Intestinal Uptake of Uridine in Suckling Rats: Mechanism and Ontogeny (43782)

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Abstract. **Nucleosides, essential substrates for a variety of intracellular metabolic reactions, are obtained from dietary and endogenous sources. Nucleotides (which dephosphorylate to nucleosides prior to intestinal absorption) are present in milk and have trophic effects on the developing gastrointestinal tract. The mechanism of transport of nucleosides in the developing intestine of suckling rats is unknown. To address this issue, we therefore examined uridine uptake in rat everted intestinal sacs.**

In suckling rats (15–17 days old), tissue uptake of low (5-μM) and high (60 μM) concentrations of [^{3}H]-uridine was linear for up to 2 min of incubation. Initial rate of uptake of [^{3}H]-uridine was (i) not significantly different in the jejunum and the ileum; (ii) greater in the presence of Na^+, than other cations; (iii) saturable as a function of concentration with a V_{max} of 21,044 ± 2,302 pmol/g tissue wet wt/30 sec and an apparent K_m of 33.8 ± 10.1 μM; (iv) inhibited by high concentration (500 μM) of unlabeled uridine and other nucleosides; (v) temperature-dependent; (vi) energy-dependent; and (vii) pH-sensitive. Developmental maturation was associated with a progressive decrease in the V_{max} of the uridine transport process (21,044 ± 2,302, 14,651 ± 1,679, and 8,461 ± 1,369 pmol/g tissue wet wt/30 sec for suckling, weanling, and adult rats, respectively) and a progressive increase in the apparent K_m of the uptake system (33.8 ± 10.1, 55.6 ± 13.1, and 61.7 ± 14.5 μM for suckling, weanling, and adult rats, respectively).

We concluded that uptake of uridine by the developing intestine of suckling rats involves a carrier-mediated system, which is energy- and temperature-dependent, and requires extracellular sodium. Furthermore, the uptake process was found to undergo clear ontogenic changes with maturation. [P.S.E.B.M. 1994, Vol 206]

Nucleosides are important substrates involved in many essential metabolic reactions, including lipid, carbohydrate, and nucleic acid synthesis (1, 2). They have also been used therapeutically as antiviral (3, 4), antiparasitic (5), and antitumor agents (6, 7). Cells obtain nucleosides from endogenous breakdown of nucleotides or dietary sources. Enterocytes in the small intestine have a limited capacity for *de novo* synthesis of nucleotides, and, therefore, the enterocytes rely heavily on salvaging them from nucleosides provided in the diet (via the brush border membrane) and from endogenous sources (via the basolateral membrane). The mechanism of intestinal uptake of uridine and other nucleosides has been examined in adult rats using isolated loops of jejunum (8–10), tissue rings (11), brush border membrane vesicles (12–14), and other intestinal epithelial cells (15–18), and uptake has been found to occur via concentrative or carrier-mediated, Na^+-dependent and Na^+-independent and equilibrative or facilitated diffusion processes. Similar nucleoside transport systems have also been demonstrated in other tissues including kidney (19, 20), brain (21–23), liver (24), placenta (25), and neoplastic cells (26, 27). No study, however, is available describing the mechanisms of uptake of nu-

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cleosides in the developing intestine of suckling rats and the subsequent maturation of its uptake process. Such a study is physiologically important because the efficiency, mechanism, and site of maximal uptake of many nutrients are known to be different in the developing intestine of suckling animals and undergo marked ontogenic changes (28–36). Also, nucleoside uptake has nutritional significance, since there are considerable amounts of nucleotides (which are hydrolysed to nucleosides in the intestinal lumen) found in human and animal milk (2, 37), and dietary nucleosides have been shown to have a trophic effect on the structure and digestive activities of intestines of young rats (38). Dietary nucleosides have also been shown to be associated in human infants with changes in stool microbial pattern (39), lipid metabolism (40, 41), and immune function (42, 43). Our aims in the present study were, therefore, to examine the mechanism of uptake of a dietary nucleoside in the developing intestine of suckling rats and determine whether ontogenic changes occur during maturation. We used uridine as a representative nucleoside, since it is significantly present in milk and its transport process has been well studied, and the everted sac as a technique.

