Biochemical Responses to Aflatoxins

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INTRODUCTION

Many investigations have dealt with cellular biochemical alterations associated with the toxicity response to aflatoxins in susceptible biologic systems. The general objectives of these studies has been elucidation of the mode of action of the compounds. Thus, evidence has been sought for a sequence of biochemical events initiated by interaction of the toxins with cellular constituents and leading ultimately to cytologic manifestations of toxicity. On the basis of available information, it is not possible to construct a definitive sequence of biochemical events leading to morphologic manifestations of toxicity and/or carcinogenicity. However, results of numerous investigations have revealed general patterns of reactions that are thought to be associated with the toxicity response by virtue of the time course and consistency of their occurrence, and their potential importance in cellular metabolic phenomena.

The reactions involved in these responses are concerned with alterations in nucleic acid and protein metabolism elicited by exposure to aflatoxins. Much of the available evidence has come from experiments dealing with alterations induced in liver tissue following in vivo or in vitro exposure to the toxins. Useful information has also been derived from experiments on in vitro cell cultures and cell-free systems. Effects of aflatoxin B\textsubscript{1} have been most extensively studied, owing to the greater availability and potency of this member of the complex.

Administration of the compound to rats is followed by rapid and marked inhibition of liver DNA and RNA synthesis, consequences of inhibition of DNA and RNA polymerases. Protein synthesis is also impaired, particularly under conditions where the synthetic process is strongly influenced by altered RNA metabolism. The observed inhibition of nucleic acid polymerases could result from direct action of the toxin with the enzymes themselves, or may be an indirect consequence of modified DNA template activity subsequent to toxin-DNA interaction. Although this point has not been completely resolved, available evidence tends to support the latter hypothesis. Thus, interaction of the toxins with DNA is viewed as the event initiating the series of observed phenomena. The purpose of this report is to provide a brief summary of experimental evidence for this sequence of biochemical events. The subject has been reviewed more comprehensively elsewhere (35).

INHIBITION OF DNA SYNTHESIS

Inhibition of DNA synthesis in cells exposed to aflatoxin preparation has been demonstrated under several experimental systems. Under in vivo conditions, this response is particularly evident in rat liver undergoing regeneration after subtotal hepatectomy. Frayssinet et al. (11) reported that aflatoxin B\textsubscript{1} inhibited the net synthesis of liver DNA when administered to rats prior to or immediately after hepatectomy at dose levels of 30 or 60 µg/animal. Using similar technics, de Recondo et al. (8, 9) showed that administration of the toxin at 100 µg/animal inhibited the incorporation of thymidine-\textsuperscript{3}H into liver DNA to the extent of 95% within 12 hours. They further demonstrated that the enzymes responsible for DNA synthesis remained fully active under these conditions, and concluded that the toxin exerted its inhibitory effects by interacting with DNA in such a way as to impair its ability to act as a primer for DNA synthesis. Impaired DNA synthesis in vivo has also been reported by Rogers and Newberne (28), who found that a single large dose (3 mg/kg) of aflatoxin B\textsubscript{1} given to rats caused marked reduction of thymidine-\textsuperscript{3}H labeling of liver cell nuclei, as determined autoradiographically. This response persisted for about 50 hours after dosing.

Using in vitro cultures of diploid and heteroploid human embryonic lung cells, Legator and Withrow (21) reported that the mitotic rate was reduced by the presence of aflatoxin B\textsubscript{1} in the medium at a level of 0.01 µg/ml. Subsequently, Legator et al. (22) and Legator (20) reported that the toxin inhibited incorporation of thymidine-\textsuperscript{3}H into these cells at concentrations of 0.1 to 1.0 µg/ml in the culture medium. More recently, Zuckerman et al. (38) reported similar effects of aflatoxin B\textsubscript{1} in cultures of human embryonic liver cells, in which the compound had an LD\textsubscript{50} value of 1 µg/ml.

Further experiments dealing with effects of the toxins on DNA polymerase activity are of interest. Wragg et al. (37) studied the ability of DNA isolated from Escherichia coli cultured in the presence of aflatoxin B\textsubscript{1} to act as a template for E. coli DNA polymerase. Their results indicated that the toxin not only inhibited DNA synthesis by the microorganism, but also significantly reduced primer activity of DNA isolated from cells cultured in the presence of the toxin. In this system, the actions of aflatoxin B\textsubscript{1} resembled those of mitomycin C, an antibiotic that inhibits DNA synthesis.

