Effect of Experimental Azotemia on Intestinal Transport of Butyric Acid (42842)

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Abstract. **Earlier studies have revealed an impairment of jejunal absorption of long chain fatty acids in experimental uremia. We investigated the intestinal absorption of butyric acid which is a short chain fatty acid in experimental renal failure (RF). Sprague-Dawley rats were randomized into the RF group which had subtotal nephrectomy, a sham-operated control group, and a pair-fed group. *In vivo* recirculating perfusion ($n = 5$) and *in vitro* everted sac incubation ($n = 8$) were employed. The *in vitro* experiments were repeated substituting the serosal buffer by either predialysis or postdialysis sera from uremic individuals, or normal serum ($n = 10$). The rate of *in vivo* butyric acid absorption was significantly lower while the *in vitro* absorption was significantly higher in the RF group than those observed in the sham-operated and pair-fed groups which showed comparable values. The normality of butyric acid absorption in the pair-fed animals despite comparable weight loss with the RF group tends to exclude anorexia and weight loss as a cause of altered butyric acid transport in RF animals. The disparity between the *in vivo* and *in vitro* data is suggestive of an inhibitory influence of uremic environment which is present *in vivo* and absent *in vitro*. This viewpoint was corroborated by the observed fall in butyric acid absorption by sacs containing predialysis uremic serum as compared with those containing normal or postdialysis sera. The latter further suggests that the inhibitory factor(s) is dialyzable.** [P.S.E.B.M. 1989, Vol 190]

Butyric acid is a short chain 4-carbon fatty acid which is produced from the fermentation of unabsorbed carbohydrates by anaerobic flora in the colon and distal small intestine in man (1) and in the rumen of ruminants (2). The available data concerning the mechanism of intestinal absorption of short chain fatty acids are limited and conflicting. Active transport (3), passive diffusion, and carrier-dependent transport at high luminal concentrations (4) have been suggested. In contrast to the long chain fatty acids which are carried through the lymphatic circulation, butyric acid and other short chain fatty acids are released in the portal venous system (5). Previous studies in our laboratory have shown a marked impairment of long chain polyunsaturated fatty acid transport in experimental renal failure (RF) (unpublished data). These observations prompted the present investigation of the effect of experimental RF on intestinal absorption of butyric acid which is a short chain fatty acid and has a different mode of transport than that of long chain fatty acids.

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Materials and Methods

Male Sprague-Dawley albino rats weighing 345–385 g were used. The animals were fed Purina rat chow and tap water *ad libitum*. They were randomly placed into RF and control groups. The animals placed in the RF group underwent a concurrent right nephrectomy and left subtotal nephrectomy. The subtotal nephrectomy was accomplished by resecting two thirds of the left kidney and partial cauterization of the remaining tissue. The procedure was performed under aseptic conditions and blood loss was kept to a minimum using strict hemostasis. The nephrectomies were performed extraperitoneally through a single dorsal incision to avoid intra-abdominal adhesions. The animals placed in the control group were sham operated in which the kidneys were exposed and manipulated without performing the actual nephrectomy. All surgical procedures were conducted under general anesthesia employing intraperitoneal injection of sodium pentobarbital (65 mg/kg). The animals were allowed a 14-day recuperation period prior to the absorption experiments. In an attempt to correct for uremia-induced anorexia, reduced nutrient intake, and weight loss, a group of normal animals pair-fed with the RF animals were included. This was accomplished by limiting the amount of food available to each PF animal to that consumed by the uremic counterpart during the pre-

ceding 24 hr. Serum creatinine concentration was determined before the absorption experiments using a kit supplied by Sigma Chemical Co. (St. Louis, MO).

