J. Life Science. (2000), 10(2), 1~8

JOURNAL OF
Life Science

Histochemical Study on Glycosaminoglycans of Esophageal Mucous Cells in *Agramus agramus, Inimicus japonicus, Epinephelus chlorostigma* and *Helicolenus dactylopterus*

Kwun Soon Jung, Mal Sook Hong, Gi Jin Jo and Un Bock Jo†

Department of Biology Education. Pusan National University, Pusan 609-735, Korea

Received: August 20, 2000

Abstract This experiment was performed to study the structure of esophageal mucousa and the histochemical properties of glycosaminoglycans of esophageal mucous cells in four teleostean species, i.e., *Agramus agramus, Inimicus japonicus, Epinephelus chlorostigma* and *Helicolenus dactylopterus*. To observe the structure of eosphageal mucosa, hematoxylin-eosin(H-E) staining was used. The glycosaminoglycans was stained with PAS(periodic acid schiff), alcain blue(AB) pH 2.5, AB pH 1.0, aldehyde fuchsin(AF) pH 1.7, AF pH 1.0, AB pH 2.5-PAS, AB pH 1.0-PAS and AF pH 1.7-alcian blue pH 2.5.

As for the amount and histochemical composition of glycosaminglycans in *Agramus agramus*, most of mucous secreting columnar cell and mucous cells contain large and moderate amount of neutral glycosaminoglycans. A few of mucous cells having small amount of neutral glycosaminoglycans and minimal amount of sulfated glycosaminoglycans. In *Inimicus japonicus*, the esophageal mucous cells were composed of most of medium sized and large mucous cells with moderate amount of neutral glycosaminoglycanonly only, a few of medium sized and large mucous cells and most of small mucous cells with considerable amount of neutral glycosaminoglycans and minimal to small amount of nonsulfated glycosaminoglycans, and a few of small mucous cells with small amount of neutral glycosaminoglycans and minimal amount of sulfated glycosaminoglycans. In *Epinephelus chlorostigma*, most of medium sized and large mucous cells were mixed small amount of neutral glycosaminoglycans with sulfated glycosaminoglycans, a few of which were contained with moderate or considerable amount of neutral glycosaminoglycans with sulfated glycosaminoglycans, while most of small mucous cells containing considerable amount of neutral glycosaminoglycans and small to moderate or considerable to minimal amount of nonsulfated glycosaminoglycans(sialomucin) a few of which containing a mixture of considerable amount of neutral glycosaminoglycans and considerable amount of nonsulfated glycosaminoglycans or containing minimal amount of nonsulfated glycosaminoglycans(sialomucin) only. In *Helicolenus dactylopterus*, most of medium sized and large mucous cells, mixing with moderate to considerable amount of neutral glycosaminoglycans, a few of which containing a mixture of small to considerable amount of neutral glycosaminoglycans and small to moderate amount of sulfated glycosaminoglycans, while most of small mucous cells with considerable amount of neutral glycosaminoglycans and moderate amount of nonsulfated glycosaminoglycans (sialomucin), a few of which having a mixture of considerable amount of neutral glycosaminoglycans and considerable or small amount of nonsulfated glycosaminoglycans(sialomucin).

Key words: *Glycosaminoglycans, Esophageal mucous cells, Histochemisty, Fishes*

Introduction

In fishes, the length of esophagus is shortest, and the mucosa and submucosa are complicated not only by the primary longitudinal folds but also by secondary and even tertiary foldings [1,2]. The esophagus was lined by a stratified squamous epithelium [3-7,9] or a psuedosratified squamous epithelium [23]. Especially the esophageal epithelium is consisting cheifly of goblet cell [2] or mucous cells [3-5,10-12,6-9].

The important function of teleostean esophagus is not only to conduct food stuffs to the stomach but also from pharynx to distal region of the gut. An aboundance of mucous cells indicates that esophageal glycosaminoglycans have some role in the digestive process [6].

Many researches have been conducted on the histological and histochemical property of glycosaminoglycans in the

†Corresponding author
Phone: 82-51-510-2696, Fax: 82-51-514-8576
E-mail: ubjo@hyowon.cc.pusan.ac.kr

esophageal mucous cells of many teleostean fishes, such as *Parasilurus astous* [10], eel [11,12], rock bass, bluegill sunfish, large mouth bass, black crappie, golden shiner, fathead minnow, grass pickeral, nothern pike, brown bullhead, yellow perch [6], *Clupanodon punctatus, Parasilurus asotus, Carassius auratus, Pagrosomus majar, Cantherine moderstus* [7], *Cyprinus carpio, carassius auratus, Parasilurus astous, Mugil cephalus. Siniperca scherzei, Sebastes hubbsi, Agramus agramus, Pleuronichthys cornutus* [8], *Lateolabrax japonicu, Seriola quinqueradiata, Sparus swinhonis, Sebaste inermis, Erosa erosa* [9] and reported that the size of mucous cells, distribution and property of glycosaminoglycans of mucous cells were different from each species.

The purpose of our study was to determine, using histological and histochemical technique, the characteristics of mucous cell types and, in particular, the glycosaminoglycans of mucous cells in the esophageal mucosa of four teleostean species of Family Percida, *Agramus agramus, Inimicus japonicus, Epinephelus chlorostigma* and *Helicolenus dactylopterus*.

