*J Clin Pathol* 1984;**37**:704–707

# Colonic bicarbonate output as a test of disease activity in ulcerative colitis

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SUMMARY No available test objectively measures impairment of function of the inflamed colonic mucosa in ulcerative colitis. To study function we assessed rectal bicarbonate output by rectal dialysis in the presence of water and bacterial fatty acid (n-butyrate) in 21 controls, 18 patients with acute ulcerative colitis, 12 patients with ulcerative colitis in remission, and 12 patients with other forms of colitis. In acute ulcerative colitis, compared with controls, bicarbonate output and pH was reduced ($p < 0.001$); stimulated bicarbonate output with bacterial fatty acid (incremental bicarbonate output) was reduced by 80% in acute ulcerative colitis ($p < 0.01$). Results indicate that bicarbonate output is a useful and selective test of mucosal function in acute ulcerative colitis. A diminished incremental bicarbonate output with n-butyrate supports the view of inadequate oxidation of bacterial fatty acids in vivo by the mucosa in ulcerative colitis. Whether the test will prove to be an index of prognosis or will aid choice between medical or surgical therapy requires further study.

Disease activity in ulcerative colitis is currently graded on clinical, sigmoidoscopic, or histological criteria.[1] A rapid and repeatable test of functional impairment and disease activity of the colonic mucosa is not yet available and would be useful to monitor clinical progress, results of treatment, or possibly to determine which treatment modality (medical or surgical) is best. We describe a test based on measurement of colonic bicarbonate, which normally appears in high concentrations in solutions placed in the colon.[2] Values of 45 mmol/l have been reported, measured by means of rectal dialysis.[3] Physiologically, bicarbonate exchanges for luminal chloride anion in a process that helps maintain luminal pH at 7·4.[4] Addition of bacterial fatty acids further stimulates luminal appearance of bicarbonate,[5,6] an effect which we used in our investigations.

In secretory diarrhoea due to viruses, toxigenic *Escherichia coli*,[7] or cholera, appearance of colonic bicarbonate is increased, leading to a systemic acidosis. Little is known about bicarbonate secretion in ulcerative colitis and no information is available which relates disease activity to bicarbonate output.

Accepted for publication 15 February 1984

We wished to determine by rectal dialysis whether colonic bicarbonate output was altered in ulcerative colitis, whether a change in bicarbonate output was important for ulcerative colitis or other inflammatory bowel conditions, and whether stimulated bicarbonate output was impaired in colitis.

## Material and methods

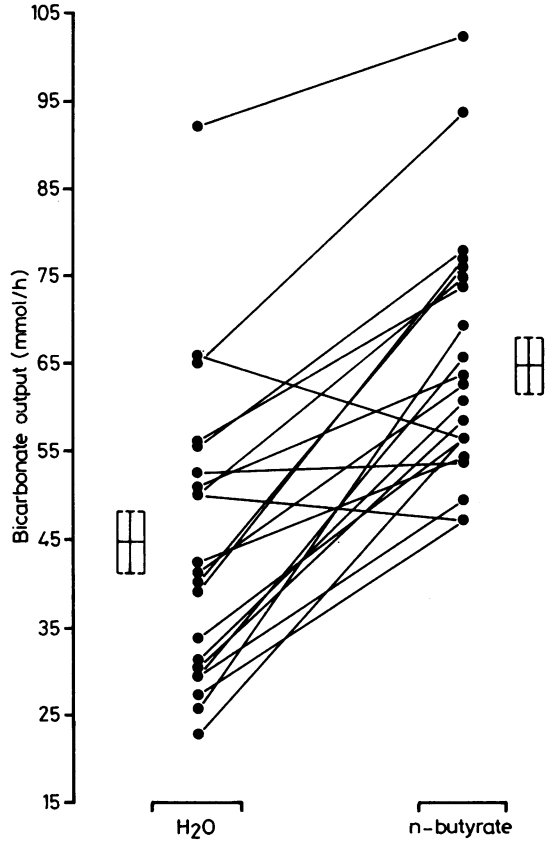
Sixty three subjects were studied: 21 control subjects with haemorrhoids or irritable bowel syndrome, in whom mucosal disease was excluded by mucosal appearances at sigmoidoscopy but not by biopsy; 30 patients with ulcerative colitis, of whom 18 were studied during an acute attack and 12 during remission. Known cases of ulcerative colitis had to have symptomatic, sigmoidoscopic, and histological features (at least mucus cell depletion, crypt abscesses, and increased inflammatory cells) before they were included in the category of acute colitis. A further 12 patients acted as disease controls: six with Crohn's disease, and three each with pseudomembranous or bacterial colitis. None of the disease control patients was taking oral or rectal steriods, but nearly all the patients with ulcerative colitis were taking sulphasalzine both during an acute attack and

