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Inhibition of Aflatoxin-Induced Serum α-Fetoprotein in Rats Fed Cauliflower (40576)

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α-Fetoprotein (AFP) is a serum protein elaborated by the fetal liver but only detected in minute amounts in normal adults. Animals and humans with chemically induced liver tumors exhibit elevated serum concentrations of AFP (1–5). Indeed, its early appearance has been shown to be present within weeks after dosing animals with hepatocarcinogens and may be useful in early detection prior to observed hepatoma development (6, 7). Experimentally and clinically aflatoxin B_1 (AB_1) is a potent hepatocarcinogen (8, 9). Although early investigators using low doses of AB_1 did not observe increased levels of serum AFP (7), later evidence indicated some elevation does occur (10). This detection has been made possible by the development of competitive binding radioimmunoassay that can quantitate rat serum levels as low as 1 ng AFP (11, 12).

Feeding cauliflower diets to rats has recently been shown to reduce AB_1-induced hepatocarcinomas and mortality (13). Natural-occurring indoles present in cauliflower, as well as in other common Cruciferae vegetables, has also recently been shown to inhibit chemically induced mammary tumors in rats (14). This preliminary study was undertaken to investigate if a relatively low level, short-term AB_1 rat feeding study would increase AFP in rat serum, and if a cauliflower-containing diet would have any effect on the level of this serum protein.

Methods. Forty weanling, male Fischer 344 Sch_f rats were divided into four equal groups and fed and watered *ad libitum* individually in raised-wire cages. The AIN-76 semipurified diet (15) or a 20% freeze-dried, finely ground cauliflower diet substituted for sucrose, both with or without the addition of 1 ppm AB_1 (Calbiochem), comprised the four dietary groups. Cauliflower was grown at the New York State Agricultural Experiment Station 1 km from the nearest highway, without pesticide sprays. At the end of 5 weeks four representative animals from each group were fasted overnight and killed by cervical dislocation and blood was obtained by cardiac puncture. AFP was measured by competitive binding radioimmunoassay (12). Hepatic aminopyrine *N*-demethylase activity was also measured (16) as previous studies have shown this mixed function oxidase (MFO), as well as others, are elevated in rats fed cauliflower diets (13).

Results. Table I shows the mean weight gain and dietary intake for the 5-week period. As seen in Fig. 1, AFP serum levels were similar in the semipurified and cauliflower-treated rats. A significant AFP increase occurred in the AB_1 treated, semipurified diet fed animals that was significantly reduced by cauliflower consumption ($P \leq 0.05$). Cauliflower + AB_1 treatment produced rats exhibiting AFP levels that were statistically similar ($P \geq 0.05$) to both control groups.

Cauliflower diets enhanced hepatic aminopyrine *N*-demethylase activity that was not changed by the addition of AB_1 treatment (Table II). The addition of AB_1 to the semipurified diet also did not significantly ($P \geq 0.05$) change the level of the hepatic MFO activity.

Discussion. The results reported here show that 1 ppm AB_1 can elevate rat serum AFP levels in 5 weeks using the highly sensitive method of competitive binding radioimmunoassay. Feeding cauliflower reduces this effect, probably via enhanced metabolism of AB_1 due to natural MFO-inducing compounds contained in this vegetable (13, 14). Although it is known that AB_1 requires metabolic activation to elicit its carcinogenic effect through epoxide and/or aflatoxicol formation (17, 18), administration of phenobarbitone, a hepatic MFO inducer, reduced hepatic malignant tumor formation and mortality in rats fed peanut meal contaminated

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with AB_1 (19). No tumors were observed in any of the rats in this study. Approximately 40 weeks are required to see AB_1 induce hepatocellular carcinomas in Fischer rats (20).

