PAPERS AND ORIGINALS

Assessment of the ^{14}C -Glycocholic Acid Breath Test

O. F. W. JAMES, J. E. AGNEW, I. A. D. BOUCHIER

British Medical Journal, 1973, 3, 191-195

Summary

The 1- ^{14}C -glycine-glycocholic-acid breath test has been performed on 104 subjects and a normal range established. Abnormal results due to bacterial deconjugation of bile salts were found not only in patients with the "contaminated bowel" syndrome and in those with ileal resection but also in a third group, patients with cholangitis. Abnormal results were also found in patients with gastrocolic fistula and staphylococcal enterocolitis, while mildly abnormal results were also found in some patients with liver disease.

Introduction

Direct demonstration of bacterial overgrowth in the small intestine is difficult. Indirect methods have been unreliable. Recently, however, a new indirect test has been described—a breath test using the radioactive-labelled bile acid 1- ^{14}C -glycine glycocholic acid. This test shows the bacterial deconjugation of bile salts by intestinal bacteria. It is based on the fact that the bile acids cholic and chenodeoxycholic acid are linked by an amide bond to their glycine or taurine conjugates and this bond may be broken only by the action of bacterial enzymes. In the test cholyglycine-1- ^{14}C (glycocholic acid) is given by mouth to the subjects. The bacterial deconjugation of the ^{14}C -glycine from the cholic acid may be caused by bacteria in the small intestine in circumstances of bacterial overgrowth, or by the normal colonic bacteria should the enterohepatic circulation of the conjugated bile salt be interrupted by ileal resection, disease, or bypass (fig. 1). When glycine-1- ^{14}C has been released it is rapidly converted to $^{14}\text{CO}_2$ either by intestinal bacterial enzymes or by tissue enzymes after absorption from the in-

FIG. 1—Principle of ^{14}C -Glycocholic acid breath test.

testine. The $^{14}\text{CO}_2$ is then transported in the blood to the lungs and exhaled. Thus the amount of $^{14}\text{CO}_2$ appearing in the breath is a direct reflection of the amount of bacterial deconjugation of bile salts which has occurred.

The value of the breath test in detecting bacterial deconjugation of bile salts has been shown by several groups (Fromm and Hofmann, 1971; Sherr *et al.*, 1971; Horchner *et al.*, 1972; James *et al.*, 1972; Parkin *et al.*, 1972). It has been well shown that the test is often abnormal in bacterial overgrowth syndromes, notably due to intestinal diverticula, and in patients with interruption of the enterohepatic circulation by terminal ileal resection.

The present study has been undertaken to confirm the usefulness of the test in clinical practice. The results of 118 tests performed on 104 subjects are presented. They include 34 control subjects who form the basis of estimation of the normal range. The present observations also provide examples of an altered breath test due to presumed bacterial deconjugation occurring in different sites in the gastrointestinal tract from the stomach to the colon. Of particular interest were positive results in patients with cholangitis and in subjects with liver disease. Comparison has been made with intestinal bacteriology in a number of these cases.

Royal Free Hospital, London WC1X 8LF

O. F. W. JAMES, M.A., M.R.C.P., Registrar, Department of Medicine

J. E. AGNEW, B.A., M.Sc., Senior Physicist

I. A. D. BOUCHIER, M.D., F.R.C.P., Reader in Medicine (From 1 August 1973, Professor of Medicine, University of Dundee)

Materials and Methods

Subjects.—The 34 control subjects comprised healthy adult volunteers and patients with conditions unrelated to the gastrointestinal tract, principally pneumonia or myocardial infarction. Informed consent was obtained from all subjects. All the 69 patients not in the control group had documented disease of the gastrointestinal tract or liver. The diagnosis was established in each case by appropriate conventional means including radiology, liver function tests, and small intestinal or liver biopsies (table).

