**N-Acetylcysteine Potentiates Inhibition of Platelet Aggregation by Nitroglycerin**

Joseph Loscalzo  
Division of Vascular Medicine and Atherosclerosis, Division of Cardiology, and Hemostasis Unit, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts 02115

**Abstract**

Platelet aggregation is currently felt to play an important role in the pathogenesis of ischemic vascular disorders. The smooth muscle relaxant, nitroglycerin, has been shown to inhibit platelet aggregation in vitro, but at concentrations that were felt to be unattainable in vivo. Because the in vivo action of nitroglycerin on smooth muscle cells has been shown to depend on the presence of reduced cytosolic sulfhydryl groups, the inhibitory effect of nitroglycerin on platelet aggregation was examined in the presence of the reduced thiol, N-acetylcysteine. Millimolar concentrations of N-acetylcysteine potentiated markedly the inhibitory effect of nitroglycerin on platelet aggregation induced by ADP, epinephrine, collagen, and arachidonate, decreasing the 50% inhibitory concentration (IC$_{50}$) ~50-fold for each of these agents. Other guanylate cyclase activators inhibited ADP-induced aggregation similarly and this inhibition was likewise potentiated by N-acetylcysteine. Platelet guanosine 3',5'-cyclic monophosphate content increased fivefold in the presence of nitroglycerin and N-acetylcysteine 2 min before maximal inhibition of ADP-induced aggregation was achieved, while simultaneously measured cyclic AMP did not change relative to base-line levels. In the absence of N-acetylcysteine, nitroglycerin induced a marked decrease in platelet-reduced glutathione content as S-nitroso-thiol adducts were produced. The synthetic S-nitroso-thiol, S-nitroso-N-acetylcysteine, markedly inhibited platelet aggregation with an IC$_{50}$ of 6 nM. These data show that N-acetylcysteine markedly potentiates the inhibition of platelet aggregation by nitroglycerin and likely does so by inducing the formation of an S-nitrosothiol adduct(s), which activate guanylate cyclase.

**Introduction**

The importance of platelets in the pathogenesis of myocardial ischemia and infarction has recently been recognized. Circulating platelet aggregates are more prevalent in patients with sudden coronary death than in patients who die of other causes (1, 2). Vasoactive substances released by platelets at sites of endothelial injury, such as thromboxane A$_2$ and lipoxygenase products, produce coronary vasospasm (3–5). Furthermore, upon platelet activation, the platelet membrane itself serves as a catalytic surface on which coagulation factors interact in the formation of fibrin (6), the presence of which further limits vessel patency.

---

**Organic nitrate vasodilators, particularly nitroglycerin, have been used for many years in the treatment of ischemic heart disease (7).** The mechanism of action of these agents remains incompletely defined (8, 9), but very likely involves direct smooth muscle relaxation in conductive (coronary) arteries (10, 11). Given the role of the platelet in the pathophysiology of coronary ischemia, several groups have investigated the effect of organic nitrate vasodilators on in vitro platelet function (12–14). Agonist-induced aggregation was uniformly inhibited by nitrates in these early studies, but the concentration of nitrates required was quite high and not achievable pharmacologically, thus calling into question the therapeutic relevance of this observation.

The work of Needleman et al. (15) demonstrated that the vasodilator action of nitroglycerin depends critically on the availability of certain essential sulfhydryl groups in vascular smooth muscle cells. These in vitro observations were extended in vivo by Horowitz et al. (16), who showed that pretreating patients with the sulfhydryl agent, N-acetylcysteine, reduced the amount of nitroglycerin required to achieve a targeted reduction in mean arterial and mean pulmonary capillary wedge pressures.

