Biomedical Research 1995; 6(2): 193-202

The innervation of *Camelus dromedarius* tongue during morpho-histogenesis

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Key words: Tongue, *Camelus dromedarius*, fetal tongue innervation

Abstract

In the present study the innervation of tongue of 58 foetuses of *Camelus dromedarius* (VRL 30-980 mm) has been studied during morpho-histogenesis. In different areas of tongue and where there are usually autonomic nerve ganglia, distribution pattern of neural network was studied for each foetal group with increasing age. The expansion of neural network into the core of vallate papllae is also described in relation to the appearance of taste buds.

Introduction

Anatomical, histological and histochemical studies on the innervation tongue and papillae during pre- and postnatal life in various domestic and laboratory animals are available for the dog (Bowne 1956, Labh and Mitra 1967), cat (Fitzgerald and Alexander 1969), goat (Labh and Mitra 1967; Qayyum et al 1975), rat (Bhargava and Agrawal 1970, Graziadei and Monti Graziadei 1978, Hamed et al 1980, Mikhail et al 1980, Prakash and Rao 1980). Much work in this field was mostly carried out in relation to lingual papillae morphogenesis and taste bud morpho-structure and differentiation (State et al 1974, Zalewski 1979, Tichý and Cerný 1987, AhPin et al 1989, Tichý 1991 *a* and *b*, Farbman and Mbieme 1991). It is known that taste bud differentiation, as observed in many species, is determined by the innervation of the mucosa and, consequently, the lateral parts of the tongue containing many taste buds are supplied with the nerves later than the *dorsum linguae*. Histochemical studies carried out by State and Bowden (1974) in the circumvallate papillae of the mouse and by Hamed et al (1980) in the foliate papillae of guinea pigs described cholinergic innervation. The enzymatic activity was seen much earlier than the intraepithelial activity related to taste bud formation, demonstrating that the cholinesterase activity pre-

ceded, in the deeper parts of the epithelial columns, the appearance of taste buds and occupied the sites of their formation.

It is known that the nerve supply of the mucous membrane of the tongue is provided, in the anterior two third, by the lingual branch of the mandibular nerve; the posterior third of the tongue receives nerve fibres from the glosso-pharyngeal nerve. Rakhaway and Bourne (1960), working on the human tongue, described a nerve plexus lying in two planes, one under epithelium and the other deeper. Mikhail et al (1980), studying the subepithelial plexus in the dog tongue, described how the ganglia in plexus were formed by bipolar nerve cells, the other ganglia in the interior of the tongue were usually formed by large multipolar nerve cells and few contained bipolar nerve cells. Furthermore, they showed some nerve fibres extending to the outermost layer of epithelium and other shorter and branched fibres in between the epithelial cells. Some fibres ended in shallow cup-shaped discs called Merkel's tactile discs. Some solitary neurons and autonomic ganglion cells have been described near the circumvallate papillae in man (Chu 1968; Ferrell and Tsuetaki 1983). Graziadei and Monti Graziadei (1978) described different autonomic neurons in rats and mice: some aggregated in the base of the papilla, others scattered through the core, along the nerve bundles and particularly near the dome. They applied the term "circumvallatae ganglion" to the entire population. In contrast with other researchers, they do not support the hypothesis that the presence of ganglion cells in circumvallate papillae has a functional relationship either with the taste buds or the taste glands, *glandulae gustatoriae*. They postulated that the nerve fibres were close to the blood vessels of the papillae to convert the tongue into an erectile organ elevating the taste buds from their deep position in the groove to a position more exposed to sapid substances. Since the distribution of the ganglion cells is near to the sunken gustatory papillae, it is possible to explain the absence of the neurons from the vicinity of the fungiform papillae which are elevated from the surface of the tongue and have their taste buds on the dorsal surface well exposed to sapid substances.

