Effect of Removal of the Eyestalks on the Growth and Maturation of the Oocytes in a Hermaphroditic Prawn, *Pandalus kessleri*[1]

By

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(*With 2 Text-figures and 1 Plate*)

Considerable work has been carried out on the physiological functions of the "eyestalk hormone" in the Crustaceans (Hanström, 1947; Brown, 1952). Its inhibitory action on ovarian growth was first demonstrated by Panouse (1943, 1944 and 1946), who found that removal of the eyestalks, or bilateral ablation of the sinus glands, accelerated unseasonable growth and maturation of the oocytes, and egg-laying, in the female shrimp, *Leander serratus*. Brown and Jones (1947 and 1949) were also successful in demonstrating similar ovarian control for the crayfish, *Cambarus immunis*, and for the fiddler crab, *Uca pugilator*. They concluded that in these animals, as in *Leander*, the sinus glands produce an ovarian-inhibiting hormonal factor, and that ovarian growth resulting from a release from this inhibition tends to vary in some manner with the size of the animal. Thus inhibition of the ovarian growth by the "eyestalk hormone" was confirmed by these authors, although subsequent observations suggested that the sinus gland serves merely as a storage and release site for material produced in other incretory organ (Bliss, 1951; Frost, Saloum and Kleinholtz, 1951; Passano, 1951a and b; Travis, 1951; Welsh, 1951).

Takewaki and Yamamoto (1951a) also obtained remarkable growth acceleration of the ovarian eggs following removal of the eyestalks in the shrimp, *Paratya compressa*, and they (1950 b) mentioned that smaller shrimps responded to the eyestalk removal more slowly than the larger ones. On this point, Otsu and Hanaoka (1951) found in the common fresh water crab, *Potamon dehaani*, that precocious differentiation of the ovaries following removal of the eyestalks took place only in animals which were heavier than 2.3 gr. in body weight.

In view of the facts above described, it seems most desirable to ascertain which organ is responsible for unseasonable growth of oocytes in eyestalkless

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animals, and whether these varying degrees of ovarian growth in different individuals are due to differing physiological conditions of the different sized animals used, or due to different physiological conditions in their incretory organs. To ascertain these problems the following experiments and observation were carried out using the prawn *Pandalus kessleri* Czerniavski, as material.

Before going further, the writers wish to express their cordial thanks to Professor Tohru Uchida, under whose guidance this work has been performed. The writers are also indebted to Dr. Kiichiro Yamamoto and Messrs. Fumio Iwata and Mitsuharu Miyawaki of the Akkeshi Marine Biological Station, Akkeshi, for their kindly aids extended to us in collecting and keeping the animals.

Material and methods

Since it has been demonstrated that *Pandalus kessleri* is a protandric hermaphrodite like many other pandalid prawns (Aoto, 1952), the experiments were performed upon either animals with male secondary sex characters or ones with female secondary sex characters. The animals used in these experiments were collected at Akkeshi Bay, on the eastern coast of Hokkaido, and were kept in large concrete tanks of about 40 cm. in depth supplied with constantly running sea water. The tanks were placed near the windows of the laboratory with indirect diurnal daylight but without electric lighting. Shortly after their being collected, animals had their eyestalks amputated bilaterally at their bases and were placed in one of the tanks other than the one containing normal control animals. They were fed with fresh fish during the course of the investigation and all showed good appetite. Mortality resulting from this operation was high in all cases ; more than about 20 percent of the experimental animals died within 24 hours after the operation. In due time, both experimental and normal control animals were sacrificed for dissection and for histological observation of the gonads. The fixative used was Bouin's fluid, and for staining Delafield's haematoxylin and eosin were employed.

Experiments

A. *Eyestalk removal in hermaphroditic males.*

On October 31, 1950, eyestalks were removed bilaterally from fifty-one animals which showed a distinct *appendix masculina*, a male secondary sex character (Aoto, 1952). The animals ranged from 13 mm. to 22 mm. in carapace length (or, 46 – 77 mm. in body length except for rostrum). Twenty-two, twenty-eight, thirty-five and forty-two days after the operation, animals were fixed and both spermatogenesis and oogenesis in the hermaphroditic gonad were examined under the microscope.

