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Changes of Glycosylation Pattern in Aging Rat Kidneys as Revealed with Lectin Conjugates

Young Gi Gil, Keun Ha Kim and Byung Tae Choi[1]*

Department of Anatomy, Medical School, Kosin University, Busan 602-703, Korea
[1]Department of Anatomy, School of Oriental Medicine, Pusan National University, Busan 609-735, Korea

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The changes of glycoconjuagates (GCs) in rat kidney due to maturation were studied from samples of fetal and postnatal kidneys by lectin histochemistry. Rat kidneys of perinatal ages and adults were fixed in 4% paraformaldehyde and were stained with nine kinds of biotinylated lectins. The immature forms of the renal developmental stage such as vesicles and ureteric bud were observed in the cortex as late as day 14 of postnatal life, but the histological appearance of the weaning kidney was similar to that observed in adults. As for histochemical properties of GCs in the glomeruli, Con A affinity tended to increase with aging, but both RCA-1 and LCA affinities showed a transient increase in immature glomeruli of neonatal rats. DBA affinity with SBA, PNA, BSL-1 and RCA-1, additional Con A one in proximal tubule, were increased in both proximal and distal tubules according to maturation. In contrast to this, transient intensive LCA affinity were demonstrated in immature proximal and distal tubule of neonatal rats. In the collecting tubules, DBA, SBA, PNA and sWGA affinities tended to increase according to maturation, but transient increase for BSL-1, RCA-1 and LCA affinities were detected in neonatal rats. The present results suggest that the mature glycosylation pattern of the kidney undergoes profound changes during maturation and is probably associated with functional maturation of the kidney.

Key words – Glycoconjuagtes, lectin, kidney, development, rat

Introduction

The kidney has an intricate structure that underlies its diverse roles of excreting waste products in the form of urine, regulating fluid and acid-base balance of the body and secreting hormones [2]. But the renal fine structure of newborn animals is different from that of adult animals [4]. Renal function is also know to be immature in newborn animals, and becomes mature in adult animals after postnatal development [5,8].

The epithelial glycoconjugates (GCs) which are observed widely in various system of the body have great functional diversity according to each organ. The adult mammalian kidney presents a regionally restricted GCs distribution reflecting the functional compartmentalization [8]. Previous histochemical evidences from the rat kidney indicate that developmental differences in its affinities appear to reflect a maturation of cellular carbohydrate components [7].

Lectins, proteins and glycoproteins that bind with an antibody-like affinity to specific oligosaccharides in specific linkage, are useful histochemical tools for more specific information about the localization and chemical structure of GCs. Lectins histochemistry seem to provide useful results to study various aspects of cells and tissue maturation of the kidney and can also distinguish between morphologically similar cells displaying functionally distinct activities during maturation [5,6].

The kidneys need the full development of the entire nephron structure and an adequate development of collecting ducts system after birth in order to be functionally efficient. But glycosylation pattern due to maturation has not been extensively studied. The present study was conducted to analyze the GCs maturation in the kidney both in developing and adult rats by histochemical methods with nine kinds of biotinylated lectins.

***Corresponding author**
Tel : +82-51-510-7379, Fax : +82-51-510-1020
E-mail : choibt@pusan.ac.kr

Materials and Methods

Animals

Female Sprague-Dawley rats from 12 to 15 weeks of age were placed overnight from 18:00 to 08:00 hours with males were examined for the presence of sperm in vaginal smear on the following morning. The day which identified

sperm in vaginal smears was designated as day 0 of gestation. Perinatal rat from bleeding were used as four period-groups, such as fetal (18 days fetus), newborn (1 and 3 days old), suckling (5, 7, and 14 days old), weaning (21 days old) and adults (105 days old) in relation to development. Five rats were used in each group. All experiments conformed to guidelines approved by the Council of the International Association for the Study of Pain in December 1982.

Histological observation

The tissue of kidneys were obtained from perinatal and adult rats under 10% chloral hydrate anesthesia (350 mg/kg i.p.) and fixed in 4% paraformaldehyde for 12 hr. Tissues were dehydrated in a graded ethanol series and embedded in paraffin. Serial 5 μm thick sections were prepared. The hematoxylin-eosin stain were used for histological observation.

