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CONTENTS

Original Articles

Evaluation of a Rapid Breath Hydrogen Analyzer for Clinical Studies of Carbohydrate Absorption

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A second-generation gas chromatograph for the analysis of H_2 in expired air has been developed. It incorporates a solid-state detector with high sensitivity for H_2 and has a small, internal pump which supplies air as the carrier gas, thus eliminating the need for a large tank of compressed gas for the carrier. A reference gas of known H_2 concentration is, of course, still required. The entire system weighs 6.0 kg and is completely portable, requiring only 120 V electric current for use. The instrument has a mean intersample interval of less than 2 min, with H_2 concentration registered on a digital display. The output has a track-hold feature which permits the output, in parts per million H_2, to be retained by the meter so the reading can be verified. The chromatogram can also be recorded on a conventional analog strip-chart recorder. The sensitivity and precision are superior to those of a thermal conductivity H_2 gas chromatograph. Moreover, the rapid response time encourages frequent calibration checks with the reference gas. This analyzer offers significant advantages over previous H_2-measuring systems for breath-analysis tests used both for diagnostic clinical practice and for field studies of nutritional assessment.

The fermentation of nonabsorbed carbohydrates exposed to certain intestinal bacteria results in the intraintestinal evolution of hydrogen (H_2) gas, a fixed portion of which is reabsorbed and excreted by the lungs (1). The collection of expired air is simple and noninvasive; it does not cause discomfort to young children or offend cultural beliefs, as might be true for blood sampling or gastrointestinal intubation. These principles and practical considerations have led to the development of H_2 breath-analysis tests for lactose, sucrose, glucose and D-(−)-xylose absorption; bacterial overgrowth; intestinal transit time; surveillance for necrotizing enterocolitis; evaluation of "tropical enteropathy"; and detection of pneumatosis cystoides intestinalis (2).

The H_2 breath test employing gas chromatography as an analytic instrument has been in use for over a decade. Relatively inexpensive gas chromatographs depend on thermal conductivity detectors for the measurement of gas concentrations. Unfortunately, they suffer from a lack of sensitivity so that concentrations less than 8 ppm are not

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measured accurately (1). In spite of that limitation, the test is used in many routine clinical laboratories (2, 3), and has even been applied to studies of lactose malabsorption in remote populations (4–8).

In 1977, we published a simple technique for measuring breath H_2 without a rebreathing system (interval sampling) (9), which has been employed by several other investigators (8, 10). We now describe the evaluation of new chromatographic instrumentation (11) which offers an even greater opportunity for widespread application of breath H_2 analysis in field investigation and in medical practice.

MATERIALS AND METHODS

The H_2 analyzer (model 12 MicroLyzer Quintron Instruments Company, 3712 W. Pierce Street, Milwaukee, Wisconsin 53215), used in this study is basically a gas chromatograph, but it has been designed as a special-purpose instrument with several specific design criteria dedicated to the measurement of breath H_2. Specifically, the system eliminates the need for a compressed carrier gas system by utilizing room air as the carrier gas and circulating it by means of a small, internal pump.

The MicroLyzer system employs a unique, solid-state gas detector consisting of an N-type, sintered SnO_2 sensor which exhibits a large change in electrical resistance when combustible or reducing gases are adsorbed on the surface of the sensor. These resistance changes are disproportionately large for small gas concentrations, thus enabling the sensor to monitor low concentrations of reducing gases accurately and with high sensitivity. The detector is almost completely specific for reducing gases, so the separation of H_2 from other gases that might interfere with its analysis is simplified. A short (38-cm) molecular sieve column markedly retards or retains all reducing gases other than H_2 (such as carbon monoxide, alcohols, ketones, etc), and the detector is insensitive to nonreducing gases which comprise most of a breath sample, ie, O_2 and N_2. Therefore, column length can be markedly shortened so that analytical time is reduced by as much as a factor of 10. A more complete description of the electronic bases of the instrument has appeared (11).

Unfortunately, the response of the sensor is nonlinear with H_2 concentration, at least at low concentrations, so a linearizing circuit is incorporated into the system to produce an output signal which is directly proportional to H_2 concentrations. The circuit also incorporates an analog track-hold section which stores the peak value of the response curve and displays it on the digital panel meter on command. This feature simplifies the calibration procedure by enabling the operator to use the gain adjustment to set the digital panel meter reading on the value of the H_2 concentration of a standard reference gas mixture. Thus, calibration is a one-step operation utilizing a reference gas of predetermined H_2 concentration.

