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EFFECTS OF INDOLE AND TRYPTOPHAN ON CYTOCHROME P-450, DIMETHYLNITROSAMINE DEMETHYLASE, AND ARYLHYDROCARBON HYDROXYLASE ACTIVITIES

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Abstract—The effects of indole and L-tryptophan feeding on the activities of two liver microsomal enzymes, dimethylnitrosamine demethylase (DMN-d) and arylhydrocarbon hydroxylase (AHH), were studied in rats and hamsters. Both indole and tryptophan increased cytochrome P-450 concentration and DMN-d activity in rats, but only indole was effective in hamsters. AHH activity in both species was induced by indole. Both indole and tryptophan increased the ratio $A_{392-500\,nm}/A_{410-500\,nm}$ obtained by *n*-octylamine difference spectroscopy of rat liver microsomes but not of hamster liver microsomes. Indole increased the absorbance difference ratio $A_{455-500\,nm}/A_{430-500\,nm}$ of rat liver cytochrome P-450 obtained by ethyl isocyanide. The opposite was true for hamsters, which showed a low band at 455 nm. Indole gave a type II binding spectrum with rat liver microsomes, but the values for absorption minimum and maximum for tryptophan were somewhat outside the reported values for type II binders. Indole, which induced liver AHH activity, protected against 7,12-dimethylbenz[a]anthracene (DMBA)-produced mammary gland carcinogenicity, whereas tryptophan was without effect.

A number of cruciferous plants induce enzymes involved in drug metabolism [1–4], due to the indole derivatives which these plants contain. In addition, several indoles have been shown to induce the activity of arylhydrocarbon hydroxylase (AHH) [1, 2], while we have already reported that the amino acid tryptophan, also an indole, induced liver dimethylnitrosamine demethylase (DMN-d) activity [5]. Microsomal activation of most chemical carcinogens is a necessary initial step in the formation of the ultimate carcinogenic species. Dimethylnitrosamine (DMN) is activated by liver microsomes to produce an active alkylating agent, a carbonium ion which is thought to be responsible for the toxic and carcinogenic effects of DMN [6]. More is known about the involvement of various cytochrome P-450 species in the activation of arylhydrocarbons than in the activation of DMN. For the activation of aromatic hydrocarbons to carcinogenic species, the first step is the formation of arene oxides by an NADPH-dependent arylhydrocarbon monooxygenase system [7].

The purpose of the present experiment was to compare the effects of indole and tryptophan on microsomal enzyme systems. Their effects on DMN-d and AHH were studied simultaneously with their effects on the concentration of cytochrome P-450 and on the chemical nature of this cytochrome, as depicted by *n*-octylamine and ethyl isocyanide difference spectroscopy. Furthermore, the effects of indole and tryptophan on mammary tumor production by 7,12-dimethylbenz[a]anthracene (DMBA) were investigated.

MATERIALS AND METHODS

Chemicals. Indole was purchased from the CalBiochem-Behring Co. (La Jolla, CA), L-tryptophan from ICN Pharmaceuticals (Cleveland, OH), dimethylnitrosamine and *n*-octylamine from the Aldrich Chemical Co. (Milwaukee, WI), ethyl isocyanide, benzo[*a*]pyrene, NADP, and NADPH from the Sigma Chemical Co. (St. Louis, MO), 3-OH-benzo[*a*]pyrene from the NCI Chemical Repository, IIT Research Institute (Chicago, IL), sodium dithionite from the Fisher Scientific Co. (Fairlawn, NJ), carbon monoxide gas from the Matheson Co., Inc. (East Rutherford, NJ), and 7,12-dimethylbenz[*a*]anthracene from the Eastman Kodak Co. (Rochester, NY).

