

The Histology of the Soft Palate of the Vervet Monkey *Cercopithecus pygerythus*

P. CLEATON-JONES

Dental Research Unit of the South African Medical Research Council and University of the Witwatersrand, Johannesburg, 2001.

Primates are frequently used today in dental research and in South Africa the primate most commonly used is the vervet monkey (*Cercopithecus pygerythus*). This paper describes the normal histology of the soft palate in this animal based on a preliminary study towards the possible use of the vervet monkey in cleft palate research.

Materials and methods

Twenty-five soft palates from adult vervet monkeys were examined. Fifteen were used for routine histology, six for nerve impregnations, and four for keratin histochemistry.

The following fixatives were used:

- (a) For most specimens 10 per cent neutral buffered formal saline¹;
- (b) for silver impregnations, de Castro's fixative,² and trichloroacetic acid-alcohol³;
- (c) for keratin histochemistry, 10 per cent neutral buffered formalin and trichloroacetic acid-alcohol.¹

After dehydration in serial alcohols the specimens were embedded in paraffin wax, except that for Von Mihalik's⁴ silver impregnation technique, the tissues were double embedded in paraffin wax and celloidin.

Serial sections were cut at 7 μ m in the sagittal, coronal and horizontal planes in most cases. For the silver impregnation studies the sections were cut at 10–15 μ m in the sagittal and coronal planes.

The tissues were stained with the following stains. Harris' haematoxylin and eosin, Masson's trichrome, Tänzer-Unna's

orcein, Gordon and Sweet's reticulin stain¹; peracetic-aldehyde fuchsin-Halmi⁵; peracetic-orcein-Halmi⁶; picro-Mallory⁷; D. D. D. reaction⁸; Chevrement and Frédéric's ferric-ferricyanide¹; Von Mihalik's silver stain⁴; Fitzgerald's nerve stains^{3, 9}.

Results

After sectioning, the soft palate was some 6 mm thick the bulk being mucous glands. The relative proportions of the various tissues are shown in Fig. 1 and each will be described in turn.

(a) Oral epithelium

Almost all of the oral surface was covered by a non-keratinized stratified squamous epithelium of some 30 cells thickness (Fig. 2). This continued onto the uvula where it was thinner (about 24 cells) and eventually merged with the nasal epithelium (Fig. 8) on the nasal surface of the soft palate. The oral epithelium contained a basal layer of cuboidal cells with occasional clear cells; all the rest of the cells in the stratum spinosum gradually flattened towards the surface. The connective tissue papillae were narrow and deep extending as much as two-thirds the thickness of the epithelium and usually at least halfway. The continuity of this oral epithelium was interrupted only by mucous duct openings.

In the anterior half of the oral surface of the soft palate there were scattered papillae usually covered by an orthokeratinized stratified squamous epithelium of about 12–15 cells thick, although occasional areas of parakeratinization could be seen (Fig. 3). Mucous ducts occasionally penetrated this epithelium and on all the papillae there were 15–20 taste buds averaging

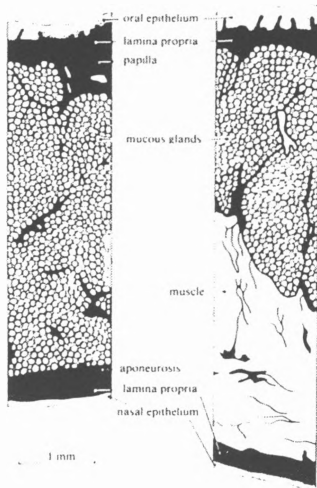


Fig. 1 Drawings of sagittal sections through the soft palate showing the tissue layers in the anterior (left) and posterior (right) halves. x 14.

54 μm long by 45 μm wide. Connective tissue papillae were wide and shallow while rete pegs were both wide and narrow (Fig. 3).

(b) *Lamina propria*

The lamina propria was usually equal in thickness to the overlying epithelium. It consisted mostly of collagen fibres, loosely packed together, arranged parallel to the epithelial surface in both the coronal and sagittal planes. Immediately deep to the epithelium the collagen fibres were more closely packed and passed into the connective tissue papillae (Fig. 2). Scattered fibroblasts and lymphocytes were present but no fat cells. Fine collagen septae passed from the lowest part for a variable distance into the underlying mucous gland layer.

