A Histological Study of the "Sternal Gland" in the Female Freshwater Prawn, *Palaemon paucidens*, a Possible Site of Origin of the Sex Pheromone[1)]

By

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(With 4 Text-figures, 2 Tables and 2 Plates)

Although accumulated data have shown that in crustaceans, as in insects, the sex pheromone from females plays an important role in initiating mating behavior in males, the site of origin of such substances has never been reported.

In the course of a study concerning the annual reproductive cycle of the female freshwater prawn, *Palaemon paucidens*, existence of a new type of rosette gland in this species came into the present author's notice. The gland, situated on the base of the last three pairs of coxopodites of pereiopods and ventrally beneath the thoracic sternum of adult females, apparently differs in regard to the site and morphology from other rosette glands such as the tegumental gland and cement gland that have been reported to occur in decapod crustaceans. In order to distinguish this gland from other glands the new gland is designated as the "sternal gland" in the present paper. The gland, in view of the annual cycle of synthesis and deposition of its secretory product, seems most likely the one closely related to the sex attractant in this species.

It is the purpose of the present paper to describe the gross anatomy and histology of the sternal gland. The growth process and annual secretory cycle of the gland are also presented of the animals during the period from April 1970 to July 1971. Further, the functional significance of the gland will be discussed.

Materials and Methods

Mature and immature specimens of the freshwater prawns, *Palaemon paucidens*, were collected in a stream in the vicinity of Sapporo, and stocked in aerated aquaria before use. The carapace length from the posterior edge of the orbit to the posterior-most point of the

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carapace was measured to represent the size of the animals. Intermolt stage of the specimens was determined according to the method previously described by the author (Kamiguchi, 1968).

For the histological study, animals of different sizes in various seasons of a year were fixed *in toto* and their sternal glands were obtained by removing posterior part of the thoracic sternum. For a comparison other rosette glands situated in various parts of the body were also studied histologically. They were embedded in paraffin and sectioned at a thickness of 8μ. Staining properties of the secretory material of these glands were investigated with the aid of following stains: 1) Delafield's hematoxylin and eosin, 2) Heidenhain's Azan, 3) Gabe's aldehyde fuchsin (AF), 4) 0.05% toluidine blue buffered at pH 4, and 5) Alcian blue buffered at pH 2.

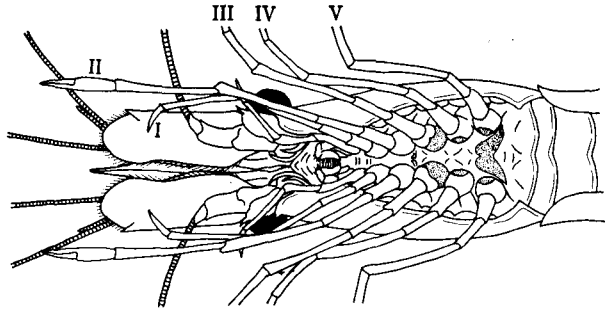
For the demonstration of lipid, these glands were fixed in Ciaccio solution followed by seven days of post-chromation period in 3% potassium bichromate. Sections were stained with Sudan black B saturated in 70% ethanol.

For the detection of glycogen, periodic acid-Schiff (PAS) reaction following pretreatment with saliva was employed after fixation in Gendre's solution.

