Aspirin has emerged as a remarkably safe, inexpensive, and effective drug for the secondary prevention of the complications of atherosclerotic disease. It acts by inhibiting the enzyme prostaglandin (PG) G/H synthase, actually a bifunctional protein that sequentially catalyzes the conversion of arachidonic acid to the highly reactive endoperoxide intermediates PGG2 and PGH2 via its cyclooxygenase (COX) and peroxidase functions. This enzyme, colloquially termed COX, has been crystallized1 and the mechanism of action of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) elucidated.2–4 The catalytic site is buried deep within the core of the enzyme and is accessed by the substrate via a hydrophobic tunnel. Aspirin irreversibly acetylates a serine residue at position 529 in the human enzyme,5 close to but not at the catalytic site, though still blocking access to it by the arachidonic acid substrate. NSAIDs, by contrast, act reversibly as competitive inhibitors at the catalytic site. Indeed, transient occupancy of that site after dosing with an NSAID may mask the serine from the effects of a subsequent dose of aspirin.6

The irreversible acetylation of Ser529 by aspirin in the vascular cells. 11 Although the isoforms differ in the capacity of platelets to generate TxA2, commonly measured as serum concentrations of its hydrolysis product TxB2. Thus, daily administration of 75 mg of aspirin takes 3 to 4 days to inhibit completely serum TxB2 ex vivo.17 Interestingly, like low-dose aspirin, 75 mg of clopidogrel also takes several days to inhibit platelet function ex vivo, perhaps owing to the gradual accumulation of a metabolite that binds to a subclass of purinergic receptors, although this has yet to be established in vivo. As in the case of aspirin, loading doses of clopidogrel have been investigated and are likely to be employed in acute settings of vascular occlusion. Studies designed to assess the potentially additive effects of aspirin with clopidogrel have been initiated. Finally, dipyridamole has been reformulated to correct prior problems with variable and transient bioavailability. This new preparation has been shown to be as effective as low-dose aspirin in the secondary prevention of stroke, and the benefit from a combination of the 2 is roughly additive.18 This has led to the US Federal Drug Administration approval of an aspirin-dipyridamole combination for this indication. Although oral inhibitors of the platelet glycoprotein IIb/IIIa have been disappointing, 3 antiplatelet drugs—low-dose aspirin, clopidogrel, and dipyrudamole—have been proven effective in the secondary prevention of cerebrovascular and/or cardiovascular disease. Their mechanisms of action suggest that their benefits might be additive, although this remains to be established in a clinical trial. This has “raised the bar” for the development of potentially novel therapies in this arena.

The advent of pharmacological inhibitors of the TP19–21 inauspiciously coincided with the initial trials establishing the efficacy of aspirin in cardiovascular disease.22–24 This confounded their development in 2 ways. First, as the effects of aspirin were attributed to inhibition of TxA2, TP antagonists had little additional benefit to offer. Perhaps preservation of the ability of the vasculature to generate other PGs such as prostacyclin (PGI2) was a plus, but this was a strictly theoretical concept. Against that, it was difficult for TP antagonists to compete against aspirin at 20 cents a tablet. Two clinical trials of TP antagonists were performed, both addressing the possibility that they might modify restenosis after angioplasty.

In the CARPORT (Coronary Artery Restenosis Prevention on Repeated Thromboxane A2 antagonism) study, the antagonist GR32191, given before percutaneous transluminal coronary angioplasty (PTCA) and for 6 months thereafter, did not differ significantly from the effects of intravenous aspirin given immediately before PTCA on luminal diameter as assessed by angiography performed 6 months after the
intervention. However, the limitations of this uncontrolled experience and of relying on an angiographic end point at a single time were highlighted by the M-HEART (Multi-Hospital Eastern Atlantic Restenosis Trial) II study. In that study, all patients received aspirin before PTCA due to its role, by then established, in the prevention of periprocedural myocardial infarction but were randomized to continuing treatment with the TP antagonist sulotroban, aspirin, or placebo. In this case, the angiographic estimate of vascular occlusion at 6 months was a secondary end point and did not differ between the groups. By contrast, continued treatment with either aspirin or sulotroban significantly reduced the later myocardial infarction rate in the 6 months after PTCA when compared with the placebo group. These observations left many unanswered questions. First, aside from there being no measure of the degree of synthesis inhibition achieved by aspirin or of TP blockade by either antagonist, the divergence of the time-integrated clinical and “snapshot” angiographic end points in the M-HEART II study illustrated the limited information available from CARPORT. Second, the hypothesis that TP blockade might be superior to aspirin was configured on the preservation of PGI₂ formation during TP blockade. PGI₂ has significant antiproliferative effects. Both virally delivered PGI synthase and a PGI₂ analogue, bera-

