Structural Components of Epithelial and Endothelial Basement Membranes*

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ABSTRACT: Basement membranes from vascular and nonvascular canine tissue were analyzed chemically and structurally. Basement membranes which derive from different embryologic cell lines were qualitatively similar but differed quantitatively in their amino acid and carbohydrate composition. In terms of residues per 1000 residues anterior lens capsule contained hydroxylysine 32, hydroxyproline 99.5, and glycine 288, compared with 20, 83.8, and 230 residues, respectively, for Descemet's membrane. Glomerular, alveolar, and choroid plexus basement membranes contained hydroxylysine 24, 6.8, and 9 residues, hydroxyproline 65, 35, and 48 residues, and glycine 229, 222, and 235 residues, respectively. The carbohydrate content was high in all, ranging from 12.5 and, 10.5% in anterior lens capsule and Descemet's membrane to 10.25, 6.7, and 7.3% in basement membranes from the glomerulus, alveolus, and choroid plexus, respectively. An acid-soluble collagen was obtained from anterior lens capsule, Descemet's membrane and glomerular basement membrane after limited digestion with pronase or pepsin. Digestion with pepsin at 4° for 16 hr yields a collagen with an intrinsic viscosity of 13 d/g, while digestion with pronase at 4° for 10 and 24 hr yields collagen fragments with intrinsic viscosities of 10 and 5.4 dl per g, respectively. An acid-insoluble collagenous component with noncollagen polypeptides still attached to it was obtained from basement membranes of the glomerulus, alveolus, choroid plexus, and Descemet's membrane.

The compositional differences among basement membranes reflect the proportion of the noncollagen polypeptides associated with the collagen and are thought to determine the functional behavior of the various basement membranes.

About 60% of glomerular basement membrane can be solubilized after treatment with pronase at 4° for 24 hr (Kefalides, 1968). From the solubilized material two major fractions are obtained. One is soluble in weak basic and acid media and precipitates with 15% KCl in 0.05% acetic acid. Its chemical composition and physical properties allow it to be classified as a collagen. It differs from other mammalian collagens in that it contains higher amount of hydroxylysine, hydroxyproline, and hexose (as glucose and galactose). The second fraction is soluble in weak basic media but insoluble in weak acetic acid. Its amino acid composition resembles that of the collagen but contains lower amounts of hydroxylysine, hydroxyproline, and glycine and its carbohydrate is composed of glucose, galactose, mannose, hexosamine, fucose, and sialic acid (Kefalides, 1968). Since vascular (capillary) basement membranes derive embryologically from different cell lines than nonvascular basement membranes (lens capsule and Descemet's membrane) and differ in their functions, it was important to investigate the chemical composition and behavior of such membranes under the same conditions of fractionation. The data suggest that basement membranes from various tissues differ quantitatively in their amino acid and carbohydrate composition and that tissue specificity determines the structural organization of these substances.

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Material and Methods

Preparation of Basement Membranes. Basement membranes were selected according to the cell type with which they are associated; they were further separated into the vascular and avascular type. Table I shows the classification of basement membranes used in this study.

Glomerular basement membrane was prepared from dog kidneys essentially by the method of Krakower and Greenspon (1951).

Bowman's capsules from dog kidneys were prepared by crushing free glomeruli between two microscope slides. The capsules were separated from the capillary tuft with the aid of tungsten wire probes under a dissecting microscope. We isolated material sufficient only for one amino acid analysis.

Anterior lens capsules were obtained from frozen dog eyes (Pel-Freez Biologicals, Rogers, Ark.), that were allowed to thaw out partially. The capsules were freed from lens and vitreous humor contamination by dissection. Anterior capsules were separated from the posterior by dissection. The capsules were shaken in 0.85% NaCl for 5 min five times and sonicated in a Raytheon Oscillator Model DF-101, 10 kc for 5 min to remove epithelial cells. The sonicated capsules were centrifuged at 1400*g* for 15 min, washed with distilled water to remove excess NaCl, and lyophilized.

Descemet's membranes were isolated from frozen sheep eyes (Swift and Co., Chicago) that were allowed to thaw out partially. The corneas were removed and the membranes separated from the stroma by blunt dissection. The isolated Descemet's membranes were further treated as described for the lens capsules.

Choroid plexus basement membrane was isolated from

TABLE I: Classification of Basement Membranes Used in This Study According to Cell-Type Association.

