

## Inhibition of platelet prostaglandin synthetase by oral aspirin.

J W Burch, ... , N Stanford, P W Majerus

*J Clin Invest.* 1978;61(2):314-319. <https://doi.org/10.1172/JCI108941>.

**Research Article**

Aspirin inhibits platelet function by permanently acetylating the cyclooxygenase that forms prostaglandins. We determined the sensitivity of platelets to aspirin in normal subjects by measuring [3H-acetyl]aspirin-susceptible cyclooxygenase in washed platelets obtained at various times after aspirin ingestion. A single 325-mg aspirin dose inactivated 89% of platelet cyclooxygenase. The inhibition persisted for 2 days suggesting that oral aspirin also inactivated megakaryocyte cyclooxygenase. Thereafter, active enzyme returned with a time-course reflecting platelet turnover (life-span 8.2±2 days). Single doses of 20-650 mg aspirin resulted in 34- greater than 95% inhibition after 24 h. Daily doses of 20-325 mg aspirin for brief periods produced 61- greater than 95% inactivation when measured 24 h after cessation of the drug. Platelet cyclooxygenase is more sensitive to inactivation by aspirin than enzyme in sheep seminal vesicles.

**Find the latest version:**

<https://jci.me/108941/pdf>



# Inhibition of Platelet Prostaglandin Synthetase by Oral Aspirin

JOHN W. BURCH, NANCY STANFORD, and PHILIP W. MAJERUS, *Division of Hematology-Oncology, Departments of Internal Medicine and Biochemistry, Washington University School of Medicine, St. Louis, Missouri 63110*

**ABSTRACT** Aspirin inhibits platelet function by permanently acetylating the cyclooxygenase that forms prostaglandins. We determined the sensitivity of platelets to aspirin in normal subjects by measuring [<sup>3</sup>H-acetyl]aspirin-susceptible cyclooxygenase in washed platelets obtained at various times after aspirin ingestion. A single 325-mg aspirin dose inactivated 89% of platelet cyclooxygenase. The inhibition persisted for 2 days suggesting that oral aspirin also inactivated megakaryocyte cyclooxygenase. Thereafter, active enzyme returned with a time-course reflecting platelet turnover (life-span 8.2±2 days). Single doses of 20–650 mg aspirin resulted in 34–>95% inhibition after 24 h. Daily doses of 20–325 mg aspirin for brief periods produced 61–>95% inactivation when measured 24 h after cessation of the drug. Platelet cyclooxygenase is more sensitive to inactivation by aspirin than enzyme in sheep seminal vesicles.

## INTRODUCTION

Platelets are involved in the generation and propagation of thrombi, particularly arterial thrombi (2, 3). They may also be important in the pathogenesis of atherosclerosis (4). A number of clinical trials have been initiated attempting to define the usefulness of antiplatelet agents in disease states associated with embolic or thrombotic phenomena (5, 6). Recent randomized controlled studies utilizing aspirin for prevention of death from a second myocardial infarction suggest that the drug may be effective although the results are not conclusive (7, 8).

Aspirin ingestion alters platelet function *in vivo*, as evidenced by a prolonged bleeding time (9), and alters

the results of some *in vitro* platelet function tests (10, 11). These effects are due to the inhibition of prostaglandin biosynthesis by aspirin (12). Aspirin irreversibly acetylates cyclooxygenase (prostaglandin synthetase), the initial enzyme in the prostaglandin biosynthetic pathway (13, 14). The aspirin concentration required for enzyme inactivation is the same as that required to inhibit platelet function (14). In platelets the product of cyclooxygenase, prostaglandin G<sub>2</sub> (PGG<sub>2</sub>),<sup>1</sup> is converted to thromboxane A<sub>2</sub> (15), which can stimulate the platelet release reaction. (PGG<sub>2</sub> can also cause platelet aggregation and release, although it is less potent than thromboxane A<sub>2</sub>.) Thromboxane A<sub>2</sub> is a potent vasoconstrictor; its release into the plasma (16) could mediate the vasospasm accompanying thrombotic episodes. Therefore, by blocking the production of PGG<sub>2</sub> and thromboxane A<sub>2</sub> in platelets, aspirin may have an antithrombotic effect. In arterial tissues one product of arachidonic acid metabolism is prostaglandin I<sub>2</sub> (PGI<sub>2</sub>, 17), a compound which is a vasodilator and which inhibits platelet aggregation *in vitro*. Prostaglandin I<sub>2</sub> may serve a protective function physiologically by preventing platelet aggregation and vasoconstriction in undamaged areas of the vascular tree. Aspirin, by blocking the production of potentially protective compounds such as PGI<sub>2</sub> in addition to thromboxane A<sub>2</sub>, could have unfavorable effects. O'Brien (10) has shown that platelet function can be inhibited by oral doses of aspirin as small as 160 mg. We recently have demonstrated that cyclooxygenase in cultured smooth muscle cells from human aorta and in skin fibroblasts is more resistant to inactivation by aspirin than the platelet enzyme (18). In theory an aspirin dose which inhibits only the platelet enzyme would be the most reasonable one to evaluate for an antithrombotic effect and should be associated with the least side effects.

