REVIEW OF THE TOXICOLOGY OF AFLATOXIN

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ABSTRACT

Most laboratory animals, with the exception of the mouse, are readily killed by the aflatoxins. Following the administration of aflatoxin B_1 to the rat, the species most extensively studied, the main lesions are seen in the liver, while kidney and adrenals show damage. The other aflatoxins are less toxic to the the rat and duckling. Aflatoxin M_1 is similar to B_1 in its acute toxicity. The acute toxicities may be modified by the nutritional state of the animal. A marginal choline diet affords protection from the hepatotoxic action B_1. The aflatoxins are widely recognized as being carcinogenic for many species. In the rat B_1 induces hepatic carcinoma at levels of 15 ppb in the diet. G_1 is less carcinogenic for the rat liver, but also induces renal carcinoma. Aflatoxin M_1 has been shown to induce carcinoma in trout liver but feeding trials in rats have been unsuccessful in inducing neoplasia. Neoplasms at sites other than the liver have been induced by the aflatoxins. The 'mycotoxin hypothesis' for the etiology of human hepatic carcinoma has not been conclusively demonstrated although the circumstantial evidence to support the hypothesis is increasing. Some control measures employed to control the hazards of mycotoxins are justified and warrant further investigation.

At this Meeting considerable emphasis has been placed on the methodology of assay of aflatoxins in food and feed, and their application to the control of mycotoxins. It would appear to be reasonable at this stage to consider what control measures are justified in making some assessment of the problem of aflatoxins. The hazards presented by mycotoxins may be conveniently divided into two classes. The first is related to the economic loss to producers of agricultural products, which will result from poor growth of livestock and subsequent failure to reach market weight. The second hazard is that to man. This may result from direct ingestion of mycotoxins from the food such as groundnuts and cereals contaminated with fungi and also from secondary contamination from eating meat from animals with residues of mycotoxins or their metabolites in the tissues. The hazards to man may be either those of acute toxicity or those of long-term chronic toxicity and carcinogenicity. In this paper I will confine my comments to aflatoxin and give some evidence which enables us to make an assessment of these hazards.

Most species are susceptible to the acute toxic action of aflatoxin when

fed what is considered a normal diet[1]. The range of susceptibility for laboratory and farm animals is great, varying from the highly susceptible young poultry to the much more resistant mouse and sheep. Within the species there are also strain and sex differences in susceptibility. For example the LD_{50} to the male Wistar rat is to the order of 7 mg kg^{-1}, while to the mature female[2] it is to the order of 14 mg kg^{-1} and the male inbred Fischer rat 2 mg kg^{-1}. Those animals which are acutely susceptible to the toxicity of aflatoxin such as the duckling, turkey poult, guinea-pig and calf are those in which field outbreaks of aflatoxicosis occurred at the time when the problem was recognized. It is now possible to recommend levels of aflatoxin in the diet for these species which will not result in loss of condition and poor growth. This however does not infer that these animals will be free from aflatoxin residues in the flesh to be used for human consumption. In the context of acute toxicity it is worth noting that monkeys are known to be susceptible to acute poisoning[3,4].

As we have heard, Patterson has investigated the correlation between the rate of metabolism of aflatoxin B_1 and the acute toxicity of the aflatoxin to various species. At present it would seem that the ability of microsomal systems to convert aflatoxin B_1 into a non-toxic metabolite is not correlated with the LD_{50}[5,6]. In *in vitro* systems the product from microsomal metabolism can be shown to be non-toxic to day-old ducklings. Patterson suggested that this metabolite when produced within an intact cell may be toxic to that cell. The other possibilities to be considered are that the aflatoxin is itself a directly acting toxin not requiring metabolism, or if metabolism is required that an alternative pathway results in the production of a so far unidentified metabolite. In this context Garner, using a modified host mediated assay system, demonstrated that the product from *in vitro* metabolism of aflatoxin B_1 is toxic to strains of *salmonella*. He concluded from his work that aflatoxin B_1 forms an epoxide at the terminal furan ring which is the toxic compound[7]. However, no direct evidence is given for this and as the incubation medium is similar to that used by Patterson one possible explanation is that a toxic intermediate is formed prior to the hemiacetyl aflatoxin.

