

Influence of Caffeine and/or Coffee Consumption on the Initiation and Promotion Phases of 7,12-Dimethylbenz(a)anthracene-induced Rat Mammary Gland Tumorigenesis¹

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ABSTRACT

The effect of caffeine and/or coffee consumption (via the drinking water) during the initiation phase and promotion phase of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary gland tumorigenesis in female Sprague-Dawley rats fed a commercial laboratory animal chow was examined. In the initiation studies, DMBA was administered once at 53-55 days of age; caffeine (100-860 mg/liter of drinking water) and/or coffee (moderate or high dose, sole source of drinking water) treatments were for 32 consecutive days, commencing 29 days prior to DMBA treatment and terminating 3 days after DMBA treatment. In the promotion studies, DMBA was administered once at 54-55 days of age; caffeine and/or coffee treatments were daily from 57-58 days of age to termination of experiments (12-21 weeks after carcinogen treatment). In the initiation studies, either moderate (100-400 mg) or high (860 mg) dose levels of caffeine or moderate to high dose levels of caffeinated coffee significantly ($P < 0.05$) reduced mammary carcinoma multiplicity (number of tumors/rat). Consumption of high or moderate dose levels of decaffeinated coffee did not significantly alter mammary carcinoma multiplicity. The addition of caffeine to the moderate dose level of decaffeinated coffee resulted in a significant ($P < 0.05$) reduction in mammary carcinoma multiplicity. In the promotion studies, prolonged consumption of moderate dose levels of caffeine or moderate or high dose levels of caffeinated coffee or decaffeinated coffee did not significantly effect mammary carcinoma multiplicity. In the early stages of promotion, however, a significant ($P < 0.05$) stimulatory effect of caffeine on mammary carcinoma multiplicity was observed; an effect that was temperate and transitory. In both the initiation and promotion studies caffeine and/or coffee consumption did not significantly affect the incidence of mammary carcinomas (percentage of rats bearing mammary carcinomas) or the mean latency period of mammary tumor appearance.

These results provide evidence that caffeine and/or caffeinated coffee consumption can significantly influence mammary carcinoma multiplicity in female rats treated with DMBA, an effect that is dependent upon the dose level, duration, and time-span of caffeine administration.

INTRODUCTION

Caffeine, a trimethylxanthine, occurs naturally in coffee, tea, and cocoa and as an additive in other beverages such as soft drinks. Caffeine is also added to many medications, notably analgesics and diet aids. The pharmacological and potential pathological activities of caffeine have received considerable attention due to its daily ingestion by a large portion of the population; caffeine is the most widely consumed drug in much of the world today.

The impact of caffeine consumption on tumorigenic processes is at present unclear; reports pertaining to the consumption of this drug and tumor development have been inconsistent and inconclusive. In humans, caffeine consumption has been reported to be associated with an increased risk for the development of urinary tract (1), pancreatic (2), ovarian (3), colon

(4), and breast tumors (5, 6). Other reports have failed to demonstrate an association between caffeine ingestion and tumor development in human populations (7-14). In studies utilizing experimental animals, it is most often reported that the chronic consumption of caffeine does not enhance the spontaneous development of tumors (15-19). In contrast, in animals exposed to various carcinogenic agents, *e.g.*, chemical carcinogens, UV light, etc., modulation (enhancement, inhibition) of tumorigenesis is often cited (20-25).

In the studies described in this communication, we report a comprehensive analysis of the influence of caffeine consumption on mammary tumorigenesis in female rats fed a commercial laboratory animal chow and treated with the chemical carcinogen, DMBA.³ More specifically, we examine the effect of caffeine consumption, at varied dose levels, on the two major components of this tumorigenic process, *i.e.*, the initiation and promotion phases of rat mammary gland carcinogenesis.

MATERIALS AND METHODS

One thousand three hundred fifty-three female Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were used in this study. All animals were housed in a temperature-controlled (24°C) and light-controlled (14 h/day) room and were given a standard commercial mouse/rat chow (Teklad; Harlan Sprague-Dawley, Inc., Winfield, IA) *ad libitum* throughout the study.

The experimental designs of these studies are summarized in Table 1. A detailed description of these studies, *e.g.*, number of rats/group, day of DMBA administration, time-span of caffeine and coffee treatments, dose levels, and time of experiment termination are provided in "Results," Tables 2-7.