Cell Type	Source of Basement Membrane	
	Vascular	Avascular
Epithelial		Lens capsule Bowman's capsule
Endothelial		Descemet's membrane[b]
Epithelial and endothelial	Capillaries of glomerulus, alveolus, and choroid plexus[a]	

[a] Adjacent to the capillary network of the choroid plexus there is an ependymal layer of epithelial cells with its own basement membrane which is separated from the capillaries by loose connective tissue (Dempsey, 1968). [b] Descemet's membrane derives from mesenchymal cells and overlies the corneal endothelium (Watzka, 1935; Neifakh, 1952).

dog brains. A sagittal incision was made into the lateral ventricles and the choroid plexus, which appears like a red cord, was carefully dissected with blunt forceps from the floor of the ventricles. The choroid plexuses were then washed by shaking with 0.85% NaCl until the washings were clear. Interstitial collagen was removed from the plexuses by extraction with 0.3 M acetic acid with gentle stirring at 4° for 24 hr and the suspension centrifuged at 1400*g* for 20 min. The extraction with 0.3 M acetic acid was repeated three more times. The insoluble residue was sonicated for 15 min in 0.85% NaCl to remove cellular elements. After sonication, the suspension was centrifuged at 1400*g* for 20 min at 4°, and the residue was washed with distilled water to remove excess salt, and lyophilized.

The lyophilized material (100 mg) was suspended in 50 ml of 0.1 M sodium acetate buffer (pH 5) and incubated with 1 mg of deoxyribonuclease (Worthington) at room temperature for 16 hr with slow stirring. After incubation, the suspension was centrifuged at 34,800*g* for 30 min and the residue was then subjected to digestion with ribonuclease (Worthington) under the same conditions. These procedures eliminated largely any contaminating deoxyribo- and ribonucleic acids. Electron microscopic studies of the final residue revealed the amorphous basement membrane but no collagen fibrils.

Alveolar basement membrane was isolated from dog lungs. Immediately after death, the lungs were perfused with 0.85% NaCl to remove any blood and were inflated by pouring 0.85% NaCl through the trachea. While inflated they were immersed in a suitable container and quickly frozen with crushed Dry Ice. After freezing, the visceral pleura was removed by scraping with a surgical blade. Sections of tissue measuring between 0.5 and 1 mm in thickness were cut from the periphery of the lung. Only a single section was cut from any given area of the lung in order to minimize removing excess connective tissue. The lung sections were minced and washed with 0.85% NaCl until the wash became clear. The tissue was then extracted with five volumes 0.3 M acetic acid at 4° for 24 hr. The extraction with acetic acid was repeated four times. The insoluble residue from the acetic acid extraction was sonicated in 0.85% NaCl (0.9 mA, 20 min) and the whole mixture was centrifuged at 1400*g* for 10 min at 4°. The supernatant suspension was allowed to stand for 3 hr at 4°. The residue which settled out was separated by decantation and was then washed three times with 0.85% NaCl followed by three washes with distilled water. The washes were separated by centrifugation at 1400*g* for 20 min at 4° and discarded. The residue was again extracted with 0.3 M acetic acid at 4° for 24 hr three times and the remaining residue was treated with deoxyribonuclease and ribonuclease as described previously. The final residue was subjected to electron microscopy and chemical studies. Electron microscopy revealed an amorphous substance with fine filaments embedded in it.

Solubility Studies. Since 0.3 M acetic acid was used to remove interstitial collagen in the purification procedure of alveolar and choroid plexus basement membranes, the final preparations of basement membranes from the above sources as well as from the glomerulus, lens capsule, and Descemet's membrane were subjected to treatment with 0.3 M acetic acid at 4° for 48 hr. On a dry weight basis, less than 1% of glomerular, alveolar, and choroid plexus basement membranes were solubilized. Descemet's membrane was solubilized to the extent of 1% and anterior lens capsule 32%.

Treatment of Basement Membranes with Enzymes. Basement membrane preparations from the various tissues were subjected to limited treatment with pronase at 4° for 24 hr according to a previous method (Kefalides, 1968) (Figure 1).

Since the collagen obtained by this method has an intrinsic viscosity which is half that obtained with skin tropocollagen, and since it could not be decided whether this was the result of cleavage of the molecule by the enzyme, we decided to reduce the time of incubation with pronase in a separate experiment, to 10 hr and in another, substitute pepsin for pronase.

In the experiment with pepsin 200 mg of anterior lens capsule was incubated with 20 mg of crystalline pepsin (Worthington) in 50 ml of 0.1 M acetic acid (pH) at 4° for 16 hr. After incubation, the mixture was centrifuged at 34,800*g* for 40 min and the supernatant was dialyzed against 0.05% acetic acid. To the retentate was added solid KCl to a final concentration of 15% and solid Na_2HPO_4 to a concentration of 0.02 M. The precipitate formed was removed by centrifugation at 34,800*g* for 40 min, was suspended in 0.05% acetic acid and reprecipitated two more times with KCl and Na_2HPO_4. The final precipitate was dialyzed against 0.05% acetic acid.

Acrylamide Electrophoresis. The method of Nagai *et al.* (1964) which separated the α, β, and γ components of denatured collagen was used. From 150 to 200 μg of protein was applied to each gel. After each run, duplicate gels were stained with Amido Black to detect protein bands and with the periodic acid–Schiff reagent to detect protein-bound carbohydrate.

