

Permeability properties of monolayers of the human trophoblast cell line BeWo

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Liu, Fei, Michael J. Soares, and Kenneth L. Audus. Permeability properties of monolayers of the human trophoblast cell line BeWo. *Am. J. Physiol.* 273 (*Cell Physiol.* 42): C1596–C1604, 1997.—The BeWo cell line (b30 clone) has been examined as a potential *in vitro* system to study transplacental transport. At the light and electron microscope level, the cells were observed to form confluent monolayers on polycarbonate filters in ~5 days and morphologically resembled the typical human trophoblast. BeWo monolayers developed a modest transepithelial electrical resistance and a molecular size-dependent permeability to hydrophilic passive diffusion markers, fluorescein, and selected fluorescein-labeled dextrans. Linoleic acid permeation across BeWo monolayers was asymmetric, saturable, and inhibited by low temperature and excess competing fatty acid. Forskolin and 8-bromoadenosine 3',5'-cyclic monophosphate treatments stimulated morphological changes in BeWo cultures and enhanced the asymmetric passage of linoleic acid across the BeWo monolayers while having minimal effects on passive permeability, affirming that the differentiation state of the cells can influence membrane transporters and transmonolayer permeability. The basic permeability properties of the BeWo monolayers suggest that the cells grown on permeable supports may be examined as a convenient *in vitro* system to evaluate some transplacental transport mechanisms.

placenta; passive diffusion; fatty acid transport

THE POLARIZED EPITHELIAL-LIKE syncytiotrophoblasts constitute the outermost layer of the placental villi and form a physical and metabolic barrier between the fetal and maternal blood circulations (25). Passive diffusion across this single layer of epithelial cells is the principal mechanism of transfer from maternal blood to fetal blood (10); however, several nutrient transport systems are also known to be present in these cells (8–11, 16, 17).

Human cytotrophoblasts have been successfully purified and cultured (14). However, in primary culture, human cytotrophoblasts spontaneously differentiate to a syncytiotrophoblast. The syncytialized trophoblasts do not form a confluent, consistent monolayer and do not permit the study of transcellular transport processes. Nutrient transport systems have been investigated with use of the primary cultures of trophoblasts but have been limited to unidirectional (maternal to fetal) uptake studies (8, 13). The derivation of the BeWo cell line from a human choriocarcinoma (19), a monolayer-forming trophoblast cell line, offers a potential alternative culture system for studying transplacental transport and metabolism *in vitro*. An *in vitro* system to study transplacental transport could potentially provide a convenient system to exploit in the investigation of those processes in the placenta regulating the distri-

bution of drugs, drugs of abuse, and pathogens between maternal and fetal compartments. The BeWo cell line is particularly attractive for this purpose because it is stable, relatively easy to maintain by passage, and grows to a confluent monolayer in a relatively short period of time. More importantly, the BeWo cell displays morphological properties and biochemical marker enzymes common to normal trophoblasts (26) and has been shown to exhibit polarized transcellular transport of transferrin (4, 5), serotonin and monoamine uptake systems (6, 21), and relevant nutrient uptake systems (17). Treated with adenosine 3',5'-cyclic monophosphate (cAMP) or forskolin, BeWo cells further differentiate and exhibit morphological characteristics similar to the fusion of cytotrophoblasts to a syncytia in primary culture (28).

In this report, we used the BeWo cell line to produce confluent monolayers on rat tail collagen-coated polycarbonate filters and in Snapwell Transwell (Fisher Scientific, St. Louis, MO) inserts. Using the Side-Bi-Side (Crown Glass, Somerville, NJ) cell diffusion systems, we systematically tested the passive permeability and carrier-mediated fatty acid transport characteristics of BeWo cell monolayers, features not previously documented for BeWo monolayers grown on permeable supports. The establishment and characterization of the basic permeability properties of monolayers grown on a permeable support establishes the basis for future application of this *in vitro* system to investigate transplacental transport and metabolism of drugs and drugs of abuse.

METHODS

Materials. Translucent polycarbonate filters (13 mm diameter, 0.4 μ m pore) and Snapwell Transwell inserts (12 mm diameter, 0.4 μ m pore size) were purchased from Fisher Scientific. Dulbecco's modified Eagle's medium (DMEM), DMEM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid modification, Hanks' balanced salt solution (HBSS) and Percoll were obtained from Sigma (St. Louis, MO). Heat-inactivated fetal bovine serum (FBS) was from JRH Biosciences (Lenexa, KS). Penicillin-streptomycin as a mixture was from GIBCO (Gaithersburg, MD). Fluorescein isothiocyanate conjugated dextrans (FITCDs), fluorescein, forskolin, and 8-bromoadenosine 3',5'-cyclic monophosphate (8-BrcAMP) were purchased from Sigma. [¹⁴C]linoleic acid was purchased from Amersham Life Science (Little, CA). [³H]dihydroalprenolol hydrochloride was purchased from DuPont-NEN Research (Boston, MA). All other standard chemicals were purchased from either Fisher Scientific or Sigma.

BeWo cell culture. The BeWo cell line was originally derived from a human choriocarcinoma (19). The BeWo clone (b30) was obtained from Dr. Alan Schwartz (Washington University, St. Louis, MO). The cells were continuously cultured in DMEM with 10% heat-inactivated FBS containing 0.37%

sodium bicarbonate and 1% antibiotics (10,000 U/ml penicillin and 10 mg/ml streptomycin). The cells were routinely maintained in 175-cm² Falcon flasks at pH 7.4 under 5% CO₂ and 95% humidity at 37°C. The cells were usually ready to be passaged after ~4 days in culture. The cells were harvested by exposure (1–2 min) to a trypsin-EDTA solution (0.25% trypsin and 0.02% EDTA in HBSS) and passed onto polycarbonate membranes coated with rat tail collagen in 60- or 100-mm Corning culture dishes for the monolayer cultures or into 12-mm Snapwell Transwell permeable polycarbonate filters. With the typical seeding density of 100,000 cells/cm², the cells formed monolayers between 4 and 6 days. The culture medium was changed every other day. All cells used in this study were from *passages 10–30*.

