

Abnormal leukotriene C₄ release by unaffected jejunal mucosa in patients with inactive Crohn's disease

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Abstract

The mucosal release of inflammatory mediators is enhanced in active inflammatory bowel disease. This study examines whether leukotriene C₄ production occurs in apparently unaffected segments of the gut. The intraluminal release of leukotriene C₄ was determined by jejunal perfusion in seven healthy controls, in nine patients with chronic ulcerative colitis, and in 13 patients with Crohn's disease (six with ileal disease, and seven with only colonic). All patients were in clinical remission and none of them had evidence of jejunal involvement. Mild intraluminal irritation with a 2.5 mmol/l deoxycholic acid solution was induced to stimulate local inflammatory mechanisms. The release of DNA (a marker of mucosal desquamation) and prostaglandin E₂ (PGE₂) was simultaneously measured. Jejunal release of DNA was higher in Crohn's disease patients than in ulcerative colitis or healthy controls. Basal release of PGE₂ was similar in the three groups of patients. Basal release of leukotriene C₄ was considerably enhanced, however, in Crohn's disease patients compared with healthy controls. In ulcerative colitis patients, basal leukotriene C₄ release was non-significantly different from controls. Bile acid perfusion stimulated PGE₂, leukotriene C₄, and DNA release in all groups studied, but leukotriene C₄ release was significantly higher in Crohn's disease patients. It is concluded that in inactive Crohn's disease there is an enhanced intraluminal release of leukotriene C₄ in apparently unaffected segments of proximal small bowel, which may reflect fundamental changes in the function of the gut mucosal barrier.

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Measurement of eicosanoids both in vitro, in biopsy specimens from patients with active inflammatory bowel disease,^{1,3} and in vivo, using dialysis bags placed in the rectal lumen of patients with active rectocolitis,^{4,5} have shown that mucosal eicosanoid synthesis is enhanced at sites of active mucosal inflammation. Whether such an increase in eicosanoid synthesis is secondary, however, to mucosal inflammation or whether it represents a fundamental change in the gut of patients with inflammatory bowel disease remains unknown. We reasoned that if eicosanoids were proinflammatory mediators fundamentally participating in the pathogenesis of inflammatory bowel disease, they would be found to be released into the bowel lumen in

increased amounts during clinical remission and even in areas of undamaged gut mucosa. There is some evidence supporting enhanced eicosanoid release by morphologically preserved mucosa in inflammatory bowel disease. Thus, inappropriate release of thromboxane B₂ comparative with prostaglandin E₂ (PGE₂) into the jejunal lumen in response to mild intraluminal irritants has been seen in clinically inactive chronic ulcerative colitis.⁶ Patients with Crohn's disease also exhibit an enhanced intraluminal release of myeloperoxidase,⁷ C₄ component of the complement,⁸ and histamine⁹ in areas of jejunum apparently unaffected by the disease. These findings may indeed reflect an ongoing activation of inflammatory mechanisms in patients with inflammatory bowel disease that may not necessarily result in clinical manifestations.

We also reasoned that biochemical mucosal defects that may not be shown under basal conditions could become evident in response to mucosal irritation. It is known that in normal human jejunum, mild intraluminal irritation by perfusion of a weak deoxycholic acid solution increases mucosal desquamation and is associated with increased release of leukotriene C₄¹⁰ and other eicosanoids.⁶ An exaggerated intestinal release of leukotriene C₄ in response to mild luminal irritation could also uncover abnormal or inappropriate inflammatory activity in the small bowel of patients with inactive inflammatory bowel disease.

