Urine Mutagenicity as an Indicator of Exposure to Dietary Mutagens Formed During Cooking of Foods

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Studies were undertaken with individuals fed fried bacon meals to determine whether fruit or vegetables, ingested along with bacon, modified uptake and subsequent excretion of bacon mutagen(s). Urinary mutagenic activity was significant in those who had consumed bacon or mixed bacon/vegetable or bacon/fruit meals within the previous 2 to 3 hr period. Although urine activity varied by a factor of 4 among 15 subjects who consumed different meals, there was no evidence from this investigation that fruit or vegetables contributed to the inherent variability in total urinary mutagenic activity. However, some differences in excretion kinetics may be attributable to vegetable or fruit supplements in mixed meals.

Introduction

Urinary excretion is one of the mechanisms the body has available to it for eradicating xenobiotics. Whether or not foreign molecules harm the body depends on their pharmacological activity, which in turn depends on tissue distribution, metabolic pathways in specific tissues, rates of metabolism, and on the balance between intake and elimination from the body.

Notwithstanding the problem of defining precise kinetics of absorption, distribution, etc., urinary excretion has been widely used in toxicology as an exposure barometer for potentially hazardous chemicals encountered in the workplace and for medicinal and other drugs (1,2).

As well as toxins, mutagenic substances may be detected in urine. Durston and Ames (3) demonstrated the mutagenicity of a urinary metabolite excreted as a glucuronide conjugate in urine of rats administered the carcinogen, 2-acetylaminofluorene. Benzidine, o-toluidine, o-tolidine, and aniline or their mutagenic metabolites were shown to occur in the urine of rats fed with these aromatic amines (4). Mice administered the drug, metronidazole, excreted a mutagenic derivative, also detected in human urine (5). Furthermore, occupational exposure of humans to chemotherapeutic agents (6), anesthetic gases (7), epichlorhydrin (8), and some industrial chemicals (9,10) has apparently contributed to excess mutagenic activity detectable in urine. Thus, measurement of urinary activity has been proposed as a means of monitoring absorption of mutagens (11,12).

The search for mutagenic excretion products, which might be related to human cancer, has inevitably led to studies of “lifestyle factors” such as cigarette smoking (13), hair dye use (14), and coffee consumption (15). We previously reported detecting urine mutagenicity following fried pork or bacon meals (16). Since then Sousa, Nath, and Ong (17) have found a similar effect following beef meals. Both studies emphasize the absorption, distribution, and excretion of biologically detectable mutagenic substances. This finding is significant in view of the fact that nutritional components are considered to play an important role in induction of a number of human cancers (18).

In the present paper we examine the effect of other dietary constituents on urine mutagen excretion. Since vitamins, minerals, peroxidases, chlorophyll, etc. can inactivate or inhibit activation of pyrolysis mutagens (19), it is possible that similar components of vegetables and fruit, eaten along with fried meat, may directly
interfere with absorption of meat mutagens and with their ultimate detection in urine.

Materials and Methods

Foods and Cooking Procedures

Bacon was obtained in 2.5 mm slices, trimmed of rind and excess fat, then fried in an open pan in its own fat at 150 to 190°C for approximately 5 min. During this time, the bacon strips were turned once and, after cooking, were drained on absorbent paper while cooling, wrapped in aluminum foil and frozen at -20°C. The frozen bacon was defrosted and heated in a microwave oven when required for eating.

Fruit (oranges, papaya) was freshly prepared for meals by being skinned and diced into 2-cm square pieces. The vegetable meal consisted of carrots and spinach, also in equal proportions (w/w). Carrots were peeled and sliced in 0.5-cm rings, stored at 4°C overnight, then added to meals as required. Spinach was freshly cut into 5-cm ribbons, and lightly cooked for 1 min in the microwave oven before being eaten.

Table 1. Dietary regimes employed in study.

| Group | Experiment          |
|-------|---------------------|
| A     | Fast                |
| B     | Vegetables          |
| C     | Fruit               |

A5 71.7 1.68 25.4 11.00 180 200.0 31.35,38 50.66 56.6 88.96
| A2   | 84.5 1.87 24.2 10.50 200 163.4 22.26,30 37.51 54.61 75.77
| A3   | 87.8 1.78 27.7 10.55 200 217.0 21.27,32 34.49 41.50 54.46
| A5   | 71.7 1.68 25.4 11.00 180 200.0 31.35,38 50.66 56.6 88.96