Materials and Methods

Materials. The following chemicals were obtained commercially: [5,6-^{3}H]-Uridine (sp act 45 Ci/mmol, radiochemical purity 98%) from Moravek, Brea, CA; and [^{14}C]-Dextran (sp act 50 Ci/mmol, radiochemical purity >98%) and other chemicals and reagents from Sigma Chemical, St. Louis, MO. Scintillation fluid (LSC) was obtained from Fisher Scientific, Tustin, CA.

Methods. Suckling (15–17 days old), male weanling (26–28 days), and male adult (80–90 days) Sprague-Dawley rats were purchased from Charles River, Wilmington, MA. All rats were fed Purina Rat Chow (Ralston Purina, St. Louis, MO) and tap water *ad libitum,* until 1 hr prior to surgery. Guidelines for the care and use of laboratory animals by Public Health Service (PHS) policy, and United States Department of Agriculture (USDA) Regulations were followed. Uptake studies were done using the everted sac technique of Wilson and Wiseman as described by us previously (36, 44). Rats were anesthetized with pentobarbital and sacrificed by cervical dislocation. The abdominal cavity was entered, the intestine was exposed, and the first 10–25 cm of small intestine were removed, starting 6, 9, and 14 cm from the pylorus of suckling, weanling, and adult rats, respectively. Ten centimeters of terminal ileum from the ileo-cecal junction were also removed and washed. Everted sacs (2.5–3 cm in length) were prepared and the serosal compartment was filled with Krebs-Ringer phosphate physiologic solution (20 m*M* NaH_2PO_4, 123 m*M* NaCl, 4.93 m*M* KCl, 1.23 m*M* $MgSO_4$, 0.85 m*M* $CaCl_2$, 5 m*M* glucose, and 5 m*M* glutamine; pH 6.5). The sacs were preincubated in a 37°C warm buffer for 5 min, and then transferred to an incubation solution (5 ml of Krebs-Ringer phosphate buffer) containing labeled and unlabeled uridine and [^{14}C]-dextran. This incubation proceeded for a defined period of time in a shaking water bath (80 oscillations/min) at 37°C. At the end of incubation, the sacs were removed, rinsed with ice-cold distilled water, drained, and the serosal fluid was collected. The tissue was weighed, digested with 0.25–0.5 ml of 2 *N* NaOH at 70°C, and counted for [^{3}H] and [^{14}C] radioactivity. Uptake of uridine was corrected for nonspecific binding (and for adherent incubation fluid remaining on the sac wall) and calculated as described by us previously (45).

In all experiments, serosal appearance of [^{3}H]-uridine represented less than 4% of the total radioactivity taken up by the everted sacs and always paralleled tissue uptake. Viability of the *in vitro* everted sacs has been previously determined in our laboratory (36, 44).

Metabolic studies were performed using the same procedures but included the following additional procedures. After washing and draining the sacs, the tissue was boiled in distilled water, cut into small pieces, homogenized in a Potter Homogenizer, and centrifuged, the pellet and supernatant were collected. The metabolic form of the [^{3}H]-radioactivity taken up by the tissue following incubation with [^{3}H]-uridine was examined using a precoated silica gel chromatographic plate and a solvent system of n-butanol and water (13:1).

Statistical Analysis. All uptake data are expressed as mean ± SEM, in terms of pmol/g tissue wet wt/unit time, and were the result of 3 to 14 everted sacs from different rats. Statistical analysis utilized the Student's *t* test and regression analysis. Kinetic parameters of uptake (the apparent K_m and V_{max}) were calculated using a computerized program of the Michaelis-Menten equation described by Wilkinson (46).

Results

Uptake of Uridine with Time. Tissue uptake of 5 and 60 μ*M* uridine was measured in three to four jejunal everted sacs of different suckling rats as a function of time. Uptake was found to be linear for up to 2 min (Fig. 1), occurring at a rate of 1,156 and 10,570 pmol/g tissue wet wt/min at 5 and 60 μ*M*, respectively. Therefore, a 30-sec incubation was used as a standard in all ensuing studies. Similar findings were obtained for uridine uptake in adult rat intestine (Fig. 1).

