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AFLATOXINS : BIOCHEMICAL TOXICITY AND METABOLISM

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OCCURRENCE OF AFLATOXINS

Aflatoxins were discovered as a consequence of epizootic outbreaks of hepatic necrosis, resulting in the deaths of thousands of turkeys poults ("Turkey X disease"), ducklings and chicks in England in 1960 and 1961. The problem was eventually traced to feed contamination, specifically a shipment of Brazilian peanut meal used as a protein supplement in poultry feed. This meal proved to be both toxic and carcinogenic and was found to be contaminated with *Aspergillus flavus (1,2)*.

The active chemicals were extracted and isolated from *Aspergillus flavus* cultures and chemically identified (3,4). Later in 1960, outbreaks of disease occurred in pigs, also apparently caused by an unknown etiology was reported in calves (5). In both cases the etiology was traced to the incorporation of Brazilian groundnut meal into the rations of the livestock. Following the reports of investigation into the cause of "Turkey X disease" in England, it was established that the same etiologic agent was present in the cotton meal used as feed which was later shown to be contaminated with aflatoxin (6,7). Lancaster *et al.* reported that rats fed the toxic groundnut meal developed hepatocarcinoma (8). In 1964, Butler and Barnes reported a series of feeding experiments with the toxic groundnut meal, assayed for aflatoxin, which showed that aflatoxin is an extremely potent carcinogen for the rat (9). This was confirmed later by using crystalline aflatoxins (10).

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Aflatoxin-producing mold strains are widely dispersed in air and soil, and are capable of growing on a variety of natural substrates. Two species of molds, the *Aspergillus* and the *Penicillium* are most important in their ability to produce toxic metabolites and the aflatoxins represent the most potent of these compounds. In *Aspergillus* group : *A. flavus*, *A. flavus* var *columnis*, *A. parasiticus*, *A. parasiticus* var *globosus* and *A. oryzae* were capable of producing aflatoxins. In *Penicillium* group : *P. puberulum*, *P. variable*, *P. frequentans* and *P. citrinum* were capable of producing aflatoxins (11). These molds generally synthetize only two or three type of aflatoxins under a given set of conditions, one of which is always aflatoxin B_1, the most potent toxin and most carcinogenic of the group. Aflatoxins in the G series almost always present in lesser amounts than aflatoxin B_1 (12). When they occur as food contaminants, aflatoxin B_1 is always present. Although aflatoxins in the G series have sometimes been found in contaminated products, they generally occur less frequently than aflatoxin B_1 amd have never been reported in the absence of aflatoxin B_1.

STRUCTURE AND CHEMISTRY OF AFLATOXINS

The aflatoxins constitute a group of highly substituted coumarins containing a fused dihydrofurofuran moiety whose characteristic physicochemical feature is their intense fluorescence under ultraviolet illumination. Structurally, the aflatoxins were grouped into two series, aflatoxin B and derivatives, and aflatoxin G and derivatives, based possibly on the blue violet fluorescence of the former and the greenish yellow fluorescence of the latter (3). Hartley *et al.* showed that aflatoxins could be resolved into four distinct compounds, namely aflatoxins B_1, B_2 and G_1, G_2 accordingly (13). Their chemical structures are shown in Fig. 1.

Structures based largely on interpretation of spectral data were proposed for aflatoxin B_1 and G_1 (4, 14), and for aflatoxins B_2 and G_2 (15, 16). The molecular formula of aflatoxin B_1 was established as $C_{17}H_{12}O_6$ (MW 312) and of aflatoxin G_1 as $C_{17}H_{12}O_7$ (MW 328) while aflatoxins B_2 and G_2 were found to be the dihydroderivatives of the parent compounds with the molecular formulae of $C_{17}H_{14}O_6$ (MW. 314) and $C_{17}H_{14}O_7$ (MW 330), respectively.

