Morphology and Ultrastructure of the Hypophysis in Bactrian Camels (*Camelus bactrianus*)

**Summary:** The morphology of the hypophysis in Bactrian camel has not been described in the literature, despite it being the master of endocrine organs in vertebrates. In the present study, we examined the morphological features of the hypophysis in Bactrian camel by means of gross anatomy, light and electron microscope. Our findings showed that the gland was a protrusion of the bottom of the hypothalamus at the base of the brain with about 1.54 g in weight and 2 cm³ in volume. The hypophysis consists of two major parts: fully developed adenohypophysis and underdeveloped neurohypophysis, the adenohypophysis consists of pars distalis and pars intermedia. Seven type cells of the pars distalis could be distinguished with immunohistochemical techniques and electron micrographs: somatotroph, mammotroph, thyrotroph, corticotroph, gonadotroph, chromophobe and stellate cells which is in accordance with most mammals. Notably, the stellate cells could be obviously distinguished from chromophobe cells in histological observation. Moreover, the corpusculum neurosecretorium (Herring bodies) were rare in the external neurohypophysis, and mainly distributed in the internal neurohypophysis, this was different from most mammals. Results from this study would provide a necessary theoretical basis for ongoing investigations for Bactrian camels and their good adaptability in arid and semi-arid circumstances.

**Keywords:** Bactrian camel; Hypophysis; Morphology; Ultrastructure.

**Introduction**

The hypophysis plays a major role in regulating important physical functions and general wellbeing. It is referred to as the body’s ‘master gland’ because its secretion regulates the activity of most other hormone-secreting glands. It senses the body’s needs and sends signals to different organs and glands throughout the body to regulate their function and maintain an appropriate environment. It secretes a variety of hormones into the bloodstream which act as messengers to transmit information from the hypophysis to distant cells, regulating their activity (Martinez-Lage, 2011; Marieb & Hoehn, 2012).

The Bactrian camels (*Camelus bactrianus*) live in the arid and semiarid areas of the northwest China and Mongolia, where feeding resources are generally scattered and poor. Although the complexity the hypophysis in Bactrian camel is considered to be most important for their adaption to extreme environment, it has received little attention. Related researches of hypophysis on large artiodactyls, such as buffalo, sheep, pigs, dig and others were often found in publications (Dorst, 1968; Li et al., 2008). Whereas, studies on the brain, hypothalamus and adrenal gland in Bactrian camel have been more detailed report (Xie *et al.*, 2006; Ye *et al.*, 2014, 2015, 2017). So we systematically investigated the hypophysis from healthy adult Bactrian camel by anatomical, histological and electron microscopical approaches, and obtained the quantitative data for comparison among artiodactyls. The purpose of this study is to provide credible anatomic and morphological data, the cellular and subcellular structures of the hypophysis, which will contribute to the future research on the physiology and pathology of hypophysis structures and will provide a theoretical basis for ongoing investigations for the relationship between environment adaptation of the Bactrian camels and their special organ structure.

**Material and Method**

**Materials.** Ten specimens of the adult Bactrian camels were obtained from the slaughterhouse of the Right Alashan
Banner Food Company in Inner Mongolia Autonomous Region, China, the average age was 9 years old. As soon as the animals were killed by exsanguination in the local slaughterhouse, four specimens were removed intact and rapidly, then the different parts of the hypophysis separated and soaked in 3 % glutaraldehyde solution and 10 % neutral formalin solution fixative respectively, for microscopic and ultrastructural study.

The remaining specimens of the head of Bactrian camels were fixed at 5 to 15 min after death. Both internal carotid arteries were isolated by blunt dissection. Cannula coupled by surgical tubing to a pressure-driven perfusion device, were inserted into each vessel. Pressure was maintained at a constant value of 130 mmHg for both the initial rinse solution, which was a mixture of 0.5 L of 0.85 % sodium chloride, 0.1 % sodium nitrite and 0.1 % sodium heparin, and the final fixative which consisted of 3 L of 0.1 M phosphate buffered 10 % formalin (pH 7.4).

