Application of a simple gas chromatographic technique for measuring breath hydrogen*

NOEL W. SOLOMONS,** FERNANDO E. VITERI, and LYLE H. HAMILTON
Guatemala City, Guatemala, and *Milwaukee, Wisc.*

A simple gas chromatographic technique for analyzing H_2 in expired air with a compact, relatively inexpensive gas chromatograph specifically adapted for H_2 analysis is described. Modifications in the basic chromatograph included increasing the capacity of the sample loop, lengthening the column, and using argon as the carrier gas to increase the sensitivity of the system. Methods for collecting and storing respiratory gas samples and for quantifying the results are described for clinical studies of carbohydrate malabsorption. Small errors in collection and quantitation are introduced by the method, but they are minor in relation to the changes in H_2 concentration which are seen with significant malabsorption. This simplification of chromatograph technology could increase the general availability of this convenient, noninvasive and well-tolerated test of intestinal absorption of carbohydrates such as lactose.

Abbreviations: hydrogen (H_2), parts per million (ppm)

The fermentation of nonabsorbed carbohydrates exposed to certain intestinal bacteria results in the intrainstestinal evolution of H_2 gas. A proportion of this gas is excreted by the lungs,[1] and therefore the increase in the pulmunary excretion of H_2 can be used clinically as an index of carbohydrate malabsorption. In their original clinical studies, Levitt and co-workers[2, 3] used a closed, continuous rebreathing system, but recent methods based on collecting breath samples at defined intervals following an oral dose of carbohydrate have been described[4–7] and standardized.[8] The measurement of breath H_2 offers the advantages of being noninvasive and sensitive enough to detect malabsorption of physiological (dietary) doses of carbohydrate.[9, 10] Moreover, in the case of lactose, the H_2 breath test has been found to be the most valid indirect method for quantifying lactose malabsorption.[9, 11] Gas chromatography, however, can be expensive and complex, and some early thermal conductivity detectors were too insensitive to measure the low concentrations of H_2 in expired air.[12] The present paper describes the adaptation of a simple, inexpensive thermal conductivity gas chromatograph for the analysis of H_2 in expired air to studies of clinical carbohydrate intolerance.

From the Division of Human Nutrition and Biology, Institute of Nutrition of Central America and Panama, Guatemala City; the Gastroenterology Section, Department of Medicine, Pritzker School of Medicine, Chicago, Ill.; Research Service, Wood Veterans Administration Hospital and the Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisc.

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Reprint request: Noel W. Solomons, M.D., Division of Human Nutrition and Biology, INCAP, Carretera Roosevelt, Zona 11, Guatemala, Central America, Apartado 11-88.

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Procedures and methods

Subjects. The subjects used in the illustrative examples were children admitted to the Clinical Research Center of the Division of Human Nutrition and Biology of the Institute of Nutrition of Central America and Panama. Informed consent in accordance with the Declaration of Helsinki was obtained from the parents or guardians.

Collection and handling of breath samples. Standard 50 to 100 cc syringes may be used to collect samples of expired air for immediate analysis. If the sample is to be stored for any length of time prior to analysis, its container must be gas-tight for H_2, the most diffusible of all gases. The Hamilton gas-tight syringe (Hamilton Co., Reno, Nev.) will serve this purpose. We generally use 3 L capacity bags of Mylar-coated foil paper* (Champion Paper Co., Chicago, Ill.) fitted with a 3 to 4 cm length of Tygon tubing (Norton Co., Plastics and Synthetics Division, Akron, Ohio). A known concentration of H_2 in these bags remained constant during storage for up to 47 days.

For preschool children, expired air was collected with a pediatric anesthesia mask attached to a 5 L gasbag via a one-way Rudolph valve. This low-resistance system is generally well tolerated by children below the age of 4 years, especially when presented as a game of "inflate the balloon." The breath samples are directly transferred to an injection syringe for analysis or to a gas-tight container. Dead space in the collection apparatus and changes in the level of ventilation during collection can introduce error in concentration determination. The dead space of our mask and valve collection apparatus, as determined by water displacement, ranged from 80 to 120 cc depending on the Rudolph valve used and upon the fit of the mask to the contours of the child's face. Since the expired volumes collected for a given sample varied from 1 to 5 L, an initial systematic error between 1.6% and 12% could be introduced by dead space. A brief flushing period of only a few breaths prior to the clamping of the outlet can eliminate the dead space in the apparatus. In practice, however, we have found that if gas volumes in excess of 3 L are collected, the constant error below 4% would not affect the reliability of the determinations. Moreover, any gas collection procedure of itself promotes slightly increased ventilation, which will wash out some H_2 from alveolar air. Such wash-out produced by minor hyperventilation is probably small and will affect estimation of basal (zero-time) as well as postcarbohydrate production of H_2 in a proportionate manner.

