Biochem. J. (1980) **186**, 177–181
Printed in Great Britain

A Simple and Fast Method for the Isolation of Basolateral Plasma Membranes from Rat Small-Intestinal Epithelial Cells

Vito SCALERA, Carlo STORELLI,* Christina STORELLI-JOSS,* Winfried HAASE and Heini MURER
Max-Planck-Institut für Biophysik, Kennedyallee 70, 6000 *Frankfurt* (*Main*) 70, *West Germany*

(*Received* 17 *May* 1979)

A method was developed for the analytical and preparative isolation of basolateral plasma membranes from rat small intestine. They were separated on a self-orientating Percoll (modified colloidal silica) gradient starting with a heavy microsomal-membrane fraction and involving centrifugation at 48000 *g* for 1 h. $(Na^+ + K^+)$-stimulated ATPase activity, used as a marker enzyme for the basolateral plasma membrane, is enriched 20-fold compared with that found in the homogenate of isolated intestinal epithelial cells.

The procedures developed so far for the isolation of basolateral plasma membranes from small-intestinal epithelial cells were lengthy, involving differential centrifugation with subsequent sucrose (or sorbitol)-density-gradient centrifugation or separation by free-flow electrophoresis (Quigley & (Gotterer, 1969; Fujita *et al.*, 1972; Douglas *et al.*, 1972; Murer *et al.*, 1974, 1976; Lewis *et al.*, 1975; Mircheff & Wright, 1976; Walling *et al.*, 1978; Mircheff *et al.*, 1979). Furthermore the methods were suitable for analytical rather than preparative purposes. Prusiner and co-workers have reported, in preliminary publications, that centrifugation of rat renal-cortical heavy microsomal-membrane fractions in the self-orientating modified colloidal silica gradient medium Percoll leads to separation of brush-border-membrane-bound enzyme activities and $(Na^+ + K^+)$-stimulated ATPase, an enzyme located in basolateral plasma membranes (Mamelock *et al.*, 1978, 1979*a*,*b*). In the present paper a simple and fast method for the isolation of basolateral membranes from rat small-intestinal epithelial cells is described, based on centrifugation in a Percoll medium.

A preliminary account of this work was given at the Spring Meeting of the German Biochemical Society (1979) in Berlin (Storelli *et al.*, 1979).

Materials and Methods

Phenylmethanesulphonyl fluoride and *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

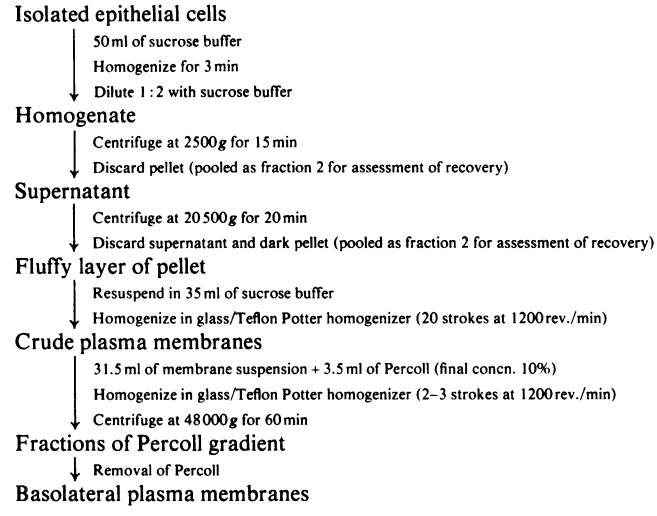
* Present address: Istituto di Fisiologia Generale, Università degli Studi, Via Amendola 165A, Bari, Italy.

Phosphoenolpyruvate, pyruvate kinase, lactate dehydrogenase, ATP (disodium salt) and NADH were purchased from Boehringer G.m.b.H. (Mannheim, Germany). Percoll was obtained from Pharmacia (Uppsala, Sweden). All other chemicals used throughout this study were obtained from Merck (Darmstadt, Germany) and were of analytical-grade purity.