Observations

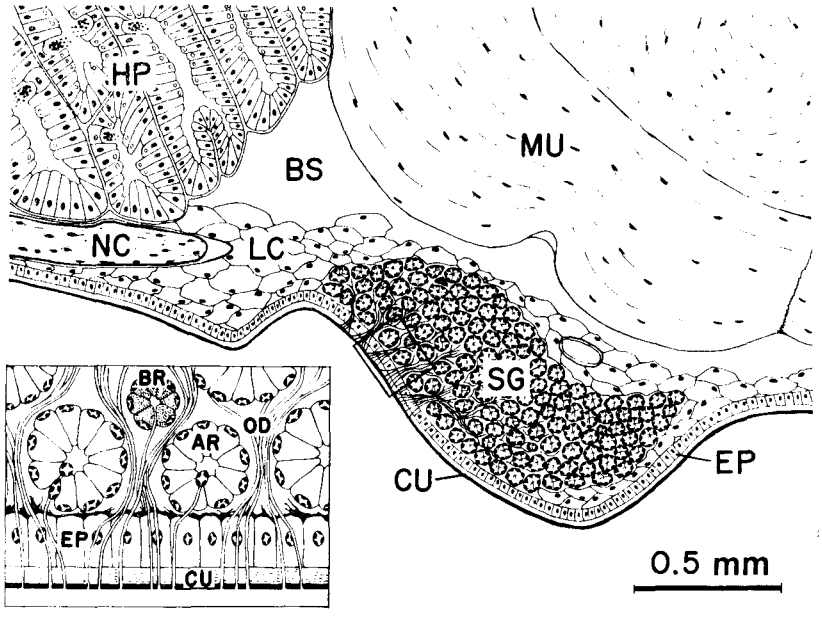
Gross anatomy and histology

The sternal gland of *Palaemon paucidens* consists of roughly 600–800 glandular structures which are made up from seven groups located separately in the inner side of the pairs of coxopodites of the 3rd, 4th and 5th pereiopods and beneath the



Text-fig. 1. Ventral view of the cephalothorax of mature female *Palaemon* in May. The sternal glands are located under stippled portions of the sternum.

thoracic sternum between a pair of 5th pereiopods (Text-fig. 1). In live specimens just before the parturial molt the gland can be identified through the cuticle by its milky white coloration (Fig. 1, Plate XIII), but it is hardly distinguishable in animals after the end of breeding season (Fig. 2). Histologically, each glandular structure or a rosette consists of ten to fourteen rosette cells that are arranged concentrically in a row and an outlet duct which extends from the central lumen



Text-fig. 2. Diagrammatic representation of the ventro-posterior part of the cephalothorax of a female *Palaemon*, 2 hours following parturial molt. Sagittal section. Anterior to the left. Inset, a higher magnification of the same section, showing a portion of the sternal gland. Note that the α-rosette is devoid of secretory product. AR, α-rosette; BR, β-rosette; BS, blood sinus; CU, cuticle; EP, epidermis; HP, hepatopancreas; LC, Leydig cells: MU, muscle; NC, ventral nerve cord; OD, outlet ducts of the rosettes.

of a rosette to outside through the epidermis and cuticle (Text-fig. 2 and Figs. 3 and 4). Of the group of glands located most posteriorly beneath the thoracic sternum, the outlet ducts tend to open in the anterior part of the gland area (Text-fig. 2), but such an assemblage of the duct openings is not observed in the remaining six groups of glands. Each rosette cell has a spherical nucleus in the periphery (Fig. 3).

Two types of rosettes (α- and β-rosette) can be distinguished in the gland according to tinctorial properties of their secretory materials (Fig. 5). Staining properties of the secretory materials of the α- and β-rosette glands and those of the cement gland are summarized in Table 1, and it is suggested that the α-rosette is rich in neutral mucopolysaccharides or mucoprotein but not rich in glycogen or lipid, whereas the β-rosette contains acid mucopolysaccharides with sulphurid group but neither glycogen nor lipid. The gland is principally composed of the α-rosettes. The β-rosettes, consisting only 5% of the whole rosette population, are seen only sparsely scattered in the gland. No intermediate form between the two types of rosettes is seen in the gland throughout the year.

