

## Delivery of Tea Polyphenols to the Oral Cavity by Green Tea Leaves and Black Tea Extract

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### Abstract

Catechins and theaflavins, polyphenolic compounds derived from tea (*Camellia sinensis*, fam. Theaceae), have been reported to have a wide range of biological activities including prevention of tooth decay and oral cancer. The present study was undertaken to determine the usefulness of green tea leaves and black tea extract for the delivery of catechins and theaflavins to the oral cavity. After holding either green tea leaves (2 g) or brewed black tea (2 g of black tea leaves in 100 ml) in the mouth for 2–5 min and thoroughly rinsing the mouth, high concentrations of catechins ( $C_{\max} = 131.0\text{--}2.2 \mu\text{M}$ ) and theaflavins ( $C_{\max} = 1.8\text{--}0.6 \mu\text{M}$ ) were observed in saliva in the 1st hour. Whereas there was significant interindividual variation in the peak levels of catechins and theaflavins, the overall kinetic profile was similar, with  $t_{1/2} = 25\text{--}44$  min and  $49\text{--}76$  min for catechins and theaflavins, respectively (average coefficient of variation in  $t_{1/2}$  was 23.4%). In addition to the parent catechin and theaflavin peaks, five unidentified peaks were also observed in saliva after black tea treatment. Hydrolysis of theaflavin gallates, apparently by salivary esterases, was observed *in vitro* and *in vivo*. These results indicate that tea leaves can be used as a convenient, slow-release source of catechins and theaflavins and provide information for the possible use of tea in the prevention of oral cancer and dental caries.

### Introduction

Tea, made from the leaves of *Camellia sinensis* (fam. Theaceae), is one of the most popular beverages worldwide. Green tea is prepared from fresh tea leaves that are pan-fried or steamed and dried to inactivate enzymes. Chemically, this

beverage is characterized by the presence of the polyphenolic catechins including: (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epicatechin (Fig. 1). Black tea is prepared by crushing withered tea leaves and allowing enzyme-mediated oxidation, commonly referred to as fermentation, to occur, leading to the formation of oligomers such as theaflavins (Fig. 1) and polymers known as thearubigins. Oolong tea is a partially fermented product that contains considerable amounts of catechins and oligomerized catechins (1).

A number of beneficial effects have been attributed to tea consumption, including the prevention of oral cancer and tooth decay (2, 3). In several animal experiments and human trials, green tea and black tea have been shown to significantly reduce plaque scores and caries index (4–8). Although tea polyphenols have been shown to have anticancer activity *in vitro* and oral cancer preventive activity in animal models (9, 10), epidemiological evidence for oral cancer prevention has been sparse and inconclusive. For example, a population-based, case-control study in Denmark has found no association between tea consumption and the development of oral squamous cell cancer (11). On the other hand, preliminary results from an intervention study have shown that oral and topical administration of a tea preparation significantly reduced the size of oral lesions and the incidence of micronucleated oral mucosa cells in leukoplakia patients compared with a nontreated group (12).

To demonstrate the effectiveness of tea and tea components as cancer preventive agents, investigators need carefully designed intervention studies. To accomplish this, a clear understanding of the bioavailability of the tea components is essential. Previous work in our laboratory has shown that after drinking brewed green tea (1.2 g of tea solids in 200 ml hot water), salivary levels of EGC and EGCG reach 11–44  $\mu\text{g}/\text{ml}$  (37–147  $\mu\text{M}$ ) and 5–22  $\mu\text{g}/\text{ml}$  (10–44  $\mu\text{M}$ ) at 20 min, respectively in human volunteers (13). These levels were two orders of magnitude greater than the plasma levels after a similar dose of tea (78 and 223 ng/ml for EGCG and EGC, respectively; Ref. 14). In practical application, chewing or holding tea leaves or extract in the mouth might be a convenient and economical method of sustained delivery of catechins and black tea polyphenols to the oral cavity.

We explored these ideas in this study and report herein the salivary levels of catechins and theaflavins achieved by holding green tea leaves or black tea extract in the mouth. These data should aid in designing proper intervention studies on the effectiveness of green and black tea in the prevention of oral cancer and tooth decay.

