

# Impaired sulphation of phenol by the colonic mucosa in quiescent and active ulcerative colitis

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## Abstract

Substantial amounts of phenols are produced in the human colon by bacterial fermentation of protein. In the colonic mucosa of animals, phenols are inactivated predominantly by conjugation with sulphate. The purpose of this study was to confirm sulphation of phenols by isolated colonocytes from man and to evaluate mucosal sulphation in inflammatory bowel disease using the phenol, paracetamol, in rectal dialysis bags. The incubation of paracetamol with colonocytes isolated from resected colon specimens (n=7) yielded a mean (SE) value of 7.0 (0.9)  $\mu\text{mol/g}$  dry weight of paracetamol sulphate after 60 minutes but virtually undetectable values of paracetamol glucuronide. Paracetamol sulphate was detected in rectal dialysates from all control subjects, with a mean (SE) value of 4.2 (0.8) nmol/hour. Sulphation was significantly impaired ( $p < 0.01$ ) in 19 patients with active ulcerative colitis (0.6 (0.2) nmol/hour) and in 17 patients with ulcerative colitis in remission (1.1 (0.4) nmol/hour). Sulphation in eight patients with Crohn's colitis (4.3 (2.1) nmol/hour) was similar to that in control subjects. Impairment of the capacity of the mucosa to sulphate phenols in quiescent and active ulcerative colitis may pose a metabolic burden on colonic epithelial cells, which are continuously exposed to endogenous phenols from the colonic lumen.

The fermentation of protein by bacteria from the human colon generates considerable amounts of phenols and phenylated short chain fatty acids such as phenyl propionate and hydroxyphenyl acetate.<sup>1</sup> Some of these volatile phenols may have adverse effects on colonocytes and harmful effects elsewhere in the body.<sup>2,3</sup> In the intact human colon, the concentration of phenols increases progressively from the caecum to the left colon, with maximal concentrations (2.0-3.0 mmol/kg) in the rectosigmoid region.<sup>4,5</sup> Studies in vitro suggest that higher concentrations could be reached under optimal fermentation conditions.<sup>6</sup> Although the functional effect of these endogenous phenols remains unclear, the use of an exogenous phenol (paracetamol) has been associated with the onset of exacerbations of ulcerative colitis.<sup>7</sup>

In experimental animals, the inactivation of phenols by the colonic mucosa is mainly achieved by conjugation with sulphate.<sup>8,9</sup> This also seems likely in humans,<sup>10</sup> although studies using colonocytes from resected colon specimens have not been undertaken. The purpose of this study was to examine the sulphation of paracetamol by human colonocytes and to evaluate

the sulphation of phenols in inflammatory bowel disease using paracetamol in rectal dialysis bags.

## Patients and methods

### STUDIES WITH HUMAN COLONOCYTES

Colonocytes were isolated from the unaffected mucosa (at least 10 cm from the tumour) of seven colon specimens resected for colonic cancer. Five specimens were from the descending colon and two from the ascending colon. Colonocytes were prepared by mechanical dissociation after incubation in a divalent calcium free solution containing ethylenediaminetetra acetic acid (EDTA).<sup>11</sup> Colonocyte suspensions containing approximately 5 mg dry weight of cells/ml suspension were incubated with paracetamol, 1 mmol/l (a concentration of phenol generally found in the colon) in the presence of sulphate (1.4 mmol/l) and [ $^{14}\text{C}$ ] butyrate (10 mmol/l) for 20, 40, and 60 minutes. The production of  $^{14}\text{CO}_2$  was determined by trapping in 10 M NaOH in the centre well of incubation flasks. After filtration through a 0.22  $\mu$  Millipore filter, paracetamol conjugates were detected by reverse phase high performance liquid chromatography (HPLC) on Spherisorb ODS2 columns as described in detail previously.<sup>10</sup> Concentrations of sulphate in the supernatant were determined by liquid chromatographic separation on a Waters IC-PAK anion column followed by detection with a Waters 430 conductivity detector. The mobile phase, which comprised sodium gluconate, boric acid, and sodium tetraborate (1.6%, 1.8%, 2.5%), was pumped through at a rate of 1 ml/minute.

### PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Of 44 patients with inflammatory bowel disease, 19 had active ulcerative colitis, 17 had quiescent ulcerative colitis, and eight had Crohn's colitis. The diagnosis had been established in all patients by accepted clinical, endoscopic, histological, and radiological criteria. Patients with ulcerative colitis ranged in age from 17 to 81 years (mean 53 years) and had a mean duration of disease of eight years. The extent of disease was categorised by sigmoidoscopy and colonoscopy as proctitis (11 patients), left sided colitis (17 patients), and total colitis (eight patients). At the time of study by rectal dialysis, the activity of colitis was assessed by clinical criteria<sup>12</sup> as well as by sigmoidoscopic findings.<sup>13</sup> Drug treatment for colitis was recorded. Patients with Crohn's colitis had a mean age of 45 years and a mean duration of disease of five years: seven of the eight patients had a normal rectum at sigmoidoscopy. There were similar numbers of men and women in both diagnostic groups.

