

Leading article

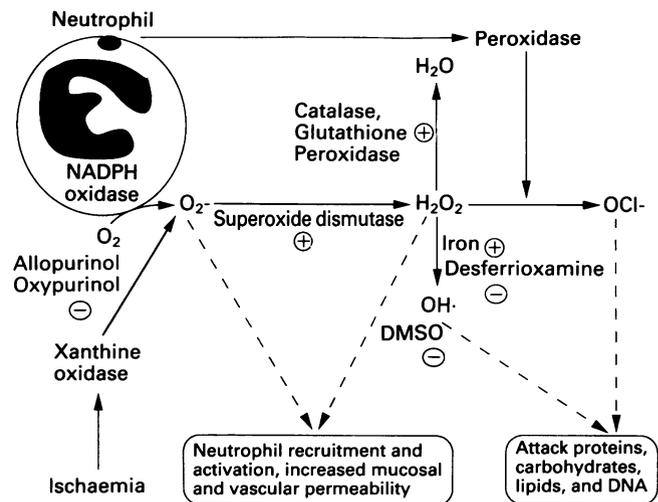
Inflammatory bowel disease – a radical view

Although the aetiology of inflammatory bowel disease remains unknown, its pathogenesis is gradually being unravelled. Increasing attention has been focused recently on the role of free radicals, in both normal metabolism and defence against disease, and also in a wide variety of conditions, for example rheumatoid arthritis, diabetes mellitus, atherosclerosis, pancreatitis, and peptic ulcer, in which oxidant stress seems to exceed homeostatic mechanisms.¹⁻⁴ Free radical activity may contribute to the pathogenesis of both inflammation and cancer. This review examines the evidence that abnormal oxidative metabolism is of central importance to active inflammatory bowel disease. The potential pathogenicity of free radicals has been emphasised by recent work which suggests that reactive oxygen metabolites are not just one of a number of mediators and cytokines involved in the inflammatory process in inflammatory bowel disease but many have a pivotal role by initiating the expression of genes controlling many other aspects of the inflammatory, immune, and acute phase response, by activation of the transcription factor NF- κ B.⁵

Free radical chemistry

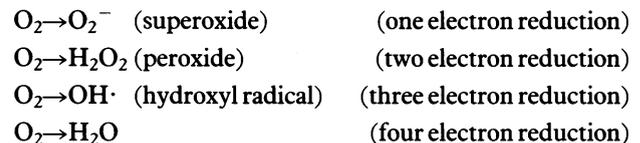
Free radical chemistry is complex and evanescent. A free radical is defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electron being defined as one that is alone in an orbital.⁶ Since electrons are more stable when paired together in orbitals, radicals are generally more reactive than non-radicals. Radicals may react with non-radicals in a number of ways: by donating the unpaired electron, or by abstracting an electron from, or combining with, another molecule. All of these reactions, however, result in the production of another radical and will usually result in a chain reaction. These reactions may be terminated by the interaction with another radical or with one of the 'chain-breaking antioxidant molecules' (such as vitamin E), or by one of the enzymatic antioxidant defences (for example superoxide dismutase, catalase, or glutathione peroxidase). An outline of reactive oxygen metabolite chemistry in biological tissues is given in the Figure.

Oxygen is a prerequisite for the efficient production of energy in aerobic organisms and yet the diatomic oxygen molecules on which we depend are themselves 'diradicals' and major promoters of free radical chemistry. Although oxygen is a good oxidising agent (atom or molecule that accepts electrons from the molecule it oxidises), restrictions on the direction of spin of the electrons it accepts means that

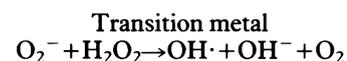


Reactive oxygen metabolites (ROM) in biological tissues. Simplified schematic representation showing the main sources and metabolic pathways involved in the production and removal of ROM. Superoxide dismutase catalyses the conversion of superoxide to hydrogen peroxide and also decreases the amount of iron available for Fenton chemistry. Catalase and glutathione peroxidase catalyse the breakdown of hydrogen peroxide to water. Allopurinol and oxypurinol inhibit xanthine oxidase, DMSO scavenges the hydroxyl radical and desferrioxamine chelates iron. ⊕ indicates catalysis, ⊖ indicates scavenging or inhibition. DMSO=dimethyl sulphoxide.

