CHAPTER 6

Food Microbiology and Public Health

6.1 FOOD HAZARDS

Although food is indispensible to the maintenance of life, it can also be responsible for ill health. A simple insufficiency will lead to marasmus (protein-energy deficiency) while over-reliance on staples low in protein, such as cassava, produces the condition known as kwashiorkor. A diet may provide adequate protein and energy but be lacking in specific minerals or vitamins giving rise to characteristic deficiency syndromes such as goitre (iodine deficiency), pellagra (nicotinic acid), beriberi (thiamine) and scurvy (ascorbic acid).

Foods are complex mixtures of chemicals and often contain compounds that are potentially harmful as well as those that are beneficial (Figure 6.1). Several vitamins are toxic if consumed in excessive amounts and many food plants produce toxic secondary metabolites to discourage their attack by pests.

Potatoes contain the toxic alkaloid solanine. Normally this is more concentrated in aerial parts of the plants and the peel which are not eaten, but high levels are also found in green potatoes and potato sprouts which should be avoided.

Cassava contains cyanogenic glycosides which produce hydrogen cyanide on hydrolysis. Similar compounds are also present in apple seeds, almonds, lima beans, yams and bamboo shoots. The body's detoxification pathway converts cyanide to thiocyanate which can interfere with iodine metabolism giving rise to goitre and cretinism. Traditional methods of preparing cassava eliminate the acute toxicity problem from hydrogen cyanide, but the increased incidence of goitre and cretinism in some areas where cassava is a staple may be a reflection of chronic exposure.

Legumes or pulses contain a number of anti-nutritional factors such as phytate, trypsin inhibitors and lectins (haemagglutinins). Many of these

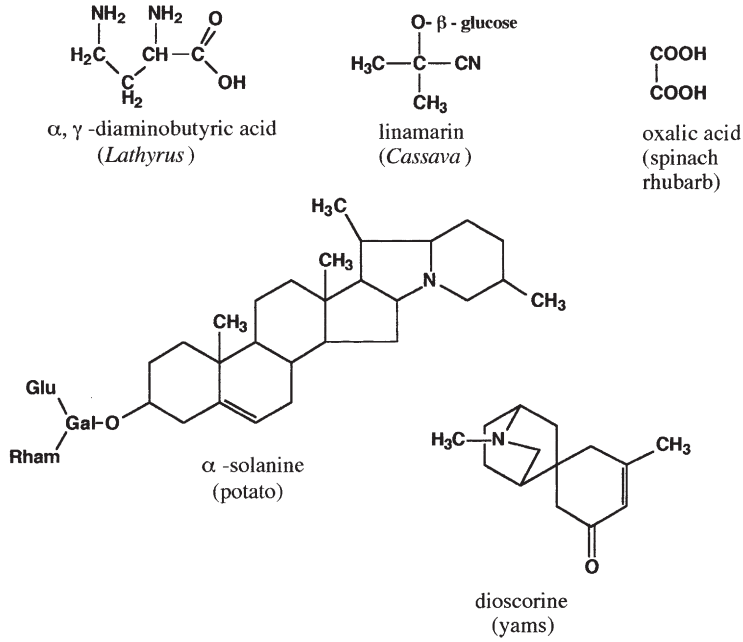

Figure 6.1 *Some natural food toxicants*

are destroyed or removed by normal preparation procedures such as soaking and cooking. Even so, red kidney beans are still responsible for occasional outbreaks of food poisoning when they have been insufficiently cooked to destroy the lectins they contain. Lathyrism is a more serious condition associated with a toxin in the pulse *Lathyrus sativa* which can be a major food item in North African and Asian communities during times of famine. In favism, an enzyme deficiency predisposes certain individuals to illness caused by glycosides in the broad bean, *Vicia faba*.

To some extent toxic or antinutritional characteristics can be bred out of cultivars intended for human consumption, although the problem cannot be eliminated completely. For affluent consumers in the developed world, particularly toxic foods can be avoided since alternatives are normally available in plenty. This is not always the case in poorer countries where these diet-related conditions are far more common.

It is however important to keep a sense of proportion in this. Eating inevitably exposes us to natural chemicals whose long-term effects on health are not known at present. Provided such foods form part of a balanced diet and are correctly prepared, the risks involved are generally acceptable, particularly when compared to the certain outcome should immoderate fear of food lead to complete abstinence.

In addition to the hazards posed by natural toxins that are an intrinsic feature of their composition, foods may also act as the vehicle by which

Table 6.1 *Possible causes of foodborne illness*

Chemical
Intrinsic, natural toxins, *e.g.* red kidney bean poisoning, toxic mushrooms
Extrinsic contamination
Algae, *e.g.* paralytic shellfish poisoning
Bacteria (infection and intoxication)
Fungi (mycotoxins)
Parasites
Protozoa
Viruses

an exogenous harmful agent may be ingested. This may be a pesticide, some other chemical contaminant added by design or accident, a micro-organism or its toxin. Various causes of foodborne illness are summarized in Table 6.1.

Here we are concerned primarily with microbiological hazards (Table 6.2). These are considered in some detail subsequently, but to justify this attention, we must first provide some assessment of their importance.

6.2 SIGNIFICANCE OF FOODBORNE DISEASE

Foodborne disease has been defined by the World Health Organization (WHO) as:

'Any disease of an infectious or toxic nature caused by, or thought to be caused by, the consumption of food or water.'

This definition includes all food and waterborne illness and is not confined to those primarily associated with the gastro-intestinal tract and exhibiting symptoms such as diarrhoea and/or vomiting. It therefore encompasses illnesses which present with other symptoms such as paralytic shellfish poisoning, botulism and listeriosis as well as those caused by toxic chemicals, but excludes illness due to allergies and food intolerances. The essential message of this section can be summarized by the conclusions of a WHO Expert Committee which pointed out that foodborne diseases, most of which are of microbial origin, are perhaps the most widespread problem in the contemporary world and an important cause of reduced economic productivity.

A number of assessments of the relative significance of hazards associated with food have concluded that micro-organisms are of paramount importance. A study conducted in the United States found that, although the attention given to different food hazards by the media, pressure groups and regulatory authorities might differ, as far as the food industry was concerned microbial hazards were the highest priority. Similarly, it has been estimated that the risk of becoming ill as a result of microbial contamination of food was 100 000 times greater than the risk from pesticide contamination.

