INTRODUCTION

One of the great challenges in crustacean physiology is the phenomenon of molting, a process which dominates their life. This dynamic metabolic event continues throughout the life of the animal, linking almost all biological activities with it.
Crustacean body is ensheathed by a thick outer 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 by the cyclic accumulation of organic and inorganic reserves through the digestive tract. The complete process of molting i.e. the protective as well as growth function, includes the recovery from preceding molt, tissue growth and preparation for the next molt as well as exuviation.

The epidermal withdrawal is a high energy demanding process which is best known in crustaceans after metamorphosis from the larval stages. In many forms, periodic molting alternates with the reproductive stages after puberty. In some forms, molting stops once the animal has reached terminal maturity (Hinsch and Al Hajj, 1975). In other decapods, such as the edible crab, Scylla, lobster etc, growth and molting appear to continue until death. The duration of the molt cycle is species specific and each stage generally lasts for days, weeks or months.
It is well known that the molting of Crustacea is under the control of a molting hormone which accelerates this process. Many of the studies on the hormonal control of molting in Crustacea had been done with brachyurans. In contrast, macrurans were less attempted in this field of study due to earlier confusion in identifying the Y-Organ and Mandibular Organ (Sochasky, et al., 1972).

Le Roux (1968) mentioned the similarity between Y-organ, mandibular organ and androgenic gland tissues. Recently, a group of Canadian investigators discovered the mandibular organ and the Y-organ in the crab, Carcinus, lobster, Homarus and crayfish, Orconectes (Sochasky, et al., 1972). They proposed that these two glands were the functional ecdysial glands.

Hormonal control of ecdysis was first postulated at the turn of the century with observations of precocious molting after eyestalk ablation (Zeleny, 1905; Megusar, 1912). Later work shows that the X-organ sinus gland complex located in the eyestalks, produces a molt inhibiting hormone (MIH) that regulates ecdysis (Brown et al., 1939; Carlisle, 1953). It had been noted
that the level of MIH in the haemolymph controls the Y-organ or mandibular organ (Passano and Jyssum, 1963), a site of ecdysteroid production (Chang and O'connor, 1977). It is not yet possible to decide which of these changes are the result of the direct action of the molting hormone and which are secondary or tertiary effects or are even more remotely connected with the hormone's primary action.

Mandibular organs were reported to be unnecessary for normal ecdysis (Sochasky, et al., 1972). Others report, however, that the mandibular organ of lobsters, shrimps and crayfishes change histologically during the molt cycle (Aoto, et al., 1974; Byard, et al., 1975; Hinsch, 1981). In some other crustaceans, the implantations of the homogenates of the mandibular organs accelerated the molting cycle (Connell, 1970; Miyawaki and Taketomi, 1970; Keller and Schmid, 1979; yudin, et al., 1980; Taketomi, et al., 1989). Furthermore, the transplantation of the mandibular organs from Callinectes into white shrimp, Penaeus setiferus shorten the length of its molt cycle (Yudin, et al., 1980). Carlisle and Connick (1973b) found that the mandibular organ contained a substance
with the biological activity of crustecdysone and that its activity was the highest in late proecdysis. Ultrastructurally, the cells of the mandibular organ of the lobster, Homarus americanus (Byard, et al., 1975) and the male crab, Libinia emarginata (Hinsch and Al Hajj, 1975) closely resemble the cytoarchitecture of cells known to synthesize steroids or lipids. Yudin, et al. (1980) stated that the mandibular organ do not store or secrete ecdysteroids although, the fine structural descriptions showed that the mandibular gland is steroidal in nature. However, the actual physiological function of this organ remains unresolved though the changes in structure is associated with molting or repeated ecdysis. Therefore, in this light of confusion in literature regarding the mandibular organ and its possible role in molting, the present investigation was carried out to see the distinguishable histological variations of the mandibular organ with the molting cycle of the prawn and crab.
MATERIAL AND METHODS

The specimens used in this current study were procured from the Kham river near Aurangabad in Maharashtra. Adult male animals of almost identical size (length of the prawns 3.5 - 4.5 Cm from rostrum to telson and crabs 40-52 Cm carapace width) were selected from the stock. Among them, actively swimming ones were separated and were used for the experimental purposes.
The molt stages of the prawn, *Macrobrachium kistnensis* was identified by microscopic examination of the outer uropod edges for evidence of apolysis (epidermal reticulation) and formation of new setae (neosetogenesis) (Drach and Tchernigovtzeff, 1967). Corkin and Rao, 1978). For crab, *Barytelphusa cunicularis* the method used was the degree of hardness of the exoskeleton (Bursey and Lane, 1971; Nagabhushanam and Vasantha, 1971 and Peebles, 1977).

