# MYCOTOXINS IN FOOD—RECENT ADVANCES IN RESEARCH IN SOUTH AFRICA*

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During the last three decades an enormous amount of research has been devoted to the study of mould metabolites in the search for antibiotics of therapeutic value. It is rather surprising that so much knowledge can have been accumulated about the production of antibiotics by moulds and so little attention paid, until recently, to the possible effects of fungal metabolites on the health of higher animals and particularly of man. This oversight is all the more surprising when it is considered that moulds have been known for more than 70 years to be capable of producing metabolites toxic to man.

Intense interest has now been aroused, however, by the mounting evidence of mould toxicity, and research is being conducted in many parts of the world, notably Great Britain, the USA, Japan, Holland and the Republic of South Africa. This interest stemmed originally from the work of Miyake *et al.*,[1] who showed that rats fed on rice infected with *Penicillium islandicum* developed certain liver abnormalities, and from the report of Sargent *et al.*[2] describing an outbreak of an obscure disease among turkey poults in Great Britain. The latter authors traced the cause of the disease to a shipment of groundnut meal and eventually to toxic metabolites of the fungus *Aspergillus flavus*, a discovery which led to the isolation and identification of the group of toxic metabolites now known as the aflatoxins.

## WIDESPREAD OCCURRENCE OF TOXIC MOULDS IN FOODSTUFFS

In South Africa, a research team of microbiologists, toxicologists and organic and analytical chemists working in close collaboration, has been functioning for some years.

The initial task undertaken was to isolate and identify moulds present on South African-grown cereals and groundnuts and to screen them for the production of metabolites toxic to Pekin ducklings. To date, Scott,[3,4] and Gouws[5] have reported strains from 25 mould species which are capable of producing metabolites toxic to ducklings. Thirteen of these also showed toxic effects when fed to weanling mice and rats. These included species of the genera *Aspergillus*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Trichoderma*, *Trichotheceum*, *Alternaria* and *Bipolaris*. Additional toxic species have been reported by other workers, and it is now recognized that there is a very wide range of fungi capable of giving rise to toxic metabolites. Among the mycotoxins recovered and identified in South Africa are aflatoxin, sterigmatocystin, tenuazonic acid and the newly-discovered ochratoxin A. The latter mycotoxin was isolated by Van der Merwe *et al.*,[6] who also elucidated its structure.

The mycotoxicoses so far known to occur in man include ergotism, which has long been recognized as a mycotoxicosis, and alimentary toxic aleukia, more recently described by Joffe.[7] Of the metabolites produced by other fungi a number, notably the aflatoxins, have been proved to be acutely toxic and also carcinogenic to laboratory animals. Aflatoxin $B_1$ has in fact been shown to be one of the most potent hepatocarcinogens yet discovered. No direct proof has yet been forthcoming, however, which implicates aflatoxin, ochratoxin or any of the other recently-studied mycotoxins as a definite cause of toxicosis or carcinogenesis in man, and the extent of the danger to

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man is consequently difficult to assess. Several countries have accepted the evidence from animal experiments as sufficient justification for legislation prohibiting the sale of foods contaminated with aflatoxin, and it is clear that the possible danger to human health represented by mycotoxins is rightly being given serious consideration.

In the East, where moulds are widely used to convert rice, soya and fish into foods of different textures and flavours, there is obviously a danger that toxic strains might be employed or toxic contaminants allowed to proliferate as a result of inadequate process control. The same danger would appear to exist in the West, where moulds are used in the manufacture of the blue cheeses. No mycotoxins have yet been recovered, however, from any of these cheeses, or from cultures of the mould strains employed in their manufacture.

A survey of cereals and groundnuts grown in South Africa recently carried out by our workers, has shown that healthy, high-quality cereals harvested and stored under satisfactory conditions are unlikely to be contaminated with aflatoxin or any other mycotoxins at the levels which can at present be detected. In some areas, however, and in many other countries of the world, primitive harvesting methods and storage facilities are still being used. Under such conditions mould growth with contamination by mycotoxins occurs readily when grain becomes damp or infested with insects.

Fermented foods form an important part of the diet of the Bantu throughout Africa. The traditional sour alcoholic beverages of the Bantu are made from soured gruels which are cooked before adding sorghum malt to effect the saccharification of some of the starch present. An alcoholic fermentation is then induced by inoculating with yeast. No aflatoxin has been found in sorghum malt produced in factories operating under controlled conditions in South Africa, but there is a possibility that moulds may grow and mycotoxins develop when malt is produced under primitive conditions. This particularly applies to the beer traditionally produced and consumed in large quantities by the Bantu. The possibility that mycotoxin contamination of food is endangering the health of human beings is therefore as real in Africa as in the Far East.