Uptake in Jejunum Versus Ileum. The uptake capacity of uridine (5 μ*M*) in the jejunum and the ileum of suckling rats was compared in this study, using 14 sacs from different rats. Uptake was found to be

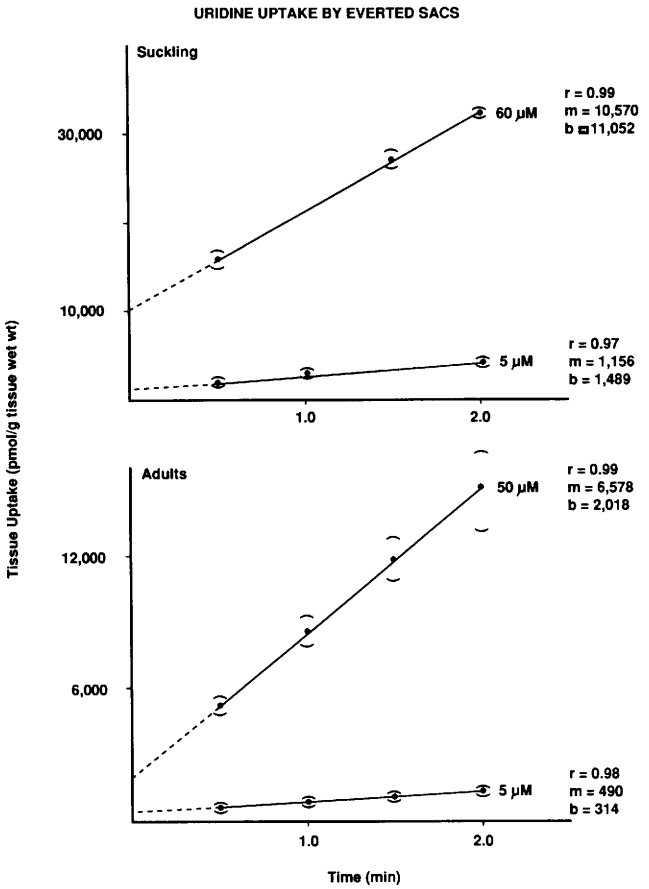

Figure 1. Tissue uptake of [^{3}H]-uridine in suckling and adult rats as a function of time. Uridine (5 and 60 μ*M* in suckling rats, 5 and 50 μ*M* in adult rats) was added to incubation medium of jejunal everted sacs at the onset experiments. Incubation was done at 37°C under continuous oxygen. Each point represents mean ± SEM of at least three separate experiments from different rats. *Y* = *mx* + *b*, where *m* = slope and *b* = *y*-intercept; *r* = correlation coefficient. In suckling rat, at 5 μ*M* uridine conc., *r* = 0.97, *m* = 1,156, and *b* = 1,489; at 60 μ*M* uridine conc., *r* = 0.99, *m* = 10,570, *b* = 11,052. In adult rat, at 5 μ*M* uridine conc., *r* = 0.98, *m* = 490, *b* = 314; at 50 μ*M* uridine conc., *r* = 0.99, *m* = 6,578, *b* = 2,018.

slightly greater in the jejunum, but the difference was not statistically significant (Table I). Similar findings were obtained in weanling and adult rat intestines (Table I).

Effect of Metabolic Inhibitors and Temperature. Significant inhibition was found in uridine uptake by the metabolic inhibitors, 2,4-dinitrophenol and sodium azide, using four everted sacs from four different rats when compared with uptake by control everted sacs prepared from the same respective rats (Table II).

Significant inhibition was found by lowering the temperature to 27°C from the physiologic temperature of 37°C (uptake of 1,094 ± 54 and 1,934 ± 100 pmol/g tissue wet wt/30 sec, respectively, $P < 0.05$). Four everted sacs from different rats were used in this study.