Forskolin was prepared in 95% ethanol and was added to the culture medium with the final concentration of 100 μM. Control cultures received the ethanol vehicle (0.6% final) in the same concentration as the forskolin-treated cultures. The 8-BrcAMP was dissolved in the culture medium and was sterilized by filtration through a 0.2-μm pore sterile filter.

Electron microscopy. BeWo cells grown on both polycarbonate filters and Snapwell Transwell insert filters were washed three times for 10 min with phosphate-buffered saline [PBS; (in mM) 129 NaCl, 2.5 KCl, 7.4 Na₂HPO₄, 1.3 KH₂PO₄, 0.63 CaCl₂, 0.74 MgSO₄, 5.3 glucose, and 0.1 ascorbic acid, pH 7.4] and fixed with 2.5% glutaraldehyde solution in PBS for 4 h at 4°C. After being fixed, membranes were washed three times for 10 min with PBS and the samples were postfixed in 1% OsO₄, dehydrated in a series of ethanol and acetone solutions, and embedded in EmBed 812. The sample was sectioned at 80 nm on a Sorvall Mt 500 Ultratome with a diamond knife. The sections were stained (10 min each) with uranyl acetate and lead citrate and examined under a Jeol 1200 EX II transmission electron microscope.

Cross sections. BeWo cells grown on both polycarbonate filters and Transwell insert filters were washed three times with PBS and were fixed in buffered 10% Formalin solution at room temperature. After fixation in 10% Formalin, tissue specimens were placed in a tissue processor for dehydration and infusion with paraffin. The specimens were then embedded in a small paraffin block to provide support for sectioning. The paraffin blocks were placed on a microtome, and very thin tissue sections were cut (~6 μm thick). Those tissue sections were then placed on a glass microscope slide. Through a series of steps, the paraffin was dissolved from around the tissue on the slide, leaving only the tissue section on the slide. The slides were then stained with the routine hematoxylin and eosin staining process.

Transmonolayer permeability. A horizontal Side-Bi-Side diffusion apparatus (Crown Glass) was used to measure the transmonolayer permeability of cells grown on the surface of 0.4-μm pored polycarbonate filters, as previously described (20, 21). PBS was added in each of the donor and receiver chambers of either the Side-Bi-Side diffusion apparatus or the Corning Costar diffusion system. The cells faced the donor chamber as the apical side, and the polycarbonate membrane faced the receiver chamber as the basolateral side. The monolayer cultures always faced the donor chamber of the diffusion systems. Polycarbonate filters treated with rat tail collagen but without cell monolayers were used as a control. The transport area was 0.636 cm² in the Side-Bi-Side diffusion system. The donor-side final concentration of the FITCDs was 10 μM for all transmonolayer permeability studies, and the donor-side final concentration of fluorescein was 0.5 μM. The water jacket surrounding the donor and the receiver chambers was thermostated at 37°C. The contents of each chamber were continuously stirred at 600 revolutions/min

with magnetic stir bars in the Side-Bi-Side diffusion system, and 5% CO₂ and 95% O₂ were slowly and continuously bubbled into the chambers. The monolayer cultures were equilibrated for 30 min at 37°C before they were undertaken in the transport studies. Either fluorescein-labeled or radiolabeled compounds at different final concentrations were added into the donor chambers. An aliquot of 100 ml from the receiver chamber was taken at the scheduled time points (0–120 or 0–180 min), and the same volume (100 μl) of fresh PBS was replaced after each sample. The samples were diluted with 0.9 ml of PBS in microcuvettes and were measured by fluorescence spectroscopy (SLA Aminco, Urbana, IL) with an excitation wavelength of 490 nm and an emission wavelength of 520 nm.

For concentration- and temperature-dependent studies of [¹⁴C]linoleic acid transport, studies were carried out as described above for fluorescein and the FITCDs, except that the concentration of fatty acid was varied and the buffer in the diffusion system contained bovine serum albumin in a ratio of 6:1 (fatty acid to albumin), according to Lafond et al. (15). To observe the effect of nonalbumin bound, unlabeled fatty acid on [¹⁴C]linoleic acid transport, we added increasing concentrations of linoleic acid containing no albumin to the donor chamber of the diffusion cell just before pulsing the chamber with a 1.7-μM pulse of [¹⁴C]linoleic acid. Radioisotope samples were added to 10 ml of ScintiVerse BD and were assayed by liquid scintillation spectrometry in an LS 6800 Beckman scintillation counter.

In other studies, 7 nM [³H]dihydroalprenolol hydrochloride was added either to the donor or to the receiver side of the diffusion apparatus. After 30 min, the buffer in the donor and receiver sides was aspirated out and the monolayers on the polycarbonate filter were removed from the diffusion apparatus and washed three times in 100 ml of ice-cold buffer (3). The monolayers were added to 10 ml of ScintiVerse containing ScintiGest and were assayed by liquid scintillation spectrometry.

Determination of transepithelial electrical resistance values. The transepithelial electrical resistance (TEER) values were measured in a chamber with planar electrodes (Endohm-Snap, World Precision Instruments, Sarasota, FL). The TEER was determined with BeWo cells grown on Snapwell Transwell filters. After three washes with PBS at room temperature, the measurement was carried out and corrected for resistance of the collagen-coated filters in the absence of BeWo cells. In other experiments, the solutions of 10 mM EDTA and 10 mM amiloride in PBS replaced the regular PBS. The cells were incubated 30 min for EDTA and 10 min for amiloride at room temperature.

Calculations and data analysis. Apparent permeability coefficients were estimated by the following equation

$$P(\text{cm/s}) = x/(A \times t \times C_d)$$

where P is the apparent permeability coefficient, x is the amount of substance (mol) in the receiver chamber at time t (s), A is the diffusion area, and C_d is the concentration of substance in the donor chamber (mol/cm³). C_d remains >90% of the initial value over the time course of the experiments. The transport of the compounds studied, expressed as the flux (mol·cm⁻²·s⁻¹), was determined as the mean ± SD from three to six different monolayers.