Materials and Methods

The species studied are as follows. Family Percida : *Agramus agramus, Inimicus japonicus, Epinephelus chlorostigma* and *Helicolenus dactylopterus*. Middle pieces of esophagi were fixed for 24 hours in a solution of 10% neutral buffered formalin, dehydrate, and embedded in paraplast (melting point 56℃) according to routine methods. Sections were cut at 6㎛ in thickness, deparaffinized and the subjected to histological and histochemical staining procedures were as fallows :

1. Hematoxylin and eosin(H-E) staining for the general observation of histological structure.
2. Periodic acid-Schiff(PAS)reaction for studying neutral glycosaminoglycans [13].
3. Alcin blue(AB) pH2.5 staining for the demonstration of acid glycosaminoglycans [14-16].
4. AB pH1.0 staining for the selective characterization of sulphated glycosaminoglycans [20,21].
5. AB pH2.5-PAS staining sequence distinguishes PAS positive(red) neutral from AB positive(blue) acid glycosaminoglycans [17,18].
6. AB pH1.0-PAS staining sequence distinguishes PAS positive(red) neutral from AB positive(blue) sulphated glycosaminoglycans [21].
7. Aldehyde fuchsin(AF) pH1.7-AB pH2.5 staining sequence distinguishes AF positive(purple) sulphated from AB positive(blue) nonsulphated glycosaminoglycans [19].

With AB pH 2.5-PAS sequence, cells containing both acid and neutral glycosaminoglycans usually showed purple, bluish purple, or reddish purple staining. In the AF pH 1.7-AB pH2.5 sequence, cells staining bluish purple containing both sulphated and nonsulphated glycosaminoglycans.

Degrees and abbreviations in the tables designate staining results as follows : B, blue; R, red; BP, bluish purple; RP, reddish purple; 4, very intense; 3, intense; 2, moderate; 1, weak; ±, trace; 0, absent.

Result

Histological structure of esophageal mucosa

The four layers of a typical vertebrate digestive tract (mucosa, submucosa, muscular coat and adventitia) were consistently present in all species. The logitudinal folds were well developed, but there differed depend on the species. In *Agramus agramus,* the entire surface of the esophagus is covered with a mucosa thrown into longitudinal folds so arranged to give a dendroid like appearance that consisting of conical primary folds and high or low secondary folds. In *Inimicus japonicus,* the esophageal longitudinal folds were consist of long conical, primary folds from which were well developed many secondary folds with irregulary tortous in shape. In *Epinephelus chlorostigma,* the esophageal folds were well developed that consisting of conical primary folds from which secondary folds with fungiform or circumvallate in short shape. In *Helicolenus dactylopeterus,* the esophageal longitudinal folds were consist of high or low conical or cylinderical primary folds from which were well developed irregular secondary folds widely.

In all species, several parts of mucosal folds, long loop, fungiform, irregular loop form of small folds were formed, which was lined with simple cuboidal epithelium and many blood vessels are distributed in the connective tissue core, the other parts of which covered with stratified squamous epithelium.

Most part of esophageal mucosa of *Agramus agramus* which was composed of stratified squamous epithelium but it is especial that considerable part of which composed of simple columnar epithelium. In all of four species, many round, spherical, elliposid form mucous cells are distributed esophageal mucous epithelium but it was distinctively different in size, morphology and distribution according to each species.

In *Agramus agramus,* mucous cells were arranged with one-two or multiple layers, the spherical or elongated ellipsoid form large mucous cells and ellipsoid form of medium sized cells were mostly distributed and spherical small mucous cells were a few (fig. 1).

In *Inimicus japonicus* which were arranged with one-two layers, according to the part which appeared three-four layers. The spherical or ellipsoid form of large mucous cells were mostly abundant and ellipsoid medium sized cells and spherical small cells are mixed. In *Epinephelus chlorostigma,* mucous cells were composed ellipsoid, spherical large mucuos cells and spherical, conical, elongated ellipsoid small mucous cells and a few of ellipsoid medium sized cells

were mixed. In *Helicolenus dectylopterus* which were round spherical large cells and spherical or ellipsoid small cells and elongated ellipsoid, spherical medium sized cells were mixed. They arranged mainly two or three layers over epithelium, but somewhere, arranged multiple layers. The numbers of esophageal mucous cells including secreting columnar epithelial cells which were highest in *Agramus agramus* but which of mucous cells were highest in *Helicolenus dactylopterus, Epinephelus chlorostigma, Inimicus japonicus* was next, and lowest in *Agramus agramus.*

The property of glycosaminoglycans of esophageal mucous cells.

The result of histochemical property of glycosaminoglycans in esophageal mucous cells of four teleostean fish species are outlined in table 1 and 2.

Agramus agramus

Esophageal columnar epithelial cells of *Agramus agramus* containing only a large amount of neutral glycosaminoglycans but large mucous cells contain considerable or a small amount of neutral glycosaminoglycans. The former is more than the latter in distribution. Medium sized and small mucous cells having a considerable or small amounts of neutral glycosaminglycans except a few cells which having a mixutre of a minimal amount of sulfated glycosaminglycans and small amount of neutral glycosaminoglycans (figs. 2 and 3).

