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Report

Cultured Buccal Epithelium: An *In Vitro* Model Derived from the Hamster Pouch for Studying Drug Transport and Metabolism

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Hamster pouch buccal epithelium (HPBE) was isolated and grown in primary cultures on rat-tail collagen-coated growth surfaces. The cultured pouch buccal epithelium (CPBE) was characterized morphologically with electron microscopy as stratified multilayers of epithelial cells with well-developed tonofibrillar–desmosomal complexes. Only the superficial layer of the cultured cells exhibited evidence of terminal differentiation. Alkaline phosphatase, alcohol dehydrogenase, and aminopeptidase activities in the primary cultured cells were determined by biochemical assays and found to be similar to those of homogenates of freshly excised hamster pouch epithelium. In addition, by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS PAGE), keratins and total proteins associated with the cultured cells were similar to those of freshly excised HPBE. The permeability characteristics of the cultured cells was determined by placing cultured cells grown on permeable polycarbonate disks in a Side-Bi-Side diffusion apparatus and quantitating the transcellular flux of tritium-labeled water, fluorescein, and fluorescein isothiocyanate dextrans (MW 3800 to 150,000). The cultured cells were least permeable on the third day of culture and were not permeable to substances with a MW greater than about 18,000. Our results indicate that primary cultures of hamster pouch epithelium exhibit biochemical properties similar to those of the excised hamster pouch epithelium from which they were derived. The morphological and permeability characteristics of cultured hamster epithelium were similar to those of nonkeratinized stratified oral epithelia typical of buccal mucosa in man, rabbit, and other species. CPBE, as described here, represents a potentially useful tool for *in vitro* drug transport, metabolism, pharmacology, and toxicology studies.

KEY WORDS: hamster pouch epithelium; cultured pouch epithelium; permeability; primary culture; fluorescein isothiocyanate dextran; diffusion; metabolism.

INTRODUCTION

Buccal epithelium forms a functional permeability barrier to the systemic absorption of exogenous substances in the oral cavity. Certain substances, however, including some drugs may rapidly cross the buccal epithelium and accumulate in pharmacologically significant levels in systemic circulation (1–4). Therefore, buccal administration represents a potentially useful route for systemic delivery of some drugs (5,6).

Due to the technical and analytical problems associated with whole-animal studies, several *in vitro* models for studying oral mucosal permeability and metabolism have been used (7–10). Most of these studies involved the use of either tissue biopsies or cultured explants of buccal, sublingual, or gingival tissue from both humans and animals. In general, these studies are limited by the availability of large amounts of viable tissue.

Hamster pouch buccal epithelium has been well characterized structurally (11) and some information is available about the biochemical properties of this tissue (10,12). In addition, hamster pouch buccal epithelium is more conveniently obtained and in larger amounts per animal than from other species. The purpose of this report is to describe the successful development and characterization of an *in vitro* model comprised of primary cultures of hamster pouch epithelium as a system for investigating buccal drug transport, metabolism, pharmacology, and toxicology.

MATERIALS AND METHODS

Chemicals

Molecular weight standards, fluorescein, fluorescein isothiocyanate dextrans (FITCD), leucine aminopeptidase (LAP) assay kit, and trypsin Type III from bovine pancreas, were purchased from Sigma Chemical Company, St. Louis, Mo. Materials for gel electrophoresis were obtained from Bio-Rad Laboratories, Richmond, Calif. Spectrapor membrane tubing, 12,000–14,000 MW cutoff, was purchased from Spectrum Medical Industries, Inc., Los Angeles, Calif. Fibronectin was obtained from Boehringer Mannheim, India-

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napolis, Ind. Heat-inactivated fetal bovine serum (FBS) and plasma-derived equine serum were obtained from HyClone laboratories, Logan, Utah. Minimal essential medium (MEM) (Eagle's modified) with Earle's salts and glutamine and without $NaHCO_3$ was obtained from Hazelton, Lenexa, Kans. Polycarbonate disks, 3-μm pore, 13-mm diameter, were purchased from Nucleopore Corporation Pleasanton, Calif. ^3H-Labeled water (1 mCi/g) was purchased from New England Nuclear, Boston, Mass., and *methyl*-^3H-thymidine in an aqeuous 2% ethanol solution (20 Ci/mmol) was purchased from Research Product International Corp., Mount Prospect, Ill. Scinti-Verse E was obtained from Fischer Scientific Company, St. Louis, Mo. All other reagents were of the highest grade commercially available.

Animals

Male Syrian golden hamsters (*Mesocricetus auratus*), 90–110 g, were obtained from Sasco Inc., Omaha, Neb.

