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Amino acids neonate
anaerobiosis transport
intestine

Metabolic Properties of Neonatal Transport

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Extract

The metabolic properties of the increased rate of transport in the 2-day-old rat intestine as compared with adult rat intestine were investigated. The intracellular accumulation of 1 mM of a prototype neutral amino acid (L-leucine) and sugar (D-galactose) was measured at 5 min in the presence of inhibitors and after preincubation with inhibitors. The intestine of the 2-day-old rat was found to be similar to adult rat intestine in its response to Na^+ dependence, sulfhydryl binders, and metabolic inhibitors under conditions of aerobiosis; however, the immature rat intestine exhibited an ability to actively accumulate amino acids to a much greater extent than adult tissue under anaerobic conditions. Transport was inhibited only 12% in the newborn intestine while adult intestine showed a 44% inhibition at initial velocities. This anaerobic transport was similarly Na^+ dependent and sensitive to metabolic inhibitors.

These results indicate that the increased transport found in newborn animals may be partially energized by anerobic metabolism, but suggest that the requirement for the maintenance of an active influx of sodium is similar in both neonates and adults.

Speculation

Anaerobic metabolism may partially account for the increased transport in newborn rats. Future studies may reveal whether the rate of anaerobic transport decreases in a pattern similar to the overall decrease in transport observed in the neonatal rat with maturation.

Intestinal transport has been observed to be maximal for both amino acids (10, 16, 22, 26) and sugars (9) immediately after birth. The decreased accumulation of neutral amino acids in adult rat small intestine appears to be due to the disappearance of transport sites found in the small intestine of newborn rats, rather than the presence in young animals of structurally modified transport carriers with a more efficient binding capacity (6, 26). It has been demonstrated that the active transport of amino acids (22) in chick small intestine soon after hatching was not completely dependent on aerobic metabolism. Measurements of glycolysis in chicken (22) and rabbit (37) small intestine have shown a decrease in the rate of anaerobic glycolysis during the first week after birth. Kidney slices from newborn rats have been shown to achieve a higher net uptake of amino acids than mature tissue after prolonged incubation (3, 4, 33, 35). Baerlocher *et al.* (5) have demonstrated an adaptive advantage in the presence of anoxia held by immature kidney over mature tissue. Therefore, it would be of primary physiologic interest to determine whether the increased rate of active intestinal transport in the newborn rat is energized by a specialized mechanism.

In continuing our study of transport in the 2-day-old rat

intestine (16, 17, 26) we are now examining the metabolic properties of the increased intracellular accumulation of a prototype amino acid (L-leucine) and sugar (D-galactose) in order to determine whether the transport properties of these metabolites in neonates are fundamentally different from those found in adult animals.

MATERIALS AND METHODS

Wistar strain rats of both sexes were used as a source of the 2-day-old intestinal segments. Male Wistar strain rats weighing 150–200 g were used as a source of adult intestine. All animals were killed by decapitation, the small intestine quickly removed, placed in saline or isotonic choline chloride, and gassed with nitrogen or oxygen. The 2-day-old intestine was split longitudinally while immersed in the isotonic medium, which yielded intestinal segments essentially free of luminal contents. The adult intestine was everted, divided into segments, randomized, and tied into sacs approximately 9 cm in length according to the method of Wilson and Wiseman (36), or everted, split longitudinally, divided into 7-cm segments, and randomized in a modification of the method of Agar *et al.* (1).

The method employed for preincubation of the 2-day-old segments was 5 ml oxygenated Krebs-Ringer-Tris buffer, pH 7.4, containing 118 mM NaCl, 25 mM Tris-HCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 1.2 mM KH_2PO_4, without or with inhibitors. The segments were preincubated at 37° for the indicated time in a water bath oscillating 50–75 times/min. After preincubation, the segments were washed in saline or isotonic choline chloride (continuously gassing with either nitrogen or oxygen), drained, and reincubated in a 37° water bath with shaking for 5 to 30 min. The medium employed for incubation, in studies without or with preincubation, was 5 ml oxygen- or nitrogen-gassed Krebs-Ringer-Tris buffer, pH 7.4, as described above, containing 8,000–15,000 cpm/ml (*methoxy*-^{3}H)inulin and ^{14}C radioactive (8,000–15,000 cpm/ml) and 1 mM nonradioactive L-leucine or D-galactose. The radioactive leucine and galactose were uniformly labeled and reported to be at least 98% pure by dilution analysis, paper chromatography, and paper electrophoresis. The everted sacs or segments were incubated for 5 min in a medium identical with that used for the 2-day-old intestinal segments.

