Kafkas Üniv. Vet. Fak. Derg.
1999, 5(2): 129-134

A HISTOCHEMİCAL STUDY ON THE SALİVARY GLANDS OF THE COTTON-TOP TAMARİNS (Saguinus oedipus oedipus)

Pamuk Tepeli Tamarin (Saguinus oedipus oedipus) Maymunlarının Tükrük Bezleri Üzerine Histokimyasal bir Çalışma

Mahmut SÖZMEN* Peter Jim BROWN**

SUMMARY

A histochemical study was performed on sections of major and minor salivary gland tissues from cotton-top tamarins (Saguinus oedipus oedipus). alcian blue/periodic acid Schiff (AB/PAS) and aldehyde fuchsin/alcian blue (AF/AB) staining techniques were used to demonstrate acid and neutral and sulphated and sialylated mucins respectively.

Staining of acinar and duct cells and ducts was assessed in the different glands. Acini in buccal, palatine, parotid and posterior sublingual glands were seromucous. Mucous acini were present in submandibular gland and anterior part of sublingual glands. Seromucous and serous demilunes were present in submandibular and palatine glands, respectively.

The patterns of histochemical staining are compared with those of other species.

Key Words: Cotton-top tamarin, Mucin, Salivary Gland.

ÖZET

Cotton-top tamarinlerin (Saguinus oedipus oedipus) major ve minor tükrük bezleri üzerinde histokimyasal bir çalışma yapıldı. Asit ve nötral musinler alcian blue/periodic acid Schiff (AB/PAS), sülfatlı ve sialilatlı musinler ise aldehyde fuchsin/alcian blue (AF/AB) ile boyandı.

İncelenen kulak altı, çene altı, dil altı, yanak ve damak tükrük bezlerinde asiner ve kanal hücrelerinin ve kanalların boyanması değerlendirildi. Yanak, damak, kulak altı ve posterior dil altı tükrük bezlerinin serömüköz karakterde olduğu gözlendi. Müköz asini, çene altı tükrük bezlerinde ve dil altı tükrük bezlerinin anterior kısımlarında tespit edildi. Çene altı tükrük bezlerindeki yarım ay asiner hücrelerinin serömüköz, damak tükrük bezlerindeki yarım ay asiner hücrelerinin ise seröz karakterde olduğu tespit edildi.

Tükrük bezlerinin histokimyasal boyanma şekilleri diğer türler ile karşılaştırıldı.

Anahtar Sözcükler: Pamuk tepeli tamarin, Musin, Tükrük bezi.

INTRODUCTION

The cotton-top tamarin (*Saguinus oedipus oedipus*) is an endangered species confined to only a small tract of tropical forests in the northwestern Columbia (1) which is invaluable as a unique model for certain types of medical research (2).

Another small primate, the common marmoset (*Callithrix jacchus*), has also been used as an experimental animal (3). There have been detailed structural studies of various cells and tissues including the gastrointestinal tract (3) of the common marmoset, but no such studies of the cotton-top tamarin.

A range of histochemical staining methods have been developed to differentiate serous or mucous; acidic or neutral and sulphated or non-sulphated mucins and are described by Leppi and Spicer (4). Despite the wide range of methods available (5-9), basically only three main types of mucin can be recognised - sulphomucins, sialomucins and neutral mucins (8).

The present study was undertaken to determine the distribution and histochemistry of different classes of mucosubstances in buccal (molar), palatine, parotid, sublingual and submandibular glands of cotton-top tamarins.

* Kafkas Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı, Kars-TÜRKİYE
** University of Bristol, School of Veterinary Science, Comparative Pathology Laboratory, Langford House, Bristol BS40 7DU-ENGLAND

Kafkas Üniv. Vet. Fak. Derg.
1999, 5(2): 129-134

MATERIALS and METHODS

Animals and Tissues

Salivary gland tissue samples from the molar, palatine, parotid, sublingual and submandibular glands were removed during the course of post-mortem examination from three cotton-top tamarins.

