Yale University
EliScholar – A Digital Platform for Scholarly Publishing at Yale

Yale Medicine Thesis Digital Library | School of Medicine

1972

Dipyridamole and aspirin tested against an experimental model of thrombosis

John Edmund Mayer Jr.
Yale University

Follow this and additional works at: http://elischolar.library.yale.edu/ymtdl

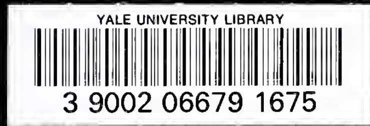
Part of the Medicine and Health Sciences Commons

Recommended Citation

Mayer, John Edmund Jr., "Dipyridamole and aspirin tested against an experimental model of thrombosis" (1972). *Yale Medicine Thesis Digital Library*. 2907.
http://elischolar.library.yale.edu/ymtdl/2907

This Open Access Thesis is brought to you for free and open access by the School of Medicine at EliScholar – A Digital Platform for Scholarly Publishing at Yale. It has been accepted for inclusion in Yale Medicine Thesis Digital Library by an authorized administrator of EliScholar – A Digital Platform for Scholarly Publishing at Yale. For more information, please contact elischolar@yale.edu.

T113
+Y12
3243



DIPYRIDAMOLE AND ASPIRIN TESTED AGAINST AN EXPERIMENTAL MODEL OF THROMBOSIS

JOHN EDMUND MAYER, JR.

1972

MUDD
LIBRARY
Medical

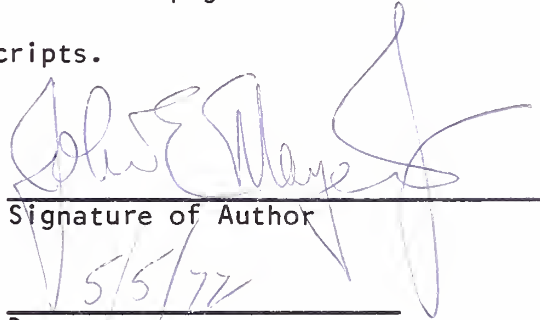
YALE

MEDICAL LIBRARY

Permission for photocopying or microfilming of " DIPYRIDAMOLE AND ASPIRIN TESTED AGAINST AN EXPERIMENTAL MODEL OF THROMBOSIS "

(TITLE OF THESIS)

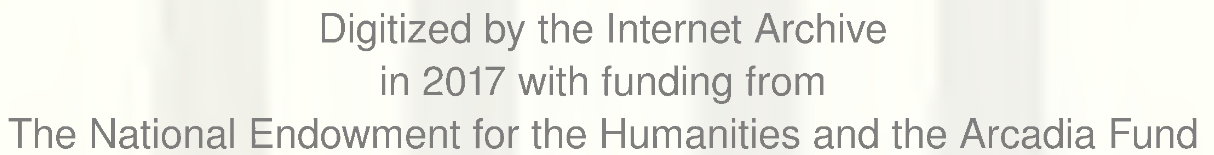
for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.



Signature of Author

5/5/72

Date


Digitized by the Internet Archive
in 2017 with funding from
The National Endowment for the Humanities and the Arcadia Fund

https://archive.org/details/dipyridamoleaspi00maye

DIPYRIDAMOLE AND ASPIRIN TESTED AGAINST AN EXPERIMENTAL MODEL OF THROMBOSIS

by

John Edmund Mayer, Jr.

B.A. Yale College, 1968

PRESENTED TO THE DEPARTMENT OF SURGERY

YALE UNIVERSITY SCHOOL OF MEDICINE

IN PARTIAL FULFILLMENT OF REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF MEDICINE

NEW HAVEN, CONNECTICUT

APRIL 1, 1972

ACKNOWLEDGMENTS

Graeme L. Hammond, M.D. for his support, guidance, and encouragement in the preparation of this work.

Mrs. Dorothy Farrell for expert assistance, advice, patience, and perpetual good humor over many months of work.

Messers. Sam Gilpin, Walter DeLuca, Spiro Condos, and Mitchell Goins for expert operative assistance and humane animal care.

And special thanks to my wife for patience, occasional operative assistance, and unfailing encouragement and optimism.

Dipyridamole was kindly supplied by Geigy Pharmaceuticals.

DIPYRIDAMOLE AND ASPIRIN TESTED AGAINST AN EXPERIMENTAL MODEL OF THROMBOSIS

Table of Contents

Page

INTRODUCTION.. 1

Review of the Literature.. 1

MATERIALS AND METHODS.. 16

RESULTS... 19

DISCUSSION.. 21

FIGURE LEGEND... 26

LITERATURE CITED.. 31

INTRODUCTION

Arterial reconstructive surgery has been attended by a modest early failure rate due to thrombosis of endarterectomized segments or vein bypass grafts.[22] With the advent of coronary artery surgery, the importance of avoiding early failure due to thrombosis assumes even greater importance as thrombosis can result not only in failure to increase blood supply to the myocardium but in mortality as well.[25] Thrombosis after surgical or other trauma to a vessel wall is most likely initiated by adherence of platelets to the site of injury.[58] Therefore, attempts to reduce the incidence of thrombosis and consequent failure of reconstructive vascular surgery can be directed at interrupting the initial platelet mediated steps. A number of pharmacologic agents have been shown in vitro and in vivo to affect platelets,[59] and an attempt has been made to apply one of these, dextran, to arterial surgery in clinical practice.[31] The present studies then were directed at the development of a model for intra-arterial thrombosis which would clot consistently and which would resemble the clinical situation. Then, the ability of two agents previously shown to have effects on platelets, dipyridamole and aspirin, to alter the rate of thrombosis were tested against this model.

Review of the Literature:

Early failure due to thrombosis constitutes an important problem in most large series of arterial reconstructive procedures for

ischemic vascular disease of the lower limb. Gutelius *et al*. reported early failure rates of 18% for saphenous vein grafts and 30% for endarterectomy in treating superficial femoral artery disease.[38] DeWeese *et al*. reported a 28% early failure rate in femoro-popliteal saphenous vein grafts[23] and 15% early failures with endarterectomy.[22] Darling *et al*. were somewhat more successful with only 7.8% early failures with femoro-popliteal vein grafts.[20] Vein grafts to tibial artery segments with both proximal and distal arterial occlusions thrombosed in 9 or 31 patients reported by Mannick *et al*.[52] In vein grafts to the distal tibial arteries at the ankle, Garret *et al*. reported 7 early failures due to thrombosis in 57 cases.[34] Harrison and his group reported a 100% patency rate at the end of hospitalization in 25 patients with superficial femoral artery disease utilizing "eversion thromboendarterectomy", but 4 (16%) required reoperation before discharge because of thrombosis of reconstructed segments.[41] Similar results were obtained by Connolly *et al*. with eversion thromboendarterectomy.[14] Each of these series of reconstructive procedures then was attended by a significant early failure due to thrombosis.

Coronary artery surgery, although less extensively reported because of its recent advent, shows similar early failure rates. Dilley *et al*. in an early report of endarterectomy for coronary disease indicated that 12% of their 25 patients died with thrombosis in the endarterectomized segment.[25] Mitchel and his group reported a series of 128 patients with saphenous vein grafts in whom 10 had thrombosed at least one graft.[56] However, 8 additional patients died without any report on the status of their grafts.[56] Green *et al*. described 6 patients

with thrombosed vein grafts among 82 cases but the total early graft failure rate was again not evaluated.[36] Early thrombosis of a graft or endarterectomized segment may be a significant problem in coronary artery surgery as it has been in peripheral vascular surgery.

Substantial evidence exists that platelets play a central role in thrombus formation after injury to the vessel wall. Bizzozero depicted the role of platelets in thrombus formation in 1882.[6] Ashford and Freiman found that simply grasping a vessel with a forceps produced disruption of the endothelium of the vessel wall and resulted in the formation of a thrombus composed primarily of platelets at the site of injury.[3] French _et al_. found that after electrical injury to the wall of a small vessel, endothelial cells were lost from the intima of the vessel wall and that platelet thrombi appeared at these sites within a few seconds.[32] Spaet and Erichson[72] and Baumgartner _et al_.[5] reported similar findings regarding the role of platelets in thrombosis after vascular injury. Zucker,[82] French _et al_.,[32] and Hovig _et al_.[47] have also emphasized the primary role of platelets in the arrest of bleeding from the end of a transected artery. The importance of platelets in thrombosis after arterial surgery has been demonstrated by Evans and Mustard who found a significant correlation between platelet adhesiveness in a glass bead column and the onset of thrombosis after dacron grafts, endarterectomy, or saphenous vein grafts.[29] In addition, Rowsell _et al_. have found that thrombocytopenic swine will not form thrombi in an extracorporeal shunt unless they are transfused with platelet rich plasma.[68] Thus, platelets play a primary role in thrombus formation after injury to the vessel wall, and it seems very likely that they are also involved in thrombosis after arterial reconstructive surgery.