Inimicus japonicus

In *Inimicus japonicus,* esophagus mucosal epithelium con-

Table 1. Histochemical properties of glycosaminoglycans in the esophageal mucous cells in four teleostean species by single stains

Species	Stains Cell types	PAS(with and without diastase)	AB pH 2.5	AB pH 1.0	AF pH 1.7	AF pH 1.0
Agramus agramus	EC	4R	0	0	0	0
	L	3R>1-2R	0	0	0	0
	M	3R>4R,!R	0>±B	0>±B	0>±P	0>±P
	S	3R.>1-2	0>±B	0>±B	0>±P	0>±P
Inimicus japonicus	L	3R	0>±B. 1B	0	0	0
	M	3R>2R	0>±B. 1B, 2B	0>±B	0>±P	0>±P
	S	3R>2R,±R	±B,1B>0,2B	0>±B	0>±P	0>±P
Epinephelus chlorostigma	L	1-2R	1B>2B,±B	±-1B>2B	±-1P>2P	±-1P>2P
	M	1-2R	1B	1B,2B	1P	1P
	S	3R	1-2B>±B,3B	0	0	0
Helicolenus dactylopterus	L	2-3R>1B	1-2B	±-1B>2B	1-2P>3P	±-1P>2P
	M	3R	2B>3B	1B-2B	1-2P	1P
	S	3R	1B>2B	0	0	0

Degree of staining : 4, Very intense ; 3, Intense ; 2, Moderate ; 1, Weak ; ±, Trace ; 0, Absent
Abbreviations : EC, Epithelial columnar cell ; L, Large mucous cell ; M, Medium Sized mucous cell ; S, Small mucous cell ; AB, Alcian blue ; AF, Aldehyde fuchsin ; R, Red ; B, Blue, ; P, Purple ; >, Most marked.

Table 2. Histochemical properties of glycosaminoglycans in the esophageal mucous cells in four teleostean species by combined stains

Species	stains cell types	AB pH2.5-PAS	AB pH1.0-PAS	AFpH1.7-AB pH2.5
Agramus agramus	EC	4R	4R	0
	L	4R>R	4R>2R	0
	M	4R>3R, 1R>2RP	4R>3R, 1R>1-2RP	0>±P
	S	4R>1-3R>2RP	4R>1-3R>1-2RP	0>±P
Inimicus japonicus	L	3R>3RP, 3BP	4R	0>±B, 1B
	M	3R>3RP, 1-2BP	4R>3RP, 3BP	0>±B, 1B,±P
	S	3RP, 2P>3R, 3BP	4R>3RP, 3BP	±B, 1B>0,±P
Epinephelus chlorostigma	L	1BP>2BP,3BP,±BP	±-1BP>2BP, 3BP	±-1P>2P
	M	1BP>2BP,3BP	1BP>2BP	1P>2P
	S	2-4P>3RP	3-4R>3RP	1-2B>±B, 3B
Helicolenus dactylopterus	L	2-3BP>1P, 1BP	2-3BP>1P, 1BP	1-2P>3P, ±P
	M	3BP>3RP	3BP	1-2P>3P, ±P
	S	3-4P, 3RP	3-4R>3RP	2B>3B, 1B

Abbreviations : BP, Bluish purple ; RP, Reddish purple. Others are the same as in the table 1.

sists of large, medium sized and small mucous cell. Most of large and medium sized mucous cells with a considerable amount of neutral glycosaminoglycans, while a few of large mucous cells having a minimal or small amount of nonsulfated glycosaminoglycans(sialomucin) in addition to a considerable amount of neutral glycosaminoglycans, and a few of medium sized mucous cells containing a mixture of both a minimal or small amount of nonsulfated glycosaminoglycans and considerable neutral glycosaminoglycans or minimal, small amount of sulfated glycosaminoglycans. Most of the small mucous cells containing minimal or small amount of nonsulfated glycosaminoglycans(sialomucin) in addition to considerable amount of neutral glycosaminglycans but a few of them containing small amount of neutral glycosaminoglycans or small amount of sulfated glycosaminoglycans in addition to neutral glycosaminoglycans (figs. 4, and 5).

Epinephelus Chlorostigma

In *Epinephelus Chlorostigma* most of medium sized and large mucous cells having a mixture of small amount of neutral glycosaminoglycans and sulfated glycosaminoglycans, while a few of them containing moderate or considerable amount of neutral glycosaminoglycans and sulfated glycosaminoglycans. Most of small mucous cells containing both considerable amount of neutral glycosaminoglycans and small or moderate amount of nonsulfated glycosaminoglycans and a few of them containing a considerable amount of nonsulfated glycosaminoglycans in addition to considerable amount of neutral glycosaminoglycans or contain small amount of nonsulfated glycosaminoglycans (figs. 6 7, and 8).