### Materials and Methods

**Chemicals and Reagents.** EGC, epicatechin, ECG,  $\beta$ -D-glucuronidase (G-7896, EC 3.2.1.31, from *Escherichia coli* with  $9 \times 10^6$  units/g solid), and sulfatase (S-9754, EC 3.1.6.1, from *Abalone entrails* with  $2.3 \times 10^4$  units/g solid) were

Received 4/30/03; revised 8/8/03; accepted 9/5/03.

**Grant support:** NIH Grant CA 88961 and facilities from National Institute of Environmental Health Sciences (NIEHS) Center Grant ES05022.

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**Notes:** Drs. Lee and Lambert contributed equally to this study.

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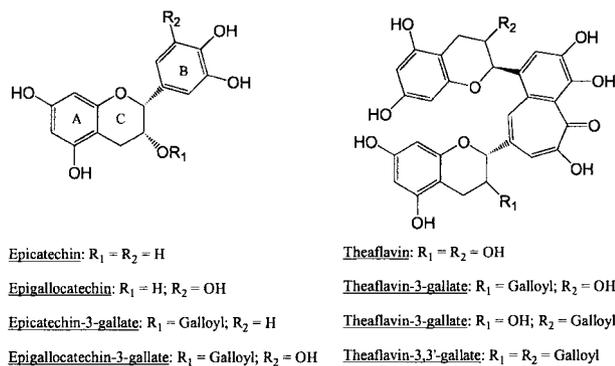


Fig. 1. Structure of catechins and theaflavins.

purchased from Sigma Chemical Co. (St. Louis, MO). Green tea leaves and black tea solids (lyophilized extract) were provided by Anhui Agricultural University (Anhui, China); EGCG and theaflavins were provided by Unilever Best Food Co. (Englewood, NJ). The mixture of theaflavins contained the following (per g): theaflavin (TF, 202 mg) theaflavin-3-gallate (TF-3-G, 331 mg), theaflavin-3'-gallate (TF-3'-G, 156 mg), and theaflavin-3,3'-digallate (TFdiG, 311 mg).

Stock solutions of EGCG, EGC, ECG, epicatechin, and theaflavins (10  $\mu\text{g}/\text{ml}$  catechins and 100  $\mu\text{g}/\text{ml}$  theaflavins) were made in 0.2% ascorbic acid-0.005% EDTA solution and stored at  $-80^\circ\text{C}$ . These standards were stable for up to 6 months. Other reagents and high-performance liquid chromatography (HPLC) solvents were of the highest grade commercially available.

**Holding of Tea and Sample Collection.** The protocol for human subjects in these studies was approved by the Institutional Review Board for the Protection of Human Subjects (Protocol no. 92-034) at Rutgers University (Piscataway, NJ). Volunteers between 25 and 50 years of age who did not smoke, drink alcohol, nor drink tea for at least two days before the start of this study were selected. After thoroughly brushing their teeth, four volunteers gently chewed 2 g of green tea leaves in the mouth for 5 min. The leaves were voided, and the subjects immediately rinsed their mouths vigorously 10 times with 50 ml water for a period of 2 min. Unstimulated saliva was collected in tubes containing 20  $\mu\text{l}$  of ascorbic acid-EDTA solution [0.4 M  $\text{NaH}_2\text{PO}_4$  buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)] before holding green tea leaves and at 2, 7, 12, 32, 62, 92, and 122 min after voiding the tea leaves and rinsing the mouth. A 500- $\mu\text{l}$  aliquot of saliva was mixed with an equal volume of 60% acetonitrile and was stored at  $-80^\circ\text{C}$  for later analysis. In a second study, volunteers held the green tea leaves in the mouth for 5 min without chewing. Samples were collected and analyzed as above.

Similar studies with black tea were conducted by having subjects hold 30 ml of black tea extract (2 g brewed in 100 ml hot water) in the mouth for 2 min. Subjects washed their mouths as above and saliva samples were collected in a similar manner.

