

Beetroot and red urine

RICHARD M. PEARCY, STEPHEN C. MITCHELL and ROBERT L. SMITH

Department of Pharmacology and Toxicology, St. Mary's Hospital Medical School, London W2 1PG, UK.

The production of a coloured urine by patients who are not prepared for such an occurrence is a disturbing event, even more so when the urine is red and reflects the possible passage of blood. Consultation with the General Practitioner regarding suspect haematuria and subsequent referral to hospital is costly both in terms of distress and finance if the cause is ultimately found to be benign. Several drugs (rifampicin, prontosil, sulphonals) as well as certain food components (beetroot, rhubarb, senna) are known to impart a reddish colouration to the urine after ingestion. This event can be quite striking in some individuals following beetroot intake but unfortunately the phenomenon of beeturia has remained little more than a poorly understood curiosity. The present study was undertaken on a quantitative basis to help clarify this situation and to increase awareness of this phenomenon thereby hopefully enabling effective and reassuring advice to be given by medical personnel to concerned patients.

Volunteers (45 male, 41 female; age 23.8 ± 7.6 (mean $\pm$ 1 s.d.) years) were recruited from amongst the staff and students of the Medical School. All were in good health and were not taking any medication one week prior to or during the study. Each volunteer ingested 125g of small whole pickled beetroot (standard batch) in the morning in the presence of the study organiser, a control urine sample being provided before ingestion and all urine voided during the subsequent 0-8hr period being collected. On completion, the urine volume and pH were measured and the sample visually examined for any pink/red colouration. An aliquot (15ml), adjusted to pH 5.0 with glacial acetic acid and centrifuged to remove any debris, was examined spectrophotometrically for absorption at 530nm against a blank of their own control urine taken through the same procedure.

Preliminary studies had shown that a 0-8hr urine sample was sufficient at this intake level to collect all detectable beetroot-related pigment. Betanin (betanine, betacyanin; the major beetroot pigment) [1] was isolated via lead acetate precipitation with subsequent pH adjustment and solvent extraction from lyophilized beetroot powder after the method of Pulcher [2]. This compound, dissolved in normal urine at pH 5, permitted the construction of calibration curves (linear from 1-500 μ g/ml) and the expression of any red colouration of the urine in terms of a betanin concentration. The spectral profile of red urine compared favourably with that obtained for betanin, but this does not exclude the presence of other coloured beetroot-related products.

On visual examination 22 (25.6%) of the subjects produced obvious red urine. These individuals were in general those who excreted larger amounts of betanin (mean value 11.4mg/0-8hr sample; population mean 5.9mg) or produced lower urine volumes (262ml; population mean 455ml), both factors giving rise to an overall greater urinary concentration of the pigment (55 μ g/ml; population mean 13.2 μ g/ml). Spectrophotometric examination revealed another 35 (40.7%) subjects who excreted measurable amounts of pigment, some 29 (33.7%) volunteers produced no detectable betanin (less than 1.5 μ g/ml) in their urine. All of those who were visually apparent were confirmed spectrophotometrically. Within the whole population the amount of betanin excreted ranged from zero (detection limit = 0.75mg in 500ml) to 43.6mg. No significant association was found between betanin excretion and sex, age or urine pH.

Previous studies have reported that anywhere between none and virtually all of their subjects produced red urine after beetroot ingestion [3-8]. The present investigation showed that a quarter of the urine samples collected were subjectively (visually) detected as positive with an additional group being confirmed objectively (spectrophotometrically) as the power of resolution increased. Consequently, the creation of separate 'excretor' and 'non-excretor' categories within this continuous spread is subjective and artificial. At certain betanin concentrations urine samples fail to appear red coloured to the human eye but this is a matter of individual subjective judgement.

The pigment betanin behaves as a pH indicator showing a maximum absorbance in an acidic environment and losing its red colouration as the urine becomes alkaline [9,10]. For comparative purposes the adjustment of urine samples to the same pH value is therefore essential, a precaution not undertaken in some studies [6]. The red colouration of samples also fades with time, being most stable when frozen at -20°C but decreasing to half its absorbance value in 15 days when stored on the bench. Repeated freezing and thawing also increased the rate of colour loss.

