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ANALYSIS OF THE PINOCYTIC PROCESS IN RAT KIDNEY

II. BIOCHEMICAL COMPOSITION OF PINOCYTIC VESICLES COMPARED TO BRUSH BORDER MICROVILLI, LYSOSOMES AND BASOLATERAL PLASMA MEMBRANES*

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SUMMARY

Pinocytic vesicles, brush border microvilli, lysosomes and basolateral plasma membranes were isolated from rat kidney cortex and their biochemical composition and membrane turnover compared. Pinocytic vesicles are devoid of marker enzymes of brush border microvilli, such as alkaline phosphatase and 5′-nucleotidase, and of lysosomes, such as acid phosphatase and β-glucuronidase. The protein pattern as revealed by polyacrylamide gel electrophoresis differs for all four membranes. Analysis of the phospholipid composition shows that pinocytic vesicles are rich in the negatively charged phospholipid phosphatidylserine and have a low content of sphingomyelin and phosphatidylethanolamine.

[^{14}C]guanido-arginine, [^{3}H]fucose and *myo*-[^{3}H]inositol were preferentially incorporated into the pinocytic vesicles. Using a double label technique with leucine also, evidence of a more rapid turnover of the pinocytic vesicle membrane proteins was obtained.

The results suggest that pinocytic vesicles are not derived from the brush border microvillous membrane but are independent entities that are newly synthesized during the pinocytic process.

INTRODUCTION

So far, renal pinocytosis has been studied indirectly by following the fate of macromolecules taken up by the cells of the proximal tubule by morphological and biochemical techniques [1–6]. As a result of these studies the following sequence of

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events was postulated for the pinocytic process: adsorption of a macromolecule to the luminal membrane, uptake into pinocytic vesicles, transfer into lysosomes and intralysosomal digestion. However, the properties of the membranes participating in pinocytosis and their interrelation remained speculative.

In the present study a direct approach to these questions was used. The three membranes probably linked to the cellular uptake of macromolecules, luminal brush border microvilli, pinocytic vesicles and lysosomes, were isolated from rat kidney cortex and their composition and membrane turnover investigated. The results obtained provide evidence that pinocytic vesicles are newly synthesized functional units, which do not originate from lateral diffusion of microvillous membrane components. Lysosomes probably originate from the fusion of pinocytic vesicles with Golgi membranes.

MATERIALS AND METHODS

1. Biological material

For all experiments male Wistar rats (180–220 g body weight) were used. After labelling of the pinocytic vesicles with horseradish peroxidase in vivo [7], the animals were killed by cervical dislocation, the kidneys were removed and placed in ice-cold buffer (0.25 M sucrose/0.07 M mannitol/0.01 M Tris · HCl, pH 7.4). The kidney cortex was dissected with a razor blade, minced and gently homogenized in a glass homogenizer with ten times its volume of sucrose buffer by ten strokes with a loose-fitting Teflon pestle (45 rev./min.)

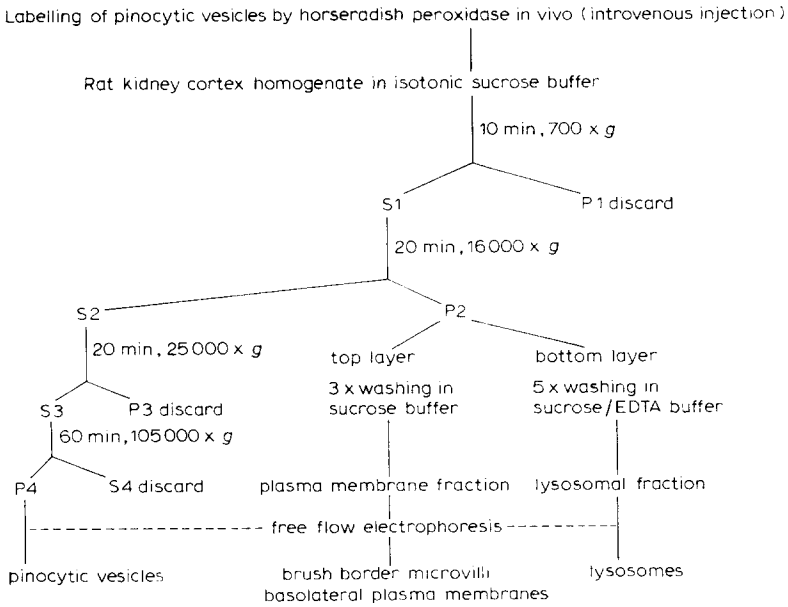
For studies on the incorporation of membrane precursors four animals were injected intraperitoneally with 0.5 ml Ringer solution containing 0.3 mCi L-[6-^{3}H]-fucose (30 Ci/mmol) or 0.3 mCi *myo*-[2-^{3}H]inositol (2.84 Ci/mmol) or 0.03 mCi L-[^{14}C]-guanido-arginine (25.9 Ci/mmol). All radiochemicals were purchased from NEN-Chemicals (G.F.R.). The animals were killed 1, 4 or 20 h after the intraperitoneal injection of the radioactive labelled membrane precursors and the kidney membrane fractions were isolated. For the double-label experiments 0.06 mCi D,L-[1-^{14}C]-leucine (24.3 Ci/mmol) and 0.25 mCi L-[4,5-^{3}H]leucine (40.7 Ci/mmol) were given to each animal. The second label was administrated 7 or 10 days after the ^{14}C injection at the same day time. 4 h later the animals were killed and the membrane fractions were isolated.

