*Chapter 2*

# *FOOD POISONING AND OTHER FOOD-BORNE HAZARDS*

## 2.1. INTRODUCTION

The term 'food poisoning' is commonly used to cover a wide variety of illnesses or clinical conditions affecting the gastrointestinal tract. The very large majority of such illnesses found in developed countries result from the consumption of contaminated food or drink, and because they are caused by infection with or the presence of bacteria, these organisms will receive the greatest attention here. However, it is necessary to consider, albeit more briefly, other forms of food poisoning and food-borne hazards since these may sometimes be of concern and pose serious health hazards in other parts of the world.

The types of food poisoning may conveniently be grouped on the basis of the causative agent as follows:

1. Bacteria
2. Fungi
3. Viruses
4. Animals
5. Plants
6. Chemicals

Other food-induced illnesses such as indigestion and sensitivity to specific foods (e.g. allergies) will not be discussed; although distressing to the individuals concerned, they can in no way be regarded as forms of food poisoning.



P. R. Hayes (ed.), *Food Microbiology and Hygiene*

## 2.2. INCIDENCE OF FOOD POISONING

In England food poisoning statistics have been published annually since the 1940s. It is well recognized that only a very small proportion, perhaps as low as 1%, of the total number of food poisoning cases figure in the annual statistics. This is due either to faulty reporting or, more usually, to the patient failing to consult his general practitioner. Figures are available from several sources including the reports of the Chief Medical Officer of Health, the Office of Population Censuses and Surveys and the Public Health Laboratory Service. The statistics which are presented in slightly different ways are never in total agreement but they suffice to show (Table 2.1) that over the five year period from 1984 to 1988 the number of cases ranged between 12000 and 26000. In the late 1980s numbers have risen dramatically to over 30000 per annum primarily due to increases in salmonellosis. Table 2.1 refers to terms such as 'general outbreaks' and 'cases' and these terms have been defined by the Department of Health and Social Security (DHSS) as:

'Food poisoning' — any acute illness attributable to the recent consumption of food.

'A case' — a person affected with food poisoning.

'Sporadic case' — an affected person whose illness is not connected with a similar illness of any other person.

'Household outbreak' — an outbreak affecting two or more persons in the same private household, not connected with any other cases or outbreaks.

'General outbreak' — an outbreak affecting two or more persons which was not confined to one private household.

### 2.2.1. The Bacteria Responsible

As can be seen from Table 2.1, *Salmonella* spp. are by far the most common cause of food poisoning in England and Wales. On average about 90% of the total number of cases can be attributed to salmonellas with *Clostridium perfringens* being responsible for a further 5–10% of cases; in 1988 salmonellas were reponsible for 93% of cases.

**Table 2.1**
Bacterial Food Poisoning in England and Wales (1984–1988)[a]

| *Causative organism* | *1984* | *1985* | *1986* | *1987* | *1988* |
|---|---|---|---|---|---|
| *Salmonella* spp | 10 603 (13 201) | 9 966 (11 172) | 12 775 (14 177) | – (18 096) | – (24 123) |
| *Clostridium perfringens* | 68 (1 716) | 64 (1 466) | 61 (906) | 51 (1 266) | 57 (1 312) |
| *Staphylococcus aureus* | 10 (181) | 13 (118) | 11 (76) | 12 (178) | 9 (111) |
| *Bacillus cereus*[b] | 28 (214) | 22 (81) | 26 (65) | 21 (137) | 20 (418) |
| Total | 10 709 (15 312) | 10 065 (12 837) | 12 873 (15 224) | – (19 677) | – (25 964) |

[a] Data from PHLS Communicable Disease Reports. Annual figures represent the total number of incidents, including general and household outbreaks, and sporadic cases.
Total number of cases in parentheses.
[b] Other *Bacillus* spp. may be included.

*Staphylococcus aureus* and *Bacillus cereus* each normally account for under 1% of cases whilst *Vibrio parahaemolyticus*, which formally accounted for a further 1%, is apparently no longer a significant problem in this country. Different types of *Escherichia coli* have been occasionally implicated in food poisoning outbreaks; the number of cases fluctuates widely but typically does not exceed 100 per annum in the UK.

Botulism, caused by *Clostridium botulinum*, is fortunately very rare in the UK as it is the most lethal type of bacterial food poisoning; a small number of cases were recorded in 1978 and 1987 and there was a general outbreak in 1989.

*Campylobacter* spp. appeared in the food poisoning statistics in the early 1980s for a few years. The Communicable Diseases Surveillance Centre reported over 6000 cases in 1978 (DHSS, 1980) and since that time cases have increased steadily until in 1989 over 32 000 campylobacter enteritis cases were reported. The exclusion of campylobacters from the food poisoning statistics is due primarily to their being regarded as a cause of food-borne infection rather than a true food poisoning (see below).

The anomalies and shortcomings of the statistics collated by different bodies have been stressed by Sheard (1981); the same author has also reviewed the general trends in food poisoning statistics (Sheard, 1986; 1987). He concluded that if the number of cases was to be reduced particular attention should be paid to all areas of the catering industry and to all outlets of raw and cooked meats; the need to monitor imported foods is also stressed as is the importance of food hygiene education.

Before leaving the statistical aspects of food poisoning it should be pointed out that there are about 100 fatal cases per annum in England; this figure is relatively low but it is one which, together with the non-fatal cases, could be substantially reduced if suitable precautions were observed.

### 2.2.2. Type of Food

The different types of food poisoning generally associate themselves with specific foods (e.g. salmonellas with meats or meat products) although full investigation of these associations may be hampered because in many outbreaks it is impossible to identify the food involved since it has often been disposed of before bacteriological tests can be initiated. However, there is ample evidence to show that the foods most commonly implicated in food poisoning in general are the various meats

including poultry. Until recently nearly three-quarters of the cases in which the food has been identified have involved re-heated or cold pre-cooked meats (principally beef, pork, ham and lamb) or poultry (chicken, turkey and duck), stews, minced meats or meat pies.

This situation changed dramatically in the UK in the mid-1980s when *Salmonella enteritidis*, phage type 4 became an ever increasingly significant cause of food poisoning. Evidence suggested that this was due to a spread of infection by this organism in chickens which also contaminated hens' eggs (PHLS Report, 1989); the latter are now apparently a major cause of food poisoning in the UK with nearly 70% of salmonellosis cases being attributed specifically to this phage type.

There are many other foods which are only infrequently implicated in food poisoning incidents; these include unpasteurized milk, other dairy products (e.g. trifles and cream cakes), fried rice and various seafoods.

## 2.3. BACTERIAL FOOD POISONING

It may be helpful to distinguish between bacterial food poisoning and food-borne bacterial infections which are considered later in this chapter. In the former the causative organism multiplies in the food and by its heavy growth induces illness by one means or another after ingestion of the contaminated food. In food-borne infections the food merely acts as a carrier for the causative organism which does not require to multiply in the food.

It is customary to divide bacterial food poisoning into the infection type and the toxin type; the distinction between them is somewhat ill-defined since presumed infective organisms have, on occasions, subsequently been found to produce exotoxins in the food. An infection food poisoning is characterized by an acute gastroenteritis (i.e. inflammation of the lining of the alimentary canal) following ingestion of food in which multiplication of bacteria has taken place, the ingested viable bacteria continuing to grow within the host's body to produce the typical symptoms. Salmonellas are principally responsible for this type of food poisoning in which endotoxins are released as the bacterial cells disintegrate. The toxin type (often termed an intoxication) is a genuine food poisoning since a poisonous substance, the enterotoxin, is present in the food having been produced by the bacteria which have grown in the food prior to consumption. The toxin also causes an acute gastro-

enteritis but ingestion of viable bacteria is usually not a prerequisite of the induction of the disease. Bacteria causing toxin food poisoning include *Clostridium perfringens* and *Staphylococcus aureus.*

### 2.3.1. Salmonellas

#### *2.3.1.1. The Organisms*

Salmonellas are short (1–2 $\mu$m), motile, Gram negative, non-sporing rods. In fact the genus *Salmonella* contains nearly 2000 different strains (termed serovars or serotypes); the number is continually increasing as new serologically distinct strains are isolated. Many serotypes are named after the place where they were first isolated, e.g. *S. newport, S. derby, S. dublin, S. heidelberg, S. montevideo*, although originally salmonellas were named in a more haphazard manner and often after the disease and affected animal (e.g. *S. typhimurium* causing typhoid in mice). Salmonellas are facultative anaerobes biochemically characterized by their ability to ferment glucose with the production of acid and gas, and their inability to attack lactose and sucrose. Their optimum growth temperature, as with most food poisoning bacteria, is about 38°C; they are relatively heat sensitive being killed at 60°C in 15–20 min and they fail to grow below about 7 or 8°C.

#### *2.3.1.2. The Disease*

Salmonellosis is the collective term used for human and animal infections caused by members of the genus *Salmonella.* As mentioned previously salmonellas induce the illness by their death following multiplication in the host's gut and their subsequent lysis with the release of a potent endotoxin. This endotoxin, a lipopolysaccharide, forms part of the membrane of the cell and is primarily responsible for the clinical symptoms. Enterotoxin(s) produced within the human intestine may well have an important role in the disease (D'Aoust, 1991).

In humans the incubation period (i.e. the period between ingestion of the contaminated food and the onset of the symptoms) varies considerably but is usually between 12 and 36 h. The principal symptoms of salmonellosis are nausea, abdominal pain, drowsiness, diarrhoea and a moderate fever; dehydration may occur resulting in great thirst. The stools are watery, greenish in colour, foul smelling and sometimes tinged with blood. The organisms can invade the blood stream and thus cause a septicaemia and in the more extreme cases the patient may go

into a coma. The mortality for the population as a whole is low, being well under 1%; susceptibility varies, however, especially with age and infants under 1 year are particularly at risk as are the elderly and infirm. The healthy adult typically needs to ingest at least a half to one million viable salmonellas to produce the symptoms whilst infants and the elderly are susceptible to only a small fraction of this number. There are circumstances, however, where very low doses can initiate the symptoms. For example, it was found that only *ca* 50 viable *S. napoli* cells needed to be ingested in the contaminated chocolate bars which caused the large food poisoning outbreak in the UK in 1982; there were 245 known cases, mainly children, whilst many adults remained unaffected at this level of contamination (Greenwood & Hooper, 1983). It would seem that the chocolate affords some protection to the organism from the stomach acids and rapid passage of the food into the intestines increases this protection.

The illness usually lasts for up to 7 days but some of the symptoms may linger for weeks or even months. During the acute stage large numbers of salmonellas are excreted in the faeces but the number gradually decreases with recovery of the patient until stools are salmonella-free. Nearly 50% of patients still show 'positive' stools after 4 weeks, 10% after 8 weeks and between 0.2 and 5% remain intermittent salmonella excreters for far longer periods. These latter patients are known as 'carriers' but there are other 'carriers' who are found to intermittently excrete salmonellas even though they have no history of any disease symptoms. Because this latter type of carrier is rarely detected before an outbreak occurs, an insidious build-up of carriers may occur within a food concern, particularly if involved with meats and poultry. A typical case was reported by the DHSS (1976).

> During the summer of 1974, outbreaks of food poisoning caused by *S. infantis* were reported in South Wales, the Midlands and the West Country. Investigations suggested that the vehicles of infection were cooked meats and pies supplied by one food factory. Sampling of various meat products and extensive swabbing of the premises failed to detect the causative organism. Stool testing of employees revealed 136 excreters of *S. infantis* dispersed throughout the factory, including members of the management and canteen staff. The reason for this high infection rate among the staff may have been the availability of the firm's products for sale both in the canteen and the firm's shop.

The carrier rate amongst the general public is likely to be much higher than expected. Harvey *et al.* (1969) analysed sewage from a modern housing estate in Pontypridd, the sewage containing no waste from food industries nor from butchers' shops. During the 14 month survey period salmonellas were regularly isolated and 35 different serotypes were confirmed, many of them on numerous occasions. In spite of these findings no salmonellosis was reported amongst the population during the survey and therefore it can be concluded that man must frequently be exposed to a wide range of salmonella serotypes albeit at low levels.

*2.3.1.3. Sources*

Mention has already been made of the predominance of meats and poultry as causes of food poisoning and this is primarily a reflection of the association of salmonellas with these foods. Since the intestinal tract of farm animals and birds is the primary habitat of *Salmonella* spp. it is to be expected that carcass meat from these sources may be contaminated with these bacteria. Rates for salmonella contamination of meats are variable but it is probable that contamination is highest in poultry. Roberts (1972) found non-host specific salmonellas in 35% of the raw chickens examined whilst Gibson (1969) quoted rates of 45% for ducklings; more recently, Gilbert (1983) reported that 79% of frozen chickens were contaminated with salmonellas. In addition, turkeys have become a common source of food poisoning which now extends throughout the year rather than being limited to the Christmas period. Such incidents are often associated with larger functions as Vernon (1977) observed:

> Catering facilities for such functions are often overstretched and consequently the turkeys may be cooked 2–3 days before they are due to be eaten. Large frozen banqueting birds, which weigh up to 35 lb and need two days defrosting, are especially likely to be insufficiently thawed and inadequately cooked. They may then be kept at ambient temperature for long periods after cooking and final reheating is often at temperatures too low to destroy bacteria. For example, about 600 persons had turkey for dinner at a club function and 209 were known to have become ill afterwards with *S. typhimurium* infection. The turkeys were large and undercooked and one batch was left overnight in the cooling ovens. They were then refrigerated, subsequently carved and reheated for only half an hour.

In view of the high rates of salmonella contamination on poultry generally it is not surprising to find poultry meat so often the cause of salmonellosis. Thus, Reilly *et al.* (1988) found in Scotland that poultry was responsible for 224 outbreaks affecting 2245 people between 1980 and 1985; this represented 52% of the total number of salmonellosis cases where the food could be identified. Again, Humphrey *et al.* (1988) found that between 1959 and 1985 in England and Wales some 43% of family and general outbreaks of salmonella food poisoning could be attributed to the consumption of poultry.

Beef cattle, pigs and lamb are also important sources of salmonellas. Contamination rates vary enormously but probably fall within the <1–10% range. Much depends on conditions at the abattoirs, many of which may, almost inevitably, encourage the spread of salmonellas from infected to non-infected animals. It is known that the duration of stay in lairages should be short as contamination levels tend to increase with time. Thus Morgan *et al.* (1987) found salmonella contamination of pig carcasses increased from 9% on day 1 to 13% (day 2) and 27% (day 3); overcrowding should also be avoided if cross-contamination is to be minimized. Following slaughter the subsequent dressing and butchery of meats increases the spread of salmonellas on meat surfaces so that by the time the meat is in retail outlets contamination levels have increased to up to 20% (Barrell, 1982; 1987).

Imported meats are often much more heavily contaminated than home-killed meats and contamination rates in excess of 20% can be expected on occasions. Particularly hazardous are the frozen packed boneless meats since the deboning process exposes wider surface areas to cross-contamination via working tops, knives, etc.

Until the 1980s hens' eggs had not been an important cause of salmonellosis in man whereas egg products had long been recognized as serious sources of infection by salmonellas. Outbreaks of salmonellosis were regularly traced to frozen, liquid and dried whole eggs and similar products. However, pasteurization, carried out principally to kill salmonellas, is now mandatory (e.g. the Liquid Egg (Pasteurization) Regulations, 1963, in the UK) and it has proved extremely effective, with very few cases emanating from these sources now being reported. For example, for liquid whole egg, the British require pasteurization at 64·4°C for 2½ min whilst in the United States 60°C for 3 min is deemed necessary; times and temperatures vary for different types of egg product (ICMSF, 1980).

Turning to hens' eggs in more detail, as stated earlier there has been an enormous increase in salmonellosis in many parts of the world

**Table 2.2**
*Salmonella enteritidis*, phage type 4 in England and Wales (1981–1988)[a]

| *Year* | *Salmonellosis—total number of identifications* | S. enteritidis *pt 4 —number of identifications* | *% S. enteritidis pt 4 to total* |
|---|---|---|---|
| 1981 | 10251 | 392 | 4 |
| 1982 | 12322 | 413 | 3 |
| 1983 | 15155 | 823 | 5 |
| 1984 | 14727 | 1362 | 9 |
| 1985 | 13330 | 1771 | 13 |
| 1986 | 16976 | 2979 | 17 |
| 1987 | 20532 | 4962 | 24 |
| 1988 to end Oct | 23038 | 10544 | 46 |

[a] From PHLS Report (1989).

specifically associated with *S. enteritidis*, phage type 4. The relevant data for England and Wales are shown in Table 2.2 but similar trends have been reported in many other countries including France, West Germany and Italy (PHLS Report, 1989). In the north-east of the United States there was a sixfold increase in reported *S. enteritidis* infections between 1976 and 1986 and this was attributed primarily to eggs or egg-containing foods (St. Louis *et al.*, 1988). There is, indeed, little doubt that much of this increase is due to salmonella contaminated eggs. A very large outbreak involving fresh eggs was described by Stevens *et al.* (1989). A total of 165/249 guests at a wedding reception together with 8 staff succumbed to *S. enteritidis* phage type 4 infection which was traced to eggs imported from Denmark. The reception menu included the widespread use of these eggs in the preparation of various lightly cooked sauces. The eggs were specially imported to reduce the risk of contamination in foods which, for religious reasons, necessitated the use of fresh eggs in egg-based foods. The authors concluded that 'despite the low risk of an individual egg being contaminated, this episode highlights the magnification of the risk when catering practices involve mixing large numbers of eggs together'.

Contamination of eggs can occur before laying in the ovary (Perales & Audicana, 1989) or after laying due probably to faecal contamination. However, the percentage of eggs that is infected *internally* is extremely low, perhaps 1 in 100000, in spite of the fact that salmonella

isolation rates from the internal organs of chicken can be *ca* 25% (Hopper & Mawer, 1988). Thus substantial contamination of chicken flesh by *S. enteritidis*, phage type 4 is highly likely so that part of the increase in phage type 4 food poisoning could well be due to contaminated chickens *per se*.

An important contributory factor in this contamination generally is salmonella infected poultry feed (see later) exacerbated by the addition of slaughtered chicken remnants, often contaminated, to the feed. Intensive rearing methods and standards of husbandry often leave much to be desired and the culling of infected laying flocks together with tight controls on poultry feeds are essential prerequisites in the control of this problem.

The pasteurization of milk has also resulted in a substantial reduction in the number of salmonellosis cases. However milk is still sometimes implicated, particularly in certain country districts where unpasteurized milk is consumed. That this practice constitutes a considerable risk was illustrated by Barrett (1986) who surveyed milk-related outbreaks occurring in 1983–4. Of 20 such outbreaks, mainly caused by *S. typhimurium*, 19 were associated with the consumption of raw milk whilst the other resulted from post-pasteurization contamination. An earlier outbreak occurred in the Brechin and Montrose districts of Scotland in 1976; over 700 persons were infected after consuming raw milk which had been contaminated with *S. dublin* (Small & Sharp, 1979). Scotland actually introduced legislation in 1983 making pasteurization of milk sold to the public compulsory. The measure has proved remarkably effective; between 1970 and 1982 there were 50 milkborne outbreaks of salmonellosis in Scotland affecting 3518 people, whereas in the 2 years following legislative change there had not been a single incident (Sharp, 1986).

Contaminated powdered milk sometimes causes outbreaks of salmonellosis such as in Britain in 1985. Baby food milk powder was contaminated with low levels of *S. ealing* and caused a big outbreak amongst an extremely susceptible group. Salmonellas are known to survive spray drying but poor factory hygiene was primarily to blame in this instance (DHSS, 1987). Cheese is another food that is occasionally involved in outbreaks of salmonellosis. A major outbreak occurred in Canada in 1984 when an estimated 10 000 persons became ill as a result of eating Cheddar cheese contaminated with low levels of *S. typhimurium* (Ratnam & March, 1986).

The involvement of chocolate as a cause of salmonellosis has already been mentioned but the *S. napoli* outbreak was only one of several where chocolate has been implicated. In the mid-1970s there were more

than 200 cases in Canada and the United States; these were mainly in young children and were caused by the relatively rare *S. eastbourne*. This serotype was found to have unusual properties in that it could be isolated in chocolate up to 15 months after manufacture (Tamminga *et al.*, 1977). The origin of the salmonella involved was traced to the cocoa beans used in the preparation of the chocolate. Another outbreak of interest involved gold-foil wrapped chocolate coins imported into Canada and the United States from Belgium (Hockin *et al.*, 1989). Children, 14 years and under, were affected; it was found the chocolate contained low levels (2–24 organisms per 100 g) of *S. nima* which again stressed the extremely low dose (<10 salmonellas) necessary to induce the clinical symptoms.

Imported desiccated coconut, used in the manufacture of biscuits, cakes and other confectionery, has been another unlikely source of these organisms and was implicated in food poisoning outbreaks in the early 1960s. Legislation was successfully introduced which improved processing and hygiene standards in the country of origin and this commodity no longer constitutes a significant health problem.

With two of the chocolate and confectionery products quoted above it was possible to trace the primary source of the salmonellas and in a similar way it has also been possible to trace an earlier source of salmonellas that may, in part, be responsible for the high incidence of these organisms in farm animals and poultry. Animal feedingstuffs, particularly those that are imported, are often contaminated with a wide variety of *Salmonella* serotypes and it has been frequently suggested that these foods are an important source of infection in the live animal (e.g. Wray & Sojka, 1977; Williams, 1981). Examples of salmonella contamination quoted for different feedingstuffs range from 5 to 27% (PHLS Working Group *et al.*, 1972; see Table 2.3) and 19% for meat and bone meal (Stott *et al.*, 1975).

**Table 2.3**
Growth of *Salmonella* from Different Ingredients of Pig Feed[a]

| *Raw material* | *Number of samples* | *% + ve* |
|---|---|---|
| Feather meal | 99 | 27 |
| Meat and bone meal | 704 | 23 |
| Fish meal | 31 | 23 |
| Fish pellets | 264 | 20 |
| Herring meal | 60 | 5 |

[a]From PHLS Working Group *et al.* (1972).