Methods

SUBJECTS

Studies were performed in seven healthy volunteers (two men and five women; median age of 23 years, interquartiles 22-24), in nine chronic ulcerative colitis patients (five men and four women; median age of 51 years, interquartiles 46-62), and in 13 Crohn's disease patients (four men and nine women; median age of 36 years, interquartiles 27-37). Chronic ulcerative colitis affected the rectum in three patients, sigmoid and left colon in four, and the whole colon in the remaining two patients. Crohn's disease affected only the colon in seven patients and the ileum in six. Diagnosis was established in each patient by conventional radiological, endoscopic, and histological criteria. At the time of study, all patients were in clinical remission according to the Truelove index for chronic ulcerative colitis¹¹ and to Harvey index for Crohn's disease.¹² The disease had been inactive for a period of one to six weeks in 17 patients and for more than six weeks in five patients. The total

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duration of the disease (that is, since a clinical diagnosis was established) ranged from 3 to 240 months for chronic ulcerative colitis patients and from 3 to 180 months for Crohn's disease patients. At the time of study three patients were receiving low dose 6-methylprednisolone and salazopyrine, nine patients low dose 6-methylprednisolone only, two patients salazopyrine, one patient 5-amino salicylic acid. The remaining seven patients had not received any treatment. The study was approved by the institutional review board, and all subjects gave written informed consent. Data from control group were included in a previous report.¹⁰

PERFUSION PROCEDURE

We used a modified double lumen perfusion technique. At 9 am, after an overnight fast, a triple lumen tube was placed in the jejunum under fluoroscopic control. The first lumen opened at the angle of Treitz and was used for infusion. The second lumen opened 60 cm distally and was used for recovery of luminal fluid by siphonage. The third lumen was connected to an inflatable balloon with a mercury bag at its tip. The balloon was used to help advance the tube into position, and was deflated thereafter. A volumetric pump (IMED 927, Milton Trading Estate, Abingdon, UK) was used to infuse a water solution containing mannitol 180 mmol/l, xylose 100 mmol/l, and polyethylene glycol (PEG) 4000 2 g/l, as a non-absorbable marker, at a rate of 5 ml/min. The osmolality of the perfusate was 280 mmol/kg and the pH was adjusted to 7.8 with 0.1 mol/l NaOH. To limit transmural jejunal water flow, the perfusate did not contain glucose or electrolytes. Deoxycholic acid (Koch-Light Laboratories, Colnbrook, Bucks, UK) was added to the solution to a final concentration of 2.5 mmol/l when appropriate. This concentration of deoxycholic acid, albeit unconjugated, is within the range of jejunal postprandial concentrations.¹³ It induces jejunal secretion without morphological epithelial injury,¹⁴ and its effect is reversible.¹⁵ Indigo blue was added to the deoxycholic acid solution to recognise its appearance and disappearance from the perfused jejunal segment. The perfusate was recovered by siphonage to preclude the local trauma, which would have otherwise occurred

with mechanical or manual suction. In every patient, a jejunal biopsy specimen was taken from the perfused segment with a Watson capsule 24 hours after the perfusion.

EXPERIMENTAL DESIGN

Every subject had two consecutive 100 minute perfusion sequences in a given day. Each sequence began with 30 minutes of intestinal washing and was followed by the test perfusion with and without deoxycholic acid for 70 minutes (30 minutes equilibration plus 40 minutes test period). The order of deoxycholic acid and deoxycholic acid free test perfusions was randomised. The total duration of the study was 200 minutes.

ANALYTICAL PROCEDURES

During each perfusion sequence aspirates were continuously collected over ice and pooled at 10 minute intervals. Aliquots of 2 ml were stored at -20°C for later analysis of PEG, a non-absorbable marker used for calculation of net water flux, and DNA to assess cellular desquamation. Indomethacin (Sigma) was added to 2 ml aliquots up to a concentration of 50 µg/ml to prevent in vitro prostanoïd generation.

Samples were analysed for PEG by the Hyden method,¹⁶ for DNA by a modification of the colorimetric method of Burton.¹⁷ Leukotriene C₄ and PGE₂ were measured by specific radioimmunoassay as described elsewhere.¹⁰ Antiserum for PGE₂ measurement was a gift from Dr J A Salmon (Wellcome Research Laboratories, Beckenham, Kent, UK). Standard leukotriene C₄ and specific antiserum were kindly donated by Dr A W Ford-Hutchinson (Merck Frosst Canada, Dorval, Quebec). Cross reactivity of leukotriene C₄ antiserum with PGE₂, thromboxane B₂, leukotriene B₄, 5-hydroxy-eicosatetraenoic acid and arachidonic acid is less than 0.1%. Cross reactivity with leukotriene D₄ and leukotriene E₄ is higher than 10%. Tritiated standards were obtained from Amersham International (Buckinghamshire, UK). Analysis of two series of samples with known amounts of standard added to either the 2.5 mmol/l deoxycholic acid or deoxycholic acid free solutions showed that the presence of deoxycholic acid in the perfusion do not interfere with the antibody binding.