Coffee/Caffeine Preparation and Administration. Coffee was prepared using a series of West Bend automatic coffee makers (model 13500; West Bend, WI). Full-strength coffee (high dose) was prepared by using 4.25 cups of coffee (Maxwell House, Auto-Drip, caffeinated or decaffeinated coffee; General Foods, Inc., White Plains, NY) and 45 cups of water in a 55-cup coffee maker. Moderate strength coffee (moderate dose) was prepared by using 2.125 cups of coffee and 45 cups of water. Decaffeinated coffee was approximately 97% caffeine free. Determination of caffeine concentration in coffee was by manufacturers specification and by gas-liquid chromatographic analysis. Periodic assessment of caffeine levels by gas-liquid chromatography in the prepared coffee indicated a maximum variation of caffeine levels, from week to week, of 9%. Coffee (caffeinated and decaffeinated, full and moderate strength) was provided as a sole source of drinking fluid. Caffeine (ICN Pharmaceuticals, Inc., Cleveland, OH), readily soluble in water, was added to the drinking water at specified levels. Caffeine and coffee solutions (drinking water) was prepared fresh three times per week (MWF).

Carcinogen Administration and Assessment of Mammary Tumorigenesis. DMBA (Eastman Kodak Co., Rochester, NY) was administered once, either *i.v.* (initiation studies, 2 mg/100 g body weight) or *i.g.* (promotion studies, 5 mg/rat). For *i.v.* administration, DMBA was dissolved in a lipid emulsion prepared by the Upjohn Co., Kalamazoo, MI. For *i.g.* administration, DMBA was dissolved in sesame oil. Begin-

³ The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; *i.g.*, intragastric.

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Table 1 Summary of experimental design of studies 1-6

Study	Objective	Before DMBA	After DMBA
1	Initiation (caffeine, high dose; coffee, full strength)	Commercial lab chow Control Caffeine Coffee Decaf coffee Decaf coffee + caffeine	Commercial lab chow
2	Initiation (coffee, moderate strength)	Commercial lab chow Control Coffee Decaf coffee	Commercial lab chow
3	Initiation (caffeine, varying doses)	Commercial lab chow Control Caffeine (3 dose levels)	Commercial lab chow
4	Promotion (caffeine, varying doses)	Commercial lab chow	Commercial lab chow Control Caffeine (7 dose levels)
5	Promotion (coffee, full strength)	Commercial lab chow	Commercial lab chow Control Coffee Decaf coffee
6	Promotion (caffeine, moderate dose; coffee, moderate strength)	Commercial lab chow	Commercial lab chow Control Caffeine Coffee Decaf coffee

Table 2 Study 1: Effect of caffeine and coffee consumption during initiation on mammary tumor incidence in female rats treated with DMBA. High dose caffeine and full-strength (dose) coffee

DMBA was administered (i.v.) once at 53 days of age. Caffeine (high dose, 860 mg/liter of drinking water) and coffee (full strength) was provided from 24-56 days of age. The experiment was terminated 18 weeks after carcinogen treatment.

Treatment	No. of rats	Percentage of rats with mammary carcinomas	Total number of mammary carcinomas	Number of mammary carcinomas per rat	Mean latency period of mammary tumor appearance (weeks)	Mean body weights (g)	
						Before treatment	After treatment
Control	41	93	266	6.5 (100) ^a	8.8	48.8	172.6
Caffeine (860 mg)	40	90	109	2.7 (42) ^b	8.8	50.4	173.2
Coffee	40	78	99	2.5 (38) ^b	8.8	48.3	171.2
Decaf coffee	41	98	199	4.9 (75)	9.2	49.7	177.7
Decaf coffee plus caffeine (860 mg)	41	90	137	3.3 (51) ^b	8.9	49.2	174.5

^a Numbers in parentheses, percentage of control.
^b vs. control, *P* < 0.05.

Table 3 Study 2: Effect of coffee consumption during initiation on mammary tumor incidence in female rats treated with DMBA. Moderate strength (dose) coffee
DMBA was administered (i.v.) once at 55 days of age. Coffee (moderate strength) was provided from 26 to 58 days of age. The experiment was terminated 12 weeks after carcinogen treatment.

Treatment	No. of rats	Percentage of rats with mammary carcinomas	Total number of mammary carcinomas	Number of mammary carcinomas per rat	Mean latency period of mammary tumor appearance (weeks)	Mean body weights (g)	
						Before treatment	After treatment
Control	40	93	220	5.5 (100) ^a	9.9	43.9	257.3
Coffee	41	93	137	3.3 (60) ^b	10.3	45.3	252.1
Decaf coffee	41	98	245	6.0 (109)	9.9	44.7	256.7

^a Numbers in parentheses, percentage of control.
^b vs. control, *P* < 0.05.

