have been made by Matsumoto (1958), Vanherp *et al.* (1977) and Nanda and Ghosh (1985) to compare the variously described NS Cell types of decapoda, but a consensus regarding the different cell types could not be established. The reason can be due to the variations

observed in the histological and staining procedures, cyclic cellular activity, species difference and even human subjectivity. In the present study the presence of Giant Neuron(G-type cell), was never noticed in the eyestalk ganglia and this is true with other penaeid prawns like Metapenaeus monoceros (Madhyastha and Ranganeker, 1976) and Penaeus japonicus (Nakamura, 1974). In its shape, size, and histological features, A type NSC of P.indicus as observed in the present study were comparable with cell type I of Caridina laevis (Pillai, 1961), type II and III cells of P.paucidens (Hisano, 1974), type III cells of P.japonicus (Nakamura, 1974), and B cells of P.serratus (Vanherp et al., 1977). The size and morpho-histological features of A-cells detected in the present investigation showed close resemblance to the A cells of P.monodon (Nanda and Ghosh, 1985), and cell types I, II, III and IV of P.stylifera (Nagabhushanam et al., 1986). B-type cells of P.indicus were found to be similar to the type IV cells of Hisano (1974), cells of Vanherp et al. (1977), B-cells of Nanda and Ghosh (1985), and cell types V, VI and VII of Nagabhushanam et al. (1986). Pear shaped C-type cells of P.indicus showed resemblance in size and cell histology to D-cells of Vanherp et al. (1977), and C-cells of Nanda and

Ghosh (1985). In P.indicus the number of C-type cells detected was comparatively less when compared to the other cell types A,B and D. D-type cells, the smallest one, corresponds in many of its features like size, shape and cell structure to Nakamura's (1974) Type V cell, D-cells of Nanda and Ghosh (1985), and Type VIII cells described by Nagabhushanam et al. (1986). The present classification of NSC types in P.indicus is in general agreement with the classification already described for NSC of other natantians by various workers (Fingerman and Aoto, 1959; Lake, 1970; Hisano, 1974; Nakamura, 1974; Diwan and Nagabhushanam, 1975; Nanda and Ghosh, 1985; Chandy & Kolwalkar 1985, and Nagabhushanam et al., 1986).

The X-organs viz. MTGXO and MEGXO in P.indicus were found to contain the combination of type A,B,C and D types of Neurosecretory cells as observed by other workers like Hisano (1974), Vanherp et al.(1977), and Nanda and Ghosh (1985). Neurosecretory cells were not detected in the medulla interna of P.indicus. This observation was supported by the findings of Nakamura (1974), Vanherp et al. (1977), and Nagabhushanam et al. (1986). While presence of X-organ in the medulla interna has been reported by workers like Hissano (1974) and Nanda and Ghosh (1985). Nagabhushanam et al. (1986) has reported two medulla

externa X-organs in P.stylifera, while other crustacean workers like Hisano (1974), Nakamura (1974), Vanherp et al. (1977), and Nanda and Ghosh (1985) have noted only one medulla interna X-organ as observed in the present study. The variations observed in the pattern and distribution of neurosecretory systems can probably be due to the species differences.

Among the X-organs detected in the present study most prominent groups found were the two X-organs in the medulla terminalis viz. MTGXO-I and MTGXO-II. Similarly Vanherp et al. (1977) in P.serratus, and Nanda and Ghosh (1985) in P.monodon, have described the two X-organs of medulla terminalis as MTGXO- I and MTGXO-II. Nagabhushanam et al. (1986) reported the presence of three X-organs viz. MTGX-I,MTGX-II and MTGX-III in the medulla terminalis of P.stylifera. It is of special mention that no sensory pore X-organ or organ of Bellonci with typical onion bodies was detected in the eyestalk of P.indicus, though this organ has been reported from natantians by various workers (Carlisle, 1959; Dall 1965b; Lake and Ong, 1970; Hisano, 1974; Vanherp et al., 1977; Nanda and Ghosh 1985).

The histological location of the sinus gland between the medulla interna and medulla externa with an abundant

supply of blood vessels indicate that the organ, in the present study, belongs to the classical type of neurohaemal organ described for many other crustaceans (Cooke and Sullivan, 1982; Fingerman, 1987).

The mode of discharge of NS material from the cells of the X-organ indicated an axonal transport, as the fuchsinophilic axonal pathway which connects the X-organ of eyestalk and sinus gland could be clearly seen during the present observation. In the current observation, neurosecretory materials were detected in the axonal tracks of NS cell types tapering towards the sinus gland, which probably indicates the transportation of this material in to the sinus gland. Similar type of observations were made by workers like Enami (1951), Pillai (1961) and Nanda and Ghosh (1985) in the crab Sesarma, in shrimps Caradina laevis and Penaeus monodon respectively. Studies of Andrew and Saleuddin (1979) showed that at least 90% of the sinus gland terminals belongs to cells whose perikarya are in the medulla terminalis X-organ with the remainder of the terminals belongs to NSC that are not associated with X-organs.

Detection of membrane bound hallowed dense core neurosecretory vesicles with diameter of 100 to 200 nm

using electron microscopy in the NSC of eyestalk indicated the production of neurosecretory materials in the neuroendocrine complex of P.indicus. Other workers like Nakamura (1974), Hisano (1976), Andrew et al. (1978) and Bellon-Humbert et al. (1981) have also described similar type of neurosecretory vesicles in the eyestalk of P.japonicus, P.paucidens, O.virilis, and P.serratus respectively.

The distribution of neurosecretory cell groups in the brain of P.indicus was found to be some what similar to those found in Caridina laevis (Pillai, 1961), in P.japonicus (Nakamura, 1974), and in P.stylifera (Nagabhushanam et al., 1986). In P.indicus, anterior median and posterior groups of neurosecretory cells have been found distributed peripherally on the dorsal and ventral aspects of the brain, as observed by the workers Pillai (1961), Nakamura (1974), and Nagabhushanam et al. (1986) in Caridina laevis, P.japonicus, and P.stylifera, respectively. But in P.indicus a dominant group of large NS cells were also detected in the central anterio-posterior plane at the dorsal surface of the brain.

When compared with brachyura, neurosecretory profile of natantian thoracic ganglia has received little

attention. In the present investigation thoracic ganglion has been identified as one of the important centres of the central nervous system, where large neurosecretory cell types of G and A were detected. Similar type of observations were made by Matsumoto (1958) in crab Eriocheir japonicus, and Nagabhushanam et al. (1986) in P.stylifera. The anterio - posteriorly stretched groups of neurosecretory cells, situated centrally on the ventral side of the thoracic ganglia may be the principal region of the production of neurosecretory material in P.indicus.

In the present study it was observed that the neurosecretory cell groups were always noticed outside the neuropiles of cerebral and thoracic ganglia in association with the rich network of blood capillaries. This probably indicates the possibility of direct release of neurosecretory material by the process of exocytosis from these NSC perikarya to the surrounding haemolymph. The diffused outer margins of active neurosecretory cells detected during the present observation also indicated the possibility of neurosecretory release from the NSC by the process of exocytosis. This phenomenon was predominantly observed in the giant neurosecretory cells of the brain and thoracic ganglia. Similar findings have been reported

by various other workers viz. Butt and Ashby (1967) in Procambarus clarkii, Weitzmamn (1969) in Gecarcinus lateralis, and Andrew and Shivers (1976) in Orconectes virilis, while working on the neurosecretory systems of the respective species.

The histochemical tests applied to the neurosecretory cells of the P.indicus revealed that the neurosecretory material is predominantly a protein, rich in cystine (-SS groups) and cysteine (-SH-group). Cystine rich neurosecretory material has also been reported in several other crustaceans viz. P.gaimardi (Lake,1970), P.paucidens (Hisano,1974), P.japonicus (Nakamura,1974), B.cunicularis (Diwan and Nagabhushnam, 1975) and P.stylifera (Nagabhushanam et al.,1986). Using chromatographic techniques, Otsu (1965), and Otsu and Sonobe (1965) have produced evidence to indicate that the chromativacting substance from the brain and thoracic ganglia of the crab, E.japonicus is polypetides rich in cystine.

PAS test to NSC, in the present work, gave positive reaction and this reaction was not fully removed by a pre-treatment with diastase which indicated the presence of carbohydrates alongwith glycogen. The presence of PAS material in the NSC cells of crabs has been observed by

Miyawaki (1956 b,c) Gabe (1966), Lake (1970), Nakamura (1974), and Nagabhushanam *et al*. (1986). Nakamura (1974) reported the strong positive nature of NSC and he even described the NSC as a PAS positive or PAS specific cell. But in the present work NSC were strongly protein specific and the reaction with PAS was only very moderate. Reaction with Sudan Black B showed that lipid component in NSC was insignificant. Being a storage site of NSM of the eyestalk, sinus gland also showed histochemical characteristics similar to that of neurosecretory cells.

The morphohistology of the Y-organ or moulting gland detected in *P.indicus* is identical to that described for *Metapenaeus sp*. (Dall, 1965b), for *Palaemon paucidens* (Aoto *et al*., 1974), and for *P.japonicus* (Bourguet *et al*., 1977). The histology of the Y-organ in *P.indicus* showed the epidermal origin of the organ which is of the typical diecdysis type (Spindler *et al*., 1980).

Histochemical tests that are employed to know the chemical nature of the Y-organ showed almost negative reaction towards carbohydrates and lipids, and fairly positive to proteins. The histochemical tests in the present study also failed to detect the steroid secretory nature of the Y-organ cells. One of the probable reasons

might be the inability of the test to detect the nanogram levels of steroid present in the Y-organ. The quantity of ecdysterone reported in the Y-organ cells of crustaceans is only in nanogram levels (Spindler et al. 1980). Furthermore, morphologically it has been confirmed that the Y-organ does not have any storage sites, therefore the material (Ecdysterone) synthesized in the cells has been considered to be released and transported continuously to the target sites (Spindler et al., 1980). However, Aoto et al. (1974) while working on the fresh water prawn P.paucidens, reported the occurrence of minute granules in the cells of Y-organ that stain with Sudan Black B in all stages of the moult cycle. Nevertheless their finding that no glycogen granules or lipid droplets were seen agrees with the present findings. Higher levels of RNA and strong cytoplasmic-basophilia detected in the cells of P. indicus Y-organ point towards the synthetic nature of the gland.

Studies dealing with the structure and functions of Mandibular organ among natantia, especially in penaeidae is very much limited. P.japonicus is the only one species which received some primary attention among penaeid group (Taketomi and Kawano, 1985). Morphohistological and cytological characteristics of the Mandibular organ observed in P.indicus found resemblance to the mandibular

organ described for P.paucidens (Aoto et al,1974), O.limosus (Burghause,1975), and P.japonicus (Taketomi and Kawano, 1985). As reported by the above workers (Aoto et al., 1974; Burghause, 1975 and Taketomi and Kawano,1985), in the present observation also the organ detected was not lobulated, and have loosely packed oval shaped cells.

Light microscopical studies and histochemistry of neuroendocrine systems in the present work during the different stages of moult showed, stage dependent cellular changes in the secretory systems of eyestalk, brain, thoracic ganglia, and Y-organ of P.indicus. But Mandibular organ did not reveal any moult linked changes.

Major neurosecretory cell types of eyestalk, brain and thoracic ganglia (Type G, A, B and C cells) observed in the current study exhibited a neurosecretory cycle during moult cycle of the prawn, with an active and passive phase. As evidenced by the histochemical tests, the active phase represents the synthetic phase in which the neurosecretory material was found to be actively produced in the NSC. At the same time in the passive phase, because it represents the resting nature of the neurosecretory cells, the production of the neurosecretory material was found to be insignificant. Appearance of

cytoplasmic vacuoles with NSM during the active phase of the secretory cycle showed the synthetic nature of the neurosecretory cell. Similar types of cyclic behaviour of the neurosecretory cells during the moult cycle of various crustaceans were reported by many workers like Durand (1956), Matsumoto (1962), Bellon-Humbert et al. (1981), and Chandy & Kolwalkar (1985).

Irrespective of the different physiological stages of the moult cycle, both active and passive cells were detected in the eyestalk, brain, and thoracic ganglia of P. indicus. But significant quantitative differences in number of cells were obtained between the active and passive neurosecretory cells during the different moult stages of the animal. In the eyestalk of postmoult and intermoult prawns majority of the (61 to 75%) neurosecretory cells were in the active phase, indicating the active synthesis of neurosecretory material. While it was of interest to note that in the eyestalk of the premoult prawns the majority of the NSC (61 to 70%) was in passive phase, showing the feeble production of neurosecretory materials. From this behaviour of neurosecretory cells it, can be assumed that in the postmoult and intermoult there is an increased production

of neurosecretory material in the eyestalk of prawn compared to the eyestalk of premoult prawn, where production of neurosecretory material was found to be very less. Similar studies of Durand (1956) and Bellon-Humbert et al. (1981) in the eyestalk of P.serratus, and Chandy and Kolwalkar (1985) in Charybdis lucifera showed that the active NSC were maximum in the postmoult and intermoult stages than in the premoult stage. Chiang and Steel (1984) in isopodes, and Chandy and Kolwalker (1985) in crab, also noted a reduced NS potential in the NSC during premoult stage, representing a reduced neurosecretory release.

Unilateral eyestalk ablation in P.indicus has not elicited any precocious moulting. This may be probably due to the fact that the moult inhibiting material removed through a single eye may not be sufficient to remove the moult inhibitory potential of the prawn (Diwan and Nagabhushanam, 1974). But bilateral eyestalk ablation accelerated the onset of proecdysis and moulting as observed in the present study. When the eyestalks were removed in the postmoult and intermoult stages, the prawn moulted faster with a significantly reduced premoult period. When the bilateral eyestalk ablation was followed by a eyestalk extract injection, acceleration of moult

process was found to be inhibitted. These observations suggested that the factor removed by the eyestalk extirpation is moult inhibitory in nature. The fact that eyestalk removal resulted in precocius moulting has been shown by many workers viz.Diwan and Nagabhushanam (1974) in crab, Freeman and Bartel (1975) in crayfish, Nakatani and Otsu (1979), Quackenbush and Herrnkind (1981), Radhakrishnan and Vijayakumaran (1984), and Snyder and Chang (1986) in lobsters, and Webster (1985) in caridian prawns. These workers described the moult inhibitory factor found in the eyestalks as the moult inhibiting hormone (MIH).

Present study also indicated that once the animal has advanced into proecdysis, the duration of moult cycle did not change significantly either by eyestalk ablation or by extract injection. Possible explanation for this observation is that during premoult, secretion of moult inhibiting materials in the eyestalk is negligible. This was indicated by detecting the passsive phase of majority of NSC during the premoult period of P.indicus in the present study. In agreement with the present observation, Freeman and Bartel (1975) in Palaemonets, and Hopkins (1982) in Uca pugilator have also observed reduced

neurosecretory activity in the premoult period of these animals. Precocious moulting observed in the present work due to bilateral eyeablation in post moult and intermoult can be attributed to the removal of dominating moult inhibiting factor present in the eyestalk during postmoult and intermoult period. This view is supported with the detection of maximum percentage of active NSC in the postmoult and intermoult stages of P.indicus. In short it can be seen that the moult inhibiting factor in the eyestalk of P.indicus fluctuated during the moult cycle with higher concentration in the postmoult, intermoult stage, and lower concentration during the premoult period.

When the neurosecretory cells of the brain and thoracic ganglia were studied it was found that percentage of active cells detected showed an opposite trend to that of eyestalk. Here the percentage of active cells was maximum (62 to 70%) during the premoult stage of the animal. In postmoult and intermoult the percentage of active cells was significantly low (29 to 30%) when compared to the passive cells (61 to 70%). Matsumoto (1962), while working on the crab Hemigrapsus, noticed remarkable increase in the activity of large neurosecretory cells of thoracic ganglia during the premoult stage. Since the secretory period of NSC of brain and thoracic ganglia of

P.indicus was almost opposite to the secretory period of eyestalk, the neurosecretory material produced by brain and thoracic ganglia cannot be moult inhibiting in nature, instead it can be a moult accelerating factor as described by Carlisle (1953), and Martin et al. (1980). Carlisle's (1953) hypothesis of moult accelerating factor in the control of moulting process in addition to the moult inhibiting factor among diecdysic crustaceans generated a wide spread interest among workers who were studying endocrine control of moulting in crustaceans.

The size and the tinctorial affinity of the Y-organ cells varied markedly during the moulting cycle of P.indicus. The volume changes noted in the Y-organ cells might be due to the increase in cytoplasmic contents of the cells during the premoult followed by a decrease in the postmoult. The strong affinity of the Y-organ cells towards RNA and haematoxylin and enlarged cell volume observed during the premoult stages in the present study probably indicate the high synthetic nature of the organ during premoult, which would result in the secretion of moult accelerating material. On the contrary, feeble affinity of the cells towards the RNA and haematoxylin and decreased cell volume during postmoult and intermoult show

poor secretion of moulting factor. These observations suggest the secretory behaviour of Y-organ, which is active during premoult, and passive during postmoult and intermoult. Findings of Aoto et al. (1974) on increased cell volume and a high affinity of cell cytoplasm towards RNA and haematoxylin during the premoult period in P.paucidens were similar to the present study.

The Y-organectomy experiments clearly showed the moult controlling function of the Y-organ in P.indicus .Among the Y-organectomised prawns the onset of premoult developments was found inhibited indicating the presence of moult inducing or accelerating factor in the Y-organ. Similarly Y-organoctomy performed by various workers like Bourguet et al. (1977) in Penaeids, Maissiat et al. (1970) in isopods, and Burghause (1975) in amphiphodes also showed that the Y-organ governs the moult controlling function in these animals. But in the present experiement some of the Y-organectomized prawns entered the premoult stage and advanced towards the ecdysis, though ecdysis was unsuccessful causing the death of the animal. Possible explanation for the initiation of moulting in such animals might be due to the incomplete removal of the Y-organs from some of the animals. As noted by Burghause (1975) in O.limoses, complete separation of Y-organ from the

surrounding tissue in P.indicus is more difficult than in brachyura, where the Y-organs are more readily discernable in the live animal. The secretion of moult accelerating material by fragments of Y-organ left in place may be sufficient to initiate premoult development. But in order to proceed towards a successful ecdysis, the presence of complete Y-gland was found very much essential (Highnam and Hill, 1979).

From the results of the present study it can be postulated that the primary control of moulting in P.indicus resides in the secretory products of X-organ sinus gland complex of eyestalk, and an endocrine gland called Y-organ(Fig.45). Observation on the secretory nature of neurosecretory cells that the moult inhibiting material secreted by the X-organ sinus gland complex was found maximum during the postmoult and intermoult of the animal, at the same time Y-organ cells showed a reduced activity. This would probably result in the dominance of moult inhibiting materials during the postmoult and intermoult resulting in the prevention of moult. On the contrary during the premoult stages the moult inhibiting materials secreted by the neurosecretory cells of the eyestalk showed decreased activity and correspondingly

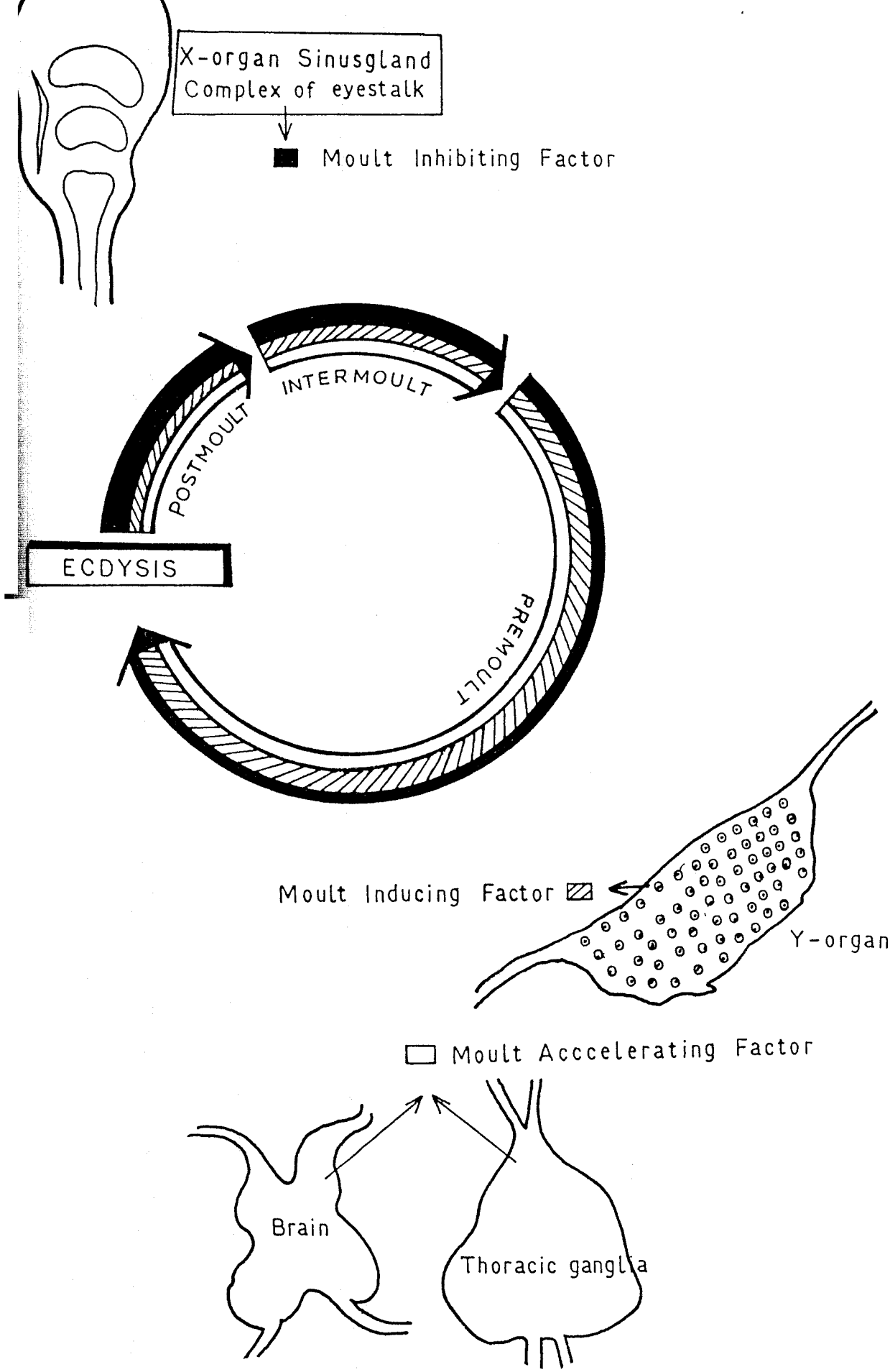


Fig. 45. Diagramatic representation of endocrine control of moulting in P. indicus

there was an increase in the secretion of the moult accelerating materials by the Y-organ cells. Evidently the increased activity of the Y-organ causes a dominance of moult inducing factor during the premoult stages inducing moulting process. The predominant secretions of neurosecretory materials from the neurosecretory cells of brain and thoracic ganglia in the premoult stages may be indicative as a source for moult promoting factor.

S U M M A R Y

Structure of neuroendocrine centres such as X-organ-sinus gland complex (Eye), brain, and thoracic ganglia, and their changes during the moult cycle were studied using histology and histochemistry. The neurosecretory cells have been classified into five differnt types on the bases of size, shape, and staining characterestic as given below: 1. Giant neuron(G-type cell) with a diameter of 75± 12/u, 2. Large oval cell (A- type cell) with a diameter of 40± 17/u, 3. Small oval cell (B-type cell) with a diameter of 23± 4/u, 4. Club shaped cell (C-type cell) with a diameter of 15±4/u, and 5. Small round cell (D-type cell) with a diameter of 9±4/u. G type cell is the largest among the five cell types while the D type cell is the smallest. Type A and B cells are the most common and widely distributed, where as the C type cell showed

limited distribution.

Histochemical tests showed that the neurosecretory material is predominantly a protein with small amount of carbohydrates and lipids. Strong positve nature of the neurosecretory cells except D type cell, to PAF, CHP, and MTP revealed the neurosecretory nature of these cells. Except D cell, all the neurosecretory cells exhibited a neurosecretory cycle, with an active neurosecretory phase and passive neurosecretory phase. Secretion and release of neurosecretory materials were seen in the active phase, while the passive phase showed the non-neurosecretory phase of the neurosecretory cell.

Ultrastructural study revealed that the neurosecretory material of the eyestalk is a hallowed-dense- core vesicle of spherical shape with 120 to 130 nm in diameter.

In the eyestalk, percentage of active neurosecretory cells was high during the postmoult and intermoult when compared to the premoult, whereas in brain and thoracic ganglia, high percentage of active neurosecretory cells was noted in the premoult, and low in postmoult and intermoult. Higher neurosecretory levels in

he eyestalk may probably result in the inhibition of ıoulting.

Eyestalk neurosecretory system in P.indicus was found :o be composed of X-organs and sinus gland. Two medulla :erminalis ganglionic X-organs (MTGXO I and MTGXO II) were ;situated in the medulla terminalis, while a single medulla ɜxterna X-organ (MEGXO) was detected in the medulla ɜxterna. The neurohaemal organ (the Sinus gland) was located between the medulla interna and medulla terminalis in the latero-longitudnal axis of the eyestalk.

Experiments on bilateral eyestalk ablation showed precocious moulting with a significantly short moult cycle duration. But bilateraly ablated prawn which recieved an eyestalk extract injection (equivalent of two eyestalks) did not show any accelarated moulting, indicating the presence of moult inhibiting factor in the eyestalk. Unilateral eyestalk ablation has not showed any significant effect on the moult cycle of the prawn.

The location and the structure of the Y-organ in Penaeus indicus have been reported for the first time through the present study. The organ is situated between the mandibular and posterior dorsoventral muscle, in close association with the hypodermis. Changes were noted in the

size of the Y-organ cells during differnt stages of the moult cycle. Y-organ removal resulted in the failure of moulting processes indicating the necessity of Y-organ for the successful completion of moult.

The mandibular organ was found out and located using histological techniques in P.indicus for the first time. The organ was detected near the posterior central base of the adductor muscle of each mandible.

CHAPTER-III ROLE OF SOME IMPORTANT METABOLITES ON MOULTING PROCESS

CHAPTER III

ROLE OF SOME IMPORTANT METABOLITES ON MOULTING PROCESS

1. INTRODUCTION

The process of dynamic event of moulting or ecdysis dominates in the life history of crustaceans. In this group of animals with a firm calcareous exoskeleton, moulting is the only means by which the tissue growth can be achieved. As the animal is forced to renew the exoskeleton during each moult, a considerable mobilisation occurs in the major organic reserves of the animals, which directly or indirectly participate in the construction of exoskeleton (Stevenson, 1985).

Passano (1960) and Yamaoka and Scheer (1970), while reviewing the principal physiological characteristics of the typical decapod moult cycle, gave primary importance to the accumulation of organic reserves in the different tissues of the animal body. Several crustacean workers who have attempted to elucidate moulting phenomenon by investigating biochemical changes associated with the moulting cycle have reported profound changes in the organic content of the major body tissues, which can be correlated with the moult cycle of the animals (Aiken, 1980)

Hepatopancreas has been identified as the primary organ for the storage of organic reserves, while haemolymph plays a secondary role as a storage site, and some accumulation of metabolites have been reported in muscle tissues of the body (Yamaoka and Scheer, 1970). The organic reserves constituted of proteins, lipids, and carbohydrates are important not only as a source of material for the construction of exoskeleton but also for the required energy during moulting.

Protein is one of the important and essential components among the organic reserves of crustaceans and considerable changes have been noticed in protein content of the haemolymph, hepatopancreas and muscle tissue in relation to moult cycle (Stevenson, 1985). Travis (1955a) while studying the physiological changes during the moulting cycle of the spiny lobster Panulirus argus, determined the blood protein in accordance with the moult cycle. Thereafter, workers like Busselen (1970) in Carcinus maenas, Lynch and Webb (1973) in Callinectes sapidus, and NageswaraRao et al (1986) in Ocypoda macrocera have reported variation in the levels of haemolymph protein with the changing stages of moult. Differences in the haemolymph protein content of crabs Carcinus

mediterraneus and Acanthony lunulatus during the moult cycle have been observed by Herberts et al. (1978) and Chaix et al (1981). Moult linked changes in the haemolymph protein of lobsters have been studied by number of workers viz. Barlow and Ridgway (1969) in Homarus americanus, Dall (1974) in Panulirus longipes, and Hepper (1977) in Homarus gammarus. Among penaeids, Bursey and Lane(1971) worked on haemolymph protein content of Penaeus duorarum in relation to different stages of moult cycle.

Protein mobilization in the whole body of P.indicus and P.esculentus during the moult cycle was studied by Read and Caulton (1980) and Barclay et al. (1983). Heath and Barnes (1970) while working on the crab Carcinus maenas determined the hepatopancreatic protein variation in relation to the moult cycle. Recent work of Barclay et al. (1983) in Penaeus esculentus revealed the protein content of hepatopancreas in relation to different stages of moult cycle.

Studies on nucleic acids in accordance with the crustacean moult cycle are very limited. By incorporating radioactive amino acids, Skinner (1966a, 1968) studied the RNA levels in the developing muscle and hepatopancreas of the land crab Gecarcinus lateralis in accordance with the

different stages of moult cycle. In the midgut gland of Orconectes virilis, Gorrel and Gilbert (1971) found an increase of RNA content during the premoult period of the moult cycle. McCarthy et al. (1976) reported RNA content and protein in relation to the moulting cycle of lobster Homarus americanus. During the moulting cycle of Orconectes sanborni, changes in epidermal DNA, protein, and protein synthesis have been described by Humphreys and Stevenson (1973). Later on, Dall and Barclay (1979) emphasized the significance of nucleic acid during the moult cycle of the western rock lobster Panulirus longipes.

The importance of lipid in the metabolic economy of most crustaceans is suggested by the percentage composition of the organism, particularly in the tissues of digestive glands (Yamaoka and Scheer, 1970). Renaud (1949) studied the lipid accumulation of Cancer pagurus during the different moult stages. Histochemical data with regard to variation in lipid content during different stages of moult cycle in Panulirus argus were furnished by Travis (1955a). In the crab Ocypoda macrocera, lipid contents of the hepatopancreas and integumentary tissues during the moult cycle were studied by Nagabhushanam and Ranga Rao (1967). While working on Geacarcinus lateralis

and Orconectes virilis, O'connor and Gilbert (1968) observed moult linked changes in the lipid content of hepatopancreas. Mobilization of lipid reserves in crab Carcinus maenas between the moult stages was studied by Heath and Barnes (1970) and later elaborated by Spindler-Barth (1976).

Lipid synthesis at various stages of moult cycle in the prawn Palaemon serratus and Palaemon paucidens have been described by Teshima et al. (1975), and Teshima and Kanazawa (1976) respectively. Cyclic histological and histochemical changes of lipid in the hepatopancreas associated with the moult cycle of the prawn Metapenaeus monoceros were reported by Madhyastha and Ranganekar (1974).

Workers like Kanazawa et al. (1976), Ando et al. (1977), and Teshima et al (1977) studied the lipid content during different stages of moult cycle of the prawn, Penaeus japonicus. Recent workers, Read and Caulton (1980), and Barclay et al. (1983) have attempted to study the lipid reserves of Penaeus indicus and Penaeus esculentus as a function of moult stages.

In crustaceans, carbohydrates serve as a precursor of

hitin and also as an energy source in the intermediary etabolism (Yamaoka and Scheer, 1970). Meenakshi and Scheer 1961) have made an earlier attempt to determine glucose evels in haemolymph of crabs Cancer magister and emigrapsus nudus in relation to moult stages. Variations f blood glucose associated with the moulting cycle of obsters were studied by Telford (1968) in Homarus mericanus, and Dall (1975) in Panulirus longipes. Later tudies of Lynch and Webb (1973) in crab Callinectes apidus, Parvathy (1970, 1971) in isopods Emerita siatica, Ligia exotica, and Telford (1974) in crayfish rconectes propinqius and Cambarus roleustus revealed the lood sugar content in relation to different moult stages f the animals. Moult linked variation of haemolymph lucose of Metapenaeus species was reported by Dall 1965c), while studying the physiology of the shrimp.

Vonk (1960) stressed the important role of lucosamine as an intermediate for chitin synthesis during he moult cycle of crustaceans. Dall (1965a) studied the lucosamine content in the haemolymph of Metapenaeus pecies in accordance with different moult stages. ecently, Stevenson (1985), while reviewing the hysiological process of cuticular synthesis, described he nature of glucosamine variation and emphasised its

importance in the building up of the cuticle.

The importance of glycogen as a main functional metabolite in crustaceans has been identified and described by Honke and Scheer (1970). Renaud (1949), and Travis (1955a) have reported the cyclic changes in the glycogen content of Cancer pagurus and Panulirus argus with different stages of moult cycle. Works of Heath and Barnes (1970) and Spindler-Barth (1976) in the common shore crab Carcinus maenas revealed moult dependant changes of glycogen in different tissues of the animal. Histochemical evidence for the glycogen variation in the hepatopancreas of Metapenaeus monoceros associated with the moult stages was furnished by Madhyastha and Rangnekar (1974).

As a main organic constituent of crustacean exoskeleton, Chitin has got an important role in the metabolic economy (Stevenson, 1985). Studies regarding chitin with reference to its role in synthesis of exoskeleton during the moulting cycle are very limited. Stevenson (1978), Hornung and Stevenson (1971), and Gwinn and Stevenson (1973), have studied the chitin synthesis in relation to different stages of moult cycle in crayfish Orconectes sanbornii and Orconectes obscurus. Parvathy

(1970) and Spindler-Barth (1976) have estimated the chitin content of the cuticle in relation to moult cycle in the isopod Ligia exotica, and the crab Carcinus maenas.

The uptake and retention of water during premoult is an integral part of the moult cycle. In crustaceans significant variation was observed in the water content of muscle and hepatopancreas during different stages of moult cycle (Passano, 1960; and Aiken, 1980). Travis (1954) in the lobster Panulirus argus, and Diwan and Nagabhushanam (1974) in the crab Barytelphusa cunicularis have observed profound changes in the water content of muscle between the early postmoult and intermoult stages of the animals. Studies of Dall and Smith (1977, 1978a, 1978b) in western rock lobster have indicated notable variation in the rate of water absorption during different stages of moult process. Estimation of the water content variation in penaeids P.indicus and P.esculentus has been made by Read and Caulton (1980), and Barclay et al (1983), respectively, in accordance with the moult stages.

Though the metabolic changes in crustaceans exhibited somewhat general pattern, significant intraspecific and interspecific variation do exist between the different species (Heath and Barnes, 1970). Hence, separate study

for each species is essential for the purpose of understanding the body metabolism. In the present study, quantitative variations in protein, RNA, DNA, lipid, glycogen, and water content of the muscle and hepatopancreas; variations in glucose, glucosamine, glycogen, protein and lipid of haemolymph, and variation in chitin content of the cuticle in the prawn Penaeus indicus, in responce to the prominent stages of the moulting cycle have been estimated.

2. MATERIALS AND METHODS

2.1. Animals

Live specimens of P.indicus for biochemical analysis were collected from the prawn culture fields of Vypeen Island near Cochin and were transported to the Central Marine Fisheries Research Institute (CMFRI) Laboratory using the transportation bags. On reaching the laboratory the prawns were transferred to fibre glass tanks of 250 litre capacity filled with filtered, and well aerated sea water. Healthy, adult prawns yet to attain reproductive maturity in the size range of 90-120 mm were selected, and housed individually in floating cages, so as to use these animals for biochemical analysis at the required stages of moulting. The moult staging identification was done

according to the method described in the chapter-I. The prawns for biochemical analysis were selected from seven moult stages viz. Early postmoult-A, Late postmoult-B, Intermoult-C, Early premoult stages-Do and D1', and Late Premoult stages-D1''' and D2-3. Haemolymph was analysed for protein, lipid, glycogen, glucose and glucosamine content. Muscle and Hepatopancreatic tissues were analysed for protein, lipid, and glycogen. Chitin was estimated from the dried samples of exoskeleton, while for the estimation of RNA,DNA, and water content, fresh tissues of muscle and hepatopancreas were used.

2.2.Haemolymph collection and tissue sampling

Prior to the collection of haemolymph, the carapace and adjacent areas of the prawns were thoroughly blotted with absorbent paper to remove excess water attached to the body surface. Haemolymph samples from the individual prawns were collected directly from the heart through the cephalothorax region of the body using hypodermic syringe fitted with a no.22 needle. The glass syringe and needle used for the haemolymph collection were rinsed in an anticoagulant (10% Trisodium citrate) prior to each collection. The collected haemolymph samples were maintained in glass vials in frozen condition at _20 °C, until use.

After the extraction of haemolymph, the prawns were sacrificed quickly and the hepatopancreas, body muscle, and exoskeleton tissues were excised out. Tissues were dried at 60 °C, till constant weights were obtained. Dried tissues were stored in desiccators with silica gel until further use.

