Light and scanning electron microscopy of the developing lingual papillae in the green iguana, *Iguana iguana*

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ABSTRACT: Reptiles have recently become a popular group of pet animals. A relatively large number of studies on the morphology of the oral cavity and method of feeding in adult individuals have been published. Nevertheless, embryological descriptions of reptile body parts or structures are rare. In this study, we describe the morphology of the developing tongue, in particular its dorsal surface, in pre-hatched green iguanas. Microscopic examination of the oral cavity of early embryos revealed that the tongue was divided into three different areas: apex, corpus and radix. The dorsal lingual surface was smooth and covered by nonkeratinised stratified squamous epithelium with slight prominences in some cases. In the underlying mesenchyme of the tongue, striated muscular tissue was formed. The epithelium thickness was reduced during formation of the lingual papillae and in later stages remained simple cuboidal. No developing taste buds could be recognised in the lingual epithelium.

Keywords: green iguana; tongue; development; morphology

The tongue is a typical organ of tetrapodes and is closely related to a terrestrial lifestyle. Fish have an elevation of mucosa in the floor of the oral cavity but this structure does not contain a voluntary controlled musculature like the tongue of land animals. The majority of adult amphibians, as well as all known reptiles, birds and mammals have a tongue (Helff, 1929). The lingual epithelium of amphibians is not keratinised, whereas in reptiles it is keratinized to varying degrees. Reptiles live in a variety of habitats, from the seashore to regions of high temperature and high or low air humidity. Keratinisation of the epithelium is considered to be a key feature of the evolution of amniotes. The different degrees of epithelial keratinisation appear to be secondary and reflect the environmental conditions of different species (Iwasaki, 2002).

Basic attributes of tongue development in reptiles (as in other amniotes) correspond to those in amphibians. A rounded swelling, named the tuberculum impar, initially originates between the mandibular and hyoid arch in all these species (Butschli, 1924). The second swelling, named the copula, is located between the hyoid arch and the following first branchial arch. The tuberculum impar is accompanied by a pair of lateral swellings, which arise from the inner surface of the mandibular arch and meet at the mid line (Torrey, 1979). The site of their junction persists post-embryonically in the form of the median sulcus of the tongue.

The tongue of squamates is connected to the floor of the oral cavity and resembles the flat and less keratinised tongue of some amphibians. Some lizards, as well as turtles, cannot protrude their tongue. Despite this fact, the apex linguae of many lizards is forked and the radix linguae also extends distally in the form of two processes (Butschli, 1924).

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Iwasaki (1990) identified three types of lingual papillae in the lizard *Gekko japonicus*. Dome-shaped lingual papillae are found on the lingual apex. Flat, fan-shaped lingual papillae are seen on the body of the tongue and long, scale-like lingual papillae are formed laterally. Surface epithelial cells of the papillae contain microvilli and microridges. A single layer of columnar epithelial cells covers the papillae on the dorsal lingual surface. These cells are filled with secretory granules. The only exception is found on the tips of the fan-shaped and scale-like lingual papillae where the epithelium is stratified squamous without cytoplasmic granules.

The tongue of the rough tailed rock agama (*Laudakia stellio*) is not forked. The lingual apex does not form any papillae and is covered by keratinised stratified epithelium. The dorsal surface of the tongue extends in the form of fungiform papillae which are covered by cells with only a small amount of keratin. The lingual body contains cylindriform papillae with serous and mucous glands in the basal portion (Koca et al., 2007).

Lingual papillae are also formed in turtles. As in other species, there is a considerable correlation between adaptation to the environment and the appearance of lingual papillae (Winokur, 1988). Turtles which live permanently or even partly in water have tongues covered by stratified epithelium. Short papillae can be formed in this case, as in *Clemmys japonica* (Iwasaki et al., 1992) and *Geoclemys reevesii* (Iwasaki, 1992). These turtles fall between the purely aquatic and purely terrestrial species. A well-developed complex of lingual papillae is found in *Testudo hermanni* which is typically terrestrial (Weisgram et al., 1989). On the other hand, the seawater olive ridley turtle (*Lepidochelys olivacea*) does not possess any lingual papillae. Only transversely orientated folds are present on the lingual body and root in this species. The epithelium which covers the tongue in this turtle species is stratified squamous with a thick layer of desquamating cells (Iwasaki et al., 1996).