CALCULATIONS AND STATISTICAL METHODS

Net water flux was calculated by a standard formula. DNA, leukotriene C₄, and PGE₂ releases were calculated using the following formula:

$$\text{release} = (\text{Pa} \times \frac{\text{PEGp}}{\text{PEGa}}) \times V$$

where Pa was the DNA, leukotriene C₄, or PGE₂ concentration measured in the jejunal aspirate, PEGp/PEGa the ratio between the concentration of PEG in the perfusion solution and in the jejunal aspirate respectively, and V was the perfusion rate of 5 ml/min.

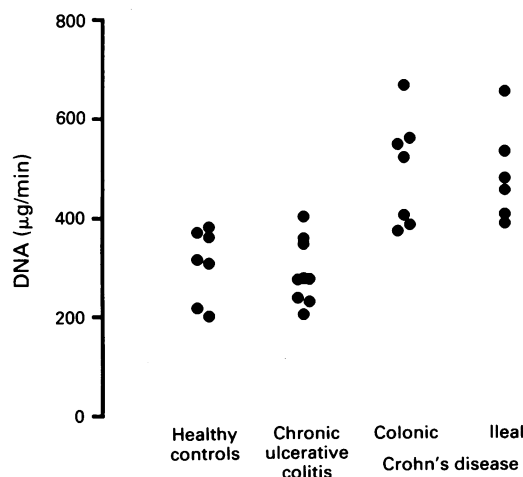


Figure 1: Individual intrajejunal DNA release as expression of the cellular desquamation. DNA loss was higher in Crohn's disease than in healthy controls ($p < 0.01$ v healthy controls and chronic ulcerative colitis patients).

TABLE I Effect of deoxycholic acid perfusion on the intrajejunal DNA release

	Healthy controls	Chronic ulcerative colitis	Colonic Crohn's disease	Ileal Crohn's disease
Basal	309 (27)	292 (22)	498 (41)*	492 (39)*
Stimulated	872 (195)†	502 (84)†	845 (61)‡	1161 (181)‡

Results are expressed as mean (SEM); * $p < 0.01$ compared with healthy controls; † $p < 0.05$ compared with basal; ‡ $p < 0.01$ compared with basal.

Results are expressed as means and standard error of the mean. Data correspond to the 20–40 minute period from either control and deoxycholic acid perfusion. Statistical differences in net water flux and DNA release were calculated by the Student's *t* test for paired or unpaired data as appropriate. Differences in leukotriene C₄ and PGE₂ release were tested by the Mann-Whitney U test.

Results

NET WATER FLUX AND MUCOSAL DESQUAMATION
Jejunal net water flux was similar in the four groups studied (mean (SEM)) (2.2 (0.6) ml/min in controls, 2.2 (1.3) in chronic ulcerative colitis, 2.7 (0.3) in colonic Crohn's disease, and 2.0 (0.3) in ileal Crohn's disease, $p = \text{NS}$). Perfusion with deoxycholic acid induced water secretion, increasing net water flux similarly in controls (4.6 (1.0) ml/min, $p < 0.01$) and all disease groups (chronic ulcerative colitis: 3.4 (0.5), colonic Crohn's disease: 3.7 (0.6), ileal Crohn's disease: 2.9 (0.2), $p < 0.05$).

Basal mean DNA release in chronic ulcerative colitis patients was similar to that seen in healthy subjects. In both colonic and ileal Crohn's disease, however, basal DNA release was higher than in controls (see Fig 1). As a consequence of the irritative effect, deoxycholic acid perfusion induced mucosal cellular desquamation, increasing luminal DNA release in healthy controls and, similarly, in chronic ulcerative colitis and Crohn's disease patients (Table I).

