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Biotin transport in rat intestinal brush-border membrane vesicles

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Transport of biotin across rat intestinal brush-border membrane was examined using the brush-border membrane vesicle (BBMV) technique. Uptake of biotin by BBMV is the result of transport of the substrate into the intravesicular space with negligible binding to membrane surfaces. In the presence of a Na^+ gradient (out > in), transport of biotin was higher with a transient 'overshoot' phenomenon. In comparison, transport of biotin in the presence of a choline gradient (out > in) was lower with no 'overshoot' phenomenon. In both jejunal and ileal BBMV, the transport of biotin as a function of concentration was saturable in the presence of a Na^+ gradient (out > in) but was linear in the presence of a choline gradient (out > in). V_{max} of the Na^+-dependent transport system was 0.88 and 0.37 pmol/mg protein per s and apparent K_t was 7.57 and 7.85 μM in jejunal and ileal BBMV, respectively. Structural analogues inhibited the transport process of biotin. Unlike the electrogenic transport of D-glucose, the transport of the anionic biotin was not affected by imposing a relatively positive intravesicular potential with the use of valinomycin and an inwardly-directed K^+ gradient, suggesting that biotin transport is most probably an electroneutral process. This suggestion was further supported by studies on biotin transport in the presence of anions of different lipid permeability. The results of this study demonstrate that biotin transport across rat intestinal brush-border membrane is by a carrier-mediated, Na^+-dependent and electroneutral process. Furthermore, transport of biotin is higher in the jejunum than the ileum.

Introduction

Biotin is an essential water-soluble vitamin which is required for normal cellular function, growth and development. Biotin acts as a coenzyme in many metabolic reactions including fatty acid biosynthesis, gluconeogenesis, and amino acid catabolism. Humans and higher mammals cannot synthesize biotin, therefore, they must obtain the vitamin by intestinal absorption. Biotin deficiency in humans leads to serious clinical complications including growth retardation, neurological disorders and skin abnormalities [1–3].

Recent studies from our laboratory have characterized the transport process of biotin in rat intestine using the everted sac technique [4,5]. The results showed that biotin transport occurs by a carrier-mediated process which is Na^+ and energy dependent and is predominant in the jejunum of adult rats [4]. Transport in the intact intestinal tissue preparation, however, represents multiple processes that include transport across two morphologically and structurally different membranes, namely the brush-border membrane (BBM) and the basolateral membrane (BLM). Because of our

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interest in characterizing the details of the biotin transport process and its regulation at different cellular levels, we designed this study to characterize the biotin transport process across the intestinal BBM of the rat using a well-validated BBMV technique.

Materials and Methods

Materials

[8,9-^{3}H]Biotin (40 Ci/mmol) and D-[1(n)-^{3}H]glucose (20 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Unlabelled biotin, biotin methyl ester, desthiobiotin and thioctic acid was from Sigma Chemical Co., St. Louis, MO. Cellulose nitrate filters (0.45 μm pore size) were from Sartorius Filters, Haywood, CA. All other chemicals and reagents were of analytical quality. The radiochemical purity of ^{3}H-biotin used in this study was 97.6 percent as determined prior to use on cellulose pre-coated TLC plates using a solvent system of butanol/acetic acid/water (4:1:5, v/v) [4–6].