In the one-humped camel, apart from many other studies, there is an apparent lack of information about the development of tongue innervation. The tongue of this species is described as a small and very mobile organ, with a prominent *torus linguae* direct rostrally to it (absent in the newborn), and a narrow *corpus linguae* which widens towards the *apex linguae* to give the tongue a spatula-shaped appearance. A thin, delicate and smooth mucous membrane runs along the ventral and lower lateral surfaces. It goes abruptly over into thick cornified mucosa which covers the borders and dorsum of the organ and has various and numerous papillae. These consist of filiform, *papillae filiformes*, conical, *papillae conicae*, flat lenticular, *papillae lenticulares*, fungiform, *papillae fungiformes* and vallate papillae, *papillae vallatae*; foliate papillae are absent (Smuts and Bezuidenhout 1987). In the one-humped camel the vallate papillae are located on the torus, along its lateral borders, and they are the greatest of the mammalia; there are 10-16 in the caudo-dorsal part of the tongue, arranged in double rows and converging to the aboral site extremity. They measure up to 20 mm. each (Tayeb 1950, Al-Bagdadi 1966, Qayyum et al 1988, Canavese et al 1990). Vallate papillae morphogenesis has been studied under LM and SEM (Canavese et al 1990).

One-humped camel tongue is richly innerved: sensory nerve fibres are supplied from lingual, glossopharyngeal and cranial laryngeal nerves; motor nerve fibres arise from the hypoglossus. The lingual nerve, *N. lingualis*, arises in common with the inferior alveolar nerve. It gives many branches, including among others, the lingual branches to the tongue. The chorda tympani of the facial nerve joins the lingual nerve close to its origin and its sensory fibres supply taste fibres to the

rostral two-thirds of the tongue. The glossopharyngeal nerve, *N. glossopharyngeus*, parts with three branches and the lingual branch, which lies distally along the caudomedial border of the hyoid bone to the root of the tongue, supplies sensory fibres to the root of the tongue, as well as taste fibres to the caudal third of the tongue. In contrast to domestic ruminants (Barone 1981), the cranial laryngeal nerve does not give rise to sensory fibres at the root of the tongue. The hypoglossal nerve, *N. hypoglossus*, motor, leaves the skull through the hypoglossal canal of the occipital bone. Proximally, it gives off the *Ansa cervicalis* to the ventral branch of the first spinal nerve and continues along the caudolateral border of the stylohyoid bone to supply the muscles of the tongue (Smuts and Bezuidenhout 1987).

Qayyum et al (1988) reported a strong positive reaction to cholinesterase close to the lateral border of the circumvallate papillae and the upper border of the fungiform papille in the adult one-humped camel. They observed, moreover, many ganglia under the lingual papillae at the posterior 1/3 portion of the tongue, a site occupied by vallate papillae. The dense cholinergic innervation in and around the blood vessels of the core of the papillae, also observed in the tongue of one-humped camel by Qayyum et al (1988) and according to Graziadei and Monti Graziadei (1978), these nerves fibres are considered to arise beneath the ganglia, as cholinergic vasodilators.

The aim of this work is to study the morphostructural changes and distribution of nerve fibres and ganglia in the tongue of *Camelus dromedarius* fetuses, and the development of the lingual papillae.

Materials and Methods

The tongues of 58 dromedary fetuses with VRL ("Vertebral-Rump Length" is the distance between the occipital protuberance and the base of the tail) between 30 and 980 mm were examined. Fetuses were divided into 4 groups according to increasing age/VRL and to similar tongue and vallate papillae histological characteristics. Foetal ages ranging 3 to 11 months were estimated from the regression equation of foetal age with VRL (Canavese and Benvenuti 1982).

The length of all the excised tongues was measured; regression and correlation coefficients (r^2) with the VRL were calculated with the SPSS^x statistical package (subroutine REGRESSION) [SPSSx 1985].