Under the raised papillae the lamina propria was at least twice as thick as elsewhere as well as more cellular (Fig. 3). These cells mainly comprised scattered lymphocytes and lymphoid follicles lying between whorled bundles of collagen fibres.

(c) *Mucous glands*

Mucous glands comprised approximately 75 per cent of the soft palate and were more numerous in the anterior half where there was little muscle. They were arranged in acini incompletely subdivided into lobules by the thin collagen septae from the oral lamina propria. No mucous glands were seen in the uvula (Fig. 4). The ducts from the glands tended to lie near the septae and passed vertically to the oral epithelial surface.

(d) *Muscle*

Striated muscle formed at least half of the posterior half of the soft palate and most of the uvula (Fig. 4). Other than the uvular muscle which inserted into the posterior edge of the palatal aponeurosis (Fig. 5), the individual named muscles could not be distinguished from each other. A few fat cells were often seen in the region of this muscle insertion.

(e) *Palatal aponeurosis*

The palatal aponeurosis was a thick collagen layer some 60 μm wide which extended from the posterior edge of the hard palate to approximately halfway along the soft palate where, alongside the midline, the uvular muscle inserted into it. It then rapidly thinned and disappeared into the nasal lamina propria.

(f) *Nasal lamina propria*

This layer contained loosely arranged collagen fibres some 180 μm wide. It was more cellular in the posterior half where there was a number of lymphoid follicles (Fig. 6). A few mixed serous and mucous glands were also present.

(g) *Nasal epithelium*

The nasal epithelium was a typical pseudo-stratified columnar

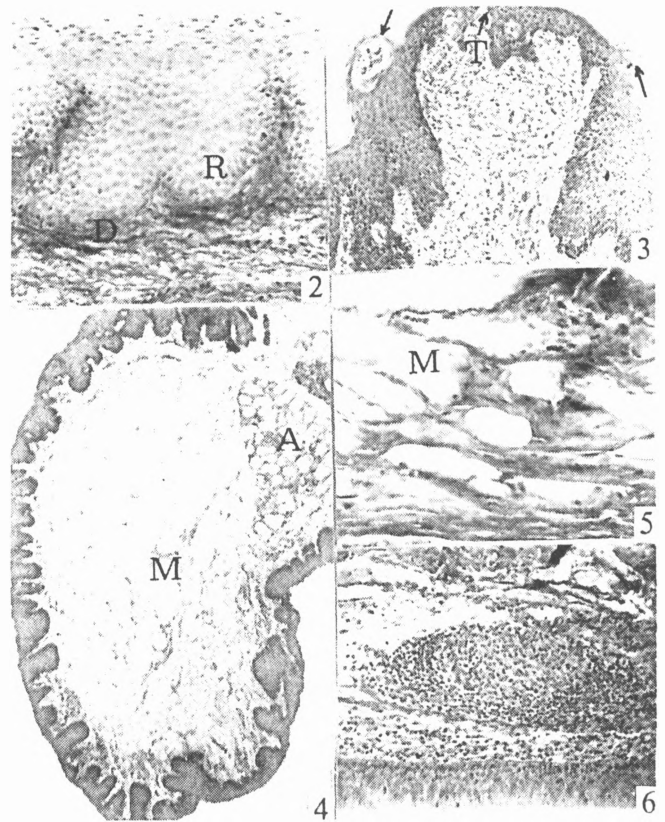


Fig. 2 Nonkeratinized stratified squamous epithelium showing progressive cell flattening towards the surface, connective tissue papillae and rete pegs (R). The dense part of the lamina propria (D) lies under the epithelium and extends into the connective tissue papillae. Below this is the less dense area above the mucous acini. *micro-Mallory x 205.*

Fig. 3 Sagittal section through an epithelial papilla in the anterior half of the soft palate. The epithelium is mainly orthokeratinized but some parakeratinization is present (arrows). Taste buds (T) are present. *H and E x 78.*

Fig. 4 Sagittal section through the uvula which consists mainly of muscle (M). A few mucous acini (A) are present at the base. *H and E x 35.*

Fig. 5 Insertion of striated muscle fibres (M) into the palatal aponeurosis. *micro-Mallory x 300.*

Fig. 6 Lymphoid follicle in the nasal lamina propria. *micro-Mallory x 185.*

epithelium lying on a 6 μm thick basement membrane (Fig. 7). The point of transition from the oral to nasal epithelium (Fig. 8) varied but was always on the nasal aspect of the soft palate.

