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Summary

Lectin histochemical studies were performed on proximal duodenum from cattle, sheep and goats to demonstrate the binding patterns in Brunner's glands, using eleven different biotinylated lectins and an avidin-biotin-peroxidase complex (ABC) method. Eight of eleven lectins showed similar binding patterns in the epithelial and gland cells of three ruminant species. Brunner's gland cells have a greater overall lectin affinity than those of epithelial cells. The Brunner's gland cells were stained moderately with Con A, WGA and sWGA, and less moderately with RCA-I, PNA, SBA, DBA, UEA-I and LTA. LPA and GS-I-B_4 poorly stained the gland cells. The DBA affinities for the duodenal gland cells showed variations, negative to moderate, in the cattle and sheep but not in the goats. UEA-I and LTA had affinity variances in the duodenal gland cells for the three ruminants. The lobules of duodenal glands of cattle showed different staining patterns for PNA, SBA and UEA-I. PNA was positive for the gland cells in the central areas of the lobules (neutral mucin secreting) but negative for those in the peripheral areas (acid mucin secreting). In contrast, SBA and UEA-I showed positive affinities for the gland cells in the peripheral areas. In the cattle duodenum, three types of DBA affinity combinations were observed: positive-positive, positive-negative and negative-positive in the epithelial cells and the Brunner's gland cells, respectively.

Brunner's glands are branched tubuloalveolar glands located in the submucosa of mammalian proximal duodenum. Glandular epithelium produce mucin and secrete it into the intestinal lumen through ducts which open at the base or sides of the crypts of Lieberkühn (1). The histological nature of the secreting cells of the Brunner's gland varies among species and generally consists of mucous cells, except for rabbits and horses, which have two cell types-mucous and serous (2-4). The characteristics of mucins in the Brunner's glands of many mammals have been widely studied using conventional methods of mucous histochemistry (3, 5-10).

Lectins are valuable histochemical probes for detecting specific terminal

carbohydrate residues or carbohydrate sequences of oligosaccharides, due to their high affinity and specificity for sugar residues (11, 12). Lectin binding localization of the gastrointestinal tract has focused on the secreting and absorptive epithelia of the large and small intestine (see reviews, 13, 14). The lectin histochemistry on duodenal absorptive cells and Brunner's gland cells has been reported in some mammals and has shown the existence of the species difference in the sugar residues of complex carbohydrates (4, 15-20).

In the present study, the lectin binding patterns of Brunner's glands in cattle, sheep and goats were characterized and compared to those in duodenal absorptive cells, using 11 different biotinylated lectins and an avidin-biotin-peroxidase complex (ABC) method.

Materials and Methods

Animals and Tissue Preparation

Crossbred sheep (five females), Saanen goats (three females and two males), and Japanese Black cattle (five steers) were used. Tissue samples were dissected out from proximal duodenum. Tissues were fixed in 10% buffered formalin, embedded in Paraplast and cut into 4 μm sections.

Histochemistry

Sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) before and after diastase treatment, alcian blue (AB, pH 2.5), AB (pH 2.5)-PAS and high-iron diamine (HID).

Lectin Staining

Lectins used in this study (E.Y. Lab. Inc., Ltd., U.S.A.), and their sugar specificities are shown in Table 1.

Endogenous peroxidase in the sections was blocked by incubation in 0.3% H_2O_2/methanol for 10 min. The sections were rinsed in phosphate buffered saline, pH 7.4 (PBS) and covered with 1% bovine serum albumin (BSA) in PBS for 30 min. After shaking off the excess BSA-PBS, the sections were incubated with one of the biotinylated lectins (25 μg/ml), except for LPA and GS-I-B_4, in 0.1% BSA-PBS in a humidity chamber, for 30 min at room temperature. LPA was diluted to 50 μg/ml in 0.05 M Tris buffer, pH 7.4, containing 0.15 M NaCl and 0.01 M $CaCl_2$, and GS-I-B_4 was diluted to 25 μg/ml in PBS containing 0.15 M NaCl and 0.1 mM $CaCl_2$. The sections were rinsed with PBS, and then incubated with ABC (Vectastain ABC Kit, Vector Lab., Inc., U.S.A.) for 30 min at room temperature. After washing with PBS, the sections were immersed in 3, 3′-diaminobenzidine-4HCl (DAB, 0.2 mg/ml)-H_2O_2 (0.005%) in PBS, for 10 min. The sections were then washed in tap water, dehydrated, and mounted.