These observations indicate that aflatoxin B\textsubscript{1} impairs DNA synthesis in a number of different experimental systems. Although the evidence regarding the mechanism of inhibition is not yet conclusive, it tends to support the hypothesis that this action is a consequence of interaction of the compound with DNA.
ALTERATIONS OF RNA METABOLISM

Altered RNA metabolism as a result of aflatoxin treatment has been demonstrated under a variety of experimental conditions. Changes of this type are among the earliest demonstrable effects in livers of rats dosed with the toxins. In vivo administration of the compound to rats or direct exposure of liver slice preparations in vitro result in rapid and dramatic inhibition of precursor incorporation into RNA, particularly in the nucleus.

These effects were first observed in regenerating rat liver following partial hepatectomy (18). It was found that aflatoxin B$_1$ at a dose of 100 ug/rat resulted in significant suppression of orotic acid-14C incorporation into hepatocyte nuclear RNA within 30 minutes after dosing. Subsequently, Clifford and Rees (5) reported that an LD$_{50}$ dose of the toxin (7 mg/kg) given to intact rats inhibited precursor incorporation into liver nuclear RNA to the extent of 80% within 3 hours after dosing. Sporn et al. (31) reported similar findings regarding incorporation of cytidine-3H into liver nuclear RNA. These investigators found that a single LD$_{50}$ dose of aflatoxin B$_1$ suppressed precursor incorporation by 92% within 70 minutes after dosing, and the inhibition was still evident (83%) 17 hours later. Simultaneously, the liver cell nuclear RNA/DNA ratio declined to 78% of the control values, indicating a net loss of nuclear RNA under these conditions.

Using similar technics, we have studied the time-course and dose-response characteristics of these effects on nuclear RNA metabolism (12, 13, 35). It was found that a single LD$_{50}$ dose of aflatoxin B$_1$ to rats strongly inhibited precursor incorporation into liver nuclear RNA in less than 15 minutes after dosing. Further, significant (63%) inhibition was still clearly evident 5 days after the treatment. Equally marked decreases in the nuclear RNA/DNA ratio occurred during the first 24 hours after treatment, but were not apparent after that time.

Similar effects of aflatoxins have been observed as a result of exposure of rat liver-slice preparations to the toxic compounds. Clifford and Rees (5) reported that orotic acid-14C incorporation into total cellular RNA was strongly inhibited when rat liver slices were incubated in vitro in the presence of aflatoxin B$_1$. Under similar conditions, these investigators (6) found that aflatoxins G$_1$ and G$_2$ also inhibited RNA synthesis, but with lower potency than B$_1$.

That the impaired RNA synthesis was attributable to inhibition of RNA polymerase activity was shown experimentally by Gelboin et al. (15). In their experiment, rats were treated with a sublethal dose (1 mg/kg) of aflatoxin B$_1$ and killed at intervals thereafter. The activity of RNA polymerase of liver cell nuclei was determined in animals killed at intervals up to 24 hours after dosing. The activity of this enzyme was inhibited (60%) as early as 15 minutes after dosing, but the inhibition had nearly disappeared 12 hours after treatment. Subsequently, however, Friedman and Wogan (13) demonstrated that a larger dose (5 mg/kg) of the toxin produced inhibition of RNA polymerase activity that persisted for several days after dosing. Clifford et al. (7) and Moule and Frayssinet (24) have also reported inhibition of RNA polymerase activity in livers of rats treated with aflatoxin B$_1$.

The interference with gene transcription caused by aflatoxin B$_1$, as indicated by its inhibition of RNA polymerase activity is presumed to be related to interaction of the compound with DNA, with the result that DNA-dependent RNA synthesis is impaired. However, as in the observed inhibition of DNA polymerase, it is possible that the action may result from interaction with the enzyme itself. Two recent experiments provide information relevant to this point. King and Nicholson (16) studied the effects of binding of aflatoxin B$_1$ to calf-thymus DNA on the ability of the preparation to act as a template for E. coli RNA polymerase. In this system, the toxin was without effect, i.e., did not alter transcription of the DNA molecule even though it was shown that aflatoxin-DNA binding had occurred. Under identical conditions, actinomycin D was highly effective in blocking RNA polymerase activity.

