

Ultrastructural localization of acetylcholinesterase (AChE) activity in the chicken Harderian gland

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Summary. Localization of acetylcholinesterase (AChE) was investigated in the chicken Harderian gland at the electron microscopic level. Nerve cells in the pterygopalatine ganglion showed AChE activity. They had a pale and large nucleus which was round or oval in shape. Reaction product of AChE was detected between the nuclear envelopes, in the cisterna of rough endoplasmic reticulum and the lumen of the Golgi lamellae, and on the plasma membrane of the nerve cell. In the interstitium of the gland, nerve fibers showing AChE activity were easily found. They were often seen in the perivascular space and between plasma cells. These nerve fibers had varicosities in contact with plasma cells and the endothelium or the smooth muscle fiber of the blood vessels. AChE-positive varicosities or terminals contained many small clear vesicles (about 50nm in diameter) and a few large dense-cored vesicles (about 100 nm in diameter). No contacts of nerve fibers with acinar cells or the ductal epithelium were observed in the present study. Our data indicate that cholinergic nerves play distinct roles in the regulation of the immune function of the chicken Harderian gland.

Key words: Harderian gland, Innervation, Acetylcholinesterase, Electron microscopy, White Leghorn chicken

Introduction

The chicken Harderian gland is the dominant orbital gland. The gland is a compound tubulo-acinar structure, and its secretory duct opens into the inferomedial portion of the conjunctival sac deep into the nictitating membrane (Wight et al., 1971a; Burns, 1976a). The gland has two important functions: production of a secretion that lubricates the nictitating membrane (Wight et al., 1971b), and protection of the ocular region from exogenous antigens (Burns, 1976b; Oláh et al., 1996). The aggregation of plasma cells is observed in the

interstitium of the chicken Harderian gland (Mueller et al., 1971; Albini et al., 1974). Plasma cells appear before and after hatching and increase their number with age (Wight et al., 1971a; Niedorf and Wolters, 1978). The cells are rich in immunoglobulins Ig A, Ig G and Ig M (Albini et al., 1974). Thus the chicken Harderian gland may contribute in an important component to the local immune system in the ocular region (Mueller et al., 1971).

A rich supply of autonomic nerves to the Harderian gland can be recognized in the domestic fowl. Histochemical studies showed the innervation of the gland by catecholamine-containing and AChE-positive nerves (Fourman and Ballantyne, 1967; Walcott et al., 1984; Walcott and McLean, 1985). AChE-positive nerves from the pterygopalatine ganglion (Gienc and Zaborek, 1984; Walcott et al., 1989) were distributed throughout the gland, especially in the interstitium. Fourman and Ballantyne (1967) suggested the regulation of the blood flow through the gland by AChE-positive nerves. Walcott et al. (1985) reported that many AChE-positive nerve fibers were seen among the plasma cells. It is supposed that cholinergic nerves play an important role in the regulation of the immune function of the chicken Harderian gland. But the relation of AChE-positive nerves to the lymphoid cell population has never been demonstrated at the electron microscopic level.

In the present study, we aim at the elucidation of the localization of AChE activity in the chicken Harderian gland at the electron microscopic level. Moreover, the neural regulation of the immune system in the chicken should be discussed.

Materials and methods

Animal and tissue preparations

Six adult male White Leghorn chickens weighing 1.5-2.5 kg were used in this study. Chickens were perfused with 2% glutaraldehyde in 50 mM sodium cacodylate buffer (pH 7.4) following 0.75% sodium chloride solution. Harderian glands were immediately removed from an orbital cave of both sides and each gland was divided into three small tissue blocks. Tissue blocks were immersed in the fresh same fixative at 4 °C

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for 6 hours. Sections at 50 μm thickness were made with a microslicer (DTK-1000, Dosaka EM, Japan) and kept in cold cacodylate buffer (pH 7.4) until next histochemical steps for AChE reaction.