Tongue samples and papillae were collected together. The material sampled was fixed in 10% neutral formalin, embedded in paraffin and longitudinal and cross sections were cut with a Leitz microtome. The 5-8 mμ thick sections were stained with hematoxylin-eosin, Mallory-Azan, Masson, Van Gieson, toluidine blue and Linder's silver method for nerve fibres. Anatomical description agrees with the "Illustrated Veterinary Anatomical Nomenclature" (1992).

Results and Discussion

The distribution of the lenghts of the foetal tongues as a function of the VRL (Table 1) and the regression equations demonstrated that there was a high and significant correlation between VRL (that is between the age of the fetuses) and the tongue length measurements (r^2 = 0.97; S.E.=6.681) and the size of the vallate papillae (r^2 = 0.898; S.E.=0.228) (Fig.1). The histological observations on the tongue and vallate papillae concerning groups *a, b, c* and *d* (Table 1) are reported here.

a. Tongues and vallate papillae from foeti with VRL 30-60 mm. (age about 2 months).

In this developmental stage, it is possible to describe small isolated branches of nerve fibres in the ventro-medial side of the tongue without a regular arrangement. Then, nerve fibres, joint with regular branches, become highly visible. From these few ascending

fibres, which collect under the epithelial layer in the lingual aponeurosis and the muscles, arise (Fig. 2). Vallate papillae are poorly definied dome-shaped forms with thin a epithelial layer. They have no taste buds and the papillae core is not yet innerved. In previous papers (Canavese et al 1986 *a* and *b*, 1987 and 1990) it was noted that papillar morphogenesis begins in foeti of 30 mm VRL and becomes more clear in 80 mm VRL when the annular pad is deeper. The appearance of a slight depression in the central part of the papillae is an interesting step: the extention and enlargement of the depression gives taste

Table 1: Biomorphometric values (mm) for one-humped camel tongue length and vallate papillae size at different developmental statges

Foetal Group (No. specimens)	V-R Length (age range-months)	Tongue Length range	Papillae Vallate range
a (3)	30-60 (< 2.0)	-	-
b (12)	130-200 (2.0-3.5)	22 - 41	0.52 - 1.03
c (15)	240-300 (3.5-5.0)	39 - 59	0.83 - 1.48
d (28)	340-980 (5.0-11.0)	64 - 165	1.27 - 3.10