Lectin histochemistry for GCs

For the lectin histochemisty, deparaffinized sections were treated with 0.3% methanolic hydrogen peroxide for 30 min to remove peroxidase remains in tissues. To prevent non-specific immunoreactions, sections were exposed to 1% bovine serum albumin for 30 min at room temperature. Nine different biotinylated lectins purchased from Vector Laboratories Inc. (Burlingame, CA, USA) were used whose concentration and oligosaccharides specification are shown in Table 1.

Sections were incubated with lectins for 18 hr at 4℃, and rinsed with phosphate buffered saline (PBS, 10 mM, pH 7.4), and then incubated with an avidin-biotin-peroxidase-complex (Vector Lab.) for 1 hr at room temperature. The horseradish peroxidase-conjugated lectin was visualized by exposure to diaminobenzidine substrate kit (Vector Lab.). Sections were rinsed with PBS and distilled water and counterstained with Mayer's hematoxylin. As controls for the lectin histochemistry, sections were incubated with horseradish peroxidase-conjugated lectins in the presence of 0.2 M inhibitory sugars.

Results

Histological observation

In the kidney of an adult rat, the glomeruli, proximal and distal convoluted tubules of mature nephron and collecting tubules were densely packed in the renal cortex region. But the perinatal periods from 18 days fetal to 14 days old rats, immature forms of renal developmental stage such as condensate cells or vesicles and ureteric bud were observed in the cortex with mature nephron. In this period, some islets of tubular structure with surrounding the collecting ducts were also observed in the medullary regions. From weaning rat on postnatal day 21, the histological appearance of the kidney was similar to that observed in adult rats.

Lectin histochemistry for GCs

The results of GCs properties in the kidney using lectin histochemistry are outlined in Table 2. As for the GCs properties in the glomeruli of nephron, three lectin affinities such as RCA-1, LCA and Con A were demonstrated in immature glomeruli of fetal or neonatal rats. The RCA-1 and LCA affinities disappeared in the mature glomeruli from weaning to adult rats, but more intensive Con A affinity was observed in the mature glomeruli of nephron (Fig. 3).

All lectin affinities examined except UEA-1 were shown in the adult proximal tubules of nephron and somewhat

Table 1. Lectins used for identifying carbohydrate residues

Lectins	Source	Major sugar specification	Concentration (μg/ml)
DBA	*Dolichos biflorus*	α-N-acetyl-D-galactosamine	10
SBA	*Glycine max*	α/β-N-acetyl-D- galactosamine	10
PNA	*Arachis hypogaea*	Galactosyl-(β-1,3)-N-acetyl-D-galactosamine	10
BSL-1	*Bandeiraea simplicifolia*	α-D-galactose	10
RCA-1	*Ricinus communis*	β-D-galactose	10
SWGA	*Triticum vulgaris*	β-N-acetyl-D-glucosamine	10
UEA-1	*Ulex europaeus*	α-L-fucose	10
LCA	*Lens culinaris*	α-D-mannose, α-D-glucose	10
ConA	*Canavalia ensiformis*	α-D-mannose, α-D-glucose	10

sWGA : succinylated WGA

Table 2. Changes of glycosylation pattern in aging rat kidney by lectin histochemistry

Lectins	Region	Fetal rat	Postnatal rat						
			Newborn		Suckling			Weaning	Adult
		18	1	3	5	7	14	21	105
DBA	GL	0	0	0	0	0	0	0	0
	PT	0	0	0	0	0-+	0-+	+	++
	DT	0	0	0	0	0-+	++	++	+++
	CD	0	0-+	+	++	++	++	+++	+++
SBA	GL	0	0	0	0	0	0	0	0
	PT	0	0	0-+	0-+	0-+	0-+	+	++
	DT	0	0	0	0	0	0	0-+	+-++
	CD	+	+-++	+-++	+-++	++	++	++	++
PNA	GL	0	0	0	0	0	0	0	0
	PT	0	0	0	0	0	0-+	0-++	0-+++
	DT	0	0	0	0	0	+-++	+-++	+-++
	CD	+	++	+	+	+	++	++	++
BSL-1	GL	0	0	0	0	0	0	0	0
	PT	0	+	++	++	++	+++	+++	+++
	DT	0	0-+	0-+	0-+	0-+	0-+	0-+	0-++
	CD	0	+	++	++	++	+++	0-+	0-+
RCA-1	GL	+-++	++	++	++	++	0	0	0
	PT	0-+	0-+	++	++	++	+++	+++	+++
	DT	0	0-+	+	+	+	+	+	+
	CD	+	++	++	++	++	+	+	+
sWGA	GL	0	0	0	0	0	0	0	0
	PT	0	+	++	+	++	++	+/+++	0-+/+++
	DT	0	0-+	0-+	0-+	0-+	0-+	0-+	0-+
	CD	0	+	+	+	+	+	++	++
LCA	GL	0	0	0	+	+	0	0	0
	PT	0	0-+	+-++	++	++	+	+	+
	DT	0	0	0-+	+	+	0-+	0-+	0-+
	CD	0-+	0-+	0-+	+	+	0-+	0-+	0-+
ConA	GL	0-+	0-+	0-+	+	+	+	+	+
	PT	0	0/+	+/++	+/++	+/++	+/++	++	++
	DT	0	0	0	0	0	0	0	0
	CD	0	0-+	0-+	0-+	0-+	0-+	0-+	0-+

