PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 160, 192–195 (1979)

In Vivo Transport of Water and Electrolytes in the Infant Rat Small Intestine[1] (40417)

M. K. YOUNOSZAI

University of Iowa, Pediatric Gastroenterology Laboratory, Iowa City, Iowa 52242

In humans (1) and rats (2, 3), several aspects of gastrointestinal function appear to have characteristic developmental patterns. Little is known of the developmental changes in transport of water and electrolytes in the small intestine. Although the basic qualities of the transport mechanisms may not change during development, quantitative differences in transport of water and electrolytes may exist between infant and older animals. The present study was designed to determine if the *in vivo* transport of water, sodium, chloride, bicarbonate and potassium were quantitatively different in the jejunum and the ileum of 2-, 3- and 8-week-old rats.

Methods and materials. Male albino rats were obtained from mothers purchased from Biolabs. The post-weaning and the mother rats were fed a regular laboratory diet. The rats were studied at 2 weeks (14–15 days), 3 weeks (21–22 days) and 8 weeks (56 days) of age. The details of the methods used for the studies have been described before (4). After anesthesia with a mixture of urethane–phenobarbital, the abdominal cavity was opened and approximately 15 cm segments of the jejunum, distal to the ligament of Treitz, and of the ileum, proximal to the ileocecal junction, were rinsed with an isotonic sodium chloride solution and cannulated. The intestine was ligated proximal and distal to the cannulated segments and placed back into the abdominal cavity. The temperature of the peritoneum was maintained at 36°–37° during the study period. The cannulated segments were perfused with solutions containing per liter: 120 mmoles of sodium chloride, 5 mmoles of potassium chloride, 25 mmoles of sodium bicarbonate, 100 μmoles of phenol red, and 25 mmoles of mannitol (−G solution), or an identical solution in which 15 mmoles of D-glucose was substituted for 15 mmoles of mannitol (+G solution). The osmolality of the solutions were 295–299 mOsm/kg. Segments of five to seven rats were perfused for each solution at each age period. The solutions were perfused at a constant rate of 0.31 ml/min for a period of 2 hr. The first hour of the perfusion was utilized to achieve steady state conditions. During the next 60 minutes the perfusates were collected over three consecutive 20-min periods. At the end of the perfusion period, the perfused segments were stripped from the mesentry and their length, wet and dry weight determined. In the perfusion solutions and perfusates, phenol red was determined as described by Schedl and Clifton (5). Sodium and potassium were determined using a Flame Photometer. Concentration of bicarbonate was calculated from the pH and pCO_2 of the solutions using the Henderson-Hasselbalch formula; pH and pCO_2 were determined using an acid base analyzer (Radiometer, Copenhagen). Chloride was measured using a chloridometer (Chloride Titrator, Radiometer, Copenhagen).

Transport of water and the electrolytes were determined using the following formulae:

$$\text{Water transport, ml/20 min} = V_i\,(1 - PR_i/PR_f)$$

$$\text{Electrolyte transport, }\mu\text{moles/20 min} = V_i\,(C_i - C_f \cdot PR_i/PR_f)$$

where V_i is volume of fluid perfused through the segments as ml/20 min. PR_i and C_i represent concentration (μmoles/ml) of phenol red (PR) and of the electrolytes (C) in the perfusion fluid before perfusion, and PR_f and C_f their respective concentrations after perfusion. The calculated transport rates, during the three consecutive 20-min perfusion periods, did not vary by more than 20% from each other, indicating that steady state conditions had been achieved. Corresponding

[1] This study was supported, in part, by Research Grant No. HDO8227; Department of Health, Education and Welfare, National Institutes of Child Health and Human Development.

0037-9727/79/020195-04$01.00/0

rates of transport for the three periods were added to obtain the rate per hr. Positive values indicated net absorption from the lumen, and negative values net secretion into the lumen. The unpaired Student's "t" test was used for statistical comparison of the differences between mean values, and p values of less than 0.05 were considered to indicate statistically significant differences between the mean values (6).

Results. Mean values of the body weight and of the measurements of the perfused intestinal segments for the rats studied are shown in Table I. The weight/cm ratio of the jejunum and of the ileum increased with age. Histologic evaluation of the segments indicated that part of the greater weight/cm ratio was due to greater mucosal (absorbing) mass in the older rats.

