The Characterization of Rat Kidney Plasma Membranes Prepared by Zonal Centrifugation

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(Received 19 May 1972)

1. Plasma membranes were isolated from a 10000g-min pellet prepared from a renal cortical homogenate in 20mM-NaHCO₃ by isopycnic centrifugation in a linear sucrose gradient in an 'A'-type zonal rotor. 2. The preparation was characterized by electron microscopy, and alkaline phosphatase, 5'-nucleotidase, L-leucine β -naphthylamidase and L-leucine *p*-nitroanilidase activities were found to be selectively associated with the renal plasma membrane. 3. The preparation had a high degree of purity, as indicated by the presence of low activities of marker enzymes associated with subcellular organelles. A preliminary chemical analysis indicated that the chemical composition resembled that of plasma membranes of other tissues. 4. Plasma membranes were also prepared from tubular fragments and their enzyme contents were found to be similar to those of plasma membranes prepared from cortical homogenates. 5. L-Leucine β -naphthylamidase, L-leucine *p*-nitroanilidase and 5'-nucleotidase were not enriched to the same extent as alkaline phosphatase in the preparation of plasma membranes from tubular fragments. A possible explanation for this finding is discussed.

Renal plasma membranes were first prepared (Coleman & Finean, 1966) from guinea-pig tissue, by using the method developed for the preparation of plasma membranes from liver by Neville (1960) and subsequently modified by Emmelot *et al.* (1964). In this procedure the kidney was homogenized in hypo-osmotic 1mM-NaHCO₃ and the plasma membranes were isolated as large sheets from a nuclear fraction by density-gradient centrifugation. A similar procedure was used by Manitiuss *et al.* (1968) to prepare plasma membranes from rat kidney. Surface-membrane fragments have also been isolated from rat kidney under iso-osmotic conditions (Fitzpatrick *et al.*, 1969; Kinne & Kinne-Saffran, 1969), whereas Thuneberg & Rostgaard (1968) and Berger & Sacktor (1970) have isolated intact brush-border fragments from rabbit kidney cortices homogenized in hyperosmotic 0.5M-sucrose. Wilfong & Neville (1970) obtained a higher yield of kidney brush-border membranes from rat kidney homogenates prepared in 20mM-NaHCO₃.

All the above procedures involve differential and density-gradient centrifugation, and the preparative procedure may be simplified and repeated washing steps avoided when a zonal rotor is used. Evans (1970) has used an 'A'-type zonal rotor to prepare mouse liver plasma membranes, and Pfeiffer *et al.* (1968) and Weaver & Boyle (1969) have prepared rat liver plasma membranes in a B XV rotor. Connock *et al.* (1971) have prepared brush-border membranes

from guinea-pig intestine by zonal centrifugation in an 'HS' zonal rotor. Binkley *et al.* (1968), using a B IV-type zonal rotor, attempted to recover plasma-membrane vesicles from a microsomal fraction prepared from a rat kidney homogenate.

The present paper describes the large-scale preparation of plasma membranes from renal cortex in an 'A'-type zonal rotor. Several enzymes known to be associated with renal plasma membranes were enriched in the preparation, whereas only trace amounts of a number of marker enzymes for subcellular organelles were detected. Sufficient purified membrane was obtained to allow a preliminary chemical analysis. Also included is some information on the enzymic characteristics of a plasma-membrane preparation obtained from tubular fragments prepared as described by Taylor *et al.* (1971).

Materials and Methods

Preparative methods

Preparation of cortical homogenates. Male Sprague-Dawley rats (200-250g) were starved overnight and killed by cervical dislocation. The kidneys were removed, decapsulated and the cortices dissected away from the medullae and homogenized at 4°C with a loose-fitting Potter-Elvehjem homogenizer (diameter clearance 0.25 mm).

Preparation of plasma membranes. The development of a preparative procedure is described in the Experimental and Results section.

Preparation of tubular fragments. The preparation

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of tubular fragments from a collagenase digest of rat kidney cortex was performed as described previously (Taylor *et al.*, 1971).