Because of the importance of platelets in the pathogenesis of myocardial ischemia, the ability of organic nitrates to inhibit platelet function in vitro (albeit at excessive concentrations), and the potentiating effect of sulfhydryl agents on nitroglycerin action in smooth muscle cells, we investigated the effect of N-acetylcysteine on the inhibition of platelet aggregation by nitroglycerin and other organic nitrates. The data presented show the following: (a) that N-acetylcysteine markedly potentiates the inhibitory action of nitroglycerin on platelet aggregation in vitro to a variety of agonists; (b) that nitroglycerin (as other nitrate vasodilators) inhibit platelet aggregation by increasing intracellular cyclic GMP (cGMP) and N-acetylcysteine markedly potentiates this effect; (c) that nitroglycerin inhibits platelet aggregation through the formation of S-nitrosothiols, these latter compounds being both potent activators of guanylate cyclase and extremely potent inhibitors of platelet aggregation; and (d) that pharmacologically achievable concentrations of nitroglycerin in vivo may act to inhibit platelet aggregation, provided that there are sufficient reduced sulfhydryl groups present in the platelet, and that, therefore, an important therapeutic action of nitroglycerin may well be inhibition of platelet activation.

**Methods**

**Materials.** Epinephrine, ADP, sodium azide, and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO). Calf skin collagen was obtained from Worthington Biochemical (Freehold, NJ). Sodium arachidonate was purchased from

---

1. Abbreviations used in this paper: DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); IC$_{50}$, inhibitory concentration 50%; PRP, platelet-rich plasma.

---

Address reprint requests to Dr. Loscalzo, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

Received for publication 11 February 1985.

© The American Society for Clinical Investigation, Inc.  
0021-9738/85/08/0703/06 $1.00  
Volume 76, August 1985, 703–708
Natrium determination. Free nitrile was assayed in the supernatant of trichloroacetic acid extracts of platelets incubated with nitroglycerin with or without N-acetylcytsteine by the method of Snell and Snell (22), which involved diazotization of sulfanilic acid and subsequent coupling with the chromophore N-(1-naphthyl)ethylenediamine. S-Nitroso-thiols were detected by assaysing free nitrite by diazotization of sulfanilic acid in the presence and absence of 0.15% HgCl₂, the latter reagent catalyzing the hydrolysis of S-nitroso bonds (23).

Preparation of S-nitro-N-acetylcytsteine. S-Nitroso-N-acetylcytsteine was prepared at 25°C by reacting equimolar concentrations of N-acetylcytsteine with NaNO₂ at acidic pH (24, 25). Solutions turned from clear to rose-colored upon completion of the reaction. The S-nitroso-thiol was identified by visible absorption spectrophotometry, having an absorption maximum of 550 nm. The completeness of the reaction was verified by measuring free nitrite in the presence and absence of 0.15% HgCl₂, as described in the above paragraph, and by measuring reduced sulphydryl groups using DTNB. Due to the instability of the S-nitroso-derivatives, S-nitroso-N-acetylcytsteine was prepared within 1 h of use, kept in acidic solution at 4°C, and diluted as necessary into aqueous buffer immediately before addition to assay systems.

Results

Inhibition of platelet aggregation by nitroglycerin. Nitroglycerin inhibited platelet aggregation induced by 11μM ADP in a typical dose-response fashion (Fig. 1). The IC₅₀ for this inhibition was 42μM. Other agonists, including 12.5μM epinephrine, 0.12 mg/ml calf skin collagen, and 0.44 mM arachidonate, were also inhibited with similar IC₅₀ values (Table I). For each agonist, the extent of aggregation at any concentration of nitroglycerin was normalized to that in the absence of nitroglycerin and N-acetylcytsteine.

Potentiation of the inhibitory effect of nitroglycerin by N-acetylcytsteine. N-Acetylcytsteine at 5.5 mM added to PRP 4 min before the addition of nitroglycerin (and 5 min before the addition of agonist) markedly potentiated the inhibitory action of nitroglycerin for each agonist tested (Fig. 1 and Table I). The IC₅₀ shifted from ~42 to 0.71 μM for ADP with similar shifts noted for collagen, epinephrine, and arachidonate (Table I). At the concentration used in these experiments, N-acetylcytsteine alone did not inhibit platelet aggregation; however, at higher concentrations, inhibition of aggregation by N-acetylcytsteine alone was apparent. Lower concentrations of N-acetylcytsteine also potentiated inhibition by nitroglycerin, the effect decreasing with decreasing concentrations of N-acetylcytsteine.

