THE PITUITARY GLAND OF THE ALEWIFE IN LAKE MICHIGAN: CYCLICAL CHANGES IN THREE ADENOHYPOPHYSEAL CELL TYPES

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The population explosion of the alewife (*Alosa pseudoharengus*, Wilson) in the Great Lakes is frequently accompanied by massive spring and early summer mortalities. The possibility of endocrine involvement in this phenomenon was first suggested by Hoar (1952). He found histological evidence of thyroid hyperplasia and exhaustion in alewives in Lake Ontario and postulated thyroid-related osmoregulatory failure as a factor in the die-offs. The lack of more recent endocrine information emphasizes the need for further study of this important regulatory system in relation to alewife physiology and mortality.

Since the pituitary gland plays a key role in a variety of hormonal activities, it is a prime target for investigation. In elucidating pituitary function a logical first step is to identify the specific types of hormone-secreting cells and to study their annual patterns of change. Investigations of this nature have been carried out in many other species of teleost fishes. The earlier literature has been reviewed by Pickford and Atz (1957). Oliverneau (1963) described six types of chromophilic cells in the teleost pars distalis on the basis of her own and other work. The tentative functions which she assigned to these cells have largely been supported by subsequent histophysiological studies. The pars intermedia of certain teleosts appears to contain two additional cell types. (Oliverneau, 1969) Thus, a total of eight kinds of cells have been identified in the teleost adenohypophysis. Among the more recent studies of cyclical pituitary changes are those of Sokol (1961), Robertson and Wexler (1962 a and b), Sathyanesan (1963), Lagos (1965), Oliverneau (1967) and Sage and Bromage (1970).

The present report includes a description of the histology and cytology of the alewife pituitary gland and an account of cyclical variations in three adenohypophyseal cell types, the gonadotrophs, thyrotrophs and corticotrophs.

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MATERIALS AND METHODS

Alewives were collected by trawl, seine or dip net from several sites in southern Lake Michigan. The collections covered a period from May 21, 1968 to April 27, 1969 with all months represented except September and October. The samples of May through August 1968 were obtained off Saugatuck, Michigan while those of November 1968 through April 1969 were taken along the western shore of the Lake between Waukegan, Illinois and Port Washington, Wisconsin. Adult specimens of both sexes were present in all collections.

Immediately after capture, the fish were placed in Bouin-Hollande fixative for at least three days. After fixation they were air dried and at this time body weight, standard length and sex were recorded and scale samples were taken for age determinations. Prior to further processing they were stored in 70% ethanol. Fifty adult specimens, all apparently healthy when captured, were selected for the pituitary study.

The dorsal portion of the head of each specimen was removed and decalcified in 5% formic acid for five days. After decalcification, extraneous tissue was trimmed away, leaving a cube about 0.5 cm. square per side containing the pituitary gland and associated brain structures. The preparations were dehydrated in ethanol, cleared in xylene and infiltrated and imbedded in paraplast. Serial sagittal sections were cut at six micra. The staining procedures employed were as follows: (1) periodic acid-Schiff reagent (PAS), lead hematoxylin and orange G (modified from MacCuain, 1956, McMunnus and Mowry, 1958 and Elftmann, 1959 a and b); (2) aldehyde fuchsin, lead hematoxylin and orange G (modified from Gomori, 1950, MacCuain, 1956 and Elftmann, 1959 a and b); (3) erythrosine, Mallory II and acid alizarine blue (modified from Herlan, 1960).

Pituitary cell types were identified by tinctorial reaction, morphology and location and by reference to other histological and histophysiological studies.

Several criteria were used in evaluating the cyclical secretory activity of gonadotrophs, thyrotrophs and corticotrophs. Nuclear diameters were measured with an ocular micrometer on midsagittal sections of glands from four fish (two males and two females) for each collection date. The mean nuclear diameter of each cell type on a given collection date was based on a total of 40 measurements. The Student–Newman–Keul multiple range test was employed to compare differences among the means for statistical significance. Results are shown graphically in Figure 1. Other criteria of secretory activity were cell size, degree of cytoplasmic granulation and vacuolation, staining intensity and nucleolar prominence.
OBSERVATIONS AND DISCUSSION

Histology and cytology.