2.3. Biochemical analysis

a. Water content

Moisture contents of the muscle and hepatopancreas were determined by keeping the preweighed wet samples at 60 °C in hot air oven till constant weights were obtained. Subsequently the tissues were desiccated over silica gel and then reweighed. The loss in weight was taken as the water content of the tissue.

b. Total Lipid

Lipid estimation was carried out as per the method of Folch et al. (1957). Preweighed tissues or a known aliquot of haemolymph were homogenized with 3 ml of Chloroform; methanol solution (2:1 V/V) in a good homogenizer. The tissue was macerated well. The homogenate and washings were pooled and centrfuged. The supernatent was transferred to a stoppered glass tube. The residue was

washed further with 1ml of chloroform; methanol mixture, centrifuged and the washing added to the previous supernatent to give a total volume of about 7 ml. Then 0.90% Sodium Chloride (for each 1 ml supernatent 0.2 ml Sodium Chloride) was added to the combined supernatent, and the mixture gently shaken. The emulsion was then collected in an amber coloured separating funnel and was allowed to stand overnight in a refrigerator to remove soluble impurities. The lower layer of the organic solvent was removed from the separating funnel. The solution containing lipid was then evaporated carefully to dryness in a water bath, just below the boiling point of Chloroform solution. The dry weight of the lipid obtained was determined gravimetrically using a Metler Monopan Balance.

Note:Filtrate obtained after lipid extraction was saved and used for the estimation of protein and glycogen.

c. Protein

Protein was estimated as total protein by the Biuret method of Gornall et al. (1949) using crystalline bovine serum albumine (Sigma) as standard. The protein from the blood was precipitated using 80% ethanol and the haemolymph protein precipitate was dissolved in 1N NaOH. In the case of muscle and hepatopancreas, the lipid

extracted fraction was dissolved in 10% NaOH using approximately 1 ml of NaOH per 15 mg dry weight of the sample. The solution was filtered and made to a final NaOH concentration of 6%. Then, 1 ml of the protein sample was pipetted out into a test tube followed by the addition of 4 ml of Biurette reagent. Colour developed was read at 540 nm using a colorimeter (Erma, Japan).

d. Nucleic Acids

RNA and DNA in the tissues, muscle and hepatopancreas were estimated as per the scheme given by Dagg and Littlepage (1972) which is based on the methods of Schmidt and Thaunhauser (1945), and Munro and Fleck (1966). Nucleic acids were extracted from fresh tissues using cold Perchloric Acid (PCA). RNA was measured directly by reading the absorbance at 260 nm. The DNA content was determined by the Indole method (Ceriotti, 1952). Absorbance was read in a UV Senior Spectrophotometer.

Scheme of Analysis: A known weight of fresh tissue of about 50-100 mg was taken. Tissue was homogenized in 4 ml of cold distilled water. The homogenate was transfered to a centrifuge tube. Any tissue remained on the sides of the homogeniser was removed by rinsing with 1 ml of ice cold

distilled water, added 2 ml of cold PCA of 0.6N, and kept in an icebath for 10 mts. Then centrifuged at 0-4 °C at 10,000 rpm for 15 mts and the supernatent was discarded. The precipitate was washed twice with (2 ml each) 0.2N cold PCA, carefully decanted the PCA, and the tubes allowed to drain out into a filter paper for 10 minutes. Four ml of 0.3 N KOH was added to the tube and incubated in a shaking waterbath at 37 °C for 1 hour to dissolve the material completely. Chilled the tubes in an icebath, and added 5ml of of 0.6 N cold PCA, centrifuged at 10,000 rpm for 15 mts at −4 °C and retained the supernatent. The precipitate was washed twice with 5 ml of cold 0.2 N PCA and centrifuged, added the supernatent, and made upto 25 ml with distilled water for RNA analysis. For RNA, the absorbance was taken at 260 nm with a blank of 0.2 PCA.

The left out precipitate after the extraction of RNA was dissolved in 5.0 ml of KOH reagent (0.3 N KOH) and kept at 37 °C in a shaking water bath overnight. Diluted to a known volume with water and 2 ml sample solution was pipetted out for DNA estimation. To the 2 ml sample, 1 ml indole reagent was added followed by 1 ml of con.HCl and the tube was placed in boiling water bath for 10 mts and cooled to room temperature under running water. Then the

solution was extracted three times with 1 ml of amylacetate to remove the interfering colour. The phases were separated by centrifuging, and the upper amylacetate layer discarded. For DNA, the absorbance was taken at 490 nm.

Standards: For RNA and DNA, standard curves were prepared by using synthetic RNA and DNA obtained from SRL and Sigma chemicals. 100 mg of RNA powder was suspended in 5 ml of distilled water. The mixture was warmed and agitated till it goes into solution. DNA standard was also prepared as in the case of RNA. Stock solutions were stable when stored under refrigeration.

e. Haemolymph glucose

Glucose estimation was carried out according to the modified methods of Somoygi (1945). Fresh haemolymph samples were used for the estimation of glucose.

Scheme of Analysis: Using a micro pipette 0.1 ml of haemolymph was added to 1.5 ml of distilled water in 10 ml glass tube and mixed well. For deproteinisation, 1 ml of 0.3N NaOH was added to the above solution and mixed with swirling movement, followed by the addition of 1 ml of 5% zinc sulphate. The solution was again mixed well and centrifuged. From the supernatent obtained, 1 ml of the

solution was pipetted out into a 25 ml graduated and stoppered test tube and added 1 ml tartaric reagent. The resultant solution was mixed well and heated for 20 minutes in a boiling water bath, covering the test tubes with glass marbles. Cooled the tubes in a pan of cold water. Then 1 ml of Arsenomolybdate reagent was added and again mixed well for the development of colour. The mixture was diluted to the mark with distilled water and the absorbance was taken at 520 nm for glucose using a colorimeter (Erma,Japan).

f.Haemolymph glucosamine

Glucosamine in the blood was determined by the method of Elson and Morgan (1953). Glucosamine standard obtained from BDH, England was used for the preparation of standard curve. Since ordinary grade acetyl acetone has not given proper colour devolopment, pure Analar grade acetyl acetone obtained from the BDH was used for the present estimation of glucosamine.

Scheme of Analysis:A known aliquot of haemolymph (0.1 or 0.2 ml) was taken in a graduated test tube and made upto 1 ml with distilled water. 1 ml of 2N HCl was added to the haemolymph sample and placed in boiling water bath for 1 hour. Then the acid was neutralized with 2N NaOH using phenophthalein as an indicator. After neutralization 1ml

of freshly prepared acetyl acetone was added to the above solution and placed in a boiling water bath for 20 mts. The solution was cooled immediately by keeping in ice-cold water. Five ml of 95% ethanol were added to the cooled solution, mixed well and added 1 ml Ehrlich reagent. After thorough mixing the solution was allowed to stand for 30-45 minutes. The purple colour developed was read at 530 nm using a colorimeter (Erma, Japan).

g. Haemolymph Glycogen

Haemolymph glycogen was estimated according to the method of Montogomery (1957). Glycogen obtained from sigma chemicals was used for making the standard curve.

Scheme of Analysis: 0.1 ml of haemolymph was pipetted out into a clean test tube using a micro pipette and made up the volume to 2 ml using distilled water. Added 0.1% of 80% phenol. 5 ml of H_2SO_4 was added to the above solution by blowing out the pipette forcefully for good mixing. Allowed to stand for 30 minutes at room temperature. For glycogen, absorbance of the solution was read at 490nm using a colorimeter (Erma, Japan).

h.Glycogen in Muscle and Hepatopancreas

Glycogen in muscle and hepatopancreas was determined using a modified anthrone method used by Spindler- Barth

(1976). Glycogen standard obtained from Sigma chemcials was used for the preparation of standard curve.

Scheme of Analysis: 100 mg of dried tissue was taken and dissolved in a known volume of KOH (10 ml KOH for 1 gm of dried tissue) by boiling the solution for 20 minutes in a water bath. After cooling, 0.5 ml sample was pipetted out into a 10 ml centrifuge tube and added 95% ethanol and kept in a fridge for 24 hours. The precipitate collected after centrifugation was extracted twice with 0.5 ml of 5% Trichloro Acetic Acid (TCA). Glycogen reprecipitated by adding 2 ml of ethanol. The pellet obtained after centrifuging was dissolved in 1 ml of distilled water. Glycogen was assayed by mixing 0.5 ml of the above solution with 1 ml of 0.2% anthrone prepared in concentrated sulphuric acid. The above mixture was kept for 20 minutes in a water bath set at 90 °C. After cooling, the absorbance of glycogen was read at 620nm using UV ECIL Senior spectrophotometer.

i. Chitin

Chitin present in the cuticle was determined gravimetrically by the modified method of Hornung and Stevenson (1971).

Scheme of Analysis: A known weight (100mg) of dried and

powdered cuticle was taken and extracted in 4 ml of 2N HCl at room temperature for 12 hours, filtered and then extracted with 4 ml of water at 100 °C for 4 hours. It was cooled, filtered, and finally extracted in 4 ml of 2N NaOH at 100 °C for 4 hours, then cooled again and filtered. The final residue remained was the chitin which was collected and again dried, till constant weight was obtained.

j. Statistical Analysis of Data

Initially the mean and standard deviation of the data were processed and analysis of variance (ANOVA) was performed to test the significance between treatments, i.e. effect of different physiological stages of moult on selected biochemical parameters studied. Later polynomial regression curves were fitted in order to derive the trend of metabolite during the moulting stages of the prawn. All the statistical tests were carried out according to Snadocor and Cochran (1968), and data were processed on PC/XT computer with suitable programs.

3. RESULTS

3.1. Protein

The nature of the protein variations observed in the haemolymph, muscle, and hepatopancreas during the different stages of the moult cycle of the prawn are given

in the Table 1.

Protein content of the haemolymph increased gradually from stage, A to stage D1', with a low value of 27.8 mg/ml in stage A, and high concentration of 99.72 mg/ml in stage D1'. A decreasing trend of blood protein was noticed from D1''' of the late premoult stage, with a sharp fall between the late premoult stage D2-3 and the first postmoult stage-A (Fig.1). ANOVA revealed that the differences in protein content in haemolymph among the different moult stages are statistically significant at 5% level.

Muscle protein values varied between a minimum of 38.9% in stage A (early postmoult) to the maximum of 63.3% in stage D1'(early promoult). Protein content exhibited a cyclic change in muscle, gradually increasing from stage A to touch the maximum in stage D1'. Thereafter muscle protein levels fell down in the late premoult D2-3, and reached the minimum in the early postmoult stage A (Fig.2). ANOVA showed that differences in the protein levels in muscle during different stages of moult cycle are statistically significant at 5% level.

Hepatopancreas showed lower values for protein,

Table - 1: VARIATION IN THE CONCENTRATION OF PROTEIN DURING THE DIFFERENT MOULTING STAGES OF PRAWN, *P. INDICUS*.

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1''	D2-3
Haemolymph (mg/ml)	N	7	7	7	7	7	7	7
	$\bar{X}$	27.8	37.05	56.86	84.18	99.72	76.14	61.90
	±SD	(2.09)	(3.51)	(6.05)	(7.89)	(5.02)	(4.99)	(3.28)
Muscle (mg/100 mg dry weight)	N	7	7	7	7	7	7	7
	$\bar{X}$	38.90	47.89	51.45	53.88	63.30	54.21	40.95
	±SD	(2.66)	(5.10)	(5.68)	(6.22)	(4.41)	(5.34)	(5.74)
Hepatopancreas (mg/100 mg dry weight)	N	7	7	7	7	7	7	7
	$\bar{X}$	8.39	12.10	14.05	15.37	18.35	16.33	14.24
	±SD	(0.66)	(1.68)	(1.01)	(1.00)	(1.18)	(0.70)	(1.08)

ANALYSIS OF VARIANCE : PROTEIN

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F.Value
Haemolymph	Treatment	6	27539.080	4589.846	191.181*
	Error	42	1008.328	24.008	
Muscle	Treatment	6	2933.195	488.866	18.595*
	Error	42	1104.180	26.290	
Hepatopancreas	Treatment	6	464.429	77.405	70.317*
	Error	42	46.233	1.101	

* Significant at 5% level ($P < 0.05$)

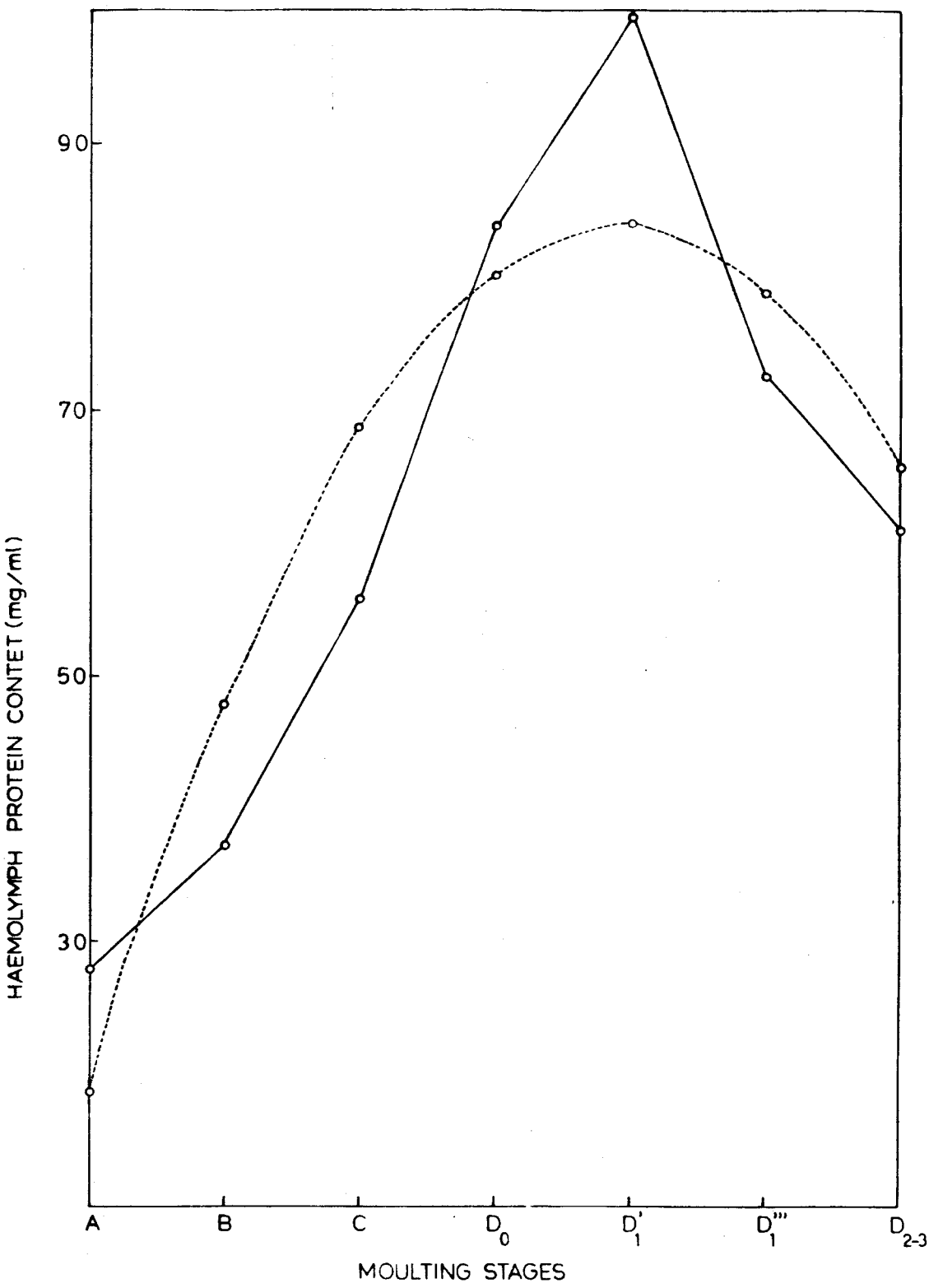


Fig. 1. Mean haemolymph protein variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = -19.6627 + 42.0704X - 4.2617\ X^2$

o——o observed value o....ostatistically analysed value

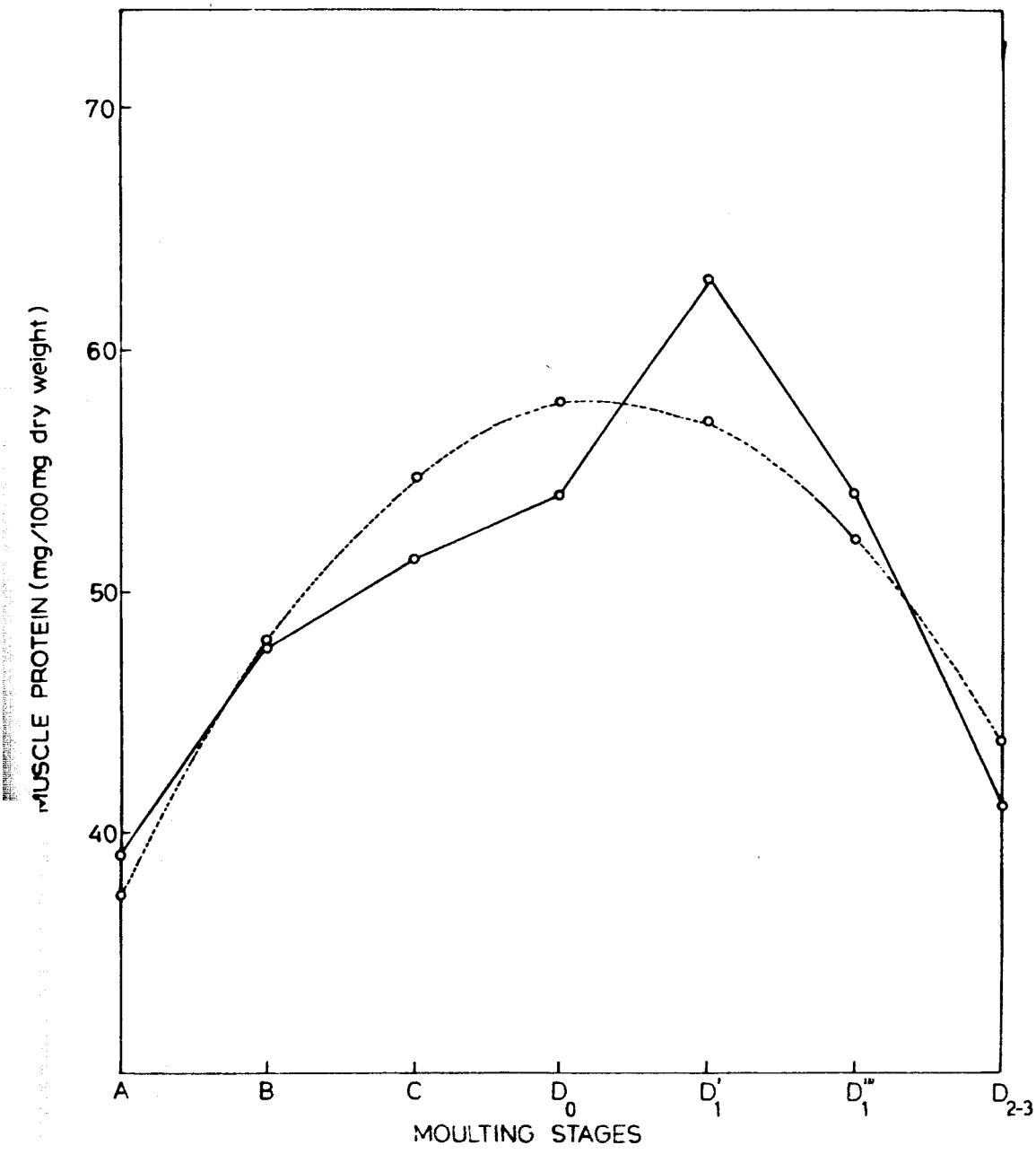


Fig. 2. Mean muscle protein variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 22.9072 + 16.3167X - 1.9040X^2$

o——o observed value o....o statistically analysed value.

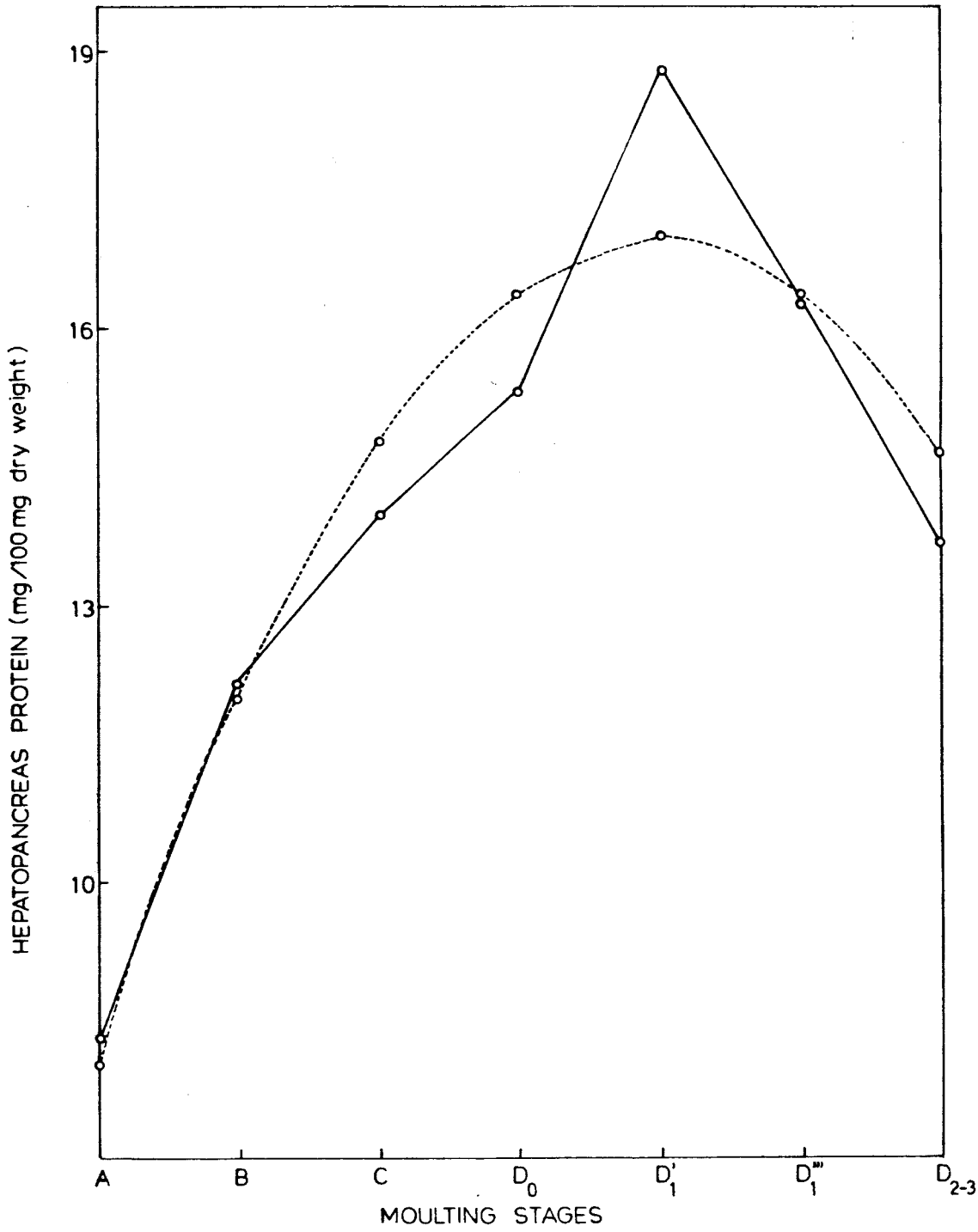


Fig. 3. Mean hepatopancreatic protein variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 3.0729 + 5.5794X - 0.5599X^2$

o——o observed value o....o statistically analysed value.

which ranged from a minimum of 8.39 mg/100 mg in stage A to a maximum of 18.85 mg/100 mg in stage D1'. From D1'protein content showed a declining trend up to the early postmoult (stage A) (Fig.3). ANOVA revealed that changes observed in protein content in hepatopancreas during different stages of moult cycle are statistically significant at 5% level.

3.2.RNA

Trend in variations of RNA values during the different stages of moult in the muscle and hepatopancreatic tissue are given in Table 2A and 2B.

RNA in the muscle was lowest in stage A (3.31/ug/mg) on wet weight basis and the maximum in stage D1' (16.98 /ug/mg). A gradual increase of RNA was noticed from early postmoult stage A to early premoult stage D1'. Similar to protein, RNA also showed a decrease after D1' to reach the low value in stage A (Fig.4A). Values of RNA/protein ratio in muscle also showed a linear increase from stage A (8.48) to early premoult stage D1'(26.84) (Fig.4B) . ANOVA showed that the fluctuations of muscle RNA values between the stages of moult are statistically significant at 5% level.

Hepatopancreas RNA content was higher than the RNA

Table - 2A: VARIATION IN THE CONCENTRATION OF RNA CONTENT DURING THE DIFFERENT MOULTING STAGES OF PRAWN, P. INDICUS

Tissue		MOULT STAGE						
		A	B	C	Do	$D1'$	$D1''$	D2-3
Muscle (µg/mg) wet weight	N	7	7	7	7	7	7	7
	$\bar{X}$	3.31	5.35	7.70	12.33	16.98	13.8	11.05
	±SD	(0.48)	(0.45)	(1.02)	(1.16)	(1.91)	(1.03)	(2.36)
Hepatopancreas (µg/mg)	N	7	7	7	7	7	7	7
	$\bar{X}$	11.43	25.37	31.77	45.33	55.8	41.88	34.33
	±SD	(3.02)	(3.13)	(2.41)	(3.34)	(4.56)	(2.08)	(3.45)

ANALYSIS OF VARIANCE: RNA

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Muscle	Treatment	6	988.342	164.724	103.185*
	Error	42	67.048	1.596	
Hepatopancreas	Treatment	6	8724.844	1454.141	162.698*
	Error	42	375.383	8.938	

* Significant at 5% level ($P < 0.05$)

Table - 2B. CHANGES IN RNA, PROTEIN RATIO IN MUSCLE DURING THE MOULTING CYCLE OF P. INDICUS

A	B	C	Do	$D1'$	$D1''$	D2-3
8.48	11.17	14.96	22.88	26.84	25.45	26.98

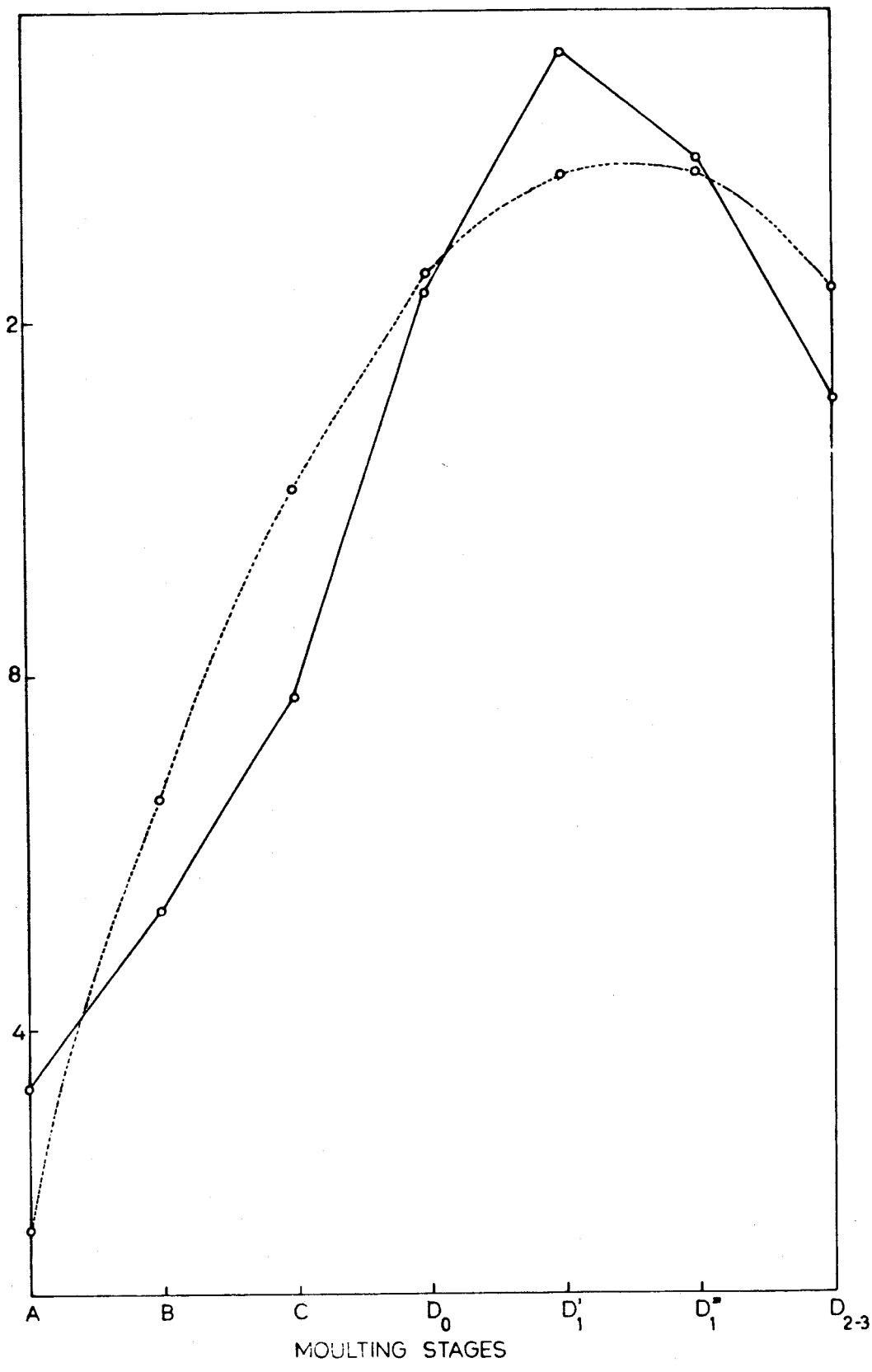


Fig. 4a. Mean muscle RNA variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = -4.3228 + 6.6575X - 0.6118X^2$

o——o observed value o....o statistically analysed value

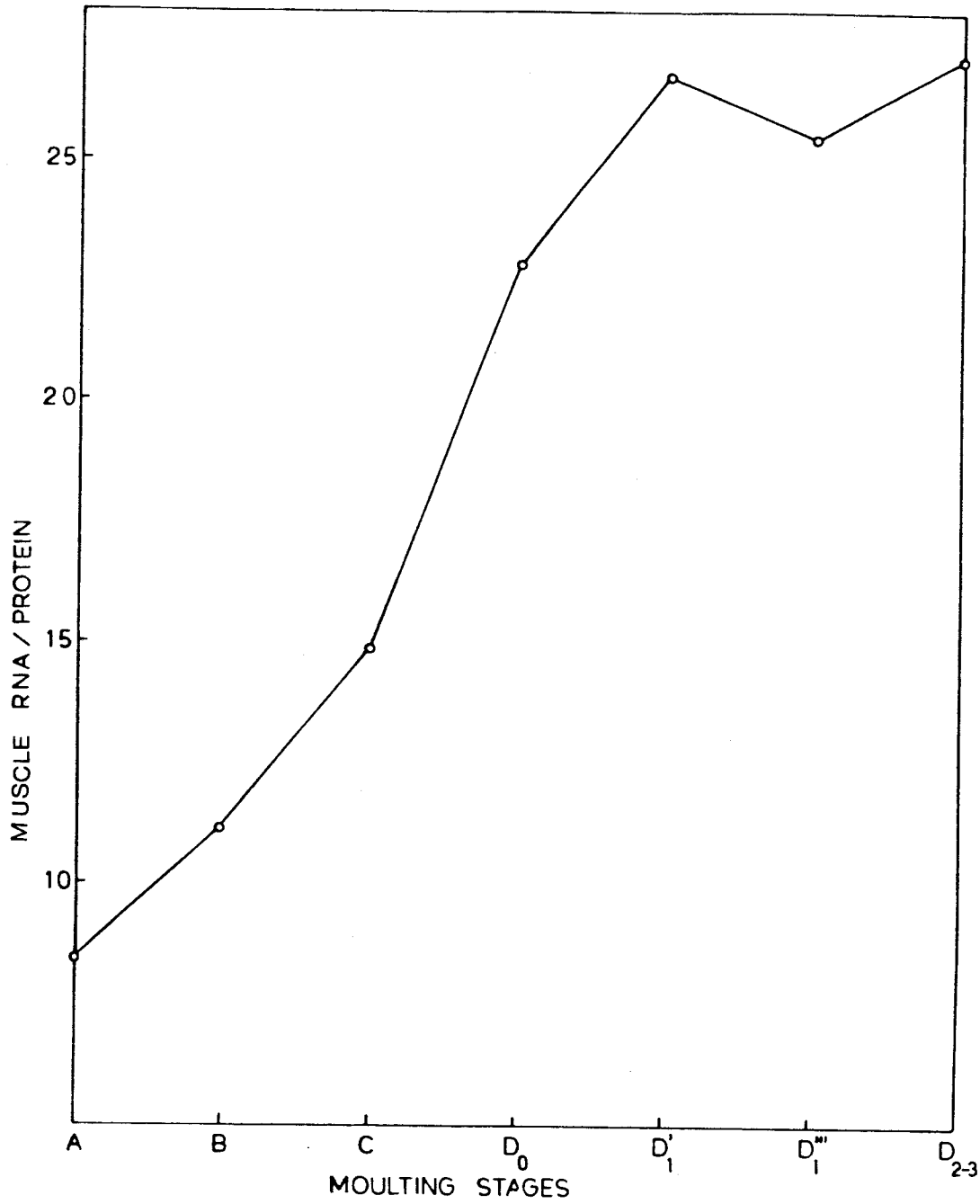


Fig. 4b. Variation of RNA/PROTEIN ratio in muscle during different moult stages of P. indicus

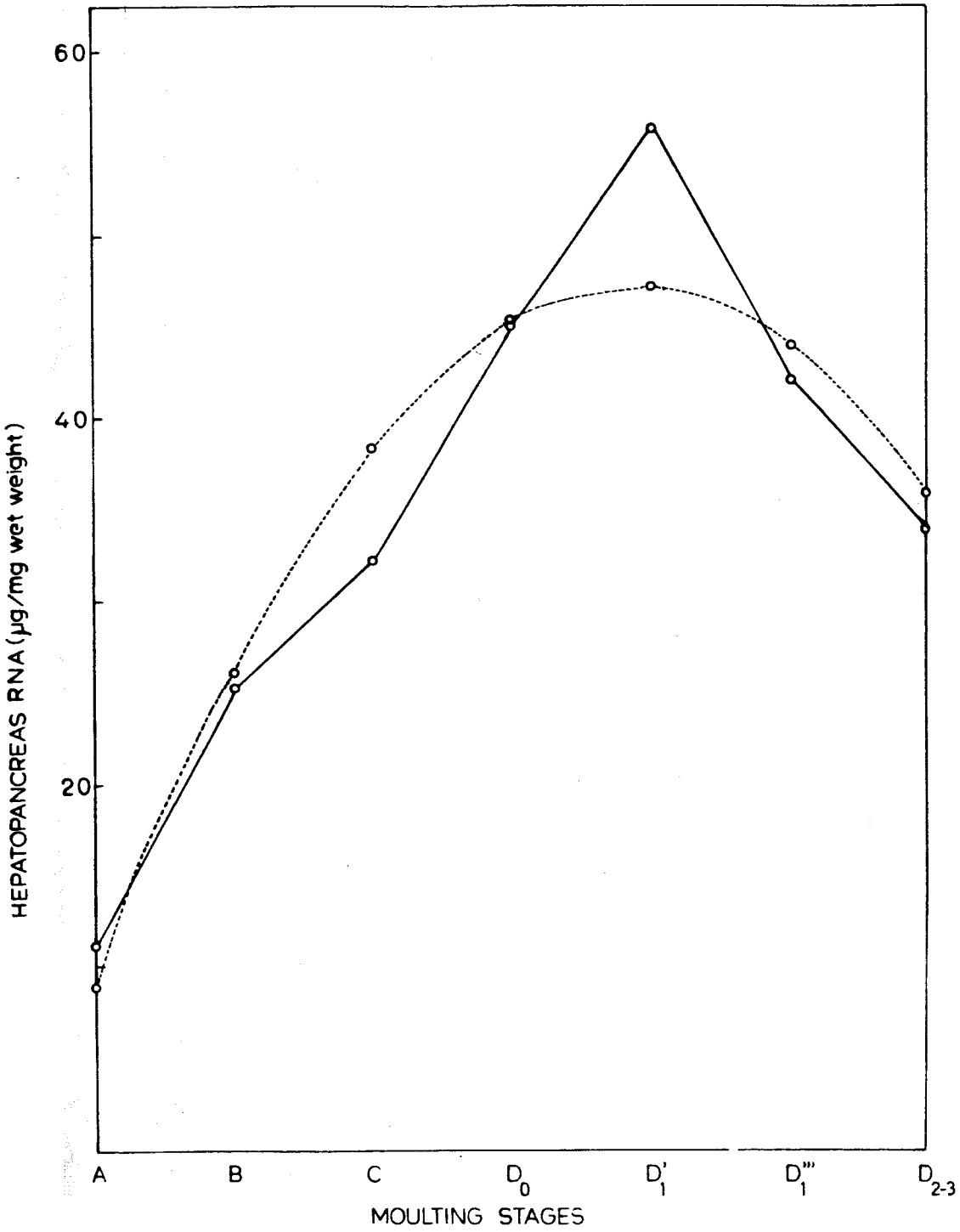


Fig. 5. Mean hepatopancreatic RNA variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = -13.83 + 25.1194X - 2.5777X^2$

o——o observed value o....o statistically analysed value

content of muscle. RNA concentration ranged from a minimum of 11.43 /ug/mg in stage A to a maximum of 55.8/ug/mg in stage D1'. During the late premoult stages of D1''' and D2-3 decreasing trend was noticed in the RNA up to the stage A (Figure 5). ANOVA showed the RNA variation in the hepatopancreas among the moult stages are statistically significant at 5% level.