(h) *Reticulin fibres*

Reticulin fibres were present throughout the soft palate but were concentrated in the oral and nasal lamina propria, particularly in the basement membrane under the epithelia (Fig. 9). There was no difference in the quantity of the fibres under the nonkeratinized and keratinized epithelia.

(i) *Elastic fibres*

In the oral lamina propria there were two groups of elastic fibres. One was just superficial to the glandular layer with the fibres running predominantly transversely (Fig. 10). This layer was constant in thickness from anterior to posterior but thinner in the midline than laterally. Between this broad band and the overlying epithelium was a second group, this time of fine fibres that passed vertically upwards towards the epithelium and into the connective tissue papillae.

The same arrangement was seen under the epithelial papillae but there were only occasional scattered elastic fibres in the uvula. Throughout its course the broad elastic band was

penetrated by mucous ducts, lymphoid follicles and deep rete pegs.

In the glandular and muscular tissue and palatal aponeurosis there were only a few scattered fibres; but deep to the nasal epithelium there was a thin dense elastic lamina (Fig. 11). No oxytalan fibres were seen in any of the regions.

(j) *Keratin histochemistry*

When the D. D. D. reaction of Barnett and Seligman⁸ was carried out for disulphide a weakly positive result was obtained in the keratinized epithelium and a negative result in the nonkeratinized. When performed for both sulphhydryl and disulphide groups a more intense positive reaction was present in the keratinized epithelium and a weakly positive reaction just below the surface of the nonkeratinized epithelium (Fig. 12).

For the ferric ferricyanide reaction a weakly positive result was obtained in the keratinized epithelium.

Thus it was shown that there was little disulphide in the keratinized epithelium but a good deal of sulphhydryl groups, a small quantity of which was also present in the nonkeratinized epithelium.

(h) *Innervation*

This part of the study was limited to the oral epithelium and oral lamina propria. In the lamina propria the main nerve bundles ran just above the glandular layer to form a subepithelial plexus.