Paper Chromatography. Carbohydrate components were identified by paper chromatography following hydrolysis of protein samples in 2 N HCl for 2 hr at 100°. The neutral sugars

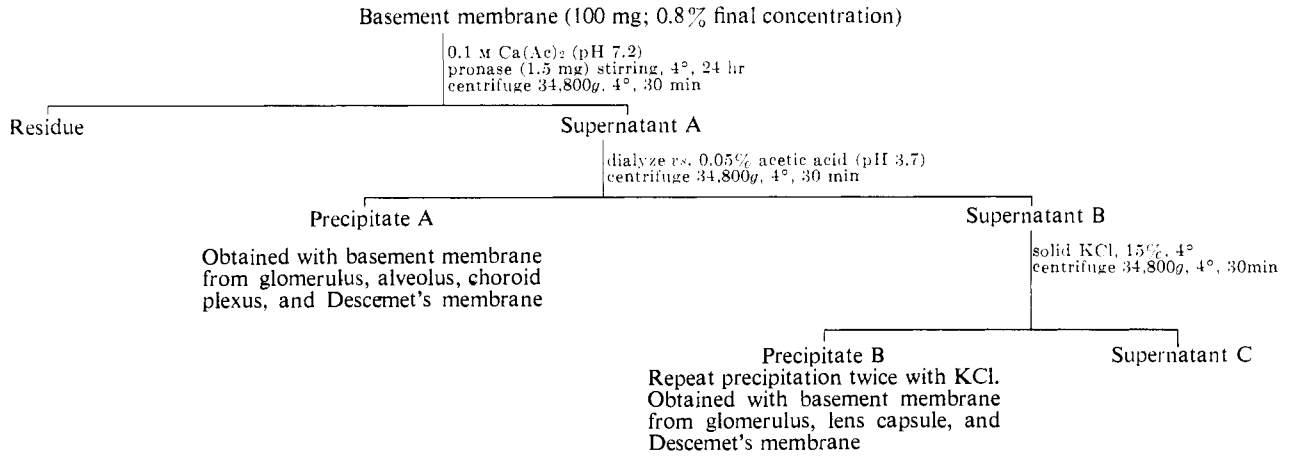

FIGURE 1: Fractionation procedure of basement membranes with pronase to isolate basement membrane collagen.

were separated from the amino sugars by the method of Boas (1953). Descending chromatography was run in ethyl acetate–pyridine–H_2O (10:4:3, v/v). Chromatograms were developed with the alkaline silver nitrate reagent.

Ultracentrifugation. Sedimentation rate constants were determined at a protein concentration of 0.5% in 0.15 M sodium citrate buffer (pH 3.65) at 4°. Schlieren optics were employed. Photographs were taken after attainment of maximum speed of 59,780 rpm.

Viscometry. Intrinsic viscosity was determined in a Ubbelohde suspended-level viscometer at 20°. The solvent was 0.15 M sodium citrate buffer (pH 3.65). Protein concentration was based on nitrogen content using the micro-Kjeldahl method.

Optical rotation was measured in a sodium vapor lamp apparatus using 0.2% solutions of protein in 0.15 M sodium citrate buffer (pH 3.65). Protein concentration was based on nitrogen content.

Electron Microscopy. Basement membrane preparations were examined in the electron microscope as described previously (Kefalides and Winzler, 1966).

Chemical Determinations. Amino acid analysis were performed in a Beckman Model 116 amino acid analyzer on protein hydrolysates (6 N HCl for 22 hr at 110°) according to the method of Moore and Stein (1954).

Total protein-bound hexose was determined by the orcinol reaction of Weimer and Moshin (1953). Fucose was measured by the method of Dische and Shettles (1948). Glucosamine and galactosamine were determined on protein samples, that were hydrolyzed in 6 N HCl for 4 hr at 100°, using the Beckman Model 116 amino acid analyzer. The resin used was the Beckman Type UR-30 in a 52 × 0.9 cm column. The sample was eluted with 0.35 M sodium buffer (pH 5.25) with a flow rate of 70 ml/hr at 55.2°. Sialic acid was estimated by the resorcinol method of Svennerholm (1957). Glucose and galactose were measured by the use of Glucostat and Galactostat reagents as described previously (Kefalides, 1968).

Results

Fractionation of Basement Membranes with Pronase. The fractionation of the various basement membranes with pronase at 4° appears in Figure 1. It is evident that the various basement membranes yield different fractions. Precipitate A which is soluble at pH 7.2 but insoluble in 0.05% acetic acid (pH 3.7) is obtained with basement membranes from the glomerulus, alveolus, choroid plexus, and Descemet's membrane but not with anterior lens capsule. On the other hand, the material which remains soluble after dialysis against 0.05% acetic acid (pH 3.7) yields a precipitate (precipitate B) with 15% KCl with basement membranes obtained from the glomerulus, Descemet's membrane and lens capsule but not with alveolar or choroid plexus basement membranes. On dry weight basis, precipitate A accounted for 17, 8, 12 and 12% with basement membranes from the glomerulus, alveolus, choroid plexus, and Descemet's membrane, respectively. Precipitate B accounted for 9, 8, and 60% with basement membranes from the glomerulus, Descemet's membrane, and anterior lens capsule, respectively.

