Mutation Research, 89 (1981) 161—177
Elsevier/North-Holland Biomedical Press

NON-MUTAGENICITY OF URINE FROM COFFEE DRINKERS COMPARED WITH THAT FROM CIGARETTE SMOKERS

H.U. AESCHBACHER and C. CHAPPUIS

Experimental Biology Laboratory, Nestlé Products Technical Assistance Company Ltd., CH-1350 Orbe (Switzerland)

(Received 18 August 1980)
(Resubmitted 13 January 1981)
(Accepted 20 January 1981)

Summary

The urine of human coffee drinkers who ingested 12 g of instant coffee per day, during 4 days in a first experiment or 12 g within 2 h in a second experiment, was fractionated by XAD-2 column chromatography.

The non-polar urine fractions so obtained were not mutagenic in the Ames Salmonella tester strains TA98 or TA100 in either experiment, either with or without β-glucuronidase treatment of the urine.

The non-polar urine fraction of smokers, who smoked 20—30 cigarettes per day during 4 days in the first experiment or 7—18 cigarettes during 7 h in the second experiment, was mutagenic when metabolically activated.

Coffee has been found to have a weak effect in the Ames test (Nagao et al., 1979; Aeschbacher and Würzner, 1980). The effect was obtained only with Salmonella TA100 and was completely deactivated by the microsomal fraction. In a further study, Aeschbacher et al. (1980) found that the deactivation of coffee was not due to protein binding but rather to metabolic deactivation. The possibility of such a deactivation was also suggested by the negative results obtained with coffee in host-mediated assays (Aeschbacher and Würzner, 1980). To extend these observations, urine fractions of human coffee drinkers were subjected to the Ames test. Because percolated coffee and instant coffees gave similar results (Nagao et al., 1979; Aeschbacher and Würzner, 1980) only instant coffee was used in the present study. The use of instant coffee permitted a more convenient preparation and allowed good standardization of the test procedure.

Abbreviations: 2-AAF, 2-acetylaminofluorene; 2-AF, 2-aminofluorene; DMSO, dimethyl sulphoxide; β-G, β-glucuronidase; 2-NPD, 2-nitro-1,4-phenylenediamine; SEM, standard error of the mean.

0165-1218/81/0000—0000/$02.50

Material and methods

First experiment

The 15 human subjects were divided into 3 groups: one group of non-coffee drinkers; one group who ingested 12 g of instant coffee per day; and one group who smoked daily on average 20–30 cigarettes and also drank coffee (12 g/day). The 2 non-smoking groups consisted of 6 subjects each (3 men and 3 women/group), whereas the third positive control group contained 3 smokers. For the group of non-coffee drinkers the results are given for 5 subjects only since one subject became ill during the experiment.

All participants followed the treatment during 4 days. One day before and for the duration of the treatment, all subjects received a standard diet. Each day the subjects received a standard breakfast composed of 2 dl orange juice and 2 "croissants". They were also given each morning 2 fruits, 2.5 dl milk and mineral water ad libitum. The 12 g of instant coffee, packed in portions, were dissolved in warm mineral water and consumed during the day at regular intervals according to the subjects' normal habits (after meals, at 10 a.m. and at 3 p.m.). The meals served for lunch were prepared in the company canteen so that each subject received portions of the same amount and composition. For supper a prepacked meal was given to be eaten at home. The calories ingested were calculated to fall in the range of 2100—2700 cal/day depending on the meal of the day.

During the 4-day treatment period all the urine was collected and stored at 4°C. Each subject received daily a 2-l dark plastic flask containing 50 ml of acetic acid for urine sampling. After 24 h all the flasks were collected, the volumes divided into 2 equal parts and then immediately stored at −20°C. The volumes collected over the whole period (4 × 24 h) ranged between 4.2 and 8.4 l per subject.