Membrane separation

Intestines from three male rats (Sprague–Dawley, 180–200 g) were rinsed with ice-cold phosphate-buffered Ringer's solution, and epithelial cells were isolated as described previously (Murer *et al.*, 1974). Cells were homogenized for 3 min in a Waring blender (type M 100-homogenizer; ESGE, Mettlen, Switzerland) in 50 ml of sucrose buffer containing 250 mM-sucrose, 10 mM-triethanolamine hydrochloride (pH 7.6) and 0.1 mM-phenylmethanesulphonyl fluoride (to inhibit proteinases). Membrane isolation was performed in a Sorvall SS34 rotor and a Sorvall RC2B centrifuge (4°C) as described in Scheme 1. The spontaneously formed Percoll gradient was fractionated from the top by pumping a 60% (w/v) sucrose solution to the bottom of the centrifuge tube via a steel cannula (approx. diam. 1 mm) with a peristaltic pump. For determination of the distribution of the enzyme activities in the gradient, the volume of the first 60 fractions collected was 0.5 ml; the remaining 5 ml was collected in 1 ml fractions (Fig. 1). If preparative membrane separation was performed (see Table 2), the top 12 ml of the gradient was collected as fraction 4, then two 3 ml fractions were collected (fractions 5 and 6). The remaining 17 ml was collected as fraction 7.

0306/3283/80/010177-06 $1.50/1



Scheme 1. *Flow scheme for the isolation of basolateral plasma membranes from rat small-intestinal epithelial cells*
Further details of the procedures are given in the text.

Since the Percoll material at higher concentration interferes with the Lowry *et al.* (1951) method for protein determination, it is necessary to remove the Percoll particles by centrifugation. For the three fractions at the top of the gradient (4, 5 and 6) this can be achieved by centrifugation in a Sorvall SS34 rotor at 48000*g* for 30 min after 3–10-fold dilution with a buffer containing 250 mM-sucrose, 10 mM-triethanolamine hydrochloride (pH 7.6) and 0.1 mM-phenylmethanesulphonyl fluoride. For the fraction at the bottom (fraction 7), centrifugation for 1 h in a swinging-bucket rotor (Christ S 40/135 Ti) at 40000 rev./min after a 3-fold dilution was performed. During these centrifugations the Percoll particles form glassy pellets. The membranous material forms a fluffy pellet on the top of the very solid Percoll pellet and was resuspended in sucrose buffer.

Protein and enzyme determination

Protein and enzyme determinations were carried out on unfrozen samples kept on ice within 9 h of the rats being killed. Aminopeptidase M (EC 3.4.11.2), γ-glutamyltransferase (EC 2.3.2.2) and alkaline phosphatase (EC 3.1.3.1) were used as marker enzymes for the brush-border membrane (Eichholz & Crane, 1965). $(Na^+ + K^+)$-stimulated ATPase (EC 3.6.1.3) was measured (Lewis *et al.*, 1975) as the marker enzyme for the basolateral membranes. Contamination by endoplasmic reticulum was monitored by analysing KCN-resistant NADH oxidoreductase activity (EC 1.6.99.2). Succinate–cytochrome *c* oxidoreductase (EC 1.3.99.1) was used as a marker for mitochondrial enzyme activity. Acid phosphatase (EC 3.1.3.2) and *N*-acetylglucosamine deacetylase (EC 3.5.1.33) were used as marker enzymes for lysosomes (Horvat & Touster, 1967; Baggiolini *et al.*, 1970). Lactate dehydrogenase activity (EC 1.1.1.27) was measured as a cytosolic enzyme.