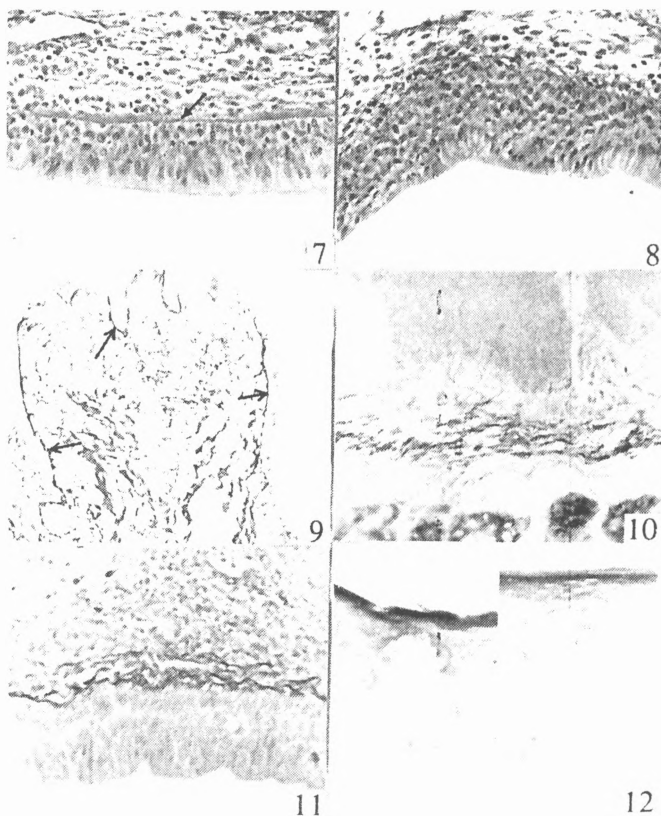


Fig. 7 Ciliated pseudostratified columnar nasal epithelium with a thick basement membrane (arrowed). Picro-Mallory x 300. Fig. 8 Junctional area between the oral epithelium (left) and nasal epithelium (right). Picro-Mallory x 300. Fig. 9 Reticulin fibres in an epithelial papilla. There is a concentration at the basement membrane (arrowed). Gordon and Sweet x 78. Fig. 10 Elastic fibres in the oral lamina propria. Orcein x 350. Fig. 11 Elastic fibres in the nasal lamina propria. Peracetic-aldehyde fuchsin-Halmi x 350. Fig. 12 D. D. D. reaction for sulphhydryl and disulphide. Positive reaction in keratinized epithelium (left) and weakly positive reaction in nonkeratinized epithelium (right). D. D. D. x 210.

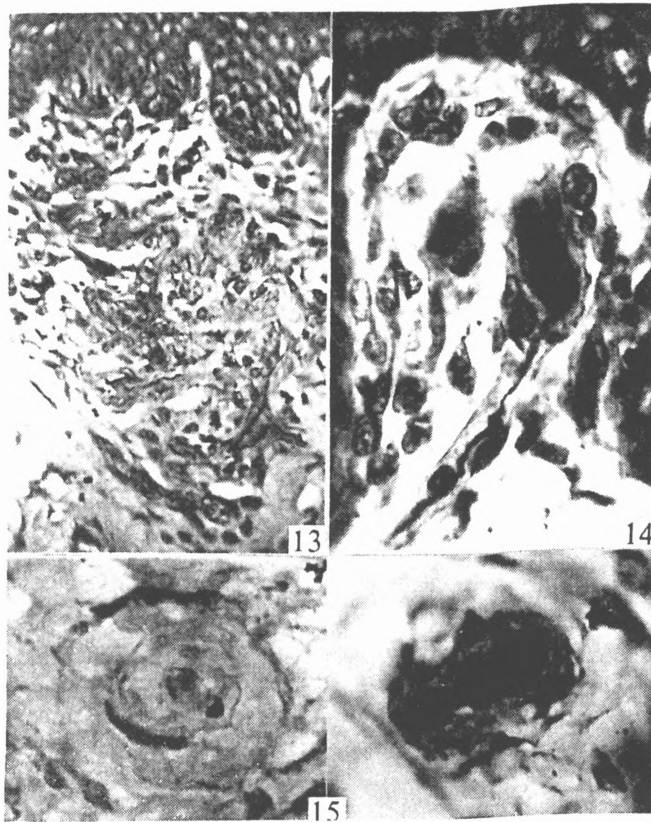


Fig. 13 Plexiform nerve ending beneath an epithelial papilla. Fitzgerald x 560. Fig. 14 Mammalian end organ in a connective tissue papilla. Von Mihalik x 1240. Fig. 15 Pacinian corpuscle (left) and a complex twisted nerve ending (right). Von Mihalik x 1060.

The most striking feature was the large amount of nervous tissue deep to the epithelial papillae. There were many nerve fibres running towards the taste buds, as well as plexiform nerve endings such as that shown in Fig. 13. No nerve fibres could be traced into the taste buds with the techniques used.

Mammalian end organs were seen in the connective tissue papillae of the non keratinized epithelium (Fig. 14) and in the lamina propria. They had an axial nerve. Pacinian corpuscles and complex coiled nerve endings were also occasionally present (Fig. 15). Free nerve endings were uncommon and when present did not penetrate the epithelium further than the stratum basale.

As a whole the nerve endings were more numerous in the anterior half of the soft palate compared to the posterior half; but appeared evenly distributed from side to side.

Discussion

The soft palate in the vervet monkey consists of the same basic layers noticed by Cohen¹⁰ in the *Macaca irus*.

A new finding in this study was the presence of both nonkeratinized epithelium covering most of the soft palate surface and the keratinized stratified squamous epithelium on the surface of the previously unreported, raised, soft palate papillae.

