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The lateral nasal gland of dog: its structure and secretory content

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INTRODUCTION

Of the 20 or more nasal glands which secrete into the nasal vestibule, the lateral nasal gland is considered to be ontogenetically and perhaps phylogenetically the oldest (Broman, 1921). Various functions have been proposed for the mammalian lateral nasal gland including the humidification of inhaled air, moistening of the nasal plane (Trautman, 1911), provision of fluid for the conduction of olfactory cues to the vomeronasal organ (Broman, 1921), enhancement of evaporative heat loss (Blatt, Taylor & Habal, 1972), and the provision of viscosity-altering substances for the mucociliary blanket (Moe & Bojsen-Møller, 1971).

The 0·1 mg protein/100 ml secretion reported present in hypo-osmotic secretions collected from dog lateral nasal glands (Blatt, Taylor & Habal, 1972) appears to be inconsistent with the histochemical (Adams & McFarland, 1972; Bast, 1924; Vidic & Greditzer, 1971) and ultrastructural (Moe & Bojsen-Møller, 1971; Vidic & Taylor, 1972) properties of lateral nasal gland acinar cells in various species. This paper describes the structure of the canine lateral nasal gland and the content of its secretions.

MATERIALS AND METHODS

Fifteen mongrel dogs were utilized in this study. For histological analyses, 12 dogs (seven adults and five 12-15 weeks old) were anaesthetized with sodium pentobarbital (i.v.), heparinized and exsanguinated. Samples of maxillary recess mucosa from sagittally sectioned heads were immersion-fixed in either buffered neutral 10% formalin (BNF) or 4% formaldehyde in Millonig's buffer (prepared from paraformaldehyde; pH 7·3; m-osmole 690). Tissue fixed in BNF was alcohol-dehydrated, paraffin-embedded, sectioned at 6 μm, and stained with either haematoxylin-eosin or alcian blue–periodic acid Schiff's (AB–PAS) reagent (Mowry, 1963); two excised maxillary recess mucosae were serially sectioned and alternately stained with alcian blue (AB) pH 2·5 and 1·0 (Spicer, 1965). Tissues fixed in formalin freshly prepared from paraformaldehyde were embedded in plastic resin, either after staining with AB or prior to staining with basic stains. Tissues stained in block with AB (pH 2·5 and 1·0) were dehydrated in alcohol/propylene oxide and embedded in Epon/Araldite and sectioned at 1 μm. Non-stained tissues were post-fixed for 1 hour in buffered 1% osmium tetroxide, dehydrated, embedded in resin, sectioned

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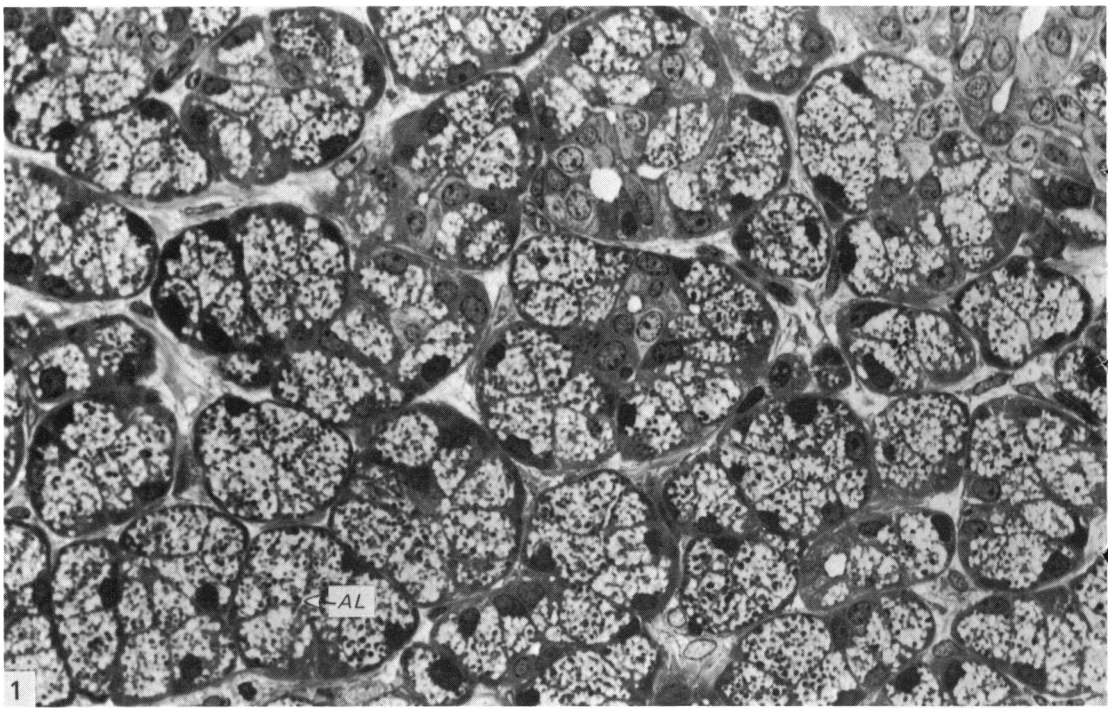


