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Gross and Microscopic Anatomy of the Extraorbital Lacrimal Gland of the Common Marmoset (*Callithrix jacchus*)

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Purpose: **The lacrimal gland is often selected for microscopic examination in toxicologic studies. However, this gland is difficult to find within the orbit in marmosets at necropsy. Therefore, we examined the extraorbital lacrimal glands in marmosets.**

Methods: **The formalin-fixed craniums of four marmosets were used in a topographic study to confirm location of the lacrimal gland, and the results were applied to a routine toxicologic study in marmosets.**

Results: **The extraorbital lacrimal gland was located on the temporal surface of the zygomaticofrontal process and was covered with the temporalis muscle. The gland was easily detached from the surrounding tissue, and its histologic features were the same as those of the intraorbital lacrimal gland.**

Conclusions: **The extraorbital lacrimal glands have been reported in some New World monkeys, but to the authors' knowledge, this is the first report in marmosets. Identification and characterization of this gland will be useful for toxicologic studies in marmosets.**

Recently, the common marmoset (*Callithrix jacchus*) has been used for toxicologic and biomedical studies as a laboratory primate, because it is easier to handle and breed, and reproduces better than does the macaque (1–3). Although the lacrimal gland is often selected for microscopic examination in toxicologic studies (4), it is difficult to find this gland in the orbit of marmosets at necropsy. The intraorbital lacrimal glands of the marmoset are smaller and less palpable than are those of the macaque. Therefore, it is important to confirm the location of the lacrimal glands in the marmoset.

We found another lacrimal gland outside the orbit by use of topographic examination. The extraorbital lacrimal gland has been reported in New World monkeys, such as the tufted capuchin (5) and squirrel monkey (6). The objective of the study reported here was to describe the gland of the marmoset, which also belongs to the New World monkey family.

The first step: A topographic study

Two male and two female marmosets, one to two years old, were studied. The animals were purchased from a commercial breeder (Clea Japan Inc., Tokyo, Japan) and were used for the immunologic study. After being sacrificed under sodium pentobarbital anesthesia, the organs, including the cranium, were preserved in neutral-buffered 10% formalin. The visceral cranium containing both eyes within the orbit was decalcified in15% formic acid formalin at room temperature for 14 days. The right orbit was cut into slices 4 to 5 mm thick vertically along the eyeball, embedded in paraffin, and sectioned at a thickness of 200 μm. These sections were deparaffinized and stained with hematoxylin and eosin (H&E). Approximately 30 specimens were obtained from the orbit.

The location of the intraorbital lacrimal glands varied in each individual animal as reported (7). In the orbit, clusters of lacrimal glands were sometimes detected in the epibulbar tissue on the dorsolateral side and the inferior side near the inner canthus. These glands had no capsule. However, outside the orbit, a glandular structure was observed beneath the orbit-forming bone, which was covered with the temporalis muscle at the outer lateral side (Figure 1). The gland was long and slender in shape, and its upper margin penetrated into the orbit through a fissure (Figures 2A–2C). The gland was surrounded by loose connective tissue and was divided by connective tissue into large lobes with many small lobules. The gland formed a compound tubuloacinar pattern of serous secretory cells, and had no intercalated portion (Figure 3). Well-differentiated excretory ducts were seen in the interstitial connective tissue. The secretory cells formed a small lumen and often contained many eosinophilic granules in the apical part of their cytoplasm (Figure 3). These

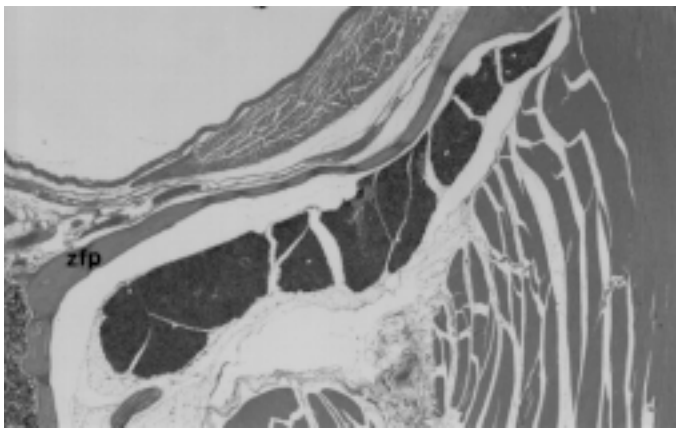

Figure 1. Photomicrograph of the extraorbital lacrimal gland beneath the zygomaticofrontal bone process (zfp). H&E stain; x14.8.

