

Effects of a Two-Phase Oil-Water Mouthwash on Halitosis

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Halitosis is caused mainly by volatile sulfur compounds (VSC), including hydrogen sulfide, methyl mercaptan, and dimethyl sulfide.^{1,2} Hydrogen sulfide and methyl mercaptan, which contains thiol (-SH) in their chemical structures, are the main components of these VSC.² Methyl mercaptan in particular, a compound derived mainly from methionine metabolism, is a primary source of pathological halitosis.³ It has been postulated that other classical malodorous compounds such as indoles and amines modify the quality of malodor rather than causing halitosis.⁴ Therefore, to improve the conditions causing halitosis, the concentration of methyl mercaptan and hydrogen sulfide in mouth air must be reduced.

A number of mouthwashes and other products have been developed to prevent bad breath. In the U.S. alone, over \$500 million is spent annually on mouthwashes, sprays, and related products.⁵ In Japan, the market for these products has increased rapidly in only a few years because the awareness of oral hygiene and bad breath has increased markedly.⁶ Despite the magnitude of this problem, many of these mouthwashes have little actual effect, other than masking odor for short periods.^{5,6}

Recently, Rosenberg introduced a novel two-phase oil-water mouthwash.⁷ As many oral microorganisms have hydrophobic outer surfaces, they would be removed by adhering to the oil droplets in such a product. One of the main actions of other mouthwashes is to remove microorganisms, but this is accomplished only by mechanical washing of the oral cavity. This two-phase mouthwash, however, is based on a new concept, utilizing the

ABSTRACT—Many oral microorganisms possess hydrophobic outer surfaces. A two-phase, oil-water mouthwash has, therefore, recently been developed to remove such oral microorganisms. The oil phase consists of olive oil and other essential oils. The aqueous phase includes cetylpyridinium chloride, which is a disinfectant that promotes the adhesion of microorganisms to oil droplets. This study determined the effects of this mouthwash on the production of volatile sulfide *in vivo* and *in vitro*. Neither rinsing with water nor brushing teeth decreased the concentration of sulfide in mouth air at 3.5 h after treatment. A reduction of only 30% of sulfide was observed when a commercial mouthwash was used. However, this study demonstrated that use of the two-phase mouthwash led to approximately 80% reduction of sulfide. Furthermore, volatile sulfide and 2-ketobutyrate productions from methionine in a saliva putrefaction system were completely inhibited by the two-phase mouthwash; and consumption of methionine was decreased by 65 percent. It is concluded that the two-phase mouthwash strongly inhibits the production of volatile sulfide.

chemical properties of microorganisms. The objective of this study was to determine the effect of this two-phase mouthwash on bad breath production *in vivo* and the production of malodor *in vitro*.

MATERIALS AND METHODS

Mouthwashes

The two-phase mouthwash (J. Morita Co., Tokyo, Japan) is comprised of an aqueous and an oil phase.⁷ The aqueous phase contains cetylpyridinium chloride (0.05%), an antibacterial agent⁸ which also promotes the adhesion of oral microorganisms to oil droplets.⁹ Sweetener and food color (blue) are

also contained in this phase. The oil phase contains olive oil, which has a high binding capacity to microorganisms, and essential oils (Fig. 1).

A dentrifice and an ordinary commercial mouthwash (Skoal,[™] Sunstar Inc., Tokyo) now on the market were also studied.

Halimeter

A Model RH17A Halimeter (Interscan Co., Ca.) was employed to determine the concentration of volatile sulfide originating mainly from hydrogen sulfide and mercaptan.¹⁰ This meter is capable of measuring molecules of hydrogen sulfide and mercaptan in parts-per-billion (ppb). For sampling, the short end of a flexible drinking straw was inserted in the inlet of a monitor. The other end of the straw was inserted either into a Teflon bag filled with sample gas or placed in the center of a subject's open mouth. The mouth was kept open, while the sample gas was drawn through the sensor by an internal pump; the highest level displayed was then read.

Subjects

Twenty male volunteers, who were non-smokers with no evidence of systemic or oral pathology, were the subjects of this study. Smokers were excluded because of their lower odor-producing potential¹¹ and because tobacco smoke itself contains VSC.¹² The volunteers were instructed not to take any oral hygiene measures, *e.g.*, oral rinsing, and not to eat or drink on the morning of the test. The evaluation of early morning mouth air was performed at 8:30 a.m. On the basis of the evaluation, 9 subjects (mean 22.4 yr.) with over 75 ppb of thiols in their mouth air, were selected for this study, since malodor can be perceived at the concentration of over 75 ppb of sulfide (data not shown).

