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A NEW METHOD FOR THE ISOLATION OF RENAL BASEMENT MEMBRANES

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Summary

A method is described for the isolation of basement membranes from rabbit renal cortex in which the detergent *N*-lauroyl sarcosine is used as the disruptive agent. The isolated membranes have been compared with membranes prepared using ultrasonication and they were comparable both in terms of purity and gross chemical composition. Glomerular and tubular basement membranes were isolated by first separating glomeruli from tubules by density gradient centrifugation followed by detergent treatment of the separated tissues.

The detergent method has the advantage that the basement membranes retained their native structure to a large degree, whereas sonicated membranes were severely fragmented. Collagen fibres were a significant contaminant in both preparations and were revealed more clearly by negative staining than by examination of thin sections. Studies with the detergent-treated membrane revealed that a few proteins, which seemed to be membrane components, were extracted with 1 M NaCl and that these proteins were lost from the basement membranes during sonication used in the conventional isolation procedure.

Introduction

Much of the interest in renal basement membrane stems from observations that it undergoes morphological changes in renal disease [1] and biochemical investigation of the basement membrane has followed from the development, by Krakower and Greenspon [2], of procedures for its isolation from glomeruli. Until now most studies have utilised these procedures which are initially to separate the renal glomeruli by sieving and, subsequently, to isolate the base-

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ment membranes by ultrasonic disruption. This latter step relies on the basement membrane's being resistant to ultrasonic disruption while the cellular elements are dispersed, so permitting the basement membranes to be recovered from the suspension by centrifugation. To assist the dispersal of nucleoprotein complexes, the disruptive step in usually conducted in 1M NaCl followed by washing of the membranes in the same medium. However, when only one method is available for the isolation of an organelle from complex biological material, there is always some uncertainty about the nature of the product. For instance, the isolated material may have undergone changes during the isolation procedure such as a loss of some of the components. Such changes can be subtle and may well be a consistent consequence of the isolation method, thus making it difficult, if not impossible, to recognise that they have occurred. Similarly, impurities may be consistently isolated with the desired product but they may remain unrecognised when there is no independent means for identifying them. In these respects, it is valuable to explore novel methods for isolation since comparisons can be made between isolates obtained using different methods. The resulting products are likely to be contaminated to different extents and to have suffered different degrees of damage, so that the comparisons can provide a basis for attempting to judge the efficacy of the isolation procedures.

In the course of studies on renal cell lipoprotein membranes, we had observed that, while these membranes were totally dispersed by detergents, the basement membranes remained apparently intact as judged by phase contrast microscopy. Consequently a method for the isolation of basement membrane was developed using detergents which permitted comparisons to be made between material so isolated and that prepared using ultrasonic disruption.

Materials and Methods

Rabbits were used throughout this study; these were Blue Bevron x Chinchilla of either sex, obtained from the Oxford University Farm and weighing 2.3—2.6 kg. Detergents and chemicals were obtained commercially; *N*-lauroyl sarcosine was purchased from the Sigma (London) Chemical Co.

Isolation of basement membrane. In initial studies, glomeruli were isolated from kidneys using the sieving procedure [2] but yields of material were found to be low and insufficient for experiment unless a large number of animals was used. Larger quantities of membrane could be obtained if tubules as well as glomeruli were utilised and a protocol was adopted whereby the mixed tubular and glomerular basement membranes (bulk membrane) could be recovered from the kidneys.

Kidneys were removed from rabbits immediately after killing with intravenous sodium pentabarbitone and plunged into ice-cold 0.15 M NaCl buffered with 0.01 M Tris/chloride, pH 7.4, (buffered saline); subsequent procedures were conducted at 0° C. The cortices were dissected from the kidneys and passed through a Climpex mincer fitted with a mesh screen (0.9 mm) to retain the larger connective tissue elements. The minced material was then washed through a sieve (150 μm spacing; Endecott Ltd., London) with a large volume of buffered saline while pressing the mince through the mesh with the base of a

beaker. This procedure fragmented the nephrons which passed through the mesh while some clumps of tubules together with connective tissue remained on the grid. The washings were centrifuged (MSE Mistral centrifuge, rotor 59560; 1000 × g_{max} for 2 min) to sediment the tubule fragments and glomeruli which were resuspended in buffered saline for the isolation of basement membrane. The sediment consisted of free glomeruli, tubular fragments, a few clumps of tissue and free cells together with nuclei.