Alkaline phosphatase and $(Na^+ + K^+)$-stimulated ATPase activities were assayed as described by Berner & Kinne (1976). Aminopeptidase M activity was measured as described by Haase *et al.* (1978). γ-Glutamyltransferase was measured by the method of Sigma Chemical Corp. (Bulletin 415). NADH oxidoreductase was measured in the presence of 0.3 mM-KCN as described by Sottocasa *et al.* (1967). Succinate–cytochrome *c* oxidoreductase was measured as described by Fleischer & Fleischer (1967). Acid phosphatase activity was measured by measuring at 405 nm the amount of *p*-nitrophenol released during incubation with *p*-nitrophenyl phosphate as substrate; 0.15% Triton X-100 in 0.1 M-sodium acetate buffer (pH 4.5) was present in the incubation mixture, and the enzyme reaction was stopped by the addition of 0.25 M-NaOH. *N*-Acetylglucosamine deacetylase was measured with *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide in 200 mM-sodium citrate buffer (pH 4.5, containing 20 mg of Triton X-100/100 ml) as substrate; the incubation was stopped by the addition of a 10-fold excess of a 13.3 mM-glycine buffer, pH 10.5. *p*-Nitrophenol released during incubation was measured at 405 nm. Lactate dehydrogenase was measured with sodium pyruvate as substrate by recording the formation of NAD^+ from NADH in the presence of 50 mM-sodium phosphate buffer, pH 7.5. Protein determinations

were carried out, after precipitation with ice-cold 10% (w/v) trichloroacetic acid, by the procedure of Lowry *et al.* (1951), with crystalline serum albumin as standard.

Results and Discussion

The distributions of aminopeptidase M, a marker enzyme for the brush-border membrane, and $(Na^+ + K^+)$-stimulated ATPase, the marker enzyme for the basolateral plasma membranes, after centrifugation for 1h at 20000rev./min in 10% (v/v) Percoll are illustrated in Fig. 1. Partial cross-contamination of the basolateral-membrane fraction by the brush-border-membrane fraction does occur, as indicated by the overlapping enzyme activities. In Tables 1 and 2 the recovered enzyme activities and the relative specific activities (enrichment factors) are given. In addition, the specific activities for the different enzymes, as measured in the initial homogenate as well as in fraction 5, are shown. During the isolation of the crude plasma-membrane fraction, total recovered enzyme activities are close to 100%, which indicates that enzyme denaturation does not occur (Table 1). Recovery of protein was also between 90 and 100% (results not shown). After pooling of the fractions from the Percoll gradient and removal of Percoll particles by centrifugation, the effective recoveries, i.e. those in relation to the enzyme activities put on the gradient, ranged between 75% for $(Na^+ + K^+)$-stimulated ATPase, 65% for alkaline phosphatase and 3.5% for lactate dehydrogenase (Table 2).

Protein recovery from the Percoll gradient was between 50 and 60% (results not shown). Since con-

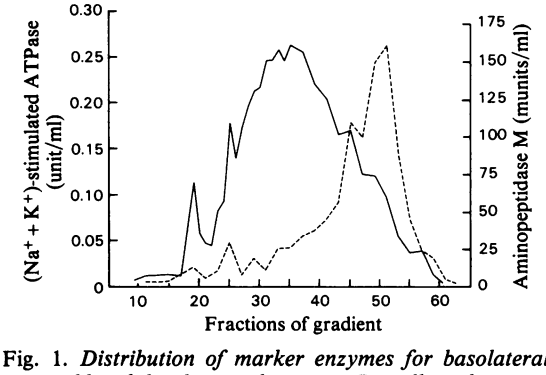


Fig. 1. *Distribution of marker enzymes for basolateral and brush-border membranes on Percoll gradient*
The gradient was separated from the top into 60 fractions of 0.5ml. The remaining 5ml of the gradient was collected as 1ml fractions (fraction 61–65). The solid line represents $(Na^+ + K^+)$-stimulated ATPase activity, and the broken line corresponds to aminopeptidase M activity.

Table 1. *Recoveries for marker enzymes in the different fractions obtained during isolation of teh crude plasma-membrane fraction*
The values represent the percentages of the enzyme activity found initially in the homogenate. The values in parentheses represent the specific activities (in munits/mg) for the different enzymes in the homogenate. The values labelled by an asterisk represent the specific activities relative to the activities found in the homogenate (enrichment factor). The results are means ± S.D. for five individual experiments. One munit represents 1nmol of substrate transformed/min.

