Mechanism of Transport of Riboflavin in Rabbit Intestinal Brush Border Membrane Vesicles (43554)

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Abstract. **Uptake of luminal riboflavin (RF) into the absorptive cells of rabbit small intestine was examined using purified brush border membrane vesicle (BBMV) preparations. These preparations were used in order to eliminate the interference of intracellular metabolism that occurs to the RF molecule during absorption. Uptake of RF by BBMV was found to be mainly (>76%) the result of transport of the vitamin into the intracellular space with less binding to membrane surfaces. All ^3H radioactivity that appeared in the intravesicular space after incubation with [^3H]RF was found to be in the form of intact RF. Uptake of RF with time was independent of the presence or absence of a Na^+ or a K^+ gradient (out >in) and occurred without transaccumulation of the substrate in the intravesicular space. Furthermore, changing the incubation buffer pH showed minimal effect on RF uptake. When examined as a function of concentration, the initial rate of RF uptake was found to be saturable both in jejunal and ileal BBMV with an apparent K_m of 7.24 ± 1.06 and 8.88 ± 0.90 μM and V_{max} of 24.31 ± 1.48 and 34.24 ± 1.55 pmol/mg protein/5 sec, respectively. Unlabeled RF and the related compounds lumiflavin, 8-aminoriboflavin, isoriboflavin, and lumichrome all inhibited (but to different degrees) the uptake of physiologic concentration of [^3H]RF. On the other hand, 8-hydroxyriboflavin, lumazine, and D-ribose all failed to inhibit [^3H]RF uptake. Similarly, the membrane transport inhibitors DIDS, SITS, and furosemide all failed to inhibit [^3H]RF uptake. The uptake of RF was found to be insensitive to changes in the transmembrane electrical potential, as shown by studies with anion substitution and valinomycin K^+-induced negative or positive intravesicular potential methodologies. These results indicate that RF uptake by rabbit intestinal BBMV occurs via a carrier-mediated system that is Na^+ independent in nature and transports the substrate by an electroneutral process. The role of this system in the overall absorption process of RF is discussed.**

[P.S.E.B.M. 1993, Vol 202]

Riboflavin (RF (vitamin B_2) in its coenzyme forms, riboflavin 5′-phosphate and flavin adenine dinucleotide, is involved in variety of metabolic reactions that are important for normal cellular biologic reactions and growth (1, 2). Deficiency of RF leads to a series of clinical abnormalities, including growth retardation, degenerative changes in the nervous system, and skin abnormalities (1–3). Frank and subclinical RF deficiencies occur in humans during periods of physiologic and pathologic stress (1–6).

Mammals must obtain RF via intestinal absorption because they cannot synthesize the vitamin. In the diet, RF exists mainly in the form of riboflavin 5′-phosphate and flavin adenine dinucleotide (1, 2), forms that are hydrolyzed to free RF in the intestinal lumen prior to absorption (7). Using a variety of intact intestinal tissue and cellular preparations (everted sacs, perfused segments, isolated cells), the transport of RF was found to involve a carrier-mediated process that was inhibited by Na^+ removal from the incubation medium (7–11). Absorption of a nutrient from the intestinal lumen to the blood across the highly polarized and metabolically active enterocyte represents transport of the nutrient across two different membranes, the brush border membrane and the basolateral membrane, as well as

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Received July 2, 1992. [P.S.E.B.M. 1993, Vol 202]
Accepted October 19, 1992.

0037-9727/93/2024-0428$3.00/0

movement across the cell. Limited studies are available describing the mechanism and regulation of the individual membrane transport event and the role of intracellular metabolism in the overall absorption process of RF. Such studies are crucial if detailed understanding of the mechanism and regulation of RF absorption under normal physiologic conditions and how certain physiologic and pathologic conditions affect the process are desired. We have recently begun a series of studies to characterize the individual transport event and its regulation. In this report, we present the results of our study on RF transport in brush border membrane vesicles (BBMV) prepared from rabbit intestine.