The dorsal lingual surface of the apex linguae in the iguana *Oplurus cuvieri* is covered by flattened columnar papillae. There are no papillae present on the lingual body which facilitates transport of food to the pharynx. Conical papillae are formed laterally on the lingual radix and present a rough area to assist swallowing. Epithelial cells on the surface of the papillae contain microvilli and invaginations. There are numerous secretory granules in the cytoplasm of these epithelial cells (Delheusy et al., 1994). Compared to this species, the forked tongue of the green iguana (*Iguana iguana*) is smooth at its apex (Abbate et al., 2008). The lingual body contains numerous columnar papillae and the radix bears conical papillae.

MATERIAL AND METHODS

The embryos of green iguanas were removed from their eggs in cooperation with Brno Zoo. Collection began after 67 days of incubation and continued every four days until hatching. The last embryo was removed after 135 days of incubation. One embryo from each interval was collected and examined. The embryos were immediately fixed in neutral formaldehyde (1.33 mol/l). During the fixation process, the heads were cut off. Due to ongoing ossification of the skull, almost all the embryos were submerged in a solution of 5.5% EDTA in 4% formaldehyde (for three weeks in the youngest decalcified embryo up to a maximum of nine months in the oldest embryo). Samples were dehydrated in a graded ethanol series (ethanol concentration in each subsequent bath was increased by 0.1 mmol/l), acetone and three baths of xylene. At the end of dehydration process, samples were infiltrated with hot paraffin and embedded in paraffin wax. Three to four μm thin sections were cut sagitally in a routine manner. The sections were dried, stained with haematoxylin and eosin, mounted and examined and photographed under an Olympus BX 51 light microscope.

The same embryonic material was used for scanning electron microscopy. Samples of specimens from 87, 95, 103, 111, 127, and 135 days of incubation were dehydrated in a graded alcohol series (0.6, 0.7, 0.96, and 1.0 mmol/l) and transferred to absolute acetone. Finally the samples were dried at the critical point and coated with gold. These samples were examined and photographed under a Tescan VEGA TS 5136 XM scanning electron microscope.

Morphological staging followed the criteria used by Wise et al. (2009) for the leopard gecko (*Eublepharis macularius*).

RESULTS

During the early developmental stages, the tongue appears as a dome-shaped prominence fixed along its entire length to the floor of oral cavity. It is covered by bilayered to stratified epithelium ros-

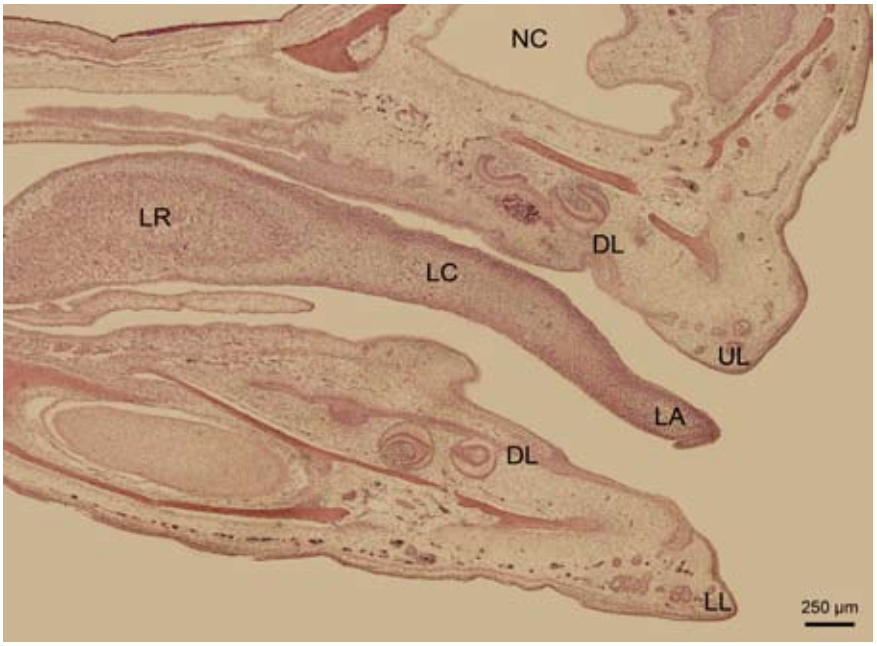

Figure 1. Sagittal section of the oral cavity and associated structures. Nasal cavity (NC), dental lamina (DL), lingual apex (LA), lingual body (LC), lingual radix (LR), upper lip (UL), lower lip (LL)

