The Intestinal Microflora in Malnutrition and Protracted Diarrhea in Infancy

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The gastrointestinal tract has a unique place in human ecology. After birth it is continually exposed to potentially harmful external agents—infective, toxic, and antigenic. In various disease states these factors upset the normally delicate ecological balance between the human host and the environment; many of these diseases are characterized by diarrhea in infancy and early childhood. This chapter concerns itself with childhood diarrheal disorders associated with abnormalities of the upper intestinal microflora, with particular emphasis on children with malnutrition and protracted diarrhea.

GASTROINTESTINAL DEVELOPMENT AND BACTERIAL COLONIZATION

*In utero*, the gastrointestinal tract develops in a protective, sterile environment, and at birth it is a sterile organ. Microbial colonization begins when the baby is exposed to its mother and her indigenous flora and to the environment. This leads to the development of a characteristically well-controlled gastrointestinal microflora. One-quarter of infants acquire their fecal flora from their mothers (1), and by the second day coliforms, lactobacilli, and enterococci make up the rapidly establishing fecal flora, with total populations about $10^8$ bacteria per gram (2). By the third day of life, *Bacteroides* sp. are becoming established and can be detected even earlier in about one-quarter of healthy babies delivered *per vaginum* and given formula feeds (3). By day 5, the characteristic Y-branched (bifidus) lactic acid bacteria are present and rapidly reach populations of about $10^8$ to $10^9$/g. Bifidobacteria predominate in breast-fed infants through the influence of the so-called "bifidus factor," which is apparently specific and helps implantation and persistence of *B. bifidum* in the infant intestine. This is important because breast-fed infants have a natural defense against *Escherichia coli*, *Bacteroides*, and *Clostridium*, which helps protect against gastroenteritis (4).

It is widely believed that *Bifidobacterium* sp. help establish local, intraluminal conditions that inhibit the growth of those potentially pathogenic bacteria. Long and Swenson (3) found *Bacteroides fragilis* in only 22% of 1-week-old breast-
fed babies compared to 61% of formula-fed infants of the same age. Mata et al. (5) also found *Bacteroides fragilis* to be uncommon in Guatemalan infants before weaning. Colonization with anaerobes seems to be at least temporarily dependent on the presence of other bacteria in the first days of life, but these interrelationships are inadequately documented and poorly understood.

Much attention has been given to normal colonization by bifidobacteria and lactobacilli. The lactic acid bacteria have been considered to be beneficial to the human gastrointestinal microflora by suppressing the proliferation of pathogenic fecal microorganisms, and preparations of lactobacilli have been used widely in therapy of gastrointestinal disorders (6). Although these organisms, particularly *Lactobacillus acidophilus*, have significant antibacterial activity, it is not clear whether this is because of production of specific antibacterial substances, reduction in the intraluminal pH through synthesis of lactate inhibiting the growth of coliforms, or inhibition of adherence and colonization, or through some other undefined mechanism such as neutralization of bacterial enterotoxins (7). Their role in microbial interactions in healthy individuals and their potential benefits in gastrointestinal disorders, including childhood gastroenteritis, are not yet firmly established.

There is an immense array of microorganisms in the gastrointestinal tract, even after the first few days of life. These include gram-positives such as staphylococci and streptococci, the enterobacteriaceae such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Pseudomonas* sp., a wide range of anaerobes and yeasts and viruses. In the first days of life, aerobic and anaerobic populations are roughly equal in feces and amount to about $10^8$ to $10^{10}$/g wet weight. After several weeks the anaerobes become the dominant group (2) and remain so in later life.