Table 6.2 *Some microbiological agents of foodborne illness*

Agents	*Important reservoir/carrier*	*Transmission*[a]			*Multiplication in food*	*Examples of some incriminated foods*
		water	*food*	*person to person*		
BACTERIA:						
Aeromonas	Water	+	+	−	+	
Bacillus cereus	Soil	−	+	−	+	Cooked rice cooked meats Vegetables, starchy puddings
Brucella species	Cattle, goats, sheep	−	+	−	+	Raw milk, dairy products
Campylobacter jejuni	Chickens, dogs, cats, cattle, pigs, wild birds	+	+	+	−[b]	Raw milk, poultry
Clostridium botulinum	Soil, mammals, birds, fish	−	+	−	+	Fish, meat, vegetables (home preserved)
Clostridium perfringens	Soil, animals, man	−	+	−	+	Cooked meat and poultry, gravy, beans
Escherichia coli						
Enterotoxigenic	Man	+	+	+	+	Salads, raw vegetables
Enteropathogenic	Man	+	+	+	+	Milk
Enteroinvasive	Man	+	+	0	+	Cheese
Entero-haemorrhagic	Cattle, poultry, sheep	+	+	+	+	Undercooked meat, raw milk, cheese
Listeria monocytogenes		+	+	+	+	Soft cheeses, milk, coleslaw, pate
Mycobacterium bovis	Cattle	−	+	−	−	Raw milk
Salmonella Typhi	Man	+	+	±	+	Dairy produce, meat products, shellfish, vegetable salads
Salmonella (non-Typhi)	Man and animals	±	+	±	+	Meat, poultry, eggs, dairy produce, chocolate
Shigella	Man	+	+	+	+	Potato/egg salads
Staphylococcus aureus) (enterotoxins)	Man	−	+	−	+	Ham, poultry and egg salads, cream-filled bakery produce, ice-cream, cheese
Vibrio cholerae O1	Man, marine life?	+	+	±	+	Salad, shellfish
Vibrio cholerae, non-O1	Man and animals, marine life?	+	+	±	+	Shellfish

Table 6.2 (*continued*)

Agents	*Important reservoir/carrier*	*Transmission*[a]			*Multiplication in food*	*Examples of some incriminated foods*
		water	*food*	*person to person*		
Vibrio para-haemolyticus	Seawater, marine life	−	+	−	+	Raw fish, crabs, and other shellfish
Yersinia enterocolitica	Water, wild animals pigs, dogs, poultry	+	+	−	+	Milk, pork and poultry
VIRUSES:						
Hepatitis A virus	Man	+	+	+	−	Shellfish, raw fruit and vegetables
Norovirus	Man	+	+	0	−	Shellfish
Rotavirus	Man	+	0	+	−	0
PROTOZOA:						
Cryptosporidium parvum	Man, animals	+	+	+	−	Raw milk, raw sausage (nonfermented)
Entamoeba histolytica	Man	+	+	+	−	Raw vegetables and fruits
Giardia lamblia	Man, animals	+	±	+	−	0
HELMINTHS:						
Ascaris lumbricoides	Man	+	+	−	−	Soil-contaminated food
Taenia saginata and *T. solium*	Cattle, swine	−	+	−	−	Undercooked meat
Trichinella spiralis	Swine, carnivora	−	+	−	−	Undercooked meat
Trichuris trichiura	Man	0	+	−	−	Soil-contaminated food

[a] Almost all acute enteric infections show increased transmission during the summer and/or wet months, except infections due to rotavirus and *Yersinia enterocolitica*, which show increased transmission in cooler months

[b] Under certain circumstances some multiplication has been observed. The epidemiological significance of this observation is not clear

+ = Yes

± = Rare

− = No

0 = No information

Adapted from WHO 1992

Foodborne diseases range from relatively mild, self-limiting gastrointestinal upsets through to life-threatening conditions such as botulism. Some foodborne infections can develop severe complications such as the haemolytic uraemic syndrome associated with about 10% of *E. coli* O157:H7 infections or the neurological disorder Guillain-Barré syndrome that follows about 0.1% of *Campylobacter* infections. Some groups of people are particularly susceptible to the more serious consequences of food borne disease. These include the elderly, infants and those who are immunocompromised as a result of illness or chemotherapy.

For otherwise healthy, well-nourished people in the developed world, most food poisoning is an unpleasant episode from which recovery is normally complete after a few days. For society as a whole though, it is increasingly being recognized as a largely avoidable economic burden. Costs are incurred in the public sector from the diversion of resources into the treatment of patients and the investigation of the source of infection. To the individual the costs may not always be calculable in strictly financial terms but could include loss of income, as well as costs of medication and treatment. Studies conducted by the Communicable Disease Surveillance Centre (CDSC) in London have even identified as a cost the 'trousseau effect', where an individual who is hospitalized incurs additional expense as a result of having to purchase items such as new night-attire for the occasion. On the larger scale, absence from work will also constitute a cost to the national economy.

A number of attempts have been made to quantify these costs and, while the errors must be large, they do at least give an idea of the magnitudes involved. Thus a study in the United States estimated that the total annual cost to the US economy of bacterial food poisoning is approaching US$ 7 billion. Substantially lower, but still considerable, costs of almost £1 million have been associated with 1482 salmonella cases in the UK in the year 1988/9 (Table 6.3). The Infectious Intestinal Disease (IID) Study conducted in England (see later) estimated the

Table 6.3 *Costs associated with 1482 Salmonella cases in 198819*

	Cost (£)	*Proportion (%)*
Investigation of cases[a]	157 162	16
Treatment of cases[b]	235 660	24
Costs to individuals and families[c]	95 962	9
Loss of production[d]	507 555	51
Total	996 339	100

[a] Local authority and laboratory costs
[b] GP and hospital services
[c] Treatment-related and incidental costs
[d] Absence from work related to illness and caring for sick individuals
Source: P.N. Sockett, PhD thesis; P.N. Sockett and J.R. Roberts (1991)

average cost of a case of IID, whatever its cause, to be £79 at 1993-1995 prices.

For the food industry, the costs can be huge and it is not unusual for the company producing a product implicated in an outbreak of food poisoning to go bankrupt as a result. Companies not directly involved in an outbreak can also suffer. There is often a general decline in demand for a product prompted by public concern that the same problem could occur with similar products from other manufacturers. There was, for instance, a marked downturn in all yoghurt sales after the hazelnut yoghurt botulism outbreak in England in 1989.

Increased vigilance by companies to ensure that the same process failures responsible for an outbreak do not occur elsewhere, also has its attendant costs. For instance, it was estimated that the costs of checking the integrity of spray-drier cladding by dried-milk manufacturers following a salmonella outbreak caused by dried milk were of the order of hundreds of thousands of pounds.

Food retailers can also be affected as a result of a decline in sales, particularly if a suspect product is associated with one particular store.

In the less developed world the consequences of foodborne illness are even more serious. Diarrhoeal disease is a major cause of morbidity and mortality in poor countries, particularly among children. It has been estimated that some 1,500 million children under 5 suffer from diarrhoea each year and that over 3 million die as a result. Diarrhoea can occur repeatedly in the same individual leading to malnutrition which in turn predisposes them to more severe diarrhoeal episodes and other serious infections. This can produce a downward spiral of increasingly poor health which can seriously impair a child's mental and physical development and can lead to its premature death. (Figure 6.2).