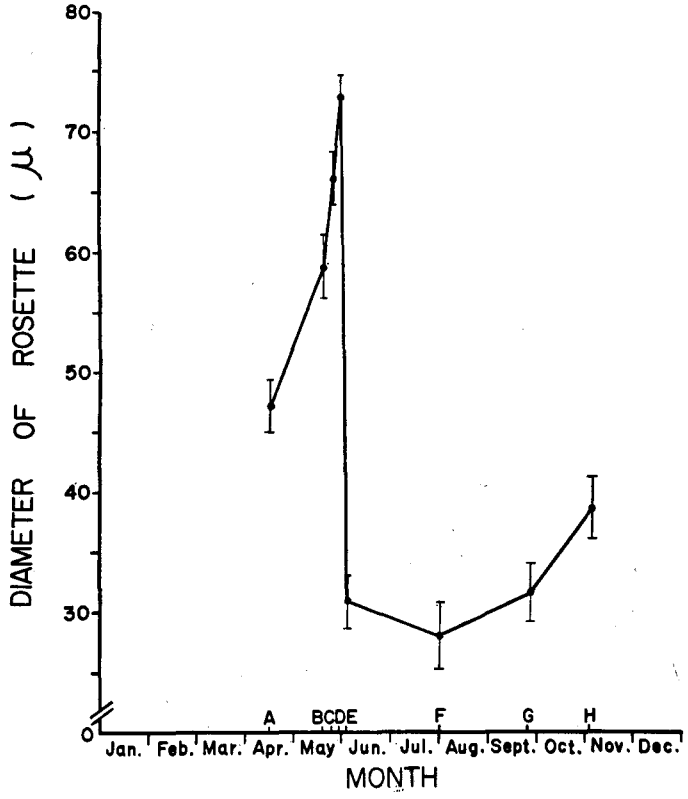
Development

The gland does not exist in males of all sizes in any season of a year. No trace of glandular structure in the corresponding portion of the body is detected in females smaller than 7 mm in carapace length. The first rosettes appear in the females, about 7–8 mm in carapace length, in June. The number and size of rosettes increase as the prawns grow. The fully developed sternal gland is seen in sexually mature females larger than 10 mm in carapace length in May, with the rosette cells containing abundant secretory granules. Growth of the gland is apparently correlated with the gonadal maturation of the animals, as will be described below.

Annual cycle of secretory activity of the gland

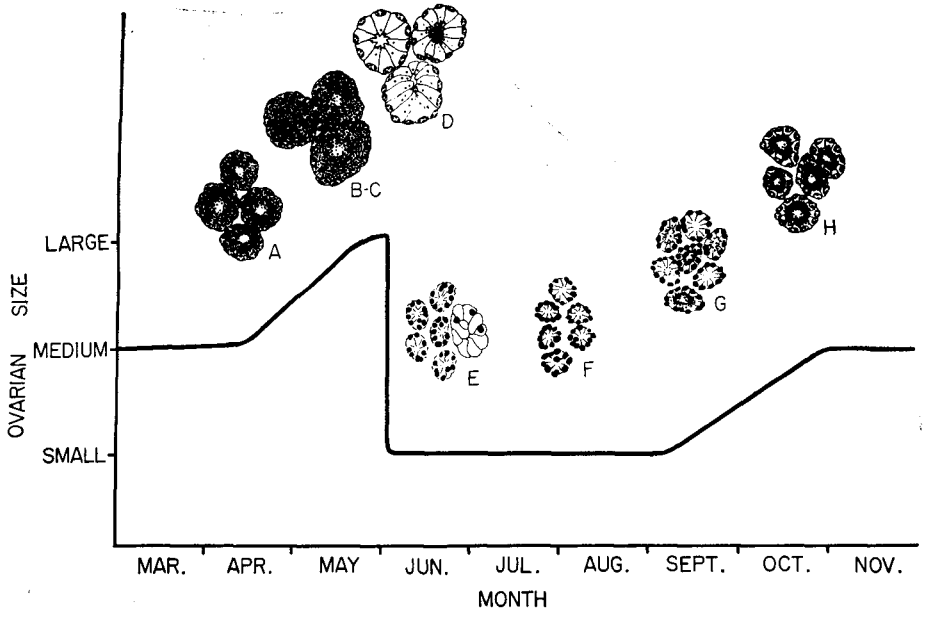
The size of the rosettes and their secretory activity were studied histologically in female prawns throughout a year except for a few winter months. Only the α-rosettes showed a clear cyclical change in secretory activity which is closely correlated with the annual reproductive cycle (Text-figs. 3 and 4). No change was found to occur in the β-rosettes throughout a year. There was no difference in histological features among the seven separate groups of glands.

