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Lectin histochemical study on the kidney of normal and streptozotocin-induced diabetic hamsters

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Summary. Lectin histochemical characteristics of the kidney of normal and streptozotocin (SZ)-induced diabetic APA hamsters were investigated. Paraffin sections of the kidney of animals killed at 1 and 3 months after SZ-injection were stained with the following 10 lectins: PNA, BPA, DBA, SBA, GSA-I, GSA-II, MPA, WGA, UEA-1 and Con A. Renal lectin binding characteristics of normal APA hamsters differed in some aspects from those in other species reported previously. In the kidney of SZ-induced diabetic APA hamsters, binding activities of some lectins increased in the affected Bowman's capsules and glomeruli as well as in degenerated epithelial cells of uriniferous tubules. Among them, GSA-II in particular exhibited strong binding activity to the degenerated epithelial cells of Bowman's capsules, distal convoluted tubules and collecting ducts.

Key words: APA hamster, Diabetes, Kidney, Lectin histochemistry, Streptozotocin

Introduction

Syrian hamsters of the APA strain, which has been maintained as a closed colony by random breeding in our laboratory, are known to develop spontaneous mesangial thickening in the renal glomeruli from an early age (Han et al., 1992a). They also develop focal and segmental glomerulosclerosis (FSG) after 6 months of age (Doi et al., 1987). Recently, Han et al. (1990) reported that streptozotocin (SZ)-induced diabetic APA hamsters developed glomerular lipidosis and FSG within a short period, and they described histopathological, ultrastructural and morphometric characteristics of the renal lesions (Han et al., 1992b; Han and Doi, 1992).

On the other hand, reports of lectin histochemistry of the kidney in either normal or diabetic Syrian hamsters are scarce, although those in other laboratory animal species have been reported, and lectin histochemical changes are seen in diabetic kidneys (Watanabe et al., 1981; Holthofer, 1983; Schulte and Spicer, 1983; Hawthorne et al., 1986; Yonezawa et al., 1986; Henninger et al., 1987; Kizaki et al., 1989). To detect changes of glycoprotein component in the Syrian hamster kidney, in this study we tried to clarify the lectin binding characteristics of the kidney in normal and SZ-induced diabetic APA hamsters.

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Materials and methods

Twenty 2-month-old male Syrian hamsters of the APA strain were injected intraperitoneally with 40 mg/kg b.w. of SZ (Lot No. 78F-0517, Sigma) dissolved in 0.1M citrate buffer (pH 4.5) according to the method of Han et al. (1990). Six age-matched male APA hamsters received citrate buffer alone and served as controls. Blood glucose levels were colourimetrically measured on blood samples collected from each animal by orbital sinus puncture at 2-week intervals.

Animals were killed by exsanguination under ether anaesthesia at 1 and 3 months after SZ-injection (MAI), respectively. Kidneys were fixed in 10% neutral-buffered formalin and 2 μm-paraffin sections were stained with haematoxylin and eosin (HE) or periodic acid-Schiff (PAS).

For lectin histochemistry, sections were also stained with *Arachis hypogaea* agglutinin (PNA), *Bauhinia purpurea* agglutinin (BPA), *Dolichos biflorus* agglutinin (DBA), *Glycine max* agglutinin (SBA), *Griffonia simplicifolia* agglutinin (GSA)-I, GSA-II, *Maclura pomifera* agglutinin (MPA), *Triticum vulgaris* agglutinin (WGA) and *Ulex europeaus* agglutinin (UEA)-I (horseradish peroxidase (HRP) conjugated form, E-Y laboratories, San Mateo, CA) as reported before (Itagaki et al., 1988) and with *Canavalia ensiformis* (Con A) (Sigma, USA) according to the method of Bernhard and Avrameas (1971). Briefly, after blocking of endogenous peroxidase, sections were incubated with 0.1% bovine

serum albumin in phosphate buffer saline (PBS) for 30 min and sequently with a solution containing 30-80 μg/ml lectin-HRP conjugate in PBS overnight at 4 °C. 3,3'diaminobenzidine was used as HRP substrate and counterstain was carried out by haematoxylin. For ConA, instead of lectin-HRP conjugate solution, sections were incubated with unconjugated Con A (50 μg/ml PBS) overnight at 4 °C and with HRP (25 μg/ml in PBS) 1hr at room temperature. Some sections were digested with 0.15% trypsin (Merck) in PBS for 30 min at 37 °C before lectin staining. Histochemical control staining was also carried out as reported before (Itagaki et al., 1988).