Diagnosis and Results in all 104 Subjects Studied

Condition	Breath Test		Bacteriology (Upper Jejunum)	
	Total No. Tested	Abnormal Results	No. Tested	Abnormal Results
Controls	35	1	12	—
Ileal resection	6	5	4	1*
Cholangitis	6	5	3	2
Primary biliary cirrhosis	12	2	6	—
Other hepatobiliary disease	17	—	6	—
Post Polya gastrectomy	4	2	2	2
Ulcerative colitis	8	—	2	—
Crohn's disease	4	1	3	—
Celiac disease	3	—	—	—
Small bowel lymphoma	2	—	2	—
Chronic pancreatitis	2	—	—	—
Nodular lymphoid hyperplasia	1	1	1	—
Gastrocolic fistula	2	2	—	—
Jejunal diverticula	1	1	1	1
Staphylococcal enterocolitis	1	1	1	1

*This patient also had a Polya gastrectomy.

Breath Test.—Five μCi of 1-(^{14}C)-glycine-glycocholic acid (Radiochemical Centre, Amersham, Bucks., specific radioactivity 19 $\mu\text{Ci}/\text{mmol}$) dissolved in 5 ml of 10% ethanol was administered by mouth to the fasting subjects. This was followed after 10 minutes by a standard Lundh test meal containing 18 g corn oil, 15 g Casilan, and 40 g glucose in 300 ml water. A hospital lunch was eaten about three hours later. Duplicate samples of expired air were obtained hourly for six to eight hours. In some subjects 24-hour samples were also collected. The air was breathed out over anhydrous CaCl_2 into a collecting tube containing 2 ml of a 0.5 molar solution of hyamine hydroxide, a monobasic alkali. The end point of neutralization was indicated by a thymolphthalein indicator. At this point one mmol CO_2 was trapped in the collecting solution; 10 ml scintillation fluid was added to this solution and the radioactivity in each sample was measured as disintegrations per minute by liquid scintillation counting. Results for each sample were expressed as the percentage of administered dose of radioactivity per mmol expired CO_2 . This was multiplied by body weight to correct for the influence of endogenous CO_2 production on the CO_2 specific activity, as suggested by Fromm and Hofmann (1971). Theoretically if there was enormous endogenous CO_2 production there would be a reduction in the CO_2 specific activity. For this reason the total $^{14}\text{CO}_2$ output has not been calculated from the product of $^{14}\text{CO}_2$ specific activity and theoretical endogenous CO_2 production. Sherr *et al.* (1971) have shown a good correlation between continuous respiration pattern analysis and the sampling method used here.

Bacteriology.—Jejunal aspiration was performed using a sterile radio-opaque tube with a mercury filled bag at the tip. The tube was screened into position until the tip had passed the duodenojejunal flexure; aspiration was then carried out. Small intestinal contents were cultured in the routine laboratory by aerobic and anaerobic means. The aspirated samples were immediately taken to the laboratory and processed on arrival. For the aerobic cultures tenfold dilutions were made from 10^{-1} per ml to 10^{-5} with tryptic soy broth, aliquots were then applied to blood agar and MacConkey. Positive

plates were subcultured. The aspirated samples were transported under liquid paraffin for anaerobic culture. One drop of the sample was taken with a "50" dropper and spread on to blood agar plates which were rapidly transferred into B.T.L. anaerobic jars and evacuated. The plates were incubated in an atmosphere of 10% CO_2 , total colony counts were made after 18 hours, and positive plates were then subcultured. The anaerobic samples were also innoculated into cooked meat medium and incubated for 18 hours at 37°C then subcultured onto blood agar and incubated as above.

