Gut, 1984, **25**, 1271–1278

Effects of sulphasalazine and disodium azodisalicylate on colonic PGE_2 concentrations determined by equilibrium *in vivo* dialysis of faeces in patients with ulcerative colitis and healthy controls

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SUMMARY The role of arachidonic acid metabolites and the mode of action of 5-aminosalicylic acid, the active moiety of sulphasalazine and disodium azodisalicylate, in ulcerative colitis remain obscure. Therefore, experiments were performed in which the effects of medication on immunoreactive prostaglandin (PG) E_2 concentrations in free faecal water were assessed using the equilibrium *in vivo* dialysis of faeces. Colonic PGE_2 concentrations in patients with active ulcerative colitis (n=11) ranged from 2035–18 000 pg/ml to be compared with a range of 103–188 pg/ml in healthy volunteers (n=10; $p<0{\cdot}001$). In all healthy volunteers PGE_2 concentrations decreased slightly ($p<0{\cdot}05$) after disodium azodisalicylate intake 2 g/day, whereas low dose disodium azodisalicylate (0·25 g/day) caused no change. In patients with ulcerative colitis in complete clinical, sigmoidoscopic, and histologic remission withdrawal of sulphasalazine (2 g/day; n=6) increased PGE_2 concentrations to values above normal levels ($p<0{\cdot}05$) which returned to pretrial values ($p<0{\cdot}05$) on disodium azodisalicylate (2 g/day; n=7). In conclusion, increased PGE_2 in free faecal water indicates an abnormality in the colonic mucosa, even in the absence of conventional signs of inflammation. We could not confirm the hypothesis that sulphasalazine and 5-aminosalicylic acid exert their therapeutic effect through promotion of endogenous cytoprotective prostaglandins. In contrast, the observation that raised PGE_2 concentrations were normalised by disodium azodisalicylate in patients with inactive ulcerative colitis suggests that subclinical disease activity was decreased by 5-aminosalicylic acid.

Both cyclooxygenase and lipoxygenase products of arachidonic acid metabolism have been implicated in the pathogenesis of ulcerative colitis. The findings of increased amounts of prostanoids in faeces and rectal mucosa of patients with ulcerative colitis[1–3] provided the hypothesis that prostaglandins may mediate the inflammatory response. The therapeutic effect of 5-aminosalicylic acid which is proved to be the major, though not necessarily the only active component of sulphasalazine,[4–6] may be related to the anti-inflammatory activity of the substance which is a salicylate and a weak prostaglandin synthesis inhibitor.[1–3] In contrast, the observation of enhanced *in vitro* production of PGE_2 and prostacyclin (PGI_2) by normal colonic mucosa in the presence of 5-aminosalicylic acid, in addition to decreased enzymatic degradation of prostaglandins by sulphasalazine,[7 8] supported the notion that a deficiency of endogenous prostaglandins promotes inflammation and that maintenance therapy with sulphasalazine may rest on cytoprotection.[7]

Thus the mode of action of sulphasalazine remains obscure, although the clinical efficacy of the drug for prevention of relapse in patients with ulcerative colitis is well established.[9 10] It is still not known whether concentrations of sulphasalazine, high enough to inhibit prostaglandin synthesis effectively, can be achieved in the colon after therapeutic doses of this weak cyclooxygenase inhibitor.[11]

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Received for publication 10 January 1984

Furthermore, it has yet to be shown that therapeutically relevant *in vivo* 5-aminosalicylic acid concentrations in free faecal water are capable of enhancing colonic prostaglandin output.[7]

The interpretation of the conflicting data concerning prostaglandins and their possible role in ulcerative colitis has been further complicated by the recent observation of increased *in vitro* leukotriene formation in the inflamed rectal mucosa.[12] Not only the lack of reliable data on colonic concentrations of sulphasalazine and its metabolites, but also the methodological problems concerning determination of prostaglandins in biological material and the choice of an experimental design, which prevents non-specific stimulation of prostaglandin biosynthesis, provide fundamental difficulties to establish the pathophysiological role of prostaglandins.[13]

