AFLATOXINS[1]

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Introduction

In trying to prepare a review article on aflatoxins for the *Annual Review of Pharmacology*, one is faced with the problem that no evidence is available that these substances possess purely pharmacological properties like some other coumarins (115). It has not been established whether aflatoxins can be photosensitizing like methoxypsoralens, oestrogenic like coumestrol, or anticoagulant like dicoumarol, etc. The high toxicities of aflatoxins no doubt complicate the picture. Special studies would be required to see whether aflatoxins can cause specific pharmacological effects in doses which are not acutely toxic.

Aflatoxins (Afs) are remarkably potent, recent additions to the ever increasing number of chemical carcinogens. The name derives from the fact that they were first identified as metabolites of the fungus *Aspergillus flavus* Link ex Fries (Aspergillus FLAvus TOXINS; Report of Interdepartmental Working Party on Groundnut Toxicity Research, D.S.I.R., 1962) isolated from a toxic groundnut meal. The initial fundamental discoveries were made in this field in Great Britain (6, 68, 101) where in 1960, the toxic meal caused the loss of 100,000 turkey poults from liver damage or turkey "X" disease (21). These discoveries were soon confirmed and extended by researchers in several other countries.

The problem of hepatotoxins and hepatocarcinogens present in some batches of groundnut meals and of certain other livestock feeds is much older than the dramatic losses of turkeys (21), of ducklings and chicks (14), of pigs (76), and of calves (77) in 1960 would suggest. Several outbreaks of liver disease occurred among guinea pigs in various laboratories in Great Britain and were suspected to be caused by some batches of diet (90). One batch of a suspected guinea pig diet was fed to rats and induced liver tumours (103). In the composition of this commercial Medical Research Council diet 18 was included 15 per cent of groundnut meal. In Morocco, as early as 1945, liver cancer was reported among pigs which were given cakes remaining after expressing the oil from cottonseeds or peanuts, while pigs fed scraps from slaughter houses did not develop liver tumours (89). The occurrence of "spontaneous" liver tumours has also been observed in French cancer institutes among Wistar rats in whose diet peanut meal was included (69). No doubt, many more such cases of "spontaneous" tumours have, in the past, remained unrecorded. In experiments designed to test the hepatocarcino-

[1] The survey of the literature pertaining to this review was concluded in August 1966.

genic action of chili (Capsicum), liver cirrhosis developed in both the experimental and the control rats. This might have been due to ardein, the protein of (toxic?) groundnuts, which was included in the semisynthetic diet fed to these animals (62).

Outbreaks of acute poisoning among livestock given mouldy feeds have been described at various times in many countries [compare (66, 123)]; whether some of these were due to aflatoxins or to some other toxic fungal metabolites would not be possible to distinguish *a posteriori*.

The extraction of the toxic constituents from the toxic groundnut meals or from cultures of *A. flavus* (6, 101), the recognition that these derive, not from the groundnuts as such, but from the contaminating fungi (101) which multiplied excessively as a result of a particularly wet harvesting season (15), the separation and purification of individual entities (42, 61, 85, 121), and the elucidation of the chemical structures of at least six toxic constituents (10, 11, 36, 51, 64, 122) have been achieved with remarkable rapidity in the intervening six years.

The success of these studies was a result of several favourable factors, besides the stimulus of the economic importance to food producers and to animal breeders, and of the possible health hazard to man. These toxins are extractable into organic solvents, they exhibit intense fluorescence by which traces can easily be recognized in ultraviolet light whether in solution, on paper chromatograms, or on thin-layer chromatoplates. They have high melting points, and because of their low solubilities, they crystallize readily from solvents. Once crystalline products became available, the modern physical methods for the study of chemical structures could be applied. The successful outcome of these studies is, however, mainly due to the perspicacity of the respective researchers and the skill in interpreting the data provided by the various physical methods, including mass spectrometry, ultraviolet and infrared spectroscopy, and in particular the nuclear magnetic resonance spectra. The structures assigned to the Afs (Fig. 1) received strong support from X-ray crystallographic studies by Cheung & Sim for aflatoxin G_1 (37) and by Van Soest & Peerdeman for aflatoxin B_1 (122a) and have been confirmed by synthesis of B_1 (23a).

The story of this rapid progress and of the contributions of the respective groups of workers has been told repeatedly, and several symposia have already been devoted to this subject (5, 15, 66, 99, 123, 124) and should be consulted. Most of the relevant data published up to April 1965 have recently been reviewed (124).