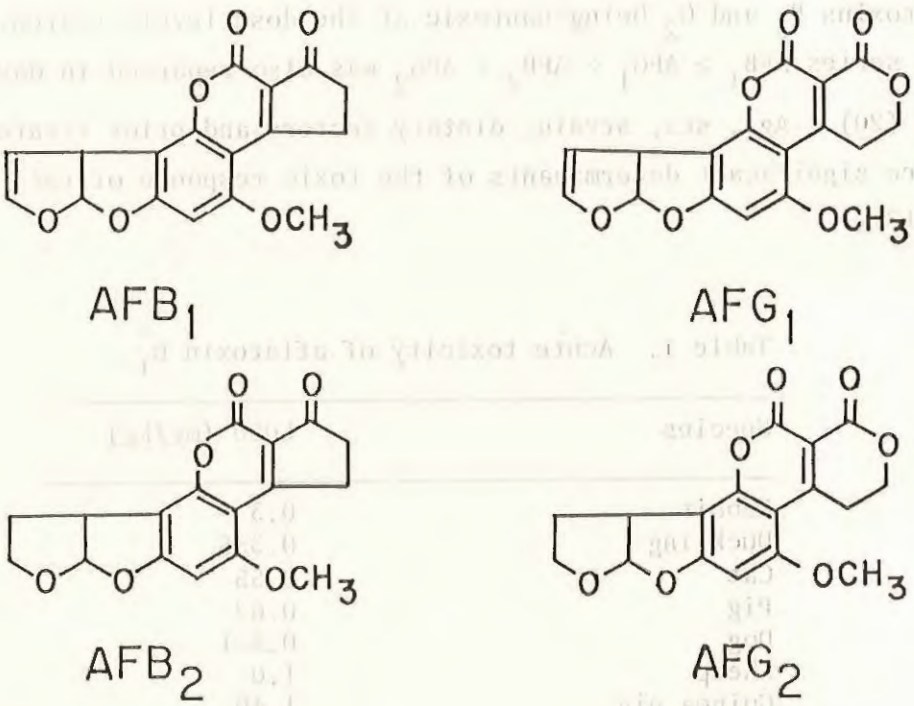

FIGURE 1. Structures of the aflatoxins.

BIOLOGICAL ACTIVITY OF AFLATOXINS

ACUTE TOXICITY OF AFLATOXINS

The aflatoxins, aflatoxin B_1 in particular, are acutely toxic to a large number of organisms, including laboratory and domestic animals (17). The toxic properties of the aflatoxins depend upon the dose, duration of exposure, and animal species. There is a wide range of susceptibility from the highly susceptible 1-day-old duckling to the more resistant mouse and adult turkey.

Aflatoxin B_1 toxicity data for rodents and nonrodent vertebrates are shown in Table 1. The acute toxicity of aflatoxin B_1 to mammals and birds may vary over two orders of magnitude between a sensitive species (rabbit, LD50 0.3 mg/kg) and an insensitive species (mouse, LD50 approximately 40 mg/kg). Aflatoxin B_1 was more toxic than aflatoxin G_1 in male rats

but aflatoxins B_2 and G_2 being nontoxic at the dose levels tested. The toxicity series $AFB_1 > AFG_1 > AFB_2 > AFG_2$ was also reported in day-old duckling (20). Age, sex, strain, dietary factors and prior treatment with drugs were significant determinants of the toxic response of rats to aflatoxin B_1 (17).

Table 1. Acute toxicity of aflatoxin B_1*.

Species	LD50 (mg/kg)
Rabbit	0.3
Duckling	0.335
Cat	0.55
Pig	0.62
Dog	0.5-1
Sheep	1.0
Guinea pig	1.40
Rat male	7.2
female	17.9
Hamster	10.2
Mouse	40.0
Chick embryo	0.025 µg/embryo

* From (18, 19)

Rats have been the most extensively studied laboratory animal. There are sex and strain differences in the susceptibility of rats with Fischer rats being considerably more susceptible than the Wistar ones. The lesion induced in both sexes and in different strains is the same, and consists of a periportal zone necrosis and biliary proliferation (18). The necrosis develops slowly over 3 days, and is accompanied by marked biliary proliferation. There is only slow recovery of this lesion.

