

Morphogenesis and morpho-structural characteristics of the circumvallate papillae in the tongue of *Camelus dromedarius*

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Introduction

The tongue of the one humped camel (*Camelus dromedarius*), the two humped camel (*Camelus bactrianus*) and other tilopods, possesses the largest circumvallate papillae (Papillae vallatae) of all mammals. In these 2 species, the tongue is relatively small and highly mobile, and has between 10 to 16 circumvallate papillae which are found in the caudo-dorsal part of the tongue, arranged in two rows at the sides of the 'torus,' such that they converge slightly in a caudal direction (Canavese and Ibrahim, 1984; Droandi, 1936; Mukasa Mugerwa, 1981; Opiel, 1900; Qayyum *et al.*, 1988; Sonntag, 1922; Tayeb, 1950). Al Bagdadi (1966) also noted that the caudal convergence of the two rows was hardly noticeable in the dromedary but highly evident in the camel. In the former, the papillae are arranged along the two sides in a single line only, whilst in the latter they tend to be arranged in double rows, with the largest papillae arranged laterally. However, Al Bagdadi (1966) has found this different arrangement between species not to be absolute, because it is also possible to find the double row arrangement in the dromedary.

It is known that circumvallate papillae are characteristic of mammals and that their number varies greatly between species, with, for example, one per side in horses, pigs and rabbits, 2 or 3 in carnivores, 4 or 5 in man, and about 10 in bovines (Barone, 1981). Even within the genus '*Camelus*', the number of these papillae is not constant, but varies between animals; the dimensions also vary from a few millimetres to about two centimetres. They are not raised above the surface of the tongue, and appear to be generally ovoid, but sometimes circular, and surrounded by a deep, narrow groove that separates them from a wide annular pad. The central part of the dorsal surface of the papillae sometimes appears depressed or tubercular. On the walls of the grooves are many taste buds. In mammals these are most frequently found on the fungiform and circumvallate papillae on the dorsal part of the tongue and on the foliate papillae at its postero-lateral margin, at the level of the 'sulcus' terminalis (Bradley, 1971).

The aim of the present study, through comparative observations made using stereo-, optical- (OM) and scanning electro microscopy (SEM), is to: (1) contribute towards a

better morpho-structural knowledge of the dromedary's circumvallate papillae; (2) document certain significant stages in the morphogenesis of the papillae, and illustrate the extent and mode of their growth by taking a series of measurements; (3) investigate the morphological development of the taste buds, and their topographical distribution in relation to the papillae.

Material and Methods

The tongues of 12 adult dromedaries and of 70 fetuses, obtained in Somalia from the slaughter-houses of Mogadishu and Agfoi, were examined. The fetuses' VR length ('Vertebral-Rump', or the distance between the occipital protuberance and the point of attachment of the tail) was between 30 and 980 mm. Taking these lengths as a base, the ages of the fetuses could be calculated using a regression curve (Canavese and Benvenuti, 1982). The rows of papillae were cut from the adults' tongues, and these together with the entire foetal tongues were immediately fixed in 10% formalin, Zenker-formol, Bouin and Dubosq-Brazil. After the fixing operation had been carried out, the samples were examined morphometrically using a stereo microscope and the following measurements were taken: length of the foetal tongues; length of the rows of papillae; maximum and minimum diameter of the papillae, taking the annular pad into account, and also the diameter of the papillae alone; average thickness of the annular pad; overall dimensions of the papillae in each row; depth of the groove on the adult tongues (tables 1 and 2).

Part of the tongue material, to be examined by optical microscopy (OM), was set in paraffin and sliced using a microtome; the sections thus obtained, between 8-10 μ m thick, were stained with hematoxylin-eosin, toluidine blue, Van Gieson and Bodian. Several papillae from the adult tongues and from the larger foetal tongues, and several of the smallest complete foetal tongues were dried using the critical point method, then impregnated with gold and examined by SEM.