In the summer months (July and August) soon after the end of the breeding season, all the fully grown females possess regressed ovaries, about one-fourth or less in size of the ones just before egg-laying. The α-rosettes in such females are the smallest throughout the year, measuring $28.0\pm2.9\mu$ in diameter, and they contain no secretory material at all (Fig. 6). The females at late September have larger rosettes ($31.6\pm2.4\mu$ in diameter) containing a small amount of secretory material in the apex of each cell (Fig. 7). Their ovaries, having no yolk granules in the oocytes yet, attain the size approximately one-half of those fully developed ones. Most females at early November possess the ovaries about one-half the size of those just before egg-laying, accumulating a small amount of yolk granules in the oocytes. These animals have the sternal gland consisted of the rosettes measuring $38.7\pm2.6\mu$ in diameter and containing a considerable amount of secretory granules (Fig. 8). In the females at late April, about a month before the beginning of breeding season, with the ovaries about two-third in size of those just before egg-laying, each rosette is $47.2\pm2.6\mu$ in diameter containing a large amount of secretory granules (Fig. 9). Females with fully developed ovaries in mid-May have rosettes measuring $58.9\pm2.6\mu$ in diameter at stage C or $66.3\pm2.2\mu$ in diameter at stage D_3 of an intermolt cycle preceding the parturial molt, both containing abundant secretory granules (Fig. 10, Pl. XIV). Five minutes after the parturial molt, the rosettes become maximum in size ($72.9\pm1.8\mu$ in diameter), but the granular appearance of their secretory material becomes obscure and their tinctorial affinity to PAS decreases considerably (Fig. 11). As the rosette's outlet duct which opens externally through the cuticle stains deeply with PAS during this particular period, the secretory material of the rosettes is most likely to be



Text-fig. 3. A change in diameter of the α-rosette in the prawns collected from April to November. Each point represents the average value obtained from five animals with standard deviation. A, females collected on April 21; B, females collected on May 16, being in stage C of an intermolt cycle just before the parturial molt; C, females collected on May 16, being in stage D_3 of a cycle just before the parturial molt; D, females 5 minutes after the parturial molt; E, females 3 days after the parturial molt; F, females collected on July 14 and August 7; G, females collected on September 28; H, females collected on November 3.

actively discharged immediately following the parturial molt. In females thirty minutes after the parturial molt, secretory product of the sternal gland is seen only in the central part of the rosette (Fig. 12). Two to eight hours following the parturial molt, the rosettes are still hypertrophied but contain no secretory material (Fig. 13). Three days after the parturial molt, the sternal gland consists of the rosettes markedly regressed in size ($30.1 \pm 2.2\mu$ in diameter) and a few larger rosettes, possibly degenerating ones (Fig. 14). In the females 6 days after the parturial molt, the gland consists of only the regressed rosettes (Fig. 15), the situation being very much similar to the gland in summer animals.



Text-fig. 4. Diagrammatic representation of the annual secretory cycle of the α-rosettes in the freshwater prawn, *Palaemon paucidens*, correlated with the annual cycle of the ovarian development. The curve of the ovarian growth is based on the results of the present author's previous study (Kamiguchi, 1971). The glands (A-H) are from the animals with corresponding letters in Text-fig. 3, respectively.

Comparison of the sternal gland with other exocrine glands

Comparison of the sternal gland and other several types of rosette glands that have been previously described in crustaceans is summarized in Table 2. In *Palaemon*, rosette structures are found in portions of the body corresponding to

Table 1. Comparison of the staining properties of the secretory products of the α- and β-rosettes, and of the cement gland cells.

Stain	*α-rosette*	*β-rosette*	*Cement gland*
Delafield's hematoxylin and eosin	pink	blue	faintly blue
Heidenhain's Azan	orange	blue	blue
Gabe's AF	+	++	++
Toluidine blue (pH 4)	blue	purple	purple
Alcian blue (pH 2)	−	+	+
PAS	++	±	+
PAS after saliva treatment	++	±	+
Sudan black B	−	−	−

Table 2. List of different types of rosette glands in Crustacea.

