Intestinal Basement Membrane of *Ascaris suum*. Preparation, Morphology, and Composition†

Benigno D. Peczon,‡ John H. Venable, Calvin G. Beams, Jr., and Billy G. Hudson*,§

ABSTRACT: Intestinal basement membrane from the helminth, *Ascaris suum*, was isolated free of cellular material by ultrasonic treatment and exhaustive washing. The basement membrane is 3.0 to 4.0 μm thick. Sections of the sonicated basement membrane examined by light and electron microscopy show a thick and thin sublamina separated by a line of denser material. The bulk of the material in the two sublamina is a fine feltwork with no identifiable strands of collagen fibrils. It is composed of 4.9% carbohydrate and 91% amino acids by weight. The monosaccharides were identified as galactose, glucose, mannose, fucose, glucosamine, and galactosamine. In contrast to basement membranes of vertebrate sources, there was no detectable sialic acid. The amino acid composition is dominated by glycine, which accounted for 15% of the amino acid residues. Proline and hydroxyproline, in a ratio of 4 to 1, accounted for 11% of the residues. Approximately 17% of the methionine residues exist in the sulfoxide form. Analyses of amide nitrogen showed that 35% of the dicarboxylic acids were in the uncharged form. An accounting of acidic and basic amino acids indicates the whole membrane has a net negative charge of 32 per 1000 amino acid residues. This invertebrate basement membrane contains the largest proportion of polar amino acids and the smallest proportion of hydroxyproline, glycine, and carbohydrate in comparison to the vertebrate membranes. Moreover, its amino acid content closely resembles that of a purified component isolated from bovine kidney glomerular basement membrane.

Basement membranes are extracellular membranes which are widely distributed in animal tissue and function as tissue support and in certain locations serve as a selective ultrafilter (Spiro, 1972). These membranes are considered to be a specialized form of collagen since they exhibit wide-angle X-ray diffraction patterns and possess several chemical properties which are characteristic of collagen (Kefalides and Winzler, 1966; Kefalides and Denduchis, 1969; Denduchis and Kefalides, 1970; Spiro, 1972).

The amino acid and carbohydrate composition has been determined for several vertebrate basement membranes including the glomerular (Spiro, 1967; Kefalides and Winzler, 1966; Westberg and Michael, 1973; Beisswenger and Spiro, 1970) and Bowman's membrane of the kidney (Kefalides and Denduchis, 1969), lens capsule (Kefalides and Denduchis, 1969; Fukushi and Spiro, 1969; Dische et al., 1967) and Descemet's membrane (Kefalides and Denduchis, 1969; Dohlman and Balazs, 1955) of the eye, choroic plexus membrane of the brain (Kefalides and Denduchis, 1969), and the alveolar membrane of the lung (Kefalides and Denduchis, 1969). Their amino acid contents are qualitatively similar but differ quantitatively. All contain significant amounts of hydroxylysine, hydroxyproline, and glycine which are characteristic amino acids of vertebrate collagen. These membranes are glycoprotein in nature and all contain the same monosaccharide constituents but in differing proportions.

The present study is the first report of the isolation and chemical characterization of an invertebrate basement membrane. The intestinal basement membrane of the helminth, *Ascaris suum*, was chosen for study because its anatomical location indicated ease of isolation in pure form. The membrane was found to be the most easily isolated of the membranes studied to date and could be prepared in rather large quantities. Moreover, it was considered possible that the membrane from a lower form of animal might have a simple structure and would serve as a model for elucidating the structure of basement membranes. Furthermore, a comparison of the detailed structure of this phylogenetically distant basement membrane to that of the mammalian membranes would define those properties which have been conserved during evolutionary development, thus providing information on the structural features required for function.

Experimental Procedure

Preparation of Intestinal Basement Membrane. Ascaris suum from porcine intestines were obtained from a local slaughterhouse and kept alive until dissection in Harpur's solution (Harpur, 1963) maintained at 37 ± 2°. Intestines from the worms were obtained by cutting 1 cm off each end, splitting open the body wall, and removing with forceps the intestine which runs with no turns from the esophagus to the anus. Cutting 1 cm from the anterior part of the worm ensures removal of the pharynx which is distinctly different from the intestine, since it remains off white on sonication, in contrast to the basement membrane which is transparent. The second cut on the tail of the worm removes the part of the intestine attached at its anus and allows pulling out the intestine intact.