After removal of the eyestalk no detectable changes took place in the testicular tissue. Seminal tubules were filled with many spermatocytes in various stages, tightly surrounding the central ovarian part. Some animals, larger than 70 mm. in body length, had testicular parts in which spermioteleosis was in progress. It is interesting to note that in these larger animals the oocytes in the ovarian part were in general comparatively large in size. This was observed in both experimental and normal control animals. Almost all of the vasa deferentia were extremely shrunk and underdeveloped. In a few specimens there were found one or two spermatozoa in the vas deferens, but these seemed to be residues of the preceding breeding season.

In order to demonstrate the degree of acceleration of ovarian growth resulting from eyestalk removal, the oocyte diameters (the longest one and the one at right angles to the former) were measured for the ten largest oocytes of each ovary. The result is summarized in Table 1. It will be observed that there is no evidence to prove any acceleration of growth and maturation of the oocytes in the hermaphroditic gonad following removal of the eyestalks. The considerable difference of size of the oocytes in both experimental and normal control animals seems to be correlated to the differences of body length in different individuals, the larger animals having, in general, larger oocytes.

Table 1. Diameter of the oocytes in the ovarian part of the hermaphroditic gonad in male animals which had their eyestalks amputated bilaterally on October 31, 1950

Date of Fixation	Days after Operation	Experimentals		Normal Controls	
		Diameter of Oocytes (μ)	No. of Animals	Diameter of Oocytes (μ)	No. of Animals
Nov. 22	22	172-382 (273)[1]	12	203-328 (248)	5
Nov. 28	28	156-330 (246)	15	140-188 (170)	5
Dec. 5	35	122-348 (242)	10	180-302 (220)	5
Dec. 12	42	130-328 (220)	14	180 (180)	4

1) In parentheses are given the average lengths of the diameter of the oocytes in each lot.

It may be concluded from these results that growth of both testicular and ovarian tissues of the hermaphroditic gonads is not affected by removal of the eyestalks when the operation has been performed soon after the end of the breeding season, or, paradoxically, that some incretory systems which is responsible for inhibiting growth of the ovary is not effective on these immature oocytes of the hermaphroditic males. However, this does not necessarily exclude the possibility of an incretory principle which in males normally inhibits the progress of spermatogenesis during other seasons. These negative results seem to be due to physiological condition of either gonad and or of the incretory organs at that time. Then the writers undertook another series of experiments using adult true females as material.

B. *Eyestalk removal in true females.*

(1) *Females soon after spawning*: Females ot *Pandalus kessleri* lay their eggs in September, carry them through winter, and hatch them during and after May

(Aoto, 1952). Eyestalks of thirty-one females were extirpated from their base bilaterally on October 17, 1951, when all the animals bore eggs that had been laid about one month before. The animals used in this series of experiment measured 30-39 mm. in carapace length, or 100-124 mm. in body length. At certain intervals the animals were fixed and then their ovaries were weighed. Some were then prepared for microscopical observation. As is shown in Table 2, the ovaries, which at autopsy weighed over 100 mg., were five in eyestalkless animals and one in normal controls. No distinct changes in the size of oocytes were found to occur during the course of the experiment, the oocytes averaging 300-400 μ in diameter, other than two exceptional cases in animals which after destalking lived 43 days and 56 days respectively; their ovarian eggs measured about 800μ in diameter.

Table 2. Weight of the ovaries in female prawns, following eyestalk removal on October 17, 1951, about one month after their egg-laying

Date of Fixation	Days after Operation	Experimentals		Normal Controls	
		Weight of Ovaries(mg.)	No. of Animals	Weight of Ovaries(mg.)	No. of Animals
Oct. 17	0	—	—	36- 65(48)	7[1]
20	3	29- 66(44)	4	33- 72(46)	4
23	6	57- 84(72)	4	35- 66(48)	4
27	10	31- 87(54)	4	28- 54(37)	4
Nov. 1	15	50-104(74)	4	30- 79(47)	4
6	20	46-188(101)	4	42- 94(61)	4
12	26	42-115(77)	4	33-118(73)	4
19	33	66- 90(73)	4	—	—
29	43	169(169)	1	—	—
19[2]	46	55(55)	1	43- 73(58)	3
29[2]	56	198(198)	1	37- 54(46)	2

1) These seven animals died within 24 hours after the operation and in this experiment were contained as control animals.

2) Two animals, of which the weight of their ovaries was measured on Nov. 19 and 29 respectively, had had their eyestalks amputated on Oct. 4.

