URINARY EXCRETION OF MUTAGENS IN PASSIVE SMOKERS*

(Mutagenicity: urine; cotinine; passive smoking)

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SUMMARY

Six healthy young volunteers with no history of active smoking were asked to keep on their Western diets avoiding the consumption of alcoholic beverages, excess coffee, any sort of medicament, and the known pro- and/or anti-mutagen-containing foods and drinks, 24 h before and during the experiments. They were exposed passively to cigarette smoke produced by 4 habitual smokers in an unventilated 48.6 m² room for 8 h. The carbon monoxide concentration was 18.85 ± 7.3 ppm during the 8-h exposure.

Frameshift mutagens were isolated from 10-h urine samples using chloroform and were tested for mutagenicity in the Salmonella/mammalian microsome assay employing Salmonella (typhi)urrum TA98. Although clearly enhanced, no significant mutagenic activity could be found with 25 ml equivalent urine/plate after passive exposure to cigarette smoke. The weak mutagenicities found were highly significant when 50 ml equivalent urine/plate was tested. No direct correlation was observed between urine mutagenicity and the urinary cotinine concentration. The results obtained are discussed with reference to inconsistent reports in the literature concerning the mutagenicity of urine after passive smoking.

INTRODUCTION

Inconsistent evidence exists in the literature concerning the recovery of mutagens excreted in urine after passive smoking. Bos et al. [1] reported, for the first time, that 12-h urine of nonsmokers staying in a smoky room for 6 h was mutagenic. In contrast, the 1985 report of Sorsa et al. [2] indicated no significant (although suggestive) difference between urine mutagenicity before and after passive smoking (their subjects were habitual smokers after a 48–72 h smoking cessation). The very recent paper of Scherer et al. [3] indicates non-mutagenicity of the urine of


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nonsmokers who were kept on a diet low in polycyclic aromatic hydrocarbons but smoked passively for 8 h.

To clarify the reason(s) for the presence of these discrepancies in reports, we performed an experiment on human subjects who smoked passively while being on a semi-controlled diet.

**MATERIALS AND METHODS**

**Volunteers**

6 healthy volunteers, 1 male and 5 female, with no smoking experience (mean age: 24.7 ± 1.5 years; mean body weight: 56.5 ± 5.2 kg) were asked to keep to normal Western diets excluding the consumption of alcoholic beverages, any sort of medication (except contraceptives), and the known pro- and/or anti-mutagens such as grilled or fried meats [4,5], ascorbic acid, tocopherol or retinol [6-8] or the beverages that contain excess amounts of these vitamins, 24 h before and during the experiments. Volunteers were also requested to avoid staying in smoky places where smokers smoke from 24 h before the experiments began. None had an industrial occupation, neither were they actively exposed to industrial chemicals.

**Smoke exposure**

4 habitual smokers began to smoke in a room of 48.6 m³ about half an hour before the nonsmokers were admitted into that experimental room (CO was about 15 ppm at the time of admission). All the volunteers sat around a table (one smoker between two nonsmokers) for an 8-h exposure during which 88 cigarettes of the same type were smoked by smokers. The cigarettes were a blond commercial filtered type of regular size (tar yield 13 mg; nicotine yield: 0.9 mg). No ventilator was installed in the room and the door and windows were kept closed during the experiment. The volunteers were allowed to leave the room repeatedly to collect their urine. Leaving the room for a rest was not allowed. They had the same meal and soft drinks during the experiment.

**Carbon monoxide determination**

CO was determined electrochemically using Ecolyzer 2400 (Energetics Science Inc., Elmsford, NY).

**Collection of urine samples**

10-h samples of the control day were collected on the weekend avoiding the first morning urine. On the day of exposure, any amount of urine that could be collected before entering the experimental room was discarded. The volunteers collected their urine during the 8-h exposure and the following 2 h when they just walked in a park to get some fresh air. The total 10-h samples were kept frozen at −20°C prior to extraction.
**Cotinine determination**

The measurement of cotinine in urine was done according to the method of Stehlik et al. [9] using capillary gas chromatography at the following conditions:

- **Column**: quartz capillary WG 11, 30m, Φ 0.32 mm, film thickness 0.2 µm.
- **Carrier gas**: nitrogen 0.5 bar
- **Split ratio**: 1:2.3
- **Detector**: nitrogen-sensitive
- **Gas flows**: hydrogen 1 ml/min, nitrogen 30 ml/min, air 10 ml/min, septum purge 1 ml/min
- **Temperature**: injector 280°C, detector 280°C, oven program: 3 min 80°C; 80-240°C at 10°C/min, held 6 min; 240-250°C at 10°C/min, held 10 min
- **Injection volume**: 1 µl
- **Time/injection**: 40 min

**Mutagenicity assays**

Urine samples were extracted with chloroform using the method described previously [10]. The extracts were tested for mutagenicity in the *Salmonella*/mammalian microsome assay using *S. typhimurium* TA98 as described elsewhere [11]. Liver homogenates were prepared after induction of liver enzymes by a single i.p. injection of Aroclor 1254 (in Mazola oil) into each 180-200 g male Sprague-Dawley Bio/l rat [11]. The assays were performed in triplicate experiments per dose.