Helicolenus dactylopterus

In *Helicolenus dactylopterus*, most of large and medium sized mucous cells containing both a small amount of sulfated glycosaminoglycans and a moderate or considerable amount of neutral glycosaminoglycans. A few of them containing a small or moderate amount of sulfated glycosaminoglycans in addition to a small or considerable amount of neutral glycosaminoglycans. But most of small mucous cells containing a considerable amount nonsulfated glycosaminoglycans. A few of them contain a considerable or small amount of nonsulfated glycosaminoglycans in addtion to a considerable amount of neutral glycosaminoglycans (figs. 9, 10 11, and 12)

Discussion

The esophagus of fishes appears short and functions solely in the transfort food from pharynx to distal region of the gut [1,2] like those of high vertebrate, large longitudinal folds were well developed [1,2] but exhibit adiversity accoding to species [7,9].

Our results on esophagus epithelium was consistent with previous reports. Esophageal longitudinal folds of *Agramus agramus* was dendroid like, *Inimicus japonicus* was cornical, *Epinephelus chlorostigma* cornical and *Helicolenus dactylopecus* were cornical or cylinderical form in shape and from which various secondary folds were formed. Esophageal mucosa of fish was pseudostratified squamous epithelium [23], or stratified squamous epithelium and in which mucous cells appeared [1,3-5,7,9,11,12,24] that assumed the form of goblet cells [2] or different form with typical goblet cells [1,7-9].

As for the esophagus mucosa of *Inimicus japonicus*, *Epinephelus chlorostigma* and *Helicolenus dactylopecus* showing stratified squamous epitehlium except *Agramus agramus* which consistent with the previous results but in *Agramus agramus* which was stratified squamous epithelium and mixed with simple columnar epithelium. And in all species secondary folds appeared in esophageal epithelium which have connective tissue cores that abundant blood vessels and covered with simple cuboidal epithelium that considered esophageal respiratory apparatus and a distinctive feature of Percida of teleostean.

Reifel and Travill [6] classified esophageal mucous cells of ten teleostean fish species A ,B, C, D, E and F type with morphology and according of hematoxylin-eosin stains and reported that type A cells appeared as spherical or oval, often pale looking cells with distinct basal, flattend nuclei, type B cells were irregular shaped or saccular with oval or spherical nunclei, and A, B type cells observed Centrachids(rock bass, bluegill sunfish and large mouth bass and black crappie), Cyprinnids(golden shiner and fathead minnow) and Perca (yellow perch), and type C cells were round to oval and often slightly basophilic with basally located ovoid or flattend nuclei, type D cells were paler, club shaped cells, both cells types observed Esocids (grass, pickeral and nothern pike), type E cells were oval to spherical and type F cells were saccular, club or goblet shaped cells. the nuclei of both types were small, dense, ovoid or cone shaped structures and observed Ictalurus(brown bullhead) and resembled goblet cells of intestine. Jo and Choi [7] reported that esophageal mucous cells of five teleostean fishes different each species, that is in *Parasilurus asotus* and *Pagrosomus major* which were large in size, especilly which appeared with several layers in esophageal mucosa of all region of esophageal fold in *Pagrosomus major*, but showed one layer just below surface epithelial cells in *Parasilurus asotus*. Esophageal mucous cells of *Carassius auratus*, *Canterines moderstus* and *Clupanodon punctatus* were small in size, which were one layer and scattering in *Carassius auratus*, and were seen a large numbers in the base and scattering in the top of esophageal fold *Canterines moderstus*, but were found a small numbers in only the base of esophageal fold of *Clupanodon punctatus*. Lee and Jo [9] reported that the shape of mucous cells of esophageal epithelim was typically round, ovoid or ellipsoid and the size of which