The present study was designed, therefore, (a) to test the effects on colonic prostaglandins of 5-aminosalicylic acid delivered by sulphasalazine or disodium azodisalicylate,[14–16] which consists of two 5-aminosalicylic acid molecules linked by an azo bond and (b) to evaluate whether 5-aminosalicylic acid might act by enhancing local formation of endogenous prostaglandins. As prostaglandins released into the intestinal lumen appear presently to provide the most reliable index of the balance between mucosal prostaglandin synthesis and degradation *in vivo* we used a slight modification of the equilibrium *in vivo* dialysis of faeces[16 17] for determination of PGE_2 in free faecal water. We chose PGE_2, the major primary prostaglandin in human colon, because this compound is considered to reflect prostaglandin production by enterocytes. To avoid the interference by prostaglandins originating in inflammatory cells, platelets, or endothelial cells the effect of oral sulphasalazine or disodium azodisalicylate on PGE_2 in free faecal water was studied in healthy volunteers and in patients with ulcerative colitis during remission in addition to patients with relapsing ulcerative colitis.

Methods

HEALTHY VOLUNTEERS AND PATIENTS

The study comprised 12 healthy medical students or nurses (six men and six women, aged 22 to 28 years) and eight patients with ulcerative colitis in remission (three men and five women, aged 29 to 64 years) maintained on sulphasalazine 2 g/day. Apart from sulphasalazine, disodium azodisalicylate, and in some cases oral contraceptives, no medication was given the last week before or during the study. The compliance was evaluated by checking the concentration of 5-aminosalicylic acid and Ac-5-aminosalicylic acid (total 5-aminosalicylic acid) in the faecal dialysate at predetermined intervals during each experiment.

In addition, the material included 11 untreated patients (five men and six women, aged 19 to 51 years) with severe ulcerative colitis according to the criteria of Truelove and Witts[18] and assigned to grade III after the classification of Baron *et al.*[19]

A diagnosis of ulcerative colitis had previously been established on the basis of symptoms, sigmoidoscopic and radiologic appearance, and histology of the rectal mucosa. Patients were considered in remission when symptom free – that is, stool frequency $\leq$2/day, without discharge of blood, pus, or mucous from the rectum – and with normal sigmoidoscopic appearance and no significant inflammation on rectal biopsy. Relapse was defined on the basis of symptoms, sigmoidoscopic appearance, and histology.

ETHICS AND SAFETY MEASURES

All participants gave their informed and signed consent. The study was conducted in accordance with the Helsinki Declaration II and approved by the Ethical Committee of Funen and Vejle Counties, Denmark. A laboratory screen included: blood haemoglobin, reticulocyte count, erythrocyte sedimentation rate, leucocyte count, leucocyte differential count, platelet count, and serum concentrations of Na, K, Ca, albumin, creatinine, urea, glutamine oxaloacetic transaminase, lactic acid dehydrogenase, alkaline phosphatase, bilirubin, and in patients with ulcerative colitis, additionally serum concentrations of orosomucoid.

FORMULATION OF DISODIUM AZODISALICYLATE

Disodium azodisalicylate was administered in gelatin capsules, each of which contained 250 mg of the powdered drug without additives.

STUDY DESIGN

Six healthy volunteers took disodium azodisalicylate (1 g bid) for three weeks. Before intake and at day three and 18, equilibrium *in vivo* dialysis of faeces was performed. Disodium azodisalicylate intake was continued until dialysis bags were recovered. Blood was drawn for the laboratory screen before the study and at day three and 18.

Another six volunteers took disodium azodisalicylate, 250 mg each morning, for at least seven days. Before taking disodium azodisalicylate and after one week, dialysis of faeces was carried out and blood was drawn for laboratory control.

In eight patients with inactive ulcerative colitis, on maintenance therapy with sulphasalazine (1 g bid), *in vivo* faecal dialysis was performed before and one

week after withdrawal. Subsequently, treatment with disodium azodisalicylate (250 mg/day) was started. Seven days later *in vivo* faecal dialysis was carried out, the dose of disodium azodisalicylate was increased (1 g bid), and after another seven days the procedure was repeated. Laboratory screen was performed on all study days.

Finally, *in vivo* faecal dialysis was performed in 11 patients with active ulcerative colitis.

EQUILIBRIUM *IN VIVO* DIALYSIS OF FAECES

We used a slight modification[16] of the method originally described by Wrong *et al.*[17] Briefly, dialysis bags (volume 1·0–1·5 ml) of Visking® tubing 8/32 were filled with Rheomacrodex® (Pharmacia, Uppsala, Sweden) containing 10% dextran in saline.