Other methods

Enzyme assays. Alkaline phosphatase (EC 3.1.3.1), L-leucine β -naphthylamidase (EC 3.4.1.1), leucine aminopeptidase (EC 3.4.1.1), 5'-nucleotidase (EC 3.1.3.5), acid phosphatase (EC 3.1.3.2) and *N*-acetyl- β -glucosaminidase activities (EC 3.2.1.30) were assayed as described by Taylor *et al.* (1971). Cytochrome oxidase (EC 1.9.3.-) activity was assayed as described by Leighton *et al.* (1968). Glucose 6-phosphatase (EC 3.1.3.9) activity was assayed by the method of Dallner (1963), the liberated phosphate being determined as described by Allen (1940). Catalase (EC 1.11.1.6) activity was determined by the procedure of Baudhuin *et al.* (1964).

Other assays. Protein was assayed in the final membrane preparation by the method of Lowry *et al.* (1951). The distribution of protein across the gradient was determined by the method of Schuel & Schuel (1967); casein was used as a standard for both methods. The density of sucrose was determined from the refractive index of the fractions by means of an Abbé refractometer (Bellingham and Stanley Ltd., London N.15, U.K.).

Electron microscopy. Membrane pellets were fixed in 3% (v/v) glutaraldehyde (TAAB Laboratories, Reading, Berks., U.K.) in 0.1M-sodium phosphate buffer, pH 7.2, for 4h at room temperature. Pellets were then washed and centrifuged three times in 0.1M-sodium phosphate buffer, and resuspended in 2% (w/v) osmium tetroxide (B.D.H. Ltd., Poole, Dorset, U.K.) in the phosphate buffer and fixed for 1h at room temperature. Pellets were then washed three times in the phosphate buffer and dehydrated through a series of aqueous ethanol solutions and finally embedded in Araldite (Ciba Ltd., Duxford, Cambridge, U.K.). Ultrathin sections were cut with an L.K.B. Ultratome, stained with uranyl acetate and lead citrate (Venables & Coggeshall, 1965) and observed with an A.E.I. EM 6B electron microscope.

Chemical analysis of the plasma-membrane preparation. A freeze-dried plasma membrane was prepared as shown in Scheme 1. The total protein content of the membrane was measured as described above. Lipids were extracted by the method of Hanson & Olley (1963) and the total cholesterol content of the extract was determined by the method of Clark *et al.* (1968). The total phospholipid content of the extract was determined by digesting a sample (1 ml) with 70% (w/v) HClO_4 and measuring the released phosphate by the method of Chalvardjian & Rudnicki (1970). The assumption was made that 25 μg of phospholipid contained 1 μg of phosphorus (Kopaczky *et al.*, 1966). Individual phospholipids were separated

by t.l.c. on silica gel F254 (0.2mm thick) on aluminium foil (E. Merck, Darmstadt, Germany) in a solvent containing chloroform, methanol, acetic acid and water (28:12:1:1, by vol.). The component phospholipids were identified by comparison with standard phospholipids under u.v. light. The individual phospholipids were eluted from the plates into chloroform and the amount of each phospholipid present was determined as described above.

The freeze-dried plasma-membrane preparation (1.0mg) was hydrolysed with 50mM- H_2SO_4 (0.5ml) at 80°C for 1h and the sialic acid content determined by the method of Aminoff (1961); *N*-acetylneuraminic acid was used as a standard.

The plasma-membrane preparation (3.0mg) was hydrolysed with 6M-HCl (1.0ml) at 100°C for 4h in a sealed tube. Total hexosamine content was then determined by the method of Gatt & Berman (1966); an equivalent mixture of glucosamine-HCl and galactosamine-HCl was used as a standard. Neutral sugars were determined by the anthrone procedure (Roe, 1955) with glucose as a standard. Nucleic acids were extracted from the plasma membranes with hot 5% (w/v) trichloroacetic acid and the DNA and RNA contents were determined by the methods of Schneider (1956).

Experimental and Results

Rat kidney cortex was separated from the medulla as a routine in all the experiments described in this paper. This procedure was adopted to minimize the number of different cell types present in the initial homogenate. The procedure developed for the isolation of plasma membranes from rat kidney cortex involved the preparation of a 10000g-min fraction (crude nuclear fraction) from the tissue homogenate, by differential centrifugation, followed by zonal fractionation.

