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Infectious diarrhoea in tropical and
subtropical regions

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Diarrhoeal diseases are among the most important health problems in the
world today. While they are generally mild, nuisance illnesses in the developed
world, where clean water and sanitation are adequate, these diseases are the
most common cause of death in young children in the developing world, where
clean water is not available and sanitation is inadequate. In these areas, up to
25% of children may never reach their fifth birthday. In addition, although the
impact on growth and nutritional status is difficult to quantify, the cumulative
toll on overall child health is enormous. A wide variety of infectious agents are
implicated in the aetiology of acute diarrhoeal illness, many of which were
unknown 20 years ago. The epidemiology, pathogenic mechanisms and
clinical presentation of the common bacterial and viral pathogens, as they are
currently understood, will be discussed in this chapter.

ESCHERICHIA COLI

Although *E. coli* are normal inhabitants of the human and animal gut, they are
also capable of causing serious diarrhoeal disease. Since the 1940s, enormous
strides have been made in understanding the role *E. coli* play in causing
intestinal disease in humans and animals, and while many controversies
remain unresolved, there currently appear to be at least three major groups of
E. coli that are associated with different types of diarrhoeal illness. These
groups, the enterotoxigenic *E. coli* (ETEC), the enteropathogenic *E. coli*
(EPEC) and the enteroinvasive *E. coli* (EIEC), are all known to cause disease
throughout the world.

Enterotoxigenic *E. coli* (ETEC)

Epidemiology

ETEC, since their recognition in the early 1970s, have been found to be one of
the leading causes of diarrhoea in children in the developing world, and may

be related to 30–40% of diarrhoea overall. The number of cases for the individual toxins seems to vary in different geographical locations, although the reasons for this variation are not clear. Heat-labile (LT), cholera-like toxin is seen in 3–44% of cases of diarrhoea in the developing world, heat-stable (ST) toxin in 2–14% and combined LT/ST in 7–9% (Guerrant et al, 1975; Black et al, 1980; Reis et al, 1982). While LT is isolated from healthy controls, ST and combined LT/ST are less frequently isolated from asymptomatic individuals. ETEC strains are also frequently isolated from travellers throughout the developing and tropical world. ETEC strains can be routinely isolated from foods and from water supplies in endemic areas.

Pathogenesis

The human enterotoxigenic *E. coli* are further subdivided into those strains that produce heat-labile (LT) toxins and those that produce heat-stable (ST) toxins. Recently, a second heat-labile toxin has been described that has the same secretory effect as the original heat-labile toxin, but is antigenically distinct. The original heat-labile toxin of *E. coli* is antigenically similar to cholera toxin and similarly produces disease by the stimulation of adenylate cyclase. The second and more recently described heat-labile toxin is antigenically distinct from cholera toxin and its effects in assays are not neutralized by anti-cholera toxin. While its effects also appear to be mediated by cyclic AMP, it does not act through the GM1 ganglioside (Guth et al, 1986).

  E. coli heat-stable toxin is a much smaller, poorly antigenic protein that binds rapidly to intestinal mucosa and promptly stimulates guanylate cyclase, resulting in increased cyclic GMP and intestinal secretion. It remains unclear what precise mechanism heat-stable toxin uses for secretion, since there is no apparent role for phospholipase A2, phospholipase C, calcium, calmodulin, oxygen radicals or substrate methylation in ST-related disease (Greenberg and Guerrant, 1985).

  A second heat-stable toxin, called STb, has been described. The mechanism of action of STb is unclear, although it does not seem to be mediated by cyclic nucleotides. STb has thus far only been found to cause disease in piglets and screening for STb in human *E. coli* strains from Brazil and Bangladesh has been unrewarding (Weikel et al, 1986).

  Heat-labile toxin and heat-stable toxin may cause disease individually, or they may be found together in a strain of *E. coli*. Combined LT/ST disease appears to be more severe than either alone, and ST disease may be more severe than LT. Adults in endemic areas appear to acquire immunity to LT, but effective immunity does not develop to the more poorly antigenic ST.

Clinical diagnosis

The severity of ETEC disease may range from totally asymptomatic infection to a syndrome approaching full-blown cholera. Abdominal cramps, abdominal pain and fever may be present. The degree of dehydration varies with the severity of the disease. Usual disease has an incubation period of 5–9 days and

a duration of 5–7 days, with as many as 10 watery stools per day at the peak of disease.

With ETEC disease, stool microscopic examination should not reveal white blood cells or erythrocytes.

Since *E. coli* are ubiquitous in the gastrointestinal tract, isolation of lactose-fermenting organisms from the stool of a symptomatic patient is not helpful. Biochemical determinants do not differentiate toxigenic from non-toxigenic strains. As disease is self limited, further diagnostic work-up is neither warranted nor cost-effective in a routine, sporadic case. Detection of LT has been classically done in 18-hour rabbit ligated loops, but may also be done in tissue culture assays. Rates of detection are similar for the Chinese hamster ovary cell elongation assay (Guerrant et al, 1974) and the Y-1 adrenal cell rounding assay (Sack and Sack, 1975). Enzyme-linked immunoabsorbent assays (ELISA) have been as successful in detecting LT as the tissue-culture assays. DNA hybridization assays by oligonucleotide probes, which are both sensitive and specific, are becoming available commercially, but would be cumbersome to perform in a routine clinical laboratory (Moseley et al, 1980).

Assays for STa have been classically done in the suckling mouse model, although rabbit ligated ileal loops at six hours will also be positive for STa. ELISA for STa is available as a research tool, as are DNA hybridization probes for both STa and STb.

Clinically the recognition of watery, noninflammatory diarrhoea is sufficient to institute appropriate therapy, which is adequate rehydration, by oral solutions if at all possible.

Enteropathogenic *E. coli* (EPEC)

Epidemiology

Disease caused by the EPEC ('attaching and effacing') organisms is seen in children, although from volunteer studies it is clear that adults may develop disease. In the developed world, outbreaks of EPEC disease are usually seen in newborn nurseries or other hospital populations. In the developing world, EPEC are associated with disease in slightly older children (aged 2–5), but actual rates of disease are difficult to determine since serotyping of strains is required to identify the EPEC strains. EPEC strains are found throughout the developing world. Immunity to these organisms appears to be acquired, since they primarily cause disease in children. Travellers may also be at risk of disease (Mathewson et al, 1985).

