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Alterations of the colonic flora and their effect on the hydrogen breath test

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SUMMARY The hydrogen breath test was performed by ingestion of 20 g lactulose and analysis of end-expiratory air. Eighteen patients undergoing colonoscopy, 17 receiving antibiotics, 12 prepared for colon surgery, and 15 controls were examined. The test was repeated under control conditions in the treated patients. Eleven of 55 subjects failed to produce significant amounts of hydrogen under control conditions. This 20% proportion of non-hydrogen producers is much higher than that reported by other investigators. The hydrogen production was very markedly depressed after preparation for colonoscopy and antibiotic therapy. The effect of neomycin and enemata as used in preparation for colon surgery was less marked. Hydrogen production by the colonic flora is thus subject to individual variations and may be affected by various therapeutic regimens. All these may cause false negative results when using the hydrogen breath test to evaluate carbohydrate absorption. The test should therefore not be performed for a considerable time after therapeutic manipulation of the colonic flora.

The introduction of the hydrogen breath test (Bond and Levitt, 1972) for the study of carbohydrate malabsorption marked an interesting and significant advance in methodology. The test is non-invasive, devoid of radioactivity, and results are available within minutes of sampling.

The amount of hydrogen produced is said to be in direct proportion to the amount of malabsorbed carbohydrate (Bond and Levitt, 1972), thus giving a semiquantitative measurement. The test can be employed to study directly the absorption of any particular sugar, while in current clinical practice the unphysiological D-xylose is used to provide information on carbohydrate absorption in general.

As with most tests, however, the problems and limitations become apparent only with time. The H_2 breath test is based on the assumption that the colonic flora is a constant factor. It is supposed to be present in practically unlimited amounts, to digest any quantity of any malabsorbed sugar, producing predictable amounts of hydrogen in the process. The purpose of the present study was to evaluate the effect of alterations in the colonic flora on the hydrogen breath test.

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Methods

SUBJECTS

Sixty-two subjects were studied from the outpatient and inpatient population of our hospital. Twenty-seven were females and 35 males, aged 13-79 years, with a mean of 52·6 ± 15·3 years. Eighteen of them were undergoing colonoscopy. Seventeen were receiving various antibiotics for at least five days. Twelve patients had preparation for colon surgery. Fifteen were control subjects. Each of the subjects in the three treatment groups was studied twice, once during the treatment and once before or at an interval after the treatment. Seven subjects receiving antibiotics could not be studied under control conditions and their data are excluded from the various graphs.

TREATMENT SCHEDULES

Preparation for colonoscopy was as follows: for two days before the examination only a low residue liquid diet was allowed. Magnesium sulphate, 15 g,

was given on the evening two days before and castor oil, 30 ml, was given at noon the day before. Tap water enemas (2½ litres) were given the evening before and on the morning of the examination. If returns of the last enema were not clear, it was repeated forthwith. No premedication was used during colonoscopy in most cases. A few subjects received less than 5 mg diazepam intravenously. The breath test was performed shortly after the colonoscopy and repeated at control conditions either before or one week after the colonoscopy. Preparation for colon surgery consisted of: 4 g neomycin per day (1 × 4) for two days, and three 2½ l tap water enemas given the morning and evening the day before and the morning of the examination. Patients were studied under control conditions in most cases before the operation and in some cases a week after the operation, which was minor in the majority.

Antibiotics

Seventeen patients were studied after a minimum of five days of treatment. The drug regimens included: ampicillin with cloxacillin, ampicillin, ampicillin with colimycin, ampicillin with streptomycin, streptomycin with oxytetracycline, oxytetracycline, and erythromycin, in commonly used dosages. Ten patients were restudied two or more weeks after therapy ended.

HYDROGEN BREATH TEST

After an overnight fast patients received 30 ml Duphalac (Philip Duphar, Amsterdam) containing 20·1 g lactulose, 3·3 g galactose, and 1·8 g lactose, diluted to 300 ml with tap water. Before taking the lactulose, and at 30 minutes intervals thereafter, air samples were analysed for H_2 concentration. Air was sampled until a decrease in H_2 concentration was recorded, or for at least four hours.

The sampling of expired air was performed using the 'end expiratory technique' (Metz *et al.*, 1975). Air was collected in a simple system constructed in our laboratory. A 80 cm long thick walled Latex tube (9 mm i.d.) was fitted with two tightly connected plastic valves at both ends. One valve was attached to a disposable mouthpiece through which the patients breathed. At the end of a forced breath, the end-expiratory air was trapped in the tube by closing both valves. Samples for analysis were collected from the tube by a syringe fitted, *via* a three way stopcock, to a 25 × 5/8 mm needle. The stopcock was used to prevent leakage during the procedure. Hydrogen concentration was analysed immediately, using a Gow-Mac thermal conductivity gas chromatograph model 69-572. Two millilitre aliquots were analysed using a gas sampling valve. The column (¼ in o.d. by 8 in) was packed with molecular sieve 5A. Nitrogen was used as carrier gas. The column and detector were kept at room temperature (about 22°-25°C). Hydrogen concentration was calculated by comparing the peak area to the corresponding peak area of a 100 ppm hydrogen standard (Minicyl calibration gas, MG Scientific, N.J.). Studies in our laboratory (unpublished) have shown that H_2 concentration in the expired air does not vary significantly with the strength of the expiration. Patients or older people unable to perform a forced expiration can thus also be studied by this method. The curves in Figs. 1, 3, 4, 5 were derived by regression analysis using the least square method.