Fig. 1. Mature dog. Light micrograph of lateral nasal gland acinus. Dense-cored vesicles and non-basophilic vesicles occupy much of the cellular volume. *AL*, acinar lumen. Epoxy/Araldite section. Basic fuchsin–azure II stain. × 635.

and stained with basic fuchsin–azure II (BF–Az). Volume ratios of tissue component were stereologically determined (Elias, Hennig & Schwartz, 1971). Thin sections were mounted on uncoated grids, stained with uranyl acetate and lead citrate, and examined in a Hitachi HU 12A electron microscope operated at 50 KV.

For biochemical analyses of lateral nasal gland secretions, three dogs (20-27 kg body weight), standardized by worming with a broad spectrum vermifuge and vaccinations for rabies, canine distemper-hepatitis, parainfluenza and leptospirosis, were housed under controlled ambient environmental conditions for 6 months prior to cannulation. The ambient temperature during the study was 20 °C. The dogs were anaesthetized with thiopental (i.v.), intubated and maintained under halothane anaesthesia during surgery. The rostral $2\frac{1}{2}$ cm of the dorsal wall of each nasal fossa was incised to expose the duct orifices (Evans, 1977); vinyl tubing (I.D. = 0·51 mm) was inserted approximately 2 cm into the lateral nasal gland excretory duct and sutured to the mucosa of the nasal vestibule. Collection vials supported by leather muzzles were maintained securely in place by Elizabethan collars. Sixty two samples were collected representing various one and two hour collection intervals during a 14 day post-cannulation period. An Auto Analyzer was utilized to measure Na^+, K^+, and Cl^- ions. IgA concentration was determined in 10 μl samples by radial immunodiffusion (Miles Lab., Inc.); each plate contained alpha chain specific anti-A serum with an accuracy of $\pm 10\%$ in the range of 16-130 mg IgA/100 ml sample. Total protein was measured spectrophotometrically using the Bio-Rad assay (Bradford, 1976); 0·1 ml samples containing 0·2-1·4 mg protein/

ml, vortexed with 5 ml dye reagent (coomassie blue), were determined at OD_{595} with Cohn fraction 5 bovine serum albumin (Sigma Chemical Co., St Louis, Mo.) as a standard. Total sialic acid content was determined by the modified thiobarbituric acid assay of Warren (Engen, Anderson & Rouze, 1974) utilizing N-acetylneuraminic acid as a standard.

To assess whether cannulae restricted secretory flow or altered secretory content: (*a*) IgA concentrations present in secretions collected from the study group were compared to those present in secretions collected from two dogs cannulated with 0·86 mm vinyl tubing; and (*b*) lateral nasal glands from one dog were prepared for histological examination eight days subsequent to cannulation with 0·51 mm tubing.