This work was presented in part at the Annual Meeting of The Association of American Physicians, Washington, D. C., 1 May 1977 (1).

Received for publication 2 September 1977 and in revised form 11 October 1977.

<sup>1</sup> Abbreviation used in this paper: PG, prostaglandin (used variously according to the identification of a given prostaglandin, i.e., PGG<sub>2</sub> or PGI<sub>2</sub>).

The acetylation of cyclooxygenase by aspirin is an irreversible reaction. Inasmuch as platelets synthesize negligible amounts of protein, the level of acetylation achieved by an *in vivo* exposure of platelets to aspirin persists for the lifetime of the platelets. Therefore, the ability of ingested aspirin to inhibit subsequent *in vitro* acetylation of cyclooxygenase in washed platelets by [<sup>3</sup>H-acetyl]aspirin (14) reflects the degree of prostaglandin synthesis inhibition achieved by various doses of aspirin. We have used this method to determine the sensitivity of platelets to oral aspirin.

## METHODS

22 normal subjects, ages 22–57, did not take aspirin for at least 14 days before each phase of the study. Several subjects were used repeatedly. Single doses of aspirin were given before breakfast to subjects who remained fasting for 1 h thereafter. Subjects were not necessarily fasting at the time of drug ingestion during the continuous dose studies. Blood was collected in 20- or 30-ml samples; maximum blood loss incurred by any one subject was 360 ml over a period of 2 mo. Informed consent was obtained from all participants.

An acetone-pentane powder of sheep seminal vesicles (19) was used in the *in vitro* experiments. [<sup>3</sup>H-acetyl]Aspirin, specific activity 177 Ci/mol, was synthesized (14). Aspirin tablets, 325 mg and 80 mg, were purchased from Parke, Davis & Co., Detroit, Mich., and Plough, Inc., Memphis, Tenn., respectively. 20-mg aspirin capsules were prepared from acetylsalicylic acid (United States Pharmacopeia powder) purchased from Merck, Sharp & Dohme Div., Merck and Co., Inc., West Point, Pa. Tablets and capsules were assayed for aspirin content (13). Diflunisal was obtained from Dr. T. Y. Shen of the Merck, Sharp & Dohme Research Laboratory, Rahway, N. J. Arachidonic acid was purchased from NuChek Prep, Elysian, Minn., and Nonidet P40 (NP-40, Shell trademark for polyoxyethylene glycol (9) *p*-*t*-octylphenol) was purchased from Particle Data Laboratories, Inc., Elmhurst, Ill. Stractan was a gift from St. Regis Paper Co., New York. All other chemicals were commercially obtained, reagent grade or better.

Venipunctures were performed with 19-gauge needles. Platelets were isolated and washed according to method II of Baenziger and Majerus (20). Platelet numbers were estimated by a "microhematocrit" method (21). Where noted below, platelets were isolated from whole blood by centrifugation through a discontinuous gradient of Stractan (22), washed twice in 0.033 M phosphate buffer, pH 6.5 (20), and resuspended in 0.015 M Tris buffer, pH 7.4 (20), before use.

