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CHEMICAL CHARACTERIZATION OF GLOMERULAR AND TUBULAR BASEMENT MEMBRANES OF CATTLE OF DIFFERENT AGES

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Summary

Glomerular and tubular basement membranes were isolated from fetal, neonatal, young and adult bovine kidneys.

An isolation method with sieves for both glomeruli and tubules from the same kidney was developed. A detergent procedure appeared to give purer glomerular and tubular basement membrane preparations than the generally used sonication method. No large differences were found in the composition of glomerular and tubular basement membrane of adult animals.

Glomerular and tubular basement membrane preparations of the four age groups showed an increase with age of hydroxylysine and both 3- and 4-hydroxyproline. The most marked increases appeared at different stages of development, that of tubular basement membrane being between fetal and neonatal stages and glomerular basement membrane between 18 weeks old and adult animals. The ratio of 3- to 4-hydroxyproline increased considerably during development. Total imino acid content was higher for both types of basement membrane from adult than from young animals, while total content of hydroxylysine plus lysine remained fairly constant.

The increase in hydroxylation of lysine was accompanied by a corresponding change in glucose and galactose content so that the ratio of galactose to hydroxylysine or glucose to galactose remained constant. Fucose content of both types of basement membranes was the same for all age groups but content of aminosugars and mannose gradually increased with age.

Introduction

The basement membranes of the nephron have clearly more than one function. The glomerular basement membrane has a role in the ultrafiltration of plasma. This process is determined by the size and charge of the structural pores

in the glomerular basement membrane, by the effective radii of the macromolecules and their charge and by haemodynamic factors [1–3]. The tubular basement membrane provides mechanical support to the tubular cells [4].

The glomerular basement membrane seems to thicken during life [5–7]. In contrast, the clearance of dextrans of various molecular size increases with age and is extended to higher molecular weight classes in normal humans [8]. In hamsters the thickness of glomerular basement membrane increases with aging and is concordant with the amount of protein excreted [7]. An increased proteinuria is found with aging in rat [9,10].

Various differences have been found in the chemical composition of glomerular basement membrane of rat, mouse and cow in relation to postnatal development and aging [11–15]. Development and aging may influence biosynthesis, degradation and turnover of the macromolecules, which compose these extracellular structures. This study supplements the investigations on the chemical composition of renal basement membranes. Firstly, in addition to young and adult also fetal and neonatal bovine kidney basement membranes were investigated. Secondly, it was possible to study both glomerular and tubular basement membranes by developing a separation method for glomeruli and tubules from the same cortical tissue. Further, a comparison is made between the sonication [16] and detergent procedure [17] for the isolation of basement membranes. The latter method appears preferable and has been used for preparing glomerular and tubular basement membranes from different age groups of animals.

Materials and Methods

Bovine kidneys of fetal (5–7 month gestational age), neonatal (0–3 weeks), young (18 weeks) and adult (3 years or older) animals were collected at local slaughterhouses. Gestational age of fetuses was estimated by the criteria of hair distribution, length and pigmentation [18]. Within 6 h after death the kidneys were frozen at −75°C. Stainless steel sieves (diameter 20 cm) were purchased from Metaalgaas Twente, Hengelo, the Netherlands, deoxyribonuclease (DNA-ase, EC 3.1.4.5), galactose dehydrogenase (EC 1.1.1.48) and chemicals for enzymatic glucose assays from Boehringer, Mannheim, Federal Republic of Germany.

Isolation procedures. All procedures are carried out at 0–4°C, except where otherwise indicated. During isolation of tubules and glomeruli the tissue is kept in 0.15 M NaCl. Kidneys are weighed after removal of fat and calyces and frozen at −20°C. A scheme for the isolation procedure of tubules and glomeruli from bovine kidneys from different age groups is shown in Fig. 1. Samples for light microscopy are taken from all sieves. The tubules are obtained by forcing the cortex through a 300 μm sieve in portions of 40 g. The material is washed through the sieve with 300 ml 0.15 M NaCl. The filtrate originating from 60 g cortex is passed over a series of sieves with 2 l 0.15 M NaCl. The pore size of the sieves varied from 180 to 38 μm. The tubules are obtained on 90, 63 and 45 μm sieves for adult animals, on 63 and 45 μm for 18-week-old animals, on 38 μm for young calves and fetuses (Fig. 1). The glomeruli are isolated by a second disruption of the tissue on the finest of those sieves, which contains

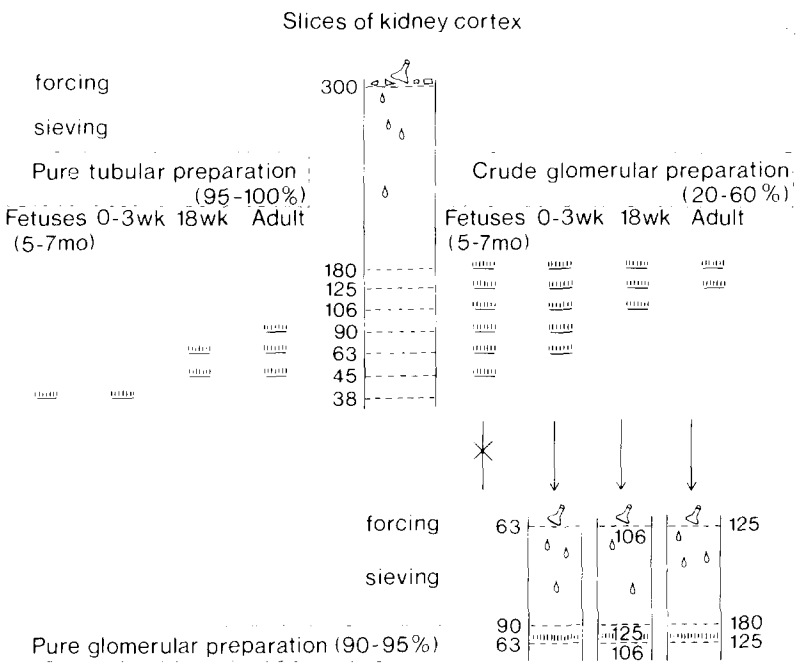

Fig. 1. Schematic representation of the isolation procedure for bovine kidney tubules and glomeruli from animals of different ages. Sieves on which pure preparations or tissue for further treatment are retained, are indicated under the age groups with vertical dashes. See text for details.

