HISTOCHEMICAL MAPPING OF GLYCOCONJUGATES IN THE EYEBALL OF THE BUFFALO *(BOS BUBALIS)*

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ABSTRACT

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In the present study, the distribution of various sugar residues in the eyeball tissues of sexually mature buffalo was examined employing fluorescein isothiocyanate- (FITC) conjugated lectins. The present observations revealed the presence of mannose (labeled by lectins ConA), galactose (labeled by PNA, GSAI, ECA), GalNAc (labeled by SBA, VVA), and GlcNAc (labeled by WGA) residues in the buffalo ocular tissues. The epithelium and stroma of the ocular tissues were labeled with mannose (ConA) and GlcNAc (WGA) binding lectins. Binding sites for WGA and PNA to the rod and cone cells of the retina were evident. The lectins Con A, WGA and GSAI bound strongly to the endothelium of blood vessels and to smooth muscle cells of the iris. In conclusion, the findings of the present study clearly indicate that the buffalo eyeball contains a wide range of glycoconjugates (bearing mannosyl, galactosyl and glucosly residues), and they lack fucosyl residues.

***Key words:** Histochemical mapping, eyeball , Buffalo*

INTRODUCTION

In recent years, lectin histochemistry has developed into a useful tool to study various aspects of cell differentiation and cell-to-cell interaction (To¨pfer-Petersen, 1999; Gabius, 2001). As well as the cell surface saccharides believed to be involved in a variety of cell functions, including development, growth regulation and cellular locomotion (Hakomori, 1981, Gabius *et al.*, 1988).

It is significant that most plant and animal lectins have been classified into a rather limited number of carbohydrate-binding groups (Goldstein and Poretz, 1986). These include the mannose/glucose-binding lectins, the galactose-binding lectins, the N-acetylgalactosamine-binding lectins, the N-acetylglucosamine-binding lectins, the L-fucosebinding lectins, sialic acid-binding lectins, and lectins with complex carbohydrate-binding sites. In the field of ocular tissue, lectins applied in for various purposes to demonstrate the importance of glycoconjugates in the morphogenesis of the corneoscleral angle where they provide some of the required signals for the differentiation of the trabecular meshwork (Beauchamp *et al.*, 1985). Lectins are at light microscopal level to investigate normal cornea in a variety of species (Holmes *et al.*, 1985; Panjwani *et al.*, 1986; Panjwani and Baum, 1988; Tuori *et al.*, 1994, Aly, 2003). As well as determine the distribution of carbohydrate residues on photoreceptor cell surfaces (Bridges, 1981, Aly, 2003). Many studies on monkey, pig, cat and rabbit, eye tissues were intensely labeled with (Kawano *et al.*, 1984).

Lectins used in eye research, mainly for human material (Prause, 1991). Lectin histochemistry is considered to be a valuable method for determining changes in the glycoconjugate content during normal and pathological conditions. To my knowledge, no data are available concerning the buffalo. Therefore, the aim of present work was to determine the changes in the sugar residues within the buffalo eyeball by means of glycohistochemical (lectin histochemical) methods.

MATERIALS and METHODS

Samples

The present study was performed on the eyeball of 10 sexually mature and apparently healthy buffalos, Specimens were taken from the cornea, sclera, iris, ciliary body, choroid, retina and optic nerve, with a side length of 0.3 - 0.5 cm.

All samples were collected within 30 min of slaughter in a local (Cairo) abattoir. Small samples of the eyeball tissue (0.5–1 cm) were fixed in Bouin's fluid for 24 h. Thereafter, fixed samples were extensively washed in 70% ethanol (3 – 24 h) to elute fixative before tissue processing to paraffin wax by routine

methods. Using a Leitz rotatory microtome (type 1521), 5-mm-thick sections were cut and mounted on both 3- aminopropyltriethoxysilane- coated and uncoated glass slides. Paraffin wax embedded sections were kept in an incubator at 40 °C until used for lectin histochemistry.

Lectin histochemistry

Distribution of sugar moieties (glycoconjugates) in the adult buffalo eyeball tissues were investigated using 9 different fluorescein isothiocyanate- (FITC) conjugated lectins, (all purchased from Sigma Aldrich Chemicals GmbH, Deisenhofen, Germany) listed in Table 1. The lectins were chosen to represent five groups: mannose-, galactose-, N-acetylgalactosamine (GalNAc)-, N-acetylglucosamine (GlcNAc)- and fucose-binding lectins. Lectin binding was revealed as follows: Sections were dewaxed (2 – 30 min) in xylene, rehydrated through descending grades of ethanol and washed under tap water for 10 min. They were then washed (3– 5 min) in 0.05M Tris-buffer, pH 6.8 and then incubated with 33 mg/ml FITC-conjugated lectin in Tris buffer in a humid chamber, at 4 °C, overnight.

Sections were then again washed under tap water for 5 min and subsequently rinsed (3 – 5 min) in Tris buffer (pH 6.8). Importantly, the hydrated sections were then taken directly from Tris-buffer and mounted with a 25:140 mixture of polyvinyl alcohol and ethylene glycol (Serva, Heidelberg, Germany) in Tris-buffer, pH 6.8. Mounted slides were stored at – 20 °C until examined using a fluorescent microscope.

Controls

Control sections were treated as described previously except that the FITC-conjugated lectins were either (1) substituted with Tris-buffer, or (2) pre-incubated with 0.4M of the corresponding hapten sugar inhibitor listed in Table 1 (Sigma, Deisenhofen, Germany) for 1 h before labeling.

Analysis of labeling Lectin-labeled eyeball tissues and their controls were evaluated using a Dialux 20 fluorescent microscope (Leitz GmbH, Wetzlar). Photomicrographs were captured using Kodak film elite 400.

