CHAPTER 8

Non-bacterial Agents of Foodborne Illness

We have seen that foods may act as a vehicle for viable bacteria such as *Salmonella*, or for pre-formed bacterial toxins such as botulinum toxin which can cause disease or illness once introduced into the body.

Foods may also act as vehicles for other disease causing agents such as helminths, nematodes, protozoa and viruses as well as toxic metabolites of fungi and algae. Each of these is a specialist area, and cannot be dealt with in the same detail as bacteria, but a food microbiologist should be aware of the occurrence and significance of these nonbacterial agents of foodborne illness.

8.1 HELMINTHS AND NEMATODES

The flatworms and roundworms are not normally studied by microbiologists but amongst these groups there are a number of animal parasites which can be transmitted to humans *via* food and water. These complex animals do not multiply in foods and they cannot be detected and enumerated by cultural methods in the way that many bacteria can. Their presence is normally detected by direct microscopic examination often following some form of concentration and staining procedure.

8.1.1 Platyhelminths: Liver Flukes and Tapeworms

In the context of foodborne parasites the two most important classes of the Platyhelminths (flatworms) are the Trematoda, which includes the liver fluke *Fasciola hepatica*, and the Cestoda which includes tapeworms of the genus *Taenia*. These organisms have complex life cycles which may include quite unrelated hosts at different stages. Thus the mature stage of the liver fluke develops in humans, sheep or cattle which may be referred to as the definitive host (Figure 8.1). It is a leaf-like animal, growing up to two and a half centimetres in length by one centimetre in width, which

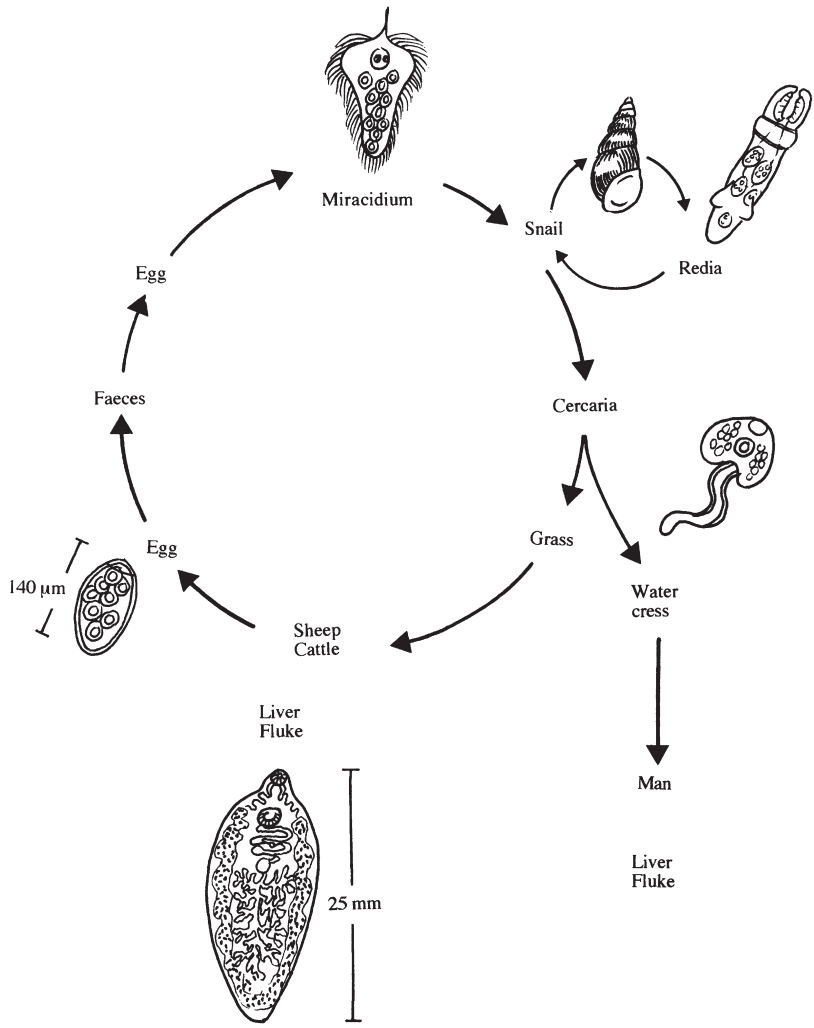

Figure 8.1 *Life cycle of the liver fluke,* Fasciola hepatica

finally establishes itself in the bile duct after entering and feeding on the liver. Having matured, it eventually produces quite large eggs ($150 \times 90\,\mu$m) which have a lid, or operculum, at one end. These are secreted in the faeces after passing from the bile duct to the alimentary tract.

The eggs hatch in water to produce a highly ciliate and motile embryo (miracidium) which cannot infect the definitive host and has to infect a species of water snail such as *Limnaea truncatula*. There are several stages in this secondary host, some leading to multiplication of the parasite, but eventually the organism is released as the final larval stage (cercaria) which encyst and may survive for up to a year depending on

their environment. Cysts will only develop further if they are swallowed by an appropriate definitive host, usually cattle or sheep in which infection can cause serious economic loss, or more rarely in humans after eating raw or undercooked watercress on which the cysts have become attached.

In people the symptoms are fever, tiredness and loss of appetite with pain and discomfort in the liver region of the abdomen. The disease is known as fascioliasis and can be diagnosed by finding the eggs in the faeces or body fluids such as biliary or duodenal fluids.

Of the Cestoda, the tapeworms *Taenia solium* associated with pork and *T. saginata* associated with beef, are best known. These long, ribbon-like flatworms have humans as their definitive host but they differ in their secondary host. The larval stages of the beef tapeworm have to develop in cattle and finally infect humans through the consumption of undercooked beef. However, the larval stages of *Taenia solium* can develop in humans or pigs. These larval stages, known as cysticerci, develop in a wide range of tissues including muscle tissue where they can cause a spotted appearance. The mature tapeworm of these species can only develop in the human intestine where it causes more severe symptoms in the young and those weakened by other diseases, than in healthy adults. The effects are fairly general and may include nausea, abdominal pain, anaemia and a nervous disorder resembling epilepsy, as well as mechanical irritation of the gut. If the latter is so severe as to cause a reversed peristalsis, so that mature segments of the tapeworm (proglottids) enter the stomach and release eggs (onchospheres), there may be an invasion of the body tissues (cysticercosis). The resulting bladder worms, or cysticerci, can especially invade the central nervous system, a situation which is often fatal. There are some species of tapeworm, such as *Diphyllobothrium latum*, which have complex life cycles involving crustacea and fish. Humans may become infected through the consumption of raw or under cooked fish.

8.1.2 Roundworms

Perhaps the most notorious of the nematodes in the context of foodborne illness, and the only one which will be dealt with here, is *Trichinella spiralis*, the agent of trichinellosis which was first recognised as a cause of illness in 1860.

This parasite has no free-living stage but is passed from host to host which can include quite a wide range of mammals including humans and pigs. Thus trichinellosis in the human population is usually acquired from the consumption of infected raw or poorly cooked pork products.

Trichinella has an intriguing life history for it is the active larval stages which cause discomfort, fever and even death. Infection starts by the

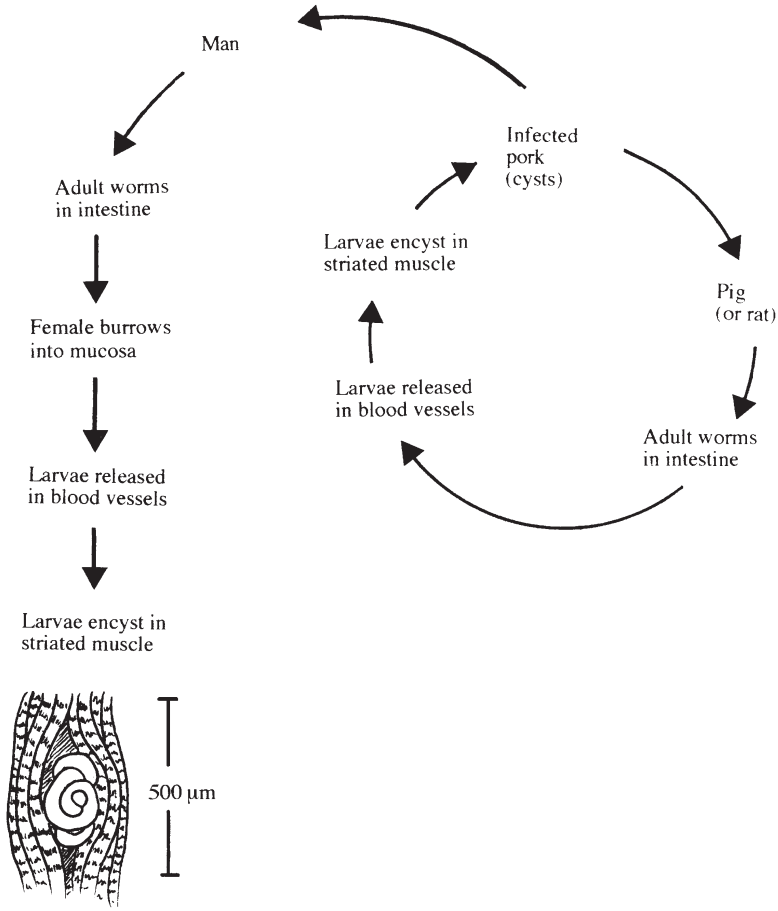

Figure 8.2 Trichinella spiralis

consumption of muscle tissue containing encysted larvae which have curled up in a characteristic manner in a cyst with a calcified wall (Figure 8.2). In this state they can survive for many years in a living host but, once eaten by a second host, the larvae are released by the digestive juices of the stomach and they grow and mature in the lumen of the intestines where they may reach 3–4 mm in length. On the assumption that uncooked human flesh is not consumed, the human host represents a dead end for nematodes such as *Trichinella*. This is unlike the situation in the Cestodes, such as *Taenia*, where passage of proglottids in human faeces can complete the cycle back to domestic animals.

The adult worms do not cause any apparent symptoms but a single female can produce more than a thousand larvae, each of which is about 100 μm long, and it is these larvae which burrow through the gut wall and eventually reach a number of specific muscle tissues in which they

grow up to about 1 mm before curling up and encysting. Such cysts were first shown to contain these tiny worm larvae by a first year medical student studying at St. Bartholomew's Hospital, London in 1835. The student was James Paget who was renowned as a Norfolk naturalist and became an eminent surgeon. He had seen, and was puzzled by, some small hard white specks in the flesh of a cadaver used in a routine post-mortem dissection.

The symptoms caused by *Trichinella spiralis* occur in two phases. The period during which the larvae are invading the intestinal mucosa is associated with abdominal pain, nausea and diarrhoea. This may occur within a few days after eating heavily infested meat or after as long as a month if only a few larval cysts are ingested. The second phase of symptoms, which include muscle pain and fever, occurs as the larvae invade and finally encyst in muscle tissue.

Prevention has to be by breaking the cycle within the pig population and by adequate cooking of pork products. The United States Department of Agriculture recommends that all parts of cooked pork products should reach at least 76.7 °C. Freezing will destroy encysted larvae but in deep tissue it may take as long as 30 days at −15 °C. Curing, smoking and the fermentations leading to such products as salami do all eventually lead to the death of encysted *Trichinella* larvae.

The control of these parasites in the human food chain is effected by careful meat inspection and the role of the professional Meat Inspector, supported by legislation is very important. Badly infected animals may be recognized by ante-mortem inspection and removed at that stage. The presence of these parasites in animals usually gives rise to macroscopic changes in tissues and organs which can be recognized by meat inspection after slaughter.

Although *T. spiralis* is the most important species of *Trichinella* several others are recognized. *T. nativa* occurs in the meat of arctic carnivores, such as polar bears and walrus, and consumption of infected meat may be responsible for trichinellosis among the Inuit people. This species is particularly resistant to low temperatures and Alaskan bear meat has been shown to be infective after 35 days at −15 °C.

8.2 PROTOZOA

Amongst the protozoa only a few genera are of special concern to the food microbiologist; the flagellate *Giardia*, the amoeboid *Entamoeba* and three sporozoid (members of the phylum Apicomplexa which contains parasitic protozoa propagated by spores) genera *Toxoplasma, Sarcocystis* and *Cryptosporidium*. Examples are known of both enteric and systemic infections.