Weaning is a particularly hazardous time for the infant. The anti-infective properties of maternal breast milk are lost or diluted and are replaced by foods which often have a low nutrient density. At the same time, the immature immune system is exposed to new sources of infection in the environment. Poor hygienic practices in the preparation of

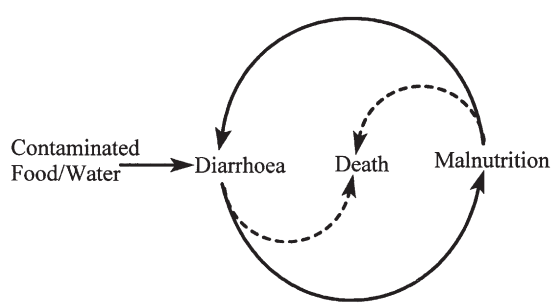

Figure 6.2 *The malnutrition and diarrhoea cycle*

weaning foods and the use of contaminated water are often implicated in weaning diarrhoea and it has been estimated that 15–70% of all diarrhoea episodes in young children are food associated.

6.3 INCIDENCE OF FOODBORNE ILLNESS

Statistics covering foodborne illnesses are notoriously unreliable. Simply quantifying the problem of those diseases initiated by infection through the gastrointestinal tract is difficult enough, but to determine in what proportion food acted as the vehicle is harder still.

Many countries have no system for collecting and reporting data on gastrointestinal infections and even where these exist the reported data is acknowledged to represent only a fraction of the true number of cases. Studies have suggested that the ratio of actual to reported cases can be between 25:1 and 100:1. One should also be circumspect about using published national statistics for comparative purposes since apparent differences can often simply reflect differences in the efficiency of the reporting system. In the United States, reporting of foodborne illness outbreaks to the Center for Infectious Diseases, is not compulsory so that some States report rates 200 times those of other States. In the early 1980s reported outbreaks of foodborne disease for the United States were roughly twice those reported by Canada which has a population only one tenth the size. It seems unlikely that Canadians are markedly more susceptible to foodborne illness or more careless about food hygiene than their neighbours; more probably the disparity reflects a higher level of under-reporting in the United States. Some support for this appears if the statistics for all gastrointestinal disease are compared. These are a much closer reflection of the relative population sizes since these figures are officially notiable in the USA.

Such statistical problems are not unique to North America. The WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe which reports data from more than 30 countries has noted the different national systems of notification and reporting. These include:

(i) notification of cases of foodborne disease without any specification of the causative agent or other epidemiologically important details;
(ii) reporting only laboratory-confirmed cases of foodborne disease collated by a central agency;
(iii) reporting cases of gastrointestinal infection which, in some cases, are regarded as being foodborne regardless of whether the involvement of food has been established;
(iv) reporting only cases of salmonellosis.

As a result, the reported incidence of foodborne illnesses can vary widely from country to country. In the countries which make up the Organisation for Economic Co-operation and Development (OECD), for example, the reported incidence of non-typhoid *Salmonella* infections ranges from 3.9 to 476.2 cases per 100,000 inhabitants and from 0.1 to 271.5 for *Campylobacter*. Some of this variation might be due to differences in food consumption habits but the WHO assumes that the overall burden of foodborne illness is probably of the same order of magnitude in most OECD countries, therefore these differences must in large part reflect the efficiency of various national data collection systems.

Most cases of foodborne illness are described as sporadic; single cases which are not apparently related to any others. Sometimes two or more cases are shown to be linked to a common factor in which case they constitute an outbreak. Outbreaks can be confined to a single family or be more generalized, particularly when commercially processed foods are involved.

In England and Wales, information on sporadic cases of foodborne disease comes from a number of different sources. The Health Protection Agency publishes statistics on clinical cases of food poisoning which comprise notifications by medical practitioners and those cases identified during the course of outbreak investigations but not formally notified by a doctor.

Although notification is statutory, *i.e.* required by law, these data are acknowledged to be incomplete as a result of significant under-reporting. Diagnosis is often made purely on the basis of symptoms, without recourse to any microbiological investigation which could establish both the causative agent and the food vehicle. Similarly, it is probably significant that the league table of the most commonly reported causes of food poisoning in England, Wales and Scotland (Table 6.4) also partially reflects the relative severity of symptoms (with the notable exception of *C. botulinum*). It is reasonable to assume that the more ill you feel the more likely you are to seek medical attention and the more likely your case is to figure in official statistics. The situation can be represented as a pyramid, where the large base reflects the true incidence of food poisoning which is reduced to a small apex of official statistics by the various factors that contribute to under-reporting (Figure 6.3).

The Infectious Intestinal Disease (IID) Study which collected data in England in the period 1993–1996 aimed to estimate some of these uncertainties. Based primarily around 70 representative doctor's practices, volunteers were recruited to notify the doctor each week whether or not they had had symptoms of gastrointestinal illness during that week and, in cases where they had been ill, to submit a faecal specimen to the laboratory. Surveys were also made on the number of people visiting the doctor complaining of IID and the proportion from which faecal specimens were taken, the long-term medical sequelae of IID and the

Table 6.4 *Outbreaks of gastrointestinal illness in England and Wales (2003, 2004) and the USA (1996, 1997)*

	Cases (outbreaks)			
	England and Wales[b]		*USA*[c]	
Agent	*2003*	*2004*	*1996*	*1997*
Salmonella	14963[a]	13125[a]	12450 (69)	1731 (60)
Clostridium perfringens	23 (2)	486 (6)	1011 (10)	255 (6)
Bacillus spp.	55 (5)	0 (0)	22 (1)	438 (4)
Staph. aureus	0 (0)	31 (2)	178 (7)	393 (9)
E. coli O157	675[a]	699[a]	325 (11)	300 (8)
Shigella sonnei	633[a]	815[a]	109 (6)	315 (10)
Clostridium Botulinum	1	2	4 (2)	2 (1)
Campylobacter	46181[a]	44294[a]	101 (5)	104 (2)

[a] Numbers of reported isolations (includes sporadic cases)
[b] Source: Health Protection Agency
[c] Source: MMWR March 17 2000 **49** No. SS-1

numbers of cases presenting to hospitals rather than to their local doctor. The results, published in the British Medical Journal (*Br. Med. J.*, 1999, **318**, 1046–1050), were broadly similar to those found in an earlier Dutch study and indicated that infectious intestinal disease occurs in 1 in 5 people each year amounting to an estimated 9.4 million cases. Of those people affected, only 1 in 6 went to the doctor. The proportion of cases that were not recorded by official statistics was large and varied widely by organism. For example, the degree of under reporting for salmonella was relatively low with 1 in 3.2 cases being reported. It was worse with campylobacter where the ratio was 1 in 7.6 cases. Under reporting of viral IID was much more severe with national surveillance picking up only 1 in 35 cases of diarrhoea caused by rotavirus and 1 in 1562 caused by small round structured viruses (Norovirus). There were also many cases for which no causative organism was identified.

An additional source of statistics is the voluntary, nonstatutory reporting system from public health and hospital laboratories of isolations of gastrointestinal pathogens. The statistics generated in this way include cases where food was not the vehicle but the pathogen was acquired by some other means such as person-to-person spread or from domestic pets.

