BREATH HYDROGEN (H_2) ANALYSIS IN SOUTHERN CHINESE CHILDREN AND INFANTS BY GAS CHROMATOGRAPHY AND A NOVEL AUTOMATIC SAMPLING SYSTEM

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ABSTRACT

Breath hydrogen (H_2) analysis was used to study lactose malabsorption in Southern Chinese children and infants. End-expired air was collected in 85 children using a modified anaesthesia bag system; while in infants, a novel automated end-expired sampling device was constructed and tested on 45 term and 27 preterm infants. Hydrogen and other respiratory gases were measured in the expired air using standard gas chromatograph equipped with a thermal conductivity detector. The system was found to have a detection limit of 0.5 ppm for H_2. Both sampling methods were found to be reproducible, with intra-individual coefficient variation of less than 10%. Using 5% carbon dioxide as the expected alveolar concentration, the samples obtained by the bag system represented 85% of the end-expired air, while those obtained by the automated machine corresponded to 75%-100% end-tidal air. Taking 20 ppm rise in breath H_2 as a cutoff criterion, the incidence of lactose malabsorption in the children was 78%; while in term and preterm infants this was 17.8% and 63% respectively.

Keywords: *breath hydrogen analysis, lactose malabsorption, sampling of expired air, Southern Chinese children and infants, gas chromatography*

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INTRODUCTION

Since the work of Levitt and his coworkers, breath hydrogen (H_2) analysis has been developed as a diagnostic test to evaluate small intestine transit time[1-3], carbohydrate malabsorption[4-7], and small intestine bacterial overgrowth[8-10]. Hydrogen production occurs as a result of dietary substrate fermentation by intestinal (usually colonic) bacteria, and there is good correlation between intestinal H_2 production and breath H_2 excretion[1]. If a well-absorbed carbohydrate, such as lactose, is administered, an increase in breath H_2 may reflect carbohydrate absorptive status of the small intestine. In fact, lactose breath H_2 test has been regarded as the most valid non-invasive method for quantifying lactose malabsorption[6,11].

The use of breath analysis for clinical diagnosis require an expired air collection method which falls into two categories: one involves collection of the total H_2 excreted in breath over a given period of time[1-3, 11-13], and the other involves a single or multiple samples of either whole breath or end-tidal fractions gathered on successive expirations[7, 12, 14-21]. Despite the more quantitative nature of the continuous collection method, the procedure is more complicated and less useful for routine clinical use. End-expired air sampling has been shown to reliably reflect carbohydrate malabsorption in infants and children using nasal prongs or catheter[7, 13], facemask[15, 22], and rebreathing techniques into a hood[23,24].

Various methods and instruments have been developed to measure trace level of H_2 (Table I)[25]. Conventionally, gas chromatography is used for this purpose. The gas is at first separated from other components by an adsorption column in a stream of carrier gas, and then subsequently quantitated by the detector installed downstream the column. Commonly employed detectors include thermal conductivity detector[25-28], helium[16] or argon ionisation detector[29,30], electron-capture detector[31, 32], and electrochemical detector[33-35]. The development of a reducing gas detector by Stevenson et al enables the determination of trace H_2 down to 0.01 ppm[18,38]. However, its great cost and requirement of expertise operation place strong limitation for routine use in a hospital.

The present study aims to determine the breath H_2 response to dietary lactose administration in Southern Chinese children and infant with a view to standardising the test procedure for routine clinical screening. End-expired air was obtained in children by a modified anaesthesia bag connected via two one-way valves to the mouthpiece, while a novel automatic breath sampling system was developed and tested for use in infants. The study was approved by the Ethical Committee of the University of Hong Kong.

MATERIAL AND METHODS

Test Subjects

Eighty-five children with ages ranging from 4 to 12 years

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Table I – Analytical methods for the detection of trace level of H_2

Chromatographic method	Helium ionisation detector[18] Thermal conductivity detector[26 28] Argon ionisation detector[29, 30] Electron capture detector[31, 32] Electrochemical detector[33-35] Electrolytic detector[36] Pyroelectric detector[37] Reducing gas detector[37 40]
Piezoelectric method	Pd coated quartz crystal[41] Sintered SnO_2 mixed with Pd, Rh, and MgO[42]
Electrochemical method	Semiconductor gas sensor[6, 43] SnO_2 + Ag[43 45] SnO_2[46] Ag on porous silica[46] $ZnO/PdCl_2$[47] Pt/SnO_2-Fe_2O_3-Al_2O_3-$PdCl_2$[48] Figaro TGS 202 sensor[49] Metal-semiconductor contact potential[50] Fuel cell[51] Field effect transistor[52-57] Clark type H_2 detector[58] Solid state proton conductor[59-61]
Other methods	Optic fibre sensor[62] Acoustic wave interaction[63] Thermochemical method[64] Mass spectrometry[65]

(mean±SD=8.3±2.5) and body weight from 18.7kg to 39.5 kg were chosen from the paediatric ward of the Queen Mary Hospital. They were all hospitalised for non-gastrointestinal conditions. Twenty-six of them have completely recovered from their illness during the tests, and the drugs prescribed for the rest did not involve antibiotics. Preliminary questioning revealed 18 of them were milk intolerant, ie they experienced intolerant symptoms (bloating, abdominal pain, flatulence) after taking a glass of milk (approximately 300ml).