AChE histochemistry

The copper-thiocholine method (Lewis and Shute, 1969) was applied as the cytochemical procedure to demonstrate AChE activity. Tissue sections were transferred into the incubation medium which contained thiocholine iodide (Sigma, USA) as an enzyme substrate and iso-OMPA (Sigma, USA) as a pseudocholinesterase inhibitor. Incubation was carried out at 4 °C for 2 hours. After the enhancement of AChE reaction with sodium sulfide medium, sections were post-fixed with 1% osmium tetroxide at room temperature for 2 hours and then embedded in Quetol 812 in the conventional manner. Ultrathin sections were made with an ultramicrotome (Super Nova, Reichert-Jung, Germany) and observed under a JEM-100sx transmission electron microscope after staining with uranyl acetate and lead compounds.

Results

Reaction products of AChE were observed on the membrane or organella of nervous elements in the chicken Harderian gland as electron-dense features.

Pterygopalatine ganglion is located along the superior margin of the Harderian gland. This ganglion contained many nerve cells showing AChE activity. AChE-positive ganglion cells have a relatively large and pale nucleus which was round or oval in shape (Fig. 1A). AChE-positive reaction was detected between the nuclear envelopes, in the cisternae of the rough endoplasmic reticulum and the lumen of the Golgi lamellae, and on the plasma membrane of the cell (Fig. 1B). Preganglionic terminals having contact with the ganglion cell showed AChE activity. These terminals contained many small clear vesicles (about 50 nm in diameter) and a few large dense-cored vesicles (about 100 nm in diameter). A few terminals contained many large dense-cored vesicles and showed AChE activity (Fig. 1C). Satellite cells in the ganglion were negative to AChE.

AChE-positive nerve bundles were frequently observed in the perivascular space (Figs. 2, 3A) and sometimes formed a dense meshwork. The nerve bundle consisted of a few or several fine nerve fibers and was found running between plasma cells in the interstitial tissue from the blood vessel (Fig. 2). Varicosity was formed along the bundle and made a contact with a plasma cell. A few large dense-cored vesicles were contained in the varicosity (Fig. 2, arrow). In the perivascular space, AChE reaction product was detected in the space between nerve terminals and plasma cells or the endothelium or the smooth muscle fiber of a blood vessel. The nerve terminals contained a few large dense-cored and many small clear vesicles (Fig. 3B).

Nerve bundles were often seen between plasma cells in the interstitial tissue. AChE activity was observed along the periaxolemma of nerve fibers in the bundle (Fig. 4). Fine AChE-positive nerve fibers were also found in the connective tissue below the epithelium of the central secretory duct (Fig. 5A). Varicosity was detected along the nerve fiber and synaptic vesicles were contained in it (Fig. 5B). AChE activity was seen in the cleft between the varicosity and a plasma cell. No nerve fibers showing AChE activity were observed in the epithelium of the secretory duct and around acini in the cortical region of the gland.

Small cells showing strong AChE activity were scattered in the interstitial tissue (Fig. 6). They had well-developed rough endoplasmic reticulum and some mitochondria as the organella. AChE activity was detected in the cisternae of the rough endoplasmic reticulum of the cell.

Discussion

In the present study we clarified that cholinergic nerves regulate the immune function of the chicken Harderian gland. The gland contains numerous plasma cells in its interstitial tissue (Wight et al., 1971a; Burns, 1975; Niedorf and Wolters, 1978). These plasma cells produce and secrete immunoglobulins in response to the local antigen stimulus (Albini et al., 1974; Burns, 1976b; Oláh et al., 1996). The Harderian gland is considered as the local immune tissue at the oculonasal region of the chicken (Mueller et al., 1971). Some studies at light microscopic level have been carried out on the cholinergic innervation of the avian Harderian gland (Fourman and Ballantyne, 1967, duck; Burns and MacKenzie, 1973, chicken; Walcott and McLean, 1985, pigeon; Walcott et al., 1985, 1989, chicken). These studies showed the dense distribution of AChE-positive nerve fibers in the gland. AChE-positive nerve fibers are related to blood vessels (Fourman and Ballantyne, 1967) and plasma cells in the interstitial tissue (Walcott and McLean, 1985; Walcott et al., 1985, 1989). No studies, however, have been available on the termination of AChE-positive nerves in the avian Harderian gland. In the present study we showed AChE-positive varicosities and terminals having a contact with plasma cells. They contained many small clear vesicles which indicate the cholinergic feature (Burnstock, 1979). These data provide the enough evidence that the cholinergic nerves regulate the immune function of the chicken Harderian gland.