Information on outbreaks is collected by the HPA from microbiologists and environmental health officials around the country. Sometimes the existence of an outbreak is impossible to ignore if it involves a large number of people or a readily defined commercial or institutional context, for example a large public reception, diners at the same restaurant or passengers in the same airliner. Sometimes the existence of an outbreak may emerge from follow-up investigations on sporadic cases. This is often possible where highly discriminating typing schemes are available that enable the pathogen strain causing an outbreak to be

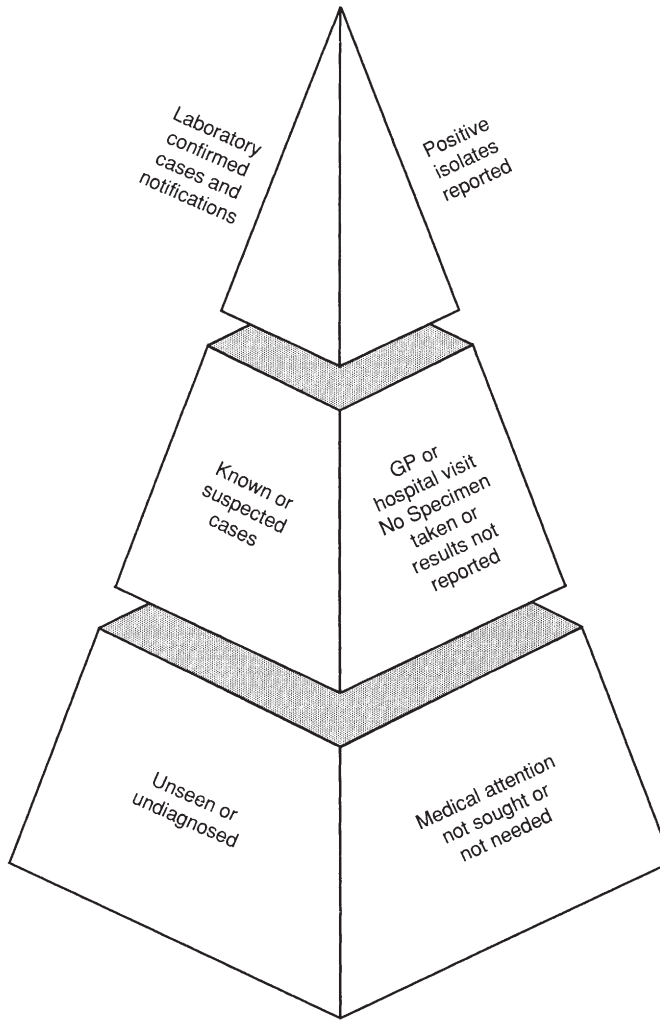

Figure 6.3 *The food poisoning pyramid* (P. Sockett, PhD thesis)

distinguished from strains responsible for the statistical background 'noise' of sporadic cases. Such schemes have also enabled international outbreaks of salmonellosis to be identified through the Enter-net surveillance network which was started by the European Union but now includes countries such as Australia, Canada, New Zealand, Japan and Mexico. Even so, it is probable that many outbreaks remain undetected, submerged in the numbers for sporadic cases.

Annual reports of statistics on food poisoning and isolations of *Salmonella* have been published for England and Wales since 1949 and showed no discernible trend until the 1980s when a steady increase was apparent (Figure 6.4). A similar but smaller increase was noted in Scotland. Nearly

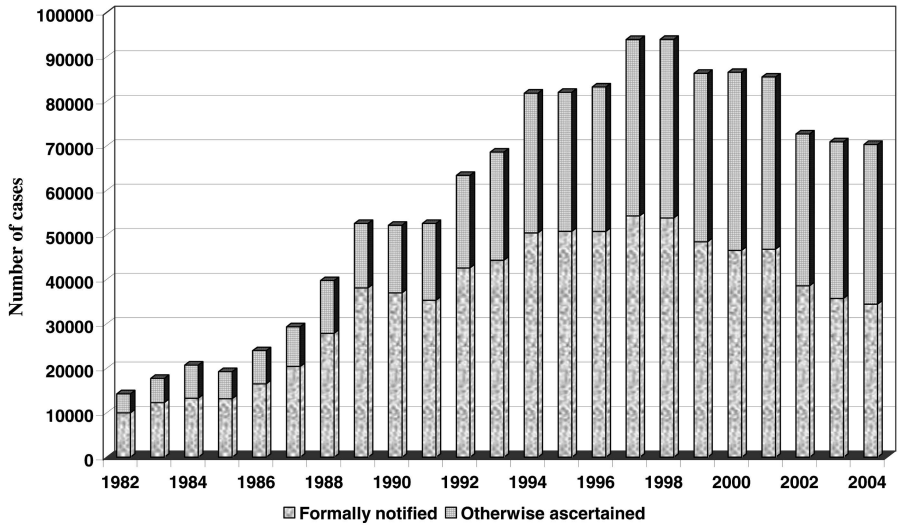

Figure 6.4 *Food poisoning in England and Wales 1982–2004*
Source OPCS

all European countries have reported an increase in foodborne illness starting in the mid-1980s, and in the United States over the period 1983–87 more cases, although fewer outbreaks, were reported. In Austria, the rate of foodborne salmonellosis has increased from 19 cases per 100 000 inhabitants in 1985 to 62 in 1989 and 89 in 1996.

Since 1997 numbers of salmonella isolations have declined throughout Europe although cases of campylobacteriosis have continued to climb. Higher numbers of foodborne disease cases may reflect improved reporting and data collection procedures, better methods of isolation and identification and heightened awareness of the problem but they are also held to reflect a real underlying increase in the incidence.

6.4 RISK FACTORS ASSOCIATED WITH FOODBORNE ILLNESS

Outbreaks of food poisoning involve a number of people and a common source and are consequently more intensively investigated than the more numerous sporadic cases that occur. Valuable information is derived from these investigations about the most common contributory factors and faults in food hygiene that lead to outbreaks of foodborne illness. Specific examples will be given in the following chapter when bacterial pathogens are considered individually, but analysis of this information does allow a number of generalizations to be made.

The foods that are most frequently incriminated in foodborne disease in Europe and North America are those of animal origin: meat, poultry,

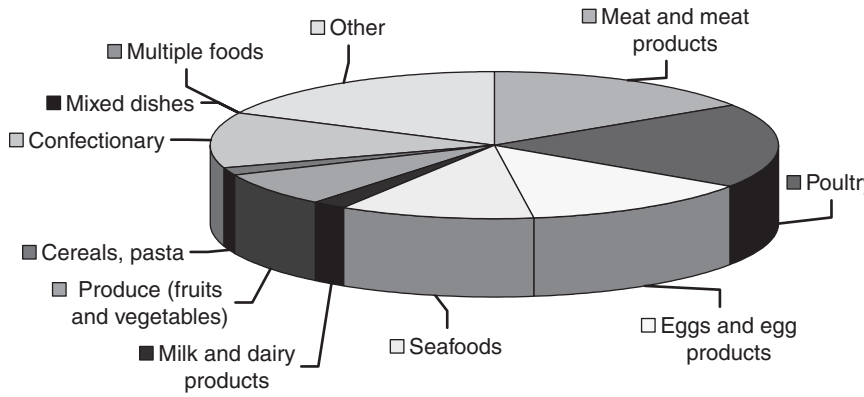

Figure 6.5 *Food implicated in foodborne disease outbreaks in the UK*

milk, eggs, and products derived from them. This is particularly true of illness caused by *Salmonella* and *Clostridium perfringens*. Data on the association of particular foods with foodborne disease outbreaks in England and Wales are presented in Figure 6.5. The same general picture is true of most industrialized countries although the relative importance of some animal products does differ. For example, in Spain between 1985 and 1989 eggs and egg products such as mayonnaise were incriminated in 62% of outbreaks for which a cause was established. In the Netherlands in 1991 and 1992 Chinese food was the most common vehicle associated with outbreaks, ahead of both poultry and eggs and other meats.