intact glomeruli. These sieves were 125, 106 and 90 μm for material from adult, 18-week- and 0—3-week-old animals, respectively. The resulting filtrate is washed with 10 l 0.15 M NaCl over two sieves, of which one is coarser than the sieve used for the second disruption and the other the disruption sieve itself. Glomeruli are obtained from the second sieve.

Isolation of basement membranes. Glomeruli and tubules of adult animals are sonicated according to Spiro [16]. The Branson Sonifier has a 0.15 inch tip and an effective output of 70 W. Sonication is carried out on crushed ice for 15-s periods with intervals of 15 s for a total sonication time of 7 min. When disruption is complete as judged by light microscopy, the suspension is washed three times with 0.15 M NaCl and three times with distilled water by centrifugation at 400 X *g* for 15 min.

A modified detergent procedure of Meezan et al. [17] is carried out at room temperature. Lysis time in distilled water and incubation time with DNAase are prolonged to 2.5 h and treatment with 4% sodium deoxycholate to 5 h. The resulting suspension is centrifuged at 20 000 X *g* for 10 min.

Washed basement membrane preparations from both isolation procedures are lyophilized and dried over P_2O_5.

Light and electron microscopy. Suspensions of sieved tissue fragments or

basement membranes are dried on an objective glass plate at 37°C, fixed in alcohol and stained with haematoxyline-eosin. The purity of the glomerular or tubular fractions is visually determined by counting 300 particles with an ocular grid at 50-fold magnification. Samples for electron microscopy are fixed in cold 2.5% glutaraldehyde in sodium-cacodylate (pH 7.4) for 2.5 h, postfixed in 1% osmium tetroxide in Palade buffer and embedded in Epon 812. Ultrathin sections are stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop 101.

Analytical procedures. Neutral sugars and hexosamines are released from basement membrane preparations with 2 M HCl (1 ml/5 mg) for 2.5 h at 100°C in sealed tubes. Glucose is determined with the glucose oxidase-perid method (Boehringer Mannheim) [19], galactose with D-galactose dehydrogenase [20] and total hexosamines with the Elson-Morgan reaction [21]. Another part of the hydrolysate is used for gas chromatographic analysis of fucose, mannose, galactose and glucose. Xylose is added as an internal standard after hydrolysis. The acid is carefully removed and sugars are reduced with $NaBH_4$ at pH 8—9 at 4°C for at least 3.5 h. After acidification with acetic acid, Dowex-50 W (H^+ form) and evaporation with methanol are used to remove sodium and borate ions, respectively. The alditols are acetylated at 100°C for 30 min with acetic anhydrid/pyridin (1 : 1, v/v). After evaporation the preparation is dissolved in chloroform, washed three times with an equal volume of H_2O and dried over P_2O_5. Analysis is carried out in a Packard gas chromatograph at 193°C with a 3% ECNSS-M column [22]. For analysis of amino acids, samples of 2—5 mg basement membranes are hydrolysed in 1 ml 6 M HCl in the presence of 0.05% phenol at 105°C for 24 h in vacuo. Norleucine (0.50 μmol/mg basement membrane) is added before hydrolysis as an internal standard. Analyses are performed with a Rank Hilger Chromaspek amino acid analyser. 3-Hydroxyproline, 4-hydroxyproline and proline are determined separately according to Guire et al. [23]. A Beckman Multichrom B amino acid analyser, with Durrum DC : A resin is used. The quantity of 3-hydroxyproline is determined by using absorbance ratios equimolar to 4-hydroxyproline. DNA is determined according to Giles and Myers [24] but with reduced temperature (13°C) and increased reaction time (48 h) in order to diminish interference of sialic acid [25]. Analysis of lipid phosphorus is carried out after extraction of 2 mg basement membrane with 5 ml chloroform methanol (2 : 1, v/v) according to the method of Fiske and Subbarow [26].

Significance of data between two age classes is determined by a two-sided Student's *t*-test. P_D values less than 0.025 were considered to be significant.

Results

Isolation of glomeruli and tubules. Mashing of bovine kidney cortex tissue on a coarse sieve (300 μm pore size) prevented damage of glomeruli to a large extent. This was essential to obtain tubular preparations virtually free of glomerular fragments with the fine sieves. Microscopical purity of the tubular preparations was at least 91% for those of the fetal group and 95% for those of the three older groups. The fractions retained on the somewhat coarser sieves (Fig. 1) could be used for preparation of a rather pure glomerular frac-

tion with a second disruption and sieving procedure. The contaminating tubules are so extensively fragmented that they are almost absent from the glomerular fraction. Increase in volume of the glomeruli with age [27] is reflected in the pore size of the sieves necessary for the isolation. Glomerular preparations showed a purity of at least 90%. However, it was not possible to isolate a pure fraction of glomeruli from fetal kidneys, probably due to the small diameter of these organ subfractions. We therefore studied preparations of glomeruli mixed with a low content of tubules (10—40%) in this age class. Capsules of Bowman were practically absent in the preparations from all age classes.