In the studies using Na^+-free incubation media, choline chloride was used as an isotonic replacement for the NaCl of the Krebs-Ringer-Tris buffer. The Na^+ concentration of the incubation media was determined by direct analysis in a Coleman flame photometer. The maintenance of an essentially Na^+-free environment during these studies was confirmed by the finding that only 1.20 ± 0.1 mM Na^+ (mean ± 1 SEM, n = 30) was present in the incubation medium after incubation.

After the incubation the everted sacs and intestinal segments were removed from the flasks and drained. The sacs were opened, the inside medium drained, and the empty sacs were washed, blotted, and weighed. Similarly, the adult and 2-day-old segments were washed, blotted, and weighed. The residual tissue was homogenized in 4 times its weight of 5% trichloroacetic acid to make a 20% homogenate. The homogenate was centrifuged, and aliquots of the supernatant were counted in a Tri-Carb liquid scintillation spectrometer (38) in a system containing xylene-dioxane-ethanol (5:5:3), naphthalene (40 g/liter), 2,5-diphenyloxazole (5 g/liter), and 1,4-bis[2-(5-phenyloxazolyl]benzene (100 g/liter). The spectrometer was adjusted to permit 60% ^{14}C efficiency, 22% ^{3}H efficiency, less than 0.01% ^{3}H efficiency on the ^{14}C channel, and 10% ^{14}C efficiency on the ^{3}H channel. An extracellular fluid space was calculated from the distribution space of (^{3}H)inulin (corrected for 10% ^{14}C contribution) in the residual tissue and expressed as percentage of tissue wet weight (28). Amino acid and sugar transport values are expressed as intracellular accumulation, which is defined as the millimolar concentration of the amino acid or sugar in the cellular water after a given incubation period. This parameter was calculated on the basis of a modification of a formula used by Crane and Mandelstam (12), which now takes the following form: millimolar (cellular water) = (millimolar homogenate supernatant X homogenate volume) – (extracellular space X tissue wet weight X 0.8 X millimolar medium)/(1 – extracellular space) X (tissue wet weight X 0.8).

The source and specific activity of the radioactive compounds used in this study were: uniformly labeled L-(^{14}C)-leucine (342 mCi/mmol), uniformly labeled D-(^{14}C)galactose (43 mCi/mmol) (39), and (*methoxy*-^{3}H)inulin (672 mCi/mmol) (40). Ethacrynic acid was used as the pure powder and was kindly supplied by Merck, Sharp, and Dohme Research Laboratories (41). The sodium iodoacetate, *N*-ethylmaleimide, dinitrophenol, sodium, arsenate, sodium fluoride, and ouabain were obtained from Sigma Chemical Company (42).

RESULTS

Since the active transport of neutral amino acids (13, 25, 27) and sugars (7, 11) in adult intestine has been shown to be Na^+ dependent, it was important to determine whether the active accumulation of leucine and galactose in 2-day-old rat intestine also requires the presence of extracellular Na^+. The comparative effect of Na^+ on the intracellular accumulation of 1 mM L-leucine and 1 mM D-galactose at 5 and 30 min is shown in Table 1. The times were chosen to represent initial velocity and steady state conditions (26). In the absence of extracellular Na^+, leucine intracellular accumulation was reduced 78% after 5 min of incubation and 82% after 30 min. The uptake of D-galactose from a Na^+-free medium was reduced 95% after 5 min of incubation and 90% after 30 min. These results suggest that the active intracellular accumulation of neutral amino acids and sugars in 2-day-old rats is a Na^+-dependent process.