The animals were selected for use according to various well defined stages of the molting cycle. The molt stages thus identified in the present investigation can be broadly classified into three groups as postmolt, intermolt and premolt. Postmolt comprises of the early postmolt stage A and late postmolt stage B while, the intermolt stage have only one stage C whereas, stage D is categorised under the premolt.

From the sacrificed animals, the samples of mandibular organs were collected and fixed in Bouin's fixative. After processing the tissues (dehydration, paraffin embedding and sectioning at 6 μ), the sections were stained with haematoxylin and eosin, cleared in xylol and mounted in D.P.X. For comparison light microscopical observations were performed on the prawn and crab.
OBSERVATIONS AND RESULTS

The experimental results received in the present probe on the mandibular organ of prawn and crab with reference to different stages of the molt cycle were emphasized using histological preparations of the gross and fine structure of the organ.

On the basis of morphological features, such as size, shape, location, number and tinctorial characteristics (staining with haematoxylin and
eosin), mandibular organ cells were classified into four types based on their region in the mandibular organ.

During all the molt stages in the prawn and crab, the mandibular organ has a centrally located median canal (MC) which is a big hollow cavity. Section was seen in this canal at B, C, and D stages of the prawn and crab. The size and shape of the canal changed at different molt stages.

The first region is the Stranded region (SR) which is seen during the B molt stage of the prawn and crab and starts right from the median canal. This region showed clear strands with darkly stained large cells and the cell population was found to be high. Moderate amount of secretion and vacuolization was also observed (Figs. 2, 6).

The second region, namely the Basophilic region (BR) was visible only at the C stage of the animals (both in prawn as well as crab). This portion took dark basophilic staining and was clearly seen in between stranded and compact regions of the prawn, while in crab it was completely
separated in the elongated median canal. The cells were closely packed and cell population was triple to that of the stranded region. Large cells were seen particularly in this region of both the animals. No secretion was noticed and vacuolization was absent (Figs. 3,7).

The third region, the compact region (CR) was found to be present only in the prawns but not in the crabs. It took light staining during the B and C stages and was distinguished by the presence of a highly compartmentalized structure encountered by the lobular region. It was observed to display diffused signs of minimal secretory activity. Moderate number of cells were present. At D stage however, high vacuolization was seen (Figs. 2,3).

The fourth region is the lobular region (LR). This region was found to have a large number of lobules towards the peripheral wall of the mandibular organ. Vacuolization was particularly common in this region and small lightly stained cells were observed. Less number of mandibular organ cells were present. The secretory material found in the lobules was in the form of patches of
deeply staining granules while, the smaller and larger lobules were visible depending upon the stage of molting in the animal (Figs. 4, 8).

Apart from the general histology, certain variations were observed in the structure of the mandibular organ in the prawn and crab at different molt stages which are mentioned below.

In prawn, the serial sections stained with haematoxylin and eosin showed that in early postmolt (Stage A), basophilic region was absent. The compact region (CR) located between the lobular and stranded regions, was highly stained and lobulated. The lobules of this region being arranged regularly with secretion. Darkly stained lobular region was found not to have any secretion in its lobules. Moderate number of cells with vacuolization were noticed in the compact and lobular regions. The stranded region was not clear but it had taken dark staining with large number of deeply stained cells, little secretion and no vacuolization was seen. The strands were present towards the median canal (Fig. 1).
Histological changes of mandibular organ during the molt cycle in prawn, *M. kistnensis*.

**Fig. 1:** Photomicrograph of MO in molting stage 'A' depicting the arrangement of CR and LR. Haematoxylin - Eosin X 100.

**Fig. 2:** Photomicrograph of MO in molting stage 'B' showing the strands. Haematoxylin-Eosin X 100.

CR = Compact region  
LR = Lobulated region  
SR = Stranded region  
MC = Median canal.
In the late postmolt (stage B), the deeply stained strands were prominent towards the median canal. The darkly stained lobular region was seen towards the peripheral wall. Secretion and vacuolization was noticed in the stranded and lobular regions. In between the stranded and lobular regions, lightly stained compact region without vacuoles and secretion was noticed. In the median canal, secretion was observed (Fig. 2).