The nonkeratinized stratified squamous epithelium appeared typical of this epithelial variety and seemed similar to the picture shown in Cohen's study in the *Macaca irus*¹⁰. It differed from that seen in human specimens (Cleaton-Jones, unpublished work) by having deeper rete pegs and connective tissue papillae.

Keratinization in the epithelium overlying the papillae was complete as shown by homogeneous staining of the stratum corneum with the Picro-Mallory stain.¹¹ It was mainly orthokeratinized but occasional areas of parakeratinization were

present at the edges of the papillae.

When the epithelia were examined with histochemical stains then further differences were shown between the nonkeratinized and orthokeratinized epithelia but not between the ortho- and parakeratinized varieties. When the D.D.D. reaction of Barnett and Seligman⁸ and ferric-ferricyanide reaction of Chèvremont and Frédéric¹ were determined the keratinization was seen to be of the soft variety and mainly sulphhydryl containing. A small amount of sulphhydryl was also found in the nonkeratinized epithelium but no disulphide was seen. These results were as had been expected.

Numerous taste buds were seen in the soft palate but only in the keratinized epithelium covering the raised papillae in the anterior half. I suggest that the papillae are probably a functional adaptation to assist taste. As taste buds appear almost always to be associated with a keratinized epithelium this possibly explains the presence of the keratinized epithelium on the surface of the taste bud containing papillae.

In the oral lamina propria the most striking features were the large amount under the raised papillae and the presence here too of lymphoid follicles. Although much of this lamina propria consisted of collagen fibres there was a significant amount of nervous tissue which contributed to the increased thickness. Why the lymphoid follicles should only be found under the papillae and not elsewhere in the oral lamina propria is obscure. Fat was absent in contrast to the large amount seen in man but the elastic tissue was fairly similar both in amount and arrangement. The fenestrated membranes reported by Duda and Provenza¹² in man, were also not present in the vervet monkey soft palate.

The glandular tissue forming the bulk of the soft palate was, as had been expected, similar to man including the uvula. In the vervet monkey the uvula is primarily muscular with only a small collection of mucous glands at its base. The septae between the glands however, were incomplete and thin compared to the strong septae seen in man.

Muscle interdigitation was confirmed by the inability to separate the various striated muscle fibres in the mid-soft palate into the named muscles other than the uvular muscle. This could be followed from the uvula to the posterior part of the palatal aponeurosis where its insertion was seen. This differed from that in man in that it was almost tendinous and the muscle fibres did not extend to the hard palate. The only fat seen in the soft palate was in the central area around this insertion.

The nasal lamina propria contained numerous lymphoid follicles which are not seen in man but less nasal glands than in human specimens. These glands were only seen in the posterior third the reason for which is unknown. The nasal epithelium with its thick basement membrane and elastic lamina beneath, was similar to that found in man.

As would be expected by the presence of collagen throughout the soft palate, reticulin was widespread.

The large amount of nervous tissue seen in the lamina propria below the raised papillae appears to be from two sources. A certain proportion of the nerve fibres probably pass to the numerous taste buds but the majority seems composed of plexiform nerve endings similar to those seen by Vij and Kanagasuntheram¹³ in the soft palate of the slow loris. These were only found below the papillae which suggests that in the vervet monkey these papillae may also play a role in sensing touch and pressure.

Winkelmann¹⁴ reported that mammalian end organs are present only in non-primate mammals. However, they have been reported in the lip of the slow loris and in the hard and soft palates of the tree shrew by Vij and Kanagasuntheram.^{13, 15} The finding of mammalian end organs in the present study adds further evidence that they are found in primates.

Ball endings, complex twisted endings and Pacinian corpuscles were similar in structure and situation to those reported by Gairns,¹⁶ Dixon,^{17, 18} and Vij and Kanagasuntheram.^{13, 15} Regarding the Pacinian corpuscles the present study is only the second report of their being present within the soft palate of a mammal.

Free nerve endings were uncommon which is in contrast to their frequent occurrence in the gibbon,¹⁵ rhesus monkey¹⁸ and man;¹⁶ but is similar to the low incidence in *Macaca fascicularis*.¹⁵ Vij and Kanagasuntheram¹⁵ felt that man had retained many primitive characteristics and hence the primitive oral innervation of the gibbon showed a close relationship to man. If this is accepted then the vervet monkey and *Macaca fascicularis* would be less closely related to man.

