New non-invasive test of gastric acid secretion for use in children

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Abstract
Loss of the gastric acid barrier may lead to recurrent enteric infections, small intestinal bacterial overgrowth, persistent diarrhoea, and thus malnutrition. To investigate this possibility, a new, non-invasive test of gastric acid secretion was developed ideal for field use in the developing world, where chronic diarrhoea and undernutrition are common. The test relies on the capacity of the kidney to retain H⁺ during gastric acid secretion, leading to a postprandial urine 'alkaline tide'. Gastric intubation studies of seven healthy adult volunteers showed a direct relation between changes in gastric acid secretion and changes in urine acid output (measured as the H⁺/creatinine molar ratio in spot urine samples). Subjects who secreted gastric acid in response to stimulation with a sham feed showed a fall in urine acid output >0.5 mmol H⁺/mmol creatinine (range −7.4 to −1·52 mean −1·12). The most reproducible decrease in urine acid output in response to normal food was observed around the time breakfast was usually eaten and was abolished by 36 hours of treatment with ranitidine. Breakfast time reductions in postprandial urine acid output in 22 healthy English children were comparable with those in healthy adults, and significantly different from values in achlorhydric adults. They were much more variable, however, in 106 Gambian children in whom values spanned both normochlorhydric and achlorhydric ranges (−2·7 to +1·8). Measuring changes in urine acid output at breakfast time provides a reliable indirect measure of gastric acid secretion that can be used in field conditions, enabling the relation between gastric acid output and the development of diarrhoeal diseases to be investigated.

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Gastric acid is the primary barrier against orally ingested enteric pathogens, and a major regulator of small bowel flora. Defective gastric acid secretion predisposes to abnormal small intestinal bacterial overgrowth and recurrent enteric infections, both conditions that may lead to the development of persistent diarrhoea and malabsorption, resulting in malnutrition and growth failure.

Children who are malnourished with chronic diarrhoea have defective gastric acid secretion. This may be a result of impaired gastrointestinal function secondary to poor nutritional status, but the role of achlorhydria in the development of malnutrition has not been investigated. Population studies of gastric acid secretion in children at risk from undernutrition are clearly not possible using conventional intubation techniques.

As parietal cells release acid into the lumen of the stomach, they release bicarbonate into the systemic circulation. Excess bicarbonate is excreted by the kidney, which means that a transient rise in urine bicarbonate excretion (the urine 'alkaline tide') immediately follows maximal gastric acid secretion. Measurement of the urine alkaline tide as an index of gastric acid output has been proposed as a non-invasive alternative to conventional gastric secretion studies. We have adapted this technique and describe a method of measuring changes in urine acid output that is suitable for use in paediatric community studies.

Subjects and methods
Five sets of studies were undertaken on adults and children in England and in The Gambia.

INTUBATION STUDIES
Seven healthy adult volunteers (six men, aged 21–43 years) underwent gastric intubation studies. At 0730, after an overnight fast, approximately 300 ml of water were drunk and a sample of all urine passed subsequently was saved. At 0830 a nasogastric tube (10 FG) was passed, and its position was ascertained by the complete aspiration of a test drink of 100 ml of water. Residual gastric contents were discarded, and basal gastric acid output was determined by collection of gastric contents at 15 minute intervals for 45 minutes. Subjects then emptied their bladders, saving a sample of urine. A sham feed, of a sandwich of the subject's own choice, was then masticated. Subjects were encouraged to chew the food well, and spit it out, rinsing their mouth out with water but not swallowing.

The gastric contents were collected at 15 minute intervals for 45 minutes to determine stimulated gastric acid output, after which the nasogastric tube was removed and subjects once more emptied their bladders. The procedure was repeated after 36 hours treatment with oral ranitidine 300 mg twice daily.

ASSESSMENTS OF NORMAL URINE ACID OUTPUT
Six healthy adult volunteers collected urine specimens throughout the course of a normal day, noting the time that food and drink were taken.

Fourteen healthy adult subjects then collected urine specimens before and within an hour after their normal breakfast, to assess the fall in urine acid output accompanying the first meal of the day.
ACHLORHYDRIC ADULTS
Nine healthy adult volunteers repeated the collections of urine throughout a morning after 36 hours’ treatment with ranitidine 300 mg twice daily. A further five subjects (four taking ranitidine, one proved to be achlorhydric by intubation studies) collected urine specimens around the time of breakfast.