At each study day five bags were swallowed. After their intestinal transit the bags were removed from the stools and cleaned with dry Kleenex tissue. Their contents were emptied in a single test tube, which was stored immediately at −20°C (faecal dialysate) for analysis. Although intestinal transit time for bags varied from 18–72 hours, they were shown radiologically to reach the colon within three hours after swallowing.

To ensure that the conditions for using the concentration of PGE_2 in the dialysate as a measure of the level in free stool water, supplementary *in vitro* studies were carried out. Intact dialysis bags were incubated with [^{3}H] PGE_2 (50 000 cpm/ml) in phosphate buffered saline (pH 7·4; 37°C). After one, three, and five hours, respectively, the radioactivities within the bags were 50%, 80%, and 95% of those measured in the external solution. Binding of PGE_2 to the dialysis membrane was negligible.

The coefficient of variation of PGE_2 concentrations in multiple dialysis bags obtained from the same stool (healthy volunteer; n=5) was less than 10%, and the contents of multiple bags recovered from a single stool were pooled for analysis.

ANALYTICAL PROCEDURES

PGE_2 was measured as previously described in detail[20] by a radioimmunological method validated by gas chromatography mass spectrometry.[21] The method included purification by extraction with ethylacetate/cyclohexane = 1:1 and chromatography on microcolumns of Sephadex® LH-20 (Pharmacia, Uppsala, Sweden) before performing the radioimmunoassay itself on the eluate fraction containing PGE_2. The values were expressed as pg/ml.

5-Aminosalicylic acid, Ac-5-aminosalicylic acid, and disodium azodisalicylate were measured by high-performance liquid chromatography as described elsewhere.[15 16]

STATISTICAL ANALYSES

The results were given as medians with their respective ranges and the data analysed by the Mann-Whitney U test for unpaired variates or Wilcoxon's test for paired variates. A p value less than 0·05 was considered significant.

Results

DRUG TOLERANCE, DROP-OUTS, AND COMPLIANCE

Disodium azodisalicylate was well tolerated by all healthy volunteers and patients with ulcerative colitis and no adverse effects or discomfort were reported. The laboratory screen revealed normal values in all cases.

In two healthy volunteers the amount of faecal dialysate collected during control conditions was insufficient for PGE_2 analysis. Two patients with inactive ulcerative colitis were excluded from the experiments on the effects of sulphasalazine, one because the dialysis bags were not collected during the study period and the other because he had not taken sulphasalazine as prescribed because of nausea. A single patient with inactive ulcerative colitis was excluded from the experiments on the effects of high dose disodium azodisalicylate because symptomatic relapse, verified by sigmoidoscopy and histology, occurred on the second day of this treatment period. She was treated with prednisolone enemas and responded well.

Concentrations of total 5-aminosalicylic acid in faecal dialysates from all healthy volunteers and the remaining patients with ulcerative colitis treated with sulphasalazine 2 g/day, disodium azodisalicylate 0·25 g/day, and disodium azodisalicylate 2 g/day were 0·8–4·3 mg/ml, 0·3–1·4 mg/ml, and 0·7–8·0 mg/ml, respectively, indicating therapeutic concentrations in free faecal water.[16]

PGE_2 CONCENTRATIONS IN HEALTHY VOLUNTEERS AND PATIENTS WITH ACUTE RELAPSING ULCERATIVE COLITIS

Figure 1 illustrates that PGE_2 concentrations were markedly increased ($p<0·001$) in faecal dialysates from all patients with active ulcerative colitis, compared with healthy volunteers. The median value in the group of patients (n=11) was 7540 pg/ml with a range of 2035–18 000 pg/ml, the corresponding value in controls (n=10) was 132 pg/ml with a range of 103–188 pg/ml.

EFFECTS OF DISODIUM AZODISALICYLATE ON PGE_2 CONCENTRATIONS IN HEALTHY VOLUNTEERS

Figure 2 illustrates that PGE_2 concentrations decreased slightly, but significantly ($p<0·05$), in healthy volunteers after 18 days of high dose

