# RESEARCH ARTICLES

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# THE PINEAL GLAND OF THE ADULT MALE SWAMP BUFFALO, BUBALUS BUBALIS: A LIGHT MICROSCOPIC STUDY

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#### Abstract

The pineal glands of the adult bull, Bos indicus, and the adult male swamp buffalo, Bubalus bubalis, were processed by Bodian or silver impregnation method and Luxol Fast Blue-periodic acid-Shiff-hematoxylin method, and studied under light microscopy. Grossly, the buffalo pineal is about 1.5 times larger than the bull pineal, has lobulated surface and central corpora arenacea. Histologically, there is an abundant connective tissue trabeculae within the buffalo pineal but very little within the bull pineal. Pineal parenchyma of the buffalo comprises primarily pinealocytes and glial cells; other components are nerve fibers, connective tissue cells, neuron-like cells, corpora arenacea, and specialized ependymal cells. The pinealocyte has characteristically spherical nucleus with prominent nucleous, and one or two cytoplasmic processes. The glial cell has oval-shaped nucleus. The nerve fibers are from two sources, one from nervi conarii and another from the epithalamus; both groups are distributed and terminated among pinealocyte processes, glial cell processes, and pericapillary spaces. The connective tissue cell has staining characteristics and morphology of the mast cell. Multipolar neuron-like cells were occasionally found. Specialized, elongated and ciliated ependymal cells line the pineal recess and contain dark granules at their apical processes. These morphological findings make the buffalo pineal distinguishable from the bull pineal. The presence of neuron-like cells and neural connection with the epithalamus pose a new evidence suggesting a possible parasympathetic innervation of the gland.

#### Introduction

In recent years the pineal gland has been elevated from its previous classification as a nonfunctional vestige to recongition as a respectable endocrine organ. This small gland, positioned on the dorsal aspect of the diencephalon, is now known to influence the reproductive system. The pineal is activated by reducing light available to the animal. Pineal antigonadotropic effects have been best demonstrated in the golden hamster and, to a lesser extent, other rodents. Cytological studies of the pineal gland have been carried out in a variety of species including in the cow, Bos taurus<sup>2</sup>. The cow pineal comprises primarily pinealocytes which have one or more processes that emerge from the cell body and terminate in or near perivascular spaces. Other components are glial, ependymal and connective tissue cells. Most species thus far examined possess unique cytological features; and there is sufficient interspecies morphological consistency to support classifying the pineal as an endocrine organ. This study concentrates on the pineal of another species closely related to the cow, the swamp buffalo (Bubalus bubalis). The purpose of this study is firstly comparative, and secondly to see if the pineal in the animal has any morphological distinction that could possibly account for the relatively low fertility in the buffalo, an animal that plays a vital role in a developing country like Thailand. This basic knowledge may also serve as baseline information for physiological and pharmacological applications of the gland in the future.

#### Materials and Methods

The pineal glands used in this study were obtained from four adult Thai bulls (Bos indicus) and six adult male swamp buffaloes (Bubalus bubalis) from a local slaughter house in Bangkok, Thailand. The buffaloes weighed between 400-500 kg and the bulls, between 200-300 kg. The animals were decapitated immediately after slaughter. By use of a hand saw, a transverse section was made just behind the eyes. A sagittal section was then made through the skull, and that portion of the brain containing the pineal was carefully removed. The pineals were separated from the epithalamus, each organ was weighed, measured, and divided into approximately three equal parts, corresponding anatomically to anterior, middle, and posterior regions.

The tissues were fixed in Bouin's fixative, paraffin-embedded, and serially sectioned at 6 µm thickness. The sections were processed according to the recommendations of Bodian or silver impregnation of nerve fibers<sup>3</sup>. Some sections were stained for myelin with Luxol Fast Blue-periodic acid-Schiff (PAS)-hematoxylin according to Margolis and Pickett<sup>4</sup>. Since the myelin component in most sections of this study was stained lightly with the latter method, the photomicrographs presented in this study, if not stated other wise, were therefore precessed with Bodian method.

