

Gastrointestinal Absorption of Ascorbic Acid in Guinea Pigs

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Gastrointestinal tract of guinea pigs was recirculated *in situ* with ascorbic acid, and absorption rate was determined in order to discuss its feasible mechanism involved. The absorption rate of ascorbic acid from the small intestine was a little larger than that from the stomach especially in relatively lower dose, and a type of saturation kinetics was observed in both sites. Apparent Michaelis constants were 1.30 mM and 1.27 mM for stomach and small intestine respectively, indicating almost the same apparent affinity to both sites. Additional absorption experiments were carried out in the presence of three metabolic inhibitors (phlorizin, ouabain, and 2,4-dinitrophenol), dehydroascorbic acid, and glucose. Phlorizin or glucose was considered to exert a competitively inhibiting effect on the small intestinal absorption of ascorbic acid, while the stomach absorption was affected neither remarkably nor competitively by almost all of the adjuvants. A difference in those inhibitory effects as well as the properties of drug moiety is fully suggestive of that in absorption mechanism between the two sites. It may be simultaneously implied, from the blood level immediately after 1 hr tests of recirculation and its curve following *i.v.* administration of ascorbic acid, that the absorbed drug will be rapidly distributed in the animal tissue cells.

Ascorbic acid is widely distributed in the animal organism as shown by chemical determinations. The biological role for this vitamin in the organism is still obscure and a number of miscellaneous functions have been suggested. In addition to the classical signs of scurvy that have been found in fibroblasts, odontoblasts, and osteoblasts losing their capacity to form collagen and in abnormalities in the blood vessels leading to localized haemorrhages, several other functions in the body seem to be impaired in its deficiency state. According to the observations by Myasnikov, ascorbic acid reduces the development of hypercholesterolemia and retards the development of experimental lipoidosis of the aorta in rabbit.³⁾ Studies along similar lines have been demonstrated in man by Samuel, Morrin, and Spittle⁴⁾ and in guinea pigs by Becker, Bolker, Banerjee, and Guchhait.⁵⁾ Man, monkey, and guinea pig are recognized as typical species unable to synthesize ascorbic acid.

It is generally known that ascorbic acid is readily absorbed from the intestinal tract. However, little is known yet with respect to the kinetics and mechanisms involved in the absorption from gastrointestinal tract. It may be, therefore, reasonably considered as great importance to evaluate the gastrointestinal absorption of this drug for a proper discussion on its physiological role.

The primary purpose of this work is to determine the rate for gastrointestinal absorption of ascorbic acid by employing *in situ* recirculation method on guinea pigs.

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Experimental

Materials—L-Dehydroascorbic acid (DAA) was prepared from the oxidation of L-ascorbic acid (AA) according to benzoquinone method reported by Hammarström.⁶⁾ AA and other compounds used were of analytical grade.

Absorption Experiments—Male Hartley guinea pigs weighing 300 to 350 g were fasted for about 15 hours prior to the experiments but water was allowed *ad libitum*. After anesthetization with pentobarbital, the stomach or the small intestine was cannulated for *in situ* recirculation, following the method of Koizumi.⁷⁾ Cannulation was applied to the tracts between cardia and pylorus for the stomach and between Treitz's ligament and ileocecal valve for the small intestine. Drug solutions were prepared to be 0.5 to 5 mM by dissolving appropriate amounts of AA in hydrochloric acid solution and phosphate buffer solution which were isotonicly adjusted at pH 1.0 and 7.4, respectively.

The stomach or the small intestine was first perfused with 100 ml of 0.9% NaCl solution and with 50 ml of drug solution under maintenance at 37°. The tubings attached to the inflow and outflow cannulae were then connected to a flask containing 50 ml of the drug solution which followed to be continuously circulated through the tract for 1 hr at 37° by means of circulation apparatus adjusted to flow at 5 ml per min. One ml of the sample solution was withdrawn periodically for 1 hr after 10 min lag of pre-recirculation. The concentration of the drug and indicator (phenol red) was determined.

Stability Tests of AA in the Perfused Solutions—Drug solutions were prepared by dissolving AA in hydrochloric acid solution and phosphate buffer solution which were recirculated independently for 1 hr prior to the tests. A volume of 50 ml, drug solution, was continuously circulated and periodically pipetted out in the same manner as described above except using a glass tubing instead of gastrointestinal tract.

Effect of Some Metabolic Inhibitors, DAA, and Glucose on the Absorption of AA—Gastrointestinal tracts were perfused with 50 ml of drug solution (0.5–5 mM) containing one of the following adjuvants: 0.01 mM of phlorizin, ouabain, and 2,4-dinitrophenol (DNP), 1 mM of DAA, and 0.1 mM of glucose.