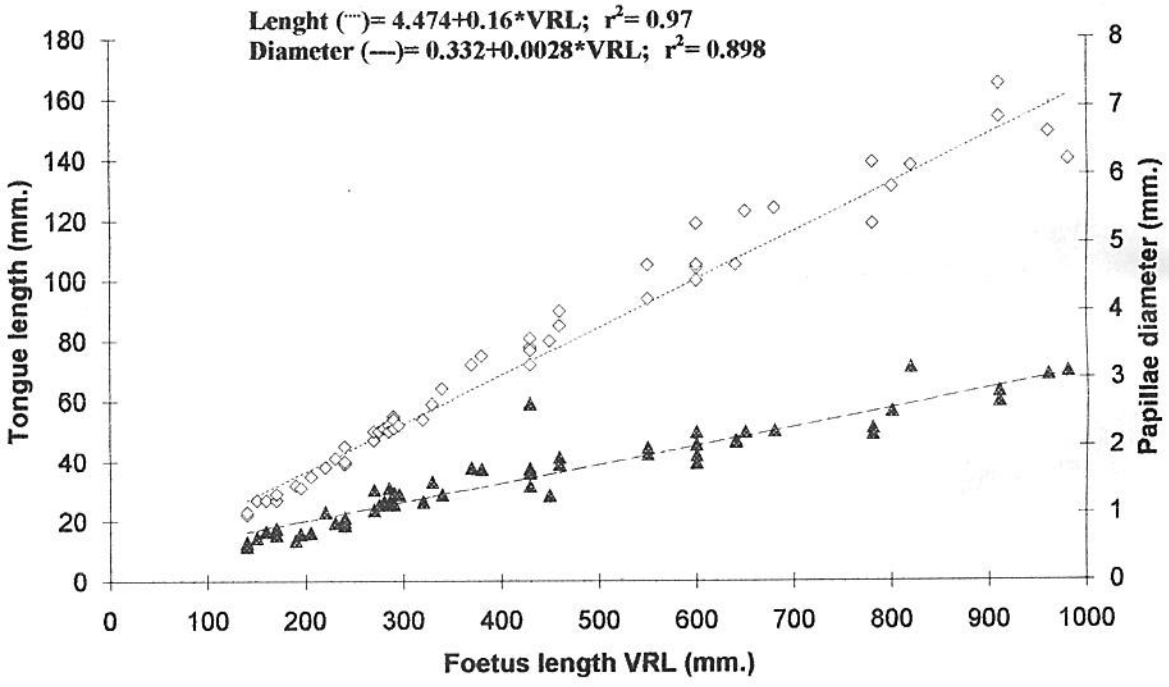