RESULTS

Histology

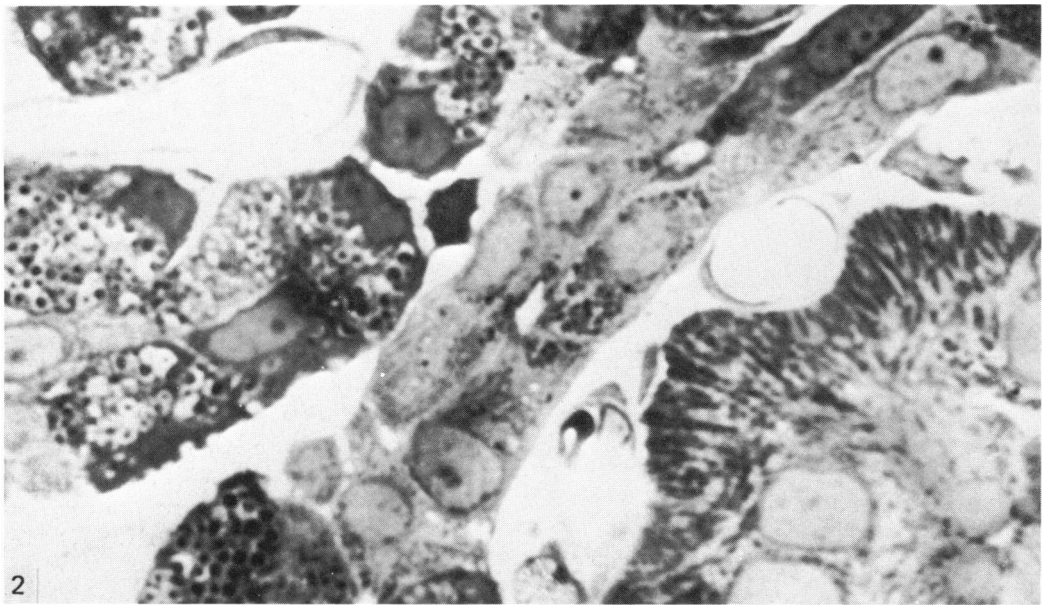
Light microscopy

Mature dog. The non-lobulated lateral nasal gland acini constituted most of the maxillary recess propria-submucosa rostrally, laterally, and ventrally (Table 1). Elongate acini, 28 μm in diameter with 1·5 μm lumina, were composed of pyramidal cells with basal, oblate nuclei. Acinar cells were distended with numerous homogenous vacuoles $\leqslant 2{\cdot}5$ μm in diameter, many of which contained basophilic cores $\leqslant 1{\cdot}8$ μm in diameter (Fig. 1). The peripheral shells of the secretory vacuoles (non-staining with basic stains) were AB-positive at pH 2·5 and negative at pH 1·0. The basophilic central portion of smaller vacuoles imparted a PAS-positive tinge to paraffin sections. Some larger basophilic granules appeared to be separate from the vacuoles. Intercalated ducts were composed of pale non-granular cells with lightly staining ellipsoidal nuclei and terminal bars were present between duct cells. Intralobar striated ducts were composed of cells 16-19 μm tall with nuclei centrally positioned. Dark duct cells present were not easily differentiated from light cells with BF–Az or AB–PAS.

Immature dog. Lateral nasal gland acini of the juvenile dog were smaller (22 μm) than those of the mature dog, constituted less of the subepithelial tissue (Table 2), had a lower cytoplasmic to nuclear volume ratio (6·4 : 1 vs. 17·5 : 1), and contained numerous smaller acidophilic vacuoles $\leqslant 2$ μm with basophilic cores (Fig. 2). As the basophilic cores were rarely larger than 1 μm in diameter, the acinar cells appeared light in plastic sections stained with basic stains. Intercalated ducts constituted a larger percentage of the lateral nasal gland in young dogs than in adult dogs (Table 1). The apical cytoplasm of many intercalated duct cells contained spherical basophilic granules $\leqslant 0{\cdot}7$ μm. A small percentage of the intercalated duct cells were dark with darkly staining nuclei. The interstitium which formed a greater portion of the propria-submucosa in the young dog than in the mature animal, contained numerous small vessels, nerves, plasma cells, fibroblasts and collagenous fibres.

