

Beeturia and colonic oxalic acid

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Summary

Beeturia is the excretion of red beetroot pigment (betalaine) in urine and faeces. It occurs in about 14% of humans. Betalaine is a redox indicator whose colour is protected by reducing agents. We investigated pigment-decolourizing systems in the intestinal tracts of beeturic and non-beeturic subjects. Betalaine was decolourized by hydrochloric acid, ferric ions and colonic bacteria preparations, but not by pancreatic or mucosal enzymes. In

animals, oral betalaine did not produce beeturia, but injection of betalaine into the peritoneum did. Oral betalaine and 1 g oxalic acid produced beeturia in non-beeturic normal humans, but passed into ileostomies without beeturia. Thus, beeturia results from colonic absorption of betalaine. Oxalic acid preserves the red colour to the colon, otherwise it is decolourized in non-beeturic individuals by non-enzymic processes in the stomach and colon.

Introduction

Beetroot (*Beta vulgaris*) is a popular vegetable food, eaten after cooking in boiling water or preserved in vinegar. Its red pigment, widely used in the food industry as a colourant,¹ is a betalaine consisting of betacyanines and betaxanthines. Of the betacyanines, 75–85% are betanine, and the majority of the betaxanthines are vulgaxanthine-1.^{2,3} About 0.5–1% of extracted beet juice solids are betalaines. In addition to the pigments, the root contains about 700–800 mg oxalic acid and 5–6 mg ascorbic acid per 100 g beetroot.⁴ The pigment is a pH and redox indicator. Eating beetroot can result in red urine and faeces in approximately 14% of the population, in 49% of individuals with iron-deficiency anaemia, (80% where no previous iron therapy had been given) and in 48% of individuals with pernicious anaemia or malabsorption.⁵

We investigated whether beeturia is due to a failure to decolourize the beetroot pigment, or decolourization of pigment is the norm, i.e. individuals with beeturia preserve the red colour, by endogenous or dietary factors, e.g. reducing agents: oxalic acid, ascorbic acid and uric acid within the gastrointestinal

contents. Further, we ask is the phenomenon dose-related, or dependent upon the coincidental intake of reducing agents with the betalaine, e.g. ascorbic acid or oxalic acid, or do bacterial flora have a role in the development of beeturia? Do individuals with beeturia metabolize oxalic acid or uric acid differently from non-beeturic subjects, or is the phenomenon dependent upon ingested reducing agents, e.g. oxalic acid? We also identified the site of betalaine absorption, using intact and ileostomy subjects.

Methods

Preparation and measurement of beetroot pigment

In *in vitro* hydrolysis experiments, the pigment was extracted from sliced beetroot with boiling water, and stored at 4°C in a dark bottle or frozen at –20°C. The pigment used in animal experiments was isolated by filtration on Sephadex G10 surrounded by aluminium foil to exclude light. The beetroot used for human experiments was either

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cooked or pickled in vinegar, and blended in a kitchen blender to a pulp before ingestion.

The ultraviolet spectrum (Unicam SP 800 recording spectrometer) of betalaine has a maximum absorption at 540 nm with additional peaks at 460, 480 and a minimum at 510 nm. The decline or stability of the pigment was measured at 540 nm, but the spectrum was recorded using recording UV absorption scanning spectrophotometry. In all experiments, the pigment solution was made to a concentration which gave a dark red colouration with a 90% full-scale deflection on the spectrophotometer.

***In vitro* experiments**

The stability of the pigment was followed in conditions simulating those of the gastrointestinal tract lumen, mucosal enzymes and bacteria.

The spectral changes of freshly prepared beetroot juice were studied in the dark at 37 °C at 15 min intervals over 6 h in distilled water at pH 7.0 and at 0.1 M HCl, pH 2.

Gastric juice and gastric mucus were separated by adding acetone to human gastric juice (pH 2.4, obtained by intubation from a fasting patient during a maximum acid output test) until there was complete precipitation. The precipitate was filtered and freeze-dried. The spectral changes of freshly prepared beetroot juice were studied over 3 h at 37 °C after addition to human gastric juice or 1% 0.1 M HCl solution, with or without freeze-dried human gastric mucus or human serum albumin.

