

Original article

Beeturia and the biological fate of beetroot pigments

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Received 11 June 1993 and accepted 11 August 1993

Beeturia, the passage of pink or red urine after the ingestion of beetroot, is said to occur in 10–14% of the population, and is more common in iron deficiency and malabsorption. A specific HPLC assay for betacyanins, the red beetroot pigments, in biological fluids was developed to study the prevalence of this apparent polymorphism in humans, and to investigate its basis in rats. Two major peaks were observed in chromatograms of extracts of unpickled beetroot. They had identical UV absorption spectra ($\lambda_{\text{max}} = 535 \text{ nm}$) by diode array analysis, and mass spectrometry indicated that one (betacyanin 1) was betanin or its epimer and the other (betacyanin 2) a dissacharide of betacyanin 1. In a population of 100 normal subjects the 0–8 h urinary recoveries after an oral dose of 60 mg beetroot extract were 0.06–0.54% for betacyanin 1 and 0.01–0.6% for betacyanin 2. The distributions of these data were skewed but not clearly bimodal by visual inspection or by kernel density analysis. Four subjects produced visibly red urine and had betacyanin recoveries at the upper end of the population range. Studies using *in situ* isolated perfused rat jejunum and liver preparations indicated a negligible absorption of the pigments after 1 h and no detectable metabolism or biliary secretion. Intact anaesthetized rats given i.v. bolus doses of beetroot extract cleared both betacyanins from plasma at the rate of $3.3 \pm 0.9 \text{ (SD) ml min}^{-1}$ ($n = 5$). The total urinary recovery of both pigments amounted to 80% of the dose, and their renal clearances approached their plasma clearances. These data suggest that beeturia does not arise from deficiencies in hepatic metabolism or renal excretion of betacyanins. After oral administration of beetroot extract to rats the betacyanin content of the stomach decreased rapidly with time but neither the intestines nor the bile duct were stained visibly red. These findings together with those showing instability of the betacyanins in acid conditions suggest that variability in the biological fate of beetroot pigments may be determined largely by gastric pH and emptying rate.

Introduction

Beeturia, the passage of pink or red urine after the ingestion of beetroot, is said to affect 10–14% of the population (Allison & McWhirter, 1956; Watson *et al.*, 1963) and is reported to be more common in iron deficiency and malabsorption (Watson *et al.*, 1963). However, the association with iron deficiency has been disputed (Forai *et al.*, 1971). Beeturia has also been linked to certain food allergies (Zindler and Colovos, 1950). Beeturia has been misdiagnosed as haematuria and is an occasional cause of unnecessary admission to hospital, especially in children.

Presented at the Joint Meeting of the British Pharmacological Society and Association Française des Pharmacologistes, Lyons, April 1991 and published as an abstract in *Fundamental and Clinical Pharmacology*, 5: 467, 1991.

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The colouration of urine caused by eating beetroot is due to the excretion of the red pigments known collectively as betacyanins (Harmer, 1980). Betanin (Fig. 1) and isobetanin, its epimer at the C15 position, are thought to constitute more than 95% of the red pigments in beetroot. These compounds are 5-O- β -glucosides of betanidin, the aglycone common to all betacyanins. Beetroot juice also contains indicaxanthin, a yellow pigment of the betaxanthin alkaloid family.

Population and family studies performed 30 years ago led to the suggestion that the ability to excrete beetroot pigments is under monogenic control in healthy subjects and that beeturia is inherited as an autosomal recessive trait (Allison & McWhirter, 1956; Watson *et al.*, 1963). However, the assessment of whether or not beetroot pigments were present in urine was subjective, relying on

visual examination of the samples by either the subjects or the investigators. The present work extends these early findings by studying the pattern of urinary excretion of beetroot pigments in a population using a specific HPLC assay. Relatively little information is available on the biological fate of the betacyanins and an additional aim of our studies was to investigate further the mechanistic basis of beeturia by a detailed study of the disposition of the betacyanins in the rat.

Materials and methods

COMPOUNDS AND REAGENTS

Beetred powder (containing betacyanin, 0.375% w/w) and Beetred liquid (containing betacyanin, 0.4% w/v) were supplied by Biocon (UK) Ltd (Kidderminster, England) and betanin powder was from Aapin Chemicals (Oxford, England).

HPLC INSTRUMENTATION

Unless otherwise stated HPLC analysis was performed at room temperature (21°C) using a radial compression column containing a C₁₈ reversed phase packing (Novapak 4 µm particle size, Millipore Ltd, Watford, UK). Detection was by UV (model LC 55 variable wavelength spectrophotometric detector, Perkin Elmer, Beaconsfield, England) at 535 nm, the wavelength of maximum absorption of the betacyanins in the visible region (Nilsson, 1970).

SEPARATION AND CHARACTERIZATION OF THE BETACYANINS

Mass spectrometric analysis

The components of beetred solution (1.3 mg ml⁻¹) were separated by semi-preparative HPLC prior to mass spectrometric analysis. The mobile phase was methanol:ammonium acetate solution (0.1 M) (13:87) at a flow rate of 2 ml min⁻¹. Mass spectra of purified components were obtained by injecting samples directly onto the heated nebulizer of a Sciex API III mass spectrometer. The nebulizer temperature was 500°C. The nebulizer gas was nitrogen at a pressure of 80 psi and a flow rate of 2 l min⁻¹. The mass spectrometer was operated in the positive ion mode (APCI atmospheric pressure chemical ionization). Scans were carried out over the mass range 150–800 amu (2 s per scan). Daughter ion spectra were also obtained using argon as the collision gas at a thickness of 350 × 10¹² molecules per cm².