#### Results

Gross Structure. The pineal of the swamp buffalo, Bubalus bubalis, is an oval-shaped gland,  $12.0 \pm 2.0$  (N = 6) mm in length,  $8.7 \pm 1.5$  (N = 6) mm in width,  $6.3 \pm 0.8$  (N = 6) mm in thickness, and weighs  $338.6 \pm 40.8$  (N = 6) mg. Its size is obviously larger than that of the bull, Bos indicus, which is  $10.0 \pm 1.2$  (N = 4) mm in length  $6.7 \pm 0.3$  (N = 4) mm in width,  $4.0 \pm 0.2$  (N = 4) mm in thickness and  $211.6 \pm 5.0$  (N = 4) mg in weight. The gland extends dorsocaudally from the epithalamus and has no stalk; thus, it could be considered as Type C pineal according to Vollrath<sup>5</sup>.

The buffalo pineal can be viewed as a gland with two surfaces, the slightly-convexed ventral surface and the arched dorsolateral surface. The ventral surface faces the superior colliculi when in situ. The dorsolateral surface is characterized by a highly lobulated feature (Figs 1A & 1B) carrying numerous nerves and blood vessels. The gland is more massive at the caudal free end than at the rostral epithalamic end; this is more clearly appreciated by viewing at its sagittal section (Fig 1C). The third ventricle extends caudally and bilaterally into the gland (Fig 1C); this portion of the third ventricle is, therefore, the pineal recess. Most portion of the gland tissue lies on top of the pineal recess. When viewed in cross section through the middle third of the gland (Fig 1D), the dorsolateral surface curves more than half-a-circle, whereas the ventral surface is only slightly convex outward. A round, single big mass of corpus arenacus or brain sand is located centrally and completely surrounded by a thin layer of the gland tissue (Fig 1D). Brain sands of various sizes are grossly seen scattering within the gland tissue; larger pieces are near the central mass and smaller pieces more peripherally.

General Microscopic Organization. The buffalo pineal is richly vascularized and encased by its capsule which is actually the pia mater. In preparation treated with Luxol Fast Blue-PAS-hematoxylin stain, several strands of connective tissue trabeculae are seen extending from the capsule into the gland tissue (Fig 2A). These trabeculae which support numerous blood vessels and nerve fibers penetrate deeply to a certain distance into the substance of the gland but do not divide it into distinctive lobules. In contrast to the buffalo pineal, the bull pineal is relatively devoid of connective tissue trabeculae (Fig 2B); its general microscopic feature is therefore rather homogeneous, containing densely packed cells and capillaries. This difference is obvious and makes the distinction between the microscopic feature of the buffalo and the bull pineal readily recognized.

The Pinealocyte and the Glial Cell. The buffalo pineal is composed of two primary cell types, the pinealocyte and the glial cell; the former being more prevalent. The pinealocyte is the larger cell with spherical nucleus of about 5-6 um in diameter (Fig 3A). In well preserved preparations it is difficult to discern their exact geometrical configuration; however, in preparations showing fixation shrinkage it is clear that the pinealocytes send out numerous cytoplasmic processes (Fig 3B), emanating in many

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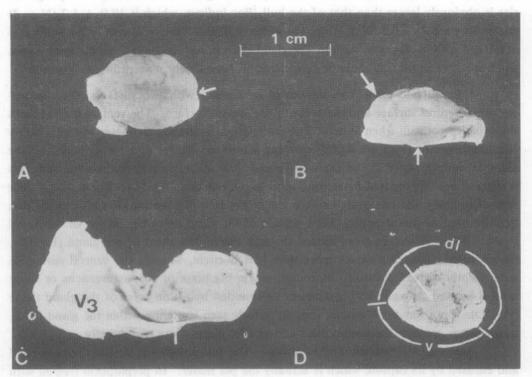


Figure 1. Gross structure of the swamp buffalo pineal. x2. A. The dorsal view: the oval-shaped gland was severed from other epithalamic structure at its rostral end, Its free caudal end is characterized by a shallow depression (arrow). B. The lateral veiw; its ventral surface (thin arrow), which anatomically lies dorsally to the midbrain, is less convexed than the dorsolateral surface. The gland is more massive at the caudal end (thick arrow) than the rostral end. The lobulated feature is clearly seen on its dorsolateral surface. C. The sagittal section of the gland showing the anatomical relationship between the third ventricle (V<sub>3</sub>) and the pineal gland. The ventricle extends deep into the pineal, and becomes the pineal recess (arrow). D. The cross section of the pineal showing a central mass of corpus arenacus (arrow) surrounded by the gland tissue. dl = dorsolateral surface. v = ventral surface.