To prepare the basement membrane, the suspended sediment was mixed with *N*-lauroyl sarcosine (0.5%(w/v) in buffered saline) employing 200 ml of detergent solution for each 10 g portion of cortex taken initially. Allowing for losses during sieving it was found that this proportion gave a detergent:protein ratio greater than 1:1 (see Results for a discussion of this). The suspended material was then vigorously homogenised for 2 min with a Silverson mixer homogeniser (Silverson Ltd., Chesham, Bucks., U.K.) both to disperse the tissue in the detergent and to disrupt the nucleoprotein gel released from nuclei. After standing for 10 min the suspension was centrifuged to sediment the basement membranes (MSE Mistral as described, 2000 × g_{max} for 2 min) and the sediment was resuspended in fresh detergent (40 ml per 10 g of cortex) and again centrifuged as described. This residue was washed once in buffered saline and suspended in unbuffered 0.15 M NaCl containing 0.01% (w/v) deoxyribonuclease 1 (Type DN 25, Sigma (London) Chemical Co.) allowing 15 ml for each 10 g portion of cortex; the suspension was incubated at 20°C for 30 min. This served to reduce the level of contaminating DNA; under the conditions used, i.e. pH 5.0—5.5, no protease activity could be detected in the enzyme preparation, using azoalbumin as the substrate. Basement membranes were sedimented by centrifugation, washed once in 0.15 M NaCl and three times in distilled water. The isolated basement membranes were stored at 0°C.

In comparing detergent-treated membrane with that prepared using ultrasonication, the suspension from sieving was divided into two equal portions, one of which was treated as described above while the other was treated by employing the sonication stage of the procedure of Krakower and Greenspon [2]. The tissue was suspended in 1.0 M NaCl by adding 40 ml for each 10 g of cortex and the suspension was subjected to sonication using an MSE transducer equipped with a 1 cm probe which provided a peak to peak amplitude of 6.5 μm. Sonication was conducted for periods of 45 s, with cooling intervals of 30 s, for a total sonication time of 8 min. The disrupted suspension was then washed four times in 1.0 M NaCl and five times in distilled water.

Separation of glomeruli from tubules. Separation of glomeruli from tubules was achieved using density gradient centrifugation based upon the method devised by Taylor et al. [3]. The residue from sieving was suspended in 43% (w/w) sucrose in buffered saline using the Silverson homogeniser; homogenisation was continued until the clumps of tissue were dispersed, normally about 1 min was required. The suspension was layered on to a discontinuous density gradient prepared in a Beckman zonal rotor (type Al-15) and which comprised 200 ml of 45% sucrose, 300 ml of 47.5% sucrose and 350 ml of 48.5% sucrose, with an underlay of 55% sucrose. All sucrose concentrations were weight for weight and were made up in buffered saline. Centrifugation was conducted at 18 000 × g_{max} for 60 min; loading and unloading the rotor was conducted at

400 × g_{max} with a pumping rate of 45 ml/min. After centrifugation the recovered fractions were examined by phase contrast microscopy and the fractions containing glomeruli or tubule fragments were separately pooled, diluted with buffered NaCl and the tissue fragments recovered by centrifugation. This procedure was found to give an effective separation of glomeruli which sedimented to the base of the 48.5% sucrose layer; the tubules, although spread out on the gradient, were restricted to the upper layers. Estimates of the purity of the fractions, obtained by counting the proportions of both tissue elements in each fraction, showed that the glomerular fraction was 90—95% pure while the tubule fraction contained only 2—3% of glomeruli.

Basement membranes were isolated from the fractions as described above.

Chemical and enzymic analysis. Nucleic acids were estimated in perchloric acid extracts of samples, prepared according to the method of Ceriotti [4]. Deoxyribonucleic acid was analysed using the method of Burton [5]; deoxyribose was used as the standard and 1 mg of sugar was taken as equivalent to 4.8 mg of DNA. Ribonucleic acid was measured by the orcinol procedure [6] with ribose as the standard; 1 mg of sugar was taken as equivalent to 4.5 mg of RNA. In most instances the RNA results were corrected for interference from DNA using the results of the DNA measurements as the basis for correction.

Protein was measured by the method of Lowry et al. [7], the basement membrane being dissolved in 0.1 M NaOH by brief heating at 80° C prior to analysis. As others have noted [8], the colour yield of basement membrane is appreciably less than that of bovine serum albumin commonly used as the standard for protein determination and the use of albumin leads to an underestimate of 25% with respect to the weight of basement membrane protein. Consequently dried bulk basement membrane was used as the protein standard.

Sugars were analysed by gas-liquid chromatography according to Clamp et al. [9]. Amino acids were measured using the method of Spackman et al. [10] employing either a Locarte or a Beckman Spinco 120B analyser. Phospholipids were extracted with chloroform/methanol (2:1, v/v) at room temperature for 20 h followed by a second extraction for the same time. The consecutive extracts were pooled and after drying were digested with $HClO_4$; inorganic phosphate was estimated by the method of King [11].

Alkaline phosphatase [12], acid phosphatase [12] and glucose-6-phosphatase [13] levels were assayed using the methods described in the appropriate reference. The effects of lauroyl sarcosine on these enzymes were judged by assaying the enzymes in whole kidney homogenate in the presence of the detergent at a 1 : 1 (w/w) detergent : protein ratio. When compared with untreated controls, the detergent was found to activate alkaline phosphatase (by 25—30%), to inhibit glucose-6-phosphatase (by 50—60%) and to have no effect on the activity of acid phosphatase.