UV Diode array analysis

Beetred solution and urine from subjects who had eaten beetroot were analysed by HPLC with diode

array detection. The mobile phase was methanol:dihydrogenphosphate solution (0.05 M) (15:85) adjusted to apparent pH 2.75 with orthophosphoric acid. The detector was a Hewlett Packard model 1040A photodiode array UV detector set to monitor continuously over the range 400–600 nm.

STABILITY OF THE BETACYANINS IN ACID CONDITIONS

The effect of acidic conditions on the stability of beetred pigments was studied by incubating beetred powder solution (0.5 ml containing 3.7 µg betacyanin 1 and 3.1 µg betacyanin 2 per ml) at 37°C after adjustment of the pH to 2.0 or 4.7 with concentrated hydrochloric acid and distilled water. An aliquot (200 µl) was injected immediately onto the HPLC.

ASSAY METHODS

Spectrophotometry

Sample (2.5 ml) was vortex mixed (1 min) with phosphate buffer (2.5 ml, 0.1 M, pH 5.5) and transferred to

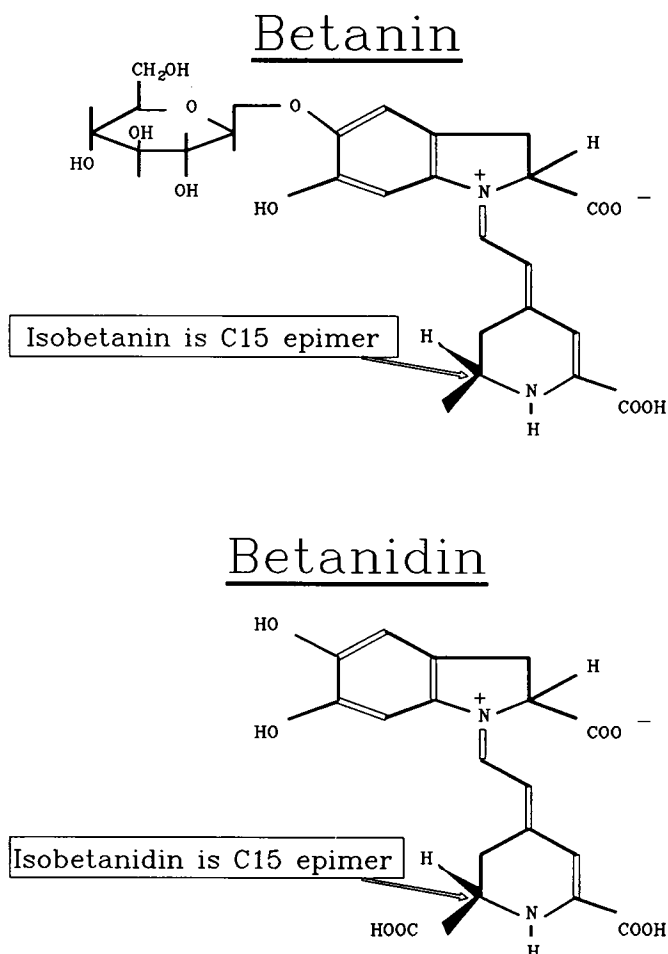


Fig. 1. Structures of betanin, isobetanimin, betanidin and isobetaniminidin.

a quartz cuvette (pathlength 1 cm). Absorbance was measured at 536 nm using a model SP-500 series 2 spectrophotometer (Pye Unicam, Cambridge, England). Samples were read against blanks of buffered urine containing no betacyanins.

HPLC: The mobile phase was methanol:ammonium acetate solution (0.1 M) (13:87 v/v) at a flow rate of 2 ml min⁻¹. Because pure samples of betacyanins were not available beetred powder was assayed for these compounds using a UV absorptivity value of 1120 dl g⁻¹ cm⁻¹ for betanin (Nilsson, 1970). Calibration graphs were constructed from standards of beetred powder containing known amounts of betacyanins.

HUMAN STUDIES

One hundred unrelated university students and staff of both sexes (age range 18–40 years) took part in the studies. All subjects were considered to be healthy on the basis of a medical history. The subjects were requested not to eat solid food for 12 h prior to the study. After emptying their bladders four subjects ingested a quantity of liquidized beetroot (prepared by homogenizing unpickled beets and filtering through a cotton cloth) containing 20, 40 or 60 mg betacyanin. Each dose was administered to each subject on separate occasions. All urine was collected in a light protected container for the following 6 h and assayed by HPLC. In a second study 100 subjects were given 200 ml of a solution containing 15 ml of beetred liquid (60 mg betacyanin), 50 ml of apple and blackcurrant juice and 135 ml of drinking water. For convenience the population study was performed overnight and all urine was collected for 8 h following dosing. After measurement of urine volumes, aliquots were stored at -20°C until analysis by spectrophotometry and by HPLC.

ANIMAL STUDIES

Whole rat

Four male Wistar rats (200–250 g) were anaesthetized with an intraperitoneal injection of sodium thiopentone (125 mg kg⁻¹). The trachea, carotid artery, jugular vein and bladder were cannulated with polyethylene catheters. To maintain an adequate urine output saline (0.9% w/v) was infused (0.1 ml min⁻¹) into the jugular vein. Beetred powder (67 mg kg⁻¹) was dissolved in saline and injected into the jugular vein. Serial blood samples (0.3 ml) were taken from the carotid artery and each sample was replaced by the same volume of heparinized saline. Plasma (0.1 ml) was mixed (15 s) with distilled water (0.1 ml) and perchloric acid (6% w/v, 0.2 ml) and

centrifuged (390 g, 30 s) in a microcentrifuge. An aliquot (0.2 ml) of the supernatant was taken for HPLC analysis. A 4 h urine collection was made and diluted to 10 ml with water prior to analysis by HPLC.

Six rats were gavaged with beetred powder solution (1 g dissolved in 5 ml distilled water) and killed by cervical dislocation at 1, 2, or 6 h after dosing. Blood was collected from the atrium and plasma samples were assayed by HPLC as described above. The stomach contents were also assayed after dilution to 25 ml with distilled water.