In general, in animals used in this experiment, no acceleration of ovarian growth and no maturation of the oocytes was observed to invariably follow removal of the eyestalks. From these results, one may make the following conclusion: the animals used in this experiment underwent egg-laying only about one month before the operation so that their oocytes were too young to respond to release from an ovary-inhibiting principle lying in the eystalks. However, another interpretation may also be possible on this point, i.e., there may be seasonal changes of physiological phases in the incretory system which is responsible for controlling ovarian growth. In the following experiment the writers intended to examine this problem using animals which had spent more than eight months after egg-laying.

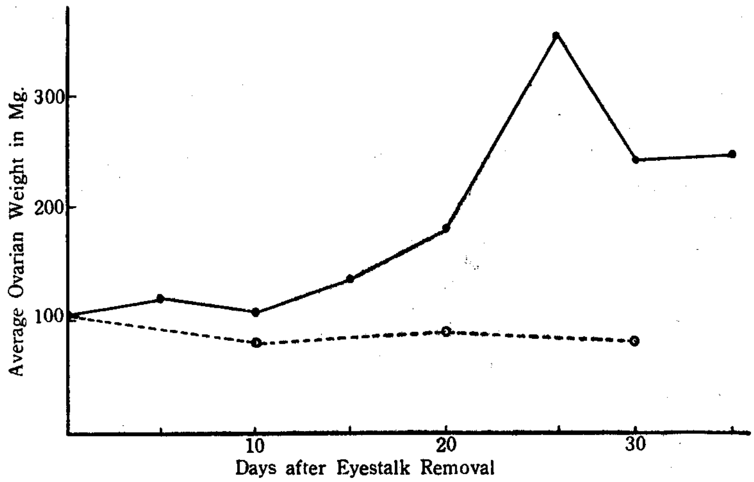
(II) *Females during early summer*: On May 28, 1952, bilateral amputation of the eyestalks was performed on forty-eight female specimens, 28–36 mm. in carapace length, or 97–119 mm. in body length. At this time all of the animals bore on their pleopods eggs laid in the preceding fall, and almost all the embryos were found to have hatched and escaped from the mother prawn's pleopods during the course of experiments which covered the following thirty-five days. Ten out of forty-eight destalked animals died in a few hours after the operation and were dissected for microscopical observation. Their ovaries were found to be in an immature state; ranging between 71–140 mg., or 104 mg. on an average, the organs as a whole were of a light yellow-pink color and had only small oocytes with no indication of deposition of yolk. Eyestalkless animals were fixed *in toto* with Bouin's fluid at approximately 5-day intervals. The ovaries were removed and placed in 70% alcohol and then weighed after being wiped softly with filter paper (Table 3). Average values of ovarian weight of the destalked

Table 3. Number of specimens, and range and averages of ovarian weight in milligram

Days after Operation	Eyestalkless			Normal Control		
	No. of Specimen	Range	Average	No. of Specimen	Range	Average
5	4	91-153	120	—	—	—
10	4	73-149	107	4	42-123	80
15	4	81-168	135	—	—	—
20	4	149-209	181	4	37-107	89
26	4	303-434	354	—	—	—
30	4	184-286	242	4	58-104	81
35	2	131-364	248	—	—	—

animals, as are shown in Text-figure 1, show that for the first twenty-six-day period the ovarian weight of the eyestalkless animals increased approximately linearly with time from an original 104 mg., a more than three-fold increase, while the normal controls showed no consistant change during the same period. One of the eyestalkless animals sacrificed after 26 days, with the ovary measuring 434 mg., had fully grown ova of bright red color. Since three normal females collected on September 12, 1951, had ovaries measuring 423 mg., 426 mg. and 486 mg. respectively, and were very close to normal egglaying, it seems very probable that in these destalked animals accelerated egglaying might occur, although it did not actually take place.

The results of these experiments appear to show that the immature (fall) oocytes are not competent to respond to eyestalk removal. Thus the question arises: is the ovarian-inhibiting hormone always present in the eyestalk or is it only present at certain times of the year (e. g., spring), thus showing its own annual cycle?



Text-figure 1. Curves showing average weight of ovaries of eyestalkless group (straight line) and normal control group (broken line).

From this point of view, the writers made some histological studies on the eyestalks of the prawn. Fixed with Bouin's fluid, or sometimes with alcoholic Bouin's fluid, eyestalks obtained monthly from April to December were cut serially at 7 or 10 μ. Removal of exoskeleton before sectioning yielded good resul s. They were stained with Delafield's haematoxylin and eosin, Mallory's triple stain, toluidine blue, or neutral red.

