rhodamine- and fluorescein-conjugated antibodies and its retardation by *n*-propyl gallate are still unclear. However, the similar protective effects by three oxygen-reducing compounds, *n*-propyl gallate, ascorbic acid, and *p*-phenylenediamine, and a similar effect of dithionite on fluorescein photobleaching in a model system (*16*) suggest that molecular oxygen is involved in the reactions that lead to the photobleaching.

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15. As an additional atmospheric oxygen-reducing compound, we tested ascorbic acid (0.1*M* to 0.2*M*) in citrate-buffered glycerol (*p*H 5.5 to 6.5). Ascorbic acid reduced by a factor of 3 to 5 the rate of photobleaching of both rhodamine and fluorescein. *p*-Phenylenediamine, applied at *p*H 8.3 as recently described (*16*), showed a similar three- to fivefold decrease in the rate of photobleaching of fluorescein. However, the latter compound did not prevent photobleaching of rhodamine, nor was it effective at acid *p*H.
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Stimulation of Colonic Secretion by Lipoxygenase Metabolites of Arachidonic Acid

Abstract. *Both 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE) increased the short-circuit current (I_{sc}) in rabbit colonic mucosa mounted in vitro in Ussing chambers. Measurements of chlorine-36 fluxes indicated that the I_{sc} response to 5-HPETE is due to stimulation of active chlorine secretion. 9-, 11-, and 12-HPETE's and leukotrienes C_4 and B_4 produced either very small increases in I_{sc} or no increase. In contrast to results in rabbit colon, no HPETE, HETE, or leukotriene was effective in rabbit ileal mucosa. The effects of 5-HPETE in the rabbit colon were unaffected by mepacrine, but could be partially blocked by indomethacin. These results suggest that drugs which block both cyclooxygenase and lipoxygenase may be effective antidiarrheals in patients with colitis.*

Diarrhea frequently develops when there is inflammation in the mucosa of the small or large bowel, whether the inflammation is acute, as in infectious enteritis (*1*), or chronic, as in inflammatory bowel disease (IBD) (*2*). One possible basis for the diarrhea is the action of inflammatory mediators on the intestinal epithelium. We previously showed that kinins, known to be produced in inflammatory lesions, stimulate secretion in both small and large intestine (*3*). Kinin receptors occur on intestinal enterocytes where they stimulate the production of prostaglandin E_2 (PGE_2), which stimulates electrolyte secretion (*3*). Kinin-induced secretion can be inhibited by drugs that inhibit PGE_2 production either by blocking cyclooxygenase (for example, indomethacin) or phospholipase A_2 (for example, mepacrine). In white cells found at inflammatory sites, however, the major route of arachidonic acid metabolism is via the lipoxygenase pathway, resulting in the release of large amounts of hydroperoxyeicosatetraenoic acids (HPETE's), hydroxyeicosatetraenoic acids (HETE's), and leukotrienes (*4*). These products affect many physiological systems including smooth muscle contractions and chemotaxis (*5*).

We report here that 5-HPETE and 5-HETE have powerful secretory effects in rabbit colon, but not ileum, suggesting that lipoxygenase products contribute to diarrhea in patients with inflammatory lesions of the colon. If this is the case, indomethacin, which blocks cyclooxygenase but not lipoxygenase (*6*), should not be an effective antidiarrheal in such patients. Indeed, clinical trials with indomethacin have not been successful (*7*). However, drugs that inhibit lipoxygenase as well as cyclooxygenase or that inhibit arachidonic acid release may be therapeutic, at least with respect to diarrhea. Since lipoxygenase products also induce chemotaxis (*4*), such drugs may also reduce inflammation. Steroids that act indirectly to inhibit phospholipase A_2 and arachidonate release are certainly effective in IBD (*8*). Nonsteroidal antiinflammatory drugs such as eicosatetraynoic acid (ETYA) (*9*) and BW755 (*6*), which inhibit both lipoxygenase and cyclooxygenase may be worth testing in patients with colitis and diarrhea.

Colonic and ileal mucosa from male New Zealand White rabbits was stripped of both muscle layers and mounted in Ussing chambers. Transepithelial potential difference (PD), short-circuit current (I_{sc}), and unidirectional Cl^- fluxes were measured as described (*10*). Serosal addition of either 5-HPETE or 5-HETE (*11*) to distal colon caused a rapid increase in PD which slowly returned to baseline (half-time decline in PD was about 80 minutes). The average tissue conductance also increased and, therefore, the change in I_{sc} was relatively greater than the change in PD. Effects of both 5-HPETE and 5-HETE were observed with concentrations as low as $5 \times 10^{-7}M$. A maximally effective concentration of 5-HPETE ($1.6 \times 10^{-5}M$) increased I_{sc} by about 120 $\mu A/cm^2$, whereas 5-HETE ($1.6 \times 10^{-5}M$) increased I_{sc} by about 90 $\mu A/cm^2$ (Fig. 1).