AFP is known to appear early in rats administered chemical carcinogens, followed by a marked decrease, then reappearance when hepatomas develop in the animals (6, 7). The actual function of this protein is not completely understood, but its production is closely coupled to cellular division and DNA synthesis in fetal development and partial hepatectomy, yet feeding low levels of chemical carcinogens insufficient to produce any detectable DNA synthesis will produce an increased level of serum AFP (21). It has been suggested that low chemical carcinogen exposure enhances AFP levels by highly selective derepression of protein synthesis that occurs following the formation of a complex between the metabolites of the carcinogen and specific chromatin loci (22).

TABLE I. WEIGHT GAIN AND MEAN DIET INTAKE OF RATS FED 20% CAULIFLOWER DIETS AND AFLATOXIN B_1 FOR 5 WEEKS

Dietary treatment	Weight gain[a] (g)	Diet intake[a] (g)
Semipurified	134 ± 8^{1}	$410 \pm 18^{1,2}$
Cauliflower	$112 \pm 5^{1,2}$	$345 \pm 13^{2,3}$
Semipurified + AB_1[b]	138 ± 4^{1}	437 ± 11^{1}
Cauliflower + AB_1	97 ± 6^{2}	312 ± 10^{3}

[a] Values are means ± SEM. Different superscript numbers indicate significantly different values ($P \leq 0.05$), within each parameter.

[b] 1 ppm aflatoxin B_1 added to each diet.

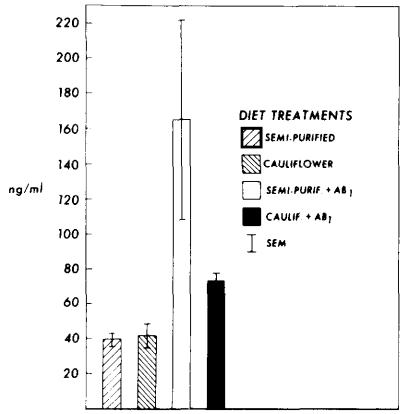

FIG. 1. Rat serum α-fetoprotein after 5-week dietary treatment of 20% cauliflower + aflatoxin B_1 (AB_1).

TABLE II. HEPATIC MICROSOMAL PROTEIN AND AMINOPYRINE *N*-DEMETHYLASE ACTIVITY[a]

Dietary treatment	*N*	Microsomal protein (mg/g liver)	Aminopyrine *N*-demethylase activity (n*M* formaldehyde/mg protein/hr)
Semipurified	4	$16.3 \pm 0.5^{1,2}$	19.5 ± 1.3^{2}
Cauliflower	4	17.6 ± 0.4^{1}	38.4 ± 6.4^{1}
Semipurified + AB_1[b]	4	17.1 ± 1.6^{1}	$25.1 \pm 1.2^{1,2}$
Cauliflower + AB_1	4	14.0 ± 0.7^{2}	39.0 ± 5.9^{1}

[a] Values are means ± SEM. Different superscript numbers indicate significantly different values ($P \leq 0.05$), within each parameter.

[b] 1 ppm aflatoxin B_1 added to each diet.

Although the cauliflower-fed rats, on the average, consumed less AB_1 than the rats fed the semipurified diet (Table I), a dose–effect may be partially responsible for the lowered serum AFP in the cauliflower-fed groups (7, 22). However, previous evidence in our laboratory has shown that dietary cauliflower inhibited AB_1 hepatocarcinoma development with a concomitant rise in hepatic MFO (13). Further evidence has shown that dosing rats with indole, a natural compound known to be present in cauliflower, reduced chemically induced mammary tumor development (14). The data reported here suggests that carcinogenic AB_1 metabolite(s) are probably metabolized more rapidly or not greatly bound to hepatic macromolecules through the action of natural constituents present in the cauliflower diet.

Summary. The highly sensitive competitive binding radioimmunoassay showed an increased level of serum AFP in Fischer male rats fed 1 ppm of AB_1 in a semipurified diet for 5 weeks. A 20% cauliflower diet significantly reduced serum AFP and increased hepatic MFO activity. The results of this study suggests that the lowered serum AFP predicts dietary cauliflower's protective action against AB_1-induced hepatocarcinoma.

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