Saliva Putrefaction System

Paraffin-stimulated whole saliva samples were taken from the subjects. Food debris was then removed through two layers of cheesecloth. One ml of saliva was mixed with 0.1 ml of 100 mM L-methionine in phosphate buffered saline (PBS) and 0.2 ml of the mouthwash solution. The mouthwash solution was prepared as follows: the emulsion of this mouthwash was diluted with water immediately after being well shaken, then the solution at the concentrations of 1%, 10%, 20%, 50% and 100% were obtained, respectively. For a control study, saliva was mixed with the methionine solution and 0.2 ml of water. The blank consisted of 1 ml water, 0.1 ml PBS and 0.2 ml mouthwash solution.

The mixtures were dispensed into individual Teflon-coated glass tubes (17.5 ml volume).⁶ Caps with a solid rubber disc lined with Teflon film

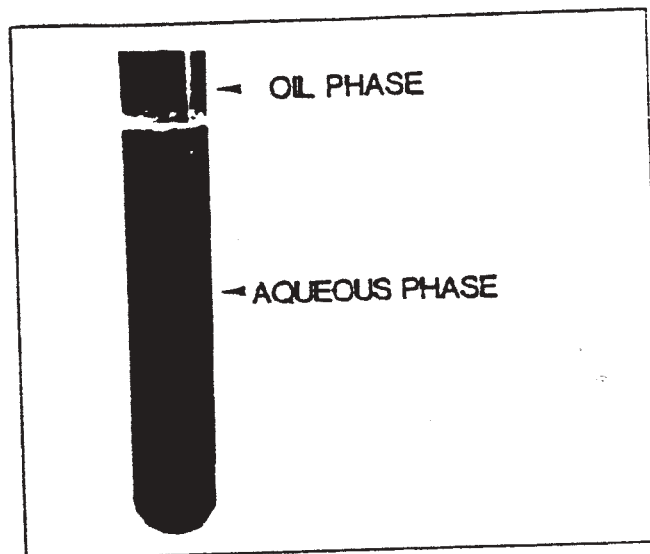


Fig. 1. The two-phase oil-water mouthwash: the oil phase is separated from the water phase. Before use, the mouthwash is shaken well.

provided an air-tight seal. Prior to incubation, head space air in the tube was replaced with nitrogen gas, and the tubes were then incubated for 18 h at 37°C. After incubation, head space air was diluted to 100 times in a Teflon bag with nitrogen gas, and the concentration of volatile sulfide was then estimated by Halimeter.[™]

Methionine Consumption in Vitro

The saliva putrefaction system mentioned above was employed. Proteins in the incubated samples were removed with Sep Pac C₁₈ (Waters Co., Ma) and methionine concentration was then determined by using a High Performance Liquid Chromatography (HPLC) system equipped with a Wakopak WS-PTC column (Wako Chemical, Osaka).¹³ The inhibition (%) of methionine consumption by the two-phase mouthwash was then obtained.

Production of 2-Ketobutyrate in Vitro

Since 2-ketobutyrate is a by-product of methionine metabolism to methyl mercaptan, production of this compound was also estimated in this study. Samples were obtained from the saliva putrefaction system outlined above, and the concentration in the sample was measured by the method of Yaegaki, employing HPLC.¹³

Effects of Removing Salivary Cellular Elements

Two ml of the mouthwash was mixed with 18 ml saliva for the control study 2 ml PBS was used instead of the mouthwash. After being mixed well, an aqueous phase was obtained; this was then centrifuged by 20,000 X g at 4°C for 20 min. The precipitate was fractionated by Percoll[™] density

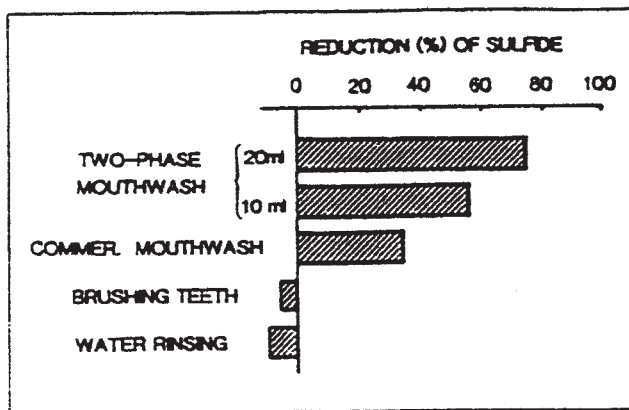


Fig. 2. Reduction (%) of sulfide in mouth air at 3.5 h after treatment.