3.3.DNA

The DNA content of muscle and hepatopancreas during different stages of the moult cycle are given in Table 3.

Highest DNA content of 5.35 /ug/mg in muscle was recorded in stage A while the lowest was noted in stage D1' (2.4 /ug/mg). From early postmoult stage A, DNA decreased gradually with low values in intermoult (stage C) and early premoult (stages Do and D1'). Thereafter it increased again in the late premoult(stages D1'''and D2-3) (Fig.6). ANOVA revealed that differences in DNA content of muscle are statistically significant during different stages of moult ($P<0.05$).

DNA concentration in hepatopancreas reached the maximum value of 4.85 /ug/mg in stage B, while the minimum value of 2.8 /ug/mg was recorded in stage D1'. Starting

Table - 3 : VARIATION IN THE CONCENTRATION OF DNA DURING THE DIFFERENT MOULTING STAGES OF PRAWN *P. INDICUS*

Tissue		MOULTING STAGES						
		A	B	C	Do	$D1'$	$D1''$	D2-3
Muscle (µg/mg) wet weight	N	7	7	7	7	7	7	7
	$\bar{X}$	5.35	4.38	2.6	2.43	2.4	3.13	4.45
	±SD	(0.49)	(0.39)	(0.8)	(0.57)	(0.46)	(0.71)	(0.48)
Hepatopancreas (µg/mg)	N	7	7	7	7	7	7	7
	$\bar{X}$	4.77	4.85	3.67	3.6	2.8	3.47	4.35
	±SD	(0.54)	(0.77)	(0.43)	(0.47)	(0.55)	(0.48)	(0.64)

ANALYSIS OF VARIANCE: DNA

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Muscle	Treatment	6	58.508	9.751	35.385*
	Error	42	11.574	0.276	
Hepatopancreas	Treatment	6	24.643	4.107	15.254*
	Error	42	11.309	0.269	

*Significant at 5% level ($P < 0.05$)

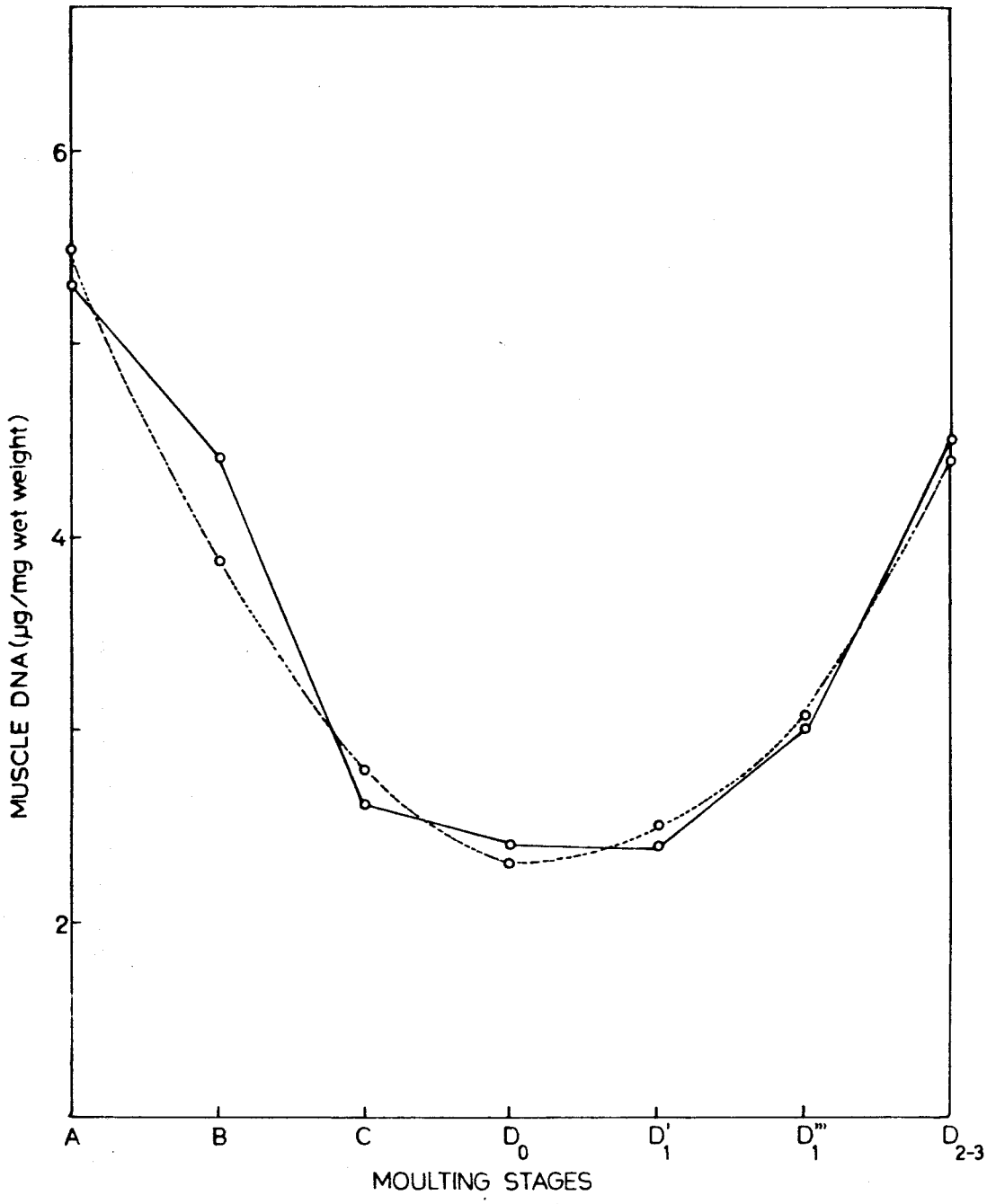


Fig. 6. Mean muscle DNA variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 7.7743 - 2.5052X + 0.289X^2$
o——o observed value o....o statistically analysed value

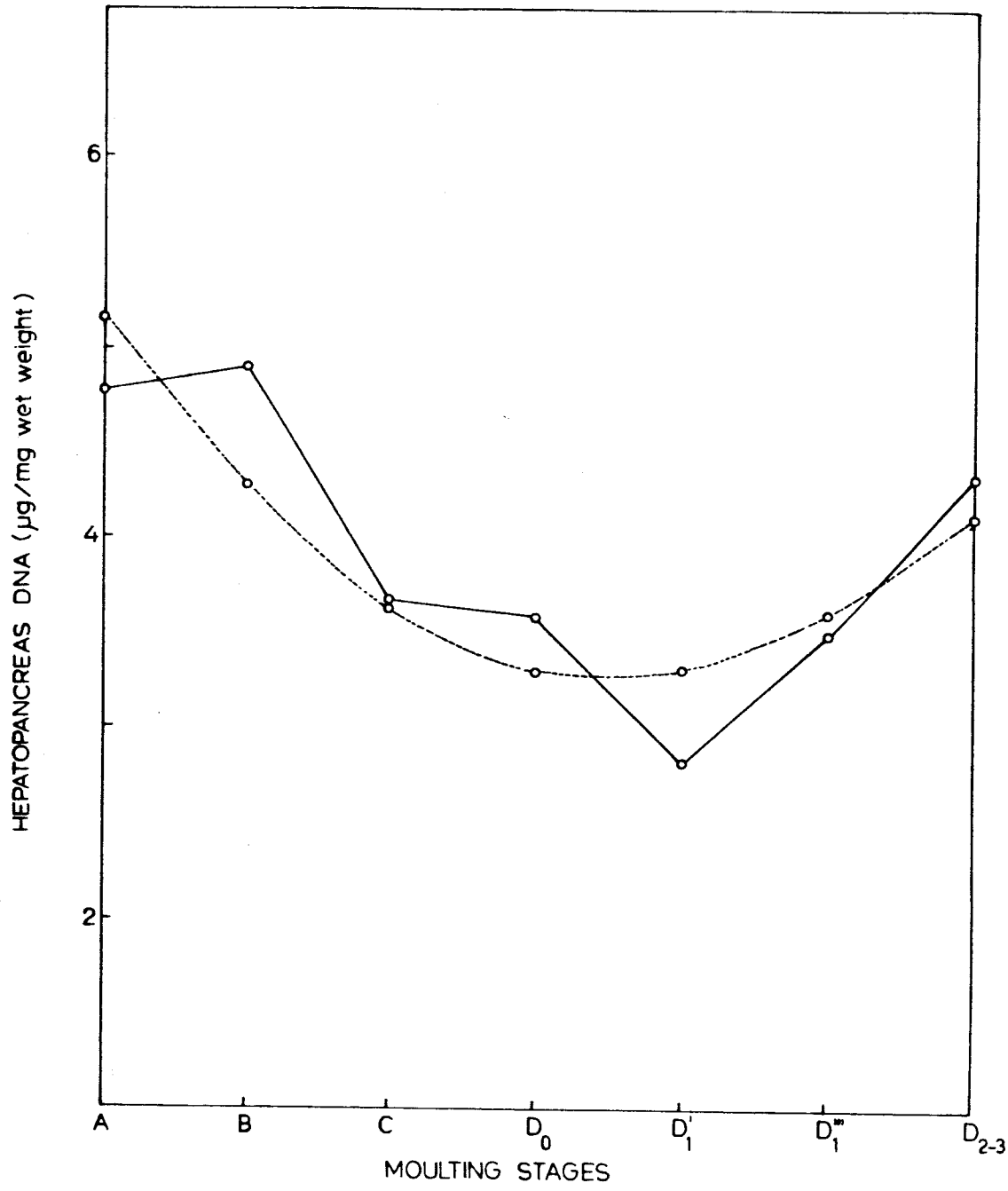


Fig. 7. Mean hepatopancreatic DNA variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 6.34 - 1.3321X + 0.145X^2$
o——o observed value o....o statistically analysed.

from the late premoult D1''', DNA showed a gradual increase upto the late postmoult stage B (Fig.7). ANOVA showed that differences in the DNA content between the moult stages are significant at 5% level.

3.4.Lipid

The trend of lipid in haemolymph, muscle, and hepatopancreas during different stages of moult cycle are given in Table 4.

In the haemolymph, the maximum lipid content of 10.34 mg/ml was recorded among the animals of stage D2-3 while stage A gave the lowest value of 7.43 mg/ml. From the early postmoult stage of A to the late premoult stage D2-3, haemolymph lipid showed a linear increase (Fig.8). ANOVA indicated that the differences in lipid content at different moult stages are statistically significant at 5% level.

In the muscle, lipid content was the maximum in stage D1' (7.49 mg/100mg) and minimum in stage A (2.72 mg/100mg). Lipid showed a linear increase from stage A to stage D1' followed by a fall in late premoult stages to reach the minimum in early postmoult (Fig. 9). Variation in lipid content during the different stages of moulting cycle in muscle tissue are statistically significant

Table - 4 : VARIATION IN THE CONCENTRATION OF LIPID CONTENT DURING THE DIFFERENT MOULTING STAGES OF PRAWN *P. INDICUS*

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1''	D2-3
Haemolymph (mg/ml)	N	7	7	7	7	7	7	7
	$\bar{X}$	7.43	8.51	8.82	9.35	9.70	9.78	10.34
	±SD	(0.34)	(0.37)	(0.17)	(0.31)	(0.37)	(0.22)	(0.51)
Muscle (mg/100 mg) dry weight	N	7	7	7	7	7	7	7
	$\bar{X}$	2.72	3.61	3.99	6.20	7.49	5.37	3.92
	±SD	(0.39)	(0.49)	(0.28)	(0.42)	(0.35)	(0.31)	(0.23)
Hepatopancreas (mg/100 mg) dry weight	N	7	7	7	7	7	7	7
	$\bar{X}$	13.02	18.60	30.43	41.72	50.14	53.25	45.72
	±SD	(2.07)	(1.37)	(3.71)	(1.61)	(1.7)	(1.92)	(1.27)

ANALYSIS OF VARIANCE: LIPID

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Haemolymph	Treatment	6	38.833	6.472	59.797*
	Error	42	4.546	0.108	
Muscle	Treatment	6	116.042	19.340	146.011*
	Error	42	5.563	0.132	
Hepatopancreas	Treatment	6	10336.610	1722.768	402.748*
	Error	42	179.656	4.278	

*Significant at 5% level ($P < 0.05$)

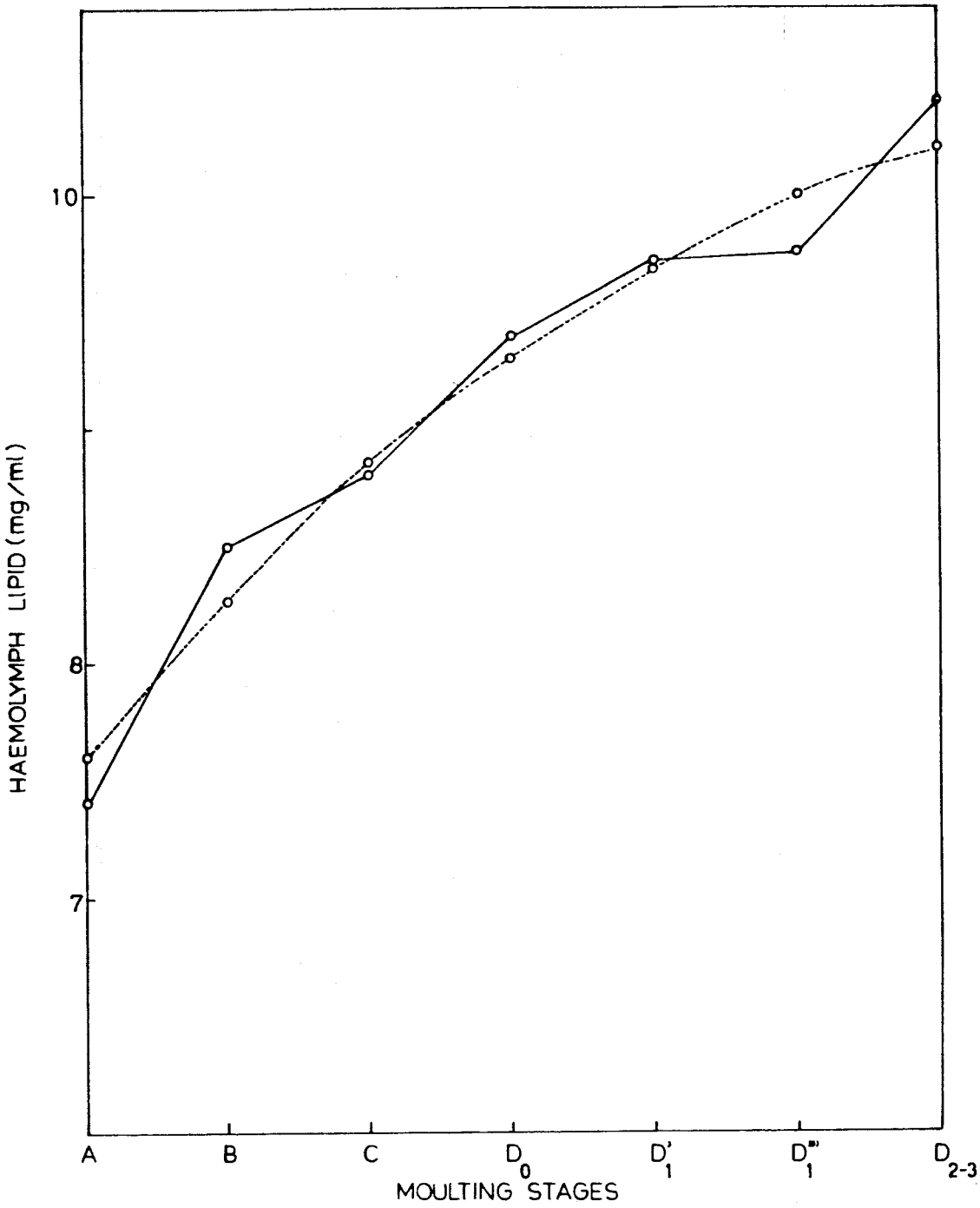


Fig. 8. Mean haemolymph lipid variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 6.8071 + 0.8281X - 0.049X^2$

o——o observed value o....o statistically analysed value.

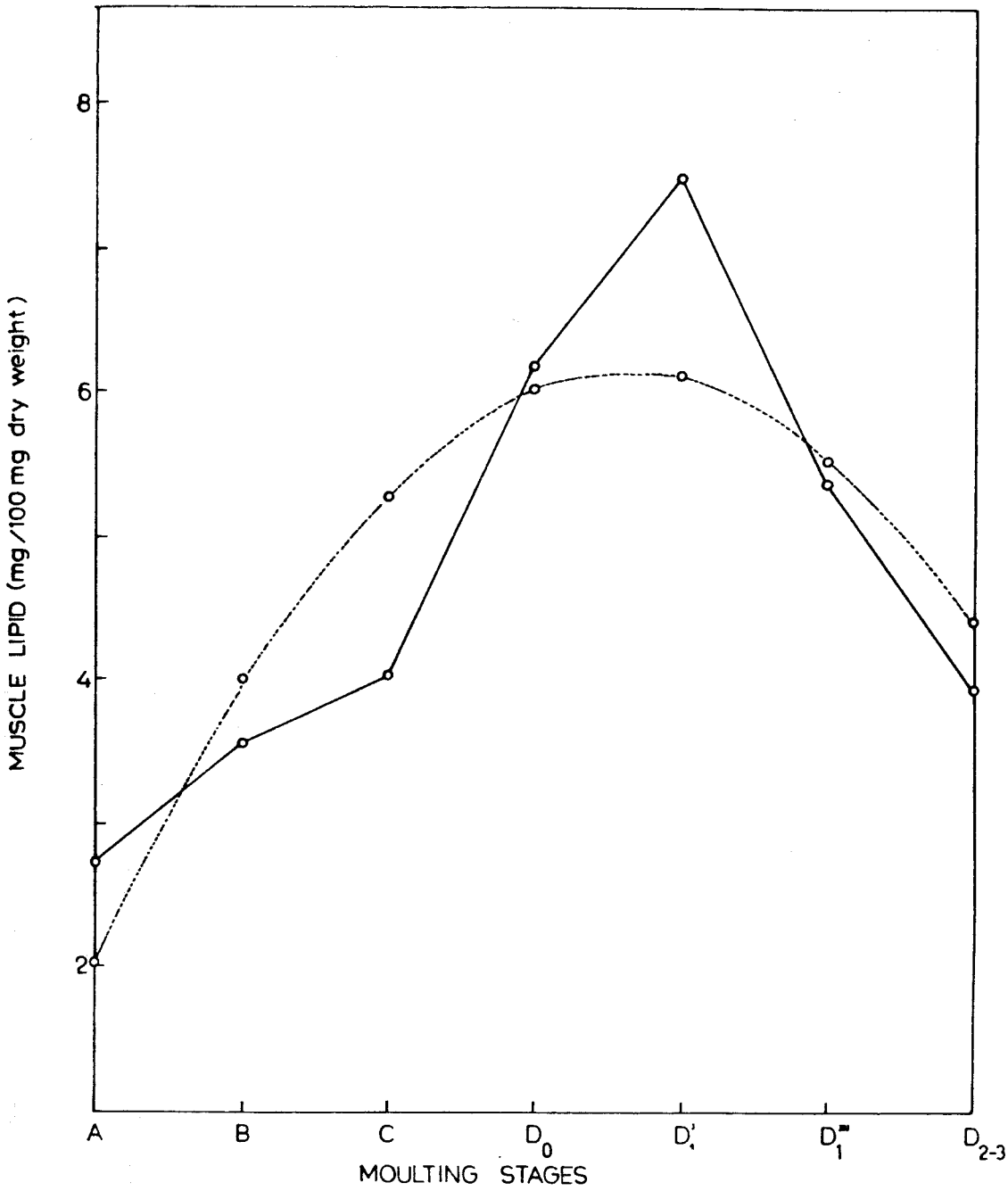


Fig. 9. Mean muscle lipid variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 0.4757 + 2.8587X - 0.3099X^2$

o——o observed value o....o statistically analysed value

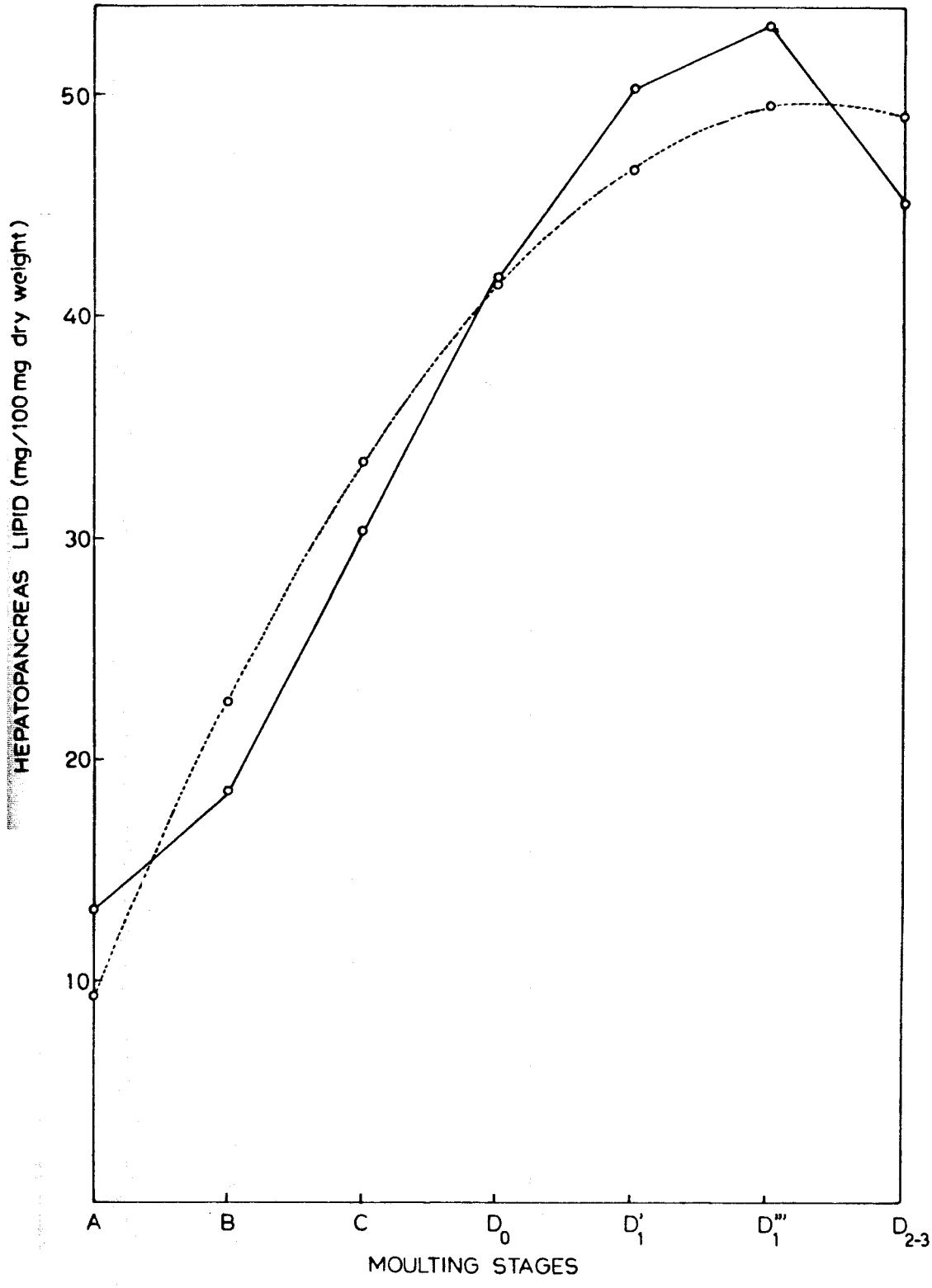


Fig. 10. Mean hepatopancreatic lipid variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 0.-6.9098 + 17.5684X - 1.3615X^2$

o——o observed value o....o statistically analysed value.

($P<0.05$).

Lipid content of the hepatopancreas during the moulting cycle ranged from a minimum of 13.02 mg/100mg in early postmoult A to the maximum of 53.25 mg/100mg in early premoult D1'. Hepatopancreatic lipid showed a gradual increase from stage A to reach the maximum value in stage D1''', subsequently a sharp fall was noted between the stages D2-3 and stage A (Fig. 10). ANOVA indicated that the variation of lipid levels during the moult cycle are statistically significant at 5% level.

3.5. Glycogen

The trend of glycogen variation in haemolymph, muscle, and hepatopancreas during differet stages of moult cycle are given in Table 5.

The high glycogen content in the haemolymph was observed in the late premoult stage of D2-3 (650.59 /ug/ml), the lowest value of glycogen of 278 /ug/ml was recorded in the late postmoult stage B. From the intermoult stage C onwards glycogen gradually increased upto the last premoult stage of D2-3, coupled with a sharp fall in concentration after ecdysis (Fig 11). ANOVA showed that fluctuation in glycogen content of haemolymph in

Table - 5: VARIATION IN THE CONCENTRATION OF GLYCOGEN DURING THE DIFFERENT MOULTING STAGES OF PRAWN *P. INDICUS*

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1''	D2-3
Haemolymph (ug/ml)	N	7	7	7	7	7	7	7
	$\bar{X}$	370.83	278	319.83	344.5	396.57	525.83	650.59
	±SD	(27.74)	(9.53)	(7.55)	(8.34)	(35.34)	(25.75)	(29.49)
Muscle (mg/g). dry weight	N	7	7	7	7	7	7	7
	$\bar{X}$	7.24	6.28	8.47	8.88	10.87	15.14	17.37
	±SD	(41)	(0.56)	(0.68)	(0.52)	(0.97)	(1.54)	(1.38)
Hepatopancreas (mg/g) dry weight	N	7	7	7	7	7	7	7
	$\bar{X}$	27.43	13.00	15.53	19.61	21.52	30.59	35.41
	±SD	(3.94)	(1.27)	(1.40)	(1.68)	(1.69)	(0.90)	(1.91)

ANALYSIS OF VARIANCE: GLYCOGEN

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Haemolymph	Treatment	6	720074.500	120012.400	250.697*
	Error	42	20106.000	478.714	
Muscle	Treatment	6	727.545	121.257	130.729*
	Error	42	38.957	0.928	
Hepatopancreas	Treatment	6	2799.828	466.638	114.223*
	Error	42	171.584	4.085	

*Significant at 5% level ($P < 0.05$)

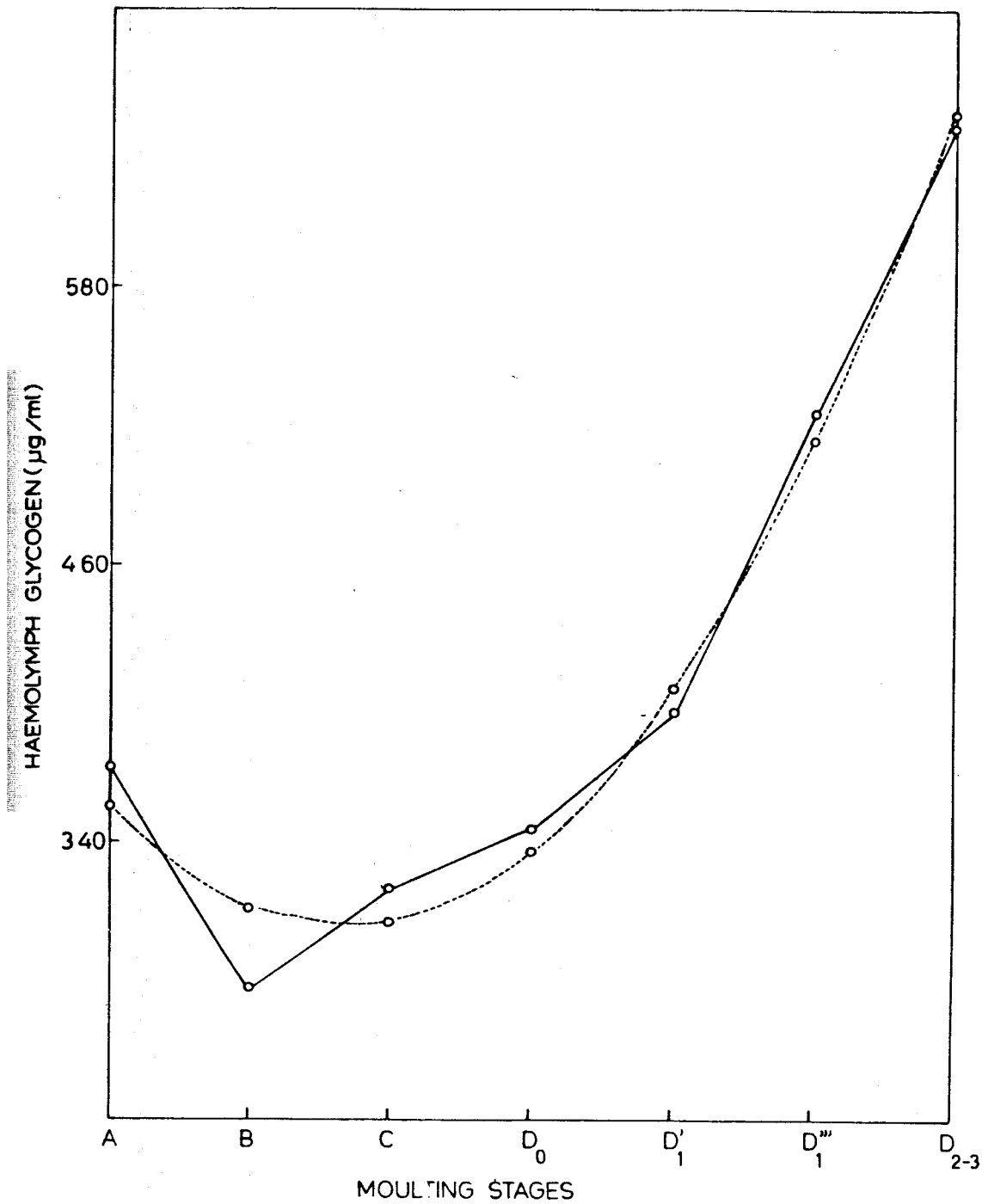


Fig. 11. Mean haemolymph glycogen variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 436.3010 - 100.0363X + 18.8072X^2$
o——o observed value o....o statistically analysed value.

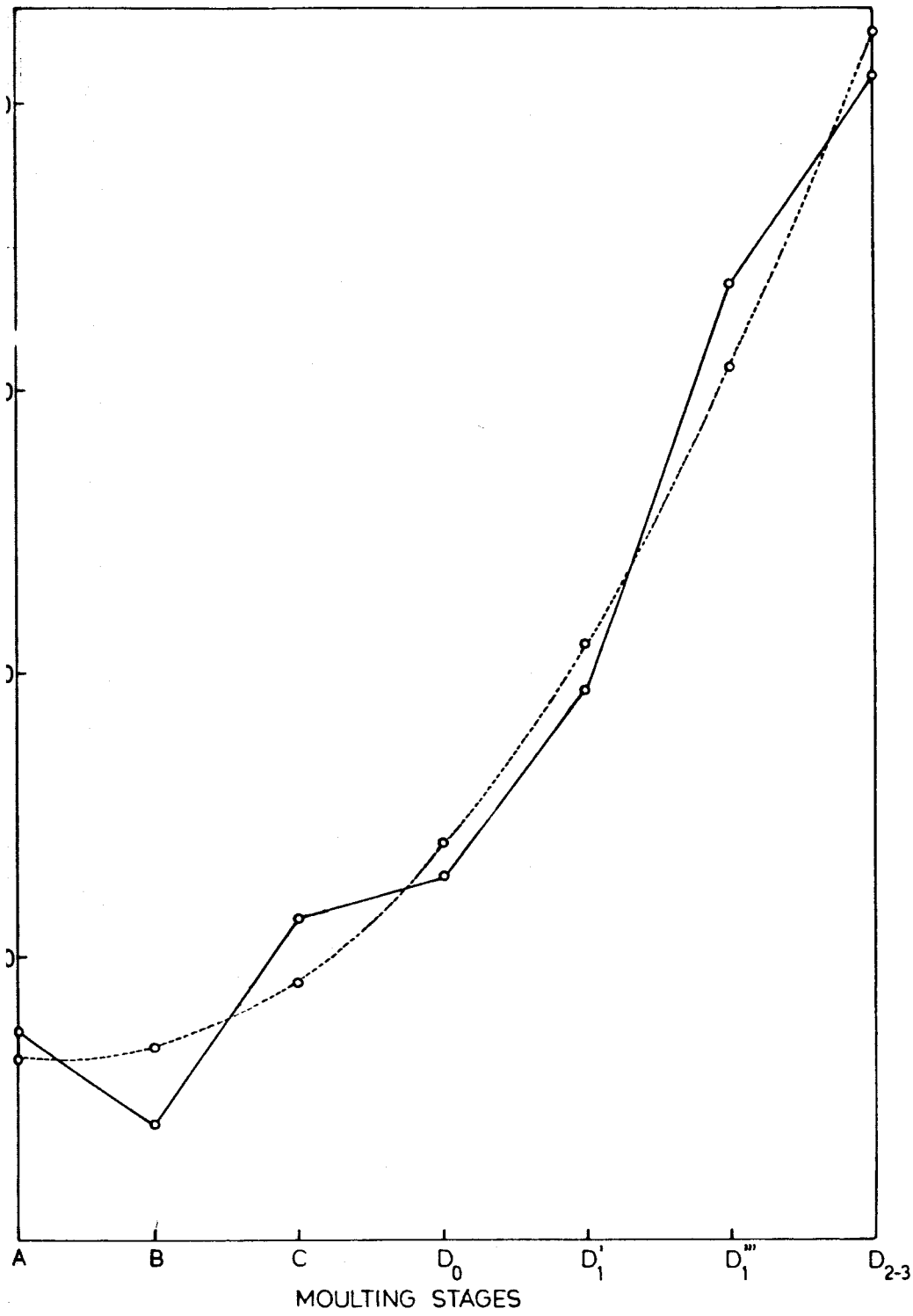


Fig. 12. Mean muscle glycogen variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 7.6214 - 1.0097X + 0.351X^2$

o——o observed value o....o statistically analysed value.