**Stability of Theaflavins.** The pH stability of the theaflavins was determined by dissolving 0.25 mg of theaflavins in 10 ml of 50 mM sodium phosphate buffer that had a pH of 6.5–9.0. The mixtures were incubated at room temperature, and samples were taken at 0, 5, 10, 40, and 60 min. The sample was stabilized by the addition of phosphoric acid (final pH 3.3) and maintained at  $4^\circ\text{C}$ . An aliquot was diluted 100 times with 30% acetonitrile and was analyzed by HPLC. To study the effect of

temperature on stability, theaflavins [0.2 mg/ml in 50 mM sodium phosphate buffer (pH 3.3)] were incubated at  $4^\circ\text{C}$ ,  $25^\circ\text{C}$ , and  $37^\circ\text{C}$  for 0, 1, 8, and 24 h and were analyzed as above.

To determine whether salivary esterases removed the galloyl group from gallated theaflavins, 5 ml of saliva were collected as above, were combined with 10 mg of TFdiG, and were incubated at  $37^\circ\text{C}$ . Aliquots were removed at 0–240 min, were diluted 100 times with 30% acetonitrile and 100  $\mu\text{l}$  of ascorbic acid/EDTA solution, and were analyzed by HPLC.

**Analysis of Tea Polyphenols.** Samples were analyzed on a HPLC system composed of an ESA Model 465 refrigerated autosampler, two ESA Model 580 dual piston pumps, and an ESA 5500 coulochem electrode array system. A Supelcosil C18 reversed-phase column (150 mm  $\times$  4.6 mm inner diameter; Supelco Co., Bellefonte, PA) was used for all applications. The column and coulochem electrode array system detector were housed in a temperature-regulated compartment maintained at  $35^\circ\text{C}$ . The autosampler was maintained at  $6^\circ\text{C}$ . For binary gradient elution, mobile phase A consisted of 1.75% acetonitrile and 0.12% tetrahydrofuran in 30 mM  $\text{NaH}_2\text{PO}_4$  (pH 3.35), and mobile phase B consisted of 58.5% acetonitrile and 12.5% tetrahydrofuran in 15 mM  $\text{NaH}_2\text{PO}_4$  (pH 3.45). The flow rate was maintained at 1 ml/min, and the eluent was monitored by coulochem electrode array system with potential settings at  $-100$ , 100, 300, and 500 mV. Gradient conditions were the same as those previously reported with the following modification (15).

For simultaneous analysis of both catechins and theaflavins, the mobile phase began with a 7-min isocratic phase of 96% A and 4% B, followed by progressive, linear increases in B to 17% at 25 min, 30% at 31 min, 44% at 50 min, 50% at 55 min, and 98% at 62 min. The mobile phase was maintained at 98% B for 5 min and then was re-equilibrated to 4% at 67.5 min.

## Results

**Analysis of Tea Polyphenols.** After subjects held 2 g of tea leaves in their mouths for 5 min, participants rinsed their mouths 10 times with 50 ml of distilled water, saliva samples were collected, and catechin levels were determined. Rinsing the mouth 10 times was found to be necessary to remove unabsorbed tea components (data not shown). Initial experiments conducted without tooth-brushing before the start of the experiment resulted in significant variation in the levels of tea polyphenols (data not shown). The addition of tooth-brushing eliminated this variation. HPLC analysis demonstrated the presence of not only the parental catechin compounds but also three unknown peaks (gA, gB, gC; Fig. 2A). Peaks gA and gC have been previously detected, although the identity of all of the peaks remains unknown (13). The retention times of EGC, epicatechin, EGCG, and ECG were 12.5, 24, 28.5, and 36 min, respectively. An analysis of blank saliva revealed no interfering or comigrating peaks compared with either the green tea leaf or black tea extract group (data not shown).

Similarly, HPLC analysis of saliva after black tea extract was held in the mouth revealed good separation of all catechin and theaflavin peaks (Fig. 2B). The retention times of EGC, epicatechin, EGCG, and ECG were 8, 16, 21.5, and 33 min, respectively; TF, TF-3-G, TF-3'-G, and TFdiG eluted at 41, 44, 45.5, and 46.5 min, respectively. In addition to the catechin and theaflavin peaks, five unknown peaks (ba–be) were observed (Fig. 2B). Whereas peak bB was found in the tea solution, peaks bA, bC, bD, and bE were formed over the time course of the