In man, the evidences of acute toxicity of aflatoxins was reported. Serk-Hansen reported the death of a boy in Uganda from acute liver damage, and it was found that the casava eaten by the boy was heavily contaminated with aflatoxin (21). In Thailand, the consumption of leftover cooked rice,

tremendously contaminated with aflatoxin, was associated with the death of Thai boy who suffered from Reye's syndrome. Reye's syndrome, in which there is an encephalopathy and fatty infiltration of the liver and the kidney, has been reproduced experimentally in monkey by feeding aflatoxin (22). Aflatoxins have also been found in autopsy specimens of Reye's syndrome by Becroft and Webster in New Zealand (23), and by Dvorackova and his coworkers in six cases in Czechoslovakia (24). All of the children consumed moldy rice heavily contaminated with aflatoxin. In Western India, Krishnamachari *et al.* reported that aflatoxin B_1 involved in the human toxicosis (25). This outbreak affected as many as 20 villages and was responsible for the death of approximately 26% (106 deaths/397 patients) of all patients admitted to the hospital. It was found that maize was contaminated with *Aspergillus flavus* and aflatoxin. The clinical features were similar to those seen in animals insulted with aflatoxin B_1.

AFLATOXINS CARCINOGENESIS

The work of Lancaster and his colleagues gave an insight into the carcinogenic potential of the aflatoxins (8). They showed a high incidence of liver tumors in rats fed with diet containing a highly mouldy peanut meal. Subsequent studies showed that aflatoxins were the carcinogenic agents in mould-infected peanuts (10, 26, 27). Aflatoxins have also been shown to have carcinogenic activity in many species of animals, including rodents, nonhuman primates, birds and fish (12, 19). Factors influencing aflatoxin carcinogenicity such as diet, hormonal status, liver injury, microsomal enzyme activity, and concurrent exposure to other carcinogenic agents have been reviewed (12). The carcinogenic potency series AFB_1 > AFG_1 > AFB_2 > AFG_2 has been verified in the rats (20).

Epidemiological surveys have revealed strong correlations between liver cancer and aflatoxin B_1 contamination in Africa, Phillipines and other countries (28, 29). Alpert *et al.* found that 40% of food samples tested in Uganda contain aflatoxin with 15% containing more than 1 ppm (30). Levels of 0.015 ppm may induce carcinoma in the rat with continuous inges-

tion. In Kenya, Swaziland, Thailand, and Mozambique, where the incidence of hepatic carcinoma is high, aflatoxins are present in human food. It was reported that the variety of fungi contaminated market foods and foodstuffs in Southeast Asia. Among these, *Aspergillus flavus* was frequently found in all foods and foodstuffs (31, 32). Natural occurrence of aflatoxins was highest in peanuts and peanut products, and responsible for human aflatoxicosis and liver cirrhosis in India (25, 33). There is, therefore, supportive evidence that man is exposed to the aflatoxins in those areas of the world in which hepatic carcinoma is prevalent. This exposure results directly from the problems of harvesting and food storage in developing countries. In order to reduce the body burden of aflatoxins, these practices must be improved.

AFLATOXINS METABOLISM

Studies on toxicity of aflatoxins have largely been focused on aflatoxin B_1 because it is the most abundant and the most toxic metabolite. It is highly hepatotoxic, hepatocarcinogenic, and teratogenic to test animals and highly mutagenic to microbial assay systems (34). Strong evidence exists to indicate that aflatoxin B_1 requires metabolic activation for its toxic, mutagenic and carcinogenic effects. For example, it requires mixed function oxidase (MFO) activation to elicit its potent mutagenicity to the bacterium *Salmonella typhimurium* (35). Also, its covalent binding to nucleic acids occurs both *in vivo* and *in vitro* in the presence of a MFO activation system (36).

Aflatoxins are primarily metabolized by the microsomal mixed function oxidase system, a complex organization of cytochrome coupled, O_2- and NADPH-dependent enzymes located mainly on the endoplasmic reticulum of liver cells, but also present in kidney, lungs, skin and other organs.These enzymes oxidatively metabolize a wide variety of foreign or xenobiotic compounds, with the net result usually being detoxication of the parent compound by the formation of various hydroxylated derivatives which, in turn, are conjugated with sulfate or glucuronic acid to form water-soluble glucuronide or sulfate esters (37). These conjugates are readily excreted in the

urine or bile. During the course of metabolism, highly reactive metabolites may be generated which have the capacity to react covalently with various nucleophilic centers in cellular macromolecules such as DNA, RNA and protein. This activation reaction poses a biological hazard to the cell and constitutes a possible theory by which certain compounds exert toxic and carcinogenic effects (38).

