Caffeine Enhancement of Chemical Carcinogen-induced Transformation of Cultured Syrian Hamster Cells

P. J. Donovan and J. A. DiPaolo

Introduction

The frequency of transformation of cultured Syrian hamster cells by chemical carcinogens can be increased by treatment of cells with X-irradiation (7), alkylating agents (4), or compounds that modify carcinogenic polycyclic hydrocarbon metabolism by affecting the aryl hydrocarbon hydroxylase enzyme system (8). The mechanism of transformation enhancement by these different agents has yet to be explained. They may affect a number of molecular processes. They may cause a modification of existing DNA and thus provide an explanation for carcinogenesis; in fact, in some systems some of these agents may also show mutagenic activity and produce chromosomal aberrations. However, although DNA is the critical site for a mutagen, the critical target(s) of chemical carcinogens is unknown. In a number of cell lines caffeine has been found to potentiate cell killing and production of chromosome aberrations by other agents such as UV light and alkylating chemicals (16, 17, 23). This synergistic effect takes place with concentrations of caffeine that, by themselves, produce minimal or no visible effect on cell survival. Caffeine has been found to affect cells only during the phase in which DNA is being synthesized (reviewed in Ref. 15). Caffeine enhanced the frequency of adenovirus transformation in Syrian hamster cells (2, 12). Enhancement by treatment of cells with caffeine was maximal when caffeine was added 5 hr after simian adenovirus 7, but it was minimal or absent when added prior to virus (2). Thus, maximal enhancement of adenovirus transformation by caffeine depended upon the sequence and timing of addition of virus and chemical. The present experiments were designed to examine the influence of caffeine on the transformation frequency produced by the known chemical carcinogens, BP, 1 AcAAF, and MNNG. These carcinogens are known to produce cell transformations that result in tumors when the cells are inoculated into hamsters (9, 10).

Materials and Methods

Transformation Assay. Details of the experimental procedures have been described previously (5, 6). Cells derived from 12- to 14-day-old Syrian hamster fetuses LVG:LAK (Lakeview Hamster Colony, Newfield, N. J.) were grown as monolayers in Dulbecco's modification of Eagle's minimal essential medium (Schwartz/Mann, Orangeburg, N. Y.) with 10% fetal bovine serum (Reheis Chemical Co.) in a humidified 10% CO2 incubator at 37°C. In all experiments, 2- to 4-day-old secondary or tertiary hamster cultures were obtained by seeding 5 x 10^6

1 The abbreviations used are: BP, benzo(a)pyrene; AcAAF, N-acetoxy-2-fluorenylacetamide; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine.
cells/100-mm Petri dish (Falcon Plastics, Oxnard, Calif.). Feeder layers were prepared by X-irradiation (4500 R) of confluent monolayers of hamster cell cultures in 5 ml of medium. After being trypsinized, feeder cells were seeded at 6 X 10⁴/60-mm dish in 2 ml of complete medium with 10% fetal bovine serum. Hamster cells, 300 cells/plate in 2 ml of medium, were seeded on the feeder layers and treatment was commenced 24 hr later. Final volume in each dish after treatment was 8 ml. The medium was removed 8 days after seeding. The cells were washed with phosphate-buffered saline, fixed with absolute methanol, and stained with Giemsa for colony counting and examination of colony morphology (stereoscopic microscope at X40). For each experimental point, 24 to 36 dishes were used. Colonies exhibiting a criss-cross pattern not seen in controls were scored as transformed. Each series of experiments was repeated a minimum of 3 times. The results were consistent; therefore only 1 complete set of data is presented.

The cloning efficiency was determined by dividing the average number of colonies per dish by the number of cells seeded per dish multiplied by 100. Transformation was calculated as the percentage of transformed colonies found in treated cultures compared to the total number of colonies scored or simply the number of transformed colonies per dish since an equal number of cells (300/dish) were seeded in all dishes. The enhancement factor is the ratio of the transformation (total colonies scored or number of transformed colonies per dish) obtained by treatment with carcinogen and caffeine divided by the corresponding results with cells treated with carcinogen only.