Pathogenesis

The mechanisms by which the EPEC strains cause diarrhoea are not well understood. Enteropathogenic strains do not produce the classical enterotoxins nor do they have invasive characteristics. Strains are recognized as EPEC when they belong to one of the following serogroups: O26, O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, O158. EPEC strains are also identified by their ability to produce characteristic lesions, which are seen

on small bowel biopsy. In these, bacteria are tightly adherent to the mucosa and cause effacement of the microvilli although they do not invade the microvilli. This attachment may correlate with specific focal adherence of certain EPEC strains to HEp-2 cells, which appears to be plasmid-mediated (Baldini et al, 1983). Whether toxin delivery occurs with this tight adherence remains unclear, although a Shiga-like toxin has been identified in some EPEC strains (Edelman and Levine, 1983). However, high levels of Shiga-like toxin production are characteristically produced by different serotypes (O157, O26) that are associated with haemorrhagic colitis and haemolytic-uraemic syndrome.

Clinical diagnosis

The clinical diagnosis of EPEC disease is difficult. Classically these strains cause a prolonged, watery diarrhoea without blood or mucus or evidence of systemic toxicity. EPEC diarrhoea may result in rather severe weight loss or failure to thrive. Fever and/or vomiting may occur. Definitive diagnosis requires serotyping of *E. coli* isolated from stool or quantitative culture from small bowel aspirates, which is not practical in a purely clinical microbiological laboratory.

Management of disease consists of adequate hydration. Antibiotic treatment may rid the small bowel of these adherent organisms and ameliorate symptoms. However, many of the EPEC strains are resistant to multiple antibiotics and susceptibility testing should be done.

Enteroinvasive *E. coli* (EIEC)

Epidemiology

Clinical disease caused by EIEC is indistinguishable from that caused by *Shigella* sp. It appears to occur rarely, although confusion with shigellosis, both clinically and biochemically, may obscure the actual incidence of disease. Outbreaks due to EIEC have occurred in the United States, associated with imported cheese. Sporadic cases have been reported throughout the world, but the true incidence of disease is not clear.

Pathogenesis

As the clinical disease caused by the EIEC is similar to that caused by *Shigella*, the pathogenesis of disease also seems to be similar. The EIEC invade the mucosal cells of the colon and multiply intracellularly. The organisms do not produce any of the enterotoxins of *E. coli*, but, like *Shigella* are positive when tested for invasiveness in the Sereny test.

Clinical diagnosis

The EIEC organisms cause a true dysentery, diarrhoeal stool mixed with blood and mucus. This may be accompanied by fever, abdominal pain, and

systemic toxicity. Microscopic examination of the stool should reveal sheets of white blood cells in clumps of mucus. When *Shigella*, *Campylobacter* or *Salmonella* species are not isolated from the stool of such a patient, the EIEC become a clear possibility. Clinical microbiology laboratories should be able to suspect EIEC by the biochemical characteristics of *E. coli* isolated from such a patient. As opposed to the majority of *E. coli* strains, the EIEC are often slow to ferment lactose. Confirmation of EIEC disease, however, would require testing isolated strains for invasiveness in the guinea pig eye or by agglutination with invasive *E. coli* serogroup type specific sera (Pai et al, 1984).

VIBRIO CHOLERAE O-1

Epidemiology

Cholera, caused by *V. cholerae* O-1, is a potentially life-threatening, acute watery diarrhoea often characterized by circulatory collapse. It has been endemic through recorded history and remains a significant cause of morbidity and mortality throughout Africa and Asia with as many as 8 million cases per year resulting in as many as 124 000 deaths. Partially protective immunity to cholera is acquired, and in endemic areas cholera is more common in children. In sporadic outbreaks or epidemics in non-endemic areas adults are also at risk of disease. Acquired immunity can be overcome by the ingestion of a sufficiently large inoculum of organisms (Levine et al, 1981). It has been difficult to associate outbreaks of cholera with isolation of the organism from water, water levels, amount of rainfall or temperature. Disease in endemic areas does, however, follow regular seasonal patterns which are remarkably consistent from year to year. Spread of disease is associated with exposure to contaminated water and consumption of fish among other factors. Only rarely does a chronic carrier state occur, although both human and animal reservoirs have been documented (Pierce et al, 1970). Cholera is rarely seen in travellers, although it does occur. There is currently an endemic focus of cholera in the United States along the Gulf coast. Increased susceptibility to disease occurs in persons who have decreased gastric acid.

The current (seventh) pandemic began in 1961 and is of the El Tor biotype. Classical cholera has reappeared in Bangladesh since 1982 (Samadi et al, 1983). Up to 80% of cases of El Tor cholera are inapparent, with a nearly 60:1 ratio of infection to serious illness. Sixty per cent of classical cholera may be inapparent and the ratio of infection to serious illness is 5:1 (Black, 1985).

Pathogenesis

After ingestion, *V. cholerae* must survive passage through the acid environment of the stomach, actively penetrate the mucus and attach to the small bowel mucosa, by a mechanism that remains unclear. After attachment and replication, which may take 12 hours to 6 days, the organism releases cholera toxin. Cholera toxin binds to a ganglioside receptor in the small bowel mucosa

via its binding (B) subunit. The active (A) subunit then crosses the cell membrane and ADP-ribosylates the regulatory protein for adenylate cyclase, leading to the production of increased cyclic AMP and the secretion of isotonic fluid and electrolytes. This results in a base deficit acidosis and potassium depletion. Depending on the severity of disease, dehydration may be mild, moderate or severe, with maximum purging rates that may exceed 1 litre per hour.

Cholera toxin alone causes diarrhoea or fluid secretion in an animal model or in human volunteers, but strains of V. cholerae which do not possess this chromosomally mediated toxin are also capable of causing similar watery diarrhoea in humans. The mechanism of fluid production by these strains, whether by an alternate, unrecognized toxin or by the act of colonization alone, is not clear (Morris et al, 1984).

Clinical diagnosis

The clinical picture of full-blown cholera (cholera gravis) is dramatic and distinctive enough to be recognized on clinical grounds alone. However, the organism may cause asymptomatic infection or mild nonspecific diarrhoea, which is clinically indistinguishable from diarrhoea caused by other agents. Clinically, fever is uncommon with cholera, although it may occur. Nausea and vomiting may be present, and while abdominal cramping may occur, tenesmus should not.

Stool from a patient with cholera is typically clear fluid with flecks of mucus (rice-water stool), without blood or significant faecal leucocytes. Rapid diagnosis is possible by dark field examination of the cholera stool, with the rapid darting motility of the V. cholerae organisms seen. Application of group-specific antisera should immobilize the organisms and confirm the diagnosis.

Diagnosis can also be confirmed by the isolation of the organism on thio-citrate-bile salt sucrose (TCBS) agar. By fermenting sucrose, V. cholerae O-1 will form yellow colonies; other vibrio species that do not ferment sucrose will appear green (Gangarosa et al, 1968).

