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GAS CHROMATOGRAPHIC QUANTITATION OF BREATH HYDROGEN AND CARBON MONOXIDE FOR CLINICAL INVESTIGATION IN ADULTS AND IN CHILDREN

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SUMMARY

A simple and suitable method of breath sampling and the gas chromatographic determination of H_2 and CO in breath of children as well as of adults are described. The analytical system allows the measurement of concentrations of H_2 and CO as low as a few ppm.

The proposed method of breath collection (tedlar bag with low resistance valve face mask) appears particularly useful for long storage purposes, because it eliminates the need for additional manipulations and the possible associated sample dilution.

Normalization of H_2 breath amounts to internal standard O_2 to obtain reliable and precise measurements is particularly useful in non-collaborating patients whose observed H_2 peak values increase by 15% after the correction

The overall procedure is fast, inexpensive and accurately recognizes adult or children lactose malabsorbers as well as subjects exposed even to low levels of CO.

INTRODUCTION

Breath analysis represents important information in laboratory diagnosis whenever one needs to assess the concentration of some components of alveolar air of endogenous origin (e.g. H_2 and CO) as well as of exogenous origin (e.g. ambient toxic gases).

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H_2 in alveolar air is derived from bacterial activity on unabsorbed carbohydrates reaching the lower gastrointestinal tract; intestinal gas diffuses into the mucosal capillary blood where it is transported to the lungs and excreted in the breath, roughly in proportion to the degree of malabsorption. The use of a breath test for H_2 determination in the expirate after oral carbohydrate loads has therefore been suggested mainly as a diagnostic non-invasive test for (a) lactose malabsorption (alternatively to sequential blood glucose determinations) [1], (b) small bowel bacterial overgrowth (alternatively to duodenal juice cultures) [2], (c) small bowel transit time (alternatively to X-ray procedures [3]. However, the results reported so far do not allow this procedure to be considered among the routine laboratory tests.

CO, besides being an environmental toxic gas to which a subject may be professionally (e.g. foundry and garage workers) or individually (smoking habits) exposed, is also produced during heme catabolism. Since CO is not catabolized in vivo and virtually 100% of CO is excreted via the lungs, the determination of CO excretion rate may be employed in a variety of clinical investigations, such as environmental poisoning and hemolytic anemias, as an alternative to CO blood level determinations. However, the CO breath test too has received little attention as a common laboratory tool [4].

It is evident that the usefulness of breath analysis, besides rapidity, is mainly due to its non-invasivity, because the specimen to be analysed is simply the expired breath. This is particularly advantageous when it is necessary to perform biological monitoring for several hours, because serial blood determinations can be avoided, thus interfering very little with the patient's normal activities.

The analysis of the breath by gas chromatography (GC) with thermoconductivity detection has proved to be the most suitable procedure for the determination of all gases present, even in concentrations as low as a few ppm.

We set up an easy and efficient system of H_2 breath collection and following analysis by means of GC, which turned out to be particularly useful in the study of lactose malabsorption. Our results confirm that H_2 determination in breath may be an alternative (and sometimes more sensitive) to blood glucose determinations. By making appropriate variations in the GC operating conditions (carrier gas, volume of the loop of the sampling valve, etc.), the analytical system described here is capable also of evaluating breath CO.

EXPERIMENTAL

Gas chromatography

The GC system used was constituted by an HP 5840 (Hewlett-Packard, Avondale, PA, U.S.A.) gas chromatograph equipped with a six-port sampling valve with a 1-ml sample loop, thermoconductivity detector and printer-plotter with integrator.

The chromatographic columns for H_2 analysis were made of steel tubing, 1 m × 0.6 mm I.D., filled with 60—80 mesh molecular sieve 5 Å. The columns operated at 60°C in the isothermal mode, the detector was at 100°C. Pure nitrogen was used as the carrier gas, with a 10 ml/min flow-rate. Instrument

sensitivity was set at 1. Before analysis, the columns were equilibrated at 300°C overnight with a nitrogen flow of 10 ml/min, in order to ensure the cleanliness of the tandem columns and to preserve the resolution characteristics of the system.

A 5 cm × 0.5 cm column, packed with magnesium chloride, was inserted in the sampling line to dry the samples.