Materials. Nonradioactive butyric acid was obtained from Sigma Chemical Co. [^{14}C]Butyric acid with the specific activity of 39 mCi/mmol and [^{3}H]inulin with the specific activity of 260 mCi/g were purchased from ICN Radiochemicals (Irvine, CA). [^{3}H]Inulin was used as a nonabsorbable marker (6). The perfusate used consisted of Krebs phosphate buffer at pH 7, unlabeled butyric acid (800 – 4000 μmol/liter), trace amounts of [^{3}H]inulin, and [^{14}C]butyric acid.

***In Vivo* Absorption Experiments.** Under general anesthesia using ether inhalation, the abdomen was opened by a midline incision. An inflow polyethylene cannula was inserted into the proximal end of the jejunum approximately 5 cm distal to the Treitz angle. An outflow L-shaped glass cannula was then introduced 15 cm distal to the inflow cannula. The cannulas were secured by ligating the jejunal section between parallel end vessels to avoid obstructing blood and lymphatic vessels. The intestinal segment was then flushed with phosphate buffer to remove any luminal residue and then flushed with air to minimize the amount of luminal fluid in the segment. The intestinal segment was then placed in the peritoneal cavity and the abdomen was sutured. The animal was allowed to awaken and was placed in a restraining cage. The inflow cannula was then connected to a reservoir containing 50 ml of the perfusate. A totally occlusive roller pump (Buhler Instruments, Inc., Fort Lee, NJ) was employed to pump the perfusate from the reservoir through the inflow cannula and into the intestinal segment at a flow rate of 1 ml/min. The outflow cannula was allowed to drain by gravity back into the reservoir. The solution in the reservoir was stirred continuously with a magetic stirrer. The animal's body temperature was closely monitored and kept at 37°C using a forced air heating device and a thermostatic temperature controller (Thermistemp model 74, Yellow Springs, OH) with a rectal probe. The rate of butyric acid absorption was calculated from disappearance of the labeled compound from the perfusate which was determined using the following equation:

$$A_t = A_o \left(\frac{I_o}{B_o} \times \frac{B_t}{I_t} \right)$$

where A_t and A_o represent the amount of butyric acid in the perfusate at times t and o, B_o and B_t equal the concentrations of labeled butyric acid at times o and t, and I_o and I_t stand for the concentrations of labeled inulin at times o and t, respectively. Accordingly, the absorption data were corrected for fluid shifts during the perfusion as reflected by changes in [^{3}H]inulin concentration (7). The perfusion was continued for 40 min and two 100-μl samples were removed from the reservoir at 10-min intervals. Each sample was mixed with 6 ml of scintillation fluid and subsequently analyzed for radioactivity. At the end of the experiment, the animal was killed by an overdose of ether and the perfused segment was removed and washed with 30 ml of phosphate buffer. A 10-g weight was attached to the most dependent portion of the segment to ensure a constant degree of stretch during the drying period. After a 24-hr drying period at 20°C, the length of the segment was measured and the tissue was further dried at 65°C for an additional 24 hr. The tissue was then crushed and solubilized with BTS 450 Tissue Solubilizer (Beckman, Fullerton, CA). Beckman Ready Organic Liquid Glacial Scintillation Cocktail was added and the solution was assayed for residual radioactivity. The absorption results were expressed in terms of dry intestinal length, since it correlates best with the intestinal surface area. The absorption studies were conducted at two different concentrations of butyric acid, i.e., low concentration (800 μM) and high concentration (4000 μM). Five animals were used in each group for each of the two perfusate concentrations. The radioactivity of ^{3}H and ^{14}C was determined by a double isotope counting method. The counting was carried to an error of ±0.5% using a liquid scintillation counter (Beckman LS-9000) with automatic quench calibration program.