Washed platelets ( $1 \times 10^9$  per ml) were incubated at 37°C for 30 min with 100  $\mu$ M [<sup>3</sup>H-acetyl]aspirin. The platelets were then sonicated (Biosonik, Bronwill Scientific, Rochester, N. Y., 45% probe intensity, 1 min) and centrifuged at 180,000 *g* for 30 min to obtain a particulate fraction. The supernate was discarded; the pellet was washed twice with distilled water and resuspended by sonication in distilled water. The suspension was then made 0.01 M in sodium phosphate, pH 7.0, and 0.5% in NP-40, and incubated at 37°C for 20 min. A second centrifugation at 180,000 *g* for 30 min removed insoluble material, and the protein concentration of the supernatant fraction was determined (23). The protein was then precipitated by the addition of 0.1 vol 50% (wt/vol) sucrose and 4 vol cold (–30°C) acetone and allowed to stand at 4°C overnight. This method results in >90% recovery of platelet cyclooxygenase (24). Under the conditions described above, cyclooxygenase is the only protein in the particulate fraction of human platelets that is acetylated by aspirin (14, 24).

The precipitate was collected by centrifugation at 15,000 *g* for 10 min and was dissolved in a mixture containing 0.01 M sodium phosphate, pH 7.4; 5% sodium dodecyl sulfate; 0.1 M 2-mercaptoethanol; 8% sucrose; and 0.01% bromophenyl blue. [<sup>3</sup>H-acetyl]cyclooxygenase content was then assayed by polyacrylamide gel electrophoresis as described previously (14).

A platelet life-span for each subject was calculated from the data presented in Fig. 1 by using the following steps. First, a "mean zero time" value for platelet cyclooxygenase activity was calculated by averaging the respective values for [<sup>3</sup>H-acetyl]aspirin acetylation obtained on days 0, 15 or 17, and 23 or 24. Next, a linear regression analysis for each subject was calculated by using data obtained on days 2, 4, and 8 or 9. Platelet life-span for each individual was estimated as the time traversed by the regression line from the cyclooxygenase activity on day 2 to the mean zero time cyclooxygenase activity for that particular individual.

*In vitro* comparison of platelet and sheep seminal vesicle cyclooxygenase acetylation was performed in 0.033 M phosphate buffer, pH 6.5 (20). Intact platelets or acetone-pentane powders of sheep seminal vesicles were incubated at 37°C with [<sup>3</sup>H-acetyl]aspirin. Periodic samples were taken, and the reaction was stopped by boiling in 5% sodium dodecyl sulfate, 0.1 M 2-mercaptoethanol. Radioactivity incorporated into cyclooxygenase with time was determined by polyacrylamide gel electrophoresis as described previously (14).

## RESULTS

Inactivation of platelet cyclooxygenase by oral aspirin was measured as a reduction in the ability of [<sup>3</sup>H-acetyl]-aspirin to acetylate the enzyme in washed platelets *in vitro*, as described in Methods. 16 subjects were given a single 325-mg aspirin dose on day 0. Tritium incorporation into platelet cyclooxygenase was measured in platelets from serial blood samples as shown in Fig. 1. We noted an initial fall in tritium incorporation to 11% of control values 6 h after aspirin ingestion. No new platelet enzyme appeared in the circulation for  $\cong 2$  days. Thereafter, unacetylated enzyme returned with a time-course consistent with platelet turnover. A platelet life-span of  $8.2 \pm 2$  days (mean  $\pm$  SD) was obtained for the 16 subjects. We interpret the "lag" in the return of unacetylated enzyme to the circulation as evidence that aspirin acetylates cyclooxygenase in the megakaryocyte. Platelet protein recovery per unit blood sample did not vary significantly (Student's *t* test, 25) at any time point; therefore, the lack of increment in tritium incorporation at 24 and 48 h implies that platelets entering the circulation during this time did not have more active cyclooxygenase than platelets in the circulation 6 h after aspirin ingestion. Furthermore, an identical pattern of enzyme recovery was seen after a single aspirin dose of 650 mg or a brief course of 325 mg per day (data not shown). Either of these latter doses will result in complete enzyme inactivation (acetylation) 6 h after ingestion.