Methods

Preparation of Intestinal BBMV. Rats (180–220 g) were purchased from Sasco, Omaha, NE and were fed Purina rat chow and tap water ad libitum. They were sacrificed by an overdose of ether, the abdomen was opened and 35 cm of the jejunum (starting 14 cm from the pylorus) or the ileum (the most distil 35 cm of the small intestine) was removed, washed and everted and the mucosa was scraped with a glass slide. Intestinal BBMV were isolated from intestinal mucosa by a modification of Kessler's divalent cation (Mg^{2+}) precipitation technique [7] as described in detail by us previously [8–10]. All preparation steps were conducted at 4°C. Using a Waring blender type homogenizer at maximum speed, the mucosal scraping was homogenized for 3 min in 60 ml of 300 mM mannitol, 5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetracetic acid (EGTA), and 12 mM tris-(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.1); 240 ml of ice-cold distilled water was then added. The homogenate was treated with 3 ml of 1 M $MgCl_2$ and centrifuged at 3000 × *g* for 15 min (Model J2-21, Beckman Instruments, Fullerton, CA). The supernatant was then centrifuged at 27000 × *g* for 30 min. The resulting pellet was resuspended in 60 ml of 60 mM mannitol, 5 mM EGTA, and 12 mM Tris-HCl (pH 7.1) and homogenized in a Potter-Elvehjem tube for 10 strokes at the highest speed. The homogenate was treated with 0.6 ml of 1 M $MgCl_2$ and centrifuged at 3000 × *g* for 15 min. The pellet was resuspended in 30 ml of 250 mM mannitol and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)-Tris buffer and centrifuged at 50000 × *g* for 15 min. With the use of tuberculin syringe with a 25-gauge needle, the pellet was resuspended in the desired volume of the transport (intravesicular) buffer (280 mM mannitol and 20 mM Hepes-Tris (pH 7.4)). The suspension was then pre-incubated at room temperature for 2 h to load the inside of the vesicles.

Transport Studies. Transport studies with BBMV were performed by a rapid-filtration technique [11] as described by us previously [8–10]. All incubations were done at 37°C. The reaction was initiated by adding a 20 μl aliquot of membrane vesicle suspension to 80 μl incubation buffer (final concentrations: 100 mM NaCl or choline chloride, 80 mM mannitol and 20 mM Mes-Tris, (pH 6.0)) containing varying amounts of radiolabelled and unlabelled substrate plus other constituents. After incubation for the desired period of time the reaction was terminated by the addition of 1 ml of ice-cold stop solution (200 mM NaCl, 100 mM mannitol, 10 mM KH_2PO_4 (pH 7.4)). The cold, diluted reaction mixture was immediately pipetted onto a prewetted filter and kept under suction. The filter was rinsed with 5 ml of ice-cold stop solution and then dissolved in 6 ml of ACS scintillation cocktail (Amersham). Radioactivity was counted in a scintillation counter (Model LS 3801, Beckman Instruments, Irvine, CA). Nonspecific binding of the substrate to the filter (background) was determined by filtering a reaction mixture that contained an identical solution but no vesicles and was subtracted from the transport data. Transport data presented in this study are the results of three experiments and are expressed as the means ± S.E. in nmol or pmol/mg protein per unit time. Data were analyzed using the Student's *t*-test and regression analysis. Protein concentration was measured by the method

of Lowry et al. [12] using bovine serum albumin as a standard.

The purity of intestinal BBMV and their suitability for transport studies has been demonstrated in previous studies in our laboratory [8–10] by morphological (electron microscope), enzymatic (marker enzymes for BBM, BLM, mitochondria and endoplasmic reticulum) and functional (transport of D-glucose and other nutrients) criteria.

Results

Effect of osmolarity

BBMV are osmotically active structures, i.e., they respond to changes in medium osmolarity. Using this characteristic of the BBMV, one can differentiate between binding of the substrate to the membrane surface and transport into the intravesicular space. This can be done by examining uptake of the substrate as a function of changing the incubation medium osmolarity. Fig. 1 shows the relationship between transport of 0.152 μM biotin into jejunal BBMV and 1/osmolarity of the incubation medium (the osmolarity was varied by changing the mannitol concentration). The relationship was linear with a correlation coefficient (r) of 0.99. Extrapolating the line to infinite osmolarity showed minimal uptake (intercept = 0.00087). These results indicate that at 90 min and under isotonic conditions almost 99 percent of the biotin taken up by the BBMV is the result of transport into the intravesicular space.

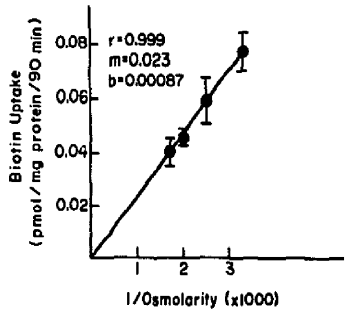

Fig. 1. Effect of incubation medium osmolarity on biotin transport. Jejunal BBMV were preloaded with 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed for 90 min at 37 °C in an incubation buffer of 100 mM NaCl, 20 mM Mes-Tris (pH 6.0) and a sufficient amount of mannitol to give the indicated osmolarity. 0.152 μM biotin was added to the incubation medium at the onset of incubation. Each point represents the mean ± S.E. of three experiments. $Y = mX + b$, where m = slope, $b = Y$ intercept. r represents the correlation coefficient.

