Absorption of vitamin C from the human buccal cavity

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(*Received* 2 *June* 1978 – *Accepted* 16 *January* 1979)

1. Ascorbic acid was absorbed across the mucosa of the human mouth.
2. Omission of sodium ions from the medium decreased the absorption of ascorbic acid.
3. The presence of D-glucose, or 3-*O*-methyl-D-glucose, increased the absorption of ascorbic acid but D-fructose had little effect and D-mannitol had no effect.
4. Calcium ions also increased ascorbic acid absorption probably by a secondary effect on Na^+ fluxes.
5. Buccal mucosa was also permeable to dehydroascorbic acid and D-isoascorbic. acid

Investigations using in vitro techniques have revealed sodium ion-dependent active transport of L-ascorbic acid from the small intestine of the guinea-pig (Stevenson & Brush, 1969) and the human (Stevenson, 1974). Both species have a nutritional requirement for vitamin C.

Transport of certain food sugars across human buccal mucosa in vivo shows stereospecificity. For the natural isomers D-glucose and D-galactose the transport is at least partly sodium-dependent (Manning & Evered, 1976).

It was the purpose of the present study to investigate the buccal absorption in vivo of L-ascorbic acid and compare it with the results obtained from small intestine. Other workers have already shown that buccal mucosa is permeable to ascorbic acid (Odumosu & Wilson, 1971, 1977).

MATERIALS AND METHODS

L-Ascorbic acid, analytical grade (Fisons Scientific Apparatus Ltd, Loughborough, England), dehydroascorbic acid (INC Pharmaceuticals Inc., Cleveland, Ohio, USA) and D-isoascorbic acid, Na salt (British Drug Houses Ltd, Poole, Dorset), were dried overnight over desiccant under vacuum. Other compounds used were analytical grade when available.

Buccal absorption was measured as follows: 25 ml sample of the ascorbic acid solution prepared in appropriate buffer solution was preincubated at 37° for 5 min. Then the solution was circulated inside the mouth by the tongue and cheeks about once a second for 5 min. The solution was expelled into a beaker and the mouth rinsed for 5 sec with 10 ml of preincubated buffer without added ascorbic acid. This was expelled into the same beaker and the volume made up to 50 ml with buffer solution. After mixing a portion was then centrifuged at 3000 ***g*** for 10 min to remove insoluble material. At the beginning of each experiment a buccal blank, consisting of 25 ml of buffer solution at 37° but without ascorbic acid, was circulated in the mouth for 5 min then treated as before. Modified Krebs–Ringer buffer at pH 6 in mM concentrations: NaCl, 118·5; KCl, 4·7; KH_2PO_4, 1·2; $MgSO_4$, 1·2; sodium citrate, 1·8; Na_2HPO_4, 6·2. For experiments with sodium-free buffer the sodium salts were substituted by potassium salts. Calcium ions were omitted otherwise calcium phosphate was precipitated. For experiments in which calcium was added (2·6 mM) the KH_2PO_4, Na_2HPO_4 and sodium citrate were omitted and glycylglycine (1·8 mM) added (Evered & Sadoogh-Abasian, 1979).

L-Ascorbic acid, dehydroascorbic acid and D-isoascorbic acid were determined colori-

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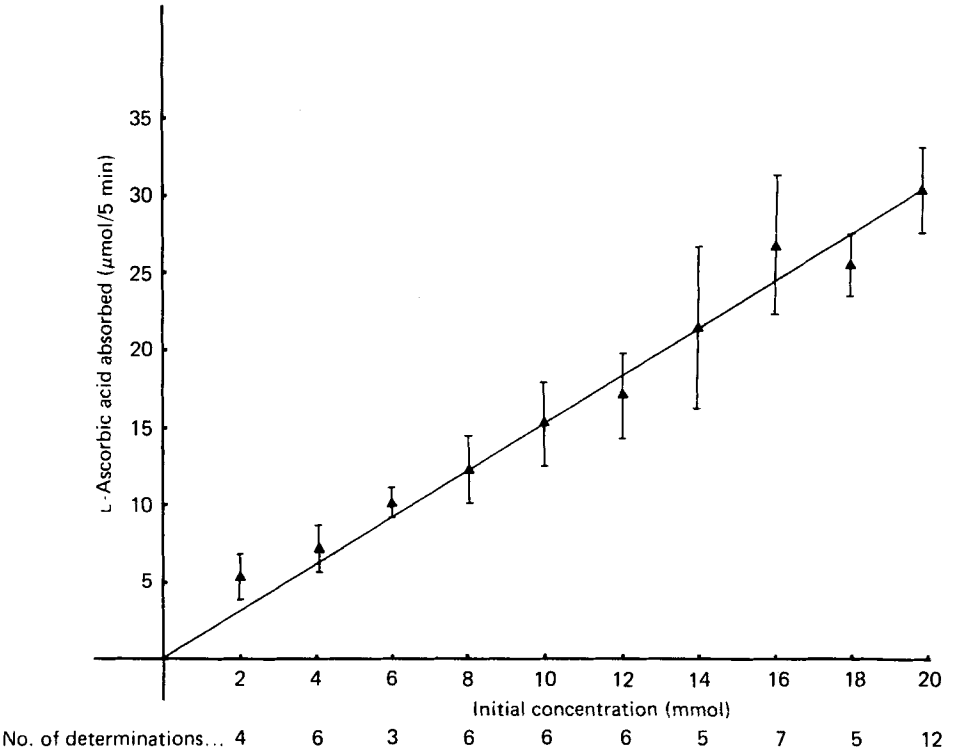

