Differentiation of mouth versus gut as site of origin of odoriferous breath gases after garlic ingestion

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Suarez, F. J., Springfield, J. Furne, and M. Levitt. Differentiation of mouth versus gut as site of origin of odoriferous breath gases after garlic ingestion. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G425–G430, 1999.—Utilizing the sulfur-containing gases of garlic as probes, we investigated the gut versus mouth origin of odoriferous breath gases. Five individuals ingested 6 g of garlic, and sulfur gases in mouth, alveolar air, and urine samples were measured. The mouth normally contained low concentrations of hydrogen sulfide, methanethiol, and dimethyl sulfide. Immediately after garlic ingestion, transient high concentrations of methanethiol and allyl mercaptan and lesser concentrations of allyl methyl sulfide (AMS), allyl methyl disulfide, and allyl disulfide were observed. With the exception of AMS, all gases were present in far greater concentrations in mouth than alveolar air, indicating an oral origin. Only AMS was of gut origin as evidenced by similar partial pressures in mouth, alveolar air, and urine. After 3 h, AMS was the predominant breath sulfur gas. The unique derivation of AMS from the gut is attributable to the lack of gut and liver metabolism of this gas versus the rapid metabolism of the other gases. Breath odor after garlic ingestion initially originates from the mouth and subsequently from the gut.

Halitosis; sulfur-containing gases; allyl methyl sulfide; methanethiol; allyl mercaptan

Material and Methods

In Vivo Study

Subjects. Five healthy subjects (4 females and 1 male, age 30–45 yr) with no problem with halitosis and no antibiotic ingestion during the previous 3 mo took part in this study. The protocol was approved by the Human Subjects Committee of the Institutional Review Board at the Minneapolis Veterans Affairs Medical Center, and all subjects gave informed consent.

Garlic intake. Subjects ingested their normal diet up to the time of the study. No garlic was ingested for 24 h before the study. The basic study consisted of two treatment periods; on one day the subjects chewed and then swallowed 6 g of raw garlic cloves and measurements were carried out over the next 4 h. As a control, on another day the subjects were studied over a similar 4-h period when no garlic was ingested. A washout period of 7 days elapsed between tests. In addition, three subjects chewed but did not swallow the 6 g of garlic.

Sample collection. Collection of mouth air. Sulfur-containing gases are extremely reactive with glass, rubber, and most plastics but unreactive with polypropylene (13, 14). A polypropylene tube (1.4 × 8 cm) was connected via stopcock to a 20-ml polypropylene syringe (All-PF Luer-Lok Syringes; Aldrich Chemical, Milwaukee, WI). Because initial studies showed that simply aspirating mouth air yielded poorly reproducible results, standardization of the collection technique was required. The following method, which yielded excellent reproducibility, was employed. The tube was placed in the mouth of the subject, and the lips were sealed around the tube. The subjects breathed through the nose for 30 s and then held their breath for an additional 15 s. Mouth air was then aspirated into the syringe, and the stopcock was closed.

Collection of alveolar air. End-alveolar breath samples were collected using a commercial device (Alveosampler; Quintron Instruments, Milwaukee, WI). Subjects held their breath for 15 s and then exhaled. This device diverts the first 500 ml of expired air, and the second 500 ml (alveolar air) are collected in a foil bag, which has been shown to be relatively nonreactive with the sulfur-containing gases.

Urine collection. Freshly collected urine (50 ml) was equilibrated for 5 min at room temperature with 50 ml of air in a sealed 100-ml vial. A sample of the gas space was removed and analyzed by gas chromatography for each of the sulfur-containing gases. The only sulfur gas detected in appreciable concentration in urine was allyl methyl sulfide (AMS). Because this gas has a solubility in water that is about 7.4 times that of air (personal observation), the partial pressure of AMS in the gas space would be predicted to be about six-sevenths of that of the unequilibrated urine sample when equal volumes of urine and gas are equilibrated.