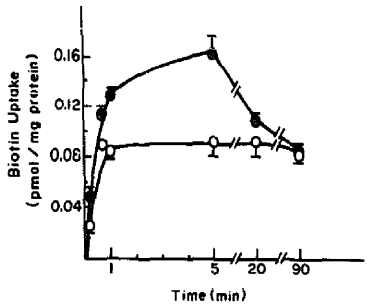

Fig. 2. Transport of biotin in the presence of a Na^+ and a choline gradient as a function of time. Jejunal BBMV were preloaded with 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed in an incubation buffer of 100 mM NaCl (●) or choline chloride (○), 80 mM mannitol and 20 mM Mes-Tris (pH 6.0). 0.17 μM biotin was added to the incubation buffer at the onset of incubation. Each point represents the mean ± S.E. of three experiments.

In another study, using the procedure described in Materials and Methods, we examined the metabolic form of the transported radioactivity into jejunal BBMV (vesicles were incubated with 0.43 μM ^{3}H-biotin for 90 min in an iso-osmolar solution). The results showed that the majority (92.4 percent) of the transported radiolabelled compound was the intact biotin molecule.

Transport with time

Fig. 2 depicts transport of 0.17 μM biotin with time in the presence of a Na^+ and a choline gradient (outside = 100 mM, inside = 0 mM). In the presence of a Na^+ gradient, transport of biotin was higher and linear for approximately 1 min of incubation with a distinct 'overshoot' phenomenon, i.e., a transient accumulation against a concentration gradient. Equilibrium was reached after 90 min of incubation. In the presence of a choline gradient, transport of biotin was lower, no 'overshoot' was observed and equilibrium was reached after approximately 1 min of incubation.

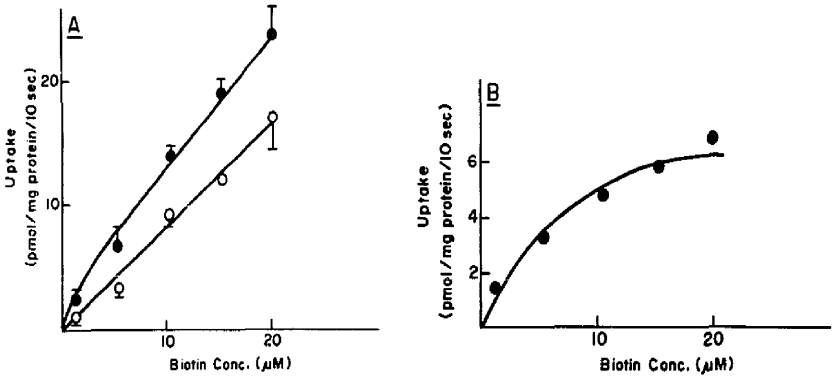

Fig. 3. Transport of biotin in jejunal BBMV as a function of concentration in the presence of a Na^+ and a choline gradient. (A) Jejunal BBMV were preloaded with 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed for 20 s at 37°C in an incubation buffer of 100 mM NaCl (●) or choline chloride (○), 80 mM mannitol and 20 mM Mes-Tris (pH 6.0) and different concentrations of biotin. Each point represents the mean±S.E. of three experiments. (B) Plot showing transport of biotin by the Na^+-dependent system determined as described in the text.

Kinetics of biotin transport

In this study we examined the transport of biotin in jejunal BBMV as a function of increasing the substrate concentration in the incubation medium in the presence of a Na^+ and a choline gradient (outside = 100 mM, inside = 0 mM). Saturation in biotin transport was observed in the presence of a Na^+ gradient but not in the presence of a choline gradient (Fig. 3A). To determine the kinetic parameters (V_{max} and apparent K_t) of biotin transport by the Na^+-dependent process,

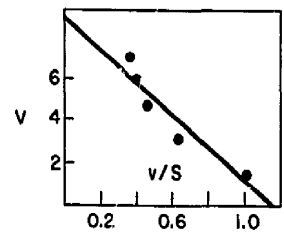

Fig. 4. Woolf-Augustinsson-Hofstee plot of the results in Fig. 3B (v against v/S). The best-fit straight line was determined by regression analysis ($Y = -7.57\ X + 8.75$; correlation coefficient (r) = 0.96).