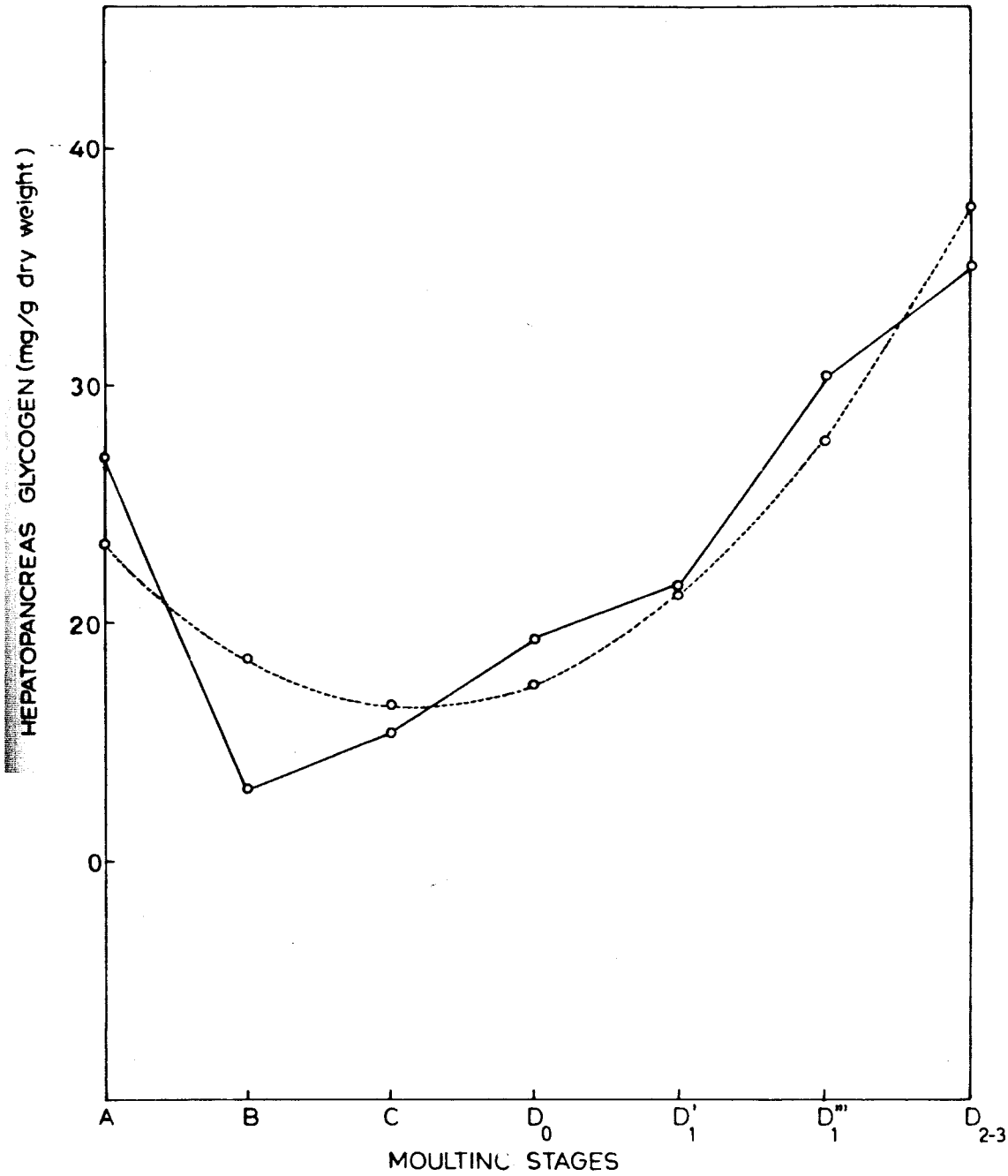


Fig. 13. Mean hepatopacreatic glycogen variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 31.7943 - 9.5384X + 1.483X^2$

o——o observed value o....o statistically analysed value.

relation to moult stages are statistically significant ($P<0.05$).

Maximum muscle glycogen was recorded in the late premoult stage D2-3 (17.37 mg/g) and the lowest from the postmoult animals of stage B(6.28 mg/g). From late premoult stage B muscle glycogen showed a gradual increse to late premoult stage D2-3. A sharp fall was noted from late premoult stage to late postmoult stage (Fig.12.). ANOVA showed that the glycogen fluctuations in muscle during different stages of moult cycle are significant at 5% level.

Glycogen in hepatopancreas varied from a minimum of 13 mg/g in stage B to the maximum (35.41 mg/g) in late premoult stage of D2-3. Similar to muscle, in hepatopancreas also glycogen gradually increased from stage B to late premoult stage D2-3, followed by a sharp fall to reach the minimum value in late postmoult stage B (Fig. 13.). ANOVA revealed that glycogen variations during different stages of moult cycles in the hepatopancreas are statistically significant at 5% level.

3.6.Glucose

Glucose content of the haemolymph during different stages of moult cycles are presented in Table 6.

Table - 6: VARIATION IN THE CONCENTRATION OF HAEMOLYMPH GLUCOSE DURING THE DIFFERENT MOULTING STAGES OF PRAWN P. INDICUS.

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1'''	D2-3
Haemolymph (µg/ml)	N	7	7	7	7	7	7	7
	$\bar{X}$	269.43	245.67	332.25	518.75	638.83	658.83	671.67
	±SD	(10.15)	(9.20)	(13.08)	(10.96)	(13.51)	(25.57)	(35.71)

ANALYSIS OF VARIANCE : GLUCOSE

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Haemolymph	Treatment	6	1514745.000	252457.500	794.665*
	Error	42	13343.000	317.691	

*Significant at 5% level

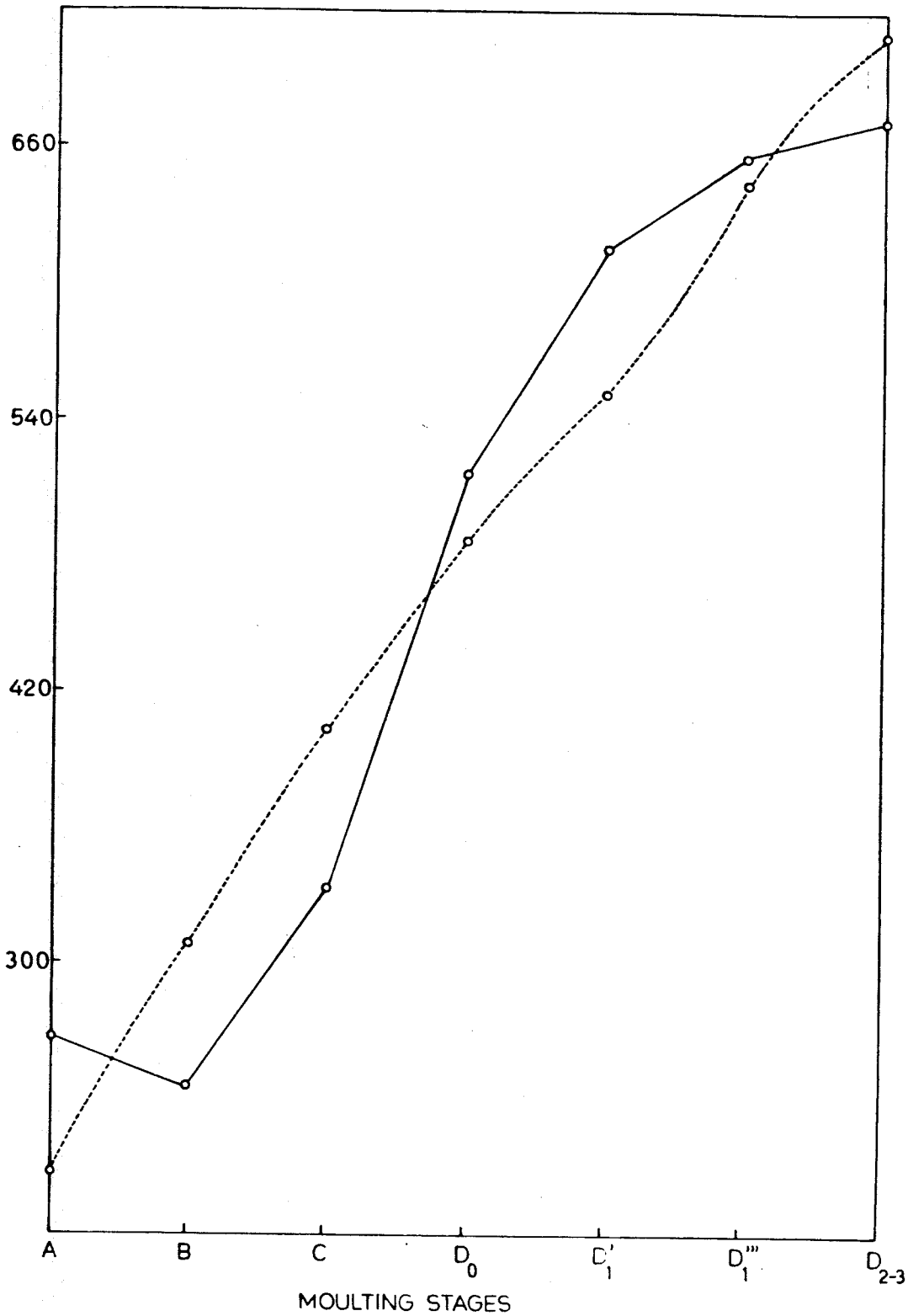


Fig. 14. Mean haemolymph glucose variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 101.8848 + 110.453X - 3.362X^2$
o——o observed value o....o statistically analysed velue.

Glucose varied widely between the different stages of moult. The lowest value was recorded in stage B, 245.67 /ug/ml and the highest was recorded in stage D2-3, 671.67 /ug/ml. From stage B onwards glucose concentration in the haemolymph increased linearly and reached the maximum in stage D2-3. A sharp fall in glucose concentration was noted soon after moulting (Fig.14). Differences in blood glucose levels during the different stages of the moult cycle are found to be statistically significant ($P<0.05$).

3.7.Glucosamine

Table 7 shows the trend of glucosamine fluctuation in haemolymph during the different stages of moult cycle.

The behaviour of glucosamine in haemolymph during the different stages of moult showed a different pattern from glucose and glycogen. Glucosamine concentration was the maximum in stage D2-3 (199.43 /ug/ml), while the minimum value was recorded in stage D1' (21.28 /ug/ml). From late premoult stage of D2-3, glucosamine content decreased gradually upto the early premoult stage of D1', and from D1''' it again showed an increasing trend (Fig. 15). ANOVA showed that the differences in glucosamine content of haemolymph between the different stages of

Table - 7 : VARIATION IN THE CONCENTRATION OF HAEMOLYMPH GLUCOSAMINE DURING THE DIFFERENT MOULTING STAGES OF PRAWN *P. INDICUS*

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1''	D2-3
Haemolymph (µg/ml)	N	7	7	7	7	7	7	7
	$\bar{X}$	153.67	118.57	71.14	25.18	21.28	56.17	199.43
	±SD	(18.06)	(5.44)	(18.21)	(7.10)	(3.82)	(20.10)	(16.78)

ANALYSIS OF VARIANCE : GLUCOSAMINE

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Haemolymph	Treatment	6	189608.800	31601.460	168.109*
	Error	42	7895.250	187.982	

*Significant at 5% level

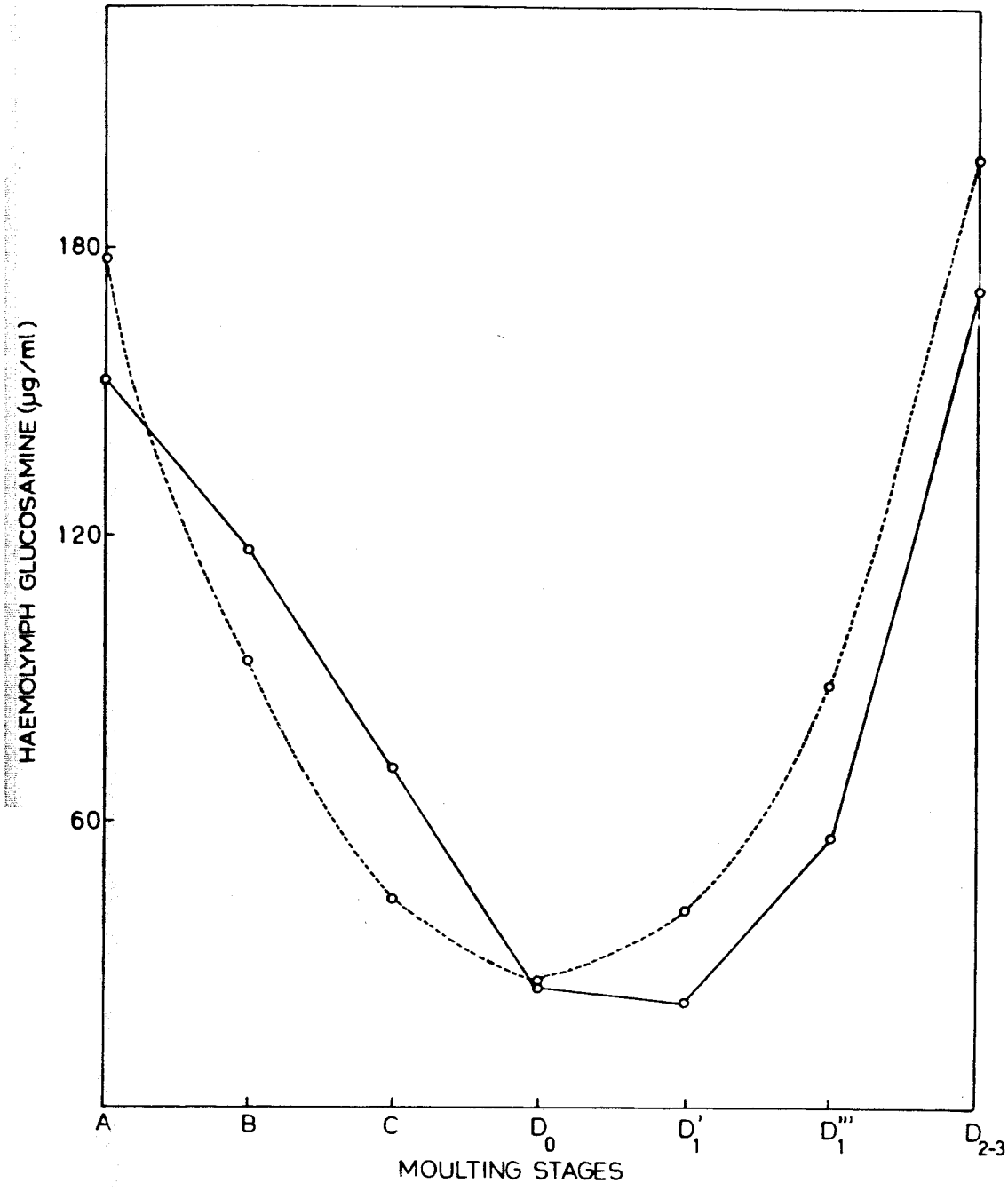


Fig. 15. Mean haemolymph glucosamine variation of P. indicus during different stages of moult cycle.

Regression equation $Y = 295.5285 - 133.2535X + 16.4893X^2$
o——o observed value o....o statistically analysed value.

moult cycle are statistically significant ($P<0.05$)

3.8. Chitin

The chitin content of the exoskeleton in the different moult stages is presented in Table 8.

In the cuticle, chitin content ranged from 13.48% to 27.83%. The maximum of 27.83% was recorded in stage A, and the lowest in stage D2-3. Estimation of chitin in exuvia gave 9.58% of chitin. From the early postmoult A, chitin showed a gradual decrease upto the late premoult D2-3 (Figure 16). Differences in chitin content among the moult stages are observed to be statistically significant ($P<0.05$).

3.9. Water content

Trend of variations of water in the muscle and hepatopancreas are given in the Table 9.

Maximum water in muscle was obtained from the animals of stage A, of the early postmoult with 78.52% of water. The lowest water content of 73.8% was recorded from prawns of early premoult stages of D1'. After ecdysis, from stage A onwards muscle water showed a decreasing trend upto D1', thereafter in the late premoult stages water content increased again facilitating the exuviation

Table - 8 : VARIATION IN THE CONCENTRATION OF CHITIN CONTENT DURING THE DIFFERENT MOULTING STAGES OF PRAWN *P. INDICUS*

Tissue		MOULT STAGES							
		A	B	C	Do	D1'	D1'''	D2-3	E
Exoskeleton (%)	N	7	7	7	7	7	7	7	7
	$\bar{X}$	27.83	22.02	18.92	18.44	18.73	16.43	13.48	9.58
	$\pm$SD	(1.66)	(1.47)	(1.13)	(1.38)	(1.39)	(1.112)	(1.190)	(1.44)

ANALYSIS OF VARIANCE : CHITIN

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Exoskeleton	Treatment	7	1454.9570	207.8510	97.8597 *
	Error	48	101.9506	2.1240	

*Significant at 5% level ($P < 0.05$)

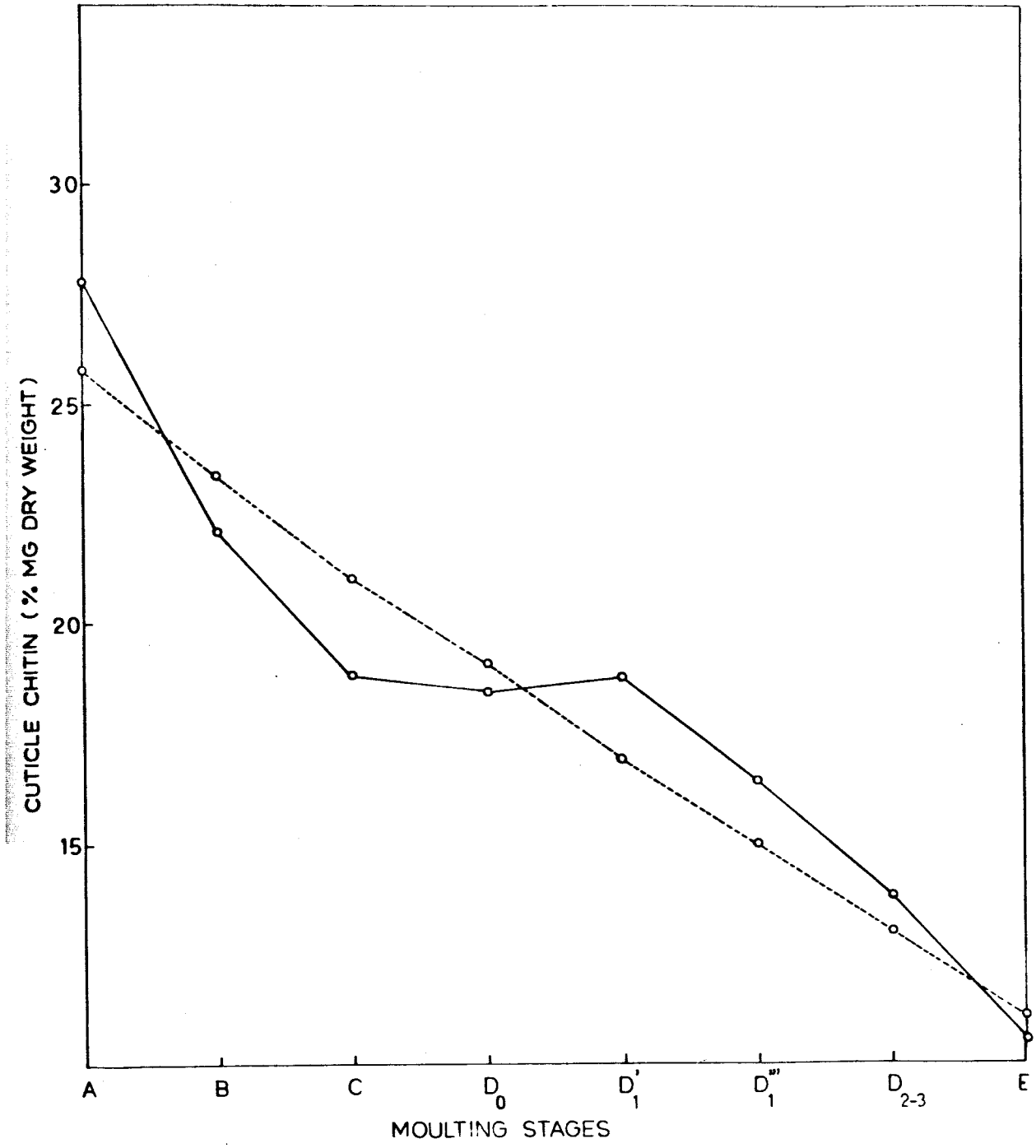


Fig 16. Mean cuticle chitin variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 28.1831 - 2.408X + 0.033X^2$

o——o observed value o....o statistically analysed value.

Table - 9: VARIATION IN THE CONCENTRATION OF WATER CONTENT DURING THE DIFFERENT MOULTING STAGES OF PRAWN, P. INDICUS

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1'''	D2-3
Muscle (%)	N	7	7	7	7	7	7	7
	$\bar{X}$	78.52	76.41	75.61	74.81	73.8	74.89	75.41
	±SD	(1.11)	(0.81)	(0.49)	(0.67)	(1.36)	(0.54)	(1.26)
Hepatopancreas (%)	N	7	7	7	7	7	7	7
	$\bar{X}$	76.86	72.07	69.31	65.03	61.93	64.22	61.35
	±SD	(2.92)	(3.03)	(3.17)	(5.93)	(3.77)	(3.4)	(3.33)

ANALYSIS OF VARIANCE : WATER CONTENT

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Muscle	Treatment	6	98.594	16.432	19.373*
	Error	42	35.625	0.848	
Hepatopancreas	Treatment	6	1371.359	228.560	170.088*
	Error	42	561.766	13.375	

*Significant at 5% level

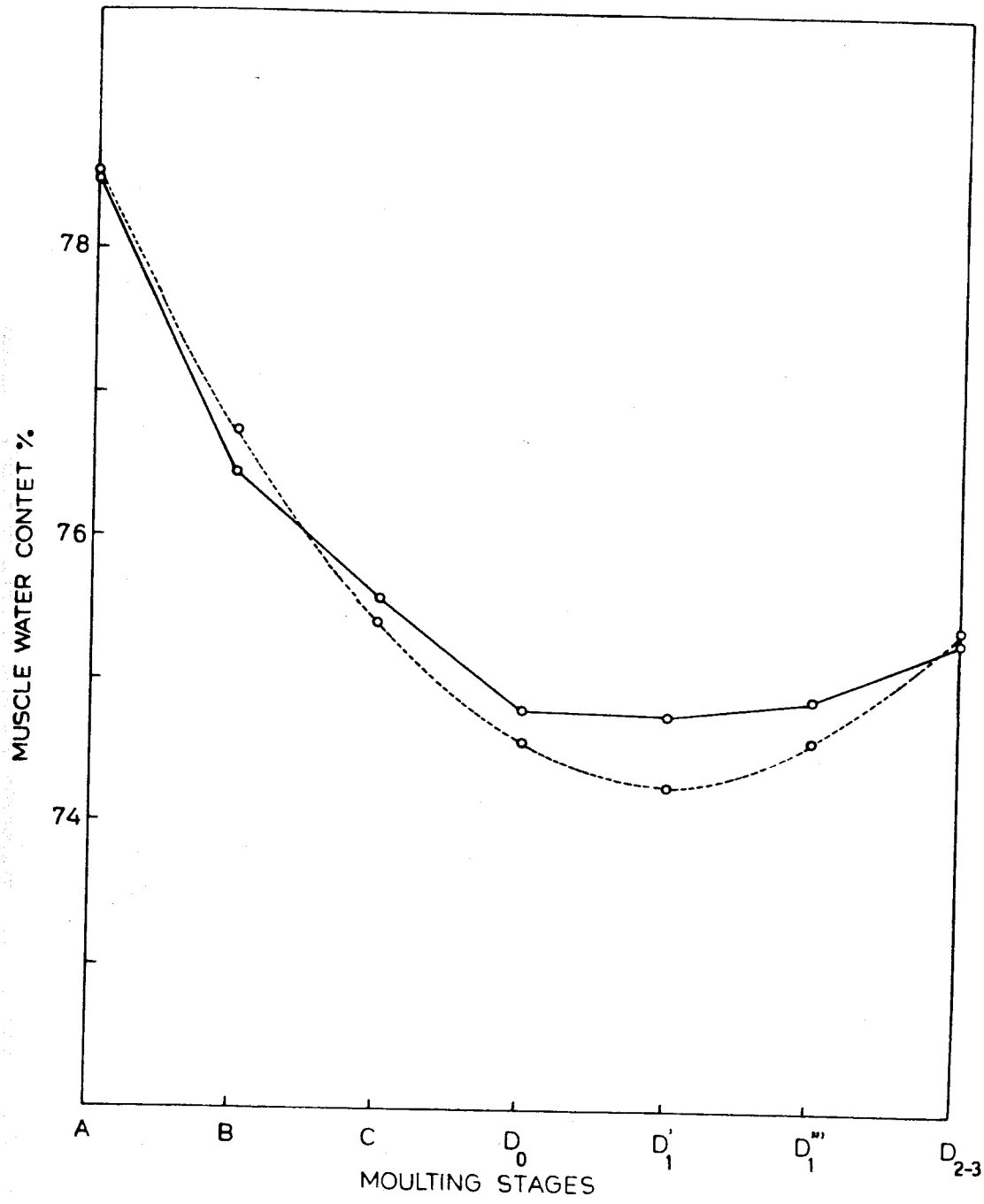


Fig. 17. Mean muscle water variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 80.9540 - 2.6467X + 0.2652X^2$
o——o observed value o....o statistically analysed value.

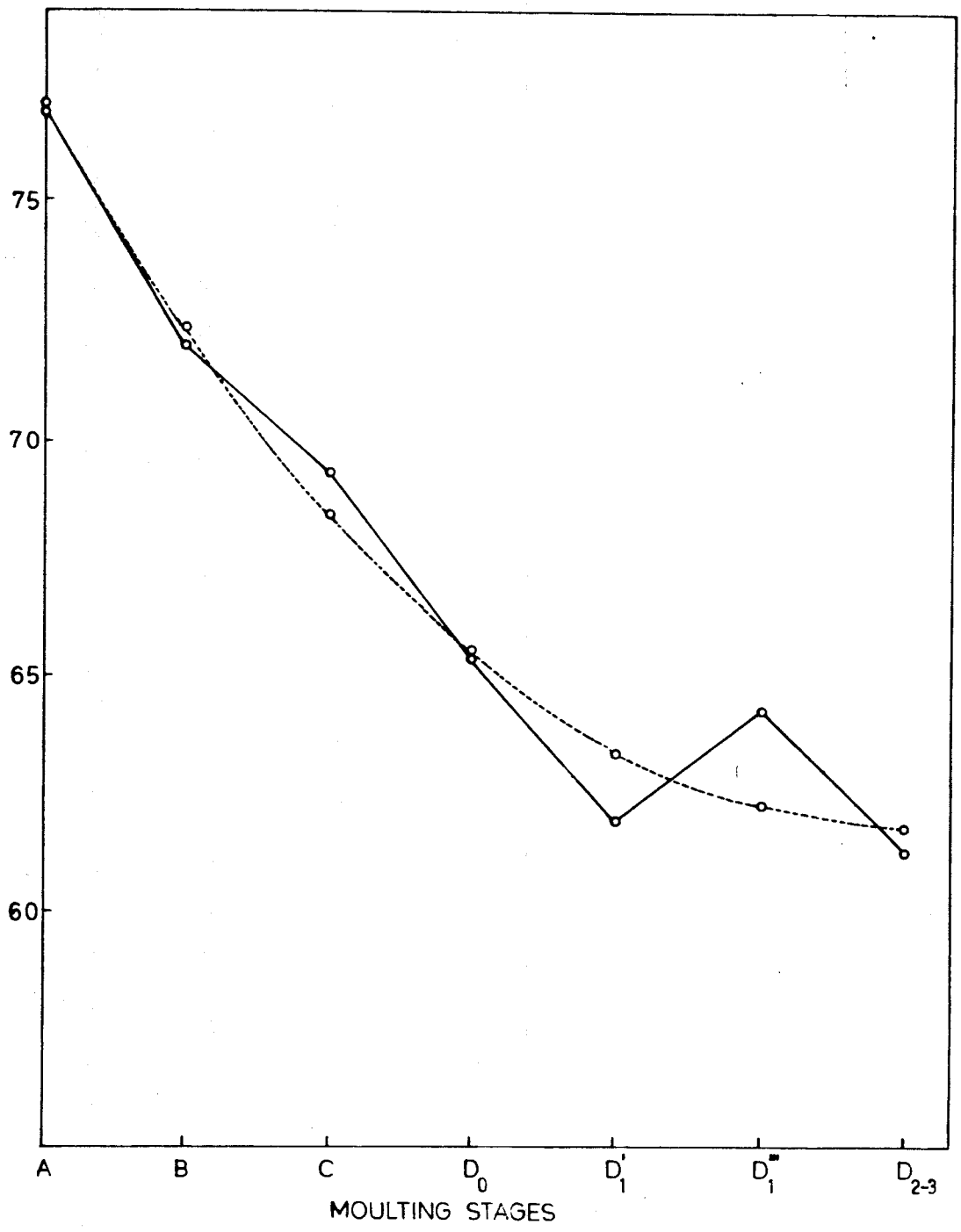


Fig.18. Mean hepatopancreatic water variation of P. indicus during different stages of moult cycle.

Regression equation, $Y = 82.4 - 5.9328X + 0.4271X^2$

o——o observed value o....o statistically analysed value.

of the animal (Fig. 17). ANOVA showed that the percentage variation of muscle water during the moulting stages are statistically significant ($P<0.05$).

The maximum water content, in the hepatopancreas, 76.86% was recorded in early postmoult stage A, while the lowest 61.35% was noted in late premoult stage D2-3. A sharp increase in the water content of the hepatopancreas was noted soon after moulting i.e. in stage A (Fig. 18). ANOVA indicated that the percentage variation of water in the hepatopancreas during the different stages of moult cycle are statistically significant ($P<0.05$).

4.DISCUSSION

Investigation on different biochemical parameters during the different moult stages of the animal showed cyclic accumulation of organic reserves in the selected major tissues of the prawn, *P.indicus*. Results of the present study on *P. indicus* indicated that the behaviour of the major organic constituents resembles, in general, with other crustaceans, exhibiting substantial changes between the premoult and postmoult stages (Passano, 1960; Yamaoka and Scheer, 1970; Aiken, 1980 and Stevenson, 1985).

Generally, crustaceans have been found to show an increase in protein contents of haemolymph, muscle and hepatopancreas during the beginning of the premoult period followed by a decrease towards the end of premoult and early postmoult periods (Renaud, 1949; Travis, 1955; Barlow and Ridgway, 1969; Spindler-Barth, 1976; Barclay et al., 1983; Smith and Dall, 1982 and Claybrook, 1985). While working on the haemolymph proteins of Panulirus argus, Travis (1955) reported an increase of blood protein content during premoult, and a decline following ecdysis. Similarly, Barlow and Ridgway (1969) in Homarus americanus, Dall (1974) in Panulirus longipes, and Hepper (1977) in Homarus gammarus reported an increase of haemolymph proteins in the early premoult stages and a subsequent fall after moulting. Comparable observations have also been reported for crabs Carcinus maenas (Robertson, 1960), and Ocypoda macrocera (Nageswara Rao et al., 1986). On line with the above observations, in the present study, protein content of haemolymph showed high value in the beginning of early premoult stage D1' and decreased levels in the late premoult stages of D1''' and D2-3. The present observation also agrees with reports of bursy and Lane (1971) in Penaeus duorarum and Smith and Dall (1982) in Penaeus esculentus, where lower protein

levels in the postmoult and higher protein levels in the premoult haemolymph of prawns were indicated. The rise in haemolymph proteins during the premoult stages has been attributed to the active resorption of the organic material present in the chitino-protein complex of the exoskeleton (Passano, 1960). Travis (1955) has suggested that the higher levels of protein in the premoult stages can be due to the active protein synthesis. According to Robertson (1960) and Passano (1960) the sharp fall of haemolymph protein after moult can be due to considerable dilution of the haemolymph as a result of increased water absorption and also due to the utilization of protein in the energy cycle and chitin synthesis.

The present study indicated that the muscle tissue of P.indicus acts as a principal storage site for proteins, where a significant protein build up was noticed during the intermoult and early premoult stages of the moult cycle. Similar observation was also made by Barclay et al. (1983) while working on the prawn P.esculentus. The build up of muscle protein in the intermoult and early premoult stages observed in the present study might be due to the tissue growth (protein synthesis) after moulting, and replacing the volume of water absorbed at the time of is. Observation of Aiken (1973) in Homarus americanus

also supports the present finding, where he accounted the protein build up during intermoult and early premoult of the animal as the real tissue growth. Stevenson and Hettick (1980) suggested that the fall of muscle protein in postmoult is due to its mobilization in the cuticular synthesis. Recently, Claybrook (1985) discussed the possibility of protein utilization from the muscle tissue for the energy requirement of the animal, which centered around the ecdysis during the late premoult and early postmoult.

When compared with the protein content of haemolymph and muscle, hepatopancreas of P.indicus showed only lesser amounts of protein. In the present study protein content of hepatopancreas showed similar changes as those in muscle and haemolymph during the moulting cycle. In agreement with the present findings, high protein turnover was reported in the hepatopancreas of Callinectes sp during the D1 stage. Skinner (1968) in Gecarcinus lateralis and Spindler-Barth (1976) in Carcinus maenas have also observed protein build up in hepatopancreas during the premoult and a subsequent fall in postmoult. The premoult accumulation of proteins in the hepatopancreas, in the current study, might be due to the

protein storage at the time of cuticular breakdown and its probable utilization subsequently during the postmoult for the cuticle synthesis resulted in lower levels of protein during the postmoult period.

Changes in RNA content during the moulting cycle in muscle and hepatopancreas of P. indicus, with lower values in postmoult and higher values in early premoult, indicated a trend similar to the protein of muscle and hepatopancreas. Compared to muscle tissue (3.31 to 16.98 /ug), hepatopancreas (11.43 to 55.8 /ug) was found to contain higher RNA content during moulting cycle of the prawn. The variation in RNA values observed in present study is found similar to the observations made by Skinner (1966, 1968), who reported a five fold increase of epidermal RNA during the premoult cycle of Gecarcinus lateralis. The premoult increase of RNA reflects the higher turnover rate of protein synthesis, which helps in the building up of tissue after ecdysis (Dall and Barclay, 1979). Works of Keller and Adelung (1970) in Orconectus limosus and Dall and Barclay (1979) in the western rock lobster Panulirus longipes during different stages of moult cycle showed lowest RNA content in postmcult and highest RNA levels in premoult stages. Dagg and Littlepage (1972) interpretted the RNA/Protein ratio as the index of

growth in Artemia salina and Euchaeta elongata. They have reported a maximum RNA/Protein ratio in early premoult stages, while in late premoult stages the ratio was erratic, indicating the breakdown of protein in the late premoult stages. Lower RNA/Protein ratio in postmoult stages and higher ratio during early premoult stages with an erratic behaviour in late premoult observed in the present study agrees well with observation of Dagg and Littlepage (1972).

DNA content in the muscle and hepatopancreas of P.indicus showed higher levels in late premoult stage D2-3 and postmoult stages (A and B). Humphreys and Stevenson (1973) while studying the DNA synthesis of cray fish Orconectes sanborni, reported the maximum DNA content between stages D3 and A in the epidermis and sub-epidermal connective tissue. Later Dall and Barclay (1979), in Panulirus longipes noticed a rise of epidermal DNA during stage D2 to stage D3 and thereafter a decline towards minimum values in intermoult. The higher DNA levels in late premoult and postmoult can be attributed to cell divison occurring in connection with the rebuilding of cuticle (Stevenson,1985).

In the present study, the lipid content of the

muscle, hepatopancreas, and haemolymph was found to increase gradually from stage A to reach maximum at stages of D1' and D1''' and then decrease during late premoult stage D2-3. These findings are in agreement with the observation of Teshima and Kanazawa (1976) who also reported an increase in the lipid content of the whole animal in Palaemon paucidens during stage D2 and then a decrease at late premoult D3-4. Work done by Teshima et al.(1975, 1977) in the whole body of Palaemon serratus and Penaeus japonicus also showed a premoult increase of lipid during different stages of moult. Further, Read and Caulton (1980) demonstrated in P.indicus that rapid synthesis of lipid in the whole animal continued till stage D2 followed by a decrease at stage D3. But O'Connor and Gilbert (1969) observed an increase in the haemolymph lipid at moult stage D1' and decrease at D4 stage in Orconectes virilis. The gradual increase of haemolymph lipid from postmoult to premoult might be due to the release of lipid reserves from the storage site such as hepatopancreas, which later in the postmoult probably utilized for chitin synthesis as stated by O'Connor and Gilbert (1969).

Ando et al.(1977) noticed the maximum lipid content in the hepatopancreas of Penaeus japonicus and reported

the most notable variation in the lipid content during the moult cycle of prawn. Renaud (1949), O'Connor and Gilbert (1968), and Heath and Barnes (1970) have also reported elevated lipid levels in the hepatopancreas at premoult and decline in late premoult and postmoult stages. A similar pattern has been reported in the penaeid prawn Metapenaeus monoceros and Penaeus esculentus with hepatopancreatic lipid accumulation during the early premoult stages, and subsequent fall during late premoult stages by Madhyastha and Ranganekar (1974), and Barclay et al. (1983), respectively. Chang and O'Connor (1985) attributed the premoult lipid increase in the midgut gland due to the fatty acid synthesis. The decline in lipid reserves during the late premoult and early postmoult as observed in the present investigation can obviously be due to the mobilization of the lipid reserves from the storage sites, for utilization in the synthesis of new exoskeleton, as suggested by Yamaoka and Scheer (1970).

Major carbohydrates such as glycogen, glucose, and glucosamine have been identified as the intermediate to chitin synthesis by Renaud (1949) and he discussed the possible role of carbohydrates as the source of energy supply at the time of cuticular synthesis. In the present observation, in P.indicus, it has been observed that the

cyclic changes of glycogen, glucosamine and chitin occur during different stages of moult cycle. Gradual increase of glycogen in the muscle from stage B(6.28 mg/g) to stage D2-3 (17.37mg/g) and its decrease soon after ecdysis at stage A (7.24mg/g) as observed in the present study is in agreement with the observation of Renaud (1949) in Cancer pagurus. Renaud (1949) reported that the glycogen content of muscle was high (0.55%) in hard crabs where as the glycogen was low (0.03%) in soft crabs. Patane (1954) in isopod Porcellio laevis, and Martin (1965) in amphipod Gammarus pulex reported high muscle glycogen levels during the premoult stages and a decline after moulting. Later, Parvathy (1971) reported comparable observation in the muscle tissue of Ligia exotica. Work of Skinner (1966) on Gecarcinus lateralis showed higher amounts of glycogen in the epidermal cells during the premoult stages of the animal. Similarly, in the crab Carcinus maenas, Spindler-Barth (1976) observed a premoult increase and postmoult fall of muscle glycogen during differnt stages of the moult cycle.