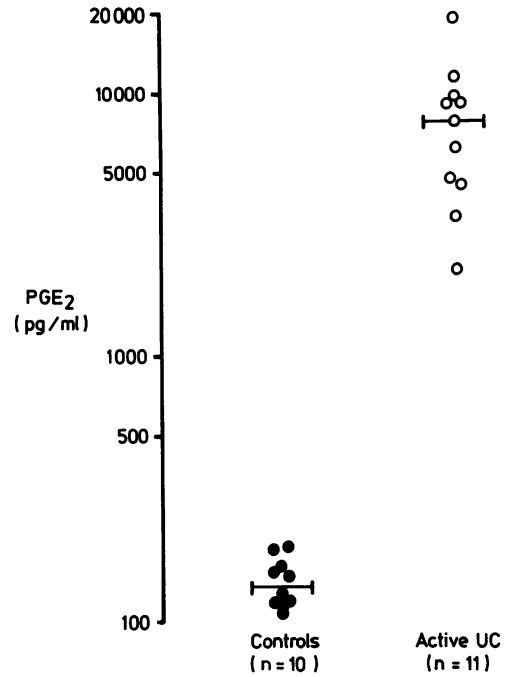

Fig. 1 *PGE_2 concentrations (log scale) in free faecal water following equilibrium* in vivo *dialysis of faeces in healthy volunteers and patients with acute relapsing ulcerative colitis.*

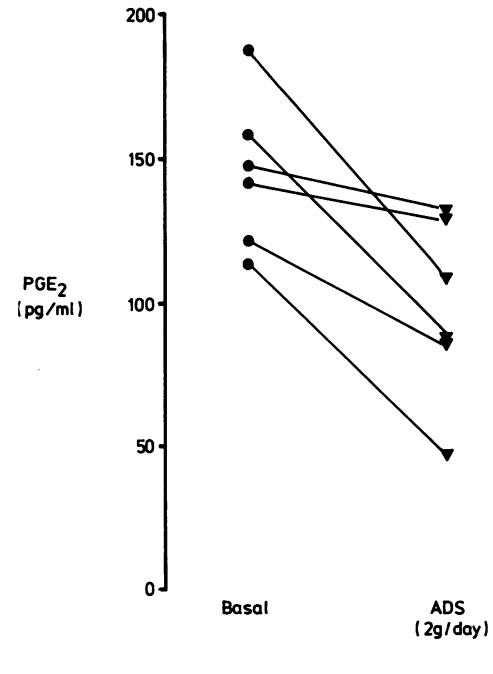

Fig. 2 *PGE_2 concentrations in free faecal water following equilibrium* in vivo *dialysis of faeces in healthy volunteers during control conditions (basal) and following 18 days of high-dose disodium azodisalicylate (2 g/day).*

disodium azodisalicylate (2 g/day) intake. As we observed no significant difference ($p>0.05$) between PGE_2 concentrations after three days (median, 76 pg/ml; range 52–148 pg/ml) and 18 days (median, 98 pg/ml; range 48–132 pg/ml) of disodium azodisalicylate intake, the effect of low dose disodium azodisalicylate (0·25 g/day) was studied at day seven. Low dose disodium azodisalicylate treatment caused no consistent change ($p>0.05$) in PGE_2 concentrations, however, compared with control conditions.

EFFECTS OF SULPHASALAZINE AND DISODIUM AZODISALICYLATE ON PGE_2 CONCENTRATIONS IN PATIENTS WITH INACTIVE ULCERATIVE COLITIS

Figure 3 illustrates that PGE_2 concentrations (n=6) in patients treated with sulphasalazine (2 g/day) were significantly ($p<0.05$) lower than those obtained after withdrawal of sulphasalazine. Low dose disodium azodisalicylate intake caused no significant ($p>0.05$) decrease in PGE_2 concentrations (n=8), the median value being 670 pg/ml with a range of 265–7070 pg/ml. PGE_2 concentrations were unchanged (6910 *vs* 7070 pg/ml) in the patient experiencing symptomatic relapse when she was on low dose disodium azodisalicylate and increased to 10 060 pg/ml during high dose disodium azodisalicylate intake, which caused a marked reduction ($p<0.05$; n=7) in all other patients with abnormally high PGE_2 concentrations. There was no significant ($p>0.05$) difference between PGE_2 concentrations in patients treated with disodium azodisalicylate and sulphasalazine, respectively, although the observed molar ratio of 5-aminosalicylic acid in free faecal water after administration of the two drugs[16] approached the calculated molar ratio of 2·30.