Electron microscopy

Acini of dog lateral nasal gland had an infra- and paranuclear array of granular endoplasmic reticular membranes with dilated cisternae (Fig. 3). Junctional complexes were present between cells adjacent to intercellular canaliculi and acinar lumina. Interdigitating cytoplasmic extensions were prominent laterally between adjacent acinar cells and over the basal surface of the cells; foveae were present in the basal surface of many acinar cells into which projected cytoplasmic processes of the same or adjacent cells (Fig. 4). The acinar cell was packed with spherical,



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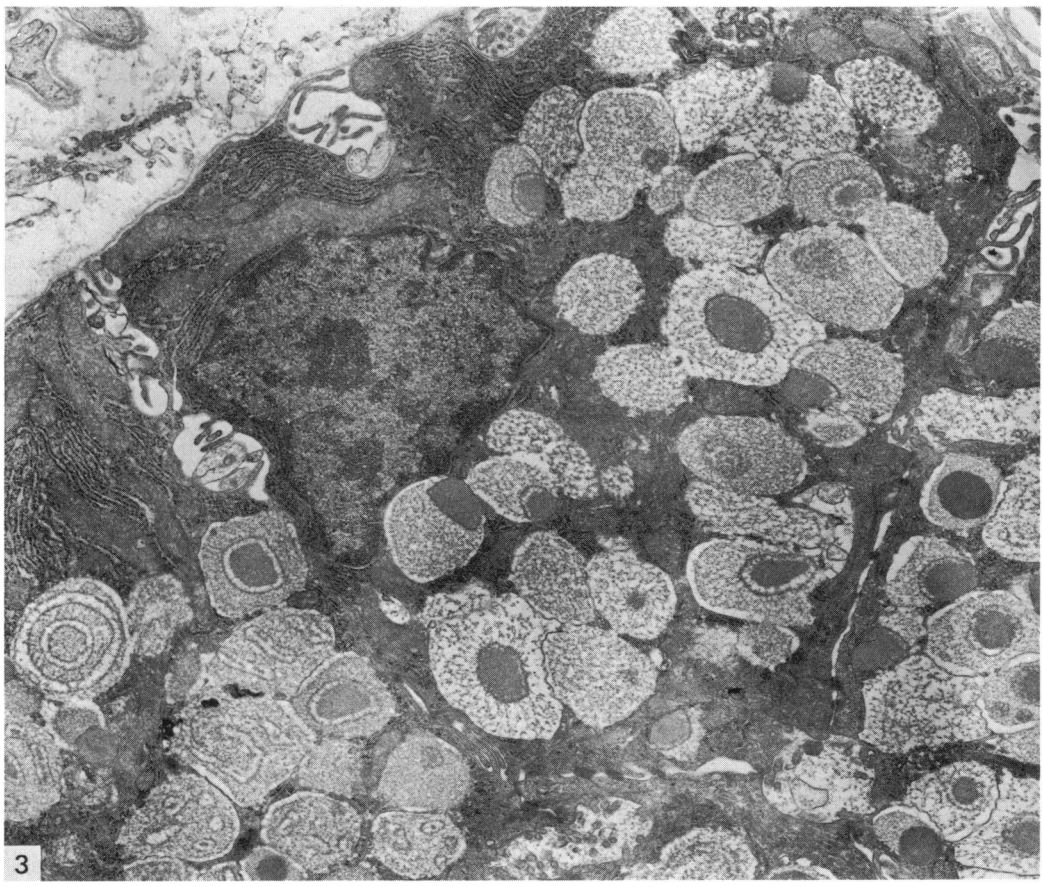

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Table 1. *Per cent composition (mean ± S.D.) of canine lateral nasal gland samples*

Age	No.	Acini	Intercalated duct	Interstitium + striated duct
12-15 weeks	5	50·1 ± 5·8	10·0 ± 1·7	39·9
Adult	3	70·4 ± 10·8	5·6 ± 2·8	24

Table 2. *Per cent composition (mean ± S.D.) of canine lateral nasal gland acini*

Age	No.	Cytoplasm	Nucleus
12-15 weeks	5	86·4 ± 1·7	13·6 ± 1·7
Adult	3	94·6 ± 1·8	5·4 ± 1·8

lucent vacuoles $\leqslant 2{\cdot}0$ μm containing electron-dense material of diverse configurations. Intercalated duct cells contained granular endoplasmic reticulum and numerous clusters of free ribosomes. Lateral cell membranes were maintained in parallel apposition by desmosomes and by a juxtaluminal junctional complex. Numerous microvilli projected into the duct lumen.