***In Vitro* Studies.** In these experiments, animals were sacrificed by cervical dislocation. The abdomen was immediately opened and proximal jejunum was removed and flushed with buffer, then divided in several 3-cm long segments. Each segment was everted and ligated at both ends to form the sac. Each sac contained 100 μl of Krebs phosphate buffer (pH 7) and was incubated in 6 ml of incubation medium. The incubation medium contained different concentrations of butyric acid (80, 400, and 1200 μmole/liter) and was prepared in a manner identical to that described for the perfusate. Eight animals were included in each group from whom sacs were prepared for experiments at each of the butyric acid concentrations described above. During the incubation period (20 min), temperature was maintained at 37°C in a shaking water bath (80 oscillations/min). At the end of the incubation, the sacs were removed and rinsed in 1 mM taurocholate solution for 15 sec, then blotted gently on paper towel in order to remove the incubation solution adherent to the sacs' surface. Sacs were drained in separate vials and then radioactivity in the drained serosal solution was determined by double counting carried to ±0.5% σ error using a liquid scintillation counter (Beckman LS-9000) with automatic quench calibration program at ambient temperature. Preincubation samples were withdrawn in duplicate (2 × 100 μl) and were used to calculate the initial specific activities of butyric acid

and inulin. The *in vitro* mucosal to serosal transport was determined from the amount of labeled butyric acid recovered in the serosal compartment following the incubation experiments.

The *in vitro* studies were repeated substituting the buffer within the sacs by pooled serum obtained from normal individuals and patients with chronic renal failure before and after hemodialysis treatment. Ten normal animals were used for preparation of sacs for these experiments. Each rat supplied an equal number of sacs for experiments with normal and uremic sera. Sacs prepared from proximal and distal sites were alternated between the two experiments in order to control for possible effect of jejunal site. These experiments were intended to mimic normal and uremic chemical environment *in vitro*. A butyric acid concentration of 800 μM was chosen for these experiments to represent the midrange of concentrations employed in the preceding *in vitro* experiments.

Statistical Analysis. All data are given as mean ± SEM. Student's *t* test was used in analysis of the data.

Results

***In Vivo* Absorption.** The rate of intestinal absorption of butyric acid at low (800 μM) and high (4000 μM) perfusate concentrations was significantly lower in the RF animals than the corresponding values observed in the sham-operated control group (Tables I and II). The amount of residual radioactivity in the tissue of the perfused segment was negligible in both groups. As expected serum creatinine concentration was significantly increased while the body weight was significantly reduced in the RF animals (Tables I and II).

The rate of butyric acid absorption in the pair-fed group was comparable with that found in the sham-operated control group and was significantly higher than that of the RF animals despite comparable food intake and weight loss (Table I).

***In Vitro* Transport.** The rate of *in vitro* mucosal to serosal transport of butyric acid showed a linear relationship with its concentration in the incubation medium (Fig. 1). In contrast to the *in vivo* results, the mucosal to serosal transport of butyric acid *in vitro* was significantly increased in the RF group as compared with both the sham-operated and pair-fed groups (Table III).

Results of the *in vitro* experiments employing normal serum and predialysis and postdialysis uremic sera in the serosal compartment are summarized in Table IV. The results showed a significant reduction in butyric acid transport by sacs containing predialysis uremic serum when compared with that obtained with normal serum. In addition, comparison of the transport rates by sacs containing predialysis serum with those containing postdialysis serum from the same uremic subjects revealed a significant rise with postdialysis sera.

Discussion

The results of the perfusion experiments *in vivo* revealed a marked reduction of intestinal absorption of butyric acid both at low and high perfusate concentrations in animals with renal insufficiency. The observed difference between the RF and the control groups is not due to the luminal factors since experimental conditions were identical in all groups. In an attempt to control for the role of uremia-induced anorexia, reduced food intake, and weight loss we included a group of normal animals pair-fed with the RF rats. Despite comparable weight loss, the pair-fed group exhibited no significant reduction in the rate of *in vivo* butyric acid absorption. This observation tends to exclude anorexia and weight loss as a major cause of the impaired *in vivo* absorption of this short chain fatty acid. In contrast to the *in vivo* results, the *in vitro* incubation studies showed a significant increase in the mucosal to serosal transport of butyric acid in the RF animals compared with the sham-operated control group and the pair-fed animals. The increased mucosal to serosal transport *in vitro* in the RF animals may suggest increased intestinal permeability to butyric acid. The reason for the observed increased *in vitro* permeability to butyric acid in animals with renal insufficiency is not known and requires further investigation. However, we have observed this phenomenon with several other compounds including