Table 4 Study 3: Effect of varied amounts of caffeine consumption during initiation on mammary tumor incidence in female rats treated with DMBA
DMBA was administered (i.v.) once at 55 days of age. Caffeine (100, 200 and 400 mg/liter of drinking water) was provided from 26-58 days of age. The experiment was terminated 12 weeks after carcinogen treatment.

Treatment	No. of rats	Percentage of rats with mammary carcinomas	Total number of mammary carcinomas	Number of mammary carcinomas per rat	Mean latency period of mammary tumor appearance (weeks)	Mean body weights (g)	
						Before treatment	After treatment
Control	41	90	186	4.5 (100) ^a	9.8	67.3	212.9
Caffeine (100 mg)	41	85	125	3.0 (67) ^b	10.2	66.4	212.6
Caffeine (200 mg)	41	93	114	2.8 (62) ^b	9.9	65.7	212.8
Caffeine (400 mg)	41	85	117	2.9 (64) ^b	9.7	66.0	212.1

^a Numbers in parentheses, percentage of control.
^b vs. control, *P* < 0.05.

Table 5 Study 4: Effect of varied amounts of caffeine consumption during promotion on mammary tumor incidence in female rats treated with DMBA

DMBA was administered (i.g.) once at 55 days of age. Caffeine (100–800 mg/liter of drinking water) was provided from 58 days of age to termination of experiment (12 weeks after carcinogen treatment).

Treatment	No. of rats	Percentage of rats with mammary carcinomas	Total number of mammary carcinomas	Number of mammary carcinomas per rat	Mean latency period of mammary tumor appearance (weeks)	Mean body weights (g)	
						Before treatment	After treatment
Control	41	46	25	0.6 (100) ^a	10.4	173.2	255.9
Caffeine (100 mg)	41	44	32	0.8 (133) ^b	10.3	174.0	271.7
Caffeine (200 mg)	40	60	35	0.9 (150) ^b	10.3	173.5	276.7
Caffeine (400 mg)	41	49	38	0.9 (150) ^b	10.7	174.1	268.2
Caffeine (600 mg)	41	51	43	1.0 (167) ^{b, c}	12.9	174.3	267.0
Caffeine (700 mg)	40	50	27	0.7 (116) ^c	10.4	173.3	258.7
Caffeine (800 mg)	41	27	16	0.4 (67) ^c	10.8	173.4	255.1

^a Numbers in parentheses, percentage of control.
^b Linear increase in mammary tumor number, $P < 0.05$.
^c Linear decrease in mammary tumor number, $P < 0.05$.

Table 6 Study 5: Effect of coffee consumption during promotion on mammary tumor incidence in female rats treated with DMBA. Full-strength (dose) coffee

DMBA was administered (i.g.) once at 55 days of age. Coffee (full strength) was provided from 58 days of age to termination of experiment (20–21 weeks after carcinogen treatment).

Treatment	No. of rats	Percentage of rats with mammary carcinomas	Total number of mammary carcinomas	Number of mammary carcinomas per rat	Mean latency period of mammary tumor appearance (weeks)	Mean body weights (g)	
						Before treatment	After treatment
Control	82	48	84	1.0 (100) ^a	15.4	175.3	267.3
Coffee	80	37	58	0.8 (80)	13.6	176.0	262.5
Decaf coffee	81	46	84	1.1 (110)	15.8	176.5	261.7

^a Numbers in parentheses, percentage of control.

Table 7 Study 6: Effect of caffeine and coffee consumption during promotion on mammary tumor incidence in female rats treated with DMBA. Moderate dose caffeine and moderate-strength (dose) coffee

DMBA was administered (i.g.) once at 54 days of age. Caffeine (moderate dose, 430 mg/liter of drinking water) and coffee (moderate strength) was provided from 57 days of age to termination of experiment (18 weeks after carcinogen treatment).

Treatment	No. of rats	Percentage of rats with mammary carcinomas	Total number of mammary carcinomas	Number of mammary carcinomas per rat	Mean latency period of mammary tumor appearance (weeks)	Mean body weight (g)	
						Before treatment	After treatment
Control	84	54	127	1.5 (100) ^a	16.7	167.1	285.8
Caffeine (430 mg)	84	59	149	1.8 (120)	15.7	167.8	294.1
Coffee	84	55	129	1.6 (107)	16.1	168.7	291.8
Decaf coffee	84	59	147	1.8 (120)	16.1	167.4	281.0

^a Numbers in parentheses, percentage of control.

ning 1 month after carcinogen treatment, all rats were palpated at 2-week intervals for the presence of mammary tumors. When mammary tumors reached 2 cm in diameter they were surgically excised, fixed in Bouin's fluid, stained with hematoxylin and eosin, and examined histologically. The excision site was sutured, and the rat was placed back in the experiment. All mammary tumors (palpable and nonpalpable) were excised and examined histologically. Termination date of each study was determined, with few exceptions, by the number and/or size of palpable mammary tumors in animal groups within each study.

