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Comparative Inhibiting Effects of Methylxanthines on Urethan-induced Tumors, Malformations, and Presumed Somatic Mutations in Mice[1]

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ABSTRACT

The inhibiting effects of methylxanthines on urethan-induced lung tumors, malformations, and presumed somatic mutations in mice were studied to determine the contribution of mutational and physiological changes to chemically induced neoplasia and malformation. When young adult or pregnant ICR/Jc1 mice were treated with urethan and then methylxanthines were given, caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) greatly suppressed urethan-induced tumorigenesis and teratogenesis, while theophylline (1,3-dimethylxanthine) did not. Of the three monomethylxanthines (methylated at positions 1, 3, or 7), 7-methylxanthine was most effective for inhibiting tumors and malformations, indicating that the methyl group at position 7 is most active. Contribution of cyclic adenosine 3′:5′-monophosphate was ruled out, since urethan-induced tumorigenesis and teratogenesis were not affected by theophylline which elevates the cellular level of cyclic adenosine 3′:5′-monophosphate by inhibiting phosphodiesterase more effectively than caffeine does; instead, tumorigenesis and teratogenesis were greatly inhibited by theobromine and 7-methylxanthine, which do not alter the level of cyclic adenosine 3′:5′-monophosphate. To test the mutational origin of cancer and malformation, the effects of caffeine on urethan induction of somatic mutations in PT × HT F_1 mice were examined, because caffeine is known to inhibit ultraviolet- and 4-nitroquinoline 1-oxide-initiated mutagenesis in *Escherichia coli* by inhibiting error-prone repair. In mice, however, caffeine did not inhibit urethan-induced somatic mutations. Furthermore, theophylline, an inhibitor of error-prone repair, did not reduce the yields of tumors and malformations. Antineoplastic and antiteratogenic effects of caffeine may be caused not by the inhibition of the mutational change but by the inhibition of the subsequent process for expressing tumors and malformations.

INTRODUCTION

It has been reported by several investigators that caffeine is antineoplastic and antiteratogenic. Caffeine suppresses the carcinogenic effect of UV (29) or cigarette smoke condensate (23) on mouse skin and of 4NQO[3] (15, 16, 18, 19) and urethan (16, 18, 19, 26) on the lung even if it is given to young adult mice 21 days after carcinogen treatment (18, 20). Furthermore, posttreatment with caffeine greatly suppressed the teratogenic effect of urethan in mice (17, 18), suggesting the similarity of the mechanism of teratogenesis and carcinogenesis. I have proposed that the antineoplastic and antiteratogenic action of caffeine may be caused by the inhibition of error-prone repair of DNA lesions produced by carcinogens, resulting in the decrease of potentially teratogenic or carcinogenic cells (17–20). In addition to the inhibition of repair mechanism, however, caffeine is known to increase the cellular level of cyclic AMP (1–3) and to show affinity to the partially denatured DNA (4). In order to analyze the mechanism of the antineoplastic and antiteratogenic action of caffeine, urethan-treated mice were posttreated with several methylxanthines, caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine), and 3 monomethylxanthines. These methylxanthines show a variety of action on repair mechanisms (4, 6, 29) and phosphodiesterase (1–3). To investigate further, I examined the effect of caffeine on urethan-induced somatic mutations in mice. This paper summarizes the data obtained from 1976 to 1979.

MATERIALS AND METHODS

Animals. Mice used were ICR/Jc1 (13) for study on tumors and malformations and PT and HT for study on somatic mutations. PT and HT mice were kindly provided by Drs. M. F. Lyon and A. G. Searle, Radiobiology Unit, Medical Research Council, Harwell, United Kingdom. The PT mouse is homozygous for the following recessive loci: *a* (non-agouti); *b* (brown); *p* (pink-eyed dilution); c^{ch} (chinchilla); *d* (dilute); *se* (short-ear); and *s* (piebald). The HT mouse is also homozygous for the following recessive alleles: *a*, *pa* (pallid); *ln* (leaden); *fz* (fuzzy); *pe* (pearl); and *bp* (brachypodism). ICR mice were maintained with Mouse Diet CA-1 (CLEA, Japan, Tokyo, Japan) (13) in a conventional mouse room at 23–25°, and PT and HT mice were maintained with Mouse Diet CRF-1 (Charles River Japan, Kanagawa, Japan) in a complete barrier system at 21–23°.