Fourman and Ballantyne (1967) claimed that AChE positive nerves were related only to blood vessels. Conventional electron microscopy by Maxwell and his coworkers (1986) showed that nerve fibers containing agranular and granular vesicles had a close contact with the endothelium of blood vessels. We also observed that nerve fibers showing AChE activity were frequently distributed in the perivascular space and found them terminating on the endothelium. It is certain that blood

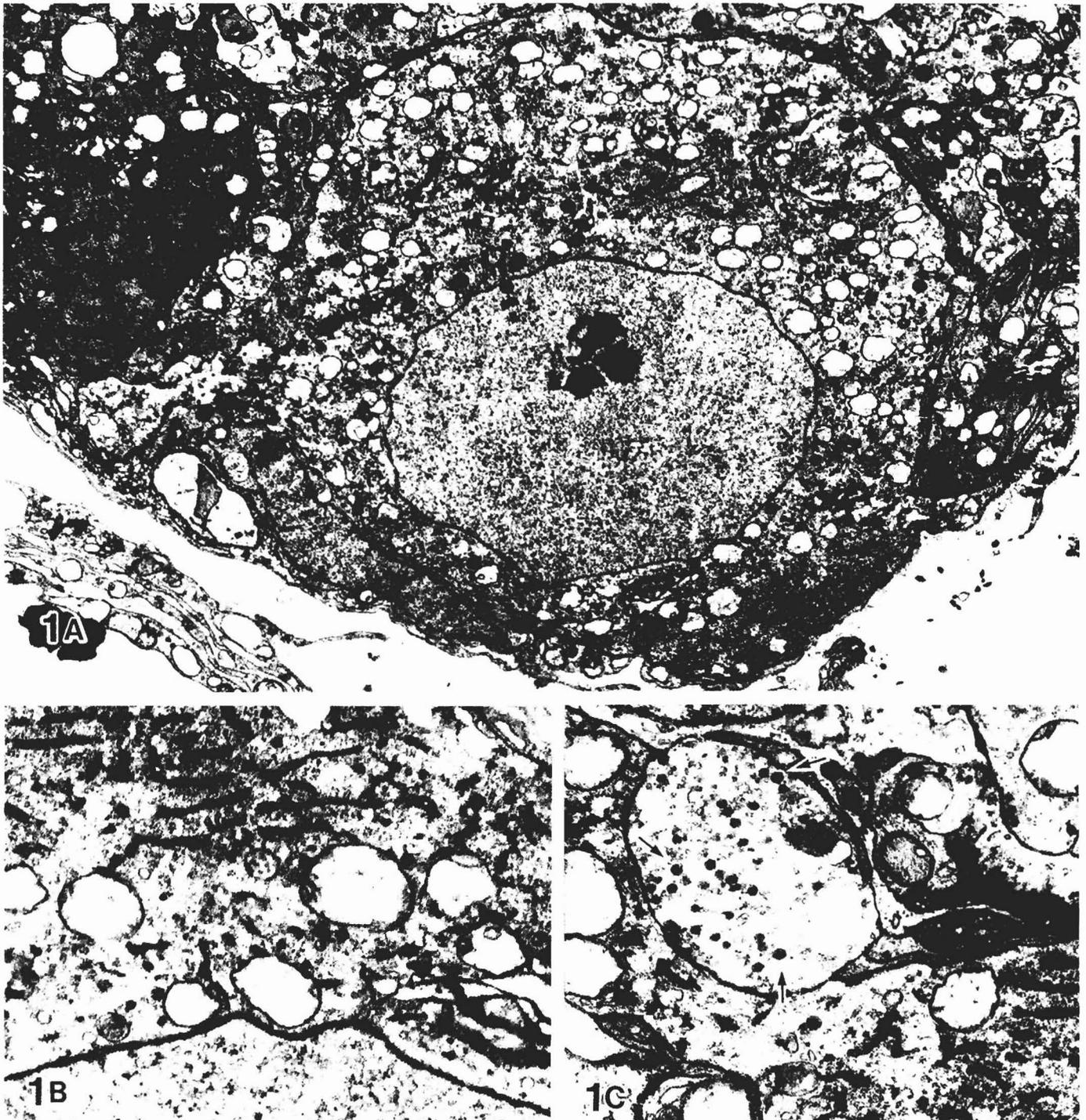


Fig. 1. Ultrastructural localization of acetylcholinesterase (AChE) activity in the nerve cell of the pterygopalatin ganglion. **A.** Low magnification view of AChE-positive ganglion cell. Reaction product is observed in the cisternae of the rough endoplasmic reticulum and Golgi lamellae, at the nuclear envelope and the plasma membrane. $\times 7,500$. **B.** High magnification view of the perikaryon. The cisternae of the rough endoplasmic reticulum is full with electron-dense reaction product. $\times 21,000$. **C.** Nerve endings which contain many large dense-cored vesicles (arrows) contact with AChE-positive ganglion cells (G). $\times 18,000$