To detect CO we used the following conditions: steel columns (3 m × 0.6 mm I.D.) filled with 60—80 mesh molecular sieve 5 Å; the columns operated at 100°C in isothermal mode and the detector operated at 80°C. Pure helium was used as carrier gas at a flow-rate of 28 ml/min. The instrument sensitivity was 3. The loop volume of the sampling valve was 3 ml.

Sampling

Expired air samples were obtained by having the subjects exhale through an anesthetic mask equipped with two low-resistance one-way valves or a Ruben valve into a 250 ml tedlar (polyvinylfluoride, Dupont de Némours International S.A., Geneva, Switzerland) vacuum empty bag (see Fig. 1). Stability of the sample on storage was determined by filling the bags with standard gas mixtures. The bags were stored at room temperature and were analysed at intervals throughout a period of 24 h.

Fig. 1. The anesthetic mask equipped with a Ruben valve connected by means of a gas tight cock, to a polyvinylfluoride bag.

During the study we compared three types of breath collection: (1) after a deep inhalation followed by a 10-sec breath-holding period, the first volume of exhalation was discarded and the last portion was collected; (2) the subject inhaled deeply, held his breath for 10 sec, and exhaled once completely into the collection bag; (3) several normal tidal volumes of breath were collected. Duration of collection of expired air was not considered crucial, because we did not need to calculate the rate of H_2 excretion, but only determine the change in H_2 concentration in serially collected samples of expired air.

Samples were introduced at atmospheric pressure into the sampling valve of the GC system through a tight connection between valve and bag, by simple compression of the bag so that the loop was filled.

Normalization of the H_2 peak

We used breath O_2 as internal standard to normalize H_2 breath amounts as proposed by Robb and Davidson [5], in order to obtain reliable and precise measurements.

Subjects

In order to test the sensitivity and the specificity of the method, we comparatively studied the H_2 excretion and the rise in blood glucose levels after an oral lactose load in 29 adult subjects without evident gastroenterological disease (age 40—50 years) and 15 children with clinical suspicion of lactose malabsorption (age 7 months to 11 years; mean 3.4 years ± 4 S.D.).

Venous blood samples were obtained in the fasting state and at 20, 40, 60 and 90 min after lactose ingestion. Plasma glucose was determined by the glucose oxidase method. Lactose malabsorption was diagnosed on the basis of an increase of blood glucose of less than 20 mg/dl [6].

Breath samples were taken simultaneously with blood and then were protracted until 240 min (at 30-min intervals) in order to consider the variability of gastric emptying and small bowel transit time.

Increases of H_2 more than 20 ppm were considered to be indicative of lactose malabsorption [8].

Breath CO was evaluated in 30 healthy male hospital workers non-professionally exposed: ten were non-smokers, ten were heavy smokers, the remainder were mild smokers.

RESULTS

Gas chromatographic determination of H_2 and CO

Figs. 2 and 3 show chromatograms of standard gas mixtures of 21 ppm H_2 in N_2 and of 50 ppm CO in N_2, respectively. A clear linearity of the measurements by examining H_2 and CO standard samples at different concentrations was found.

The limit of sensitivity that can be reached using this procedure is 1.0 ppm and 1.5 ppm, respectively, for H_2 and CO analysis.

The intra-assay reproducibility of the method for H_2 measurements was tested by collecting and analysing five breath samples of a patient delivered in rapid succession; the mean values, standard deviation, and coefficient of

variation were 41 ppm, 1.4 ppm and 3.4%, respectively. The inter-assay variability was determined by analysing aliquots of the same sample containing 21 ppm of H_2 on ten occasions over a period of 20 days in the same operating conditions. A mean peak area* of 2.27 mV sec, a standard deviation of 0.03 mV sec and a coefficient of variation of 1.3% were obtained.

The intra- and inter-assay variability for CO measurements showed coefficients of variation of 3.9% and 1.8%, respectively.

The calculation of the peak area was automatically integrated using the external standard method.