An analog output signal is available for hard copy of the chromatogram with a strip-chart recorder; however, the built-in digital panel meter on the unit provides a direct display of H_2 in ppm after the machine is calibrated. When they were used, analog recordings were made with a 10-inch strip-chart recorder (model 101, Quintron Instrument Co.). In these instances, the H_2 concentrations were calculated from peak height measurements by conventional methods (12).

For this evaluation, the MicroLyzer was operated in three locations: Milwaukee, Wisconsin; Boston, Massachusetts; and Guatemala City, Guatemala. Four different gas mixtures containing trace concentrations of H_2 in compressed room air were used as reference gases to calibrate the instrument. They were from the following sources: 99.8 ppm (SupelCo, Inc., Bellefonte, Pennsylvania); 50, 53, 55, and 118 ppm (the Specialty Gas Division of Linde, Union Carbide, East Chicago, Illinois); and 52 ppm (Matheson, Gloucester, Massachusetts). Calibration was accomplished by introducing an 8-cc sample of reference gas by use of the integral sampling valve, following a flush of the loop with 35 cc or more from a standard disposable syringe having 60 or 70 cc capacity. After the response was registered, the meter was put on "hold," and the output gain was adjusted so the recalled peak reading on the digital panel meter displayed the established value of H_2 concentration (in ppm) for the reference gas. The samples are routinely flushed through a small (2 cc) drying tube containing Drierite® to desiccate gas prior to its introduction into the sample loop. A larger drying tube is attached online to the air inlet to dehumidify the aspirated room air carrier-gas propellant.

End-expiratory breath samples for analysis were taken directly into a plastic syringe, or into Mylar® impregnated, foil gas envelopes (Champion Paper Co., Columbus, Georgia), described in our earlier publication (9). The nonabsorbable carbohydrate was a lactulose syrup containing 10 g/15 ml (Cephulac®, Merrill-National, Cincinnati, Ohio). For comparative studies, a Carle model 111 gas chromatograph (Carle Co., Anaheim, California) was employed.

RESULTS

Stability and Analytical Reproducibility. The baseline is affected by the temperature of the sensor. It became apparent from our earliest experiences with the MicroLyzer that maximum baseline stability required about 2 hr of warm-up time with the sensor turned on and the carrier-gas flow established, ie, the pump also turned on. It is heated by applied voltage while temperature equilibrium is established due to heat loss from conduction by the carrier gas. Both processes must proceed for about 2 hr to establish temperature equilibration and a stable baseline. Depending on the stability of the ambient temperature, some small baseline drift in either direction of about 1–2 ppm/1–2 hr may be observed, related to room temperature change. This is not a problem since the meter should be checked and reset at 000 at the beginning of each analysis.

TABLE 1. REPRODUCIBILITY OF SEQUENTIAL H_2 CONCENTRATION READINGS FROM DIGITAL DISPLAY AFTER SINGLE CALIBRATION*

Time (hr)	*Minutes elapsed*	*H_2 (ppm)*
1500	0	99
1530	30	100
1600	60	99
1630	90	103
1700	120	101
1730	150	100
1800	180	98
1830	210	96
1900	240	92

*Calibrated with a 99.8 ppm standard reference gas at 1450 hr.

TABLE 2. SHORT-TERM REPRODUCIBILITY OF CONSECUTIVE H_2 CONCENTRATION READINGS FROM DIGITAL DISPLAY OF MICROLYZER

Time (hr)	*H_2 (ppm)*	*Time (hr)*	*H_2 (ppm)*
1600	52	1635	100
1605	57	1640	102
1610	57	1645	101
1615	58	1650	98
1620	59	1655	103
1625	59	1700	106
Mean	57.0		101.7
SD	2.61		2.73
SEM	1.06		1.12
Coeff. var.	4.4%		2.7%

*The machine was calibrated with a 52 ppm standard (Matheson) reference gas at 1555, and with a 99.8 (SupelCo) gas at 1630 hr.

