Upper intestinal bacterial flora during transpyloric feeding

H D DELLAGRAMMATICA, B I DURDEN, AND R D G MILNER

Department of Paediatrics and Department of Medical Microbiology, University of Sheffield, Children's Hospital, Sheffield

SUMMARY Samples from the pharynx, stomach, duodenum or jejunum, and faeces were collected on 7 days between 1st and 28th day from neonates weighing less than 1·5 kg at birth who were fed by transpyloric tube. These were cultured on selective and non-selective media, and the results were expressed in a semi-quantitative manner. The number of bacterial species and the density of their growth increased with the patient's age; this was particularly noticeable with Gram-negative bacteria and the ratio of Gram-negative to Gram-positive organisms increased steadily in specimens from all sites with increasing age. The upper small intestine was more heavily colonised than the stomach early in life and the microflora present was predominantly faecal in nature. The species isolated from all sites were mainly aerobes or facultative anaerobes; strict anaerobes did not form a significant proportion of the microflora in these infants. Necrotising enterocolitis developed only after heavy jejunal colonisation with Gram-negative bacilli.

Since its introduction in 1970,1 transpyloric feeding has become an established alternative to gastric feeding for neonates. Challacombe, Richardson, and Anderson2 used a polyethylene nasojejunal tube in infants without gastrointestinal disease and found that prolonged intubation of the upper small intestine resulted in qualitative changes in the microflora, and in particular in a significant increase in the number of coliforms present.

The purpose of the present study was to examine the bacterial flora of the upper small intestine qualitatively and semi-quantitatively in ill neonates of very low birthweight who were fed transpylorically, to compare with their pharyngeal, gastric, and faecal flora, and to compare the results with the clinical condition of the patient. Particular attention was paid to necrotising enterocolitis, which has been reported to be more common with this method of feeding.3-5

Materials and methods

Subjects. All 19 ill neonates with birthweights <1500 g who were admitted to the intensive care unit of the Jessop Hospital for Women between November 1980 and April 1981 and in whom transpyloric feeds were started within 12 hours of birth were included in the study. All infants received systemic antibiotic therapy; other clinical details are given in Table 1. The study did not interfere with the routine clinical care of the babies, as it was standard practice for very low birthweight infants to be fed transpylorically.

Feeds. All babies mainly received a humanised formula (Cow and Gate Premium Milk). Some babies were also offered feeds of pooled heat-sterilised expressed breast milk, but for the purposes of the study they were regarded as being artificially fed.

The milk was given via a Silicon nasojejunal tube FG-5 (Vygon Ltd) as a continuous infusion controlled by a syringe pump. The syringes containing the milk were disposable and were changed every 4 hours. The nasojejunal tubes were inserted within approximately 30–40 minutes6 and were changed routinely every 4 days. The timing of insertion was determined by the clinician. The position of the tip of each nasojejunal tube was checked radiologically if a radiograph was indicated for other reasons, or by pH determination and the presence of bile in the aspirate. An orogastric tube was passed at the same time.

Bacteriological samples. Sets of four samples were collected from each baby: (1) pharyngeal swab/secretions; (2) gastric aspirate; (3) duodenal/jejunal aspirate; (4) rectal swab/meconium/faeces. This set
Table 1 Clinical characteristics of the study group