2. Isolation of membrane fractions

Brush border microvilli, pinocytic vesicles, lysosomal membranes and basolateral plasma membranes were isolated from the same starting material according to the isolation scheme presented in Table I. The procedure combines slightly modified techniques for the isolation of brush border microvilli, pinocytic vesicles and basolateral plasma membranes which have been previously published by our laboratory [7, 8] with the isolation procedure used by Henning and Heidrich for rat liver lysosomes [9]. After free flow electrophoresis in every fraction protein content and marker enzyme activities were determined. The fractions with the highest specific activities were combined and centrifuged for 150 min at 105 000 $\times g$. Pinocytic vesicles and lysosomes were freeze-thawed prior to centrifugation to remove the intravesicular content and to obtain only isolated membranes. The pellets containing the membrane fragments were used for further analyses.

TABLE I

ISOLATION OF BRUSH BORDER MICROVILLI, PINOCYTIC VESICLES, LYSOSOMES AND BASOLATERAL PLASMA MEMBRANES FROM RAT KIDNEY CORTEX



3. Enzyme and protein determination

The enzyme assays were performed as described previously [7] except that for the determination of acid phosphatase (EC 3.1.3.2) 20 mM glycerophosphate was used as substrate in the presence of 10 mM cysteine chloride and the inorganic phosphate released from the substrate was determined [10]. β-Glucuronidase (EC 3.2.1.31) was determined as described by Just [11] with phenolphthalein-glucuronide as substrate.

The protein content was measured after precipitation of the membranes in 10 % trichloroacetic acid and dissolution in M NaOH according to the method of Lowry et al. [12]. Bovine albumin was used as a standard solution.

4. Lipid analysis

For lipid analysis the membranes were extracted with chloroform/methanol (1 : 1, v/v) according to Dodge et al. [13] as modified by Peter and Wolf [14]. Lipid phosphorus was determined according to Bartlett [15] and Fiske and SubbaRow [10] after conversion of the organic phosphorus into inorganic phosphate by incineration with $Mg(NO_3)_2$ in a platin crucible. The lipids were separated by thin-layer chromatography on silica gel plates (Sil G UV Polygram, Machery and Nagel, Düren, G.F.R.) and the components were measured quantitatively by a photodensitometric analysis (Kontron densitometer). Neutral lipids were separated according to Pfleger [16] and identified with bichromate sulfuric acid. Phospholipids were resolved according to Skipski [17] and identified by the phosphate staining procedure of Dittmer [18] using pure standards as references. Two solvent systems were used: chloroform/

methanol/acetic acid/water (120 : 60 : 18 : 4, by vol.) and (120 : 60 : 18 : 7.5, by vol.). The latter system was used to separate phosphatidylserine from phosphatidylinositol.

5. Determination of sialic acid and hexosamine

Sialic acid and hexosamine content of the membranes was analyzed in pooled membrane fractions containing at least one mg of protein per sample following the method of Warren [19] and Winzler [20] as modified by Glossmann and Neville [21].

6. Polyacrylamide gel electrophoresis

About 100 μg of membrane protein were dissolved in 1 % sodium dodecyl sulfate and 1 % mercaptoethanol at pH 8.3 by incubation for 10 min at 80 °C. Then the samples were centrifuged for 15 min at 15 000$\times g$. The membrane components present in the supernatant were separated in 7.5 % polyacrylamide running gels at pH 8.9 after stacking at pH 6.9 in 2.5 % polyacrylamide stacking gels. The gels and the electrode buffers contained 0.1 % sodium dodecyl sulfate and were prepared according to Maurer [22]. Bromphenolblue was used as an indicator dye. The separation was performed at room temperature with 2 mA per gel for 2 h. The gels were stained afterwards with 0.1 % Coomassie blue [23] and scanned at 550 nm in a Leitz-Unicam spectrophotometer equipped with a gel-scanning unit. Glycoproteins were stained according to Zacharius [24] as modified by Glossmann and Neville [21].

