Effect of various naphthalene compounds on aryl hydrocarbon (benzo[a]pyrene) hydroxylase in vitro (Units/mg protein $\pm 10^{-2}$)

Additions	Untreated		Induced	
	10^{-5} M	10^{-4} M	10^{-5} M	10^{-4} M
B(a)P[a]		42 ± 7[b]		423 ± 12
B(a)P + Naphthalene	43 ± 2(+ 2)	42 ± 4(± 0)	423 ± 10(± 0)	423 ± 13(± 0)
B(a)P + Naphthol —(1)	42 ± 1(± 0)	43 ± 3(+ 2)	419 ± 14(— 1)	402 ± 11(— 5)
B(a)P + Naphthol —(2)	42 ± 6(± 0)	41 ± 4(— 2)	415 ± 9(— 2)	398 ± 15(— 6)
B(a)P + Naphthonitril —(1)	40 ± 8(— 5)	39 ± 5(— 7)	424 ± 15(± 0)	419 ± 10(— 1)
B(a)P + Naphthonitril —(2)	38 ± 3(—10)	36 ± 6(—14)	415 ± 13(— 2)	402 ± 15(— 5)
B(a)P + Naphthylphosphordicloridat —(1)	36 ± 2(—14)	36 ± 4(—14)[c]	338 ± 12(—20)	330 ± 9(—22)[c]
B(a)P + Naphthylphosphordicloridat —(2)	35 ± 2(—17)	33 ± 6(—21)[c]	343 ± 16(—19)	330 ± 16(—22)[c]
B(a)P + 2-Methyl-β-Naphthothiazol	34 ± 6(—20)	19 ± 2(—45)[c]	381 ± 12(—10)	190 ± 8(—55)[c]

Microsomal preparations from 3-Methylcholanthrene pretreated rats. [a] BP = benzo[a]pyrene. [b] Mean + S.D. of duplicate or triplicate determination from two separate experiments. [c] Significantly different from control ($P < 0.001$) values in parenthesis give the percentage of inhibition (—) or stimulation (+)

aryl hydrocarbon (benzo[a]pyrene) hydroxylase by 2 isomers of naphthyl-phosphordicloridate in the control and methylcholanthrene-induced microsomes can probably be explained by the relative instability of the 2 molecules which, in the water phase, decompose easily liberating the halogen radicals which probably act directly on the enzyme. The inhibition of 2-methyl-β-naphthothiazol can probably be explained by its occupation of the benzo[a]pyrene hydroxylation enzyme site.

The naphthalene compounds studied in this work are not known for carcinogenic activity. Comparison of these results and those obtained for certain carcinogens and noncarcinogens[9] confirm the hypothesis that there is no correlation between the absence or presence of carcinogenic activity and the ability to alter benzo[a]pyrene hydroxylation. The noncarcinogenic 2-methyl-β-naphthothiazol markedly inhibits the enzyme as do the known carcinogens 3-methylcholanthrene (51%), dimethylbenz[a]anthracene (32%), or the noncarcinogenic dibenz (a, c) anthracene (40%)[9], whereas the other naphthalene substances shown in the Table have a negligible effect. Moreover, these substances do not show a marked selective effect between the controlormethylcholanthrene-induced microsomes as has been described for some inhibitors[10]. However, the microsomal enzyme system from control and methylcholanthrene-treated rats can be affected differently by a variety of other hydrocarbons[8], which suggests that there are at least 2 forms of this enzyme complex[7,9].

Résumé. L'effet du naphthalène et certains de ses dérivés a été étudié in vitro sur l'action de l'aryl hydrocarbon (benzo[a]pyrene) hydroxylase. Les isomères naphthylphosphordicloridat-(1), naphthylphosphordicloridat-(2) et 2-methyl-β-naphtothioazol inhibent l'enzyme dans les microsomes des rats controles et des rats traités avec le méthylcholanthrène, sans un effet différentiel. Cette inhibition suggère l'occupation du site commun sur l'enzyme. Cependant le naphtalène, le naphthol-(1), le naphthol-(2) le naphthonitrile-(1), le naphthonitrile-(2) ont un effet negligeable sur l'activité enzymatique.

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Metabolism of Aflatoxins B_1 and G_1 by *Aspergillus parasiticus*

Four principal pathways have been clearly recognized for the metabolism of aflatoxins by various animal species[1]. These involve hydroxylation at 2 or 4 position, reduction of the cyclopentenone carboxyl to the hydroxyl group or demethylation of aflatoxin B_1. Many strains of *Aspergillus flavus* and *A. parasiticus* are known to form small amounts of aflatoxins B_2, G_2, B_{2a}, G_{2a}, M_1, M_2, GM_1 and parasiticol. As no strain forming aflatoxin G alone has been reported, it is presumed that all the aflatoxins are derived from aflatoxin B_1. This possibility was tested by using various (^{14}C) labelled aflatoxins as substrates for in vitro incubation with a homogenate prepared from *A. parasiticus*.