Table 2 demonstrates the effect of a 5-min incubation with

Table 1. *Effect of Na^+ on intracellular accumulation of L-leucine and D-galactose in intestine of 2-day-old rats*[1]

		Intracellular accumulation			
	118 mM Na^+	mM/5 min	Inhibition, %	mM/30 min	Inhibition, %
L-Leucine, 1 mM	+	4.330 ± 0.353		8.618 ±0.640	
L-Leucine, 1 mM	–	0.961 ±0.068	78	1.560 ±0.207	82
		$P < 0.001$		$P < 0.01$	
D-Galactose, 1 mM	+	2.118 ±0.130		3.557 ±0.186	
D-Galactose, 1 mM	–	0.117 ±0.005	95	0.351 ±0.031	90
		$P < 0.001$		$P < 0.001$	

[1] Intestinal segments were incubated for 5 or 30 min at 37° in a Krebs-Tris-Na^+ medium or a Krebs-Tris-choline medium containing 1 mM L-leucine or 1 mM D-galactose. Each value represents the mean ± 1 SEM from at least four individual experiments. A paired-difference *t* test was used to obtain the probability values and a *P* of 0.05 or less was interpreted as indicating a significant inhibition. The percentage of inhibition values were obtained by dividing the average uptake in the Krebs-Tris-choline chloride medium by the average uptake in the Krebs-Tris-Na^+ medium and subtracting from 100%. The percentage of inhibitions and *P* values represented paired experiments. Control values as given may represent values from several experiments.

Table 2. *Effect of metabolic inhibitors on intracellular accumulation of 1 mM L-leucine and 1 mM D-galactose in intestine of 2-day-old rats*[1]

Inhibitor	Intracellular accumulation					
	Leucine, mM/ 5 min	Inhibition, %	*P*	Galactose, mM/ 5 min	Inhibition, %	*P*
None	4.863 ±0.248			1.978 ±0.073		
Sodium iodoacetate, 1.0 mM	3.451 ±0.224	15	0.05	1.709 ±0.114	10	
N-Ethylmaleimide, 1.0 mM	3.389 ±0.436	38	0.001	1.538 ±0.120	17	0.05
Dinitrophenol, 0.4 mM	3.207 ±0.180	41	0.001	1.420 ±0.123	23	0.01
Sodium arsenate, 10.0 mM	3.423 ±0.224	16	0.05	1.747 ±0.056	8	
Sodium fluoride, 10.0 mM	3.798 ±0.218	7		1.867 ±0.075	2	
Ouabain, 1.0 mM	4.067 ±0.201	29	0.01	1.611 ±0.154	21	0.05

[1] Intestinal segments were incubated for 5 min at 37° in a Krebs-Tris medium containing 1 mM L-leucine or 1 mM D-galactose without or with the various inhibitors at the indicated concentrations. Each value represents the mean ± 1 SEM from at least six individual experiments. Percentage of inhibition and probability values were obtained as described in Table 1.

various metabolic inhibitors on the intracellular accumulation of leucine and galactose in 2-day-old rat intestine. The sulfhydryl inhibitors, iodoacetate and *N*-ethylmaleimide, reduced the intracellular accumulation of leucine 15% and 38%, respectively. Galactose uptake was inhibited by *N*-ethylmaleimide 17%, but was not significantly decreased by iodoacetate. Dinitrophenol, a potent inhibitor of oxidative phosphorylation, reduced the intracellular accumulation of leucine 41% and galactose 23%. The inhibitors of glycolysis, sodium arsenate (2) and sodium fluoride (32), decreased the uptake of L-leucine 16% while galactose uptake was not significantly inhibited. Ouabain was used as an inhibitor of the (Na^+, K^+)-dependent ATPase (23) and inhibited leucine uptake by 29% and galactose uptake 21%. This inhibition was substantially less than the observed in a Na^+-free medium. These results may be a reflection of the relative inaccessability of the serosal membrane in intact intestine, as ouabain has been observed to produce a much larger inhibition of leucine uptake in isolated intestinal epithelial cells than that produced by the absence of Na^+ (27).

As shown in Table 1, the intracellular accumulation of amino acids and sugars in 2-day-old rat intestine is extremely Na^+ dependent. The inhibition of active uptake of amino acids in adults by metabolic inhibitors is thought to be ultimately a reflection of the inhibition of ATP production required to maintain the Na^+ gradient across the cell membrane (30). In order to allow sufficient time for the Na^+ gradient across the cell to be dissipated a study was made of the effect of preincubating with the inhibitors at varying time periods (Tables 3 and 4). Generally, the percentage of inhibition of uptake of both leucine and galactose increased with time of preincubation. Ethacrynic acid (2 mM) was used in addition to ouabain as an inhibitor of (Na^+, K^+)-dependent ATPase (23). Ethacrynic acid has been shown to inhibit Na^+ absorption in the Loop of Henle (18) and in hamster intestine (8) and to inhibit the extrusion of Na^+ from guinea pig kidney cortex slices (23). All inhibitors tested inhibited L-leucine and D-galactose uptake at least 50% after a 30-min preincubation except ouabain, which inhibited leucine 38% and galactose 37%. The results suggest strongly that the energy required for the intracellular accumulation of leucine and galactose is derived in part from aerobic metabolism and is consistent with the Na^+ gradient hypothesis for the energization of active transport.