Groups of subjects were given single aspirin doses of 20, 80, 160, and 650 mg. Unacetylated enzyme was measured at 24 h after ingestion and compared to levels measured immediately before ingestion (i.e., each sub-

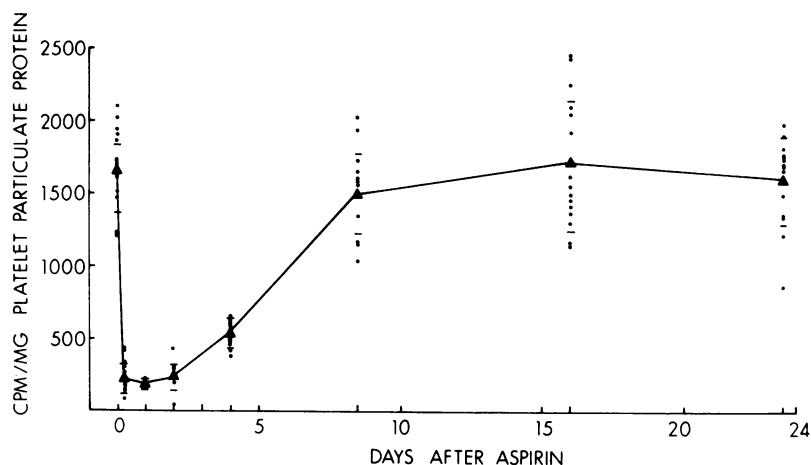


FIGURE 1 Reappearance of active (unacetylated) cyclooxygenase in the circulation after a single 325-mg aspirin dose. Values are presented as tritium incorporation (counts per minute) per milligram platelet particulate protein (i.e., that solubilized by NP-40; see Methods). Protein recovery did not vary significantly at the different time points. Triangles represent mean values; bars represent 1 SD from the mean; dots are individual values. The mean ( $\pm$ SD) value for all subjects before aspirin ingestion was  $1647 \pm 436$ . Values of  $<100$  cpm per mg platelet particulate protein ( $<5\%$  residual activity, 20–60 cpm per gel over background) are considered indistinguishable from zero.

ject served as his own control). We found a direct relationship between the degree of enzyme inactivation and the amount of aspirin ingested (Table I). In vitro, the acetylation of cyclooxygenase by aspirin follows pseudo-first order kinetics; that is, the degree of inactivation is directly proportional to the dose of aspirin and the time of exposure (see below). This relationship does not strictly hold when degree of acetylation is compared to oral aspirin dose, inasmuch as the relationship between ingested aspirin dose and effective plasma aspirin concentration is undoubtedly complex.

We also gave groups of subjects daily doses of 20, 80, and 325 mg per day for 6, 5, and 7 days, respectively, and measured acetylation 24 h after the last aspirin ingestion (Fig. 2). Again a direct relationship of dose to enzyme inhibition was seen. 20 mg aspirin per day

resulted in 61% inhibition of enzyme activity; 325 mg aspirin per day reduced enzyme activity to levels below that detectable with our assay.

Platelet isolation by method II of Baenziger and Majerus (20) results in approximately 30–50% yields of platelets from whole blood. To insure that we were not

TABLE I  
Platelet Cyclooxygenase Activity 24 Hours after Oral Aspirin in a Single Dose

Experiment	Aspirin dose	Subjects	Cyclooxygenase acetylation after aspirin	Inactivation
	mg	n	cpm/mg protein	%
A	20	7	$1,061 \pm 218$	34
B	80	4	$318 \pm 53$	73
C	160	4	$204 \pm 62$	82
D	325	16	$184 \pm 21$	89
E	650	3	$78 \pm 31$	$>95$

Mean  $\pm$  1 SD.

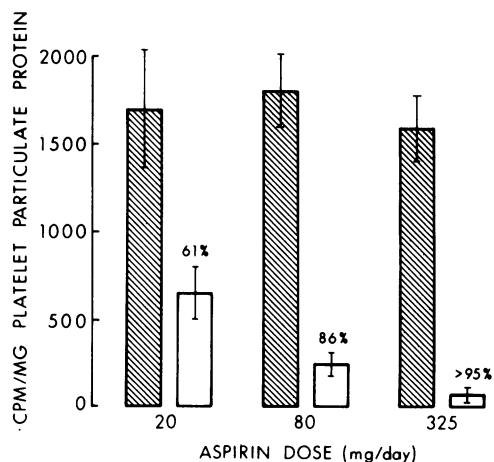


FIGURE 2 Effect of daily aspirin on platelet cyclooxygenase. Mean values  $\pm$  one SD are plotted. Cross-hatched bars represent mean cyclooxygenase level before aspirin ingestion. Solid bars represent mean cyclooxygenase level 24 h after cessation of drug. Percentage figures above each solid bar indicate the percent inhibition attained by the respective aspirin dose. Number of subjects participating in the experiment were 6, 8, and 6 for the 20-, 80-, and 325-mg doses, respectively.