Freshly prepared beetroot juice solution was left at room temperature over 24 h with or without ascorbic acid (200 mg), final volume 10 ml, and spectral changes observed.

Freshly prepared beetroot juice was placed in 0.1 M HCl (total volume 10 ml) at 37 °C for 3 h with either 0, 100, 200, 1000 and 2000 mg/l of ascorbic acid or oxalic acid. Spectral changes were monitored.

The above was repeated with either ferric chloride or ferrous sulphate (5–10 mg/10 ml), rather than oxalic/ascorbic acid.

***In vivo* rodent experiments**

Beetroot juice pigment was added to the drinking water of four 150 g male Wistar rats. One week later, beetroot pigment in sterile water (1 ml) was injected into the same animal's anterior abdominal wall. The animals were housed in wire-bottomed cages over filter paper to show the colour of the voided urine.

Four adult male rats and two adult male guinea pigs were killed by cervical dislocation. Tissues (Table 1) were homogenized and incubated using standard conditions.^{6,7} The tissues were incubated at 37 °C for 3 h, after which the protein was precipitated

with ammonium sulphate. Red pigment was measured by UV recording spectroscopy. Simultaneous control experiments used boiled tissues.

Small intestinal absorption of beetroot pigment was examined by the everted sac technique⁷ (Krebs Ringer bicarbonate solution with 5% CO₂ in oxygen bubbling through the solution). The concentration of red pigment on the serosal and mucosal side was measured by UV recording spectroscopy.

Rat caecal contents: eight Wistar rats, fed from birth on a chow diet, were killed by ether overdosage. Their pooled caecal contents (15 g) were added to 100 ml buffer (10 mmol Tris maleate) at pH 7. Fibrous particles were removed by filtration through a fine metal mesh and Whatman paper (qualitative grade 1). The filtrate was broken up using an ultrasound sonicator for 10 min, ensuring that the temperature did not rise above 37° during the process. The sample was centrifuged to remove fine debris, diluted (v/v) with buffer, separated into 10 ml aliquots and stored at –20 °C. The control was buffer with no bacterial cell preparation present. The bacterial preparation was warmed to 37 °C and beetroot pigment was added. The UV spectrum was read (540 nm) at 10 min intervals over 3 h.

The experiment was repeated with the addition of chelating agents: 0.1 g EDTA or 0.1 g or 0.2 g desferrioxamine (11 ml flask volume).

Human experiments

Preliminary experiments

A non-beeturic male collected urine looking for beeturia after eating on separate non-consecutive days: (i) an aqueous extract of pigment from 3 kg beetroot; (ii) beetroot juice from 500 g beetroot with 1 g ascorbic acid; (iii) beetroot juice from 500 g beetroot with 1 g oxalic acid—urinary oxalic acid⁸ and red beet pigment in urine and faeces were measured; (iv) 500 g beetroot pulp after a 3-day course of neomycin (500 mg qid) to reduce the colonic bacterial mass.

Group studies

We recruited 15 healthy subjects, median age 35 years (range 18–58 years) and three ileostomy patients (30–55 years) who had undergone proctocolectomy for ulcerative colitis some years previously and who were in good health. Six of the normals but none of the ileostomy patients had previously had beeturia. All were well and were receiving no medical care at the time of study, and none had current gastrointestinal disease.

We measured 24-h oxalic⁸ or uric acid⁹ excretion from 15 individuals with (9) or without (6) beeturia. No beetroot was eaten during this collection period. We then measured 8-h urinary oxalic acid and uric

acid excretion from nine individuals with (5) or without (4) beeturia after ingestion of boiled beetroot (oxalic acid content 4.6 mmol/l). On the first day, the subjects ate their usual meals, but no beetroot. This was repeated on a separate day, but 150–340 g pulped cooked beetroot was eaten after an overnight fast. Usual meals were eaten thereafter. The 8 h urine collection was analysed for beet pigment and oxalic acid⁸ and uric acid⁹ content were measured.