Further information is provided by the results of Moule and Frayssinet (24) who investigated aflatoxin-induced alterations of transcription in rat liver cells by in vitro technics. In their experiments, a deoxyribonucleoprotein (DNP) template preparation was prepared from livers of rats treated with aflatoxins. The effects of aflatoxin treatment on the ability of this template to be transcribed by Micrococcus lysodeikticus RNA polymerase were investigated. Template activity under these conditions was not affected by aflatoxins, nor, in fact, by actinomycin D. However, in vitro exposure of the DNP preparation to aflatoxins resulted in varying degrees of inhibition of transcription. The unanticipated findings indicated that inhibition was maximal with very impure aflatoxin mixtures and insignificant when highly purified aflatoxin B$_1$ was used. In view of these findings, it remains uncertain as to whether the observed in vivo inhibition of RNA and DNA polymerase activities is attributable to altered template activity or direct inhibition of the enzymes involved.

In view of the marked effects of the toxins on nuclear RNA synthesis, it might be anticipated that cytoplasmic RNA metabolism would be affected as well. Such findings have been reported (12, 32). A single sublethal dose of aflatoxin B$_1$ to rats resulted in a decrease of approximately 50% in total liver RNA content over the 72 hours following treatment. Under similar conditions, marked alterations in polyribosome profiles take place. We have recently reported effects of aflatoxin B$_1$ on liver polyribosome profiles of rats treated with a single sublethal (3 mg/kg) dose of the compound (26). This treatment resulted in large increases in monomer and dimer fractions and decreases in the polyribosome areas that were apparent within 3 hours after dosing. Polysomal disaggregation was maximal at 12 hours and completely reversed after 48 hours. The pattern of disaggregation and reaggregation after toxin treatment suggested that the observed effects are related to alterations in both RNA and protein metabolism.

EFFECTS ON PROTEIN SYNTHESIS

Based on present knowledge of the mechanisms of gene transcription and translation, alterations in DNA-dependent RNA synthesis would be expected to result in changes in protein metabolism. The evidence summarized in the preceding sections indicates that exposure of liver tissue to aflatoxin B$_1$ results in marked alterations in gene transcription as evidenced by impaired synthesis of nuclear RNA. Associated
with this effect are significant and persistent losses of cytoplasmic RNA and polyribosomal disaggregation. Under these circumstances, changes in protein metabolism, particularly impaired synthesis, would be anticipated. The effects of aflatoxins on this aspect of cellular metabolism have been investigated with somewhat paradoxical results. Impaired synthesis of total liver proteins has been observed upon exposure of liver slices to aflatoxins in vitro. However, under in vivo conditions, synthesis of specific proteins (inducible enzymes) is inhibited by the toxin, whereas total liver protein synthesis is not markedly affected.

Inhibition by aflatoxins of amino acid incorporation into rat and duckling liver slice preparations was first reported by Smith (30). Similar results were reported by Clifford and Rees (5, 6) for rat liver slices exposed to aflatoxin B1. In contrast, incorporation in vivo of leucine-14C into rat liver proteins was not affected by prior treatment of animals with LD50 doses of aflatoxin B1 (6, 29).

It has been demonstrated that the in vivo synthesis of certain liver enzymes is inhibited in animals treated with aflatoxin B1. Characteristics of inhibition in these instances suggest that the observed effects on protein synthesis are secondary to inhibition of RNA synthesis. These conclusions are based on studies of toxin effects on the inducibility of tryptophan pyrrolase, an enzyme present in mammalian liver at low levels. Liver tryptophan pyrrolase activity of rats can be increased by the injection of large doses of hydrocortisone or of tryptophan, the substrate for the enzyme. Either treatment results in a 5- to 15-fold increase in enzyme activity (10). Although both agents produce a qualitatively similar result, it is now known that they effect increased enzyme activity through entirely different mechanisms. Induction of the enzyme by corticosteroids results from de novo synthesis of enzyme protein, whereas tryptophan is thought to increase levels by stabilization of existing enzyme, i.e., prevention of degradation.

Several characteristics of this induction process make it a useful experimental tool in determining the site of action of inhibitors such as aflatoxins. The time course of liver tryptophan pyrrolase induction by cortisone is such that enzyme activity increases during a 4- to 6-hour period following injection of the inducer, reaches a maximum at 6 hours, then returns to pretreatment levels by 10 to 12 hours. Because the induction process involves de novo synthesis of enzyme protein, administration of inhibitors of protein synthesis (e.g., puromycin) at any point during the induction phase (0 to 6 hours) blocks further increase in enzyme activity and initiates degradation of the molecule (14). Thus, the induction phenomenon remains puromycin-sensitive over the entire induction phase.