Materials and Methods

Materials. [^{3}H(G)]Riboflavin (sp act 44.6 Ci/mmol; radiochemical purity 98%) was obtained from American Radiolabeled Chemicals Inc., St. Louis, MO. Cellulose nitrate filters (0.45-μm pore size) were obtained from Milipore Corp., Bedford, MA. 8-Aminoriboflavin (8-NH_2-RF), isoriboflavin (iso-RF), and 8-hydroxyriboflavin (8-OH-RF) were kindly supplied by Dr. Vincent Massey of the University of Michigan School of Medicine at Ann Arbor. All other chemicals and reagents were obtained from commercial sources and were of analytical quality.

Methods. The jejunum (or ileum) of adult New Zealand White rabbits (2.5–3.5 kg) was removed and flushed with ice-cold saline solution, and the mucosa was scraped. The scraping was frozen at −70°C and BBMV were prepared using a Mg^{2+} precipitation method (12). The isolation procedure has been described in detail by us previously (13, 14). The final pellet was suspended in "transport buffer" of 280 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4) to achieve a final protein concentration of 3–5 mg/ml. All vesicle preparations were used fresh on the day of isolation and triplicate transport measurements were performed at each point. Transport studies were performed at 37°C by a rapid-filtration technique (15) as described by us before (13, 14). The reaction was started by mixing 20 μl of vesicle suspension with 80 μl of incubation buffer, which usually contained 100 m*M* NaSCN or KSCN, 80 m*M* mannitol, and 20 m*M* Hepes/Tris (pH 6.4). Riboflavin (labeled and unlabeled) and other constituents were added to the incubation medium at the beginning of the experiment. At the end of incubation, the reaction was terminated by adding 1 ml of ice-cold stop solution (100 m*M* NaCl, 100 m*M* mannitol, and 10 m*M* KH_2PO_4, pH 7.4). The mixture was immediately filtered on a vacuum manifold and washed with 5 ml of ice-cold stop solution, and the filter was dried and counted for radioactivity in a Beckman liquid scintillation counter. Protein concentration was measured by the method of Lowry *et al.* (16) using bovine serum albumin as the standard. Each data point presented in this paper is the mean ± SE (pmol/mg protein/unit time) of multiple transport measurements from at least two different BBMV preparations from different rabbits. Slight quantitative, but not qualitative, differences were seen in the absolute amount of RF taken up by some BBMV preparations. In such cases, data were compared with simultaneously performed controls using the same vesicular preparation and the results are presented as percentage relative to those controls.

The metabolic form of the transported substrate following incubation of jejunal BBMV with 0.11 μ*M* [^{3}H]RF for 60 min at 37°C was investigated using silica gel, precoated, thin layer chromatography plates and a solvent system of ethanol:water (9:1 v/v) (9) and found to be >97% in the form of intact RF.

Results

General Characteristics of RF Uptake by Rabbit Intestinal BBMV. Figure 1 depicts the results on the effect of incubation medium osmolarity (adjusted with mannitol) on the uptake of 0.11 μ*M* RF. Incubation was done for 60 min at 37°C. The results showed an inverse and linear (r = 0.99) relationship between RF uptake and incubation medium osmolarity. When the uptake line was extended to infinite osmolarity (i.e., zero intravesicular space), it intersected the y axis at 0.292. From these results, it could be calculated ([y-