Effect of Na^+. The effect of lowering the Na^+ concentration in the incubation medium (from 145 m*M* to 20 m*M*) on the tissue uptake of 5 μ*M* [^{3}H]-uridine was measured. The removed Na^+ was replaced by equimolar concentration of K^+, choline, and mannitol compensating for osmolarity. The results demonstrated that decreasing the Na^+ concentration led to a significant decrease in uridine uptake (Table III). Six to ten everted sacs from different rats were used in this study.

Uptake of Uridine as a Function of Concentration. The initial rate of uridine uptake by three to four jejunal everted sacs from different suckling rats was studied as a function of increasing the substrate concentration (1–120 μ*M*) in the incubation medium. At lower concentrations uridine uptake increased linearly, but at higher concentrations, it reached saturation (Fig. 2). Kinetic parameters of the uptake process were calculated, the apparent K_m and V_{max} were found to be 33.8 ± 10.1 μ*M* and 21,044 ± 2,302 pmol/g tissue wet wt/30 sec, respectively.

Changes in uridine uptake kinetic parameters with maturation were examined. The apparent K_m and V_{max} of uridine uptake in weanling and adult rats were compared with the results with those described above for suckling rats. The results showed a statistically insignificant but progressive increase in the apparent K_m of uridine uptake with maturation (33.8 ± 10.1, 55.6 ± 13.1 [$P < 0.25$], and 61.7 ± 14.5 [$P < 0.1$] μ*M* for suckling, weanling, and adult rats, respectively), while the V_{max} showed a significant progressive decrease (21,044 ± 2,302, 14,951 ± 1,679 [$P < 0.05$], and 8,461 ± 1,369 [$P < 0.05$] pmol/g tissue wet wt/30 sec for suckling, weanling, and adult rats, respectively).

Table I. Uptake of [^{3}H]-Uridine by Jejunal and Ileal Everted Sacs of Rats of Different Ages

Site	Uptake (pmol/g tissue wet wt/30 sec)		
	Suckling rats	Weanling rats	Adult rats
Jejunum	1,768 ± 270 (14)*	449 ± 46 (3)	199 ± 30 (9)
Ileum	1,671 ± 174 (14)	363 ± 69 (3)	197 ± 20 (10)

Note. Intestinal everted sacs were incubated for 30 sec in 5 ml of Krebs-Ringer phosphate buffer containing 5 μ*M* uridine at 37°C.
* Parentheses indicate the number of experiments (everted sacs) from different rats.

Table II. Effect of Metabolic Inhibitors on Uptake of [^{3}H]Uridine in Everted Sacs of Suckling Rats

	Uptake (pmol/g tissue wet wt/30 sec)	
Control	1,872 ± 318 (4)*	
2,4-Dinitrophenol (1 m*M*)	894 ± 124 (4)	$P < 0.025$**
Azide (10 m*M*)	1,102 ± 126 (4)	$P < 0.05$

Note. Everted sacs were incubated for 30 sec in Krebs-Ringer phosphate buffer containing 5 μ*M* uridine and 1 m*M* 2,4-dinitrophenol or 10 m*M* azide.
* Parentheses indicate the number of experiments (everted sacs) from different rats.
** *P* values calculated using the Student's *t* test. Comparison was made with simultaneously performed controls.

Table III. Effect of Na^+ on Uptake of [^{3}H]-Uridine by Jejunal Everted Sacs of Suckling Rats

	Uptake (pmol/g tissue wet wt/30 sec)	
Control (143 m*M* Na^+)	1,704 ± 135 (10)*	
123 m*M* K^+ + 20 m*M* Na^+	861 ± 59 (6)	$P < 0.01$**
123 m*M* Choline + 20 m*M* Na^+	1,224 ± 131 (9)	$P < 0.025$
246 m*M* Mannitol + 20 m*M* Na^+	1,175 ± 125 (6)	$P < 0.01$

Note. Incubation conditions as in Table I, except that NaCl was replaced isoosmotically with either KCl, choline chloride, or Mannitol in Krebs-Ringer buffer.
* Parentheses indicate the number of experiments (everted sacs) from different rats.
** *P* values calculated using the Student's *t* test. Comparison was made with simultaneously performed controls.

