Indican excretion in the elderly

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Summary

It has been suggested that up to a half of elderly hospitalized patients excrete an abnormally high concentration of indican compared with young individuals. Thus, the commonly used colorimetric qualitative test for urinary indican excess may be invalid in the aged. However, of 21 young control subjects, 18 healthy elderly subjects, and 23 disabled, elderly, long-stay patients, no individual had levels of indican detectable by the standard colorimetric qualitative test. The use of a quantitative assay demonstrated that urinary indican concentration is similar in young and elderly individuals. A diurnal variation in urinary indican concentration was discovered which may affect the interpretation of the qualitative test.

KEY WORDS: indican, urinary excretion, bacterial overgrowth, diurnal variation.

Introduction

Urinary indican concentration may be elevated in small bowel bacterial overgrowth. It has been suggested that this is a common finding in the elderly and cases with elevated urinary indican have been reported in elderly patients who had no demonstrable anatomical lesions of the small bowel (Roberts, James and Jarvis, 1977; Holden *et al.*, 1978). A recent report claimed that up to 50% of a group of elderly patients had a positive screening test for excess urinary indican (Pearce, 1980). Such a report implies either a high rate of false positive tests of excess indican excretion or a high prevalence of bacterial overgrowth in the elderly.

Indican is produced by bacterial deconjugation of dietary tryptophan to indole which is absorbed at all levels of the intestine, but principally from the small bowel (Tabaqchali *et al.*, 1966; Tomkin and Weir,

1972). Indole is hydroxylated in the liver and the indican formed is then excreted by the kidneys (Posner, Mitomer and Udenfriend, 1961; Fordtran, Scroggie and Polter, 1964). The metabolism of tryptophan to indican may be altered in the elderly. There is evidence that liver hydroxylation and renal function diminish with age (Irvine *et al.*, 1974; McLachlan, 1978; Epstein, 1979). Ageing is associated with a decrease in the acidity of upper gastrointestinal secretions, which may enhance the ability of bacteria to deconjugate tryptophan (Baron, 1963; Neale, Lambert and Gorbach, 1969). Many strains of *Escherichia coli* and *Klebsiella* produce tryptophanases capable of initiating the production of indican. These bacteria can be present in the oral cavity of elderly patients (Neale and Tabaqchali, 1966; Valenti, Trudell and Bentley, 1978) and this may indicate a different flora in the upper gastrointestinal tract of elderly subjects compared with young individuals. These factors may affect the rate of indican production and excretion in the elderly and thereby invalidate the commonly used qualitative test.

Materials and methods

Three groups of subjects were studied. Twenty-one healthy young controls, 18 healthy elderly controls, and 23 ill elderly, long-stay patients. Young control subjects were 29 ± 4 years old. None had a history of gastrointestinal disease or abdominal surgery, and they were on no medication. The healthy elderly subjects were 66 ± 4 years old, lived independently, did not have gastrointestinal symptoms or signs, had never undergone abdominal surgery, and were not taking drugs known to affect gastrointestinal function. Both groups of healthy subjects collected a 24-hr urine specimen. The healthy elderly group also collected a first morning spot specimen, which was used for comparison with the ill elderly patients. The

long-stay patients were 81 ± 7 years old; many were confused and incontinent, and most were unable to collect 24-hr urine specimens. First morning spot specimens were collected. None of these patients was taking antibiotics or laxatives.

The 24-hr urine specimens were preserved with thymol. Indican excretion was quantitatively measured in triplicate with internal standards by the method of Curzon and Walsh (1962). The method is based on the Ehrlich reaction of indoxyl sulphate with para-dimethylaminobenzaldehyde in acid conditions. The addition of 40% NaOH causes precipitation which is extracted into petroleum spirit and the extinction of the yellow extract measured colorimetrically at 464 m μ .

The intraassay coefficient of variation was less than 10%. The interassay coefficient of variation was 11%. The minimum detectable concentration was less than 0.05 mmol/litre. To determine whether there was a diurnal variation in urinary indican concentration a young subject collected timed urine specimens for 2 days. The sensitivity of the qualitative colorimetric indican test in common use (Varley, Gowenlock and Bell, 1980), was assessed by performing the test on a range of prepared indican standards. The tubes were read 2 hr after the addition of hydrochloric acid.

Values are expressed as mean $\pm$ 1 s.d. Statistical comparisons were by Student's *t*-test. The results from the timed collection in the young subject were analysed by the method of Fort and Mills (1979). The project was approved by the Hospital Ethical Committee and informed consent was given by all participants.

Results

The young and elderly healthy subjects excreted on average 0.17 ± 0.14 and 0.18 ± 0.13 mmol of indican/24 hr, respectively. The mean concentration of indican in the 24-hr urine specimens was 0.13 ± 0.12 mmol/litre in the young and 0.12 ± 0.11 mmol/litre in the elderly healthy subjects. The mean concentration of indican of first morning spot urine specimens was 0.20 ± 0.11 mmol/litre in the healthy elderly and 0.21 ± 0.11 mmol/litre in the long-stay elderly patients. There were no positive results from the qualitative test on any individual urine specimen. The higher concentration in the healthy elderly subjects' first morning specimens prompted us to look for a diurnal variation in urinary indican concentrations, which was confirmed in a young subject and is shown in Fig. 1. The colorimetric qualitative test became positive when the indican concentration exceeded 0.5 mmol/litre (125 mg/litre) in the standard preparations.

FIG. 1. Histogram of the hourly concentration of urinary indican in a healthy young male with fitted sine curve (---). The sine function was fitted to the data by the method of Fort and Mills (1979). Indican concentration (mmol/litre) = $0.09 \times \sin [\pi/12 \times (t + 0.6)] + 0.23$ t = time (hr).

Discussion

There has been some debate about the reliability of the qualitative indican test for bacterial overgrowth. Early reports indicated that the test was sensitive and specific (Neale and Tabaqchali, 1966; Tabaqchali *et al.*, 1966). Later studies concluded that the test was not valid (Hamilton *et al.*, 1970; Beekin and Karich, 1973; Mayer and Beekin, 1975). Other reports maintained that the test remains clinically useful (Tomlin and Weir, 1972; Aarbakke and Schjonsky, 1976; Patney *et al.*, 1979). Although some doubt remains about the value of the test, the colorimetric test is inexpensive, non-invasive, and is much simpler to perform in elderly patients than breath tests or upper gastrointestinal intubation.

The finding of a diurnal variation in indican concentration (Fig. 1) with high morning values may explain the higher concentration found in the spot specimens compared with the overall 24-hr urine collections. This also suggests that a false positive result in spot samples may be more likely to occur in the morning, and a false negative result in the evening. A concentration of 0.4 mmol/litre (100 mg/litre) or higher is considered abnormal (Aarbakke and Schjonsky, 1976; Patney *et al.*, 1979). However, because this qualitative test has a sensitivity which is time-dependent, variable results may be obtained from laboratory to laboratory. With long incubation periods (3–2 hr), we found that the qualitative test was more likely to become positive.

Since none of the subjects in this study excreted detectable amounts of indican as measured by the qualitative test, the probability that 50% of elderly subjects have a positive test is less than 1% (Siegel, 1956). The observation by Pearce (1980) that half of an elderly population had significant indicanuria

may have been a result of selection of patients, might have taken too low a concentration of indican as indicative of disease, may have been the result of the testing of spot morning urine specimens, or could have been a result of a prolonged incubation time. It is concluded that the concentration of urinary indican in healthy elderly is similar to that in the young and that the qualitative test has the same value in both age groups if suitable precautions are followed.

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