Intestines were split with scissors, cut into 1 in. sections, and batches from 8 to 10 worms were pooled in 0.85% NaCl

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for sonic disruption of the cells attached to the basement membrane. For complete sonic disruption, the membranes were sonicated at least six times with a Branson Model S125 sonicator equipped with a probe terminating to 0.5 in. diameter. Each burst was limited to 30 sec. Temperature was maintained at 2°. Cell debris was decanted after each sonic burst.

The sonicated material from 50 worms was pooled and centrifuged at 12° for 10 min at 5000*g* in a 30-ml centrifuge tube. The sediment was washed twice with 20-ml portions of 0.85% NaCl followed by five washes with cold deionized water. The conductivity of the final wash approached that of deionized water as measured with a Radiometer (Copenhagen) type CDM sd conductance meter. The sediment was lyophilized and then dried in a vacuum oven at room temperature for 2 days. This drying procedure reduced the water content of the membranes to 4.6 ± 2%, as determined by more extensive drying of two samples at 75 ± 5° in a vacuum oven over a period of several days.

Microscopic Examinations. Specimens of the intestine of *Ascaris* at its midpoint were fixed in Bouin's fixative for light microscopic study and embedded and sectioned in paraffin by standard methods. Sections 5 μm thick were stained with Harris' hematoxyline and eosin, and studied and photographed.

The purity of basement membranes cleaned by sonication was evaluated by phase-contrast or dark-field microscopy.

Specimens for study by electron microscopy were fixed in a 2:3 dilution of Karnovsky's (1965) glutaraldehyde–formaldehyde fixative in a 0.2 *M* sodium cacodylate buffer. Dow epoxy resins were used for embedding (Locke and Krishnan, 1971). Gold sections, approximately 10 nm thick, stained with hot uranyl acetate (Lockwood, 1970) were studied with a Phillips EM 200 electron microscope using a 30-μ objective aperture, a microelectron gun with double condensor, and a liquid nitrogen anticontamination device.

Analysis of Amino Acid. Intestinal basement membrane was hydrolyzed with glass distilled constant boiling HCl under reduced pressure in sealed tubes at 105° for 24, 48, and 72 hr. The amino acid contents of the hydrolysates were determined on a Beckman 120C amino acid analyzer by the method of Guire et al. (1974).

The total half-cystine content of the membrane was determined as cysteic acid after oxidation of the protein with performic acid and hydrolysis with 6 *N* HCl for 24 hr by the method of Schram et al. (1954).

Methionine sulfoxide content was determined by the direct method of Ray and Koshland (1962). Analyses were performed on the amino acid analyzer using the accelerated program described by Odell et al. (1974).

Tryptophan analyses were performed according to the procedure of Hugli and Moore (1972). Samples (1–2 mg) were hydrolyzed in 0.6 ml of 4.2 *N* NaOH containing 25 mg of starch for 16 hr at 105°. Other samples were hydrolyzed for an additional 80 hr to determine the degree of completion of the alkaline hydrolysis.

Analysis of Monosaccharides. The membrane was hydrolyzed with 2 *N* H_2SO_4 (1–2 mg/ml) in sealed tubes for 4 hr at 100° and the hydrolysate passed through coupled columns of Dowex 50 and Dowex 1 resin (Spiro, 1966). The neutral monosaccharides present in the effluent and wash were identified by descending paper chromatography on Whatman No. 1 paper run in pyridine–ethyl acetate–acetic acid–water (5:5:3:1) for 20 hr (Fisher and Nebel, 1955). Monosaccharides were located on the chromatogram by the silver nitrate method (Trevelyan et al., 1950; Benson et al., 1952). Quantitation of neutral sugars, as well as verification of identification based on elution times, was performed on the Technicon Model SC-1 autoanalyzer as described by Lee et al. (1969). Corrections were made for hydrolytic destruction of the sugars by treatment of known quantities of sugars in the presence of bovine serum albumin under identical conditions of hydrolysis. Neutral sugars were also quantitated by this analytical procedure after hydrolysis of the membrane by a slight modification of the hydrolysis procedure of Lehnhardt and Winzler (1968) in which the volume of Dowex 50 suspension in 0.01 *N* HCl was increased from 0.05 to 0.2 ml. Fucose was independently analyzed by means of the Dische-Shettles cysteine–sulfuric acid reaction as employed by Spiro (1966). The hexosamines were determined on the short column of the amino acid analyzer after hydrolysis of the membrane in 4 *N* HCl at 100° for 6 hr. Sialic acid analyses were made by the thiobarbituric acid assay of Warren (1959) after hydrolysis of the membrane in 0.1 *N* H_2SO_4 at 80° for 1 hr. Hexuronic acid analyses were made by the procedure of Dische (1947) with glucuronic acid as the standard. Corrections for the contributions of fucose, mannose, galactose, glucose, glucosamine, and galactosamine to the absorbance at 530 nm were made.