Discussion

The findings that the concentrations of PGE_2 in stool water from patients with active ulcerative colitis are much greater than in health and return to normal concentrations during clinical remission are in agreement with previous studies by Gould and coworkers, who found raised concentrations of

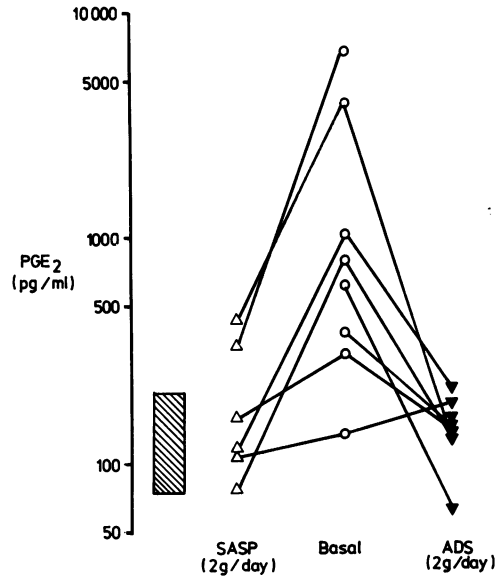

Fig. 3 *PGE_2 concentrations (log scale) in free faecal water following equilibrium* in vivo *dialysis of faeces in healthy controls (shading area; 95% confidence limits), and in patients with inactive ulcerative colitis during treatment with sulphasalazine 2 g/day (△), without treatment (○), and during treatment with disodium azodisalicylate 2 g/day (▽).*

prostaglandin-like material measured by bioassay[1] and radioimmunoassay[22] (PGE_2 20–37 ng/g) in stools from patients with an acute attack of ulcerative colitis and raised urinary excretion of PGF metabolites determined by gas chromatography mass spectrometry.[22] Similarly, numerous *in vitro* studies have shown that the amount of PGE_2[2] and the activities of prostaglandin synthetase[23] measured in fresh biopsies of rectal mucosa, as well as the generation of PGE_2[2] and the stable breakdown products of PGI_2 and thromboxane A_2[3] by cultures of rectal mucosa from patients with acute relapsing ulcerative colitis, were significantly higher than those obtained in material from healthy controls. Finally, Rampton *et al*[24] have observed a 13 fold increase in rectal PGE_2 release in active disease compared with controls and patients in remission using non-equilibrium *in vivo* dialysis. Both the cause and the source of enhanced prostanoid synthesis in ulcerative colitis remain obscure, however, and it should be emphasised that increased prostaglandin formation may simply be the result of non-specific stimulation caused by mucosal damage and that increased amounts of prostaglandins generated may originate in inflammatory cells, platelets, or vessel endothelium, in addition to epithelial cells.[13] The simple atraumatic method of equilibrium *in vivo* dialysis of faeces used in the present study obviates most of the named potential sources of error, when the mucosa is not the site of active inflammation. Although the age range of the controls in the present study differs from that of the patients with ulcerative colitis this defect in composition of the material was not considered important because significant age related differences in prostaglandin concentrations have never been reported.

There has also been considerable discussion as to whether the therapeutic effects of sulphasalazine and/or 5-aminosalicylic acid rely on inhibition of the cyclooxygenase activity, suppression of the activity of prostaglandin-degrading enzymes, or reduction of the lipoxygenase activity. Our results in healthy volunteers suggest that the slight, but significant, reduction in PGE_2 concentrations obtained during high dose disodium azodisalicylate intake represents a weak cyclooxygenase inhibition by 5-aminosalicylic acid, rather than by disodium azodisalicylate which is completely split in the colon.[16] This observation is in agreement with previous *in vitro* studies reporting that 5-aminosalicylic acid inhibits prostaglandin biosynthesis.[2 3 25 26] The drug concentrations used by most investigators,[2 3 7 25] however, were a factor 10–1000 less than those measured in free faecal water in the present *in vivo* study. This point may be important because Hoult and Page,[7] who observed a similar inhibition of prostaglandin synthesis *in vitro* only when using concentrations as measured in the present study, suggested that 5-aminosalicylic acid exerts its therapeutic effect through promotion of endogenous cytoprotection because prostaglandin formation was found to be enhanced in the presence of low or subpharmacological concentrations – that is, 0·007–0·07 mg/ml *vs* 0·8–8·0 mg/ml – of 5-aminosalicylic acid. As the administration of disodium azodisalicylate in low doses in the present study caused concentrations of total 5-aminosalicylic acid also exceeding those leading to increased prostaglandin concentrations *in vitro* the above mentioned low concentrations cannot be considered clinically relevant. Furthermore, it is well established that the optimal therapeutic benefit is proportional to the dose used and as for sulphasalazine is limited only by adverse effects.[27]

