Ascorbate Stimulation of Salicylate Absorption in Isolated Rat Intestine

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Abstract □ Salicylate transport across the rat intestine was measured using both *in vitro* and *in vivo* procedures. After the steady state for labeled salicylate was reached, the addition of ascorbate stimulated tracer flux with the establishment of a new steady state. The tissue permeability had a saturable dependence on ascorbate concentration. Also, ascorbate stimulated the tissue short-circuit current.

Keyphrases □ Ascorbate—effect on GI absorption of salicylate, rat intestine *in vivo* and *in vitro* □ Salicylate—GI absorption, effect of ascorbate, rat intestine *in vivo* and *in vitro* □ Absorption, GI—salicylate, effect of ascorbate, rat intestine *in vivo* and *in vitro* □ GI absorption—salicylate, effect of ascorbate, rat intestine *in vivo* and *in vitro*

Ascorbic acid effects on metabolic activity as well as on nutrition are the subject of substantial scientific activity (1). The effect of sex on metabolic availability of ascorbic acid is just one example of the basic questions under study (2). Recently, the modification of salicylamide metabolism in the male by ascorbic acid taken orally was reported (3). Followup work with crossover studies on rats using oral and intramuscular administrations demonstrated that the ascorbic acid interaction was strongest for oral drug administration. This interaction may occur during the first pass of the drugs through both the liver and the intestinal tissue.

Ascorbate interaction with salicylate transport across the isolated rat intestine is reported here as determined by *in vitro* and *in vivo* techniques. The absorption is clearly stimulated, and the *in vitro* experiment also demonstrates an increase in the tissue short-circuit current.

EXPERIMENTAL

In vitro salicylate flux across the rat jejunum was measured by a reported procedure (4) using the technique of Ussing and Zerhan (5) as modified by Schultz and Zalusky (6). The tissue chamber[1] consisted of two conical half chambers with a 1.13-cm^2 cross-sectional area. Each half chamber was connected to a gas lift perfusion apparatus containing 10 ml of buffer solution and was heated to 37° by water jackets.

The composition of the buffer, in milliequivalents per liter, was: Na, 143.3; K, 6; Mg, 2.4; Ca, 5; Cl, 128.3; HCO_3, 25; H_2PO_4, 1.2; SO_4, 2.4; and glucose, 25. The pH was adjusted to 7.4 with carbon dioxide, and solution mixing was effected by 95% O_2–5% CO_2. Salt bridges, consisting of 4% agar in buffer solution, were connected to silver–silver chloride electrodes located approximately 22 mm from the mounted tissue while calomel electrodes were placed *via* salt bridges at about 2 mm from the tissue.

Sprague–Dawley male rats, 300–400 g, were sacrificed by decapitation. Four tissue sections were excised from each animal for mounting on the cells by cutting along the mesenteric line from the segment of intestine 25–50 cm distal to the stomach. ^{14}C-Salicylic acid, 2 μCi and 0.1 m*M*, was introduced on the mucosal side of the tissue; the increasing concentration in the serosal bathing solution was measured at 10-min intervals from 20 min after the label was added until 110 min. The ascorbate solution, made fresh with buffer and pH adjusted with sodium hydroxide and carbon dioxide, was added to the serosal solution at 50 min.

The salicylate flux before ascorbate addition, J, was determined by a linear least-squares fit to the data points from 20 to 50 min. The flux after ascorbate treatment, J^c, was determined by a fit to the data from 70 to 110 min. The spontaneous potential difference across the tissue was continuously recorded. External current was applied periodically to determine the current needed to force the tissue potential to zero.

In vivo salicylate absorption was measured using the same animals and buffer. The labeled salicylic acid was 7 μ*M*, and the ascorbate was initially 28 m*M*. The procedure of Schanker *et al.* (7), as modified (8), was used. The small intestine was cannulated, using polyethylene 320 tubing, at the ligament of Trietz and at a point 10 cm proximal to the ileocecal junction to form an *in situ* loop of intestine. Perfusion of the loop at a rate of 1.5 ml/min was carried out in the direction of normal GI flow by means of a peristaltic pump.