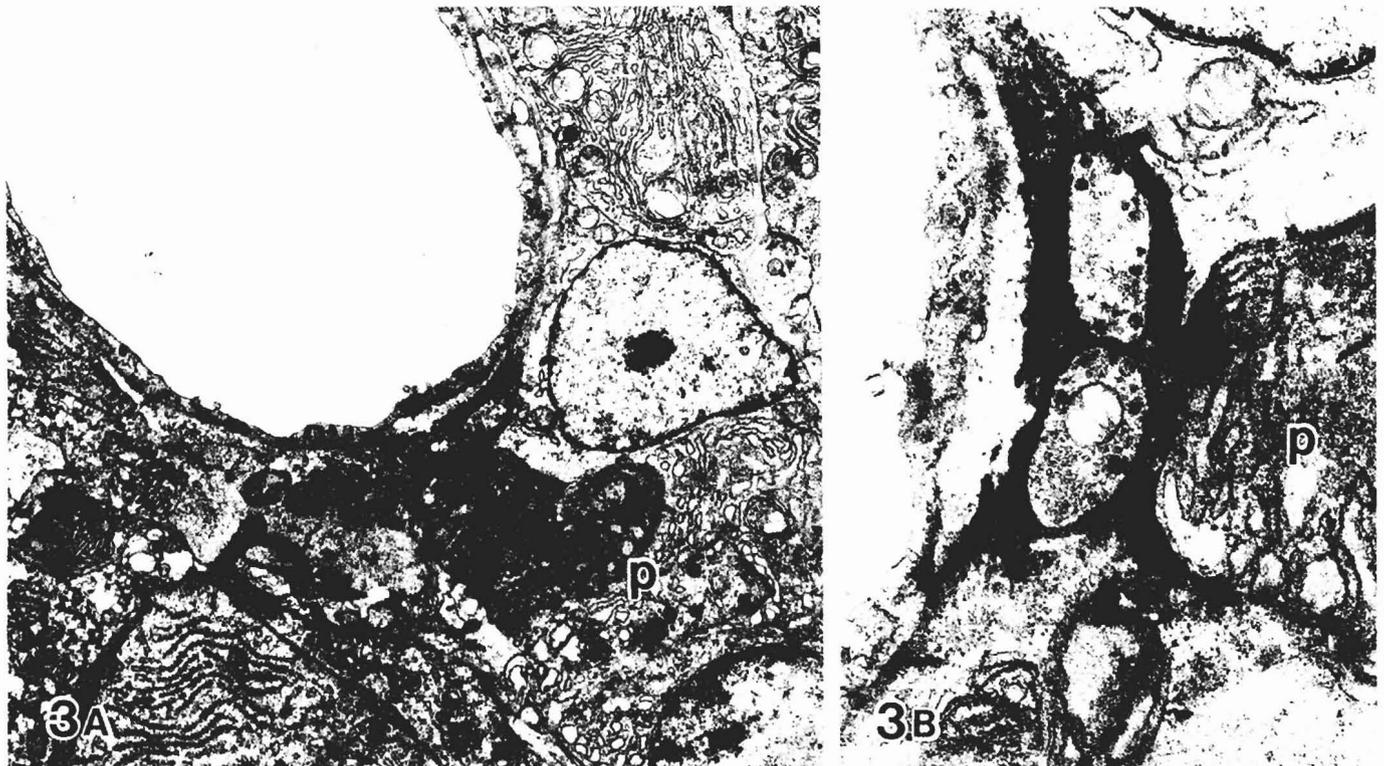