The evolution of a platelet thrombus after vascular injury involves a fairly well understood sequence of events from the initial sticking of platelets to the vessel wall through growth of the thrombus, stabilization, and ultimately organization. Anatomic studies[3,32,72] have shown that the initial event in thrombus formation is the adherence of platelets to subendothelial structures after loss of the endothelial cells, or to perivascular connective tissue fibers after transection of small vessels. The substance in the perivascular tissue which induces platelet aggregation has been reasonably certainly identified as collagen by electron microscopy.[32,47] In vitro studies[45,80,83] have clearly demonstrated the ability of collagen to induce platelet aggregation. The loss of native structure by heating, by treatment with collagenase, or by blocking the epsilon amino groups of lysine of the collagen molecule results in the loss of the ability of collagen to induce platelet aggregation.[80] The nature of the substance in the subendothelial layer of the vessel wall which induces platelet aggregation after loss of the endothelium is still somewhat in doubt. French and his co-workers described platelets adhering to elastic tissue and fine fibers.[32] Spaet and Erichson concluded, however, that collagen was the active substance in the subendothelial layer as suspensions of elastic tissue did not induce platelet aggregation in vitro.[72] Baumgartner _et al_. more recently have described the adherence of platelets to a non-collagenous subendothelial structure after diffuse injury to the aorta in an experimental animal.[5] Hughes and Mahieu have shown that basement membrane prepared from renal tubules can induce platelet aggregation in vitro and have hypothesized that the basement membrane in vessels is

the important substance in bringing about platelet aggregation in vivo.[5] Several investigators[3,32,47,72] have stated that fibrin formation does not seem to precede platelet adherence to any of these structures. The initial step in thrombus formation then is the adherence of platelets to one or more subendothelial or perivascular structures.

The exposure of platelets to these subendothelial and perivascular structures results not only in the adherence of platelets, but also in morphologic changes in the platelets and the release of specific active substances to induce further platelet aggregation. Platelets in contact with collagen fibers in vivo show formation of pseudopodia,[76] loss of granules,[32] and breaks in the cell membrane,[32] and similar changes have been demonstrated in vitro with collagen induced platelet aggregation.[45] These morphologic changes are associated with the release of compounds which show further aggregating activity toward platelets, including adenosine diphosphate (ADP),[46,54,73] serotonin,[54] and epinephrine,[59,75] in a calcium[73] and energy dependent process.[50] ADP was initially demonstrated to aggregate platelets in vitro by Gaarder et al.[33] and Born,[7] and has subsequently been shown to produce changes in platelet shape similar to those found in a developing platelet plug.[11] The application of ADP to areas of minor injury in vessels leads to the enhancement of white thrombus formation,[43,44] and the intravenous infusion of ADP can increase the amount of thrombus formed in an extracorporeal shunt, decrease the clotting time, transiently decrease the platelet count, and cause the formation of platelet aggregates in the vasculature of a variety of different organs.[60] In addition, ADP in sufficient concentration can cause the release of more ADP and serotonin

from platelets.[54] Serotonin has also been shown to be capable of producing platelet aggregation,[62] and of increasing the rate of thrombus formation in areas of minor injury to a vessel wall.[43,44] Along with ADP and serotonin, catecholamines have been shown to have potent effects on platelet aggregation. Epinephrine and norepinephrine can induce platelet aggregation in vitro,[54,63] and can bring about the release of ADP and serotonin from platelets.[54] Epinephrine can also markedly enhance the ability of collagen,[75] thrombin,[75] and ADP[2] to induce platelet aggregation. Of these three active agents released by the platelet collagen interaction, it is currently believed that ADP is the most important physiologically.[59] The most compelling evidence for this hypothesis comes from the observations that inhibitors of ADP aggregation can inhibit aggregation induced by a variety of different agents including serotonin,[62] epinephrine,[62] thrombin,[42,62] and collagen,[73] while antagonists of catecholamines,[59] serotonin,[43] and thrombin[83] do not affect ADP mediated platelet aggregation. Thus, the initial stages of thrombus formation and growth after vascular injury result from a specific interaction between collagen or other substances in the vessel wall and platelets from the blood. This leads, in turn, to specific morphologic changes in the platelets and the release of active compounds which then induce more platelets to aggregate around the site of injury.

The future of the platelet thrombus at the site of injury then depends in part on the activation of the plasma coagulation system. The aggregation of platelets in vitro by collagen, ADP, epinephrine, and serotonin is associated with platelet factor 3 becoming available to activate the plasma coagulation system.[39] This "factor" is thought

to be a lipoprotein moiety which remains attached to the platelet membrane and serves as a catalytic site for the interaction of plasma coagulation factors.[39,53] The activation of the plasma coagulation factors results in the generation of thrombin and then fibrin,[53] which can in turn influence the fate of the platelet plug.[53,58] Thrombin has actions similar to those of collagen in inducing platelet aggregation, producing morphologic changes, and causing the release of active substances.[42,54,62,73] In addition to its direct effects on platelets, thrombin catalyses the conversion of fibrinogen to fibrin.[53] Both Zucker[82] and Hovig and his group[47] have demonstrated that the formation of fibrin is necessary for the stabilization of the platelet plug. In experimental animals with clotting factor deficiencies who do not form fibrin, the initial arrest of bleeding from the transected end of a vessel by the formation of a platelet plug is followed by a high incidence of re-bleeding.[47,82] The plasma coagulation system then is important in the growth and stabilization of the platelet thrombus.

There is also some inconclusive evidence that the fibrinogen component of the coagulation system is important in primary platelet aggregation as well. Cross found in vitro that the addition of fibrinogen or plasma to washed platelets led to a marked increase in the response of platelets to ADP.[16] Similar findings have been reported by Weiss and Rogers in the study of two patients with cogenital afibrinogenemia.[79] However, Gugler and Luscher reported normal platelet aggregation in a similar patient.[37] The exact role of fibrinogen in the primary processes of platelet aggregation remains somewhat unclear.

With functioning platelet aggregation and plasma coagulation systems, the final determinant of the extent of the thrombus formed after vascular injury depends on the patterns of blood flow in the area of the injury.[58] Mustard _et al_. theorized that laminar flow would favor the restriction of further thrombus growth by diluting the ADP, serotonin, epinphrine, and thrombin as they are formed from the developing platelet plug.[58] Disturbed flow with eddy formation and areas of stagnation would allow the buildup of these active substances and favor the further growth of the thrombus.[58] This reasoning has been verified by this group who found that diffuse injury to the aorta of a pig resulted in the buildup of thrombus at areas of disturbed flow such as the bifurcation, but caused only a thin layer of thrombus formation in the areas of laminar flow.[58] This group of workers also reported similar findings regarding the formation of thrombi in an extracorporeal shunt.[60] These observations correlate with clinical observations that the chances for the success of saphenous vein grafts are increased when they are inserted into non-diseased portions of the vessel with good runoff and with the finding that endarterectomy has a higher success rate when this procedure leaves a smooth transition between the endarterectomized segment and the remainder of the vessel wall.[23] With grafts inserted into diseased segments of vessel and in endarterectomies without a smooth transition, turbulent flow with eddy formation favors the accumulation of platelet aggregating substances. Similarly, poor runoff favors the buildup of these substances because of sluggish flow. Thus, the pattern of blood flow through the injured segment of vessel inlfuences the ultimate growth of the thrombus.

After the initial platelet-fibrin thrombus is formed, it is gradually replaced with fibrin.[53,58] The platelets gradually dissolve, and over a period of time ranging up to 14 days the fibrin and platelet remnants are phagocytosed by mononuclear cells and replaced by a mass rich in smooth muscle and collagen covered by endothelium.[53] However, if the thrombus has completely occluded the lumen the resolution of the thrombus may leave a non-patent fibrous cord.