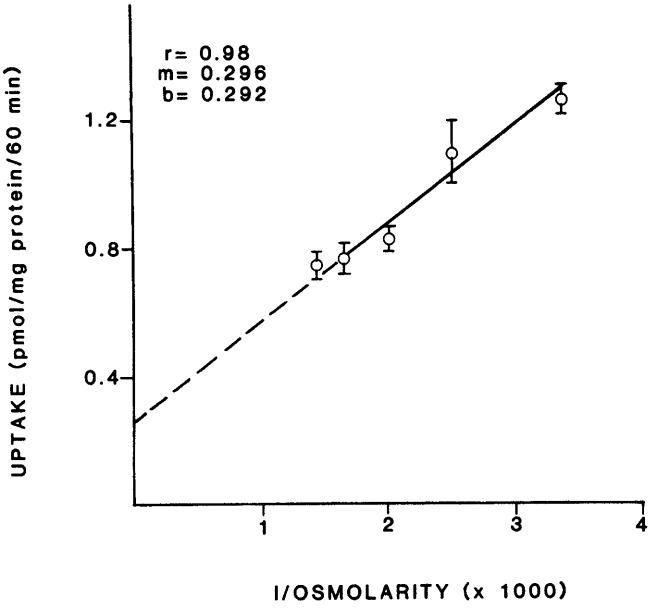

Figure 1. Effect of incubation medium osmolarity on uptake of RF by rabbit jejunal BBMV. Vesicles were preloaded with a buffer of 280 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4) and incubated in a buffer of 100 m*M* NaSCN, 20 m*M* Hepes/Mes (pH 6.4), and a variable amount of mannitol to give the indicated osmolarity. Incubation was done for 60 min at 37°C in the presence of 0.11 μ*M* [^{3}H]RF. Each data point is the mean ± SE of seven to nine separate transport measurements from three different BBMV preparations. $Y = 0.296X + 0.292$; $r = 0.99$.

intercept/uptake at 300 mOsm] × 100) that the major portion (>76%) of RF taken up by these vesicles under iso-osmolar condition is the result of transport of the substrate into an osmotically active intravesicular space and the rest is due to binding to membrane surfaces.

Figure 2 shows the results on the uptake of 0.11 μM RF with time in BBMV incubated in the presence of an Na^+ gradient, in the presence of a K^+ gradient (outside = 100 m*M*, inside = 0 m*M*), or in the absence of cation and the presence of mannitol. Uptake was found to be similar (not significantly different) under these three different incubation conditions and was rapid and linear for approximately 20 sec of incubation. Uptake rate was leveled off thereafter, reaching equilibrium sometime between 40 sec and 60 min. No transaccumulation of RF inside the BBMV and no stimulation by Na^+ was seen. The uptake of 20 μM RF with time was similarly found to be linear for 10 sec of incubation but decreased thereafter, and was similar in the presence of an Na^+ and a K^+ gradient (out > in) (data not shown). In contrast to the lack of effect of Na^+ on RF transport, transport of D-glucose (0.1 m*M*) showed its usual Na^+-gradient dependency with an "overshot" phenomenon in the presence of an inwardly directed Na^+, but not K^+, gradient (in the presence of Na^+, transport was [in pmol/mg protein] 675 ± 71 [n = 4], 685 ± 94 [n = 4], 503 ± 54 [n = 4], 243 ± 7 [n = 3], and 132 ± 15 [n = 4] at 5, 10, 20, and 60 sec and 5 min, respectively; in the presence of K^+, transport was 108 ± 17 [n = 3], 93 ± 5 [n = 4], 98 ± 2 [n = 4], 94 ± 6.3 [n = 4], and 131 ± 7.9 [n = 3] at 5, 10, 20, and 60 sec and 5 min, respectively).

We also examined the effect of changing the incubation buffer pH on the uptake of 0.11 μM RF. The results (Fig. 3) showed minimal effect of pH on the initial rate of RF uptake.

Uptake of RF in Jejunal and Ileal BBMV as a Function of Concentration. In this study, we examined and compared the uptake of RF as a function of concentration (0.11–20 μM) in jejunal and ileal BBMV. Incubation was done for 5 sec at 37°C. In both types of BBMV, uptake of RF was found to include a saturable component. Uptake by this component was determined at each concentration by subtracting nonspecific uptake (calculated from the slope of the uptake line between 100 μM and the point of origin) from total uptake (Fig. 4). Transport kinetic parameters were then determined using a computerized program of Michaelis-Menten equation as described by Wilkinson (17). An apparent K_m of 7.24 ± 1.06 and 8.88 ± 0.90 μM and V_{max} of 24.31 ± 1.48 and 34.24 ± 1.55 pmol/mg protein/5 sec were found for jejunal and ileal BBMV, respectively.

Effect of Unlabeled RF and Related Compounds on the Uptake of [^{3}H]RF. The effects of the addition to the incubation medium of high concentrations of