**Chemical Treatment.** For each experiment, fresh solutions of carcinogens were prepared in acetone. BP (10 mg/ml of acetone), from Eastman Kodak Co., Rochester, N.Y., was added to 100 ml warm medium with serum to make a stock solution, which was further diluted with complete medium to obtain the required concentration. All procedures with BP were done in a darkened room illuminated by red light. MNNG (from Aldrich Chemical Co., Milwaukee, Wis.) and AcAAF (from Drug Research and Development Branch, National Cancer Institute, Bethesda, Md.) were dissolved in acetone (1 mg/ml acetone), and immediately diluted with cold medium without serum to the desired concentration; 2-ml aliquots were added to each dish, followed by 2 ml of medium with 20% fetal bovine serum. In all cases, final concentration of acetone solvent was less than 0.02%. The final concentration per ml medium of each carcinogen was 2.5 µg for BP, 1 µg for AcAAF, and 0.25 µg for MNNG.

Caffeine (Calbiochem, La Jolla, Calif.) was dissolved in medium without serum at a concentration of 5 mg/ml and sterilized through a Millipore filter; at time of use, caffeine solution was diluted with complete medium and added to each dish in 0.4 ml of medium.

**Survival Assay.** The sensitivity of hamster cells to the various chemicals was first determined by quantitating the proportion of cells that formed colonies following 8 days of incubation. The cell number was varied to obtain approximately the same number of colonies per dish. The effect of varied concentrations of caffeine on untreated cells was assessed first. Caffeine incubation was for 2 or 7 days. In the former case, the medium with caffeine was replaced with fresh medium and incubation was continued for 5 days. The results were normalized by designating as 100% the cloning efficiency of the control cells. The influence of caffeine on survival of carcinogen-treated cells was also determined following either 2 or 7 days of incubation. Results were expressed relative to the cloning efficiency obtained with carcinogen only, which was designated as 100%.

**Enhancement Assay.** One hr after carcinogen treatment, caffeine (50 to 100 µg/ml medium) was added. After an exposure period of 48 hr, the medium was replaced with fresh medium. In 1 set of experiments, the treatment was reversed and caffeine at 50 µg/ml medium was added 1 hr prior to AcAAF; the total exposure to the combination was 48 hr. Controls that had been treated with carcinogen only were similarly treated.

In some experiments utilizing a constant concentration of AcAAF, the effect of varied concentrations of caffeine on transformation and toxicity was assessed.

To assess the temporal effects of caffeine on carcinogen-induced transformation, cells were treated with a fixed concentration of either AcAAF or MNNG; caffeine was added at different time intervals. For comparison, cells treated with carcinogen only were treated with complete medium and manipulated in the identical way as carcinogen- and caffeine-treated dishes.

**RESULTS**

Survival curves of hamster cells treated or not treated with caffeine, carcinogen or combinations of both agents were first established (Chart 1). The percentage of cells capable of multiplying on hamster feeder layers was determined by the criterion of colony formation. Results were plotted relative to the cloning efficiency obtained with untreated controls or cells treated with the carcinogens used: BP, AcAAF, and MNNG, at 2.5, 1, and 0.25 µg/ml of medium, respectively. When caffeine, at concentrations (0.25 to 1.0 mM) of 50 to 200 µg/ml of medium, was incubated with cells for either 2 or 7 days, no toxicity was observed, i.e., the cloning efficiency obtained was identical to that for untreated controls (32%). Furthermore, no gross morphological differences between caffeine-exposed cells and untreated control cells were revealed by observation with a phase contrast microscope. Ordinarily, carcinogen treatment at the concentrations used for 7 days results in a 25 to 33% reduction of cloning efficiency relative to controls. In these experiments, the cloning efficiency of untreated controls was 32% and those obtained with BP, AcAAF, and MNNG were 24, 23, and 24%, respectively.

The addition of caffeine at various concentrations to cells treated 1 hr previously with carcinogen resulted in the potentiation of the toxicity of all 3 carcinogens. When caffeine was removed after 48 hr and incubation was continued for an additional 5 days in complete medium without carcinogen, relative cloning efficiency was greater for all 3 carcinogens compared to the exposure of carcinogen and caffeine for 7 days. In increasing order of toxicity potentiation the classification appears to be BP, MNNG, and AcAAF; however, on a molar basis at the doses of potentiated toxicity the order is BP, AcAAF, and MNNG. With all 3 carcinogens and 50 µg...
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Chart 1. Cloning efficiency (survival) of secondary hamster embryo cultures seeded to form colonies. Twenty-four hr after seeding, cells were treated with caffeine, carcinogen, or combination of carcinogen followed by caffeine 1 hr later. Incubation was for a total of 2 or 7 days. When carcinogen and caffeine treatment was for 2 days, the medium was replaced with fresh medium for an additional 5 days. Results were normalized by considering as 100% the cloning efficiency of untreated controls and carcinogen only treatment. Open symbols on solid lines indicate 2-day exposures. BP (2.5 μg/ml), o, MNNG (0.25 μg/ml), o, AcAAF (1.0 μg/ml), o, Caffeine only for 7 days, X—X.