At the end of the experiment, the animal was sacrificed, the intestine was separated from the mesentery, and the length of perfused intestine was measured. Samples were removed from the reservoir at fixed times during the experiment and were assayed by liquid scintillation spectrometry. Apparent absorption rate constants, calculated as the disappearance rate per centimeter of perfused intestine, were measured before, k_a, and after, $k_a{}^c$, ascorbate administration, thus allowing each animal to serve as its own control.

RESULTS AND DISCUSSION

The flux data from the *in vitro* studies are presented in Table I. The flux of labeled salicylate from the mucosal to the serosal side of the tissue was measured before, J, and after, J^c, ascorbate, [C], was added to the serosal solution. Salicylate reached a new steady-state flux after ascorbate addition over a concentration range of 1–57 m*M*. For example, for [C] = 6 m*M*, the average salicylate flux, $\bar{J}$, before ascorbate addition was 6.69 nmoles/cm^2/hr with a standard error of the mean of ±0.59, using 14 separate tissues taken from seven different animals. The stimulatory effect of ascorbate resulted in an average salicylate flux ratio, $\overline{(J^c/J)}$, of 1.24 for these 14 tissues, thus showing an average 24% increase in the transport rate at this concentration. The paired t-test applied to these data showed that the $\overline{(J^c/J)}$ was different from unity at a significance level of 99.95%. For the studies reported in Table I, five different concentrations of ascorbate were used on 42 tissues from 11 animals. The difference in the $\bar{J}$ values is a reflection of the large range of flux values usually found in this type of measurement. It was imperative, therefore, to use each tissue as its own control, thus allowing application of paired t-test statistics, to observe the flux change due to ascorbate. In 39 out of the 42 cases, the treatment with ascorbate resulted in an increased flux. At [C] = 57 m*M*, one tissue had no flux change; at 6 m*M*, two of the 14 tissues had a 4% decrease. From J averaged over all 42 tissues and for the salicylate concentration of 0.1 m*M*, the average permeability of the jejunum for salicylate transport from the mucosal to the serosal side of the tissue was P_{ms} = 0.592 cm/hr.

Table I—Average Salicylate Flux[a] before and after Addition of Serosal Ascorbate

[C], m*M*	$\bar{J}$	$\overline{(J^c/J)}$	$p<$	Tissues, Animals
1	6.50 ± 0.38	1.19 ± 0.05	0.0025	8, 4
6	6.69 ± 0.59	1.24 ± 0.05	0.0005	14, 7
17	6.00 ± 0.65	1.34 ± 0.05	0.0005	8, 4
28	4.80 ± 0.85	1.67 ± 0.14	0.0025	6, 3
57	5.59 ± 1.08	1.60 ± 0.22	0.01	6, 3
				42, 11

[a] Flux units are nanomoles per square centimeter per hour ± *SEM*.

[1] E. W. Wright, Inc., New Haven, Conn.

Table II—Effect of Ascorbate on Apparent Absorption Rate Constants for Salicylate

	k_a, $\times 10^6$ cm^{-1} hr^{-1}	$k_a{}^c$, $\times 10^6$ cm^{-1} hr^{-1}
	1.76	2.82
	1.56	2.21
	1.90	2.49
	1.41	1.86
Mean	1.66	2.34[a]

[a] $p < 0.01$.

