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Determination of Sucralose in Splenda and a Sugar-Free Beverage Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

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Sucralose is a chlorinated carbohydrate nonnutritive sweetener of food and beverage products. The determination of sucralose in food and beverages is important to ensure consistency in product quality. Sucralose was determined in two commercial products without sample preparation using high-performance anion-exchange (HPAE) chromatography coupled with pulsed amperometric detection (PAD). Sucralose was determined with a 10 min isocratic separation. To determine sucralose and other carbohydrates (e.g., dextrose) simultaneously, a gradient separation was developed. The linear range of electrochemical response extended over 3 orders of magnitude, from 0.01 (LOD) to 40 μM (16 μg/mL; 25 μL injection). High precision, high spike recovery, and method ruggedness were observed for both samples.

KEYWORDS: Sucralose; anion-exchange chromatography; HPAE-PAD; pulsed amperometric detection; integrated pulsed amperometric detection; HPLC; carbohydrates; artificial sweeteners; Diet Rite; Splenda

INTRODUCTION

Sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside) is used in the manufacture of artificially sweetened food and beverage products. Sucralose, and most other carbohydrates, lack a good chromophore and therefore require high sample concentrations to be detected by UV absorbance. Refractive index detection also requires high sample concentrations (*1*, *2*). Many food and beverage ingredients either are chromophoric or have refractive properties and can interfere with the direct detection of sucralose by these detection methods. Carbohydrates, glycols, alcohols, amines, and sulfur-containing compounds can be oxidized and therefore directly detected by pulsed amperometric detection (PAD). This detection method is specific for those analytes that can be oxidized at a selected potential, leaving all other compounds undetected. PAD also has a broad linear range and very low detection limits.

High-performance anion-exchange chromatography (HPAE) is a technique capable of separating most carbohydrates and their analogues (*3*, *4*). For complex samples containing sucralose, such as foods and beverages, the high resolving power of HPAE and the specificity of PAD allow the determination of sucralose with little interference from other ingredients (*5*−*7*).

This paper describes the development of HPAE-PAD methods to determine sucralose in an artificial sweetener and a beverage. We use a different anion-exchange column than that used in previously published HPAE-PAD methods to enable faster separations, and we demonstrate the use of a gradient method to simultaneously determine dextrose and sucralose. The previously published HPAE-PAD methods (*5*−*7*) for sucralose determinations used an older electrochemical program (triple-potential waveform). A newer waveform for carbohydrate detection developed by Rocklin et al. (*8*, *9*) provides a greater long-term reproducibility of electrochemical response and is recommended by the instrument manufacturer. Here we use the improved waveform with the recently introduced disposable gold working electrodes. The disposable gold working electrodes have greater electrode-to-electrode (and system-to-system) reproducibility than the permanent gold working electrodes, thereby further improving the method reproducibility. Another waveform that has been used for simultaneous determinations of amino acids and carbohydrates (*AAA-Direct* is a registered trademark of Dionex Corporation) (*10*) was also studied. We evaluated the performance of the gradient and isocratic separations using the recommended waveform for carbohydrate determinations and the waveform for simultaneous determination of amino acids and carbohydrates. These methods were evaluated with Red Raspberry Diet Rite (Diet Rite is a registered trademark of Dr. Pepper/Seven Up, Inc.) and Splenda (Splenda is a registered trademark of McNeil-PPC, Inc.). Red Raspberry Diet Rite is a soft drink artificially sweetened with sucralose. This beverage also contains natural and artificial flavors, a preservative (potassium benzoate), an acidulant system (citric acid/potassium citrate), food dye, and another nonnutritive sweetener (acesulfame potassium). Splenda is a solid artificial sweetener used as a sugar substitute, consisting of dextrose, maltodextrin, and sucralose. Sucralose was separated from the other ingredients in these samples and quantified. We present detection limits, linear ranges, ruggedness, and results of spike recovery experiments using these methods.

