Intestinal Absorption of L-Ascorbic Acid in Rats with Renal Failure (42928)

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Abstract. **We studied L-ascorbic acid absorption in rats subjected to subtotal nephrectomy (renal failure (RF) group) and compared the results with those obtained in sham-operated normal animals and those pair-fed with their azotemic counterparts (PF group). *In vivo* recirculating perfusion and *in vitro* everted sac techniques were employed. The *in vitro* experiments were repeated substituting buffer within the serosal compartment with pooled sera from uremic and normal individuals. L-Ascorbic acid absorption *in vivo* in the RF group was significantly lower than those found in normal control and PF groups. In contrast, the *in vitro* mucosal to serosal transport was increased in the RF and PF groups when compared with the normal control group, suggesting increased permeability to L-ascorbic acid in the former groups. The disparity between *in vivo* and *in vitro* results in the RF animals is indicative of some inhibitory influence present in the intact uremic animals. However, experiments comparing the effect of uremic with normal sera on *in vitro* transport failed to reveal any suppressive effect of uremic chemical environment. In addition, serum ascorbic acid was reduced in PF and RF groups when compared with the normal control animals, thereby excluding elevated blood level as a cause of impaired absorption in intact animals with RF. In conclusion, *in vivo* jejunal absorption of L-ascorbic acid is impaired in rats with RF despite evidence of increased *in vitro* permeability. The latter appears to be mediated by reduced nutrient intake and weight loss. The inhibitory influence present *in vivo* could not be reproduced by incubation with uremic sera *in vitro*.** [P.S.E.B.M. 1989, Vol 191]

Ascorbic acid is a water-soluble vitamin which plays a major role in collagen formation, iron absorption, and oxidation-reduction reactions. Previous studies have shown a marked impairment of intestinal absorption of several vitamins in experimental uremia (1–3). However, the effect of renal failure on intestinal transport of ascorbic acid has not been previously investigated. Therefore, we undertook this study to address this issue.

Materials and Methods

Male Sprague-Dawley rats weighing 335–400 g, fed Purina Rat Chow and tap water *ad libitum*, were randomly placed into renal failure (RF) and control groups. Animals placed in the RF group underwent a concurrent right nephrectomy and a left subtotal nephrectomy. The latter was accomplished by resecting two-thirds of the left kidney with partial cauterization of the remaining tissue. The nephrectomies were performed extraperitoneally through a single dorsal incision to avoid intra-abdominal adhesions. The mortality rate in this group was approximately 60%. Animals placed in the control group were sham-operated by exposing and manipulating the kidneys without performing the actual nephrectomy. All surgical procedures were conducted under general anesthesia employing an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and under aseptic conditions with blood loss kept to a minimum using strict hemostasis. 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 (PF) with the azotemic animals were included. Serum creatinine concentration was determined before the absorption experiments using a kit supplied by Sigma Chemical Co. (St. Louis, MO). Serum L-ascorbic acid was measured using spectrophotometric assay by Smith Kline Bio-Science Laboratories (Van Nuys, CA).

Materials. Unlabeled L-ascorbic acid and pyruvic acid were obtained from Sigma Chemical Co. L-[^{14}C]-Ascorbic acid with the specific activity of 10.0 mCi/m*M* and [^{3}H]inulin with the specific activity of 260

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mCi/g were purchased from New England Nuclear Research Products Inc. (Boston, MA). [^{3}H]inulin was used as a nonabsorbable marker (4). The perfusate used consisted of Ringer's buffer (NaCl, 140 m*M*; $KHCO_3$, 10 m*M*; K_2HPO_4, 1.2 m*M*; $CaCl_2 \cdot 2H_2O$ 1.2 m*M*; $MgCl_2$, 1.2 m*M*; pyruvic acid, 5 m*M*) at pH 7.2, to which unlabeled L-ascorbic acid, trace amounts of [^{3}H]-inulin, and L[^{14}C]ascorbic acid were added.