Preparation of 10000g-min fractions

To compare the utility of the three homogenization media that have been used in other laboratories in the preparation of kidney plasma membranes, 10000g-min fractions were prepared from cortices (1 cortex/15ml of the medium) homogenized in either 0.25M-sucrose, pH 7.4, 1mM- NaHCO_3 , pH 7.4, or 20mM- NaHCO_3 , pH 8.1. Homogenates were prepared with a Potter-Elvehjem homogenizer (diameter clearance, 0.25mm), filtered through two layers of muslin, and then centrifuged at 1000g for 10min at 4°C. The 10000g-min fractions were assayed for a number of enzyme activities, the recoveries of which are presented in Table 1. About 40% of the initial activities of alkaline phosphatase, 5'-nucleotidase and L-leucine β -naphthylamidase was recovered in the 10000g-min fraction when 20mM- NaHCO_3 was used

Scheme 1. Preparation of plasma-membrane fragments from rat kidney cortex

Table 1. *Percentage of the total enzyme activities recovered in the 10000g-min pellets obtained from cortical homogenates prepared in different media*

Three homogenization media were used: 0.25M-sucrose, pH7.4; 1mM-NaHCO₃, pH7.4; and 20mM-NaHCO₃, pH8.1. The results shown are of a typical experiment, and are the total activities recovered in the 10000g-min fractions expressed as percentages of the total activities in the homogenate. The total activities recovered for all the enzymes assayed were in the range 90–110% of the total activity of the initial homogenate.

Enzyme	Enzyme activity recovered (%) in the 10000g-min pellets after homogenization in:		
	0.25M-sucrose	1mM-NaHCO ₃	20mM-NaHCO ₃
Alkaline phosphatase	13.4	18.0	40.2
5'-Nucleotidase	14.0	17.5	38.8
L-Leucine β -naphthylamidase	14.5	17.8	42.0
Cytochrome oxidase	41.6	27.7	35.1
Acid phosphatase	32.8	6.3	13.0
N-Acetyl- β -glucosaminidase	48.0	5.0	10.5
Catalase	27.8	5.0	5.3
Glucose 6-phosphatase	20.8	6.6	8.2

as the homogenizing medium. Lower activities (20%) of these enzymes were recovered when the homogenization was performed in 1mM-NaHCO₃. A greater degree of contamination of the 10000g-min fraction by lysosomal and peroxisomal enzymes was found when 0.25M-sucrose was used as the homogenization medium. The percentage recovery of cytochrome oxidase activity indicated that there was equal contamination of the 10000g-min fractions prepared in the three homogenization media. The greatest contamination by intracellular membranes, as measured by the recovery of glucose 6-phosphatase activity, was in the 10000g-min fraction prepared in 0.25M-sucrose. As the best recoveries of the marker enzymes for plasma membranes were found when 20mM-NaHCO₃ was used as the homogenization medium, this was used for the subsequent large-scale preparation of plasma membranes.

Separation of plasma membranes from a 10000g-min fraction

(a) *Rate zonal separation.* Details of the preparation of the 10000g-min fraction from homogenates prepared in 20mM-NaHCO₃ and the conditions used in the rate-zonal procedure are given in Scheme 1. After centrifugation in an 'A'-type zonal rotor for 1½h, fractions (35ml) were collected and the distribution of protein and several enzyme activities across the gradient are shown in Fig. 1. Three protein peaks are present. Peak 1 at the light end of the gradient contained small vesicles, membrane fragments and soluble protein. Acid phosphatase and catalase activities were detected in this peak, which also contained some alkaline phosphatase activity. A second protein peak (peak 2), which occurs at 30–40% (w/w)

sucrose, contained mainly mitochondria, although some sheets of plasma membrane were present. The overlap of cytochrome oxidase and alkaline phosphatase activities indicates that the separation of mitochondria from plasma membranes was only partially complete. Alkaline phosphatase, 5'-nucleotidase and L-leucine β -naphthylamidase activities had identical distributions across the gradient and for simplicity only alkaline phosphatase activity has been included in Fig. 1. No enzymic activity was associated with the third protein peak found at the heavy end of the gradient.

