PLATELETS are derived from bone marrow megakaryocytes by extension of cytoplasmic processes which undergo attenuation, develop constrictions at the distal ends, and then rupture, thereby releasing platelets.1 During its life span of approximately 10 days the unstimulated platelet functions in some unknown manner to maintain vascular integrity. The platelet is also the only blood cell component involved in the uptake and transport of serotonin (5-HT). If blood vessel continuity is interrupted, the vessel contracts and concomitantly platelets at the site are activated. Activation can also take place intravascularly by pathological stimuli such as endotoxin and immune complexes generated in certain disease states.2 Adhesion of platelets in proximity to the subendothelium occurs and this is accompanied by aggregation (cohesion) of additional platelets which have been "recruited" into the microenvironment. This sequence (primary hemostasis) is modulated by adhesion of platelets to subendothelial collagen, formation of thromboxane A2 (TXA2), mobilization of intraplatelet calcium, release of adenosine diphosphate (ADP) and 5-HT (fig. 1). Platelet activation also results in exposure of specific surface receptors which bind fibrinogen and this bound fibrinogen is a cofactor for aggregation. Such exposure is inhibited when platelet cAMP levels are elevated by agents such as prostacyclin (PGL2).

The stimulated platelet undergoes a unique morphological change from that of a disc to a spiny sphere* and the surface membrane phospholipoprotein develops the capacity to catalyze interactions between activated coagulation proteins, culminating in thrombin formation and fibrinogen polymerization (secondary hemostasis). The process of clot retraction is initiated when platelets form pseudopodia which adhere to fibrin strands at points where the strands cross one another. The platelet pseudopodia then contract and draw the sides of injured vessels together. Clot retraction requires ATP, glucose, calcium and normal fibrin formation. Formation of the hemostatic platelet plug is then complete.

Stimulated platelets also secrete proteins which were originally synthesized in the megakaryocyte.4 Among these are platelet factor 4 (PF-4), which has anti-heparin properties and can react with heparan sulfate in the vessel wall. The platelet-derived growth factor (PDGF) which stimulates smooth muscle cell proliferation is also released. PDGF has been implicated in the atherosclerotic process.4

Platelets and Thrombosis

Arterial thrombi resemble hemostatic plugs in that they form via an interaction of platelets with an injured vascular surface. Morphologically such thrombi contain mainly adherent platelets at the interface and a mixture of leukocytes, fibrin and erythrocytes in the distal portion. These observations prompted the use of pharmacologic agents capable of suppressing platelet aggregation, release and adhesion.5 In the early 1970’s clinical trials were initiated with the overall goal of attempting to prevent or reduce platelet accumulation in diseased vessels of the heart, brain, extremities and on vascular prostheses.5-8 To date analyses of almost every clinical trial have been fraught with interpretive difficulties.5-8

Attempts were initially made to prolong platelet survival with platelet inhibiting drugs plus anticoagulants in patients with valve prostheses and with aortocoronary bypass grafts, since shortened platelet survival as measured by isotopic techniques correlated with malfunctioning prostheses and thromboembolic phenomena.5 In arterial thromboembolism, increased con-
Thrombin - Collagen - Epinephrine

**FIGURE 1.** Simplified scheme of events following platelet stimulation. Platelet stimuli vary in their ability to initiate the process of TXA2 synthesis. Formation of TXA2 is followed by calcium mobilization, and the presence of free calcium inhibits platelet adenylate cyclase. Such inhibition initiates events leading to aggregation. Free calcium also stimulates dense granule secretion. Among the most important secreted products are fibrinogen (which is a co-factor for aggregation), serotonin and ADP. Released thromboxane and ADP then serve to "recruit" other platelets into the hemostatic plug or thrombus — as the case may be. In contrast, platelet aggregation and release are inhibited by prostaglandins I2, D2 and E1 (which is not a natural product). The latter prostaglandins stimulate adenylate cyclase to varying degrees which results in elevations in platelet cAMP, consequences of which include inhibition of both phospholipase A2 and calcium mobilization. The sequence shown here is mainly hypothetical because the events following exposure to a platelet stimulus are so rapid that currently available techniques cannot monitor them accurately. (Courtesy of the Upjohn Co.)

The purpose of this review is to summarize current concepts of arachidonic acid (20:4) metabolism in platelets and neutrophils and to discuss new information on interactions between different cell types involving this pathway. Some of these phenomena may be directly applicable to problems in pharmacologic modification of platelet function.