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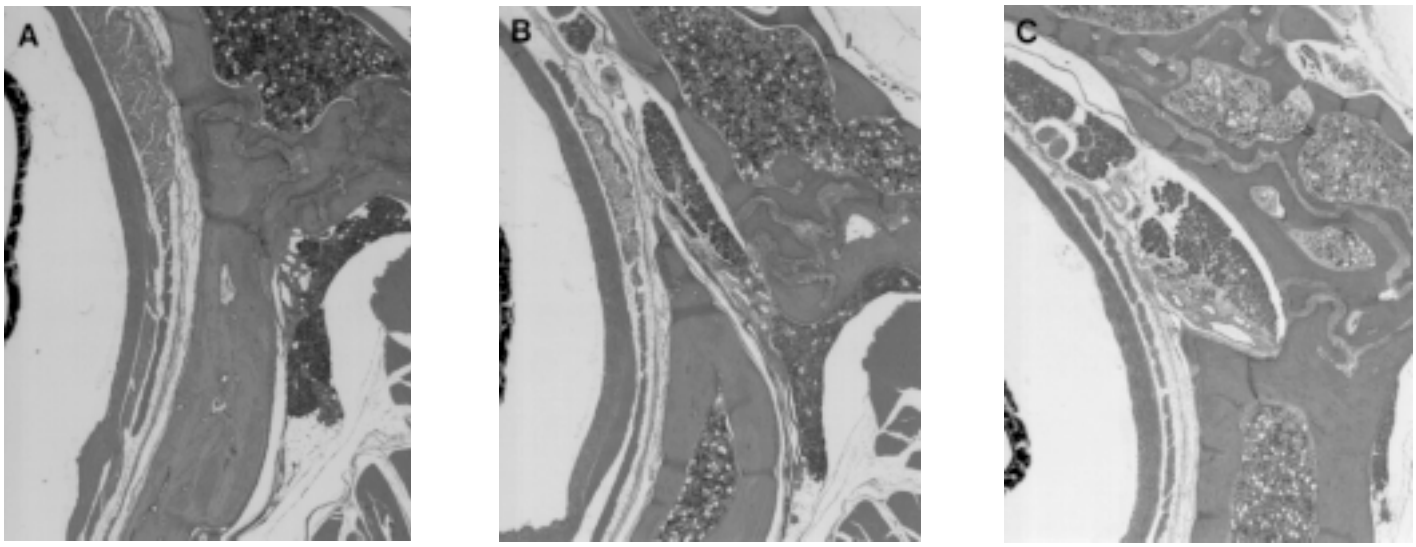

Figure 2. Photomicrographs of part of the extraorbital lacrimal gland penetrating into the orbit through the zygomatico-sphenoid suture. Serial sections from **(A)** to **(C)**. H&E stain; x20.

structural findings were the same as those of the intraorbital lacrimal gland. We also performed a gross anatomic examination of the remaining left orbit. After removing the temporalis muscle in the depths of the fossa temporalis, a gray-colored, flat gland approximately 10 mm long and 2 mm wide was detected on the temporal surface of the zygomaticofrontal process. The upper portion of this gland penetrated the zygomaticosphenoid suture. The gland was easily detached from the surrounding tissue, and it was confirmed histologically to be a lacrimal gland.

The second step: Application to a toxicologic study

Next, we applied this method in a routine toxicologic study for 52 weeks in marmosets. Twenty-five males and twenty-five females, 10 to 14 months old, obtained from the same breeder as described previously, were studied. The study was performed in accordance with the Good Laboratory Practice system and conformed to the guidelines for animal experimentation (the Animal Care and Use Committee of Mitsubishi Chemical Safety Institute, Ltd., 1995). The animals were housed individually in stainless steel cages (30 x 60 x 65 cm) in a room maintained at a temperature of 25 to 28°C, with relative humidity of 40 to 70% and a 12-hour light/dark cycle. They were fed 80 g of a basic diet (CMS-1, Clea Japan Inc.) mixed at the rate of 1,000 g of CMS-1, 200 ml of water, and 1 g of ascorbic acid daily, and were allowed ad libitum access to sterilized water. They were administered the test substance or vehicle alone by use of gastric gavage tubes daily for 52 weeks. The test substance had no effect on the lacrimal glands. We were able to collect the lacrimal glands from all marmosets (Figure 4), except one, the cranium of which was not tested. The tissues of lacrimal glands were microscopically examined in detail. In addition to the routine study, selected sections were stained with periodic acid-Schiff (PAS) with or without use of the diastase digestion method, and alcian blue at pH 2.5 and 1.0 (Table 1). They were used for histochemical examination of the intracytoplasmic granules of the acinar cell (8).