TABLE 1. *Lectins and Their Specificities*

Lectins	Acronym	Major sugar specification
Canavalia ensiformis	Con A	α-D-Man > α-D-Glc > GluNAc
Wheat germ	WGA	GlcNAc(β-1-4-GlcNAc)$_{1-2}$ > βGlcNAc > NeuNAc
succinylated WGA	sWGA	GlcNAc(β-1-4-GlcNAc)$_{1-2}$
Ricinus communis I	RCA-I	β-D-Gal > αGal ≫ GalNAc
Peanut	PNA	β-Gal(1-3)-GalNAc > α, βGal
Dolichos biflorus	DBA	GalNAc-α-1-3-GalNAc ≫ αGalNAc
Soybean	SBA	α-D-GalNAc > β-D-GalNAc > Gal
Ulex europaeus I	UEA-I	α-L-Fuc
Lotus tetragonolobus	LTA	α-L-Fuc
Limulus polyphemus	LPA	NeuNAc
Griffornia simplicifolia I-B$_4$	GS-I-B$_4$	α-Gal

Man : mannose, Glc : glucose, Gal : galactose, Fuc : fucose, GluNAc : N-acetylglucosamine, GalNac : N-acetylgalactosamine, NeuNAc : N-acetyl neuraminic acid (sialic acid)

Controls

The specificity of lectin binding was confirmed by incubating the sections with biotinylated lectins in the presence of hapten sugars. The sugars were 0.2 M α-methyl-D-mannoside for Con A, 0.2 M N-acetyl-D-glucosamine for WGA and sWGA, 0.2 M lactose for RCA-I, 0.1 M D-galactose for PNA, 0.1 M N-acetyl-D-galactosamine for SBA and DBA, 0.1 M L-fucose for UEA-I and LTA, 0.2 M sialic acid for LPA, and 0.2 M α-methyl-D-glucoside for GS-I-B$_4$. The sections were treated with ABC and DAB-H_2O_2 to confirm the absence of nonspecific binding for ABC. The endogenous peroxidase activity was also checked by incubating with DAB-H_2O_2 alone.

Results

Histology and Histochemistry

Brunner's glands in the three ruminant species studied were mostly located in the submucosa, and were found occasionally in lamina muscularis and lamina propria, in which the gland cells contact with the crypts of Lieberkühn. The glands consisted of numerous tubuloalveolar structure and their size were different between the three ruminant species. There was a succession in the width of gland lumen (narrow to wide), and in the cell height (low to high), for cattle, sheep and goats, respectively. The Brunner's glands of cattle and goats showed lobules separated by connective tissues, but those of sheep did not show lobules.

TABLE 2. *Results of Histochemical Study*

	PAS*	AB(pH 2.5)	AB-PAS	HID
Cattle				
Brunner's gland cells				
central areas	⧺	−/±	R	−
peripheral areas	⧺	⧺	B, BP	+/⧺
Goblet cells	⧺	⧺	RP	⧺
Sheep				
Brunner's gland cells	⧺	+	RP	−
Goblet cells	⧺	⧺	R, P, B	⧺
Goats				
Brunner's gland cells	⧺	+	B, BP	+
Goblet cells	⧺	⧺	R, P, B	⧺

* before and after diastase treatment.
Staining intensity: −, negative; ±, slight; +, week; ⧺, moderate; ⧺, strong
Colour of reaction: R, red; B, blue; RP, red purple; BP, blue purple; P, purple