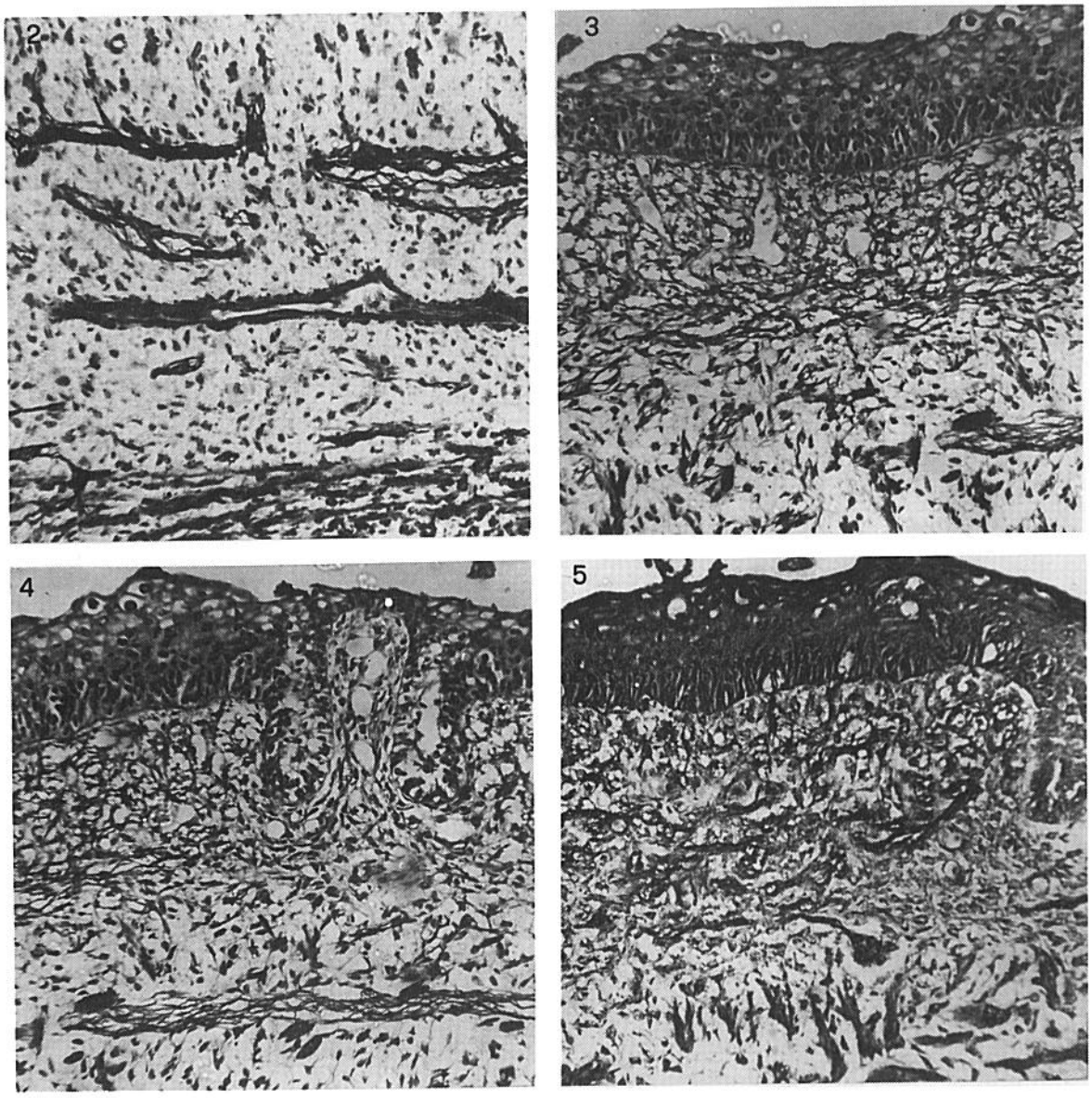


