FU is not selective for the tumor tissues, and its host toxicity follows a very steep dose-toxicity relationship (1). The tissue selectivity of dFUR depends on the site of its activation and the subsequent fate of FU. When the FU concentration in blood is increased, the host tissue toxicity will be increased and the target specificity of dFUR will be diminished. The molar dosage of dFUR used clinically (19) is 20–30 fold higher than the maximally tolerated dose of FU (1). Thus, even a minor variability in the dFUR disposition may alter the systemic FU concentrations and therefore its selectivity and therapeutic efficacy. There is a growing interest in developing FU prodrugs with an improved selectivity (1). The kinetic relationship between the tissue clearances of a prodrug and the systemic availability of FU may serve as guidelines for future prodrug development.

References

(1) Chabner, B. A. (1982) In Pharmacologic Principles of Cancer Treatment (Chabner, B. A., ed.) W. B. Saunders Co., Philadelphia.
(2) Cook, A. F., Holman, M. J., Kramer, M. J., Trown, P. W. (1979) J. Med. Chem. 22, 1330–1335.
(3) Armstrong, R. D., Diasio, R. B. (1981) Cancer Res. 41, 4891–4894.
(4) Au, J. L.-S., Rustum, Y. M., Minowada, J., Srivastava, B. I. S. (1983) Biochem. Pharmacol. 32, 541–546.
(5) Bollag, W., Hartmann, H. R. (1980) Eur. J. Cancer 16, 127–132.
(6) Ishitsuka, H., Miwa, M., Takemoto, K., Kukuoka, K., Itoga, A., Maruyama, H. B. (1980) Gann 71, 112–123.
(7) Rustum, Y. M., Au, J. L.-S. (1983) Proc. Am. Assoc. Cancer Res. 24, 1198.
(8) Au, J. L.-S., Walker, J. S., Rustum, Y. (1983) J. Pharmacol. Exp. Therap. 227, 174–180.
(9) Baker, D. E. J. (1979) In: The Laboratory Rat, Vol. I, biology and diseases (Baker, H. J., Lindsey, J. R., Weisbroth, S. H. eds.) Academic Press, New York.
(10) Rowland, M., Tozer, T. N. (1982) Clinical pharmacokinetics: concepts and application, Lea and Febiger, Philadelphia.
(11) Cleland, W. W. (1979) In: Methods of Enzymology 63, 103–138
(12) Wilkinson, G. R., Shand, D. G. (1975) Clin. Pharmacol. Ther. 18, 377–390.
(13) Kono, A., Hara, Y., Sugaia, S., Karubu, Y., Maisushima, Y., Ishusuka, H. (1983) Chem. Pharm. Bull. (Tokyo) 31, 175–178.
(14) Bivin, W. S., Crawford, M. P., Brewer, N. R. (1979) In: The Laboratory Rat, Vol. I, biology and diseases (Baker, H. J., Lindsey, J. R., Weisbroth, S. H., eds.) Academic Press, New York.
(15) Boxenbaum, H. (1980) J. Pharmacokin. Biopharm. 8, 165–176.
(16) Gustavason, B. G., Brandberg, A., Regarth, C. G., Almersjo, O. E. (1979) J. Pharmacokin. Biopharm. 7, 665–673.
(17) Pang, K. S., Strobl, K., Gillette, J. R. (1979) Drug Metab. Disp. 7, 366–372.
(18) Plaa, G. L. (1971) In: Fundamentals of drug metabolism and drug disposition. (LaDu, B. N., Mandel, H.-G., Way, E. L. eds.). Williams and Wilkins Co., Baltimore.
(19) Abele, R., Alberto, P., Seematter, R. J., Germano, G., Heintz, R., Bollag, W. (1982). Cancer Treat. Rep. 66, 1307–1313.

In Vitro Drug Absorption Models. I. Brush Border Membrane Vesicles, Isolated Mucosal Cells and Everted Intestinal Rings: Characterization and Salicylate Accumulation

Iwona Osiecka[1], Patty A. Porter[2], Ronald T. Borchardt[1], Joseph A. Fix[2,3], Colin R. Gardner[2]

Received: March 22, 1985; accepted: June 22, 1985.

Abstract: Brush border membrane vesicles, isolated mucosal cells and everted rings from rat intestine were compared for their suitability for drug uptake studies. Vesicles from brush border membranes were judged to be metabolically and morphologically functional on the basis of biochemical and microscopic criteria. With the use of a collagenase-vascular-perfusion method, populations of villus, mid villus and crypt cells were separated. An alternative approach that is based on an EDTA-dissociation procedure afforded fractions enriched in villus and crypt cells. Although several enzymatic and metabolic activities of these two cell preparations were comparable, cell viability based on the Trypan Blue dye exclusion test, ultrastructural appearance and glucose uptake more closely conformed to *in vivo* values for cells isolated according to the EDTA-dissociation method. These cells were chosen as a model for drug transport investigation. The morphological and functional integrity of everted rings was verified by histological examination, extracellular space estimation and assessment of glucose transport ability. Sodium salicylate uptake studies using brush border membrane vesicles and isolated mucosal cells were highly variable, whereas everted segments exhibited good reproducibility in uptake experiments. Time dependence of salicylate uptake was demonstrated with membrane vesicles and everted rings. Time dependence was not observed in mucosal cell uptake studies, probably because of the time required to separate the cells from the incubation solution. Based on ease of preparation, technical aspects of *in vitro* incubation and reproducibility of results, everted intestinal rings were considered to be a good potential model for *in vivo* drug absorption. Brush border membrane vesicles were generally regarded as unacceptable because of variations after storage and between experiments. Isolated cells offered certain advantages, but the utility of cells as an *in vitro* model remains equivocal.

[1] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66045.
[2] INTERx Research Corporation, Merck Sharp and Dohme Research Laboratories, 2201 W 21st, Lawrence, Kansas 66046.
[3] Address all correspondence to: Joseph A. Fix, Ph.D., INTERx Research Corporation, 2201 W 21st, Lawrence, KS 66046.

Intestinal absorption is essential for the bioavailability of orally administered drugs. Several methods have been developed for studying intestinal uptake *in vitro*, including everted intestinal sacs (1), everted segment-rings (2), cell suspension (3–8) and membrane vesicles (9). However, a direct comparison of the different *in vitro* systems and an examination of correlation with *in vivo* drug uptake studies have not been adequately detailed.

Most of the procedures for the isolation of epithelial cells employ mechanical agitation (3), scraping (4), hydrolytic enzymes (5) or chelating chemicals (6). It is not clear which of these procedures causes less damage to the cells. We have characterized and compared cell populations prepared by two different methods: Weiser's-EDTA-intestinal perfusion (7) and Hartmann and coworkers' intravascular collagenase perfusion (8). Everted intestinal rings and brush border membrane vesicles (BBMV) were also prepared and tested.

The aim of this study was to compare the three *in vitro* systems as potential models for studying drug transport mechanisms. The factors included in the analysis were technical ease of preparation, conditions required to maintain a viable system, reproducibility of transport data, and demonstration of *in vitro* drug transport.

Experimental

Materials

D-[^{3}H]-Glucose (sp. act. 15.0 Ci/mmole), L[^{3}H]-glucose (sp. act. 10.7 Ci/mmol), D-[^{14}C]-glucosamine (sp. act. 7.0 mCi/mmol) and [methyl-^{3}H]-thymidine (sp. act. 20.0 Ci/mmol) were purchased from New England Nuclear. [^{3}H]-3-*O*-Methyl-D-glucose (sp. act. 65.0 Ci/mmol) was from ICN; ^{3}H-inulin (sp. act. 2.42 Ci/mmol) and L-[4,5-^{3}H] leucine (sp. act. 130 Ci/mmol) were from Amersham. All other chemicals were from Sigma Chemical Company.