Amino Acid Composition. The amino acid composition of the various intact basement membranes appear in Table II. Anterior lens capsule and Bowman's capsule which are both epithelial avascular basement membranes show many similarities and differences. Both are characterized by high hydroxylysine content—32 and 29 residues per 1000 residues, respectively. The lysine content of Bowman's capsule is twice that of lens capsule. Hydroxyproline accounts for 99.5 residues/1000 residues in lens capsule and 106 residues in Bowman's capsule. However, the sum of hydroxyproline and proline is similar in both but lower than that found in mammalian collagen from skin or tendon (Kefalides and Winzler, 1966). The glycine content is high in both types of basement membrane, but it does not account for one-third of the amino acid residues. Half-cystine is present in both.

The amino acid composition of Descemet's membrane differs significantly from that of anterior lens capsule in those amino acids which characterize collagen. The hydroxylysine content of Descemet's membrane is about two-thirds the amount found in lens capsule while the lysine content is more than twice that found in lens capsule. However, the sum of lysine and hydroxylysine is almost identical in both. Although a small difference exists in the hydroxyproline content, the difference in proline content is significant—Descemet's mem-

TABLE II: Amino Acid Composition of Basement Membranes from Various Tissues. Residues/1000 Residues.

Amino Acid	Anterior Lens Capsule[a]	Bowman's Capsule[b]	Descemet's Membrane[a]	Choroid Plexus[a]	Glomerulus[c]	Alveolus[d]
Hydroxylysine	32.0	29.0	20.0	9.0	24.0	6.8
Lysine	11.0	23.0	24.0	41.0	26.0	41.5
Histidine	12.0	14.0	11.6	16.0	14.4	12.6
Arginine	38.3	36.0	39.5	61.0	48.2	50.0
3-Hydroxyproline	19.5	18.0	6.8	Trace	8.5	Trace
4-Hydroxyproline	80.0	88.3	77.0	48.3	56.5	35.0
Aspartic	57.8	59.6	58.0	68.0	70.0	68.5
Threonine	31.0	36.0	36.4	39.0	40.5	38.8
Serine	48.0	41.3	42.1	52.8	49.0	57.7
Glutamic	95.0	91.0	94.0	94.0	97.0	78.0
Proline	66.0	63.0	95.0	69.0	69.8	77.1
Glycine	288.0	260.0	230.0	235.6	229.0	222.6
Alanine	46.1	50.5	53.0	104.5	65.0	97.8
Half-cystine	18.0	22.3	11.0	10.0	22.7	12.2
Valine	35.0	28.3	45.0	37.0	36.0	57.7
Methionine	6.2	6.1	8.2	9.4	6.0	9.4
Isoleucine	30.0	28.3	28.5	22.5	28.1	22.5
Leucine	56.5	58.1	75.2	50.0	60.2	50.0
Tyrosine	11.0	13.7	21.0	12.0	22.0	10.4
Phenylalanine	29.0	33.4	25.0	21.5	26.8	29.3

[a] Average of three runs. [b] Single run. [c] Average of 15 runs. [d] Average of two runs.

TABLE III: Carbohydrate Composition of Basement Membranes from Various Tissues (g/100 g).

Carbohydrate	Anterior Lens Capsule	Descemet's Membrane	Choroid Plexus	Glomerulus	Alveolus
Hexose[a]	10.5	8.2	4.0	6.2	4.8
Glucose	5.0	3.5	1.4	2.3	1.8
Galactose	5.1	3.7	1.85	2.4	2.0
Mannose[b]	0.4	2.0	0.75	1.5	1.0
Glucosamine	0.85	1.2	1.1	1.1	0.7
Galactosamine	0.15	0.3	0.5	0.3	0.2
Fucose	0.6	0.6	0.2	0.75	0.2
Sialic acid	0.4	0.7	1.5	2.0	0.8

[a] Corrected for contribution of fucose. [b] Value obtained by difference between total hexose and the sum of glucose and galactose.

brane contains 50% more. There are 288 residues/1000 residues glycine in lens capsule compared with 230 residues in Descemet's membrane. Significant differences also exist in the half-cystine, valine, leucine, and tyrosine content. Similarities are found in the histidine, arginine, aspartic acid, and glutamic acid content.

Significant differences were noted in the amino acid composition among basement membranes associated with vascular tissue. In terms of residues per 1000 residues glomerular basement membrane contains hydroxylysine 24, hydroxyproline 65, proline 69.8, and glycine 229 compared with 9, 48.3, 69, and 235.6 residues for choroid plexus and 6.8, 35, 77.1, and 222.6 residues for alveolar basement membrane, respectively. The low hydroxylysine content of choroid plexus and alveolar basement membrane is reflected in their higher lysine content. The alanine content, although similar in the basement membranes from choroid plexus and alveolus, is higher by 50% in these membranes compared with that of the glomerulus.