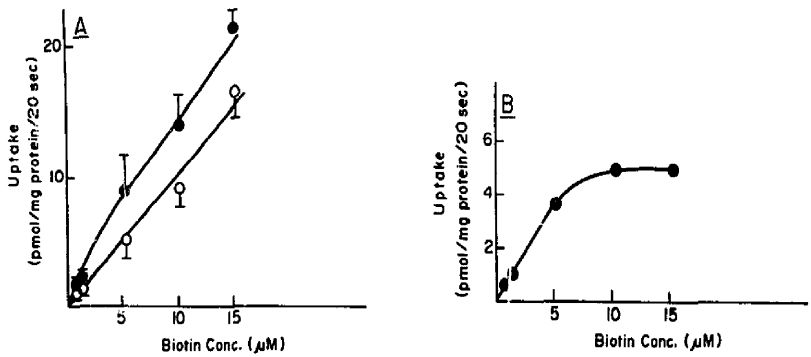

Fig. 5. Transport of biotin in ileal BBMV as a function of concentration in the presence of a Na^+ and a choline gradient. (A) Ileal BBMV were preloaded with 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed for 20 s at 37°C in an incubation buffer of 100 mM NaCl (●) or choline chloride (○), 80 mM mannitol and 20 mM Mes-Tris (pH 6.0) and different concentrations of biotin. Each point represents the mean±S.E. of three experiments. (B) Plot showing transport of biotin by the Na^+-dependent system determined as described in the text.

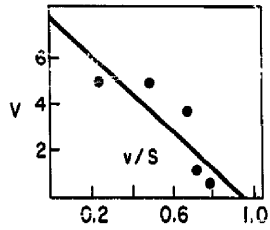

Fig. 6. Woolf-Augustinssion-Hofstee plot of the results in Fig. 5B (v against v/S). The best-fit straight line was determined by regression analysis ($Y = -7.85\ X + 7.47$; $r = 0.84$).

we first determined the amount of biotin transported by this system at each concentration. This was done by subtracting biotin transport in the presence of a choline gradient from transport in the presence of a Na^+ gradient (Fig. 3B). We then calculated the transport kinetic parameters of the Na^+-dependent system from the Woolf-Augustinsson-Hofstee plot (Fig. 4). V_{max} and apparent K_m of 0.88 pmol/mg protein per s and 7.57 μM were obtained, respectively.

Similarly, the transport of biotin in ileal BBMV was saturable in the presence of a Na^+ gradient but was linear in the presence of a choline gradient (Fig. 5A). Transport of biotin by the carrier-medited system (Fig. 5b) was then calculated as described above. The V_{max} and apparent K_m of the Na^+-dependent transport system of biotin was calculated from the Woolf-Augustinsson-Hofstee plot (Fig. 6) and found to be 0.37 pmol/mg protein per s and 7.85 μM, respectively.

TABLE I

EFFECTS OF STRUCTURAL ANALOGUES AND RELATED COMPOUNDS ON BIOTIN TRANSPORT

Jejunal BBMV were preloaded with 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed for 20 s at 37°C in an incubation buffer of 100 mM NaCl, 80 mM mannitol and 20 mM Mes-Tris (pH 6.0). 0.17 μM biotin and different concentrations of the structural analogues or the related compound under study were added to the incubation medium at the onset of the experiment. Each point is the mean ± S.E. of at least three experiments. P values were calculated using the Student's t-test.