Five beeturic and five non-beeturic individuals ate pickled (in vinegar) beetroot pulp after fasting overnight (340 g) and urine was collected for 3 h. Urinary beetroot pigment, oxalate and urate were measured.

Subjects with an ileostomy collected ileostomy effluent and urine over 8 h before and after ingestion of beetroot. The presence of beetroot pigment and amounts of oxalic acid and uric acid were measured.

The above time periods of 8 h and 3 h were chosen to see if the beetroot pigment was excreted in urine concurrently with the reducing agents. All excreta, urine, faeces, and ileostomy effluent were collected into plastic containers covered in tin foil to avoid decolourization by light before measurement.

Results

In vitro experiments

Beetroot pigment immediately decolourizes in alkaline solutions, and regains redness at neutral or acid pH. During alkaline decolourization, the maximum peak shifts to 390 nm. The red appearance persists until 70% of UV spectral absorption is lost.

Newly prepared beetroot-juice red pigment left in the dark at 37 °C over 6 h at pH 7 in water shows no decrease in A_{540} , but in 0.1 M HCl, pH 2, it is decreased to 30–40% of the original.

There was no change in the red pigment spectrum over 6 h in a solution of 1% mucus (from human gastric juice) or 1% serum albumin in 0.1 M HCl. Gastric juice, pH 2.4, resulted in <10% decolourization over 6 h. Gastric juice protein was precipitated by boiling for several minutes. The filtrate (pH 2) totally decolourized beetroot pigment over 60 min. The addition of 9 ml 100, 200, 1000 and 2000 mg/l ascorbic acid to 0.1 M HCl (total volume 10 ml) protected against decolourization in a concentration-dependent manner.

Beetroot juice at room temperature for 18 to 20 h completely decolourizes. Resistance to such decolourization is conferred by prior addition of 9 ml 2000 mg/l ascorbic acid or oxalic acid (total volume 10 ml).

Fresh beetroot pigment oxidised by 2 drops per 100 volume of hydrogen peroxide in 100 ml of

beetroot pigment extract is immediately decolourized when 1 ml is added to 10 ml 0.1 M HCl. This decolourization is prevented by 100 mg oxalic acid in 10 ml.

Beetroot pigment (10 ml) decolourizes immediately when 5–10 mg ferric chloride, but not ferrous sulphate, is added.

In vivo rodent experiments

Rats given beetroot juice pigment in their drinking water excreted yellow-coloured urine with no evidence of red beet pigment. Following pigment injection into the anterior abdominal wall, beeturia was observed within 2–3 h.

None of the rodent tissues or gastrointestinal fluids tested decolourized beetroot pigment. The mucosal tissue showed activity for enteric enzymes, e.g. sucrose (Table 1).

In everted rat jejunal sac experiments, <10% of the pigment passed from the mucosal to the serosal side over 3 h.

Rat caecal extract incubated for 18 h at 37 °C with red beetroot pigment resulted in loss of red pigment to 40% of original. This property persisted after boiling the inoculum at 100 °C. Oxalic acid at concentrations ranging from 3–8 mmol/l in the incubation system resulted in 90% of red pigment being retained.

The incubation experiment using the caecal bacterial extract was repeated with added 0.1 g of EDTA or 0.1 g or 0.1 g desferrioxamine in 11 ml in the flask. The untreated samples reduced in colour by 55% over 18 h. In the presence of EDTA, the red colour reduction was 74% over 18 h. In the presence of desferrioxamine (0.1 g and 0.2 g in 11 ml) the redness decreased by 28% and 20%, respectively, from the initial value.

Human experiments

None of the human tissues or gastrointestinal fluids (Table 1) decolourized the beetroot pigment.

Preliminary experiments

An individual with no previous history of beeturia had neither beeturia or red faeces following ingestion on separate days of (i) an aqueous concentrate of pigment (from 3 kg beetroot), or (ii) beetroot juice from 500 g beetroot plus 1 g ascorbic acid.

