Structure and Development of the Perianal Gland of the Dog

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Abstract. The development and structure of the perianal gland were studied histologically in 74 dogs of age 0 day to 16 years. The gland was seen already in 14-day-old puppies of both sexes, and in dogs younger than 9 months old it opened to the hair follicle through an excretory duct. The glandular tissues of adult dogs developed flourishingly around semi-closed duct-like structures which fused with each other forming a "tortoise-shell" lobules without connection to hair follicles. In electron microscopy, the glandular cells were divided into two types, light and dark cells, and the former type of cells had well-developed vacuoles. No secretory granules were contained in both types of cells. In larger lobules of adult male dogs, intralobular cysts developed occasionally showing sometimes a central necrosis. This might result from a rapid and successive conversion of tubular cells to glandular cells. Some glandular tissues comparable to the perianal gland were detected also at the loin, the prepuce, the dorsal and ventral parts of the tail, and the groin. Administration of testosterone propionate to 60-day-old puppies induced a significant development of the glandular tissues which became to have size and structure comparable to those in adult dogs.

The normal structural and functional development of the perianal gland have been studied long since. In 1875, Siedamgrotzky first described this glandular structure consisting of a long duct and compactly arranged polyhedral glandular cells [24]. Schaffer, who described this gland as "Hepatoid Drüsens", observed that presecretory granules were present in the glandular cells and that serous materials were secreted through intracellular canaliculi and intercellular pathways resembling those in the bile secretion in the liver [21–23]. Parks studied in detail on structural development of the perianal glandular tissues as well as histochemical observations of the cytoplasmic granules, suggesting the secretion of proteinous watery materials [20].

On the other hand, many cases of neoplasms are known to arise from the perianal gland in old dogs of both sexes, suggesting that sex hormones might play a role in the occurrence of tumors [1–7, 25–27]. The systemic or local administration of estrogensic hormones to the tumor cases has been made successfully as well as the surgical excision and X-ray irradiation [19]. To disclose the mechanism of tumor development in the perianal area it is needed to study in detail the structural and functional development of the gland with age. However, much remained in these respects and there is no experimental informations on the effect of sex hormones.

The present paper deals with light and electron-microscopic observations of the development, distribution and histochemical characteristics of perianal glands as well as
effects of testosterone propionate (TP) upon them.

Materials and Methods

Forty-four male and female dogs of various strains 0 day to 16 years of age were examined. Specimens were obtained from perianal area by cutting the skin vertically or horizontally to the anus. Besides, from dogs of age 0 and 7 months and those of age 3 and 8 years specimens were harvested from other parts of the skin. They were fixed with 10% buffered (pH 7.2) or non-buffered formalin, absolute alcohol, Zenker's solution, Baker's formol calcium solution, Regaud's solution, Carnoy's fixative or Susa's solution. After embedded in paraffin, 5 to 6 μm and 8 to 10 μm serial sections were made for routine or histochemical observations. Frozen sections were also prepared. Sections were stained with hematoxylin-cosin, periodic acid-Schiff reaction (PAS) and Masson's trichrome. Also the following histochemical stainings were applied; Sudan Black B and Sudan Black III for whole lipids, Sudan Black B on paraffin embedded section for fat solvent-resistant lipids, luxol fast blue, Landing's phosphomolybdic acid, Baker's acid hematein test, Regaud's hematoxylin, Altmann's acid fuchsin for phospholipids, Schultz's method for cholesterol, PAS for carbohydrate, ninhydrin-Schiff reaction for protein, toluidine blue for metachromasia, Feulgen reaction and methyl green pyronin stain for nucleic acids.

For electron microscopy, specimens of perianal areas were obtained from 22 dogs of both sexes of age from 3 months to 5 years and they were fixed with 1% OsO₄ in Millonig's solution and embedded in EPON 812. Sections were made using a ultramicrotome LKB-1 and observation was made with JEM-7A.

