^{14}C -glycocholate test in Crohn's disease—its value in assessment and treatment

J. H. B. SCARPELLO AND G. E. SLADEN

From the Academic Division of Medicine, Royal Hospital, West Street, Sheffield

SUMMARY The ^{14}C -glycocholic acid test (^{14}C -GCA) has been assessed in 27 patients who have had resection of the ileum and colon for Crohn's disease and in 19 patients with unoperated stable Crohn's disease. The incidence of increased breath output of $^{14}\text{CO}_2$ and faecal output of ^{14}C was significantly greater in operated patients. Half the unoperated patients had normal results and, of the others, a modest increase in faecal ^{14}C was the usual finding. There was no correlation between the ^{14}C -GCA test, the Schilling test, and the extent and severity of the radiological signs in the unoperated patients. Metronidazole therapy was poorly tolerated and had little or no influence on symptoms but did decrease the excretion of $^{14}\text{CO}_2$ in the breath when the ^{14}C -GCA test was repeated. Cholestyramine therapy was beneficial in the majority of resected patients with diarrhoea and an increased faecal ^{14}C excretion and, on repeat testing, there was a significant increase in the output of breath $^{14}\text{CO}_2$. The beneficial effect was less marked in the unoperated patients and the breath $^{14}\text{CO}_2$ output remained normal in those retested. Interruption of the enterohepatic circulation of bile acids did not seem to make an important contribution to the symptoms of patients with stable unoperated (and uncomplicated) Crohn's disease, even when the ileum was extensively involved.

The ^{14}C -glycocholic acid (^{14}C -GCA) test (Fromm and Hofmann, 1971) may be abnormal in patients with Crohn's disease as a result of either small intestinal bacterial overgrowth proximal to strictures or fistulae or bile acid malabsorption associated with ileal disease or resection. Increased bile acid deconjugation in the small gut or an excess of bile acids in the colon may contribute to symptoms, especially diarrhoea (Hofmann, 1967). The ^{14}C -GCA test may be useful in assessing the extent and severity of ileal disease and may provide a guide to therapy, either with antibiotics, or bile acid sequestering agents.

We have assessed the test in both resected and non-resected Crohn's patients. Those with troublesome diarrhoea were given a trial of cholestyramine or metronidazole and the effects on bowel function and the ^{14}C -GCA test were studied. Metronidazole was used because of its specific anaerobic bactericidal properties (Nastro and Finegold, 1972), as anaerobic bacteria—and especially the bacteroides—are largely responsible for deconjugation of bile acids (Shimada *et al.*, 1969; Lewis and Gorbach, 1972).

Methods

Forty-six patients with Crohn's disease were studied. The diagnosis was based on the usual clinical, laboratory, radiological, and histological criteria. Twenty-seven patients had had an ileal (and in some cases colonic) resection and had a mean age of 45.4 years. They were taken from a larger series of ilectomised patients, described in detail in the accompanying paper (see Scarpello and Sladen, 1977, Table 3). Six of these patients had evidence of recurrent Crohn's disease at the time of study.

Nineteen unoperated patients were studied, mean age 31.3 years. As with the previous group, the patients were normally studied as outpatients on uncontrolled food intake. They were not acutely ill and their general health and routine laboratory tests (haemoglobin, albumin, ESR) were stable. Six were taking continuous low-dose prednisolone (less than 10 mg daily). The extent of ileal and colonic involvement was based on scrutiny of the most recently available barium contrast examination. Severity was assessed subjectively as moderate (+) or severe (++).

Received for publication 24 February 1977

No patient had received antibiotics for at least one month and all antidiarrhoeal medication was stopped for a minimum of 24 hours before the test. All patients underwent simultaneous ¹⁴C-GCA and Schilling tests as described previously (Scarpello and Sladen, 1977). Creatinine clearance was also measured to aid interpretation of an abnormal Schilling test and no patient had a clearance of below 50 ml/min.

Patients with an abnormal ¹⁴C-GCA test and troublesome bowel symptoms were offered treatment with either cholestyramine (4 g three times daily) or metronidazole (200 mg three times daily) for a period of one month. Cholestyramine was given to patients with an increased faecal ¹⁴C output or if antibiotics had failed. Metronidazole was used where breath ¹⁴CO₂ excretion was increased or if cholestyramine had not proved beneficial. The response to therapy was assessed from the bowel history and also by observation of a 24 hour stool collection before and after therapy. After treatment, whenever possible, the ¹⁴C-GCA test was repeated.