LUMINAL LEUKOTRIENE C₄ RELEASE

Leukotriene C₄ was released into the jejunal lumen in measurable quantities in all subjects studied (Fig 2). Basal mean release of leukotriene C₄ in healthy controls was below 20 ng/min.

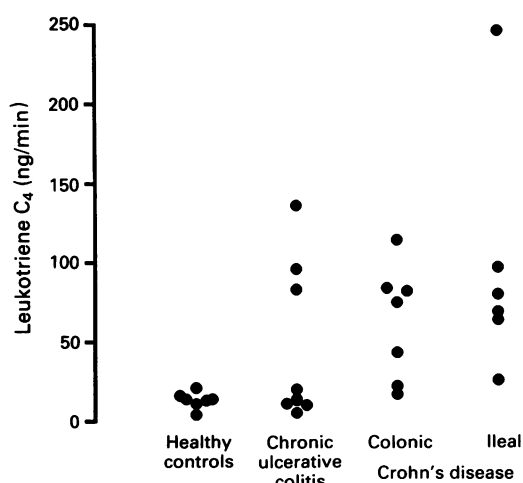


Figure 2: Basal intrajejunal release of leukotriene C₄. The output of leukotriene C₄ was considerably enhanced in colonic and ileal Crohn's disease ($p < 0.05$ v healthy controls).

Chronic ulcerative colitis patients showed a trend towards higher basal leukotriene C₄ release than controls (43 (16) ng/min), but the difference was not significant largely because of three individual chronic ulcerative colitis patients with a high basal leukotriene C₄ release: one patient with widespread colitis and two with left sided colitis. They had been in remission for 2 to 56 weeks, and treated with low doses of methylprednisolone (two patients) or salazopyrine (one patient). From the six chronic ulcerative colitis patients with low leukotriene C₄ release, two had not received any drug treatment and four were receiving low dose methylprednisolone.

In Crohn's disease patients, basal leukotriene C₄ release was considerably and significantly enhanced (colonic Crohn's disease: 63 (14) ng/min, ileal Crohn's disease: 97 (31), $p < 0.01$ v healthy controls). Individual values of basal leukotriene C₄ release in every Crohn's disease patient were above the range seen in healthy controls.

Deoxycholic acid irritation induced a significant increase in leukotriene C₄ release over basal in healthy controls in both chronic ulcerative colitis and Crohn's disease patients ($p < 0.05$ for each group). Stimulated leukotriene C₄ release in Crohn's disease was significantly higher than in healthy controls ($p < 0.05$, Table II). The release of leukotriene C₄ significantly correlated with that of DNA ($r = 0.58$, $p < 0.01$).

LUMINAL PGE₂ RELEASE

PGE₂ was released in measurable quantities in all the subjects studied (Fig 3). Basal mean release of PGE₂ in healthy controls was 8 (1) ng/min. Basal mean PGE₂ release in chronic ulcerative colitis patients (7 (2)) and in Crohn's disease patients (ileal Crohn's disease: 7 (2); colonic Crohn's disease: 5 (1)) was similar to that seen in healthy controls.

Deoxycholic acid stimulation also induced a significant increase in PGE₂ release in healthy controls (39 (23), $p < 0.05$ v basal) in chronic ulcerative colitis patients (21 (6), $p < 0.05$ v basal) and in Crohn's disease patients (colonic Crohn's disease: 30 (11); ileal Crohn's disease: 26 (12), $p < 0.05$). There were no differences, however, in stimulated PGE₂ release among the groups studied.

JEJUNAL HISTOLOGICAL EXAMINATION

All jejunal biopsy specimens taken from Crohn's disease patients showed no signs of inflammation or granulomas and were considered normal jejunal mucosa.