Caffeine per ml medium, cloning efficiency was approximately 20 to 30% less than that obtained with carcinogen only. Therefore, this caffeine concentration, which caused minimal reduction in cloning efficiency, was utilized in most of the combination experiments. Potentiation of cell lethality was greater from incubation of carcinogen and caffeine for 7 days than from 2-day exposures, but the order of potentiation remained the same.

When 50 or 100 μg caffeine per ml medium were added 1 hr after carcinogen and allowed to remain for 48 hr before being replaced with fresh complete medium for an additional 5 days of incubation, the number of transformed colonies per dish increased relative to the number obtained by carcinogen treatment only (Chart 2A). The average numbers of transformations per dish with BP, MNNG, or AcAAF alone were 1.19, 0.5 and 0.9, respectively. The transformations per dish with 100 μg of caffeine per ml medium was slightly greater than that obtained with 50 μg caffeine per ml medium, with BP (3.49 versus 3.27), and MNNG (2.6 versus 2.0). With AcAAF the results differed from those obtained with the BP and MNNG in that with AcAAF the number of transformations per dish was less with the higher concentration of caffeine than with the lower, 2.0 versus 3.7. The absolute number of transformations per dish using AcAAF with 100 μg caffeine per ml medium is approximately twice that of AcAAF alone but only 50% of that which was obtained with AcAAF and 50 μg caffeine per ml. If enhancement is expressed on the basis of transformed colonies (Chart 2B) relative to the total colonies thus considering the phenomenon of potentiation of toxicity by caffeine, the enhancement still parallels the results obtained on the basis of average transformations per dish. Transformation enhancement was highest with MNNG and either 50 or 100 μg caffeine per ml medium. As expected, the enhancement of AcAAF transformation with 100 μg caffeine per ml medium was reduced relative to the results obtained with 50 μg caffeine per ml medium post AcAAF, while with BP and combinations the transformation frequency was increased 4- and 5-fold with 50 and 100 μg caffeine per ml, respectively.

When the sequence of addition of AcAAF and caffeine (50 μg/ml medium) was reversed (caffeine added first), the results were similar to those expected from AcAAF only. No cell lethality could be attributed to caffeine since the cloning efficiency was 20.3% for the combination and 20.7% for AcAAF only. In terms of transformation, no significant enhancement occurred; relative to AcAAF only, the enhancement was 1.25 calculated on a Petri dish basis and 1.2 on a colony basis.

The effect of varying concentrations of caffeine added 1 hr post-AcAAF was studied in terms of toxicity, transformation, and enhancement (Charts 3 and 4). Increasing caffeine concentration resulted in a concurrent increase in toxicity as indicated by a reduction in cloning efficiency. The addition of 0.5 to 200 μg caffeine per ml of medium 1 hr after AcAAF caused a linear decrease in the cloning efficiency relative to AcAAF as illustrated by the semilog plot. There was a linear increase in average transformation per dish with increased concentration of caffeine up to 100 μg caffeine per ml of medium. The average transformation per dish was highest, 3.2, following 100 μg caffeine per ml and lowest, 1.0, following 0.5 μg caffeine per ml of medium. At a level (200 μg) at which the cloning efficiency was 20% of that obtained with AcAAF only, the average number of transformations per dish was 1.5. In this experiment, AcAAF only gave a transformation frequency per dish of 0.64. When this factor is applied to the transformation per dish obtained with the lowest concentration of caffeine utilized, 0.5 μg/ml of medium (average transformation per dish was 1.03), an enhancement of 1.6 resulted. With the use of enhancement data (transformation per dish basis), a linear relationship was obtained with a maximum of 5-fold resulting from the addition of 100 μg caffeine per ml of medium. Thus, increasing caffeine concentration produced an increased enhancement on a transformation-per-dish basis independent of toxicity. When the transformation frequencies obtained with varying concentrations of caffeine added 1 hr post-AcAAF were plotted as transformation rate (transformed colonies/total colonies % versus caffeine concentration) (Chart 4), a maximum transformation of 13% was obtained. There was no threshold for caffeine, and only a limited number of cells could be transformed. AcAAF alone caused a transformation frequency of 0.87 on the basis of transformed colony per total colonies. Although the transformation frequency obtained after 200 μg/ml of medium is less than that obtained with 100 μg caffeine, enhancement is still significant since the ratio is greater than 11. The enhancement obtained on a colony basis commencing with 5, 10, 50, and 100 μg caffeine was 3.5, 8, 9.3, and 15, respectively.