8.2.1 *Giardia lamblia*

Although usually associated with water, or transmission from person to person by poor hygiene, a number of outbreaks of the diarrhoeal disease caused by *Giardia lamblia*, which may also be known as *G. intestinalis* or *Lamblia intestinalis*, have been confirmed as foodborne outbreaks. The organism survives in food and water as cysts but, although it can be cultured in the laboratory, it does not normally grow outside its host. The infective dose may be very low and once ingested the gastric juices aid the release of the active flagellate protozoa, known as trophozoites, which are characterized by the possession of eight flagella and two nuclei (Figure 8.3). The organism is not particularly invasive and it is not clear how the symptoms of diarrhoea, abdominal cramps and nausea are caused but it is possible that a protein toxin is involved.

Giardia cysts have been found on salad vegetables such as lettuce and fruits such as strawberries and could occur on any foods which are washed with contaminated water or handled by infected persons not observing good hygienic practice. Confirmed foodborne outbreaks have implicated home-canned salmon and noodle salad but the difficulty of demonstrating low numbers of cysts in foods may be one reason why foods are rarely directly implicated.

Although the cysts are resistant to chlorination processes used in most water treatment systems, they are killed by the normal cooking procedures used in food preparation.

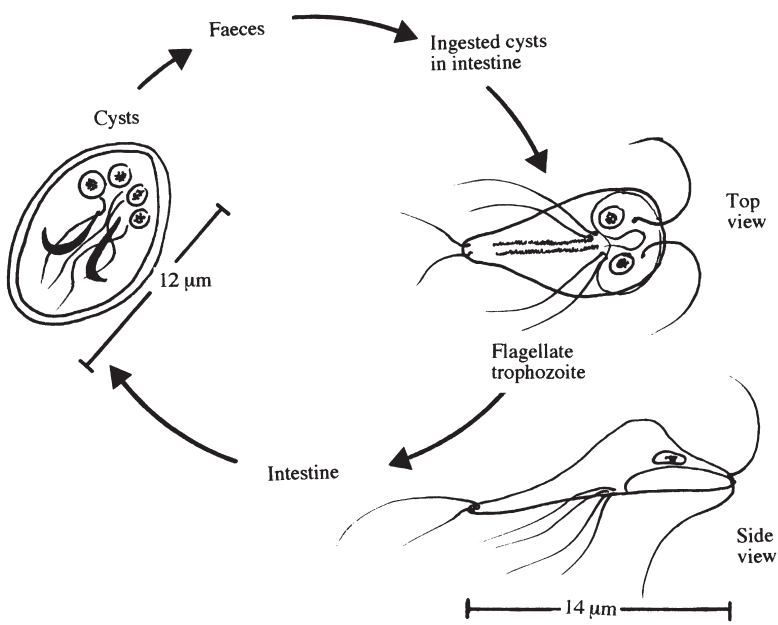

Figure 8.3 Giardia lamblia *trophozoite*

8.2.2 *Entamoeba histolytica*

Amoebic dysentery can be very widespread wherever there is poor hygiene, for it is usually transmitted by the faecal–oral route. Although outbreaks from food are also documented they are surprisingly rare. The organism is an aerotolerant anaerobe which survives in the environment in an encysted form. Indeed, a person with amoebic dysentery may pass up to fifty million cysts per day. Although most infections remain symptomless, illness may start with the passing of mucous and bloody stools, due to ulceration of the colon, a few weeks after infection and progress to severe diarrhoea, abdominal pain, fever and vomiting. *Entamoeba histolytica* infection is endemic in many poor communities in all parts of the world but there has been a steady decline in reported incidence in the United Kingdom over the past twenty years.

8.2.3 Sporozoid Protozoa

Cryptosporidiosis appears to be an increasing cause of diarrhoea and, although the disease is normally self-limiting, it can become a serious infection in the immunocompromised such as AIDS patients. It is very uncommon for food to be directly implicated in cryptosporidiosis but this may reflect the difficulties in detecting small numbers in foods and the low infective dose required to cause disease. Indirect evidence from epidemiological studies does suggest that certain foods such as raw sausages are a risk factor. However, as a waterborne threat *Cryptosporidium* can be very serious and caused what might have been the largest documented outbreak of gastrointestinal disease in America when an estimated 403,000 cases were reported in Milwaukee, Wisconsin in 1993.

Although it is complex, the whole life cycle of *Cryptosporidium parvum* can take place in a single host which may be human or a species of farm animal such as cattle or sheep. *Cryptosporidium meleagridis*, previously associated with birds, is also known to infect humans.

Cyclospora, closely related to *Cryptosporidium* in the phylum of protists known as the Alveolata, has been recognised since the early 1990s as a causative agent of a few gastrointestinal outbreaks associated with unprocessed fresh food products such as soft fruits and vegetables. Although there are several species associated with different animals, only *Cyclospora cayetanensis*, first recognised in 1977, is found in humans and seems to be restricted to the human host. Symptoms include non-bloody diarrhoea, loss of appetite, weight loss, stomach cramps, nausea, vomiting, fatigue and fever. Outbreaks occurring in North America during the late 1990s were often associated with the consumption of fresh raspberries imported from Guatemala. By contrast, species of *Sarcocystis* are obligately two-host parasites, the definitive host in which

sexual reproduction of the parasite takes place being a carnivore such as cats, dogs or humans, and an intermediate host such as cattle, sheep or pigs in the tissues of which the asexual cysts are formed.

Two species can infect humans: *S. hominis*, which infects cattle, and *S. suihominis* from pigs. Although symptoms are usually mild, they can include nausea and diarrhoea. Beef and pork which have been adequately cooked lose their infectivity.

In the case of *Toxoplasma gondii*, the definitive host is the domestic or wild cat but many vertebrate animals including humans are susceptible to infection by the oocysts shed in their faeces. Thus herbivores can become infected by eating grass and other feedstuffs contaminated by cat faeces and, once infected, their tissues may remain infectious for life. Although foodborne infection in humans may be rare, it could occur through consumption of raw or undercooked meat, especially pork or mutton.

Toxoplasmosis is usually symptomless or associated with a mild influenza-like illness in healthy humans, but infection can be serious in immunocompromised people.

8.3 TOXIGENIC ALGAE

Although strictly speaking the term algae should now be used as a collective term for a number of photosynthetic eukaryotic phyla, for the purposes of this section the prokaryotic cyanobacteria, or blue-green algae, will also be included.

A number of planktonic and benthic algae can produce very toxic compounds which may be transported to filter-feeding shellfish such as mussels and clams, or small herbivorous fish which are food for larger carnivorous fish. As these toxins pass along a food chain they can be concentrated and it may be the large carnivorous fish which are caught for human consumption which are most toxic. In the case of shellfish, the toxins may accumulate without apparently harming the animal but with potent consequences for people or birds consuming them.

A number of distinct illnesses are now recognized including PSP (paralytic shellfish poisoning), NSP (neurotoxic shellfish poisoning), DSP (diarrhoeal shellfish poisoning), ASP (amnesic shellfish poisoning) as well as ciguatera fish poisoning. The toxins implicated in the various forms of shellfish poisoning are not only undetectable organoleptically but are also generally unaffected by cooking.

8.3.1 Dinoflagellate Toxins

Planktonic dinoflagellates may occasionally form blooms containing high numbers of organisms when environmental conditions such as

temperature, light and nutrients are appropriate. *Gonyaulax catenella* and *G. tamarensis* (now both referred to the genus *Alexandrium*) are the best known of a number of dinoflagellates responsible for paralytic shellfish poisoning.

This can be a serious illness with a high mortality rate. The toxic metabolites of these algae, which include saxitoxin and gonyautoxin (Figure 8.4) block nerve transmission causing symptoms such as tingling and numbness of the fingertips and lips, giddiness and staggering, incoherent speech and respiratory paralysis. Saxitoxin, so named because it was isolated directly from the Alaskan butter clam, *Saxidomas giganteus*, before it was recognized that it is actually a dinoflagellate metabolite, is a very toxic compound having a lethal dose for the mouse of as little as 0.2 μg. Recent studies have even thrown doubt on whether saxitoxin is, in fact, a dinoflagellate metabolite for it has been shown to be produced by a species of bacteria of the genus *Moraxella* isolated from the dinoflagellates.

To control paralytic shellfish poisoning, the collection and sale of bivalves in areas affected by algal blooms is banned.

Neurotoxic shellfish poisoning is less severe and less common than paralytic shellfish poisoning and is associated with the brevitoxins, which are complex cyclic polyethers produced by the dinoflagellate *Ptychodiscus brevis* (=*Gymnodinium breve*) one of the species responsible for the phenomenon known as a 'red tide'.

A third quite different form of poisoning associated with eating shellfish which have accumulated dinoflagellates is diarrhoeal shellfish poisoning caused by lipophilic toxins such as dinophysistoxin produced by *Dinophysis fortii* (Figure 8.5). The major symptoms, which usually occur within an hour or two of consuming toxic shellfish, include diarrhoea, abdominal pain, nausea and vomiting and may persist for several days.

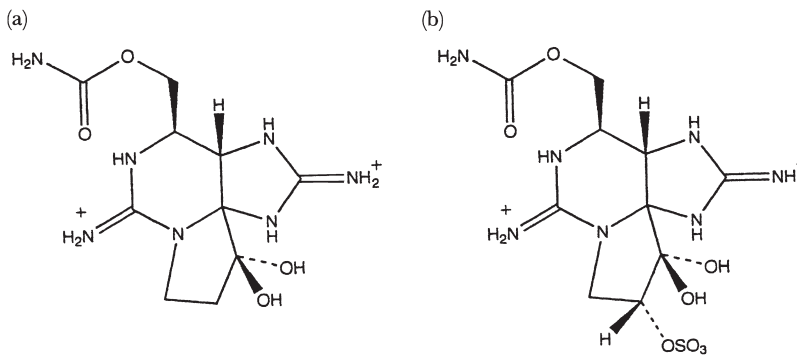

Figure 8.4 *Toxic metabolites of algae (a) saxitoxin and (b) gonyautoxin 2*

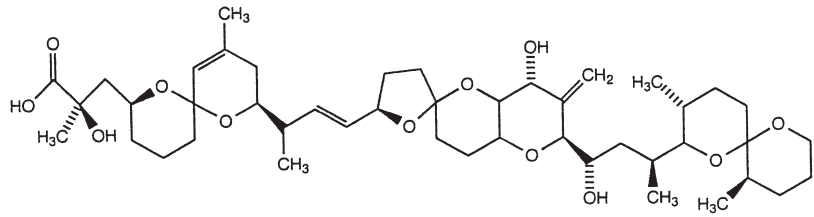

Figure 8.5 *Dinophysistoxin*

For many years a strange type of poisoning occurring after eating a number of different species of edible fish, including moray eel and barracuda, has been known as ciguatera poisoning. It can be a serious problem in tropical and subtropical parts of the world. Nausea, vomiting and diarrhoea may be accompanied by neurosensory disturbances, convulsions, muscular paralysis and even death.

After many years research it has been shown that the toxins responsible for these symptoms, which include ciguatoxin $C_{60}H_{86}O_{19}$, a polyunsaturated polycyclic ether, are produced by a benthic or epiphytic species of dinoflagellate known as *Gambierdiscus toxicus*. The toxins are concentrated along a food chain starting with the consumption of algae by herbivorous and detritus-feeding reef fish which themselves are consumed by the larger carnivorous fish caught for human consumption.

8.3.2 Cyanobacterial Toxins

Although the blue–green algae are prokaryotes, their photosynthesis, during which oxygen is liberated from water, is characteristic of that shown by the eukaryotic algae and higher plants. Several genera of freshwater cyanobacteria, especially species of *Microcystis, Anabaena* and *Aphanizomenon*, can form extensive blooms in lakes, ponds and reservoirs and may cause deaths of animals drinking the contaminated water. The presence of such cyanobacteria in public water supplies has also been implicated in outbreaks of human gastroenteritis.

The cyanoginosins, toxic metabolites of *Microcystis aeruginosa*, are cyclic polypeptides containing some very unusual amino acids (Figure 8.6) and are essentially hepatotoxins.

