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EFFECT OF BARBITURATE ON THE DISTRIBUTION OF ASCORBIC ACID IN THE GASTROINTESTINAL TRACT

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Abstract—Ascorbic acid levels in the gastrointestinal tract were highest in the mucosal layer of the duodenum. Barbiturate anaesthesia accentuated a difference between mucosal and serosal layers, whereas cervical dislocation decreased this difference, mainly by increasing the serosal concentration. Prolonged barbiturate dosage also increased the serosal concentration to a greater extent than the mucosal whether the animals were anaesthetized before the experiment or killed by cervical dislocation.

In previous experiments it has been shown that the ascorbic acid content of lung is labile; much of it behaved as though it were extracellular and it appeared to function as an extracellular antioxidant [1–3]. The high concentration in the lung has suggested that it may act as a detoxicating agent. This suggestion has gained support through the reduction in toxicity offered by ascorbic acid against some forms of oxidative damage [4, 5], as well as the protective effect offered against agents credited as being carcinogenic [6, 7]. The mucosal lining of the gastrointestinal (GI) tract appeared to be a similar external site, liable to come in contact with an array of toxic material. It seemed possible that here also ascorbic acid may be required in high concentration. The present paper presents differences in the level of ascorbic acid of the mucosal and serosal layers of the small intestine. In the course of this investigation it was found that the concentration of ascorbic acid in the intestinal tissues was altered by the method of anaesthesia. Accordingly, a more detailed study was made of the effect of barbiturate treatment on the distribution of ascorbic acid.

METHODS

Animals. Male albino rats weighing 150–200 g (University of Queensland Central Animal Breeding House strain) were fed a commercial breeding ration containing no ascorbic acid, and allowed food and water *ad lib*. up to the time of the experiment. Some rats were given sodium pentabarbitone (30 mg/day) by stomach tube for 5 days before the experiment. These rats showed no changes in body weight from untreated controls.

Anaesthesia. Rats were given an intraperitoneal dose of sodium pentabarbitone (100 mg/kg) and were kept unconscious for 20–30 min before tissue was removed.

Tissue preparation. Anaesthetized rats or rats killed by cervical dislocation were bled by severing the jugular veins. The GI tissue was removed and the small intestine was divided into six equal lengths, termed segments I–VI, from the duodenum to the ileum. Segment I consisted of the duodenum and a small portion of the jejunum, segments II and III were the rest of the jejunum and segments IV, V and VI were the ileum. The GI tissue was washed with 0.9% NaCl to remove contents, blotted with filter paper and sampled. Mucosal strips were prepared by the method of Dickens and Weil-Malherbe [8] and Crane and Mandelstam [9]. A portion of each segment of intestine was opened lengthwise, washed with 0.9% NaCl, blotted, spread mucosa-side-up on a glass plate and the mucosal layer scraped off with a microscope slide. This procedure was carried out rapidly at room temperature and took no more than 1–2 min. Histological examination showed that the portion remaining, designated serosal layer, consisted mainly of muscle. The villi and underlying glands were removed almost completely as the mucosal layer.

Extraction and chemical assay. Weighed tissue samples were extracted with cold metaphosphoric acid (5% w/v) and assayed for reduced ascorbic acid using the 2 : 4 dinitrophenylhydrazine procedure of Roe and Kuether [10] with the modification described by Bolin and Book [11]. DNA was estimated by the diphenylamine method of Burton [12].

RESULTS

Localization of ascorbic acid along GI tract of anesthetized rats. The concentration of ascorbic acid in entire segments of GI tract which contained mucosal, serosal and muscle elements was higher in the oesophagus and upper half of the small intestine than in the stomach and lower half of the small intestine. There was a gradual decrease in the concentration from jejunum to terminal ileum (segments II–VI). The results were expressed in terms of DNA in order to minimize the effects of fluctuations in water content of the tissue (Table 1). When the results were expressed in terms of wet weight, the range of concentrations was considerably greater; similar differences were observed between upper and lower small intestine but the concentration in

Table 1. Concentration of ascorbic acid in the gastrointestinal tract of ten anaesthetized rats*

Tissue		μg Ascorbic acid/mg DNA*
Oesophagus		47.0 ± 4.9
Stomach		38.2 ± 1.6[n,x]
Small intestine	I	46.2 ± 3.8[a,m]
	II	50.0 ± 5.3[a,m]
	III	47.4 ± 3.8[a,y]
	IV	42.8 ± 2.6
	V	35.9 ± 3.4[n]
	VI	33.7 ± 1.9[b]

* The superscript pair (a,b) denotes a statistical difference between the means at $P < 0.01$. The superscript pairs (m,n) and (x,y) denote a statistical difference between the means at $P < 0.05$. Results are expressed as means ± S.E.

oesophagus and stomach was about half that of the duodenum (Table 2).

Distribution of ascorbic acid in the layers of the GI tract. When tissue was examined from rats suddenly killed, the ascorbic acid in the mucosal layer was found to be significantly higher than that of the serosal layer ($P < 0.01$). The concentration of ascorbic acid remained fairly constant throughout the length of the small intestine in both mucosal and serosal layers (Table 3).

The difference in ascorbic acid level between intestine taken from anaesthetized rats and that taken from killed rats is obvious; an analysis of variance shows a highly significant difference between the treatments ($P < 0.001$). For anaesthetized rats, the mucosal layer was found to have a significantly higher ascorbic acid level than the serosal layer ($P < 0.001$). This difference was greatest in the duodenal region and decreased towards the end of the ileum (normal in Table 3). Except for segment I, the level of ascorbic acid in the serosal layer remained almost constant throughout the small intestine. The mucosal layer of the duodenal segment contained the highest level of ascorbic acid.