Forty-five term (gestation: 38-43 weeks) and 27 preterm infants (25-37 weeks) of Southern Chinese origin were also studied in the maternity ward and the Special Care Baby unit of the hospital. Informed consent was obtained from their parents before the test. All infants were on their routine formula feeding (S26, Wyeth) at three-hourly intervals. None of them have been receiving antibiotics.

Collection of expired air

In children, end-expired air was obtained in duplicate using a modified anaesthesia bag schematically shown in Fig 1. It was constructed of a 1-litre latex bag (Hudson Co.) connected via a one-way valve to a T-joint (IMV Manifold, Hudson Co.), one end of which was attached with a self-sealing silicone rubber septum (SU-850, Gallenhamp) and the other connected via a one-way valve to a disposable mouthpiece. The children were asked to breathe deeply into the mouthpiece to inflate the bag until he or she was exhausted. The end-expired fraction of the air was trapped by the two one-way valves (estimated volume 37 ml) and then transferred to a water-sealed all-glass syringe (2ml, Dovebrand, Shanghai) via the septum on the T-joint.

In newborn baby and infant, end-expired air sample was collected using a newly developed automatic sampling system which has been validated recently by the authors[66]. The system has three main parts: the sensing probe, a controller, and a syringe driver (Fig 2). The sensor is an electronic device consisting of a

Fig 1 – Schematic diagram of the anaesthesia bag system used for end expired air collection in children

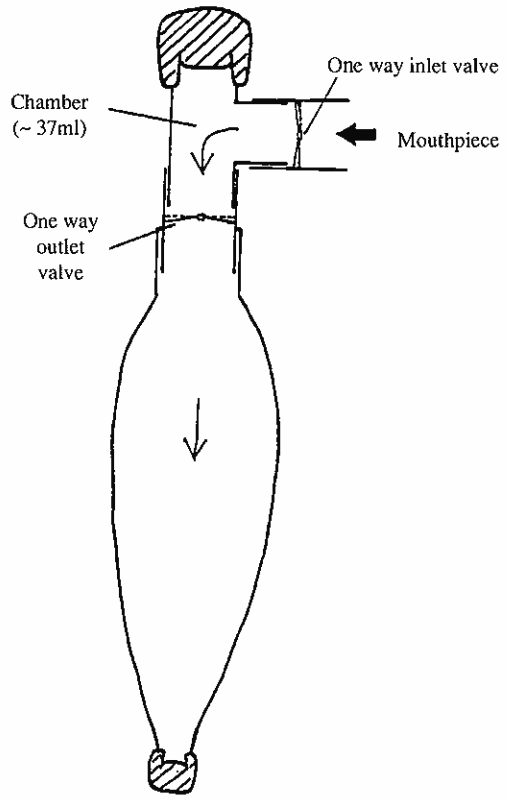
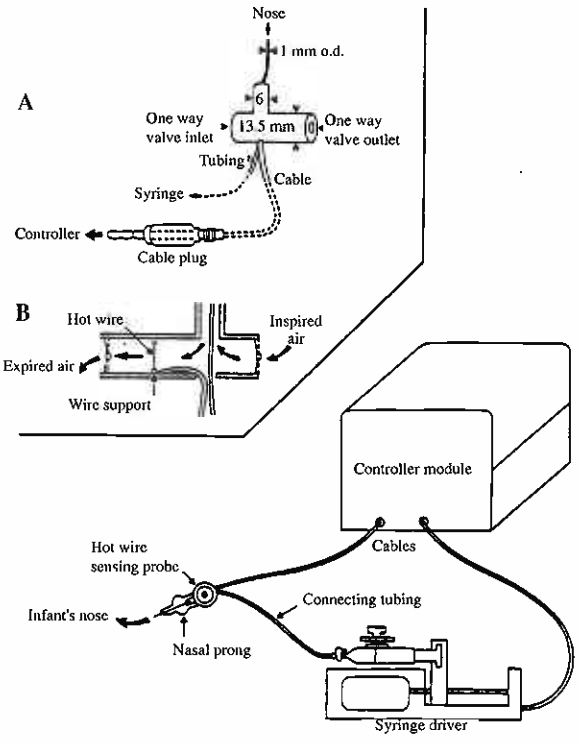


Fig 2 – The novel automatic end-expired air sampling device used for breath collection in infants. Inset shows accessories to the sensior (A) the sensor probe with the fine tubing for air sampling, and (B) internal arrangement of probe (o.d. denotes outer diameter)



tungsten hot wire of diameter 5 μm. A fine catheter (outer diameter 1 mm), with one end connected to the needle of a glass syringe on the driver, is inserted into the probe, which protrudes slightly into the nasal cavity through one of the two limbs of an infant nasal prong. The nasal prong (Argyle Co Ltd, St Louis, USA) prevents direct contact of the infant with the electronic part of the system. Cooling of the sensor by the expired air flow causes imbalance of the Wheatstone bridge circuit, and this signal is despatched to the controller. The controller, which is a microcontroller based system to integrate and interpret messages, picks up expiratory signals from the sensor. Above a certain preset threshold value of the output signal, the controller can be triggered to drive the piston of the syringe to collect a sample of expired air. The driving stops as soon as the signal falls below the threshold. A variable time switch is incorporated in the controller so that the end-fraction of expired air can be collected, and the time between the sensing of expiration and the driving of the syringe can be set from a minimum of 0 ms to a maximum of 990 ms at 10 ms intervals. The syringe driver is electronically operated by the controller. Upon expiration and as soon as the delay-time has been completed, the motor is automatically triggered to drive the piston of the mounted syringe. Since only 0.25 ml sample is needed for breath H_2 analysis, cumulative samples to a total of 1 ml are collected from successive expirations. The detailed operational procedure has been described in detail elsewhere[66]. Previous experiment revealed that the sampler could obtain 87% of the end-expired air sample as indicated by the respiratory gases concentrations (oxygen [O_2], nitrogen [N_2] and carbon dioxide [CO_2])[25, 66].