For adults and children old enough to co-operate fully, a single-breath, end-expiratory sample of expired air, which accurately reflects H_2 concentration in alveolar air,[8] was collected directly into a syringe or foil gasbag. It should be remembered that because of an anatomical dead space of about 30% of the tidal volume, *mixed air* samples such as those collected in young children with the use of multiple respirations and nonrebreathing systems will have an H_2 concentration of approximately 70% of a corresponding *alveolar* concentration.

Transfers of samples to the sample loop of the chromatograph from gas-tight foil bags or compressed gas cylinders were made with a 60 cc syringe adapted with Tygon tubing (Norton Co.) To minimize dead space error in the transfer, a triple aspiration technique was used. The first two aspirations filled any pre-existing dead space with sample gas. The third aspiration therefore contained an undiluted sample of the original gas being transferred for analysis.

Chromatographic analysis. Samples of mixed or alveolar air were analyzed on a gas chromatograph (Model S; QuinTron Instruments Co., Inc., Milwaukee, Wisc.), which measures gas concentrations by differences in thermal conductivity from that of the carrier gas. Gas components were separated on a ¼ inch (O.D.) aluminum column packed with 13× molecular sieve activated at 350° C for 4 hr. Argon was used as the carrier gas, and the flow rate was varied from 15 to 22 cc/min as measured with a soap-film flow meter. The standard chromatograph was adapted specifically for the analysis of H_2 with two simple modifications. (1) The capacity of the larger loop of the two-loop sampling valve[13] was increased from 8 to 16 ml, and (2) the molecular sieve column length was increased from 152 cm (60 inches) to 366 cm (144 inches) to isolate the H_2 peak from the initial artifact related to sample introduction and from the subsequent oxygen peak. Gas samples of between 25 and 60 cc were delivered into the injection port to ensure complete flushing of the sample loops. Ten minutes were required for complete elution of a gas sample at a carrier gas flow rate of 18 cc/min, the H_2 peak registering within the first 5 min. With careful spacing of sample introduction while the major gas components, O_2 and N_2, were still being eluted, it was possible to overlap samples and thus minimize waiting periods. Even without such a tight introduction schedule, however, six samples could be analyzed per hour at this carrier-gas flow rate.

*These foil gas bags were designed and courteously supplied by Dr. D. H. Calloway, Department of Nutrition, University of California at Berkeley.

