STUDIES ON THE JAPANESE CHARS, THE IWANA
(GENUS SALVELINUS)

II. THE HYPOTHALAMIC NEUROSECRETORY SYSTEM OF THE
NIKKO-IWANA, SALVELINUS LEUCOMAENIS
PLUVIUS (HILGENDORF)

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Introduction

Enami (1957), Sano (1956) and their collaborators have demonstrated details of
the caudal neurosecretory system of many Japanese teleosts and suggested some
functions of the system. However, little is known about the morphology and role
of the cranial secretory system of Japanese fishes, although a relationship between
this system and gonadal maturation of fish has been indicated by several European
and American endocrinologists (von Brehm, 1958; Öztan, 1963; and Billenstein,
1963, etc.).

In previous paper the authors have described variations found in some of the
endocrine glands, such as the pituitary, thyroid, adrenal cortical tissue, and gonads
of the Japanese char, the Nikkō-iwana (Honma and Tamura, 1965), and the land-
locked salmonoid fish, the Koayu (Honma and Tamura, 1963). However, these
studies failed to elucidate details of any seasonal secretory cycle of neurosecretory
cells or their products stored in the neurohypophysis. The present paper deals with
the anatomy and histological changes of the hypothalamo-hypophysial neurosecretory
system of the Nikkō-iwana, Salvelinus leucomaenis pluvius (Hilgendorf).

A variety of staining methods have been used to follow the secretory activity of
the nucleus preopticus and nucleus lateralis tuberis, and the distribution of secretory
material in relation to cyclic changes in the pituitary and gonadal maturation.

Materials and Methods

Specimens were obtained throughout 1963 by the junior author (E. T.) by
angling in the mountain tributaries of the Seki-gawa River near the foot of Mt.
Myoko, a part of Jō-Shinêtsu Plateau National Park.

The condition of these fifty specimens used is presented in Table 1. After
immersion in Bouin's fixative, a block of tissue including the hypothalamo-hypophysial
region was removed from the brain, embedded in paraffin and "Tissue mat" mixture, cut serially at 5 to 6 μ in sagittal or transverse directions, and stained
chiefly with chrome alum hematoxylin (CH)-acid fuchsin, and paraldehyde fuchsin.
Table 1. Salvelinus leucomaenis pluvius (Hilgendorf) from Jô-Shînêtsu Plateau National Park, south-western part of Niigata Prefecture, the Japan Sea side.

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Body length (cm)</th>
<th>Weight of body (g)</th>
<th>Sex and number of fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 13–28, 1963</td>
<td>6.4–17.4</td>
<td>8.0–69.4</td>
<td>5 4</td>
</tr>
<tr>
<td>May 11–23, 1963</td>
<td>9.5–17.4</td>
<td>13.0–93.0</td>
<td>1 3</td>
</tr>
<tr>
<td>June 13–21, 1963</td>
<td>3.6–18.5</td>
<td>0.7–86.3</td>
<td>2 3</td>
</tr>
<tr>
<td>July 4–14, 1963</td>
<td>4.7–19.5</td>
<td>1.7–116.8</td>
<td>2 2</td>
</tr>
<tr>
<td>August 10–15, 1963</td>
<td>4.7–17.3</td>
<td>1.9–91.2</td>
<td>2 1</td>
</tr>
<tr>
<td>September 11–18, 1963</td>
<td>6.6–14.5</td>
<td>4.5–50.6</td>
<td>3 5</td>
</tr>
<tr>
<td>October 4–14, 1963</td>
<td>10.4–17.3</td>
<td>17.4–76.0</td>
<td>2 8</td>
</tr>
<tr>
<td>November 11–28, 1963</td>
<td>11.7–16.7</td>
<td>24.3–68.7</td>
<td>3 3</td>
</tr>
<tr>
<td>December 4, 1963</td>
<td>11.3</td>
<td>22.4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>21 50 29</strong></td>
</tr>
</tbody>
</table>

(AF)-azan trichrome methods. These procedures, which differentially stained the neurosecretory materials of the nucleus preopticus and nucleus lateralis tuberis were recommended by Billenstein (1962 and 1963) in her study of the Eastern brook trout.

