Biosynthetic Origin of Aflatoxin G₁: Confirmation of Sterigmatocystin and Lack of Confirmation of Aflatoxin B₁ As Precursors

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The origin of aflatoxin G₁ was studied using mutant strains of *Aspergillus parasiticus* blocked early in the pathway and by tracing ¹⁴C-labelled aflatoxin B₁ (AFB₁) in wild-type *A. flavus* and *A. parasiticus* strains. Sterigmatocystin (ST) was a precursor of AFB₁, AFG₁ and AFG₂ in the four mutants examined. The identity of AFG₁ was confirmed by mass spectrometry. No evidence for conversion of AFB₁ to AFG₁ was found. A rigorously controlled study of conversions of radioactivity based on preparative thin-layer chromatography of aflatoxins demonstrated that low levels of aflatoxin interconversions previously reported in the literature might actually be artifacts.

INTRODUCTION

The aflatoxins are a family of potent mycotoxins produced by certain strains of the common moulds *Aspergillus flavus* and *A. parasiticus*. Aflatoxin contamination of foods and feeds presents an important toxicological hazard (Goldblatt, 1969; Betina, 1984). The major naturally occurring aflatoxins are aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂). Most toxigenic strains of *A. parasiticus* produce AFB₁, AFB₂, AFG₁ and AFG₂, while toxigenic strains of *A. flavus* produce only AFB₁ and AFB₂. No strains that produce the G aflatoxins without the B aflatoxins have been isolated from nature or from laboratory mutants (Klich & Pitt, 1985; Bennett & Deutsch, 1986); however, a number of strains that produce AFB₂ and little or no AFB₁ are known (Dutton *et al.*, 1985).

Considerable research has been done on aflatoxin biosynthesis (Maggon *et al.*, 1977; Heathcote & Hibbert, 1978; Steyn, 1980; Applebaum & Marth, 1981; Bennett & Christensen, 1983). Known aflatoxin precursors include five anthraquinones (norsolorinic acid, averantin, averufanin, averufin and versicolorin A) and the xanthenes sterigmatocystin (ST) and *O*-methylsterigmatocystin (Bhatnagar *et al.*, 1987; McCormick *et al.*, 1987) with the general sequence, acetate→anthraquinones→xanthenes→aflatoxins. Biotransformation experiments with blocked mutants, radiotracer studies with ¹⁴C-labelled precursors and stable isotope studies with ¹³C-labelled precursors have emphasized the biosynthetic origin of AFB₁ while the biosynthetic origins of AFB₂, AFG₁ and AFG₂ have received less attention. It is generally assumed that AFB₁ is a precursor of the other aflatoxins (Thomas, 1965; Biollaz *et al.*, 1970), and several reviews include Figures illustrating an AFB₁ to AFG₁ conversion (Berger & Jadot, 1976; Heathcote & Hibbert, 1978; Bu'Lock, 1979).

Experimental verification of the role of AFB₁ as the precursor of AFG₁ is based on two studies. Maggon & Venkatasubramanian (1973) used [¹⁴C]AFB₁ as a substrate for *in vitro* incubation with a cell-free homogenate of *A. parasiticus* and reported recovery of low levels of ¹⁴C label in AFB₂, AFG₁ and AFG₂; Heathcote *et al.* (1976) reported similar results using an *in*

Abbreviations: AFB₁, B₂, G₁, G₂, aflatoxins B₁, B₂, G₁ and G₂; ST, sterigmatocystin; GM, growth medium; LSRM, low-sugar replacement medium; EMW, ethanol/methanol/water.

vivo system with wild-type *A. flavus*. However, recent work in our laboratory raises questions about the metabolic origin of AFG₁. Using a mutant blocked in the aflatoxin pathway at versicolorin A we were unable to biotransform AFB₁ or AFB₂ into AFG₁ or AFG₂ (Floyd *et al.*, 1987).

In this report we re-examine the final stages of aflatoxin biosynthesis by doing rigorously controlled experiments on biotransformation of ST, AFB₁ and AFG₁ with four blocked mutants, as well as by tracing [¹⁴C]AFB₁ in wild-type *A. flavus* and *A. parasiticus*.

METHODS

Organisms. The wild-type strain of *A. parasiticus* was SU-1 (ATCC 56775); the wild-type strain of *A. flavus* was SRRS 2002, isolated by L. S. Lee from aflatoxin-contaminated cottonseed. Four anthraquinone-accumulating mutants of *A. parasiticus* were used: a norsolorinic acid-accumulating mutant, *nor-1* (ATCC 24690); an averufin-accumulating mutant, *avr-1* (ATCC 24551); a versicolorin-A-accumulating mutant, *ver-1* (ATCC 36537); and an averantin-accumulating double mutant, *avn-1 ver-1* (ATCC 56774).