gradient centrifugation, as described previously by the authors.^{14,15}

RESULTS

Effects on Bad Breath *in Vivo*

Nine subjects abstained from oral hygiene, including mouth rinsing, and from eating and drinking in the morning until completing the experiment. Prior to treatment the strength of oral malodor was evaluated by the Halimeter™ at 8:30 a.m. Immediately after shaking the two-phase mouthwash, 10 or 20 ml of it was used for rinsing the mouth for 30 sec. For the controls, the mouth was rinsed with 20 ml distilled water. Also, teeth were brushed with dentifrice for 3 min. Although the subjects received instructions regarding the scrubbing method of brushing, the others were not instructed. Rinsing with commercial mouthwash (Skoal™) followed the instruction. Each experiment was carried out on different days with the same subjects. At 3.5 h after treatment, the strength of malodor was evaluated again.

Our preliminary study had indicated that the smell of this mouthwash, the dentifrice, and the commercial mouthwash did not obstruct the measurement of sulfide at 3.5 h after treatment (data not shown). Since meals usually decrease VSC concentration in physiological oral malodor by around 90%,¹⁶ and as the interval between meals or tea-times would normally be 3-4 h, the deodorant effect of a mouthwash must last longer than 3 h. The time of 3.5 h after treatment was therefore selected for evaluation of the mouthwash.

Results were expressed as percentages compared with primary concentrations before treatments. Mouth rinsing with either 10 or 20 ml of this mouthwash strongly inhibited the production of malodor. The commercial mouthwash lost its effect at 3.5 h after rinsing. Neither rinsing with water

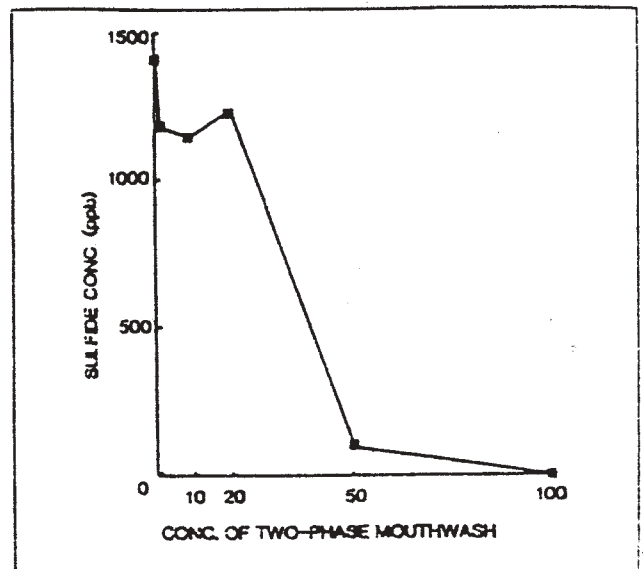


Fig. 3. The effects of the two-phase, oil-water mouthwash on the production of volatile sulfide in a saliva putrefaction system. Averages from 5 experiments at each concentration of the mouthwash are shown.

nor brushing teeth was very effective in controlling malodor (Fig. 2).

Effects on Malodor Production *in Vitro*

The effects on the production of volatile disulfide were determined by employing the saliva putrefaction system. With a 100% concentration of the mouthwash solution, the production of volatile sulfide was completely inhibited. However at concentrations of less than 50%, sulfide production rapidly increased, and no significant differences were observed in comparison with controls (Fig. 3).

Consumption of Methionine and Production of 2-Ketobutyrate

As L-methionine is one of the sources of oral malodor, the consumption of methionine was estimated in the saliva putrefaction system, employing a 100% concentration of the two-phase mouthwash. It was demonstrated that methionine consumption was decreased dramatically in the test group. Furthermore, 2-ketobutyrate, which is a by-product of methionine metabolism,¹⁷ was not detected, although 770.5 nM/ml of this compound was found in the sample from the control group. The control's compound was incubated with PBS instead of the mouthwash (Table 1).

Effects of Removing Salivary Cellular Elements

Salivary cellular elements are a very important source of oral malodor. Hence we determined whether the two-phase mouthwash removed these cellular elements from saliva. Figure 4 shows that

Table 1
Effects on 2-Ketobutyrate Production
and L-Methionine Consumption

	2-Ketobutyrate (n mol/ml)	L-Methionine Consumption	
		μ mol/ml	Reduction (%)
Control *	770.5	8.8	—
Test *	N.D. **	3.1	65.4

* n = 5; ** not detectable; a saliva putrefaction system was employed.

the middle layer (density:1.051-1.076 g/ml), which contains the moderately degraded cells,¹⁴ was decreased in the test. Around 32% of disulfide is involved in this fraction.¹⁴ However, intact cells or highly degraded cells involved in the upper (density:1.019-1.051 g/ml) or lower layer (density:1.076-1.139 g/ml) were not affected by the two-phase mouthwash (Fig. 4).

