Title:

Biological behavior of myoepithelial cells in the regeneration of rat atrophied sublingual glands following release from duct ligation

Authors:

S. Takahashi[1,*], T. Kohgo[2], S. Nakamura[3], A. K. S. Arambawatta[1], T. Domon[1], T. Yamamoto[1], & M. Wakita[1]

Affiliations of authors:

[1]Department of Oral Health Science, Hokkaido University Graduate School of Dental Medicine, Sapporo, 060-8586, Japan

[2]Department of Oral Pathobiological Science, Hokkaido University Graduate School of Dental Medicine, Sapporo, 060-8586, Japan

[3]Department of Oral Physiology, Showa University School of Dentistry, Tokyo, 142-8555, Japan

Short Title:

Myoepithelial cells in sublingual gland regeneration of the rat

Summary

The present study aimed to clarify how myoepithelial cells behave during regeneration of an atrophied sublingual gland by investigating cell proliferation and ultrastructure. Atrophy of rat sublingual glands was induced by unilateral ligation of the excretory duct near the hilum with metal clips, which were then removed after one week of ligation for regeneration. The sublingual glands 0 to 14 days after unligation were examined with single immunohistochemistry for actin as a marker of myoepithelial cells, double immunohistochemistry for actin and proliferating cell nuclear antigen (PCNA) as a marker of proliferating cells, and transmission electron microscopy (TEM). The single immunohistochemistry and TEM showed that myoepithelial cells surrounded residual ducts in the atrophied glands and immature and mature acini in the regenerating glands. Although PCNA-positive myoepithelial cells were identified during regeneration, PCNA labeling indices of myoepithelial cells were low at all time points except at day 7. Ultrastructurally, myoepithelial cells showing bizarre shaped structures in the atrophy changed with maturation of differentiating acinar cells and appeared normal in the regenerated glands. There was no differentiation of the remaining duct cells to myoepithelial cells. These observations suggest that proliferation of myoepithelial cells and differentiation to myoepithelial cells do not commonly participate in the regeneration of atrophied sublingual glands and that the bizarre shaped myoepithelial cells in the atrophied sublingual glands recover the original shapes with acinar cell regeneration.

Introduction

Numerous studies have investigated the atrophy and regeneration of salivary glands, as it is both important and interesting to understand this from clinical aspects in oral medicine. According to previous reports, duct cells increase and acinar cells disappear in atrophic salivary glands (Walker & Gobé 1987, Burford-Mason et al. 1993, Cummins et al. 1994, Burgess & Dardick 1998, Scott et al. 1999, Takahashi et al. 2000, 2002). In the regeneration, acinar cells differentiate from duct cells remaining in the atrophied glandular tissue and then actively proliferate (Burford-Mason et al. 1993, Cummins et al. 1994, Takahashi et al. 2004a, 2005). Although these phenomena have all been observed in the three major salivary glands, there are a number of differences among the three glands. For instance, most acinar cells disappear by apoptotic cell death in the atrophy of parotid glands (Walker & Gobé 1987, Scott et al. 1999, Ihrler et al. 2004) and submandibular glands (Takahashi et al. 2000) but by de-differentiation from acinar to duct cells and necrosis in addition to apoptosis in sublingual gland atrophy (Takahashi et al. 2002). In the regenerative process of parotid (Scott et al. 1999) and submandibular glands (Takahashi et al. 2004a), apoptosis occurs rarely, like in the normally functioning glands, however apoptosis actively participates in sublingual gland regeneration, remodeling the regenerated glandular tissue (Takahashi et al. 2005). These suggest that care must be shown when applying the results obtained from one salivary gland to other salivary glands.

Many data concerning the behavior of acinar and duct cells in the atrophy and

regeneration have been reported, but myoepithelial cells have not been examined much because of the difficulties in identifying them histologically (Tandler et al. 1970, Redman & Ball 1979, Redman et al. 1980). Previous investigations using transmission electron microscopy (TEM) showed that myoepithelial cells remaining in the atrophic parotid and submandibular glands commonly take on bizarre appearances with cell processes extending to the interstitial spaces and redundant folds of basal lamina (Emmelin et al. 1974, Garrett & Emmelin 1979, Takahashi et al 2001). The changes in the appearance of myoepithelial cells in sublingual gland atrophy and regeneration have not been reported.

Formerly, myoepithelial cells were understood to be unable to proliferate mitotically due to the terminally differentiated cell state (Batsakis et al. 1989). However, using double immunohistochemistry with actin and proliferating cell nuclear antigen (PCNA) Burgess et al. (1996) found that myoepithelial cells are able to proliferate in the atrophy and regeneration of parotid glands, and the proliferative activity of myoepithelial cells has also been identified in atrophic (Takahashi et al. 2001) and regenerative (Takahashi et al. 2004b) submandibular glands and in atrophic sublingual glands (Takahashi et al. 2003). There were differences in the degree of proliferative activity of myoepithelial cells among these cases, and it still remains unclear how myoepithelial cells proliferate in the regeneration of sublingual glands.