Comparison of sonication and detergent method for the isolation of basement membranes. Preparations of glomeruli or tubules from adult animals were treated in equal portions according to the sonication method or the detergent method, as described in Materials and Methods. Yields of basement membranes expressed in mg dry weight per 100 g cortex wet weight were, in the case of glomerular basement membrane, about 20 and 11 and, in the case of tubular basement membrane, about 25 and 16 for preparations obtained by sonication and detergent treatment, respectively.

It appeared necessary to extend the periods of lysis, DNAase and detergent treatment to eliminate cellular (haematoxyline-positive) material. Under the conditions used no protease activity could be found using azoalbumine as a substrate. Light microscopy of glomerular basement membrane preparations, obtained by sonication, shows few glomeruli just before lyophilization. In the glomerular basement membranes obtained by detergent treatment and in all tubular basement membrane preparations no haematoxyline-positive material was detected. Ultrastructurally, sonicated glomerular basement membrane preparations show cellular debris and collagen fibres between the basement membranes (Fig. 2A). In the detergent-treated glomerular basement membrane preparations (Fig. 2C) and all tubular basement membrane preparations (Fig. 2, B and D) no cellular debris was noted; however, a few collagen fibres could be detected in the glomerular basement membrane preparations. Sonicated tubular basement membranes show a more or less frayed appearance, while the detergent-treated tubular basement membranes are very regular.

Preparations of both types of basement membranes obtained with the detergent method have a very low lipid phosphorus content in comparison to the preparations of the sonication procedure (Table I). The DNA content of tubular basement membrane is much lower in detergent-treated than in sonicated preparations, but with glomerular basement membrane no difference was noted. Total residue weight of amino acids in sonicated and detergent-treated preparations was, in the case of glomerular basement membrane, 87.2 and 86.5% and of tubular basement membrane 86.7 and 93.2% of the dry weight, respectively. Glomerular and tubular basement membrane preparations isolated with the detergent method show smaller variations in composition. In detergent-treated preparations the content of aspartic acid, threonine, valine, leucine (data not shown) and lysine was markedly lower and that of 3- and 4-hydroxyproline, proline, glycine, hydroxylysine, galactose and glucose higher than in sonicated preparations, while other amino acids (such as alanine) and other carbohydrates did not show marked differences between the two methods. The morphological and chemical data indicate that preparations from

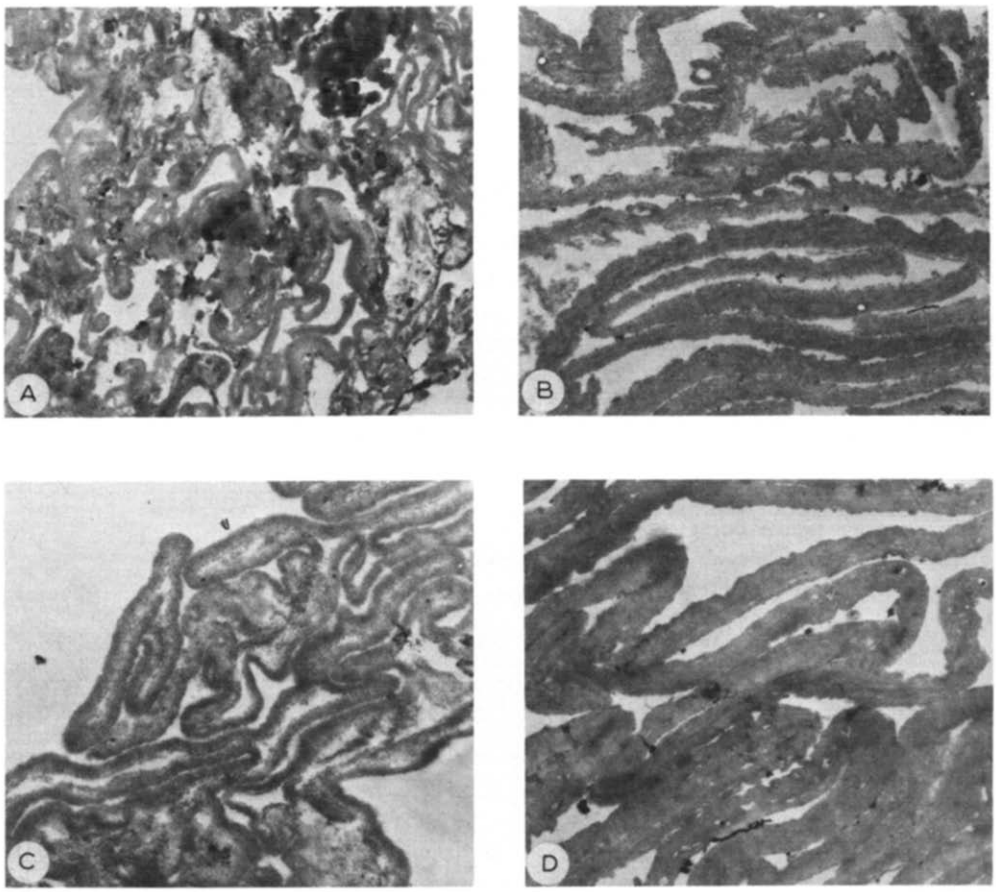

Fig. 2. Electron micrographs of (A) glomerular and (B) tubular basement membrane preparations obtained by the sonication method, and of (C) glomerular and (D) tubular basement membrane preparations obtained by the detergent method. All specimens were prepared from adult bovine kidneys. In A cellular debris and small fibrils can be detected between the basement membrane fragments. These contaminants were less visible in the glomerular basement membrane preparations isolated with the detergent method (C). Both tubular basement membrane preparations (B and D) shown almost no contaminants. Note the serrations on one side of the tubular basement membrane fragments in B. A, ×4500; B, ×5500; C, ×7500; D, ×5000.

the detergent procedure are purer than those from the sonication method.