Polyacrylamide gel electrophoresis was conducted essentially according to the method of Weber and Osborn [14]. Gels were polymerised in tubes of 7.5 mm internal diameter and were prepared from 7% (w/v) acrylamide, 0.09% (w/v) bis-acrylamide polymerised with $(NH_4)_2S_2O_8$ in 0.1 M sodium phosphate buffer, pH 7.2, containing 1% (w/v) sodium dodecyl sulphate (final concentrations); the running buffer was 0.1 M sodium phosphate buffer, pH 7.2, containing 1% sodium dodecyl sulphate. Samples of basement membrane were extracted with

5% sodium dodecyl sulphate, containing 1% (v/v) 2-mercaptoethanol, prior to electrophoresis by heating at 100°C for 10 min, which rendered 90% of the membrane protein non-sedimentable. After electrophoresis the gels were stained with Coomassie-Brilliant Blue (0.1%) in propan-2-ol/acetic acid/water (25 : 10 : 65, v/v) and cleared in propranol-2-ol/methanol/acetic acid/water (5 : 10 : 25 : 50, v/v). Cytochrome *c*, aldolase, bovine serum albumin, phosphorylase and rabbit muscle myosin were used as molecular weight standards.

Electron microscopy. Samples for thin section microscopy were prepared by fixing with glutaraldehyde, followed by O_sO_4. They were embedded in epoxy resin and post-stained with uranyl acetate and lead citrate [15]. Negative staining was effected using 1% sodium phosphotungstate, pH 7.2. When measurements were taken, the narrowest regions of the sample were measured as these were assumed to be sectioned at right angles.

Results and Discussion

The choice of detergent. In initial studies various detergents were used in order to establish which detergent might be the most effective for the isolation of basement membrane from glomeruli and tubule fragments. The non-ionic detergents Nonidet P40, Tween 80, Lubrol WX and Triton X-100 all proved unsatisfactory in that even at high detergent : protein ratios cellular residues could be seen to be associated with the basement membrane when they were examined by microscopy. Anionic detergents were found to be more effective in removing cellular material from the basement membrane. Sodium dodecyl sulphate and *N*-lauroyl sacrosine yielded membranes with lower phospholipid contents than did sodium deoxycholate at comparable protein to detergent ratios. However, sodium dodecyl sulphate gave lower protein recoveries than lauroyl sarcosine since it dissociated protein from the membrane at concentrations necessary to disperse the cellular material. *N*-lauroyl sarcosine was therefore selected as being the most suitable detergent for the isolation of undegraded but cell-free basement membrane.

Conditions for the isolation of basement membrane using detergent

Experiments were conducted to ascertain the optimal concentration of lauroyl sarcosine required for dissociation of cellular material from the glomeruli and tubular fragments. Samples of sieved tissue were suspended in increasing volumes of 0.5% lauroyl sarcosine dissolved in buffered saline in order to increase progressively the detergent : protein ratio. After 10 min at 0°C all the samples were centrifuged to sediment the basement membrane and the protein contents and alkaline phosphatase activities of the residues and supernatants were measured. As the detergent was increased with respect to protein, the amounts of both alkaline phosphatase and protein in the residue diminished while the levels in the supernatant increased commensurately. At detergent : protein ratios greater than 1 : 1 (w/w) no further loss of protein from the residue was observed and the alkaline phosphatase activity of the residue attained a very low constant level; under these conditions approx. 5% of the protein and 0.1% of the alkaline phosphatase remained in the residue. Analysis of the nucleic acid content of the residue showed a high level of DNA which was removed

by treatment with DNAase. This treatment further reduced the protein and alkaline phosphatase present in the residue as a result of the degradation of the nucleoprotein gel. These findings led to the protocol described under Materials and Methods.

Yields of bulk basement membrane protein, expressed as mg protein per g wet weight of cortex, were 1.8 ± 0.4 mg for detergent-treated membranes and 1.2 ± 0.3 mg for the ultrasonicated membrane. Yields for separated tubules and glomeruli, respectively, were 1.2 ± 0.2 and 0.7 ± 0.2 mg for detergent treatment and 0.8 ± 0.2 and 0.5 ± 0.2 mg for ultrasonication.

Purity of basement membranes isolated using either detergent treatment or ultrasonic disruption

The purities of the detergent and sonicated preparations were compared by measuring the levels of markers for likely contaminants (Table I). The levels of DNA, RNA and phospholipid were comparable in the two preparations. Acid phosphatase which is found in glomeruli as well as in tubular cells [3] was not detected, neither was glucose-6-phosphatase. Membrane preparations were white indicating the absence of cytochromes. The only appreciable difference was that the specific activity of alkaline phosphatase was ten times greater in the sonicated membranes as compared with the detergent-treated material. Since alkaline phosphatase is present in plasma membranes [12], ultrasonication can be judged to have been less efficient in removing these elements from the basement membrane. Taking the alkaline phosphatase specific activity in isolated rabbit kidney brush borders as 1.7 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ [12], the amount of membrane protein residual in the basement membrane preparations was estimated as 13.8 and 1.7 μg per mg of ultrasonicated and detergent-treated membrane, respectively.