Compound	Concn. (μM)	Biotin transport (pmol/mg protein per 20 s)	P value
Control		0.199 ± 0.057	
Biotin methyl ester	25	0.081 ± 0.013	< 0.05
	50	0.064 ± 0.009	< 0.05
Thioctic acid	25	0.065 ± 0.005	< 0.05
	50	0.056 ± 0.006	< 0.025
Desthiobiotin	50	0.070 ± 0.015	< 0.05

Effect of structural analogues

The effect of the structural analogues biotin methyl ester and desthiobiotin and of the related compound thioctic acid on the transport of 0.17 μM biotin was examined. Incubation was performed for 20 s. All compounds examined caused significant inhibition in biotin transport into jejunal BBMV (Table I).

Effect of transmembrane electrical potential

The effect of imposing an electrical potential across the BBM on the transport of the anionic biotin was examined using previously-described methods [8,9,13–15]. The methods used were: (a) valinomycin-induced K^+ diffusion electrical potential, and (b) anion substitution method.

In the first method we examined the transport of 0.17 μM biotin in the presence of an inwardly-directed K^+ gradient and the presence of the K^+ ionophore valinomycin (10 μg/mg protein) (Expt. I) (incubation buffer was 90 mM NaCl, 50 mM potassium gluconate and 20 mM Mes-Tris, (pH 6.0); transport buffer was 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4)). The results were compared to that of a simultaneously-performed experiment (Expt. II) in the absence of a K^+ gradient ($K_o^+ = K_i^+ = 50$ mM) and the presence of valinomycin (10 μg/mg protein), i.e., a 'voltage clamp' condition (incubation buffer was as above; transport buffer was 180 mM mannitol, 50 mM potassium gluconate and 20 mM Hepes-Tris (pH 7.4)). The rapid diffusion of K^+ in the first experiment will generate a transmembrane potential with a relatively positive intravesicular compartment thereby affecting any electrogenic component of biotin transport. The results of these two experiments are shown in Fig. 7. No significant difference in biotin transport was observed in the two experiments at any time point examined. These results suggest that biotin transport is most probably an electroneutral process. Unlike the transport of biotin, the transport of D-glucose by the Na^+-dependent system was inhibited by the above

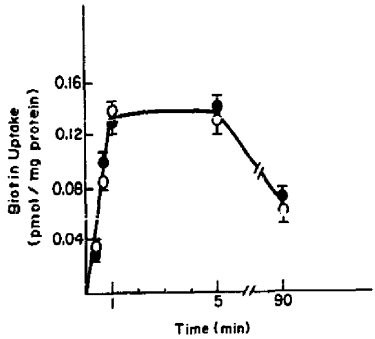

Fig. 7. Effect of valinomycin-induced K^+ diffusion transmembrane electrical potential of biotin transport. Jejunal BBMV were preloaded with either 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4 ((●)) or 50 mM K-gluconate, 180 mM mannitol and 20 mM Hepes-Tris (pH 7.4 (○)). In both cases incubation was performed for different periods of time at 37°C in an incubation buffer of 90 mM NaCl, 50 mM potassium gluconate and 20 mM Mes-Tris (pH 6.0) in the presence of valinomycin (10 μg/mg protein). 0.17 μM biotin was added to the incubation medium at the onset of incubation. Each point represents the mean ± S.E. of three experiments.

treatments, i.e., by imposing a relatively positive intravesicular compartment with the use of valinomycin and a K^+ gradient (incubation conditions were identical to those described above with biotin) (Fig. 8).

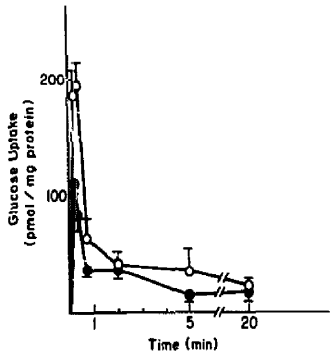

Fig. 8. Effect of valinomycin-induced K^+ diffusion transmembrane electrical potential on D-glucose transport. Incubation conditions and buffers were identical to those described in the legend of Fig. 7. 0.1 mM D-glucose was added to the incubation medium at the onset of incubation. Each point represents the mean ± S.E. of three experiments. As seen in the presence of valinomycin and an inwardly-directed K^+ gradient (●), there was a decrease in the magnitude of the 'overshoot' as compared to that in the presence of valinomycin but in the absence of a K^+ gradient (○).

