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Lectin Binding Pattern of Neuroepithelial and Respiratory Epithelial Cells in the Gerbil Nasal Cavity

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In this study, we applied a panel of lectins for histochemical mapping of carbohydrates in the various types of epithelia in the nasal cavity of the gerbil. Lectin histochemistry at the light microscope level was used to determine the distribution of sugar residues on cell surface.

Seven 20-day gerbils were decapitated and sectioned through nose with 3mm thickness. A panel of seven biotinylated lectins (Con A, WGA, RCA I, PNA, SBA, DBA, UEA I) were applied to the samples and the lectins were demonstrated by avidine-biotin-horseradish peroxidase complex technique with 0.05% DAB staining. In the nasal respiratory epithelium, the cilia reacted with all lectins except PNA. The lateral nasal wall cilia reacted with UEA I, but the medial wall did not react with UEA I. The cytoplasm of the epithelium did not react with WGA, PNA and UEA I. The goblet cells reacted with all the lectins except Con A. The submucosal glands in the respiratory epithelium did not react with DBA. For Con A and DBA, there existed 2 groups of reaction and non-reaction in the submucosal glands. In olfactory neuroepithelium, the ciliary layer reacted with the lectins except UEA I and the receptor neurons and olfactory nerve fibers did not react with Con A and UEA I. The Bowman gland did not react with Con A and PNA. In vomeronasal organ, the surface reacted with all the lectins. The receptor neurons and nerve fibers did not react with PNA and DBA.

Thus, the presence of different sugars on the surface of the different types of epithelia in the gerbil nasal cavity was demonstrated indirectly by lectin binding patterns. With these basic results from gerbil nasal mucosa, we expect to be able to perform further nasal functional and morphological studies in the gerbil nasal mucosa.(Ajou Med J 1997; 2(2): 119~125)

Key Words: *Lectin, Nasal Mucosa, Gerbil*

INTRODUCTION

Lectins are sugar binding proteins or glycoproteins of non- immune origin extracted from plants or animals and have a charactristic property to bind with specific carbohydrates. Thus, the epithelium can be classified by the lectin-binding patterns of tissues.

The nasal epithelium of the mammals can be grouped as squamous and respiratory epithelium and neuroepithelium and mammalian neuroepithelium in the nasal mucosa can be divided into 2 types; olfactory and vomeronasal organ neuroepithelium. In the human nasal mucosa, the vomeronasal organ is rudimentary, however in the rodents it is prominent.

So we could compare easily the nasal neuroepithelium in the rodents by lectin-binding patterns which have been used to study the different carbohydrate residues on cell surfaces. Also in this study, we attemped to demonstrate indirectly the functional differences by lectin-binding patterns in the various types of epithelia including respiratory epithelium of the gerbil nasal cavity.

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MATERIALS AND METHODS

Seven 20-day mongolian gerbils were decapitated and sectioned through nose with 3 mm thickness. Fixation with 10 % formalin and decalcification with 5% nitric acid for 36 hours was done.

Tissues were dehydrated with ethanol and embedded in the paraffin. Frontal sections with 6 micron-thickness of the decapitated specimen was done and slide mounting and drying at 58°C for 1 hour for lectin histochemistry were prepared. After deparaffinization and hydration, the slides were immersed in 0.3% H_2O_2 in methanol for 30 minutes to reduce intrinsic peroxidase activity. One percent of bovine serum albumin (BSA) in PBS was applied on the slides for 5 minutes at room temperature. A panel of biotinylated lectins (Vector laboratory, CA, USA) was applied to the specimens in a moist chamber for 30 minutes at room temperature.

For the demonstration of the biotinylated lectins, avidine-biotin-horseradish peroxidase complex (Vectastain ABC kit, Elite PK-610, standard, Vector laboratories, CA, USA) was used. A solution of 0.05% diaminobenzene hydrochloride (DAB, Sigma, MO, USA) staining for 10 minutes and washing in distilled water for 10 minutes were done. The slides were counter-stained with hematoxyline and dehydrated for the examination.

We analysed lectin-binding patterns for histochemical mapping of carbohydrates in the various types of epithelia in the nasal cavity of gerbil including respiratory epithelium and neuroepithelia of olfactory and vomeronasal organ (Fig. 1). Receptor neurons of olfactory and vomeronasal epithlium were analysed separately.