Second experiment

In this experiment, 18 human subjects (12 non-smokers and 6 smokers) received 1 l of water and a week later 1 l of water containing 12 g of coffee to drink within 2 h after a standard breakfast. They were not allowed to eat or drink anything else during the 7 h following the breakfast on the day of the test. During this time, the urine was collected (on average about 1 l/subject) in dark plastic bottles containing 25 ml of acetic acid/l, stored at 4°C and then at the end of the collection period at −20°C. Hence, each subject served as his own control. The groups were made up of equal numbers of males and females. The smokers consumed 7—18 cigarettes of various brands (about the same number per subject on the 2 occasions) during the 7-h period.

For both experiments the volunteers, 24—44 years old, did not ingest anything other than the test substances or the standard meals provided. They were also urged to avoid medication.

Urine extraction

For the first experiment, half of the total volumes (4 times 0.5 volume of the 24-h collection) were thawed at 4°C, pooled and subjected to XAD-2 column chromatography. The same procedure was carried out on the remainder

of the urine that was incubated with β-glucuronidase (β-G) before extraction (see below).

Plastic columns, of 14.5 cm × 3.5 cm, were packed with 18 g purified XAD-2 resin and adjusted to give a flow speed of 3 ml urine per minute. The columns were rinsed with 100 ml water before, and with 50 ml water after, the passage of urine. The columns were then eluted with 100 ml acetone which was later evaporated to dryness at about 45°C in a rotary evaporator (Buchi, Flawil, Switzerland). The residue was taken up in 3 ml of DMSO, immediately diluted and used in the Ames test.

In the second experiment, all the urine of the non-smokers and the two halves of the urine of smokers (half treated with β-G, the other half untreated) were subjected to column chromatography as described above with the following modifications. The columns were packed with 10 g XAD-2 resin, 50 ml of acetone was used and the residue was taken up in 0.4 ml DMSO per 100 ml urine. This was done to adapt to the smaller urine volume obtained in the second assay.

For the rat study, 30 ml of urine per group was passed through a 1.5 cm × 10 cm glass column packed with 6 g XAD-2 resin, rinsed with 50 ml water before and after the passage of urine and finally eluted with 30 ml acetone. The eluate was evaporated as described above and taken up in 0.75 ml of DMSO.

Mutagenicity testing

The standard Ames test was done according to Ames et al. (1975), with the exception of the cofactor solution which was as described by Mattern and Greim (1978). The S9 mix contained 50 μl of liver supernatant (Yamasaki and Ames, 1977) prepared from livers of male Sprague–Dawley rats that had been treated with Aroclor 1254 (Ames et al., 1975). The S9 and *Salmonella typhimurium* TA100 and TA98 were stored at −80°C and regularly checked for the microsomal enzymic activity of the S9 by the aminopyrine demethylase method (Dalton and Di Salvo, 1972), or for strain specificity as described by Ames et al. (1975).

TABLE 1

EFFECT OF β-G ON 2-AAF MUTAGENICITY IN RAT URINE USING SALMONELLA TA98

Treatment	DMSO containing urine fraction (μl/plate)	−S9 mix	+S9 mix
DMSO	100	38	53
Untreated rats	25	35	57
	100	43	51
2-AAF-treated rats.	25	43	175
Urine treated without β-G	100	59	407
2-AAF-treated rats.	25	88	697
Urine treated with β-G	100	308	1872

The values represent the mean number of revertants of 4 plates.

TABLE 2

INHIBITORY EFFECT OF β-G-TREATED URINE ON MUTAGENIC INDUCTION OF 2-AF ON TA98 WITH METABOLIC ACTIVATION (+S9 MIX)

Volume of DMSO containing the urine fraction (μl/plate)	Urine volume (ml)		
	0.5	1.5	3.0
25	323	323	43
50	360	213	[a]
100	348	105	[a]
200	245	[a]	[a]

The values represent the mean numbers of revertants of 4 plates.

[a] No revertant colonies occurred because of the bactericidal effect of the DMSO containing the urine fraction.