Statistical Analysis, Studies 1–6. Percentage of rats bearing mammary carcinomas was analyzed by chi-square analyses. Error variance for mean number of mammary carcinomas per rat (mammary carcinoma multiplicity), mean latency period of mammary tumor appearance, and mean body weights were computed by analysis of variance. Means for experimental groups, compared with controls, were analyzed using Dunnett's *t* test. Mammary carcinoma multiplicity, as a function of caffeine dose levels, in Study 4 was analyzed by regression analysis using a piecewise linear curve.

RESULTS

Effect of Caffeine and Coffee Consumption on the Initiation Phase of DMBA-induced Rat Mammary Gland Carcinogenesis

When a high dose level of caffeine (860 mg) was administered to rats (via drinking water) prior to, during, and shortly after

carcinogen treatment, a 58% reduction in mammary carcinoma multiplicity (number of mammary carcinomas per rat) was observed ($P < 0.05$) (Table 2); a moderate dose level of caffeine (100–400 mg) reduced mammary carcinoma multiplicity by 33–38% ($P < 0.05$) (Table 4). During the same time period, the consumption of full strength and moderate strength caffeinated coffee reduced mammary carcinoma multiplicity by 62 and 40% ($P < 0.05$), respectively (Tables 2 and 3). Decaffeinated coffee (full or moderate strength), in contrast, did not significantly effect mammary carcinoma multiplicity (Tables 2 and 3). The addition of caffeine to the decaffeinated coffee sharply reduced mammary carcinoma multiplicity ($P < 0.05$) (Table 2). Caffeine and/or coffee consumption did not significantly effect the percentage of rats with mammary carcinomas or the mean latency period of mammary tumor appearance.

Effect of Caffeine and Coffee Consumption on the Promotion Phase of DMBA-induced Rat Mammary Gland Carcinogenesis

When moderate, but varying dose levels of caffeine (100–600 mg) were administered to rats (via drinking water) commencing 3 days after carcinogen treatment and for 12 weeks postcarcinogen treatment, a linear increase in mammary carcinoma multiplicity was observed ($P < 0.05$) (Table 5). A linear decrease in mammary carcinoma multiplicity was observed in rats ad-

ministered high dose levels of caffeine (600–800 mg) during the same period of time ($P < 0.05$) (Table 5). After prolonged consumption of moderate doses of caffeine (for 18 weeks after carcinogen treatment), no significant effect in mammary carcinoma multiplicity was observed (Table 7), although the number of palpable mammary tumors in the caffeine treated rats from 6–12 weeks after carcinogen tumor was greater ($P < 0.05$) than controls (data not shown).

Prolonged consumption of high (Table 6) and moderate (Table 7) dose levels of caffeinated coffee did not significantly effect mammary tumor multiplicity. However, in rats treated with moderate dose levels of caffeinated coffee, an apparent increase in number of palpable mammary tumors was observed in the first few weeks postcarcinogen treatment but this increase did not quite reach the 5% level of statistical probability (data not shown). No significant effect of decaffeinated coffee, at moderate or high dose levels, on mammary carcinoma multiplicity was observed (Tables 6 and 7). Caffeine and/or coffee consumption did not significantly effect the percentage of rats with mammary carcinomas or the mean latency period of mammary tumor appearance.

Effect of Caffeine and/or Coffee Consumption on Body Weight Gains

Initiation Studies. When young rats (24–26 days old) were given moderate dose levels of caffeine (100–400 mg), or moderate dose levels of coffee, a slight but insignificant decrease in body weight gains was observed during the first week of caffeine and/or coffee consumption. Thereafter, body weight gains increased slightly (compared to controls) and by the time of carcinogen treatment body weights were essentially identical to that observed in control animals (Tables 3 and 4). In contrast, when high dose levels of caffeine (860 mg) or coffee were given to young rats (24 days old), a significant ($P < 0.05$) reduction in body weight gains was observed during the first week. Consequently, we decided to halve the dose of caffeine and/or coffee during the period of Weeks 2 and 3. During this period of time, body weight gains in these animals increased substantially (compared to controls). During Week 4 (and to treatment termination), these rats received the same dose level of caffeine (860 mg) and coffee (full strength) as provided in Week 1. At the time of carcinogen treatment, no significant difference in mean body weights among these groups of rats was observed (Table 2). Nontumor-related mortality was negligible in both control and experimental (caffeine or coffee) groups.

