

## LETTERS TO THE EDITOR

### Colonic mucus and colitis

SIR,—I found the review on mucus and colitis by J M Rhodes (*Gut* 1989; 30: 1660–6) most interesting, but I was surprised by the absence of several pertinent references. It must surely be relevant that about 8% of the general population show a constitutional lack of the O acetylated, sialidase resistant form of sialic acid.<sup>1,2</sup> These people do not appear to show an increased proneness to ulcerative colitis.<sup>3</sup> Patchy alteration in sialic acid structure (loss of O acetyl groups) is seen in hyperplastic epithelium in colitic biopsy material. Such mucosal alterations are likely to be secondary to inflammatory injury.<sup>3</sup> Thus mucin heterogeneity, whether genetically determined or acquired, appears to be unimportant in the aetiology of ulcerative colitis. Perhaps these observations should be added to the other negative findings catalogued by J M Rhodes. I suggest that they reduce the likelihood of a 'mucus/bacteria' hypothesis.

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- 2 Fuller CE, Davies RP, Williams GT, Williams ED. Crypt restricted loss of sialomucin O-acetylation in the human colon – a marker of somatic mutation. *J Pathol* 1989; 157: 174A.
- 3 Jass JR, Sugihara K, Love SB. Basis of sialic acid heterogeneity in ulcerative colitis. *J Clin Pathol* 1988; 41: 388–92.

### Reply

SIR,—I am sorry that Professor Jass's excellent papers were not referred to in my leading article. This was, however, intended as a presentation of hypotheses to act as stimuli for further studies rather than as a comprehensive review of published work. As pointed out in the article a change in the O acetylation of mucin sialic acids is just one of many potential alterations in mucin structure that could have an effect on its interaction with bacteria. Others include alterations in sulphation, sialylation, length, and branching of oligosaccharide side chains and changes in the expression of carbohydrate receptors for bacterial adherence lectins.

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### Scavenger effect of sulphasalazine (SASP), 5-aminosalicylic acid (5-ASA), and olsalazine (OAZ)

SIR,—We read with interest the paper by Williams and Hallett<sup>1</sup> on the action of SASP and 5-ASA on toxic oxygen metabolite production by neutrophils. The authors conclude by suggesting a scavenger effect is induced by both drugs, as previously reported.<sup>2,4</sup>

We have recently concluded a similar experiment, evaluating the influence of SASP, its

metabolites (sulphapyridine and 5-ASA) and OAZ, on the generation of superoxide anions (O<sub>2</sub><sup>-</sup>) by activated neutrophils and by a cell free xanthine-xanthine oxidase system. Human neutrophils were prepared from heparinised peripheral blood of healthy volunteers by using a combined dextrane/Ficoll-Hypaque separation procedure and hypotonic lysis to remove contaminating erythrocytes. The resulting cells (>95% neutrophils) were washed twice in phosphate buffer (pH 7.4) and then activated using 0.1 µg/ml of phorbol myristate acetate.

The production of O-radicals, generated by the catalysed reaction xanthine oxidase upon xanthine, was induced by incubating 0.05 IU/ml of dialysed xanthine oxidase in 100 mM k-phosphate buffer (pH 7.8) containing 0.1 mM EDTA and 0.5 mM xanthine.

O<sub>2</sub> generation was measured either after spectrophotometrically reducing cytochrome c (cyt c) at 550 nm in a cuvette maintained at 37°C, or monitoring the luminol dependent light emission at 37°C on a Perkin-Elmer luminescence spectrophotometer.

