BREATH-HYDROGEN TEST FOR SMALL-INTESTINAL BACTERIAL COLONISATION

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Summary Breath-hydrogen production after oral glucose administration was examined in patients suspected of having small-intestinal colonisation and compared with the ^{14}C-glycine-cholate breath test (^{14}C-G.C.) and with bacteriological examination of the small intestine. Of 17 patients, 12 had bacteriological evidence of small-intestinal colonisation. Each breath test showed 8 of the 12 patients to be colonised, but only 5 patients gave positive results with both tests. Nevertheless, using both tests only 1 patient out of 12 with small-intestinal colonisation would have been missed. There were no false-positive results in the 5 bacteriologically normal patients when the breath-hydrogen test was used. It is concluded that simultaneous use of these two relatively simple breath tests may improve the indirect diagnosis of small-intestinal colonisation.

INTRODUCTION

THERE is considerable interest in the detection and clinical relevance of small-intestinal colonisation.[1] However, sampling of intestinal contents is an invasive technique which is often unacceptable to the patient. Furthermore, bacteriological examination of the intestinal contents is both difficult and expensive, particularly since bacteroides and other anaerobic bacteria are of prime importance.[2,3]

For these reasons various attempts have been made to develop an indirect test for small-intestinal colonisation. The ^{14}C-glycine-cholate breath test (^{14}C-G.C.) was developed for the detection of bacteria in the small intestine able to deconjugate bile acids.[4,5] However, high false-positive and false-negative rates detract from its value as an absolute indicator of bacterial colonisation of the small intestine.[6] Thus the detection of bacterial metabolic products of substrates other than bile-acids may improve diagnostic precision. Most bacteria are able to ferment carbohydrates with the evolution of hydrogen (H_2), which can be detected in the breath.[7] We studied H_2 production after glucose ingestion and compared the results with those of the ^{14}C-G.C. test and culture of jejunal aspirates.

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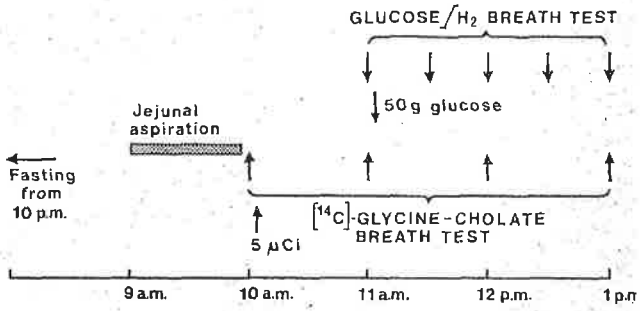
MATERIAL AND METHODS

The following 17 patients, suspected of having small-intestinal colonisation were studied:

Diagnosis	*Number*
Polya partial gastrectomy	7
Billroth-1 partial gastrectomy	1
Vagotomy and drainage	3
Jejunoileal bypass	1
Jejunal diverticuli	1
Irradiation enteropathy	1
Infective enteropathy	1
Small-bowel Crohn's disease	2

In most cases 3 tests were performed on the same morning following a standard protocol:



After an overnight fast, a sterile tube was passed to the region suspected of being colonised, luminal fluid was aspirated, and 0·5 ml transferred to each of 4 sample bottles containing glycerol transport broth, which were frozen rapidly and stored at −20°. These specimens were cultured aerobically and anaerobically on a series of selective and non-selective media.[8] A routine ^{14}C-G.C. test was begun with the patient swallowing 5 μCi of radiosiotope bile salt. Hourly breath samples were collected for three hours and carbon-14 dioxide ($^{14}CO_2$) was measured.[9] After the first hour the glucose/H_2 test was started (see figure). In this test the patient forcibly expired through a modified Haldane Priestley tube and at the end of expiration a 30 ml breath sample was drawn into a plastic syringe.[10] An initial breath sample was obtained, then 50 g glucose in 250 ml of water was drunk and half-hourly breath samples were collected for two hours (see figure).

A peak H_2 concentration of 20 p.p.m. was taken as indicating abnormal H_2 production. In normal subjects H_2 production does not increase after ingesting 100 g glucose. Patients with hypolactasia, when challenged with 50 g of lactose produce more than 20 p.p.m. H_2 in their breath by two hours. Therefore we arbitrarily took values greater than 20 p.p.m. H_2 after 50 g oral glucose as indicating abnormal H_2 production. A positive ^{14}C-G.C. test was taken as >0·0007%.[9] Small-intestinal colonisation was defined as more than 10^3 bacteria per ml of aspirate representing any of the bacterial groups commonly found in blind-loop patients.[3,8] Bacteria normally present in the small intestine, such as lactobacilli and streptococci, were not included in the colony-counts.