trally, whereas the caudal regions are covered by simple epithelium. In addition, the number of layers decreases laterally so that the tallest epithelium covers the dorsal area of the lingual apex. There are no mucosal prominences present in the early stages so the dorsal lingual surface remains completely smooth. However, the developing musculature in the underlying connective tissue becomes more prominent, and at the 83 day stage of intra ovo development (stage 38), the muscle fibres lie in horizontal, vertical and longitudinal directions. No lingual glands have yet been formed.

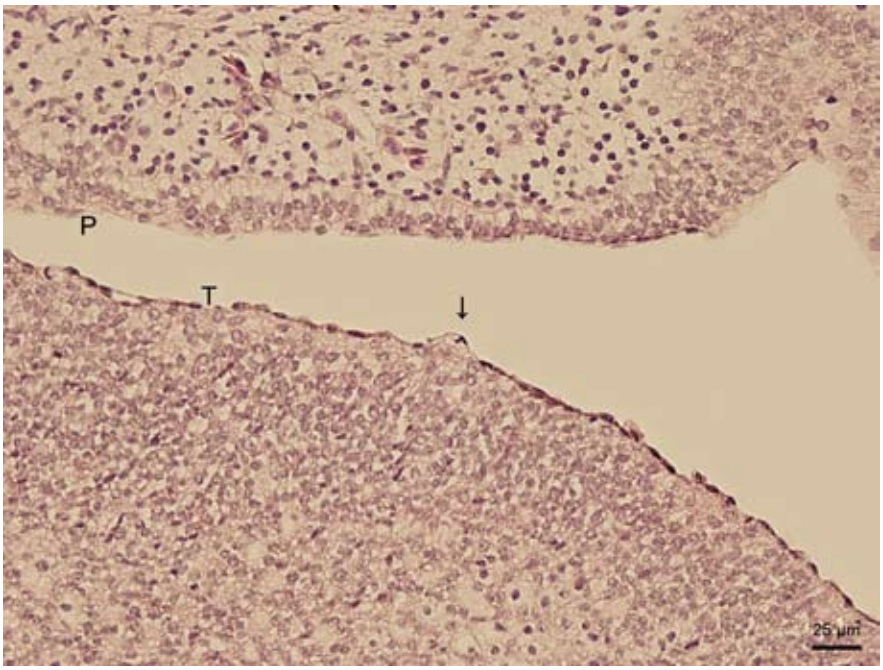

Figure 2. Sagittal section of the oral cavity. Palate (P), tongue (T), uppermost epithelial cells forming sessile prominences (↓)