Wistar male weanling rats and Syrian golden hamsters were obtained from Charles River Breeding Laboratories. They were kept in plastic filter-top cages, five to a cage, in an animal room on a 12-hr light–dark cycle. Indole and tryptophan were mixed with the semipurified diet, which contained 18% casein as a protein source. Such diets were fed the animals from receipt until they were killed. At the age of 30 days animals were decapitated. Livers were homogenized in 4 vol. (w/v) 0.1 M potassium phosphate buffer, pH 7.4, and centrifuged for 20 min at

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Table 1. Protein concentration of liver microsomes and liver weight expressed as percentage of body weight*

	Rat			Hamster		
	Number of animals	Microsomal protein [mg·(g liver)$^{-1}$]	Liver weight (% of body weight)	Number of animals	Microsomal protein [mg·(g liver)$^{-1}$]	Liver weight (% of body weight)
Control	33	22 ± 0.6	4.5 ± 0.09	26	20 ± 1.0	6.2 ± 0.20
Indole (1%)	15	25 ± 2.4	4.9 ± 0.17†	13	27 ± 1.1†	6.8 ± 0.25
Indole (0.5%)	25	24 ± 1.3	4.8 ± 0.12†	17	23 ± 1.3	6.8 ± 0.27
Tryptophan (1%)	19	26 ± 1.4†	4.9 ± 0.12†	14	23 ± 1.0	5.6 ± 0.30

* Each value is the mean ± S.E.
† Denotes statistically significant difference ($P < 0.05$) from control.

10,000 *g*. The supernatant fraction was centrifuged at 105,000 *g* for 1 hr. The microsomal pellet was resuspended in 0.1 M phosphate buffer and the centrifugation at 105,000 *g* was repeated. DMN-d activity was determined as described earlier [5] using the method of Venkatesan *et al.* [8]. Substrate concentration was 4 mM. AHH activity was determined according to Wiebel and Gelboin [9]. Cytochrome P-450 was determined by the method of Omura and Sato [10], using 91 $mM^{-1} \cdot cm^{-1}$ as the molar extinction coefficient for the reduced cytochrome P-450–CO complex. *N*-Octylamine difference spectroscopy was performed as described by Jefcoate *et al.* [11] and Nebert and Gielen [12]. Ethyl isocyanide difference spectra were measured in microsomes reduced with sodium dithionite at pH 7.4. Ethyl isocyanide was added to the sample cuvette to a final concentration of 2.8 mM. For spectral measurements, the Aminco DW-2 spectrophotometer was used. Protein was determined by the method of Lowry *et al.* [13].

For the study of the influence of indole and tryptophan on mammary tumor production by DMBA, ninety female Sprague–Dawley rats from the National Institutes of Health animal production facilities were used. The animals were fed with 0.5% indole or 1% tryptophan in Wayne meal for 1 week before, and 2 weeks after, the administration of DMBA. At the age of 30 days, 8 mg DMBA in 1 ml corn oil was administered to the animals by stomach tube; control animals received corn oil only. Animals were checked once a week for occurrence of mammary tumors and were killed at the age of 33 weeks.

RESULTS

Feeding indole at 1% in the diet increased the microsomal protein in hamster liver, while tryptophan had the same effect in rat microsomes (Table 1). Both indole and tryptophan increased the liver

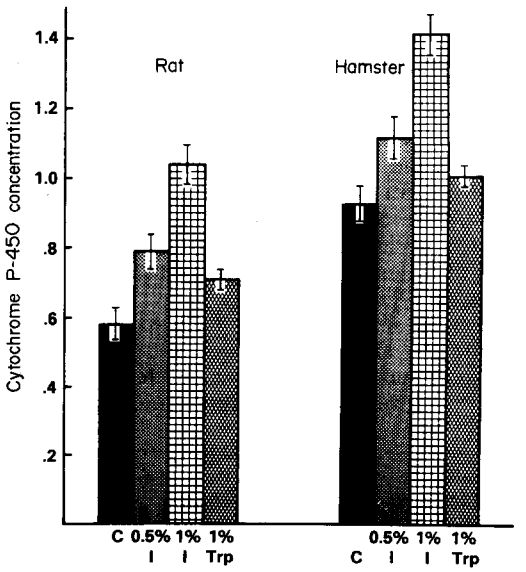


Fig. 1. Cytochrome P-450 concentration (nmoles/mg microsomal protein) in rat and hamster liver microsomes. Key: I = indole-fed animals; Trp = tryptophan-fed animals.