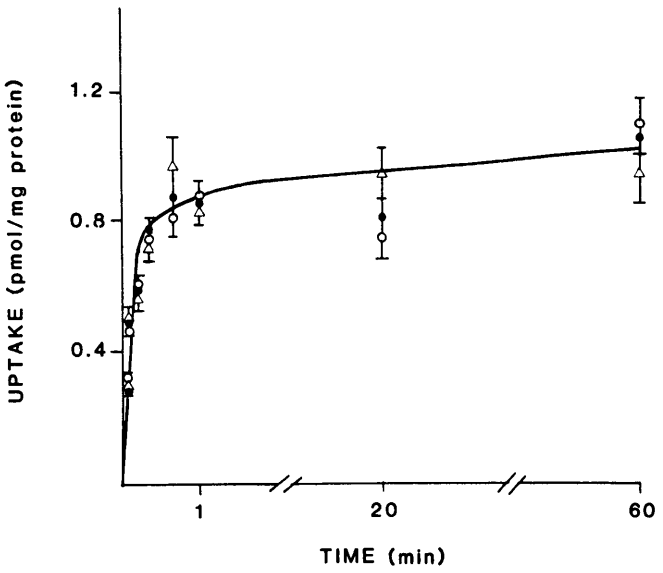

Figure 2. Uptake of RF by rabbit jejunal BBMV as a function of time in the absence and presence of an Na^+ and a K^+ gradient. Vesicles were preloaded with a buffer of 280 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4). Vesicles were then incubated at 37°C in a buffer of 100 m*M* NaSCN (●) or KSCN (○) or 200 m*M* mannitol (△), 80 m*M* mannitol and 20 m*M* Hepes/Mes (pH 6.4). RF (0.11 μM) was added to the incubation medium at the onset of incubation. Each data point is the mean ± SE of three to seven separate transport determinations from two to three different BBMV preparations.

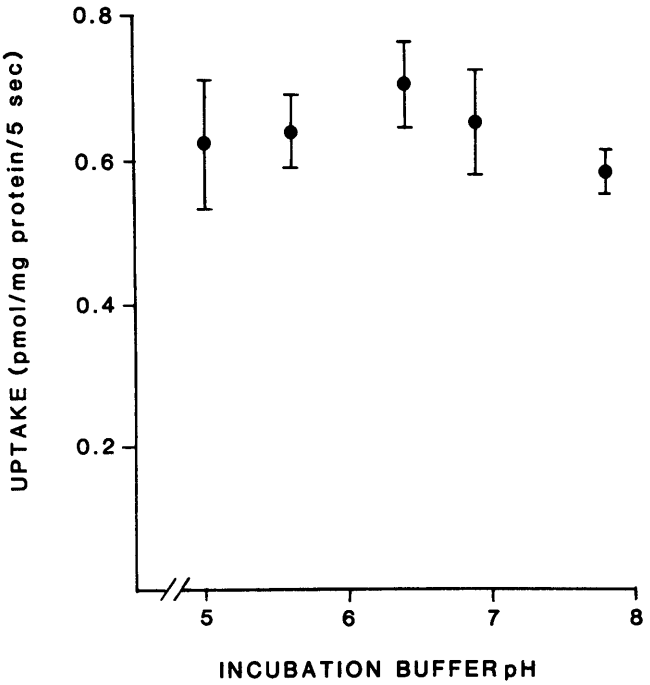

Figure 3. Effect of incubation buffer pH on uptake of RF by rabbit jejunal BBMV. Vesicles were preloaded with buffer of 280 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4) and incubated for 5 sec at 37°C in buffer of 100 m*M* NaSCN, 80 m*M* mannitol, and 20 m*M* of either Hepes/Tris or Hepes/Mes of different pH. RF (0.11 μM) was added to the incubation buffer at the onset of incubation. Each point is the mean ± SE of six different measurements for two separate BBMV preparations.