To determine the stability of an initial calibration, the machine was calibrated with the 99.8 ppm (SupelCo) standard mixture, again after 3 hr of equilibration, and this same gas was measured at 30-min intervals over 4 hr (Table 1). No significant deterioration in stability was registered until the ninth determination at 240 min. In routine use, a requirement to recalibrate the machine after several hours of use has been consistently observed and is probably related to a change in the operating temperature. Changes in ambient temperature alter the sensitivity of the instrument as well as the baseline.

To test the short-term reproducibility of the machine, after a 3-hr warm-up, standard gas samples of 52 ppm (Matheson) and 99.8 (SupelCo), each calibrated internally, were measured at 5-min intervals over a 30-min period. The entire procedure was completed in 1 hr. The consecutive repetitions had coefficients of variations of 4.4% for the 52 ppm gas and 2.7% for the 99.8 ppm standard (Table 2). Other tests in different laboratories provided similar values for reproducibility.

Linearity of H_2 Detection Response and Comparative Validation. After adequate warm-up and calibration of the machine with the 99.8 ppm H_2 standard mixture (SupelCo), a syringe with 60 cc capacity was filled. Half the volume was injected into the sample loop. The syringe was filled with 40 cc of standard gas and diluted to 60 cc volume with room air, to produce a theoretical concentration of 67 ppm. Half of this sample was analyzed, and the remaining 30 cc of gas was, in turn, diluted to 60 cc. This procedure of a 50% dilution was repeated successively. Thus, theoretical sample concentrations of 99.8, 67, 33.5, 17.7, 8.8, 4.4, and 2.2 were produced. Figure 1 shows the results of the analyses of the dilutions in relation to the theoretical slope. This procedure was repeated on numerous occasions in two laboratories. The slope was consistently between 1.010 and 0.990.

The machine was taken to the Laboratory of Pediatric Gastroenterology of the Massachusetts General Hospital, in which a more sophisticated thermal conductivity gas chromatograph (Carle model 111) was in operation. Using various random dilutions of the 99.8 standard gas in room air and dividing the same sample between the Carle instrument and the MicroLyzer, simultaneous comparisons of the analyses were made. As shown in Figure

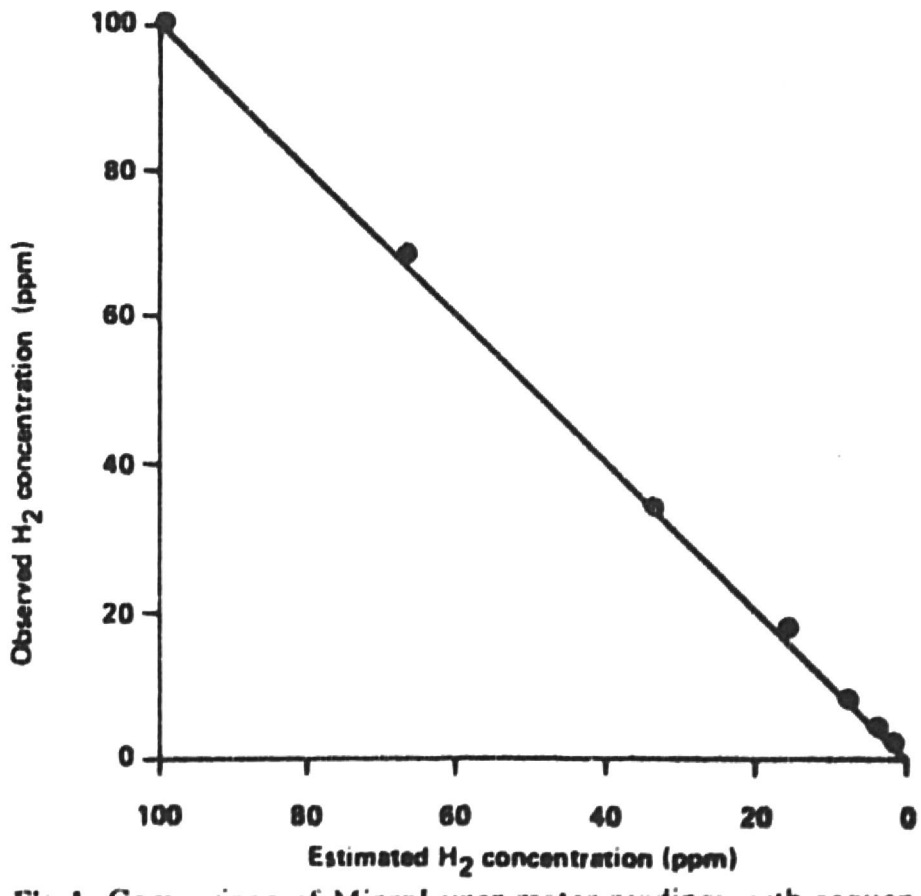


Fig 1. Comparison of MicroLyzer meter readings with sequential, known dilutions with room air of 99.8 ppm standard H_2 gas mixture.