Results

Normal Range (34 Subjects).—The normal range of $^{14}\text{CO}_2$ exhaled is shown in fig. 2. The control subjects excreted little $^{14}\text{CO}_2$ in the first four hours of the test (under 0.14% dose/mmol CO_2). At later collections recovery of the $^{14}\text{CO}_2$ in the breath increased. The exhaled $^{14}\text{CO}_2$ activity did not rise above 0.1% dose/mmol CO_2 in most normal subjects, but in a few it reached values of up to 0.32% dose/mmol CO_2 during the latter part of the test. Among the normal subjects who were also tested 24 hours after ingestion of the ^{14}C -glycocholic acid a few also showed some increase in excretion of the $^{14}\text{CO}_2$ —up to 0.22% dose/mmol CO_2 . Small intestinal bacterial culture performed on 10 of the control subjects showed no abnormal growth.

FIG. 2—Results obtained from 34 subjects in normal range and false positive test in one subject.

Reproducibility.—Six control subjects were retested from 6 to 15 months after their initial test. Only small and insignificant changes occurred.

Ileal Resection (Six Patients).—There were five patients with abnormal results in this group. This finding is in agreement with previous studies (Fromm and Hofmann, 1971, Sherr *et al.*, 1971). While all five abnormal results had become so within two hours of starting the test, the maximal level of exhaled $^{14}\text{CO}_2$ in four of these was reached only at the fourth or subsequent hours after the start of the test (fig. 3). The fifth patient in whom an abnormal result occurred had also undergone a Polya gastrectomy. Small intestinal samples from this patient showed significant growths of *Streptococcus faecalis* and coliforms. Small bowel bacterial culture in three other subjects in this group was negative.

Cholangitis (Six Patients).—There were five positive test re-

FIG. 3—Results in five patients with ileal resection in whom test was abnormal. Normal range is indicated by shaded area.

FIG. 4—Results in five patients with cholangitis in whom test was abnormal. Normal range is indicated by shaded area.

sults in this group (fig. 4). Three patients showed maximal abnormality one hour after the start of the test, the other two had a mildly abnormal result. The one with a normal result had received antibiotics in the month before the test. Bacterial culture of duodenal contents from three of the patients with an abnormal result showed significant numbers of coliform organisms in two. In one patient in whom cholecystectomy was performed the breath test and the bacteriology had reverted to normal two weeks after the operation.

Liver Disease (29 Patients).—Three of 12 patients with primary biliary cirrhosis had $^{14}\text{CO}_2$ counts above normal after the first hour; two remained abnormal on one or more subsequent occasions and were thus regarded as having an abnormal breath test result (fig. 5). Bacterial culture in all three was negative. In no other patient with liver disease was there an abnormal finding. These comprised four with alcoholic cirrhosis, one with a hepatic abscess, and two each with one of the following diagnoses: secondary biliary cirrhosis, cryptogenic cirrhosis, active chronic hepatitis, portosystemic encephalopathy after colonic exclusion, bile duct carcinoma, and infective hepatitis.

FIG. 5—Results in two patients with primary biliary cirrhosis with abnormal tests.

FIG. 6—Abnormal tests found in a variety of conditions, where bacterial deconjugation of bile salts is thought to have occurred.

Postgastrectomy (Four Patients).—Two out of four patients who had a Billroth II gastrectomy gave abnormal test results (fig. 6). In both patients positive small bowel bacterial cultures were obtained; heavy growths of micrococci, staphylococci, and haemophilus were found in the first, and a heavy growth of coliforms and klebsiella in the second. In both cases the breath test and bacteriology reverted to normal after use of antibiotics.

Miscellaneous Diagnoses (24 Patients).—Among eight patients with malabsorption not due to bacterial overgrowth (three coeliac disease, two small bowel lymphoma, two chronic pancreatitis, and one lymphoid nodular hyperplasia) an abnormal test result was found only in the case of lymphoid nodular hyperplasia. Bacterial culture in four of these subjects, including the patient with lymphoid nodular hyperplasia, was negative. None of the eight patients with ulcerative colitis who were tested gave an abnormal result. One of four subjects with Crohn's disease but no ileal resection had an abnormal result; small bowel bacterial culture in this patient was negative. In this patient the test was repeated during antibiotic treatment for a perianal abscess and was

found to be normal. One case of gastrocolic fistula due to a gastric carcinoma and one patient with a duodenocolic fistula, cause unknown, showed abnormal results, each markedly positive after one hour (fig. 6). A subject with multiple diverticula of the jejunum also had an abnormal result, jejunal bacteriology showing high counts of veillonellae and coliforms. In one patient with staphylococcal enterocolitis the test remained normal for the first three hours but became very abnormal thereafter (fig. 6).