Inhibitory effect of other guanylate cyclase activators on

![Figure 1.](image-url)
**Table I. Inhibition of Platelet Aggregation by Nitroglycerin and Potentiation of Inhibition by N-Acetylcysteine**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>IC50 −N-Acetylcysteine</th>
<th>IC50 +N-Acetylcysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (11 μM)</td>
<td>42±2 μM</td>
<td>0.71±0.10 μM</td>
</tr>
<tr>
<td>Epinephrine (12.5 μM)</td>
<td>34±2 μM</td>
<td>0.20±0.08 μM</td>
</tr>
<tr>
<td>Collagen (0.12 mg/ml)</td>
<td>36±2 μM</td>
<td>0.60±0.15 μM</td>
</tr>
<tr>
<td>Arachidonate (0.44 mM)</td>
<td>45±6 μM</td>
<td>1.00±0.10 μM</td>
</tr>
</tbody>
</table>

*Aggregation of platelets in PRP was induced by addition of particular agonists after incubating platelets at 37°C with various concentrations of nitroglycerin ±5.5 mM N-acetylcysteine for 5 min. IC50 of each represent the mean±SEM for three experiments done in duplicate. For further details, see Methods.

**Table II. Inhibition of Platelet Aggregation by Guanylate Cyclase Activators and Potentiation of Inhibition by N-Acetylcysteine**

<table>
<thead>
<tr>
<th>Activator</th>
<th>IC50</th>
<th>IC50 −N-Acetylcysteine</th>
<th>IC50 +N-Acetylcysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium azide</td>
<td>1.3 μM</td>
<td>0.026 μM</td>
<td></td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>42 μM</td>
<td>0.80 μM</td>
<td></td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>10 μM</td>
<td>0.46 μM</td>
<td></td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>59,000 μM</td>
<td>680 μM</td>
<td></td>
</tr>
</tbody>
</table>

*Aggregation of platelets in PRP was induced by addition of 11 μM ADP after incubating platelets at 37°C with various concentrations of azide, nitroglycerin, nitroprusside, or nitrite±5.5 mM N-acetylcysteine for 5 min. For further details, see Methods.

**Table III. Increase in Platelet cGMP by Nitroglycerin and Potentiation by N-Acetylcysteine**

<table>
<thead>
<tr>
<th>[Nitroglycerin]</th>
<th>cGMP pmol/10⁸ platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>3.9 x 10⁻¹⁰</td>
<td>0.26±0.02</td>
</tr>
<tr>
<td>3.9 x 10⁻⁶</td>
<td>0.32±0.04</td>
</tr>
<tr>
<td>3.9 x 10⁻⁵</td>
<td>0.54±0.08</td>
</tr>
<tr>
<td>3.9 x 10⁻⁴</td>
<td>0.80±0.04</td>
</tr>
</tbody>
</table>

*Platelets in PRP were incubated for 5 min at 37°C with a range of nitroglycerin concentrations±5.5 mM N-acetylcysteine. Each point represents the mean±SEM of three experiments done in duplicate. For further details, see Methods.

**ADP-induced platelet aggregation and potentiation by N-acetylcysteine.** Nitroglycerin is a potent activator of guanylate cyclase (26) and, given that this activation requires the presence of reduced thiol groups (26), other guanylate cyclase activators were tested for their ability to inhibit ADP-induced platelet aggregation in the presence and absence of N-acetylcysteine. In Table II are listed the IC50s for sodium azide, sodium nitroprusside, sodium nitrate, and nitroglycerin in the presence and absence of 5.5 mM N-acetylcysteine. These data show that while the IC50s ranged widely (1.3 μM for sodium azide to 59,000 μM for sodium nitrate), in each case N-acetylcysteine potentiated the inhibition of ADP-induced aggregation, reducing the IC50, on average, 50-fold (ranging from 22- to 87-fold for nitroprusside to nitrite, respectively).