In a separate series of experiments six rats were dosed orally as described above and urine was collected for 6 h. After measurement of the volume, the urine sample was centrifuged (390 g, 1 min) and an aliquot (200 µl) was injected onto the HPLC.

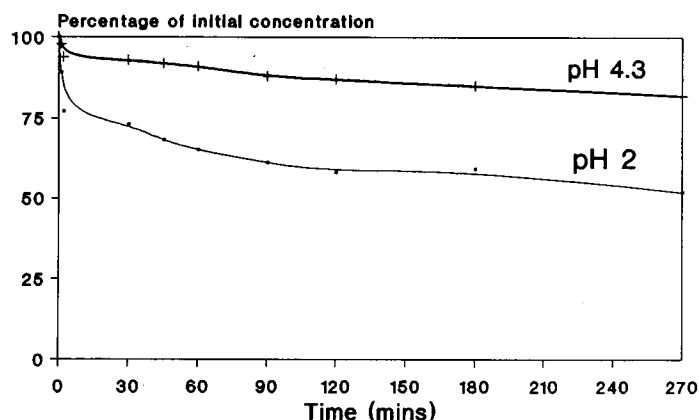
Isolated perfused rat jejunum

The *in situ* preparation of Castle et al. (1985) was used to study the absorption of the betacyanins and the dose-dependency of this process. The intestinal vasculature of four rats was perfused in a recirculating mode with semisynthetic medium comprising physiological saline, dialysed bovine serum albumin (3% w/v), washed bovine erythrocytes (haematocrit 39%) and D-glucose (4.45 mM) at a pH of 7.4 and a flow rate of 1.5 ml min⁻¹. The lumen was perfused in a single pass configuration with physiological saline containing D-glucose (3.3 mM) at a pH of 5.8–6.8 and a flow rate of 1.5 ml min⁻¹. Beetred powder was either added to the intestinal lumen (dose = 27 mg, *n* = 4 rats) or to the vascular perfusate reservoir (dose = 40 mg, *n* = 4 rats). Lumen samples taken at 5, 10, 15, 20, 30, 45 and 60 min after dosing, were centrifuged (390 g, 2 min) and an aliquot (150 µl) was injected onto the HPLC. Samples of vascular perfusate taken at 5, 10, 15, 20, 30, 45 and 60 min after dosing, were centrifuged (390 g, 5 min) and a sample (0.5 ml) of the resulting supernatant was deproteinized with perchloric acid (0.2 ml, 6% w/v). During the development of this method it was noted that degradation of both betacyanin 1 and 2 occurred after addition of perchloric acid (41–47% loss after 100 min at room temperature; 33% after storage at 4°C overnight). Therefore, to minimize decomposition aliquots (200 µl) were injected onto the HPLC exactly 2 min after addition of perchloric acid.

Isolated perfused rat liver

Three male Wistar rat (250 g) livers were perfused with a recirculating medium as described by Lennard et al. (1983). Three experiments were also performed with the liver excluded from the circuit. Perfusate

Stability of Betacyanin 1 In acid conditions



Stability of Betacyanin 2 In acid conditions

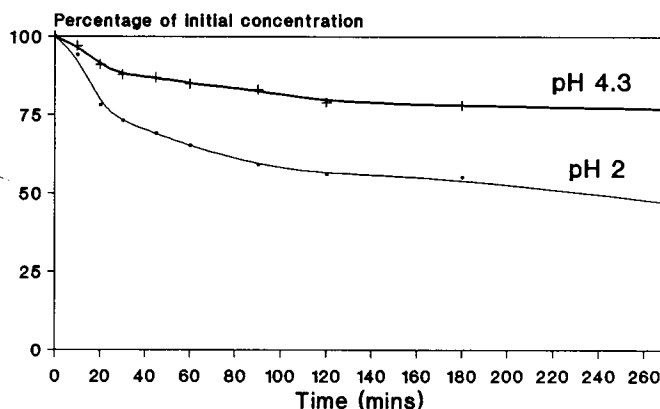


Fig. 2. The affect of acidic conditions on the stability of the betacyanins. The initial concentration of betacyanin 1 was $3.7 \mu\text{g ml}^{-1}$ and that of betacyanin 2 was $3.1 \mu\text{g ml}^{-1}$.

samples were assayed for the betacyanins as described for jejunum perfusate.

DATA ANALYSIS

Human population study

The frequency distributions of the urine recovery of betacyanins were tested for the presence of multimodality by the kernel density method described by Silverman (1981) and Jackson *et al.* (1989).

Intact rat study

The area under the plasma betacyanin concentration-time curve (AUC) was estimated using the linear trapezoidal rule with extrapolation to infinity (concentration at 240 min per elimination rate constant). The elimination rate constant (k) was calculated by log-linear regression of the terminal concentrations. Total clearance (CL) was calculated from dose/AUC and renal clearance from feCL , where fe is the fraction of the dose recovered unchanged in the urine. The terminal elimination half life was calculated from $0.693/k$.

Results

Characterization, analysis and stability of beetred pigments

Two compounds absorbing at 536 nm with retention times of 4.2 (betacyanin 1) and 6.0 min (betacyanin 2) were identified from the analysis of beetred solution by HPLC. The complete loss of both compounds following hydrolysis with β -glucosidase or hydrochloric acid (data not shown) indicated the presence of one or more glucoside groups in the

molecules. Furthermore, incubation with β -glucosidase or hydrochloric acid caused the beetred solution to turn from red to yellow indicating a change in the chromophore.