The glycogen content of hepatopancreas in the present study showed identical pattern to that of muscle glycogen. Travis (1955), while working on P.argus, found an

increased activity of hepatopancreatic glycogen at premoult, and a reduced activity in postmoult stages. Continuous increase of hepatopancreatic glycogen during premoult stages and thereafter a decline in postmoult stage of crab Cancer pagurus were recorded by Renaud (1949). Similarly Madhyastha and Ranganekar (1974) in Metapenaeus monoceros who noted a build up of glycogen in the hepatopancreas during the premoult period and gradual reduction of the same as the prawn enters into postmoult period. Observation of Heath and Barnes (1970) and Spindler-Barth (1976) also showed a gradual increase of hepatopancreatic glycogen through the intermoult and early premoult stages to attain the maximum levels in late premoult stage followed by a minimum value in postmoult stages of the crab Carcinus maenas.

Spindler-Barth (1976) reported that the blood of Carcinus maenas stores glycogen in premoult stages, and get mobilized during the postmoult for the cuticular synthesis. During the present investigation, haemolymph glycogen of P. indicus showed moult dependent changes with the maximum glycogen content in late premoult stage. Bauchau and Mengcot (1978) discussed the utilization of blood glycogen as a source of energy at the time of ecdysis and indicated the role of glycogen as source of

glucose, the precursor of chitin synthesis.

The premoult accumulation of glycogen in haemolymph, muscle and hepatopancreas as observed in the present study might be due to the increased glycogen synthesis and storage at the time of tissue growth, as described by Stevenson (1985). Scheer and Scheer (1951) were also of the view that the principal role of glycogen present in hepatopancreas and other tissue in crustacea is in the synthesis of chitin. As a precursor of chitin synthesis, the stored glycogen is utilized in the postmoult stages for the synthesis of new cuticle resulting in the postmoult fall of glycogen(Yamaoka and Scheer, 1970).

Glucose being the primary and major sugar of crustaceans, has got a prime role among the organic constituents which take part in the construction of exoskeleton (Florkin, 1960; Honke and Scheer, 1970 and Chang and O'Connor, 1985). Profound changes of haemolymph glucose noticed during different moult stages of P. indicus followed a similar pattern to that of blood glycogen. Various workers (McWhinne and Scheer, 1958; Riegel, 1960; Lynch and Webb, 1973; Dall, 1974; Telford, 1968, 1974 and Spindler-Barth, 1976) who have studied the variation of haemolymph glucose during the moult cycle of crustaceans,

reported increased levels of glucose in premoult stages followed by a significant decrease after ecdysis and recoming to normal levels in intermoult stage. Results of the present observation agree well with the above works, and the premoult build up and postmoult utilization of haemolymph glucose seem to be typical of decapod moult cycle. The significance of premoult accumulation of haemolymph glucose is that sugars are mobilized in preparation for chitin synthesis which occurs at the time of moulting and immediately thereafter (Florkin, 1960). The mobilization of glucose for the building up of new cuticle results in the sharp fall of glucose in the postmoult stages (Honke and Scheer, 1970).

One of the important processes taking place at the time of moulting, involving sugars, is the synthesis of new chitinous cuticle. During the present study it was observed that percentage of chitin content in the exoskeleton decreased steadily to a relatively low point during the hardening stages of intermoult and early premoult. This can probably be due to the increasing calcium salt deposition for the purpose of strengthening the exoskeleton as described by Drach and Tchernigovtzeff (1967) and Lockwood (1968). The fall of cuticular chitin

during the stages D1''' and D2-3 in the present case might be due to the breakdown of chitin to chitin precursors to be used in the neocuticular synthesis (Honke & Scheer, 1970 and Hornung and Stevenson, 1971). The resorption value of chitin during moulting process in P.indicus has been determined by comparing the chitin content of casted exoskeleton (exuvia) with that of intermoult prawns of similar size, and from the results obtained it was estimated that about 50% of cuticular chitin was reabsorbed. Parvathy (1970) and Spindler-Barth (1976) have discussed the reutilization of glucosamine as a breakdown product of chitin in the late premoult and early postmoult stages. Further studies by Hornung and Stevenson (1971) and Gwinn and Stevenson (1973a, 1973b) in Orconectes hanborni and Orconectes obscurus showed that the rate of chitin synthesis rised progressively during early premoult from a minimum level and reached its peak in the early postmoult soon after ecdysis after which it showed a gradual decline to attain the minimum level in the intermoult and early premoult stages.

Renaud (1949) is of opinion that glycogen is first hydrolysed to glucose, which is then aminated yielding glucosamine, and the glucosamine further undergoes polymerization to yield chitin. Renaud's opinion of chitin

formation has been supported by later workers like Dall (1965a), and Stevenson (1978). Higher concentration of chitin precursors viz. glycogen, and glucose during the stages of intermoult and premoult, and their subsequent fall in the postmoult stages indicated that nature of chitin variation in relation to different moult stages in P.indicus is in agreement with the observations made by Renaud (1949), Dall (1965a) and Stevenson (1978). The haemolymph glucosamine concentration of P.indicus was higher during the late premoult stage D2-3, and postmoult stages A and B. Glucosamine being the immediate precursor of chitin, higher glucosamine content of haemolymph indicated that in P.indicus deposition of chitin is taking place mainly in latepremoult and postmoult stages as observed in other crustaceans (Hornung and Stevenson, 1971). Since the total amount of glycogen utilized in the cuticular build up is not sufficient to account for chitin synthesized in cuticle, many workers have suggested the possible contribution of major organic reserves like lipid and protein in chitin formation (Renaud, 1949; Vonk, 1960; Honke and Scheer, 1970 and Stevenson and Hettick, 1980). The build up of protein and lipid in the premoult stages and the subsequent fall in the postmoult stages of P.indicus, as observed in the present study indicate the

utilization of these reserves also at the time of chitin deposition.

The body volume increase during ecdysis in crustaceans has been attributed to water uptake, and considerable variation in the water content of the tissues takes place during the moulting cycle due to intake of water and consequent hydration of tissues (Passano, 1960 and Yamaoka and Scheer, 1970). Travis (1954) observed that during moulting cycle of Panulirus argus the water content reached a level of 81.3% prior to moult and 71% at the time of intermoult stage. Diwan and Nagabhushanam (1974) reported highest quantity of water during the early postmoult stage and thereafter the water content declined till early premoult and again increased during the late premoult while working on Barytelphusa cunicularis. The cyclic changes of water content observed in the muscle and hepatopancreas of P. Indicus in relation to the moult cycle agree well with the observation of Diwan and Nagabhushanam (1974). Later, Dall and Smith (1977 , 1978a, 1978b) in Panulirus longipes, and Barclay et al. (1983) in P.esculentus recorded comparable observations showing high water content in postmoult and low water content in the early premoult stage of the animals. Increased water

absorption and tissue hydration at the time of ecdysis has been understood to help in the exuviation of the animal (Dall and Smith, 1978). In the present study, major metabolites like protein, lipid, and carbohydrate in muscle hepatopancreas, and haemolymph gave high values when the water content was minimum, i.e. in the intermoult and early premoult stages, where as low values were obtained when the water content was maximum, i.e. in late premoult stage D2-3, and early postmoult stages of A and B. This inverse relationship of water and organic reserves indirectly reflects the mechanism of tissue growth in P.indicus, i.e. the water absorbed between the moults is later replaced by the deposition of organic materials resulting in an increase in the dry weight of the body.

S U M M A R Y

Biochemical changes in P.indicus associated with different moult stages were carried out. Some of the metabolites were estimated in selected tissues such as haemolymph, muscle,hepatopancreas, and cuticle. General trend of metabolic variation observed was their minimum values in the postmoult stages and an increase to the maximum values in the premoult stages. Protein, RNA, Lipid, Glycogen, and Glucose in different tissues such as haemolymph, muscle, and hepatopancreas followed this

trend. But DNA values in muscle and hepatopancreas, Glucosamine in heamolymph, Chitin in cuticle and water content in muscle and hepatopancreas showed higher values in the postmoult and lower values in the premoult. The premoult increase of metabolites in body tissues can be due to the absorption from the food, synthesis, and active resorption of the organic material from the chitino-protein complex of the exoskeleton, while postmoult fall of metabolites can be attributed to the utilisation of these in the energy cycle, chitin synthesis during the process of ecdysis, and neocuticular synthesis.

CHAPTER-IV DISTRIBUTION AND MOBILIZATION OF SOME MINERALS IN RELATION TO MOULT CYCLE

CHAPTER IV

DISTRIBUTION AND MOBILIZATION OF SOME MINERALS IN RELATION TO MOULT CYCLE

1.INTRODUCTION

The crustacean exoskeleton is extensively mineralized with calcium carbonate, in the form of calcite, which is the principal inorganic component of the exoskeleton (Passano, 1960). Magnesium and phosphate salts are relatively minor components of the integument (Richards, 1951). The mineral load in the exoskeleton is in a constant state of flux, since most of the decapods have to mineralize the newly built exoskeleton following the moult and again demineralize the old skeleton in preparation for the next moult (Huner, et al., 1979).

The role of minerals in cuticle formation in crustaceans had been reviewed by Travis (1960, 1963), and Passano (1960). Much attention has been devoted to the cuticular mineralization of the heavily mineralized decapods such as, the crayfish, lobster, and brachyurans (Travis, 1955a, 1960a and b, 1963 and 1965; Chaisemartin, 1962; Welinder, 1974, 1975a, 1975b; Greenaway, 1976, 1983, 1985; Huner et al., 1976; Mills and Lake, 1976; Mills et al.,1976; Vigh and Dendinger, 1982 and Sheets and

Dendinger, 1983). However, very little attention has been paid on natantian prawns which are less mineralized. In addition, trace minerals such as magnesium and phosphorus have been largely ignored in all taxas of crusatacea.

Among natantian, Dall (1965a) studied the calcium metabolism in Metapenaeus sp. Thereafter Bursey and Lane (1971), and Welinder (1974) studied the calcium changes during the moulting cycle of the prawn Penaeus duorarum. Cuticular mineralization of calcium, magnesium and phosphorus in relation to different moult stages of the prawn Penaeus californiensis has been done by Huner et al.(1979). Seasonal changes in the calcium content of muscle, haemolymph, and exoskeleton of P.indicus have been studied by Rao et al. (1983).

The extent to which hardening of the cuticle takes place with calcification showed great variation not only between different crustaceans, but also between different regions of the same animal (Dennel, 1960). Investgation dealing with the distribution of important minerals in the exoskeleton of crustacea have apparently received little attention Drach and Laffon (1942) provided the basis for the 'topographic variation' of calcium in the exoskeleton of the crabs Carcinus maenas, Cancer pagurus, and Maia

quinado. Several observations of calcium distribution on limited number of exoskeletal areas have been reported or the decapod crustacea Homarus americanus (Hayes and rmstrong, 1961), Clibanarius oluvaceous (Chockalingam, 971), and Lirceus brachyurus (Hawkes and Schraer, 1973). ater on a comprehensive analysis of calcium of all the ajor areas of the exoskeleton of the crayfish Astacus luviatilis and A.pallipes has been made by Chaisemartin 1962, 1967). Similarly Mills and Lake (1976) and Mills et l. (1976) studied the calcium distribution in the ifferent regions of the exoskeleton of crayfish .fluviatilis, Parastacoides tasmanicus and crayfish of he genera Engaeus and Geocharax, respectively. There are o reports available on the exoskeletal distribution of agnesium and phosphorus.

The properties of exoskeleton are conditioned by ther tissues of the body like haemolymph, hepatopancreas, nd muscle (Erribabu, et al.,1985). Hence, the periodic eplacement of the exoskeleton necessitates many changes n these body tissues (Dennel, 1960). Haemolymph calcium ariation in Sesarma dehani during the moulting cycle was tudied by Numanoi (1939). Workers like Robertson (1960), naway (1976), and Sheets and Dendinger (1983) worked

on the haemolymph calcium variations during the moulting cycle of crab C.maenas, C.pagurus and Callinectes sapidus, respectively. Work done by Travis (1955b) in lobster and Greenaway (1972, 1974b, 1974c), and Wright (1979, 1980) in crayfish also revealed the nature of calcium variation in relation to the moult cycle of these species. Haemolymph calcium variation in relation to moult cycle in prawns P. duorarum and M.rosenbergii have been reported by Bursey and Lane (1971) and Fieber and Lutz (1982). Mineral changes of hepatopancreas in relation to moult cycle were studied in crab M.squinado (Drach, 1939), and C.sapidus (Sheets and Dendinger, 1983). Sather (1967), and Brannon and Rao (1979) also studied the hepatpancreatic mineral variation during the moult cycle of Podoththalmus vigil and Palaemonetes pugio.

In the present investigation, studies were carried out to understand quantitative changes of important minerals like calcium, magnesium, and phosphorus in different body tissues Viz. exoskeleton, muscle, hepatopancreas, and haemolymph during the moult cycle of P.indicus. The mapping of calcium, magnesium, and phosphorus in different regions of the exoskeleton were also carried out to know the distribution pattern of the selected minerals in the exoskeleton of the prawn.

2. MATERIALS AND METHODS

Healthy adult prawns yet to attain reproductive maturity in the size range of 80-120 mm were selected for the present study. Methods of collection, maintenance, and moult staging were the same as described in the chapter-I.

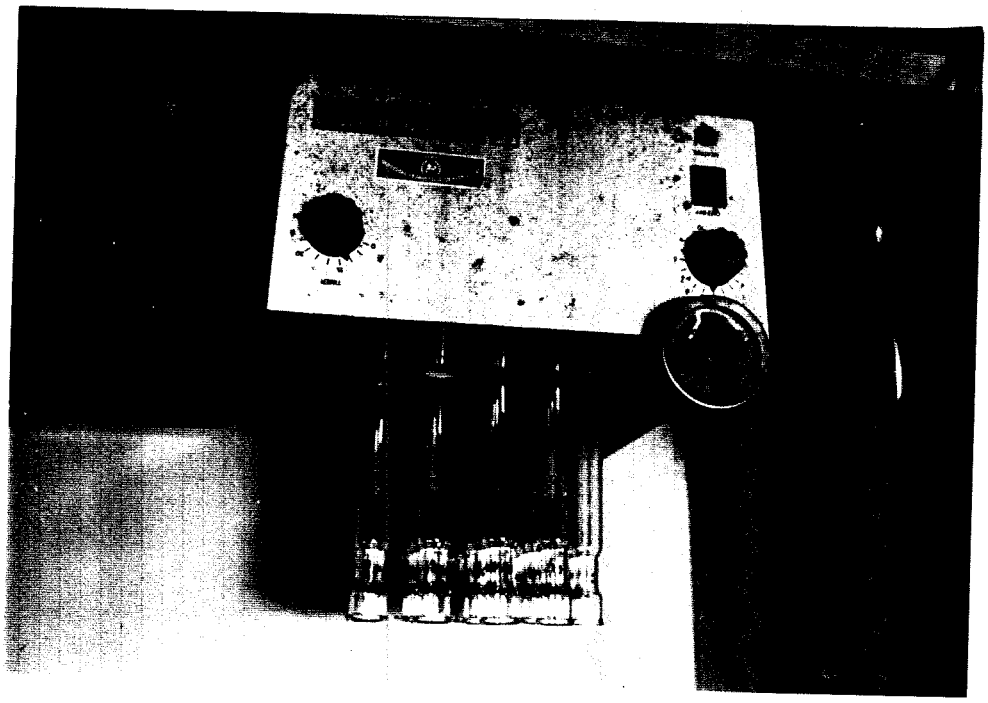
A: Analysis of calcium, magnesium and phosphorus in different tissues

P.indicus of different moult stages viz. A(early post moult), B(Late postmoult), C(intermoult), Do(early premoult), D1'(early premoult), D1'''(late premoult) and D2-3(late premoult) were selected for the present investigation. Methods adopted for haemolymph collection and tissue sampling were the same as given in chapter-III.

Preparation of tissues for mineral analysis'

Known weight(50-100 mg) of dried and finely powdered tissues or known volume of (0.2-0.5 ml) haemolymph were taken in 250 ml digestion tubes. One ml of double distilled water and 4ml of Analar HNO_3 were added to each tube and wet digestion was performed using a digestion block (Fig.1), fitted with an automatic temperature control unit. Initially the mixture and the digestion tube was heated to 150 °C to dissolve the tissues. Digestion

Fig.1: Digestion Block.



was continued until a little HNO_3 remained in the tube. Then, the tubes were taken out and allowed to cool to the room temperature. One ml of $HClO_4$ was added to the cooled tubes and kept again in the digestion block. Dig estion was continued at 300 °C, so that very little acid remained in the tubes. The tissues were removed from the digestion block, cooled and made upto the required volume using dilute HNO_3 (0.5N). For the samples of calcium estimation, Lanthanum oxide (0.5%) was added to the madeup solution to reduce the interference. The blank contained all the reagents used in the sample preparation. Digested samples were stored in tefflon capped glass vials, under refrigeration until used.

Working standards for calcium and magnesium were prepared fresh every time, using commercial standard stock solution obtained from BDH, England.

Estimation of Calcium and Magnesium

Perkin-Elmer 2380 atomic absorption spectrophotometer incorporated with automatic curve correction was utilised for the analysis of calcium and magnesium. The light source used was an intesitron hollow cathode lamp and air-actylene was the oxidant-fuel combination used.

Phosphorus

Phosphorus in haemolymph and various tissues (muscle, hepatopancreas, and exoskeleton) was digested using wet digestion method as described earlier and estimated by the method of Lowery et al. (1954) using phosphomolybdate and ascorbic acid. To the known volume of wet ashed sample, mixture of ammonium molybdate and ascorbic acid solution were added and mixed well. The tubes were placed at 37 °C for two hrs. Then the samples were cooled and the absorbance was recorded using ECIL senior Spectrophotometer at 882 nm.

B: Mapping of calcium, Magnesium and phosphorus in exoskeleton.

Prawns belonging to intermoult stage C were used for the mapping of minerals. For this purpose, different regions of the selected exoskeleton as illustrated in Fig.2 were dissected out. The exoskeletal parts were washed in double distilled water and dried at 80 °C for twelve hrs. After cooling, the samples were stored in a desiccator, and weight of each sample was taken to the nearest 0.1 mg. After recording the weight, each sample was digested as described earlier for the analysis of calcium, magnesium, and phosphorus. Digested samples were stored in tefflon capped glass vials under refrigeration

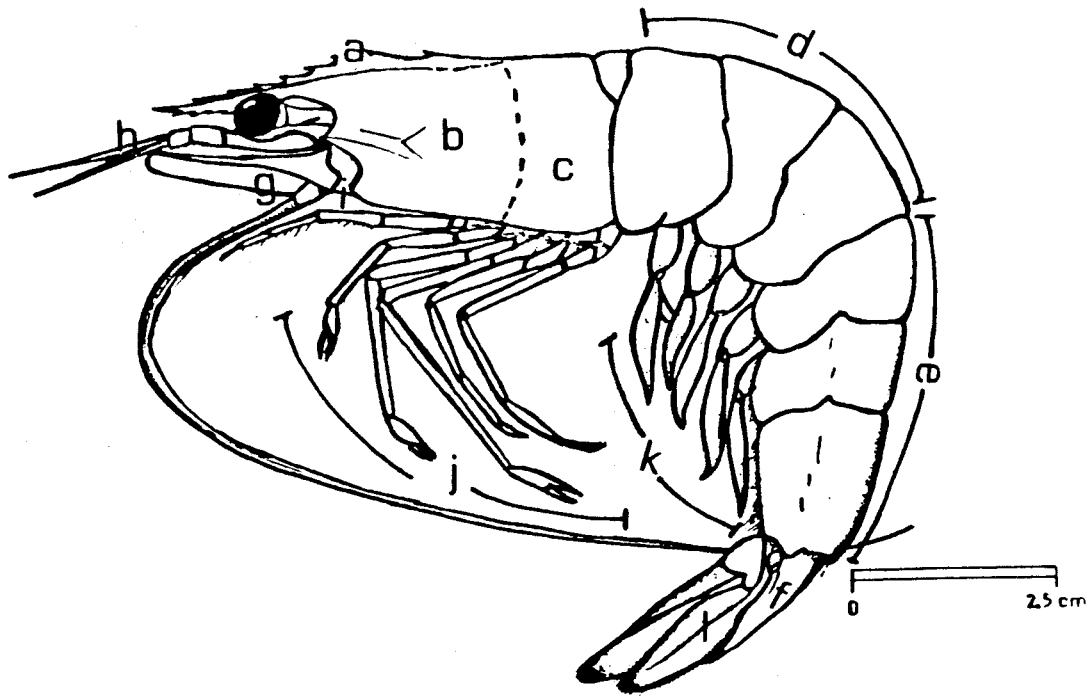


Fig. 2. Exoskeletal areas of P. indicus selected for mineral mapping.

a. Rostrum
b. Upper region of Carapace
c. Lower region of Carapace
d. Upper abdomen
e. Lower abdomen
f. Telson
g. Antenna
h. Antennule
i. Mouth parts and Maxillipeds
j. Walking legs
k. Swimmerets
l. Uropod

until used. Calcium and magnesium were estimated using AAS, and phosphorus was estimated using spectrophotometer as described in the previOus case.

Statistical analysis

Means and standard deviations were calculated for all estimations. Analysis of variance (ANOVA) was carried out to test the significance between treatments, i.e., effect of different moulting stages on selected minerals. All the statistical tests were carried out according to Snadcor and Cochran (1968) using IBM PC/XT computer.

3.RESULTS

A: Distribution and Mapping of calcium, Magnesium, and Phosphorus in the intermoult exoskeleton.

The results of the analysis of mineral distribution in the exoskeletal areas of the species, P.indicus are given in the Table 1. Diagramatic representations of the calcium, magnesium, and phosphorus distribution over the exoskeletal regions are presented in Figs. 3,4, and 5.

The results of the present study showed that the major areas of the exoskeleton varies considerably in their calcium, magnesium and phosphorus concentrations. In the case of calcium the range of distribution varied from

Table 1. *DISTRIBUTION OF CALCIUM, MAGNESIUM AND PHOSPHORUS IN THE MAJOR EXOSKELETAL AREAS OF P. INDICUS

Area	Area Symbol	Calcium (mg/g)	Magnesium (mg/g)	Phosphorus (mg/g)
1. Rostrum	a	393.75 ±35.82	28.91 ±4.8	10.27 ±2.7
2. Upper region of Carapace	b	176.32 ±17.44	18.75 ±3.76	10.41 ±1.03
3. Lower region of Carapace	c	82.81 ±12.50	9.44 ±2.57	12.87 ±2.01
4. Upper abdomen	d	136.35 ±18.10	12.55 ±1.99	13.71 ±1.11
5. Lower abdomen	e	165.09 ±19.27	14.43 ±2.33	14.42 ±2.05
6. Telson	f	138.72 ±26.8	9.71 ±1.36	11.76 ±1.15
7. Antenna	g	153.59 ±23.07	7.62 ±0.98	13.08 ±2.04
8. Antennule	h	148.64 ±13.4	8.04 ±1.02	11.64 ±1.40
9. Mouth parts and Maxillipeds	i	152.86 ±22.72	10.69 ±2.12	9.93 ±0.99
10. Walking legs	j	278.14 ±26.68	14.23 ±2.08	10.37 ±1.04
11. Swimmerets	k	29.32 ±8.92	3.99 ±0.49	14.48 ±1.22
12. Uropod	l	58.55 ±11.31	4.87 ±1.01	11.95 ±1.07
Mean exoskeletal Concentration		159.52	11.94	12.07

*Values expressed are means of five specimens of P. indicus of intermoult stage C

minimum of 29.32mg/g in the swimmerets to the maximum of 393.75 mg/g (Fig.3) in the rostrum. The main body parts of the exoskeleton viz. rostrum, carapace, abdomen, and telson showed higher levels of calcium (average 182.72 mg/g), while average calcium content of the appendages was comparitively low with 136.85 mg/g. Variation in the levels of calcium content was also noted between the two areas of the same exoskeletal regions such as carapace and abdomen. The upper region of the carapace found to contain more calcium (176.32 mg/g), whereas the calcium concentrations of the lower regions of the carapace was fairly low (82.81 mg/g). Similarly, the upper and lower regions of the abdominal cuticle varied in their calcium content with 136.35 mg/g and 165.09mg/g respectively. The average calcium content of the total exoskeleton in P. indicus was 159.52 mg/g (15.95 %).

The pattern of distribution of magnesium on the exoskeletal areas was similar to that of calcium distribution. Magnesium content of the exoskeletal regions varied from 3.99 mg/g (swimmerets) to 28.91 mg/g (rostrum) (Fig.4). The main body parts of the exoskeleton viz. rostrum, carapace, abdomen and telson also showed higher concentrations of magnesium (average, 15.63 mg/g), while

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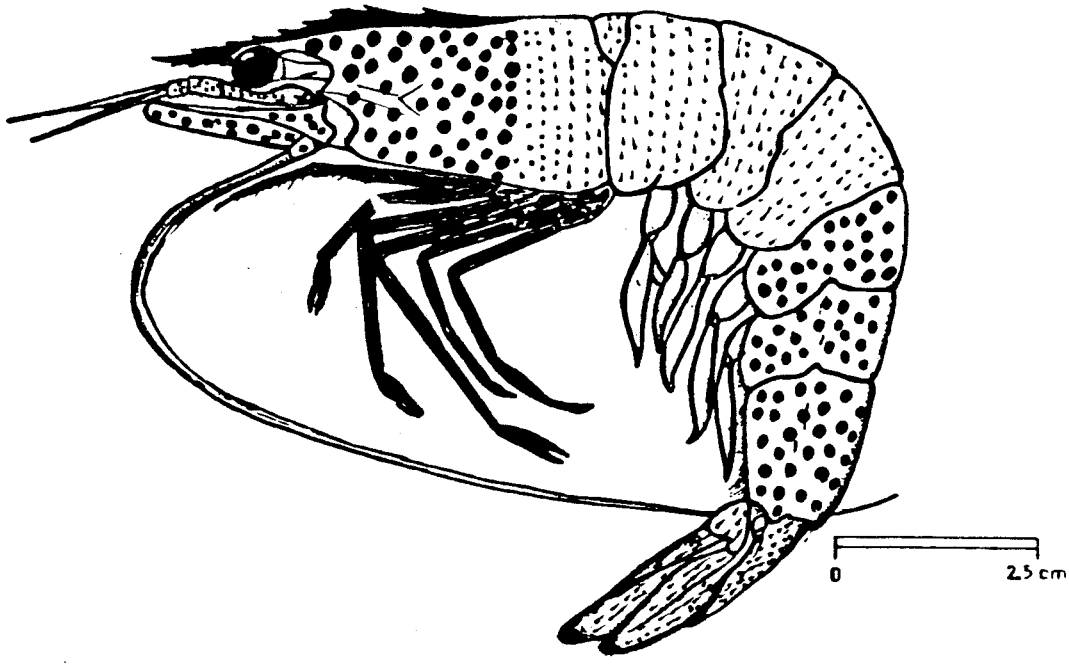


Fig. 3. The pattern of calcium distribution in the exoskeleton of P. indicus

- 0 - 50 mg/g
- 50 - 150 mg/g
- 150 - 250 mg/g
- >- 250 mg/g

e appendages were found to contain considerably lesser vels (average, 8.24 mg/g). Two areas of the carapace and domen showed variations in their magnesium content. The ncentration in the upper region of the carapace was .75 mg/g, whereas the lower region of the carapace ntained only 9.44 mg/g. Similarly, upper abdominal egion of the exoskeleton showed concentration of 12.55 g/g when compared to 14.43 mg/g in the lower abdominal egion. The average magnesium concentration of the xoskeleton recorded in P.indicus was 11.94 mg/g 1.19%).

Unlike calcium and magnesium, phosphorus has not xhibited any pattern in its distribution over the xoskeletal regions. Remarkable differences in the hosphorus levels were not detected between the different egions of the exoskeleton. The phosphorus content in the xoskeletal regions varied between 9.93 mg/g to 14.48 mg/g (Fig.5). The average concentration recorded in the xoskeleton was 12.07 mg/g (1.2%).

B: Mobilization of minerals in different tissues in elation to moult stages

Calcium:

Calcium variations in haemolymph, muscle,

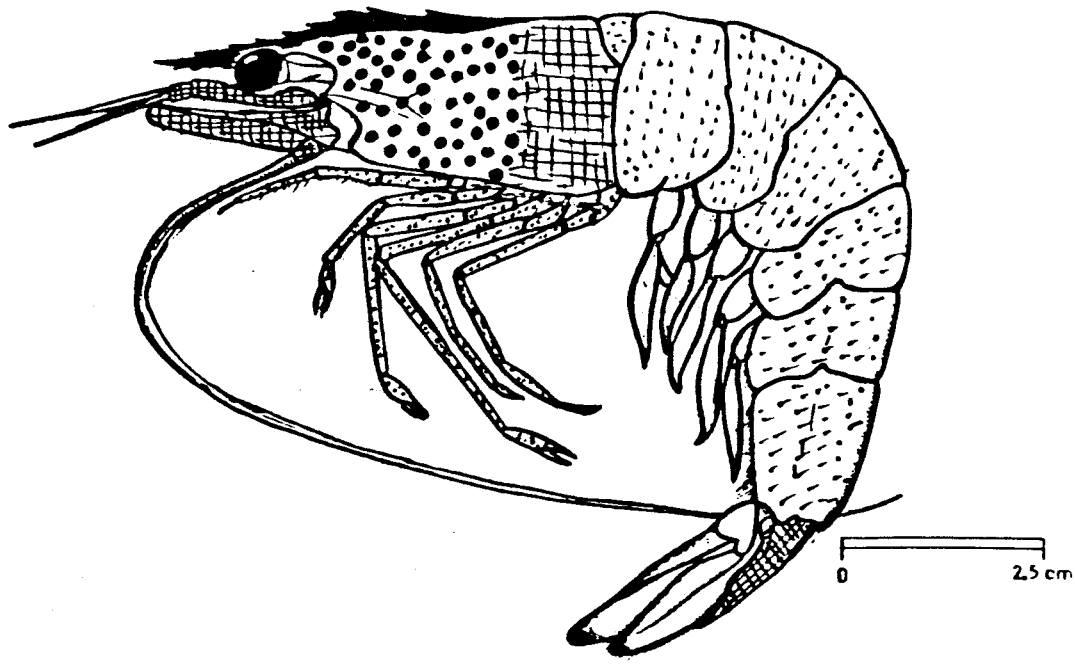


Fig. 4. The pattern of magnesium distribution in the exoskeleton of P. indicus

0 - 5 mg/g

5 - 10 mg/g

10 - 15 mg/g

15 - 25 mg/g

>25 mg/g

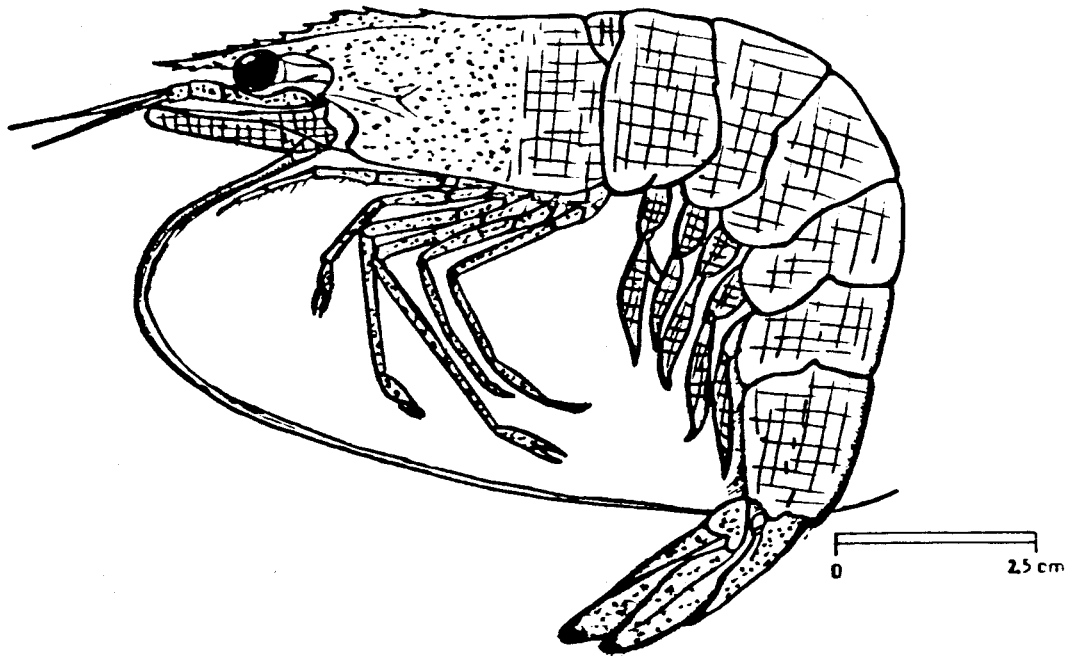


Fig. 5. The pattern of phosphorus distribution in the exoskeleton of P.indicus

0 - 12 mg/g

> 12 mg/g

hepatopancreas, and exoskeleton during different stages of the the moult cycle are given in the Table 2.

Calcium concentration in the haemolymph followed a well defined pattern with the maximum concentration in last premoult stage (Fig.6). Haemolymph calcium content showed rapid rise from the early premoult stages (0.78 mg/ml) to its peak value (1.88 mg/ml) at D2-3, followed by a decline through the postmoult stages to reach the minimum values during the intermoult and early premoult stages (0.78 to 0.83 mg/ml). The variations in haemolymph calcium during different moult stages were highly significant as determined by analysis of variance ($P<0.01$).

Muscle tissue showed the minimum calcium levels (1.75 mg/g) in early postmoult stage A and a maximum during the last premoult stage D2-3 (4.74 mg/g). There was a gradual and steady increase in calcium levels from the early postmoult A to the last premoult D2-3 (Fig.7). Variation seen in calcium content of muscle during the different moult stages are found to be statistically significant ($P<0.01$).