**Gross anatomy.** The brains and hypophysis were removed from the cranium at one week after death. The hypophysis was kept intact and weighted. The length parameters were measured by vernier caliper, and photos were taken by digital camera.

**Microstructure.** Samples of hypophysis for light microscope (LM) were fixed in 10 % formaldehyde for 72 h, dehydrated, cleared and embedded in paraffin. Embedded tissues of were cut into 7 mm thick sections and stained with hematoxylin and eosin (HE) or special stain (SS) (Wang et al., 2006).

**SS: form step 1 to 3**

Step 1. Alcian blue staining: tissue sections of 7 μm’s thick were dewaxed in xylene, soaked into the solution of formic acid (88 % formic acid 40 ml + H₂O₂ 4 ml + H₂SO₄ 0.5 ml) for 5 min, and then washed 10 min. The samples were stained by alcian blue (alcian blue 2 g + 2 N H₂SO₄ 100 ml) for at least 12 h, and then washed 10 min.

Step 2. Periodic acid-Schiff staining (PAS): The samples were soaked into 1 % periodic acid for 5 min, water rinsed several times, then stained by Schiff reagents (water 200 ml + Basic fuchsin 1 g + 1 N HCl 20 ml + potassium metabisulfite 1 g + activated carbon 2 g) for 10 min of water rinse.

Step 3. Azocarmine and orange G staining: The samples stained by 0.5 % azocarmine for 30 s, water rinsing, stained by 2 % orange G (orange G 2 g + phosphotungstic acid 1 g + water 100 ml) for 30 min. The sections were dehydrated through increasing concentrations of ethanol and xylene, coverslip slides using permount.

**Ultrastructure.** The samples were prepared for Transmission electron microscope (TEM) with three major procedures, post processing, semi-thin sectioning and ultra-thin sectioning. In post processing, small pieces were first fixed in 3 % glutaraldehyde buffer for 1 week; second, they were washed in 0.1 M phosphate buffer; third, the pieces were cut into 1 mm³ lumps and post-fixed with osmium tetroxide for 1 h; fourth, the samples were washed twice in 0.1 M phosphate buffer and then dehydrated in ascending grades of ethanol before being embedded in epon 812. Semi-thin sections of different samples were collected and stained with toluidine blue. Ultra-thin sections were then collected on copper grids. The ultra-thin sections were stained with a saturated solution of uranyl acetate for 30 min, followed by lead citrate for 7 min in a carbon dioxide-free environment. The end-products were examined under a transmission electron microscope (JEOL, JEM-1230).

**Data analysis.** All the sections were photographed and analyzed by MOTIC Images Advanced 3.0 and the data obtained were statistically analyzed using the SPSS in version 19.

**RESULTS**

**Anatomy.** The hypophysis of Bactrian camel rests upon the hypophysial fossa of the sphenoid bone in the center of the middle cranial fossa attaching to the hypothalamus by hypophysis stalk. And it was surrounded by sella turcica covered diaphragma sellae. The gland was oval in shape with the same size of a peanut. The terminal of hypophysis was a tiny protuberance, which was the terminal of neurohypophysis. The hypophysis was about 1.54 g in weight with the weight ratio of 0.0045 g/kg to body (AGW/BW). Its volume was about 2~3 cm³ with different radial lines of 1.68'1.43'1.14 cm. Moreover, the hypophysis stalk was 0.55 cm in diameter and 1.09 cm in length. The hypophysis could be divided into two parts, the outer adenohypophysis and the inner neurohypophysis (Table I) (Figs. 1 and 2).