Simultaneously, the condition of the gonads was determined by staining with hematoxylin-eosin and azan triple stain.

**Observations**

The hypothalamic neurosecretory center of the Nikkô-iwana, consist of two pairs of cell bodies similar to those of other bony fishes which have been described. They are defined mainly by their staining reactions and their morphology.

**Nucleus preopticus**

Each nucleus preopticus, which is stained by CH or AF is situated at the side of the third ventricle just dorso-anterior to the optic chiasma (Plate, I, fig. 1). The neurosecretory cells are usually polygonal, having round or ovoid nuclei in their central region, and one or two axons arise from each cell. The cells are distributed roughly into two groups, the larger ones (ca. 18 µ in diameter) being in the more ventricular region while the smaller (ca. 10 µ in diameter) are nearer to the chiasma. The former may be referable to the pars magnocellularis and the latter to the pars microcellularis, although there is no distinct boundary between the two.

The neurosecretory axons of the nucleus preopticus of either side first extend laterally, then turn ventrally and posteriorly along the ventral side of the hypothalamus as the tracts. The tracts enter the pituitary stalk and finally reach the bottom of the digits of the neurohypophysis. The path of the axons can be delineated easily by tracing CH or AF stainable granules from their origin in the nuclei. There are also indications that some axons pass to the third ventricle, but the authors
Fig. 1. A line of the nucleus preopticus located near the third ventricle, just dorso-anterior to the optic chiasma. Sagittal section. ×100

Fig. 2. Nucleus preopticus of the adult fish caught late in April. CH-positive coarse granules are seen in the cell. ×750

Fig. 3. A small quantity of CH-positive neurosecretory material in the neurohypophysis of the fish caught late in April. ×500

Fig. 4. Nucleus preopticus of the fish taken in prespawning season (September). Pericaryon and axon are filled with rich CH-positive neurosecretory granules. ×500

Fig. 5. An active secretory phase of the nucleus preopticus of the fish in the spawning season. Vacuoles in the cell are detected. ×500

Fig. 6. Rich CH-positive neurosecretory granules stored in the digits of neurohypophysis of the fish in the spawning season. ×500

Fig. 7. A heavy vacuolized nucleus preopticus in the postspawning spent fish. ×500

Fig. 8. A small quantity of CH-positive neurosecretory material in the postspawning spent fish. ×500
have been unable to trace these along there complete length.

There is no noticeable change in the size of the neurosecretory cells of the nucleus preopticus of adult fish throughout the year. Although most of the cells are usually in a state of secretory activity, seasonal variations in the size, number, and staining affinity of the secretory granules, and number and quantity of vacuoles in the pericaryons are encountered. The highest level of activity is reached in the breeding season of the fish. However, the authors have been unable to determine whether the neurosecretory granules are produced within the nuclei of the neurosecretory cells.

The pericaryon of cells of the pars magnocellularis of adult fish caught late in April is filled with fairly rich CH- or AF-positive granules, and the quantity of neurosecretory material in the neurohypophysis is rather small (Plate I, figs. 2, 3). On the other hand, in specimens taken just prior to the spawning season (September), a considerable number of small vacuoles appear in a part of the pericaryon near the axon, and many secretory granules are found inside the axon (Plate I, fig. 4). The dispersed vacuoles occupy nearly half the space of the pericaryon. In the spawning season, about the middle of October, a large part of the secretory cell is occupied by the vacuoles, suggesting that it is very active (Plate I, fig. 5). Parallel with this change, the quantity of neurosecretory substance stored in the digits of the neurohypophysis increases (Plate I, fig. 6). The granules in the neurohypophysis become concentrated in the region of the capillaries that supply this region of the hypophysis. Up to the end of November the vacuoles within the secretory cells continue to unite together, indicating continued release of secretory products. An even more conspicuous state of vacuolization is detected in the cells of the nucleus preopticus of the spent fish (Plate I, fig. 7). The number of cells in the pars magnocellularis without CH- or AF-stainable granules increases remarkably after the breeding season, but some of the cells do not show such activity. The quantity of neurosecretory material in the neurohypophysis decreases after spawning (Plate I, Fig. 8).