A progressive dependence on aerobic metabolism for the transport of L-valine has been demonstrated in the maturing chick intestine (22). As adult rat intestine has been shown to be extremely sensitive to anaerobiosis (24, 27), it was of interest to investigate whether 2-day-old intestinal tissue differed from adult tissue in its capacity to transport amino acids anaerobically. The effect of anaerobiosis on the intracellular accumulation of 1 mM leucine by everted intestinal sacs and segments from adults and 2-day-old rat intestinal segments at 5 and 30 min is compared in Table 5. Leucine intracellular accumulation in adult sacs was shown to be inhibited 44% and 39% in adult segments after an incubation time of 5 min, whereas uptake in the 2-day-old rat intestine was inhibited only 12%. All were inhibited more than 70%, however, after 30 min of incubation.

The anaerobic transport of amino acids in intestine is apparently unique to immature animals (Table 5) (22). Therefore, to further elucidate the characteristics of the

Table 3. *Effect of preincubation with metabolic inhibitors on intracellular accumulation of 1 mM L-leucine in intestine of 2-day-old rats*[1]

Inhibitor	Intracellular accumulation					
	5-min preincubation		15-min preincubation		30-min preincubation	
	mM/5 min	Inhibition, %	mM/5 min	Inhibition, %	mM/5 min	Inhibition %
None	5.197 ±0.201		5.164 ±0.204		4.817 ±0.269	
Sodium iodoacetate, 1.0 mM	4.479 ±0.133	21	3.142 ±0.215	38	2.332 ±0.117	52
	$P < 0.01$		$P < 0.01$		$P < 0.01$	
N-Ethylmaleimide, 1.0 mM	2.345 ±0.217	56	1.144 ±0.086	78	0.769 ±0.060	84
	$P < 0.001$		$P < 0.01$		$P < 0.001$	
Dinitrophenol, 0.4 mM	3.799 ±0.301	28	2.625 ±0.224	50	1.968 ±0.179	59
	$P < 0.02$		$P < 0.001$		$P < 0.001$	
Sodium arsenate, 10.0 mM	3.739 ±0.159	34	2.445 ±0.154	52	1.607 ±0.078	77
	$P < 0.01$		$P < 0.001$		$P < 0.001$	
Sodium fluoride, 10.0 mM	3.737 ±0.374	34	2.745 ±0.071	46	2.286 ±0.072	53
	$P < 0.02$		$P < 0.001$		$P < 0.01$	
Ethacrynic acid, 2.0 mM	3.646 ±0.211	21	2.280 ±0.076	58	1.324 ±0.058	71
	$P < 0.05$		$P < 0.001$		$P < 0.001$	
Ouabain, 1.0 mM	3.191 ±0.199	39	3.114 ±0.211	40	2.942 ±0.126	38
	$P < 0.01$		$P < 0.01$		$P < 0.01$	

[1] Intestinal segments were preincubated at 37° in a Krebs-Tris medium without or with the inhibitors at the indicated concentrations for 5, 15, or 30 min. At the end of the preincubation the segments were removed, washed in oxygenated isotonic NaCl and reincubated for 5 min in a Krebs-Tris medium containing 1 mM L-leucine. Each value represents the mean ± 1 SEM from at least six individual experiments. Percentage of inhibition and probability values were obtained as described in Table 1.