Abbreviations: GL, glomeruli; PT, proximal tubules; DT, distal tubules; CT, collecting ducts.; /, proximal convoluted tubules/proximal straight tubules.
Degree of staining: +++, intense, ++, moderate; +, weak; 0, absent.

stronger ones such as BSL-1 and RCA-1 were observed than others. Besides, more intensive sWGA and Con A affinities were shown in the proximal straight tubules than proximal convoluted tubules. DBA affinity with SBA, PNA, BSL-1, RCA-1 and Con A were increased in amount with maturing of the proximal tubules. In contrast to this, sWGA and LCA affinities were increased temporarily in suckling or weaning period of aging kidneys(Fig. 1, 2 and 4).

The changes of lectin affinities in the distal tubules of nephron showed a similar pattern to those in the proximal tubules, however, significant differences such as the lacking affinity for Con A was revealed. DBA, SBA, PNA, BSL-1 and RCA-1 affinities tended to decrease with maturation in the distal tubules, but LCA affinity were increased temporarily in the neonatal period as shown in proximal tubules(Fig. 1 and 2).

All lectin affinities examined except UEA-1 were shown in the collecting tubules of kidneys. Affinities for DBA, SBA, PNA and sWGA were increased in the collecting tubules according to maturation, but BSL-1, RCA-1 and LCA

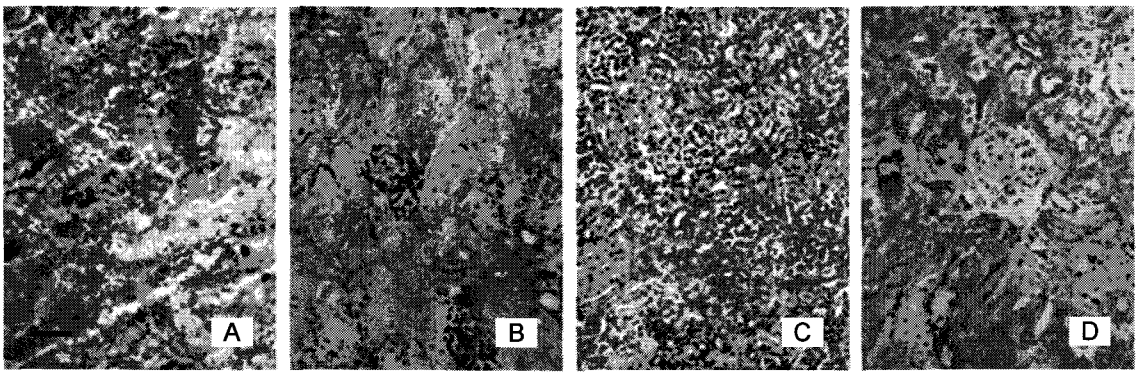

Fig. 1. DBA binding pattern in the rat kidney of 1 day old newborn (A), 5 days (B) and 14 days old (C) suckling and 105 days old adult rats (D). Note the strong reactivities in the mature proximal and distal tubules of the adults compared with immature tubules from fetal to suckling rat. Bar scale = 100 μm