Fraction	Alkaline phosphatase	$(Na^+ + K^+)$-ATPase	Amino-peptidase M	γ-Glutamyl-transferase	Cytochrome *c*–succinate oxidoreductase	NADH oxido-reductase	Lactate dehydrogenase	Acid phosphatase	*N*-Acetyl-glucosamine deacetylase
Homogenate	100 (968.6 ± 470.5)	100 (160.0 ± 30.0)	100 (59.3 ± 21.4)	100 (15.6 ± 2.6)	100 (50.1 ± 20.4)	100 (272.0 ± 160.0)	100 (3950 ± 2640)	100 (27.2 ± 11.0)	100 (50.3 ± 26.5)
Pooled fractions for recovery	96.8 ± 10.4	59.2 ± 10.5	82.2 ± 20.8	82.8 ± 7.7	96.2 ± 31.8	72.4 ± 19	83.2 ± 5.5	78.2 ± 18	89.5 ± 6.7
Crude plasma membranes	12.5 ± 2.6 0.93 ± 0.29*	34.8 ± 6.7 2.57 ± 1.24*	14.2 ± 2.8 1.06 ± 0.38*	12.5 ± 5.1 1.14 ± 0.31*	12.6 ± 3.6 1.32 ± 0.80*	24.6 ± 11.9 2.10 ± 1.58*	7.5 ± 2.4 0.58 ± 0.18*	17.1 ± 6.2 1.04 ± 0.58*	9.2 ± 2.3 0.76 ± 0.32*

Table 2. *Recoveries, enrichment factors and specific activities for marker enzymes in the different pooled fractions of the Percoll gradient*

Fractions were collected and Percoll was removed as described in the text. The total enzyme activity recovered after the Percoll-removal step was taken as 100%, and the enzyme activity found in the different fractions was expressed as a percentage (numbers given in the first line) of total recovered enzyme activity. The value labelled by an asterisk represents the specific activity relative to the enzyme activity found in the homogenate (enrichment factor). The values in parentheses represent the specific activities (in munits/mg of protein) for the different enzymes in fraction 5. 'Effective recovery' means total activity recovered from the gradient in comparison with the amount of enzyme activity put on the gradient. Means ± S.D. for five experiments are given. One munit represents 1 nmol of substrate transformed/min.

Fraction	Alkaline phosphatase	$(Na^+ + K^+)$-ATPase	Amino-peptidase M	γ-Glutamyl-transferase	Cytochrome *c*–succinate oxidoreductase	NADH oxido-reductase	Lactate dehydrogenase	Acid phosphatase	*N*-Acetyl-glucosamine deacetylase
4	12.9 ± 4.6	11.1 ± 4	8.6 ± 5.3	11.4 ± 6	5.8 ± 1.3	6.4 ± 4.4	20.8 ± 5.8	9.3 ± 4.4	8.7 ± 4.5
	1.67 ± 0.67*	9.41 ± 3.32*	1.31 ± 0.17*	1.60 ± 0.13*	0.53 ± 0.28*	1.05 ± 0.57*	0.12 ± 0.07*	1.54 ± 0.07*	0.48 ± 0.06*
5	8.8 ± 2.8	25.5 ± 8.6	7.1 ± 3.5	9.5 ± 3.2	9.0 ± 3.4	13.6 ± 5	5.9 ± 1.6	13.4 ± 5.3	4.4 ± 1.9
	2.49 ± 0.71*	20.6 ± 2.3*	2.12 ± 0.60*	2.33 ± 0.10*	1.16 ± 0.76*	1.7 ± 1.47*	0.04 ± 0.02*	2.49 ± 1.62*	0.30 ± 0.10*
	(2520 ± 1110)	(3340 ± 420)	(124 ± 39)	(37.4 ± 4.1)	(55.9 ± 37.2)	(462 ± 318)	(158 ± 92)	(61.6 ± 32.0)	(14.1 ± 5.2)
6	13.5 ± 2.5	28.4 ± 2.4	11.9 ± 4.1	13.7 ± 3.5	18.8 ± 3.5	14.1 ± 5.8	10.1 ± 0.7	16.3 ± 5.5	6.6 ± 3.
	2.92 ± 1.14*	18.9 ± 5.5*	2.74 ± 0.74*	2.92 ± 0.28*	1.11 ± 0.57*	2.22 ± 1.62*	0.05 ± 0.01*	2.52 ± 1.71*	0.38 ± 0.
7	69.2 ± 9.4	40.1 ± 10.5	75.6 ± 9.1	71.2 ± 10.1	66.4 ± 6.2	64.9 ± 20	63.5 ± 6.7	61.4 ± 14.4	80.4 ± 9.4
	2.28 ± 1.53*	3.59 ± 2.31*	2.31 ± 1.03*	2.42 ± 1.84*	2.57 ± 1.60*	3.42 ± 1.45*	0.06 ± 0.02*	1.78 ± 0.83*	1.06 ± 0.67*
Effective recovery (%)	64.3 ± 23	75.2 ± 22	47.6 ± 11	44.9 ± 1.4	31.3 ± 26	60 ± 36	3.5 ± 3	42.6 ± 11.8	23.1 ± 14.3