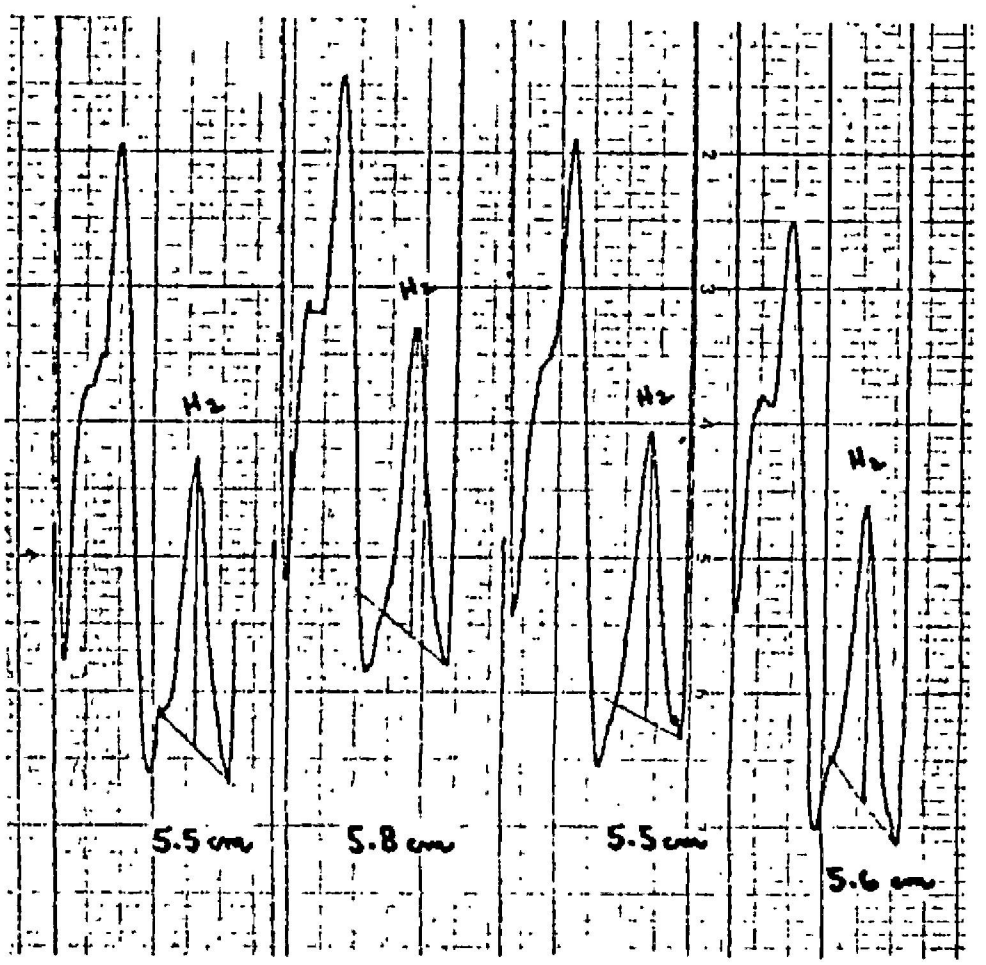


Fig. 1. Chromatogram showing four consecutive determinations of H_2 concentration from a 16 cc sample of a precalibrated standard reference gas containing 55 ppm of H_2 in room air. The earliest appearing peak is an artifact from the sample introduction and is followed by the H_2 peak and the O_2-N_2 peak.

Chromatograms were recorded on a single-pen recorder (Model L 101; Quintron). Usual paper speed was 40 cm/hr, and a full-scale deflection of 25.4 cm (10 inches) was produced by a 1 mV input signal. When the pen deflection was greater than full-scale, a built-in, line-operator recorder range extender[14] (a zero-suppression circuit which changes the polarity of the recorder input) was operated to keep the pen on the chart without decreasing the sensitivity of the instrument.

Quantitation of H_2 concentration and H_2 excretion volumes. A standard gas mixture (reference gas; Union Carbide Corp., Linde Division, Chicago, Ill.) of analytically determined concentration, in this case 55 ppm of H_2 in room air, was used to identify the elution time for H_2 among the various component peaks on the chromatogram and to determine the unknown concentration of H_2 in an expired air sample. The latter was calculated by the "peak height" method.[15] The maximum excursion of the response curve above baseline (peak height) of an unknown gas can be translated into concentration by comparison to the peak height of the pre-calibrated reference gas, as follows:

$$C_{(unknown)} = \frac{C_{(reference)}}{\text{Peak height}_{(reference)}} \times \text{Peak height}_{(unknown)} \quad (1)$$

Due to a sloping baseline at the time the H_2 peak is eluted, a baseline extrapolation is usually required. The best estimation of the point where the pen would have been had no H_2 been present (baseline) is the arithmetic mean of the point of origin and the point of termination of the H_2 peak.[16]

A study period of 6 hr following the ingestion of the test dose has been routinely used, with usual sampling intervals of 1 hr for adults and ½ hr for preschool children. The volume of H_2 in excess of that predicted by the basal, pre-carbohydrate-load production rate excreted during the observation period can be estimated by integrating the area under the interval concentration curve with the use of an approximation for total expired volume.

Total ventilation for a given collection interval is estimated by multiplying minute ventilation (tidal volume × respirations per minute) by the time in the interval, i.e., 30 or 60 min. The effect of the collection procedure on ventilation prevents its use as a measure of ventilation, and so we have used the nomogram of Radford et al.[17] to calculate the predicted tidal volumes, making appropriate