Red faeces (without beeturia) were excreted within 12 h of the ingestion of beetroot juice (500 g beetroot) after 3 days treatment with neomycin (500 mg qid) to reduce the colonic bacterial mass.

Both beeturia and red faeces were recorded following beetroot juice (500 g beetroot) plus 1 g oxalic acid.

Table 1 Rat and human secretions and tissues used

	Buffer	pH	Notes
Human saliva	Na citrate	4.8 6.8	
Rat gastric mucosa	Phosphate	7	
Rat small intestinal Muscle tissue	Phosphate	7	
Rat colon mucosa	Phosphate	7	
Rat jejunal brush border	Tris Malate Phosphate	7–9 6 7–9	} Shown to have sucrase activity
Guinea pig jejunal brush border	Tris	7–9	
Human jejunal brush border	Sodium citrate phosphate buffer	3–8.1	
Duodenal juice	Phosphate Phosphate	7 9	
Pancreatic lipase	Phosphate	7	Sigma

Group studies

Urinary 24 h oxalate and uric acid excretion were measured on two separate days in nine individuals (history of beeturia) and six individuals (no history of beeturia). Subjects ate their habitual diet, without beetroot during this period. The median and range total urinary oxalate in the beeturic group was 0.33 (0.29–0.44) mmol/24 h and in the non-beeturic group was 0.27 (0.2–0.4) mmol/24 h (no significant difference). The mean and range concentrations (24 h urine) of oxalic acid were 0.33 (0.14–0.61) mmol/l in the beeturic group and 0.15 (0.11–0.27) mmol/l in the non-beeturic group. This difference is significant

($p < 0.05$; Wilcoxon Rank sum test) and persisted when corrected for body surface area.¹² Total urate and urate concentration (24 h), were similar in the two groups. (Table 3).

Urinary oxalic and uric acid (8 h) were measured in nine individuals (5 beeturic) after ingesting 150 g pulped cooked beetroot or 340 g beetroot pickled in vinegar following an overnight fast. There was no difference between the 8 h urinary oxalate and urate excretion (total or concentration) in the non-beeturic and beeturic groups before and after beetroot challenge. All of those with a history of beeturia had beeturia after the ingestion of the beetroot. (Tables 2 and 3).

Table 2 Total oxalic acid (mmol) in urine and ileostomy effluent

	Beeturic subjects	Non-beeturic subjects	Ileostomy patients
3 h urine, no beetroot	0.05 (5) (0.04–0.058)	0.05 (5) (0.02–0.09)	
3 h urine after 340 g beetroot (P)	0.07 (5) (0.05–0.08)	0.06 (5) (0.03–0.10)	
8 h urine, no beetroot	0.11 (5) (0.06–0.36)	0.15 (4) (0.12–0.017)	0.05 (3) (0.04–0.07)
8 h urine after 150 g beetroot (B)	0.13 (5) (0.03–0.3)	0.20 (4) (0.10–0.24)	0.10 (3) (0.08–0.016)
8 h ileostomy effluent, no beetroot			0.4 (3) (0.1–0.7)
8 h ileostomy effluent, 150 g beetroot (B)			1.2 (3) (1.2–1.95)

B, boiled beetroot; P, pickled beetroot.
Data are medians (no. of subjects) with (range).

Table 3 Total uric acid excretion (mmol) in urine and ileostomy effluent

	Beeturic subjects	Non-beeturic subjects	Ileostomy patients
3 h urine, no beetroot	0.1 (5) (0.01–0.2)	0.2 (5) (0.1–0.5)	
3 h urine after 340 g beetroot (P)	0.2 (5) (0.1–0.6)	0.4 (5) (0.2–0.6)	
8 h urine, no beetroot	0.6 (5) (0.2–0.8)	0.65 (4) (0.4–1.9)	0.2 (3) (0.2–0.4)
8 h urine after 150 g beetroot (B)	0.6 (5) (0.4–0.9)	0.75 (4) (0.7–1.8)	0.3 (3) (0.2–6.3)

B, boiled beetroot; P, pickled beetroot.
Data are medians (n) with (range).