Data obtained from eight individuals who undertook a repeat study within one week showed that the characteristic was reproducible (product moment correlation coefficient $r_c = 0.86$) but four subjects who ingested the same quantity of beetroot on five separate occasions spaced over several months showed variation ranging from good to poor excretors. This suggests that within any population study a spread of excretion capacities will always be found but during repeat investigations the characteristics of any one particular individual within the group may change.

Preliminary studies had indicated that the type of beetroot (variety, preparation, source) used influenced the phenomenon; susceptible individuals who produced an intense red colouration with one brand gave virtually normal urine with a different brand. The betanin concentration of the 'Detroit Rubidus' variety is twice that of 'Firechief' and times of planting and harvesting greatly influence the pigment content of the crop [11,12]. Varying the amount of beetroot eaten (up to 1kg) suggested that this may be a dose-dependent phenomenon with different individuals possessing different absorption capacities and excretory thresholds.

Beeturia as a phenomenon is well established [13] and it has been proposed that this is a characteristic controlled by a single autosomal gene for which man is polymorphic [4]. Such a sweeping statement has not gone without criticism [14,15]. It has also been suggested that beeturia is related to food allergies [3] or linked to iron absorption [5,16], although the latter association has been contested [8]. Evidence against beeturia being caused by underlying hepatic metabolic differences was provided when a volunteer known to produce a non-rubric urine after ingesting betanin was shown to excrete red urine when given betanin intravenously [5]. Similarly, anthocyanins (betanin was originally thought to possess an anthocyanin structure) are excreted in the urine after intravenous injection but not after oral administration to rats or dogs [17]. It appears probable that, in the main, beeturia is dependent upon intrinsic differences in absorption existing between individuals and influenced by such factors as the amount and betanin content of the beetroot ingested, other food substances within the gut and transient gastrointestinal dysfunctions. Differences in metabolism brought about by intestinal microflora or by gut wall mucosal enzymes which may influence the absorption processes must also be considered.

The authors would like to acknowledge Dr R Ayesh for his help in part of this study.

- Mabry, T.J., Taylor, A. & Turner, B.L. (1963) *Phytochemistry* 2, 61-64.
- Pulcher, G.W., Curtis, L.C. & Vickery, H.B. (1938) *J. Biol. Chem.* 123, 61-75.
- Zindler, G.A. & Colovos, G.C. (1950) *Ann. Allergy* 8, 603-617.
- Allison, A.C. & McWhirter, K.G. (1956) *Nature* 178, 748-749.
- Watson, W.C., Luke, R.G. & Inall, J.A. (1963) *Br. Med. J.* II, 971-973.
- Saldanha, P., Magalhaes, L.E. & Horta, W.A. (1960) *Nature* 187, 806.
- Forrai, G., Vágútfalvi, D. & Bölckey, P. (1968) *Acta Paed. Acad. Sci. Hung.* 9, 43-51.
- Forrai, G., Vágútfalvi, D., Lutter, J., Benedek, E. & Soós, E. (1971) *Folia Haemat.* 117, 424-426.
- Huang, A.S. & Van Elbe, J.H. (1987) *J. Food Sci.* 52, 1689-1693.
- Durupthy, A. & Durupthy, O. (1987) *Bull. Union Physicians* 81, 665-670, 694.
- Mazza, G. & Chubey, B.B. (1985) *Canadian Inst. Food Sci. Technol. J.* 18, 332-334.
- Wolyn, D.J. & Gabelman, W.H. (1986) *Hort. Sci.* 21, 1339-1340.
- Johnstone Jervis, J. (1914) *Brit. Med. J.* 1, 1408.
- Penrose, L.S. (1957) *Brit. Med. J.* 1, 282.
- Saldanha, P., Frota-Pessoa, O. & Peixoto, L.I.S. (1962) *J. Hered.* 53, 296-298.
- Tunnessen, W.W., Smith, C. & Oski, F.A. (1969) *Am. J. Dis. Child.* 117, 424-426.
- Horwitt, M.K. (1935) *Proc. Soc. Exp. Biol. Med.* 30, 949-951.