Animal by-products, with salmonella contamination levels of up to 80%, are widely used in animal feeds (ICMSF, 1980); this practice significantly increases the chance of home-reared animals becoming infected. The use of these products in pet foods has been an additional hazard and some cases of salmonellosis have been traced to domestic pets.

*2.3.1.4. The Relationship Between Salmonella Source and Salmonellosis in Man*

Many examples could be cited of unusual serotypes found in animal and pet foods being subsequently isolated from the animals feeding thereon. Jones *et al.* (1982) reported an outbreak where *S. mbandaka* was isolated from raw milk obtained at 3 separate farms. It transpired that the dairy herds were all infected by a vegetable supplement feed from the same supplier; samples taken from 13 bags of the unopened feed were all found to contain *S. mbandaka.*

Other links in an contamination/infection chain have also been observed but it is more difficult to demonstrate the total relationship: contaminated animal feed → infected animal → contaminated meat → salmonellosis in man.

The problem is a difficult one with complications arising through: (1) the contamination of feedingstuffs; (2) the movement of animals around the country increasing the chances of cross-contamination and the introduction of newly purchased infected animals to previously salmonella-free herds; and (3) the delay after salmonellosis has occurred before bacteriological investigations can be completed. However, Hobbs & Hugh-Jones (1969) reported on a hospital outbreak of salmonellosis caused by patients consuming infected turkeys which had been fed with *S. senftenberg* contaminated meal; the serotype was isolated at all stages of the chain. Similar evidence involving animal feed, pigs and outbreaks of human salmonellosis in the Cardiff area was found by Harvey (1973). Indirect evidence supporting the relationship comes from the PHLS Working Group *et al.* (1972) who attributed the narrow range of serotypes found in pigs and humans in Denmark to the rigorous standards required in the manufacture of animal feeds in that country. Perhaps the most conclusive evidence for this contamination sequence was that supplied by the remarkable increase in *S. agona* isolations in the early 1970s (DHSS, 1974) which stated:

> *S. agona* is now the second most common salmonella serotype to be isolated from humans in this country. In 1972 there were 570

isolations of this serotype from human cases and excretors and in 1971 there were 765 yet prior to 1970 it was rarely isolated. It is interesting to note the epidemiological background to this remarkable rise. *S. agona* was isolated from a consignment of fishmeal which is widely used in feeds for pigs, chickens and other farm animals imported into this country from South America in 1970. Since then, carriage of *S. agona* in animals and human food, particularly chickens and pork products, has become more common until in 1972 this serotype came second to *S. typhimurium* in isolations from human food. Similar epidemiological features of *S. agona* have been observed over the same period of time in the United States, Israel and the Netherlands. Austria, Finland, Hungary, Italy, Luxembourg and Jugoslavia all reported isolations of *S. agona* for the first time in 1970, though in these cases the source was not known. This epidemiological picture suggests that fishmeal may have been the original vehicle for the wide dissemination of *S. agona* noted since 1969–70, and it demonstrates once again that unusual strains of microorganisms present in animal feedingstuffs can on occasions rapidly become established among farm animals and poultry, with subsequent spread to the human population.

There is clear evidence, therefore, that human and animal salmonellosis can be related to contaminated feeds on occasions. However, there are many other sources of *Salmonella* spp. and many means of spreading them (e.g. rodents and herring gulls) so that the inter-relationships between all these factors are very complicated and the size of any food poisoning outbreak is extremely variable (Oosterom, 1991).

Human salmonellosis is normally seen in the form of small family outbreaks and one of the final stages in the contamination chain is usually the cross-contamination of cooked food by raw food or by dirty working surfaces, the cooked food being subsequently left at room temperature for a number of hours.

#### *2.3.1.5. Control Measures*

These are only discussed briefly here but are considered more fully in appropriate chapters later. Some of the recommendations are long-term objectives and may be almost impossible to achieve but if salmonellosis is to be reduced every effort should be made to:

1. Ensure animal feedingstuffs are salmonella-free and imported feeds are suitably heat treated.
2. Eliminate salmonellas in poultry breeding stock.
3. Improve hygiene standards in abattoirs and broiler houses.
4. Avoid cross-contamination risks, particularly of cooked by raw foods, in processing factories and kitchens.
5. Ensure adequate heating of foods, followed by rapid cooling where foods are to be stored.
6. Refrigerate foods at below 5°C where possible and avoid leaving foods at room temperature for lengthy periods.
7. Ensure food handlers are not salmonella carriers.
8. Control rodents, birds and pests in and around factory premises.
9. Increase salmonella surveillance, particularly of cooked foods.

### 2.3.2. *Clostridium perfringens*

#### *2.3.2.1. The Organism*

*Clostridium perfringens*, also known as *Clostridium welchii*, is a large (2–8 $\mu$m long and 1 $\mu$m wide), non-motile, Gram positive, sporing rod. The spores which are rarely seen are oval and subterminal (Fig. 2.1).

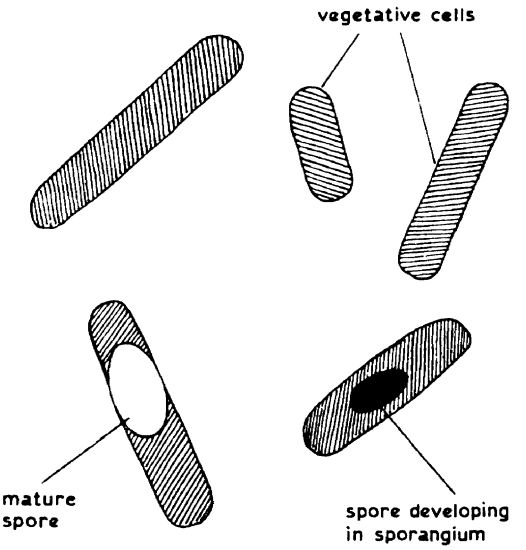


**Fig. 2.1.** Diagram of *Clostridium perfringens*.

Although classed as an obligate anaerobe, *C. perfringens* will grow in the presence of low levels of oxygen. The organism has a high optimum temperature for growth (45°C) and is capable of growing down to about 15°C. Five types of *C. perfringens*, A, B, C, D and E, are distinguished on the basis of various toxins produced. Of these only type A has been implicated in food poisoning outbreaks in the UK while type C causes a more serious condition called necrotic enteritis. It is the heat-resistant strains of type A, the spores of which can resist boiling for 1 to 5 h, which are responsible for the majority of outbreaks of food poisoning caused by this organism although heat-sensitive strains may sometimes be implicated.

*2.3.2.2. The Disease*

The symptoms of *C. perfringens* food poisoning first appear after an incubation period of 6 to 22 h. The symptoms are characterized by severe abdominal cramps, nausea and profuse diarrhoea; there is normally no vomiting or fever. Recovery is usually rapid and within 12 to 24 h although the period may be extended in elderly people for a further 1 or 2 days. The mortality rate is again very low and only very few deaths are reported annually in the UK.

For many years it was assumed that this was an infection form of food poisoning although it is now known that an enterotoxin is involved. Relatively large numbers of viable cells, more than one million, need to be ingested and the cells continue to multiply in the small intestine where sporulation occurs. The enterotoxin is produced in the sporulating cells and is released on cell lysis; it is this enterotoxin which is responsible for the typical symptoms (Skjelkvale & Uemura, 1977) although it has been demonstrated that enterotoxin production can be induced by non-sporulating cultures of *C. perfringens* (Goldner *et al.*, 1986).

*2.3.2.3. Sources*

*C. perfringens* is very widely distributed and is probably the most common of all pathogenic bacteria on the earth's surface. Type A is the most frequently isolated type and can be readily found in soils, natural waters and the intestinal contents of man and animals. Heat-sensitive strains of *C. perfringens* type A are always found in the intestinal contents of man but heat-resistant strains are much less frequently isolated, less than 10% of normal human faeces samples being positive ; rates are much higher for a short period following a food poisoning outbreak.

*C. perfringens* type A has been isolated from a wide variety of foods, most frequently from raw meats and poultry. The extensive contamination in meats led Smart *et al.* (1979) to suggest that strains of *C. perfringens* capable of causing food poisoning are present on every commercial carcass of raw meat, even if only in low numbers; similar results have been obtained for poultry (Nakamura & Schulze, 1970).

*2.3.2.4. Conditions Leading to Food Poisoning Outbreaks*

As might be expected, the majority of *C. perfringens* food poisoning outbreaks result from the ingestion of meat and poultry dishes which have been subjected to incorrect temperature treatment. This type of food poisoning is generally associated with catering malpractices and, as is shown in Table 2.1, the number of individuals affected per outbreak (15–25) is, on average, greater than that for other forms of food poisoning. These outbreaks frequently occur in institutions, hospitals, canteens and similar catering establishments where they are often the principal type of food poisoning (Sharp *et al.*, 1979).

The problem of temperature control needs amplification. Foods can be inadequately heated so that the vegetative cells of the organism are destroyed but the heat-resistant spores remain viable. Even during inadequate cooking oxygen is driven off and conditions in the food become anaerobic thus enabling spore germination to commence once the meat has cooled to 50°C. Slow cooling between 50 and 25°C is particularly hazardous with this organism as growth rates are high at these temperatures and large numbers of vegetative cells may therefore develop within a few hours. The problem is exacerbated if the foods, such as cooked meats, stews and gravies, are then stored at room temperature before being eaten as further growth will occur. The effect of cooking and cooling on *C. perfringens* in meat was studied by Sutton *et al.* (1972) who showed that even vegetative cells may not be killed towards the centre of the larger joints of meat. Cooling at 15°C was ineffective in controlling the growth of the organism and even at 4°C it would take 4½ h to reduce the temperature to 15°C, the minimum growth temperature for *C. perfringens* (Table 2.4). In catering, foods are often prepared on the day prior to consumption and eaten after warming up. This practice can be dangerous as the foregoing shows and, in addition, the final reheating is often only sufficient to stimulate further growth of the organisms rather than destroy them. A typical outbreak was described by Vernon (1977):

**Table 2.4**
Heat Penetration and Cooling Data for 4.5 kg Joints of Beef Cooked in Oven at 213°C for 3½ h and Cooled at Three Different Temperatures for 7 h[a]

| | | | | |
|---|---|---|---|---|
| *Heat penetration data* | | | | |
| Depth of thermocouple (mm) | 76 | 51 | 6 | 3 |
| Time to reach 60°C (min) | 220 | 145 | 40 | 37 |
| Temperature at end of cook (°C) | 55 | 64 | 90 | 96 |
| Maximum temperature (°C) | 65 | 71 | 90 | 96 |
| *Cooling data* | | | | |
| Temperature of cooling (°C) | 15 | 4 | −30 | |
| Depth of thermocouple (mm) | 51 | 76 | 76 | |
| Temperature at end of cooling (°C) | 22 | 14 | −5 | |
| Time to reach 15°C (min) | >420 | 395 | 220 | |

[a]From Sutton *et al.* (1972).

126 of 230 persons had diarrhoea and abdominal pain 12–14 hours after eating turkey at a staff canteen Christmas dinner. On this occasion frozen turkeys each weighing about 20 lb had been thawed, and after being stuffed were roasted for only 4 hours. They were then left for 24 hours unrefrigerated in a warm kitchen before being carved. The meat was held for a further day at room temperature, reheated to 200°F and then it finally remained on a hotplate for another hour before consumption. Heat resistant *C. welchii* were isolated from all patients tested and from the remains of the turkeys.

Perhaps rather surprisingly, boiled salmon was implicated in 5 large *C. perfringens* food poisoning outbreaks, as reported by Hewitt *et al.* in 1986. In total over a thousand persons were affected and in each instance boiled salmon, served cold as an hors d'oeuvre was incriminated. The two largest outbreaks occurred at a major London hotel over a weekend. In each instance frozen salmon was purchased by the hotel 2 days before consumption. The fish was filleted into slices approximately 1·25 cm thick and placed in a cold water bouillon. The fish was brought to the boil and kept boiling for 15–30 min. It was left to cool for 2–3 h at room temperature, drained, and transferred to trays for overnight refrigerated storage. The following afternoon the salmon fillets were portioned, the dishes made up and again refrigerated until the next night when the fish was served. Approximately 75% of the 1100 guests became ill with typical symptoms after an incubation period of 6–30 h; all guests recovered within 3 days.

*2.3.2.5. Control Measures*

Obviously because of its widespread occurrence on foods as well as in the environment generally it would be impractical to eliminate *C. perfringens*. Carcass meat and poultry will inevitably be contaminated with this bacterium and survival of the heat-resistant spores after cooking can also be anticipated. The control measures should therefore be aimed at restricting both spore germination and the subsequent proliferation of vegetative cells whilst the food is cooling and during storage of the food.

Thus the following measures are recommended:

1. Where possible the food should be cooked and eaten immediately.
2. Where food is to be held over after cooking it should be cooled *as rapidly as possible* (to below 20°C within 1 h) and refrigerated; placing hot foods in a refrigerator should be avoided as cooling is likely to be too slow so that blast coolers should be used.
3. Partial or complete cooking of foods on one day with re-heating the next should preferably be avoided but where it is necessary it is important to ensure that the food is thoroughly heated before being consumed.
4. Cross-contamination of cooked foods by raw or by dirty working surfaces, equipment and utensils should be avoided.

### 2.3.3. *Staphylococcus aureus*

*2.3.3.1. The Organism*

*Staphylococcus aureus* is a small (0·5–1 μm in diameter), spherical, Gram positive, non-motile organism typically forming irregular clusters of cells like bunches of grapes. These organisms are facultative anaerobes growing better in the presence of air with an optimum growth temperature around 37°C and capable of growing down to 8°C or slightly below. *S. aureus* is unusual in being able to tolerate low water activity levels ($a_w$ 0·86 minimum) and thus it grows in fairly high salt concentration levels. All *S. aureus* strains are coagulase positive (i.e. possess an enzyme coagulating blood plasma) but only about 30% of strains are able to produce the enterotoxins associated with food poisoning. Six enterotoxins, A, B, C1, C2, D and E, have been identified with types A and D being most commonly involved in food poisoning (Halpin-Dohnalek & Marth, 1989). It has been found that about two-thirds of

the enterotoxin-producing strains form one toxin only, usually type A and the majority of the remainder form only two. The enterotoxins are exotoxins, so-called because they are produced within the intact cell and are released into the food or culture medium. The enterotoxins produced by *S. aureus* have the unusual property of being markedly heat-resistant. Most are able to withstand boiling in a food for up to 30 min with type B toxin showing the greatest stability (Bergdoll, 1970). Typically there is a 60–70% loss of activity within a few minutes when enterotoxin B is heated above 80°C but the remaining activity is lost much less rapidly; indeed, reactivation can occur during prolonged storage at room temperature or by heating to a higher temperature (Reichart & Fung, 1976).

According to the statistics, *S. aureus* only accounts for 0·5–1 % of food poisoning cases in the UK. However, it is likely that this is the most common form of poisoning but because of its relatively mild nature few people report the illness. Surprisingly, in the United States it accounted for well over 50% of the reported cases for many years although more recently this figure has dropped to about 10% (Cliver, 1987).

*2.3.3.2. The Disease*

After ingestion of the contaminated food the symptoms appear quickly, within 1–6 h, with an average of about 3 h. The most predominant and severe symptom is vomiting which is preceded by a feeling of nausea. Vomiting can be very frequent and is followed in the later stages by retching. Other common symptoms are abdominal cramps and diarrhoea. The symptoms usually last for 1–2 days and mortality is extremely low although fatal cases have been recorded.

It is known that large numbers of *S. aureus* must be present in foods for them to be hazardous but the precise number necessary to produce enough enterotoxin to induce the symptoms is not certain; figures in excess of 1 million per gram have been suggested (Halpin-Dohnalek & Marth, 1989), a number that roughly corresponds to an enterotoxin level in the food consumed of 1 $\mu$g. This is sufficient to cause illness in man but in school children the dose may only be about 0·2 $\mu$g such as was found by Evenson *et al.* (1988) when investigating a large outbreak of staphylococcal food poisoning involving chocolate milk.

*2.3.3.3. Sources*

The most important source of *S. aureus* is probably the human body, the principal reservoir being the nose. Between 30 and 40% of healthy

individuals carry *S. aureus* and many of these nasal carriers inevitably also harbour the organism on their hands and other parts of their body. Many lesions such as boils, carbuncles, septic cuts and abrasions abound with *S. aureus* whilst another fruitful source of the organism is hair where carriage rates may be as high as those for the nose (Bryan, 1976).

Animals can also be important sources of *S. aureus*. Dairy cows commonly carry the organism on the udder and teats, and an infection, a form of bovine mastitis, can be set up by the organism. This close association with the udder inevitably means that milk becomes infected but *S. aureus* can also be spread from the infected region to milking equipment, other utensils and the hands of workers; thus a more general infection of the herd can be initiated. *S. aureus* is also commonly found on poultry and, to a lesser extent, on carcass meat. Its presence is not particularly significant even though fairly high numbers, including enterotoxin producing strains, can be isolated from poultry (Harvey *et al.*, 1982).

*2.3.3.4. Foods Involved in Staphylococcal Food Poisoning*

Cooked foods that have been handled by a *S. aureus* carrier, especially those with septic lesions, and subsequently stored under warm conditions for lengthy periods are the main cause of this form of food poisoning. Cured cooked meats, especially hams, which are made up into sandwiches or otherwise eaten cold are commonly implicated as are other cold meats and poultry. Other foods less frequently involved in producing outbreaks include egg products such as custards, artificial cream filled confectionery products and trifles, and cooked peeled prawns.

An interesting outbreak associated with dried Italian egg lasagne was reported by Woolaway *et al.* (1986). In January 1984 a food poisoning outbreak involving one family in Florence was traced to lasagne originally produced in Parma. Numerous outbreaks followed in Luxembourg which were traced to the same source. A month later there was a public warning issued by the DHSS in the UK since lasagne of the same brand had been imported recently. Nine food poisoning incidents were subsequently reported, involving 47 cases, following consumption of the lasagne. Examination of 50 unopened packets of lasagne revealed that 41 were heavily contaminated with *S. aureus* (counts of $10^4$ to $10^8$ per g) and enterotoxin A was also found. The contamination was traced back to inadequately pasteurized liquid egg that had been used in the preparation of the lasagne; the counts of *S. aureus* increased during factory preparation of the product.

Outbreaks of staphylococcal food poisoning from raw or pasteurized milk in the UK are rare but raw milk and raw milk products such as cream and cheese have given rise to outbreaks in many other countries. In the latter cases *S. aureus* can originate from a cow suffering from bovine mastitis but this contamination route is the exception rather than the rule. A more unusual source of staphylococcal food poisoning is canned foods. Canned processed peas, corned beef and other meats have been incriminated in outbreaks after *S. aureus* from infected hands had passed through minute holes in the seams of cans handled whilst still wet with cooling water.

Vernon (1977) described a number of food poisoning outbreaks caused by *S. aureus* as follows:

> In one outbreak, 47 of 95 passengers on a river trip in June developed vomiting and diarrhoea 3–6 hours after a meal which included chicken pieces that had been kept warm after cooking. Similar strains of staphylococci were isolated from a patient, from the chicken and from a dressing on the finger of one of the food-handlers; all the strains produced enterotoxin A. In another outbreak, 30 of 70 guests at a wedding vomited 1–2 hours after a reception at which chicken was served; the cooked chicken had been kept overnight in an unrefrigerated van. A count of $1{\cdot}4 \times 10^{10}$ *S. aureus* per g. was obtained from the chicken, which had been prepared by a nasal carrier of staphylococci of a similar type.
>
> There were two large outbreaks in schools. In one 129 of 348 pupils vomited 3 hours after eating custard; similar enterotoxin producing strains of *S. aureus* were isolated from a patient, the custard and two food-handlers. In the second, chicken had been sliced by hand and then left at room temperature: the patients, the chicken and the nose of a food-handler yielded similar strains of *S. aureus* producing enterotoxin A.

The presence of large numbers of *S. aureus* in foods does not necessarily mean that enterotoxin has been produced. Scheusner & Harmon (1973) found that whilst all pre-inoculated samples of various commercial foods which contained enterotoxin also contained high populations of *S. aureus*, enterotoxin was not detected in pre-inoculated samples of meat and fish pies containing similar high final numbers of the organism. There are many factors affecting enterotoxin production including type of food, pH (little if any production below pH 5·0), temperature

(optimal production at 37°C but wide range tolerated), presence of oxygen (poor enterotoxin production under anaerobic conditions) and the presence of other organisms whose main effect is to inhibit the growth of *S. aureus* rather than affect enterotoxin production *per se* (Notermans & van Otterdijk, 1985; Halpin-Dohnalek & Marth, 1989). There are, therefore, many inter-related factors determining whether or not *S. aureus* food poisoning occurs and these have been succinctly described by Bryan (1976):

1. A source of an enterotoxigenic strain of *Staph. aureus* must be in a food production, processing or preparation environment.
2. The organism must be transferred from the source to a food.
3. The food must be contaminated with thousands of *S. aureus* per g, or, more usually, the food must be heated before it becomes contaminated, or it must contain high levels of salt or sugar.
4. The organism must survive in the food; it must not be outgrown or inhibited by competing organisms or killed by heat, low pH, or other adverse conditions before it can produce enterotoxin.
5. The food, after it becomes contaminated, must support the growth of *S. aureus*.
6. The contaminated food must stay within the temperature range that is suitable for proliferation of *S. aureus* long enough for this organism to multiply and produce enterotoxin.
7. A sufficient quantity of enterotoxin-bearing food must be ingested to exceed the enterotoxin susceptibility threshold of persons eating the food.