Results and Discussion

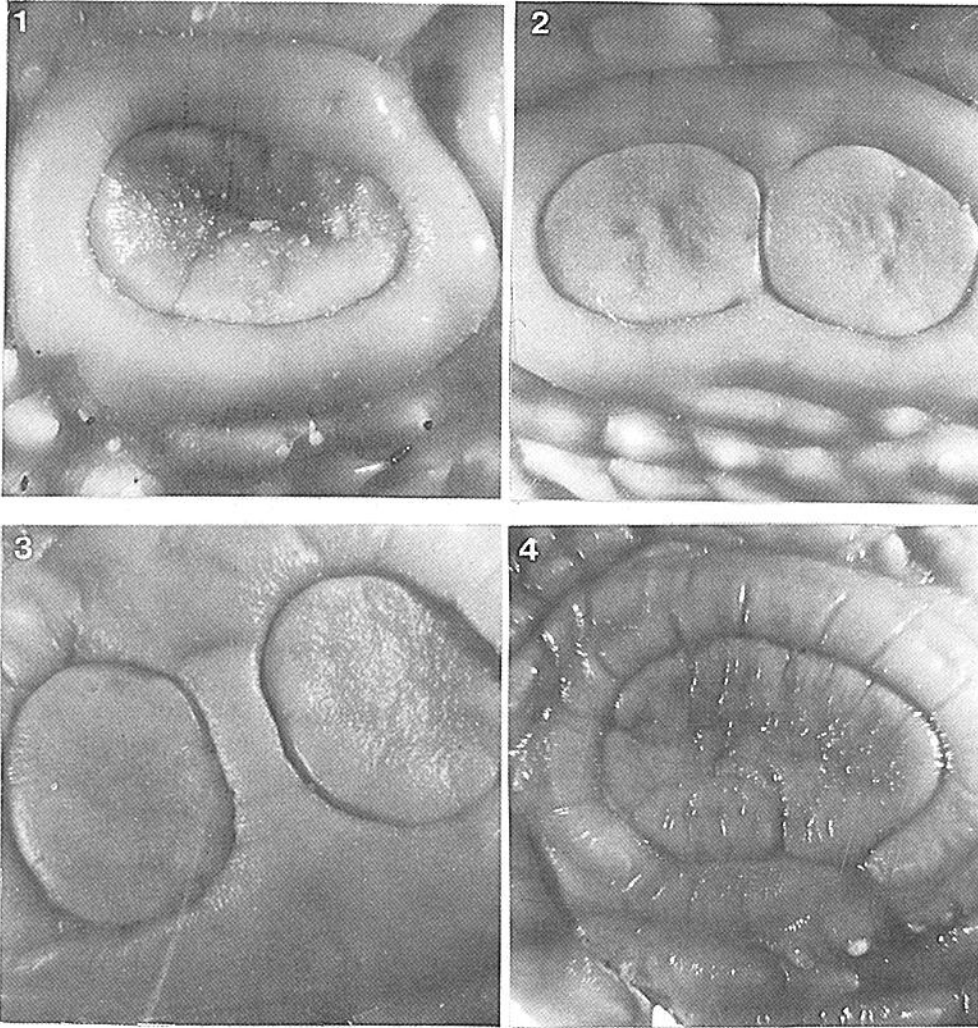
a) Circumvallate papillae of the adult dromedary:

In this species, according to our estimates, adults have about 10-15 papillae, and this is in fact supported by past findings (Droandi, 1936; Mukasa Mugerwa, 1981; Sonntag, 1922). Even though they are not raised above the surface of the tongue, they are a significant feature of it, due to their dimensions: ovoid or circular in shape, with varying dimensions of between a few millimeters and about 2 cm, and surrounded by a groove 1-2 mm in depth, which separates them from an annular pad which is itself about 2.5 mm in width and slightly more raised above the tongue surface than the papillae (Table 1).

Table 1. Circumvallate papillae of adult dromedary. Average \pm standard deviation of the values (mm) of the measurements taken.

	x	s	n
Diameter of papillae	6.03 \pm	1.38	91
Diameter of papillae and annular ring	10.64 \pm	1.75	91
Width of annular ring	2.30 \pm	0.38	91
Depth of groove	1.38 \pm	0.24	68