Basal and lateral cell membranes of striated ducts were folded; numerous basally situated, elongate mitochondria with tubular cristae were present between the membrane infoldings of the light duct cell. Juxtaluminal junctional complexes were present between duct cells.

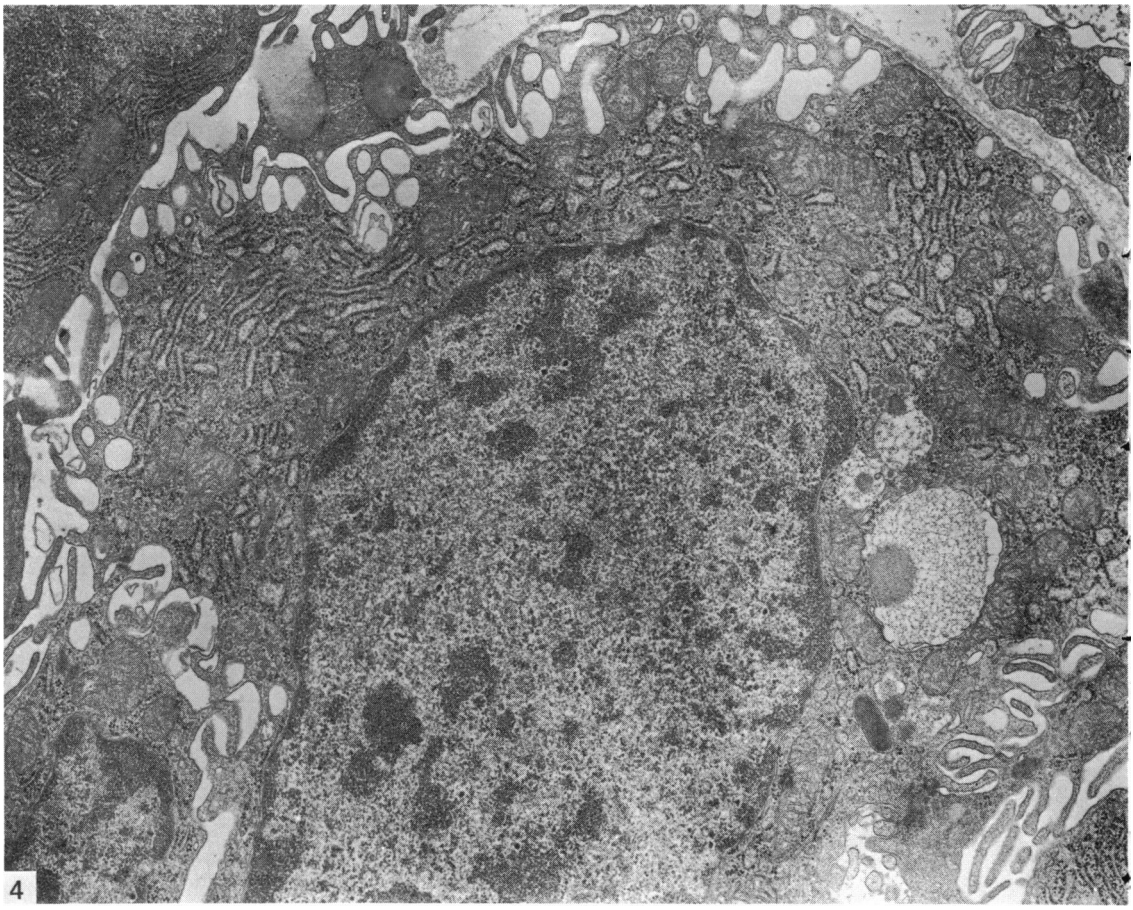
Biochemical analysis of secretions

Some dilatation present in intercalated ducts and widened subluminal intercellular spaces of striated ducts of a cannulated lateral nasal gland provided evidence of a degree of abnormalcy in secretory flow. However, secretory granules in acini from a chronically cannulated lateral nasal gland appeared similar to those in gland acini from non-cannulated dogs (Fig. 5). Mean IgA concentration values of secretions collected from 0·86 and 0·51 mm cannulae were similar.

