



Title	Histological and Ultrastructural Studies on the Y Organ and the Mandibular Organ of the Freshwater Prawn, <i>Palaemon paucidens</i> , with Special Reference to Their Relation with the Molting Cycle (With 2 Text-figures, 1 Table and 6 Plates)
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**Histological and Ultrastructural Studies on the Y Organ  
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*Palaemon paucidens*, with Special Reference to  
Their Relation with the Molting Cycle**

By

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(With 2 Text-figures, 1 Table and 6 Plates)

Since Gabe's first description of "l'organe Y" in some malacostracan crustaceans and his implication of this organ as an ecdysial gland that is comparable to the one in insects (Gabe, 1953, 1954), a number of investigations have been carried out in an effort to clarify physiological significance, if any, of this tiny structure (for references, see Gabe, 1966; Herman, 1967). Thus, the Y organ from more than 100 different species of crustaceans has been described (Gabe, 1956; Echali er, 1959). Unfortunately, however, so little has been reported of anatomical and cytological details of this organ that there has been a considerable confusion concerning its exact location and identification among the authors. Meanwhile, Le Roux (1968) discovered in the cephalothorax of a crab *Carcinus maenas* a pair of glandular structure which, structurally so different from the Y organ, he proposed to name "the mandibular organ". But, he, too, failed to give any photomicrographic or even a schematic detail to it, although he claimed that homologs of the mandibular organ were observed in the reptantians and natantians.

Recently, a group of Canadian investigators found two sets of organs, the mandibular organ and the "Y organ" (a structure which they believed to be "a functional ecdysial gland" or what they proposed the phrase "the molting gland of Echali er") in the crab *Carcinus*, the lobster *Homarus*, and the crayfish *Orconectes* (Sochasky *et al.*, 1972). They pointed out that the mandibular organ in crayfish is much more conspicuous than the potential Y organ and that there is a considerable similarity between the histological appearance of "the molting gland of Echali er" and other structures in the cephalothorax. They hence concluded that these are the causes which, together with the meagerness of histological and cytological informations of these structures presently available, are responsible for much of the Y organ confusions.

The situation is never the better in the natantians. Although the "Y organ" has been described in *Metapenaeus* sp. (Dall, 1965) and *Pandalus danae* (Hoffman, 1967), no structure comparable to the mandibular organ has been described by these authors in these animals. Le Roux (1968), on the other hand, referred to the presence of the mandibular organ homolog in *Palaemon squilla* but he made no mention of the possible existence of the Y organ in this prawn.

The present paper deals with the anatomy and histology of the Y organ and the mandibular organ in the freshwater prawn, *Palaemon paucidens*. Further, evidence is presented to show an ultrastructural cyclic change of these organs which runs parallel with the molting cycle of the animal. For comparison, light microscopical observation was carried out on the prawn *Pandalus kessleri* and the crayfish *Procambarus clarkii*.

### Materials and Methods

The freshwater prawns, *Palaemon paucidens*, used in this investigation were collected in a small lake in the vicinity of Sapporo from April to November, 1972. They were stocked in aerated aquaria in our laboratory, fed twice a week with pieces of boiled fish paste, and were selected for use according to the stage of molting cycle.

The prawns *Pandalus kessleri*, used for comparison, were collected in October, 1970, at Akkeshi Bay. The crayfishes *Procambarus clarkii* were obtained through a dealer in Tokyo. They were sacrificed without regard to their molting cycle.

*Procedures for histology:* For histological study, small pieces of tissue containing the Y organ or the mandibular organ were dissected out under a stereoscopic dissecting microscope with the aid of a pair of Wecker's scissors and were fixed in Bouin's fluid. They were embedded in paraffin, sectioned at a thickness of 6  $\mu$ , and stained with Delafield's hematoxylin-eosin, Heidenhain's Azan, toluidine blue (buffered at pH 6.0) or Gabe's aldehyde fuchsin (AF). For the demonstration of glycogen or lipid, the materials fixed in Gendre's solution or Ciaccio's solution were stained with periodic acid-Schiff (PAS) or Sudan black B.

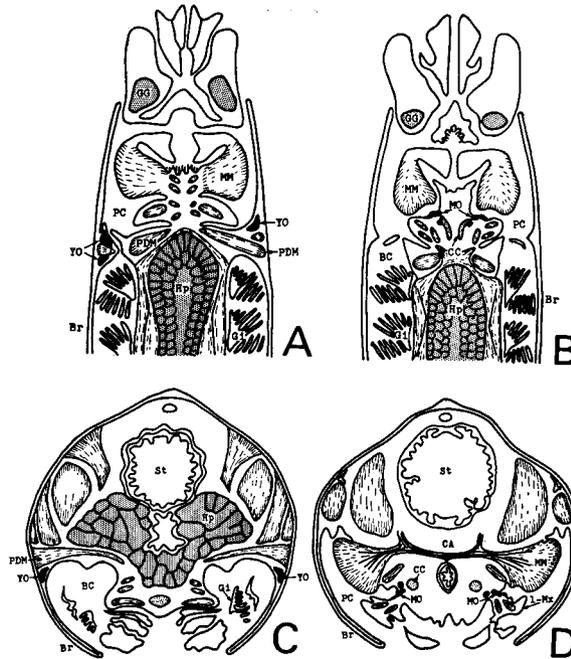
To compare the nucleus/cytoplasm ratio of the two organ cells, the number of nuclei per unit area of sectioned materials was counted of each organ at various stages of a molting cycle, and the amount of cytoplasm at each stage relative to that at the intermolt stage (stage C) was calculated. The stage of each specimen was determined according to the method previously described by Kamiguchi (1968).

*Procedures for electron microscopy:* For an electron microscopic study, fully grown females (12–20 mm in carapace length) were exclusively used, because excision of these two minute organs was encountered by great difficulties. The tissues containing each organ were dissected out and put into the ice-cold (4°C) 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) with or without 7% sucrose. The same solution in 0.1 M cacodylate buffer (pH 7.3) was also used in some cases. Tissues, trimmed rapidly in these solutions, were allowed to be fixed in renewed 5% glutaraldehyde for about 2 hr at 0–4°C. Then, they were rinsed in buffer solution followed by 1% OsO<sub>4</sub> post-fixation for about 2 hr at 0–4°C. They were dehydrated through a graded series of acetone and embedded in Epon 812. Ultra-thin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome, stained with lead citrate with or without being followed by uranyl acetate, and examined in a Hitachi HS-7 electron microscope.

## Observations

## Light microscopy

Survey in the prawns *Palaemon paucidens* and *Pandalus kessleri* proved that they possess two kinds of glandular organs, the Y organ and the mandibular organ, in the thorax. Gross anatomy and histology of these organs will be described in the following. Topographic situation of these two organs is shown in Text-fig. 1.



Text-fig. 1. Diagrammatic representation of horizontal (A, B) and transverse (C, D) sections of the anterior cephalothorax through the levels of the Y organ (A, C) and of the mandibular organ (B, D) in the prawn *Palaemon paucidens*. BC, branchial chamber; Br, branchiostegite; CA, cephalic apodeme; CC, circumesophageal connective; GG, green gland; Gi, gill; Hp, hepatopancreas; MM, mandibular muscle; MO, mandibular organ; 1-Mx, first maxilla; PC, prebranchial chamber; PDM, posterior dorso-ventral muscle; St, stomach; YO, Y organ.