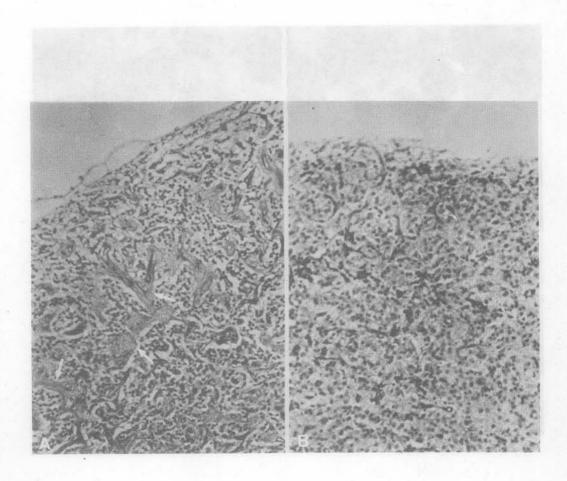


Figure 2. General microscopic feature of the swamp buffalo pineal (A), compared to that of the bull pineal (B). Numerous connective tissue trabeculae (arrows) are seen within the parenchyma of the buffalo pineal but not in the bull pineal. Luxol Fast Blue-PAS-hematoxylin. x 100.

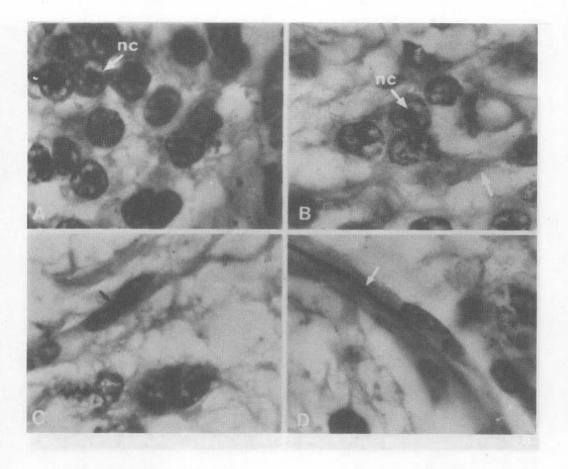


Figure 3. Two main types of cells in the buffalo pineal the pinealocyte (A & B) and the glial cell (C & D). The pinealocytes may stay closely together in some area (A) or loosely in other (B). Some pinealocyte processes are clearly seen (B, arrow). Note the conspicuous nucleolus (nc) in the pinealocyte nucleus which is consistent in this cell type. The glial cell nucleus, which contains scattered heterochromatin is elongated and some of them are indented (c, arrow). Some glial cells are associated with nerve fibers (D, arrow). x 1,000.

directions, thereby rendering the cell a delicately stellate form. Its spherical nucleus contains a conspicuous, centrally located nucleolus surrounded by many heterochromatin (Figs 3A & 3B). The features described are identical in most of the pinealocytes; no distinctive morphological difference among individual pinealocytes under these preparations can be made.

The glial cell is characterized by its rod-shaped or elongated nucleus, with one or two small nucleoli, and cytoplasm with a few processes (Figs 3C & 3D). The nuclei of certain glia cells are sligthly indented (Fig 3C) and some are closely associated with nerve fibers (Fig 3D). The cells disperse among pinealocytes; some are located within the connective tissue teabeculae.

Nerve Fibers. Many silver-stained positive nerve fibers are seen as bundles or as a single fiber penetrating, ramifying, and ending within the gland tissue. These fibers come at least from two sources; one as bundles entering its dorsolateral surface and another as nerve tracts from the epithalamic area, penetrating the gland at the pineal-epithalamic junction. All nerve fibers were stained negatively with Luxol Fast Blue-PAS-hematoxylin preparation, a preferential techique for viewing myelin; thus, the result indicates that these nerve fibers are non-myelinated.

