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Contents

Preface

During 1975, five hundred million episodes of diarrhoea were likely to occur among the babies and small children of Asia, Africa and Latin America; and the disease would kill between five and eighteen million of them. Despite the tremendous advances made in medicine over the last few decades, gastroenteritis remains largely responsible for the high death rate in infancy and early childhood in many tropical countries and poorer communities and it is still a danger within prosperous societies.

Professor Otto Wolff and Dr John Harries of the Institute of Child Health in London suggested that the Ciba Foundation should bring together work on childhood diarrhoea of bacterial origin with the new and fast developing work on diarrhoea due to viral infections in infants and young children. Their idea gave rise to the symposium recorded in this book. We received valuable help in the planning also from Dr Geoffrey Sharp, Dr T. H. Flewett, Dr W. E. van Heyningen and Professor Ralph Hendrickse.

Many factors contribute to acute diarrhoea in early childhood, and it was not possible to explore in detail all of them at the symposium. Protozoal causes were deliberately omitted as formal topics, but are mentioned as contributory pathogens with interactions which require further research. Initially the symposium concentrates on identifying bacterial and viral causative agents, including the analysis of the mode of action of enterotoxins and the transmission of enterotoxin production by plasmids in *Escherichia coli*. The complex pathogenesis of overt diarrhoea and the implications for its clinical management are discussed, considerable stress being laid on protection by colostrum and breast milk.

Viral gastroenteritis affects the young of all mammals. Before successful preventive therapy can be developed, more research is needed into the transport defects and immunological mechanisms involved.

The symposium ends with accounts of diarrhoea among children in the developing world where deprivation complicates the picture. Poor nutrition, unsafe water supplies and frequent exposure to intercurrent infections combine with understandable ignorance to create conditions in which gastroenteritis becomes a constant extra and often final hazard. The disease induces a state of fluid electrolyte malnutrition. If simple guidelines are followed which match fluid intake to thirst and stool output, oral rehydration using a glucose-electrolyte solution is a simple, cheap and effective remedy. Professional training is not required for this treatment of diarrhoeal disease, and it will save many young lives if it can become common.

But further research is needed at every level. Diarrhoea must be looked at both in the micro-environment of the host-pathogen interaction and in the macro-environment of man in his society. And workers at all levels should be constantly alert to potentially useful exchanges of ideas in order that, as Dr Jon Rohde urges at the end of the symposium, science may be taken to where the diarrhoea is.

KATHERINE ELLIOTT

The problem of bacterial diarrhoea

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Abstract The reported incidence of 'pathogenic' bacteria, as judged by serotype, in the stools of children with acute diarrhoea has varied from 4 to 33% over the last twenty years. Techniques such as tissue culture provide a means for detecting enterotoxin-producing strains of bacteria, strains which often do not possess 'pathogenic' serotypes. 'Pathogenicity' requires redefinition, and the aetiological importance of bacteria in diarrhoea is probably considerably greater than previous reports have indicated.

Colonization of the bowel by a pathogen will result in structural and/or mucosal abnormalities, and will depend on a series of complex interactions between the external environment, the pathogen, and the host and its resident bacterial flora. Enteropathogenic bacteria may be broadly classified as (*i*) invasive (e.g. *Shigella*, *Salmonella* and some *Escherichia coli*) which predominantly affect the distal bowel, or (*ii*) non-invasive (e.g. *Vibrio cholerae* and *E. coli*) which affect the proximal bowel. *V. cholerae* and *E. coli* elaborate heat-labile enterotoxins which activate adenylate cyclase and induce small intestinal secretion; the secretory effects of heat-stable *E. coli* and heat-labile *Shigella dysenteriae* enterotoxins are not accompanied by cyclase activation.

The two major complications of acute diarrhoea are (*i*) hypernatraemic dehydration with its attendant neurological, renal and vascular lesions, and (*ii*) protracted diarrhoea which may lead to severe malnutrition. Deconjugation of bile salts and colonization of the small bowel with toxigenic strains of *E. coli* may be important in the pathophysiology of the protracted diarrhoea syndrome.

The control of bacterial diarrhoea requires a coordinated political, educational, social, public health and scientific attack. Bacterial diarrhoea is a major health problem throughout the world, and carries an appreciable morbidity and mortality. This is particularly the case during infancy, and in those developing parts of the world where malnutrition is common. This paper is concerned mainly with acute bacterial diarrhoea, and reviews the problem as a whole.