Comparison of basement membrane preparations from animals of different age. Isolation of basement membrane preparations of the four age classes with the detergent method yielded comparable quantities of basement membrane material: about 10 mg glomerular basement membrane and about 15 mg tubular basement membrane per 100 g wet weight of cortex. Electron microscopic examination indicated that the glomerular basement membrane preparations contained some fibrillar material between the basement membrane fragments. This material was absent in the tubular basement membrane specimens. The tubular basement membrane showed a marked thickening with age. This phenomenon was not observed with glomerular basement membrane.

Amino acid and carbohydrate composition of glomerular and tubular base-

TABLE I

CHEMICAL CHARACTERISTICS OF BASEMENT MEMBRANE PREPARATIONS FROM KIDNEYS OF ADULT CATTLE ISOLATED WITH SONICATION OR DETERGENT METHOD

Values of amino acids and carbohydrates (μmol/100 mg dry weight) and of lipid-phosphorus and DNA (mg/100 mg dry weight) are means ± S.D. The number of preparations analysed is given in parentheses. For lipid-phosphorus and DNA content only 3—5 preparations of each group were analysed. Galactose and glucose were enzymatically determined.

Compound	Glomerular basement membrane		Tubular basement membrane	
	Sonication method (6)	Detergent method (6)	Sonication method (6)	Detergent method (10)
3-Hydroxyproline	7.7 ± 3.0	12.6 ± 1.2	7.3 ± 1.8	11.2 ± 1.8
4-Hydroxyproline	40.4 ± 12.6	59.1 ± 8.9	47.4 ± 8.9	68.1 ± 6.8
Proline	45.0 ± 4.9	52.7 ± 5.4	42.9 ± 8.3	50.2 ± 7.4
Glycine	127.5 ± 24.6	163.4 ± 11.2	142.3 ± 17.9	179.2 ± 10.5
Alanine	44.7 ± 3.7	42.9 ± 3.4	43.5 ± 1.8	43.9 ± 3.7
Hydroxylysine	17.3 ± 5.2	24.0 ± 1.7	20.7 ± 3.9	28.6 ± 3.9
Lysine	22.3 ± 5.6	13.7 ± 1.0	18.2 ± 3.2	14.9 ± 2.1
Glucose	9.9 ± 2.5	14.8 ± 1.3	12.5 ± 2.1	17.4 ± 1.5
Galactose	13.6 ± 3.8	19.2 ± 1.1	15.8 ± 2.8	20.5 ± 1.5
Lipid-phosphorus	0.26 ± 0.12	0.03 ± 0.01	0.21 ± 0.11	0.03 ± 0.01
DNA	1.09 ± 0.64	0.96 ± 0.91	0.15 ± 0.12	0.04 ± 0.05

ment membrane preparations are given in Tables II—IV. Total residue weight of amino acids and carbohydrates is comparable for both types of basement membranes from the different age groups and varies from 85 to 100% of the dry weight. Chemical analyses of tubular and glomerular basement membrane preparations (mainly originating from glomeruli) from fetuses do not show significant differences in the amino acid and carbohydrate content. Although the fetal glomerular basement membrane preparations were isolated from glomeruli fractions containing various amounts of contaminating tubules, no clear correlation was found between their chemical composition and percentage of tubular contamination. Therefore, we used the impure glomerular basement membrane preparations of the fetal group for comparison with glomerular basement membrane preparations of the other age groups. Only for the content of some amino acid and carbohydrate components we found significant differences between the age groups, as will be described hereafter.

In the case of glomerular basement membrane the content of 3- and 4-hydroxyproline, hydroxylysine, total imino acid, galactose and glucose is higher in the adult than in the 18-week-old animals (Tables II and III). In addition, the 4-hydroxyproline/4-hydroxyproline + proline ratio and the hydroxylysine/hydroxylysine + lysine ratio were found to be higher in the adult animals than in the 18-week-old animals. The 3-hydroxyproline/4-hydroxyproline ratio increases during development. Significant differences between some succeeding age groups are also present in the relative content of aspartic acid, alanine, glycine and half-cystine.

For the tubular basement membrane preparations the content of both 3- and 4-hydroxyproline appeared to be significantly higher in the 0—3-weeks-old than in the fetal animals and in the adult compared to the 18-week-old animals

TABLE II

AMINO ACID COMPOSITION OF GLOMERULAR BASEMENT MEMBRANE PREPARATIONS OF CATTLE OF DIFFERENT AGE

Values (in residues per 1000 amino acid residues) are means ± S.D. The number of preparations analysed is given in parentheses. Statistical significance (P_D) is indicated for differences between components of two succeeding age groups. The ratios given are molar ratios.