Rates of transport of water, sodium, chloride, bicarbonate, potassium and glucose are shown in Table II. Rates of transport are expressed on the basis of the dry weight of the segments to normalize for the differences in weight and water content of the jejunum and of the ileum between the 2-, 3- and 8-week-old rats. Rates of transport based on the wet weight of the segments had the same relationships as those based on the dry weight of the segments.

Transport of water. During perfusion of the glucose free solution, minimal amount of water was secreted into both segments of the 2-week-old rats, while water was absorbed in both segments of the 3- and of the 8-week-old rats. Glucose significantly enhanced net water absorption in both segments at all three age periods. The magnitude of the increase was greater in the 2- than in the 8-week-old rats, and greater in the jejunal than in the ileal segment.

Transport of sodium. During perfusion of the glucose free solution in the 2- and 3-week-old rats, net absorption of sodium was two fold greater in the jejunal than in the corresponding ileal segments, however, the differences did not achieve statistical significance ($0.1 > p > 0.05$). Glucose significantly enhanced net absorption of sodium in both segments of the 2- and the 3-week-old, but only in jejunal segment of the 8-week-old rats ($p < 0.05$).

Transport of chloride. During perfusion of the glucose free solution in the jejunal segment, net absorption of chloride was similar at 2- and 3-weeks of age, and significantly lower at 8- than at 2- and 3-weeks of age (Table II). In the ileal segment, net absorption rates were similar at all three age periods. Glucose had no effect on absorption of chloride, except in the jejunal segment of the 8-week-old rats where absorption rate was enhanced ($p < 0.01$).

Transport of bicarbonate. During perfusion of the glucose free solution in the jejunal segment, absorption of bicarbonate was greater at 2- weeks than at 8-weeks of age. In the ileal segment, transport of bicarbonate was minimal and varied between net secretion and net absorption (Table II). Glucose had no effect on net transport of bicarbonate in either the jejunal or the ileal segments.

Transport of potassium. During perfusion of the glucose free solution, potassium was secreted into both segments of the rats at all three age periods; however, secretion rates were significantly greater at 2- than at 8-weeks of age ($p < 0.05$). Glucose changed net secretion of potassium to net absorption in the segments of the 8-week-old rats (Table

TABLE I. AGE, BODY WEIGHT AND MEASUREMENTS OF THE PERFUSED SEGMENTS OF THE RATS STUDIED[a].

	Values and means SE					
Age, Weeks	2		3		8	
Body weight, g	27 ± 1		37 ± 1		205 ± 6	
Segment perfused	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Length, cm	13.9 ± 0.9	14.9 ± 1.0	15.7 ± 0.8	16.6 ± 1.3	17.5 ± 1.0	13.3 ± 1.0
Wet weight,						
mg	207 ± 16	231 ± 20	316 ± 25	341 ± 22	1334 ± 123	992 ± 83
mg/cm	14.9 ± 0.5	13.8 ± 1.3	20.8 ± 1.3	21.0 ± 1.3	75.6 ± 3.3	76.4 ± 6.9
H_2O Content						
mg/100 mg wet weight	79.7 ± 0.5	81.5 ± 0.5	79.9 ± 0.3	81.1 ± 0.4	80.4 ± 0.3	79.9 ± 0.4

[a] Two studies were conducted. Each study involved perfusion of the segments of 5–7 rats, at each age period. Since body weight and measurements of the perfused segments were similar, the combined mean values of the two groups are presented.

TABLE II. NET TRANSPORT OF WATER AND ELECTROLYTES IN THE PERFUSED SEGMENTS OF THE RATS STUDIED.