Table I. *In Vivo* Intestinal Absorption of 800 μM Butyric Acid in Azotemic (RF), Sham-Operated, and Pair-Fed Groups[a]

	RF group	*P*	Sham group	*P*	Pair-fed group
Creatinine (mg/dl)	3.2 ± 0.6	<0.001	0.74 ± 0.19	NS[b]	1.04 ± 0.3*
Body weight (g)					
Initial	372 ± 8	NS	368 ± 14	NS	368 ± 22
Final	264 ± 24	<0.001	385 ± 13	<0.001	254 ± 21
Absorption rate (μmole/100 cm/hr)	0.59 ± 0.04	<0.01	0.79 ± 0.09	NS	0.76 ± 0.07*
	$n = 5$		$n = 5$		$n = 5$

[a] Data are given as mean ± SEM.
[b] NS, Not significant.
*$P < 0.001$ compared with the RF group.

ascorbic acid, pyridoxin, and vitamin E (unpublished data).

The observed impairment of butyric acid absorption *in vivo* and its increased transport *in vitro* in RF animals points to the presence of some inhibitory or depressive influence(s) in intact animals with renal insufficiency. Occasional secretion to the lumen of short chain fatty acids including butyric acid has been demonstrated in normal human volunteers (3). A marked butyric acid secretion in the uremic group can theoretically explain the observed reduction of its *in vivo* absorption in these animals. However, this is unlikely, since the absorption rates were determined from the disappearance of radiolabeled butyric acid. Consequently, addition of newly secreted unlabeled butyric acid to the lumen would not have been detected in the experimental system employed here. It should be noted that a substantial backleak of the absorbed butyric acid in RF animals cannot be excluded as a possible culprit, since labeled and unlabeled butyric acid would be returned to the lumen in the same proportion. However, this scheme is difficult to reconcile with the observed increased butyric acid transport *in vitro* unless the presumed backleak is promoted by the physicochemical factors only present in the intact azotemic animals.

Table II. *In Vivo* Intestinal Absorption of 4000 μM Butyric Acid in Azotemic (RF) and Sham-Operated Groups[a]

	Group		*P*
	RF	Sham	
Creatinine (mg/dl)	2.9 ± 0.8	0.58 ± 0.13	0.001
Body weight (g)			
Initial	359 ± 10	368 ± 15	NS[b]
Final	239 ± 22	380 ± 20	<0.001
Absorption rate (μmole/100 cm/min)	2.86 ± 0.15	3.82 ± 0.21	<0.01
	n = 5	*n* = 5	

[a] Data are given as mean ± SEM.
[b] NS, Not significant.

In an attempt to determine whether the reduction in the *in vivo* absorption in the RF animals was due to a defect in the transport of butyric acid from enterocytes to the portal circulation, we compared the amount of radioactivity remaining in the perfused segments after proper rinsing. The results showed no trapping of butyric acid in either group which tends to exclude a possible enterocyte to capillary transport defect as the main abnormality.

In order to examine the effect of uremic chemical environment, we repeated the *in vitro* studies substituting serosal buffer solution with pooled sera from either normal volunteers or uremic patients before and after dialysis. Addition of predialysis uremic serum markedly reduced butyric acid transport by sacs from both uremic and control animals when compared with results obtained using normal serum. These observations suggest that uremic serum contains some inhibitory factor(s) capable of depressing butyric acid transport. Examination of the data obtained using postdialysis serum from