Observation from the median sagittal plane, the hypophysis in Bactrian camel is bilaterally symmetrical. The cavum infundibular visibly extended to the distal of infundibular stalk, with a portion deep inside of the hypophysis. The hypophysis in Bactrian camel could be divided into three parts: pars nervosa, pars distalis and pars intermedia. The pars distalis was biggest, located in ventral surface of the hypophysis, and surrounding the pars inter-
media excluding the top of the hypophysis. The hypophyseal cleft was obvious and big between pars distalis and pars intermedia, and it was filled with gelatinous substances. The pars intermedia is a narrow region between the pars distalis and pars nervosa, which surrounding the pars nervosa excluding hypophysis stalk and the tiny protuberance at the terminal of pars nervosa. The pars nervosa was water-drop shape, located in the centre of the gland, and surrounded by the pars intermedia and the pars distalis. Furthermore, the pars tuberalis that surrounds the neural stalk, was an intrusion of the pars distalis to the hypothalamus. The neurohypophysis consisted of the pars nervosa, the infundibular stalk and the median eminence. The infundibular stalk and the pars tuberalis together comprise the hypophysis stalk. Besides, on the dorsum of the hypophysis, filled with gelatinous substance, a big cavity formed between the capsule and the pars intermedia, or between the pars intermedia and the pars nervosa (Figs. 2 and 3).

<table>
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<td>Thickness (cm)</td>
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<td>0.25</td>
<td>0.84-1.25</td>
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<td>Length of pituitary stalk (cm)</td>
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AGW/BW: the ratio of the hypophysis gland weight / body weight.
**LM and TEM.** The hypophysis was covered by a thinner layer of capsule with connective tissue, which consisted of mainly collagen fibers and reticular fiber. The parenchyma had two anatomically distinct zones, the outer adenohypophysis and the inner neurohypophysis, which had different histological and cytological structures in LM and TEM.

**Adenohypophysis.** The adenohypophysis constituted 80% of the volume of the hypophysis with fasciculate or irregular anastomosing reticulum, less connective tissue and affluent blood sinuses present. The cells of adenohypophysis arranged in clusters, and formed the following parts: the pars distalis and the pars intermedia (Figs. 3 and 4).

**Pars distalis (PaD).** The pars distalis was the largest portion of the whole gland. Clusters of cells were arranged here, fasciculately or reticulumly, with blood sinuses affluenty mingled (Figs. 4A and 4B). According to the colors after HE staining, the cells could be divided into chromophobe cells, acidophil cells and basophil cells. The cytoplasmic of the acidophil cells were red, and the nuclei were deep blue. The cytoplasmic of the basophil cells were light blue, and the nuclei were deep blue. Moreover, the chromophobe cells were hard staining by HE, the cytoplasmic were light blue or colorless, and the nuclei were light blue. In general, the chromophobe cells accounts for above 50% of the cells of pars distalis, the acidophil cells were 40%, and the basophil cells were less than 10% (Figs. 4C-L). Furthermore, a thin layer of cells that was similar with ependymal cells located in the interior marginal area of the pars distalis, and margin on hypophyseal cleft. The cells were triangle, round or ovoid in shape, and palisading closely. The nuclei were rounded or oval and the nucleo-cellula ratio (N/C ratio) was larger (Fig. 4K).

**Chromophobe cell (CC).** Half and more of the pars distalis are the chromophobe cell, which arranged in clusters. The chromophobe cells were small in size with rounded or polygonal-shaped and with a lighter HE staining (Figs. 4C-L). Some publication indicated that chromophobe cells were...
undifferentiated to reserve for transforming into acidophilic cell and basophilic cell. Ultrastructural examinations confirmed that the chromophobe cells were rounded or polyhedral, whose nuclei were rounded or oval with a larger N/C ratio. It is worth noting that underdeveloped organelles and absence of secretory granules was the most prominent feature of the chromophobe cells (Fig. 5A).

**Acidophil cell (AC).** The majority of acidophil cells were located in the lateral and central parts of the pars distalis, and the minority were in the interior part. The acidophil cells were triangle, round, or ovoid in shape, and medium-sized or somewhat larger. The nuclei were rounded or oval, dyed with various shades, and usually one or two were in a cell. The cytoplasmic of acidophil cells could be stained by eosin at HE. The results of azocarmine and orange G staining could demonstrate the difference of two types of acidophil cells: Orange somatotrophic cells and nacarat lactotrophic cells (Figs. 4C-L). Further, a relationship of the secretory granules identified by the electron microscopy has also been demonstrated (Ciocca et al., 1979).