Morphological examination of the preparations by phase contrast microscopy showed no evidence of contamination by cellular material and while the sonicated material was highly fragmented the detergent-treated material showed relatively intact basement membrane ghosts from tubules and glomeruli (Fig. 1).

TABLE I

COMPARISON OF THE PURITY OF BASEMENT MEMBRANES PREPARED BY EITHER DETERGENT TREATMENT OR BY ULTRASONICATION

Basement membranes were prepared as described in the text and were analysed for the marker substances below as described under Materials and Methods. DNA and RNA values are given as μg/mg protein. Enzyme specific activities are $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein. Phospholipid values are given as μg atoms P/mg protein; n.f. indicates not found. The results are the means of at least four separate experiments and the standard deviations are shown.

Marker substance	Detergent-treated membrane	Ultrasonicated membrane
DNA	8.5 ± 6.0	10.5 ± 4.2
RNA	4.5 ± 2.2	4.1 ± 2.3
Alkaline phosphatase	0.003 ± 0.001	0.024 ± 0.009
Acid phosphatase	n.f.	n.f.
Glucose-6-phosphatase	n.f.	n.f.
Phospholipid	0.047 ± 0.007	0.041 ± 0.012

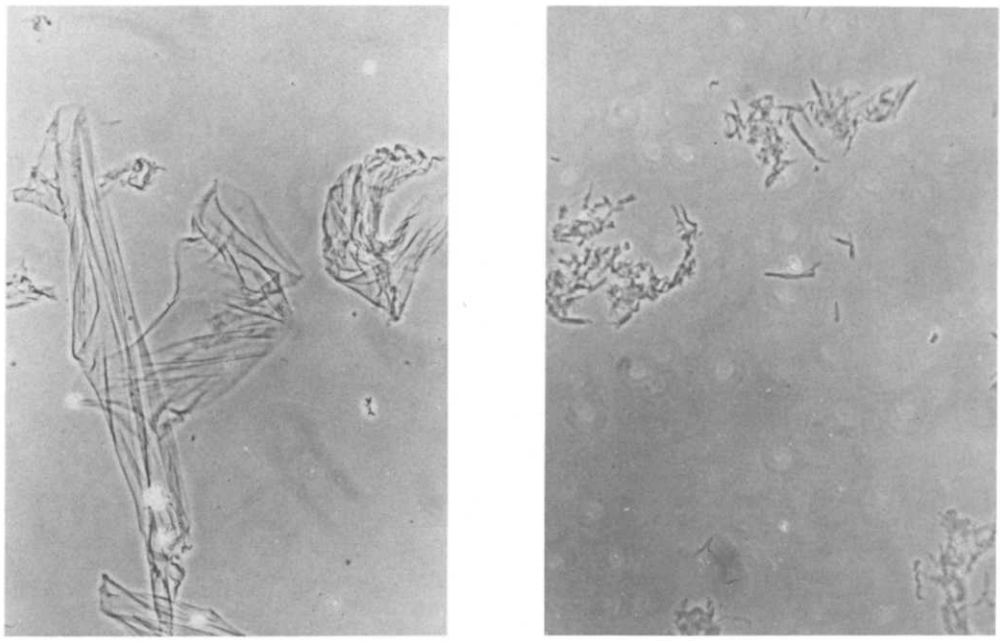


Fig. 1. Phase contrast micrographs of renal basement membranes prepared (a) using detergent and (b) using ultrasonication as described in the text. (X950).

Comparison of the ultrastructures of the basement membranes

Electron microscopic examination of thin sections of basement membrane prepared using detergent from glomeruli and tubules (Figs. 2a and 2b) showed the membranes to possess a felt-like structure commonly described for renal basement membranes when viewed in whole tissue sections [16]. The membranes were not frayed although sonicated membranes show this kind of damage [17]. The glomerular membranes were single membranes 85 nm wide and fine branched filaments could be seen associated with one face of the membrane. The tubular membranes consisted of two coplanar sheets with widths of 50 and 100 nm; the sheets were separated by an electron-lucent space which contained collagen fibres in some regions.

When detergent-treated bulk membranes were examined, no recognisable cellular elements could be seen but collagen fibres were present (Fig. 2c). Similar contamination was observed in bulk membrane preparations obtained using sonication (Fig. 2d). Collagen contamination of glomerular basement membrane preparations has not been widely reported to occur and it seemed likely that separation of the glomeruli before the preparation of the membranes might be the reason for this. Careful examination of membrane samples from isolated glomeruli showed some contamination but it seemed less obvious than that observed in bulk membrane preparations.