Promotion Studies. When 58-day-old rats were given moderate or high doses of caffeine (100–800 mg) no significant effect on body weight gains was observed. Body weights tended to be slightly increased in rats consuming moderate doses of caffeine (100–600 mg); less in rats receiving high dose levels of caffeine (700–800 mg) (Table 5). Coffee consumption, either at moderate doses or high doses did not significantly effect body weight gains (Tables 6 and 7). Because high dose levels of caffeine and/or coffee did not effect body weight gains in the promotion studies (unlike the initiation studies), no adjustments in caffeine and/or coffee ingestion were necessary. The volume of fluid consumed (water, caffeinated water, and coffee) by each rat was monitored only in the promotion studies. Volume of fluid ingestion in control rats was similar to that in rats treated with moderate dose levels of caffeine or moderate-strength coffee. In rats treated with high dose levels of caffeine (600–800 mg) or full-strength coffee, fluid consumption decreased (10 to 40%, compared to controls) during the first weeks of fluid consumption; thereafter, fluid consumption returned to normal control

levels. Nontumor-related mortality was negligible in both control and experimental (caffeine or coffee) groups.

Histopathology of Mammary Tumors

In these studies, nearly 3000 mammary tumors were analyzed for histopathological characteristics. Greater than 98% of these tumors were mammary adenocarcinomas. The remaining mammary tumors (<2%) were benign fibroadenomas; the benign tumors were excluded (because of insufficient numbers) from data computation. The only exception to this was in the assessment of mean latency period of mammary tumor appearance; in the computation of these data we did not differentiate between carcinomatous and benign mammary tumors.

DISCUSSION

The results of this study provide evidence that caffeine consumption can significantly influence chemical carcinogenesis of the rat mammary gland. Caffeine consumption consistently inhibits the initiation phase of this carcinogenic process, at moderate or high dose levels. In contrast, when caffeine is consumed for prolonged periods of time during the promotion phase of this carcinogenic process, at moderate dose levels, no significant effect of caffeine in mammary tumorigenesis was observed. An apparent stimulatory effect of caffeine on mammary tumorigenesis was observed in the early stages of promotion; this effect was not pronounced, *i.e.*, it was temperate and transitory, just reaching the 5% level of statistical probability.

We are aware of only two previous studies which examined the effect of caffeine consumption on mammary tumorigenesis in rats treated with chemical carcinogens. In a preliminary study, utilizing a small number of rats, Minton *et al.* (26) did not observe a significant effect of caffeine on DMBA-induced mammary gland tumorigenesis in rats maintained on a standard laboratory chow; in rats treated with caffeine and fed a high fat diet, a significant enhancement of this tumorigenic process was observed. The study by Minton *et al.* is difficult to evaluate due to the limited number of animals utilized in their study and the uncertainty as to whether or not caffeine was administered during the initiation and/or promotion phases of this tumorigenic process. The second study which examined the relationship between caffeine and chemical carcinogenesis of the rat mammary gland was reported recently by our laboratory (27). In this study, moderate dose levels of caffeine was found to enhance the promotion phase of this tumorigenic process, albeit, the observed promoting effect of caffeine was not dramatic. Indeed, to achieve a significant ($P < 0.05$) enhancing effect of caffeine on the promotion phase required combining the results of three separate experiments. This positive but weak association between caffeine consumption and the enhancement of promotion of DMBA-induced rat mammary gland tumorigenesis provided the impetus for the present study. The results provided in this communication confirm and extend our earlier report supporting the concept that moderate dose levels of caffeine, when administered for a short duration, may temporarily enhance the early promotion phase of chemical carcinogenesis of the rat mammary gland. However, prolonged consumption of caffeine during promotion does not appear to significantly influence this carcinogenic process.

In our previous study (27), cited above, we did not observe a significant effect of caffeine on the initiation phase of DMBA-induced rat mammary gland tumorigenesis. This lack of an observed effect of caffeine on this phase of tumorigenesis was

due, in all probability, to the generation of insufficient numbers of mammary carcinomas in control and caffeine-treated animals (only 23–27 mammary carcinomas/animal group). In the present study, with large numbers of mammary carcinomas per group, a clear inhibitory effect of caffeine (both at moderate and high dose levels) on the initiation phase of DMBA-induced rat mammary gland tumorigenesis was consistently observed.

