Lack of Mutagens in Urines of Operating Room Personnel

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Mutagenic activity of urines obtained from operating room personnel was assayed in the Ames Salmonella/mammalian microsome system using three strains of histidine-dependent S. typhimurium, TA1535, TA1538, and TA100. Two procedures were employed. In the first, 100- and 200-μl aliquots of urine obtained from 28 subjects working in either scavenged or unscavenged operating rooms were tested. In the second, urine samples obtained from 13 physicians before and after starting an anesthesia residency, as well as 250-fold concentrates of these samples, were assayed. There was no statistically significant difference in urinary mutagenic activities between individuals working in scavenged and those working in unscavenged operating rooms. Furthermore, urines of anesthesiologists collected before and after beginning training had similar mutagenic activities. Only heavy smokers had mutagenic urine. It was concluded that the majority of operating room workers do not excrete mutagens in the urine. (Key words: Anesthesiologists. Anesthetics, gases: trace concentrations. Anesthetics, volatile: trace concentrations. Bacteria: mutagenicity. Biotransformation: liver homogenate. Toxicity: mutagenicity; carcinogenicity.)

Certain hazards, such as increased risk of malignancy and adverse reproductive effects, are possibly associated with working in the operating room suite.1,2 If so, the repeated exposure to waste inhalational anesthetics and their subsequent in-vivo metabolism to mutagens/carcinogens may be responsible for some of these effects. Metabolites excreted in the urine can be assayed for mutagenicity in an adaptation of the Ames Salmonella/mammalian microsome test.3 In such a study, McCoy et al.4 reported that urines of a small test group of anesthesiologists were mutagenic. Because the Ames system is approximately 90 per cent accurate in detecting carcinogens as mutagens,5 McCoy’s results implied that anesthesiologists were being exposed to chemicals capable of producing genetic damage and possibly cancer. Furthermore, because only uncentracted samples of urine were assayed, their findings indicated that the excreted mutagens/carcinogens were very potent. In the present study, we have attempted to confirm the findings of McCoy and colleagues by examining the urines of anesthesiologists and other operating room personnel working in either scavenged or unscavenged operating rooms. In addition, we have examined concentrated as well as uncentracted urines of physicians both before and after beginning training as anesthesiologists.

Materials and Methods

Procedure 1

A single afternoon urine sample was obtained from 28 people working in the operating rooms of three hospitals. Urine samples of nine individuals who had never worked in an operating room were used as a control. The test individuals were separated into two groups according to whether they worked permanently in scavenged or unscavenged operating rooms. Each test group comprised four anesthesiologists and ten operating room nurses. Test individuals had been working in the operating suite continually for at least three months before their urines were collected. On the average, individuals spent 30 hours per week in an operating room. The only inhalational anesthetics used in the three hospitals were nitrous oxide, halothane, and enflurane.

Urine samples were filtered through Whatman #1 filter papers and stored at −20 C. In the urine mutagenicity assay, Salmonella typhimurium strains TA1535 and TA100 were exposed to 100- and 200-μl aliquots of thawed urine with and without an Aroclor 1254-pretreated rat metabolic activation system (S-9) as previously described.6 After 48 hours of incubation at 37 C, revertant colonies were counted. 2-amino anthramine (20 μg/plate) was used as a positive control. Triplicate plates were prepared at each urine concentration.

Procedure 2

Thirteen male physicians who were about to begin an anesthesia residency were given a simple questionnaire concerning smoking habits, use of over-the-counter or prescription medications, and chemical exposure, either at work or at home. Two or three urine specimens were then taken from each resident on consecutive afternoons before assignment to

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operating room duties. Urine specimens were again obtained from each resident approximately 11 and 15 months later. During the period of study, residents worked for as long as two months per year outside the operating room. Urine samples were collected, however, only after residents had been back in the operating suite for at least three weeks. Nitrous oxide levels were measured on numerous occasions in 11 operating rooms in which residents worked. Time-weighted concentrations in breathing zones near the anesthesiologists averaged 50 ± 5 ppm when endotracheal techniques were employed.\textsuperscript{7} Concentrations were higher when mask techniques were used. Concentrations of halothane and enflurane, the only volatile anesthetics used in the 11 operating rooms, were about one-sixtieth and one-thirtieth of the nitrous oxide levels, respectively. For positive controls, single urine samples were obtained from five heavy smokers who did not work in the operating room.