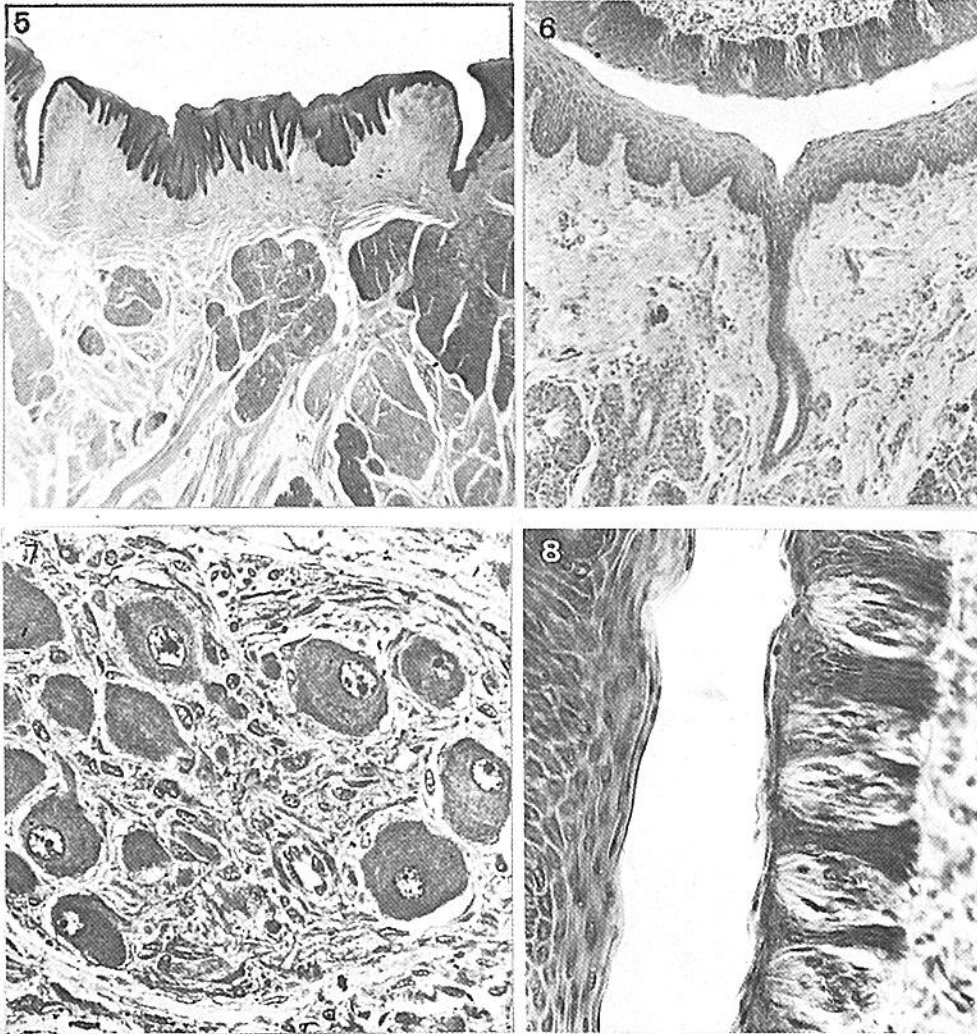
The form, dimensions and arrangement of the papillae in the rows are not constant. The most common papillae in the rows are ovoid shaped, normally surrounded by a well-defined annular pad. These are known as 'simple' papillae (Fig. 1). Often, however, papillae of a more complex form are found, such as, for example, 'double' papillae, in which two papillae are found together, each surrounded by an annular pads, forming a figure '8' (Fig. 2). Sometimes two papillae are found together surrounded by a single, larger than usual, ovoid annular pad (Fig. 3). There is also a small percentage of very small papillae, which have an almost rudimentary appearance, with a central diameter averaging 2.5 mm and a proportionally wider than usual annular pad with a width



Figures 1 - 4: Circumvallate papillae of adult dromedary.

1. 'Simple' circumvallate papillae viewed by stereo microscope. X 13.
- 2 & 3. 'Double' circumvallate papillae viewed by stereo microscope. X 13.
4. Circumvallate papillae with incomplete annular ring. X 13.

averaging 2 mm. At times, the annular pad is incomplete, forming a more or less open 'C' shape, (Fig. 4). This type of papilla is usually found to be of large dimensions, as stated by Al Bagdadi (1966). The arrangement of the papillae in the row is not always linear, sometimes they are displaced medially or laterally. Sometimes, where there a main row of large papillae, the small, more rudimentary papillae already described above,