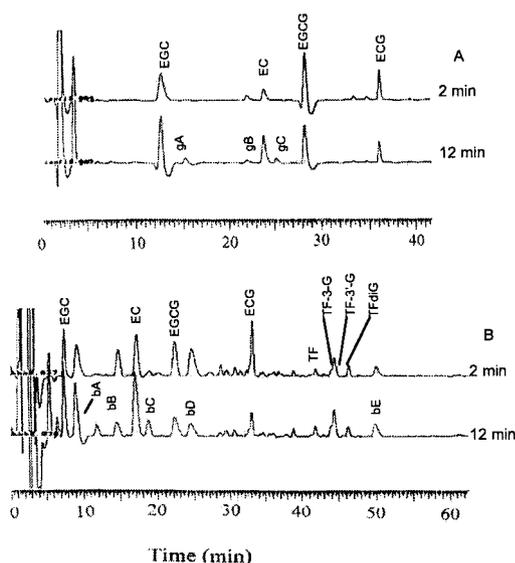


Fig. 2. A, representative high-performance liquid chromatography (HPLC) profile of saliva at 2 and 12 min after holding green tea leaves (2 g) for 5 min. B, representative HPLC profile of saliva at 2 and 12 min after holding black tea extract (50 ml) for 2 min. Samples were collected after voiding of the tea leaves and rinsing. Detection at 100 mV. For clarity, the unknown peaks are marked on the 12 min chromatogram.

experiment. These peaks are currently being identified by liquid chromatography/mass spectrometry and nuclear magnetic resonance. The concentrations of peaks gA–gC and bA–bE were estimated by assuming that the compounds had similar electrochemical properties to the catechins and theaflavins, respectively. Peak heights were compared with the peak heights of standards of known concentration.

**Salivary Levels of Green Tea Polyphenols.** The efficiency of chewing tea leaves in the mouth for extracting catechins was compared with that of successively boiling the leaves for 10 min in 200 ml of water. It was interesting to note that chewing 2 g of green tea leaves in the mouth for 20 min extracted EGCG, ECG, and epicatechin from the leaf matrix as effectively as boiling green tea leaves in 200 ml of water for four 10-min periods (Table 1). A gC peak was not observed when

Table 2 Catechin peak levels and elimination half-lives after holding green tea leaves in the mouth for 5 min

Compound	Peak level ( $\mu\text{g/ml}$ )		$t_{1/2}$ (min)
	Chewing	Holding	Holding
EGCG	31–90	7.8–60	$33.6 \pm 2.2$
ECG	11–28	2.0–20	$35.7 \pm 2.2$
EGC	14–160	9.5–80	$43.9 \pm 13.3$
Epicatechin	5.7–28	2.0–18	$37.0 \pm 7.3$

the tea leaves were boiled and are apparently formed during the chewing process.

Initially, it was hypothesized that chewing green tea leaves would result in a higher salivary level of catechins compared with holding the leaves without chewing. Chewing did lead to an increase in the peak levels of catechins in the saliva of 2–4-fold (Table 2). However, in some subjects, at later time points, there was a second increase in the concentration of the catechins (Fig. 3A). In experiments to accurately determine the kinetics of the catechins in the saliva, however, we elected to have the subjects hold the leaves in their mouths without chewing.

Salivary levels of catechins after holding tea leaves in the mouth without chewing were maximal in the first 10 min and decreased in a time-dependent manner (Fig. 3B). EGC and EGCG were found in the highest concentrations ( $39 \pm 31$  and  $31 \pm 21 \mu\text{g/ml}$ , respectively). The  $t_{1/2}$  for the catechins was 34–44 min (Table 2). The unknown peaks gA and gC, but not gB, increased as a function of time, reaching a maximal level (estimated  $C_{\text{max}} = 2.5\text{--}4.0 \mu\text{g/ml}$ ) at 50 and 22 min, respectively, followed by a time-dependent decrease (Fig. 3C). For cA, cB, and cC, the  $t_{1/2}$  was  $34.8 \pm 2.6$ ,  $29.5 \pm 2.6$ , and  $26.5 \pm 13$  min, respectively.