Results

Changes in blood glucose levels

Diabetes developed in SZ-injected animals on and after 2 weeks (blood glucose levels: >350 mg/dl) while no significant change in blood glucose level was recorded in control ones.

Histological findings of the kidney of control animal

Control animals developed slight expansion of mesangial area of spontaneous nature in the renal glomeruli at 3 MAI (Fig. 1a).

Lectin histochemical findings of the kidney of control animal

Lectin reactivities of the kidney at 3 MAI are summarized in Table 1. The lectin binding characteristics were almost common to animals killed at 1 and 3 MAI, and trypsin pretreatment had little enhancing effects on the lectin stainability.

Renal corpuscle: The epithelia of Bowman's capsules were always stained with WGA, DBA, GSA-I (Fig. 1b),SBA and MPA and only sometimes with PNA and BPA. GSA-I (Fig. 1b), MPA and BPA bound weakly or moderately to some of the capillary loops but no lectins had binding sites in the mesangium.

Proximal convoluted tubule: WGA, GSA-I (Fig. 1b),

Table 1. Lectin reacitivy in each segment of nephron in APA hamsters.

		WGA	UEA-I	DBA	GSA-I	GSA-II	PNA	SBA	MPA	BPA	Con A
Renal corpuscle											
P. Bow.[1]		1-2	0	1-2	1-2	0	0-1	1-3	1-2	0-2	0
V. Bow.[2]		1-2	0	0-1	1-2	0	0-1	0-2	1-2	0-2	0
Cap.[3]	0	0	0	0	0-2	0	0	0	0-1	0-2	0
Mes.[4]	0	0	0	0	0	0	0	0	0	0	0
Proximal tubules											
Conv.	B.B.[5]	1-2	0	0	2	0-1	0-2	1-3	1-2	1-2	0-1
	Cyt.[6]	0-2*	0	0-2*	2*	2*	0-1*	0-1*	2*	2*	0-2*
Str.[7]	B.B.	1	0	0	0-3	0-2	0-2	1-2	1	0-2	0-1
	Cyt.	1	0	0	0-1	0-1	0-1	1	1	1	1
Loop of Henle											
Desc.[8]	A.B.	2	1-2	1-2	2	0-1	2-3	2	2	2	0
	Cyt.	0	0	0	0	0	0	0	0	0	0
Asc.[9]	A.B.	2-3	1-3	1-3	1-2	0-2	0-2	1-2	1	1-2	1
	Cyt.	1	0-1	1-2	0	0-1	0-2	1	0	0-1	0
Distal tubules											
	A.B.[10]	3	3	3-4	1-3	0-2	0-3	3	0-3	2-3	0
	Cyt.	1-3	1-4	1-3	0-3	0-2	0-3	1-2	0-2	0-3	0-2
Collecting Ducts											
Cort[11]	A.B.	3	3	3-4	1-2	0-1	0-3	2-3	0-2	2-3	0
	Cyt.	1-2	1-4	1-3	0-2	0-1	0-1	1	0-1	0-1	0-1
Med.[12]	A.B.	3	1-3	0-3	0	0-2	0-3	1-3	0-1	0-3	0
	Cyt.	1-3	1-2	0-3	0-3	0-2	0-3	0-2	0-2	0-3	0-1

[1]: Parietal layer of Bowman's capsule; [2]: Visceral layer; [3]: Capillary loops; [4]: Mesangium; [5]: Brush border of proximal convoluted tubule cells; [6]: Cytoplasm; [7]: Straight portion; [8]: Descending loop of Henle; [9]: Ascending loop of Henle; [10]: Apical border; [11]: Cortical collecting ducts; [12]: Medullary collecting ducts. Numbers indicate staining intensity on a subjective scale from 0, unreactive, to 4, most reactive. A 0-1, 0-2, 0-3 or 0-4 indicate heterogeneity in staining, with a subpopulation of cells being unstained and the remainder varying in reactivity from 1-4. *: Binding to each lectin was seen as a granular pattern.

SBA (Fig. 1c), MPA and BPA uniformly stained brush borders of epithelial cells, to which GSA-II (Fig. 1d), PNA and Con A showed heterogeneous stainability. In addition, all lectins except for UEA-I exhibited granular staining pattern within the cytoplasm of epithelial cells.

Straight proximal tubule: All lectins except for UEA-I and DBA bound moderately to both the brush border and cytoplasm of some epithelial cells.