Study Participants and Experimental Meals

This study involved 21 nonsmoking male and female volunteers whose heights and weights were determined and from whom 24 hr dietary histories were collected. Information was also sought on vitamin supplements and medication taken. Participants fasted from 9 PM the previous night and were randomly divided into three groups, A, B, and C, who submitted to various dietary regimens next day (Table 1). The first experiment provided baseline data after fasting or after meals of vegetables or fruit alone. In the second experiment, a test meal of meat of 2 g fried bacon/kg body weight with or without spinach/carrots or oranges/papaya was consumed by all participants. Vegetables and fruit were eaten at 4 g/kg body weight. Each individual drank two glasses of water (250 mL each) with breakfast, then a glass/hr until experiment completion 8 hr later.

Urine Mutagenicity Assays

Salmonella assays were performed as described previously (16). In brief, strain TA 1538 was used throughout this study. Assays were conducted “blind” with urine extracts, both in the presence and absence of liver S9 mix, and results were not decoded until all assays had been completed.

Urines were individually collected in randomly numbered 500-mL polypropylene bottles (Kayline) and an aliquot was removed for creatinine determination be-

Table 2. Study participant and urine excretion data following a fried bacon meal.

| Subject | Weight, kg | Height, m | BMIa | Time, hr | Vol., mL | Creatinine, g/100 mL | Urine extract (µL)/plate | TA 1538 revertants/platea |
|---------|------------|-----------|------|----------|----------|----------------------|--------------------------|--------------------------|
| A1      | 71.1       | 1.69      | 24.9 | 11.20    | 200      | 97.6                 | 31.5,38                  | 46.56                    | 71.72                    |
| A2      | 84.5       | 1.87      | 24.2 | 10.50    | 200      | 163.4                | 22.26                    | 37.51                    | 54.61                    |
| A3      | 87.8       | 1.78      | 27.7 | 10.55    | 200      | 217.0                | 21.27                    | 34.49                    | 41.50                    |
| A5      | 71.7       | 1.68      | 25.4 | 11.00    | 180      | 200.0                | 31.35                    | 50.66                    | 56.65                    |
| A6      | 65.8       | 1.76      | 21.2 | 11.30    | 400      | 214.5                | 20.25                    | 36.45                    | 37.38                    | 40.78                    |

a In the presence of S9; in the absence of S9 and without urine extract, mean and standard deviation of TA 1538 revertants were 18 ± 5/plate (n = 36).

b BMI (Body Mass Index) = \( \frac{W(\text{kg})}{H^2(\text{m}^2)} \); overweight > 25; obese > 30; underweight < 20; wasted < 16; normal range is 20–25.
fore storing at −20°C for 1 to 2 weeks. Amounts of up to 200 mL urine were thawed, filtered through Whatman No. 1 paper, then applied to the tops of 8 cm XAD-2 columns.

After washing with water to eliminate histidine, extracted material was eluted from the XAD-2 using acetone, dried in the usual manner, taken up in 800 μL of DMSO and assayed in aliquots of 10, 25, 50, or 100 μL/plate. Results were expressed as revertant colonies/plate.

Creatinine Assays

Determination of mutagen excretion in relation to creatinine excretion may be a useful way of monitoring differences between individuals and dietary groups, particularly since creatinine output is not readily affected by diuresis (20).

Creatinine levels were determined using a Roche Diagnostica kit. This utilizes the Jaffé reaction, in which creatinine, in an alkaline medium with picric acid, produces a yellowish-red salt, 2,4,6-trinitrocyclohexadiene. The intensity of the color developed in this procedure is directly proportional to the creatinine concentration, and test/standard absorbances were measured at 545 nm. Results were determined as μmole/L.

Results

There was very little excess mutagenic activity in urines of fasting individuals or of those who had eaten vegetables or fruit alone (data not shown). The only exceptions were with the first morning urines, which sometimes gave 60 to 70 revertants/100 μL urine extract (+S9). In some cases this activity could have been due to foods eaten on the previous day. The dietary histories indicated A4, A7, and B9 had eaten cooked meat for the previous evening meal. However, A6, B14, and C16 also exhibited high activity in the first urine sample but had not eaten meat.

Investigations of urine mutagenicity revealed clear evidence of activity in urines following meals of fried bacon, supplemented or unsupplemented with fresh vegetables or fruit (Tables 2, 3, and 4). This activity was much in excess of that detected after nonbacon diets and was only evident in the presence of S9. Without S9, assays of 193 urines exhibited near background levels of TA 1538 revertants (data not shown).