The specific activities of a number of marker enzymes in a plasma-membrane preparation isolated by rate zonal centrifugation are shown in Table 2. The specific activity of the plasma-membrane marker enzymes increased five- to six-fold compared with their activity in the cortical homogenate. Some 10% of the cortical activity of these enzymes was recovered in the membrane preparation. The specific activities of acid phosphatase, N-acetyl- β -glucosaminidase and cytochrome oxidase were unaltered, whereas the specific activities of both glucose 6-phosphatase and catalase decreased relative to the cortical homogenate.

(b) *Isopycnic zonal centrifugation.* Extension of the run time (see Scheme 1) to 18h resulted in a redistribution of the protein across the gradient (Fig. 1). A major protein peak (6) was found at the heavy end of the gradient, which corresponded with the peak of cytochrome oxidase activity, indicating that the mitochondria had moved further into the gradient than in the rate zonal separation. The plasma-membrane sheets were associated with protein peak 5 and all the activities of the marker enzymes for plasma membranes measured were elevated in these frac-

Fig. 1. Distribution of some enzyme activities and protein in fractions obtained from a 10000g-min fraction prepared from cortical homogenates

For details of the preparative procedure and zonal centrifugation see Scheme 1. Enzyme profiles are expressed as relative activity (arbitrary units). Enzymes and protein were assayed as described in the Materials and Methods section. (a), (b) and (c) are profiles obtained from a typical rate separation after 1½ h. (d), (e) and (f) are profiles obtained from a typical isopycnic separation after 18 h. The continuous line shows the sucrose gradient (% w/w); ●, protein (mg/ml); ▲, alkaline phosphatase; △, cytochrome oxidase; ■, catalase; ○, acid phosphatase.

Table 2. *Enzyme activities in plasma membranes isolated by rate zonal centrifugation*

Details of the centrifugation procedure are given in Scheme 1. The results shown are of a typical preparation. Plasma membranes were prepared from the three fractions with the highest alkaline phosphatase activity (see Fig. 1*b*). Specific activity is expressed as μmol of substrate hydrolysed/h per mg of protein.

Enzyme	Specific activity		Relative specific activity $\left(\frac{b}{a}\right)$
	Homogenate (a)	Plasma membranes (b)	
Alkaline phosphatase	0.2	1.2	6.0
5'-Nucleotidase	5.5	30.2	5.5
L-Leucine β -naphthylamidase	2.1	10.8	5.2
Cytochrome oxidase	7.8	8.0	1.0
Acid phosphatase	0.4	0.4	1.0
Catalase	440.0	15.0	0.03
Glucose 6-phosphatase	0.8	0.3	0.38

Table 3. *Enzyme activities in plasma membranes isolated by isopycnic zonal centrifugation*

Details of the centrifugation procedure are given in Scheme 1. The results shown are of a typical preparation, with 12 kidneys. Plasma membranes were prepared from the four fractions with the highest alkaline phosphatase activity (Fig. 1*e*). (a) Specific activities, expressed as μmol of substrate hydrolysed/h per mg of protein. (b) Total activities, expressed as $\mu\text{mol/h}$. The percentage of the total activity of the original homogenate recovered in the plasma membranes is given in parentheses. Relative specific activity is the specific activity of the plasma membranes divided by that of the homogenate. Recovery (%) represents the percentage of the total activity of the original homogenate recovered in all fractions. Abbreviation: N.D., not determined.