it usually accepts these one at a time as shown in the following equations:



Transition metals, such as iron, are important promoters of free radical reactions, and in particular of the formation of the extremely reactive hydroxyl radical (OH·) via the Fenton reaction. The overall reaction scheme is as follows:



Superoxide and hydrogen peroxide increase mucosal and vascular permeability, are involved in the recruitment and activation of neutrophils,⁷ and are precursors of the more

damaging hydroxyl radical (via Fenton chemistry) and hypochlorite (via the action of myeloperoxidase released by activated neutrophils). The hydroxyl radical can attack and damage almost every molecule found in living cells, including proteins, carbohydrates, lipids, and DNA. It is for this reason that the production of free radicals is normally tightly controlled and that concentrations of free transition metals are virtually non-existent. Hypochlorite is a powerful oxidant that may react directly against membrane associated targets or indirectly by forming less reactive chloramines that may diffuse across the membrane and attack cytosolic components.⁸ Since neither hydrogen peroxide nor hypochlorite have an unpaired electron (free radical) we shall henceforward use the term 'reactive oxygen metabolites' (ROM) which encompasses all the species so far described.

ROM in inflammatory bowel disease

Several points merit consideration in the evaluation of the pathogenic role of ROM in inflammatory bowel disease. Firstly, even in cell free systems, the measurement of ROM is complicated by rapid reactions and interconversions between them, the wide variety of reactions possible, and the relative non-specificity of many of the methods used to detect ROM and of the scavengers used to identify particular species. These problems are intensified in biological experiments. Secondly, most of the work to date relating to free radicals in inflammatory bowel disease has concentrated on ROM, but these are not the only radicals found in living cells: others, for example nitrogen, carbon, and sulphur centred radicals, may be as important. Lastly, as in the evaluation of the contribution to the inflammatory response of other mediators such as eicosanoids,⁹ it is crucial to assess the pathogenic importance of ROM using the criteria resembling those originally set out by Vane as follows¹⁰:

DETECTION OF ROM AT THE SITE OF INFLAMMATION

Increased mucosal production of ROM related to disease activity has been shown in colorectal biopsy specimens^{11,12} and stimulated mucosal phagocytes¹³ from patients with inflammatory bowel disease compared with controls. Quantification is difficult, but evidence that sufficient ROM are produced to cause mucosal damage is supported by the findings of increased lipid peroxides in rectal biopsy specimens of patients with active ulcerative colitis.¹⁴ Low values of mucosal trypsin inhibitor,¹⁵ superoxide dismutase, metallothionein,¹⁶ and glutathione,¹⁷ and of circulating superoxide dismutase¹⁸ and glutathione peroxidase,¹⁹ and increased activities of circulating leukocyte elastase²⁰ and colorectal mucosal collagenase²¹ may also reflect the activity of ROM in patients with active inflammatory bowel disease.

What are the sources of ROM production in the gut? There are two main candidates (Figure). Firstly, phagocytes are prominent in the mucosa of patients with active inflammatory bowel disease and can produce ROM via both the respiratory burst and prostaglandin and leukotriene metabolism.^{13,22} Secondly, xanthine oxidase, which produces superoxide, is formed from xanthine dehydrogenase during ischaemia and is implicated in the pathogenesis of ischaemia-reperfusion injury³; this seems a likely route of ROM production in Crohn's disease if multifocal gastrointestinal infarction does indeed contribute to its pathogenesis.²³ Conversion of xanthine dehydrogenase to xanthine oxidase, however, can also take place in the presence of various pro-inflammatory substances such as formyl-leucyl-methionyl phenylalanine (FMLP) and tumour necrosis factor alpha, and can be induced by activated neutrophils.²⁴ Superoxide and hydrogen peroxide are not only precursors of more harmful substances, but, by increasing mucosal perme-

ability²⁵ and recruitment and activation of further neutrophils,⁷ may help establish a vicious cycle of inflammation and tissue damage.

