Mycotoxins are toxic metabolites produced by fungi, especially from saprophytic moulds growing on foodstuffs or animal feeds. They must always have represented a hazard to man and domestic animals, but their effects have been largely overlooked until the past 20 years. Although poisonous mushrooms are carefully avoided, moulds have generally been considered to cause unsightly spoilage of food, without being dangerous to health. However, it is now well established that mycotoxins (diseases caused by mycotoxins) have been largely overlooked until the past 20 years. They must always have represented a hazard to man and domestic animals, at least during recent history. The most important have been ergotism, which has killed hundreds of thousands of people in Europe in the last millennium; alimentary toxic aleukia (ATA) which brought slow death to tens of thousands of people in the USSR between 1941 and 1947, stachybotryotoxicosis, which killed tens of thousands of horses and cattle in the USSR in the 1930s; and aflatoxicosis, which killed 100,000 young turkeys in England in 1960 and has caused death or disease in many other animals — and perhaps man as well. Each of these diseases is now known to have been caused by growth of specific moulds which produced one or more potent toxins in a particular commodity. An important distinction must be made between bacterial toxins and mycotoxins. The classic bacterial toxin is a protein which swiftly produces antibody reactions with characteristic symptoms. Fungal toxins are almost all low molecular weight chemical compounds and hence are unsuspected and insidious in their action. Mycotoxins can be acutely or chronically toxic, or both, depending on the dose and the kind of toxin. In animals, symptoms of acute toxicity include liver and kidney damage, attack on the CNS, skin disorders, and hormonal-like effects. Nerve toxins may induce trembling or death without apparent cause. Skin disorders may be manifest as open lesions or as photosensitivity, while the manifestation of hormonal effects include abortion in cattle, vulvovaginitis in pigs, and variety of ill-defined disorders such as vomiting, feed refusal and ill thrift. Toxins producing liver and kidney damage are even more insidious: levels much lower than those producing acute effects are often carcinogenic. Ingested in small quantities in the diet, they can cause cancer in experimental animals long after the time of ingestion. It is probable that man can be affected in the same way.

### Significant mycotoxins

#### Table 1

<table>
<thead>
<tr>
<th>Date</th>
<th>Disease</th>
<th>Toxin</th>
<th>Cause</th>
<th>Diagnosis</th>
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<tr>
<td>1954</td>
<td>Ergotism</td>
<td>ergot alkaloids</td>
<td>Claviceps purpurea in rye</td>
<td>1800: fungal cause suspected 1850: fungal cause demonstrated</td>
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<td>1910</td>
<td>Acute cardiac beriberi</td>
<td>citre-viridin</td>
<td>Penicillium citreocfrum in rice</td>
<td>1910: yellow rice-sale banned 1969: fungal cause demonstrated</td>
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<td>1965-66</td>
<td>'Bobali-beer' (cereals/crathy)</td>
<td>T-2</td>
<td>Fusarium sp. in barley</td>
<td>1980: fungal origin proposed</td>
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<tr>
<td>1974</td>
<td>Hepatitis</td>
<td>aflatoxin</td>
<td>Aspergillus flavus in maize</td>
<td>1975: fungal cause demonstrated</td>
</tr>
<tr>
<td>Current</td>
<td>Pellagra</td>
<td>T-2</td>
<td>Fusarium sp. in maize</td>
<td>1980: fungal origin proposed</td>
</tr>
<tr>
<td>Current</td>
<td>Reye's syndrome</td>
<td>aflatoxin</td>
<td>Aspergillus flavus in nuts and maize</td>
<td>1977: fungal origin proposed</td>
</tr>
<tr>
<td>Current</td>
<td>Kwashiorkor</td>
<td>aflatoxin</td>
<td>Aspergillus flavus in nuts and cereals</td>
<td>1983: fungal involvement proposed</td>
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</tbody>
</table>