8.3.3 Toxic Diatoms

An outbreak of food poisoning, known as amnesic shellfish poisoning (ASP) or domoic acid poisoning, following consumption of cultivated mussels from farms in Canada, which involved more than 100 cases and three deaths, was shown to be due to a glutamate antagonist in the

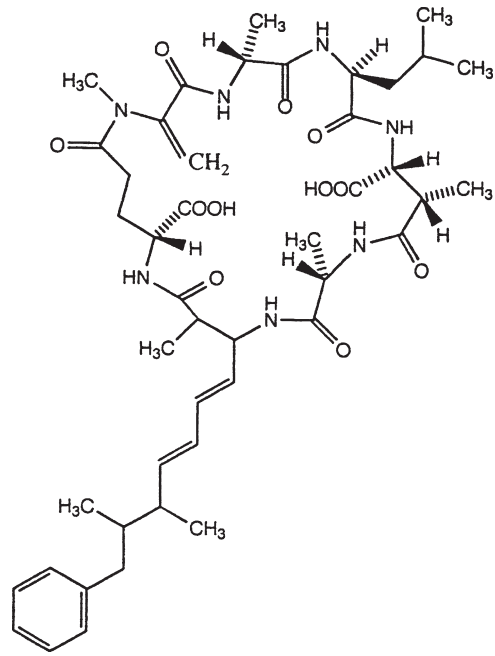

Figure 8.6 *Cyanoginosin*

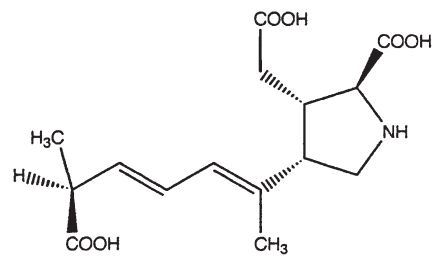

Figure 8.7 *Domoic acid*

central nervous system known as domoic acid (Figure 8.7). This compound had been produced by *Nitzschia pungens*, a chain-forming diatom of the phytoplankton which is now referred to the genus *Pseudonitzschia*.

8.4 TOXIGENIC FUNGI

The fungi are heterotrophic and feed by absorption of soluble nutrients and although many fungi can metabolize complex insoluble materials, such as lignocellulose, these materials have to be degraded by the secretion of appropriate enzymes outside the wall. A number of fungi are parasitic on both animals, plants and other fungi, and some of these

parasitic associations have become very complex and even obligate. However, it is the ability of some moulds to produce toxic metabolites, known as mycotoxins, in foods and their association with a range of human diseases, from gastroenteric conditions to cancer, which concerns us here.

The filamentous fungi grow over and through their substrate by processes of hyphal tip extension, branching and anastomosis leading to the production of an extensive mycelium. Some species have been especially successful in growing at relatively low water activities which allows them to colonize commodities, such as cereals, which should otherwise be too dry for the growth of micro-organisms.

Frequently, when moulds attack foods they do not cause the kind of putrefactive breakdown associated with some bacteria and the foods may be eaten despite being mouldy and perhaps contaminated with mycotoxins. Indeed, some of the changes brought about by the growth of certain fungi on a food may be organoleptically desirable leading to the manufacture of products such as mould-ripened cheeses and mould-ripened sausages using species of *Penicillium*.

8.4.1 Mycotoxins and Mycophagy

The vegetative structures of the filamentous fungi are essentially based on the growth form of the spreading, branching, anastomosing mycelium and have a relatively limited morphological diversity. However, the structures associated with spore production and dispersal give rise to the developmental and morphological diversity of the filamentous fungi. Many are microscopic and conveniently referred to as moulds, but amongst the basidiomycetes and ascomycetes there are species producing prodigiously macroscopic fruit bodies, the mushrooms and toadstools, which have evolved as very effective structures for the production and dispersal of spores. These two aspects of fungal morphology have led to two distinct branches in the study of fungal toxins.

The mycotoxins are metabolites of moulds which may contaminate foods, animal feeds, or the raw materials for their manufacture, and that happen to be toxic to humans or their domestic animals. The study of mycotoxins, and the legislation associated with their control, are based on them being considered as adulterants of foods or animal feeds.

On the other hand, mushrooms and toadstools have provided a traditional source of food in many parts of the world for many thousands of years. Unfortunately, this group of fungi includes a number of species which produce toxic metabolites in their fruiting bodies but, because the toxins are a natural constituent of fruiting bodies deliberately ingested, usually as a result of mistaken identity, they are not considered as mycotoxins (Table 8.1). This is a somewhat arbitrary

Table 8.1 *Toxic compounds of some 'toadstools'*

Toxin	*Species*	*Toxic effects*
Coprine	*Coprinus atramentarius*	Considerable discomfort when consumed with alcohol
Illudin	*Omphalotus olearius*	Gastrointestinal irritation Vomiting
Amatoxin	*Amanita phalloides*	Liver and kidney damage, death unless treated
Orellanin	*Cortinarius orellanus*	Irreversible kidney damage, death or very slow recovery
Psilocybin	*Psilocybe cubensis*	Hallucinogenic
Muscarine	*Inocybe patouillardii*	Vomiting and diarrhoea

distinction based on human behaviour and not on the chemistry, biochemistry or toxicology of the compounds.

There are relatively few species of agarics which can be considered as deadly poisonous but they include the deathcap, *Amanita phalloides*, a quarter of a cap of which can be lethal to a healthy adult, and species of *Cortinarius* which are still foolishly mistaken for edible wild fungi (Figure 8.8). In both these cases the toxins cause irreversible damage to the liver and kidneys and death may follow several weeks after the initial consumption of the poisonous fungi.

It is worth emphasizing a major difference between the toxic metabolites of fungi and the toxins of most of the bacteria associated with food poisoning. The former are relatively low molecular weight compounds, although their chemistry may be very complex, while the latter are macromolecules such as polypeptides, proteins or lipopolysaccharides. An exception to this generalization is an unusual bacterial food poisoning associated with a traditional food produced in parts of Indonesia; a form of tempeh is made by inoculating coconut flesh with moulds such as *Rhizopus* and *Mucor*. Occasionally the process becomes contaminated with the bacterium *Burkholderia cocovenenans*, previously known as *Pseudomonas cocovenenans*, which produces at least two low molecular weight toxic metabolites, bongkrekic acid and toxoflavin (see Chapter 9).

Although there are many genera of moulds which include toxigenic species three stand out as especially important – *Aspergillus, Penicillium* and *Fusarium*.

8.4.2 Mycotoxins of *Aspergillus*

8.4.2.1 The Aflatoxins. In 1959 a very singular event occurred which initiated the international interest which now exists in mycotoxins. This was the deaths of several thousand turkey poults and other poultry on farms in East Anglia and, because of the implications for the turkey

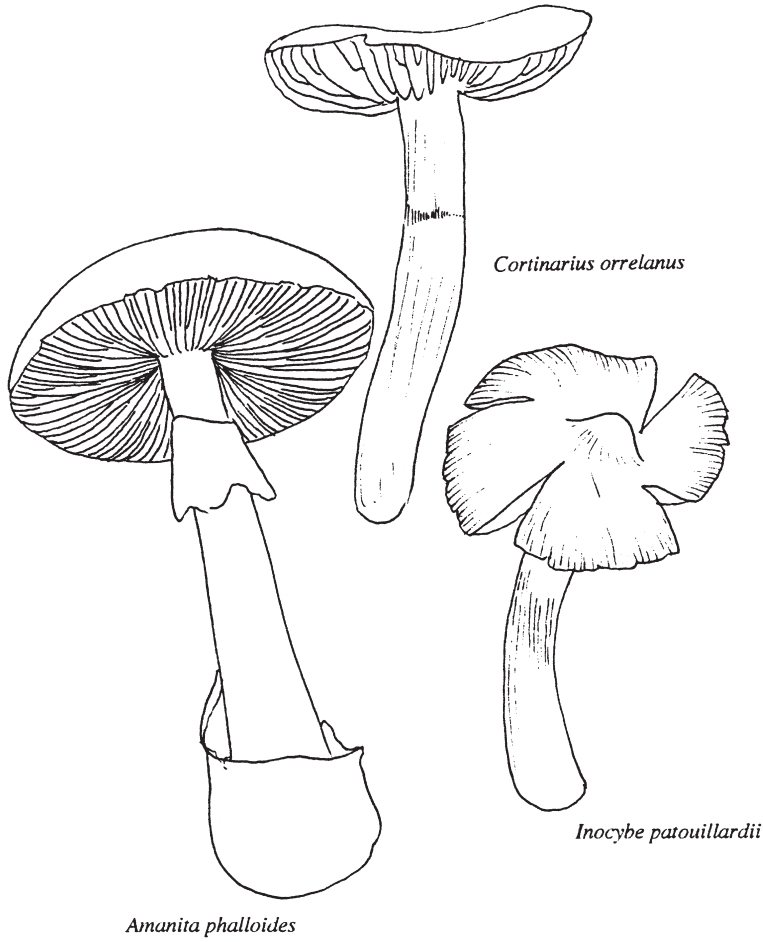

Figure 8.8 *Toxic agarics*

industry and the manufacture of pelleted feed which supported it, a considerable effort was put into understanding the etiology of this major outbreak of what was initially referred to as turkey X disease. Although the name implies a disease such as a viral infection, it was shown that the birds had been poisoned by a contaminant in the groundnut meal used as a protein supplement in the pelleted feed. The contaminant, which was called aflatoxin, fluoresces intensely under ultra-violet light and was shown to be produced by the mould *Aspergillus flavus* growing on the groundnuts.

Aflatoxin is not only acutely toxic but, for the rat, it is amongst the most carcinogenic compounds known. The demonstration of the potential carcinogenicity of aflatoxin made it possible to rationalize the etiology of diseases such as liver carcinoma in rainbow trout and

Table 8.2 *Some reports of aflatoxins during 1996 and 1997*

Commodity	*Country (year) reported*	*Incidence (%)*	*Range ($\mu g\,kg^{-1}$)*
Maize	Argentina (1996)	20	5–560
Peanuts	India (1996)	45	5–833
Pistachios	Netherlands (1996)	59	2–165
Wheat	Uruguay (1996)	20	2–20
Cottonseed meal	UK (1997)	71	5–25
Maize	India (1997)	45	5–666
Rice	Equador (1997)	9	6.8–40

From Pittet, *Revue Médecine Vétérinaire*, 1998, **149**, 479

hepatitis X in dogs which had been described nearly a decade earlier but had remained a mystery. Very sensitive analytical methods for aflatoxins were developed which led to the demonstration that their occurrence was widespread in many agricultural commodities, especially groundnuts and maize, much of which may be destined for human consumption.

Aflatoxins are still reported from a wide range of foods and animal feeds (Table 8.2) but, whereas the concentrations which cause acute toxic symptoms would be measured in $mg\,kg^{-1}$, today's analytical procedures make it possible for quantitative detection of $\mu g\,kg^{-1}$.

In 2005 the European Commission was still expressing anxiety about imports of pistachios, peanuts and brazil nuts from a number of producing countries because they were contaminated with unacceptable levels of aflatoxins. The aflatoxins are produced predominantly by two closely related species of mould, *Aspergillus flavus* and *A. parasiticus*, both of which are especially common in the tropics and subtropics. More recently three more species have been recognised as aflatoxigenic, *A. nomius, A. pseudotamarii* and *A. ochraceoroseus*, but the frequent reports in the early literature of the production of aflatoxins by other species, even belonging to different genera of moulds, are usually the result of artefacts or mistakes.

Initially, it was considered that aflatoxin contamination was essentially a problem of poor storage of commodities after harvest allowing the growth of storage fungi such as aspergilli and penicillia with consequent formation of mycotoxins. Indeed, conditions of high humidity and warm temperatures can give rise to the highest levels of aflatoxin in food often exceeding the upper limit initially established by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) of $30\,\mu g\,kg^{-1}$ in foods for human consumption. It has to be recognized that these agencies faced a hard dilemma when setting these limits and this is reflected in the observation that 'clearly the group would have preferred a lower figure, but felt that the danger of malnutrition was greater than the danger that aflatoxin would produce liver cancer in man'. Meanwhile, many developed countries had set even more

stringent legislative or guideline levels, some of the more recent of which are shown in Table 8.3.