Steroid induction of tryptophan pyrrolase is also inhibited by inhibitors of RNA synthesis (e.g., actinomycin D), but only under restricted conditions. Administration of actinomycin D simultaneously with, or up to 2 hours after, the inducer (i.e., during the first 2 hours of the induction phase) blocks the induction process. However, after 2 hours the process becomes insensitive to actinomycin D (14). Because this agent is known specifically to inhibit the synthesis of messenger RNA, its effects on tryptophan pyrrolase induction are taken to indicate that (a) cortisol induction of the enzyme requires DNA-dependent RNA synthesis, and (b) sufficient messenger RNA is synthesized during the first 2 hours to permit complete induction of the enzyme even when messenger RNA synthesis is inhibited after that time.

We have utilized this experimental system in investigating the effects of aflatoxin B1 (12, 36). In these experiments, rats were injected with hydrocortisone at 0 time, and groups of animals in addition received aflatoxin B1 (3 mg/kg) at 0 time, or at 1 to 4 hours after the inducer. The animals were then killed at hourly intervals and the liver tryptophan pyrrolase levels determined. The results showed that aflatoxin B1 effectively blocked the enzyme induction when administered simultaneously with the inducer or up to 2 hours thereafter. However, at 3 and 4 hours, the induction process was no longer sensitive to the inhibitory effects of the compound. These effects were virtually identical with those of actinomycin D studied in parallel experiments, and it was concluded that the two compounds acted through similar mechanisms, presumably inhibition of messenger RNA synthesis.

Clifford and Rees (5, 6) also demonstrated that aflatoxin B1 inhibited cortisone induction of rat liver tryptophan pyrrolase in animals treated with the toxin prior to induction.

The duration of effects of aflatoxin B1 on this process have also been investigated (36). Rats were injected with one LD50 dose (5 mg/kg) of the toxin, thereafter receiving no further treatment with the compound. The ability of hydrocortisone to induce the enzyme was then evaluated 3, 5, and 10 days after toxin administration. The results showed that the induction process was completely inhibited at all three time intervals. These results indicate that aflatoxin B1 exerts an inhibitory effect on the inducibility of this enzyme which persists long after many of its other observed actions have disappeared. It is not yet clear whether this prolonged action is attributable only to inhibition of gene transcription.

Inhibition by aflatoxin B1 of inducibility of rat liver enzymes has also been demonstrated in a different experimental system. Pong and Wogan (25) showed that the induction of zoxazolamine hydroxylase (a microsomal drug-metabolizing enzyme of rat liver) by benzpyrene was completely blocked by simultaneous administration of aflatoxin B1 and the inducer. When the toxin was administered at intervals after the inducer, the system eventually became insensitive to the inhibitory effects in a manner similar to the tryptophan pyrrolase system described above.

Taken together, the results of these experiments further illustrate the parallelism between the effects of actinomycin D and aflatoxin B1 noted previously and provide additional evidence that the action of aflatoxin B1 in vivo involves suppression of the synthesis of specific liver proteins through its alteration of RNA metabolism.

NUCLEOLAR MORPHOLOGY

Alterations of nuclear RNA metabolism resulting from treatment with aflatoxin B1 are associated with changes in the morphology of nucleoli as observed by electron microscopy of affected cells. Ultrastructural lesions in liver cell nucleoli of rats treated with aflatoxin B1 were described by Bernhard et al. (2). Lesions consisted of segregation of the granular and
fibrillar components of the organelle, with the formation of so-called nucleolar "caps." The structural change was developing within 30 minutes after administration of the toxin (0.5 mg/kg), but proved to be reversible, since the nucleolar morphology was essentially normal 24 hours after injection.

Lafarge et al. (19) described similar effects of the toxin, and studied simultaneously the development of the nucleolar lesion and nuclear RNA synthesis. They concluded that inhibition of nucleolar RNA synthesis preceded development of the morphologic lesion, as the former process was essentially maximal 20 minutes after treatment with the toxin, whereas the latter took longer to develop. Similarities in the observed changes with those induced under comparable conditions by actinomycin D were noted by both groups.

