The Source of the Ovarian Inhibiting Hormone in the Eyestalks of the Crab, *Potamon dehaani*

By

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(*With 4 Text-figures*)

The ovarian inhibiting hormone in the sinus gland of crustaceans was first indicated by Panouse ('46) who found that the removal of the eyestalks in *Leander serratus* was followed by the rapid development of the ovaries. Conversely the transplantation of the sinus gland into eyestalkless animals had the reverse effect in reducing the heightened rate of the ovarian development to normal levels. Later experiments showed that the similar influence of eyestalks on the development of ovaries is observable in a variety of shrimps and crabs, *Cambarus immunis, Uca pugilator* (Brown and Jones '49), *Paratya compressa* (Takewaki and Yamamoto '50). The present writers (Ōtsu and Hanaoka '51) found in *Potamon dehaani* that the removal of the eyestalk affects the ovaries only in adult females, no promoting influence on the ovarian development being detected in immature animals. Ōtsu and Hanaoka ('55) have also reported that the water extract of the eyestalks of *Potamon dehaani* contains some substance which affects the growth rate of decapitated coleoptiles of *Avena* and that a synthesized plant growth promoting substance, α-naphthyl acetic acid, tends more or less to suppress the ovarian development in eyestalkless animals. Recently Carlisle ('54) confirmed by injection experiments that in *Lysmata* the ovarian inhibiting hormone is detectable in pars ganglionaris x-organi and in the sinus gland, but neither in pars distalis x-organi nor in the distal ganglia of the eyestalk, and stated that the ovarian inhibiting hormone is produced in the neurosecretory cells of pars ganglionaris.

Incidental to the experimental studies of the ovarian inhibiting hormone in crustaceans, the present writers had an opportunity to make an observation on the neurosecretory cells in the eyestalks of *Potamon dehaani*. The following investigation was undertaken to elucidate the relationships between the activity of the ovarian inhibiting hormone and the cytological changes which are manifested by the neurosecretory cells in the eyestalks of this form.

Observations

In the eyestalks of adult females preserved in Bouin's solution and stained

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with Mallory's triple stain, three groups of secretory cells were observed. Those of the first group are neurosecretory cells which are distributed over the wide area of the dorsal surface of the central nerve axis. The cells of the second group cover the ventral surface of medulla interna, and the cells of the third one constitute a small glandular body which presumably corresponds to the Y-organ reported by Matsumoto ('55) in this form (*Potamon dehaani*). In the cells of the latter two groups, however, neither change of cytological feature suggestive of seasonal differences of secretory activities, nor any connection of nerve tract to the sinus gland was detected. They may be considered as playing no important rôle in the production of ovarian inhibiting hormone. The following description, therefore, was confined to the observations of the neurosecretory cells of the first group.

In the females which were collected during the sexually quiescent season, the cells of the first group were relatively large in size as well as in number (Fig. 1, nc). Their nuclei are stained yellow with orange G and contain fine granules on the periphery. The cytoplasm is opaque and homogeneous in appearance and takes fuchsin and anilin blue remarkably well. These cells are located over the wide area of the medulla interna but no variation of cytological character suggestive of local difference of secretory activity has been detected among them. The neurosecretory cells of this type are presumably homologous to the cells of the x-organ, but they differ from those of the hitherto reported forms in their wide distribution over the nerve axis.

The axons of the neurosecretory cells of the x-organ run together along the dorsal surface of the nerve axis to form the x-organ sinus gland tract (Fig. 1, sgt). At the end of this tract these axons enlarge amid a frame work of connective tissue and make up the sinus gland (Fig. 1, sg). The sinus gland consists of a number of lobes and contains a colloidal substance which stains uniformly with orange G. Granules of secretory products of the x-organ can be traced along the x-organ sinus gland tract to the sinus gland.

During the period of rapid ovarian development, i.e., when the ovarian inhibiting hormone of eyestalks is inactive, the neurosecretory cells of the x-organ exhibit characteristic figures which suggest a low secretory activity. Their nuclei stain faintly with orange G and assume spherical, elliptical or crescent shapes. The granular inclusions in the nucleus are much depleted. The cytoplasm loses the affinity with dyes, the cell boundary becomes indistinct and the whole neurosecretory cells form a loose syncytium-like structure (Fig. 3, nc). The granules of secretory substance in the x-organ sinus gland tract completely disappear. The sinus gland is reduced in size, and clear spaces appear between the lobes (Fig. 2). Each lobe contains many small droplets which stain irregularly with fuchsin and orange G.