It is now realized that aflatoxins are not simply a problem of poor storage, but they can be produced in the growing crop before harvest. Aflatoxigenic species of *Aspergillus* can establish an endophytic relationship with the healthy plant and produce low, but significant, amounts of aflatoxin when the plant is stressed, such as occurs during a drought.

Like many microbial secondary metabolites, the aflatoxins are a family of closely related compounds, the most toxic of which is referred to as aflatoxin B1 (Figure 8.9). The precise nature of the response to aflatoxin is dependent on species, sex and age, in general the male is more sensitive than the female. Some animals, such as the day-old duckling and the adult dog, are remarkably sensitive to the acute toxicity of aflatoxin B1 with LD_{50} values of 0.35 and 0.5 mg kg^{-1} body weight respectively, while others, such as the adult rat and the mouse, are more resistant (LD_{50} *ca.* 9 mg kg^{-1}). Not all animals respond to the carcinogenic activity of aflatoxin but for the rat and the rainbow trout aflatoxin B1 is one of the most carcinogenic compounds known.

Table 8.3 *Some maximum tolerated levels[a] for aflatoxin in foodstuffs*

Country	*Commodity*	*Tolerance* (μg kg^{-1})
Australia	Peanut products	15
Belgium	All foods	5
Canada	Nuts and nut products	15
China	Rice and other cereals	10
France	All foods	10
	Infant foods	1
India	All foods	30
United Kingdom	Nuts and nut products	2[a]
United States	All Foods	20

[a] On 16 July 1998 the European Commission set maximum tolerated levels for a number of food commodities (EC Regulation 1525/98 EC) and Member States were allowed a period of time to comply. UK Statutory Instrument, Contaminants in Food (Amendment) Regulations 1999 (SI No. 1999/1603) came into force on 30 June 1999 reflecting UK compliance with the EC regulation

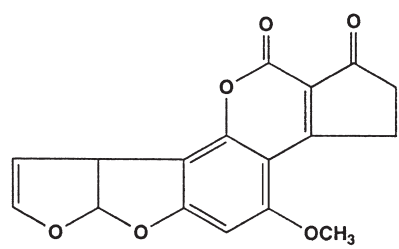

Figure 8.9 *Aflatoxin B1*

What about humans? Are they as sensitive as the dog or as resistant as the rat to the acute toxicity and does aflatoxin cause liver cancer in humans?

A particularly tragic demonstration of the acute human toxicity of aflatoxin was reported in India in 1974 when a large outbreak of poisoning occurred involving nearly 1000 people of whom nearly 100 died. From the concentrations of aflatoxins analysed in the incriminated mouldy maize it is possible to estimate that the LD_{50} of aflatoxin B1 in humans lies somewhere between that for the dog and the rat. During 2004 another large outbreak of aflatoxicosis occurred in a rural part of Kenya resulting in 317 cases and 125 deaths. Locally produced maize was shown to be the cause and a subsequent survey of 65 markets in Kenya showed that 55% of maize samples were contaminated with aflatoxin levels exceeding the Kenyan regulatory level of $20\,\mu g\,Kg^{-1}$, 35% exceeded $100\,\mu g\,Kg^{-1}$ and 7% exceeded $1{,}000\,\mu g\,Kg^{-1}$.

Although aflatoxin may be considered amongst the most carcinogenic of natural products for some animals, it is still not clear whether it is a human carcinogen. Liver cancer in some parts of the world, such as the African continent, is complex and the initial demonstration of a correlation between exposure to aflatoxin in the diet and the incidence of liver cancer has to be considered with caution. It is known that a strong correlation occurs between the presence of hepatitis B virus and primary liver cancer in humans and it now seems clear that these two agents act synergistically.

Although liver cancer may be attributable to exposure to aflatoxin in parts of Africa, it is necessary to ask why liver cancer is not also more prevalent in India where dietary exposure to aflatoxin also occurs. In India, cirrhosis of the liver is more common and there is still a lot to learn about the role of aflatoxin in liver cancer and liver damage in different parts of the world.

A diverse range of responses to the toxic effects of a compound may occur because the compound is metabolized in the animal body and the resulting toxicity is influenced by this metabolic activity. This is certainly the case with aflatoxin B1 from which a very wide range of metabolites are formed in the livers of different animal species (Figure 8.10). Thus the cow is able to hydroxylate the molecule and secrete the resulting aflatoxin M1 in the milk, hence affording a route for the contamination of milk and milk products in human foods even though these products have not been moulded.

The formation of an epoxide could well be the key to both acute and chronic toxicity and those animals which fail to produce it are relatively resistant to both. Those animals which produce the epoxide, but do not effectively metabolize it further, may be at the highest risk to the carcinogenic activity of aflatoxin B1 because the epoxide is known to

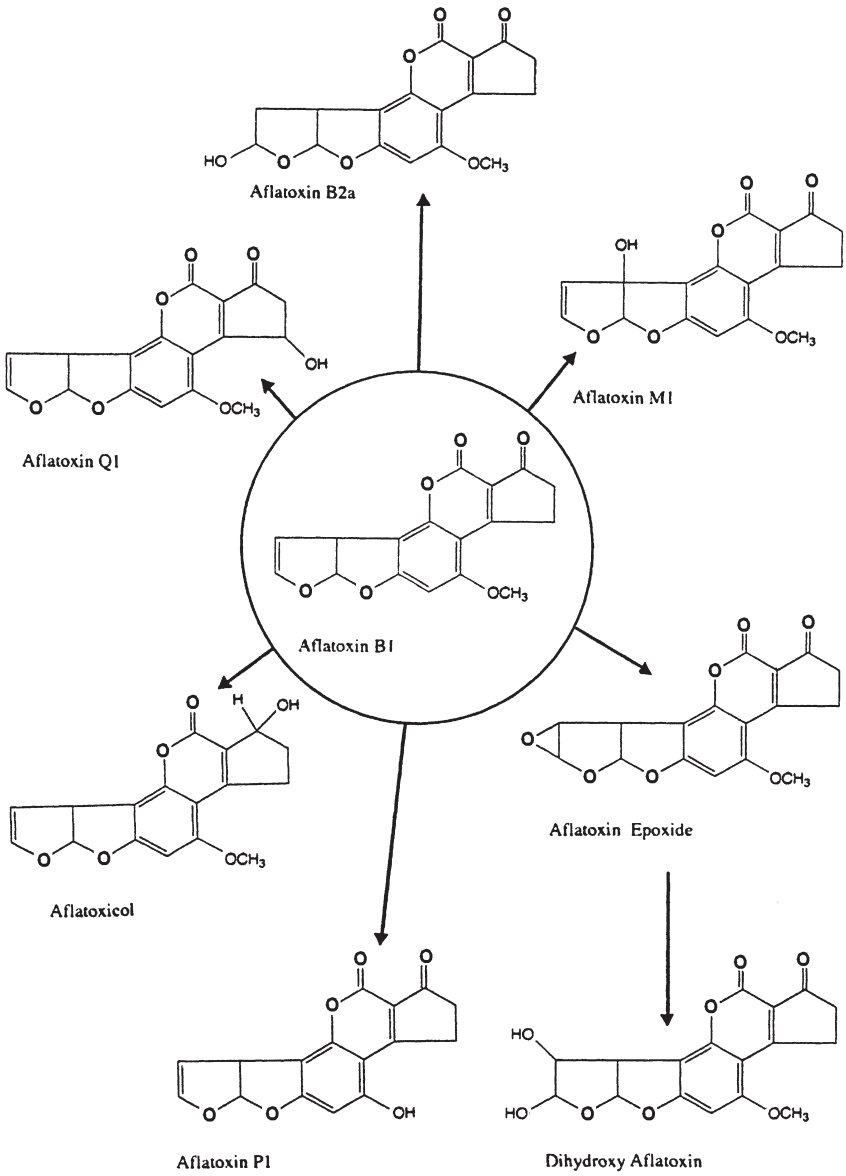

Figure 8.10 *Metabolites of aflatoxin B1*

react with DNA. Those animals which not only produce the epoxide but effectively remove it with a hydrolase enzyme, thus producing a very reactive hydroxyacetal, are most sensitive to the acute toxicity. The hydroxyacetal is known to react with the lysine residues in proteins.

It is now known that aflatoxin B1 epoxide reacts rather specifically with guanine residues of DNA at a number of hot spots, one of which is

codon 249 of the *p53* gene. The product of this gene is involved in processes which normally protect against cancer and it is known that the hepatitis B virus binds to the *p53* gene product. Thus with aflatoxin B1 and hepatitis B interacting with *p53* in different ways it is easy to see that they could act synergistically.

The parent molecule may thus be seen as a very effective delivery system having the right properties for absorption from the gut and transmission to the liver and other organs of the body. It is, however, the manner in which the parent molecule is subsequently metabolized *in vivo* which determines the precise nature of an animal's response. Information available about the metabolic activity in the human liver suggests that humans are going to be intermediate in sensitivity to the acute toxicity and may show some sensitivity to the chronic toxicity of aflatoxin B1, including carcinogenicity.

Several studies have demonstrated that very young children may be exposed to aflatoxins even before they are weaned because mothers, consuming aflatoxin in their food, may secrete aflatoxin M1 in their milk. There is no doubt about the potential danger of aflatoxin in food and every effort should be made to reduce or, if possible, eliminate contamination.

8.4.2.2 The Ochratoxins. Ochratoxin A (Figure 8.11), which is a potent nephrotoxin, was first isolated from *Aspergillus ochraceus* in South Africa, but it has been most extensively studied as a contaminant of cereals, such as barley, infected with *Penicillium verrucosum* in temperate countries such as those of northern Europe. This is because it is known to be a major aetiological agent in kidney disease in pigs and, because it is relatively stable, ochratoxin A may be passed through the food chain in meat products to humans.

A debilitating human disease known as Balkan endemic nephropathy, the epidemiology of which is still a mystery, may be associated with the presence of low levels of nephrotoxic mycotoxins, such as ochratoxin, in the diet of people who have a tradition of storing mould-ripened hams for long periods of time. The presence of ochratoxin in foods of tropical

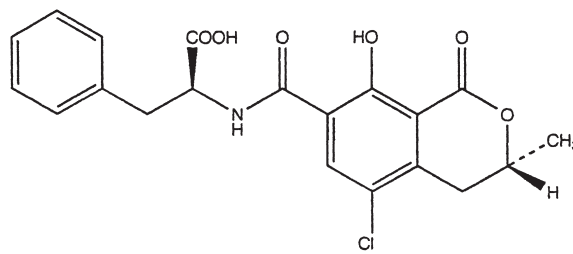

Figure 8.11 *Ochratoxin A*

and subtropical origin, such as maize, coffee beans, cocoa and soya beans is usually due to contamination by *Aspergillus* species.

It is now appreciated that ochratoxin A is quite widespread at low levels in foods and it is necessary to add wine, beer, grape juice and dried fruits (on which the black-spored *A. carbonarius* is implicated) to the list. The most recent toxicological assessment of ochratoxin indicates that it is not only an acute nephrotoxin but may also cause cancer of the kidneys and Member States of the European Union have been engaged in discussions about setting regulatory limits for this mycotoxin. Draft proposals were published by the European Commission in 1999 but it was not until 2002 that the following levels were agreed and set out in Commission Regulation (EC) No 472/2002. For raw cereal grains the maximum level is set at 5 $\mu g\,Kg^{-1}$ whereas for cereal products, and cereal grains intended for direct human consumption, a more stringent level of 3 $\mu g\,Kg^{-1}$ was set. For dried vine fruit, such as currants, raisins and sultanas, the level was set at 10 $\mu g\,Kg^{-1}$.

8.4.2.3 Other Aspergillus *Toxins.* Sterigmatocystin (Figure 8.12), a precursor in the biosynthesis of aflatoxins, is produced by a relatively large number of moulds but especially by *Aspergillus versicolor*. It is not considered to be as acutely toxic, or as carcinogenic, as aflatoxin but it is likely to be quite widespread in the environment and has been isolated from a number of human foods such as cheeses of the Edam and Gouda type which are stored in warehouses for a long period of time. In this situation the moulds grow only on the surface and sterigmatocystin does not penetrate beyond the first few millimeters below the surface.