Nucleolar ultrastructural changes were also observed in livers of rats and monkeys treated with aflatoxin B1 by Svoboda et al. (32). Following acute administration of the compound, nucleolar segregation similar to that described above was found to occur in both species. However, in contrast to the reversibility of the lesion reported by earlier investigators, Svoboda et al. (32) found the lesion induced by a single dose of toxin to persist up to 72 hours in rats and up to 48 hours in monkeys. The latter investigators also studied liver nucleolar morphology in rats after chronic feeding of diets containing aflatoxin B1 at levels of 1 to 2 ppm, which is a carcinogenic regimen. No nucleolar abnormalities were found in the liver cells of animals consuming these diets for periods up to 52 weeks, at which time liver tumors had developed.

Unuma et al. (34) recently compared the morphology of liver nucleoli from rats treated with aflatoxin B1 with nucleoli from several types of hepatoma cells. After acute dosing with the compound (0.75 mg/kg), they found morphologic changes similar to those reported previously. However, when rats were treated chronically (5 to 30 days) with daily doses of the toxin, little change was observed in the ultrastructure of liver nucleoli.

On the basis of this information, it would appear that the marked alterations in nucleolar morphology induced by single doses of aflatoxin B1 are associated with the acute effects of the toxin but do not occur after chronic treatment even with equivalent doses.

INTERACTIONS WITH CELLULAR CONSTITUENTS

In the sequence of biochemical reactions to aflatoxins described in the preceding sections, the initiating event giving rise to the sequential changes is envisioned as interaction of the toxic compounds with DNA. Most of the available evidence for aflatoxin-DNA interaction is based upon experiments in which in vitro binding has been demonstrated.

Several criteria have been applied in demonstrating binding, including alterations in the aflatoxin absorption spectrum that occur upon interaction with DNA. Sporn et al. (31) reported a shift in absorption maximum and hypochromism at 362 m
ds upon binding of aflatoxin B1 to calf-thymus DNA when the two compounds were equilibrated in phosphate buffer solutions. These criteria were utilized in demonstrating that aflatoxin B1 bound to native calf-thymus DNA and, to a lesser extent, to heat-denatured calf-thymus DNA and native E. coli RNA. By the same criteria, aflatoxin B1 did not bind to bovine serum albumin, calf-thymus histone, or to enzymatically hydrolyzed calf-thymus DNA.

Sporn et al. (31) also estimated the molar binding of aflatoxin B1 to DNA. Solutions of B1 in the presence or absence of calf-thymus DNA were dialyzed until equilibrium distribution was reached. Under these conditions, it was calculated that 600 moles of native DNA-phosphorus bound 1 mole of aflatoxin B1. Under similar conditions, denatured DNA bound aflatoxin B1 in a molar ratio of 170:1.

Clifford and Rees (5, 6) also demonstrated interactions between aflatoxins and DNA by measuring the spectral shift induced by the binding. By the same technics Clifford et al. (7) compared the interactions of aflatoxin B1, G1, and G2 with DNA. Qualitatively similar spectral shifts were induced by DNA binding of the three compounds. However, the shifts were quantitatively different, being greatest with aflatoxin B1 intermediate with G1, and smallest with G2. The extent of the toxicity and biochemical potency of the compounds were qualitatively related to the magnitude of spectral shift induced by DNA binding.

Black and Jirgensons (3) using equilibrium dialysis technics demonstrated that aflatoxin B1 bound to DNA and also to two highly purified lysine-rich histone preparations under the conditions used. They further reported that binding was associated with increased viscosity of histone and DNA solutions and concluded that aflatoxin binding resulted in gross conformational changes in the histone and DNA molecules.

It is clear from these investigations that aflatoxins are capable of interacting with DNA under conditions in which the two compounds are brought into contact in vitro. Interactions with cellular constituents under in vivo conditions have been studied in only one series of experiments (23). Injection of tritiated aflatoxin B1 into rats resulted in incorporation of radioactivity into liver RNA, DNA, and protein; the protein fraction was most highly labeled. Radioactivity disappeared from the RNA fraction, but was still present in DNA and protein fractions after one month. It is of considerable interest that the same investigators reported that radioactivity from tritiated aflatoxin G1 was incorporated into liver proteins but not DNA or RNA, in experiments done under comparable conditions. The nature of the interactions involved and also the relationship, if any, of in vivo interactions to in vitro binding described above are not clear. It remains for further experimentation to establish that, under in vivo conditions, the compounds interact with DNA in such a manner as to alter its transcription and, further, that this interaction is directly responsible for the observed biochemical sequelae.