## I. Y organ

*Gross anatomy:* The Y organ in *Palaemon* is identified as a pair of slender strip of tissues located in the junction of the prebranchial and the branchial chambers, just beneath the inner wall of branchiostegite (Text-fig. 1, A and C). It is translucent in a live specimen. In a fully-grown animal (15 mm in carapace

length), it measures about 2.0 mm long and 0.3 mm wide. The anterior part of the tissue, about 0.8 mm long, lies in an epidermal shelf which, projecting inwards from the inner wall of the branchiostegite, extends antero-horizontally from the posterior end of the prebranchial chamber. The posterior part of the organ, about 1.2 mm long, is located between the lateral end of the posterior dorso-ventral muscle where it is attached to the cuticle and the anterior dorsal roof of the branchial chamber, forming a slender crescent. In *Pandalus* the organ is located in the position corresponding to that in *Palaemon*, forming a fine strip of tissue (about 6 mm long and 0.5 mm wide) in an ovigerous female, 35 mm in carapace length. But, the epidermal shelf in which the organ is inserted is rather obscure. In the crayfish *Procambarus*, the organ is also elongated and found in the corresponding position as in the prawns.

*Histology:* Although the *Palaemon* Y organ tissue is closely associated with the epidermis, it is clearly distinguishable from the epidermal cells throughout the whole length of the tissue, excepting for a few little portions in the posterior part (Fig. 1). It is bathed freely in the surrounding blood sinus and has neither lumen nor secretory canal. No direct supply of the nerves is evident in the tissue in spite of our careful observations. It is composed of many lobulated cell masses. Each lobulette consists of ten to twenty cells with a poor cytoplasm, their cell boundaries being hardly detectable. The nucleus is 7–8  $\mu$  in diameter, spherical or ovoid in shape and contains several markedly aggregated chromatins. No mitotic figure is observed in cells of any animals examined at various stages of a molting cycle during a period from April to November. The cytoplasm is slightly acidophilic, containing neither glycogen nor lipid droplets but frequently many minute granules that stain with Sudan black B. Further, with the stains employed in this study, no secretory product is detected in the organ cell at any molting stages or in any seasons of a year. In the crayfish, histological features of the organ are more or less similar to that in *Palaemon*. The *Pandalus* Y organ (Fig. 2), however, shows the histological appearance considerably different from that of the *Palaemon* Y organ. In this species the organ is denoted as the folded invagination of the epidermis with many lumens. The organ cell has an elongated nucleus and a rare cytoplasm. Similar aspects have also been reported in the "Y organ" of the prawn, *Pandalus danae* (Hoffman, 1967).

## II. Mandibular organ

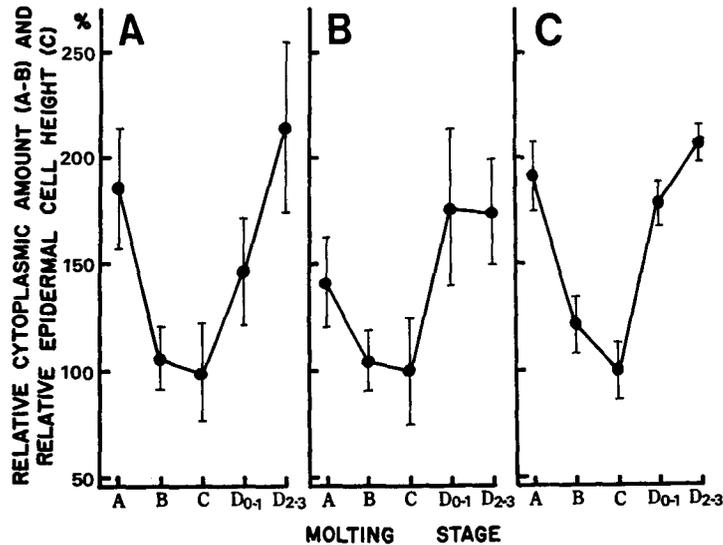
*Gross anatomy:* The mandibular organ in *Palaemon* is protruded inwards from the epidermis at the joint of the mandibular and the first maxillary segments (Text-fig. 1, B and D). The organ is surrounded by several muscles: anteriorly by the mandibular muscle, posteriorly by the first maxilla promotor muscle, and interio-laterally by the lateral adductor muscle of the first maxilla. It is in a triangular sail shape, measuring 0.8–1.0 mm long and 200–300  $\mu$  wide at the broadest portion in a fully-grown animal. In the fresh material the organ is

slightly whitish in color and hardly distinguishable from other surrounding tissues. The *Pandalus* mandibular organ is situated in the corresponding position as in *Palaemon* with a similar shape. In *Procambarus*, on the other hand, the organ is rather irregular in shape and yellowish in color, occupying the position homologous to that in the prawns.

*Histology:* The mandibular organ tissue is closely associated with the epidermis by fine collagenous fibers and supplied by surrounding blood sinus. It is not lobulated and has neither lumen nor secretory canals (Fig. 3). No innervation is observed in the tissue. The organ cell is about  $20\ \mu$  wide and  $35\ \mu$  long with slightly acidophilic cytoplasm. Nucleus is spherical or ovoid (about  $8\text{--}10\ \mu$  in diameter). Without regard to the molting stage or the season, no secretory product is found in the cytoplasm with the stains used. Besides these organ cells, in the organ tissue found is another type of cell with a smaller nucleus (about  $6\text{--}7\ \mu$  in diameter) containing markedly agglutinated chromatin and a rare cytoplasm. Occurrence of this cell type on the surface of the tissue suggests that the cell is a supportive one. The *Pandalus* and *Procambarus* mandibular organs are almost the same as the *Palaemon* organ in that they show no secretory product in the organ cell cytoplasm (Fig. 4). It is noteworthy that in *Procambarus* the organ cells contain one or two azo-carmin-positive granules. These organelles seemed to correspond to the lysosome-like multivesicular body observed in our electron microscopic study.

### III. Histological changes of the Y organ and the mandibular organ in *Palaemon* during the molting cycle

In view of implications by several authors of the Y organ as an ecdysial gland, the Y organ and the mandibular organ were studied histologically in *Palaemon* at various stages of a molting cycle to see if there is a correlation between one or both of these organs and the molting. Both organs consist of the cells that contain no secretory product at any stages of the cycle, but marked changes in the amount of cytoplasm and tinctorial affinity of their nuclei were noticed (Text-fig. 2, A and B). In the Y organ cell during the intermolt stage (St. C) the cytoplasm was smallest in amount, the nucleus stained deeply and rather uniformly by hematoxylin, and many inter-lobular spaces were observed at this stage (Fig. 5). The cytoplasm started to increase in size at early premolt (St.  $D_0\text{--}D_1$ "), reaching the maximum (approximately twice as much as that at stage C) at late premolt (St.  $D_3\text{--}4$ ) when chromatin became granulated and no inter-lobular space was seen any longer (Fig. 6). The amount of cytoplasm decreased again at postmolt (St. A-B). A similar cyclic change was seen in the amount of cytoplasm of the mandibular organ cell during the molting cycle, being increased during the premolt stage, neither increased nor decreased significantly during the early and late premolt stage (Fig. 7), and decreased again at the postmolt stage (Fig. 8). On the other hand, in the staining property of the mandibular organ cell no remarkable change



Text-fig. 2. Changes in the amount of cytoplasm of the Y organ cells (A) and the mandibular organ cells (B) and in the epidermal cell height (C) of the prawn *Palaemon paucidens* during the molting cycle. Each plotted point represents the mean value with standard deviation relative to the value of the intermolt (St. C) animal, which is calculated as 100%.

was noticed during the molting cycle.