Based on the apparent association between metabolism and toxicity of aflatoxin B_1, attempts have been made to quantitate the relative susceptibility of aflatoxin B_1 as a function of respective *in vitro* metabolic profiles of aflatoxin B_1 (39). *In vitro* metabolic parameters of aflatoxin B_1 for various animal species were then determined and compared with animal susceptibilities to aflatoxin toxicity. The same metabolic parameters were also determined for human tissue preparations and used to estimate the unknown human susceptibility based upon the correlations established for the test animals. The distribution of metabolic products formed showed that aflatoxin B_1 is biotransformed *in vitro* by all the species tested to aflatoxin metabolites. The major metabolites produced by hamster, monkey and human *in vitro* was aflatoxin Q_1, while rat and duck showed a relatively higher conversion to aflatoxicol. A similarity in metabolic profile was observed between hamster and human. Based on the maximal overall *in vitro* metabolic activity of aflatoxin B_1, Patterson has calculated the time required by the whole liver preparations of an animal to completely metabolize one LD50 dose of aflatoxin B_1 (40). According to Patterson's correlation, the fast metabolizers such as rabbit and duck are more prone to acute rather than chronic or carcinogenic aflatoxicosis, whereas the slow metabolizers such as sheep and rat are more susceptible to chronic rather than acute effects. Man and monkey were found to be fast metabolizers, and are typically more susceptible to acute aflatoxicosis but are relatively resistant to carcinogenic effects.

Aflatoxin B_1 is biotransformed into several kinds of metabolites by cytosol and microsomal enzyme systems as shown in Fig. 2. There are at least four types of metabolic reactions characteristic of aflatoxin B_1 :

reduction, hydroxylation, O-demethylation and epoxidation leading to the respective formation of aflatoxicol (AFL), aflatoxin M_1 (AFM_1) and aflatoxin Q_1 (AFQ_1), aflatoxin P_1 (AFP_1) and the aflatoxin B_1-2, 3-oxide (AFB_1-2, 3-oxide). Except for the epoxide, all the metabolites have been isolated and characterized.

Aflatoxin M_1 : Ring hydroxylation of aflatoxin B_1 at the 4 position produces aflatoxin M_1. This metabolite was first detected in the milk of cows ingesting aflatoxin B_1 (41). Aflatoxin M_1 has also been detected in the urine of humans consuming aflatoxin B_1-contaminated peanut butter (42). It was formed *in vitro* by liver preparations from a variety of species, including human, and it has been found in milk, tissues and urine of animals and people ingesting aflatoxin B_1 (43, 44, 45). The degree of acute toxicity of aflatoxin M_1 appears to be equivalent to that of aflatoxin B_1 but is much less carcinogenic (46, 47).

Aflatoxin Q_1 : Ring hydroxylation of the carbon atom β to the carbonyl function of the cyclopentenone ring produces aflatoxin Q_1. It was identified as major *in vitro* aflatoxin B_1 metabolite using monkey liver and human liver microsomes (48, 49). It was also produced *in vitro* by liver microsomes of various animals (44). This seems to be a minor pathway *in vivo* and free aflatoxin Q_1 has not been found in tissues or excreta of any animal exposed to aflatoxin B_1 (43). This metabolite was nontoxic to chicken embryo (50), and was only 1-2% as mutagenic as aflatoxin B_1 in the Ames bacterial mutagenesis assay (45).

Aflatoxin P_1 : This metabolite was produced by the O-demethylation of aflatoxin B_1, and was the major excretory product in the urine of aflatoxin B_1-treated rhesus monkey (51). It was slightly formed *in vitro* by mouse, rat, monkey and human microsomes (44, 52). Aflatoxin P_1 was nontoxic to chicken embryos (53), and inactive in the Ames assay (54).

Aflatoxicol : Reduction of the cyclopentenone carbonyl function of aflatoxin B_1 by an NADPH-dependent cytoplasmic enzyme produced aflatoxicol. Rabbit

and bird liver homogenates were active aflatoxicol producers, whereas rodent and sheep preparations were inactive (55). Aflatoxicol was nontoxic to the day-old duckling (56), and the relatively high mutagenic potential and carcinogenicity in trouts may be due to its metabolism to aflatoxin B_1 (54).