To each plate, 0.2 μg 2-AF was added and then the volumes of DMSO containing the urine fractions, as given above, were added.

The urine samples were prepared as described above and diluted with DMSO. The amounts of the DMSO solution used are indicated in the tables. 4 petri plates were used per concentration except in rare instances where only 3 petri plates per concentration could be used owing to the restricted DMSO volume available. The inoculated petri plates were incubated for 3 days at 37°C and then counted with a Biotran II.

The positive control without metabolic activation (—S9 mix) was obtained

TABLE 3

FIRST EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA98: NON-SMOKERS, NON-COFFEE DRINKERS

Urine hydrolase	—β-G							
Microsomal activation	—S9 mix				+S9 mix			
DMSO containing the urine fraction (μl/plate)	0	25	50	100	0	25	50	100
Subject number								
3	23	26	29	21	35	35	34	43
5	18	20	29	28	34	35	43	39
6	23	28	28	23	34	34	42	43
11	18	29	24	29	34	42	38	37
12	24	24	29	24	40	35	35	37
Mean number ± SEM	21 ± 1	25 ± 2	28 ± 1	25 ± 2	35 ± 1	36 ± 1	38 ± 2	40 ± 1
2-NPD, 1 mg/plate		869 ± 75						
2-AF, 10 μg/plate						+		

Footnotes to tables 3—12

The results represent the average numbers of revertants of 4 plates per concentration and per subject.

No revertant colonies, owing to the bactericidal effect of the urine fraction in DMSO, is indicated as —.

The mean values of all subjects ± SEM are indicated at the bottom of each table.

2-NPD, used to check inducibility without S9 mix

2-AF, used to check inducibility with S9 mix with a spot test

+, strong mutagenic effect with 2-AF.

with 1 mg of 2-nitro-1,4-phenylenediamine (2-NPD)/plate. When S9 mix was used for activation, 2-aminofluorene (2-AF) was used to check inducibility. Because 2-AF is a potent mutagen, paper discs containing 10 μg 2-AF, prepared in advance, were applied per plate to avoid repeated handling of the mutagen and hence reduce the risks.

Dimethyl sulphoxide (DMSO), 2-NPD and 2-AF were obtained from Fluka A.G., Buchs (Switzerland).

β-Glucuronidase treatment

The urine was adjusted to pH 6, and then 2 ml of a solution containing 5 mg β-G/ml was added per 100 ml urine. β-G Type IX (Sigma, St. Louis, U.S.A.) with a specific activity of about 175 000 modified Fishman units per gram was used.

The β-G-treated urine was incubated for 24 h at 37°C in a shaking water-bath and then subjected to column chromatography.

For the first assay, half of each subject's urine, which ranged in volume from 2.1 to 4.2 l, was incubated with β-G, whereas the other half of the urine was not treated with the enzyme.

For the second assay, half of the smokers' urine was treated with β-G; the other half remained untreated while all the urine of non-smokers was treated with the enzyme.

As a check of β-G activity, a rat trial was carried out with 2-acetylaminofluorene (2-AAF), which had been shown to be suitable for this purpose (Durston and Ames, 1974). Urine of 6 untreated Sprague—Dawley rats (250 g),

+β-G							
—S9 mix				+S9 mix			
0	25	50	100	0	25	50	100
22	25	20	19	43	36	36	26
23	24	29	42	31	42	42	37
22	25	36	35	43	38	51	53
23	24	22	28	31	33	39	45
30	25	—	—	39	41	32	—
24 ± 3	25 ± 1	27 ± 4	31 ± 5	37 ± 3	38 ± 2	40 ± 3	40 ± 6
	715 ± 40						
					+		

Because of the clear-cut results, no statistical evaluations were necessary for the treatment of the results of Tables 3—12, except for the results of Tables 9 and 10 which were compared by using the paired *t* test. No significant difference between urine extracts of non-coffee-drinking or coffee-drinking smokers (—β-G) was observed. No statistical analysis could be done on +β-G-treated urine because only 4 subjects yielded enough urine for this treatment (Table 10). The results with treated urine (+β-G), however, were not different from those of untreated urine.