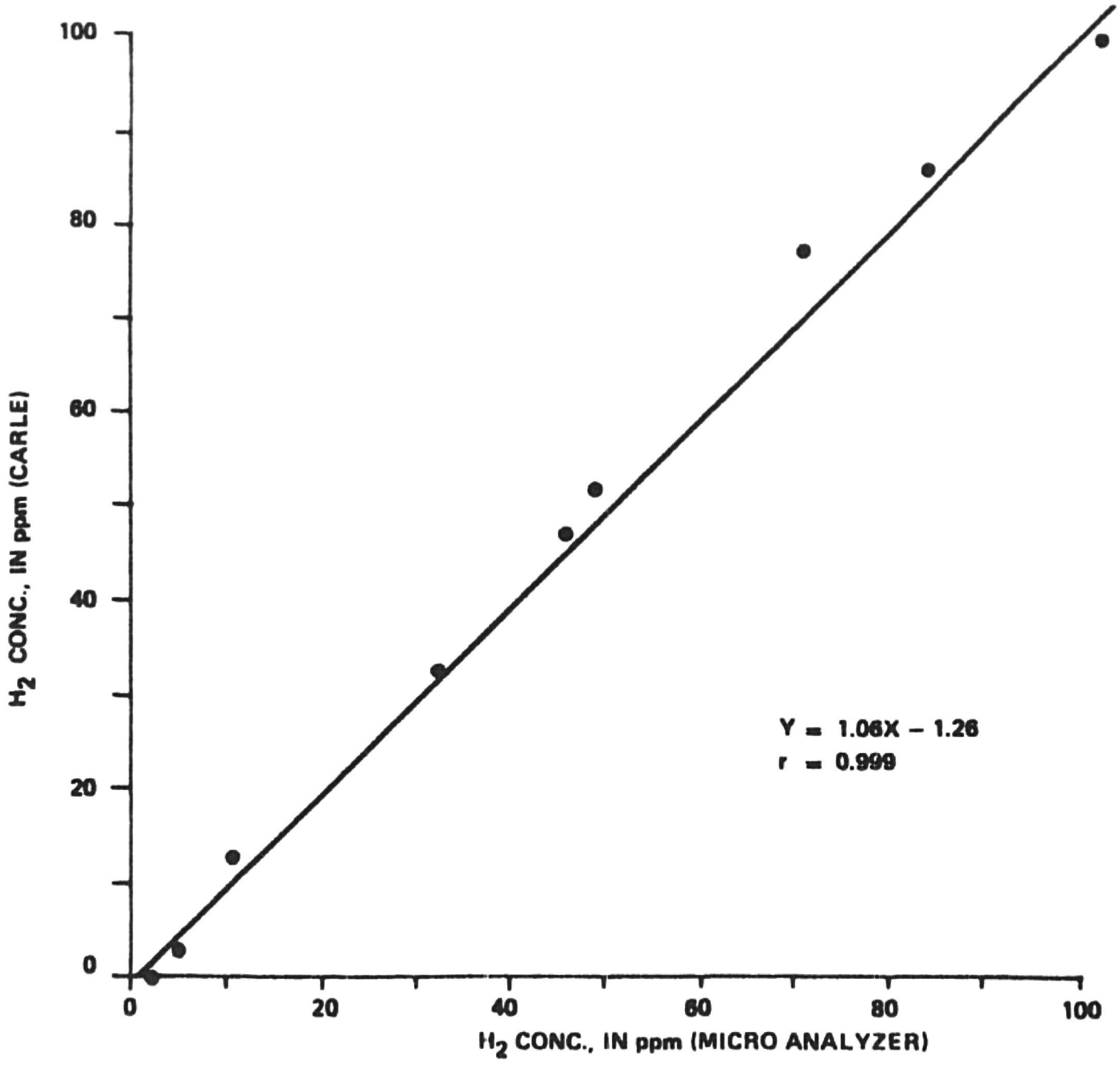


Fig 2. The simultaneous comparison of analyses of various random dilutions of a 99.8 ppm or a 52 ppm standard gas from the same gas sample as determined on a Carle chromatograph (model 111) and on the MicroLyzer.