Other Chemical Analyses. Ribonucleic acid content was estimated from ribose content. Ribose was extracted according to the Schmidt-Thannhauser procedure (Volkin and Cohn, 1954) and analyzed with the Technicon Sugar Autoanalyzer.

Deoxyribonucleic acid content was estimated from deoxyribose content. Deoxyribose was analyzed by the diphenylamine reaction of Burton (1956) after hydrolysis of the membrane in 0.5 *N* $HClO_3$ at 90° for 15 min.

Inorganic phosphorus was determined according to the procedure of Chen et al. (1956) after digestion of the membrane according to the procedure of King (1932). Inorganic phosphate and adenosine monophosphate standards run with the sample established percentage recovery.

Amide nitrogen was determined enzymatically as ammonia (Tabor, 1970) after hydrolysis of 2 to 3 mg of membrane in 0.4 ml of 2 *N* HCl in sealed tubes at 100° and trapping of NH_3 in Conway microdiffusion cells (Conway and Byrne, 1933).

Lipids were extracted from 10 mg of membrane according to the procedure of Folch et al. (1957), methylated according to the procedure of Mason and Waller (1964), and detected and quantitated on a Perkin-Elmer Model 990 gas–liquid chromatograph, using a 6 ft long Gas-Chrom A column coated with 5% diethylene glycol succinate. The gas–liquid chromatograph was operated isothermally at 190°.

Results

Morphology, Isolation, and Purity of the Basement Membrane. The wall of the intestine of *Ascaris suum* consists of a single layer of high columnar epithelial cells resting on a thick basement membrane (Figure 1). This membrane is a uniform 3–4 μm in thickness and is relatively tough. Shaking ribbons of the intestine in a suspension of fine glass beads removes most of the cellular material without tearing the membrane but cellular debris remains. An electron micrograph of a cross section of a basement membrane prepared in this manner is shown in Figure 2. The debris arises from the basal flutings of the columnar cells. The luminal surface of the membrane can be cleaned completely

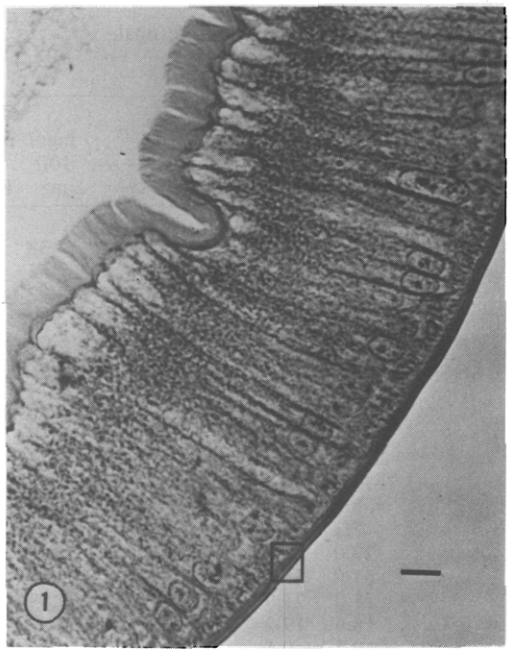

FIGURE 1: A longitudinal section of the intestinal wall viewed by light microscopy. The lumen of the intestine appears at the upper left and shows some detritus. The pseudocoelomic cavity appears at the lower right. The intestinal wall consists of palisade-like high columnar cells, each cell extending from the thick basal lamina to the lumen. The luminal surface of these cells is crowned by microvilli. A small black rectangle in the lower part of the micrograph frames the basal lamina which runs from its upper right to its lower left corners. Figure 2 illustrates this framed view at higher magnification; ×523.5; bar equals 10 μ.

FIGURE 2: An electron micrograph of a mechanically isolated basement membrane. The orientation and field match that of the black rectangle of Figure 1. Fragmented membranes from the basal in-foldings of the intestinal cells remain adhered to the luminal side. The basement membrane is primarily a fine feltwork forming a thick and a thin sublamina. The thick sublamina shows inhomogeneous zones with coarser materials woven into the finer feltwork. A thin basal sublamina borders the pseudocoelomic cavity and is devoid of the coarser materials; ×193,500; bar equals 1 μ.

of cellular debris by sonication of ribbons of the intestine. The cleaned surface (Figure 3) is undulating in profile but shows no particular structural modification of its components. A dark-field micrograph of a randomly selected unsectioned fragment of membrane isolated by sonication is shown in Figure 4. The fragment is wadded in the center of the field but its sheet-like character is seen in a partially unfolded part at the right. Smoothness and lack of light scattering from a single thickness suggest that the membrane is free of cellular debris.