The effect of caffeinated coffee consumption on the initiation phase of chemical carcinogenesis of the rat mammary gland essentially paralleled that of caffeine consumption, *i.e.*, at moderate or high dose levels, caffeinated coffee inhibited this phase of DMBA-induced rat mammary gland tumorigenesis. Decaffeinated coffee, in contrast, did not influence the initiation phase of this tumorigenic process. When caffeine was added to the decaffeinated coffee, a striking inhibition of the initiation phase of DMBA-induced mammary tumorigenesis was observed. Thus, the inhibiting effect of coffee on the initiation phase of DMBA-induced mammary gland tumorigenesis appears to be due to the caffeine content of this beverage. That other factors contained in coffee may effect this tumorigenic process cannot be ignored (28, 29). In contrast, the consumption of caffeinated or decaffeinated coffee did not significantly effect the promotion phase of this carcinogenic process. Clearly, there was no evidence that prolonged consumption of moderate or high doses of coffee (caffeinated or decaffeinated) during the promotion phase of this carcinogenic process increases mammary gland tumorigenesis.

In the initiation studies, consumption of high dose levels of caffeine or coffee, while suppressing mammary tumorigenesis, also caused a reduction in body weight gains. Consequently, the dose level of caffeine and the strength of the coffee was reduced by one-half during Weeks 2 and 3 of the initiation phase. High dose caffeine and full-strength coffee was administered once again during Week 4. At the end of this period (day of carcinogen treatment) mean body weights of control and experimental animals were nearly identical. It is difficult to assess what influence this temporal decrease in body weight gains had upon this phase of mammary tumorigenesis. It is well known that suppression of body weight gains can significantly inhibit chemical carcinogenesis of the rat mammary gland (30). In rats treated with moderate dose levels of caffeine or coffee during initiation, no reduction in body weight gains was observed. Thus, caffeine and/or coffee can inhibit the initiation phase of DMBA-induced mammary gland tumorigenesis in rats having normal body weight gains. This problem was not encountered in the more mature rats of the promotion studies; no marked effect of caffeine and/or coffee consumption (moderate or high dose) on body weight gains was observed.

In both the initiation and promotion studies, differences among group treatments were demonstrated solely in mammary carcinoma multiplicity, *i.e.*, the number of mammary carcinomas per rat. No significant differences among group treatments were observed in the percentage of rats bearing mammary carcinomas or in mean latency period of mammary tumor appearance. It has been our experience over the years that mean number of mammary carcinomas per rat is the most reliable index of mammary tumorigenesis in this experimental animal model. Only by lowering the dose of the carcinogen or by reducing substantially the length (duration) of the experiment can one provide the potential for modulation of the percentage of rats with mammary carcinomas as a function of treatment.

In the initiation studies, the carcinogen (DMBA) was always administered *i.v.* During initiation, group treatments were provided prior to, during and shortly (3 days) after carcinogen

treatment. By administering the carcinogen *i.v.*, one eliminates the potential problem of interference by group treatments (caffeine and/or coffee) of carcinogen absorption from the gastrointestinal tract. In the promotion studies, group treatments were begun 3 days after carcinogen administration. Interference by group treatments of carcinogen absorption (from the gastrointestinal tract) is not a problem thus the carcinogen, in these studies, was administered solely *i.g.* All the initiation and promotion studies were conducted over a 3-year period. During this period of time, mammary carcinoma incidence varied from experiment to experiment. The major reason for the variance in tumor incidence is the variability in time of experiment termination (12–21 weeks after carcinogen treatment). Other factors, albeit of lesser importance, may also be involved. For example, although the carcinogen (DMBA) was always purchased from the same supplier (Eastman Kodak Co.), carcinogen lot numbers differed from experiment to experiment. Another explanation could be the variability in responsiveness of the experimental animals to the carcinogen. Although all rats were purchased from the same supplier (Harlan Sprague-Dawley, Inc.) differences in carcinogen responsiveness in rats of the same strain, but of different stock (particularly Sprague-Dawley), are frequently encountered. These problems underscore the need and importance of the use of large numbers of experimental animals in each experimental group when using this experimental animal model and when examining a temperate modulator of tumorigenesis such as caffeine.

In these studies, the rats received daily doses of caffeine ranging from 100 to 860 mg (per liter of drinking water). The 430-mg dose level was intermediate and we arbitrarily designated this level as a moderate dose level. Based upon the average daily volume of drinking water consumed by each rat, we estimated that 12.9 mg of caffeine was the average amount of caffeine consumed daily by rats receiving the moderate dose level of caffeine (430 mg). This is equivalent, on a body weight basis, to a daily consumption of 3096 mg caffeine by a 60-kg woman. If one uses values corrected according to metabolic body weight (31), then 12.9 mg of caffeine per rat per day is equivalent to 1376 mg of caffeine per 60-kg woman per day. The mean caffeine content of a cup of caffeinated coffee is \approx 100 mg (32). Thus, the intermediate dose level of caffeine used in these studies was not unreasonably high, as it was equivalent to the consumption of 13–14 cups of coffee per day. Our lowest dose level of caffeine used in these studies, *i.e.*, 100 mg (per liter of drinking water), would be equivalent to only 3–4 cups of caffeinated coffee per day.