The apparent permeability coefficient for the BeWo monolayers, P_e , was calculated from the following relationship

$$1/P_t = 1/P_e + 1/P_c$$

where P_t is the apparent permeability coefficient for the collagen-coated polycarbonate membranes in the presence of BeWo cell monolayers, and P_c is the apparent permeability coefficient for collagen-coated polycarbonate membranes alone (1). Michaelis-Menten parameters were estimated by Sigma Plot (Version 2.0).

In all experiments, data were expressed as means \pm SD for at least four replicates. A one-way analysis of variance followed by Dunnett's multiple-comparison test was applied to analyze the significance of differences between treatment and control groups of raw data at the 0.05 level of significance.

RESULTS

BeWo cells (b30) have been continuously subcultured and were routinely maintained in the 175-cm² flasks. In the presence of 10% FBS, the BeWo cells consistently developed confluent monolayers between *days 4* and *6*. A typical confluent monolayer grown on a rat tail collagen-coated polycarbonate filter is shown in Fig. 1A. BeWo cells were also grown on Snapwell Transwell inserts to facilitate TEER measures. Because the cell cultures on the Snapwell Transwell inserts were not visible under the light microscope, culture samples were observed in cross section to verify formation of monolayers. Figure 1B is a representative cross section of BeWo cells grown on the Transwell filter at *day 5* in culture. The importance of monitoring growth of BeWo cells on the Transwell filter is illustrated in Fig. 1C, in which higher seeding densities and longer growth times allow for formation of multiple layers of cells. Figure 2 shows a scanning electron photomicrograph of BeWo cell cultures on polycarbonate filters. The cells generally exhibit a flattened polygonal shape with microvilli and close apposition with adjacent cells. Transmission electron micrographs confirmed the presence of apical microvilli, close cell apposition, and the presence of a single layer of cells (not shown).

The cell seeding density was found to directly affect BeWo monolayer permeability. The relationship between different seeding densities and permeability to a FITCD (FD-04, average mol wt 4,400) is shown in Fig. 3. By *day 5* in culture, seeding densities of 100,000 cells/cm² and 200,000 cells/cm² provided for lowest dextran permeability. Because the higher seeding density resulted in formation of multiple layers (similar to the condition shown in Fig. 1C), usually within 24 h and most readily in the Transwell insert, the seeding density of 100,000 cells/cm² was chosen to be the optimal density for reproducibly establishing BeWo monolayer cultures.

At the established seeding density of 100,000 cells/cm², the passage of selected passive permeability markers across the BeWo monolayers was determined for cells grown on polycarbonate filters. These filters were placed in the side-by-side diffusion apparatus to facilitate permeability studies with fluorescein (mol wt 376) and FITCDs varying in molecular weight (average mol wt 4,400, 20,000, and 70,000). Figure 4 shows the day-to-day change in BeWo monolayer permeability to fluorescein and the different FITCDs. The permeation of the hydrophilic fluorescein and FITCDs across BeWo

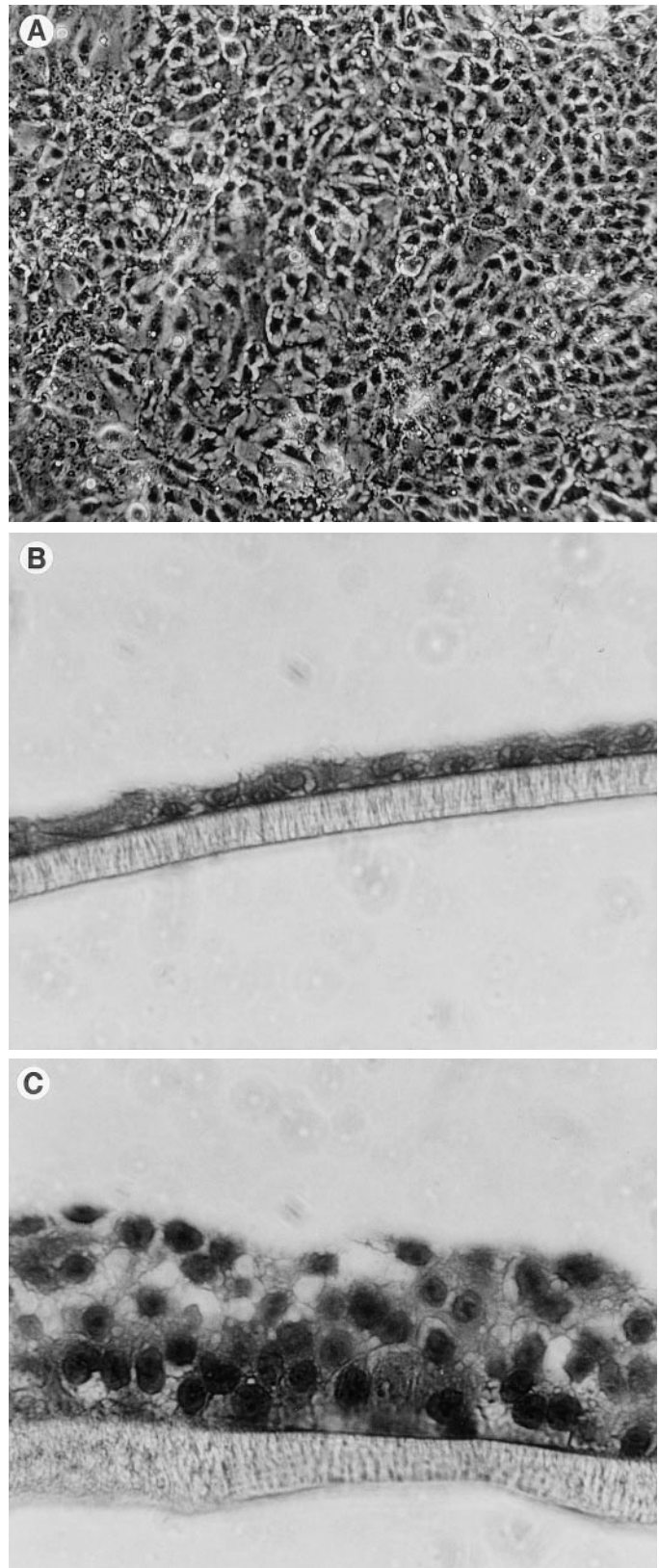


Fig. 1. A: 5-day-old confluent BeWo monolayer grown on a rat tail collagen-coated polycarbonate in 60-mm dish ($\times 200$). B: 5-day-old confluent BeWo monolayer grown on a rat tail collagen-coated Snapwell Transwell insert ($\times 400$). C: 7-day-old BeWo cell culture of multiple layers grown on rat tail collagen-coated Snapwell Transwell insert ($\times 400$).