INCIDENCE, DISTRIBUTION AND PATHOGENIC SPECIES

The reported incidence of 'pathogenic' bacteria cultured from the stools of

children (mainly less than two years old) with acute diarrhoea has varied from 4 to 33% over the last 20 years (Cramblett *et al.* 1971), the commonest organisms being 'enteropathogenic' strains of *Escherichia coli* (EPEC), *Salmonella* and *Shigella*. Stools were not cultured from control children in all of these studies, and the true pathogenicity of the isolated bacteria is therefore not always clear. It is now clear that enterotoxin-producing strains of *E. coli* can cause diarrhoea in both adults and children, and also that the somatic serotypes of these strains are often not the recognized EPEC (Gorbach & Khurana 1972; Gorbach *et al.* 1975; Sack *et al.* 1975). The term 'enteropathogenic' as applied to serotypes of *E. coli* requires redefinition. The detection of enterotoxin-producing strains of bacteria by the use of techniques such as the rabbit ligated loop and tissue culture will probably show that the aetiological importance of bacteria in diarrhoeal illness in children is considerably greater than previous reports have indicated.

Table 1 shows the geographical distribution of the established bacterial pathogens in man, and the age groups mainly affected. Mortality and morbidity is much greater under the age of two years than in older children, and the commonest pathogens during this critical period of physical and intellectual development are *E.coli*, *Salmonella* and *Shigella*. Curiously, cholera is rare in infants under the age of one year (Mosley *et al.* 1968). Toxigenic strains of staphylococci are ubiquitous and most foods cannot fail to become contaminated with small numbers of viable organisms. They produce enterotoxins in food and disease results from ingestion of preformed toxin; the majority of outbreaks of food poisoning caused by staphylococci are due to the coagulase-positive species. *Vibrio parahaemolyticus* differs from *V. cholerae* in being a marine organism, and has been found mainly in Japan where it has been a major cause of outbreaks of food poisoning (Zen-Yoji 1968); this is probably related to the Japanese custom of eating uncooked fish. Other vibrios such as

TABLE 1

Distribution of established bacterial pathogens in man, and age groups mainly affected

Bacterial species	*Distribution*	*Age group mainly affected*
E. coli	Global	0–5 years
Salmonellae	Global	All
Shigellae	Global	0–8 years
Vibrio cholerae	Asia and contiguous areas	All
Vibrio parahaemolyticus	Japan	Adults
Clostridium perfringens	Global	All
Staphylococci	Industrialized nations	All

V. fetus, or related vibrios, have been implicated as the cause of infantile diarrhoea (Mandel & Ellison 1963). *Clostridium perfringens* is one of the commonest causes of food-borne diarrhoea (Center for Disease Control 1970), and can produce a fatal enteritis known as 'Darmbrand' in Germany (Jeckeln 1947) and 'pig-bel' in the highlands of New Guinea (Murrell *et al.* 1966). It is present in faeces, water and soil and can contaminate most commercially available meat and poultry. Other bacterial species which have been implicated as occasionally causing diarrhoea are *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Bacillus cereus* and *Bacillus subtilis* (Grady & Keusch 1971*a*). The role of these species in the causation of diarrhoea, however, is far from clear and further carefully controlled studies are required.

DISEASE DETERMINANTS

Colonization of the bowel by sufficient numbers of pathogenic bacteria results in disease. Colonization will depend on a complex series of interactions between the external environment, the pathogen, and the host and its resident bacterial flora. Knowledge on many aspects of these interactions is fragmentary and a clearer understanding is of fundamental importance for the control of diarrhoeal disease.

The external environment

The most urgent and important factors which require attack lie in the environment. At the 5th Caribbean Health Ministers Conference in 1973 a strategy and plan of action to combat gastroenteritis and malnutrition in children under two years of age was formulated (1975). This is an important document and should serve as a model for other parts of the developing world. The plan includes improvement of environmental health services (e.g. safe water supplies, sewage disposal, and solid waste disposal), the development of infant welfare clinics, campaigns to encourage breast-feeding, family planning advice, improved management and follow-up of gastroenteritis and malnutrition, health and nutrition education of the public, and economic and agricultural measures. Malnutrition is probably the single most important predisposing factor to the development of bacterial diarrhoea.

Interactions between the host and bacteria

The ecology of the gut flora reflects intricate relationships between the host and bacteria, and is of fundamental importance in determining bacterially induced disease. Before considering mechanisms available to the host for the control of the bacterial flora of its alimentary tract, I shall review the available information on the flora of normal subjects.