Masson's trichrome stain was used to determine the amount of connective tissue. The labeled streptavidin-biotin method (Dako LSAB Kit; Dako Co., Carpinteria, CA) was used for immunohistochemical analysis of the lacrimal glands, submandibular glands, parotid glands, and submandibular lymph nodes of the same animal. As the organs were immersed in neutral-buffered 10% formalin for only 24 hours, microwave treatment for antigen retrieval was omitted. The specimens were incubated for 10 minutes with rabbit antiserum (Dako Co.) to human lysozyme (9) as the primary antibody at the optimal dilution of 1:300, followed by sequential 10-minute incubations with biotinylated second antibody and peroxidase-labeled streptavidin. Visualization of the bound antibody was performed using diaminobenzidine (Dako Co.). Slides were counterstained with methyl green. Histiocytes in the lymph nodes served as internal positive markers for intracellular lysozyme. Small pieces of the lacrimal gland taken from one animal of each sex were examined by electron microscopy. The samples were fixed in a 2.5% glutaraldehyde solution for 2 hours, and further fixed in 1% osmium tetroxide solution for another 2 hours at 4°C. Samples were embedded in epoxy resin, and ultrathin sections were cut, stained with uranyl acetate and lead citrate, and observed, using a JEM 100CX electron microscope (JEOL, Tokyo, Japan).

The granules in the apical parts of the secretory cells reacted positively to PAS staining (Table 1). About half of these granules reacted strongly; the others showed weak staining. The reactivity did not change after diastase digestion. In a few of the secretory cells, the granules were barely stained with alcian blue at pH 2.5, and none were stained with alcian blue at pH 1.0 (Table 1). By use of electron microscopy, pyramid-shaped acinar cells with poorly differentiated microvilli at the luminal surface were seen to contain various amounts of secretory granules in the cytoplasm (Figure 5). The granules varied in size and had homogeneous contents of variable electron density. Dark acinar cells were tightly packed with uniformly enlarged granules. The ovoid nucleus, mitochondria, and rough endoplasmic reticulum were often located in the basolateral part of the cells. Intercellular secretory canaliculi were observed linking pairs of acinar cells (Figure 5). The granules have been reported to contain neutral polysaccharides with a small quantity of sialic acid (7). It also was reported that the differences in histochemical reactions and electron density of the granules are due to maturation

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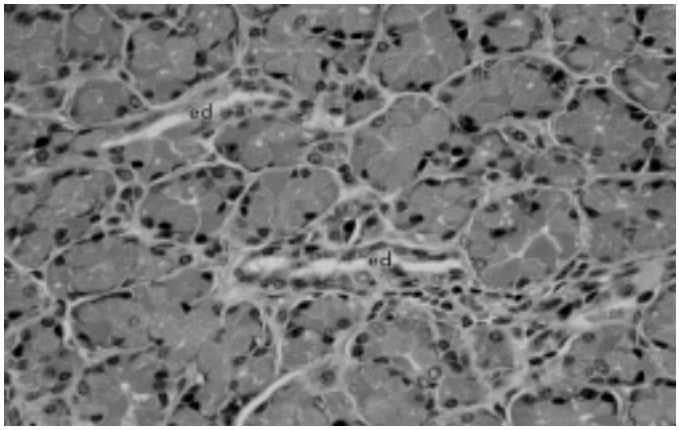

Figure 3. Higher magnification of the extraorbital lacrimal gland. The gland forms a compound tubuloacinar pattern of serous secretory cells with well-differentiated excretory ducts (ed) in the interstitium. H&E stain; x296.

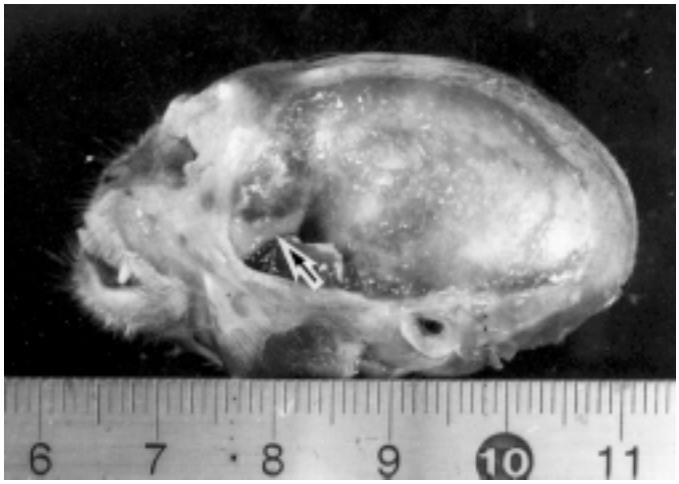

Figure 4. Gross observation of a lateral view of the marmoset skull. The extraorbital lacrimal gland (arrow) is adjacent to the temporal surface of the zygomaticofrontal process. The temporal muscle has been removed.