Aflatoxin B_1 -2, 3-oxide : Although generally remaining unidentified, the most reactive and labile metabolites are thought to be the "ultimate" toxins in their respective biochemical lesions. The metabolite of greatest current interest is the proposed aflatoxin B_1 -2, 3-oxide. Even though aflatoxin B_1 -2, 3-oxide has not been isolated, its formation and toxic action can be measured indirectly by the Ames mutagen assay. Using mutagenesis in the test bacterium *Salmonella typhimurium* as the model biochemical lesion, differing aflatoxin B_1 mutagenic responses elicited by post-mitochondrial liver fractions prepared from animals of different susceptibilities to aflatoxin B_1 carcinogenicity indicate that net epoxide formation appears to be another metabolic parameter associated with animal susceptibility (39).

Structural-activity evaluations using mutagenicity and DNA-binding activity as model biological lesions support the involvement of the 2, 3-vinyl-ether double bond in toxicity, with the epoxide as the active species (45, 59).

MACROMOLECULAR BINDING OF AFLATOXIN

The covalent incorporation of an aflatoxin moiety into nucleic acids and proteins is now considered to be an important mechanism by which aflatoxin B_1 initiates its toxic and carcinogenic effects. The aflatoxin B_1-2, 3-oxide has been proposed by various investigators as the active form or ultimate carcinogen of aflatoxin B_1 (60, 61). It was later shown that the predominant metabolite of aflatoxin B_1 that binds to DNA in animal and human tissues is the aflatoxin B_1,-2, 3-oxide (36, 62).

Once aflatoxin B_1 -2, 3-oxide formed, it can either exert electrophilic attack on the target nucleophiles such as in nucleic acids and proteins to cause biochemical lesions; or be conjugated by reduced glutathione

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(GSH) and be detoxified (63,64); or be hydrolyzed to form the 2, 3-dihydrodiol of aflatoxin B_1 (58). After incubation the mixture of aflatoxin B_1, DNA, microsomes and NADPH, and following chemical analysis of the modified DNA, the major aflatoxin B_1 -DNA adduct was identified as 8,9-dihydro-8-(7-guanyl)-9-hydroxy-aflatoxin B_1 or 2, 3-dihydro-2-(N^7-guanyl)-3-hydroxy-aflatoxin B_1 (Fig.3) (65). This adduct was also the major product formed *in vivo* in rat liver (63,66). Aside from this major adduct, more than ten minor products were detected, and one of them was identified as an imidazole ring-opened derivative of aflatoxin B_1-N^7-Guanine (Fig.3) (67).

Attempts in isolating the highly reactive 2, 3-oxide have been unsuccessful, although it can be chemically generated by oxidation of aflatoxin B_1 and trapping the epoxide as nucleic acid or nucleoside adducts with DNA (68). Although the epoxide itself has not been isolated because of its great reactivity, a more stable model compound, aflatoxin B_1-2, 3-dichloride has been synthesized (61). This electrophilic analog of the epoxide was considerably more potent than aflatoxin B_1 in the Ames mutagenesis assay and in the induction of various rat and mouse tumors. The aflatoxin B_1 -2, 3-dichloride also reacted spontaneously with DNA, RNA, protein and amino acids with nucleophilic centers, such as cysteine, histidine and lysine. Thus it seems likely that the epoxidation pathway may represent an important activation step in aflatoxin metabolism. Furthermore, it is generally agreed that this metabolite is probably produced by the mixed function oxidase enzyme system.

The relative biological hazard posed by the formation of aflatoxin adducts with different classes of cellular macromolecules is unknown. However, in studies comparing *in vivo* macromolecular binding of aflatoxin B_1 (toxic) and aflatoxin B_2 (relatively nontoxic) to rat liver DNA, RNA and protein, it was noted that aflatoxin B_2 bound to the nucleic acids approximately 1% of that bound by aflatoxin B_1, whereas protein binding was 35 to 70% of that observed with aflatoxin B_1 (61). This suggested that aflatoxin-protein adducts were relatively unimportant in the manifestation of toxic and carcinogenic effects. There has been very good correlation, with certain parameters studied, between the toxicity and carcinogenicity of afla-