Blood Level of Total AA—One ml of cardiac blood was withdrawn immediately after 1 hr perfusion and periodically for 2 hr after the intravenous administration of AA (10 and 50 mg per kg) to another group of the animals. The concentration of total AA was determined as described below.

Analytical Procedures—In the absorption and stability tests, the concentration of AA was determined by spectrophotometric measurement at 250 nm of *n*-butylalcohol layer which was shaken with the supernatant solution of the mixture, 1 ml of sample, 2 N HCl, and 6% trichloroacetic acid. This organic phase was also used to determine the concentration of phenol red at 501 nm. On the other hand, the blood level of total AA (AA plus DAA transformed) was determined according to the 2,4-dinitrophenylhydrazine method reported by Fujita.⁸⁾

Results

Gastrointestinal Absorption of AA

Degradation of AA in 1 hr perfusates of stomach and small intestine obeyed apparently pseudo first order kinetics. The logarithm of remaining AA in the lumen, appropriately corrected by the periodical change of concentration of phenol red as the indicator, was plotted against time. Straight lines obtained in Fig. 1 show that the absorption of AA from guinea pig stomach and small intestine is also pseudo first order. Since the degradation and absorption are considered to occur simultaneously in the lumen, absorption ratio (C_2/C_0) is given in the function of disappearance ratio ($1-C/C_0$) of AA as indicated in Eq. 1:

$$C_2/C_0 = [k_2/(k_1 + k_2)](1 - C/C_0) \quad (1)$$

where k_1 and k_2 are rate constants for degradation and absorption respectively, C_0 , C , and C_2 are concentrations of initial, remaining and absorbed drug respectively. Absorption ratio in 1 hr perfusion is summarized in Table I including the rate constants obtained for degradation and absorption. It is comparatively suggested that AA is as readily absorbed from small intestine as from stomach of guinea pigs in spite of a great difference in the proportion of non-ionized form between these sites. The pH of drug solution was not changed significantly during the recirculation, resulting in pH 0.9 to 1.1 and pH 7.2 to 7.5 for stomach and small intestine respectively.

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TABLE I. Rate Constants for Degradation and Absorption and Absorption Ratio of AA in Gastrointestinal Recirculation

In stomach				In small intestine			
C_0 (mM)	k_1 (hr ⁻¹) ^a	k_2 (hr ⁻¹) ^b	Absorption ratio(%) ^c	C_0 (mM)	k_1 (hr ⁻¹) ^a	k_2 (hr ⁻¹) ^b	Absorption ratio(%) ^c
0.42	0.19 ± 0.03	0.40 ± 0.05	29 ± 5	0.42	0.26 ± 0.04	0.49 ± 0.04	33 ± 6
0.78	0.14 ± 0.02	0.38 ± 0.04	30 ± 5	0.75	0.16 ± 0.02	0.50 ± 0.03	33 ± 4
2.5	0.057 ± 0.01	0.18 ± 0.02	17 ± 3	2.4	0.11 ± 0.03	0.20 ± 0.03	18 ± 4
5.2	0.048 ± 0.01	0.13 ± 0.01	14 ± 2	5.3	0.075 ± 0.01	0.13 ± 0.02	15 ± 4

a) degradation rate constant
 b) absorption rate constant
 c) Percentage obtained in 1 hr recirculation

Absorption rates ($v = k_2 C_0$) were plotted normally and reciprocally against initial drug concentration in Fig. 2 and 3. It is strongly suggested, from these results, that a typical saturation kinetics may be applied to the gastrointestinal absorption of AA in guinea pigs. Straight lines illustrated in Fig. 3 are considered to satisfy the following type of Lineweaver-Burk's Equation:

$$1/v = 1/V_{max}' + (K_m'/V_{max}')1/C_0 \quad (2)$$

where V_{max}' and K_m' are apparent maximum rate and Michaelis constant, respectively. Table II summarizes these kinetic parameters. Apparent affinity to the absorption site was implied to be almost the same in both sites.