Urine fractions from smokers (±coffee), on the other hand, provoked a significant (analysis of variance) dose-dependent increase in the number of revertants.

TABLE 4

FIRST EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA98: NON-SMOKERS, COFFEE DRINKERS

Urine hydrolase	—β-G							
Microsomal activation	—S9 mix				+S9 mix			
DMSO containing the urine fraction (μl/plate)	0	25	50	100	0	25	50	100
Subject number								
1	23	26	24	27	35	40	36	43
7	24	26	27	28	40	42	39	40
8	24	28	28	23	40	30	40	40
9	18	24	26	25	34	39	34	42
10	24	23	30	22	40	35	36	42
14	23	25	28	26	36	38	39	39
Mean number ± SEM	23 ± 1	25 ± 1	27 ± 1	25 ± 1	37 ± 1	37 ± 2	37 ± 1	41 ± 1
2-NPD, 1 mg/plate		908 ± 50						
2-AF, 10 μg/plate						+		

housed individually in metabolic cages, was collected for 24 h into dark bottles containing 0.3 ml acetic acid. Urine of the same rats was again collected for 24 h but this time after i.p. injection of 10 mg of 2-AAF/kg. For each collection period, urine of all rats was pooled, and 30-ml portions were prepared. 30 ml urine of untreated rats and 30 ml of 2-AAF-treated rats were used without β-G, whereas 30 ml of 2-AAF-treated rats were incubated with 10 mg β-G/100 ml urine as described above. The urine was then subjected to column chromatography as above. The results given in Table 1 confirm the correct functioning of the methods used.

TABLE 5

FIRST EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA98: SMOKERS, COFFEE DRINKERS

Urine hydrolase	—β-G							
Microsomal activation	—S9 mix				+S9 mix			
DMSO containing the urine fraction (μl/plate)	0	25	50	100	0	25	50	100
Subject number								
13	18	26	26	24	41	38	83	140
15	27	22	25	23	35	74	140	257
16	24	27	28	26	40	53	118	221
Mean number ± SEM	23 ± 3	25 ± 2	26 ± 1	24 ± 1	39 ± 2	55 ± 10	114 ± 16	206 ± 35
2-NPD, 1 mg/plate		941 ± 43						
2-AF, 10 μg/plate						+		

+β-G							
−S9 mix				+S9 mix			
0	25	50	100	0	25	50	100
22	26	28	29	43	39	41	38
30	8	—	—	39	39	26	—
30	21	—	—	39	18	36	21
23	27	29	29	31	39	34	—
23	—	—	—	31	34	27	—
22	27	—	—	43	32	—	—
25 ± 2	22 ± 4	29 ± 1	29 ± 0	38 ± 2	34 ± 3	33 ± 3	30 ± 8
	649 ± 39						
					+		

Effect of β-glucuronidase-treated urine on Salmonella typhimurium

Throughout the experiments the β-G-treated urine exerted an inhibitory effect on revertant colonies which resulted in some cases in complete bactericidity. The reducing effect of β-G-treated urine fractions on induced colonies of revertants (smokers' urine) was mainly observed with Salmonella TA98 (Table 5) whereas a bactericidal effect due to the β-G treatment was more often observed with TA100 (Tables 6—8).

