Belg. J. Zool., 134 (1) : 25-30 January 2004

# Microscopic studies of the paraphysis of the turtle *Trachemys (scripta) dorbigni* (Duméril & Bibron, 1835)

**Elisa C. Winkelmann[1,3], Marilda C. Fernandes[2], Andrea P. Jackowski[3], Antonio G. Severino[3] and Felipe L. Schneider[1,3]**

[1] **Universidade Luterana do Brasil, Rua Miguel Tostes 101, CEP : 92420280, Canoas, RS**

[2] **Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Sarmento Leite 245, CEP : 900150 170, Porto Alegre, RS**

[3] **Universidade Federal do Rio Grande do Sul, Sarmento Leite 500, CEP : 900150 170, Porto Alegre, RS**

Correspondence author : Elisa Cristiana Winkelmann-Duarte, e-mail : ijawinkel@yahoo.com.br

ABSTRACT. A microscopic investigation was conducted on the paraphysis of the turtle *Trachemys dorbigni*. The paraphysis is a highly vascular neuroepithelial structure lined by a simple cuboidal epithelium. The prominent ultrastructural features observed were many dense bodies, mitochondria and lipid droplets. The cells of the paraphysis exhibit extensive microvillar borders, large intercellular spaces, many mitochondria, dense bodies and lipid droplets. Few PAS (periodic acid of Schiff) positive granules were found in the cytoplasm of the epithelial cells. Large intercellular spaces were seen when samples were fixed by perfusion. Scanning electron microscopy revealed that surface epithelial cells have microvilli and cilia distributed in tufts in the center of the cell. Macrophagic cells were frequently seen on the surface of the epithelial cells. The connective tissue of the paraphysis presented many sinusoid vessels, mast cells, fibroblasts, and collagen fibers. Intra-arterial administration of Evans blue showed the absence of the blood-brain barrier. This is probably related to the presence of a fenestrated endothelium, which characterizes this structure as a circumventricular organ (CVO). The presence of vesicles in the cytoplasm of epithelial cells, fenestrations, and macropinocytosis vesicles in the vascular endothelium suggest absorption and secretion functions.

KEY WORDS : paraphysis, turtle, morphology, electron microscopy.

## INTRODUCTION

The paraphysis is a branchial and tubular sacciform structure of the posterior telencephalic roof. It is considered a circumventricular organ similar to the choroid plexus (Oksche, 1973). With few exceptions, this specialized neuroepithelial structure is common to all vertebrates, including humans, in an embryonic stage (Shuangshoti & Netsky, 1966; Ebbesson & Schroeder, 1975; Kondziolka & Bilbao, 1989).

In reptiles as well as in amphibians, the paraphysis is well developed and occupies a great subdural area located between the posterior borders of the cerebral hemispheres (Kelly, 1964; Ebbesson & Schroeder, 1975). Studying green sea turtles, Owens & Ralph (1978) demonstrated that the paraphysis was a continuation of the choroid plexus of the third ventricle, slightly to the enlarged distal end of the pineal gland. A fluid-filled cavity was formed inferior to the large pineal-paraphyseal complex.

Studies on the ultrastructural features of the paraphysis in different species (*Lampreta* sp. : Tsuneki, 1986; *Chondricthyes* : Shuangshoti & Netsky, 1966; *Bufo bufo* L. 1758 larvae : Farnesi et al., 1994; *Necturus* sp. : Kelly, 1964, Ebbesson & Schroeder, 1975; *Hyla versicolor* Le Conte, 1825 : Kemnitz et al., 1990 : *Natrix maura* Laurenti, 1768 : Fernández-Llebrez et al., 1982) showed that it consists of a single or pseudostratified epithelium surrounded by highly vascular connective tissue.

The most important functions related to this structure are the production of the cerebral spinal fluid (CSF) in cooperation with the choroid plexus, and the exchange of substances between the blood and the CSF (Kelly, 1964; Owens & Ralph, 1978; Fernández-Llebrez et al., 1982; Kemnitz et al., 1990; Hinton et al., 1990; Farnesi et al., 1994). Paraphysectomy in *Rana* species also showed that a substance discharged by the paraphysis, by the choroid plexus or by both structures seems to be involved in calcium regulation. It resulted in hypocalcemia, motor neuron degeneration, abnormal increased weight, and a tendency to form cysts in the parathyroid gland (Ueno et al., 1984; Nelson et al., 1985).