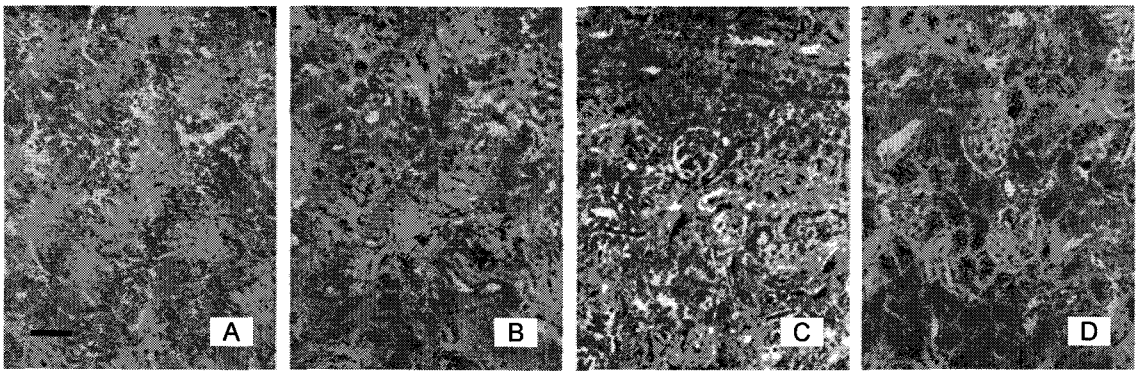

Fig. 2. BSL-1 binding pattern in the rat kidney of 1 day old newborn (A), 5 days (B) and 14 days old (C) suckling and 105 days old adult rats (D). Note the strong reactivities in the mature proximal and distal tubules, especially in the proximal tubules, of the adults compared with immature tubules from fetal to suckling rat. Bar scale = 100 μm

affinities were temporarily increased in the neonatal period.

Discussion

The structural and functional unit of the kidney is the nephron which consists of renal glomeruli, the proximal tubule, the loop of Henle and the distal tubule, and then the nephron empty into a complex system of collecting ducts [2]. The newborn kidney is functionally non-efficient and these functional characteristics extend after the post weaning period [13]. The present study described age-related changes of GCs in the renal glomeruli, proximal and distal tubule and collecting ducts with morphological changes.

The fetal and newborn kidney is very immature and all stages of renal glomerular and tubular development can be visualized in the histological sections [3,10]. From two-stage process of kidney development, the first involves inductive interaction between ureteric bud and metanephric mesenchyme and occurs until around postnatal day 10. The second stage is marked by rapid division of tubular epithelial cells and is present as late as day 20 of postnatal life [10].

In the present study, immature forms of renal developmental stage such as vesicles and ureteric bud were observed in the cortex from fetal to 14 days old rat between mature nephron and collecting ducts. After postnatal day 21, the histological appearance of the kidney was similar to that observed in adult rats. These morphological results were consistent with previous researchers who reported that an active process of cortical cell proliferation and differentiation occur as late as postnatal day 20 [10].

Along the nephron, various cell types can be distinguished fulfilling specific functions of filtering, reabsorption and secretion [11,14]. Lectins, a new group of cell type-specific markers, have been introduced to the study of renal structure and function based on their unique property to stain selectively various epithelial cell

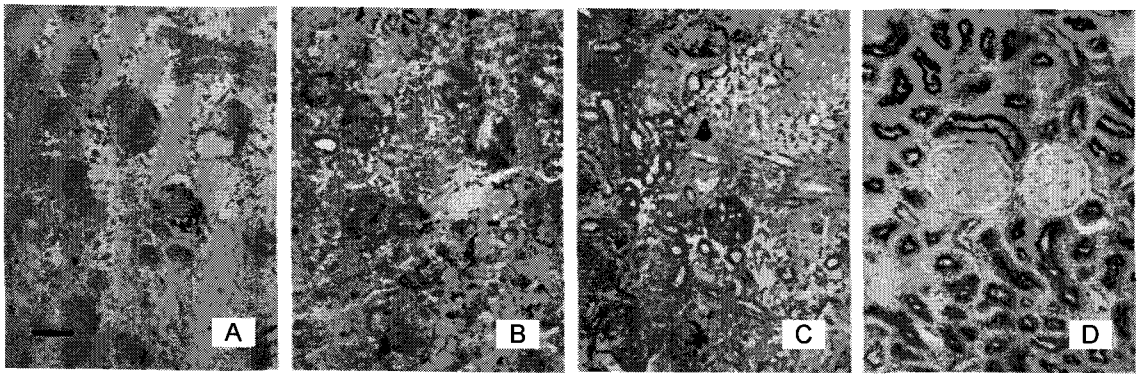

Fig. 3. RCA-1 binding pattern in the rat kidney of 18 days old fetal (A), 1 day old newborn (B) and 7 days old (C) suckling and 105 days old adult rats (D). Note the strong reactivities in the immature glomeruli from fetal to sucking rats and the disappearance of those reactivities in mature glomeruli of adult rats. In contrast to this, the reactivity of proximal tubules tended to increase with maturation. Bar scale = 100 μm.

