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# MODE OF ACTION AND HUMAN HEALTH ASPECTS OF AFLATOXIN CARCINOGENESIS

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Abstract - The toxicity and carcinogenicity of the aflatoxins have been studied extensively in the rat. In this species these compounds are recognized as some of the most active hepatocarcinogens. There is now increasing evidence of both acute and chronic toxicity in man supporting the 'mycotoxin' hypothesis. The study of the mechanism of action of the aflatoxins on rat liver indicate that the pattern of events is similar to other hepatic carcinogens and that an irreversible change is induced at an early stage.

At the First I.U.P.A.C. sponsored Symposium on Mycotoxins in Foodstuffs in 1973 various aspects of toxicology and carcinogenicity of the aflatoxins and other mycotoxins was discussed (Ref.1). At the same time there was considerable discussion on methodology, e.g. the assay of aflatoxin and the application of methods to control mycotoxin contamination of foodstuffs. In a review of the toxicology and carcinogenicity of the aflatoxins, evidence was presented demonstrating why such world wide concern was justified. Since that time extensive epidemiological studies have been reported, yielding increasing evidence that man is susceptible to the acute toxicity of those compounds (Ref.2) as well as their carcinogenic activity (Ref.3). The aetiology of human hepatic neoplasia and its possible connection with the mycotoxin hypothesis (Ref.4) was formulated at a time when the carcinogenicity of these compounds was recognised but there was only superficial circumstantial evidence as to their role in human disease. Information is now available of detailed epidemiological surveys correlating the incidence of hepatic carcinoma with the daily intake of aflatoxin (Ref.5). In this review we wish to discuss some of the experimental studies being undertaken to investigate the mode of action of aflatoxins which it is hoped might give direct evidence of the susceptibility of man.

The aflatoxins were originally isolated following outbreaks of acute disease in many species of animals all having liver lesions as their common factor (Ref.6 & 7). Considering the known presence of these toxic substances in the environment coupled with the circumstantial evidence implicating them in chronic disease, it would be surprising if there was not also evidence of acute toxicity in man. However, the evidence for this is still sparse and little of it is conclusive. The first report was from Uganda (Ref.8) of a young male dying with acute liver disease. Histologically centrilobular necrosis was seen. Investigation of the food consumed, casava, demonstrated heavy contamination with aflatoxin. In recent years Reyes' syndrome, which presents as an encephalopathy in children with fatty degeneration of the liver and kidneys, has been associated with acute aflatoxicosis. In a series of studies from Thailand, children dying of Reyes' syndrome, which has a seasonal variation in incidence, have been shown to have increased amounts of assayable aflatoxin in their tissues compared with unaffected children dying from other causes (Ref.9). Although the causal relationship between aflatoxin ingestion and the induction of the full clinical syndrome is somewhat tenuous, it has been possible to reproduce the encephalopathy in the macaque (Ref.10). Further there have been reports from India of an outbreak of disease involving nearly 400 patients with a 20% mortality. This has been discussed in detail elsewhere in these proceedings but it is worth noting that the disease was characterized by jaundice and evidence of portal hypertension. Furthermore the dogs which shared the food from the affected household also died with jaundice. The aflatoxin levels reported in the food ranged from 6.25 to 15.6 ppm. It is of interest that some of the earliest outbreaks of disease which retrospectively were traced to aflatoxin, occurred in dogs, indicating their extreme sensitivity to the toxin (Ref.2).

The carcinogenicity of the aflatoxins is too well known to require presentation of the evidence (Ref.11). However, it is worth recalling that, in the rat, levels as low as 0.015 ppm continuously or short term higher dosage is sufficient to induce a high incidence of hepatic carcinoma. The aflatoxins are also carcinogenic for trout (Ref.12),

duck (Ref.13), monkey (Ref 14) and the neonatal mouse (Ref.15). It is of interest that the adult mouse appears not to be susceptible to the carcinogenic action of aflatoxin. Aflatoxin has also been shown to induce neoplasia in other sites, notably the kidney (Ref.16), stomach (Ref.17) and the colon (Ref.18). At present the main interest lies in investigating the mechanism of action for these compounds and further elucidating their role in human disease, notably hepatocarcinoma.