As described above, the behavior of myoepithelial cells during regeneration of sublingual glands is little understood. The aim of the present investigation was to elucidate how myoepithelial cells behave during the regeneration of the atrophied sublingual gland especially paying attention to cell proliferation and ultrastructure. To achieve this,

myoepithelial cells were examined during regeneration of the atrophied rat sublingual gland following release from duct ligation with double immunohistochemistry of actin and PCNA, and TEM.

Materials and Methods

Animals

Seven-week-old male Wistar rats, weighing 190-220g, were provided (Hokudo Co., Sapporo, Japan) and housed at the Animal Facility of Hokkaido University Graduate School of Dental Medicine during the experimental period. The experimental protocol was approved by the Animal Experimental Committee and carried out in accordance with the Guide for the Care and Use of Laboratory Animals of Hokkaido University Graduate School of Dental Medicine.

Ligation and release of the excretory duct

All operations were performed under general anesthesia with inhalation of ether. In the experimental rats, the right sublingual gland and its excretory duct were dissected through a ventral incision in the neck. The right excretory duct was doubly clipped with Ligaclips (Ethicon Endo-Surgery Inc., Cincinnati, OH) near the hilum to induce gland atrophy, and the skin closed with silk sutures. The atrophy of the sublingual glands was caused by duct obstruction and damage to the corda tympani running on the excretory duct in the rodents (Harrison & Garrett 1972, Harrison et al. 2001). After one week of ligation, the clips were removed under the same operative procedures. The experimental animals were killed at 0, 1, 2, 3, 4, 5, 7, 10 or 14 days after removal of the ligation.

Four animals which were not subjected to any of the operations were used as controls.

Immunohistochemistry

The experimental animals at each time point and the control animals were killed with deep inhalation of ether. The fresh sublingual glands were immediately removed, embedded in OCT compound (Miles Scientific, Naperville, IL), and frozen in liquid nitrogen. The 5 μ m thick frozen sections made with a cryostat were fixed in 4% paraformaldehyde for 2 min and then immersed in 0.3% hydrogen peroxide to inhibit endogenous peroxidase. The sections were reacted with anti-PCNA mouse monoclonal antibody, PC-10 (DakoCytomation, Kyoto, Japan) as a marker for the proliferating cells (Miyachi et al. 1978), biotinylated anti-mouse rabbit polyclonal antibody (DakoCytomation, Kyoto, Japan), and streptavidin-biotin horseradish peroxidase complex (HRP) (DakoCytomation, Kyoto, Japan) in turn. The immunoreaction of PCNA was developed with 3,3'-diaminobenzidine as brown nuclei. Then, the sections were incubated with HRP-labeled anti-alpha smooth muscle actin mouse monoclonal antibody, 1A4 (DakoCytomation, Kyoto, Japan) as a marker of myoepithelial cells (Norberg et al. 1992, Takahashi et al. 1997). The immunoreaction of actin was visualized with a VIP Substrate kit (Vector Laboratories, Burlingame, CA) as purple cytoplasm. After immunohistochemical staining, the sections were lightly counterstained with hematoxylin.

Single immunochemistry for actin was also carried out, omitting the part with PCNA from the above immunohistochemical double staining.

Normal mouse serum was substituted for both primary antibodies as a negative control.

Calculation of the PCNA labeling index in myoepithelial cells

Four experimental rats at each time point and the four control rats were used for the calculations of the PCNA labeling index in the myoepithelial cells. Five sections, doubly immunostained with PCNA and actin were randomly selected for each animal. Approximately 200 myoepithelial cells were observed at a magnification of x400 (BH-2, Olympus, Tokyo, Japan) and the percentage of double-positive cells were calculated in each section. The average of the percentages of the five sections was used as the labeling index for that animal, and then the mean and standard error of the mean (SEM) were calculated for the four experimental animals at each time point and for the four controls. These data were analyzed statistically by one-way ANOVA, followed by the Fisher's protected least significant difference (PLSD) post hoc test, using StatView 4.5 statistical software (Abacus Concepts, Berkeley, CA). Values of $P<0.05$ were considered statistically different.

Transmission electron microscopy

The experimental animals at each time point and the control animals were anesthetized with sodium pentobarbital given by intraperitoneal injection at a dose of 40 mg/kg body weight. They were perfused with 2% paraformaldehyde-1.25% glutaraldehyde buffered at pH7.4 with 0.05M sodium cacodylate from the ascending aorta through the heart for 15 min. The right sublingual glands were collected and immersed in the same fixative following perfusion. Then the tissue was post-fixed in 1% osmium tetroxide and embedded in Epon 812. The ultrathin

sections were made with an ultramicrotome and stained with uranyl acetate and lead citrate. The prepared sections were observed with a transmission electron microscope (H-7000 electron microscope, HITACHI, Tokyo, Japan).