Several study participants could not be included in this report because of insufficient urine for processing (A4), loss of urine sample (A7), nonlinear data (A4, B12), and significant toxicity (B13, C17, C20). Urine toxicity was noted with B13, C17, C19, and C20 in experiments with and without bacon, but only with B12 in the second experiment. There was a possible association between urine toxicity and Mystecin used by participant C20; dietary histories did not indicate any common food constituents, eaten the previous day, which might have contributed to the toxicity detected.

In order to quantify results, slopes of the activity curves were determined using the negative control and the two lower dose levels of urine concentrate. In general, the curves did not exhibit straight line dose-response relationships. Instead, they assumed a biphasic character, indicative of inhibition at higher concentration of the urine extract (Fig. 1a). Thus, for 57/58 urines, the slope of activity determined at lower concentrations was assumed to be a more accurate reflection of the mutagenic component(s) of the urine concentrate. How-

| Subject | Weight, kg | Height, m | BMI b | Time, hr | Vol., mL | Creatinine, g/100 mL | TA 1538 revertants/plate a |
|---------|------------|-----------|-------|----------|---------|---------------------|--------------------------|
| B8      | 60.8       | 1.68      | 21.5  | 11.00    | 180     | 141.4               | 22,26,34, 44,51, 53,61, 45,71 |
|         |            |           |       | 13.30    | 280     | 48.4                | 31,35,38, 48,69, 73,78, 67,82 |
|         |            |           |       | 14.50    | 210     | 29.2                | 26,30,31, 45,49, 54,60, 69,82 |
|         |            |           |       | 16.00    | 320     | 22.4                | 28,28,31, 33,48, 37,38, 46,46 |
| B9      | 67.6       | 1.72      | 22.8  | 12.20    | 210     | 190.6               | 22,26,30, 56,57, 89,92, 113,133 |
|         |            |           |       | 14.05    | 90      | 173.4               | 24,28,33, 116, 148, - |
|         |            |           |       | 15.25    | 250     | 46.2                | 28,29,31, 35,45, 58,62, 81,92 |
|         |            |           |       | 16.10    | 200     | 19.6                | 20,25,42, 31,35, 41,45, 34,42 |
| B10     | 45.6       | 1.62      | 17.4  | 12.00    | 130     | 150.6               | 22,26,34, 45,49, 60,66, 42,51 |
|         |            |           |       | 14.26    | 310     | 21.2                | 22,26,30, 34,40, 46,47, 47,52 |
|         |            |           |       | 15.45    | 320     | 15.0                | 21,27,32, 30,33, 41,53, 39,40 |
|         |            |           |       | 16.17    | 310     | 12.6                | 20,24,30, 29,33, 35,37, 24,40 |
| B11     | 60.7       | 1.61      | 23.4  | 10.30    | 110     | 131.8               | 28,28,31, 38, - 43, - 51, - |
|         |            |           |       | 14.30    | 250     | 106.4               | 20,25,42, 70,84, 123,128, 140,149 |
|         |            |           |       | 15.55    | 300     | 20.4                | 22,27,33, 38,39, 39,42, 44,62 |
|         |            |           |       | 16.25    | 300     | 15.0                | 20,24,30, 34,40, 36,42, 20,38 |
| B14     | 65.1       | 1.66      | 23.6  | 12.15    | 220     | 92.0                | 31,35,38, 49,59, 58,85, 81,92 |
|         |            |           |       | 15.10    | 220     | 98.6                | 28,30,31, 55,64, 64,68, 105,110 |
|         |            |           |       | 16.10    | 180     | 5.6                 | 28,30,31, 32,40, 47,50, 60,64 |

a,b See Table 2.
ever, one urine (A2, 12.35 hr) yielded much higher colony numbers at the 100 μL extract/plate level (Fig. 1b). In this case, slope was determined using results at the two upper dose levels, since the activity of this urine would otherwise have been overlooked.

Knowing slopes of the dose-response curves, and knowing volumes of individual urine samples voided over the 8-hr period, it was possible to express results as revertants/urine sample. Urine excretion predominantly occurred over a 2 to 7 hr period with a peak between 3 and 5.5 hr following a meal of fried bacon (Fig. 2a).

A similar pattern of excretion occurred with subjects on bacon/vegetable and bacon/fruit regimes (Figs. 2b, 2c). Considerable individual variability in excretion kinetics was evident, both within and between the three experimental groups. In one case, mutagen excretion peaked as early as 3 hr post-meal (B9) while in others, peak excretion occurred around 6 hr (B14, C15). Some subjects yielded high peak values (A1, A3, A5, A6, B11), whereas others showed flattened excretion curve profiles (A2, B10, B14). In terms of kinetics, these high peak values appear to be more characteristic of individuals who had consumed fried bacon alone.