Numerous attempts have been directed at attempting to define agents which would inhibit platelet aggregation. The first group of agents discovered were the structural analogues of ADP, adenosine and 2 chloroadenosine. Born[8] found that the addition of either compound to an in vitro system before the addition of ADP inhibited the normal aggregation by ADP, and Born _et al_. also reported that the intravenous infusion of adenosine or 2 chloroadenosine prevented the formation of a white thrombus at the site of an arterial injury.[10] Clayton, Born, and Cross then investigated other structural analogues of adenosine and found that adenosine monophosphate (AMP) also inhibited ADP-mediated platelet aggregation. However, other compounds with small structural changes in the adenosine moiety were lacking in activity.[13] Born _et al_. also noted that adenosine and 2 chloroadenosine had potent vasodilator action[10] and subsequently investigated the relationship between the vasodilating actions of adenosine analogues and their ability to inhibit ADP mediated platelet aggregation. Their findings showed a close correlation between vasodilator and inhibitory activities.[9] Emmons _et al_. then investigated dipyridamole because of its vasodilator actions and because an earlier report by Bunag had shown that dipyridamole could in-

crease the adenosine level in whole blood.[12] This group found that incubation of dipyridamole with platelet rich plasma decreased aggregation by ADP.[27] In addition, they found that an intravenous infusion of dipyridamole markedly decreased the formation of thrombi at sites of vessel injury.[28] However, long term human ingestion did not affect platelet aggregation in vitro induced by ADP, serotonin, or norepinephrine.[27] Didisheim subsequently showed that dipyridamole decreased the rate of occlusion of mesenteric vessels after electrical injury in the rat.[24] Further studies have revealed that dipyridamole inhibits platelet aggregation induced by collagen and thrombin in vitro,[17,59] and inhibits the release of ADP[17,59] and serotonin[17,59,84] by these platelet aggregating agents. Dipyridamole given in vivo inhibited platelet aggregation induced in vitro by collagen and thrombin and increased the bleeding time from the cut end of a mesenteric vessel in rabbits.[17] These studies have also indicated that dipyridamole decreases the uptake of glucose,[17] adenosine[17,69] and serotonin[17] by platelets, and it has been hypothesized that its actions result from this generalized metabolic inhibition.[69] Dipyridamole has very recently been shown to decrease the rate of thrombosis after mechanical endarterectomy in dogs,[18] to decrease the platelet turnover[40] and incidence of thromboembolic complications[74] in patients with prosthetic heart valves, and to decrease the amount of platelet aggregation in a heart lung pump oxygenator.[28]

In addition to dipyridamole, Emmons _et al_. also investigated prostaglandin E_1 because of its vasodilator effects.[26] They found that PGE_1 inhibited ADP, serotonin, thrombin and norepinephrine induced platelet aggregation, and decreased the amount of thrombus formation in

injured vessels.[26] Further studies have shown that collagen induced aggregation is also inhibited by PGE_1, and that PGE_1 will decrease the release of radioactively labeled serotonin from platelets exposed to collagen and thrombin.[51] Kinlough-Rathbone _et al_. suggested that these effects on platelets might result from the actions of PGE_1 to increase intra-platelet cyclic AMP,[51] but Ball _et al_. could not find a correlation between platelet cyclic AMP and inhibition of collagen induced platelet aggregation.[4] However, they did find that theophylline, a methyl xanthine which inhibits the phosphodiesterase enzyme which breaks down cyclic AMP, enhanced the effects of PGE_1 to inhibit collagen induced platelet aggregation.[4] Salzman has concluded in a recent review that the role of cyclic AMP in platelet aggregation and in its inhibition by compounds such as PGE_1 is not clear and bears further investigation.[70]

Because of the evidence of the importance of catecholamines in platelet aggregation, various inhibitors of the cardiovascular actions of the catechols have been investigated. Phentolamine, an alpha blocking agent, has been shown to inhibit epinephrine induced platelet aggregation.[63] In addition, alpha blocking agents can reduce the ability of collagen and thrombin to induce platelet aggregation.[75] Significantly, the beta blocking agent propranolol was much less effective in inhibiting platelet aggregation.[75] Thus, an alpha receptor has been postulated to reside on the platelet membrane which is closely involved in platelet aggregation.[75]

The non-steroidal anti-inflammatory drugs constitute another class of agents which have effects on platelets. Mustard _et al_. found

that sulfinpyrazone could increase the platelet survival in rabbits although it did not significantly affect the formation of thrombus in an extracorporeal shunt.[61] Packham _et al_. found that the addition of phenylbutazone or sulfinpyrazone to platelet rich plasma decreased the platelet aggregation induced by collagen, and decreased the amount of ADP and serotonin released.[65] They also found that phenylbutazone could prolong the bleeding time from cut ends of mesenteric vessels.[65] However, they could demonstrate no change in the response of platelets to thrombin.[65] In addition to these pyrazole anti-inflammatory compounds, aspirin has been shown to have marked effects on platelets and hemostasis. Gast reported that aspirin decreased platelet "stickiness", increased the bleeding time, and increased the incidence of post tonsillectomy hemorrhage from 0.1% to 8.7%.[35] Weiss and Aledort then reported that an oral dose of 3 grams of aspirin per day inhibited platelet aggregation induced by a connective tissue suspension in vitro and increased the bleeding time, but did not affect ADP-induced aggregation of platelets.[77] Evans _et al_. then showed that aspirin added to platelet rich plasma inhibited aggregation induced by collagen, thrombin, and antigen antibody complexes, and simultaneously reduced the release of ADP and serotonin in response to these aggregating substances.[30] They also found that aspirin administration prolonged the platelet survival and reduced the amount of thrombus forming in an extracorporeal shunt.[30] Zucker and Peterson confirmed the effects of aspirin on serotonin release and also found that aspirin decreased the availability of platelet factor 3 after exposure of platelets to aggregating stimuli.[85] Weiss _et al_. reported similar findings regarding

the effects of aspirin on aggregation and release of ADP by connective tissue, and also found that aspirin reduced platelet aggregation in response to epinephrine.[78] Danese _et al_. found that aspirin reduced the amount of thrombus formed after chemical and mechanical injury to the vessel wall in dogs.[18,19] The mechanism by which aspirin inhibits platelet aggregation has been investigated by Al-Mondhiry _et al_., and this group concluded that it may result from acetylation of active sites on the platelet membrane.[1]

An additional class of agents which affect the biological membranes has been shown to inhibit platelet aggregation. Included in this group are a variety of antihistamines including promethazine and diphenhydramine. Both of these agents have been reported to decrease platelet aggregation in response to ADP, epinephrine, and collagen.[55] A second group of membrane active drugs with effects on platelets are the tranquilizers and antidepressants. Chlorpromazine, amitryptiline, imipramine, nortryptiline, and norimipramine have all been shown to inhibit ADP, epinephrine, and collagen induced platelet aggregation as well.[55] The local anesthetics comprise a third group of membrane active drugs with lidocaine, cocaine, and nupercaine reported to inhibit platelet aggregation in vitro.[64] The precise mechanisms of action by which these various drugs act are unknown, but they have been classified together by Mustard and Packham because each has been demonstrated in some systems to have effects on biological membranes.[59] They have hypothesized that this common feature may underly their ability to alter platelet aggregation.[59]

Despite the large number of agents demonstrated in a variety of experimental situations to affect platelets, only two agents have received any clinical application in arterial surgery. Heparin has been employed because of its effects on plasma coagulation factors, but it has also been shown to have effects on platelets.[59] It can inhibit thrombin induced aggregation of platelets and release of platelet constituents,[59] but it is ineffective against the actions of collagen on platelets except at concentrations greater than 10 units/ml (10 mg/100 ml).[59,71] This latter dosage is equivalent to approximately 8 mg/kg which is almost three times the generally accepted dose for full heparinization. In addition, heparin does not inhibit ADP induced platelet aggregation even at these high doses.[59] However, heparin does prolong platelet survival in man.[59] Salzman has reported that patients with "difficult situations" in peripheral artery reconstructions were not aided by the addition of heparin to the regimen.[71] Somewhat better results have been achieved with the use of dextran. Moncrief _et al_. employed clinical dextran (average molecular weight 70,000) with autologous end to end vein grafts to the femoral arteries of dogs. This group reported an increase of over 100% in the patency rate with dextran.[57] Winfrey and Foster utilized low molecular weight dextran (average molecular weight 40,000) during removal of the intima by mechanical means over a 2 cm length of artery with almost 5 times as many vessels patent in the dextran as compared to the control group.[81] Foster _et al_. have reported that the employment of low molecular weight dextran immediately post operatively has significantly increased their success rate in peripheral arterial reconstructions.[16] Recent work has

demonstrated clinical dextran (macrodex) with an average molecular weight of 70,000 could increase the bleeding time and decrease platelet adhesiveness when given to patients.[15] However, Cronberg and his group were unable to reproduce the effects on platelet adhesiveness when macrodex was added to platelets in vitro.[15] Similar but less potent effects were observed with low molecular weight dextran by this group.[15] Macrodex also has been shown to markedly reduce factor VIII activity in the blood, but the importance of this finding remains unclear as the role of factor VIII in platelet aggregation is not known.[15]

In summary, then, peripheral and coronary arterial reconstructive surgery are complicated by a recognized early failure rate due to thrombosis in most series, and it is likely that this thrombosis is initiated by platelets. The events in arterial thrombosis are reasonably well defined with platelet adherence to collagen or other subendothelial substance leading to the release of platelet aggregating substances from these adherent platelets. The platelet thrombus is then stabilized with fibrin via the plasma coagulation system with the pattern of blood flow being an important determinant of the ultimate extent of the thrombus. Several agents have been shown to affect platelets and to alter thrombus formation after experimental injury via a variety of mechanisms. Relatively few attempts, however, have been made to apply these clinically to arterial reconstructive surgery.