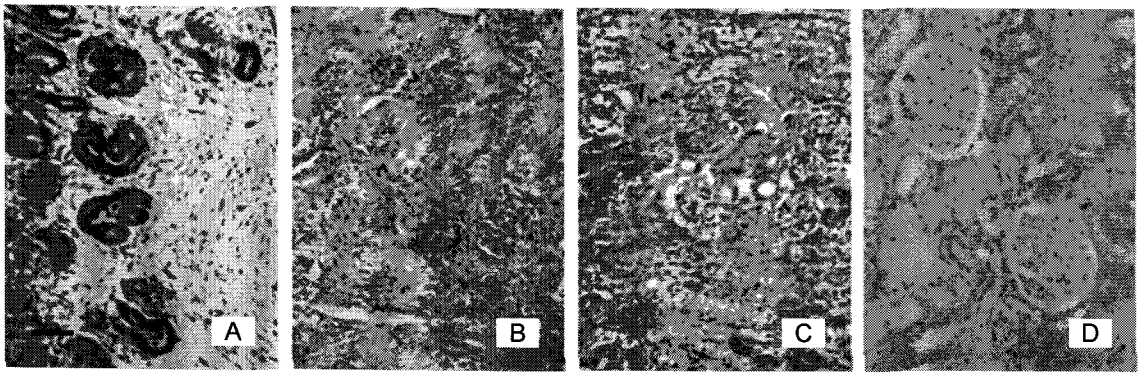

Fig. 4. LCA binding pattern in the rat kidney of 18 days old fetal (A), 1 day old newborn (B) and 7 days old (C) suckling and 105 days old adult rats (D). Note a transient increase affinity in immature proximal tubules of newborn and suckling rat compared with mature proximal tubules of adults. Bar scale = 100 μm.

types along the nephron [5]. The histochemical lectin-binding studies have revealed a high degree of cell type-specific GCs expression and compartmentalization along the nephron by their specific reaction to the cell coat [1,2].

The affinities with different types of lectin have been shown to indicate alterations of kidney glycosylation that are probably attributed to physiological function [12]. However, the newborn kidney has low glomerular filtration rate, a limited capacity to reabsorb sodium and a low ability to concentrate urine and these no-efficient functional characteristics extend as late as the postnatal weaning period [10,13].

Thus, the adult kidney has been subjected to studies on cell type-specific GCs levels present in glycoproteins. Previous histochemical evidence from the adult rat kidney indicates the presence of specific reaction to cell glycosylation within nephron. The glomeruli are positive for BSL-1 and the proximal tubule react with DBA, BSL-1 and WGA. The positive for DBA, SBA, BSL-1 and PNA shows in the distal tubule [1,10]. In the present study, the results of kidney GCs properties showed that portions of nephron and collecting ducts differ from one another with regard to their lectin binding properties in accordance with previous findings.

The development of protein glycosylation in the kidney remains undifferentiated until the postnatal period and exhibit a dynamic expression pattern during nephron morphogenesis [14]. As for the immature forms of renal developmental stage, the ureteric bud of the early metanephros of the mouse reacts with Con A, LCA, RCA-1, SBA and PNA [9]. The ureteric bud of the rat also presents apical BSL-1 reactivity which disappears thereafter [10].

In the glomeruli, the maturation of the podocyte sialoglycoprotein coat and glomerular basement membrane are multiphasic processes that continue late into postnatal development [8]. Early mouse glomeruli expressed heteroge-

neously terminal galactosyl and N-acetylgalactosaminyl moieties in the podocyte, but later these sites disappeared and were apparently covered by sialic acid [9].