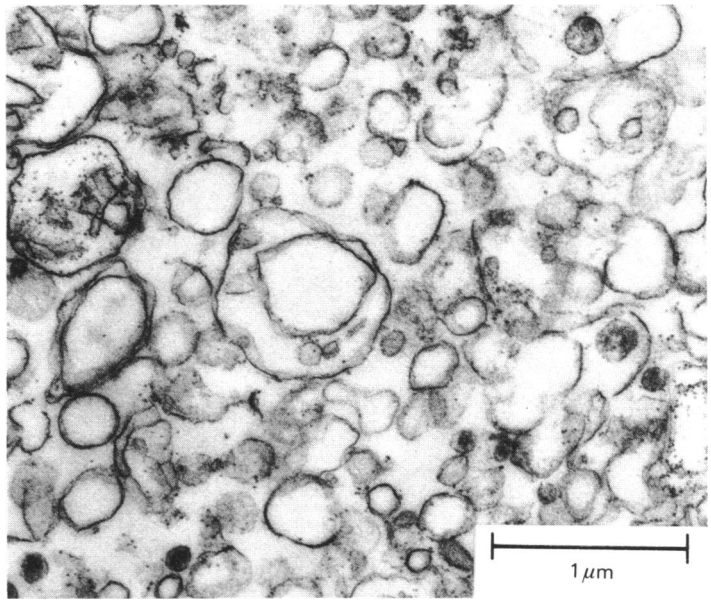
trol experiments demonstrated the absence of any inhibitory effect of the Percoll material on the enzyme activities, the loss of enzyme activity as well as the loss of protein corresponds to a loss into the supernatants or into the glassy Percoll pellets during the Percoll-removal step. In order that the samples be analysed within a few hours of the fractionation and because of other experimental difficulties (dilute supernatants, solid pellets), we confined the analyses of enzyme activities and protein content to those samples that were of particular interest and importance. Therefore Table 2 shows only the recovery of the enzymes as a percentage of the total enzyme activities recovered in the membranous fractions.

On the basis of the enzyme-recovery data (Table 2), a closer examination of the membrane material present in fraction 5 seems to be of interest for the purpose of preparative basolateral-membrane isolation. In fraction 5 the relative specific activity (enrichment of enzyme activity over that in the homogenate) of $(Na^+ + K^+)$-stimulated ATPase is approx. 20-fold, whereas marker enzymes for the brush-border membrane are enriched by a factor of 2. Contamination with mitochondria and with endoplasmic reticulum is also tolerable, as indicated by enrichment factors considerably lower than 2. Practically no lactate dehydrogenase activity is found in fraction 5. An interesting pattern is found for the lysosomal markers, acid phosphatase and *N*-acetylglucosamine deacetylase. Acid phosphatase is enriched by a factor of more than 2, whereas *N*-acetylglucosamine deacetylase shows a very low enrichment factor. Mircheff & Wright (1976) have postulated that acid phosphatase might be partially located in basolateral plasma membranes. Our finding of a non-parallelism between these two 'lysosomal' enzyme activities and an increased activity in the fraction containing basolateral membranes seems at first to be in agreement with an extralysosomal location of acid phosphatase in the small-intestinal epithelial cells. However, differential absorption of lysosomal enzymes to basolateral plasma membranes is an equally likely explanation.

Plate 1 shows the morphological appearance of the isolated basolateral plasma-membrane fraction. Predominantly closed membrane vesicles seem to be present, which appear as closed rings. The dark electron-dense particles seen occasionally that look like ribosomes could be identified in control experiments as remaining Percoll particles.