isolating an especially sensitive subpopulation of platelets by our procedures, platelets were isolated by density gradient centrifugation in Stractan gradients, a method which yields >90% of platelets from whole blood (22). Platelet yields from 30 ml whole blood by our usual procedures averaged  $3.4 \times 10^9$ ; by using the Stractan method, yields averaged  $9.0 \times 10^9$ . No difference in residual enzyme activity was noted in platelets isolated by the two methods in two subjects after the ingestion of 325 mg aspirin daily for 6 days and 160 mg aspirin daily for 6 days, respectively; in both cases >95% inhibition was seen. In another experiment after a single 325-mg aspirin dose, there were 17% residual enzyme in platelets isolated by the standard method and 10% residual enzyme in platelets isolated using Stractan gradients. Therefore, our methods do not induce sampling errors that could account for the aspirin sensitivity noted above.

To assess further the relative sensitivity of platelets to aspirin, we evaluated cyclooxygenase activity in washed platelets exposed to aspirin *in vitro* and compared the results to cyclooxygenase in acetone-pentane powders of sheep seminal vesicles. These experiments were performed in the presence of a large excess of aspirin, resulting in pseudo-first order kinetics for the acetylation reaction<sup>2</sup>. In the presence of 100  $\mu\text{M}$  aspirin, the inactivation of sheep seminal vesicle enzyme proceeded with a  $t_{1/2}$  of 10 min yielding a  $k_{\text{apparent}}$  of  $0.069 \text{ min}^{-1}$ . In the presence of 10  $\mu\text{M}$  aspirin, platelet enzyme was inactivated with a  $t_{1/2}$  of 3.2 min and a  $k_{\text{apparent}}$  of  $0.217 \text{ min}^{-1}$ . We define a potency term as  $k_{\text{apparent}}/(\text{aspirin concentration})$ . The potency of aspirin toward sheep seminal vesicles is therefore  $690 \text{ min}^{-1}\text{M}^{-1}$ ; toward intact platelets it is  $21,700 \text{ min}^{-1}\text{M}^{-1}$ . This represents a 31-fold greater sensitivity of platelets relative to sheep seminal vesicles. The greater sensitivity of platelet enzyme is not due to transport of the drug by platelets because the same differences were observed when aspirin was added to disrupted rather than intact platelets. The same difference was also seen when fresh seminal vesicle microsomes were used instead of the acetone-pentane powder.

## DISCUSSION

We have determined the dose of aspirin required to inactivate cyclooxygenase by directly measuring the degree of acetylation of the enzyme. This method is

<sup>2</sup> A plot of  $\log$  [fraction unacetylated enzyme] versus time is linear. The slope of this line equals the apparent rate constant,  $k_{\text{apparent}}$ , and is determined as  $\ln 2$  divided by time to half acetylation. The apparent rate constant is directly proportional to aspirin concentration (13). Therefore, the "potency" term (see below), defined as  $k_{\text{apparent}}$  divided by aspirin concentration, reflects the rate constant of the reaction with various tissues.

much more sensitive than *in vitro* assays of platelet function or assays of products of prostaglandin synthesis such as malonaldehyde, and allows measurement of low levels of residual activity. Small single doses of aspirin cause prompt inactivation of enzyme; no new enzyme appears in circulating platelets for approximately 2 days indicating that megakaryocyte cyclooxygenase is also inactivated. O'Brien (10) initially suggested that megakaryocytes were altered by aspirin because of a similar lag period in recovery of *in vitro* platelet aggregation after aspirin. Several other studies of aspirin's effect on platelets are consistent with the hypothesis that megakaryocyte enzyme is inactivated (26–28). Alternative theories, such as an aspirin-sensitive nonexchangeable pool of new platelets in the spleen (29) would also explain a 2-day lag period; however, this seems to us to be a less likely possibility. Assuming a 2-day lag before enzyme recovery, we measured a platelet life-span of  $8.2 \pm 2$  days. This is consistent with other estimates of platelet life-span obtained by radioisotope methods (30) or by *N*-ethylmaleimide induced malonaldehyde formation after aspirin ingestion (31).