Discussion

Leukotriene C₄ is a sulphopeptide leukotriene synthesised by mast cells by the lipoxygenase pathway of arachidonic acid metabolism. Acting through a specific receptor, it is a well known mediator of the inflammatory response. This study performed by perfusion of histologically normal jejunum of patients with clinically inactive inflammatory bowel disease, shows that in Crohn's disease, but not in chronic ulcerative

TABLE II Effect of deoxycholic acid perfusion on the intrajejunal release of leukotriene C₄

	Healthy controls	Chronic ulcerative colitis	Colonic Crohn's disease	Ileal Crohn's disease
Basal	14 (2)	43 (16)	63 (14)*	97 (31)*
Stimulated	101 (53)†	115 (26)†	240 (47)*‡	558 (158)*‡

Results are expressed as mean (SEM); * $p < 0.05$ compared with healthy controls; † $p < 0.05$ compared with basal.

colitis, there is an enhanced intraluminal release of leukotriene C₄. Furthermore, this study shows that increased release of leukotriene C₄ is related to mucosal desquamation and that both leukotriene C₄ release and desquamation are also abnormally increased in Crohn's disease when the jejunal mucosa is irritated by a weak bile acid perfusate. The unique character of the leukotriene response is underscored by the fact that basal and stimulated PGE₂ release were similar in healthy controls, chronic ulcerative colitis patients, and in Crohn's disease patients.

In the small intestine, sulphidopeptide leukotrienes induce inflammatory phenomena, such as oedema because of an increased vascular permeability, electrolytes secretion, and intestinal smooth muscle contraction.^{18,19} Previous investigators who have measured prostanoid and leukotriene generation at sites of active bowel inflammation in patients with relapsing inflammatory bowel disease, have usually found increased generation of both lipo-oxygenase and cyclo-oxygenase metabolites. When leukotriene C₄ has been specifically measured in inflamed rectal mucosa it has been found increased.²⁰ A comparison of the effects of selective inhibition of either cyclo-oxygenase or lipo-oxygenase activity in patients with ongoing chronic ulcerative colitis, suggests that leukotrienes are more important than prostaglandins as mediators of the inflammation in this condition.²¹⁻²⁴ In this context, our finding of baseline increased release of leukotriene C₄ in the non-inflamed small bowel is persuasive evidence that in Crohn's disease proinflammatory mechanisms in the intestinal mucosal are permanently activated. The fact that leukotriene C₄ release is enhanced while PGE₂ is similar to that of controls, may suggest that mast cells or macrophages are activated as, compared with, polymorphonuclear cells these cells can generate sulphidopeptide leukotrienes. This finding is particularly

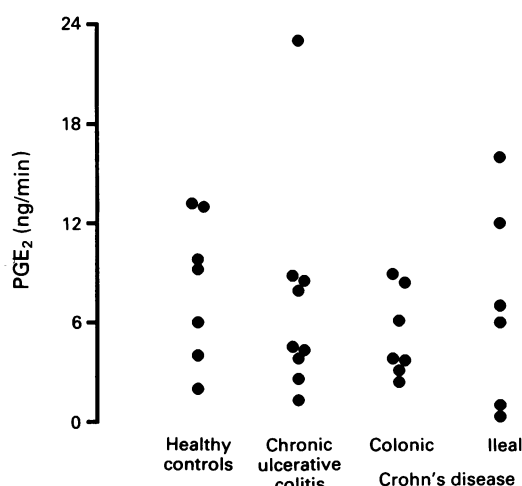


Figure 3: Basal intrajejunal release of PGE₂. The intrajejunal PGE₂ release was similar among the four groups of subjects studied.

relevant in the light of recent data showing that leukotriene C₄ generation is associated with the development of intestinal mucosal damage²³⁻²⁵ and that, conversely, its decrease coincides with a reduction of inflammatory mucosal lesions.²⁸

A potential flaw of our study could be the lack of a group of untreated patients. Studying such a group would pose, however, obvious difficulties by the discomfort of intubating patients and withdrawing maintenance treatment at least for a week before the study. In reality, the enhanced release of leukotriene C₄ seen in our Crohn's patients cannot be attributed to medical treatment with salazopyrine or steroids because these drugs, if anything, would depress leukotriene C₄ release because they are active inhibitors of lipo-oxygenase metabolite formation.^{20,22-24} We are therefore reporting a pathophysiological process that remains operational and apparent despite therapeutic pharmacological intervention.

