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Mutagens in Coffee and Other Beverages

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A cup of coffee contains mutagens which produce about 5 × 10^4–10^5 revertants of *Salmonella typhimurium* TA 100 without S9 mix. One of the mutagens was identified to be methylglyoxal. Methylglyoxal was present in various beverages such as black tea, whisky, and brandy. Methylglyoxal itself induced tumors in rats when administered by subcutaneous injection. However, the mutagenic properties of coffee were different from those of methylglyoxal. The mutagenicity of coffee was suppressed by catalase, and coffee was found to contain hydrogen peroxide. Furthermore, coffee solution was found to have a hydrogen peroxide-generating system. Instant coffee (15 mg/mL) contains 130 μM hydrogen peroxide immediately after the dissolution of coffee powder in water at room temperature. The concentration of hydrogen peroxide increased with time. The mutagenicity of methylglyoxal was increased by the copresence of hydrogen peroxide. A maximum of 30-fold enhancement was observed. The mutagenicity of black tea but not that of whisky was suppressed by catalase.

Mutagenicity of Coffee

Coffee, instant and freshly brewed, is mutagenic to *S. typhimurium* TA 100, TA 104, and TA 102 and *E. coli* WP2 *uvrA*/pKM101 without metabolic activation by S9 mix (*1*). One cup of instant coffee ordinarily prepared contains mutagens which produce 5 × 10^4 revertants of *S. typhimurium* TA 100 (*2*) by the preincubation method (*3*), which is a modification of the method of Ames et al. (*4*). It also induced λ prophage lysogenized in *E. coli* (*5*). These genotoxic activities were provoked by roasting coffee beans.

The extracts of green coffee beans prepared in the same way as roasted beans showed no genotoxicity by induction of mutation in *S. typhimurium* TA 100, *E. coli* WP2 *uvrA*/pKM101 or induction of λ prophage in *E. coli* (*5*).

Instant coffee induced diphtheria toxin-resistant mutation in Chinese hamster lung cells *in vitro*. At the concentration of 4 to 6 mg/mL, instant coffee induced 30 to 75 mutants per 2.5 × 10^5 survivors (*6*). Since the concentration of instant coffee ordinarily prepared is about 15 mg/mL, the concentration of coffee which induces mutation in Chinese hamster lung cells is low enough to raise the question of whether coffee is hazardous to humans.

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Mutagenic Dicarbonyls

The mutagenicity of coffee was found to be suppressed by sulfite or bisulfite (*7*), and dicarbonyl compounds were suspected of being mutagens in coffee. Coffee reacted with *o*-phenylene diamine, and quinoxaline derivatives produced were analyzed by GC-MS using authentic compounds of quinoxaline, methylquinoxaline, ethylquinoxaline and dimethylquinoxaline (*8*). It was found that the powder of a brand of instant coffee contained 23, 100, 20, and 46 μg of glyoxal, methylglyoxal, diacetyl, and ethylglyoxal per gram, respectively. The mutagenicity of glyoxal and diacetyl were reported by Bjeldanes and Chew (*9*). Among those dicarbonyls, methylglyoxal was the strongest in mutagenicity as shown in Table 1, and 1 mg of methylglyoxal induced 100,000 revertants of *S. typhimurium* TA 100. Guanine base modified with methylglyoxal is known to produce 3,5,6,7-tetrahydro-6,7-dihydroxy-6-methylimidazo[1,2-*a*]purine-9(8H)one (*10*).

Table 1. Mutagenicity of dicarbonyls to *S. typhimurium*.[a]

Dicarbonyls	Mutagenicity, revertants/μg
Glyoxal	20
Methylglyoxal	100
Ethylglyoxal	25
Propylglyoxal	35
Diacetyl	0.4
Acetol	0

[a] *S. typhimurium* TA 100 was used without S9 mix, and a preincubation method (*3*) was employed.

Table 2. Amounts of dicarbonyls in various beverages and foods.

	Glyoxal, μg/mL	Methylglyoxal, μg/mL	Ethylglyoxal, μg/mL
Bourbon whiskey	0.39	1.5	0.42
Apple brandy	0.33	0.32	0.43
Wine	0.97	0.57	0.92
Japanese sake	0.29	0.26	0.70
Instant coffee[a]	0.34	1.6	0.70
Brewed coffee[b]	0.87	7.0	1.9
Black tea[c]	0.02	0.05	0.1
Green tea[d]	Trace	Trace	0.34
Soft drink	—	1.4	—
Bread	0.3[e]	0.79[e]	0.4[e]
Toast	0.5[e]	2.5[e]	0.7[e]
Soy sauce	4.9	8.7	8.4
Soy bean paste	4.2[e]	5.1[e]	4.2[e]

[a] Prepared by dissolving 1.5 g of coffee powder in 100 mL of water.
[b] Prepared from 10 g of ground coffee beans and 150 mL of boiling water.
[c] Prepared from 4 g of tea leaves and 100 mL boiling water.
[d] Prepared from 5 g of tea leaves and 20 mL of hot water.
[e] μg/g.

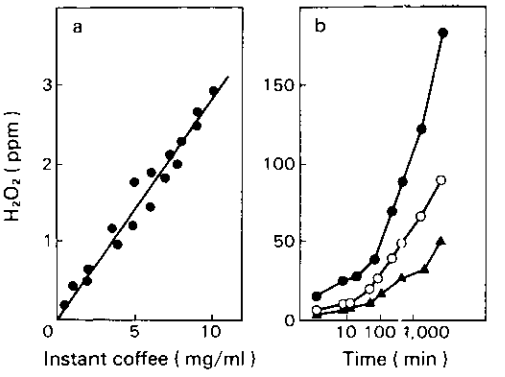

FIGURE 1. Concentration of hydrogen peroxide in instant coffee. (a) Content of hydrogen peroxide measured immediately after the dissolution of instant coffee powder. (b) Time course of the concentration of hydrogen peroxide of instant coffee solution: (▲) 15 mg/mL; (○) 30 mg/mL; (●) 100 mg/mL.