The results of the conventional mucous histochemical staining are summarized in Table 2. Brunner's glands of the three ruminants were strongly stained by PAS before and after a diastase treatment. In cattle, Brunner's gland cells in the peripheral areas of the lobules showed moderate PAS and AB, and weak HID staining, whereas those in the central areas were strongly stained by PAS but negatively stained by AB or HID (Figs. 1-3). With AB-PAS staining, the

FIG. 1. Cattle duodenal gland stained with PAS-hematoxylin. Cells in the central areas of the lobules show stronger staining than those in the peripheral areas.

FIGS. 2 and 3. Cattle duodenal gland stained with AB (pH 2.5)-hematoxylin (Fig. 2) and with HID (Fig. 3). Cells in the peripheral areas of the lobules show positive staining, but cells in the central areas are negative.

FIG. 4. Cattle duodenal gland stained with PNA. Cells in the central areas of the lobules show positive binding, but cells in the peripheral areas of the lobules are negative.

FIGS. 5 and 6. Cattle duodenal gland stained with SBA (Fig. 5) and UEA-I (Fig. 6). Cell in the peripheral areas of the lobules show stronger affinity than those in the central areas.

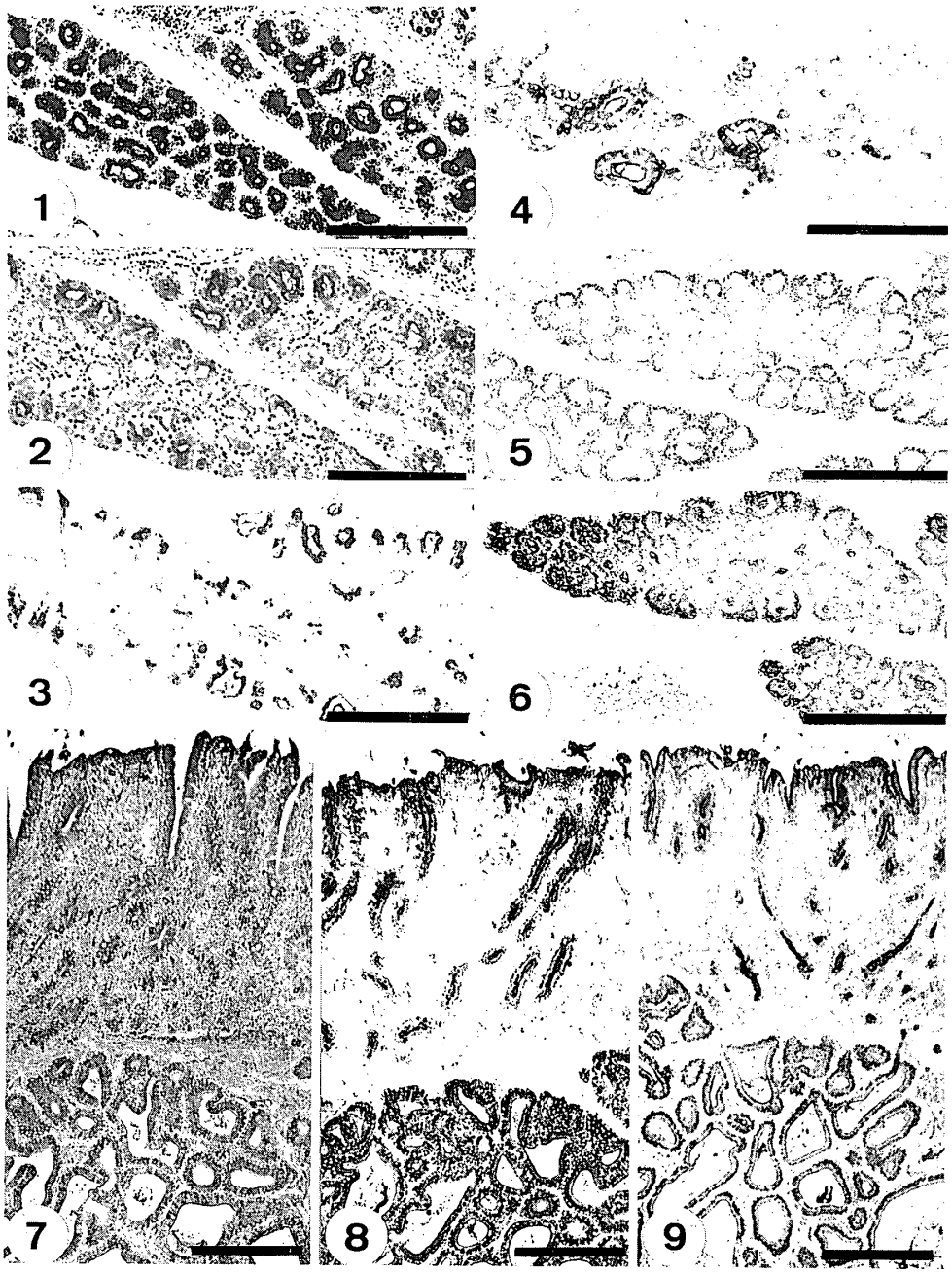
FIG. 7. Goat duodenum stained with Con A. Epithelial cells and Brunner's gland cells show moderate affinities. Connective tissues of lamina propria also show positive affinities.