After collection, urine was immediately filtered and stored at −20°C. Urine samples were subsequently thawed and assayed directly as described above, as well as in a concentrated state. The concentration method was that of Ames and Yamasaki.\textsuperscript{9} Briefly, plastic Bio-Rad\textsuperscript{®} columns (0.7 cm ID × 4 cm) were packed with washed XAD-2 resin. A 100-ml urine sample was loaded onto each column and effluent flow of 2 ml/min was regulated by means of a stopcock. The components adsorbed onto the column were then eluted into test tubes with 10 ml methyl alcohol. The eluted fraction was evaporated to dryness under a nitrogen atmosphere using a heating block at 60°C. The dried eluted fraction was redissolved in 0.4 ml dimethyl sulfoxide to yield a 250-fold urine concentrate. To test for mutagenicity, Salmonella typhimurium strains TA1538 or TA100 were exposed to 25, 50, and 100 μl of urine concentrate on plates with S-9 and β-glucuronidase.\textsuperscript{9} At the end of 40 hours of incubation at 37°C, revertant colonies were counted. Student $t$ tests for unpaired and paired data were used for statistical comparisons; $P < 0.05$ was considered significant.

### Results

In the 28 operating room workers, there was no significant increase in mean reversion rates of S. typhimurium strains TA1535 or TA100, with or without S-9, for any urine sample tested. By contrast, plates containing S-9 and the positive control, 2-amino anthramine, showed highly significant ($P < 0.001$), 750 per cent average increases in reversion rates for both strains. There was no group related difference in urinary mutagenic activities among individuals not working in the operating suite, those working in scavenged operating rooms, and those working in unscavenged operating rooms (table 1).

The results from the questionnaire indicated that none of the 13 residents was taking medication or was exposed chronically to chemicals other than trace concentrations of waste anesthetics. When assayed in the unconcentrated state, no urine sample from any anesthesia resident was mutagenic to strains TA1588 or TA100. When concentrates of urine were assayed, that from one resident, the only heavy smoker, was mutagenic to strain TA1588 but not TA100 (table 2). Concentrates of urine from other residents were negative. Strain TA1588 is most sensitive to frame-shift mutagens such as aromatic hydrocarbons contained in cigarette smoke, whereas strain TA100 is most sensitive to base-pair mutagens. Although each urine sample from the heavy smoker showed a dose-dependent increase (maximum fivefold) in mutagenic activity, the response was the same for his urine whether obtained before or after the start of anesthesia training. Similarly, results with urines from the other anesthesia residents were statistically the same before and after the start of training (table 3).

Results from the five non-operating room personnel who were heavy smokers were consistent with those from the single resident who smoked heavily; a dose-
dependent increase in mutagenic activity of concentrates of urine was seen with strain TA1538.

**Discussion**

McCoy and colleagues previously reported that unconcentrated urine samples of 15 anesthesiologists were mutagenic to *Salmonella* strains TA1535 and TA100 but not to strain TA1538. Several of the anesthesiologists investigated had worked in the operating suite for less than a year, while others had been exposed continually to waste anesthetic gases for many years. The exact concentrations of anesthetics to which they were exposed, however, were not measured. In contrast, we could find no mutagenic activity in unconcentrated urine specimens collected from eight anesthesiologists and 20 nurses working in either scavenged or unscavenged operating rooms.