It is not always easy to observe collagen fibres in thin sections, particularly if the fibres are dispersed. Accordingly, samples were also examined by negative staining and this technique revealed that dispersed collagen fibres were a more extensive contaminant of isolated glomerular and tubular membranes than was apparent from thin sections. Isolated tubular membranes showed dispersed col-

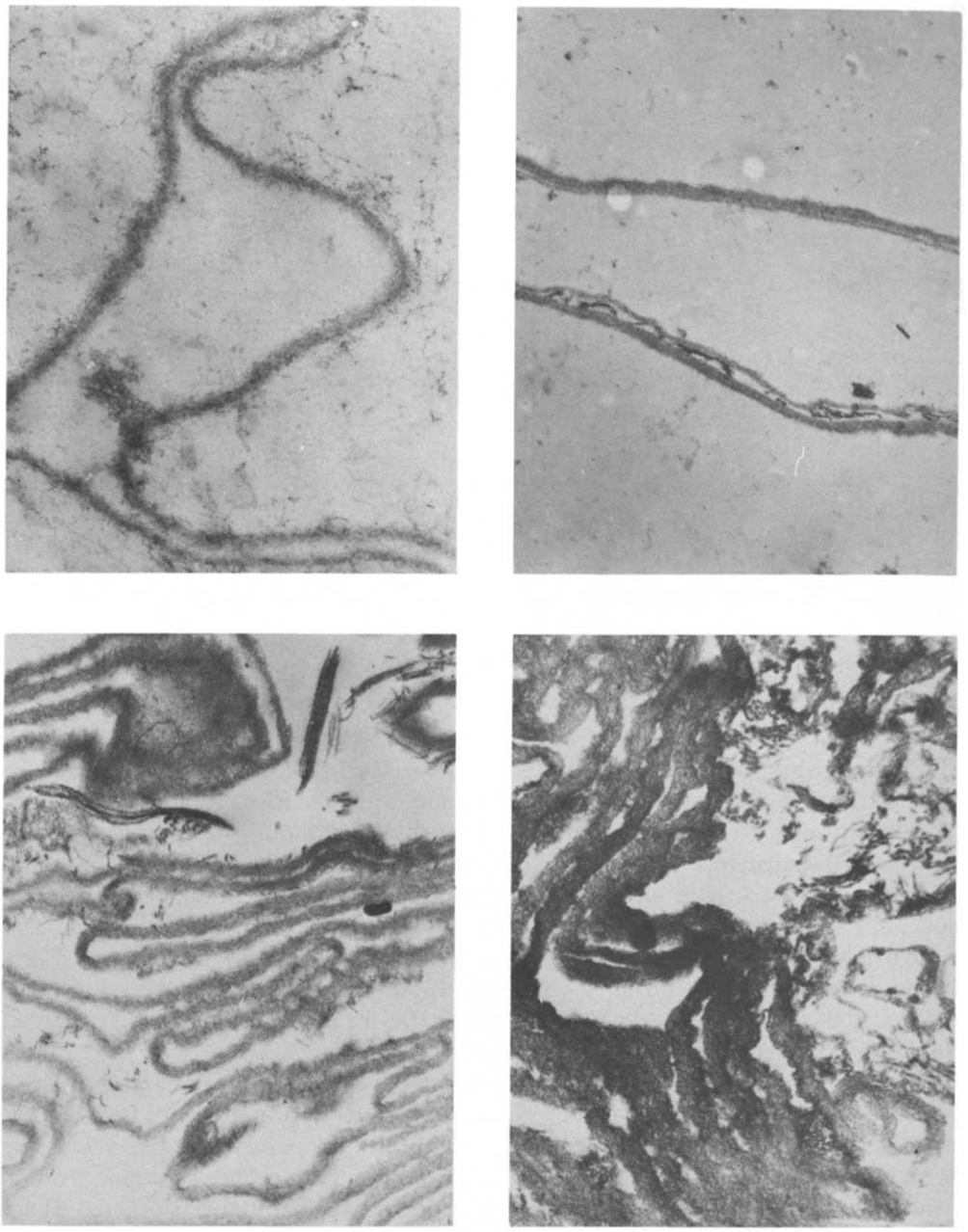
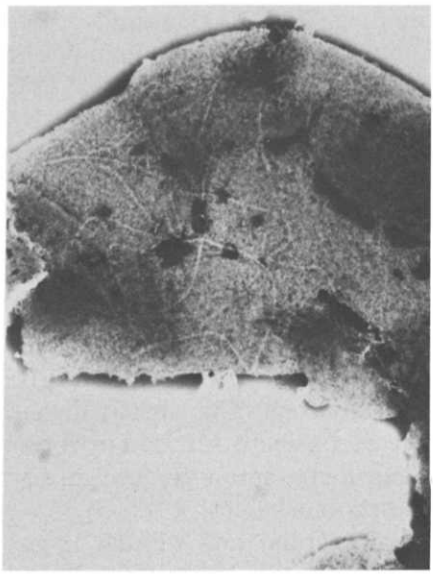