A number of laboratories have examined the effect of caffeine and/or coffee consumption on tumorigenesis in experimental animals. It is most often reported that chronic consumption of caffeine and/or coffee does not enhance the spontaneous development of tumors in these animals (15–19). In contrast, in laboratory animals exposed to an array of chemical or physical carcinogens, caffeine and/or coffee is often reported to have a modulating effect on tumorigenic processes, enhancing and inhibiting depending upon the experimental conditions (20–25). With regard to the relationship between caffeine and/or coffee consumption and experimentally induced mammary tumors, the only report that we are aware of, other than the two previously cited (26, 27), was that by Petrek *et al.* (33) who recently examined the effect of caffeine on the genesis of mammary tumors in female ACI rats treated chronically with diethylstilbestrol. In their study, chronic caffeine treatments (1 and 2 g/liter of drinking water) significantly reduced the incidence of mammary carcinomas in the diethylstilbestrol-treated

animals. This report is consistent with the results of our study in which our highest dose level of caffeine (800–860 mg/liter of drinking water) inhibited the initiation and early promotion phases of DMBA-induced rat mammary gland carcinogenesis.

In this communication, we do not intend to discuss the potential mechanisms by which caffeine ingestion might influence rodent mammary gland tumorigenesis; that will be discussed in a sequacious report (34). Instead, our intent is to provide evidence as to whether or not caffeine consumption can influence mammary tumorigenesis in female rats treated with a chemical carcinogen. Our results demonstrate that caffeine ingestion can indeed influence this carcinogenic process, an effect which is decisively dependent upon the dose-level and time-span of caffeine administration.