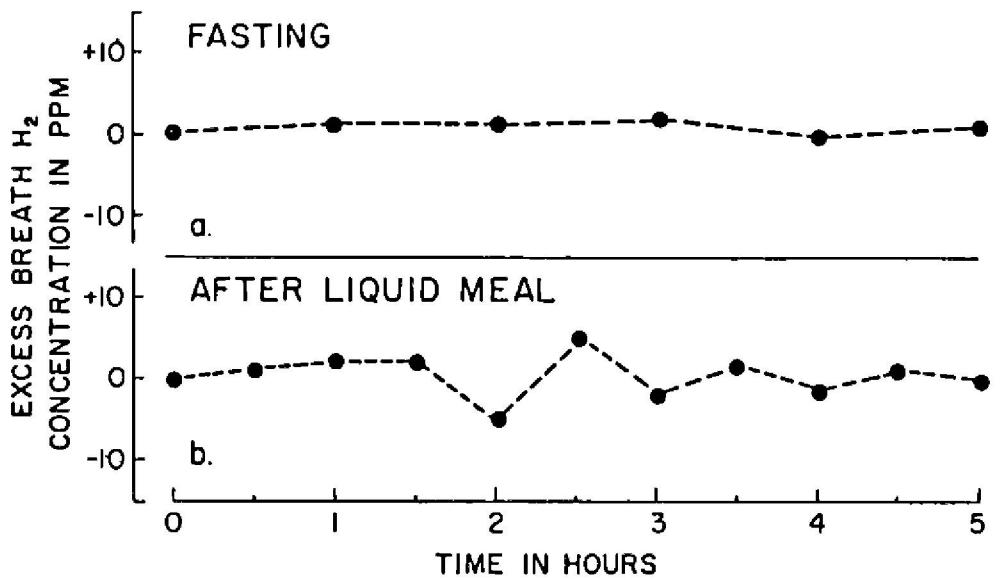


Fig. 2. a, Change in breath H_2 concentration in ppm relative to the zero-time sample during a 5 hr fast with breath samples analyzed at 60 min intervals. *b*, Change in breath H_2 concentration after ingestion of a liquid meal of casein, sucrose, vegetable oil, and water in the same individual. Breath samples taken at 30 min intervals.

correction for the altitude above sea level. The excess volume of H_2 excreted during the 6 hr period can be estimated with the following formula:

$$\text{Excess } H_2 \text{ volume in cc} = \frac{(T_0-T_0)+(T_I-T_0)}{2}+\frac{(T_I-T_0)+(T_{II}-T_0)}{2}+ \tag{2}$$

$$\frac{(T_{II}-T_0)+(T_{III}-T_0)}{2}+\cdots+\frac{(T_{n-1}-T_0)+(T_n-T_0)}{2}\times$$

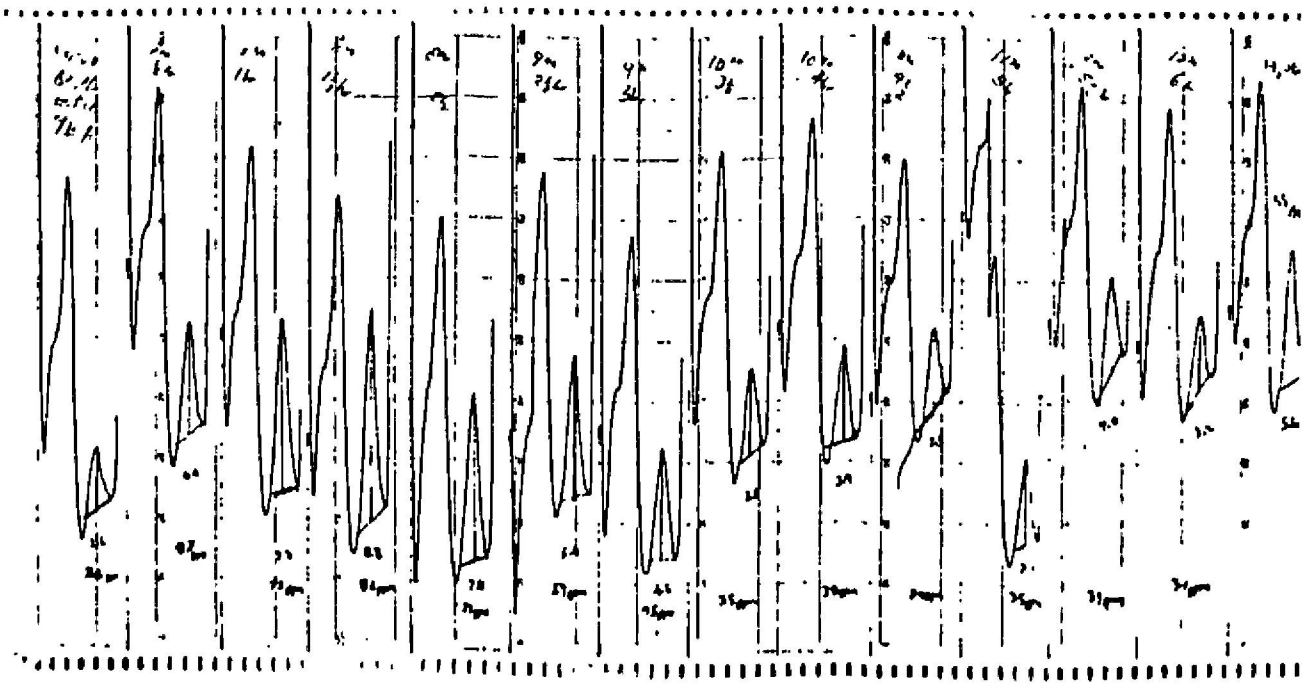
$$\text{respirations/min} \times \text{min/interval} \times \text{tidal volume in cc (corrected)} \times 10^{-6}$$