Ergotism

The association of one human illness with a fungus has been known for a long time. Ergotism occurred throughout the last millennium in central Europe, and has certainly killed hundreds of thousands of people. The relationship between ergotism and the formation of ergots in maturing grain was established by the 17th century. By 1750, it was known that ergot result from the growth of the fungus Claviceps purpurea in the ovaries of grains, especially rye. During milling, ergots are not readily separated from sound grain and as a result become fragmented and dispersed throughout the flour. The first symptom of ergotism is a feeling of coldness in the hands and feet, followed by a sensation of intense burning. In extreme cases, gangrene, necrosis and death may follow. In the Middle Ages the disease was known as 'St Anthony's Fire', because it was believed that travelling to the shrine of St Anthony would relieve the burning sensation. Modern medicine is more likely to attribute the curative effect of this trip to the victim moving away from his contaminated environment. The toxic principles in ergots are now known to be a range of alkaloids, all derivatives of syringeic acid, which have a wide spectrum of biological activities. Some have been used in low doses for many years to induce childbirth. The last known outbreak of ergotism occurred in the French village of Pont-St-Esprit in 1954. More than 200 people became ill and four died from cardiovascular collapse as a result of muscular spasms. This well-documented mycotoxicosis was due to gross negligence by a miller. It was notable because many people suffered from hallucinations, screaming that they were on fire or were being chased by wild beasts. Fuller suggests that the alkaloid responsible for these symptoms may have been lysergic acid diethylamide (LSD).

Ergotism can now be regarded as a disease of the past since stringent controls on levels of ergot in grain have been established throughout the world.

Acute cardiac beriberi

Another human mycotoxicosis of significance is acute cardiac beriberi. It was a common disease in Japan, especially in the second half of the 19th century. The first symptoms of acute cardiac beriberi are heart distress and palpitation, with rapid breathing. After a few hours, breathing becomes laboured, nausea and vomiting are experienced, and within two to three days, anguish, pain, restlessness and sometimes maniacal behaviour occur. In extreme cases, progressive paralysis leading to respiratory failure may cause death. Beriberi is the general name given to a vitamin B, deficiency resulting from the consumption of polished rice. However, painstaking and pioneering work by Uraguchi established that acute cardiac beriberi probably was not an avitaminosis but a mycotoxicosis. In 1910, the incidence of acute cardiac beriberi suddenly decreased. Uraguchi points out that this coincided with implementation of a government inspection scheme which dramatically reduced the sale of mouldy rice in Japan. The incidence of true beriberi was falling. It is notable that the victims of acute beriberi were often young adults without any history of disease.

Alimentary toxic aleukia

A third disease caused by a mycotoxin is known as alimentary toxic aleukia (ATA). From 1942 to 1948 this disease caused the deaths of many people in Russia, especially in the Orenburg district north of the Caspian Sea. In some localities, mortality was as high as 65% of those afflicted and up to 10% of the population. The Russian authorities have apparently never released a figure for the total mortality, but it must have been at least one hundred thousand. Records show that ATA was pre-
The following set of mycotoxicoses suggested a possible correlation with mycotoxins in the food supply. Field studies were initiated on an international basis. Epidemiological data were coupled with analyses of those foods that form the staple diet of stable indigenous populations. Stability in both diet and population is essential in studies of this kind because of the long induction period (10-20 years) for human liver cancer.

Such studies were carried out in Kenya, Swaziland, Uganda, Thailand, Mozambique and rural south-eastern United States. Great care is needed to obtain meaningful results in such studies, but by 1976 sufficient data existed to allow plotting and statistical analysis. These indicated a positive correlation between the logarithm of aflatoxin intake and the occurrence of primary human liver cancer, at least in Africa and south-east Asia.

Epidemiological studies in the USA have produced results differing from those above. Stollf and Friedman estimated that children in rural communities in the southern states of the USA may ingest as much as 40 ng aflatoxin per kg of body weight per day, mostly from maize. Van Rensburg’s figures, such as the level should produce 4–10 deaths from primary liver cancer per 105 population per year. The actual level encountered, however, was about one. That is less than in some other regions of the USA, such as Wisconsin and California, where aflatoxin is unlikely to be ingested in significant amounts.