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The control group consisted of 22 patients with either haemorrhoids or colon cancer (three patients), but no inflammatory bowel disease. They ranged in age from 49 to 79 years (mean 63 years). Informed consent was obtained from all subjects. The study was approved by the Ethics of Research Committee of The Queen Elizabeth Hospital.

#### RECTAL DIALYSIS

The technique of rectal dialysis *in vivo* has been described in detail previously.<sup>14</sup> Briefly, dialysis tubing (Visking 8/32) was filled with 1.5 ml of distilled water containing 10 mmol/l sodium butyrate, 1 mmol/l paracetamol, and [<sup>14</sup>COOH]-Dextran ( $4 \times 10^4$  counts/ml) as a volume marker. The tubing was tied off at both ends and left in the rectum for one hour. Bicarbonate output was determined by microelectrode analysis within three to five minutes of sample collection. Paracetamol conjugates were identified and analysed by reverse phase HPLC as described above. Samples were analysed in batches after storage at  $-20^\circ\text{C}$ .

#### STATISTICS

The Mann-Whitney U test for non-parametric data was used for statistical comparisons. Correlation coefficients were determined by the Spearman method.

### Results

#### STUDIES WITH HUMAN COLONOCYTES

Paracetamol sulphate was detected in supernatants from all incubations with human colonocytes. The sulphate conjugate increased to a mean (SE) of 3.5 (0.7)  $\mu\text{mol/g}$  dry weight of colonocytes at 20 minutes, 6.0 (0.7)  $\mu\text{mol/g}$  at 40 minutes, and 7.0 (0.9)  $\mu\text{mol/g}$  at 60 minutes. Glucuronide conjugates were undetectable in colonocyte cultures from six colon specimens but were present at low values in cultures from one specimen. There was no apparent decrease in inorganic sulphate in the incubation medium over the period of study. During the incubation, colonocytes remained viable as assessed by the linear utilisation of butyrate (results not shown).

#### RECTAL DIALYSIS

Paracetamol sulphate was detected in dialysates from all control subjects, with mean (SE) values of 4.2 (0.8) nmol/hour. As shown in Figure 1, sulphation was significantly impaired ( $p < 0.01$ ) in patients with quiescent ulcerative colitis (1.1 (0.4) nmol/hour) and in patients with active colitis (0.6 (0.2) nmol/hour). Patients with Crohn's colitis showed similar sulphation values (4.3 (2.1) nmol/hour) to those observed in control subjects. Seven of the eight patients with Crohn's disease had a normal rectum at sigmoidoscopy.

Paracetamol glucuronide was detected infrequently; low values were found in seven control subjects (median 0, mean 0.14 nmol/hour), two subjects with active colitis (median 0, mean

0.09 nmol/hour), three with inactive colitis (median 0, mean 0.19 nmol/hour), and two with Crohn's colitis (median 0, mean 0.06 nmol/hour).

The mean (SE) bicarbonate output in dialysates was 44.0 (4.1)  $\mu\text{mol/hour}$  in control subjects, 23.2 (4.3)  $\mu\text{mol/hour}$  in active ulcerative colitis, 42.6 (9.7)  $\mu\text{mol/hour}$  in quiescent ulcerative colitis, and 33.7 (5.1)  $\mu\text{mol/hour}$  in Crohn's colitis. Values in active colitis were significantly lower ( $p < 0.01$ ) than those in control subjects, confirming results from a previous study.<sup>12</sup>

Paracetamol sulphation did not correlate significantly with rectal bicarbonate output in 36 patients with active or quiescent ulcerative colitis ( $r = 0.02$ ) or in the subgroup with active colitis ( $r = -0.42$ ). Furthermore, there was no apparent relation between paracetamol sulphation and disease activity as assessed by clinical criteria, although patients with more severe inflammation at sigmoidoscopy tended to have lower sulphation values (Fig 2). Although patients with proctitis had higher mean values of paracetamol sulphate (1.55 nmol/hour) than those with left sided colitis (0.26 nmol/hour) and total colitis (0.93 nmol/hour), differences between groups did not reach statistical significance. Similarly, mean sulphation values in seven patients without drug treatment (0.19 nmol/hour) did not differ significantly from mean values in 22 patients receiving sulphasalazine alone (1.0 nmol/hour) or seven receiving sulphasalazine and prednisolone (0.66 nmol/hour).