In our group of patients with chronic ulcerative colitis, leukotriene C₄ release was not significantly enhanced compared with controls. As mentioned in the results section, three patients receiving corticosteroids or sulphasalazine showed an abnormally high release of leukotriene C₄. Six other chronic ulcerative colitis patients, however, showed a low leukotriene C₄ release even if two of them were not receiving any drug treatment. In contrast, all the 13 patients with Crohn's disease showed a high release of leukotriene C₄. These data suggest that some chronic ulcerative colitis patients show an abnormal activation of intestinal mast cells or macrophages even in segments of the small intestine. Further studies in chronic ulcerative colitis patients are needed to clarify this point.

The fact that healthy controls were younger than patients with Crohn's disease might suggest that enhancement of jejunal leukotriene C₄ is an age related phenomenon. If an older population produces more leukotriene C₄ than younger people, however, than our chronic ulcerative colitis group should have had the highest release of leukotriene C₄.

In our study, the degree of irritation induced by deoxycholic acid perfusion was assessed by changes in net water flux (water exorption) and increased cellular desquamation (enhanced luminal DNA output). We selected deoxycholic acid as the agent to induce mucosal irritation for several reasons. Firstly, it was known to reversibly stimulate water secretion, impair absorption of monosaccharides, and induce mucosal desquamation and epithelial damage. Secondly, it was known that bile acids increase mucosal generation of PGE₂ in experimental animals²⁹⁻³¹ and hence activate arachidonic acid metabolism. In fact, although increased production of mucosal prostaglandin has been postulated to mediate the bile acid effects, there must be a concomitant prostaglandin independent mechanism because indomethacin inhibits prostaglandin synthesis without changing bile acid effects.^{30,32,33} Thirdly, in a study with human volunteers we obtained evidence that jejunal bile acid perfusion enhanced leukotriene C₄ release into the lumen.¹⁰ Our study shows that in Crohn's disease patients mucosal irritation by bile acids increases leukotriene C₄ production

above the already raised basal production. This finding suggests that in Crohn's disease the intestinal mucosal leucocytes maintain their capacity to produce leukotriene C₄ even over a basal enhanced production.

In contrast with leukotrienes that are pro-inflammatory eicosanoids, PGE₂ exerts a protective effect on intestinal mucosa against noxious agents. Thus, it has been shown that exogenous PGE₂ protects the small bowel from indomethacin induced ulceration³⁴ and also protects the colon from a variety of damaging agents including ethanol, indomethacin, and trinitrobenzenesulphonic acid.³⁵⁻³⁷ In addition, rabbits immunised with PGE₂ that produce circulating antibodies develop ulcers both in the stomach and small intestine, suggesting that endogenous PGE₂ also plays an active part in the defence of intestinal mucosa.³⁸ More importantly, it seems that one mechanism for PGE₂ mediated intestinal mucosal protection could be inhibition of parietal leukotriene production.^{34,35} Our results would thus suggest that in Crohn's disease the PGE₂ mediated intestinal mucosal protection is preserved (normal PGE₂ generation) but that the defence balance is upset by the predominance of proinflammatory leukotriene C₄ production. Why intestinal leukotriene C₄ production is higher in Crohn's disease than in ulcerative colitis cannot be answered by our studies. Crohn's disease patients exhibit higher basal and luminal leukotriene C₄ outputs after irritation than chronic ulcerative colitis patients. The different magnitude may reflect greater underlying inflammatory activity in Crohn's disease (a disease of ubiquitous gastrointestinal involvement) or could reflect a more fundamental discrepancy in pathogenic mechanism between these two forms of inflammatory bowel disease.

In conclusion, our results show that the intraluminal release of leukotriene C₄ and DNA in the unaffected jejunum of patients with quiescent Crohn's disease is enhanced and that its capacity to increase in response to mucosal irritation is preserved. These findings suggest that in Crohn's disease even when the disease is clinically inactive there is a widespread and sustained activation of intestinal proinflammatory mechanisms. These changes may represent fundamental changes in the function of the gut mucosal barrier.