FIG. 8. Goat duodenum stained with sWGA. Epithelial cells and Brunner's gland cells show strong affinities.

FIG. 9. Goat duodenum stained with RCA-I. Epithelial cells and Brunner's gland cells show moderate affinities.
Bars in Figs. 1-9 = 200 μm

Brunner's gland cells in the cattle exhibited a red colour in the central areas and blue to blue purple colors in the peripheral areas. Brunner's gland cells of sheep were positive to AB (pH 2.5) and negative to HID. Those of goats were stained positively by AB (pH 2.5) and HID. When stained with AB-PAS, Brunner's gland cells were red-purple and blue to blue-purple in sheep and goats, respectively. Sexual differences in the duodenal glands of goats were not observed in these stainings.

Goblet cells of three ruminant species reacted with PAS, AB (pH 2.5) and



HID. With AB-PAS staining, the mucous of goblet cells of cattle duodenum exhibited a red-purple colour, but those of sheep and goats revealed differences between individual goblet cells, which stained either red or blue or a combination of the two colours. Goblet cells in the lower crypts were stained more strongly than those of the upper villi. Columnar absorptive cells showed weak PAS and AB staining at Golgi areas and brush border.

Lectin Histochemistry

The relative lectin staining-intensities of the absorptive cells and Brunner's gland cells in the duodenum of the three ruminants are schematically illustrated as histograms in Fig. 16. Lectin staining patterns, exclusive of DBA, UEA-I and LTA, were almost the same in the three ruminant species. In the goats of either sex, there were no differences of the lectin staining in the Brunner's gland and epithelial cells. In the three ruminants, lectins showed a greater overall affinity for the Brunner's gland cells than for the absorptive and goblet cells of the mucosa, with the exception of LPA which, in some cattle, showed weaker affinities for the duodenal gland cells than for the epithelial cells (Fig. 16).

The Brunner's gland cells of the three ruminant species were stained moderately with Con A (Fig. 7), WGA and sWGA (Fig. 8), and less moderately with RCA-I, PNA, SBA (Figs. 9–11) and DBA (Figs. 13, 14). sWGA intensely stained more than other lectins examined. The affinity for DBA showed individual differences, with negative to moderate binding in the glandular cells of cattle and sheep (Fig. 16), but not in goats. UEA-I (Fig. 12) and LTA exhibited weak to moderate affinities for the gland cells of the sheep, but only produced slight staining in those of the goats. However, these two lectins showed different affinities for the gland cells of the cattle. LPA and GS-I-B_4 poorly stained the gland cells of the three ruminants. In the Brunner's glands of the cattle, PNA weakly or moderately stained the gland cells in the central areas of the lobules and was found in negative or trace amount in the peripheral areas of the lobules (Fig. 4). In contrast, for the cattle, SBA (Fig. 5) and UEA-I (Fig. 6) showed positive affinities for the cells in the peripheral areas of the lobules but negative or trace affinities for those in the central areas of the lobules.