Animals

Male Sprague-Dawley rats (200–250 g) were used in all experiments. Animals were fed commercial rat chow *ad libitum* or fasted overnight prior to experiments (16 h).

Preparation and Storage of BBMV

BBMV were prepared from cell homogenates or scrapings by the $CaCl_2$ precipitation method of Schmitz (10) as modified by Kessler and coworkers (11). The final pellet was resuspended (1 mg/ml) in 10 mM Tris/Hepes buffer (pH = 7.5) containing 50 mM mannitol. Vesicle preparations were frozen with liquid nitrogen and stored at −80°C according to Stevens and coworkers (12).

Isolation of Epithelial Cells

Two procedures of cell isolation were followed:
1) The procedure developed by Weiser (7) which involves the exposure of the mucosal surface to EDTA in order to dissociate the cells.
2) The method of Hartmann and coworkers (8) which includes intravascular perfusion of the intestine with collagenase.

In both methods, sequential collection of cells at specific time intervals during the incubation or perfusion of the intestine afforded separation of villus cells from crypt cells. To improve cell viability, the following modification was introduced: freshly isolated cells were suspended in Krebs-Henseleit buffer with 10 mM glucose or in Eagle culture medium and kept on ice in an atmosphere of 95 % O_2/5 % CO_2 for 15 min). Cells exposed to nutrients exhibited increased viability, even after the media had been washed out and replaced by glucose-free buffer.

Cell Viability

The viability of the freshly prepared cells was determined with the Trypan Blue dye exclusion test. Preparations with viability greater than 80 % were used for all studies.

Preparation of Everted Intestinal Rings

Segments of small intestine were rinsed with cold saline, everted over a glass rod and cut into rings weighing approximately 30–50 mg. The rings were immediately placed in cold Krebs-Henseleit buffer or Eagle culture medium and continually gassed with 95 % O_2/5 % CO_2.

Estimation of Extracellular Space

Extracellular space in preparations of everted intestinal rings was calculated using ^{3}H-inulin according to Rosenberg and coworkers (13). There was no measurable trapping of ^{3}H-inulin in cells pelleted by centrifugation through a sucrose layer; therefore, extracellular space adjustments were not required with the isolated cell experiments.

Electron Microscopy Studies

Cells and BBMV were pelleted by centrifugation and resuspended in cold Karnovsky's fixative in 0.1 M cacodylate buffer, pH = 7.4. They were fixed for four hours, washed in the same buffer overnight, postfixed for two hours in 1 % OsO_4, dehydrated in ethanol, and embedded in Spurr Plastic. Thin sections were stained with lead citrate and uranyl acetate (14).

Histology

Tissue sections of rat intestine were placed in Bovin-Dubosque fixative. Following embedding of the tissue in parafin, 7 μm sections were prepared and stained with hematoxylin and periodic acid-Schiff reagent (15).

Enzyme Assays

Cells were suspended in the appropriate buffer for the determination of enzymatic activity and were frozen and thawed three times to release intracellular enzymes. The resulting lysate was centrifuged at 20,000 g for 20 min and supernatant assayed for enzymatic activity.

BBMV were suspended in the appropriate buffer for the enzyme assays. Alkaline phosphatase activity was determined with *p*-nitrophenyl phosphate as reported by Weiser (7). Sucrase activity was measured using the Tris/glucose oxidase reagent (16). Na/K-ATP-ase was determined by the method of Proverbio and Castillo (17). Inorganic phosphate was measured according to Fiske and Subbarow (18).

^{3}H-Leucine Incorporation

Cells (10^6 cells/ml) were resuspended in Eagle culture medium and incubated in an atmosphere of 95 % O_2/5 % CO_2 at 37°C in the presence of ^{3}H-leucine (7.7 μM). At the end of the incubation, samples were mixed with 20 % trichloroacetic acid (TCA) and the precipitate collected by filtration (Whatman GF/C glass fiber filters). Radioactivity was measured in a liquid scintillation counter (Beckmann LS 3133T) using 3a70 (RPI) scintillation cocktail and employing an external standard for quench correction. For protein determination, the precipi-

tate was collected by centrifugation (300 × g, 15 min) and dissolved in 1 ml of 2 % Na_2CO_3 in 0.1 N NaOH.

^{14}C-Glucosamine Incorporation

Cells (10^6 cells/ml) were resuspended in Eagle culture medium and incubated under O_2/CO_2 in the presence of ^{14}C-glucosamine (71.4 μM). Incorporation experiments were terminated by filtration through Whatman GF/C glass fiber filters. Radioactivity and protein were measured as described above.

^{3}H-Thymidine Incorporation

^{3}H-Thymidine (100 μCi) was injected into rats intraperitoneally and cells isolated three hours later (allowing sufficient time for nucleic acid synthesis). Radioactivity and protein were measured in material precipitated from cells by the TCA procedure.

Glucose Uptake Studies

BBMV (1 mg protein/ml) were incubated at 25°C in 10 mM Hepes/Tris pH 7.5, 100 mM NaCl in the presence of 1 mM D-[^{3}H]-glucose (sp. activity 10 mCi/mmol) or 1 mM L-[^{3}H]-glucose (sp. activity 10 mCi/mmol). Iso-osmolarity was maintained with D-mannitol. Cells (10^6 cells /ml) and intestinal rings (20–50 mg) were incubated at 37°C in an atmosphere of 95 % O_2/5 % CO_2 in Krebs-Henseleit buffer, pH 7.4, in the presence of 1 mM 3-*O*-methyl-[^{3}H]-glucose or 1 mM L-[^{3}H]-glucose. Phlorizin, an inhibitor of glucose transport (19), was included in the incubations at 0.5 mM where indicated.

Salicylate Uptake Studies

BBMV were suspended in 10 mM Tris/Hepes buffer (pH = 7.5) with 100 mM mannitol and incubated in the presence of the drug at 25°C. Uptake was terminated by a rapid filtration technique using Sartorius filters (0.45 μm pore size). Filters were extracted with a mixture of water and methanol (1:1) for 12 h (87 % recovery). Extracts were assayed for the presence of drug by high pressure liquid chromatography (HPLC).

Cells were resuspended in Eagle culture medium or in Krebs-Henseleit buffer, incubated under O_2/CO_2 in the presence of substrate, and then separated by centrifugation through 12 % sucrose with 2.5 % BSA or by rapid filtration (Whatman GF/C glass fiber filters). Pellets and filters were extracted with 1 N perchloric acid or with a mixture of water and methanol (1:1), respectively, for 12 h (85 % recovery). Extracts were assayed for the presence of drug by HPLC.

Rings were incubated under O_2/CO_2 in the presence of drug. At the end of the incubation, rings were blotted on Whatman No. 1 filter paper and placed in 1 N HCl or perchloric acid. After 12 h (90–95 % recovery), aliquots of the resulting tissue extracts were assayed for the presence of drug.

Assay of Salicylate

Salicylate content in vesicle, cell and ring extracts was determined by high pressure liquid chromatography employing UV detection at 303 nm. A Brownlee reverse phase C18 column (5 micron particle size) was used with an 18 % acetonitrile, 0.04 % ammonium, 0.4 % formic acid mobile phase. The detection limit was 0.1 μg/ml with a coefficient of variation of 5.6 %.

Protein Assay

Protein was measured by the method of Lowry and coworkers (20) with bovine serum albumin as the standard.