Considering the wide range of aerobic and anaerobic bacteria to which the gut is exposed from the environment, there is a remarkably constant pattern of colonization in neonates, which suggests a finely organized regulatory mechanism; the oxidation–reduction potential ($E_h$) may play an important role in this mechanism. In neonates, the $E_h$ is positive (+200 mV), but later it becomes negative and in adults varies from $-250$ to $-350$ mV, so the environment is strictly anaerobic, and only energetic reactions possible at this low level will occur. Bacteria such as *Escherichia coli* and the enterococci can grow in positive redox levels, and their growth and fermentation would result in a drop of the $E_h$ to a negative level, at which other, mostly anaerobic, bacteria would colonize. Undoubtedly, there are other factors that help determine colonization patterns in the early days of life. For example, inhibition of growth of *Shigella* by *E. coli* occurs through production of acid by *E. coli*, and *Bacteroides* sp. produce bacteriocins, which inhibit growth within their own species (9). *Bacteroides* sp. are also able to degrade and ferment mucins, glycogen, and plant polysaccharides, which are important in the patterns of development of the microecology within the gut lumen.
MICROBIAL AND HOST INTERACTIONS

There are numerous, very complex interactions between microorganisms within the gut lumen and between those microorganisms and the human host. These interactions involve stimulation of the local immunity in the gastrointestinal tract. Berg (10) has shown that the very high rate (100%) of translocation of viable \textit{E. coli} from the gut to mesenteric lymph nodes in gnotobiotic mice monocontaminated with \textit{E. coli} was reduced drastically (to 0%) when animals were colonized with \textit{E. coli} plus the whole cecal flora from specific pathogen-free mice. Treatment of animals with antibiotics (penicillin, metronidazole, or clindamycin) increased gut populations of enteric bacteria such as \textit{E. coli} and promoted their translocation to the mesenteric nodes. The indigenous microflora thus has an important defense mechanism helping to confine microorganisms to the gut lumen. Immunosuppression (e.g., by methotrexate, cytosine arabinoside, cyclophosphamide, and prednisone) also helped to promote bacterial translocation to the mesenteric nodes. Thymectomy had a similar effect on the movement of aerobes and anaerobes; similarly, athymic (\textit{nu/nu}) mice have many more gut microorganisms in their mesenteric nodes, spleens, livers, and kidneys than do heterozygous (\textit{nu/+}) mice.

Microorganisms also stimulate the production of specific antigens and antibodies (e.g., blood-group-specific antibodies), and although protective antibodies transferred from mother to newborn may prevent illness, it must not be assumed that the fetus and neonate are immunologically incompetent (11). The extent and efficacy of passively acquired immunity are not well understood, but there is some evidence that infants infected, or challenged, with coliforms are able to stimulate antibody production by their mother's mammary glands (12). Other interactions to be considered include the transfer of plasmids between bacterial cells and strains and the effect of enteric pathogens on the composition of the normal gastrointestinal microflora (13).

DIET

Diet, not surprisingly, has a profound effect on the intestinal flora, particularly in early life. As long ago as 1900, it was shown that breast-fed babies harbor bifidobacteria whereas artificially fed infants have mainly gram-negative bacteria (14,15). By the end of the first week of life, the fecal flora of breast-fed babies has more than 90% \textit{Lactobacillus bifidus}; in addition, they also have coliform bacteria in similar numbers to those found in normally fed older children and adults. Other bacteria, including gram-negative anaerobes and gram-positive bacteria, are present in relatively small numbers. When infants are changed from breast to bottle feeding, a marked change occurs in their fecal flora; the main difference is the emergence of \textit{Bacteroides} sp., clostridia, and \textit{E. coli} in large numbers; aerobic enterobacteriaceae (e.g., \textit{Klebsiella}, \textit{Enterobacter}), en-
terococci, and staphylococci are consistently present in larger numbers than they are in breast-fed infants (16,17). Breast milk also favors the growth of bifidobacteria including *B. bifidum*, *B. longum*, and *B. infantis* in *vitro*; this is a selective influence of human milk, which does not occur with other mammalian milks and is considered to be related to a specific factor known as BB factor (4). This factor is stable to heat and irradiation and, thus, is probably nonprotein by nature.