Fish and shellfish are less commonly implicated but can be an important vehicle in some countries, often reflecting local dietary habits. In Japan in the year 2000 seafoods were responsible for 25% of outbreaks where a causative food was identified.

Outbreaks can result from the distribution of a contaminated food product or from situations where meals are being produced for large numbers of people. Evidence from numerous countries has shown that mass-catering is by far the most frequent cause of outbreaks, whether it comes under the guise of restaurants, hotels, canteens, hospitals or special events such as wedding receptions. There are a number of reasons why this should be, but inadequacies of management, staff training and facilities are often identified.

Analyses of the specific failures in food hygiene that have contributed to outbreaks have been conducted on a number of occasions and results of two of these, from the United States and from England and Wales are presented in Table 6.5. Comparing the two is not entirely straightforward since, in most outbreaks more than one contributory factor has been identified so that the columns do not add up neatly to 100%. Also, the surveys differ in the categories used and even where they are nominally the same they may still not be equivalent in all respects. Even

Table 6.5 *Factors contributing to outbreaks of food poisoning*

Factor	*England and Wales*[a]	*USA*[b]
Preparation too far in advance	57	29
Storage at ambient temperature	38	63
Inadequate cooling	32	
Contaminated processed food	17	n.i.
Undercooking	15	5
Contaminated canned food	7	n.i.
Inadequate thawing	6	n.i.
Cross contamination	6	15
Food consumed raw	6	n.i.
Improper warm handling	5	27
Infected food handlers	4	26
Use of left overs	4	7
Extra large quantities prepared	3	n.i.

[a] 1320 outbreaks between 1970 and 1982 from Roberts 1985
[b] Outbreaks occurring between 1973 and 1976 from Bryan 1978
n.i. category not included in analysis
Figures are expressed as percentages. Since several factors may contribute to a single outbreak columns do not total 100%

so, inspection of Table 6.5 reveals two major contributory factors; temperature and time. Failure to cool foods and hold them at temperatures inimical to microbial growth, or to heat them sufficiently to kill micro-organisms, coupled with prolonged storage giving micro-organisms time to multiply to dangerous levels. An interesting difference between the two sets of data is the lower incidence of infected food handlers contributing to illness in England and Wales.

6.5 THE CHANGING SCENE AND EMERGING PATHOGENS

So far we have tried to present a brief overview of the current situation with regard to foodborne disease. It is important to remember however that what we see now is just a snapshot of a dynamic situation. Major food hazards such as *Listeria monocytogenes* and *Campylobacter* were simply not recognised as such 40 years ago and other "new" pathogens such as Verotoxin-producing *E. coli* and *Enterobacter sakazakii* have emerged even more recently. This has led to some organisms being designated emerging pathogens. These are not necessarily entirely new species (though they can be) but can also be old favourites in new guises. In their broadest definition emerging pathogens are organisms causing illnesses that have only recently appeared or been recognised in a population, or organisms that are well recognised but are rapidly increasing in incidence or geographical range.

A number of factors contribute to this evolving pattern of foodborne disease:

Changes in farming practices – there are constant economic pressures to increase agricultural productivity and this can impact on food safety as

when, for example, intensified animal production contributes to the spread of zoonotic pathogens or pressure on land use results in contamination of field crops from manure.

Increased international trade in foods – countries increasingly source their food on a global basis and this can pose problems with control of foodborne hazards. High standards and efficient control methods in one country can be undermined by importation from a country where such standards do not apply. There have been a number of international outbreaks of foodborne disease cause by imported foods and these are likely to increase with the level of international trade.

Changes in food processing – there is increasing reliance on refrigeration and the cold chain as a way of extending the shelf life of fresh foods and this has contributed to the emergence of psychrotrophic pathogens such as *Listeria monocytogenes* as important concerns.

Increased international movement of people – this can take the form of refugees from wars, social conflict or economic hardship as well as movement of those from more prosperous regions for leisure or business purposes. Both offer new opportunities for the acquisition or transmission of foodborne and other diseases. In Sweden, 90% of *Salmonella* cases are estimated to be imported cases.

Changing character of the population – the very young, the old, the very sick and the immunocompromised are all more at risk from foodborne diseases. As a result of improvements in nutrition and healthcare, the proportion of the population in some of these groups is increasing.

Lifestyle changes – increased affluence, urbanisation and other social changes can lead to increased consumption of exotic or unusual foods or meals prepared away from the home. This can result in changes in the incidence and nature of foodborne illness.

Microbial evolution – micro-organisms are constantly changing their characteristics as a result of evolutionary processes. If these changes affect the virulence or pathogenicity of an organism then a new hazard can emerge. Such changes can occur completely independently of human activity but the latter can sometimes provide selective pressures enabling new strains to thrive.

6.6 THE SITE OF FOODBORNE ILLNESS. THE ALIMENTARY TRACT: ITS FUNCTION AND MICROFLORA

In most of the cases of foodborne illness we consider, the pathogenic (disease producing) effect occurs in the alimentary tract giving rise to symptoms such as diarrhoea and vomiting. Since these are essentially a dysfunction of the gut, a useful starting point would be to outline its normal operation and the role micro-organisms play in this process.