Table 1: FITC-labeled lectins used for investigation of sugar moieties in the buffalo ocular tissue

Lectin group	Lectin source (Latin name)	Common name	Acronym	Sugar specificity	Binding inhibitor
I. D-Mannose (D-Glucose)-binding lectins	*Canavalia ensiformis Agglutinin*	Jack bean	Con A	α-D-Man > α-D-Glc	Man
II. D-Galactose-binding lectins	*Arachis hypogaea Agglutinin*	Peanut	PNA	β-D-Gal-(1-3)-D-GalNAc	Gal
	Griffonia simplicifolia I Agglutinin	Griffonia or Bandeiraea	GSA-I	Terminal α-Gal	Gal
	Erythrina Cristagalli Agglutinin	Coral tree	ECA	α -D-Gal-(1-4)-GlcNAc	Gal
III. N-acetyl-D-galactosamine (GalNAc)-binding lectins	*Glycine max Agglutinin*	Soybean	SBA	D-GalNAc	GalNAc
	Visea villosa Agglutinin	Hairy vetch	VVA	D-GalNAc	GalNAc
IV. N-acetyl-D-glucosamine (GlcNAc)-binding lectins	*Triticum vulgaris Agglutinin*	Wheat germ	WGA	GlcNAc(β1-4GlcNAc) $_{1-2,}$ NeuNAc	GlcNAc
V. L- Fucose-binding lectins	*Ulex europaeus –I Agglutinin*	Gorse seed	UEA-I	α-L-Fuc	α-L-Fuc
	Lotus tetragonolobus Agglutinin	Asparagus pea	LTA	α-L-Fuc	α-L-Fuc

Man, mannose; Glc, glucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; NeuNAc, N-acetylneuraminic acid (sialic acid); α-L-Fuc, α-L-Fucose.

RESULTS

Slight differences in lectin labeling to reveal different sugar moieties were seen in different ocular tissues. The results are summarized in (Table 2) and described in detail below.

1. Mannose-binding lectins

The lectin Con A recognise glycan structures bearing terminal mannose residues. ConA bind to α-D-mannose and showed intense labelling epithelium of cornea, iris, ciliary body and retina (Fig. 1&2&3&4). Moreover, moderately strongly labeled the stroma of cornea, iris, ciliary body (Fig. 1&2&3) and optic nerve (Fig.5). No Con A labeling was seen in the sclera and nerve fibre layer of the optic nerve (Fig.5).

2. Galactose-binding lectins

PNA, GSA-I and ECA recognise glycans with terminal galactose residues.

PNA recognises α-D-galactose and β-D-galactoseβ (1→3)-D-N-acetylgalactosamine. PNA labeling was only observed in epithelium of the cornea, retinal pigmented epithelium and layer of rod and cone cells of the retina (Fig. 6) as well as the epithelium stromal blood vessels and dilatator muscle of the iris (Fig. 7). Other constituents of the ocular tissues remained moderately reacted or completely unlabeled. GSA-I recognises α-D-galactose and α-D-N- acetylgalactosamine.

GSA-I strongly labeled the blood vessels in the iris, ciliary body, choroids, retina and optic nerve (Fig. 8&9&10&11) while labeling was undetectable in all other ocular tissues. ECA recognises α-D-galactose and β-D-galactoseβ (1→4)-D-N-acetylgalactosamine. ECA weakly labeling the blood vessels of the ocular tissues (Fig. 12).

3. N-acetylgalactosamine (GalNAc)-binding lectins

Although SBA and VVA are both preferentially bind to α-D-N-acetylgalactosamine residues, they showed a different distribution of labeling pattern in the buffalo eye. No SBA labeling was detected in the ocular tissue in the buffalo. VVA labelling the vascular tunic of the buffalo eye mainly the stroma, muscle and blood vessels of the iris, ciliary body and choroid as well as the pigmented epithelium and rod and cone cells of the retina and optic nerve seen in (Fig.13)

4. N-acetylglucosamine (GlcNAc)-binding lectins

WGA preferentially binds to galactose-β (1→4)–N-acetylglucosamine, α-D-N-acetylglucosamine and neuraminic acid. WGA labelling was widely distributed throughout buffalo ocular tissues. WGA strongly labelling the epithelium and stroma of cornea (Fig.14), iris, ciliary body and retina as well as the rod and cone cells of the retina. WGA moderately labelling the muscles of the iris and ciliary body in addition to blood vessels (Fig.15) distributed in ocular tissues of the buffalo.

5. Fucose-binding lectins

UEA-I and LTA bind to α-L-fucose. UEA-I but not LTA weakly labeled the corneal epithelim while the other constituents of the ocular tissues were unlabeled.