In contrast neither 5-HPETE nor 5-HETE increased I_{sc} when added to rabbit ileal mucosa. Other HPETE analogs produced little effect in either colon or ileum. Small increases in I_{sc} were observed in the colon with 11-HPETE (20.2 $\mu A/cm^2$ at $3.2 \times 10^{-5}M$) and 12-HPETE (12.2 $\mu A/cm^2$ at $3.2 \times 10^{-5}M$). 9-HPETE ($3.2 \times 10^{-5}M$) and leukotrienes B_4 and C_4 ($10^{-7}M$) had no effect in either colon or ileum. The leukotriene C_4 and D_4 antagonist FPL 55712 did not affect the response to 5-HPETE, indicating that either leukotriene A_4, 5-HETE, or 5-HPETE itself were effecting the change in I_{sc}. To establish the ionic basis of the electrical response to 5-HPETE and 5-HETE we determined the I_{sc} responses in the absence of both Cl^- and HCO_3^- and also measured Cl^- fluxes. Replace-

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Table 1. Effect of 5-HPETE on Cl^- fluxes across short-circuited rabbit colonic mucosa. The values for four paired experiments are expressed as microequivalents per hour per square centimeter (± standard error) except for conductance, G, which is expressed as millisiemens per square centimeter.

Treatment	Unidirectional mucosal to serosal Cl^- flux	Unidirectional serosal to mucosal Cl^- flux	Net Cl^- flux	I_{sc}	G_t
Control	6.7 ± 0.6	6.2 ± 0.7	0.5 ± 0.4	1.4 ± 0.2	5.0 ± 0.2
5-HPETE (1.6×10^{-5} *M*)	6.4 ± 0.8	9.8 ± 0.7*	−3.4 ± 0.7*	5.0 ± 0.7*	7.9 ± 1.0

*$P < .05$ (Student's paired t-test).

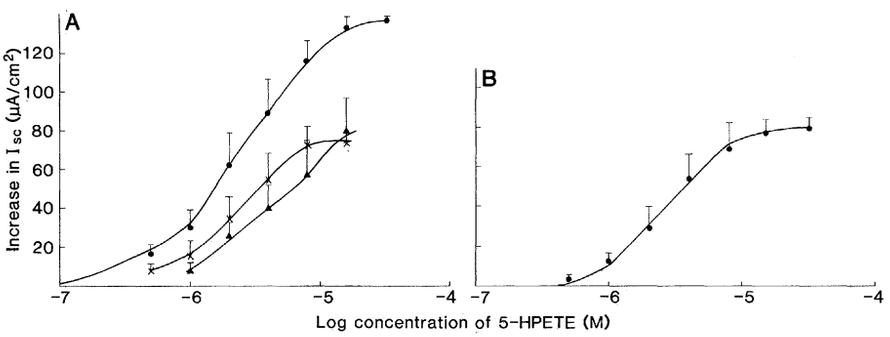

Fig. 1. Dose response curves for 5-HPETE and 5-HETE on short-circuit current (I_{sc}) in rabbit colonic mucosa. Values shown are means ±1 standard error for five to six determinations. (A) Effects of 5-HPETE (●), 5-HPETE plus $5 \times 10^{-6}M$ indomethacin (X), and 5-HPETE plus $5 \times 10^{-5}M$ indomethacin (▲). (B) Effects of 5-HETE.

ment of both Cl^- and HCO_3^- with gluconate nearly abolished the response to 5-HPETE and 5-HETE (*12*), suggesting that these agents stimulate electrogenic anion secretion. Measurements of unidirectional Cl^- fluxes indicate that 5-HPETE stimulates net secretion of Cl^- (Table 1). The change in net Cl^- flux is about equal to the change in I_{sc}. The mucosa-to-serosa flux did not change significantly, but the serosa-to-mucosa flux increased, accounting for the change in net flux.

The action of 5-HPETE on the colon was unaffected by treatment of the tissue with mepacrine ($5 \times 10^{-5}M$). This concentration of mepacrine inhibits the secretory effect of bradykinin in the same tissue (*3*). Thus 5-HPETE does not appear to act by stimulating phospholipase A_2 and the subsequent metabolism of arachidonic acid by the cyclooxygenase pathway. Indomethacin ($5 \times 10^{-6}M$), however, produced a significant inhibition of the effect of 5-HPETE (Fig. 1), but had no effect on the response to 5-HETE. A higher concentration of indomethacin ($5 \times 10^{-5}M$) did not further inhibit the effect of 5-HPETE. It is known that in addition to being able to inhibit cyclooxygenase, indomethacin can also inhibit the conversion of 12-HPETE to 12-HETE by a peroxidase (*13*). The inhibitory effect of indomethacin suggests, therefore, that the action of 5-HPETE is partially a result of its conversion to 5-HETE.

We cannot ascertain the molecular basis of the action of 5-HPETE and 5-HETE from our observations. We have shown that the secretory effect of 5-HPETE is not dependent on extracellular calcium and is not associated with an increase in the cellular concentrations of adenosine 3′, 5′-monophosphate (cyclic AMP) or guanosine 3′, 5′-monophosphate (cyclic GMP) (*14*). It has been observed that 5-HETE is rapidly incorporated into cell membrane components such as phospholipids (*15*). This could lead to an alteration of cell membrane function. However, the striking structural and tissue specificity observed in the present studies is certainly suggestive of a receptor-mediated action.

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11. The HPETE's were prepared by singlet oxygen oxidation and separated by high-performance liquid chromatography (HPLC). The HETE's were formed by triphenylphosphine reduction of the HPETE's followed by HPLC purification. For details of synthesis see N. A. Porter, R. A. Wolf, E. M. Yarborough, H. Weenan *Biochem. Biophys. Res. Commun.* **89**, 1058 (1979); N. A. Porter, J. Logan, V. Kantoyiannidou, *J. Org. Chem.* **44**, 3177 (1979).
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