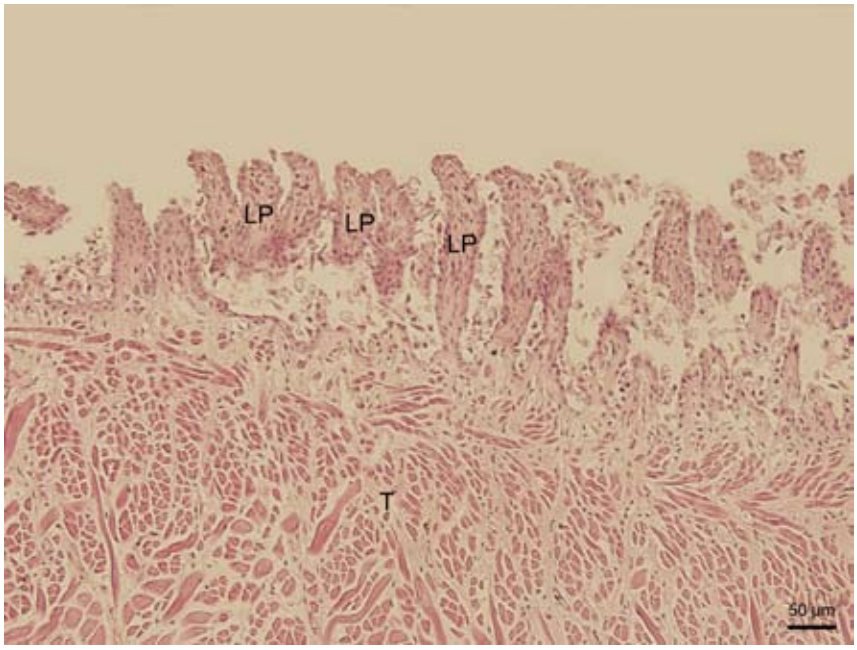

Figure 3. Sagittal section of the tongue (T) in the area of the lingual body. Lingual papillae on the dorsal lingual surface (LP)

During development, the lingual apex, corpus and radix can be distinguished (79 days of intra ovo development, stage 37; Figure 1). The apex and radix are bifurcated. The tongue is completely covered by stratified squamous epithelium, which appears lower on the ventral lingual surface. The uppermost epithelial cells form sessile prominences in some developmental stages. These structures are found not only on the dorsal lingual surface (79 and 83 days of intra ovo development, stage 37 – Figure 2, and 38) but also on the ventral surface of the tongue (87 days of intra ovo development, stage 39).

The lingual corpus bulges dorsally during subsequent development. The lingual apex becomes elongated. At day 95 of intra ovo development (stage 40), prominences of different shapes and

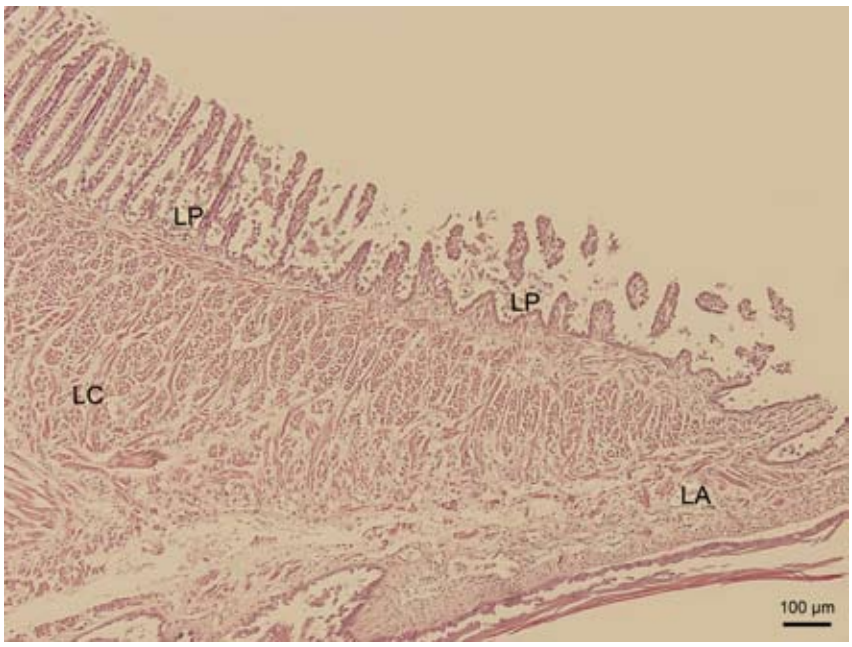

Figure 4. Sagittal section of the tongue. Lingual papillae (LP) on the lingual apex (LA) and the lingual body (LC)

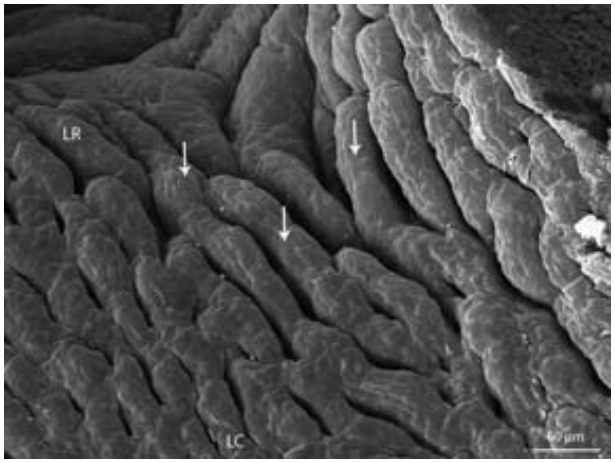

Figure 5. Dorsal lingual surface in the area of the lingual body (LC) and the lingual radix (LR). Tortuous mucosal strips (↓)