Generally a serological response is seen within 3–5 days of infection and peaks at 10 days. A four-fold rise in serological titre is confirmatory of acute infection, although titre rises with disease are generally much higher. Vibriocidal antibody titres rise in spite of early treatment with antibiotics; the antitoxin antibody titre rise may be blunted by treatment (Cash et al, 1974).

As with all diarrhoeal illness, rapid and sufficient rehydration is life saving. With adequate rehydration, the mortality rates from cholera have fallen from 40–80% to less than 1%. In severe cases, intravenous rehydration may be used; oral rehydration therapy should be instituted as soon as is clinically feasible. Early oral antibiotic treatment does shorten the course of disease and may halve fluid losses. Tetracycline is the drug of choice, although chloramphenicol and erythromycin can be used. Furazolidone should be used in pregnant women and children under the age of ten. V. cholerae can develop resistance to tetracycline, chloramphenicol and erythromycin, so local sensitivity patterns should be ascertained (Mhalu et al, 1979).

NON-O-1 *VIBRIO CHOLERAE*

There are strains of *V. cholerae* that do not agglutinate O-1 antisera and are known as the non-O-1 vibrios or non-agglutinating vibrios. While these organisms are capable of causing diarrhoeal illness, they are generally associated with milder disease than that caused by the O-1 vibrios.

Epidemiology

Non-O-1 vibrios are, like O-1 vibrios, found in salt water and are concentrated by filter-feeding shellfish, such as oysters, and in other shellfish, such as crabs. Diarrhoeal disease associated with these organisms is generally linked to the consumption of raw or inadequately cooked shellfish. Disease may also be seen in travellers and is found in cases of diarrhoea from Africa, Asia, Europe, and North and South America.

Pathogenesis

A minority of the non-O-1 vibrios produce cholera toxin or have the genetic structure necessary to produce cholera toxin. Strains which do not produce cholera toxin may still have activity in animal model bioassays, such as the rabbit ligated ileal loop (as whole organisms) or the suckling mouse. While this suggests the existence of other enterotoxins, further identification or characterization of such toxins has not been done, and mechanisms of disease are not clear (Morris, 1986).

Diagnosis

Non-O-1 *V. cholerae* can cause disease other than diarrhoea, although approximately 50% of isolates are associated with diarrhoeal illness. When diarrhoea occurs, it may be mild or severe and it may contain blood or leucocytes. Abdominal cramping, fever, nausea and vomiting may be present. As with *V. cholerae*, if the non-O-1 organisms are to be isolated from stool, selective (TCBS) agar should be used, and the clinical suspicion of disease from travel, or seafood ingestion must be strong enough to pursue the diagnosis (Morris et al, 1981).

NON-CHOLERA VIBRIOS

Vibrios other than *V. cholerae* are capable of causing diarrhoeal illness. These include: *V. parahaemolyticus*, *V. fluvialis*, *V. mimicus*, *V. hollisae*, *V. furnissii* and *V. vulnificus*.

Epidemiology

All non-cholera vibrios are also found in salt water and in various shellfish. *V. parahaemolyticus* is the cause of up to one-quarter of all food-borne

diarrhoeal illness in Japan and has been found in other parts of Asia as well as coastal areas of the United States. *V. fluvialis*, which may be confused with the *Aeromonas* species, has also been found in patients with diarrhoeal disease in Asia, the Middle East and the United States. As with the other non-cholera vibrios, *V. mimicus*, *V. hollisae* and *V. furnissii* are generally associated with diarrhoeal disease after the consumption of raw or undercooked shellfish. *V. vulnificus* has been isolated from the stools of patients with diarrhoea, but it is more typically associated with sepsis and wound infections in patients with liver dysfunction.

Pathogenesis

V. parahaemolyticus produces a haemolysin that appears to be associated with diarrhoeal disease (Kanagawa phenomenon). This haemolysin is lethal for mice and has enterotoxic activity in suckling mice. *V. fluvialis* appears to produce toxins that are active in rabbit ligated ileal loops and in infant mice, and which cause elongation of Chinese hamster ovary cells. Cholera toxin or the gene for its production is not present in *V. fluvialis*. Some strains of *V. mimicus* produce a heat-labile toxin that is antigenically similar to cholera toxin (Shandera et al, 1983).

Clinical diagnosis

Diagnosis requires clinical suspicion from a history of raw seafood ingestion or travel to an endemic area, and the plating of stool specimens on appropriate selective media to allow isolation of the organisms. *V. parahaemolyticus* is blue green on TCBS agar; *V. fluvialis* will grow on TCBS but strains which are isolated should also be screened for the ability to grow in 6–7% salt to differentiate *V. fluvialis* from *Aeromonas* species. *V. mimicus* will also grow on TCBS, but also does not ferment sucrose and will be blue green, not yellow. *V. hollisae* grows poorly on TCBS but will grow on blood agar (Bonner et al, 1983).

AEROMONAS

The *Aeromonas* complex (*A. hydrophila*, *A. caviae*, *A. sobria*), which are associated with disease in humans, are members of the genus *Aeromonas* within the family Vibrionaceae. The *Aeromonas* complex is also associated with water, but unlike the vibrios, it may be isolated from either fresh or salt water. *Aeromonas* has been documented in diarrhoeal stools since the 1960s, but its role in causing diarrhoea remains controversial since it is also frequently isolated from stools of healthy controls (0.7% in Europe to 27% in Thailand). Data have been conflicting, with a study in Thailand showing enterotoxigenic strains isolated as frequently from controls as from patients (Pitarangsi et al, 1982), as opposed to a study from Australia which found enterotoxigenic strains only in children with diarrhoea and nontoxigenic strains in both patients and controls (Gracey et al, 1982).

Pathogenesis

Aeromonas species produce a number of enterotoxins that are active in animal model bioassays, including both a heat-stable and a heat-labile enterotoxin. These strains are also often haemolytic, and haemolysins may also have a role as virulence factors in diarrhoeal disease (Ljungh et al, 1977).

Clinical diagnosis

In those series in which it appeared that *Aeromonas* did play a causative role in diarrhoeal illness, the spectrum of disease ranged from a more common, watery diarrhoea to a dysentery syndrome with blood and mucus in the stool. Again, selective media, with antibiotics (such as ampicillin–blood agar or CIN agar), is required to isolate the organisms from stool. Any isolates growing on these plates must also be tested for oxidase, to differentiate the oxidase-positive *Aeromonas* from ampicillin-resistant, oxidase-negative organisms, such as a resistant *E. coli*.