The time relationship between AcAAF and subsequent...
Chart 2. Average transformations per dish (A) and enhancement of transformation (transformed colonies/total colonies) (B) obtained with carcinogen (CARC.) followed by caffeine divided by transformation frequencies obtained by carcinogen alone. Twenty-four hr after cells were seeded for colony formation, they were treated with BP, AcAAF, or MNNG and 1 hr later with 50 or 100 µg caffeine per ml medium. After 48 hr of incubation the medium was replaced with fresh, complete medium; incubation was continued for an additional 5 days. Open bar, carcinogen alone; black and cross-hatched bars, addition of 50 or 100 µg caffeine per ml of medium, respectively.

Chart 3. The average transformation per dish and cloning efficiency (C.E.) relative to AcAAF obtained with treatment of 1 µg AcAAF per ml medium followed at 1 hr by varying concentrations of caffeine for 48 hr. Subsequently, the cells were returned to complete fresh medium for an additional 5 days.

treatment at various time intervals with a constant amount of caffeine (50 µg/ml) for 48 hr was determined. The results of 3 independent experiments calculated on the basis of transformed colonies to total colonies for double treatment compared to AcAAF treatment at each time interval are shown (Chart 5). The overall shape of all 3 curves was similar. The maximum enhancement (10.5 to 17-fold) in all experiments occurred when caffeine was added 4 hr post-AcAAF. The experiment with the greatest enhancement at 4 hr also had higher enhancement than the other experiments at 1 through 6 hr. For example, when caffeine was added 1 hr post-AcAAF, enhancement was 3-, 4-, and 7-fold. Calculation of the ratio of the enhancements obtained at 4 and 1 hr for all 3 experiments results in a ratio of approximately 2.5.

The complete data on caffeine enhancement of 1 AcAAF experiment shown in Chart 3 are given in Table 1. In these experiments in which caffeine was added 14, 24, or 48 hr post-AcAAF, corresponding dishes with carcinogen only were given medium change at the same time to compensate for any pulse in growth that might be associated with fresh medium and serum. Caffeine potentiation of toxicity increased when caffeine was added 1, 2, 3, or 4 hr postcarcinogen; subsequently, potentiation decreased until at 24 hr there was no difference in cloning efficiency between AcAAF-caffeine treated cells and AcAAF only-treated cells. At the point of greatest enhancement, the toxic effect as indicated by reduction in cloning efficiency was also the greatest; nevertheless, even in this situation the cloning efficiency was reduced by only two-thirds of that obtained with AcAAF alone. The results were still based on a significant number of colonies (Table 1). Furthermore, either before or after maximum enhancement, at the 3- and 6-hr time points, the cloning efficiency was in the same range as that obtained at 4 hr while the enhancement was 70 and 30% of the maximum. Analysis of the results on the basis of transformed colonies per dish paralleled results obtained on the basis of transformed colonies per total colonies. Furthermore, in both situations, the enhancement disappeared when caffeine was added 24 hr postcarcinogen treatment. At this point, there was no difference in cloning efficiency between AcAAF-caffeine- and AcAAF only-treated cells.

The differences resulting from adding caffeine for 48 hr at 1, 2, 3, and 4 hr post-AcAAF were not due to the difference at the end of the exposure period. The double treatment was extended for the 1st 3 situations to 51, 50, and 49 hr, respectively, to coincide with the end of the treatment that commenced at 4 hr post-AcAAF. Under these circumstances, maximum enhancement occurred only when caffeine was added 4 hr post-AcAAF.

After it had been established that maximum enhancement occurred when caffeine was added at 4 hr, experiments were done to determine minimum incubation required for maximum enhancement. Caffeine was added 4 hr post-AcAAF and the combination was removed 4, 8, 12, 24, or 48 hr later. Analysis revealed that the transformation frequency was similar to AcAAF only after 4 or 8 hr of caffeine exposure, 1.1 and 1.3%, respectively (see also Chart 5). When the caffeine incubation was extended to 12 hr or longer, the transformation frequency increased to 4.5%. After 24-hr incubation the transformation frequency was 3.7%, and after 48 hr maximum transformation was 8%. Comparison of enhancement on a per dish basis resulted in a ratio of 5:4:5.5, commencing with the 12-hr point.