As summarized in Fig. 16 and shown in Figs. 7–12, the absorptive cells were weakly or moderately stained with Con A, WGA, sWGA, RCA-I and SBA, and slightly or weakly stained with PNA, DBA, UEA-I and LTA. They were slightly or negatively stained with LPA and GS-I-B_4. DBA and UEA-I showed differences between individuals or between the ruminant species (Fig. 16). The mucous of goblet cells showed slightly positive affinities to SBA, WGA, sWGA, RCA-I and DBA. The cytoplasm of goblet cells of the three ruminant species were weakly or moderately stained by Con A, WGA, sWGA and RCA-I, and slightly stained by DBA, SBA and UEA-I.

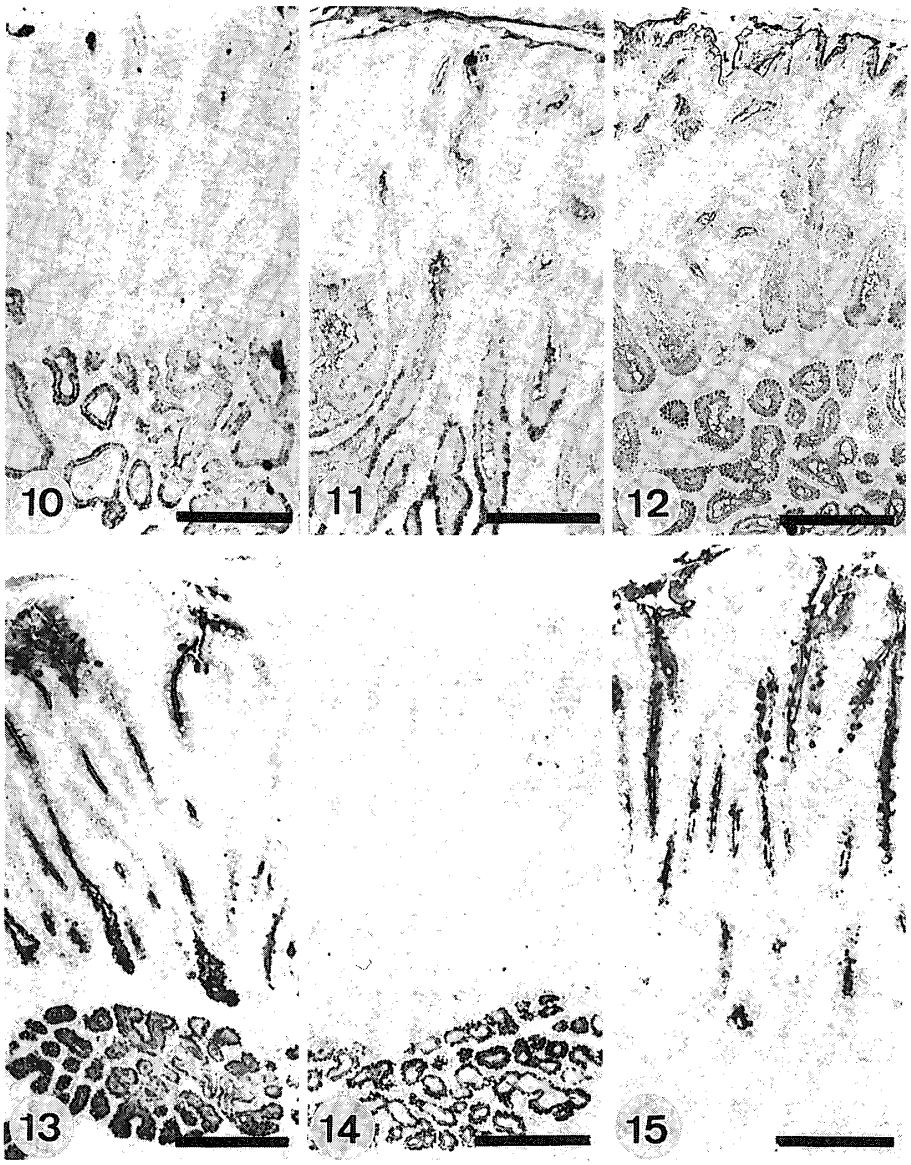


FIG. 10. Goat duodenum stained with PNA. Epithelial cells show negative or trace binding, and Brunner's gland cells indicate moderate affinity.

FIG. 11. Sheep duodenum stained with SBA. Epithelial cells and Brunner's gland cells show very weak and moderate affinities, respectively.

FIG. 12. Sheep duodenum stained with UEA-I. Epithelial cells and Brunner's gland cells show weak affinities.

FIGS. 13-15. Cattle duodenum stained with DBA. Both epithelial cells and Brunner's gland cells are positive (Fig. 13). Brunner's gland cells are positive but duodenal mucosa are negative (Fig. 14). Epithelial cells and Goblet cells are positive, but Brunner's gland cells are negative (Fig. 15).
Bars in Figs. 10-15=200 μm

In the duodenum of the Japanese Black cattle, DBA showed very unique and different staining patterns. The duodenal tissue of one animal showed positive staining in both absorptive and Brunner's gland cells (Fig. 13). Those of three