Isolation and Culture of Hamster Buccal Epithelial Cells

Hamsters were sacrificed by CO_2 asphyxiation and each cheek pouch was everted, excised, and washed according to McCoy (13). Each pouch weighed about 0.392 ± 0.04 g (wet weight). One longitudinal incision was made in each pouch. The pouches were then incubated in serum-free Eagle's medium containing 0.25 g/100 ml trypsin Type III for 30–45 min at 37°C. After incubation, the pouches were washed with Eagle's medium containing serum. The epithelial sheets were separated with forceps and shredded (24). Each pouch yielded 10–15 × 10^6 cells as counted by crystal violet nucleus staining (14). Approximately 15–20 × 10^4 cells/cm^2 were seeded in 100 or 60-mm plastic culture dishes containing polycarbonate disks coated with cross-linked rat-tail collagen and fibronectin (15). The cells were incubated at 95% humidity, 5% CO_2 at 37°C in a culture medium consisting of MEM containing either 15% fetal bovine serum or 10% plasma-derived horse serum, 100 μg/ml penicillin G, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin B, 300 μg/ml ascorbic acid, 2.2 g/liter sodium bicarbonate, 10 m*M* Hepes, pH 7.4. The medium was changed three times weekly.

Enzyme Assays

After the incubation in trypsin as described above, the buccal epithelial sheets were separated with forceps and shredded, washed with phosphate-buffered saline (PBS) buffer, pH 7.4, containing 27 m*M* sodium citrate, and centrifuged for 10 min at 200*g*. The pellets were suspended in hypotonic PBS (HPBS), pH 7.4, for *p*-nitrophenylphosphatase (PNPP) assays and in HPBS containing 0.24 *M* sucrose for alcohol dehydrogenase assays. The suspensions were then homogenized for 1 min (15 strokes), sonicated for 1 min, and freeze (−70°C)–thawed three times. These samples were used for enzyme assays, protein assays, and keratin extraction or sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of total proteins.

PNPP activity was routinely measured in HEPES buffer (45 m*M* HEPES, pH 7.4, 100 m*M* KCl, 1% 2-mercaptoethanol) and followed by the increase in absorbance at 410 nm at 37°C due to release of *p*-nitrophenyl. The concentration of *p*-nitophenyl phosphate as substrate was 10 m*M*. NADH was determined according to Koivula *et al.* (16) with 10 m*M* ethanol as substrate. One unit of activity was defined as the amount of enzyme catalizing the formation of 1 μmol NAD/min under the assay conditions.

Aminopeptidase activity was assayed as described by Greenberg (17) and Sigma assay kit No. 251. The amount of cellular protein was determined by the method of Lowry *et al.* (18).

Keratin Extraction and Electrophoresis

The tissue homogenate (see Enzyme Assays) was used to extract keratins for SDS electrophoresis according to the method described by Winter *et al.* (19) and Regnier *et al.* (20). Gel electrophoresis was performed according to the method of Laemmli (21) and Sun and Green (22) with 5% acrylamide in the stacking gel, 12.5% acrylamide in the separating gel, and 3% polyacrylamide in both sections. Molecular weight standards were as follows: bovine serum albumin, 66K; ovalbumin, 45K; glyceraldehyde-3-phosphate dehydrogenase, 36K; carbonic anhydrase, 29K; trypsinogen, 24K; trypsin inhibitor, 20K; and α-lactalbumin, 14.2 K.

Chromatography

The size distribution of the dextrans in the receptor compartment of the diffusion apparatus after 60 min was examined by gel chromatography. Samples of an 800-μl mixture of FITCDs were applied on a 32 × 1.5-cm column of Sephadex G150-120 equilibrated with PBS buffer, pH 7.4. The column was eluted with the same buffer, at the rate of 28 ml/cm^2/hr (the void volume was 50 ml), 45 fractions of 1ml were collected, and the fluorescence of each fraction was measured with a Gilson absorbance detector at 520 nm.

Light and Electron Microscopy

Hamster pouch buccal epithelium and cultured pouch buccal epithelium, grown on polycarbonate disks, were fixed in 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.4, for 2–3 hr. Two buffer washes of 5 min each were used prior to postfixation with 1% OsO_4 in 0.1 *M* sodium cacodylate buffer, pH 7.4, for 1 hr. Following washings, samples were stained *en bloc* in 1% aqueous uranyl acetate for 1 hr, dehydrated in an ethanol series, embedded in Spurr's resin, and sectioned for electron microscopy. Thin sections were stained with 2% uranyl acetate and lead citrate and examined in a Philips EM 300 at an accelerating voltage of 60 keV. Hamster epithelial layers were separated according to McCoy (13) and stained with a solution composed of equal volumes of 1% methylene blue in 1% sodium borate and 1% azure II. The staining solution was placed on the sections and heated at 40°C for 3 min, rinsed with water, and photographed. Cultured cells were stained with hematoxylin at various times according to Pitt *et al.* (23) to examine cell integrity.