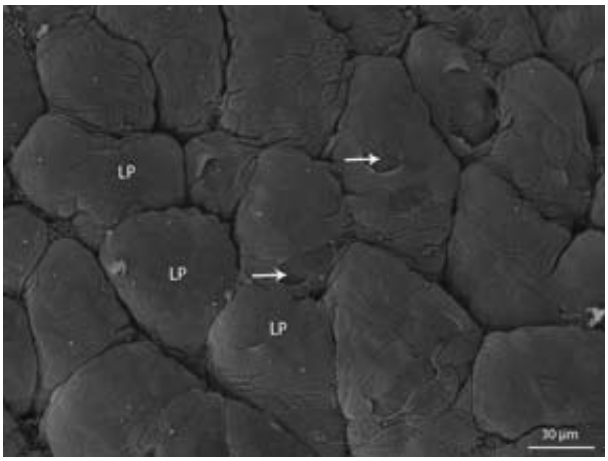

Figure 6. Dorsal lingual surface with less differentiated lingual papillae (LP). Desquamated epithelial cells (→)

sizes develop on the lingual body. The lamina propria forms numerous finger-like processes in the remaining areas of the body of the tongue so that the base of the epithelium in these areas is not straight. Since the stratified epithelium flattens the extensions of the lamina propria, only the prominences previously mentioned can be observed. Two more structures develop during this period: a thickening of the lingual apex which is covered by lower keratinised squamous epithelium and the hyaline cartilage plate in the lingual musculature.

Development in the last weeks before hatching is irregular. The most differentiated embryonic stages from all the data processed are those of the 111 and 119 days of intra ovo development (end of stage 42 and end of stage 42). The dorsal lingual surface extends in the form of papillae which resemble mechanical lingual papillae in mammals. They appear as slender mucosal processes, the length of which decreases caudally. The free papillary endings proximally on the lingual body tend to bend caudally, therefore papillae on the lingual radix and distally on the lingual body are similar to conical papillae (Figure 3), and papillae found proximally on the lingual body resemble filiform papillae (Figure 4) in mammals. Some papillae on the lingual apex bifurcate. Compared to these two clearly differentiated stages, the mucosa of the dorsal lingual surface in younger stages (95 and 103 days of intra ovo development, stage 40 and 41; Figure 5) constitutes tortuous mucosal strips, whereas in older embryonic categories (127 and 135 days of intra ovo development, start of stage 42 – Figure 6, and start of stage 42) these strips develop into less differentiated papillae. These papillae appear as rounded prominences with a wide base.

The dorsal lingual surface is covered by nonkeratinised stratified squamous epithelium in the early stages, the tongue of which is more or less smooth. The flat surface cells have a mosaic arrangement (Figure 7) and contain numerous bizarre formations (microvilli) and droplets of secreta. The height of epithelium decreases with the formation of lingual papillae. In these cases, the epithelium is simple cuboidal. The apical surface of the epithelial cells is dome-shaped and these cells also have a mosaic arrangement (Figure 8). The only area of the tongue where the epithelium remains stratified is at the thickening of the lingual apex. Moreover, keratinisation of this structure is more prominent during the ongoing embryonic development.

Several mucous secreting glandular tubules are present in the connective tissue stroma of the ventral lingual surface at day 127 of intra ovo development (start of stage 42). The epithelial cells differentiate later and form more layers by day 135 of intra ovo development (start of stage 42). These glandular tubules open on the ventral lingual surface between the papillary processes of the mucosa.

No developing taste buds were found in the lingual epithelium of the investigated green iguana embryonic material.