Sections of the basement membrane show visible lamination even by light microscopy. Two bands are particularly evident: the basal boundary layer and a middle lamina (Figure 1). Viewed by electron microscopy (Figures 2, 3, and 5) this membrane exhibits a thick sublamina (basal lamella) just beneath the base of the cells and a thin sublamina (mesenterial membrane), approximately 0.15 μm thick, bordering the pseudocoelom. This agrees with earlier descriptions of the membrane (Rudall, 1955; Kessel et al., 1961; Sheffield, 1964) and it is reasonable to conclude that the sonication procedure for isolating the membrane does not alter its morphology. The junction between the thick and thin sublamina is marked by a line of collected denser material in the former (Figure 5). Strands of this coarse material also form a zone of concentration deeper within the thick sublamina, perhaps related to the middle dense lamina seen in light micrographs (Figure 1). The bulk of material in the two sublamina is a fine feltwork composed of strandlike elements less than 5 nm thick.

Relatively large amounts of membrane are obtained by our procedure. Approximately 1 mg of dry membrane is obtained from each adult worm.

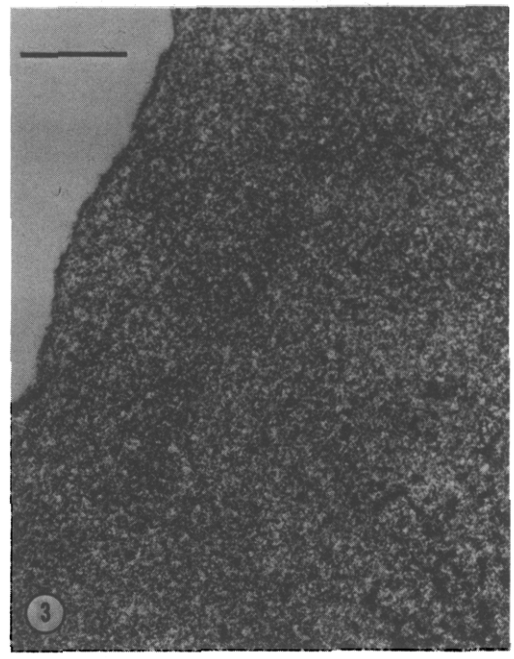

FIGURE 3: An electron micrograph of the luminal surface of the basement membrane showing the surface freed of membranous debris after sonification. The fineness of the primary feltwork is illustrated plus an increasing inhomogeneity of structure toward the pseudocoelomic side (lower right); ×140,250; bar equals 0.1 μ.

Since sonic disruption does not separate the sublamina, all compositional data that are reported in this article are in reference to the whole basement membrane.

DNA and RNA analyses on the sonicated basement

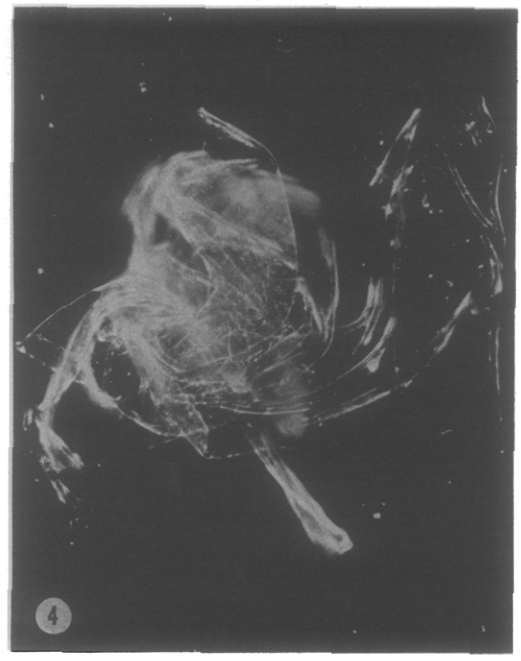

FIGURE 4: A dark-field light micrograph of an unsectioned, isolated fragment of the basement membrane. The fragment is wadded in the center of the field but its sheet-like character is seen in a partially unfolded part at the right. Smoothness and lack of light scattering from a single thickness correspond to the membrane free of cellular debris; ×112.5.