**Increase in platelet cGMP by nitroglycerin and potentiation by N-acetylcysteine.** Mellion et al. (27) showed that sodium nitroprusside and nitric oxide inhibited ADP-induced platelet aggregation and that this inhibition was preceded by an early, large, and partly transient increase in intracellular cGMP. For this reason, the effect of nitroglycerin on platelet cGMP was assessed and the influence of N-acetylcysteine on this effect examined. Total platelet cGMP was measured by radioimmunoassay in quiescent platelets and platelets incubated with nitroglycerin with and without N-acetylcysteine. Platelets in PRP were incubated with various concentrations of nitroglycerin with or without 5.5 mM N-acetylcysteine for 5 min at 37°C, after which the platelets were processed as described previously (14) and cGMP determined. Table III lists the values obtained and indicates that nitroglycerin increased resting cGMP from baseline levels fourfold without N-acetylcysteine (from 0.20±0.04 to 0.80±0.04 pmol/10⁸ platelets) and up to 19-fold with N-acetylcysteine (to 4.08±0.26 pmol/10⁸ platelets) at the highest nitroglycerin concentrations used (390 μM).

The incubation time used in this experiment was chosen because inhibition by nitroglycerin of platelet aggregation in the presence of N-acetylcysteine is maximal by 4 min and because subsequent experiments evaluating the time course of changes in platelet aggregation were carried out for as long as 5 min after addition of reactants (see below). As had been shown previously for nitroglycerin (14), no change in platelet cAMP from basal levels of 3.5±0.5 pmol/10⁸ platelets occurred in these experiments.

**Time course of increase in platelet cGMP and inhibition of platelet aggregation.** The temporal relationship between the increase in platelet cGMP and the decrease in ADP-induced platelet aggregation is shown in Fig. 2. Platelets in PRP were incubated at 37°C with 39 μM nitroglycerin for 1 min and 5.5 mM N-acetylcysteine was added at time zero. The incubation mixture was sampled at frequent times after addition of N-acetylcysteine to determine the maximal rate of platelet aggregation in response to 11 μM ADP, a value that typically was derived from no more than the first 15 s of the aggregation tracing after addition of ADP. (Please note: the extent of aggregation was not measured in these experiments because the time required to achieve the maximal extent of aggregation was prohibitively lengthy [up to 2 min] relative to the time course of changes in cGMP that were being simultaneously measured.)

The concentration of nitroglycerin used (39 μM) was chosen to permit measurement of aggregation rates that were less than completely inhibited, thereby permitting assessment of changes in rates at early times. Since the earliest time point sampled after addition of nitroglycerin was 1 min, the large and transient increase in cGMP described by Mellion et al. (27) at very early times (<30 s) after addition of nitroprusside or nitric oxide would not have been detected if it occurred in platelets exposed to nitroglycerin. Only the stable, late-appearing
maximal rate of aggregation (●) (maximal change in percent transmittance per second) determined. Each point represents the mean±SEM of two or three experiments done in duplicate. Note that the ordinate is inverted for the rate of aggregation.

(i.e., beyond 1 min) elevations of cGMP were measured in this experiment in order that they might be correlated better with changes in aggregation rate.

Fig. 2 shows that by 30 s after addition of 5.5 mM N-acetylcysteine to platelets incubated with 39 μM nitroglycerin, cGMP levels increased fourfold to 0.90 pmol/10^8 platelets. Maximal inhibition of platelet aggregation rate (plotted with a reversed ordinate for purposes of comparison with the cGMP plot) lagged behind and was not attained until 2.5 min after addition of N-acetylcysteine. Thus, this experiment demonstrates that the increase in platelet cGMP induced by nitroglycerin in the presence of N-acetylcysteine preceded the attainment of maximal inhibition of platelet aggregation.

Effect of nitroglycerin on reduced glutathione concentration in platelets. Nitroglycerin undergoes denitrosation in smooth muscle cells and hepatocytes through the action of an organic nitrilotransferase (28). This denitrosation requires GSH, the thiol undergoing oxidation in the process. GSH levels were measured in the quiescent platelet and found to be 4.0 × 10^{-17} mol GSH/platelet, a value comparing favorably with published values (29). Incubating platelets with increasing concentrations of nitroglycerin led to a reduction in this intracellular GSH to <10% of the base-line levels at ~1 μM nitroglycerin (Fig. 3).