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MATERIALS AND METHODS

Materials. All chromatography eluents, standards, and samples were prepared using 18 MΩ·cm deionized water, free of electrochemically active impurities. NaOH (50% (w/w)) was purchased from Fisher Scientific (Pittsburgh, PA) or J.T. Baker Chemical Co. (Phillipsburg, NJ). Anhydrous sodium acetate used to make eluent was obtained from Dionex Corp. (Sunnyvale, CA; P/N 059326) certified for *AAA-Direct* use. Other sources of sodium acetate may cause detection problems when using the *AAA-Direct* waveform. Dextrose was purchased from Pfanstiehl Labs (Waukegan, IL), MALTRIN maltodextrins M040 was obtained from Grain Processing Corp. (Maltrin is a registered trademark of Grain Processing Corporation, Muscatine, IA), and sucralose was obtained from McNeil Nutritionals, Inc. (Fort Washington, PA). Solid sucralose and dextrose standards were dissolved in deionized water to a 10 mg/mL concentration. These were mixed and diluted with water to yield the desired concentrations. The solutions were maintained frozen at −5 °C until needed. Red Raspberry Diet Rite beverage was diluted 50-fold, or as needed, in deionized water and analyzed directly. Splenda sugar substitute was prepared at a concentration of 100 mg/mL with deionized water. This solution was diluted to 100 μg/mL and analyzed directly.

Chromatography System. The chromatography system used for this work was a Dionex BioLC microbore (2 mm) system with degas option, ED50 Electrochemical Detector, AS50 Autosampler and AS50TC Thermal Compartment. The anion-exchange column set used was the CarboPac PA20 (3 × 150 mm, P/N 060142, Dionex) with its guard (3 × 30 mm, P/N 060144, Dionex). (BioLC and CarboPac are registered trademarks of Dionex Corporation).

Two electrochemical waveforms were investigated: *AAA-Direct* waveform and carbohydrate waveform.

AAA-Direct Waveform. +0.13 V from 0.00 to 0.04 s, +0.33 V from 0.05 to 0.21 s, +0.55 V from 0.22 to 0.46 s, +0.33 V from 0.47 to 0.56 s, −1.67 V from 0.57 to 0.58 s, +0.93 V at 0.59 s, and +0.13 V at 0.60 s using the pH reference mode; current was integrated between 0.21 and 0.56 s for detection.

Carbohydrate Waveform. +0.10 V from 0.00 to 0.40 s, −2.0 V from 0.41 to 0.42 s, +0.6 V at 0.43 s, −0.10 V from 0.44 to 0.50 s using the Ag/AgCl reference mode; current was integrated between 0.20 and 0.40 s for detection.

We used *AAA-Direct-Certified* disposable gold working electrodes (P/N 060082) with the *AAA-Direct* Waveform (replaced every 7 days), and *Carbohydrate-Certified* disposable gold working electrodes (P/N 0600139) with the Carbohydrate Waveform (replaced every 14 days) (*11*). Eluents (water in channel A, 250 mM NaOH in channel B, 1 M sodium acetate in channel C) were prepared as recommended by the manufacturer (*12*). Sucralose was separated using a flow rate of 0.50 mL/min and a column temperature of 30 °C with a 10 or 25 μL injection. The isocratic separation method (40 mM NaOH and 75 mM sodium acetate) was produced by allowing the pump to proportion 76.5% A, 16% B, and 7.5% C. This method had a run time of 9.55 min. Alternatively, the 40 mM NaOH and 75 mM sodium acetate eluent may be prepared as a single solution without pump proportioning. The column set was washed periodically with 40 mM NaOH/800 mM sodium acetate (16% B, 80% C) for 30 min to restore retention times to their initial values. The gradient method (used for simultaneous determinations of dextrose and sucralose) used 40 mM NaOH (16% B) for 5 min, followed by a 10 min gradient of 40 mM NaOH to 40 mM NaOH (16% B)/75 mM sodium acetate (7.5% C) with the final concentration held for an additional 5 min, followed by a step to 40 mM NaOH (16% B)/800 mM sodium acetate (80% C) for 10 min to wash the column, and then a return to starting conditions for 15 min to prepare for the next injection.

RESULTS AND DISCUSSION

Chromatographic Separation. Figure 1 shows the isocratic separation of sucralose using a CarboPac PA20 column set. Using this method, sugar alcohols (alditols), glycols, and mono- and disaccharides are not retained and elute near the void volume while sucralose is retained and typically elutes at about 5.6 min.