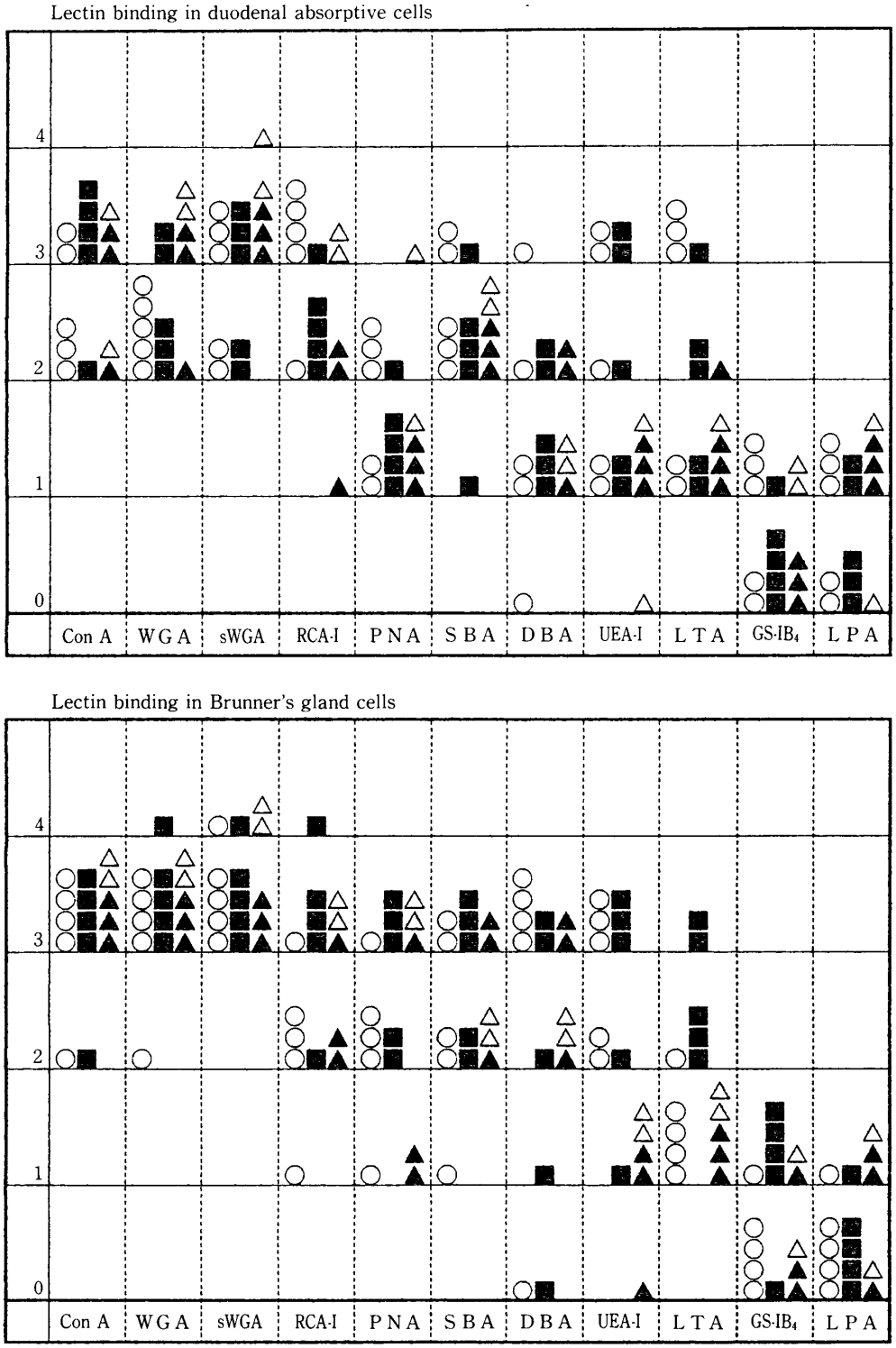


FIG. 16. Lectin staining scores in duodenal mucosal absorptive cells and Brunner's gland cells of cattle, sheep and goats.
○: Cattle, ■: Sheep, △: Male Goats, ▲: Female Goats
Staining intensity: 0=negative, 1=slight, 2=weak, 3=moderate, 4=strong

other steers showed moderate staining in the Brunners's gland cells but very slight or negative staining in the absorptive cells (Fig. 14). Those of the last animal showed a positive affinity only in the absorptive cells (Fig. 15).

Controls

When each lectin solution contained hapten sugars, they produced negative and weaker stainings than the originals (WGA, sWGA, GS-I-B_4). The nonspecific binding of ABC and endogenous peroxidase activity in the epithelial and gland cells were not observed.

Discussion

Histology and Histochemistry

Brunner's glands show great species specific difference in the width of glandular lumen and in the height of epithelial cells of the glands (21, 22). We observed in this study, the sccession in the width of gland lumen (narrow to wide), and in the cell height (low to high), for cattle, sheep and goats, respectively. These observations were identical with previous reports (22, 23). Cattle and goats showed gland lobules in Brunner's glands separated by connective tissues. Krause studied the duodenal glands of eight ungulate animals native to North America, and reported a lobular structure in the glands of American bison but not in the other seven ruminants (6). We confirmed the presence of Brunner's glands in the lamina proplia in the three domestic ruminants, and they are similar to what has been observed in humans and dogs (19).

The Brunner's glands of the three domestic ruminants produced specific reactions using conventional mucous staining methods (Table 2), showing that there are secretions of both neutral and acidic mucins. The duodenal gland cells of sheep and goats predominantly secrete neutral and acidic mucins, respectively. The Brunner's glands of Japanese Black steers produce neutral mucins in the central area of the lobules and acidic mucin in the peripheral area of gland lobules, as reported in Holstein cows (20). The acidic mucin of the gland cells of cattle and goats contain sulphomucin (HID positive). Takehana et al. (20) considered these localization in the lobules of duodenal glands peculiar to ruminant duodenal glands based on results of Holstein cows. Brunner's glands of goats, however, show the lobular structures but non of the histochemical features as had been observed in bovines. Furthermore, this unique staining pattern was reported only in American bison (6). Therefore, the histochemical characteristics of the duodenal glands of bovines are not peculiar to ruminants.

