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Developmental Maturation of Riboflavin Intestinal Transport in the Rat

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ABSTRACT. The intestinal transport of riboflavin in the immature intestine of the suckling rat (14 day old) and its subsequent maturation in weanling (22 day old) and adult (90 day old) rats were investigated using the intestinal everted sac technique. The mucosal-to-serosal transport of 0.5 μM riboflavin was linear with time for 30-min incubation and occurred at a rate of 4.6, 3.6, and 1.6 pmol/g initial tissue wet wt/min in suckling, weanling, and adult rats, respectively. The transport of 0.5 μM riboflavin was higher in the jejunum than the ileum in all age groups. The transport system of riboflavin in all age groups was saturable, energy-, temperature-, and Na^+-dependent. Kinetic parameters of the transport process were different. Apparent K_t of the transport process was the same in suckling and weanling rats (0.12 and 0.11 μM, respectively) but tripled in adult rats (0.35 μM). On the other hand, a progressive decrease in V_{max} from 166 to 122 to 54 pmol/g initial tissue wet weight/30 min was observed in the suckling, weanling, and adult rats, respectively. The present study demonstrates that the characteristics of the transport process of riboflavin is similar in suckling, weanling, and adult rats and occurs by an energy-, temperature-, and Na^+-dependent carrier-mediated process. However, the affinity and the activity (or the number) of the transport carriers of riboflavin decrease with maturation. (*Pediatr Res* 19: 1175–1178, 1985)

Concern over the long-term effects of malnutrition in early infancy has led to a burgeoning of interest in fetal and neonatal intestinal function, structure, and maturation. Intestinal absorption of nutrients undergoes maturational changes during the early stage of life with respect to mechanism, efficiency, and site of maximal transport (1). These changes, however, do not follow a unified pattern. In the rat, the intestinal transport of folate (2), amino acids (3, 4), and calcium (5) is higher in the suckling period and decreases toward adulthood. On the other hand, transport of glucose (6) and bile salts (7, 8) is lower in the suckling period and increases toward adulthood. Furthermore, the mechanism of transport shows maturational changes. For example, bile salt and calcium transport in suckling rats occurs by a diffusion process which evolves with age to become an active process in adulthood (5, 7, 8). Moreover, absorption of certain nutrients occurs along the small intestine in the suckling animal but becomes confined to specific locations with maturation (9, 10).

Recent studies from our laboratory and elsewhere have shown that the intestinal transport of physiological concentrations of riboflavin is a carrier-mediated process which is Na^+, energy- and temperature-dependent process (11–15). No study is available, however, describing the mechanism, efficiency, and site of maximal transport of riboflavin in the immature intestine of suckling animals and its subsequent maturation. Such a study is of physiological and nutritional importance because riboflavin is essential for normal growth and development. The present study was designed to examine the mechanism, efficiency, and site of maximal transport of riboflavin in suckling rats and to determine its subsequent maturation.

MATERIALS AND METHODS

Two days after birth Sprague-Dawley rat pups (Sasco, Omaha, NE) were distributed among mothers to maintain a litter size of nine to 10 pups until the time of the study. Adult Sprague-Dawley rats (3 months old) were purchased directly from the same supplier. Mothers, weanling, and adult rats were fed Purina Rat Chow (St. Louis, MO) and tap water *ad libitum*. The National Council's guidelines for the care and use of laboratory animals were followed.

Transport studies were performed in suckling (14 day old), weanling (21 day old), and adult (3 month old) rats using the everted sac technique (16). Rats were killed by an overdose of ether. The abdomen was opened and 12 cm of the jejunum (starting 6, 9, and 14 cm from the pylorus of suckling, weanling, and adult rats, respectively) and 8 cm of the terminal ileum were removed and washed with ice cold phosphate buffer. Everted sacs (4 cm in length) were then prepared as described previously (16). Everted sacs were incubated in 10 ml Erlenmeyer flasks containing 6 ml of continuously oxygenated (100% O_2) Krebs-Ringer's phosphate buffer, pH 6.5. The buffer solution contained (unless otherwise stated) 20 mM NaH_2PO_4, 125 mM NaCl, 4.93 mM KCl, 1.23 mM $MgSO_4$, 0.85 mM $CaCl_2$, and 10 mM glucose. The serosal compartment was filled with the same buffer used for incubation. Incubation was carried out at 37° C (unless otherwise mentioned) in a shaking water bath (80 oscillations per min) in a darkened room to avoid possible decomposition of riboflavin. At the end of incubation, sacs were removed and washed, and the serosal medium was drained into a scintillation vial containing 6 ml of the scintillation cocktail and analyzed for radioactivity.