In order to investigate the effect induced by anaesthetic in the level and distribution of ascorbic acid in the GI tract, rats were treated orally with sodium

Table 2. Concentration of ascorbic acid in the gastrointestinal tract of ten anaesthetized rats*

Tissue		μg Ascorbic acid/g tissue*
Oesophagus		170 ± 57
Stomach		131 ± 9[e]
Small intestine	I	295 ± 33[d,x]
	II	352 ± 39[a,d]
	III	311 ± 24[d,x]
	IV	248 ± 12[c]
	V	186 ± 24[b,y]
	VI	181 ± 25[b,y]

* The superscript pairs (a,b) and (d,e) denote a statistical difference between the means at $P < 0.01$. The superscript pairs (a,c) and (x,y) denote a statistical difference between the means at $P < 0.05$. Results are expressed as means ± S.E.

Table 3. Comparison between period of anaesthesia and cervical dislocation on ascorbic acid in small intestine*

	Anaesthetized (μg ascorbic acid/mg DNA)				Cervical dislocation (μg ascorbic acid/mg DNA)			
	Normal		Barbiturate treated		Normal		Barbiturate treated	
Segment	Mucosa	Serosa	Mucosa	Serosa	Mucosa†	Serosa†	Mucosa	Serosa
I	56 ± 6	32 ± 5	53 ± 3	37 ± 5	63 ± 4	46 ± 3	64 ± 3	51 ± 2
II	38 ± 2	22 ± 2	61 ± 9	40 ± 4	56 ± 4	46 ± 2	68 ± 4	57 ± 2
III	33 ± 3	22 ± 2	51 ± 7	37 ± 3	55 ± 5	51 ± 5	65 ± 3	53 ± 4
IV	32 ± 3	22 ± 4	49 ± 5	40 ± 5	56 ± 5	52 ± 6	61 ± 4	52 ± 2
V	28 ± 5	21 ± 7	40 ± 6	36 ± 2	52 ± 6	46 ± 5	57 ± 3	53 ± 4
VI	30 ± 8	23 ± 6	38 ± 5	39 ± 2	48 ± 6	45 ± 4	49 ± 3	57 ± 6

* Results are expressed as means ± S.E. Five values contribute to each mean except in columns marked † where 10 values were used.

pentabarbitone. Table 3 shows that rats treated with barbiturate before anaesthesia had a significantly greater concentration of ascorbic acid in their GI tracts than the normal anaesthetized rats ($P < 0.001$). The concentration of ascorbic acid was higher in the mucosal layer than in the serosal layer ($P < 0.001$). Except for segment I, both layers had increased ascorbic acid levels which were about 20–100 per cent higher than those of normal rats. Both mucosal and serosal layers tended to become more uniform in concentration and were similar to those found in tissues from rats killed by cervical dislocation.

Treatment with barbiturate prior to cervical dislocation produced only minor variation in the mean values for ascorbic acid in either mucosa or serosa from those of normal rats similarly killed.

DISCUSSION

Results for the distribution of ascorbic acid in the GI tract show that there is considerable variation between regions. Those with higher levels, such as duodenum, have ascorbate equivalent to that of brain, lung or liver [13]. Where concentrations are lower, as in stomach and ileum, the level of ascorbate is still higher than in skeletal muscle and non-organ tissues. When comparison is made between mucosal and serosal layers, it is apparent that the mucosa has a greater concentration of ascorbic acid than the serosa, where the levels of ascorbate are greater than those reported for other muscular tissues such as skeletal or cardiac muscle [13].

The levels of ascorbic acid throughout the GI tract were very variable, conforming with the findings of Litwornia [14], who measured ascorbate in the guinea-pig gut, and of other workers [15–17] who found fluctuation in the levels of other GI tissue components. Part of this variation may be due to the range of water content associated with the tissues. Parsons [18] refers to water, perhaps entrained with villi, which may amount to more than a third of the total tissue water. Results expressed on a cellular basis reduced, but did not overcome, the variability in results. The great range of metabolic activity in the GI tract may account for this variation in cellular components.

The high level of ascorbic acid in the mucosa of the small intestine may be a reflection of its requirements in that area. If ascorbic acid functions in the small intestine either as an extracellular antioxidant or as a detoxicating agent, in a manner similar to that in lung [3], it is possible that this role is greatest in the mucosa which is exposed to the external environment. The duodenal region, where the concentration is highest, may require additional ascorbic acid for synthesis of substances such as collagen and serotonin which takes place in greater amounts in this region due to greater cell renewal [19] and larger numbers of enterochromaffin cells [20].

The fall in the ascorbic acid levels of rats anaesthetized with barbiturate from the levels in those which had been suddenly killed may be due to several causes. Barbiturate may induce a massive urinary release of ascorbate, similar to that reported by Smythe and King [21] for a range of materials, during the period of anaesthesia. Intense sympathetic stimulation at the moment of sudden death [22] may result in decreased intestinal blood flow and frequently results in increased intestinal motility, effects which are the reverse of those in barbiturate anaesthesia where tone and motility of the GI tract are depressed [23]. As blood ascorbic acid levels are low [13], differences in diffusion of ascorbic acid out of the tissue could account for the different tissue levels.

Treatment of rats with barbiturates increases ascorbic acid production in the rat liver markedly and also increases the level in the tissues [24]. It appears that GI tissue reaches a plateau concentration of ascorbic acid and this is demonstrated by either dosage of barbiturate or sudden death. Combined treatments did not raise this plateau which may be influenced by circulatory changes during killing.

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