The biochemical environment in the gut lumen also has an important influence on the development of the bifidus flora. β-Lactose of human milk seems to be most important, and a lactose concentration of at least 1.2% is crucial. The addition of 1 to 2% lactulose to artificial formulas encourages the growth of a flora similar to that in breast-fed infants, so that artificial milk preparations resembling human milk promote a similar flora (18).

Breast feeding also influences other components of the intestinal flora. Breast-fed babies have fewer *E. coli* serotypes and apparently lack *E. coli* containing the K1 antigen; there are also fewer types of *Klebsiella* and other enterobacteriaceae (19). This probably helps to protect breast-fed infants from a range of potential bacterial pathogens.

The perinatal gastrointestinal microflora can, therefore, be seen as the end result of numerous interacting factors involving the neonate, its gastrointestinal tract, and the microbes with which they come in contact continually. The factors involved in maintaining this delicate balance in health are discussed below in more detail. It is important to realize that at least some of these, e.g., gastric acid secretion and intestinal peristalsis, are inadequately developed in early life. This makes even more remarkable the success with which the balance between the gut flora and its host is kept in healthy individuals.

**CONTROL OF THE GASTROINTESTINAL MICROFLORA**

After becoming established in the first days and weeks of life, the gut microflora is strictly controlled, and there are important regional variations in the distribution of bacteria along the length of the alimentary tract. After meals there is a temporary wave of ingested microorganisms (20), but in the fasting state there are some broad generalizations that can be made after these “transients” are cleared from the lumen.

The upper limit of the bacterial populations in upper intestinal secretions is generally accepted as $10^3$ to $10^4$/ml, i.e., “relatively sterile.” Gram-positive bacteria, such as streptococci, staphylococci, and diphtheroids, and yeasts predominate in the upper intestinal lumen. Distally along the small intestine, the total microbial populations increase, and coliforms appear, with the total population in the terminal ileum about $10^5$ to $10^8$/ml. At the lower end of the small intestine the ileocecal valve acts as an effective “trap door,” and distal to it, the bacterial populations increase dramatically to $10^9$ to $10^{11}$ organisms per gram of feces;
here anaerobes predominate, e.g., Clostridia, Bacteroides, and anaerobic lactobacilli (21,22).

**Gastric Acid**

Secretion of hydrochloric acid by the gastric mucosa is an important factor controlling the gastrointestinal microflora. Achlorhydria can be associated with bacterial overgrowth in the upper gut and a tendency to gastrointestinal infections (23,24). Gastric acid secretion is one of several human gastrointestinal functions that are poorly developed in the first days and weeks of life, so relative hypochlorhydria may predispose young and premature infants to intestinal bacterial overgrowth and gastrointestinal infections. Malnourished infants have low fasting gastric acid secretion and very reduced secretory responses to stimulation of the gastric mucosa by pentagastrin (25); this is associated with chronic atrophic gastritis similar to the histologic abnormalities that occur in the jejunal mucosa in malnourished children in developing countries (26).

Reduced fasting and poststimulation gastric acid responses also occur in experimental protein deprivation (27) and may be important in poorly nourished, small-for-dates babies by removing one of the normal controlling influences over the upper gut flora and allowing excessive colonization in the upper intestinal lumen.

**Gastrointestinal Motility**

The normal intestinal motility is a major factor controlling the upper intestinal microflora and preventing bacterial overgrowth (21,28,29). Intestinal stasis and overgrowth in the so-called “blind loop syndrome” (30) is the classic example of the consequences of loss of this normal physiologic “housekeeper,” which keeps the intestinal lumen clean by the regular, propulsive activity of the interdigestive motor complex (31,32). Vantrappen and his colleagues (33) have shown that normal humans have a readily identified uninterrupted burst of rhythmic contraction waves that progress rapidly down the intestine to be followed by a period of quiescence. In patients with upper intestinal bacterial overgrowth, there appears to be a specific disorder of motility characterized by complete or almost complete absence of the interdigestive motor complex. Coordinated intestinal peristaltic activity develops late in utero and may not be fully organized until the eighth month (34); this may be an important factor in microbial colonization and overgrowth in premature and newborn babies.