<table>
<thead>
<tr>
<th>Gender</th>
<th>Birthweight* (g)</th>
<th>Gestational age† (weeks)</th>
<th>Diagnoses</th>
<th>IPPV</th>
<th>Outcome (study period)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>640</td>
<td>26</td>
<td>RDS, apnoeic attacks, NEC, jaundice</td>
<td>YES</td>
<td>Survived</td>
</tr>
<tr>
<td>M</td>
<td>800</td>
<td>28</td>
<td>RDS, ABO isoism (E-T), acute renal failure</td>
<td>YES</td>
<td>Died</td>
</tr>
<tr>
<td>M</td>
<td>840</td>
<td>28</td>
<td>PROM, NEC</td>
<td>YES</td>
<td>Died</td>
</tr>
<tr>
<td>M</td>
<td>850</td>
<td>26</td>
<td>PROM, <em>S. albus</em> septicemia, meconium ileus</td>
<td>YES</td>
<td>Died</td>
</tr>
<tr>
<td>M</td>
<td>940</td>
<td>27</td>
<td>RDS, jaundice, IVH</td>
<td>YES</td>
<td>Died</td>
</tr>
<tr>
<td>M</td>
<td>980</td>
<td>27</td>
<td>RDS, IVH</td>
<td>YES</td>
<td>Died</td>
</tr>
<tr>
<td>M</td>
<td>1100</td>
<td>33</td>
<td>Apnoeic attacks, <em>S. albus</em> septicemia</td>
<td>NO</td>
<td>Survived</td>
</tr>
<tr>
<td>M</td>
<td>1120</td>
<td>28</td>
<td>RDS, pneumonia, NEC</td>
<td>YES</td>
<td>Died</td>
</tr>
<tr>
<td>M</td>
<td>1160</td>
<td>28</td>
<td>RDS, pulmonary haemorrhage, jaundice</td>
<td>YES</td>
<td>Survived</td>
</tr>
<tr>
<td>M</td>
<td>1180</td>
<td>28</td>
<td>RDS, <em>E. coli</em> septicemia, IVH</td>
<td>YES</td>
<td>Died</td>
</tr>
<tr>
<td>M</td>
<td>1200</td>
<td>28</td>
<td>RDS, pneumothorax, apnoeic attacks, PDA, jaundice</td>
<td>YES</td>
<td>Survived</td>
</tr>
<tr>
<td>F</td>
<td>1220</td>
<td>29</td>
<td>RDS, <em>E. coli</em> septicemia, jaundice</td>
<td>YES</td>
<td>Survived</td>
</tr>
<tr>
<td>F</td>
<td>1220</td>
<td>29</td>
<td>NEC</td>
<td>NO</td>
<td>Survived</td>
</tr>
<tr>
<td>F</td>
<td>1280</td>
<td>29</td>
<td>PROM, apnoeic attacks</td>
<td>YES</td>
<td>Survived</td>
</tr>
<tr>
<td>M</td>
<td>1280</td>
<td>30</td>
<td>Congenital pneumonia; jaundice, small IVH</td>
<td>NO</td>
<td>Survived</td>
</tr>
<tr>
<td>M</td>
<td>1320</td>
<td>29</td>
<td>RDS, pneumothorax, NEC, jaundice, PDA</td>
<td>YES</td>
<td>Survived</td>
</tr>
<tr>
<td>M</td>
<td>1340</td>
<td>30</td>
<td>Apnoeic attacks, <em>S. albus</em> septicemia, PDA, small IVH</td>
<td>YES</td>
<td>Survived</td>
</tr>
<tr>
<td>F</td>
<td>1380</td>
<td>30</td>
<td>RDS, NEC, jaundice</td>
<td>NO</td>
<td>Survived</td>
</tr>
<tr>
<td>F</td>
<td>1480</td>
<td>32</td>
<td>Apnoeic attacks, <em>S. aureus</em> septicemia, jaundice</td>
<td>YES</td>
<td>Survived</td>
</tr>
</tbody>
</table>

RDS = respiratory distress syndrome, NEC = necrotising enterocolitis, PROM = prolonged rupture of membranes, IVH = intraventricular haemorrhage, PDA = patent ductus arteriosus, E-T = exchange transfusion.

*Mean birthweight 1123 (640-1480) g; †mean gestational age 28-6 (20-33) weeks.

A total of samples was collected on days 1, 2, 3, 7, 14, 21, and 28 of life between 0900 and 1100 hours. The first set of samples was obtained before giving the first milk feed.

In order to reduce contamination of gastric and duodenal or jejunal samples with organisms proliferating within the lumen of the tube, two initial aspirates, each equal to the tube volume, were discarded from each tube on each sampling day. The samples were taken to the laboratory for culture within 10 minutes of collection. Stuart's transport medium with charcoal was used for pharyngeal and rectal swabs but not for aspirates or faeces.

**Laboratory procedure.** Each sample was seeded on to a variety of non-selective and selective media for the semi-quantitative recovery and identification of both commensal organisms and potential pathogens. The media were blood agar, MacConkey's agar, Mitis-Salivarius agar, and Sabouraud's agar incubated aerobically; heated (Cholatone) blood agar incubated in air plus CO₂; BM-kanamycin-kanamycin agar, reinforced-clostridial cotton blue agar, and Rogosa agar incubated anaerobically. The swab or loopful of the aspirate was seeded on to a sector of each plate and a sterile loop was used to streak the specimen over the plate in a standard manner. The density of growth was scored on a scale 0-5+ according to the extent of growth of each species around the streaks. The method was developed and evaluated in this laboratory for the semi-quantitative study of the bacterial flora of neonates. Growth was assessed after incubation for 24 and 72 hours. All strains isolated were purified and identified by standard conventional bacteriological methods.

