Bacterial Contamination of the Small Intestine as an Important Cause of Chronic Diarrhea and Abdominal Pain: Diagnosis by Breath Hydrogen Test

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ABSTRACT. Unsuspected bacterial contamination of the small intestine was indicated by breath hydrogen testing in nine patients aged 2 to 34 months during physical examinations for chronic diarrhea and abdominal pain. Elevated bacterial counts of questionable significance were found in duodenal aspirates before and after antibiotic treatment. There was no evidence of bile salt deconjugation or structural changes in the small intestine by light or electron microscopy. This may indicate that the site of colonization is distal to the biopsy site. Breath testing indicated lactose malabsorption in all patients, and four of five patients tested also malabsorbed sucrose. Duodenal disaccharidase levels in all patients were within the normal ranges, but in eight patients the lactase-sucrase ratio was greatly elevated (0.80 ± 0.36; normal <0.45). Dietary restriction alone did not cause complete cessation of symptoms, whereas all patients responded dramatically to oral antibiotic therapy. When patients were well, the lactase-sucrase ratio had returned to normal in those tested, and all nine had normal lactose and lactulose breath hydrogen tests. Unsuspected bacterial contamination of the small intestine, which is easily detected using the breath hydrogen test, may be more commonly associated with chronic diarrhea in children than has been previously realized. In such cases, therapy should be directed at removing the contamination. *Pediatrics* 1984;74:229–235; bacterial contamination, chronic diarrhea, abdominal pain, breath hydrogen, intestinal biopsy.

Diarrhea, abdominal pain, and malabsorption secondary to bacterial overgrowth in the small intestine, in the absence of an anatomic lesion or severe motility disorder, have only rarely been convincingly demonstrated. This condition has been suspected as a cause of diarrhea in the pediatric age group, and there have been several reports of its association with protracted diarrhea and carbohydrate intolerance. In the past, the diagnosis of small bowel overgrowth has relied on the culture of abnormal bacterial flora and/or demonstration of deconjugated bile salts in intestinal fluid. Both require intestinal intubation and the results may be falsely normal due to the difficulty of culturing anaerobic organisms, the possibility of more distal colonization, the rapid absorption and dilution of free bile acids, or inadequate bacterial contact time with new bile acid secretions before aspiration. The advent of the breath hydrogen test (BHT) has provided a simple, rapid, and noninvasive method to detect bacterial overgrowth, especially in children. The use of different test sugars enables various aspects of carbohydrate malabsorption to be studied, with lactose and sucrose used to determine primary and secondary malabsorption. The unabsorbable sugar lactulose is the most suitable for detecting bacterial overgrowth because it traverses the entire bowel unabsorbed, and thus, it can titrate upper small bowel and colonic bacterial groups.

We have modified the BHT to improve its sensitivity and reliability, and we have used it as part of our investigative assessment of children with chronic diarrhea and abdominal pain. This paper reports our findings from nine patients with a BHT suggesting bacterial overgrowth.

PATIENTS AND METHODS

All nine patients were referred to the Gastroenterology Unit of the Adelaide Children’s Hospital.

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The age range and symptoms at examination are shown in Table 1. All children except one had four to seven sloppiness, often offensive, bowel actions daily. The child with abdominal pain had a normal bowel habit. Because of lack of associated growth failure, it was felt that these children had a chronic nonspecific diarrhea. No evidence of steatorrhea was noted on stool microscopy findings. There were no associated diseases except in patient 8 who had cystic fibrosis. The infant with bloody diarrhea had normal stools prior to the onset of symptoms. A rectal biopsy excluded Hirschsprung's disease. Control subjects for the BHT were ten children of hospital staff members who had no gastrointestinal symptoms at the time of testing. The mean age of 36 months was higher for the control children than for the patients.