SHIGELLA

Epidemiology

Dysentery caused by *Shigella* is seen throughout the world and is probably the most communicable of the bacterial diarrhoeas. Volunteer studies have demonstrated that disease can be caused by ingestion of 10–100 viable *Shigella dysenteriae* I or a few thousand *Shigella flexneri* 2a. The small infectious inoculum helps to explain the ease of transmission. Consequently person-to-person transfer as well as environmental spread by faecally contaminated food and water or by flies appear to be important in transmission of shigellosis. The importance of personal hygiene in disease transmission is shown in studies, which document a significant reduction of the incidence of disease with the provision of soap and encouragement of hand washing as well as sanitary disposal of faeces and sufficient quality and quantities of available water (Tjoa et al, 1977; Boyce et al, 1982).

In the developing world, *Shigella* dysentery is most common in children, appearing after weaning and being common until the age of ten. Adults in endemic areas may acquire disease from contact with children. Shigellosis may also be transmitted sexually, especially between homosexual males. Travellers are also at risk of disease from contaminated food or water. Chronic carriage of *Shigella* seems to occur in a small percentage of cases, which may provide a reservoir for the organism (Levine et al, 1973).

Pathogenesis

There are four species in the genus *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. These are separable serologically on the basis of somatic O antigens into four groups: A, B, C and D respectively. All are pathogenic for

humans. The disease often begins with mild, watery diarrhoea and may develop into frank dysentery, perhaps reflecting the progression of infection from the proximal small bowel to the colon and the different pathophysiological capabilities of the organism in different portions of the bowel. *Shigella* toxin causes secretion in rabbit jejunum. However, the classical dysenteric syndrome, with small volumes of blood and mucus-containing stool, arises from invasion of the colonic epithelium by the organism. In addition to colonic epithelial cells, *Shigella* are capable of invading vaginal epithelium, guinea pig, rabbit, and rat corneal cells, as well as various cell-culture lines. This invasiveness is an essential virulence trait in pathogenic strains (Sereny, 1955; Keusch, 1986). After invasion, the organism multiplies intracellularly and spreads to adjacent cells, causing cell death and marked inflammatory response. Histologically, vascular congestion, haemorrhage, oedema, mono- and polymorphonuclear cell infiltrates and epithelial cell shedding are seen in the colon, with the formation of micro-ulcers and an overlying bloody exudate. Grossly, the infected colon appears erythematous and friable with an adherent greyish-white exudate. A recent study of colonic function in patients with acute shigellosis showed diminished net absorption of water and chloride and increased potassium secretion in the colon without changes in small bowel function (Butler et al, 1986).

It is not clear what role, if any *Shigella* toxin plays in these pathogenic changes. Nor are the pathogenic mechanisms of *Shigella*-associated complications (leukaemoid reaction, haemolytic-uraemic syndrome, seizures) well delineated (Ashkenazi et al, 1983 Butler et al, 1984; Koster et al, 1984). Recent work has suggested that these complications are more frequent in patients with higher concentrations of *Shigella* toxin in stool (Bennish et al, 1986). Some have postulated that immune complexes form with endotoxin or *Shigella* toxin in the pathogenesis of haemolytic-uraemic syndrome (Koster et al, 1978). As with any inflammatory colitis, reactive arthritis and Reiter's syndrome may follow *Shigella* dysentery in persons with HLA-B27.

Studies of shigellosis which measure alpha-1-antitrypsin in the stool document loss of up to 100–500 ml of plasma per day into the stool from damaged mucosa, providing a potential causative link between shigellosis and protein-loss malnutrition.

Clinical diagnosis

Bacillary dysentery should be considered when any patient presents with acute diarrhoeal illness associated with blood and mucus and signs of systemic toxicity. However, dysentery caused by *Shigella* cannot be distinguished clinically from dysentery caused by *Salmonella*, *Campylobacter*, *Yersinia*, *Vibrio parahaemolyticus* or amoebae. Diarrhoea caused by *Shigella* may also be watery without dysentery, especially when *S. sonnei* is involved, and the differential diagnosis should include cholera, toxigenic *E. coli* or rotavirus diarrhoea.

The development of convulsions in a child with dysentery should suggest *Shigella* as the aetiological agent, although it is not clear whether the neurotoxic properties of *Shigella* toxin play a role in these symptoms.

A leukaemoid reaction may occur in a patient with shigellosis, and especially infection with *S. dysenteriae* I. White blood cell counts over 66 000 were seen in 3.8% of hospitalized patients with shigellosis in Bangladesh (Butler et al, 1984). The development of haemolytic-uraemic syndrome in a patient with dysentery should suggest *S. dysenteriae* I infection. Other syndromes, such as meningismus, reactive arthritis or Reiter's syndrome, may occur in shigellosis.

The laboratory diagnosis should include microscopic examination of the stool, which should reveal sheets of leucocytes in the typical patient with dysentery. However, the only way to confirm the diagnosis of shigellosis is to recover the organism from stool culture. Selective media, with a high concentration of bile to inhibit growth of Gram-positive organisms and other coliforms, and thiosulphite to allow distinction of *Salmonella* from *Shigella*, is required. The invasive potential of strains can be assessed in the Sereny test (keratoconjunctivitis in guinea pigs with the inoculation of organisms).

When any patient with shigellosis is to be treated with antibiotics, sensitivity testing of the strain should be considered, as multiply-resistant *Shigella* are increasingly common throughout the world.

SALMONELLA

The *Salmonella*, of which there are over 2000 serotypes, may cause gastroenteritis, enteric fever, focal infections or prolonged bacteraemia. On biochemical testing, they can be placed in three major groups for simplicity's sake. Group I, which contains all but two of the serotypes, causes a variety of diarrhoeal and systemic illnesses throughout the world. Group II, serotype choleraesuis, is associated with both porcine and human infection and causes both local infections and diarrhoea in humans. Into Group III falls *Salmonella typhi*, the causative organism of most typhoid fever (Hornick, 1984).

Group I: enteritidis serotypes

Epidemiology

Diarrhoeal disease is caused by a large number of *Salmonella* serotypes. In the United States, the most common serotype is *S. typhimurium*. Many of the other serotypes are named after the geographical locale from which they were first isolated (*S. heidelburg, S. miami, S. newport, S. senegal*) (Black et al, 1960). *Salmonella* organisms have been isolated from outbreaks associated with pet turtles, carmine red dye, poultry, meat, eggs, water, milk (including a large outbreak in the Chicago area in 1985 involving 16 285 cases of *S. typhimurium* enteritis) and marijuana. While turtle-borne disease in the United States has decreased with stricter import regulations, turtles are still associated with 12–17% of cases in infants in Puerto Rico. The number of reported cases has steadily increased in recent years, despite decreased active surveillance. At increased risk of serious disease are infants under the age of 6

months, the elderly, and immunocompromised persons, including those with the acquired immunodeficiency syndrome (AIDS). Any decrease in gastric acid, for whatever reason, places an individual at higher risk of acquiring disease. Salmonella have been isolated in up to 7% of patients with traveller's diarrhoea.