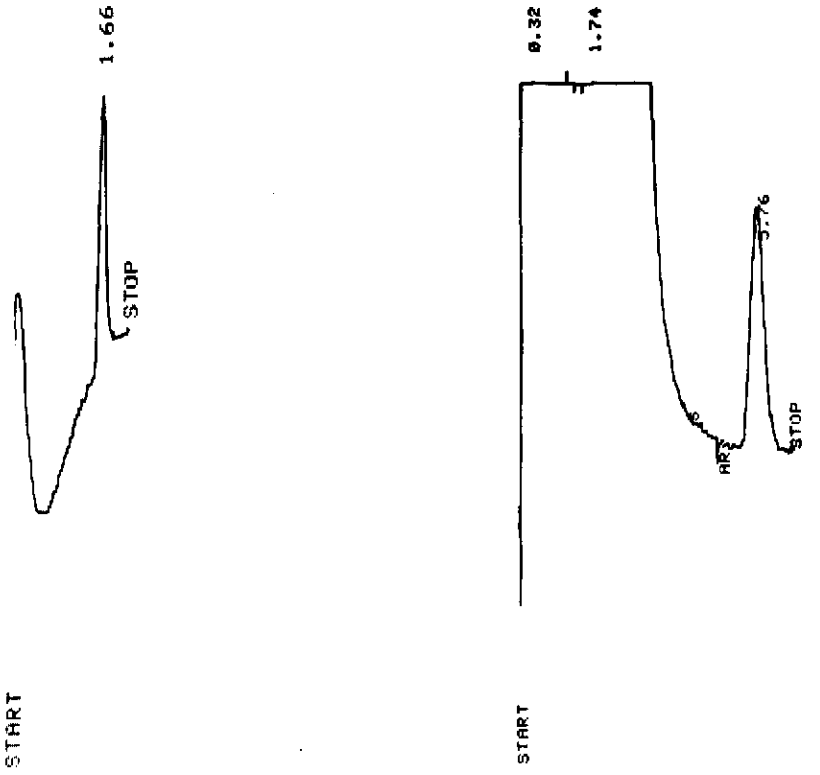

Fig. 2. Chromatogram of a standard gas mixture of 21 ppm H_2 in N_2. Oven temperature 60°C, detector temperature 100°C. Sensitivity 1; attenuation 2^1.

Fig. 3. Chromatogram of a standard gas mixture of 50 ppm CO in N_2. Oven temperature 100°C, detector temperature 80°C. Sensitivity 3; attenuation 2^1.

Sample collection and normalization of the H_2 peak

We observed that types "a" and "b" breath collection gave H_2 concentrations 10% higher than "c" type collection. For this reason, in adult collaborating patients the "a" type of breath collection was routinely utilized, whereas in

*Area units: 1 count = 3.2 μV sec.

non-collaborating children the "c" type of breath collection was utilized with correction of the H_2 concentration to internal standard. The correction to O_2 concentrations in fact increased the observed H_2 peak values by 10—15% only in children, no substantial increase being possible to detect in adult patients. The data reported in the tables are in any case all normalized to O_2 concentration. We stress, however, that the normalization may not be necessary in adults who can exhale alveolar air. It must be noted that the correction to O_2 levels is mainly useful to obtain reproducible results in the same subjects but it does not guarantee the accuracy of the results.

Sample storage

In Table I are reported the analysis of H_2 and CO standard gas mixtures stored in the tedlar bags. The results, expressed as mean percentage original value of five consecutive analyses for each sample at every time, document the good stability of the stored samples.

TABLE I

STABILITY OF H_2 AND CO LEVELS IN STANDARD GAS MIXTURES STORED IN TEDLAR BAGS

Hours	Percentage of original value			
	H_2 21 ppm	H_2 60 ppm	CO 20 ppm	CO 50 ppm
0	100	100	100	100
12	92.2	93.4	100	100
24	86	89.7	99	98
48			96	97
72			96	96

H_2 Excretion in breath after oral lactose load

In Table II we compare the results of the H_2 breath test with those of the blood glucose level increments in the subjects tested.

Evaluation of carbon monoxide in breath

In Table III are reported the results of breath samples of 30 smoker subjects in order to point out the possibility of determining simply and quickly CO in the breath of subjects professionally or individually exposed to toxic gases. These results are in agreement with literature data obtained using other analytical methods [4, 7].

No normalization of observed CO was performed because the levels of CO in both the working and analytical areas were considered too low to alter the determinations significantly.

TABLE II

COMPARISON OF H_2 BREATH TEST AND GLUCOSE BLOOD LEVEL INCREMENT FOR THE DIAGNOSIS OF LACTOSE MALABSORPTION

Results are expressed as mean ± S.D. and range.