Stool specimens were cultured for bacteria and viruses and examined by electron microscopy. Sweat tests were performed on four patients; findings were positive only in patient 8. Duodenal intubation and biopsy were carried out in all patients following sedation with quinalbarbital and using metaclopromide to increase gastric emptying and upper gastrointestinal motility. A sterile No. 8 French feeding tube was attached to the intestinal biopsy capsule tubing, and duodenal juice of pH 7 or greater was collected on ice for aerobic and anaerobic bacterial culture and measurement of bile acids. The average time taken for the biopsy and juice collection was 15 minutes. Routine culturing was done on horse blood agar, MacConkey agar, chocolate horse blood agar (incubated in 5% CO₂), and anaerobic blood agar incubated under anaerobic conditions. For colony counting, cultures were inoculated onto horse blood agar plates, incubated in 5% CO₂ for two days, and then read according to the method of Miles et al.⁹ Duodenal mucosa was obtained using a Watson pediatric biopsy capsule as described by Townley and Barnes,¹⁰ and was divided for histology, disaccharidase estimation, and electron microscopy. When assessing the results of microbial culture, the level considered by ourselves and others as abnormally high at the site sampled was ≥10⁶ colonies per milliliter.¹¹⁻¹⁵

Individual bile acids and their glycine and taurine conjugates were assayed using high-performance thin-layer chromatography.¹⁶ Disaccharidase levels were measured using a modification of the method of Dahlgvist.¹⁷ The normal range for lactase activity was 14 to 132 U/g of protein, and sucrase activity was 32 to 228 U/g of protein. These values were obtained from disaccharidase estimations on duodenal biopsy material from 200 children who had been examined for chronic diarrhea, failure to thrive, short stature, or suspected celiac disease and

**TABLE 1. Clinical Features, Examination Results, and Therapy in Nine Patients with Bacterial Contamination of Small Intestine**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Symptoms</th>
<th>Culture of Duodenal Juice (Organism/mL)</th>
<th>Lactase-Sucrase Ratio (Normal &lt;0.45)</th>
<th>Therapy</th>
<th>Source Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diarrhea 6 wk, steeming 5 mo</td>
<td>3.5 x 10⁴ Lactobacillus sp, 7.5 x 10⁶ Pseudomonas aeruginosa</td>
<td>70/71 (0.77)</td>
<td>+</td>
<td>Diet/lincocin, trimethoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>2</td>
<td>Diarrhea 6 mo, steeming 5 mo</td>
<td>3.2 x 10⁴ E.coli, 7.5 x 10⁶ Staphylococcus aureus</td>
<td>94/91 (1.03)</td>
<td>+</td>
<td>Diet/lincocin, trimethoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>3</td>
<td>Diarrhea 7 mo, steeming 5 mo</td>
<td>6 x 10⁶ Enterococci</td>
<td>29/33 (0.93)</td>
<td>+</td>
<td>Diet/lincocin, trimethoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>4</td>
<td>Diarrhea 8 mo, steeming 5 mo</td>
<td>1.9 x 10⁶ Enterococci</td>
<td>39/45 (0.88)</td>
<td>+</td>
<td>Diet/lincocin, trimethoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>5</td>
<td>Diarrhea 9 mo, steeming 5 mo</td>
<td>5 x 10⁶ Staphylococcus aureus</td>
<td>30/37 (0.86)</td>
<td>+</td>
<td>Diet/lincocin, trimethoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>6</td>
<td>Diarrhea 10 mo, steeming 5 mo</td>
<td>1.3 x 10⁶ Enterobacteriaceae, 7.5 x 10⁵ Aeromonas hydrophila</td>
<td>39/47 (1.33)</td>
<td>+</td>
<td>Diet/lincocin, trimethoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>7</td>
<td>Diarrhea 11 mo, steeming 5 mo</td>
<td>3 x 10⁵ Serratia marcescens</td>
<td>40/54 (0.85)</td>
<td>+</td>
<td>Diet/lincocin, trimethoprim/sulfamethoxazole</td>
</tr>
</tbody>
</table>

*Child with cystic fibrosis.*

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in whom no mucosal abnormality had been found. The lactase-sucrase ratio was compared with our own data for the 200 children examined for chronic diarrhea, failure to thrive, etc, and the data of Kerry and Townley for heterozygotes examined for su-
crase-isomaltase deficiency.