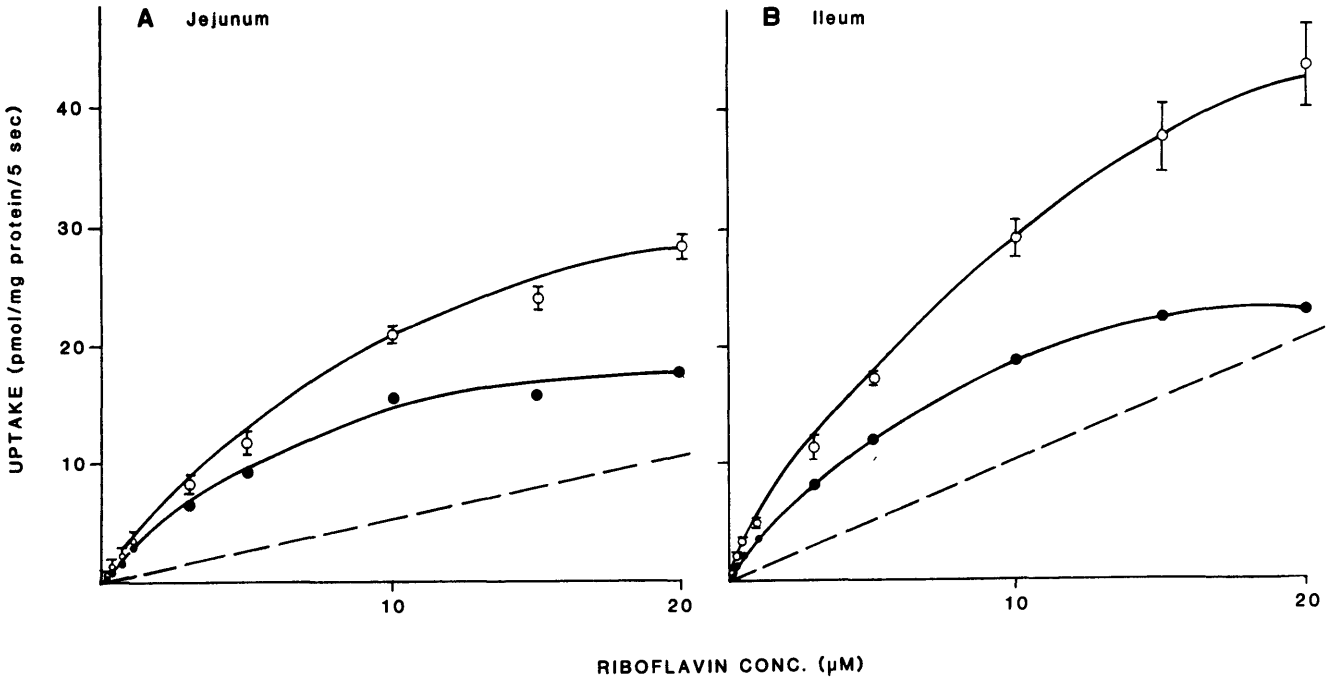

Figure 4. Uptake of RF as a function of concentration in BBMV isolated from rabbit (A) jejunum and (B) ileum. Jejunal and ileal BBMV were preloaded with a buffer of 280 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4). Incubation was done for 5 sec (initial rate) at 37°C in a buffer of 100 m*M* NaSCN, 80 m*M* mannitol, and 20 m*M* Hepes/Mes (pH 6.4). Different concentrations of RF (1–20 μM and 100 μM) were added to the incubation medium at the onset of incubation. Each point is

unlabeled RF and the related compounds lumiflavin, 8-NH_2-RF, iso-RF, 8-OH-RF, lumichrome, lumazine, and D-ribose on the uptake of 0.11 μM [^{3}H]RF were examined in this study. Our aims were to further confirm the existence of a carrier-mediated system for RF uptake by rabbit intestinal BBMV and to determine the structural-activity relationship between the carrier system and its substrate. Incubation was done for 5 sec at 37°C and the results are shown in Table I. Unlabeled RF caused significant ($P < 0.01$) and concentration-dependent inhibition in [^{3}H]RF uptake. Similarly, lumiflavin inhibited the uptake of [^{3}H]RF ($P < 0.01$) and the degree of inhibition was similar to that caused by unlabeled RF. 8-NH_2-RF, iso-RF, and lumichrome also inhibited ($P < 0.05$–0.01) the uptake of [^{3}H]RF and to a similar extent, but the inhibition was less than that seen with unlabeled RF and lumiflavin. 8-OH-RF, lumazine, and D-ribose, on the other hand, failed to cause significant inhibition in [^{3}H]RF uptake.

Unlabeled RF and lumiflavin (100 μM) caused a similar inhibition in the uptake of 0.11 μM [^{3}H]RF in vesicles incubated in the presence of an Na^+ gradient, and in the absence of Na^+ but presence of mannitol; also under these conditions, the inhibition was similar whether incubation was done at pH 6.4 or 7.8 (at pH 6.4, unlabeled RF and lumiflavin reduced [^{3}H]RF uptake to 17.23 ± 1.25% [$n = 5$] and 17.69 ± 2.0% [$n = 5$] that of simultaneously performed controls, respectively; at pH 7.8, unlabeled RF and lumiflavin reduced [^{3}H]RF uptake to 17.5 ± 1.67% [$n = 4$] and 18.67 ± 3.33% [$n = 3$] to that of simultaneously performed controls, respectively).