Fig. 2. Electron micrographs of basement membranes isolated by detergent treatment or by ultrasonication. (a) Detergent-treated glomerular membrane (X23 600). (b) Detergent-treated tubular membrane (X 10 300). (c) Detergent-treated bulk membrane showing collagen contamination (X15 500). (d) Sonicated bulk membrane showing collagen contamination (X15 500).

lagen fibres apparently adhering to the membrane surface (Figs. 3a and 3b) regardless of the method of preparation and glomerular membrane was similarly contaminated (Fig. 3c).



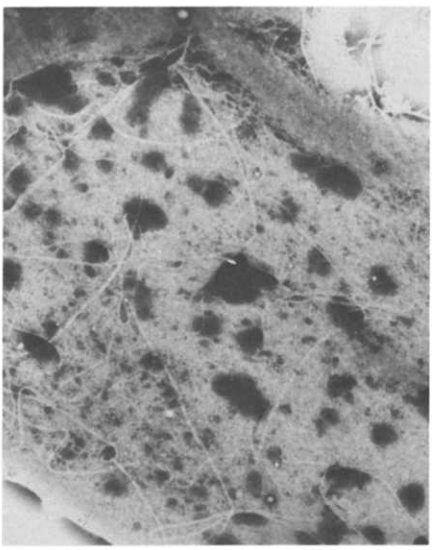


Fig. 3. Negatively stained basement membrane prepared from separated tubules and glomeruli using either ultrasonication or detergent. (a) Tubular basement membrane fragment from a sonicated preparation, collagen fibres are apparent on the membrane surface (X22 500). (b) Tubular basement membrane fragment from a detergent preparation, collagen fibres are again seen (X24 000). (c) Glomerular basement membrane fragment from a sonicated preparation showing clusters of collagen fibres (X9 400).

It is clear from these results that collagen fibres survive both sonication and detergent treatment and that prior separation of the glomeruli and tubules does not eliminate the contamination although it may reduce it. Since all previous authors have used thin section microscopy rather than negative staining in assessing the purity of their preparations, the contamination has probably

remained unrecognised. It should be noted here that Kefalides has argued that collagen is a component of basement membrane [18] on the basis of studies in which collagen was isolated from basement membrane preparations [19]. However, the yields of collagen from glomerular membranes were low (9% by weight) and it is conceivable that this may have been derived from unrecognised contamination. This view has recently been expressed by Sato and Spiro [20] deduced from studies of the composition of glomerular basement membrane.

Chemical analyses

The amino acid and carbohydrate compositions of bulk basement membrane, prepared using detergent or sonication, are compared in Tables II and III. The values recorded were similar for the two preparations. The slightly higher levels of hydroxyproline, hydroxylysine, glucose and galactose found in detergent-treated samples could indicate a higher collagen contamination but no significant differences were noted in the glycine and proline values.

Comparison of the amino acid contents of glomerular and tubular membranes (Table II) showed no significant differences except possibly for lysine

TABLE II

AMINO ACID COMPOSITIONS OF BASEMENT MEMBRANE FROM RABBIT KIDNEY CORTEX

Bulk basement membrane (i.e. tubular plus glomerular) was isolated from cortex using either ultrasonication or treatment with *N*-lauroyl sarcosine. Tubules and glomeruli were separated from cortex using density gradient centrifugation as described under Methods and the basement membranes were isolated using *N*-lauroyl sarcosine. Amino acid analysis was carried out using hydrolysates prepared at 105°C under vacuum in constant boiling HCl for 24 h and the values reported were corrected for losses during hydrolysis. The results are given as residues per 1000 and show standard deviations. The results for bulk membranes are taken from four experiments and for tubular and glomerular membranes from three experiments. Recovery of amino acids was 86.5 ± 4.5% of the dry weight of the membrane samples.