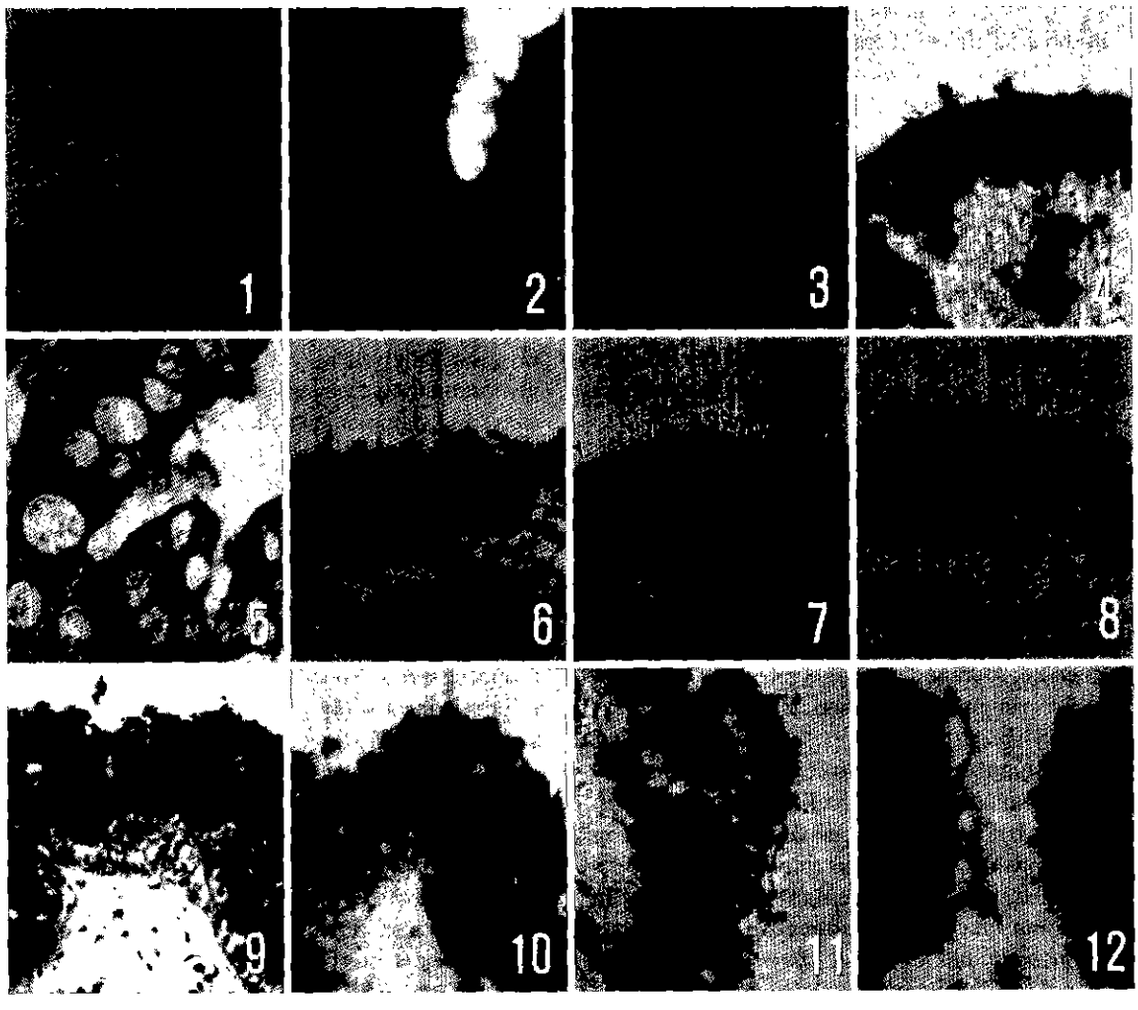

Fig. 1. Esophagus of *Agramus agramus* stained with hematoxylin-eosin showing mucosa consisting of stratified squamous epithelium with various mucous cells. Mucous cells were mixed with large(L), medium sized(M) and small(S) in size. ×400.
Fig. 2. Esophagus of *Agramus agarmus* stained with PAS. Epithelial columnar cells(arrow) show very intense red, but most mucous cells show intense red, while a few of them show moderate or weak red. ×400.
Fig. 3. Esophagus of *Agramus agramus* stained with alcian blue pH1.0.Epithelial columnar cells and mucous cells show not stained, but a few of medium sized and small cells show trace blue. ×400.
Fig. 4. Esophagus of *Inimicus japonicus* stained with PAS. Most mucous cells show intense red. while a few of medium sized, small cells show moderate red. ×400.
Fig. 5. Esophagus of *Inimicus japonicus* stained with alcian blue pH2.5. Most large, medium sized cells show not stained, while a few of them show trace of weak blue. Small cells show moderate or weak blue. ×400.
Fig. 6. Esophagus of *Epinephelus chlorostigma* stained with PAS. Large and medium sized show weak or moderate red but small cells show intense red. ×400.
Fig. 7. Esophagus of *Epinephelus chlorostigma* stained with alcian blue pH1.0. Most large and medium sized cells show modrate or weak blue, while small cells show not stained (arrowheads) ×400.
Fig. 8. Esophagus of *Epinephelus chlorostigma* stained with aldehyde pH1.7 alcian blue blue pH 2.5. Most large and medium sized cells show moderate or weak purpple, but small cells show weak or moderate blue. ×400.
Fig. 9. Esophagus of *Helicolenus dactylopterus* stained with alcian blue pH2.5. Most large and medium sized cells show moderate or intense blue, but small cells show weak or moderate blue. ×400
Fig. 10. Esophagus of *Helicolenus dactylopterus* stained with alcian blue pH2.5-PAS. Most large and medium sized cells show moderate or intense bluish purple, while small cells show intense purple or reddish purple. ×400.
Fig. 11. Esophagus of *Helicolenus dactylopterus* stained with alcian blue pH1.0-PAS. Most large and medium sized cells show moderate or intense bluish purple, but small cells show intense red or intense reddish purple. ×400.
Fig. 12. Esophagus of *Helicolenus dactylopterus* stained with aldehyde fuchsin pH1.7- alcian blue pH2.5. Most large an medim sized cells show moderate or intense purple. ×400.

was the largest in *Lateolabrax japonicus* and *Sparas swinhonis*, in the other fishes large and small mucous cells were mixed. In *Sparus Swinhonis* the mucous cells appeared in several layers throughtout the entire esophageal mucosa and in *Sebastes inremis* they appeared one or two layers over the most part of esophageal mucosa but in *Seriola quinqueradiata*, *Erosa erosa* and *Latedabrax japonicus* they appeared one layer of esophageal mucosa.