7. Determination of radioactivity

In single label experiments the radioactivity incorporated into the membranes was counted after precipitation of the membranes with 5 % cold trichloroacetic acid, Millipore-filtration and an additional washing with 3$\times$ 5 ml 5 % cold trichloroacetic acid, in 10 ml Insta Gel (Packard) with the Packard scintillation counter 3380.

In double label experiments the isolated membrane fractions were treated according to Meldolesi [25]. They were precipitated three times with 5 % cold trichloroacetic acid, incubated in 5 % trichloroacetic acid for 90 min at 90 °C, then extracted twice in chloroform/methanol/ether (1 : 1 : 1, by vol.) and finally dissolved in 0.5 M NaOH and counted as described above.

Radioactivity in polyacrylamide gels was measured after slicing the gels into 30 slices. The slices were dissolved in 1 ml H_2O_2 at 60 °C overnight and counted using Insta Gel scintillation fluid. Usually two slices had to be combined in one scintillation vessel due to the low amount of radioactivity present in the gels.

RESULTS

Origin of the membrane fractions

In this study a procedure was developed to isolate brush border microvilli, pinocytic vesicles, lysosomes and basolateral plasma membranes simultaneously from the same starting material. This should allow direct comparison of the membranes because they are derived from cells in an identical functional state and, this is important for turnover studies, are synthesized from the same intracellular pool of precursors. It is reasonable to assume that this pool represents the cells of the proximal tubule since kidney cortex is used as starting material which contains about 4 times as many cells of the proximal as of the distal tubule. In addition lysosomes are more

TABLE II

ENZYME ACTIVITIES IN BRUSH BORDER MICROVILLI (I), PINOCYTIC VESICLES (II), LYSOSOMAL MEMBRANES (III) AND BASOLATERAL PLASMA MEMBRANES (IV) ISOLATED FROM RAT KIDNEY CORTEX

The activities are expressed as specific activity in μmol/h per mg protein with the exception of β-glucuronidase, whose activity is given in munits/mg protein. The enrichment factor is defined as the ratio of the specific activity found in the fraction compared to the specific activity of the enzymes measured in the starting material, the rat kidney cortex homogenate. The number of experiments is given in brackets. n.d. = not determined.

Enzyme	I			II			III			IV		
	Specific activity		Enrichment factor	Specific activity		Enrichment factor	Specific activity		Enrichment factor	Specific activity		Enrichment factor
Alkaline phosphatase	233.45	(6)	14.50	11.07	(8)	0.58	13.85	(3)	0.86	2.09	(4)	1.33
Aminopeptidase	12.50	(3)	3.82	0.85	(3)	0.26	n.d.	—	—	n.d.	—	—
5'-Nucleotidase	25.00	(3)	3.13	6.48	(3)	0.81	5.56	(4)	0.80	0.22	(2)	0.03
Acid phosphatase	0.096	(4)	0.67	0.068	(5)	0.48	1.93	(11)	12.90	0.07	(4)	0.5
β-Glucuronidase	n.d.	—	—	0	(3)	0	63.80	(2)	3.50	n.d.	—	—
$(Na^+ + K^+)$-ATPase	1.01	(5)	0.30	12.14	(4)	5.70	0	(4)	—	53.87	(4)	16.0
Succinate dehydrogenase	0.012	(4)	0.10	0.003	(5)	0.07	0.029	(4)	0.46	0.017	(4)	0.14
Glucose-6-phosphatase	0.127	(6)	1.20	0.192	(6)	1.81	0.045	(4)	0.30	0.07	(4)	0.7

abundant in the proximal tubule [26]. Furthermore, the basolateral plasma membranes contain an adenylate cyclase which is highly sensitive to parathyroid hormone which acts predominantly on the proximal tubule [27].

Purity of the membrane fractions

As shown in Table II the combination of differential centrifugation and free flow electrophoresis leads to fractions which show a low cross contamination and in which in addition the contamination with other cellular membrane systems is reduced. In all fractions the specific activity of succinate dehydrogenase is decreased compared to the starting material; the glucose-6-phosphatase which is localized in the endoplasmic reticulum is only increased slightly, if at all.