Fig. 1. The effect of initial concentration (mM) of ascorbic acid on absorption (μmol/5 min) across buccal mucosa at pH 6 in man. Points represent mean values, with their standard errors represented by vertical bars. Number of determinations at each concentration in parentheses. For details of procedures, see p. 15.

metrically (Bessey *et al.* 1947). Monosaccharides interfered with this assay so a titrimetric method (Barakat *et al.* 1955) was used for ascorbic acid assays when sugars were added.

The vitamin C saturation test (Varley, 1967) was performed on the experimental subjects to assess their nutritional status with respect to vitamin C. None were vitamin C deficient by this criterion. The vitamin C saturation test was normal throughout the study with male subject 1. The amount of vitamin C absorbed through the buccal cavity is negligible nutritionally. The amount given in each saturation test, approximately 0·7 g, outweighs by many magnitudes the buccal absorption. For studying inter-sex differences the subjects were apparently healthy Caucasian adults aged between 25 and 50 years with no dentures. The remaining experiments were with one male subject, designated male 1, who was 25 years of age.

RESULTS

The uptake of L-ascorbic acid, from aqueous buffer solution, measured as loss from the buccal cavity was studied over the concentration range 2–20 mM (Fig. 1). Experiments were at pH 6, the average pH of the buccal contents.

Uptake was measured with a few different subjects to establish that the inter-subject variation was small (Table 1). Absorption averaged 13·1 μmol/5 min at 10 mM initial concentration. The vitamin C saturation tests were within the normal range.

Absorption of dehydroascorbic acid and isoascorbic acid at 10 mM concentration was compared with that of L-ascorbic acid (Table 2).

Table 1. *Inter-subject variation in the buccal absorption (μmol/5 min) of vitamin C at* 10 mM *in apparently healthy adult subjects*

(Mean values with their standard errors; number of determinations in parentheses)

Male			Female			Statistical significance of difference
15·1 ± 2·2 (6)	11·7 ± 1·6 (5) 12·5 ± 0·98 (15)*	9·8 ± 0·81 (4)	11·9 ± 0·66 (4)	13·9 ± 0·0 (2) 13·8 ± 0·71 (9)*	16·5 ± 2·0 (3)	NS

NS, Not significant.
* Mean ± SEM.

Table 2. *Buccal absorption (μmol/5 min) of dehydroascorbic acid and isoascorbic acid compared with that of* L-*ascorbic acid at* 10 mM *in an apparently healthy adult subject*

(Mean values with their standard errors; number of determinations in parentheses)

Control (L-ascorbic acid)	Dehydroascorbic acid	D-Isoascorbic acid	Statistical significance of difference between treatments*
13·8 ± 1·1 (6)	12·9 ± 1·0 (6)	—	NS
13·0 ± 1·4 (8)	—	13·0 ± 0·74 (12)	NS

NS, Not significant.
* Student's *t* test.

Absorption of L-ascorbic acid in the presence of D-glucose, 3-*O*-methyl-D-glucose, D-fructose, D-mannitol and dehydroascorbic acid was also investigated (Table 3). Buccal absorption of L-ascorbic acid at 10 mM was partially inhibited by 2 mM Amytal (Eli Hilly & Co. Ltd, Basingstoke): control 16·6 ± 3·5 (*n* 6) μmol/5 min; with Amytal 10·5 ± 3·1 (*n* 6). The difference is not statistically significant ($P < 0{\cdot}1$).

The effect of omitting Na^+ by replacement with K^+ was tested and also the presence or absence of Ca^{2+} (Table 4).

According to Fig. 1, 10 mM is an intermediate concentration in investigating the downhill transport of L-ascorbic acid. That is why it was chosen for the experiments for investigating the effect of different ions. The effect of different sugars was investigated with 4 mM L-ascorbic acid in order to avoid osmotic problems when the concentration of sugar is five times that of L-ascorbic acid.

An experiment was performed to investigate whether there was any metabolic loss of ascorbic acid during buccal experiments.