where T_0 = H_2 concentration in ppm at time zero (before dose), T_I = H_2 concentration in ppm at the end of first interval, T_{II} = H_2 concentration in ppm at the end of second interval, T_{III} = H_2 concentration in ppm at the end of third interval, T_{n-1} = H_2 concentration in ppm at the end of penultimate interval, and T_n = H_2 concentration in ppm at the end of final interval.

Results

Reproducibility of H_2 concentration measurements. A 16 cc sample of the 55 ppm H_2 reference gas was measured 47 times during a 2-month period during which the flow rate was maintained constant at 18 cc/min. The mean peak height was 5.6 ± 0.40 cm, with a coefficient of variation of 7%. Fig. 1 shows a chromatogram demonstrating four consecutive analyses of the reference gas. Under normal operating conditions with minimum attenuation of the chromatograph, 1 mV full-scale deflection of the pen recorder, a 16 cc sample volume, and an argon flow rate of 18 cc/min, the signal provided approximately 1 mm deflection for each part per million of H_2 in the sample. Nonetheless, variation in ambient operating conditions was large enough that frequent reference gas measurements are recommended to maintain high accuracy for the analysis of H_2 concentration in the unknown sample of expired air.

Range of concentration of breath H_2. The range of H_2 concentration measured during the analysis of over 1,500 samples of expired air in our laboratory has been 11 to 284 ppm. Metz et al.[4] reported that increases in breath H_2 concentration of 20 ppm suggested a significant degree of carbohydrate malabsorption when 50 Gm of lactose are administered to a lactose-intolerant adult. Such increments would produce changes