Very little overlapping is observed between brush border membranes, lysosomal membranes and basolateral membranes. The fraction containing the pinocytic vesicles is also not contaminated by brush border microvilli and lysosomes but the enrichment of $(Na^+ + K^+)$-ATPase indicates some contamination of this fraction with basolateral plasma membranes. Therefore in this study data on this membrane are given in addition to the results concerning the three membranes which are involved directly in the pinocytic process.

Protein and glycoprotein pattern of brush border microvilli, pinocytic vesicles, lysosomal membranes and basolateral plasma membranes

As can already be anticipated from the different enzyme activities found in the various membranes, the protein pattern as revealed by polyacrylamide gel electrophoresis differs from membrane to membrane (Fig. 1). In the microvilli and the basolateral plasma membranes proteins ranging from $> 200\,000$ to $< 20\,000$ in their apparent molecular weight and a high number of glycoproteins can be detected without any obvious predominance of one protein species. The pinocytic vesicles show a simpler protein pattern, strong staining is observed in a region of the gel corresponding to $> 300\,000$, approx. 112 000–105 000 and approx. 23 000–20 000 apparent molecular weight. These proteins seem not to be present in microvilli nor in basolateral plasma membranes and thus can be considered as constituents of the pinocytic vesicles, whereas some faint bands are observed both in pinocytic vesicles and in basolateral plasma membranes, probably due to some cross contamination. The proteins of approx. 112 000 and approx. 105 000 apparent molecular weight show a strong glycoprotein staining, indicating that part of the major proteins of the pinocytic vesicles are glycoproteins. Their individual carbohydrate content is probably higher than the value of 20 % found for brush border glycoproteins such as alkaline phosphatase [28], because the carbohydrate content of the pinocytic membrane (Table III) is about twice as high as that of the brush border microvilli, while the number of glycoproteins in the pinocytic vesicles is smaller than in the brush border microvilli.

The lysosomal membrane is characterized by proteins with a low molecular weight. The major bands correspond to a molecular weight of approx. 135 000, approx. 128 000 and approx. 35 000.

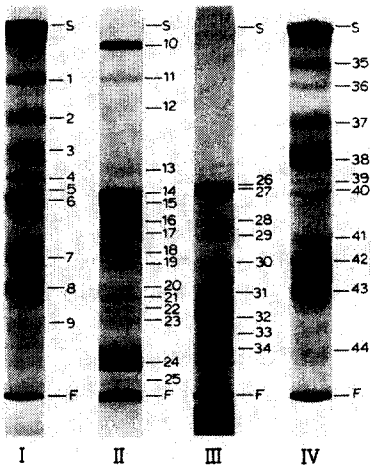


Fig. 1. Protein and glycoprotein pattern of brush border microvilli (I), pinocytic vesicles (II), lysosomal membranes (III) and basolateral plasma membranes (IV) as revealed by polyacrylamide gel electrophoresis. S = start, F = front, the apparent molecular weight (M_r) is derived from an equilibration curve using DNA polymerase, trypsin inhibitor and bovine serum albumin as standards (Boehringer, Mannheim). The values represent means of two or three determinations. n.d., not determined; +, glycoprotein positive; ! +, glycoprotein strongly positive.

	Band No.	M_r			Band No.	M_r	
I	1	>300000	+	II	23	34000	
	2	243000			24	23–21000	
	3	176000			25	19000	
	4	133000	!				
	5	120000		III	26	135000	n.d.
	6	107000			27	128000	n.d.
	7	60200	+		28	91600	n.d.
	8	37000	+ +		29	78500	n.d.
	9	28000	+		30	59500	n.d.
					31	47000	n.d.
II	10	>300000			32	34900	n.d.
	11	>300000			33	29500	n.d.
	12	241000			34	25000	n.d.
	13	142000					
	14	112000	+ +	IV	35	>300000	+
	15	105000	+ !		36	>300000	
	16	88500			37	243000	
	17	76700			38	171000	+
	18	64400			39	141000	+
	19	57500			40	120000	
	20	46700			41	77500	
	21	42000			42	59000	+ +
	22	39000			43	45000	
					44	25400	

TABLE III

BIOCHEMICAL COMPOSITION OF BRUSH BORDER MICROVILLI (I), PINOCYTIC VESICLES (II), LYSOSOMAL MEMBRANES (III) AND BASOLATERAL PLASMA MEMBRANES (IV) ISOLATED FROM RAT KIDNEY CORTEX

The results represent mean values ±S.E.M. derived from *n* determinations and are expressed as μg/mg protein. Usually about two preparations of brush border microvilli and basolateral plasma membranes, four preparations of pinocytic vesicles and ten preparations of lysosomal membranes were combined for each determination.