The aim of this work was to investigate the morphological features of the paraphysis of the turtle *Trachemys (scripta) dorbigni* (Duméril & Bibron, 1835) using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and light microscopy.

## MATERIAL AND METHODS

Twenty-four adult turtles *Trachemys dorbigni* (twenty-one females and three males) weighing between 950g and 2,500g were captured over the year and used in this study with the permission of IBAMA (Registration numbers 047/97; 026/98; 004/99). The animals were fed with ground meat ad libitum.

**Light Microscopy (LM)**

Six animals (one male and five females) were anesthetized with 35% chloral hydrate and perfused with a saline solution followed by 4% paraformaldehyde in sodium phosphate buffer (pH. 7.4). Sections were stained with haematoxylin/eosin (HE). Evans Blue at 0.01% was added to the saline solution (adapted from Eberhardt, 1971) in two animals (females) to demonstrate the vascular permeability of the paraphysis.

**Gomori's Acid Phosphatase (**six females)

Cryostat sections were incubated in a solution of 0.1M trismaleate buffer (pH 5) and 1.25 % sodium β-glycerophosphate. Lead nitrate (0.2%) was added to this solution under constant stirring, followed by heating at 37º C for 5 minutes and subsequent filtration. Sections were incubated for 30 minutes at 37º C, rinsed in distilled water (three times), developed in a solution of 5% ammonium sulfide for 5 min, rinsed in distilled water and mounted in Kaiser's medium. As a control, a few sections were incubated in a medium with 1% sodium fluoride (NaF) (Pearse, 1968).

**PAS** (one male and four females)

After fixation in Bouin's solution, the paraphysis was dehydrated, embedded in paraffin and cut into 8 µm-thick sections, which were then deparaffinized, hydrated and subjected to 0.5% periodic acid for 15 min. After distilled water rinsing, sections were immersed in Schiff's reagent for 30 min, rinsed again in distilled water, submitted to three sulfurous baths, rinsed again in distilled water, and subsequently stained with Mayer's hematoxylin, dehydrated, and mounted with Entellan®. For control of the specificity of reaction we used sections incubated in 0.5% α-amylase at 37 °C for 3 hours (Pearse, 1968).

**Electron Microscopy**

The paraphyses of three females and one male were fixed by immersion and perfusion with 3% glutaraldehyde, 1.5% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). For post-fixation 2% $OsO_4$ was used in the same buffer. Ultrathin sections were collected on copper grids, contrasted with uranyl acetate and lead citrate (Reynolds, 1963), and examined in a JEOL 1200 EX II electron microscope at 80 KV.

The paraphyses of four females were dehydrated in acetone and dried by the critical point technique using carbon dioxide (Balzer CPD 030), sputter-coated with carbon and gold (Balzer SCD 050), and examined under a scanning electron microscope (JEOL 5800 and Cambridge Stereoscan).

## RESULTS

The paraphysis of the turtle *Trachemys dorbigni* is a well-developed, often tubular organ. It is located in the interhemispheric fissure (Fig. 1). At the superior and posterior faces (Fig. 2) it is bordered by the proximal and distal portions of the pineal gland, laterally by the cerebral hemispheres, and by the third ventricle at the inferior side. However, below the pineal-paraphysis complex there is a CSF-filled cavity connected to the third ventricle by an orifice (Fig. 3).

Histochemical study showed a few PAS-positive granules throughout the cytoplasm (Fig. 4). The basal membranes of the endothelium and epithelium of the paraphysis became stained after treatment with Schiff's reagent. Pre-treatment with α-amylase reduced the number of PAS-positive granules in the epithelium, indicating the presence of glycogen. The presence of lysosomes was demonstrated through Gomori's acid phosphatase technique (Fig. 5).

The paraphysis is lined with a simple, cuboidal epithelium, the cells of which have a round, central nucleus (5.3 to 7.5 µm in diameter) occupying a large part of the cytoplasm. The (usually singular) nucleolus is very prominent and spherical (0.08-0.12µm in diameter) (Fig. 7). The cytoplasm of paraphyseal cells contained several mitochondria with transversal crests and dense bodies (0.8-1 µm in diameter) (Fig. 6).