**Somatotrophic cell (SC).** The somatotrophic cells constitute over 50% of the acidophil cells, and arranged in clusters along the capillary. The cytoplasmic of somatotrophic cells could be stained orange by orange G (Figs. 4F and 4J). In electron micrographs, the somatotrophic cells were rounded or polyhedral, the nuclei were round and located centrally. Many secretory granules ultrastructurally visible were electron-dense, uniformly spherical and generally 240 to 410 nm in diameter, with no gap between membrana limitans and endocrine (Fig. 5B).

**Lactotrophic cell (LC).** The lactotrophic cells always arranged singularly, and tended to be randomly distributed within the pars distalis. The cytoplasmic of lactotrophic cells could be stained nacarat by azocarmine and orange G (Figs. 4C-L). Further, a relationship of the secretory granules distinguished under light microscopy. In electron micrographs, the lactotrophic cells showed small and less secretory granules measuring generally 70 to 150 nm in diameter, and the secretory granules were the smallest. The secretory granules were electron-dense and uniform, spherical, and located in the cytoplasm marginal area (Fig. 5D).

**Basophil cell (BC).** In general, the basophil cells were slightly larger than the acidophil cells, and round, ovoid or polygon in shape. The cytoplasmic could be stained light blue by HE, and the nuclei were dark blue. Three types of basophil cells could be distinguished with immunohistochemical techniques and electron micrographs: Thyrotrropic cells, gonadotrophic cells and corticotrophic cells (Figs. 4C-L and 5D-F).

**Thyrotrropic cell (TC).** The thyrotrropic cells were round or polyhedral in shape, and medium-sized or somewhat larger. They often formed small nests occupying a fairly area of the anterior-median portion of the pars distalis. The nuclei were rounded or oval with a small N/C ratio. After the procedure of SS staining, the cytoplasmic were dark blue, especially the cytoplasm marginal area, and the nuclei were light red (Figs. 4F and 4J). Ultrastructurally, the thyrotrropic cells showed small and less secretory granules measuring generally 70 to 150 nm in diameter, and the secretory granules were the smallest. The secretory granules were electron-dense and uniform, spherical, and located in the cytoplasm marginal area (Fig. 5D).

**Gonadotrophic cell (GC).** The gonadotrophic cells were round, ovoid or long strip in shape, larger-sized, and medium-sized, and widely distributed throughout the pars distalis. By SS staining, the cytoplasmic were fuchsia, and the nuclei were crimson (Figs. 4F and 4J). In electron micrographs, a lot of secretory granules were electron-dense, spherical, and visible in the cytoplasm. The size of secretory granules varies considerably, generally divided into large granules and small granules. The diameter of large granules generally were 200 to 250 nm, small granules were about 80 to 150 nm (Fig. 5E).

**Corticotrophic cell (ACTH).** The corticotrophic cells were ovoid in shape, smaller-sized, amount was less, and usually located within the central part of the pars distalis. After SS staining, the cytoplasmic were light blue, and the nuclei were light red (Figs. 4F and 4J). Ultrastructurally, these cells showed small and less secretory granules in diameters of generally 80–250 nm. The secretory granules were electron-dense and nacarat, spherical locating in the cytoplasm marginal area (Fig. 5F).

**Stellate cells.** The stellate cells could not be clearly distinguished under light microscopy. In electron micrographs, the stellate cells looked similar to the chromohoepe cells, as they were absence of secretory granules. Their differences were that the chromohoepe cells were rounded or polygonal-shaped arranging in clusters, but the stellate cells were irregular-shape, with long protuberance arranging as a single cell. Furthermore, the organelles of chromohoepe cells were more developed than the chromohoepe cells (Fig. 5G).