Two animals were female (aged nine months and nine years) the other was a four year old male. In all cases, sodium pentothale euthanasia was performed because of progressive weight loss and/or chronic diarrhoea. In the young female there was mild pneumonia. In both adult animals post-mortem examination revealed chronic colitis and carcinoma of the large intestine; such an association of colitis and neoplasia in the colony has already been described in detail (10). In all cases the salivary glands were macroscopically and microscopically normal.

The tissues were fixed in 10 percent neutral buffered formol saline for 1-2 days at room temperature before being routinely processed and embedded in polywax at 60 °C. Sections were cut at 5 μ thickness and mounted on slides.

Histochemistry

Representative sections were stained with haematoxylin and eosin and in the following ways:

AB/PAS procedure (5)

Sections were immersed in 1% alcian blue (pH2.5) solution for 5 minutes at room temperature; rinsed and treated with 1% periodic acid for 5 minutes; rinsed and exposed to Schiff's reagent for 15 minutes; rhinsed and nuclei stained with Mayer's haematoxylin; dehydrated, cleared in xylene and mounted.

AF/AB procedure (11)

Sections were treated with ripened aldehyde fuchsin solution for 20 min; rinsed in 70% alcohol, then in tap water and treated with alcian blue (1%) solution (pH 2.5) for 5 min; washed in water, dehydrated and mounted.

In all cases, the staining procedures were controlled by using samples of dog colon as positive control tissue.

RESULTS

Histology

Buccal (Molar) salivary glands: The acini consisted of clusters of pyramidal and cuboidal cells. The acinar cell cytoplasm varied in appearance; smaller acini were less vacuolated, with more eosinophilic cytoplasm. Basal nuclei had granular basophilic chromatin and prominent nucleoli.

Aldehyde Fuchsin / Alcian Blue

A granular mixture of AF-AB cytoplasmic staining was confined to some coarsely vacuolated acinar cells. There was similar mixed staining of the apical surface of other acinar cells and of secretion in acinar lumina.

Alcian Blue / PAS

A range of mixed AB-PAS staining occured in some coarsley vacuolated cells surrounding acinar lumen; other cells were unstained. There was similar mixed staining of the apical surface of other acinar cells and of secretion in acinar lumina.

Palatine salivary gland: The acini consisted of clusters of pyramidal cells with basal rounded nuclei, with finely granular chromatin. Acinar cell cytoplasm was clear and vacuolated. In two animals there were also scattered basophilic cells appearing as demilunes. Excretory ducts included numerous goblet cells.

Aldehyde Fuchsin / Alcian Blue

Acinar cells were stained predominantly with alcian blue. In most acini, virtually the entire cell cytoplasm was intensely-stained, with a coarsely-lobulated pattern; faint AF-staining was confined to the periphery of acinar cells. A small number of acinar cells were stained with both AF and AB. Demilune cells were unstained, indicating their serous nature.

Kafkas Üniv. Vet. Fak. Derg.
1999, 5(2): 129-134

Goblet cells observed in excretory ducts were stained mostly with AB. In a single animal, some goblet cells stained purple with AF or exhibited a fine mixture of blue and purple staining.

Alcian Blue / PAS

A complete range of AB and/or PAS staining occurred in different acini (Figure 1). Most acini contained AB-positive cells; many of the cells were also stained with PAS. A few cells were stained with PAS alone; the latter were present particularly at the periphery of acini. Most duct epithelial cells stained with PAS. Goblet cells in the excretory ducts were stained with AB, as was the duct luminal content.

Parotid salivary gland: The parotid salivary gland consisted of lobules of pyramidal epithelial cells with basal nuclei which were large, and rounded, with finely-granular chromatin. The acinar cell cytoplasm was clear and vacuolated, speckled with dense basophilic granules. Ducts were lined by columnar cells with pale, eosinophilic cytoplasm. Nuclei were located in the centre of the cells and were rounded, with finely granular chromatin.

Aldehyde Fuchsin/Alcian Blue

The parotid salivary gland tissue was generally poorly-stained. There was no AF-positive staining in acinar or duct cells. Alcianophilia was confined to a few coarser granules in larger acinar cells and duct epithelial cells.