Results

Figure 1 depicts the mean H_2 concentration in the expired air plotted against time in 51 subjects studied under control conditions. Four subjects were excluded because they had high H_2 concentrations at zero time.

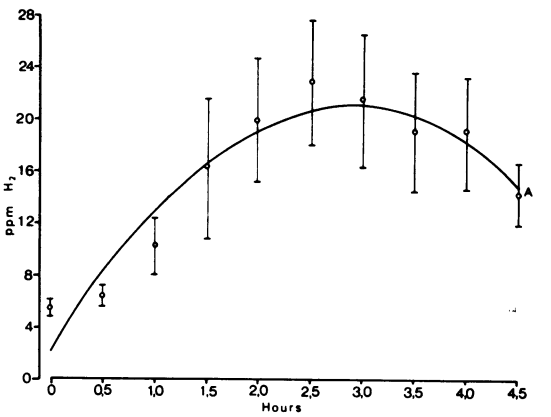

Fig. 1 *Hydrogen breath test: mean values of 51 subjects studied under control conditions. Vertical axis—hydrogen concentration (ppm). Horizontal axis—time (hr). Vertical lines indicate two standard errors.*

Eleven of these 55 subjects produced very little H_2 after lactulose ingestion. Their H_2 concentration was below two standard errors of the mean at all points throughtout the test (Fig. 2).

The mean age of these 11 subjects was 59·7 ± 13 years. Eight of them were above the age of 50 years, of whom four were above 70 years. Eight of them were males and three females. The mean age of the 40 hydrogen producers was 50·5 ± 12 years. Twenty-two were above 50 years, of whom six were above the age of 70 years. Twenty-one were males and 19 females.

Figure 3 shows the data of 18 subjects studied

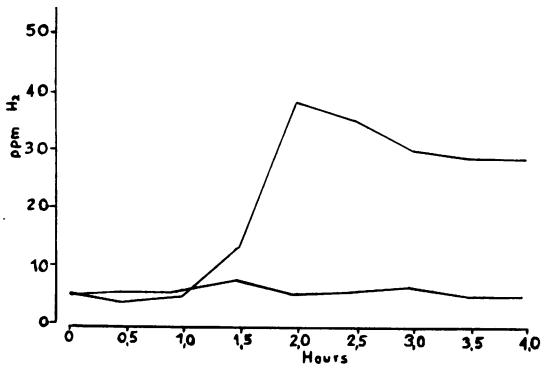

Fig. 2 *Hydrogen breath tests of two subjects under control conditions. A hydrogen producer (upper curve) and a non-producer (lower curve).*

under control conditions (A), and after colonoscopy (B). Their control data are within the range of the greater control group (51 subjects). After the bowel preparation for colonoscopy, the production of hydrogen was very markedly depressed ($P < 0.05$ for all points after 30 minutes).

Figure 4 shows the effect of antibiotic therapy in 10 subjects. Again there is a very marked depression of hydrogen production ($P < 0.05$ for all points except ½, 3½, and four hours). When the data were plotted for all 17 subjects receiving antibiotics, the curve was almost identical.

Figure 5 shows the effect of neomycin and enemas (as in preparation for colon surgery) in 12 subjects. The depression in hydrogen production is much less marked and the difference from control is not significant at most points (except for two and 2½ hours).

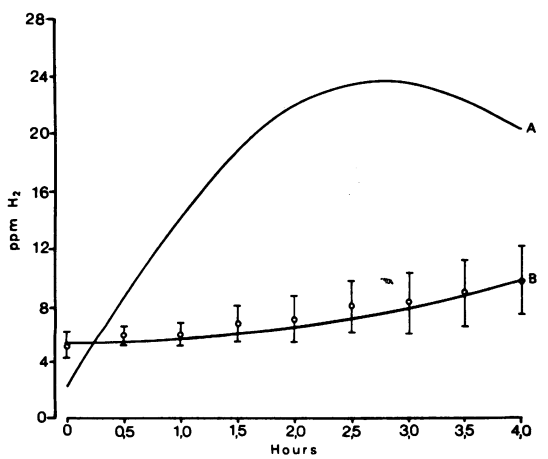

Fig. 3 *Effect of preparation for colonoscopy in 18 subjects. A: control study; B: after preparation.*