	I	*n*	II	*n*	III	*n*	IV	*n*
Total lipid phosphorus	11.5± 1.6	7	25.6±1.1	3	6.5	2	11.8±2.1	6
Cholesterol	114 ±35	3	195	2	75	2	108 ±6.9	4
Cholesterolester	26	2	34	2	0	2	30	2
Hexosamines	36.5± 1.2	4	75.6±8.9	4	not determined		28.2±1.7	4
Sialic acid	8.9± 0.5	4	11.0±1.8	6	not determined		9.0±0.8	4

Lipid composition of brush border microvilli, pinocytic vesicles, lysosomal membranes and basolateral plasma membranes

The phospholipid and cholesterol content of the various membranes is compiled in Table III. Compared to brush border microvilli and basolateral plasma membranes which are very similar in lipid content, the phospholipid/protein and the cholesterol/protein ratio is about two times higher in the pinocytic vesicles and about two times lower in the lysosomal membranes. The higher lipid/protein ratio in the pinocytic vesicles probably mirrors the reduction of catalytically active proteins, which was already indicated by the lack of enzymatic activities in the pinocytic vesicles.

The composition of the phospholipids in the membranes is given in Table IV. The most striking finding is the high content of acid phospholipids (phosphatidylinositol+phosphatidylserine) in the pinocytic vesicles, which is accompanied by a decreased sphingomyelin and phosphatidylethanolamine content. Lysosomes also contain low amounts of sphingomyelin; however, the content in acid phospholipids is similar to that of the microvilli and basolateral plasma membranes. Compared to pinocytic vesicles the lower content in acid phospholipids is compensated by an increased content in phosphatidylcholine and phosphatidylethanolamine.

Thus the analysis of enzyme activities, protein, glycoprotein and lipid composition of the brush border microvilli, pinocytic vesicles and lysosomes reveals unique properties of the pinocytic vesicles and lysosomes compared to the microvilli.

Incorporation of labelled membrane precursors into brush border microvilli, pinocytic and basolateral plasma membranes

In order to study further the relation between pinocytic vesicles and brush border microvilli the rates of turnover of membrane components were compared. Two sets of experiments were chosen. First the incorporation of glycoprotein, protein and phospholipid precursors was studied during relatively short time periods in which no appreciable reutilisation of the label should occur. In addition, where possible, a precursor was selected which cannot be reutilized, such as guanido-arginine as protein

TABLE IV

PHOSPHOLIPID COMPOSITION IN BRUSH BORDER MICROVILLI (I), PINOCYTIC VESICLES (II), LYSOSOMAL MEMBRANES (III) AND BASOLATERAL PLASMA MEMBRANES (IV) ISOLATED FROM RAT KIDNEY CORTEX

The values are given as percent of total lipid phosphorus and represent mean values ±S.E.M. derived from *n* determinations performed in quadruplicate. Usually about two preparations of brush border microvilli and basolateral plasma membranes, four preparations of pinocytic vesicles and ten preparations of lysosomal membranes were combined for each determination. In all cases the recovery of phosphorus was 90–100 %. Lysophosphatidylethanolamine was present only in traces (approximately 4 % of total lipid phosphorus) and therefore did not interfere significantly with the phosphatidylcholine determination. Other lysophospholipids, cardiolipin and phosphatidic acid were not detected.

	I	*n*	II	*n*	III	*n*	IV	*n*
Sphingomyelin	28.9±0.5	(3)	12.4±1.9	(4)	14.8±5.2	(3)	22.5±1.4	(5)
Phosphatidylcholine+lysophosphatidylethanolamine	18.9±2.7	(3)	21.5±5.8	(4)	29 ±1.6	(3)	24.8±2.0	(5)
Phosphatidylethanolamine	23.5±0.9	(3)	14.4±4.0	(4)	26.8±2.3	(3)	22.0±1.0	(5)
Phosphatidylinositol+phosphatidylserine	28.1±3.6	(3)	51.7±8.5	(4)	28.6±3.5	(3)	32.4±2.0	(5)
Thereof								
Phosphatidylinositol	6.7	(2)	11.3	(2)	14.1	(2)	8.1 ±0.3	(3)
Phosphatidylserine	19.5	(2)	40.6	(2)	11.2	(2)	21.2±1.1	(3)

TABLE V

INCORPORATION OF LABELLED MEMBRANE PRECURSORS INTO DIFFERENT MEMBRANE FRACTIONS ISOLATED FROM RAT KIDNEY

In the first line the radioactivity found in the pinocytic vesicles is given as cpm/mg protein, the other results are expressed as the ratio of radioactivity/mg protein found in one fraction divided by the radioactivity/mg protein found in the other fraction. Mean values of 3 experiments are given.