Table 4. *Effect of preincubation with metabolic inhibitors on intracellular accumulation of 1 mM D-galactose in intestine of 2-day-old rats*[1]

Inhibitor	Intracellular accumulation: 5-min pre-incubation mM/5 min	Inhibition, %	15-min pre-incubation mM/5 min	Inhibition, %	30-min pre-incubation mM/5 min	Inhibition %
None (18)	2.106 ±0.105		2.151 ±0.076		2.336 ±0.096	
Sodium iodoacetate (6), 1.0 mM	2.052 ±0.111	4	1.496 ±0.121	38	1.160 ±0.086	54
			$P < 0.001$		$P < 0.001$	
N-Ethylmaleimide (6), 1.0 mM	1.132 ±0.072	46	0.706 ±0.121	64	0.553 ±0.021	77
	$P < 0.01$		$P < 0.001$		$P < 0.001$	
Dinitrophenol (11), 0.4 mM	1.653 ±0.103	21	1.425 ±0.088	29	1.120 ±0.096	50
	$P < 0.01$		$P < 0.001$		$P < 0.001$	
Sodium arsenate (8), 10.0 mM	1.694 ±0.146	22	1.536 ±0.203	37	1.093 ±0.094	56
	$P < 0.01$		$P < 0.01$		$P < 0.001$	
Sodium fluoride (11), 10.0 mM	1.609 ±0.138	24	1.213 ±0.126	46	0.953 ±0.134	59
	$P < 0.01$		$P < 0.001$		$P < 0.001$	
Ethacrynic acid (4), 2.0 mM	1.337 ±0.101	33	1.234 ±0.186	36	0.709 ±0.103	63
	$P < 0.05$		$P < 0.02$		$P < 0.01$	
Ouabain (8), 1.0 mM	1.659 ±0.083	22	1.441 ±0.072	30	1.531 ±0.103	37
	$P < 0.02$		$P < 0.01$		$P < 0.001$	

[1] The methodology is the same as in Table 3 with the exception of the substitution of 1 mM D-galactose for L-leucine in the incubation medium. The number of individual experiments is shown in parentheses.

Table 5. *Anaerobic intracellular accumulation of 1 mM L-leucine in rats as function of time*[1]

Atmosphere	2-day-old mM/5 min	Inhibition, %	Adult (sacs) mM/5 min	Inhibition, %	Adult (segments) mM/5 min	Inhibition, %
O_2	4.330 ±0.353		2.349 ±0.178		2.106 ±0.142	
N_2	3.801 ±0.427	12	1.316 ±0.053	44	1.328 ±0.094	39
	$P < 0.05$		$P < 0.001$		$P < 0.01$	
	mM/30 min		mM/30 min		mM/30 min	
O_2	8.839 ±0.459		2.529 ±0.183		4.348 ±0.077	
N_2	1.722 ±0.129	81	0.681 ±0.069	73	1.057 ±0.031	76
	$P < 0.001$		$P < 0.001$		$P < 0.001$	

[1] Intestinal segments from 2-day-old rats and everted sacs and segments from adult rats were incubated 5 or 30 min at 37° in a Krebs-Tris medium containing 1 mM L-leucine under an O_2 or N_2 atmosphere. Each value represents the mean ± 1 SEM from at least seven individual experiments. Percentage of inhibition and probability values were obtained as described in Table 1.

Table 6. *Effect of Na^+ on anaerobic intracellular accumulation of L-leucine and D-galactose in intestine of 2-day-old rats*[1]

	118 mM Na^+	Intracellular accumulation: mM/5 min	Inhibition, %	mM/30 min	Inhibition, %
L-Leucine, 1 mM	+	3.801 ±0.427		1.722 ±0.129	
L-Leucine, 1 mM	–	0.935 ±0.083	75	0.739 ±0.040	57
		$P < 0.001$		$P < 0.001$	
D-Galactose, 1 mM	+	1.817 ±0.196		1.816 ±0.178	
D-Galactose, 1 mM	–	0.161 ±0.008	91	0.366 ±0.019	80
		$P < 0.001$		$P < 0.001$	

[1] Intestinal segments were incubated for 5 or 30 min at 37° in a Krebs-Tris-Na^+ medium or Krebs-Tris-choline medium containing 1 mM L-leucine or 1 mM D-galactose under a N_2 atmosphere. Each value represents the mean ± 1 SEM from at least seven individual experiments. Percentage of inhibition and probability values were obtained as described in Table 1.

anaerobic intracellular accumulation of leucine and galactose, the effect of Na^+ on this anaerobically energized process was studied (Table 6). In the Na^+-free medium after 5 min of incubation anaerobic leucine uptake was reduced 75% and did not reach values greater than unity in the absence of extracellular Na^+. Galactose uptake was inhibited 90% after 5 min of incubation. In a Na^+-free medium, the intracellular accumulation of leucine and galactose was reduced significantly after a 30-min anaerobic incubation (Table 6).