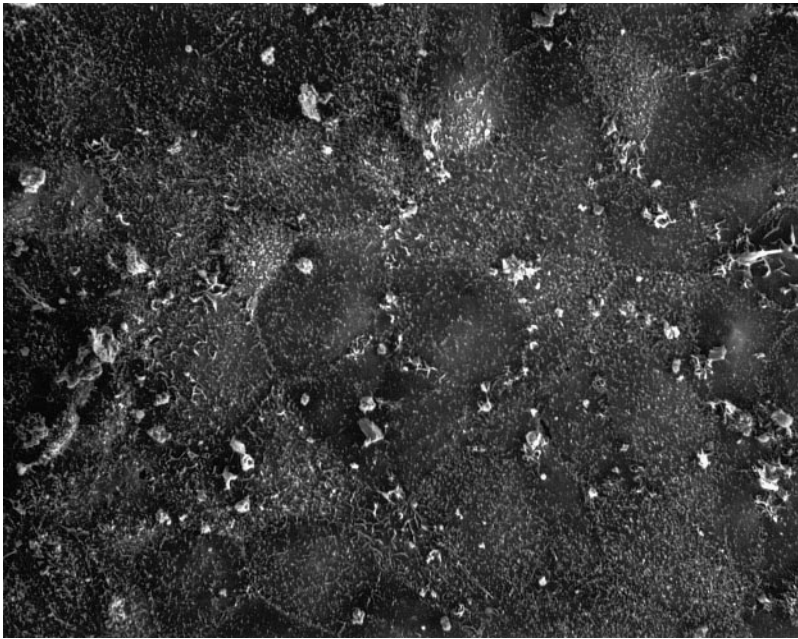


Fig. 2. Scanning electron photomicrograph of BeWo cell cultures on polycarbonate membranes ($\times 350$).

monolayers was dependent on the molecular weight and the age of the cultures. The lower-molecular weight marker, fluorescein, was the most sensitive to development of confluent monolayers but reached the minimum permeability at *day 5* in culture or approximately the same time as the higher-molecular weight markers. The largest dextran (FD-70) showed very little change in permeability with day in culture. Figure 5 illustrates the relationship between the decreased permeation of the selected markers across the BeWo monolayers and an increase in molecular size. These results generally

illustrated that passive permeation across BeWo monolayers was sensitive to molecular size.

The TEER developed by the BeWo monolayer was observed to be a maximum of $\sim 70\Omega \cdot \text{cm}^2$. Figure 6 shows the development of electrical resistance as a function of day in the culture postseeding. The maximal TEER in the Snapwell Transwell filters developed at approximately *day 5* in culture and was consistent with the overall development of sieving properties of the monolayers with respect to fluorescein and the FITCDs described above. The BeWo monolayer TEER was sub-

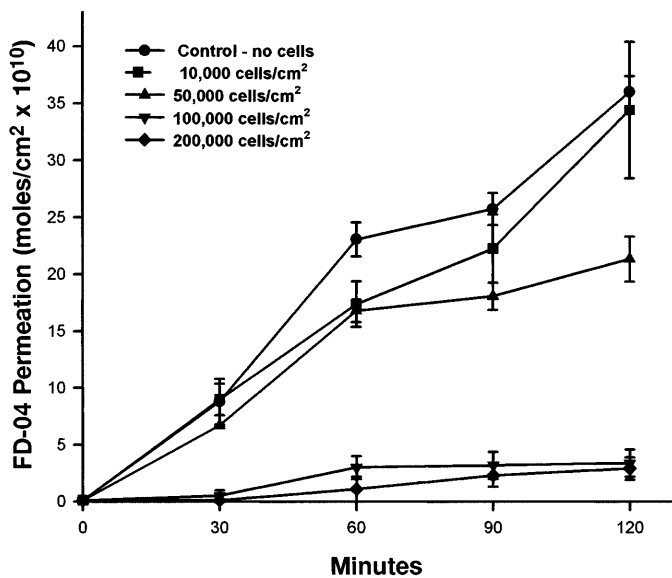


Fig. 3. Effect of cell seeding density on permeation of fluorescein isothiocyanate-conjugated dextran (FITCD) FD-04 (average mol wt 4,400) across 5-day-old cultures of BeWo monolayers at 37°C. Control represents FD-04 permeation across collagen-coated polycarbonate membrane in absence of BeWo cells. Data represent means \pm SD from quadruplicate samples.

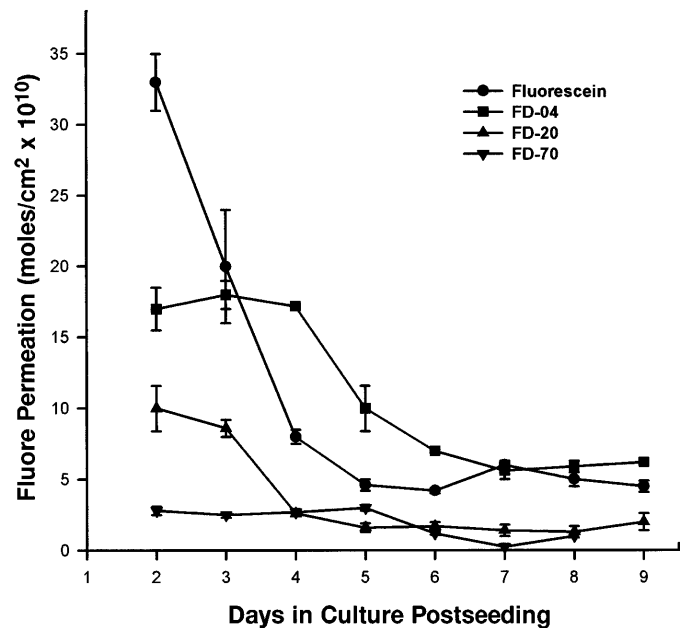


Fig. 4. BeWo monolayer permeability to fluorescein (Fluore, mol wt 376) and FITCDs (average mol wt: FD-04, 4,400; FD-20, 20,000; FD-70, 70,000) at selected days postseeding in culture at 37°C. Data represent means \pm SD from quadruplicate samples.