Results

Details of the histological changes during regeneration of sublingual glands following release from duct ligation have been reported previously (Takahashi et al. 2005). In summary, the experimental sublingual glands showed marked atrophy after one week of ligation (day 0, the day of removal of clips). Most acini had disappeared and many ducts remained. At day 3, newly differentiating immature acini had appeared. Thereafter the acini matured and increased in number, while the number of ducts decreased. After 10 days, the histology of the experimental sublingual glands was indistinguishable from that of the controls.

Immunohistochemical observations

At day 0, the single immunohistochemical staining for actin showed that in the atrophic sublingual glands there were many myoepithelial cells surrounding the remaining ducts except at some ducts where there were no myoepithelial cells (Figure 1A). The immature acini differentiating from the residual ducts and maturing acini were also surrounded by myoepithelial cells (Figure 1B). After 10 days, the myoepithelial cell distribution in the experimental sublingual glands were quite similar to that in the control glands; myoepithelial cells were identified around acini and intercalated ducts, but not around striated and interlobular ducts (Figure 1C).

In the double immunohistochemistry, positive reactions for PCNA and for actin were recognized as brown nuclei and purple cytoplasm, respectively. The PCNA-positive

myoepithelial cells were identified at the periphery of ducts (Figure1D), immature acini (Figure1E) and mature acini (Figure1F) at every time interval during regeneration. In the controls, PCNA-positive myoepithelial cells were also identified and the PCNA labeling index of myoepithelial cells in the control glands was 0.93%. Figure 2 and Table 1 demonstrate the change in the PCNA labeling indices of myoepithelial cells. The highest labeling index was 2.09% at day 7 and the lowest was 0.58% at day 1. Only the labeling index at day 7 showed a statistically significant difference from that of the control ($p=0.008$). During regeneration, the index of myoepithelial cells were lower than that of acinar cells, which was reported previously (Takahashi et al. 2005).

Negative control sections for single and double immunohistochemical staining showed no positive reactions.

Ultrastructural observations

In the atrophic sublingual glands at day 0, myoepithelial cells were oval and often extended small cell processes of bizarre appearances into the interstitial spaces. The basal lamina tended to be arranged in many irregular folds (Figure 3A). In the regenerating sublingual glands, myoepithelial cells at the periphery of transitional duct-acinar structures showed an oval shape. However, cell processes into the interstitial spaces and irregular folds of the basal lamina were few. The basal lamina was along the basal cell membrane of the myoepithelium (Figure 3B). Myoepithelial cells around immature acini were generally flatter (Figure 3C), and the myoepithelial cells extending long and thin cell processes embracing acinar cells at

the periphery of mature acini were extremely flat (Figure 3D). Myoepithelial cells around the ducts recovered their normal shapes more rapidly according as the differentiation to intercalated ducts progressed. Therefore, myoepithelial cells around ducts tended to be more normal than those around immature acini at the same time point during regeneration.

During the regeneration of sublingual glands, the remaining ducts and newly differentiating acini were commonly surrounded by myoepithelial cells except at some ducts where this was not the case. And transformation that the undifferentiated peripheral cells of ducts gradually acquired the characteristics of myoepithelial cells such as myofilaments, dense bodies, and caveolae was not identified.

No apoptosis of myoepithelial cell was ultrastructurally observed in the regenerating sublingual glands.

Discussion

The present study used PCNA for immunohistochemical identification of proliferating cells, since the species specificity of PCNA is very low and as there is no need for a pre-treatment which often makes immunohistochemical double staining difficult. It is known that PCNA expression is involved both in cell proliferation and also in DNA repair (McCormick & Hall 1992). However, Burford-Mason et al. (1993) noted that DNA repair is not a significant factor and that PCNA-positive cells reflect proliferating cells in the atrophy and regeneration of salivary glands. In addition, many mitotic figures as well as PCNA-positive cells in the parenchyma have been observed in the experimental model used in the present study (Takahashi et al. 2005). This makes it allowable to conclude that PCNA is an appropriate marker for proliferating cells in the present study.

During the regeneration of sublingual glands, the PCNA labeling indices for myoepithelial cells except for those at day 7 were around 1% and were not show statistically significantly different from the controls. It has been also reported that myoepithelial cells in the atrophy of sublingual glands do not proliferate actively and that their proliferative activity maintains the level of the controls during atrophy (Takahashi et al. 2003). These facts suggest that the proliferative activity of myoepithelial cells in the sublingual gland is unchanged and stays low under a variety of pathological circumstances. According to Burgess et al. (1996), observing the experimental atrophy and regeneration of the rat parotid gland, the proliferative activity of myoepithelial cells in atrophy is very high, however in regeneration it shows a

level similar to the controls. In the rat submandibular gland, myoepithelial cells both in atrophy and regeneration proliferate more actively than in normal glands (Takahashi et al. 2001, 2004b). The previous reports above and the present study show that the three major salivary glands differ in the proliferative activity of myoepithelial cells under the induced pathological conditions. Since myoepithelial cells participate in the histogenesis of several kinds of salivary gland tumors (Batsakis et al. 1983, Dardick & Nostrand 1987, Dardick & Burford-Mason 1993, Redman 1994), it is possible to consider that the differences in proliferative activity of myoepithelial cells among the three major salivary glands are related to the differences in the frequencies and in the types of tumors of the salivary glands developing in the three glands. It has been speculated that the differences in the proliferative activity of myoepithelial cells originate in different distributions of myoepithelial cells (Takahashi et al. 2001, 2004b) or in different mechanisms of acinar cell deletion in the gland atrophy (Takahashi et al. 2003). However, these two hypotheses are insufficient to explain the differences fully and there may be additional factors. It is difficult to discuss this problem with the data in the present study and a further study will be needed.