2, an excellent correlation, $r = 0.999$, and a displacement of only 1 ppm was achieved.

Fidelity of Digital Display and Analysis Response Time. Hydrogen concentration can be recorded either from the digital display or from an analog chart record. We connected the MicroLyzer to a single-pen recorder (model 101), and simultaneously made graphic recordings and notations of the digital reading. Random dilutions of a standard gas (Linde) and a single breath sample were analyzed. The digital results were manually recorded as registered by one of us (N.W.S.), and the corresponding graphic strip (Figure 3) was submitted blind to another (L.H.H.) for calculation by the peak-height method (12). A strict agreement between the two methods of quantitation was observed.

As determined by stopwatch or by evaluating the recording (Figure 4), the time required for a return to the zero baseline after the injection of a sample was 117 sec in the system evaluated. Thus, 30 samples can be analyzed per hour with the MicroLyzer, as compared to 4–6 samples with the previous system (9). If the MicroLyzer is left undisturbed after the analysis of a given sample and the instrument left in the "hold" mode, the memory display decays at a constant rate of about 1 ppm every 3 min. The electronic memory requires a peak of about 10 ppm in order to erase and reset the memory stage and record the new concentration. Thus, for all but low concentrations of H_2 in unknown samples, the peak reading can be determined by activating the "hold" mode within 3 min of sample analysis.

Longevity of the Column. Moisture will inactivate the molecular sieve chromatographic column and impair the flow characteristics and gas separation