**Pars intermedia (PaI).** The pars intermedia of the hypophysis in Bactrian camel was narrow situated between the pars distalis and the pars nervosa, approximately 730 um in thickness, and composed of basophil cells and many crevices with different sizes. These cells arranged in cords or clusters. There was an obvious and big cleft between pars distalis and the pars intermedia, which named hypophyseal cleft. There was also a cleft between the pars intermedia and the pars nervosa, which was smaller than the
hypophyseal cleft. The pars intermedia was developed and surrounding the pars nervosa except hypophysis stalk and the tiny protuberance at the terminal of pars nervosa. The basophil cells were the only type observed after HE and SS staining in the pars intermedia, the cells were irregular polygon, larger-sized, and the nuclei were round or oval (Fig. 6).

**Neurohypophysis (PN).** The neurohypophysis consists of the pars nervosa, the infundibular stalk and the median eminence. They contained large numbers of unmyelinated nerve fibres, pituicytes, rich in capillaries and connective tissue. The nerve fibre bundles environ the capillarie, which was located centrally, surrounded by connective tissue and unmyelinated nerve fibers, radiated out from internal neurohypophysis to external neurohypophysis, and were dyed deep by SS. By SS staining, the nerve fibres were deep blue, the connective tissues were red, and the pituicytes were red or blue and tended to be randomly distributed within the nerve fibres. The corpusculum neurosecretorium (Herring bodies) were rare in the external neurohypophysis, and mainly distributed in the internal neurohypophysis (Figs. 7 and 9).

**Nerve fibres.** The nerve fibers of the neurohypophysis mainly started from the hypothalamus, and branchily radiated out from internal neurohypophysis to external neurohypophysis. The pituicytes and some narrow clefts were randomly distributed within the nerve fibre bundles. The cells of internal neurohypophysis were loose, and filled with a lot of blood sinus and cavity. The corpusculum neurosecretorium were different in size and shape, and mainly distributed in the internal neurohypophysis, which were stained deep eosinophilic masses by HE (Figs. 7A-D and 9) (Dellmann & Rodriguez, 1970; Vazquez & Amat, 1978).

**Pituicyte.** Pituicytes were glial cells with irregular shapes and some protuberances. Their cytoplasm often contains lipid droplets and pigments. Two types of pituicytes could be distinguished by HE and TEM: protoplasmic pituicytes and fibrous pituicytes (Figs. 7E-F and 8).

**Protoplasmic pituicyte (PP).** The protoplasmic pituicyte constitute most of the pituicyte with larger size, different protuberances, and long elliptic in the nuclei. In electron micrographs, the cells and its nuclei were irregular in shape. The cytoplasm contained numerous lipid droplets. many pigments with electron-dense and irregular shapes which were visible in the cytoplasm (Figs. 7E-F and 8A).

**Fibrous pituicyte (FP).** The fibrous pituicyte constitute a handful of pituicyte, with smaller size and less protuberances. The nuclei were irregular round or ovoid, and the marginal area was dyed deep by HE. In electron micrographs, the cells and nuclei were irregular in shape with a larger N/C ratio. A lot of lipid droplets with irregular shapes were visible in the cytoplasm. Furthermore, the cytoplasm of fibrous pituicyte included numerous ribosomes, abundant round or oval mitochondria, and developed endoplasmic reticulum (Figs. 7E-F and 8B).
DISCUSSION

Although the hypophysis showed great differences in morphological characters in mammals, Bactrian camel possessed one similar to Chinese buffalo (Kang & Liu, 1992). There were great differences in the gland weights of mammals.
and generally large body with big one but a small ratio of AGW/BW (Table II) (Francis & Mulligan, 1949; Dorst; Kang & Liu; Takano et al., 1999; Li et al.). The hypophysis of Bactrian camel was about 1.54 g in weight, its AGW/BW was 0.0045 g/kg, and it was similar with herbivores that had the similar body size to Bactrian camel. The weight and morphological characters of the hypophysis would be greatly influenced by seasons, age and sex (Dent, 1961).
The Bactrian camel had fully developed adenohypophysis that constituted 80% of the volume of the hypophysis. It was worth noting that the pars intermedia was more developed than in humans and in most domestic animals (Hewitt, 1950; Dorst). We noticed an interesting phenomenon: some mammals that live in arid and semi-arid areas had developed pars intermedia, like rodent and Bactrian camel, while undeveloped ones existed in humans, primates and domestic animals, or were even missing in elephants and whales. We hypothesized that the evolution level of pars intermedia might be related to thirst resistance ability. However, the neurohypophysis was undeveloped, which was related to low fertility of Bactrian camel.

