Although the total amount of label in 2 samples may not be very different, the qualitative distribution of the label may be such as to permit identification of specific patterns[1]. In the double-labelling experiments, induced and uninduced cells were reacted with $^{3}H$ NEM and $^{14}C$ NEM respectively; both isotopes were distributed uniformly throughout the gel. A uniform distribution was obtained as well when the cells were reacted with unlabelled NEM in the presence of substrate, followed by $^{3}H$ NEM after the substrate was removed by washing. Thus, in spite of satisfactory functional protection, no evidence for specific labelling of a component of the $C_4$-transport system was found.

Fox and KENNEDY[2] have estimated that although there is a significant difference in the amount of NEM incorporated between protected and unprotected fractions, 0.15 nmoles $mg^{-1}$ respectively, only about 20% of this difference can be ascribed to labelling on the M protein. KARLIN et al.[9] have calculated that only 10–20% of the labelling of Electroplax electric organ by an alkylating substrate analogue was on the acetylcholine receptor, and that the amount of bound label was not significantly decreased by prior treatment with unlabelled alkylating reagent.

In spite of these difficulties, 3 specific membrane proteins have now been labelled and apparently located electrophoretically[4,5,10]. The reason for the present negative are not known[11].

*Résumé.* Le transporteur des C-4 acides est inhibé par le N-éthylmaléimide. L'inhibition est partiellement bloquée par le substrat, mais ce n'est pas évident en regardant la définition spécifique du transporteur.

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## Effect of some Derivatives of Naphthalene on Aryl Hydrocarbon (Benzo[a]pyrene) Hydroxylase in vitro

The inducible enzyme system, aryl hydrocarbon (benzo[a]pyrene) hydroxylase, is found in many tissues of numerous mammalian species. It was first described in rat liver by CONNEY and MILLER[1] and called benzo[a] pyrene hydroxylase. The enzyme converts polycyclic hydrocarbons to phenols, dihydrodiols, quinones and epoxides[2,3]. In addition to its function as a detoxification agent, this enzyme complex has been found to activate polycyclic hydrocarbons to toxic and carcinogenic metabolites[4]. The enzyme also catalyzes the formation of covalently bound complexes of hydrocarbon with DNA[5,6], RNA[5] and protein[6]. In this work, we examined the affinity of certain isomeric derivatives of naphthalene for this enzyme and studied their effect on benzo[a]pyrene hydroxylation. The inhibition shown by some compounds suggests competition for the benzo[a]pyrene hydroxylation enzyme site[7].

All chemicals were obtained from Calbiochem or Sigma. The naphthalene compounds were purified by recrystallization. Male Wistar rats (Commentry strain) weighing from 100 to 120 g were injected i.p. with methylcholanthrene (20 mg/kg) in 0.5 ml corn oil. Control animals received corn oil only. Animals were sacrificed by decapitation and the livers were quickly removed, chilled at 0 °C and homogenized with 0.25 *M* sucrose in 0.05 *M Tris*-HCl, pH 7.5 in a Perspex homogenizer. Microsomes were obtained from the 10% homogenate according to SCHNEIDER[8]. Aryl hydrocarbon (benzo[a]pyrene) hydroxylase was assayed as described by GELBOIN[7]. Alkali-extractable metabolites of benzo[a]pyrene were measured with a Jobin-Yvon Model spectrophotofluorometer to determine enzyme activity. Enzyme activities were determined in duplicate or triplicate. The activity was compared to a blank to which acetone had been added prior to incubation. The various naphthalene hydrocarbons were dissolved in ethanol and added in 0.010 ml amounts to yield a final concentration equimolar or 1/10 equimolar to B(a)P. This blank gave no fluorescent readings. The addition of ethanol containing the various polycyclic hydrocarbons (0.010 ml) to the reaction mixture after the incubation period did not affect the subsequent fluorometric measurements.

As shown in the Table, the addition of naphthalene and its 4 derivatives (naphthol-(1), naphthol-(2), naphthonitril-(1), naphthonitril-(2)), at equimolar or 1/10 equimolar concentrations to the benzo[a]pyrene substrate, had no significant effect on benzo[a]pyrene hydroxylation when the medium contained either control or induced microsomes.

The 2 isomers, naphthyl-phosphordicloridat- (1) and naphthyl-phosphordicloridate-(2), inhibit hydroxylation of benzo[a]pyrene incubated with control microsomes by 14% and 21% respectively, and 2-methyl-$\beta$-naphthothiazol by 45%. These 3 compounds also inhibit aryl hydrocarbon (benzo[a]pyrene) hydroxylase in methylcholanthrene-induced microsomes (22%, 22%, and 55% respectively). The addition of other compounds to control or methylcholanthrene-induced microsomes resulted in either negligible or no inhibition of benzo[a]pyrene hydroxylation. With the exception of 2-methyl-$\beta$-naphthothiazol, and the 2 isomers of naphthyl-phosphordicloridate, the results suggest that none of the other compounds studied has an affinity for the benzo[a]pyrene hydroxylation site equal to benzo[a]pyrene. The inhibition of

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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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