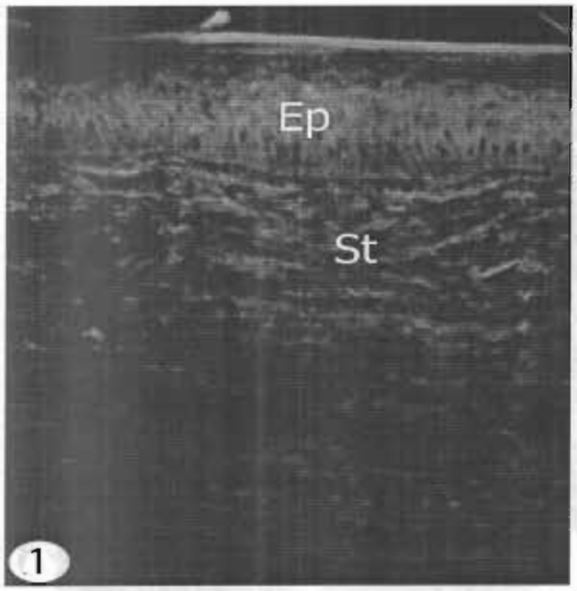
Controls

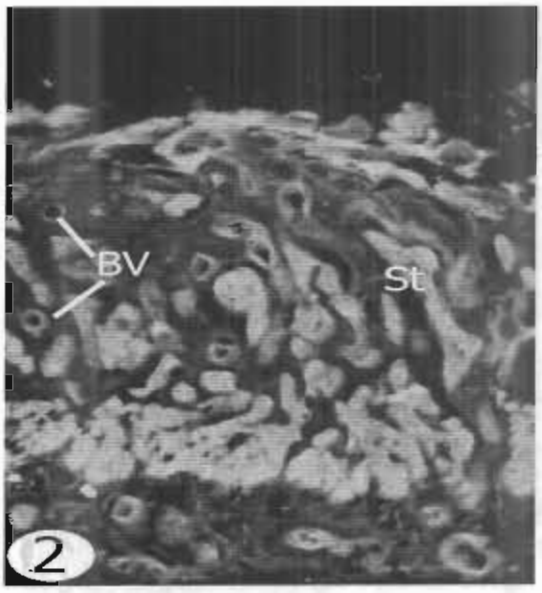
None of the negative control sections that were performed by omission of the lectin showed positive reactions. Similarly, all of the sections that were treated with the pre-incubated corresponding hapten sugar inhibitor showed no binding affinity except with WGA and PNA. With both of them, the specific reaction products decreased but did not disappear completely as with other lectins. This probably due to the fact that both WGA and PNA could recognise more than one sugar (PNA recognises α-D-galactose and β-D-galactoseβ (1→3)-D-N-acetylgalactosamine, while WGA binds to galactose-β (1→4)–N-acetylglucosamine, α-D-N- acetylglucosamine and neuraminic acid) and in the inhibition experiment, we have used the major specific sugar as an inhibitor (showed in table 1). These data may support the finding that some lectins could bind more than one sugar with different degree.

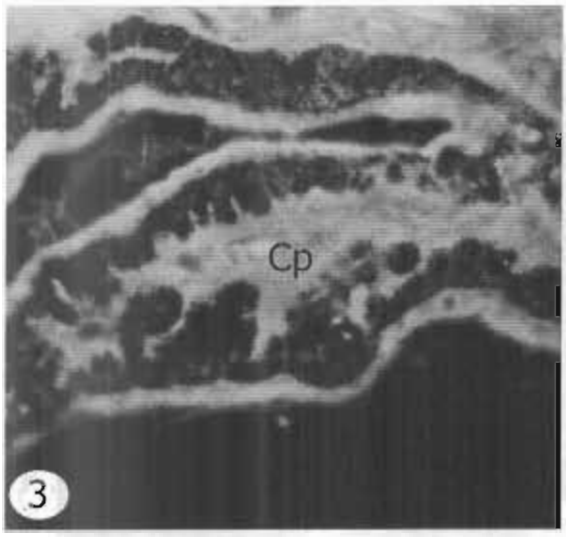
Table 2: Lectin binding sites in the buffalo eyeball fixed with Bouin's solution.

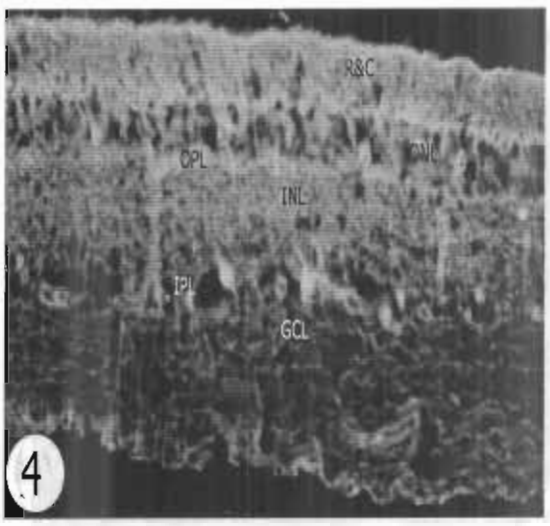
Sites	*Con A*	*LTA*	*ECA*	*SBA*	*VVA*	*WGA*	*PNA*	*GSA I*	*UEA I*
Cornea									
Ep	+++	+	+	+/-	+/-	+++	+++	+	++
Bm	++	-	-	-	-	++	++	-	-
St	++	-	+/-	-	-	++	+/-	-	-
Dm	++	+/-	+	-	+/-	++	+/-	-	-
En	++	+	+/-	+/-	+/-	++	+/-	-	-
Sclera	-	-	+	-	+/-	+/-	-	-	-
Iris									
PE	+++	-	+/-	-	-	+/++	-	-	-
St	++	-	++	-	++	++	+	-	+
BV	+/++	+/-	+/++	+/-	++	+/++	++	+++	-
SM	++	-	+	-	+/++	++	-	-	-
Ciliary body									
CM	+	-	-	-	-	+	+	-	-
NPE	++	+	+	+	+	++	++	+	-
Choroid									
BL	++	-	++	-	++	++	-	-	-
BV	++	-	++	-	++	++	++	++	-
Retina					++/++	++/++			
RPE	++/+++	-	-	-	+	+	++/+++	+/-	-
R&C	++/+++	-	-	-	++/++	++/++	++/+++	++	-
ELM	++	-	-	-	+	+	++	-	-
ONL	+	-	-	-	-	++	-	-	-
OPL	+	-	-	-	-	+	+	-	-
INL	+	-	-	-	-	+	-	-	-
IPL	+	-	-	-	-	+	+	-	-
GCL	+	-	-	-	-	+	-	-	-
NFL	+	-	-	-	-	-	+	-	-
ILM	++	-	-	-	+	+	++	-	-
BV	+	-	-	-	-	++	+	+++	-
					+	+			
Optic nerve									
NF	-	+	+	-	+	-	+	-	+
S	++	+	++	-	-	++	+	-	-
BV	++	-	++	++	+	++	-	++	-

