Effect of chronic hypo and hypervitaminosis C on the brush border enzymes and the intestinal uptake of glucose and alanine

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Summary. Brush border sucrase and alkaline phosphatase activities are considerably enhanced in the intestine of ascorbic acid deficient guinea-pigs. Similar increase in the uptake of D-glucose and L-alanine also occurs in chronic vitamin C deficiency. However the permeability of D-glucose and L-alanine in the intestine of animals fed with large doses of vitamin C is severely depressed, with a reduction in the levels of sucrase and alkaline phosphatase activities.

The involvement of ascorbic acid in various metabolic processes has been well characterized in recent years. Enhancement in the level of various lysosomal enzymes in the liver and the impairment of the collagen biosynthesis in different epithelial tissues has been observed in vitamin C deficiency[1,2]. Also scurvy is known to be associated with the various endocrine disturbances of the body[3-6]. In view of the fact that the role of vitamin C in the molecular physiology of the intestine has not been amply investigated, we studied the effect of chronic latent scurvy and hypervitaminosis C on the intestinal uptake of D-glucose and L-alanine and on the brush border sucrase and alkaline phosphatase activities.

Materials and methods. Male guinea-pigs (200-250 g) were used in these studies. The animals were kept for a week on a stock diet consisting of soaked gram, green grass and scorbutic diet, as described by Banerjee[3]. Those animals which grew well on this diet were selected for these experiments. Animals were divided into 3 groups and were given the following diets: 1. Control group: scorbutogenic diet + 10 mg ascorbic acid daily. 2. Chronic scorbutic group: latent chronic scurvy was produced in the animals as described by Ginter et al.[7].

3. Hypervitaminosis C group: scorbutogenic diet + 50 mg ascorbic acid per day. After 3 months, animals were sacrificed, intestines removed, flushed with cold saline and everted. Liver, spleen and adrenals were also taken out for the determination of ascorbic acid levels. There were 6-8 animals in each group.

Measurement of glucose and alanine uptake. Intestinal uptake of glucose and alanine was determined using tissue accumulation technique[8] as described earlier[9]. Briefly, everted rings (0.5 cm) from the jejunal portion of the gut were incubated in 5 ml of the Krebs Ringer buffer, pH 7.4, containing 5 mM D-glucose or 5 mM L-alanine with a trace of U-^{14}C, glucose or U-^{14}C, alanine (Atomic Research Center, Trombay, Bombay) respectively. All incubations were for 5 min at 37 °C. At the end of the incubation, tissues were removed, gently blotted, weighed and the radioactivity taken up by the tissues was determined by digesting the tissues with KOH as described by Robinson and Alvarado[10].

Enzyme assays. Sucrase and alkaline phosphatase were assayed in the jejunal part of the intestine following the methods of Dahlqvist[11] and Eichholz[12]. Ascorbic acid in the tissues was estimated as described by Roe and Kuether[13]. Protein was determined by the method of Lowry et al.[14].

Results and discussion. The results presented in table 1 indicate that feeding of the scorbutogenic diet for 3 months to guinea-pigs lead to a considerable depletion of the tissue ascorbic acid reserves. The significantly lower content of ascorbic acid in various tissues of the deficient group compared to controls is suggestive of the development of scurvy in these animals. The animals in the hypervitaminosis C group showed a marked accumulation of the vitamin C in liver, spleen and adrenals compared to control animals. As shown in table 2, in vitamin C deficiency there occurs an appreciable increase in the activities of brush border sucrase and alkaline phosphatase compared to the control group. But in animals exposed to large doses of the vitamin, the levels of these enzymes were significantly depressed. Hoehn and Kaufer[1] recently reported enhanced activities of lysosomal enzymes in the liver of scorbutic animals; thus it suggests a similarity in the mode of action of vitamin C in these 2 tissues.

Similar to the effect of ascorbic acid on the brush border enzymes, the permeability of glucose and alanine in the intestine is also enhanced in vitamin C deficiency, and it is considerably reduced in the hypervitaminosis C group (table 3). These results are in agreement to the earlier reports, where an increase in the intestinal transport of glucose and methionine was demonstrated in scorbutic guinea-pigs using everted sac technique[15,16]. Thus both the uptake and transport of these nutrients is elevated in the intestine of vitamin C deficient animals.

Table 1. Ascorbic acid content of various tissues in control, chronic scorbutic and hypervitaminosis C groups of guinea-pigs

Group	Liver	Spleen	Adrenals
	μg per g wet weight (mean ± SD)		
Control	50 ± 11	204 ± 41	376 ± 61
Chronic scorbutic	15[b] ± 3	40[b] ± 10	108[b] ± 44
Hypervitaminosis C	65[a] ± 5	434[b] ± 35	660[b] ± 53

[a] $p < 0.05$; [b] $p < 0.001$, compared to control.