Diagnosis by H_2 breath level increment (ppm H_2)	Diagnosis by glucose blood level increment (mg/dl glucose)			
		Absorbers	Malabsorbers	Borderline
		31 ± 10 (20 — 54)	12.8 ± 6.6 (0 — 23)	(20—25)
Absorbers				
5.4 ± 7.0 (0 — 19)	Children (*n*)	5	—	—
	Adults (*n*)	7	—	—
Malabsorbers				
64 ± 40 (21 — 24)	Children (*n*)	—	10	2
	Adults (*n*)	—	20	4

TABLE III

CO IN BREATH OF SMOKERS AND NON-SMOKERS

Smoking habits	CO in breath
Non-smokers	4.5 ppm
Mild smokers (10—20 cigarettes/day)	18.0 ppm
Heavy smokers (> 20 cigarettes/day)	42.5 ppm

DISCUSSION

Expired breath, one of the most readily available biological materials, has been poorly utilized for clinical investigation until recent times. We experimented with methods for collecting, storing, measuring and quantitating H_2 and CO in expired air for clinical studies of carbohydrate malabsorption and CO production or exposure.

Compared to other methods already published, our procedure shows some advantages. The breath collection in a squeezable bag connected with a low-resistance face mask (especially suitable for children) allows easy, direct introduction of the sample into the GC apparatus by means of a sampling valve (reproducibility of the injected volume). Moreover, it is possible to store the sample in the bag itself, thus eliminating the need to transfer the samples into storage containers, which usually produces a loss of at least 10% of the gases [8]. Storage in tedlar bags has been found useful in all cases when it is not possible to perform the gas analysis immediately after sampling. Furthermore,

our method shares several advantages with the recently reported technique of Christman and Hamilton [9]. In fact, it is possible to examine non-hospitalized patients by means of the collecting bags, the H_2 elution time (approx. 90 sec) is short enough for our purposes, and, finally, operating procedures are just as simple. Our method has the advantage of a linear signal—concentration curve, and the particular GC versatility allows other breath gases to be determined simultaneously, thus permitting the use of O_2 as internal standard in cases where peak normalization is necessary.

Our results show a good agreement between the H_2 breath test and the standard lactose tolerance test (glucose blood level increment). According to clinical symptoms (abdominal malaise, diarrhoea with positive clinitest), to a H_2 production of more than 20 ppm and to a glucose blood level increment of less than 20 mg/dl it has been possible to separate lactose absorbers from lactose malabsorbers.

However, it must be noted that six subjects (four adults and two children) were lactose malabsorbers on the basis of H_2 breath test and clinical response, but they showed a borderline (20—25 mg/dl) glucose blood level increment. The finding of these borderline cases may be explained by the lower reliability of the glucose blood level determination and possibly decreased glucose utilization such as in subclinical diabetes mellitus [10]. No false negative H_2 breath test was found, in agreement with the opinion [11] of the rarity of the absence of colonic bacteria capable of producing H_2 from non-absorbed lactose.

We found a good correlation between CO in the breath and amount of smoked tobacco. Thus the CO breath test may be considered a rapid, efficient and non-invasive method to evaluate exposure to environmental CO also.

As far as normalization of the H_2 peak is concerned, it is known that breath samples are representative of alveolar air only in adults, and in children old enough to cooperate fully [4] in order to give an end-expiratory sample in a single breath. Therefore some authors [5, 8] have proposed the normalization of the observed breath H_2 levels to one of the other respiratory gases, for preschool children. In respect of this suggestion we do not find it necessary to correct for breath H_2 values against CO_2 amounts [10] because (1) we used columns packed with molecular sieve (which traps CO_2 molecules), (2) the alveolar CO_2 concentration is physiologically widely variable, and (3) CO_2 is an end-product of lactose metabolism after oral load. The normalization to respiratory nitrogen [5] proposed in order to avoid variability of the sampling volume was not considered because our GC system is equipped with a gas sampling valve, and we used nitrogen as carrier gas.

We used breath O_2 as internal standard to normalize H_2 breath amounts as proposed by Robb and Davidson [5] in order to obtain reproducible measurements in non-collaborative subjects.

No normalization of the CO peak was applied because all examined subjects were adults.

The method discussed here may be particularly suitable for epidemiological studies of lactose absorption [12] as well as for monitoring subjects exposed to CO even at low levels, in adults and children. Our data contribute to validate the few results produced up to now by this procedure.

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