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Note

Simultaneous Determination of Hydrogen, Methane and Carbon Dioxide of Breath Using Gas-Solid Chromatography†

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Summary The analysis of respiratory hydrogen and methane was estimated as a useful index of intestinal fermentation of undigestible carbohydrate. A simultaneous and precious analysis of these gases as well as carbon dioxide was studied. A gas-impermeable multi-laminated film bag metalized by aluminum vapor was fitted to use as a storage bag; its impermeability was verified by measuring the residual rate of hydrogen after 3 months' storage. Hydrogen, methane and carbon dioxide of the breath gas even at 1 ppm could be determined simultaneously by using a gas-solid chromatography installed with a photoionization detector and active carbon column. To observe the genesis of hydrogen and methane after carbohydrate ingestion, pectin, a typical water-soluble dietary fiber, was fed to healthy volunteers. Increasing excretion of pulmonary hydrogen or methane showed the sign of intestinal fermentation as the results of carbohydrate malabsorption.

Key Words hydrogen, methane, carbon dioxide, pectin, dietary fiber, gas chromatography, photoionization detector, intestinal fermentation, respiration

It is considered that water-soluble dietary fibers such as pectin and guar gum retard the increasing of serum cholesterol value and of blood sugar level that are caused by the ingestion of diets. As these dietary fibers are indigestible carbohydrates, they reach the large intestine without breakdown. Intestinal microorganism

† The outline of this study has been already reported at the 43rd Annual Meeting of Jpn. Soc. Nutr. & Food Sci. (1989, May).

ferments them to produce mainly short chain fatty acids and gases such as carbon dioxide, hydrogen and methane (*1*). These gases are partially absorbed from the large intestine and then exhausted through exhalation (*1–4*). Neither hydrogen nor methane is synthesized in human cells (*5*). The measurement of these gases is used as a simple and reliable method to assess the fermentation rate of dietary fibers in intestines. There are many reports concerning the breath gas determination with gas-solid chromatograph (GSC) installed with a thermal conductivity detector (TCD) (*2–4, 6–10*). However, TCD is not so sensitive to detect a very small amount of hydrogen and methane in alveolar air. It is difficult to measure directly the exhausted hydrogen and methane in the breath with TCD.

It is preferable to establish the analytical method with sufficient detectability for simultaneous measurement of a large amount of carbon dioxide and a very small amount of hydrogen or methane. Only in the case of a large amount of these three gases produced by *in vitro* fermentation, the simultaneous analysis of the gases is successfully performed by using GSC with TCD (*11*). Although more sensitive photoionization detector (PID) than TCD was developed, it has not been used for determination of breath gas.

The purpose of this study is to evaluate the possibility of simultaneous determination of carbon dioxide, hydrogen and methane with a GSC installed with a PID. We found that the GSC installed with a PID is useful for this purpose. Also, we reconfirmed that the measurement of hydrogen and methane exhausted in breath is useful for the assessment of intestinal fermentation.

MATERIALS AND METHODS

Sample and reagent. Highly methylated pectin for medical treatment (BROWN NF: Ester value, 65%; Unipectine Co., Ltd., Paris, France), hydrogen, methane and carbon dioxide (99.9% respectively, Gasukuro Kougyo Inc., Tokyo) as the standard gases, and nitrogen gas and helium gas (99.99% respectively, Nihon Sanso Inc., Tokyo) were used.

Subjects. Two healthy male volunteers (age: 25 and 47 years), who have no anamnesis concerning gastroenteric disorder and carrier not administered any antibiotic for 3 months before the experiment, participated in the study. This study was conducted according to the Declaration of Helsinki approved in the World Medical Assembly.

Breath sampling. Each subject was instructed to fast for 13 h following their meal at 19 : 00 the day before the experiment. Five grams of pectin dissolved in 50 ml of water was orally administered at 7 : 59 the following morning. A breath collection as background level was done from 8 : 00 to 8 : 10, then collected for exactly 10 min at intervals of 30 min for 6 h into a Douglas-type bag. After measuring the total gas volume with a wet-type gas meter (W-NK-5A: Shinagawa Seiki Co., Ltd., Tokyo), the collected sample was transferred into a gas-impermeable multi-laminated film bag (volume: 1 liter, OPP15 μ/PE13μ/aluminum