Glands	*Animals*	*Location of the gland*	*Possible function*	*Authors*
Cement gland	Crayfish, *Cambarus affinis*	ventrally beneath the abdominal sterna and in the pleopods	production of the cement substance to attach the eggs on the pleopods	Andrews (1904)
Tegumental gland	Lobster, *Homarus vulgaris*	beneath the integument of the entire body	secretion of epicuticular materials	Yonge (1932)
Circum-orbital gland	Prawn, *Pandalus kessleri*	in the eyestalks just beneath the eye proper, surrounding the lamina ganglionalis	unknown	Aoto & Nishida (1956)
Mucopolysaccharide gland	Isopod, *Armadillidium vulgare*	in the tips of the mouthparts	secretion of lubricating materials to aid swallowing	Stevenson & Murphy (1967)
Sternal gland	Prawn, *Palaemon paucidens*	in the inner side of coxopodites of the 3rd–5th pereiopods and beneath the thoracic sternum between a pair of 5th pereiopods	production of the sex pheromone	Kamiguchi (present paper)

loci of the tegumental, the circum-orbital and the mucopolysaccharide glands. However, all of these rosette glands are present in both sexes, and they show no cyclical change of activity related to female reproductive cycle, staining blue with Azan or metachromatically with toluidine blue. Further, a rosette gland that seemingly corresponds to the cement gland is located in the pleopods of female *Palaemon* and it does show a cyclical change of activity. But, the staining properties (Table 1) and, more significantly, the time of release of the secretory product apparently differ between the two. The secretory material of the sternal gland is discharged almost completely within about 30 minutes after the parturial molt, while most of the secretory material is retained in the cement gland even 5 hours after the parturial molt (Fig. 16). In the latter, the product is discharged gradually within the following two days and, containing no secretory material the gland is entirely regressed 6 days after the parturial molt (Fig. 17). These results indicate that the sternal gland differs structurally, and therefore functionally, from other rosette glands.

Discussion

The facts that the sternal gland contains maximum amount of secretory product just prior to the parturial molt and that the whole product is released

within about 30 minutes after the parturial molt by the female may indicate that the gland exerts its function in regard to a certain reproductive event which takes place during this particular period. It has been demonstrated that male *Palaemon* are attracted to and copulate with the females soon after the parturial molt, suggesting release of the sex attractant from such females (Kamiguchi, 1972). The exuvial fluid, supposedly diffusing in water from a female at the ordinary molt, is not effective as a sex attractant, so that there must be a gland which is responsible for production and liberation of such substance. This hypothetical gland in copulation-ready females must have a secretory activity which is closely related to the parturial molt. Also its product must be released to external medium within about 30 minutes after the parturial molt, since the males are attracted to the female only for this limited period of time. The sternal gland which has been described here is just the one that satisfies these conditions.

In our preliminary experiments to confirm the role of this gland as the site of production of sex attractant, it was tried to either cauterize the gland or seal it with paraffin to block the outflow of the secretory product of the sexual mature females. However, because of the gland's complicated arrangement the operations were not performed successfully and when the male's copulatory response to such females was tested no conclusive results were obtained. In another preliminary experiment, crude extracts of the gland from mature females were prepared and the male animal's copulatory response to the extracts was tested. However, not only the test animals (males) but also the control animals (females) were attracted to the extracts, suggesting that the Phase I response (searching) to the extracts was brought about by their non-sexual, perhaps nutritional, factor rather than by the pheromone supposedly contained in the extracts. A further purification of the secretory product would be necessary for verification of the physiological function of the sternal gland as the site of synthesis and release of the sex pheromone.

The term "sternal gland", according to Lüscher and Müller (1960), was first used by Grassé (1949) to denote an exocrine gland that is situated in the fifth abdominal sternite of the termites. In *Kalotermes* and *Zootermopsis* the gland is said to secrete a trail pheromone (Lüscher and Müller, 1960). It is interesting to note that the crustacean sternal gland, as designated from its position in female *Palaemon*, is also very likely the site of production and release of a special kind of trail pheromone, the sex attractant.