Alcian Blue / PAS

In acinar epithelial cells there were many fine granules of AB-positive staining and some larger PAS positive granules. Some duct epithelial cells contained a few small PAS-positive granules as well as more diffuse cytoplasmic staining and staining of luminal contents.

Sublingual salivary gland: Acini consisted of groups of cuboidal and pyramidal cells with basal nuclei. Acinar cell cytoplasm varied in appearance in anterior and posterior parts of the gland. Anterior gland cells were typical of serous cells, with small amounts of finely granular basophilic cytoplasm; posterior gland cells had larger amounts of cytoplasm containing many large, basophilic apical granules and resembled mucous cells.

Aldehyde Fuchsin / Alcian Blue

In mucous acinar cells in the posterior gland tissue, there was moderate AB staining of some larger apical granules; some cells showed AF positivity of the peripheral cytoplasm.

Fine AF positive granules were also present in serous cells in the anterior glands. There were also some smaller, scattered AB positive cytoplasmic granules. Staining of ducts was confined to AF-positivity of the luminal third of the epithelial cells.

Alcian Blue /PAS

Anterior gland serous cells contained PAS-positive cytoplasm; the size and number of cytoplasmic granules varied. A few of the larger mucous cells present in the posterior glands contained large cytoplasmic granules which stained with either AB-positive (Figure 2) or stained with both AB and PAS. Staining of duct luminal contents varied in anterior and posterior glands and reflected the difference in AB and PAS staining of secretory tissue.

Submandibular salivary gland: Acini consisted of a mixture of mucous and serous cells. Mucous acinar cells were cuboidal and pyramidal, with basal nuclei and cytoplasm containing dense basophilic granules. Serous acinar cells were pyramidal cells present at the periphery of mucous acini; they had basal nuclei which were large and rounded with finely granular chromatin and abundant pale cytoplasm.

Aldehyde Fuchsin / Alcian Blue

Alcianophilia was confined to clusters of mucous acinar cells and slight staining of the luminal surface of ducts. Serous demilunar cells were weakly stained with AF.

Alcian Blue / PAS

AB-staining was confined to mucous acinar cells. Peripheral demilunar cells contained fine

Kafkas Üniv. Vet. Fak. Derg.
1999, 5(2): 129-134

PAS-positive granules (Figure 3). Some duct epithelial cells contained a few PAS-positive granules and there was diffuse PAS-positive staining of the apical cytoplasm; there was some AB-positive staining of luminal secretion.

DISCUSSION

The histological structure of salivary gland has been studied in various species including ruminants (12), human (6), carnivores (including cats and dogs) (13), rodents (14) and primates (*Saimiri sciureus* and *Macaca mulatta*) (4). Primate salivary glands have been subjected to a series of investigations including histochemistry (4,15) and ultrastructure (16-20). The tamarin salivary glands examined in this study contained a variety of histochemically demonstrable neutral glycoconjugates, sulphated and sialylated glycoconjugates according to the classification of Spicer et al (7) and Leppi and Spicer (4).

Most histological studies of salivary glands have investigated the submandibular and parotid major salivary glands (14,15). The submandibular glands in most mammals contain both serous and mucous cells; seromucous acini have been observed in Rhesus monkeys (*M.mulatta*) (13) and Squirrel monkeys (*Saimiri sciureus*) (4,16). In the cotton-top tamarins described here, submandibular, palatal and posterior sublingual gland acinar secretory cells were mucous in character with sialomucin predominating. Sialomucins were also detected in the tamarins by lectin-binding (Sozmen and Brown, unpublished) and sialic acid containing mucins also predominated in Philippine macaques (*Macaca irus*) (15,21) and rhesus and squirrel monkeys (4). In contrast, guinea pig and giant rat submandibular glands contain only serous acini (22). Demilunar cells in the tamarin submandibular gland were seromucous. Seromucous demilunes have also been detected in submandibular glands of squirrel monkeys (*Saimiri sciureus*) (4,16), Rhesus monkeys (*Macaca mulatta*) (13), Mindanao tarsier (*Tarsius syrichta*) (23) and other macaques (*M. cyclopis* and *M. fuscata*) (24), while submandibular demilunes are composed of serous cells in common marmosets (*Callithrix jacchus*) (25) and Philippine macaques (*Macaca irus*) (15,21).