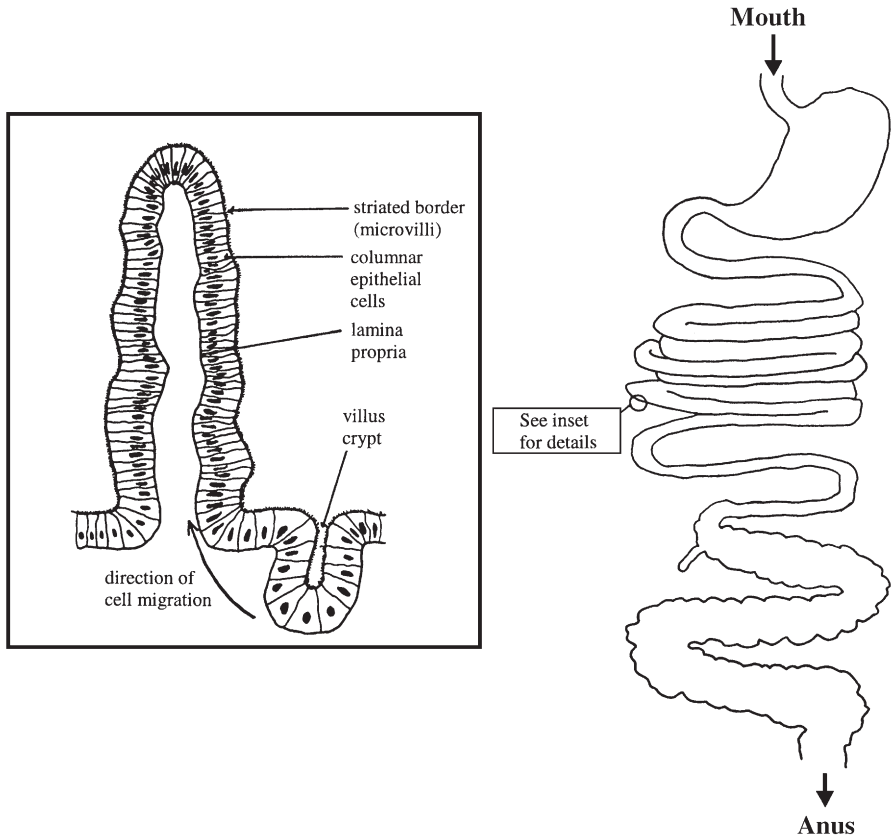

Figure 6.6 *The gastrointestinal tract. Inset: expanded view of inner surface of small intestine*

The alimentary or gastrointestinal tract is not an internal organ of the body but a tube passing through it from the mouth to the anus (Figure 6.6). Its principal functions are the digestion and absorption of food and the excretion of waste. Unlike most of the body's other external surfaces, it is not lined with a dry protective skin and so, although it possesses some protective features, it offers a more congenial environment for micro-organisms and an easier route by which they can penetrate the body.

In the mouth, food is mixed with saliva and broken down mechanically to increase the surface area available for attack by digestive enzymes. Saliva is an alkaline fluid containing starch-degrading (amylase) enzyme and the antimicrobial factors immunoglobulin (IgA), lysozyme, lactoferrin and lactoperoxidase. It provides lubrication to assist chewing and swallowing and performs a cleansing function, rinsing the teeth and mouth to remove debris. On average, an adult secretes and swallows about 1.5 l of saliva each day.

The variety of foods consumed and the range of micro-environments in the mouth result in a diverse and continually changing microflora. On the teeth, bacteria are associated with the formation of dental plaque – an organic film in which bacteria are embedded in a matrix derived from salivary glycoproteins and microbial polysaccharides. The microbial composition of plaque varies with its age but filamentous *Fusobacterium* species and streptococci are common components. Plaque offers a protective environment for bacteria and its development is often a prelude to conditions such as dental caries and periodontal disease.

Swallowed food descends *via* the oesophagus into the stomach; a bulge in the alimentary tract which serves as a balance tank from which food is gradually released into the small intestine for further digestion.

In the stomach, food is blended with gastric juice, an acidic fluid containing hydrochloric acid. Stomach pH can range from 0.8–5.0 (typically 2.0–3.0) and has a marked effect on ingested micro-organisms, killing most. Normally only acid-tolerant vegetative cells and spores survive and the microbial count in the stomach is low, although lactobacilli are frequently found in association with the stomach wall. Gastric acidity generally provides very effective protection for subsequent sections of the intestine but is not, as we shall see, an invulnerable defence. Bacteria can evade prolonged exposure to the acid by being sheltered in food particles or as a result of accelerated passage through the stomach as occurs, for instance, when the stomach is full. Alternatively, acidity may be neutralized by the food or absent as a result of illness.

The digestive functions of the stomach are not confined to those of a mechanical churn with antimicrobial features. Proteases, such as pepsin, and lipase which can operate at low pH partially digest the stomach contents. The gastric mucosa also secretes a protein responsible for efficient absorption of vitamin B_{12}. Little absorption of nutrients occurs in the stomach, with the notable exception of ethanol, but some material transfer is often necessary to adjust the osmotic pressure of the stomach contents to ensure they are isotonic with body fluids.

From the stomach, small quantities of the partially digested mixture of food and gastric juice, known as chyme, are released periodically into the small intestine. In this muscular tube over 6 metres long most of the digestion and absorption of food occur. Its internal lining is extensively folded and the folds covered with finger-like projections or villi which are themselves covered in microvilli. This gives the inner surface the appearance and texture of velvet and maximizes the area available for absorption (Figure 6.6).

In the first section of the small intestine, the duodenum, large-scale digestion is initiated by mixing the chyme with digestive juice from the pancreas and bile from the gallbladder which neutralize the chyme's

acidity. The pancreatic juice also supplies a battery of digestive enzymes, and surfactant bile salts emulsify fats to facilitate their degradation and the absorption of fat soluble vitamins. Further digestive enzymes that break down disaccharides and peptides are secreted by glands in the mucous lining of the duodenum called, with evocations of a Gothic horror, the crypts of Lieberkühn.

The duodenum is a relatively short section of the small intestine, accounting for only about 2% of its overall length. Food is swept along by waves of muscle contraction, known as peristalsis, from the duodenum into the jejunum and thence into the ileum. During this passage, nutrients such as amino acids, sugars, fats, vitamins, minerals and water are absorbed into capillaries in the villi from where they are transported around the body. Absorption is sometimes a result of passive diffusion, but more often involves the movement of nutrients against a concentration gradient; an active process entailing the expenditure of energy.

The gut is home to a huge population of bacteria comprised of more than 400 different species with total numbers estimated at around 10^{14}, far more than the number of cells in the human body. The microbial population increases down the length of the small intestine: counts of 10^2–10^3 ml^{-1} in the duodenum increase to around 10^3–10^4 in the jejunum, 10^5 in the upper ileum and 10^6 in the lower ileum. This corresponds with a decreasing flux of material through the small intestine as water is absorbed along its length. In the higher reaches of the duodenum, the flow rate is such that its flushing effect frequently exceeds the rate at which micro-organisms can multiply so that only those with the ability to adhere to the intestinal epithelium can persist for any length of time. As the flow rate decreases further along the small intestine, so the microbial population increases, despite the presence of antimicrobial factors such as lysozyme, secretory immunoglobulin, IgA, and bile.

In the healthy individual, the microflora of the small intestine is mainly comprised of lactobacilli and streptococci, although, as we shall see, other bacteria have the ability to colonize the epithelium and cause illness as a consequence.

Extensive microbial growth takes place in the colon or large intestine where material can remain for long periods before expulsion as faeces. During this time active absorption of water and salts helps to maintain the body's fluid balance and to dry faecal matter. Bacterial cells account for 25–30% of faeces, amounting to 10^{10}–10^{11} $cfu\,g^{-1}$, the remainder is composed of indigestible components of food, epithelial cells shed from the gut, minerals, and bile.