Chemicals. The following solutions were prepared just before use: 10, 5, 2.5, and 1% aqueous solution of urethan (ethyl carbamate; Wako Pure Chemical Industries, Osaka, Japan); 0.49 and 0.10% aqueous solutions of caffeine (Nakarai Chemical Industries, Kyoto, Japan); 0.45 and 0.09% aqueous solutions of theophylline (Sigma Chemical Co., St. Louis, Mo.); 0.45 and 0.09% aqueous solutions of theobromine (Sigma); 0.42 and 0.08% aqueous suspensions of 1-methyl-, 3-methyl-, and 7-methylxanthines (Fluka AG Chemical Industry, Buchs, Switzerland); 0.38% aqueous suspension of xanthine (Wako Pure Chemical Industries). Monomethylxanthines and xanthine were ground to fine powder and suspended in 0.9% NaCl solution. When these suspensions were injected i.p., fine crystals disappeared from the peritoneal cavity about 6 hr after injection, indicating that these agents were absorbed within 6 hr. When suspensions of these agents were administered s.c., however, these remained at the injected site for about 3 days. Higher doses of these 6 methylxanthines given in the following manner correspond to half-maximum tolerated doses to young adult and pregnant mice.

Examination of Effects of Methylxanthines on Urethan-induced Carcinogenesis. A single s.c. injection of 0.1 mg of urethan per g of

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[3] The abbreviations used are: 4NQO, 4-nitroquinoline 1-oxide; cyclic AMP, cyclic adenosine 3′:5′-monophosphate.

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body weight was given to 25-day-old female ICR/Jc1 mice. Then, 7 i.p. injections of 0.05 μmol of methylxanthines and xanthine per g of body weight were given at 6-hr intervals during the period of 0 to 36 hr after urethan treatment.

In addition, a single s.c. injection of a high dose (1.0 mg/g body weight) of urethan was given to 25-day-old female mice. Then, 5 i.p. injections of 0.25 μmol of monomethylxanthines per g of body weight were given daily starting 5 days after urethan treatment. This experiment was scheduled to rule out direct interaction between urethan and methylxanthines and also to analyze which methyl group is effective in suppressing tumors. The carcinogenicity of monomethylxanthines (0.25 μmol/g daily for 5 days) was also tested.

Mice were killed 5 months after urethan treatment. Gross pathological lesions, especially for tumors, were examined as described previously (14, 22), and specimens were examined microscopically. Most of the induced tumors were in the lung (papillary adenomas). Rarely, lymphocytic leukemias were observed. Lung tumor frequency was chosen for the statistical comparison, because scoring the number of tumor nodules in the lung makes it easy to analyze quantitatively the difference in the yields of tumors among experimental groups (14, 25).

Examination of Effects of Methylxanthines on Urethan-induced Teratogenesis. An estrous female ICR/Jc1 mouse (9 to 12 weeks old and weighing 29 to 32 g) was placed in a cage with a breeder male in the evening, and the next morning the vaginal plug was checked to determine Day 1 of gestation (22). Pregnant mice received a single s.c. injection of urethan (1.0 mg/g of body weight) at 2 p.m. on Day 10, and then 5 i.p. injections of 0.25, 0.05, or 0.025 μmol of methylxanthines per g of body weight were given at 6-hr intervals during the period of 0 to 24 hr after urethan treatment. Mice were killed on Day 19 by cervical dislocation. By hysterectomy, their fetuses and fetal appendages were removed, weighed, and recorded. Implants, early deaths (deaths before the completion of placenta, *i.e.*, before Day 9), and late deaths (deaths after Day 10) were checked, and the external appearance of living fetuses and fetal appendages was examined, especially for malformations (13).