Therefore, further investigations were made using TA98 on the inhibiting capacity of the β-G-treated urine on the mutagen 2-AF 6 l of urine were col-

+β-G							
−S9 mix				+S9 mix			
0	25	50	100	0	25	50	100
23	24	26	36	31	39	65	62
22	25	17	12	43	69	90	90
30	—	—	—	39	—	—	—
25 ± 3	25 ± 1	22 ± 5	24 ± 12	38 ± 4	54 ± 15	78 ± 13	76 ± 14
	659 ± 70						
					+		

TABLE 6

FIRST EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA100: NON-SMOKERS, NON-COFFÉE DRINKERS

Urine hydrolase	−β-G							
Microsomal activation	−S9 mix				+S9 mix			
DMSO containing the urine fraction (μl/plate)	0	25	50	100	0	25	50	100
Subject number								
3	121	133	134	132	141	147	128	133
5	138	133	127	119	153	139	151	152
6	121	135	135	127	141	142	137	148
11	138	125	116	126	153	129	136	126
12	130	127	121	117	118	117	108	118
Mean number ± SEM	130 ± 4	131 ± 2	127 ± 4	124 ± 3	141 ± 6	135 ± 5	132 ± 7	135 ± 6
2-NPD, 1 mg/plate		755 ± 7						
2-AF, 10 μg/plate						+		

lected from 6 non-smokers as described above, pooled and adjusted to pH 6 with NaOH. The urine was then divided into volumes of 0.5, 1.5 and 3 l, treated with 2 ml of a β-G solution (5 mg/ml) per 100 ml urine and incubated for 24 h at 37°C. The urine was then extracted and dried as described above, and resuspended in 3 ml DMSO. The DMSO solutions so obtained were added at

TABLE 7

FIRST EXPERIMENT: EFFECTS OF URINE FRACTIONS ON SALMONELLA TA100: NON-SMOKERS, COFFEE DRINKERS

Urine hydrolase	−β-G							
Microsomal acitvation	−S9 mix				+S9 mix			
DMSO containing the urine fraction (μl/plate)	0	25	50	100	0	25	50	100
Subject number								
1	121	131	122	124	141	141	150	—
7	130	119	113	120	118	126	112	120
8	130	131	116	115	118	119	118	116
9	128	124	128	125	118	130	125	142
10	128	126	116	121	153	119	110	130
14	121	138	133	118	141	133	156	140
Mean number ± SEM	126 ± 2	128 ± 3	121 ± 3	121 ± 2	133 ± 6	128 ± 3	129 ± 8	130 ± 5
2-NPD, 1 mg/plate		756 ± 7						
2-AF, 10 μg/plate						+		

+β-G							
—S9 mix				+S9 mix			
0	25	50	100	0	25	50	100
129	79	—	—	124	121	113	—
117	118	131	128	120	156	142	162
129	128	129	101	124	139	166	180
117	114	113	110	120	140	135	141
147	—	—	—	145	139	—	—
128 ± 5	110 ± 11	124 ± 6	113 ± 8	127 ± 5	139 ± 6	139 ± 11	161 ± 1
	764 ± 8						
					+		

volumes of between 25 and 200 μl/plate (Table 2) to petri plates which all contained 0.2 μg 2-AF/plate.

Table 2 shows that the β-G treatment of increasing urine volumes caused a volume-dependent decrease in the number of 2-AF-induced revertants which finally resulted in complete bactericidity. These observations led to the conclusion that β-G treatment yields urine constituents that are bactericidal.

+β-G							
—S9 mix				+S9 mix			
0	25	50	100	0	25	50	100
129	90	—	—	124	127	107	—
147	29	—	—	145	82	—	—
147	51	—	—	145	67	—	—
129	113	93	—	124	138	127	—
137	—	—	—	120	—	—	—
129	—	—	—	124	—	—	—
136 ± 4	71 ± 19	93 ± 0	—	130 ± 5	104 ± 17	117 ± 10	—
	773 ± 7						
					+		

TABLE 8

FIRST EXPERIMENT: EFFECTS OF URINE FRACTIONS ON SALMONELLA TA100: SMOKERS, COFFEE DRINKERS

Urine hydrolase	−β-G							
Microsomal activation	−S9 mix				+S9 mix			
DMSO containing the urine fraction (μl/plate)	0	25	50	100	0	25	50	100
Subject number								
13	133	124	148	132	153	156	176	195
15	121	137	123	131	141	193	225	392
16	130	131	138	156	118	146	217	362
Mean number ± SEM	130 ± 5	131 ± 4	136 ± 7	140 ± 8	137 ± 11	165 ± 14	206 ± 15	316 ± 61
2-NPD, 1 mg/plate			759 ± 11					
2-AF, 10 μg/plate							+	