On this study, classify esophageal mucous cells according to distribution, size and morphology. Although numbers of distribution there was a difference in each species, in all species large, medium sized and small mucous cells were mixed but in *Agramus agramus* mucous secreting columnar cells also appeared in esophageal epithelium.

The morphology of large mucous cells was spherical, ellipsoids in *Agramus agramus, Inimicus japonicus* and *Epinephelus chlorostingma* but in *Helicolenus dactylopterus* it was round or spherical form. That of medium sized mucous cells in *Helicolenus dactylopterus* was elongated ellipsoid or spherical form but in the rest species it was ellipsoids. The shape of small mucous cells was spherical in *Agramus agramus*, spherical, pyramids or elongated ellipsoids in *Epinephelus* chlorostigma and spherical or ellipsoids in *Helicolenus dactylopterus*.

Concerning the number of mucous cells, if including mucous secreting columnar epithelial cells it was highest in *Agramus agramus* but that of mucous cells was highest in *Helicolenus dactylopterus, Epinephelus chlorostigma, Inimicus japonicus* was next, and lowest in *Agramus agramus*. In the most epithelium mucous cells arranged one-two layers in *Agramus agramus* and *Inimicus japonicus* but which distributed two-three layers in *Epinephelus chlorostigma* and *Helicolenus dactypterus*.

Our results conistent with the previous result such as Reifel and Travill [6], Jo and Choi [7], Lee and Jo [9], which indicated that the difference of morphology, size, local distribution and number of mucous cells may be a specificity of each species.

It was reported that the property of glycosaminoglycans in the mucous cells of teleostean esophagus different in each species as if mucous cells appeared in the other organ. That is, Kim [10] reported that esophageal mucous cells of *Misgurnus anguilicaudatus* contained acid glycosaminoglycans and eel, *crucian carp* and *sheat fish* contained both acid glycosaminoglycans and neutral glycosaminoglycans but those of *snakehead* contained only neutral glycosamnoglycans. Jo [12] reported that the histochemical composition of acid glycosaminoglycans in the esophageal mucous cells of eel contained only nonsulfated glycosaminoglycans and in *cyliorhinus torazame* which composed of sulfated glycosaminoglycans and nonsulfated glycosaminglycans but the fomer was more than the latter. Im and Kwun [8] reported that the esophageal mucous cells of *Parasilurus asotas*, *Mugil cephalusand* and *Agramus agramus* contained only neutral glycosaminoglycans. those of *Pleuronichthys cornutus* contained mixture of small amount of neutral glycosaminoglycans, large amount of sulfomucin (sulfated glycosaminoglycans) and sialomucin (nonsulfated glycosaminoglycans), in *Ophicephalus argus* some cells contained neutral glycosaminoglycans only and others with mixture of neutral glycosaminoglycans, sulfomucin(sulfated glycosaminoglycans) and sialomucin (nonsulfated glycosaminoglycans). In the esophageal mucous cells of *Carassius auratus*, *Siniperca scherzeri* and *Sebastes hubbsi* some cells with neutral glycosaminoglycans and sulfomucin (sulfated glycosaminoglycans) and others with mixure of neutral glycosaminoglycans, sulfomucin(sulfated glycosaminoglycans) and sialomucin (nonsulfated glycosaminoglycans). In the esophagus of *Cyprinus carpio* were identified cells with a large amount of neutral glycosaminoglycans and sulfomucin (sulfated glycosaminoglycans) and some sialomucin (nonsulfated glycosaminoglycans) and cells with a large amount of neutral glycosaminoglycans and sialomucin (nonsulfated glycosaminoglycans) and some sulfomucin (sulfated glycosaminoglycans). The glycosaminoglycans in the esophageal mucous cells of *Mugil cephalus* were reconized as consisting mainly of sulfomucin (sulfated glycosaminoglycans) with a small amount of neutral glycosaminoglycans and sialomucin (nonsulfated glycosaminoglycans). Jo and Choi [7] reported that the esophageal mucous cells of both *Parasiulrus asotus* and *Cantherine mederstus* were contained only neutral glycosaminoglycans. Most mucous cells of *Carassius auratus* were consisting of large amount of acid glycosaminoglycans and neutral glycosaminoglycans and a few mucous cells with small amount of acid glycosaminoglycans and neutral glycosaminoglycans. While in *Clupanodon punctatus* and *Pagrosomus major* showed the presence of acid glycosaminoglycans and neutral glycosaminoglycans, with most of the mucous cells being relatively more prominent of acid glycosaminoglycans and a few mucous cells more prominent of neutral ones. Reifel and Travill [6] reported that teleostean esophagus exhibit a diversity of mophologically and histochemically recoginzable type of mucous cells, with each esophagus producing at least two different glycosaminoglycans. that is, type A mucous cells of rock bass, blue gill sunfish, large mouth bass and black crappie contained combination of sialidase resistant sialomucin and a weakly acidic sulfomucin, type A mucous cells of golden shiner and fathead minnow contained only sialomucin (nonsulfated glycosaminoglycans). Type B mucous cells of rock bass, blue gill, sunfish, large mouth bass and black crappie contained combination of neutral glycosaminoglycans and some of sialomucin (nonsulfated glycosaminoglycans) but that of golden shiner and fathead minnow contained combination of a little of sialomucin (nonsulfated glycosaminoglycans) and strong sulfomucin (sulfated glycosaminoglycans). Type D and F cells contained only neutral glycosaminoglycans. Lee and Jo [9]