A comparison between lens capsule and Descemet's

TABLE IV: Amino Acid Composition of Fractions Obtained from Basement Membranes after Treatment with Pronase at 4° for 24 hr.[a]

Amino Acid	Residues/1000 Residues					
	Anterior Lens Capsule Ppt B[b]	Descemet's Membrane		Glomerulus		Alveolus Ppt A[c]
		Ppt A[c]	Ppt B[b]	Ppt A[c]	Ppt B[b]	
Hydroxylysine	41.0	37.6	40.0	36.6	42.0	18.0
Lysine	11.0	28.0	15.0	25.0	7.0	25.3
Histidine	9.0	16.5	7.0	16.0	9.2	12.1
Arginine	33.8	34.0	29.8	47.0	27.0	44.3
3-Hydroxyproline	23.0	8.0	6.0	16.7	18.0	Trace
4-Hydroxyproline	118.7	81.0	135.7	83.1	134.0	77.0
Aspartic	51.6	61.0	41.1	65.8	52.0	69.0
Threonine	23.0	43.0	21.3	37.2	23.1	35.3
Serine	38.0	47.0	31.4	50.0	42.8	49.6
Glutamic	80.0	86.0	83.5	86.3	79.0	86.5
Proline	65.5	85.0	88.0	65.6	62.0	80.0
Glycine	313.0	220.0	310.0	236.0	322.4	284.0
Alanine	32.0	50.0	36.0	50.4	33.4	80.0
Half-cystine	9.3	18.0	4.3	31.3	6.0	28.5
Valine	24.0	45.0	31.0	38.4	27.0	21.1
Methionine	9.4	7.0	7.1	7.0	7.6	5.0
Isoleucine	26.1	31.0	27.9	28.0	27.3	18.5
Leucine	58.0	65.0	57.9	54.2	51.0	39.0
Tyrosine	6.3	12.0	5.5	13.9	4.0	9.0
Phenylalanine	27.7	25.0	22.3	27.9	25.2	16.3

[a] See Figure 1. [b] Fraction which is soluble at pH 3.7 and 7.2 and precipitates with 15% KCl. [c] Fraction which remains insoluble at pH 3.7, but soluble at pH 7.2.

membrane on the one hand and the basement membranes from the glomerulus, choroid plexus and alveolus on the other shows again many quantitative differences. Anterior lens capsule contains considerably more hydroxylysine, hydroxyproline, and glycine than any of the capillary basement membranes. In terms of the above amino acids, Descemet's membrane bears a closer resemblance to glomerular basement membrane. If we assume the hydroxylysine and hydroxyproline content to indicate the relative amount of collagen present in basement membranes, then choroid plexus and alveolar basement membranes must contain the least, anterior lens capsule the most, and Descemet's membrane and glomerular basement membrane intermediate amounts.

Carbohydrate Composition. The carbohydrate composition of the various basement membranes appears in Table III. The same sugars are present in all basement membranes. However, significant differences are noted among them not only in terms of total carbohydrate but also in the relative amounts of the individual sugars. Neutral hexose is higher in anterior lens capsule compared to Descemet's membrane (10.5% compared with 8.2%). Descemet's membrane contains twice as much hexose as does choroid plexus basement membrane. Glomerular basement membrane contains one-third more hexose than does alveolar basement membrane. The ratio of glucose to galactose varies slightly from 1 in all basement membranes. Both glucosamine and galactosamine are present. Glucosamine accounts for about 80% of the total hexosamine content. Fucose does not vary significantly among basement membranes from lens capsule, Descemet's membrane and glomerulus; however, the choroid plexus contains about one-third the amount found in the above basement membranes. Significant variability was noted in the sialic acid content.

Characterization of the Fractions Obtained by Digestion of Basement Membranes with Pronase. AMINO ACID COMPOSITION. Precipitate B, which is soluble in 0.05% acetic acid and is obtained after repeated precipitation with 15% KCl, meets the chemical criteria for collagen (Kefalides, 1968). It is characterized by a high content of hydroxylysine regardless of the source of basement membrane (Table IV). Hydroxylysine accounts for 4.1% of all amino acid residues; it is a unique property of this mammalian collagen. The lysine content is proportionately low and the sum of these two amino acids accounts for about 5% of the total. The remaining basic amino acids do not vary greatly among the basement membrane collagens from lens capsule, Descemet's membrane, and glomerulus. The hydroxyproline content is high in all and varies between 141.7 and 152 residues per 1000 residues. The hydroxyproline content of the collagen from anterior lens capsule and Descemet's membrane is identical. The proline content is higher in the collagen from

TABLE V: Carbohydrate Composition of Fractions Obtained from Basement Membranes after Treatment with Pronase at 4° for 24 hr.[a]