Results

As shown in Fig. 1 A, freshly prepared brush border membranes were obtained in a vesicular form. Some contamination by mitochondrial particles was visible. The orientation of the vesicles appeared to be right side out, as indicated by estimation of the sucrase activity in the presence and absence of Triton X-100 (Table I). Sucrase active sites are located on the outer surface of the mucosal membrane (11). The hydrolysis of sucrose was not increased when vesicles were lysed with detergent (Table I). BBMV preserved in liquid nitrogen (12) were used in a majority of experiments. Their initial morphology was identical to freshly prepared vesicles. After approximately five days to three weeks of storage at −80°C, vesicles tended to convert into sheets which coincided with the loss of transport properties (data not shown).

The final preparation of vesicles from brush border membranes was purified approximately ten-fold as compared to crude cell homogenates (Table I). Both brush border membrane marker enzymes were obtained with approximately 20 % recovery. High specific activity of the basolateral membrane marker enzyme, Na/K-ATP-ase, shows some contamination by contraluminal membrane fragments.

Table I Activity of Marker Enzymes in BBMV Preparations.

Enzyme	Cell Homogenate μmol/min/mg protein (mean ± S.E.)	Vesicles	Purification Factor	Recovery % (mean ± S.E.)
Alkaline Phosphatase	3.53 ± 0.05	33.75 ± 1.0	9.56	22.56 ± 0.70
Sucrase	0.19 ± 0.02	2.00 ± 0.28	10.60	18.52 ± 1.22
	0.18 ± 0.01[a]	1.99 ± 0.03[a]		
	nmol/min/mg protein			
Na^+/K^+-ATPase	153	187	1.22	2.18

Vesicles (1 mg protein/ml) were suspended in 0.1 M Tris HCl buffer pH 7.4 for alkaline phosphatase assay, in 0.1 M sodium maleate buffer pH 6.0 for sucrase assay, or in 0.1 M Tris HCl buffer pH 7.0 for ATPase assay. Osmolarity of the reaction mixture was adjusted with D-mannitol. Results are based on 3–5 determinations. The activity of Na^+/K^+ATPase was estimated only for one preparation.

[a] Activity measured in the presence of 0.25 % Trition X-100.

Table II. Comparison of the Weiser-EDTA Incubation and Hartmann-Collagenase Vascular Perfusion Procedures for Isolated Mucosal Cell Preparation: Cell Viability

Cell Preparation Procedure	Initial Mucosal Cell Viability (%) (Mean ± S.E.)
Weiser	
Villus Enriched Cell Population	81 ± 7.7
Hartmann	
Villus	50 ± 7.8*
Mid villus	54 ± 11.5*
Crypt	51 ± 8.5*

Cells obtained by the Weiser procedure were a mixture of villus, midvillus and crypt cells. Immediately after isolation and washing, cell viability was determined by Trypan blue dye exclusion with a minimum of 100 viable cells being observed. * Significantly less than Weiser preparation viability ($p < 0.05$, n = 3).

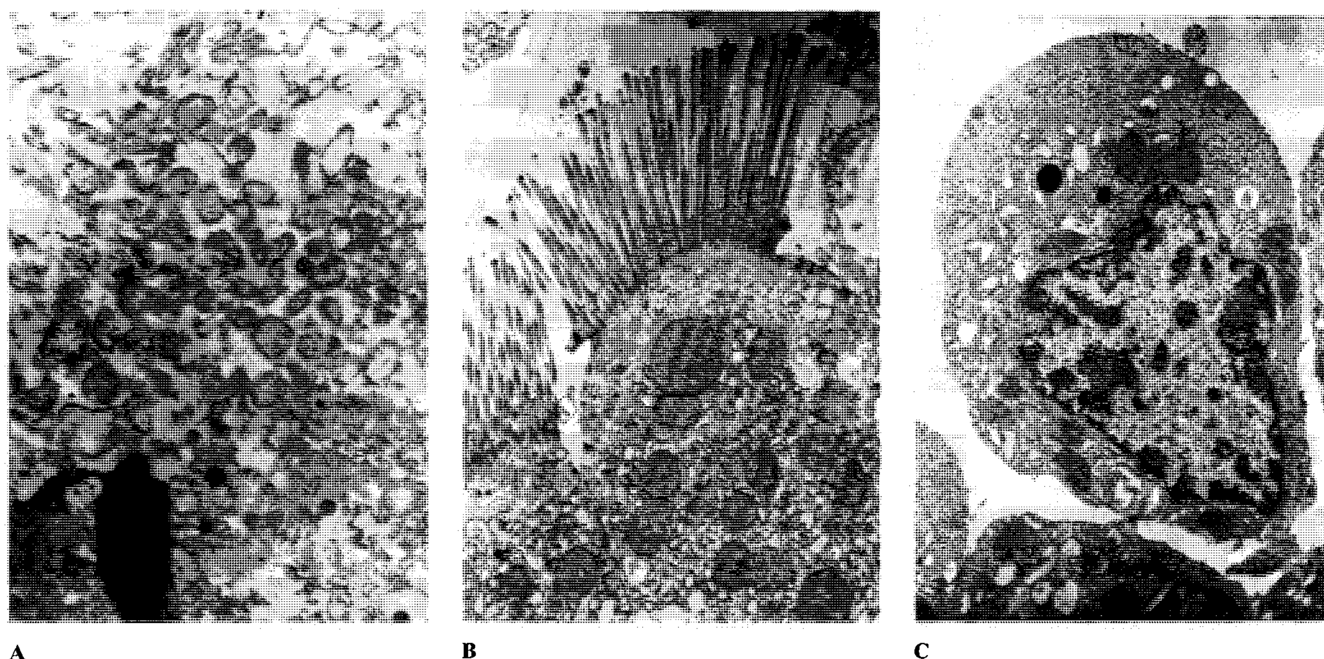

Fig. 1 Electron micrographs of brush border membrane vesicles (A, ×33,000), villus cell (B, ×10,000) and crypt cell (C, × 10,000).