The mixed seromucous character of the parotid gland of cotton-top tamarins described here is similar to that observed in squirrel monkeys (*Saimiri sciureus*) (17) and marmosets (*Callithrix jacchus* and *C. penicillata)* (25,26). In contrast, predominantly serous acini have been reported in parotid glands in humans (6,27) and in some other primate species including macaques (*Macaca irus*) (21), spider monkey (*Ateles paniscus*) (28), squirrel monkey (*Saimiri sciureus*) (17), and olive baboon (*Papio anubis*) (29). In a parallel lectin-binding histochemical study the parotid gland contained sialic acid (Sozmen and Brown, unpublished). The failure to demonstrate sialic acid by AF/AB histochemical staining reported here, may relate to interference with binding of cationic dyes due to masking by protein (30); a similar discrepancy has been described in humans (31).

In both rhesus and squirrel monkeys, sialic acid-containing mucins were present in sublingual glands as well as in submandibular glands. Rhesus monkey sublingual glands consist of two different type of acinar cells (mucous in the anterior part and seromucous in the posterior region) whereas squirrel monkey sublingual gland contained only mucous acini (4). Two distinct secretory patterns were present in the cotton-top tamarin sublingual glands; serous cells were mainly present anteriorly whereas mucous cells predominated in the posterior part of gland. The variation in the secretory end pieces observed in rhesus monkey (*Macaca mulatta*) sublingual glands relates to the presence of demilune cells in posterior lobules (8). In the cotton-tamarins described here, anterior and posterior gland tissue did not contain demilune cells. In contrast orangutan (*Pongo pygmaeus*) sublingual glands contained only large mucous acinar cells (8).

Salivary glands other than the submandibular and parotid have received less attention. Minor salivary glands secrete saliva spontaneously even in the absence of nervous stimulation (32) and variation in histochemical staining might reflect asynchrony of secretory activity of a population of similar secretory cells. There have been only of few reports of seromucous secretory tissue in minor salivary glands in various domestic animals (33), including ruminants (12) and in human (9).

Kafkas Üniv. Vet. Fak. Derg.
1999, 5(2): 129-134

In relation to palatal gland mucopolysaccharide secretion, differences exist between primates. Some primate palatal glands (*M. irus, M. mulatta, M. fasciculatta*) (34) have been considered as mucous whereas tufted capuchine monkey palatine glands (*Cebus apella*) were serous in nature (20). Cotton-top tamarin palatine salivary glands were seromucous in character, containing a mixture of neutral and acidic, sulphomucin and sialomucin; demilunar cells were predominantly serous.

Duct epithelial staining varied in different tamarin glands. Striated ducts were present in all glands except sublingual glands; a lack of striated ducts in sublingual glands was also observed in cats (35). In the excretory ducts of tamarin palatine glands, goblet cells were present but were absent in other glands. The goblet cells contribute glycoproteins to the saliva that is produced by that particular gland and similar goblet cells have been observed in excretory ducts in submandibular glands in Japanese monkey (*Macaca fuscata*) (19) and rhesus monkey (*Macaca mulatta*) (30), in parotid glands of rhesus monkeys (36) and in sublingual glands of crab-eating monkeys (*Macaca fascicularis*) and African green monkeys (*Cercopithecus aethiops*) (15).

ACKNOWLEDGEMENTS

Mahmut Sozmen was supported by a grant form the Turkish Higher Educational Council.