The pituitary gland of the alewife is very similar to that of its relative the herring (*Clupea harengus, L*) described by Buchmann (1940). The alewife gland is ovoid in shape with a tapering anterior region that ends in a narrow hypophyseal duct. According to Buchmann the duct is open to the pharynx in young herring but closed in adults. No pharyngeal connection was observed in adult alewives, although the duct extends for some distance in an anteroventral direction toward the pharynx. The major pituitary regions, neurohypophysis and adenohypophysis, are readily distinguishable in histological sections (Fig. 2).

The neurohypophysis consists largely of fiber tracts that originate in the hypothalamus and extend through the infundibular stalk to the posterior dorsal part of the gland. Here, the greatest concentration of neurohypophyseal tissue is located. From this area, fiber bundles of varying size ramify into the other pituitary regions and form interdigitations with groups of adenohypophyseal cells. Numerous glial cells with ovoid nuclei are scattered among the fibers. Aldehyde fuchsin-positive globules, presumed to be products of neurosecretion, are frequently present, especially in...
the posterior dorsal region. The neurohypophysis is well vascularized and small vessels are abundant in close proximity to adenohypophyseal cells.

The adenohypophysis is subdivided into the three regions characteristic of most bony fishes. From anterior to posterior they are: rostral pars distalis (pro-adenohypophysis), proximal pars distalis (meso-adenohypophysis) and pars intermedia (meta-adenohypophysis).

The rostral pars distalis constitutes about 40% of the adenohypophysis. The cells are arranged for the most part in follicles of varying size and shape. (Fig. 3). With the techniques used, little stainable material is seen in the follicular lumina. Neurohypophyseal fibers are interspersed among the follicles and the latter are bounded by basement membranes.

Examination of serial sections reveals that the follicles are not isolated units, but instead, have interconnecting lumina. Furthermore, every lumen is in contact directly or indirectly with the lumen of the hypophyseal duct. Thus, the follicle cells form a continuous, folded epithelium surrounding passages that are essentially ramifications of the hypophyseal duct. The functional significance of this morphological pattern is not clear.

The follicle wall consists principally of a layer of large columnar cells whose outer surfaces are adjacent to the basement membrane. Their nuclei are either basal in position or centrally located. The cytoplasm usually contains granules that stain with erythrosine and orange G although agranular, poorly stained cells are not
uncommon. Substantial evidence from several species of euryhaline teleosts indicates that these cells produce a prolactin-like hormone that promotes sodium retention in low saline environments (reviewed by Ball and Baker, 1969). An extremely thin layer of non-secretory squamous cells lines the follicular lumina and covers the apical borders of the prolactin cells. This layer is continuous with the lining of the hypophyseal duct.

A second secretory cell type occurs in inconspicuous clusters between the prolactin cell follicles and the neurohypophyseal fiber tracts in the dorsal part of the rostral pars distalis. The cells are small and round or polyhedral in shape with central nuclei. Their cytoplasm is scanty and contains granules that stain weakly with lead hematoxylin. (Figs. 8 and 9). Similar cells in *Poecilia latipinna* and *Anguilla anguilla* show hyperactivity under the influence of the adrenocortical inhibitor, metopirone, suggesting that they are ACTH-producing corticotrophs. (Ball and Oliverneau, 1966).

The proximal pars distalis is situated ventral and posterior to the rostral pars distalis. (Fig. 2). It comprises from 20% to 30% of the adenohypophysis, attaining its greatest size before and during the spawning period. The cells are arranged in cords or masses around neurohypophyseal terminations. Three kinds of cells can be identified.

One of the cell types is distinguished by the presence of fine orange G-positive granules in the cytoplasm. These cells are relatively small, round or polyhedral in shape with large spherical nuclei. They are most concentrated dorsally along the neurohypophyseal branches but are also scattered throughout the re-
mainder of the proximal pars distalis. In certain teleosts, changes in these cells during the normal growth cycle (Olivereau, 1963) or as a result of experimental alterations in growth (Sage, 1967) suggest that they are somatotrophs which produce growth hormone.

A second cell type shows variations closely associated with the reproductive cycle. These are large round or irregularly shaped cells with large nuclei (Figs. 4 and 5). They are most abundant in the centers of cell cords in the posterior and central areas of the proximal pars distalis. Characteristically, the cytoplasm stains strongly with the PAS technique and with aldehyde fuchsin, but under certain circumstances cytoplasmic degranulation and vacuolation are widespread. It is highly probable that these cells are gonadotrophs since their cyclical activity, and tinctorial reactions are essentially like those reported for this type of cell in other teleost studies (Sokol, 1961, Robertson and Wexler, 1962a and b, Sathyanesan, 1963, Olivereau and Herlant, 1964, Lagios, 1965, and Olivereau, 1967 and 1969).