#### *2.3.3.5. Control Measures*

As with *C. perfringens*, measures aimed at eliminating *S. aureus* would be totally impracticable due to its widespread occurrence. Therefore control measures should aim to limit contamination and the subsequent growth of the organism in foods. Vital control measures recommended are:

1. Keep handling of cooked foods to a minimum. Particular care should be taken with warm cooked foods which should preferably be cooled to below 20°C where subsequent handling is essential.
2. Personnel with septic lesions should not handle foods; because of the high nasal carriage rate in humans it would be impracticable to prohibit such carriers from handling foods but disposable gloves should be worn by all operatives.

3. Adequate heat treatment of the food is essential followed by prompt cooling to 10°C or below where foods are to be stored.
4. Minimize cross-contamination from raw to cooked foods and from dirty working surfaces, equipment and utensils.

### 2.3.4. *Bacillus cereus*

#### *2.3.4.1. The Organism*

*Bacillus cereus* is a large (3–5 μm long and 1 μm wide), Gram positive, spore-forming rod (Fig. 2.2). The spores, which are elliptical and central, are much less heat resistant than those of *C. perfringens* and are destroyed at 100°C within 5 to 30 min. *B. cereus* is a facultative anaerobe typically growing over a temperature range of 10–48°C and strains have an optimum growth temperature of 35–45°C; strains are occasionally isolated growing down to 4°C but failing to grow above 37°C (Van Netten *et al.*, 1990).

Two different toxins can be produced by *B. cereus* inducing two distinct types of food poisoning. The first of these toxins, an enterotoxin, is secreted into the food during logarithmic growth. This enterotoxin is relatively heat sensitive and is destroyed in 30 min at 56°C (Goepfert *et*

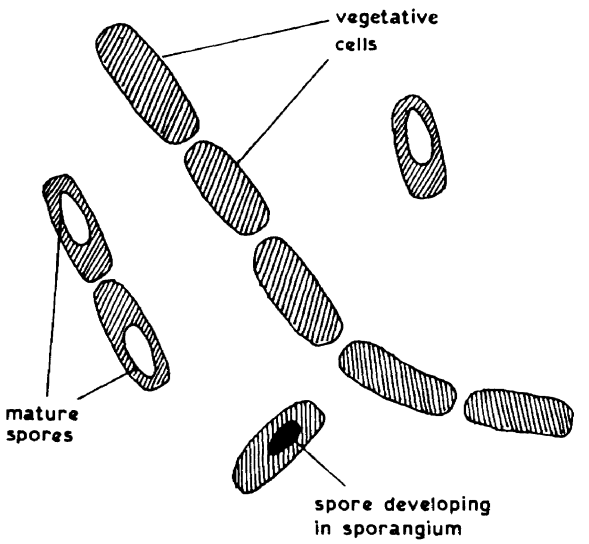


**Fig. 2.2.** Diagram of *Bacillus cereus*.

*al.*, 1973); the illness induced is a 'diarrhoeal syndrome'. The second toxin is again preformed in the food but is much more heat resistant being unaffected by 90 min heating at 126°C (Melling & Capel, 1978); this toxin induces a 'vomiting syndrome'.

*2.3.4.2. The Disease*

As stated above, evidence has accumulated that *B. cereus* is responsible for two distinct types of food poisoning. In the first type, which is similar to *C. perfringens* food poisoning, the incubation period is about 8–16 h. The symptoms are characterized by acute abdominal pain and profuse diarrhoea; nausea, vomiting and fever are rare and the symptoms usually last for less than 24 h. This form of the disease has been reported in several European countries, the United States and Canada. Many different foods have been implicated including meat and poultry products and sauces.

The second type of food poisoning, which is similar in character to that produced by *S. aureus*, has a much shorter incubation period of only 1–5 h. The symptoms are acute nausea and vomiting; diarrhoea is comparatively rare. The duration of the illness is from 6 to 24 h. This form of the disease was first reported in the UK in 1971 and since then there have been many cases always associated with the consumption of cooked rice; similar incidents have been reported in other parts of the world (Gilbert, 1979).

*2.3.4.3. Foods Involved in B. cereus Food Poisoning*

*B. cereus* is found commonly in soil and water and can be readily isolated from a wide variety of plant foods including cereal dishes, mashed potatoes, vegetables and vegetable soups. These foods together with various cooked meat dishes have been responsible for the diarrhoeal form of food poisoning common in several European countries. It has been reported as a common form of food poisoning in the Netherlands (Beckers, 1988) and has been associated with meat dishes that are frequently well seasoned with spices; it should be noted that spices often contain large numbers of aerobic spore-bearing bacilli including *B. cereus*. The cooking of these foods was clearly inadequate allowing the spores to survive so that the warm storage conditions after cooking resulted in spore germination and subsequent heavy growth of vegetative cells (Ormay & Novotny, 1969). In most outbreaks large numbers of *B. cereus*, between $10^5$ and $10^8$ per gram, were isolated from the incriminated foods; examples of outbreaks have been cited by Gilbert & Taylor (1976).

As already mentioned, the second type of *B. cereus* food poisoning appears to be associated almost exclusively with the consumption of fried rice prepared in Chinese restaurants or 'take-away' shops. In these establishments portions of boiled rice have been allowed to 'dry-off' at room temperature for periods of up to 24 h or even longer. In this way boiled rice may take many hours to cool thus creating ideal conditions for the germination of any surviving spores and for the subsequent multiplication of vegetative cells. When required the rice is fried with beaten egg for about 1 min and kept warm until served. The frying process reduces bacterial numbers substantially but the final holding period, preserving, can again be extensive and further heavy growth of bacteria may take place (Gilbert *et al.*, 1974).

Beckers (1988) reported a typical food poisoning episode in which large numbers of *B. cereus* were isolated from fried rice:

> Three people, a man and his two children aged 2 and 6, became ill about 3–5 h after eating fried rice. The symptoms were nausea, vomiting, abdominal cramps, palpitations and severe tightness of the chest. The mother had not eaten any of the fried rice and did not fall ill. Tests carried out on the remains of the fried rice showed it to contain about $10^7$ *B. cereus*/g and 0·96% monosodium glutamate. No fecal samples were tested. The case history and the test results, and the fact that the remains of the fried rice had been stored in the refrigerator, pointed to *B. cereus* as the cause of the food poisoning. Its effect may have been reinforced by the presence of a considerable quantity of monosodium glutamate, which probably caused the palpitations and tightness of the chest.

*2.3.4.4. Control Measures*

Gilbert *et al.* (1974) have suggested the following preventative measures:

1. Rice should be boiled in smaller quantities on several occasions during the day, thereby reducing the storage time before frying.
2. After boiling the rice should either be kept hot, at not less than 63°C, or cooled quickly and transferred to a refrigerator within 2 h of cooking. The cooling of rice, especially large bulks of boiled rice, will be hastened by dividing the product into separate portions or by spreading the bulk in clean shallow containers.
3. Boiled or fried rice must not be stored under warm conditions and never at a temperature between 15° and 50°C. Under no

circumstances, therefore, should cooked rice be stored at kitchen temperature for more than 2 h.

4. The beaten egg used in the preparation of fried rice should be freshly prepared.

### 2.3.5. *Vibrio parahaemolyticus*

#### *2.3.5.1. The Organism*

*Vibrio parahaemolyticus* is a short, slender (1·5–2·5 μm by 0.5 μm), curved, Gram negative rod which is motile by means of a single polar flagellum (Fig. 2.3). The organism is facultatively anaerobic. It is slightly halophilic growing best in the presence of 2–4 % NaCl but tolerating salt concentrations of up to 8%. The temperature range of growth is 10–44°C and optimum growth occurs at 37°C at which temperature cell division can occur extremely rapidly (every 10–12 min). A further unusual property of this organism, and related vibrios, is its preference for alkaline conditions and some strains exhibit optimal growth even as high as pH 9·0. *V. parahaemolyticus* is very heat-sensitive and is readily

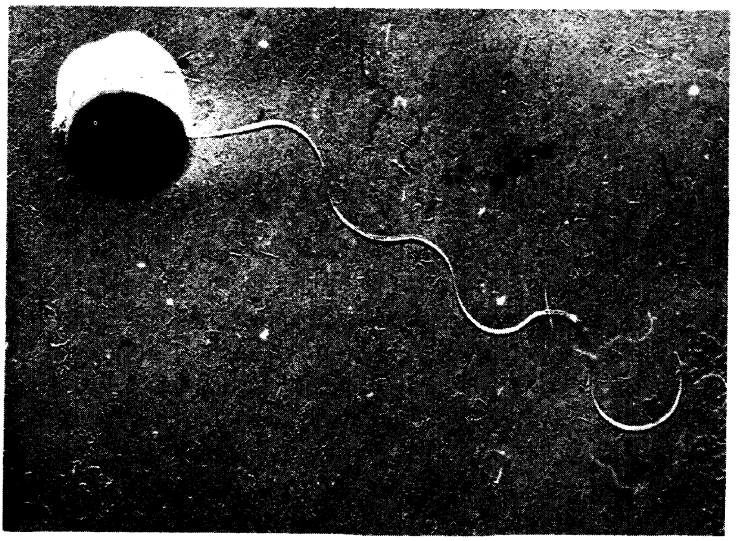

**Fig. 2.3.** Electron micrograph of a cell of *Vibrio parahaemolyticus* showing single polar flagellum (× 15000).

killed at temperatures above 50°C. Not all strains are pathogenic for man but those that are almost invariably give a positive Kanagawa reaction (i.e. lyse red blood cells). These pathogenic strains appear to possess a 'toxic factor' which is released on lysis of the bacterial cells (Brown *et al.*, 1977); however, in spite of extensive studies into the properties of the Kanagawa haemolysin and pathogenic mechanisms in *V. parahaemolyticus*, no explanation as to how the illness is induced has been forthcoming (Sakazaki & Shimada, 1986).

*2.3.5.2. The Disease*

The symptoms of *V. parahaemolyticus* food poisoning usually appear 10–18 h after ingestion of the contaminated food although incubation periods varying from 2 to 48 h have been reported. The main symptoms are nausea, vomiting, abdominal pain and diarrhoea. Mild fever occurs on occasion but the duration of the illness is usually short, from 1 to 4 days; the mortality rate is very low, most deaths being associated with the elderly and infirm. Carriers of the organism have not been reported.

*2.3.5.3. Foods Associated with V. parahaemolyticus Food Poisoning*

This organism is almost exclusively found in the marine environment. It is most abundant during the summer months in inshore areas such as shallow coastal and estuarine waters when the temperature of these waters is at its warmest. It has also been isolated from coastal marine sediments and from various marine animals, especially bivalve shellfish but also from crustaceans and fish. *V. parahaemolyticus* is particularly prevalent in Far Eastern waters and hence natural levels of contamination can be high; Chan *et al.* (1989) found mussels and oysters to contain, on average, 4·6 and $3{\cdot}4 \times 10^4$ per g respectively.

In Japan, where raw fish is commonly eaten, *V. parahaemolyticus* accounts for approximately 60% of food poisoning cases. For many years it was thought that the organism was restricted to the Far East but more recently it has been isolated from many other regions of the world. A survey (Ayres & Barrow, 1978) undertaken to determine the distribution of *V. parahaemolyticus* around Britain showed isolation rates for the organism of 11, 18 and 14%, respectively, from coastal waters, sediments and shellfish, i.e. oysters, cockles and mussels; however, all isolates gave a Kanagawa negative reaction and the authors concluded that these organisms were unlikely to pose any serious health hazard. Similar isolation rates for European waters have been reported elsewhere although van den Broek *et al.* (1979) found that as many as

26% of their samples of mussels from estuarine waters in The Netherlands were contaminated with *V. parahaemolyticus*. Higher isolation rates have been reported in other parts of the world, e.g. Thompson & Vanderzant (1976) found 60% of oysters from the northern Gulf of Mexico to be contaminated.

*V. parahaemolyticus* is thus principally associated with seafoods and it is these foods which have been responsible for most of the outbreaks of this type of food poisoning. In European countries and the United States, unlike Japan, most seafoods are cooked before consumption and therefore the organism is invariably killed. However, oysters are an exception but they are rarely implicated in food poisoning outbreaks, probably because the numbers of *V. parahaemolyticus* present in this mollusc are too low to initiate the illness. Foods that have been involved in food poisoning outbreaks in the UK and United States include dressed crab, lobster, prawns and shrimps, usually imports from the Far East. Typically there used to be about 100 cases a year reported in the UK but this number was reduced to almost zero by the latter half of the 1980s.

Outbreaks appear to have been caused in two ways, either by inadequate cooking or by recontamination after cooking, both of which left viable organisms in the food which were capable of developing into vast numbers during subsequent storage at room temperature. A typical outbreak was reported by Hooper *et al.* (1974):

> In July 1973, the nurse at a holiday camp on the south coast of England told the general practitioner in charge of the medical services that five people had reported to her that morning with acute gastroenteritis and that there seemed to be a common source of infection ... [as] ... all had eaten dressed crab the previous evening. Each of the four families concerned had purchased crab meat on the same day from the same fishmonger in the town.
>
> Symptoms developed 16–18 hours after the crab meat had been eaten. Severe cramp-like abdominal pains were followed soon afterwards by violent diarrhoea accompanied by weakness and fever. Two patients also reported vomiting before diarrhoea started. All recovered in 24–48 hours. ...
>
> The outbreak was described in the local, and later in the national, press, and as a result six people in a further three families reported that they had also had a similar illness on the same date after meals of dressed crab ... purchased from the same fishmonger.

The crab had been caught locally and none remained available for laboratory examination but faecal samples obtained from six patients yielded *V. parahaemolyticus*. Further investigations led to the conclusion that cross-contamination of cooked by live crabs or from dirty working surfaces was the primary cause of this outbreak.

*2.3.5.4. Control Measures*

Once again this is primarily a storage and cross-contamination problem so control measures should aim to:

1. Ensure adequate heat treatment of cooked seafoods.
2. Ensure rapid cooling and refrigeration of cooked seafoods if they are to be stored and ensure raw seafoods are refrigerated at all times.
3. Avoid cross-contamination of cooked by raw seafoods or by surfaces which have come into contact with the raw food.
4. Where possible, attempt to import seafoods in the raw state so that final processing can be monitored.

## 2.3.6. Botulism

*2.3.6.1. The Organism*

*Clostridium botulinum*, the causative organism of botulism, is an obligately anaerobic, motile, Gram positive, spore-forming rod; the rods are large (4–8 $\mu$m by 0·9–1·2 $\mu$m), although type E (see below) is more slender (width 0·3–0·7 $\mu$m). Seven types of *C. botulinum* are now recognized, designated A to G on the basis of the toxins they produce; however strains producing two toxins (e.g. A and B or A and F) have been isolated so that this strict classification no longer holds true. Types A, B and E produce exotoxins that are almost entirely responsible for botulism in man. Types C and D are important as causes of botulism in animals, including birds, although type C has been implicated in a few outbreaks of human botulism. Types F and G have also caused human botulism on a few occasions.

The optimum temperature for growth of the organism varies from 25° to 37°C depending upon the strain; the minimum growth temperature for the majority of the types varies between 10°C and 20°C but psychrotrophic strains of types B,E and F have minimum growth temperatures of 3–5°C. The spores of *C. botulinum* are markedly heat-resistant and those of types A and B can resist boiling for up to 6 h. The spores of types C and D are rather less heat-resistant whilst type E spores are inactivated at 80°C in 15 min.

The exotoxins produced by *C. botulinum* are extremely poisonous to man and a fatal dose for an adult of type A toxin, the most potent, can be as little as $1 \times 10^8$ g; in other words, 1 g could kill 100 million people. The toxins which are preformed in the food before ingestion are normally absorbed in the small intestine, transferred via the bloodstream and finally absorbed into the nervous system. The toxins are classed as neurotoxins since their action is specifically directed to nerves, especially the peripheral nerves of the involuntary muscles of the body. The toxins are not very resistant to heat, types A and B being inactivated by exposure to 80°C for 10 min whilst type E is destroyed within 5 min at 60°C (Riemann, 1969; Sakaguchi, 1969).

*2.3.6.2. The Disease*

The first symptoms usually appear some 18–36 h after ingestion of food containing the toxin but they may be delayed a further 36 h or even longer, considerable variation occurring in individual cases. In rare cases the first symptoms may occur within a few hours, the earlier the onset the less favourable the prognosis. The first symptoms are usually nausea, vomiting and possibly diarrhoea accompanied by fatigue, headache and dizziness. Persistent constipation is a common feature following the early symptoms and this is usually accompanied by blurred vision and difficulty in swallowing and speaking. Extreme muscular weakness is another common feature. There is no fever and the temperature is frequently subnormal. In the more severe cases the involuntary muscles become paralysed and the paralysis spreads to the respiratory system and heart, death finally resulting from respiratory failure or cardiac arrest. The severest symptoms last for up to 10 days with death most common between the third and sixth day after ingestion of the food. With survivors recovery is extremely slow but is complete within 6 months. The mortality rate is high being quoted at 65% in the United States but the rate is generally much lower in European countries.

Treatment of patients includes the administration of antitoxin specific for the type of toxin involved, together with the use of modern life-support techniques which have helped to reduce the previously high mortality rates.

*2.3.6.3. Sources*

*C. botulinum* is widely distributed in soils although the types causing human botulism are more prevalent in the nothern hemisphere than in the southern. Concentrations are low, probably less than 1 per gram of

soil. *C. botulinum* can be isolated readily from many fresh and marine waters, their sediments and from fish, but such isolates are typically the psychrotrophic strains of types B, E and F; their original source may well be terrestrial. In the UK about 5 % of soil samples contain *C. botulinum*, mainly type B, but isolation rates from aquatic environments are substantially higher. Smith *et al.* (1978) found 35% of samples to be positive; type B was again the most widespread (30% positive) whilst types C, D and E were isolated occasionally (1–3% positive).

The predominance of type B is common in many European countries but in Scandinavia and the Baltic type E is widespread. Type E is also relatively common in marine waters, sediments and fish in other areas of the northern hemisphere such as Alaska and the Pacific coast of the United States, the Soviet Union and Japan (Hobbs,1976). In the United States mesophilic rather than psychrotrophic strains tend to predominate but geographical location affects the distribution pattern. Thus from the Rocky Mountains to the Pacific coast type A is more common than B whereas the reverse is true to the east of the Mississippi River (Sugiyama & Sofos, 1988).

*2.3.6.4. Foods Implicated in Botulism*

A wide variety of foods has been involved in human botulism reflecting the world distribution of the predominant types and the eating habits of the populations affected.

In the UK no substantiated outbreak of botulism was recorded until 1922 when the Loch Maree, Gairloch, tragedy occurred. This has been described in detail by Dewberry (1959) but the following is a summary:

> A party of eight visitors staying at a local hotel went out fishing on the 14th August and during the day ate duck paste sandwiches. About 3 am on the 15th the first of the party was taken ill and late that evening three died; in the following week the remaining members of the party also died. Samples of the duck paste, as well as one of the sandwiches, were examined and found to contain *C. botulinum*, type A, together with lethal quantities of type A toxin.

After that outbreak there were no further cases in the UK until 1935 when four deaths occurred in London. Three cases occurred in 1944 and five in 1947. In 1955 two Mauritian students became victims of type A poisoning after eating pickled fish brought from Mauritius; both patients recovered after treatment with type A antitoxin. Four old-age

pensioners in Birmingham contracted type E botulism in the 1978 outbreak described by Ball *et al.* (1979) of which the following is a summary:

> After eating a tin of canned salmon, originally processed in Alaska, for tea on Sunday, 30th July the first couple began to show symptoms of vomiting and severe diarrhoea at 2 am on the Monday. They were moved to hospital in acute distress a few hours later where they were examined and found to have blurred vision and had difficulty in speaking. Police were alerted to the second couple who shared the meal and called at their house to find them desperately ill and too weak to seek help. They were rushed to hospital where they deteriorated rapidly and by 10 am they were unable to breathe as their muscles became paralysed. All four victims were placed in the intensive care unit and treated with antitoxin but in spite of the latest life-support techniques the first couple admitted died 17 and 23 days after eating the contaminated food. Death was due to cardiac arrest and respiratory failure. *C. botulinum* type E was isolated from the remains of the salmon in the tin.

It was later ascertained that the can had a construction fault which enabled it to become contaminated during the air cooling of cans at the Alaskan factory. It was alleged that operatives handling raw fish dried their overalls on the cooling cans during breaks thus allowing *C. botulinum* spores to gain entry into any faulty cans; if true, this is a perfect example of the results of cross-contamination of cooked food by raw.

The latest outbreak of botulism in the UK occurred in the summer of 1989 associated with hazlenut yoghurt (O'Mahony *et al.*, 1990). Twenty-seven cases were recorded all showing the typical symptoms; although many required intensive care treatment there was only one fatality. *C. botulinum* type B toxin was detected in cans of hazlenut puree, used in the preparation of the yoghurt, but toxin levels must have been minimal for only one death to have been recorded.

Outbreaks in Europe have been principally associated with various meats and meat products, often home-preserved, in which type B toxin has been formed. Historically botulism is associated with blood and liver sausages and the name botulism is derived from the Latin word for sausage *botulus*; smoked ham, pork brawn and various pâtés have also been implicated. Fish, and to a lesser extent other marine foods, in which type E toxin has been produced have been a common cause of

botulism in northern European countries, the Soviet Union and Japan. In the latter country during the period 1951–83 there were 189 outbreaks, almost all type E botulism associated with fish (Sugiyama & Sofos, 1988). Such outbreaks can usually be explained on the basis of poor home-processing or the consumption of uncooked foods.