Effect of Membrane Transport Inhibitors, Folic Acid, and Probenecid on the Uptake of [^{3}H]RF. The effect of the membrane transport inhibitors DIDS, SITS, and furosemide (all at 1 m*M*), that of folic acid and its derivative, methotrexate (0.1 m*M*), and probenecid (5 m*M*) on the uptake of 0.11 μM [^{3}H]RF was examined in this study. The results (Table II) showed that these compounds have either a minimal or no effect on [^{3}H]RF uptake.

Effect of Electrical Membrane Potential on RF Uptake. The effect of transmembrane electrical potential on RF uptake in jejunal BBMV was examined using two well-established methods: the anion substitution method and the valinomycin-induced K^+ diffusion method (13, 14, 18–20). In the first method, anions of different membrane permeabilities were used (SCN^- > NO^-_3 > gluconate$^-$) in the incubation medium, and initial rate of uptake (5 sec) of 0.11 μM [^{3}H]RF into BBMV preloaded with regular transport buffer (see Materials and Methods) was examined. Incubation with a highly permeable anion would generate a greater transient negative intravesicular potential than a less permeable anion. The results showed similar RF uptake in the presence of SCN^-, NO^-_3, and gluconate$^-$ (in pmol/mg protein/5 sec, 0.449 ± 0.44 [$n = 6$], 0.474 ± 0.029 [$n = 6$], and 0.462 ± 0.072 [$n = 6$], respectively).

Table I. Effect of Unlabeled RF and Related Compounds on the Uptake of [^{3}H]RF by Rabbit Intestinal BBMV[a]

Condition	Concentration (μM)	Uptake (% of simultaneously performed controls)
Control		100
Unlabeled RF	20	37.76 ± 3.39 (15) ($P < 0.01$)
	50	27.63 ± 2.56 (10) ($P < 0.01$)
	100	12.45 ± 3.37 (30) ($P < 0.01$)
Lumiflavin	50	34.19 ± 4.85 (5) ($P < 0.01$)
	100	17.18 ± 2.67 (8) ($P < 0.01$)
8-NH_2-RF	50	78.67 ± 8.89 (6) ($P < 0.05$)
	100	47.56 ± 20.0 (6) ($P < 0.025$)
Iso-RF	50	73.33 ± 9.33 (6) ($P < 0.025$)
	100	53.33 ± 8.89 (6) ($P < 0.01$)
Lumichrome	50	78.97 ± 3.85 (5) ($P < 0.01$)
8-OH-RF	50	107.11 ± 20.0 (6) (NS)
	100	91.11 ± 11.11 (6) (NS)
Lumazine	50	115.75 ± 6.56 (6)
D-Ribose	50	116.93 ± 6.56 (6)

[a] Intestinal BBMV were preloaded with a buffer of 280 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4), and incubated for 5 sec (initial rate) at 37°C in a buffer of 100 m*M* NaSCN, 80 m*M* mannitol, and 20 m*M* Hepes/Tris (pH 6.4), in the presence of 0.11 μM [^{3}H]RF and the indicated concentrations of the unlabeled compound. Data are presented as percentage relative to simultaneously performed controls. Numbers in parentheses represent the number of separate transport determinations from at least two separate BBMV preparations. *P*-values were calculated using Student's *t* test.

Table II. Effect of Membrane Transport Inhibitors, and Folic Acid, Methotrexate, and Probenecid on the Uptake of [^{3}H]RF by Rabbit Intestinal BBMV[a]

Condition	Concentration (m*M*)	Uptake (% of simultaneously performed controls)
Control		100
DIDS	1	98.84 ± 1.05 (6) (NS)
SITS	1	92.81 ± 4.15 (6) (NS)
Furosemide	1	99.07 ± 8.35 (6) (NS)
Folic acid	0.1	109.28 ± 8.82 (6) (NS)
Methotrexate	0.1	106.25 ± 1.11 (4) (NS)
Probenecid	5	103.67 ± 6.56 (5) (NS)

[a] For details, see footnote to Table I. The mixing of folic acid and methotrexate with RF was done immediately before the start of the uptake reaction.