Diffusion Studies

Horizontal Side-Bi-Side diffusion cells (Crown Glass Company, Inc., Somerville, N.J.) were used for transport

studies. Collagen-coated polycarbonate disks with or without cells were placed in the diffusion cells such that the collagen matrix or epithelial cells always faced the donor chamber of the diffusion cell. The diameter of the diffusion area was 9 mm. The water jacket surrounding the sample chambers was thermostated at 37°C with a circulating water bath. The volume of the diffusion-cell sample chamber (i.e., both donor and receptor) was maintained at 3.0 ml with PBSA buffer (129 m*M* NaCl, 2.5 m*M* KCl, 7.4 m*M* Na_2HPO_4, 1.3 m*M* KH_2PO_4, 0.63 m*M* Ca Cl_2, 0.74 m*M* $MgSO_4$, 5.3 m*M* glucose, pH 7.4) during experiments and continually stirred with magnetic stir bars driven at a constant speed (600 rpm) by an external drive console (Crown Glass Company, Inc., Somerville, N.J.).

The donor chamber was pulsed with ^{3}H-thymidine (0.5 μCi/ml) or ^{3}H- water (0.5 μCi/ml) or contained 1 μ*M* fluorescein or a FITCD. A 0.1-ml aliquot of sample was removed from the receptor chamber at various times and diluted with either 1 ml of PBSA buffer for fluorometry or 10 ml of Scinti-Verse E with scintillation spectrometry. The fluorescence of samples was measured with an excitation of 490 nm and an emission of 520 nm in an SLM-4800 spectrofluorometer. Samples were assayed for ^{3}H with a Beckman 6800 scintillation counter (Beckman Instrument, Inc., Fullerton, Calif.). In order to maintain a constant volume in diffusion cells, an aliquot (0.1 ml) of fresh assay buffer was added back to the receptor chamber after each withdrawal.

Viability of the cultured epithelial cells was checked periodically by ^{3}H-thymidine uptake. A 1.5-μCi ^{3}H-thymidine pulse was added to the donor chamber at the same time as an FITCD. After a 1-hr period, the disks containing epithelial cells were carefully separated, then washed with 1 ml of PBS buffer in a dish, 200 μl of 1 *N* KOH was added, and incubation was carried out at 37°C for 30 min. To these samples was added 10 ml of scinti-Vers E, and then they were assayed by scintillation spectrometry.

Calculations

Apparent permeability coefficients were estimated by the following relationship:

$$P \text{ (cm/sec)} = X / (A \times t \times C_d)$$

where P is the apparent permeability coefficient, X is the amount of substance (mol) in the receptor chamber at time t (sec), A is the diffusion area (i.e., 0.636 cm^2), and C_d is the concentration of substance in the donor chamber (mol/cm^3) (in these studies C_d remains >90% of the initial value over the time course of the experiments). The flux (mol/cm^2/sec) of substances across the tissues was calculated as the linearly regressed slope through linear data (i.e., time points of 5 to 60 min) shown in the figures and used for calculation of the permeability coefficients reported.

RESULTS

Isolated hamster pouch buccal epithelium (HPBE) was successfully grown on either 100-mm culture dishes or polycarbonate discs (3.0-μm pore) precoated with rat-tail collagen and fibronectin. After 3 days in culture, the epithelial cells form confluent, stratified multilayers of cells in a 100-mm dish when seeded at a density of 1.5 to 2.0 × 10^5 cells/cm^2. Figures 1A and B show typical hamster epithelial cells after 1 and 3 days in culture, respectively. In Fig. 2, a cross section shows stratified multilayers of cultured cells. Generally, about four or five layers of cells form after 3 days in culture with the uppermost layer, the only layer showing signs of terminal differentiation (i.e., loss of cell organelles, nucleus, etc.). Other typical stratified epithelial-cell characteristics, extensive interdigitation, and tonofibrillar–desmosomal complexes are frequent and obvious (Fig. 2). After approximately 5 days in culture the superficial layers of cells were observed by electron microscopy to begin sloughing off (not shown) and corresponding changes in permeability result as described below.