Mouth and alveolar air samples were collected immediately before garlic consumption and then after 5, 30, 60, 120, 180, 185, and 240 min. Urine samples were collected before garlic ingestion and after 1, 2, 3, and 4 h. Immediately after the 3-h collection, subjects brushed their teeth using Mentadent, a toothpaste that contains baking soda and hydrogen peroxide (Chesebrough-Ponds, Greenwich, CT). The same payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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collection and mouth-cleansing procedures were utilized during the 4-h control (nongarlic) period.

In Vitro Studies

Sulfur gases in garlic. Raw garlic (1 g) was diced and placed inside a 20-ml polypropylene syringe. The garlic was then crushed by applying pressure with the plunger, and 15 ml of air were then added to the syringe, which was sealed with a stopcock. After incubation at room temperature for 10 min, a gas sample was removed for analysis.

Tissue metabolism of garlic gases. The ability of various rat tissues to metabolize some of the major sulfur-containing gases observed after garlic ingestion was assessed as follows. Rats (Sprague-Dawley, male) were anesthetized with Nembutal (40 mg/kg), and samples of liver and mucosa from the stomach, small intestine, and cecum were obtained. The tissues were homogenized using a dual Teflon homogenizer in RPMI buffer (pH 7.4, 1 mg tissue to 16 µl of buffer). Aliquots (32 µl) of each homogenate were then incubated at 37°C with 10 ml of air containing various sulfur-containing gases. The initial concentrations of methanethiol and allyl mercaptan were approximately 40 parts per million (ppm). Because of the inability to demonstrate metabolism of AMS at this concentration, additional studies with AMS were carried out at a concentration of ~5 ppm. The rate of metabolism of the various gases was determined from the rate of disappearance of the gas when incubated with tissue, minus the disappearance rate observed during incubation with buffer. Measurements were obtained at 0, 30, 60, and 90 min of incubation, and the results were expressed as picomoles of the sulfur gas metabolized per minute per milligram of tissue.

Gas analysis. Sulfur-containing gases. Initially, the identities of the sulfur-containing gases in raw garlic and in human breath samples obtained after eating garlic were established using gas chromatography-mass spectroscopy (GC-MS; Kratos MS25, 70 eV), using a cross-linked silicone capillary column (HP-1, 30 m × 0.32 mm; Hewlett-Packard, Palo Alto, CA) maintained at 80°C. Subsequently, the gases were identified via their GC retention time and quantified via the peak areas compared with those of authentic standards (Aldrich). Gas chromatographic measurements were obtained using an HP-5890A GC (Hewlett-Packard) equipped with a sulfur chemiluminescence detector specific for sulfur compounds. A 0.5-ml gas sample was injected onto a column (2.4 m, 3.1 mm OD Teflon packed with Chromosil 330; Supelco, Bellefonte, PA) maintained at 120°C. The carrier gas was nitrogen at a flow rate of 25 ml/min.

Carbon dioxide. The CO₂ content of mouth and breath samples was determined using Medical Gas Analyzer LB-2 (Beckman Instruments, Anaheim, CA). Mouth samples had <1.5% CO₂, whereas breath samples had at least 4% CO₂.

Statistical analysis. Data are expressed as means ± SE (15). Significance of differences between treatments or days was determined by paired t-test.

RESULTS

Figure 1 shows a GC tracing of the sulfur-containing gases released from raw garlic. Gases identified were hydrogen sulfide (H₂S), methanethiol, allyl mercaptan, AMS, allyl methyl sulfide, and allyl disulfide.

Figures 2–5 compare the concentrations of various sulfur gases observed in the mouth air, alveolar air, and urine during the control period (when no garlic was ingested) versus the concentrations following garlic
During the control period, only three sulfur-containing gases (H$_2$S, methanethiol, and dimethyl sulfide) were detectable (>2 parts per billion (ppb)). H$_2$S was found in alveolar, mouth, and urine samples, with the highest concentrations in the mouth (Fig. 2). Methanethiol was found only in mouth samples (Fig. 3) as were very low concentrations of dimethyl sulfide (data not shown).