Effect of S-nitroso-N-acetylcysteine on platelet aggregation. S-nitroso-N-acetylcysteine, synthesized from N-acetylcysteine and NaNO_2 as described in Methods, markedly inhibited platelet aggregation in response to 11 μM ADP, as depicted in Fig. 4. Incubating PRP for 5 min with S-nitroso-N-acetylcysteine inhibited aggregation with an IC_{50} of 6 nM; it is important to note that at equivalent concentrations of N-acetylcysteine or NaNO_2 alone, no significant inhibition was noted (Fig. 1 and Table II), which supports the hypothesis that the adduct itself (and/or a metabolic product thereof) was the active species. Similar effects of S-nitroso-N-acetylcysteine were noted when using epinephrine (12.5 μM) and calf skin collagen (0.12 mg/ml) as agonists (Table IV).

Formation of S-nitroso-thiols by platelets on incubation with nitroglycerin. Platelets in PRP were incubated with 2.2 mM nitroglycerin with or without 5.5 mM N-acetylcysteine for 5 min at 37°C and extracted with TCA. The concentration of total nonprotein S-nitroso-thiol was determined by assaying free nitrite using diazotization and aromatic derivatization in the presence and absence of HgCl_2 as described in Methods. Mercuric ions catalyze the hydrolysis of the nitroso moiety from the nitroso-thiol adducts and, in so doing, permit the determination of nitroso-thiols. In the absence of N-acetylcysteine, 1.5±0.5 nmol/10^8 platelets of S-nitroso-thiol was detected, while with N-acetylcysteine, this value increased to 5.1±1.0 nmol/10^8 platelets. No nitroso-thiol was detected in the absence of nitroglycerin. The effect of lesser concentrations of nitroglycerin was not assessed because sensitivity of the assay was limited and prohibitively large amounts of platelets would have been required. Protein-associated S-nitrosothiols were not measured in these experiments because only adducts to which the plasma membrane is permeable appear to be important for the effect of other organic nitrates noted in previous studies (27).

Discussion

These experiments demonstrate that the reduced thiol, N-acetylcysteine, markedly potentiates inhibition of platelet aggregation by nitroglycerin and other organic nitrate vasodilators.
Table IV. Inhibition of Platelet Aggregation by S-Nitroso-N-Acetylcysteine*

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Inhibition Concentration (IC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (11 µM)</td>
<td>0.006±0.0005</td>
</tr>
<tr>
<td>Epinephrine (12.5 µM)</td>
<td>0.100±0.001</td>
</tr>
<tr>
<td>Collagen (0.12 mg/ml)</td>
<td>0.036±0.001</td>
</tr>
</tbody>
</table>

* Aggregation of platelets in PRP was induced by addition of agonist after incubating platelets at 37°C with various concentrations of S-nitroso-N-acetylcysteine for 5 min. Each point represents the mean±SEM of two experiments done in duplicate. For further details, see Methods.

N-Acetylcysteine was chosen for these experiments in place of other thiols because of its proved safety in humans and because of its demonstrated efficacy in potentiating the hypotensive effect of nitroglycerin in vivo (16). In smooth muscle cells, the relaxing effect of nitroglycerin was felt initially to be mediated by a nitroglycerin "receptor" (30), through which specific tolerance could be induced by prolonged exposure to nitroglycerin and reversed by reduced thiols (15). Further studies by Ignarro’s group (31) suggested that the effect of nitroglycerin on smooth muscle cells was more complex than this and that cGMP was an essential mediator of nitroglycerin-induced vasodilatation. In these experiments, increases in smooth muscle cGMP in response to incubation with nitroglycerin preceded decreases in vascular tone, supporting the importance of the cyclic nucleotide.

In platelets, the inhibition of aggregation by nitroglycerin and other guanylyl cyclase activators (nitroprusside, azide, and nitrite) suggests that cGMP is a crucial mediator of this inhibition (27). The fact that the maximal increase in platelet cGMP preceded the maximal inhibition of aggregation further supports this hypothesis. Controversy exists in the platelet literature about the role of cGMP in the aggregation response. Chiang and colleagues (32, 33), White et al. (34), and Glass et al. (35) demonstrated that platelet aggregation is associated with a rise in platelet cGMP levels, while the more recent studies of Claesson and Malmsten (36) and Weiss et al. (37) showed that cGMP either had no effect on platelet aggregation or else, at higher concentrations, inhibited aggregation. The data presented here show that, at least as far as inhibition of platelet aggregation by nitroglycerin is concerned, platelet cGMP levels maximally increase before maximal inhibition of aggregation, thereby arguing that elevation of cGMP is associated with inhibition of aggregation.