In the second method, the effect of inducing a negative or a positive intravesicular potential with the use of valinomycin (10 μg/mg protein) and an outwardly and an inwardly directed K^+ gradient, respectively, on the uptake of 0.11 μM RF was examined. The results are shown in Table III. Uptake of RF was found to be similar whether a negative or a positive intravesicular potential was induced compared with voltage clamp control.

Table III. Effect of Valinomycin-Induced K^+ Diffusion Transmembrane Potential on RF Uptake by Rabbit Jejunal BBMV[a]

[K+] in/out	Uptake (pmol/mg protein/5 sec)
a) 50/50 m*M* "voltage clamp"	0.51 ± 0.050 (10)
b) 0/50 m*M* (inside positive)	0.53 ± 0.066 (9)
c) 50/0 m*M* (inside negative)	0.45 ± 0.066 (9)

Intestinal BBMV were preloaded with a buffer of 50 m*M* potassium gluconate, 80 m*M* mannitol, and 20 m*M* Hepes/Tris (pH 7.4) (a and c) or 280 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4) (b) and were preincubated with valinomycin (10 μg/mg protein) for 10 min at room temperature. Incubation was performed for 5 sec at 37°C in an incubation buffer of 90 m*M* sodium gluconate, 50 m*M* potassium gluconate, and 20 m*M* Hepes/Tris (pH 6.4) (a and b), or 90 m*M* sodium gluconate, 80 m*M* mannitol, and 20 m*M* Hepes/Mes (pH 6.4) (c). [^{3}H]RF (0.11 μM) was added to the incubation mixture at the onset of incubation. The numbers in parentheses indicate the number of separate transport assays from three separate BBMV preparations.

Discussion

The first step in the absorption process of RF from the intestinal lumen to the blood is its internalization into the absorptive cell by uptake across the brush border membrane of the enterocyte. Thus, characterization of this event is essential for detailed understanding of the overall absorption process. To do so with a substrate such as RF, which is known to undergo some degree of enzymatic metabolism inside the enterocyte (9, 21, 22), caution must be used to eliminate the interference of intracellular metabolism with the uptake measurements. This can be done with the use of isolated BBMV because this preparation lacks intracellular components. When used in this study, this preparation was indeed found to have no effect on the metabolic form of the transported substrate after incubation with [^{3}H]RF for 60 min.

Uptake of RF by rabbit intestinal BBMV was found to be mainly (>76%) the result of transport of the substrate into an osmotically active intravesicular space with less binding to membrane surfaces. Uptake of RF with time was similar in the presence of Na^+, K^+, and mannitol in the incubation medium and occurred with no transaccumulation of the substrate in the intravesicular space (i.e., no overshoot), indicating lack of active transport. This is in contrast to the clear Na^+ dependency and overshoot seen in the transport of D-glucose in this vesicular preparation. The lack of effect of Na^+ on RF uptake in rabbit intestinal BBMV is similar to the recent findings in our laboratory with rat, dog, and pig intestinal BBMV (unpublished observations). It is, however, in contrast to the inhibition seen in RF transport in intact tissue of rat intestine after Na^+ removal (8, 10, 11). These findings taken collectively would suggest that the Na^+ role in RF transport in the intact intestinal tissue of these animal species is indirect (i.e., secondary) in nature and does not involve interaction of Na^+ with the brush border membrane RF carrier system.

The initial rate of uptake of RF was found to be saturable as a function of concentration in both the jejunum and the ileum. These results indicate the involvement of a carrier-mediated system in the RF uptake process. It is interesting to notice that while the uptake system has similar K_m (i.e., affinity) in the two areas of the small intestine, the V_{max} was found to be slightly higher in the ileum than in the jejunum. This finding suggests that the distribution (and/or activity) of the RF uptake system per mg brush border membrane protein is slightly higher in the ileum than in the jejunum. It also demonstrates that RF uptake is efficient along the length of the small intestine. It is interesting to mention here that others, who have studied RF transport by examining its uptake into the intestinal tissue compartment or by isolated cells, have also observed somewhat higher uptake in the ileum than the jejunum (9, 15), whereas those who have studied serosal or blood appearance (11; see also Ref. 2 for review) have found the opposite. A recent, unpublished study in our laboratory has indicated that all these reports might be correct. We found that ileal tissue is capable of taking up a somewhat larger amount of RF than jejunal tissue of the same rats, but releases less into the serosal compartment.