To characterize the cultured cells it was pertinent to compare their biochemical properties with hamster pouch. Electrophoresis of keratins from CPBE and HPBE showed common bands at molecular weights of 46, 50, and 54 kD (Fig. 3A). SDS-PAGE of total (water-soluble and water-insoluble) proteins of both CPBE and HPBE also showed very similar bands between 45 and 66 kD (Fig. 3B). The specific activity of aminopeptidase, alcohol dehydrogenase, and alkaline phosphatase in CPBE verified that a similar metabolic activity of buccal epithelium was retained and expressed in primary cultures (Table I).

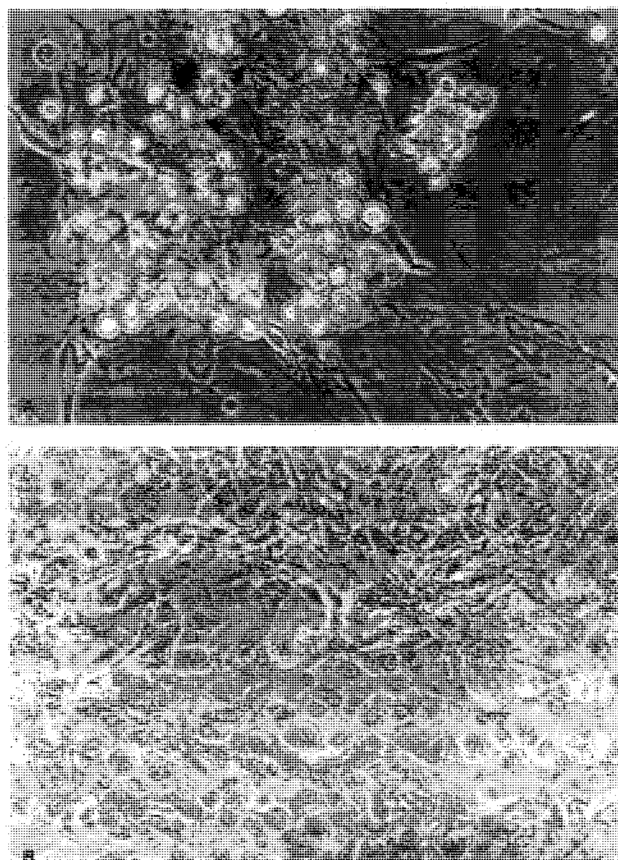

Fig. 1. Light micrographs of primary cultures of hamster pouch buccal epithelium. (A) Cultured hamster pouch buccal epithelial cells seeded into culture: first day; (B) third day. ×200; reduced 35% for reproduction.

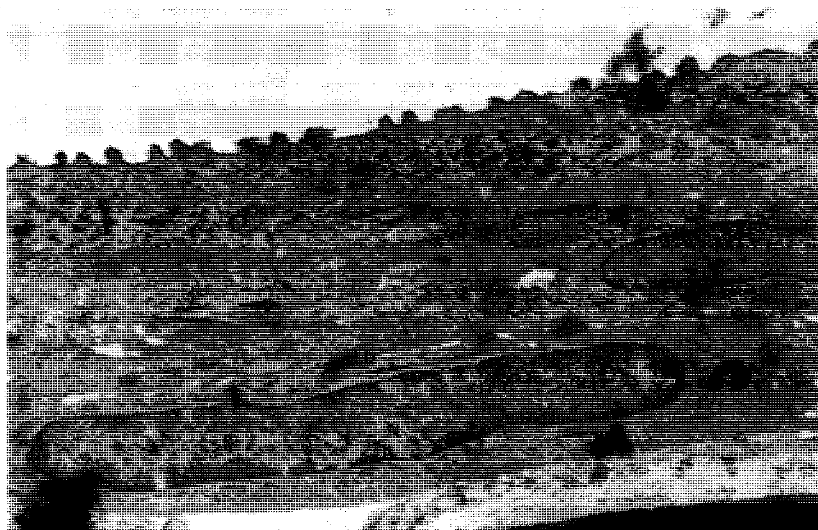

Fig. 2. Electron micrograph of cultured hamster pouch buccal epithelium in cross section. Cultured cells after the third day. K, keratinized layer; N, nucleus; D, desmosomal complexes. ×6800; reduced 35% for reproduction.