The bibliography on Afs, which already includes more than 400 papers published since 1960, is increasing rapidly. Many papers deal with the agricultural aspects, with the methods of detection and estimation of Afs in various foods and animal feeding stuffs, with the conditions which favour or which prevent the production of Afs, and with attempts, so far only partly successful, at detoxification (40); only a few of these papers will be mentioned here.

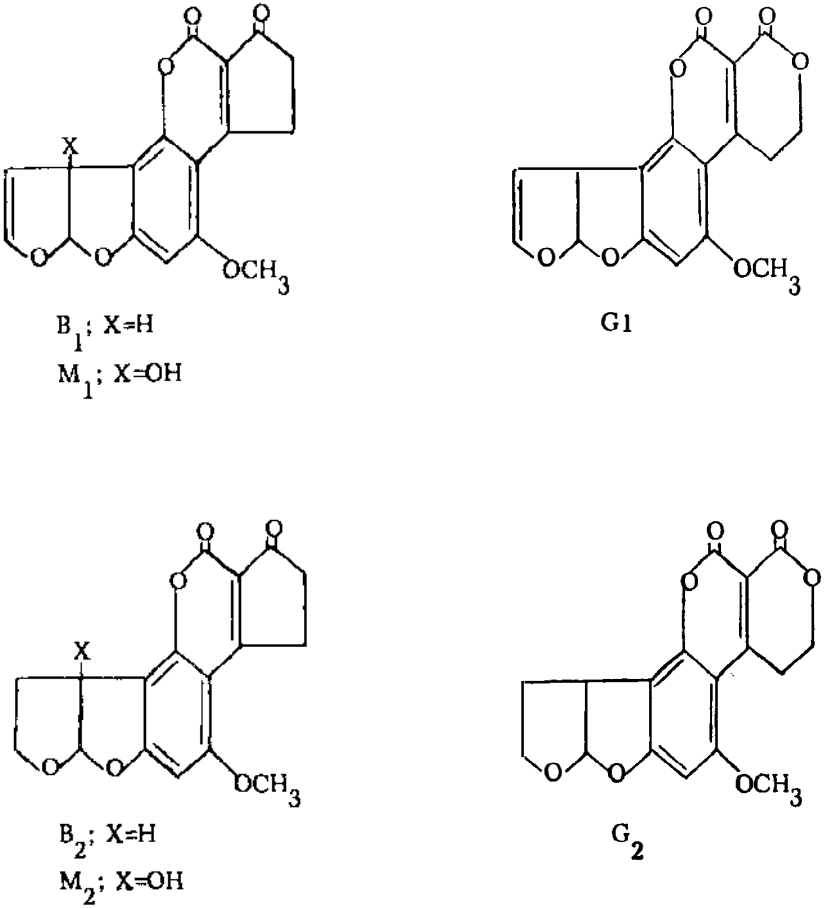

FIG. 1. Structures of the aflatoxins.

This article deals mainly with the toxic and carcinogenic effects of Afs, their mode of action, and with the role which they may play in the aetiology of human liver diseases.

PRODUCTION, ISOLATION, AND ESTIMATION OF AFLATOXINS

From the studies of the last few years, much evidence has been accumulated on the frequent fungal contamination of various animal and human foodstuffs (34, 66), on the almost ubiquitous distribution of *Aspergillus flavus* and of other Aspergilli through the world (15, 96), and on the conditions which favour the production of Afs by various strains or isolates of the *A. flavus oryzae* group (15, 39, 106, 107). Certain other fungi, e.g., *Penicillium puberulum*, can also produce Afs (63). It has been recognized that contamina-

tion with *A. flavus* does not necessarily imply the presence of Afs and that there is no parallelism between abundant growth of the fungus and high concentrations of these metabolites. The latter depend not only on the potential ability of the fungus to produce Afs, but to a great extent on the conditions of its growth, such as the temperature, oxygenation, appropriate nutrient and moisture content in the medium, and on the state of growth of the mycelium. Conditions of growth can also affect the relative amounts of the various Afs (13, 106). *A. flavus*, even when present in the soil, will not usually invade the growing plants or the intact seeds or nuts, but does so when the protective shell, husk, or membrane become damaged, especially during harvesting or storage (13, 107). Individual kernels invaded by the fungus may contain up to 3000 ppm of Afs; even a small number of such kernels can render the meals toxic. The nature of the protective factors present in the external coverings is still to be discovered. While warm, humid weather favours the growth of the fungus, the optimal conditions for the production of Afs by the mycelium are more stringent.