Obligate anaerobes such as *Bacteroides* and *Bifidobacterium* make up 99% of the flora of the large intestine and faeces. Members of the Enterobacteriaceae, most commonly *Escherichia coli*, are normally present at around $10^6\,g^{-1}$, enterococci around $10^5\,g^{-1}$, *Lactobacillus*,

Clostridium and *Fusobacterium*, 10^3–10^{5-1}g, plus numerous other organisms, such as yeasts, staphylococci and pseudomonads, at lower levels.

The interaction between the gut microflora and its host appears to have both positive and negative aspects and is the subject of much current research and conjecture. Addition of antibiotics to feed has been shown to stimulate the growth of certain animals, suggesting that some gut organisms have a deleterious effect on growth.

A normal gut microflora confers some protection against infection. One example of this effect is the inflammatory disease pseudomembranous colitis caused by *Clostridium difficile*. Normally this organism is present in the gut in very low numbers, but if the balance of the flora is altered by antibiotic therapy, it can colonize the colon releasing toxins. Similarly, the infective doses of some other enteric pathogens have been shown to be lower in the absence of the normal gut flora.

It appears that protection is not simply a result of the normal flora occupying all available niches, since enterotoxigenic *E. coli* adheres to sites that are normally vacant. Some direct antagonism through the production of organic acids and bacteriocins probably plays a part, but stimulation of the host immune system and its capacity to resist infection also appear to be factors.

In monogastric animals such as humans, gut micro-organisms do not play the same central role in host nutrition as they do in ruminants. Some facultative anaerobes found in the gut, such as *E. coli* and *Klebsiella mobilis* (previously known as *K. aerogenes* and *Enterobacter aerogenes*) are known to produce a variety of vitamins *in vitro* and studies using animals reared in a germ-free environment and lacking any indigenous microflora have shown that *in vivo* vitamin production by micro-organisms can be important on certain diets. In humans, however, the evidence is less convincing. Some have questioned the efficiency of absorption of vitamins produced in the large intestine pointing to the fact that vegans have developed vitamin B_{12} deficiency despite its production in the gut and excretion in the faeces. It appears that an adequate balanced diet will probably meet all the body's requirements in this respect and that, short of coprophagy, which is practised by some herbivores such as rabbits, access to vitamins produced *in situ* is limited.

6.7 THE PATHOGENESIS OF DIARRHOEAL DISEASE

Several foodborne illnesses, such as typhoid fever, botulism and listeriosis, involve body sites remote from the alimentary tract which serves simply as the route by which the pathogen or toxin gains entry to the body. The pathogenesis of these conditions will be discussed under the individual organisms concerned.

Table 6.6 *Clinical classification of infections diarrhoea*[a]

Type	*Symptoms*	*Typical causative organisms*
Acute watery	diarrhoea	Loose or watery stools without visible blood. Duration generally less than 7 days
Vibrio	*cholerae*, Enterotoxigenic *E. coli*. Small round structured viruses	
Acute bloody	diarrhoea	Loose or watery stools with visible blood. Duration generally less than 7 days
Shigella,	*Campylobacter jejuni* Enteroinvasive *E. coli*. Small round structured viruses	
Persistent	diarrhoea	Loose or watery stools with or without visible blood with a duration of 14 days or more
	Multifactorial: enteric infection, malnutrition, impaired immunity, lactose intolerance	

[a] Definition: passage of loose or watery stools three or more times in a 24 hour period

A more common conception of foodborne illness, often described as food poisoning, is where symptoms, like the causative agent, are confined to the gut and its immediate vicinity. The patient presents an acute gastroenteritis characterized by diarrhoea and vomiting. Individual pathogens will be described in some detail subsequently, but for now we will consider some common features of the mechanisms involved.

Diarrhoea is the excessive evacuation of too-fluid faeces (see Table 6.6). Any process which seriously interferes with the gut's capacity to absorb most of the 8–10 l of fluid it receives each day, or increases secretion into the intestinal lumen, will produce this condition. Consequently, the aetiology of diarrhoea can be quite complex and a number of different mechanisms have been identified.

The ability to cause illness is generally the result of a combination of properties that enable a micro-organism to damage its host. These are called virulence factors and include not only those factors most directly responsible for damage such as toxins, but others such as the ability to evade host defence mechanisms e.g. stomach acidity and bile in the gut, the ability to adhere to mucosal surfaces, secrete proteins and, where necessary, invade host cells. Many of these properties are encoded on relatively large, discrete segments of DNA known as pathogenicity islands. *Salmonella* for example possesses at least 5 pathogenicity islands. These are incorporated into the bacterial chromosome but have a G + C content markedly different from the surrounding chromosome. This and other characteristics suggest that they have been acquired by horizontal transfer from a foreign source. Acquisition of relatively large segments of DNA can promote genetic variability and play and important role in

microbial evolution. When these encode virulence factors, they allow sudden rapid changes in pathogenicity an the emergence of new hazards.

Toxins are frequently the direct cause of diarrhoea. As their nomenclature often causes students some confusion, one or two preliminary definitions are probably in order. *Exotoxin* is the term used to describe toxins that are released extracellularly by the living organism. These include:

enterotoxins which act on the intestinal mucosa generally causing diarrhoea;
cytotoxins which kill host cells;
neurotoxins which interfere with normal nervous transmission.
Endotoxins are pyrogenic (fever producing) lipopolysaccharides released from the outer membrane of the Gram-negative cell envelope by bacterial lysis.

Bacterial food poisoning can be divided into three principal types.

(1) *Ingestion of pre-formed toxin*. Toxins may be produced in and ingested with the food as in *Staphylococcus aureus* food poisoning and the *Bacillus cereus* emetic syndrome. Botulism is similar in this respect though in this case gastrointestinal symptoms are of minor importance. The absence of person to person spread and a relatively short incubation period between ingestion of food and the onset of symptoms are usual characteristics of this type of food poisoning.
(2) *Non-invasive infection*. In a non-invasive infection, viable bacteria ingested with food, colonize the intestinal lumen. This is principally associated with the small intestine where competition from the endogenous microflora is less intense. To prevent their removal by the flushing action of the high flow rates in this section of the gut, the pathogen generally attaches to and colonizes the epithelial surface. It does this by producing adhesins, molecules often associated with fimbriae on the bacterial cell surface, which recognize and attach to specific receptor sites on the microvilli. Loss of the ability to adhere to the gut wall will dramatically reduce a pathogen's virulence – its ability to cause illness.

Once attached, the pathogen produces a protein enterotoxin which acts locally in the gut changing the flow of electrolytes and water across the mucosa from one of absorption to secretion. Several enterotoxins act by stimulating enterocytes (the cells lining the intestinal epithelium) to over-produce cyclic nucleotides.