Validation of breath sample storage in syringes

Since the study required the temporary storage or collection of breath samples in capped syringes, the following experiment was undertaken to assess the extent of gas leakage from these devices, with a view to confirming that the problem would not interfere with the experimental results. All-glass syringes (2 ml, Dovebrand) were tested in the study. The luer tip of each syringe was fitted with a three-way teflon stopcock. The plunger-barrel space of the syringe was sealed with water, and such technique has been shown to retain 85%-90% of H_2 for a storage period of 16 hours[25]. A total of thirty-eight glass syringes were filled up with standard gases as follows: of the 38 glass syringes, half were filled with 111.9 ppm H_2 in N_2 (HK Oxygen) while the rest with 19.99% CO_2 in N_2 (HK Oxygen), up to the 2-ml mark. One syringe from each of these two groups was analysed immediately as the zero-time value, while the rest were left undisturbed at room temperature. Three from each group were analysed periodically as follows: 1, 2, 4, 8, 16 and 32 hours.

Analysis of sample by gas chromatography

Samples collected were sent as soon as practicable for analysis in the laboratory close to the ward. The Shimadzu GC8-APT gas chromatograph (Shimadzu Co, Japan) was used for determining the contents of the samples. It was equipped with a thermal conductivity detector (TCD), and to increase the sensitivity of detection, a chopping amplifier (Shimadzu Amp-7, Japan) was incorporated into the system. The output signal was then read on a chart recorder (Shimadzu R111, Japan) connected in parallel with a data processor (Shimadzu CR3A Chromatopac, Japan). Extra pure grade argon (HK Oxygen) was used as the carrier gas. It was passed through a molecular sieve trap (pre-filter) to remove any trace of moisture and residual gases which would deteriorate the performance of the separating column.

The sample was introduced into the gas chromatograph by a 0.25-ml gas tight syringe (Hamilton Co Ltd, USA). Interfering components in the sample were separated by the stainless steel molecular sieve column (13X, 80/100 mesh, 6' x 0.125") for H_2, O_2 and N_2. The quantity of the component was measured by integration of the TCD signal output with results expressed in both peak height and peak area. The results were then compared with that obtained with standard gas mixture (111.9 ppm H_2 in N_2), and the concentrations were then printed out on the data processor. Parameters such as column temperature, filament current, and flow rate were then optimised to give a sensitive measurement of H_2 content. Using the same operational conditions, another 0.25 ml of the sample was injected into channel 2, where a silica gel column (80/100 mesh, 6' x 0.125") was used to separate out the CO_2 peak.

Breath H_2 Test (BHT)

The BHT methodology was basically as follows: in children, a basal breath sample was collected after an overnight fast (at least 8 hours), and a test meal of isotonic lactose solution (*ca.* lactose 1g per kg body weight) was then administered. Further breath samples were collected at half-hourly intervals thereafter, for a total of 4 hours. In infants, in order to attain maximal co-operation from the mothers and minimal interruption to nursing procedures, prolonged fasting was not implemented for the infants. The basal sample was taken just before meal time ie 3 hours from the last meal. All infants were given a test meal of 7% freshly prepared aqueous lactose solution (*ca.* lactose 1g per kg body weight). Hydrogen and carbon dioxide analyses were performed within 15 minutes of breath sample collection. Each H_2 value was then normalised to a CO_2 concentration of 5%, representing the normal end-expired concentration[67].

Blood Glucose Test

In order to study the BHT against the conventional blood lactose tolerance test, twenty-four of the aforementioned children were simultaneously monitored for their blood glucose levels during the test procedure. Blood for glucose analysis was drawn from fingertips using a lancet. Samples were drawn at 0, 15, 30, 60, 90 and 120 minutes. Glucose in the capillary samples was determined using the Beckman Glucose Analyser 2 (Beckman) which is based upon the oxygen rate method employing an oxygen electrode. The method has been shown to be accurate and a precision of 0.8% was attained with the manual sampling pipettes[66]. All samples were immediately analysed after collection to avoid storage instability.

RESULTS

The syringe/stopcock system maintained H_2 concentration at 90%-98% after 8 hours, and at 56%-63% after 32 hours. Since all samples were done within 15 minutes of the breath collection, the problem of gas leakage did not seem to pose a false negative error to the experiment (Table II).

Using a filament current of 70 mA, and a column temperature of 70°C, the optimum flow rate for the measurement of H_2 as shown from the Van-Deemter plot of height-equivalent-to-theoretical-plate (HETP) versus flow rate (Fig 3) was around 20-30 ml/min. Under these operating conditions, the H_2 content was measured with a detection limit of 0.5 ppm and at a retention time of 0.45 minute. The linearity curve of H_2 was calculated from regression analysis and was shown in Fig 4 (a, b). Carbon dioxide was well separated from its preceding peak (mixture) and had a retention time of 1.6 minutes. The precision of measurements as documented from the standard gas were found to be less than 2%.