All chemicals used in this study were of analytical quality. Unlabeled riboflavin was obtained from Sigma Chemcial Co.; D-2-^{14}C-riboflavin (specific activity 46.5 mCi/mmole) and the scintillation cocktail were obtained from Amersham (Arlington Heights, IL). The purity of ^{14}C-labeled riboflavin was determined before use on silica gel pre-coated thin-layer chromatography

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plates using pyridine:glacial acetic acid:water (10:1:40) as a solvent system (17). The compound was found to be 97% radiochemically pure. Using similar chromatography procedure, we have previously shown (11) that the radioactivity that appears in the serosal compartment of jejunal everted sacs prepared from adult rats and incubated for 30 min with 0.5 μM ^{14}C-riboflavin to be the intact ribofloavin molecule.

Statistical analysis. Each group of data presented is the result of five to eight separate experiments from five to eight different rats and is expressed as mean ± SEM. Data were analyzed using Student's *t* test and regression analysis.

RESULTS

Transport with time. The mucosal-to-serosal transport of 0.5 μM riboflavin as a function of time was examined at buffer pH 6.5 in jejunal everted sacs prepared from suckling, weanling, and adult rats in order to determine the linearity of the transport process (Fig. 1). After 2- to 3-min delay in the appearance of riboflavin in the serosal compartment, the transport of riboflavin proceeded in a linear manner for 30-min incubation. Transport rates given by the slopes of the lines were 4.6, 3.6, and 1.6 pmol/g initial tissue wet wt/min for suckling, weanling, and adult rats, respectively.

Effect of concentration. Mucosal-to-serosal transport of riboflavin as a function of increasing substrate concentration within the physiological range (0.1 to 1 μM) in the mucosal medium

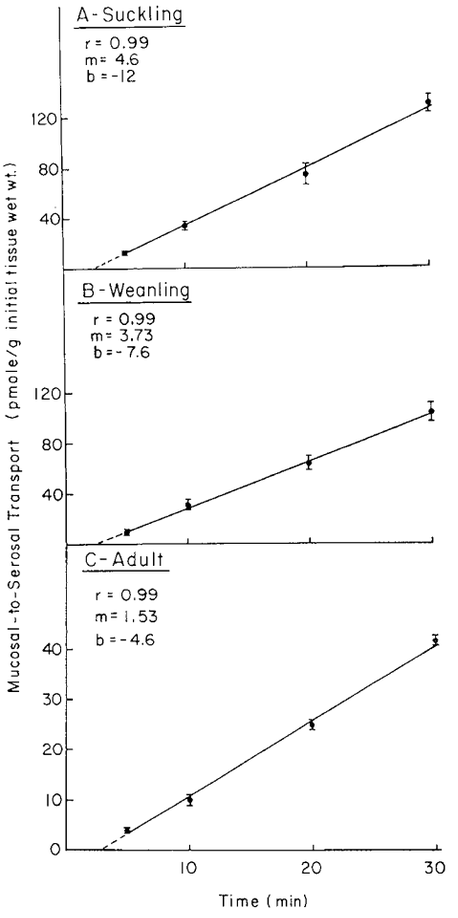

Fig. 1. Mucosal-to-serosal transport of riboflavin in suckling (*A*), weanling (*B*) and adult (*C*) rats as a function of time. Riboflavin (0.5 μM) was added to the incubation medium of jejunal everted sacs at the beginning of the experiment and incubation was performed at 37° C under continuous oxygenation. Each *point* represents mean ± SEM of at least five separate experiments from five different rats. Y = mx + b; where m = slope, b = y intercept, and r = correlation coefficient.

