The Microbiological Assay of Aflatoxin

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ABSTRACT

A number of possible bases for a biological assay for the aflatoxins were examined, of these two were selected for further study. An inhibition assay using Bacillus megaterium was developed in combination with thin layer chromatographic analysis. This method was capable of detecting 0.1 µg of aflatoxin B_1 and of differentiating between various mycotoxins.

An induction assay using a lysogenic strain of Bacillus megaterium was also developed. This assay has a sensitivity of 2-4 µg/ml aflatoxin B_1 and is claimed to be more specific than the inhibition assay.

A number of other mycotoxins were examined to test the versatility and specificity of the assays. The inhibition assay was able to detect and differentiate between most of the toxins. The induction assay detected only those mycotoxins reported or suspected to be carcinogenic.

PREFACE

It has been known for many years that certain fungi produce substances toxic to human beings and livestock. Toxic Basidiomycetes and ergotism have been responsible for the loss of many lives. It is only comparatively recently however that attention has been directed to the imperfect fungi as sources of toxins. In particular, a dramatic outbreak of an unknown disease among poultry in the United Kingdom, called Turkey X Disease, was eventually traced to a substance produced by Aspergillus parasiticus. This active principle was named 'aflatoxin' after the Aspergillus flavus-oryzae group to which Aspergillus parasiticus belongs.

'Aflatoxin' was soon shown to be in fact a number of related compounds which were designated B_1, B_2, G_1 and G_2 due to the blue or green colour of their fluorescence under ultra-violet light and the relative positions they reach on thin layer chromatographic plates. Not all the aflatoxins are of equal potency, B_1 being the most potent and G_2 the least.

The aflatoxins are of especial interest since they have been shown to be carcinogenic in very low concentrations in addition to their hepatotoxic effect at higher concentrations. Aflatoxins have been reported from a wide variety of fungi, chiefly Aspergillus and Penicillium species though other fungi, including a Rhizopus species, have been implicated. It was also shown that the toxins could be formed on a wide range of substrates and could easily occur in human foodstuffs if the fungus was allowed to grow on them.

Chemicophysical methods of detection and quantitation have been developed, based mainly on extraction by an organic solvent followed by concentration, separation on thin layer chromatographic plates and fluorodensitometric estimations of the spots.

Chemical assays are rapid, sensitive and very precise but may not always accurately reflect the biological activity of the substance under test. This is particularly the case where very small chemical differences

can make very large changes in activity. There have been shown to be at least nine different aflatoxins with biological activities varying from extreme toxicity to practically no effect. It is therefore desirable to have a biological assay to complement the results of the chemical assay.

The ideal bioassay would be simple, speedy, sensitive, specific and, of course, reproducible. Early bioassay methods were based on the toxicity of aflatoxin to vertebrates; the most sensitive and specific assay being that using ducklings. Fish larvae and fertile eggs have also been used. Later an inhibition-zone microbiological bioassay using Bacillus megaterium was developed and put forward as a standard method in the Journal of the Association of Official Analytical Chemists. There have been reports in the literature of other effects of aflatoxin which might be suitable for a bioassay system. Among these are tissue-culture abnormalities, induction of lysogenic bacteria and suppression of chloroplast development in germinating cress seeds.

The assay based on ducklings is relatively specific but requires skill to perform and is not nearly as rapid or sensitive as chemical methods. The inhibition assay using B. megaterium is more rapid and easier to perform but is not very specific and is also less sensitive than chemical assays though it requires much less material than does the duckling assay.

For these reasons it was considered worthwhile to look for an assay as specific as the duckling assay but with the advantages of a microbiological assay. A number of possibilities had already been suggested by various authors and it was these that I proposed to investigate.

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