***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 jejunum approximately 5 cm distal to the Treitz angle. An outflow L-shaped glass cannula was then introduced 20 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 flushed with Ringer's buffer to remove luminal contents and then air to minimize residual fluid in the lumen. The bowels were placed in the peritoneal cavity and the abdomen was sutured. The animal was placed in a restraining cage and allowed to awaken. The inflow cannula was then connected to a reservoir containing 50 ml of the perfusate with a 10 m*M* concentration of L-ascorbic acid. The reservoir was stirred continuously with a magnetic stirrer. 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 0.5 ml/min. The outflow cannula was allowed to drain by gravity back into the reservoir. The animals' body temperature was closely monitored with a thermostatic temperature controller (Thermistemp Model 74; Yellow Spring, OH) with a rectal probe and kept at 37°C using a forced air heating device. The rate of L-ascorbic acid absorption was calculated by determining the rate of its disappearance from the perfusate. Water shifts were measured using [^{3}H]inulin which served as a non-absorbable marker and the absorption rate was corrected accordingly (4). We confirmed the validity of inulin as a nonabsorbable marker in the RF model using *in vitro* everted sac incubation technique which showed negligible mucosal-to-serosal transport of [^{3}H]-inulin in all models studied. The perfusion was continued for 30 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 sacrificed by an overdose of ether and the perfusate intestinal segment was removed and washed with 30 ml of Ringer's buffer. The length of the segment was measured after it was suspended with a 10-g weight attached to its most dependent portion. The tissue was dried for 24 hr at 20°C and subsequently processed for determination of residual radioactivity. The absorption results were expressed in terms of intestinal length and dry weight. The radioactivity of ^{3}H and ^{14}C was determined by double isotope counting which 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 an overdose of ether. The abdomen was immediately opened, the proximal jejunum removed, and then divided into eight 3-cm segments. Each segment was everted and ligated at both ends after introducing 100 μl of Ringer's buffer (pH = 7.2). The sacs were placed in vials containing 6 ml of incubation medium with different concentrations of L-ascorbic acid (0.2, 1.0, 2.0, 4.0, 8.0, and 10.0 m*M*) and were incubated for 10 min in a 37°C shaking water bath (80 oscillations/min). At the end of the incubation, the sacs were removed, rinsed in Ringer's buffer, then blotted gently on paper towel. They were then drained and the radioactivity of the drained solution was determined by a double isotope counting method. The counting was 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 L-[^{14}C]ascorbic acid and [^{3}H]inulin.

The *in vitro* studies were repeated substituting the buffer within the sacs with serum samples obtained from three normal individuals and three uremic patients. At least three normal animals were employed in each group. The intestinal segments were processed in the same fashion as described under the *in vivo* experiments.

Statistical Analysis. All data are given as ± SE. Data are expressed as μmol/g of dry weight/min and μmol/100 cm/min. Analysis of variance (ANOVA), Student's *t* test, and linear regression were used as appropriate (5).

Results

***In Vivo* Absorption.** The results are depicted in Table I. As expected, serum creatinine concentration in the RF group was significantly increased when compared with the normal and PF control groups. Both RF and PF animals exhibited comparable losses in body and intestine weights. No significant difference was found in the tissue water content (estimated from the dry and wet weights of the perfused segments) between RF animals and the control groups (81.3% for RF animals, 83.1% for PF animals, and 81.7% for the normal control animals). The rate of intestinal absorption of 10 m*M* L-ascorbic acid was significantly lower in the RF animals than that found in the normal control group. Likewise, comparison with the PF group showed a significantly lower absorption rate in the renal failure group, despite comparable losses of body and intestine

weights in the two groups. The observations mentioned above held true whether the rate of absorption was expressed per length or dry weight.

In comparison to normal controls, the PF animals showed a tendency for greater rates of absorption especially when calculated for intestine weight. The differences, however, did not attain statistical significance. The residual radioactivity present in the tissue of the perfused segment was negligible in all groups. Serum ascorbic acid concentration was comparable in the RF and PF animals who showed significantly lower values than those found in the normal controls.

***In Vitro* Transport.** The rate of *in vitro* mucosal to serosal transport of L-ascorbic acid at concentrations of 0.2–10 mM in the incubation medium showed a linear relationship to its concentration ($r = 0.88$, $P < 0.05$ in RF animals; $r = 0.95$, $P < 0.01$ in normal controls; and $r = 0.95$, $P < 0.01$ in PF group). In contrast to the *in vivo* results, the rates of *in vitro* mucosal to serosal transport expressed per unit of intestine weight were higher in both the RF and PF groups as compared with those found in the normal control group. However, when calculated for unit length, analysis of variance showed no significant difference among the three groups (Table II).