Conclusions

The method for the isolation of basolateral plasma membranes from rat small-intestinal epithelial cells as described here offers several advantages over the methods published so far: (1) the method is fast; (2) the yield obtained is high enough to allow transport studies; (3) the purification factor (enrichment) is



EXPLANATION OF PLATE 1

Electron micrograph of a thin-sectioned preparation of basolateral plasma membranes (fraction 5)
Membranes were fixed with 2.5% glutaraldehyde in 0.1 M-sodium cacodylate buffer, pH 7.3, for about 2 h. Afterwards they were washed in cacodylate buffer and post-fixed in 1% OsO_4/0.05 M-HCl/veronal buffer at pH 7.4. After being washed in water, membranes were stained with 2% uranyl acetate for 1 h. Magnification ×26 000.

(*facing p.* 180)

high; (4) only simple laboratory equipment (no ultracentrifuge, no free-flow electrophoresis) is needed.

Note Added in Proof (Received 16 November 1979)

The distribution pattern of marker enzymes obtained on Percoll gradients might be slightly altered by the use of different Percoll stock solutions. Therefore Percoll concentrations and/or centrifugation times may need to be varied slightly to obtain optimal conditions for separation.

We express our gratitude to Dr. S. B. Prusiner and his colleagues for stimulating discussions, which enabled us to develop this method.

References

Baggiolini, M., Hirsch, J. G. & De Duve, C. (1970) *J. Cell Biol.* **45**, 586–597

Berner, W. & Kinne, R. (1976) *Pflügers Arch.* **361**, 269–277

Douglas, A. P., Kerley, R. & Isselbacher, K. J. (1972) *Biochem. J.* **128**, 1329–1338

Eichholz, A. & Crane, R. K. (1965) *J. Cell Biol.* **26**, 687–692

Fleischer, S. & Fleischer, B. (1967) *Methods Enzymol.* **10**, 406–432

Fujita, M., Ohta, H., Kawai, K., Matsui, H. & Nakao, M. (1972) *Biochim. Biophys. Acta* **274**, 336–347

Haase, W., Schäffer, A., Murer, H. & Kinne, R. (1978) *Biochem. J.* **172**, 57–62

Horvat, A. & Touster, O. (1967) *Biochim. Biophys. Acta* **148**, 725–740

Lewis, B. A., Elkin, A., Michell, R. H. & Coleman, R. (1975) *Biochem. J.* **152**, 71–84

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mamelock, R. D., Groth, D. F. & Prusiner, S. B. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1398

Mamelock, R. D., Bildstein, C. L. & Prusiner, S. B. (1979*a*) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 244

Mamelock, R. D., Prusiner, P. E., Bildstein, C. L. & Prusiner, S. B. (1979*b*) *Clin. Res.* **27**, 498A

Mircheff, A. K. & Wright, E. M. (1976) *J. Membr. Biol.* **28**, 309–333

Mircheff, A. K., Sachs, G., Hanna, S. D., Labiner, C. S., Rabon, E., Douglas, A. P., Walling, M. W. & Wright, E. M. (1979) *J. Membr. Biol.* in the press

Murer, H., Hopfer, U., Kinne-Saffran, E. & Kinne, R. (1974) *Biochim. Biophys. Acta* **345**, 170–179

Murer, H., Aamann, E., Biber, J. & Hopfer, U. (1976) *Biochim. Biophys. Acta* **433**, 509–519

Quigley, J. P. & Gotterer, G. S. (1969) *Biochim. Biophys. Acta* **173**, 456–464

Sottocasa, G. L., Kuylenstierna, L., Ernster, L. & Bergstrand, A. (1967) *J. Cell Biol.* **32**, 415–438

Storelli, C., Scalera, V., Storelli-Joss, C., Hildmann, B. & Murer, H. (1979) *Hoppe-Seyler's Z. Physiol. Chem.* **360**, 383

Walling, M. M., Mircheff, A. K., Van Os, C. H. & Wright, E. M. (1978) *Am. J. Physiol.* **235**, E539–E545