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# SPECIAL REPORTS AND REVIEWS

## Reactive Oxygen and Nitrogen Metabolites as Mediators of Secretory Diarrhea

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Secretory diarrhea is a common symptom associated with inflammation of the intestinal mucosa, regardless of the factor(s) responsible for initiation of the inflammation.[1] Reactive metabolites of oxygen and nitrogen, through oxidation and free radical reactions, are believed to contribute to the diarrhea by acting as secretagogues.[2] The purpose of this report is to succinctly review the evidence in support of the probability that highly reactive oxygen- and nitrogen-containing molecules produced in the inflamed intestinal mucosa contribute to secretory diarrhea.

Patients with ulcerative colitis can have fecal excretion weights of 234 to as much as 1500 g/day,[3–5] thus exceeding values that define diarrhea clinically. Harris and Shields[6] and Head et al.[7] reported that the colon of patients with proctocolitis and Crohn's disease, respectively, absorbed water and electrolytes at a reduced rate compared with healthy subjects. Similar findings were noted by other investigators in studies in vivo[8–11] and in vitro.[12,13] Impaired fluid absorption in the jejunum occurs in patients with ulcerative colitis,[14] and the colonic mucosa from infants with colitis secretes NaCl when studied in vitro.[15]

It is not possible from studies in patients with inflammatory bowel disease (IBD) to define with certainty the cause of the intraluminal fluid accumulation. There is little compelling evidence from human studies that active electrolyte secretion, rather than inhibition of absorption in the small bowel or colon, causes the diarrhea.[16] The apparent reduction of sodium absorption may be due to electrolyte movement from the blood to the lumen. Studies in vivo, using $^{22}Na$ and $^{36}Cl$ or other suitable radiolabeled solutes, have not been performed in patients to confirm a mechanism of serosal-to-mucosal transport (i.e., active secretion). Activation of a secretory flux has been inferred, mostly from in vitro short-circuit current studies in which inflammatory mediators evoke secretion in normal mucosa.[17,18] However, in inflamed mucosa, the secretory response is less vigorous than in healthy tissue.[19] Thus, additional factors, such as increased mucosal permeability[2,16,20–23] and interference with $Na^+,K^+$–adenosine triphosphatase and epithelial cell absorptive processes,[24] probably contribute to luminal fluid accumulation in IBD. As for potential secretagogues, a plethora of candidates is possible, including neuropeptides,[25] kinins,[26] C5a,[27,28] platelet activating factor,[29] biogenic amines, cytokines, eicosanoids, and reactive metabolites of oxygen and nitrogen.[18,30,31]

Other inflammatory conditions of the gut are also associated with secretory diarrhea. For example, patients with food allergy often present with diarrhea, presumed to be primarily caused by intestinal secretion.[30] The mucosal response is associated with an eosinophilic enteritis.[30,32] Rodent models have been developed based on immediate hypersensitivity reactions.[33] On challenge, sensitized animals show a mucosal inflammatory response characterized by increased permeability,[34] inhibition of electrolyte absorption, or stimulation of secretion.[30,35–37] It is unlikely that a single mediator is responsible for these effects.

There are a variety of factors associated with induction of an immune response by the intestinal mucosa. Most obvious among these are enteric infections. *Salmonella, Shigella*, and *Escherichia coli* (O157:H7) infections are especially associated with mucosal inflammation and secretory diarrhea.[38] *E. coli* O157:H7 reduces sodium absorption and stimulates secretion of chloride ions in the distal colon of rabbits, and these effects are related to infiltration of the tissue by neutrophils.[39] The parasite *Trichinella spiralis* also initiates an inflammatory response in the mucosa, which has been well characterized and serves as a model for investigating immune-mediated intestinal secretion in rats.[37] In rabbits infected with *Yersinia enterocolitica,* inhibition of short-chain fatty acid absorption due to reduced $Na^+$ uptake ($Na^+/H^+$ exchange) was sug-

*Abbreviations used in this paper:* AAPH, 2,2′-azo-bis (2-amidinopropane) dihydrochloride; cNOS, constitutive nitric oxide synthase; iNOS, inducible nitric oxide synthase; L-NAME, $N^G$-nitro-L-arginine methyl ester; ROM, reactive oxygen metabolites; SNP, sodium nitroprusside.