Examination of Effects of Caffeine on Urethan-induced Somatic Mutations. An estrous PT female was mated with HT males in the evening, and the next morning the vaginal plug was checked to determine Day 1 of gestation. Mice were exposed to light from 4 a.m. to 6 p.m., which resulted in ovulation at about 2 a.m. (7), indicating that fertilization occurs at about 2 a.m. (7) of Day 1. Pregnant mice received a single s.c. injection of 1.0 mg of urethan per g of body weight at 2 p.m. of Day 11. Consequently, PT × HT F_1 embryos at 10.5 days after fertilization were treated with urethan. F_1 offspring were examined for spots and malformations every week and killed 6 to 8 weeks after birth to make permanent preparations of the coat. F_1 embryos were heterozygous for the 7 recessive coat color alleles, *b*, c^{ch}, *d*, *In*, *p*, *pa*, and *pe*, and homozygous for *a*. Presumed somatic mutations were detected as a colored spot on the black coat, which derived from a mutated pigment cell. White spots on the ventral midline (white midventral spots) are thought to be caused by killing or specific division delay of melanoblasts (12, 24). Hair was plucked from the affected areas, and altered gene loci were determined microscopically. Since hundreds of precursor pigment cells exist in Day 11 embryos, somatic mutations are detected hundreds of times more frequently than germinal mutations (21, 24). Procedures are given in a scheme (Fig. 1), and details for the procedures will be published elsewhere. Preliminary results with urethan showed a linear dose-response relationship in mutation frequency at doses of 0.25, 0.5, and 1.0 mg/g (Chart 1). Immediately after treatment with 1.0 mg of urethan per g, 5 i.p. injections of 0.25 μmol of caffeine per g of body weight were given to pregnant mice at 6-hr intervals, and frequencies of presumed somatic mutations and malformations were compared against urethan-alone controls.

RESULTS

Effects of Methylxanthines on Urethan-induced Lung Neoplasia. Yields of lung tumors in young adult mice which had received a low dose of urethan (0.1 mg/g) were significantly reduced by posttreatment with caffeine (0.05 μmol/g) (Table 1), as in the case of previous reports, (16, 18, 20) with a higher dose of urethan (1.0 mg/g). The level of inhibition was about 70%. The same level of inhibition of urethan-induced lung tumorigenesis was observed with theobromine, while theophylline did not reduce tumor yields (Table 1). This suggests that the methyl group at position 7 may be active in reducing tumor yields. In fact, only 7-methylxanthine among 3 monomethylxanthines reduced tumor yields significantly (Table 1). In order to analyze the difference more strictly, higher doses of monomethylxanthines were given 5 days after treatment with 1.0 mg of urethan per g. As shown in Table 2, posttreatment with 7-methylxanthine greatly reduced lung tumorigenesis. Sig-