Experimentally high yields of Afs with preponderance of aflatoxin B_1 have been obtained by growing *Aspergillus parasiticus* Spears, or certain other aflatoxin-producing isolates, on sterilized crushed moist groundnuts (39), wheat (36), rice (110), or certain other cereals, at 28–30° C, for four to seven days. Liquid media, such as Czapek-Dox broth, fortified with certain sugars, groundnut or yeast extract, or corn steep liquor (106) have also been used for the production of aflatoxins.

For the isolation of Afs from contaminated foodstuffs or from fungal cultures, extraction with solvents (methanol, chloroform, aqueous acetone, etc.) is followed by the concentration of the extracts, and by the removal of oil (if present) with petroleum ether, in which Afs are not soluble. Further purification has been achieved by column, paper, or thin-layer chromatography using various adsorbents and various solvent systems.

A convenient screening (46) and a simple estimation procedure (71) have recently been described. The procedure using thin-layer chromatography on Kieselgel (Merck) and the solvent system, chloroform: methanol (98:2 v/v) resolves the crude mixture of Afs into a series of brightly fluorescent spots of various R_F's and of various shades of fluorescence; violet, blue, greenish, and golden yellow, visible on inspection of such chromatoplates in ultraviolet light (43). In some instances, at least 12 distinct spots have been seen (114). By scraping off the respective areas from the chromatoplates, elution with solvents, and crystallization, the major constituents have been obtained in pure form for structural studies.

The isolated Afs represent a group of closely related substituted bifuranocoumarins, the structures of which are shown in Figure 1. They have in common the central 5-methoxycoumarin moiety (10, 11, 36, 51, 64) which is the feature responsible for the similarity in the intense fluorescence and ultraviolet absorption of these compounds (Table I), and also for their photosensitivity (74).

On the basis of the colour of the fluorescence exhibited on thin-layer

TABLE I

PHYSICAL DATA OF AFLATOXINS

Aflatoxin	Molecular formula	Molecular weight	Melting	$[\alpha]_D$	UV absorption 265 mμ	362 mμ	(mμ)	R_F[a]
B_1	$C_{17}H_{12}O_6$	312	268–269 decomp.	−558[b]	13,400	21,800	425	0.56
B_2	$C_{17}H_{14}O_6$	314	286–289 decomp.	−430[b]	11,000	20,800	425	0.53
G_1	$C_{17}H_{12}O_7$	328	244–246 decomp.	−556[b]	10,000	16,100	450	0.48
G_2	$C_{17}H_{14}O_7$	330	237–240 decomp.	−473[b]	11,200	19,300	450	0.46
M_1	$C_{17}H_{12}O_7$	328	299 decomp.	−280[c]	11,600	19,000[d]	425	0.40
M_2	$C_{17}H_{14}O_7$	330	293 decomp.	—	10,900	21,000[d]	-	—

[a] Silica gel
[b] In chloroform
[c] In dimethylformamide
[d] At 357 mμ

chromatoplates, two of the Afs have been assigned the letter B (for Blue) and two the letter G (for Green) (61, 85). Recently, evidence has been obtained that the green fluorescence of aflatoxin G_1 is probably due to a yellow impurity, which can be removed, and that the pure compound exhibits a blue fluorescence (74).

Although their fluorescence is blue or violet, two hydroxylated Afs have been assigned the letter M. Originally, the letter M was intended to indicate a toxic metabolite isolated from the Milk of cows fed toxic groundnut meals (3, 44) and found also in the milk (44) and liver (31) of rats given pure aflatoxin B_1. Aflatoxin M is thus a Metabolite of aflatoxin B_1, and the retention of the designation M is doubly justified. It is also present in crude aflatoxin mixtures, in which it can sometimes represent significant proportions of the toxic constituents. Indeed, a batch of contaminated groundnuts contained sufficient quantities of aflatoxin M for its isolation and resolution into two components, M_1 and M_2 (64). This has been achieved by using chromatography on paper impregnated with formamide-water (85:15) and the solvent system ethyl acetate-benzene (9:1), on which M_1 showed a blue-violet fluorescent spot, R_F 0.34, and M_2 a violet fluorescent spot, R_F 0.23. The fluorescence intensities of the aflatoxins M_1 and M_2 have been reported to be three times stronger than those of the respective aflatoxins B. The presence of the hydroxyl has been deduced from the infrared absorption peak at 3425 cm^{-1} (Nujol) and its position from the nuclear magnetic resonance spectrum. Aflatoxin M_2 has been identified as a dihydro derivative of M_1, from which it has been obtained by catalytic hydrogenation (Pd on carbon in acetic acid).