In the statistical assessment of observed differences, both parametric (Student's *t* test) and non-parametric (Wilcoxon) methods were used. In all cases identical values for *P* were obtained.

metric (Wilcoxon) methods were used. In all cases identical values for *P* were obtained.

Results

RESECTION PATIENTS (Table 1)

These results have been described in the accompanying paper (Scarpello and Sladen, 1977), but for ease of comparison the relevant data are shown again in Table 1, together with information about their major symptoms. Patients 2-7 had increased breath ¹⁴CO₂ but normal faecal ¹⁴C output, patients 8-19 had increased output of isotope in both breath and faeces, whereas patients 20-27 had increased faecal ¹⁴C output only.

NON-RESECTION PATIENTS (Table 2)

Faecal ¹⁴C measurements were made in only 13 of the 19 patients, since five had no bowel action during the 24 hour collection period and one passed an unacceptably small stool.

Marker-corrected faecal ¹⁴C was increased above our normal range in seven patients (cases 13-19). All of these had 24 hour faecal volumes of greater

Table 1 Results in resected Crohn's patients

No.	Age (yr)	Sex	Bowel symptoms	Length of resected bowel (cm)		Breath ¹⁴ CO ₂ (%) [*]	Faecal ¹⁴ C (%) [†]	24 hour faecal weight (g)	Schilling (%) [‡]
				Ileum	Colon				
1	52	M	NFS	14	11	3.7	1.6	145	23.1
2	53	F	WD	60	10	11.1	—	10	7.8
3	41	M	NFS	120	15	17.4	2.3	160	5.6
4	41	M	NFS	50	15	15.1	9.2	290	2.9
5	55	F	NFS	40	Nil	6.9	8.8	115	8.1
6	51	F	WD	35	5	15.6	8.6	870	0.2
7	53	M	Nil	30	NK	11.1	—	70	0.6
8	40	F	NFS	75	11	8.0	58.3	370	1.6
9	41	F	NFS	35	Nil	4.4	14.1	315	12.9
10	54	F	NFS	50	BP	12.9	34.3	545	1.8
11	49	F	WD	NK	15	16.6	17.9	490	1.5
12	57	M	NFS	50	8	20.3	14.8	90	2.4
13	69	M	NFS	100	12	21.2	24.1	445	0.4
14	32	M	NFS	36	15	5.9	57.9	275	4.1
15	37	F	NFS	45	10	9.8	43.6	545	15.9
16	33	M	NFS	200	22	10.6	17.8	940	0.7
17	57	F	Nil	60	15	13.5	17.7	105	1.5
18	29	F	NFS	35	12	7.5	70.8	725	1.0
19	26	F	Nil	30	15	8.0	22.9	235	14.5
20	21	F	AP	22	15	0.8	16.9	510	17.4
21	35	M	NFS	45	15	1.5	19.8	250	19.8
22	75	F	WD	35	BP	0.8	56.4	1000	0.9
23	47	F	NFS	40	50	0.4	40.3	530	0.9
24	68	F	NFS	5	15	2.0	15.5	740	1.5
25	44	F	NFS	80	15	3.9	56.5	660	4.9
26	29	F	NFS	17	BP	1.2	21.9	470	8.1
27	36	M	WD	30	35	3.3	28.6	300	15.7

NFS: non-formed stool.

WD: watery diarrhoea.

AP: abdominal pain.

*Normal range <4.0%.

†Normal range <10.3%.

‡Normal range >8.0%.

NK: length unknown.

BP: bypass.

Table 2 *Non-resected Crohn's patients*

No.	Age (yr)	Sex	Bowel symptoms	Breath $^{14}\text{CO}_2$ (%)*	Faecal ^{14}C (%)†	24 hour faecal weight (g)	Schilling (%)‡	Site of disease	
								Ileum	Colon
1	38	M	Nil	2.4	6.4	180		++	+
2	57	F	Nil	3.0	—	0	5.9	++	—
3	42	M	Nil	2.4	—	0	18.6	+	—
4	22	M	Nil	2.6	—	0	4.0	++	+
5	27	F	Nil	0.8	—	25	9.4	++	—
6	50	F	Nil	1.7	10.2	170	18.0	—	+
7	23	F	Nil	1.2	—	0	30.2	+	++
8	29	F	NFS	0.8	3.3	135	13.9	+	++
9	33	F	AP	0.6	8.6	135	4.0	++	—
10	18	F	AP	3.5	—	0	21.7	—	++
11	44	F	Nil	5.9	1.6	120	11.3	++	—
12	26	M	NFS	6.0	1.5	125	9.4	—	++
13	26	M	NFS	1.1	16.9	430		++	+
14	25	M	NFS	0.5	15.5	520	9.0	+	+
15	34	F	NFS	2.7	13.0	270	10.0	+	++
16	24	M	NFS	0.8	12.7	250	20.9	—	++
17	26	F	WD	1.1	13.6	495	3.1	++	++
18	27	F	WD	0.2	14.4	400	5.5	++	—
19	25	F	Nil	1.9	12.8	235	25.6	++	++

Key: see Table 1.