In the United States the picture is rather different and most cases of botulism have been caused by canned or bottled fruits or vegetables which have been inadequately processed in the home. In the period from 1930 to 1949 when home-canning was at its height there were 255 outbreaks attributed to that cause. Green beans and sweetcorn have been the foods most frequently implicated and where toxin identification has been possible type A has predominated. Botulism continues to be relatively common in the United States with 203 cases occurring between 1978 and 1983; home prepared foods were again mainly responsible (Sugiyama & Sofos, 1988). Commercially canned foods have a much better, although not blameless, record with 60 outbreaks and just over 100 fatalities occurring from the turn of the century to 1970. Occasional outbreaks still occur and commercial canned products such as vichyssoise, beef stew and canned mushrooms were responsible for small outbreaks with fatalities in the 1970s (Lynt *et al.*, 1975).

As already indicated, *C. botulinum* type E is primarily isolated from the aquatic environment and hence fish and other seafoods predominate in outbreaks of this type. This is also true for the United States where the majority of type E outbreaks have been associated with these foods (Sugiyama & Sofos, 1988). Particular interest was aroused in the early 1960s by two outbreaks of type E botulism in which 12 people died following the consumption of vacuum-packed smoked fish. Until these episodes it had been assumed that the combination of vacuum-packaging and refrigerated storage was a safe method of preserving fish products. It was found that toxin production in fish contaminated with type E spores occurred not only under vacuum-packaged conditions but may also be produced in certain species of fish (e.g. herring) regardless of the method of packaging (Abrahamsson *et al.*, 1965).

Instances of an alternative form of botulism, 'infant botulism' have been frequently reported in more recent years (Sugiyama & Sofos, 1988). This infection is caused by *C. botulinum*, typically either type A or type B, growing in the intestinal tract of infants aged under 9 months; whilst growing the toxin is produced and this form of illness is therefore similar to *C. perfringens* food poisoning in that respect. The organism is transmitted in honey, a food that has been found to contain

low numbers of *C. botulinum* spores on occasions (Hauschild *et al.*, 1988); such spores are probably initially picked up by bees. It has been recommended that honey should not be given to infants under 1 year old since below that age the infant has not yet developed a competitive gut microflora which would prevent the germination of the *C. botulinum* spores (Sugiyama & Sofos, 1988).

*2.3.6.5. Factors Affecting Toxin Formation in Foods*

Toxin formation in foods is obviously dependent on the spores withstanding the processing treatment, or on a post-process contamination. Subsequently conditions must allow for spore germination, growth of the vegetative cells and toxin production. Conditions affecting this chain of events include the composition of the food, the temperature and period of storage, as well as the moisture content, pH, oxygen availability and OR potential, and salt content of the food.

Although many types of food have been implicated in outbreaks, the foods do have common features. Most have been subjected to some form of preservation, they have been stored under conditions permitting toxin to be formed and they have been either eaten without further cooking or the reheating has been insufficient to destroy the toxin.

Temperature is an important factor but toxin formation is possible over the whole growth temperature range. However other factors such as the degree of acidity and the salt content of the food become more effective growth inhibitors as the temperature is reduced so that lower storage temperatures (15°C as a maximum) are to be preferred. Again, toxin production is possible at all moisture and pH values at which growth occurs although toxin is unstable at pH values above 7 (Baird-Parker, 1971). No growth or toxin production can occur at pH 4·5 or below under normal conditions.

In some foods, such as canned vegetables, toxin production is unlikely unless air is excluded. Foods like meats and fish have a strong reducing tendency so that growth and toxin formation are quite possible even in the presence of air; the example of herring has been mentioned previously.

Strains of *C. botulinum* differ considerably in their sensitivity to salt in foods. Some, mainly strains of types A and B, will tolerate levels of up to 10% sodium chloride whilst type E strains are inhibited at 4·5–5 % (Sugiyama & Sofos, 1988). The situation is complex because sensitivities to sodium chloride alter at different pH values and at different sodium nitrite levels if the latter is included as a preservative in the food.

*2.3.6.6. Control Measures*

There are many facets to this problem but the following points are crucially important:

1. Ensure that the heat treatment for canned and bottled foods (pH >4·5) is sufficient to destroy the most heat-resistant *C. botulinum* spores.
2. Use the cleanest possible chlorinated water for cooling cans after processing. Where air cooling is used ensure that cross-contamination from raw materials or dirty equipment, or by handling is avoided during cooling.
3. Where milder heat processes are applied to foods ensure that suitable inhibitory compounds are introduced or that the pH is low enough to prevent growth of *C. botulinum.*
4. Ensure adequate heat treatment with semi-processed or processed vacuum-packed foods and store at a maximum temperature of 3°C.
5. Never taste a food that is suspect unless it is pre-heated to 100°C.

## 2.3.7. Listeriosis

*2.3.7.1. The Organism*

*Listeria monocytogenes*, the causative organism of listeriosis, is a facultatively anaerobic, motile (at 20–25°C), short Gram positive rod of regular appearance; cells are 0·5–2·0 μm in length and 0·4–0·5 μm in diameter. The optimum temperature of growth of the organism is typically about 35–37°C but strains can exhibit wide growth temperature ranges of 1–45°C; in fact the majority of strains may well grow at *ca* 1°C (Junttila *et al.*, 1988). However *L. monocytogenes* also exhibits a surprising resistance to heat and it has been suggested that it survives the minimum pasteurization heat treatment (72°C for 15 s) required by many countries for raw milk (Fernandez Garayzabel *et al.*, 1987). Apparent differences in heat sensitivity have been highlighted depending on the methodologies employed. Thus Bradshaw *et al.* (1987) claimed that normal pasteurization treatments were sufficient to destroy *L. monocytogenes.* This has been questioned by Doyle *et al.* (1987) who stressed the need to use suitable enrichment procedures in order to facilitate the recovery of metabolically injured cells (see Chapter 3). This point is illustrated in Table 2.5 which shows that *L. monocytogenes* was only isolated after 48 h refrigeration of the milk following pasteurization.

**Table 2.5**
Counts of *Listeria monocytogenes* in Milk (Initial Count $10^8$ per ml) Stored at 4°C for 5 d Following Pasteurization (72°C/15 s)[a]

| | *Days* | *Count per ml* |
|---|---|---|
| Immediately after pasteurization | 0 | 0 |
| Storage at 4°C | 1 | 0 |
| | 2 | 40 |
| | 3 | 150 |
| | 4 | 800 |
| | 5 | 2 500 |

[a]From Fernandez Garayzabel *et al.* (1987).

The virulence mechanism has to be elucidated but *L. monocytogenes* strains lyse red blood cells (i.e. haemolytic strains) and this appears to be associated with pathogenicity; likewise *L. monocytogenes* kills chick embryos when cells are injected, which non-pathogenic *Listeria* spp. fail to do (Schönberg, 1989).

*2.3.7.2. The Disease*

*Listeria monocytogenes* is a low grade pathogen, the ingestion of low numbers of viable cells causing no clinical manifestations in healthy adults. Certain groups of humans are more susceptible to the organism and these include infants, the elderly, persons immunocompromised by drugs or disease and pregnant women. With such groups *L. monocytogenes* causes, in particular, septicaemia and meningitis but such cases are also reported occasionally in apparently healthy adults, possibly where large numbers of organisms have been ingested. Listeriosis is of especial concern in pregnant women; infections produce 'flu-like symptoms and may cause premature labour, stillbirth or early death of the newly-born infant. Mortality is very high, 50% in infants, and at least 25% with the other groups. The incubation period is lengthy, usually about 14 days, although it can vary from 4 to 21 days.

*2.3.7.3. Sources of Listeria and Foods Involved in Listeriosis*

*Listeria* spp. are widely distributed in the environment and the same is true, to a lesser extent, of *L. monocytogenes*. Thus the latter can be isolated from soil, silage, water, vegetation, a wide range of foods and the faeces of many animals including humans. Cox *et al.* (1989), in an examination of listerias in food processing and other environments, found 3·5% of samples taken to contain *L. monocytogenes* whilst Charlton *et*

*al.* (1990) found *Listeria* spp. in 75/597 samples (12·6%) taken from milk processing plants; a half of these isolates were identified as *L. monocytogenes*. These typical findings stress the comparative ubiquity of this organism.

Raw milk is recognized as an important source of *L. monocytogenes*. Hayes *et al.* (1986) found 12% of over 100 such samples examined in the United States to harbour the organism; contamination rates of up to 55% for listerias in general have also been reported in certain parts of that country (Dirksen & Flagg, 1988). Thus Gitter *et al.* (1980) had stressed earlier that the excretion of *L. monocytogenes* in milk was an important factor in the transmission and epidemiology of listeriosis, whilst Slade *et al.* (1989) concluded that 'the ubiquity of *Listeria* spp. in the rural environment makes their control and eradication from the agricultural niche, and hence from raw milk supplies, a daunting, somewhat improbable, task'.

It has already been mentioned that there is conflicting evidence concerning the ability of *L. monocytogenes* to withstand pasteurization treatments used for milk. This problem was highlighted earlier, in 1983, in a big outbreak of listeriosis in the United States which was directly attributed to the consumption of pasteurized milk. However, recently it has been shown unequivocally that the *L. monocytogenes* strain involved in that outbreak could not survive correct pasteurization treatment (Lovett *et al.*, 1990). The presence of *L. monocytogenes* in 1% of over one thousand pasteurized milk samples examined in the UK indicates that post-pasteurization contamination or inadequate processing remain a problem (Greenwood *et al.*, 1991).

Soft Mexican-style cheese was incriminated in a further outbreak in the United States in which there were 103 reported cases of whom, 18 adults and 29 unborn infants died. Some doubt exists about the exact cause but it was most likely due to some form of post-pasteurization contamination (Dirksen & Flagg, 1988). This incident illustrates the inevitable association of dairy products in general being causes of listeriosis; thus cheeses, particularly soft cheeses, made either from pasteurized or unpasteurized milk have been implicated. An isolated case in the UK was reported by Bannister (1987):

> A 36-year-old woman was admitted to hospital on 9th January with an 18 hours' history of fever, back pain, aching legs and increasing headache with neck stiffness..... She was not pregnant. On examination she was distressed and drowsy with a temperature of 38·7°C..... *Listeria monocytogenes* appeared in CSF (i.e. cerebral spinal fluid) culture..... (It was found that) she had purchased two

soft French cheeses for Christmas, and had kept them refrigerated for about 2 weeks. She had eaten both types of cheese during the week before her illness but her husband and son had not done so. Remaining portions of the cheese were examined ..... One of them, a Camembert, did not yield any growth of significance, but a soft country cheese produced a heavy growth of *L. monocytogenes*. Both the strain isolated from the CSF and the one isolated from the cheese .....were shown to be of (the same serotype).

The frequency of listeriosis has increased in England, Wales and Northern Ireland (see Table 2.6) and concern has been expressed about this trend. More recent data suggest the peak may have been reached. However, it is reasonable to assume that, since listeriosis is not notifiable in the UK, the official figures are a considerable underestimate of the true position (Lacey & Kerr, 1989). Foods are the most likely means of acquiring the infection (WHO Working Group, 1988) so that much attention is necessarily being paid to the possible role of food in the spread of listeriosis. Most studies have been published on cheeses: these have included surveys on the natural incidence of *L. monocytogenes* in cheeses and the behaviour of deliberately inoculated strains during the manufacture and ripening of cheeses. Beckers *et al.* (1987) found 14·5% of soft cheeses imported into the Netherlands to contain *L. monocytogenes* with recoveries of the organism of up to $10^6$ per g cheese. Pini & Gilbert (1988) in a survey of home produced and imported soft

**Table 2.6**
Listeriosis in England, Wales and Northern Ireland (1973–1991)[a]

| *Year* | *No. Cases*[b] | *Year* | *No. Cases*[b] |
|---|---|---|---|
| 1973 | 25 | 1983 | 119 (115[c]) |
| 1974 | 28 | 1984 | 91 (115) |
| 1975 | 39 | 1985 | 124 (149) |
| 1976 | 31 | 1986 | 113 (137) |
| 1977 | 47 | 1987 | 204 (259) |
| 1978 | 87 | 1988 | 239 (291) |
| 1979 | 70 | 1989 | (250) |
| 1980 | 75 | 1990 | (117) |
| 1981 | 86 | 1991 | (130) |
| 1982 | 77 | | |

[a]Based on data from PHLS Communicable Disease Reports.
[b]Unedited annual totals.
[c]Numbers in parentheses show data enhanced by additional information from the PHLS Laboratory of Microbiological Reagents.

cheeses in the UK found similar isolation rates (10%) and counts of up to $10^5$ per g; one interesting conclusion drawn from this study was that it made little difference whether the cheeses had been prepared from pasteurized or non-pasteurized milk.

When *L. monocytogenes* is deliberately inoculated into pasteurized milk used in the manufacture of different cheeses the fate of the organism is determined by the type of cheese and, in particular, its pH. Thus Ryser & Marth (1987) have shown that, when *L. monocytogenes* is inoculated at $5 \times 10^2$ per ml, with Camembert cheese there is an initial slight increase in numbers followed by a decrease to < 10–100 per g during the first 18 days' ripening; counts of the organism then increase to $1 \times 10^6 - 5 \times 10^7$ per g, these increases reflecting the higher pH (6–7) of the cheese. No such increases are seen with Cheddar cheese whilst with cottage cheese decreases and possible elimination of the organism are possibly due to the low pH (*ca* 5) of the latter (Ryser *et al.*, 1985). One can safely conclude from these studies that the presence of significant numbers of *L. monocytogenes* in milk used in the manufacture of soft cheeses is a potential health hazard.

Concern has been expressed about raw chickens and meats as an important source of *L. monocytogenes*. Pini & Gilbert (1988) found 60% of fresh and frozen birds in the UK to be contaminated with the organism; Bailey *et al.* (1989) reported that 23% of broiler carcasses harboured *L. monocytogenes* in the United States. Isolation rates for *L. monocytogenes* from raw meats tend to be somewhat lower (0–45%) although for such a ubiquitous organism it is not surprising that it can normally be isolated from raw beef, pork and lamb (Johnson *et al.*, 1990).

Studies involving the deliberate inoculation of the organism onto chicken carcasses have included that of Harrison & Carpenter (1989). They used high inoculum levels ($10^6$–$10^7$ per g) onto chicken breasts and found that heating them to an internal temperature of 82°C was insufficient to eliminate totally *L. monocytogenes*, which recovered to $10^7$ per g following post-heating storage at 10°C for 10 days; storage at 4°C for 4 weeks following heating to an internal temperature of 77°C allowed the organism to recover to $10^5$ per g. These results indicate that a recommended final temperature of 71°C will not eliminate *L. monocytogenes* although the authors point out that the inoculum level was far higher than would be expected. Glass and Doyle (1989) studied the survival and growth of the organism in a range of foods during storage at 4°C for up to 12 weeks. Growth of *L. monocytogenes* was best on sliced chicken and turkey, counts increasing from $10^3$ to $10^5$ per g in 4 weeks;

there were smaller increases on other foods (e.g. ham, bratworst) with levels of $10^4$ per g being established after 6 weeks whilst on cold roast beef only slight growth occurred over the full 12 weeks. The authors again concluded that pH was crucial in determining growth patterns; above pH 6 growth was most rapid whereas *L. monocytogenes* grew poorly or not at all on products with a <5 pH. However, George *et al.* (1988), using laboratory media rather than foodstuffs, found *L. monocytogenes* was capable of growing down to pH 4·4 (20°C incubation), pH 4·6 (10°C) and only down to pH 5·2 at 4°C suggesting that storage temperature is an important determinant of growth control when pH is considered.

Coleslaw was responsible for an outbreak of listeriosis in Canada in 1981 and celery, tomatoes and lettuce have been implicated in hospital outbreaks; in fact, *L. monocytogenes* can be isolated readily from fresh vegetables (Heisick *et al.*, 1989). Growth of food poisoning bacteria on these and similar foods would be unexpected and little work has been published. However, Steinbruegge *et al.* (1988) found that *L. monocytogenes* inoculated onto lettuce increased 10-fold in numbers over 14 days' storage at 5°C and from $10^3$ to $10^6$ per g over the same period at 12°C. These results again stress the remarkable facility of this pathogen to grow at relatively low temperatures on unexpected foodstuffs. The safety of cook-chill foods has therefore been questioned. Lacey & Kerr (1989) found 25% of such foods at retail outlets to be contaminated with *L. monocytogenes* and, in consequence, proposed that the maximum storage time for cook-chill foods should be limited to 3 days at a temperature below 3°C.

*2.3.7.4. Control Measures*

Control measures should surmount the problems associated with the ability of *L. monocytogenes* to withstand relatively high temperatures, its ability to grow down to just over 0°C and its ability to so effectively repair from the effects of metabolic injury at low temperatures. It is therefore essential to:

1. Ensure that milk is adequately pasteurized and there is no post-pasteurization contamination.
2. Use only *L. monocytogenes*-free milk in the manufacturing of soft cheeses.
3. Ensure foods, particularly poultry, are heated to a minimum internal temperature of 72°C.
4. Where chill storage of pre-heated foods like poultry is required;

(a) ensure higher internal temperatures (minimum 75°C) are reached,
(b) limit the shelf life of all products, heated or unheated, where growth of *L. monocytogenes* is feasible to a maximum of 5 days,
(c) ensure storage temperatures are maintained at a maximum of 4°C for such products.

### 2.3.8. Enteritis due to *Campylobacter* spp.

#### *2.3.8.1. The Organism*

Two *Campylobacter* species have been implicated as enteric pathogens in humans. These are *C. jejuni* and *C. coli* although the former is responsible for over 90% of cases. Campylobacters used to be classified as vibrios but valid differences between these two groups are now recognized. Campylobacters are small, spiral or curved Gram negative rods which are motile with a single polar flagellum at one or both ends of the cell. They differ from vibrios in being strictly microaerophilic and will thus only grow in reduced oxygen levels. *C. jejuni* and *C. coli* are able to grow at 42°C but not at 25°C and they are often loosely described therefore as 'thermophilic campylobacters'. Their pathogenic mechanism has yet to be fully elucidated but it is known that campylobacters elaborate a lipopolysaccharide endotoxin rather like that found with salmonellas; in addition, it has been claimed that an enterotoxin, similar to cholera toxin, may be formed (Hoffman & Blankenship, 1986) as well as a cytotoxin which assists the invasiveness of the organism (Guerrant *et al.*, 1987). Feeding experiments by Robinson (1981) have demonstrated that the infective dose in healthy adults is below 500 cells; a fraction of this number may suffice since numbers in foods are low.

#### *2.3.8.2. The Disease*

Campylobacters are a relatively recent addition to the list of organisms causing gastroenteritis; in fact, Skirrow (1977) described it as a 'new' disease although infections in man caused by these organisms had been recognized some years earlier. The incubation period varies from 2 to 11 days. The most common symptoms are diarrhoea and abdominal cramps but these are often preceded by a fever which lasts for up to 24 h. Diarrhoea is profuse for up to 3 days and may be accompanied by bloody stools; vomiting is unusual. The duration of the illness varies from 3 to 14 days.

*2.3.8.3. Foods Involved*

It is now recognized that campylobacters are responsible for more cases of acute infective diarrhoea in developed countries than any other bacteria. In the UK there are now over 30 000 reported cases per year but, because these bacteria do not multiply to any extent in the food, campylobacter infections are not regarded as a true bacterial food poisoning.

There is little doubt that both *C. jejuni* in particular, and *C. coli* are widely distributed both in foods and the environment generally but contaminated poultry and raw milk have been the main causes of infections in humans. Campylobacters can be readily isolated from the gut contents of poultry and poultry carcasses are, in consequence, often contaminated with these organisms. Thus Lammerding *et al.* (1988), in a survey of different raw foods in Canada, found 74% of turkeys and 38% of chickens harboured campylobacters; lower isolation rates for *C. jejuni* and *C. coli* were found for beef (23%) and pork (17%) whilst veal (43%) was more heavily contaminated. These results were largely substantiated by Fricker & Park (1989) who, in a detailed study covering a two-year period, again found the highest recoveries of these campylobacters from poultry; these and recoveries from other sources are shown in Table 2.7. Both Lammerding *et al.* (1988) and Fricker & Park (1989) also reported that whereas *C. jejuni* was predominant (> 90%) in most foods and the environmental samples *C. coli* predominated (95%) on pork.

Genigeorgis *et al.* (1986) detected that the level of contamination in poultry increased steadily during growth of the birds. Only 2% of

**Table 2.7**
Recoveries of *Campylobacter* spp. from Environmental and Food Sources[a]

| *Type of Sample* | *No. examined* | *No. positive* | *Percentage positive* |
|---|---|---|---|
| Sewage | 436 | 424 | 96·6 |
| River water | 345 | 105 | 30·4 |
| Poultry | 758 | 421 | 55·5 |
| Beef | 127 | 30 | 23·6 |
| Pork | 158 | 29 | 18·4 |
| Lamb | 103 | 16 | 15·5 |
| Seafood | 89 | 13 | 14·6 |
| Cooked meats | 86 | 2 | 2·3 |
| Salads | 106 | 0 | — |

[a]From Fricker & Park (1989).

10-day old chicks carried *C. jejuni* but this figure had risen to 82% by day 50. These authors suggested that transmission from one generation to another was feasible through contaminated litter or equipment but they argued that the development of *C. jejuni*-free flocks was now possible.

Food poisoning outbreaks involving poultry are usually due to the campylobacters surviving cooking. An unusual outbreak involving cadets at a military camp in the Netherlands was described by Brouwer *et al.* (1979):

> ... live chickens were obtained for the evening meal of 123 first-year cadets ... (on a survival exercise). The chickens were killed and skinned by each cadet for himself and prepared over a wood fire.... A large number (89) of the cadets became ill in the following week with symptoms of enteritis.... Faeces samples from 104 cadets ... (yielded 34 positive cultures) ... for *Campylobacter fetus* ssp *jejuni*. Seven of these were isolated from cadets who never showed symptoms whereas 27 originated from the 89 men who became ill.
>
> We believe that this extensive outbreak of enteritis was a consequence of eating chicken contaminated with *Campylobacter fetus* ssp *jejuni*. The method of preparation left the chickens partially unheated so that the campylobacters could survive the preparation of the food. Moreover, by handling, cooking and eating the chickens with bare hands, reinfection of the adequately cooked parts of the chickens could not be excluded.