In the tubules of the paraphysis (Fig. 8), epithelial cells presented an apex with numerous microvilli (3.5 µm long and 0.2 µm in diameter). Cilia (6.5-9 µm long and 0.18-0.23 µm in diameter) were usually detected at the central surface of the cell (Fig. 9). The numbers of cilia varied from four to 11 per cell. Cells with a morphology resembling macrophage cells were detected on the ventricular surface of these cells.

Mast cells were found in the connective tissue located under the epithelium (Fig. 6). Collagen fibers and fibroblasts processes were also detected (Fig. 10).

The paraphysis presents a large number of blood vessels in the connective tissue. By injecting Evans Blue intra-arterially, we observed that the paraphysis allowed the passage of this dye to its interior, showing that this structure has no blood-brain barrier (Fig. 1). The vascular endothelium presents many fenestrations from 50 to 75 nm in diameter, covered by a thin diaphragm (Fig. 11). Cytoplasmic projections of the endothelium were observed in both apical and basal portions. On the luminal face, these projections formed macropinocytosis vesicles with diaphragms. The presence of these vesicles was clearly seen in the cytoplasm (Fig. 12). The basal layer of these capillaries could be traced along large extensions, even if in a few points it was discontinuous.

## DISCUSSION

Analysis of the serial sections of the paraphysis (sagittal, coronal, and horizontal) showed that this structure of the turtle *Trachemys dorbigni* is formed by a tubular cavity which is enlarged in the proximity of the pineal gland and is covered by epithelial cells and meninges. This cavity decreases its diameters through the communication with the third ventricle. A similar cavity was reported by Owens & Ralph (1978) in marine turtles. Due to the close relationship between this cavity, the pineal gland and the paraphysis, Owens & Ralph called it pineal-paraphyseal cavity. In the snake *Natrix maura*, this cavity was also observed and was named central ventricular cavity (Fernández-Llebrez et al., 1982).

According to several authors (KAPPERS, 1956; SHUANGSHOTI & NETSKY, 1966; EBBESSON & SCHROEDER, 1975; KEMNITZ et al., 1990; FARNESI et al., 1994), the lining epithelium is continuous between the paraphysis and the choroid plexus. In our study we confirmed this finding and also observed a communication between the lumen of the paraphysis and the third ventricle. This communication, which occurs through a small orifice, had also been described in other animals, such as the salamander *Amblystoma mexicanum* Shaw, 1789 (KAPPERS 1950), the frog *Hyla versicolor* (KEMNITZ et al., 1990) and larvae of the toad *Bufo bufo* (FARNESI et al. 1994). These findings demonstrate that the paraphysis is not an isolated organ but is connected to the CSF of the other ventricular cavities.

In the cytoplasm we observed an accumulation of mitochondria, dense bodies (probably secondary lysosomes), and lipid droplets, particularly in the apical pole of epithelial cells. These lipid droplets were also found in the basal portion of the cell in the salamander *Necturus* sp. (KELLY, 1964; SHUANGSHOTI & NETSKY, 1966; EBBESSON & SCHROEDER, 1975).

Few histochemical assays have been done on the paraphysis. The most common studies are those investigating the presence of glycogen, especially those describing the paraphysis in amphibians and reptiles. Histochemical and biochemical studies demonstrated that the quantity of glycogen in the cells of the paraphysis and in other cells of the CNS is related to environmental conditions and to the different seasons of the year. From spring through winter there is a gradual increase in the total amount of glycogen in the cells of the CNS as well as in the paraphysis (FERNÁNDEZ-LLEBREZ et al., 1982; PARTATA & MARQUES, 1994). With TEM we observed few glycogen granules in the paraphysis of *Trachemys dorbigni.* There were no variations in the amount of glycogen in animals prepared in spring as compared to autumn. Our results are in disagreement with the findings of KAPPERS (1956), who described the epithelium of the paraphysis of salamanders as being rich in glycogen. The presence of glycogen in the paraphysis was also described in fishes, amphibians, and other reptiles (WOLFF, 1962; SHUANGSHOTI & NETSKY, 1966; MCNULTY, 1976), but in *Natrix maura* it was not detected (FERNÁNDEZ-LLEBREZ et al., 1982).