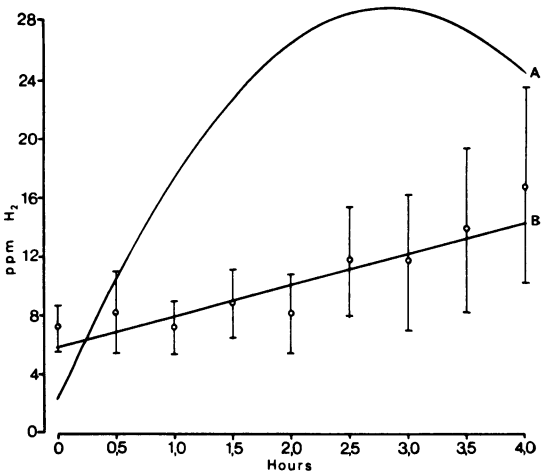

Fig. 4 *Effect of antibiotic therapy in 10 subjects. A: control study; B: during therapy.*

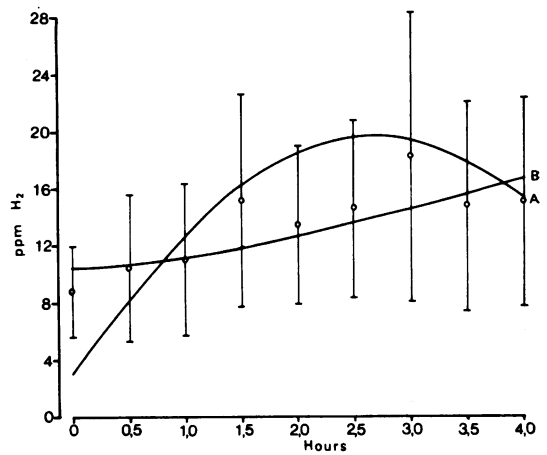

Fig. 5 *Effect of surgical preparation (neomycin and enemas) in 12 subjects. A: control study, B: after preparation.*

In presenting the results, the 11 subjects that did not produce hydrogen were not excluded from the various groups (colonoscopy, three; antibiotics, three; surgical preparation, three; and control group, two). The differences between treatment and control data are therefore more marked than appears in the graphs.

After all three modes of bowel preparation the typical bell-shaped curve of hydrogen concentration was replaced by a plateau which continued to rise throughout the four to 4½ hours of the test.

Discussion

The hydrogen breath test in our study was per-

formed by the method of Metz *et al.* (1976b) the 'end expiratory technique'. In this technique, unlike the original method of Levitt (1969), no quantitative air collection is required and the test is simple and easy to perform. We have evaluated both techniques (unpublished) and found that the results are comparable.

A striking finding in our study was the high proportion of control subjects (20%), who produced very little H_2 after lactulose ingestion. Metz *et al.* (1976a) considered a rise of 20 ppm H_2 as borderline; therefore, all our 11 cases would have been considered flat also by their criteria, as a maximal rise of more than 12 ppm at 2½ hours was within the normal range in our study. However, as they used 50 g lactose (of which a portion is absorbed) and a single sampling at two hours, the data are not strictly comparable. Assuming that lactulose is not absorbed, this has to mean that the bacterial flora of our 11 subjects were unable to produce H_2 using lactulose as substrate. Five of these 11 subjects were studied one to six weeks after antibiotic therapy and it cannot be excluded that in these patients there was a carry over of the antibiotic effect. Bond and Levitt (1975) found only two out of 42 non-H_2 producers in their studies. Levitt and Donaldson (1970) found two out of 55; Metz *et al.* (1976c) reported one out of 52 such subjects, given 33 g lactulose. Newcomer *et al.* (1975) found none among 25 lactase deficient subjects given 50 g lactose. All the above studies were performed in the USA and the United Kingdom. The differences between their results and ours may be due to geographical variations in the composition of the prevalent colonic flora, possibly in relation to different dietary habits, or other factors may be involved. In any case, henceforth, it will not be possible to exclude carbohydrate malabsorption on the basis of a flat H_2 breath test without concurrent evidence that H_2 is produced after a lactulose load, at least in our population. Even this would be valid only if all carbohydrates are handled similarly by the colonic flora as suggested by the data of Levitt and Donaldson (1970). Even the H_2 producing colonic flora can be markedly affected by various treatment regimens as demonstrated in our study.

Mechanical cleansing of the colon by a combination of laxatives and enemata—as in preparation for colonoscopy—depressed H_2 production very markedly. This is probably a quantitative effect reflecting the reduced number of bacteria remaining in the colon. The effect of antibiotics was also very striking: interestingly, this effect was produced by different antibiotics, having variable spectra of activity. Thus, the previous use of antibiotics, laxatives, and enemata may be a cause of false negative hydrogen breath tests. The duration of this effect is presently unknown and is probably worth studying. It is noteworthy that the combination of neomycin and enemata (surgical preparation) caused a much lesser depression of hydrogen production. Murphy and Calloway (1972) studied the effect of various antimicrobial agents on bean-induced flatulence, including the composition of the flatus. Neomycin was found to have an unremarkable and variable effect. Iodochlorohydroxyquin markedly depressed and succinylsulfathiazole markedly increased flatus and hydrogen production.

We have no data on the suppression of the colonic flora achieved by the various therapeutic regimens. This does not permit us to correlate the observed effects with any specific type of bacteria.

The H_2 breath test remains an interesting and possibly useful tool for the evaluation of carbohydrate malabsorption. However, more data will have to be obtained on the limitations inherent in this test.

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