Results

First experiment

With Salmonella strains TA98 and TA100 (±S9 mix) the number of revertants observed with the non-polar urine fraction of non-coffee drinkers and coffee drinkers was in the range of the spontaneous revertants (Tables 3, 4 and 6, 7). Urine fractions of smokers, on the other hand, induced a dose-dependent increase in the number of revertants with strain TA98 and also in TA100 but only in the presence of S9 mix (Tables 5 and 8). This effect was partially inhibited by extracts of urine treated with β-G. Such an inhibitory effect on mutagenic activity with extracts of large volumes of β-G-treated urine was confirmed with the mutagen 2-AF in strain TA98 as described under Methods. (See also Table 2.)

The highest concentration of β-G-treated urine fractions also caused a bactericidal effect (Tables 3—8). This effect was more pronounced in TA100 than in TA98 and occurred more frequently in urine fractions of coffee drinkers than of non-coffee drinkers (Tables 4 and 7 or 3 and 6 respectively).

Second experiment

When the same subjects drank water, or one week later coffee, their urine extracts did not increase the number of revertants with or without S9 mix in Salmonella TA100 (Tables 11 and 12 respectively). In this experiment, all the urine of non-smokers (Tables 11 and 12) was treated with β-G because this treatment yielded toxic (bactericidal) compounds in the first assay, and hence might possibly form mutagenic compounds.

All urine was treated to obtain high enough concentrations of the fractions because the volume available was limited. In the smokers' urine, a weak but significant increase in revertants was observed in TA98 in the presence of

+β-G							
−S9 mix				+S9 mix			
0	25	50	100	0	25	50	100
117	134	119	131	120	151	150	156
129	—	—	—	124	105	—	—
147	—	—	—	145	—	—	—
131 ± 9	134 ± 0	119 ± 0	131 ± 0	130 ± 8	128 ± 23	150 ± 0	156 ± 0
	768 ± 13						

S9 mix (± β-G) (Tables 9 and 10). The paired *t* test showed that the effect was not significantly different whether the smokers drank coffee or water (see legend to Tables 9 and 10). Here too, as with the non-smokers, a choice had to be made to obtain a strong enough concentration of the urine fraction. Again, based on the observations in the first experiment, Salmonella TA98 with S9 mix was chosen. This allowed the verification of the inhibitory effect of β-G treatment of urine.

The strong inhibitory effect of β-G-treated urine on revertant colonies observed in the first experiment was less pronounced in the second experiment. This was attributed to the lower urine volume extracted in the second experiment which was also confirmed with the 2-AF experiment mentioned under Methods (Table 2).

Discussion

Urine of human coffee drinkers who had ingested 48 g (or an equivalent of about 24 cups) of instant coffee was collected during 4 days. The urine volume was divided into halves, one of which was treated with β-G, the other half remaining untreated. β-G treatment liberates possibly mutagenic compounds from urine conjugates that otherwise are not detected by the Salmonella test system (Yamasaki and Ames, 1977). The 2 halves of the urine were fractionated by XAD-2 column chromatography and subjected to the Ames tester strains TA98 and TA100.