Amino acid	Fetal (7)	P_D	0–3 weeks (6)	P_D	18 weeks (3)	P_D	Adult (6)
3-Hydroxyproline	8 ± 2		11 ± 3		11 ± 2	<0.001	18 ± 3
4-Hydroxyproline	69 ± 10		62 ± 11		63 ± 4	<0.001	82 ± 10
Aspartic acid	67 ± 7		69 ± 4		73 ± 3	<0.025	62 ± 10
Threonine	33 ± 4		39 ± 4		39 ± 2		32 ± 6
Serine	43 ± 4		48 ± 4		47 ± 3		46 ± 2
Proline	77 ± 10		73 ± 8		72 ± 1		74 ± 9
Glutamic acid	91 ± 8		102 ± 14		91 ± 3		94 ± 8
Glycine	239 ± 24	<0.01	199 ± 23		212 ± 6	<0.001	225 ± 2
Alanine	71 ± 2	<0.001	61 ± 2		60 ± 5		60 ± 2
Half-cystine	27 ± 4	<0.01	34 ± 4	<0.025	38 ± 1	<0.001	27 ± 4
Valine	36 ± 3		39 ± 4		37 ± 4		33 ± 2
Methionine	8 ± 2		9 ± 1		10 ± 1		8 ± 2
Isoleucine	26 ± 2		29 ± 2		30 ± 1		28 ± 2
Leucine	53 ± 4		62 ± 4		59 ± 3		60 ± 4
Tyrosine	15 ± 3		18 ± 2		18 ± 2		15 ± 2
Phenylalanine	26 ± 2		27 ± 2		27 ± 2		29 ± 2
Histidine	20 ± 4		26 ± 4		26 ± 2		26 ± 1
Hydroxylysine	25 ± 3		26 ± 5		27 ± 4	<0.01	34 ± 2
Lysine	25 ± 7		29 ± 4	<0.001	23 ± 1	<0.001	19 ± 2
Arginine	44 ± 7		48 ± 6		45 ± 1		42 ± 4
Total imino acids	155 ± 20		144 ± 14		147 ± 5	<0.001	174 ± 12
Hyl + Lys	51 ± 6		54 ± 6		50 ± 5		53 ± 3
3-Hyp/4-Hyp ratio	0.10 ± 0.03		0.15 ± 0.05		0.15 ± 0.03		0.18 ± 0.04
4-Hyp/4-Hyp + Pro ratio	0.47 ± 0.02		0.46 ± 0.05		0.46 ± 0.01	<0.001	0.53 ± 0.05
Hyl/Hyl + Lys ratio	0.51 ± 0.08		0.47 ± 0.07		0.53 ± 0.03	<0.001	0.64 ± 0.02

TABLE III

CARBOHYDRATE COMPOSITION OF GLOMERULAR BASEMENT MEMBRANE PREPARATIONS OF CATTLE OF DIFFERENT AGES

Values (in μmol per 100 mg dry weight) are means ± S.D. The number of preparations analysed is given in parentheses. Statistical significance (P_D) is indicated for differences between two succeeding age groups. Values were obtained by gaschromatography except those indicated with (a) enzymatically or (b) spectrophotometrically. The ratios given are molar ratios.

	Fetal (7)	0—3 weeks (6)	18 weeks (6)	P_D	Adult (7)
Fucose	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.2		1.0 ± 0.4
Mannose	2.5 ± 0.2	3.3 ± 0.5	3.6 ± 0.7		4.1 ± 0.6
Galactose [a]	13.1 ± 2.0	13.4 ± 2.6	15.1 ± 1.7	<0.001	19.2 ± 1.1
Galactose	12.0 ± 1.8	13.8 ± 2.9	15.6 ± 1.9	<0.01	18.8 ± 1.1
Glucose [a]	11.3 ± 2.0	11.0 ± 1.6	12.1 ± 1.0	<0.025	14.8 ± 1.3
Glucose	10.5 ± 1.6	11.8 ± 2.7	13.1 ± 2.8		15.9 ± 0.9
Hexosamines [b]	3.7 ± 0.8	5.4 ± 0.8	6.3 ± 0.8		7.0 ± 1.3
Glc/Gal ratio [a]	0.86 ± 0.11	0.83 ± 0.06	0.81 ± 0.06		0.78 ± 0.05
Glc/Gal ratio	0.87 ± 0.06	0.86 ± 0.04	0.84 ± 0.09		0.84 ± 0.01
Gal/Hyl ratio [a]	0.74 ± 0.10	0.85 ± 0.09	0.81 ± 0.05		0.80 ± 0.07

(Tables IV and V). Aspartic acid, glycine and valine contents are lower in the 18-week-old than in the 0—3-week-old animals. The ratio of 3-hydroxyproline to 4-hydroxyproline increases during development. The hydroxylation grade of proline is markedly higher in the 0—3-week-old than in fetal age group. The hydroxylation grade of lysine is higher in the 0—3-week-old than in the fetal animals and still higher in the 18-week-old animals. Mannose, galactose, glucose, and hexosamine contents are higher in the 0—3-week-old than in the fetal age group.

The chemical analyses of glomerular and tubular basement membrane preparations from adult cattle show significant differences only for the glucose content and the glucose/galactose ratio (P_D values < 0.025 and 0.010, respectively).

Discussion

Both tubular and glomerular preparations could be obtained with a high degree of purity from cortex of bovine kidneys by a combination of two separate procedures, one for the isolation of glomeruli [16] and another for the isolation of tubules [28]. The procedure used also appeared to be applicable to porcine and bovine kidneys. In literature, two reports mention the simultaneous isolation of tubules and glomeruli from kidney cortex with a sieving procedure, one for rat kidneys [29] and the other for human kidneys [30]. After mashing and sieving of the cortical tissue these investigators obtained a tubular preparation on a rather coarse sieve (125 μm pore size) which did not retain the glomeruli. We were unable to isolate pure tubules from bovine and human kidneys with the method of Mahieu and Winand [30]. In contrast, we retained our tubular preparations on sieves with smaller pore size (90 μm and smaller).

TABLE IV

AMINO ACID COMPOSITION OF TUBULAR BASEMENT MEMBRANE PREPARATIONS OF CATTLE OF DIFFERENT AGES

Values (in residues per 1000 amino acid residues) are means ± S.D. The number of preparations analysed is given in parentheses. Statistical significance (P_D) is indicated for differences between two succeeding age groups. The ratios given are molar ratios.