Table 2. Effects of indole and L-tryptophan on rat and hamster liver dimethylnitrosamine demethylase activity

	Rat		Hamster	
	Number of animals	DMN-d [nmoles HCOH ·(mg microsomal protein)$^{-1}$·hr^{-1} mean ± S.E.]	Number of animals	DMN-d [nmoles HCOH ·(mg microsomal protein)$^{-1}$·hr^{-1} mean ± S.E.]
Control	25	68 ± 2.6	26	249 ± 9.3
Indole (1%)	4	148 ± 12.9* (+118)†	13	365 ± 27.7* (+47)
Indole (0.5%)	25	110 ± 5.4* (+62)	14	319 ± 14.9* (+28)
Tryptophan (1%)	15	86 ± 4.1* (+26)	12	225 ± 10.4

* Denotes statistically significant difference ($P < 0.05$) from control.
† Numbers in parentheses are the per cent enzyme induction by indole or by tryptophan.

weights in rats, but in hamsters the differences were not significant. Both indole and tryptophan feeding increased DMN-d activity in rats, but in hamsters only indole was effective (Table 2). The relative increase in the enzyme activity induced by indole was always higher in the rat liver than in the hamster liver. Tryptophan did not have any effect on AHH activity, whereas indole increased the activity in both species (Table 3).

Figure 1 shows the cytochrome P-450 concentration of microsomes expressed in nanomoles per milligram of microsomal protein. Indole increased cytochrome P-450 concentration in both rat and hamster, but tryptophan was effective only in rats. The slight increase in hamster liver by tryptophan was not statistically significant. No shift from the absorption maximum at 450 nm could be demonstrated.

Figure 2 (panels a and b) shows the characteristic *n*-octylamine difference spectra for rat and hamster. The shape of the spectra obtained by *n*-octylamine when the microsomal preparation from indole-induced rat liver was used was different from that of control rats (Fig. 2a). Both indole and tryptophan increased the absorption difference between 392 and 500 nm more than between 410 and 500 nm. Table 4 gives the average ratios when absorption differences between 392 and 500 were divided by the absorption differences between 410 and 500 nm. In rats, indole feeding produced a 55 per cent increase, and tryptophan feeding a 27 per cent increase, in this ratio when compared to the control animals. In hamster liver, the increase in this ratio was not statistically significant.

When ethyl isocyanide was added to the sodium dithionite reduced microsomes, only indole significantly increased the height of the 455 peak in rats when compared to the control animals, whereas in hamsters the effect was the opposite (Table 5). The difference ratio $A_{455-500\,nm}/A_{430-500\,nm}$ decreased when 1% indole was fed.

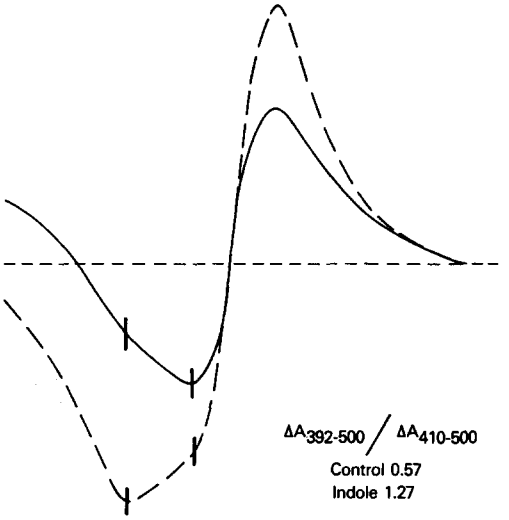
A typical type II spectrum was obtained when indole was added to the control rat microsomes with absorption minimum at 390 and absorption maximum 423 (Fig. 3). The corresponding values for tryptophan were 413 and 434. The values for tryptophan are outside the values given for type II binder; the range for absorption minimum was 390–410 nm and for absorption maximum it was 425–430 nm.