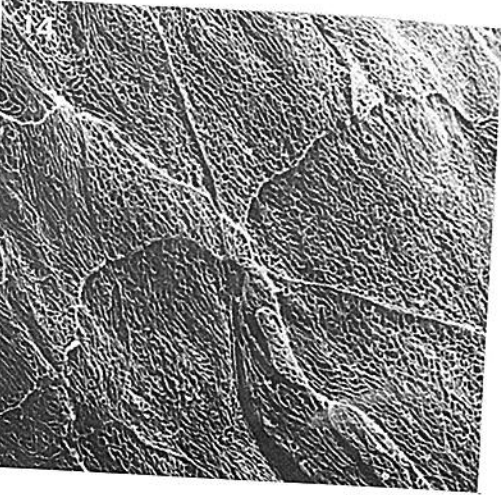
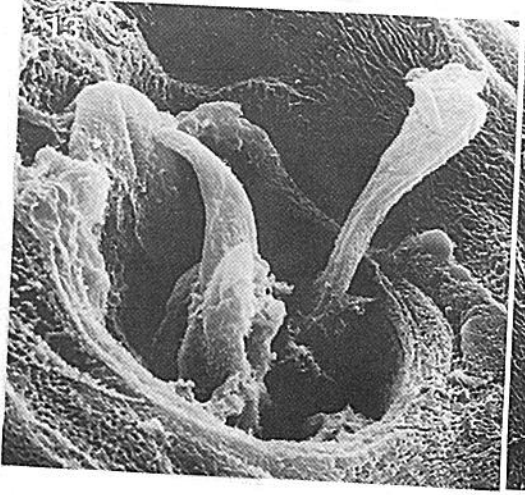
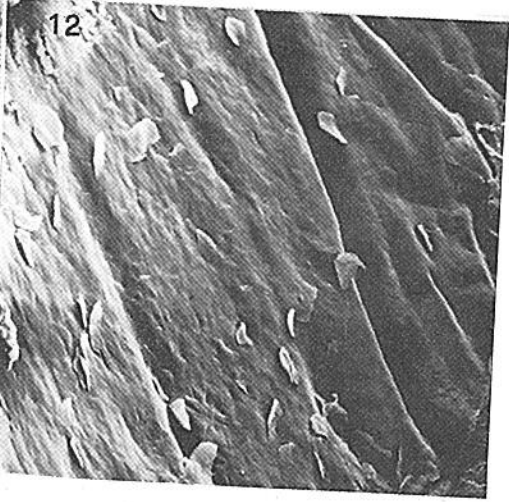
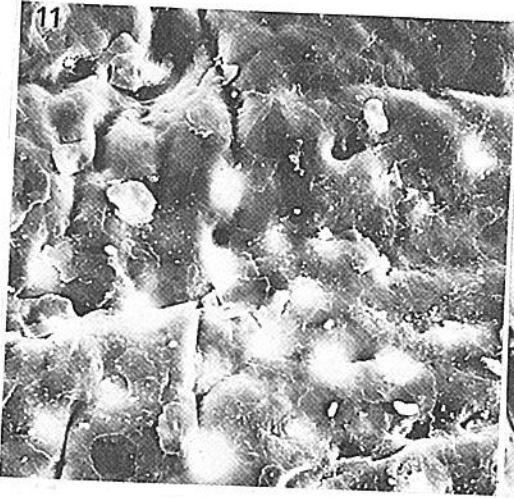
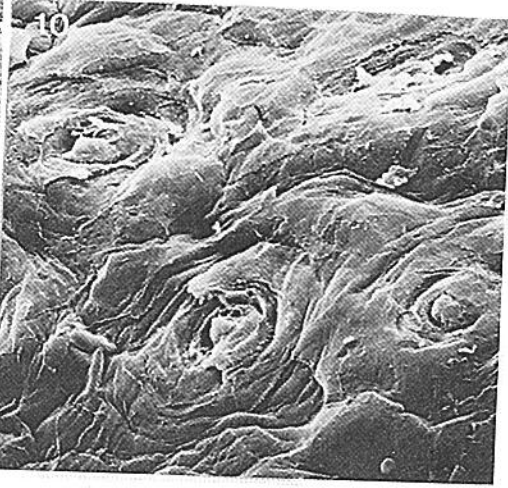
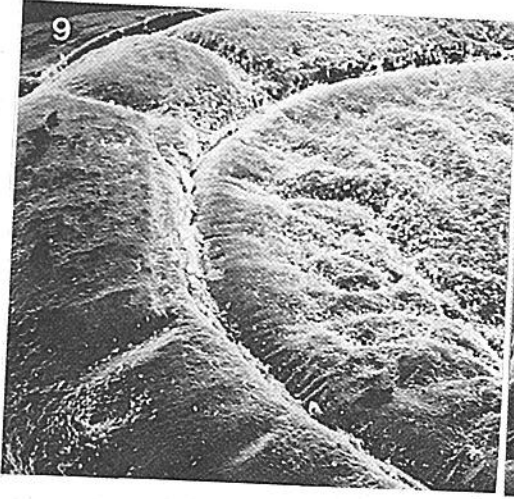
Figures 5 - 8: Circumvallate papillae of adult dromedary.

5. Circumvallate papilla section. Note the different appearances of the epithelium in the various areas: annular pad, groove, papilla. Hem. - eos. X 25.