The average flux ratios are plotted in Fig. 1 as a function of the ascorbate concentration. With the average tissue permeability to salicylate, P_{ms}, and fitting of the data in Fig. 1, the permeability as a function of ascorbate concentration, $P_{ms}{}^c$, is reported for a salicylate concentration of 0.1 m*M*. The computer fit was forced to unity for no added ascorbate and fits well to the "best" experimental point at [*C*] = 6 m*M*. While fitting is not precise, it does allow an estimate to be reported:

$$P_{ms}{}^c = P_{ms}\{1 + V[C]/(K + [C])\} \qquad \text{(Eq. 1)}$$

where $V = 0.74 \pm 0.17$ (*SD*) and $K = 10.5 \pm 7.9$. *In vivo* studies (9) showed that P_{ms} has a linear dependence on the salicylate concentration over a considerable range but nothing is yet known about the dependence for $P_{ms}{}^c$.

The tissue potential difference, V_t, was recorded continuously throughout each experiment. At frequent set intervals, the tissue potential was forced to zero by applying an external current momentarily to obtain, in addition to V_t, the tissue resistance, R, and the short-circuit current, I_{sc} (10). In Fig. 2, the top graph shows a normal monotonically decreasing tissue potential, V_t, as a function of time. The ascorbate was introduced at 50 min but showed no perceptible perturbation to V_t.

The tissue electrical resistance, R, the middle graph in Fig. 2, showed usual behavior as compared to earlier work and responded to the ascorbate treatment at 50 min with a substantial decrease. The short-circuit current, I_{sc}, is the electrical parameter often used to characterize biological tissues. The bottom graph of Fig. 2 shows a 13% increase in I_{sc}, with onset immediately after ascorbate was added, which leveled to a constant value at 70 min.

The electrical effect of ascorbate is different than that observed with either theophylline[2] or ouabain[3] on the same type of tissue. For ascorbate, the increase in I_{sc} was essentially due to the decrease in R. For theophylline, I_{sc} increased due to both an increase in V_t and a decrease in R. For ouabain, I_{sc} decreased because of a decrease in V_t and an increase in R. With the continuous recording of V_t, it was possible to ascertain a more rapid, distinct response for both ouabain and theophylline when added to the serosal solution, thus supporting the claim that the serosal side of the tissue is the more responsive for the action of both drugs.

In vivo absorption studies were carried out to determine if the effect of ascorbate on salicylate flux was an *in vitro* artifact or a biological phenomenon. Table II gives apparent absorption rate constants before,

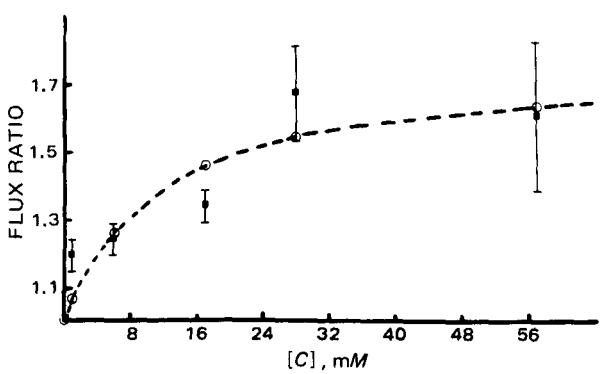

Figure 1—*Averaged individual tissue flux ratios as a function of ascorbate concentration. The solid squares are the average values, and the bars are the standard errors of the mean for each ascorbate concentration. The circles connected by the dotted curve is the mathematical fit to the experimental data by the BMD nonlinear computer program (11).*

[2] G. Barnett, unpublished work.

[3] D. W. Adair, doctoral dissertation, University of California, San Francisco, Calif., in preparation.