Summary

1) A histochemical study was made on a new type of exocrine rosette gland situated in the inner side of paired coxopodites of the 3rd-5th pereiopods and beneath the thoracic sternum between a pair of 5th pereiopods in the freshwater prawn, *Palaemon paucidens*.

2) The gland, designated as the "sternal gland", is present neither in males of all sizes nor in immature females smaller than 7 mm in carapace length.

3) The gland is first seen in females about 7–8 mm in carapace length, enlarges as the animals grow, and develops fully in sexually mature females measuring 10 mm or larger in carapace length.

4) The gland consists of two types of rosettes, the α- and the β-rosettes, with different tinctorial properties. The α-rosettes, occupying about 95% of all the rosette population of the gland, show a clear annual cycle of secretory activity, whereas the β-rosettes do not indicate such a cyclic change throughout the year.

5) The α-rosette cells are in regressed state during the summer months, containing no secretory material. The amount of secretory material increases gradually through the fall months, reaches the maximum during the breeding season (May and June), and again becomes nil when the material is liberated into surrounding water within about 30 minutes following the parturial molt.

6) Possibility that the gland is the site of production and release of a sex pheromone which attracts males to copulation-ready females is argued.

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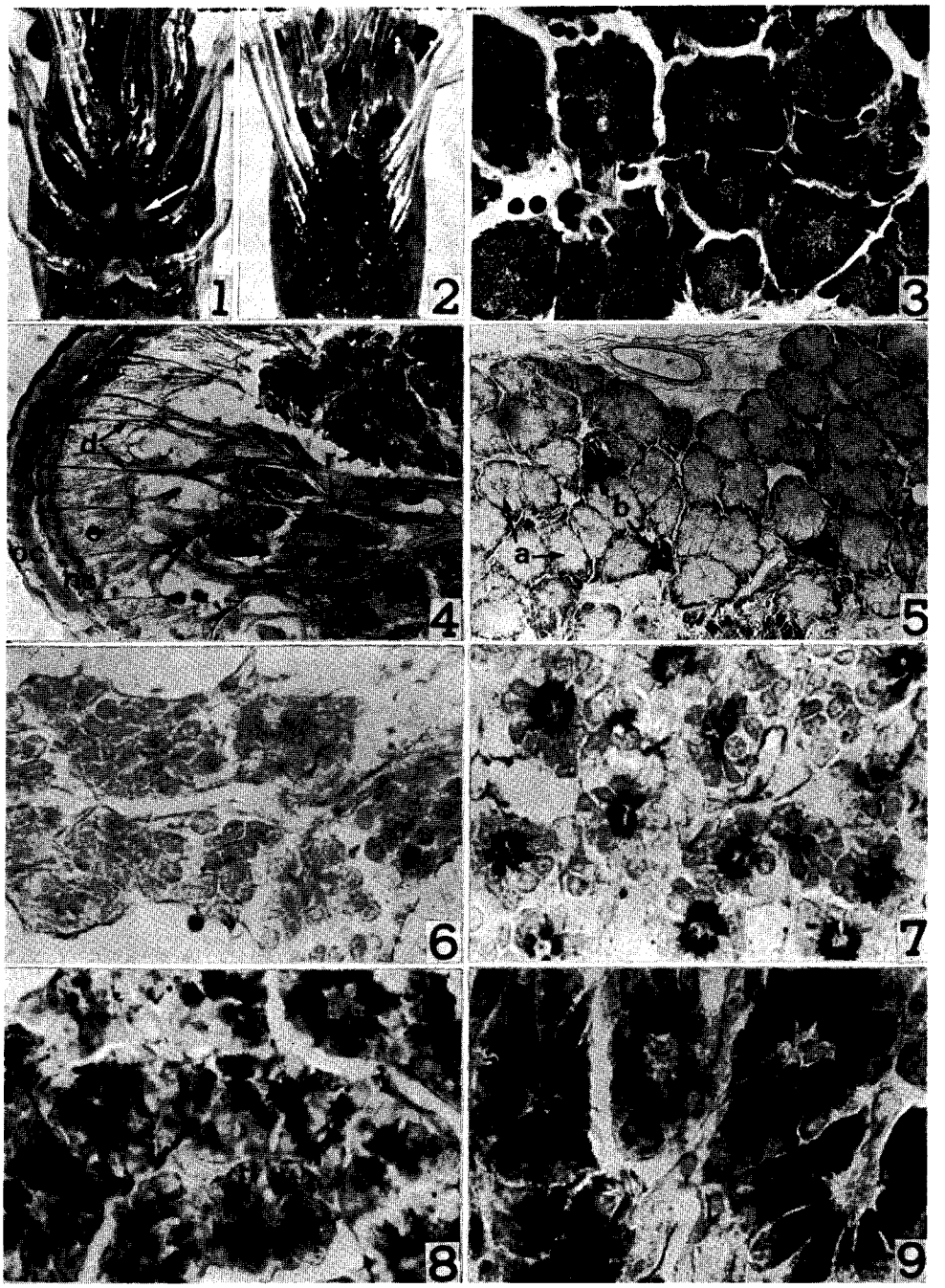