The time dependency of addition of caffeine also occurred.
Chart 4. Transformation frequency (transformed colonies/total colonies) obtained with 1 μg AcAAF per ml medium followed at 1 hr by varying concentrations of caffeine (0.5 to 200 μg/ml medium) for 48 hr. At the end of treatment, the medium was replaced with fresh medium, and incubation was continued for an additional 5 days.

Chart 5. Caffeine enhancement of transformation of hamster embryo cultures exposed to 1 μg AcAAF per ml medium 24 hr subsequent to cloning and followed by a 48-hr exposure to 50 μg caffeine per ml medium added at the time intervals indicated. The number of transformed colonies from caffeine-treated cells was compared with AcAAF only-treated cells cultured for the same length of time. Results of 3 different experiments are shown.

Chart 6. Enhancement of transformation frequency of hamster embryo cultures exposed to 1 μg AcAAF per ml medium 24 hr subsequent to cloning and followed by varying concentrations of caffeine (0.5 to 200 μg/ml medium) for 48 hr. At the end of treatment, the medium was replaced with fresh medium, and incubation was continued for an additional 5 days.

with cells that had been pretreated with MNNG; enhancement was maximum when caffeine was added 4 hr post MNNG (Chart 6). Caffeine addition 6 hr post-MNNG resulted in the lowest enhancement factor, 1.7, obtained during the 72-hr course of the experiment. Subsequently, enhancement increased, and caffeine at 12 hr post-MNNG produced an enhancement in the range of that obtained with AcAAF at 14 hr; transformation rose to 5.8 at 24 and 48 hr. At 72 hr there was still a substantial enhancement of 2.6. By contrast, with AcAAF, later addition of caffeine at 24 and 48 hr reduced the enhancement to 1.

The cloning efficiency and transformation data as well as other information, including the average number of transformed colonies per dish for a smaller experiment similar to the one in Chart 6, are given to show the variability that may occur between experiments as well as confirmation of enhancement at 24 and 48 hr (Table 2). In this experiment the enhancement obtained by the addition of caffeine at 4, 24, and 48 hr was slightly greater than that shown in Chart 6. The cloning efficiency of cells that received double treatment was always less than comparable controls with MNNG only. When cells were given caffeine either 12 or 72 hr post-MNNG, the least enhancement occurred; but cloning efficiency was within the same range as when maximum enhancement occurred.

DISCUSSION

These experiments demonstrate that caffeine potentiation of transformation and lethality of Syrian hamster cells by diverse carcinogens is dependent upon the concentration of caffeine, the time of addition of caffeine, the length of exposure, and the specific carcinogen used.

The influence of caffeine on cell survival in this study confirms and extends the reports previously made for mouse L-cells, Hela cells, and some Chinese hamster cell lines (16, 19). The potentiation of carcinogen toxicity by caffeine on secondary and tertiary Syrian hamster cells was obtained with caffeine concentrations probably lower than any previously reported, although caffeine concentrations as high as 1 mM alone is non-lethal to hamster cells in terms of colony formation. Increasing the doses of caffeine or increasing the incubation period can potentiate carcinogen toxicity. A comparison of the toxicity with caffeine added 1 hr postcarcinogen with toxicity at times such as 4 hr postcarcinogen is extremely important with even the relatively low caffeine concentrations. It is possible that its presence in conjunction with carcinogens with a relatively short half-life may potentiate toxicity until survival eventually approaches only 1% of that obtained with carcinogen only. Interestingly, the addition of caffeine prior to the AcAAF did not influence toxicity or transformation as occurred with AcAAF.

At higher concentrations then used in the present experiments, caffeine when present during the S phase of the cell cycle produces chromosome breaks and kills cells as well as potentiating the killing of cells or production of chromosomal aberrations by other agents (11, 13). In the current experiments, however, which utilized lower concentrations of
Caffeine Enhancement of Hamster Cell Transformation

Table 1
Transformation of hamster fetal cells: AcAAF treatment followed by caffeine

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<th>Caffeine added post-AcAAF (hr)</th>
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<th>Enhancement/dish (transformed colonies)</th>
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a We seeded 300 cells (60-mm dish) for colony formation; 24 hr later the cells were treated with 1 µg AcAAF followed by 50 µg caffeine per ml medium at the time intervals indicated; 48 hr after addition of caffeine, the medium was replaced by fresh medium without caffeine; corresponding dishes with AcAAF only (—) were also refed.

b Ratio of frequency of transformed colonies obtained with AcAAF and caffeine to colonies obtained with AcAAF only. The latter by definition is one.

c CE%, cloning efficiency, determined by dividing the average number of colonies per plate by the number of cells seeded per plate multiplied by 100.