In order to characterize the possible energization of the anaerobic uptake of leucine and galactose, the intestinal segments from 2-day-old rats were preincubated for 15 min aerobically with various inhibitors before a 5-min anaerobic incubation with leucine or galactose and the resultant accumulation was compared with that of segments preincubated in the Krebs-Tris medium alone, *i.e*, without inhibitors (Table 7). These results demonstrated an inhibition of anaerobic uptake that was generally of the same magnitude as the inhibition of intracellular accumulation under aerobic conditions when segments were preincubated with the same inhibitors for 15 min (compare Tables 3 and 4, 15-min preincubation). The greatest percentage of inhibition was produced by the sulfhydryl binder, *N*-ethylmaleimide, a compound which would be expected to act directly on the cell membrane rather than through a cellular mechanism.

DISCUSSION

The main objective of this study was to ascertain whether the increased transport in the 2-day-old rat intestine as compared with adult intestine is indicative of a vastly different energization process. Generally, the intracellular accumulation of leucine and galactose in 2-day-old rat intestine was shown to be the same as found in adult animal intestine in its response to metabolic inhibitors, sulhydryl binders, and in its Na^+ dependence (7, 12, 14, 24, 27). The most significant difference observed between the transport characteristics of adult intestine and those of 2-day-old rat intestine was the level of active transport maintained by the young animal under anaerobic conditions. The inhibition of dinitrophenol and sodium arsenate suggests that, in part, the transport of leucine and galactose in the small intestine of the neonate is coupled

Table 7. *Effect of preincubation with metabolic inhibitors on anaerobic intracellular accumulation of L-leucine and D-galactose in intestine of 2-day-old rats*[1]

Inhibitor	Intracellular accumulation L-leucine		Intracellular accumulation D-galactose	
	mM/5 min	Inhibition, %	mM/5 min	Inhibition, %
None	3.529 ±0.220		1.780 ±0.101	
N-Ethylmaleimide, 1.0 mM	1.126 ±0.117	70	0.901 ±0.044	50
	$P < 0.001$		$P < 0.001$	
Dinitrophenol, 0.4 mM	1.616 ±0.153	57	1.072 ±0.046	41
	$P < 0.001$		$P < 0.001$	
Sodium arsenate, 10.0 mM	1.339 ±0.107	57	0.758 ±0.037	56
	$P < 0.001$		$P < 0.001$	
Sodium fluoride, 10.0 mM	1.598 ±0.109	49	1.074 ±0.106	38
	$P < 0.01$		$P < 0.01$	
Ethacrynic acid, 2.0 mM	2.320 ±0.125	39	1.229 ±0.058	32
	$P < 0.01$		$P < 0.001$	
Ouabain, 1.0 mM	2.703 ±0.234	29	1.483 ±0.079	18
	$P < 0.02$		$P < 0.01$	

[1] Intestinal segments were preincubated at 37° in an oxygenated Krebs-Tris medium without or with the inhibitors at the indicated concentrations for 15 min. At the end of the preincubation, the segments were removed, washed in saline or isotonic choline chloride under a N_2 atmosphere and reincubated for 5 min in N_2-gassed media containing 1 mM L-leucine or 1 mM D-galactose. Each value represents the mean ± 1 SEM from at least six individual experiments. Percentage of inhibition and probability values were obtained as described in Table 1.

to oxidative phosphorylation. However, the maintenance of active transport in neonates under conditions of anaerobiosis suggests that glycolysis can provide some of the energy necessary for transport. Even after an anerobic incubation as long as 30 min, 2-day-old intestine is still capable of actively accumulating leucine. The active accumulation of amino acids under conditions of anaerobiosis has also been demonstrated in intestine of newborn chicks (22) and the kidney cortex of newborn Long-Evans rats (5). Other studies have shown that anaerobic metabolism can support transport under selective situations such as rat renal papillary slices (20) and glomeruli (21). Lowenstein *et al.* (20), who investigated the *in vitro* uptake of amino acids in renal papilla of rats, found that the accumulation of α-aminoisobutyric acid fell only slightly in O_2-deficient media and the accumulation of lysine remained the same, which indicated that the papilla was able to accumulate amino acids actively *in vitro* under conditions which simulate its *in vivo* environment, *i.e.*, low O_2 tensions and high sodium concentrations.