Observations

A. *General appearance*: There is a definite black-pigmented spot on the dorsolateral surface of the eyestalk, just adjacent to the compound eye; and on the opposite site to this spot on the eyestalk is a thinning of the cuticle — the sensory pore. In the core of the eyestalk are the primary optic centers (*lamina ganglionaris, medulla externa, m. interna, and m. terminalis*), which are surrounded by a loose connective tissue. Arising just beneath the sensory pore, a stalk of rather solid connective tissue intrudes toward *m. terminalis*, and at the inner end of this stalk is a mass of secretory cells, termed the X organ (Hanström, 1939), [the X organ distalis (Carlisle and Passano, 1953)].

The sinus gland of *Pandalus kessleri* is situated ventrolaterally between the *medulla interna* and *m. externa*, just on the opposite side of the X organ.

The gland, as a whole, is irregular in shape and composed of tissues in a sac-structure which stain in pink color with eosin. In most preparations, however, we could not find cellular boundaries, the nuclei being very few in number and stained in the same manner as the nuclei of neurilemma. There was no evidence of cyclic changes in the sinus gland.

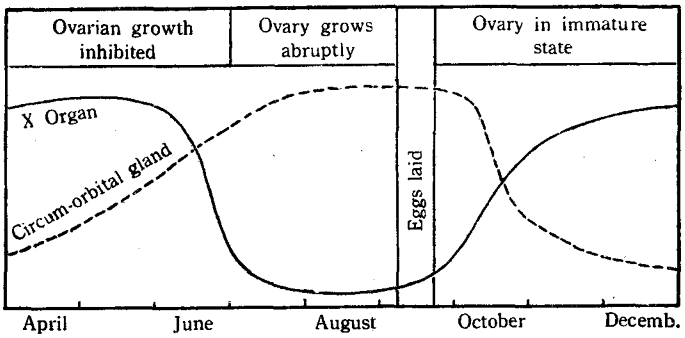
B. *X organ and its secretory activity* : The X organ of *Pandalus kessleri* is, as described in other forms of Crustaceans, a grape-cluster structure composed of several cells. The nucleus is round in shape and has several nucleoli within it. During April, May, June and early July, the secretory granules, staining in metachromatic color (basophilic) with toluidine blue, are found in abundance in the cytoplasm of the cell (Pl. XVIII, Fig. 2). At first, these secretory granules can be seen in the small vacuoles of the cytoplasm scattered near the nucleus. These vacuoles gradually become larger and connected each other to form large vacuoles, and at the same time the secretory granules increase in size.

This process of granule- and vacuole-formation can be observed more or less throughout the year. However, in late July and August the granules are not stained with toluidine blue, but are only noticed by their blue color stained with Mallory's triple staining ; acidophilic in nature (Pl. XVIII, Fig. 3). At that time, the nucleus of the X cell is stained in blue but its nucleoli are not distinct. During September and October, immediately after the animals lay eggs, the secretory granules recover their metachromatic nature. Identically staining granules are seen along the nerve fibers between the X organ and the *medulla terminalis*. Throughout the year the writers could not find any changes in staining reaction of the nucleus to toluidine blue.

C. *Another glandular structure in eyestalk* : During our study on the histology of the *Pandalus* eyestalk, we found a glandular structure, which surrounds the *lamina ganglionalis* just beneath the eye proper (the present writers termed this the "circum-orbital gland"). The gland consists of several groups of glandular cells, of which about six cells are radially arranged in one group. The cell has a round nucleus and one large nucleolus. The cytoplasm contains many secretory granules and their chemical nature changes in seasons, showing annual physiological cycle of the gland.

During April there is found a large nucleolus in the center of the nucleus, stained in deep cobalt blue with toluidine blue (Pl. XVIII, Fig. 4). When stained with Delafield's haematoxylin and eosin, it clearly shows an acidophilic nature. Also there are found in the cytoplasm of the circum-orbital gland cell some secretory granules, which stain metachromatically with toluidine blue. Acidophilic nature of the nucleolus is gradually lost as time passes and, at last, it becomes basophilic in nature, stained blue with toluidine blue. The secretory granules increase in number and attain their maximal number in July and August, while the nucleolus loses its acidophilic nature and becomes inconspicuous, and the chromatin of the nucleus is stained deeply with toluidine blue during the same period (Pl.