**Mobilization and Metabolism of Arachidonic Acid**

The compounds depicted in figs. 2 and 3 are derived from oxygenation of arachidonic acid. They are formed mainly via enzymatic pathways and the major end products are known as "eicosanoids." These include "classical" prostaglandins (PGD2, PGE2, PGF2α), hydroxy acids, thromboxanes, leukotrienes and prostacyclin. Since there is no free arachidonate in cells, it must initially be released from cell phospholipids by the action of phospholipase(s). In prevention and treatment of thrombosis, a phospholipase inhibitor might be useful for blocking this initial step in the arachidonic acid cascade. In cells which synthesize protein (in contrast to platelets), steroids induce synthesis of macrocortin — a polypeptide which inhibits phospholipase activity. As will be mentioned subsequently, more platelet arachidonate is released than is utilized for eicosanoid formation and some of this released 20:4 can be taken up and metabolized by other stimulated cells such as neutrophils.

**The Cyclooxygenase Reaction**

Cyclooxygenase is a particulate enzyme which is preferentially activated by free arachidonate. Endoperoxides — the initial oxygenation products are formed...
rapidly (platelet oxygen burst\textsuperscript{12}), and in platelets, endothelial cells and other tissues are the precursor molecules for prostaglandins, TX\textsubscript{A\textsubscript{2}}, and PGI\textsubscript{2}. Since non-steroidal antiinflammatory agents such as aspirin inhibit cyclooxygenase the reaction is of great clinical significance. In platelets the inactivated enzyme is not replaced because of the absence of protein synthesis. Inhibition of platelet cyclooxygenase by aspirin and its consequent clinical effects on platelet function due to the absence of thromboxane formation was the major justification for initiating clinical trials in thrombosis prevention. In re-evaluating results of most clinical trials to date, the question has arisen as to whether beneficial effects observed with aspirin were due to effects other than cyclooxygenase inhibition.\textsuperscript{5-8, 13} The latter also applies to sulfinpyrazone.\textsuperscript{6, 7} For example in some studies women did not benefit from aspirin administration.\textsuperscript{5-8, 13} This observation is difficult to comprehend if only cyclooxygenase inhibition was involved. It would have been of interest to know whether the females who did not respond demonstrated any alterations in bleeding time after aspirin ingestion. Sulfinpyrazone is a weak, reversible cyclooxygenase inhibitor and its beneficial effect may be in preventing endothelial injury and cardiac arrhythmia.\textsuperscript{5, 7} Aspirin therapy was effective in venous thrombosis\textsuperscript{13} — which is also difficult to explain since platelets are not the major component of venous thrombi and thrombin-fibrin formation is involved.

In human vascular endothelium endoperoxides are converted mainly to PGI\textsubscript{2} which induces strong vasodilation and inhibits platelet aggregation via elevation of cAMP. Under conditions which favor close cell contact, endothelial cells can utilize platelet endoperoxides for PGI\textsubscript{2} production.\textsuperscript{14} Some investigators have proposed that a balance may exist between thromboxane and prostacyclin production in the maintenance of blood fluidity, but this has become controversial.\textsuperscript{6, 13} Although aspirin-treated endothelium can renew PGI\textsubscript{2} production via protein synthesis, low doses of aspirin have been recommended in order to inhibit only the more sensitive platelet cyclooxygenase.\textsuperscript{6} Against this hypothesis are several instances in which aspirin in doses of 1 gm or more per day were utilized with successful results.\textsuperscript{13, 15-19}

**The Lipoxygenase Reaction**

In contrast to cyclooxygenase, the initial products of which (endoperoxides PGG\textsubscript{2} and PGH\textsubscript{2}) are common to all tissues, lipoxygenases are tissue–specific with regard to positional specificity of the oxygenation step. Platelets contain a 12-lipoxygenase which catalyzes insertion of a hydroperoxy group at position 12 of arachidonate followed by reduction by a peroxidase to a hydroxyl group. The platelet lipoxygenase can be substrate-activated in the absence of other stimuli. In contrast neutrophils contain a 5-lipoxygenase which is not substrate-activated and requires cell stimulation leading to formation of eicosanoids such as leukotrienes and other hydroxy acids (figs. 2 and 3). Although platelet 12-HETE (fig. 3) is chemotactic for other cells, a direct effect on platelet function by its lipoxygenase products has not as yet been demonstrated. However lipoxygenases are not inhibited by aspirin and platelet lipoxygenase products have been found to interact with leukocytes by various mechanisms.\textsuperscript{11, 20} Such information on cell-cell interactions was not available when aspirin trials were initiated, and we do not know whether these phenomena result in beneficial or detrimental effects, if any.