Nucleus lateralis tuberis

The nucleus lateralis tuberis of the Nikkō-iwana is large, and is situated in the most ventral region of the hypothalamus, behind the optic chiasma and just on either side of the third ventricle (Plate II, figs. 1, 2). The shape of the secretory cell is usually polygonal and polymorphous with a round nucleus in its central region. A single axon (in some cases two to three) arises from each cell. The neurosecretary granules in the pericaryon and in the axon give a positive reaction with acid fuchsirn, phloxine and azocarmine. These red axonal tracts are found in the pituitary stalk in the more dorsal part of the neurohypophysis, while the CH- or AF-positive axons and substances are in the more ventral part of the neurohypophysial digits that project into the pars intermedia. The extremities of the red
Fig. 1. Nucleus lateralis tuberis in the most ventral region of the hypothalamus, just on either side of the third ventricle. Cross section. ×100

Fig. 2. Nucleus lateralis tuberis in the most ventral region of the hypothalamus, just behind the optic chiasma. Sagittal section. ×100

Fig. 3. Extremities of the red axons in the neurohypophysis, reaching nearly to the rostral and proximal parts of the pars distalis of hypophysis. ×500

Fig. 4. Nucleus lateralis tuberis of the fish taken in April. Vacuoles in the cytoplasm are hardly visible. ×750

Fig. 5. Nucleus lateralis tuberis of the fish taken in June. Indication of formation of vacuoles in the cytoplasm are slightly detected. ×500

Fig. 6. Nucleus lateralis tuberis of the fish taken in the spawning season. Vacuoles in the cytoplasm are well detected. ×500

Fig. 7. Neurohypophysial digits into the pars distalis. Large gonad stimulating bosophilic cells with rich coarse granules are seen. ×500

Fig. 8. Higher magnification of the neurohypophysial digit, showing red axons and Herring bodies. ×500
axons reach nearly to the rostral and proximal parts of the pars distalis of the adenohypophysis, although the exact route of the axon has not been determined (Plate II, figs. 3, 8). There is no noticeable change in the size of the secretory cells (ca. 20 μ in diameter) of the adult fish throughout the year. On the other hand, a seasonal change in the activity of the cell can be recognized in mature fish that have gonad-stimulating cells in the proximal part of the pars distalis.

The pericaryon of the nucleus in specimens taken in April contain acid fuchsin or azocarmine positive neurosecretory products, but the vacuoles are hardly visible (Plate II, fig. 4). In May to June the vacuoles are still difficult to detect in the neurosecretory cells, occupying only 10–20% of the pericaryon (Plate II, fig. 5). Thus, in spring and early summer, the secretory activity of the nucleus lateralis tuberis is not high and, correspondingly, there are few acid fuchsin positive neurons in the dorsal area of neurohypophysis, although at this time of the year the ovarian eggs are in the peripheral nucleolus stage, and there are spermatogonia in the testis. In September the nuclei are fairly active, with many vacuoles in the peripheral region of the pericaryon and in the region near axon. The vacuolization proceeds more markedly as the gonads mature (Plate II, fig. 6). The vacuoles unite with each other, and occupy the greater part of the pericaryon.

As already reported in a previous paper (Honma and Tamura, 1965) spermatozoa and eggs in the yolk globule stage are found by October, accompanying a noticeable increase in the number of gonad-stimulating basophilic cells in the adenohypophysis (Plate II, figs. 7, 8). The spawning season extends from late October to early November. In the nuclei of the spent fish, acid fuchsin or azocarmine positive neurosecretory substance disappears, and the cytoplasm of the cell is difficult to stain. The cells are extremely vacuolized.