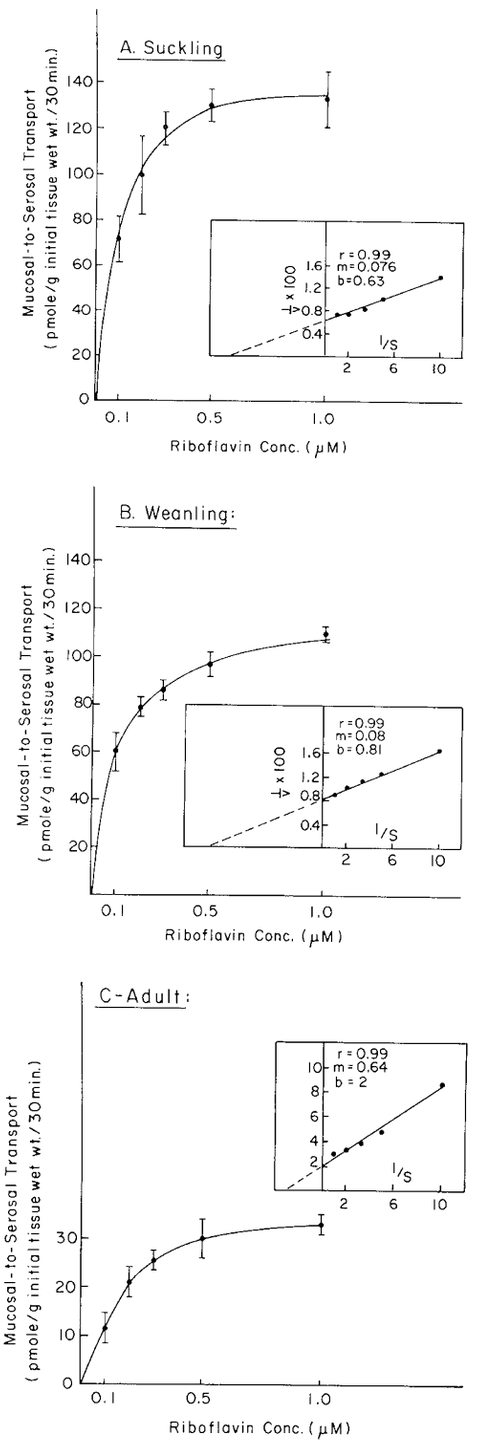

Fig. 2. Mucosal-to-serosal transport of riboflavin in suckling (*A*), weanling (*B*), and adult (*C*) rats as a function of increasing mucosal medium riboflavin concentration. Jejunal everted sacs were incubated for 30 min at 37° C under continuous oxygenation. Each *point* represents mean ± SEM of at least five separate experiments from at least five different rats. Inset is 1/v (reciprocal of the amount of riboflavin transported) against 1/s (reciprocal of riboflavin concentration in the mucosal medium). Y = mx + b; where m = slope, b = y intercept, and r = correlation coefficient.

was examined in jejunal everted sacs of suckling, weanling, and adult rats. In all age groups, saturation in riboflavin transport process was observed (Fig. 2). Apparent K_t of 0.12, 0.11, and 0.35 μM and V_{max} of 166, 122, and 54 pmol/g initial tissue wet

Table 1. *Effect of Na^+ on the mucosal-to-serosal transport of riboflavin**

Total Na^+ concentration	Mucosal-to-serosal transport (pmol/g initial tissue wet wt/30 min)		
	Suckling	Weanling	Adult
145 mM Na^+ (control)	130 ± 7	105 ± 8	42 ± 3
20 mM Na^+ + 125 mM K^+	41 ± 4†	52 ± 3†	17 ± 2†
20 mM Na^+ + 125 mM choline	34 ± 3†	24 ± 2†	15 ± 2†

* Jejunal everted sacs were incubated in the presence of 0.5 μM riboflavin for 30 min at 37°C. Values are expresssed as mean ± SEM of at least five separate experiments.

† Significantly lower than control values of the same group ($p < 0.01$).

Table 2. *Effect of metabolic inhibitors on mucosal-to-serosal transport of riboflavin**

Metabolic Inhibitor	Mucosal-to-serosal transport (pmol/g initial tissue wet wt/30 min)		
	Suckling	Weanling	Adult
Control	130 ± 7	105 ± 8	42 ± 3
2,4-Dinitrophenol (1 mM)	26 ± 2†	32 ± 3†	15 ± 2†
Azide (10 mM)	26 ± 3†	32 ± 1†	14 ± 1†

* Jejunal everted sacs were incubated in the presence of 0.5 μM riboflavin for 30 min at 37° C. Values are expressed as mean ± SEM of at least five separate experiments.

† Significantly lower than control values of the same group ($p < 0.01$).

weight/30 min were calculated for suckling, weanling, and adult rats, respectively (see insets of Fig. 2).