Table 2. Effect of hypo- and hypervitaminosis C on the intestinal brush border sucrase and alkaline phosphatase activities

Group	Sucrase (μmoles glucose/min/g protein at 37 °C)	Alkaline phosphatase (μmoles Pi/min/g protein at 37 °C)
Control	62.00 ± 7.65	275.42 ± 12.37
Chronic scorbutic	87.22[a] ± 8.56	330.74[a] ± 30.39
Hyr ervitaminosis C	42.83[a] ± 4.59	247.32[b] ± 7.53

[a] $p < 0.001$; [b] $p < 0.01$, compared to control. Values are mean ± SD of 6-8 duplicate determinations.

Table 3. Effect of hypo- and hypervitaminosis C on the uptake of glucose and alanine by guinea-pig intestine

Group	D-glucose (μmoles taken (up/h/g tissue)	L-alanine (μmoles taken up/h/g tissue)
Control	13.68 ± 2.17	14.14 ± 1.86
Chronic scorbutic	18.87[a] ± 1.43	20.32[a] ± 2.89
Hypervitaminosis C	8.96 ± 3.49	9.70[a] ± 1.39

[a] $p < 0.01$ compared to control. Values are mean ± SD of 6 experiments each.

Hyperactivities of the thyroid and adrenal glands has been shown to occur in ascorbic acid deficiency[16]. Recently Clenano et al.[17] demonstrated that cortisone or tri-iodothyronine injections to female rats can elicit the precocious appearance of the disaccharidases in intestine. Therefore the changes observed in the digestive functions of the small intestine could possibly be related to the hormonal imbalances associated with vitamin C deficiency. There is ample kinetic evidence which emphasizes a close functional relationship between the brush border disaccharidases and the sugar transport system at the mucosal surface of the enterocytes[18,19]. The similarities observed in the alterations of brush border sucrase and glucose absorption in intestine in vitamin C deficiency and after feeding excessive doses of the vitamin, suggests a common control mechanism for these systems under the 2 nutritional statuses. The results of the present study provide indirect evidence for such a parallelism between the 2 systems.

Whether ascorbic acid is directly involved in the intestinal digestive and absorptive mechanisms, or is linked to these processes indirectly by inducing metabolic alterations, is not clear at present; nevertheless the hyperactivities of the brush border enzymes and the enhanced permeability of sugars and amino acids in intestine in scurvy seems to be a characteristic of this derangement. Excessive intake of vitamin C as an antidote for common cold and other ailments has often been advocated[20]. In view of the observed changes in the intestinal functions after feeding large doses of vitamin C, such a vitamin therapy needs a further careful exploration.

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Positive alliesthesia after insulin

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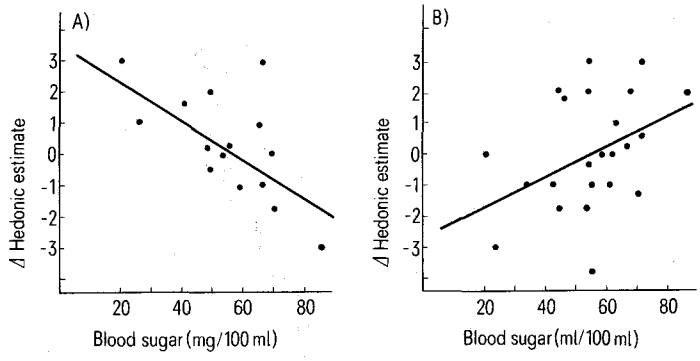
Summary. Volunteers experienced sucrose solution as more pleasant 36–48 min after insulin, than after saline control. These changes in affective estimates correlate negatively with blood sugar at 30 min and positively at 50 min after the insulin injection.

The same stimuli can be perceived as pleasant or unpleasant according to the internal state, that is, the homeostatic situation. This phenomenon has been called by Cabanac[1] alliesthesia and plays an important role in the reinforcement of the behavior and behavioral regulation of some homeostatic functions. For instance, in the case of olfactogustative stimuli, the alliesthesia parallels hunger and satiety. Since, inspite of being often investigated the role of insulin in hunger and satiety is not yet clear[2], and because insulin is a major metabolic hormone supposed to influence homeostatic energetic balance, we wanted to see if insulin produces some changes in the evaluation of the taste of sucrose.

In double-blind experiments, 85 healthy young volunteers were asked, in the morning before breakfest, to rate the pleasantness or unpleasantness which they felt about the taste of 5 sucrose solutions before and after an i.m. injection of 0.15 units/kg wt of normal insulin or isotonic saline.



Correlations between the blood sugar 30 min (A) and 50 min (B) after i.m. injection of 0.15 units/kg b.wt and changes in hedonic estimates of sucrose solutions tasted 36–48 min after the injection. Each point represents the differences between the affective estimates before and after the injection. *A* $y = -0.059\ x + 3.610$, $r = -0.595$, $p < 0.02$; *B* $y = 0.048\ x - 2.67$, $r = 0.416$, $p < 0.05$.