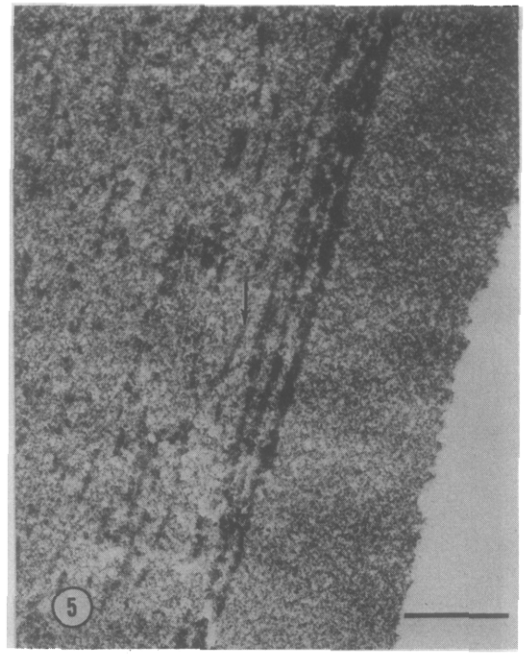

FIGURE 5: An electron micrograph of the pseudocoelomic surface of the basement membrane. A homogeneous basal sublamina, 0.15 μ thick, borders the pseudocoelom. A thick, coarse strand associated with the thicker part of the membrane is marked by an arrow. The individual finer strands of the primary feltwork are visible within the black circle; ×140,250; bar equals 0.1 μ.

membrane indicate the virtual absence of these components. DNA and RNA are estimated to account for 0.06 and 0.03%, respectively, of the dry weight of the basement membrane. Total elemental phosphorus is estimated to be 0.06% of the dry weight.

Amino Acid Composition. The amino acid composition of the basement membrane is summarized in Table I. Most

Table I: Composition of *Ascaris suum* Intestinal Basement Membrane.

Component	μmol/100 mg after Hydrolysis for 24 hr	48 hr	72 hr	Av[a]	mg[c]/100 mg	Residues per 1000 AA Residues
Amino acid						
Lys	18.3	20.0	17.5	18.6	2.38	21.8
His	18.3	19.1	18.3	18.6	2.55	21.8
Arg	47.3	57.0	57.2	57.1[b]	8.92	66.9
4-Hyp	16.6	19.8	20.7	20.2[b]	2.28	23.7
Asp	71.0	70.7	67.2	69.6	8.01	81.5
Thr	38.4	41.5	39.4	39.8	4.02	46.6
Ser	44.0	44.6	40.0	42.9	3.74	50.2
Glu	100.3	105.7	111.4	105.8	13.66	123.9
Pro	78.5	69.1	74.4	74.0	7.18	86.7
Gly	126.2	126.4	128.5	127.0	7.25	138.8
Ala	48.9	54.4	52.4	52.9	3.70	60.9
Val	38.1	44.1	45.8	45.0[b]	4.46	52.7
Met[d]	7.1	8.8	9.8	9.3[b]	1.22	10.9
Ile	29.0	31.2	30.9	30.4	3.44	35.0
Leu	51.0	55.0	50.5	52.2	5.91	61.2
Tyr	21.2	21.9	22.8	22.0	3.59	25.8
Phe	16.6	19.2	15.9	17.2	2.53	20.1
Hyl	9.5	9.5	9.2	9.4	1.35	11.0
Half-cystine				32.1	3.31	37.6
Trp				10.4	1.94	12.2
Amide N				(61.2)		(71.6)
Met sulfoxide				(1.54)		(1.80)
	Subtotal			853.6	91.44	
Monosaccharides						
Man				2.18	0.35	2.56
Fuc				2.70	0.39	3.16
Gal				11.38	1.84	13.33
Glc				9.01	1.46	10.56
GlcN				3.21	0.52	3.76
GalN				1.84	0.30	2.15
	Subtotal			26.38	4.86	
	Total				96.3	

[a] Unless otherwise indicated, the values represent an average of the analysis obtained at 24, 48, and 72 hr. [b] Values are averages of 48- and 72-hr hydrolyses. [c] Calculated using residue molecular weight of amino acid and monosaccharides. [d] Values include methionine sulfoxide.

of the amino acids were released from the protein within 24 hr hydrolysis time. Significantly more arginine, hydroxyproline, valine, and methionine were released by extended times of hydrolysis. No significant loss of serine was detected with increasing time of hydrolysis. Determinations of half-cystine as cysteic acid gave consistently higher values than those determined directly from normal amino acid analysis of the acid hydrolysate.