After garlic ingestion the concentration of H$_2$S in the mouth increased from about 50 to 150 ppb, whereas breath and urine concentrations remained roughly constant (Fig. 2). Methanethiol concentrations in mouth samples increased dramatically 5 min after garlic intake to >2,000 ppb and then fell precipitously to about 200 ppb at 30 min (Fig. 3). Negligible increases in the concentration of methanethiol in alveolar air and urine were observed.

The sulfur-containing gas present in highest concentration after garlic intake was allyl mercaptan, which was present in the mouth at a mean concentration of 15,900 ± 5,750 ppb 5 min after garlic ingestion (Fig. 4). This concentration declined by greater than 97% by 1 h. As was the case with methanethiol, negligible alterations of the concentration of this gas were observed in alveolar air and urine.

As shown in Fig. 5, immediately after garlic ingestion the mouth contained concentrations of AMS (mean 71 ± 26 ppb) that were well below that observed for methanethiol and allyl mercaptan. However, in contrast to the latter two gases, the mouth concentration of AMS did not decline over 3 h. The partial pressure of this gas rose appreciably in both alveolar air and urine samples such that mouth, alveolar air, and urine samples had roughly comparable partial pressures. After 3 h the concentrations of AMS in mouth and alveolar samples (mean 110 ppb) exceeded that of any of the other sulfur-containing gases. To determine if this concentration was detectable by the human nose, three volunteers blindly compared the smell of air containing 100 ppb of AMS and room air. All three subjects readily detected an unpleasant, “garlic-like” odor in the AMS-containing gas.

When subjects chewed but did not swallow the garlic, the concentration of all the sulfur gases in mouth air was comparable to that observed when the garlic was swallowed (data not shown). However, AMS was never detectable in urine and was present, in low concentrations, in alveolar air samples only for the initial 30 min. The mouth concentrations of two other gases (allyl methyl disulfide and allyl disulfide) increased to levels
of 200–500 ppb 5 min after garlic ingestion and then fell precipitously (data not shown). These gases were not observed in urine nor alveolar samples.

The effect of brushing the teeth and mouth with toothpaste was studied at 3 h. A >80% decrease in H₂S and methanethiol in mouth air occurred immediately after brushing, and the concentrations of these gases then tended to rise over the next hour. In contrast, oral cleansing resulted in only about a 45% decrease in the mouth concentration of AMS, and no change in the concentration of this gas in alveolar air was observed. When two subjects brushed their teeth and mouth immediately after garlic ingestion, reductions of >90% in the concentrations of methanethiol and allyl mercaptan were observed in mouth gas, whereas only a minor, transient decrease in AMS concentrations was observed (data not shown).

The ability of rat tissues to metabolize various sulfur gases is shown in Fig. 6. Each of these tissues rapidly metabolized allyl mercaptan and methanethiol, whereas metabolism of AMS was not significantly different from zero (metabolism in 4 studies averaged 0.7 ± 2.1%). The metabolism of allyl mercaptan and methanethiol was not associated with the appearance of AMS. The ratio of the disappearance rate of a sulfur gas from the gas phase when incubated with buffer to that with tissue in buffer averaged 1:12, 1:6, and 1:1 for methanethiol, allyl mercaptan, and AMS, respectively.

**DISCUSSION**

In the present study garlic was used as a probe to distinguish the relative importance of the mouth versus the gut in the origin of malodorous breath gases. Although there have been allusions to the possibility that malodorous breath gases may originate from the gut (8), we are unaware of any rigorous previous study of this question.