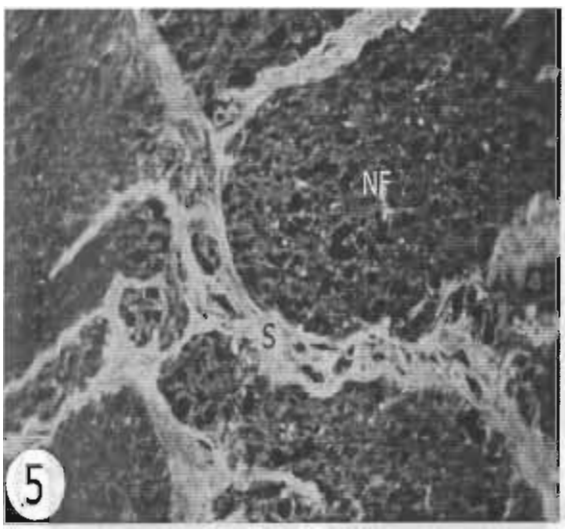
- = negative reaction + = week reaction ++ = moderate reaction +++ = strong reaction
Ep = Epithelium, Bm = Bawman's membrane, St = Stroma, Dm = Descemet's membrane, En = Endothelium, PE = Pigmented epithelium, NPE = Non pigmented epithelium, BV = Blood vessels, SM = Sphincter muscle, CM = Ciliary muscle, BL = Basal lamina, RPE = Retinal pigmented epithelium, R&C = Rods and cones, ELM = External limiting membrane, ONL = Outer nuclear layer, OPL = Outer plexiform layer, INL = Inner nuclear layer, IPL = Inner plexiform layer, GCL= Ganglion cell layer, NFL = Nerve fiber layer, ILM = Internal limiting membrtane, S = Septa, NF = Nerve fiber.