Figures 4 and 5 show the results for two separate experiments where the effects of tryptophan and indole on mammary tumor production by DMBA were studied. Tryptophan first increased tumor production by DMBA, but by 23 weeks there was no difference in tumor incidence among control and tryptophan-fed animals. On the contrary, indole feeding reduced the tumor incidence. The number

Table 3. Effects of indole and L-tryptophan on rat and hamster liver arylhydrocarbon hydroxylase activity

	Rat		Hamster	
	Number of animals	AHH [pmoles 3-OH-BP ·(mg microsomal protein)$^{-1}$·min^{-1} mean ± S.E.]	Number of animals	AHH [pmoles 3-OH-BP ·(mg microsomal protein)$^{-1}$·min^{-1} mean ± S.E.]
Control	16	76 ± 4.3	10	114 ± 10.2
Indole (1%)	8	197 ± 11.8* (+159)†	6	343 ± 26.3* (+200)
Indole (0.5%)	9	165 ± 9.7* (+117)	9	175 ± 17.8* (+54)
Tryptophan (1%)	4	59 ± 10.1	8	104 ± 7.5

* Denotes statistically significant difference ($P < 0.05$) from control.
† Numbers in parentheses are the percent enzyme induction by indole.



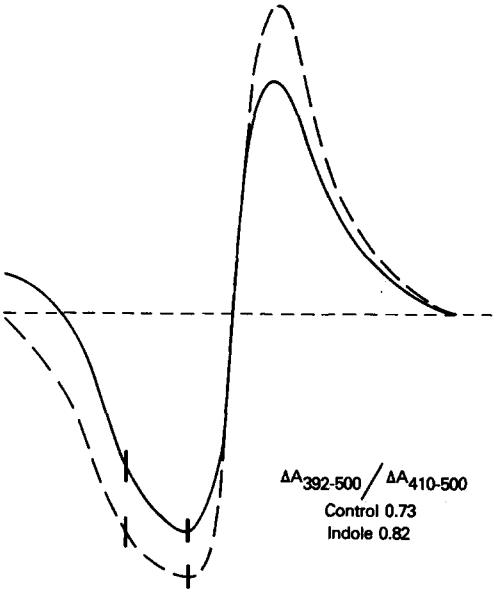


Fig. 2. *n*-Octylamine difference spectra of cytochrome P-450 in indole-treated (- - - -) and control (———) rats (A) and hamsters (B). Spectra were taken in 0.1 M potassium phosphate buffer (pH 7.4) containing 50% glycerol. Ten microliters of *n*-octylamine in methanol was added to the sample cuvette to make the final concentration 2 mM, and 10 microliters of methanol alone was added to the reference cuvette. The vertical lines on the left mark wavelength 392 and the ones on the right 410 nm.

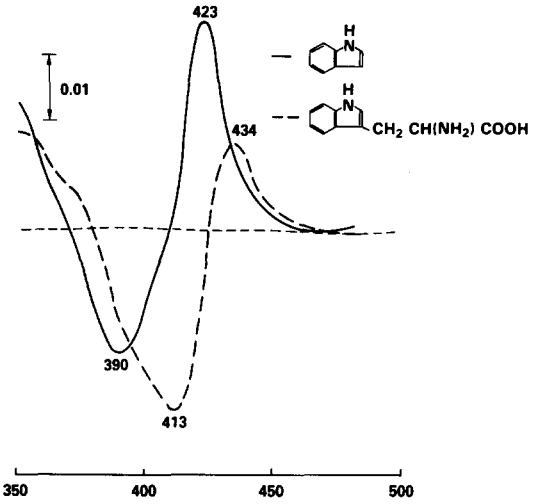


Fig. 3. Binding spectra of indole and tryptophan to rat liver microsomes.

of tumors per tumor bearing animal among the controls was 3.32 and among the indole-fed animals only 1.75.