Ten millilitres of pH 6 buffer after pre-incubation at 37° was circulated in the mouth for 5 min then added to 25 ml of 10 mM ascorbic acid solution in buffer. After diluting to 50 ml the mixture was incubated for 10 min. After centrifugation the amount of ascorbic acid in the supernatant fraction was assayed. Standard ascorbic acid solution was incubated alongside this test solution. Since the assays were identical it was concluded that there was no metabolic loss. Absorption of ascorbic acid into the deposited cells is negligible and has been fully studied by Odumosu & Wilson (1977).

With the titrimetric method only L-ascorbic acid is assayed. The spectrophotometric method assays the total dehydroascorbic acid after oxidation of ascorbic acid. Hence the results of ascorbic acid absorption using the titrimetric determination (Table 3) are somewhat lower than using the spectrophotometric method (Fig. 1) comparing the results at 4 mM.

Table 3. *L-Ascorbic acid absorption (μmol/5 min) at 4 mM in the presence of various compounds (20 mM) in an apparently healthy adult subject*

(Mean values with their standard errors; number of determinations in parentheses)

Control (L-ascorbic acid)	Glucose	3-*O*-Methyl-D-glucose	Fructose	Mannitol	Dehydroascorbic acid	Statistical significance of difference between treatments*
3·6±0·26 (4)	8·4±0·87 (4)	—	—	—	—	$P < 0·005$
4·8±0·47 (6)	—	7·4±0·1 (6)	—	—	—	$P < 0·005$
4·6±0·1 (6)	—	—	5·8±0·14 (6)	—	—	$P < 0·005$
4·4±0·36 (4)	—	—	—	4·5±0·41 (4)	—	NS
4·06±0·25 (6)	—	—	—	—	4·8±0·46 (6)	$P < 0·1$

NS, Not significant.
* Student's *t* test.

Table 4. *The effect of calcium and sodium ions on ascorbic acid absorption (μmol/5 min) at 10 mM from the mouth in apparently healthy adult subjects*

(Mean values with their standard errors; number of determinations in parentheses)

Absence of Ca^{2+}	Presence of 2·6 mM Ca^{2+}	Statistical significance of difference between treatments*
10·1±1·6 (7)	13·7±0·93 (7)	$P < 0·05$
Absence of Na^{+}	Presence of 133 mM Na^{+}	
7·4±1·9 (22)	12·8±1·3 (22)	$P < 0·01$

* Student's *t* test.

DISCUSSION

For drugs which pass across the buccal mucosa permeability is increased by increasing lipid solubility or the percentage of unionized molecules present. It may be calculated that the ascorbate anion is only 1·6% unionized at pH 6, the pH at which our buccal experiments were performed. Furthermore, ascorbic acid is very soluble in water and poorly soluble in lipid. Other workers have studied the effect of pH on the buccal absorption of vitamin C. They showed that by increasing the pH from 3·4 stepwise up to pH 9 there is a gradual decrease in buccal absorption and also uptake into buccal mucosal cells (Odumosu & Wilson, 1977). It can be calculated that vitamin C is only 13·7% ionized at pH 3·4 but almost fully ionized (> 99·99%) at pH 9. This would imply transport of the unionized molecule and the authors interpreted their experimental results to indicate passive diffusion of vitamin C. They also showed that the process was not stereospecific since both the natural form, L-ascorbic acid, and the unnatural D-isomer, were transferred across buccal mucosa at similar rates.

According to our experiments there was no statistical difference between male and female subjects (Table 1) in contrast to the results of Odumosu & Wilson (1971). The absorption rate of ascorbic acid did not reach a maximum with increasing concentration up to 20 mM. This can be interpreted to indicate either passive diffusion or a carrier-mediated transport with a very high dissociation constant k_t. Transport occurred with the concentration gradient in our experiments. Investigations could not be conducted with the present experimental system to study transport against the concentration gradient. The assay procedure was not sensitive enough to measure accurately transport of ascorbic acid below the plasma level of approximately 5 μmol (Barkham & Howard, 1958). The level in stimulated saliva is approximately one-tenth of that concentration (Eastoe, 1961). In our study high 'blank' values, presumably due to aldehydic or ketonic compounds reacting with 2,4-dinitrophenylhydrazine,

precluded accurate assay of ascorbic acid at these low levels. Possibly a radioisotope would be the best way to carry out such assays as in studies on intestinal absorption of ascorbic acid in vitro (Stevenson, 1974) or in animals (Spencer *et al.* 1963). Such experiments with healthy humans in vivo are precluded by safety regulations.