MATERIALS AND METHODS

The initial efforts in this study were directed at developing an arterial model which would parallel as closely as possible the events in clinical arterial surgery but which would also provide a consistently clotting system against which various agents could be evaluated. The adult mongrel dog was chosen as the experimental animal, and all dogs weighed between 22 and 28 kg. Intrabenous sodium pentobarbital (25 mg/kg) was employed for anesthesia, and all animals were placed supine on the operating table, intubated, and maintained on a volume respirator. Intravenous gallamine in 10 mg doses provided supplemental anesthesia.

In Group I, consisting of 25 vessels in 13 dogs, 4 cm segments of femoral artery between the inguinal ligament and the knee, or 4 cm segments of common carotid artery were isolated under sterile conditions and the side brances were ligated and divided. All vessels measured between 4 and 5 mm in external diameter after dissection. Blood flow was interrupted between atraumatic vascular clamps and a 3 cm logitudinal arteriotomy was created with Potts arterial scissors. A mechanical endarterectomy was then performed using an ordinary chemical spatula to develop a plane of dissection. The arteriotomy was then closed with a running 6-0 everting suture. Prior to the last stitches both proximal and distal occlusions were temporarily removed to flush blood clot forming secondary to stasis.

Group II consisted of 11 vessels in 7 dogs and was identical to Group I except that the brachial arteries were employed. Vessels in this group measured 2 to 3 mm in diameter after dissection.

In Group III, consisting of 10 vessels in 7 dogs, 4 cm segments of femoral artery were isolated and the side branches were ligated and divided under sterile conditions. The following procedure was then employed to remove the intimal and muscularis layers of the vessel wall. Blood flow was interrupted between vascular clamps, and a solution containing 1 gram of the proteolytic enzyme pronase in 400 cc of normal saline (0.25%) was prepared. This solution of enzyme was then perfused for thirty minutes through the isolated segment of artery at 200 cc per minute via canulas inserted into two small longitudinal arteriotomies at either ends of the isolated segment. After perfusion, the vessels were flushed thoroughly with normal saline to remove residual pronase solution. The arteriotomies were then closed with 6-0 everting sutures and blood flow restored.

Group IV consisted of 6 vessels in 4 dogs. The procedure in this group was identical to that performed in Group III except that the perfusate consisted of normal saline instead of pronase. All vessels were removed at 6 hours after perfusion.

In Group V, consisting of 10 vessels in 5 dogs, the procedure was identical to that in Group III except that the dogs were given 250 mg of dipyridamole by mouth each day for 4 days prior to operation and then continued until the time of sacrifice at intervals from 6 hours to 6 weeks after operation.

Group VI consisting of 10 vessels in 5 dogs was similar to Group V except that 40 grains of aspirin (approximately 2.6 grams) was given to the animals the day preceding operation and each day thereafter until sacrifice 6 hours to 10 days after operation.

Patency of the vessel was determined in vivo by palpation of the femoral artery pulse distal to the site of perfusion, and at sacrifice by direct examination of the vessels. In addition, two microscopic sections were made of all vessels. One was stained with hematoxylin and eosin, the other with Masson trichrome.

The effects of pronase on the plasma coagulation system were evaluated by adding 4 drops of the 0.25% pronase solution to a plain glass test tube and to a citrated tube of venous canine blood. Lee White, prothrombin, and partial thromboplastin times were determined in the hospital laboratory.

RESULTS

In Group I, of the 25 femoral and carotid arteries subjected to endarterectomy, 9 clotted within 6 hours after the restoration of blood flow. Microscopy demonstrated that the intima, internal elastic membrane, and part of the muscularis layers had been removed by the mechanical endarterectomy. In all cases there were platelets adherent to the damaged areas of the vessel wall, but in 16 vessels this was not sufficient to occlude the lumen of the vessel.

In Group II, of the 11 brachial arteries endarterectomized, 6 clotted within 6 hours. Microscopic findings were similar to those found in Group I.

In Group III, all 10 vessels perfused with pronase showed loss of pulsations distal to the perfusion site within 3 hours after the restoration of blood flow. Gross inspection of the vessels showed clot occluding the lumen in the area of perfusion. Microscopy demonstrated that the intima and part of the muscularis layers of the wall had been removed by the proteolytic action of the pronase. The thrombus occluding the lumen was composed of a platelet mass containing fibrin with trapping of red and white blood cells, and was confined to the area perfused with pronase. (Figure 1).

In Group IV, none of the vessels perfused with saline became occluded in 6 hours, at which time the dogs were sacrificed. Two of these 6 vessels were in dogs which had perfusion of pronase in the contralateral femoral artery which led to thrombus formation. Microscopy of the saline treated vessels revealed no disruption of the vessel wall and no thrombus formation. (Figure 2).

In Group V, of the 10 vessels perfused with pronase in animals treated with dipyridamole, 3 clotted acutely, 2 were patent at sacrifice 6 hours postoperatively, and 5 remained patent until sacrifice at 2 to 6 weeks after operation. In the vessels studied at 6 hours after perfusion, a slight amount of clot was seen grossly, and microscopic examination showed a thin layer of platelets and fibrin along the portion of the vessel perfused with pronase. (Figure 3). In the vessels studied at 2 to 6 weeks no clot was seen grossly and the inside of the vessel was covered with a smooth shiny lining. On microscopic examination, no platelet fibrin clot was seen. The luminal surface of the vessel wall at 2 weeks after the time of perfusion showed a fibroblastic proliferation in the intimal area which appeared to be covered by a layer of endothelial cells (Figure 4). No unusual problems with hemostasis were noted in this group of dogs receiving dipyridamole.

In Group VI, of the 10 vessels perfused with pronase in animals receiving aspirin, 9 remained patent until sacrifice at 6 hours to 10 days after operation. With this dose of aspirin there appeared to be a marked hemostatic defect. Even the smallest arterioles and venules required ligation or cautery for hemostasis. One animal not included in the above results died from massive and uncontrollable hemorrhage from arteriotomy suture lines despite all measures. A second animal, included in the series, developed large hematomas in both groins after operation. At 6 hours after perfusion pulses were palpable and a moderate amount of clot was visible on the vessel wall on gross examination. The microscopic findings were similar to those in the vessels examined 6 hours postoperatively in Group V with a layer of platelet fibrin thrombus adherent to the damaged areas of the vessel wall. The findings in longer term vessels were similar to Group V vessels.

DISCUSSION

Groups I and II demonstrated that mechanical endarterectomy over a 3 cm length of vessel can produce a significant early thrombosis rate. The thrombosis rate is clearly related to the size of the vessel involved as femoral and carotid vessels measuring 4 to 5 mm in diameter became occluded with thrombus 36% of the time while the incidence of occlusion increased to 60% in the 2 to 3 mm brachial arteries. The rates of occlusions, however, are less than those reported by Winfrey and Foster, who had a 95% occlusion rate after similar procedures in dogs[81] with 3.5 mm vessels. Microscopic examination in this study showed that platelets were involved in the thrombus formation in accordance with previously cited observations of the events following injury to the inner layers of the vessel wall.[3] Because these mechanical endarterectomies did not provide a model of arterial thrombosis which would consistently lead to occlusion of the vessel a second model was explored.