The lectin-binding pattern of the different structures were classified as −: negative, −/+: partial weak positive, +: weak positive, ++: strong positive, +++: very strong positive (Fig. 2). This classification was decided by comparing the DAB staining activity to the specimen as no stain (−), very lightly stained (+), lightly stained (++), stained in brown color (+++), and stained in dark-brown color (++++).

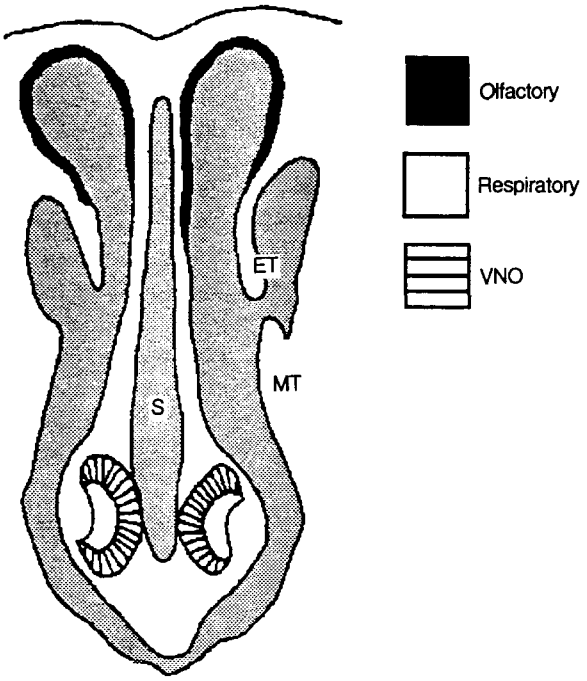

Fig. 1. Sxhematic drawing of gerbil nasal cavity. VNO: vomeronasal organ, ET: ethmoturbinal, MT: maxilloturbinal, S: septum

Table 1. Lectins used in study

Name	Abbreviations	Major specificity to carbohydrates
1) Concanavalia ensiformis	Con A	Mannose>Glucose
2) Triticum vulgare	WGA	N-acetylglucosamine> Sialic acids
3) Ricinus communis agglutinin	RCA I	βGalactose>αGalactose
4) Arachis hypogaea	PNA	Galctoseβ1>3GalNAc> Galactose
5) Glycin max	SBA	N-Acetylgalactosamine> Galactose
6) Dolichos biflorus agglutinin	DBA	N-acetylgalactosamine>> Galactose
7) Ulex europaeus agglutinin	UEA I	Fucose

RESULTS

In the respiratory mucosa (Table 2 and Fig. 3), the cilia reacted with all the lectins except PNA. The lateral nasal wall cilia reacted with UEA I, but in the medial wall, the cilia did not react with UEA I. Even in the same type of the repiratory mucosa, lectin binding patterns were different depending on the location for UEA I lectin. The cytoplasm of the repiratory epithelium did not react with WGA, PNA and UEA I. The goblet cells reacted with all the lectins except Con A.

In the olfactory epithelium (Table 3 and Fig. 4), the ciliary layer reacted with the lectins except UEA I. The DBA reacted strongly on the ciliary layer than other lectins. The The receptor neurons and olfactory nerve fibers did not react with Con A and UEA I.

In the vomeronasal oran epithelium, the surface reacted with all the lectins. The DBA reacted partially on the surface. The receptor neurons and nerve fibers did not react with PNA and DBA(Table 4 and Fig. 5).

The submucosal secretory glands in the respiratory epithelium did not react with DBA(Table 5 and Fig. 3). For Con A and DBA, there existed 2 groups; reaction and