PRODUCTION OF MUCOSAL DAMAGE BY ROM

Stimulated neutrophils are cytotoxic to cells *in vitro*.²⁶⁻²⁹ Hydrogen peroxide has been used as an enema or as a cleaning agent for endoscopes and may cause mucosal damage when applied to the surface of the gut wall. 'Hydrogen peroxide enteritis' can mimic an acute ulcerative, ischaemic, or pseudomembranous colitis and ranges from a reversible, clinically silent process to an acute, toxic fulminant colitis associated with perforation and death.³⁰ In anecdotal reports of exacerbation of inflammatory bowel disease by iron supplementation, this may be mediated by hydroxyl radical production by the Fenton reaction. Millimolar concentrations of stable oxidants (H₂O₂, OCl⁻, and monochloramine) cause a dose dependent increase in mucosal permeability in animal models and are toxic to intestinal epithelial cells.²⁵ Whether these concentrations of ROM resemble those found in the mucosa in inflammatory bowel disease *in vivo* is unclear. An animal model of colitis, however, has been developed recently using the free radical initiator, 2,2'-azobis(2-amidinopropane) dihydrochloride³¹ and trinitrobenzene sulphonic acid, which also induces colitis experimentally and is metabolised to superoxide and hydrogen peroxide by rat colon homogenates or isolated colonocytes.³²

There is considerable interest in the relation between oxidant stress and the development of cancer.³³ While there is no doubt that ROM can cause oxidative DNA damage leading to base changes, strand breaks, and enhanced expression of proto-oncogenes, and that oxidative stress can induce malignant transformation in cell culture,³⁴ the relation between these observations and the development of malignancy *in vivo* is more complex and will depend on other factors such as the rate of damage, antioxidant defences, DNA repair mechanisms, and the necessity for multiple steps (initiation, promotion, and progression). The constitutive and oxidant induced activity of adenosine diphosphate ribosyl transferase (ADPRT), an enzyme involved in DNA repair, is reduced in patients with inflammatory bowel disease and also in those with colon cancer.³⁵ This may be the result of genetic variation in factors determining the redox cycling of reduced glutathione,³⁶ and correlates well with the low glutathione values found in the colonic mucosa of patients with inflammatory bowel disease.¹⁷ Although the finding of oxidative DNA damage is remote from establishing a causal link between excess ROM production and cancer in patients with inflammatory bowel disease, it does suggest further possible lines of research.

PREVENTION OR AMELIORATION OF MUCOSAL DAMAGE BY AGENTS WHICH PREVENT THE RELEASE OR BLOCK THE EFFECTS OF ROM

Agents blocking the release or effects of ROM, for example, xanthine oxidase inhibitors, superoxide dismutase or its mimetic WR 2721, catalase, the glutathione peroxidase mimetic PZ 51, dimethyl sulphoxide, and desferrioxamine (Figure), all decrease inflammation in animal or intestinal cell culture models of colitis,^{28,29,37-41} and preliminary studies show promise for the use of allopurinol in pouchitis⁴² allopurinol and DMSO in ulcerative colitis⁴³ and superoxide dismutase injections in refractory Crohn's disease.⁴⁴

The aminosalicylates are far less specific agents, and it is not known which of their many pharmacological actions explains their therapeutic effect in inflammatory bowel disease.⁴⁵ The aminosalicylates scavenge ROMs *in vitro* at low

concentrations (IC_{50} for scavenging superoxide 10–20 μM)⁴⁵ and *in vivo*.¹⁴ They also inhibit myeloperoxidase and chelate iron at low concentrations (IC_{50} 20 μM and 300 μM respectively).⁴⁵ These concentrations are of the same order as those found in the plasma of patients taking 5-ASA preparations orally (10–15 μM)⁴⁶ and are far lower than those required to inhibit cyclo-oxygenase, lipoxygenase, or the binding of FMLP to neutrophils (IC_{50} 10, 6, and >5 mM respectively)⁴⁵ or those found within the colonic lumen of these patients (10–20 mM).⁴⁶ The finding of 5-ASA metabolites identical to those formed by the reaction of 5-ASA with the hydroxyl radical in extracts of faeces from patients with inflammatory bowel disease treated with sulphasalazine, but not in those with rheumatoid arthritis also receiving the drug, suggests that ROM scavenging is a clinically important mechanism of action in inflammatory bowel disease.¹⁴