Perfusion of segments of artery with a 0.25% pronase solution had previously been shown to remove the intima and a variable amount of media from the vessel wall depending on the length of perfusion.[49,67] These findings were confirmed by Group III of this study. After the restoration of normal blood flow, all vessels perfused with pronase developed thrombi which completely occluded the lumen within 3 hours. In accordance with previously cited observations of thrombosis following arterial injury[3] microscopy showed platelets adherent to the damaged area of the vessel wall with the formation of fibrin and the trapping of

red and white blood cells. The effect of pronase on the vessel wall is similar to that resulting from mechanical endarterectomy in that the intima and part of the muscularis layers are removed. The 100% thrombosis rate after this enzymatic endarterectomy is clearly much higher than that resulting from mechanical endarterectomy. However, as vessels perfused with saline in Group IV did not show any thrombus formation, it was felt that the occlusion rate in Group III did not result from technical factors. Pronase has been shown in vitro to induce platelet aggregation by Davey and Luscher,[21] and it is possible that a small amount of pronase remaining on the vessel wall would have a direct effect on the blood to enhance thrombus formation. This effect was believed to be minimized by flushing the vessel with saline several times after the perfusion with pronase and before the restoration of blood flow. In addition, it should be noted that the concentration of pronase used by Davey to induce platelet aggregation was four times that used in the initial perfusate in the present experiments. After flushing with saline and restoring blood flow, the pronase would likely be diluted even further, so that it seems unlikely that the direct action of pronase on platelets was responsible for the 100% thrombosis rate. The finding that the Lee White, prothrombin, and partial thromboplastin times were not altered by the addition of small amounts of pronase solution to samples of venous blood is some evidence that the thrombosis did not result from a direct effect of pronase on the blood. It would appear that pronase produces some change in the vessel wall which makes it more thrombogenic than a vessel after mechanical endarterectomy although the nature of this change is not clear.

Dipyridamole given at a dose of 250 mg per day to the animals in Group V appeared to play a role in preventing thrombotic occlusion in a significant percentage (70%) of the vessels perfused. This effect most likely results from its effects on platelet aggregation. The findings in this group correlate well with previous studies demonstrating the ability of dipyridamole to reduce thrombus formation in vivo.[17,18,24,28] In particular, Danese and Haimov reported that 100 mg of dipyridamole in 16 kg dogs would decrease the rate of occlusions 4 hours after endarterectomy.[18] However, Danese, Voleti, and Weiss found that 200 mg dipyridamole per day did not decrease the rate of occlusion 2 days after endarterectomy or injury with sulphuric acid.[19] The reasons for the latter findings are unclear, but in neither of these reports was a 100% rate of occlusive thrombus formation utilized as a control. The model using pronase in the present study was employed to provide this type of consistently clotting arterial mocel. Significantly dipyridamole did not inhibit thrombus formation completely as a layer of thrombus was demonstrated on the vessel wall 6 hours after restoration of blood flow. The effect seemed to be to reduce the growth of the thrombus and thus to prevent occlusion of the lumen of the vessel. This correlates with the in vitro findings that dipyridamole does not affect platelet adhesion to collagen but does decrease the release of ADP and serotonin from platelets exposed to collagen as well as decreasing the ability of platelets to aggregate in response to ADP.[17]

Aspirin administration to the dogs in Group VI also provided significant protection against the development of thrombotic occlusion after perfusion with pronase. Both of the series reported by

Danese et al.[18,19] demonstrated significant protection by aspirin against thrombotic occlusion after endarterectomy or chemical injury. The dose of aspirin employed in the present study, however, was much higher than that used by Danese et al., and may be responsible for the marked hemorrhagic tendency found. In vitro studies have shown marked effects of aspirin on platelet aggregation,[30,78,84,85] and thus it is likely that the protection afforded by aspirin in Group VI is also mediated by the effects of this drug on platelets. Similar to the effects of dipyridamole, aspirin did not inhibit thrombus formation but did decrease the rate of complete occlusion.

The ability of dipyridamole and aspirin to reduce the incidence of thrombotic occlusion after enzymatic removal of intima and muscularis, as shown in this study, may have clinical application. In arterial reconstructive surgery, especially in small vessels, the use of either or both of these agents may reduce the early failure rate due to thrombosis. As discussed previously, Foster et al. reported that the use of low molecular weight dextran immediately postoperatively increased the early patency rate in their series of peripheral vascular reconstructions. Similar use of either aspirin or dipyridamole might be employed on an experimental basis, ideally in a double blind prospective study with patients matched as closely as possible. Such a study seems warranted in view of the in vitro findings of the activities of these drugs on platelets,[59] the experimental animal work in the present study and in those of Danese et al.,[18,19] and the findings of the beneficial effects of these agents on platelet turnover and embolic complications of prosthetic heart valve replacement.[40,74] The problems with the use

of such agents, especially in surgical patients, are obviously those of hemostasis. With the systemic administration of a drug with potent effects on the primary means of defense against hemorrhage, there is certainly risk of significant morbidity and mortality during surgical procedures. A more ideal means of preventing thrombosis in a local area, such as in reconstruction of a particular arterial system, is to apply an agent in that local area which would have effects only in that area. Two agents described in the literature would fit these criteria, adenosine and Prostaglandin E_1. Both agents have very potent effects on platelets and both are rapidly degraded in the blood stream. In this way, the effects of either agent applied via an intra-arterial canula to a particular arterial system might be restricted to that arterial system. Unfortunately, dogs will not tolerate an indwelling canula over any length of time and it was not possible to test this idea against the present model of thrombosis. Ultimately, however, the latter approach may be more applicable than the induction of a generalized hemostatic effect to reduce thrombus formation in a limited area.

FIGURE LEGEND

Figure 1 — Longitudinal section through one side of vessel wall (35X) showing thrombus (T) adherent to the media (M) after digestion of the intima and inner layers of media with pronase. (A) indicates the adventitial surface.

Figure 2 — Longitudinal section through one side of vessel wall (100X) after perfusion with saline. (I) indicates the intact intimal layer. (M) represents media. (L) is the lumen of the vessel.

Figure 3 — Longitudinal section through one side of vessel wall (100X) from animal treated with dipyridamole 6 hours after perfusion with pronase. The amount of thrombus (T) is markedly reduced compared to that occurring after perfusion with pronase without prior administration of dipyridamole. (L) and (A) represent luminal and adventitial surfaces. There is hemorrhage into the medial layer (M) of the vessel wall.

Figure 4 — Longitudinal section through one side of vessel wall (430X) two weeks after perfusion with pronase in animal treated with dipyridamole. (F) refers to an area of fibroblastic proliferation on the luminal (L) surface of the vessel wall. (M) denotes media.