Our method of hydrogen ($H_2$) collection and analysis procedure has been described previously. An important aspect of the methodology is sample quality correction of $H_2$ values based on expired oxygen levels. By correction to a common oxygen level, $H_2$ responses between individuals can be quantitatively compared. Without such a normalization procedure, small $H_2$ changes and early transient peak $H_2$ productions tend to be lost in the highly variable values obtained with various quality breath samples. This is especially so in children in whom cooperation and constancy of breath samples is not always possible.

The lactose BHT used 2 g/kg of lactose (maximum 20 g) in 100 mL of water with 30-minute interval sampling. The sucrose BHT used 30 g of sucrose in 100 mL of water, a level we have found is normally absorbed readily even in young infants.

Bacterial overgrowth was indicated by an early transient $H_2$ increase preceding a later $H_2$ increase when unabsorbed carbohydrate reached the colon (Fig 1). The magnitude of the $H_2$ responses was unimportant, as only the characteristic double peak was used to detect patients with suspected overgrowth. In most patients, the early transient $H_2$ increase was first noted during a routine lactose BHT, and the suggestive overgrowth result was then demonstrated the next day by a second BHT using unabsorbable lactulose (10 mL of Duphalac syrup) in 100 mL of water. Samples were collected every 20 minutes for the first 60 minutes, and at 30-minute intervals thereafter with lactulose testing. Because all patients showed the same shaped curve, a mathematical mean from all nine patients was constructed (Fig 2) after correcting $H_2$ values to a common oxygen level (16.9%). The time base for each person was adjusted to align the early and late $H_2$ increases, and the $H_2$ values were expressed as a percentage of the average maximum early $H_2$
peak, which served as a common reference point. A similar mathematical mean curve was constructed for ten normal children after the same lactulose dose, and it was fitted to the same time base as was used for patients with overgrowth. Relative $H_2$ for the normal children was expressed as percent of the average first $H_2$ increase.

**RESULTS**

Electron microscopy and culture of stools failed to detect pathogenic organisms in any child. Findings from a barium meal and follow-through examination were normal in all but two children (patients 7 and 9) in whom the features were entirely nonspecific but suggested a pattern consistent with malabsorption.

Bacterial cultures of duodenal aspirate yielded a variety of microorganisms as shown in Table 1. The organisms most commonly isolated were of an oral type, ie, they were species regarded as normal flora and included streptococci of the viridans group, *Staphylococcus albus*, *Staphylococcus aureus*, *Lactobacillus* sp (aerobic and anaerobic), and *Diplococcus pneumoniae*. Organisms regarded as being of fecal type were isolated in numbers $\geq 10^4$ organisms per milliliter which is a significant level of growth in the upper small intestine. Oral-type flora alone were cultured from only one patient; the remaining patients had a mixture of both oral and fecal flora.

Deconjugated bile acids were not detected in duodenal juice from any patient.

The duodenal mucosal architecture as viewed by both light and electron microscopy was normal in all patients. Disaccharidase levels for lactase and sucrase and the corresponding lactase-sucrase ratios are shown in Table 1. All children had normal lactase levels, but the BHT indicated lactose malabsorption and bacterial overgrowth. In five patients with bacterial overgrowth, the fasting $H_2$ level was elevated (Table 2). A base-line sample 30 minutes later showed a decrease in all cases. This is in keeping with our previous experience that elevated fasting levels continue to decrease to normal levels over time. Patients 2 and 8 had low sucrase levels, but only patient 8 was shown to malabsorb sucrase by BHT. Patients 4, 7, and 9, who had normal sucrase levels, were shown to malabsorb sucrase.