In the second method we examined the transport of 0.17 μM biotin into jejunal BBMV in the presence of anions with different lipid permeabilities ($SCN^- > SO_4^{2-}$). Incubation with a relatively lipid-permeable anion (SCN^-) will create a relatively greater negative intravesicular compartment than a poorly-permeable anion (SO_4^{2-}), thereby affecting any electrogenic component of biotin transport. No significant difference in biotin transport was observed in the presence of these anions (transport of 0.127 ± 0.02 ($n = 4$) and 0.122 ± 0.01 ($n = 4$) pmol/mg protein/20 s were reported for SCN^- and SO_4^{2-}, respectively) further suggesting that biotin transport is probably an electroneutral process.

Discussion

The present study examined biotin transport in BBMV of rat small intestine. First, we examined biotin uptake as a function of incubation medium osmolarity. Our aim here was to differentiate between binding of the substrate to membrane surfaces and transport into an active intravesicular space. The results showed that biotin uptake by BBMV is the result of transport of the substrate into the intravesicular space with negligeable binding to membrane surfaces. Transport of biotin with time in the presence of a Na^+ gradient (outside > inside) was rapid and showed a transient 'overshoot' phenomenon indicating an uphill movement of biotin against a concentration gradient. In the presence of a choline gradient (outside > inside) transport of biotin was slow and no 'overshoot' was observed. These results indicate that biotin transport is Na^+ dependent. The importance of Na^+ in biotin transport observed in this study with BBMV is similar to those observed previously by us in everted sacs of rat intestine [4,5].

The transport of biotin as a function of concentration was saturable in the presence of a Na^+ gradient but was linear in the presence of a choline gradient, both in jejunal and ileal BBMV. This observation suggests that, in the presence of Na^+, biotin is transported by a carrier-mediated system. The involvement of a carrier-mediated system in

biotin transport in BBMV is further supported by the finding that structural analogues and a related compound inhibit biotin transport. The apparent K_m of the Na^+-dependent transport system of biotin was similar in jejunal and ileal BBMV. On the other hand, the V_{max} of the transport process was approx. 2.35-fold higher in the jejunum than in the ileum (V_{max} of 0.88 and 0.37 pmol/mg protein per s, respectively). This findings suggests that the number (i.e., density) and/or the activity of the transport system of biotin is higher in the jejunum than the ileum. Naturally the lower surface area available for absorption in the ileum as compared to the jejunum will lead to a further decrease in biotin transport in that segment of the small intestine in the intact tissue preparation [4].

The transport of biotin by the Na^+-dependent system was not affected by inducing a relatively positive intravesicular space with the use of valinomycin and an inwardly-directed K^+ gradient. This is unlike the transport of D-glucose by the Na^+-dependent system (a known electrogenic process [16,17]) which was inhibited by similar treatment. Furthermore, transport of biotin was not affected by inducing a relatively negative intravesicular space with the use of anions of different lipid permeation. These observations suggest that biotin transport is most probably an electroneutral process. Transport of the anionic biotin by a carrier-mediated, Na^+-dependent and electroneutral process is probably occurring through a $biotin^-/Na^+$ cotransport mechanism. It is possible that the movement of Na^+ down its concentration gradient provides the energy required for the uphill movement of biotin against a concentration gradient. Under normal in vivo conditions an inwardly-directed Na^+ gradient across the intestinal BBM is maintained by the activity of the Na^+/K^+-ATPase 'pump' located at the basolateral membrane of the enterocyte. Thus, the above-proposed mechanism for biotin transport across rat intestinal BBM could easily function in the intact enterocyte.

The present findings on biotin transport in rat intestinal BBMV are similar to those recently described by us in human intestine BBMV [9]. This clearly demonstrates that the rat is an excellent animal model to be used to project the mechanism and regulation of biotin transport in the human intestine. Having a suitable animal model to study the details of biotin intestinal transport and its regulation is important, since it has been previously reported that species differences may exist in the way animals' small intestine absorb biotin [6]. In summary, the present study shows that biotin transport in rat intestinal BBMV is by a carrier-mediated process which is, Na^+-dependent and appears to be electroneutral in nature. Furthermore, transport of biotin is higher in the jejunum than in the ileum.

Acknowledgments

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