In a recent communication (51), the identification of two other aflatoxins, B_{2a} and G_{2a}, isolated from *A. flavus* cultures has been described; these are said to represent hydroxylated B_2 and G_2 aflatoxins, in which the hydroxyls are situated at the position 2 of the terminal tetrahydrofurano moiety (and not at the position 4 of the same ring as reported for M_1 and M_2). In support of the proposed structure, aflatoxin B_{2a} has been acetylated and proved to give a compound identical with the known acetoxydihydro derivative of aflatoxin B_1.

Several procedures have been used for the estimation of Afs in appropriately purified extracts from suspected foodstuffs or fungal cultures (22, 43). Estimations based on acute toxicity in susceptible animals [one day old ducklings (6, 35) or young trout (12, 111)] can be done by using relatively crude extracts, and are more relevant to the assessment of hazard to animal health than those obtained by physical methods. The latter [compare (75)] have been based on the estimation of fluorescence or of ultraviolet absorption of the isolated, usually preponderant and most toxic constituent, aflatoxin B_1. However, fluorescent but nontoxic compounds of R_F, similar to aflatoxin B_1, may be present in some plant and fungal materials, while the neglected aflatoxins M or G have been found sometimes to represent a high proportion of the aflatoxin mixtures (64, 106). It is not unlikely that the good correlation between the results obtained with the physical and the biological methods were often caused by the cancelling out effects of such factors.

Other biological methods, which have been suggested for the detection of Afs, whether based on the death of chick embryos in fertilized eggs (94), on the inhibition of animal or human cells in tissue culture (41, 57, 65, 72, 126), on the inhibition of the growth and of chlorophyll production in plants (105), or on some biochemical criteria, though extremely sensitive, can be considered only indicative and have to be checked by toxicity tests in whole animals.

Susceptibility of Various Species to the Acute Action of Aflatoxins

Aflatoxin-containing meals have been shown to be hepatotoxic to many species of mammals, birds, and fish. Susceptibility to the toxic effects of Afs vary greatly with age, sex, nutritional status, and in particular with the species and even breed of animal. In all the species so far tested, the very young prove more susceptible than adults, and mature males more susceptible than mature females, except at particular stages of pregnancy when the latter appear to become more susceptible (32, 70). A dose of aflatoxin equal to only a quarter of the LD_{50} given on the 16th day of pregnancy or later can cause liver damage in the mother rat and retard the growth of the foetus (32, 70). Susceptibility to Afs [like that to the hepatotoxic pyrrolizidine alkaloids (102)] appears to depend on the state of sexual hormones in the animal. Foetal abnormalities have also been induced by injecting hamsters with aflatoxin B_1 on the eighth day of pregnancy (51a).

Low protein (4 per cent) diet has been found to increase the susceptibility of rats to Afs; the livers of these rats became very fatty (79), similar to those seen in rats maintained on a low (6 per cent) protein diet and given the pyrrolizidine alkaloid, lasiocarpine (102). Rats given a diet devoid of protein are even more susceptible to Afs (81).

Depending on their susceptibility to Afs, the animal species so far tested can be roughly divided into:

(*a*) very susceptible—for which the LD_{50} of aflatoxin B_1 is of the order of 1 mg/kg body weight or less: duckling (Khaki-Campbell or Pekin) (9, 25, 35,

85), rainbow trout (*Salmo gairdnerii*) (59), guinea pig (27, 91), rabbit (124), dog (124), newly-born rat (124), turkey poult (14);

(*b*) susceptible—but which require up to ten times higher doses: pig (60), rat (124), monkey (17, 80, 120), calf (5, 7), pheasant (1), chick (14), ferret (1), hamster (124), cow (7), mink (67), quail (Coturnix) (9), Coho salmon (*Oncorhynchus kisutch*) (59), and certain breeds of chicken (1, 58);

(*c*) resistant—which can tolerate relatively large doses of Afs without ill effects, like mice (92) and sheep (5).