Fig. 1: Distribution and regression plots of the tongue length and papillae diameter from 36 dromedary foeti in comparison with V-R length.

buds their definite site, in the groove of the internal wall. Some significant changes in the innervation and vascularization are related with the morpho-histogenesis steps. However, the ganglia at this developmental stage could not be traced.

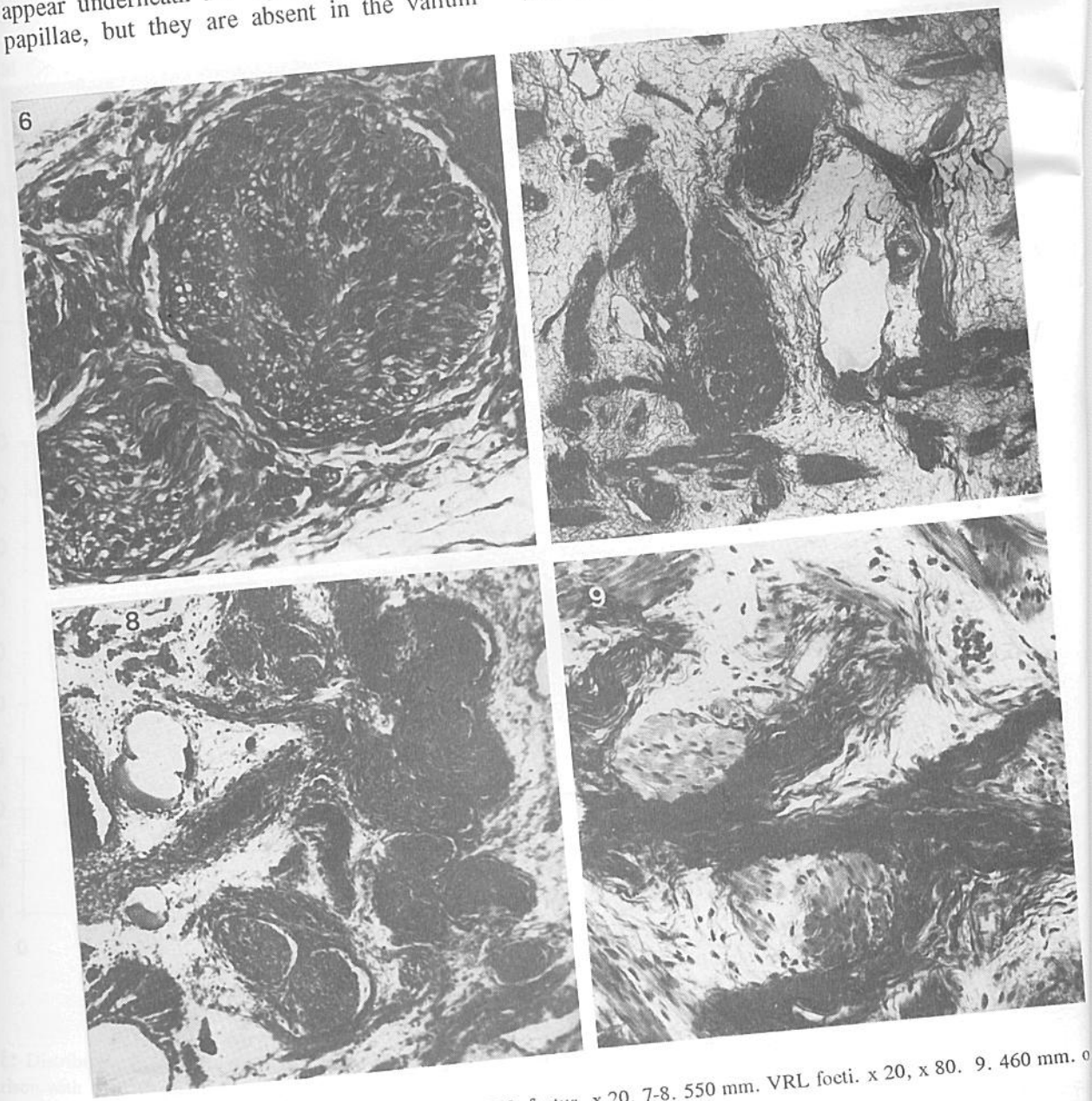
b. Tongues and vallate papillae from foeti with VRL 130-200 mm (age about 2.0-3.5 months).