In the next, the change in the cell height was examined of the epidermis during a cycle (Text-fig. 2, C). It was found that the epidermal cell height, lowest in stage C, started to increase during stages D<sub>0</sub>-D<sub>1</sub>, reaching the maximum at stages D<sub>3-4</sub>. Apparently, a clear parallelism was noticed between the epidermal cell height and the amount of cytoplasm of the Y organ and the mandibular organ.

### Electron microscopy

#### I. The fine structure of the Y organ cell and its changes during the molting cycle

The Y organ cell has a spherical or ovoid nucleus and a poor cytoplasm which contains mitochondria, endoplasmic reticula, inconspicuous Golgi complexes and several kinds of cytoplasmic inclusions. It has neither glycogen granules nor lipid droplets. The cell border is complicated by tight cellular junctions of pseudopodial processes. At the organ periphery, there are observed marked infoldings of cell surface, which may serve to facilitate exchange of metabolites between the cell and surrounding blood sinus (Fig. 9, arrows). Ten to twenty cells enclosed by a basal lamina constitute a lobulette. Sometimes, expanded intercellular spaces are observed among the organ cells. Capillaries are also observed occasionally between the lobules. Frequently, a small number of cells with highly electron dense cytoplasm are found in the tissue without regard to the

molting cycle (Fig. 15). The high electron density is due to increase of population density of free ribosomes, but its functional significance is unknown. There occur many lysosome-like inclusions in these cells, suggesting that the cells may be regressive ones.

*Nucleus:* The Y organ cell nucleus, about 7–8  $\mu$  in diameter, contains one or two nucleoli and agglutinated chromatins which are in contact with the nuclear membrane. Sometimes, there are seen a small number of ribosomes attached to the nuclear membrane. In some nuclei expanded perinuclear space is observed with no regard to the molting cycle (Fig. 9). Through our present work, no mitotic figure was seen in the nucleus at any stage of a cycle.

*Mitochondria:* The Y organ cell possesses many mitochondria in the cytoplasm. Their size and internal structure show remarkable changes during the molting cycle. In the mid to late premolt (St.  $D_1'''$ – $D_4$ ) and postmolt (St. A-B) periods, mitochondria are the smallest in size and have the rod or ellipsoid shape (about 0.9–1.3  $\mu$  in length and 0.5  $\mu$  in width) (Fig. 10). They contain a small number of tubular cristae arranged in all directions. Mitochondrial matrix has a considerably high electron density, including a few intra-mitochondrial granules (Fig. 10). In the intermolt (St. C) and early premolt (St.  $D_6$ – $D_1''$ ), on the contrary, almost all mitochondria turn into a round or elliptic shape (about 1.8  $\mu$  in length and 1.6  $\mu$  in width), being greatly increased in size (roughly 15 times larger in volume than those in the mid to late premolt or postmolt periods (Fig. 11). In addition, the number of tubular cristae increases remarkably and the mitochondrial matrix becomes electron lucent in these enlarged mitochondria.

*Endoplasmic reticulum (ER):* Most of the endoplasmic reticula of the Y organ cell are smooth ER, and only in rare occasions a few ribosomes are detected on them. Most ribosomes are scattered freely throughout the cytoplasm. Typical rough ER in modest amounts are also observed in the cytoplasm, setting apart from the smooth ER areas. The smooth ER show a marked structural change during the molting cycle. They take an undulated tubular form during the mid to late premolt (St.  $D_1'''$ – $D_4$ ) and the postmolt (St. A-B) periods (Fig. 13), and sometimes, especially at stage  $D_2$ , the ER consist of lamellar cisternae showing anastomosing network occasionally. Almost all of these smooth ER become much vesiculated in the intermolt and early premolt periods (Fig. 11).

*Golgi complex:* Golgi complexes are rather inconspicuous in the Y organ cell (Fig. 10), and they show no ultrastructural change during the molting cycle. No evidence was available to indicate production of the secretory granules in this study.

*Other cytoplasmic inclusions:* Besides ordinary cell organelles already mentioned, four kinds of inclusions (types I-IV) are noticeable in the Y organ cell. Type I inclusion is spherical in general (about 0.4  $\mu$  in diameter) and has an electron

dense matrix enclosed by a limiting membrane (Fig. 10). Inclusions of this type tend to occur abundantly in the cells situated in organ periphery. They are more abundant in the prawns at intermolt (St. C) and early premolt (St. D<sub>0</sub>-D<sub>1</sub>"') stages than in those at other molting stages. Type II inclusion, about 0.1  $\mu$  in diameter, is constituted of two components. The first one, a cap-like structure made of one or two crescent-shaped bodies with high electron density, covers the second one, which is a round vesicle with much less electron dense matrix. Sometimes, the vesicular element is connected to neighboring smooth ER (Fig. 14, arrow). The type II inclusion shown in Fig. 14 is an extraordinarily large one (about 3.2  $\mu$  in diameter). Type III inclusion is bounded by a limiting membrane, containing concentrically arranged membrane systems in the matrix with moderate electron density (Fig. 15, arrows). These whirls of membrane systems seem to represent brought-in residues of degenerated organelles so that type III inclusion may be the lysosomes. Although the type III inclusions are encountered more frequently in the cells with electron dense cytoplasm, no quantitative change is noticed of the occurrence of this type of inclusions during the molting cycle. Type IV inclusion is delimited by the double membranes and has a finely granular matrix (Fig. 12). Their size and shape are highly variable, but no evidence is available to show any correlation between their occurrence and the molting cycle. Sometimes, intermediate forms of these four types of inclusions, especially those intermediate ones between type I and type II inclusions, are observed, but it is not certain whether one type of these inclusions can be transformed to another. Changes in the above mentioned organelles and inclusions during the molting cycle are summarized in Table 1.

Table 1. Changes in the organelles of the Y organ cell in the molting cycle of the freshwater prawn, *Palaemon paucidens*

Molting stages Organelles	Postmolt (St. A-B)	Intermolt (St. C)	Early premolt (St. D <sub>0</sub> -D <sub>1</sub> "')	Mid to late premolt (St. D <sub>1</sub> '''-D <sub>4</sub> )
Mitochondria	Small in size (1.1 $\mu$ $\times$ 0.5 $\mu$ ). Rod- or round shape. With a few tubular cristae and electron dense matrix.	Large in size (1.6 $\mu$ $\times$ 1.8 $\mu$ ). Round shape. With many tubular cristae and electron lucent matrix.		Small in size (1.1 $\mu$ $\times$ 0.5 $\mu$ ). Rod- or round shape. With a few tubular cristae and electron dense matrix.
Endoplasmic reticulum	Tubular form.	Mostly vesicular and a few tubular in form.		Cisternal and a few tubular one.
Inclusions Type I Type II	+* #	## +	+ +	+ ##

\* The frequency of occurrence is graded in arbitrary units from + to ##.