differences in a secretory cycle (8, 10–12). Although most mammals have no intercellular secretory canaliculi (8), Marback et al. (7) documented their presence in the marmoset by light microscopy, and we confirmed these observations by electron microscopy in this study. Immunohistochemically, the cytoplasm, other than the granules, in most of the secretory cells and in some of the ductular epithelium exhibited granular labeling with an antibody to lysozyme (Table 1). The reactivity was more marked than that in histiocytes. The acinar cells in the salivary glands rarely reacted to the antibody. These immunohistochemical results were based on the previous observation that significant concentrations of the enzyme are present within tears as a bacteriolytic element (13). A few lymphocytes and plasma cells infiltrated the perivascular or periductal connective tissue of the glands unilaterally or bilaterally in 7 of 25 males and 6 of 24 females. Hematopoietic cells were also detected in the interstitium of one female. This cellular infiltration is observed spontaneously in the intraorbital lacrimal glands of cynomolgus (14) and rhesus (14, 15) monkeys.

Table 1. Histochemical reactions of the extraorbital lacrimal gland in the marmosets

	Acinar cell		
Stain	Granules	Other cytoplasm	Ductular epithelium
PAS without diastase digestion	Half strongly positive, half weakly positive	No reaction	No reaction
PAS with diastase digestion	Same reaction as above	No reaction	No reaction
Alcian blue at pH 2.5	Scarcely positive in a few cells	No reaction	Rarely
Alcian blue at pH 1.0	No reaction	No reaction	No reaction
Anti-human lysozyme immunostaining	No reaction	Strongly positive	Some were positive

PAS = periodic acid-Schiff.

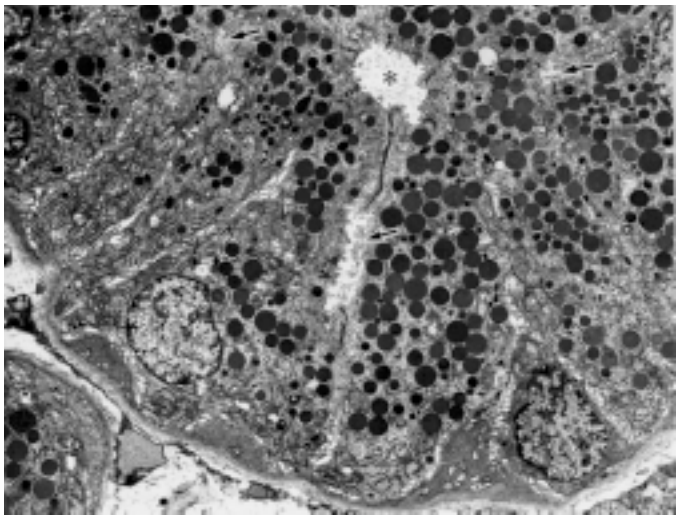

Figure 5. Electron micrograph of the acinar cells, Notice cytoplasm contains various amounts of secretory granules. Intercellular secretory canaliculi (arrows) are observed. *Lumen. Magnification = 3,714.8.

In this study, we found that the marmoset has a larger lacrimal gland located outside the orbit, similarly to that of the tufted capuchin (5) and squirrel monkey (6). This gland was similar histologically to those in the orbits of monkeys (10, 12, 15) and humans (11). Maitchouk et al. (6) surgically removed the extraorbital lacrimal gland to examine tear flow of the accessory lacrimal glands in squirrel monkeys. It should be possible to weigh the gland if necessary, because it is covered with a thin layer of connective tissue and can be dissected easily from the surrounding tissue.

The extraorbital lacrimal gland is well developed in rodents, and opens into the lateral edge of the conjunctiva with the long secretory duct (8). On the other hand, the extraorbital lacrimal gland of marmosets was joined directly to the intraorbital gland. This anatomic structure does not resemble that of rodents, but that of rabbits (i.e., the accessory lacrimal gland is located at the caudal and ventral orbital margins [16]). Due to the less well developed zygomatic process, the orbit communicates with the fossa temporalis in rabbits. However, the process separates the orbit from the fossa in primates, including the marmoset. Therefore, we believe that part of the lacrimal gland is located outside the orbit. Sakai (8) reviewed the comparative anatomy and histology of the mammalian lacrimal glands, and reported that primates have only the intraorbital lacrimal gland located in the outer superior canthus (*Glandula lacrimalis superior*). As

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New World monkeys such as marmosets are evolutionarily more primitive than are anthropoid primates, the anatomic location of their lacrimal glands might be closely allied to that of mammals, such as rabbits, sited at lower taxonomic positions.

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