The acute toxicity of the aflatoxins may be modified. Newberne demonstrated that rats maintained on a marginal choline diet for two weeks are resistant to the necrogenic action of aflatoxin B_1 for the liver[8]. The LD_{50} changes from between 1 and 2 mg kg^{-1} to something over 15 mg kg^{-1}. This protection is against both the lethal action of aflatoxin and the necrogenic action. Protein-depleted animals are in the case of aflatoxin less resistant to the necrogenic action[9]. In contrast to this DDT treatment induces resistance. A more drastic way of modifying the acute susceptibility to aflatoxin is that of hypophysectomy[10]. Hypophysectomized animals are considerably more sensitive to the acute toxicity of aflatoxin. This will be referred to again when discussing the carcinogenic action of aflatoxin.

Much work has been published on the comparative toxicities of the major aflatoxins and their metabolites and will not be further discussed here except to note that aflatoxin M_1, which is one of the principal metabolites and of concern in public health, has a very similar acute toxicity to that of aflatoxin B_1[11].

There is very little evidence as to the acute susceptibility of man to the aflatoxins. There has been a report from Uganda in which a boy of 14 years died of acute hepatic necrosis and it was found on subsequent examination that the diet (cassava) consumed by this boy contained large amounts of aflatoxin[12]. Further, recently, Shank and Wogan and their colleagues in Thailand have described an acute lesion of encephalopathy in children which has a seasonal incidence and is associated with damage to both liver and kidney[13]. Although they did not have direct evidence that this was indeed induced by aflatoxin the compound was found in the viscera of the children. Further they were able to reproduce the encephalopathy in Macaque monkeys mimicking the human disease[14].

Earlier in this Meeting we heard from Dr Hayes something of the evidence that rubratoxin is a teratogen. With aflatoxin there is a report from Di Paolo, Elis and Erwin[15] that it is teratogenic for the hamster. In the rat an extensive series of both feeding and acute single-dose experiments failed to demonstrate teratogenicity[16]. When single doses of aflatoxin were given in the early stages of pregnancy there was no demonstrable effect upon the foetus. When given in the later part of pregnancy from day 16 there was considerable foetal stunting on day 21, and when maintained for their life-span there was no evidence of an increased incidence of neoplasm attributable to aflatoxin in either male or female rats. The possible implications for humans is that if aflatoxin is used as a supplemental food for pregnant women they may be more accurately susceptible to the aflatoxin. It is known that pregnant women are more susceptible to other hepatoxic agents during pregnancy.

However, it is the carcinogenicity of the aflatoxins which necessitates continuing the control measures which have been instituted. Aflatoxin B_1 or mixed aflatoxins have been shown to be carcinogenic to mammals, birds and fish[1]. Aflatoxin G_1[17], M_1[18] and possibly B_2[19] are also carcinogenic in some situations. It is however worth pointing out at this stage, as Dr Goldblatt mentioned in his introductory talk, that one can only consider the carcinogenicity of these compounds within the experimental protocol used.

At the present time it is uncertain that hepatic tumours may be induced in monkeys. Monkeys have been demonstrated to be chronically susceptible to aflatoxin and the experiments from Glaxo Laboratories showed very interesting change within the livers of monkeys fed aflatoxin for 3 years[20]. Unfortunately these experiments were not prolonged further and no unequivocal hepatic neoplasms were seen.

The levels upon which the regulatory authorities base their recommendations are those derived from our data and that of Professor Newberne[1]. In our hands, using Wistar–Porton strain rats, it is possible to show that to the rat liver aflatoxin is the most potent carcinogen known[21]. It was also possible to demonstrate a dose response and a sex difference in the incidence of tumours. At high level of dosage 100 per cent of both males and females develop hepatic carcinoma. However, at lower doses the female is less susceptible which is similar to the observations following the feeding of other carcinogens to the rat and also that which is observed in the human situation. The experiments of Wogan and Newberne[22], using inbred Fischer rats, demonstrated that levels as low as 15 ppb of aflatoxin B_1 in the diet

may induce 100 per cent incidence of hepatic neoplasia. This information has been published extensively and will not be given again in detail.

As in the case of the acute toxicity the carcinogenic action of aflatoxin may be modified. Earlier I referred to the fact that hypophysectomy increases the susceptibility of rats to the acute toxic action of the aflatoxin. In contrast to this, long-term feeding of aflatoxin to hypophysectomized animals indicated that these animals are resistant to the carcinogenic action of aflatoxin[23]. In the experiments which we have described the animals ate aflatoxin at dietary levels which would have resulted in a high incidence of neoplasia. Newberne and Williams[24] have demonstrated that diethyl stilboesterol administered to rats affords some protection against the carcinogenic action of aflatoxin, and McLean and Marshall[25] have shown that phenobarbitone also affords some protection while a marginal choline diet appears to enhance the carcinogenic action of these compounds[8]. At this Meeting Dr Purchase discussed some of the problems of extracting aflatoxin from various foods. He showed that for peanuts methanol extraction was one of the more efficient methods. However, it is worth noting that in the original experiment describing the carcinogenesis of choline-deficient diets, hepatic tumours arose in animals fed choline-deficient diets in which the protein supplied was peanut protein extracted with methanol. In this same experimental situation if one ensures that all the aflatoxin is extracted along with the choline no neoplasia is induced in the liver.