Calcium content of the hepatopancreas during the moulting cycle ranged from a minimum of 7.50 mg/g in stage

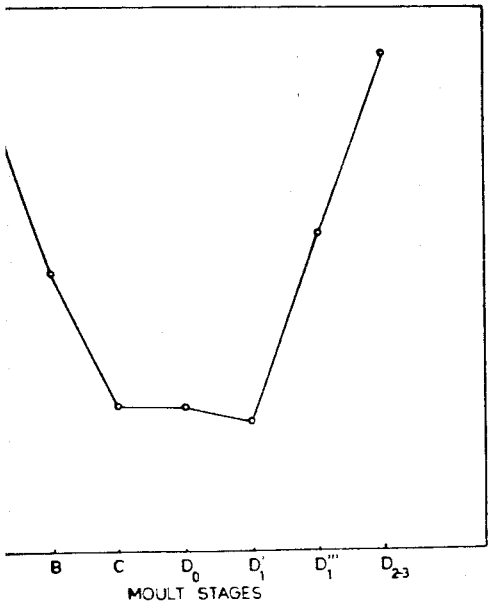
Table 2. VARIATION IN THE CONCENTRATION OF CALCIUM DURING THE DIFFERENT MOULT STAGES OF PRAWN *P. INDICUS*

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1''	D2-3
Haemolymph (mg/ml)	N	8	8	8	8	8	8	8
	$\bar{X}$	1.738	1.232	0.834	0.826	0.781	1.346	1.884
	±SD	0.158	0.228	0.062	0.099	0.078	0.134	0.234
Muscle (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	1.745	1.778	2.608	3.627	4.628	4.686	4.737
	±SD	0.366	0.331	0.426	0.450	0.501	0.553	0.631
Hepatopancreas (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	14.202	11.674	7.506	8.052	8.395	10.623	12.7
	±SD	2.222	2.044	1.223	1.354	1.144	1.607	2.39
Exoskeleton (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	51.167	99.742	161.592	166.642	168.984	153.850	147.657
	±SD	9.362	13.804	9.583	7.850	6.820	9.986	6.605

ANALYSIS OF VARIANCE : CALCIUM

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Haemolymph	Treatment	6	9.7635	1.6272	67.0755*
	Error	49	1.1887	0.0243	
Muscle	Treatment	6	88.0451	14.6742	64.8308*
	Error	49	11.0910	0.2263	
Hepatopancreas	Treatment	6	314.4077	52.4013	16.6278*
	Error	49	154.4202	3.1514	
Exoskeleton	Treatment	6	93175.6200	15529.2700	174.8960*
	Error	49	4350.7810	88.7914	

*Significant at 1% level ($P < 0.01$)



i. Mean haemolymph calcium
ion of P. indicus during different
of moult cycle

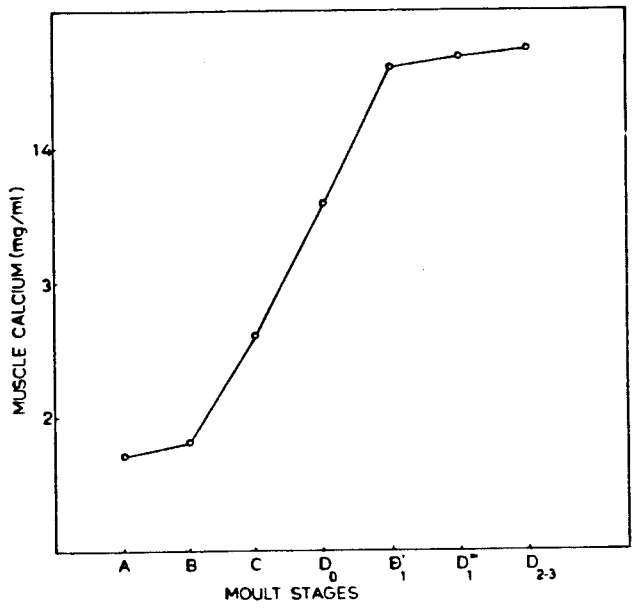
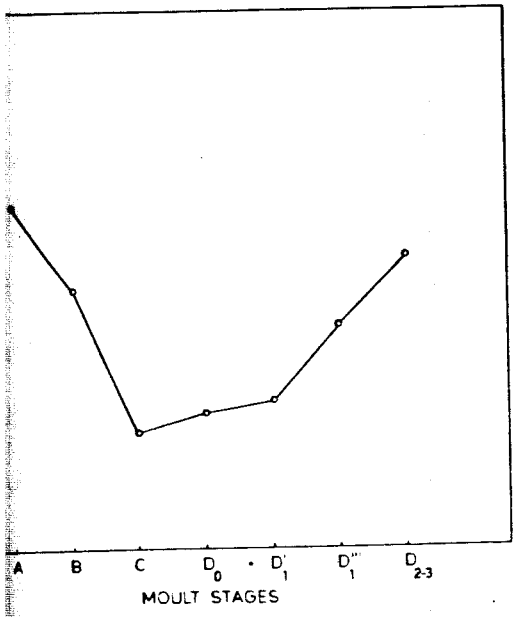


Fig. 7. Mean muscle calcium variation of P. indicus during different stages of moult cycle



8. Mean hepatopancreas calcium
ation of P. indicus during different
es of the moult cycle

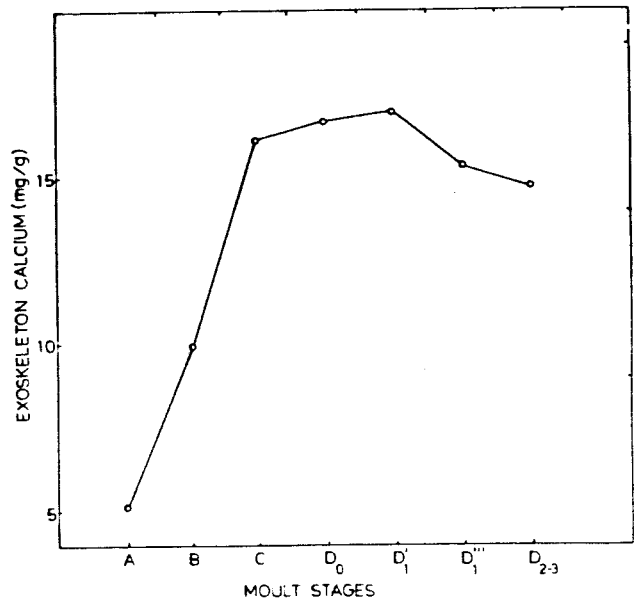


Fig. 9. Mean exoskeletal calcium variation of P. indicus during different stages of moult cycle

C to the maximum of 14.20 mg/g in stage A. From the intermoult stage C, hepatopancreatic calcium showed an increasing trend to reach its maximum value in stage A. After moulting, the levels started decreasing from stage B and remained low in intermoult and early premoult stages (Fig.8). ANOVA indicated that the variations of calcium during different stages of the moulting are statistically significant ($P<0.01$).

The lowest calcium content recorded in the exoskeleton was in stage A (51.17 mg/g). From stage A onwards, exoskeletal calcium content showed a linear increase up to early premoult stage of D1' (168.98 mg/g). Thereafter the level started falling down in the late premoult stages and the lowest recorded was in early postmoult stage A. A very sharp fall in the calcium concentration was noted between the stages D2-3 and A, while a sharp rise in the exoskeletal calcium was recorded between the stages B and C (Fig.9). Analysis of variance showed that the calcium fluctuations in the exoskeleton during the different stages of moult cycle are significant at 1% level.

Magnesium:

Changes in the magnesium levels in haemolymph,

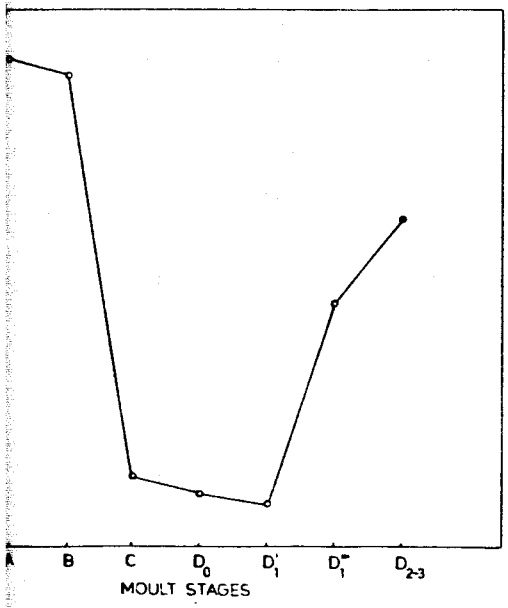
Table 3 : VARIATION IN THE CONCENTRATION OF MAGNESIUM DURING THE DIFFERENT MOULTING STAGES OF PRAWN, *P. INDICUS*

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1''	D2-3
Haemolymph (mg/ml)	N	8	8	8	8	8	8	8
	$\bar{X}$	0.422	0.416	0.176	0.163	0.155	0.276	0.328
	±SD	0.013	0.021	0.011	0.002	0.009	0.009	0.017
Muscle (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	0.887	0.749	1.339	1.446	1.481	1.486	1.437
	±SD	0.029	0.015	0.046	0.035	0.019	0.027	0.031
Hepatopancreas (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	4.184	3.319	2.758	2.663	2.715	3.669	3.867
	±SD	0.230	0.248	0.176	0.152	0.184	0.126	0.580
Exoskeleton (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	7.232	9.561	12.474	14.077	14.358	13.149	11.223
	±SD	0.417	0.495	0.409	0.534	0.536	0.499	0.726

ANALYSIS OF VARIANCE : MAGNESIUM

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Haemolymph	Treatment	6	0.6480	0.1080	603.9624*
	Error	49	0.0088	0.0002	
Muscle	Treatment	6	4.5775	0.7629	815.8346*
	Error	49	0.0458	0.0009	
Hepatopancreas	Treatment	6	18.2501	3.0417	38.5954*
	Error	49	3.8617	0.0788	
Exoskeleton	Treatment	6	321.3667	53.5611	193.3924*
	Error	49	13.5708	0.2770	

*Significant at 1% level ($P < 0.01$)



10. Mean haemolymph magnesium
ation of P. indicus during different
es of moult cycle

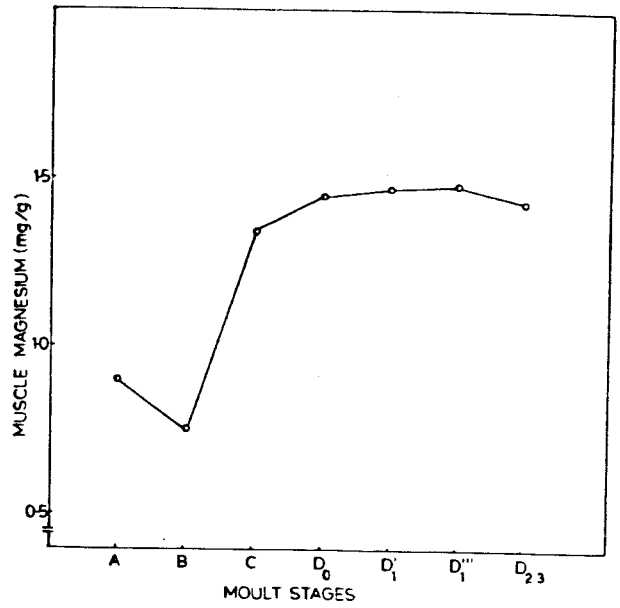
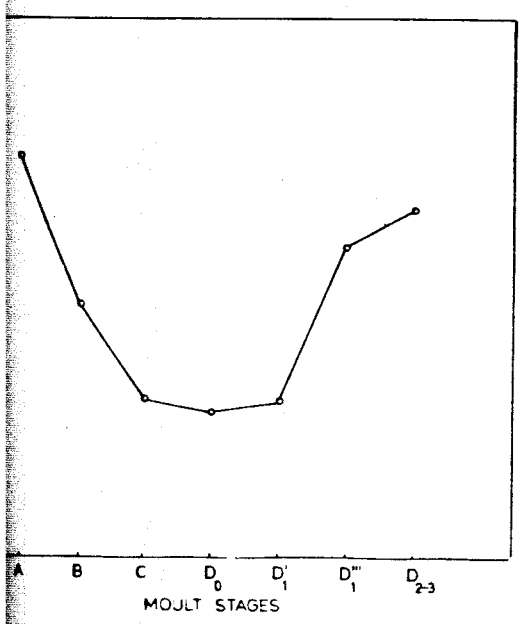


Fig. 11. Mean muscle magnesium variation of P. indicus during different stages of moult cycle.



12. Mean hepatopancreas magnesium
ation of P. indicus during different
es of moult cycle.

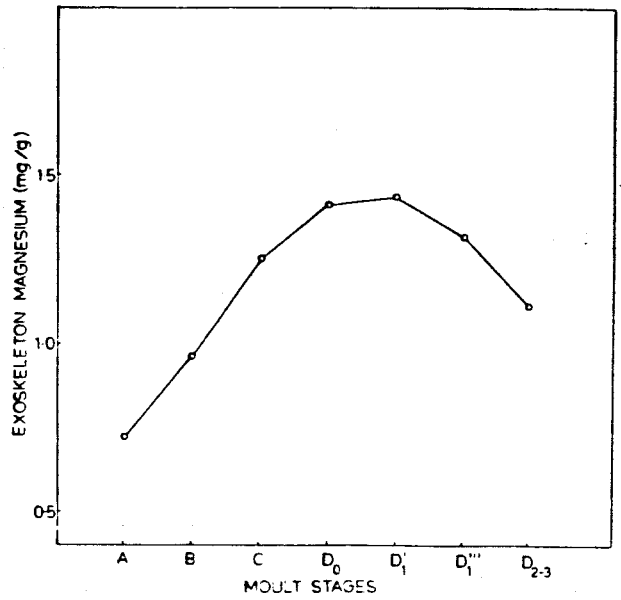


Fig. 13. Mean exoskeletal magnesium variation of P. indicus during different stages of moult cycle

muscle, hepatopancreas, and exoskeleton during different stages of the moult cycle are presented in the Table 3.

The lowest magnesium concentration in the haemolymph was in premoult stage D1' (0.16 mg/g). Thereafter, concentration rose gradually to last premoult stage D2-3, while the maximum haemolymph magnesium was recorded in stage A (0.42 mg/g). From stage A, concentration declined gradually to the minimum in stage D1' (Fig.10). ANOVA revealed that magnesium concentrations during different moult stages in the haemolymph are statistically significant at 1% level.

Postmoult stage B, showed minimum level of magnesium in the muscle tissue, i.e. 0.75 mg/g. From stage B onwards there was a linear increase till the stage D1''' with a value of 1.49 mg/g. A sharp increase was recorded between the stages B and C, while a sharp fall was noted between stages D2-3 and A (Fig.11). The variations of muscle magnesium were significant between different moult stages as determined by ANOVA ($P<0.01$).

In the hepatopancreas, maximum magnesium concentration was recorded in stage A (4.18 mg/g). From stage A it decreased gradually and reached the minimum

value of 2.66 mg/g in stage Do. In the late premoult stages the level increased again and remained high till the stage B after the ecdysis (Fig.12). Differences in the magnesium content among the moult stages are found to be statistically significant ($P<0.01$).

Magnesium changes in the exoskeleton followed a similar pattern to that of calcium. The minimum concentration in the exoskeleton (7.23 mg/g) was in stage A and thereafter the concentration showed a linear increase to the maximum value (14.36 mg/g) in the early premoult stage D1'. Late premoult stages showed a sharp fall in the concentration, while the fall between the last premoult stage D2-3 and early postmoult stage A was considerable (Fig.13). ANOVA indicated that the variation of exoskeleton magnesium levels during the moulting cycle are statistically significant ($P<0.01$).

Phosphorus:

Changes in the phosphorus concentration of haemolymph, muscle, hepatopancreas, and exoskeleton during different stages of moult cycle are given in Table 4.

Phosphorus concentration in the haemolymph during the moult cycle ranged from the minimum of 0.042 mg/g in stage D1' to the maximum of 0.108 mg/g in stage D2-3. Late

Table 4 : VARIATION IN THE CONCENTRATION OF PHOSPHORUS DURING THE DIFFERENT MOULTING STAGES OF PRAWN, *P. INDICUS*

Tissue		MOULT STAGES						
		A	B	C	Do	D1'	D1''	D2-3
Haemolymph (mg/ml)	N	8	8	8	8	8	8	8
	$\bar{X}$	0.098	0.072	0.050	0.044	0.042	0.081	0.108
	±SD	0.006	0.008	0.008	0.006	0.005	0.009	0.006
Muscle (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	5.461	5.231	4.722	4.437	5.096	5.628	6.706
	±SD	0.507	0.432	0.316	0.283	0.325	0.312	0.774
Hepatopancreas (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	1.460	1.635	1.799	1.808	2.077	1.867	1.756
	±SD	0.090	0.055	0.105	0.094	0.107	0.084	0.123
Exoskeleton (mg/g)	N	8	8	8	8	8	8	8
	$\bar{X}$	19.186	18.565	12.475	13.793	14.349	15.265	26.319
	±SD	0.544	3.054	2.389	1.262	1.467	1.764	4.009

ANALYSIS OF VARIANCE : PHOSPHORUS

Tissue	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Haemolymph	Treatment	6	0.0334	0.0056	99.1827*
	Error	49	0.0028	0.0001	
Muscle	Treatment	6	25.8575	4.3096	21.1680*
	Error	49	9.9759	0.2036	
Hepatopancreas	Treatment	6	1.7643	0.2940	33.3389*
	Error	49	0.4322	0.0088	
Exoskeleton	Treatment	6	1077.8440	179.6406	32.8670*
	Error	49	267.8184	5.4657	

*Significant at 1% level ($P < 0.01$)

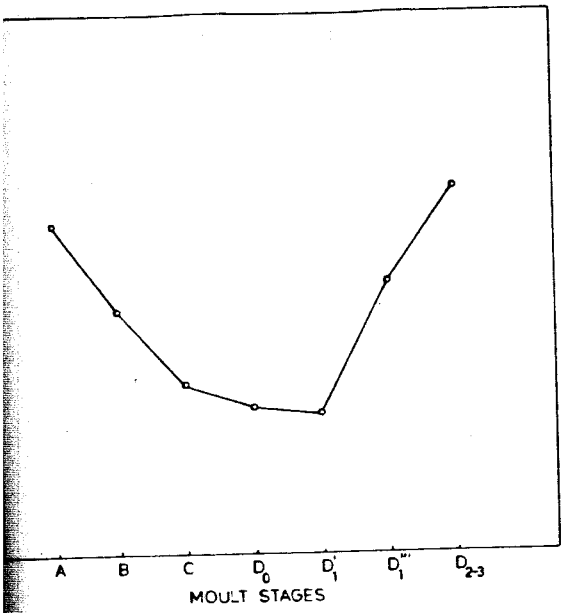


Fig. 14. Mean haemolymph phosphorus variation of P. indicus during different stages of moult cycle.

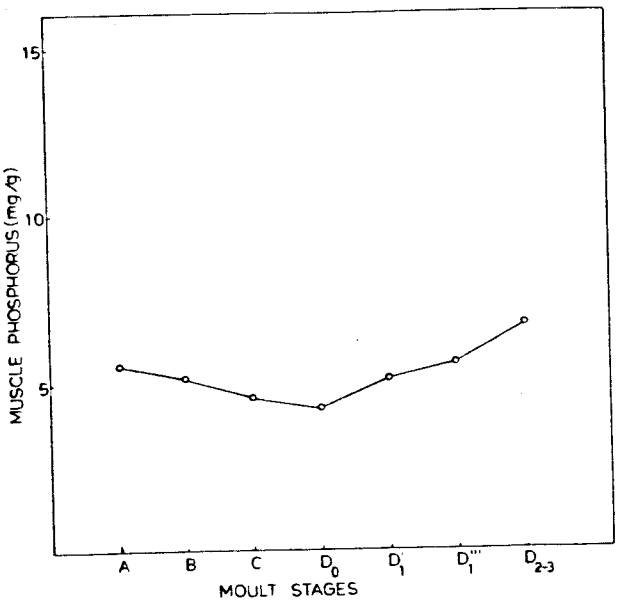


Fig. 15. Mean muscle phosphorus variation of P. indicus during different stages of moult cycle

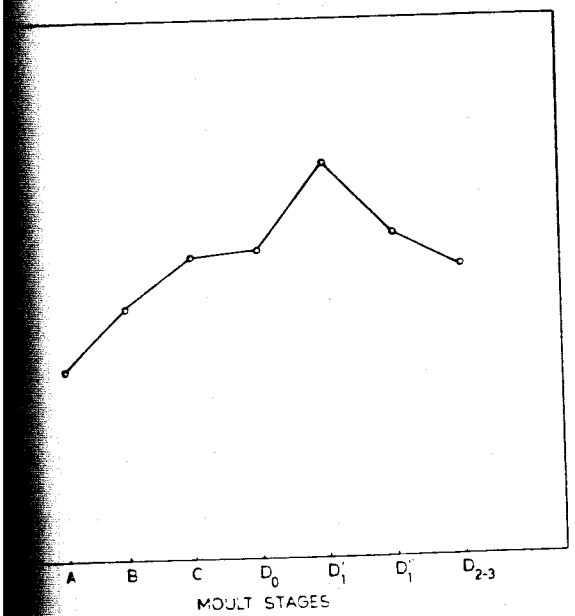


Fig. 16. Mean hepatopancreas phosphorus variation of P. indicus during different stages of moult cycle.

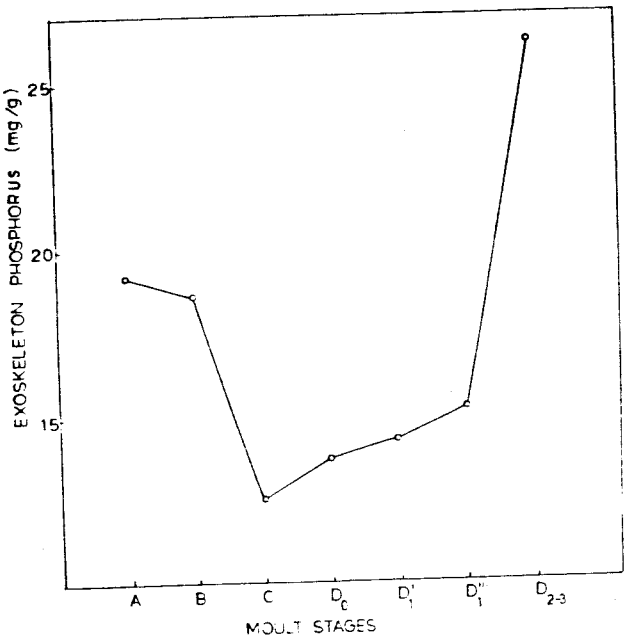


Fig. 17. Mean exoskeletal phosphorus variation of P. indicus during different stages of moult cycle.

premoult stage D1''' and D2-3, and postmoult stages A and B were found to contain higher levels of phosphorus when compared to intermoult (stage C) and early premoult (stages Do) (Fig.14). Differences in phosphorus content during different stages of the moult cycle are found to be statistically significant ($P<0.01$).

Muscle phosphorus content increased from a minimum concentration of 4.44 mg/g in stage Do to the maximum concentration of 6.71 mg/g in last premoult stage D2-3. A fall in the level was noted after moulting to reach the minimum during stage Do (Fig.15). ANOVA indicated that the differences in muscle phosphorus levels between the different moult stages are statistically significant ($P<0.01$).

Hepatopancreas phosphorus concentration during the moulting cycle varied between 1.46 mg/g in stage A to 2.08mg/g in stage D1'. Phosphorus content of the hepatopancreas showed a gradual increase from stage A to stage D1' and a fall thereafter during the late premoult stages (Fig.16). Differences in the hepatopancreas phosphorus content during the different stages of the moult cycle are found to be statistically significant at [illegible] level.

Phosphorus content of the exoskeleton during the moult cycle ranged from 12.48 mg/g in stage C to 26.32 mg/g in stage D2-3. From the intermoult stage C, the level gradually increased and touched the maximum value in last premoult stage D2-3, prior to the moulting (Fig.17). After moulting the concentration showed a declining trend in postmoult stages A and B, and the minimum value of 12.48mg/g was reached in the intermoult stage C. The variaton in the phosphorus content of the exoskeleton were significant as determined by the analysis of variance ($P<0.01$).

4.DISCUSSION

The concentrations of calcium in the exoskeleton of the prawn species used in the present investigation are low when compared with those of highly mineralized crustacean species like Astacus pallipes-20% (Chaisemartin, 1962), Orconectes virilis-20-25% (Travis, 1963 and McWhinnie et al. 1969), and Callinectes sapidus-29.1%(Cameron and Wood, 1985). The calcium concentration obtained in the present study (15.95%) was, however, comparable to the values obtained by Welinder (1974) in P.duorarum (16%), and Huner et al.(1976a) in P.californiensis (12.14%), while Dall (1965a) reported,

19% of calcium in the exoskeleton of Metapenaeus sp. Mills and Lake (1976) suggested that the differences in concentration of exoskeletal calcium may be related to the amount of available calcium in the waters inhabited by the species, while Huner et al. (1976c) attributed the exoskeletal calcium variation among crustaceans due to the species differences.

Contributions of magnesium and phosphorus in intermoult exoskeleton of P.indicus are found to be fairly low (1.19-1.2%), when compared to calcium (15.95%), in the present investigation. Studies dealing with the exoskeletal magnesium and phosphorus in crustaceans are very much limited. Huner et al.(1976b) reported the magnesium levels in the intermoult carapaces of three species of fresh water crayfishes Orconectes virilis, Procambarus alleni, and P.clarkii as 0.408%, 0.428%, and 0.421% respectively. Magnesium levels in the carapace of Metapenaeus sp was estimated by Dall (1965a), who recorded a concentration of 0.135% . Exoskeletal magnesium reported by the above wokers are comparatively low, when compared to the magnesium levels (1.24%) recorded in the exoskeleton of the prawn P.californiensis (Huner et al., 1979), while the magnesium values detected during the

present investigation (1.19%) shows close similarity with the observation of Huner et al. (1979). The relatively low concentrtion of exoskeletal magnesium (1-2%) observed by Dall (1965a), and Huner et al.(1976b, 1979) suggests little importance for this mineral in the overall structural integrity of crustacean exoskeleton. Exoskeletal phosphorus content of Metapenaeus sp and P. californiensis were studied by Dall (1965a) and Huner et al (1979) and their values 0.5% and 0.7% were comparatively lower than the phosphorus value (1.2%) observed in the present study.

Analysis of areas of the exoskeleton for calcium in several crustacean species have shown that there are no differences in patterns of exoskeletal calcium distribution. Drach and Laffon (1942) had shown that each region of the exoskeleton of the crab Maia squinado varied in the calcium levels. Chaisemartin (1962, 1967) has examined the exoskeleton of the crayfish Astacus fluviatilis, and A.pallipus for calcium and observed considerable variation in the calcium concentration among different regions of the exoskeleton. In the crayfish, the range of calcium was from 122 mg/g in the walking legs to 265.7 mg/g in the rostrum (Chaisemartin, 1962,1967). No attempt has been made by these authors (Drach and Laffon,1942 and Chaisemartin, 1962,1967) to explain the

observed pattern of calcium distribution in the species studied other than in terms of structural rigidity.

Later on detailed study was conducted by Mills and Lake (1976) in Parastacoides tasmanicus and Astacopsis fluviatilis on calcium distribution in different regions of the exoskeleton. Studies of Mills and Lake (1976) revealed notable variations (52.74 mg/g to 869.44mg/g) in the calcium content of crayfish exoskeleton. Further studies of Mills et al. (1976) in crayfish genera Engaeus and Geocharax also showed variations of calcium levels between the different exoskeletal regions such as carapace, abdomen, telson and appendages. The differential distribution of calcium over different regions of the exoskeleton in crayfishes has been attributed to the burrowing habits of the species (Mills and Lake, 1976 and Mills et al.,1976). Hardening of the terminal areas of the chelepeds in crayfish has been related to use made of this appendage as a weapon in defence, aggression, and also to a lesser extent in excavating activities (Mills and Lake, 1976). In penaeid prawn P.californiensis, Huner et al.(1979) recorded high calcium concentration in the rostrum (14.78%) followed by carapace (13.1%), and abdomen (12.35%). Results of the present investigation fall in

line with the observation of Huner et al.(1979). Relative differences in calcium distribution of exoskeletal regions observed in the present study find similarity with the calcium content of exoskeletal areas reported for crayfish (Chaisemartin, 1962; Welinder, 1975a; Mills and Lake, 1976 and Mills et al., 1976), although P.indicus is considerably less calcified than crayfish. Regional differences noted in the exoskeletal calcium content of P.indicus may be due to the conservation of calcium by restricting distribution to areas where it is most beneficial in terms of rigidity. Calcium distribution may also be influenced by environmental factors and nature of habitat where the animal is living (Rao et al.,1983)

Magnesium distribution in the exoskeletal regions in P.indicus showed very close resemblance to that of calcium distribution. Other than the work of Huner et al. (1979), no literaure is available on magnesium distribution in the exoskeletal regions. Huner et al. (1979) recorded the highest magnesium levels in the rostrum (1.31%) followed by carapace (1.13%), and abdomen (1.01%). In the present study unlike calcium and magnesium, phosphorus did not show much variation between the different regions of the exoskeleton. Huner et al. (1979) while working on P.californiensis did not observe much variation in the

distribution of exoskeletal phosphorus. More or less uniform phosphorus level in the exoskeleton probably indicates its insignificant role in the hardening of the exoskeleton.

Studies on the mobilization of calcium, magnesium, and phosphorus in selected tissues of P.indicus viz. haemolymph, muscle, hepatopancreas, and exoskeleton showed substantial variations between the moult stages of the prawn. Among the minerals studied (calcium, magnesium and phosphorus), the most notable changes were exhibited by calcium. Passano (1960) and Greenaway (1985) discussed the importance of calcium in the body metabolism of crustaceans and they have described the calcium as the principal inorganic material of the exoskeleton. The demand for calcium is particularly high in crustacea as the exoskeleton is shed regularly to allow increase in body size (Drach, 1939 and Passano, 1960).

From the literature it can be seen that other than the works of Hagerman (1973) who reported a fall in the concentration of haemolymph calcium during the premoult stage in Crangon vulgaris, other crustacean workers reported increased calcium levels in the premoult stages and a subsequent fall after moulting (Numanoi,

939; Travis, 1955b; Robertson,1960; Haefner, 1964; Bursey nd Lane, 1971; Charmantier, 1972; Greenaway, 972,1974b,c,1976; Fieber and Lutz, 1982, and Sparkes and reenaway, 1984). In the present study also remarkable hanges in the haemolymph calcium were observed with a eak value in last premoult, and low value after moulting n the intermoult and early premoult stages. Just prior to cdysis, the concentration of total calcium in the aemolymph of P.indicus increased to about the double of he intermoult value. Similarly in Panulirus there was 5% increase (Travis, 1955b), in Sesarma catenata 66.5% ncrease (Hecht, 1975), and in Macrobrachium rosnbergii 0% increase (Fieber and Lutz, 1982), while in the two pecies of Sesarma studied by Numanoi (1939), and in oloyhuisana transversa studied by Sparkes and Greenaway 1984), a rise of haemolymph calcium to about 150 times to hat of intermoult value was recorded.

A rise in the haemolymph calcium concentration is ited as an evidence for mineral absorbtion prior to the oult (Robertson, 1937; Roer, 1980). According to Passano 1960), Haefner (1964), and Greenaway (1983) the higher evels of haemolymph calcium in premoult of crustacea are ue to the resorption of calcium from the exoskeleton

rior to the event of moultimg. Postmoult fall of calcium s resorted to the utilization of haemolymph calcium after oulting, in the mineralization of the newly formed xoskeleton (Passano, 1960; Greenaway, 1985). Drach 1939), Travis (1957), and Haefner (1964) reported the ontinuous withdrawal of calcium from the haemolymph uring the postmoult by active transport into the ypodermal cells and their deposition in the exoskeleton or hardening.

In P. indicus the trend of variation exhibited by the haemolymph magnesium and phosphorus were similar to that of calcium. The present obervation is in line with the findings of Sheets and Dendinger (1983), who reported an increase of haemolymph magnesium concentration during the premoult, and a decline during the postmoult in Callinectes sapidus.

Literature reveals little information to show the relationship of muscle calcium, magnesium, and phosphorus to the moulting cycle of crustaceans. In the present study, a slow but gradual increase was noted in the calcium content of muscle from postmoult to premoult. As pointed out by Rao et al. (1983), and Greenaway (1985), a part of the calcium absorbed by the prawn from the food

and water may be depositing in the muscle tissue until the occurrence of moult, resulting in the gradual build up of muscle calcium. Notable reduction of the muscle calcium in P.indicus after the moult probably indicates the mobilization of muscle calcium for the hardening of the newly formed exoskeleton, as described by Passano (1960). Magnesium levels of muscle in P.indicus showed more or less similar behaviour to that of muscle calcium. In contrast to calcium and magnesium, fall of phosphorus content in muscle after moulting was not significant in the present study.

Passano (1960) has pointed out the importance of hepatopancreas as a temporary storage site for inorganic substances resorbed from the old exoskeleton prior to the event of ecdysis. In the present study hepatopancreas calcium levels were observed rising gradually from late postmoult stage B to late premoult and reached the maximum in early postmoult stage A. Accumulation of calcium was reported to occur between the moult stage C4 and D1' in the crab M.squinado (Drach, 1939) while Sather (1967) observed the accumulation of calcium in the hepatopancreas of Podothalmus vigil until the late premoult stage D4. Brannon and Rao (1979) obseved a sharp rise in the calcium content of the hepatopancreas of Palemonetes pugio during

the premoult stage Do, but didn't examine the later stages. Recently, Sheets and Dendinger (1983) reported a higher concentration of hepatopancreatic calcium just before and after ecdysis and lower values in the intermoult and early premoult stages of blue crab C.sapidus. The trend of hepatopancreatic magnesium during the moult cycle of P.indicus followed the same pattern to that of hepatopancreatic calcium. A similar observation of the magnesium variation has been reported by Sheets and Dendinger (1983) in C.sapidus. The accumulation of calcium and magnesium in the hepatopancreas of P.indicus from the intermoult stages up to the early postmoult indicated the storage nature of the organ. The fall of calcium and magnesium after stage A, indicated the mobilization of the minerals for the postmoult mineralization of the exoskeleton. A slow but steady increase of hepatopancreas phosphorus from early postmoult to early premoult and its fall immediately before and after moult as observed in the present work, probably indicate the build up of phosphorus during the tissue growth and its subsequent use at the time of moult as an energy source as described by Huner et al.(1979).

Exoskeletal calcium and magnesium of P.indicus showed

similar pattern of variation during the different stages of the moult cycle. The pattern of marked increase up to the intermoult stage from the lowest value in postmoult stages and a further decline in the late premoult follows closely the basic pattern reported for calcium fluctuation in heavily mineralized crabs (Robertson, 1960; Greenaway, 1976 and Sheets and Dendinger, 1983) and crayfish (Travis, 1960,1963,1965; Chaisemartin, 1962; Greenaway, 1974a,b,c; Adegboye ,1975 and Huner et al.,197º). The present observation on exoskeletal calcium and magnesium are in accordance with the findings of Drach and Lafon (1942) in Cancer pagurus and Maia squinado, Welinder (1975a) in Astacus fluviatilis, and Sheets and Dendinger (1983) in C.sapidus who also observed a rapid mineralization soon after moulting in postmoult and intermoult, followed by a somewhat longer period of slow mineralization up to the early premoult stages. Huner et al. (1979)studied the postmoult mineraliztion of exoskeleton in the juvenile prawn P.californiensis and recorded a rapid rise in the concentration of calcium and magnesium immediately after postmoult. The reduction in calcium and magnesium in premoult stage D2-3 is expected since this is the period when maximum demineralization takes place, and a new exoskeleton is formed beneath the old exoskeleton

(Passano,1960). It is not unusual that magnesium which is believed to substitute for calcium in the mineral matrix of crustacean exoskeleton showed the same trend observed for calcium (Richard,1951). In contrast to the exoskeletal calcium and magnesium, phosphorus values showed maximum concentration in late premoult and postmoult.

S U M M A R Y

Distribution and mobilisation of calcium, magnesium and, phosphorus in relation to different stages of moult cycle were studied in the prawn P.indicus. Calcium is detected as the principal inorganic component of the exoskeleton (15.95%), while magnesium (1.19%) and phosphorus(1.2%) were relatively minor components of the exoskeleton. Different region of the exoskeleton showed variation in calcium, magnesium, and phosphorus concentrations. Variation of calcium, magnesium, and phosphorus content in haemolymph muscle, hepatopancreas, and exoskeleton showed substantial difference between the moult stages of the animal. All the three minerals in haemolymph gave the maximum values in late premoult stages, followed by a decline through the postmoult to reach the minimum values in intermoult and early premoult stages. In muscle, a gradual and linear increase of mineral (calcium, magnesium, and phosphorus) accumulation

was noted from the postmoult to the premoult stage. Hepatopancreatic calcium and magnesium remained high in late premoult and early postmoult stages, while phosphorus gave lower values in late premoult stages. Minimum values for calcium and magnesium in heapatopancreas were recorded in stage C and Do, where as minimum phosphorus values were obtained in stage A. In the exoskeleton, the lowest concentrations of calcium and magnesium were recorded in stage A, which showed a rapid increase from stage B to stage C to touch the maximum values in intermoult and early premoult stages. A very sharp fall in the calcium concentration was noted between the stages D2-3 and A. In the case of exoskeletal phosphorus, values remained high in late premoult and early postmoult stages, and minimum in the intermoult stage C.

CHAPTER-V SOME ENVIRONMENTAL FACTORS AND CONTROL OF MOULTING

CHAPTER V

SOME ENVIRONMENTAL FACTORS AND CONTROL OF MOULTING

INTRODUCTION

As the animals have to move in space or time through a range of environmental variables such as temperature, pH, salinity, and light, these factors exert significant effect on the physiological system of the animals (Aiken, 1978). Crustaceans are not an exception for the effect of environmental factors as they are distributed in a wide range of ecological habitats. The effect of environmental factors are highly variable depending on the species and their nature of habitats.

In the life history of Crustaceans, moulting is the end point of a series of physiological events to achieve general body growth (Passano, 1960). The effect of the environmental factors primarily reflects on the moult cycle, and there by the act of moulting in crustaceans, in the natural habitats, becomes very critical (Aiken, 1978). Control of moulting due to the exogenous influence can be direct or indirect. Exogenous factors viz. temperature, salinity, pH, and light may affect together or separately on general metabolic activities or trigger some specific exogenous behaviour so as to influence the moulting process (Aiken, 1978). The extent of the effects

of environmental factors over moulting varies among the species (Conan, 1985). Therefore, investigation on the influence of some important environmental factors like temperature, salinity, pH and light on moulting process are of utmost importance.

Among the exogenous factors, temperature plays a prime role in the regulation of crustacean moulting and growth (Conan, 1985). Influence of temperature on the frequency of moult and growth rate of crab Eriocheir sinensis, Cancer irroratus and Callinectes sapidus was studied by DeLeersnyder (1972), Haefner and VanEngel (1975), and Winget et al.(1976), respectively. Templeman (1936) made an earlier attempt to study effect of temperature on the larval growth rate of lobster Homarus americanus. Further studies of McLeese (1956) and Aiken and Waddy (1975) in H.americanus and Chittleburough (1975) in Panulirus longipus also revealed the effect of temperature on the moulting and growth of these animals. The moulting and growth of euphausids (Fowler et al.,1971), and brown shrimp (Carlberg and Vanolst, 1976) were found to be influenced by temperature. Involvement and influence of temperature on the growth and moulting of Palemon serratus and Pandalus jordani were reported by

Richard (1978), and Rothilsberg (1979), respectively. Experiments conducted by Zein-Eldin and Aldrich (1965) showed that the growth process of Penaeus aztecus was greately influenced by the temperature.