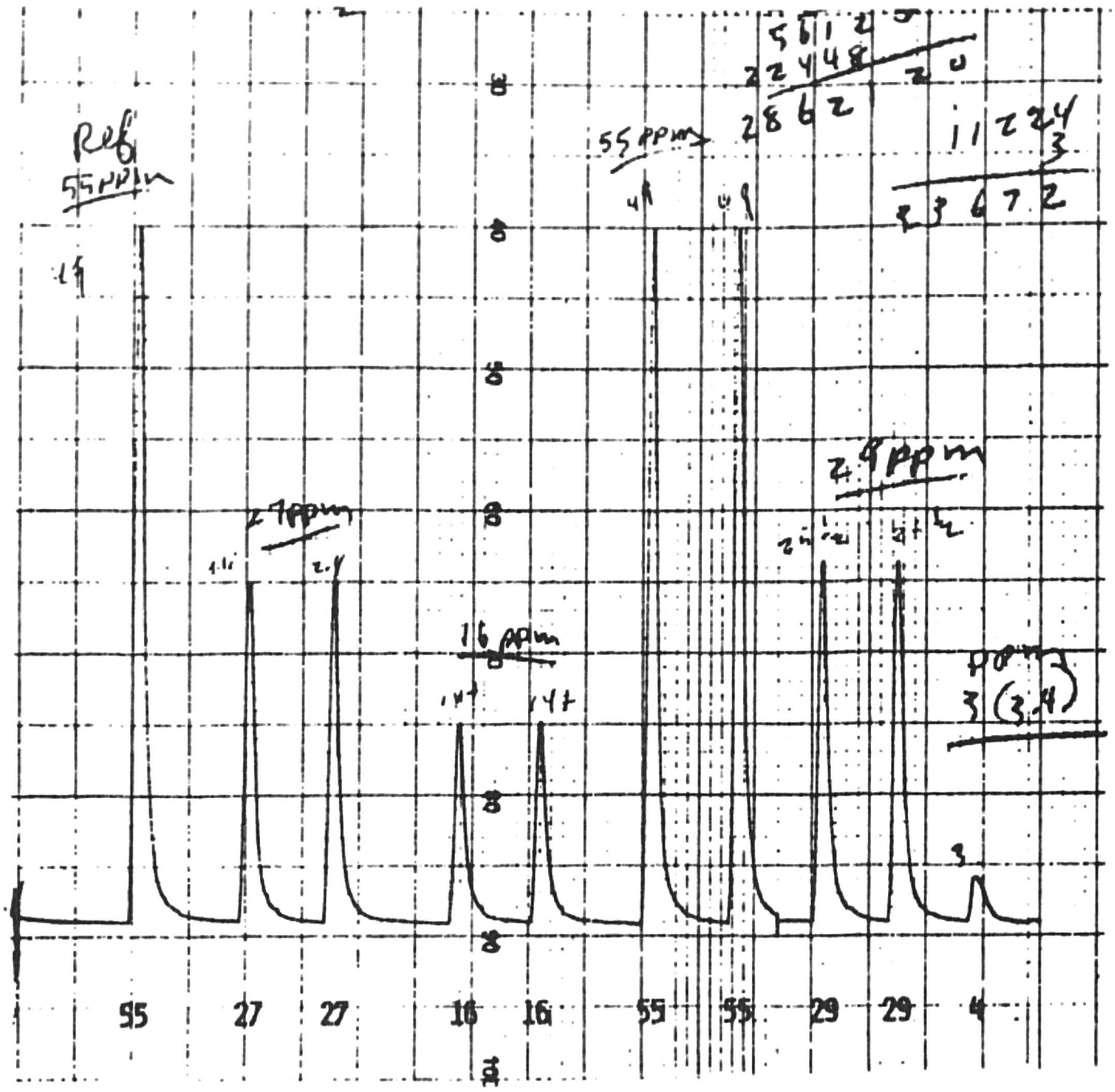


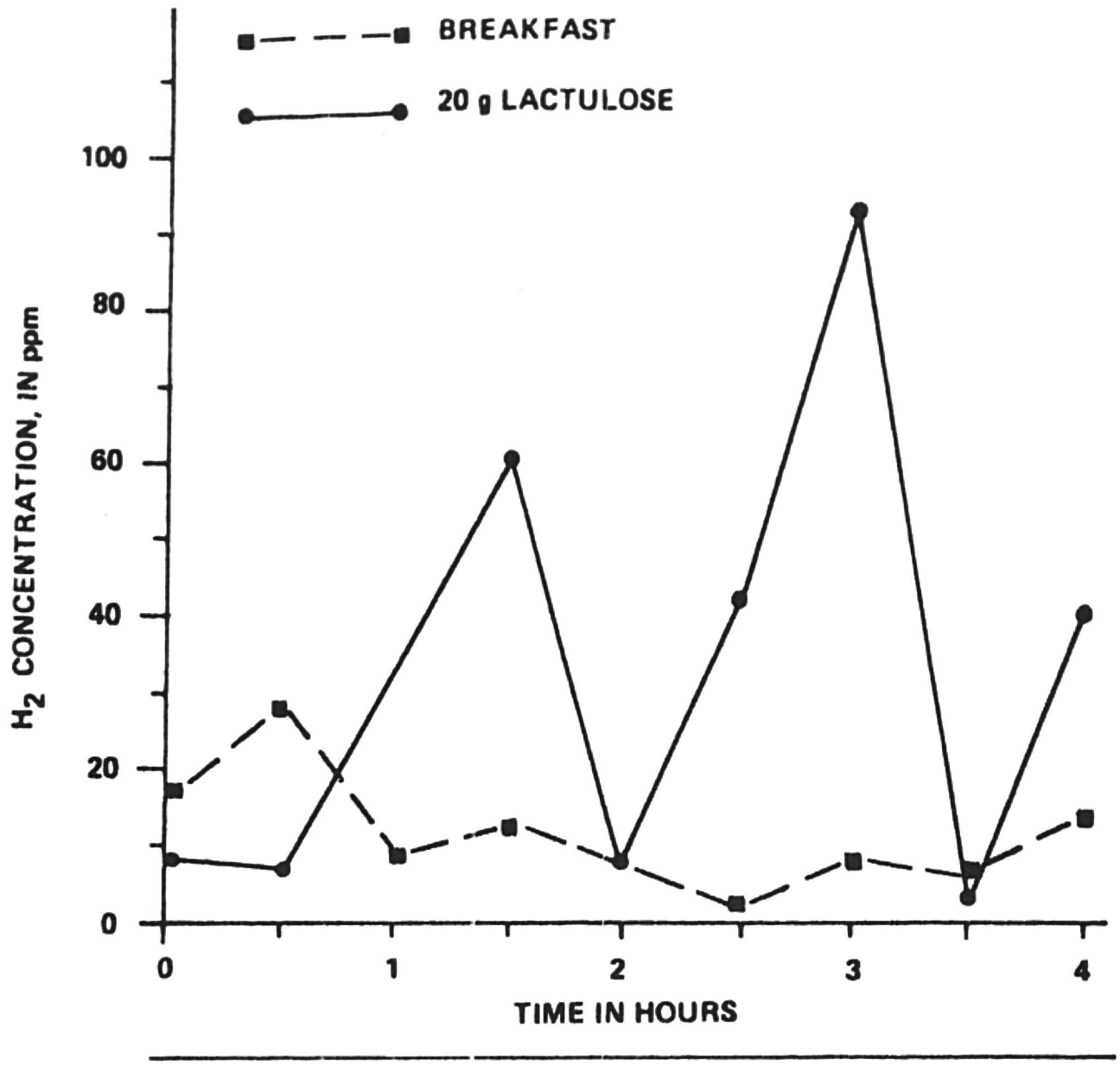
Fig 3. Actual section of graph paper in which recordings of various dilutions of H_2 in room air were recorded from the digital panel meter of the MicroLyzer by one of us (N.W.S.) as shown by the typed numbers below. The calculations based on peak height (ref 12) measured by the other (L.H.H.), without knowledge of the digital readout, are handwritten above the respective peaks.