When the activities/urine sample are summed, the overall 8 hr excretion totals also demonstrate considerable within- and between-group variability over a 4-fold range (Table 5). Those for group A appear to be greater than for groups B and C, until taking into account the fact that the group A subjects were heavier than groups B or C. Thus, they consumed more bacon per person. However, there was no direct relationship between the amount of bacon ingested and the 8 hr excretion values, using a statistical correlation test. Nor was there any relationship between mutagen excretion
and the Body Mass Index or between mutagen and creatinine excretion (Tables 2, 3, and 4).

Correcting excretion values and expressing these as urine activity/100 g bacon consumed shows that there is no statistically significant difference between the three groups (Table 5); nor could any difference be demonstrated when mutagenic activity was expressed as a factor of creatinine excretion.

### Discussion

Most of the mutagenic activity detected in the urines of the 21 individuals surveyed here with *Salmonella* TA 1538 was directly attributable to ingested fried bacon (16). There was no evidence of mutagen excretion after meals of fruit or vegetables alone. Having controlled for tobacco smoking and for diet, there appear to be no other environmental factors markedly contributing to urinary mutagenic activity in these individuals. Nor was there any evidence of histidine-related growth factors (21), despite the use of XAD-2 ion exchange techniques.

Dose-response curves of nearly all the urine concentrates indicate the possible presence of inhibitors of urine-mediated mutagenesis. These inhibitors could not be derived from vegetables or fruit since they appeared to be active in 57/58 urine extracts. They may be related to porphyrins (22) or to certain fatty acids (23) which are able to suppress activation of fried meat mutagens.

In theory, the amount of any mutagen detected in urine should be directly related to the quantity of a substance or its precursor ingested, inhaled or absorbed across the skin. This will clearly depend on the rate of liver metabolism and other variables, which may remain relatively constant within one individual, but which may vary considerably between individuals. In the present study, the wide variation in mutagen excretion seen among 21 people is presumably an inherent characteristic of human populations, due to individual differences in digestive and metabolic processing. This could explain the different levels of dietary mutagen excretion previously reported in separate studies (16,24). It would

### Table 5. Total urine mutagenic activity over the 8 hr experimental period.

| Subject | TA 1538 revertants per 8 hr urine collection | Corrected activity per 100 g bacon eaten | Mean and standard deviation |
|---------|---------------------------------------------|-----------------------------------------|-----------------------------|
| A1      | 7534                                        | 5291                                    | 4232 ± 1105*                |
| A2      | 4599                                        | 2721                                    |                             |
| A3      | 6864                                        | 3909                                    |                             |
| A5      | 7679                                        | 5355                                    |                             |
| A6      | 5114                                        | 3886                                    |                             |
| B8      | 4033                                        | 3317                                    |                             |
| B9      | 4814                                        | 3561                                    |                             |
| B10     | 3355                                        | 3679                                    |                             |
| B11     | 5315                                        | 4378                                    |                             |
| B14     | 3198                                        | 2456                                    |                             |
| C15     | 1898                                        | 1300                                    |                             |
| C16     | 4146                                        | 4146                                    |                             |
| C18     | 3426                                        | 2685                                    | 3370 ± 1362*               |
| C19     | 7844                                        | 4592                                    |                             |
| C21     | 5499                                        | 4128                                    |                             |

*No significant differences between groups A, B, and C with the t-test.*
also explain why there is no correlation between the amount of bacon eaten and the total urinary mutagenic activity/person/8 hr. A similar lack of correlation has been reported between the number of cigarettes smoked and mutagen levels in smokers' urines (25). From this study, urine activity can be used as an indicator of exposure, but not as a dosimeter of the extent of exposure, to dietary mutagens. Thus, urinary excretion may be used to investigate inhibitory or protective factors in the diet, provided individual variation is taken into account. There is no evidence here that overall urinary clearance of bacon mutagen(s) was modified by fruit or vegetables, eaten with bacon, despite the evidence of inactivation and adsorption of pyrolysate mutagens by vegetable constituents (20,26).

This and similar studies point to the need to take into account individual variability, the contribution of dietary mutagens and of inhibitors of mutagenesis as potential confounding factors in using urine assays for monitoring environmental or occupational exposures.

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