Carbohydrate	g/100 g					
	Anterior Lens Capsule	Descemet's Membrane		Glomerulus		Alveolus
	Ppt B	Ppt A	Ppt B	Ppt A	Ppt B	Ppt A
Hexose[b]	10.4	11.5	10.0	9.4	10.4	9.3
Glucose	5.0	3.5	5.0	3.2	5.1	2.2
Galactose	5.2	6.0	5.2	3.4	5.25	5.5
Mannose[c]	Trace	2.0	0.0	2.8	0.00	1.6
Glucosamine	0.2	6.4	Trace	2.2	Trace	1.0
Galactosamine	0.0	1.6	0.0	0.4	0.0	0.4
Fucose	0.0	0.2	0.0	0.1	0.0	0.2
Sialic Acid	0.0	1.5	0.0	3.0	0.0	2.3

[a] See Figure 1. [b] Corrected for contribution of fucose. [c] Value obtained by difference between total hexose and the sum of glucose and galactose.

Descemet's membrane and it reflects the high amount found in the intact membrane. The sum of hydroxyproline and proline varies between 207 and 229 residues per 1000 residues. These values fall within the range expected for mammalian collagens (Gross, 1963). The glycine content varies between 310 and 322 residues per 1000 and accounts for almost one-third of the amino acid residues. Variable amounts of half-cystine is present in all. Small differences exist in the content of the remaining amino acids.

The fraction which precipitates in 0.05% acetic acid (precipitate A) appears to be a mixture of at least two protein moieties. Although it contains significant amounts of hydroxylysine, hydroxyproline, and glycine, their content is much lower than that in precipitate B. There are greater similarities in the amino acid composition between precipitate A from Descemet's membrane and glomerulus than between these two tissues and alveolus.

Carbohydrate Composition. The carbohydrate composition of precipitate B from three basement membranes appears in Table V. Striking similarities are present in the hexose content which accounts for about 10% of dry weight. Glucose and galactose exist in almost equimolar amounts and account for all the hexose. No other sugars are present. Precipitate B from lens capsule contains about 0.2% hexosamine.

The carbohydrate composition of precipitate A from three basement membranes differs qualitatively and quantitatively from precipitate B. In addition to glucose and galactose, mannose, hexosamine, fucose, and sialic acid are present. Furthermore, differences are noted in the total hexose content and the relative amounts of the various sugars of precipitate A among the three types of basement membranes. The hexosamine content of this fraction from Descemet's membrane is three to five times higher than that from the other two sources while the sialic acid varies from 1.5 to 3%.

Paper Chromatography of Sugars. Paper chromatography of acid hydrolysates of the collagen (precipitate B) from glomerulus, lens capsule, and Descemet's membrane revealed only glucose and galactose. Precipitate A revealed glucose, galactose, mannose, and fucose.

Physical Characteristics. The physical characteristics of the collagen fractions (precipitate B) isolated from various basement membranes are given in Table VI. The collagen fraction which was obtained after digestion with pronase at 4° for 24 hr had a specific optical rotation $[\alpha]^{24}$ of −360°. Treatment of anterior lens capsule with pepsin at 4° for 16 hr yields a fraction with a specific optical rotation of −384°. The intrinsic viscosity of the collagen fractions obtained after digestion with pronase for 24 hr is lower than that observed with pronase-treated calfskin tropocollagen (Davison and Drake, 1966). However, when the time of incubation with pronase is reduced to 10 hr then the collagen isolated has an intrinsic viscosity of 10 dl/g, a value identical with that reported by Davison and Drake (1966) for pronase-treated calfskin tropocollagen. Similarly, when anterior lens capsule is treated with pepsin at 4° for 16 hr the collagen obtained has an intrinsic viscosity of 13 dl/g, a value very

TABLE VI: Physical Characteristics of Basement Membrane Collagens.

	Lens Capsule	Descemet's Membrane	Glomerulus
$[\alpha]^{24}D$ (deg)	−360 −384[b]	−360	−360
Intrinsic viscosity (dl/g)	5.4 10[a] 13[b]	5.4	5.2
Sedimentation coefficient, $s_{20,w}$ (S)	3.6	3.6	3.5

[a] After treatment with pronase at 4° for 10 hr. [b] After treatment with pepsin at 4° for 16 hr.

close to 14 dl/g obtained by Davison and Drake (1966) for pepsin-treated calfskin tropocollagen.

The sedimentation coefficient, $s_{20,w}$, determined at 4° is about 3.6 S for the collagen fraction obtained after digestion with pronase for 24 hr.

Acrylamide Electrophoresis. The electrophoretic pattern of basement membrane collagen from the glomerulus, lens capsule, and Descemet's membrane appears in Figure 2. It separates into three fast components, two components with intermediate mobility and one with the least mobility. All bands stain for protein and carbohydrate. The similarities among the collagens from three different sources is evident. Treatment of the collagen with collagenase followed by electrophoresis abolished the staining for protein and carbohydrate.