NORMAL ENGLISH CHILDREN
Twenty two healthy children (age range 1–8 years) collected two urine specimens—one before and one 20 minutes to two hours after breakfast for determination of the change in the H⁺/creatinine molar ratio. A wide range of personal preferences in children across this age range made it impossible to use an absolutely standardised breakfast. Most children, however, had breakfast cereal with milk, with the addition, or in some cases substitution, of buttered toast.

WEST AFRICAN CHILDREN
Children between 3 and 5 years of age from Keneba village, the site of a Medical Research Council Nutritional Research Station in The Gambia (n = 106), collected two urine specimens, one before and one within two hours after a standard breakfast consisting of fried dough, fresh bananas, and water.

DETERMINATION OF GASTRIC AND URINE ACID OUTPUT
The acid content of gastric juice was determined by titration to pH 7-4 with NaOH. Acid output was calculated by allowing for the amount of juice produced in each time interval, and was expressed as mEq/h. Urine specimens were processed as soon as possible after collection, both in the UK and in the Gambia, and titratable acidity was determined by titration of each urine specimen to pH 7-4.

Creatinine concentrations in urine were determined using the modified Jaffé reaction (Roche diagnostics) on a COBAS BIO centrifugal fast analyser in the UK, and using a bench spectrophotometer in the Gambia. Urine acid output was expressed as the urinary H⁺/creatinine ratio for each sample tested.

Results

INTUBATION STUDIES
In six of the seven subjects basal acid output ranged between 2-2 and 13 mEq/h (mean 5-5), and stimulated acid output ranged between 8 and 34-2 mEq/h (mean 17-9). These six subjects underwent a further intubation test after three doses of ranitidine 300mg over 36 hours. In one subject intubation failed at the second study, and in the other five subjects basal acid output was 0 to 2-2 mEq/h (mean 0-55) and stimulated acid output rose to between 0-1 and 1-2 mEq/h (mean 0-68).

The remaining subject was achlorhydric during the first intubation study (basal acid output 0 mEq/h, stimulated acid output 0-36 mEq/h), and a further test after histamine receptor blockade was not therefore undertaken.

The sham feed produced a transient rise in gastric acid output that was most marked in the 30 minutes after feeding (Fig 1). The change in gastric acid secretion, between basal acid secretion and stimulated acid secretion, that occurred during the first 30 minutes after sham feeding was compared with the change in urine acid output (ΔUAO) over the same period. AUAO was calculated as the difference between the urine H⁺/creatinine molar ratio of the urine sample collected after basal acid secretion, and that of the first urine sample passed after gastric stimulation.

Pooled results from a total of 12 test days in seven subjects, which include results from the same subjects before and after treatment with ranitidine, are shown in Figure 2. The relation between the change in gastric acid secretion and ΔUAO was log/linear, and two discrete populations were seen. An arbitrary cut off of ΔUAO = −0.5 separated achlorhydric from normal responses.

Figure 1: Changes in gastric acid output after sham feeding in seven subjects, showing mean (SEM) at two points in the 60 minutes before the sham feed, and then at 15 and 30 minutes thereafter.

Figure 2: Comparison of changes in gastric and urine acid outputs (ΔUAO) compared with basal values, which occurred during the first 30 minutes after gastric stimulation by sham feeding for seven subjects on a total of 12 test days.
NORMAL URINE ACID OUTPUTS IN ADULTS

In six adult volunteers who collected urine specimens throughout the day (noting times of passing urine and of all food consumed), fasting urine acid outputs ranged from 1·46 to 2·14 mmol H⁺/mmol creatinine (mean 1·74). All subjects showed a fall in UAO after breakfast. The changes in UAO in relation to food are shown in Figure 3. The most consistent fall in UAO was seen after breakfast, and suggested that a light meal after a relatively long fast provided the ideal stimulus for observing changes in UAO that reflect gastric acid output.