Ochratoxin In the early 1970s, observers in Denmark noted a high incidence of nephritis (kidney inflammation) in pigs at slaughter. A search for

### Table 2. Chronic mycotoxicoses of human significance.

<table>
<thead>
<tr>
<th>Date</th>
<th>Disease</th>
<th>Toxin</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>1903:</td>
<td>aflatoxin</td>
<td>Aspergillus flavus in foods</td>
<td></td>
</tr>
<tr>
<td>1979:</td>
<td>fungal cause unknown</td>
<td>Aspergillus flavus in foods</td>
<td></td>
</tr>
<tr>
<td>1979:</td>
<td>fungal cause suspected</td>
<td>Aspergillus flavus in foods</td>
<td></td>
</tr>
<tr>
<td>1973:</td>
<td>fungal cause suspected</td>
<td>Aspergillus flavus in foods</td>
<td></td>
</tr>
<tr>
<td>1972:</td>
<td>fungal cause suspected</td>
<td>Aspergillus flavus in foods</td>
<td></td>
</tr>
</tbody>
</table>

### Other possible acute mycotoxicoses

Table 3 outlines some acute mycotoxicoses, some of which may be of limited acute significance. However, Stollf and Friedman estimated that children in rural communities in the southern states of the USA may ingest as much as 40 ng aflatoxin per kg of body weight per day, mostly from maize. Van Rensburg’s figures, such as the level should produce 4–10 deaths from primary liver cancer per 105 population per year. The actual level encountered, however, was about one. That is less than in some other regions of the USA, such as Wisconsin and California, where aflatoxin is unlikely to be ingested in significant amounts.

### Ochratoxin

In the early 1970s, observers in Denmark noted a high incidence of nephritis (kidney inflammation) in pigs at slaughter. A search for

### Figure 3: Colonies of Fusarium graminearum on carnation leaf agar and potato dextrose agar. Note the deep red pigment. The possible causes eventually showed the presence of ochratoxin A, a mycotoxin originally reported from the common mould Aspergillus ochraceus. Analysis of pig feeds showed that 50% of samples contained ochratoxin A at levels up to 27 mg/kg. The mould responsible was found to be Penicillium viridicatum, which often occurs in Danish barley.

### Figure 2: Orange-yellow reverse colonies of Aspergillus flavus when grown on Aspergillus flavus and parasiticus agar for 2 days at 30°C.

The disease remains baffling, but the absence of any known causative agent and the presence of the peculiar renal syndrome suggest that a mycotoxin may be to blame. Studies are continuing.

### Assaying mycotoxins

Mycotoxins can be assayed by either chemical or biological techniques. The most common chemical technique is thin layer chromatography of solvent extracts, followed by visualisation by ultraviolet light or chemical reagents. Quantitative techniques have been developed for most common mycotoxins. However, these techniques have proved to be difficult to assay by this technique. The best procedures rely on such sophisticated techniques as gas-liquid chromatography coupled with mass spectrometry. Although of great value for the assay of specific compounds, chemical methods cannot provide a guarantee of non-toxicity because the existence of unknown toxic compounds cannot be taken into account. Biological tests have the advantage that definite answers on the toxicity of a food or feed can be provided. However, they are non-specific, rarely providing information on other mycotoxicoses and are slow and very expensive.

### Detecting mycotoxicogenic fungi

The detection of mycotoxigenic fungi is also difficult because so many species cause mycotoxicoses and the identification of fungi remains a specialist task. Ideally, a selective medium should be available for each toxigenic fungus in the same way that selective media have been formulated for specific food poisoning bacteria. Development of such media is in its infancy, at this time only two media of this type exist, one for Aspergillus flavus and A parasiticus, and one for Penicillium viridicatum, which make ochratoxin and some other less well known toxins. The medium for A flavus, known as AFPA media, was first described by Friedman. It was developed from the observation that these fungi produce a characteristic orange-yellow reverse colour in the presence of ferric citrate. AFPA contains antibodies which control the growth of spreading fungi and an antibiotic to eliminate bacterial contamination. Petri dishes prepared in these media are incubated at 30°C for
Listeria monocytogenes, first described in 1926 by Murray, Webb and Horsfall, 4 is still a baffling micro-organism for the epidemiologist. Animals, fish, birds and humans are all susceptible to infection but, despite the organism's widespread distribution in nature, human infections are comparatively rare. Furthermore, the mode and source of infection are often obscure.