## II. The fine structure of the mandibular organ cell and its changes during the molting cycle

The mandibular organ cell has a round nucleus and a rich cytoplasm with many mitochondria, endoplasmic reticula, Golgi complexes, free ribosomes, microtubules and several kinds of cytoplasmic inclusions. It contains neither glycogen granules nor lipid droplets. Rosette-forming free ribosomes (polysomes) are scattered throughout the cytoplasm. Interdigitation between contiguous mandibular organ cells is very conspicuous (Fig. 17), although infoldings of its cell surface are almost unrecognizable (Fig. 19).

*Nucleus:* The nucleus of the mandibular organ cell is spherical or ovoid (about 8–10  $\mu$  in diameter) and contains one or two rather irregular nucleoli and aggregated chromatin (Fig. 16). They show no ultrastructural changes in parallel with the molting cycle. No mitotic figure is observed in all the animals examined.

*Mitochondria:* The mandibular organ cell has a great number of mitochondria which are highly variable in shape. They are usually elliptical or rod-shaped (about 1.5  $\mu$  in length and 0.7  $\mu$  in width) and have the matrix with low electron density, containing no intra-mitochondrial granules. Generally, they have the rod-shaped cristae arranged irregularly, but sometimes concentrically arranged cristae are also seen in the mitochondria (Fig. 16). In addition to these mitochondria, small and somewhat electron dense mitochondria (about 0.4–1.1  $\mu$  in length and 0.4  $\mu$  in width) are observed in animals at the late premolt and postmolt periods. Their tubular cristae tend to run in parallel with the long axis of the mitochondria (Fig. 17). On the other hand, in some preparations fixed at early premolt period (St. D<sub>0</sub>–D<sub>1</sub>) there are extraordinarily hypertrophied mitochondria (about 15 times larger in volume than those in the intermolt period) in which most cristae have disappeared, with only a few remaining at the periphery (Fig. 19). The drastic change in appearance of these mitochondria gives an impression that they are not the physiologically activated ones but the degenerative ones.

*Endoplasmic reticulum (ER):* Both the rough and the smooth ER are present in the mandibular organ cell. They are vesicular or short-tubular in shape and sparsely dispersed throughout the cytoplasm (Fig. 17). They show no cyclic change in parallel with the molting cycle. It is interesting to note that the ER turn into the indented vesicles in the early premolt cells that contain extraordinarily swollen mitochondria (Figs. 18 and 19).

*Golgi complex:* Golgi complexes are more conspicuous in the mandibular organ cell than in the Y organ cell. Besides the ordinary Golgi complexes, ring-shaped ones are occasionally observed (Fig. 16). Sometimes Golgi cisternae are vacuolated, containing modest amounts of thready materials. No noticeable change is seen in the complex throughout the molting cycle.

*Other cytoplasmic inclusions:* A variety of cytoplasmic inclusions of different

sizes are seen in the organ cell. Some of them, with a fairly granular matrix of moderate electron density, are frequently observed to contain electron dense materials of various shapes (Fig. 20). Other inclusions have a highly electron dense matrix bounded by a limiting membrane. The larger ones among such inclusions (about 2–3  $\mu$  in diameter) contain amorphous materials and several electron dense granules in the matrix (Fig. 21), whereas the smaller ones, granules of about 0.2–0.4  $\mu$  or less in diameter, are found in clusters (Fig 21, arrow). None of these organelles carry out ultrastructural changes during the molting cycle.

### Discussion

Two facts emerge from our studies. First, the three species of macruran decapods used in the present study, *Palaemon paucidens*, *Pandalus kessleri* and *Procambarus clarkii*, possess two kinds of paired organs in the cephalothorax region, of which one is obviously homologous to the Y organ and the other to the mandibular organ both originally described in brachyuran decapods. Second, both of these organs show histologically a clear cyclic change in parallel to the molting cycle. Experimental data have been accumulated to support the view that crustecdysone or its analogues are responsible for completion of molting in many crustaceans, but at present we lack the information on the site of synthesis of such substances. Our studies on the ultrastructure of these two organs seem to give a clue to this question.

The mandibular organ cells manifested no remarkable changes during the molting cycle but some extraordinarily hypertrophied mitochondria and the indented vesicular form of the smooth ER in some cells of early premolt *Palaemon*. The Y organ cells, on the other hand, showed marked changes during the cycle. In the intermolt (stage C) to early premolt (stage D<sub>0</sub>–D<sub>1</sub>" ) cells, the size of mitochondria and the number of cristae increased greatly and the smooth ER turned into vesicular in form. As these are the characteristics considered to show a high cellular activity (Watanabe, 1957; Kahri, 1968), the Y organ cells in early premolt stages are thought to be in their highest state of activity. In view of the fact that these ultrastructural changes in the Y organ precede all the morphological and physiological events that occur in the epidermis and hepatopancreas of *Palaemon* at the premolt stage, it is most likely that the Y organ is the site of synthesis of the molting hormone.

The Y organ cells in *Palaemon* are characterized by mitochondria with tubular cristae, highly developed smooth ER, rich free ribosomes and rather inconspicuous Golgi complexes, and have a close resemblance to some mammalian steroid synthesizing cells such as the testicular interstitial cells (Christensen, 1965), the adrenocortical cells (Friend and Brassil, 1970) and the lutein cells (Enders, 1973). Moreover, type II inclusions found in the Y organ cells are very similar to the "cap-like structure" observed in guinea pig interstitial cells, which seems to participate in the steroid synthesis (Christensen, 1965). The Y organ in crustaceans,

therefore, is highly probable to be the site of synthesis of a steroid, molting hormone.

It is noteworthy, however, that the smooth ER in the Y organ cells take either vesicular or undulated tubular form, somewhat different in appearance from the branched tubular form of that in the mammalian steroid synthesizing cells. The difference may reflect the fact that the mammalian steroid hormone is synthesized *de novo* with an aid of several enzymes contained in the smooth ER (Christensen, 1965), whereas in arthropods steroids are synthesized by conversion of the cholesterol from outside (see Herman, 1967). King (1969) is of the opinion that the function of the ecdysial gland is to supply the blood with necessary enzyme(s) which are involved in the peripheral conversion of the precursor substance to the active molting hormone, crustecdysone. However, in the *Palaemon* Y organ cells development of the rough ER is much less extensive throughout the molting cycle so that the possibility of enzyme production of this organ is not feasible. Nevertheless, our results support the view that the Y organ and not the mandibular organ is the site of synthesis of molting hormone in *Palaemon*.

If this view is the correct one, further studies are needed for elucidation of the releasing and storing mechanism of the hormone from this gland. Recently, Carlisle and Connick (1973) found in the crayfish *Orconectes propinquus* that a gland which is situated in the antennary segment contained a substance with the biological activity of crustecdysone, and that its activity was the highest in late proecdysis. Our examination of the antennary segment area resulted in finding no such a "gland" but a pair of green glands in all the three macrurans used. If their gland is actually a portion of the excretory organ, then it would be natural that the hormone that had 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.

### Summary

1) Two kinds of paired organs, the Y organ and the mandibular organ, were described in three macruran decapods, *Palaemon paucidens*, *Pandalus kessleri* and *Procambarus clarkii*. The former is located in the junction of the two cephalothoracic chambers, the prebranchial and the branchial ones, and the latter near the joint of the mandibular and the first maxillary segments.