During intermolt (stage C), the four regions were clearly demarked. Basophilic region was particularly prominent at this stage. No secretory material was traceable at any region. Vacuolization was observed in the stranded and lobular regions. The cell number and size were high in both basophilic as well as stranded region, moderate in the compact region and less in the lobular region (Fig. 3).

During premolt (stage D), vacuolization was high and irregularly arranged lobules were seen. Some amorphous deposition of unknown nature was found in the lobular and compact regions. Basophilic region was absent at this stage. Less number of cells noticed (Fig. 4).
Fig. 3: Photomicrograph of MO in molting stage 'C' showing the BR, CR, LR and SR. Haematoxylin-Eosin X 100.

Fig. 4: Photomicrograph of MO in molting stage 'D' depicting the irregular CR and LR. Haematoxylin-Eosin X 100.

BR = Basophilic region
CR = Compact region
LR = Lobulated region
SR = Stranded region
MC = Median canal.
In crab, at early postmolt (stage A), the lobules in the lobular region were compactly arranged with full secretion. Some strands were noticed around the median canal. The median canal was not well developed. Little vacuolization was observed. Moderate number of darkly stained cells were seen (Fig. 5).

At late postmolt (stage B), the strands were prominent towards the median canal and large number of darkly stained cells were seen in the stranded region. The lobules of the lobular region had a secretion. Vacuolization was observed in the lobular region (Fig. 6).

During intermolt (stage C), the median canal was well developed. A highly stained basophilic region was separately distinct at the centre of the elongated median canal with cells arranged compactly and the cell size was also large. The lobular region was found to have deeply stained cells with secretion. Vacuolization too was noticed (Fig. 7).
Histological changes of the mandibular organ during the molt cycle in crab, *B. cunicularis*.

**Fig. 5:** Microphotograph of MO in molting stage 'A' showing the packedly arranged lobules. Haematoxylin-Eosin X 100.

**Fig. 6:** Light micrograph of MO in molting stage 'B'. Note the SR. Haematoxylin-Eosin X 100.

LR = Lobulated region  
SR = Stranded region  
MC = Median canal.
During premolt (stage D), the lobules in the lobular region had become enlarged with secretion. The basophilic and stranded regions were absent in this region. Vacuolization was noticed. Large number of deeply stained cells were observed (Fig.8).
Fig. 7: Light micrograph of MO in molting stage 'C' The BR is visible in the MC Haematoxylin - Eosin X 100.

Fig. 8: Photomicrograph of MO in molting stage 'D' revealing the LR. Haematoxylin - Eosin X 100.

BR : Basophilic region
LR = Lobulated region
MC = Median canal.
DISCUSSION

The present comparative study of the mandibular organ in relation to molting on the prawn, *Macrobrachium kistnensis* and crab, *Barytelphusa cunicularis* revealed many interesting features. Our observations confirm that like other endocrine organs, mandibular organ of the prawn and crab is also susceptible to molting rhythms. The presence of the median canal in the centre of the
mandibular organ clearly suggests that its secretions first come into the canal and are then released into the haemolymph. Moreover, changing structure of the mandibular organ during molt cycle may further be explained on the basis that the prawn and crab like other crustaceans exhibit rhythmic changes corresponding to their molting.