Pathogenesis

Group I salmonella are associated with a variety of syndromes, from asymptomatic infection to watery diarrhoea or full-blown dysentery. These syndromes generally reflect the portion of the gastrointestinal tract involved in infection, with the upper small bowel characteristically involved in watery diarrhoea, the terminal ileum in patients who present with pasty stools, and the colon in the dysenteric presentations.

The infectious dose usually required in volunteer studies is 100 000 organisms. Salmonella are susceptible to gastric acid, although their passage through the stomach may well be buffered by the food or water with which they were ingested (Giannella et al, 1973a). The organisms attach to the gut mucosa and within 8–48 hours penetrate the epithelial cells to enter the lamina propria, eliciting a polymorphonuclear response. Attachment may be prevented by the presence of appropriate antibody in the gut.

The diarrhoea caused by salmonella may be associated with the release of toxins; a heat-labile, cholera-like toxin has been identified in some strains. An alternative pathogenic mechanism proposed is the stimulation and release of prostaglandins by the inflammatory exudate resulting from the penetration of the epithelial surface causing increased cyclic AMP and fluid secretion.

Persons who are at risk of more severe disease include those that have defective cell-mediated immunity or defects in phagocytic killing of ingested organisms.

Clinical diagnosis

The incubation period of salmonella diarrhoea is brief, 8–48 hours. A prodrome of malaise, abdominal pain and fever may precede diarrhoea. The diarrhoea often lasts 2–5 days and, while debilitating, is not clinically distinguishable from that caused by other enteric pathogens. In children 8–15% may have associated bacteraemia, but the clinical course of these children is no different than the course of children without bacteraemia. However, because of the bacteraemia, complications that do not generally occur with other diarrhoeal pathogens may be seen, such as meningitis in infants, osteomyelitis in patients with sickle-cell disease, and endothelial infections, in the walls of aortic aneurysms or intravascular prostheses.

Faecal leucocytes are often seen on microscopic examination of fresh stool. Confirmation of the aetiology of the diarrhoea requires isolation of salmonella on selective agar. The organisms are generally non-lactose-fermenting and are distinguished by their ability to produce hydrogen sulphide gas.

Diarrhoea due to enteritidis serotypes of salmonella should be managed as all diarrhoeal illness—by the maintenance of adequate hydration, via the oral

route if at all possible. In general, treatment of salmonella diarrhoea with antibiotics only prolongs excretion of the organism and is contraindicated (Aserkoff and Bennett, 1969). However, infants under the age of six months, the elderly, and those with intravascular prostheses are at greater risk of focal infection and should probably receive antibiotic treatment when enteritidis serotypes are isolated from stool.

Many *Salmonella* are multiply antibiotic-resistant and sensitivity testing of isolates should be done (Holmberg et al, 1984). Prevention of much *Salmonella* disease is possible with careful personal hygiene, handwashing and the avoidance of raw milk. The multiplicity of serotypes would necessitate a broad-spectrum vaccine.

Group II: choleraesuis serotype

The *Salmonella choleraesuis* serotype causes bacteraemia and localized infections (abscesses) in elderly patients and diarrhoeal disease in children. As with many salmonella infections, isolation of *S. choleraesuis* from a blood culture should raise the distinct possibility of abscess or focal infection in a patient.

Group II: enteric fever

Epidemiology

Salmonella typhi causes most cases of enteric fever, although this syndrome can be caused by serotypes from group I or by serotype *S. choleraesuis* (especially in children). One-third to one-half of the 500 cases of typhoid fever seen yearly in the United States have occurred in travellers. In contrast to the large animal reservoir of non-typhi salmonella, *S. typhi* only infects humans, so chronic carriers are the major reservoir of disease. Chronic carriage is often associated with gallbladder infection, and women are three times as likely as men to be chronic carriers. Carriers may contaminate food directly or indirectly by faecal contamination of water. Persons with genitourinary *Schistosoma haematobium* infections may have chronic recurrent salmonella bacteraemia as the caecum of the schistosome may carry *S. typhi*.

Typhoid fever remains a serious health problem in the developing world, although actual numbers of cases are difficult to determine.

Pathogenesis

The steps in initiation of the disease are similar to those in Group I salmonella. However, the bacteraemic phase, after the invasion of the lamina propria, plays a much more prominent role in typhoid fever. The organism is taken up into the reticuloendothelial system from the blood and replicates intracellularly. As with other salmonella, the infectious dose of *S. typhi* is 10^5; higher doses are associated with higher attack rates and shorter incubation periods. In naturally acquired disease the mean incubation period is 12 days.

In the gut, much of the disease is localized initially to the distal ileum, particularly to the Peyer's patches. High concentrations of bacteria are presumed to locally release quantities of endotoxin, which may contribute to local inflammation, haemorrhage and bowel wall necrosis. Endotoxin also causes release of endogenous pyrogen; however, circulating endotoxin has not been found in natural infections.

Since salmonella live intracellularly, they are protected from many host defences and may continue to seed the bloodstream from sites in the liver and bone marrow. Relapses, which occur in up to 15% of patients, probably also arise from these foci (Hornick et al, 1970).

When diarrhoea is seen later in the course of typhoid fever, it may be caused by local inflammation and destruction of the villi in the distal small bowel or by a second enteric pathogen.

Clinical diagnosis

The presentation of typhoid fever begins with nonspecific fever, headache and abdominal pain. Abdominal pain and constipation may be associated with an ileus caused by inflammation of the distal small bowel. Evanescent small raised non-tender lesions—rose spots—which are the result of a small vessel vasculitis, may be seen on the chest and abdominal wall near the end of the first week of illness. The typical clinical presentation also includes a pulse–temperature deficit, a relative bradycardia, and temperatures up to 104 °F. Intestinal perforation may occur after two or more weeks of illness. The current paucity of disease in the United States has led to a delay in diagnosis and appropriate treatment, with a resultant increase in case mortality (Tolaymat et al, 1979). Clinical suspicion of typhoid fever is of paramount importance to proper diagnosis.

Salmonella typhi may be recovered from the blood in 85% of cases. Culture of rose spots, liver or bone marrow should also yield the organism. When blood cultures remain negative and clinical suspicion of disease is high, liver or bone marrow cultures may be indicated.