NFS: non-formed stools.

AP: abdominal pain.

WD: watery diarrhoea.

Radiological involvement: + moderate, ++ severe.

Table 3 *Response to cholestyramine therapy*

Group	Before cholestyramine			After cholestyramine			
	Breath $^{14}\text{CO}_2$ (%)*	Faecal ^{14}C (%)†	24 hour faecal weight (g)	Subjective effect	Breath $^{14}\text{CO}_2$ (%)*	Faecal ^{14}C (%)†	24 hour faecal weight (g)
Resected							
8	8.0	58.3	370	Improved	14.5	—	25
11	16.6	17.9	490		17.1	—	0
15	9.8	43.6	545		10.5	46.8	225
18	7.5	70.8	725		17.5	16.7	450
21	1.5	19.8	250		4.6	7.2	165
23	0.4	40.3	530	Slight improvement	8.7	16.3	200
24	2.0	15.5	740		21.2	21.7	60
5	6.9	8.8	115	Slight improvement	14.5	22.7	105
10	12.9	34.3	545	Slight improvement	6.6	29.7	295
16	10.6	17.8	940	Severe constipation		ST	
26	1.2	21.9	470	Severe constipation		ST	
4	15.1	9.2	290	No change		ST	
6	15.6	8.6	870	No change		ST	
27	3.3	28.6	300	No change		ST	
2	11.1	—	10	Unable to tolerate drug		ST	
9	4.4	14.1	315	Unable to tolerate drug		ST	
Non-resected							
14	0.5	15.5	520	Improved	0.6	21.2	430
19	1.9	12.8	235	Improved	0.4	14.3	180
16	0.8	12.7	250	Slight improvement	0.5	9.4	190
17	1.1	13.6	495	Slight improvement	1.4	21.8	245
18	0.2	14.4	400	No change	0.2	7.3	200

Key as in Table 1.

ST: stopped treatment.

than 200 g and none had an abnormal breath $^{14}\text{CO}_2$ output. The remaining patients had faecal ^{14}C recoveries within the normal range and faecal volumes of less than 200 g. Two of these (cases 11, 12) had increased breath $^{14}\text{CO}_2$ output.

The Schilling test was unequivocally abnormal in only five patients, all of whom had extensive ileal

disease. Only one patient (case 17) had a combination of watery diarrhoea, increased faecal ^{14}C , and an abnormal Schilling test.

PATIENTS TREATED WITH CHOLESTYRAMINE (Table 3)

Sixteen resected and five non-resected patients

Table 4 Response to metronidazole therapy

Group	Before antibiotics			After antibiotics			
	Breath ¹⁴ CO ₂ (%)*	Faecal ¹⁴ C (%)†	24 hour faecal weight (g)	Subjective effect	Breath ¹⁴ CO ₂ (%)*	Faecal ¹⁴ C (%)†	24 hour faecal weight (g)
Resected							
10	12.9	34.3	545	Slight improvement	5.1	57.3	475
2	11.1	—	10	No change	4.4	ST	210
4	15.1	9.2	290			ST	
5	6.9	8.8	115			ST	
11	16.6	17.9	490			49.0	
27	3.3	28.6	300	Increased diarrhoea		ST	
6	15.6	8.6	870			ST	
8	8.0	58.3	370			ST	
9	4.4	14.1	315			ST	
13	21.2	24.1	445	Severe lethargy		ST	
Non-resected							
12	6.0	1.5	125	No change	5.0	—	60
17	1.1	13.6	495	Slight improvement	0.9	25.7	395
18	0.2	14.4	400	Sore tongue		ST	

Key as in Table 1.

ST: stopped treatment.

received treatment. Fourteen completed therapy and underwent a repeat ¹⁴C-GCA study. Of these, 13 described some degree of improvement in their bowel symptoms. A further two resected patients (cases 26, 16) experienced severe constipation and abdominal colic within 48 hours of starting the drug and treatment was discontinued. Four had no change in symptoms and two of these (cases 6, 4) had a normal faecal ¹⁴C output. Two were unable to tolerate the drug because of nausea.