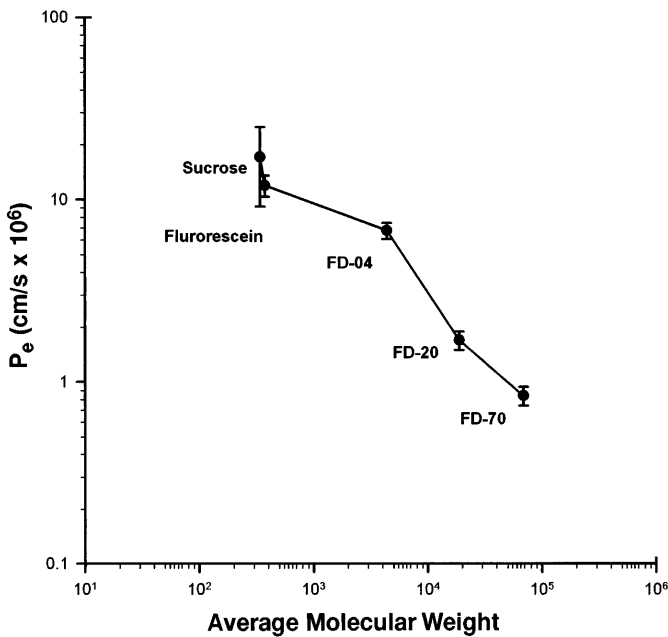


Fig. 5. Relationship between 5-day-old BeWo monolayer permeability to fluorescein (mol wt 376) and FITCDs (average mol wt: FD-04, 4,400; FD-20, 20,000; FD-70, 70,000) and average mol wt of markers at 37°C. Apparent permeability coefficients (P_e) have been adjusted for effects of collagen and polycarbonate filter as described in METHODS. Data represent means \pm SD from quadruplicate samples.

stantially reduced when the cells were subsequently exposed to EDTA (10 mM), whereas the resistance was only partially attenuated in the presence of amiloride (10 μ M), a sodium channel blocker.

To further define some of the nutrient transport properties of the BeWo monolayers, we examined whether

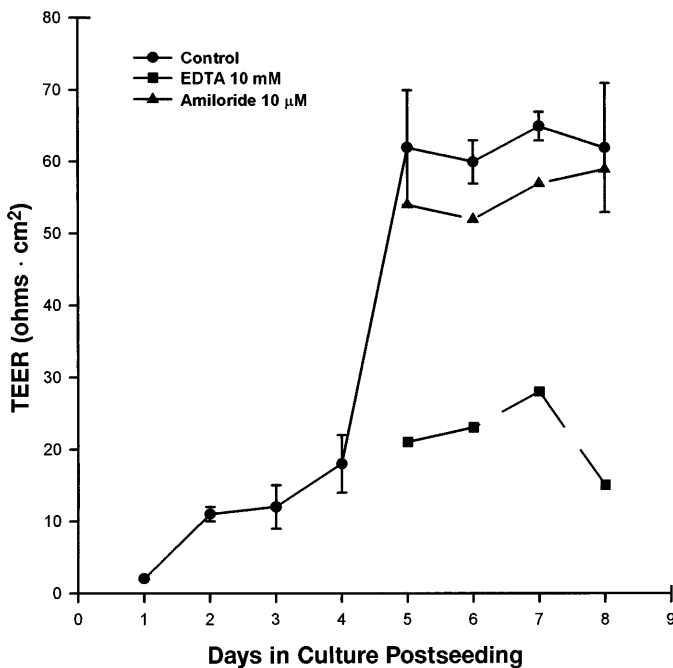


Fig. 6. Development of transepithelial electrical resistance (TEER) by BeWo monolayer cultures and effects of amiloride and EDTA at room temperature (\sim 25°C). Data represent means \pm SD from quadruplicate samples.

the cell line retained a transporter for fatty acids. The passage of linoleic acid across the cells was found to be asymmetric, with the permeation rate greater in the apical-to-basolateral direction, as shown in Fig. 7. As affirmed in Fig. 8A, the passage of linoleic acid across the monolayers was saturable [Michaelis constant (K_m) = 324 μ M and maximum velocity = 14.6 fmol/min] in the presence of albumin (6:1 fatty acid-to-albumin ratio), inhibited by low temperature, and enhanced in the presence of albumin (data not shown). The K_m for the fraction of the estimated free or unbound linoleic acid available for transport (15) was 7.4 μ M. Figure 8B illustrates the inhibition of [¹⁴C]linoleic acid transport in the presence of increasing concentrations of albumin-free linoleic acid (50% inhibitory concentration = 30 μ M). The asymmetry of the monolayers was further supported by an approximately sevenfold greater binding of [³H]dihydroalprenolol hydrochloride on the basolateral surface (e.g., 1.6 ± 0.50 pmol/cm² total bound on the basolateral surface, 0.22 ± 0.03 pmol/cm² total bound on the apical surface) of the BeWo monolayers.