	[^{3}H]fucose			[^{14}C]guanido-arginine			*myo*-[^{3}H]inositol		
	1 h	4 h	20 h	1 h	4 h	20 h	1 h	4 h	20 h
Pinocytic vesicles	36306	20875	26905	1705	1692	1471	38269	59640	41538
Pinocytic vesicles/brush border microvilli	1.66	1.26	1.16	1.35	1.39	1.83	1.71	2.18	3.30
Pinocytic vesicles/basolateral plasma membranes	2.21	1.47	1.21	1.38	1.35	1.45	1.33	1.44	1.60
Basolateral plasma membranes/brush border microvilli	0.75	0.86	0.96	0.98	1.03	1.26	1.29	1.51	1.89

precursor. As a second approach the double-label technique of Arias et al. [29] was applied. In these experiments the animals were injected first with [^{14}C]-leucine and after 7–10 days with [^{3}H]leucine. The ratio $^{3}H/^{14}C$ found in the membranes is a function both of the rate of decay of the ^{14}C content and of the rate of incorporation of the ^{3}H label, but is independent of the absolute amount of leucine present in the membrane proteins.

Table V shows the results obtained when the amount of the protein precursor ([^{14}C]guanido-arginine), the glycoprotein precursor ([^{3}H]fucose) and the phosphatidylinositol precursor (*myo*-[^{3}H]inositol) present in the membranes after different periods of incorporation were determined. In the table, the three membranes are compared with each other by calculating the ratio between the radioactivity/mg protein found in each membrane. Thereby the membranes from each experiment are compared directly and the specific activities in the cellular pool which vary from experiment to experiment do not influence the results. It is obvious from Table V that at each time point the amount of protein, glycoprotein and phospholipid precursor per mg membrane protein is higher in the pinocytic vesicles than in the brush

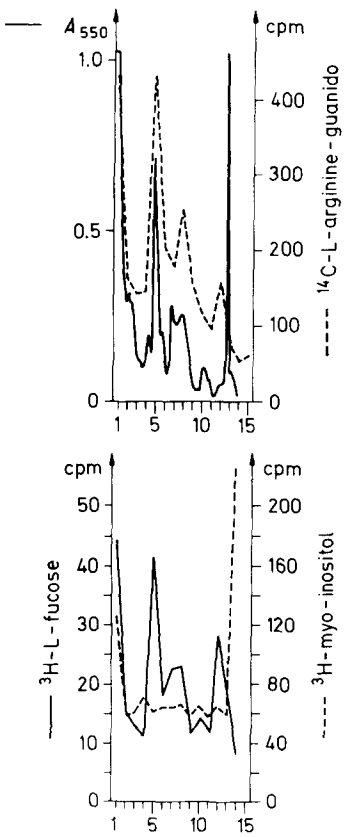


Fig. 2. Distribution of protein, [^{3}H]fucose, [^{14}C]guanido-arginine and *myo*-[^{3}H]inositol in polyacrylamide gels of pinocytic vesicles. One typical experiment after an incorporation time of 4 h is given. For further experimental details see Methods.

border microvilli and in the basolateral plasma membranes. Unfortunately no data on lysosomes could be obtained because the amount of lysosomal membranes derived from the four animals used for each time point was too small to allow accurate determination of radioactivity.

All [^{14}C]arginine and [^{3}H]fucose was found by polyacrylamide gel electrophoresis to be associated with membrane proteins as shown in Fig. 2. *myo*-[^{3}H]Inositol was incorporated only in the phospholipid fraction of the membranes, therefore it migrates mainly in front of the indicator dye. The radioactivity remaining on top of the running gel probably represents aggregated membrane fragments which have not been resolved by sodium dodecyl sulfate.

The higher amount of radioactivity found in the pinocytic vesicles can either be due to a higher turnover of the membrane components or mirror a higher concentration of the membrane component having a similar rate of turnover as in other membranes. The latter explanation is probably true for *myo*-inositol and fucose, since pinocytic vesicles contain more phosphatidylinositol and more carbohydrates than the brush border and the basolateral plasma membranes. Analysis of the amino acid composition of the membranes revealed an identical content of arginine, thus the results obtained with [^{14}C]guanido-arginine may point to a higher turnover of pinocytic proteins.