ACHLORHYDRIC ADULTS

The mean change in urine acid output that occurred at breakfast time in 14 normal adults was −0·88 mmol H⁺/mmol creatinine (95% confidence interval (CI) of mean −0·71, −1·0 mmol H⁺/mmol creatinine). Full results are shown in Figure 4. This change was abolished in all 10 subjects who were treated with ranitidine 300 mg for three doses (mean change after ranitidine −0·05 mmol H⁺/mmol creatinine, 95% CI 0·33, −0·34 mmol H⁺/mmol creatinine). An arbitrary cut off of −0·5 mmol H⁺/mmol creatinine (roughly equidistant from the 95% CI of both means, and which also corresponded to the cut off value obtained from intubation studies) was chosen to discriminate between normochlorhydric and achlorhydric individuals. Within this small population of 24 tests in adults, including 10 tests in subjects rendered achlorhydric by ranitidine treatment, the detection of a fall in UAO at breakfast time of greater than 0·5 mmol H⁺/mmol creatinine demonstrated normochlorhydria with a positive predictive value of 96% and a negative predictive value of 100%.

NORMAL ENGLISH CHILDREN

The mean AUAO at breakfast time in 22 British children was −1·43 mmol H⁺/mmol creatinine (95% CI −0·47, −2·39 mmol H⁺/mmol creatinine). These values are shown in Figure 4 beside adult values for 14 normochlorhydric and 10 achlorhydric tests. The results in children are similar to those in normal adults (test of hypothesis that groups are different, t = 1·32, p = 0·27) but significantly different from those in achlorhydric adults (t = 2·084, p = 0·046).

WEST AFRICAN CHILDREN

Changes in UAO before and after breakfast were measured in 106 Gambian children. The range of AUAO obtained was much greater than for any other group (Fig 4) (maximum value 1·8, minimum −2·8, mean (SD) 0·04 (1·05)).

Discussion

We have described a series of studies that establishes the use of the measurement of changes in UAO at breakfast time from two spot urine samples, as a reliable guide to gastric acid secretion in response to food in populations with a high prevalence of achlorhydria. This test is eminently suitable for use under field conditions in developing countries.

Johnson et al suggested that changes in urine and gastric acid output were linearly related. Our use of creatinine molar ratios has removed the source of experimental error involved in collecting total urine volumes over a relatively short period, and has revealed the existence of a log/linear relation between concomitant changes in gastric acid secretion and UAO.

This technique allows study of gastric acid secretion in response to physiological stimuli, particularly that of food. All other established techniques involve the measurement of luminal acidity in response to varied stimuli, none of which can be considered to be truly physiological. Our interest lies in measuring the gastric secretory response to food, the major source of ingested bacteria. We have found our technique...
most useful when the stimulus of a light meal follows fasting, a state that naturally occurs in most people at breakfast time. We have shown that the fall in breakfast time UAO is a reliable predictor of gastric acid secretion in adult populations with a high prevalence of achlorhydric subjects. Further studies are planned to assess the predictive values of this test in populations where achlorhydria is less prevalent.

Normal British children form a population whose range of AUAOs is similar to that in normochlorhydric adults but distinct from that of achlorhydric adults. Gambian children, however, form a more heterogeneous group with a wider spread of results (Fig 4), despite the fact that these children consumed a standard breakfast, compared with the British children who exhibited a wide range of individual preferences for breakfast foods. Comparison of these results with those from English children suggests that substantial numbers of rural Gambian children may be achlorhydric. There are two possible explanations for this. Young children in this environment are prone to recurrent febrile illnesses, known to inhibit gastric acid secretion and the incidence of acute *Helicobacter pylori* infection in this particular community is approaching 50% in the first year of life. Taylor suggests that in more than 50% of cases of acute *H pylori* infection, hypochlorhydria lasts for several weeks. If this is true, many young children in The Gambia will have achlorhydria secondary to *H pylori* infection.

In summary, we have developed a non-invasive test which predicts accurately gastric acid output in populations with high prevalences of achlorhydria and is suitable for field use for population studies of young children in developing countries. The first field study using this technique suggests that in a population in which persistent diarrhea and growth failure are endemic, achlorhydria is common. This observation will be followed by further direct measures of gastric acidity, but should stimulate renewed interest in the potentially crucial relationship between the gastric acid barrier and intestinal flora.

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