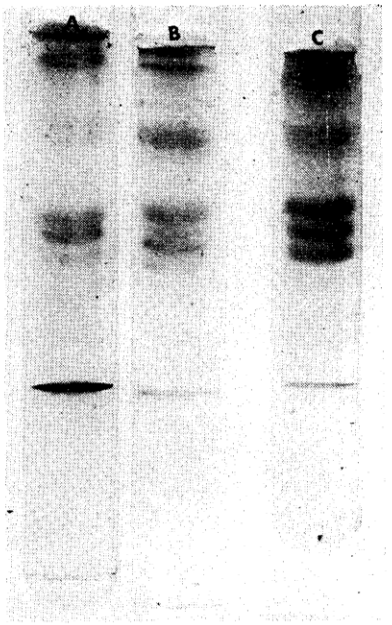

FIGURE 2: Disk electrophoresis of basement membrane collagen–0.04 M acetic acid–0.23 M glycine buffer (pH 4.0). (A) From Descemet's membrane, (B) from lens capsule, and (C) from glomerulus.

Discussion

Since basement membranes derive embryologically from different cells and are found in tissues with different functions, it is not surprising to find that they differ in their chemical composition and structure (Kefalides and Winzler, 1966; Spiro, 1967, 1969; Kefalides, 1968, 1969a).

In early embryonic life glomerular capillary basement membrane is laid down as two distinct layers from epithelial and endothelial cells (Vernier, 1964; Rhodin, 1964). In later embryonic life the two fuse into a single layer. It is not clear at present whether in the adult only the endothelial or only the epithelial cell or both are involved in the biosynthesis of basement membrane of the glomerular capillary. Studies in growing rats suggest that the epithelial cell contributes toward newly synthesized glomerular basement membrane (Kurtz and Feldman, 1962). It is generally agreed, however, that both types of cells play a role in its synthesis (Farquhar, 1964).

The basement membrane of the alveolar septa is associated with both the capillary endothelium and the alveolar epithelium. For the most part the two basement membranes are fused into one but at intervals an intermembranous space is formed, resulting in the separation of the basement membrane into an epithelial and an endothelial component (Karrer, 1956).

The basement membrane of the choroid plexus is associated with the capillary endothelium. A space containing loose connective tissue separates the capillary basement membrane from that of the ependymal layer composed of epithelial cells (Dempsey, 1968).

Descemet's membrane derives from mesenchymal cells and overlies the corneal endothelium (Watzka, 1935; Neifakh, 1952), while anterior lens capsule overlies a single layer of epithelial cells which are of ectodermal origin (McKeehan, 1951).

Although the carbohydrate composition of lens capsule was reported earlier (Pirie, 1951; Dische and Borenfreund, 1954; Dische, 1964; Dische *et al.*, 1967), no data were available on the complete amino acid composition of this structure. At the time this paper was submitted for publication, Spiro (1969) reported on the chemistry of bovine lens capsule. The amino acid and carbohydrate composition of bovine lens capsule is very similar to that of the canine lens capsule reported here. Similarly, studies on the chemical composition of Descemet's membrane were reported by Krause (1934) and Dohlman and Balazs (1955) but the complete amino acid analysis of this structure was again lacking. The hydroxyproline and glycine content of both these structures was reported to be sufficiently high to suggest that collagen was their major protein component (Pirie, 1951; Dohlman and Balazs, 1955).

All basement membranes examined in this study contain significant amounts of hydroxylysine, hydroxyproline, proline, and glycine. The content of these amino acids varies among the various basement membranes. Distinct differences were noted between anterior lens capsule and Descemet's membrane, both from avascular tissue, but one of epithelial and the other of mesenchymal cell origin. Differences between these two basement membranes are also reflected in their carbohydrate composition. Anterior lens capsule contains more neutral hexose but less hexosamine and sialic acid. Anterior lens capsule and Descemet's membrane behave differently when treated with pronase under the same conditions. Anterior lens capsule yields only one type of collagen molecule in the fraction which is solubilized with pronase. This fraction is soluble at pH 7.2 and 3.7 (precipitate B). However, Descemet's membrane yields an additional fraction (precipitate A) which precipitates out during dialysis against 0.05% acetic acid. This acid-insoluble fraction represents a mixture of collagen in combination with noncollagen-type polypeptides. This is suggested not only from the lower hydroxyproline and glycine content but also from the high amounts of hexosamine and sialic acid. It should be noted that the acid-soluble collagen fraction (precipitate B) from anterior lens capsule is very similar to that from Descemet's membrane in terms of amino acid and carbohydrate composition. These data indicate that epithelial and endothelial cells resemble each other in terms of the type of collagen they synthesize but differ in that a larger

portion of a noncollagen glycoprotein is produced by the endothelial cell which synthesizes Descemet's membrane.

Differences in amino acid and carbohydrate composition were noted between Descemet's membrane and choroid plexus basement membrane. Furthermore, choroid plexus basement membrane yields no acid-soluble collagen when treated with pronase. Their differences in function could be a reflection of their compositional and structural differences.