One of the arguments used against a significant ROM scavenging role for the aminosaliculates is that 4-ASA, while clinically effective, is less efficient in scavenging ROM than 5-ASA.⁴⁷ While this is certainly true for some radicals, both 4-ASA and 5-ASA scavenge hypochlorite and inhibit myeloperoxidase at low concentrations.⁴⁵ Indeed, hydroxyl radical scavenging is an unlikely mode of action *in vivo* for most drugs because the drugs are never present in tissues at sufficiently high concentrations to compete with biological molecules for the hydroxyl radicals generated *in vivo*.⁴⁸ Whether the ability to chelate free iron and so prevent the formation of the hydroxyl radical is important remains to be seen.

Conclusions

It is now clear that ROM are produced in excess in active inflammatory bowel disease. That they are rather more than irrelevant epiphenomena, at least in experimental colitis, is indicated by the anti-inflammatory effects of specific antioxidants. While the known antioxidant actions of the aminosaliculates are compatible with the proposal that ROM play a major role in the pathogenesis of inflammatory bowel disease, proof of the hypothesis must await the outcome of controlled trials in the human disease of more specific agents that interfere with oxidative metabolism. This treatment may prove valuable not only in ameliorating the inflammatory response, but also in chronic ulcerative colitis, and it may even find a place in preventing colonic cancer.

Samuel Butler declared a century ago that 'few radicals have good digestions'.⁴⁷ We believe that this axiom should now be updated: 'excess radicals – have bad digestion'.

N J SIMMONDS
D S RAMPTON

Gastrointestinal Science Research Unit,
The London Hospital Medical College,
26 Ashfield Street, London E1 2AJ.

- Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, et al. Oxygen radicals and human disease. *Ann Intern Med* 1987; 107: 526–45.
- Braganza JM. Towards antioxidant therapy for gastrointestinal disease. *Current Medical Literature in Gastroenterology* 1989; 8: 99–106.
- Parks DA, Bulkley GB, Granger DN. Role of oxygen-derived free radicals in digestive tract diseases. *Surgery* 1983; 94: 415–22.
- Davies GR, Simmonds NJ, Stevens TRJ, Grandison A, Blake DR, Rampton DS. Mucosal reactive oxygen metabolite production in duodenal ulcer disease. *Gut* 1992 (in press).
- Schreck R, Rieber P, Bauerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF- κ B transcription factor and HIV-1. *EMBO J* 1991; 10: 2247–58.
- Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. 2nd ed. Oxford: Clarendon Press, 1989.
- Grisham MB, Granger DN. Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. *Dig Dis Sci* 1988; 33: 6S–15S.
- Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989; 320: 365–76.
- Hawkey CJ, Rampton DS. Prostaglandins and the gastrointestinal mucosa: are