Cells of the third type also react positively to PAS and aldehyde fuchsin, but they differ from gonadotrophs in several other ways. They are usually smaller and cone-shaped or spindle-shaped with eccentric nuclei. (Figs. 6 and 7). They are less numerous than gonadotrophs, and are confined mainly to the anterior ventral zone of the proximal pars distalis. Cytoplasmic granulas are finer in texture and cyclical changes in granulation and vacuolation are less pronounced. Histophysiologial studies showing changes in similar cells under the influence of hypothyroidism and hyperthy-

![Figure 4. Pre-spawning gonadotrophs, March (X2164), g, granulated cell, d, deganulated cell; v, blood vessel.](image-url)

The pars intermedia makes up 40% or less of the alewife adenohypophysis. It is closely associated with the main trunk of the neurohypophysis in the posterior part of the gland (Fig. 2). The cells are aggregated in masses around short, broad neurohypophyseal terminations. Two types of small, faintly acidophilic cells are recognizable, one type, spherical, with a central nucleus, and the other, angular, with an eccentric nucleus. Olivereau (1969) described two types of pars intermedia cells in Leuciscus rutilus.
The functions of these cells are unknown although in fishes as in other vertebrates the pars intermedia is assumed to produce the hormone, intermedin.

Cyclical pituitary changes.

Variations in pituitary cytology are evident when glands from different collection dates are compared. The gonadotrophs, thyrotrophs and corticotrophs were selected for detailed studies of these changes. To facilitate description the annual pituitary cycle is arbitrarily subdivided into three periods related to reproductive activity. They are the pre-spawning phase from early January to mid April, the spawning phase from late April to early August and the post-spawning phase from mid August to late December.

Annual variations in mean nuclear diameters of gonadotrophs, thyrotrophs and corticotrophs are shown in Figure 1. In the three cell types studied, nuclear size is at a maximum during the pre-spawning phase in March and at a minum near the end of the post-spawning phase in December. The differences between maxima and minima for all cell types are statistically significant. Gonadotroph nuclear diameters increase to the pre-spawning peak and then gradually decrease during the remainder of the year. Nuclear size in thyrotrophs is somewhat more variable. A decline occurs in April followed by a rise in May, but neither is statistically significant. Thereafter, mean nuclear diameters decrease with minor fluctuations to the December minimum. Mean diameters of corticotroph nuclei show the greatest variability. The annual maximum in March as well as two secondary size peaks in May and November all are significantly greater than mean nuclear diameters for preceding and succeeding months.
The more subjective cytological variations are considered separately for each cell type. The gonadotroph cycle parallels the reproductive cycle, but precedes it by several weeks. Maximum cell size is attained during the pre-spawning phase in March (Fig. 4). Coarse, intensely stained granules are abundant in the cytoplasm. Relatively few cells show cytoplasmic degranulation and vacuolation. At the peak of spawning activity during June and July, gonadotrophs are much more variable. Large heavily granulated cells, partially degranulated cells and agranular vacuolated cells may be found closely associated. This variability may indicate functional differences in individual cells as regards hormone production, storage and depletion. Decreasing size and increasing degranulation and vacuolation are characteristic of late spawning and early post-spawning gonadotrophs. Some nuclear pycnosis is present, but widespread cellular degeneration as noted by Robertson and Wexler (1962b) in Pacific salmon does not occur. The post-spawning picture is incomplete because of the lack of September and October specimens, but during November and December cell size reaches a minimum. The cytoplasm shows typical PAS and aldehyde fuchsia staining reactions although granules are either very small or absent (Fig. 5).

Thyrotroph size is also at a maximum during the pre-spawning period in March. Most cells are elongated and cone-shaped (Fig. 6). Partial cytoplasmic degranulation is widespread. This condition has often been related in previous pituitary studies to a high level of thyrotroph activity. During the spawning phase thyrotrophs vary widely in size, although the characteristic shape is main-

**Figure 8.** Pre-spawning corticotrophs, March, showing large size, reticular cytoplasm and large vacuole (X2164).
tained. The more elongated cells are about twice the length of the shorter ones. Cytoplasmic degranulation is rare, indicating a lower level of activity than during the pre-spawning phase. Unlike gonadotrophs during the spawning phase, vacuolation does not take place. Except for a general size decrease, little change is seen in post-spawning thyrotrophs. (Fig. 7).