The changes in the pattern of innervation and vascularization are noteworthy in this fetal group. The organization of nerve bundle in the areas mentioned in *a* has become well



Figs. 2-5: Dromedary foeti. Linder's staining. **2.** 60 mm VRL foetus x 63. **3-4:** 130 mm VRL foeti x 55. **5.** 245 mm VRL foetus x 63

defined. In the foeti of 130-140 mm VRL, where vallate papillae are developed, the deeper nerve plexus increases and it is locateddeep into the submucosal connective tissue of papillar cores next to the lingual aponeurosis. Dense and faint nerve fibres appear underneath the epithelium of vallate papillae, but they are absent in the vallum connective tissue (Fig. 3). The scattered innervation under the epithelium seems to precede the taste bud formation which is defined in the 240 mm VRL one-humped camel foeti (Canavese et al 1990). As suggested by Tichý and Cerný (1987) the taste bud differentation is initiated by the contact of epithelial cells with a nerve fibre in the pig and, according

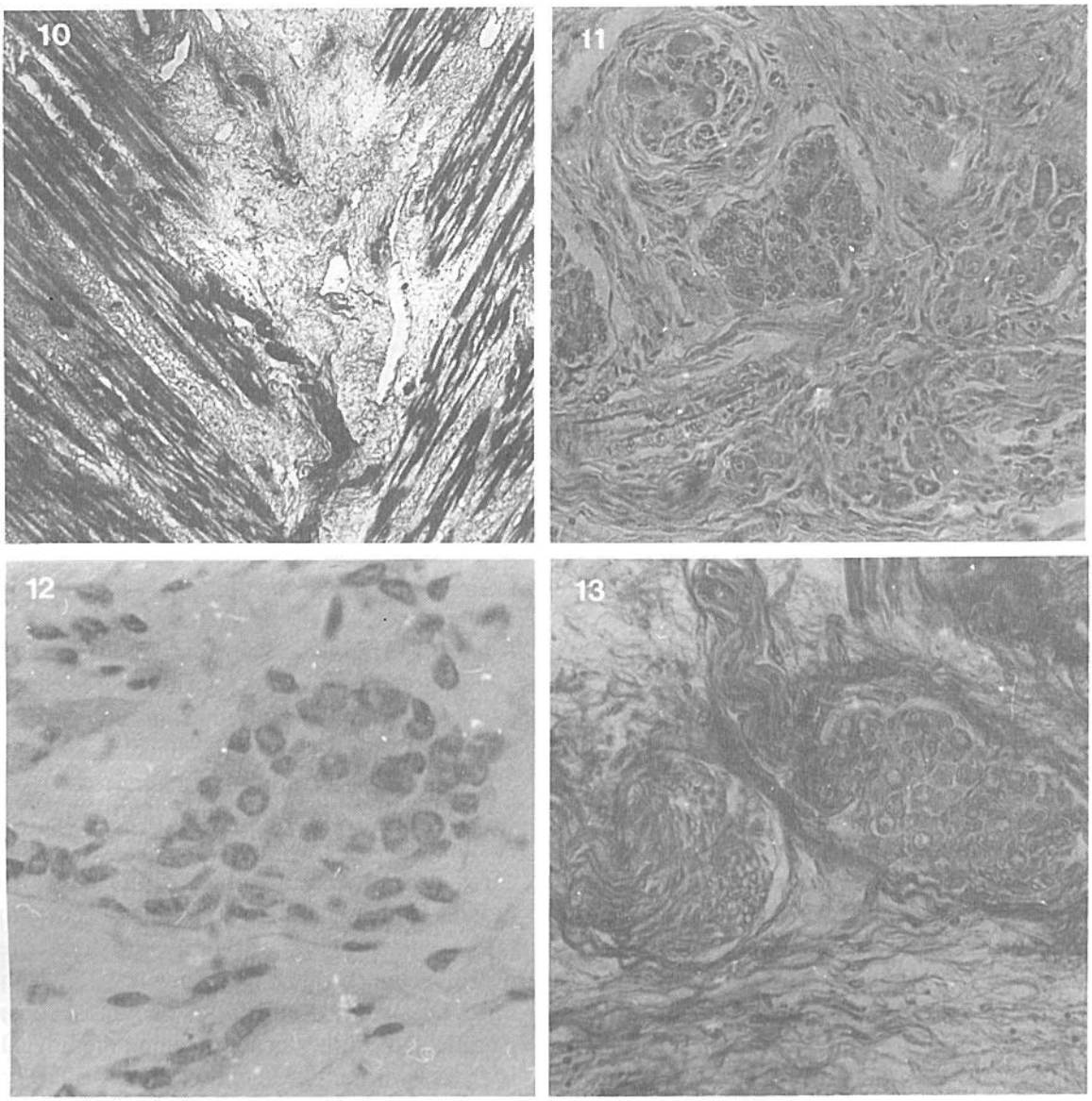


Figs. 6-9. Dromedary foeti. 6. 460 mm. VRL foetus. x 20. 7-8. 550 mm. VRL foeti. x 20, x 80. 9. 460 mm. o
postnatal life. In the cross sections sliced VRL foetus. x 20.