Amino acid	Fetal (3)	P_D	0—3 weeks (6)	P_D	18 weeks (7)	P_D	Adult (9)
3-Hydroxyproline	7 ± 1	<0.025	10 ± 3		11 ± 2	<0.01	14 ± 2
4-Hydroxyproline	62 ± 4	<0.025	75 ± 11		77 ± 10	<0.025	89 ± 8
Aspartic acid	72 ± 8		68 ± 4	<0.01	61 ± 4		62 ± 4
Threonine	38 ± 3		35 ± 2		34 ± 2		33 ± 3
Serine	50 ± 1		47 ± 4		44 ± 4		43 ± 4
Proline	74 ± 7		59 ± 12		66 ± 6		65 ± 9
Glutamic acid	105 ± 15		99 ± 12		91 ± 6		97 ± 9
Glycine	222 ± 11		224 ± 18		240 ± 9		233 ± 12
Alanine	66 ± 1		63 ± 5	<0.025	57 ± 3		57 ± 5
Half-cystine	21 ± 4		26 ± 3		25 ± 2		24 ± 4
Valine	36 ± 1		39 ± 3	<0.01	35 ± 1		35 ± 3
Methionine	8 ± 3		10 ± 3		10 ± 2		10 ± 3
Isoleucine	28 ± 2		30 ± 3		30 ± 2		28 ± 2
Leucine	59 ± 3		63 ± 5		60 ± 2		60 ± 2
Tyrosine	16 ± 4		17 ± 3		16 ± 2		15 ± 2
Phenylalanine	27 ± 5		29 ± 2		31 ± 1		29 ± 3
Histidine	22 ± 9		21 ± 3		24 ± 4		23 ± 4
Hydroxylysine	22 ± 4	<0.025	29 ± 4	<0.001	38 ± 3		37 ± 5
Lysine	28 ± 4		24 ± 5		20 ± 3		19 ± 2
Arginine	47 ± 1		43 ± 3		40 ± 6		42 ± 5
Total imino acids	143 ± 11		145 ± 16		154 ± 8	<0.01	169 ± 11
Hyl + Lys	50 ± 8		54 ± 5		58 ± 4		56 ± 6
3-Hyp/4-Hyp ratio	0.10 ± 0.01		0.12 ± 0.03		0.13 ± 0.01		0.14 ± 0.02
4-Hyp/4-Hyp + pro ratio	0.45 ± 0.01	<0.001	0.56 ± 0.07		0.54 ± 0.05		0.58 ± 0.05
Hyl/Hyl + Lys ratio	0.44 ± 0.01	<0.001	0.55 ± 0.08	<0.025	0.65 ± 0.04		0.65 ± 0.03

TABLE V

CARBOHYDRATE COMPOSITION OF TUBULAR BASEMENT MEMBRANE PREPARATIONS OF CATTLE OF DIFFERENT AGE

Values (in μmol per 100 mg dry weight) are means ± S.D. The number of preparations analysed is given in parentheses. Statistical significance (P_D) is indicated for differences between two succeeding age groups. Values were obtained by gaschromatography except those indicated with (a) enzymatically or (b) spectrophotometrically. The ratios given are molar ratios.

	Fetal (7)	P_D	0—3 weeks (6)	18 weeks (8)	Adult (10)
Fucose	0.7 ± 0.3		0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2
Mannose	2.5 ± 0.1	<0.01	3.1 ± 0.4	3.0 ± 0.2	3.4 ± 0.4
Galactose [a]	11.8 ± 1.1	<0.001	16.9 ± 3.0	20.2 ± 2.0	20.5 ± 1.5
Galactose	12.5 ± 2.1	<0.01	16.9 ± 2.6	19.4 ± 2.6	20.0 ± 2.5
Glucose [a]	10.5 ± 1.2	<0.001	15.0 ± 2.0	17.4 ± 1.8	17.4 ± 1.5
Glucose	10.2 ± 1.0	<0.025	15.4 ± 3.1	18.4 ± 1.9	19.4 ± 2.9
Hexosamines [b]	3.6 ± 0.7	<0.025	5.2 ± 1.2	5.4 ± 0.3	6.0 ± 0.6
Glc/Gal ratio [a]	0.89 ± 0.14		0.90 ± 0.08	0.86 ± 0.04	0.85 ± 0.06
Glc/Gal ratio	0.85 ± 0.11		0.91 ± 0.08	0.95 ± 0.04	0.97 ± 0.05
Gal/Hyl ratio [a]	0.84 ± 0.21		0.86 ± 0.08	0.75 ± 0.11	0.74 ± 0.13

The morphological and chemical differences found between the basement membrane preparations isolated either with the sonication or the detergent method favoured the latter procedure (absence of cellular debris, low lipid phosphorus content, high hydroxylation grade of lysine and proline, high content of 3-hydroxyproline, glycine, galactose and glucose, smaller variations in chemical composition). Our results confirm the findings of Meezan et al. [17] who compared the chemical composition of their basement membrane preparations obtained with a detergent procedure to results reported in literature of basement membranes isolated by sonication. The constancy of this detergent method has advantages over the variable mechanical and kinetic conditions of the sonication method. The time of ultrasonication of glomeruli influences the chemical composition and the purity of glomerular basement membrane preparations [31,32]. Some reports mention the resistance of a part of the glomeruli to sonication [33—36]. In the case of human kidneys these structures were ascribed to the possible presence of hyalinized glomeruli which had to be removed by an extra sieving procedure [33].