Media and culture conditions. Stock cultures were maintained on potato dextrose agar (Difco) containing 0.5% yeast extract. Synthetic growth medium (GM) was modified by substituting sucrose for glucose but otherwise was formulated according to Adye & Mateles (1964). The low-sugar replacement medium (LSRM) was also formulated according to Adye & Mateles (1964). Approximately 10⁶ spores were used to inoculate 100 ml GM. Cultures were incubated at 27 °C in the dark on a gyratory shaker (model G76, New Brunswick) operating at 180–200 r.p.m.

Biotransformation experiments. Wild-type or mutant strains were grown for 48 h at 27 °C on the shaker in GM. Resultant mycelial pellets were harvested on cheesecloth and rinsed with LSRM. Pellets were weighed on sterile, tared paper and 1 g (wet wt) was added to 9.8 ml LSRM. For biotransformation studies, 555 nmol unlabelled ST, AFB₁, AFB₂, AFG₁ or AFG₂, or 2 and 4 nmol [¹⁴C]AFB₁, dissolved in 200 µl acetone were added to the 9.8 ml LSRM cultures.

Controls consisted of adding 200 µl acetone without aflatoxin; in some controls, mycelia were also omitted. After transfer, cultures were incubated for an additional 24 h, then extracted.

Extraction of metabolites. Extraction procedures were essentially those used by Lee *et al.* (1976). Briefly, entire cultures (mycelium plus culture filtrate) were first extracted with aqueous acetone (70%, v/v). The mycelial pellets were then removed by filtration and the culture filtrate was extracted twice with chloroform in a separating funnel. The pooled, non-aqueous fractions from entire cultures were filtered through sodium sulphate, then concentrated in a Buchi rotary evaporator or air-dried in a chemical hood. Dried samples were resuspended in 1 ml chloroform for subsequent TLC. For preparative TLC the resuspended samples were concentrated to approximately 500 µl under nitrogen.

Quantification of metabolites. Initial TLC on silica gel G plates in ether/methanol/water (EMW) (96:3:1, by vol.) was done to obtain a visual estimate of the appropriate amount of extract to spot for densitometric readings, as recommended in the Official Methods of the American Oil Chemists' Society (Walker, 1983). Prescored 250-µm-thick silica gel G plates (20 × 20 cm) (Analtech) were spotted, developed in EMW, and scanned for fluorescent materials using a Shimadzu model CSW 9-10 recording densitometer; excitation was at 360 nm for aflatoxins and at 540 nm for ST. The quantities of aflatoxins and ST were calculated from the respective integrated areas of standards run on the same plate.

Preparative TLC. Samples were concentrated under nitrogen to approximately 500 µl and then streaked at the origin of 500-µm-thick Absorbosil Plus One (Alltech) silica gel plates (20 × 20 cm) which were poured in the laboratory. A separate spot of mixed aflatoxin standards was placed adjacent to each streak; plates were developed in EMW. Zones of sample corresponding to the *R_F* values of aflatoxin standards were visualized by examining the plate under low-wattage UV and then scraped from the plate. Aflatoxins were extracted from the silica with acetone through a sintered glass filter. *A. parasiticus* SU-1 culture extracts alone and with added ST were used to establish preparative TLC conditions and to determine the expected *R_F* zones for aflatoxins and ST. The methods were standardized further by analysing the extracts again by TLC. In the samples containing ¹⁴C, zones corresponding to the *R_F* values of aflatoxins were scraped directly into vials of scintillation fluid for counting radioactivity.

Confirmation of identity of AFG₁. Ten separate cultures (1 g wet wt mycelium) of the double mutant *avn-1 ver-1*, grown for 48 h in GM, were incubated for 24 h in LSRM supplemented with 555 nmol ST. Chloroform extracts of the cultures were fractionated into individual aflatoxins by preparative TLC. The purity of each recovered AFG₁ sample was assessed by TLC.

The pooled, purified AFG₁ was analysed by HPLC using a Waters model 480 high pressure liquid chromatograph equipped with a 300 × 4 mm (inside diameter) normal phase silica gel column (10 µm particle size) by the method of the American Oil Chemists' Society (Walker, 1983). Further, the AFG₁ was identified by MS with a Finnigan MAT quadrupole mass spectrometer.