reported that according to amount and histochemical composition of glycosaminoglycans in mucous cells there was a difference in each species. As for the histochemical property, in *Sebastes jnermis* three kinds of mucous cells were appeared, which having only strong sulfated glycosaminoglycans or mixutre of small amount of strongly sulfated glycosaminoglycans and minimal to small amount of neutral glycosaminoglycans but mucous cells with small moderate amount of neutral glycosaminoglycans were existed in a few number of cells. The mucous cells of *Spararus swinhonis* esophagus were noted two types, most mucous cells were contained small amount of strongly sulfated glycosaminoglycans and neutral glycosaminoglycans while a few cells appeared to contain small amount of neutral glycosaminoglycans and minimal amount of weakly sulfated glycosaminoglycans. In *Seriola quinqueradiata*, three types of esophageal mucous cells were existed. Most of which contained small amount of strongly sulfated glycosaminoglycans and neutral glycosaminoglycans, a few of them having a mixutre of small amount of neutral glycosaminoglycans and minimal amount of weakly sulfated glycosaminoglycans. In *Seriola quinqueradiata*, three types of mucous cells were existed, most of which contained small amount of strongly sulfated glycosaminoglycans or small amount of exclusively nonsulfated glycosaminoglycans. In *Erosa erosa*, three types of cells were existed and some cells have mixture of small amount of nonsulfated glycosaminoglycans (sialomucin) and minimal amount of neutral glycosaminoglycans or some cells have mixture of small amount of weakly sulfate glycosaminoglycans and minimal amount of neutral glycosaminoglycans but mucous cells with small to moderate amount of neutral glycosaminoglycans and small amount of strongly sulfated glycosaminoglycans were existed in a few number. Mucous cells of *Lateolabrax japonicus* were three types which mixture of moderate amount of weakly sulfated glycosaminoglycans and a small amount of neutral glycosaminoglycans.

In the present study, *Agramus agramus* contained two kinds of mucous cells which different histochemical property from each other. Regardless of size, mucous cells were distringuished epitehlium columnar cells and mucous cells secreting large or considerable amount of neutral glycosaminoglycans and mucous cells which contain mixture of small amount of neutral glycosaminoglycans and minimal amount of sulfated glycosaminoglycans the former was the most, the latter was a few.

Such result was different from the property of esophageal mucous cells of *Agramus agramus* of Im and Kwun [2]. In the case of *Inimicus japonicus*, there was three kinds of mucous cells. Most of medium sized and large mucous cells contain only considerable amount of neutral glycosaminoglycans, a few of medium sized and large mucous cells and most of small mucous cells contain the mixture of considerable amount of neutral glycosaminoglycans and minimal to small amount of nonsulfated glycosaminoglycans(sialomucin), a few of small mucous cells contain only small amount of neutral glycosaminoglycans and minimal amount of sulfated glycosaminoglycans. In the *Epinephelus chlorostigma* two kinds of mucous cells exist and of which medium sized and large mucous cells contain mixture of small amount or moderate to cosiderable amount of neutral glycosaminoglycans and sulfated glycosaminoglycans. Small mucous cells with consisting of considerable neutral glycosaminoglycans, small to moderate, considerable amount or minimal amount of nonsulfated glycosaminoglycans(sialomucin). In the *Helicolenus dactylopterus* mucous cells were two kinds that medium sized and large mucous cells with small or moderate amounts to considerable amount of neutral glycosaminoglyans and small amount of sulfated glycosaminoglycans and small mucous cell with considerable amount of neutral glycosaminoglycans and moderate, small amount or considerable amount of nonsulfated glycosaminoglycans(sialomucin). As for the morphology and property of glycosaminoglycans of mucous cells *Epinephelus chlorostigma* similar to *Helicolenus dactylopterus* but *Agramus agramus* differ from *Inimicus japonicus*.

Such result of the size and distribution of mucous cells of teleostean but also the property of glycosaminoglycans different from each species and it was consistent with the reports of Reifel and Travill [6], Kim [10], Jo [12], Jo and Choi [7], Im and Kwun [8], Lee and Jo [9]. Chan [25] suggested that such a presence of two or more carbohydrate types in an epithelium could indicates two level of maturation in the formation of the mucous secretion. Based upon a study of human salivary gland mucous cells, Eversole [26] hypothesized that each individual mucous cell synthesized a neutral glycoprotein which is subsequently carboxylated, and finally sulfated. Freeman [27] reported that protein synthesized from rough endoplasmic reticulum and it combined acid glycosaminoglycans and became glycoprotein and finally it turned mucin granule that can be observed through electron microscope. And Reifel and Travill [6] suggested that the presence of two types of esophageal mucous cells in each species of perhaps represents two stage of cell maturity, but such differences in maturation are difficult to apply to the species whose mucous cells change their carbohydrate content from sulfomucin to neutral glycosaminoglycans in a rostral to distal direction.