By virtue of all the conclusions from the studies of pineal innervation in mammals, it is most likely that those fibers entering the dorsolateral surface are postganglionic sympathetic fibers specifically innervating the pineal named the nervi conarii. Branches of the nervi conarii accompanied by Schwann cells enter the gland capsule as a single (Fig 4A) or as a neurovascular (Fig 4B) bundle. Within the capsule, the fibers disperse widely in all directions before penetrating deep into the gland tissue (Fig 4C). Many fibers are located within the connective tissue trabeculae (Fig 4D), whereas some penetrate directly from the capsule and ramify in the gland parenchyma.

The nerve tracts originating from the epithalamic area enter the gland from its ventricular surface. As they pass deep into the substance of the pineal, their numbers appear to decrease considerably since many fibers leave their main courses to ramify among pinealocytes (Fig 5A). Many fibers seem to terminate at pinealocyte processes (Figs 5C & 5D). Some fibers are in close contact with but not terminate at the process (Fig 5B). Contact with some "neuron-like" cells are accasionally seen (Fig 5B); the cell has dark cytoplasm, vesicular nucleus containing a small dark nucleolus making it an owl-eyed appearance, typical of a neuron. Some fibers are seen to connect between two pinealocytes (Fig 5C).

In addition to its distribution among pinealocytes and glial cells, the nerve fibers are also found preferentially in the vicinity of pineal capillaries (Fig 6A & 6B). Most fibers are seen within the pericapillary spaces (Fig 6C).

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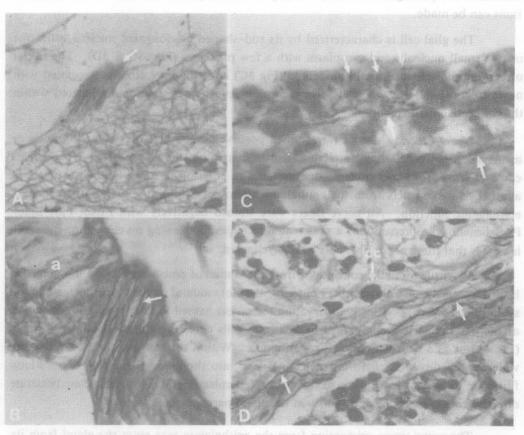


Figure 4. Nerve fibers entering the buffalo pineal. A. A branch of the nervi conarii is entering the gland capsule; Schwann cells (arrow) accompany the nerve. x 100. B. A bundle of silver stained-positive fibers (arrow) accompanies an arteriole (a) as neurovascular bundle before entering the gland. x 400. C. Numerous nerve fibers in cross sections (small arrows) and longitudinal sections (large arrows) are located within the pineal capsule. x 400. D. The nerve fibers run through the connective tissue trabecula (arrow). cc = connective tissue cell. x 400.

Other Components. In addition to pinealocytes and glial cells, there are other structures which are not strictly belonged to the pineal. These are the nouron-like cells, the connective tissue cells, the specialized opendymal cells, and the corpora arenacea or brain sands (Fig. 7).

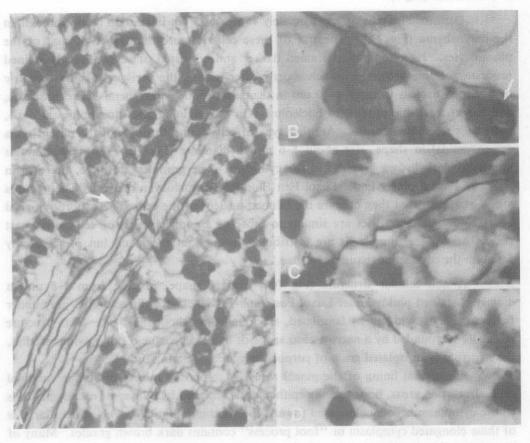


Figure 5. Nerve fibers from the epithalamus penetrating deep into the pineal tissue. A. A bundle of nerve fibers is seen along its path within the gland. The number of nerve fibers decreases as they penetrate deeper into the gland tissue. Some fibers are leaving their main path to ramify among pinealocytes (arrows). x 400. B. A nerve fiber lies in close contact with neuron-like cell (arrow) and the pinealocytes. x 1,000. C. A nerve fiber connects two pinealocytes. x 1,000. D. Termination of a nerve fiber at the pinealocyte process is frequently observed. x 1,000.