Enzyme		Homogenate	Plasma membranes	Relative specific activity	Recovery (%)
Alkaline phosphatase	(a)	0.20	1.98	9.90	
	(b)	120.0	24.7 (20.6)		101
5'-Nucleotidase	(a)	6.05	56.28	9.30	
	(b)	3630	704 (19.4)		N.D.
L-Leucine β -naphthylamidase	(a)	2.34	20.71	8.85	
	(b)	1404	259 (18.4)		N.D.
L-Leucine <i>p</i> -nitroanilidase	(a)	1.20	10.31	8.59	
	(b)	720	129 (17.9)		N.D.
Acid phosphatase	(a)	0.39	0.28	0.72	
	(b)	234.0	3.5 (1.5)		104
<i>N</i> -Acetyl- β -glucosaminidase	(a)	0.81	0.41	0.51	
	(b)	486.0	5.1 (1.0)		109
Cytochrome oxidase	(a)	8.20	2.13	0.26	
	(b)	4920	27 (0.6)		87
Catalase	(a)	420.0	11.0	0.03	
	(b)	252000.0	137 (0.05)		98
Glucose 6-phosphatase	(a)	0.84	0.21	0.25	
	(b)	504.0	2.6 (0.5)		100
Protein (mg)		600.0	12.5 (2.1)		99

tions. Fig. 1 shows that this procedure gave a better separation of mitochondria and plasma membranes than the rate separation.

Characterization of the plasma-membrane preparation

The plasma-membrane fragments separated by isopycnic zonal centrifugation were situated between

38 and 41 % (w/w) sucrose, indicating that they were isopycnic with a density of 1.17–1.18 g/ml (at 20°C).

(a) *Enzyme activity.* The specific and total activities of a number of enzymes in a plasma-membrane preparation prepared by isopycnic zonal centrifugation as described in Scheme 1 are given in Table 3. There was a fourfold decrease in the cytochrome oxidase

activity present in this preparation compared with the rate zonal preparation (Table 2). An eight-to-tenfold increase in the relative specific activities of alkaline phosphatase, 5'-nucleotidase, L-leucine β -

Table 4. *Chemical analysis of rat kidney plasma-membrane preparation*

Results are expressed as % (by weight) of the freeze-dried membrane and $\mu\text{g}/\text{mg}$ of membrane protein. Nucleic acid is expressed as μg of nucleic acid and phosphorus/mg of protein and as μg of nucleic acid/mg of protein (assuming nucleic acid to be 8% phosphorus). The molecular weight of phospholipid was assumed to be 780, and neutral sugars present were determined with glucose as a standard. The values in parentheses are the phosphorus contents.

	Content	
	[% (by wt.) of membrane]	($\mu\text{g}/\text{mg}$ of protein)
Protein	51.2	
RNA (RNA P)	0.4	7.0 (0.55)
DNA (DNA P)	0.2	5.0 (0.4)
Total carbohydrate	7.4	144
Hexosamine	1.1	21.3
Sialic acid	0.7	13.0
Neutral sugars	5.6	110.0
Total phospholipid	28.4	546.0
Total cholesterol	8.1	158.0
Cholesterol/phospholipid molar ratio = 0.59		

naphthylamidase and L-leucine *p*-nitroanilidase was found and the recovered activities of these enzymes represented 20% of their activity in the cortical homogenate. The low recoveries of marker enzymes indicated that there was only slight contamination of the plasma membranes by lysosomes, peroxisomes and endoplasmic reticulum. No nuclear contamination was detected by electron microscopy and analysis for nucleic acids. The residual acid phosphatase activity present in the preparation may be of lysosomal origin, as it is possible that this enzyme has been reabsorbed by the plasma membranes (Baccino *et al.*, 1971).

(b) *Morphology*. Examination of the membrane preparation in the electron microscope demonstrated that it consisted predominantly of vesicular structures of various sizes and of large sheets of membrane. Structures resembling brush-border fragments with attached microvilli were frequently observed. Mitochondrial structures were rarely encountered and recognizable nuclear material was never seen.

(c) *Chemical composition*. Details of a preliminary chemical analysis of the freeze-dried membrane preparation are given in Table 4. Some 50mg of membrane was obtained from 40 rat kidneys by the isopycnic procedure (Scheme 1). Protein, phospholipid and cholesterol were the major constituents of the membrane, which contained some carbohydrate (7.5%), but 5% of the membrane remained unaccounted for.