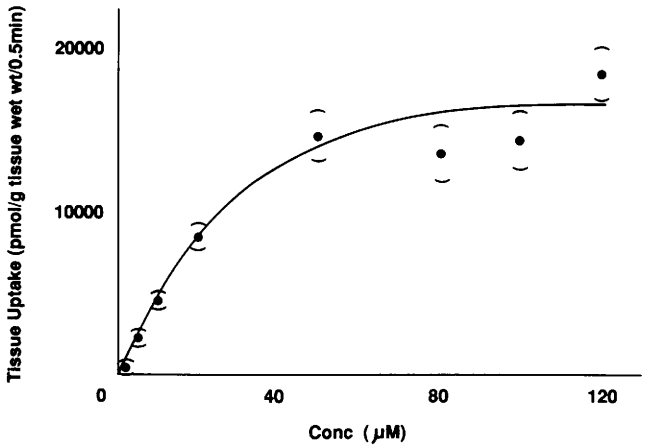

Figure 2. Initial rate of tissue uptake of uridine in suckling rats as a function of concentration. Uridine (1–120 μ*M*) was added to the incubation medium of jejunal everted sacs at the beginning of the experiments. Incubation was performed for 30 sec at 37°C under continuous oxygen. V_{max} and K_m for the carrier-mediated system were calculated as described in the text.

Effect of Unlabeled Uridine and Other Nucleosides on the Uptake of [^{3}H]-Uridine. Significant inhibition was shown in the uptake of [^{3}H]-uridine by high concentration of uridine, adenosine, and guanosine (Table IV). High concentration of thymidine and cytidine did not cause significant inhibition (Table IV). Three to four jejunal everted sacs from different suckling rats were used in this study.

Effect of pH. Uridine uptake was significantly lower at pH 5.0 than uptake at physiologic pH 6.5, and uptake at pH 8.0 was similar to uptake at pH 6.5. Tissue uptake of uridine was 1,924 ± 195, 1,998 ± 148, and 1,536 ± 168 pmol/g tissue wet wt/30 sec, at pH of 8.0, 6.5, and 5.0, respectively. Three to four jejunal everted sacs from different suckling rats were used in this study.

Metabolic Form of the Uridine Taken up by the Intestine. The metabolic form of the radioactivity taken up by the jejunal everted sacs was determined at 0.5 and 2 min following incubation with 5 μ*M* [^{3}H]-uridine at 37°C. At both times, 97% of the radioactivity taken up by tissue was found to be in the form of intact uridine.

Table IV. Effect of Unlabeled Uridine and other Nucleosides on Uptake of [^{3}H]-Uridine by Jejunal Everted Sacs of Suckling Rats

	Uptake (pmol/g tissue wet wt/30 sec)	
Control	1,872 ± 318 (4)*	
Uridine	635 ± 63 (4)	$P < 0.01$**
Adenosine	477 ± 34 (4)	$P < 0.01$
Guanosine	551 ± 104 (4)	$P < 0.01$
Thymidine	1,409 ± 112 (3)	NS
Cytidine	1,533 ± 142 (4)	NS

Everted sacs were incubated for 30 sec in Krebs-Ringer phosphate buffer containing 5 μ*M* uridine and 500 μ*M* unlabeled uridine, adenosine, guanosine, thymidine, or cytidine.
* Parentheses indicate the number of experiments (everted sacs) from different rats.
** *P* values calculated using the Student's *t* test. Comparison was made with simultaneously performed controls.

Discussion

Nucleotides are present in both human and animal milk, and, following their hydrolysis in the intestinal lumen, are absorbed as nucleosides. Dietary nucleosides have been shown to be associated with trophic effects on the gastrointestinal tract and with favorable changes in the gastrointestinal tract flora, lipid metabolism, and the immune system. The mechanism for uptake of dietary nucleosides in the developing intestine and its maturational changes are not known and were the subject of this investigation, using uridine as the substrate to address these issues.