	Bulk membrane (detergent treated)	Bulk membrane (ultra-sonicated)	Tubular membrane (detergent treated)	Glomerular membrane (detergent treated)	Glomerular membrane (Sachot et al. [21])
Hydroxyproline	71.1 ± 9	59.5 ± 6	82.0 ± 6	70.5 ± 10	39
Proline	74.5 ± 3	72.5 ± 5	72.8 ± 2	71.2 ± 5	65
Hydroxylysine	27.3 ± 4	18.8 ± 3	20.6 ± 1	19.9 ± 2	19
Lysine	24.8 ± 4	28.5 ± 6	22.4 ± 3	36.6 ± 6	36
Histidine	18.4 ± 2	11.7 ± 1	12.1 ± 2	15.0 ± 2	19
Arginine	48.5 ± 2	48.0 ± 1	39.8 ± 1	44.0 ± 5	57
Threonine	36.7 ± 2	43.0 ± 6	35.5 ± 4	31.9 ± 2	51
Serine	43.0 ± 2	49.3 ± 3	44.6 ± 3	42.8 ± 1	61
Aspartic acid	70.7 ± 3	69.8 ± 3	67.6 ± 5	79.6 ± 17	79
Glutamic acid	93.8 ± 4	98.0 ± 3	96.9 ± 2	93.6 ± 2	105
Glycine	217.7 ± 15	211.7 ± 16	256.8 ± 10	233.8 ± 7	192
Alanine	75.4 ± 6	70.0 ± 3	62.3 ± 2	65.8 ± 3	72
Valine	38.3 ± 4	43,5 ± 3	35.3 ± 2	43.4 ± 4	38
Isoleucine	28.4 ± 2	35.8 ± 2	29.4 ± 2	28.1 ± 2	33
Leucine	59.3 ± 3	64.1 ± 3	60.4 ± 3	60.0 ± 5	76
Tyrosine	19.5 ± 1	18.5 ± 1	12.9 ± 2	12.2 ± 2	14
Phenylalanine	31.3 ± 2	28.1 ± 2	29.4 ± 2	25.2 ± 1	28
Methionine	7.7 ± 1	9.7 ± 1	9.6 ± 1	9.1 ± 2	2
Half-cystine	15.5 ± 2	14.4 ± 2	11.4 ± 1	12.2 ± 1	15

TABLE III

THE CARBOHYDRATE COMPOSITION OF BASEMENT MEMBRANES PREPARED USING DETERGENTS OR ULTRASONICATION

Membranes were isolated as described in the text and analysed for their carbohydrate content by gas-liquid chromatography. The results are the means from at least three separate isolates, each analysis being conducted in duplicate. The recoveries of carbohydrate by weight were 9.0 and 8.3%, respectively, for the detergent-treated and ultrasonicated membranes. Results are expressed in nmol/mg protein.

Carbohydrate	Detergent-treated membranes	Ultrasonicated membranes	Rabbit glomerular membrane [21]
Fucose	20.9 ± 1.2	17.6 ± 1.7	28.1
Mannose	42.8 ± 1.8	40.3 ± 6.3	58.8
Galactose	176.9 ± 12.1	153.8 ± 10.4	132.3
Glucose	145.8 ± 11.7	129.4 ± 12.3	139.4
N-Acetylgalactosamine	14.8 ± 3.2	16.2 ± 4.8	12.2
N-Acetylglucosamine	79.5 ± 4.7	84.2 ± 6.2	60.8

and glycine. The results for the separated membranes were similar to those obtained for bulk membranes but lower glycine values were recorded for the latter samples.

When the amino acid composition of the glomerular membranes was compared with that reported by Sachot et al. [21] for rabbits, the proline, threonine, serine and methionine values were found to differ appreciably but the remaining results were in reasonable agreement. Similarly the carbohydrate compositions were in broad agreement with those reported by Sachot et al. [21] but no separate analysis of composition of glomerular membranes was undertaken in the present study.

Comparison of the bulk membrane preparations by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulphate revealed very similar patterns (Fig. 4) except that two bands (apparent molecular weights of 115 000 and 96 000) were less obvious in the sonicated preparation.

The effects of ultrasonication on detergent-treated membranes and of detergent treatment on sonicated membranes

A further comparison of the bulk membrane isolates was conducted by subjecting the preparations to the alternative purification procedure. Thus membranes prepared with detergent were ultrasonicated and vice versa.

When detergent membranes were ultrasonicated in 1 M NaCl, 15% of the original protein remained soluble after centrifuging the sonicated suspension at 100 000 $\times g_{max}$ for 30 min. A similar degree of extraction was obtained when membranes were stirred in 1 M NaCl overnight. Thus the protein extraction results from the exposure of the membranes to strong salt solution rather than from the effects of ultrasonication. Polyacrylamide gel electrophoresis of the extract demonstrated four or five minor components and a major component with an apparent molecular weight of 85 000 (Fig. 4). This material was presumably derived from those bands, molecular weights 115 000 and 96 000, which were more obvious in the detergent-treated membranes when analysed by electrophoresis. Indeed electrophoresis of the salt-extracted detergent membranes showed that the intensities of these bands had diminished but it is not clear