For comparison with previous studies, the actual equivalent of coffee present in the highest concentration of the urine fraction applied per plate was calculated. In the first experiment the highest concentration used (0.1 ml/plate) represented an equivalent of 0.8 g coffee/plate. Treatment with or without β-G gave no mutagenic effect in either strain with urine of non-coffee or coffee

TABLE 9

SECOND EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA98: NON-COFFEE DRINKERS, SMOKERS

Urine hydrolase	−β-G						+β-G					
Microsomal activation	+S9 mix						+S9 mix					
DMSO containing the urine fraction (μl/plate)	0	12	25	50	100	200	0	12	25	50	100	200
Subject number												
15	43	47	40	53	58	75	43	46	51	71	82	101
16	34	37	46	47	73	31	—	—	—	—	—	—
17	33	33	36	39	44	49	33	35	28	24	27	40
20	40	35	35	41	45	50	40	40	42	41	51	55
21	41	40	39	43	50	45	41	40	44	37	40	112
24	29	29	29	41	51	73	29	27	31	41	38	38
Mean ± SEM	37 ± 2	37 ± 3	37 ± 2	44 ± 2	54 ± 4	54 ± 7	37 ± 3	38 ± 3	39 ± 4	43 ± 8	48 ± 9	69 ± 16
2-AF, 10 μg/plate			+							+		

TABLE 10

SECOND EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA98: COFFEE DRINKERS, SMOKERS

Urine hydrolase	−β-G						+β-G					
Microsomal activation	+S9 mix						+S9 mix					
DMSO containing the urine fraction (μl/plate)	0	12	25	50	100	200	0	12	25	50	100	200
Subject number												
15	43	42	41	48	60	88	43	44	42	53	60	43
16	34	43	42	45	66	83	—	—	—	—	—	—
17	33	30	29	27	39	31	—	—	—	—	—	—
20	40	33	34	37	42	47	40	34	37	34	—	—
21	41	44	41	44	50	59	41	49	39	48	69	87
24	29	26	39	37	50	97	—	—	—	—	—	—
Mean ± SEM	37 ± 3	36 ± 3	38 ± 2	40 ± 3	51 ± 4	68 ± 11	41 ± 1	42 ± 4	39 ±	45 ± 6	64 ± 5	65 ± 22
2-AF, 10 μg/plate				+						+		

TABLE 11

SECOND EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA100: COFFEE DRINKERS, NON-SMOKERS

Urine hydrolase	+β-G						+β-G					
Microsomal activation	—S9 mix						+S9 mix					
DMSO containing the urine fraction (μl/plate)	0	12	25	50	100	200	0	12	25	50	100	200
Subject number												
1	104	95	108	98	109	116	112	109	109	88	103	99
5	92	109	92	—	—	—	124	111	106	109	104	—
6	104	119	116	121	107	104	112	117	99	114	110	111
7	92	119	107	117	115	109	124	114	122	112	123	113
8	137	119	116	110	112	84	129	148	135	121	109	111
9	124	137	119	136	126	124	126	139	138	130	135	123
10	137	—	—	—	—	—	129	136	136	134	133	—
18	131	128	150	143	138	127	126	122	128	134	142	125
19	124	118	125	110	121	107	126	135	129	137	125	128
22	126	119	129	130	146	126	129	131	127	137	146	133
23	126	125	117	128	124	133	129	137	126	143	127	135
25	131	119	122	123	99	108	126	129	129	122	117	117
Mean ± SEM	119 ± 5	119 ± 3	118 ± 4	122 ± 4	120 ± 4	114 ± 5	124 ± 2	127 ± 4	124 ± 4	123 ± 5	123 ± 4	120 ± 4
2-NDP, 1 mg/plate			775 ± 86									
2-AF, 10 μg/plate									+			