The CO_2 contents of the breath samples obtained by the modified anaesthesia latex bag were found to be 4.25±1.50% (SD, n=1330), corresponding to 85% of the alveolar

Table II – Retentivity of glass syringes for H_2 and CO_2

Time (hour)	Percent retained (%) Hydrogen (111.9 ppm)	Carbon dioxide (19.99%)
0	100.0	100.0
1	99.9	98.9
	99.5	99.9
	99.6	99.0
	(99.7)*	(99.3)
2	99.5	99.1
	99.0	99.0
	99.4	98.5
	(99.3)	(98.9)
4	98.8	98.0
	98.1	97.5
	99.0	97.6
	(98.6)	(97.7)
8	90.6	95.9
	92.8	96.6
	93.5	96.0
	(92.3)	(96.2)
16	89.8	91.8
	87.6	90.8
	86.0	92.7
	(87.8)	(91.8)
32	55.8	86.9
	63.2	90.0
	60.4	82.9
	(59.8)	(86.6)

*Data in parenthesis show the mean value.

Fig 3 - Van-Deemter plots of height-equivalent-to-theoretical-plate (HETP) versus carrier gas (argon) flow rate

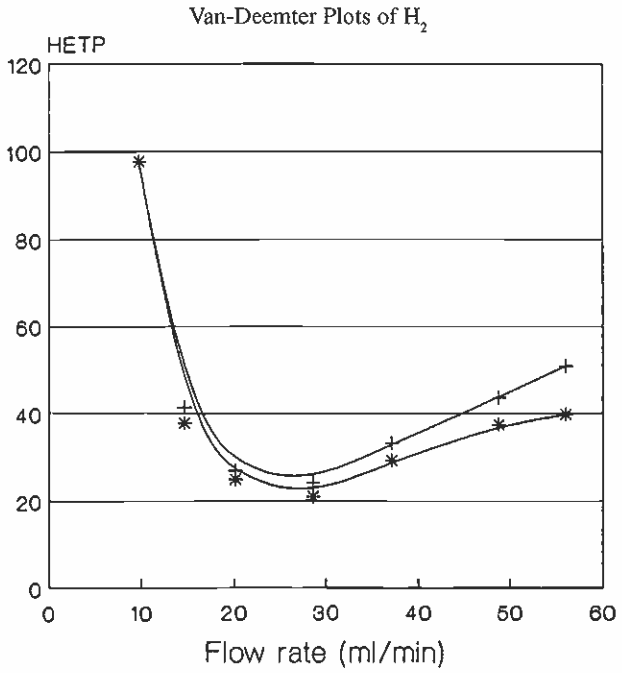


Fig 4 – Response curve of GC-TCD to trace concentrations of hydrogen: (A) gas chromatograms of trace levels of hydrogen diluted with ambient air.

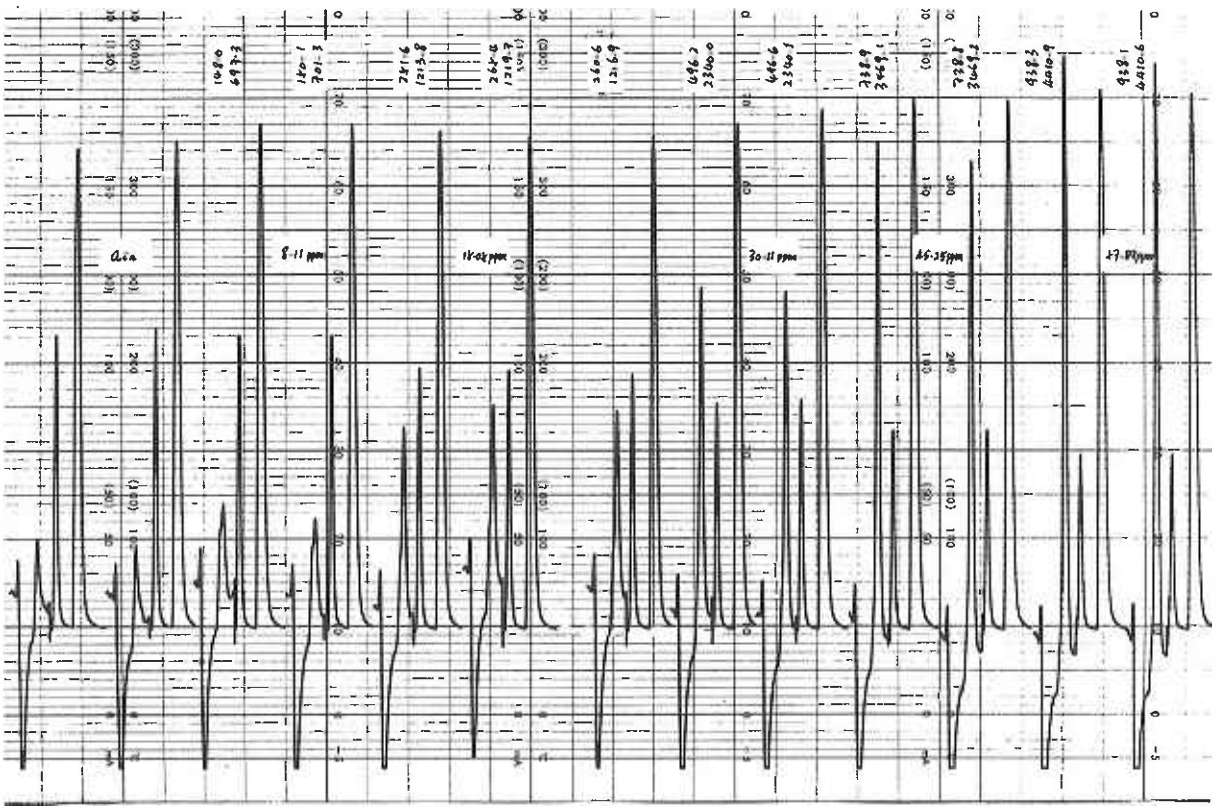
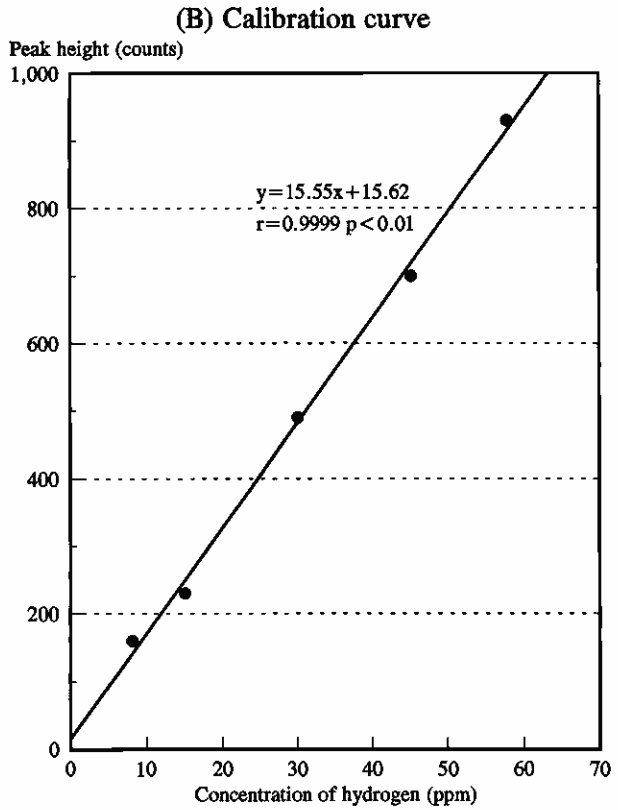