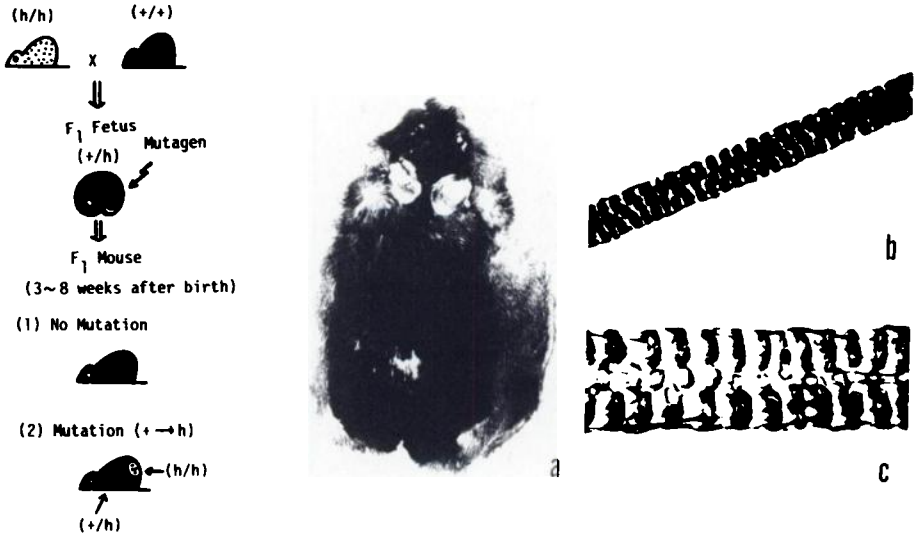

Fig. 1. Scheme of procedures detecting somatic mutations in PT × HT F_1 mice. Mutagens were given to PT × HT F_1 embryos which are heterozygous at the recessive coat color gene (*h*) with wild allele. Colored spot derived from a mutated pigment cell (*h*/*h*) was observed during the period of 3 to 8 weeks after birth. Details are given in "Materials and Methods." *a*, permanent preparation of the coat. Light-colored (thin brown) spot is seen at the left low back. *b*, microscopic view of normal heterozygous hair. × 50. *c*, microscopic view of affected hair. × 100. Extensive loss of black pigments indicates the alterations at the pink-eyed dilution locus.

nificant reduction of lung tumorigenesis was also observed with 3-methylxanthine, but the level of inhibition was one-half that by 7-methylxanthine. There were no differences in the size and histological patterns of induced tumors among experimental groups. Incidence of lymphocytic leukemias and ovarian tumors seems to be reduced by all monomethylxanthines, but the results are not conclusive because of small sample size. These monomethylxanthines were not carcinogenic in this strain of mice (Table 2).

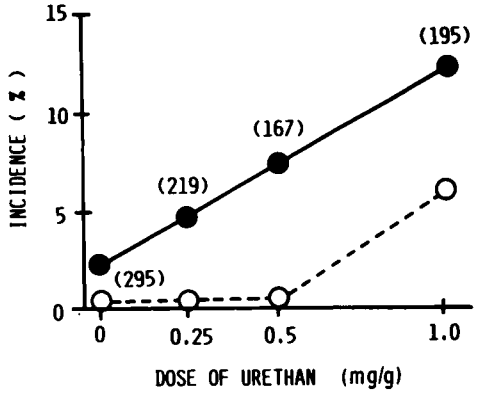

Chart 1. Dose-response relationship of urethan-induced somatic mutations in PT × HT F_1 mice. PT × HT F_1 embryos were treated with 0.25, 0.5, and 1.0 mg of urethan per g by treating pregnant mice on Day 11. Details for the experimental procedure are given in "Materials and Methods." ●, colored spots; ○, white midventral spots. *Numbers in parentheses*, numbers of PT × HT F_1 offspring examined for spots. A part of the work was presented at the Third International Conference of Environmental Mutagens at Tokyo (21).

Effects of Methylxanthines on Urethan-induced Teratogenesis. In parallel to the inhibiting effects on lung neoplasia, urethan-induced malformations were also greatly reduced by posttreatments with 0.25 μmol of caffeine or theobromine per g, while an equivalent dose of theophylline did not reduce them (Table 3). The incidence of malformation-bearing fetuses was significantly reduced by posttreatments with theobromine at one-fifth the dosage (0.05 μmol/g), but malformations were not reduced by an equivalent dose of caffeine. Although theophylline and a low dose of caffeine did not reduce the incidence of fetuses with various kinds of malformations, the incidence of polydactyly was significantly reduced by these 2 agents [$p < 0.01$ (Table 3)]. All monomethylxanthines significantly reduced urethan-initiated teratogenesis, while unmethylated xanthine did not (Table 3). Of the 3 monomethylxanthines, 7-methylxanthine was most effective in reducing the incidence of malformations (Table 3) as in the case of urethan-induced lung neoplasia (Table 2).

Effects of Caffeine on Urethan-induced Somatic Mutations. As shown in Table 4, a single s.c. injection of urethan (1.0 mg/g) induced significant incidence of the presumed somatic mutations indicated by colored spots ($p \ll 0.001$ by χ^2 against controls), killing of melanoblasts indicated by white midventral spots (see "Materials and Methods") ($p < 0.001$),

Table 1

Comparative inhibiting effects of methylxanthines (0.05 μmol/g) on lung neoplasia induced by a low dose of urethan

Methylxanthines were given i.p. at 6-hr intervals during the period of 0 to 36 hr after urethan treatment. Details are given in "Materials and Methods." The χ^2 test was applied with Yates' correction, and a t test was made after testing the variance ratio. No tumors were found other than those in the lung.