Results of the *in vitro* experiments employing normal and uremic sera in the serosal compartment are summarized in Table III. The results revealed no significant difference in L-ascorbic acid transport by normal sacs containing either uremic or normal sera.

Table I. The *In Vivo* Absorption of 10 m*M* L-Ascorbic Acid in Renal Failure (RF), Pair-Fed (PF), and Normal Control (NL Control) Groups

	RF	NL control	PF	*t* test (*P*)
Creatinine	2.51 ± 0.20[a]	0.98 ± 0.17	0.92 ± 0.04	<0.001[b]
Body weight (g)				
Initial	349.8 ± 6.1	349.3 ± 5.4	380.4 ± 6.3	NS[b]
Final	280.0 ± 9.7	357.3 ± 4.8	293.1 ± 12.8	<0.01[b]
Dry weight (3 cm)				
Intestinal segments (g)	0.037 ± 0.001	0.045 ± 0.002	0.035 ± 0.001	<0.01[b]
Serum ascorbic acid (m*M*)	0.033 ± 0.007	0.079 ± 0.012	0.035 ± 0.003	<0.05[b]
Absorption rate				
μmol/100 cm/min	3.36 ± 0.76	7.91 ± 1.17	9.80 ± 1.30	<0.005
μmol/g/min	32.7 ± 8.18	73.67 ± 14.03	107.11 ± 18.18	<0.01

[a] Data are given as mean ± SE, a minimum of six animals were included in each group.
[b] RF vs NL control.

Table II. *In Vitro* Mucosal to Serosal Transport of L-Ascorbic Acid at 0.2, 1, 2, 4, 8, and 10 m*M* Concentrations in Renal Failure (RF), Normal Control (NL control), and Pair-Fed (PF) Animals

	RF	NL Control	PF	*t* test (*P*)
Serum creatinine (mg/dl)	2.39 ± 0.14[a]	0.67 ± 0.05	0.88 ± 0.08	<0.001[b]
Body weight (g)				
Initial	346.3 ± 6.2	356.4 ± 5.4	379.7 ± 8.9	NS[b]
Final	282.3 ± 5.4	369.9 ± 6.0	275.9 ± 8.3	<0.001[b]
Mucosal-serosal transport				ANOVA
μmol/g/10 min				(*P*)
0.2 m*M*	0.0059 ± 0.001	0.0032 ± 0.001	0.0179 ± 0.003	<0.001
1.0 m*M*	0.0287 ± 0.007	0.0206 ± 0.003	0.0540 ± 0.008	<0.01
2.0 m*M*	0.0658 ± 0.006	0.0478 ± 0.009	0.2525 ± 0.083	<0.02
4.0 m*M*	0.1559 ± 0.036	0.0400 ± 0.009	0.2200 ± 0.034	<0.01
8.0 m*M*	0.1574 ± 0.016	0.0589 ± 0.006	0.5725 ± 0.116	<0.001
10.0 m*M*	0.2472 ± 0.043	0.2165 ± 0.062	0.6690 ± 0.058	<0.001
μmol/100 cm/10 min				
0.2 m*M*	0.0090 ± 0.001	0.0060 ± 0.001	0.0087 ± 0.0014	NS
1.0 m*M*	0.0633 ± 0.019	0.0335 ± 0.005	0.0572 ± 0.0050	NS
2.0 m*M*	0.1077 ± 0.018	0.0848 ± 0.015	0.1090 ± 0.0231	NS
4.0 m*M*	0.3050 ± 0.061	0.2183 ± 0.056	0.2953 ± 0.0329	NS
8.0 m*M*	0.2939 ± 0.030	0.2243 ± 0.051	0.4063 ± 0.1120	NS
10.0 m*M*	0.9200 ± 0.340	0.3858 ± 0.090	0.8416 ± 0.0730	NS

[a] Data are given as mean ± SE, a minimum of four animals were included in each group.
[b] RF vs NL control.

Table III. *In Vitro* Mucosal to Serosal Transport of 4 m*M* L-Ascorbic Acid by Everted Sacs from Normal Rats Containing Pooled Sera from Normal Individuals and Uremic Patients before Dialysis[a]

	Normal serum	Uremic serum	*P*
Transport			
μmol/g/10 min	$5.8 \times 10^{-5} \pm 1.0 \times 10^{-5}$	$8.0 \times 10^{-5} \pm 2.0 \times 10^{-5}$	NS
μmol/100 cm/10 min	$7.5 \times 10^{-5} \pm 1.1 \times 10^{-5}$	$10.0 \times 10^{-5} \pm 1.6 \times 10^{-5}$	NS

[a] Data are given as mean ± SE.