Fig 4 – Response curve of GC-TCD to trace concentrations of hydrogen: (B) Calibration linearity curve

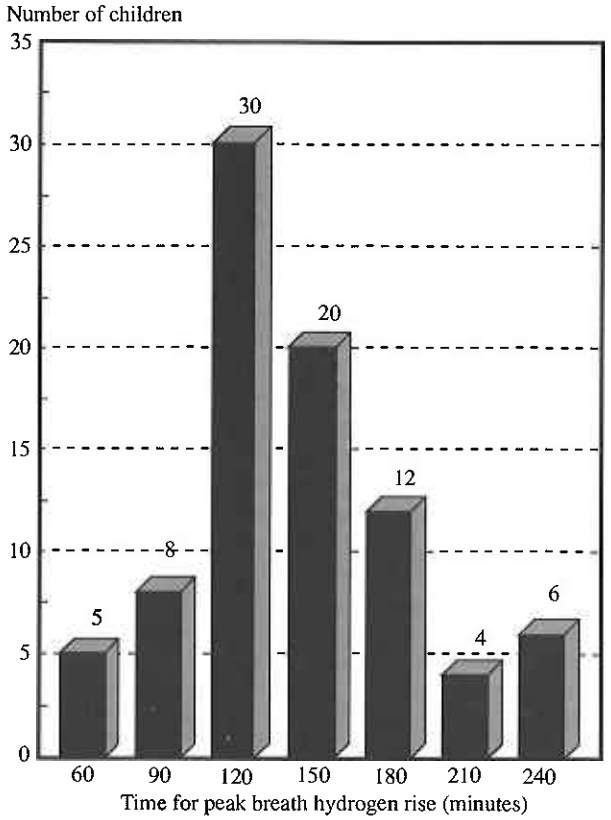


concentration[67,68]. The intra-individual sampling precision was estimated from the coefficient of variation (CV) of the duplicate samples and was calculated to be less than 10%. Similarly, the automatic end-tidal air sampling device offers a precise sampling method with reproducibility of more than 90%, as measured by intra-individual CVs of the concentration of respiratory gases. When comparing with the expected concentration of CO_2 of 5%, the values of breath H_2 obtained correspond to 72%-100% of the end-expired air.

Fig 5 shows the timing of maximal H_2 concentration in the 85 breath H_2 tests. The peak H_2 excretions ranged from 60 to 240 minutes after the lactose meal. Seventy-two of the 85 children had the peak H_2 rise at 120 minutes or later. Fig 6 (a, b, c, d) show the distributions of the rise in breath H_2 at different time after the lactose administration. If using 20 ppm (v/v) rise in breath H_2 as the diagnostic criterion, then at 90-minute, only 3.5% of the children were classified as lactose malabsorbers. The incidence was increased to 78.8% when diagnostic time was taken as 120 minutes. Fig 7 (a, b) summarise the breath H_2 profiles of the 85 children. Their fasting values range from 4 to 22 ppm with a mean of 10.6 ppm. The fasting H_2 concentrations of the malabsorbers were found to be significantly higher than those of the absorbers ($p<0.005$).