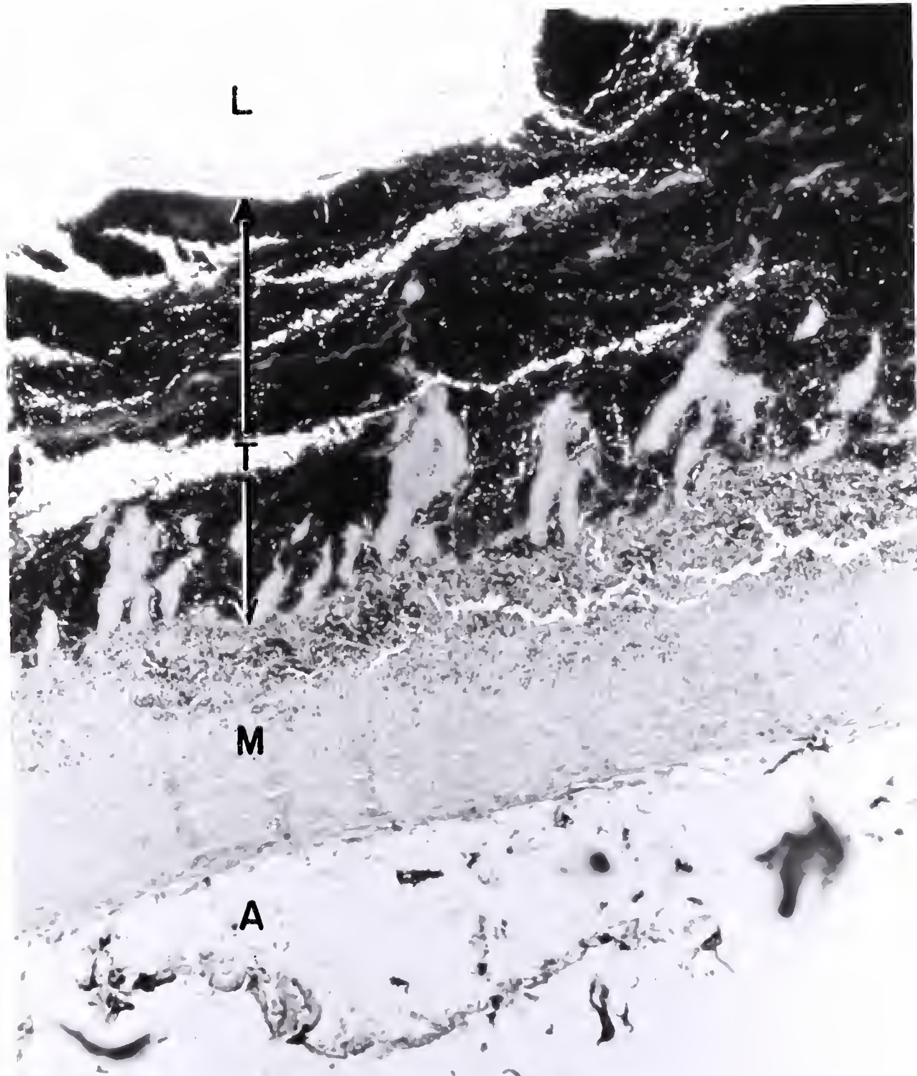


Figure 1.