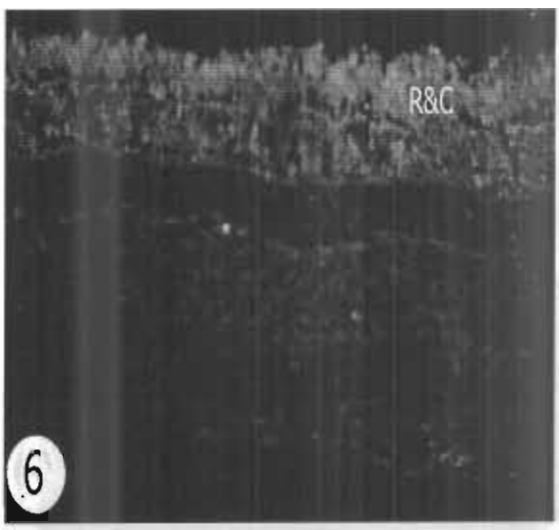

Ep
St
1

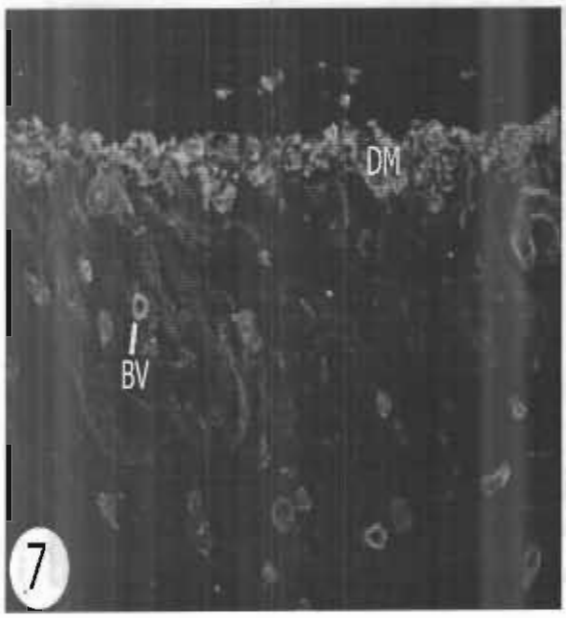

BV
St
2

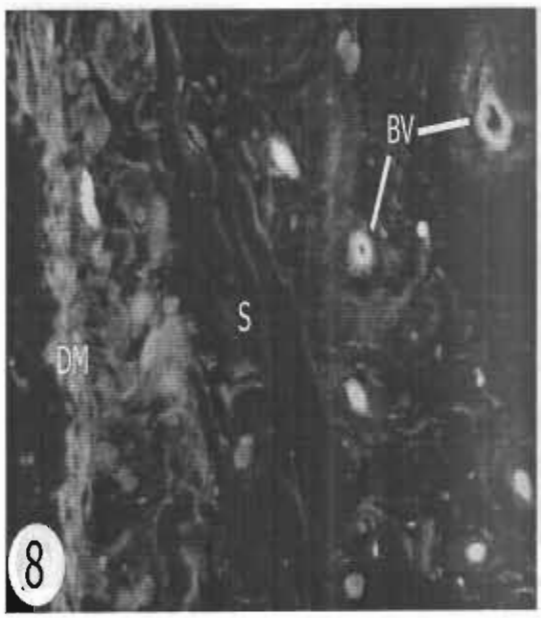

Cp
3


R&C
OPL
INL
IPL
GCL
4


NF
S
5


R&C
6


DM
BV
7


BV
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DM
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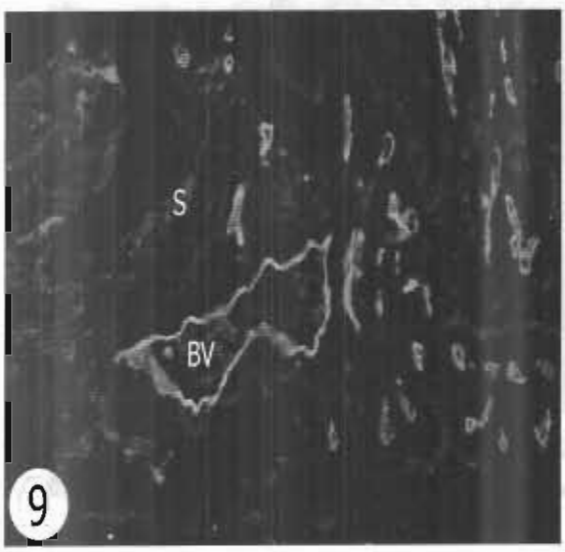
S
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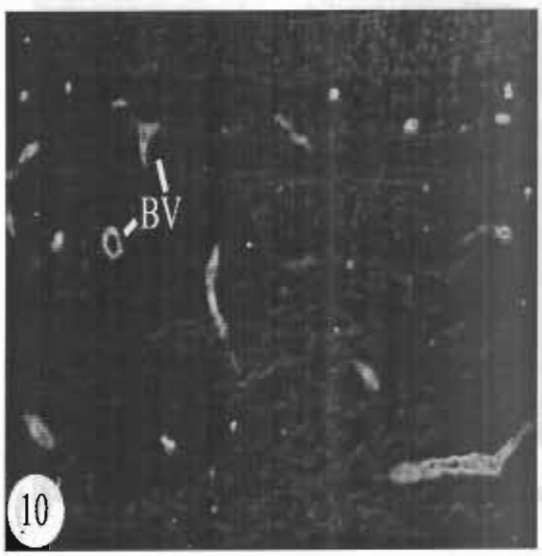
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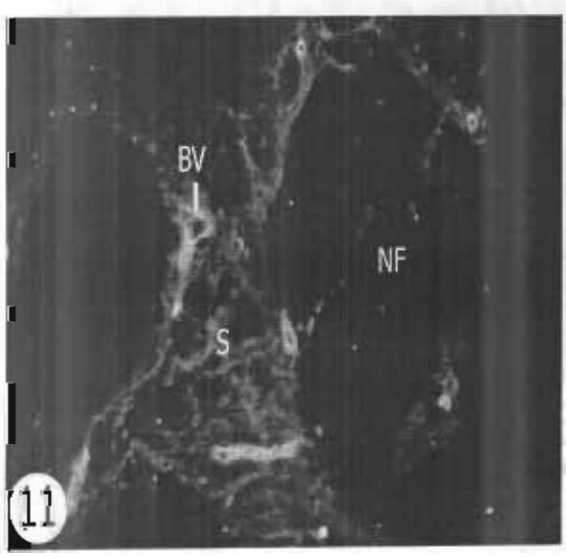
BV
NF
S
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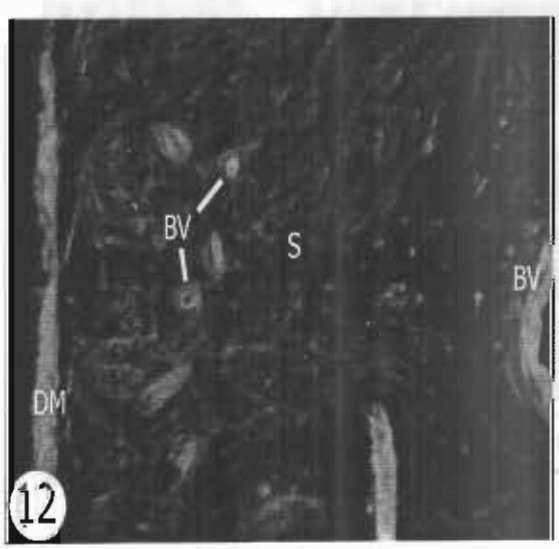
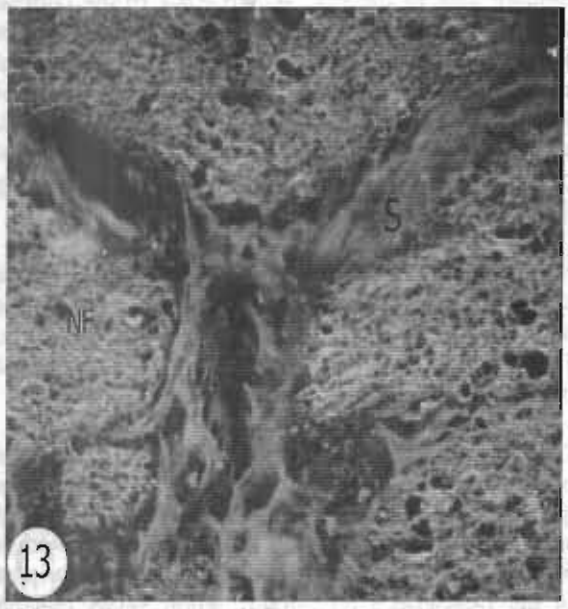
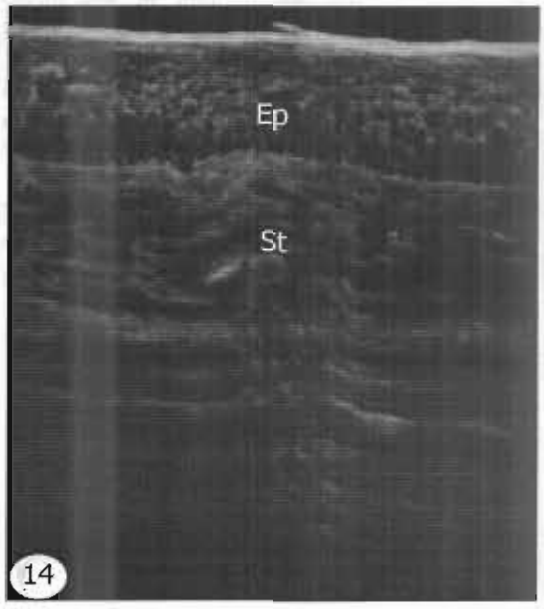
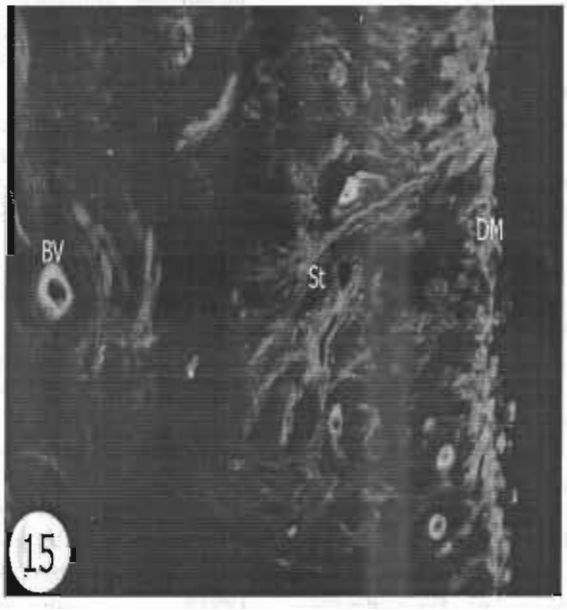