Tissue uptake of low and high concentrations of uridine was found to be linear. Similar results were found in adult rats. Uptake of uridine in the jejunum was not found to be statistically different from that in the ileum. Similar findings were obtained when uridine uptake was examined in weanling and adult rats. The uptake of uridine, however, was significantly greater in younger animals when compared with older animals (suckling > weanling > adult).

Uridine uptake by the developing intestine of suckling rats was energy- and temperature-dependent in nature, as shown by the significant inhibition in uridine uptake caused by metabolic inhibitors and by lowering the incubation temperature, respectively. Uptake was also found to be pH sensitive, with highest uptake at pH 6.5 and a considerable decrease at lower pH.

Uridine uptake by suckling (as well as adult) rat intestine was found to depend on the presence of Na^+ in the incubation medium, since lowering the Na^+ concentration led to a significant decrease in uridine uptake. These results suggest the existence of a Na^+:uridine co-uptake system. Indeed, recent studies in our laboratory have confirmed this suggestion using

intestinal brush border membrane vesicles (unpublished observations).

Uptake of uridine by the developing intestine was found to be carrier-mediated in nature, as indicated by saturation of uridine uptake as a function of concentration and the ability of high-concentration unlabeled uridine and other nucleosides to inhibit the uptake of low-concentration [^{3}H]-uridine. Similar results have been demonstrated using cultured kidney cells (48).

Clear maturational changes were found in the kinetic parameters of the carrier-mediated uptake system. These findings are consistent with uptake studies in many other animals. The V_{max} was found to decrease gradually with maturation (suckling to weanling to adult), indicating a decrease in the number and/or activity of the uptake carriers. The apparent K_m was found to increase gradually with maturation, indicating a decrease in the affinity of the uptake carrier. The cause of these changes is not known at present but could be due to factors such as hormonal changes, changes in the thickness and resistance of the unstirred water layer, and/or physicochemical changes in the composition and fluidity of the enterocyte membrane (47). Similar maturational changes in intestinal uptake have been seen with folate, riboflavin, valine, and other nutrients (28–36).

The observation that uridine uptake in everted sacs of suckling rats was significantly inhibited by high concentration of some nucleosides, adenosine and guanosine, but not by other nucleosides, cytidine and thymidine, suggests that an interrelated uptake system exists for some of the nucleosides, in addition to independent uptake systems for each nucleoside. Similar findings were observed for nucleoside uptake in liver (24) and kidney cells (48).

In summary, our findings indicate that uridine uptake in the intestine of developing suckling rats is greater than that in weanling and adult rats, but is similar in nature, being carrier-mediated, energy- and temperature-dependent, and requiring extracellular Na^+. Ontogenic changes appear to involve a decrease in number and/or activity and affinity of the uptake system with maturation.