One of the most interesting characteristics of the anaerobic active accumulation of amino acids and sugars by the intestine of the 2-day-old rat was its complete dependence on extracellular Na^+. It appears that active transport can be maintained both anaerobically and aerobically in the 2-day-old as opposed to a complete dependence on aerobic metabolism in the adult intestine, but both of these transport systems are equally Na^+ dependent.

Many of the characteristics of transport in the neonatal rat may be elucidated from these and previous studies (3–5, 16, 17, 26, 33–35). Studies which involve the ontogeny of amino acid transport in rat kidney cortex have demonstrated short term amino acid transport to be lower in the kidney of newborn Wistar and Long-Evans rat (3, 4, 34), although Segal (29) did not observe this in the Sprague-Dawley strain. However, the steady state ratios were shown to be greater in neonates than in adult kidney for all three strains (3, 4, 29, 34). There is evidence that these results reflect an impaired efflux in the newborn kidney (5, 29, 35). This may be explained by the changing specific activity of transport sites during postnatal maturation concomitant with increasing membrane area (15, 19).

Two major transport systems for amino acids have been observed in intestines of 2-day-old rats (16, 17). The most active of these systems has the highest affinity for neutral L-amino acids with lipophilic side chains. The second transport pathway has affinity for basic amino acids. The pathways mediating the transport of the acidic amino acids, imino acids, and *N*-substituted amino acids, however, have been shown to have minor activity in the neonatal rat (17).

The highest rate of active intestinal transport of both amino acids and sugars (9, 10, 16, 22, 26) occurs immediately after birth and decreases as a function of age. On the basis of kinetic studies (26), the increase in intracellular accumulation of amino acids in the 2-day-old rat intestine can best be explained by the presence of more transport sites rather than by a more efficient binding by the carrier. The progressive decrease in transport activity may be a result of the disappearance of the widespread and locationally nonspecific transport sites found in the intestine of newborn rats (6, 26).

In this study, the metabolic properties of the increased rate of transport in the 2-day-old rat intestine were found to be similar to those of adult intestine. The uptake of leucine and galactose in the small intestine of the newborn rat was shown to be maintained concentratively under anaerobic conditions. Future studies may elucidate further characteristics of the energization and ontogeny of this system.

SUMMARY

The rate of transport for both amino acids and sugars has been observed to be greatest immediately after birth. The main objective of this study was to ascertain whether this increased transport in the 2-day-old rat intestine as compared with adult intestine is indicative of a basically different energization process. The intracellular accumulation of 1 mM of a prototype neutral amino acid (L-leucine) and sugar (D-galactose) was measured at 5-min initial velocities in the presence of inhibitors and after preincubation with inhibitors. The 2-day-old rat intestine was found to exhibit a similar Na^+ dependence to that found in the adult. Sulfhydryl binders (1 mM Na iodoacetate, 1 mM *N*-ethylmaleimide) and inhibitors of aerobic and glycolytic metabolism (0.4 mM dinitrophenol, 10 mM Na arsenate, 10 mM Na fluoride) produced a similar decrease in the adults and 2-day-olds. The most significant difference between the transport characteristics of 2-day-olds and adults was the level of active transport maintained by the young animals under anaerobic conditions. Transport was inhibited only 12% in the newborn intestine, whereas adult intestine showed a 44% inhibition at initial velocities. This anaerobic transport was similarly Na^+ dependent and sensitive to the metabolic inhibitors. The results indicate that the increased transport found in newborn animals can be partially energized by anaerobic metabolism, but that the requirements for the maintenance of an active influx of sodium is similar in both neonates and adults.

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Corrigendum

Aromatic Acids in Urine of Healthy Infants, Persistent Hyperphenylalaninemia, and Phenylketonuria, before and after Phenylalanine Load

By S. Rampini *et al.*

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p. 704——Under *Subjects and Methods*, weight of the premature infants at examination should have appeared as 1,990–2,500 g rather than 2,990–2,500 g. Furthermore, the healthy full term infants studied were seven.

p. 707——Under *Aromatic Acids in Urine,* the final sentence should have appeared as "The reported values for phenylacetic acid do not include phenylacetic acid originating from phenylacetylglutamine."