### Discussion

The present observations show that colonic epithelial cells have a considerable capacity to sulphate phenol, a feature broadly predicted to

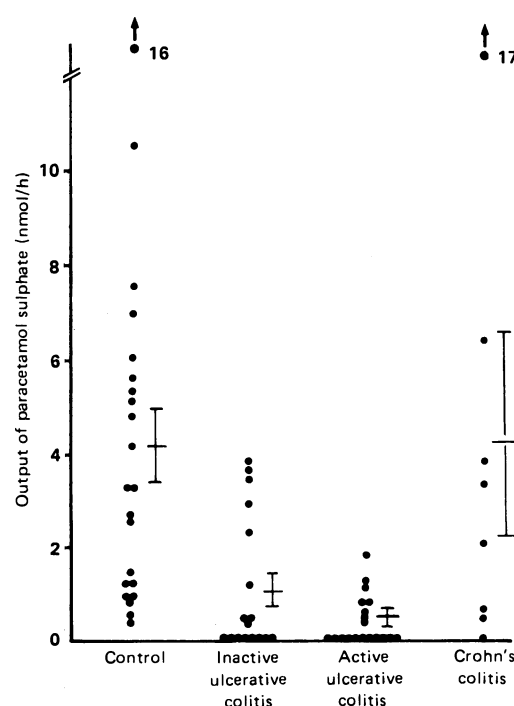


Figure 1: Values of paracetamol sulphate in rectal dialysates from control subjects, patients with quiescent and active ulcerative colitis, and patients with Crohn's colitis. Bars are mean (SE).

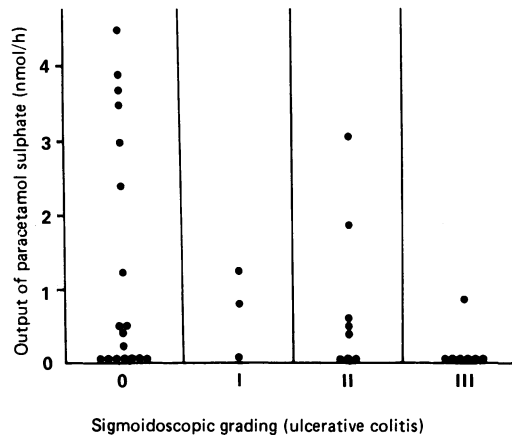
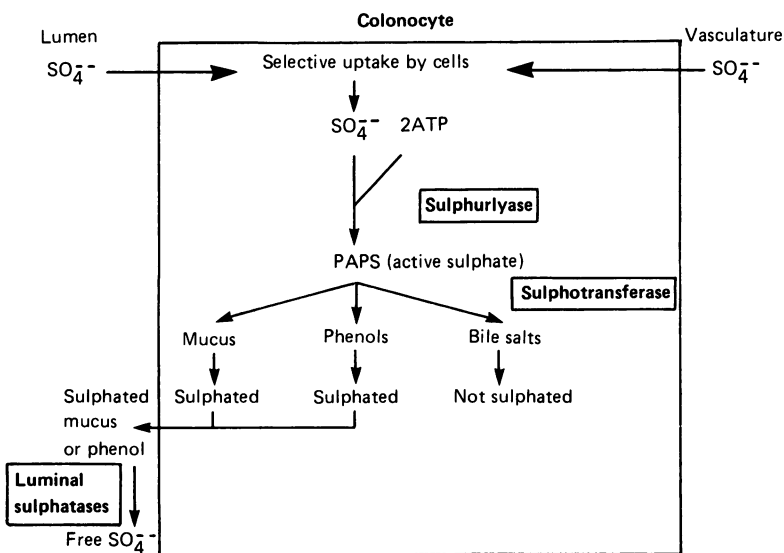


Figure 2: Values of paracetamol sulphate in rectal dialysates in relation to degrees of rectal inflammation as assessed at sigmoidoscopy.

occur in the gastrointestinal tract of animals<sup>7 15 16</sup> and now shown specifically for the human colonic mucosa in vivo. Other known means whereby colonic epithelial cells inactivate foreign compounds are by acetylation,<sup>18</sup> hydroxylation,<sup>19</sup> and S-methylation.<sup>20</sup> Conjugation by glucuronidation seems to be poorly developed in colonic epithelial cells.<sup>21</sup> Sulphation of phenols such as 1-naphthol and hydroxycoumarin has been observed in isolated mucosal sheets and in colonocytes from the rat and guinea pig<sup>8 9</sup> while sulphation of 1-naphthol was shown with human colonic mucosa in culture in vitro.<sup>22</sup> The present investigations show that sulphation of another phenol, paracetamol, occurs in epithelial cells of the mucosa in vivo. These observations suggest that the colonic mucosa has the capacity to sulphate a number of phenols but whether the results are applicable to sulphation of phenols produced by bacterial fermentation of protein substrates has not been established.