We have heard at this Meeting of Dr Sinhuber's work demonstrating that aflatoxin M_1 is carcinogenic for the trout liver[18]. We have attempted to demonstrate the carcinogenicity of milk containing aflatoxin M_1. This work was carried out in collaboration with Dr Ruth Allcroft, then of Central Veterinary Laboratory, Weybridge. She fed cows high levels of contaminated peanut meal, the milk was collected and roller dried. On assay this dried milk contained 30 ppb of aflatoxin M_1. Initially this was fed as 50 per cent of the diet to our colony of Wistar–Porton rats for 2 years. In those animals there was no evidence of tumour induction attributable to aflatoxin. This experiment was then repeated using inbred Fischer rats similar to those used by Newberne. The milk, which was toxic to day-old ducklings, was fed to the rats for 18 months. When the diet was exhausted the animals were returned to normal diet, and maintained for their life-span. In these animals there is still no evidence of neoplasia induced by aflatoxin.

Considerable emphasis has been placed on the hepatic tumours induced by these mycotoxins. However, there is evidence now that although the liver may be the principal site of induction of neoplasia other tumours may be induced. These are principally of the alimentary tract (oesophagus, glandular stomach, duodenum and colon in the rat) and also possibly salivary gland and Harderian gland[26]. Kidney-epithelial tumours may also be induced by aflatoxin G_1[17]. In our experiments aflatoxin G_1 produces a high incidence of such tumours, while they are rarely seen following aflatoxin B_1. These results are somewhat contrary to those reported by Epstein, Bartus and Farber[27] who showed strain differences in the susceptibility of B_1, in that one strain of rats resulted in the induction of renal tumours in preference to liver tumours.

In view of this data, upon which regulations are based, what control

measures are justified? For man, the hypothesis for the induction of liver cancer by mycotoxins still requires proof[28], though there is increasing circumstantial evidence that the high incidence of chronic liver disease in various parts of the world may be mycotoxin induced. It would appear justified on the evidence to assume, at least in part, that the mycotoxins were responsible for this hepatic neoplasia. Further one must consider the extrahepatic tumours. These are common in both Western Europe and North America and the possible role of mycotoxins should be considered in the induction of these neoplasms.

In the United States, although the Delaney Amendment was designed for food additives, the principle of a nil tolerance for carcinogens in the food seems to be widely held. I would suggest that with present information this is unfortunate as it tends to prevent rational discussion of problems. Although Western European countries may not necessarily have such legislation a similar principle is often applied. At present the WHO/PAG recommend that supplemental foods for under-nourished children should not contain more than 30 ppb aflatoxin B_1 in the supplement. This can be shown to be a level having long-term effects on experimental animals, but in the light of present knowledge it would appear to be a reasonable level in those developing countries where protein malnutrition is a problem.

However, these observations should not prevent one considering the effects of any recommendations which regulatory authorities may make in the developed countries. What practical levels can be recommended and what are the likely effects of these recommendations? The United States is a consumer and a producer of peanuts. Europe is a consumer and not a producer, but many of the developing countries are producers. Dr Goldblatt in his opening address said that paradoxically the recognition of aflatoxin has resulted in higher quality peanuts being available to the consumer in the United States. This is probably also true of Western Europe. However, the converse may well be true of the developing countries, in which there are economic difficulties and food shortages. If consumer countries make only extremely low levels of aflatoxin permissible in the diet this will have two effects. Firstly the producing countries, because of the difficulties in agriculture will be unable to sell their product, leading to economic loss. Secondly these are countries in which there may be protein malnutrition. The contaminated peanuts, will of necessity, be eaten by the population so presenting an increasing health problem. In view of this I think that it is only reasonable that consideration is given to the effects of the recommended levels. Until the agricultural conditions in the developing countries can be improved, enabling them to produce peanuts of a quality which is considered safe by the regulatory authorities of the developed countries, it would appear reasonable that some form of assistance is made to the developing countries enabling them to institute improved agricultural methods. In this way the paradox of higher quality peanuts following the discovery of aflatoxin will be apparent, not only to us in the developed countries, but also to those in the developing countries.

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