Caffeine, no conclusion can be made as to the dependency of the toxicity with the number of cells actually being damaged during the S phase, particularly when one compares results with AcAAF and MNNG. In the former case cell survival increases when caffeine is added to intervals greater than 4 hr post-AcAAF, while in the latter case damage again increased as indicated by a decrease in cloning efficiency with applications of caffeine at intervals as late as 1 day postcarcinogen. These results raise the question as to whether repair or expression of damage is cell cycle dependent.

The increase in absolute number of transformations following different concentrations of caffeine reached a maximum with either 50 or 100 µg caffeine per ml of medium, and cell lethality increased linearly. Concentrations of caffeine greater than 100 µg/ml medium further increased lethality and potentiated a relatively smaller increase in number of transformations than 50 µg/ml medium but still produced a significant enhancement. When transformation and enhancement were determined on a colony basis, a correction factor of 3 was required to take into consideration cell lethality. These results are interesting to compare with mutagenesis experiments that utilized caffeine in mammalian cells. Trosko and Chu (20) have reported that caffeine depressed UV mutagenesis whereas Arlett (1) reported that caffeine enhances UV mutagenesis. The 2 groups used different techniques and caffeine concentrations. Trosko and Chu used 1 mM, approximately twice the concentration of caffeine used by Arlett's group and 4 times that used in many of our experiments. These concentrations kill 30 to 90% of the cells. More recently, Roberts and Sturrock (18) have reported the enhancement by caffeine of N-methyl-N-nitrosourea-induced mutations as well as chromosomal aberrations in Chinese hamster cells. The induction of mutations was dose dependent and observed only with toxic concentrations of N-methyl-N-nitrosourea and 0.75 mM, concentration of caffeine. These results suggest that toxicity, mutations, and DNA damage are amenable to a common DNA repair mechanism. Although it is not clear how the mutagenic or antimutagenic effects of caffeine are related to the mechanism of action at the biochemical level, concentration and time of application are extremely important.

Caffeine effects are poorly understood. Recently, it has been shown to minimize irradiation-induced division delay (possibly through cyclic 3', 5'-AMP) but not to effect response associated with ultimate death (24). Caffeine also affects enzymes, binds to denatured DNA (22), and may induce DNA synthesis in smaller units (14).

Caffeine has been reported to influence viral transformation on April 13, 2017. © 1974 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from
in vitro and in UV experiments utilizing rodents. The studies with 6-acetoxy-BP and simian adenovirus 7 indicated that viral transformation enhancement depended upon the sequence of addition of virus and chemical (2). UV treatment produced 95% tumor incidence on left mouse ears and a 50% incidence on right ears that had been pretreated with 80 µg caffeine (25). This indicates the importance of concentration and the time of application of caffeine.

The detailed studies with AcAAF and MNNG demonstrate that some of the differences in the enhancement are probably due to the type of carcinogen used. AcAAF cannot be repaired by excision in deficient xeroderma cells and thus is considered as X-ray type damage (3). Recently, caffeine was shown to affect synergistically AcAAF sedimentation profiles of newly synthesized DNA of Chinese hamster cells (21). The results were interpreted as lending support to the concept that repair processes have time-dependent expression of damage, similar to early and late repair. This indicates the importance of concentration and the time of application of caffeine.

### Table 2
Transformation of hamster fetal cells: MNNG treatment followed by caffeine

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<sup>a</sup> We seeded 300 cells (60-mm dish) for colony formation; 24 hr later the cells were treated with 0.25 µg MNNG followed by 50 µg caffeine per ml medium at the time intervals indicated; 48 hr after addition of caffeine, the medium was replaced by fresh medium without caffeine; corresponding dishes with MNNG only (–) were also refed.

<sup>b</sup> Ratio of frequency of transformed colonies obtained with MNNG and caffeine to colonies obtained with MNNG only. The latter by definition is 1.

<sup>c</sup> CE%, cloning efficiency, determined by dividing the average number of colonies per plate by the number of cells seeded per plate multiplied by 100.

### REFERENCES


Caffeine Enhancement of Chemical Carcinogen-induced Transformation of Cultured Syrian Hamster Cells

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