Ten individuals (5 beeturic) ate pickled beetroot pulp (340 g) after fasting overnight, and urine was collected for 3 h. Urinary beetroot pigment, oxalate and urate were measured. The urine of the beeturic group was uniformly red. Urine from two of the non-beeturic group was light pink. However, urinary oxalate and urate (total or concentration) were unchanged in both these groups (Tables 2 and 3). In all instances where the subject had beeturia, there was also a reddening of the faeces.

The three ileostomy subjects ate: (i) an oral dose of 250 g pulped cooked beetroot, when in all three the ileostomy effluent was bright red with no accompanying beeturia; (ii) 150 g boiled beetroot. Here 8 h urinary oxalate and uric acid were unchanged following beetroot ingestion (Tables 2 and 3). Oxalate output through the ileostomy over 8 h was: pre-beetroot, median 0.4 mmol (range 0.1–0.7) and post-beetroot median 1.2 mmol (1.2–1.95). In each instance, the post-beetroot ileostomy effluent was bright red.

Discussion

Little is known of the metabolic fate of the natural chemicals present in vegetables. Similarly, little is known of the factors which influence metabolism and absorption from the colon. The pigments of beetroot are widely available, both as the vegetable and as a dye in the food industry.^{1–3} In some individuals, the pigment passes intact along the gastrointestinal tract. A proportion is absorbed and excreted in the urine, the remainder passes out in the stool. This phenomenon is known as beeturia.⁵ There is a variable frequency in the occurrence of beeturia in some individuals, others consistently experience it after eating beetroot. Occasionally non-beeturic subjects show pink urine after ingesting beetroot. This study was suggested by a colleague who had beeturia for the first and only time after ingesting beetroot with oysters, which are rich in oxalic acid.

Zindler and Colovos¹² suggested that beeturia was secondary to food allergy. Allison and McWhirter¹³ thought that beeturia had a genetic origin. They tested 104 subjects for beeturia, ten of whom were positive. A second small study demonstrated beeturia in each of four couples, whose children were beeturic. They proposed that the characteristic was controlled by one single autosomal gene, with a frequency of 43% of the population being heterozygous for the gene. They quote R.B. Fisher in unpublished experiments as showing that the pigment is readily absorbed by a rat intestinal preparation. This was not our finding using a different preparation to study absorption. Allison and McWhirter suggested

that the phenomena of beeturia and excretion of methanethiol from asparagus fell within the definition of polymorphism. This was, however, based on a very small sample of four couples and six offspring.

The variable occurrence of beeturia suggests an underlying mechanism related to the availability of a protective factor. An alternative would be a decolourizing system which is variable in activity, e.g. an inducible enzyme. In the absence of an identifiable human or animal enzyme system, it is difficult to envisage such a system.

Watson and colleagues⁵ suggested that there was an intestinal block to beetroot pigment absorption. They showed that 80% of individuals with iron-deficiency anaemia had beeturia. This fell to 48.6% after treatment. Of patients with pernicious anaemia or non-specified intestinal malabsorption, 48% had beeturia. This association with malabsorption may well involve a non-absorbed substance with protective properties, e.g. oxalate. Iron deficiency may affect the decolourizing system in the colon. However, this is also difficult to envisage, as decreased iron stores in the host are unlikely to affect bacterial iron status.

Betalaine (betacyanine and betaxanthine) is both a redox and a pH indicator. The unstable red pigments are readily decolourized to a brown pigment by light, heat, alkali and exposure to hydrochloric acid. The beet contains betacyanine and betaxanthine decolourizing enzymes, which are anatomically separate from the epidermal portion of the beet where the pigment is stored.¹⁰ The red colour is stabilized by reducing agents, including oxalic, ascorbic and uric acids. There is a substantial amount of oxalic acid and ascorbic acid in beetroot.⁴ The red form of betalaine could be maintained by oxalic acid and ascorbic acid.