Other Components. In addition to pinealocytes and glial cells, there are other structures which are not strictly belonged to the pineal. These are the neuron-like cells, the connective tissue cells, the specialized ependymal cells, and the corpora arenacea or brain sands (Fig 7).

Occasionally, cells of approximately 15 um x 25 um in size are found throughout the parenchyma (Fig 7A). The size of these cells is more than twice of that of the pinealocytes. Its cytoplasm is stained darker and consisted of several darkly stained processes. Some processes branch shortly after leaving the perikaryon. Its eccentric oval-shaped nucleus is about 8-10 um in diameter, contains peripheral heterochromatin, and has at least one prominent nucleolus. On this staining and morphological basis, these cells resemble neurons. Another type of cells found throughout the parenchyma are pyramidal in shape, size about 5-6 um in diameter (Fig 7B). The cell contains numerous dark-brown granules in the cytoplsm. The granules are large, uniform in size, and stained pinkly under Luxol Fast Blue-PAS-hematoxylin method. The granules are so abundant that they obscure the bilobed nucleus of the cell. These morphological and staining characteristics are similar to those of a connective tissue cell called mast cell. These pineal mast cells are distributed throughout the gland but preferentially found in the connective tissue trabeculae.

A non-cellular structure (Fig 7C), oval in shape, ranging from 3-8 um in diameter, stained pinkly under Luxol Fast Blue-PAS-hematoxylin and blue-grey under Bodian methods, and usually lamellated, are found throughout the gland. This structure is usually surrounded by a narrow clear space or halo. By virtue of all previous studies, the structure is an isolated grain of corpora arenacea or brain sand.

Ependymal lining of the pineal recess is composed mostly of simple cuboidal cells. In certain areas, however, the epithelium is replaced by a layer of ependymal cells whose cytoplasm become elongated toward the ventricle (Fig 7D). The ventricular side of these elongated cytoplasm or "foot process" contains dark brown graules. Many of these cells also contain a number of cilia extending into the ventricular space.

#### Discussion

The mammalian pineal begins its development as a single evagination from the roof of the diencephalon, lined with ependymal cells. As differentiation proceeds, the enlage commences to diverticulate, folds extensively, and produces minor lamina within the organ. Initially, these small diverticulae are rosetted structure with lumen connected to the third ventricle; further cell growth obliterates the lumen, resulting in severance from their connection with the third ventricle. During and after diverticulation, the gland is invaded by the connective tissue of the ensheathing pia mater. Both the blind end of each diverticulum and the interdiverticular space are invaded by connective tissue trabeculae carrying capillaries and nerve fibers<sup>6</sup>.

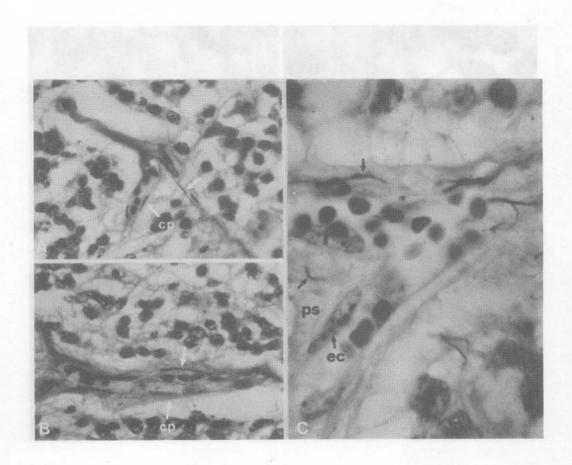


Figure 6. Nerve fibers at the pineal capillaries. A & B Nerve fibers (arrows) lie closely to the capillaries (cp). x 400. C. Many nerve fibers (arrows) are located within the pericapillary space (ps). ec = endothelial cell of the capillary. x 1,000.