	Posttreatment		Tumor-bearing mice		Tumors/lung	
Urethan (mg/g)	Methylxanthine	Period (hr)	Incidence	p	Mean ± S.E.	p
0.1	None		31/59 (52.5)[a]		1.07 ± 0.17	
0.1	Caffeine	0–36	7/32 (21.9)	<0.01	0.31 ± 0.11	<0.005
0.1	Theophylline	0–36	25/43 (58.1)	NS[b]	1.36 ± 0.27	NS
0.1	Theobromine	0–36	11/56 (19.6)	<0.001	0.28 ± 0.08	≪0.001
0.1	1-Methylxanthine	0–36	18/36 (50.0)	NS	0.86 ± 0.18	NS
0.1	3-Methylxanthine	0–36	26/44 (59.1)	NS	1.02 ± 0.18	NS
0.1	7-Methylxanthine	0–36	18/48 (37.5)	≒0.12	0.56 ± 0.13	<0.05
0.1	Xanthine	0–36	26/40 (65.0)	NS	1.02 ± 0.20	NS

[a] Numbers in parentheses, percentage of tumor-bearing mice.
[b] NS, not significant.

Table 2

Comparative inhibiting effects of monomethylxanthines (0.25 μmol/g) on urethan-induced neoplasia

Monomethylxanthines were given i.p. once a day during the period of 5 to 9 days after urethan treatment. Statistical analysis was performed against urethan-alone controls or untreated controls. Details are given in the text and legends to Table 1.

	Posttreatment		Tumor-bearing mice		Tumors/lung		
Urethan (mg/g)	Methylxanthine	Period (days)	Incidence	p	Mean ± S.E.	p	Other than lung tumors
1.0	None		49/53 (92.5)[a]		12.3 ± 1.3		4 L,[b] 2 OC
1.0	1-Methylxanthine	5–9	39/43 (90.7)	NS	11.1 ± 1.5	NS	1 L
1.0	3-Methylxanthine	5–9	50/53 (94.3)	NS	8.4 ± 1.1	<0.02	None
1.0	7-Methylxanthine	5–9	43/62 (69.4)	<0.01	4.8 ± 0.6	≪0.001	None
0.0[c]	1-Methylxanthine	5–9	3/47 (6.4)	NS	0.06 ± 0.04	NS	None
0.0	3-Methylxanthine	5–9	2/47 (4.3)	NS	0.04 ± 0.03	NS	None
0.0	7-Methylxanthine	5–9	1/37 (2.7)	NS	0.03 ± 0.03	NS	None
None	None		7/181 (3.9)		0.04 ± 0.01		None

[a] Numbers in parentheses, percentage of tumor-bearing mice.
[b] L, lymphocytic leukemia; OC, ovarian cystoma; NS, not significant.
[c] An equal volume of distilled water was given instead of urethan solution.

and malformations ($p \ll 0.001$). Posttreatments with caffeine did not suppress but rather slightly but not significantly increased the incidence of colored spots ($p \fallingdotseq 0.35$) and white midventral spots ($p \fallingdotseq 0.1$), while tail anomalies and polydactylies were almost completely suppressed to the level of the control value (Table 4). A microscopic view of the affected hair showed no substantial differences in the affected loci of coat color genes between caffeine and caffeineless groups. Although small numbers of mice were tested, caffeine was not mutagenic by this method (Table 4).

DISCUSSION

Effects of posttreatments with methylxanthines on urethan-induced neoplasms, malformations, and somatic mutations are summarized in Table 5. Not only caffeine (1,3,7-trimethylxanthine) but also theobromine (3,7-dimethylxanthine) greatly reduced yields of tumors and malformations induced by urethan, while theophylline (1,3-dimethylxanthine) did not. Of the 3 monomethylxanthines tested, 7-methylxanthine showed the strongest antitumorigenicity and antiteratogenicity (Tables 1 to 3), suggesting that methyl group at position 7 is the most active site for inhibition of urethan-induced teratogenesis and tumorigenesis. Direct interaction between urethan and methylxanthines and altered metabolism of urethan by methylxanthines are ruled out, because urethan is short acting (14) and caffeine and 7-methylxanthine inhibited tumorigenesis even when these were given 5 to 10 days after urethan treatment (Table 2; Ref. 20).