The experiment with an individual, whilst lacking statistical strength, suggests that beeturia is not a dose-related phenomenon. There was no beeturia after ingestion of 3 kg beetroot. Nor does the ingestion of 1 g ascorbic acid with the beetroot induce beeturia. Presumably the ascorbic acid is absorbed from the jejunum, and does not pass to the colon.

The acid conditions of the stomach might be expected to decolourize the red pigment. Presumably the red beetroot pigment passes through the gastric juice stabilized by oxalic and ascorbic acid and gastric mucoproteins. It is not impossible that this protective effect of mucoprotein may persist along the gastrointestinal tract.

There appear to be no enteric mucosal or secreted decolourizing enzyme systems. There is modest (10%) small intestinal mucosal transfer of the beet pigment, as shown in the rat experiments. There is, however, urinary excretion of the red pigment when injected into the anterior abdominal wall, thereby

bypassing enteric decolourizing systems. Similar results have been obtained in the rat and in man.⁵ Anthocyanin from concord grapes is excreted in the urine of rat and dog when given intravenously but not enterally.¹¹

The human ileostomy experiments show that both red beetroot pigments and oxalic acid may pass through the small intestine to the colon as demonstrated by the ileostomy experiments. There was no relationship between the amount or concentration of the endogenous urinary reducing agent uric acid and beeturia. Whether or not an individual has beeturia or not appears to be determined in the colon.

Boiled beetroot does not predictably lead to beeturia, whereas pickled beetroot does. The acid vinegar may alter the physical state of oxalic acid. After the ingestion of ¹⁴C-labelled oxalate added to oxalate-rich meals containing spinach or rhubarb, as either calcium and sodium oxalate, urinary oxalate excretion increased over 1 to 8 h following small intestinal absorption. Yet over 95% of the ¹⁴C was excreted in faeces.¹⁴ Earlier work¹⁵ using chemical assays suggested that calcium salts of oxalic acid are absorbed less readily than the free acid. There is variability in the absorption of oxalate in various conditions,^{16,17} for example, following jejunioileal bypass, where the urinary excretion of ¹⁴C extends over 38 h. Where there is reduced absorption of oxalate from the small intestine, as in malabsorption, more oxalate would pass to the colon to protect the pigment.

The *in vitro* experiments indicate that the decolourizing systems are bacterial. The decolourization by ferric but not ferrous iron and the inhibition of decolourization by the bacterial preparation by desferrioxamine suggests that ferric iron is involved. The pigment is decolourized by some form of iron, presumably ferric and of bacterial origin. The decolourized pigment may then be absorbed or excreted in the urine or faeces unnoticed because of the indistinct colouration. Another variable distinguishing beeturic from non-beeturic subjects may be the quantity or enzymic properties of the colonic bacteria. This is difficult to prove or disprove. There are some 500 species of bacteria in the colon whose enzymes can be induced. It is possible that beeturia depends upon the presence or absence of a bacterial decolourizing system. Our results suggest a decolourizing system which is inhibited in subjects with beeturia.

The relationship between beetroot pigment and oxalate is probably not unique. The phenomenon of coincidental reducing agents stabilizing unstable plant pigments is also seen in the faeces of birds in the autumn after eating coloured berries. The berry pigment is excreted unchanged through the cloaca, presumably stabilized by the avian urinary nitrogen end-product uric acid. There may well be other, as

yet unsuspected, interactions between dietary and endogenous substances. These may not be so obvious as the excretion of a pigment, but may be of nutritional and metabolic importance.

The distinction between a non-beeturic individual and a beeturic individual appears to rest with oxalic acid in the colon. This protects the red beetroot pigment before absorption and subsequent urinary excretion. This concentration will depend on the residue of the enteric oxalic acid not absorbed from the small intestine. Individual variation in extent of absorption may determine whether red beetroot pigment reaches the colon. Another variable is the colonic concentration of oxalic acid and colonic bacterial metabolism. Urinary oxalic acid content probably has no influence on beeturic status, and little relationship with the colonic concentration. In individuals with malabsorption with beeturia, the small intestinal absorption of oxalic acid may be such that sufficient oxalic acid passes to the colon to induce beeturia.

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