In the developing salivary glands, the myoepithelial cells arise from the outer cells of end-buds and adjacent ducts (Cutler & Chaudhry 1973, Redman & Ball 1979, Redman et al. 1980) and are considered to be of epithelial origin because of the fact that myoepithelial cells are always observed within the basal lamina (Garrett & Emmelin 1979). In the regeneration of salivary glands after partial damage, newly regenerating myoepithelial cells also arise from the peripheral cells of the epithelial cell clusters and ducts (Hanks & Chaudhry 1971,

Takahashi et al. 1997). Acquiring the characteristics of myoepithelium such as myofilaments, dense bodies, caveolae and others, the undifferentiated peripheral cells differentiate to myoepithelial cells in development (Cutler & Chaudhry 1973, Redman & Ball 1979, Redman et al. 1980) and regeneration (Hanks & Chaudhry 1971, Takahashi et al. 1997). Therefore, the peripheral region of the remaining ducts and immature acini were observed attentively with TEM in the present study. However, the remaining ducts and immature acini were commonly surrounded by mature myoepithelial cells, and there were no undifferentiated cells at their periphery. No differentiation from peripheral cells to myoepithelial cells with the above transformation was identified. Further the number of proliferating myoepithelial cells identified with immunohistochemical examination was small. These findings suggest that the myoepithelial cells in the regenerated sublingual glands are mainly residual myoepithelial cells from the atrophic sublingual gland. In addition, taking into consideration that myoepithelial cells do not increase mitotically during atrophy of the sublingual gland (Takahashi et al. 2003), it is suggested that myoepithelial cells in the regenerated sublingual gland originate in the undisturbed sublingual gland before induction of atrophy. The biological significance of stable population of myoepithelial cells in the atrophy and regeneration may be to contribute to the resistance of sublingual gland to the pathological factors.

In this study, myoepithelial cells often displayed bizarre appearances with protrusion of cell processes into interstitial spaces and irregular folds of basal lamina. A similar appearance of myoepithelial cells has previously been reported in the experimental atrophy of

other salivary glands (Emmelin et al. 1974, Garrett & Emmelin 1979, Takahashi et al. 2001), in human sialadenitis (Harrison & Badir 1998), and in the involution of mammary glands (Radnor 1972, Walker et al. 1989). These suggest that bizarre shaped myoepithelial cells are common in regressive exocrine glands. The bizarre shaped myoepithelial cells have morphologically changed with the transformation from duct cells to acinar cells during sublingual gland regeneration and have become typical myoepithelial cells at the periphery of matured acini like in the undisturbed sublingual glands. It is possible to consider that this morphological alteration of myoepithelial cells is induced by acinar cells becoming plumper with their differentiation and maturation, since the bizarre appearances were caused by atrophy and loss of underlying cells (Garrett & Emmelin 1979). And the biological significance of the bizarre shape of myoepithelial cells in the atrophy may be to contribute to the rapid recovery in accordance with underlying cell maturation.

In conclusion, the proliferation of myoepithelial cells and the differentiation to myoepithelial cells rarely participate in the regeneration of atrophied rat sublingual glands following release from duct ligation, and the bizarre shaped myoepithelial cells in the atrophied sublingual glands recover the original shapes with acinar cell regeneration.

Acknowledgments

This study was supported by Grant for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (No. 17390499).

References

Batsakis JG, Kraemer B, Sciubba JJ (1983) The pathology of head and neck tumors: the myoepithelial cell and its participation in salivary gland neoplasia. Part17. Head Neck Surg 5: 222-233.

Batsakis JG, Regezi JA, Luna MA, El-Naggar A (1989) Histogenesis of salivary gland neoplasms: a postulate with prognostic implications. J Laryngol Otol 103: 939-944.

Burford-Mason AP, Cummins MM, Brown DH, MacKay AJ, Dardick I (1993) Immunohistochemical analysis of the proliferative capacity of duct and acinar cells during ligation-induced atrophy and subsequent regeneration of rat parotid gland. J Oral Pathol Med 22: 440-446.

Burgess KL, Dardick I, Cummins MM, Burford-Mason AP (1996) Myoepithelial cells actively proliferate during atrophy and regeneration of rat parotid gland. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 82: 674-680.

Burgess KL, Dardick I (1998) Cell population changes during atrophy and regeneration of rat parotid gland. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85: 699-706.

Cummins M, Dardick I, Brown D, Burford-Mason A (1994) Obstructive sialadenitis: a rat model. J Otolaryngol 23: 50-56.