XVIII, Figs. 5 and 6). In September, the secretory products, stained in metachromatic color with basic dyes, are seen outside of the cells. Moreover, there are seen among the glands several holes with no cellular structure, which are thought to be resulted from outflowing of the secretory product of the circum-orbital gland. In October, soon after the breeding season, the chromatin loses its capacity to be stained with toluidine blue in deep, the nucleolus becomes again visible in the nucleus, and the secretory granules in the cytoplasm decrease in number (Pl. XVIII, Fig. 7).



Text-figure 2. Secretory activity of two incretory systems in the *P. kessleri* eyestalk through the year.

From the the results of histological studies, we could roughly draw two curves demonstrating secretory activity of the X organ and the circum-orbital gland (Text-figure 2). It is interesting to note that the curve representing secretory activity of the circum-orbital gland is closely related with developmental degree of the ovaries, while the curve representing the X organ shows tendency to counter it.

Pyle (1943) found cyclic secretory phenomena associated with the molting in the sinus gland but not in the X organ of three species of Crustacea (*Homarus americanus*, *Pinnotheres maculatus* and *Cambarus virilis*). The difference of *P. kessleri* from those other species investigated by Pyle may be due to their molting behavior. Female *Pandalus* lay their eggs in September and carry them more than eight months until the eggs hatch in and after May of the following year ; molting, therefore, can occur only during the four months between late May and September.

Discussion

In 1939, Brown and Cunningham found that eyestalkless *Leander* died within several days following removal of their eyestalks while nomal controls survived under the same cirucumstance. Later, Roberts (1944), measuring the rate of oxygen consumption in normal and eyestalkless crayfish, *Cambarus virilis*, found that the requirements for food and oxygen were much greater where eyestalks were absent, and he concluded that eyestalks either directly or indirectly inhibit metabolic rate of the crayfish. On this point, Edwards (1950) was successful in confirming that bilateral eyestalk extirpation caused an increase in oxygen consumption of the fiddler crab, *Uca*. Frost, Saloum and Kleinholz (1951) also measured the rate of oxygen consumption in *Astacus* and stated that "a factor in the eyestalks, *outside of the sinus gland*, in responsible for the marked increase in oxygen consumption following eyestalk ablation." At the very same time, several other workers, from their histological and experimental studies on different crustacean species (Welsh, '51, and Bliss, '51, in *Gecarcinus lateralis*; Travis, '51, in *Panulinus argus*; and Passano, '51a and b, in some crabs) came to the conclusions that "the sinus gland itself is a sac of loose connective tissue, showing no evidence of local secretory activity," and that the so-called 'eyestalk hormone' is "produced by neurosecretory cells of the X organs and carried through their axons to the 'sinus gland' which serve as reservoirs". The evidences presented here reveal that it is true of *P. kessleri*, too ; the sinus gland shows no secretory activity at all, while the X organ cells exhibit constant high level of secretory activity through the year except during the summer months.

In the present experiment, unseasonable growth and maturation of the ovary following bilateral ablation of the eyestalk took place only in the case where the experiment was carried out during May and June, when the secretory granules are normally most abundant in the X cells. Also the change in chemical nature of the granules from basophilic to acidophilic during late July and August coincides exactly the time when the ovarian eggs grow rapidly of female *Pandalus* in their natural habitat. These facts make us conclude that, 1) the X organ secretes ovarian-inhibitory hormone through the year except during late July and August, 2) that in the normal female reproductive cycle the ovary, free from inhibitory control of the X organ during this period, grow rapidly until egg-laying occurs in early September, and 3) that eyestalk ablation during May and June is most effective in accelerating ovarian growth, because during this period the inhibitory factor is most powerful and might be balanced with increased growth potential of the ovary. The last concept is supported by histological evidence.

As described before, accelerated ovarian growth following eyestalk removal took place in larger specimens of the shrimp, *Paratya compressa*, more easily (Takewaki and Yamamoto, 1950b) and in big specimens of the fresh water carb, *Potamon dehaani*, which measure not less than 2.3 gr. in body weight (Otsu and Hanaoka, 1951). Stephens (1951) made destalking experiments using female *Cambarus virilis* in different seasons and found that percentage of animals having laid eggs after eyestalk removal was highest in the November- and the January-groups and considerably lower in the September- and the October-groups. These results suggest that irresponsiveness of some ovaries in the present experiment of destalking is due to physiological condition in the ovaries themselves - immaturity. It should be noted, however, that considerably low secretory activity of the *Pandalus* X organ soon after the breeding season and low temperature of the sea water may be other causes for irresponsiveness of young ova which are released from inhibitory systems.