6. Circumvallate papilla section through the base of a groove. Note the outlet of a glandular excretory duct. X 250.

7. Sympathetic neurons, which collectively form the circumvallate ganglion. Bodian. X 800.

8. Taste buds on the internal groove wall. Hem. - eos. X 500.



are found at the sides of the row.

The OM examinations revealed that the layered epithelium which covers the papillae has different characteristics in different areas. It is very often on a level with the annular pad, although less so at the outer wall of the groove, and is relatively thin at the groove's internal wall, where it is composed of 6-8 layers of cells which contain within them the taste buds. On the dorsal surface of the papillae where there are sometimes irregular depressions, the epithelium is thick and penetrated by numerous papillae of the underlying connective tissue. This tissue appears richly vascularised and contains, in its depths, groups of glands mainly of the mucous type (Von Ebner's glands) surrounded by small strands of striated muscular fibres (Fig. 5). The excretory ducts from these glands open out at the base of the groove (Fig. 6). Also in the connective tissue are found scattered groups of neurons from the autonomic nervous system, which collectively form the circumvallate ganglion (Fig. 7). Observed for the first time by Drasch (1887), and Fusari and Panasci (1889/90), more recently they have been described by Graziadei and Monti Graziadei (1978). Numerous well defined taste buds were seen in the sections. They were ovoid or barrel-shaped, measuring 50-65 x 35-45 μm (Fig. 8).

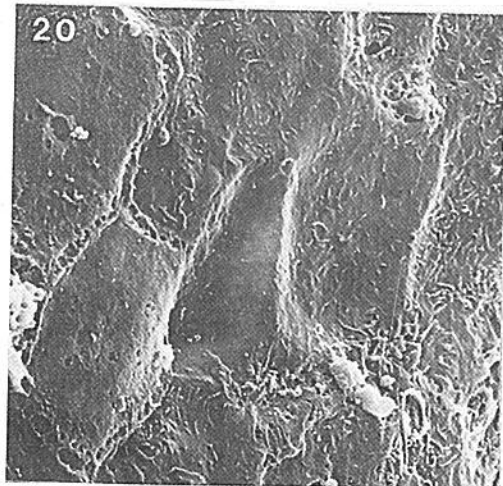
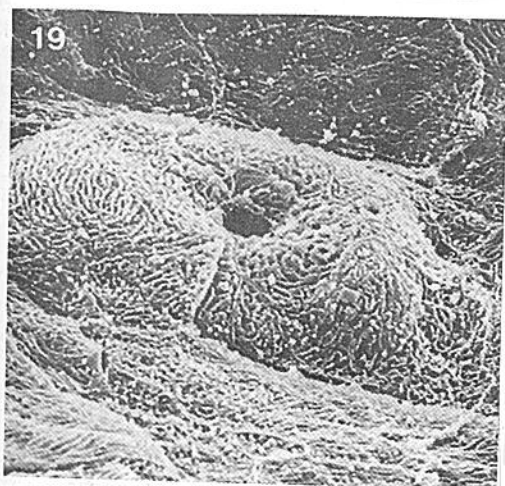
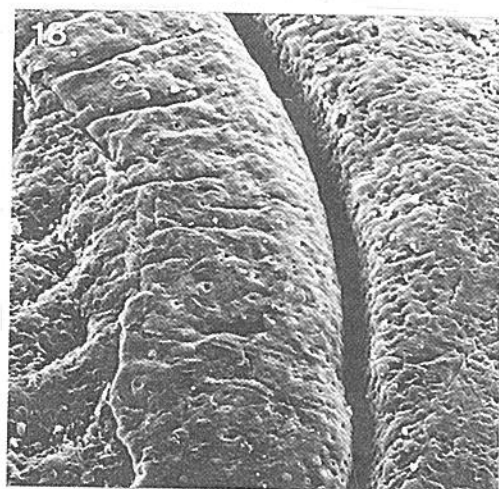
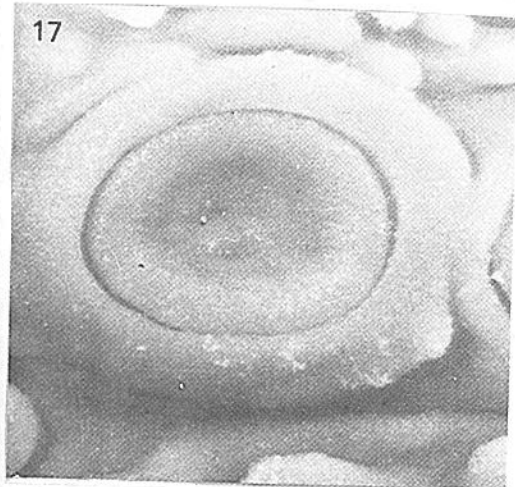
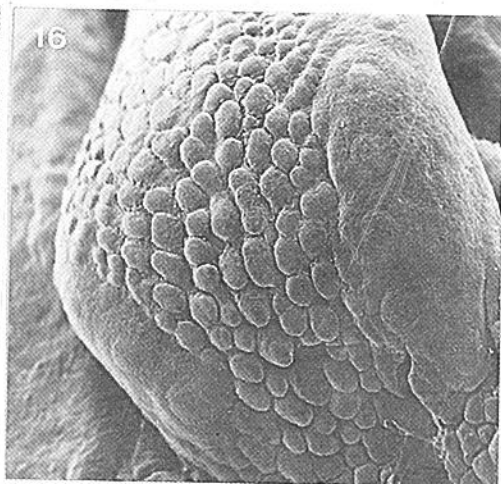
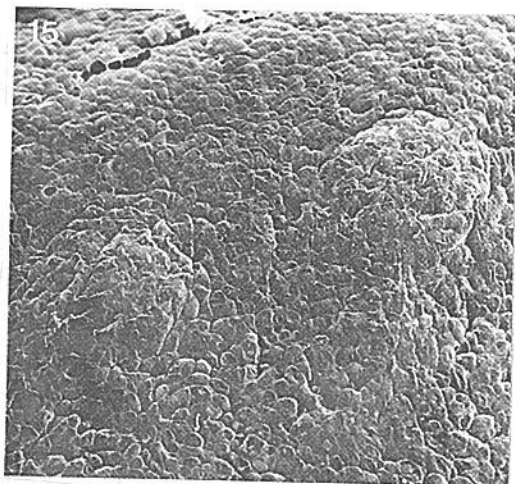
Using SEM, the various components of the papillae could be both viewed as a whole and analysed in detail. The surface of the internal groove wall reveals numerous, well-defined taste - bud pores, which are however completely absent from the external groove wall and from the dorsal part of the papillae and annular pads. The dorsal surfaces of the papillae and annular pads often contain depressions (Figs. 9-12). SEM also clearly reveals the different characteristics of the internal and external groove walls. The internal wall contains taste - bud pores which are seen to be slightly hollow structures (Fig. 11) from which tufts of microvilli extend (Fig. 13). The external wall appears by comparison rather smooth except for a certain number of cellular structures undergoing desquamation (Fig. 12). The details of the cellular surfaces show, in both cases, a well-developed microplications (Figs. 13 & 14).