DISCUSSION

Halitosis has not usually been examined or treated in dental clinics although approximately 50% of the adult population suffers from halitosis.⁴ Only gas chromatography equipped with a flame photometric detector² has been a reliable method of evaluating bad breath. Therefore, compact detectors of bad breath such as the Halimeter™¹⁰ and others (GC Co., Tokyo; Tokuyama Soda Co., Tokyo) have recently been introduced clinically because of a compelling need to establish a more adaptable analyzer of halitosis for dental clinics. Hence, it is now possible for clinicians to evaluate bad breath objectively and scientifically. On the other hand, there are few methods of treatment for halitosis other than periodontal treatment and tongue scraping. Therefore the practical application of a very effective mouthwash for halitosis is much anticipated.

The two-phase oil-water mouthwash is based on a new concept and is completely different from the conventional mouthwashes now on the market. This new mouthwash not only has a disinfecting effect, but also the aggressive effect of removing oral microorganisms which are absorbed into its oil droplets. Our results demonstrated that this two-phase mouthwash has more deodorant activity than other mouthwashes and brushing of teeth. Hence, we consider that it is possible to introduce this mouthwash clinically.

Our *in vitro* study demonstrated that the production of 2-ketobutyrate, which is a by-product of methyl mercaptan production from L-methionine,¹⁸ was completely inhibited by the two-phase mouthwash. However, some consumption of methionine

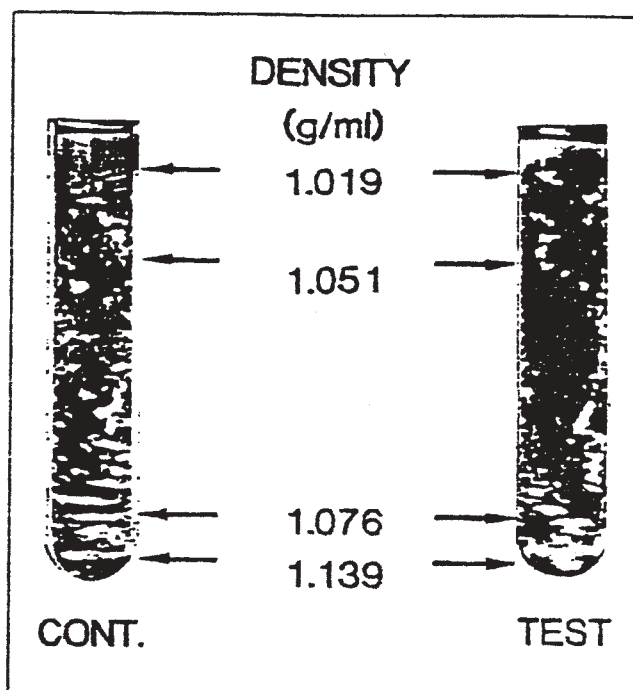


Fig. 4. Effects on salivary cellular elements.

was found, although it was decreased by 65% (Table 1). Hence we concluded that the remaining bacterial activity might metabolize methionine, although the pathway for methyl mercaptan production was completely blocked. If this is indeed the case, this mouthwash might have a specific inhibiting effect on methyl mercaptan production, besides its general effects on bacteria.

Sulfur-containing amino acids, originating from proteins or the amino acid pool are a source of VSC.^{1,18} Salivary cellular elements, consisting mostly of epithelial cells, provide bacteria with such sources.^{18,19} This study showed that the two-phase mouthwash removed the degraded cells involved in the middle layer of a Percoll™ density gradient from the aqueous phase which contains the saliva treated with the mouthwash. Hence, it is presumed that the oil droplets in the mouthwash can remove not only oral microorganisms but also VSC sources, such as degraded cells.

Based on an epidemiological study that employed gas chromatography, the methyl mercaptan concentration at 85 percentile in mouth air was found to be 10 ng/10 ml air.⁴ An objectionable concentration of methyl mercaptan is 0.5 ng/10 ml air. Therefore, it would be anticipated that an ideal mouthwash would reduce sulfide by more than 95% at 3 or 3.5 h after treatment; however, such a mouthwash or other related products have not been reported. Hence, if the two-phase mouthwash could be improved by, for example, the addition of zinc chloride or some other disinfectant,^{6,16} a highly effective mouthwash, close to an ideal mouthwash,

might thus be obtained.

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