**Salivary Levels of Black Tea Polyphenols.** Similar experiments were attempted using black tea leaves; however, it was observed that black tea leaves were too brittle and that particles of black tea leaves remained in the mouth even 60 min after vigorous rinsing. This led to variable levels of tea polyphenols in the saliva. The experiments, therefore, were conducted using black tea extract. The composition of black tea extract was determined by boiling 2 g of leaves in 100 ml of water for 10 min before decanting off the liquid. An aliquot was combined

Table 1 Amounts of green tea polyphenols extracted from 2 g green tea leaves by four successive boiling periods of 10 min in 200 ml of water and percentage extracted by chewing green tea leaves

The catechin extraction efficiency of chewing green tea leaves was compared with that of boiling an equal amount of tea leaves in water. Green tea leaves (2 g) were boiled four times for 10 min each in 200 ml of water. An aliquot of this extract was then diluted and analyzed by high-performance liquid chromatography (HPLC). In another experiment, green tea leaves (2 g) were gently chewed for 20 min. The leaves were voided (5–10 ml of saliva), and the subjects washed the mouth vigorously 10 times with water (total volume = 350 ml) in a 2-min period. The leaves and washout were combined and centrifuged. The clear supernatant was then diluted and analyzed by HPLC.

Compound	Hot water extractable (mg)					Saliva washout (mg/ml)	Total saliva amount (mg)	% saliva extractable
	1st	2nd	3rd	4th	Total			
EGCG	9.6	9.1	7.5	8.2	34.5	4.5	33.8	98.1
ECG	3.5	3.1	2.8	3.3	12.7	1.6	12.1	95.1
EGC	17.0	7.7	4.6	4.2	33.4	2.4	18.2	54.6
Epicatechin	5.1	3.1	2.4	1.8	12.4	1.6	12.2	98.3
gA <sup>a</sup>	0.75	0.61	0.41	0.21	1.98	1.0	7.4	373
gB <sup>a</sup>	4.4	2.7	2.0	0.89	10.0	0.24	1.8	17.7
gC <sup>a</sup>	0	0	0	0	0	0.06	0.4	n/a

<sup>a</sup> These amounts are based on the assumption of similar electrochemical behavior between the unknown compounds and the catechins. The amounts were determined by comparing the peak height of the unknowns to the peak heights of a standard catechin mixture.

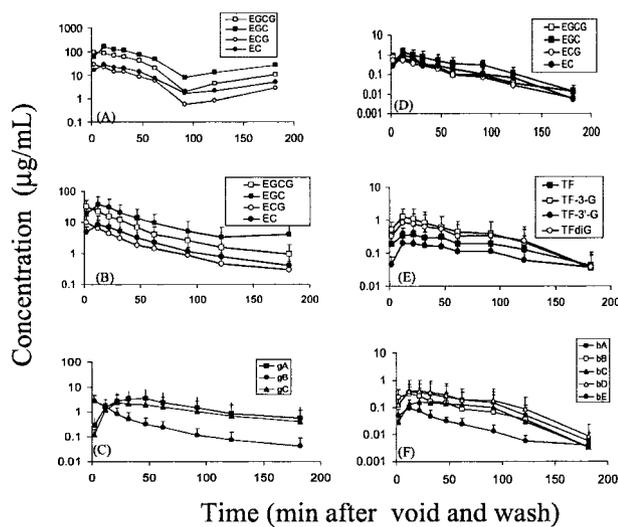


Fig. 3. Salivary levels of tea polyphenols after placing tea in the mouth. A, levels of catechins in the saliva of one subject over the course of the experiment after gentle chewing of green tea leaves for 5 min. Salivary levels (B) of catechins or unknown peaks (C) over the course of the experiment after the holding of green tea leaves. Salivary levels of catechins (D), theaflavins (E), or unknown peaks (F) over the course of the experiment after the holding of black tea extract. All of the samples were collected after voiding tea leaves or solution and after washing of the mouth vigorously for 2 min with water. Detection at 100 mV.

with an equal volume of 60% aqueous acetonitrile and was analyzed by HPLC as above (Table 3). TFdiG and EGCG were the most abundant components at 40.7 and 74.0  $\mu\text{g/ml}$ , respectively.