Further evidence for the existence of a carrier-mediated system for RF uptake by rabbit BBMV came from the studies showing that unlabeled RF and certain related compounds cause significant inhibition in the uptake of physiologic concentration of [^{3}H]RF. It is interesting to note that uptake of 0.11 μM [^{3}H]RF was inhibited by 87% by 100 μM unlabeled RF, which indicates that uptake of such physiologic concentration of RF by rabbit BBMV is mostly via the carrier-mediated process. The studies with different RF analogs have shown that replacement of the CH_3- group at Position 8 of the isoalloxazine ring with an NH_2 group (as in the case of 8-NH_2-RF) or the movement of this CH_3- group to Position 6 of the ring (as in the case of iso-RF) causes a decrease in the ability of these compounds to inhibit the uptake of [^{3}H]RF compared with equimolar concentrations of unlabeled RF. Replacing the same CH_3- group with an OH− group (as in the case with 8-OH-RF), however, caused disappearance of the inhibitory effect of the new compound on [^{3}H]RF uptake. This is most likely due to the fact that such replacement leads to changes in the physiochemical property of the new molecule due to the ionization of the OH− group; in fact, the pKa of the new molecule, i.e., the 8-OH-RF, is 4.8 compared with pKa of the parent RF molecule of 10.2 (23). The pKa values of 8-NH_2-RF and iso-RF, however, are similar to that of RF. It is interesting to mention here that mammalian lives were also found to be unable to phosphorylate 8-OH-RF and that this compound poorly inhibited RF phosphorylation (24). Another interesting observation was the finding that replacing the ribityl side chain at Position 10 of the isoalloxazine ring by a CH_3- group (as in the case of lumiflavin) did not decrease the ability of the new compound to inhibit the uptake of [^{3}H]RF, whereas its total removal (as in the case of lumichrome) led to a sharp decrease in its inhibitory effect.

It has been reported that RF might behave as an anion when it comes to transport across biologic membranes (25, 26). However, the inability of the anion transport inhibitors DIDS and SITS (27) as well as the anionic probenecid to inhibit RF uptake argues against such a concept in the present preparation. Another membrane transport inhibitor, furosemide (inhibitor of Na^+ cotransport processes; 28), and folic acid and methotrexate (compounds that have been reported to affect RF membrane transport in other systems; 29) also failed to affect RF uptake. No role for transmembrane potential in RF uptake by rabbit intestinal BBMV was found, as indicated by the results of the anion substitution study and the valinomycin K^+-induced negative and positive intravesicular potential studies.

A question may arise from the results of the present study about how the small intestine efficiently absorbs and accumulates RF from a moving diet where the vitamin exists in minute quantities without the existence of an active uptake system. The answer to this might be provided by a concert afford of the uptake system of the BBM described in the present study and the RF intracellular metabolizing system (9, 21, 22). According to this scheme, RF is extracted from the diet by this high-affinity uptake system, which is distributed along the length of the small intestine; some of it is then converted in the cell to riboflavin 5′-phosphate and flavin adenine dinucleotide (9, 21, 22), which then becomes trapped (i.e., metabolic trapping) in the cell. Such events could ensure efficient uptake and accumulation of RF from the diet.

The above findings on the existence of a carrier-mediated system for RF transport in rabbit intestinal BBMV and its inhibition by structurally related compounds are similar to those seen with human intestinal BBMV (30). In the latter preparation, however, RF transport was found to be partially dependent upon Na^+. Whether this is an indication of the existence of species variation in the requirements of the RF transport carrier is not clear and requires further investigation.

Our studies demonstrate that RF uptake by rabbit intestinal BBMV is via a facilitated carrier-mediated system. This system is distributed along the length of the small intestine, is independent of Na^+, and transports the vitamin by an electroneutral process.

This study was supported by the Medical Research Service of the Department of Veterans Affairs and by NIH Grant DK-39501 (to H. M. S.).

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