The estimates of susceptibility in many species derive mostly from feeding aflatoxin-containing meals, and need exact evaluation with individual pure aflatoxins. Only small amounts of pure compounds have as yet been available; their LD_{50} has been established in ducklings (9, 35, 36, 64, 124). Aflatoxin B_1 has proved to be the most toxic (LD_{50} about 0.4 mg/kg). Aflatoxin M_1 has been reported to be almost as active as aflatoxin B_1 (64) while aflatoxin G_1 has been only about half as active (35). The dihydro aflatoxins B_2, M_2, and G_2, were only about one quarter as active as the respective aflatoxins B_1, M_1, and G_1, which have a double bond in the terminal bifurano moiety of their molecules (35, 36, 64). No data have as yet been reported on the biological properties of aflatoxins B_{2a} and G_{2a}.

Except for the difference in dose levels, the acute effects caused by the various Afs were similar, and were indistinguishable from those due to the ingestion of toxic groundnut meals (35, 88). In animals which die a few days after dosage, liver necrosis and bile duct proliferation are the lesions most commonly present; these are occasionally accompanied by kidney lesions (86). Pure aflatoxin B_1 has been tested in the duckling (35, 88), rat (26, 124), guinea pig (27, 124), trout (12), dog, hamster, and rabbit (124). It is of interest that while in the duckling and in the rat liver necrosis is periportal, it is centrilobular in the guinea pig (27). In animals which survive longer, liver necrosis is not seen, but the bile duct proliferation may become more prominent, the parenchymal cells and their nuclei increase in size, and hyperplastic nodules and a certain amount of fibrous tissue may be present. These subacute liver lesions are similar to those described in animals given pyrrolizidine alkaloids (102). In the monkey, fatty livers and periportal fibrosis are the predominant subacute lesions (80, 120).

Carcinogenic Action of Aflatoxins

It has already been mentioned that the occurrence of liver tumours in laboratory rats (and in hatchery rainbow trout) has been traced to toxic batches of groundnut or of other meals included in the diet of these animals. It would be of interest to know whether the conditions in the hatcheries in which liver tumours developed in rainbow trout were favourable for the production of aflatoxins. Even slight contamination of the diet with the appropriate strain of fungi might then have been sufficient to induce the liver tumours (64a).

Experimental feeding of toxic meals or of mixtures of Afs induced

liver tumours, hepatoma, and cholangioma in rats (18, 29, 68, 69, 86, 98, 103, 124), ducklings (33), trout (12, 111), and guinea pigs (16). The last three species, which are more susceptible than rats to the acute toxic action of Afs, require appropriately smaller doses than the rat for the development of liver tumours. It is interesting to note that mice which are resistant to aflatoxin-containing diet [(92) Schoental, unpublished results] developed sarcomata at the site of subcutaneous injections of Afs (49).

Continuous feeding of Afs is not necessary; rats developed liver tumours when given Afs for 89 (18) or 30 days only (124). It is not yet known whether a single dose of Afs could induce hepatomata as do certain pyrrolizidine alkaloids (102). Dose-response studies in rats showed that when the dosage is appropriately reduced, liver tumours tend to appear after longer time intervals, the animals survive longer, and some develop tumours also at sites other than the liver, such as the glandular stomach (29, 30, 50), kidney (29, 50, 98), lacrimal duct (29, 50), etc.

In rats, crystalline Afs proved to be carcinogenic at the site of administration [giving sarcoma at the site of repeated subcutaneous injections (48–50) and tumours of the trachea on intratracheal intubation (50)] and also to induce tumours in organs which are remote from the site of administration (liver, kidney, etc.). This might indicate that Afs are carcinogenic per se, and that they can exert their carcinogenic action in various organs of the body to which they are carried by the blood stream. It is not yet known whether a hydroxylated aflatoxin, like M_1, is formed from B_1 only in the liver, or whether it can be formed also in other tissues and represent the proximal carcinogen.

It has been pointed out that many carcinogens can interact with thiols without the need of enzymes and that they are, or become through metabolic transformation, multifunctional agents (104). This could apply to aflatoxins. Hydroxylation of aflatoxin B_1 to M_1 does not affect its toxicity, but increases the number of functional sites. Aflatoxin M_1 would be expected to react with thiols more readily than aflatoxin B_1 (47) and to be a proximal carcinogen. The possibility that thiols might be involved in the action of Afs has received support from the evidence that cysteine and glutathione prevent the inhibition by Afs of the activity of the amino acid-activating enzyme in both liver and *Escherichia coli* preparations (113a).