in remission.

Dialysis tubing (Visking ¼"), tied off at each end with 0 silk, was placed by proctoscope in the rectum, which was cleared of faecal contents with cotton swabs. Segments of tubing, 3 cm, contained either 1·5 ml of distilled water or 1·5 ml of 40 mM sodium n-butyrate together with $^{14}$COOH—dextran (4 × $10^4$ counts/ml) as a non-dialysable marker for volume correction at the end of a 60 min rectal placement. This time interval was chosen as previous reports indicated that equilibration occurred over this period.[3 8] Wherever possible bicarbonate output was determined with water and n-butyrate in each case. If this was not possible patients were studied with butyrate alone. Dialysate was discarded when faecal pigments were observed, which indicated excessive bacterial presence. pH and $pCO_2$ of the dialysate were measured by micro gas analysis within 3 min of collection, and bicarbonate concentration was derived from these values. Radioactivity was measured by scintillation counting, and volume was calculated from initial and final counts. Bicarbonate output was expressed as mmol/h (volume after 60 min × bicarbonate concentration) and the incremental bicarbonate output designated the difference between bicarbonate output obtained with water and that with n-butyrate.

## Results

The procedure was tolerated well by all patients and none of them sustained macroscopic mucosal damage. The mean bicarbonate output with distilled water was 45·1 ± 3·6 (n = 21), which increased significantly to 65·3 ± 3·1 ($p < 0·01$, Wilcoxon's paired rank sum test) with n-butyrate (Figure). The pH increased from 7·53 ± 0·03 (n = 21) with distilled water to 7·64 ± 0·03 with butyrate in the dialysis bag ($p < 0·01$ Wilcoxon's paired rank sum test).




*Increased bicarbonate output in rectal dialysate with n-butyrate in the control group. Wilcoxon's paired rank sum test $p < 0·01$ (water v butyrate).*

Bicarbonate output was significantly lower in patients with active ulcerative colitis compared with healthy and disease controls (Table). The incremen-

*Bicarbonate output (mmol/h), pH, and incremental bicarbonate output with butyrate (40 mmol/l) in healthy and disease controls and in patients with colitis*

| | *Bicarbonate output* | *pH* | *Incremental bicarbonate output* |
|---|---|---|---|
| Healthy controls | 65·3 ± 3·1 (21)* | 7·64 ± 0·03 (21) | 19·9 ± 3·2 (21) |
| Disease controls | 51·3 ± 7·7 (12) | 7·43 ± 0·06 (12) | 9·0 ± 6·9 (10)† |
| Acute ulcerative colitis | 23·3 ± 1·7 (18) | 7·15 ± 0·04 (18) | 3·8 ± 1·4 (16)‡§ |
| Quiescent ulcerative colitis | 57·1 ± 3·7 (12) | 7·56 ± 0·06 (12) | 13·9 ± 4·8 (9)† |

Values given as mean ± SEM of the number of cases in parentheses.
*One tailed analysis of variance
$F = 24·27$; $df_1 = 3$ $df_2 = 59$
healthy control v disease control $p < 0·001$.
acute colitis v disease control $p < 0·001$.
healthy control v quiescent colitis $0·1 < p < 0·001$.
disease control v quiescent colitis $0·5 < p < 0·01$.
Compared with healthy controls (Wilcoxon's rank sum test)
†Not significant.
‡$p < 0·01$.
Compared with disease controls (Wilcoxon's rank sum test)
§Not significant.

tal bicarbonate output was notably smaller in patients with acute ulcerative colitis than in controls. A trend towards lower values of bicarbonate output was also noted in the disease controls, but this was not significant.