After detergent treatment glomerular basement membranes retain their spherical form when viewed by light microscopy, a phenomenon which was not found after sonication. This intactness may result from the DNA content being unavailable to DNAase in comparison to that in ultrasonicated glomerular basement membrane preparations. When compared to ultrasonicated preparations Ligler et al. [37] found no differences in DNA, RNA and phospholipid content of mixed preparations of glomerular and tubular basement membranes isolated with the detergent *N*-lauroyl sarcosine.

The amino acid and carbohydrate composition of our detergent-treated glomerular basement membrane preparations from adult bovine kidneys is comparable to that reported for sonicated preparations [12,16,34]. In these preparations the content of 4-hydroxyproline, glycine, hydroxylysine, galactose and glucose appeared to be somewhat higher and that of lipid-phosphorus

and lysine lower. Our detergent-treated tubular basement membrane preparations from adult cattle contained a little more proline and hydroxylysine but somewhat less glucose, galactose and mannose than the preparations of Ferwerda et al. [28].

Different changes in the chemical composition of basement membranes have been reported to be associated with age. An increase in the hydroxylation grade of lysine and proline and of the content of glucose and galactose was found between glomerular basement membrane preparations of neonatal and adult mice [13]. Rat glomerular basement membrane showed an increase with age of 4-hydroxyproline, but not of hydroxylysine, and an increase of the glucose and galactose content [11,14], while other workers [15] report an increase in hydroxylysine and both isomers of hydroxyproline but no change in glycosylation of the hydroxylysine residues. The degree of hydroxylation of lysine (bot not of proline) and glycosylation of hydroxylysine of bovine glomerular basement membrane significantly increases between 4-month- and 2.5-year-old animals, but not between those of 2.5 and 8 years [12]. We found for bovine glomerular basement membrane between 18 week old and adult animals a significant increase in the hydroxylation of both lysine and proline and in the glucose and galactose content. However, no change was observed in the glucose to galactose ratio and the galactose to hydroxylysine ratio, which indicates that there is no change in the glycosylation of hydroxylysine, but only in the relative number of hydroxylysylglycoside residues. Bovine tubular basement membrane showed a similar increase in the degree of hydroxylation of proline and lysine and in the glucose and galactose content between the fetal and neonatal period.

Whole glomerular basement membrane from rat kidneys [14,15] showed an analogous change in total imino acid content and constancy of the total of hydroxylysine and lysine residues with age as with both bovine glomerular and tubular basement membranes. These data are controversial if one assumes an equal distribution of both types of amino acids over the basement membrane peptides. This discrepancy may be possible if hydroxylysine and lysine residues are distributed to a greater extent in one part of a peptide chain and the imino acids in another part. Sato and Spiro [36] suggest a structural model for the basement membrane in which the peptide chains vary largely in the proportion of helical segments and polar regions. These different parts may have different rates of synthesis or degradation. An alternative explanation may be that there are several peptide chain types, one of which contains more imino acids. The relative amount in this latter chain may increase during development. Such an increase and the change in hydroxylation of lysine and proline could be the result of differences in the biosynthetic or degradative processes of the basement membrane at different stages of development and/or aging. A retarded folding of the procollagen polypeptides into a triple helix at higher age could be responsible for a more complete hydroxylation and glycosylation of lysine [39], although activities of hydroxylases and glycosyltransferases are lower in adult than in fetal or young animal tissues [40—42]. Metabolic transformations of three collagen modifications of rat skin and tendon appeared to be age dependent [43]. These modifications may differ in amino acid composition and/or in extent of hydroxylation and glycosylation.

The sugars of the heterosaccharide moiety of basement membranes also show marked changes in their content with age. Lens capsules from calves contain more mannose, hexosamine and sialic acid than those of adults [38] and rat glomerular basement membrane shows a decrease of hexosamine and sialic acid [11]. However, adult mouse glomerular basement membrane contains more fucose, hexosamine and sialic acid than neonatal glomerular basement membrane [13]. We have also found in both glomerular and tubular basement membrane, that the content of mannose and hexosamine gradually increases with age. The differences between the content of these carbohydrates and of glucose and galactose may be useful in determining which type of carbohydrate chain is involved in or influences the antigenic reactivity, as has been noted for glomerular basement membrane of different age groups in mice [13].

Our results show that in tubular basement membrane the increase of the content of glucose and galactose and the hydroxylation of proline and lysine appear in an earlier stage of life than is the case for glomerular basement membrane. It does not seem likely to be a result of impurities which are retained during the isolation of basement membrane from animals of different age groups. Lipid phosphorus content of glomerular basement membrane is similar for 18-week-old and adult animals and the glycine content also remains fairly constant in both tubular and glomerular basement membranes of all age groups. These two facts indicate that cellular membrane components or extracellular fibrillar collagen do not contribute to changes observed during development. The differences in age dependency could be due to the fact that glomerular basement membrane has its origin from more than one cell type [44] since collagen molecules of different tissues differ in metabolism [43]. These differences may also be related to the different physiological functions of both basement membrane types.

The functional aspects of the changes in the content of glycosylated hydroxylysine in basement membranes are not clear. The presence of these saccharides may disturb the arrangement of collagen molecules to fibrils [45] but intermolecular bonds may presumably be formed since crosslinks to which a saccharide is probably coupled were isolated [46]. The high hydroxylation grade of lysine could reflect the presence of a large number of stable crosslinks, which are derived from hydroxyallysine and not from allysine [47].

The nature and degree of hydroxylation, glycosylation and crosslinking could play a role in the permeability of the basement membrane for macromolecules. Filtration experiments with filter pads of glomerular and tubular basement membranes of animals of different age could give some information on a possible relation between their structure and permeability properties [48].