2) Both organs showed a cyclic change, with their organ cell cytoplasm being increased in amount during the premolt (St. D) and decreased during the postmolt (St. A-B) stage.

3) The Y organ cells exhibit during the period from the intermolt (St. C) stage to the early premolt (St. D<sub>0</sub>-D<sub>1</sub>"") stage remarkable changes such as a great increase in size of mitochondria and in number of their cristae, and the transformation of smooth ER from tubular to vesicular form. On the other hand, only minor changes are noticed in some mandibular organ cells at early premolt (St. D<sub>0</sub>-D<sub>1</sub>"") stage.

4) These results are discussed in relation to the possible site of production of the molting hormone in macruran crustaceans.

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## Explanation of Plates XVII-XXII

Fig. 1. Anterior part of the Y organ tissue of *Palaemon* at postmolt stage (St. A), which is situated in the epidermal shelf of inner wall of the branchiostegite. Sagittal section. Hematoxylin-eosin.  $\times 200$ . BS, blood sinus; Ep, epidermis; YO, Y organ.

Fig. 2. The Y organ tissue of *Pandalus* at intermolt stage (St. C<sub>b</sub>). Note that the tissue is infolded and contains many lumens. Sagittal section. Hematoxylin-eosin.  $\times 200$ .

Fig. 3. Horizontal section through the mandibular organ of *Palaemon* at postmolt stage (St. A). Anterior is to the bottom. Hematoxylin-eosin.  $\times 100$ . BS, blood sinus; Ep, epidermis; MO, mandibular organ.

Fig. 4. The mandibular organ tissue of *Pandalus* at intermolt stage (St. C<sub>b</sub>), showing close resemblance to that of *Palaemon* (Figs. 7 and 8). Hematoxylin-eosin.  $\times 400$ .

Fig. 5. The Y organ tissue of *Palaemon* at intermolt stage (St. C<sub>a</sub>). Note that the organ cell has a nucleus stained deeply with hematoxylin and a rare cytoplasm. Many inter-lobular spaces are also noticed (arrows). Hematoxylin-eosin.  $\times 400$ .

Fig. 6. The Y organ tissue of *Palaemon* at late premolt stage (St. D<sub>3</sub>). Organ cells are much increased in size and no interlobular space is seen (compare with those in Fig. 5). Hematoxylin-eosin.  $\times 400$ .

Fig. 7. The mandibular organ cells of *Palaemon* at late premolt stage (St. D<sub>3</sub>). Amount of the cytoplasm is most conspicuous (compare with the cells shown in Fig. 8). Hematoxylin-eosin.  $\times 400$ .

Fig. 8. The mandibular organ tissue of *Palaemon* at postmolt stage (St. A). Hematoxylin-eosin.  $\times 400$ .

Figs. 9-21. Electronmicrographs of the Y organ cell and the mandibular organ cell in *Palaemon*. BL, basement lamina; BS, blood sinus; CM, cell membrane; ER, endoplasmic reticulum; G, Golgi complex; In-I, type I inclusion; In-II, type II inclusion; IS, intercellular space; Mt, mitochondria; N, nucleus; Nu, nucleolus.

Fig. 9. The Y organ cell at postmolt stage (St. A). Arrows point infolding of the cell membrane.  $\times 9,000$ .

Fig. 10. The Y organ cell at mid premolt stage (St. D<sub>2</sub>), containing tubular endoplasmic reticula (ER) and rod to round mitochondria (Mt).  $\times 13,300$ .

Fig. 11. Large round mitochondria (Mt) and vesicular endoplasmic reticula (ER) in a Y organ cell at intermolt stage (St. C<sub>a</sub>).  $\times 16,500$ .

Fig. 12. Type IV inclusion (arrow) in a Y organ cell at late premolt stage (St. D<sub>3</sub>).  $\times 11,900$ .

Fig. 13. The Y organ cell at mid premolt stage (St. D<sub>2</sub>), containing cisternal endoplasmic reticula (ER) and rod-shaped mitochondria (Mt). Almost all the ribosomes are separated from the membrane.  $\times 20,900$ .

Fig. 14. Type II cytoplasmic inclusion (In-II) in a Y organ cell at mid premolt stage (St. D<sub>2</sub>). Note its point of connection to the endoplasmic reticula (ER) (arrow).  $\times 11,900$ .

Fig. 15. Type III inclusions (arrows) in the Y organ cells with dark cytoplasm at mid premolt stage (St. D<sub>1</sub>'').  $\times 11,200$ .

Fig. 16. The mandibular organ cell at postmolt stage (St. B). Note that some mitochondria (Mt) have concentric cristae (arrows).  $\times 9,000$ .

Fig. 17. Interdigitation of the contiguous mandibular organ cells. Cristae of the mitochondria run parallel to the long axis.  $\times 13,300$ .

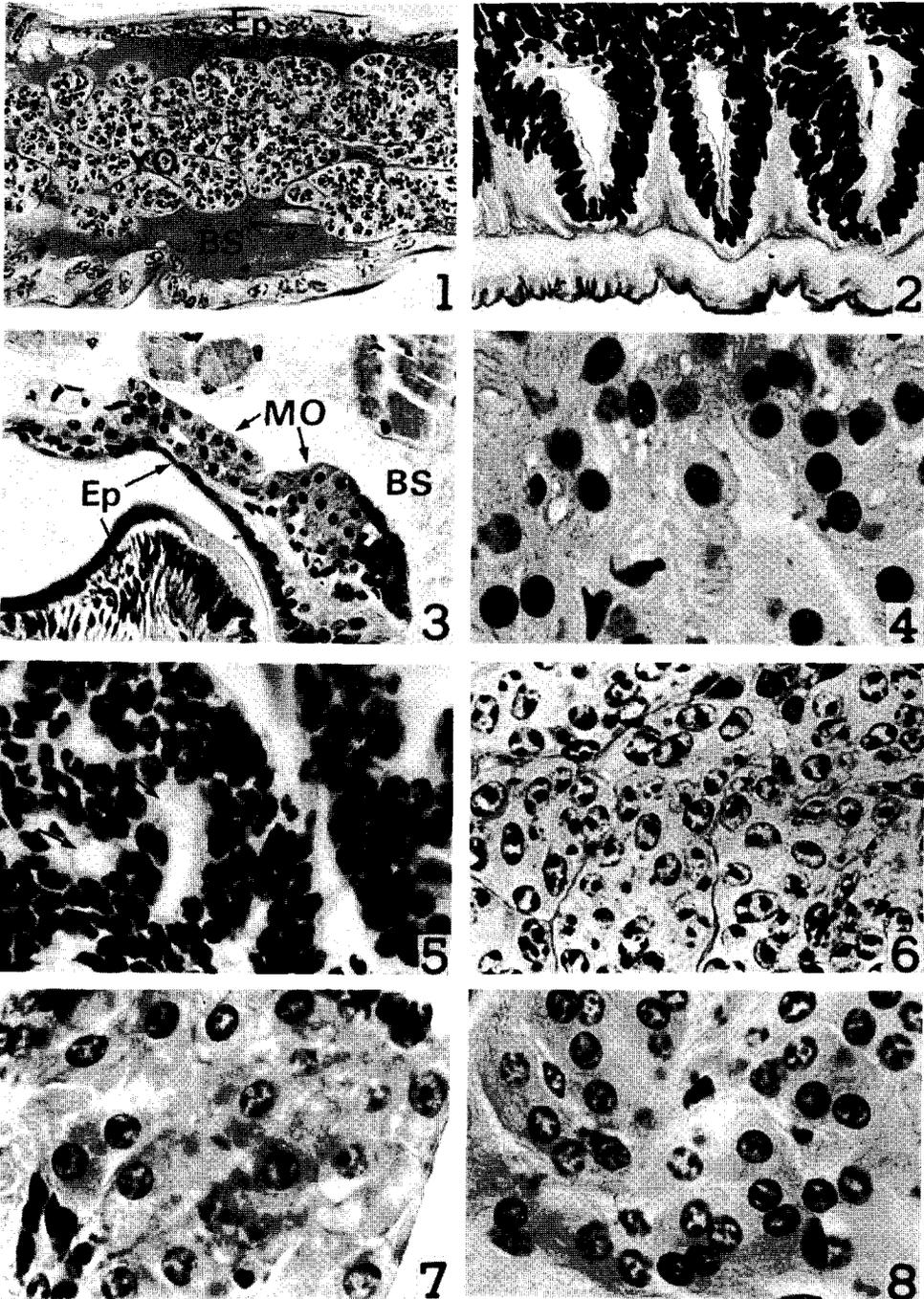
Fig. 18. The indented endoplasmic reticula (arrows) in a mandibular organ cell at early premolt stage (St. D<sub>1</sub>').  $\times 14,200$ .