Seven type of cells in pars distalis could be distinguished by immunohistochemical techniques and electron micrographs, and the constitution was similar with the most of mammals (Moriarty, 1973). Among them, the stellate cells were contradictory due to the absence of secretory granules, some scholars think it belonged to the chromophobe cells, but others disagreed with it (Soji & Herbert, 1989; Allaerts et al., 1996). The difference between them was that the chromophobe cells were rounded or polygonal-shaped with arrangement of clusters, but the stellate cells were irregular-shape, usually had long protuberance, and were always arranged in a single cell. Furthermore, the organelles of stellate cells were more developed than the chromophobe cells. Therefore, they could easily be differentiated in terms of morphology, but the functions of the former were unclear. Soji & Herbert found that the stellate cells could transmit signals via the network of protuberance in rats. It was reported that: the dendritic cells were visible in the adenohypophysis, and its functions were not only the antigen presenting, but also could regulate the development, hormone secretion and feedback, etc. (Allaerts et al.; Hoek et al., 1997).

The neurosecretory granules are transported axonally down to the pars nervosa, and they often accumulate in the dilated portions of the axons near their terminals named corpusculum.
neurosecretorium. The corpusculum neurosecretorium, in Bactrian camel which was different from most mammals, were rare in the external neurohypophysis, and mainly distributed in the internal neurohypophysis.

Our study would provide a comprehensive assessment of hypophysis in Bactrian camel, and all of these morphological characteristics of the hypophysis, might have evolved to assist the camel in adapting to a particular environmental niche. However, further studies are necessary to better understand the biological characteristics and regulation mechanisms of this organ.

ACKNOWLEDGEMENTS. This study received financial support from National Natural Science Foundation of China (39300097), and Open Foundation of Chinese Educational Department Key Laboratory of Arid and Grassland Agroecology. The authors are also grateful to Dr. Lei Zhu, Chun Yang and Zhongtian Bai for the collection of specimens.


RESUMEN: La morfología de la hipófisis en el camello bactriano no ha sido descrita en la literatura, a pesar de ser el maestro de los órganos endocrinos en los vertebrados. En el presente estudio, examinamos las características morfológicas de la hipófisis del camello bactriano por medio de anatomía general, microscopía de luz y microscopía electrónica. Nuestros hallazgos mostraron que la hipófisis es una protuberancia ubicada en la porción inferior del hipotálamo, en la base del cerebro, con aproximadamente 1,54 g de peso y 2 cm³ de volumen. La hipófisis consta de dos partes principales: adenohipófisis, completamente desarrollada, y neurohipófisis, poco desarrollada; además, la adenohipófisis consta de una pars distalis y una pars intermedia. Con técnicas inmunohistoquímicas y micrografías electrónicas en la pars distalis se pudieron distinguir siete tipos de células: somatotrofas, mamotróficas, tirotrofas, corticotrofas, gonadotrofas, cromófobas y estrelladas, lo que es similar a la mayoría de los mamíferos. En la observación histológica las células estrelladas se pueden distinguir naturalmente de las células cromófobas. Además, es rara la presencia de corpusculum neurosecretorium (Cuerpos de Herring) en la neurohipófisis externa, hallándose distribuidos principalmente en la neurohipófisis interna, esto es diferente a lo encontrado en la mayoría de los mamíferos. Los resultados de este estudio proporcionarían una base teórica necesaria para las investigaciones en curso de los camellos bactrianos y su buena adaptabilidad en circunstancias áridas y semiáridas.

PALABRAS CLAVE: Camello bactriano; Hipófisis; Morfología; Ultraestructura.

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Accepted: 07-08-2018

Received: 26-06-2018