The experimental induction of hepatic carcinoma has been studied using a variety of inducing chemicals. Most work has been done using acetylaminofluorine and its derivatives, butter yellow (2,4-dimethylaminoazobenzene), ethionine, the nitrosamines and aflatoxin. The first three of these compounds are not now considered to present a hazard to man but there is increasing evidence of environmental exposure of man to low levels of nitrosamines and aflatoxin. There appears to be a common pattern of events prior to the recognition of unequivocable hepatocarcinoma. The pattern may be somewhat confused if the dose of the inducing chemical is close to its hepatotoxic dose. However, in the absence of extensive necrosis and fibrosis which is best studied using low levels of nitrosamines or aflatoxin there is a similar sequence of events.

We have studied the carcinogenic process using a schedule in which a high incidence of hepatic carcinoma is induced by feeding 5 ppm aflatoxin for 6 weeks followed by return to normal diet. At the end of the 6 weeks feeding period an irreversible change has occurred within the liver in that no further action is required to induce carcinoma. Morphological examination of the liver at this time demonstrates multiple foci of cells which are characterized by the presence of starvation resistant glycogen and a loss of glucose-6--phosphatase activity (Ref.19). This is similar to that reported in nitrosamine carcinogenesis (Ref.20). However, these foci are present throughout the liver and it is difficult to know, which, if any, of them are related to the final neoplasm or indeed the fate of those which do not develop into carcinoma. It is hoped that if it proves possible to characterize and identify the various sub-populations of liver cells resulting from the carcinogenic feeding regime, then it may be possible to use this as the basis for a predictive test for man.

The proliferative response resulting from feeding aflatoxin to rats has been studied by measuring the incorporation of tritiated thymidine into DNA. It has been shown that following an early inhibition, lasting for about 3 weeks, there is subsequently an increased incorporation of thymidine, indicating a proliferative response (Fig. 1). The mitotic index reflects this increased incorporation of thymidine but it is of interest that both the foci of cells, containing starvation glycogen, and the more normal lobular parts of the liver have an increased mitotic index (Arora, Butler and Neal, unpublished observations). It has been shown that if the estimated daily dietary intake of aflatoxin is given as a single i.p. injection there is a marked inhibition of both RNA and DNA synthesis (Fig. 2). It is worth noting that the inhibition of DNA synthesis is considerably greater than that of RNA. This parallels the observations during the early stages of the 6 weeks feeding cycle. It has also been shown that at the end of the 6 weeks feeding cycle rats become resistant to the acute toxicity of aflatoxin. The $LD_{50}$ is increased from 0.6 mg/kg to more than 1.5 mg/kg (Ref.21).

Tissue culture studies have shown that it is possible to produce lines of hepatic parenchymal cells from both control and treated rats. These lines grow after an initial period of maintenance culture which is considerably shorter in the case of cells isolated from those animals treated with aflatoxin. In parallel with the results of experiments in the whole animal, the cells derived from aflatoxin treated animals are also resistant to the acute toxicity of aflatoxin when treated in culture (Ref.21). However, it is not known at present whether it is the cells from the proliferative foci or the more normal parts of the liver lobule which form the cell lines in the continuous cultures from aflatoxin-treated rats.

In all the studies on experimental hepatocarcinogenesis utilizing aflatoxin as the inducing agent it is apparent that the neoplasm usually arises in a liver in the absence of cirrhosis. The experience in man is that in Europe and North America most hepatocarcinomas arise in cirrhotic patients (Ref.22). This has led to the view that cirrhosis predisposes to carcinoma. However, in those areas where hepatocarcinoma is a relatively common disease, many arise in livers showing no cirrhosis. Also there is little evidence that in those areas there is a corresponding increase in the incidence of cirrhosis. It is possible that in areas of high incidence of hepatic neoplasia multiple factors influence the development of the carcinoma and it has been shown that animals fed low lipotrope diets have an increased susceptibility to hepatocarcinomas (Ref.23).