A total of 72 breath H_2 tests were performed in the infants. Eight of the 45 term infants (17.8%) showed an elevation of normalised breath H_2 greater than 20 ppm, while 63.0% (17 in 27) of the preterm infants showed such rise. The basal breath samples of the term infants had H_2 content ranging from 1.41 ppm to 26.32 ppm, with a mean of 9.085±4.809(SD) ppm. Correlation of excretion of H_2 after lactose administration with the basal H_2 value was found to be significant ($p<0.01$) with a regression coefficient r=0.28316. The mean basal breath H_2 concentration of the preterm infants was 16.20±12.82(SD) ppm, ranging from 2.87 to 67.7 ppm. A linear correlation was also

Fig 5 – Distribution of postprandial time for peak hydrogen rise in 85 children after a lactose load of 1 g/kg



observed between the postprandial rise in normalised breath H_2 and the basal breath H_2 with a regression coefficient of 0.60480 ($p < 0.0001$).

Table III shows the cross-tabulation of breath H_2 test by blood glucose test, weighted by frequency, on 24 children. In the blood glucose test, a rise of blood glucose content ≥ 1.1 mmol/L after lactose challenge is regarded as normal[6] and is defined as negative for lactose malabsorption, while a rise < 1.1 mmol/L indicates malabsorption and is defined as positive. Based upon these criteria, the sensitivity and specificity of the BHT measured against the conventional blood lactose tolerance test were calculated respectively as 12/13 = 0.923 and 8/11 = 0.730.

DISCUSSION

Gas chromatography equipped with a thermal conductivity detector has been used to determine trace H_2 content in expired air[25-27]. It is in fact not only a detection method, but also involves a molecular sieve column that separates H_2 from the diluent and hence removes the possible interference. Moreover, the fast recovery time of the detector, as well as the stable analytical condition attained by the system would make it particularly more suitable than other modes of detection for routine clinical use in hospitals.

The present study shows that the Shimadzu system can detect H_2 level with a limit of 0.5 ppm. Incorporation of the Amp-7 chopping amplifier affords a stable amplification of the TCD signal while permitting the use of a low filament current (70 mÅ). This helps to maintain a longer service life of the alloy filaments, since a low thermal conductive carrier gas (argon) has been used to increase the sensitivity of H_2. Moreover, the use of a low flow rate (20-30 ml/min) and a 6 ft molecular sieve column allow the H_2 peak to be detected at a reasonably fast

Fig 6 – Distribution of rise in breath hydrogen in 85 children at (A) 90 min, (B) 120 min, (C) 150 min, and (D) 180 min after a lactose load of 1 g/kg