Tryptophan analysis on a 16-hr alkaline hydrolysate gave a value of 0.06 μmol/mg of dry membrane. Extending the hydrolysis time to 96 hr released 0.104 μmol of tryptophan per mg of dry membrane (Table I).

Amide nitrogen analysis gave a value of 0.612 μmol present in the amide form.

Methionine sulfoxide analysis (Table I) indicates that 16.6% of the methionine residues are present in the sulfoxide form.

Carbohydrate Composition. Identification of neutral monosaccharides by paper chromatography and from elution profiles on the autoanalyzer revealed the presence of mannose, galactose, glucose, and fucose in the membrane. Glucosamine and galactosamine were identified from their elution times on the amino acid analyzer.

The amounts of these monosaccharides are given in Table I. The total carbohydrate represents 4.9% of the dry weight of the membrane. Quantitative analysis of the neutral monosaccharides released on hydrolysis with 2 *N* H_2SO_4 yielded values 6% lower than those obtained by hydrolysis with Dowex 50, with the exception of galactose where hydrolysis with 2 *N* H_2SO_4 yielded an average value lower by 27%. The values shown in Table I are those obtained after hydrolysis in Dowex 50. Fucose values determined separately by the Dische-Shettles cysteine–sulfuric acid reaction (Spiro, 1966) closely agreed with those obtained by the above procedure.

Analyses for sialic acids and hexuronic acids gave values of less than 0.1% for each one.

Lipid Composition. The major lipid component of the membrane was identified as cholesterol and accounted for 2% of the dry weight. Triglycerides were not detected in significant quantities. Assuming a phospholipid molecular weight of 750 and correcting for the phosphorus content of the DNA and RNA content found in the membrane on the basis of the phosphorus analysis, total phospholipid content is less than 2.0%.

Discussion

The *Ascaris suum* intestinal basement membrane used in this study was essentially free of cellular material, as clearly shown by microscopic examination and verified by chemical analyses for DNA, RNA, and phosphorus.

Amino acids account for 91.4% of the weight of the intestinal basement membrane (Table I). Noteworthy is the presence of hydroxyproline, hydroxylysine, and a relatively large amount of glycine. These amino acids are characteristic constituents of vertebrate basement membranes. A portion of the methionine residues, 16.6%, is present in the sulfoxide form. Methionine sulfoxide is also a constitutent of vertebrate basement membranes but it is not known whether the oxidation of methionine occurs in vivo or during the isolation procedure (Spiro, 1972).

Since substantially more tryptophan was released on increasing the length of alkaline hydrolysis from 16 to 96 hr., the membrane most likely contains -Ile-Trp-or -Val-Trp- sequences. These two sequences have been shown to be particularly resistant to alkaline hydrolysis (Levene et al., 1932; Muramatsu et al., 1963).

A comparison of the amino acid composition of the intestinal basement membrane with that of bovine glomerular basement membrane (Table II), a representative vertebrate basement membrane, reveals close similarities and certain notable differences in several classes of the amino acids. These differences mainly reflect a decrease in the amount of hydroxyproline, hydroxylysine, and glycine and an increase in acidic amino acids of the intestinal membrane as compared to the glomerular membrane. A portion of the methionine residues exist in the sulfoxide form as has been reported for glomerular basement membrane and lens capsule (Hudson and Spiro, 1972). Based on the amount of acidic and basic amino acids and amide groups (Table I), it is calculated that the intestinal membrane contains an excess of 32 anionic groups per 1000 amino acids in contrast to 14 cationic groups in the glomerular membrane (Spiro, 1967a,b).

A further comparison of the amino acid composition of the intestinal membrane to that of other vertebrate basement membranes (Westberg and Michael, 1973; Spiro, 1972; Kefalides and Winzler, 1966; Kefalides and Denduchis, 1969) shows that it contains the largest proportion of polar amino acids and the smallest proportion of hydroxyproline and glycine. It is particularly striking that the amino acid composition of this invertebrate basement membrane closely resembles that of a component isolated from vertebrate glomerular basement membrane (Ohno et al., 1975).

Table II: Comparison of Amino Acid Composition of Intestinal Basement Membrane with Glomerular Basement Membrane and *Ascaris* Cuticle and Muscle Layer Collagen.