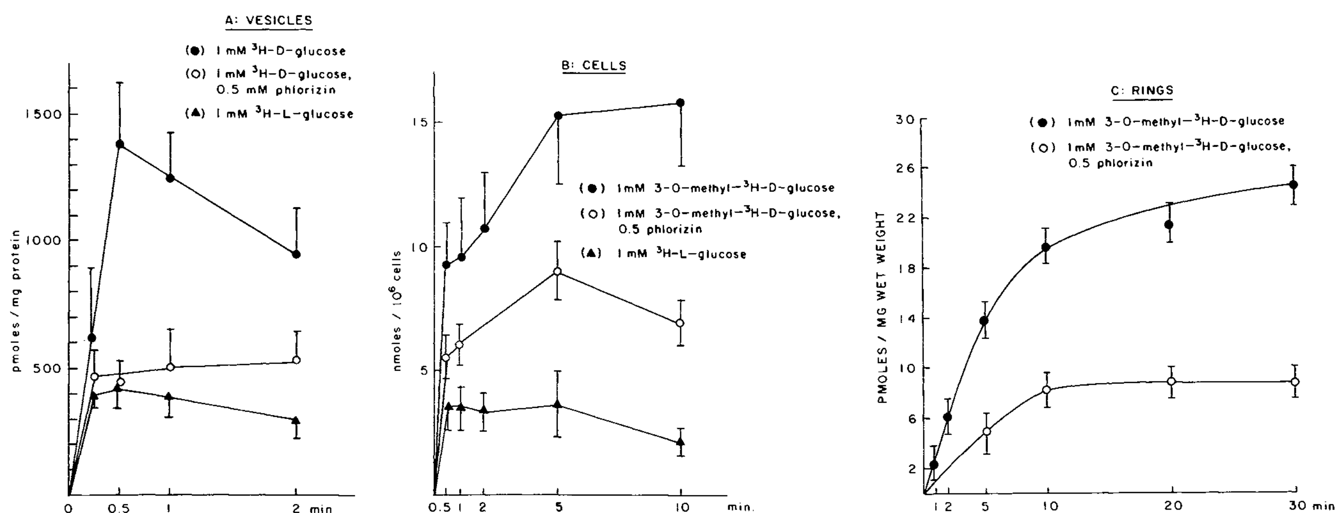

Fig. 2 Time course of glucose uptake in brush border membrane vesicles (A), isolated mucosal cells (B) and everted intestinal rings (C). All results are the mean ± S.D. from 3–6 experiments. Phlorizin was included in the incubations at 0.5 mM where indicated. Vesicles (1 mg protein/ml) were incubated at 25°C in 10 mM Hepes/Tris pH 7.5, 100 mM NaCl in the presence of D-[^{3}H]-glucose (sp. activity 10 mCi/mmol) or L-[^{3}H]-glucose (sp. activity 10 mCi/mmol). Iso-osmolarity was maintained with D-mannitol. Cells (10^6 cells/ml) and intestinal rings (20–50 mg) were incubated at 37°C in an atmosphere of 95 % O_2/5 % CO_2 in Krebs-Henseleit buffer, pH 7.4, in the presence of 3-*O*-methyl-[^{3}H]-glucose or L-[^{3}H]-glucose.

Characteristics of the BBMV sugar transport system are presented in Fig. 2 A. The uptake of D-glucose by BBMV shows a typical overshoot caused by the initial Na^+ gradient. Accumulation of D-glucose was decreased in the presence of phlorizin. L-glucose equilibrated slowly, showing no temperature or phlorizin dependence.

An increase of medium osmolarity up to 800 mOsM resulted in a decrease of the glucose uptake. This effect indicates that the uptake corresponds to transport into an osmotic space rather than to nonspecific absorption on the surface of the membrane. The data support the existence of an intact, Na^+ dependent, active glucose transport system in the BBMV.

Weiser's procedure for cell isolation (EDTA intestinal perfusion) yielded 5×10^8 cells per rat small intestine, whereas only half of this amount was obtained with the Hartmann preparation (collagenase vascular perfusion). The Weiser procedure resulted in two enriched cell fractions: villus and crypt cells. The Hartmann procedure allows the separation of three relatively distinct cell populations: villus (tip), midvillus and crypt cells. The viability of cells prepared according to Hartmann and coworkers was significantly lower than those prepared by Weiser's procedure (Table II).

The initial viability of Weiser's cells was about 80 % as estimated by Trypan Blue dye exclusion. In the original

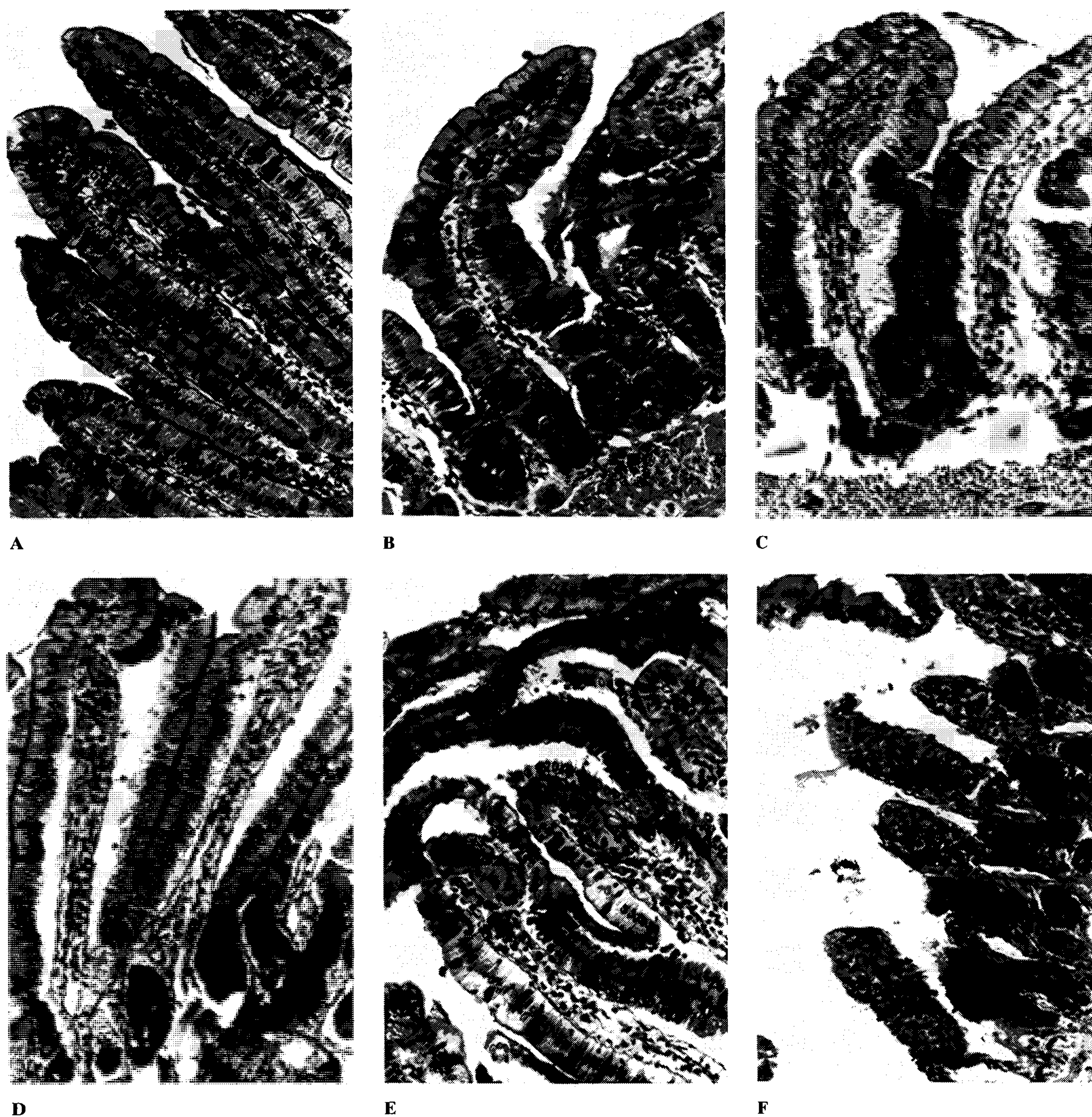

Fig. 3 Cross-sections of rat small intestine rings after incubation at 37°C (magnification ×160). A "0" time, B 5 min, C 10 min, D 20 min, E 40 min, F 60 min.

method, cells were collected and suspended in phosphate-buffered saline. Krebs-Henseleit buffer or Eagle's culture medium was found to be more suitable for storage and incubation of the isolated cells.

As shown in Table III, cells prepared by EDTA perfusion (Weiser's procedure) retained higher viability when incubated at 37°C for 15 min if they were initially stored (4°C) in either Krebs with glucose or Eagle's medium. Freshly isolated cells suspended in Krebs buffer without glucose showed rapid loss of viability when incubated at 37°C in the same buffer. Cells suspended initially in Krebs buffer containing glucose or in Eagle's medium and then transferred into the glucose-free medium for incubation at 37°C exhibited the same viability as cells incubated in Krebs buffer with glucose or in Eagle's medium. The exposure of cells to nutrients such as glucose and amino acids in the preliminary storage phase at 0–4°C apparently allows cells to maintain their viability even after these media had been washed out. This observation is consistent with the results of Crafstrom and coworkers (4).