REFERENCES

- Cole, P. Coffee-drinking and cancer of the lower urinary tract. *Lancet* *1*: 1335–1337, 1971.
- MacMahon, B., Yen, S., Trichopoulos, D., Warren, K., and Nardi, G. Coffee and cancer of the pancreas. *N. Engl. J. Med.*, *304*: 630–633, 1981.
- Hartge, P., Lasher, L. P., McGowan, L., and Hoover, R. Coffee and ovarian cancer. *Int. J. Cancer*, *30*: 531–532, 1982.
- Phillips, R. L., and Snowdon, D. A. Association of meat and coffee use with cancers of the large bowel, breast and prostate among Seventh Day Adventists: preliminary results. *Cancer Res.*, *43*: 2403s–2408s, 1983.
- Minton, J. P., Foecking, M. S., Webster, D. J., and Matthews, R. H. Caffeine, cyclic nucleotides, and breast disease. *Surgery (St. Louis)*, *86*: 105–109, 1976.
- Boyle, C. A., Berkowitz, G. S., LiVolai, V. A., Ort, S., Merino, M. J., White, C., and Kelsey, J. L. Caffeine consumption and fibrocystic breast disease: case-control epidemiology study. *J. Natl. Cancer Inst.*, *72*: 1015–1019, 1984.
- Wynder, E. L., Hall, N. E. L., and Polansky, M. Epidemiology of coffee and pancreatic cancer. *Cancer Res.*, *43*: 3900–3906, 1986.
- Shedlofsky, S. Coffee and cancer of the pancreas. *N. Engl. J. Med.*, *304*: 1604, 1981.
- Jick, H., and Dinan, B. J. Coffee and pancreatic cancer. *Lancet*, *2*: 92, 1981.
- Cramer, D. W., Welch, W. R., Hutchison, G. B., Willet, W., and Scully, R. E. Dietary animal fat in relation to ovarian cancer risk. *Obstet. Gynecol.*, *63*: 833–838, 1984.
- Lawson, D. H., Jick, H., and Rothman, K. J. Coffee and tea consumption and breast disease. *Surgery (St. Louis)*, *90*: 801–803, 1981.
- Marshall, J., Graham, S., and Swanson, B. A. Caffeine consumption and benign breast disease: a case-control comparison. *Am. J. Public Health*, *72*: 610–613, 1982.
- Lubin, F., Ron, E., Wox, Y., and Modan, B. Coffee and methylxanthines and breast cancer: a case-control study. *J. Natl. Cancer Inst.*, *74*: 569–573, 1985.
- Rosenberg, L., Miller, D. R., Helmrick, S. P., Kaufman, D. W., Schottenfeld, D., Stolley, P. D., and Shapiro, S. Breast cancer and the consumption of coffee. *Am. J. Epidemiology*, *122*: 391–399, 1985.
- Takayama, S., and Kuwabara, N. Long-term study on the effect of caffeine in Wistar rats. *Gann*, *73*: 365–371, 1982.
- Bauer, A. R., Rank, R. K., Kerr, R., Straley, R. L., and Mason, J. D. The effects of prolonged coffee intake on genetically identical mice. *Life Sci.*, *21*: 63–70, 1977.
- Wurzner, H. P., Lindstrom, E., Vuatag, L., and Luginbuhl, H. A two-year feeding study of instant coffees in rats. II. Incidence and types of neoplasms. *Food Cosmet. Toxicol.*, *15*: 289–296, 1977.
- Macklin, A. W., and Szot, R. J. Eighteen month oral study of aspirin, phenacetin and caffeine in C57BL/6 mice. *Drug Chem. Toxicol.*, *3*: 135–163, 1980.
- Mohr, U., Althoff, J., Ketkar, M. B., and Conradt, P. The influence of caffeine on tumor incidence in Sprague-Dawley rats. *Food Cosmet. Toxicol.*, *22*: 377–382, 1984.
- Rothwell, K. Dose-related inhibition of chemical carcinogenesis in mouse skin by caffeine. *Nature (Lond.)*, *252*: 69–70, 1974.
- Nomuro, T. Diminution of tumorigenesis initiated by 4-nitroquinoline-1-oxide by post-treatment with caffeine in mice. *Nature (Lond.)*, *260*: 547–549, 1976.
- Armuth, V., and Berenblum, I. The effect of caffeine on 2-stage skin carcinogenesis and on complete systemic carcinogenesis. *Carcinogenesis (Lond.)*, *2*: 977–979, 1981.
- Nomura, T. Timing of chemically induced neoplasia in mice revealed by the antineoplastic action of caffeine. *Cancer Res.*, *40*: 1332–1340, 1980.
- Theiss, J. C., and Shimkin, M. B. Inhibition effect of caffeine on spontaneous and urethan-induced lung tumors in strain A mice. *Cancer Res.*, *38*: 1757–1761, 1978.
- Denda, A., Yokose, Y., Emi, Y., Murata, Y., Ohara, T., Sunagawa, M., Mikami, S., Takahoshi, S., and Konishi, Y. Effects of caffeine on pancreatic tumorigenesis by 4-hydroxyamino-quinoline 1-oxide in partially pancreatectomized rats. *Carcinogenesis (Lond.)*, *4*: 17–22, 1983.
- Minton, J. P., Abou-Issa, H., Foecking, M. K., and Sriram, M. G. Caffeine and unsaturated fat diet significantly promotes DMBA-induced breast cancer in rats. *Cancer (Phila.)*, *51*: 1249–1253, 1983.
- Welsch, C. W., Scieszka, K. M., Senn, E. R., and DeHoog, J. V. Caffeine (1,3,7-trimethylxanthine), a temperate promoter of DMBA-induced rat mammary gland carcinogenesis. *Int. J. Cancer*, *32*: 479–484, 1983.
- Sugimura, T., and Sato, S. Mutagens—carcinogens in foods. *Cancer Res.*, *43*: 2415s–2421s, 1983.
- Wattenberg, L. W. Inhibition of neoplasia by minor dietary constituents. *Cancer Res.*, *43*: 2448s–2453s, 1983.
- Welsch, C. W. Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. *Cancer Res.*, *45*: 3415–3443, 1985.
- Gilbert, R. M. Caffeine as a drug abuse. *In*: R. J. Gibbins, Y. Israel, H. Kolant, R. E. Popham, W. Schmidt, and R. G. Smart (eds.), *Recent Advances in Alcohol and Drug Problems*, Vol. 3, pp. 49–75. New York: John Wiley & Sons, 1976.
- Bunker, M. L., and McWilliams, M. Caffeine content of common beverages. *J. Am. Diet. Assoc.*, *74*: 28–32, 1979.
- Petrek, J. A., Sandberg, W. A., Cole, M. N., Silberman, M. S., and Collins, D. C. The inhibitory effect of caffeine in hormone-induced rat breast cancer. *Cancer (Phila.)*, *56*: 1977–1981, 1985.
- Welsch, C. W., DeHoog, J. V., and O'Connor, D. H. Influence of caffeine consumption on carcinomatous and normal mammary gland development in mice. *Cancer Res.*, *48*: 2078–2082, 1988.