The apparent permeability coefficient of the CPBE to tritiated water decreased with the age of the cultured cells up to the third day (i.e., $2 \times 10^{-4} \pm 0.14 \times 10^{-4}$ cm/sec) and then gradually increased through day 7 (Fig. 4). The permeability coefficient of the whole hamster pouch to tritiated water (Fig. 4) was lower, $1.2 \times 10^{-4} \pm 0.19 \times 10^{-4}$ cm/sec. The relationship of the molecular weight of different FITCDs (MW 3860–148,900) to their flux across polycarbonate disks without cells during a 1-hr time period was a function of molecular weight (data not shown). The permeability of CPBE to FITCDs with different molecular weights at different days of culture was also related to molecular weight and was minimal in 3-day-old cultures. Gradual increases in the permeability of the cultured cell were observed after 3 days in culture (Figs. 5A–C). The differences among apparent permeability coefficients for polycarbonate disks with and without cells (Figs. 6A and B) indicated that the cultured cells form the major barrier to FITCD penetration, and not the collagen or polycarbonate disks. The plateauing of the apparent permeability coefficients (Fig. 6B) for the FITCDs was accounted for by low MW impurities (MW <18,000) associated with the high molecular weight dextrans. Gel chromatography (Fig. 7) of FITCD mixtures crossing the collagen-coated polycarbonate disks with or without CPBE indicates that only substances with MWs less than about 18,000 penetrate the cultured-cell barrier.

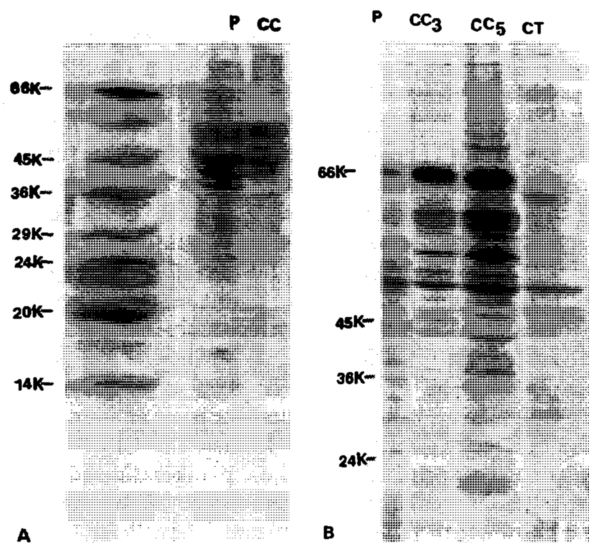

Fig. 3. SDS gel electrophoresis of hamster pouch buccal epithelial (A) keratins and (B) proteins. Hamster pouch, P; Cultured pouch buccal epithelium (CPBE) after 3 days in culture, CC3; CPBE after 5 days in culture, CC5; connective tissues separated from pouch serosal side, CT.

Viability of the cultured cells was examined by thymidine uptake. Thymidine uptake by CPBE under the experimental conditions (i.e., exposure to either transendothelial assay buffer or dextrans; 9254 ± 1937 cpm ^{3}H-thymidine uptake after 60 min) was always >90% of that under control conditions (i.e., exposure to growth medium; 9360 ± 2040 cpm ^{3}H-thymidine uptake after 60 min). In other control experiments, thymidine uptake by collagen-coated polycarbonate disks was negligible (data not shown).

DISCUSSION

In this study we have reported a technique for isolation and primary culture of HPBE. Seeded onto rat-tail collagen and fibronectin-coated surfaces, isolated buccal epithelial cells attach and spread. After 3 days in culture, the entire growth surface is covered with a continuous stratified layer of epithelial cells. Typically, the stratified system was comprised of up to five layers of epithelial cells, with only the superficial layer of cells exhibiting the morphology of terminally differentiated characteristics of keratinocytes (i.e., loss of cell organells, nucleus, etc.). The cultured cells also maintained other morphological characteristics of stratified epi-

Table 1. Specific Activity (nmol/mg/min) of Selected Enzymes from Hamster Pouch Epithelium and Cultured Pouch Epithelium[a]

Enzyme	Cultured cells[b]	Pouch	Literature (Ref. No.)
Alkaline phosphatase	0.97 ± 0.22	1.27 ± 0.26	—
Alcohol dehydrogenase	18.33 ± 2.6	19.4 ± 2.5	21.9 ± 2.7 (10)
Tyrosine aminopeptidase	0.76 ± 0.13	0.61 ± 0.23	—
Leucine aminopeptidase	2.63 ± 0.32	2.39 ± 0.50	1.5 ± 0.27 (12)

[a] All values are means ± SD for five separate preparations.
[b] No significant statistical differences between the specific activity of enzymes associated withh cultured cells and that of enzymes associated with pouch epithelium was determined by Student's t test.

thelium including frequently observed tonofibrillar desmosomal complexes (11,24).