Discussion

NORMAL RANGE

The provision of a large number of control subjects has enabled a well defined normal range for the test to be established. As will be seen in fig. 2 there was no difference between results obtained from healthy volunteers (all hospital personnel) and from patients with disease unrelated to the gastrointestinal tract. Most of the control subjects provided very low values for $^{14}\text{CO}_2$ specific activity in expired air for the first four hours of the test but higher values were obtained after five, six, and seven hours in a number of apparently normal subjects. The readings obtained in all control subjects except one fell below 0.03% of the dose of radioactivity per mmol $\text{CO}_2 \times \text{kg}$ body weight at the first hour; all fell at or below 0.05% at the second hour.

The distribution of the results was not statistically normal, nor did it appear to become so after making a simple logarithmic transformation. An abnormal test result was therefore defined as one in which $^{14}\text{CO}_2$ levels higher than those exhaled in any control subject were measured on *two* or more occasions during the test.

Our results for control subjects are more extensive than those in other series and show wide individual variation. Repeat tests in control subjects showed excellent reproducibility. The causes for the variations between individual subjects are uncertain and there may be several explanations. One possibility is that since bacteria may be found in the small intestine in a proportion of "normal" subjects (Martini *et al.*, 1957; Kalser *et al.*, 1966; Hamilton *et al.*, 1972), these may be capable of deconjugating a part of the labelled bile salt. Thus one control subject who had no history of gastrointestinal disease gave results far outside the range found in all other controls (fig. 2). This individual has not been included as being within the normal range but is regarded as having a "false" abnormal result. A repeat test one month after the first test showed a result within the normal range. An alternative explanation is that in some subjects a greater proportion of the labelled bile salt may be allowed to enter the colon due either to a minor inefficiency of the enterohepatic circulation or to wide variations in the bile salt pool size.

CHOLANGITIS

The abnormal test result in patients with cholangitis was of interest for two reasons: it supported the work of Scott and Khan (1968) who showed appreciable bacterial activity in the small intestine of a high proportion of patients with cholangitis; in addition the test offers another way for diagnosis in cholangitis, particularly in patients with long-standing biliary tract disease.

LIVER DISEASE

The observation that $^{14}\text{CO}_2$ specific activity was abnormal in two out of 12 patients with primary biliary cirrhosis is of

interest. A third patient with primary biliary cirrhosis showed raised $^{14}\text{CO}_2$ specific activity at the first hour only. Among the seven other patients with cirrhosis there was no abnormal result, though one patient with alcoholic cirrhosis showed a value above the normal range at the third hour only. No other subject with hepatobiliary disease showed any abnormality. These minor abnormalities in patients with primary biliary cirrhosis are difficult to interpret. It is possible that are bacteria which others have shown to be present in subjects with liver disease may cause some degree of bacterial deconjugation on an already reduced pool of bile salts (Martini *et al.*, 1957; Cerlek *et al.*, 1965; Badley *et al.*, 1969; Lal *et al.*, 1972). This may contribute further to an intraluminal bile salt deficiency and thus exacerbate the steatorrhoea which is a feature of the disease.

The normal findings in two patients with colonic exclusion and chronic portosystemic encephalopathy, who at the time of the test were experiencing an exacerbation of their symptoms and who were not taking antibiotics, suggests that, in these patients at least, bacterial colonization of the small intestine by deconjugating organisms was not contributing to the worsening of the encephalopathy.