Most extensively studied in this respect is the cholera toxin produced by *Vibrio cholerae*. The toxin (MW 84 000) comprises five B subunits and a single A subunit. The B subunits bind to specific ganglioside (an acidic glycolipid) receptors on the enterocyte surface. This creates a hydrophilic channel in the cell membrane through which the A unit can pass. Once

inside the cell, a portion of the A unit acts enzymically to transfer an ADP-ribosyl group derived from cellular NAD to a protein regulating the activity of the enzyme adenylate cyclase. As a result, the enzyme is locked into its active state leading to an accumulation of cyclic adenosine monophosphate (cAMP) which inhibits absorption of Na^+ and Cl^- ions while stimulating the secretion of Cl^-, HCO_3^- and Na^+ ions. To maintain an osmotic balance, the transfer of electrolytes is accompanied by a massive outflow of water into the intestinal lumen. This far exceeds the absorptive capacity of the large intestine and results in a profuse watery diarrhoea (Figure 6.7).

A number of other enterotoxins have been shown to act in the same way as the cholera toxin including the heat labile toxin (LT) produced by some types of enterotoxigenic *E. coli*. Other toxins such as the heat stable toxin of *E. coli* are similar in the respect that they stimulate the production of a cyclic nucleotide in enterocytes. In this case it is cyclic guanosine monophosphate (cGMP) which differs slightly from cAMP in its activity but also produces diarrhoea as a result of electrolyte imbalances.

A different enterotoxin is produced by *Clostridium perfringens* as it sporulates in the gut. The toxin binds to receptors on the surface of cells of the intestinal epithelium, producing morphological changes in the

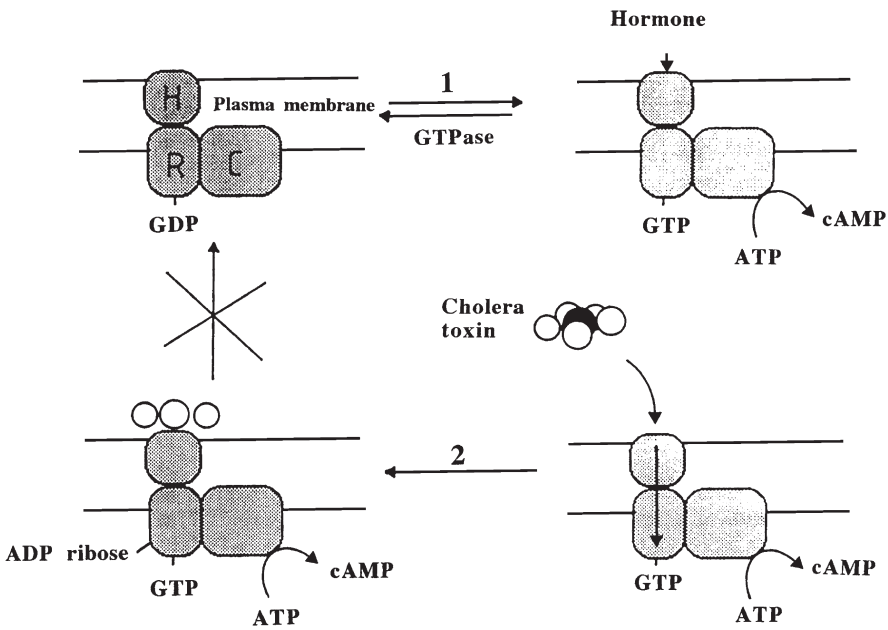

Figure 6.7 *Cholera toxin and its mode of action. (1) Reversible physiological activation of adenylate cyclase (C) through hormone binding to receptor (H) and (2) cholera toxin binding and translocaton of A subunit leads to ADP ribosylation of regulatory subunit (R) and irreversible activation*

membrane which affect absorption/secretion processes thus precipitating diarrhoea. It does not increase intestinal cAMP levels.

A traditional method of analysing for the presence of enterotoxins is based upon their *in vivo* action. The ileum of a rabbit under anaesthesia is tied off to produce a number of segments or loops which serve as test chambers. These are injected with cultures, culture filtrates or samples under test. If an enterotoxin is present it produces, after about 24 h, an accumulation of fluid in the loop which becomes distended. A number of alternative assays, based on the effects of enterotoxins on cells in tissue culture are also used. These have the advantages of being more economical, more humane and easier to quantify than the ligated ileal loop assay but are less directly related to the clinical action of the toxin.

(3) *Invasive infection*. Other diarrhoea-causing pathogens invade the cells of the intestinal epithelium but do not normally spread much beyond the immediate vicinity of the gut. Some, such as *Salmonella* preferentially invade the ileum to produce a profuse watery diarrhoea. Bacterial cells invade and pass through the epithelial cells to multiply in the *lamina propria*, a layer of connective tissue underlying the enterocytes. The precise mechanism of fluid secretion into the intestinal lumen is not known and is probably multifactorial. A heat-labile enterotoxin which stimulates adenylate cyclase activity has been identified in some salmonellas as well as a cytotoxin. It has also been suggested that the local acute inflammation caused by the infection and responsible for the fever and chills that are often a feature of salmonellosis, causes an increase in levels of prostaglandins, known activators of adenylate cyclase.

Other enteroinvasive pathogens like *Shigella* and enteroinvasive *E. coli* invade the colonic mucosa and produce a dysenteric syndrome characterized by inflammation, abscesses and ulceration of the colon and the passage of bloody, mucus-and pus-containing stools. Bacterial cells adhere to the enterocytes *via* outer membrane protein adhesins. They are then engulfed by the enterocytes in response to a phagocytic signal produced by the bacterium and multiply within the cytoplasm invading adjacent cells and the underlying connective tissue. The strong inflammatory response to this process causes abscesses and ulcerations of the colon.

Invasiveness can be diagnosed by examination of the fluid accumulated and the mucosal surface in rabbit ileal loops. A less definitive test for invasiveness is the Sereny test which measures the ability of an organism to cause keratoconjunctivitis in the eye of guinea pigs or rabbits.

Some shigellas also produce a protein exotoxin, known as Shiga toxin, which has a range of biological activities. It inhibits protein synthesis by inactivating the 60S ribosomal subunit and is a powerful cytotoxin. It

has neurotoxic activity causing paralysis and death in experimental animals and is an enterotoxin capable of causing fluid accumulation in ligated rabbit ileal loops. As an enterotoxin, it appears unrelated to cholera toxin since it does not stimulate adenylate cyclase or cross-react with antibodies to cholera toxin. Its role in the pathogenesis of shigellosis is unclear since strains incapable of producing Shiga toxin remain pathogenic. Enteroinvasive *E. coli* causes a similar syndrome but does not produce Shiga toxin.

Some authors have linked the enterotoxin activity of Shiga toxin with the watery diarrhoea which often precedes dysentery. Interestingly, a similar sequence of watery diarrhoea with supervening bloody diarrhoea is seen with enterohaemorrhagic *E. coli*. This organism, which both colonizes the epithelial surface in the colon and multiplies in the *lamina propria*, produces a number of Shiga-like toxins, sometimes known as verotoxins because of their activity against Vero cells in culture.

Common features have been identified among the various diarrhoea-causing toxins and a number of bacterial exotoxins important in other diseases such as diphtheria. Each consists of five, linked B units which are able to bind to the target cell and facilitate transport of the active A unit into the cell.

Having discussed some of the general features of foodborne diseases, in the next chapter we will look more closely at some of the bacterial agents responsible for them.