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1. Lehninger AL. Biochemistry. New York: Worth Publishers, pp729–747, 1975.
2. Uauy R. Dietary nucleotides and requirements in early life. In: Lebenthal E, Ed. Textbook of Gastroenterology and Nutrition in Infancy (2nd Ed). New York: Raven Press, pp265–280, 1989.
3. Dahlberg JE, Mitsuya H, Blam S, Broder S, Aaronson SA. Broad spectrum antiretroviral activity of 2′,3′-dideoxynucleosides. Proc Natl Acad Sci USA **84**:2469–2473, 1987.
4. Yarchoan RY, Broder S. Development of antiviral therapy for the acquired immunodeficiency syndrome and related disorders: A progress report. N Engl J Med **316**:557–564, 1987.
5. El Kouni MH, Messier NJ, Cha S. Treatment of schistosomiasis by purine nucleoside analogues in combination with nucleoside inhibitors. Biochem Pharmacol **36**:3815–3821, 1987.
6. Chu MY, Zuckermann LB, Sato S, Crabtree GW, Bogden AE, Lim M, Klein RS. 9-Deaza-adenosine, a new potent antitumor agent. Biochem Pharmacol **33**:1229–1234, 1984.
7. Elion GB. The purine path to chemotherapy. Science **244**:41–47, 1989.
8. Bronk JR, Lister N, Lynch S. Absorption of 5-fluorouracil and related pyrimidines in rat small intestine. Clin Sci **72**:705–716, 1987.
9. Bronk JR, Hastewell JG. The transport and metabolism naturally occurring pyrimidine nucleosides by isolated rat jejunum. J Physiol **395**:349–361, 1988.
10. Bronk JR, Hastewell JG. The specificity of pyrimidine nucleoside transport and metabolism by rat jejunum *in vitro*. J Physiol **408**:405–411, 1989.
11. Bronk JR, Hastewell JG. The transport of pyrimidines into tissue rings cut from rat small intestine. J Physiol **382**:475–488, 1987.
12. Jarvis SM. Characterization of sodium-dependent nucleoside transport in rabbit intestinal brush-border membrane vesicles. Biochim Biophys Acta **979**:132–138, 1989.
13. Betcher SL, Forrest JN Jr., Knickelbein RG, Dobbins JW. Sodium-adenosine cotransport in brush-border membranes from rabbit ileum. Am J Physiol **259**(Gastrointest Liver Physiol 22): G504–G510, 1990.
14. Roden M, Paterson ARP, Turnheim K. Sodium-dependent nucleoside transport in rabbit intestinal epithelium. Gastroenterology **100**:1553–1562, 1991.
15. Schanker LS, Tocco DJ. Active transport of some pyrimidines across the rat intestinal epithelium. J Pharmacol Exp Ther **128**:115–121, 1960.
16. Jakobs ES, Paterson ARP. Sodium-dependent, concentrative nucleoside transport in cultured intestinal epithelial cells. Biochem Biophys Res Commun **140**:1028–1035, 1986.
17. Vijayalakshmi D, Belt JA. Sodium-dependent nucleoside transport in mouse intestinal epithelial cells. J Biol Chem **263**:19419–19423, 1988.
18. Jakobs ES, Van Os-Corby DJ, Paterson ARP. Expression of sodium-linked transport activity in monolayer cultures of IEC-6 intestinal epithelial cells. J Biol Chem **265**:22210–22216, 1990.
19. Williams TC, Doherty AJ, Griffith DA, Jarvis SM. Characterization of sodium-dependent and sodium-independent nucleoside transport systems in rabbit brush-border and basolateral plasma-membrane vesicles from the renal outer cortex. Biochem J **264**:223–231, 1989.
20. Le Hir M. Evidence of separate carriers for purine nucleosides and for pyrimidine nucleosides in the renal brush border membrane. Renal Physiol Biochem **13**:154–161, 1990.
21. Lee CW, Jarvis SM. Nucleoside transport in rat cerebral-cortical synaptosomes. Evidence for two types of nucleoside transporters. Biochem J **249**:557–564, 1988.
22. Johnston ME, Geiger JD. Sodium-dependent uptake of nucleosides by dissociated brain cells from the rat. J Neurochem **52**:75–81, 1989.
23. Wu X, Yuan G, Brett CM, Hui AC, Giacomini KM. Sodium-dependent nucleoside transport in choroid plexus from rabbit. Evidence of a single transporter for purine and pyrimidine nucleosides. J Biol Chem **267**:8813–8818, 1992.
24. Holstege A, Gengenbacher H, Jehle L, Hoppmann J. Facilitated diffusion and sodium-dependent transport of purine and pyrimidine nucleosides in rat liver. Hepatology **14**:373–380, 1991.
25. Barros LF, Bustamante JC, Yudilevich DL, Jarvis SM. Aden-