This tendency could also be detected by the double label technique. As shown in Table VI the ratio of ^{3}H/^{14}C in the pinocytic vesicles is 20–40 % higher than in the brush border microvilli and 12–25 % higher than in the basolateral plasma membranes. The difference is not due to a higher turnover of plasma proteins, which are taken up by the vesicles in vivo and which are also labelled with the radioactive amino acid. This possibility was excluded by removing the content of the pinocytic vesicles by freeze thawing prior to the sedimentation of the membranes. Furthermore, lower incorporation of protein precursors into the soluble protein fraction of the homogenate than into the membrane components was observed.

TABLE VI

INCORPORATION OF [^{14}C]LEUCINE AND [^{3}H]LEUCINE INTO DIFFERENT MEMBRANE FRACTIONS ISOLATED FROM RAT KIDNEY (DOUBLE-LABEL TECHNIQUE)

The values are given as the ratio cpm^{3}H/cpm^{14}C in the membrane protein after administration of [^{14}C]leucine 7 (left column) or 10 (right column) days before [^{3}H]leucine.

Fraction	^{3}H/^{14}C ratio		
	7 days		10 days
Brush border microvilli	5.65	5.95	45.82
Basolateral plasma membranes	6.14	6.67	52.52
Microsomal fraction*	6.75	7.41	63.74
Pinocytic vesicles	6.94	7.49	65.87
Ratio pinocytic vesicles/brush border microvilli	1.22	1.25	1.43
Ratio pinocytic vesicles/basolateral plasma membrane	1.13	1.12	1.25
Ratio basolateral plasma membrane/brush border microvilli	1.09	1.12	1.14

* P_4 in Table I.

DISCUSSION

In the understanding of the pinocytic process two main problems are still unsolved. One concerns the recognition of the macromolecules and the triggering of the pinocytosis. From studies on different tissues and cells it is known that preferentially positively charged macromolecules are pinocytized suggesting the presence of some receptor sites in the membrane [30, 31]. The other problem concerns the part of the cell from which the membrane of the pinocytic vesicles originates and how lysosomes are related to pinocytic vesicles. We approached these questions in the present study by comparing the biochemical composition and properties of three membrane elements related to pinocytosis: the apical cell membrane (brush border microvilli) at which the recognition takes place, the pinocytic vesicles which have a transport function for macromolecules and the lysosomes which contain hydrolytic enzymes for protein degradation.

Function and origin of the components of pinocytic vesicles

In comparing the chemical composition of pinocytic vesicles with that of brush border microvilli it was found that the pinocytic vesicles have two characteristics; one is the high concentration of acid phospholipids, and the other glycoproteins, which are probably absent from the brush border microvilli. It is tempting to speculate that these components of the pinocytic vesicles are directly related to the specific function of the pinocytic vesicles. One could envisage that as already described for the erythrocytes, the phospholipids of the pinocytic vesicles are unevenly distributed across the membrane [32] in such a way that the acid phospholipids are facing the inside of the vesicle, i.e. the lumen-directed side, before the vesicle is pinched off. The negatively charged headgroups of the phospholipids might act thereby as binding sites for positively charged macromolecules, which are preferentially pinocytized. The electrostatic binding of the molecules to the lipids would then lead to a distortion of the membrane surface and this might initiate the pinocytic process [33]. Also, the glycoproteins might be involved in the recognition step of pinocytosis. It is known that dextran derivates are avidly pinocytized by the kidney [34]; for these molecules an interaction with the sugar moiety of the glycoprotein could be the initial step for pinocytosis.

The assumption that the components of the pinocytic vesicles are related to the recognition step of pinocytosis would imply that the apical portion of the luminal membrane (the microvilli) and the crypts between the microvilli, where pinocytosis is induced, are primarily different from each other.

This different composition has some consequences in regard to the question of the origin of the membrane material which constitutes the pinocytic vesicles. Essentially two different modes of formation of endocytic vesicles can be distinguished. One hypothesis postulates a continuous flow of membrane material from the microvilli into the pinocytic vesicles, the membrane pool being identical for both membranes. The other possibility involves a synthesis of new membrane material during the formation of the pinocytic vesicles; in this case microvilli and pinocytic vesicles would have a different turnover. Our observation of a different biochemical composition of the two membranes would favour the latter possibility.