After the black tea solution was held in the mouth for 2 min, followed by vigorous rinsing, both catechins and theaflavins could be detected (Fig. 2B). The levels of black tea polyphenols increased and then decreased with a similar profile in all of the subjects (Fig. 3, D and E), although the peak levels of these compounds were quite variable among the subjects. EGC (0.45–2.8  $\mu\text{g/ml}$ ), epicatechin (0.20–2.5  $\mu\text{g/ml}$ ), TF-3-G (0.5–2.6  $\mu\text{g/ml}$ ), and TFdiG (0.34–2.0  $\mu\text{g/ml}$ ) were present at the highest concentrations. The  $t_{1/2}$  for the theaflavins and catechins were 49–76 min and 25–31 min, respectively (Table 3). Compared with the results from holding green tea leaves, the  $t_{1/2}$  of the catechins is slightly shorter but the difference is not statistically significant. In addition to the expected theaflavin and catechin peaks, five unknown peaks (bA–bE) were observed in the saliva after holding black tea extract in the mouth (Fig. 2B). Compound bB was present in the black tea extract, whereas the other compounds were found only in the saliva and

Table 3 Levels of catechins and theaflavins in black tea solutions and elimination half-lives after holding black tea extract in the mouth for 2 min<sup>a</sup>

Compound	Black tea extract ( $\mu\text{g/ml}$ )	Peak level ( $\mu\text{g/ml}$ )	$t_{1/2}$ (min)
EGCG	74.0	0.33–1.9	30.4 $\pm$ 5.6
ECG	36.7	0.25–1.6	24.9 $\pm$ 3.4
EGC	19.3	0.45–2.8	31.2 $\pm$ 1.9
Epicatechin	16.0	0.20–2.5	27.6 $\pm$ 8.1
TF	24.6	0.10–0.80	60.1 $\pm$ 46.0
TF-3-G	29.3	0.50–2.6	48.5 $\pm$ 31.2
TF-3'-G	21.3	0.05–0.6	75.8 $\pm$ 73.5
TFdiG	40.7	0.34–2.0	67.9 $\pm$ 65.1

<sup>a</sup> These data were obtained from the same experiment shown in Fig. 3, D–F.

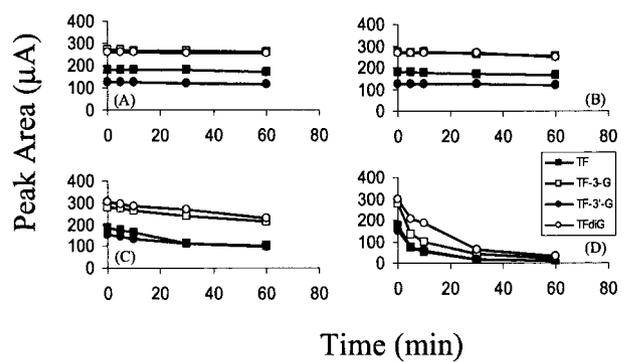


Fig. 4. Stability of theaflavins as a function of pH. Incubations were in 50 mM sodium phosphate buffer. With 0.25 mg of theaflavins, pH 6.5 (A), pH 7.0 (B), pH 7.5 (C), pH 9.0 (D).

seem to have formed while the extract was held in the mouth. The  $C_{\text{max}}$  of bA–bE ranged from 0.10 to 0.49  $\mu\text{g/ml}$ ;  $t_{1/2}$  for bA, bB, bC, bD, and bE were  $33.4 \pm 4.3$ ,  $25.9 \pm 5.7$ ,  $37.7 \pm 15.1$ ,  $34.2 \pm 10.7$ , and  $38.5 \pm 6.1$  min, respectively (Fig. 3F).

After pure theaflavins (0.18 mg/ml) were held in the mouth, higher peak salivary levels (TF, 2.5–23  $\mu\text{g/ml}$ ; TF-3-G, 8.9–85  $\mu\text{g/ml}$ ; TF-3'-G, 1.9–40  $\mu\text{g/ml}$ ; TFdiG, 2.5–43  $\mu\text{g/ml}$ ), but similar kinetics of elimination, were observed (data not shown). Peaks bA–bE were not observed when pure theaflavins were held in the mouth.