Descending loop of Henle: All lectins except for Con A and GSA-II uniformly bound to the apical border of epithelial cells, while no lectins had binding sites in their cytoplasm.

Ascending loop of Henle: All lectins exhibited binding activities with varying intensity to the apical border of epithelial cells. WGA, DBA and SBA bound weakly but constantly to the cytoplasm of all epithelial cells, but

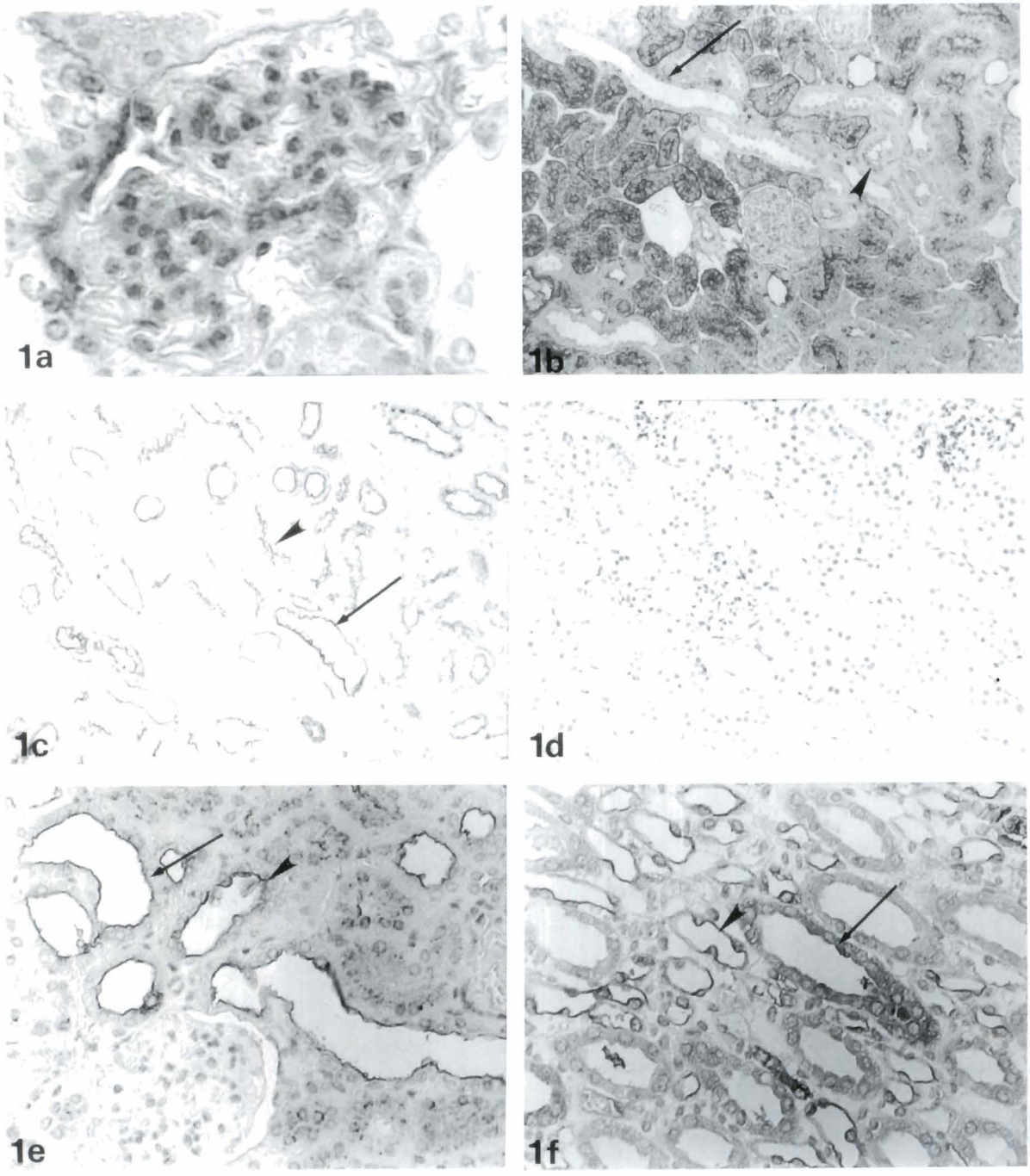

Fig. 1. A kidney of control APA hamster at 3 MAI. Except for a, all are lectin histochemically stained.
a. Slight expansion of mesangial area. PAS x 600.
b. Epithelia of Bowman's capsule, proximal and distal convoluted tubules (arrowhead) and cortical collecting ducts (arrow) are positive for GSA-I. x 125.
c. Epithelia of proximal (arrowhead) and distal tubules (arrow) are positive for SBA. x 125.
d. GSA-II reactivity to epithelia of proximal and distal tubules is very variable, and no positive reaction is seen in this case. x 125.
e. Epithelia of distal convoluted tubules (arrowhead) and cortical collecting ducts (arrow) are positive for PNA. x 300.
f. Epithelia of ascending loops of Henle (arrowhead) and medullary collecting ducts (arrow) are positive for PNA. x 300

UEA-I, GSA-II, PNA and BPA only bound sporadically to the epithelial cell cytoplasm.

Distal convoluted tubule and cortical collecting duct: DBA, UEA-I, WGA and SBA (Fig. 1c) bound strongly to the apical border and moderately or strongly to the cytoplasm of epithelial cells. On the other hand, GSA-I (Fig. 1b), GSA-II (Fig. 1d), PNA (Fig. 1e), MPA and BPA bound to the apical border and/or cytoplasm of epithelial cells with varying intensity.

Medullary collecting duct: All lectins bound to epithelial cells with varying pattern (Fig. 1f). Among them, WGA and UEA-I bound moderately or strongly to both the apical border and cytoplasm of epithelial cells.

Histological findings of the kidney of diabetic animal

Lesions in the uriniferous tubules were characterized by marked vacuolization of epithelial cells in the proximal convoluted tubules and by necrosis and desquamation of epithelial cells in the distal convoluted tubules and collecting ducts (Fig. 2a). On the other hand, lesions in the renal glomeruli were characterized by FSG showing segmental expasion of mesangial area with lipid droplets and foam cells at 3 MAI (Fig. 2b). In this connection, mild expansion of mesangial area was already observed in some glomeruli in the juxtamedullary cortex at 1 MAI.