Table 1. Production of aflatoxins by wild-type and blocked mutants of *A. parasiticus* with and without ST

Mycelia were grown in GM for 48 h at 27 °C on a gyratory shaker, after which 1 g (wet wt) of mycelium was transferred to 10 ml LSRM with or without 555 nmol ST and incubated for an additional 24 h. Results are means of triplicate samples; SD values are given in parentheses. ND, None detected; tr., <1 nmol (g mycelium)⁻¹.

Strain	ST	Aflatoxin [nmol (g mycelium) ⁻¹]				ST recovered [nmol (g mycelium) ⁻¹]
		B ₁	B ₂	G ₁	G ₂	
Control (medium only)	+	ND	ND	ND	ND	500 (106)
<i>nor-1</i>	—	tr.	ND	tr.	tr.	
	+	449 (27)	ND	15 (3)	0.5 (0.25)	96 (22)
<i>avn-1 ver-1</i>	—	ND	ND	ND	ND	
	+	439 (56)	ND	10 (3)	2.8 (0.9)	71 (16)
<i>avr-1</i>	—	253 (94)	ND	ND	ND	
	+	488 (45)	ND	8 (2)	0.15 (0.07)	185 (47)
<i>ver-1</i>	—	2 (1)	ND	tr.	ND	
	+	417 (9)	ND	19 (4)	1.0 (0.85)	219 (94)
SU-1 (Wild-type)	—	577 (29)	8 (2)	397 (10)	11(2)	
	+	1002 (53)	ND	414 (48)	ND	287 (76)

Quantification of radioactive label. Radioactivity was determined by liquid scintillation counting in a Beckmann LS 1800 scintillation counter interfaced to a Hewlett Packard 2617IA printer to record c.p.m., efficiency of ¹⁴C detection and d.p.m. ¹⁴C standards were run for every 50 sample counts, and both silica gel and solvent controls were included. The scintillation fluid for non-aqueous samples was Kodak 'Ready to Use I'; for aqueous extracts Kodak 'Ready to Use II' was used.

Autoradiography. Developed TLC plates from radioactive tracer studies were marked under longwave UV light, then covered with an unexposed X-OMAT AR film (Eastman Kodak) and placed into a cassette type exposure holder (Kodak). Exposure time at room temperature was 5 weeks. In some experiments, TLC plates were first sprayed with Enhance, then exposed at -10 °C for 5–10 d. All films were developed according to manufacturer's protocol with GBX developer and fixer (Kodak). The developed autoradiogram was interpreted by superimposing the film onto the corresponding TLC plate.

Chemicals and solvents. All the chemicals used were of American Chemical Society quality. Aflatoxin standards, [¹⁴C]AFB₁ [uniformly ring-labelled, specific activity 50 Ci mol⁻¹ (1.85 TBq mol⁻¹)] and ST were purchased from Sigma.

RESULTS

The role of ST as a precursor of aflatoxins was examined in four mutants blocked in the aflatoxin pathway prior to ST. The effect of exogenous ST on aflatoxin production by wild-type *A. parasiticus* was also examined (Table 1). Three of the mutants produced aflatoxins in the absence of added ST: *nor-1* and *ver-1* produced trace to low levels of aflatoxin [2 nmol (g mycelium)⁻¹] while *avr-1* produced 253 nmol AFB₁ (g mycelium)⁻¹. Only the averantin-accumulating double mutant *avn-1 ver-1* was entirely blocked, producing no detectable aflatoxins under the conditions of these experiments. In the presence of ST, all four mutants produced similar levels of AFB₁ [417–488 nmol (g mycelium)⁻¹], AFG₁ [8–19 nmol (g mycelium)⁻¹] and AFG₂ [0.5–2.8 nmol (g mycelium)⁻¹]. No detectable AFB₂ was produced by any of the mutant strains in the presence or absence of ST. When exogenous ST was added to wild-type *A. parasiticus* SU-1, the level of total aflatoxin increased and shifted from production of all four aflatoxins to production of predominately AFB₁ and AFG₁.