On mass spectrometric analysis betacyanin 1 gave m/z ions of 419, 257 and 161. Betanin and isobetanin (M.Wt. 550) undergo thermal degradation resulting in loss of their three carboxylic acid groups to give a species of molecular weight 418, which is consistent with a protonated molecular ion ($M+1$) of 419. Cleavage of the glycoside bonds would result in fragments of molecular weight 257 and 161. These data are compatible with betacyanin 1 being betanin or isobetanin or a mixture of the two. The absence of an ion at m/z 419 for betacyanin 2 indicates that it is not a stereoisomer of betanin. The presence of ions at m/z 582 and 604 is consistent with MH^+ and MNa^+ ions of molecular weight 581, suggesting that betacyanin 2 might be the diglucoside of betanadin, which has a molecular weight of 580. Diode array analysis of betacyanins 1 and 2 gave similar UV spectra (λ_{max} 540 nm). Analysis of urine from subjects who had eaten beetroot gave two peaks with the same retention times and spectra as betacyanins 1 and 2.

Calibration graphs for the HPLC assay of betacyanins 1 and 2 were linear over the ranges $0.05\text{--}2.0 \mu\text{g ml}^{-1}$ (urine) and $0.1\text{--}5.0 \mu\text{g ml}^{-1}$ (plasma and perfusate) ($r^2 > 0.99$), and passed through the origin. Coefficients of variation for the standards at the lower end of the range were 5% or less.

It was observed that urine samples from beeturic subjects following ingestion of beetroot became progressively pinker on exposure to sunlight. On HPLC

analysis of freshly collected urine exposed to long wavelength (366 nm) UV light for 5 min, a third peak was detected (betacyanin 3). The concentration of betacyanins 1 and 2 were unaffected by UV light exposure. Betacyanin 3, the appearance of which was not influenced by the redox state of the samples (data not shown), was not present in control urine from subjects who had not eaten beetroot nor in control urine to which beetroot extract had been added.

At pH 2.0 the concentration of both betacyanins 1 and 2 decreased markedly with time, whereas at pH

4.7 the pigments were much less susceptible to decomposition (Fig. 2).

Human Studies

The time-course and dose-dependency of the urinary excretion of beetroot pigments was studied in four subjects. Betacyanins were detected spectrophotometrically in urine within 30 min of eating solid beetroot. There was considerable interindividual variation in the rates of elimination of betacyanins, but in all subjects excretion peaked between 1 and 4 h and the

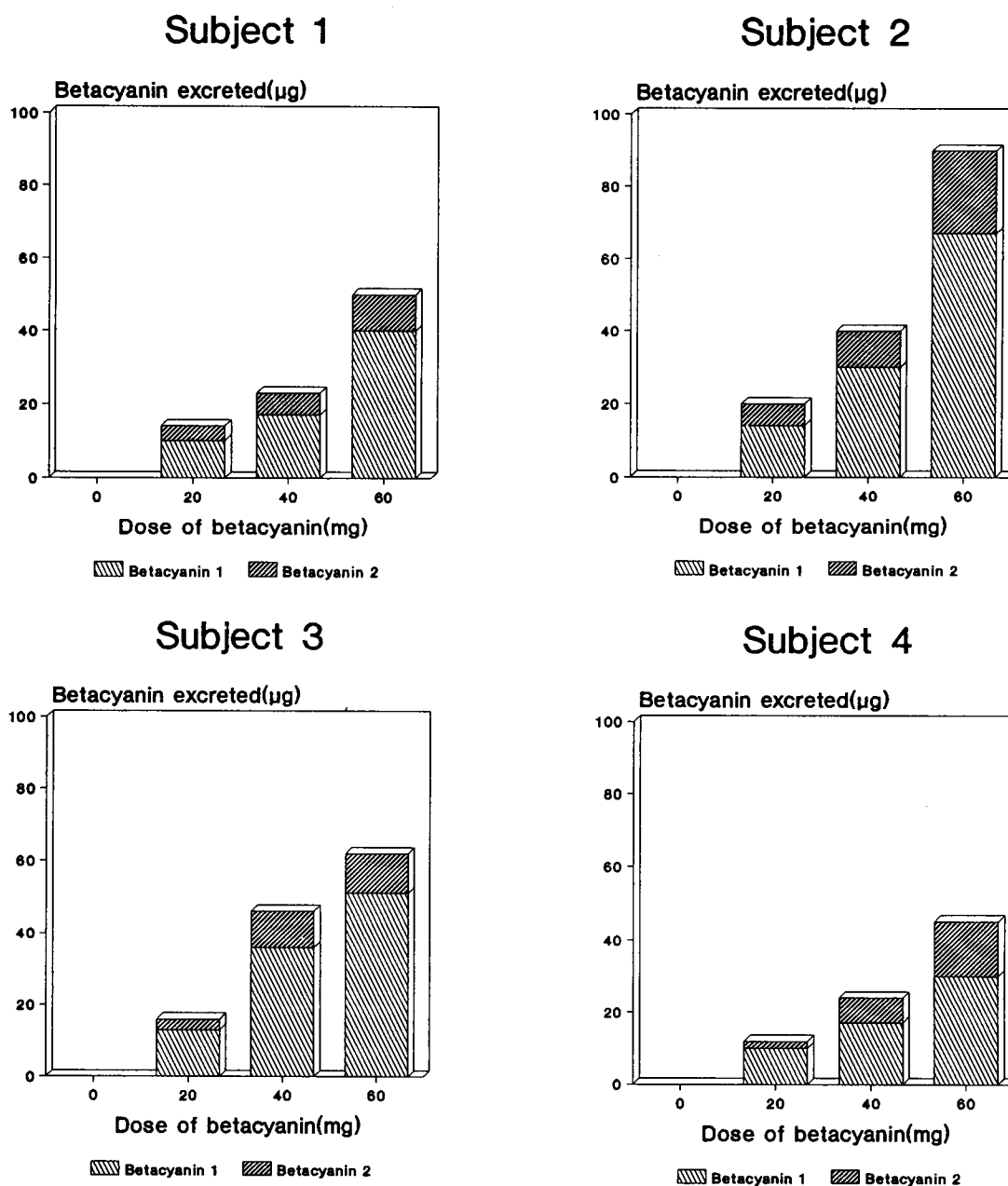
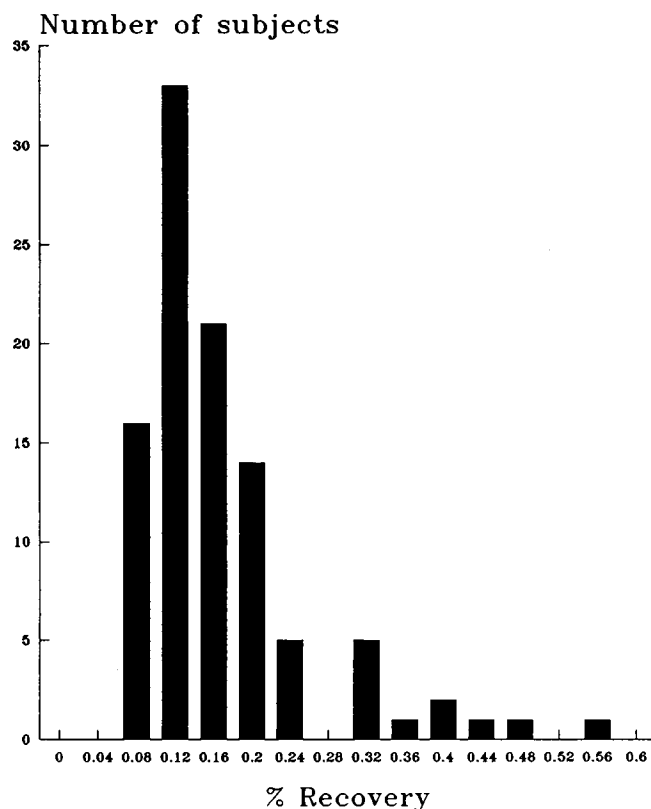