Routine laboratory tests are nonspecific. A mild anaemia and leucopenia are usual. Elevations of alkaline phosphatase and the transaminases are consistent with granuloma in the liver. The Widal test is nonstandardized and may reflect infection with salmonella other than *S. typhi* or previous vaccination. However, paired sera with a four-fold titre rise or a single markedly elevated O agglutinin titre is suggestive of typhoid fever.

Treatment of typhoid fever requires antibiotic therapy with chloramphenicol, ampicillin, trimethoprim-sulphamethoxazole or possibly with a third-generation cephalosporin, depending on strain sensitivity. Clinical improvement should be seen within 3–5 days and treatment should be continued for 12–14 days. Relapses should also be treated with antibiotics. The organism may only be eradicated from chronic carriers by the surgical removal of the nidus of infection, such as a nonfunctioning gallbladder.

Prevention of disease is possible with sanitation and protected water supplies, and to a certain extent, by vaccination. A new live attenuated vaccine

appears to be more effective than the older, killed vaccines. Killed vaccines may be associated with transient decreases in cell-mediated immunity and probably should not be used during epidemics.

CLOSTRIDIUM DIFFICILE

Epidemiology

Clostridium difficile may be a member of the normal colonic flora in 3% of healthy adults and 40–60% of children under the age of one year. The organism generally contributes to disease after disturbance of the balance of normal flora and is seen most commonly after antibiotic usage, but also after cancer chemotherapeutic agents, heavy metal ingestion, or abdominal surgery. Clindamycin, ampicillin, and the cephalosporins are antibiotics often associated with the development of disease. In areas of the developing world where antibiotics are freely available, it remains unclear what role *C. difficile* disease plays in community acquired diarrhoea. In both developed and developing areas *C. difficile* is a significant pathogen in hospitalized patients (Gupta and Yadav, 1985). There is good evidence that *C. difficile* is transmitted nosocomially and via environmental sources (Bender et al, 1986; Heard et al, 1986).

Pathogenesis

C. difficile is a Gram-positive, anaerobic bacillus with subterminal spores; it consistently produces two toxins (A and B) that contribute to colitis. Toxin A causes fluid secretion and mucosal damage in both the large and small bowel of several animal models (Lyerly et al, 1985; Lima et al, 1986a; Mitchell et al, 1986). A recent study showed a possible carbohydrate-binding site for toxin A on intestinal membrane brush border that has a Gal α1-3 Gal β1-4GlcNAc nonreducing terminal (Krivan et al, 1986). Toxin A also has a cytopathic effect in tissue culture monolayers. Toxin B is a much more potent cytotoxin than toxin A, but does not cause fluid secretion in animal models (Lima et al, 1986b). Toxin B produces the cytotoxic changes which are measured in the tissue culture assay.

Disease in humans occurs predominantly in the colon, especially the rectosigmoid area. The ileum may rarely be involved. Grossly, the colitis may be mild and nonspecific or severe, with a granular, ulcerating mucosa studded with nodules or confluent pseudomembranes of fibrin, mucus, necrotic epithelial cells and leucocytes adherent to the underlying inflamed tissues.

Clinical diagnosis

Initially, most patients present with diarrhoea and abdominal pain. Symptoms may develop at any time during a course of antibiotics or up to 6–8 weeks after the discontinuation of antibiotics. The usual time for disease to appear is within two weeks after beginning a course of antibiotics. As with most enteric

pathogens, there is a spectrum of severity of disease, from mild, nonspecific diarrhoea to severe disease with 20 or more bloody stools per day, lasting for weeks. Patients may present with toxic megacolon or colonic perforation instead of diarrhoea. Fever may or may not be present, and laboratory findings are nonspecific. Microscopic examination of fresh stool samples should reveal leukocytes in at least 50% of patients.

The differential diagnosis includes Crohn's disease, idiopathic ulcerative colitis, and ischaemic colitis, especially if the patients have nonspecific colitis. Other intestinal pathogens should be considered, such as *Salmonella, Shigella,* invasive *E. coli, Campylobacter, Yersinia, Entamoeba histolytica* and *Strongyloides.*

Endoscopy of the colon may reveal classic pseudomembranes, but may also demonstrate only nonspecific colitis. Isolation of the organism on selective agar is not sufficient to confirm the diagnosis; direct evidence of the toxin, either in the stool or from isolated organisms, is required. Unfortunately the counterimmunoelectrophoresis method lacks both sensitivity and specificity. The most sensitive and specific toxin assay remains the tissue culture assay, in which toxin must not only be shown to have a cytopathic effect on tissue culture cells but also be neutralized by specific *C. difficile* antitoxin. A latex agglutination assay that appears to correlate with but not identify toxin A may be useful in laboratory settings that do not have tissue culture capabilities.

Treatment requires clinical suspicion of disease and discontinuation of any nonessential antibiotics along with rehydration. Persistent or worsening illness may necessitate oral vancomycin or metronidazole therapy.

CLOSTRIDIUM PERFRINGENS

Epidemiology

Clostridium perfringens causes several types of diarrhoeal illness, including food poisoning and enteritis necroticans (also known as 'Darmbrand' or 'pig-bel')—probably caused by *C. perfringens* type C.

C. perfringens type A food poisoning is characterized by high attack rates involving roasted, boiled, stewed or steamed meats or poultry. 'Darmbrand' was first described in epidemics in northern Germany after World War II, and was related to the consumption of rancid meat. A similar clinical syndrome ('pig-bel') was seen after ritual pig kills and pork meals among the highland Melanesians in New Guinea.

Pathogenesis

C. perfringens is a Gram-positive bacillus with aerotolerant spores. There are five subgroups of *C. perfringens*, A–E, based on the production of four toxins: alpha, beta, epsilon and iota. Alpha toxin, produced by *C. perfringens* type A, appears to be related to the production of food poisoning and is a structural

component of the spore coat. This toxin causes fluid secretion in rabbit ligated loops as well as contributing to protein loss in the intestinal lumen.

Beta toxin appears, by the development of an apparently protective antibody against it, to be implicated in the production of 'pig-bel' or 'Darmbrand' (Murrell et al, 1966; Lawrence et al, 1979). However, an enterotoxin similar to alpha toxin has been isolated from *C. perfringens* type C. Whether this toxin or beta toxin is responsible for necrotizing enteritis remains unclear. The type A food poisoning toxin does not seem to contribute to necrotizing enteritis in experimental animal models, whereas toxins produced by type-C organisms do produce necrotizing enteritis in the animal model (Lawrence and Cooke, 1980).