The phospholipids present in the plasma-membrane preparation were separated by t.l.c. Of the total phospholipid recovered 45% was phosphatidylcholine, 30% sphingomyelin, 13% phosphatidylethanolamine, and the remaining 12% was phosphatidylserine and

Table 5. *Enzyme activities in plasma membranes isolated by isopycnic zonal centrifugation from tubular fragments*

Enzyme activities of plasma membranes are compared with the activities of the collagenase digest and a homogenate of tubular fragment in 20mM- NaHCO_3 . Results are for a typical preparation. Specific activity is expressed as μmol of substrate hydrolysed/h per mg of protein. Abbreviation: N.D., not determined.

Enzyme	Specific activities			Relative specific activity $\left(\frac{b}{a}\right)$
	Collagenase digest (a)	Tubular fragments	Plasma membranes (b)	
Alkaline phosphatase	0.26	1.09	2.06	8.0
5'-Nucleotidase	3.26	9.31	18.78	5.8
L-Leucine β -naphthylamidase	0.90	1.82	4.21	4.7
L-Leucine <i>p</i> -nitroanilidase	0.44	0.85	1.98	4.5
Cytochrome oxidase	6.30	15.26	3.82	0.61
Acid phosphatase	0.73	1.68	1.35	1.8
N-Acetyl- β -glucosaminidase	0.88	2.25	0.62	0.7
Glucose 6-phosphatase	0.69	1.72	0.7	1.0
Catalase	N.D.			

Table 6. *Effect of collagenase on some renal enzyme activities*

A single kidney cortex was minced with a razor and incubated with stirring in each medium. Each suspension was forced through a no. 1 serum needle (Luer), and after 1 h was homogenized finely in a Potter-Elvehjem homogenizer (diameter clearance, 0.1 mm). The four homogenates were assayed for five enzyme activities and protein as described in the text. Results are of a typical experiment and specific activities are expressed as μmol of substrate hydrolysed/h per mg of protein. Allowance was made for the additional protein present owing to the collagenase.

Cortex homogenized in ... Enzyme	Specific activity			
	0.45 M-Sucrose, pH 7.4	0.9% NaCl, pH 7.4	0.9% NaCl, pH 8.5	0.05% Collagenase in 0.9% NaCl, pH 8.5
Alkaline phosphatase	0.25	0.21	0.26	0.24
5'-Nucleotidase	7.40	7.52	7.48	4.31
L-Leucine β -naphthylamidase	3.32	3.26	2.35	1.29
L-Leucine <i>p</i> -nitroanilidase	1.70	1.71	1.22	0.61
Acid phosphatase	0.44	0.48	0.52	0.68

phosphatidylinositol. All the spots on the t.l.c. plates were accounted for by marker lipids.

The low concentration of nucleic acids present indicated that there was only minimal contamination by ribosomal and nuclear material.

Isolation of plasma membranes from tubular fragments by isopycnic zonal centrifugation

Tubular fragments prepared from a collagenase digest of rat kidney cortex were homogenized in 20 mM-NaHCO₃ and a 10000g-min fraction was prepared. Plasma membranes were then isolated from this fraction by the isopycnic zonal-centrifugation procedure (Scheme 1). The protein and enzyme profiles across the gradient were similar to those shown for the isopycnic separation in Fig. 1. The specific activities of a number of enzymes present in the plasma-membrane preparation are shown in Table 5. Slightly higher specific activities of glucose 6-phosphatase, cytochrome oxidase and acid phosphatase were found (Table 5; cf. Table 3). There was a greater increase in the relative specific activity of alkaline phosphatase in this membrane preparation than of 5'-nucleotidase, L-leucine β -naphthylamidase and L-leucine *p*-nitroanilidase. This finding contrasts with the results shown in Table 3, where these enzymes show approximately equal enrichment, and it was decided to investigate further the effect of collagenase on the enzyme activities associated with the plasma membrane.

Effect of collagenase on plasma-membrane enzyme activity

The results presented in Table 6 indicate that the specific activity of alkaline phosphatase was not significantly affected by the use of collagenase. In

contrast the specific activities of 5'-nucleotidase, β -naphthylamidase and *p*-nitroanilidase were decreased. Conversely, the acid phosphatase activity was elevated. A possible explanation of these results is given in the Discussion section.