**Stability of Theaflavins.** Previously, it has been shown that catechins are unstable under neutral and alkaline conditions (16), but no reports have been made concerning the stability of theaflavins. Here, we addressed this issue to determine the optimal experimental conditions and to determine what the effects of physiological pH and temperature would have on the stability of theaflavins. Theaflavins were incubated at pH 6.5, 7.0, 7.5, and 9.0 at room temperature. Fig. 4 shows that theaflavins were stable at pH 6.5 but slowly degraded at pH 7.0 and 7.5. At pH 9.0, degradation occurred very rapidly. Whereas it has been reported that EGCG and EGC are less stable than epicatechin and ECG in alkaline pH, we did not observe any significant differences in stability among the four theaflavins. This may indicate that degradation occurs primarily at the benzotropolone moiety.

Analysis of the effect of temperature on theaflavin stability at pH 3.3 showed that, in the absence of ascorbic acid, incubation of theaflavins at 37°C results in a 20% loss of the starting amount of theaflavins after 24 h. The addition of ascorbic acid-EDTA and decreased temperatures greatly reduced the rate of decomposition. Theaflavins in the presence of 0.01% ascorbic acid were stable for at least 3 days and 6 months at 6°C and –80°C, respectively.

The effect of salivary catechin esterase activity on TFdiG stability was studied by incubating pure TFdiG in the presence of human saliva at 37°C. The level of TFdiG decreased over time (25% decrease after 60 min) with a concomitant rise in the amount TF-3-G and TF-3'-G, suggesting that TFdiG was hydrolyzed to form these two compounds. This is similar to our previous observations with EGCG, which was hydrolyzed by salivary esterases (13).

## Discussion

In previous studies, we have shown that oral consumption of green tea, or holding brewed green tea in the mouth, results in

salivary tea catechin levels that are two orders of magnitude greater than plasma levels from similar doses of tea (13). Here we show that high salivary levels of tea polyphenols can be achieved by holding either green tea leaves or black tea extract in the mouth. Whereas there is significant interindividual variation, holding green tea leaves in the mouth results in a higher  $C_{\max}$  (2.0–80  $\mu\text{g/ml}$ ) and longer  $t_{1/2}$  (33.6–43.9 min) compared with previous results ( $C_{\max} = 1.8\text{--}44 \mu\text{g/ml}$ ;  $t_{1/2} = 11\text{--}15 \text{ min}$ ; Ref. 13).

Initially, it was hypothesized that chewing green tea leaves would result in the higher salivary levels of catechins compared with holding the leaves without chewing. Chewing did lead to an increase in the peak levels of catechins in the saliva of 2–4-fold (Table 2). However in some subjects, at later time points, there was a second increase in the concentration of the catechins (Fig. 3A). This increase may be the result of a decrease in the volume of saliva produced after long periods of chewing tea leaves. When subjects consumed large volumes of water immediately before the start of the experiment, these later increases were not observed (data not shown). It is also possible that tea leaf particles become trapped in the spaces between the teeth. These fine particles may get into the saliva samples at later time points and affect the measurements of catechin levels. For practical application, chewing tea leaves may be more efficient for delivering high concentrations of tea catechins to the oral mucosa. In experiments to accurately determine the kinetics of the catechins in the saliva, however, we elected to have the subjects hold the leaves in their mouths without chewing.

Holding pure theaflavins (0.18 mg/ml) in the mouth results in 10–20-fold higher salivary levels of these compounds than did holding black tea extract, which has a similar concentration of theaflavins: there was no effect on elimination kinetics. These results may indicate that other components in black tea interfere with the transfer of theaflavins from the solution into the saliva and the oral mucosa.

In addition to the theaflavin and catechin peaks, we observed several unknown peaks in saliva after holding green tea leaves or black tea extract in the mouth. Compounds gC, bA, and bC–bE were not present in the tea leaves or black tea extract and appeared to form while the leaves were held in the mouth: compound gA was present in the green tea leaves and increased in the saliva over time before decreasing because of elimination. On the basis of comparisons with pure catechin and theaflavin standards, these peaks reached high ng/ml to low  $\mu\text{g/ml}$  levels and may represent metabolites created in the oral milieu by either human enzyme systems or oral microflora. Additional experiments are needed to identify these peaks and determine their origin. The concentrations of these compounds can then be accurately measured by comparison with standards. When pure theaflavins were held in the mouth, compounds bA–bE were not observed, indicating that the compounds are derived from some component of black tea other than theaflavins. Moreover, because the identity of these compounds remains unknown, we cannot comment on their potential contribution to the biological effects of green or black tea in the oral cavity.