Age, Weeks	2		3		8	
Segment perfused	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Net water transport ml/hr per g dry wt						
−G[1]	−2.1 ± 2.3	−2.2 ± 1.3	4.3 ± 1.6[a]	3.2 ± 1.4[a]	1.4 ± 0.6	2.0 ± 0.6[a]
+G[2]	12.7 ± 1.6[b]	5.2 ± 2.3[b,c]	9.7 ± 1.8[b]	6.2 ± 1.2[b,c]	6.6 ± 0.6[a,b]	3.8 ± 0.5[b,c]
Net sodium transport mmoles/hr per g dry wt						
−G	0.50 ± 0.38	0.25 ± 0.35	0.88 ± 0.27	0.45 ± 0.29	0.46 ± 0.11	0.58 ± 0.16
+G	1.46 ± 0.20[b]	1.20 ± 0.25[b]	1.43 ± 0.29[b]	0.92 ± 0.09[b]	1.14 ± 0.12[b]	0.96 ± 0.22
Net chloride transport mmoles/hr per g dry wt						
−G	1.71 ± 0.81	1.66 ± 0.63	1.76 ± 0.83	1.58 ± 0.47	0.19 ± 0.10[a]	1.33 ± 0.09[c]
+G	1.14 ± 0.36	1.35 ± 0.35	0.92 ± 0.39	1.46 ± 0.48	0.58 ± 0.06[a,b]	1.14 ± 0.14[c]
Net bicarbonate transport mmoles/hr per g dry wt						
−G	1.21 ± 0.42	0.23 ± 0.35[c]	0.70 ± 0.32	0.21 ± 0.15[c]	0.43 ± 0.16[a]	−0.39 ± 0.16[c]
+G	0.89 ± 0.15	0.37 ± 0.30	1.35 ± 0.13	−0.16 ± 0.27[c]	0.73 ± 0.09	−0.11 ± 0.09[c]
Net potassium transport μmoles/hr per g dry wt						
−G	−32.1 ± 15.7	−31.6 ± 22.6	−39.1 ± 11.8	−24.1 ± 21.1	−6.7 ± 5.0[a]	−4.2 ± 4.1[a]
+G	−15.3 ± 7.1	−22.2 ± 7.1	−44.1 ± 9.3	−48.9 ± 16.6	21.8 ± 4.9[a,b]	1.7 ± 4.7[a,c]
Glucose transport mmoles/m per dry wt	1.19 ± 0.11	0.70 ± 0.09[c]	1.11 ± 0.10	0.74 ± 0.11[c]	0.40 ± 0.01[a]	0.21 ± 0.01[a,c]

* The segments of this group of rats were perfused with a near isotonic physiologic solution of NaCl, KCl, $NaHCO_3$ and mannitol (20 mmole/l), which did not contain glucose (−G).

†† The segments of this group of rats were perfused with solution identical to that in the −G group with the exception that 15 mmoles of glucose was substituted for 15 mmoles of mannitol (+G).

[a] Mean value significantly different than corresponding mean value in the 2-week-old rats, $p < 0.05$–<0.01.

[b] Mean value in +G group significantly different than corresponding mean value in −G rats, $p < 0.05$–<0.01.

[c] Mean value of ileal segment significantly different than mean value of corresponding jejunal segments, $p < 0.05$–<0.005.

II); however, only the values in the jejunal segment achieved statistical significance ($p < 0.01$).

Absorption of glucose. Rate of absorption of glucose was significantly greater in the 2- than in the 8-week-old rats ($p < 0.01$) and significantly greater in the jejunal than in the corresponding ileal segments at all three ages ($p < 0.05$).

Discussion. The above findings suggested that under the experimental conditions in the small intestine, the magnitude of transport of water and of the electrolytes changed with age in the rat. In general, the mucosa of the jejunum and ileum appeared to be more permeable to water and electrolytes in the young than in the older rats. Because net absorption and net secretion rates are the sum of the unidirectional fluxes, lumen-to-plasma and plasma-to-lumen, it is possible that an alteration in the magnitude of one or both of these fluxes were responsible for the observed developmental changes. The results of the present study are in agreement with those of our previous studies which showed that when the intestinal lumen was perfused with hypertonic solutions the permeability of the small intestinal mucosa to water decreased with increasing age (7).