Fig. 19. Highly swollen mitochondria (Mt) and indented endoplasmic reticula (ER) in the mandibular organ cells at early premolt stage (St. D<sub>1</sub>').  $\times 4,750$ .

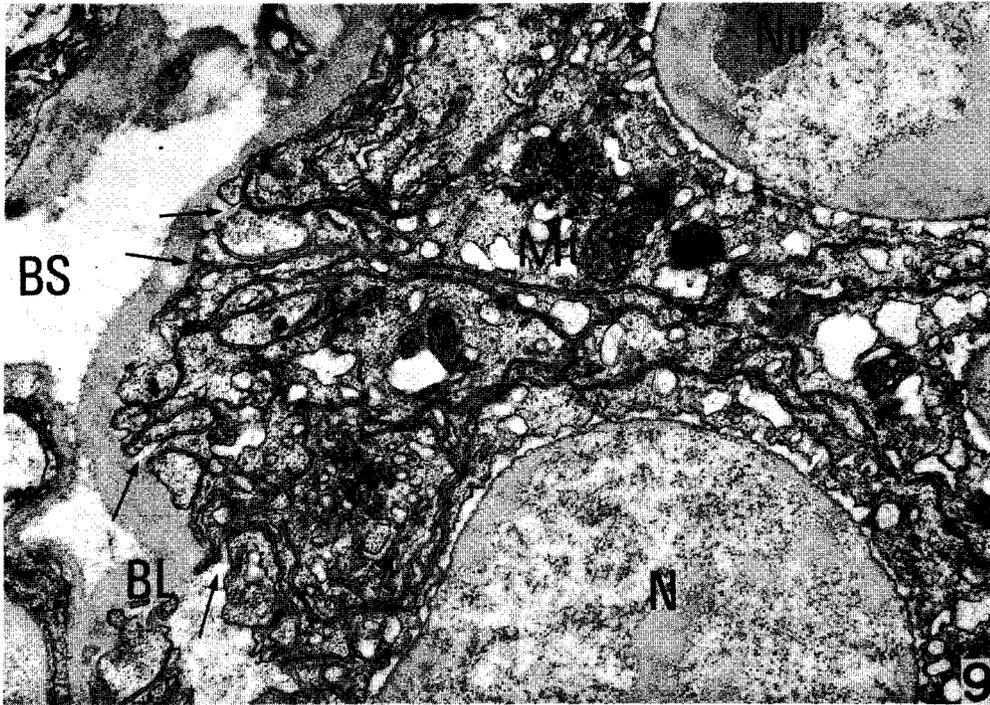
Fig. 20. A mandibular organ cell at postmolt stage (St. B), which includes a variety of cytoplasmic inclusions of different sizes (arrows). For further explanation, see text.  $\times 25,700$ .

Fig. 21. A mandibular organ cell at intermolt stage (St. C<sub>a</sub>). Note a large electron-dense inclusion (LDI) and a nearby cluster of small granules (arrow).  $\times 11,400$ .