In epidemic studies, it is a number of clinical manifestations — characterised initially by a febrile-like syndrome of which abdominal pain and vomiting are the most common. In addition, abortion, meningitis, oedema, arthritis, internal and external abscesses, subacute bacterial endocarditis and opportunistic infection in immunosuppressed patients have been reported.

Transmission
It is not known whether or not there is a single reservoir of infection. The organism is widely distributed in a range of environments — from dust, water, soil, fodder and silage, and many species of birds, fish, animals and insects, but little is known about the mode of transmission. In the toxigenic strain, it is usually considered that the infection is transmitted orally, by handling contaminated material, i.e., milk and milk products or meats. Oral or venereal infection and insect vectors have also been suggested as routes of possible transmission. 5 The isolation rate of L. monocytogenes from human stools, for example, can be as high as 29%.

Epidemiology
The epidemiology of Listeria infections is poorly understood and there is a shortage of information relating to the total number of bacteriologically proven cases which occur world wide. Seeliger et al. 6 found that the peak of the animal infections occurred in the spring, whereas the peak of the human infections was reached in the autumn months. A similar pattern was reported by Bush et al. 7 in the USA. 8 It is of interest that the peaks of infection in humans and animals coincide with the autumn months. A similar peak of infection in humans and animals occurs world wide, i.e., the autumn as high as 29%.

Infections is poorly understood and transmission is unknown. Isolation rates in humans and animals are comparatively rare. 8 However, there is a possible peak of infection in the spring, whereas the peak of infection in humans and animals is known. This peak of infection in humans and animals is known to occur world wide, i.e., the spring and autumn.

Listeria monocytogenes species, the principal producers of listeriose, are most readily recognised by their large, crescent shaped spores and to pink red reverse colour on potato dextrose agar (Figure 3). The spores are not always formed on laboratory media, however, and some listeriose producers do not make these reverse colours. A great deal of further work is needed in the formulation of media for mycotoxigenic fungi.

Conclusion
Improved living standards, higher quality food supplies and a more varied diet have helped to virtually eliminate the risk of acute mycotoxins in man in most Western countries. However, there is ample evidence that mycotoxins can, and still do, cause human disease in some developing countries, particularly in rural populations whose diet is based on a single, staple commodity such as corn, rice or peanuts.

Human disease only represents the tip of the iceberg, however, considering the effects caused by mycotoxins. The world-wide cost of debilitating diseases and death in domestic animals due to ingestion of mould-contaminated feed must be enormous. Such problems can never be completely overcome, but with a better understanding of the physiological effects of mycotoxins, and the fungi which produce them, they can, perhaps, be minimised.

References
1. Fuller, J.G. (1968) The Day of St Anthony's Fire
 CONTINUED FROM OVERLEAF

method depends on the fact that the organisms are arranged in layers in the colony and collectively act as a diffusing grating. The size of the colony is such that under the lighting conditions used it reflects the blue part of the spectrum back to the observer. The temperature range for growth of Listeria monocytogenes is 37-45°C with an optimum temperature at 30°C. At 4°C Listeria is believed to multiply more rapidly than other species. This property has been used by Gray and by other workers to recover Listeria species from heavily contaminated materials and cultures. Listeria growth at this temperature is apparent to the naked eye after about 10 days; the organisms are motile and are pathogenic to laboratory animals.