Cutler LS, Chaudhry AP (1973) Differentiation of the myoepithelial cells of the rat submandibular gland in vivo and in vitro: an ultrastructural study. J Morphol 140: 343-354.

Dardick I, Burford-Mason AP (1993) Current status of histogenetic and morphogenetic concepts of salivary gland tumorigenesis. Crit Rev Oral Biol 4: 639-667.

Dardick I, Nostrand AWP (1987) Morphogenesis of salivary gland tumors: a prerequisite to improving classification. Pathol Ann 22: 1-53.

Emmelin N, Garrett JR, Ohlin P (1974) Secretory activity and the myoepithelial cells of salivary glands after duct ligation in cats. Arch Oral Biol 19: 275-283.

Garrett JR, Emmelin N (1979) Activities of salivary myoepithelial cells: a review. Med Biol 57: 1-28.

Hanks CT, Chaudhry AP (1971) Regeneration of rat submandibular gland following partial extirpation. A light and electron microscopic study. Am J Anat 130: 195-208.

Harrison JD, Garrett JR (1972) Mucocele formation in cats by glandular duct ligation. Arch Oral Biol 17: 1403-1414.

Harrison JD, Badir MS (1998) Chronic submandibular sialadenitis: ultrastructure and phosphate histochemistry. Ultrastruct Pathol 22: 431-437.

Harrison JD, Fouad HMA, Garrett JR (2001) Variation in the response to ductal obstruction of feline submandibular and sublingual salivary glands and the importance of the innervation. J Oral Pathol Med 30: 29-34.

Ihrler S, Blasenbreu-Vogt S, Sendelhofert A, Rössle M, Harrison JD, Löhrs U (2004) Regeneration in chronic sialadenitis: an analysis of proliferation and apoptosis based on double immunohistochemical labelling. Virchows Arch 444: 356-361.

McCormick D, Hall PA (1992) The complexities of proliferating cell nuclear antigen.

Histopathol 21: 591-594.

Miyachi K, Fritzler MJ, Tan EM (1978) Autoantibody to a nuclear antigen in proliferating cells. J Immunol 121: 2228-2234.

Norberg L, Dardick I, Leung R, Burford-Mason AP, Rippstein P (1992) Immunogold localization of actin and cytokeratin filaments in myoepithelium of human parotid salivary gland. Ultrastruct Pathol 16: 555-568.

Radnor CJP (1972) Myoepithelium in involuting mammary glands of the rat. J Anat 112: 355-365.

Redman RS, Ball WD (1979) Diffrenetiation of myoepithelial cells in the developing rat sublingual gland. Am J Anat 156: 543-566.

Redman RS, Sweney LR, Mclaughlin ST (1980) Differentiation of myoepithelial cells in the developing rat parotid gland. Am J Anat 158: 299-320.

Redman RS (1994) Myoepithelium of salivary glands. Microsc Res Tech 27: 25-45.

Scott J, Liu P, Smith PM (1999) Morphological and functional characteristics of acinar atrophy and recovery in the duct-ligated parotid gland of the rat. J Dent Res 78: 1711-1719.

Takahashi S, Domon T, Yamamoto T, Wakita M (1997) Regeneration of myoepithelial cells in rat submandibular glands after yttrium aluminium garnett laser irradiation. Int J Exp Pathol 78: 91-99.

Takahashi S, Nakamura S, Suzuki R, Islam N, Domon T, Yamamoto T, Wakita M (2000) Apoptosis and mitosis of parenchymal cells in the duct-ligated rat submandibular gland.

Tissue Cell 32: 457-463.

Takahashi S, Nakamura S, Shinzato K, Domon T, Yamamoto T, Wakita M (2001) Apoptosis and proliferation of myoepithelial cells in atrophic rat submandibular glands. J Histochem Cytochem 49: 1557-1563.

Takahashi S, Shinzato K, Nakamura S, Domon T, Yamamoto T, Wakita M (2002) The roles of apoptosis and mitosis in atrophy of the rat sublingual gland. Tissue Cell 34: 297-304.

Takahashi S, Shinzato K, Domon T, Yamamoto T, Wakita M (2003) Proliferation and distribution of myoepithelial cells during atrophy of the rat sublingual gland. J Oral Pathol Med 32: 90-94.

Takahashi S, Shinzato K, Nakamura S, Domon T, Yamamoto T, Wakita M (2004a) Cell death and cell proliferation in the regeneration of atrophied rat submandibular glands after duct ligation. J Oral Pathol Med 33: 23-29.

Takahashi S, Shinzato K, Domon T, Yamamoto T, Wakita M (2004b) Mitotic proliferation of myoepithelial cells during regeneration of atrophied rat submandibular glands after duct ligation. J Oral Pathol Med 33: 430-434.

Takahashi S, Nakamura S, Domon T, Yamamoto T, Wakita M (2005) Active participation of apoptosis and mitosis in sublingual gland regeneration of the rat following release from duct ligation. J Mol Histol 36: 199-205.