When used as a storage medium, Eagle's culture medium was more effective than Krebs-Henseleit buffer with 10 mM glucose in preventing cells from deteriorating during incubation at 37°C (Table III). Therefore, Eagle's medium was chosen to store and incubate isolated mucosal cells in all

experiments (except for the glucose uptake experiments where Krebs buffer without glucose was used).

The gross morphology of freshly isolated cells was routinely examined by light microscopy. Many single cells retained their polarity, others became spherical. Occasional sheets or clusters of cells were also observed. No major morphological differences were observed between the two methods of cell isolation at the light microscope level.

Transmission electron micrographs of the cells prepared according to Weiser revealed a good retention of the subcellular structure. A typical villus cell with intact microvilli is shown in Fig. 1 B. Figure 1 C shows a typical crypt cell, characterized by the large nucleus.

Cells prepared by the method of Hartmann and coworkers did not exhibit a satisfactory preservation of the ultrastructure. Many of the cells were seriously damaged, while others had a fragmented brush border region, swollen nuclei and swollen mitochondria (data not shown).

Alkaline phosphatase and sucrase are located in the brush border membrane of enterocytes. Both enzyme activities were shown to be higher in the villus cell population, regardless of the method of cell preparation (Table IV). The crypt to villus gradient of brush border enzyme activities confirms observations of other investigators (7, 8), reflecting the process of cell differentiation during movement to the tip of the villus. The lower alkaline phosphatase activity observed in cells prepared by the Weiser procedure may be due to residual EDTA in the cell suspensions. Small amounts of this chelating agent may interfere with divalent cation activation of the brush border enzymes. The presence of divalent cations in the incubation buffer should, however, minimize this possibility.

The incorporation of glucosamine into the acid precipitable material (Table V) was highest in villus cells, regardless of the method of cell preparation. The uptake of glycoprotein precursors coincides with development of the brush border during migration from the crypt to the villus region. Again, a specific crypt to villus gradient was observed. This was further illustrated by differences in the extent of incorporation of ^{3}H-leucine and ^{3}H-thymidine, markers of protein and nucleic acids synthesis, respectively. Incorporation was much higher in crypt cell populations, which consist of rapidly dividing, poorly differentiated enterocytes. The results presented in Table V indicate expected metabolic activity and good separation of the different cell populations.

Glucose is known to be absorbed in the intestine via an active transport system. Therefore, this activity of the isolated mucosal cells was of the greatest importance with respect to evaluation of the suitability of the cell system for drug transport studies. As shown in Fig. 2 B, cells prepared according to the Weiser procedure were able to accumulate 3-*O*-methyl-D-glucose. This process was inhibited in the presence of phlorizin, a specific blocker of the glucose carrier. The uptake of L-glucose was much lower, indicating stereospecificity of the transport system.

No glucose uptake was observed with the use of cells prepared by the method of Hartmann and coworkers, indicating poor viability of this preparation.

The structural integrity of everted intestinal rings was verified under experimental conditions. Histological examination of rings immediately after eversion ("0 time" specimens) showed no morphological changes produced by the mechanical manipulation (Fig. 3 A). Incubation at 37°C for 5 min in oxygenated Eagle's medium did not affect the structure of the mucosa (Fig. 3B). However, the appearance of the tissue

Table III. Effect of Storage Time at 4°C in Krebs with and without Glucose Buffer and Eagles Medium on Mucosal Cell Viability

Time in Storage Buffer (4°C) (min)	Per cent Initial Viability	Mucosal Cell Viability (Per cent) Incubation Buffers (37°C): Krebs Without Glucose	Krebs With Glucose	Eagles
Krebs without Glucose				
15	79	52	63	79
Krebs with Glucose				
15	87	82	76	81
60	87	56	60	48
Eagles				
15	88	84	85	75
60	88	82	75	86

Cells were prepared by the Weiser-EDTA incubation procedure. Freshly isolated cells (with the indicated initial viabilities) were stored in the indicated storage buffers at 4°C for either 15 or 60 min. Following the storage period, cells were washed twice with the appropriate incubation buffers and incubated in the same buffers at 37°C for 15 min. Viability was then determined by Trypan blue dye exclusion.

Table IV. Alakaline Phosphatase and Sucrase Activity in Intestinal Mucosal Cells

Cell Preparation		Alkaline Phosphatase µmol/min/mg protein (mean ± S.E.)	Sucrase µmol/min/mg protein (mean ± S.E.)
Weiser	Villus	4.84 ± 0.02	0.244 ± 0.04
	Crypt	2.32 ± 0.12	0.108 ± 0.02
Hartmann	Villus	13.93 ± 0.65	0.272 ± 0.01
	Mid Villus	10.27 ± 1.30	0.180 ± 0.01
	Crypt	2.47 ± 0.17	0.122 ± 0.01

After isolation, cells were suspended in 0.5 M Tris/HCl buffer pH 7.4 for alkaline phosphatase assay and in 0.1 M sodium maleate buffer pH 6.0 for sucrase assay. Results are based on 3–5 determinations and are expressed as µmol of *p*-nitrophenol (phosphatase) and glucose (sucrase) released per milligram protein per minute.

Table V. ^{14}C-Glucosamine, ^{3}H-Leucine and ^{3}H-Thymidine Incorporation by Isolated Mucosal Cells from Rat Jejunum

Cell Preparation		Total Incorporated Radioactivity % (mean ± S.E.): ^{14}C-glucosamine	^{3}H-leucine	^{3}H-thymidine
Cells isolated by the method of Weiser	Villus	74.88 ± 1.8[a]	27.12 ± 4.0[a]	29.33 ± 0.26[c]
	Crypt	25.16 ± 1.8[a]	72.87 ± 4.2[a]	70.66 ± 0.26[c]
Cells isolated by the method of Hartmann et al.	Villus	52.06 ± 5.3[a]	12.79 ± 0.77[b]	7.33 ± 0.51[c]
	Mid villus	36.63 ± 5.5[a]	28.67 ± 4.25[b]	31.20 ± 5.05[c]
	Crypt	11.26 ± 2.3[a]	58.37 ± 3.58[b]	61.47 ± 4.56[c]

[a] Experiment performed *in vitro*, cells (10^6 cells/ml) were incubated for 60 min in the presence of ^{14}C-glucosamine (71.4 µM); n = 3.
[b] Experiment performed *in vitro*, cells (10^6 cells/ml) were incubated for 30 min in the presence of ^{3}H-leucine (7.7 µM); n = 6.
[c] Experiment performed *in vivo*, cells were isolated three hours after intraperitoneal injection of ^{3}H-thymidine (100 µCi/rat); n = 6.

changed markedly after 10 min of incubation at 37°C. The preparations lost color and the consistency was less firm. The nuclei of these epithelial cells showed initial signs of swelling (Fig. 3 C). After 20 min of incubation under the same conditions, the tissue was very pale with significant edema of the villi (Fig. 3 D). Incubation for 40 min caused serious disruption of the mucosal tissue (Fig. 3 E). The epithelial lining disappeared completely after 60 min (Fig. 3 F).

The disruption and hydration of intestinal segments following incubation at 37°C have been reported by a number of authors (21). These results are in disagreement with the report of Shaw and coworkers (2), however, who have not observed any changes in extracellular space between 5 and 60 min of incubation at 37°C. In our studies, extracellular space increased from 8 % at 2 min to 16 % at 20 min of incubation. The increase in extracellular space was linear with time ($r = 0.9992$ by linear regression, $y = 0.366x + 6.36$) from 2 min to 60 min of incubation at 37°C.