Keratins of certain molecular weights (e.g., 50- and 58-kD keratins in human epidermal cells) are reported to be specific markers for stratified squamous epithelium (25). Major keratin molecular weight bands vary, however, with the tissue origin of the stratified epithelium (22,25). In this study, a comparison by SDS-PAGE between keratins of HPBE and those of CPBE demonstrated common profiles including common major bands at molecular weight 46, 50, and 54 kD. Similarly, one-dimensional SDS-PAGE comparisons of total proteins between HPBE and CPBE were comparable including major bands at 45 and 66 kD. In addition, other biochemical characteristics, the specific activities of alkaline phosphatase, alcohol dehydrogenase, and aminopeptidase, were similar in the CPBE relative to the HPBE. The specific activities observed in the primary cultures and the excised tissue were also consistent with values reported in the literature (10,12). The choice of alcohol dehydrogenase and leucine aminopeptidase for this study was deliberate since other researchers have documented the specific activity of these enzymes in buccal epithelium. The consistency of enzyme activities among excised tissue, observations in the literature, and the cultured epithelial cells suggests that the primary culture development does not alter enzyme expression in buccal epithelium. However, this study was not broad enough to conclude, unequivocally, that the expression of *all* enzyme systems may be similar in both excised buccal epithelium and the primary culture system. Retention of similar biochemical characteristics, keratins, and enzymes in primary culture compared to corresponding tissues *in vivo* is consistent with observations in other cell culture systems (10,22,25).

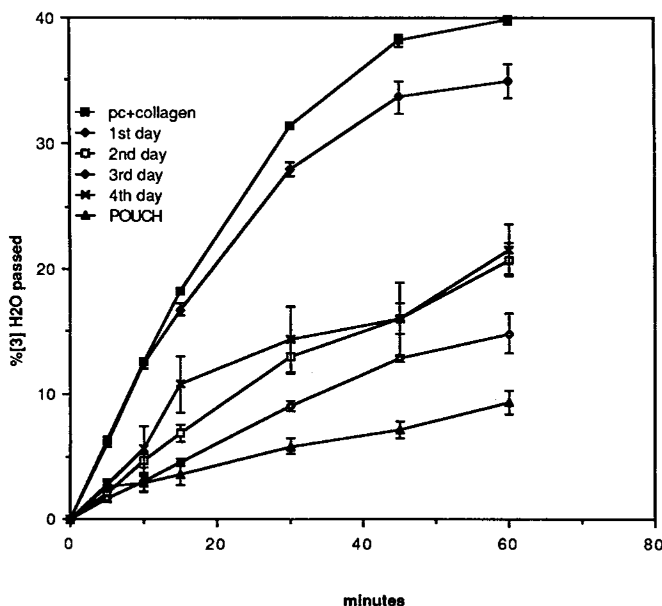

Fig. 4. Time-dependent flux of ^3H-H_2O across cultured pouch buccal epithelium and excised hamster pouch epithelium. The flux ^3H-H_2O across cultured pouch buccal epithelium was determined after indicated days in culture as described under Materials and Methods.

The permeability of cultured HPBE to ^3H-water, fluorescein, and FITCDs was followed initially as function of the days in culture. The permeability of the primary CPBE was observed to decrease gradually with the time in culture (i.e., minimal at 3 days) before gradually increasing (i.e., maximal at 7 days). The decreased permeability corresponds with the formation of up to five stratified layers of cultured cells at day 3. After approximately 5 days in culture, however, the cells begin to slough off, as occurs naturally and continually *in vivo* (24), accounting for the increased permeability of cultured cells at this time.

In comparative studies, the permeability of excised HPBE to water was lower than that observed with the CPBE. Although not likely because of our isolation technique, this difference could be due to the presence of additional factors (e.g., connective tissue, smooth muscle, etc.) associated with excised tissue that could not be efficiently removed in the excision. Alternatively, and more likely, the CPBE does not appear to be as differentiated as HPBE *in vivo* (26). Whether totally or partially keratinized, the barrier to transepithelial diffusion resides primarily in the intercellular junctions of the outer one-third of stratified oral epithelium (27). In general, keratinized oral epithelium is less permeable than nonkeratinized oral epithelium (6). The HPBE possesses essentially a strateum corneum-like (i.e., keratinized or terminally differentiated) superficial keratinocyte layer. In contrast, the superficial layer of CPBE does not differentiate to a stratum corneum-like layer as shown in this study by electron microscopy. Rather, the CPBE appears similar to the nonkeratinized (i.e., parakeratinized or partially differentiated) human (9) and rabbit (28) buccal epithelia, both of which retain a significant intercellular barrier in the superficial layers (27). These differences probably account, at least in part, for differences in the permeability of CPBE versus HPBE to water. In this regard, it is more desirable and relevant to generate an *in vitro* model that mimics the nonkeratinized human buccal epithelium for drug deliv-