The homogenate was prepared from 7-day-old *A. parasiticus* (ATCC 15517) mycelium grown in yeast extract sucrose medium at 27 °C as stationary cultures, by the method of RAJ et al.[2]. Labelled aflatoxins were obtained by growing the fungus, in 100 ml of yeast extract sucrose medium containing 10 μCi of sodium (1–^{14}C) acetate under the same conditions. Radioactive aflatoxins were extensively purified by column chromatography[3] and repeated preparative thin layer chromatography[4]. Labelled aflatoxins, after evaporation of the solvent, were incubated at 30 °C with 1 ml of homogenate in 2 ml of 0.05 *M* phosphate buffer (pH 6.5) and cofactor (2 mg NAD^+) for 2 h on a reciprocating shaker. Aflatoxin

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B_1 had 88500 cpm and G_1 gave 9580 cpm, when used for incubation. At the end of the incubation period, 5 ml chloroform was added and aflatoxins extracted and separated by repeated preparative TLC[4]. The radiopurity of aflatoxins before and after in vitro incubation was checked as recommended by AYRES et al.[5]. The radioactivities of the various aflatoxins were determined in Beckman LS 233 liquid scintillation spectrometer having 100% efficiency for C^{14} counting using 10 ml of the scintillating fluid. The scintillation cocktail was prepared by dissolving 4 g of diphenyloxazole (PPO) and 200 mg of 1–4-*bis*-(2-4-methyl-5-phenyl-oxazolyl) benzene (dimethyl POPOP) in 1 l of toluene. Suitable controls were provided to check any chemical transformation.

In the second set of experiments, 233 µg of aflatoxin B_1 was incubated with 1 ml of homogenate plus 2 ml of phosphate buffer and various cofactors (NAD^+, NADH, $NADP^+$) added individually under similar conditions. The results of incubation of radioactive aflatoxins B_1 and G_1 with *Aspergillus parasiticus* homogenate and their conversion into other aflatoxins are presented in Tables I and II. The values presented are the average of 4 separate experiments.

Table I. Metabolism of aflatoxins B_1 and G_1 by *Aspergillus parasiticus*[a]

Substrate used	Product formed		
aflatoxin	Aflatoxin	cpm	Incorporation (%)
B_1 (cpm 88500)	B_2	710	0.800
	G_1	1850	2.900
	G_2	685	0.780
	M_1	50	0.057
	B_{2a}	60	0.068
G_1 (cpm 9560)	Parasiticol[b]	86	0.90

[a] Radioactivity was not detected in aflatoxins B_1 and G_1 when ^{14}C labelled B_{2a}, G_{2a} and M_1 were used as substrates.
[b] Identified by TLC and UV-absorption spectra.

Table II. Conversion of aflatoxin B_1 to G by *Aspergillus parasiticus*

Substrate	Aflatoxins in the homogenate (µg)			
	Before incubation		After incubation	
	B	G	B	G
Aflatoxin B_1 + NAD (2 mg)	0.71	0.88	154.0	2.99
Aflatoxin B_1 + NADH (1 mg)	0.71	0.88	133.6	2.46
Aflatoxin B_1 + NADP (1 mg)	4.00	3.33	147.4	6.66

It is clear from these results that aflatoxin B_1 is the biogenetic precursor of all the aflatoxins including aflatoxin G_1. These conversions are enzymatic in nature since the boiled homogenate failed to bring about any such conversion. Furthermore, since the aflatoxin content of the homogenate did not change appreciably with time, the possibility of some precursors of aflatoxin G being present in the homogenate and thereby giving rise to aflatoxin G, can be neglected during the incubation period.

The conversion of labelled aflatoxins G_1 and B_2 to G_2 could not be demonstrated due to the low radioactivity of aflatoxin G_2 formed. Similarly the conversion of aflatoxins B_2 and G_2 to M_1, M_2, B_{2a} and G_{2a} and that of M_1 to M_2 could not be proved conclusively.

The conversion of aflatoxins B_1 to G_1 may probably involve a keto-lactonase system similar to the one observed by HEDEGAARD and GUNSALUS[6] in *Pseudomonas putida*, affecting the conversion of camphor to 1–2-campholide. According to DETROY et al.[7] the proportions of secondary fungal metabolites like aflatoxins can be altered in favour of any one of the metabolite by appropriate precursor pressure. Thus it is possible to explain the high amounts of either aflatoxins B_2 and G_1, observed under some particular conditions[8–10], as due to the cumulative effects of various factors, favouring the formation of these metabolites. The nature of such factors is not clearly understood at present but temperature, shaking and aeration play some role in these conversions[7].

ELSEWORTHY et al.[11] reported that aflatoxin B_2 and G_2 are biogenetic precursors of aflatoxins B_1 and G_1. Their conclusion was based on the higher radioactive incorporation of methoxyl side chain labelled 5-hydroxydihydrosterigmatocystin, into aflatoxins B_2 and G_2 compared to aflatoxins B_1 and G_1. Since the demethylation processes were not studied and only the methoxy side chain was labelled, the higher incorporation of aflatoxins B_2 and G_2 could only represent the methylating efficiencies of thes various aflatoxins. Our findings obtained with afla toxin labelled in the ring fail to corroborate the suggestion of ELSEWORTHY et al.[11]. Since no radioactivity was observed in aflatoxins B_1 or G_1 when labelled aflatoxins M_1, B_{2a} and G_{2a} were used as substrates, the possibility of these minor aflatoxins being the precursors of B_1 and G_1 can be ruled out.

Résumé. Un examen préliminaire fait avec une préparation d'*Aspergillus parasiticus* est rendu évidente la conversion de l'aflatoxine B en G. Il est probable que l'aflatoxine B_1 est le précurseur biogénétique d'autres aflatoxines produite par le champignon.

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