The most interesting feature concerning incretory systems in *Pandalus kessleri* is the circum-orbital gland, which is found in eyestalk connective tissue surrounding the *lamina ganglionalis*. The gland consists of several radially arranged cells which have clear seasonal cycle through the year; their secretory activity becomes higher during spring and maximal number of secretory granules in the cytoplasm is counted in July and August. This cyclic change of the circum-orbital gland is in quite different from that of the X organ. During May, June and early July, when the secretory activity is at a maximum in the X organ, that in the circum-orbital gland gradually becomes higher, attaining its maximal point during late in July and August, and at that time the X organ starts a sudden degeneration. In view of these facts, an antagonistic relationship between these two incretory systems is conceivable, although further study is needed on this problem.

Also, whether these incretory organs, in deteriorate stages, produce a special substance which plays a physiological role in the reproductive process should be determined in the future.

What factor is controlling the cyclic change of these incretory system? Stephens (1952), making a study of the female reproductive cycle in the crayfish, found that the tendency to respond to eyestalk removal by the laying of eggs increased markedly during winter. Thus, although the cyclic phenomenon in incretory organs was not studied by her histologically, it could well be assumed that the photoperiodicity is the main factor controlling reproductive cycle, causing directly physiological changes in incretory systems.

As to the relation between sex-reversal and hormonal factors, Carlisle (1954) suggested that the ovarian inhibiting hormone in *Lysmata* is probably the agent responsible for the inhibition of sex reversal, because "the rate of sex-reversal is strictly correlated with rate of ovarian development". During the observation on the normal course of sex-reversal in *P. kessleri*, one of the present writers (Aoto, 1952) found that oocytes of the hermophroditic males during sex reversal are about 280–350μ while other mature males have oocytes of 130–170 μ in diameter during and after the discharge of spermatozoa. It seems probable to us that the sudden growth of oocytes in the central ovarian part of hermaphroditic gonads after the last discharge of spermatozoa is not necessarily due to hormonal factors from the eyestalk but due to being released from either mechanical pressure or sexual hormone from the surrounding testicular tissue which is almost completely diminished in animals changing sex.

Summary

1) The eyestalks of *Pandalus kessleri* were removed bilaterally from three different groups of animals; hermaphroditic males, and true females, soon after the breeding season, and mature females during early summer.

2) Unseasonable growth and maturation of the ovarian eggs following eyestalk removal took place only when the operation was performed on females during early summer.

3) The X organ, which is thought to be responsible for producing the ovary-inhibiting hormone, was found to have a clear seasonal cycle — its secretory activity is constantly high except for during late July and August.

4) Another secretory organ (termed the "circum-orbital gland" in the present paper) was found in the eyestalk. Its secretory activity increases during spring, attains its maximal level during summer, and becomes degenerated in Octocer — soon after the breeding season.

5) Histological evidence suggests an antagonistic relationship between two incretory systems.

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Addendum

The circum-orbital gland was first described under the term "Y-gland" by the present writers (Kagaku, vol. 24, no. 6, 1954). However, it was found recently that a French author used the term "organe Y" on an entirely different organ, which was found in malacostracan crustaceans (Gabe, M., Compt. rend. acad. sci., tom. 257, 1953). To avoid confusion the writers abandoned the name of "Y-gland", and, instead, give a new term, "the circum-orbital gland," which was kindly suggested by Dr. L. M. Passano of Yale University, U. S. A. to whom the writers are much obliged.

Also the writers acknowledge the invaluable criticism given to our manuscript by Dr. F. A. Brown, Jr. of Northwestern University and Dr. L. H. Kleinholz of Reed College, both of the U. S. A.

Explanation of Plate XVIII

Fig. 1. Ovaries of *Pandalus kessleri*. Four normal controls fixed on June 17 (a), and four experimentals fixed on June 23, twenty-six days after eyestalk removal.