The 0·33 ml/hour secretory rate ranged from 0·14-0·42 in the three dogs and from 0·19-0·41 for four different time periods (Table 3). IgA constituted 14% of the total protein present (a $2\frac{1}{2}$ times higher value may be more correct as serum IgA standards were used; Mygind & Thomsen, 1976). Electrolyte levels present (mean ± SE) were: Na^+-14·2 ± 1·0 mequiv/L ($N = 26$); K^+ − 29·6 ± 1·0 mequiv/L

Fig. 2. Twelve weeks old dog. Light micrograph of lateral nasal gland. Dense-cored vesicles are present in acinar cells. The apices of intercalated duct cells contain basophilic, electron-dense granules. Epoxy/Araldite section. Basic fuchsin–azure II stain. × 2436.

Fig. 3. Fifteen weeks old dog. Electron micrograph of lateral nasal gland acinus. Granular vacuoles with and without dense cores occupy much of the supra- and paranuclear cytoplasmic space. Cytoplasmic extensions are present laterally and basally. Non-myelinated nerve fibres are positioned in caveolae of the acinar cell. × 11 763.



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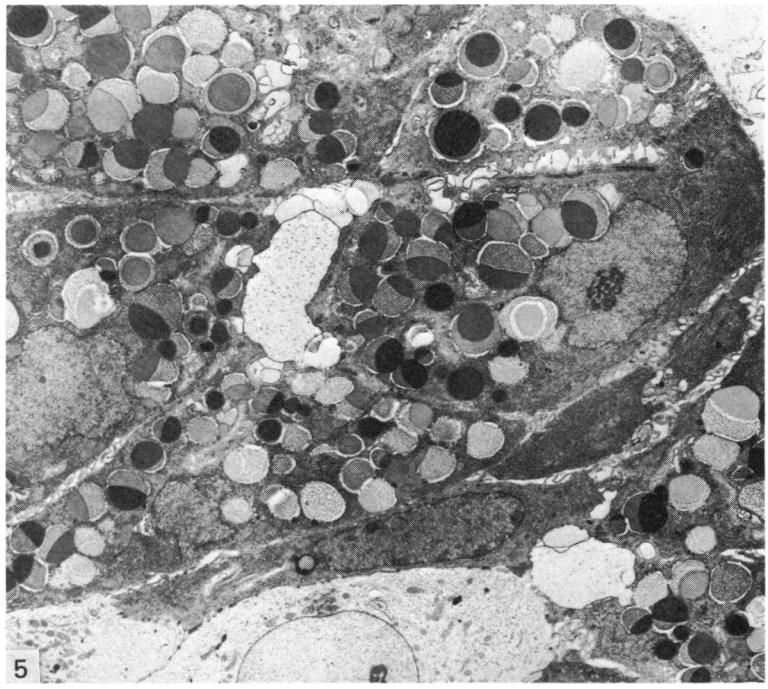

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Table 3. *Secretory rate and content of the dog lateral nasal gland*

Time period	Secretory rate (ml/hr)		Total protein (mg/ml)*		IgA (mg/100 ml)†		Sialic acid (mg/100 ml)‡	
	N	Mean ±S.E.	*N*	Mean ±S.E.	*N*	Mean ±S.E.	*N*	Mean ±S.E.
3 a.m.-9 a.m.	8	0·28±0·07	11	2·93±0·51	6	30·6±7·8	8	27·00±2·17
9 a.m.-3 p.m.	19	0·41±0·07	23	2·02±0·19	8	29·9±6·7	19	24·78±1·65
3 p.m.-9 p.m.	12	0·38±0·11	16	2·20±0·26	5	45·2±7·8	12	21·63±1·79
9 p.m.-3 a.m.	11	0·19±0·04	12	2·26±0·52	8	29·9±5·9	12	23·90±2·34
Mean (3 dogs)	50	0·33±0·04	62	2·35±0·17	27	32·9±3·5	50	24·21±1·01

* Total protein determined by Bio-Rad dye at OD_{595}.
† Immunoglobulin content determined by radial immunodiffusion.
‡ Sialic acid determined by modified thiobarbituric acid assay.