All drug uptake data have been corrected for the extent of extracellular space, based on the results of the present study.

Everted intestinal rings were shown to accumulate 3-*O*-methyl-D-glucose via an active, phlorizin sensitive transport system (Fig. 2 C). Similar results were seen with vesicles and cells.

In order to estimate potential drug accumulation in the muscle layer, the distribution of glucose between mucosa and muscle was determined. After 2 min of incubation, rings were rinsed with cold saline and the mucosa was carefully removed by scraping. Approximately 85 % of absorbed glucose was associated with epithelial cells. Similar experiments with sodium salicylate showed only 60 % of the drug being associated with the mucosal layer.

BBMV, isolated mucosal cells and everted intestinal segments were employed for drug transport studies. The results of sodium salicylate uptake illustrate the drug transport capability of all three *in vitro* systems (Table VI).

Freshly prepared BBMV exhibited time dependent salicylate uptake. The amount of the absorbed drug, however, was dependent on the length of storage of the vesicles at −80°C prior to use and was highly variable between different BBMV preparations.

Isolated mucosal cells did not show time dependent salicylate uptake. This may be explained by a technical difficulty in accurately determining the uptake at short time intervals (centrifugation requires 30–60 sec to completely separate cells from the reaction solution).

Table VI. Time Dependence of Salicylate Absorption in BBMV, Mucosal Cells and Everted Intestinal Rings

Time (Min)	Salicylate Uptake (Mean ± S.E.) BBMV[a] (μg/mg protein)	Cells[b] (μg/mg protein)	Rings[c] (μg/mg wet wt.)
0	–	19.0 ± 0.6	–
0.25	7.8 (n=1)	–	–
0.50	14.1 ± 1.1	–	–
1.0	27.9 ± 2.3	–	–
2.0	21.8 ± 1.4	17.5 ± 0.8	1.15 ± 0.05
5.0	15.4 ± 0.7	–	2.61 ± 0.10
10.0	–	18.4 ± 1.6	3.50 ± 0.16
20.0	–	–	3.80 ± 0.18

A mixture of villus, mid villus and crypt cells, prepared by the Weiser-EDTA incubation procedure, was used for cellular salicylate absorption studies. BBMV were prepared as previously described. Intestinal rings (2–3 mm) were prepared from everted rat jejunum. All results are based on incubations at 37°C, pH 7.2 (n = 3–6).
Salicylate concentration in incubation buffer:
[a] 1.6 mg/ml, [b] 5.0 mg/ml, [c] 20.0 mg/ml

Everted intestinal segments absorbed sodium salicylate in a time dependent manner. This was observed in all experiments performed with rings, which indicated a high reliability of the system. The variability among the samples from a single rat was very low (< 5 %), whereas the inter-animal variability (30–35 %) was significantly greater.

The high extent of drug accumulation in the muscle layer of intestinal segments (approximately 40 %) and lack of the temperature effect (22) suggested a passive diffusion mechanism for salicylate transport.

Discussion

The majority of the information available on intestinal drug absorption is based on experiments performed *in vivo*, which does not allow a detailed examination of membrane transport mechanisms. The use of *in vitro* systems to study intestinal absorption is not a new concept, although the specific application in the current studies affords advantages over previous work. The major application of *in vitro* systems has been the study of nutrient absorption, primarily glucose and amino acids (5, 23). Many studies, however, have been confined to one *in vitro* model, and have not examined the relative advantages or disadvantages of other systems.

In the study reported here, three *in vitro* systems have been examined: everted intestinal rings, isolated mucosal cells and BBMV. The systems were evaluated on the basis of ease of preparation, viability or sustained functional capacity, day-to-day variation in preparations, techniques required for drug transport studies, reproducibility of drug absorption and projected correlation with known *in vivo* drug absorption. It should be noted that, as *in vitro* systems become further removed from the normal *in vivo* situation, there is a balance which must be achieved between increased experimental control and potential decreased correlation with the true *in vivo* system.

As an example, brush border membranes are the site of nutrient and drug uptake in the intestine. One way to increase understanding of the mechanism of luminal absorption is to dissect the cell system into smaller components, such as isolated membrane vesicles. The use of membrane preparations allows control of both external and internal composition of the vesicles, as well as the driving forces in the transport process. Complicating effects of cellular metabolism can also be excluded. However, since controlling physiological functions of an intact cell are not present, processes that are influenced by cellular enzymatic or metabolic activity may not be accurately reflected by membrane vesicle studies. The results from studies with BBMV, which appear to be well suited for drug absorption studies (24), must be interpreted with care.

Kessler's modification (11) of Schmitz method (10) is a simple, rapid and efficient isolation procedure. The procedure yielded large numbers of mostly closed, relatively pure vesicles, capable of accumulating sugars and amino acids. The effect of sodium phlorizin and L-glucose on the uptake of D-glucose suggested that a Na-dependent, highly specific glucose-carrier remained intact in these membranes. However, in contrast to Kessler and coworkers (11) who reported an excellent stability of their preparations, occasional populations

of freshly isolated vesicles were not functionally active (glucose transport was not observed). When stored in liquid nitrogen at −80°C, some preparations remained unchanged for up to three weeks, while others lost their activity after several days.

With sodium salicylate as a model compound for drug absorption studies, BBMV were found to exhibit time dependent drug accumulation, with peak absorption occurring at 1 min (Table VI). The "overshoot" in salicylate accumulation and subsequent decrease in drug content may be caused by the initial Na^+ driving force or gradient, similar to that observed with glucose (25).

The data shown in Table VI for salicylate accumulation in BBMV was obtained on a single, freshly prepared population of vesicles. As mentioned previously, drug accumulation varied considerably between different preparations and on storage. This high variability, often exceeding 50%, makes it very difficult to reliably compare drug absorption data obtained on different days. The variability observed is probably a combination of two main factors: day-to-day variation in vesicle preparations (mentioned above) and variability due to technical aspects of drug uptake experiments. With a rapid filtration technique, incubations can be terminated as soon as 5 sec. Even if the filtering and washing require only 2–3 sec, the variability introduced can be large for the very early time points which are required. Approximately 98% of the protein in a sample was routinely recovered in the filters, indicating that vesicles were not being lost by passing through the membrane. This does not, however, preclude the possibility of leakage of drug from the vesicles during filtration.

In general, BBMV offer the advantages of relatively easy preparation, large quantities available for *in vitro* studies and the ability to accumulate drugs, as salicylate. However, the daily variation in structural integrity, viability and drug transport, as well as changes during storage, tend to offset these positive aspects. While vesicles may be useful for some detailed studies of membrane transport, their general utility as a tool for drug absorption studies appear limited.

Isolated mucosal cells offer a greater degree of complexity than is present in vesicles since the metabolic machinery of the cell is intact. The main advantages of isolated cells are their closer resemblance to the true cellular barrier and the ability to obtain relatively homogenous cell populations (villus versus crypt cells). This latter advantage offers the potential to study drug absorption as a function of cell type on the villus projections.