Previous identification of ST as a precursor of AFG₁ was based solely on TLC assay (Singh & Hsieh, 1977; Floyd *et al.*, 1987). We verified the precursor status of ST with respect to AFG₁ by analysing a pooled sample of AFG₁ isolated after biotransformation of ST by the totally blocked double mutant *avn-1 ver-1*. HPLC analysis yielded a peak with the same retention time (8.4 min) as that of an authentic standard of AFG₁. The identity of AFG₁ was confirmed by MS. The

Table 2. *Recovery of aflatoxins B₁, B₂, G₁ and G₂ after incubation in replacement medium with and without wild-type and mutant mycelia of A. parasiticus*

Mycelia were grown for 48 h in GM; 1 g (wet wt) was transferred to 10 ml LSRM and incubated with 555 nmol aflatoxin; in controls aflatoxins were incubated in LSRM alone. Results are means of triplicate samples; SD values are given in parentheses. ND, None detected.

Strain	Aflatoxin added (555 nmol)	Aflatoxin recovered (nmol)			
		B ₁	B ₂	G ₁	G ₂
Control (no mycelium)	None	ND	ND	ND	ND
	AFB ₁	446 (45)	ND	ND	ND
	AFG ₁	ND	ND	416 (18)	ND
SU-1 (Wild-type)	None	1930 (83)	2.4 (0.72)	132 (22)	0.7 (0.35)
	AFB ₁	2660 (530)	2.5 (4)	125 (20)	61 (19)
	AFG ₁	1200 (240)	ND	304 (19)	ND
<i>avn-1 ver-1</i> (Averantin mutant)	None	ND	ND	ND	ND
	AFB ₁	288 (25)	13 (4)	ND	ND
	AFG ₁	ND	ND	245 (15)	ND

sample from the *avn-1 ver-1* double mutant had ions at *m/e* 328 (*M* +), *m/e* 256, *m/e* 241 and *m/e* 213, corresponding to *m/e* values of authentic AFG₁ and the published spectrum (Cole & Cox, 1981).

In an attempt to demonstrate interconversion of AFB₁ to AFG₁, individual samples of AFB₁ and AFG₁ were added separately both to strain SU-1 and to the *avn-1 ver-1* double mutant (Table 2). Since the *avn-1 ver-1* double mutant makes no detectable aflatoxins, recovery of aflatoxins other than the aflatoxin presented to the mutant would be evidence for metabolic conversion. When AFB₁ and AFG₁ were incubated with the *avn-1 ver-1* double mutant, or in controls lacking mycelia, they were recovered in good yields (AFB₁, 52 and 80%; AFG₁, 44 and 75%, respectively). Low levels of AFB₂ (13 nmol) were detected, suggesting a possible biotransformation of AFB₁ to AFB₂; however, there was no evidence of an AFB₁ to AFG₁ biotransformation. In general, the incubation of exogenous aflatoxins with the wild-type strain SU-1 resulted in a simple additive recovery over control values. No increase in AFG₁ was observed with added AFB₁, although production of AFG₂ was higher. When AFG₁ was added to the wild-type, recovery of AFB₁ was lower and neither AFB₂ nor AFG₂ were detected.

Since it is possible that exogenous aflatoxins were not taken up by mycelia in replacement cultures, exogenous [¹⁴C]AFB₁ (4 nmol) was fed to wild-type *A. parasiticus* before incubation for 24 h. The distribution of ¹⁴C label was then measured before and after extraction. When separate samples of culture filtrates and mycelia were placed directly into scintillation fluid, all the original label was recovered (Table 3). When the entire culture (medium plus mycelia) was extracted, 71% of the label was recovered in the chloroform-soluble fraction. When the mycelia were separated from the culture filtrate and washed, 2% of the label was associated with the water-soluble mycelial fraction and 16% with the chloroform-soluble mycelial fraction, indicating probable uptake of 0.6 nmol [¹⁴C]AFB₁. An autoradiogram of a TLC plate of chromatographed mycelial extract indicated that the majority of the ¹⁴C label recovered was associated with a spot corresponding to AFB₁ (not shown).

Since published reports of conversion of AFB₁ into other aflatoxins were based on recovery of ¹⁴C label in biotransformation experiments with wild-type strains, we studied the metabolic conversion of exogenous [¹⁴C]AFB₁ added to both wild-type *A. parasiticus* (SU-1) and wild-type *A. flavus* (SRRC 2002). In addition, [¹⁴C]AFB₁ was incubated in LSRM in the absence of mycelia. All cultures were extracted and subjected to preparative TLC according to the aforementioned procedures. The TLC plate was divided into zones corresponding to the chromatographic mobilities of the different aflatoxins and entire silica gel fractions were

Table 3. Recovery of ^{14}C label from mycelia of wild-type *A. parasiticus* incubated with exogenous [^{14}C]AFB₁

Strain SU-1 was grown for 48 h in GM; 1 g (wet wt) mycelium was transferred to 10 ml LSRM and incubated with 4 nmol [^{14}C]AFB₁ for 24 h. Data are means of four independent observations; SD values are given in parentheses.