Sterigmatocystin (Fig. 2), a metabolite of *Aspergillus versicolor* (Vuillemin) Tiraboschi, is a dihydrobifuranomethoxyxanthone derivative (24). Part of its structure is similar to that of aflatoxin B_1 but it has no lactone ring. Sterigmatocystin has been found to be carcinogenic (50); it induced sarcomata at the site of repeated subcutaneous injections and also liver tumours. Though the dosage was much higher than that used for the induction of sarcomata by Afs (500 μg/rat per dose against 2.0 μg/rat per dose of Afs), this result indicates that the unsaturated lactone ring can be replaced by a γ-pyrone, without abolishing the carcinogenic activity.

The role of the methoxy group on the aromatic ring, which might be expected to be essential for the carcinogenic action, is not yet known. A

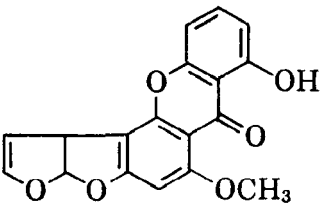

FIG. 2. Sterigmatocystin.

demethylated aflatoxin B_1 has not been prepared. It might be a precursor in the biosynthesis of aflatoxins.

THE METABOLISM OF AFLATOXINS

The metabolism of Afs has so far been studied in two species: the rat (31, 44, 52, 109), moderately susceptible to the acute toxicity and readily responding to their carcinogenic action, and in the sheep (8, 82) which appears resistant to aflatoxins.

When fluorescence is used as a guide in the search for metabolites, only such products can be detected in which the chromophoric moiety, the aromatic ring conjugated to the double bond of the α, β-unsaturated lactone, remains intact. In the sheep, only 8 per cent of the total single dose of mixed Afs was recovered in the excreta in the form of unchanged B_1 and G_1, 6.4 per cent in the urine, 1.6 per cent in the faeces, and 0.1 per cent in the milk. Aflatoxin M_1 was excreted in relatively large amounts in the urine; it was also present in the faeces and the milk (82).

When toxic cow's milk was treated with rennet (4), its toxicity was found in the precipitated casein fraction, which included also the fat, while the other milk proteins and the supernatant were not toxic. There is little doubt that cheese or butter made from such toxic milk will retain its toxicity. It is not yet certain whether the toxicity of rennet-precipitated casein can be quantitatively removed by extraction with alcohol, which is the usual procedure for the preparation of vitamin-free casein. In the case of toxic peanut meals, extraction with hot methanol was not sufficient to remove all its toxicity (86, 98). It seems now possible that contaminated milk and casein might have been responsible for much confusion in nutritional studies. The following examples illustrate the point. It has been reported that liver necrosis developed in rats maintained on a diet of dried milk (53, 54), and liver tumours have been found in obese rats given a diet of milk and egg yolk (84). Liver necrosis has been seen in rats fed a diet prepared with a batch of casein derived from New Zealand milk; but not in rats given a similar diet containing casein derived from English milk (83).

When rats were given pure aflatoxin B_1, aflatoxin M was detected in extracts of the blood and liver (31). The peak values were reached in about one to two hours and declined rapidly. Besides aflatoxin M, other faintly fluorescent spots were seen on thin-layer chromatoplates (31). Several fluorescent constituents have also been seen in extracts of bile after intravenous injection

of Afs to rats. Some of these might have been conjugated metabolites or photodecomposition products of Afs (52). However, no toxicity could be detected in the liver, meat, and eggs of animals fed aflatoxin-containing meals (1, 3, 93).

Using ^{14}C-labelled aflatoxin B_1, the distribution of radioactive carbon has been studied in rat tissues and excreta (109, 124). About 25 per cent of the total administered dose of the ^{14}C-methoxy-labelled compound [obtained by growing *A. flavus* in cultures containing ^{14}C-methylmethionine (2)] was excreted in 24 hours in the expired CO_2, about 20 per cent in the urine and about 25 per cent in the faeces. Of the 30 per cent left in the body, the liver contained about 7 per cent of the dose. When ring-labelled aflatoxin B_1 was used [obtained by growing *A. flavus* in the presence of 1-^{14}C-acetate (2)], almost no activity was found in the expired CO_2, about 18 per cent was excreted in the urine and about 65 per cent in the faeces. It is of interest that the proportion retained by the liver was practically the same (about 9 per cent) as with the O-methyl-labelled compound. This might indicate the presence of the intact methoxy group and of the undegraded ring structure in the retained material.