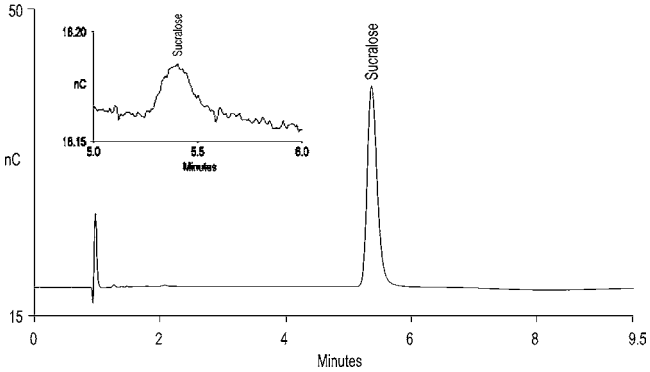

Figure 1. Separation of sucralose (10 μM, 25 μL injection) using a CarboPac PA20 column set with 40 mM NaOH and 75 mM sodium acetate at 0.5 mL/min at 30 °C. (Inset) Sucralose at the lower limit of detection of 0.28 pmol (0.01 μM, 25 μL injection).

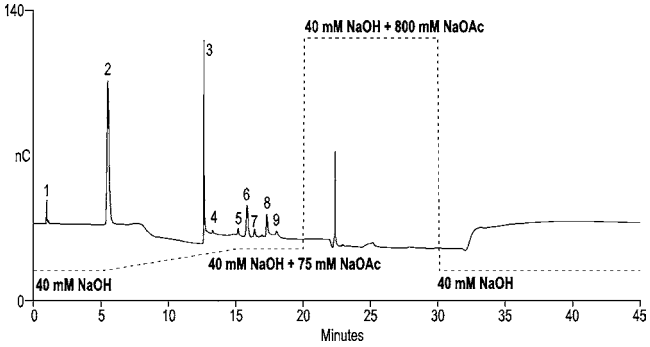

Figure 2. Simultaneous determination of 10 μM dextrose (glucose) and sucralose using a gradient method (40 mM NaOH for 5 min, then a linear gradient to 40 mM NaOH 75 mM sodium acetate over 10 min) using the CarboPac PA20 analytical and guard columns at 0.5 mL/min and a 30 °C column temperature, 25 μL injection. Peaks: (1) void, (2) dextrose, (3–5, 7–9) system peaks, (6) sucralose.

Run times of 10 min were routinely used for this method. The use of this column set and eluent condition reduced the run time for sucralose determinations from 15 min (*5*−*7*) using the CarboPac PA1 column set to 10 min.

Simultaneous determination of monosaccharides (e.g., dextrose) and sucralose was performed using a gradient separation method. Figure 2 shows the analysis of a standard mixture of 10 μM dextrose (peak 2; 5−6 min) and sucralose (peak 6; 16 min). Peak 1 was the void, and peaks 3, 4, 5, and 7−9 were found in a blank (water) injection and the result of trace impurities in the water injected and accumulation of impurities in the eluent prior to application of the acetate gradient. Peaks eluting during the acetate gradient also occurred in the absence of any injection, and the extent and profile of these peaks varied with the length of the time period following the method's column wash step and the quality of reagents used for eluent preparation. Generally, peaks detected by PAD using the two waveforms investigated in this paper are compounds containing hydroxyl, amino, and thio groups. These peaks did not interfere with sucralose determinations.

Sucralose (10 μM) chemically degraded in the 40 mM NaOH eluent at a rate of 0.11%/min (25 °C). This decomposition rate was insignificant for the short interval of time (5.6-min isocratic or 16-min gradient separations) that sucralose was exposed to NaOH during chromatography. Because the exposure time was the same for calibration standards and the unknown samples, any minor decomposition is self-correcting. When sucralose was treated with NaOH for 48 h, seven reaction product peaks were

resolved, eluting at 1.01, 1.18, 1.50, 1.98, 2.23, 2.49, and 4.10 min using the isocratic method. These peaks did not interfere with sucralose determination and increased in amount with a concomitant decrease in the sucralose peak during treatment with NaOH. The reaction products were not identified and probably resulted from simple displacement (i.e., alcohols) and alkylation (i.e., cyclic ethers) reactions with NaOH. Sucralose standards and samples should not be prepared in NaOH.