Gastrointestinal flora in normal subjects There is no information on the bacterial flora along the whole gastrointestinal tract of the normal child. In normal adults the same bacterial species are found throughout the gastrointestinal tract, but the relative numbers show marked variation according to the sampling site (Williams & Drasar 1972). In the fasting state the stomach is virtually sterile but immediately after a meal counts of up to 10^5/ml (streptococci, enterobacteria, bacteroides and bifidobacteria from the mouth and meal) are found; as gastric pH falls bacterial counts fall, and relatively few are grown below pH 3. Streptococci, lactobacilli, bifidobacteria and occasional bacteroides (10^3–10^4/ml) occur in the fasting proximal small gut, whilst counts of 10^5–10^7/ml are seen in the distal ileum; these bacteria are 'transients' from the mouth. In contrast to the small gut, 99% of bacteria in the colon and faeces are anaerobes (predominantly bacteroides and bifidobacteria) in counts of 10^{10}–10^{11}/g. The dominant aerobes in faeces are enterobacteria (mainly *E. coli*), enterococci (e.g. *Streptococcus faecalis*) and lactobacilli. Many favourable interactions occur between the resident bacterial flora of the alimentary tract and are important in maintaining the normal ecology (Bryant 1972); e.g. (*i*) certain bacteria such as *Bacteroides ruminicola* produce branched-chain organic acids which are essential for the growth of other bacteria, (*ii*) lactate-fermenting bacteria probably derive lactate from other bacteria, and (*iii*) H_2 and CO_2 produced by some bacteria are necessary for the growth of others.

Studies on the faecal flora of breast-fed babies (Bullen & Willis 1971) have shown a relative and absolute preponderance of bifidobacteria over *E. coli*/coliforms, whereas the reverse is the case in bottle-fed babies. Faeces from breast-fed babies never yielded bacteria other than bifidobacteria, bacteria of the *E. coli*/coliform complex and strains of anaerobic streptococci; faeces from bottle-fed babies, however, commonly contain clostridia, bacteroides and proteus species, and *Pseudomonas aeruginosa*. During weaning the flora becomes similar to that of the adult (Mata *et al.* 1972). The predominant bifidobacteria may play a contributory role in the low frequency of *Shigella* and other enteropathogens during breast-feeding (Mata *et al.* 1972), and form a good example of bacterial interactions favourable to the host.

TABLE 2

Mechanisms available to the host for the control of the bacterial flora of its alimentary tract

Gastric juice:	$[H^+]$
	Inhibitory substance
Small gut motility	
Resident bacterial flora:	Substrate competition
	Maintenance of $[H^+]$ and redox potential
	Production of short-chain organic acids
	Synthesis of colicins
	Inhibition of *Shigella*
Immune systems	
Diet	
Others:	Regulation of cell turnover and brush-border enzymes
	Production of lysozyme by Paneth cells
	Synthesis and interbacterial transfer of plasmids

Table 2 lists some of the mechanisms available to the host for the control of its gut flora.

Gastric juice The protective role of the intact stomach in bacterial enteritis is supported by abundant evidence of acid sensitivity among pathogens; also salmonellosis is commoner in post-gastrectomy cases (Waddell & Kunz 1956), and patients with hypochlorhydria are more likely to get cholera (Hurst 1934; Sack *et al.* 1970). An unidentified inhibitory substance may also contribute to the stomach's protective influence (Smith 1966).

Small gut motility Peristalsis is probably the most important factor in maintaining the relative sterility of the small intestine. Inhibitory studies using opiates, ganglion blockers, antiperistaltic pouches and ligation provide good evidence on how reduced motility provides favourable conditions for pathogens to colonize the small bowel (Grady & Keusch 1971*b*); this may explain why opium addicts are said to be more susceptible to severe attacks of cholera (Gorbach 1975). Impaired motility may also result in overgrowth of the small intestine by bacteria not normally considered pathogenic and, as a result of substrate metabolism, such as bile salt degradation (Guiraldes *et al.* 1975), may produce diarrhoea.

Resident flora The resident bacterial flora possesses a number of mechanisms which protect the host from pathogens, and maintain the normal ecosystem (Grady & Keusch 1971*b;* Bryant 1972); for example (*i*) substrate competition, (*ii*) maintenance of $[H^+]$ and redox potential, which discriminate against invading pathogens, (*iii*) the production of short-chain organic acids which have

bactericidal properties in protonated form, (*iv*) synthesis of colicins which are bactericidal to certain strains of *E. coli*, and (*v*) growth of *Shigella* is inhibited by indigenous flora of the mouse intestine.