The BeWo cells are not capable of spontaneous differentiation in culture (28). Development of culture conditions that facilitate BeWo cell differentiation and inhibit cell proliferation potentially may make the monolayer system morphologically similar to the primary trophoblast cultures and to the in vivo placental barrier. Figure 9 shows the morphological changes of BeWo cell cultures (5 days) after 4 days forskolin treatment. Addition of 8-BrcAMP has a similar effect on the cells (not shown). The morphology of the treated BeWo cells was similar to the primary trophoblast cultures (Fig. 9C), including the presence of the large openings between the cells, making them unuseable for transport studies.

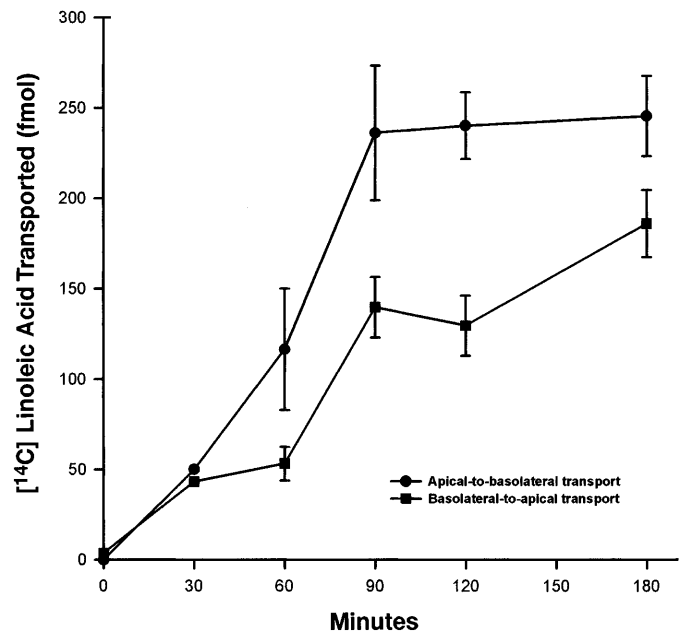


Fig. 7. Bidirectional passage of [¹⁴C]linoleic acid across 5-day-old BeWo monolayers at 37°C. Data represent means \pm SD from quadruplicate samples.

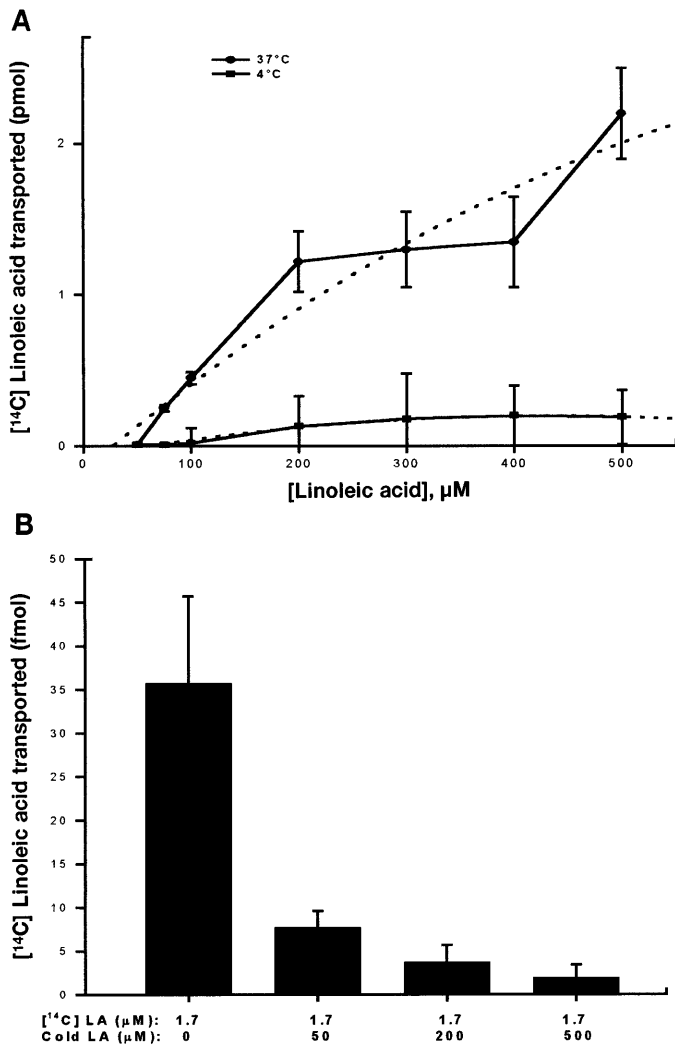


Fig. 8. *A*: concentration and temperature dependence of apical-to-basolateral [¹⁴C]linoleic acid transport across 5-day-old BeWo monolayers in presence of albumin. Ratio of fatty acid to albumin was 6:1. Data represent means ± SD from quadruplicate samples taken at 1 h. Dotted line represents best-fit nonlinear regression through data points. *B*: effect of increasing unlabeled linoleic acid (LA) concentration (cold LA) on apical-to-basolateral [¹⁴C]linoleic acid transport across 5-day-old BeWo monolayers at 37°C in absence of albumin. Data represent means ± SD from quadruplicate samples taken at 1 h.

The effects of forskolin and 8-BrcAMP on BeWo cell growth and morphology were significant and suggested that monolayer permeability might be altered in the presence of agents that stimulate formation of a syncytia. Table 1 summarizes experiments in which the permeability of the BeWo monolayers in the presence of either forskolin or a cAMP was monitored with fluorescein before formation of syncytia. Except for a small but statistically significant increase with the 3-day treatment with forskolin, no substantial changes in the permeability of the BeWo monolayers to fluorescein were observed after a 2- or 3-day treatment with either forskolin or 8-BrcAMP. Table 2 summarizes similar data for linoleic acid for a 3-day treatment with forskolin. Statistically significant trends of increased linoleic acid transport were observed after forskolin pretreatment, with the greater effects of cell differentiation on