There is an apparent parallelism in the response to methylxanthines between urethan-induced teratogenesis and tumorigenesis, although 1-methylxanthine showed only antiteratogenicity (Table 5). There might be a similar process in the mechanism of chemically induced tumorigenesis and teratogenesis. One possible explanation is that cyclic AMP promotes cell differentiation (3), resulting in decrease of tumors and malformations, because caffeine is known to increase cellular level of cyclic AMP by inhibiting phosphodiesterase (1–3). However, contribution of cyclic AMP for inhibiting teratogenesis and carcinogenesis will be ruled out, because yields of urethan-induced tumors and malformations were not reduced by theophylline which elevates cellular level of cyclic AMP more effectively than does caffeine (1, 2). Furthermore, theobromine and 7-methylxanthine, which do not inhibit phosphodiesterase, greatly reduced yields of tumors and malformations (Table 5).

The other possibility is that a mechanism similar to mutagenesis may be involved in urethan-induced teratogenesis and carcinogenesis, because caffeine is known to suppress UV- and 4NQO-induced mutations in a specific strain of *Escherichia coli* by inhibiting error-prone repair (9, 10, 27, 28). However, there is a serious discrepancy in the hypothesis. Theophylline, an inhibitor of error-prone repair (29), did not reduce yields of lung tumors and malformations, although Zajdela and Latarjet (29) reported that the incidence of UV-induced skin tumors was suppressed by the presence of either caffeine or theophylline. In order to make clear the *in vivo* action of caffeine, I tested the effects of posttreatment with caffeine on urethan-

Table 3

Comparative inhibiting effects of methylxanthines on urethan-induced malformations

Methylxanthines were given i.p. at 6-hr intervals during the period of 0 to 24 hr after urethan. Details are given in "Materials and Methods." For statistical analysis, a χ^2 test was applied against urethan-alone controls.

Treatment					Late deaths		Living fetuses		Malformation-bearing fetuses			
Urethan (mg/g)	Methylxanthines (μmol/g)	No. of mice	No. of implants	No. of early deaths	No.	%[a]	No.	%[a]	No.	%[b]	p	Details
1.0	None	14	172	8	9	5.5	155	94.5	82	52.9		36 CP,[c] 32 T, 32 PD
1.0	Caffeine (0.25)	9	112	6	6	5.7	100	94.3	7	7.0	$\ll$0.001	4 CP, 3 PD
1.0	Caffeine (0.05)	11	134	3	5	3.8	126	96.2	55	43.7	NS	30 CP, 24 T, 5 PD
1.0	Theophylline (0.25)	10	126	6	8	6.7	112	93.3	57	50.9	NS	14 CP, 41 T, 9 PD
1.0	Theobromine (0.25)	8	99	5	3	3.2	91	96.8	12	13.2	$\ll$0.001	5 CP, 7 T, 2 PD
1.0	Theobromine (0.05)	15	183	7	5	2.8	171	97.2	61	35.7	<0.01	20 CP, 36 T, 22 PD
1.0	1-Methylxanthine (0.05)	19	238	8	14	6.1	216	93.9	52	24.1	$\ll$0.001	8 CP, 24 T, 21 PD
1.0	3-Methylxanthine (0.05)	12	157	1	13	8.3	143	91.7	51	35.7	<0.01	15 CP, 31 T, 10 PD, 1 Ex
1.0	7-Methylxanthine (0.05)	13	155	6	6	4.0	143	96.0	12	8.4	$\ll$0.001	3 CP, 4 T, 5 PD
1.0	7-Methylxanthine (0.025)	8	91	3	5	5.7	83	94.3	20	24.1	<0.001	12 CP, 13 T, 3 PD
1.0	Xanthine (0.05)	10	132	5	9	7.1	118	92.9	59	50.0	NS	25 CP, 30 T, 20 PD
None	None	28	351	18	13	3.9	320	96.1	1	0.3		1 Ex

[a] Percentage of survivors (*i.e.*, implants minus early deaths) at the time of urethan treatment (Day 10).
[b] Percentage of living fetuses.
[c] CP, cleft palate; T, tail anomaly (kinky and/or short); PD, polydactyly; NS, not significant; Ex, exencephalus.