The process of sulphation involves a number of steps including extraction of sulphate from the circulation, conversion of sulphate to 'active sulphate', and the presence of sulphotransferase enzymes that transfer sulphate from 'active sulphate' to specific compounds such as phenols or mucus (Fig 3). These steps have all been shown in colonic mucosa. For example, Jennings and

Figure 3: Steps involved in the sulphation of mucus and phenols in colonic epithelial cells (collated from references 17, 24, 25, 26, and 30).



Florey<sup>23</sup> showed rapid extraction of circulating sulphate by colonic epithelial cells, while others showed greater extraction of sulphate by colonic cells than cells from the small intestine.<sup>24</sup> The sulphate extracted by cells is converted to 'active sulphate' (3-phosphoadenosine 5'-phospho-sulphate), of which about 20% is available for mucus sulphation and the remainder available for other sulphation processes such as phenolic sulphation.<sup>25 26</sup> Sulphotransferases for phenol have been found in the human colon and show maximal activity in the distal colon.<sup>27</sup>

While colonic epithelial cells promote sulphation, sulphate can also be removed from a number of substances, including sulphated mucin, by the activity of sulphatases of bacterial or lysosomal origin.<sup>28 29</sup> Excessive activity of sulphatases seems unlikely to account for the current observations, however, as a previous study showed normal faecal sulphatase activity in patients with inflammatory bowel disease.<sup>30</sup>

The current study showed impaired sulphation of phenols in both active and quiescent ulcerative colitis. The possibility of a degree of improvement in sulphation with resolution of colitis will only be clarified by serial studies in individual patients, but in this study paracetamol sulphate values in quiescent disease were similar to those in active disease and there was no significant correlation between sulphation and output of bicarbonate. Furthermore, sulphation did not seem to be influenced by the extent of colitis or by drug treatment. The specificity of impaired sulphation for ulcerative colitis has not been addressed as yet, but sulphation seems to be normal in patients with Crohn's colitis without rectal inflammation at sigmoidoscopy. In colon specimens with cancer, histochemical studies have shown impaired sulphation of mucins, particularly in the region of the tumour,<sup>31 32</sup> but the relation between this change and rectal sulphation of phenols remote from tumours remains unclear. In this study, the three control subjects with colon cancer had paracetamol sulphate values above the mean value for the group as a whole (4.2 nmol/hour).

Assuming an adequate supply of sulphate, impaired sulphation of phenols could be due to a specific enzyme defect (sulphotransferase or sulphurylase) or to a diminished supply of adenosine triphosphate. At present, there is no evidence to support or refute the possibility of a specific enzyme defect. A diminished supply of adenosine triphosphate, however, could account for the observations, since colonocytes in ulcerative colitis show low concentrations of adenosine triphosphate and defects in oxidative metabolism.<sup>33 34</sup>

The diminished capacity for phenolic sulphation in quiescent and active ulcerative colitis raises questions relevant to the pathogenesis of the disease. For example, the ingestion of paracetamol has been associated with the onset of exacerbations of ulcerative colitis, an association which became more striking with higher doses of the drug.<sup>7</sup> While the concentrations of paracetamol attained in the colonic lumen are not known, it is possible that unsulphated phenols may be toxic to colonic epithelial cells in a similar way to the toxic effect of excess paracetamol on

liver cells. Whether dietary phenols,<sup>1,2,6</sup> especially those ingested from high protein diets, are injurious to patients with ulcerative colitis is likewise unknown. Results from this study, however, raise the possibility that dietary provocative studies or dietary exclusion may establish a more precise relation between phenolic ingestion and exacerbations of colitis.

A further potential consequence of reduced sulphation in ulcerative colitis is the metabolism of excess unsulphated phenols by liver cells. Hepatic sulphation occurs largely in periportal regions<sup>35,36</sup>; sites frequently involved with inflammatory changes in ulcerative colitis.<sup>37</sup> The possibility that unsulphated phenols, absorbed from the intestinal tract, contribute to these inflammatory changes may be a fruitful area for further study.

In conclusion, rectal dialysis has shown that detoxification of paracetamol occurs in the human colon, a function that is reduced or absent in active colitis and found to be absent in 47% of patients with quiescent colitis. Loss of this detoxification mechanism in ulcerative colitis may be related to exacerbations of colitis or may put an additional burden on detoxification processes in the liver.

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