CONTAMINATED BOWEL SYNDROME

The finding of abnormal test results and abnormal small bowel bacteriology in two of the four patients who had Billroth II gastrectomies supports the concept of bacterial deconjugation in the afferent loop of these patients. In both treatment with antibiotics not only caused return of the breath test and bacteriology to normal but cut faecal fat excretion from over 20 g daily to less than 10 g daily. The abnormality in the patient with multiple jejunal diverticula confirms the results of Parkin *et al.*, 1972.

TIMING OF ABNORMAL RESULTS

Though it has been claimed that the time at which the test becomes most abnormal gives no indication of the site of bacterial deconjugation (Fromm and Hofmann, 1971; Hofmann and Fromm, 1971), the present investigation suggested that this may not always be so. There was maximally raised $^{14}\text{CO}_2$ specific activity in the breath after one hour in the patients with cholangitis. In the subjects with ileal resection and in the patient with staphylococcal enterocolitis the maximal $^{14}\text{CO}_2$ specific activity was at the fourth or a subsequent hour. This suggested that the site of maximal bacterial deconjugation may, to some extent, be determined by the time when the maximum amount of $^{14}\text{CO}_2$ is exhaled. This would seem to be of little clinical consequence in an individual patient except possibly to give an indication as to the best site for aspiration of intestinal contents when investigating any bacterial overgrowth situation. It should be stressed that aspiration for bacterial cultures in this series was obtained only from the upper jejunum.

In this investigation all positive test results had become abnormal on at least two occasions up to and including the four-hour collection except in the patient with enterocolitis. This implies that as a practical screening test for the presence of the contaminated bowel syndrome or for bacterial activity in cholangitis the breath test need only be performed for four hours after ingestion of ^{14}C -glycocholic acid. The test in this way could be used as an outpatient procedure.

Reversion of an abnormal result to normal after a course of antibiotics does not necessarily imply the presence of a small bowel contamination syndrome which has been successfully treated. The test finding also reverted to normal in subjects with Crohn's disease, ileal resection, and staphylococcal enterocolitis after antibiotic treatment. Anti-

biotics appear to stop bacterial deconjugation of bile salts not only in the small intestine but also in the colon.

Conclusion

The ^{14}C -glycocholic acid breath test is a useful and simple screening test for the detection of bacterial deconjugation of bile salts. This may be of value not only in the diagnosis of bacterial overgrowth syndromes but also in the evaluation of cholangitis. Facilities for elaborate anaerobic bacterial culture are not generally available; however, this test may be performed in any hospital with liquid scintillation counting equipment. It may be used on an outpatient basis and is fairly inexpensive (total cost about £5 per patient).

References

- Badley, B. W. D., Murphy, G. M., and Bouchier, I. A. D. (1969). *Lancet*, 2, 400.
 Cerlek, S., Mustrovic, F., and Govorcic, B. (1965). *Medicinski Pregled*, 18, 247.
 Fromm, H., and Hofmann, A. F. (1971). *Lancet*, 2, 621.
 Hamilton, J. D. *et al.* (1972). *Quarterly Journal of Medicine*, 39, 265.
 Hofmann, A. F., and Fromm, H. (1971). *New England Journal of Medicine*, 279, 686.
 Horchner, P. *et al.* (1972). *Folia Medica Neerlandica*, 15, 186.
 James, O., Agnew, J. E., Lydford, R., and Bouchier, I. A. D. (1972). *Gut* 13, 845.
 Kalser, M. H. *et al.* (1966). *New England Journal of Medicine*, 274, 500, 558.
 Lal, D., Gorbach, S. L., and Levitan, R. (1972). *Gastroenterology*, 62, 275.
 Martini, G. A., Phear, E. A., Ruebner, B., and Sherlock, S. (1957). *Clinical Science*, 16, 35.
 Parkin, D. M. *et al.* (1972). *Lancet*, 2, 777.
 Scott, A. J., and Khan, G. A. (1968). *Gut*, 9, 187.
 Sherr, H. P. *et al.* (1971). *New England Journal of Medicine*, 279, 656.