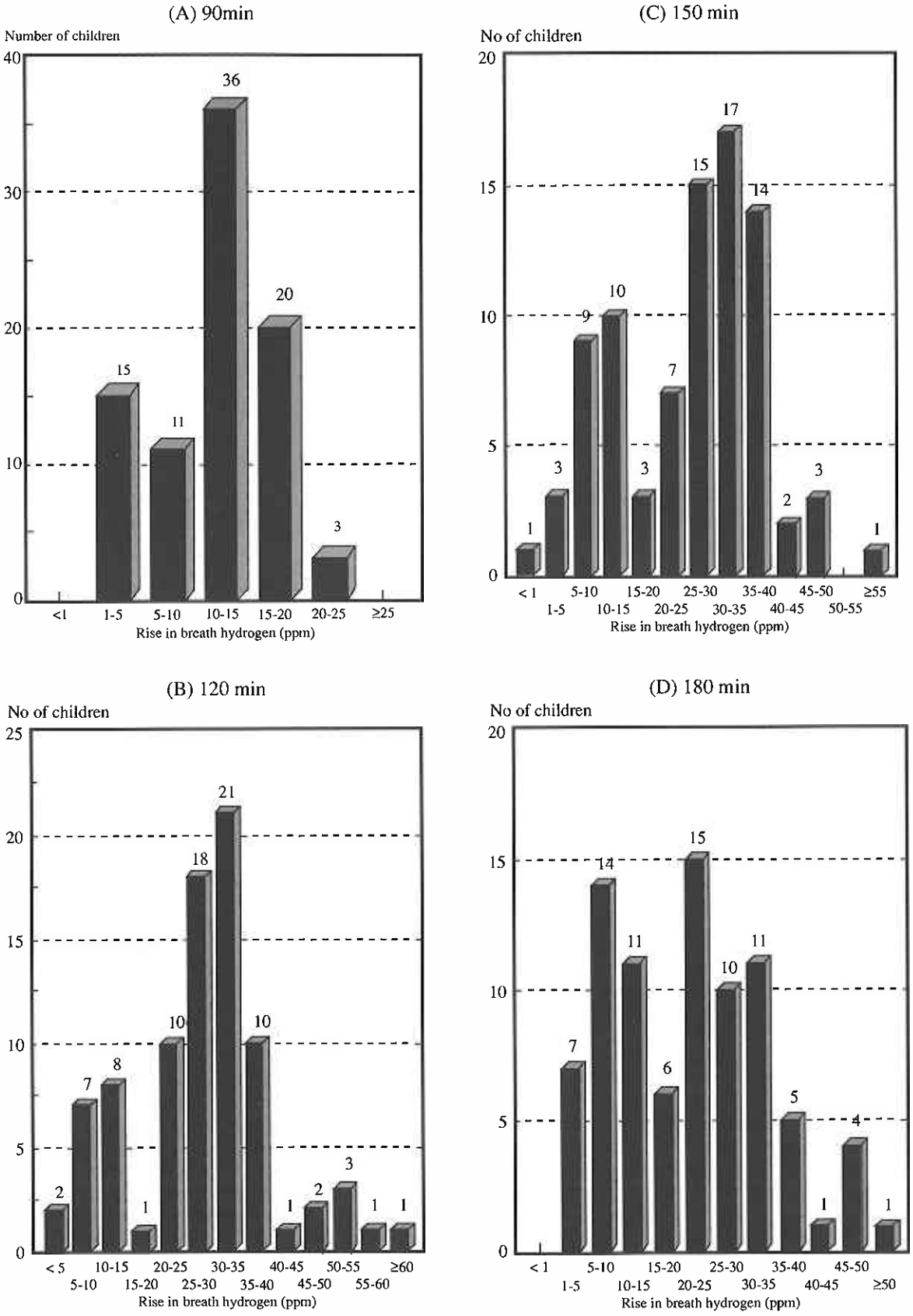


Fig 7 – Breath hydrogen profile of the 85 children. A rise of 20 ppm hydrogen is used as criterion to distinguish (A) malabsorber from (B) absorber

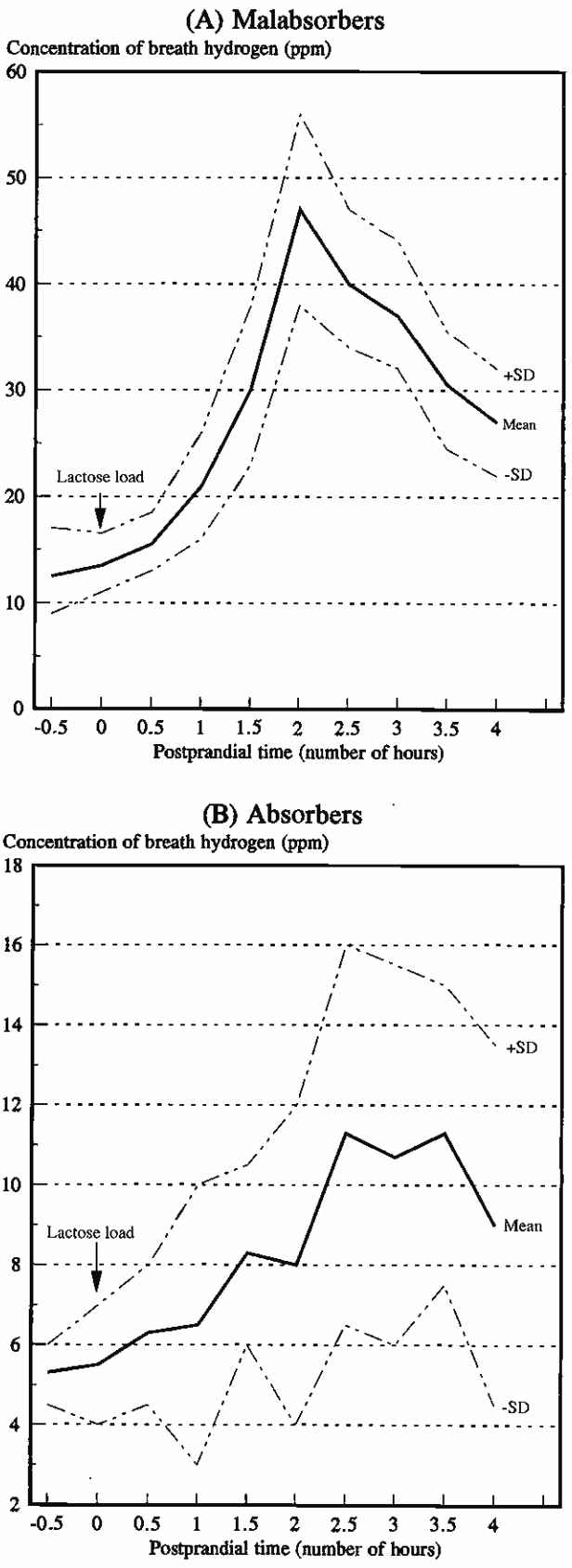


Table III – Cross tabulation of breath H_2 test (BHT) by blood lactose tolerance test (LTT)

	LTT Positive	LTT Negative	Row Total
BHT Positive			
Count	12	3	15
Row percent	80.0	20.0	62.5
Column percent	92.3	27.3	
Total percent	50.0	12.5	
BHT Negative			
Count	1	8	9
Row percent	11.1	88.9	37.5
Column percent	7.7	72.7	
Total percent	4.2	33.3	
Column	13	11	24
Total	54.2	45.8	100.0

time and with sufficient resolution.

Table IV shows a comparison among different detectors used for determining trace H_2. Although TCD is not the most sensitive detector, its large linearity range and fast response simplify the analytical work, and it also avoids the use of radioactive source as in the case of helium ionisation detector or argon ionisation detector. Moreover, it allows simultaneous determination of O_2, N_2 and CO_2 contents in the breath sample, thus facilitating the use of them as internal standard. It also avoids the hazard of mercury vapour that may be connected in the use of the reducing gas detector.

Since breath is not a homogenous sample, its components are expected to be continuously diluted by the anatomical dead space as they come out of the respiratory tract in the expired air. Hydrogen as an exogenous gas produced in the colon and brought to the alveoli by the bloodstream, is expected to have its concentration rising in expiring air until a plateau is reached. Such plateau value has been regarded as the best estimate of the alveolar concentration[69, 70]. In the present study, the strategic approach of sampling this end-expired air was used to develop the sampling systems for use in children and infants.