The question now arises whether these structural changes are incidental to the molt cycle or whether they reflect a direct participation of the mandibular organ principles responsible for the change process related to molting? To answer this question the present probe was undertaken. Despite extensive efforts to understand the molting physiology, two explanations are possible for such a change encountered in the mandibular organs during molting period in the present probe. Vacuolization is particularly common in the pre and post molt. The increased vacuolization is suggestive of an activity of a secretory nature with unknown product(s). These instances of apparently synchronous occurrence of molting with secretion, premolt etc. suggests the possibility that the mandibular organ is involved in molting activities.
Sochasky, et al. (1972) described the mandibular organs and Y-organs as the ecdysial glands in crustaceans which closely resemble each other at the level of the light microscope. Comparative fine structural studies of the mandibular organ and Y-organ in macrurans revealed changes in activity during the molt cycle. The view is that the crustecdysones or its analogues are responsible for completion of molting in many crustaceans. Crustecdysone, the crustacean molting hormone is a steroid and the cytoarchitecture of the mandibular organ cells was similar to that found in vertebrate cells associated with steroid secretion (Christensen and Gillim, 1969; Hinsch and Al Hajj, 1975). Couch and Hagion (1983) reported that the mandibular organs may produce steroids other than ecdysteroids such as progesterone-like. At present, we lack information on the site of synthesis of such substances from the mandibular organ. However, eyestalk ablation resulted in precocious ecdysis and growth (Passano, 1953) in numerous crustaceans and had indicated that the eyestalk neurosecretory system produced a molt inhibiting hormone (MIH). The action of MIH is found to be that of inhibiting the mandibular organ or Y-organ.
Aoto, et al. (1974) demonstrated that in the mandibular organ cells during molt cycle, some extraordinarily hypertrophied mitochondria and an indented vesicular form of the smooth endoplasmic reticulum (SER) in some cells of the early premolt Palaemon paucidens were denoted. No secretory product at any stage of the cycle was observed in the mandibular organ cells but, marked changes in the amount of cytoplasm and tinctorial affinity of their nuclei were noticed. The amount of cytoplasm which increased during the premolt stage, neither increased nor decreased significantly during the early and late premolt stages but decreased again at the post-molt stage. In the staining property of the mandibular organ cells, no remarkable change was seen during the molt cycle. The change in the cell height at the epidermis during the cycle was lowest in stage C, started to increase during stage $D_0 - D_1$, reached the maximum at stage $D_3 - D_4$ (Ato, et al., 1974).

Byard, et al. (1975) in Homarus americanus observed that the agranular reticulum appears to proliferate in both sexes during premolt, especially
in late premolt resulting in large areas filled with agranular reticulum to the exclusion of other organelles. In some cases, the CER (cisternal) formed extensive layers around a microbody. These "whorls" were often large and probably correspond to the round pale areas of cytoplasm with acidophilic centers. These whorls occurred at all molt stages but were more common in premolt in both sexes. Towards the central region of the mandibular organ, the cells gradually enlarged, nuclei measured up to 10 μm and the amount of acidophilic granular cytoplasm increased, resulting in spherical and polygonal cells up to 30 μm in diameter. A high degree of vacuolization seen in cells of the mandibular organ at the time of molting suggested a role in molting and perhaps in the production of ecdysones or their conversion from some precursor (Byard, et al., 1975).

The Y-organs appear to change most dramatically during the late intermolt whereas, the mandibular organs increase in activity during premolt (Aoto, et al., 1974; Byard, et al., 1975). After injections of the proposed molting hormone,
20-hydroxyecdysone, the mandibular organs of crayfish developed extensive net works of agranular endoplasmic reticulum, modified mitochondria and giant granules (Miyawaki and Taketomi, 1970) because the Y-organ was the source of ecdysteroid (Chang and O’Connor, 1977) and the mandibular organ appears to show activity soon after Y-organ activity. There may be a relationship between the Y-organ, the mandibular organ and ecdysis (Yudin, et al., 1980). More work is necessary to determine the functional activity of the mandibular organs including the possibility of interaction with other neuroendocrine glands in molting.

Connell (1970) commented that implanting mandibular organs into shrimp decreased the time between molts and implantation into crabs accelerated proecdysis. If the mandibular organ does promote molting, it does not seem to do so by storing or secreting ecdysteroid, as indicated by the lack of significant amounts of radioimmunoassay (RIA) active material in the medium of the cultured glands. These results were similar to those obtained in crayfish following molt induction.
(Keller and Schmid, 1979). The experiments of Yudin, et al. (1980) indicated that the mandibular organs did not synthesize an ecdysone precursor or trophic hormone to stimulate production of ecdysone by the Y-organ and he quoted that the mandibular organs did not store or secrete ecdysteroids. Implantation of the mandibular organs of Callinectes into Penaeus setiferus accelerated molting in the shrimp. Hence, he guessed that the mandibular organ then must synthesize a steroid other than ecdysones to fulfil its apparent endocrine function. Perhaps, the ecdysone produced by the Y-organ affects the activity of the mandibular organ which in turn may influence ecdysis. Another possibility is that the substance produced by the mandibular organ is involved in the reproductive cycle which was closely related to ecdysis.

Taketomi, et al. (1989) examined that the homogenates of the mandibular organs from the crayfish, Procamburus clarkii, when injected into the freshwater shrimp, Caridina denticulata, accelerated
the molting cycle of the shrimp which strongly suggests that the mandibular organs of decapod crustaceans either produce or secrete molting hormone(s) and thereby accelerate the molting process or produce a factor that activates the Y-organs. This indicates that mandibular organ function in molting may not be direct but may reflect a change in it.