Lectin histochemical findings of the kidney of diabetic animal

Lectin reactivities in the kidney of diabetic animals at 3 MAI are shown in Table 2. Apart from an increase in the intensity of lectin reactivity at 3 MAI, the lectin binding characteristics were almost common to hamsters killed at 1 and 3 MAI.

Renal corpuscle: DBA and GSA-I (Fig. 2c) reactivities to parietal epithelial cells became stronger, and GSA-II bound strongly to both the parietal and visceral epithelial cells at 3 MAI (Fig. 2d).

GSA-I (Fig. 2c), GSA-II, PNA, SBA, MPA, BPA and Con A exhibited granular stainability in the mesangium and foam cells, especially at 3 MAI.

Proximal convoluted tubule: The cytoplasm of degenerated epithelial cells was granularly stained with all lectins except for UEA-I, and their stainabilities increased at 3 MAI.

Straight proximal tubule: Lectin binding characteristics of epithelial cells in diabetic animals did not differ from

Table 2. Lectin reactivity in each segment of nephron in 3 MAI SZ-induced diabetic APA hamsters.

	WGA	UEA-I	DBA	GSA-I	GSA-II	PNA	SBA	MPA	BPA	Con A
Renal corpuscle [1]										
P. Bow.	1-2	0	2-3	2-3	2-3	0-2	1-3	1-2	1-3	0-2
V. Bow.	1-3	0	0-1	0-2	0-3	0-1	0-1	1-2	0-1	0
Cap.	0	0	0	0-3	0	0	0	1-2	0-2	0
Mes.	0	0	0	1	1	1	1	1	1	1
Proximal tubules										
Conv. B.b.	0-1	0	0-1	1-3	0	0-4	2-3	0-1	1-3	0
Cyt	0-2*	0	0-2*	2-4*	0-2*	2-3*	2*	0-3*	2-3*	0-3*
Str. B.B.	1	0	0	0-3	0-1	1-2	0-3	0-1	1-3	0-1
Cyt.	1	0	0	0-1	0-1	0-1	1	1	1	1
Loop of Henle										
Desc.A.B.	2-3	1-2	2	1-2	0-1	1-3	2	2-3	2	0
Cyt.	0	0	0	0	0-1	0-3	0	0	0	0
Asc.A.B.	2-3	1-2	2-3	0-2	0-2	0-3	2-3	1-2	1-3	0-1
Cyt.	1	0-1	1-2	0-2	0-2	1-3	1	0-2	0-1	0-1
Distal tubules										
A.B.	3-4	0-3	3-4	0-3	0	3-4	3-4	0-4	2-4	0
Cyt.	1-3	0-4	0-4	0-4	3	0-4	1-3	0-4	0-3	0-2
Collecting ducts										
Cort. A.B.	3-4	2-3	2-3	0-3	0-2	2-3	3	1-2	1-3	0
Cyt.	1-3	0-4	0-2	0-3	0-2	0-1	0-3	0-2	0-1	0-1
Med. A.B.	3-4	2	0-4	0	0-2	0-3	1-3	0	0-3	0
Cyt.	2-4	1-4	0-3	0-2	0-2	1-4	1-3	0-3	0-3	0-2