## Discussion

Our study has confirmed that there is normally considerable bicarbonate secretion into the colon of man, the rate of which we have termed "bicarbonate output." Values of bicarbonate secretion in our control cases were slightly higher than those reported by McNeil *et al*[3]; this may be due to differences between volume corrections obtained with a radioactive, non-dialysable marker and those obtained by weighing dialysis bags.

In ulcerative colitis the bicarbonate output was significantly reduced, and this is in keeping with previous investigations. Breuer *et al*[9] found higher levels of chloride anion in the colons of patients with ulcerative colitis compared with controls, indicating that the normal luminal to mucosal exchange of chloride for bicarbonate was impaired. In another study[10] with orally ingested dialysis bags the luminal pH in ulcerative colitis was low in the presence of normal concentrations of bacterial fatty acids. Excess bacterial fatty acids has in the past been implicated as the cause of acidic stools, especially in infant diarrhoea.[11] Our findings of low pHs indicate that a failure of bicarbonate secretion rather than excessive bacterial fermentation is the cause of lowered luminal pH of the colon with ulcerative colitis.

Bicarbonate output in other forms of colitis was noticeably different. In Crohn's colitis Breuer *et al*[9] found that the luminal levels of chloride were within the normal range, which, together with our results, suggests an unimpaired exchange of chloride for bicarbonate. In contrast with our pH values in the large bowel in disease controls, the pH of the small bowel in patients with Crohn's disease was higher than in patients with healthy mucosa.[12] Thus in general bicarbonate secretion, at least in Crohn's disease, is not grossly altered, though some impairment of mucosal production does exist as our stimulated bicarbonate output was not optimal. Overall, a lowered bicarbonate output in ulcerative colitis appears to occur mainly in the acute condition.

We have confirmed that bicarbonate output is stimulated by n-butyrate, an observation first shown in man with acetate by McNeil *et al*[3] and Ruppin *et al*.[6] Whether stimulation of bicarbonate output was maximal needs to be established by further work. Bicarbonate secretion is mediated by carbonic anhydrase,[13] which is located in the columnar epithelial cells of the superficial epithelium of the human colon.[14] Butyrate could either alter the activity of this enzyme directly or exert its action indirectly through metabolism of n-butyrate to $CO_2$ in the colonic epithelium.[15] The $CO_2$ on which carbonic anhydrase acts is usually considered to be of metabolic origin, though generation of $CO_2$ by bacteria could be an alternative source. We tried to exclude bacterial $CO_2$ as much as possible by performing dialysis in rectums cleared of luminal contents. Previous in vitro investigations with isolated epithelial cells of the colon showed diminished production of $CO_2$ from n-butyrate in ulcerative colitis.[15] We have confirmed these findings in vivo by showing a reduced incremental output of bicarbonate in response to butyrate. Observations of the present and other studies show that the colonic mucosa in ulcerative colitis is unable to oxidise short chain fatty acids.

The value of bicarbonate output in determining prognosis of ulcerative colitis is unclear. We did not correlate bicarbonate output with histological activity, but 12 of 18 patients had moderate disease activity on sigmoidoscopy, indicating that diminished bicarbonate output occurred in the early phase of acute ulcerative colitis. Two of the lowest bicarbonate values were recorded within two weeks of urgent surgery for previously controlled colitis. We are hoping to determine by long term follow up whether low bicarbonate output is an indicator of prognosis in ulcerative colitis.

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