Cyclopiazonic acid (Figure 8.13) gets its name because it was first isolated from a mould which used to be called *Penicillium cyclopium* (now known as *P. aurantiogriseum*) but it has subsequently been isolated from *Aspergillus versicolor* and *A. flavus*. In the latter, it is formed primarily in the sclerotia and there has always been a suspicion that some of the symptoms ascribed to the ingestion of food contaminated by *A. flavus* may be due to the presence of this compound as well as to the presence of aflatoxins.

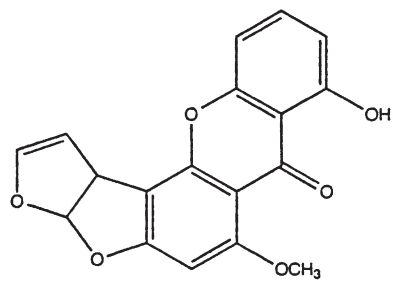

Figure 8.12 *Sterigmatocystin*

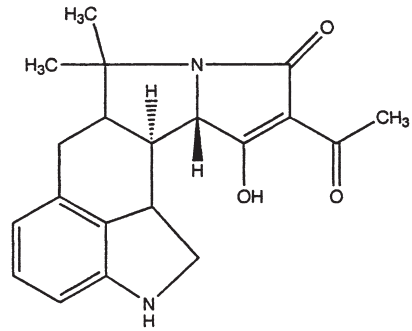

Figure 8.13 *Cyclopiazonic acid*

In parts of India a disease known as kodua poisoning occurs following the consumption of kodo millet (*Paspalum scrobiculatum*) which is both a staple food and an animal feed. *Aspergillus flavus* and *A. tamarii* have been isolated from incriminated samples of millet and both species are able to synthesize cyclopiazonic acid. Poisoning in cattle and humans is associated with symptoms of nervousness, lack of muscle co-ordination, staggering gait, depression and spasms and, in humans, sleepiness, tremors and giddiness may last for one to three days.

Some of these symptoms are reminiscent of a problem in intensively reared farm animals known as staggers in which complex indole alkaloid metabolites (tremorgens) are implicated. One of these metabolites, aflatrem, is also produced by some strains of *A. flavus*.

8.4.3 Mycotoxins of *Penicillium*

Penicillium is much more common as a spoilage mould in Europe than *Aspergillus* with species such as *P. italicum* and *P. digitatum* causing blue and green mould respectively of oranges, lemons and grapefruits, *P. expansum* causing a soft rot of apples, and several other species associated with the moulding of jams, bread and cakes. Species which have a long association with mould-ripened foods include *P. roquefortii* and *P. camembertii*, used in the mould ripened blue and soft cheeses respectively.

The mycotoxin patulin (Figure 8.14) is produced by several species of *Penicillium*, *Aspergillus* and *Byssochlamys* but is especially associated with *P. expansum* and was first described in 1942 as a potentially useful antibiotic with a wide spectrum of antimicrobial activity. It was discovered several times during screening programmes for novel antibiotics and this is reflected in the many names by which it is known including claviformin, clavicin, expansin, penicidin, mycoin, leucopin, tercinin and clavatin.

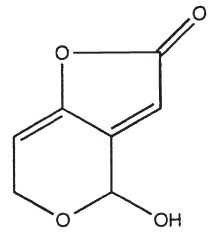

Figure 8.14 *Patulin*

It was not until 1959 that an outbreak of poisoning of cattle, being fed on an emergency ration of germinated barley malt sprouts, alerted the veterinary profession to patulin as a mycotoxin. In this instance the producing organism was *Aspergillus clavatus* but the same toxin has been implicated in several outbreaks of poisoning from such diverse materials as apples infected with *P. expansum* to badly stored silage infected with a species of *Byssochlamys*. As far as humans are concerned, it is the common association of *P. expansum* with apples, and the increasing consumption of fresh apple juice as a beverage, which has caused some concern.

Patulin is not a particularly stable metabolite. It is stable at the relatively low pH of apple juices, although it is destroyed during the fermentation of apple juice to cider. Even if there was no concern over the toxicity of patulin, the demonstration of its presence in a fruit juice is a useful indicator that very poor quality fruit has been used in its manufacture. However, at least 33 countries have now set limits on patulin in fruit and fruit juices, the most common being 50 ppb.

The nephrotoxic metabolite citrinin, produced by *P. citrinum*, was also first discovered as a potentially useful antibiotic but again rejected because of its toxicity. It is probably not as important as ochratoxin, produced by *P. verrucosum* as well as *Aspergillus ochraceus*, although it may be implicated in the complex epidemiology of 'yellow rice disease'.

8.4.3.1 Yellow Rice Disease. A complex of disorders recognized in Japan a number of times since the end of the last century has been associated with the presence of several species of penicillia and their toxic metabolites on rice. This moulded rice is usually discoloured yellow and several of the toxic metabolites implicated are themselves yellow pigments. There was an early awareness that moulds may be responsible for cardiac beriberi and in 1938 it was demonstrated that *Penicillium citreoviride* (*P. toxicarium*) and its metabolite citreoviridin were responsible. The most toxic of the species of penicillia associated with yellow rice disease is *P. islandicum* which produces two groups of toxins, hepatotoxic chlorinated cyclopeptides such as islanditoxin, as well as the much

less acutely toxic, but potentially carcinogenic, dianthraquinones such as luteoskyrin.

8.4.4 Mycotoxins of *Fusarium*

Some species of *Fusarium* cause economically devastating diseases of crop plants such as wilts, blights, root rots and cankers, and may also be involved in the post-harvest spoilage of crops in storage. The genus is also associated with the production of a large number of chemically diverse mycotoxins both in the field and in storage.

8.4.4.1 Alimentary Toxic Aleukia. Outbreaks of this dreadful disease, which is also known as septic angina and acute myelotoxicosis, occurred during famine conditions in a large area of Russia. A particularly severe outbreak occurred during the period 1942–47 but there had been reports of the disease in Russia since the 19th century.

Studies in Russia itself demonstrated that the disease was associated with the consumption of cereals moulded by *Fusarium sporotrichioides* and *F. poae* but the nature of the toxin remained unknown. Studies of dermonecrosis in cattle in the United States showed it to be caused by a *Fusarium* metabolite called T-2 toxin (Figure 8.15) which is one of the most acutely toxic of a family of compounds called trichothecenes.

There is good evidence that T-2 toxin was a major agent in the development of alimentary toxic aleukia in humans, the first symptoms of which are associated with damage of the mucosal membranes of mouth, throat and stomach followed by inflammation of the intestinal mucosa. Bleeding, vomiting and diarrhoea, which are all associated with damage of mucosal membrane systems, were common but recovery at this stage was possible if the patient was given a healthy, uncontaminated, vitamin-rich diet. Continued exposure to the toxin, however, led to damage of the bone marrow and the haematopoietic system followed by anaemia and a decrease in erythrocyte and platelet counts. The occurrence of necrotic tissue and skin haemorrhages were further characteristics of the disease.

As well as giving rise to this sequence of acute symptoms, the trichothecenes are known to be immunosuppressive and this undoubtedly contributed to victims' sensitivity to relatively trivial infectious agents. Indeed, many people died of bacterial and viral infections before succumbing from the direct effects of the toxin itself. Unlike aflatoxin, the acute toxicity of T-2 toxin is remarkably uniform over a wide range of animal species (Table 8.4) and it is reasonable to assume that the human LD_{50} will be in the same range. Although improved harvesting and storage has eliminated alimentary toxic aleukia from Russia this

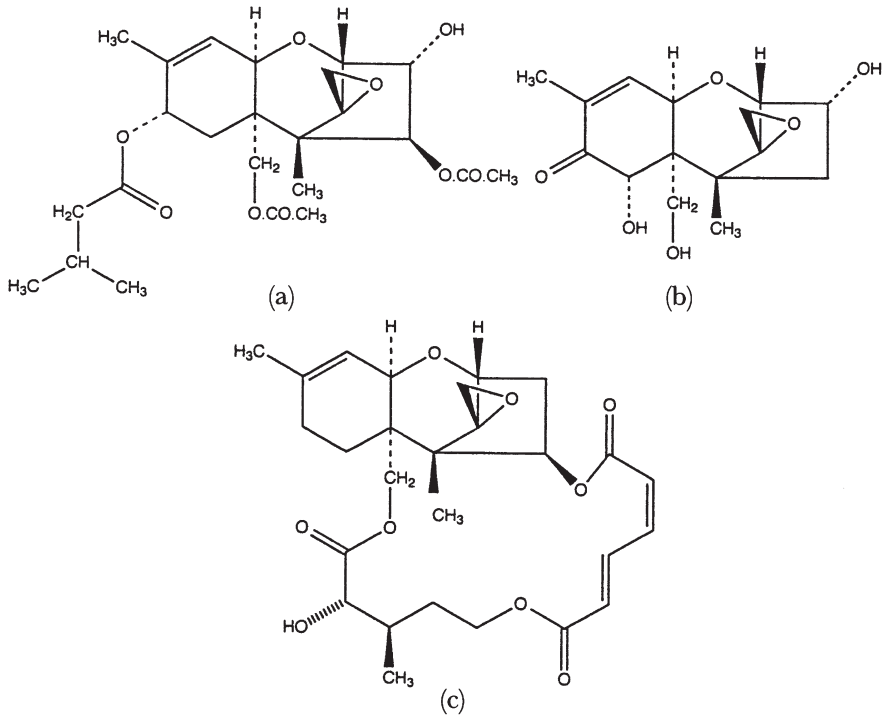

Figure 8.15 *Trichothecenes. (a) T-2 toxin, (b) deoxynivalenol, and (c) verrucarin A*

Table 8.4 *Some LD_{50} values for T-2 toxin*

Species	*LD_{50} (mg kg^{-1})*
Mouse	5.2 (intraperitoneal)
Rat	5.2 (oral)
Guinea pig	3.1 (oral)
7-Day-old chick	4.0 (oral)
Trout	6.1 (oral)

disease may still occur in any part of the world ravaged by war and famine.

Three of the most important mycotoxins, aflatoxin, ochratoxin and T-2 toxin, are immunosuppressive but react differently against the immune system. All three inhibit protein biosynthesis, aflatoxin by inhibiting transcription, ochratoxin by inhibiting phenylalanyl tRNA synthetase, and T-2 toxin by inhibiting translation through binding with a specific site on the eukaryote ribosome. One consequence of these distinct modes of activity is that mixtures of such mycotoxins are likely to be synergistic in activity and this has been shown experimentally in the case of aflatoxin and T-2 toxin. This observation is significant in the context of the probability that a food which has gone mouldy will probably be infected

by several species of mould and may thus be contaminated by several different mycotoxins.

8.4.4.2 DON and Other Trichothecenes. In Japan an illness known as red-mould disease involving nausea, vomiting and diarrhoea has been associated with the consumption of wheat, barley, oats, rye and rice contaminated by species of *Fusarium*. The species most frequently incriminated was *Fusarium graminearum*, although it had been misidentified as *F. nivale*, and the trichothecene toxins isolated from them were called nivalenol and deoxynivalenol. It is now realized that *F. nivale* itself does not produce trichothecenes at all, indeed it may not even be a *Fusarium*.

Deoxynivalenol (Figure 8.15), also known as DON and vomitoxin, was also shown to be the vomiting factor and possible feed-refusal factor in an outbreak of poisoning of pigs fed on moulded cereals in the United States. Deoxynivalenol is much less acutely toxic than T-2 toxin, having an LD_{50} of $70\,mg\,kg^{-1}$ in the mouse.

Nevertheless, it is more common than T-2 toxin especially in crops such as winter wheat and winter barley. In 1980 there was a 30–70% reduction in the yields of spring wheat harvested in the Atlantic provinces of Canada due to infections with *Fusarium graminearum* and *F. culmorum*, both of which may produce DON and zearalenone. It is not clear whether DON and other trichothecenes are as immunosuppressive as T-2 toxin but it seems prudent to reduce exposure to a minimum. Several countries have set legislative limits for DON and zearalenone in cereals and the E.C. implemented regulatory limits for the European Union in 2006. For DON they range from $200\,\mu g\,Kg^{-1}$ in processed cereal based foods for infants and ingredients used in the manufacture of food for infants to $1750\,\mu g\,Kg^{-1}$ in durum wheat and oats. For zearalenone they range from $20\,\mu g\,Kg^{-1}$ in processed cereal based products for infants to $100\,\mu g\,Kg^{-1}$ in unprocessed cereals except maize. Maximum limits for maize are likely to be implemented during 2007.