Detection of mutagens in human urine by the Ames *Salmonella* system is limited because human urine contains sufficient histidine to interfere with the assay. Therefore, only a small amount of urine may be added to each plate, and only highly concentrated or potent mutagens will be detected. Because modern anesthetic agents are not mutagenic when tested directly, and the present study with unconcentrated urine is negative, it is possible that the finding of McCoy and colleagues was related to causes other than exposure to waste anesthetic gases.

A more sensitive method for detecting urinary mutagens is to concentrate certain urinary components while removing histidine by XAD-2 resin adsorption. The resin absorbs drugs, and their metabolites, which can then be extracted using a small quantity of solvent such as methyl alcohol. Urines obtained from anesthesia residents before and after periods of working in the operating suite for as long as 15 months were concentrated in this manner and then tested. However, there was no relationship between mutagenic activity and time spent in the operating suite. The only mutagenic activity seen was with concentrates of urine from heavy cigarette smokers, in agreement with results of Ames and Yamasaki.

In assessing results of the present study, several points should be considered. First, since only 15 residents were tested, there is a reasonable chance that in a large survey, mutagens might be found in urine of an occasional individual. For example, a hypothetical individual producing a mutagenic metabolite because of a rare anesthetic metabolic pathway would probably not be included in our small test group. Second, none of the residents was taking medication. Thus, it was not possible to determine the influence of agents such as enzyme inducers on production of mutagenic metabolites. Third, all concentrated urines tested were from male residents, whereas the only increased incidences of malignancy in operating room personnel reported have been in females. Therefore, despite the present-day difficulties of sampling a sufficient population of starting female anesthesia residents, testing of such a sample would be desirable. Finally, the average anesthetic concentration to which residents were exposed was low, because all operating rooms at the institutions where they worked were scavenged. Nitrous oxide levels in operating rooms at one institution averaged approximately 50 ppm; this value was presumably representative of all the institutions. These levels are well below those expected in unscavenged operating rooms or in dental offices, where average concentrations of 1,000 ppm or higher have been recorded occasionally. For the first test procedure, in which unconcentrated urines were assayed, some samples were obtained from individuals working in a hospital with unscavenged operating rooms. Soon after the sampling period, that hospital installed scavenging devices, and efforts to obtain additional specimens for concentrating were not considered worthwhile. Thus, there still exists the possibility that those who work in more contaminated environments would excrete mutagenic metabolites.

### Table 2. Mutagenic Activity of Concentrated Urine: Heavy Smoker

<table>
<thead>
<tr>
<th>Concentration (µl/Place)</th>
<th>Strain TA1555</th>
<th>Strain TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22 ± 3</td>
<td>122 ± 17</td>
</tr>
<tr>
<td>25</td>
<td>41 ± 4F</td>
<td>156 ± 20</td>
</tr>
<tr>
<td>50</td>
<td>65 ± 5F</td>
<td>137 ± 14</td>
</tr>
<tr>
<td>100</td>
<td>107 ± 11</td>
<td>150 ± 17</td>
</tr>
</tbody>
</table>

* Averaged over the three sampling periods.
† Significantly greater than control (0 µl/plate), *P < 0.01.*

### Table 3. Mutagenic Activity of Concentrated Urine: Thirteen Anesthesia Residents before and during Training

<table>
<thead>
<tr>
<th>Concentration (µl/Place)</th>
<th>Number of Revertant Colonies, Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain TA1558</td>
<td>Before Training</td>
</tr>
<tr>
<td>0</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>25</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>50</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>100</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>Strain TA100</td>
<td>197 ± 18</td>
</tr>
<tr>
<td>0</td>
<td>188 ± 19</td>
</tr>
<tr>
<td>25</td>
<td>217 ± 28</td>
</tr>
<tr>
<td>50</td>
<td>217 ± 28</td>
</tr>
<tr>
<td>100</td>
<td>195 ± 21</td>
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In spite of the limited scope of the present study, the results are encouraging, for they indicate that the majority of operating room personnel working under normal conditions do not have detectable mutagenic activity in the urine.

References