Small frownish grammics (arrow) are located at the

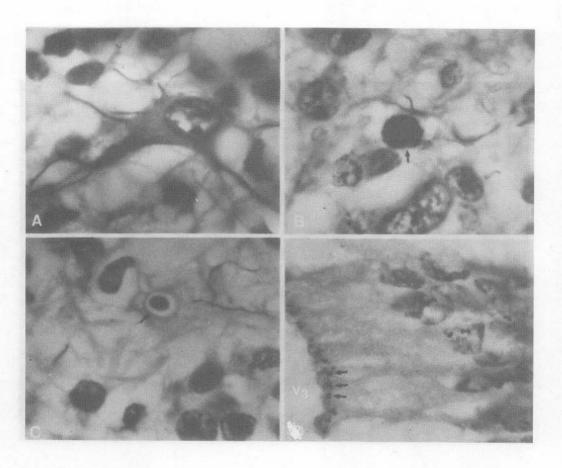


Figure 7. A. A neuron-like cell, approximately 15 um x 25 um in size, is occasionally seen throughout the buffalo pineal parenchyma. The cell has multiple branching processes, dark cytoplasm, eccentric nucleus, and one or two necleoli. x 1,000. B. A connective tissue cell (arrow) with many regular, brown granules obscuring its bilobed nucleus. Its morphology resembles that of the mast cell. x 1,000. C. An isolated brain sand (arrow) is characterized by its blue – stained oval stucture surrounded by narrow clear space. x 1,000. D. A row of elongated ependymal cells lining the pineal recess. Small brownish granules (arrow) are located at the ventricular side of their cytoplasmic processes. x 1,000.

The general microscopic feature of the buffalo pineal apparently follows the developmental scheme outlined above. The connective tissue trabeculae are abundant and within them are numerous nerves and blood vessels. These incomplete connective tissue septa originating from the capsule, likewise, renders the pineal surface many small lobulated areas. In the bull pineal, this connective tissue feature is poorly-developed, rendering it the homogeneous appearance and also the smooth surface. This outstanding difference may be due to either in developmental process of the pineal according to phylogenetic evolution or difference in age of the animals. Unforfunately, the exact age at which the animals were slaughtered can not be obtained: most cattles are slaughtered at age beyond 3 years. However, it is quite possible that the buffalo might be older in age, since most buffaloes are kept working in the rice field longer than the bull before being slaughtered.

The two major cell types, pinealocytes and glial cells, are also found in the pineal of many species. Pinealocytes, the major cell type, are, in some species, further classified into two or three other cell types according to their morphology<sup>7</sup>. In the rabbit, there are dark and light pinealocytes according to dark and light appearance in the cytoplasm. The difference is due to the amount of secretory granules within the cytoplasm; the more granules the darker it becomes. Whether there are two distinctive types of pinealocytes with different functions or this only represents a single cell type with difference in functional stage remains an open question. In the cow, only one population of pinealocytes was reported<sup>2</sup>. In the buffalo, using both staining techniques, only one population of pinealocytes has also been detected. Pinealocytes of the buffalo and the bull are nondistinguishable, and comprise of spherical nucleus with conspicuous nucleolus and a few cytoplasmic processes. The only difference between the two is that the size of the nucleus is somewhat smaller in the buffalo. The surface area of the pinealocyte is greatly enlarged by the presence of many complexly intertwined cytoplasmic processes which are so difficult to resolve with light microscope.

In most species, the glial cell is designated protoplasmic or fibrous astrocyte-like cell<sup>8</sup>. However, without the star-shaped characteristics of astrocyte as revealed by Golgi method it is inadequate to state that the glial cell in this study is astrocyte-like.

In many species, the neural element of the pineal is quite extensive and comprises myelinated and nonmyelinated nerve fibers and, in some species, a group of neurons. In rhesus monkey, its volumetric estimations have shown that as much as 30-50% of its total volume is occupied by the nervous tissue<sup>9</sup>. In most species, nerve fibers enter the pineal at two different points<sup>10</sup>. A bundle of mostly nonmyelinated nerve fibers, the nervi conarii, penetrates the pineal at its distal end, while nerve fibers, which are mostly myelinated enter the pineal proximally through its stalk from the habenular and posterior commissures. Pineal innervation of the buffalo also follows this general scheme. Nerve fibers of both sources ramify among pinealocytes, glial cells, and pericapillary spaces.