**Mucosal Defense Mechanisms**

There are several nonspecific local mechanisms that help to protect the mucosa against microbial invasion. These include regeneration of mucosal cells and
regular replacement of the surface glycoproteins (35). The glycocalyx is mostly carbohydrate attached to surface enzymes that are vulnerable to degradation by proteolytic or detergent activity (36), which may be reflected in mucosal damage and depression of mucosal enzyme activities in many diarrheal diseases of infants and children. Some bacteria are able to remove surface enzymes from the surface membrane (37); Bacteroides sp., for example, secrete proteases that can markedly decrease human brush border sucrase and maltase, although many other bacterial species, including Clostridium, anaerobic lactobacilli, E. coli, Klebsiella sp., and Proteus sp. apparently lack this ability (38). The enzymes involved appear to be bacterial proteases with elastase-like activity, characteristic of enzymes that split disaccharidases from the brush border (39,40).

Mucus secretion by the intestinal mucosa is an important local nonspecific protective factor that acts against physicochemical and microbiological injury. Other factors include products of bacterial metabolism and bile salts, which are known to inhibit the growth of bacteria, including anaerobes, in vitro, although their physiological role in vivo is unclear (41).

Immune Mechanisms

All classes of immunoglobulins are present in intestinal secretions, but the dominant one is IgA in its dimeric form. Secretory antibodies are important in opsonization, complement fixation, and immune exclusion at the mucosal surface (42). These secretory, mucosal antibodies have been referred to as "antiseptic paint," protecting the body at the intestinal mucosa from infective, toxic, and antigenic agents from the environment. Williams and Gibbons (43) showed impaired adhesion of Streptococcus viridans to epithelial cells after exposure of the organisms to specific secretory IgA antibodies, which apparently block specific binding sites on the bacterial cell wall and thereby inhibit bacterial adherence to epithelial surfaces. This would inhibit colonization and assist clearance of the bacteria by the surface secretions.

Intestinal antibodies also protect against toxic bacterial metabolites, such as enterotoxins. Secretory antitoxins complexing with cholera toxin can prevent binding of the toxin to mucosal receptors and thus interfere with activation of mucosal adenylate cyclase, which mediates toxin-induced net fluid and electrolyte secretion, which causes diarrhea (44). Many microorganisms are now known to be enterotoxigenic, and some enteric bacteria, including E. coli, elaborate a heat-labile toxin (LT) that is immunologically similar to cholera toxin (CT). This immunological cross reactivity affords protection to toxins produced by other bacterial species, e.g., Aeromonas sp. (45), at least experimentally. This suggests that it might be possible to achieve immunological protection against a broad range of enterotoxigenic diarrheas. These responses are related to the nature of the antigenic stimulus and its mode of administration; direct immunization is much more effective than parenteral immunization, and the stomach significantly impairs priming by toxins via the oral route. A variety of
cell-mediated immune responses in the gastrointestinal mucosa are important in antibacterial protection; these have been reviewed elsewhere (45a).

THE CONTAMINATED SMALL BOWEL SYNDROME IN CHILDREN

Upper intestinal bacterial overgrowth occurs in a range of clinical situations, which can be classified according to the type of defect and disorder and its associated underlying abnormality (see Table 1).

Major clinical features of the contaminated small bowel syndrome include diarrhea, intestinal malabsorption, and, in children, failure to thrive. The syndrome has been reviewed recently by several authors (46-51). Rather than commenting on all the clinical settings and variations, further discussion focuses on intestinal bacterial overgrowth in malnourished children with diarrhea and its possible role in the chronic, nonspecific diarrheal disorders that are included in the spectrum of diseases called by some “intractable diarrhoea of infancy” (52).