A daily dose of 20 mg aspirin per day will result in >50% enzyme inactivation. A variety of evidence suggests that other tissues may be less sensitive to aspirin. Thus, analgesic and antiinflammatory doses of aspirin range from 325 mg to 4 g per day (32). Halushka et al. (33) have shown that large doses (30 mg/kg IV) of aspirin in dogs completely block platelet aggregation and platelet arachidonic acid metabolism but have no effect on myocardial synthesis of immunoreactive prostaglandin E-like material. Paterno et al. (34) measured prostaglandin production in human synovial tissue and in intact platelets perfused with aspirin *in vitro*. They found that platelets were six-fold more sensitive to aspirin than synovium, whereas the tissues were equally sensitive to indomethacin and fenoprofen. Collier and Flower (35) and Horton et al. (36) have shown decreased, but persistent, prostaglandin content in human semen after aspirin doses of up to 7.2 g per day. Prostaglandin E content in human semen dropped by 58% (35) and 56% (36) after 2.4 and 3.6 g aspirin per day, respectively, compared to 61% enzyme inactivation which we observe in platelets after 20 mg aspirin per day. The results indicate wide *in vivo* differences in aspirin sensitivity. Comparison of the potency of aspirin in inhibiting sheep seminal vesicle cyclooxygenase vs. platelet enzyme *in vitro* showed that the platelet enzyme was 31-fold more sensitive to aspirin. We obtained similar results comparing cultured intact diploid human fibroblasts and human arterial (medial) smooth muscle cells to platelets (18). Whereas the *in vivo* differences in aspirin sensitivity of various tissues are probably due, in part, to drug distribution, the *in vitro* studies indicate that additional unknown factors con-

tribute to the difference. The sensitivity of platelets seems relatively specific for aspirin, because we have also studied an aspirin analog, diflunisal (5-[2,4-difluorophenyl]salicylic acid), and have found that seminal vesicle and platelet enzyme are equally inhibited by this drug (37).

Gryglewski et al. (17) have proposed that prostaglandin I<sub>2</sub>, which inhibits platelet aggregation and is a vasodilator, functions to prevent thrombosis and platelet plug formation. If this hypothesis is correct, aspirin may have opposing effects, i.e., to prevent thrombosis by interfering with platelet thromboxane A<sub>2</sub> production and to stimulate thrombosis by blocking PGI<sub>2</sub> production in arteries. They further speculate that arteries in vivo may form PGI<sub>2</sub> from endoperoxides secreted by platelets (38) as well as from arachidonic acid via arterial cyclooxygenase (39). It is presently unknown whether or not cyclooxygenase in human coronary arteries and other pertinent tissues is less sensitive to aspirin than the platelet enzyme. If so, the most effective antithrombotic dose of aspirin would be that which inhibits enzyme in platelets but not in other tissues. If vascular tissue depends on endoperoxides secreted by platelets to produce PGI<sub>2</sub>, then aspirin may not be useful as an antithrombotic agent.

The hemostatic defect caused by aspirin in normal subjects is a minor one, resulting in mild prolongation of the bleeding time. In fact, a patient with complete cyclooxygenase deficiency and only mild clinical symptoms has been described (40). These data and the fact that aspirin has only subtle effects on thrombin-induced platelet reactions indicate that metabolism of arachidonic acid via cyclooxygenase is not essential to platelet function. Rather, this pathway probably serves to modulate the reactions involved in platelet function, perhaps by altering the threshold stimulus necessary to initiate aggregation or release (41). By irreversibly blocking this pathway at low doses, aspirin would be a useful antithrombotic agent, assuming that the loss of this modulatory pathway favorably affects pathological thrombosis. Only forthcoming controlled clinical trials can verify this assumption.

The low level of side effects attributable to aspirin among occasional users and the fact that common major toxicities are dose-related (42) suggest that chronic aspirin ingestion in the dosage range of <325 mg per day may be well tolerated. Small doses of aspirin will inhibit platelet prostaglandin production to a high degree. It is even conceivable that doses of 20 mg per day might have antithrombotic action inasmuch as the degree to which cyclooxygenase must be inhibited to alter thrombosis is unknown.