TABLE 12

SECOND EXPERIMENT: EFFECT OF URINE FRACTIONS ON SALMONELLA TA100: NON-COFFEE DRINKERS, NON-SMOKERS

Urine hydrolase	+β-G						+β-G					
Microsomal activation	—S9 mix						+S9 mix					
DMSO containing the urine fraction (μl/plate)	0	12	25	50	100	200	0	12	25	50	100	200
Subject number												
1	104	123	107	128	126	117	112	107	115	111	104	95
5	92	99	105	95	100	107	124	113	113	99	108	103
6	104	105	107	104	84	80	112	115	104	110	98	96
7	92	103	101	100	97	92	124	116	111	106	112	100
8	137	118	109	—	—	—	129	132	124	123	107	—
9	124	114	117	122	119	103	126	114	128	123	133	118
10	137	—	—	—	—	—	129	129	127	121	116	—
18	131	137	144	141	119	55	126	128	132	130	131	132
19	124	130	114	116	126	129	126	140	121	122	135	131
22	126	131	125	115	123	129	129	130	133	119	113	118
23	126	117	115	111	114	139	129	127	120	117	114	122
25	131	45	—	—	—	—	126	135	125	110	55	—
Mean ± SEM	119 ± 5	111 ± 8	114 ± 4	115 ± 5	112 ± 5	105 ± 9	124 ± 2	124 ± 3	122 ± 3	116 ± 3	111 ± 6	113 ± 5
2-NDP, 1 mg/plate			713 ± 74									
2-AF, 10 μg/plate										+		

drinkers. The absence of a mutagenic effect of these urine fractions, especially in Salmonella TA100, confirms the detoxifying effect of the mammalian metabolizing system (Nagao et al., 1979; Aeschbacher and Würzner, 1980; Aeschbacher et al., 1980).

The positive results with urine fractions of smokers which corresponded to a consumption of 40—60 cigarettes (half of the total smoked), when tested in the presence of a metabolic activation system in Salmonella TA98, agree well with the results of Yamasaki and Ames (1977).

Because the urine of human subjects was collected for 4 days under normal physiological conditions, a relatively high urine volume had to be extracted and then maximally concentrated to obtain a high enough concentration of possibly mutagenic compounds. With the high volume extracted, β-G treatment hydrolysed urine conjugates into compounds that were bactericidal or inhibited the mutagenic effect of cigarette smokers' urine fractions. It must be remembered that coffee consumption is strongly associated with smoking, and that none of the smokers agreed to stop drinking coffee for the 4-day test period. A possible interaction between coffee drinking and smoking therefore cannot be totally excluded. To investigate these problems, a second assay was undertaken wherein the subjects ingested 1 l of water or coffee within 2 h, with urine collection for the following 7 h. Each subject received water and 1 week later received the coffee treatment; hence he served as his own control. The β-G-treated urine fractions of non-smoking coffee drinkers who ingested 12 g of coffee, 6 cups, which represents an equivalent of about 0.6 g coffee/plate at the highest concentration (200 μl/plate), were not mutagenic in Salmonella TA100. The fractions that were suspected of containing toxic substances due to the hydrolysing activity of the enzyme (β-G) were only tested with strain TA100, the only strain that had previously responded with coffee (Nagao et al., 1979; Aeschbacher and Würzner, 1980).

For the smokers smoking 7—18 cigarettes per 7 h a small but significant increase of revertants occurred in strain TA98 with S9 mix, but no significant difference was observed between the urine samples collected during consumption of water or coffee. A synergistic effect of coffee can therefore be excluded.

In contrast with the first experiment, the mutagenic activity of smokers' urine was less depressed by β-G-treated urine fractions. This is due to the smaller volumes of urine used, which is confirmed by the volume-dependent inhibition of 2-AF mutagenicity.

Although the extraction method used in the present study might lead to a loss of some excreted coffee constituents through elution with water (e.g. methylxanthines, which are not mutagenic in the Ames test, McCann and Ames, 1976), the non-polar compounds that are suspected of containing mutagens (Yamasaki and Ames, 1977) would be retained and would be subjected to the mutagenicity testing. The XAD-2 method had been established to overcome the presence of histidine in urine that would interfere with the Ames test.

The present investigations led to the conclusion that urine fractions of heavy coffee drinkers were not mutagenic, whereas those of heavy cigarette smokers showed mutagenic activity.

Acknowledgements

We thank Drs. R. Aeschbach, R. Stalder and the Mathematics Section for their help and advice, Ms. R. Acheson for editorial help, and Ms. L. Müller and E. Ruch for technical assistance.

We are also greatly indebted to the volunteers who participated in this trial.

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