Through Gomori's acid phosphatase method we observed the presence of positive granules in the paraphyseal epithelium, mainly in areas close to the cell apex. They appear to be lysosomes and dense bodies. The elements that reacted with acid phosphatase are likely to be the same ones that had reacted with PAS, and this is in agreement with KOENING & BARROW (1962), who demonstrated that Schiff's reagent also reacts with lysosomes. EBBESSON & SCHROEDER (1975), in a study involving the paraphysis of *Necturus* sp., reported the presence of granules similar to lysosomes in the apical regions of cells of the paraphyseal epithelium.

The examination of the surface of the paraphyseal epithelium of *Trachemys dorbigni* by SEM showed that this surface is covered by numerous short microvilli and cilia distributed in tufts. Besides these structures, we also found macrophagic cells similar to the epiplexus cells reported by LING et al. (1998) in rats. These authors proposed that a typical cell of the epiplexus has from three to five cytoplasmic processes emanating from the cell body. In our study the cells observed on the ventricular surface of the paraphysis usually had three processes.

In the literature there are no SEM data of the paraphysis. From our observations we highlight the presence of dilations at the end of both microvilli and cilia. We suspect that such dilations are artifacts of the preparations, since they were not detected in the TEM examination.

Elements of the conjunctive tissue that are notable in the formation of the tubules of the paraphysis can vary between different species of amphibians and reptiles. The most prominent structures found in the turtle *Trachemys dorbigni* are mast cells, collagen fibers, and fibroblast processes. Mast cells were numerous in the dorsal region of the paraphysis lying closer to the pineal gland. No macrophages were found, nor were any nerve fibers.

The paraphysis is a highly vascular structure presenting many fenestrated sinusoid capillaries, indicating possible absence of the blood-brain barrier. This absence was demonstrated through its permeability to Evans Blue. The sinusoidal vascularization was described in the amphibians *Ambystoma* sp. (ROOFE, 1936; KAPPERS, 1950, 1956; EBBESSON & SCHROEDER, 1975), *Necturus* sp. (KELLY, 1964), *Rana catesbiana* Schreber, 1782 and *R. pipiens* (HINTON *et al*., 1990), the turtle *Chrysemys picta marginata* Agassis, 1857 (WARREN, 1911), and the snake *Natrix maura* (FERNÁNDEZ-LLEBREZ et al., 1982). The presence of fenestrations in the endothelium, which confirm the blood-brain barrier absence, was described in *Necturus* sp. (KELLY, 1964), *R. catesbiana* and *R. pipiens* (HINTON et al., 1990), larvae of *Bufo bufo* (FARNESI et al., 1994), and in *Natrix maura* (FERNÁNDEZ-LLEBREZ et al., 1982).

Circumventricular organs (CVOs) of the brain are characterised as highly vascular structures, with no blood-brain barrier, and in contact with one of the cerebral ventricles (WEINDL et al., 1972; LOW, 1982, PETERS et al., 1991). Based on these criteria, we consider the paraphysis of the turtle *Trachemys dorbigni* to be a CVO. A number of authors (FERNÁNDEZ-LLEBREZ et al., 1982; TSUNEKI, 1986; KEMNITZ et al., 1990) also described the paraphysis as a CVO, due to its pronounced vascularization and its contact with the cerebral ventricular system.

The connection with the ventricle and some morphological features suggest possible functions of the paraphyse. The presence of vesicles in the cytoplasm of the epithelial cells, the fenestrations and the macropinocytosis vesicles in the vascular endothelium are indications of substance exchange activities, may be also secretion. In this way, the paraphysis may be able to release substances into the cerebro-spinal fluid of the ventricular system. At present there are few data in the literature about the functions of the paraphysis. When its physiological role has been determined by further studies, it may become possible to explain the atrophy of this structure in humans (KONDZIOLKA & BILBAO, 1989).

## REFERENCES

EBBESSON, S.O.F. & D.M. SCHROEDER (1975). The choroid plexus and paraphysis in nonhuman vertebrates. In : NETSKY