To study the effect of TP on the perianal gland, 11 mongrel puppies of the same sex and 60 days of age from 4 litters were used. TP was dissolved in acetone and an emulsion was made in camellia-oil so as to contain 5 mg TP in 0.1 ml. Control dogs were given camellia-oil at 0.2 ml/kg/day. Different doses were injected subcutaneously at the neck daily for 14 days as follows; Litter I---6 μg (TP 10 mg/kg/day) and 6 μg (Control); Litter II---6 μg (TP 10 mg/kg/day) and 6 μg (Control); Litter III---6 μg (TP 10 mg/kg/day) and 6 μg (Control); Litter IV---6 μg (TP 5 mg/kg/day) and 6 μg (Control). On the next day of the last injection, dogs were killed by electricity and materials for histological observation were harvested.

Results

The perianal gland was seen in neither fetuses nor 11-day-old puppies, and it was detected in 14-day-old mongrel puppies of both sexes. The alveoli were connected with the outer-root sheath of hair follicles though the duct having no myoepithelial cells (Fig. 1). The glandular cells near the duct contained many lipid droplets, as those of the sebaceous gland, but at a distal portion, they appeared to contain many cytoplasmic granules 0.3--0.5 μm in size. In puppies of age 20 days or younger, the ducts were not completely closed, but in 2-month-old puppies a small space was seen only at the proximal opening having a solid epithelial lining with some lipid-laden cells. In dogs of age 3 to 7 months a few vesicles (50--80 μm in size) were seen occasionally in the course of the solid ducts, which contained sudanophilic substances surrounded by a thin keratin layer. In 9-month-old dogs, the proximal end of the duct disappeared into the connective tissue around the hair follicle and in older ones any connection between the duct and the hair follicle could be detectable. In adult animals, some "non-sebaceous cysts" described by Parks [20], 50 to 200 μm in diameter were usually enclosed by a concentric arrangement of flattened cells as well as a keratin layer, containing a quantity of sudanophilic amorphous masses (Fig. 2). These structures were seen most frequently in male adult dogs but occasionally in younger ones of age 3 to 8 months. There were two types of duct cells differentiating into glandular and reserve cells, respectively. Duct cells shifting gradually to glandular cells formed acini.

By electron microscopy, the duct cells were similar to those of the outermost zone of the outer-root sheath of hair follicles.
Those cells had a cuboidal nucleus being smooth in outline, a small number of elongated mitochondria, endoplasmic reticulum, tonofilaments, and half-desmosomes between the basement membrane. In young dogs the following four layers were seen in “non-sebaceous cysts”. An outermost layer consisted of cells commonly seen also in other parts of the duct and it was slightly interdigitating with cells of the median layer. The cells of the median layer were rich in glycogen granules and many of them had in their cytoplasm widely distributing thick tonofilaments that were conjugated tightly to well-developed desmosomes. The cells of the inner layer contained many ribosomes as well as numerous Odland bodies, keratohyalin granules and tonofilbrils, and they surrounded the central cyst filled with keratin masses and lipid droplets (Fig. 2). In adult dogs, the inner layer was lacking. At the distal portion, the duct cells increased in number and a few vesicular cells were seen occasionally in the median layer, having a number of large ovoidal mitochondria, and ribosomes as well as well-developed Golgi apparatus and agranular endoplasmic reticulum and abundant vacuoles. Tonofilaments characteristic of the common duct cells were also observed (Fig. 3). Such vacuolated cells increased in number towards the end of the ducts.

The acini budding from a short secondary ductule as a grape cluster were enclosed by reserve cells resembling basket cells of the salivary gland. They were surrounded by a thin layer of connective tissues with dense capillary networks and some nervous ends. In 2-month-old puppies, most acini at the distal portion of the duct were packed densely with eosinophilic polyhedral glandular cells, although a proximal portion resembled those of 14-day-old ones. In older animals, acini at the proximal portion of the duct were shrunk with disappearance of the duct, and in dogs older than 9 months, only a few acini were seen near the hair follicle. The acini at the distal portion of the duct were enlarged and fused each other with the adjacent acini forming a “tortoise shape” lobus (Fig. 4).