DISCUSSION

It is well documented that AHH activity is cytochrome P-450 dependent and that at least two different classes of cytochrome P-450 are involved. One P-450 form is induced by phenobarbital, while the other form, induced by methylcholanthrene, shows an absorption peak at 448 instead of 450 nm [14–16]. The cytochrome P-450 dependency has been questioned recently for DMN-d [17–19]. The dependency of DMN-d metabolism on cytochrome P-450 is supported by its sensitivity to CO [20], lack of bacterial mutagenicity when a CO-treated microsomal preparation was used [21], lack of incorporation of labeled methyl groups from DMN into protein in the absence of microsomes [22], and as shown recently, nearly quantitative production of molecular nitrogen from metabolism of DMN [23].

There is a definite difference between the activation mechanisms for AHH and DMN-d. Both methylcholanthrene and phenobarbital are potent inducers of AHH, whereas the activity of the low

Table 4. Average ratios of $A_{392-500\,nm}/A_{410-500\,nm}$ obtained by *n*-octylamine difference spectroscopy*

	Rat		Hamster	
	Number of animals	$A_{392-500\,nm}/A_{410-500\,nm}$	Number of animals	$A_{392-500\,nm}/A_{410-500\,nm}$
Control	12	0.62 ± 0.21	4	0.68 ± 0.049
Indole (0.5%)	8	0.96 ± 0.044†	6	0.83 ± 0.051
Tryptophan (1%)	8	0.79 ± 0.058†		

* Microsomes equivalent to 3 mg protein/ml 0.1 M potassium phosphate buffer containing 50% glycerol (pH 7.4) were used. Ten microliters of *n*-octyl amine in methanol was added to the sample cuvette (final concentration, 2 mM), and 10 μl methanol only was added to the reference cuvette. Values are means ± S.E.

† Denotes statistically significant difference ($P < 0.05$) from control.

Table 5. Ethyl isocyanide difference ratios expressed as $A_{455-500\,nm}/A_{430-500\,nm}$*

	Rat		Hamster	
	Number of animals	Ratio	Number of animals	Ratio
Control	14	0.600 ± 0.0229	5	0.361 ± 0.0087
Indole (1%)	15	0.718 ± 0.0137†	8	0.336 ± 0.0054†
Tryptophan (1%)	7	0.620 ± 0.0273	3	0.374 ± 0.0127

* Baseline was obtained after addition of sodium dithionite to both cuvettes. Ethyl isocyanide at a final concentration of 2.8 mM was added to the sample cuvette. Microsomal preparation equivalent to 2 mg protein/ml 0.1 M potassium phosphate buffer (pH 7.4) was used. Values are means ± S.E.

† Denotes statistically significant difference ($P < 0.05$) from control.

substrate-level DMN-d is inhibited [24]. In the present experiment, we showed that indole induces both AHH and DMN-d activities in rats and hamsters. Arcos *et al.* [25] did not find any induction of DMN-demethylase I by indole, whereas tryptophan feeding induced AHH. In their experiment, they injected indole in trioctanoin at 117 mg/kg body weight level 24 hr before killing the animals. We also found that indole injected once at 100 mg/kg body weight did not induce DMN-d. It seems that a longer period of time is needed for induction of DMN-d by indole. The absence of induction of AHH by tryptophan in our experiments may be due to our use of a different strain of rats, or to different feeding times. Indole seems to belong to a category of enzyme inducers different than that of either methylcholanthrene or phenobarbital. Therefore, we extended our study to the effect of indole on cytochrome P-450, which is one component of the mixed-function oxidase system. In rats, both indole and tryptophan increased microsomal cytochrome P-450 and the difference in absorption between 392 nm and 500 nm when *n*-octylamine was used as a ligand for the oxidized form of cytochrome P-450. This difference may reflect the high spin form of cytochrome P-450 [12, 26]. In rat, indole also increased the absorbance difference at 550 nm when ethyl isocyanide was used as ligand for reduced cytochrome P-450 (Table 5). When hamster liver microsomes were used, however, the increase in the absorbance difference ratio by the *n*-octylamine method was not significant and by the ethyl isocyanide method the ratio 550/530 decreased, rather than increased. The exact chemical nature of these two ethyl isocyanide–cytochrome P-450 complexes is not well understood. It is known that the ratio of intensity at 455 nm to that at 430 nm is increased by prior treatment of rats with methylcholanthrene, but it remains the same after treatment with phenobarbital [14].