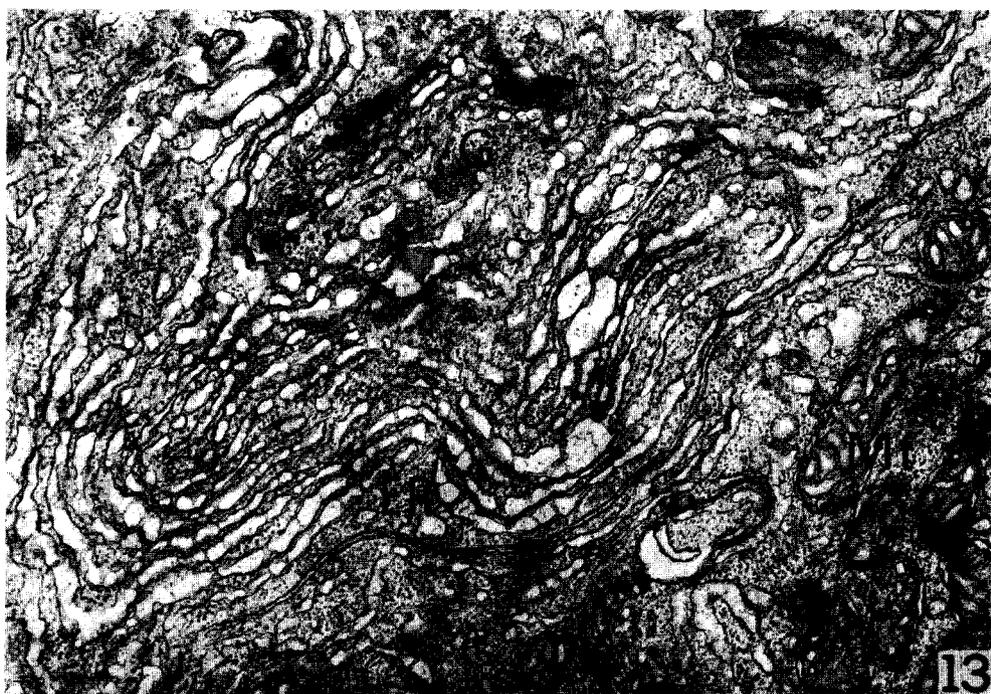
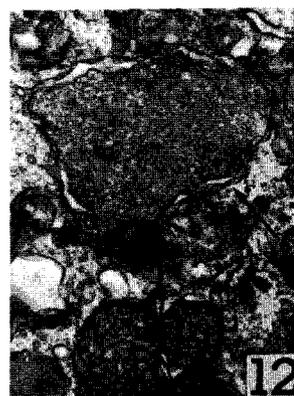
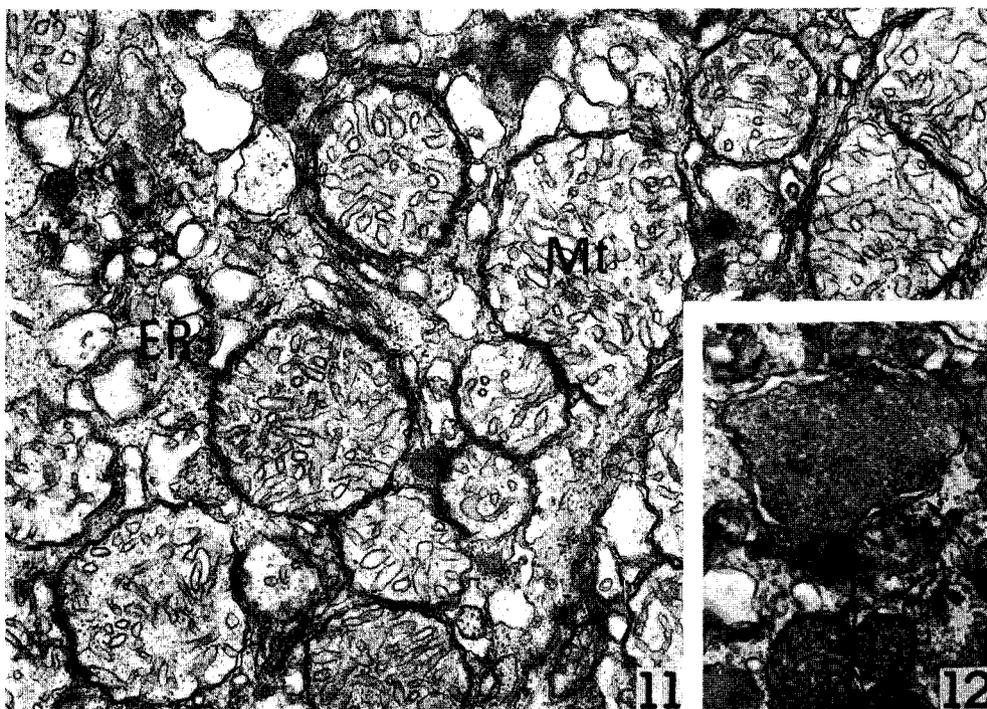
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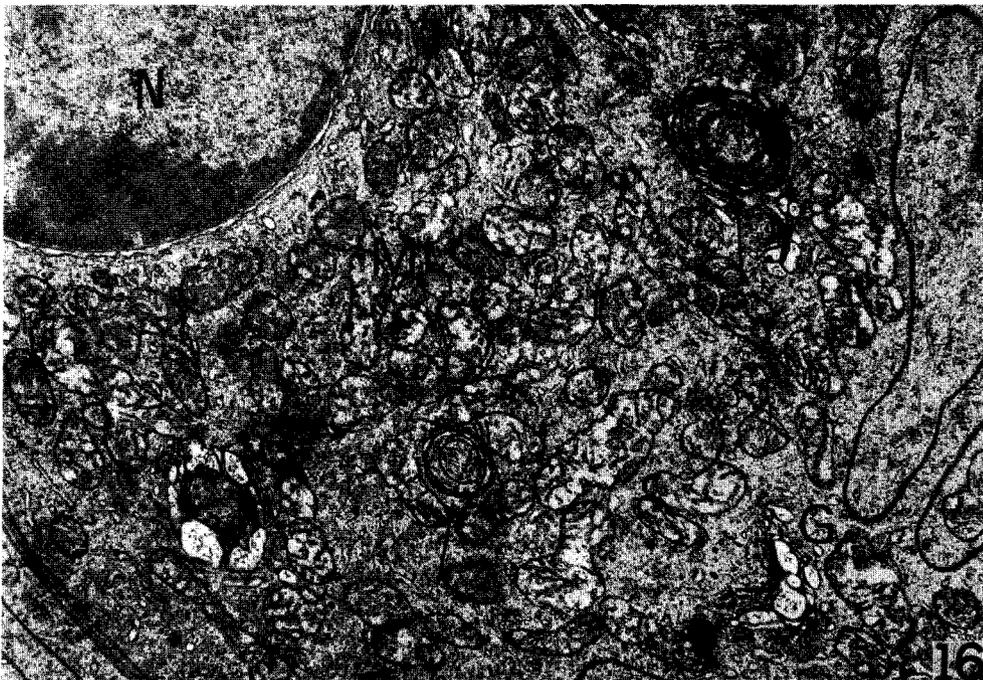
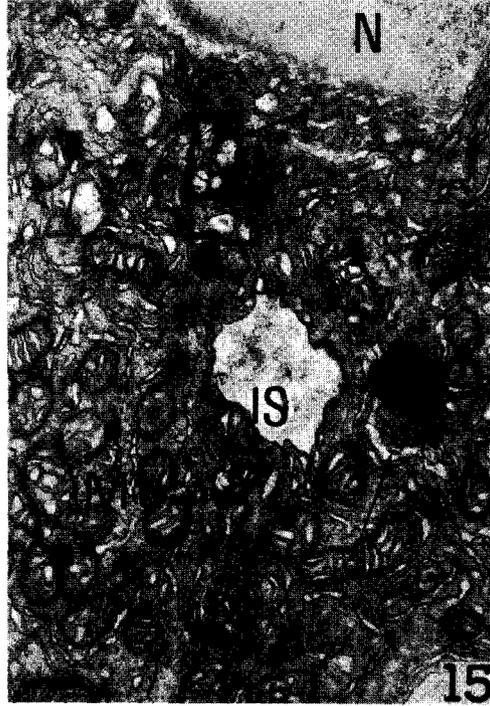
*T. Aoto, Y. Kamiguchi and S. Hisano: Y Organ and Mandibular Organ*