<table>
<thead>
<tr>
<th>Type of defect</th>
<th>Underlying abnormality</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Congenital</td>
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<tr>
<td>Obstruction</td>
<td>Total</td>
<td>Agenesis, atresias, volvulus</td>
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<td></td>
<td>Partial</td>
<td>Diaphragms, bands, volvulus</td>
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<td></td>
<td>Other lesions favoring stasis</td>
<td>Diverticula</td>
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<td></td>
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<td>Duplications</td>
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<td>Cysts</td>
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<td>Acquired</td>
<td>Surgical</td>
<td>Functional postoperative stasis</td>
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<td></td>
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<td>Anatomical postoperative obstruction (e.g., adhesions)</td>
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<td></td>
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<td>Short-gut syndrome</td>
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<td></td>
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<td>Fistulous connections between the small and large intestines</td>
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<td>Interruption of the enterohepatic bile salt circulation</td>
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<td></td>
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<td>Removal of the ileocecal valve</td>
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<tr>
<td></td>
<td>Others</td>
<td>Benign or malignant tumors obstructing the gut or mesentery</td>
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<td></td>
<td></td>
<td>Chronic infections (e.g., TB) causing intestinal or mesenteric obstruction</td>
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<td></td>
<td>Portal venous congestion (?) with chronic liver or cardiac disease</td>
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<tr>
<td>Infective</td>
<td>Bacterial by-products (e.g. bile salts and enterotoxins)</td>
<td>Acute gastroenteritis in small infants</td>
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<td></td>
<td></td>
<td>Postgastroenteritis malabsorption</td>
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<tr>
<td></td>
<td></td>
<td>Protein–energy malnutrition</td>
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<tr>
<td>Nutritional</td>
<td>Multifactorial</td>
<td>Protein–energy malnutrition</td>
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<td></td>
<td></td>
<td>(?) Prematurity and intrauterine growth retardation</td>
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From Gracey (41), with permission.
Childhood Malnutrition

Twenty-five years ago Smythe (53) demonstrated changes in the intestinal bacterial flora in kwashiorkor. In the mid-1960s, children dying from malnutrition were shown to have very large numbers of bacteria in their upper intestinal contents, but those studies were done on postmortem specimens (54). In 1972, studies on jejunal aspirates of malnourished Australian Aboriginal and Guatemalan children showed that they had significant bacterial contamination in vivo (55,56). This finding has been confirmed in other intubation studies done in Africa (58,59) and southeast Asia (60). However, James et al. (61) found no correlation between the numbers or types of bacteria in jejunal contents of Jamaican subjects in relation to their nutritional state, but this may be the result of methodological differences in collection, storage, and transportation of their specimens, which were flown to England for microbiological studies. It is extremely difficult to get adequate “control” specimens of jejunal contents from healthy children, e.g., of the elite, living in good conditions in developing countries (62). The question, therefore, remains unanswered whether the upper gut microflora in such children is comparable to the reported “control” populations studied in Western countries, where the environmental conditions are so different from those in developing countries. This is an important area for further study, because environmental factors are likely to be very important in determining patterns of microbial colonization in the upper reaches of the gastrointestinal tract.

We have previously shown that oropharyngeal secretions from undernourished children living in grossly contaminated environments have abnormally high rates of isolation of fecal organisms and enteric pathogens (63,64). This probably reflects constant exposure to fecal contamination, since enteric pathogens such as Salmonella can be frequently isolated from water supplies in contaminated environments (65). Studies from Africa (58,66,67) and our own studies of undernourished Aboriginal communities living in remote, semidesert areas in the tropical north of Western Australia show that contamination of weaning foods and water supplies are important problems in such populations where undernutrition is common. This probably helps to explain the presence of large numbers of fecal bacteria in oropharyngeal and upper intestinal secretions.