Respiratory Disease in Young Adults: Influence of Early Childhood Lower Respiratory Tract Illness, Social Class, Air Pollution, and Smoking

J. R. T. COLLEY, J. W. B. DOUGLAS, D. D. REID

British Medical Journal, 1973, 3, 195-198

Summary

The prevalence of cough during the day or night in winter has been studied in 3,899 20-year-olds. These were members of a cohort born in England, Scotland, and Wales in the last week of March 1946. The prevalence of cough and current smoking habits have been related to events recorded during infancy and childhood—that is, exposure to air pollution, social class of father, and lower respiratory tract illness under 2 years of age. At age 20, of these factors cigarette smoking was found to have the greatest effect on symptom prevalence, followed by a history of a lower respiratory tract illness under 2 years of age. Social class and air pollution had little effect.

Introduction

There is still doubt about the long-term consequence of early childhood chest illness. Paediatric opinion has tended to regard the improvement in respiratory experience that occurs as children grow up as indicating permanent cure. On this view, what happens later in adult life—for example, the development of symptoms and signs of chronic bronchitis—would be quite unrelated to childhood experience. There is evidence from a number of epidemiological studies suggesting that this view may not be true. From these studies it seems that childhood experience is relevant to respiratory disease in early adult life (Colley, 1971).

We present the respiratory findings in a birth cohort followed until the age of 20 years. We have studied the relations of social class, lower respiratory tract illness under 2 years of age, air

pollution, and smoking to the frequency of respiratory symptoms at the age of 20 years.

Methods

The material forming the basis of this paper is part of that collected during the follow-up of the cohort of children who were all born in England, Wales, and Scotland in the last week of March 1946. The objectives of the original study and the sampling methods used are described elsewhere (Douglas and Blomfield 1958; Douglas 1964; Douglas *et al.*, 1968).

The original population consisted of 13,687 infants born alive in the last week of March 1946. The sample so obtained was not the entire births in that period. This was owing to the non-operation of a few maternal and child welfare authorities and to late registration of some births. There is, however, no reason to assume that any serious biases have been introduced into the sample by these losses. As only limited funds were initially available for the study twins and illegitimate children were excluded from the sample of 13,687 infants. This left a total of 12,468 from which the follow-up sample was drawn. All births to non-manual workers and all births to agricultural workers were included but only one out of every four births to manual workers. The final sample size was 5,362 of whom 4,701 were living in Great Britain in 1964.

In 1946 there was no intention to follow these infants for more than a few years. In the event most have been followed for more than 20 years. During the follow-up period information has been collected at different times on social factors, environmental exposure, illness, educational attainment, and other aspects of the development of these children. The following specific items were studied.

Lower Respiratory Tract Infection Under Two Years of Age.—This history was obtained at interview by health visitors from the parents when the child was 2 years old. The question asked was, "Has this baby ever had a lower respiratory infection—that is, bronchitis, bronchopneumonia, or pneumonia?"

Social Class Classification.—This is based on the 1957 occupation of the father of the survey child, on the education of both parents, and the occupation of both grandfathers. Where the 1957 occupation is not known the 1946 occupation has been used. Four social class groups are used: (1) upper middle class, (2) lower middle class, (3) upper manual working

London School of Hygiene and Tropical Medicine, London WC1E 7HT

J. R. T. COLLEY, M.D., B.Sc., Senior Lecturer in Epidemiology
 D. D. REID, M.D., F.R.C.P., Professor of Epidemiology

M.R.C. Unit for the Study of Environmental Factors in Mental and Physical Illness, London School of Economics, London WC2
 J. W. B. DOUGLAS, B.M., B.Sc., Director