King, et al. (1969) was of the opinion that the function of the ecdysial gland is to supply the blood with necessary enzyme(s) which were involved in the peripheral conversion of the precursor substance to the active molting hormone, Crustecdysone (its activity was the highest in late proecdysis). Aoto, et al. (1974) also mentioned that if this gland is actually a portion of the excretory organ, then it would be natural that the hormone which has been liberated into circulation but not utilized during late proecdysis was either accumulated in the excretory apparatus before excreted as a waste or "stored" there for re-use. Anger and Dawirs (1981) speculated that in decapod crustaceans cholesterol or related substances which may serve as precursors of the molting hormone might
be the critical constituent settling the PRS (Point of Reserve Saturation - The minimum time in which enough reserves are accumulated for successfully completing the instar without food). Further studies are needed for elucidation of the releasing and storing mechanism of the hormone from the mandibular gland.

Adelung (1971) recognised that the high amount of molting hormone was released at stage D₃ in Carcinus maenas. Furthermore, it apparently does not contain molt inducing substances (Connell, 1970; Carlisle and Connick, 1973b). Keller and Schmid (1979) showed that in vitro mandibular organs of the macruran Orconectes limosus in contrast to Y-organs under identical experimental conditions at no stage produce ecdysones while the Y-organs synthesize x-ecdysone. The mandibular organ of the crustaceans might be considered to be a production site of β-ecdysone, either by synthesis or by conversion from X-ecdysone in analogy with the ecdysocytes in insects. Role of mandibular organ in molting is documented in the literature by many authors nevertheless, no attempts had been made, hitherto, to understand hormonal basis of mandibular organ.
The miracles of mandibular organ are packed with exciting puzzles to exercise our mental muscles, because lobsters deprived of their mandibular organs have survived and continued to molt for up to two years (Aiken unpublished, Byard, et al., 1975). If so then what about the above all manifestations? On the other hand, Sochasky, et al. (1972) whose preliminary observations on Homarus, mandibular organ had not revealed any prominent role in molt control. Byard, et al. (1975) reported that there were no major ultrastructural changes in the mandibular organs during the molt cycle. Kawano and Taketomi (1985), however, did not find any changes in the ultra structure of the mandibular organ during the molt cycle in the shrimp, Penaeus japonicus which suggests that the mandibular organs do not have a direct role in controlling ecdysis in this species.

A histological survey of serially sectioned mandibular organ of the prawn and crab, stained with Haematoxylin and Eosin in the present study showed four different types of regions i.e. lobular region
(LR), compact region (CR), stranded region (SR), basophilic region (BR) and a median canal (MC). The median canal is well developed at all stages of molting in the prawn and crab. The lobular region is particularly common at D stage in both species. The compact region is very distinct at B and C stages of the prawn but, it is absent in the crab. The stranded region is accumulated around the median canal at B stage of the prawn and crab. The basophilic region is present only at C stage of both prawn and crab. In the prawn, the basophilic region is in between the stranded region and compact region however, in the crab it is separate distinctly in the median canal. The cell population of the mandibular organ varies depending on the region. In the stranded and basophilic regions the cells are found to be high whereas, in the compact and lobular regions, moderate number of cells are present. The disposition and distribution of secretory material is observed. The secretion with vacuolization is noticed particularly in the pre and post-molt stages.
Based on the visual observations it is found that the present findings gain support from the earlier observations of Aoto, et al. (1974) and Byard, et al. (1975). Hence, our findings raise the possibility to draw firm conclusions in this regard that the mandibular organ is a site of molt hormone synthesis and hence, it is designated as "molting gland".

Studies on the mandibular organ mechanisms regulating the rhythms related to the molt cycle are limited. The nature of the stimulus for molt cycle is not known but, it may act by mandibular organ. Unfortunately, no other statistics regarding the structure and function of this gland are available. We should not neglect the role of mandibular organ here and forward the statement that the actual process of molting is initiated when secretion of mandibular organ starts because secretion of molting hormone causes ecdysis.

After disclosing its function and structure, we can comment that the mandibular organ too, presumably, is highly adaptive and constitutes an
indispensable clock regulating periodic activities such as molting. Further chemical analysis of this gland will help in solving the riddle of its functional significance.