[1]: See the footnotes in Table 1 about abbreviation of each site. Numbers indicate staining intensity on a subjective scale from 0, unreactive, to 4, most reactive. A 0-1, 0-2, 0.3 or 0-4 indicate heterogeneity in staining, with a subpopulation of cells being unstained and the remainder varying in reactivity from 1-4. *: Binding to each lectin was seen as granular to vacuolar pattern.

those in control ones.

Descending and ascending loop of Henle: There was no significant difference in the lectin reactivity of epithelial cells between diabetic and control animals.

Distal convoluted tubule: PNA showed strong binding activity to the apical border of epithelial cells at 3 MAI (Fig. 2e). In addition, SBA and MPA bound to the apical border and PNA (Fig. 2e), GSA-II (Fig. 2d), SBA and MPA to the cytoplasm of degenerated epithelial cells, respectively.

Cortical collecting duct: The binding activity of GSA-II to the cytoplasm of degenerated epithelial cells increased at 3 MAI (Fig. 2d).

Medullary collecting duct: The cytoplasm of epithelial cells was moderately or strongly stained with PNA (Fig. 2f), SBA and MPA at 3 MAI.

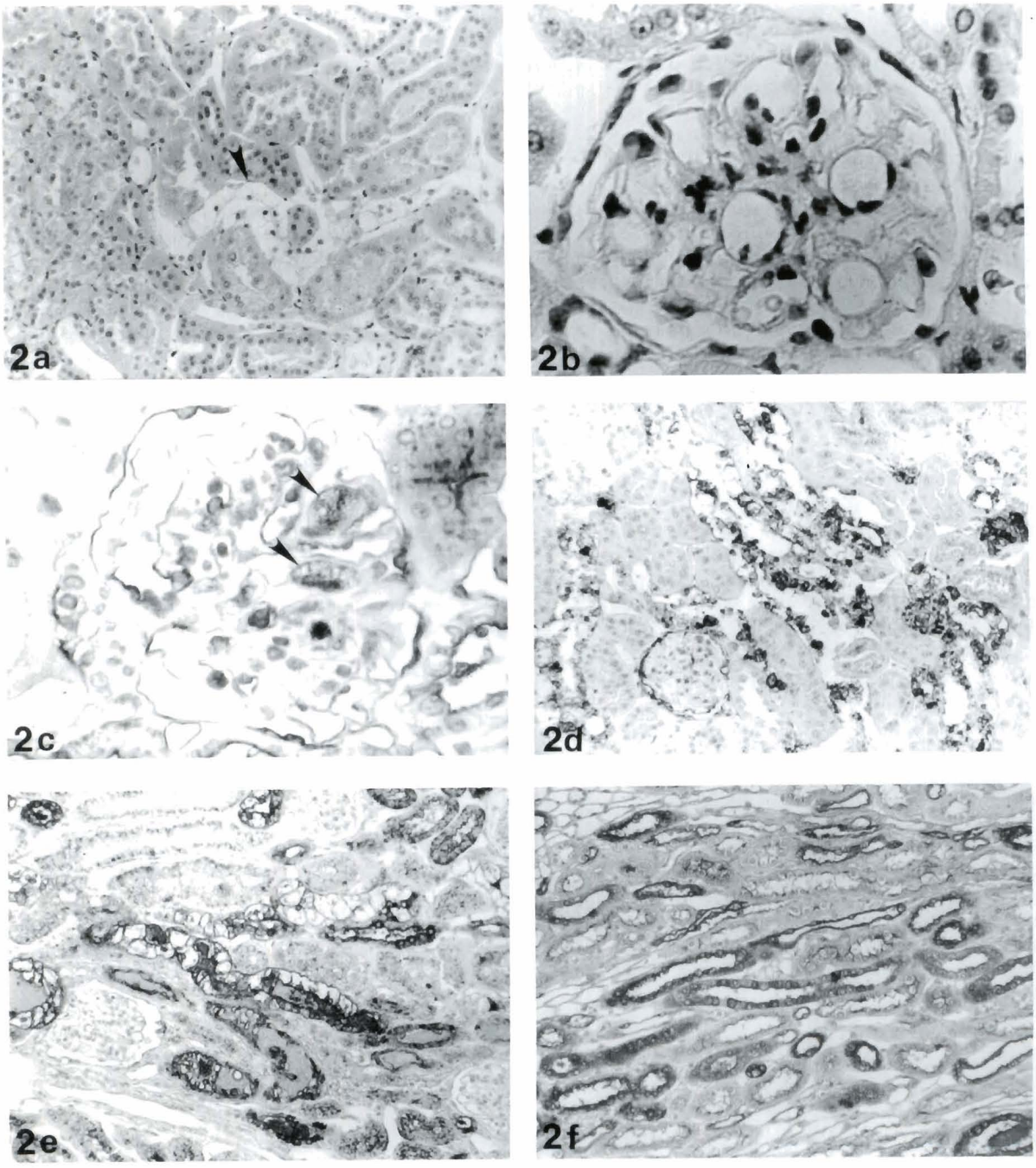

Fig. 2. A kidney of SZ-induced diabetic APA hamster at 3 MAI. Except for a and b, all are lectin histochemically stained. **a.** Necrosis in distal convoluted tubule epithelia (arrowhead). HE x 150. **b.**Segmental expansion of mesangial area with lipid droplets and foam cells. x 600. **c.** Cytoplasm of foam cells shows granular stainability for GSA-I (arrowheads), x 500. **d.** Degenerated epithelia of distal convoluted tubules and cortical collecting ducts are strongly positive for GSA-II. x 150. **e.** Degenerated epithelia of distal convoluted tubules are strongly positive for PNA. x 150. **f.** Epithelia of medullary collecting ducts are positive for PNA. x 150

Discussion

In the present study, we clarified the lectin binding characteristics of the kidney of normal and SZ-induced diabetic APA hamsters, of which histological and histopathological findings were the same as those previously reported by Han et al. (1992b).

Except for WGA stainability, lectin binding characteristics of the normal APA hamsters were almost similar to those of normal rats (Le Hir and Dubach, 1982; Murata et al., 1983). Namely, WGA bound to neither glomerular capillary loops nor mesangium in APA hamsters.