Immune systems The precise role of immune mechanisms in the control of the gut flora has not been clearly defined. IgA levels are high in intestinal secretions, suggesting that secretory IgA may be of importance; however, in patients with selective IgA deficiency the bacterial flora of the small intestine and faeces is normal (Brown *et al.* 1972). In contrast, patients with hypogammaglobulinaemia have moderate to excessive numbers of anaerobic bacteria in the small intestine (Brown *et al.* 1972). The role of immunodeficiency, whether primary or acquired, as a predisposing factor in acute bacterial diarrhoea is not at present clear.

Both *V. cholerae* and shigellae stimulate the production of serum bactericidal and agglutinating antibodies, but parenteral immunization with killed bacteria confers only short-term protection (Mosley *et al.* 1970; Higgins *et al.* 1955). The relative importance of systemic and local immunity to host resistance is discussed in detail elsewhere (see Pierce, pp. 129–143 and McNeish, pp. 181–190).

Diet A high-carbohydrate diet increases the relative numbers of bifidobacteria, whilst a high-fat diet favours bacteroides (Hoffmann 1964). People eating a mixed 'western' diet have more bacteroides and fewer aerobes than those eating the native largely vegetarian diet in Uganda, South India or Japan (Hill *et al.* 1971). *Sarcina ventriculi* is virtually confined to vegetarians in whom faecal counts may reach 10^8/g (Crowther 1971). The significance of these observations in relation to diarrhoeal illness is not known.

Breast-fed babies are much less likely to develop enteritis than bottle-fed babies (Gerrard 1974), and the present decline of breast-feeding throughout the world is particularly disturbing. The mechanism(s) of the protection provided by breast-feeding are probably multifactorial, and include reduced risks of contamination, immunoglobulins (Gerrard 1974) and iron-binding proteins (Bullen *et al.* 1972; Bullen, this volume, pp. 149-162) in colostrum and milk, and the preponderance of bifidobacteria in association with a low pH in the faeces (Bullen & Willis 1971; Bullen, this volume, pp. 149–162).

Other interactions Bacteria may play a role in regulating turnover rates of epithelial cells, and in influencing brush-border enzyme activity (Savage 1972). The lysozyme of the succus entericus is, at least partly, synthesized in the Paneth cells of the crypts and probably contributes to intestinal defence mechanisms

(Peeters & Vantrappen 1975). Plasmids are non-chromosomal genetic elements of certain bacteria which can be transferred from one bacterial strain to another by sexual conjugation. They have an important regulatory role in the biosynthesis of a wide variety of bacterial products that play a part in the survival of bacteria, and in their interactions with their host and other bacteria. Of particular importance is the demonstration that the synthesis of both heat-stable and heat-labile *E. coli* enterotoxins is under plasmid control, and the possibility that toxin pathogenicity may be a transferable factor (Gyles 1972).

PATHOPHYSIOLOGICAL MECHANISMS

Enteropathogens may be broadly classified as invasive or non-invasive. Invasive organisms (i.e. *Salmonella*, *Shigella*, and certain strains of *E. coli*) penetrate the mucosa of the distal small intestine and colon to produce morphological abnormalities and dysentery; studies on *Salmonella* and *Shigella* diarrhoea in the rhesus monkey have shown the jejunal mucosa to be intact, but in a secretory state with respect to the transport of fluid and electrolytes (Rout *et al.* 1974; Rout *et al.* 1975). Thus, dysentery results from mucosal disruption, and diarrhoea from jejunal secretion superimposed on the absorptive defect in the distal bowel. Except for *Sh. dysenteriae* (Keusch *et al.* 1972) invasive pathogens are not known to elaborate enterotoxins.

The non-invasive organisms elaborate heat-labile (*V. cholerae* and *E. coli*) and heat-stable (*E. coli*) enterotoxins in the small bowel, and induce secretion without affecting mucosal structure. The transport defect is confined to the small bowel and diarrhoea results from the normal absorptive capacity of the colon being overwhelmed. The secretory effects of the heat-labile enterotoxins of *V. cholerae* and *E. coli* are mediated by activation of the adenylate cyclase system, and these molecular interactions are considered in detail elsewhere (see van Heyningen *et al.*, pp. 73–82; Flores & Sharp, pp. 89–103; Field, pp. 109–122).

COMPLICATIONS

The two most important complications of acute diarrhoea are (*a*) hypernatraemic dehydration, and (*b*) protracted diarrhoea.

Hypernatraemic dehydration

Hypernatraemic dehydration (i.e. serum sodium $>$ 150 mequiv./l) is the most important complication during the acute phase with a reported incidence of