Detection. Two commonly used electrochemical detection waveforms, a quadruple-potential carbohydrate waveform and the *AAA-Direct* waveform, were compared for suitability with this analytical method. The quadruple-potential carbohydrate waveform has two advantages for this application: (1) the response for most amino acids and similar compounds with amino groups is low while response for carbohydrates is high and (2) the *Carbohydrate-Certified* disposable electrode used with this waveform can be used for at least twice as long (14 days) as the *AAA-Direct-Certified* disposable electrode used with the *AAA-Direct* waveform. The *AAA-Direct* waveform has the advantage of having a 2−3-times greater signal-to-noise ratio for sucralose peak area or height response. We used the carbohydrate waveform with corresponding electrodes because it allowed longer use of each disposable Au working electrode and had better day-to-day peak area reproducibility. For applications where greater sensitivity is required, the *AAA-Direct* waveform should be considered. Either waveform, with its corresponding working electrode, produces stable detector response for common sugars, such as sucrose, fructose, and glucose, with no temporary or long-term electrode fouling (*10*, *13*).

Linearity. The region of highest linearity of peak area response to amount injected was estimated to extend from 1.24 to 250 pmol per injection ($r^2 = 1.000$). The corresponding mean peak area response factor (slope) for this range was 17.0 nC·min/pmol). In this paper we define the linear concentration range to be that region where the peak area response factor (slope) was within ±20% of this highly correlated linear region. The mass (pmol) of sucralose injected having peak area response factors below 13.6 nC·min/pmol were considered outside the upper linear range. Our results showed peak area linearity ($r^2 = 0.9958$) extended from the lower limit of detection (LOD) up to 1000 pmol (40 μM for 25 μL injection). The peak height was linear ($r^2 = 0.9942$) from the LOD to 760 pmol (30 μM for 25 μL injection). High linearity throughout the lower concentration range indicated that sucralose was sufficiently stable under the alkaline eluent conditions used in this method.

Lower Limits of Detection and Quantification. Baseline peak-to-peak noise was measured in maximum/minimum peak height units (pC) over 1-min intervals. Throughout this study period, lasting several months, baseline noise for the isocratic separation method ranged from 5.3 to 112.5 pC (mean ± SD; 15.4 ± 11.3, $n = 1690$ injections) using the carbohydrate waveform. The concentration (or mass injected) of sucralose at the lower limit of detection (LOD) was calculated from 3 times the average peak-to-peak noise, divided by the average peak height response factor for sucralose within its linear region. Similarly, the lower limit of quantification (LOQ) is the concentration (or mass injected) calculated from 10 times the average peak-to-peak noise. The estimated LOD using the carbohydrate waveform was found to be 0.28 pmol (0.01 μM for 25-μL injection), and the LOQ was 0.93 pmol (0.04 μM for 25-μL injection). Figure 1 (inset) shows the sucralose peak at the LOD where the peak height is about 3 times above the noise.

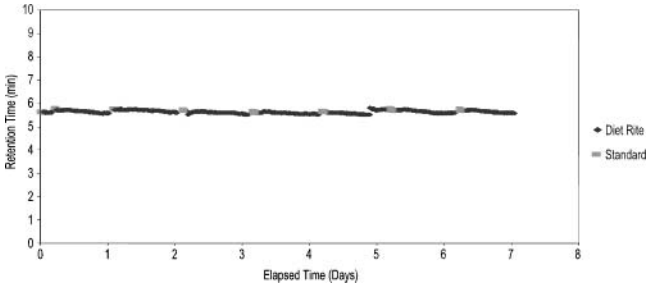

Figure 3. Reproducibility of sucralose retention times for a standard (A) and for sucralose in a 50-fold dilution of Red Raspberry Diet Rite beverage (B) over 7 days of consecutive injections (836) using the separation conditions in Figure 1.