The modified anaesthesia bag offers a convenient method of breath sampling in children. The low resistance of the valves as well as the high elasticity of the bag allows the children to blow throught it without difficulty. End-expired air is automatically trapped inside a small chamber (about 37 ml) sealed by the two one-way valves, and direct transfer of the sample to a water-sealed glass syringe can be readily accomplished at the self-sealing rubber stopper. The samples obtained are reproducible, with intra-individual CVs less than 10%; and represent about 85% of the expected alveolar concentration using CO_2 as an estimate. The inflating character of the bag also gives a more real indication of breath collection during sampling as compared with the facemask system or PVC collecting tube system used by previous workers[15, 17, 21]. However, cooperation of the test subject is still required and thus the system is generally applicable for children aged 3 and upwards.

Due to the lack of cooperation, breath sampling in infants can only be done either in a flow-through system[12, 13], or by intubation[12, 19]. However, the dead space of the flow-through system is too large and the available gas chromatographic method is not sensitive enough to detect the diluted sample. Intubation

Table IV – Comparison among different gas chromatographic detectors for the determination of trace hydrogen (H_2)

Detector	Carrier Gas	Sample Volume	Detection Limit	Possible Interference	Linearity Range
TCD	Ar	0.25 ml	0.5-1 ppm	He, Ne	0-500 ppm
HID	He	0.25 ml	< 1 ppm	Ne	0-10ppm > 10 ppm significant quadratic curvature
AID	Ar	1-2 ml	1 ppm	O_2, H_2O	30-150 ppm
ECD (SECS)	N_2 doped with 20 ppm N_2O	5.11 ml	0.1-1 ppm	CO_2, CH_4	–
Microlyzer	Room air	8 ml	2-3 ppm	CO	0-120 ppm nonlinear require linearizer
GMI Analyzer	Room air or N_2	20 ml	0.25 ppm	CO	0-100ppm
Reducing gas detector	Room air or N_2	2.5 ml	0.01 ppm	CO_2,CH_4	0.5-2 ppm

Note: TCD: Thermal Conductivity Detector
HID: Helium Ionization Detector
AID: Argon Ionization Detector
ECD: Electron-capture Detector
SECS: Selective electron-capture sensitization

is too invasive to the infant and consent from parents can hardly be obtained. Even if a catheter is successfully inserted into the pharyngeal region, the subsequent sampling by the plastic syringe in coordination with the chest movement of the infant, as described by previous workers[19], can hardly be a standardised procedure for routine breath collection. In this paper (see also reference 25) a new concept was tested to collect automatically a small fraction of the expired air, notably the end-expired fraction, of each breath upon the sensing of the expiratory signal; and subsequently collecting the composite sample for analysis. The automated end-tidal sampling system was then constructed. The results showed that the breath samples obtained represented 72%-100% of the end-tidal air sample using CO_2 as the estimate. The breath collection was reproducible and the nasal prong was found to be well tolerated by the infants.

The finding that the peak H_2 excretion occurred within 120-150 min interval after the lactose meal (1 g/kg) in the children may suggest the importance of collecting postprandial samples during this period if simplified procedure of the test is to be performed in field study[67]. Moreover, twenty-one of the children studied have reported intolerant symptoms during the tests (loose stool, abdominal pain, flatulence and/or bloating). They all had breath H_2 rise greater than 30 ppm. Some children however had breath H_2 rises greater than 30 ppm but were found to be tolerant to the lactose meal. This may indicate a poor correlation of the breath H_2 rise with the occurrence of intolerant symptoms.

The study in infants confirms earlier observations, based on the finding of excessive reducing substances (>0.5%) and acidity in the stools, that many neonates, particularly the preterm infants, have some degree of lactose malabsorption[71-73]. Although the incidence of lactose malabsorption is relatively low in our term infants (17.8%), in the preterm infants the value was significantly higher at 63.0%. Such significantly higher incidence of lactose malabsorption in preterm infants may be related to the intestinal disaccharidases with advancing gestation, as suggested by previous workers[74-76]. In humans, the disaccharidases are detected early in gestation and increase rapidly after 20 weeks and except for lactase, they reach adult level of activities by 27-28 gestational weeks. However, the activity of lactase tends to lag behind that of sucrase and does not reach "normal" or term levels until 36 weeks of gestation. The results thus suggested that malabsorption of pure lactose and increased excretion of H_2 in the breath is a normal event for the healthy infants, particularly the premature infants. It cannot be taken as *prima facie* evidence of lactose intolerance, but is more likely indicative of a desirable adaptation of the faecal flora to a physiologic malabsorption of the carbohydrate. Indeed as MacLean has suggested earlier that the decision whether lactose is being tolerated by the infant is a clinical one, based mainly on symptoms of abdominal distention, stool output, and rate of weight gain[19], an elevated breath H_2 concentration should not be the cause for a change in the dietary management so long as the infant is doing well.

In conclusion, the BHT is an effective test for the diagnosis of lactose malabsorption. Its simplicity and non-invasiveness allows it to be adopted in field and clinical studies with much acceptance. The conventional cut off level (20 ppm) has been proven with an accuracy of 92.3% for detecting lactose malabsorption. Despite the recent study by Davies et al[77] which showed that an interfeed interval of 4 hours in babies is insufficient to cause breath H_2 levels to fall in a predictable way, our results suggest that a preprandial fasting for 3-4 hours would suffice to avoid high basal values in infants, and the postprandial 120-180 min interval could be the best postprandial breath sampling time for maximum rise in H_2 concentration.

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