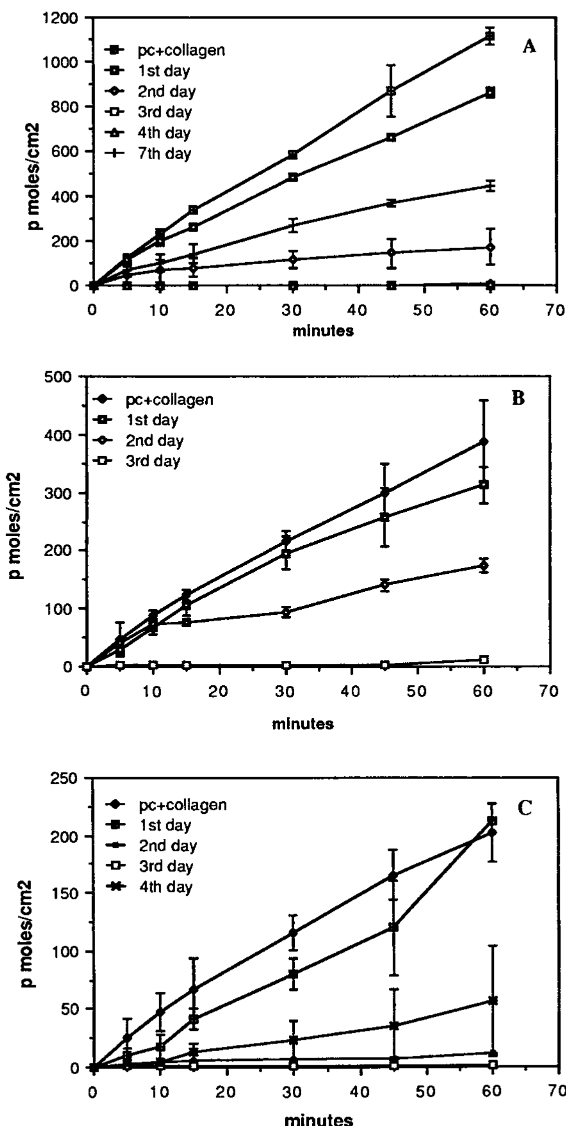

Fig. 5. Time-dependence flux of fluorescein isothiocyanate dextrans across cultured hamster pouch buccal epithelium. (A) Fluorescein; (B) FITCD, MW 3860; (C) FITCD, MW 18,900. The flux of the selected substances across the cultured cells was determined after the indicated days in culture as described under Materials and Methods.

ery studies. Appropriate comparative biochemical and permeability studies between nonkeratinized CPBE and human buccal epithelium remain a goal of future studies in our laboratory.

FITCDs, biologically inert molecules of various molecular weights, were used in permeability studies to determine the role of molecular size in determining penetration of CPBE as previously described for *in vitro* rabbit buccal models by Tolo and Jonsen (9). With increasing molecular weight; the apparent permeability coefficients of the FITCDs was observed to decrease. At a molecular weight of approximately 18,000 the apparent permeability coefficients become similar, because of the existence of low molecular weight impurities associated with the FITCDs shown here by gel chromatography and by others (29). We conclude

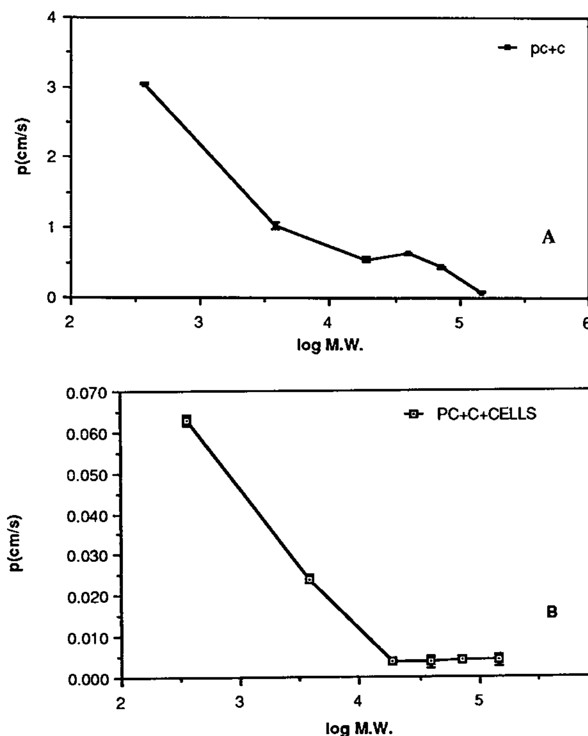

Fig. 6. Relationship of the apparent permeability coefficient ($p \times 10^4$ cm/sec) for FITCD penetration of cultured hamster buccal epithelium. (A) Collagen-coated polycarbonate disks alone; (B) Cultured pouch epithelium grown on collagen-coated polycarbonate disks.