The results of the membrane turnover might also be considered to provide

some evidence of a different turnover, although the differences observed between brush border membranes and pinocytic vesicles are small and their interpretation is complicated by the assumptions made for the application of the double-label technique to renal membranes. As pointed out by Arias et al. [29] certain conditions have to be fulfilled to obtain valid results. The first is that the experiments should be terminated after the peak of incorporation of the second pulse label. Assuming that leucine is not incorporated more slowly than arginine, the maximum incorporation of leucine should occur 1 h after the injection (see Table V). On the other hand first-order kinetics of the decay are required. Our limited amount of data for arginine provide evidence for first-order kinetics in the time periods investigated in this study. Quirk et al. [35] on the other hand observed a biphasic incorporation of labelled leucine into brush border fragments from rabbit kidney. The membrane fraction used by these authors was very inhomogeneous compared to the fractions used in our study; it contained, in addition to microvillous membranes, pinocytic vesicles and the membrane areas where the pinocytosis was induced. Thus the biphasic kinetic of incorporation might, as already speculated by Quirk et al., be composed of a rapid component which can be attributed to the membrane regions involved in pinocytosis, whereas the slow component represents the turnover of the brush border membrane. A relatively slow turnover rate of the brush border membrane comparable to that in the kidney could also be demonstrated in the small intestine [36].

Origin of lysosomal membrane components

For the isolation of renal lysosomes we could successfully apply the method developed by Henning and Heidrich [9] for the isolation of liver lysosomes. By the combination of differential centrifugation and free-flow electrophoresis a high degree of purification is obtained which is similar to the results reported by several authors using different techniques [1, 37–41].

The isolated lysosomes represent secondary lysosomes which according to electron microscopic studies are thought to stem from the fusion of pinocytic vesicles with primary lysosomes [42]. Primary lysosomes are thought to be components of the Golgi apparatus, which store and modify the hydrolytic enzymes synthesized in the endoplasmic reticulum [43]. Direct evidence for a transfer of components from the site of protein synthesis to the lysosomes can be derived from the observation of Goldstone and Koenig [44, 45] that there is a common glycoprotein in rough endoplasmic reticulum, Golgi fraction and in the soluble fraction of lysosomes isolated from rat kidney cortex.

Our experiments additionally provide some evidence for a transfer of membrane material from the pinocytic vesicles to the lysosomal membranes. If the lipid composition of pinocytic vesicles and lysosomal membranes is compared to the composition of Golgi membrane lipids studied recently by Zambrano et al. [46] (see Table VII) it appears that the lysosomal membrane represents a mixture of the lipids of pinocytic vesicles and Golgi membranes. For example, the phosphatidylserine content of lysosomes might be lower than in pinocytic vesicles because the Golgi membranes have a very low content of phosphatidylserine. On the other hand, the phosphatidylcholine content of the lysosomes might be higher than that of pinocytic vesicles because the Golgi membranes are rich in phosphatidylcholine. Although this tentative explanation should be considered with careful reserve because results from

TABLE VII

COMPARISON OF THE LIPID COMPOSITION OF PINOCYTIC VESICLES AND LYSOSOMES (TABLE IV) WITH THAT OF THE GOLGI APPARATUS FRACTION ISOLATED ZAMBRANO ET AL. [44]

The values are given as percent of total lipid phosphorus.

	Pinocytic vesicles	Golgi	Lysosomal membranes
Sphingomyelin	12.4	6.7	14.8
Phosphatidylcholine	21.5	52.1	29
Phosphatidylethanolamine	14	23.1	26.8
Phosphatidylinositol	11.3	7.1	14.1
Phosphatidylserine	40	5.4	11.2

different laboratories are compared, the data suggest that transfer of lipids from pinocytic vesicles into the lysosomal membranes is a very probable process.

With regard to the transfer of protein components from the membranes of the pinocytic vesicles to the lysosomal membranes, it appears that the main proteins of the pinocytic vesicles are not present in the lysosomes, at least not in an unaltered form. However, it is easily conceivable that alterations such as partial degradation and/or changes in the carbohydrate and sialic acid content might occur during the period of fusion and therefore no definite conclusions can be drawn from the protein and glycoprotein pattern obtained in polyacrylamide gel electrophoresis.

At a first view our results seem to be at variance with results obtained from studies in rat liver [47, 48], where a great similarity in the lipid pattern of plasma membranes and tritosomes (lysosomes isolated after application of Triton WR-1339 to the animal [49]) has been observed. However, this discrepancy can be explained by the different experimental models used. The tritosomes seem not to be related to pinocytosis but rather to a different cellular process, namely autophagocytosis. Liver plasma membranes severely impaired by the detergent Triton are taken up into autophagocytic vacuoles, which contribute to the tritosomal fraction [47]. Therefore, tritosomes appear to be "plasma membrane-like" organelles, whereas normal lysosomes are "endoplasmic reticulum-like" organelles [9] because they contain more components of primary lysosomes. Thus, in liver as in kidney, under normal circumstances lysosomes seem to be the result of the fusion between enzyme-containing Golgi vesicles (primary lysosomes) and pinocytic vesicles, the participation of plasma membranes in this process seems to be negligibly small.

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