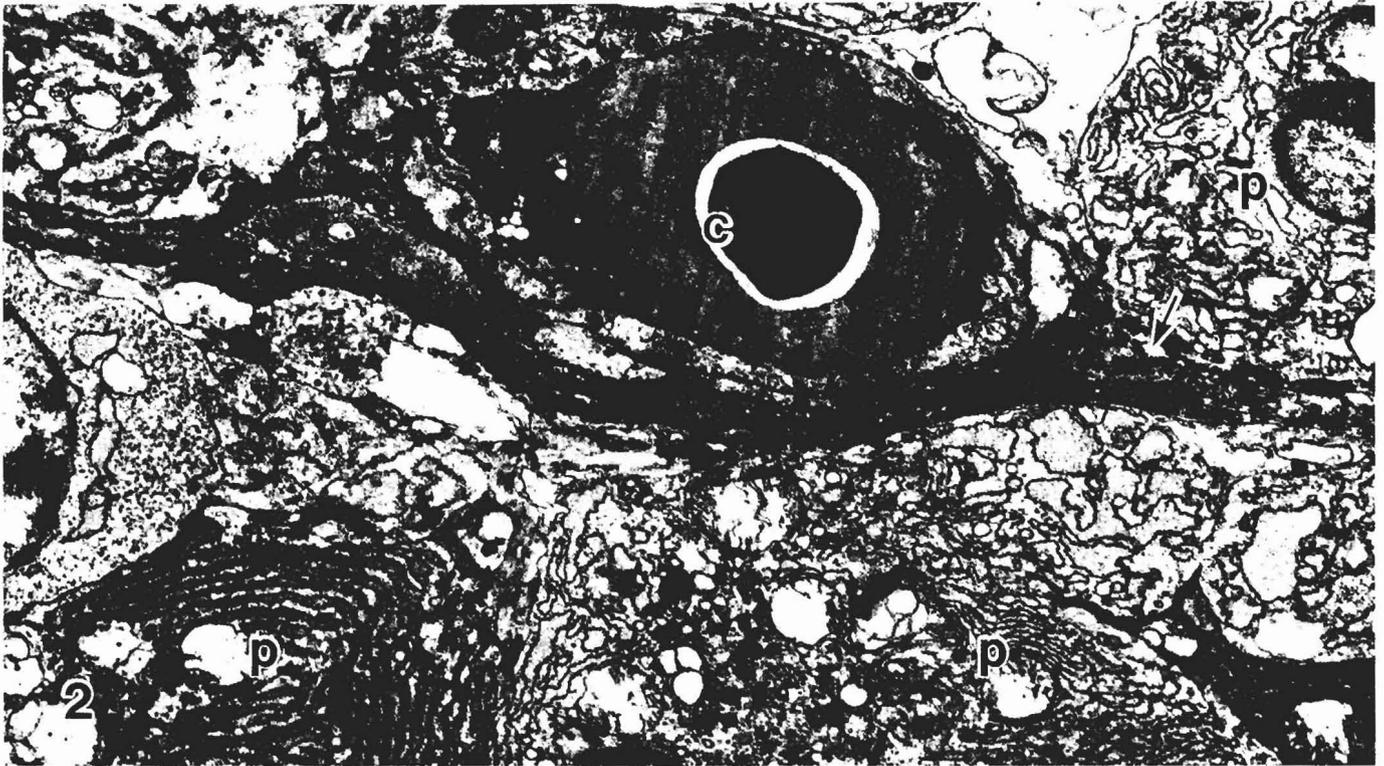
AChE in the chicken Harderian gland