Another important source of infection is unpasteurized milk and in this case the bacteria originate from the bovine faeces or possibly infected udders (Hoffman & Blankenship, 1986). As well as poultry many other types of animal faeces contain campylobacters. Humphrey & Beckett (1987) found 72% of bovine faecal samples to be positive whilst Beumer *et al.* (1988), in the Netherlands, reported that 22% of such samples yielded *C. jejuni*; however only 4·5% of raw milks were positive and these authors suggested that an enzyme, lactoperoxidase, naturally present in milk tends to kill-off the campylobacters. However, because of the low infective dose, enteritis outbreaks emanating from unpasteurized milk are often reported. Robinson *et al.* (1979) described an outbreak involving 63 people in Cumbria and 14 in Bradford, and Porter & Reid (1980) reported on an outbreak involving 148 patients in Kincardineshire and Angus. A large outbreak involving 2500 primary school children in the Luton area was described by Jones *et al.* (1981);

the children became seriously ill after drinking free school milk which was almost certainly contaminated. Large numbers of campylobacters were isolated from the faeces of patients in these outbreaks although direct isolations from the milk were not effected. In the last example the milk should have been pasteurized but faulty operation of a by-pass valve probably allowed the milk to pass through unheated; campylobacters are readily killed by pasteurization (Waterman, 1982).

Although other animal foods often harbour campylobacters they are not regarded as a significant source of infection. Dogs probably account for nearly 5% of human sporadic cases and puppies, in particular, have been implicated (Miller *et al.*, 1987).

#### *2.3.8.4. Control Measures*

*C. jejuni* and *C. coli* tend to die-off in foods held at ambient temperatures and growth, if at all possible, is always slow. But campylobacters are important enteric pathogens and control methods are very necessary. These include:

1. Milk pasteurization.
2. Introduction of campylobacter-free poultry flocks.
3. Thorough cooking of poultry.
4. Separation of raw and cooked meats and poultry.

### 2.3.9. Miscellaneous Bacterial Food Poisoning

#### *2.3.9.1. Escherichia coli*

*E. coli* is a short, typically motile, Gram negative rod with many characteristics similar to those of salmonellas. One of the principal features differentiating *E. coli* from salmonellas is its ability to attack lactose and sucrose with the production of acid and gas. Non-pathogenic *E. coli* strains can be isolated in massive numbers from the intestines of warm-blooded animals. However, many strains of *E. coli* are pathogenic to humans to varying degrees and these strains can be divided into four groups (Olsvik *et al.*, 1991).

The first group, enteropathogenic *E. coli* (EPEC) cause severe diarrhoea in infants but the pathogenic mechanism remains unknown although certain EPEC strains produce one or more cytotoxins. The second group, enterotoxigenic *E. coli* (ETEC), also cause diarrhoea in humans, both infants and adults, the latter group usually succumbing to the world-wide illness known as traveller's diarrhoea. ETEC strains pro-

duce enterotoxins of two distinct types: these are a heat-labile toxin, inactivated at 60°C in 30 min, and a heat-stable toxin, resistant to 100°C for 15 min (Scotland, 1988). ETEC strains have been implicated in several large outbreaks of infection with water as well as a wide range of foods (e.g. various meats and poultry, mashed potatoes, milk and cheese) as sources. MacDonald *et al.* (1985) described a multistate outbreak in the United States caused by ETEC contaminated imported Brie and Camembert cheeses produced at the same factory. One hundred and sixty adults were affected by nausea, vomiting, diarrhoea and, typically, abdominal cramps following an incubation period of roughly 44 h after consuming the cheese; similar outbreaks involving the same factory were also reported in many European countries. Riordan *et al.* (1985) reported an outbreak in England where cold curried turkey mayonnaise was implicated; 27 staff and visitors at a school function were affected by severe diarrhoea some 36 h after consuming the food which had been left at ambient temperature for 8 h following preparation. Faeces samples taken from patients contained strains producing both heat-labile and heat-stable toxins.

The third group of pathogenic *E. coli*, enteroinvasive *E. coli* (EIEC) produce a cytotoxin and often induce rather more severe illnesses like colitis and a form of dysentery, accompanied by fever and bloody stools. Many outbreaks have been attributed to EIEC strains, with cheeses, milk and meats the foods most frequently incriminated. Imported French cheese was again responsible for a large outbreak in the United States (Marrier *et al.*, 1973); on this occasion at least 387 persons contracted gastroenteritis.The EIEC strain was isolated from the stools of patients, from various samples of the cheese and from the French factory where the cheese had been prepared. The final group, enterohaemorrhagic *E. coli* (EHEC) also produce cytotoxins to give more severe symptoms. EHEC strains have been implicated in a number of outbreaks in nursing homes in the United States, with hamburgers the food most often involved. Ryan *et al.* (1986) described such an outbreak affecting 34 patients, 19 of whom had severe haemorrhagic colitis. A more serious disease caused by EHEC strains is haemorrhagic-uraemic syndrome, an often fatal condition in young and elderly patients characterized by anaemia and renal failure.

#### *2.3.9.2. Yersinia enterocolitica*

*Y. enterocolitica* is a small Gram negative rod which has the unusual property of being non-motile at 37°C but motile, with peritrichous

flagella, below this temperature. Another unusual feature is its ability to grow at 4°C with most strains growing down to 1°C or even below although its optimum growth temperature is probably about 33°C.

*Y. enterocolitica* has been associated with a variety of clinical conditions, particularly a form of gastroenteritis in which abdominal pain and diarrhoea are the main symptoms; less frequently observed are vomiting and a mild fever. In another enteric condition the symptoms mimic acute appendicitis and in this case fever and vomiting are more common (Nilehn, 1969).

*Y. enterocolitica* is widely distributed and can be isolated from many foodstuffs (Swaminathan *et al.*, 1982); however, only a small number of strains (i.e. serotypes) are associated with human disease. The main source of *Y. enterocolitica* is undoubtedly pigs which are known to carry the specific pathogenic strains on occasions. De Boer *et al.* (1986), in a detailed survey of a variety of foods, found the highest isolation rates on raw pork (73%), unpasteurized egg (43%), raw vegetables (43%) and raw beef (42%), with a surprisingly low recovery (10%) of *Y. enterocolitica* from raw milks, often implicated in outbreaks of yersiniosis. These authors stressed that only 4% of the isolates from foods were recognized pathogenic strains, these all being obtained from pig samples; on the other hand, Anderson (1988) reported that 25% of pig carcasses were contaminated with human pathogenic *Y. enterocolitica.*

There has been a marked increase in the number of reported cases of yersiniosis world-wide in the last decade, but this may be at least partly attributable to our greater awareness of the pathogenic nature of the organism. A very large outbreak involving 16000 people occurred in the United States in 1982 (Dirksen & Flagg, 1988); contamination was traced to a dairy in Tennessee from which outdated milk was transported, in crates, to a pig farm where the milk was unloaded. On return the crate washing proved inadequate and the crates contaminated milk carton exteriors on which the yersinias were shown to survive for up to 21 days.

Many yersinias produce a heat-stable toxin similar to that produced by ETEC strains. Toxin production by *Y. enterocolitica* has been demonstrated in milk at 25°C but it is not a common property amongst yersinias; furthermore, such production is suppressed at chill temperatures (Walker & Gilmour, 1990). It has therefore been suggested that enterotoxin production may not be important in pathogenesis (Schiemann, 1988) but our knowledge of the epidemiology of this organism is less well understood than with most food poisoning bacteria.

*2.3.9.3. Enterococci*

The enterococci, also known as the faecal streptococci, comprise two species *Streptococcus faecalis* and *S. faecium* although it is the former which has been mainly implicated in occasional food poisoning outbreaks. Both species are small (0·5–1 $\mu$m in diameter), spherical, Gram positive organisms which occur mostly in pairs or short chains. Both species contain a few strains which are motile, both exhibit a fairly wide temperature range of growth, from 10 to 45°C and both are isolated from human and other warm-blooded animal faeces.

The incubation period of the illness ranges from 2 to about 20 h and the main symptoms are vomiting, abdominal pain and diarrhoea. Foods involved in outbreaks include cheeses, various beef dishes, pasteurized canned hams, Vienna sausage, turkey products and evaporated milk. Whilst high numbers of enterococci were found in these foods, much of the evidence implicating these organisms as food poisoning agents is circumstantial. No toxins have ever been identified and attempts to reproduce the symptoms in human volunteers fed massive doses of enterococci have produced inconsistent and unconvincing results (Bryan, 1979); doubt therefore remains as to whether or not these organisms are capable of causing food poisoning.

*S. zooepidemicus*, although not an enterococcus, has been implicated in a small number of isolated incidents and in a severe food-borne outbreak in Yorkshire due to unpasteurized milk (Edwards *et al.*, 1988); of 11 persons taken to hospital 7 died and it is almost certain that others were taken ill in the area. All patients were affected with septicaemia and a proportion with meningitis. The authors concluded that this was a clear example of the perils of drinking unpasteurized milk!

*2.3.9.4. Aeromonas spp.*

The genus *Aeromonas* is composed of species that are Gram negative motile rods with, typically, a single polar flagellum. Two aeromonads, *A. hydrophila* and *A. sobria* are now recognized as potential pathogens which can cause diarrhoea in humans. *A. hydrophila* is widely distributed and is very common in water so that, in the past, it has often been assumed that infections are water-borne. However, many foods carry this bacterium and *A. sobria* to a smaller extent; Fricker & Tompsett (1989) reported carriage rates for the two aeromonads of 69% (for poultry), 21% (for both pork and raw salads), 17% (for beef) and 15% (for fish) and they concluded that both raw and cooked foods were potential sources of human infection. Both the aeromonads produce

enterotoxins and cytotoxins which are similar to those of *E. coli* but there is no clear evidence that they have a role in pathogenicity (Todd *et al.*, 1989).

It has not been demonstrated that growth of either *S. zooepidemicus* or the aeromonads in food is a necessary prerequisite for induction of the illness. Thus these organisms cannot be regarded as true food poisoning bacteria. However in the remaining illnesses described the food does act primarily as a carrier of the causative organism, growth being unnecessary. Examples of these food-borne illnesses are briefly described.

*2.3.9.5. Bacillary dysentery*

The disease known as bacillary dysentery or shigellosis is caused by *Shigella* species of which *S. sonnei* and *S. flexnuri* are the most important. Shigellas are similar to salmonellas in many respects but are non-motile and fail to produce gas from carbohydrates. They are often transmitted by means other than the ingestion of food but where food-borne shigellosis is concerned the incubation period is relatively short, some 7–36 h. The principal symptoms are diarrhoea (often heavily blood-stained), fever, nausea and abdominal cramps. Bacillary dysentery is usually caused by contaminated drinking water but, where foods have been implicated, milk and various salads are the most common vehicles. It is likely that poor personal hygiene is a contributory cause in the spread of this illness and most outbreaks have incriminated infected food handlers at some point in the chain of infection.

*2.3.9.6. Cholera*

Cholera is a disease originating in the East and its symptoms were first described many centuries ago. The causative organism, *Vibrio cholerae*, also known as *V. comma*, is a curved Gram negative rod similar to *V. parahaemolyticus*. A recent form of cholera, El Tor cholera, has become widespread in the last 30 years or so. It originated in Indonesia and spread through the Indian sub-continent into the Middle East and Africa and reached Western Europe in the early 1970s. The incubation period varies from 6–8 h up to 2 or 3 days and the illness is characterized by a severe inflammation of the intestinal tract resulting in the almost continuous discharge of liquid ('rice-water') stools containing blood and mucus. As a consequence of this fluid loss the patient becomes seriously dehydrated and death may follow if the fluid is not replaced; an enterotoxin is primarily responsible for the symptoms

although non-toxin producing strains can cause a relatively mild form of gastroenteritis. Generally cholera is a spasmodic infection transmitted by person-to-person contact but where water, in particular, is contaminated with sewage epidemics may occur. Where foods have been incriminated it has usually followed contact with contaminated water. Fish, oysters and mussels, infected by the water in which they live and which have been eaten raw or after inadequate cooking, have been responsible for outbreaks; salad foods, fruits and vegetables washed in contaminated water have also been implicated.

*2.3.9.7. Typhoid*

Typhoid fever is caused by *Salmonella typhi*, a member of the genus *Salmonella* described earlier but distinguished by its inability to produce gas from carbohydrates; in many respects, therefore, it is similar to the shigellas. Typhoid is the most severe of the diseases caused by salmonellas. After a lengthy incubation period of 7 to 21 days the disease sets in with a general feeling of malaise and during the first week the body temperature steadily rises. About the seventh to tenth day a rash appears and during the second week the fever is at its highest. Death may occur from the severity of the disease at this stage but in the less severe cases there is a gradual improvement in the third or fourth week. *S. typhi* is excreted in the faeces in large numbers during the illness and, as with salmonellosis, a carrier problem exists with patients who have recovered and with symptomless excreters. Sewage-contaminated water is the most common source of infection but chlorination of water supplies has largely eliminated this source in developed countries. The last big waterborne outbreak in the UK was in Croydon in 1937 when 431 people were infected by water that had been contaminated by a symptomless excreter repairing the mains system.

Two foods that used to be frequently incriminated in typhoid fever outbreaks were raw milk and ice-cream but the heat treatment regulations introduced in the UK in the 1940s overcame this source of infection. Raw milk was incriminated in an extensive outbreak in Bournemouth in 1938 when over 700 people were infected and 70 died. Ice-cream was the vehicle of infection in Aberystwyth in the same year when 210 cases were recorded. In both these examples the infection again stemmed from symptomless carriers and this stresses the problems that such people often unknowingly pose. Other foods associated with the disease have been shellfish including oysters, infected by the contaminated water in which they were living. Various canned meats have been

responsible for a number of outbreaks in more recent years in the UK, the last major one being in Aberdeen in 1964 when 515 cases were reported. The original source of the organism was a South American tin of corned beef which had been cooled in contaminated water after cooking; *S. typhi* was undoubtedly spread to other meats in the shop by handling and by equipment — this outbreak was an excellent example of the problems created by cross-contamination!

*2.3.9.8. Brucellosis*

Members of the genus *Brucella* cause brucellosis which is also known as undulant fever and Malta fever. The causative organisms, *B. abortus, B. suis* and *B. melitensis*, are all small Gram negative ovoid rods although coccal forms are common. These three species are predominant in cattle, pigs, and goats or sheep, respectively, although other animals such as horses, rabbits, chickens, dogs and cats may become infected. These animals therefore serve as a source of infection and human brucellosis almost always results from contact with infected animals or from ingestion of their products.

Clinically the incubation period lasts for 1–3 weeks and the onset of the illness is gradual with increasing fever, general weakness and pains, and chills. The fever tends to vary in intensity, often over a number of weeks or even months; hence the description of the illness as 'undulant fever'. The normal length of the illness is between 2 and 3 months but in many cases weakness lasting from the infection can persist for a year or more. The disease is widely distributed and infection usually occurs following the consumption of raw milk from infected cows or goats, or cheese prepared from similarly infected milk. At one time about 20% of dairy cows in Europe and the United States were infected with *B. abortus* but incidents of brucellosis have been substantially reduced by the introduction of techniques which have enabled the infected animal to be detected and removed from the herd. Another important factor contributing to the decrease is the general consumption of pasteurized rather than raw milk, the brucellas being readily killed by this heat treatment.

## 2.4. MYCOTOXICOSES

Mycotoxicoses are caused by the ingestion of poisonous metabolites (mycotoxins) which are produced by fungi growing in food. The list of

fungi and the mycotoxins produced by them which may be detrimental to human health is an extensive one; much of the evidence linking mycotoxins with illness is circumstantial and only those where a clear relationship has been established will be discussed. Of these, the aflatoxins are the most important and are considered first.

### 2.4.1. Aflatoxins

In 1960 in the UK a mouldy peanut meal fed to turkeys produced a loss of appetite and general weakness followed by death within a week; because the cause was unknown it was called 'turkey X disease'. The feed was found to be infected with the mould *Aspergillus flavus* which had formed a poisonous toxin subsequently given the name 'aflatoxin' in view of its origin. Almost simultaneously there were similar diseases involving ducklings in Kenya and Uganda, and infections of hatchery-reared rainbow trout in Europe and the United States. *A. flavus* contaminated feeds were found to be responsible for the incidents and toxic factors were successfully isolated and identified. There are four main aflatoxins designated $B_1$, $B_2$, $G_1$ and $G_2$ by the blue (B) or green (G) fluorescence given when viewed under a UV lamp. Chemically the four compounds are very similar and they are classed as heterocyclic compounds, or, more specifically, substituted coumarins. All are heat-resistant and prolonged heating at 100°C is necessary to destroy their potency; they are unaffected by long-term storage, remaining stable in peanut butter stored at 23°C for over two years. The structure of aflatoxin $B_1$ is given in Fig. 2.4.

It is now known that about 30% of *A. flavus* strains produce aflatoxins (mainly $B_1$ and $B_2$) and that a second species, *A. parasiticus*,

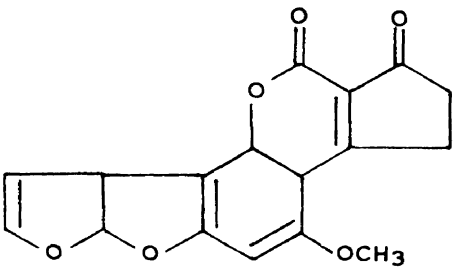


**Fig. 2.4.** Chemical structure of aflatoxin $B_1$.

produces both B and G aflatoxins. Both these species are commonly isolated from the soil and are found on living and dead plants and animals throughout the world.

*2.4.1.1. Biological Effects*

The main target organ for aflatoxins is the liver where they can cause either tissue damage or tumours. In many animal species aflatoxin is a highly active liver carcinogen and tumours may be produced by the administration of minimal amounts of aflatoxin. On the other hand, other animal species are relatively resistant to the cancer-inducing effects but may be sensitive to the acute effects. Thus the rainbow trout affected in the early outbreaks were found to have hepatomas (i.e. cancer of the liver) whereas only tissue damage was found in the livers of the turkeys (Barnes, 1970).

The main interest in aflatoxins at the present time is in determining whether or not they are a cause of hepatomas in humans and evidence is accumulating in support of this relationship (Bullerman, 1979). It has been known for many years that there is a high incidence of hepatomas in African Bantu tribes who rely to a considerable extent on fermented cereal-based foods, often mould-contaminated, as a protein source. Extensive liver cancer also occurs in many Asiatic communities. The incidence of hepatomas in Indonesia is amongst the highest in the world and a fermented peanut 'cake', a very popular food in this area, usually contains measurable amounts of aflatoxin. Many deaths occur annually amongst Thai children and the tissues have been found to contain high levels of aflatoxin. In 1974 in India there were several hundred deaths linked to the consumption of corn contaminated with aflatoxin and it is becoming increasingly apparent that the global distribution of liver carcinoma is related to the consumption of foodstuffs which support the growth of aflatoxin-producing moulds.

*2.4.1.2. Aflatoxins and Their Production in Different Foods*

Many foods have been found to support the growth of aflatoxin-producing strains of *A. flavus* and *A. parasiticus*. Various nuts, and in particular peanuts, are susceptible to infection. Studies with peanuts have shown that infection occurs after harvesting. Damaged nuts are most prone to infection and mould growth is optimal under conditions of high humidity and high temperature, conditions normally found in those areas where hepatomas are common. Nowadays great care is taken to prevent infected nuts from being included in consignments to

manufacturers. Electronic screening to eliminate discoloured and potentially infected nuts has been introduced, and many other testing and selection procedures are routinely performed. Control measures in the field include the possible use of insecticides to minimize insect damage to nuts. Storage of nuts below 5°C or in modified atmospheres (e.g. 20% $O_2$: 60% $CO_2$: 20% $N_2$; see p.124) totally suppresses aflatoxin production by the aspergilli (Paster & Bullerman, 1988). Antifungal agents such as sorbic acid, potassium sorbate and propionic acid have been shown to exert control of growth and toxin production by *A. flavus* and *A. parasiticus* (Ray & Bullerman, 1982; Bullerman *et al.*, 1984); however much of the experimental work has been performed under laboratory conditions rather than in the field where reduced efficiency of these mould inhibitors may be experienced.

There are many foods on which mould growth is common, such as cheeses, and concern has been expressed about the possibility of toxin formation in such foods. Aspergilli have been readily isolated from commercial Cheddar and Swiss cheeses, but aflatoxin-producing strains were rarely noted (Bullerman & Olivigni, 1974; Bullerman, 1976). Toxin formation was only demonstrated under laboratory conditions and no aflatoxin was demonstrated in any purchased cheese. Whilst it is possible to induce aflatoxin formation by artificially inoculating aflatoxin-producing strains into cheeses, the risk of aflatoxin being present in commercial products appears remote; nevertheless, checks must be continued in view of the occasional isolation of aflatoxin-producing strains. With regard to cheeses produced by 'starter' moulds such as Roquefort and Camembert the evidence suggests that mycotoxins, if formed, are not a health hazard in such cheeses (Scott, 1981); furthermore, aflatoxins have never been detected. Mould growth is also common on cured and fermented meats and the situation with these foods is similar to that of cheese. Aflatoxin-producing moulds have been isolated from aged cured meats (country cured hams and 'salami type' sausages) but aflatoxins and other mycotoxins have not been demonstrated in these foods (Wu *et al.*, 1974). Soya beans, various ground spices and dried fruits, rice, maize and spaghetti are other foods in which aflatoxins have been found.