BV
S
BV
DM
12







LEGEND

Fig. 1: Lablling of the epithelium (Ep) and stroma (St) of the buffalo cornea with ConA (×600).

Fig. 2: Lablling the endothelium of the blood vessels (BV) of the stroma (St) of the buffalo iris with Con A (×600).

Fig. 3: Lablling of the stroma of ciliary process (Cp) of the buffalo ciliary body with Con A (×600).

Fig. 4: Labelling of the layer of rods and cones (R&C), outer nuclear layer (ONL), outer plexiform layer (OPL), Inner nuclear layer (INL), inner plexiform layer (IPL) and Ganglion cell layer (GCL) of the buffalo retina with Con A (×600).

Fig. 5: Labelling of the nerve fiber (NF) and connective tissue septa (S) of the buffalo optic nerve with Con A (×600).

Fig. 6: labelling of the layer of rods and cones (R&C) layer of the buffalo retina with PNA (×600).

Fig. 7: Lablling the endothelium of the blood vessels (BV) and iridal dilatators musscle (DM) of the stroma of the buffalo iris with PNA (×600).

Fig. 8: Lablling the endothelium of the blood vessels (BV) and iridal dilatators musscle (DM) of the stroma (S) of the buffalo iris with GSA-I (×600).

Fig. 9: Lablling the endothelium of the blood vessels (BV) of the stroma (S) of the buffalo ciliary body with GSA-I (×600).

Fig. 10: Lablling the endothelium of the blood vessels (BV) of the buffalo retina with GSA-I (×600).

Fig. 11: Lablling the endothelium of the blood vessels (BV) and the connective tissue septa (S) of the buffalo optic nerve with GSA I (×600).

Fig. 12: Lablling the endothelium of the blood vessels (BV) and iridal dilatators musscle (DM) of the stroma (S) of the buffalo iris with ECA (×600).

Fig. 13: Labelling of the nerve fiber (NF) and connective tissue septa of the buffalo optic nerve with VVA (×600).

Fig. 14: Lablling of the epithelium (Ep) and stroma (St) of the buffalo cornea with WGA (×600).

Fig. 15: Lablling the endothelium of the blood vessels (BV) and iridal dilatators musscle (DM) of the stroma (S) of the buffalo iris with WGA (×600).

DISCUSSION

Glycoproteins occur mainly intracellular and may also be found in cell membranes with a variety of important biological functions. Lectins are sugar binding protein that can be useful for the localization of glycoproteins in cells. This could contribute to a better interpretation of the physiological and pathological processes in the corneal tissue (Bonvicini *et al.*, 1983).

Lectin histochemistry enables the morphological evaluation of the distribution of the saccharide residues within the tissue sections (Spicer and Schulte, 1992). Thus the present data, demonstrating the lectin binding sites in the buffalo eyeball, propose a basis for further analyses of the role of saccharide residues in the eye under different experimental and pathological conditions.

The pattern of lectin binding in the corneal epithelium suggests the presence of glycoconjugates containing terminal α-mannose, N-acetylglucosamine and sialic acid residues and sparse terminal α-galactose and β-N-acetylgalactosamine residues (Panjwani *et al.*, 1986; Rittig *et al.*, 1990; Bishope *et al.*, 1991 and Lawrenson *et al.*, 1998). This generally agree with my results. Lectin binding to the cornea in different species, e.g. calf has been studied previously by Panjwani and Baum, 1989. The results of this study are generally in accordance with their findings but there are also some differences: Panjwani and Baum, 1989 mentioned that Con A and PNA binds to the apical corneal epithelium, whereas in my results show that Con A binds throughout all epithelial layers of the cornea. My results are different to the findings of Tuori *et al.*, 1994 who reported no binding also of the Con A and PNA to the corneal epithelium. Tuori *et al.*, 1994. demonstrated that WGA, UEA-I and GSA-I bound to the corneal epithelium and that apical cells of the epithelium of the cornea displayed more α-GalNAc, GlcNAc, sialic acid and α-L-Fucose residues than the basal cells. This agrees with my results but is weakly binding by UEA-I.

Furthermore, Con A and WGA bound to the corneal stroma in my experiment, but no binding of this lectins was seen in the study of Panjwani and Baum, 1989 who reported that PNA is the only lectin that binds to the corneal stroma.