Although temperature is generally thought to have a more significant effect on the physiology of animals, salinity probably through its osmotic effects, also plays an important role in controlling the physiological state of the animals (Zein- Eldin and Aldrich, 1965). Salinity assumes importance among the abiotic factors especially for those animals, which have a dual life cycle between the sea and estuaries. Influence of salinity on growth and survival of prawns has been reported by several workers (Gunter, 1961; Venkataramiah et al., 1972; Nair and Kutty, 1975; and Raj and Raj, 1980). In the case of Crustaceans, especially on prawns, only limited literature is available relating the effect of salinity with moulting process and growth. Zein-Eldin and Aldrich (1965) studied the combined effect of temperature and salinity on the postlarvae of Penaeus aztecus. Later on, the work done by Bookhout (1972) in Pagurus alatus, Rothilsberg (1979) in Pandalus jordani, and Stirts and Turner (1981) in Emerita talpodia showed the influence of salinity on the moulting and growth process of the respective species.

During the inshore or estuarine phase of life cycle, many species of penaeid prawns experience considerable changes in the ionic content of water. Wickins (1984) pointed out the possibility of pH fall in the natural environment to nearly neutral values following the influx of fresh water after rain, with a corresponding reduction in salinity and alkalinity to adversly affect the normal body physiology of the animals. Studies of Wickins (1984) in Penaeus monodon revealed the effect of reduced pH on the exoskeletal mineralization of the prawn. Waters varying widely in the ionic content have provided much scope for the study of environmental influence on mineralization in decapods (Dodd, 1967; Gibbs and Bryan, 1972). Later Malley (1980), and Wickins (1984) have signified the importance of pH on growth physiology of Crayfish Orconectes and prawn P.monodon respectively. From the literature it is apparent that many aquatic organisms are physiologically unable to tolerate conditions of extreme pH variations (Havas.1981)

Studies on the effect of light over the moulting and growth of crustaceans are confusing due to the contradictory results (Aiken, 1978). Effect of light can be expressed either directly or indirectly. Direct

influence of light will affect the behaviour of the animals and its activity like feeding (Richard, 1978) and indirectly the effect of light operates through the central nervous system to control physiological process such as moulting (Aiken, 1969). Effect of photoperiod on the moulting process has been studied in crab (Diwan and Nagabhushanam, 1974), in crayfish (Stephens, 1955; Aiken, 1969; Mason, 1978), in lobster (Donahue, 1954; Aiken and Waddy, 1976), and in isopods (Moceguard et al., 1976). Later work done by Bishop and Herrnkind (1976), Descouterelle (1976, 1978), Richard (1978), Benayon and Fowler (1980), and Emerson (1980) has revealed the influence of light on the growth process of caridean and penaeid prawns.

It is known that removal of limbs from crustaceans will induce precocious moulting (Bliss, 1956). This observation has been confirmed later by the works of several investigators who have studied the effect of autotomy on the moulting and growth, among crabs (Rao, 1966; Adelung, 1971; Skinner and Graham, 1972; Diwan and Nagabhushanam, 1974; Fingerman and Fingerman, 1974, and Hopkins, 1982), in lobster (Chittleborough, 1975), and in crayfish (Bittner and Kopanda, 1973 and Nakatani and Otsu,

1979). In contrast to the great deal of work on autotomy and moulting in reptantians, studies on natantian group on these aspects are fragmentary. Investigations of Stoffel and Hubschman (1974) in Palaemonetes kadiakensis and Webster (1985) in Palaemon elegans have revealed some informations regarding the effect of autotomy on the prawns.

Adelung (1971) suggested that moult frequency is determined by the rate of tissue growth following ecdysis. If so, feeding influences growth primarily by controlling the moult frequency. Starvation experiments conducted by Adelung (1971), Marsdon et al. (1973), and Dawirs (1984) in Carcinus maenas have revealed the influence of feeding on the growth process of crab. Moulting and growth of lobsters (Templeman, 1936; Chitteleburough, 1974, and Anger et al., 1985), of crayfish (Rao et al., 1977), and of isopod (Strong and Daborn, 1980) were also found affected by the rate of food intake. Importance of feeding on the growth of prawn P.japonicus and P.serratus was reported by Cuzon et al. (1980), and Papathanassion and King (1984), respectively.

P.indicus, being a candidate species used in the brackish water prawn farming suffers significant stress

from environmental parameters through out their life cycle. The brackish water environments, where these animals are traditionally farmed, often encounter wide and rapid variation of abiotic factors like temperature, salinity and pH. Therefore, in the present work, experiments were conducted in the laboratory to find out the effect of some important exogenous factors viz. temperature, pH, salinity, light, and other biological factors like autotomy and starvation, on the moulting and growth of the prawn P.indicus.

2.MATERIALS AND METHODS

Experiments were designed to study the effect of some important environmental factors viz. temperature, pH, salinity, light, and other important biological factors like starvation and autotomy on the moult cycle of P.indicus. The influence of environmental factors on moulting was assessed by taking into consideration of the moult cycle duration and growth increments in terms of weight and length. The effect of environmental factors used in the present experiments encompasses all the possible levels of environmental factors experienced by P.indicus in the natural habitats. All the experiments were conducted at the Marine Prawn Hatchery Laboratory,

Narakkal.

1.Temperature

The temperature levels selected and simulated for the experiment were 26±1 °C (ambient temperature), 31 °C, 32.5 °C, and 37 °C (Simulated experimental temperatures). Hatchery raised early juveniles of P.indicus belonging to the same brood (26-27 mm in total length) were selected at random for the experiment. A group of 12 prawns were exposed to each selected temperature levels. Initial length and weight of the prawns were noted before introducing the animal into the system. Prawns were held individually in 2000 ml beakers with 1000 ml of filtered sea water. The beakers with the prawns were kept in water bath of required experimental temperature. The temperature in the waterbath was maintained at the required levels using a temperature control unit, composed of a Jumo thermometer, a heating coil, and a temperature relay system (Fig. 1A and 1B). The water temperature in the waterbath was made uniform by continuously mixing the water by bubbling the air. Feeding was done during the evening hours with pelletized feed at the rate of 15% body weight of the animal (Ali, 1982). Every morning 3/4 of the water was changed using preheated water of the desired

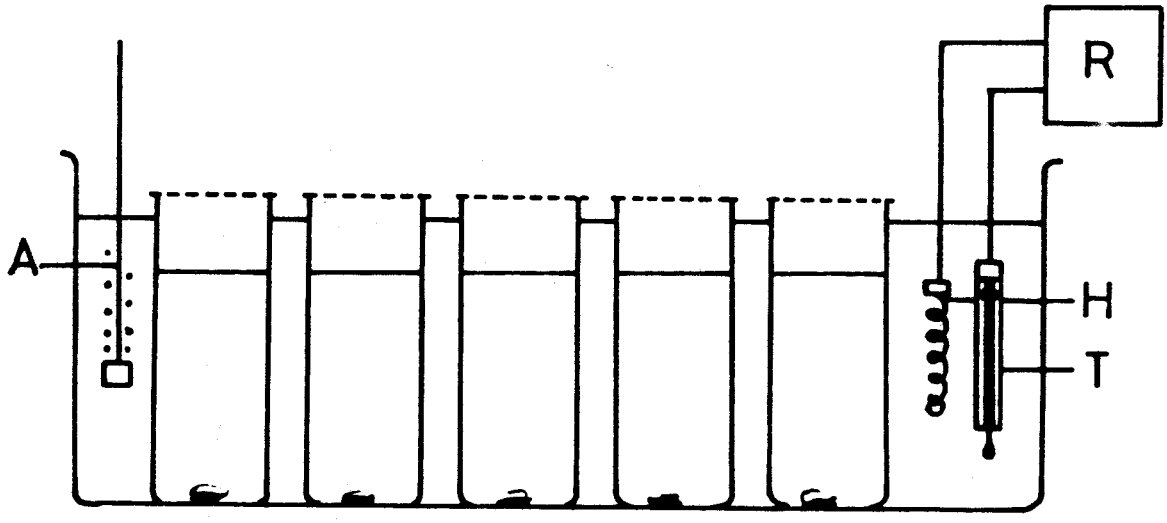


Fig.18: Diagramatic representation, experimental set up for temperature experiment. A - Aerator; H - Heating Coil; R - Automatic Temperature Relay; T - Jumo thermo meter.

temperature, and leftover feed and faecal matters were siphoned out. During experimental period, other parameters like salinity (17±2.5‰), pH (8±0.2), and light (dark and light phases of 12/12 hrs) were maintained constant. Experiment was continued for a period of 20 days. Details of ecdysis of each animal were monitored and recorded daily. At the termination of the experiment, total length and weight of the animal were recorded. Experiments at each temperature were repeated atleast thrice, and average values were taken.

2. pH

pH levels selected for experiments were two acidic pH of 5±0.2 and 6±0.2, a neutral pH of 7 ±0.2 and three alkaline pH of 8±0.2, 9±0.2, and 10±0.2. Media of different experimental levels of pH were prepard in separate fibreglass tank of 250 litres capacity, and kept for atleast three days for stabilization. Everyday the pH was adjusted and brought to the required level. Media of lower ranges of pH were prepared by using 1N HCl, and higher ranges were prepared by using 1N NaOH solution. The freshly prepared media of pH 9±0.2 and 10±0.2 were turbid and not clear. In such cases solutions were allowed to settle completely and only the clear

supernatent was used for the experiment. Six groups of prawns, each consisting 17 prawns of size 26-27 mm in total length, were exposed to each pH level selected for the experiment. In each experimental set up, animals were maintained individually in 3000 ml transparent plastic jars containing 2000 ml of required pH media (Fig.2). The initial length and weight of each prawn was recorded before the introduction of the animal into the experimental system. For aeration purpose airstones were not used to avoid the possible interaction of the stones with pH media, instead aeration was given directly through plastic aeration tubes. Feeding and changing of water media were done everyday as described earlier. Experiment was continued for a period of 20 days. Moulting details of each prawn were observed daily and records were kept. In the pH experiments other parameters like temperature (30±1 °C), salinity (17.5±2.5‰) and light were maintained constant. Each experiment was repeated thrice and average values were taken.

3.Salinity

Salinity levels selected for the experiment were 3‰, 5‰, 15‰, 25‰, 35‰, and 45‰. Required salinities for the experimental purpose were prepared either by diluting

seawater with tap water or by partial freezing of sea water. Five groups of prawn, each group containing 17 prawns of size 26-27 mm in total length, were selected for the experiment and acclimatized to the respective salinities before the introduction of the animals to the experimental salinities. The initial length and weight of each prawn were also recorded. Animals were kept individually in transparent plastic jars of 3000 ml capacity with 2000 ml of water of required salinity (Fig.2). Proper aeration was given to each jar during expeimental period. Prawns were fed with MPHL pelletized feed at the rate of 15% body weight. Every morning 3/4 of the water from each jar was changed using fresh saline water prepared earlier, and the leftover feed and faecal matter were siphoned out. Experimental period was for 20 days. Moulting details of the each animal were monitored and recorded daily. After the termination of the experiment, total length and weight attained by the prawns were recorded separately. In the salinity experiments other parameters like temperature (30 ± 1 °C), pH (8 ± 0.2), and light were simulated to natural conditions. Each experiment was repeated thrice and the average values were taken.

4.Light:

Three different light regimes were selected for the present experiment, viz. 24hr light, 12hr light/ 12hr dark, and OOhr light (total darkness). Three groups of prawns, each consisting of 12 prawns of size 26-27 mm in total length, were exposed to each selected light phase. The initial length and weight of the prawns were recorded before introducing the animal into the experimental system. Prawns were maintained individually in transparent plastic jars of 3000 ml capacity with 2000 ml of water. Electric light from four 100 watts bulb was the source of light in the experiment, and complete darkness was created by keeping the plastic jars in 3ft diameter plastic pool, where the incoming light rays were prevented by covering the pool with wooden plank and black canvas. Feeding and changing of water media were done as described earlier. Experiment was continued for a period of 20 days. In the light experiment other parameters like salinity (17.5 ± 2.5‰), temperature (30 ± 1 °C), and pH (8 ± 0.2) were maintained constant. Moulting details of each prawn during the experimental period were recorded daily. When the experiments were terminated total length and weight attained by animals were recorded. Each experiment was repeated thrice and the average values were taken.

5.Autotomy:

Effect of autotomy on the moult cycle of P.indicus was studied using subadult prawns of size 70-80 mm in total length. In the five experimental groups comprising of 2 leg, 4 leg, 6 leg, 8 leg, and 10 leg (I,II,III,IV and V pair of walking legs), autotomy was performed. In each group 17 prawns were used for autotomy. Animals used for the autotomy were either in the intermoult stage or in the late postmoult stages. A group of 17 intact prawns were kept as controls. The walking legs of the prawns were removed at the junction between the body and the leg using an Electrocautery apparatus. Prawns were maintained individually in floating plastic cages in 3ft diameter pools as described in the I-Chapter (Fig.3). Prawns were fed with MPHL pelletized diet at the rate of 15% body weight of the prawn. Faecal matters and leftover feed was removed every morning. Moult cycle details of the autotomized animals and intact controls were observed daily and records were maintained. Prawns were kept in the experimental system for observation till they completed atleast two moult cycles.

6.Starvation:

Starvation experiments were conducted to elucidate the effect of feeding on the moulting process. Animals of 60-80 mm (TL) were selected for the experiments. Starvation experiments constituted three sets of experiment viz. 1. starved from postmoult stages of A and B., 2. starved from intermoult and early premoult stages of C and Do., and 3. starved from late premoult stages of D1''' and D2-3. Prawns of required moult stages were selected and each group comprised of 17 prawns. Animals were maintained in the floating plastic cages as described earlier in chapter-I (Fig.3). Seventeen properly fed prawns, maintained along with the experimental group served as controls. Prawns were kept in the experimental condition till each prawn completed at least one moult cycle, i.e. from ecdysis to ecdysis. Moult stage developments of the prawns were observed daily using setogenisis and records of moulting data of the individual prawns were maintained.

Statistical Analysis

Analysis of variance (ANOVA) was performed to test the significance between treatments, i.e. the effect of environmental parameters on the moulting and growth of the

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Statistical Analysis

Analysis of variance (ANOVA) was performed to test the significance between treatments, i.e. the effect of environmental parameters on the moulting and growth of the

prawns. Experimental results of autotomy and starvation were tested using student 't ' test.

3. RESULTS

1. Temperature

Results of the experiments conducted to study the effect of temperature on moulting are given in Table 1, and Figure 4.

Among the tested temperatures, higher rate of moulting and growth observed in the group of prawns which were exposed to 31 °C, and in those exposed to the lower temperature of 26±1 °C, the moulting frequency and growth rate observed were considerably less. When the temperature was increased to 32.5 °C and 35 °C, animals moulted faster, but a corresponding increase in growth was not recorded. Animals exposed to 37 °C did not survive and this temperature was found lethal to the animals.

In the present study, higher levels of temperature were found to accelerate the moult cycle of the prawn. The increase was linear from the low temperature of 26±1 °C to the high temperature of 35 °C. At 26±1 °C the recorded moult cycle duration was the lengthiest, of

Table - 1: EFFECT OF TEMPERATURE ON MOULTING AND GROWTH IN *P. INDICUS* JUVENILES

		Temperature (^{o}C)			
		26 + 1	31	32.5	35
Increase in length (mm)	N	12	12	12	12
	$\bar{X}$	6.9	11.9	8.33	4.4
	$\pm$SD	(1.2)	(0.8)	(0.9)	(0.8)
Increase in wet weight (mg)	$\bar{X}$	162.3	204.7	159.9	56.4
	$\pm$SD	(7.0)	(2.6)	(2.4)	(3.0)
Moult cycle duration (hrs)	$\bar{X}$	138.0	96.0	96.0	84.0
	$\pm$SD	(10.8)	(0.0)	(0.0)	(12.5)

ANALYSIS OF VARIANCE

Parameter	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Length	Treatment	3	354.057	118.019	138.2*
	Error	44	37.563	0.584	
Weight	Treatment	3	142024.663	47341.354	2882.1*
	Error	44	722.750	16.426	
Moult cycle	Treatment	3	20916.000	6732.000	97.95*
	Error	44	3024.000	68.727	

*Significant at 1% level ($P < 0.01$)

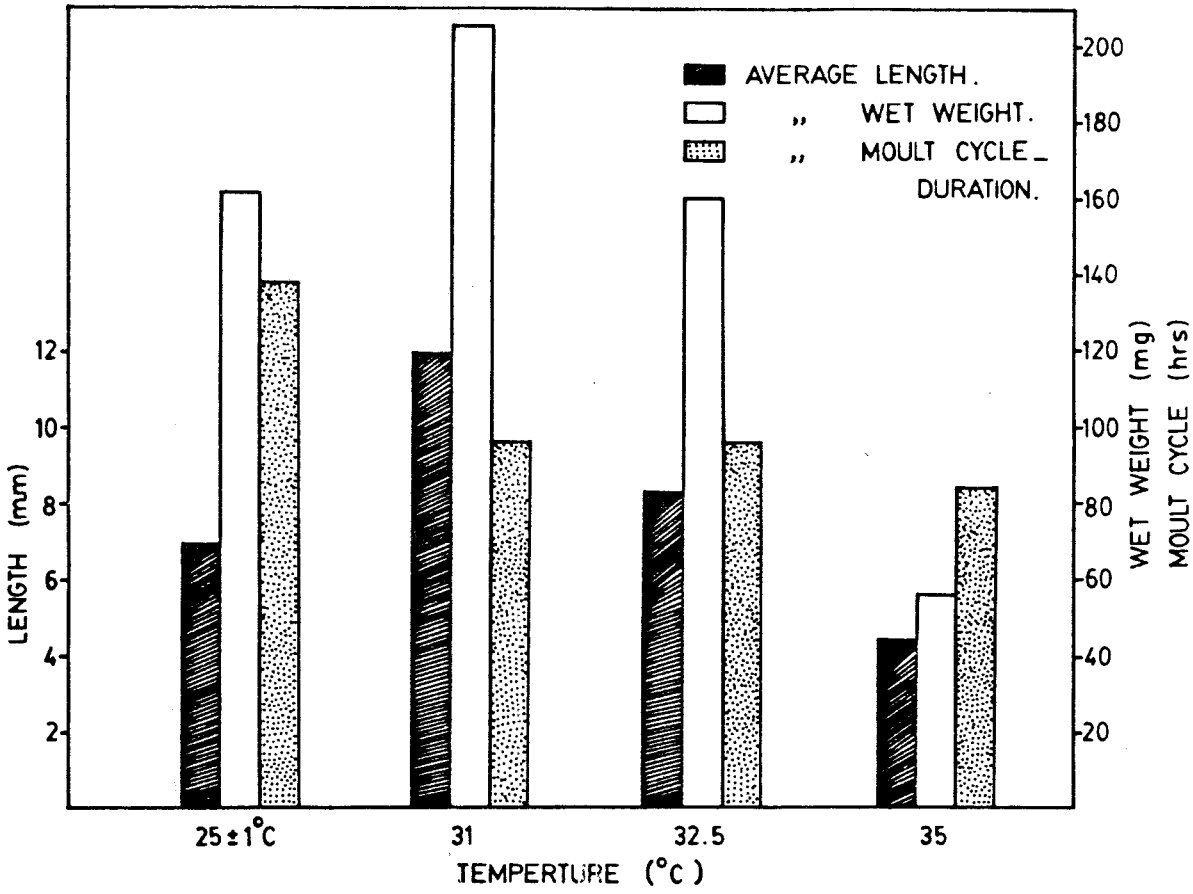

Fig. 4. Mean increase in total length, total wet weight, and average time duration for one moult cycle of P. indicus juveniles exposed to different temperature levels for a period of 20 days.

138±0.8hrs. At 31 °C and 32.5 °C moult occurred faster with a reduced moult cycle duration of 96 hrs. The shortest moult cycle was recorded among the animals exposed to 35 °C. But the precocious moult cycle, coupled with a corresponding growth gain in terms of length and weight was observed only upto a temperature of 31 °C. Beyond 31 °C, i.e. at 32.5 °C and 35 °C, moult cycle was faster but it was not reflected in terms of increase in length or weight. The effect of various temperatures on the moult cycle of the animals observed was found to be statistically significant ($P<0.01$).

A gain in length of 11.9 mm was recorded at 31 °C. At the same time length gain at 26±1 °C was only 6.9 mm and at 32.5 °C it was 8.33 mm. The minimum increment of length, 4.4mm, was recorded at 35 °C. In terms of wet weight, the highest weight increment of 204.7 mg was recorded at 31 °C. It was interesting to note that for the other ranges of temperature both lower and higher, the growth obtained was significantly lower ($P<0.01$) to that at 31 °C. The lowest growth rate in terms of weight was recorded at the higher temperature of 35 °C. When the duration of moulting was considered with increase in length and weight among the juvenile prawns, the most

favourable temperature observed was at 31 °C. The length and growth increments obtained at different temperature levels showed significant variation ($P<0.01$).

2. pH

In the acidic pH of 5±0.2, and higher alkaline pH of 10±0.2 all the prawns died within a 24 hrs. In acidic pH of 6±0.2 majority of the prawns failed to survive through the experimental period of 20 days. In pH 9±0.2, 30% of the animals died in the course of the experiment. Prawns exposed to extreme acidic and alkaline pH were very weak and feeding was also poor. Abnormal swimming pattern and muscle necrosis were common in these animals. 80% of the dead animals were found in the state of a half exuviated condition. The results of the pH experiments are presented in the Table 2, and Figure 5.

At pH 8±0.2 and 7±0.2, all experimental animals moulted successfully and were healthy and active. Average moult cycle duration for pH 8±0.2 was 96 hrs. In the case of higher pH 9±0.2, an extended moult cycle period of 109.3 hrs was recorded. The moult cycle duration observed for the neutral pH 7±0.2 was 91.6 hrs. From these results, it is evident that variations in pH do have a

Table - 2: EFFECT OF pH ON MOULTING AND GROWTH IN *P. INDICUS* JUVENILES

		pH		
		7 ± 0.2	8 ± 0.2	9 ± 0.2
Increase in length (mm)	N	11	17	12
	$\bar{X}$	4.9	10.9	3.1
	±SD	(0.8)	(0.9)	(0.3)
Increase in wet weight (mg)	$\bar{X}$	155.5	194.9	48.7
	±SD	(2.6)	(0.39)	(4.5)
Moult cycle duration (hrs)	$\bar{X}$	91.6	96.0	109.3
	±SD	(9.7)	(0.0)	(12.6)

ANALYSIS OF VARIANCE

Parameter	Source	D.F.	Sum of SQRS	Mean SQRS	F. Value
Length	Treatment	2	438.708	219.354	345.9*
	Error	34	21.563	0.634	
Weight	Treatment	2	126774.427	63387.213	4541.9*
	Error	34	474.492	13.956	
Moult cycle	Treatment	2	1669.346	834.673	12.77*
	Error	34	2222.545	65.369	

*Significant at 1% level ($P < 0.01$)

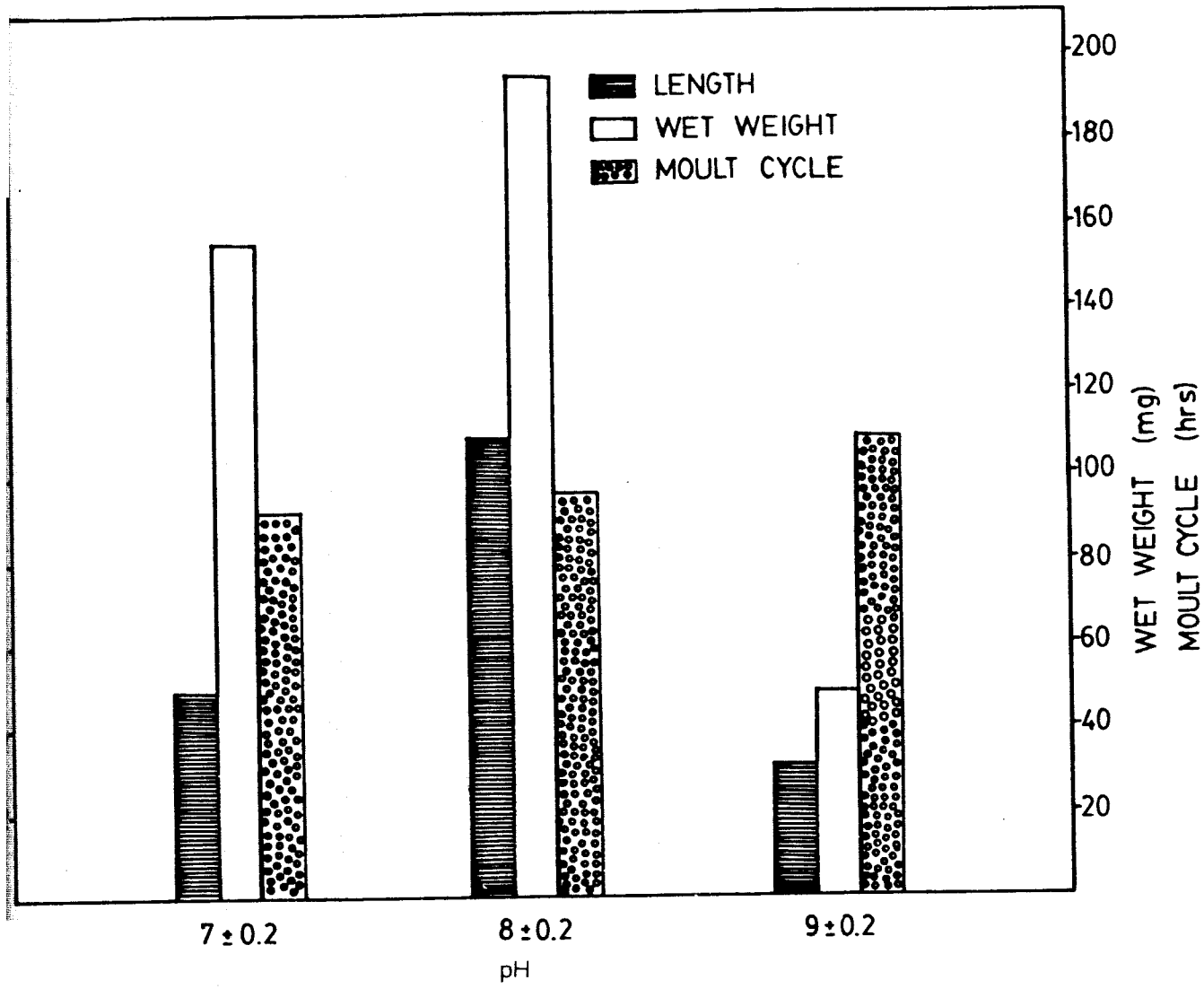

Fig. 5. Mean increase in total length, total wet weight, and average time duration for one moult cycle of P. indicus juveniles exposed to different pH levels for a period of 20 days.

significant effect ($P<0.01$) on the moult period of the prawn.

The maximum length increment of 10.9 mm was recorded in prawns exposed to pH 8±0.2, while the lowest length increment of 3.1 mm was noted among the prawns kept at pH 9±0.2. Though the prawns exposed to pH 7±0.2 performed normal moulting behaviour, the growth attained at this pH in terms of length, 4.9 mm, was considerably less to that at pH 8±0.2.

Wet weight gained by the animal at different pH levels was highly significant ($P<0.01$). pH 8±0.2 gave the highest growth of 194.9 mg, at the same time at pH 9±0.2, weight gained by the prawn was considerably less (48.7mg). At pH 7±0.2 though the animals moulted successfully the average growth increment attained, was lesser (155.5mg) when compared to that at pH 8±0.2.

Comparison of tested pH showed that the higher growth in terms of length and weight, coupled with a fast moult cycle was obtained at pH 8±0.2.

3. Salinity

Moult experiments conducted at different levels of salinity showed the ability of juvenile *P.indicus* to

		Salinity (%)				
		5	15	25	35	45
Increase in length (mm)	N	17	17	17	15	11
	$\bar{X}$	7.5	11.8	9.6	4.6	2.9
	$\pm$SD	(0.8)	(0.6)	(0.8)	(0.7)	(1.0)
Increase in wet weight (mg)	$\bar{X}$	133.4	212.5	181.7	75.7	29.5
	$\pm$SD	(8.4)	(5.8)	(23.7)	(6.0)	(4.8)
Moult cycle duration (hrs)	$\bar{X}$	104.5	96.0	98.1	160.8	130.9
	$\pm$SD	(11.8)	(0.0)	(4.7)	(41.8)	(16.5

ANALYSIS OF VARIANCE

Parameter	Source	D.F.	Sum of SQRS	Mean SQRS	F.Value
Length	Treatment	4	746.981	186.745	301.7*
	Error	72	44.597	0.619	
Weight	Treatment	4	314486.914	78621.729	496.89*
	Error	72	11392.443	158.228	
Moult cycle	Treatment	4	47046.483	11761.621	28.48*
	Error	72	29737.309	413.018	

*Significant at 1% level ($P < 0.01$)

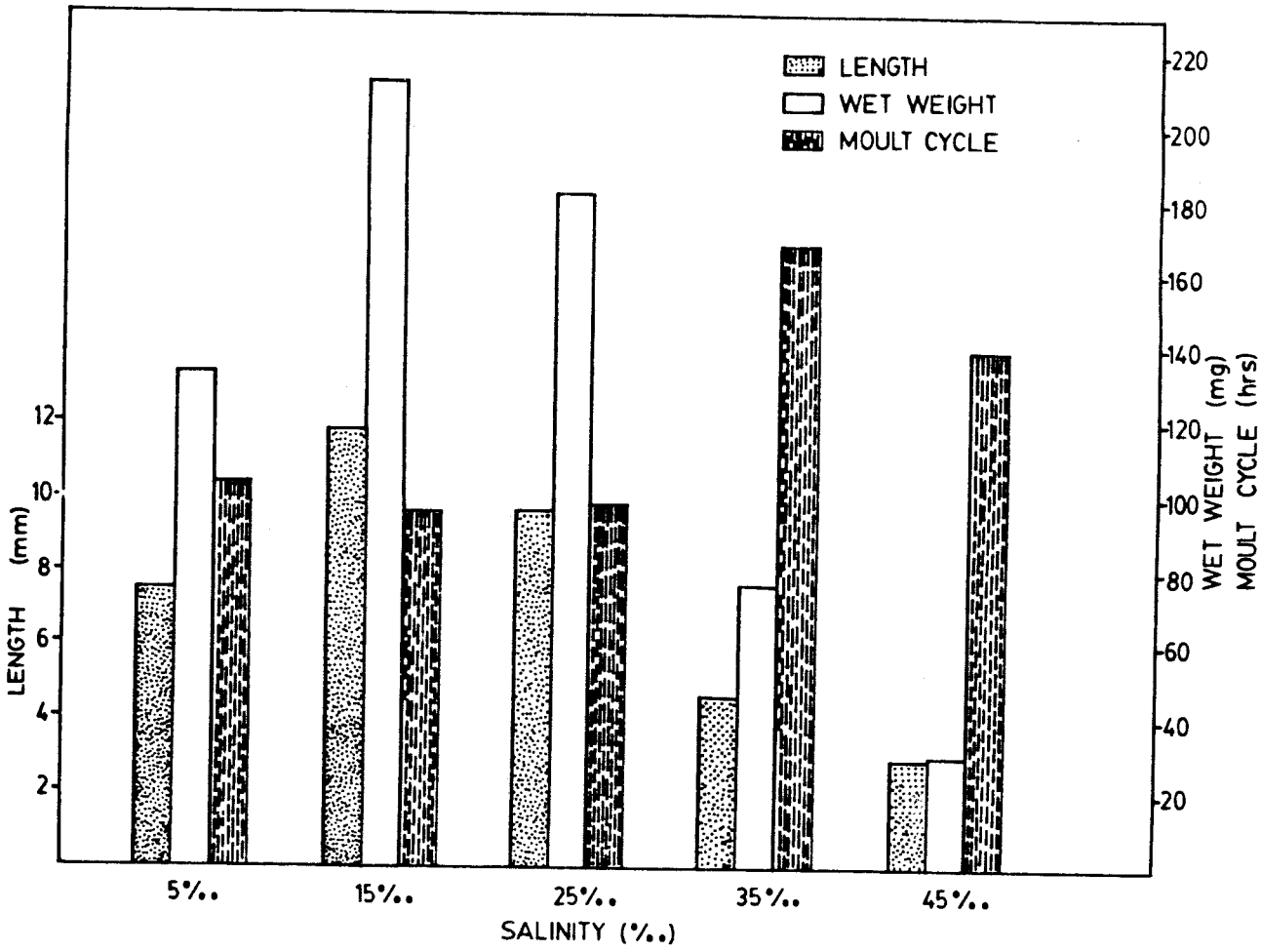

Fig. 6. Mean increase in total length, total wet weight, and average time duration for one moult cycle of P. indicus juveniles exposed to different salinity levels for a period of 20 days.

perform moulting in a wide range of salinity, i.e. 5-45‰. Though the animals performed the moult in such a wide range of salinities, the growth achieved in terms of length and weight varied considerably in different levels of salinities. The experimental data are summarized in the Table 3, and Figure 6.

At salinities of 15‰ and 25‰, prawns performed normal moult with short moult cycle period of 96 hrs, and 98.1 hrs, respectively, while at the lower salinity of 5‰ and higher salinity of 45‰ prawns showed extended moult period of 104±5 hrs, and 130.9±16.5 hrs, respectively. Majority of the prawns (80%) exposed to the lower salinity of 3‰ died in the initial stage of the experiment itself.

The animals exposed to 35‰, and 45‰ salinities developed muscle necrosis. This was very much evident at 45‰. Out of 17 prawns exposed to 45‰, 6 prawns died in the course of the experiment. The dead animals were found in half moulted condition. The most lengthy moult cycle duration was noted in the prawns exposed to 35‰ salinity i.e. 160.8 hrs. Faster moult cycle along with high growth was recorded at 15‰ salinity, and at the same time in extreme lower and higher salinities both moulting and

growth were found less. ANOVA revealed that moult cycle duration varied significantly with different levels of salinity ($P<0.01$).

In terms of length increment, the highest growth of 11.8 mm was recorded among the prawns which were exposed to the salinity of 15‰. At the highest salinity (45‰), the length gain recorded was the lowest (2.9mm). Among the other tested salinities of 5‰ and 35‰ also the length increment attained was considerably less i.e. 7.5 mm and 4.6 mm, respectively when compared to that at 15‰ (11.8 mm). ANOVA showed the growth in length attained by the animal at different levels of salinity was statistically significant ($P<0.01$).

Growth increment in terms of wet weight also showed similar trend to that of length increment, in different experimental salinities. In the present study, the highest weight gain of 212.5 mg was recorded at 15‰, while the lowest was noted at 45‰ (29.5 mg). ANOVA indicated significant ($P<0.01$) variations in weight gain between the different tested salinities.