Fig. 3. The effect of dose on the 0–6 h urine recovery of betacyanins in four subjects. The pigments were administered in the form of filtered liquidized beetroot.

BETACYANIN 1



BETACYANIN 2

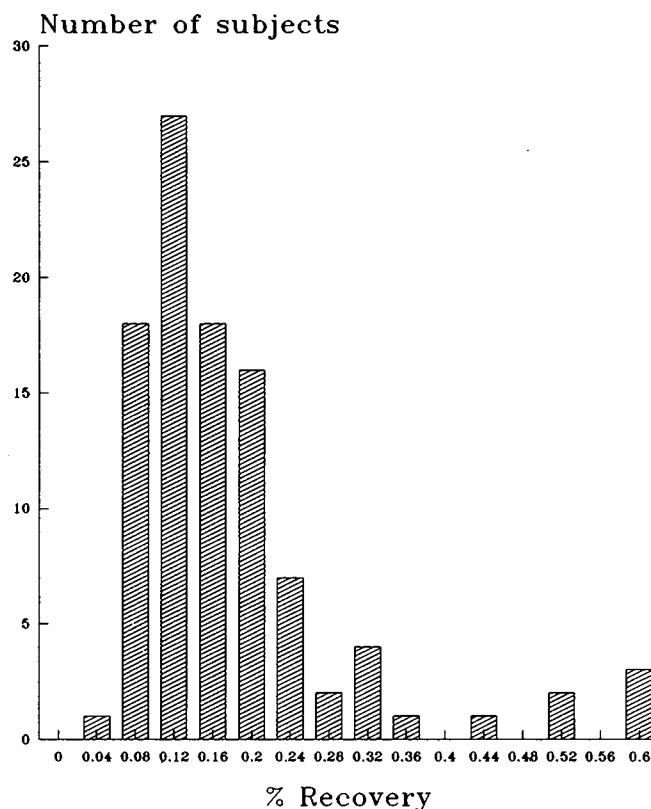


Fig. 4. Frequency distributions of the 0–8 h urine recoveries (% of dose) of betacyanins in 100 subjects after consumption of 15 ml beetred liquid containing 60 mg betacyanin.

pigments were undetectable after 6 h (data not shown). Betanin (betacyanin 1) was the major component (65–80%) of the two pigments detected by HPLC. After consumption of liquidized beetroot the excretion of betacyanins was clearly dose-dependent (Fig. 3). The lowest dose used (20 mg betacyanin) approximated to that contained in a standard portion of beetroot eaten during a meal.

Visual inspection of the urine samples obtained from a population of 100 subjects 6 h after oral administration of beetred liquid showed a range of colour from that of normal urine to a distinct red tinge observed in four subjects. The urinary recovery of betacyanin 1 ranged from 0.06–0.54% of the betacyanin dose and that of betacyanin 2 from 0.01–0.6% of the dose. The frequency distributions of the percentage recoveries of both betacyanins 1 and 2 were skewed (Fig. 4). Bimodality in either distribution was not clearly apparent from visual inspection and the distributions were found to be unimodal based on kernel density analysis. The four subjects who had visibly red

urine also had the highest urinary excretion of betacyanins.

Animal Studies

Following intravenous administration of a solution of beetred powder (67 mg kg^{-1}) to intact rats plasma betacyanin concentrations fell rapidly and biexponentially (Fig. 5). Mean ($\pm$ SD) ($n = 5$ rats) values of the pharmacokinetic parameters were: terminal elimination half-life $18(\pm 3)$ min (betacyanin 1), $18(\pm 3)$ min (betacyanin 2); plasma clearance $3(\pm 1) \text{ ml min}^{-1}$ (betacyanin 1), $3(\pm 1) \text{ ml min}^{-1}$ (betacyanin 2); renal clearance $3 \pm 1 \text{ ml min}^{-1}$ (betacyanin 1), $2 \pm 0.5 \text{ ml min}^{-1}$ (betacyanin 2).