As report of Lee and Jo [9] and Reifel and Travill [6], the diversity of mucous cells morphology, distribution and histochemical composition of glycosaminoglycans in teleostean esophagus indicates additional function, provably digestive and adaptation of injuring factor broughted inhabitation and feeding.

Reference

1. Reifel C. W. and A. A. Travill. 1978. Gross morphology

of the alimentary canal in 10 teleostean species, *Anat. Anz.*, **144**, 441-449.
2. Curry, E. 1939. The histology of the digestive tube of the CARP(Cyprines Carpio commuins), *J. Morph.*, **65**, 53-65.
3. Andrew, W. 1959. Textbook of comparative histology, New York ; Oxford University Press, pp 229-285.
4. Patt D. I. and G. R. Patt. 1969. Comparative vertebrate histology, New York, Harper and Row, pp. 146-205.
5. Andrew, W. and C. P. Hickman. 1974. Histology of the vertebrates, Saint Louis, Mosby Co., pp. 264-291.
6. Reifel, C. W. and A. A. Travill. 1977. Structure and carbohydrate histochemistry of the esohpagus in ten telostean species, *J. Morph.*, **152**, 303-314.
7. Jo U. B. and I. J. Choi. 1983. Histochemical study of the properties of mucosubstances on the esophageal mucous cells in five teleostean species. *Pus. Uni. J. Sci.*, **35**, 239-247.
8. Im M. S. and H. S. Kwun. 1983. Histochemical study on mucosubstances of the mucous cells in teleostean esophagus. J. Catholic Med. Colledge., Vol. 36, No. **4**, 803-812.
9. Lee S. H. and U. B. Jo. 1986. Histochemical study on mucosubstances of the esophageal mucous cells in the *telosts. Pus. Uni. J. Mol. Biol.* Vol., **1**, 29-39.
10. Kim Y. K. 1972. Comparative histochemical study on the mucosa of the digestive tract of fresh water fishes. *Pus. Uni. J. Sci.*, **14**, 135-160.
11. Jo U. B. and H. C. Park. 1972. Comprative study on the digestive mucosa of frogs and fishes. Pus. Uni. J. **13**, 383-403.
12. Jo U. B. 1974. Histochemical studies on the acid mucosubstances in the mucosa of alimentary Tract of Lower Vertebrates. Kor. J. Anat., Vol. 7 No.1: 1930.
13. McManus, J. F. A. 1984. Periodic acid Schiff's reaction, Stain Techn, **23**, 99-108. (AFIP modification)
14. Mowry, R. W. 1956. Alcian blue technics for the histochemical study of acidic carbohydrates, *J. Histochem, Cytochem,* **4**, 407.
15. Johnson, F. B. 1960. Manual of histologic special staining technics. 2nd ed., P.141, McGraw Hill Book Co.
16. Spicer S. S., R. G. Horn and T. J. Leppi. 1967. Histochemistry of connective tissue mucopolysaccharides In ; Wagner B. M., Smith D. E., The connective tissue , Williams & Wilkins, Baltimore, pp. 251-303.
17. Mowry, R. W. 1960. Alcian blue and alcian blue-periodic acid Schiff's stains for carbohydrates. Manual of histologic special stain technics. 2nd ed., p.142, McGraw Hill Book Co.
18. Mowry, R. W. 1963. The special value or method that color both acidic and vicinal hydrate groups in the histochemical study of mucin. *Ann. N.Y. Acad. Sci.*, **106**, 402-423.
19. Spicer, S. S and D. B., Meyer. 1960. Histochemical differentiation of acid mucopolysaccharides by means of combined aldehyde fuchsin-alcian blue staining. *Am. J. U. path.*, **33**, 453-459.
20. Pearse, A. G. E. 1968. Carbohydrates and mucosubstances. In ; Histochemistry. The oretical and applied, Vol. 1. 3rd ed., Churohill Livingstion, Edinburgh London New York, pp. 294-380.
21. Lev, R. and S. S., spicer. 1964. A histochemical comparison of human epithelial mucin in normal and in hypersecretory state including pancreatic cystic fibrosis. *Amer. J. path,* **46**, 23-47.
22. Johnson, W. C., F.B. Johnson and E. B., Herwig. 1962. Effect of varying the pH on reaction for acid mucopolysaccharides. *J. Histochem. cytochem.*, **10**, 684.
23. Bucke, D. 1971. The anatomy and histology of the alimentary tract of the carnivorous fish, the pike Esoxlucius *J. Fish. Biol.*, **3**, 421-431.
24. Kim Y. K. 1972. Histochemical study on the mucosa of the digestive tract of the Loach (Misguranus anguillicaudatus), *Pus. Uni. J.*, **13**, 237-247.
25. Chan, V. M. 1941. The histology of the alimentary tract of the deep-water gurnard, peristedion longispatha, Univ. Nebraska Stud, *Sci. Tech.*, **1**, 7. (cited from 6)
26. Eversole, L. R. 1972. The mucoprotein histochemistry fo human mucous acinar cell containing salivary glands : submandibular and sublingual glands. *Arch, Oral Biol.*, **17**, 43. (Cited from 8).
27. Freeman, J. A. 1966. Goblet cell structure, *Anat. Rec.* **154**(1), 121-448.