Effect of Some Metabolic Inhibitors, DAA, and Glucose on the Absorption of AA

Application of double reciprocal plots in a similar manner to Fig. 3 to the absorption rates of AA in the presence of these adjuvants resulted in that a typical effect of competitive inhibition was only revealed on the small intestinal absorption by phlorizin or glucose. Other effects than these were found to be neither competitive nor significant in the gastrointestinal

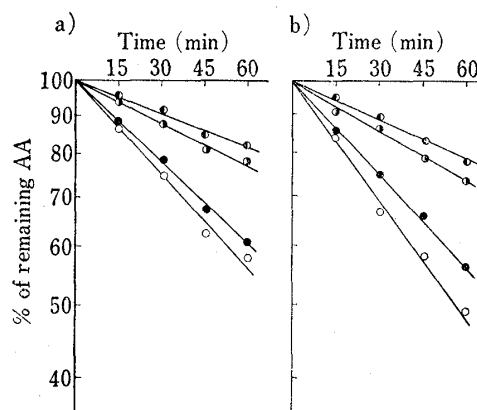


Fig. 1. Percentage of Remaining AA in Gastrointestinal Recirculation

a) in stomach
 ○: 0.42 mM
 ●: 0.78 mM
 ◐: 2.51 mM
 ◑: 5.19 mM

b) in small intestine
 ○: 0.42 mM
 ●: 0.75 mM
 ◐: 2.38 mM
 ◑: 5.30 mM

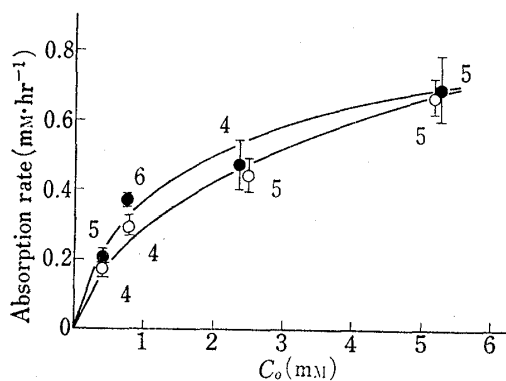


Fig. 2. Effect of Initial Concentration on Gastrointestinal Absorption of AA

○: in stomach ●: in small intestine
 vertical bar: standard deviation
 number in parenthesis: number of runs

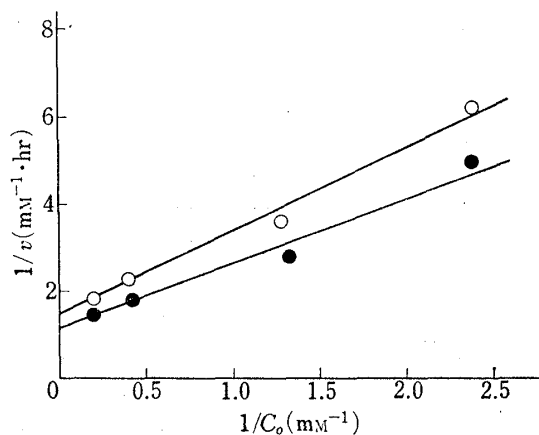


Fig. 3. Lineweaver-Burk Type Plots for Gastrointestinal Absorption of AA

○: in stomach ●: in small intestine

tracts. Percentages of inhibition at 1 mM of AA are compiled in Table III. Although ouabain and 2,4-DNP did not act competitively, they showed apparently similar degrees of inhibitory effects on the small intestinal absorption of AA (1 mM) to those of phlorizin and glucose, respectively. Small effects of phlorizin or ouabain on the stomach absorption and of DAA on the small intestinal absorption were neither competitive nor non-competitive. The remarkable differences in the effect of phlorizin and glucose between stomach and small intestine are fully suggestive of an unlikeness of the absorption mechanisms involved in each site.

Blood Level and Elimination Rate of Total AA

Blood levels measured immediately after gastrointestinal absorption tests of 1 hr are indicated in Table IV. These values represent net and total AA which was found as the sum of AA and DAA in the whole blood. A dose dependence, which is similar to but not so significant as that of absorption rate, was found in the blood level immediately after the perfusion experiments. However, these levels are accounted to be considerably lower than expected from the gross estimation of total blood volume in guinea pigs.

TABLE II. Kinetic Parameters for Lineweaver-Burk Type Plots

Parameters	In stomach	In small intestine
V_{max}' (mm·hr ⁻¹)	0.78	0.87
K_m' (mM)	1.30	1.27

TABLE III. Inhibitory Effect of Some Adjuvants on Gastrointestinal Absorption of AA Loaded as 1 mM

Adjuvants (mM)	% of inhibition	
	In stomach	In small intestine
Phlorizin(0.01)	13.1±2.4	82.3± 7.2
Ouabain(0.01)	24.6±4.8	78.4± 8.1
2,4-DNP(0.01)	0.8±0.2	61.4± 4.9
DAA(1)	1.4±0.3	10.2± 2.6
Glucose(0.1)	3.1±0.8	65.1±10.3