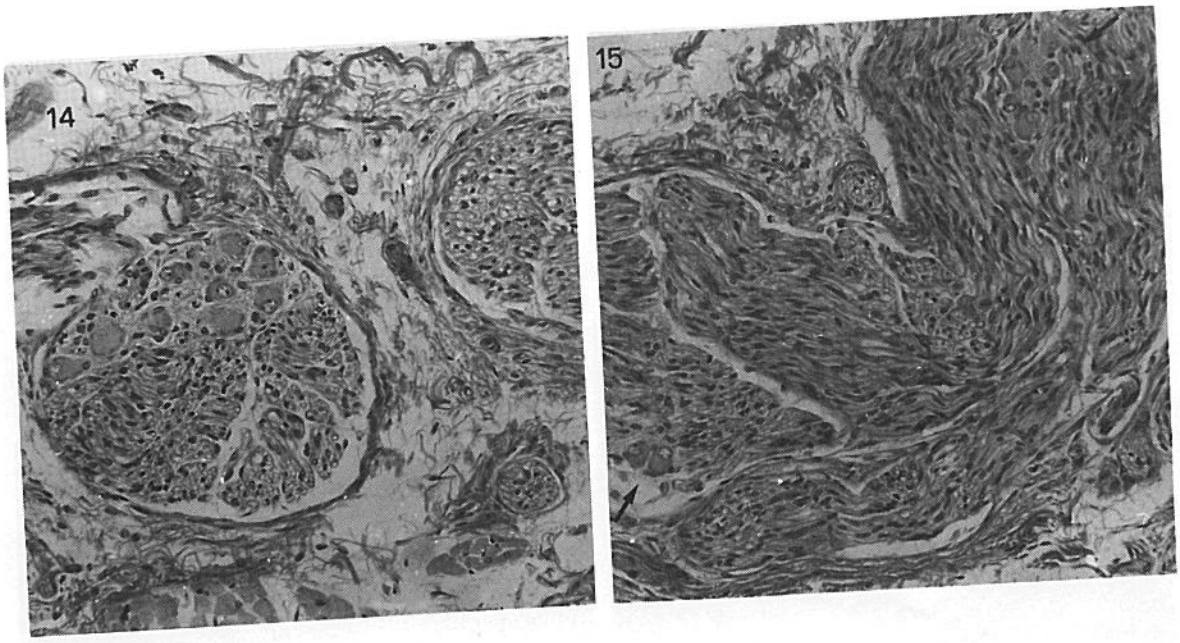
to Farbman Mbiene (1991), the tongue epithelium would exert a general trophic effect on growing axons of sensory nerves. It is possible to observe that the vascular network is well developped and rich in the whole papillae where there are many capillaries and large vascular spaces, such as next to the neural network as in the vallum connective tissue (Fig. 4).

c. Tongues and vallate papillae from foeti with VRL 240-300 mm (age about 3.5-5.0 months).

The arrangement of lingual innervation remains but becomes more pronounced than in *b*. In particular, the faint nerve bundle-network in the core of vallate papilla rearrang-



Figs. 10-13. ***10.*** *780 mm. VRL foetus. x 65.* ***11.*** *830 mm. VRL foetus. x 63 .* ***12.*** *220 mm. VRL foetus. x 200.* ***13.*** *460 mm. VRL foetus. x 80.*



Figs. 14-15: *Dromedary foeti.* **14.** *780 mm. VRL foetus. x 50.* 15. *780 mm. VRL foetus. x 50, solitary neurons (arrows).*

es and larger nerve bundles appeared; the plexiform organization remains only under the lateral margins of the papilla next to the taste buds in the internal wall of the groove (Fig.5). The glandular organization has proceeded and it is already possible to observe the deeper network expanding in the connective tissue among the glandular *acini*.

d. Tongues and vallate papillae from foeti with VRL 340-980 mm. (age about 5.0-11.0 months).

In this foetal group, larger than previous one, the lingual innervation of one-humped camel is found to be similar in distribution and some topographic characteristics to those through the body and root of the tongue, the innervation is supported by two thick lingual branches formed by other smaller branches (Fig.6). They lie on the ventral side of the tongue, in both lateral borders of the medial part, *septum linguae*, and in the ventral position as for lingual vessels. The small nerve branches during their route towards the lingual apex of the tongue, give rise to other branches which lead to the dorsal part of the tongue running next to the septum or, with lateral-obliquus direction, in the connective tissue layer around lingual vessels (Figs.7-9). The intrinsic muscles of the tongue are innerved by nerve fibres which arise from the last ones (Fig. 10).

A large plexiform network of nerve fibres takes shape in the mucosa connective tissue layer next and under the aponeurosis, many other bundles run along the connective tissue septa around various glandular *acini* (Fig.11). A faint neura network in the papillae vallate cores arises from this larger plexus which derives from ascending nerves bundles next to the connective tissue septum. Along the neural network there are many ganglia with different sizes and number of neurons. Some ganglia and solitary neurons along the lingual branches, in the deeper plexus and on the its secondary branches (Figs.12-15), while they are not along nervous fibres of the muscles.

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