The 24 hour stool output after therapy was significantly decreased in the resected patients ($P < 0.01$) and tended to decrease in the non-resected group, although not significantly ($P > 0.05$).

There was a significant increase in the breath ¹⁴CO₂ output of the resected patients after therapy ($P < 0.05$) and three patients (cases 21, 23, 24) had initially normal results which became positive after treatment. However, the breath ¹⁴CO₂ remained normal in the non-resected patients. Changes in faecal ¹⁴C were very variable.

PATIENTS TREATED WITH METRONIDAZOLE (Table 4)

Ten resected and three non-resected patients were studied. Only four completed therapy and only two of these described any improvement. Two (cases 6, 8) had increased diarrhoea and three (cases 9, 13, 18) experienced severe side-effects (lethargy, malaise, sore tongue) and stopped treatment. The remainder described no change in bowel function.

Breath ¹⁴CO₂ excretion after therapy was reduced in the four cases where a repeat study was possible and in two (cases 10, 11) these changes were substantial.

Discussion

The results of the ¹⁴C-GCA test differ considerably between the two groups of patients. An increased breath ¹⁴CO₂ output was found in only two (10.5%) of the non-resected patients compared with 18 (66%) of the others. Similarly, faecal ¹⁴C recovery was increased in only seven (36.8%) of the non-resected group compared with 20 (74%) of the resected patients. Breath ¹⁴CO₂ output, faecal ¹⁴C recovery and stool volumes are all significantly greater in the resected patients ($P < 0.01$).

In the resected patients there was a positive correlation between length of ileum resected and breath ¹⁴CO₂ excretion (see Scarpello and Sladen, 1977). There was no similar correlation with faecal ¹⁴C. Previous studies are at variance with these results. Fromm *et al.* (1973) found no correlation between either breath or faecal ¹⁴C and ileal resection, while Lenz (1975) found a correlation with faecal, but not breath, ¹⁴C output. By contrast, in the non-resected patients there was no significant correlation between either breath or faecal ¹⁴C excretion and the extent or severity of ileal or colonic disease. Similarly, there was no relation between impaired vitamin B₁₂ absorption, bowel symptoms and the ¹⁴C-GCA test in these patients.

In both groups treatment with metronidazole was disappointing. Only two patients described any improvement and the incidence of troublesome side-effects was high. As breath ¹⁴CO₂ output decreased after treatment, a degree of bactericidal activity can be assumed. The increased faecal ¹⁴C output observed after treatment supports the concept (Garbutt

et al., 1970) that bacterial degradation favours bile acid absorption.

Our patients had no frankly obstructive features either clinically or radiologically which could predispose to bacterial overgrowth in the small gut. In view of the negative response to metronidazole it would seem that excessive bacterial overgrowth probably did not contribute significantly to symptoms in these patients. Recently, metronidazole has been advocated in the management of Crohn's disease (Ursing and Kamme, 1975). However, these studies involved acutely ill, steroid-dependent patients, and so far no controlled trials have been reported.

Treatment with cholestyramine was successful in many of our patients. Improvement of bowel function was more marked in the resected patients where faecal ^{14}C output was greatest. By the criteria of Hofmann and Poley (1972) and Fromm *et al.* (1973), 10 resected patients (8, 10, 11, 15, 16, 18, 21, 23, 24, 26, Table 3) and four non-resected patients (14, 16, 17, 19, Table 3) had good evidence of symptomatically relevant bile acid malabsorption, confirmed by response to cholestyramine (Hofmann and Poley, 1969). The lack of response in four patients (4, 6, 27, 18, Table 3) is difficult to explain; they did not have extensive ileal resections (greater than 100 cm) nor had marked steatorrhoea (more than 70 mmol/day) been documented in the past. There was no apparent correlation in this series between response to cholestyramine and length of remaining colon as suggested by Hofmann and Poley (1969).

The increase in breath $^{14}\text{CO}_2$ excretion observed in the resected patients after cholestyramine was usually accompanied by a reduction in faecal ^{14}C output and probably reflects slowed colonic transit which enables further deconjugation and absorption of ^{14}C -glycine to occur. Conversion of a normal to increased breath $^{14}\text{CO}_2$ in three patients (cases 21, 23, 24, Table 3) supports the view (Scarpello and Sladen, 1977) that the normal results observed before treatment are attributable to rapid transit. If this is so, it is noteworthy that the $^{14}\text{CO}_2$ output remained very low in the non-resected patients after cholestyramine, even in the two patients whose symptoms definitely improved. This raises doubts about the interpretation of raised faecal ^{14}C in those patients as a whole. The increase was modest (maximum = 16.9%) compared with that seen in many patients after resection. The observations suggest that bile acid malabsorption is of little clinical significance in unoperated Crohn's disease, but there is no direct information based on faecal bile acid output to support this.