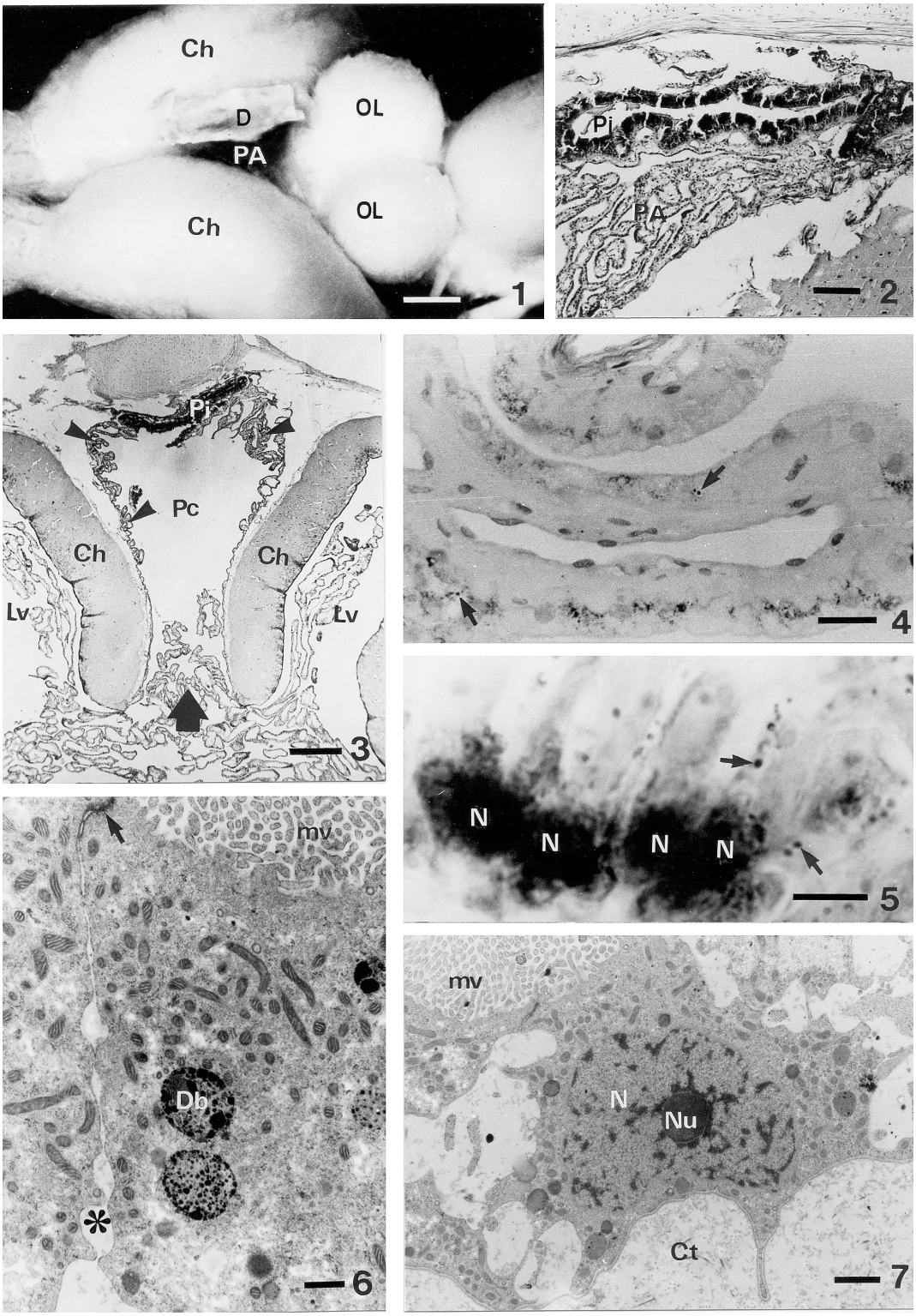
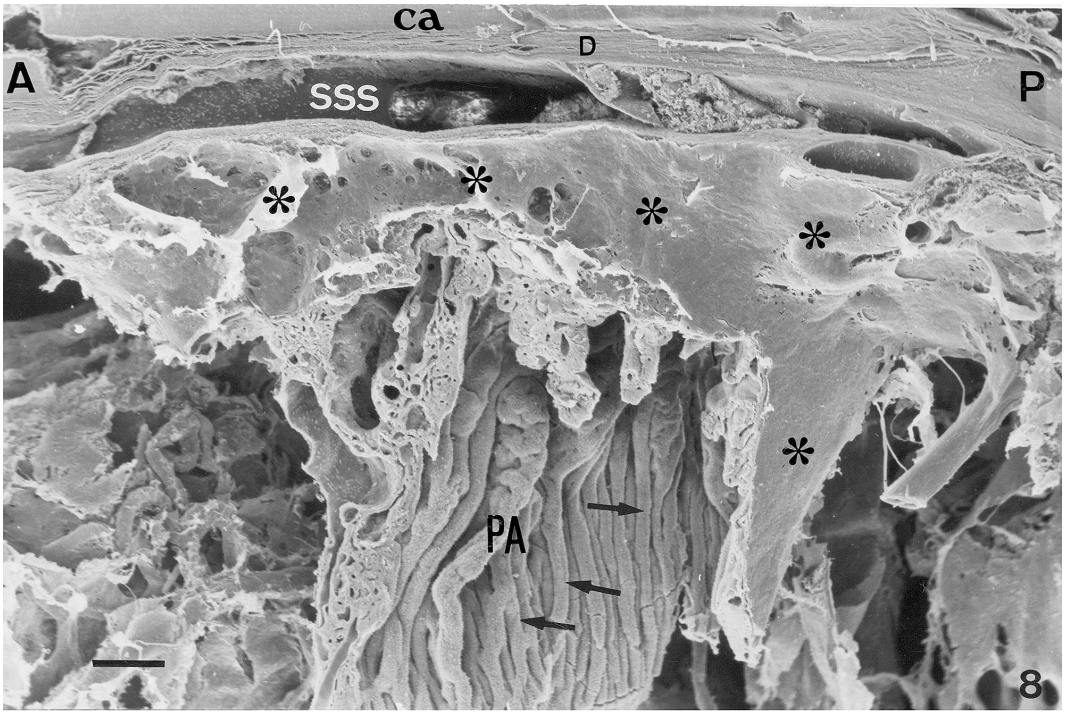
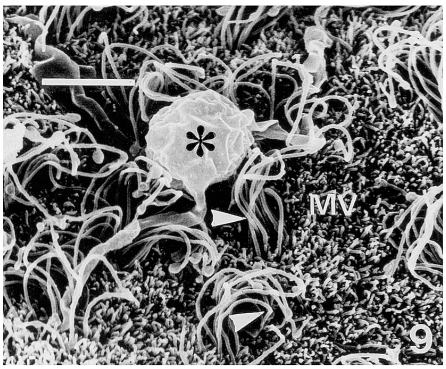
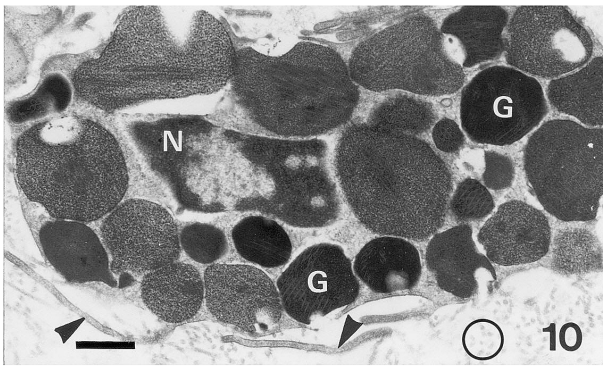
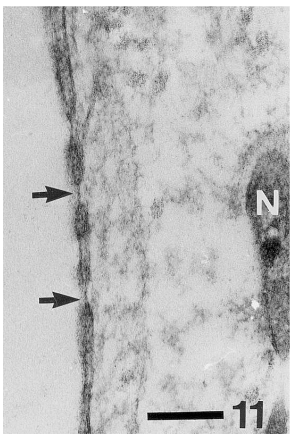