Holthofer (1983) reported that SBA had affinity to the proximal tubule epithelial cells in humans, pigs and rats while SBA receptors were found preferentially in the distal tubule epithelial cells in mice, rats, guinea pigs and rabbits. SBA stainability in APA hamsters was similar to that in the latter animal species. In addition, UEA-I was considered to be a marker of distal tubule epithelial cells in APA hamsters as reported in rabbits, guinea pigs and pigs by Holthofer (1983).

Thus APA hamsters shared many lectin binding characteristics in the kidney with other animal species, and this suggests that the main nephron functions are basically the same in all animal species though there are some structural differences among them.

As to the changes in lectin binding activities of the kidney of diabetic animals, Hawthorne et al. (1986) reported an accumulation of WGA-positive substance in the glomerular basement membrane and mesangium in diabetic rats. In addition, Yonezawa et al. (1986) reported that GSA-I A4 had binding sites in the sclerotic areas of glomeruli in diabetic mice. In the kidney of diabetic APA hamsters, as compared with control ones, the lectin reactivity generally increased especially at 3 MAI, and granular deposition, positive for many lectins, was observed in the thickened mesangium, foam cells and degenerated epithelial cells of the proximal convoluted tubule. This suggests that glycocompounds accumulated in such affected renal structures as mentioned above probably due to long-lasting hyperlipidemia as well as hyperglycemia as reported in SZ-injected APA hamsters by Han et al. (1992b).

Furthermore, GSA-II bound strongly to the degenerated parietal cells of Bowman's capsules and it increased its reactivity to the degenerated epithelial cells in the distal convoluted tubules and collecting ducts in diabetic APA hamsters at 3 MAI. Such a change in GSA-II stainability has not been reported in other diabetic models, and GSA-II may be useful as an indicator of the development of lesions in the distal tubule epithelia in the case of SZ-induced diabetic APA hamsters.

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