Mouth to cecum transit times using lactulose were significantly longer for patients with bacterial overgrowth compared with those of normal children and patients being examined because of chronic diarrhea (Table 3). The transit time difference is still significant after allowing for possible overestimation from midpoint estimates.

### TABLE 2. Hydrogen Production (ppm) from Patients with Bacterial Overgrowth

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Test Sugar</th>
<th>Base-Line $H_2^*$</th>
<th>1st Peak $H_2$</th>
<th>2nd Increase $H_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactulose</td>
<td>4</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>Lactulose</td>
<td>17</td>
<td>22</td>
<td>$&gt;52$</td>
</tr>
<tr>
<td>3</td>
<td>Lactulose</td>
<td>6</td>
<td>28</td>
<td>$&gt;127$</td>
</tr>
<tr>
<td>4</td>
<td>Sucrose</td>
<td>10</td>
<td>21</td>
<td>$&gt;29$</td>
</tr>
<tr>
<td>5</td>
<td>Lactose</td>
<td>17</td>
<td>65</td>
<td>$&gt;77$</td>
</tr>
<tr>
<td>6</td>
<td>Lactulose</td>
<td>0</td>
<td>15</td>
<td>$&gt;16$</td>
</tr>
<tr>
<td>7</td>
<td>Lactulose</td>
<td>0</td>
<td>19</td>
<td>$&gt;26$</td>
</tr>
<tr>
<td>8</td>
<td>Lactulose</td>
<td>52</td>
<td>74</td>
<td>$&gt;105$</td>
</tr>
<tr>
<td>9</td>
<td>Lactulose</td>
<td>37</td>
<td>54</td>
<td>$&gt;87$</td>
</tr>
</tbody>
</table>

* High base lines were shown to be falling before administration of the test sugar.

### TABLE 3. Transit Times

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Small Bowel Transit Time (min)</th>
<th>Large Bowel Transit Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overgrowth, lactulose</td>
<td>7</td>
<td>$24 \pm 13$</td>
</tr>
<tr>
<td>Overgrowth, lactose</td>
<td>9</td>
<td>$27 \pm 15$</td>
</tr>
<tr>
<td>Normal lactulose</td>
<td>24</td>
<td>$69 \pm 43$</td>
</tr>
<tr>
<td>Chronic diarrhea, lactulose</td>
<td>40</td>
<td>$70 \pm 30$</td>
</tr>
</tbody>
</table>

* $P < .01$ compared with control subjects or patients with chronic diarrhea.

The lactase-sucrase ratios were greater than normal in all but one patient (mean $\pm SD$ 0.80 $\pm 0.36$), and were significantly different from those of 16 normal subjects ($0.40 \pm 0.16 P < .01$) and seven obligate heterozygotes for sucrase-isomaltase deficiency ($1.33 \pm 0.34$). Mean lactase levels in patients with bacterial overgrowth ($45 \pm 30$) were not significantly different from those of control patients ($37 \pm 22$). Mean sucrase levels in patients with overgrowth ($64 \pm 41$), although appearing lower than those of control patients ($91 \pm 42$), were not significantly different.