REFERENCES

1. Hernandes-Camacho J, Cooper RW: The nonhuman primates of columbia. In: Thorington RW, Heltne PG (eds): Neotropical Primates: Field studies and conservation. National Academy of Science, Washington D.C. 35-69, 1976.
2. Epstein MA: A prototype vaccine to prevent Epstein-Barr virus-induced tumours. Proceedings of the Royal ociety of London B, 221: 1-20, 1984.
3. Jablonski O, Gossrau R: Ultrastructure of the gastrointestinal epithelium in the marmoset (Callithrix jaccus). Zeitschrift fur Mikroskopisch-Anatomische forschung, 103: 190-220, 1989.
4. Leppi TJ, Spicer SS: The histochemistry of mucins in certain primate salivary glands. American Journal of Anatomy, 118: 833-859, 1966.
5. Mowry RW: Alcian blue techniques for the histochemical study of acidic carbohydrates. Journal of Histochemistry and Cytochemistry, 16: 530-539, 1956.
6. Harrison JD, Auger DW, Paterson KI, Rowley PSA: Mucin histochemistry of submandibular and parotid salivary glands of man: Light and electron microscopy. Histochemical Journal, 19: 555-564, 1987.
7. Spicer SS, Leppi TJ, Stoward PJ: Suggestions for a histochemical terminology of carbohydrate-rich tissue components. Journal of Histochemistry Cytochemistry, 13: 559-603, 1965.
8. Pinkstaff CA: The cytology of salivary glands. International Review of Cytology, 63: 141-261, 1980.
9. Eversole LR: The histochemitry of mucosubstances in human minor salivary glands. Archives of Oral Biology, 17: 1225-1239, 1972.
10. Kirkwood JC, Pearson GR, Epstein MA: Adenocarcinoma of the large bowel and colitis in captive cotton top tamarins (Saquinus o. oedipus). Journal of Comparative Pathology, 96: 507-515,1986.
11. Spicer SS, Meyer DB: Histochemical differentation of acid mucopolysaccharides by mean of combined aldehyde fuchsin-alcian blue staining. American Journal of Clinical Pathology, 33: 453-460, 1960.
12.. Parida S, Das LN: Histochemical characterization of the minor salivary glands of domestic ruminants. Indian Journal of Veterinary Anatomy, 4: 1-8, 1992.
13. Shackleford JM, Wilborn WH: Structural and histochemical diversity in mammalian salivary glands. Alabama Journal of Medical Science, 5: 180-203, 1968.
14. Munhoz COG: Histochemical classification of acini and ducts of parotid glands from artiodactyles, carnivores and rodents. Acta Histochemica, 39: 302-317, 1971.
15. Jacobsen N, Form SH: Mucous and serous components of the submandibular and sublingual glands of Macaca irus and Cercopithecus aethiops. Acta Histochemica, 46: 281-287, 1973.
16. Cowley LH, Shackleford JM: An ultrastructural study of the submandibular glands of the squirrel monkey, Saimiri sciureus. Journal of Morphology, 132:117-135, 1970.
17. Cowley LH, Shackleford JM: Electron microscopy of squirrel monkey parotid gland. Alabama Journal of Medical Science, 7: 273-282, 1970.
18. Ichikawa M, Ichikawa A: light and electron microscopic histochemistry of the serous secretory granules in the salivary glandular cells of the mongolian gerbil (Mongolian meridianus) and Rhesus monkey (Macaca irus). Anatomical Record, 189: 125-139, 1977.
19. Sato A, Miyoshi S, Toh H, Ohmori T: Fine structure of the duct systems in the japanese monkey, Macaca fuscata, submandibular gland. Japanese Journal of Oral Biology, 27: 86-95, 1985.
20. Watanabe L, König Jr B: Ultrastrusture of palatine salivary glands of the tufted capuchine monkey (Cebus apella linnaeus, 1758). Zeistschift fur Mikroskopiseh-Anatomische Fosrchung, 102: 590-597, 1988.
21. Clemencki J, Zajac S: Structure and topography of the great salivary glands in Macacus cynomolgus. Folia Morphologica (Warszawa), 27: 91-96, 1968.
22. Asojo TA, Aire TA: Microstereological and histochemical studies of the salivary glands of the giant rat (Cricetomys gambianus, waterhouse). Acta Anatomica, 117: 65-72, 19863.
23. Smith AA: Major salivary glands of the philippine tarsier. Folia primatologica, 10: 113-130, 1969.
24. Kagayama M: The fine structure of the monkey submandibular gland with a special reference to intra-acinar nerve endings. American Journal of Anatomy, 131: 185-195, 1971.
25. De Araujo Sacramento N, Miraglia T: Structure and distribution of polysaccharides in the salivary glands of the common marmoset (Callithrix jacchus). Acta Anatomica, 82: 368-382, 1972.