RESULTS

12 patients had positive jejunal cultures. Of these, 8 were glucose/H_2 positive and 8 were ^{14}C-G.C. positive, with 5 patients being positive in both tests and 1 patient negative in both. Thus of the 4 colonised patients who were ^{14}C-G.C. negative, 3 produced H_2 and grew non-bile-salt deconjugating bacteria. Therefore each test failed to diagnose 4 cases, but the combination of the two breath tests diagnosed 11 out of 12 colonised patients. In the 5 non-colonised patients only the ^{14}C-G.C. test gave false-positive results in 4. These included 2 patients with abnormal barium-meal appearances suggestive of Crohn's disease, which is compatible with a glucose/H_2 negative, ^{14}C-G.C.-positive result.

DISCUSSION

End-expiratory breath sampling of H_2 can be used to demonstrate colonic H_2 production in patients with hypolactasia[11] and hyposucrasia.[12] In the only previous report of small-intestinal H_2 production, ingestion of 10 g of glucose in a patient with small-intestinal bacterial overgrowth resulted in "only minimal quantities of H_2 in the small intestine".[13] This observation does not seem to have been followed up, possibly because it was inferred that the yield might be very low. Although this hypothesis is supported by the knowledge that bacterial-counts in small-intestinal colonisation are usually several hundredfold less than colonic counts, nevertheless the small-intestinal bacteria would have a much greater substrate load. About one sixth of the H_2 produced in the large bowel by fermentation of carbohydrate was absorbed and excreted in the breath, the remainder passing as flatus.[7] In contrast, H_2 produced in the upper small intestine would be almost entirely absorbed and expired in the breath.

In this study 4 out of 12 colonised subjects did not produce H_2. There are three possible reasons for this. First, not all bacteria produce H_2—e.g., some strains of bacteroides and anaerobic streptococci. Second, the stagnant segment could be below the level at which all the glucose has been absorbed, as in the patient with pelvic irradiation. Finally some bacterial species may produce insufficient H_2 to be detected in the breath. Although there were no false-positive glucose/H_2 tests these could theoretically occur from rapid small-intestinal transit of glucose to the cæcal bacteria. In this situation a sustained H_2 rise over several hours is seen, whereas in small-intestinal fermentation, H_2 production reaches a peak early and is declining or even finished by two hours.[13]

The ^{14}C-G.C. test has previously been compared with jejunal bacterial counts,[14] and although an increased count was usually associated with a positive ^{14}C-G.C. test, about a third of colonised patients had a normal ^{14}C-G.C. test, which may be because the type of bacteria—e.g., enterobacteria and some enterococci, lacking the deconjugase enzyme for splitting off ^{14}C-glycine—produce a false-negative result. Another pitfall in the interpretation of the ^{14}C-G.C. test in small-intestinal colonisation is the false-positive results caused by distal ileal mucosal disease.[15] Thus in cases of Crohn's disease or irradiation enteropathy[9] it may be impossible to say, without also performing fæcal counting, whether the positive ^{14}C-G.C. is due to small-intestinal colonisation, distal ileal mucosal disease, or both.[16 17]

The combination of the two breath tests correctly diagnosed 11 out of 12 colonised patients. It would seem therefore that the combination of these two breath tests, which can be performed concurrently and without discomfort to the patient, will considerably improve the indirect diagnosis of small-intestinal colonisation. Testing for both H_2-producing and bile-salt-deconjugating bacteria may help in the choice of a suitable antimicrobial agent with which to treat the patient. Furthermore, with the non-isotopic glucose/H_2 test, monitoring treatment or relapse in the patients would be possible. Unfortunately if both of the tests are negative this does not exclude colonisation. Although we feel that these preliminary results are encouraging, a study in depth together with prospective studies on individual patients with and without antibiotics are required before these tests become established for the diagnosis of small-intestinal colonisation.

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APLASTIC ANÆMIA: EVIDENCE FOR AN IMMUNOLOGICAL MECHANISM

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Summary The soft agar culture assay (C.F.U.-c) has been used in vitro as a measure of hæmopoietic capacity of bone-marrow. In a patient with aplastic anæmia pretreatment of the patient's bone-marrow with horse anti-human-thymocyte globulin and complement (A.T.G. + C) prior to culture led to a dramatic increase in ability to form colonies in the soft agar assay; and co-culturing marrow from a normal donor and from the patient resulted in a distinct reduction in the number of expected C.F.U.-c. These findings point to an immunological or autoimmune mechanism in this patient by selective destruction of the suppressing cells in the patient's marrow with A.T.G. and by suppression of normal myelopoiesis following addition of the patient's marrow to normal marrow.

INTRODUCTION

APPROXIMATELY 50% of the aplastic anæmias are considered to be idiopathic[1] and an autoimmune mechanism has been postulated for some of these.[2 3] Clinical evidence supporting this concept comes from the results of bone-marrow transplantation. Some patients have been