The amount of high spin iron present in cytochrome P-450 is less in rat microsomes than in hamster microsomes [27]. Cytochrome P-450 containing high spin iron might be involved in the metabolism of DMN, for DMN-d activity is always higher in hamster liver than in rat liver microsomes. According to model studies the high spin state of cytochrome P-450 is five-coordinated [28]. DMN has been shown to produce a ligand binding spectra with oxidized cytochrome P-450 [29]. The consequence of this binding for the metabolism of DMN, however, is still unknown. It is possible that the high spin state hemoprotein induced by methylcholanthrene is different from that induced by indole and that only the latter species is active in the monooxygenase system which is involved in the metabolism of DMN.

The first step in the metabolism of polycyclic

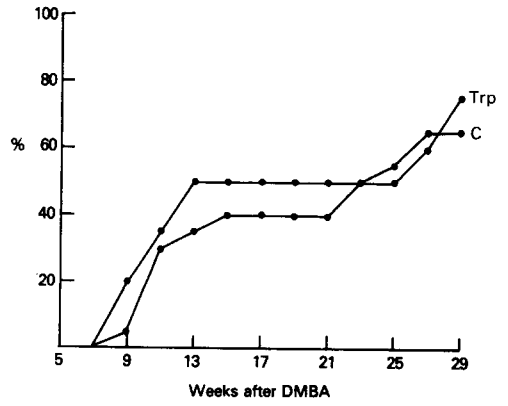


Fig. 4. Effect of tryptophan feeding on mammary tumor production by DMBA. Twenty animals (100%) were included in each group.

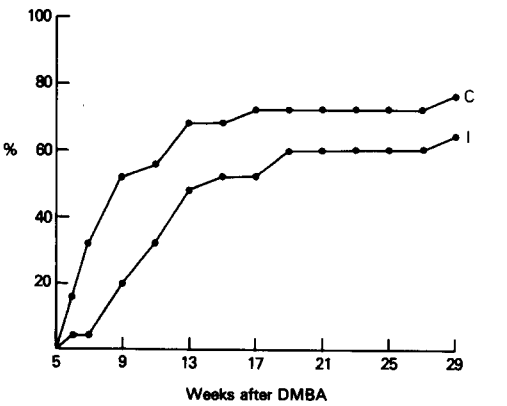


Fig. 5. Effect of indole feeding on mammary tumor production by DMBA. Twenty-five animals (100%) were included in each group.

hydrocarbons is the formation of arene oxides by an NADPH-dependent aryl hydrocarbon monooxygenase system [7, 30]. There appears to be a positive correlation between arylhydrocarbon hydroxylase activity and total hydrocarbon metabolism [31, 32]. Thus, indole which increased enzyme activity decreased mammary tumor production from DMBA. It is possible that a smaller dose of DMBA reaches the mammary gland when the liver has an active microsomal enzyme system, because more is metabolized to water-soluble products and excreted. Active liver enzyme system seems to protect the peripheral tissue against tumor production by carcinogens [33]. In accord, it was shown earlier that indole derivatives protect the animals against mammary tumor production by DMBA [34]. On the other hand, tryptophan did not induce AHH activity and increased rather than decreased mammary turmor formation.

These data emphasize the importance of investigating the joint effects of carcinogens and other environmental influences, a more realistic situation than studying the action of potent carcinogens alone. Practically, they also point toward the benefits to be gained by including certain vegetables in the diet.

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