Recently Borgeat and associates isolated a new metabolite of arachidonic acid which formed following addition of 12-HETE to leukocytes which were stimulated with ionophore A23187.\textsuperscript{21, 22} This product was identified as 5 S, 12 S-diHETE which is a stereoisomer of leukotriene B\textsubscript{4}. The full spectrum of biologic activity for diHETE has not as yet been determined, but it appears to be a major product in platelet-neutrophil interactions.\textsuperscript{11}

In an extension of previous studies of cell-cell interactions\textsuperscript{14} we directly demonstrated that aspirin-treated platelets provided precursors for the formation of neutrophil leukotrienes and diHETE. Platelets radiolabeled with arachidonic acid were stimulated with ionophore A23187 in the presence of unlabeled neutrophils. Several radiolabeled products not produced by platelets alone were detected. These included: LTB\textsubscript{4}, diHETE, and 5-HETE. The results indicated that platelet-derived arachidonate served as precursor for LTB\textsubscript{4} and 5-HETE synthesized by the neutrophil. Platelet-derived 12-HETE was converted to diHETE by the neutrophil.\textsuperscript{11}

Thus, stimulated platelets from a subject who has ingested aspirin (or other non-steroidal anti-inflammatory agents) remain capable of serving as sources of compounds synthesized by other cells. These substances possess biological activities such as chemotaxis, neutrophil activation and smooth muscle contractibility. Perhaps previously unexplained beneficial effects of aspirin on ischemic vascular diseases may eventually be attributable to products produced in cell-cell interactions from precursors which are quantitatively increased due to diversion of arachidonate away from the cyclooxygenase pathway.

**Concluding Remarks**

The clinical trials involving aspirin and sulfinpyrazone were devised because of the ability of these compounds to induce a platelet functional defect. Clinically, the aspirin defect is manifested as a prolongation of
Figure 2. Pathways of arachidonic acid metabolism in human platelets and endothelial cells. Free arachidonate is metabolized by two different enzymatic mechanisms. Formation of the endoperoxides PGG₂ and PGH₂ is catalyzed by cyclooxygenase. The endoperoxides are pivotal compounds in all tissues in which they form. Thus, in platelets they are converted to thromboxane A₂ and in the endothelial cell PGI₂ is formed. In addition endothelial cells synthesize PGF₂α, PGE₂ and PGD₂. The cyclooxygenase pathway is inhibited irreversibly by aspirin and reversibly by sulfipyrazone. The lipoxygenase step results in formation of 12-HPETE and 12-HETE from arachidonate and is slightly delayed but not inhibited by aspirin. (Courtesy of the Upjohn Co.)

Figure 3. Leukotriene synthesis from arachidonic acid. In leukocytes there is a 5-lipoxygenase which converts arachidonic acid into 5-HPETE which can then be converted to LTA₄, a common precursor of leukotrienes B₄, C₄, and D₄. Leukotrienes possess numerous biological properties which may play an important role in allergic and inflammatory diseases. Neutrophils process LTA₄ mainly to LTB₄ which appears to be a regulator of neutrophil function. In mononuclear cells and basophils LTA₄ combines with glutathione in a reaction catalyzed by glutathione-S-transferase which results in the formation of leukotrienes C₄ and D₄. The latter are components of the slow-reacting substance of anaphylaxis (SRS-A) and have the ability to induce smooth muscle contraction and increase vascular permeability in small blood vessels. The lipoxygenase pathway described here is not affected by aspirin and may account for some of the side-effects of aspirin ingestion. Neutrophils can utilize metabolic intermediates from aspirin-treated platelets for the production of leukotrienes and other hydroxy acids. (Courtesy of the Upjohn Co.)