Discussion

Since its first description in a teleost by E. Scharer (1928), the hypothalamic neurosecretory system has been identified histo-morphologically in the diencephalic nuclei of all classes of vertebrates. In the higher vertebrates, such as reptiles, birds and mammals, two pairs of CH or AF stainable neurosecretory nuclei are found: the nucleus supraopticus and nucleus paraventricularis. In lower vertebrates, such as amphibia, fishes and cyclostomes, only a single pair of neurosecretory nuclei called nucleus preopticus can be distinguished. In his extensive work, Charlton (1932) described the morphology and position of the nucleus preopticus, pars magnocellularis, and nucleus lateralis tuberis in about 140 species of fish and fish-like animals extending three classes (Cyclostomata, Chondrichthyes and Osteichthyes). Although the latter neurosecretory center has been demonstrated only in teleosts, it has not been found in all species of this group. Details of the nucleus lateralis tuberis and its efferent pathway have been described by Stahl (1957) and Stahl and
Leray (1962).

**Nucleus preopticus**

Like those of other bony fish the nucleus preopticus of the Nikkō-iwana consists of two major parts: ventrally the nucleus preopticus pars microcellularis located near the chiasma opticus, and dorsally the nucleus preopticus pars magnocellularis located near the third ventricle. The tinctorial response of the nucleus is CH- or AF-positive, as in other vertebrate species. In the platyfish Öztan (1963) has shown that the great majority of the axonal tracts originating from the pars magnocellularis and pars microcellularis join together and are seen to proceed towards the hypophysial stalk, although some of the neurosecretion-bearing axons terminate beyond the neurohypophysis and the third ventricle. In the Nikko-iwana, we have confirmed that the axonal tracts enter the neurohypophysis through the hypophysial stalk, but their possible pathway to the third ventricle and adenohypophysis is indistinct. The neurosecretory granules in the neurohypophysis are distributed in the region of the digits which extend behind the pars intermedia (meta-adenohypophysis), and are concentrated close to the capillary walls. The arrangement mentioned above was also found in the Eastern brook trout by Billenstein (1962 and 1963), although Stahl and Leray (1962) have found that fibers filled with neurosecretory material from the nucleus preopticus penetrate between the acidophil and basophil cells of the proximal zone of pars distalis (meso-adenohypophysis) in several teleostean species. Öztan (1963) has suggested that since the neurohypophysis of sterile platyfish lacks hypophysial gonadotropic cells and is full of preoptic neurosecretory material, there is no direct relation between the hypophysial gonadotropic function and the secretion of the nucleus preopticus pars magnocellularis. Arvy, Fontaine and Gabe (1955) reported abundant storage of neurosecretory substance in the neurohypophysis of the Atlantic salmon and inactivity of the nucleus preopticus in smolt stage individuals captured in the fresh water, or in “mended” individuals that have survived after spawning and returned to the sea. On the other hand, mature salmon taken during the upstream migration and fish caught at the spawning place showed scanty substance in the neurohypophysis and a very active phase of the secretory nuclei. In the Nikko-iwana, we have also found a correlation between the activity of the nucleus preopticus pars magnocellularis and the amount of the neurosecretory substance stored in the neurohypophysis. In this fish, axonal transport of substance through the hypothalamus becomes marked from the prespawning season onwards.

Frideberg and Olsson (1959) concluded from their study on the stickleback (*Gasterosteus aculeatus*) that the preoptic neurosecretory substance is concerned with osmoregulation and possibly other fields of function. However, the Nikko-iwana is unlike the stickleback in that it does not move from one osmotic environment to another during the prespawning period, and there is, therefore, no reason to suppose
that the increased neurosecretory activity of this fish is associated with a change in osmoregulation. The results presented here suggest that the intimate relationship between the preoptic neurosecretory substances and spawning may be better explained in relationship to the ejection of ripe gametes, involving perhaps such substances as isotocin (Acher, 1963) or arginine vasotocin (Lederis, 1962).