Finally, the situation of the subepithelial plexus is similar to that seen in the rhesus monkey by Dixon.¹⁹

Summary

In this study the basic histological structure of the soft palate of the vervet monkey has been described and resembles that found in man. Striking features are the presence of taste buds on the surfaces of raised epithelial papillae covered with a keratinized stratified squamous epithelium; the amount of nervous tissue beneath these papillae and histochemical differences between the keratinized and nonkeratinized epithelia.

I thank the Poliomyelitis Research Foundation, especially Dr P. A. D. Winter and Mr C. Brandt, who kindly supplied all the monkey material; and also the skilled technical assistance of Mrs D. Banks, Mrs B. Friedrich, Mrs L. Szal, Miss E. Vieira, and Mrs H. Wilton-Cox.

Received August 1, 1975.

- ¹ Pearse, A. E. E. (1968). *Histochemistry Theoretical and Applied*. 3rd edn. Churchill, London.
- ² Culling, C. F. A. (1963). *Handbook of Histopathological Techniques*. 2nd edn. Butterworths, London.
- ³ Fitzgerald, M. J. T. (1964). The double impregnation silver technique for nerve fibres in paraffin sections. *Q. J. Microsc. Sci.*, 105, 359.
- ⁴ Von Mihalik, P. (1940). Untersuchungen über die Entwicklung des sympathischen Nervensystems. *Anat. Anz.*, 89, 241.
- ⁵ Fullmer, H. M. and Lillie, R. D. (1958). The oxytalan fibre, a previously undescribed connective tissue fibre. *J. Histochem. Cytochem.*, 6, 425.
- ⁶ Fullmer, H. M. (1959). The peracetic orcein Halmi stain: a stain for connective tissues. *Stain Technol.*, 34, 81.
- ⁷ Postgraduate Medical School of London (1952). Department of Pathology. *Morbid Histology Technical Methods*.
- ⁸ Barnett, R. J. and Seligman, A. M. (1952). Demonstration of protein-bound sulphhydryl and disulphide groups by two new histochemical methods. *J. Nat. Cancer Inst.*, 13, 215.
- ⁹ Fitzgerald, M. J. T. (1963). A general-purpose silver technique for peripheral nerve fibres in frozen sections. *Stain Technol.*, 38, 321.
- ¹⁰ Cohen, L. (1967). The histology of the oral mucosa of *Macaca irus*. *Lab. Anim.*, 1, 65.
- ¹¹ Weinmann, J. P., Meyer, J. and Medak, H. (1960). Correlated differences in granular and keratinous layers in the oral mucosa of the mouse. *J. Invest. Derm.*, 34, 423.
- ¹² Duda, M. and Provenza, D. V. (1966). Elastic fibres in the human soft palate. *J. Balt. Coll. Dent. Surg.*, 21, 5.
- ¹³ Vij, S. and Kanagasuntheram, R. (1969). Innervation of oral tissue in some primates. *Folia primat.*, 11, 289.
- ¹⁴ Winkelmann, R. K. (1964). Nerve endings of the North American opossum (*Didelphia virginiana*). A comparison with nerve endings of primates. *Amer. J. Phys. Anthropol.*, 22, 253.
- ¹⁵ Vij, S. and Kanagasuntheram, R. (1970). Sensory nerve terminations in the oral tissues of the tree shrew (*Tupaia glis*) and gibbon (*Hylobates agilis*). *Archs. oral Biol.*, 15, 1047.
- ¹⁶ Gairns, F. W. (1955). The sensory nerve endings of the human palate. *Q. J. Exp. Physiol.*, 40, 40.
- ¹⁷ Dixon, A. D. (1961). Sensory nerve terminations in the oral mucosa. *Archs. oral Biol.*, 5, 105.
- ¹⁸ Dixon, A. D. (1962). The position, incidence and origin of sensory nerve terminations in oral mucous membrane. *Archs. oral Biol.*, 7, 39.
- ¹⁹ Dixon, A. D. (1963). Nerve plexuses in the oral mucosa. *Archs. oral Biol.*, 8, 435.