Two procedures which allow a separation of crypt and villus cells have been examined. Among various methods of enterocyte isolation tested by other authors (27), that of Weiser (7) with EDTA as a dissociating factor has given the best results. The procedure is simple and rapid with no added proteases or other enzymes and minimal risk of mechanical overmanipulation. On the other hand, the method developed recently by Hartmann and coworkers (8) which includes intravascular perfusion of the intestine with collagenase appeared to offer some advantages. The maintenance of temperature, oxygenation and nutrients in the perfused intestine and avoidance of mechanical stress are important aspects of this method. Unfortunately, it was found to be technically complicated, time consuming and yielded preparations of poor viability (as estimated by the Trypan Blue dye exclusion test and examination of the ultrastructure). Among the several criteria of cell viability, the preservation of subcellular structure appeared to be the most sensitive. The results of electron microscopy studies demonstrated that the majority of cells obtained by the method of Hartmann and coworkers did not exhibit satisfactory retention of their normal structure. These authors themselves report the existence of ultrastructural changes in cell preparations, including swelling of mitochondria, dilatation of cisternae of the rough endoplasmic reticulum, fragmentation of microvilli and vacuolization. In contrast to Hartmann's procedure, cells isolated according to Weiser were characterized by excellent viability and unchanged ultrastructure.

Enzymatic and metabolic characteristics of both cell preparations did not show any significant differences. Brush border membrane marker enzymes were more active in tip villus cells, with a decrease of the activity along the length of villus, regardless of the method of cell isolation. The level of glucosamine, leucine and thymidine incorporation was identical in both preparations. The observation that glucosamine incorporation was highest in villus cells agrees with that of Weiser (7), but differs from the data of Hoffman and Kuksis (28) and Hartmann and coworkers (8), who reported the highest glucosamine uptake in crypt cells. The suggested explanation (28) that these discrepancies are due to different viabilities of crypt cells obtained by different methods seems unlikely, since the two cell preparations have been compared under the same conditions in this study and glucosamine uptake was examined in cells with comparable viabilities. Weiser's conclusion that the more highly differentiated upper villus cells incorporate more sugar precursors into membrane glycoproteins associated with membrane bound enzymes may be a reasonable interpretation. Moreover, the peak of glucosamine incorporation coincided with peaks of sucrase and alkaline phosphatase, which have been shown to be glycoproteins (29, 30). The incorporation of leucine into proteins and thymidine into DNA demonstrated a typical crypt to villus gradient in both cell preparations, which agrees with the data of other investigators (7, 8, 31).

The active transport of glucose, an important functional criterion of cell viability, was observed only with cells prepared according to Weiser's procedure. Based on their glucose transport ability and higher viability, Weiser's preparations were considered more suitable for drug transport studies.

The selected method yields sheets of columnar cells as well as individual or cluster forming cells that lose their polarity rapidly. It is very difficult to obtain a homogeneous distribution of rat enterocytes in suspension because of high mucus secretion, resulting in cell clumping (26, 27). Other species are known to produce less mucus, and thereby reduce cell clumping (32). However, rats were pursued as an experimental model since most *in vivo* drug absorption data have been obtained with rats. The high variability observed in the results of this study may have been partly due to unequal sampling of clumped or clustered cells. Dithiothreitol was added to the isolation medium to reduce mucus, but clustered cells were still observed. Other anti-clustering agents, such as tetraphenylborate (6) and albumin (5), were avoided to minimize possible interferences with membrane transport processes.

Membrane polarity (luminal versus serosal) is a distinctive feature of mucosal cells. The loss of this polarity in isolated cells presents another factor that can change membrane transport as compared to *in vivo* conditions, and is one of the most important disadvantages of the cell suspension model. Quaroni and coworkers (33) have maintained rat enterocytes in culture, but these are nondifferentiated crypt cells which do not grow in monolayers. The availability of both the basolateral membrane and the brush border membrane region to drug absorption in isolated cell studies complicates interpreta-

tion of the data. Drugs that utilize a specific transport system in the brush border region may be adequately studied in isolated cell systems. However, compounds that passively cross the membrane barrier by diffusive mechanisms present a more complicated analysis. The use of isolated cell systems assumes equal diffusivity across the brush border and basolateral regions, which may not be true for all compounds.

When sodium salicylate was employed to examine drug accumulation in isolated mucosal cells, drug uptake was observed but time dependence could not be demonstrated. Based on the membrane vesicle work which demonstrated peak salicylate absorption by 1 min of incubation, it seems likely that the inability to observe time dependence with isolated cells was a technical problem. Cells were too fragile to be separated by the rapid vacuum filtration technique used with vesicles. Instead, a separation and washing method was used where the cells were centrifuged through a dense sucrose layer to separate them from the incubation solution. While this effectively separates cells from drug solution, the process requires at least 1 min to totally remove the cells. With drugs such as salicylate, with very rapid absorption rates, the time required to terminate the reaction may preclude any demonstration of time dependent drug uptake. This is not a major disadvantage if equilibrium drug levels are desired, but kinetic data would be more difficult to accurately determine.

The use of everted intestinal rings affords a significant advantage in that the structural integrity of the mucosal barrier is preserved. Epithelial cells retain a number of highly differentiated properties including anatomical and functional polarization due to the presence of tight junctions and normal cell-to-cell contact. This results in a predominant access of the drug to the brush border membrane, similar to *in vivo* conditions. Everted rings did not exhibit any morphological changes caused by manipulation of the tissue. They contained a fully active D-glucose transport system. Since the preparation of the rings is extremely rapid and technically simple, they provide a good model for screening studies of drug uptake.

The results of salicylate uptake experiments with everted rings had the highest reproducibility of all three systems examined. The first detectible levels of salicylate in the rings was observed at 2 min of incubation. The levels gradually increased to 10 min and appeared to reach nearly constant levels at 10 to 20 min. Standard errors were less than 5 % indicating good reproducibility between samples. The use of everted intestinal rings suffers from the small number that can be obtained from one animal. It is possible to mix and randomize rings from several rats, but since inter-animal variability was relatively high, this reduces the reproducibility of the studies.

All data have to be interpreted carefully, taking into consideration disadvantages of the use of intestinal rings for transport studies (2, 34). There are two intestinal surfaces, luminal and serosal, accessible to the drug under experimental conditions. The presence of other than mucosal cell types complicates interpretation of the uptake. In the case of compounds absorbed through a passive diffusion mechanism, a high level of accumulation in the muscle layer compared to transport into mucosal cells was observed. For actively absorbed compounds, such as glucose or amino acids, approximately 85 % of recovered substrate was associated with mucosa, indicating that uptake was occurring across the luminal rather than serosal side.

The high extent of tissue degradation during incubation at 37°C, confirmed by histological examination and measurements of extracellular space, represents another limitation of this system. As such, only drug uptake over short time intervals should be investigated. From this point of view, all data based on incubations exceeding 20–30 min (2) are questionable.

On the basis of the direct comparison of BBMV, isolated mucosal cells and everted intestinal rings, it seems apparent that mucosal cells and everted intestinal rings merit further examination as potential *in vitro* models for intestinal drug absorption. BBMV, while allowing precise control of experimental conditions and availability of numerous samples from a single animal, demonstrate variability and time dependent changes which limit their use for routine uptake studies. Although isolated cells and everted rings each possess their own particular disadvantages, their ability to accumulate drug and serve as *in vitro* models are worth pursuing. For simple routine screening procedures with a limited sample number, everted rings may offer the simplest and most efficient model. More detailed investigations might best be performed with a combination of the isolated cell and everted ring approaches. The utility of isolated mucosal cells and everted intestinal rings as models for drug absorption has been examined in a subsequent study (22).

Acknowledgements

The authors wish to thank Linda Frost for her technical assistance in the assay of salicylate levels in this study.