Thirty-two random milk samples were also examined. Each was collected at the end of a 4-hour cycle, just before the syringe was due to be changed. They were cultured and assessed in the same manner.

**Results**

Twelve (63%) of the 19 neonates survived the study period. Sixteen (84%) neonates were ventilated and all received at least one course of antibiotics. Six (31%) neonates developed necrotising enterocolitis, 2 of whom died. Eight neonates completed the full 7 sampling days. Altogether 372 bacteriological samples were collected. Details of the organisms isolated are shown in Table 2.

The pattern of colonisation of the four sites was essentially the same. The number of different species isolated increased from all four sampling sites with increasing age as also did the density of growth. This was particularly pronounced for Gram-negative organisms. Moreover the ratio of the number of Gram-negative strains isolated to the number of Gram-positive isolates rose progressively from 0.2 to 1.25 as the samples were collected from the pharynx through to the colon and as age increased; lower scores were obtained on days 1-3 and higher scores on days 7-28 (Table 3). About half the specimens collected on day 1 (54%) and day 2 (40%) were sterile, but only 8% of those collected on days 7-28 were sterile; all the later sterile specimens were from the stomach or small intestine. If growth occurred, most of the semi-quantitative scores for specimens collected on days
Table 2  Bacterial species isolated from the gastrointestinal tract of preterm infants

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of samples from which the species was isolated</th>
<th>Pharyngeal samples</th>
<th>Gastric samples</th>
<th>Duodenal or jejunal samples</th>
<th>Rectal or faecal samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1  2  3  7  14  21  28</td>
<td>Day 1  2  3  7  14  21  28</td>
<td>Day 1  2  3  7  14  21  28</td>
<td>Day 1  2  3  7  14  21  28</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. albus</td>
<td></td>
<td>6  8  8  6  8  7  7</td>
<td>3  5  5  4  3  2</td>
<td>1  4  6  4  4  1  2</td>
<td>2  4  6  7  3  3</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td>2  3  1  1</td>
<td>2  3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>α-haemolytic streptococci</td>
<td></td>
<td>4  3  1  1  2  4</td>
<td>3  1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. faecalis</td>
<td></td>
<td>4  3  1  1  2  4</td>
<td>3  1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Non-haemolytic streptococci</td>
<td></td>
<td>1  1  2  1  1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. sulfarius</td>
<td></td>
<td>2  2  1  1  1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gram-negative aerobes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>4  3  3  3  6  6  9</td>
<td>4  2  2  5  5  7  5</td>
<td>4  3  2  5  6  9  8</td>
<td>4  2  4  8  9  9  8</td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em></td>
<td></td>
<td>2  1  1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td></td>
<td>1  2  1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anaerobes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusobacterium sp.</td>
<td></td>
<td>1  1  1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bifidobacterium sp.</td>
<td></td>
<td>1  1  1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clostridia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionibacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida sp.</td>
<td></td>
<td>1  1  3  2  1  1</td>
<td>1  1  1  1  1  1</td>
<td>1  1  1  1  3  3  2</td>
<td>1  1  1  3  3  3  2</td>
</tr>
<tr>
<td>Sterile</td>
<td></td>
<td>8  5  2</td>
<td>1  1  1  1  1  1</td>
<td>8  5</td>
<td></td>
</tr>
<tr>
<td>Total number of samples</td>
<td></td>
<td>19  17  14  12  10  11  10</td>
<td>19  17  14  12  10  11  10</td>
<td>19  17  14  12  10  11  10</td>
<td>19  17  14  12  10  11  10</td>
</tr>
</tbody>
</table>
Table 3. Ratios of Gram-negative to Gram-positive organisms isolated from the four sampling sites during the 7-28 days postnatal period.