b) The circumvallate papillae of the dromedary foetus:

Foetuses with a VR of about 30 mm already have roughly defined papillae, which appear as slightly dome-shaped forms, as yet without annular pads (Fig. 15). These appear later on and become more clearly defined in 80 mm VR foetuses (Fig. 16), whilst it is only at this stage that the grooves begin to deepen. Next, the central part of the papillae forms a slight depression which becomes slowly more accentuated, whilst at the same time, a sort of 'ring-doughnut' appears between the centre of the papillae and the annular pad (Fig. 17). On the surface of these 'ring-doughnuts,' the taste buds can be seen, and it is only at a later stage that they appear on the internal groove wall. In fact, the 'ring doughnuts' are only temporary structures, and disappear as the foetuses and their papillae

Figures 9-14 Circumvallate papillae of adult dromedary.

9. Circumvallate papilla seen as a whole by SEM. X 20.
10. Annular pad surface. Note numerous depression. SEM. X 43.
11. Internal groove wall with numerous bud pores (arrows. SEM. X 390.
12. External groove wall, smooth, with a few flaking cells. SEM. X 200.
13. Cellular surface of internal groove wall with microplications. Note the taste bud pores from which numerous microvilli extend. SEM, X 2340.
14. Cellular surface of external groove wall with microplications. SEM. X 2470.



develop (Fig. 18). Whilst positioned on the 'ring-doughnuts', the taste bud pores are found at the top of dome-shaped structures which have a basal diameter of about 25 μm (Fig. 19). No taste buds were found on the internal wall of the ocellus in foetuses (Fig. 20). It was noted that the number of papillae already existing in 30 mm VR foetuses was almost always the same as in adults. Even if the number differed, the difference was not significant.