Tandler B, Denning CR, Mandel ID, Kutscher AH (1970) Ultrastructure of human labial glands; III. myoepithelium and ducts. J Morphol 130: 227-246.

Walker NI, Benett RE, Kerr JFK (1989) Cell death by apoptosis during involution of the

lactating breast in mice and rats. Am J Anat 185: 19-32.

Walker NI, Gobé GC (1987) Cell death and cell proliferation during atrophy of the rat parotid gland induced by duct obstruction. J Pathol 153: 333-344.

Table 1

	Myoepithelial Cell	Acinar Cell	Duct Cell
Control	0.93±0.35	1.28±0.11	0.28±0.10
Day 0	0.78±0.26	2.15±0.03	3.18±0.41
Day 1	0.58±0.21	3.88±0.26	4.34±1.38
Day 2	0.60±0.19	3.99±0.83	3.94±0.96
Day 3	0.71±0.22	20.55±6.27	6.30±1.22
Day 4	1.65±0.39	35.72±4.03	3.86±0.69
Day 5	1.44±0.23	24.62±1.90	2.29±0.58
Day 7	2.09±0.37	13.58±4.92	1.27±0.48
Day10	1.41±0.33	12.65±1.57	0.86±0.05
Day14	0.98±0.23	4.13±0.34	0.65±0.04

Legends

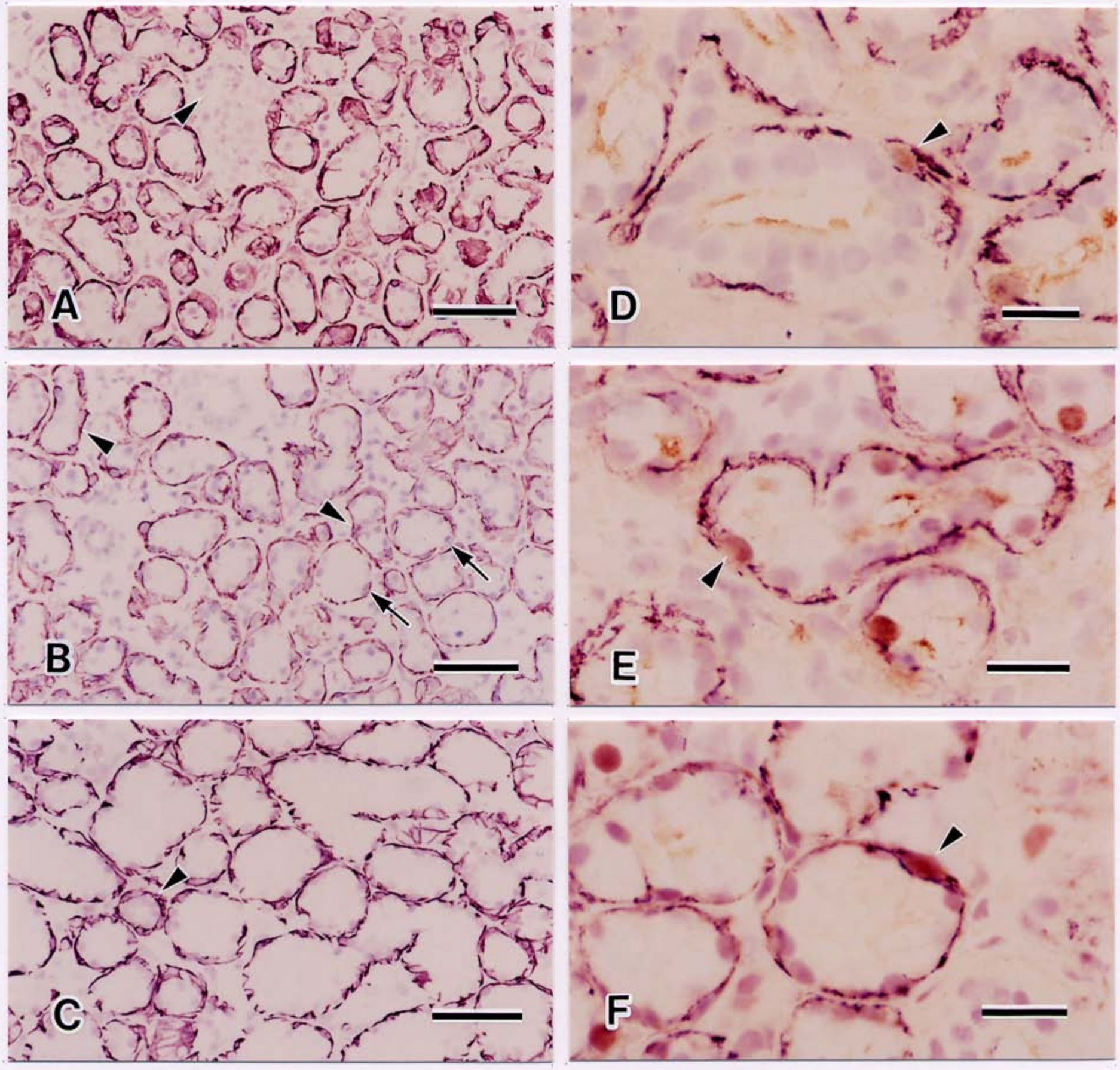
Figure 1. Single immunohistochemistry for actin (A-C, scale bars=50 μ m) and double immunohistochemistry for proliferating cell nuclear antigen (PCNA) and actin (D-F, scale bars=20 μ m). (A) Day 0. Myoepithelial cells at the periphery of the remaining ducts except at one duct (arrowhead). (B) Day 4. Immature acini (arrows) and residual ducts (arrowheads) surrounded by myoepithelial cells. (C) Day 14. Myoepithelial cells around mature acini and an intercalated duct (arrowhead). (D) Day 5. A myoepithelial PCNA-positive cell (arrowhead) in the vicinity of a duct. (E) Day 5. A PCNA-positive myoepithelial cell (arrowhead) around an immature acinus. (F) Day 10. A double positive cell (arrowhead) at the periphery of a mature acinus.