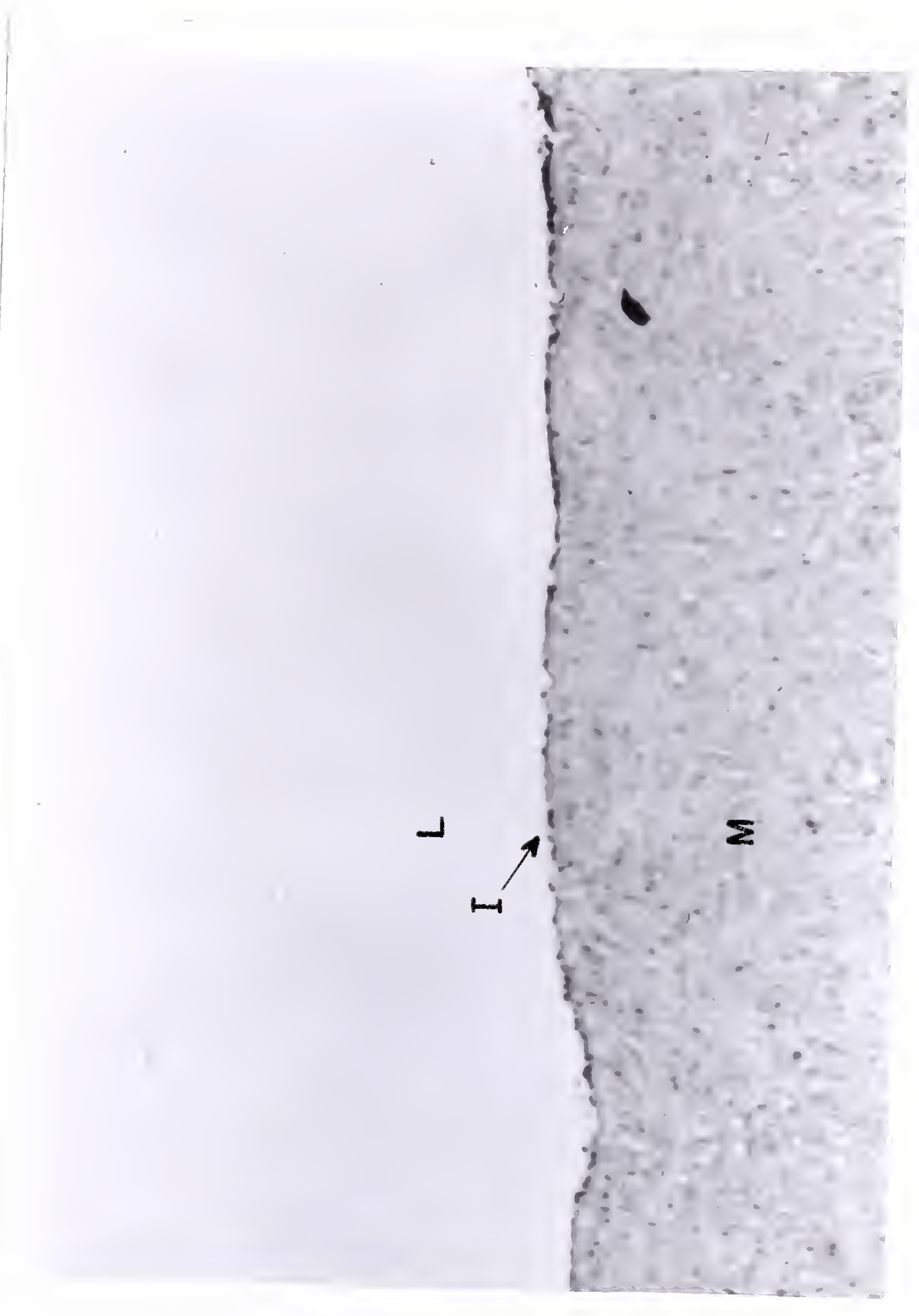


Figure 2

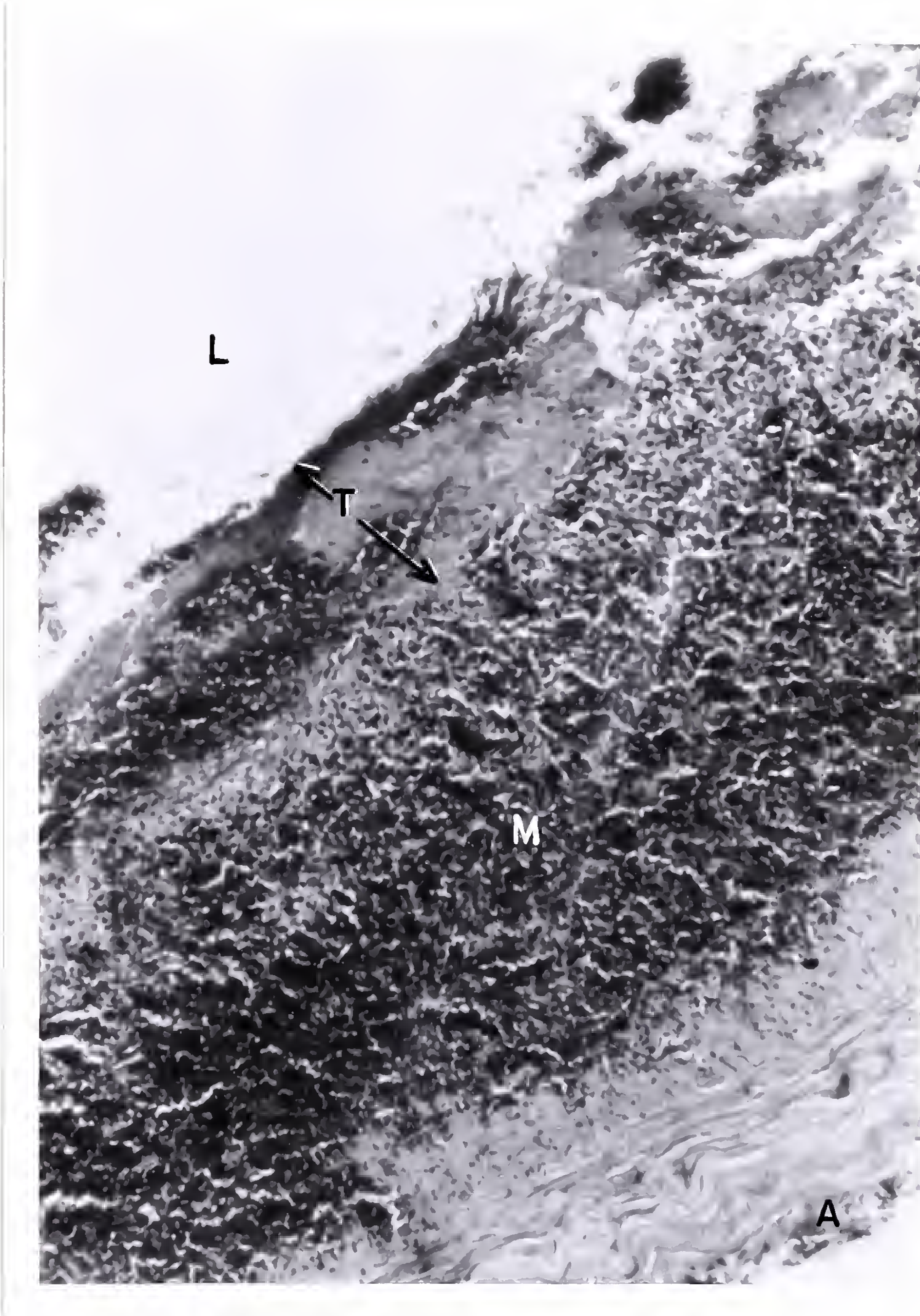


Figure 3

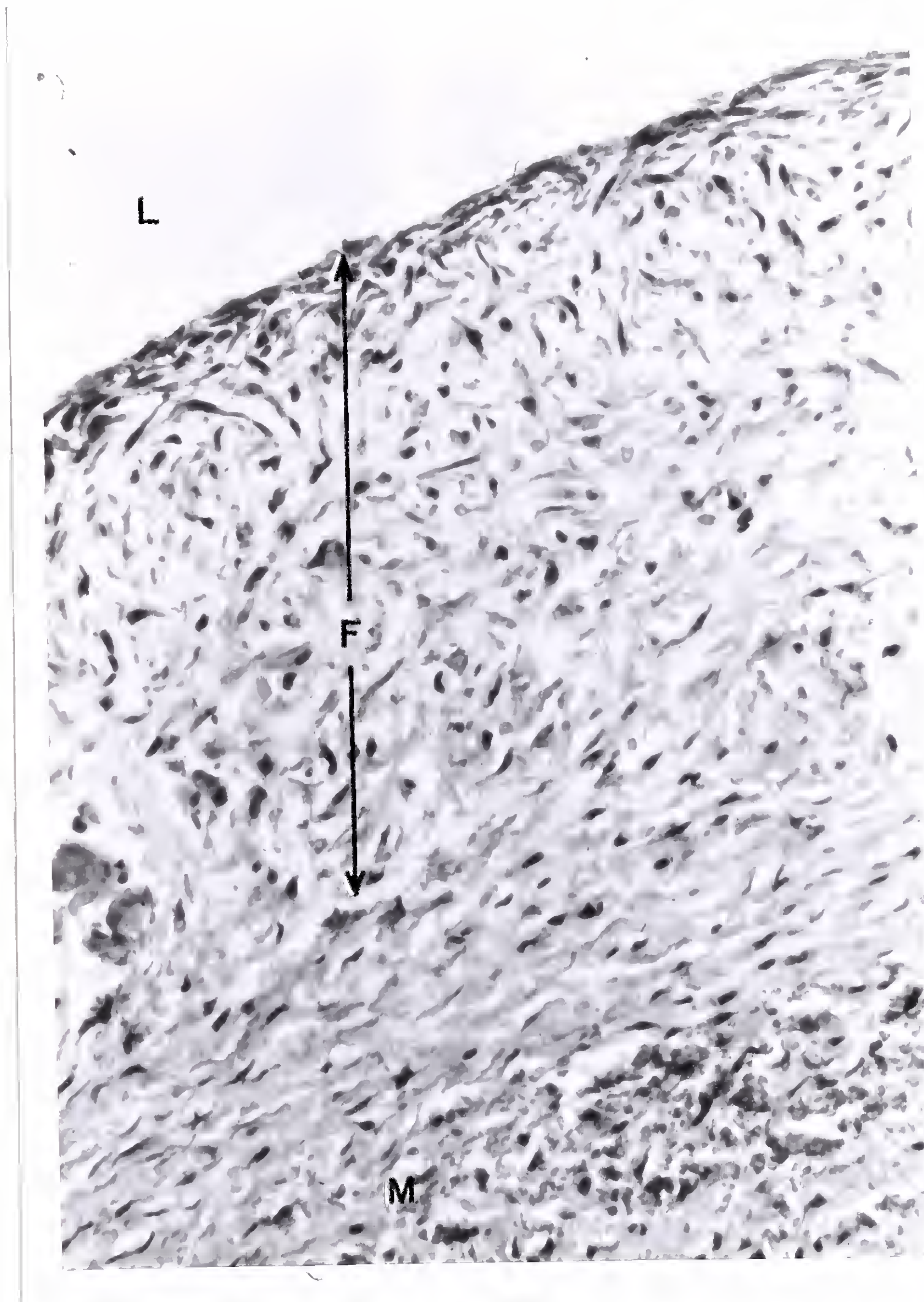


Figure 4

LITERATURE CITED

1. Al-Mondhiry, H., A.J. Marcus and T.H. Spaet 1970. On the Mechanism of Platelet Function Inhibition by Acetylsalicylic Acid. Proc. Soc. Exp. Biol. Med., 33: 632.

2. Ardlie, N.G., G. Grew and C.J. Schwartz 1966. Influence of Catecholamines on Nucleotide Induced Platelet Aggregation. Nature, 212: 415.

3. Ashford, T.P. and D.G. Freiman 1967. The Role of the Endothelium in the Initial Phase of Thrombosis. Amer. J. Path., 50: 257.

4. Ball, G., G.G. Brereton, M. Fulnwood, D.M. Ireland and P. Yates 1970. Effects of Prostaglandin E_1, Alone and in Combination with Theophylline or Aspirin on Collagen Induced Platelet Aggregation and on Platelet Nucleotides Including Adenosine 3'5' Cyclic Monophosphate. Biochem. Jour., 120: 709.

5. Baumgartner, H.R., M.B. Stemerman and T. Spaet 1971. The Adhesion of Blood Platelets to Subendothelial Surfaces: Distinct from Adhesion to Collagen. Experientia, 27: 283.

6. Bizzozero, J. 1882. Ueber einen neuren Formbestand theil des Blutes und Dessen Rolle bei der Thrombose und der Blutgerinnung. Virchows Arch., 90: 261.

7. Born, G.V.R. 1962. Aggregation of Blood Platelets by Adenosine Diphosphate and its Reversal. Nature, 194: 927.

8. Born, G.V.R. 1964. Strong Inhibition by 2-Chloroadenosine of the Aggregation of Blood Platelets by Adenosine Diphosphate. Nature, 202: 95.

9. Born G.V.R., R.J. Haslam, M. Goldman and R.D. Lowe 1965. Comparative Effectiveness of Adenosine Analogues as Inhibitors of Blood Platelet Aggregation and as Vasodilators in Man. Nature, 205: 678.

10. Born, G.V.R., A.J. Honour and J.R.A. Mitchell 1964. Inhibition by Adenosine and by 2-Chloroadenosine of the Formation of and Embolization of Platelet Thrombi. Nature, 202: 761.

11. Bull, B.S. and M.B. Zucker 1965. Changes in Platelet Volume Produced by Temperature, Metabolic Inhibitors, and Aggregating Agents. Proc. Soc. Exp. Biol. Med., 120: 296.

12. Bunag, R.D., C.R. Douglas, S. Imai and R.M. Berne 1964. Influence of a Pyrimidopyrimidine Derivative on Deamination of Adenosine by Blood. Circ. Research, 15: 83.

13. Clayton, S., G.V.R. Born and M.J. Cross 1963. Inhibition of the Aggregation of Blood Platelets by Nucleosides. Nature, 200: 138.

14. Connolly, J.E., E.A. Stemmer, R.B. Doering 1968. Eversion Endarterectomy: Autograft Replacement of Aorta, Iliac, and Femoral Arteries. Surgery, 63: 128.

15. Cronberg, S., B. Robertson., M. Nilsson and J.E. Nilehn 1966. Suppressive Effect by Dextran on Platelet Adhesiveness. Thromb. Diath. Haemorrh., 16: 384.

16. Cross, M.J. 1964. Effect of Fibrinogen on the Aggregation of Platelets by Adenosine Diphosphate. Throm. Diath. Haemorrh., 12: 524.

17. Cucuianu, M.P., E.E. Nishizawa and J.F. Mustard 1971. Effect of Pyrimido-Pyrimidine Compounds on Platelet Function. J. Clin. Lab. Med., 77: 158.

18. Danese, C.A. and M. Haimov 1971. Inhibition of Experimental Arterial Thrombosis in Dogs with Platelet Deaggregating Agents. Surgery, 70: 927.

19. Danese, C.A., C.D. Voleti and H.J. Weiss 1971. Protection by Aspirin Against Experimentally Induced Arterial Thrombosis in Dogs. Thromb. Diath. Haemorrh., 25: 288.