26. Miraglia T, Moura CS, Neves HB: Histochemical data on the parotid glands of the marmosets, Callithrix jacchus and Callithrix penicillata. Acta Anatomica, 87: 334-344, 1974.
27. Munger BL: Histochemical studies on seromucous and mucous secreting cells of human salivary glands. American Journal of Anatomy, 115: 411-430, 1964.
28. Leeson CR: The fine structure of the parotid gland of the spider monkey (Ateles paniscus). Acta Anatomica, 72: 133-147, 1969.
29. Tandler B, Erlandson RA: Ultrastructure of baboon parotid gland. Anatomical Record, 184: 115-132, 1976.
30. Scott JE, Dorling J: Differential staining of acid glycosaminoglycans (Mucopolysaccharides) by alcian blue in salt solutions. Histochemie, 5: 221-233, 1965.
31. Mandel ID: Human submaxillary, sublingual and parotid glycoproteins and enamel pellicle. In: Horowitz MI, Pigman W (Eds): The glycoconjugates, Vol. I: Mammalian glycoproteins and glycolipids. Academic Press, London, 153-179, 1977.
32. Emmelin N: Nervous control of salivary glands. In: Handbook of physiology. Section 6: Alimentary canal, Vol II: Secretion, American Physiological Society, Washington D.C., 595-632, 1967.
33. Stinson AW, Calhoun ML: Digestive system. In: Dellmann HD, Brown EM (Eds): Textbook of Veterinar Histology. 3th ed. Lea and Febiger, Philadelphia,209-263, 1987.
34. Klein PB, Weilemann WA, Schroeder HE: Structure of the soft palate and composition of the oral mucous membrane in monkeys. Anat Embryol, 156: 197-215, 1979.
35. Tandler B, Poulsen JH: Ultrastructure of the cat sublingual gland. Anatomical Record, 187: 153-172, 1977.
36. Shackleford JM Klapper CE: Structure and carbohydrate histochemistry of mammalian salivary glands. American Journal of Anatomy, 111: 25-47, 1962.

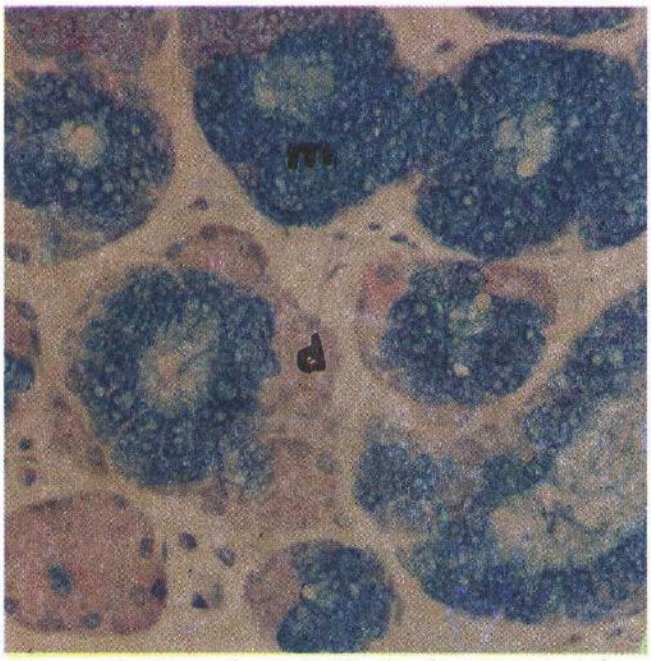

Figure 1. Tamarin palatal gland: mucous acinar cells (m) are densely stained (with AB). Serous demilunar (d) cells are also stained (with PAS). (AB/PAS X 400).
Resim 1. Tamarin damak tükrük bezi: Müköz asiner hücrelerde (m) koyu boyanma (AB). Seröz yarım ay hücrelerinde (d) açık renkte boyanma (PAS). (AB/PAS x 400)