Following the oral administration of beetred powder solution the betacyanin content of the stomach decreased with time and the pigments were virtually undetectable at 6 h (Fig. 6). However, it was striking that neither the intestines nor the bile duct were stained visibly. Furthermore, betacyanins were not detectable in blood samples taken at 1, 3 and 6 h after dosing, although small quantities of both betacyanin 1

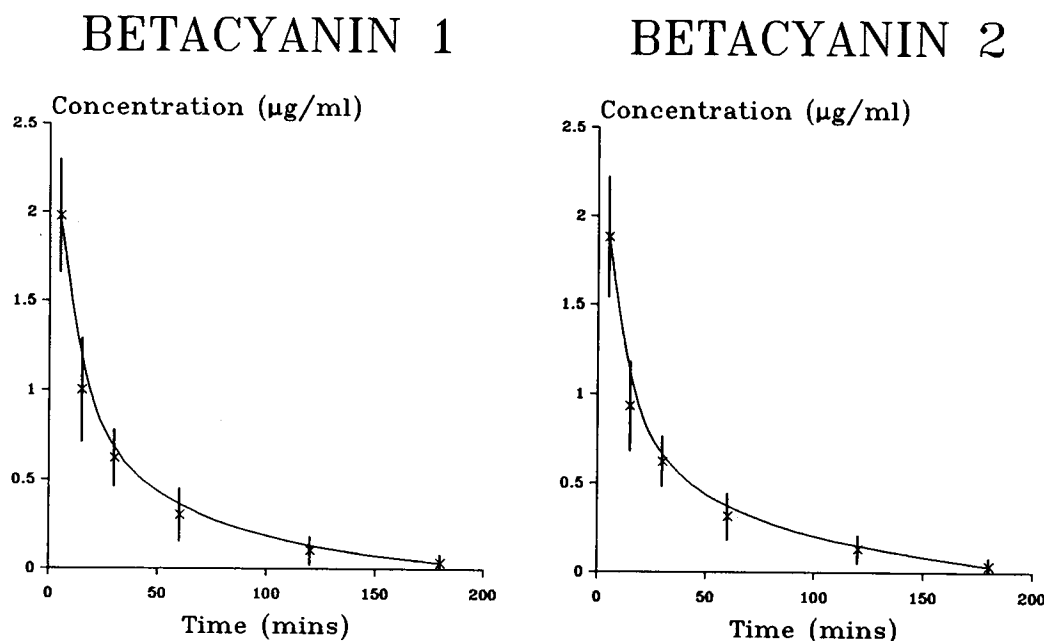


Fig. 5. Plasma concentrations of betacyanins 1 and 2 after the intravenous administration of beetred powder (67 mg kg^{-1}) dissolved in saline (0.9%, 0.5 ml) to anaesthetized rats. Each point is the mean ($\pm$ SD) value for four rats.

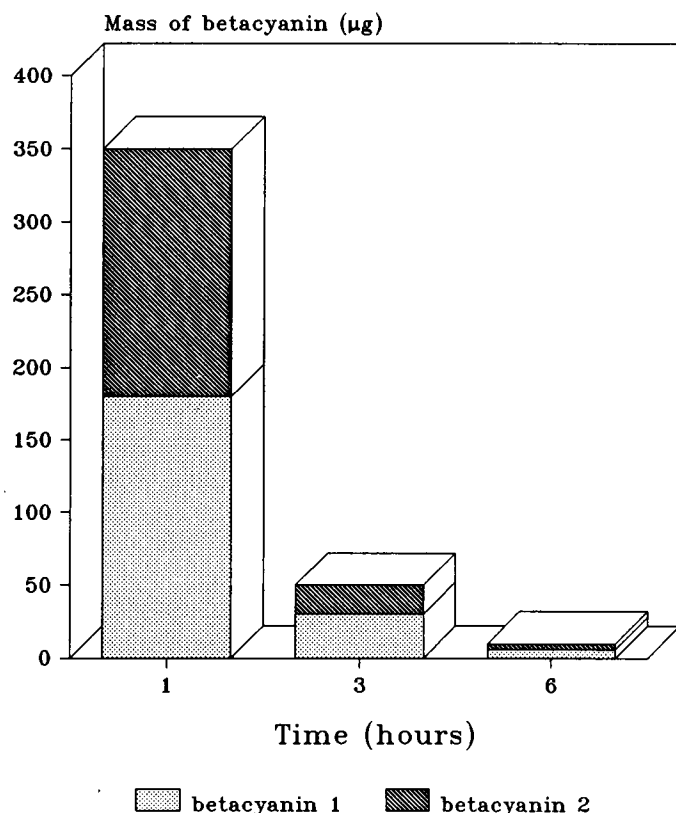


Fig. 6. The betacyanin content of the stomach at various times after the oral administration of beetred powder (1 g dissolved in 5 ml distilled water) to intact rats. The data are the mean values from two rats studied at each time point.

($0.59 \pm 0.10\%$ of dose, $n = 6$) and betacyanin 2 ($0.62 \pm 0.16\%$) were recovered in the urine after 6 h.

After addition of beetred powder (27 mg) to the intestinal lumen reservoir of the isolated perfused rat jejunum preparation, the lumen concentrations of betacyanins 1 and 2 fell to about 80% of their initial values (Fig. 7A). Betacyanins were not detected in any of the vascular perfusate samples. In experiments where beetred (40 mg) was added to the vascular perfusate reservoir small decreases in the vascular perfusate concentrations of betacyanins 1 and 2 were observed after 60 min (Fig. 7B). No betacyanins were detected in jejunum perfusate at any sampling time. Similar results were obtained using higher doses of beetred (data not shown).