Capillary basement membranes associated with both epithelial and endothelial cells found in different organs showed marked differences in their amino acid and carbohydrate composition. Glomerular basement membrane, for example, contains three times as much hydroxylysine and twice as much hydroxyproline as does alveolar basement membrane. Glomerular basement membrane contains more total hexose, hexosamine, and sialic acid than alveolar basement membrane. Their differences are also reflected in their behavior after treatment with pronase. Glomerular basement membrane yields both the acid-soluble and -insoluble collagen fractions while alveolar basement membrane yields only the latter. In many respects, glomerular basement membrane resembles Descemet's membrane while alveolar basement membrane resembles that from choroid plexus.

This study and previous reports from this laboratory (Kefalides, 1968, 1969a) have demonstrated the presence of a unique collagen in basement membranes from lens capsule, Descemet's membrane and capillaries of various tissues. The most unique feature of this collagen is the high content of hydroxylysine and hexose. Furthermore, it contains about 50% more hydroxyproline and significant amounts of cystine compared to mammalian collagens described thus far (Gustavson, 1956; Gross, 1963). Minor tissue-specific differences in the degree of hydroxylation of proline have been reported in collagens isolated from rat skin and tendon collagen (Bornstein, 1967). Several studies have demonstrated that in mammalian embryonic tissue the hydroxyproline and hydroxylysine in collagen are synthesized by hydroxylation of proline and lysine after they have been incorporated into large polypeptide precursors of collagen (Stetten, 1949; Sinex *et al.*, 1959; Peterkofsky and Udenfriend, 1963; Prockop and Juva, 1965; Kivirikko and Prockop, 1967). It would appear from the present study that cells which are involved in the biosynthesis of basement membrane collagen possess an enzyme system which is capable of hydroxylating more prolyl and lysyl residues on the "protocollagen" peptide chain. Differences in activity of hydroxylating enzymes for collagen among different issues of the same species could be the result of differences in cofactor or substrate specificities for hydroxylation in fibroblasts, on the one hand, and epithelial or endothelial cells, on the other hand. The studies of Kivirikko and Prockop (1967) and Fujimoto and Prockop (1969) indicated that the chicken embryo proline hydroxylase preferentially hydroxylates proline in the third position of the sequence Gly-Pro-Pro-. Control of hydroxylation of prolyl and lysyl residues could, therefore, be dependent on a number of factors including sequence and tissue specificity.

The collagen fraction which is obtained from basement membrane after treatment with pronase for 24 hr differs in its physical parameters from other mammalian collagens (Kefalides, 1968). Although it exhibits a high negative optical rotation and a circular dichroism pattern identical with that of skin tropocollagen, it has a length and molecular weight which correspond to about two-thirds of the values for other mammalian collagens. It appears that the low intrinsic viscosity is the result of enzyme cleavage of the collagen molecule proper since treatment of basement membranes with pronase for 10 hr yields a collagen with an intrinsic viscosity of 10 dl/g and treatment with pepsin for 16 hr results in a collagen with an intrinsic viscosity of 13 dl/g. The amino acid and carbohydrate composition of the collagens with the high viscosity values does not differ from that of the collagen with the low viscosity (unpublished data).

Recent studies on the immunochemistry of the antigenic components of basement membranes (Kefalides, 1969b) suggest that, when whole basement membrane is used as immunogen, the antigenic determinants are located on peptides with an amino acid and carbohydrate composition unlike that of the collagen molecule proper. Digestion of glomerular basement membrane with collagenase produced four main soluble fractions which cross-reacted upon immunodiffusion with one of the antigenic components of whole basement membrane. Two of these soluble fractions contain hydroxyproline and hydroxylysine and all four contain glucose, galactose, mannose, fucose, sialic acid, and hexosamine. In addition to the soluble fraction, an insoluble, undigestible fraction is obtained after digestion with collagenase. This fraction lacks hydroxyproline and hydroxylysine, elutes with the void volume on Sephadex G-200 and cross-reacts with the second antigenic component of whole basement membrane. A fraction with the same chemical and immunologic properties as the undigestible fraction after collagenase was obtained after treatment of glomerular and Descemet's membrane with 8 M urea (unpublished data).

These data would suggest that two types of noncollagen polypeptides rich in carbohydrate are associated with the collagen molecule in basement membranes. One of these is a large molecular weight glycoprotein which interacts with the collagen molecule by hydrogen bonds. A second type of noncollagen polypeptide also rich in carbohydrate interacts with the collagen molecule by covalent bonds. During digestion with pronase or pepsin sufficient amounts of these noncollagen polypeptides could be cleaved and thus render a portion of the collagen molecules soluble in acetic acid. However, since the digestion is carried out at a low temperatures and for limited periods of time, not all the pronase susceptible material is digested and this could result in the soluble fraction which represents a complex of collagen and noncollagen proteins. Precipitate A would correspond to such a complex. Furthermore, the relative proportion of the noncollagen polypeptides and the nature of their interaction with the collagen in a given basement membrane could also determine the proportion of acid-soluble collagen (precipitate B) that will be obtained after proteolytic digestion.

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