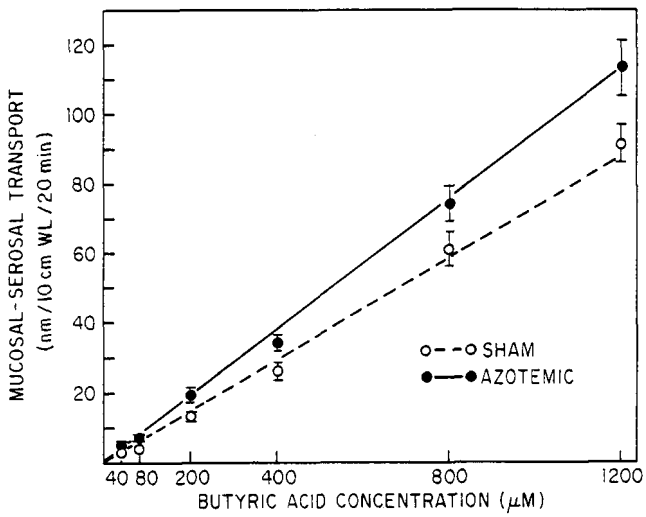

Figure 1. Relationship between butyric acid concentration and its mucosal to serosal transport in azotemic and control groups. Each point represents the mean transport rate at a specific concentration. Vertical bars represent SEM. Eight animals were used in each group.

Table III. *In Vitro* Mucosal to Serosal Transport of Butyric Acid at 80, 400, and 1200 μM Concentrations in Azotemic (RF), Sham-Operated, and Pair-Fed Animals[a]

	RF group	*P*	Sham group	*P*	Pair-fed group
Serum creatinine (mg/dl)	3.39 ± 0.6	<0.001	0.83 ± 0.1	NS[b]	1.28 ± 0.14*
Body weight (g)					
Initial	369 ± 12	NS	367 ± 18	NS	384 ± 20
Final	252 ± 28	<0.001	385 ± 19	<0.001	261 ± 17
Mucosal to serosal transport (nmole/10 cm/20 min)					
80 μM	6.29 ± 0.65	<0.02	4.07 ± 0.50	NS	3.85 ± 0.71*
400 μM	34.12 ± 1.75	<0.01	23.28 ± 2.97	NS	23.25 ± 4.60*
1200 μM	113.32 ± 2.50	<0.01	91.16 ± 4.10	<0.01	51.81 ± 13.86*
	n = 8		*n* = 8		*n* = 4

[a] Data are given as mean ± SEM.
[b] NS, Not significant.
* $P < 0.01$ compared with the azotemic group.

Table IV. *In Vitro* Mucosal to Serosal Transport of 800 μM Butyric Acid by Everted Sacs from Normal Animals Filled with Pooled Serum from Three Normal Individuals and Three Uremic Patients before and after Dialysis[a]

	Normal serum	*P*	Uremic serum		
			Predialysis	*P*	Postdialysis
Serum creatinine (mg/dl)	0.8 ± 0.1	<0.001	10.20 ± 1.10	<0.001	4.7 ± 0.2*
Mucosal to serosal transport (nmole/10 cm/20 min)	20.43 ± 2.10 $n = 10$	<0.001	15.93 ± 2.16 $n = 10$	<0.05	22.20 ± 2.03 $n = 10$

[a] Data are given as mean ± SEM.
* $P < 0.001$ when compared with normal serum.

the same subjects revealed marked reversal of the observed inhibitory influence. It thus appears that the inhibitory factor(s) responsible for reduced butyric acid transport must be a dialyzable substance(s). Interestingly, the mean value for mucosal to serosal transport by sacs containing postdialysis serum was greater (although not significantly) than that observed with normal serum. This observation suggests that the inhibitory factor(s) may be a constituent of normal serum whose concentration rises above normal in uremia and falls below normal after dialysis.

The linearity of the relationship between *in vitro* transport rates and butyric acid concentration at 40–1200 μM concentration range in both RF and normal animals suggest simple diffusion as the mode of transport in this concentration range. This is consistent with earlier studies employing normal subjects (4).

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