In the tubules of nephron, terminal fucosyl residues which character mature proximal tubules appear postnatal development in mouse [9]. The intensity of binding begins to acquire the adult pattern from postnatal day 20 in the proximal and distal tubules of nephron of the rat kidney [10]. As for the lectin binding in the collecting ducts, DBA reactivity are appeared by day 13 in mouse [9]. The positive for DBA and VVA and negative for BSL-1 shows in the fetal rat kidney, and positive ones decrease to amounts found in the adults kidneys [5,10].

These results present that the mammalian kidney exhibit a dynamic expression pattern during nephron morphogenesis. Hanai et al. [4] categorized the changes of lectin affinities in aging kidney into four groups: (a) consistent positive affinities, (b) positive ones during gestation but become negative with aging, (c) negative ones in the gestational stage but become positive with aging and (d) consistent negative ones. In the present study, the GCs changes of the developing kidney could be classified into two groups with different lectin affinities.

The first tended to increase according to maturation and the other showed a transient increase in specific developmental stage such as fetal or newborn rats. According to maturation, DBA affinity recognizing α-N-acetyl-D-galactosamine, SBA one recognizing α/β-N-acetyl-D-galactosamine and PNA one recognizing galactosyl-(β-1,3)-N-acetyl-D- galactosamine are increased in both tubules of nephron and collecting ducts and Con A one recognizing α-D-mannose and glucose in glomeruli. In contrast to this, transient intensive LCA affinity recognizing α-D-mannose and glucose were demonstrated in immature tubule of nephron and collecting ducts of neonatal rats.

The present results suggest that the compartmentalized expression of cell GCs in the adult rat kidney is acquired in a sequential manner during kidney maturation. Although the understanding of GCs function in the developing kidney still remains unclear, such sequential appearance of the mature glycosylation pattern probably reflects functional changes in maturation progresses of the nephron. Nonetheless, we demonstrated in this study some alterations in GCs distribution of the kidney with aging and these histochemical results may reflect different functions between immature and mature structure of the kidney.

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초록 : 성장과정 중 흰쥐 신장의 복합당질 변화에 대한 연구

길영기 · 김근하 · 최병태[1]*

(고신대학교 의과대학 해부학교실, [1]부산대학교 한의학전문대학원 해부학교실)

성장과정 중 흰쥐 신장에서 나타나는 복합당질의 변화를 알아보기 위해 18일 태자부터 성체에 이르는 신장을 형태적 관찰과 더불어 9 가지 lectin (SBA, DBA, PNA, BSL-1, RCA-1, sWGA, UEA-1, LCA 및 Con A)으로 검색하였다. 신장 발생단계에서 성숙한 신원구조와 함께 미성숙한 구조물 즉 소포와 요관아 등이 생후 14일에 이르기까지 관찰되었으며 생후 21일에 이르러 성체와 유사한 구조적 특성을 보였다. 복합당질의 변화를 보면 사구체에서 RCA-1, LCA 및 Con A에 반응을 나타내며 RCA-1 및 LCA는 태자와 신생쥐에서 일시적으로 증가하다 성체에서 관찰되지 않으나 Con A는 성장과 더불어 증가하였다. 근위곡요세관은 UEA-1을 제외한 모든 lectin에 반응하며 DBA, SBA, PNA, BSL-1, RCA-1 및 Con A반응이 성장과 더불어 증가하며 특히 RCA-1과 BSL-1반응이 현저하였다. 이에 비해 sWGA와 LCA반응은 성장과정에 일시적으로 증가하며 성체에 이를수록 감소하였다. 원위곡요세관도 근위곡요세관 유사하게 DBA, SBA, PNA, BSL-1 및 RCA-1반응은 성숙과 함께 증가하나 LCA반응은 성숙과정에 일시적으로 증가하며 성체에서 감소하였다. 집합관에서는 DBA, SBA, PNA, sWGA반응이 성숙과 동시에 증가하나 BSL-1, RCA-1, LCA반응은 미성숙관에서 일시적으로 증가하였다. 이상의 반응으로 보아 신장발생과정에서 형태적 기능적 성숙과 함께 다양한 복합당질의 변화를 보이는데 대체로 성숙에 따라 반응이 증가하는 복합당질군과 미성숙기에 일시적으로 증가하며 성체에서 감소하는 복합당질군으로 대별할 수 있었다. 이러한 출생전후 복합당질의 변화는 신장의 기능적 성숙과정과 연관성을 가지며 발생과정에서 현저한 변화를 나타내는 복합당질은 정상 신장발생에 대한 표지인자로 유용할 것이다.