At the center of these lobules glandular cells were 10–18 μm in diameter having vesicles and cytoplasmic granules both 1–4 μm in diameter while in periphery there were large and spindle shaped cells 18–25 μm in diameter having smaller granules within a rather basophilic cytoplasm. These cytoplasmic granules were stained with Sudan black B (Fig. 6), luxol fast blue, Landing’s phosphomolybdic acid, acid hematein, Regaud’s hematoxylin and Altmann’s anilin acid fuchs, while they were negative to Schiff, PAS, ninhydrin-Schiff, Feulgen, toluidine blue or methyl green pyronin stain. In adult males, “intralobular cysts” 50–200 μm in diameter, described by Parks [20], were common at the center of these lobules, containing mostly sudanophilic keratinous materials occasionally with some nuclear or cytoplasmic residues (Fig. 5). The glandular cells surrounding the cysts were flattened and seen to be gradually keratinized towards the lumen. The perianal extent of glandular tissues depended upon the size of animals, and in larger breeds such as Shephered, the gland extended to 3.5 cm distant from the anus and 1.5 cm deep from the surface of the skin. In dogs 7 to 16 years of age, the glands were usually compressed in irregular shape within connective tissues but some basophilic duct cells seemed to differentiate into the glandular tissue. Some glandular tissues comparable to the perianal gland were seen also at the loin, the prepuce, the dorsal and ventral parts of the tail, and the groin, but not in other
parts of the skin. The acini of these portions were small and usually not fused each other, but their duct were partially plugged by amorphous keratinous materials similar to those found in the perianal area.

In electron microscopic observations, the acini consisted mostly of many light glandular cells having a number of vacuoles, and among light cells masses there were a few non-vacuolated “dark” cells (Fig. 7). The nucleus of light cells were located usually near the center of the cells containing one or two distinct nucleoli. Chromatin was precipitated near the nuclear membrane that was smooth in young animals but invaginated sometimes in aged ones. The cytoplasmic organelles developed well. Mitochondria were cuboidal, spheroidal or gourd-shaped, 1–4 μm in diameter or length with poor cristae. In old dogs they showed various regressive changes such as the fusion of cristae and accumulation of dense materials, lipid droplets or myelin deposits. Around Golgi apparatus there were many small vesicles 100 μm in diameter. Most of rough endoplasmic reticulum appeared with cystic dialation and ribosomes were partially detached from their surface. A large number of ribosomes were scattered diffusely in the cytoplasm. Well-developed vacuoles, usually 2–3 μm in diameter containing some dense fibrinous substances, were most characteristic of light cells. Intracellular canaliculi could not be detected, although some narrow spaces could be seen occasionally around the light cells in young dogs. In adults, the glandular cells were lined more compactly without any intercellular spaces. Lysosomal dense bodies 0.5–4.0 μm in diameter were common showing various shapes and amounts of tonofilaments were very variable in each cells. Glycogen granules could not be detected in the light cell. In the dark cells, invagination of nuclear membrane was frequent with dense cytoplasmic matrix. Mitochondria showed almost the same findings as those of the light cells. Golgi apparatus were scattered diffusely in the cytoplasm, and lysosomal dense bodies and tonofilaments were rather rich on the dark cells. Transitional cells to the light cell could not be detected.

The glandular cells having highly dense and several thick tonofilament bundles surrounded an intralobular cyst. Occasionally these cysts developed towards the periphery of the lobules and the contents were assumed to exudate in the connective tissues through a leak of the reserve cells.

After administration of TP in 60-day-old puppies, glandular tissues were shown to develop extensively becoming almost comparable to those of adult animals (Figs. 8-a, 8-b). Microscopically, alveoli composed of several glandular cells were connected with the excreatory ducts forming a grape cluster-like structure which transformed rapidly to the glandular tissues in a distal portion near the hair follicle (Fig. 9). An active development of reserve cells was one of the most characteristic appearance of test group, as seen in malignant perianal gland tumors. The prostate of the treated animals was enlarged to some degree. Also some effects were detectable in secreting portions of the apocrine sweat gland and anal sac, while the sebaceous gland seemed not to be affected. There were no effects in other organs of both sexes.