DISCUSSION

In the presented study we focused on the second half of embryogenesis, i.e., 63rd to 135th day of incu-

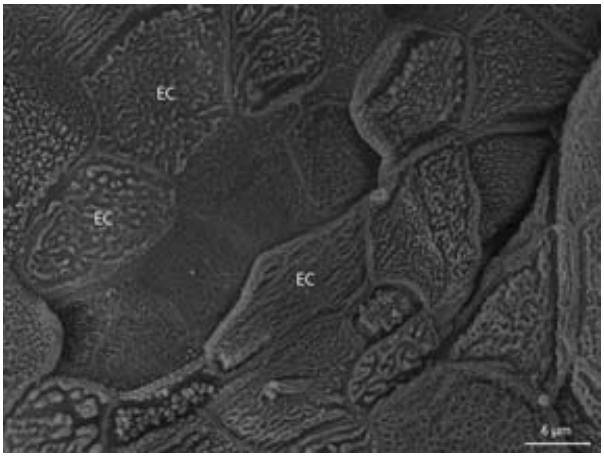

Figure 7. Dorsal lingual surface, detail of Figure 6. Epithelial cells (EC) of the lingual papilla with numerous formations (microvilli)

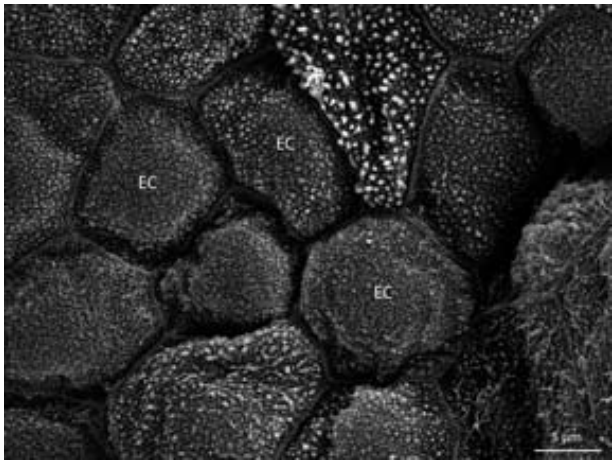

Figure 8. Dorsal lingual surface in the area of the lingual radix. Dome-shaped epithelial cells (EC)

bation under our conditions. We assume that maturation and differentiation of the tongue in this period is insignificant and that differences between individual embryonic stages are small. Since temperature and air humidity are essential factors in the development of the oviparous green iguana, fluctuations in these values are reflected as a discontinuity in ontogenesis. In other words, variable external conditions are probably responsible for the accelerated development in some earlier stages in comparison to later stages prior to hatching. Moreover, these external conditions also affected the relatively extended development of embryos in our egg yield, which exceeded almost twice the breeder's standard.

The tongue is only mildly differentiated in the early stages at the beginning of our observation. The surface of the lingual mucosa is smooth. Developing lingual papillae, which were described in adults of the green iguana (Abbate et al., 2008), were not present. The lingual epithelium constitutes sessile formations in some later stages but these structures do not correlate with the further differentiation of the lingual papillae as we suggest. These epithelial prominences are found not only on the dorsal lingual surface (at 79 or 83 days of intra ovo development, stage 37 and 38) but also on the ventral surface (at 87 days of intra ovo development, stage 39). There are no lingual papillae formed ventrally on the tongue of adult green iguanas (Cizek, 2004). It seems that these formations originate as a result of uneven differentiation of the lingual epithelium.

Lingual papillae, regarded as connective tissue prominences covered by epithelium, were observed at 111 days of development (end of stage 42). The appearance of the lingual papillae is in fact connected with their function in food intake. On the other hand, the epithelium covering the lingual papillae which contains secretory cells, with droplets of secreta on the surface, as well as glands at the cores of the papillae, hint at the possibility that lingual papillae have more than a purely mechanical function in the future animal. It appears that they play an important role in food prehension and also in its digestion (Cizek, 2004). Thickening of the lingual apex, which is formed together with lingual papillae, represents a protective adaptation that allows safe contact of the tongue with structures in the environment. Glandular complexes of the tongue follow similar developmental dynamics. Their differentiation accelerates shortly before hatching. Our findings can be compared only with results from different reptile species (Rabinowitz and Tandler, 1991). We obviously assume that there is a lower occurrence of glands on the dorsal lingual surface. We believe that the different diet and subsequently different specialisation of the lingual glands in certain areas of the dorsal lingual surface makes, in our case, the glandular apparatus of the tongue species-specific. The form of the ingested food can substitute for an insufficient number of lingual glands resulting in a reduced amount of saliva. It is also possible that a considerable expansion of the glands on the dorsal lingual surface takes place postnatally. However, we did not find an expansion of glandular parenchyma in our previous studies (Cizek, 2004).

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