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References

1 Ryan, G.B. and Karnovsky, M.J. (1976) Kidney Int. 9, 36–45
2 Rennke, H.G. and Venkatachalam, M.A. (1977) Fed. Proc. 36, 2619–2626
3 Brenner, B.M., Bohrer, M.P., Baylis, C. and Deen, W.M. (1977) Kidney Int. 12, 229–237
4 Welling, L.W. and Welling, D.J. (1978) Am. J. Physiol. 234, F54–F58
5 Bloom, P.M., Hartmann, J.F. and Vernier, R.L. (1959) Anat. Rec. 133, 251
6 Ashworth, C.T., Erdmann, R.R. and Arnold, N.J. (1960) Am. J. Pathol. 36, 165–171
7 McNelly, N.A. and Dittmer, J.E. (1976) Exp. Geront. 11, 49–55
8 Arturson, G., Groth, T. and Grotte, G. (1971) Clin. Sci. 40, 137–158
9 Couser, W.G. and Stilmant, M.M. (1975) Lab. Invest. 33, 491–501
10 Bolton, W.K., Benton, F.R., Maclay, J.G. and Sturgill, B.C. (1976) Am. J. Pathol. 85, 277–300
11 Lui, S. and Kalant, N. (1974) Exp. Mol. Pathol. 21, 52–62
12 Cruz, A., David, H. and Oliveira, M.H. (1974) Pathol. Biol. 22, 721–724
13 Blue, W.T. and Lange, C.F. (1976) Immunochemistry 13, 295–298
14 Kalant, N., Satomi, S., White, R. and Tel, E. (1977) Can. J. Biochem. 55, 1197–1206
15 Hoyer, J.R. and Spiro, R.G. (1978) Arch. Biochem. Biophys. 30, 496–503
16 Spiro, R.G. (1967) J. Biol. Chem. 242, 1915–1922
17 Meezan, E., Hjelle, J.T. and Brendel, K. (1975) Life Sci. 17, 1721–1732
18 Richter, J. and Götze, R. (1950) in Lehrbuch der Tiergeburtshilfe, Schoetz, Berlin
19 Werner, W., Rey, H.L. and Wielinger, H. (1970) Fresenius Z. Anal. Chem. 252, 224–228
20 Wallenfels, K. and Kurtz, G. (1966) in Methods in Enzymology (Wood, W.A., ed.), Vol. IX, pp. 112–116, Academic Press, New York
21 Boas, N.F. (1953) J. Biol. Chem. 204, 553–563
22 Sawardekar, S., Sloneker, J.H. and Jeanes, A.R. (1965) Anal. Chem. 37, 1602–1604
23 Guire, P., Riquetti, P. and Hudson, B.G. (1974) J. Chromatogr. 90, 350–353
24 Giles, K.W. and Myers, A. (1965) Nature 206, 93
25 Croft, D.N. and Lubran, M. (1965) Biochem. J. 95, 612–620
26 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–471
27 Darmady, E.M., Offer, J. and Woodhouse, M.A. (1973) in Textbook of Geriatric Medicine and Gerontology (Brocklehurst, ed.) Churchill Livingstone, London
28 Ferwerda, W., Meyer, J.F.M., van den Eijnden, D.H. and van Dijk, W. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 976–984
29 Krisko, I., De Bernardo, E. and Sato, C.S. (1977) Kidney Int. 12, 238–243
30 Mahieu, P. and Winand, R.J. (1970) Eur. J. Biochem. 12, 410–418
31 Kefalides, N.A. (1974) J. Clin. Invest. 53, 403–407
32 Tryggvason, K. (1977) Eur. J. Clin. Invest. 7, 177–180
33 Westberg, N.G. and Michael, A.F. (1970) Biochemistry 9, 3837–3846
34 Sato, T., Musiakata, H., Yoshinaga, K. and Yoshizawa, Z. (1975) Tohoku J. Exp. Med. 115, 299–306
35 Sachot, N., Sternberg, M., Sjwarlo, L., Rebeyrotte, P. and Lague, G. (1975) Comp. Biochem. Physiol. 50A, 575–579
36 Sato, T. and Spiro, R.G. (1976) J. Biol. Chem. 251, 4062–4070
37 Ligler, F.S., Robinson, G.B. and Byrne, J. (1977) Biochim. Biophys. Acta 468, 327–340
38 Fukushi, S. and Spiro, R.G. (1969) J. Biol. Chem. 244, 2041–2048
39 Uitto, W.J., Uitto, J., Kao, W.W.Y. and Prockop, D.J. (1978) Arch. Biochem. Biophys. 185, 214–221
40 Spiro, R.G. and Spiro, M.J. (1971) J. Biol. Chem. 216, 4919–4925
41 Risteli, J. and Kivirikko, K.J. (1976) Biochem. J. 158, 361–367
42 Anttinen, H., Oikarinen, A. and Kivirikko, K.J. (1977) Clin. Chim. Acta 76, 95–101
43 Niedermüller, H., Skalicky, M., Hofecker, G. and Kment, A. (1977) Exp. Geront. 12, 159–168
44 Misra, R.P. (1971) Pathobiology Annu. 1, 325–351
45 Morgan, P.H., Jacobs, H.G., Segrest, J.P. and Cunningham, L.W. (1970) J. Biol. Chem. 245, 5042–5048
46 Robins, S.P. and Bailey, A.J. (1974) FEBS Lett. 38, 334–336
47 Bailey, A.J. and Robins, S.P. (1976) Sci. Prog. 63, 419–444
48 Robinson, G.B. and Brown, R.J. (1977) FEBS Lett. 78, 189–193