Five individuals ingested 6 g of raw garlic, and the origin of the sulfur-containing gases responsible for breath malodor was then determined by comparison of partial pressures of sulfur gases in mouth, alveolar air, and urine samples obtained over the subsequent 4 h. Our method of collecting mouth air might have resulted in minor contamination with alveolar air. However, a primary mouth origin of some of the sulfur gases was clearly demonstrable by the finding of concentrations in mouth samples that were orders of magnitude greater than that of alveolar air. Similarly, the alveolar air samples, having passed through the mouth, would carry along some mouth contamination; however, once again the enormous discrepancy between mouth and alveolar samples for several gases showed that this contamination was relatively minor. Nevertheless, it is difficult to clearly identify absorption from the gut as the sole source of a breath gas. Oral tissue is exposed to gases of gut origin via both expired air and the circulation. In fact, if diffusion from the blood to the oral cavity were sufficiently rapid, the partial pressure of the sulfur gases in mouth and alveolar air would be expected to be identical (i.e., equal to the partial pressure in arterial blood). Therefore, we utilized analyses of urine as an additional indicator that breath gases represented compounds that were absorbed from the gut and delivered to the lungs via the circulation.
To determine which of the sulfur-containing gases in breath originated from garlic, the concentrations of sulfur gases during a 4-h control period when no garlic was ingested were compared with a similar period after garlic ingestion. Only three sulfur-containing gases (H\textsubscript{2}S, methanethiol, and dimethyl sulfide) were detected during the control period. The much higher concentrations of these gases in mouth versus alveolar air samples indicated that these gases were originating primarily from the mouth.

Our analysis showed that six different sulfur-containing gases (H\textsubscript{2}S, methanethiol, allyl mercaptan, AMS, allyl methyl disulfide, and allyl disulfide) were identifiable in raw garlic. With the exception of H\textsubscript{2}S, the breath concentration of each of these gases was appreciably greater after garlic intake versus the control period. Immediately after garlic ingestion, two gases (methanethiol and allyl mercaptan) were present in very high concentrations in mouth air, whereas alveolar air and urine concentrations remained negligible. The mouth concentrations of these two gases dropped by about 10- to 20-fold over the first 30 min and were negligible at 2 h. When garlic intake was immediately followed by brushing of the teeth and tongue there was a marked decrease (15- to 20-fold) in sulfur-containing gases in the mouth. This finding suggests that these gases originated from particles of garlic that were trapped in the oral cavity rather than gases dissolved in the oral mucosa.

Only one gas (AMS) had a partial pressure in alveolar air and urine that approximated that of mouth air. In contrast to the other major garlic gases, AMS concentrations remained relatively constant throughout the 4-h period of the study. Thus this gas apparently was absorbed from the gut into the blood, with delivery to both the alveolar air and the urine. This concept was supported by the observation that when garlic was chewed, but not swallowed, AMS did not appear in the urine.

By 3 h AMS was the predominant sulfur gas on the breath. This gas has a characteristic garlic-like odor that was readily detectable at the breath concentrations observed in our study. We conclude that most of this gas originates from the gut (rather than the mouth) and this gas is likely to account for the well-known persistence of malodorous breath long after garlic ingestion.

Given the apparent gut absorption of AMS the question arises as to why the other sulfur-containing gases (which were present in much higher concentrations in garlic than was AMS) appeared to be absent from blood, as evidenced by their negligible concentrations in urine. The explanation appears to be that gut mucosa and liver tissue rapidly metabolize most garlic gases such as methanethiol and allyl mercaptan (see Fig. 6). Presumably, this metabolism removes virtually all of these gases during the “first-pass,” such that negligible quantities reach the systemic circulation. In contrast, we were unable to demonstrate metabolism of AMS by either gut or liver tissue and hence the appearance of this gas in the systemic circulation.

As would be expected cleansing the teeth and mouth (via brushing with toothpaste) had a variable effect on the sulfur-containing gases, depending on their origin. The concentration of the sulfur gases that originated in the mouth dropped sharply and then gradually rose over the subsequent hour. In contrast, with AMS, a gas that originates from the gut, oral hygiene resulted in only a minor, transient decline in the mouth concentration and no change in alveolar air or urine concentrations. Thus brushing of the teeth and mouth would not be expected to reduce the breath odor that remains several hours after garlic ingestion.

The results of this study demonstrate that halitosis may be of oral and/or gut origin. A rational approach to the treatment of this common problem presumably should begin with the identification of the malodorous gas and its site of origin. The simple techniques utilized in the present study make it possible to differentiate between a mouth versus gut origin. For gases of mouth origin, oral hygiene is the treatment of choice. When the gut is the source of the malodorous gases, oral hygiene will be ineffective, and reduction of the breath concentration of these gases presumably will require manipulation of the diet and/or the gut flora.

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