**RESULTS**

The CO concentration at the beginning of the experiments when the nonsmokers entered the experimental room was about 15 ppm. Later on, the CO concentration throughout the 8-h exposure to cigarette smoke was \(18.85 \pm 7.3\) ppm. Non-mutagenicities of the urine samples collected before passive smoking are documented in Table I. A fairly clear dose-response relationship was detected when the urine samples collected after passive exposure to cigarette smoke were tested for mutagenicity (Table I). In this connection, although 25 ml equivalent urine per plate clearly enhanced the number of revertants, using the standard of Ames et al. [11] for interpretation of the mutagenicity data, no doubling of the revertants was observed. Doubling this latter amount of urine/plate resulted in more than 100% increase in the mutation frequency of the tester strain (Table I).

Exceptionally, testing the urine of volunteer E did not result in detection of a significant mutagenic activity, although cotinine was found in her urine in a high quantity (Table I).

Comparing the urine concentrations of cotinine before and after passive smoking, the urine samples collected after exposure contained 1.1-5.4-fold more cotinine than
Table 1

MUTAGENIC ACTIVITIES AND COTININE CONCENTRATIONS OF THE URINE SAMPLES OF 6 HEALTHY VOLUNTEERS BEFORE AND AFTER PASSIVE EXPOSURE TO CIGARETTE SMOKE

The samples were analyzed for cotinine by a gas chromatographic method, and were extracted with chloroform to be tested for mutagenicity. The extracts were tested by the *Salmonella*/mammalian microsome assay employing *Salmonella typhimurium* TA98 in the presence of NADPH-generating system and Aroclor-induced rat liver homogenates, in triplicate experiments per dose.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Cotinine</th>
<th>Mutagenicity (mean revertants/plate)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before exposure µg per ml urine</td>
<td>After exposure µg per ml urine</td>
</tr>
<tr>
<td>A</td>
<td>0.018</td>
<td>0.020</td>
</tr>
<tr>
<td>B</td>
<td>0.007</td>
<td>0.017</td>
</tr>
<tr>
<td>C</td>
<td>0.015</td>
<td>0.037</td>
</tr>
<tr>
<td>D</td>
<td>0.007</td>
<td>0.020</td>
</tr>
<tr>
<td>E</td>
<td>0.006</td>
<td>0.027</td>
</tr>
<tr>
<td>F</td>
<td>0.010</td>
<td>0.027</td>
</tr>
</tbody>
</table>

\(^a\)100 µl extract represents 25 ml of urine.

\(^b\)Spontaneous mutation frequency: 34.0 ± 2.3 revertants/plate.
the samples collected before (Table I). An exception was the urine of subject A, which contained more cotinine before than after exposure. In contrast, only the urine collected after exposure was mutagenic.

**DISCUSSION**

Controversies are seen among the results of various authors [1-3], which do not permit a conclusion as to whether urine of passive smokers is mutagenic. Our results indicate that the inconsistency of the reports is possible due to some methodical difficulties of extraction and testing procedures and/or the experimental designs. Comparing the results of our mutagenicity assays with those of the cotinine measurements, no clear correlation can be found between urine mutagenicity and the enhancement of cotinine in urine after passive exposure to cigarette smoke.

Our data, in agreement with the findings of Bos et al. [1], indicate that detectable mutagens are excreted in urine after passive smoking. The mutagenicities found with the urine of passive smokers (Table I; last column) are comparable with those of nonsmokers after active smoking of 4 or 5 cigarettes [10]. Thus the previous negative reports of other authors [2,3] might hint at minute shortcomings of the experimental designs such as testing low volumes of urine [3] or admitting smoke-experienced volunteers to play the unexperienced nonsmokers’ roles during a short smoking cessation [2]. Urinary excretion of mutagens depends on a variety of factors, the diet being one of them [5,12]. This latter includes both pro- and anti-mutagens [4-8,12-14]. A simple difficulty might arise in the recovery of urinary mutagens when the test person’s consumption of dietary anti-mutagens is disregarded. Additionally, endogenous formation or detoxification of carcinogenic/mutagenic compounds should not be disregarded as the contributing factors.

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**REFERENCES**