Our data showing abnormally high luminal PGE_2 concentrations in untreated patients with ulcerative colitis in complete remission provide direct evidence against the hypothesis that a deficiency of cytoprotective prostaglandins is important in the pathogenesis of ulcerative colitis.[7 11] In this connection it appears important that low dose disodium azodi-

salicylate did not increase local PGE_2 concentrations, which were almost normalised, both by sulphasalazine and by high dose disodium azodisalicylate. Similar observations were made by Gould *et al*[22] in two patients with ulcerative colitis, in whom withdrawal of sulphasalazine was associated with an increased urinary excretion of prostaglandin F metabolites. In addition, Rampton *et al*[28] observed that withdrawal of sulphasalazine in patients with inactive ulcerative colitis had a detrimental effect on rectal fluid and electrolyte absorption, previously reported to be negatively correlated to the release of PGE_2.[24] They were unable to find any statistically significant change in rectal PGE_2 production,[28] however, probably because they used non-equilibrium dialysis, meaning that the bags were removed from the rectum before sufficient concentrations for determination of a true difference were achieved. Finally, administration of the synthetic prostanoid, 15-(R)-15 methyl PGE_2, failed to maintain remission in patients with ulcerative colitis.[29]

Although our results in healthy volunteers indicate that 5-aminosalicylic acid is a weak inhibitor of cyclooxygenase activity, as discussed above, the therapeutic effect of 5-aminosalicylic acid in patients with inactive ulcerative colitis, as reflected by a marked decrease in PGE_2 concentrations, is not necessarily related only to this mode of drug action (Figs. 2 and 3). Thus the PGE_2 concentrations off treatment are much higher in ulcerative colitis than in controls and the apparent inhibitory effect of the 5-aminosalicylic acid during disodium azodisalicylate or sulphasalazine intake appears to be correspondingly greater than in the controls. There are two possible explanations for this. Either inflamed mucosa is in some way more sensitive to inhibition of PGE_2 synthesis by 5-aminosalicylic acid (for which proposition there is no evidence), or the increased synthesis of PGE_2 is a secondary consequence of disease activity which is normalised by 5-aminosalicylic acid. This second possibility seems the most likely and is indirectly supported by the clinical observation that non-steroidal anti-inflammatory compounds, such as indomethacin and flurbiprofen, which are stronger cyclooxygenase inhibitors than 5-aminosalicylic acid and sulphasalazine, are without therapeutic effect in both active and inactive ulcerative colitis.[30–32] In contrast, non-steroidal anti-inflammatory compounds may worsen inflammatory activity,[33] or even provoke a relapse.[34 35] The named clinical effects might be explained by a diversion of arachidonic acid metabolism along the lipoxygenase pathway resulting in enhanced production of potent non-prostaglandin-hydroxyacids and leukotrienes. This mechanism of drug action would explain the paradox of sulphasalazine induced exacerbation of ulcerative colitis,[36] sulphasalazine being a weak cyclooxygenase inhibitor. Thus the lipoxygenase metabolites are potent chemotactic agents which have powerful secretory effects in rabbit colon,[37] at least at supraphysiological concentrations, and constitute major arachidonic acid metabolites produced by colonic mucosa from patients with active ulcerative colitis.[12 38] Both sulphasalazine and 5-aminosalicylic acid block the lipoxygenase pathway in leucocytes,[39] and soybean,[40] but the significance of these observations for the mode of drug action are unresolved as yet and require that the therapeutical effect is shown in controlled clinical trials with selective lipoxygenase inhibitors in patients with ulcerative colitis.

The determination of PGE_2 by equilibrium *in vivo* dialysis of faeces appear to be more sensitive than any other clinical or conventional laboratory method for assessment of disease activity because abnormally high luminal concentrations of PGE_2 were observed, not only in active ulcerative colitis, but also in the absence of inflammation. PGE_2 in free faecal water may, therefore, be used not only as a marker of subclinical disease activity, but also as a predictor of relapse in patients with ulcerative colitis. Furthermore, *in vivo* faecal dialysis may provide a tool for the detection of other arachidonic acid metabolites in ulcerative colitis and their response to treatment.

We would like to thank the volunteers who submitted themselves for study. We are grateful to Pharmacia AB, Uppsala, Sweden, for supplies of disodium azodisalicylate and for performing the drug analyses and also wish to thank Anne Rasmussen and Lene Persson for their valuable technical assistance.

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