Impaired gastric acid secretion has been observed in malnourished children in association with chronic atrophic gastritis (25), which resembles, histologically, the appearance of the damaged upper intestinal mucosa in childhood malnutrition. This removal of the normal gastric acid defense mechanism against invading microorganisms and the high rate of exposure to fecal bacteria from the environment help to explain the occurrence of upper intestinal bacterial contamination in malnutrition. Other defense mechanisms mentioned above will also be compromised and contribute to the establishment of abnormal bacterial populations in the upper gut; these include local immune mechanisms and the integrity of the gut mucosa itself, which is often extensively damaged.
by repeated and chronic bacterial, viral, parasitic, and fungal infections. Post-enteritis protein intolerance might also contribute to mucosal abnormalities. The suggestion has also been made that the presence of an intestinal infection, e.g., viral gastroenteritis, can help the propagation of different enteric pathogens such as *E. coli* (68).

In all, the available information suggests that numerous interacting factors combine in malnourished children to encourage microbial contamination of the upper gut as another expression of the well-known synergism of malnutrition and infection (63). Malnutrition itself causes histological abnormalities in the upper gut mucosa and alterations to its immunological defenses; constant infectious challenge from the environment, particularly at the time of weaning, and the damaging effects of gut infections and postgastroenteritis mucosal damage set the stage for bacterial colonization, diarrhea, and malabsorption to become characteristic of childhood malnutrition.

**CHRONIC UNEXPLAINED DIARRHEA OF INFANCY**

Many infants and young children have diarrheal illnesses without an easily identifiable cause. These are included in the so-called "intractable diarrhoea" group, which has been linked with almost 40 underlying diseases (52). It must be realized, then, that we are not dealing with specific diarrheal diseases but a group of illnesses in which diarrhea is not readily explained.

Many of these illnesses will be infective by nature; recently the spectrum of infectious diarrheas has been widened by the discovery of additional causes of these diseases. Major advances were made by showing that viruses and enterotoxigenic bacteria caused previously undiagnosed episodes of childhood diarrhea. Their role as causes of previously unexplained chronic childhood diarrhea is yet to be determined. Techniques are now available to allow this to be done using carefully planned epidemiological studies combined with comprehensive microbiological investigations.

In 1965 Valerie Burke and her colleagues (69) investigated 12 babies with "refractory diarrhea" following an illness characteristic of acute gastroenteritis; repeated intestinal biopsies were done on nine of these patients. All patients had sugar intolerance when diarrhea was present, and initial biopsies showed variable degrees of histological damage with villous shortening, blunting, and inflammatory infiltration of the lamina propria. Assays showed depression of mucosal enzyme activity, particularly of lactase. Diarrhea subsided following exclusion of lactose from the diet, and the mucosal abnormalities and lactase activity recovered after an interval of 1 to 18 months. This was the first published report of secondary disaccharidase deficiency with intestinal disaccharidase assays. Many subsequent reports have confirmed temporary mucosal damage and disaccharidase depression following gastroenteritis.

Barnes and Townley (70) made similar observations in 31 infants with gastroenteritis, and the same group (71) found overgrowth of the upper intestinal
secretions by *Candida albicans* in patients with depressed lactase activity. Studying a group of infants with "persistent postenteritis diarrhea," Zoppi et al. (72) found mucosal damage with clubbing and shortening of villi and mild to severe infiltration of the lamina propria with lymphocytes and plasma cells. Rossi et al. (73) also found mucosal injury with depressed disaccharidases in a group of 30 infants, two-thirds of whom took 6 months to recover.