With the gradual development of the papillae, the first taste buds appear. In mammals, these are normally situated in the lingual epithelium, and begin their development at an early stage in the growth of the embryo. In some species, such as man, their development seems to be complete before birth, but in other species, such as the mouse and rabbit, it is completed only after birth (Farbman, 1971). In the dromedary, the first indication of the presence of taste buds is in 240 mm VR foetuses. At this stage, they are rather widely spaced and irregular, and in height do not yet occupy the complete thickness of the epithelium. Their presence is restricted to the periphery of the dorsal surface of the papillae, and they are as yet absent from the internal groove wall, which is already outlined but not yet well-defined (Figs. 21 & 22).

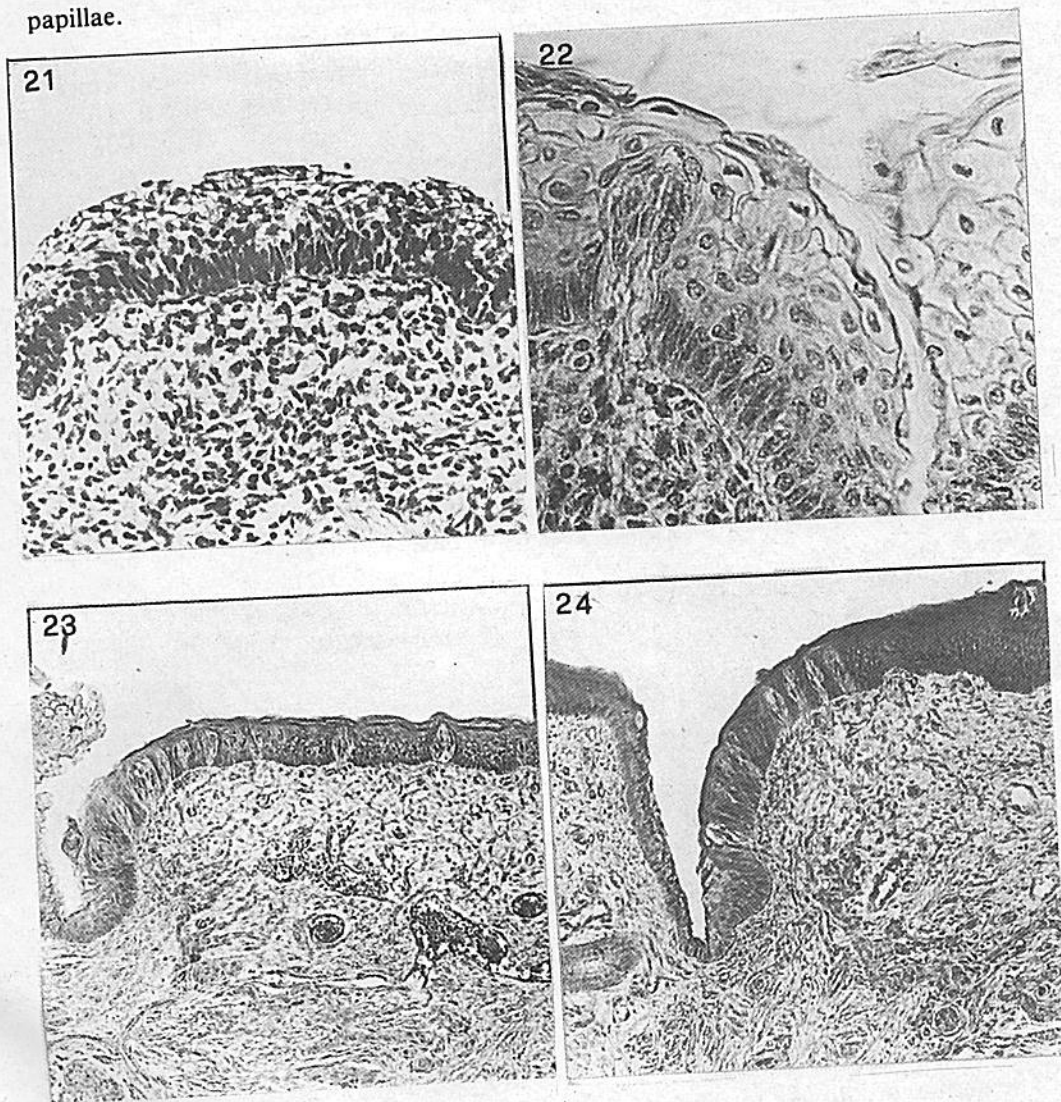
With continuing foetal development (i.e. in foetuses of between 330 and 680 mm VR) the taste buds slowly become more numerous and occupy a significant part of the periphery of the papilla dorsal surface, also becoming established on the internal groove wall. The buds at this point occupy, in height, the total thickness of the epithelium, and the underlying connective tissue is richly vascularised. The boundary between the epithelium and the lamina is still normally in the form of a straight line. In 830-960 mm VR foetuses, the position of the taste buds gradually becomes limited to the internal groove wall, which is by now well-defined. A few buds can still be seen here and there on the dorsal part of the papillae, but these are by now of a degenerative form. The epithelium covering the dorsal surface of the papillae is at this stage much thicker, and the boundary line between it and the underlying connective tissue has become more irregular, a sign of the complex and deep connections which are to develop between the epithelium and the connective tissue later on, and which are characteristic of the adult dromedary circumvallate papillae (Figs. 23 & 24).