It has also been suggested that hepatitis B plays a significant role in the induction of hepatic carcinoma. In many areas of the world where hepatocarcinoma is common, there is a higher incidence of hepatitis B antigen in those patients with carcinoma and also an increased incidence in the population as a whole (Ref.22). However, this correlation shows considerable variation throughout the world. The mechanism is not understood at present,

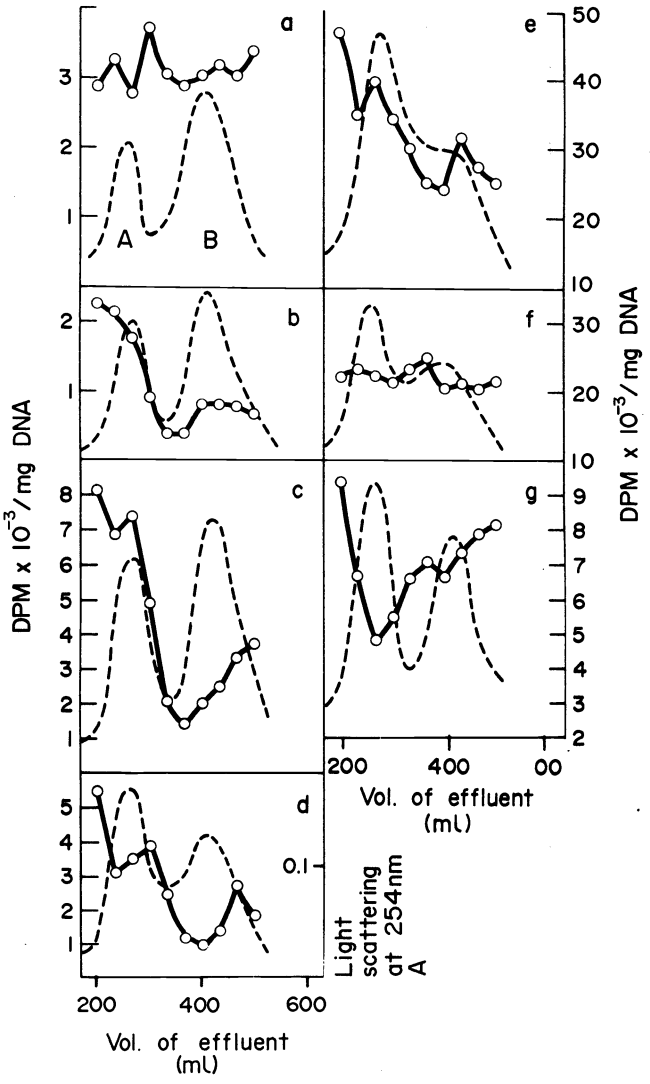


Fig. 1. Effect of feeding low level of aflatoxin on hepatic ploidy and DNA synthesis in vivo. Zonal centrifugation, nuclear profiles and [$^3$H] thymidine incorporation into nuclei during 6 weeks of feeding aflatoxin-containing diet. a) controls, b) 1 week's feeding, c) 2 weeks' feeding, d) 3 weeks' feeding, e) 4 weeks' feeding, f) 5 week's feeding g) 6 weeks' feeding. O [$^3$H] incorporation into nuclei (24 h after [$^3$H] thymidine injection 20 µCi/rat - - - light scattering at 254 nm A = zone occupied by diploid nuclei; B = zone occupied by tetraploid nuclei. Zonal centrifugation of isolated nuclear fractions carried out on 20-50% sucrose gradient in M.S.E. Zone 'A' rotor.