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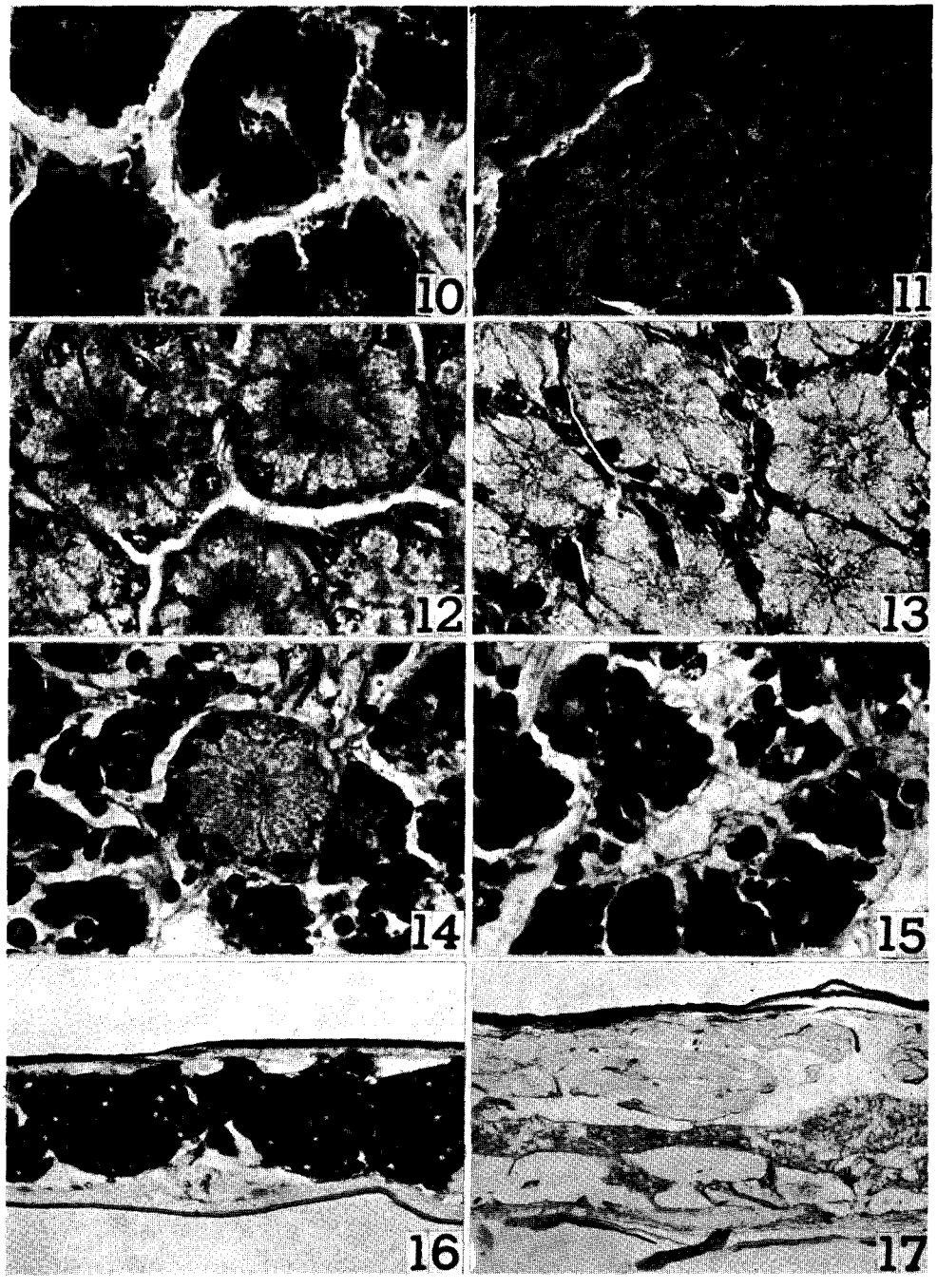