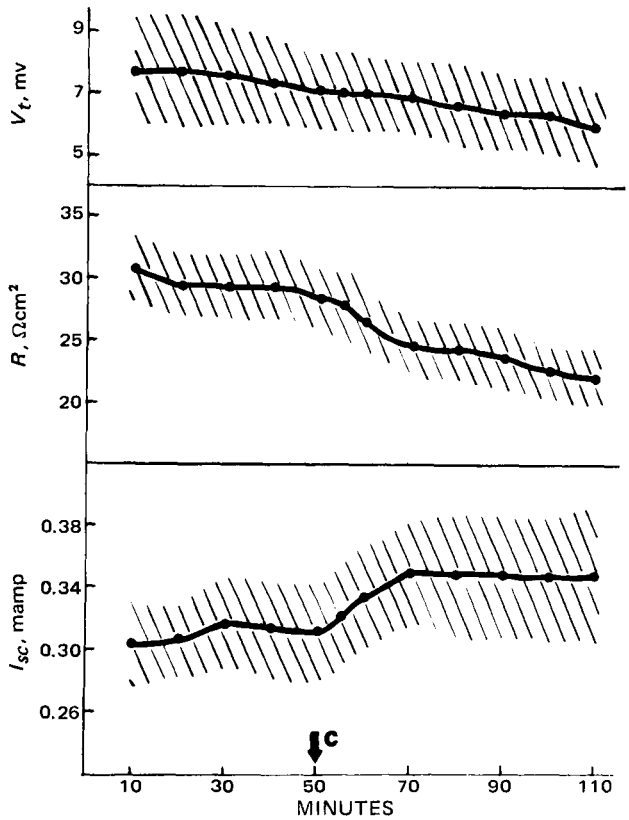

Figure 2—*Tissue electrical parameters as a function of time. The top graph is the tissue potential difference,* V_t; *the middle graph is the tissue resistance,* R; *and the bottom graph is the tissue short-circuit current,* I_{sc}. *Each point is an average of 13 tissues, reported in Table I for the case* [C] = 6 m*M*, *where the shaded region extends over ± 1* SEM. *Ascorbate, 6 m*M, *was added to the serosal bathing solution at 50 min, as noted by the arrow.*

k_a, and after, $k_a{}^c$, the introduction of ascorbate into the solution which perfused the mucosal tissue surface. Each of the four experiments showed increased absorption, while the mean value gave a 40% increase in the rate constant; by the paired t-test, this increase is significant at a 99% level. From Fig. 1, it is observed that the *in vitro* procedures gave roughly a similar result.

Clinical work has indicated that ascorbic acid affects both the absorption rate and various metabolic processes. In this precisely defined, and thus rather theoretical, system, ascorbate enhanced the absorption of tracer quantities of salicylate across the intestine of the laboratory rat. Tissue electrical resistance decreased for the 6 m*M* ascorbate treatment and, based on these tentative results, showed the same effect at higher ascorbate levels as well. The electrical response is considerably different than that due to treatment with the metabolic inhibitor ouabain or the antidiuretic hormone-like behavior of theophylline.

The estimate of the salicylate tissue permeability reported here suggests a saturable dependence on the ascorbate concentration. The average flux after ascorbate treatment, $\bar{J}^c$, appears to be independent of either the ascorbate concentration or the initial average flux, $\bar{J}$. The range for $\bar{J}^c$ was 7.6–8.0, while the average for all 42 tissues was 7.82 ± 0.27 nmoles/cm^2/hr. This constant, or perhaps maximum, salicylate flux in the presence of exogenous ascorbate, observed in averaged data, is not explained, although it has been suggested that perhaps the treatment results in a return of each tissue to a state of preexperimental integrity[4].

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Metabolic Studies of the Nonnutritive Sweeteners Cyclopentylmethylsulfamate and Cyclopentylsulfamate: Determination of Metabolites in Rat Urine

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Abstract □ The *nonnutritive* sweetener sodium cyclopentylmethylsulfamate was fed to Wistar albino rats. The urine was collected for 3 days, combined, and examined (GLC) for the metabolites cyclopentylmethylamine and cyclopentylmethanol. The percent conversion to these metabolites was 0.077 and 0.0102, respectively. The percent conversion to cyclopentylmethylamine was the lowest conversion to amine observed when compared to the metabolism of three other sweet sulfamates, cyclopentylsulfamate, cycloheptylsulfamate, and cyclooctylsulfamate, previously administered to rats. The average excretion of unmetabolized sulfamate was 15.4%. Sodium cyclopentylsulfamate was fed to rats over 9 days, and an analysis was carried out for the metabolites cyclopentylamine, cyclopentanone, and cyclopentanol. A decrease in the level of metabolites occurred after the first 3 days.