Con A and WGA bound to the Descemet's membrane of the buffalo cornea. This result is generally in accordance with the studies of Panjwani and Baum, 1989 and Tuori *et al.*, 1994, but with a little difference. Con A reacted with the anterior part of the Descemet's membrane, while WGA reacted mainly with the posterior border of the Descemet's membrane. Heterogeneity in the distribution of the glycoproteins within the Descemet's membrane has been demonstrated previously (Gordon, 1990; Ljubimov *et al.*, 1995 and Lawrenson *et al.*, 1998). The thin basement membranes showed a marked presence of N-acetylgalactosamine residues, whereas a low concentration of these sugar residues was found in thick basement membranes (Salamat *et al.*, 1993).

The observations of the present study showed binding of Con A and WGA to the corneal endothelium. This finding is not in accordance with the results of Panjwani and Baum, 1989 and Tuori *et al.*, 1994 who reported that the corneal endothelium tend to bind only GSA-I-B4. Some of the results from previous lectin binding studies in the human cornea are somewhat different in various investigations (Bonvicini *et al.*, 1983; Panjwani *et al.*, 1986; Brandon *et al.*, 1988 and Bishop *et al.*, 1991).

Brandon *et al.*, 1988 suggested that some of the variation in the histochemical lectin binding studies is due to different staining procedures or post-mortem changes of the tissues. This could also explain the difference observed between my investigation and the studies of Panjwani and Baum, 1989 and Tuori *et al.*, 1994.

PNA is a lectin which preferentially detects α-galactose. It is also used as a biological marker to detect the T antigen. Due to the neoexpression of T antigen in malignant cells, PNA and other lectins of the same specificity have been used as tools in the diagnosis of cancer. In my work PNA reacted weakly with the buffalo cornea.

Contrary Con A, WGA and PNA showed a distinct reactivity with Bowman's membrane, showing α-mannose, N-acetylglucosamine and α-galactose residues in this structure.

The binding of lectins to the blood vessels of the anterior uvea demonstrates the presence of glcoconjugates containing terminal N-acetylglucosamine and α-galactose in the vascular endothelium. The weak staining with these lectins revealed also the presence of some α-mannosyl, N-acetylgalactosamine and sialic acid residues in the endothelium. The vascular endothelium has been previously studied using some of these lectins in bovine tissues (Alroy *et al.*, 1987, Tuori *et al.*, 1994). My demonstration of the presence of α- and β-galactose and sialic acid and the absence of fucose are in agreement with the results of Alroy *et al.*, 1987, and Tuori *et al.*, 1994. My result found mannose residues in the endothelium similar to finding of Tuori et al., 1994 and in contrast to the results of Alroy *et al.*, 1987. Previous studies have suggested that GSA-I-B4 is an endothelial marker in mouse tissues (Laitinen, 1987) in the same way as UEA-I is for human tissues (Holthöfer *et al.*, 1982). The present results show that GSA-I is and endothelium marker in buffalo tissues and these findings are in accordance with observations of Tuori *et al.*, 1994.

The stroma of the iris is abundant in collagen fibers. They were stained by Con A, ECA, VVA, WGA and PNA. The presence of sialic acid and β-galactose has been noticed previously by Pena *et al.*, 1981. Similar results were also obtained by Tuori *et al.*, 1994.

The posterior pigmented epithelial cell membrane of the iris and the non-pigmented epithelium cell of the ciliary body have α-mannose and N-acetylglucosamine residues. This is in accordance with the studies of Tuori *et al.*, 1994. α-mannosyl and N-acetylglucosamine residues are abundant in the buffalo iridal and ciliary muscle, whereas the Gal-(β1,3)-N-GalNAc residues are also present in the ciliary muscle. Identical results were obtained by Tuori *et al.*, 1994. Lectin histochemistry has been applied to human and rat skeletal muscle previously (Pena *et al.*, 1981) and it has been shown that Con A reacted with muscle cells whereas UEA-I was negative. However, the other lectins used in the present study (PNA and WGA) stained iridal and ciliary smooth muscle cells differently.

The results of my study show clearly that specific structures in the buffalo retina can be stained with different lectins (Table 2). The binding of the retinal structures was dependent on the sugar-binding specificities of the different lectins, demonstrating the presence of different glycoconjugates in specialized parts of the retina.

The binding of Con A and WGA to buffalo retinal structures is in agreement with previous studies in the frog (Bridges, 1981), in the monkey (Uehara *et al.*, 1983a) and in human (Söderström, 1988). However, there are some differences in the staining pattern of PNA and UEA-I that may result from species differences or from variation in tissue preparation, which are known to affect lectin histochemistry (Brasitus *et al.*, 1982 and Söderström *et al.*, 1984).

In my study, normal buffaloretinal pigmented epithelium showed lectin binding sites for Con A, WGA, VVA and PNA. These results are in accordance with the findings of (Bopp *et al.*, 1992) in human retinal pigmented epithelium. The biochemical role of lectin binding sites in RPE still remains unclear, especially the significance of the presence or absence of certain cellular sugar residues for structure and function of RPE-cells (Bopp *et al.*, 1992). Con A also binds with high affinity to rhodopsin, the photoreceptor molecules of the rods (Fukuda *et al.*, 1979; Liang *et al.*, 1979 and Bridges and Fong, 1980). Rhodopsin contains a special oligosaccharide-chain GlcNAcβ1-2Manα1-3(Manα1-6) Manβ1-4GlcNacβ1-4GlcNac-Asn (Fukada *et al.*, 1979 and Liang *et al.*, 1979). Its high content of both terminal N-acetylglucose and α-mannose residues explain the binding of Con A to the rods in buffalo retina. Thus, the distribution of Con A-binding sites in buffalo rods might reflect the distribution of rhodopsin within these cells. However, there are also other glycoconjugates in the rods, such as the outer segment protein, with a molecular weight of 291,000 (Dreyer *et al.*, 1972; Bownds *et al.*, 1974) and the glycoprotein of the interphotoreceptor matrix (Adler and Klucznik, 1982) that bind Con A and WGA.