**Discussion**

In studies on the dogs from at birth to 14 days of age, the perianal gland could be seen at first in 14-day-old puppies and the result was compatible to the observation by Parks [20], although Cotchin [1–4] and Nielsen [19] described that it appeared already
at birth. The glandular tissues had same structure in various strains of dogs in the present study.

Whereas Siedamgrotzky [24] stated that the canine perianal gland was derived from the sebaceous gland, the present observations suggested that it was assumed to derive from the hair follicle after formation of the apocrine sweat gland and the sebaceous gland. The detection of the gland in the prepuce, the dorsal and ventral parts of the tail and the groin, is also compatible with such origin of the gland.

The duct cells might be changed first into lipid-laden cells leaving lipid in the lumen after cellular death. And then, neighbouring cells seemed to become keratinized making an epithelial pearl structure. At the terminal end of the duct, small vesicles and vacuoles were frequently seen in the cytoplasm. The duct cells were also connected with reserve cells which enclosed acini as basket cells in the salivary gland.

The lobules of the perianal gland were composed of cosinophilic, polyhedral and granular cells having no lumen. In electronmicroscopic observation, the cells were divided into two types, light and dark cells. Most of the glandular cells were light cells containing many small vesicles, mitochondria, vacuoles, well-developed Golgi apparatus and wide spread ribosomes, suggesting active secretion of mucous materials. Dark cells occasionally seen among the light cells having a characteristics of serous secretion. However, there were no granules nor canalicii within the cytoplasm in both type cells. Schaffer [21–23] and Parks [20] described the presence of presecretory granules 1–2 μm in diameter containing some proteinous materials. These granules however, were considered to be mitochondria in the present electron microscopic observations. Since the ducts have no lumen in adult dogs, except for non-sebaceous cysts, the secretion never can attain the hair follicle even if there is some activity of secretion. Intra-lobular cysts encountered in the central zone, enclosed by the cornified glandular cells, and having lipid droplets, keratin and some cellular debris, are assumed to be derived from degenerated glandular cells due to malnutrition.

The function of the perianal gland of the dog remains still unknown. The treatment of 60-day-old puppies with TP resulted in well-developed and widely distributed perianal glands as those in adult dogs. Such effect of the androgenic hormone was shown more dramatically on perianal gland than sebaceous and apocrine sweat glands. Then, the perianal glands of dogs might be supposed to secrete some attractive substance in breeding season. On the other hand, the dog has a pair of anal sacs which become active in breeding season secreting sexual odor. According to Schaffer [21–23], waxy materials are secreted to maintain the humidity around the anus. However, dogs as well as in other mammals have a large number of sweat and sebaceous glands at the perianal area which are enough to supply water and waxy materials.

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References


Explanation of Figures

Fig. 1. Glandular tissue of a 14-day-old male dog. Ducts opening to a hair follicle. H-E, ×400.

Fig. 2. Fine structure of a non-sebaceous cyst of a 5-month-old male dog. OL; Outer-layer, ML; Median-layer, IL; Inner-layer, C; Central mass. ×5000.

Fig. 3. Fine structure of a vacuolated cell which appeared at the distal portion of the duct of 6-month-old male dog containing a large number of mitochondria (mt), vacuoles (ve), and tonofilaments (to). ×7000.

Fig. 4. Lobules of a 5-year-old male dog showing a "tortoise shell" appearance. H-E, ×100.

Fig. 5. An intra-lobular cyst of a 4-year-old male dog. H-E, ×100.

Fig. 6. Cytoplasmic granules of glandular cells. Sudan-black B stain. ×1000.

Fig. 7. Fine structure of glandular cells. A dark cell is surrounded by vacuolated light cells having well-developed mitochondria. ×5000.

Fig. 8. Glandular tissues of a dog, one day after treatment with TP (10 mg/kg/day for 14 days) (8-a), and non-treated control (8-b). H-E, ×20.

Fig. 9. Glandular cells proliferating vigorously in the dog shown in Fig. 8-a. H-E, ×100.