#### ACKNOWLEDGMENTS

This research was supported by grants HL 14147 (Specialized Center for Research in Thrombosis), HL 07088, and HL 16634 from the National Institutes of Health.

#### REFERENCES

1. Burch, J. W., N. Stanford, and P. W. Majerus. 1977. Inhibition of platelet cyclo-oxygenase by oral aspirin. *Clin. Res.* **25**: 513A.
2. Thomas, D. P. 1972. The platelet contribution to arterial and venous thrombosis. *Clin. Haematol.* **1**: 267-282.
3. Mustard, J. F. 1976. Function of blood platelets and their role in thrombosis. *Trans. Am. Clin. Climatol. Assoc.* **87**: 104-127.
4. Ross, R., and J. A. Glomset. 1976. The pathogenesis of atherosclerosis. *N. Engl. J. Med.* **295**: 369-377, 420-425.
5. Verstraete, M. 1976. Are agents affecting platelet functions clinically useful? *Am. J. Med.* **61**: 897-914.
6. Weiss, H. J. 1976. Antiplatelet drugs—a new pharmacologic approach to the prevention of thrombosis. *Am. Heart J.* **92**: 86-102.
7. Elwood, P. C., A. L. Cochrane, M. L. Burr, P. M. Sweetnam, G. Williams, E. Welsby, S. J. Hughes, and R. Renton. 1976. A randomized controlled trial of acetyl salicylic acid in the secondary prevention of mortality from myocardial infarction. *Br. Med. J.* **1**: 436-440.
8. The Coronary Drug Project Research Group. 1976. Aspirin in coronary heart disease. *J. Chronic Dis.* **29**: 625-642.
9. Mielke, C. H., Jr., M. M. Kaneshiro, I. A. Maher, J. M. Weiner, and S. I. Rapaport. 1969. The standardized normal Ivy bleeding time and its prolongation by aspirin. *Blood.* **34**: 204-215.
10. O'Brien, J. R. 1968. Effects of salicylates on human platelets. *Lancet.* **1**: 779-783.
11. Weiss, H. J., L. M. Aledort, and S. Kochwa. 1968. The effect of salicylates on the hemostatic properties of platelets in man. *J. Clin. Invest.* **47**: 2169-2180.
12. Smith, J. B., and A. L. Willis. 1971. Aspirin selectively inhibits prostaglandin production in human platelets. *Nat. New Biol.* **231**: 235-237.
13. Roth, G. J., N. Stanford, and P. W. Majerus. 1975. Acetylation of prostaglandin synthase by aspirin. *Proc. Natl. Acad. Sci. U. S. A.* **72**: 3073-3076.
14. Roth, G. J., and P. W. Majerus. 1975. The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. *J. Clin. Invest.* **56**: 624-632.
15. Hamberg, M., J. Svensson, and B. Samuelsson. 1975. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci. U. S. A.* **72**: 2994-2998.
16. Smith, J. B., C. Ingeman, and M. J. Silver. 1976. Persistence of thromboxane A<sub>2</sub>-like material and platelet release-inducing activity in plasma. *J. Clin. Invest.* **58**: 1119-1122.
17. Gryglewski, R. J., S. Bunting, S. Moncada, R. J. Flower, and J. R. Vane. 1976. Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins.* **12**: 685-713.
18. Baenziger, N. L., M. J. Dillender, and P. W. Majerus. 1977. Cultured human skin fibroblasts and arterial cells produce a labile platelet-inhibitory prostaglandin. *Biochem. Biophys. Res. Commun.* **78**: 294-301.
19. Wallach, D. P., and E. G. Daniels. 1971. Properties of a novel preparation of prostaglandin synthetase from sheep seminal vesicles. *Biochim. Biophys. Acta.* **231**: 445-457.
20. Baenziger, N. L., and P. W. Majerus. 1974. Isolation of human platelets and platelet surface membranes. *Methods Enzymol.* **31**: 149-155.
21. Tollefsen, D. M., J. R. Feagler, and P. W. Majerus. 1974.