	Residues/1000 Amino Acid Residues			
Amino Acid	*Ascaris*[a] Intestinal Basement Membrane	Bovine[b] Glomerular Basement Membrane	*Ascaris*[c] Cuticle Collagen	*Ascaris*[d] Muscle Layer Collagen
Nonpolar aliphatic	359	395	396	480
Ala	60.9	60.7	78	63
Leu	61.2	59.3	20	54
Ile	35.6	29.1	10	24
Val	52.7	38.2	27	13
Gly	148.8	208	261	326
Imino–amino acids	110	138	310	225
Pro	86.7	68.8	291	103
Hyp	23.7	68.8	19	122
Hydroxy amino acids	157	202	56	197
Ser	50.2	55.3	17	16
Thr	46.6	37.4	16	13
Tyr	25.8	17.8	4	6
Hyl	11.0	22.3		40
Hyp	23.7	68.8	19	122
Acidic amino acids	205	164	136	137
Asp	81.5	67.9	64	57
Glu	123.9	96.2	72	80
Basic amino acids	122	115	91	101
Lys	21.8	27.0	49	19
Hyl	11.0	22.3		40
Arg	66.9	49.0	32	39
His	21.8	16.4	10	3
Aromatic amino acids	58.1	51.6	14	20
Phe	20.1	27.9	10	14
Tyr	25.8	17.8	4.0	6
Trp	12.2	6.0		
Sulphur-containing amino acids	48.5	44.8	19.8	9
Met	10.9	14.2	3.8	6
Half-cystine	37.6	30.6	16	3

[a] Data from Table I. [b] Data from Spiro, 1967a. [c] Data from Josse and Harrington, 1964. [d] Data from Fujimoto, 1968.

The amino acid composition of *Ascaris* intestinal basement membrane differs greatly from that of cuticle collagen and muscle layer collagen from the same organism (Table II). These differences are prominent in the proportion of those amino acids characteristic of collagen as well as the other amino acids. The membrane contains much less glycine, proline, and hydroxyproline than the collagens. In addition, hydroxylysine is present in the membrane and in muscle layer collagen but absent in the cuticle collagen. These differences presumably reflect structural features that are required for their respective functions.

Carbohydrates account for 4.9% of the weight of the membrane (Table I). The monosaccharides were identified as mannose, galactose, glucose, fucose, glucosamine, and galactosamine. Hexuronic acid, a minor component of lens capsule (Kefalides and Denduchis, 1969; Fukushi and Spiro, 1969), is absent in the intestinal basement membrane. The presence of glucose, galactose, and hydroxylysine in approximately the same proportions suggests the

Table III: Comparison of Carbohydrate Composition of Intestinal Basement Membrane with Glomerular Basement Membrane and *Ascaris* Cuticle and Muscle Layer Collagen.[a]

Monosaccharide	Residues/1000 Amino Acid Residues			
	Ascaris Intestinal Basement Membrane	Bovine Glomerular Basement Membrane	*Ascaris* Cuticle Collagen	*Ascaris* Muscle Layer Collagen
Mannose	2.56	5.1		
Fucose	3.26	1.6		
Galactose	13.33	20.2		
Glucose	10.56	16.4		
Glucosamine	3.76	10.0		
Galactosamine	2.15	1.4		
Sialic acids		4.4		
Total carbohydrate	35.5	59.1	2.3[b]	77[c]

[a] References identical with those in Table II. [b] Total hexoses calculated assuming 99.6% protein in cuticle collagen. [c] Total hexoses calculated from the data of Fujimoto (1968).

presence of the glucosylgalactosylhydroxylysine carbohydrate unit which has been characterized from vertebrate basement membrane (Spiro, 1967a,b). A distinctive feature of the composition is the absence of sialic acid which is a common constituent of vertebrate membranes. It is noteworthy that the studies of Mohos and Skoza (1970) suggest that sialic acid found in the glomerular membrane comes from the plasma membranes of the epithelial cells lining the basement membrane. Their studies, however, do not rule out the possibility that the glomerular membrane proper contains sialic acid in amounts insufficient for histochemical demonstration. Moreover, sialic acid was recently shown to be a constituent of a glycoprotein component of glomerular basement membrane (Ohno et al., 1975).

The carbohydrate composition of intestinal basement membrane is qualitatively similar to glomerular basement membrane, with the exception of sialic acid, but differs in the relative proportions of the individual monosaccharides as well as the total amount of carbohydrate (Table III). The total carbohydrate content of the intestinal basement membrane is the lowest of the basement membranes reported so far (Kefalides and Winzler, 1966; Kefalides and Denduchis, 1969; Spiro, 1972), greater than that of *Ascaris* cuticle collagen, and less than that of *Ascaris* muscle layer collagen (Table III).