Initial treatment in these children was dietary modification by lactose withdrawal in all patients and added sucrase withdrawal when indicated by BHT. Because of incomplete resolution of symptoms in all patients, oral antibiotic therapy was tried as outlined in Table 1. The response to this treatment was often dramatic, leading to complete resolution of symptoms within 24 hours. The antibiotics were chosen according to the sensitivity of the organisms cultured from duodenal juice except in three patients (patients 5, 8, and 9) who were given antibiotics for other indications, otitis media, pneumonia, and colitis, respectively. All children were able to tolerate disaccharides after antibiotic treatment with no evidence of malabsorption either clinically or by BHT. Lactulose BHT levels returned to normal in all children after antibiotic therapy and showed a normal colonic $H_2$ increase.
TABLE 4. Pretreatment and Posttreatment Examination Results in Three Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (mo)</th>
<th>Lactase-Sucrase Ratio (Normal &lt;0.45)</th>
<th>Duodenal Juice Culture</th>
<th>Treatment</th>
</tr>
</thead>
</table>
| 2           | 6        | 22/26 (0.85)                         | 5 × 10⁴ Pseudomonas sp  | 1. Lactose, sucrose-free diet  
|             |          |                                     | 2. Trimethoprim         |           |
| 7           | 14       | 21/34 (0.62)                         | 5 × 10⁴ *Enterobacter hafniæ*, 2 × 10⁴ Staphylococcus albus | 1. Lactose, sucrose-free diet  
|             |          |                                     |                        | 2. Lincomycin               |
|             | 22       | 21/85 (0.25)                         | ...                    | 1. Lactose, sucrose-free diet  
|             |          |                                     |                        | 2. Flucloxacillin           |
| 8           | 14       | 26/17 (1.53)                         | 3 × 10⁹ Pseudomonas capacia | 1. Lactose, sucrose-free diet  
| 17*         | 2/4 (0.5)| ...                                 |                         | 2. Flucloxacillin           |
| 18          | 10/62 (0.16) |                                    | 2 × 10⁸ Pseudomonas aeruginosa | 1. Lactose, sucrose-free diet  

* Meconium ileus equivalent requiring surgical correction.

from the unabsorbed carbohydrate without the early transient increase seen prior to treatment.

Three children had their biopsies repeated when they were asymptomatic and the BHT level had returned to normal (Table 4). The lactase-sucrase ratios had altered, sucrase levels had increased, but significant bacterial colonies were still present in the two duodenal juice samples cultured. Patient 8 had a lower lactase level after treatment due to the development of meconium ileus equivalent, which markedly lowered all disaccharidase levels before surgical correction.

DISCUSSION

The children in this study were examined because of chronic diarrhea or abdominal pain. The diagnosis of bacterial contamination of the small intestine was not previously suspected and may have been undetected without the use of the BHT. In four children the fasting breath H₂ level was elevated; this is an unusual finding and may be a useful indicator of the presence of bacterial overgrowth.

The finding of equally elevated bacterial counts after treatment in the two patients studied probably reflects the inadequacy of the sampling site in that the pathogenic organisms may have been present more distally. The presence of both oral- and fecal-type flora indicates that some of the contamination was due to organisms passing into the upper intestine during intubation. The lack of clearance of the organisms after antibiotic therapy in the face of obvious clinical improvement would seem to make it unlikely that these organisms were the cause of the diarrheal illness.

Using sensitive methodology, we were unable to detect the presence of deconjugated bile salts in the duodenum in any patient. Our findings do not support the suggestions of Gracey et al.°-21 Chalacombe et al.° and Kibby et al.° who were also unable to find evidence of bile salt deconjugation in patients with carbohydrate intolerance. It is possible that the sampling site influenced our findings and that deconjugated bile salts may have been found more distally. Rapid absorption of free bile acids, luminal dilution below detection limits, or inadequate bacterial contact time for new secretions prior to aspiration may have prohibited our finding free bile acids.°

The duodenal mucosa was normal in each patient as was the anatomy of the small intestine as determined by barium studies in the majority of children. Gracey et al.° had suggested that ultrastructural changes detected in rats with blind loops may be specific for the condition. We feel, however, that these structural changes may be artifactual as we found them to be present both before and after successful therapy.