performance. Scrupulous care must be exercised to protect the column from humidity. With the carrier gas (prepump) filter in place and the injection filter interposed between the syringe and the sample loop, chromatographic columns have functioned without signs of degeneration for 4–6 months, even in the humid environment during the rainy season in the highlands of Guatemala.

Application to Human Studies. To demonstrate the performance of the H_2 MicroLyzer under clinical conditions, a subject underwent serial half-hourly determinations of breath H_2 concentration after a routine breakfast of pancakes with or without a 20-g dose of lactulose. At each collection interval, a 70 cc sample of air was collected, and 35-cc aliquots were analysed in duplicate. The absolute levels of mean breath H_2 concentrations are shown in Figure 4. Because Tadesse and Eastwood (13) showed that cigarette smoking produces an artifactual rise in breath H_2, we have used this model to determine the limits of the digital meter response. Readings of 165–385 have been seen with the cigarette-smoke-contaminated breath samples, and serial dilution of such samples have shown a linear response analogous to that of the reference gas shown in Figure 1.

DISCUSSION

The second-generation H_2 chromatograph (MicroLyzer) offers significant advantages over our previous system for measuring breath H_2 (9). The baseline (zero) drift of the MicroLyzer is minimal, and our signal is free of the pressure artifact dis-



DATA

time (min)	H_2 in ppm	time (min)	H_2 in ppm
0	17, 17	0	8, 8
30	29, 26	30	6, 6
60	10, 7	60	32, 31
90	12, 12	90	60, 60
120	9, 8	120	8, 7
150	2, 2	150	41, 42
180	9, 7	180	93, 93
210	6, 5	210	2, 2
240	14, 14	240	40, 41

Incap 81–219

Fig 4. Serial, 30-min-interval determinations of breath H_2 concentration with the Micro-Lyzer in the same individual on two occasions. On the first, he consumed a breakfast of coffee with sugar, orange juice, three 5-in. pancakes, and imitation maple syrup; on the second he ingested the same meal plus a 20-g dose of lactulose (Cephulac®). Each 70-cc sample was divided in half and analyzed in duplicate. Displayed below are the values registered on the digital panel meter of the instrument.

played when the sample is introduced to our earlier unit and to most thermal conductivity chromatographs. A displacement of the baseline at the time when H_2 is eluted reduces accuracy, particularly at low H_2 concentrations. The solid-state detector system—essentially sensitive only to reducing gases—eliminates these background signals. The greater baseline stability and reduced response to other

components in the sample allow a much more accurate measurement of H_2 concentrations, particularly in the range of 0–10 ppm.