The most virulent group of trichothecenes are those with a macrocyclic structure attached to the trichothecene nucleus such as the satratoxins, verrucarins and roridins produced by *Stachybotrys atra* (Figure 8.15). This species has been implicated in a serious disease of horses, referred to as stachybotryotoxicosis, fed on mouldy hay. It seems that species of *Fusarium* do not produce such toxins.

8.4.4.3 Zearalenone. Zearalenone (Figure 8.16) is an oestrogenic mycotoxin which was first shown to cause vulvovaginitis in pigs fed on mouldy maize. Pigs are especially sensitive to this toxin and, although its acute toxicity is very low, it is common in cereals such as maize, wheat and barley being produced by *Fusarium graminearum, F. culmorum* and other species of *Fusarium*. The toxin was called zearalenone because of

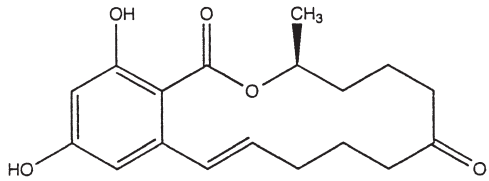

Figure 8.16 *Zearalenone*

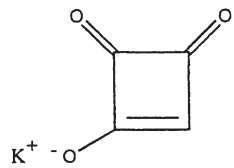

Figure 8.17 *Moniliformin*

its initial isolation from *Gibberella zeae*, the perfect stage of *F. graminearum*.

In gilts, the vulva and mammary glands become swollen and, in severe cases, there may be vaginal and rectal prolapse. In older animals there may be infertility, reduced litter size and piglets may be born weakened or even deformed. There is concern about the long-term exposure of the human population to such an oestrogen.

Zearalenone, and the corresponding alcohol zearalenol, are known to have anabolic, or growth promoting activity, and, although its use as a growth promoting agent is banned in some countries, it is permitted in others. This can lead to problems in international trade because zearalenone can be detected in the meat of animals fed on diets containing it.

8.4.4.4 Oesophageal Cancer. In parts of Northern China, and the Transkei in Southern Africa, there are regions of high incidence of human oesophageal carcinoma and the epidemiology of the disease fits the hypothesis that the consumption of moulded cereals and mycotoxins are involved. *F. moniliforme* (by the strict code of biological nomenclature this should now be called *F. verticillioides*), which belongs to a distinct group of the genus which do not produce trichothecenes, seems to be the most likely fungus to be involved. Strains of this species are associated with a disease of rice which has been a particular problem in China and other, probably distinct, strains are commonly isolated from maize grown in Southern Africa and many other parts of the world. *F. moniliforme* is a very toxigenic species and its occurrence in animal feeds is associated with outbreaks of a disease known as equine leukoencephalomalacia in horses and liver cancer in rats.

One of the first mycotoxins to be isolated during the study of these diseases was called moniliformin (Figure 8.17) because it was presumed

to have been produced by *F. moniliforme*. It is now known that moniliformin is actually produced by strains of the related species *F. subglutinans* and not *F. moniliforme*. However, it is the latter which is especially associated with human oesophageal cancer and a number of complex metabolites have been isolated and characterized from cultures of this species, including fusarin C, which is mutagenic, and the fumonisins which are carcinogenic (Figure 8.18). However it would probably be wise to be cautious about extrapolating laboratory tests demonstrating carcinogenic activity to a human disease. Nevertheless, since the discovery of the fumonisins, reported in 1988, they have become the focus of a considerable amount of interest. Once the analytical problems had been overcome it was realized that they are widespread wherever maize is grown. In a survey in the UK in 1998, 97% of the 67 samples of maize examined were found to contain fumonisins at levels ranging from 25 000–27 000 $\mu g\,kg^{-1}$. Similar levels of contamination have been found

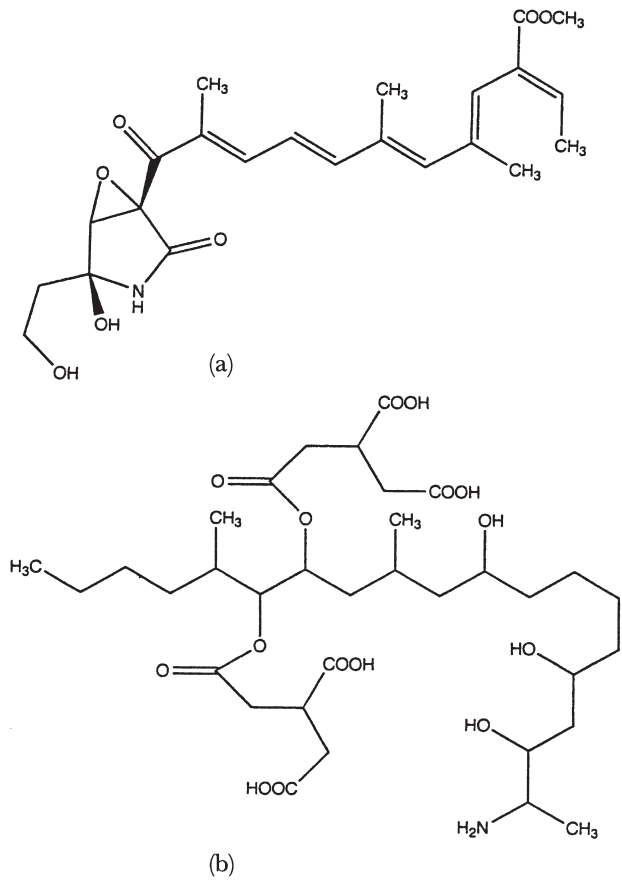

Figure 8.18 *Complex metabolites from* Fusarium moniliforme. *(a) Fusarin C and (b) fumonisin B1*

in other parts of Europe and in South America, Africa, India and the USA.

Fumonisin B1 has been confirmed to cause equine encephalomalacia, porcine pulmonary oedema, kidney damage in rodents and hepatic cancer in rats. It is known to cause apoptosis in tissue cell cultures and has cancer promoting activity in several experimental systems. At the molecular level it is a potent competitive inhibitor of ceramide synthase, blocking the biosynthesis of complex sphingolipids and leading to the accumulation of sphinganine.

Despite all of this information it is still not clear whether the fumonisins are responsible for human oesophageal carcinoma, but clearly it is important to determine its significance to human health.

8.4.5 Mycotoxins of Other Fungi

Ergotism has been documented as a human disease since the middle ages but its aetiology remained a mystery until the mid-19th century when it was demonstrated to be caused by a fungus, *Claviceps purpurea*. This fungus is a specialized parasite of some grasses including cereals and, as part of its life cycle, the tissues of infected grains are replaced by fungal mycelium to produce a tough purple brown sclerotium which is also known as an ergot because it looks like the spur of a cockerel (Figure 8.19). The biological function of the sclerotium is to survive the adverse conditions through the winter in order to germinate in the following spring. Ergots contain alkaloid metabolites which may be incorporated into the flour, and eventually the bread, made from the harvested grain.

Ergotism, or St Anthony's fire, is infrequent in human beings. The toxicity of the ergot alkaloids is now well understood and one aspect of their activity is to cause a constriction of the peripheral blood capillaries leading, in extreme cases, to fingers and toes becoming gangrenous and necrotic. Different members of this family of mould metabolites may also have profound effects on the central nervous system stimulating smooth muscle activity.

Plant–fungal interactions can be complex and there are instances where a toxic plant metabolite is produced in response to fungal attack. Thus, when the sweet potato, *Ipomoea*, is damaged by certain plant pathogens it responds by producing the phytoalexin ipomeamarone. This antifungal agent is produced to limit fungal attack but it is also an hepatotoxin to mammals. Further complexity arises when other moulds, such as *Fusarium solani*, degrade ipomeamarone to smaller molecules such as ipomenol which can cause oedema of the lung (Figure 8.20).

A disease of sheep in New Zealand known as ryegrass staggers may cause an estimated loss of hundreds of millions of dollars in some years. It is caused by an intimate association of perennial ryegrass (*Lolium*

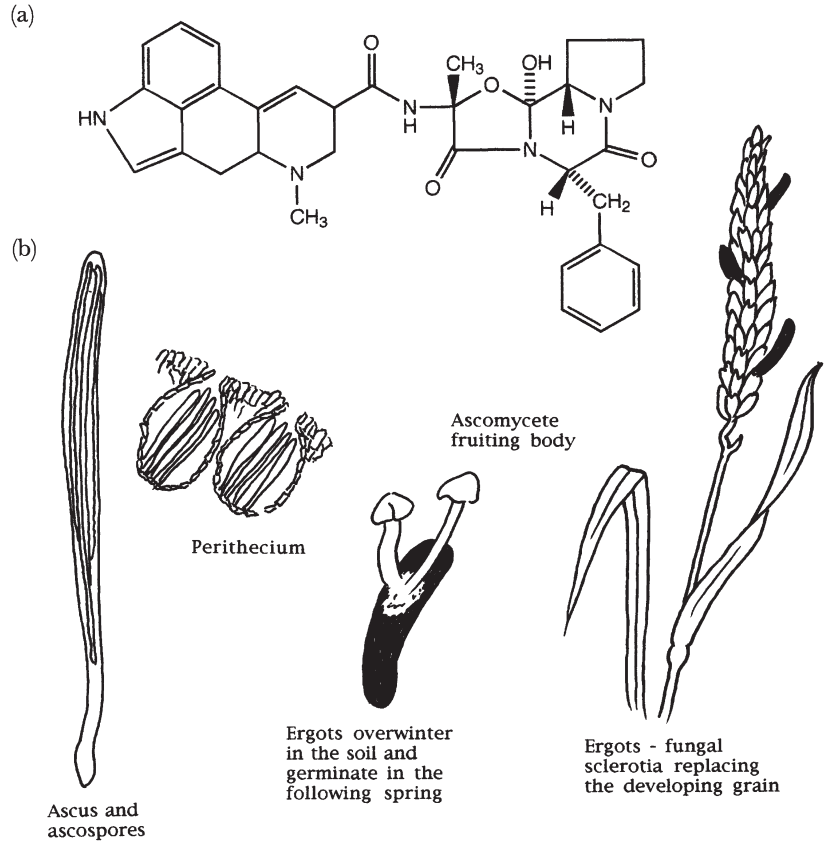

Figure 8.19 *Claviceps purpurea. (a) The alkaloid ergotamine, and (b) aspects of development*

perenne) and an endophytic fungus, *Acremonium loliae*. The endophyte–plant association results in the production of complex tremorgenic mycotoxins known as the lolitrems (Figure 8.21) which are responsible for the staggering response and possible collapse of sheep under stress. The endophyte is seedborne and completes its whole life cycle within the plant, although it can be cultured with difficulty in the laboratory.

It is possible to eliminate the endophyte by careful heat treatment of seed but, in New Zealand, the planting of endophyte-free ryegrass provides pastures which are very susceptible to insect damage such as that caused by the stem weevil *Listronotus bonariensis*. It is almost certain that the role of the endophyte in controlling insect damage is not due to the production of lolitrems so the possibility remains that a genetically engineered strain of *Acremonium loliae*, which no longer produces lolitrems, could be used to replace the wild strain in perennial ryegrass.