For testing the scavenger effect of SASP and OAZ, we could not use the chemiluminescence method, because of its intense yellow colour in solution, which might have interfered with the light emission. We therefore used the reduction of cyt c assay for evaluating the effect of SASP, OAZ and also for sulphapyridine, but we could not use the same assay for 5-ASA. In fact 5-ASA caused a direct chemical reduction of cyt c effect, already reported by Neal *et al.*<sup>5</sup> Therefore, investigating the action of 5-ASA, we adopted the chemiluminescence method. In our study, 5-ASA, OAZ, and SASP produced a dose dependent inhibition of superoxide anions in both the neutrophils and cell free xanthine-xanthine oxidase system, 5-ASA being the most powerful (>50% of inhibition at 10 µM, the lowest concentration used). In contrast, sulphapyridine showed a dose dependent inhibitory effect on the cellular system, not modifying the activity of xanthine oxidase.

As can be seen, our data are only partly in agreement with the findings of Williams and Hallett. In our opinion, this study is not without certain methodological limitations. The authors, in their experiment, did not report the interference of SASP on light emission, because of its intense yellow colour in solution, therefore the inhibition on chemiluminescent response determined by SASP in their experiment could be partly attributed to the quenching effect on light emission by this drug. Finally, the authors did not highlight the direct chemical reduction provoked by 5-ASA on cyt c, as already mentioned. This, again, might determine a limitation of their results.

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### Reply

SIR,—Gionchetti *et al.*, although providing evidence which supports our contention that sulphasalazine and particularly 5-aminosalicylate are scavengers of a neutrophil derived oxygen metabolite, have questioned two methodological points in our paper.<sup>1</sup>

The first point raised was that the inhibition of luminol dependent chemiluminescence (LDCL) by sulphasalazine was because of its 'intense yellow colour', presumably by absorbing emitted photons, rather than by scavenging a luminol reactive molecule. We do not think the absorbance of sulphasalazine can account for our results for two reasons: (i) at the emission wavelength for luminol (425 nm) in our luminometer (maximum photon path-length 0.8 cm) a concentration of sulphasalazine which inhibited the peptide-induced neutrophil LDCL by 50% (16.5 µM) would reduce detection of photons by a maximum of only 8%; (ii) absorbance of photons alone could not account for the differential effect of sulphasalazine on the LDCL response induced by the peptide f-met-leu-phe and phorbol myristate acetate (PMA) (Fig 1b). At high concentrations of sulphasalazine, however, its absorbance would be expected to cause interference. This probably accounts for part of the small inhibition of the PMA induced response we observed with 50 µM sulphasalazine (expected reduction in photon detection about 20%). The important point, however, is that absorption of photons cannot account for the inhibition by 5-aminosalicylate, as this compound would produce no significant reduction of transmission at concentrations which totally inhibit peptide induced LDCL.

The second point raised concerned the use of cytochrome c reduction as an assay of superoxide production. As cytochrome c readily accepts electrons, only the reduction of cytochrome c which can be inhibited by superoxide dismutase can be defined as being due to superoxide.<sup>2</sup> At the concentrations of 5-aminosalicylate we used (0.5 µM–50 µM) interference was not a problem. Neal *et al.*<sup>5</sup> were unable to use 5-aminosalicylate in this assay but were using considerably higher concentrations of 5-aminosalicylate (up to 1000 µM). The widely recognised problems with cytochrome c reduction<sup>1</sup> led us to measure oxygen consumption also and so determine whether regeneration of oxygen as a result of superoxide scavenging had occurred. As we detected no inhibition of oxygen consumption (nor did Neal *et al.*<sup>5</sup> in concentrations up to 1000 µM) this confirmed our conclusion that 5-aminosalicylate did not scavenge superoxide. We therefore suggested that the inhibition of LDCL by 5-aminosalicylate resulted from reaction with another oxygen metabolite which triggers luminol chemiluminescence, namely hypochlorite.<sup>5</sup> We have also produced more direct evidence for this. In chemically generating hypochlorite systems (xanthine/xanthine oxidase plus peroxidase and peroxidase plus hydrogen peroxide) or hypochlorite alone, the accompanying LDCL was inhibited by 5-aminosalicylate.<sup>6</sup> Furthermore, the fluores-