The short column allows repetition of successive samples without requiring a backflush system. If the number of patients, or samples from a given patient, are excessive, one need no longer devise elaborate storage systems (evacuated tubes, foil gas envelopes, etc) required with chromatographs having longer analytical times. This is especially relevant to the newer applications of H_2 breath-test technology attempting to define upper-intestinal bacterial overgrowth (14, 15), or gastrointestinal transit time (16–18), both of which require a sampling frequency greater than the half hour (19, 20), one hour (21), or two hours (4–6, 22) used in lactose absorption tests. The MicroLyzer allows breath sampling and analysis at 2-min intervals, providing an almost continuous record of changes in breath H_2 concentration, if needed. If it is desired to store the samples for subsequent analysis, they can be kept in the Mylar-impregnated bags or transferred to a plastic syringe. The digital display and memory eliminates the necessity for the operator to master the tedious and exacting procedure of calibrating with peak heights from the recorded response curves. Thus, operating simplicity is even greater than with the former model (9), and medical personnel (physicians) with no prior experience in chromatography have been able to operate the MicroLyzer with less than 30 min of instruction.

The simplicity of the MicroLyzer also contributes to its portability. Unlike the previous field surveys (4–8), in which gas was collected at one site and analyzed at a central laboratory, the MicroLyzer permits on-site analyses in remote situations provided only that a source of electric power is available and sufficient warm-up time (2 hr) is allowed. The large, heavy argon cylinder used for the carrier gas is no longer necessary, and the need to store and transport gas samples is reduced or eliminated. Despite its manageable size and light weight, the MicroLyzer offers improved stability and sensitivity comparable to more elaborate, immobile, conventional gas chromatographs.

The demonstration of the generation of H_2 in tobacco smoke is relevant to the breath H_2 test. Not only must smoking be avoided during tests for sugar malabsorption, but, since ambient air is used as the carrier gas, smoking should be avoided in the area of the MicroLyzer when it is being used for analyses. We have also observed that sometimes the environment contains vapors (eg, organic solvents) which are not detected by odor, but which will change the baseline of the MicroLyzer output. Introducing outside air to the inlet tube through the external drying column returns the baseline to its original reading. The various drying tubes are important to its operation. Not only do they preserve the life of the column by protecting it from contamination from water vapor, but the injection of the sample of moist expired air through a drying tube reduces the small error introduced by having a dry gas as the reference mixture and a humid gas as the unknown.*

This study, conducted in three widely separated laboratories with many standard mixtures for reference gases, demonstrated the possible source of discrepancies among reference gases. Inconsistencies between reference gases from different suppliers and even from the same supplier were seen more often than not, with discrepancies of as much as 10 ppm seen with a set of gases certified to be 55 ppm and 118 ppm by the manufacturer. The accuracy of the stated value certified by the suppliers of reference gases is ±5% for SupelCo and Linde Specialty Gases Division and ±2% or 5% (depending on concentration) by Matheson. Apparently not all reference standards which were used met those criteria. Most discrepancies, however, were in the expected ranges, and the analytical errors caused by the reference gas variability would not cause problems in the clinical application of the technique.

Ironically, having the system very specific for H_2 can prove to be a disadvantage in one aspect. Niu et al (23) have suggested the utility of simultaneous measurement of another gas in the sample, such as carbon dioxide, to adjust breath samples for leakage or altered ventilation induced by sleep or crying in

*If for some reason the sample is not dried before it is introduced into the sampling valve, but the reference gas is dry, a small correction factor can be applied to account for the effect of water vapor on the sample size. The correction factor is applied to the apparent concentration of the H_2 in the reference gas:

$$\text{Ref gas [ppm]} \times \frac{P_B - P_{H_2O}\ \text{room temperature}}{P_B} = \text{Apparent ref gas [ppm]}$$

Since P_{H_2O} is usually near 25 mg Hg at room temperature and P_B something over 700 mm Hg, the correction factor is less than 3.5%, which amounts to about 3 ppm for each 100 ppm H_2 in the reference gas.

infants. The use of such gas as an internal reference, a facility offered by more complex gas chromatographic systems, is not available with the MicroLyzer.

The technology for H_2 breath analysis has passed from an investigative novelty to a mainstream clinical test for the hospital or office and a useful noninvasive field instrument for anthropological and nutritional surveys (3). The MicroLyzer incorporates a series of features which are ideally suited to these applications.

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