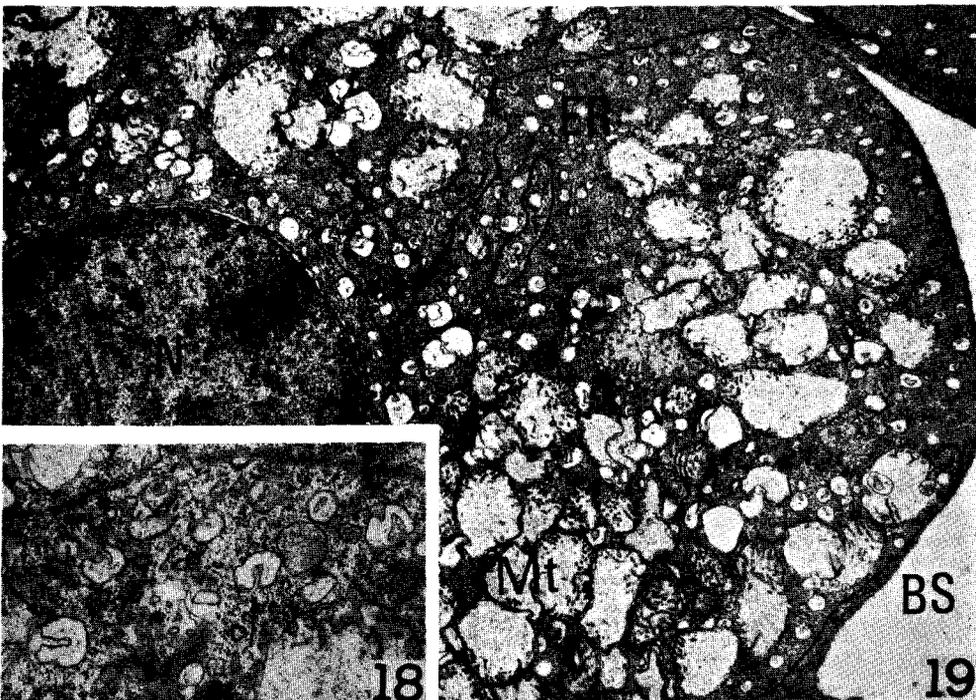
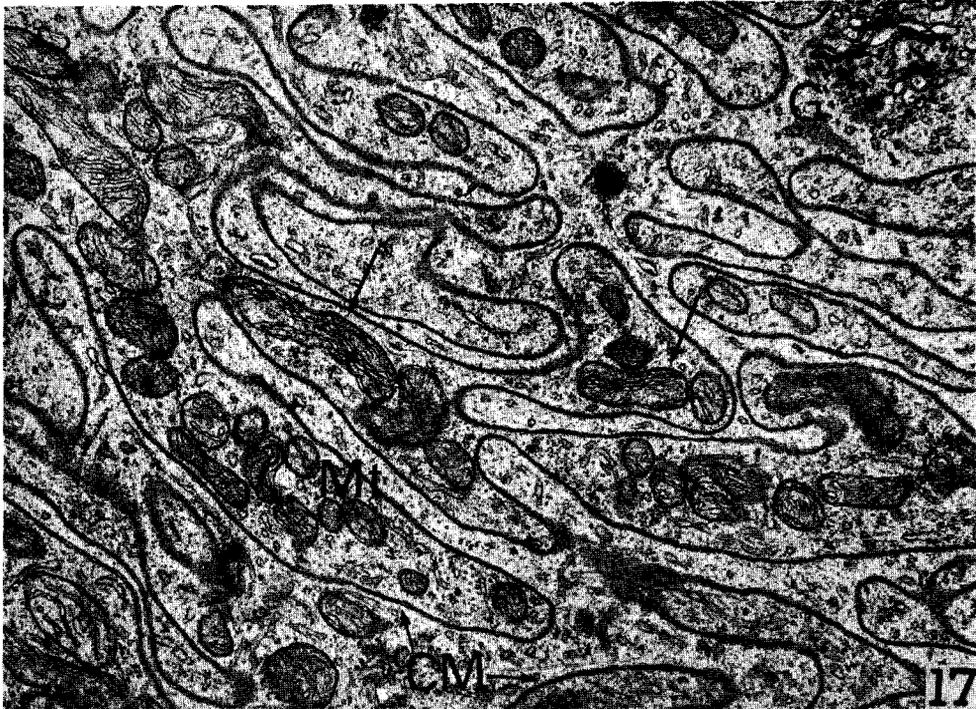
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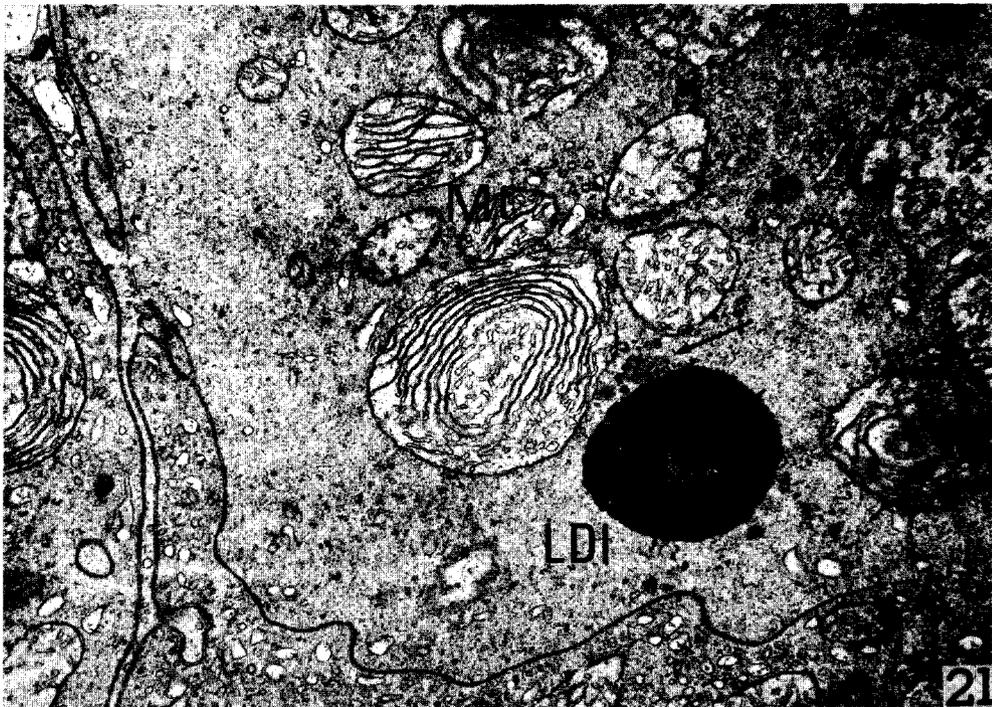
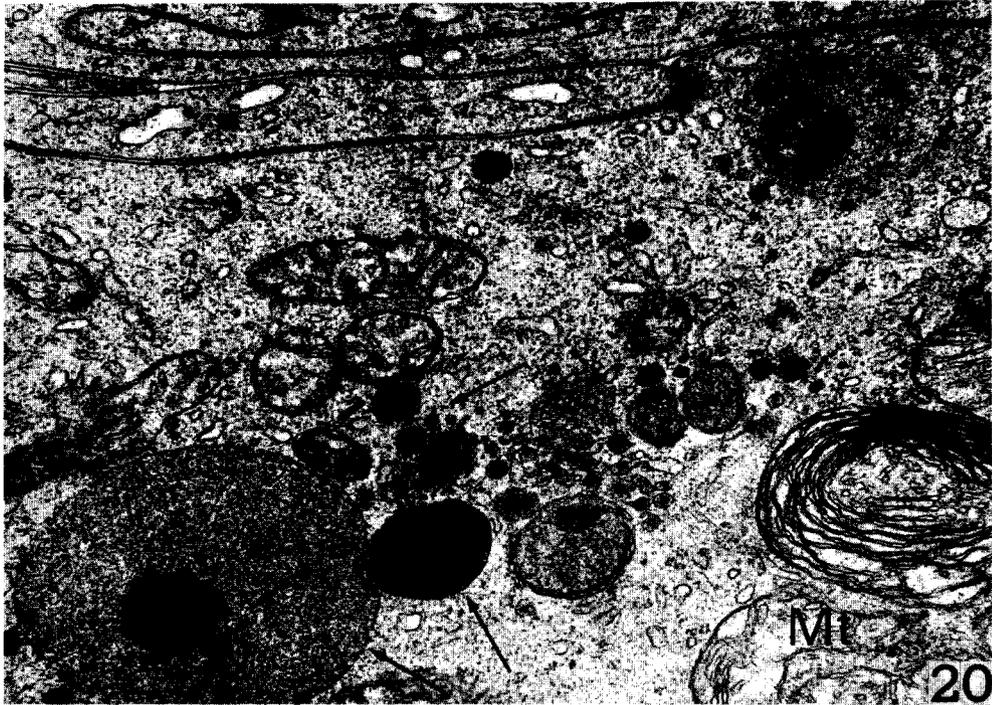
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