The present study also demonstrates that chewing green tea leaves is as effective at extracting EGCG, epicatechin, and ECG from the leaf matrix as is repeatedly boiling the leaves. Previously, Khafif *et al.* (17) reported that the growth of cell lines derived from oral leukoplakia patients and oral cancer patients was inhibited by EGCG after a 5-day treatment, with  $IC_{50}$  equal to 8–18  $\mu\text{M}$ . Treatment with EGCG, EGC, or epicatechin, at concentrations of 80  $\mu\text{M}$ , also resulted in growth

inhibition of oral squamous cell carcinoma cells (10). These concentrations are within the range achievable in the saliva by holding (2–131  $\mu\text{M}$ ) or chewing (12–260  $\mu\text{M}$ ) green tea leaves. Although a single administration of these concentrations of tea polyphenols is probably not sufficient to elicit an anticancer effect, it is possible that, by holding tea leaves in the mouth regularly over the course of a day, effective levels of catechins or theaflavins could be maintained in the oral cavity. The salivary levels presented in this report were measured after the leaves were voided from the mouth. The amount of EGCG available to oral mucosa when the tea is still in the mouth should be similar to the saliva washout (9.8 mM) or black tea extract (162  $\mu\text{M}$ ). The total concentration of theaflavins in the black tea extract was 161  $\mu\text{M}$ . These data support the potential usefulness of holding tea leaves in the mouth as a means of oral cancer prevention.

In the present study, we also studied the stability of theaflavins. Our purpose was two-fold. First, we wanted to establish that these compounds were stable under our experimental conditions and would not degrade during analysis. Theaflavins are stable under acidic conditions and in the presence of ascorbic acid. Second, the stability of theaflavins in the oral cavity and gastrointestinal tract may affect the bioavailability of these compounds. Theaflavins have been shown previously to have very low bioavailability. Mulder *et al.* (18) found that the maximum plasma concentration of theaflavins was 1 ng/ml in volunteers after oral ingestion of 700 mg of theaflavins (equivalent to 30 cups of black tea). We demonstrate that the theaflavins are stable under acidic conditions (pH 6.5) but degrade at neutral and basic pH (pH >7.0). The pH of the oral cavity is usually 7.0–7.4 and it is expected that under physiological conditions, some degradation of theaflavins will occur. Once the compounds enter the gastric environment, they are probably stable. We have observed that theaflavins are stable in simulated gastric juice (pH 1.7) at 37°C for greater than 4 h (data not shown). Likewise, the slightly acidic (pH 6.4) and largely anaerobic environment of the small intestine disfavors theaflavin degradation (19). In the colon, however, the pH becomes more basic (pH ~8.0), and the potential for degradation may arise, but the low oxygen tension disfavors oxidative degradation of theaflavins. On the basis of our results, the low bioavailability of theaflavins is probably not due to pH or temperature-dependent degradation in the gastrointestinal tract. In contrast, the low bioavailability of these compounds is probably due to the high molecular weight and large polar surface area of these compounds (20). The hydroxyl groups on theaflavins likely form a large hydration shell that gives the compounds greater apparent surface area and prevents movement through the plasma membrane (21).

In summary, this report demonstrates that holding green tea leaves or black tea extract in the mouth represents a method to achieve locally high levels of tea polyphenols. Black tea leaves proved to be too brittle and resulted in the deposition of plant material in the mouth, which confounded attempts at determining theaflavin and catechin levels in this study. Similarly, chewing green tea leaves resulted in significant variation in the data but higher levels of catechins than holding tea leaves. In practical application, however, chewing or holding green or black tea leaves probably results in the most effective delivery of these compounds to the oral cavity, and is the most convenient and economical sustained delivery system. Development of sustained release products such as chewing gum may aid in the esthetic acceptance and standardization of tea polyphenol delivery. The data presented here should prove useful in designing quality intervention trials to determine the effective-

ness of tea as an oral cancer preventive and anticariogenic substance.

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# Cancer Epidemiology, Biomarkers & Prevention

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*Cancer Epidemiol Biomarkers Prev* 2004;13:132-137.

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