- they important in its function, disease, or treatment? *Gastroenterology* 1985; 89: 1162–8.
- Vane JR. Prostaglandins in the inflammatory response. In: Lepow IH, Ward PA, eds. *Inflammation: mechanisms and control*. New York: Academic Press, 1972: 261–79.
 - Simmonds NJ, Allen RE, Stevens TRJ, van Someren RNM, Blake DR, Rampton DS. Chemiluminescence assay of reactive oxygen metabolites in inflammatory bowel disease. *Gastroenterology* 1992; 103: 186–96.
 - Keshavarzian A, Sedghi S, Kanofsky J, List T, Robinson C, Ibrahim C, Winship D. Excessive production of reactive oxygen metabolites by inflamed colon: analysis by chemiluminescence probe. *Gastroenterology* 1992; 103: 177–85.
 - Williams JG. Phagocytes, toxic oxygen metabolites and inflammatory bowel disease: implications for treatment. *Ann R Coll Surg Engl* 1990; 72: 253–62.
 - Ahnfelt-Ronne I, Nielsen OH, Christensen A, Langholz E, Binder V, Riis P. Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. *Gastroenterology*. 1990; 98: 1162–9.
 - Playford RJ, Freeman T, Quinn C, Calam J. Deficiency of a mucosal trypsin inhibitor in patients with ulcerative colitis. *Gut* 1990; 31: A1166.
 - Mulden TPJ, Verspaget HW, Janssens AR, de Bruin PAF, Pena AS, Lamers CBHW. Decrease in two intestinal copper/zinc containing proteins with antioxidant function in inflammatory bowel disease. *Gut* 1991; 32: 1146–50.
 - Inauen W, Bilzer M, Rowedder E, Halter F, Lauterburg BH. Decreased glutathione (GSH) in colonic mucosa of patients with inflammatory bowel disease: mediated by oxygen free radicals? *Gastroenterology* 1988; 94: A199.
 - Verspaget HW, Pena AS, Weterman IT, Lamers CBHW. Diminished neutrophil function in Crohn's disease and ulcerative colitis identified by decreased oxidative metabolism and low superoxide dismutase content. *Gut* 1988; 29: 223–8.
 - Burdelski M, Oellerich M, Pippenger CE, Meng X, Rodeck B, Latta A, Kucher K. Free radical scavenging enzyme activities in erythrocytes of paediatric patients with Crohn's disease. In: Williams CJ, ed. *Trends in inflammatory bowel disease therapy*. Lancaster: Kluwer Academic Publishers, 1990: 453–4.
 - Adeyemi EO, Neumann S, Chadwick VS, Hodgson HJF, Pepys MB. Circulating human leucocyte elastase in patients with inflammatory bowel disease. *Gut* 1985; 26: 1306–11.
 - Sturzaker HG, Hawley PR. Collagenase activity in rectal and colonic biopsies. *Proc R Soc Med* 1975; 68: 33.
 - Blake DR, Allen RE, Lunec J. Free radicals in biological systems – a review oriented to inflammatory processes. *Br Med Bull* 1987; 43: 371–85.
 - Wakefield AJ, Dhillon AP, Rowles PM, Sawyerr AM, Pittilo RM, Lewis AAM, Pounder RE. Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. *Lancet* 1989; ii: 1057–62.
 - Friedl HP, Till GO, Ryan US, Ward PA. Mediator-induced activation of xanthine oxidase in endothelial cells. *FASEB J* 1989; 3: 2512–8.
 - Grisham MB, von Rotter C, Granger DN. The effects of neutrophil-derived oxidants on ileal mucosal permeability and epithelial cell viability. *Gastroenterology* 1989; 98: A186.
 - Dull BJ, Salata K, Van Langenhove A, Goldman P. 5-aminosalicylate: oxidation by activated leukocytes and protection of cultured cells from oxidative damage. *Biochem Pharmacol* 1987; 36: 2467–72.
 - Dallegrì F, Ottonello L, Ballestrero F, Ferrando F, Patrone F. Cytoprotection against neutrophil derived hypochlorous acid: a potential mechanism for the therapeutic action of 5-aminosalicylic acid in ulcerative colitis. *Gut* 1990; 31: 184–6.
 - Baker SS, Campbell CL. Rat enterocyte injury by oxygen-dependent processes. *Gastroenterology* 1991; 101: 716–20.