Precision and Reproducibility. The peak area and retention time precision were determined for replicate injections of a sucralose standard (10 μM for 10 μL injection) over 4.7 days (619 injections) using the isocratic separation method with the carbohydrate waveform. The sucralose retention time ranged from 5.49 to 5.67 min (mean ± SD; 5.60 ± 0.04 min, 0.7% RSD). No upward or downward trend was observed for at least 4 days when analyzing a relatively pure sucralose standard. For complex samples such as the Red Raspberry Diet Rite beverage, the same method resulted in a gradual loss of sucralose retention time. This was corrected by using periodic column washes (40 mM NaOH/800 mM sodium acetate; 30 min every 24 h, 0.5 mL/min). Figure 3 shows sucralose retention times over 7 days for the following sequence of injections: six injections of sucralose standard (B), followed by 120 injections of a 50-fold diluted beverage (A), followed by a daily 30 min wash with 40 mM NaOH/800 mM sodium acetate at 0.5 mL/min. This sequence was repeated 7 times. Retention times ranged from 5.51 to 5.83 min with a trend to shorter retention times within each day. Sucralose retention time was restored to its original value after each 30 min column wash. The intraday precision (RSD) within each of the seven 24 h periods range from 0.1% to 1.2% for standards (10 μM, $n = 6$ injections per day) and from 0.5% to 1.2% for the Diet Rite beverage (7.8 μM measured concentration, $n = 120$ injections per day).

The peak area and peak height RSDs for a sucralose standard (10 μM for 10 μL injection) in the 4.7 day experiment above (619 injections) were 2.7% (1.340 ± 0.037 nC·min) and 3.1% (7.09 ± 0.22 nC), respectively. No increasing or decreasing trends were observed, as expected for the carbohydrate waveform (*8*). The peak area RSDs for the sucralose standard and the sucralose measured in a 50-fold diluted Red Raspberry Diet Rite over 7 days were 2.4% and 3.0%, respectively. Daily peak area RSDs ranged from 0.3% to 2.9% for the standard and from 1.6% to 2.6% for the sample. The high retention time and response reproducibility indicate that this method is suitably rugged for this application.

Determination of Sucralose in Red Raspberry Diet Rite Beverage. The Red Raspberry Diet Rite beverage product contains carbonated water, citric acid, natural and artificial flavors, sucralose, caramel color, potassium citrate, potassium benzoate, acesulfame potassium (Sunett Brand, nonnutritive sweetener), (Sunett is a registered trademark of Nutrinova, Inc.), and Red Dye #40. The freezing and thawing of this beverage for sample storage essentially eliminated all carbonation. Figure 4A shows the isocratic separation of beverage. Sucralose was well resolved from the other detected ingredients. Most ingredients, including acesulfame, are not detected by PAD. The measured concentration of sucralose in a 25 μL injection of a 50-fold dilution of this beverage was 7.8 μM. Corrected for

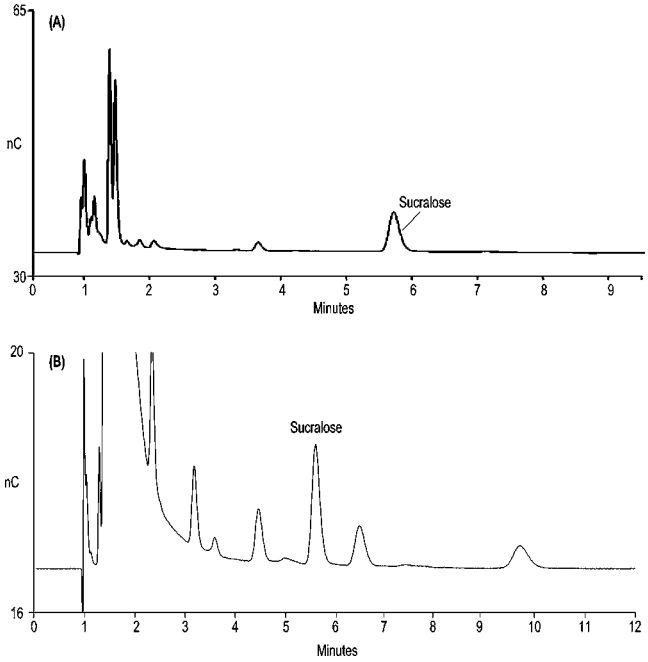

Figure 4. Determination of sucralose in (A) Red Raspberry Diet Rite beverage (1–50 dilution) and (B) 100 μg/mL Splenda using the method in Figure 1.