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gested to limit colonic cell energy supply and contribute to fluid loss in this acute colitis.[40] The etiopathophysiology of infectious diarrhea fits the emerging paradigm for the role of immunocyte products as secretagogues.[41]

Pelvic irradiation is another cause of mucosal inflammation, malabsorption, and diarrhea. Infiltration of inflammatory cells, edema, and epithelial cell loss can occur in up to 75% of irradiated patients.[42,43] Radiotherapy to the pelvis is associated with an increase in the mucosal release of the eicosanoids leukotriene $B_4$, thromboxane $B_2$, and prostaglandin $E_2$.[42] Goodner et al.[44] recognized that whole body irradiation of rats caused the movement of electrolytes and water from the blood into the intestinal lumen and impaired absorption. Using a more direct method of intestinal perfusion, other authors confirmed that radiation interferes with the normal absorptive processes in the small intestine.[45] The radiation may damage crypt cells, preventing their migration and maturation into absorptive cells. In the rabbit ileum, serosal-to-mucosal flux of chloride has been shown after gamma irradiation.[46] Radiation-induced changes in responsiveness of rat ileal mucosa to neural stimulation have been linked to decreased mast cell histamine content, but this occurred in the absence of obvious inflammation.[47]

## Biological Oxidants and IBD

There is substantial evidence for the central importance of free radicals such as nitric oxide and other reactive oxygen metabolites (ROM) in IBD.[48–50] Support for this concept comes from clinical research and secondary studies in animal models of IBD. Monocytes from patients with Crohn's disease[51] and polymorphonuclear leukocytes from patients with ulcerative colitis[52] have an increased capacity to generate ROM. Macrophages are also increased in the mucosa of patients with active IBD, and they undergo more robust respiratory burst activity than macrophages isolated from normal mucosa.[53,54] Likewise, monocytes from patients with Crohn's disease are more sensitive to induction or respiratory burst by bacterial wall products than normal cells.[55] Other investigators extended the findings in isolated inflammatory cells to mucosal biopsy specimens from patients with IBD.[56]

### Sources of Free Radicals and Oxidants

Superoxide radical ($O_2^{\bullet-}$) is pivotal in the formation of other oxidants that have greater stability and are more potent. Dismutation of $O_2^{\bullet-}$ to $H_2O_2$ yields HOCl (hypochlorous acid) in the presence of myeloperoxidase and chloride ions.[48] The source of $O_2^{\bullet-}$ is the inflammatory cells. Superoxide dismutase catalyzes the conversion of $O_2^{\bullet-}$ to $H_2O_2$ (which is metabolized by catalase to $H_2O$ and $O_2$). Myeloperoxidase catalyzes the formation of HOCl from $Cl^-$ and $H_2O_2$. In the presence of primary amines or ammonia (present in the gut from products of digestion and bacterial metabolism), *N*-chloramines such as monochloramine ($NH_2Cl$) are generated by reaction with HOCl (Figure 1).

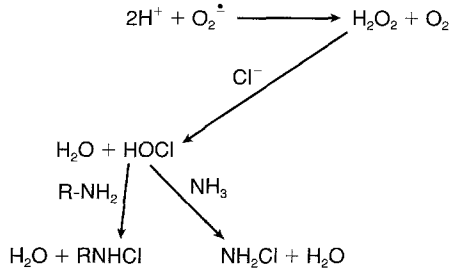


**Figure 1.** Reactions leading to production of *N*-chloramines.

The estimated reactive half-lives of these radicals are 10–20 seconds for $O_2^{\bullet-}$ and 1.0 nanosecond for $^{\bullet}OH$. $H_2O_2$, HOCl, and RNHCl are considered to be "stable" in the absence of catabolic enzymes or antioxidants.[49] Histamine chloramine (formed from histamine plus HOCl) has effects when added exogenously to the gut,[57,58] but it has not been proven that this substance is formed in the inflamed intestine.

$NO^{\bullet}$ is synthesized by endothelial cells, neurons, macrophages, and epithelial cells[59–61] and participates in the immune response.[62] The effects of this free radical are both beneficial and detrimental to the mucosa.[63–66] It is formed in picomole amounts by many types of cells from the action of a calcium/calmodulin–dependent constitutive $NO^{\bullet}$ synthase (cNOS), but on stimulation by toxins such as bacterial lipopolysaccharides, nanomole amounts are produced by an inducible form of the enzyme (iNOS).[49,59] cNOS and iNOS are inhibited by $N^G$-nitro-L-arginine methyl ester (L-NAME), $N^G$-monomethyl-L-arginine, and $N^G$-nitro-L-arginine. Because $NO^{\bullet}$ is unstable in biological systems (3–5 seconds), its by-products, L-citrulline, $NO_2^-$ and $NO_3^-$, are commonly measured.

### Studies in Humans

Roediger et al.[67] detected increased nitrite levels in the colonic dialysate of patients with ulcerative colitis, providing indirect evidence that $NO^{\bullet}$ may be produced in the mucosa of patients with IBD. Even though $NO^{\bullet}$ may have protective effects on the mucosa under certain circumstances,[64–66] it can also lead to epithelial cell injury.[61,63,64] Other ROM derived from or produced by the metabolism of $NO^{\bullet}$, such as peroxynitrite, may have effects on epithelial cell membrane lipid peroxidation.[68,69] This could alter normal physiological regulation