Keyphrases □ Cyclopentylsulfamate and cyclopentylmethylsulfamate—metabolism in rats □ Metabolism—various sulfamates in rats □ Sulfamates, various—metabolism in rats compared □ Sweeteners, nonnutritive—various sulfamates, metabolism in rats compared

The artificial sweetener cyclamate (*N*-cyclohexylsulfamate) has been the subject of many investigations, including the feeding of the compound to different animal species to study its distribution and metabolism (1–4). Until 1966, it was generally considered that the compound, whether administered orally, intravenously, or intraperitoneally, was excreted entirely unchanged. However, in 1966, Kojima and Ichibagase (5) found cyclohexylamine in the urine of dogs and humans who had been fed cyclamate. Various toxicological studies (6–9) then showed that certain animals fed cyclamate developed lesions, chromceromal breaks, and tumors of the urinary bladder. The presence of cyclohexylamine as a metabolite and the possibility of cyclamate being a carcinogen resulted in the sweetener being banned in 1969 in the United States and Britain.

The purposes of this investigation were to study the level of excretion of sweet-tasting sulfamates related to cyclamate, to determine whether metabolic breakdown occurs, and, if so, to compare the levels of breakdown with the levels reported for cyclamate. Cycloheptylsulfamate and cyclopentylsulfamate were administered to rats and rabbits, and the results were reported previously (10, 11). The recent surprising finding (12) that sodium cyclopentylmethylsulfamate is sweet and the suggestion that its amine metabolite may be less carcinogenic than, for example, cyclohexylamine prompted study of this compound.

This paper describes the results of *in vivo* administration of cyclopentylmethylsulfamate to rats and the extended feeding of cyclopentylsulfamate to rats.

EXPERIMENTAL

Reagents and Chemicals—Cyclopentylmethylamine, bp 140–143°, was prepared by the following route: cyclopentanone[1] → cyclopentanol (13) → cyclopentylbromide (14) → cyclopentylcyanide (15) → cyclopentylmethylamine (16). This amine was sulfamated by the method of Audrieth and Sveda (1) and recrystallized (twice) from ethanol.

Anal.—Calc. for $C_6H_{12}NNaO_3S$: C, 35.82; H, 5.97; N, 6.96. Found: C, 35.85; H, 5.45; N, 6.81.

Sodium cyclopentylsulfamate was prepared as previously described (10). Cyclopentylmethylamine, cyclopentylamine[1], cyclopentylmethanol[1], cyclopentanol[1], cyclopentanone[1], and dichloromethane[2] were redistilled before use. *p*-Benzoquinone was sublimed; *n*-dodecane[3], analytical reagent grade sulfosalicylic acid[4], chloroform, ethanol, and 1,4-dioxane (reagent grade) were used as obtained.

Feeding Experiments—Female Wistar albino rats, ~300 g, were kept on solid food and water in metabolism cages[5]. Prior to feeding, the urine of each rat was collected and monitored using both detection methods (described later), and the rats were then starved of water for 24 hr. Sodium cyclopentylmethylsulfamate (1450 mg/kg) was administered orally in aqueous solution (25–30 ml of water). The extended feeding of sodium cyclopentylsulfamate was carried out over 9 days as follows: 200 mg in 20 ml of water was fed for the first 5 days, sulfamate was not given for the 6th and 7th days, and feeding was resumed for the 8th and 9th days.

The urine of the rats fed cyclopentylmethylsulfamate was collected

[1] Aldrich Chemical Co.
[2] May and Baker.
[3] British Drug Houses.
[4] AnalaR grade, May and Baker.
[5] NKP cages, Kent, England.