Although there can be no doubt about the potential for the presence of mycotoxins in food to cause illness and even death in humans, there are

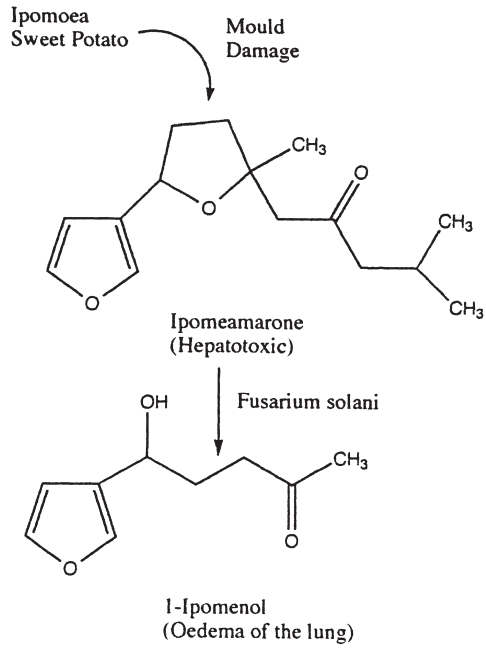

Figure 8.20 *Ipomeamarone and ipomenol*

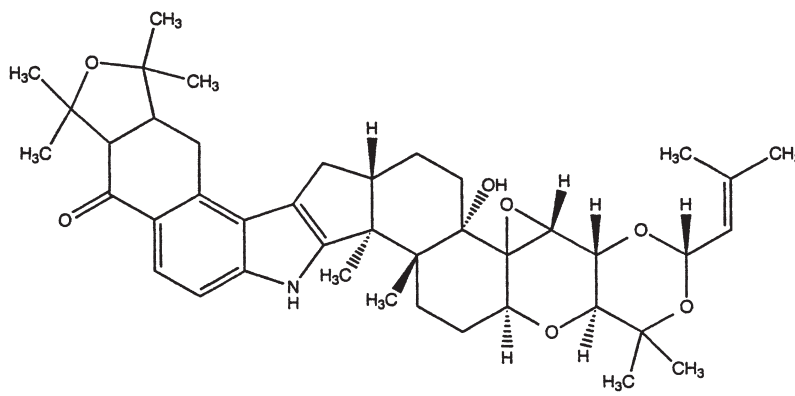

Figure 8.21 *Lolitrem B*

far more overt mycotoxicoses in farm animals throughout the world. Thus facial eczema in sheep in New Zealand, caused by the saprophyte *Pithomyces chartarum* growing on dead grass, slobbers in cattle in the United States, caused by *Rhizoctonia leguminicola* parasitic on red clover, and lupinosis of farm animals in Australia, caused by *Phomopsis leptostromiformis* growing on lupins, do not have a direct effect on humans. However, there can be no denying the impact that such

outbreaks of mycotoxin poisoning have on economics through losses in productivity.

Recognition of the potential to cause harm in humans, by the imposition of maximum tolerated levels of mycotoxins such as aflatoxin, can also have a major impact on economics by rendering a commodity unacceptable in national or international trade. Thus, a major problem occurred for Turkey, the world's most important exporter of dried figs, during the Christmas of 1988.

Several European countries imposed a ban on the import and sale of dried figs following the demonstration of aflatoxin in 30% of samples of figs analysed. Fearing the loss of 50 000 jobs in the fig-drying and packing industry, Turkey was vigorous in her diplomatic efforts to have the bans lifted. This was done fairly soon after they had been imposed and an international symposium on 'Dried Figs and Aflatoxins' was held in Izmir, Turkey, in April 1989. More recently, in 1996, the first reports of aflatoxin contamination of pistachios imported into Europe caused some concern, but the producing country has taken appropriate action to reduce the level of contamination.

In 1980, nearly 66% of random samples of maize from North Carolina had concentrations of aflatoxins in excess of 20 μg kg^{-1} giving rise to an estimated loss to producers and handlers of nearly 31 million dollars. It is rare that the losses and costs arising from mycotoxin contamination can be calculated but these three isolated and very different examples indicate that on a world-wide basis they must be considerable. In at least two of these examples aflatoxin was probably formed in the commodity during growth and development in the field. Under these conditions aflatoxin formation is usually relatively low and in neither case was there any evidence of harm to human beings. However, it is when commodities are improperly stored that really high concentrations of mycotoxins may be formed and it is in these situations that human suffering can occur.

8.5 FOODBORNE VIRUSES

Viruses differ profoundly from other types of micro-organism. They have no cellular structure and possess only one type of nucleic acid (either RNA or DNA) wrapped in a protein coat or capsid. They are also extremely small, with diameters generally in the range 25–300 nm (1 nm = 10^{-3} μm), so that most are invisible using conventional light microscopy and can only be viewed with the electron microscope. Some viruses (for example HIV) are enveloped by an outer lipid membrane, but these cannot be transmitted *via* food since they are relatively fragile and are destroyed by exposure to bile and acidity in the digestive tract.

As obligate intracellular parasites, viruses cannot multiply other than in a susceptible host cell whose machinery and metabolism they hijack

for the purpose of viral replication. Consequently, virus multiplication will not occur in foods which can act only as a passive vehicle in the transmission of infection.

In recent years viruses have been increasingly recognized as an important cause of foodborne illness. There are currently more than 100 human enteric viruses recognized (Table 8.5), and since these are spread by the faecal–oral route, food is one potential means of transmission. Broadly speaking there are two types of pathogenic enteric viruses which differ in their target tissues. Both enter the body *via* the gut, but gastroenteritis viruses remain confined there while others, such as the polio and hepatitis viruses, cause illness once they have migrated to other organs.

8.5.1 Polio

The genus *Enterovirus* is made up of small (28 nm), single-stranded RNA viruses, and includes poliovirus, which was at one time the only virus known to be foodborne. Polio can be a transient viraemia with an incubation period of 3–5 days and characterized by headache, fever and sore throat, but in a minority of cases it can progress to a second stage where the virus invades the meninges causing back pain and headaches. In the worst cases the virus may spread to neurons in the spinal chord causing cell destruction and various degrees of paralysis. Ascent of the infection to the brain may cause death.

Like other enterovirus infections, poliovirus is more likely to produce an asymptomatic infection in very young children. From about the turn of the century, however, improvements in hygiene and sanitation in industrialized countries meant that early infection and acquisition of immunity became less common. As a result, the disease changed from endemic to epidemic and was widely feared as it became more frequent in older children and young adults where it was likely to be much more severe.

Poliomyelitis is now virtually eradicated in developed countries due to the availability of very effective live and inactivated vaccines. At the time that mass-vaccination programmes were introduced in the 1950s, food was no longer important as a vehicle. Previously, contaminated milk had been the principal source of foodborne polio but this route of infection had been controlled by improvements in hygiene.

8.5.2 Hepatitis A and E

A similar story applies to another enterovirus, Hepatitis A, the cause of infectious hepatitis. Improvements in public hygiene and sanitation in

Table 8.5 *Human enteric viruses*

Family	*Features*	*Viruses*	*Associated diseases*
Adenoviridae	Icosahedral particles with fibres. 100 nm, DNA.	Group F adenovirus Serotypes 40 and 41 (AdV).	Gastroenteritis
Astroviridae	28 nm particles with surface 'star' motif. ssRNA.	Human astrovirus, 7 serotypes (HAs + V)	Mild gastroenteritis
Caliciviridae	34 nm particles with cup-shaped depressions on surface. ssRNA.	Sapovirus 5 or more serotypes	Gastroenteritis
	Less distinct surface features.	Norovirus 4–9 serotypes.	Gastroenteritis
Parvoviridae	22 nm featureless particles. ssDNA.	Parvovirus, *e.g.* Ditchling and Cockle agent.	Gastroenteritis, normally shellfish associated.
Picornaviridae	Featureless 28 nm icosahedral particles. ssRNA.	Poliovirus types 1–3.	Meningitis, paralysis fever.
		Echovirus types 1–65. Enterovirus now viruses numbered 68–71.	Meningitis, rash, diarrhoea, fever, respiratory disease.
		Coxsackie A types 1–23.	Meningitis, herpangia, fever, respiratory disease.
		Coxsackie B types 1–6.	Myocarditis, congenital heart anomalies, pleurodynia, respiratory disease, fever, rash, meningitis.
		Hepatovirus (Hepatitis A).	Infectious hepatitis.
Reoviridae	Double shelled capsids. 70–80 nm segmented as RNA.	Reovirus	No disease associations known.
	Outer shell appears as 'spokes of a wheel'. 70 nm.	Rotavirus. Mainly Group A, occasionally B and C in humans.	Gastroenteritis.
Coronaviridae[a]	Fragile, pleomorphic, enveloped particles with prominent club-shaped spikes. SsRNA.	Human enteric coronavirus (HECV).	Gastroenteritis, possibly neonatal necrotizing enterocolitis.
Unclassified		Hepatitis E virus* (Enterically transmitted, non-A, non-B hepatitis), (HEV).	Infectious hepatitis

(M. Carter)

[a] Potential agents not confirmed as human pathogens

the developed world have reduced exposure to the virus so that, when it does occur, it tends to be later in life when the illness is more severe.

The incubation period varies between two and six weeks. During this period the virus multiplies in the cells of the gut epithelium before it is carried by the blood to the liver. In the later part of the incubation period the virus is shed in the faeces. Early symptoms are anorexia, fever, malaise, nausea and vomiting, followed after a few days by symptoms of liver damage such as the passage of dark urine and jaundice.

Like other enteric viruses, hepatitis A is transmitted by the faecal–oral route. Primarily it is spread by person-to-person contact but food-and waterborne outbreaks do occur. Milk, fruits such as strawberries and raspberries, salad vegetables such as lettuce, and shellfish are common food vehicles. With the exception of those caused by shellfish, common source outbreaks are usually due to contamination by an infected food handler. The long incubation period of the illness often makes identification of the source extremely difficult. For the same reason, it is difficult to say with any accuracy what proportion of hepatitis A cases are transmitted by food, although it has been estimated that about 3% of cases in the United States are food or waterborne.

The agent of enterically transmitted non-A, non-B hepatitis has now been designated hepatitis E virus and molecular biology studies indicate it is a calici-like particle with an unusual RNA structure. It too is transmitted by the faecal–oral route and produces illness after an incubation period of 40 days.

8.5.3 Gastroenteritis Viruses

A number of different viruses have been implicated in gastroenteritis by their presence in large numbers (up to 10^8–$10^{10}\,g^{-1}$) in diarrhoeal stools. In most cases it has not proved possible to culture the virus thus preventing their full characterization. As a result, classification has been based largely on morphology and geographical origin.

Although other, better characterized, viruses such as rotavirus, calicivirus and astrovirus are also known to cause diarrhoea, it is these less well-defined agents that are responsible for most outbreaks of foodborne gastroenteritis where a virus is identified. In the United States they were originally known as Norwalk-like agents after the virus which caused an outbreak of gastroenteritis in schoolchildren in Norwalk, Ohio in 1968. In the UK, they were described as small round structured viruses (SRSVs) based on the fact that, when viewed in the electron microscope, they are particles about 25–30 nm in diameter possessing an amorphous structure lacking geometrical symmetry (Figure 8.22). They are now classified as a distinct genus, Norovirus (NoV), within the family Caliciviridae.

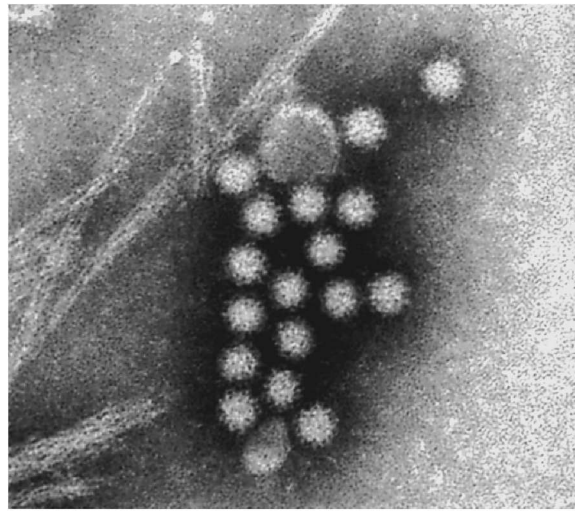

Figure 8.22 *Small round-structured viruses (SRSVs), magnification × 200 000 (Photo: H. Appleton)*

Foodborne viral gastroenteritis is characterized by an incubation period of 15–50 h followed by diarrhoea and vomiting which persists for 24–48 h. The infectious dose is not known. Studies in model systems have suggested that doses as low as one cell culture infectious unit can produce infection but in polio vaccination an oral dose of 100 000 infectious units is given to ensure a success rate of at least 90%.

The onset of symptoms such as projectile vomiting may be very sudden and unexpected and this can contribute to the further spread of illness (see below).

8.5.4 Sources of Food Contamination

The importance of viral gastroenteritis is clear from the huge under reporting revealed by the Infectious Intestinal Disease Study in England. The degree to which this is foodborne is uncertain since a considerable amount of human to human transmission must also occur. Estimates of the proportion of viral gastroenteritis which is foodborne, made in the UK, Australia and the USA, vary between 10% and 40%.