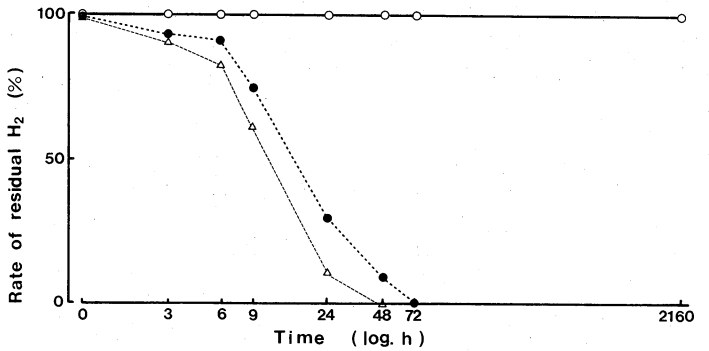

Fig. 1. Airtightness of packing-sheet materials made up storage gas bag as a function of time. ○, multi-laminated film bag; ●, polyethylene bag; △, rubber balloon.

vapor metalized PET12 μ/PE13 μ/CPP20 μ) as a storage bag. Gas impermeability of the storage bag was verified by measuring the residual of hydrogen after 3 months' storage in comparison with a polyethylene bag (thickness, 20 μ) and a rubber balloon, as illustrated in Fig. 1.

Assay procedures. Standard mixture gases were prepared by using hydrogen, methane and carbon dioxide in nitrogen. After clearing with nitrogen, a gas-sampling glass bottle (Gasukuro Kougyo Inc., Tokyo) was filled with nitrogen up to the level of 100%. An aliquot of each standard gas was injected into the gas-sampling bottle with a gas tight syringe.

After the injection of 1 ml of gas into gas chromatograph, the compositions of the standard gas and the sample of cellected breaths were measured. The concentration of each constituent in the collected breath was measured by employing the extra standard curve determined from the standard curve determined from the standard gases.

In case that the concentrations of constituent are 0 to 7%, a linear relationship between detectable output area and the concentration was observed. Therefore, in this study three kinds of standard gases were used. They were A (hydrogen by 10 ppm, methane by 10 ppm, carbon dioxide by 2%), B (hydrogen by 50 ppm, methane by 50 ppm, carbon dioxide 4%) and C (hydrogen by 2%, methane by 2%, carbon dioxide 6%) on the basis of nitrogen, respectively. Each standard gas and sample gas were measured in triplicate. The detectable limit of each gas examined in this assay procedure was 1 ppm.

GSC conditions were as follows,

Gas chromatograph:	Hitachi 263-50 Gas chromatograph
Detector:	Hitachi photoionization detector
Packing and column:	Glass column (3 mmϕ × 200 cm) packed with active carbon (60/80 mesh, Gasukuro Kougyo Inc., Tokyo)
Column temperature:	90°C

Injection temperature: 100°C
Carrier gas: Helium (99.99%) 50 ml/min
Bridge current (PID): 750 V
Integrator: Hitachi D-2500 data processor

RESULTS

The gas chromatogram of hydrogen, methane and carbon dioxide in the prepared gas mixture is illustrated respectively in Fig. 2. It took about 7 min for the analysis of one sample.

In preliminary experiment, it was observed that no dietary intake gradually decreased the breath excretion of hydrogen or methane from 2–3 to 1 ppm, and carbon dioxide from 2.5 to 2% of both subjects under the same condition of this study. The concentrations of hydrogen, methane and carbon dioxide in collected breaths after pectin feeding were 2 to 13.2 ppm, 0 to 4.6 ppm and 2.5 to 3.0%, respectively. Changes of pulmonary gas excretion of subjects A and B are shown in Fig. 3.

The expiration of hydrogen gas was increased following the oral ingestion of pectin in both subjects, attained the maximum level around 1 or 2 h following the

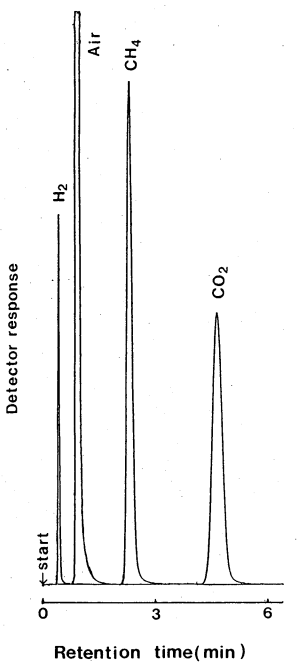

Fig. 2. Gas chromatogram of standard mixture gas. A mixture gas is composed from three gases-hydrogen, methane and carbon dioxide-in nitrogen as a standard.