Biochemical identification
Carbohydrate fermentation patterns of L. monocytogenes performed in meat extract broth with bromocresol purple indicator, incubated at 35°C for 24-72 hours are usually characteristic. Acid reactions occur with dextrose, maltose, di-lactulose, salicin, ascuulin, deoxyn and trehalose. No acidification occurs with mannitol, arabinoside, dulcitol, adonitol, raffinose or inulin.

Seeleger14 reported that L. monocytogenes does not produce indole, nor does it reduce nitrates or hydroxide. The production of acetylmethylcarbinol is usually positive, and the methyl red test and catalase are both positive. Protelytic activity is absent.

Using selective agents to isolate L. monocytogenes
To aid recovery of L. monocytogenes from heavily contaminated environments many workers have suggested the use of various agents to suppress growth of other bacterial species—especially Gram-negative organisms—and to allow the bulk of the Gram-positive species to flourish. One of the first practical observations made in this direction was the use of temperature as a selective agent. Gray et al15 reported that L. monocytogenes not only survived at 4°C for long periods but actually grew at this temperature more rapidly than other bacteria.

Chemicals used as selective agents for the isolation of L. monocytogenes were first recommended by Schoer16 who suggested the use of potassium tellurite as an inhibitor of Gram-negative organisms. This finding was confirmed by Gray et al15 in spite of a report that potassium tellurite could exert an inhibiting effect on L. monocytogenes depending upon the constituents of the broth medium.17 Substitutes for potassium tellurite were then sought. After a thorough investigation of media selective for L. monocytogenes, a combination of thallous acetate and nalidixic acid was recommended.18,19 Leighton20 confirmed this finding.

The use of dyes has received little attention, perhaps because of the wide variation which occurs in the manufactures. Pancheo and Santos21 tested nine strains of L. monocytogenes against 31 different dyes; the most effective of these as selective agents being nigrosin, methyl violet, crystal violet, methyl green, Sudan III, basic fuchsin and water soluble cosin. Interest in the use of dyes has been revived with reports of tryptophan acting as a selective agent,22 and of a medium containing polymyxin, nalidixic acid and demethyl blue.23

The construction of media selective for L. monocytogenes is not straightforward, as many of the decisive selective agents suggested can inhibit the growth of Listeria. Furthermore, it is also apparent from the literature that a selective medium which is efficient in the hands of one group of workers may prove to be totally inadequate in other hands.

Tests for pathogenicity
Pathogenic strains of L. monocytogenes can be identified by the Antox test. Two to three drops of an overnight culture suspended in 5ml of distilled water are introduced into the conjunctival sac of a rabbit or guinea-pig. A purulent conjunctivitis develops in 2-5 days with the organisms being demonstrated in direct films of pus. Ampicillin drops in the eye rapidly removes the infection. Pathogenic strains may also be recognised in the in vitro tests described by Grove and Welchman.24 A positive CAMAGREF reaction, acid reaction with thiamine and non-acid reaction with xylose were associated with pathogenic L. monocytogenes.

Antigenic structure
The current somatic and flagellar antigen distribution is shown in Table 2. Serotypes 1/2a and 4b are associated with human and animal infections. Serotype 5 L. (Listeria ivanovii) has been reported as the infective agent in ovine infections.

Conclusions
It appears from literature surveys that microbiologists are becoming increasingly aware of the role of L. monocytogenes as an infective agent. With the improvement in isolation techniques, for example the use of media containing thallous acetate and nalidixic acid, and the use of culture characteristics, there should be an apparent increase in the number of diagnosed human cases of listeriosis. However, the picture will not be complete until the epidemiologists insist that L. monocytogenes infections are notified.

References

Table 2. The current somatic and flagellar antigen distribution for Listeria spp.

<table>
<thead>
<tr>
<th>Designation Pattern</th>
<th>Seeliger-Donker Voset</th>
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<tr>
<td>O-Antigens</td>
<td>H-Antigens</td>
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<tr>
<td>1/2a</td>
<td>AB</td>
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<tr>
<td>1/2b</td>
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