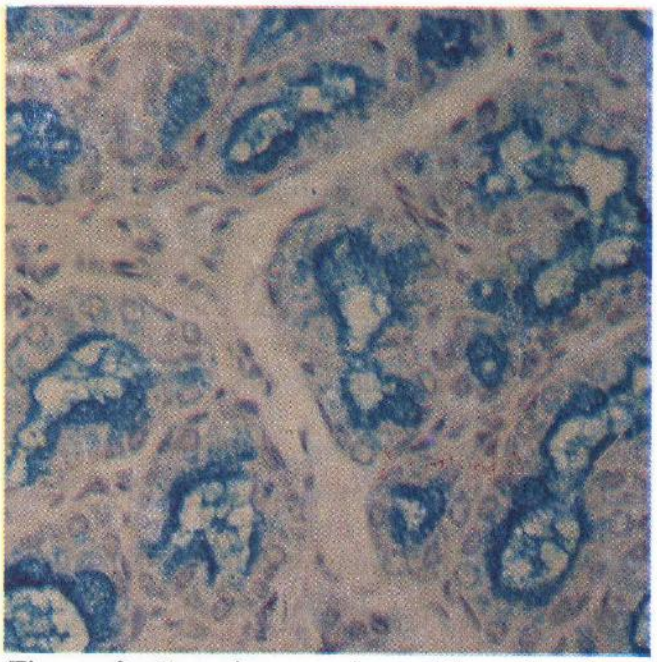

Figure 2. Tamarin posterior sublingual salivary gland: staining (AB-positive) is visible only of the luminal aspect and content of acinar cells. (AB/PAS X 400).
Resim 2. Tamarin ön dilaltı tükrük bezi: AB-pozitif boyanma asiner hücrelerin yalnızca luminal yüzeyinde ve lumen içeriğinde mevcut. (AB/PAS x 400)

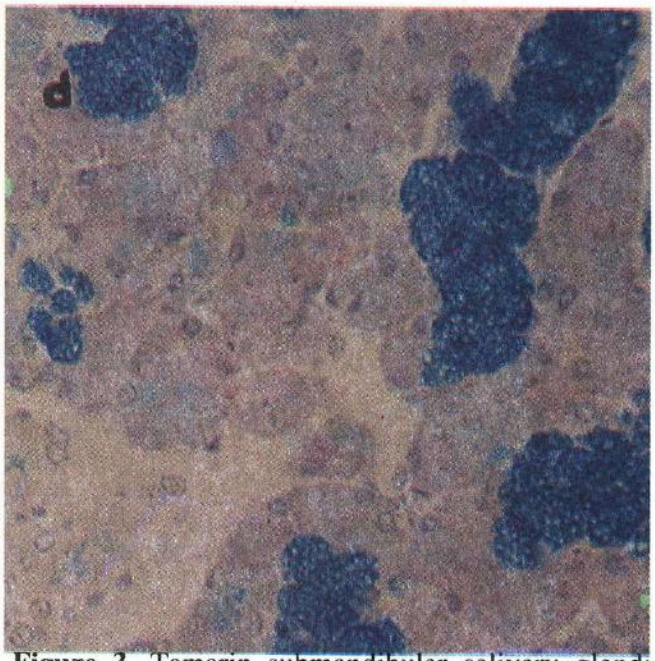

Figure 3. Tamarin submandibular salivary gland: mucous acinar cells are densely stained (with AB). Serous demilunar (d) and acinar cells contain fine PAS positive granules. PAS positive staining is also present of luminal aspect of striated ducts. (AB/PAS X 400).
Resim 3. Tamarin çenealtı tükrük bezi: Müköz asiner hücrelerde koyu boyanma (AB). Seröz yarımay (d) ve asiner hücrelerde PAS ile granüler tarzda boyanma. Çizgili kanalların luminal yüzeylerinde PAS pozitif boyanma (AB/PAS x400).