Y. Kamiguchi: Sternal gland in Palaemon

Explanation of Plates XIII and XIV

Figs. 1–2. Ventral view of the thoracic sterna of two female *Palaemon paucidens*. Live specimens. ×2.5.

Fig. 1. A prawn at stage D_3 of an intermolt cycle, just before the parturial molt, collected on May 16. The sternal glands (arrows) are distinguished through the cuticle for its milky white coloration.

Fig. 2. A prawn collected on July 14, about a month after the end of the breeding season. The sternal glands are not distinguishable.

Fig. 3. Section of a well-developed sternal gland of an animal in May. It consists of several rosette cells with the cytoplasm in granular appearance and a peripheral nucleus. Delafield's hematoxylin and eosin. ×200.

Fig. 4. Photomicrograph of a portion of sternal gland showing many outlet ducts (d) running from the α-rosettes (r) through the epidermis (e) and the newly formed cuticle (nc) under thick old cuticle (oc). Aldehyde fuchsin. × 200.

Fig. 5. The sternal gland of a prawn 1 hour after the parturial molt, containing a few β-rosettes (b) sparsely distributed among empty α-rosettes (a). Toluidine blue. × 100.

Figs. 6–15. Photomicrographs of the sternal gland at various stages of development. Figs. 6–10, PAS; Figs. 11–15, PAS-Mayer's hematoxylin. × 400.

Fig. 6. The gland of a female collected on July 14, consisted of regressive rosettes with no secretory material.

Fig. 7. The gland of a female collected on September 28, with the rosettes containing a small amount of secretory granules.

Fig. 8. The gland of a female collected on November 3, with the rosettes containing an increased amount of secretory granules.

Fig. 9. The gland of a female collected on April 21, consisting of hypertrophied rosettes with rich secretory granules.

Fig. 10. The gland of a prawn at stage D_3 of an intermolt cycle, collected on May 16, just before the parturial molt. The rosettes are full of secretory granules.

Fig. 11. The gland of a female five minutes after the parturial molt. Note that the rosettes are almost devoid of secretory granules and that their tinctorial nature is drastically changed.

Fig. 12. In a female 30 minutes after the parturial molt, the rosettes are almost empty, containing weakly PAS-positive materials in the apex of each rosette cell.

Fig. 13. In a female two hours after the parturial molt, the gland consists of still hypertrophied rosettes with no secretory material.

Fig. 14. In a prawn three days after the parturial molt, most of the rosettes are regressed except for a few less regressed ones.

Fig. 15. Six days after the parturial molt, all the female have regressive sternal gland.

Fig. 16. The cement gland of a female 5 hours after the parturial molt, containing a large amount of AF-positive material. Aldehyde fuchsin (AF). × 100.

Fig. 17. The cement gland of a female 6 days after the parturial molt, showing the regressive rosettes with no secretory material. AF. × 100.