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- Gould S. Prostaglandins, ulcerative colitis and sulfasalazine. *Lancet* 1975; ii: 988.
- Sharon P, Ligumsky M, Rachmilewitz D, Zor U. Role of prostaglandins in ulcerative colitis. Enhanced production during active disease and inhibition by sulfasalazine. *Gastroenterology* 1978; 75: 638-40.
- Ligumsky M, Karmeli F, Sharon P, Zor U, Cohen F, Rachmilewitz D. Enhanced thromboxane A₂ and prostacyclin production by cultured rectal mucosa in ulcerative colitis and its inhibition by steroids and sulfasalazine. *Gastroenterology* 1981; 81: 444-9.
- Lauritsen K, Hansen J, Bytzer P, Bukhave K, Rask-Madsen J. Effects of sulfasalazine and disodium azodisalicylate on colonic PGE₂ concentrations determined by equilibrium in vivo dialysis of faeces in patients with ulcerative colitis and healthy controls. *Gut* 1984; 25: 1271-8.

- Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. Effects of topical 5-aminosalicylic acid and prednisolone on equilibrium in vivo dialysis of rectum in relapsing ulcerative colitis. *Gastroenterology* 1986; 91: 837-44.
- Casellas F, Guarner F, Rodriguez R, Salas A, Tallada N, Malagelada J-R. Abnormal jejunal prostanoid release in response to mucosal irritation in chronic ulcerative colitis. *Eur J Gastroenterol Hepatol* 1991; 3: 667-73.
- Hallgren R, Colombel JF, Dahl R, Fredens K, Kruse A, Jacobsen NO, et al. Neutrophil and eosinophil involvement of the small bowel in patients with celiac disease and Crohn's disease: studies on the secretion rate and immunohistochemical localization of granulocyte granule constituents. *Am J Med* 1989; 86: 56-64.
- Ahrestedt O, Knutson L, Nilsson B, Nilsson-Ekdahl K, Odling B, Hallgren R. Enhanced local production of complement components in the small intestine of patients with Crohn's disease. *N Engl J Med* 1990; 19: 1345-9.
- Knutson L, Ahrestedt O, Odling B, Hallgren R. The jejunal secretion of histamine is increased in active Crohn's disease. *Gastroenterology* 1990; 98: 849-54.
- Casellas F, Guarner F, Rodriguez R, Malagelada J-R. Human jejunal LTC₄ response to luminal irritants. *Eur J Gastroenterol Hepatol* 1991; 3: 393-7.
- Truelove SC, Witts LJ. Cortisone in ulcerative colitis. Final report on a therapeutic trial. *BMJ* 1955; 2: 1041-8.
- Harvey RF, Bradshaw JM. A simple index of Crohn's disease activity. *Lancet* 1980; i: 514.
- Tangerman A, Schaik A, Van Der Hoek EW. Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal subjects. *Clin Chim Acta* 1986; 159: 123-32.
- Harries JT, Sladen GE. The effects of different bile salts on the absorption of fluid, electrolytes and monosaccharides in the small intestine of the rat in vivo. *Gut* 1972; 13: 596-603.
- Teem MV, Phillips SF. Perfusion of the hamster jejunum with conjugated and unconjugated bile acids: inhibition of water absorption and effects on morphology. *Gastroenterology* 1972; 62: 261-7.
- Hyden S. A turbidimetric method for the determination of higher polyethylene glycols in biological materials. *Ann R Agric Coll Sweden* 1955; 22: 139-45.
- Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956; 62: 315-23.
- Smith PL, Montzka DP, McCafferty GP, Wasserman MA, Fondacaro JD. Effect of sulfidopeptide leukotrienes D₄ and E₄ on ileal ion transport in vitro in the rat and rabbit. *Am J Physiol* 1988; 18: G175-83.