4. Light

Experiment with different light period has not

TABLE 4.1 EFFECT OF LIGHT ON MOULTING AND GROWTH IN *P. INDICUS* JUVENILES

		Light (hrs)		
		24	12	0
Increase in length (mm)	N	12	12	12
	$\bar{X}$	9.0	9.2	10.1
	$\pm$SD	(0.9)	(1.0)	(1.0)
Increase in wet weight (mg)	$\bar{X}$	180.7	181.3	183.3
	$\pm$SD	(3.5)	(3.2)	(3.4)
Moult cycle duration (hrs)	$\bar{X}$	102.0	100.0	96.0
	$\pm$SD	(10.9)	(9.34)	

ANALYSIS OF VARIANCE

Parameter	Source	D.F.	Sum of SQRS	Mean SQRS	F. V
Length	Treatment	2	8.167	4.083	4.4
	Error	33	30.583	0.927	
Weight	Treatment	2	44.125	22.063	1.9
	Error	33	371.125	11.246	
Moult cycle	Treatment	2	224.000	112.000	1.6
	Error	33	2256.000	68.364	

*Values of 'F' are not significant at 1% level ($P > 0.01$)

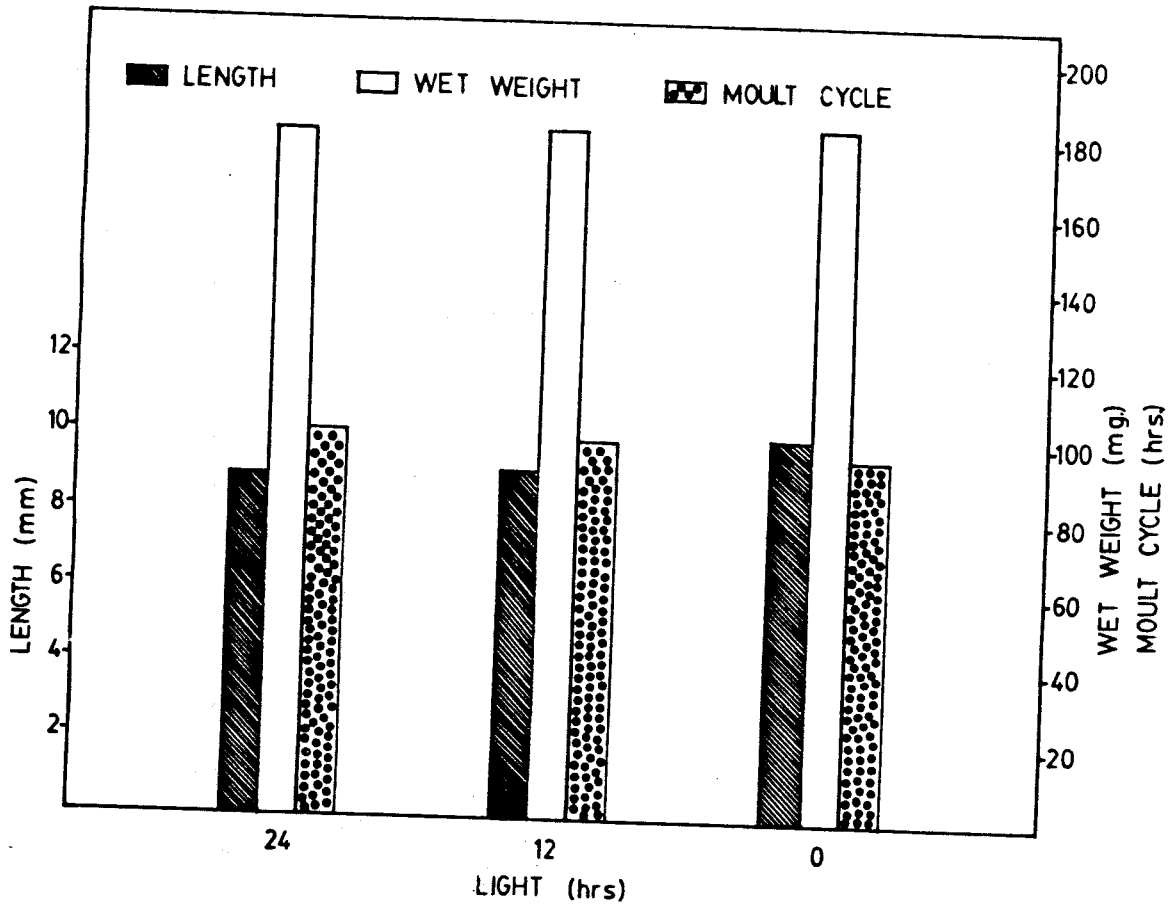

Fig. 7. Mean increase in total length, total weight, and average time duration for one moult cycle of P. indicus juveniles exposed to different light periods for a period of 20 days.

revealed any significant effect over the moult cycle of the prawn. The data obtained are summarized in Table 4, and Figure 7.

It was observed that prawns kept in complete darkness showed a slight accelerated moult period (96 hours), which when compared to the other light regimes, 12 hr light / 12 hr dark (96hrs), and 24 hr light and 24 hr dark (102 hours), was not statistically significant ($P>0.01$). Growth increment obtained under different light regimes also didn't reveal any significant variation.

5.Autotomy

In all the five sets of experiment, moult cycle of the animal was not affected by the autotomy of walking legs. Moult cycle duration observed among the autotomized prawns has not showed any significant variation from that of intact controls ($P>0.01$). A very meagre modification of premoult period was observed in the case of 8 and 10 leg removed groups. But it was not significant compared to the control groups (Table 5). The premoult period observed for the control group with intact walking legs was 120 ± 19.6 hrs, while among the 8 and 10 legs removed prawns the average premoult period recorded was 126.9hrs

Table 5: EFFECT OF LEG AUTOTOMY ON THE MOULT CYCLE OF *P. INDICUS*

Group	No.	Operation	Premoult Period (hrs)	't' test
I	17	2 legs removed	120 ± 19.6	
II	17	4 legs removed	123.4 ± 16.6	
III	17	6 legs removed	120 ± 13.9	P >0.05
IV	17	8 legs removed	116 ± 16.6	
V	17	10 legs removed	126.9 ± 18.1	
VI	17	Intact Controls	120 ± 19.6	

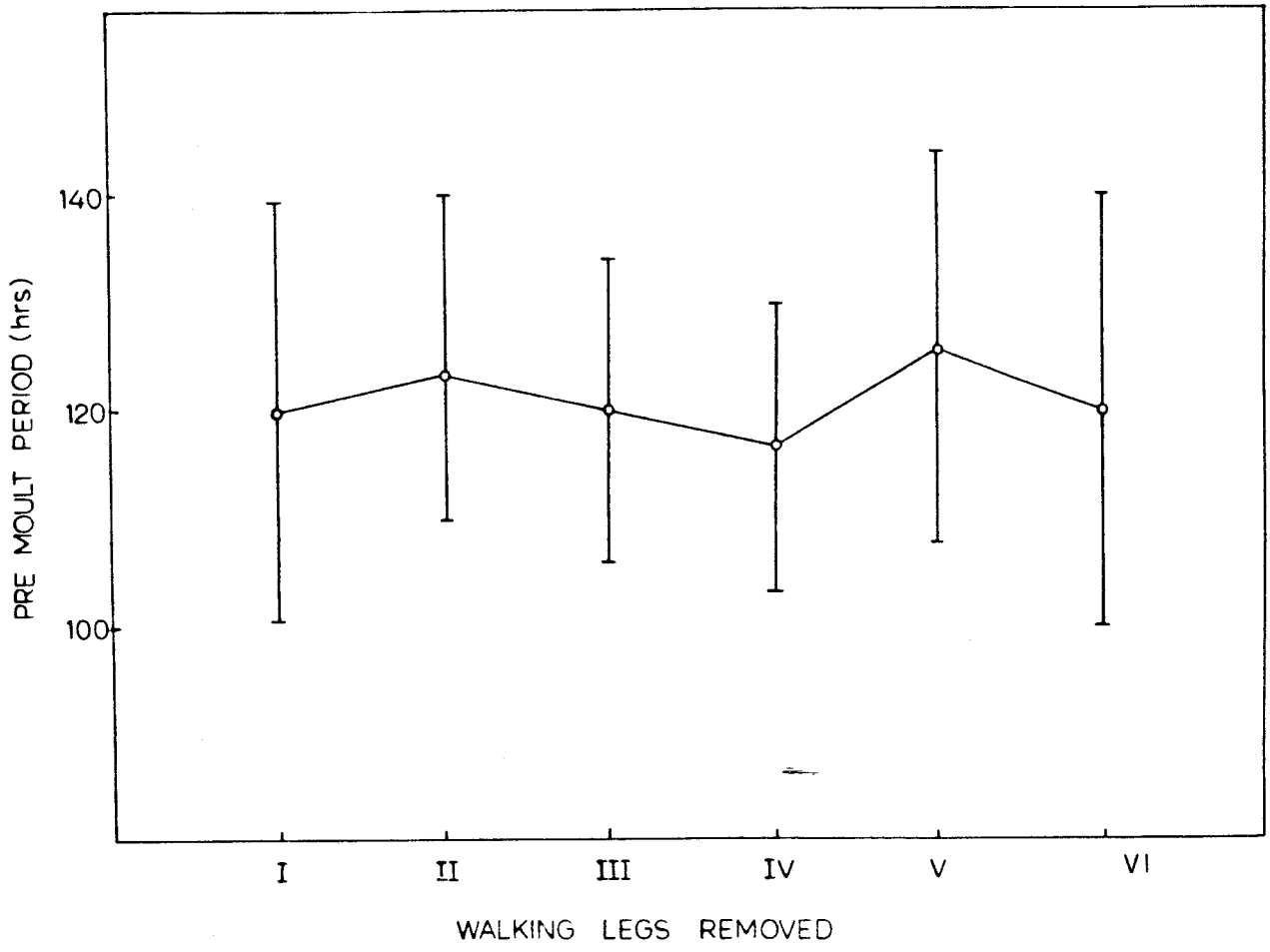

Fig. 8. Effect of limb removal in P. indicus, expressed in terms of the premoult period.

I. Prawns from which 2 walking legs are removed.

II. Prawns from which 4 walking legs are removed.

III. Prawns from which 6 walking legs are removed.

IV. Prawns from which 8 walking legs are removed.

V. Prawns from which 10 walking legs are removed.

VI. Intact controls.

and 116.6 hrs (Fig.8).

6. Starvation

Results of the starvation experiments are presented in Table 6, and Figure 9.

Starved from postmoult stages (A and B):

The prawns starved from the postmoult stages of A and B, reached the intermoult stage C. Thereafter the progression from stage C to Do was very slow. Animals were very weak and inactive. Prawns stayed in the early premoult stage (Do) for many days without further progress towards moult with an average premoult period of 219.4±16.6 hrs. All the prawns starved from the postmoult stages advanced up to the premoult stage (Do), and after that they died without performing ecdysis. At the same time all the control animals which were fed properly, moulted successfully with a premoult period of 130.3±12.8 hrs.

Starved from intermoult (C) and early premoult (Do):

All the animals of these groups progressed towards the moult and entered the premoult stage of D1'. But after this stage the moult development was retarded and the prawns showed a highly extended premoult period of

Table 6 : EFFECT OF STARVATION ON THE MOULT CYCLE OF *P. INDICUS*

Group	No.	Treatment	Intermoult (hrs)	Premoult (hrs)	't' test
I	17	Starved from Postmoult Stages A & B	54.9 ± 11.7	219.4 ± 16.6	
II	17	Starved from intermoult & early premoult (Stage C and Do)	34.3 ± 12.8	233.1 ± 11.7	
III	17	Starved from late Premoult (Stages D1''' & D2-3)	34.3 ± 12.8	219.4 ± 16.6	$P < 0.05$
IV	17	Property fed controls	30.9 ± 11.7	130.3 ± 12.8	

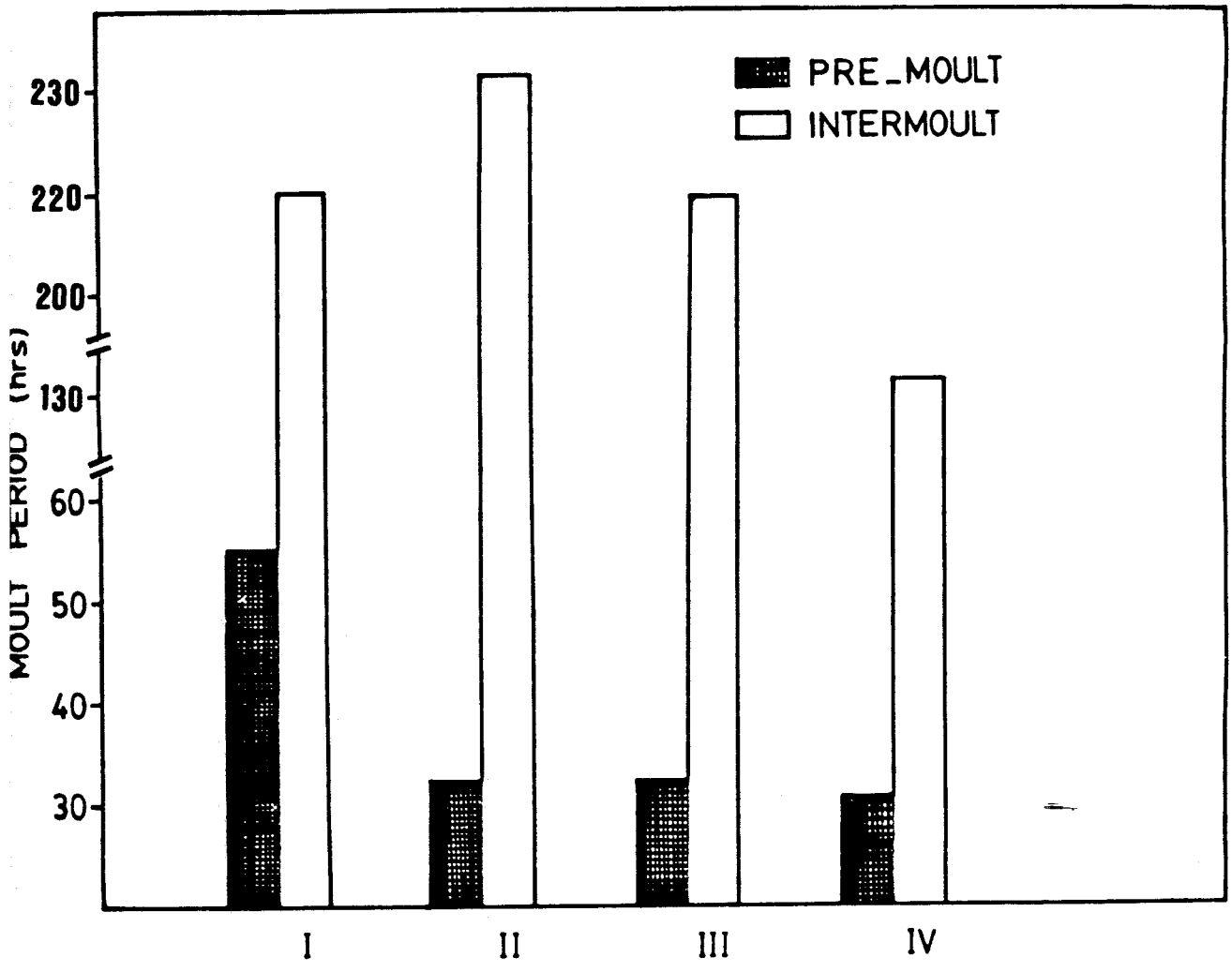

Fig. 9. Effect of starvation in P. indicus expressed in terms of the premoult period.

I. Prawns starved from moult stages A and B.

II. Prawns starved from moult stages C and Do

III. Prawns starved from moult stages D1''' and D2-3.

IV. Properly fed controls.

233.1±11.7hrs. Out of seventeen prawns starved, 3 died at the time of moulting. At the same time in the control groups all the prawns moulted normally with a premoult period of 130.8±12.8 hrs.

Starved from late premoult (D1''', and D2-3):

The prawns which were forced to starve from the late premoult stage completed their first moult cycle successfully. However, in the subsequent moult cycle the animals found difficulty to proceed towards moult. Once the animals reached the early premoult period, no further moult development was noticed. All the prawns remained in the premoult condition for a long period of 219.4±16.6 hrs. Finally, they died either at the time of ecdysis or soon after moulting, whereas all the properly fed control prawns completed their moult successfully with a significantly short premoult period of 130.8±12.8 hrs.

4.DISCUSSION.

Temperature, pH, salinity, light, autotomy, and starvation are the important exogenous factors known to influence the moulting and growth process in crustceans (Passano, 1960; Aiken, 1980 and Conan, 1985). Distinct works dealing with the role of exogenous factors over the

physiological process of moulting, especially on tropical prawns are limited. Therefore, the mode of interaction of these factors over the moulting and growth of the animal P.indicus was taken for the present investigation. From the present work it is observed that the dynamic event of moulting and its subsequent effect on growth has got a direct relationship with environmental factors such as temperature, pH, salinity, and food availability.

The temperature exhibited a direct effect on the moult cycle and growth of the animal. Moulting frequency of P.indicus was found increasing with a corresponding increase in temperature. Similarly in P.aztecus, Zein-Eldin and Aldrich (1965) reported a linear relation between the temperature and growth. The faster growth attained by P.aztecus at 32 °C with a rapid moult cycle was considerably higher when compared to the growth at 25 °C. Ringo (1965) noted an apparent spurt of growth in brown shrimps when the water temperature exceeded 20 °C, and the growth was linear upto 31 °C. Similarly Fowler et al. (1971) noted an accelerated moulting cycle with the increasing temperature in euphausids. Workers like Rothlisberg (1979), and Schluter (1979) also found moulting as a dependent factor of temperature in Pandalus jordani and Argulus foliaceus, respectively. Though a

positive relationship was found between the moulting frequency and temperature in P.indicus the relationship was not linear in terms of the growth of the animal. In the present study, co-ordinated increase of moulting frequency and tissue growth was noted only upto the level of 31 °C. Beyond this temperature, though an increase in further reduction of moult period was observed, correspondingly no tissue growth was recorded in the animal.

Conan (1985) has discussed about a specific range of temperature for each species in which relationship of temperature and growth was linear. According to him (Conan, 1985), within a specific temperature range, intervals between the ecdysis are usually shortened with a corresponding increase in tissue growth until a threshold level of temperature is reached. Beyond this threshold temperature, moulting of the animal becomes rather erratic. This is very much true in the case of P.indicus. The temperature threshold for the animal was only upto 31 °C. Above this temperature, growth was not achieved by the animal irrespective of a more shortened moult cycle. At 35 °C, prawns had faster moult cycle with an average duration of 84hrs, than that at 31 °C

(i.e. average 96hrs). In the present observation the tissue growth attained at 35 °C was significantly less compared to that at 31 °C, where the prawns attained maximum tissue growth. Observation of Richard (1978) indicated reduced growth of P.serratus in extreme low temperature as well as in extreme high temperatures. Zein-Eldin and Griffth (1966), in P.aztecues, also noted a linear relationship between growth and temperature in 15-32 °C range, growth rate, however, decreased markedely at 35 °C.

When compared to the lower temperature, higher temperature levels were found to be more harmful to the animals. In P.indicus, higher temperature of 35 °C was found harmful especially at the time of moult, which even lead to the death of the animal. Work done by McLeese (1956) in American lobsters, and Chittleborough (1975) in Panulirus longipes also showed an adverse effect of higher temperatures on moulting and growth of the respective species.

The effect of temperature on growth can be due to the direct effect on the body metabolism (Perkins, 1972 ; Brandford, 1978), or indirect by stimulating the animals to secrete more growth hormones (Aiken and Waddy, 1975).

In both the cases, there will be a physiological limit to which the animal can successfully respond with the dynamic event of moulting to attain tissue growth. The lower growth rate and moulting frequency noticed in P.indcus at the lower temperature of 26±1 °C may be due to the slow rate of metabolism. Animals were comparatively less active and their feeding performance was also poor. Aiken (1980) observed indefinite blocking of moult at lower temperatures in American lobsters. Templeman (1936, 1940) in Homarus americanus, Dall (1965c) in Metapenaeus sp, and Winget et al. (1973) in crabs also noted the slow rate of moulting at lower temperature. In P.indicus the optimum temperature range for moulting and growth was 29 to 31 °C, with a thermal threshold temperature of 31 °C.

Informations pertaining to pH studies on moulting and growth of crustaceans are very limited. In the present observation the pH was found to have a direct control over the moulting process of P.indicus. In order to perform successful moult cycle the prawn preferred an optimum range of pH. In the present study both lower (acidic) and higher (alkaline) pH were found as limiting factors for moulting and growth. Havas and Hutchinson (1982), and Leivestad et al. (1976) observed that the low pH was harmful to the life of crustaceans. From the

literature it is apparent that aquatic organism are unable to tolerate conditions of unfavourable pH in the acidic or the alkaline range. Probably, the physiological functions of the animal can be affected, resulting in the depressed growth. Pillai et al. (1983) have reported mass mortalily of fishes and crustaceans in Vembanad lake due to the effect of lower pH. Abdul Aziz and Balakrishnan Nair (1978) have reported the absence of crustacean fauna from the Ashtamudi lake due to the lower pH resulted by the coconut husk retting in these areas. Wickins (1984) studied the effect of reduced pH on the mineralization process in P.mondon, and found that the sub optimal pH in the acidic range adversly affect the mineralization of the prawn. Malley (1980) also reported decreased survival and calcium uptake in the cray fish Orconects virils in lower pH levels. Since one third of the prawn exoskeleton is calcium carbonate, under normal conditions a major share of calcium is taken up from the sea water together with bicarbonate, possibly in exchange for H^+ ions to maintain electrical neutrality (Dall, 1965a and Greenaway, 1974). Therefore, a successful mineralization of the exoskeleton will depend on the optimum ionic content of the surrounding waters, and in the ability to take up ions (Wickins, 1984). In the present study it was clearly

observed that an optimum pH of 7.8 to 8.2 is necessary for successful moult and growth. Outside the optimum range, both higher and lower pH adversely affected the moulting physiology of the prawns.

Brackishwater and intertidal environments are probably the most demanding and stressful aquatic biotope, where the prawns like P.indicus complete the growth phase of their life. Due to the cyclic changing pattern of the salinity in these areas, the ecological incidence of osmoregulation is essentially important in the life cycle of the prawns belonging to the area (Gilles and Pequeux, 1983). Reports are there on the effects of different salinity levels on growth and survival of penaeid larvae and juveniles (Zein-Eldin, 1963; Zein-Eldin and Griffth, 1966, Grajecer and Neal, 1972; Venketaramiah et al., 1972; Nair and Kutty, 1975, and Raj and Raj, 1980). But among crustaceans especially in prawns, available literature is meagre to substantiate the effect of salinity on moulting physiology and growth. In the present study, moulting and growth in juveniles of P.indicus were found to be affected by the varying levels of salinity. Prawns exposed to lower (5‰) and higher (45‰) salinities showed signs of stress and muscle necrosis. Lakshmi et

al. (1978), in P.aztecus, observed muscle necrosis in the prawns exposed to sub optimal and supra optimal salinity ranges and has described the development of muscle necrosis as an indication of stress on the animals. In P.indicus moult occurred at a faster rate at 15‰ with an accelerated growth when compared to all other tested salinities. Hence, 15‰. was considered as the optimal salinity. It was difficult to elucidate any specific relationship between the moulting cycle and salinity. But in other salinities than the optimal, the moult cycle observed was erratic with considerably reduced growth rate. The observations made here point towards the fact that, though the prawns moult in wide range of salinities, the growth increment attained was comparatively less. Venkataramaiha et al. (1972) in his studies on P.aztecus stated that although young shrimps can survive in a wide range of salinities, the best growth can be attained only in optimum salinities. It is mentioned that, most of the euryhaline invertebrates though survive in a wide range of salinities, the actual growth takes place in a restricted narrow range only (Kinne, 1970). This is true in the case of P.indicus, where the animal survived in a wide range of salinities i.e. 5‰ to 45‰., with the highest growth in the salinity of 15‰.

There is no concrete literature relating the moulting cycle and the moult controlling function of the light. In the present investigation using three light phases, i.e. 24hr light, 12hrlight/12hr dark, and 24 hr dark (complete darkness), no significant effect was noticed either on moulting period or on growth. Skinner and Graham (1972) noticed precocious moulting in animals kept in darkness when compared with those exposed to light. But they (Skinner and Graham, 1972) accounted the effect as due to the privacy rather than to the amount of exposure to darkness. In the present case also the small accelerated moult period noticed among the prawns under complete darkness was not significant to draw any conclusion. The observed effect of photoperiod on crustaceans was generally inconclusive and contradictory in nature. Studies of Barnes and Stone (1974) have not revealed any effect of light on the process of moulting. Cobb (1970) in H.americanus, and Forster (1973) in Palaemon serratus, reported enhanced growth rate in continuous darkness, while in Panulirus longipes (Chittleborough, 1975) inhibition of growth was recorded in the darkness. In the present study no correlation was obtained between the light period and moulting frequency of P.indicus. Similarly, many other workers like Winget et al. (1973),

Kracht (1974), Bishop and Herrnkind (1976), and Benayoun and Fowler (1980) have not recorded any relationship between photoperiod and the moulting of the animals.

Precocious moulting due to autotomy of pereopods or cheleped has been reported in many species of crabs (Skinner and Graham, 1970,1972; Bennet, 1973; Fingerman and Fingerman, 1974, and Hopkins, 1982, and 1983), lobsters (Chittleborough, 1975), and in crayfish (Bittner and Kopanda, 1973, and Nakatani and Otsu (1979), but there are only very few reports of autotomy among the prawns. The present experiments of autotomy in P.indicus have not provided any positive results in relation to the moult cycle period. Autotomized prawns exhibited same moulting duration as that of intact controls.

Stoffel and Hubschman (1974) in Palaeomonetes kadiakensis noticed precocious moulting as a result of multiple autotomy. In the case of P.indicus even the complete removal of the walking legs has not showed precocious moulting. Webster (1985) has not observed any moult acceleration after the autotomy of walking legs in Palaemon elegans. According to the hypothesis of Skinner and Graham (1972) no moult inhibiting factor is present in the limbs of crustacea, and the precocious moult is

stimulated by severing of a critical number of nerves. Therefore, the stimuli can be nervous and related to the number of nerves cut during the autotomy. However, in the case of P.indicus, in the present study, it can be presumed that the nervous stimuli produced as a result of leg autotomy was not sufficient to cause any precocious moulting in the animals.

Adelung (1971) suggested that the rate of tissue growth is determined by the frequency of moult. In order to achieve tissue growth, timely availability of suitable food as the source of energy is unavoidable. Hence, feeding influences crustacean growth, primarily controlling the moulting frequency. Starvation experiments conducted in the present study clearly indicated the importance and prime necessity of proper feeding for the completion of successful moult cycle. Under starved conditions prawns failed to meet the metabolic demand required for the occurrence of moulting and growth leading to the death of the animal. Cuzon et al.(1980), and Chittleborough (1975) observed a depressed growth increment and delayed moulting in starved Panulirus longipes. Experiments conducted by Adelung (1971), and Dawiris (1984) in crab, and Rao et al. (1972)

in crayfish have also showed the inhibition of growth under conditions of starvation. In the case of P.indicus also proper food supply was imperative for proper moulting and growth, otherwise the moult cycle was extended considerably resulting in depressed growth. Continuous starvation blocked the moulting cycle finally leading to the death of the prawn.

Among the abiotic factors studied, temperature was found to have the prime influence over the moulting cycle of the prawn. Other factors like salinity and pH also exerted significant control over the moulting physiology of P.indicus, while light has not exhibited any notable control over moulting frequency of the Prawn. Like any other crustacean, the biotic factor, starvation, resulted in the direct modification of moulting causing stunted growth, and even death. At the same time, autotomy has not made any alteration in the moult cycle of the prawn.

S U M M A R Y

Effect of important environmental factors like temperature, pH, salinity, light, and other biological factors viz. autotomy and starvation on moult cycle of the prawn P.indicus were studied. Among the exogenous factors, temperature was found to have the major influence on

moulting process. Salinity and pH also influenced the physiology of moulting, but light did not show any notable effect on the moult cycle.

Both lower and higher temperatures were found not to favour the occurrence of normal moulting. Prawns kept in high temperature of 37 °C died in a few hours. A fast growth with short moult cycle duration was observed among the prawns exposed to 31 °C (optimum temperature recorded in the present study), low temperature (26±1 °C) gave poor growth with considerably extended period of moult cycle.

In the extreme pH of 5±0.2 (acidic) and 10±0.2 (alkaline) all the experimental prawns died within 24hrs. Prawns exposed to a pH of 6±0.2, and 9±0.2 became very weak, and 50% of the animals died during their effort in exuviation. In the present experiment, the optimum pH which favoured normal moulting and good growth was found to be at 8±0.2.

Prawns were allowed to moult in a wide range of salinities i.e. from 5‰ to 45‰ . A salinity of 15‰ was found to be ideal for the animals, with shortest moult cycle duration and fast growth. In salinities of 5‰ and

35‰, the moult cycle duration was extended and growth poor when compared to 15‰. Most of the animals kept in 3‰ and 45‰ salinity failed in their moultimg efforts.

Experiment with different light periods and autotomy have not revealed any significant effect over the moult process of the species. Starvation experiments conducted in the present work indicated the importance and prime necessity of proper feeding for the successful moulting process. Prawns made to starve showed considerably extended moult cycle. Continuous starvation blocked the progress of moult, leading to the death of the organisms.

SUMMARY AND CONCLUSIONS

S U M M A R Y A N D C O N C L U S I O N S

The physiology of moulting in the Indian white prawn Penaeus indicus has been investigated by comprehensive approach to the problem. The major aspects of the study include, Detailed classification of the moult cyc'e, Neuroendocrine control of moulting process, Variations in the biochemical constituents in relation to moult cycle, Distribution and mobilization of important minerals during moulting cycle, and Role of important environmental factors over moult cycle of the prawn.

The prawns for the present investigation were collected from the traditional prawn farms of Vypeen Island and Marine Prawn Hatchery Laboratory, Narakkal, Cochin.

The salient findings from the present study are given below.

1. Characterization and classification of complete moult cycle of Penaeus indicus have been worked out on the bases of setal development and epidermal retraction in the uropods (setogenisis). Based on the setal morphology, the moult stages were identified as Postmoult (stages A and B), Intermoult (stage C), and Premoult (stages

Do,D1',D1'',D1''' and D2-3).

2. The setogenic moult staging was verified on the basis of structural changes in integumentary tissue, during the moult cycle. On the basis of cuticle histology moult stages A and B (postmoult), C (intermoult), and D1''' and D2-3 (late premoult) were identified. Moult staging used in the present study was found to be a rapid and simple technique for determining the different stages of the moult cycle. Since excision of appendage is not required, this technique is non-destructive and permits repetitive moult staging of an individual.

3. The average time duration of one moult cycle with relative duration of each stage was determined in the adult P.indicus. Premoult occupied the major part of the moult cycle (71%), followed by intermoult (18.35%), and postmoult (10.45%).

4. A linear relationship was observed between the size or age of the prawn and the moult cycle duration. The duration of the moult cycle was found to be more with increase in size or age of the prawn.

5. Observation on the moulting behaviour showed that majority of the prawns moulted during the late hours of

the night, especially between 00 and 04 hrs. Prawns were found very active prior to moult and the actual process of ecdysis was very short which lasted only for 30-50 seconds.

6. Structure of neuroendocrine centres such as X-organ sinus gland complex of eye, brain, and thoracic ganglia, and their structural changes during the moult cycle were studied using histology and histochemistry. Neurosecretory cells of different sizes and shapes were observed in the above centres. The neurosecretory cells have been classified into five different types on the bases of size, shape, and staining characterestics as 1. Giant neuron(G-type cell) with a diameter of 75$\pm$ 12/u, 2. Large oval cell (A- type cell) with a diameter of 40$\pm$ 17/u, 3. Small oval cell (B-type cell) with a diameter of 23$\pm$ 4/u, 4. Club shaped cell (C-type cell) with a diameter of 15$\pm$4/u, and 5. Small round cell (D-type cell) with a diameter of 9$\pm$4/u. G type cell is the largest among the five cell types while the D type cell is the smallest. Type A and B cells are the most common and widely distributed, where as the C type cell showed limited distribution.

7. Histochemical tests showed that the neurosecretory material is predominantly a protein with small amount of

carbohydrates and lipids. Strong positive nature of the neurosecretory cells except D type cell, to PAF, CHP, and MTP revealed the neurosecretory nature of these cells. Electronmicroscopical study revealed that the neurosecretory material is made up of hallowed-dense-core vesicle of spherical shape with 120 to 130 nm in diameter.

8. Except D cell, all the neurosecretory cells exhibited a secretory cycle, with an active neurosecretory phase and passive neurosecretory phase. Secretion and release of neurosecretory materials were seen in the active phase, while the passive phase showed the non-neurosecretory phase or inactive phase. In the eyestalk, percentage of active neurosecretory cells was high during the postmoult and intermoult when compared to the premoult, whereas in brain and thoracic ganglia, high percentage of active neurosecretory cells was noted in the premoult and low in postmoult and intermoult.

9. Eyestalk neurosecretory system in P.indicus was found to be composed of X-organs and sinus gland. The central axis of the eyestalk was found to consist of an optic ganglia, which was further divided into three different medullae viz.medulla terminalis, medulla interna, and medulla externa. Two medulla terminalis ganglionic X-

organs (MTGXO I and MTGXO II) were detected in the medulla terminalis, while a single medulla externa X-organ (MEGXO) was detected in the medulla externa. The neurohaemal organ (the Sinus gland) was located between the medulla interna and medulla terminalis in the latero-longitudinal axis of the eyestalk.

10. Experimental evidence has been obtained in the role of neurosecretory system of eyestalk on moulting in P. indicus through extirpation of the eyestalk. Bilateral eyestalk ablation elicited precocious moulting with a significantly short moult cycle duration. But bilateraly ablated prawns which received an eyestalk extract injection (equivalent of two eyestalks) did not show any accelarated moulting, indicating the presence of moult inhibiting factor in the eyestalk. Unilateral eyestalk ablation has not showed any significant effect on the moult cycle.

11. The location and the structure of the Y-organ in Penaeus indicus have been reported for the first time through the present study. The organ is situated between the mandibular and posterior dorsoventral muscle, in close association with the hypodermis at the junction of prebranchial and branchial chambers. The gland cells have rare cytoplasm with nuclei of oval to spherical shape.

Changes were noted in the size of the Y-organ cells during differnt stages of the moult cycle. Y-organectomy resulted in the failure of moulting processes indicating the necessity of Y-organ for the succesful completion of moult.

12. The mandibular organ was found and located using histological techniques in P.indicus for the first time. The organ was detected near the posterior central base of the adductor muscle of each mandible.

13. Changes in the biochemical constituents associated with different moult stages were carried out. General trend of metabolic variation observed was their minimum levels in the postmoult stages and an increase to the maximum concentrations in the premoult stages. Protein, RNA, Lipid, Glycogen, and Glucose in different tissues such as haemolymph, muscle, and hepatopancreas followed this trend. DNA values in muscle and hepatopancreas, Glucosamine in heamolymph, Chitin in cuticle and Water content in muscle and hepatopancreas showed higher values in the postmoult and lower values in the premoult. The premoult increase of metabolities in body tissues can be due to the absorbtion from the food, synthesis, and active resorption of the organic material from the chitino-

protein complex of the exoskeleton, while postmoult fall of metabolites can be attributed to the utilisation of these in the energy cycle, chitin synthesis during the process of ecdysis, and neocuticular synthesis.

14. Distribution and mobilisation of calcium, magnesium, and phosphorus in relation to different stages of moult cycle were studied in the prawn P.indicus. Calcium is detected as the principal inorganic component of the exoskeleton (15.95%), while magnesium (1.19%) and phosphorus (1.2%) were relatively minor component of the exoskeleton. Different regions of the exoskeleton showed variation in calcium, magnesium, and phosphorus concentrations. Variation of calcium, magnesium, and phosphorus content in haemolymph, muscle, hepatopancreas, and exoskeleton showed substantial difference betwen the moult stages of the animal.

15. All the three minerals in haemolymph gave the maximum values in late premoult stages, followed by a decline through the postmoult to reach the minimum values in intermoult and early premoult stages. In muscle, a gradual and linear increase of mineral (calcium, magnesium, and phosphorus) accumulation was noted from the postmoult to the premoult stage. Hepatopancreatic calcium and magnesium

remained high in late premoult and early postmoult stages, while phosphorus gave lower values in late premoult stages. Minimum values for calcium and magnesium in hepatopancreas were recorded in stage C and Do, where as minimum phosphorus values were obtained in stage A.

16. In the exoskeleton, the lowest concentrations of calcium and magnesium were recorded in stage A, which showed a rapid increase from stage B to stage C to touch the maximum values in intermoult and early premoult stages. A very sharp fall in the calcium concentration was noted between the stages D2-3 and A. In the case of exoskeletal phosphorus, values remained high in late premoult and early postmoult stages, and minimum in the intermoult stage C.

17. Effect of important environmental factors like temperature, pH, salinity, light, and other biological factors viz. autotomy and starvation on moult cycle of the prawn P.indicus were studied. Among the exogenous factors, temperature was found to have the major influence on moulting process. Salinity and pH also influenced the physiology of moulting, but light did not show any notable effect on the moult cycle.

18. Both lower and higher temperatures were found not to favour the occurrence of normal moulting. Prawns kept in high temperature of 37 °C died in a few hours. A fast growth with short moult cycle duration was observed among the prawns exposed to 31 °C (optimum temperature recorded in the present study), while low temperature (26±1 °C) gave poor growth with considerably extended period of moult cycle.

19. In the extreme pH of 5±0.2 (acidic) and 10±0.2 (alkaline) all the experimental prawns died within 24hrs. Prawns exposed to a pH of 6±0.2, and 9±0.2 became very weak, and 50% of the animals died during their effort in exuviation. In the present experiment, the optimum pH which favoured normal moulting and good growth was found to be at 8±0.2.

20. Prawns were allowed to moult in a wide range of salinities i.e. from 5‰ to 45‰. A salinity of 15‰ was found to be ideal for the animals, with shortest moult cycle duration and fast growth. In salinities of 5‰ and 35‰ the moult cycle duration was extended and growth poor when compared to 15‰. Most of the animals kept in 3‰ and 45‰ salinity failed in their moultimg efforts.

21. Experiment with different light periods has not revealed any significant effect over the moult process of the species.

22. Starvation experiments conducted in the present work indicated the importance and prime necessity of proper feeding for the successful moulting process. Prawns made to starve showed considerably extended moult cycle. starving condition blocked the progress of moult, finally leading to the death of the organisms. In the present study autotomy has not revealed any significant effect on the moult cycle of the prawn.

It is well known that moulting in crustaceans is the indirect version of growth process. Hence to achieve faster growth rate and higher production of animals in aquaculture techniques, a good knowledge of different aspects of moult process and its control is very much necessary. In this context the new informations generated in the present investigation on moulting physiology of Penaeus indicus would certainly help the scientific prawn farmers in enhancing prawn production by better management.

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