Fig. 2. AChE-positive nerve bundle running between plasma cells (p). Varicosity (arrow) is formed along this bundle. A few large dense-cored vesicles are seen in the varicosity. C: capillary. x 9,000

Fig. 3. AChE-positive nerve fibers in the perivascular space of the chicken Harderian gland. **A.** Low magnification view of AChE-positive nerve fibers. Two fine fibers are localized between the blood vessel and the plasma cell (p). x 6,900 **B.** High magnification view of AChE-positive fibers. Reaction product is observed between nerve fibers and the plasma cell (p). Many small clear vesicles are found in nerve fibers. x 23,000

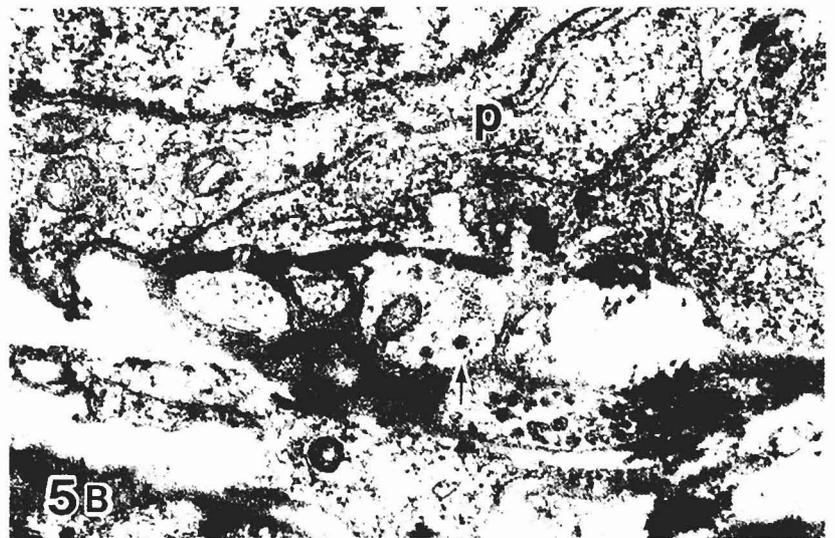
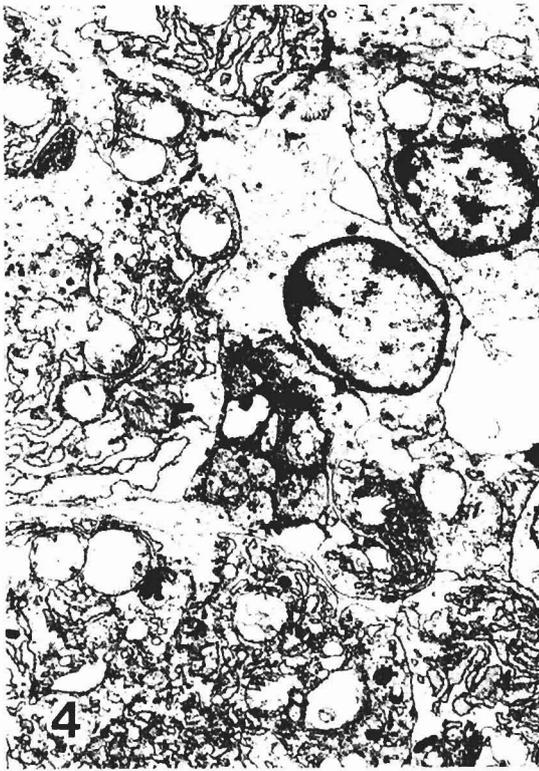