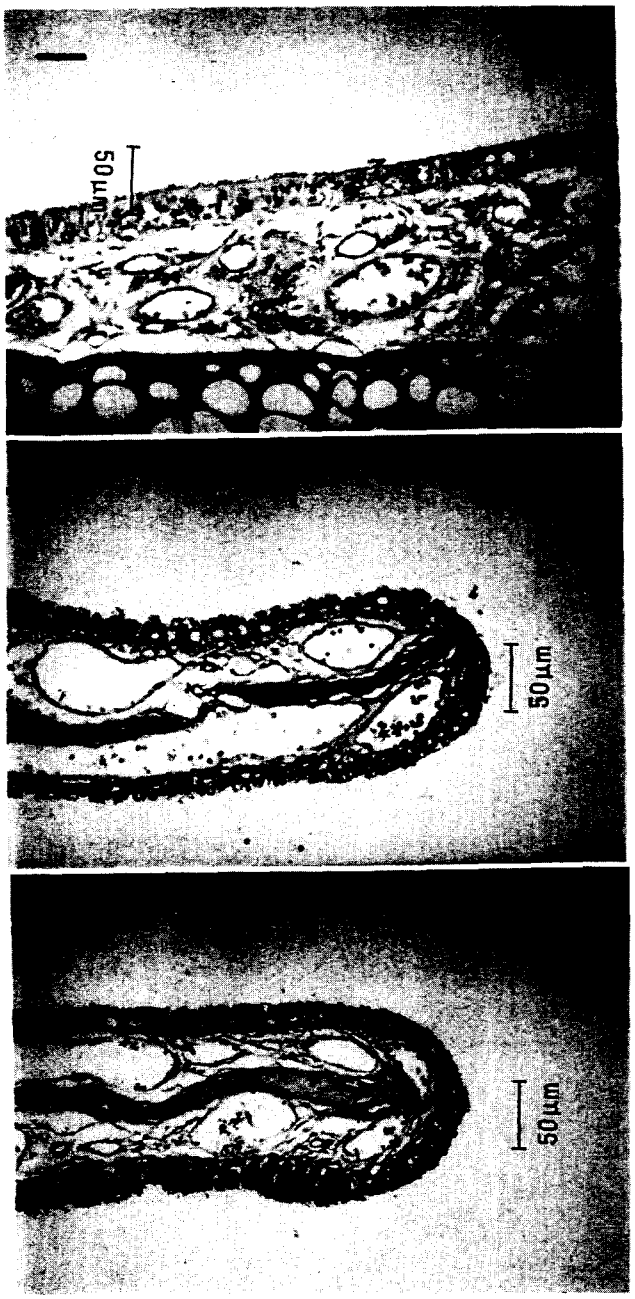

Fig. 2. Examples of lectin-biding activity (X200).
− : respiratory mucosa epithelium of medial nasal wall (UEA I)
+ : olfactory mucosa epithelium of medial nasal wall (UEA I)
++ : respiratory mucosa of ethmoturbinal (WGA)
+++ : respiratory mucosa of ethmoturbinal (DBA)

Table 2. Lectin binding pattern in respiratory epithelium

Lectins	Ciliated cells		Goblet cell
	Cilia	Cytoplasm	
Con A	+	+	−
WGA	++	−	+
RCA I	++	+	+
PNA	−	−	+
SBA	++	+	+
DBA	+++	+	+
UEA I	−/+	−	+

Table 3. Lectin binding pattern in olfactory neuroepithelium

Lectins	Olfactory epithelium		Olfactory nerve fiber
	Cilia	Receptor-neuron	
Con A	+	−	−
WGA	++	+	++
RCA I	++	+	+
PNA	−/+	+	++
SBA	++	+	+
DBA	+++	++	++
UEA I	−	−	−