Figure 4. Metabolic fate of released arachidonate as currently understood. Following its release from phospholipid, some arachidonate can leave the cell. It can then be processed by a cell in the immediate microenvironment. In the absence of aspirin a portion of the 20:4 is rapidly converted to endoperoxide by cyclooxygenase. The lipoxygenase also acts on arachidonate and continues to do so until the substrate is no longer available. In the presence of aspirin, arachidonate is diverted away from cyclooxygenase toward the other pathways (fig. 4). As already mentioned, free arachidonate and hydroxy acids can also react with other cells in the microenvi-
vironment. We do not know the effects of excess free arachidonate in the aspirin-treated platelet on reacyla-
tion reactions or binding to albumin. On theoretical
grounds a platelet phospholipase inhibitor might be
effective, since all pathways of arachidonate metab-
olism would be blocked. Thromboxane synthetase in-
hibitors are now under study since they may also pro-
mote PGi₂ production in the absence of TXA₂ via
endoperoxide transfer from platelets to endothelial
cells.¹⁴

Unfortunately this report must be concluded in a
paradoxical and rather unscientifc manner. We still
recommend low-dose aspirin and dipyridamole as a
therapeutic modality for patients with ischemic vascu-
lar diseases, but only because they appear to be better
than nothing and may not be harmful. Nevertheless
one should keep in mind³⁵ the statement by Sir Karl
Popper: "Science really is nothing more than a con-
tinuous abandonment of ideas shown by objective,
scientific inquiry, to be untenable." Thus, further
clinical trials at this time would not seem to be indicat-
ed because we do not know: a) which patient sub-
groups to select, b) which mechanisms of thrombosis
predominate in a given patient — thrombin formation
or aggregation as induced by ADP and thromboxane,
c) the optimal dose to employ, and d) the implications
of cell-cell interactions via the arachidonate pathway.

References
1. Radley JM, Haller CJ. The demarcation membrane system of the
2. Bradlow BA. Intravascular coagulation and fibrinolysis. S Afr J
3. Grober SE, Hawiger J. Evidence that changes in platelet cyclic
AMP levels regulate the fibrinogen receptor on human platelets. J
Biol Chem 257: 14606–14609, 1982
4. Niewiarowski S, Varma KG. Biochemistry and physiology of se-
creted platelet proteins. In: Colman RW, Hirsh J, Marder VJ,
Saltzman EW, eds. Hemostasis and thrombosis: Basic principles
5. Didisheim P, Fuster V. Actions and clinical status of platelet-
6. Packham MA, Mustard JF. Pharmacology of platelet-affecting
7. Mustard JF, Packham MA. Role of platelets in stroke and transient
ischemic attack. In: Conn HL, Jr, DeFelice E, Kuo PT, eds. Pro-
8. Weiss HJ. Platelets: Pathophysiology and antiplatelet drug ther-
apy. New York: Alan R. Liss, 1982
9. Bradlow BA. The role of platelets in ischaemic cerebro-vascular
Persico P. Macrocortin: a polypeptide causing the anti-phospholi-
han CN, Rutherford LE, Korchak HM, Weissmann G. Formation
of leukotrienes and other hydrox y acids during platelet-neutrophil
interactions in vitro. Biochem Biophys Res Comm 109: 130–137,
1982
12. Bressler NM, Broekman MJ, Marcus AJ. Concurrent studies of
oxygen consumption and aggregation in stimulated human plate-
14. Marcus AJ, Weksler BB, Jaffe EA, Broekman MJ. Synthesis of
prostacyclin from platelet-derived endoperoxides by cultured hu-
307: 73–78, 1982
of the toes with palpable peripheral pulses. Response to
17. McKenna R, Galante J, Bachmann F, Wallace DL, Kaushal SP,
Meredith P. Prevention of venous thromboembolism after total
knee replacement by high-dose aspirin or intermittent calf and thigh
Kurland LT. Effect of aspirin on prevention of coronary and cere-
brovascular disease in patients with rheumatoid arthritis. Mayo
19. Davis RF, Engleman EG. Incidence of myocardial infarction in
20. M. Souf J, Fruteau de Laclos B, Borget P. Stimulation of leuko-
triene biosynthesis in human blood leukocytes by platelet-derived
12-hydroperoxy-icosatetraenoic acid. Proc Natl Acad Sci USA 79:
6042–6046, 1982
Corey EJ. Studies on the mechanism of formation of the 5S,12S-
dihydroxy-6,8,10,14(E, E, E, Z)-icosatetraenoic acid in leukocytes.
Prostaglandins 23: 713–724, 1982
of arachidonic acid by human platelet lipoygenase. Biochem
Biophys Res Comm 95: 1090–1097, 1980
tions.
25. Eting R. The principle of inverse irreversibility. New Scientist
96: 808–810, 1982
Recent progress in the role of platelets in occlusive vascular disease.

A J Marcus

Stroke. 1983;14:475-479
doi: 10.1161/01.STR.14.4.475

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/14/4/475.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/