Rod and cone discs are formed by infolding of the plasma membrane. In the rods these infoldings are sealed off to form stacks of flattened bimembranous discs, so that the oligosaccharide layer that normally resides on the extracellular surface (Hirano *et al.*, 1972) is sequestrated into the disc interior. Con A stained material in the buffalo rods seemed to be intracellularly located and its concentration was higher in the outer segments than in the inner segments.

There are membranous disks inside the rod outer segment that contain rhodopsin (Jan and Revel, 1974 and Basinger et al., 1976). It has been suggested that the rod outer segments bind fucosyl and galactosyl residues prior to disk shedding and phagocytosis by the cells of the pigment layer (McLaughlin and Wood, 1980; O'Brien, 1976). In this way the cells of the pigment layer can recognize the differences between shed and intact disks. Previous studies have given conflicting results concerning this theory. In

the monkey retina, RCA I, which identifies terminal galactosyl residues, binds to the outer segments of the rods, indicating the presence of galactosyl residues. However, in the monkey retina, UEA I and LTA which identify fucosyl residues bind only to the inner segments of the rods and cones (Bunt and Klock, 1980 and Uehara et al., 1983a).

In my study we could not find any binding of ECA, UEA I or LTA in the rods. These findings are in accordance with those of Bridges, 1981 and could be due to species differences, indicating a different processing of rhodopsin in different species. However, in the rods of the frog, RCA I staining is seen on the surface of the outer segments (Bridges and Fong, 1979). Those studies were done with cell suspensions in which the cell surface is easier to study. In the present investigation tissue sections were used. The stained material is primarily intracellular. If the cell surface is only slightly stained, it may be difficult to observe it in tissue sections. Sheets and occasional chunks of this matter were sometimes found on the detached retina and probably represent the interphotoreceptor matrix, which is known contain fucose residues (Feeney, 1973).

Since Con A and WGA also bind to the outer segment of the cones, their visual pigments may contain sugar sequences that resemble those found in rhodopsin, but show different lectin affinities. For instance, rhodopsin and iodopsin both bind to Con A columns but they are eluted at different concentrations of α-methyl-mannopyranoside (Fager and Fager, 1978).

WGA is bound to terminal GlcNac or sialic acid residues (Goldstein and Hayes, 1978). WGA dose not bind to rhodopsin (Yamamoto *et al.*, 1983), so the binding of WGA to the retina must be explained by the presence of other glycoconjugates. These are present in the cytoplasm of the rods and cones but are not seen in the internal segment of the cones. Quite interesting is the band-like staining by WGA within the outer plexiform layer, for which no clear morphological equivalents can be seen with conventional staining methods.

Lectin PNA is known to have high affinity for α-D-galactose and N-acetylgalactosamine (Lotan et al., 1975). It is, therefore, possible to assume that these carbohydrate residues are present at the terminal oligosaccharides of membrane glycoconjugates in rods and cones. Concerning the binding sites of PNA, it is noticeable that the labelling was uniform throughout the outer and inner segments of rods and cones.

In the monkey retina, PNA also selectively stained the cones, but the binding was concentrated to the outer segment (Uehara *et al.*, 1983b). Their was also found in human, (Söderström, 1988) in pig and cat and in rabbit retina (Kawano *et al.*, 1984). These observations are different to my results. We showed that PNA bound strongly with rods and cones layer. My finding agree with results which reported by Kawano *et al.* (1984) that mammals including rat and bovine and non-mammals including birds and goldfish strongly bound PNA to rods and cones.

In addition to the layer of the rods and cones, Con A stained many other structures of the retina. The external and internal limiting membrane was only slightly stained whereas in the outer nuclear layer, where the nuclei of the rods and cones are located. The cell surfaces of the neurons in the inner nuclear layer were marked. Both inner and outer plexiform layers were diffusely stained with Con A. This finding agrees with results in the human retina (Söderström, 1988).

The internal and external limiting membranes and the wall of the retinal vessels are labelled with PNA. The vessel wall contains collagen fibrils (Hogan *et al.*, 1971) which are abundant in α-galactose (Muir and Lee, 1969).

In summary, my study shows that the normal buffalo eye contains a distinct distribution pattern for several lectins. These results on the normal buffalo eye may form the basis for future studies concerning changes in lectin staining occurring in different diseases of the eye.

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دراسة هستوكيميائية للسكريات المرتبطة في مقلة العين بالجاموس

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الغرض من هذا البحث هو دراسة توزيع بقايا السكريات في مقلة العين في الجاموس باستخدام اللاكتين. أظهرت الدراسة وجود بقايا سكريات المرتبطة في جميع أنسجة العين في الجاموس بدرجات متفاوتة علي النحو التالي، حيث لوحظ وجود بقايا سكريات المانوز والجلاكتوز في الطبقة الطلائية والليفية لأنسجة مقلة العين في الجاموس فيما عدا طبقة الشبكية التي لوحظ بها وجود بقايا سكريات المانوز والجلوكوز. كما لوحظ وجود بقايا سكريات المانوز و وجلاكتوزأمين والجلاكتوز في الأوعية الدموية المنتشرة داخل مقلة عين الجاموس وخاصة في طبقة القزحية كما لوحظ وجود نفس هذه السكريات العضلة البسيطة الموجودة داخل القزحية. ومن هذه الدراسة نستخلص أن مقلة العين بالجاموس تحتوي علي مدي واسع من السكريات المرتبطة ما عدا سكر الفيوكوسيل.