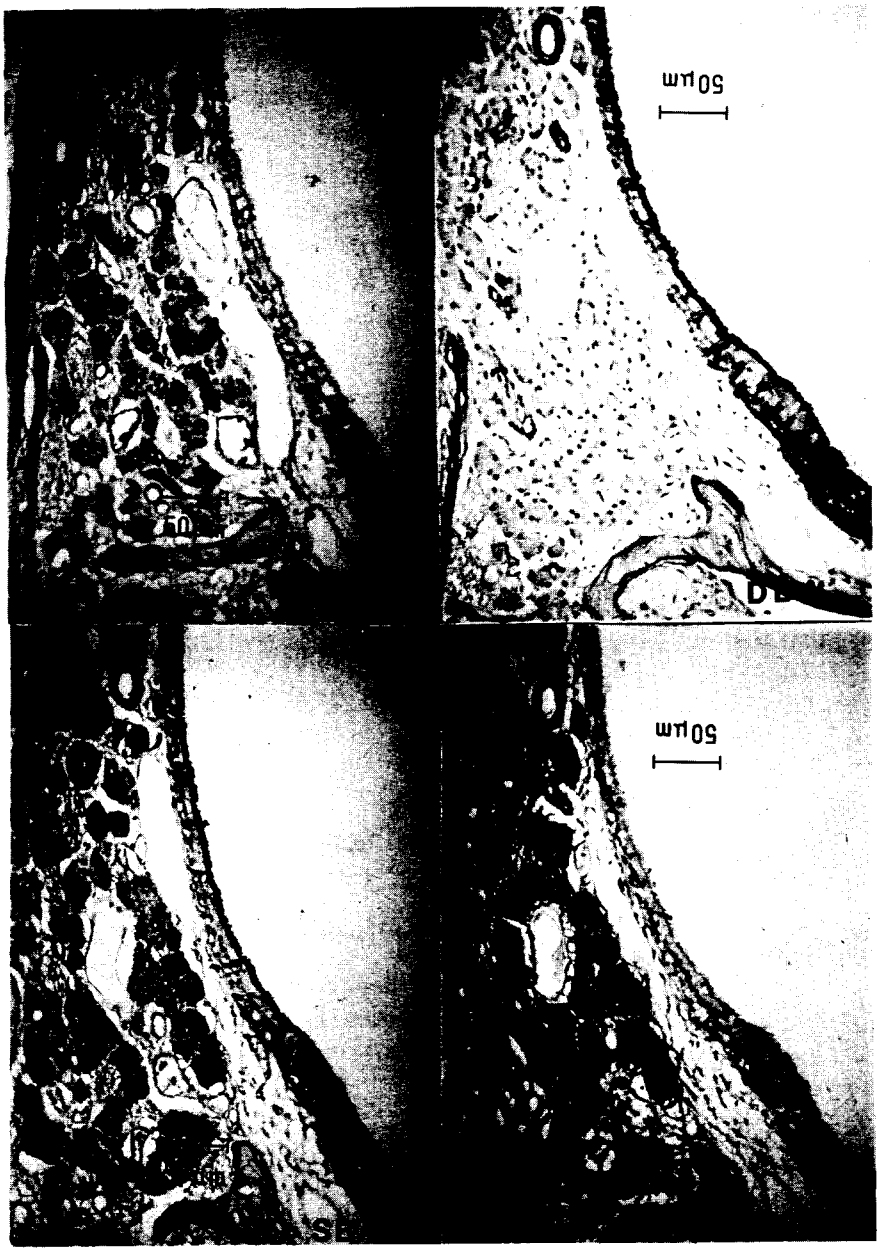

Fig. 3. Lectin binding to the repiratory epithelium (X200).
PNA: binding with submucosal galnds and goblet cells
DBA: binding with cilia and cytoplasm except submucosal glands
SBA: binding with cilia, cytoplam and submucosal glands
UEA I : binding with goblet cells and submucosal glands

non- reaction. In the olfactory epithelium, the Bowman gland did not react with Con A and PNA (Table 5 and Fig. 5).

DISCUSSIONS

Lectins are sugar binding protein or glycoprotein used for the studies of wound healing process, developmental differentiation of respiratory epithelium and detection of malignant tumors[1]. In the nasal epithelium, the carbo-