In recent years concern has been expressed about the presence of hydroxylated aflatoxins in milk and milk products. These toxins are produced by lactating mothers and animals which have previously ingested foods contaminated with aflatoxin $B_1$, this compound being converted to the hydroxylated form (aflatoxin $M_1$). In underdeveloped countries

there have been reports of very young children being exposed to the effects of these toxins through their mothers' milk before weaning (Moss, 1989). In developed countries it is cows' milk and products derived from it which have been under the closest scrutiny and evidence has accumulated that milk often contains extremely low levels of aflatoxin $M_1$ (e.g. Blanco *et al.*, 1988). The efficiency of aflatoxin conversion in cows is poor; Frobish *et al.* (1986) reported that under 2% of aflatoxin $B_1$, deliberately added to grain fed to lactating animals, was converted to the hydroxylated form. Again, although many different types of cheeses can contain aflatoxin $M_1$, it is almost invariably at such low levels that no danger to human health exists. Detailed studies have been performed to determine the fate of aflatoxin $M_1$ in different cheeses but similar results have been reported. For example, Brackett & Marth (1982) used aflatoxin $M_1$ contaminated milk to prepare cheddar cheese and processed cheese slices; they found that although there were variations in toxin levels during a 1-year storage period little overall change occurred in both types of cheese. The cheese slices were prepared from the semi-matured cheddar cheese and processing included heating the cheese to 87°C, a temperature that had no effect on the toxin levels; this shows that the M forms of aflatoxin are again heat stable. This stability is again in evidence with yoghurts. When prepared from skimmed milk naturally contaminated with aflatoxin $M_1$, Wiseman & Marth (1983) demonstrated that the toxin remained stable in yoghurt over a 6-week storage period at 7°C.

Since the source of aflatoxin $M_1$ is foods contaminated with aflatoxin $B_1$, stringent standards have either been introduced or at least recommended in developed countries for animal feedingstuffs. For example, within the European Community regulations for different categories of animal food vary from 10 to 50 $\mu$g per kg maximum, specifically for aflatoxin $B_1$; there are also regulations concerning the movement of contaminated animal feeds between Member States (see Chaper 13).

In conclusion, there is little doubt that the risk of exposure to aflatoxin in the western world is remote. The most likely source is peanuts or peanut butter but control measures are extremely rigorous and apparently effective. However, peanut butter obtained from health food shops does contain low levels of aflatoxin on occasion, the absence of suitable preservatives in such foods permitting mould growth. Legislation has been recommended in the UK allowing an upper limit of 10 $\mu$g per kg for *total* aflatoxin in nuts and nut products; limits of 5–20 $\mu$g per kg have been enforced in many other countries. Unfortunately the situation in developing countries is very different and there is little doubt that much aflatoxin is

being unwittingly consumed to produce its serious long-term effects. It was even suggested (Marth & Calanog, 1976) that 'toxic metabolites of moulds constitute the single greatest food-borne hazard to human health worldwide'; this remains true today.

### 2.4.2. Miscellaneous Mycotoxins

#### *2.4.2.1. Ergot Alkaloids*

Probably the first mycotoxicosis described was ergotism, an illness resulting from the ingestion of rye and less commonly other grains infected with the mould *Claviceps purpurea*. The fungus tissue grows into a hard mass, the ergot, which contains a number of alkaloids, all derivatives of lysergic acid, which are used medicinally when purified. One of the derivatives is lysergic acid diethylamide (LSD), a hallucinatory drug. Although ergots have a medicinal value, the unpurified alkaloids are poisonous and may produce two kinds of ergotism, the gangrenous and the convulsive. Convulsive ergotism is the more common form and is characterized by a variety of symptoms including vomiting, diarrhoea, disorderly speech, convulsive fits, hallucinations and delirium with death ensuing in severe cases. Nowadays outbreaks are rare and the last recorded in the western world, which resulted in five deaths, was in France in 1951; in the UK there was a mild although extensive outbreak in Manchester in 1927.

#### *2.4.2.2. Fusarial Toxins*

Outbreaks of a mycotoxicosis called alimentary toxic aleukia (ATA) have been recorded in the USSR since the last century but there are no reports of its occurrence elsewhere. ATA is caused principally by toxin-producing strains of *Fusarium sporotrichioides* which grow in stored grains (wheat, millet and barley) held in poor conditions during winter months. Many of these strains grow down to −10°C and toxin formation is most active just above 0°C. The toxin produced, sporofusarin, is remarkably heat-stable withstanding 125°C for 30 min. There was a series of outbreaks in the USSR between 1942 and 1947, probably the result of war and its aftermath, when tens of thousands of people are thought to have died from ATA. Initially the symptoms are relatively mild and consist of vomiting and diarrhoea but after an asymptomatic period of 1–12 months the patient's condition becomes serious. The later symptoms include a severe rash, mouth and throat lesions, angina and leukaemia, and mortality can be in excess of 50%.

*2.4.2.3. Ochratoxins*

*Aspergillus ochraceus* and *Penicillium viridicatum* are able to produce ochratoxins which, chemically, are substituted coumarins like the aflatoxins. Both species have been frequently isolated from mouldy grain, particularly barley, and nuts amongst other foods and concern has been expressed about possible health hazards. It has been found that ochratoxin A, the most potent of these toxins, causes severe tissue damage in human and animal kidneys. There is evidence that a fatal chronic kidney disease, Balkan endemic nephropathy, common in rural areas of Bulgaria, Romania and Yugoslavia is caused by the consumption of cereal products which are frequently contaminated (10%) with ochratoxin A. This level of contamination is higher than that reported in other areas of the world and strongly suggests that the toxin is responsible for this disease (Pavlovic *et al.*, 1979).

*2.4.2.4. The 'Yellow Rice' Toxins*

Members of the genus *Penicillium* produce a number of mycotoxins in food, particularly rice. This commodity is liable to rapid mould spoilage in the humid, warm conditions prevailing in the East and rice that is heavily contaminated with mould appears yellow. Three species of *Penicillium* are known to produce mycotoxins in rice which are hazardous to health; these are *P. islandicum* (mycotoxins cause liver damage), *P. citrinum* (kidney damage) and *P. citreoviride* (paralysis of central nervous system). Although no large-scale outbreaks have been reported and the evidence relating to such illness remains circumstantial, the vast quantity of rice consumed together with its frequent contamination suggest that it is probably the cause of much human disease; in fact, acute cardiac beriberi, common throughout Asia, may well be linked to the consumption of 'yellow rice' toxins.

## 2.5. VIRUS FOOD POISONING

Viruses are unable to multiply in food and therefore food can only act as a vehicle for the transmission of these organisms, much in the same way that it does with typhoid fever and bacillary dysentery. Many different viruses have been isolated from foods of different kinds but their significance in the food is often poorly understood.

Viruses which infect by being ingested are termed intestinal viruses. They multiply in the intestines of the infected person and large numbers

of them (up to $10^{12}$/g of faeces) may be excreted. In view of their high degree of host specificity (Chapter 1) it is obvious that the primary source of infection in food-borne outbreaks must be of human origin. Two viruses which are important agents of human infection and which are transmitted via food are infective hepatitis and poliomyelitis.

### 2.5.1. Infective Hepatitis

Infective hepatitis, caused by hepatitis A virus, is the most common of the food-associated virus diseases; some 100 outbreaks involving many thousands of cases have been reported in the last 25 years. The most common vehicle of transmission has been shellfish (oysters and clams), previously contaminated by polluted water, which have been eaten either raw or after very light cooking. In many cases it has proved impossible to ascertain the food implicated since the disease has a lengthy incubation period, varying from 10 to 50 days, and therefore by the time an outbreak is detected the food is no longer available for examination. Many foods other than shellfish have been implicated occasionally and these include raw milk, dairy products, various cold meats and watercress, probably all contaminated initially by food handlers or polluted water (Cliver, 1979).

### 2.5.2. Poliomyelitis

It has long been recognized that poliovirus, the agent responsible for poliomyelitis, can be spread by food and in nearly every case the food implicated has been raw milk. The original source of the virus, where it was determined, proved to be man, again underlining the high degree of host specificity of these agents. One large outbreak occurred in Broadstairs, Kent, in 1927 when 62 cases were reported over a 16-day period. Children in local boarding schools contracted the illness after consuming raw milk obtained from a single dealer although the original source of the virus was never traced. Because of the introduction of pasteurization and the widespread use of vaccines, outbreaks of poliomyelitis attributable to milk are rarely reported in developed countries although pasteurized milk has been implicated in two outbreaks; this was presumably caused by improper pasteurization or post-pasteurization contamination.

### 2.5.3. Gastroenteritis

There are certain viruses, including enteroviruses and parvoviruses as well as some which are as yet uncharacterized, which cause gastroenteritis in man. In recent years there have been frequent outbreaks of gastroenteritis of this type following the consumption of raw oysters but other shellfish have also been implicated on occasions. One such outbreak was recorded as follows (DHSS, 1977):

> Shortly before Christmas the Department was informed of a number of outbreaks of food poisoning after the eating of cockles.... In an incident in one restaurant, of a large number of people who ate cockles, over 90% became ill while other people in the same restaurant who had eaten no cockles, but otherwise had the same food, remained well. Twenty to thirty hours after eating the cockles the victims developed diarrhoea, vomiting, abdominal pains and giddiness; there were no deaths but a number of cases were admitted to hospital.... The evidence suggested that all the cockles concerned came from Leigh-on-Sea in Essex and that the infection was due to a virus which had been picked up by the cockles from sewage-contaminated water and had not been destroyed in processing, due to inadequate heat treatment. Over 800 people were eventually known to have been affected.

The shellfish often grow in polluted waters but it is possible to purify them by transplanting them to clean water; in the clean water they cleanse themselves of harmful bacteria and most viruses by their normal feeding and respiratory activities.

### 2.5.4. Bovine Spongiform Encephalopathy

Bovine spongiform encephalopathy (BSE) was first described in the UK in 1987 and it has been mainly restricted to this country although there have been isolated incidents reported in Eire and France. There were 46 cases in the UK in 1987 but since that time numbers have risen steeply so that in 1991 a total of 18000 BSE cases was reported (House of Commons answer, 20 January, 1992).

BSE is an infection of cattle manifested by total loss of coordination of movement, and extreme apprehension. BSE is probably a form of scrapie, a virus-like (perhaps a prion) infection of sheep, which has been transmitted to cattle orally, that is, by the inclusion of infected sheep

brains in cattle feedingstuffs or possibly through infected grazing land. The host specificity so typical of viruses has thus been lost undermining the so-called 'species-barrier'. There is mounting evidence that the 'prion' can be transmitted from certain animals to others but vulnerability to infection appears to be extremely variable (Dealler & Lacey, 1990). Furthermore the 'prion' may change its protein structure when passing from one animal species to another and in so doing the range of animal species it can subsequently infect may alter. Thus the scrapie particle cannot infect rhesus monkeys directly from sheep but if an intermediate host (e.g. mink) is infected with the particle, the 'prion' from mink is then able to infect rhesus monkeys. It should be pointed out that many of the 'species-barrier' experiments are artificial in that the various infective particles are often inoculated by abnormal routes (e.g. massive doses directly into the brains of test animals) rather than orally.

In cattle there is a lengthy incubation period of 2–5 years during which time the scrapie-like particles spread to the brain and viscera, a prelude to the appearance of the symptoms. Thus the disease only manifests itself in adult animals, i.e. mainly dairy cows. However, it is likely that symptomless younger livestock carries the infective particle. This carriage could be due to transmission from one generation to another, but infection via pasture land is perhaps more feasible.

The fundamental question to be asked is whether a further species barrier can be breached allowing humans to become infected. A number of epidemiological studies have failed to produce evidence of any link between exposure to scrapie and Creutzfeld-Jakob disease, the form of infection in humans (Taylor, 1989); this is a progressive and fatal encephalitis with an extremely lengthy incubation period of 5–15 years. But the spectrum of animals that can be affected apparently depends on the animal the particle last infected so that conclusions drawn on scrapie to human infection may not hold true for BSE to human infection. Furthermore, infection of humans by the oral route has been clearly suggested by earlier studies on another human spongiform encephalopathy, kuru, which was common in remote parts of New Guinea earlier this century (Critchley *et al.*, 1972). This encephalopathy was apparently caused by the traditional consumption of human brains, mainly by young women, who predominantly suffered from the illness in later years. Thus the suggested preventative measures that all animal brains should be excluded from human food together with prohibition of milk and certain tissues from BSE infected cattle in such food are eminently sensible.

### 2.5.5. General Control Measures

The source of infection in food-borne virus infections is normally human faeces. Faecal contamination can occur directly by means of an infected food handler and in this case control could prove difficult. Alternatively, contamination of food can be indirect principally by means of polluted water. This source of infection can be controlled by cleansing shellfish in chlorinated or pure water, the shellfish purging themselves of the contaminating virus particles. Furthermore, most viruses are relatively heat-sensitive, being inactivated at 80°C in a few minutes, so adequate cooking can be relied upon as an additional safeguard.

## 2.6. ANIMAL TOXINS AND PARASITIC INFECTIONS

Certain freshwater and marine animals are toxic to man even when eaten fresh (natural toxicity). In addition, many marine foods may become toxic either after consuming other forms of marine life which are themselves toxic or as a result of microbial action after death (secondary toxicity). Finally, a wider range of foods, including pork and beef as well as marine foods, may be infested with animal parasites which are harmful to man and which can be readily transmitted by ingestion of the food (parasitic infections).

### 2.6.1. Animals that are Naturally Toxic to Man

#### *2.6.1.1. Puffer Fish Poisoning*

Poisonous fish are widely distributed in warm oceans but are particularly numerous in the Central and South Pacific Oceans and in the Caribbean. Probably the most poisonous are the puffer fish of which there are many different species. They are commonly found along the coasts of Japan, China and Indonesia and they have been responsible for many deaths in these countries. The toxin responsible is termed tetrodotoxin and it is found in the highest concentration in the viscera of the fish. It is fairly heat-stable and will resist boiling for 10 min. Most deaths occur following the consumption of fish roe rather than the flesh itself. The onset of symptoms is rapid occurring within a few minutes of consuming the food; the symptoms include nausea, vomiting, and numbness or a tingling sensation in the throat and limbs. Within a further short period muscular

weakness and progressive paralysis become apparent and, in over 60% of cases, this is followed by death, all within a few hours of eating the food.

*2.6.1.2. Moray Eel Poisoning*

Moray eels are large animals, about 3 m long, which inhabit tropical reefs. They are frequently caught and eaten by natives of the Far East where they have caused widespread poisoning. In Moray eel poisoning the toxins are present in the flesh of the fish and about 10% of the cases prove fatal. The symptoms are similar to puffer fish poisoning although the paralysis is normally less severe.

*2.6.1.3. Miscellaneous Poisoning*

Certain indigenous species of whelks, squids, crabs and turtles, which are eaten in certain parts of the world, are known to be inherently toxic and have caused food poisoning outbreaks.

### 2.6.2. Secondary Toxicity

*2.6.2.1. Paralytic Shellfish Poisoning (PSP)*

Certain marine dinoflagellates, a type of plankton, synthesize a toxin, saxitoxin, that is harmful to man. These toxin-containing dinoflagellates are normally present in low numbers in the sea and the toxin level is consequently low. However in certain climatic conditions planktonic blooms can occur and shellfish (oysters, mussels and clams) feeding in affected water then accumulate the toxin to such an extent that humans who consume the shellfish contract PSP. Outbreaks of PSP have been reported in many parts of the world but most commonly in North America where numerous deaths have been attributed to this form of poisoning. The symptoms of the illness generally develop within 5–30 min after ingestion of the affected food and they are again similar to, although less severe than, those associated with puffer fish poisoning. The duration of the illness is from 1 to 3 days and the mortality rate has been variously reported as between 1 and 20%. The first recorded outbreak in the UK was described (DHSS, 1969) as follows:

> On 30th May, 1968 a general practitioner with a woman patient who complained of tingling in her hands and fingers, a feeling in her mouth like a dental anaesthetic and weakness and inco-ordination of her arms and legs, sent her to hospital with a diagnosis of mussel poisoning. She had indeed cooked and eaten, 1½ hours

> before her symptoms began, some fresh mussels which were part of a large consignment gathered between 19th and 29th May. In all 78 people were affected. ... happily nobody was seriously ill, possibly because the retailer discarded the liquid in which the mussels were boiled. Samples of the mussels were examined ... to determine the concentration of toxin in the flesh of the shellfish. The toxin was shown ... to be derived from a minute dinoflagellate organism ... found in high concentration in sea water over more than 300 miles off the East Coast. Its toxin had been taken up in the muscle tissue of mussels, cockles, scallops and clams, whose method of feeding is by filtering out the plankton from sea water. The mussels concentrated the most toxin and soft clams the least.

Control measures should ensure that shellfish are not harvested in waters containing heavy growth of dinoflagellates.

*2.6.2.2. Ciguatera Poisoning*

This form of poisoning was first recognized in the West Indies. It often results from a sequence of events commencing with toxic algae commonly found in warm waters near coral reefs or sea shores. Herbivorous fish feed on the algae and they in turn are eaten by carnivorous fish such as sea bass and barracuda. It is these latter fish which typically cause ciguatera poisoning in man although over 300 species of fish have been incriminated. The symptoms, which develop between 30 min and 4 h after ingestion of the food, are, once again, similar to puffer fish poisoning except that paralysis is seen only in severe cases. The mortality rate is from 2 to 7% and in these cases death occurs within 1–24 h. It is of little importance in the UK although one case has been reported following the consumption of imported fish.

*2.6.2.3. Scombrotoxin Poisoning*

Scombrotoxin poisoning is generally regarded as a chemical intoxication resulting from eating scombroid fish, particularly mackerel and tuna; the exact causative agent has yet to be determined. Scombroid fish contain unusually large amounts of histidine, an amino acid, which is converted to histamine by certain spoilage bacteria including, of greatest significance, *Proteus morganii*. Histamine production only occurs by this means at fairly high temperatures (above 10°C) so that it is in fish stored at higher temperatures (15–25°C) that histamine accumulates rapidly (Hobbs, 1987). Symptoms, which usually appear a few minutes

after eating the food, are characteristic of histamine poisoning, and include dizziness, vomiting, diarrhoea, difficulty in swallowing and large itchy red blotches on the skin; the combination of symptoms manifested can vary in each case but they usually disappear within 8–12 h and fatalities are rare. The relationship between histamine levels and clinical symptoms is rather tenuous and there have been many cases recorded where non-scombroid fish (e.g. herring and sardines) have been implicated; thus it has been suggested that more than one causative agent may be involved (Hobbs, 1987).

It has been claimed that commercially canned scombroids are safe to eat although it is known that the toxin is heat-resistant: a major outbreak in the United States in 1973, involving 232 people who had eaten canned tuna, appears to refute the claim. Between 1979 and 1986 there were over 250 suspected incidents of scombrotoxic fish poisoning in the UK but the number has decreased substantially in the last few years suggesting that greater care is being taken over storage temperatures for fish.

### 2.6.3. Parasitic Infections

Many foods may act as carriers of parasites which can be ingested by man to initiate infections. Many of these infections have a worldwide distribution whilst others are limited to certain geographical regions. The parasites responsible for these infections are varied in character and many have complex life cycles passing through different stages in different animal hosts. They commonly exhibit a free-living stage in soil or water and an internal parasitic stage in the gut or other tissues of man and/or other animals. It is not within the scope of this book to discuss the life cycles of these parasites; more detailed information on these and other aspects can be obtained from appropriate references listed in the Bibliography. The principal parasitic infections of man are outlined in Table 2.8 and some additional information on these infections is given in the following paragraphs.

#### *2.6.3.1. Protozoa*

Amoebic dysentery, caused by *Entamoeba histolytica*, is a widespread disease but primarily associated with tropical and sub-tropical areas. It has been claimed that 10% of the world's population is affected but outbreaks in temperate zones, usually resulting from sewage-contaminated

**Table 2.8**
Principal Parasitic Infections of Man

| *Phylum* | *Species* | *Illness produced in man* | *Principal symptoms* | *Principal source of infection* | *Intermediate animal hosts* | *Number of cases in England (1980)*[a] |
|---|---|---|---|---|---|---|
| Protozoa (single-celled organisms) | *Entamoeba histolytica* | Amoebic dysentery | Variable. May be limited to diarrhoea but in severe cases ulceration of organs of the body and possible death. | Vegetables and fruits contaminated by handlers, flies etc. Contaminated water. | None | 543 |
| | *Toxoplasma gondii* | Toxoplasmosis | Hydrocephalus and blindness in children. Less severe in adults. often chronic with possible involvement of the eyes. | Undercooked or raw meats (pork, lamb, beef, poultry). Domestic cats. | Domestic cats | 790 |
| Trematodes (non-segmented flatworms, the flukes) | *Fasciola hepatica* | Fascioliasis | Inflammation, abscesses and haemorrhages in the intestines. | Watercress. | Snails and sheep or cattle | 4 |
| | *Clonorchis sinensis* | Clonorchiasis | Infections of bile duct, gall bladder and liver. | Undercooked or raw freshwater fish. | Snails and freshwater fish | 28 |
| Cestodes (segmented flat - worms, the tapeworms) | *Taenia solium* and *T. saginata* | Taeniasis | Loss of weight or more severe symptoms of vomiting, abdominal pain and, in extreme cases, death. | Undercooked pork (*T. solium*) or beef (*T. saginata*) | Pigs and cattle, respectively | 2<br>74 |
| | *Diphyllobothrium latum* | Diphyllobothriosis | Asymptomatic in 50% of cases. Diarrhoea, vomiting, dizziness and general weakness in remainder. | Undercooked or raw fish. | Freshwater fish and water-fleas | 3 |
| Nematodes (long, cylindrical unsegmented worms, the roundworms) | *Trichinella spiralis* | Trichinosis | Majority of cases asymptomatic. In others vomiting and diarrhoea in first few days. Followed by muscular pains, oedema and fever. | Undercooked pork or pork sausages. | Pigs | — |
| | *Anisakis* sp. | Anisakiasis or 'herring-worm' disease | Variable. May be limited to stomach pains and vomiting. Stomach ulcers in severe cases. | Undercooked or raw fish, often lightly salted. | Marine fish including herring | — |

[a] Communicable Disease Reports, Quartely Editions (PHLS).

drinking water, are uncommon (Table 2.8). The transmissible stage of the life cycle is termed the 'cyst' but it is not very resistant and remains viable only if kept moist. It is destroyed at 55°C and will not resist freezing for more than 24 h.