The ability of the reduced thiol, N-acetylcysteine, to potentiate the inhibitory effect of nitroglycerin further supports the importance of guanylyl cyclase activity in the inhibition of platelet aggregation in these experiments. Preincubation with reduced thiol was essential for the expression of partially purified hepatic guanylyl cyclase activity (38). In addition, the fact that hepatic and pulmonary (38) guanylyl cyclase are rapidly inactivated by molecular oxygen, sulfhydryl oxidants, and thiold alkylation agents suggests that the redox state of sulfhydryl groups on guanylyl cyclase determines its activation. Reduced thiols have also been found to stimulate activation of purified guanylyl cyclase by nitroglycerin, among which are included cysteine, GSH, penicillamine, and dithiothreitol (26).

In the metabolism of nitroglycerin, denitritification occurs in a process requiring that GSH be catalyzed in liver by an organic nitrate reductase (30); the specific enzyme that serves this function is probably a glutathione-S-transferase (39). Denitritification is probably responsible not only for the metabolic fate of nitroglycerin, but also for its role as an activator of guanylate cyclase. Nitrite ion hydrolyzed from nitroglycerin reacts with reduced thiol to form S-nitroso-thiol compounds that, themselves, are very potent activators of guanylate cyclase (26), vasodilators (24), and, as shown both in this study for S-nitroso-N-acetylcysteine and in a recent study for other S-nitroso-thiols (40), extremely potent inhibitors of platelet activation. The loss of GSH and the appearance of S-nitroso-thiols in platelets incubated with nitroglycerin supports the existence of this mechanism in platelets. The chemical identity of the S-nitroso-thiol(s) that form intracellularly has yet to be determined, but the possible candidates include S-nitroso-glutathione, S-nitroso-cysteine, or, perhaps, S-nitroso-N-acetylcysteine itself.

What N-acetylcysteine does in this system is, as yet, not fully explained: it may serve as a source of thiol-reducing equivalents for the GSH system in the platelet; it may directly activate guanylate cyclase; it may serve as a reduced thiol source in the enzyme-catalyzed denitritification reaction; or it may serve as a substrate directly in the formation of S-nitroso-thiols. Further studies are currently underway to clarify these issues.

In summary, the data presented here show the following: that (a) nitroglycerin and other organic nitrates inhibit platelet aggregation and that this inhibition is potentiated markedly by the reduced thiol, N-acetylcysteine; that (b) nitroglycerin, as other guanylyl cyclase activators, inhibits platelet aggregation by inducing an increase in platelet cGMP levels that rise maximally before maximal inhibition of platelet aggregation; that (c) S-nitroso-thiols form when platelets are incubated with nitroglycerin; that (d) S-nitroso-N-acetylcysteine is an extremely potent inhibitor of platelet aggregation; and that (e) such S-nitroso-thiols adducts are the active forms of nitroglycerin and other organic nitrates.

These data provide new insights into the possible effects of nitroglycerin on the vascular bed. At concentrations that are pharmacologically attainable in vivo, nitroglycerin reacts with GSH and/or other cellular thiols to form S-nitroso-thiols that not only vasodilates directly, but also markedly inhibit platelet aggregation. Inhibition of platelet activation and aggregation prevents synthesis of platelet-derived thrombomodulin A₂₇, and thereby further potentiates local direct vasodilating effects. In light of these data, the use of S-nitroso-N-acetylcysteine or other S-nitroso-thiols as antihypertensive or antiplatelet agents deserves further study.

Acknowledgments

The author wishes to express his appreciation to Drs. Andrew Schafer and Robert I. Handin for helpful discussions and to Ms. Jane Freedman for excellent technical support.

This work was supported by a Clinician-Scientist Award from the American Heart Association and, in part, by National Institutes of Health grants HL35014 and HL17513.
Atherosclerosis.