Unseasonable rain at the time of harvesting and unsatisfactory storage conditions may lead to mould contamination in groundnuts. Sellschop[8] furthermore maintains that *Aspergillus flavus* can be introduced into the maturing pod and kernel by termites, and that infestation of the harvested pods by the larvae of the boll-worm can cause mould contamination during curing. Contamination due to insect infestation before or immediately after harvesting is difficult to prevent or control. However, research into insect control and methods of harvesting and storage may yet provide the answer to the problem of preventing mycotoxin contamination in groundnuts.

The recovery of toxic moulds from widely-used foods and animal feeds indicates that there is an urgent need to assess the extent to which food and feeds may be contaminated with mycotoxins. We shall also have to determine to what extent this contamination constitutes a hazard to the health of man and his domestic animals and to what extent our knowledge is still inadequate in order to cope with the problem.

## IDENTIFICATION OF TOXIC MOULDS AND METABOLITES AND NEED FOR RELIABLE ASSAY METHODS

As already stated, a great number of mould species have been proved to form toxic metabolites under certain circumstances. We have no hesitation in predicting that more such moulds will be found. It is therefore essential that the toxicity of moulds isolated from foodstuffs should continue to be investigated. A valid criticism which may be levelled at the work done to date, is that reliance has been placed on acute oral toxicity tests performed on small animals, particularly ducklings.

Whether this method of screening is adequate is a question that still has to be decided. Moulds may also produce metabolites which are not acutely toxic, but which have deleterious long-term effects. Ethionine, which can be formed through microbial activity, may be quoted as an example; its toxicity is low but it is carcinogenic.[9] Screening for the presence of such substances is time-consuming and costly, but will have to be done if the danger associated with the ingestion of mould metabolites is to be fully assessed.

In every case where the ingestion of mould-infested material has been shown to give rise to acute or chronic toxic effects, the substances responsible should be isolated and identified. This basic research is essential, but is not in itself sufficient. It will have to be followed-up by research aimed at developing reliable and practicable techniques for the quantitative determination of proven mycotoxins in foodstuffs and feeds. The need for reliable methods suitable for routine use is emphasized by the fact that in South Africa, nearly 4,000 samples of groundnuts alone are required to be assayed annually for their aflatoxin content. Until recently, the only methods available for the determination of mycotoxins and in general use were those developed for the assay of aflatoxin in groundnuts and cereals. Research workers in South Africa have now, however, developed a method for the assay of ochratoxin in cereals[10] and an improved technique for the determination of aflatoxin M in milk.[11] Routine screening for these toxins should now also be considered, and it is gratifying that assay methods for mycotoxins are now being scrutinized by the International Union of Pure and Applied Chemistry.

The problem still faces us, however, that while the currently available assay techniques may be sensitive enough to detect quantities of toxin capable of producing acute damage, we cannot rely on them to disclose the presence of smaller quantities which, when ingested over long periods, may cause pathological changes resulting in impaired health and possibly malignancy. Aflatoxin and its metabolites are not present in detectable quantities in meat and other products obtained from animals dosed at low levels with aflatoxin,[12] but we have recovered them within a few days from the liver and blood of animals given a single large dose by injection. It is conceivable, therefore, that aflatoxin may be present in small but significant quantities, currently undetectable, in meat obtained from animals which have ingested aflatoxin.

A further barrier to effective control is the fact that it is extremely difficult to obtain representative samples of foods such as cereals and groundnuts for the purpose of analysis. Individual groundnut kernels which have become mould-infested have been found by us to contain as much as 1,500 μg. of aflatoxin/G, while overseas workers have reported levels as high as 4,000 μg./G for individual kernels.[13] Calculations based on these findings indicate that a single mouldy kernel could be responsible for an aflatoxin content of 45 μg./kg. in 200 lb. of groundnut meal, making it unacceptable for use in animal feeds according to the standards that have been laid down in South Africa. The possibility of missing such heavily-infected kernels in the sampling process, when they are scattered and few in number, is obviously very great.

*Studies on Toxicity*

Much has already been learnt from observations on animals about the toxic effects of aflatoxins B and G and the nature of the lesions caused by these substances, and investigations aimed at obtaining similar information about ochratoxin A and aflatoxin M, are at present in progress in our laboratories. The effects of these and the other known mycotoxins on man, however, remain completely unknown.