References

(1) Tepperman, K., Finer, R., Donovan, S., Elder, R. C., Doi, J., Ratliff, D., Ng, K. (1984) Science 255, 430–432.
(2) Shaw, R. D., Li, K., Hamilton, J. W., Shug, A. L., Olsen, W. A. (1983) Am. J. Physiol. 245, G376–G381.
(3) Koster, A. S., Borm, P. J. A., Dohmen, M. R., Nordhoek, J. (1984) Cell Biochem. Function 2, 95–101.
(4) Grafstrom, R., Moldeus, P., Anderson, B., Orrenius, S. (1979) Med. Biol. 57, 287–293.
(5) Sepulveda, F. V., Burton, K. A., Brown, P. D. (1982) J. Cell. Physiol. 3, 303–308.
(6) Harrison, D. D., Webster, H. L. (1969) Exp. Cell Res. 55, 257–260.
(7) Weiser, M. M. (1973) J. Biol. Chem. 248, 2536–2541.
(8) Hartmann, F., Owen, R., Bissel, D. M. (1982) Am. J. Physiol. 242, G147–G155.
(9) Selhub, J., Rosenberg, J. H. (1981) J. Biol. Chem. 256, 4489–4493.
(10) Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J., Crane, R. K. (1973) Biochim. Biophys. Acta 323, 98–112.
(11) Kessler, M., Acuto, O., Storelli, C., Murer, H., Mueller, M., Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154.
(12) Stevens, B. R., Wright, S. H., Hirayama, B. S., Gunther, R. D., Ross, H. H., Harms, V., Nord, E., Kippen, J., Wright, E. M. (1982) Membr. Biochem. 4, 271–282.
(13) Rosenberg, L. E., Blair, A., Segal, S. (1961) Biochim. Biophys. Acta 54, 479–488.
(14) Glauert, A. M. (1975) Practical Methods in Electron Microscopy, North Holland Publishing Company, Amsterdam.
(15) Humason, G. L. (1972) Animal Tissues Techniques, Freeman and Company, San Francisco.
(16) Dahlquist, A. (1961) Methods in Enzymology VIII, 584–591.
(17) Proverbio, F., Castillo, R. (1981) Biochim. Biophys. Acta 646, 99–108.
(18) Fiske, C. H., Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400.
(19) Alvarado, F., Crane, R. K. (1962) Biochim. Biophys. Acta 56, 170–171.

(20) Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
(21) Levine, R. R., McNary, W. F., Kornguth, P. J., LeBlanc, R. (1970) Eur. J. Pharmacol. 9, 211–219.
(22) Porter, P. A., Osiecka, I., Borchardt, R. T., Fix, J. A., Frost, L., Gardner, C. R. (1985) Pharm. Res. 293.
(23) Stevens, B. R., Kaunitz, J. D., Wright, E. M. (1984) Ann. Rev. Physiol. 46, 417–433.
(24) Murer, H., Kinne, R. (1980) J. Membrane Biol. 55, 81–95.
(25) Yokota, K., Nishi, Y., Takesue, Y. (1983) Biochem. Pharmacol. 32. 3453–3457.
(26) Pinkus, L. M. (1981) Methods in Enzymology 77, 154–162.
(27) Hegazy, E., del Pino, V. L., Shwenk, M. (1983) Eur. J. Cell Biol. 39, 132–136.
(28) Hoffman, A. G. D., Kuksis, A. (1979) Can. J. Physiol. Pharmacol. 57, 1393–1400.
(29) Semenza, G., Tannenbaum, G., Kessler, M., Toggenburger, G., Wahlgren, L. (1977) in Biochemistry of Membrane Transport, FEBS Symposium 42, (G. Semenza, F. Carafoli, eds.), 269–279, Springer-Verlag, Berlin.
(30) Saini, P. K., Done, J. (1970) FEBS Lett. 7, 86–89.
(31) Bronstein, A. D. Leleiko, N. S., Munro, H. N. (1983) Biochim. Biophys. Acta 739, 334–343.
(32) Evans, E. M., Wrigglesworth, J. M., Burdett, K., Pover, W. F. R. (1971) J. Cell. Biol. 51, 452–464.
(33) Quaroni, A., Wands, J., Trelstad, R. L., Isselbacher, K. J. (1979) J. Cell Biol. 80, 248–265.
(34) Munck, B. G. (1981) in Physiology of the Gastrointestinal Tract, (L. R. Johnson, ed.), 1097–1122, Raven Press, New York.

In Vitro Drug Absorption Models. II. Salicylate, Cefoxitin, α-Methyldopa and Theophylline Uptake in Cells and Rings: Correlation with *In Vivo* Bioavailability

Patty A. Porter[2], Iwona Osiecka[1], Ronald T. Borchardt[1], Joseph A. Fix[2,3], Linda Frost[2], Colin Gardner[2]

Received: March 22, 1985; accepted: June 22, 1985.

Abstract: Isolated mucosal cells and everted intestinal rings have been examined as potential *in vitro* models for intestinal drug absorption. The uptake of salicylate, cefoxitin, α-methyldopa and theophylline was characterized on the basis of time, concentration and temperature dependence and compared to *in vivo* drug absorption. Theophylline was well absorbed in all systems. Biochemical studies supported a passive transport mechanism, although a significant temperature dependence was observed. Salicylate, cefoxitin and α-methyldopa demonstrated time- and concentration-dependent absorption. The uptake of α-methyldopa was temperature-dependent in both the isolated cell and ring studies. With all drugs, cellular uptake exhibited greater variability than drug accumulation in rings. A comparison of *in vitro* and *in vivo* absorption demonstrated a good correlation between the data from *in vivo* studies and intestinal rings. Cellular drug uptake did not completely mimic that observed *in vivo*. On the basis of technical aspects of preparation, reproducibility of results, and correlation with *in vivo* drug bioavailability, everted intestinal rings were judged to be the best *in vitro* model for intestinal drug absorption.

Drug absorption studies have typically been performed using whole animal models. Serum, plasma or urine drug levels are monitored following administration of an experimental dosage form, and conclusions based on pharmacokinetic analysis are formed. While this approach provides valuable information and is the ultimate test for drug absorption, there are certain disadvantages or restrictions with this method. Experiments are costly and time consuming, and a detailed analysis of drug interaction with the mucosal barrier membrane is difficult. Experimental variables are not easily controlled since the biological system is constantly adjusting to maintain homeostasis.

In attempts to study nutrient, ion or drug transport on a more detailed level, various *in situ* and *in vitro* models have been employed. *In situ* intestinal perfusions (1–3), isolated segments (4), everted sacs (5), intestinal rings (4), isolated cell suspensions (6–8) and membrane vesicles (9) have all been employed with varying degrees of success. Often, these systems have been investigated independently, without suitable comparisons between models or without comparison to known *in vivo* transport properties of the compounds under investigation.

In a previous report (10), we described the isolation and characterization of rat brush border membrane vesicles, mucosal cells and everted intestinal rings. Based on structural and functional integrity, reproducibility of results and demonstrable drug transport, everted intestinal rings and isolated mucosal cells were chosen for further evaluation as potential *in vitro* models for oral drug absorption. The transport of cefoxitin, salicylate, α-methyldopa and theophylline were examined in these *in vitro* systems and compared to *in vivo* absorption. The four model drugs were chosen to provide a series of test compounds with varying physical properties and anticipated differences in intestinal absorption profiles. Projections as to the utility of mucosal cells and isolated rings as suitable models for studying intestinal drug absorption are presented. The

[1] Department of Pharmaceutical Chemistry School of Pharmacy University of Kansas, Lawrence, Kansas 66045
[2] INTERx Research Corporation, Merck Sharp and Dohme Research Laboratories, 2201 W. 21st, Lawrence, Kansas 66046
[3] Address all correspondence to: Joseph A. Fix, Ph. D.
INTERx Research Corporation 2201 W. 21st
Lawrence, Kansas 66046