Discussion

Although Evans (1970) was able to isolate rat liver plasma membranes by a rate zonal-centrifugation procedure, this type of separation was found to be unsuitable for the preparation of rat kidney plasma membranes because of the difficulty encountered in separating mitochondria from the plasma-membrane fragments. Mitochondrial contamination of the plasma-membrane preparation was considerably decreased when the centrifugation time was extended to allow the mitochondria to approach their isopycnic density. The isopycnic zonal procedure has a number of advantages when compared with the previously published methods for the preparation of plasma membranes from kidney tissue. High yields of purified membranes can be obtained by a simple three-stage procedure, and the use of an 'A'-type zonal rotor enables large quantities of material to be processed and avoids repeated washing steps.

A number of marker enzymes have previously been shown to have high specific activities in rat kidney plasma membranes. The eight-to-ten-fold enrichment of 5'-nucleotidase, L-leucine β -naphthylamidase, L-leucine *p*-nitroanilidase and alkaline phosphatase compares favourably with that obtained for these enzymes in previous preparations of plasma membranes from kidney (Coleman & Finean, 1966; Wilfong & Neville, 1970; Berger & Sacktor, 1970) and we have demonstrated that the disaccharidase maltase is also enriched in rat kidney plasma membranes (Taylor *et al.*, 1972). The results for leucine

aminopeptidase (Tables 3, 5, and 6) strongly suggest by their similarity that the same enzyme is involved in the hydrolysis of both substrates.

Morphological studies of the plasma-membrane fraction demonstrated that the preparation was free of contaminating cytoplasmic organelles. Structures resembling brush-border membranes were present in the preparation and in this respect the preparation was similar to that of Wilfong & Neville (1970).

A sufficient amount (50mg) of membrane was obtained from a single preparation to allow a detailed chemical analysis to be carried out. The cholesterol/phospholipid molar ratio, the classes of phospholipids present and the carbohydrate content are very similar to those reported for hepatic (Touster *et al.*, 1970; Coleman *et al.*, 1967; Dod & Gray, 1968) and intestinal (Eichholz, 1967) plasma membranes. The values obtained for the phospholipid and cholesterol content of our preparation are similar to those published by Glossman & Neville (1971) for a brush-border membrane preparation from rat kidney. These authors found a slightly lower amount of protein in their preparation but significantly less carbohydrate. This latter observation may be explained by the fact that their carbohydrate and protein values were determined in a delipidated membrane.

Recently a large-scale procedure has been devised for the preparation of tubular fragments from a collagenase digest of rat kidney cortex (Taylor *et al.*, 1971). These fragments represent a less heterogeneous starting material than whole kidney cortex, as they are free of glomerular contamination, cell debris and erythrocytes. The isopycnic procedure can be used successfully to prepare plasma membranes from tubular fragments, and the enrichment of alkaline phosphatase was similar to that found in the plasma membranes obtained from whole cortical homogenates. The preparation of plasma membranes from defined regions of the nephron will allow an easier correlation of the enzyme activities of the plasma membranes with the physiological functions of the renal tubules. The use of collagenase as a disaggregating agent led to the interesting finding that the various marker enzymes for the plasma membranes were not enriched to the same extent (Table 5), and lower activities of 5'-nucleotidase, L-leucine β -naphthylamidase and L-leucine *p*-nitroanilidase were obtained in homogenates prepared with collagenase in the medium. This result might be explained by the fact that alkaline phosphatase is located in a different region of the plasma membrane from the other marker enzymes, and evidence for this possibility has been described recently by Thomas & Kinne (1972). These authors found that the aminopeptidase activity of rat renal cortex was associated with the surface of the microvillus matrix, whereas alkaline phosphatase was located in the matrix of the microvilli.

We acknowledge a Science Research Council studentship to D. G. T. We are indebted to Mr. D. Border, Department of Biochemistry, Imperial College, London for providing facilities for electron microscopy. The work was supported by grants from the Medical Research Council, National Kidney Research Fund and the University of London Central Research Fund.

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