chain, which is usually oriented in an L-shaped configuration in the hydrophobic channel. Mutation to their COX-1 equivalents of 3 residues predicted to be critical to the spatial and flexibility differences in the COX-2 channel abolishes this feature in the mouse enzyme. Both COX isoforms are present, with remarkably similar distributions, in human atherosclerotic plaque. Although this may also be true in the atherosclerotic mouse, it is unknown whether 15(R)-HETE has any biological effects in aspirin-treated animals. Thus for now, the role of TP activation by HETEs in explaining the discrepancy between the effects of aspirin and the TP antagonist in the study of Cayette and colleagues remains quite speculative. Perhaps the explanation is more mundane. The relationship between inhibition of the capacity of platelets to generate TxA₂—estimated by serum TxB₂—and inhibition of Tx-dependent platelet activation is remarkably nonlinear. Just 5% residual capacity is sufficient fully to sustain function. In the present study, the aspirin regimen suppressed serum TxB₂ by only 70% to 75%, and TP-dependent platelet activation is likely to have been unimpaired. Clearly, platelets may be a source of growth factors relevant to atherogenesis, but activation of TP s in other cells may also be relevant. In tissues other than the platelet, we have no information as to the relationship between biosynthetic capacity and function.

Cayette and colleagues raise the possibility that the discrepant results between aspirin and S 18886 might also be explained if isoprostanes (iPs), free radical–catalyzed products of arachidonic acid, played an important role in TP activation. Indeed, at least 2 iPs—iPF₂α-III (also known as 8-iso-PGF₂α) and iPE₂-III—exert potent effects on platelet function and vascular tone, and these actions are prevented by TP antagonists and absent in TP-deficient mice. Generation of iPs is increased in both humans with atherosclerosis and in apoE-KO mice, and iP suppression with vitamin E retards atherogenesis in this model. Perhaps the use of TP antagonists should be reconsidered in other syndromes in which oxidant stress and COX activation coincide, such as occlusion/reperfusion and inflammation. We are at a very early stage in elucidating the biological actions of iPs and related compounds, some of which may act as incidental ligands at other PG receptors.

Where do the observations of Cayette and colleagues lead us? It would certainly be interesting to confirm the effects of the compound in murine models more reminiscent of the human disease. It would also be reassuring to confirm that similar effects are observed with structurally distinct antagonists and TP deletion. It remains a formal possibility that the observed effects relate to properties of S 18886 that are unrelated to TP antagonism. Such studies will also increase our sense of the magnitude of the effects we might anticipate with TP antagonism. This seems consistent, but somewhat modest, in the apoE-KO mouse. Should such studies reinforce our confidence, modern imaging modalities such as electron beam CT of the coronary or ultrasonography of the carotid arteries afford noninvasive approaches to assess the impact of TP antagonism on plaque progression in humans. Reagents are now available for the study of TP expression in human disease, and model systems have implicated TP.
activation in settings previously not considered when these drugs were under development. Finally, TP antagonists may even be worth considering as alternative platelet inhibitors to aspirin in certain circumstances. For example, because platelets express COX-1 only, selective inhibitors of COX-2 do not afford cardioprotection. However, in the absence of any actual clinical data, the empiric combination of these drugs with low-dose aspirin has some theoretical limitations. Even low-dose aspirin causes gastrointestinal side effects, which may erode the postulated benefit of selective COX-2 inhibition on the gastrointestinal tract. By contrast, TP antagonism has been shown to protect against NSAID-induced enteropathy in the rat. Similarly, selective COX-2 inhibitors, NSAIDs, and even low doses of conventionally formulated aspirin depress PGI2 biosynthesis in healthy individuals. Combination of TP antagonists with COX-2 inhibitors would afford at least similar cardioprotection to that of aspirin while sparing PGI2 formation from further suppression.

In summary, the provocative studies of Cayette et al.22 draw attention to a class of compounds that were previously abandoned for reasons that were rational at the time. Now that we know more about the breadth of potential TP ligands and are increasingly informed of the biology of PGs as a result of what we know more about the breadth of potential TP ligands and are increasingly informed of the biology of PGs as a result of

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