The content of glyoxal, methylglyoxal, and ethylglyoxal in various beverages and foods are summarized in Table 2. Among the various beverages, coffee contains the highest amount of methylglyoxal, and the intake of methylglyoxal by drinking 2 to 3 cups of coffee can be calculated to be as much as 1 mg/day. The content of methylglyoxal in soy sauce was comparable to that of brewed coffee, but the average intake of soy sauce per person per day is 30 mL in Japan (*11*).

Carcinogenicity of Methylglyoxal

Doses of 0.2 mL of a solution of methylglyoxal in saline at a concentration of 10 mg/mL were injected subcutaneously twice a week for 10 weeks into 8 male and 10 female Fischer 344 rats. A control group of 21 males and 19 females received only saline in the same way.

After 20 months, 3 males and 1 female of the methylglyoxal-treated rats had tumors at the injection site, but no tumors were observed in the control group. The size of the largest tumor was 35 × 35 × 20 mm. Histologically, the tumors were fibrosarcomas. A carcinogenicity experiment on methylglyoxal by oral administration is going on in our laboratory by giving 0.5% methylglyoxal in drinking water.

Characteristics of the Mutagenicity of Coffee

Mutagenicity of coffee was suppressed by the addition of cytosol fraction of rat liver (*1*). This mutagen-inactivating factor was partially purified. The fraction contained hemoproteins and showed catalase activity. Bovine liver catalase (Sigma C-100) (*12*) and horse radish peroxidase (Sigma Type X) suppressed the mutagenicity of instant coffee, when they were added into a mixture of instant coffee and bacterial tester strain.

However, the mutagenicity of methylglyoxal, one of the mutagens in coffee, was not affected by catalase or peroxidase. These results suggested that methylglyoxal itself is not a major mutagen in instant coffee and that coffee contained hydrogen peroxide, which might be involved in the enhancement of the mutagenicity of methylglyoxal.

The concentration of hydrogen peroxide in instant coffee was measured by oxygen electrode (Oritector, Oriental Electric Co.). As shown in Figure 1, the concentration of hydrogen peroxide in instant coffee solution immediately after the dissolution of coffee powder was increased with an increase of the concentration of

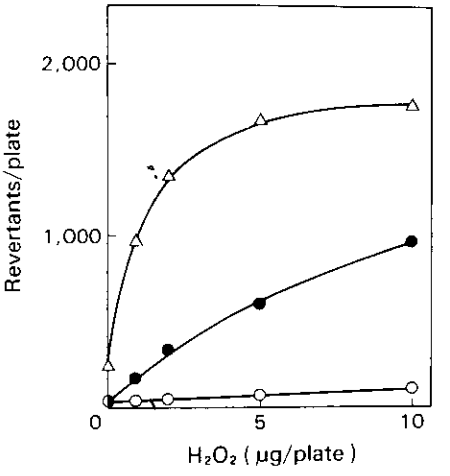

FIGURE 2. Effect of hydrogen peroxide on the mutagenicity of methylglyoxal. The mutagenicity was tested with TA 100 without S9 mix by the preincubation method. Hydrogen peroxide was added to the preincubation mixture that contained (○) 0, (●) 1.5 μg, and (△) 3.75 μg of methylglyoxal.

coffee powder. The concentration of hydrogen peroxide increased with the length of the incubation time up to 48 hr at 37°C. This meant that instant coffee had a hydrogen peroxide generating system. The presence of a hydrogen peroxide generating system was also clearly shown by the fact that when, after incubation of coffee solution with rat catalase for 30 min, the catalase was inactivated by anti-rat catalase rabbit immunoglobulin, and mutagenicity reappeared (*1*).

The effect of the temperature of the water used to dissolve instant coffee powder was also examined, since the concentration of oxygen was decreased with the increase of the temperature. The highest concentration of hydrogen peroxide was obtained at around 80°C. The temperature-dependent profile of the production of hydrogen peroxide seemed to be an integration of the concentration of oxygen in water and the reaction rate at a specific temperature.

Enhancement of the Mutagenicity of Methylglyoxal by Hydrogen Peroxide

Figure 2 shows the effect of hydrogen peroxide on the mutagenicity of methylglyoxal. A level of 1.5 μg of methylglyoxal, which corresponds to the amount in 15 mg of instant coffee powder, showed almost no mutagenicity. By adding hydrogen peroxide to the preincubation mixture, the mutagenicity was tremendously enhanced and reached 30-fold with 10 μg of hydrogen peroxide. These concentrations of hydrogen peroxide in the preincubation mixture also correspond to those of hydrogen peroxide in instant coffee. Methylglyoxal and hydrogen peroxide could account for most of the mutagenicity of coffee.

The mutagenicities of black tea, green tea, and a herb tea (Mate leaves) also were suppressed by the addition of catalase.

Coffee was clearly shown to be genotoxic *in vitro*, and its ingredient, methylglyoxal was shown to be carcinogenic by subcutaneous injection. Hydrogen peroxide, another compound present in coffee, was also suggested to be carcinogenic (*13*). However, reports of the *in vivo* carcinogenicity of coffee in animals have so far all been negative (*14,15*). To obtain the information necessary for the risk evaluation of coffee to humans, however, long-term animal experiments should be carried out by administering the purified mutagens from coffee.

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