Experiments of various kinds are being carried out on laboratory animals in South Africa, where particular attention is being given to the possibility that the development of primary liver cancer in man may be related to the ingestion of mycotoxins. As is well known, the incidence of primary carcinoma of the liver is much higher in the Bantu population of Southern Africa than in any of the other population groups, and no satisfactory explanation of the racial selectivity displayed by this tumour has yet been found. We have found no evidence that the maize meal which reaches the consumer through commercial channels and forms the staple food of the Bantu contains any mycotoxins, nor are we aware that the Bantu have any preference for mouldy cereals, as has been claimed by Kraybill and Shimkin.[14] It is possible, however, that maize contaminated with moulds as a result of storage under primitive conditions may be consumed by the producer, if food is in short supply. Epidemiological studies have indicated that the incidence of hepatoma is higher in certain areas than in others, but a study of the climatic conditions in these areas has failed to prove on climatic grounds that mould infestation is more liable to occur in the areas where the incidence is highest. In addition, samples obtained at autopsy from the cadavers of liver-cancer victims have not yielded any evidence that aflatoxin is present in the liver in this disease. Much of the work done so far has therefore produced negative results, but these results are inconclusive and a great deal more work will have to be done, before we shall be able to say with certainty whether or not aflatoxin or any other mycotoxin is a causative factor in the aetiology of liver cancer in South Africa.

*Additional Investigations done in our Laboratories*

The indirect contamination of food of animal origin with mycotoxins was first reported by Allcroft and Carnaghan[12] and by De Iongh.[15] Aflatoxin present in the feed of cows appears to be metabolized by these animals and partially excreted in their milk, from which it can be recovered in the intermediate hydroxylated form. This metabolite was given the name of aflatoxin M and was stated to be toxic to ducklings. Though recoverable from milk it could not be detected in eggs or meat obtained from hens and other animals fed on aflatoxin-containing rations.

These findings have been confirmed in our laboratories and sufficient quantities of aflatoxin M isolated from milk to enable its structure to be established[16] and to formulate a reliable assay method.[11] It was found that the toxic fraction consisted of 2 compounds which were named aflatoxin $M_1$ and $M_2$.[16]

As stated earlier, failure to detect the presence of aflatoxins in meat is not a guarantee of their absence. The fact that with our improved technique we have already found concentrations of aflatoxin in milk considerably higher than any previously reported, indicates that the amount of aflatoxin which can be considered permissible in animal feeds is a question that needs careful reconsideration.

Even at the levels of contamination now permitted in animal feeds, the utilization of aflatoxin-contaminated groundnut presscake presents a very real problem. If these levels were to be lowered, the amount of contaminated presscake that could be incorporated into animal feeds would be substantially reduced and large quantities of this otherwise valuable protein-rich product wasted, unless some means could be found of detoxifying the contaminated material.

We have found in our laboratories[17] that if the hexane normally used in the process of oil extraction is replaced by a mixture of hexane and acetone, the aflatoxin content of groundnut presscake can be reduced to very low levels. An aflatoxin content of 5,000 μg./kg. in a sample of groundnuts was for instance brought down to 60 μg./kg. in the presscake by extracting the sample for 10 hours in a Soxhlet extractor with an azeotropic mixture of hexane, acetone and water.

The envisaged cost of converting existing plants, together with certain problems connected with the recovery of the solvents, have so far kept the industry from evincing any serious interest in the application of this technique. The time may come, however, when we shall be forced to resort to this or some other method of eliminating excess aflatoxin, and it is obvious that further research in this direction is essential.

## SUMMARY AND CONCLUSIONS

With the discovery of the mycotoxins, a vast new range of urgent problems has been uncovered and presented to the food technologist, the microbiologist, the chemist and the toxicologist for solution. It has been proved beyond doubt that a variety of fungi capable of elaborating toxic metabolites can grow on foodstuffs under favourable conditions. Some of these metabolites have been shown to be acutely toxic and some carcinogenic to laboratory animals. No direct evidence of deleterious effects in man that can be ascribed definitely to the newly-isolated mycotoxins has yet been forthcoming, but the results of animal experiments have been so alarming that several countries have shown their concern by prohibiting the sale of aflatoxin-contaminated foodstuffs. This step has necessitated the routine use of chemical assay techniques which have many drawbacks in practice.

A partial solution to the problem of mycotoxin contamination may lie in effective decontamination methods. Far more effective control will undoubtedly be achieved by developing more reliable and practicable analytical techniques for routine use, in order to cope with the enormous amount of assay work involved in the control of a steadily increasing number

of proven mycotoxins. Obviously, however, the most practical solution to the problem would lie in realistic practices aimed at the prevention of mould growth on foods and feeds at all stages before consumption.

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