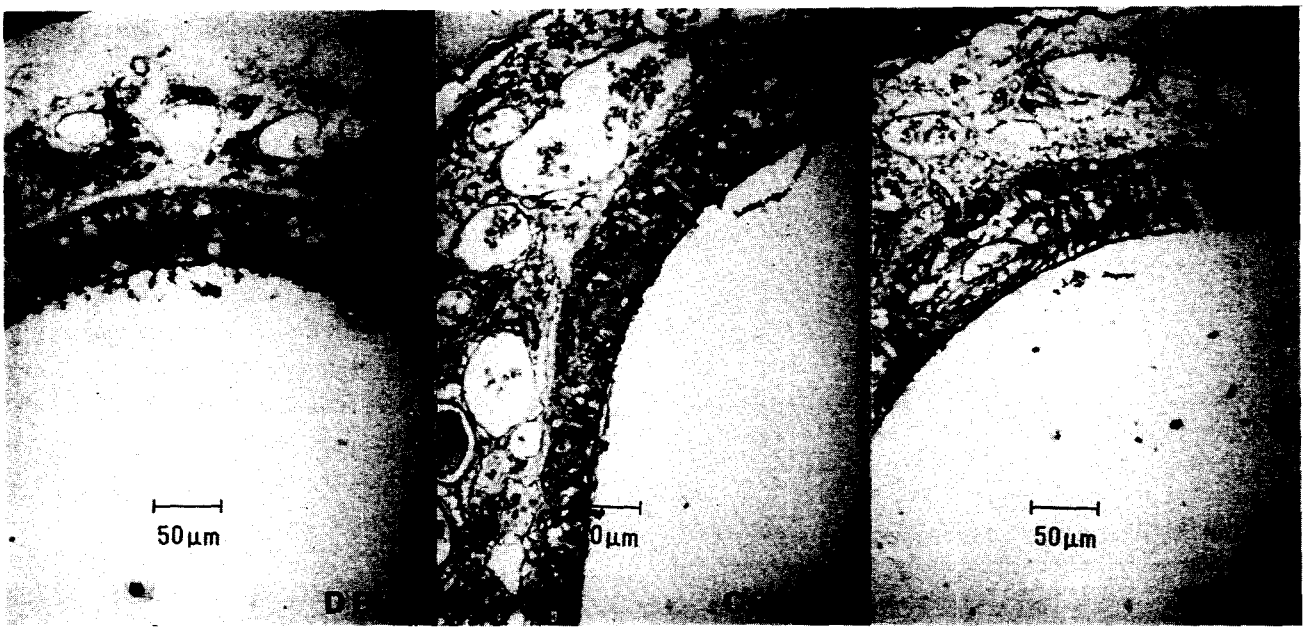

Fig. 4. Lectin binding to the olfactory epithelium (X200).
DBA: strong binding with cilia, receptor-neurons and nerve fibers
Con A: binding with cilia
WGA : binding with cilia, receptor neurons and nerve fibers

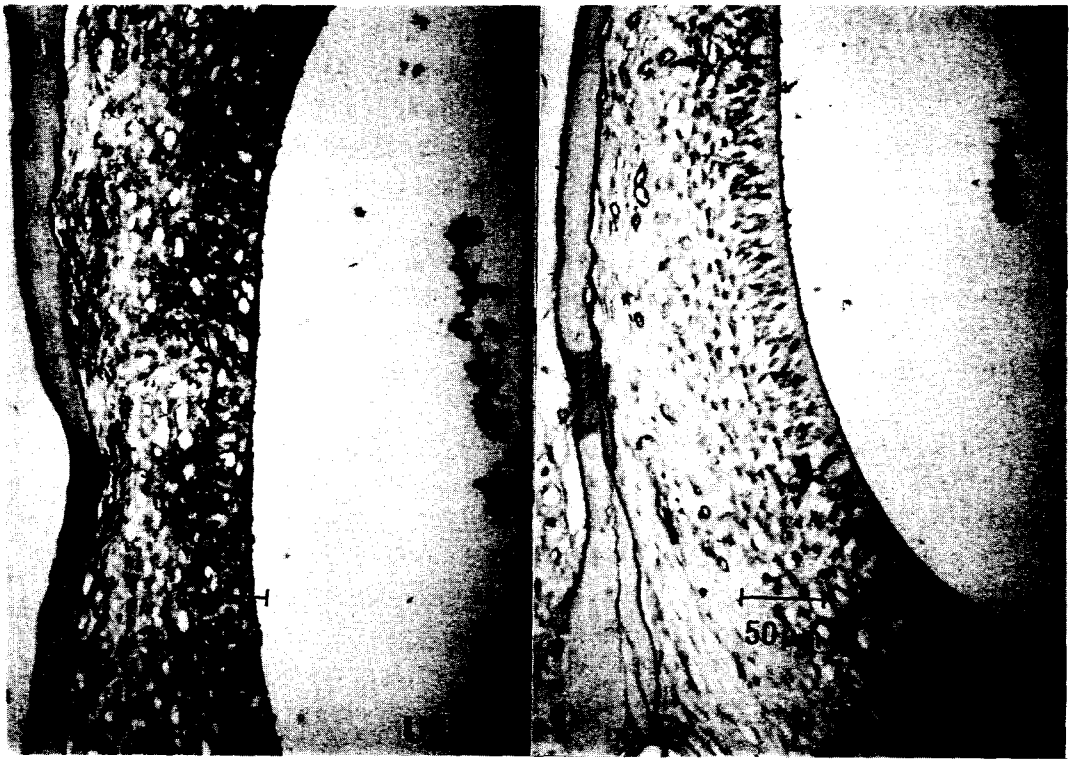

Fig. 5. Lectin binding to the vomeronasal organ (X200).
UEA I: strong binding with surface, receptor-neurons and nerve fibers
PNA: binding with surface only