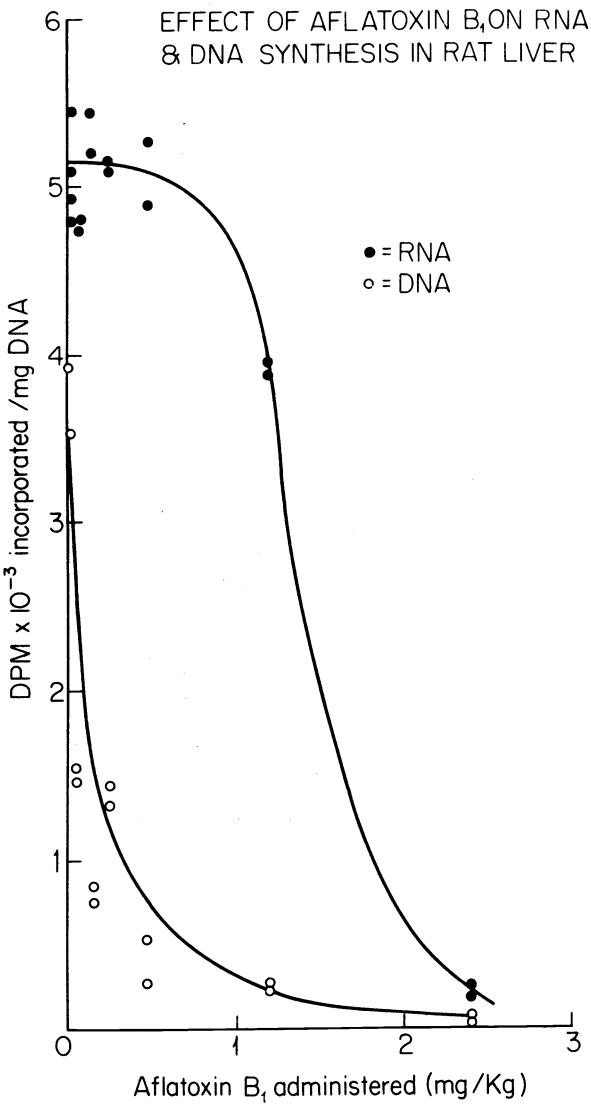


Fig. 2. The effect of aflatoxin $B_1$ injected into rats on RNA and DNA synthesis in vivo. Groups of 2 male Fischer rats (approx. 250 g body wt) injected i.p. with aflatoxin $B_1$ dissolved in DMSO (200 µls) 1.5 h after these injections 20 µCi me-[$^3$H] thymidine (21 Ci/mmole) and 0.5 µCi 6-[$^{14}$C] orotic acid (60.8 m Ci/mmole) injected i.p. in 0.25 ml saline. Animals killed 1 h later, nuclear fractions prepared and incorporation of label into acid insoluble form determined.

but it is possible that there are synergistic effects between environmental factors such as the aflatoxins and the exposure to hepatitis B antigen.

The recognition of the aflatoxins as hepatocarcinogens led to the development of the mycotoxin hypothesis for the aetiology of hepatic carcinoma in man. We do not wish to reiterate the epidemiological evidence for this but the surveys which have been reported from Kenya, Thailand, Swaziland and Mozambique demonstrate a good correlation between the ingestion of aflatoxin and the incidence of hepatic carcinoma. Although such investigations do not prove the hypothesis, the evidence is such that this is the best hypothesis available for those areas of the world with a high incidence of carcinoma. However, in other areas such as Western Europe or North America where exposure to aflatoxin is slight it is necessary to consider the effect of other agents such as alcohol and certain drugs used for long term treatment.

It is obvious from the many studies that have been undertaken investigating the mechanism of action, the experimental induction of carcinoma and environmental factors, it is unlikely that man is exposed to only a single hepatocarcinogenic agent or indeed that the same group of factors will induce the same pattern of disease in all communities. As mentioned above it has been shown that animals on low lipotrope diets have increased susceptibility to aflatoxin hepatocarcinogenesis while in contrast vitamin 'A' deficient animals have an increased incidence of colonic carcinoma also induced by aflatoxin (Ref.18). While the epidemiological surveys have at present focused on hepatic neoplasia such experimental data surely indicates that the scope of future investigations must be broadened to include other neoplastic disease.

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