(N = 26); and $Cl^- - 23{\cdot}8 \pm 1{\cdot}5$ mequiv/L (N = 15). No significant difference in rate of secretion, total protein, IgA, or sialic acid concentrations were present between the four different time periods analysed (Table 3).

DISCUSSION

Although the lateral nasal gland is well developed in fetal pigs with acini both PAS-and AB-positive (Bojsen-Møller), 1967), acinar volume of 15 weeks old dogs is approximately 70% that of mature dogs. The secretory content of the lateral nasal gland acinar cells is predominantly sialomucin; the vesicles contain a protein core with a peripheral layer of sialomucin similar to vacuoles of salivary glands (Ichikawa & Ichikawa, 1975). Myoepithelial cells, not present in the proximal acini of rat anterior medial nasal septal glands (Tandler & Bojsen-Møller, 1978), are also not observed in dog lateral nasal gland acini.

Augmentation of secretory flow from a dog anticipating being fed sausage during this study suggests that psychological factors may be important to the study of lateral nasal gland secretions. The processes of harvesting collection vials and animal care most probably induce differing secretory contents and rates. Beta adrenergic receptors, thought to be the primary mediators of increased mucous secretion (Bogart & Picarelli, 1978; Gallagher *et al.* 1975), may regulate the secretory protein content of lateral nasal glands. In work demonstrating a substantial increase in secretion of sialic acid from adrenergically stimulated rat submandibular gland tissue, Bogart & Picarelli (1978) proposed the presence of cholinergic and of alpha and beta adrenergic receptors in the membrane of acinar cells.

Although the composition and function of proteinaceous nasal secretions remains largely unknown (Tandler & Bojsen-Møller, 1978), the presence of large amounts

Fig. 4. Fifteen weeks old dog. Electron micrograph of basal cellular processes and cytoplasmic extensions from acinar cells of the lateral nasal gland. Dilated cisternae of basally positioned granular endoplasmic reticular membranes are evident. × 17524.

Fig. 5. Mature dog. Electron micrograph of an acinus from a lateral nasal gland whose duct was cannulated with 0·51 mm ID tubing for 8 days. The dilatation of acinar lumen and increased nuclear translucency present, indicative of increased synthesis, may have resulted from an altered secretory flow. × 3310.

of sialic acid and immunoglobulin A in secretions which discharge into the nasal vestibule is indicative of a protective function. The pathway by which IgA reaches the lumen of the nasal gland may be similar to the epithelial transmission as described by Bradley, Bourne & Brown (1976) and Brandtzaeg (1974). The proximity of plasma cells to the basal lamina and the complexity of cytoplasmic extensions from the base of acinar cells may contribute to the transfer of the IgA dimer. IgG and IgM contribute little to the protein content as they are present in quantities too low to be measured by the radial immunodiffusion technique utilized in this study (< 3 mg IgM and 25 mg IgG/100 ml secretion).

The non-IgA component of secretory protein, 86% of the total, not characterized in this study might include lysozyme, peroxidase or other bactericidal/virucidal substances.

SUMMARY

Lateral nasal glands of 12-15 weeks old pups are immature. Gland acini of pups and mature dogs contain numerous electron-lucent vacuoles with basophilic, electron-dense cores. The vacuoles contain both acid and neutral glycoproteins, sialated glycoproteins being the dominant acidic moiety. Lateral and basal cytoplasmic extensions of the acinar cytoplasm greatly increase the cell surface area. Electron-dense granules, smaller and less numerous than the lucent vacuoles of acini, occur in intercalated duct cells.

Secretions collected from the excretory duct of the lateral nasal gland from conscious dogs contain 235 mg protein/100 ml secretory fluid. Immunoglobulin A accounts for 14% of the secretory protein. Total sialic acid content is 0·024% of the secretion by weight.

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