**Nucleus lateralis tuberis**

Although some investigators report that the nucleus lateralis tuberis is stained with oxidized CH or AF (Öztan (1963) in the platyfish, Barrington (1960) in the minnow and Enami (1957) in the Japanese cat fish, etc.), the nucleus of the Nikko-iwana has a tinctorial positive response to acid fuchsin, phloxine and azocarmine, but not to CH or AF. It is more likely that the secretory material of this nucleus is CH or AF negative as exemplified by many investigators (Bargman, 1953; Fridberg and Olsson, 1959; von Brehm, 1958; Billenstein, 1962 and 1963; Stahl and Leray, 1962; etc.). Charlton (1932) demonstrated four related nuclei, grouped together in the anterior, posterior, intermediate and lateral regions, but the last one is most constantly present. Öztan (1963) also distinguished two groups within the nuclei; large cells lying antero-ventro-laterally and postero-ventro-laterally, and smaller cells laterally. However, it is not possible to make an absolute distinction between these in the Nikko-iwana, since there is a transition from the larger to the smaller cells.

Seasonal variation in the secretory activity of the nuclei in association with the reproductive phenomena or gonadotropic function of the hypophysis were observed in *Tinca* (von Brehm, 1958), Eastern brook trout (Billenstein, 1962), *Mugil* (Stahl and Leray, 1962) and *Platypoecilus* (Öztan, 1963). We find a similar situation in the Nikko-iwana. As in the Eastern brook trout (Billenstein, 1962, 1963) the axons bearing red neurosecretory substance are only found just dorsal to the adenohypophysis, and, in particular, near the proximal zone of the pars distalis. The markedly active phase of these axons follows the increase in number of gonad-stimulating cells at the time of yolk globule formation in the ovarian eggs and of spermiogenesis, i.e., early in October (Honma and Tamura, 1965). Thus, the nucleus lateralis tuberis seems to be concerned with the control of gonadotropin secretion by the cyanophil cells distributed in the proximal zone of the pars distalis. However, there is no direct experimental demonstration of this role. Furthermore, whether or not there is definite contact of axons with the capillaries which penetrate into the region of the glandular cells (such as the gonad-stimulating cells) has not been determined. It is conceivable that this could be elucidated by electron microscopic studies.

**Summary**

The anatomy and the seasonal secretory cycle of the hypothalamo-hypophysial neurosecretory system in the Japanese char, the Nikko-iwana, *Salvelinus leucomaenis pluvius*, were examined histologically. The results obtained were as follows.
1. Using Billenstein’s (1963) staining methods, the neurosecretory substances of the nucleus preopticus lateralis are stained differentially. The former is CH- or AF-positive and the latter is acid fuchsin, azocarmine and phloxine positive.

2. The blue or purple axonal tracts of the nucleus preopticus reach the more ventral part of the neurohypophysis, via the hypothalamus, and the secretory substance is stored in the digits that penetrate into the pars intermedia. On the other hand, the red axons are distributed in the more dorsal part of the neurohypophysis, and some are found near the proximal zone of the pars distalis.

3. Although a picture of secretion in the nucleus preopticus is recognized throughout the year, the activity becomes more marked in the prespawning season, and decreases after spawning. It seems probable that the neurohormonal substance stored in the neurohypophysis plays a role in the ejection of gametes.

4. In adult fish that have gonad-stimulating cells in the adenohypophysis, the secretory phase of the nucleus lateralis tuberis is also recognized throughout the year. The activity of the nucleus is correlated with the increase in number of the gonadotrophs and ripeness of the gametes. The secretory substance may be concerned with control of the gonad-stimulationg cells.

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