Immunohistochemical Investigation of S-100 Protein and PGP9.5 in the Dental Pulp of Green Iguana (Iguana iguana)

Kunihiro Suzuki,¹,² Hiroyuki Mishima,¹ Shuhei Torii,¹ and Yukishige Kozawa¹,²

Department of ¹Histology, Cytology and Developmental Anatomy, and ²Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan

Abstract
In this study, innervation of dental pulp in the tooth of green iguana (Iguana iguana) was investigated. Cryosections of dissected maxillae and mandibles were examined by free-floating technique with anti-PGP9.5 antibodies and anti-S-100 protein antibodies, and identified by electron microscopy. Immunoreactive fibers were observed in the pulp. They appeared to be a mixture of myelinated and unmyelinated fibers with small varicosities and were distributed in the pulp along the gap between the tooth and the jaw bone. Dental pulp of polyphyodonts has not been investigated much as replacement of teeth occurs in succession. These findings may promote investigations that could improve our understanding of the function of dental pulp in reptiles.

Keywords:
dental pulp, innervation, reptile, iguana

Introduction
Ankylosis is the prevalent form of attachment in reptiles. Most iguanas and all true lizards have the pleurodont type ankylosis (1–3). They have a continual succession of teeth, i.e. they are polyphyodont. Crocodiles are polyphyodonts and have gomphosis similar to mammals. They have a rich nerve distribution and nerve endings were reported in the periodontal ligament, however innervation of the dental pulp has not been confirmed so far (4, 5). In this study, innervation of dental pulp was observed in the tooth of green iguana (Iguana iguana) by S-100 protein (6) and PGP9.5 (7) immunohistochemical and electron microscopic examination.

Materials and Methods
In this report, green iguanas (n=4), weighing 12–20 g and 20–25 cm long, were used to study the enamel, dentin, dental pulp and periodontal tissue.

Under anesthesia the animals were euthanized and their mandibles and maxillae were removed. The specimens were fixed with 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for transmission electron microscopic (TEM) observation. For immunohistochemical observations, the specimens were fixed with 4% paraformaldehyde buffered with 0.1 M phosphate buffer at pH 7.4. Some specimens were demineralized with 10% EDTA (pH 7.4) for a month.

5 μm sections embedded in paraffin were stained with hematoxylin and eosin, and examined using a light microscope (LM) (Nikon Microflex HFX-IIA). 10 μm cryo-sections were reacted with anti–PGP9.5 antibodies and anti–S-100 protein antibodies (Vector Lab.). For TEM observation, the specimens were post-fixed for two hours at 4°C in 2% osmium tetroxide. They were dehydrated in graded alcohol and embedded in epoxy embedding resin (Epon 812, TAAB). Semi-thin sections of approximately 0.5 m thickness were cut using an ultramicrotome and stained with 0.75% toluidine blue (pH 7.4) and examined using LM. Ultra-thin sections were conventionally stained with 4% uranyl acetate for 10–15 min, followed by Reynold’s lead citrate for 10–15 min. The specimens were observed by TEM (JEOL 1200EX).

All procedures in this study were conducted according to the animal experiment guide, approved by the Animal Experiment Committee of Nihon University, School of Dentistry at Matsudo.
Results and Discussion

The teeth of green iguana, seen erupted in the oral ridge of the specimen, were flat and serrated at the margins. They were arranged in a single row, and the size and density of dental pulp of adjacent teeth alternated gradually (Fig. 1). A layer of coarse textured tissue was observed between the tooth and bone, termed bone of attachment or pedestal bone, which contained lacunae and tubules (2, 8). The bases of the teeth, in the later stages of eruption, were found attached to the jaw bone. The pulp of ankylosed teeth continued to the outer periodontal tissues since it was not completely enclosed by bone and dentin (1, 3).

A dense population of cells was observed at the periphery of the pulp, identified as the odontoblast layer (Fig. 1). The cell free zone and cell rich zone were also clearly distinguished. Predentine and mineralized dentine were separated by an undulating line. Blood vessels were observed in the central pulp region.

S-100 protein reaction appeared in the dental pulp (Fig. 2). Immunoreactive thick fibers ran through the central pulp and the bundles extended towards the cuspal direction. PGP9.5 immunoreactive fibers were also observed in the dental pulp (Fig. 3). Immunoreactive thick fibers which occupied the gap between the tooth and jaw ran through the central pulp towards the cuspal direction. The branches diverging from the thick bundles were darkly stained with beads or varicosities of varying size. The presence of plexus was not confirmed in the subodontoblastic area. Thin fibers near the odontoblastic layer formed small bead like or varicosity like structures (Fig. 4).

Electron micrograph clearly confirmed nerve bundles, a mixture of myelinated and nonmyelinated nerves, in the pulp near the gap between the jaw and dentine (Fig. 5).

Dental pulp in the teeth varied in density according to the developmental stage. Reaction to both S-100 protein and PGP9.5 in certain stages of pulp development suggests that these bundles and fibers are nerve elements, though S-100 protein has been identified in a variety of cell types of neuroectodermal origin and
Fig. 3. PGP9.5 immunoreactive fibers in the dental pulp. Immunoreactive thick fibers ran through the central pulp. The branches diverging from the thick bundles were darkly stained with beads or varicosities of varying size.

Fig. 4. Magnified image of the PGP9.5 immunoreactive fibers. Thin fibers near the odontoblastic layer had small beads and varicosities.

Fig. 5. Electron microscopy of the immunoreactive fibers. Nerve bundles at the base of pulp near the gap between jaw and dentine showing a mixture of myelinated and non-myelinated nerves.

in non-neuroectodermal cells (9). Immunoreactive fibers which were observed at the restricted stage of pulp development shows that the degeneration of the pulpal cells and innervation results in loss of the capacity to express S-100 protein and PGP9.5.

Dental pulp of polyphyodonts has not been studied in detail since replacement of teeth occurs in succession. Innervation of the dental pulp of crocodile has not been confirmed in previous studies (4, 5). Crocodile, which is the only living reptile with gomphosis, has a different mode of tooth attachment compared to iguana, though both are polyphyodonts. Our findings might promote investigations towards the better understanding of the functions of dental pulp in reptiles. Further studies on the relation between innervation of tooth and mode of tooth attachment between polyphyodonts and diphyodonts are required.

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References