Following the addition of beetred (112 mg) to the reservoir of an isolated perfused rat liver preparation, the mean ($\pm$ SD) percentage of the dose recovered did not differ in experiments in which livers were present (betacyanin 1 = $91 \pm 3\%$; betacyanin 2 = $90 \pm 5\%$, $n = 3$) compared to those when livers were excluded from the circuit (betacyanin 1 = $90 \pm 5\%$; betacyanin 2 = $91 \pm 4\%$, $n = 3$) ($p > 0.05$). The amounts present in bile were at the lower limit of detection of the HPLC assay.

Discussion

Following the HPLC analysis of beetred solution, two compounds absorbing at 536 nm were separated.

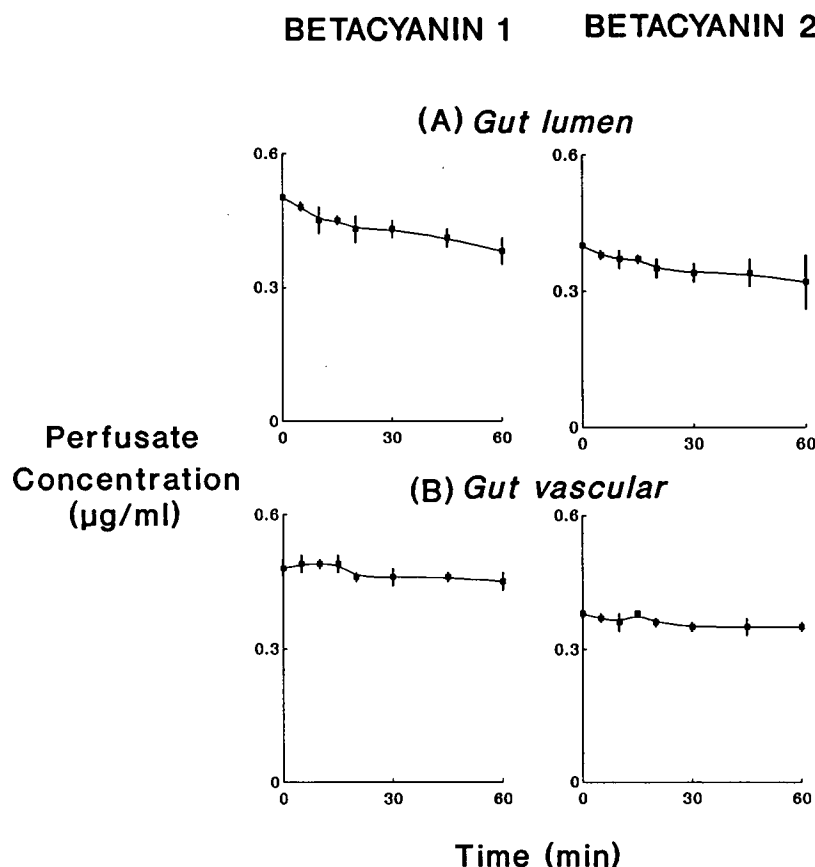


Fig. 7. (A) Intestinal lumen perfusate betacyanin concentrations after the addition of beetred powder (27 mg) to the lumen of an isolated perfused rat jejunum preparation. Each data point represents the mean ($\pm$ SD) result of four experiments. (B) Vascular perfusate betacyanin concentrations after the addition of beetred powder (40 mg) to the vascular perfusate reservoir of an isolated perfused rat jejunum preparation. Each point is the mean ($\pm$ SD) of four experiments.

Based on data from hydrolysis with β -glucosidase and from mass spectral analysis, the compounds were putatively identified as: 1) betanin or isobetanin or a mixture of the two; and 2) the diglucoside of betanidin or isobetanidin or a mixture of the two. For the purposes of the present discussion these red pigments are referred to as betacyanins 1 and 2. These findings are at variance with those of Schwartz & von Elbe (1980) who reported the HPLC separation of four compounds absorbing at 535 nm and identified them as betanin, isobetanin, betanidin and isobetanidin, respectively. However, these authors did not make clear the nature and source of their beetroot product and did not employ mass spectral structural analysis.

The HPLC method developed for the assay of betacyanins 1 and 2 was applied to their quantitative analysis in human urine and a range of rat tissues. The demonstration by diode array UV detection that urine from subjects who had eaten beetroot gave two peaks with identical spectra to those observed on analysis of beetred extract, confirmed that humans

excrete small quantities of betacyanins after beetroot ingestion.

To devise a standard protocol for the investigation of betacyanin excretion in a large population, the time-course and dose-dependency of its excretion was studied in four subjects using direct UV spectrophotometry. Betacyanins were detected within 30 min of eating beetroot suggesting that absorption was rapid. At pH 2 betacyanin is unionized and thus some absorption may be occurring from the stomach. Excretion was essentially complete after 6 h, and an overnight urine collection was chosen for the population study.

The urinary excretion of betacyanins following the ingestion of solid beetroot showed considerable intra-individual variation and no relationship between the dose and the amounts excreted was evident (data not shown). The extent to which beetroot is chewed may affect the dissolution of the pigments in the gastrointestinal fluids leading to inconsistent absorption. An additional factor may be the significant variation in the betacyanin content of individual varieties or

'brands' of beetroot (Wolyn and Gabelman, 1986). However, in the present work the same 'brand' of solid beetroot was used and the variation in pigment content of individual beets was found to be small (data not shown). A much lower intra-individual variation in betacyanin excretion was obtained with juice prepared by homogenization and filtration of pooled beets or beetred liquid obtained from a commercial source. Using liquidized beetroot, the excretion of betacyanins was dose-dependent and saturation was not evident over the range of doses studied. The urinary recovery of betacyanins represented only a minor proportion of the dose (less than 1%). Betacyanin 1 was the predominant red pigment excreted in the urine.