osine transport and nitrobenzylthioinosine binding in human placental membrane vesicles from brush-border and basal sides of the trophoblast. J Membrane Biol **119**:151–161, 1991.
26. Crawford CR, Ng CYC, Belt JA. Isolation and characterization of an L1210 cell line retaining the sodium-dependent carrier *cif* as its sole nucleoside transport activity. J Biol Chem **265**:13730–13734, 1990.
27. Crawford CR, Belt JA. Sodium-dependent, concentrative nucleoside transport in Walker 256 rat carcinosarcoma cells. Biochem Biophys Res Commun **175**:846–851, 1991.
28. Batt E, Schacter D. Developmental pattern of some intestinal transport mechanisms in newborn rats and mice. Am J Physiol **216**:1064–1068, 1969.
29. Borowitz SMJ, Ghishan FK. Developmental maturation of jejunal phosphate (P) transport by brush border membranes in the rat. (abstract) Pediatr Res **18**:190A, 1984.
30. Fitzgerald JF, Reiser S, Christiansan PA. Developmental pattern of sugar and amino acid uptake in the postnatal rat small intestine. Pediatr Res **5**:698–703, 1971.
31. Ghishan FK, Jenkins JT, Younszai MK. Maturation of calcium transport in the rat small and large intestine. J Nutr **110**:1622–1628, 1980.
32. Ghishan FK, Wilson FA. Developmental maturation of D-glucose transport by rat brush-border membrane vesicles. Am J Physiol **248**(Gastrointest Liver Physiol 11):G87–G92, 1985.
33. Little JM, Lester R. Ontogenesis of intestinal bile salt absorption in the neonatal rat. Am J Physiol **239**(Gastrointest Liver Physiol 2):G319–G323, 1980.
34. Younoszai MK, Komnick K. Maturation of small intestine: Absorption of L-valine in rats. Pediatr Res **16**:756–760, 1982.
35. Younoszai MK, Lynch A. *In vivo* D-glucose absorption in the developing rat small intestine. Pediatr Res **9**:130–133, 1975.
36. Said HM, Ghishan FK, Murrell JE. Ontogenesis of intestinal transport of 5-methyltetrahydrofolate in the rat. Am J Physiol **249**(Gastrointest Liver Physiol 12):G567–G571, 1985.
37. Janas LM, Picciano MF. The nucleotide profile of human milk. Pediatr Res **16**:659–662, 1982.
38. Uauy R, Stringel G, Thomas R, Quan R. Effect of dietary nucleosides on growth and maturation of the developing gut in the rat. J Pediatr Gastroenterol Nutr **10**:497–503, 1990.
39. Gil A, Corral E, Martinez A, Molina JA. Effects of the addition of nucleotides to an adapted milk formula on the microbial pattern of feces in at term newborn infants. J Clin Nutr Gastroenterol **1**:127–132, 1986.
40. Sanchez-Pozo A, Pita ML, Martinez A, Molina JA, Sanchez-Medina F, Gil A. Effects of dietary nucleotides upon lipoprotein pattern of newborn infants. Nutr Res **6**:763–771, 1986.
41. DeLucchi C, Pita ML, Martinez A, Molina JA, Uauy R, Gil A. Effects of dietary nucleotides on the fatty acid composition of erythrocyte membrane lipids in term infants. J Pediatr Gastroenterol Nutr **6**:568–574, 1987.
42. Carver JD, Cox WI, Barness LA. Dietary nucleotide effects upon murine natural killer cell activity and macrophage activation. JPEN **14**:18–22, 1990.
43. Carver JD, Pimentel B, Cox WI, Barness LA. Dietary nucleotide effects upon immune function in infants. Pediatrics **88**:358–363, 1991.
44. Said HM, Redha R. Ontogenesis of intestinal transport of biotin in the rat. Gastroenterology **94**:68–72, 1988.
45. Said HM, Greene HL, Moore MC, Ghishan FK. Developmental maturation of D-glucose active transport system in rat intestine. Digestion **36**:195–200, 1987.
46. Wilkinson GN. Statistical estimations in enzyme kinetics. Biochem J **80**:324–332, 1961
47. Murer H, Kinne R. The use of isolated membrane vesicles to study epithelial uptake processes. J Membr Biol **55**:81–95, 1980.
48. Griffith DA, Doherty AJ, Jarvis SM. Nucleoside transport in cultured LLC-PK_1 epithelia. Biochem Biophys Acta **1106**:303–310, 1992.