Fraction used for scintillation counting	^{14}C label recovered	
	$10^{-3} \times \text{d.p.m.}$	%*
Unextracted culture filtrate†	357 (10)	83
Unextracted mycelium‡	100 (5)	23
Chloroform extract of culture filtrate plus mycelium	308 (15)	71
Chloroform extract of mycelium§	68.8 (13)	16
Water extract of mycelium	9.3 (3)	2

* (d.p.m. recovered/d.p.m. introduced) $\times$ 100.

† Samples of culture filtrate and mycelia placed directly into the scintillation vial.

‡ Both culture filtrate and mycelium extracted into aqueous acetone and partitioned into chloroform.

§ Mycelia separated from culture filtrate, washed, extracted with aqueous acetone and partitioned into chloroform.

|| Aqueous phase of §.

Table 4. Recovery of ^{14}C label from preparative TLC plates of chloroform extracts after addition of [^{14}C]AFB₁ to wild-type mycelia of *A. parasiticus* and *A. flavus*, and to controls without mycelia

Mycelia were grown for 48 h in GM; 1 g (wet wt) was transferred to LSRM and incubated for 24 h with [^{14}C]AFB₁ [4 nmol (431×10^3 d.p.m.) added to control and *A. parasiticus*; 2 nmol (215×10^3 d.p.m.) added to *A. flavus*]. For *A. parasiticus* and *A. flavus*, results are means of two independent observations; SD values are given in parentheses. In controls, [^{14}C]AFB₁ was incubated in LSRM alone.

R_f of preparative silica gel zone*	Description of zone	^{14}C label recovered					
		Control (no mycelium)		Wild-type <i>A. parasiticus</i>		Wild-type <i>A. flavus</i>	
		$10^{-3} \times \text{d.p.m.}$	%	$10^{-3} \times \text{d.p.m.}$	%	$10^{-3} \times \text{d.p.m.}$	%
0-0.05	Origin	1.9	0.4	—	—	5.1 (0.5)	2.3
0.05-0.18	Below G_2	10.1	2.3	—	—	3.6 (0.3)	1.7
0.18-0.22	G_2	3.0	0.7	3.5 (6.4)	0.8	3.0 (1.1)	1.4
0.22-0.25	G_1	12.0	2.8	8.8 (0.9)	2.0	7.7 (2)	3.6
0.25-0.26	B_2	3.0	0.7	6.4 (0.9)	1.5	2.2 (0.4)	1.0
0.26-0.36	B_1	47.0	10.9	95.0 (2.8)	22.0	42.0 (5.6)	19.5
0.36-1.0	Above B_1	5.9	1.4	—	—	7.7 (2.1)	3.6
Total ^{14}C label recovered			19.2		26.3		33.1

* Developing solvent was ethanol/methanol/water (96:3:1, by vol.).

scraped into scintillation vials and counted. Total d.p.m. recovered (Table 4) from cultures containing mycelia ranged from 26–32%; only 18% of the label was recovered from medium alone. With each species most of the label was associated with the zone corresponding to AFB₁. The level of label associated with AFB₂, AFG₁ and AFG₂ was similar for controls lacking mycelia and for *in vivo* studies.

Autoradiograms prepared from chromatographed control extracts of [^{14}C]AFB₁ incubated with LSRM showed smearing of radioactivity from the origin to an R_f corresponding with AFB₁. Extracts from wild-type *A. flavus* (SRRRC 2002) showed similar smearing on autoradiograms.

DISCUSSION

Most published studies on aflatoxin biosynthesis have utilized isotopic labels to measure the incorporation of label from putative precursors into AFB₁ (Heathcote & Hibbert, 1978; Steyn, 1980; Bennett & Christensen, 1983). We examined the biosynthetic origin of AFG₁ using *in vivo* (whole cell) biotransformations with mutant strains that were blocked in the aflatoxin pathway but were derived from wild-type *A. parasiticus* that originally produced both B and G aflatoxins. Analysis of end-product aflatoxins by fluorodensitometric procedures permitted reproducible quantification of aflatoxin levels as low as 2–5 ng.