Enteric viruses may be introduced into foods either as primary contamination, at source where the food is produced, or as secondary contamination during handling, preparation and serving. It is possible that salad vegetables fertilized with human excrement or irrigated with sewage polluted water could be contaminated with viruses while in the field. Salads and fruits such as raspberries have been implicated in outbreaks, though in some cases this could also have been the result of

secondary contamination during preparation. Evidence of unequivocal primary contamination is largely restricted to bivalve molluscan shellfish, such as clams, cockles, mussels and oysters, which have been involved in numerous outbreaks of hepatitis and gastroenteritis. In the UK between 1976 and 1987 there were several large outbreaks involving cockles from the Essex coast in which more than 2000 people were affected. Large outbreaks have also been reported from Australia and the United States, but these pale beside the outbreak of hepatitis A in Shanghai in 1988 when almost 300 000 were reported ill and contaminated clams were identified as the source of infection.

The problem arises because these shellfish are grown in shallow, inshore, coastal waters that are often contaminated with sewage. Since they feed by filtering sea water to extract suspended organic matter, they also tend to concentrate bacteria and viruses from the surrounding environment. Pumping rates can be quite substantial as an oyster will filter up to four litres of seawater per hour and concentrate microorganisms in their gut by up to a thousand-fold.

It is possible to decontaminate shellfish by relaying them in clean waters (if these can be found) or removing them to special depuration plants where they are encouraged to filter water that is recirculated and purified, usually by treatment with UV light or ozone. Depuration procedures have proved very successful for removing bacterial pathogens; coliform bacteria have been shown to be removed within 24–48 hours. However the rate at which viruses are cleansed is much slower and less predictable. This is probably due to the small size of the virion compared with the bacterial cell, the relative strength of its attachment to the gut wall and on its ability to penetrate into deeper tissues. It has been suggested that virus particles ingested by the shellfish are taken up by macrophages and transported from the gut to tissues that are remote from the depuration process, though there is little evidence for this.

The problem is compounded by the fact that some shellfish, such as oysters, are consumed without any cooking and those that are cooked receive only a mild, relatively uncontrolled heat process in order to prevent the flesh assuming the consistency of rubber. Studies on the heat inactivation of hepatitis A virus have led to the introduction of guidelines in the UK for the cooking of cockles which recommend that the internal temperature of the meat should reach 85–90 °C for 1.5 min. It is not known whether these guidelines provide an acceptable safety margin with regard to NoV since they cannot be cultured *in vitro* for their heat sensitivity to be determined.

Secondary contamination by infected food handlers is an alternative source of infection, particularly with those food items that are subject to extensive handling in their preparation and are consumed without reheating. Usually the food handler is suffering from viral gastroenteritis

at the time. One outbreak in the UK provides a graphic illustration of secondary contamination and also how the sudden onset of symptoms can catch victims unaware and exacerbate the problem.

The outbreak occurred at a hotel in the UK where over 140 people were ill. One chef vomited in the changing room lavatory and then immediately returned to food preparation. Later that day he had an episode of diarrhoea. Two days later another member of the kitchen staff vomited into a bin outside the kitchen door and the following day two staff vomited in the kitchen itself. The epidemiological evidence strongly implicated the cold foods prepared by the chef who was found to be excreting the NoV 48 h after his symptoms subsided. However, it is clearly possible that several other foods may have been contaminated by droplets from the vomitus from other affected kitchen workers.

In 1982 a huge outbreak in the Twin Cities area of Minnesota was caused by a baker who was working during an episode of diarrhoeal illness. Despite claiming to have washed his hands thoroughly after a visit to the toilet, he transferred sufficient virus to the butter cream that he mixed by hand to cause an outbreak which affected at least 3000 people. Shortly after, in the same area, a second outbreak affecting 2000 people occurred in which a food handler contaminated salads during banquet catering.

There is no evidence yet of a persistent symptomless carrier state for these viruses. They are no longer apparent in patient's stools shortly after recovery but this may simply reflect the insensitivity of electron microscopy as a detection method.

8.5.5 Control

The problems of monitoring and control of foodborne viruses are very different to those posed by bacteria. Testing foods for the presence of pathogenic viruses is not possible since many cannot be cultured and the numbers present are too low to be detected by techniques such as electron microscopy. An alternative would be to use more readily cultured viruses that are shed in the faeces, such as the vaccine polio strain, as indicator organisms for the presence of pathogenic enteric viruses. However, current extraction methods are very inefficient and the culture techniques, based on observation of a cytopathic effect in cell monolayers or plaques in cell monolayers under semi-solid medium, are far more complex and expensive than bacteriological testing. Already though, techniques based on immunoassay and nucleic acid probes with the polymerase chain reaction promise to improve both the sensitivity and speed of virus detection.

An interesting approach is to use coliphage, a bacteriophage which infects the enteric bacterium *E. coli*, as a viral indicator. Coliphages do

not require expensive tissue culture techniques for their enumeration since they can be detected through their ability to form plaques in a lawn culture of a suitable strain of *E. coli*. The problem of extraction of the coliphage from food remains however, and interpretation of the significance of their presence in foods is uncertain.

As with other problems of microbiological food safety, control of viral contamination is most effectively exercised at source. Primary contamination can be controlled by avoiding the fertilization of vulnerable crops with human sewage and the discharge of virus-containing effluents into shellfish-harvesting waters. Secondary contamination is even harder to detect microbiologically and can only be controlled by the strict observance of good hygienic practices in the handling and preparation of foods.

Prospects are poor for a vaccine against the gastroenteritis viruses since immunity following infection appears to be short lived. Volunteers who were made ill by ingesting a faecal extract containing the Norwalk agent became ill again a year later when given the same extract a second time. A new vaccine against hepatitis A, based on normal hepatitis A virus inactivated with formaldehyde, was licensed for use in the UK in 1992.

8.6 SPONGIFORM ENCEPHALOPATHIES

Spongiform encephalopathies (SEs) are degenerative disorders of the brain that occur in a number of species. They are recognized by the clinical appearance of the affected animal and the characteristic histological changes they produce in the brain. Microscopic examination reveals the presence of vacuoles in the neurons giving the grey matter the appearance of a section through a sponge.

Scrapie, the disease of sheep and goats, has been known since the 18th century but was first described scientifically in 1913. Its name is derived from one of the symptoms; an itching which causes the infected animal to scrape itself against objects.

The agent of scrapie and other SEs have been described as 'slow viruses' due to their long incubation periods. However, it is now thought that the infectious agent, known as a prion, is neither a bacterium nor a virus. It is invisible in the electron microscope, cannot be cultured in media or cell cultures and does not provoke the formation of specific antibodies in infected animals. It is also very resistant to heat, irradiation and chemical treatments such as formalin. In sheep, the illness is transmitted both vertically and horizontally and other animals have been infected as a result of intraperitoneal or intracerebral injection of infected tissue preparations. The evidence available suggests that these illnesses are intoxications rather than infections. The prion contains a protein PrP^{Sc} which is also a major component of the plaques formed in

the brains of affected individuals. PrP^{Sc} is a modified version of the protein PrP^{C} found normally on the outer surface of neurons. Differences in its tertiary structure make it resistant to proteolytic degradation and removal when its useful life is over. It is thought that prion PrP^{Sc} finds its way to the neuron surface where its interaction with PrP^{C} leads to production of further PrP^{Sc}, a process known as 'recruitment'. This initiates a chain reaction, the accumulation of PrP^{Sc}, plaque formation, and the onset of neurological symptoms.

Four human SEs are known, Gerstmann–Sträussler–Scheinker syndrome, Fatal familial insomnia, Creutzfeldt–Jacob disease and kuru, though the last two may in fact be the same disease. Kuru, first described in 1957, is restricted to the Fore people of Papua New Guinea where it was the major cause of death among Fore women. It was shown to be transmissable to chimpanzees by intracerebral inoculation with brain extracts from dead patients and it was eventually decided that human transmission was in fact foodborne, albeit of a rather special kind. The Fore tribe have a tradition of cannibalism and it was the tribal custom for women and children to eat the brains of the dead as a mark of respect. Since this practice was suppressed in the late fifties incidence of the disease has declined.

Creutzfeldt–Jacob disease is more widespread than kuru and in the UK has an incidence of 1 per 2 000 000 inhabitants. Accidental transmission by injection of contaminated pituitary extracts, corneal grafting and implantation of contaminated electrodes has occurred but oral transmission has not been described.

Much attention has been focused on these conditions since the emergence of bovine spongiform encephalopathy (BSE), or mad cow disease, in cattle in Britain in 1985/6. The illness is thought to result from the scrapie agent crossing the species barrier and being transmitted to cattle by scrapie-infected sheep protein fed to cattle. Its emergence at this time has been linked with changes in the commercial rendering processes used in the production of animal proteins such as reduced processing temperatures and abandonment of the use of solvents to extract tallow. Control measures introduced in the UK include prohibition of the sale of bovine offal from animals more than six months old and banning the feeding of ruminant-derived protein to ruminants and the slaughter of all cattle over 30 months of age. In 1996 a ban on the use of all mammalian-derived animal protein in feed for all farm animals was introduced.

Incidence of BSE in cattle peaked in 1992 with 36,680 cases and by 2005 the annual total had declined to less than 150 cases. By and large the epidemic seems to have been confined to the UK as reported cases in other countries totalled around 600 at the end of 1997, mostly in Switzerland (256) and Ireland (224). This is a little surprising since

thousands of tons of the same meat and bone meal thought to have caused BSE in the UK were also exported in the period 1985–1990, as were large numbers of British breeding cattle, nearly 58 000 to the EU alone. An alternative hypothesis is that the epidemic was caused by contaminated meat and bone meal from Africa imported into the UK.

The concern was that if the agent can cross the species barrier from sheep to cattle and be acquired through food, it could do so again–infecting humans. Initially, the concensus of expert opinion was that this was highly unlikely. There is no evidence linking human occupational exposure to potentially scrapie-infected tissues with degenerative encephalopathy, and sheep and sheep products have been eaten for longer than scrapie has been known without any evidence of it causing a similar human illness. It may be that the ease with which ingested abnormal prion protein can recruit the normal prion protein is reflected in the similarity of their amino acid compositions. If this is the case then recruitment of normal human prion by the BSE prion is likely to be relatively inefficient. The cattle and sheep prions differ at 7 positions whereas the cattle and human prions differ at more than 30 positions. The BSE agent has however been shown to cause illness in cats and some primates. Results of experiments with transgenic mice expressing PrP^{C} also indicate that induction of human prion produced by bovine prions is inefficient. In 1996, however, a new variant of CJD (vCJD) in humans was described which appears to be linked to BSE. It is thought that those affected acquired the agent through consuming beef products before the offal ban was imposed in 1989. The number of vCJD cases reported in the UK has increased from 1995 to a peak of 28 cases in 2000 and has since declined (Figure. 8.23).

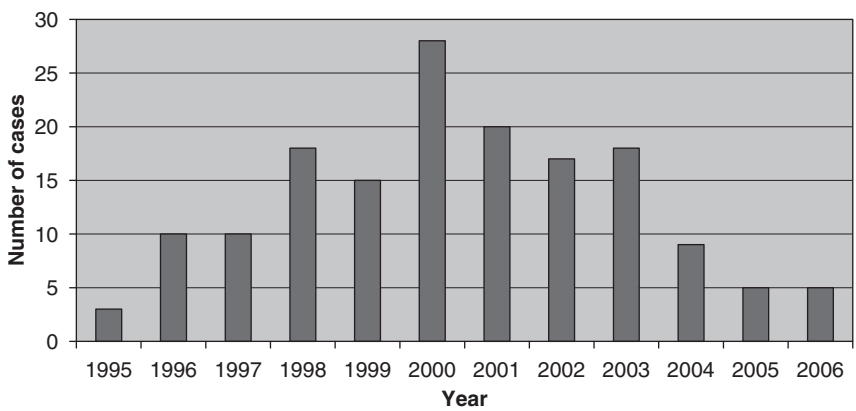

Figure 8.23 *vCJD cases in UK*
(data from Health Protection Agency)