Other workers have shown similar abnormalities in the ^{14}C -GCA test in patients with Crohn's disease.

Pedersen *et al.* (1973) described nine non-resected Crohn's patients, all of whom had an increased faecal ^{14}C , while breath $^{14}\text{CO}_2$ was raised in only five. Lenz (1975) in a series of 38 patients with Crohn's disease (26 resected, 12 non-resected), found an increased faecal ^{14}C in all but one of the resected patients, while breath $^{14}\text{CO}_2$ was normal in almost half. Of the non-resected patients six had increased faecal ^{14}C and only one increased breath $^{14}\text{CO}_2$. In these earlier reports, the response to treatment with antibiotics or bile acid binding agents was not assessed and the practical value of the results in relation to management was not discussed.

Our conclusion would be that the ^{14}C -GCA test is of value in assessing the functional ileal deficit in patients after ileal resection, provided faecal ^{14}C is measured, and can be used to predict the response to cholestyramine therapy. In practice, an assessment of faecal volume and a therapeutic trial will generally suffice. Abnormalities of the test in unoperated patients were less frequent, applied mainly to faecal ^{14}C , and did not correlate well with radiological changes or the Schilling test. The clinical response to cholestyramine in non-resected patients was modest, further suggesting that bile acid malabsorption contributed little, if at all, to their symptoms. Anaerobic bacterial overgrowth in the small gut did not appear to contribute to symptoms in either the resected or the unoperated patients in this study.

We would like to thank the many clinicians of the Sheffield Hospitals who have allowed us to study their patients, especially Dr C. D. Holdsworth and Professor H. L. Duthie, and also Mr P. Cloke for technical assistance. J.H.B.S. was supported by a research grant from the Trustees of the Former United Sheffield Hospitals.

References

- Fromm, H., and Hofmann, A. F. (1971). Breath test for altered bile acid metabolism. *Lancet*, **2**, 621-625.
- Fromm, H., Thomas, P. J., and Hofmann, A. F. (1973). Sensitivity and specificity in tests of distal ileal function: prospective comparison of bile acid and vitamin B_{12} absorption in ileal resection patients. *Gastroenterology*, **64**, 1077-1090.
- Garbutt, J. T., Wilkins, R. M., Lack, L., and Tyor, M. P. (1970). Bacterial modification of taurocholate during enterohepatic recirculation in normal man and patients with small intestinal disease. *Gastroenterology*, **59**, 553-566.
- Hofmann, A. F. (1967). The syndrome of ileal disease and the broken enterohepatic circulation: choleraic enteropathy. *Gastroenterology*, **52**, 752-757.
- Hofmann, A. F., and Poley, J. R. (1969). Cholestyramine treatment of diarrhea associated with ileal resection. *New England Journal of Medicine*, **281**, 397-402.
- Hofmann, A. F., and Poley, J. R. (1972). Role of bile acid malabsorption in pathogenesis of diarrhea and steat-

- orrhea in patients with ileal resection. *Gastroenterology*, **62**, 918-934.
- Lenz, K. (1975). An evaluation of the 'breath test' in Crohn's disease. *Scandinavian Journal of Gastroenterology*, **10**, 665-671.
- Lewis, R., and Gorbach, S. (1972). Modification of bile acids by intestinal bacteria. *Archives of Internal Medicine*, **130**, 545-549.
- Nastro, L. J., and Finegold, S. M. (1972). Bactericidal activity of five antimicrobial agents against *Bacteroides fragilis*. *Journal of Infectious Disease*, **126**, 104-107.
- Pedersen, L., Arnfred, T., and Thaysen, E. H. (1973). Rapid screening of increased bile acid deconjugation and bile acid malabsorption by means of the glycine-1-(¹⁴C) cholyglycine assay. *Scandinavian Journal of Gastroenterology*, **8**, 665-672.
- Scarpello, J. H. B., and Sladen, G. E. (1977). Appraisal of the ¹⁴C-glycocholate acid test with special reference to the measurement of faecal ¹⁴C excretion. *Gut*, **18**, 742-748.
- Shimada, K., Bricknell, K. S., and Finegold, S. M. (1969). Deconjugation of bile acids by intestinal bacteria: review of literature and additional studies. *Journal of Infectious Diseases*, **119**, 273-281.
- Ursing, B., and Kamme, C. (1975). Metronidazole for Crohn's disease. *Lancet*, **1**, 775-777.