The duodenal mucosal disaccharidase levels in our patients were well within the normal range except for sucrase depression in two patients. Despite this, all children showed biochemical evidence of lactose malabsorption by the BHT, and four of five showed malabsorbed sucrose by breath testing. We believe this demonstrates the increased sensitivity of the BHT compared with the measurement of disaccharidase levels. The BHT measures the ability of the whole small intestine to absorb carbohydrate, whereas the duodenal biopsy samples only a minute area of intestine.°

An unusual finding and one that is difficult to explain is the alteration of the lactase-sucrase ratio which differed significantly from that of normal children and also from obligate heterozygotes for sucrase-isomaltase deficiency. It is probable that...
the altered ratio is directly related to bacterial contamination because the ratio returned to normal in three patients when well. An alteration of the lactase-sucrase ratio may be an indicator of bacterial colonization of the upper intestine.

We believe this study demonstrates the value of the BHT in the diagnosis of bacterial contamination in the small intestine of children. The BHT is simple, rapid, noninvasive, and removes the necessity for duodenal intubation or anaerobic culture facilities to make the diagnosis. We have not tested for other parameters thought to reflect bacterial overgrowth, e.g., Schilling test or fat absorption, due to their reported negligible predictive value for detecting patients with high colony counts.36

It seems likely that bacterial contamination played an important role in the pathogenesis of the diarrhea in these children. After finding biochemical evidence of sucrose and/or lactose malabsorption and bacterial overgrowth, dietary manipulation alone was tried with partial improvement but without totally relieving symptoms. However, antibiotic therapy brought immediate relief in all patients, suggesting that sterilization of the upper intestine was an important factor in bringing about clinical recovery. In all patients, the BHT results for lactose and lactulose returned to normal when the patients were well. It seems unlikely that the symptoms resolved spontaneously as these children had been symptomatic for many weeks prior to treatment. The cause of the bacterial contamination in these previously healthy children remains obscure.

We did note that the transit time from mouth to cecum was significantly increased in this group compared with values for normal children or other children with diarrhea. It may be that there is some degree of stasis and this, together with an initiating insult, allows the establishment of an abnormal flora which then causes symptoms to persist.38 Coello-Ramirez et al14 have suggested that proliferation of bacteria within the small bowel of infants with diarrhea may be related to the presence of undigested carbohydrates that were present in our patients. It is possible that bacterial overgrowth may be the reason for continuing symptoms and may represent one of the causes or consequences of chronic diarrhea. Hopefully, our findings will increase the suspicion of bacterial overgrowth in young children and infants with chronic diarrhea who have been unresponsive to dietary carbohydrate restriction.

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SOME ENGLISH STATISTICS CONCERNING INFANTS FOUND DEAD IN BED (1855)

Under the section entitled “Medical Jurisprudence,” the Lancet of January 27, 1855 noted that “...we believe it may be stated as a fact that not one child out of two hundred who has been found dead in bed has lost its life in consequence of having been overlaid.” In 1855 it was commonly accepted that “overlaying” was a frequent cause of what we now term the “sudden infant death syndrome.”

Also included in this issue of the Lancet were the following statistical data concerning infants found dead in bed.

These lifeless little bodies are discovered, in at least ninety-five instances out of a hundred, after three o’clock in the morning. Not one out of a hundred such bodies is discovered dead between nine and twelve at night.

The greatest number of such bodies found dead are discovered in the months of December, January, and February; the next greatest number in September, October, and November. The spring months—namely, March, April, and May, exhibit them in the third degree; and beyond all question, the least number are found in the summer months—June, July, and August.

Of the days of the week when such bodies are found dead, the greatest number are seen on Sunday mornings, next on Monday mornings, and the fewest on Saturday mornings.

An experience of fourteen years, in a Coroner’s jurisdiction embracing between eight and nine hundred thousand souls, many portions of which are densely populated, has established the accuracy of these statements by proofs which admit of no dispute. Equally true is that out of hundreds of examples of infants found dead in bed, only two instances have been seen in which proof was conclusive that the little creature had been destroyed by the pressure of persons who had been lying with them in bed. Even in one of those cases the question might have been fairly raised, whether the signs of pressure visible on the body had not resulted from contact after death with the person who had slept with the deceased infant.

Noted by T.E.C., Jr, MD

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