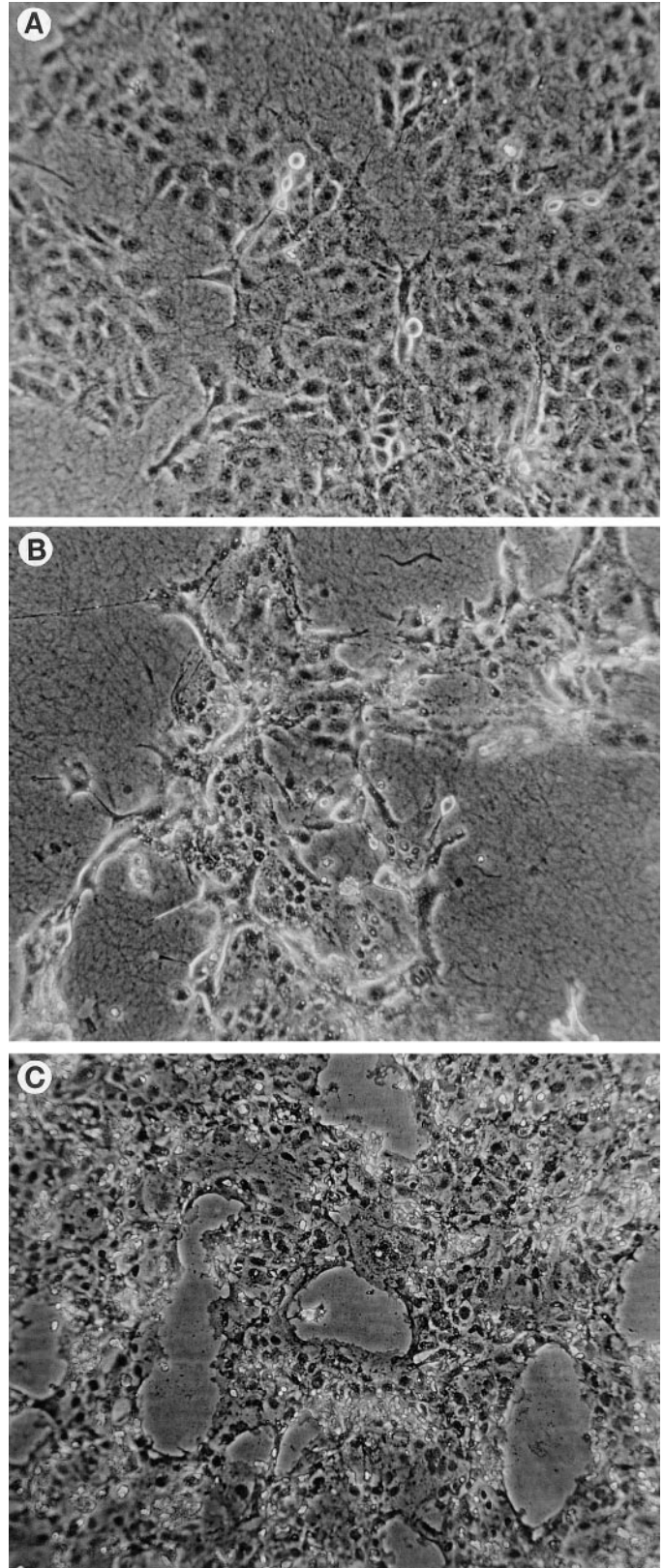


Fig. 9. *A*: 4-day-old BeWo culture (×200). *B*: 5-day-old BeWo culture after 4 days of treatment with forskolin (×200). *C*: 6-day-old primary culture of human cytotrophoblasts that have aggregated and fused (×200).

Table 1. Effects of 10 μ M 8-BrcAMP or 100 μ M forskolin on apparent permeability coefficients for fluorescein passage across BeWo monolayers at 37°C

Treatment	Fluorescein Permeability Coefficients, cm/s $\times 10^5$	
	+8-BrcAMP	+Forskolin
Control (vehicle)	3.4 \pm 0.15	3.4 \pm 0.1
2-Day	3.3 \pm 0.4	3.9 \pm 0.3
3-Day	3.8 \pm 0.9	4.7 \pm 0.1*

Data represent means \pm SD from quadruplicate samples. 8-BrcAMP, 8-bromoadenosine 3',5'-cyclic monophosphate. * Different from respective control, $P < 0.05$.

the apical-to-basolateral transport. After a 4-day exposure to forskolin, the permeability of the treated monolayers dramatically increased and was quite variable (not shown), and this was likely because of the sloughing and aggregation of cells, which created large intercellular openings (see Fig. 9).

DISCUSSION

The BeWo cells form a monolayer of cells with regular microvilli on the apical surface, consistent with the general characteristics of the human trophoblast (19, 27, 28). The cell line is readily passaged, and the stability of the BeWo cell line has been previously noted (20). A few reports also exist on the growth of BeWo monolayers on a semipermeable membrane (4, 5). However, variations in cell culture density, passaging methods, splitting ratios, and extracellular matrix will significantly influence the cellular composition of a cell line (2). In particular, the growth and composition of the BeWo cell line have been shown to be sensitive to the make up of the extracellular matrix (12). In this study, we have described the experimental conditions for the growth of BeWo cells on a porous substrate and placement in a side-by-side diffusion apparatus (2) for the purpose of developing a model to study transplacental transport and metabolism in vitro.

Passive diffusion is the primary mechanism by which xenobiotics cross the placental barrier (7, 22, 25). An appropriate in vitro system would be expected to provide at least a qualitative representation of the trophoblast sieving of molecules observed in the placenta. BeWo monolayers form a molecular weight-selective barrier after \sim 5 days in culture. The passive perme-

Table 2. Effects of 100 μ M forskolin on permeation of 100 μ M linoleic acid across BeWo monolayers

	Linoleic Acid Flux, mol/s $\times 10^{17}$
Apical to basolateral	
Control (vehicle treatment)	5.7 \pm 2.5*
3-Day treatment with forskolin	15.7 \pm 0.1†
Basolateral to apical	
Control (vehicle treatment)	3.9 \pm 0.4
3-Day treatment with forskolin	5.7 \pm 0.1†

Data represent means \pm SD from quadruplicate samples incubated at 37°C for 1 h. * Different from basolateral-to-apical control, $P < 0.05$; † different from respective control, $P < 0.05$.

ation of selected hydrophilic markers across the BeWo monolayers was found to be linearly related to molecular size. The calculated P_e was also proportional to the water diffusion coefficients for the markers (not shown), consistent with the trans-trophoblast passage of small hydrophilic solutes in the placenta through aqueous paracellular routes (7, 22, 25).