- The binding of thrombin to the surface of human platelets. *J. Biol. Chem.* **249**: 2646–2651.
22. Corash, L., H. Tan, and H. R. Gralnick. 1977. Heterogeneity of human whole blood platelet subpopulations. I. Relationship between buoyant density, cell volume, and ultrastructure. *Blood.* **49**: 71–87.
  23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 264–275.
  24. Stanford, N., G. J. Roth, T. Y. Shen, and P. W. Majerus. 1977. Lack of covalent modification of prostaglandin synthetase (cyclo-oxygenase) by indomethacin. *Prostaglandins.* **13**: 669–675.
  25. Remington, R. D., and M. A. Schork. 1970. Statistics with Applications to the Biological and Health Sciences. Prentice-Hall, Inc., Englewood Cliffs, N. J. 148–191.
  26. Kocsis, J. J., J. Hernandez, M. J. Silver, J. B. Smith, and C. Ingberman. 1973. Duration of inhibition of platelet prostaglandin formation and aggregation by ingested aspirin or indomethacin. *Prostaglandins.* **3**: 141–144.
  27. Schwartz, A. D. 1974. A method for demonstrating shortened platelet survival utilizing recovery from aspirin effect. *J. Pediatr.* **84**: 350–354.
  28. Jafari, E., A. Saleem, B. S. Shaikh, and L. D. Demers. 1976. Effect of aspirin on prostaglandin synthesis by human platelets. *Prostaglandins.* **12**: 829–835.
  29. Shulman, N. R., S. P. Watkins, Jr., S. B. Itscoitz, and A. B. Students. 1968. Evidence that the spleen retains the youngest and hemostatically most effective platelets. *Trans. Assoc. Am. Physicians.* **81**: 302–313.
  30. Abrahamsen, A. F. 1968. Platelet survival studies in man, with special reference to thrombosis and atherosclerosis. *Scand. J. Haematol. Suppl.* **3**: 1–53.
  31. Stuart, M. J., S. Murphy, and F. A. Oski. 1975. A simple nonradioisotope technique for the determination of platelet life-span. *N. Engl. J. Med.* **292**: 1310–1313.
  32. Woodbury, D. M., and E. Fingl. 1975. Analgesic-antipyretics, anti-inflammatory agents, and drugs employed in the therapy of gout. In *The Pharmacological Basis of Therapeutics*. L. S. Goodman and A. Gilman, editors. Macmillan Publishing Co., Inc. New York. 5th edition. 325–358.
  33. Halushka, P. V., H. B. Daniell, W. L. Miller, Jr., and H. Thiboudeaux. 1977. Increased coronary sinus prostaglandin E-like material (iPGE) during myocardial infarction (MI): beneficial effects of aspirin (ASA). *Clin. Res.* **25**: 225A.
  34. Patrono, C., G. Ciabattini, F. Greco, and D. Grossi-Belloni. 1976. Comparative evaluation of the inhibitory effects of aspirin-like drugs on prostaglandin production by human platelets and synovial tissue. *Adv. Prostaglandin Thromboxane Res.* **1**: 125–131.
  35. Collier, J. C., and R. J. Flower. 1971. Effect of aspirin on human seminal prostaglandins. *Lancet.* **II**: 852–853.
  36. Horton, E. W., R. L. Jones, and C. G. Marr. 1973. Effects of aspirin on prostaglandin and fructose levels in human semen. *J. Reprod. Fertil.* **33**: 385–392.
  37. Majerus, P. W., and N. Stanford. 1977. Comparative effects of aspirin and diflunisal on prostaglandin synthetase from human platelets and sheep seminal vesicles. *Br. J. Clin. Pharmacol.* **4**: 155–185.
  38. Moncada, S., R. J. Gryglewski, S. Bunting, and J. R. Vane. 1976. A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. *Prostaglandins.* **12**: 715–737.
  39. Bunting, S., R. Gryglewski, S. Moncada, and J. R. Vane. 1976. Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins.* **12**: 897–913.
  40. Malmsten, C., M. Hamberg, J. Svensson, and B. Samuelsson. 1975. Physiological role of an endoperoxide in human platelets: hemostatic defect due to platelet cyclooxygenase deficiency. *Proc. Natl. Acad. Sci. U. S. A.* **72**: 1446–1450.
  41. Majerus, P. W. 1976. Why aspirin? *Circulation.* **54**: 357–359.
  42. Boston Collaborative Drug Surveillance Program. 1974. Aspirin use in patients with major upper gastrointestinal bleeding and peptic-ulcer disease. *N. Engl. J. Med.* **290**: 1158–1162.