### *2.6.3.2. Toxoplasma gondii*

This is the causative organism of toxoplasmosis and can be found in a wide range of animals including wild cats, rodents, dogs, foxes, hedgehogs and birds, as well as those listed in Table 2.8. The worldwide incidence of this disease is not known but estimates put the number of cases well in excess of 1 million. In the United States infection rates in the newborn are 1 in 10000 and nowadays it is the most commonly reported parasitic infection in the UK. The transmissible stage is a cyst which is destroyed at 60°C and also by freezing. Transmission of the disease to humans is normally either by ingestion of contaminated raw or undercooked meats or through the domestic cat. The former mode can be effectively blocked by adequate cooking and the more likely method of transmission is by contamination with the infected faeces of domestic cats through direct handling of cats or cat litter.

### *2.6.3.3. Trematodes*

The fluke *Fasciola hepatica* is a common parasite of sheep and cattle and causes human infections, principally in Mediterranean and South American countries. Occasional outbreaks in Western European countries including Britain have been reported. The life cycle of this fluke is complex and includes snails as an intermediate host alternating with the above-named herbivores or man. Human cases almost always result from the contamination of watercress beds by snails infected with the fluke. The snail releases a free-living stage of the fluke which becomes encysted and in this form is ingested, with the raw watercress, by man.

Clonorchiasis, caused by *Clonorchis sinensis*, is the most important human disease caused by flukes. More than 40 species of freshwater fish may become infected by the fluke and humans are, in turn, infected by consuming inadequately cooked, salted, dried or raw fish. This practice is common in the Far East where this disease is prevalent, and it has been estimated that, in this region, some 20 million people are infected. The cysts are readily destroyed at temperatures over 50°C but they are resistant to salting and drying.

*2.6.3.4. Cestodes*

Two cestode worms are responsible for taeniasis, *Taenia solium*, the pork tapeworm, and *T. saginata*, the beef tapeworm; both have a worldwide distribution. Their life cycles are similar with man acting as the primary host. Infection results from the ingestion of undercooked or raw meats containing the cysts. Infected pork or beef is often described as 'measly'. The cyst develops by hooking onto the intestinal wall when growth of the tapeworm begins. The adult tapeworm, which may reach several metres in length, is composed of hundreds of segments which intermittently break off and are voided in the faeces. The eggs released from the segments are comparatively resistant to environmental extremes and the life cycle is completed by the infection of animals grazing in pastures which have been treated with contaminated manure. In a small proportion of cases with *T. solium* the life cycle may follow a different pathway. Humans can accidently ingest the voided eggs which later hatch in the intestines to produce oncospheres which, in turn, are carried to all parts of the body by circulating blood. The oncospheres develop into mature worms causing human cysticercosis; many cases of this form of infection associated with the eye, heart, liver, lungs and brain have been reported although cerebral cysticercosis is probably the most common. Prevention of taeniasis, and hence cysticercosis, is easily achieved by heating infected meat to 60°C or by freezing.

The third tapeworm infecting man is *Diphyllobothrium latum* which is found in a variety of freshwater fish including trout, perch and pike. Two intermediate hosts are required in the life cycle, the fish becoming infected by eating copepods (water-fleas) which are themselves infected from human sewage containing the eggs. Other fish-eating animals including dogs, cats and pigs can also be infected. Like the other tapeworms it is readily killed by heating or freezing and like them is widely distributed.

*2.6.3.5. Nematodes*

Trichinosis, caused by *Trichinella spiralis*, is probably the most important of all the food-borne parasitic infections although cases in the UK are now rare; its distribution is worldwide and many animals can act as a host and cause an infection in man although pigs are recognized as the major source of infection. The life cycle is relatively simple and, unlike many of the other parasites, the whole life cycle can be completed in one host. After ingestion of the cyst-contaminated meat, the roundworms develop in the intestine finally producing larvae which pass by

way of the blood vessels into muscles where fresh cysts are formed. Adequate cooking of pork to a temperature of 58°C will destroy the parasite; so also will freezing (-15°C for 20 days) or curing.

Anisakiasis, caused by *Anisakis* spp. and related genera, has only been recognized as a parasitic infection for the past 25 years or so. It was first described in The Netherlands when several hundred people were infected after eating raw, slightly salted herring and similar outbreaks have been reported subsequently in Japan and South America; mackerel and cod have also been implicated. Heating to 50°C, freezing, curing or marinating to a pH below 4 will kill the organisms.

## 2.7. POISONOUS PLANTS

Many plants are known to be inherently toxic to man but the majority are not normally eaten and therefore need not be considered here. Only a small number of examples will be mentioned and readers requiring more information should consult the appropriate references listed in the Bibliography.

Cultivated mushrooms have become an important part of the human diet but in Europe, in particular, many exotic edible fungi are collected and eaten. Unfortunately, poisonous mushrooms are sometimes mistaken for edible forms and food poisonings of this kind are the most frequent attributed to plants.

There are about a dozen poisonous fungi found in the UK of which *Amanita phalloides*, the death cap or death angel mushroom, is by far the most important; it is responsible for about 90% of deaths caused by poisonous fungi. *A. phalloides* contains a number of toxins (phallotoxins and amatoxins) which act on the liver and kidney causing severe tissue damage. After ingestion of the mushroom the first symptoms, vomiting, diarrhoea and severe abdominal pain, appear after 6–24 h and are followed by a rapid loss of strength and convulsions; death occurs in over 50% of cases, usually within 2–8 days. The toxins are extremely resistant to boiling and frying so that normal cooking processes afford little protection.

*Amanita muscaria*, the fly agaric or scarlet fly cap mushroom, is another of the amanitas which is poisonous to man. The toxic principle is muscarin, an alkaloid producing hallucinatory effects which are evident in the most severe cases. However, this fungus is less toxic to humans than *A. phalloides*.

Probably the next most important form of plant poisoning is that resulting from the ingestion of berries, usually by children. The dark purple berries of *Atropa belladonna*, the deadly nightshade, contain a mixture of poisonous alkaloids including atropine and the consumption of three or four berries may be sufficient to cause death, usually within 24 h. Symptoms include vomiting, a rapid heartbeat, double vision and hallucinations. *Solanum dulcamara*, the woody nightshade, forms red berries containing solanine, another alkaloid. Vomiting, diarrhoea and convulsions in the more serious cases are the principal symptoms and fatalities have been occasionally reported in children. The berries produced by bryony (*Bryonia dioica*), black nightshade (*Solanum nigrum*), wood laurel (*Daphne laureola*) and spurge olive (*Daphne mezereum*) and the seeds of the laburnum (*Laburnum anagyroides*) are amongst others that have caused fatalities in children.

The consumption of raw red kidney beans (*Phaseolus vulgaris*) was responsible for a number of food poisoning outbreaks reported in the UK in the late 1970s. Kidney beans and certain others (e.g. lima beans) contain a number of potential toxic factors of which lectins are the most important; they sensitize tissues in the small intestine leading to gastrointestinal disturbances. In the above outbreaks, following a short incubation period of 1–3 h, those affected suffered severe nausea and vomiting followed by diarrhoea at a later stage; recovery was rapid. It was reported that by eating 4–5 raw beans the symptoms can be induced (Noah *et al.*, 1980).

Finally, mention should be made of three plants that are commonly eaten by man which have poisonous parts to them. Thus: (1) the seeds of apples can be fatal if consumed in large numbers; (2) the tuber sprouts and peelings of potatoes contain solanine (see above) and can also prove fatal; and (3) the leaves and stalks of rhubarb, which contain oxalic acid, may again result in death.

## 2.8. CHEMICAL POISONING

Chemical poisoning through the ingestion of food is rare in the UK. In spite of this, foods are regularly consumed that contain chemicals that would prove harmful if ingested in substantially greater amounts. Thus there is a threshold level in man for these chemicals and provided that this level is not exceeded no harm is done as the chemicals are excreted or rendered innocuous in some other way.

The most common form of chemical poisoning is metallic poisoning; it is characterized by an extremely rapid onset of symptoms, often within a minute or two of consumption of the food, the principal features being vomiting and acute abdominal pain. In the past, metallic poisoning was relatively common but the introduction of legislation controlling the use of poisonous metals has virtually eliminated this hazard. The metals mainly implicated have been arsenic, lead, mercury, antimony, cadmium and zinc, and these will be discussed briefly. Other information including details of outbreaks of historical interest can be found in Dewberry (1959).

*Arsenic* has probably caused most metallic poisoning in humans and yet it occurs in trace amounts in marine foods. A large outbreak of arsenical food poisoning occurred in Lancashire and surrounding counties at the turn of the century when over 600 people were poisoned, 70 fatally, after drinking beer contaminated with arsenic. Until the introduction of organically based pesticides, poisonings were occasionally reported which resulted from the ingestion of inadequately washed fruits treated with arsenical sprays.

*Lead* is another metal that occurs naturally in many foods in minute quantities but it causes toxic symptoms if the threshold level is exceeded. At one time lead poisoning was not uncommon due to the use of lead in cooking utensils, food processing machinery, pipework, pottery glazes, artificial food-colouring materials and fruit sprays. However, this form of poisoning has been virtually eliminated by restrictive legislation.

Poisoning caused by *mercury* is rare but there have been one or two serious outbreaks in comparatively recent times. Mercury, discharged as effluent into the sea around Japan, was converted by bacteria into methyl mercury and deposited in the flesh of fish. Methyl mercury attacks the nervous system and in the Minamata Bay outbreak, which covered a period from 1953 to 1961, there were 48 fatalities resulting from the ingestion of contaminated fish; this form of poisoning is now known as 'Minamata disease'.

The remaining three metals are all associated with a similar poisoning sequence, i.e. the release of the toxic agent through the reaction of an acid food with a plated food utensil. Thus *antimony* caused outbreaks of food poisoning, particularly in the 1920s and 1930s, when cheap enamel-coated utensils were attacked by the acids contained in such foods as lemon juice and vinegar; in this way the antimony was dissolved out of the enamel and consumed with the food. Similarly,

*cadmium*, another metal that has been used to plate utensils in which acid foods have been held, has caused some poisoning outbreaks. Finally, *zinc*, an element that is widely distributed in foods in trace amounts, has been responsible for isolated outbreaks of metallic food poisoning which have been attributed to the use of galvanized iron vessels for the storage of fruits, e.g. apples, plums and rhubarb.

## BIBLIOGRAPHY

Fenlon, D. R. (1981). Seagulls (*Larus* spp.) as vectors of salmonellae: an investigation into the range of serotypes and numbers of salmonellae in gull faeces. *Journal of Hygiene, Cambridge,* **86**, 195–202

Franco, D. A. (1988). *Campylobacter* species: considerations for controlling a foodborne pathogen. *Journal of Food Protection*, **51**, 145–53.

Genigeorgis, C. A. (1989). Present state of knowledge on staphylococcal intoxication. *International Journal of Food Microbiology*, **9**, 327–60.

Gross, R. J. & Rowe, B. (1985). *Escherichia coli* diarrhoea. *Journal of Hygiene, Cambridge*, **95**, 531–50.

Healy, G. R. & Juranek, D. (1979). Parasitic infections. In: *Food-Borne Infections and Intoxications,* 2nd edn. Eds H. Riemann and F. L. Bryan, New York, Academic Press.

Mackey, B. M. & Bratchell, N. (1989). A review. The heat resistance of *Listeria monocytogenes. Letters in Applied Microbiology*, **9**, 89–94.

McLauchlin, J. (1987). A review. *Listeria monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in humans. *Journal of Applied Bacteriology*, **63**, 1–11.

Moreau, C. (1979). *Moulds, Toxins and Food.* Chichester, John Wiley & Sons.

Moss, M. O. (1987). Microbial food poisoning. In: *Essays in Agricultural and Food Microbiology*. Eds J. R. Norris and G. L. Pettipher, Chichester, John Wiley and Sons.

Park, R. W. A., Griffiths, P. L. & Moreno, G. S. (1991). Sources and survival of campylobacters: relevance to enteritis and the food industry. In: *Pathogens in the Environment.* Ed. B. Austin. Journal of Applied Bacteriology Symposium Supplement No. 20, 97S–106S.

Pusztai, A. (1986). The role in food poisoning of toxins and allergens from higher plants. In: *Developments in Food Microbiology—2.* Ed. R. K. Robinson, London, Elsevier Applied Science Publishers.

Roberts, D. (1982). Factors contributing to outbreaks of food poisoning in England and Wales 1970–1979. *Journal of Hygiene, Cambridge*, **89**, 491–8.

Samarajeewa, U., Sen, A. C., Cohen, M. D. & Wei, C. I. (1990). Detoxification of aflatoxins in foods and feeds by physical and chemical methods. *Journal of Food Protection*, **53**, 489–501.

Scott, H. G. (1969). Poisonous plants and animals. In: *Food-Borne Infections and Intoxications*. Ed. H. Riemann, New York, Academic Press.

Smith, J. L. (1987). *Shigella* as a foodborne pathogen. *Journal of Food Protection*, **50**, 788–801.

## REFERENCES

Abrahamsson, K., De Silva, N. N. & Molin, N. (1965). Toxin production by *Clostridium botulinum* type E, in vacuum-packed, irradiated fresh fish in relation to changes of the associated microflora. *Canadian Journal of Microbiology*, **11**, 523–9.

Anderson, J. K. (1988). Contamination of freshly slaughtered pig carcasses with human pathogenic *Yersinia enterocolitica*. *International Journal of Food Microbiology*, **7**, 193–202.

Ayres, P. A. & Barrow, G. I. (1978). The distribution of *Vibrio parahaemolyticus* in British coastal waters: report of a collaborative study 1975–6. *Journal of Hygiene, Cambridge*, **80**, 281–94.

Bailey, J. S., Fletcher, D. L. & Cox, N. A. (1989). Recovery and serotype distribution of *Listeria monocytogenes* from broiler chickens in the southeastern United States. *Journal of Food Protection*, **52**, 148–50.

Baird-Parker, A. C. (1971). Factors affecting the production of bacterial food poisoning toxins. *Journal of Applied Bacteriology*, **34**, 181–97.

Ball, A. P. *et al.* (1979). Human botulism caused by *Clostridium botulinum* type E: the Birmingham outbreak. *Quarterly Journal of Medicine*, **48** (New Series), 473–91.

Bannister, B. A. (1987). *Listeria monocytogenes* meningitis associated with eating soft cheese. *Journal of Infection*, **15**, 165–8.

Barnes, J. M. (1970). Aflatoxin as a health hazard. *Journal of Applied Bacteriology*, **33**, 285–98.

Barrell, R. A. E. (1982). Isolations of salmonellas from human, food and environmental sources in the Manchester area: 1976–1980. *Journal of Hygiene, Cambridge*, **88**, 403–11.

Barrell, R. A. E. (1987). Isolations of salmonellas from humans and foods in the Manchester area: 1981–1985. *Epidemiology and Infection*, **98**, 277–84.

Barrett, N. J. (1986). Communicable disease associated with milk and dairy products in England and Wales: 1983–1984. *Journal of Infection*, **12**, 265–72.

Beckers, H. J.(1988). Incidence of foodborne diseases in the Netherlands: annual summary 1982 and an overview from 1979 to 1982. *Journal of Food Protection*, **51**, 327–34.

Beckers, H. J., Soentoro, P. S. S. & Delfgou-van Asch, E. H. M. (1987). The occurrence of *Listeria monocytogenes* in soft cheeses and raw milk and its resistance to heat. *International Journal of Food Microbiology*, **4**, 249–56.

Bergdoll, M. S. (1970). Enterotoxins. In: *Microbial Toxins III. Bacterial Protein Toxins.* Eds T. C. Montie, S. Kadis and S. J. Ajl, New York, Academic Press.

Beumer, R. R., Cruysen, J. J. M. & Birtantie, I. R. K. (1988). The occurrence of

*Campylobacter jejuni* in raw cows' milk. *Journal of Applied Bacteriology*, **65**, 93–6.

Blanco, J. L. *et al.* (1988). Presence of aflatoxin $M_1$ in commercial ultra-high-temperature treated milk. *Applied and Environmental Microbiology*, **54**, 1622–3.

Brackett, R. E. & Marth, E. H. (1982). Fate of aflatoxin $M_1$ in cheddar cheese and in process cheese spread. *Journal of Food Protection*, **45**, 549–52.

Bradshaw, J. G. *et al.* (1987). Thermal resistance of *Listeria monocytogenes* in dairy products. *Journal of Food Protection*, **50**, 543–4.

Brouwer, R., Mertens, M. J. A., Siem, T. H. & Katchaki, J. (1979). An explosive outbreak of *Campylobacter* enteritis in soldiers. *Antonie van Leeuwenhoek*, **45**, 517–9.

Brown, D. F., Spaulding, P. L. & Twedt, R. M. (1977). Enteropathogenicity of *Vibrio parahaemolyticus* in the ligated rabbit ileum. *Applied and Environmental Microbiology*, **33**, 10–14.

Bryan, F. L. (1976). *Staphylococcus aureus*. In: *Food Microbiology: Public Health and Spoilage Aspects*. Eds M. P. Defigueiredo and D. F. Splittstoesser, Westport, Avi Publishing Co.

Bryan, F. L. (1979). Infections and intoxications caused by other bacteria. In: *Food-Borne Infections and Intoxications*, 2nd edn. Eds H. Riemann and F. L. Bryan, New York, Academic Press.

Bullerman, L. B. (1976). Examination of Swiss cheese for incidence of mycotoxin producing moulds. *Journal of Food Science*, **41**, 26–8.

Bullerman, L. B. (1979). Significance of mycotoxins to food safety and human health. *Journal of Food Protection*, **42**, 65–86.

Bullerman, L. B. & Olivigni, F. J. (1974). Mycotoxin producing potential of moulds isolated from cheddar cheese. *Journal of Food Science*, **39**, 1166–8.

Bullerman, L. B., Schroeder, L. L. & Park, K-Y. (1984). Formation and control of mycotoxins in food. *Journal of Food Protection*, **47**, 637–46.

Chan, K.-Y., Woo, M. L., Lam, L. Y. & French, G. L. (1989). *Vibrio parahaemolyticus* and other halophilic vibrios associated with seafood in Hong Kong. *Journal of Applied Bacteriology*, **66**, 57–64.

Charlton, B. R., Kinde, H. & Jensen, L. H. (1990). Environmental survey for *Listeria* species in California milk processing plants. *Journal of Food Protection*, **53**, 198–201.

Cliver, D. O. (1979). Viral infections. In: *Food-Borne Infections and Intoxications*, 2nd edn. Eds. H. Riemann and F. L. Bryan, New York, Academic Press.

Cliver, D.O. (1987). Foodborne disease in the United States, 1946–1986. *International Journal of Food Microbiology*, **4**, 269–77.

Cox, L. J. *et al.* (1989). *Listeria* spp. in food processing, non-food and domestic environments. *Food Microbiology*, **6**, 49–61.

Critchley, M., O'Leary, J. L. & Jennett, B. (1972). *Scientific Foundations of Neurology*. London, Heinemann.

D'Aoust, J-Y. (1991). Pathogenicity of foodborne *Salmonella*. *International Journal of Food Microbiology*, **12**, 17–40.

Dealler, S. F. & Lacey, R. W. (1990). Transmissable spongiform encephalopathies : the threat of BSE to man. *Food Microbiology*, **7**, 253–79.

De Boer, E., Seldam, W. M. & Oosterom, J. (1986). Characterization of *Yersinia enterocolitica* and related species isolated from foods and porcine tonsils in the Netherlands. *International Journal of Food Microbiology*, **3**, 217–224.

Dewberry, E. B. (1959). *Food Poisoning*, 4th edn, London, Hill.

DHSS (1969). *On the State of Public Health for the Year 1968*. London, HMSO.

DHSS (1974). *On the State of Public Health for the Year 1972*. London, HMSO.

DHSS (1976). *On the State of Public Health for the Year 1974*. London, HMSO.

DHSS (1977). *On the State of Public Health for the Year 1976*. London, HMSO.

DHSS (1980). *On the State of Public Health for the Year 1978*. London, HMSO.

DHSS (1987). *On the State of Public Health for the Year 1986*. London, HMSO.

Dirksen, J. & Flagg, P. (1988). Pathogenic organisms in dairy products; cause, effects and control. *Food Science and Technology Today*, **2**, 41–3.

Doyle, M. P. *et al.* (1987). Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurization. *Applied and Environmental Microbiology*, **53**, 1433–8.

Edwards, A. T., Roulson, M. & Ironside, M. J. (1988). A milk-borne outbreak of serious infection due to *Streptococcus zooepidemicus* (Lancefield Group C). *Epidemiology and Infection*, **101**, 43–51.

Evenson, M. L., Hinds, M. W., Bernstein, R. S. & Bergdoll, M. S. (1988). Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. *International Journal of Food Microbiology*, **7**, 311–6.

Fernandez Garayzabel, J. F. *et al.* (1987). Survival of *Listeria monocytogenes* in raw milk treated in a pilot plant pasteurizer. *Journal of Applied Bacteriology*, **63**, 533–7.

Fricker, C. R. & Park, R. W. A. (1989). A two-year study of the distribution of 'thermophilic' campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. *Journal of Applied Bacteriology*, **66**, 477–90.

Fricker, C. R. & Tompsett, S. (1989). *Aeromonas* spp. in foods: a significant cause of food poisoning? *International Journal of Food Microbiology*, **9**, 17–23.