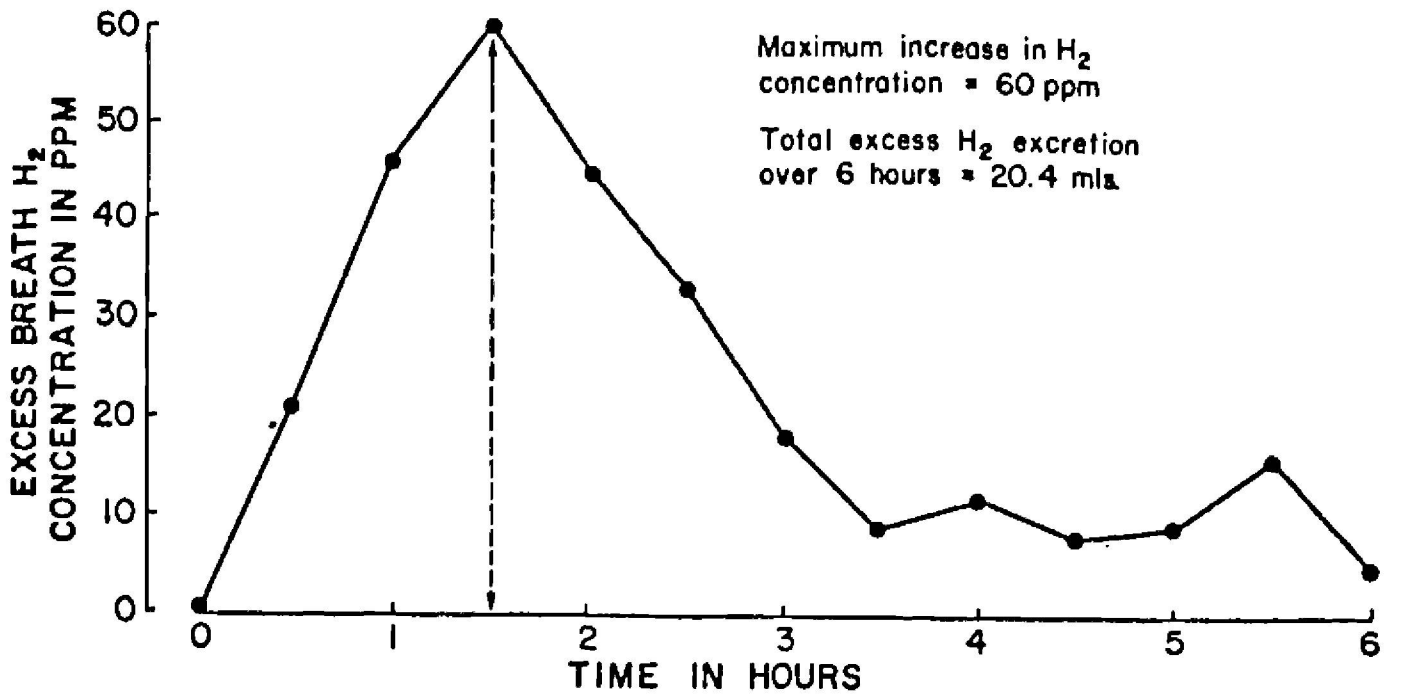


Fig. 3. Actual chromatogram (above) and graphical representation of the change in breath H_2 concentration (below) in a lactose-intolerant child who received an oral dose of 1.75 Gm/kg lactose. Samples were collected at 30 min intervals over 6 hr. The maximum increment in H_2 concentration was 60 ppm at 90 min; integration of the area under the curve using equation 2 estimated an excess H_2 excretion of 20.4 cc.

equivalent to a 2 cm increase in peak height above that for the baseline sample under the conditions specified for our system.

H_2 concentrations in expired air under basal conditions. Breath H_2 concentrations during waking hours have been found to be reasonably constant. Graphs of the change in H_2 concentration in the breath of a normal preschool child during a 5 hr morning fast is shown in Fig. 2, *a*. The response to a lactose-free liquid meal consisting of casein, sucrose, and vegetable fat in water in the same subject is illustrated in Fig. 2, *b*.

Carbohydrate malabsorption. Carbohydrate malabsorption such as that seen in lactose intolerance is associated with a significant increase in breath H_2 concentration following the oral administration of a standard test dose of lactose. Fig. 3 shows the original chromatogram and the H_2 concentration curve over 6 hr in a lactose-intolerant preschool child after ingestion of a standard pediatric dose of 1.75 Gm. of lactose per kilogram of body weight. The maximum rise in breath H_2 concentration of 60 ppm was seen 1½ hours after administration of the lactose. The net excess production of H_2 as estimated by equation 2

was 20.4 cc, assuming an expired volume of 73.5 L during each 30 min interval of the study, according to the Radford nomogram.[17] The corresponding change in blood glucose was less than 20 mg/dl over the first 2 hr after ingestion. The subject experienced no obvious symptoms from the test carbohydrate.

Discussion

Methods for collecting, storing, measuring, and quantifying H_2 in expired air for clinical studies of carbohydrate malabsorption have been presented. The analysis can be performed adequately with a simple, compact, and relatively inexpensive thermal conductivity gas chromatograph specifically adapted for the measurement of H_2 in trace concentrations. Simplicity of operation is such that even an unskilled technician can be trained to operate the chromatograph within a matter of hours. The technique is versatile and provides physiologically meaningful results because the doses of carbohydrate administered need not be excessive. Analysis time is rapid, and the results can be made available to the patient and physician at the end of the test period. The apparatus is light-weight and portable and can be used in field studies in remote areas, as well as for routine studies in the clinic or laboratory. The carrier gas, argon, is inexpensive, and usually is readily available in most countries, since commercial grade compressed gas is suitable.

The only associated discomfort is that which occasionally arises from symptoms due to the malabsorption of the test carbohydrate, and these are usually mild and transient. Patient acceptance and safety of this noninvasive, interval collection method is better than for those procedures requiring repeated blood sampling, intestinal intubation, use of radioisotopes, or confinement of the patient in a closed rebreathing system as employed by alternative methods for estimating carbohydrate intolerance.[9, 11] In this regard it is especially well suited for use in infants and children. Errors may be introduced with a collection procedure using interval sampling[4] and with the baseline correction in peak height calculations,[16] but these are small in comparison with the magnitude of the changes above basal H_2 concentration expected with malabsorption of carbohydrates and do not invalidate the method. Thus the simplified gas chromatographic techniques described herein should increase the general availability of carbohydrate absorption tests based upon the determination of H_2 gas in expired air.

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