dilution, the concentration of sucralose in the beverage was determined to be 390 μM (155 μg/mL). Spike recovery (10 μM sucralose in 50-, 100-, 500-, 1000-, 10 000-fold dilutions of the beverage) ranged from 92% to 96%. The high recovery observed in this study is consistent with the high recoveries observed by Ichiki, Kobayashi, and Kishi for a number of food samples (*5*−*7*), including coffee, tea, apple juice, biscuit, chocolate, pudding, yogurt, fruit jam and jelly, miso, ketchup, cheesecake, and chewing gum. We speculate that the sample preparation procedures described for these foods are compatible with the chromatography methods presented here because these previously published methods used a CarboPac column similar to the one used here with PAD.

Determination of Sucralose in Splenda. Splenda is a granular no calorie sweetener added to food and beverages in a manner similar to granulated table sugar. The ingredients listed on the product label include dextrose, maltodextrin, and sucralose. Maltodextrins are partial hydrolysates of starch. Figure 4B presents the isocratic separation of 100 μg of Splenda/mL (10 μL injection). The sucralose peak was resolved from other ingredients, while the dextrose peak eluted at the column void volume. Some maltodextrin peaks eluted before and after sucralose, while other peaks were poorly detected due to their low concentration or eluted during the periodic column wash.

Dextrose and sucralose were determined in the same injection using a gradient method that first eluted dextrose at a low hydroxide concentration (40 mM), followed by an acetate gradient that eluted the more strongly retained sucralose peak from the stationary phase. Figure 5 shows the separation of both dextrose and sucralose in Splenda using a gradient separation where the dextrose peak (peak 3) is displayed full scale, and the sucralose peak appears as a minor peak (peak 10). The inset to Figure 5 expands the signal response of the chromatogram after dextrose elutes to better display the sucralose peak. Besides dextrose (peak 3) and sucralose (peak 10), peaks 4, 7, 8, 11, 15, and 17 are not found in the blank or standards. Peaks 7, 8, 11, 15, and 17 are maltodextrins based on retention time identity using MALTRIN maltodextrins M040 as a standard. The

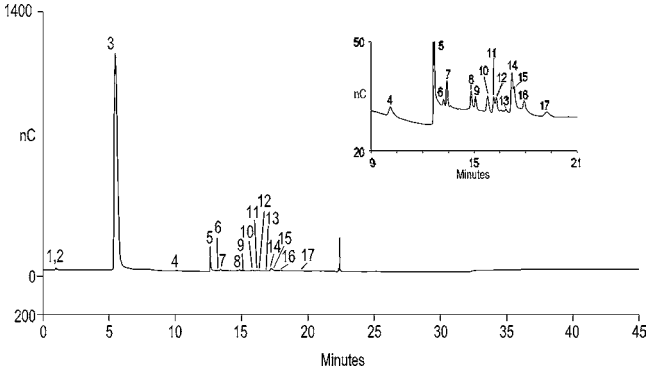

Figure 5. Determination of dextrose (peak 3) and sucralose (peak 10) in 100 μg/mL Splenda using the method in Figure 2.

dextrose concentration was determined to be 474 μM (85.3 μg/mL) for a 100 μg/mL solution of Splenda, while sucralose was 3.6 μM (1.4 μg/mL) for the same solution of Splenda. Although dextrose is about 130-times more abundant (on a molar basis) than sucralose, sucralose is known to be about 320−1000 times sweeter than sucrose (*14*), and therefore, a much lower concentration achieves the desired sweetness.

CONCLUSION

HPAE-PAD was used to determine sucralose in Splenda and a beverage. The linear range of electrochemical response extended over 3 orders of magnitude, from 0.01 (LOD) to 40 μM (16 μg/mL; 25 μL injection). High precision, method ruggedness, and high spike recovery were demonstrated for these complex sample matrices. Sucralose determinations are possible in under 10 min per sample injection. Mixtures of sucralose and other carbohydrates (e.g., dextrose) can be determined simultaneously with longer methods. These determinations, which only required sample dissolution and/or dilution, were shown to be rugged for routine analyses.

ABBREVIATIONS USED

PAD, pulsed amperometric detection; HPAE, high-performance anion-exchange.

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