Many reports show the tendency for duodenal gland cells of carnivores to produce neutral mucin, and for those of herbivores to secrete both neutral and acidic mucins, with the exception of a few animals (3, 5-7). Omnivores including

primates show no clear tendencies. In ruminant duodenal glands, serous cells were not found as had been reported in those of horses and rabbits (2-4). It is interesting that horses and rabbits have simple stomachs and are herbivorous hindgut-fermenters; they differ from ruminants which are foregut-fermenters.

Lectin Histochemistry

The three species of domestic ruminants showed similar binding patterns to each lectin, except for DBA, UEA-I and LTA, in duodenal epithelial cells and Brunner's gland cells. Brunner's gland cells had a greater overall lectin affinity than in absorptive and goblet cells of the mucosa (Fig. 16). The same phenomena were observed in the duodenum of humans, monkeys, cats and dogs (19).

In the duodenum of Japanese Black steers, three unusual patterns of DBA binding were observed. The three types were combinations of different DBA affinities for epithelial cells and Brunner's gland cells: positive-positive, positive-negative and negative-positive, respectively (Figs. 13-15). Takehana et al. (20) did not observe the staining pattern of DBA in the duodenum of Holstein cows. So, it is not clear that the different binding patterns of DBA observed in duodenum of Japanese Black steers are peculiar to bovines. In sheep duodenal glands, DBA affinity showed individual differences, from negative to moderately positive (Fig. 16). In the esophageal epithelium of pigs, the binding patterns of DBA, PNA and UEA-I showed individual differences (23).

The affinities of Brunner's gland cells for UEA-I and LTA, both of which have a binding specificity for fucose (24), showed variable affinities between the three domestic ruminants, and furthermore, both lectins did not show the same staining affinities among cattle (Fig. 16). Differences between UEA-I and LTA binding were noted in the Brunner's gland cells of other animals (16, 18). These affinity differences between UEA-I and LTA support their linkage differences in tissues, as had been indicated by precise experiments in vitro (25).

In cattle, the duodenal gland cells in the central area of lobules showed stronger affinity for PNA and weaker affinities for SBA and UEA-I than the cells in the peripheral areas of lobules (Figs. 4-6). PNA was almost negative in the peripheral areas. These binding patterns were almost comparable to the patterns of conventional histochemical staining (Table 2). These binding features of PNA and UEA-I were also observed in the duodenal glands of Holstein cows (20). Two types of gland cells, containing neutral or acidic mucin, show zonal distribution in the duodenal glands of hamsters (9), but they did not show any differences with lectin staining (18). Crescenzi et al. (15) reported two different types of neutral mucins in the human duodenal glands, and the lectin binding patterns showed mutually exclusive reaction patterns with SBA positive cells unreactive to LPA and vice versa. In duodenal gland cells of horses, RCA-I showed no binding in both mucous and serous cells (4). So, the differences of lectin binding affinities

have no relation with the mucous character shown by conventional histochemical staining like as AB-PAS.

Many reports have suggested the existence of species and individual difference in the sugar residues of complex carbohydrates in glandular cells of the Brunner's gland (4, 15-20). N-acetylglucosamine were found to be dominant sugar residues in the Brunner's gland cells and absorptive cells of the three species of domestic ruminant examined. Also, mannose, N-acetylgalactosamine and fucose residues appeared to be rather common. Fucose residue was markedly less in goat duodenum when comparing the three ruminants. In contrast, α-galactose and N-acetyl neuramic acid were markedly less or absent. Although AB and HID staining results indicate the presence of sialomucin in the glandular cells and goblet cells, LPA which specifically binds to sialyl residues did not bind to these secretory cells. This shows that the sialyl residues in the tissues in question, are not freely accessible for this lectin, as has been suggested by Geleff and Böck (16).

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