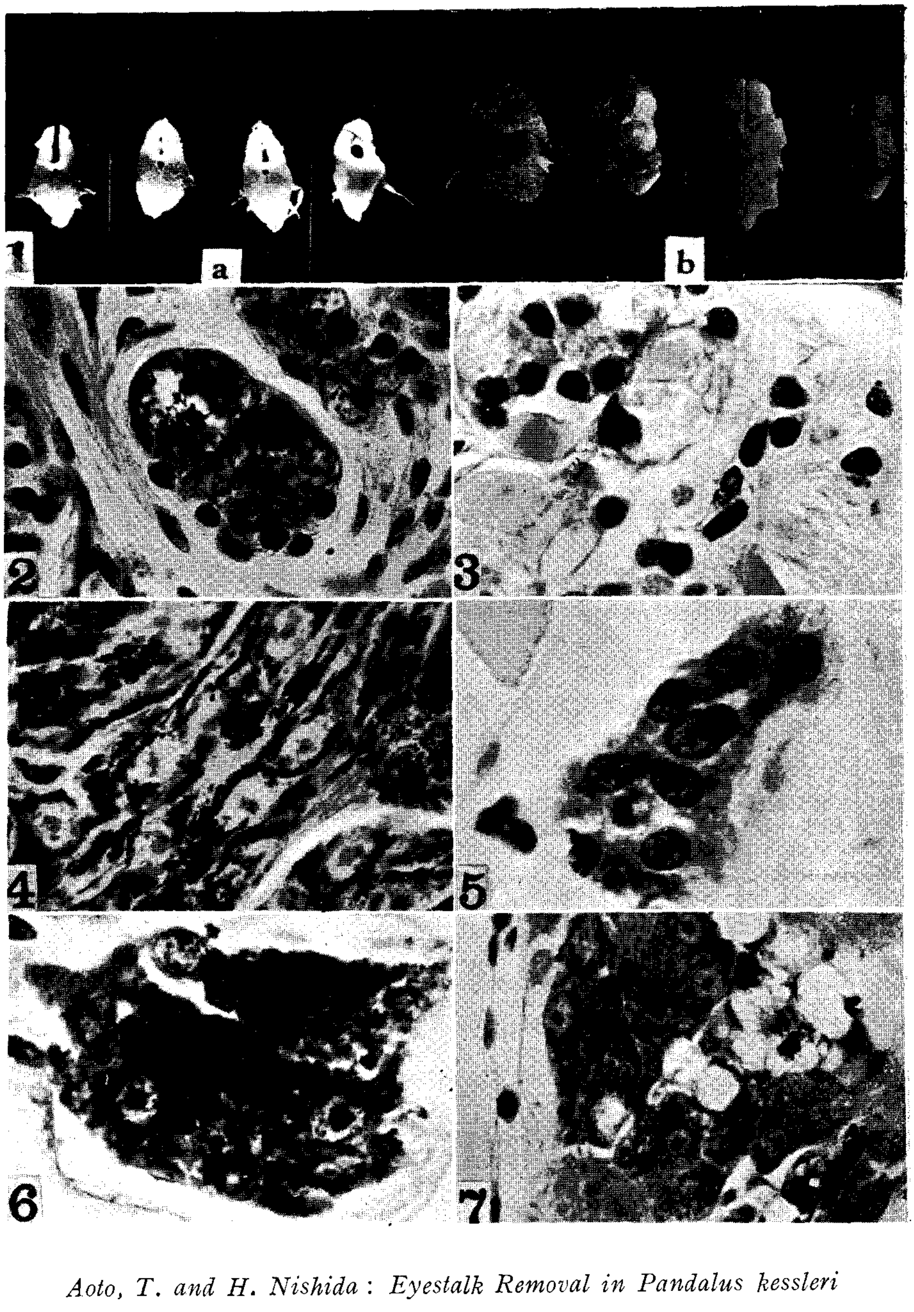
Fig. 2. The X organ in April. The cells from a cyst and contain lots of secretory granules which show metachromatical color reaction. The cells in the upper corner are in the early stages of secretory activity, ×400.

Fig. 3. The X organ in July. The cytoplasm of the X organ cells does not show metachromatical color reaction. ×400.

Fig. 4. The cricum-orbital gland in April. Secretory activity is low and chromatins are few. The nucleolus is quite large. ×400.

Figs. 5-6. The circum-orbital gland in July. The nuclei stain deep with toluidine blue. Secretory granules are seen in abundant in Fig. 5. ×400.

Fig. 7. The circum-orbital gland in October. The chromatins are few and nucleolus is large. Secretory granules are seen collected in the center of the group of cells. Many holes outside of the cells are thought to be residue of secretory product, which are partly seen among the holes. As a whole, the gland just starts the course of atrophy. ×400.



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