Table 4

Effects of caffeine on the presumed somatic mutations and malformations induced by urethan in PT × HT F_1 offspring

Posttreatment				Live offspring		Colored spots		White midventral spot		Malformation-bearing offspring		
Urethan (mg/g)	Caffeine (μmol/g)	Period (hr)	No. of pregnant mice	No.	Mean ± S.E.	Incidence	%	Incidence	%	Incidence	%	Details
1.0	None		40 (11)[a]	200	6.9 ± 0.4	25/195[b]	12.8	12/195	6.2	49/195	25.1	40 T,[c] 19 PD
1.0	0.25	0–24	30 (6)	162	6.8 ± 0.5	24/148	16.2	16/148	10.8	5/148	3.4[d]	3 T, 2 PD
None	0.25	0–24	4 (0)	35	8.8 ± 1.0	0/ 35	0.0	0/ 35	0.0	2/ 35	5.7	2 T
None	None		45 (6)	304	7.8 ± 0.37	6/295	2.0	1/295	0.3	8/295	2.7	7 T, 1 DW

[a] Numbers in parentheses, number of mice that resulted in abortion and cannibalism.
[b] Numbers in parentheses, number of mice that survived more than 3 weeks.
[c] T, tail anomaly; PD, polydactyly; DW, dwarf.
[d] Significantly different from the value of urethan-alone controls at $p \ll 0.001$ by χ^2 test.

Table 5

Comparative inhibiting effects of methylxanthines on urethan-induced tumors, malformations, and somatic mutations in relation to their effects on the level of cyclic AMP and error-prone postreplication repair

Methyl groups	Inhibiting effects of methylxanthines on				
	Tumors	Malformations	Mutations	Phosphodiesterase[a]	Error-prone repair[b]
1, 3, 7	+[c]	+	−	+	+
1, 3	−	−	NT	+	+
3, 7	+	+	NT	−	NT
1	−	+	NT	+	NT
3	+	+	NT	+	NT
7	+	+	NT	−	NT
None	−	−	NT	−	NT

[a] Refs. 1, 2, and 3.
[b] Refs. 6, 9, 10, 27, 28, and 29.
[c] +, significantly inhibited; −, no effects; NT, not tested.

induced somatic mutations in mice, since all experiments on the molecular mechanism of caffeine have been done with *E. coli* (9, 10, 27, 28) and cultivated mammalian cells (5, 6, 8), and there are no data for mice which can develop tumors and malformations. As shown in Chart 1 and Table 4, urethan induced significant yields of presumed somatic mutations in mice (21), as it did in *Drosophila* sperm (19), while mutagenicity has not been detected in the *Salmonella* tester system even with enzymatic activation by liver homogenates and S-9 fraction (11). Although caffeine suppresses UV and 4NQO-induced mutations in *E. coli* (9, 10, 27, 28), and also urethan-induced recessive lethal mutations in *Drosophila melanogaster* (19), caffeine posttreatment did not suppress but instead slightly increased urethan-induced somatic mutations in coat color, while the incidence of malformed offspring was almost completely suppressed by caffeine (Table 4). Consequently, the mechanism causing tumors and malformations might be different from that for mutagenesis in somatic cells. Caffeine, theobromine, and 7-methylxanthine may suppress subsequent processes expressing tumors and malformations, although such action has not been studied. Alternatively, xanthines possessing a methyl group at position 7 may show affinity to DNA damaged by urethan, resulting in selective killing of such cells with urethan damage responsible for tumorigenesis and teratogenesis, but not for mutagenesis, because purine analogues are known to show strong affinity to the partially denatured DNA (4). We must await more information about the molecular mechanism of methylxanthines.

The most important reservation is the method used here for detecting somatic mutations in mice. Since F_1 embryos heterozygous at recessive coat color genes with wild alleles were treated, colored spots might indicate somatic recombination or deficiency of a chromosomal segment other than forward gene mutation or deletion of the wild allele (12, 24). Consequently, a new method *e.g.*, a method to detect reverse mutation in somatic cells,[4] may contribute to a more accurate analysis of the relationship between mutagenesis, teratogenesis, and carcinogenesis.

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[4] T. Nomura, unpublished data.

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