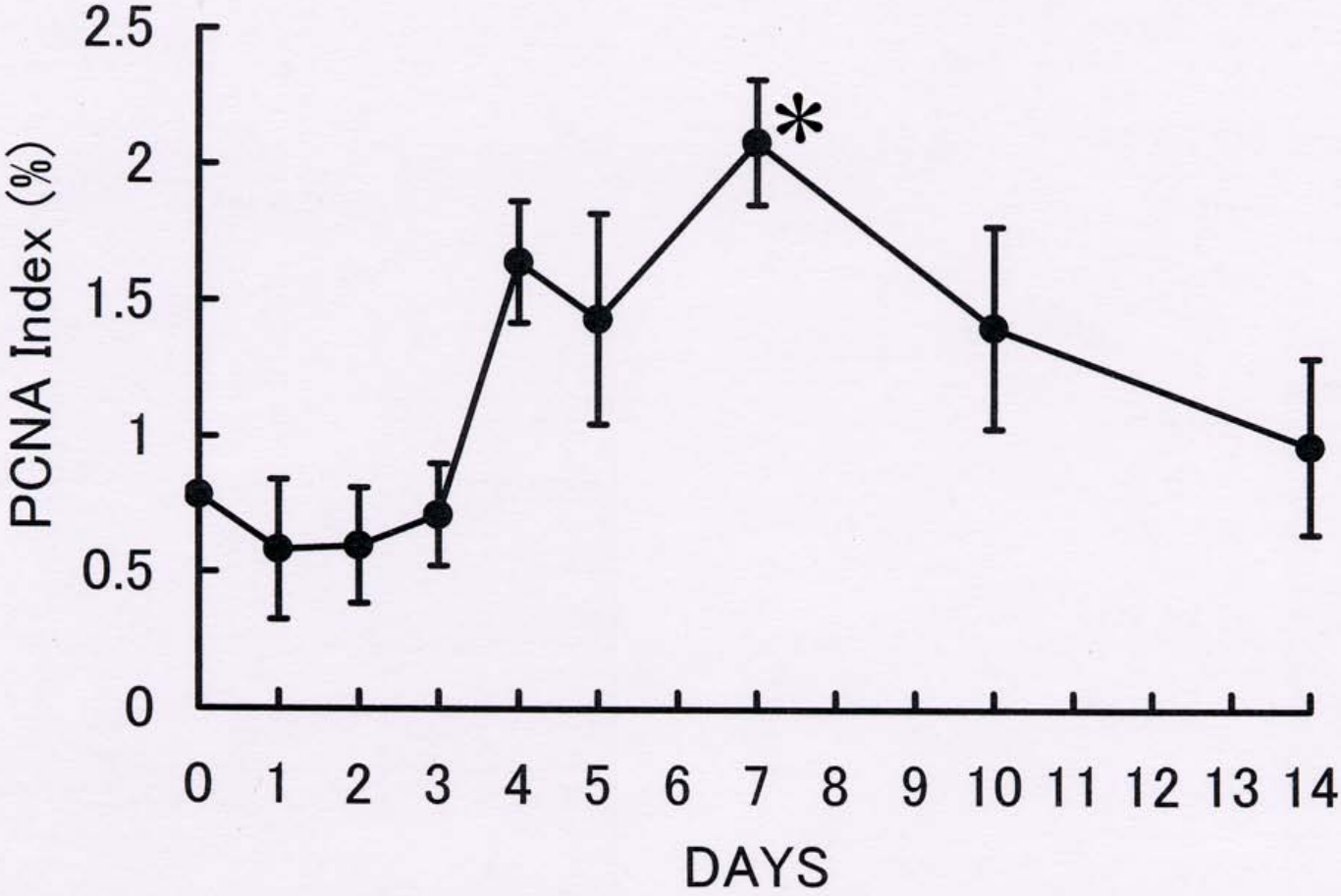
Figure 2. PCNA labeling index of myoepithelial cells in the experimental sublingual glands (n=4). The results are expressed as mean±SEM. The asterisk stands for statistically significant differences from the control (n=4) ($p<0.05$). The labeling index in the controls is 0.95% (not shown).