In some species, particularly among rodents, the nerve fibers entering from the pineal stalk do not seem to terminate within the pineal parenchyma, but rather follow the extension of the stalk into the gland tissue and return toward the epithalamus without making functional contact with pineal structures<sup>11</sup>. In the buffalo, innervation from the epithalamus may not follow this "loop phenomenon" since the fibers entering its epithalamic side penetrates deep into the gland, branch, and are also diminished in number as they progress deeper. Some nerve fibers are also seen ramifying among pinealocytes. It is possible that these fibers may have functional contact with pinealocytes.

The presence of neurons in the pineal is found consistently in primates<sup>12</sup>, and many nonprimates<sup>13</sup>. Cholinesterase activity has also been reported in the pineals of cow, sheep, pig and rat<sup>7</sup>. In this study, some multipolar neuron-like cells are present within the buffalo pineal. Traditionally, pineal gland is innervated almost exclusively by sympathetic nervous system from the superior cervical ganglia via either the nervi conarii or along with blood vessels. The sympathetic innervation increases the secretory function of pinealocytes via adenylate cyclase system<sup>14</sup>. What role the "pineal neurons" play is still unknown. Some investigators raised a possibility of parasympathetic inner vation of the gland as opposed to its sympathetic innervation<sup>11</sup>.

In general, the intraparenchymal sympathetic fibers end in close appositional contact with processes of pinealocytes and their terminal buds but not on the somata of these cells; no morphological characteristics of true synaptic junctions are observed, except those that have been illustrated by Wartenberg and Kappers in, respectively, the cat and the rat pineal<sup>7</sup>. In rodents, a number of terminal boutons have been found among pinealocyte and glial cell processes as well as within the pericapillary space<sup>15</sup>. In the buffalo, many nerve terminals are among pinealocyte processes and pericapillary spaces. Whether there is any neuronal synapse is difficult to resolve under light microscopy. The function of nerve terminals at the pericapillary spaces, has been suggested as to regulate blood flow to the pineal, thus, creating another way of controlling the pineal function<sup>11</sup>.

Mast cells are frequently observed in the buffalo pineal. Developmentally, the gland is invaded by mesodermal tissue including mast cell which contain heparin, histamine, slow-reacting substance of anaphylaxis (SRS-A), and eosinophil chemotactic factor of anaphylaxis (ECF-A) in their granules. Mast cells are mainly involved in immunologically mediated "immediate hypersensitivity" reactions and are located in connective tissue of many organs. Whether they have any specific function within the pineal remains unknown.

Brain sands or corpora arenacea are commonly encountered in pineal glands of many species. There has been a notable theory, without experimental bases, however, that corpora arenacea represent atrophic processes within the gland. There are certain

studies suggesting that this theory is probably wrong. For example, Wurtman et al. <sup>16</sup> have shown that there is no correlation between the degree of calcification in the human pineal and the activity of certain pineal enzymes. When pineal tissue of individuals 1 to 80 years of age was analyzed, the activity of hydroxyindole- O-methyltransferase, monoamine oxidase, and histamine-N-methyltransferase remained essentially constant. Reiter<sup>17</sup>, based on the secretory mechanism of certain endocrine glands, proposed just an opposite theory that the more calcified deposits, the more likely the gland is hyperactive. In the buffalo, the histological feature of the pineal revealed no involution process despite the presence of a relatively large central piece of corpora arenacea.

The specialized elongated ependymal cells which have cilia at their apical ends line the buffalo pineal recess. These so called "tanycytes" have been reported in many species including the cow<sup>2</sup>. The presence of dense granules within their apical ends have also been described and suggested to be certain secretory products of pinealocytes<sup>18</sup>. The products might be transferred from the pinealocyte to the tanycyte and subsequently released into the CSF. This secretion via the third ventricle has been hypothesized as another mode of pineal hormone secretion.