Frobish, R. A. *et al.* (1986). Aflatoxin residues in milk of dairy cows after ingestion of naturally contaminated grain. *Journal of Food Protection*, **49**, 781–5.

Genigeorgis, C., Hassuneh, M. & Collins P. (1986). *Campylobacter jejuni* infection on poultry farms and its effect on poultry meat contamination during slaughtering. *Journal of Food Protection*, **49**, 895–903.

George, S. M., Lund, B. M. & Brocklehurst, T. F. (1988). The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. *Letters in Applied Microbiology*, **6**, 153–6.

Gibson, E. A. (1969). Salmonella infection in farm livestock in Britain. In: *Bacterial Food Poisoning*. Ed. J. Taylor, London, Cox and Wyman.

Gilbert, R. J. (1979). *Bacillus cereus* gastroenteritis. In: *Food-Borne Infections and Intoxications*, 2nd edn. Eds H. Riemann and F. L. Bryan, New York, Academic Press.

Gilbert, R. J. (1983). Food-borne infections and intoxications—recent trends and prospects for the future. In: *Food Microbiology: Advances and Prospects*. Eds T. A. Roberts and F. A. Skinner, London, Academic Press.

Gilbert, R. J., Stringer, M. F. & Peace, T. C. (1974). The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. *Journal of Hygiene, Cambridge*, **73**, 433–44.

Gilbert, R. J. & Taylor, A. J. (1976). *Bacillus cereus* food poisoning. In: *Microbiology in Agriculture, Fisheries and Food.* Eds F. A. Skinner and J. G. Carr, London, Academic Press.

Gitter, M., Bradley, R. & Blampied, P. H. (1980). *Listeria monocytogenes* infection in bovine mastitis. *The Veterinary Record*, **107**, 390–3.

Glass, K. A. & Doyle, M. P. (1989). Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Applied and Environmental Microbiology*, **55**, 1565–9.

Goepfert, J. M., Spira, W. M., Glatz, B. A. & Kim, H. U. (1973). Pathogenicity of *Bacillus cereus*. In: *The Microbiological Safety of Food.* Eds B. C. Hobbs and J. H. B. Christian, London, Academic Press.

Goldner, S. B., Solberg, M., Jones, S. & Post, L. S. (1986). Enterotoxin synthesis by nonsporulating cultures of *Clostridium perfringens. Applied and Environmental Microbiology*, **52**, 407–12.

Greenwood, M. H. & Hooper, W. L. (1983). Chocolate bars contaminated with *Salmonella napoli*: an infective study. *British Medical Journal*, **286**, 1394.

Greenwood, M. H., Roberts, D. & Burden, P. (1991). The occurrence of *Listeria* species in milk and dairy products: a national survey in England and Wales. *International Journal of Food Microbiology*, **12**, 197–206.

Guerrant, R. L. *et al.* (1987). Production of a unique cytotoxin by *Campylobacter jejuni. Infection and Immunity*, **55**, 2526–30.

Halpin-Dohnalek, M.I. & Marth, E. H. (1989). *Staphylococcus aureus*: production of extracellular compounds and behavior in foods—a review. *Journal of Food Protection*, **52**, 267–82.

Harrison, M. A. & Carpenter, S. L. (1989). Survival of large populations of *Listeria monocytogenes* on chicken breasts processed using moist heat. *Journal of Food Protection*, **52**, 376–8.

Harvey, J., Patterson, J. T. & Gibbs, P. A. (1982). Enterotoxigenicity of *Staphylococcus aureus* strains isolated from poultry: raw poultry carcases as a potential food-poisoning hazard. *Journal of Applied Bacteriology*, **52**, 251–8.

Harvey, R. W. S. (1973). Salmonella contaminated animal feed in relation to infection in animals and man. In: *The Microbiological Safety of Food.* Eds B. C. Hobbs and J. H. B. Christian, London, Academic Press.

Harvey, R. W. S., Price, T. H., Foster, D. W. & Griffiths, W. C. (1969). Salmonellas in sewage. A study in latent human infection. *Journal of Hygiene, Cambridge*, **67**, 517–23.

Hauschild, A. W. H., Hilsheimer, R., Weiss, K. F. & Burke, R. B. (1988). *Clostridium botulinum* in honey, syrups and dry infant cereals. *Journal of Food Protection*, **51**, 892–4.

Hayes, P. S. *et al.* (1986). Isolation of *Listeria monocytogenes* from raw milk. *Applied and Environmental Microbiology*, **51**, 438–40.

Heisick, J. E., Wagner, D. E., Nierman, M. L. & Peeler, J. T. (1989). *Listeria* spp. found on fresh market produce. *Applied and Environmental Microbiology*, **55**, 1925–7.

Hewitt, J. H. *et al.* (1986). Large outbreaks of *Clostridium perfringens* food poisoning associated with the consumption of boiled salmon. *Journal of Hygiene, Cambridge*, **97**, 71–80.

Hobbs, B. C. & Hugh-Jones, M. E. (1969). Epidemiological studies on *Salmonella senftenberg*. 1. Relations between animal foodstuff, animal and human isolations. *Journal of Hygiene, Cambridge*, **67**, 81–8.

Hobbs, G. (1976). *Clostridium botulinum* and its importance in fishery products. *Advances in Food Research*, **22**, 135–85.

Hobbs, G. (1987). Microbiology of fish. In: *Essays in Agricultural and Food Microbiology*. Eds J. R. Norris and G. L. Pettipher, Chichester, John Wiley & Sons.

Hockin, J. C. *et al.* (1989). An international outbreak of *Salmonella nima* from imported chocolate. *Journal of Food Protection*, **52**, 51–4.

Hoffman, P. S. & Blankenship, L. C. (1986). Significance of *Campylobacter* in foods. In: *Developments in Food Microbiology—2*. Ed. R. K. Robinson, London, Elsevier Applied Science Publishers.

Hooper, W. L., Barrow, G. I. & McNab, D. J. N. (1974). *Vibrio parahaemolyticus* food poisoning in Britain. *Lancet*, **1**, 1100–2.

Hopper, S. A. & Mawer, S. (1988). *Salmonella enteritidis* in a commercial laying flock. *Veterinary Record*, **123**, 351

Humphrey, T. J. & Beckett, P. (1987). *Campylobacter jejuni* in dairy cows and raw milk. *Epidemiology and Infection*, **98**, 263–9.

Humphrey, T. J., Mead , G. C. & Rowe, B. (1988). Poultry meat as a source of human salmonellosis in England and Wales. *Epidemiology and Infection*, **100**, 175–84.

International Commission on Microbiological Specifications for Foods (ICMSF) (1980). *Microbial Ecology of Foods*, Vol 2 (Food Commodities). New York, Academic Press.

Johnson, J. L., Doyle, M. P. & Cassens, R. G. (1990). *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products. A review. *Journal of Food Protection*, **53**, 81–91.

Jones, P. H. *et al.* (1981). *Campylobacter* enteritis associated with the consumption of free school milk. *Journal of Hygiene, Cambridge*, **87**, 155–62.

Jones, P. W., Collins, P., Brown, G. T. H. & Aitken, M. (1982). Transmission of *Salmonella mbandaka* to cattle from contaminated feed. *Journal of Hygiene, Cambridge*, **88**, 255–63.

Junttila, J. R., Niemela, S. I. & Hirn, J. (1988). Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic listeria. *Journal of Applied Bacteriology*, **65**, 321–7.

Lacey, R. W. & Kerr, K. G. (1989). Opinion. Listeriosis—the need for legislation. *Letters in Applied Microbiology*, **8**, 121–2.

Lammerding, A. A. *et al.* (1988). Prevalence of *Salmonella* and thermophilic *Campylobacter* in fresh pork, beef, veal and poultry in Canada. *Journal of Food Protection*, **51**, 47–52.

Lovett, J. *et al.* (1990). High-temperature short-time pasteurization inactivates *Listeria monocytogenes*. *Journal of Food Protection*, **53**, 734–8.

Lynt, R. K., Kautter, D. A. & Read, R. B. (1975). Botulism in commercially canned foods. *Journal of Milk and Food Technology*, **38**, 546–50.

MacDonald, K. L. *et al.*(1985). A multistate outbreak of gastrointestinal illness caused by enterotoxigenic *Escherichia coli* in imported semisoft cheese. *Journal of Infectious Diseases*, **151**, 716–20.

Marrier, R. *et al.* (1973). An outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported French cheese. *Lancet*, **2**, 1376–8.

Marth, E. H. & Calanog, B. G. (1976). Toxigenic fungi. In: *Food Microbiology: Public Health and Spoilage Aspects*. Eds M. P. Defigueiredo and D. F. Splittstoesser, Westport, Avi Publishing Co.

Melling, J. & Capel, B. J. (1978). Characteristics of *Bacillus cereus* emetic toxin. *FEMS Microbiology Letters*, **4**, 133–5.

Miller, I. S., Bolton, F. J. & Dawkins, H. C. (1987). An outbreak of *Campylobacter* enteritis transmitted by puppies. *Environmental Health*, **95** (July), 11–4.

Morgan, I. R., Krautil, F. L. & Craven, J. A. (1987). Effect of time in lairage on caecal and carcass contamination of slaughter pigs. *Epidemiology and Infection*, **98**, 323–30.

Moss, M. O. (1989). Mycotoxins of *Aspergillus* and other filamentous fungi. In: *Filamentous Fungi in Foods and Feeds*. Eds M. O. Moss, B. Jarvis & F. A. Skinner. Journal of Applied Bacteriology Symposium Supplement No. 18, 69S–81S.

Nakamura, M. & Schulze, J. A. (1970). *Clostridium perfringens* food poisoning. *Annual Review of Microbiology*, **24**, 359–72.

Nilehn, B. (1969). Studies on *Yersinia enterocolitica* with special reference to bacterial diagnosis and occurrence in human acute enteric disease. *Acta Pathologica et Microbiologica Scandinavica*, Supplement, **206**, 1–48.

Noah, N. D., Bender, A. E., Reaidi, G. B. & Gilbert, R. J. (1980). Food poisoning from raw red kidney beans. *British Medical Journal*, **281**, 236–7.

Notermans, S. & van Otterdijk, R. L. M. (1985). Production of enterotoxin A by *Staphylococcus aureus* in food. *International Journal of Food Microbiology*, **2**, 145–9.

Olsvik, Ø., Wasteson, Y., Lund, A. & Hornes, E. (1991). Pathogenic *Escherichia coli* found in food. *International Journal of Food Microbiology*, **12**, 103–14.

O'Mahony, M. *et al.* (1990). An outbreak of foodborne botulism associated with contaminated hazelnut yoghurt. *Epidemiology and Infection*, **104**, 389–95.

Oosterom, J. (1991). Epidemiological studies and proposed preventive measures in the fight against human salmonellosis. *International Journal of Food Microbiology*, **12**, 41–52.

Ormay, L. & Novotny, T. (1969). The significance of *Bacillus cereus* food poisoning in Hungary. In: *The Microbiology of Dried Foods*. Eds E. H. Kampelmacher, M. Ingram and D. A. A. Mossel, Bilthoven, International Association of Microbiological Societies.

Paster, N. & Bullerman, L. B. (1988). Mould spoilage and mycotoxin formation in grains as controlled by physical means. *International Journal of Food Microbiology*, **7**, 257–65.

Pavlovic, M., Plestina, R. & Krogh, P. (1979). Ochratoxin A contamination of foodstuffs in an area with Balkan (endemic) nephropathy. *Acta Pathologica et Microbiologica Scandinavica*, **87B**, 243–6.

Perales, I. & Audicana, A. (1989). The role of hens' eggs in outbreaks of salmonellosis in north Spain. *International Journal of Food Microbiology*, **8**, 175–80.

PHLS Report. (1989). Memorandum of evidence to the Agriculture Committee inquiry on salmonella in eggs. *PHLS Microbiology Digest*, **6**, 1–9.

PHLS Working Group, Scovgaard, N. & Nielsen, B. B. (1972). Salmonellas in pigs and animal feeding stuffs in England and Wales and in Denmark. *Journal of Hygiene, Cambridge*, **70**, 127–40.

Pini, P. N. & Gilbert, R. J. (1988). The occurrence in the UK of *Listeria* species in raw chickens and soft cheeses. *International Journal of Food Microbiology*, **6**, 317–26.

Porter, I. A. & Reid, T. M. S. (1980). A milk-borne outbreak of *Campylobacter* infection. *Journal of Hygiene, Cambridge*, **84**, 415–19.

Ratnam, S. & March, S. B. (1986). Laboratory studies on salmonella—contaminated cheese involved in a major outbreak of gastroenteritis. *Journal of Applied Bacteriology*, **61**, 51–6.

Ray, L. L. & Bullerman, L. B. (1982). Preventing growth of potentially toxic molds using antifungal agents. *Journal of Food Protection*, **45**, 953–63.

Reichart, C. A. & Fung, D. Y. C. (1976). Thermal inactivation and subsequent reactivation of staphylococcal enterotoxin B in selected liquid foods. *Journal of Milk and Food Technology*, **39**, 516–20.

Reilly, W. J. *et al.* (1988). Poultry-borne salmonellosis in Scotland. *Epidemiology and Infection*, **101**, 115–22.

Riemann, H. (1969). Botulism-types A, B and F. In: *Food-Borne Infections and Intoxications*. Ed. H. Riemann, New York, Academic Press.

Riordan, T. *et al.* (1985). An outbreak of food-borne enterotoxigenic *Escherichia coli* diarrhoea in England. *Journal of Infection*, **11**, 167–71.

Roberts, D. (1972). Observations on procedures for thawing and spit-roasting frozen dressed chickens, and post-cooking care and storage: with particular reference to food-poisoning bacteria. *Journal of Hygiene, Cambridge*, **70**, 565–88.

Robinson, D. A. (1981). Infective dose of *Campylobacter jejuni* in milk. *British Medical Journal*, **282**, 1584.

Robinson, D. A. *et al.* (1979). *Campylobacter* enteritis associated with consumption of unpasteurized milk. *British Medical Journal*, **1**, 1171–3.

Ryan, C.A. *et al.* (1986). *Escherichia coli* 0157:H7 diarrhoea in a nursing home: clinical, epidemiological, and pathological findings. *Journal of Infectious Diseases*, **154**, 631–8.

Ryser, E. T. & Marth, E. H. (1987). Fate of *Listeria monocytogenes* during the manufacture and ripening of Camembert cheese. *Journal of Food Protection*, **50**, 372–8.

Ryser, E. T., Marth, E. H. & Doyle, M. P. (1985). Survival of *Listeria monocytogenes* during manufacture and storage of cottage cheese. *Journal of Food Protection*, **48**, 746–50.

Sakaguchi, G. (1969). Botulism-type E. In: *Food-Borne Infections and Intoxications*. Ed. H. Riemann, New York, Academic Press.

Sakazaki, R. & Shimada, T. (1986). *Vibrio* species as causative agents of food-borne infection. In: *Developments in Food Microbiology—2*. Ed. R. K. Robinson, London, Elsevier Applied Science Publishers.

Scheusner, D. L. & Harmon, L. G. (1973). Growth and enterotoxin production by various strains of *Staphylococcus aureus* in selected foods. *Journal of Food Science*, **38**, 474–6

Schiemann, D. H. (1988). Examination of enterotoxin production at low temperature by *Yersinia* spp. in culture media and foods. *Journal of Food Protection*, **51**, 571–3.

Schönberg, A. (1989). Method to determine virulence of *Listeria* strains. *International Journal of Food Microbiology*, **8**, 281–4.

Scotland, S. M. (1988). Toxins. In: *Enterobacteriaceae in the Environment and as Pathogens*. Eds B. M. Lund, M. Sussman, D. Jones and M. F. Stringer. Journal of Applied Bacteriology Symposium Supplement No. 17, 109S–129S.

Scott, P. M. (1981). Toxins of *Penicillium* species used in cheese manufacture. *Journal of Food Protection*, **44**, 702–10.

Sharp, J. C. M. (1986). Milk and dairy products. *PHLS Microbiology Digest*, **3**, 28–9.

Sharp, J. C. M., Collier, P. W. & Gilbert, R. J. (1979). Food poisoning in hospitals in Scotland. *Journal of Hygiene, Cambridge*, **83**, 231–6.

Sheard, J. B. (1981). Food poisoning in England during 1979. Another year of high incidence. *Environmental Health*, **79**, 204–9.

Sheard, J. B. (1986). Food poisoning in England and Wales during 1983. A new title but still the same problems. *Environmental Health*, **94**, 57–62

Sheard, J. B. (1987). Food poisoning cases nearly double the level of 1970. *Environmental Health*, **95**, 10–15.

Skirrow, M. B. (1977). *Campylobacter* enteritis: a 'new' disease. *British Medical Journal*, **2**, 9–11.

Skjelkvale, R. & Uemura, T. (1977). Experimental diarrhoea in human volunteers following oral administration of *Clostridium perfringens* enterotoxin. *Journal of Applied Bacteriology*, **43**, 281–6.

Slade, P. J., Fistrovici, E.C. & Collins-Thompson, D. L. (1989). Persistence at source of *Listeria* spp. in raw milk. *International Journal of Food Microbiology*, **9**, 197–203.

Small, R. G. & Sharp, J. C. M. (1979). A milk-borne outbreak due to *Salmonella dublin*. *Journal of Hygiene, Cambridge*, **82**, 95–100.

Smart, J. L., Roberts, T. A., Stringer, M. F. & Shah, N. (1979). The incidence and serotypes of *Clostridium perfringens* on beef, pork and lamb carcasses. *Journal of Applied Bacteriology*, **46**, 377–83.

Smith, G. R., Milligan, R. A. & Moryson, J. (1978). *Clostridium botulinum* in aquatic environments in Great Britain and Ireland. *Journal of Hygiene, Cambridge*, **80**,431–8.

St.Louis, M.E. *et al.* (1988). The emergence of Grade A eggs as a major source of *Salmonella enteritidis* infections. *Journal of the American Medical Association*, **259**, 2103–7.

Steinbruegge, E. G., Maxcy, R. B. & Liewen, M. B. (1988). Fate of *Listeria monocytogenes* on ready to serve lettuce. *Journal of Food Protection*, **51**, 596–9.

Stevens, A. *et al.* (1989). A large outbreak of *Salmonella enteritidis* phage type 4 associated with eggs from overseas. *Epidemiology and Infection*, **103**, 425–33.

Stott, J. A., Hodgson, J. E. & Chaney, J. C. (1975). Incidence of salmonellae in animal feed and the effect of pelleting on content of Enterobacteriaceae. *Journal of Applied Bacteriology*, **39**,41–6.

Sugiyama, H. & Sofos, J. N. (1988). Botulism. In: *Developments in Food Microbiology—4*. Ed. R. K. Robinson, London, Elsevier Applied Science.

Sutton, R. G. A., Kendall, M. & Hobbs, B. C. (1972). The effect of two methods of cooking and cooling on *Clostridium welchii* and other bacteria in meat. *Journal of Hygiene, Cambridge*, **70**,415–24.

Swaminathan, B., Harmon, M. C. & Mehlman, I. J. (1982). A review: *Yersinia enterocolitica*. *Journal of Applied Bacteriology*, **52**, 151–83.

Tamminga, S. K., Beumer, R. R., Kampelmacher, E. H. & Van Leusden, F. M. (1977). Survival of *Salmonella eastbourne* in milk chocolate prepared with artificially contaminated milk powder. *Journal of Hygiene, Cambridge*, **79**, 333–7.

Taylor, D. M. (1989). Bovine spongiform encephalopathy and human health. *Veterinary Record*, **125**, 413–15.

Thompson, C. A. & Vanderzant, C. (1976). Relationship of *Vibrio parahaemolyticus* in oysters, water and sediment, and bacteriological and environmental indices. *Journal of Food Science*, **41**, 117–22.

Todd, L. S., Hardy, J. C., Stringer, M. F. & Bartholomew, B. A. (1989). Toxin production by strains of *Aeromonas hydrophila* grown in laboratory media and prawn puree. *International Journal of Food Microbiology*, **9**, 145–56.

Van Den Broek, M. J. M., Mossel, D. A. A. & Eggenkamp, A. E. (1979). Occurrence of *Vibrio parahaemolyticus* in Dutch mussels. *Applied and Environmental Microbiology*, **37**,438–42.

Van Netten, P. *et al.* (1990). Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. *Journal of Applied Bacteriology*, **69**, 73–9.

Vernon, E. (1977). Food poisoning and salmonella infections in England and Wales, 1973–75. *Public Health, London*, **91**, 225–35.

Walker, S. J. & Gilmour, A. (1990). Production of enterotoxin by *Yersinia* species isolated from milk. *Journal of Food Protection*, **53**, 751–4.

Waterman, S. C. (1982). The heat sensitivity of *Campylobacter jejuni* in milk. *Journal of Hygiene, Cambridge*, **88**, 529–33.

WHO Working Group. (1988). Food-borne listeriosis. *Bulletin of the World Health Organisation*, **66**, 421–8.

Williams, J. E. (1981). Salmonellas in poultry feeds—a worldwide review. Part I. *World's Poultry Science Journal*, **37**, 6–19.

Wiseman, D. W. & Marth, E. H. (1983). Behavior of aflatoxin $M_1$ in yogurt, buttermilk and kefir. *Journal of Food Protection*, **46**, 115–18.

Woolaway, M. C. *et al.* (1986). International outbreak of staphylococcal food poisoning caused by contaminated lasagne. *Journal of Hygiene, Cambridge*, **96**, 67–73.

Wray, C. & Sojka, W. J. (1977). Reviews of the progress of Dairy Science: bovine salmonellosis. *Journal of Dairy Research*, **44**, 383–425.

Wu, M. T., Ayres, J. C. & Koehler, P. E. (1974). Toxigenic aspergilli and penicillia isolated from aged, cured meats. *Applied Microbiology*, **28**, 1094–6.