Biochemical Effects of Aflatoxins

Much effort is now being devoted to the study of the early biochemical and ultrastructural changes induced by Afs in the liver cells, in the hope of finding the primary lesions involved in the carcinogenic action. Like many other hepatotoxins, Afs have been found to inhibit the incorporation of labelled precursors into DNA (45, 78), RNA (38, 55, 56, 116), and proteins, especially into the inducible enzyme proteins, both *in vivo* (38, 56, 95, 108, 125) and *in vitro* (38, 113), and also in plants (20). The bathochromic effect of DNA on the ultraviolet absorption spectrum of Afs *in vitro* has been interpreted as an indication of binding of the latter to DNA (38, 116), possibly interfering with the DNA-dependent messenger RNA and affecting the synthesis of proteins (38). Histochemically, the decrease of several enzymatic activities has been observed, while acid phosphatase activity was increased (1, 23, 199). Of interest is the striking decrease of the vitamin A content in the livers of pigs and calves (7).

In partially hepatectomized rats, inhibition of the biosynthesis of DNA and RNA could be seen after very small doses of Afs (45, 55, 70). The French workers postulate that the inhibition of mitosis in such regenerating rat liver is the result of inadequate production of nuclear materials.

Parallel electron microscopic studies (19) indicate that the first changes, already seen 30 minutes after aflatoxin B_1, occur in the nucleolus and involve the redistribution of its content, resembling the formation of nucleolar "caps" after actinomycin D. This has been confirmed in intact rats (117) and can also be seen after administration of the pyrrolizidine alkaloid lasiocarpine. Cytoplasmic changes follow somewhat later and consist of the dislocation and decrease of ribosomes, proliferation of the smooth endoplasmic reticulum, loss of glycogen, and degeneration of mitochondria (19, 28,

118). Unless the cells undergo necrosis, all the changes appear to be reversible, and their significance for the carcinogenic process is not clear.

Changes have been reported in the various cellular membranes of the duckling parenchymal liver cells, especially at the site of the penetration of extravasated red cells (118, 119), which the South African workers consider of primary importance.

Aflatoxin inhibits the mitoses stimulated in rat liver *in vivo* by dimethylsulphoxide (97). Inhibition of cell division in tissue culture can lead to giant cells (72), and this effect as well as the induction of chromosomal aberrations in *Vicia faba* (73) has been interpreted as mutagenic action of aflatoxins.

Relevance of Aflatoxins to Human Liver Diseases and Primary Liver Cancer

At present no direct evidence is available as to the degree of susceptibility of man to aflatoxins. The ill effects seen in a few patients given large amounts of bread made from peanut meal, which caused death and liver lesions in dogs, is difficult to interpret (14a). Though the majority of the animal species tested, including primates (17, 80, 120), were susceptible, the mouse and the sheep showed surprisingly great resistance to aflatoxins. The factors responsible for this difference are not known. It has been suggested that there is some correlation between the susceptibilities to Afs of cells grown in tissue culture and of the animals from which the cells derive (57). On this basis (57, 72, 126), man would be expected to be susceptible. However, as cells of the resistant species have not yet been tested, caution is indicated in applying this criterion.

Primary liver cancer may result from the cumulative effects not only of several carcinogenic but also of cocarcinogenic agents (112). Genetic and nutritional factors can modify the effects of the ingested toxins of plant or fungal origin, and the outcome will depend on their interplay. Sudden change from a low nutritional level to an abundant, high protein diet may stimulate a precancerous dormant liver condition to flare up into cancer (87). This could apply in the case of young Africans working in goldmines.

When an adult is suffering from a chronic liver disease, the disentangling of the various factors to which he might have been exposed during his life is not easy; the aetiology of liver disease in the infant and the newly-born may be easier to investigate, as the mothers may still remember any unusual features of food or medicine taken during pregnancy or lactation.

Groundnuts consumed whole can be selected free from contamination by hand picking. Processes used for the refining of oils will inactivate any extracted aflatoxins. The meals, however, are difficult to detoxify without diminishing their nutritional value and are subject to additional contamination during storage and transport. Aflatoxins are likely to remain a serious hazard to the health of animals and possibly of man until suitable means are found either to control growth of fungi by appropriate fungicides or to increase resistance to their action.

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