Fig. 4. Nerve bundle running in the interstitial space of the chicken Harderian gland. Several nerve fibers showing AChE activity are observed in this nerve bundle. x 6,900

Fig. 5. AChE-positive nerve fibers in the interstitial space of the chicken Harderian gland. **A.** Fine AChE-positive nerve fibers (arrow) are observed beneath the epithelium (E) of the duct. x 8,100. **B.** High magnification view of AChE-positive nerve ending. Reaction product is observed between nerve fibers and the plasma cell (p). Nerve endings contain many small clear vesicles and few large dense-cored vesicles (arrow). x 20,000

Fig. 6. AChE-positive cell in the interstitial space of the chicken Harderian gland. Rough endoplasmic reticulum is well developed in this small cell. x 8,100

vessels are one of the target tissues of AChE-positive nerves and that the blood flow of the Harderian gland is under the control of the cholinergic system. On the other hand, no nerve fibers were found around acini of the gland in this study. Walcott et al. (1989) also indicated that AChE-positive nerve fibers surrounding epithelial acini were uncommon. So the exocrine acini may be regulated by another system.

Several studies have shown the peptidergic innervation of the Harderian gland. Neuropeptides such as vasoactive intestinal polypeptide (VIP), substance P (SP), galanin and pituitary adenylate cyclase-activating polypeptide (PACAP), have been detected in nerve elements of the chicken Harderian gland (Walcott et al., 1985, 1989; Hiramatsu et al., in preparation). In this study nerve terminals showing AChE activity contained a few large dense-cored vesicles indicating the peptidergic feature. It is possible that neuropeptides mentioned above and acetylcholine colocalize in the same nerve terminals in the gland. These nerve terminals may release both acetylcholine and neuropeptides as the immunoregulators.

Recently many studies have shown the interactive actions between the nervous system and the immune system (cf. following reviews: Cavagnaro, 1986; Bellinger et al., 1990; Hori et al., 1995; Qui et al., 1996). The immune system may signal the nervous system by means of chemical messengers like cytokines. The nervous system modulates the immune system functions through releasing classical neurotransmitters, acetylcholine and noradrenaline, and neuropeptides. Plasma cells in the chicken Harderian gland have a contact with nerve fibers which are cholinergic in nature. It is supposed that the cholinergic system may regulate the production and the secretion of immunoglobulins by plasma cells. As mentioned above, the cholinergic nerves may release neuropeptides with acetylcholine. It has been shown that neuropeptides like VIP and SP affect immunoglobulin production (Stanisz et al., 1986). So it is supposed that acetylcholine and neuropeptides coregulate the immunoglobulin production in the chicken Harderian gland.

We found AChE-positive cells in the interstitium of the gland. These cells were smaller than nerve cells observed in the pterygopalatine ganglion and had well-developed rough endoplasmic reticulum. This feature is very similar to that of plasma cells which is described in the previous studies (Rothwell et al., 1972; Maxwell et al., 1986). But there is a smaller number of AChE-positive cells in the interstitium than plasma cells. T lymphocytes in the human peripheral blood show AChE activity and it is speculated that this enzyme has a regulating role in the cellular response (Szelényi et al., 1982; Páldi-Haris et al., 1990). It is unknown that AChE-positive cells in the chicken Harderian gland are plasma cells or other type of lymphocytes. This enzyme, however, may regulate the microenvironment around the cells and lose its activity during the cell maturation. Further experiments are necessary about this problem.

In conclusion, the cholinergic system regulates the blood flow and the immune function of the chicken Harderian gland.

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