## **Concluding Remark**

Generally, histological feature of the buffalo pineal is similar to the pineal of many species. However, the finding of neuron-like cells and a possible neural connection with the epithalamic structures are relatively new feature described in only a few species thus far examined. The histological features described (numerous pinealocytes with vesicular nuclei and conspicuous nucleoli, numerous intertwined capillaries, high amounts of corpora arenacea, the presence of tanycytes, the possible existence of intramural neurons, and relatively abundance of neuronal connections) indicate a very actively endocrinological role of the gland. If the primary function of the buffalo pineal is to produce hormones which antagonize the reproductive function like the pineal of all species examined thus far, the active features described may partly explain the relatively low fertility in the buffalo.

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### บทคัดย่อ

ต่อมไพเนียลของควายหนุ่มตัวผู้ (Bubalus bubalis) มีขนาดใหญ่เป็นประมาณ 1.5 เท่าของต่อมไพเนียล ของวัวหนุ่มตัวผู้ (Bos indicus) เมื่อนำมาผ่านกรรมวิธีทางจุลกายวิภาคศาสตร์, ย้อมด้วยสีเฉพาะเพื่อศึกษาดูเส้นใย ประสาทและเซลล์ทั่ว ๆ ไป, พบว่าต่อมไพเนียลของควายมีเนื้อเยื่อเกี่ยวพันมากกว่าของวัวอย่างเห็นได้ชัด เซลล์และ องค์กรต่าง ๆ ที่พบในต่อมไพเนียลของควายได้แก่ เซลล์ไพเนียล (pinealocyte) เซลล์พี่เลี้ยง (glial cell), เส้นใยประสาท. เซลล์ของเนื้อเยื่อเกี่ยวพัน. เซลล์ที่มีรูปร่างคล้ายเซลล์ประสาท, เม็ดทรายที่เกิดในต่อมไพเนียล (corpora arenacea), และเซลล์บุโพรงสมองที่อยู่ติดกับต่อมไพเนียล (ependymal cells) เซลล์ไพเนียลมีลักษณะเฉพาะตัวคือ มีนิวเคลียสกลม มีนิวคลีโอลัสเห็นเด่นชัด และมี 1 หรือ 2 ระยางค์ ของไซโตพลาสม (cytoplasmic process) เซลล์พี่เลี้ยงมีนิวเคลียส เป็นลักษณะรูปไข่ เส้นใยประสาทมาจากแหล่งกำเนิด 2 แหล่ง คือจากเนอไวโคนาริไอ (nervi conarii) และจากสมอง ส่วนอิปิทาลามัส (epithalamus) เส้นใยประสาททั้งสองนี้กระจายอยู่ทั่วไประหว่างระยางค์ของเซลล์ไพเนียล, เซลล์ พี่เลี้ยง, และช่องว่างข้างหลอดเลือดฝอย (pericapillary spaces) เซลล์เนื้อเยื่อเกี่ยวพันมีลักษณะเหมือนมาสต์เซลล์ (mast cell) ทั้งทางรูปร่างและลักษณะการติดสีย้อม เซลล์ที่มีรูปร่างคล้ายเซลล์ประสาทพบอยู่กระจัดกระจายทั่วไป แต่ ไม่มากนัก เซลล์บุโพรงสมองมีลักษณะพิเศษกว่าเซลล์บุโพรงสมองทั่วไปคือ มีรูปร่างยาวมีขน (cilia) ที่ปลาย และ ยังมีจุดดำ ๆ (granules) กระจัดกระจายอยู่ที่ปลายไซโตพลาสมด้วย ดังนั้นลักษณะทั่ว ๆ ไปของต่อมไพเนียลของควาย จึงคล้าย ๆ กับต่อมไพเนียลในสัตว์เลี้ยงลูกด้วยนมอื่น ที่ต่างกันก็คือ การพบเชลล์ที่คล้าย ๆ เชลล์ประสาทและเส้นใย ประสาทที่มาจากบริเวณอิปีทาลามัสซึ่งลักษณะทั้งสองประการนี้พบได้ในสัตว์บางประเภทเท่านั้น สนับสนุนว่าต่อมไพเนียลของควายอาจถูกควบคุมโดยระบบประสาทอัตโนมัติ ทั้งประเภท parasympathetic และ sympathetic nervous system.