Table 4. Lectin binding pattern in vomeronasal organ

Lectins	Vomeronasal epithelium		Vomeronasal nerve fiber
	Surface	Receptor-neuron	
Con A	+	+	+
WGA	++	+	++
RCA I	++	+	+
PNA	++	−	−
SBA	++	++	+
DBA	−/+	−	−
UEA I	++	++	++

Table 5. Lectin binding pattern in secretory glands

Lectins	Submucosal glands	Bowman glands
Con A	−/+	−
WGA	+	+
RCA I	−/+	+
PNA	+/++	−
SBA	+	++
DBA	−	+
UEA I	++	+

hydrate terminal of the cell membrane binding characteristic of the lectin can distinguish functional difference[2]. The physiologically important glycoproteins are distributed on the cell surface and cytoplasm in the nasal cavity. These glycoproteins are thought to be related with cell adhesion, cell differentiation and information changes between cells.[3] Especially the mucopolysaccharides secreted from the nasal mucosa are thought to be closely related with density of nasal seceretions, mucosal edema, mucociliary transport function and adhesion for viruses.[2,4~6]

There exist variable lectin-binding patterns depending on the species on the same type of epithelium. There have been several studies about lectin binding of the nasal cavity mucosa including olfactory and respiratory epithelium in various species; human,[5~8] mouse,[2] and rat.[9~11] Ueno et al.[11] studied differences in terminal carbohydrate structures of sialomucin in the murine nasal cavity. They suggested that different carbohydrate structures of sialomucin in the nasal cavity may reflect differences in susceptibility to bacterial colonization and viral infection between respiratory and olfactory epithelium, and that different carbohydrate structure influence rheological properties of nasal secretion. In the rodents, the lectin-binding patterns of the nasal cavity mucosa were found to be different between each species. But there has been no report about the lectin-binding pattern of the gerbil nasal cavity.

Our study may suggest a functional difference by different lectin-binding patterns in the epithelia and secretory glands. The submucosal glands may secrete different mucopolysaccharides, because of the Con-A, RCA I and PNA staining patterns (Table 5). So the functional difference of the secretory glands were demonsted by lectin-binding patterns.

There has been also a report that some neurotrophic viruses which attack the olfactory epithelium could be explained by viral affinity to some type of lectins on the epithelium. Lundh et al.[2] studied the lectin binding patterns of various epithelium in the mouse nasal cavity, and suggested that the different sugars are exposed on the surface of the different types of epithelia in the nasal cavity, thereby providing a basis for selectivity in microbial attacks on these areas. Also Hofmann et al.[12] suggested that the different lectin-binding patterns in the olfactory epithelium reflect functionally different subtypes of olfactory epithelial cells.

In the present study, we demonstrated different lectin-binding patterns in the nasal neuroepithelia. This may suggest a functional difference beetween olfactory and vomeronasal organ epithelium and also different susceptability to certain vitus can be explained by different types of terminal sugar of the neuroepithelium.

Pastor et al.[9] carried out studies on the glycoconjugates of the nasal mucosa of rat and guinea pig using conventional techniques with peroxidase-labelled lectins. The lectin-binding patterns were different between rat and guinea pig. When compared the results of Pastor et al.[9] and Lundh et al.[2], the nasal lectin- binding patterns of the gerbil were found to be different rat, guinea pig or mouse.

It is suggested that these results with lectin can be usefully exploited for further study when functional or morphological studies with gerbil nasal cavity are carried out.

CONCLUSIONS

Difference in the lectin-binding capacity of gerbil between neuroepithelia and the respiratory epithelium in the nasal cavity showed that there exist differences in terminal sugars in the various types of epithelia and secretroy glands. Functional difference between the nasal respiratory, olfactory and vomeronasal organ epithelium could be explained by different lectin-binding patterns of the cell surfaces and cytoplasms of each epithelium with these results from gerbil nasal mucosa on hand, we expect to be able to perform further functional and morphological studies with gerbil nasal mucosa.

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