DISCUSSION

Evidence summarized in the preceding sections indicates that aflatoxin B1 causes dramatic alterations in nucleic acid and protein synthesis in liver when administered in acute doses to rats. The observed inhibitions of DNA synthesis, nuclear RNA synthesis and alteration of gene transcription appear quickly after the compound is administered. The time course and other characteristics of these responses are consistent with the hypothesis that they are initiated as a result of interaction of
the toxic material with DNA in such a way as to interfere with its transcription. Although binding of DNA and aflatoxin B1 has been amply demonstrated under in vitro conditions, evidence that comparable interactions occur in vivo is much less extensive. It is not yet clear whether the interaction involves direct binding of the unaltered aflatoxin molecule or whether interaction of a type (e.g., alkylation) requiring metabolic conversion of aflatoxin B1 is involved. The compound causes similar effects in widely different systems, which would seem to favor the hypothesis that it acts directly, without metabolic conversion, since it is unlikely that all of these systems would possess the necessary metabolic capabilities for activation. However, the possibility of metabolic activation cannot yet be ruled out, particularly as regards the long-term effects of the toxin.

It is also unknown as to what proportion of an administered dose of aflatoxin B1 reaches the nuclei of liver cells, and to what extent the material interacts with other nuclear constituents (e.g., histones) that might influence gene transcription. Similarly, it remains to be determined whether the lesser biochemical effectiveness of the other aflatoxins (B2, G1, G2) is attributable solely to their varying capabilities to interact with DNA.

Similarities between the biochemical actions of aflatoxin B1 and actinomycin D have been noted by many authors. These similarities are particularly striking regarding in vitro binding to DNA, alteration of nucleolar morphology, inhibition of nuclear RNA synthesis, and inhibition of enzyme induction. However, in spite of analogous effects of the two compounds, it is not possible at this time to conclude that they act through entirely analogous mechanisms, nor that all of the biochemical effects of aflatoxin B1 can be attributed to those pathways apparently shared by both substances. Such conclusions are not possible because of important dissimilarities in certain of their biochemical and biologic properties. Several differences can be illustrated by examples relevant to this discussion.

A single dose of aflatoxin B1 to rats or other animals causes parenchymal cell necrosis, bile duct proliferation, and other histologic changes in liver, whereas actinomycin D causes no similar lesions even when administered at lethal dose levels. Thus, if hepatocellular necrosis developed solely as a consequence of the known biochemical effects of these compounds, they should be equally effective as necrogenic agents. In contrast, actinomycin D is highly effective in suppressing messenger RNA synthesis in microbial systems (27), while aflatoxin B1 has only weak antibiotic activity against a few microorganisms (1, 4). Possibly the greatest dissimilarity between the two compounds is in their carcinogenic activities. Aflatoxin B1 is a potent hepatocarcinogen for several animal species; tumor induction by actinomycins has been reported in only one experiment (17) dealing with subcutaneous sarcoma induction in mice.

It is therefore difficult to construct functional generalizations based upon the early effects of the compounds upon nucleolar metabolism. This is especially evident with regard to the changes in nucleolar morphology elicited by aflatoxin B1. Similar changes have been reported after administration of actinomycin D, mitocycin, and 4-nitroquinoline-N-oxide (2) and also by certain pyrrolizidine alkaloids (33). This alteration is therefore not specific to aflatoxins, and, because the other compounds causing the lesion are thought to act by widely different mechanisms, the ultrastructural change would seem not to be related to specific biochemical changes. The relevance of this change to the carcinogenic process is also uncertain since the lesion appears to be reversible after a single dose of aflatoxin B1 (2) and does not appear after multiple repeated doses of the compound (34).

Considerable further experimental data will therefore be required in order to relate the observed biochemical effects of aflatoxins directly to the subcellular and cellular events ultimately manifest in toxicity (necrosis) or in tumor induction. On the basis of presently available evidence, it would appear that the biochemical changes induced by single doses of aflatoxins are associated with the acute (toxicity) response to the compounds. Association of these effects with the chronic processes leading to tumor formation is less certain. The need for further investigation of the biochemical events associated with carcinoma induction by aflatoxins is clearly indicated. In such studies, the aflatoxins will undoubtedly provide useful model compounds, and their investigation may provide additional insights into the mechanisms underlying the carcinogenic process.

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