As in the other two cell types, the peak of corticotroph activity occurs during the pre-spawning phase in March. At this time cell size is at a maximum. (Fig. 8). Nucleoli attain their greatest degree of prominence. The relatively abundant cytoplasm presents a reticular appearance suggestive of degranulation. Occasional very large cytoplasmic vacuoles are present. Spawning and post-spawning corticotrophs show decreases in size, nucleolar prominence and cytoplasmic reticulation and vacuolation (Fig. 9). Subjective evaluation of corticotroph activity is difficult because of their small size and sparse granulation. Variation in mean nuclear diameters over the annual cycle appears to be a more reliable indicator of the corticotroph functional state.

From the foregoing cytological observations it may be inferred that high levels of gonadotrophic, thyrotrophic and adrenocorticotrophic hormones are secreted by the alewife pituitary gland just prior to the spring spawning migration. The expected result is stimulation of the appropriate target organs, the gonads, thyroids and interrenals. This is obviously true of the gonads which undergo marked growth and increased functional activity. Histological evidence of thyroid stimulation in alewives during the spring was noted by Hoar (1952) and Boyles (unpublished communication, 1970).\(^1\) In certain other teleosts, increased thyroid function parallels

\(^1\) Dr. Marcia Boyles, Biology Department, Grand Valley State College, Michigan.
reproductive activity (Berg, et al., 1959, Bromage and Sage, 1968). There is no published information concerning the alewife interrenal but in Pacific salmon, extreme interrenal hyperplasia and elevated plasma levels of 170H corticosteroids were found during the spawning migration (Robertson and Wexler, 1959, Hane and Robertson, 1959). In the alewife and other migratory fishes, the increased endocrine activity associated with spawning may be partly an adaptive response to environmental changes encountered during their shoreward migration.

The possible effects of hormonal variations on alewife mortality may now be considered. It seems unlikely that pituitary gonadotrophins and gonadal steroids are primarily involved since sexually immature fish and adults in spawning condition are both abundantly represented in the spring dieoffs (Brown, 1968).

Changing levels of thyroid hormones may be more significant. Thyroid function in fishes is not well understood, but effects on osmoregulation, growth and migratory and motor behavior have been noted (Gorbman, 1969). The original suggestion of Hoar (1952) that thyroid induced osmoregulatory failure may be a factor in alewife mortality should receive further attention.

Changes in interrenal function may be even more pertinent. Corticosteroids cause electrolyte and fluid shifts in fishes but their exact roles in normal osmoregulation are not clear. These hormones also mediate responses to stress. (Chester Jones, et al., 1969). Stanley (1969) found significant shifts of sodium from plasma to muscle in apparently healthy alewives taken from Lake Michigan in June and July. In a laboratory study, Stanley and Colby (1971, in press) demonstrated that cold shock lowered plasma sodium levels in alewives maintained in fresh water. Holzer (1971) obtained similar results, and in addition found plasma sodium depletion and tissue hydration in dying alewives. These changes may denote partial or complete osmoregulatory failure possibly related to interrenal insufficiency.

In the present study, there are cytological indications of decreases in thyrotroph and corticotroph activity after pre-spawning peaks in March. These may signify reduced TSH and ACTH secretion during the spawning migration. A resulting decline in thyroid and interrenal function could, therefore, contribute to the mass alewife mortalities in June and July.

Since firm conclusions cannot be drawn from cytological evidence alone, it is obvious that endocrine-related physiological data must be obtained before the role of hormones in alewife mortality can be fully evaluated.
SUMMARY

Alewives were collected from Lake Michigan during all seasons to study annual variations in pituitary cytology. The alewife pituitary gland like those of other isospondylons teleosts has a hypothalamic duct and a follicular type rostral pars distalis. A total of seven adenohypophyseal cell types were recognized, two in the rostral pars distalis, three in the proximal pars distalis and two in the pars intermedia. Detailed studies of gonadotrophs, thyrotrophs and corticotrophs reveal basically similar annual patterns of change in secretory activity. Maximal stimulation is indicated in March just prior to the shoreward spawning migration and minimal activity occurs in December after the fish have returned to deep water. Thyrotrophs and corticotrophs are somewhat more variable in their cyclical patterns than gonadotrophs. Decreasing thyrotroph and corticotroph activity during the spawning run may lead to thyroid and interrenal deficiencies that are related to the annual spring and early summer mortalities.

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