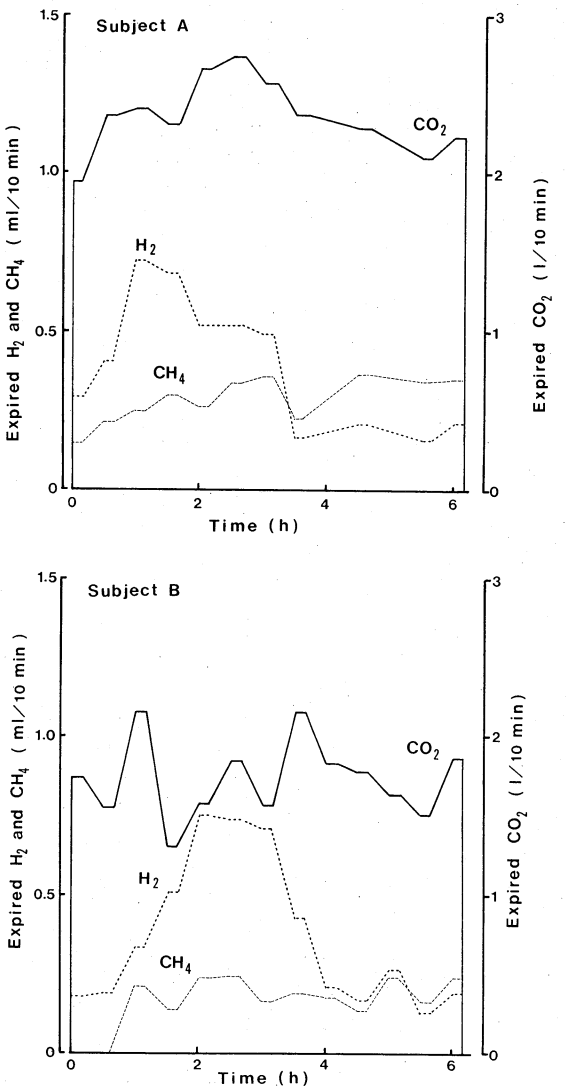

Fig. 3. Change of breath gases expiration (Subjects A and B). ·····, hydrogen; ----, methane; ——, carbon dioxide.

ingestion respectively, and returned to the initial levels within 4 h after the ingestion. This suggests that the 5 g of pectin was catabolized partially into short chain fatty acids, carbon dioxide and hydrogen by intestinal microflora within about 4 h after ingestion. The total volume of hydrogen produced by the ingestion of 5 g of pectin was 326 μl in subject A and 313 μl in subject B. The volume of hydrogen that appeared in subject A attained the maximum level more rapidly than in subject

B, which may depend on the individual difference of the subject's intestinal microflora. Otherwise, since the total volume of hydrogen and methane exhausted was little different between the two subjects, it is supposed that hydrogan was partially converted to methane by intestinal microorganisms (*12*).

While the volume of methane exhaled was very little in both subjects, a constant tendency concerning the exhausted methane was not detectable. However, a slight increase of the expiratory methane was observed in subject A. It is suggested that methane-productive bacterium inhabit the gastrointestinal tract of subject A.

Since the breath carbon dioxide, being different from hydrogen or methane, is produced not only by the fermentation of pectin in the large intestine but also by energy metabolism in organs, it is impossible to determine whether the origin of the carbon dioxide was due to the ingested pectin or not. However, it was observed that the volume of exhausted carbon dioxide from subject A increased following the pectin ingestion and returned to the initial level after 5 h. On the other hand, the increment of expiratory carbon dioxide was not detectable in subject B.

DISCUSSION

Human breath is mainly composed of oxygen, nitrogen, carbon dioxide and a bit of hydrogen and methane. The carbon dioxide is produced mostly through oxidative metabolism in our body and exhausted through the respiration system. Furthermore, carbohydrates that are difficult to absorb are catabolized to a bit of carbon dioxide and short chain fatty acids by intestinal fermentation. The latter is mostly absorbed to be oxidized and to be changed to carbon dioxide. Thus all of the carbon dioxide is mixed and exhausted in relatively high concentration of 2 to 3%.

Hydrogen and methane are produced only by the fermentation of carbohydrate in large intestine of hind-gut animals like man. It is said that the hydrogen is exhausted in the concentration of 1 to 200 ppm in breath and that, on the average, the methane is exhausted in the concentration of less than 1 ppm in 2/3 of persons and 2 to 60 ppm in 1/3 of the persons (*3*).

Christman and Hamilton (*3*) have already reported a new sensitive chromatographic method for measuring trace concentrations of breath hydrogen, but not methane or carbon dioxide. Throughout this study, it was found that the present analytical method by using GSC installed with a PID could determine simultaneously the concentration of exhausted hydrogen, methane and carbon dioxide. This method could detect quantitatively the slight difference of breath composition between the subjects A and B as illustrated in Fig. 3, and was sufficiently useful for the time course analysis of breath gas through study of energy metabolism.

Namely, it is supported that the present analytical method is not only suitable for the analysis of hydrogen and methane in breath to assess the fermentation of dietary fiber in large intestine but also useful for the analysis of gases in fecal fermentation experiment *in vitro*.

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