Transport in the ileum. Mucosal-to-serosal transport of 0.5 μM riboflavin in ileal everted sacs of suckling, weanling, and adult rats was examined and the results were compared to that of jejunal everted sacs prepared from the same rats. Transport in the ileum of 50 ± 7, 40 ± 2, and 16 ± 1 pmol/g initial tissue wet weight/30 min compared to transport in the jejunum of 130 ± 7 ($p < 0.01$), 105 ± 8 ($p < 0.01$), and 42 ± 3 ($p < 0.01$) pmol/g initial tissue wet weight/30 min were reported for suckling, weanling, and adult rats, respectively.

Effect of Na^+. Na^+ requirement of the transport system of riboflavin in suckling, weanling, and adult rats was examined by lowering the total Na^+ concentration in the incubation medium from 145 mM (control) to 20 mM (osmolarity was compensated with choline or K^+) (Table 1). Significant inhibition ($p < 0.01$ for all) in the mucosal-to-serosal transport of 0.5 μM riboflavin was observed upon decreasing Na^+ concentration in the incubation medium. The inhibition was similar whether Na^+ was replaced by choline or K^+.

Effect of metabolic inhibitors and temperature. Effects of 1 mM 2,4-dinitrophenol and 10 mM azide on the mucosal-to-serosal transport of 0.5 μM riboflavin into jejunal everted sacs of suckling, weanling, and adult rats were examined and the results were compared to simultaneously studied untreated controls (Table 2). All metabolic inhibitors examined caused significant inhibition ($p < 0.01$) in the transport of riboflavin in all age groups.

In another experiment, the Q_{10} values (the ratio of transport rate at 37° C/transport rate at 27° C) of 0.5 μM riboflavin were determined. Q_{10} values of 4.5, 4.0, and 3.5 were calculated for suckling, weanling, and adult rats, respectively.

DISCUSSION

Riboflavin is a water-soluble micronutrient which participates as a coenzyme in many metabolic reactions including oxidation-reduction, tricarboxylic acid cycle, dehydrogenase reactions, and fatty acid oxidation. Humans and higher mammals cannot synthesize riboflavin. Therefore, the only source of this micronutrient is from diet by absorption through the small intestine. The present study examined the developmental aspects of riboflavin intestinal transport by determining the mechanism, efficiency, and site of maximal transport of the vitamin in suckling, weanling, and adult rats. At first, we examined the transport of riboflavin as a function of time. The results showed a linear increase in the amount of riboflavin transported into the serosal compartment during 30-min incubation in all age groups. Riboflavin transport rate, however, was highest in suckling and lowest in adult rats (4.6, 3.6, and 1.6 pmol/g initial tissue wet weight/min for suckling, weanling, and adult rats, respectively).

Transport of riboflavin appeared to be higher in jejunum than in ileum of rats of all age groups. The transport of riboflavin as a function of concentration was saturable in all age groups (Fig. 2). Transport kinetic parameters, however, were different. The apparent K_t riboflavin transport process was similar in the suckling and weanling rats (0.12 and 0.11 μM, respectively) but tripled in adult rats (0.35 μM). V_{max}, on the other hand, showed a progressive decrease from suckling to weanling to adult rats (160 to 122 to 54 pmol/g initial tissue wet weight/30 min for suckling, weanling, and adult rats, respectively). These data suggest that the affinity of the carrier sites for the riboflavin transport process (as represented by the apparent K_t) decreases after the weanling age. The data also suggest that the number and/or the activity of the carrier sites of the riboflavin transport process (as represented by V_{max}) decrease progressively with maturation, with V_{max} reaching its lowest value in adult rats. The lower K_t and the higher V_{max} of the riboflavin transport process at younger ages would explain the mechanism by which the intestine of the rapidly growing young animal meets the high demand for the vitamin.

The transport process of riboflavin appears to be dependent on normal intracellular energy production, since metabolic inhibitors and low temperature significantly inhibit the riboflavin transport process. The transport process of riboflavin is also Na^+ dependent. Decreasing the total Na^+ concentration in the incubation medium from 145 to 20 mM caused significant inhibition in the transport of riboflavin. This inhibition occurred despite the fact that the osmolarity of the incubation medium was compensated for by the addition of the monovalent cations, choline, or K^+.

Our present results on the mechanism of transport of physiological concentrations of riboflavin confirm the recent observations from our laboratory and elsewhere on the existence of a carrier-mediated process for the vitamin in rat small intestine (11–15). In summary, the present study demonstrate that the mechanism of intestinal transport of riboflavin is similar in suckling, weanling, and adult rats and involves a carrier-mediated system which is energy, temperature, and Na^+ dependent. The study also shows that the affinity and the activity (or the number) of the transport carriers are higher in suckling rats compared to adult rats.

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