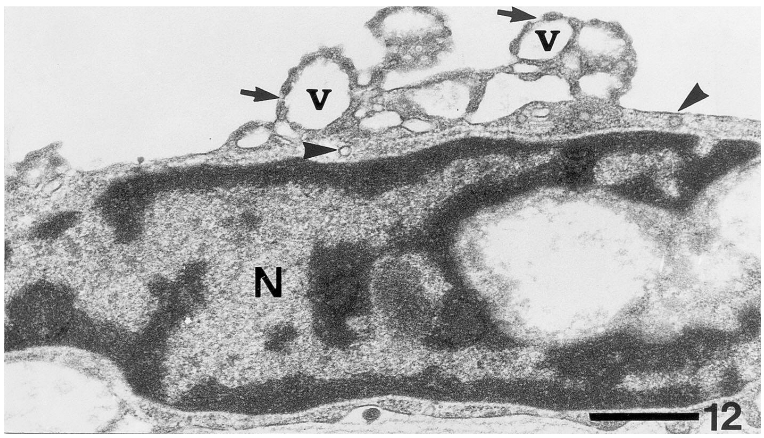

Fig. 1-7. – **1.** Micrograph of the *Trachemys dorbigni*'s brain (dorsal view) intra-arterially injected with Evans Blue, showing that the paraphysis (PA) presents no blood-brain barrier : cerebral hemisphere (Ch), optical lobe (OL), dura mater (D); bar : 0.16 mm. – **2.** Photomicrograph of a sagittal section showing the location of paraphysis and the structures to which it is anatomically related. Pineal (Pi) and paraphys¬is (PA). LM section, material included in paraffin and stained with HE; bar : 50 µm. – **3.** Coronal section of brain showing the relation between the paraphysis, lateral ventricles, and the third ventricle. Orifice (arrow) that links the paraphyseal cavity (Pc) with the third ventricle, cerebral hemisphere (Ch), pineal (Pi), paraphysis (arrowheads) and lateral ventricle (Lv). LM section, stained with HE; bar : 250 µm. – **4.** Paraphysis sections processed for PAS technique; PAS-positive granules (arrows) are very scantily found in the paraphyseal epithelium. LM; bar : 30µm. – **5.** Photomicrograph showing lysosome staining through the technique of Gomori's acid phosphatase; Lysosomes (arrows), nucleus (N). LM; bar : 10 µm. – **6.** Electron micrograph of a section of the paraphyseal epithelium showing cytoplasmic structures : dense bodies (Db) and intercellular spaces (*). Desmosome (arrow), microvilli (mv). TEM; bar : 500nm. – **7.** Electron micrograph showing the nucleus (N) and the nucleolus (Nu) of an epithelial cell of paraphysis. In the apical portion of the cell are microvilli (mv). Underlying the epithelium is the layer of connective tissue (Ct). TEM; bar : 1µm.




Figs 8-12. – **8.** Para-sagittal section of paraphysis (SEM) : anterior (A) and posterior (P) part of paraphysis (PA); in the top see the location of the pineal gland (*). Dura-mater (D), superior sagittal sinus (SSS), cartilage (ca), tubules of paraphysis (arrows). SEM; bar : 200 µm – **9.** Surface of paraphysis observed under SEM : macrophagic cell (*), cilia (arrowheads), microvilli (MV). SEM; bar : 5 µm. – **10.** Mast cells in the cytoplasm of paraphyseal cells. Granules (G), nucleus (N), fibroblasts processes (arrowheads), cluster of collagen fibrils (circle). TEM; bar : 500 nm. – **11.** Vascular endothelium. Note diaphragms (arrows) in the fenestrations along the endothelium of epithelial cells of paraphysis. Nucleus (N). TEM; bar : 200 nm. – **12.** Vascular endothelium showing the nucleus (N). On the luminal surface note the presence of macropinocytosis vesicles (v) and diaphragms (arrows). Also, note the presence of macropinocytosis vesicles close to the nucleus (arrowhead). TEM; bar : 500 nm.

& Shuangshoti (eds). *The choroid plexus in health and disease*. Great Britain : John & Sons, pp. 162-174.

Eberhardt, H.G. (1971). Supravitale Farbstoffversuche zur Frage der Stoffverteilung im ZNS der Ratte, besonders in Hypothalamus und Infundibulum. *Z. Mikr. Anat. Forsch*., 84 : 525-534.

Farnesi, R.M., S. Tei, D. Vagnetti, B. Santarella & P. Pollacci (1994). Ultrastructure of the paraphysis in *Bufo bufo* larvae. *J. Morphol*., 219(1) : 7-13.

Fernández-Llebrez, P., J. Becerra, & F. Marin Giron (1982). Ultrastructure of the paraphysis cerebri of the water snake *Natrix maura* L. *J. Comp. Neurol*., 208 : 345-351.

Hinton, D.A., S.R. Nelson & V.H. Gattone (1990). Vasculature of the paraphysis cerebri of the frog. *J. Submicrosc. Cytol. Pathol.,* 22(3) : 345-51.

Kappers, J.A. (1950). The development and structure of the paraphysis cerebri in Urodeles with experiments on its function in *Amblystoma mexicanum*. *J. Comp. Neur*., 92 : 93-122.