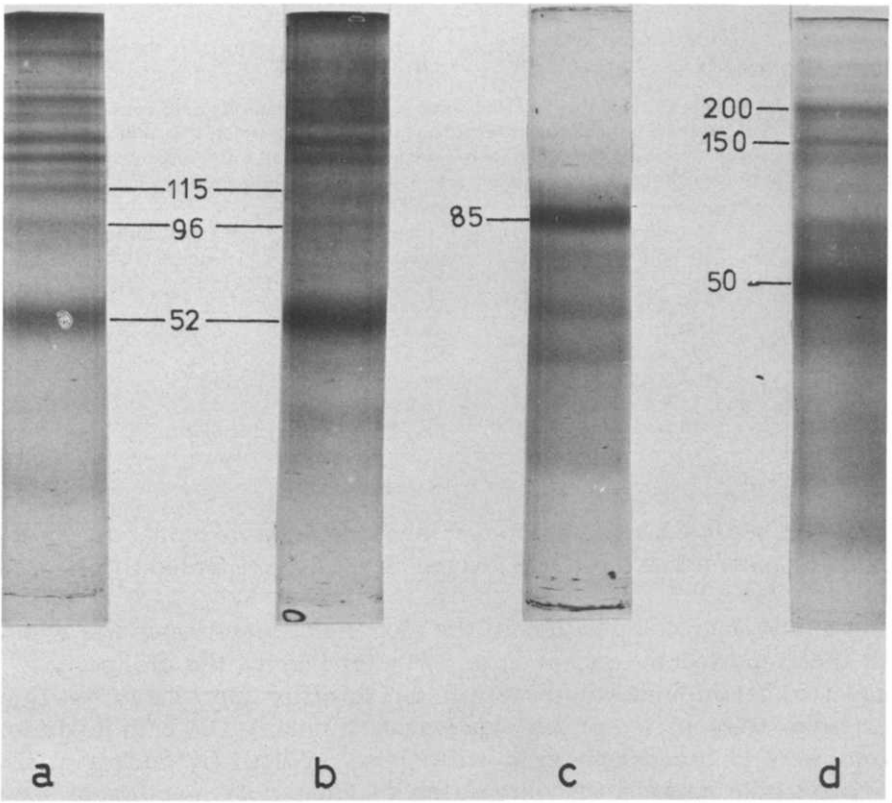


Fig. 4. Polyacrylamide electrophoresis gels of basement membrane proteins run in the presence of sodium dodecyl sulphate and stained for protein with Coomassie Blue. (a) Detergent-treated bulk membrane. (b) Sonicated bulk membrane. (c) Protein extracted from detergent membrane using 1 M NaCl. (d) Protein extracted from sonicated membranes by *N*-lauroyl sarcosine. All the gels were loaded with 100 μg of protein but only gels a and b are directly comparable since the extracted protein, gels c and d, was only 10—15% of the total basement membrane protein. Migration of proteins on the gels varied slightly; the numbers indicate the apparent molecular weights of the bands. Gels of detergent-treated bulk membrane which had been extracted with 1 M NaCl were indistinguishable from gels of sonicated membrane (gel b) and so are not included here.

why the extracted protein should have exhibited a more rapid mobility on electrophoresis.

Extraction of sonicated membrane with *N*-lauroyl sarcosine at a detergent: protein ratio of 2 : 1 (w/w) removed 8—10% of protein. Electrophoresis of this material on polyacrylamide gels revealed multiple bands. The more prominent components had apparent molecular weights of 200 000, 150 000, 100 000, 80 000 and 50 000 (Fig. 4). The levels of alkaline phosphatase in the sonicated membranes were reduced by detergent extraction to those reported for detergent-treated membrane in Table I. Thus at least a part of the protein extracted by detergent can be regarded as contaminant.

Conclusions

The results reported here demonstrate that basement membranes can be isolated from the kidney cortex using the detergent *N*-lauroyl sarcosine to disrupt

cellular structures. On gross analysis, the membranes isolated using detergent are found to differ little from those obtained using the more conventional sonication procedure. An advantage of the detergent method is that there is little physical disruption and the membranes obtained retain their native physical structure to a large degree and so may be used in morphological studies and perhaps in micro-perfusion experiments. There is no reason why this procedure should not be used in isolating basement membrane from other soft tissues and in preliminary experiments we have obtained membranes from tissues such as intestine and lung. While this work was in progress a method was reported in which sodium deoxycholate was used as the disruptive agent [22]. While we made no exhaustive study of this agent we did not find it as satisfactory as *N*-lauroyl sarcosine in preliminary experiments.

Two points arise from our findings that should be stressed. The observation that collagen fibres contaminated both detergent-treated and sonicated preparations and were clearly revealed by negative staining but not by thin section microscopy leads us to suggest that authors should include the use of negative staining when assessing the purity of basement membrane samples. When this is not done, collagen contamination may remain unrecognised and so result in a misinterpretation of findings. The discovery that 1 M NaCl effects a selective extraction of components from basement membrane isolated using detergents indicates that salt solutions at high concentrations may remove actual components from the membranes. This possibility needs to be examined more closely since sonication in 1 M NaCl has been widely used as an isolation procedure. It is equally possible that detergent treatment removes component membrane proteins, but the finding that lauroyl sarcosine extracts a variety of proteins, including alkaline phosphatase, from sonicated membranes suggests that a random array of contaminating proteins is extracted by this agent.

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