Clinical diagnosis

C. perfringens type A food poisoning presents as watery diarrhoea with abdominal pain, but without vomiting, after an incubation period of 8–24 hours. Signs of systemic illness or toxicity do not occur and the illness usually lasts only about 24 hours.

In contrast, necrotizing enteritis is a much more serious disease accompanied by vomiting, severe abdominal pain, bloody diarrhoea and ultimately shock. The mortality rate of this disease approaches 40%. The differential diagnosis should include acute food poisoning syndromes, antibiotic-associated colitis, acute shigellosis and acute ulcerative colitis.

To confirm the diagnosis, clinical suspicion of disease must be high enough to ensure that both stool and sample food specimens are plated on the proper selective medium, an egg-yolk-free tryptose sulphite cycloserine agar. *C. perfringens* is occasionally a part of normal flora and isolated organisms must be serotyped to ensure that they belong to pathogenic groups. In an outbreak of disease, serotypes of organisms isolated from affected individuals as well as from contaminated food should agree.

Treatment of *C. perfringens* food poisoning is supportive, with fluids and electrolytes. Treatment for necrotizing enteritis requires supportive careful rehydration, and surgical resection of involved bowel must be considered when signs of toxaemia and complications appear.

CAMPYLOBACTER

The genus *Campylobacter* contains four species known to cause disease in humans. *C. jejuni* and *C. coli* are associated with diarrhoeal disease. *C. fetus* more commonly causes systemic infection in immunocompromised hosts although it has been associated with diarrhoeal disease. *C. laridis* is a rare human pathogen. In addition, campylobacter-like organisms (CLO) (*C. cinaedi* and *C. fennelliae*) have been recently identified and are associated with proctocolitis in homosexual men (Quinn et al, 1984). Recent work has also associated the CLO *C. pylori* with histological gastritis (Marshall and Warren, 1984).

Epidemiology

Diarrhoeal disease caused by *C. jejuni* or *C. coli* is seen throughout the world. Its frequency may correlate with the lack of available clean water and generally poor hygiene in these areas as well as crowded conditions and close contact with animals, which may serve as reservoirs of infective organisms. The exact mechanism of transmission in the developing world is not clear.

In addition to a higher rate of disease caused by campylobacter, there is an associated increase in asymptomatic carriage of the organism in the developing world. While campylobacter isolation rates in diarrhoea in Sweden were 7% with a 0.25% asymptomatic carriage rate (Walder, 1982), diarrhoeal rates were 7% in Brazil, 10% in the Gambia and 12% in Bangladesh, and reported isolation rates from asymptomatic individuals were as high as 16% in South Africa (Bokkenheuser et al, 1979), 25% in South India (Mathan et al, 1984) and 39% in one-year-olds in Bangladesh. However, a significant number of these Bangladeshi children had had recent diarrhoeal illness (Blaser et al, 1980). The organism was cultured from children with diarrhoeal disease, but not from controls, in Rwanda (Butzler, 1973) and Zaire (DeMol et al, 1978). Work in the Gambia and Nigeria could not confirm a suspected increase in infection with campylobacter in malnourished children as rates of isolation from well-nourished and malnourished children in both countries were equivalent (Lloyd-Evans et al, 1983). Type-specific protective immunity appears to develop after exposure (in volunteer studies) which may explain the predominance of the disease in children.

The variation in numbers of infections in different geographical locations may reflect differences in climate, humidity, rainfall and population density, as well as poor hygiene. Campylobacter have been isolated from various foodstuffs (poultry, meat, milk, cheese) in both the developed world and the developing world. Unchlorinated water supplies have also been associated with outbreaks of disease in Vermont, Sweden and Great Britain. Campylobacter can be routinely cultured from sick puppies and kittens and from healthy dogs, cats, fowl, goats, cattle, pigs, birds and wild animals. Travellers are at risk of campylobacter diarrhoea.

Pathogenesis

The mechanisms by which *C. jejuni* and *C. coli* cause disease remain unclear. Since the organisms do cause a variety of syndromes, it is likely that a variety of virulence factors can be implicated in the production of disease. The high number of asymptomatic infections in the developing world may reflect carriage of strains which do not have or have lost these virulence characteristics.

Ingestion of as few as 500 organisms in volunteer studies can cause disease, but not in all individuals. The *Campylobacter* species are extremely susceptible to gastric acid. If the organisms survive passage through the stomach, they can attach and multiply in the jejunum and ileum. Small bowel mucosal attachment may be mediated by flagella or by LPS and appears to be partially blocked by L-fucose. After a 3–5 day incubation period, it is hypothesized that

attached organisms may produce disease by the release of one of a number of toxins. An LT-like enterotoxin has been identified in strains from the United States, Canada, Belgium, India and Japan. This toxin binds to GM1 ganglioside and is neutralized by anti-LT antibody, but does not contain gene-probe detectable LT gene sequences. In addition, cytotoxins that have in vitro activity have been identified.

There is also clearly colonic involvement in *Campylobacter* disease, with mucosal oedema, hyperaemia and friability seen on colonoscopy. These changes could be related to the recognized ability of some *Campylobacter* to invade HeLa tissue culture cells (Pearson et al, 1985).

Clinical diagnosis

Four clinical diarrhoeal syndromes are seen with *C. coli/jejuni* infection:
1. Acute, mild diarrhoea
2. Classical dysentery
3. Relapsing or prolonged diarrhoea
4. Proctocolitis (most often seen in homosexual men)
Asymptomatic infection may also occur, more often in tropical developing areas.

In the mild diarrhoea, symptoms may last only 24 hours and be indistinguishable from viral gastroenteritis. A more severe, watery diarrhoea, indistinguishable from ETEC disease, may also occur. In dysenteric disease, symptoms not only vary with the severity of the infection but with the age of the patient. Fever and abdominal pain are usually seen with bloody diarrhoea in patients over the age of one year (Naqvi et al, 1983). Fever may be absent in infants, although they may have abdominal distension. Disease is generally self-limited, but will last more than seven days in 10–20% of patients. An additional 5–10% of patients may develop relapsing disease (Blaser et al, 1979). In these patients it is important to ensure that no other pathogen is present, as *Campylobacter* disease from the developing world is often part of a mixed infection. Seventy per cent of patients will have cleared the organism from their stool by four weeks, virtually 100% by seven weeks. Chronic carriage of *Campylobacter* is unusual.

Microscopic examination of the stool may reveal white blood cells and erythrocytes, depending on the presentation of the disease. Phase microscopy or Gram stain of fresh stool specimens may reveal organisms consistent with *Campylobacter* in 65% of culture-positive cases (Sazie and Titus, 1982).