Discussion

The results of the *in vivo* perfusion experiments revealed a marked reduction of intestinal absorption of L-ascorbic acid in animals with renal insufficiency. The observed difference between the renal failure and the control groups did not appear to be due to luminal factors as experimental conditions were identical in both groups. In an attempt to control for the uremia-induced anorexia, reduced food intake, and weight loss, a group of normal animals pair-fed with the renal failure rats was included in the study. Despite comparable weight loss, the pair-fed group exhibited no significant reduction in L-ascorbic acid absorption *in vivo*. In fact, the rate of absorption expressed per unit of weight in the pair-fed animals was insignificantly greater than that found in the normal control group. These observations tend to exclude anorexia and the associated reductions in body and intestine weights as a major cause of impaired *in vivo* transport of this vitamin. In fact, the *in vitro* incubation studies showed a significant increase in the mucosal to serosal transport of L-ascorbic acid (per unit of intestine weight) in the renal failure and pair-fed animals when compared with that of the normal control group. These observations are indicative of increased intestinal permeability to L-ascorbic acid *in vitro* in both uremic and pair-fed animals. Reduced food intake and the resultant fall in body weight common to both groups appear to have contributed to the observed increased permeability.

The observed impairment of L-ascorbic acid absorption *in vivo* in renal failure animals but not in the pair-fed group in the face of the demonstrated increased *in vitro* permeability is of considerable interest. These observations point to the presence of some inhibitory or depressive influence(s) in azotemic animals which is present *in vivo* and absent *in vitro*. It is possible that backleak of both labeled and unlabeled vitamin C could explain the observed reduction in absorption in the *in vivo* study. While possible, this scheme is difficult to reconcile with the observed increased L-ascorbic acid transport *in vitro* unless the presumed backleak is promoted by physicochemical factors only present in the intact azotemic animals. Normally ascorbic acid is partly excreted in the urine (6) and as such renal failure may lead to elevation of its serum concentration. Since L-ascorbic acid is passively absorbed in the rat intestine (7, 8), elevated levels of this vitamin may impair its mucosal to serosal transport by narrowing the concentration gradient. It should be noted, however, that in the present study serum ascorbic acid levels in renal failure and pair-fed groups were significantly lower than those found in the normal control group. Accordingly, a narrow ascorbic acid concentration gradient was not responsible for decreased L-ascorbic acid in the azotemic animals. In fact, low serum ascorbic acid level and a wide concentration gradient may be responsible for the slight increased absorption in the pair-fed animals.

To determine whether the reduction in the *in vivo* absorption in the azotemic animals was due to a defect in the transport of L-ascorbic acid from enterocytes to the portal circulation, we compared the residual radioactivity in the perfused segments after proper rinsing. The results showed no trapping of L-ascorbic acid in either group, excluding such a possibility.

In an attempt to examine the effect of the uremic chemical environment, we repeated the *in vitro* studies substituting serosal buffer solution with pooled sera from either normal volunteers or uremic patients before dialysis. Addition of uremic serum did not alter the L-ascorbic acid transport by sacs from normal rats, thereby excluding a rapid effect of uremic chemical environment on ascorbic acid absorption *in vitro*. It thus appears that physiologic factors only present in intact azotemic animals are responsible for the observed impairment of ascorbic acid transport. Moreover, these physiologic factors are not related to the observed uremia-induced anorexia and weight loss since the pair-fed group did not manifest a similar reduction in ascorbic acid absorption. Likewise, the observed abnormality is not caused by the alteration of serum ascorbic acid level in renal failure animals since serum ascorbic acid was reduced in this group which should have enhanced rather than suppressed its absorption. It is conceivable that hemodynamic alterations present in animals with renal failure may have somehow contributed to the observed functional abnormality.

The linearity of the relationship between *in vitro* absorption rates and L-ascorbic acid at 0.2–10 m*M* concentration range in both renal failure and normal animals suggest simple diffusion as the mode of transport in this concentration range. This confirms earlier studies employing normal rats (7).

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