TABLE IV. Blood Level of Total AA Following Gastrointestinal Recirculation

Dose (mM)	Blood level (μg/ml) following	
	Stomach recirculation	Small intestine recirculation
0.5	3.1±0.2	4.1±0.4
1	4.4±0.3	5.7±0.5
3	5.6±0.2	7.2±0.3
5	5.2±0.4	7.3±0.4

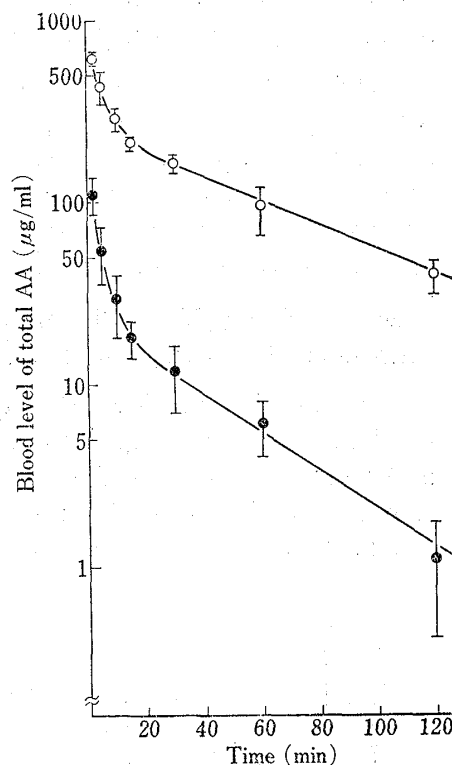


Fig. 4. Blood Level Curve of Total AA Following Intravenous Administration
 —○— : 50 mg/kg —●— : 10 mg/kg

Fig. 4 shows blood levels of total AA following intravenous administration to guinea pigs. The profile of rapid decrease indicates that the absorbed AA may be promptly and ubiquitously distributed in body tissues of the animal. There seems to be a type of dose dependence also in elimination rate, but further inspection would be necessary to approve it. Half-life for distribution phase was approximately 3 min and those for elimination phase were about 30 and 45 min following the injection of 10 and 50 mg per kg, respectively.

Discussion

It has been well established that AA and DAA form a readily reversible oxidation-reduction system and both forms of the vitamin are equally effective as antiscorbutic agents, though the relative instability of the former has been well known in neutral to basic pH range and that of the latter in highly acidic or neutral pH range has been demonstrated.⁹⁾ In this experiment, AA was found to be rather stable in small intestinal perfusate than it was expected at pH 7.4. Since the pK_a value is known to be 4.17 (pK_1) and 11.57 (pK_2), non-ionized and monoanionic species are predominant in stomach and small intestinal pH, respectively. Nevertheless, the absorption of AA from guinea pig small intestine was revealed to be a little higher than from the stomach. It is reasonably considered that the absorption mechanisms and/or processes of this drug are essentially different between these sites. Hornig has recently suggested that AA is actively absorbed from the upper part of small intestine in man and guinea pig but not in rat.¹⁰⁾ A typical saturation profile observed in this experiment for small intestinal absorption was well analogous to those reported in man and guinea pigs^{10,11)} and is sufficiently indicative of a specific mechanism similar to active transport. This suggestion of active transport and also the difference in the absorption mechanism between gastrointestinal tracts should be approved with certainty when a notable effect of inhibition by phlorizin and glucose was thoroughly discussed. It may be reasonably proposed, on account of competitive inhibition by these agents, that the small intestinal absorption of monoanionic AA occurs in a specific, active transport mechanism similar to that of sugars. No reports have been found to describe the exact mechanism for the stomach absorption of AA either in man or in experimental animals. Though neither competitively nor non-competitively, ouabain or phlorizin exhibited a significant inhibitory effect on the stomach absorption of AA. In the stomach, however, there was no indication of such inhibitory effects as those of phlorizin and glucose in the small intestine. From the inhibitory profile of several adjuvants, the stomach absorption of non-ionized AA is supposed to proceed in some other and more complicated mechanisms than the active transport which is discussed in the small intestinal absorption.

It has been well known that AA is partially destroyed and partially excreted by the body in maintaining a renal threshold. This vitamin is readily excreted by the kidney in large amounts only when the plasma level exceeds this threshold, which is approximately estimated to be 1.4 mg% in man.¹²⁾ When the tissues are not saturated and the plasma level is low, the ingestion of AA results in little or no renal excretion, for the tissue cells avidly take up the vitamin as it is absorbed and thus the concentration in the plasma is prevented from reaching threshold values. Although the value for guinea pigs have not been reported yet, a typically rapid distribution would be responsible for the low blood levels of this vitamin observed after 1 hr perfusion tests. This was confirmed by a considerably short half-life for the distribution phase following *i.v.* administration of AA.

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