Confirmation of disease requires isolation of the organism from the stool. Selective media, such as Skirrow's, Butzler's or Campy-BAP with antibiotics, are necessary. *Campylobacter* are strictly microaerophilic organisms, and require reduced oxygen conditions to grow. This may be provided by a mixture of 5–10% oxygen with 3–10% carbon dioxide and nitrogen, by a candle jar or by an Alka-Seltzer (or its equivalent) and steel wool combination in a more primitive laboratory setting (Pennie, 1984). A temperature of 42 °C is optimal for the growth of *C. jejuni* and *C. coli*. A Gram stain of a fresh culture should reveal the characteristic gull-wing shape of *Campylobacter*, but serial passage of organisms will cause them to lose this morphology.

Other *Campylobacter* species

C. fetus and *C. laridis* are only rarely associated with diarrhoeal illness. While
C. pyloridis is not associated with diarrhoea, it is frequently isolated from
patients with histological evidence of gastritis. Other CLOs are associated
with proctocolitis in homosexual males. Culture of these species requires the
same microaerophilic atmosphere but a temperature of 37 °C for a longer
period of time, as well as media that do not contain cephalosporin antibiotics.

Viral diarrhoea

Cumulative data from epidemiological studies have found bacterial or
parasitic aetiologies for only 20–50% of cases of diarrhoea worldwide,
suggesting that viral agents may cause many of the remainder. In the last two
decades direct and immune electron microscopy have demonstrated two
major groups of enteric viruses in association with acute diarrhoea in both
temperate and tropical areas: rotavirus, Norwalk and Norwalk-like virus. The
latter, often named after the location of the outbreak from which they were
identified, include: Hawaii, Ditchling, Snow mountain, MC, W, Cockle,
Parramatta. Since these agents cannot be cultured, it is difficult to know what
their presence in faecal specimens means, and available information about
pathogenic mechanisms is largely from volunteers infected with bacteria-free
stool filtrate. Rotaviruses, which have been cultured with some difficulty and
have been studied in animal models, often infect young children in the first 2–3
years of life. Recently, the use of direct detection techniques has found
additional viral agents that may cause diarrhoea, such as enteric adenoviruses,
caliciviruses and astroviruses, as well as enteric coronavirus-like particles and
small round virus like-particles, both suspected to produce this syndrome
(Chiba et al, 1980; Brandt et al, 1985; Gerna et al, 1985; Bishai et al, 1986).
However, the epidemiological importance of these agents remains to be
established.

Epidemiology

Rotavirus

Rotaviruses have been found worldwide in the stools of children with
diarrhoea. Most disease is seen in children between the ages of 6 and 24
months. In the developed world, rotavirus is considered the major cause of
infectious diarrhoea in children. In the developing world, 21% (in Brazil and
Nigeria) to 46% (in Bangladesh) of childhood diarrhoea is associated with
rotavirus (Black et al, 1980; Guerrant et al, 1983; Fagbami et al, 1985). In
Bangladesh, in the 6–24-month age group, rotavirus is seen more frequently
than enterotoxigenic *E. coli*. In contrast to older infants who are usually
symptomatic, up to 50% of neonates may be asymptomatically infected with
rotavirus.

Transmission appears to be primarily person to person, although water-
borne and airborne spread have been postulated. Community outbreaks

appear to be rare, but nosocomial transmission has been described (Noone and Banatvala, 1983; Hjelt et al, 1985). Crowded conditions and poor hygiene increase the likelihood of disease. In temperate areas, disease seems to predominate in cooler, winter months. In tropical areas, disease occurs throughout the year, although in Northeastern Brazil 71% of rotaviral disease occurred in the dry season, i.e. June to October (Guerrant et al, 1983).

Norwalk

Norwalk agent, as the prototype of the uncultivable viral agents, also occurs worldwide. In the developed world, disease is seen in older children and adults. In the developing world, antibody to Norwalk agent is acquired earlier. Nosocomial spread has been seen in institutions and nursing homes; community outbreaks have been seen in schools and camps and have been traced to contaminated swimming and drinking water, shellfish and cake frosting (Kaplan et al, 1982). Transmission has been assumed to be faecal–oral or by faecal contamination, although the rapidity of secondary spread suggests that aerosols may play a role.

Pathogenesis

Most of the information about the effects of rotaviral diarrhoea is derived from the histology of biopsy specimens or from animal models. In humans, histology shows mononuclear infiltration of the lamina propria, mitochondrial swelling, and sparse and irregular microvilli. The development of these changes has been followed in animal models, with replacement of the columnar epithelium by cuboidal epithelium preceding the villus shortening. Following these morphological changes there was impaired glucose-coupled sodium transport, sucrase activity was diminished, and thymidine kinase activity was increased (Davidson et al, 1977; Rodriguez et al, 1977). Volunteers given the Norwalk or Hawaii agent began to show jejunal villus blunting, with many vacuolated cells, within 24–48 hours (Dolin et al, 1975). The lamina propria is infiltrated with mononuclear cells and polymorphonuclear leucocytes. Accompanying these morphological changes are physiological abnormalities, including enzyme deficiencies (alkaline phosphatase, sucrase and trehalase) and transient impairment of carbohydrate and fat absorption (Blacklow et al, 1972; Agus et al, 1973). Alterations in adenyl cyclase are not seen in either rotaviral or Norwalk agent disease, suggesting that cyclic nucleotides are not involved in mediation of fluid secretion (Levy et al, 1976; Davidson et al, 1977).

Clinical diagnosis

The viral diarrhoeas are usually acute, self-limited diseases, often accompanied by vomiting. A low-grade fever is occasionally present, as it is with many other diarrhoeal agents. Dehydration from vomiting and diarrhoea may be more severe in rotaviral disease than in most bacterial illnesses. In rotaviral disease, vomiting may appear before diarrhoea, but does not last as

long (2.6 versus 5 days) (Rodriguez et al, 1977). Upper respiratory symptoms may accompany the diarrhoea. Other signs and symptoms include malaise, lethargy or irritability in infants and young children. While there are some differences in the clinical manifestations of the diseases among agents, these do not permit a specific aetiological diagnosis. Rotaviral, Norwalk and Norwalk-like viral diarrhoea is characteristically non-inflammatory; microscopic examination of the stools shows few if any leucocytes.

Confirmation of rotavirus as the aetiological agent of disease may be done by a commercially available monoclonal antibody ELISA, by serology, or by demonstration of the classic wheel-like morphology of the virus on electron microscopic examination of the stool. The other agents, such as Norwalk, require RIA, ELISA or electron microscopy examination of the stool, in which each agent is identified by agglutination in convalescent immune serum. The specific aetiological diagnosis of these illnesses remains a research tool, however, and is rarely indicated clinically.

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