The various morphometric parameters which were considered in relation to the foetuses (Table 2), when compared to VR and tongue length, enabled the calculation of correlation coefficients which proved to be highly significant. The relevant regression curves were also calculated (Table 3). It can be concluded that the increase in values of the various

Figures 15-20: Circumvallate papillae of dromedary foetus.

15. Two very roughly defined papillae from a 30 mm VR foetus. SEM. X 300.
16. Base of the tongue of an 85 mm VR foetus. Note the two rows of circumvallate papillae along the edges. SEM. X 27.
17. Circumvallate papillae of a 600 mm VR foetus seen through a stereomicroscope. Note the 'ring-doughnut' formation on the central papilla. X 11.
18. Detail of circumvallate papilla of an 830mm VR foetus. From left to right: papilla centre; edge of papilla centre with 'ring-shaped doughnut' formation, on which are numerous taste bud pores; groove; annular ring. SEM. X 70.
19. 830 mm VR foetus. Internal groove wall and taste bud pores on top of dome-shaped forms. SEM. X 3370.
20. 830mm VR foetus. External groove wall. Note absence of taste buds. SEM. X 3510.

parameters during foetal development is regular and continuous. Also to be noted is that the level of growth of the annular pad is proportionally greater than that of the papillae.



Figures 21-24: Circumvallate papillae of dromedary foetus.

- 21. Circumvallate papillae from a 95 mm VR foetus. Note absence of taste buds. OM.X 120.
- 22. Detail of circumvallate papilla from a 330 mm VR foetus. Note rough outline of a taste bud. OM.X 192.
- 23. Circumvallate papillae from a 680mm VR foetus. Note the numerous taste buds present on the dorsal epithelium and on the developing groove wall. OM.X 48.
- 24. Circumvallate papillae from a 960 mm VR foetus. Note that at this stage the taste buds are only present on the internal groove wall. OM.X 48.

Table 2. Grouping of fetuses by VR length, and relative minimum/maximum values (mm) of the various measurements taken.

	Group I 15 fetuses V-R 140-240	Group II 24 fetuses V-R 270-550	Group III 16 fetuses V-R 600-980
	mm	mm	mm
Length of tongue	22-45	47-119	100-165
Average length of papilla row	3.3-6.9	7-20.5	16-29
Average papilla size	0.45-0.85	0.88-1.61	45-2.58
Average annular ring size	0.07-0.185	0.18-0.375	0.313-0.695
Total papilla size	4.95-9.48	8.08-19.32	12.88-27.5

Table 3. Correlations coefficients and regression equations for pairs of measured values.

	r	y = a + bx	n
V-R LENGTH			
Length of tongue	0.9858**	y = 4.4739 + 0.1636x	55
Average length of papilla row	0.9728	y = -0.1669 + 0.0314x	55
Average papilla size	0.956	y = 0.2932 + 0.00218x	55
Average annular ring size	0.969	y = 0.01363 + 0.000655x	55
Total papilla size	0.9411	y = 2.916 + 0.0242x	55
TONGUE LENGTH			
Average length of papilla row	0.977	y = 1.412 + 0.191x	55
Average papilla size	0.952	y = 0.2513 + 0.0131x	55
Average annular ring size	0.9378	y = 0.01156 + 0.00378x	55
Total papilla size	0.9404	y = 2.419 + 0.1458x	55

** P < 0.01 for all the parameters considered.

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Summary

The authors describe the morphological characteristics and reveal aspects of the morphogenesis of dromedary circumvallate papillae. They also examine the appearance of the first taste buds, and their topographic arrangement in relation to the papillae. Scanning electron microscopy and morphometric evaluation was used so that it was possible to examine in detail the papillae from 30 mm VR fetuses, and compare the growth of this organelle with the general development of the tongue.

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