The transport of D-glucose across buccal mucosa was inhibited by D-galactose and 3-*O*-methyl-D-glucose suggesting that they share at least one common carrier system (Manning & Evered, 1976). Hence the rationale of testing the influence of glucose on ascorbic acid transport. It was surprising to find that glucose enhanced ascorbic acid transfer across the buccal mucosa but a similar effect has been described with human ileum in vitro. It was interpreted to be due to glucose acting as an energy metabolite (Stevenson, 1974). 3-*O*-Methyl-D-glucose also enhanced ascorbic acid transport and since this sugar is non-metabolizable and cannot be phosphorylated to act as an energy source in mammals (Campbell, 1952; Campbell & Young, 1952), our results suggest an alternative explanation.

Ascorbic acid transport across small intestine is Na^+-dependent in the guinea-pig (Stevenson & Brush, 1969) and in the human (Stevenson, 1974). Our experiments showed the Na^+-dependence of ascorbic acid absorption from the mouth by substituting Na^+ ions by K^+ ions. However even within the 5 min test period the 25 ml of test solution became diluted with perhaps 5–6 ml saliva. This contributed Na^+ and therefore one cannot conduct the experiment in vivo with a total absence of Na^+. Resting saliva contains approximately 15 mM Na^+ ions and wax-stimulated saliva approximately 45 mM (Eastoe, 1961). Hence the rate of Na^+-dependent absorption is not totally abolished in the buccal experiments. However, ascorbic acid transport across buccal mucosa was enhanced by the presence of D-fructose. Transport of this sugar is independent of Na^+ in both the small intestine (Sigrist-Nelson & Hopfer, 1974) and buccal mucosa (McMullan *et al.* 1977). Hence this phenomenon merits further investigation.

Conversely, transport of D-glucose and 3-*O*-methyl-D-glucose is Na^+-dependent in both mucosal systems (Schultz & Curran, 1970; Manning & Evered, 1976). Finally lack of enhancement of L-ascorbic acid absorption in the presence of D-mannitol (a sugar alcohol) indicates that enhancement in the presence of the sugars previously mentioned is because of their secondary effect on Na^+ fluxes. From this indirect evidence of the different effects of different sugars on ascorbic acid transport, it may be inferred that ascorbic acid absorption across the buccal mucosa is Na^+-dependent and occurs by a carrier-mediated process. The influence of glucose and its 3-*O*-methyl derivative on this transport can be interpreted as a secondary effect on Na^+ fluxes and not an effect on energy metabolism.

The increase in L-ascorbic acid absorption shown in the presence of Ca^{2+} ions was also found with some sugars. This phenomenon was also interpreted to be a secondary effect, stimulation of Na^+ fluxes (McMullan *et al.* 1977).

Our experiments are compatible with carrier-mediated transport of L-ascorbic acid across mucosa of the buccal cavity as with small intestine. Poor inhibition of transport by the barbiturate metabolic inhibitor, Amytal, suggests active transport since barbiturates inhibit the electron-transport chain. Possibly this result is due to the known poor transport of barbiturates across buccal mucosa (Moffat, 1968) The effect of increasing pH decreasing ascorbic acid transport found by other workers (Odumosu & Wilson, 1977) could be interpreted as poor affinity of the carrier for the highly charged ascorbate anion at high pH values.

Dehydroascorbic acid at five times the molar concentration, had no inhibitory effect on ascorbic acid absorption. This result suggests that ascorbic acid and dehydroascorbic acid, though interconvertible by oxidation-reduction, are not absorbed using the same carrier. Possibly dehydroascorbic acid is absorbed by passive diffusion. Dehydroascorbic acid penetrates some other cell membranes faster than ascorbic acid. Examples are the brain (Patterson & Mastin, 1951) and mammalian erythrocytes (Christine *et al.* 1956; Hughes & Maton,

1968). Since in these cells there is an efficient reduction of dehydroascorbic acid to ascorbic acid this will tend to promote absorption of dehydroascorbic acid. Outward flow of ascorbic acid from erythrocytes will also promote absorption of dehydroascorbic acid. This evidence suggested absorption across a 'pore' but the poor uptake of dehydroisoascorbic acid into erythrocytes was surprising (Hughes & Maton, 1968).

In rat intestine oxidation of ascorbic acid to dehydroascorbic acid did not significantly affect its absorption (Davis *et al.* 1977). This is consistent with our suggestion that dehydroascorbic acid and D-isoascorbic acid penetrate buccal mucosa at the same rate as L-ascorbic acid at equal concentrations.

Thus, ascorbic acid joins the list of compounds which are absorbed from the human buccal cavity in a rather similar way to absorption from the small intestine. Many drugs (reviewed by Beckett & Hossie, 1971), well-absorbed monosaccharides (Manning & Evered, 1976), and a poorly absorbed disaccharide, lactulose (Evered & Sadoogh-Abasian, 1979) all show this striking correlation.

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Printed in Great Britain