The intestinal basement membrane can be classified as a specialized form of collagen along with the vertebrate membranes since it exhibits a wide-angle diffraction pattern characteristic of collagen (Rudall, 1955). This criterion is considered to be the most reliable index for inclusion of a protein in the collagen family (Spiro, 1972). The presence of appreciable amounts of hydroxyproline, hydroxylysine, and glycine which are characteristic components of vertebrate collagen further supports this classification. However, this membrane, analogous to the vertebrate basement membranes, does not exhibit under the electron microscope the characteristic 640-Å periodicity of classical collagens.

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A Magnetic Resonance Study of Concanavalin A. Identification of a Lanthanide Binding Site†

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ABSTRACT: The solvent proton relaxation enhancement technique has been utilized to demonstrate that the plant lectin concanavalin A (Con A) exhibits a single tight binding site ($K_D \cong 15\ \mu M$) per 27,000 molecular weight subunit for the paramagnetic lanthanide Gd^{3+}. It was also found that Gd^{3+} could not be competitively displaced from its binding site on demetallized Con A by the addition of Zn^{2+} to occupy the transition metal ion site S1, nor by the subsequent addition of excess Ca^{2+} to occupy site S2. When both Mn^{2+} and Gd^{3+} were added to demetallized Con A, a combined solvent relaxation enhancement effect was observed. The individual contributions of each ion to the combined enhancement were extracted from the data and were found to be identical with their contributions in the absence of the other ion. The Ca^{2+} effect on the relaxation enhancement properties of the Con A bound Mn^{2+} was shown to be completely intact in the presence of Gd^{3+}. Thus, Gd^{3+} binds to a separate site on Con A, distinct from the transition metal ion site (S1) and the Ca^{2+} binding site (S2). We propose to designate this Gd^{3+} binding site as S3. Gd^{3+} binding to site S3 was found to be effectively competed for by the lanthanides Sm^{3+}, Eu^{3+}, and Tb^{3+} as well as Pb^{2+}. On the basis of the crystallographic locations previously determined for the Pb^{2+} binding sites and also the Sm^{3+} binding sites on Con A, a tentative proposal is advanced for the location of the single tight Gd^{3+} binding site observed in solution. The location of the Gd^{3+} binding site on the Con A monomer provides a second paramagnetic metal ion reference point on the protein structure which can be utilized in high resolution nuclear magnetic resonance studies of this lectin in solution.

In view of the numerous recent applications of the plant lectin Con A[1] as a probe of cell surface structure and dynamics (Nicolson, 1971, 1973; Inbar and Sachs, 1973; Edelman et al., 1973), there is now considerable interest in the detailed structural features of the protein and exactly how these relate to its interaction with a cell surface receptor (Gunther et al., 1973; Edelman et al., 1973; Cuatrecasas, 1973).

It has been established that Con A is a metalloprotein (Agrawal and Goldstein, 1968; Kalb and Levitzki, 1968; Shoham et al., 1973) requiring the prior occupation of two metal ion binding sites in order to exhibit specific monosaccharide binding activity (Kalb and Levitzki, 1968). The "site induction" model for the metal ion requirements of Con A was first advanced by Kalb and Levitzki (1968). They proposed that each Con A monomer has a site (labeled S1) able to bind a variety of transition metal ions (Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}) which must be occupied before the second metal ion site (S2), specific for Ca^{2+}, is available. It was also found that both sites S1 and S2 must be occupied in order for Con A to bind methyl α-D-glucoside. A structural rationale for the induction of site S2 by the occupation of S1 is offered by the recent X-ray crystal structure of Con A (Becker et al., 1975; Hardman and Ainsworth, 1972), in which the Mn^{2+} (site S1) and Ca^{2+} are found to be approximately 5 Å apart and sharing two aspartate residue ligands (Asp-10 and -19). Recent results obtained in this laboratory (Barber and Carver, 1973, 1975) indicate that nuclear magnetic resonance (NMR) techniques can be usefully applied to characterize such ligand-induced (i.e., Ca^{2+} and methyl α-mannoside) structural perturbations for Con A in solution.

In this paper we wish to report a third metal ion binding site per Con A monomer, most highly specific for Gd^{3+}, but which also binds the lanthanides Tb^{3+}, Eu^{3+}, and Sm^{3+} less tightly. We propose to refer to this site as site S3 in keeping with the metal ion site nomenclature of Kalb and Levitzki

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[1] Abbreviations used are: Con A, concanavalin A; PRE, solvent proton relaxation enhancement.