In the present study in the 2-week-old rats the net secretion of water into the intestinal lumen, when the perfusion fluid was free of glucose, appeared to be against an osmotic pressure gradient. In rats, serum osmolality at this age was 306 ± 1 mOsm/kg (7) and luminal fluid osmolality was 295 mOsm/kg, thus the movement of water would be expected to have been from lumen to blood. The reason for this apparent discrepancy remains unknown. As expected, presence of glucose in the intestinal lumen enhanced net absorption of water. The greater magnitude of enhancement of absorption of water in the segments of the younger rats was most likely due to the higher rates of absorption of glucose in the segments of the younger than of the older rats (Table II). Glucose also enhanced the rate of absorption of sodium from the intestinal lumen. Although the enhancement in the rate of absorption of sodium was of greater magnitude in segments of the 2- than of the 8-week-old rats, the differences did not achieve statistical significance. Transport rates of chloride and bicarbonate were greater and more variable in segments of the 2- and the 3-week-old than in the corresponding segments of the 8-week-old rats. The finding that both bicarbonate and chloride were absorbed in the ileal segment of the 2-week-old rats (Table II) suggested that the postulated bicarbonate-chloride exchange mechanism in the ileal segment was less active in the 2- than in the 8-week-old rats.

Under the experimental conditions (15 mmoles/l of glucose in the perfusion solution) in this and previous studies, the rate of absorption of glucose was significantly greater in the intestine of the 2-week-old than older rats (4). However, when using solutions containing concentrations of glucose over 55 mmoles/l, the rates of absorption were significantly greater in the older animals (4).

The basic physiologic phenomenon responsible for the observed differences in transport in segments of the 2-, 3- and 8-week-old rats cannot be determined from results of the present study. Since the tight junctions and lateral intercellular spaces appear to provide the major pathway for transepithelial water absorption, either coupled to net solute absorption or resulting from osmotic gradients (8–12), it is possible that the dimension or the hydraulic conductivity and/or ion selectivity of the tight junctions and intercellular spaces altered in rats during the age periods studied. Such age-related variations could have been the result of changes in the biochemical composition of the epithelial membranes in the rats during development (13).

Summary. Using an in vivo onepass perfusion technique, transport of water (H_2O), sodium (Na), chloride (Cl), bicarbonate (HCO_3) and potassium (K) was studied in segments of the jejunum and ileum in normally growing 2-, 3- and 8-week-old rats. From solutions containing in mmole/l, NaCl 120, KCl 5, $NaHCO_3$ 25, Phenol Red 100 μmoles, glucose 0 or 15 and mannitol 25 or 10, quantitative differences in the net transport rates (μmoles/hr per g dry weight of segment) were observed. In general permeability of the epithelium of the perfused segments to H_2O, Cl, HCO_3 and K appeared to be greater in the 2 than in the 8-week-old rats. Glucose had the expected effect of enhancing absorption rates of water and Na in the segments of the rats at all three age periods.

1. Younoszai, M. K., *in* "Infant Nutrition" (S. J. Fomon, ed.), Second Edition, p. 95. W. B. Saunders Co., Philadelphia, London, Toronto (1974).
2. Koldovský, O., "Development of the Functions of the Small Intestine in Mammals and Man," S. Karger, Basel (Switzerland), New York (1969).
3. Deren, J. J., *in* "Handbook of Physiology" (C. F. Code and W. Heidel, eds.), Vol. III, Section 6, American Physiological Society, Washington, DC (1968).
4. Younoszai, M. K., and Lynch, A., Pediatr, Res. **9**, 130 (1975).
5. Schedl, H. P., and Clifton, J. A., Gastroenterology **41**, 491 (1961).
6. Huntsberger, D. V., and Leaverton, P. E., "Statistical Inference in the Biomedical Sciences," Allyn and Bacon, Inc., Boston (1970).
7. Younoszai, M. K., Sapario, R. S., and Laughlin, M., J. Clin. Invest. **62**, 271 (1978).
8. Schultz, S. G., Frizzell, R. A., and Nellans, H. N., Ann. Rev. Physiol. **36**, 51 (1974).
9. Frömter, E., and Diamond, J., Nature (New Biol.) **235**, 9 (1972).
10. Tai, Y. H., Desjeux, J. F., Danisi, G., and Curran, P. F., J. Membrane Biol. **31**, 189 (1977).
11. Binder, H. J., *in* "Gastrointestinal Physiology II" (R. K. Crane and A. C. Guyton, eds.), Vol. 12, p. 286, University Park Press, Baltimore, London, Tokyo (1977).
12. Podesta, R. B., and Mettrick, D. F., Amer. J. Physiol. **232**, E62 (1977).
13. Raul, F., Simon, P., Kedinger, M., and Haffen, K., Cell. Tiss. Res. **176**, 167 (1977).

Received March 27, 1978. P.S.E.B.M. 1979, Vol. 160.