 - Ma TY, Hollander D, Freeman D, Nguyen T, Krugliak P. Oxygen free radical injury of IEC-18 small intestinal epithelial cell monolayers. *Gastroenterology* 1991; 100: 1533–43.
 - Bilotta JJ, Waye JD. Hydrogen peroxide enteritis: the 'Snow White' sign. *Gastrointest Endosc* 1989; 35: 428–30.
 - Tamai H, Levin S, Gaginella TS. Induction of colitis in rats by 2–2'-azobis (2-amidinopropane) dihydrochloride. *Inflammation* 1992; 16: 69–81.
 - Grisham MB, Volkmer C, Tso P, Yamada T. Metabolism of trinitrobenzenesulfonic acid by the rat colon produces reactive oxygen species. *Gastroenterology* 1991; 101: 540–7.
 - Weitzman SA, Gordon LI. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood* 1990; 76: 655–63.
 - Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. *FEBS Lett* 1991; 281: 9–19.
 - Markowitz MM, Rozen P, Pero RW, Tobi M, Miller DG. Hydrogen peroxide induced adenosine diphosphate ribosyl transferase (ADPRT) response in patients with inflammatory bowel disease. *Gut* 1988; 29: 1680–6.
 - Pero RW, Roush GC, Markowitz MM, Miller DG. Oxidative stress, DNA repair and cancer susceptibility. *Cancer Detect Prev* 1990; 14: 555–61.
 - Fretland DJ, Widmoski DL, Anglin CP, Walsh RE, Levin S, Riley D, Weiss RH, Gaginella TS. Superoxide dismutase (SOD) modulates acetic-acid induced colitis in rodents. *Gastroenterology* 1991; 100: A581.
 - Burakoff R, Zhao L, Joseph I, Koo H, Rosenfeld W. SOD prevents colitis and attenuates eicosanoid release and motility changes in a trinitrobenzenesulfonic acid rabbit colitis. *Gastroenterology* 1991; 100: A565.
 - Keshavarzian A, Doria MI, Sedghi S, Kanofsky JR, Hecht D, Holmes EW, et al. Mitomycin-C-induced colitis in rats – a new model of acute colonic inflammation implicating reactive oxygen species. *J Lab Clin Med* 1992; 120: 778–91.
 - Keshavarzian A, Haydek J, Zabih R, Doria M, D'Astice M, Sorenson JRJ. Agents capable of eliminating reactive oxygen species: catalase, WR-2721, or, Cu(II)₂(3,5-DIPS)₄ decrease experimental colitis. *Dig Dis Sci* 1992 (in press).
 - Keshavarzian A, Morgan G, Sedghi S, Gordon JH, Doria M. Role of reactive oxygen metabolites in experimental colitis. *Gut* 1990; 31: 786–90.
 - Levin KE, Pemberton JH, Phillips SF, Zinsmeister AR, Pezim ME. Role of oxygen free radicals in the aetiology of pouchitis. *Dis Colon Rectum* 1992; 35: 452–6.
 - Saum AS. Role of oxygen-delivered free radical scavengers in the management of recurrent attack of ulcerative colitis: a new approach. *J Lab Clin Med* 1992; 119: 710–7.
 - Emerit J, Pelletier S, Tosoni-Verlignue D, Mollet M. Phase II trial of copper

- zinc superoxide dismutase (CuZnSOD) in treatment of Crohn's disease. *Free Radic Biol Med* 1989; 7: 145-9.
- 45 Yamada T, Volkmer C, Grisham MB. Antioxidant properties of 5-aminosalicylic acid: potential mechanism for its anti-inflammatory activity. In: Williams CJ, ed. *Trends in inflammatory bowel disease therapy*. Lancaster: Kluwer Academic Publishers, 1990: 73-84.
- 46 Christensen LA, Fallingborg J, Abildgaard KA, Jacobsen BA, Sanchez G, Hansen SH, *et al.* Topical and systemic availability of 5-aminosalicylate comparisons of three controlled release preparations in man. *Aliment Pharmacol Therap* 1990; 4: 523-33.
- 47 Ireland A, Jewell DP. Mechanism of action of 5-aminosalicylic acid and its derivatives. *Clinical Science* 1990; 78: 119-25.
- 48 Halliwell B. Free radical scavengers and inflammatory bowel disease. *Gastroenterology* 1991; 101: 872.
- 49 Butler S. Mind and Matter. Indigestion. In: Festing Jones H, ed. *The notebooks of Samuel Butler* 1912.