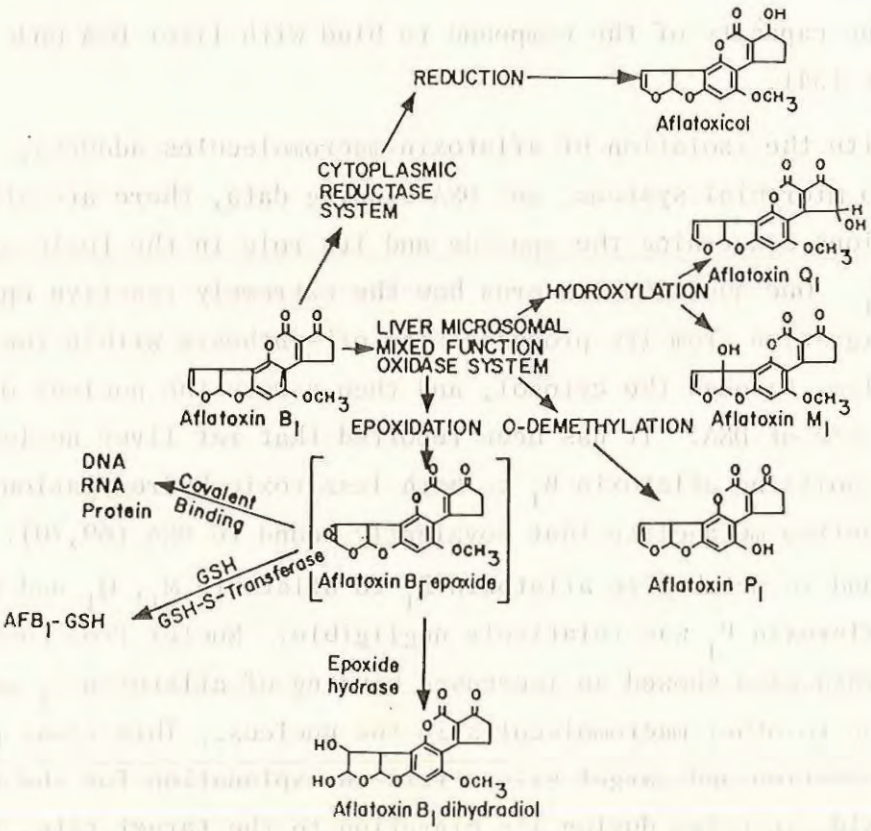
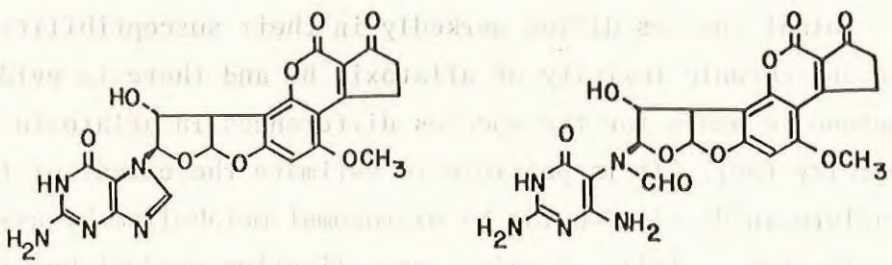


FIGURE 2. Aflatoxin B_1 and its metabolic pathway (From Ueno, 1985; Moss and Neal, 1985) (57,58)



8,9-Dihydro-8-(N^7-guanyl)-9-hydroxy-aflatoxin B_1
(AFB_1-Gua)

8,9-Dihydro-8-(N^5-formyl-2',5',6'-triamino-4'-oxo-N^5-pyrimidyl)-9-hydroxy-aflatoxin B_1
(AFB_1-triamino-Py)

(57)

FIGURE 3. Aflatoxin B_1-DNA adducts (From Ueno, 1985)

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toxin B_1 and the capacity of the compound to bind with liver DNA under *in vivo* conditions (34).