The results from the population study indicated a wide interindividual variation in the ability to excrete betacyanins. Betacyanins were detected in the urine of all subjects. No evidence was found for bimodality in the frequency distributions of the urinary recoveries of betacyanins 1 and 2. Thus, using a specific HPLC method the early evidence that the ability to excrete beetred pigments is polymorphic (Allison & McWhirter, 1956) could not be confirmed. However, our data indicate that beeturic subjects are at the top of the range of the population distribution with respect to betacyanin excretion. Based on visual and spectrophotometric analysis of urine, Percy *et al.* (1991) have reported similar findings.

To determine whether beeturia could be attributed to a difference in the absorption, metabolism, or excretion of beetred pigments, a series of studies in the rat were performed. The observation that the rat also excretes only small quantities of the betacyanins in urine following oral ingestion of beetroot (Krantz *et al.*, 1980 and present work) suggested that this species may be a suitable model for man with respect to the fate of beetred pigments.

After intravenous dosing of beetred liquid to rats, about 80% of the dose was excreted renally as betacyanins 1 and 2, indicating that the kidney is the major route of elimination of beetroot pigments. These findings confirm those of Krantz *et al.* (1980), who used a non-specific UV spectrophotometric assay for these compounds. The high renal clearances of the betacyanins in the rat together with an anecdotal report of extensive urine recovery of the pigments in a single human subject after intravenous injection (Watson *et al.*, 1963) suggest that the excretion of betacyanins by the kidney is not likely to be subject to significant interindividual variability in man.

The slow rate of decline of the betacyanins from the perfusate of an isolated perfused rat liver preparation

(Krantz *et al.*, 1980 and present work) indicates that hepatic metabolism plays a minor role in the disposition of these compounds.

Since the betacyanins are subject to rapid renal clearance but undergo little hepatic metabolism, interindividual variation in their urinary excretion would seem to be largely determined by gastrointestinal absorption and/or metabolism. When rats are given beetred solution orally, the absence of betacyanins in the blood together with recovery of only small quantities of these compounds in the urine indicates that little reaches the systemic circulation, which is in accordance with the physicochemical properties of these compounds. The results of experiments in which beetred powder was added to the lumen of a perfused rat jejunum preparation showed that the betacyanins were not absorbed extensively by the jejunum or metabolized on either the mucosal or serosal side of the jejunal wall over the perfusion period studied. However, Krantz *et al.* (1980) observed that suspensions of the stomach wall, the small intestine and the colon deactivated 75, 35 and 60%, respectively, of the added betacyanin, although no metabolism occurred in suspensions of intestinal or caecal contents.

The marked decrease in the stomach concentration of betacyanins 6 h after oral administration of beetred solution to the intact rat, the lack of visible purple staining of the intestines and the absence of significant amounts of betacyanins in blood or bile, suggest that the stomach is mainly responsible for decolourizing beetroot. Betanin is known to be unstable at the extremes of pH (Vonelbe *et al.*, 1974). To determine whether degradation of the betacyanins might occur in the stomach, incubations were carried out at two gastric pH conditions. Incubation of beetred solutions with acid caused a colour change from red to yellow, a reaction which was irreversible, since on adjustment of the pH to 5.5 prior to spectrophotometric analysis, the red colour did not reappear. Experiments performed at pH 2, the mean fasting gastric pH (Dressman *et al.*, 1990), resulted in 40–45% decomposition of betacyanins 1 and 2 after 2 h. Considerable intra- and interindividual variations in human gastric pH have been reported. In a study of 12 healthy subjects the ratio of highest to lowest median absolute deviation was found to be 17:1 for gastric pH in the fasted state with marked fluctuation within an individual (Dressman *et al.*, 1990). During a meal gastric pH rose to a median of 5.0 but there was considerable inter-subject variation in the rate of return to pre-meal values. Thus, the time taken for gastric pH to decrease from 5 to 2 ranged from 8 to 240 min. Wide variation in the rate of gastric emptying between individuals is

well documented. In a recent study of six subjects the time taken to empty 80% of the stomach content ranged from 27 to 120 min in the fasting state (Riley *et al.*, 1992). If the acid-catalysed degradation of beetroot pigments in the stomach is a major determinant of their fate in man, the occurrence of beeturia may be more common in individuals who, after eating beetroot, show a slow decrease in stomach pH to fasting levels coupled with a rapid rate of gastric emptying.

In achlorhydria gastric pH rises to a mean value of 4.7. At this pH much less decomposition of betacyanins occurred during incubation *in vitro* and, therefore, a much higher proportion of ingested beet-red pigments should be available for absorption. Achlorhydria is associated with iron deficiency and pernicious anaemia, the two pathological conditions in which Watson *et al.* (1963) reported the highest incidence of beeturia. These authors attributed the occurrence of beeturia in their patients to a deficiency of iron but did not consider the possible role of gastric pH.

In conclusion, the bioavailability of beetred pigments and, therefore, the extent to which they are excreted in the urine, show wide interindividual variation. The fate of beetroot pigments may be determined largely by gastric pH and rate of gastric emptying. Percy *et al.* (1991) have also concluded that beeturia is probably due to differences in absorption and not metabolism. The hypothesis that beeturia is predominantly a function of the gastric processing of beetred pigments could be tested by pharmacological manipulation of stomach conditions.

Acknowledgements

This work was the basis of undergraduate projects carried out by A.R.W. (BMedSci) and S.L.M. (BSc). It was supported by the Swann Morton Foundation. P.V. Macrae and D. K. Walker (Pfizer Central Research) are thanked for the mass spectral analysis and I. Marsh (Department of Clinical Chemistry, Royal Hallamshire Hospital) for the UV diode array analysis. We are also grateful to A.

Rostami Hodjegan for performing the kernel density analysis.

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