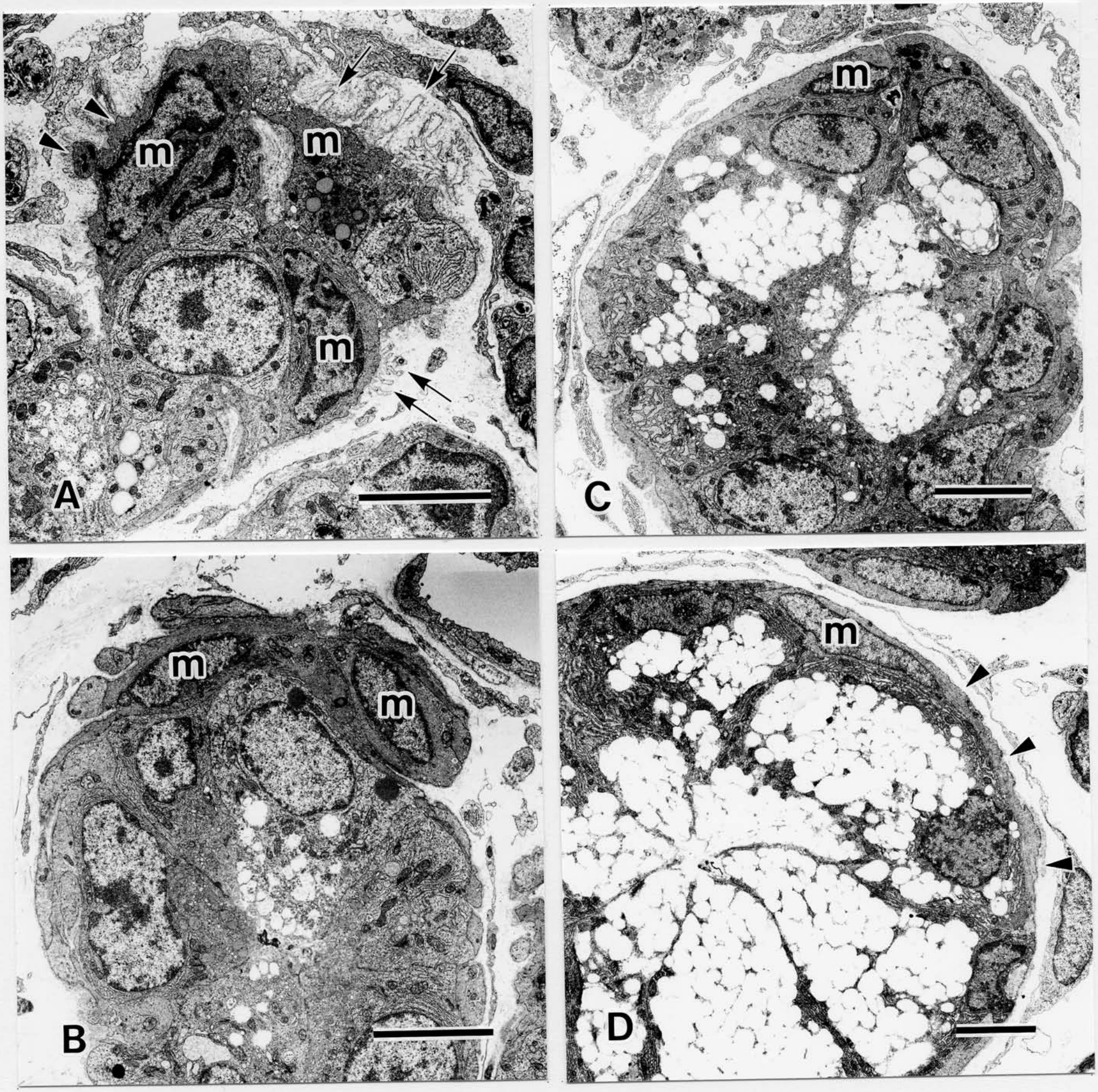
Figure 3. Electron micrographs of experimental sublingual glands. Scale bars=5 μ m (A) Day 0. Irregular folds of the basal lamina (arrows) and cell processes of the myoepithelium (m) into the interstitial space (arrowheads). (B) Day 4. Myoepithelial cells (m) at the periphery of the transitional duct-acinar structure. (C) Day 4. Myoepithelial cell (m) around an immature

acinus. (D) Day 10. Myoepithelial cell (m) with long and thin cell process (arrowheads) around a mature acinus.

Table 1. PCNA labeling indices (%) of myoepithelial cells, acinar cells and duct cells (±SEM). Values for acinar and duct cells have been reported previously (Takahashi et al. 2005).


A
B
C
D
E
F


PCNA Index (%)
2.5
2
1.5
1
0.5
0
*
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
DAYS


m
m
m
A
m
C
m
m
B
m
D