<table>
<thead>
<tr>
<th>Days</th>
<th>Pharyngeal aspirate</th>
<th>Duodenum or jejunal aspirate</th>
<th>Rectal washout</th>
<th>Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>1/2 (0.5)</td>
<td>2/3 (0.67)</td>
<td>3/4 (0.75)</td>
<td>4/5 (0.8)</td>
</tr>
<tr>
<td>4-7</td>
<td>1/3 (0.33)</td>
<td>2/3 (0.67)</td>
<td>3/4 (0.75)</td>
<td>4/5 (0.8)</td>
</tr>
<tr>
<td>8-14</td>
<td>1/4 (0.25)</td>
<td>2/3 (0.67)</td>
<td>3/4 (0.75)</td>
<td>4/5 (0.8)</td>
</tr>
<tr>
<td>15-21</td>
<td>1/5 (0.2)</td>
<td>2/3 (0.67)</td>
<td>3/4 (0.75)</td>
<td>4/5 (0.8)</td>
</tr>
<tr>
<td>22-28</td>
<td>1/6 (0.16)</td>
<td>2/3 (0.67)</td>
<td>3/4 (0.75)</td>
<td>4/5 (0.8)</td>
</tr>
</tbody>
</table>

Discussion

The gastrointestinal tract at birth is thought to be sterile, but within a few days, the mouth and the colon acquire a normal flora. The presence of a normal flora is essential for the normal development of the infant. We were unable to recover significant numbers of bacteria from the ileum or rectum of the very preterm infant, as the ileum is the very preterm infant, and the rectum is the mature infant. We were also unable to recover significant numbers of bacteria from the ileum or rectum of the very preterm infant, as the ileum is the mature infant. The ileum was the only site where significant numbers of bacteria were recovered from the very preterm infant.

Our results show that in the very preterm infant, the ileum is the predominant site for bacterial growth. This is in contrast to the mature infant, where the duodenum and jejunum are the predominant sites for bacterial growth. The ileum is the predominant site for bacterial growth in the very preterm infant, but the duodenum and jejunum are the predominant sites for bacterial growth in the mature infant.

In the very preterm infant, the intestinal flora is predominantly anaerobic, with a predominance of Gram-negative bacteria. This is in contrast to the mature infant, where the intestinal flora is predominantly aerobic, with a predominance of Gram-positive bacteria.

The growth of bacteria in the ileum is thought to be dependent on the presence of a factor in the ileum, which is not present in the mature intestine. This factor is likely to be a growth factor for bacteria, but its exact nature is unknown.

The ileum is the only site where significant numbers of bacteria were recovered from the very preterm infant, as the ileum is the mature infant. The ileum was the only site where significant numbers of bacteria were recovered from the very preterm infant, as the ileum is the mature infant. The ileum was the only site where significant numbers of bacteria were recovered from the very preterm infant, as the ileum is the mature infant.
study faecal flora in term healthy neonates in
whom a luxuriant growth of anaerobic organisms
was obtained. The experience of Graham et al. was similar. They found that in ill low birthweight
neonates anaerobic organisms did not achieve the
importance found in normal infants.

A significant resident upper intestinal Gram
negative flora could lead to impaired assimilation of
feeds. This has been reported in nasojejunal-fed babies but other investigators did not substi-
tuate this finding. However, the Gram-negative flora could play a contributory role in the
previously reported increased incidence of necrotis-
ing enterocolitis in these babies. In the present
study group, the incidence of necrotising enterocolitis was high for reasons which are not immediately
apparent. This group of babies was essentially
artificially fed but the use of unheated raw human
milk may offer some immunological advantages.

In preterm infants the feeding of raw human milk
does not reduce the numbers of colonies in the
faeces, but other factors acting in the gut lumen—
for example the anti-adherent effect of secretory IgA
on \textit{E. coli}—may be due to the beneficial effects of
raw human milk. The very slow, cautious initia-
tion of enteral feeds during the acute illness in
these neonates may also be important in preventing the
condition.

More studies on the upper gut flora in neonates
of very low birthweight are necessary as until now
all conclusions and assumptions have been extra-
polated from studies on older infants and children
under very different conditions from those en-
countered by the very low birthweight newborn baby.

We thank the microbiology laboratory staff,
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care unit, Jessop Hospital for Women, Sheffield, for
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the babies.

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Hospitals.

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Correspondence to Dr H D Dellagrammaticas,
2nd Department of Paediatrics, University of
Athens, Aglaia Kyriakou Children’s Hospital,
Athens 617, Greece.

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