Kappers, J.A. (1956). On the presence of periodic acid Schiff positive substances in the paraphysis cerebri, the choroid plexuses and the neuroglia of *Amblystoma mexicanum*. *Experientia*., 12 : 187-188.

Kelly, D.E. (1964). An ultrastructural analysis of the paraphysis cerebri in newts. *Z. Zellforsch*., 64 : 778-803.

Kemnitz, C.P., L.M. Fox & J.A. Mcnulty (1990). Ultrastructure and the organization of circumventricular organs and endolymphatic tubules in the treefrog, *Hyla versicolor. J. Morphol*., 204(2) : 197-208.

Koenig, H. & K. Barrow (1962). Glycolipoprotein granules as lysosomes. *J. Histochem. Cytochem*., 10 : 684-685.

Kondziolka, D. & J.M. Bilbao (1989). An immunohistochemical study of neuroepithelial (colloid) cysts. *J. Neurosurg*., 71(1) : 91-7.

Ling, E., C. Kaur & J. Lu (1998). Origin, nature and some functional considerations of intraventricular macrophages, with special reference to the epiplexus cells. *Micros. Res. Tech*., 41 : 43-56.

Low, F. N. (1982). The central nervous system in scanning electron microscopy. *Scan. Electron. Microsc*., (Pt 2) :869-90.

Mcnulty, J.A. (1976). A comparative study of the pineal complex in the deep -sea fishes *Bathylagus Wesethi* and *Nezumia liolepis*. *Cell. Tissue. Res.,* 172(2) : 205-25.

Nelson, S.R., F.M. Foltz, P. Camarata & M.P.J. Sarras (1985). Occurrence of bone fractures and parathyroid hyperplasia in paraphysectomized frogs (*Rana catesbeiana*). *Anat. Rec.,* 211(3) : 311-7.

Oksche, A. (1973). Circumventricular structures and pituitary functions. In J. Ariëns Kappers (ed) : Proceedings of the fourth international Congress of Endocrinology, Washington D.C., 1972. Amsterdam : Excerpta Medica, pp. 73-79.

Owens, D.W. & C.L. Ralph (1978). The pineal-paraphyseal complex of sea turtles. I. Light microscopic description. *J. Morphol*., 158(2) : 169-179.

Pearse, A.G.E. (1968). Histochemistry, theoretical and applied. 3 ed. v. I, Edinburg London New York : Churchill Livingstone.

Partata, W. & M. Marques (1994). Effects of fasting and seasonal variations in brain glycogen disposition in the turtle (Chrysemys dorbigni). *Comp. Biochem. Physiol*., 107a : 727-730.

Peters, A, S.L. Palay & H. Webster (1991). The fine structure of nervous system : neurons and their supporting cells. Oxford University Press, Inc., 328-343.

Reynolds, E.S. (1963). The use of lead citrate at high pH as an eletroplaque stain in electron microscopy. *J. Cell Biol*., 17 : 208-212.

Roofe, P.G. (1936). The histology of the paraphysis of *Amblystoma*. *J. Morphol*., 59 : 1- 10.

Shuangshoti, S. & M.G. Netsky (1966). Choroid plexus and paraphysis in lower vertebrates. *J. Morph.,* 120 : 157-188.

Tsuneki, K. (1986). A survey of occurrence of about seventeen circumventricular organs of various vertebrates with special reference to lower groups. *J. Hirnforsch*., 27(4) : 441-70.

Ueno, S., H. Umar, H.J. Bambauer & M. Ueck (1984). Ultracytochemical localization of $Ca^{++}$- ATPase activity in the paraphyseal epithelial cells of the frog, *Rana esculenta*. *Cell. Tissue. Res.*, 235(1) : 3-11.

Warren, J. (1911). The development of the paraphysis and pineal region in Reptilia. *Am. J. Anat*., 11 : 313-392.

Weindl, A., R.J. Joynt & N.Y. Rochester (1972). Ultrastructure of the ventricular walls. Three-dimensional study of regional specialization. *Arch. Neurol.*, 26(5) : 420-7.

Wolff, F. (1962). Funtionell-histolosche Studien am Plexus Choroideus von *Rana temporaria* L. unbter besonder Berücksichtigung der Sekretionsfrage. *Z. Zellforsch*., 57 : 63-105.

*Received : April 9, 2003*
*Accepted after revision : November 22, 2003*