Despite the isolation of aflatoxin-macromolecules adducts, mutagenicity to microbial systems, and DNA-binding data, there are still unsolved questions concerning the epoxide and its role in the toxic action of aflatoxin B_1. One question concerns how the extremely reactive epoxide survives the migration from its proposed site of synthesis within the endoplasmic reticulum, through the cytosol, and then across the nuclear membrane to the target site of DNA. It has been reported that rat liver nuclei were capable of metabolizing aflatoxin B_1 to both less toxic hydroxylation metabolites and reactive metabolite that covalently bound to DNA (69,70). Nuclei were found to metabolize aflatoxin B_1 to aflatoxin M_1, Q_1 and P_1; the formation of aflatoxin P_1 was relatively negligible. Nuclei from phenobarbital-treated rats also showed an increased binding of aflatoxin B_1 metabolites to DNA and to other macromolecules in the nucleus. This close proximity of the activation and target site offers an explanation for the survival of the epoxide *in vitro* during its migration to the target site, and may stimulate activation of the nuclear membrane and subsequent DNA binding *in vivo*.

Animal species differ markedly in their susceptibilities to both the acute and chronic toxicity of aflatoxin B_1 and there is evidence supporting a metabolic basis for the species differences in aflatoxin B_1-induced hepatotoxicity (58). It is possible to estimate the extent of the activation to aflatoxin B_1 -2, 3-oxide by microsomal metabolism by assaying aflatoxin B_1 dihydrodiol (71). A major detoxification product has recently been identified as a glutathione conjugate of the epoxide aflatoxin B_1 (2, 3-dihydro-2-(S-glutathionyl)-3-hydroxy-aflatoxin B_1 (72). Both activation and deactivation pathways have been investigated and would account for the relative susceptibilities to aflatoxin B_1 toxicity. Glutathione conjugation (AFB_1-GSH) has also been associated with reduced macromolecular binding *in vivo* and *in vitro* via cytosolic GSH-S-transferase enzymes (64,73,74).

Another pathway of the inactivation of aflatoxin B_1 -2, 3-oxide

is hydrolysis of this active intermediate by epoxide hydrase. Based on previous evidence that 1,2,3-trichloropropane oxide, a potent inhibitor of epoxide hydrase, increases the binding of aflatoxin B_1 to nuclear DNA *in vitro*, it is presumed that aflatoxin B_1 -2, 3-oxide is inactivated by this enzyme (57). In conclusion, two major pathways that can detoxified aflatoxin B_1 -2, 3-oxide are

a) hydration to the aflatoxin B_1 dihydrodiol by hepatic microsomal epoxide hydrase.

b) conjugation with glutathione to form 2, 3-dihydro-2-(S-glutathionyl)-3-hydroxy aflatoxin B_1 which catalyzed by glutathione-S-epoxide transferase in soluble fraction.

BIOCHEMICAL EFFECTS OF AFLATOXIN

Many investigations have difficulty with the biochemical responses elicited by aflatoxin B_1 in a variety of experimental systems with the goal of identifying those reactions essential to the toxic and carcinogenic activity of the compound. The vast majority of the *in vivo* studies have been carried out using acutely toxic single doses of aflatoxin B_1, therefore the resulting biochemical responses were probably more relevant to mechanisms underlying the toxicity of aflatoxin B_1 rather than its carcinogenicity. No recent comprehensive experiments on the biochemical effects of aflatoxin B_1 are available.

Inhibition of DNA synthesis was one of the first events of aflatoxin B_1 intoxication to be reported. Lafarge and Frayssinet reported an inhibition of DNA synthesis after administration of aflatoxin B_1 and the inhibition was maximal between 2 and 24 hours postdosing in rats (75). The mouse, which was more resistant to the toxic and carcinogenic effects of aflatoxin B_1, was much more resistant to the effects of aflatoxin B_1 on DNA synthesis. An aflatoxin B_1 (2.5 mg/kg) resulting in 92% inhibition of DNA synthesis in rats, only caused a 13% reduction in DNA synthesis in mice(76).

When aflatoxin B_1 acts directly on the DNA molecule its consequence

is the inhibition of the ability of the nucleic acid to act as a primer for RNA synthesis. Nucleolar RNA synthesis was inhibited by afaltoxin B_1 both *in vivo* and *in vitro* (75,77). RNA synthesis was maximally inhibited from 15 min to 12 hours. Complete recovery of RNA synthesis was attained by 36 hours. Inhibition of RNA polymerase has been demonstrated by several investigators in various biological systems (78,79). The direct addition of aflatoxin B_1 to nuclear preparations from the livers of untreated animals did not inhibit RNA synthesis indicating that metabolisms of aflatoxin B_1 was required for the expression of these effects (78,80).