The pathogenesis of childhood gastroenteritis has become better understood in the past decade through studies of the infecting microorganisms and how they cause diarrhea. Enterotoxigenic bacteria cause disease through several mechanisms. One is production of a colonization factor antigen (CFA), which facilitates bacterial adherence to the intestinal mucosal surface. Candy et al. (74) used the buccal epithelial cell (BEC) assay to study an outbreak of acute diarrhea in children. A known adhesive strain of *E. coli* (01:K1:H7) adhered significantly more frequently to BEC from infants with protracted diarrhea than from children with acute diarrhea, healthy infants, and healthy adults. They also found that enterobacteria isolated from the patients with protracted diarrhea were more likely to adhere to their own BEC than to the BEC from healthy adults. Preliminary studies with intestinal biopsy specimens suggest that the enterocytes from patients with protracted diarrhea are also more adhesive, and this may help to explain why bacterial overgrowth of the small gut is common in infants with protracted diarrhea. A recent, detailed case study has shown that some *E. coli* that are not invasive or enterotoxigenic may damage the gastrointestinal epithelium by adhering to it, causing villous blunting and inflammatory infiltration in the lamina propria (75); perhaps this occurred with some of the original patients studied by Burke et al. (69).

Many *E. coli* produce either a heat-labile enterotoxin (LT) or a heat-stable toxin (ST), and some bacteria are capable of producing both. Cholera toxin and LT stimulate intestinal mucosal adenylate cyclase, which leads to increased mucosal levels of cyclic AMP and thus to net fluid and electrolyte loss and diarrhea; there is some evidence that with ST, guanylate cyclase is involved (76). Identification of enterotoxigenic bacteria has been hampered by the lack of simple, reliable, portable diagnostic methods and has needed complex techniques such as cell culture, the suckling mouse assay, or ligated ileal loop methods, which are mostly unavailable in developing countries except for some research institutions. However, reports from several tropical centers (77–80) suggest that toxigenic bacteria may be a major cause of the diarrheal diseases of children seen in those regions.

The scope for toxin production by intestinal bacteria is likely to be much more extensive than recognized only a few years ago and is certainly not confined to *E. coli*. An illustrative example is the recent finding that enterotoxigenic *Aeromonas* sp. are important, previously unrecognized, causes of bacterial diarrheas (81). They were isolated from more than 10% of children with diarrhea and less than 1% of children without diarrhea in a prospective study involving over 1,000 children with gastroenteritis in Perth, Australia, and are the com-
monest causes of bacterial diarrheas in children in our community. Enterotoxigenic strains of \textit{E. coli}, \textit{Aeromonas hydrophila}, and other enterobacteria isolated from intestinal contents of malnourished children have been shown in experiments in our laboratory to interfere with intestinal fluid and electrolyte transport \cite{80}; this is likely to be a significant cause of watery diarrhea and, perhaps, protracted diarrhea in children with malnutrition. Other newly found bacterial causes of gastroenteritis include \textit{Campylobacter}, \textit{Yersinia}, and \textit{Clostridium difficile}.

Apart from specific gastrointestinal infections, infants with protracted diarrhea have an abnormally profuse, mixed microbial flora in the upper gut, predominantly coliforms \cite{82-84}. The precise pathogenic significance of this microbiological abnormality is uncertain. It has been suggested that microbial degradation of bile salts might be an important mechanism because of the harmful effects of deconjugated bile salts on intestinal absorption \cite{82,85}, but it is likely that the capacity of many enteric bacteria to produce toxins might be just as important \cite{80}, particularly in relation to watery diarrhea.

At this stage we should recall that bacterial contamination of upper intestinal secretions has extensive, deleterious effects on intestinal digestion and absorption and on the integrity of the small intestinal mucosa; these and their pathogenesis have been the subject of several recent reviews \cite{21,22,41,46,47}. The main digestive and absorptive abnormalities are steatorrhea, carbohydrate malabsorption, hypoproteinemia, vitamin B\textsubscript{12} malabsorption, and net intestinal loss of fluid and electrolytes. Combined, these abnormalities contribute to diarrhea and malabsorption in the contaminated small bowel syndrome. In children with undernutrition and protracted diarrhea, they are potent causes of wastage of scarce nutrients and worsening of the nutritional state.

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