The four characterized anthraquinone-accumulating mutants, *nor-1*, *avr-1*, *ver-1* and *avn-1 ver-1* are all blocked in aflatoxin biosynthesis prior to ST. In feeding studies with ST, after 24 h incubation, all four blocked mutants produced AFB₁, AFG₁ and AFG₂, but no detectable AFB₂. Similar results for 24 h incubations were reported by Floyd *et al.* (1987) for *avr-1*, *ver-1* and *avn-1 ver-1*; when incubations with ST were continued for 96 h AFB₂ was detected. Floyd *et al.* (1987) used autoclaved mycelia as controls; in this study living mycelia without ST were used. The controls with living mycelia demonstrated that the *nor-1*, *avr-1* and *ver-1* mutants are 'leaky'; i.e. they produce low levels of aflatoxin in the absence of ST. The strain *avn-1 ver-1* is a double mutant, isolated after nitrosoguanidine treatment of *ver-1* (Bennett *et al.*, 1980). This double mutant strain has never produced detectable aflatoxins in our laboratory and is therefore the mutant of choice for studying the late stages of the aflatoxin pathway.

The production of AFG₁ from ST has been reported before (Singh & Hsieh, 1977; Floyd *et al.*, 1987). In those studies, AFG₁ was identified by TLC assay and biotransformation was effected by the 'leaky' *ver-1* mutant. We have verified the role of ST as a precursor of AFG₁. The 'non-leaky' *avn-1 ver-1* double mutant was incubated with ST; the resultant AFG₁ was purified and confirmed by HPLC using fluorescent detection and by MS.

The mutant bioconversion strategy was then attempted in order to confirm the role of AFB₁ as the precursor of AFG₁. Exogenous unlabelled AFB₁ and AFG₁ were presented to replacement cultures of the *avn-1 ver-1* double mutant, and after 24 h incubation cultures were extracted (Table 2). No interconversion of AFB₁ to AFG₁ or AFG₂ was found, nor did AFG₁ demonstrate interconversion in this study. There was evidence for a slight conversion (2%) of AFB₁ to AFB₂ but this would have to be established by further studies and could be attributed to streaking. This confirms the lack of AFB₁ to AFG₁ bioconversion reported by Floyd *et al.* (1987) using *ver-1*.

It is possible that aflatoxins are not permeable through the fungal cell wall and hence not available at the biosynthetic site. To test this hypothesis we measured the fungal uptake of ¹⁴C label from exogenous [¹⁴C]AFB₁, and found that 16% of the label was associated with the chloroform-soluble portion of the mycelia, indicating uptake of [¹⁴C]AFB₁. To our knowledge this is the first report of movement of AFB₁ from the outside to the inside of the cell. It is well-known that aflatoxins synthesized in the mycelium readily cross the cell wall into the culture medium (Shih & Marth, 1973). Therefore, our inability to biotransform exogenous aflatoxins cannot be dismissed as a mere problem of permeability.

The two published reports concerning AFB₁ to AFG₁ conversions utilized [¹⁴C]AFB₁. Incorporation of label into aflatoxin was measured after preparative TLC. Maggon & Venkatasubramanian (1973) used cell-free homogenates and reported incorporation of label from [¹⁴C]AFB₁ into AFB₂ (0.8%), AFG₁ (2.9%) and AFG₂ (0.8%). Heathcote *et al.* (1976) used whole cell biotransformations with wild-type *A. parasiticus*. Five separate trials were reported: label from [¹⁴C]AFB₁ was recovered in AFB₂ (0.5–3.5%), AFG₁ (0.3–3.7%) and AFG₂ (0.6–1.8%). In our studies, two wild-type strains were selected for biotransformations. Controls consisted of exogenous [¹⁴C]AFB₁ incubated and extracted from LSRM in the absence of mycelium. In our controls, recovery of radioactivity from preparative TLC zones corresponding with AFB₂ was 0.7%; with AFG₁, 2.6%; and with AFG₂, 0.7%. These control values were within the range of recovery of label for our incubations with mycelia and with the values given by Maggon & Venkatasubramanian (1973). At one end of the range, the incorporation values reported by Heathcote *et al.* (1976) are higher. In our experiments autoradiography revealed smearing of radioactivity on the preparative TLC plates. We conclude that the low levels of

radioactivity found in aflatoxins other than AFB₁ after preparative TLC can represent artifacts of the method rather than metabolic interconversion.

In summary, ST serves as a precursor of both B and G aflatoxins. No AFB₁ to AFG₁ conversion could be demonstrated either in feeding studies with a blocked mutant or in radiotracer studies with wild-type strains. These results suggest independent pathways from ST to the B and G aflatoxins. AFB₁ is not an intermediate, but rather an end-product of the aflatoxin biosynthetic pathway.

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