Protein synthesis is also inhibited by aflatoxin B_1 treatment, although the effect is neither as rapid nor as extensive as the inhibition of DNA and RNA synthesis in comparable model systems. *In vivo* liver protein synthesis in monkeys was inhibited by 3.5 to 13 hours after an oral dose of aflatoxin B_1, though no effects were observable 1 hour postdosing (81). Hence, aflatoxin B_1-induced disruption of protein synthesis was not necessarily a consequence of the primary inhibition of DNA/RNA synthesis. Polysome disaggregation is consistently associated with, and may be the basis for, the inhibition of protein synthesis by aflatoxin B_1 treatment. Polysome disaggregation has been demonstrated after aflatoxin B_1 treatment in liver preparations from rats and monkeys (81,82). Polysome reaggregation was apparent by 36 hours, and the normal profile was again obtained 5 days after aflatoxin B_1 treatment.

Several miscellaneous biochemical processes, including alterations in cellular enzyme activities, have also been shown to be affected by aflatoxin B_1 treatment. Generally these mechanisms have not been investigated as extensively as those concerned with macromolecular biosynthesis. This does not necessarily imply that these studies are less significant in terms of understanding the mode of action of aflatoxins, although many of these aflatoxin-induced effects may be expected to be derived from and secondary to the disruption of nucleic acid or protein synthesis. The observations that aflatoxin B_1 may also function to some degree as a membrane-active toxin, is suggested by its effects on mitochondrial function and lysosome

permeability. These responses may have validity in explaining at least some of the acute toxic effects of the aflatoxins.

An early observation that a low orally administered dose of aflatoxin B_1 inhibited oxygen consumption and phosphorylation in rat liver mitochondria indicating that mitochondria were also a sensitive target of aflatoxin action (83). Results from previous studies have established that acutely toxic doses of aflatoxin B_1 inhibit mitochondrial electron transport between b and c or C_1 (site II) (84,85). However, it has also been suggested that the degree and sites of inhibition by aflatoxin B_1 depend on the toxin concentrations. Aflatoxin B_1 also inhibited at the cytochrome oxidase level (86,87).

It is significant that the concentration of aflatoxin B_1 which induced a higher percentage inhibition of guinea fowl liver mitochondrial respiration was about a hundred times lower than that reported for the rat. This probably accounts for the greater susceptibility of the avian species to aflatoxin hepatotoxicity (88). Obidoa and Siddiqui reported that aflatoxin B_1 inhibition of guinea fowl liver mitochondrial respiration is not localized at coupling site II but may involve inhibition around site I (86). These findings explain the greater susceptibility of the avian species to aflatoxin toxicity.

Breakdown of lysosomes and the release of degradative lysosomal enzymes into the surrounding tissues is obviously implicated in the hepatic necrosis and hemorrhage consistently observed with acutely toxic doses of aflatoxin B_1. A broad range of rat lysosomal enzyme activities were increased in whole liver homogenates after an oral LD50 dose of aflatoxin B_1 (89). As an example, there was about a 3-fold increase in acid DNAase activity 48 hours after aflatoxin B_1. Much of the enzymatic activity was present in the supernatant, indicating that the lysosomal enzymes had been released. *In vitro* treatment of rat liver lysosomal preparations with aflatoxin B_1 also produced a dose-dependent release of the marker enzymes (90).

Recent studies have demonstrated that many compounds undergo bio-

transformation in the liver to chemically reactive metabolites which covalently bind to hepatic macromolecules, particularly DNA, resulting in hepatic injury (91). The isolation and structural identification of the major aflatoxin B_1 -DNA adduct *in vivo* and *in vitro* strongly suggested that the microsomal oxidase-catalyzed epoxidation of aflatoxin B_1 at the 2,3 double bond is the mechanism by which aflatoxin residues are covalently bound to nucleic acids and may coincidentally explain the relative biological potency of aflatoxin B_1 (65,67). The mechanism responsible for the inhibition of mitochondrial function and the potentiation of lysosomal enzymes release are not as yet clear.

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