- Gieske TH, Sabol JS, Raddatz R, MDL 28.753 an agonist of LTD₄ but an antagonist of LTC₄ in longitudinal muscle of guinea pig ileum. *J Pharmacol Exp Ther* 1990; 254: 192-7.
- Peskar BM, Dreyling KW, Peskar BA, May B, Goebel H. Enhanced formation of sulfidopeptide-leukotrienes in ulcerative colitis and Crohn's disease: inhibition by sulfasalazine and 5-aminosalicylic acid. *Agents Actions* 1986; 18: 381-3.
- Boughton-Smith NK, Hawkey CJ, Whittle BJR. Biosynthesis of lipoxygenase and cyclo-oxygenase products from ¹⁴C-arachidonic acid by human colonic mucosa. *Gut* 1983; 24: 1176-82.
- Sharon P, Stenson WF. Enhanced synthesis of leukotriene B₄ by colonic mucosa in inflammatory bowel disease. *Gastroenterology* 1984; 86: 453-60.
- Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. In vivo effects of orally administered prednisolone on prostaglandin and leukotriene production in ulcerative colitis. *Gut* 1987; 28: 1095-9.
- Peskar BM, Dreyling KW, May B, Schaarschmidt K, Goebel H. Possible mode of action of 5-aminosalicylic acid. *Dig Dis Sci* 1987; 32: 51-6S.
- Zipser RD, Nast CC, Lee M, Kao HW, Duke R. In vivo production of leukotriene B₄ and leukotriene C₄ in rabbit colitis. Relationship to inflammation. *Gastroenterology* 1987; 92: 33-9.
- Perdue MH, Ramage JK, Burget D, Marshall J, Masson S. Intestinal mucosal injury is associated with mast cell activation and leukotriene generation during *Nippostrongylus* induced inflammation in the rat. *Dig Dis Sci* 1989; 34: 724-31.
- LeDuc LE, Nast CC. Chemotactic peptide-induced acute colitis in rabbits. *Gastroenterology* 1990; 98: 929-35.
- Eliakim R, Karmeli F, Rachmilewitz D. Ketotifen effectively prevents mucosal damage in two models of experimental colitis. *Gastroenterology* 1991; 100: A578.
- Beubler E, Juan H. Effect of ribonoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon. *J Pharm Pharmacol* 1979; 31: 681-3.
- Rampton DS, Breuer NF, Vaja SG, Sladen GE, Dowling RH. Role of prostaglandins in bile salt induced changes in rat colonic structure and function. *Clin Sci* 1981; 61: 641-8.
- De Rubertis FR, Craven PA, Saito R. Bile salt stimulation of colonic epithelial proliferation. Evidence for involvement of lipoxygenase products. *J Clin Invest* 1984; 74: 1614-24.
- Karlstrom L. Mechanisms in bile salt-induced secretion in the small intestine. An experimental study in rats and cats. *Acta Physiol Scand* 1986; 126 (suppl 549): 1-48.
- Argenzio RA, Henrikson CK, Lincos JA. Effect of prostaglandin inhibitors on bile salt-induced mucosal damage of porcine colon. *Gastroenterology* 1989; 96: 95-109.
- Robert A. An intestinal disease produced experimentally by a prostaglandin deficiency. *Gastroenterology* 1975; 69: 1045-7.
- Wallace JL, Whittle BJR, Boughton-Smith NK. Prostaglandin protection of rat colonic mucosa from damage induced by ethanol. *Dig Dis Sci* 1985; 30: 866-76.
- Schumert R, Nast CC, Cominelli F, Zipser R. Effects of 16,16-

- dimethyl prostaglandin E₂ and indomethacin on leukotriene B₄ and inflammation in rabbit colitis. *Prostaglandins* 1988; **36**: 565-75.
- 37 Allgayer H, Deschryver K, Stenson WF. Treatment with 16,16-dimethyl prostaglandin E₂ before and after induction of colitis with trinitrobenzenesulfonic acid in rats decreases inflammation. *Gastroenterology* 1989; **96**: 1290-300.
- 38 Redfern JA, Blair AJ, Lee E, Feldman M. Gastrointestinal ulcer formation in rabbits immunized with prostaglandin E₂. *Gastroenterology* 1987; **93**: 744-52.