Although the TEER of a cell monolayer is poorly correlated with permeability to larger hydrophilic markers (1), very reproducible and maximal TEER values were also observed for BeWo 5 days postseeding at a density of 100,000 cells/cm². The TEER values of this system were low compared with reports of other laboratories (4); however, differences in growth substrates, temperature, buffers, instrumentation, and other lab-to-lab variables can account for the differences. As expected, EDTA treatment of the cells, likely chelating extracellular free calcium and disrupting intercellular junctions, substantially decreased the TEER value. In contrast, the inhibition of sodium channels by amiloride did not seem to play an important role in TEER of BeWo monolayers. Generally, these results appeared consistent with placental ion-transfer characteristics described in previous reports (24).

A few studies (4, 5) have examined the morphological and functional polarity of BeWo monolayers grown on permeable supports. In those studies, polarized transport of transferrin by BeWo monolayers has been reported (4, 5). Functional polarized transport of the fatty acid linoleic acid has also been demonstrated in plasma membrane vesicles prepared from human syncytiotrophoblasts by Lafond et al. (15) but not in BeWo monolayers. We were able to demonstrate a polarized transport of linoleic acid across the BeWo monolayers favoring the apical-to-basolateral direction. The transport was saturable, enhanced in the presence of albumin, and could be inhibited by excess unlabeled fatty acid and low temperature, all features of a carrier-mediated process and consistent with the work of Lafond et al. (15). In fact, the estimated K_m of \sim 7.4 μ M for unbound linoleic acid transport by BeWo monolayers here was in close agreement with the 7.9 μ M reported by Lafond et al. (15) with human brush border vesicles. In other studies, we were also able to demonstrate apparent preferential binding of [³H]dihydroalprenolol to the basolateral surface of the BeWo monolayers, an observation that was in agreement with the \sim 10-fold greater binding of dihydroalprenolol on basolateral membranes of the human placental barrier (3). Collectively, the studies of earlier researchers (12, 14) and our results here affirmed that BeWo monolayers exhibit morphological and functional polarity when grown on permeable supports. Moreover, these studies indicated that the BeWo monolayers may have a number of applications in characterizing the nature and role of polarized carrier mechanisms at the placental barrier.

BeWo cells are not capable of spontaneously differentiating to the syncytiotrophoblast in culture (23). This is in contrast to primary cultures of cytotrophoblasts, which aggregate and form a syncytia typical of the in

vivo placental barrier (14). Forskolin and 8-BrcAMP, stimulants of differentiation and inhibitors of proliferation, were added to the BeWo cultures to assess the role of differentiation on monolayer permeability. The treatment of BeWo monolayer cultures with forskolin and 8-BrcAMP did stimulate BeWo cell differentiation, and morphologically the BeWo cells did take on an appearance similar to the syncytialized primary cultures. These morphological changes were consistent with earlier reports (28). The forskolin or 8-BrcAMP-induced alteration of the differentiation of the BeWo cells may not only alter morphology but could also regulate transport systems. Our studies suggested that induction of the differentiation of BeWo cells did not substantially alter passive transport of fluorescein. By contrast, the forskolin pretreatment enhanced the transport of linoleic acid and developed more polarity in the transport process. Longer treatments with forskolin resulted in cell sloughing and an increase in monolayer permeability resulting from syncytia formation. There have been no reports for comparison on the effects of forskolin on the passive permeability of trophoblasts in culture. With respect to nutrient transporters, in BeWo cellular or monolayer uptake studies, forskolin has been reported to increase transferrin uptake transport (26); however, there was no effect on L-alanine transport (17). Forskolin treatment of BeWo cells does stimulate the development of asymmetry in the neutral amino acid transporter, suggesting that trophoblast fusion and differentiation is accompanied by significant membrane specialization (8). The altered passage of linoleic acid across the BeWo monolayers in this study provides further indication that, in addition to neutral amino acid carriers, other transporters may be similarly regulated during trophoblast fusion and differentiation. In future studies, the effects of forskolin on BeWo transporters might be considered a routine part of the process of characterizing carrier mechanisms.

In conclusion, the human choriocarcinoma cell line BeWo (b30) was cultured on semipermeable membranes, formed confluent monolayers in ~5 days, and was morphologically similar to primary cultures of human trophoblasts, except in the extent of differentiation. The passive sieving properties of the BeWo monolayers were defined by establishing the permeability with respect to fluorescein and FITCDs of varying molecular weight. The BeWo monolayers developed a reproducible TEER indicative of ion transport, and the monolayers were functionally polarized with respect to linoleic acid transport. BeWo cells grown on permeable supports could be induced to undergo differentiation in the presence of forskolin and 8-BrcAMP. Under our growth and experimental conditions, we have extended some of the observations of other laboratories (5, 6, 17, 21) and support the notion that BeWo monolayers might be further exploited as a polarized trophoblast layer to examine transplacental transport and metabolism. In current studies, we have established that BeWo monolayers also retain inducible cytochrome P-450 enzymes, peptidases, and surface lectin binding typical of the human placenta (F. Liu, X. Zhang, M.

Soares, and K. L. Audus, unpublished observations). Ongoing research in our laboratory also includes application of this in vitro system to the study of transport and metabolism of drugs and drugs of abuse. On the basis of these results, the BeWo cell monolayers appear to be an appropriate in vitro system to investigate the transplacental transport of certain nutrients and xenobiotics.

We gratefully acknowledge Dr. Jerome F. Strauss III for valuable comments and assistance in establishing the primary human trophoblast cultures.

This study was supported by the National Institute of Drug Abuse (N01DA-4-7405). Support for the Cellular and Molecular Biopharmaceutics Handling Laboratory was provided by Corning Costar.

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Received 31 March 1997; accepted in final form 21 July 1997.

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