from these experiments that the permeability of the primary CPBE is dependent on molecular weight for substances with molecular weights less than 18,000. Further, substances with molecular weights greater than 18,000 will not cross primary CPBE. The permeability of our primary cultures of HPBE to FITCDs, then, was lower than reported for excised rabbit buccal mucosa (9) and cultured rabbit mucosa (28), which exclude molecules greater than 40,000 and 70,000, respectively. As described above, HPBE is considered a keratinized epithelium, while the buccal epithelium of human and rabbit, for example, is less keratinized and, likely, more permeable (6,9,28). Observations here appear consistent with reported differences in keratinization between the hamster and the rabbit (9,28). Although our cultures do not exhibit complete keratinization, other differences between the barriers developed in culture probably exist. The slightly greater permeability (i.e., to water) of the cultured cells versus excised tissue shown here was also observed in the rabbit system (9,28).

To conclude, we have described the establishment of a primary culture system for HPBE. Establishment of this culture system substantially reduces animal requirements (e.g., up to 20 times more experiments from one excised hamster pouch) and provides a model more amenable than the whole animal to manipulation of experimental conditions (e.g., temperature and pH) that are useful in characterizing biochemical processes. The biochemical characteristics of the cultured cells were similar to those of HPBE *in vivo;* morphological and permeability characteristics of CPBE appear more typical of nonkeratinized buccal mucosa observed in

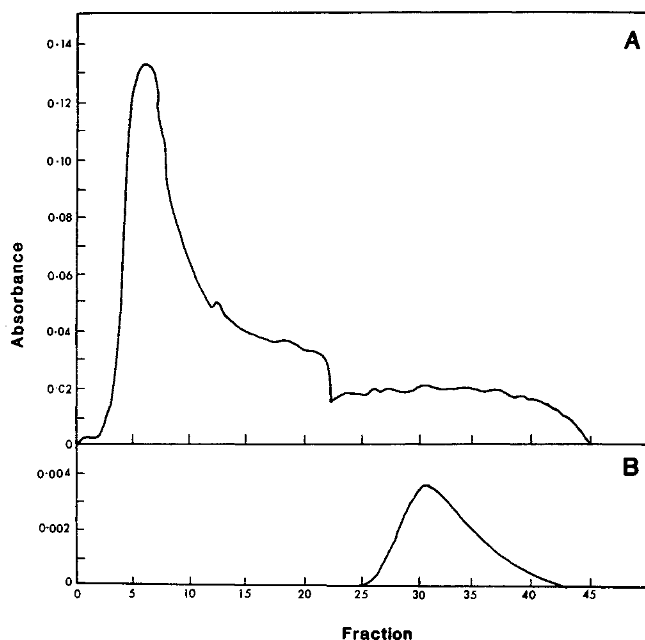

Fig. 7. Gel chromatography of a mixture of fluorescein and FITCDs (MW 376 to 148,900) before and after crossing cultured pouch buccal epithelium. Samples were loaded onto a 32 × 1.5 Sephadex G150-120 column equilibrated and eluted with PBS buffer, pH 7.4. The void volume was 50 ml and 45 fractions of 1 ml were collected. (A) Eight-tenths milliliter of standard solution containing a mixture of fluorescein and FITCDs. Absorbance in early fractions corresponds to that in dextrans with high molecular weights. (B) An 0.8-ml aliquot taken from the receptor side of a diffusion apparatus 60 min after the donor chamber was pulsed with an FITCD mixture. Peak fractions represent dextrans with a MW of about 18,000. The diffusion apparatus contained 3-day-old cultured pouch buccal epithelium on collagen-coated polycarbonate disks.

man and other species. This *in vitro* system represents a potentially useful model for investigation of buccal drug transport, metabolism, pharmacology, and toxicology. Current efforts are directed at using this established methodology to characterize buccal peptide transport and metabolism and to develop a suitable *in vitro* human buccal epithelium model for similar investigations.

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