In the females fixed a few weeks after ovulation the neurosecretory substances make their appearance again in the x-organ sinus gland tract, and in the sinus

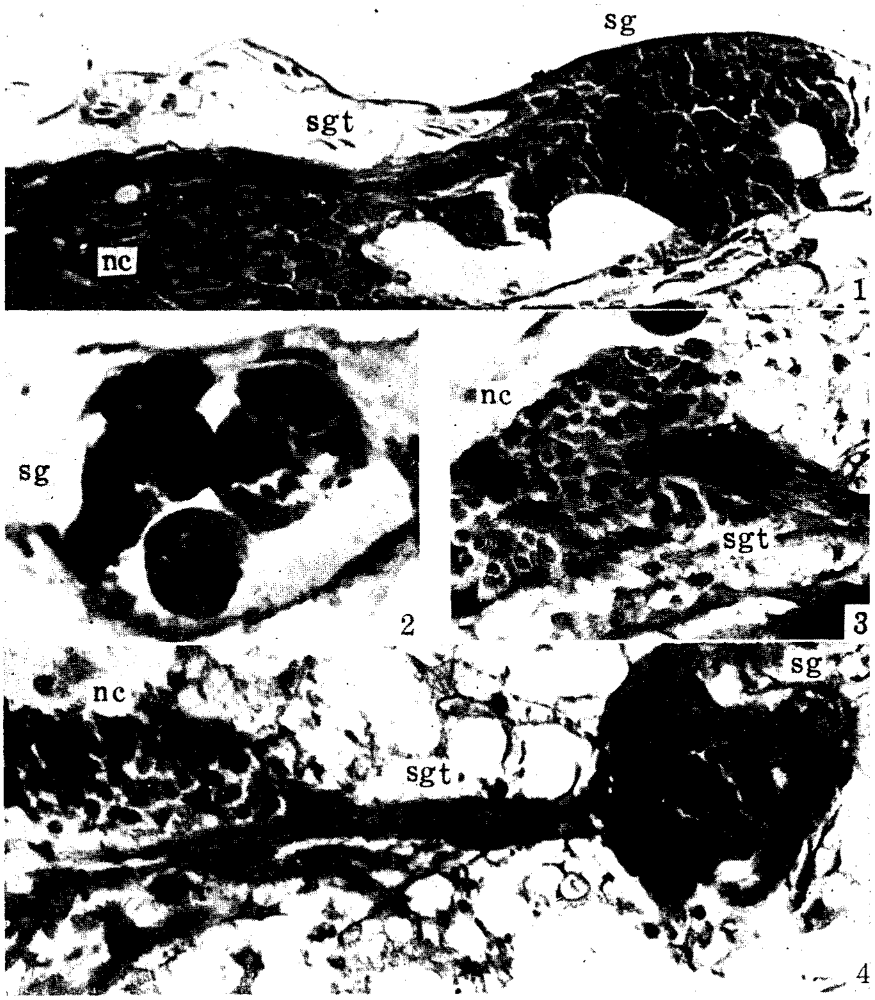

Fig. 1. X-organ sinus gland system of a female preserved during the sexually quiescent period. The cytoplasm of the neurosecretory cells is opaque and the sinus gland contains masses of a colloidal substance. Fig. 2. X-organ of a female with rapidly growing ovaries. The neurosecretory cells with coarse cytoplasm form a syncytium-like tissue. Fig. 3. Sinus gland of a female with rapidly growing ovaries. The lobes which constitute the sinus gland are separated by clear spaces. Each lobe contains many dark droplets stained with dye. Fig. 4. X-organ sinus gland system of a female preserved a few weeks after ovulation. Fine granules appear again in the sinus gland tract and the sinus gland regain its original shape. nc, Neurosecretory cell. sgt, Sinus gland tract. sg, Sinus gland.

gland the small droplets unite into large spherical bodies (Fig. 4). The fuchsinophilic nature of the storage substance gradually disappears and thus the cyclic changes of the neurosecretory activity of the x-organ sinus gland system are completed.

Discussion

The observations described in the preceding section reveal that the cytological changes of neurosecretory cells of the x-organ sinus gland system coincide pretty well with the seasonal activities of the ovarian inhibiting hormone. The appearance of granular substance in the sinus gland tract is probably the cytological manifestation of the production of the ovarian inhibiting hormone in the neurosecretory cells of the x-organ. It is highly probable that the ovarian inhibiting hormone is produced by the neurosecretory cell of the x-organ and passes along the axons of the sinus gland tract to the sinus gland where it is stored and finally released.

It is obvious that the study of the ovarian development must take into its scope a consideration of moult which follows the removal of eyestalks in close connection with the development of ovaries. In some way, therefore, the neurosecretory cells responsible for the production of the moult inhibiting hormone must be expected to exist in the eyestalks. Indeed, Carlisle ('54) reported on the bases of his injection experiments in *Lysmata* that the moult inhibiting hormone is detectable in pars distalis x-organi, while the moult accelerating hormone and the ovarian inhibiting hormone are contained in the pars ganglionaris x-organi and the sinus gland. The present observations could neither offer any evidence suggestive of the local difference of secretory activities in the x-organ nor the seasonal changes of the cytological features in the entire x-organ which are correlated with moulting. Of course, these facts do not deny the possibility that the neurosecretory cells of the x-organ in *Potamon dehaani* are concerned with the production of hormonal substances other than the ovarian inhibiting hormone. Cells with similar morphological characters may have different secretory functions or may produce distinct substances at the different seasons of the year.

Considering all of these facts, however, it may be assumed that the neurosecretory cells of the x-organ which were observed in the eyestalks of *Potamon dehaani* are at least the site of the production of the hormone responsible for the inhibition of ovarian development. The local or seasonal difference of their secretory activities will be discussed in another report.

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