20. Darling, R.C., R.R. Linton and M.A. Razzule 1967. Saphenous Vein Bypass Grafts for Femoropopliteal Occlusive Disease: A Re-appraisal. Surgery, 61: 31.

21. Davey, M.G. and E.F. Luscher 1967. Actions of Thrombin and Other Coagulant and Proteolytic Enzymes on Blood Platelets. Nature, 216: 857.

22. DeWeese, J.A., H.B. Barner, E.B. Mahoney and C.G. Rob 1966. Autogenous Venous Bypass Grafts and Thromboendarterectomies for Atherosclerotic Lesions of the Femoropopliteal Arteries. Ann. Surg., 163: 205.

23. DeWeese, J.A., R. Terry, H.B. Barner and C.G. Rob 1966. Autogenous Venous Femoropopliteal Bypass Grafts. Surgery, 51: 28.

24. Didisheim, P. 1968. Inhibition by Dipyridamole of Arterial Thrombosis in Rats. Thromb. Diath. Haemorrh., 20: 257.

25. Dilley, R.B., J.A. Cannon, A.A. Kattus, R.N. MacAlpin and W.P. Longmire 1965. The Treatment of Coronary Occlusive Disease by Endarterectomy. Jour. Thor. and CV Surg., 50: 511.

26. Emmons, P.R., J.R. Hampton, M.J.G. Harrison, A.J. Honour and J.R.A. Mitchell 1967. Effect of Protaglandin E_1 on Platelet Behavior in Vitro and in Vivo. Brit. Med. Jour., 2: 468.

27. Emmons, P.R., M.J.G. Harrison, A.J. Honour and J.R.A. Mitchell 1965. Effect of Dipyridamole on Human Platelet Behavior. Lancet., 2: 603.

28. Emmons, P.R., M.J.G. Harrison, A.J. Honour and J.R.A. Mitchell 1965. Effect of a Pyrimidopyrimidine Derivative on Thrombosis Formation in the Rabbit. Nature, 208: 255.

29. Evans, G. and J.F. Mustard 1968. Platelet Surface Reaction and Thrombosis. Surgery, 64: 273.

30. Evans, G., M.A. Packham, E.E. Nishizawa, J.F. Mustard and E.A. Murphy 1968. The Effect of Acetylsalicylic Acid on Platelet Function. Jour. Exp. Med., 128: 877.

31. Foster, J.H., D.A. Killen, P.C. Jolly and J.H. Kirtley 1966. Low Molecular Weight Dextran in Vascular Surgery: Prevention of Early Thrombosis Following Arterial Reconstruction in 85 Patients. Annals of Surgery, 163: 764.

32. French, J.E., R.G. MacFarlane and A.G. Sanders 1964. The Structure of Haemonstatic Plugs and Experimental Thrombi in Small Arteries. Brit. Jour. Exp. Path., 45: 467.

33. Gaarder, A., J. Jonsen, S. Laland, A. Hellem and P.A. Owren 1961. Adenosine Diphosphate in Red Cells as a Factor in the Adhesiveness of Human Blood Platelets. Nature, 192: 531.

34. Garrett, H.E., P.I. Kotch, M.T. Green, E.B. Diethrich and M.E. DeBakey 1968. Distal Tibial Artery Bypass with Autogenous Vein Grafts: An Analysis of 56 Cases. Surgery, 63: 90.

35. Gast, L.F. 1964. Influence of Aspirin on Hemostatic Parameters. Ann. Rheumatic Diseases, 23: 500.

36. Green, G.E., F.C. Spencer, D.A. Tice and S.H. Stertzer 1970. Arterial and Venous Microsurgical Bypass Grafts for Coronary Artery Disease. Jour. Thor. and CV Surg., 60: 491.

37. Gugler, E. and E.F. Luscher 1965. Platelet Function in Congenital Afibrinogenemia. Thromb. Diath. Haemorrh., 14: 361.

38. Gutelius, J.R., S. Kreindler and J.C. Luke 1965. Comparative Evaluation of Autogenous Vein Bypass Graft and Endarterectomy in Superficial Femoral Artery Reconstruction. Surgery, 57: 28.

39. Hardisty, R.M. and R.A. Hutton 1966. Platelet Aggregation and the Availability of Platelet Factor 3. Brit. Jour. Haem., 12: 764.

40. Harker, L.A. and S.J. Slichter 1970. Studies of Platelet and Fibrinogen Kinetics in Patients with Heart Valves. New Eng. Jour. Med., 283: 1302.

41. Harrison, J.H., W.D. Jordan and A.R. Perez 1967. Eversion Thromboendarterectomy. Surgery, 61: 26.

42. Haslam, R.J. 1964. Role of Adenosine Diphosphate in the Aggregation of Human Blood Platelets by Thrombin and by Fatty Acids. Nature, 202: 765.

43. Honour, A.J. and J.R.A. Mitchell 1964. Platelet Clumping in Injured Vessels. Brit. Jour. Exp. Path., 45: 75.

44. Honour, A.J. and J.R.A. Mitchell 1963. Platelet Clumping in Vivo. Nature, 197: 1019.

45. Hovig, T. 1963. Aggregation of Rabbit Blood Platelets Produced in Vitro by Saline Extract of Tendons. Thromb. Diath. Haemorrh., 9: 248.

46. Hovig, T. 1963. Release of a Platelet Aggregating Substance (Adenosine Diphosphate) from Rabbit Blood Platelets Induced by Saline Extract of Tendons. Thromb. Diath. Haemorrh., 9: 265.

47. Hovig, T., H.C. Rowsell, W.J. Dodds, L. Jorgensen and J.F. Mustard 1967. Experimental Hemostasis in Normal Dogs and Dogs with Congenital Disorders of Blood Coagulation. Blood, 30: 636.

48. Hughes, J. and P. Mahien 1970. Platelet Aggregation Induced by Basement Membrane. Thromb. Diath. Haemorrh., 24: 395.

49. Kabemba, J.M. and G.L. Hammond (in press) Chemical Degradation of the Intima from Normal Canine and Altherosclerotic Human Vessels. Jour. Surg. Research.

50. Kinlough, R.L., M.A. Packham and J.R. Mustard 1969. Glucose and Platelet Aggregation. Fed. Proc., 28: 509.

51. Kinlough-Rathbone, R.L., M.A. Packham and J.F. Mustard 1970. The Effect of Prostaglandin E_1 on Platelet Function in Vitro and in Vivo. Brit. Jour. of Haem., 19: 559.

52. Mannick, J.J., B.T. Jackson, J.D. Coffman and D.M. Hame 1967. Success of Bypass Vein Grafts in Patients with Isolated Popliteal Artery Segments. Surgery, 61: 17.

53. Marcus, A.J. 1969. Platelet Function. New Eng. Jour. Med., 280: 1213.

54. Mills, D.C.B., I.A. Robb and G.C.K. Roberts 1968. The Release of Nucleotides, 5 Hydroxytryptamine, and Enzymes from Human Blood Platelets during Aggregation. Jour. Physiol., 195: 715.

55. Mills, D.C.B. and G.C.K. Roberts 1967. Membrane Active Drugs and the Aggregation of Human Platelets. Nature, 213: 35.

56. Mitchel, B.F., M. Adam, C.J. Lambert, U. Sungu and S. Shiekh 1970. Ascending Aorta-to-Coronary Artery Saphenous Vein Bypass Grafts. Jour. Thor. and CV Surg., 60: 457.

57. Moncrief, J.A., J.C. Darin, P.C. Canizaro and R.B. Sawyer 1963. Use of Dextran to Prevent Arterial and Venous Thrombosis. Ann. Surg., 158: 553.

58. Mustard, J.R., L. Jorgensen, T. Hovig, M.F. Glynn and H.C. Rowsell 1966. Role of Platelets in Thrombosis in Pathogenesis and Treatment of Thromboembolic Diseases. Thromb. Diath. Haemorrh. Suppl., 21: 131.

59. Mustard, J.F. and M.A. Packham 1970. Factors Influencing Platelet Function: Adhesion, Release and Aggregation. Pharm. Rev., 22: 97.

60. Mustard, J.F., H.C. Rowsell, F. Loly, B. Hegardt and E.A. Murphy 1966. The Effect of Adenine Nucleotides on Thrombus Formation, Platelet Count, and Blood Coagulation. Jour. Exp. Molec. Path., 5: 43.

61. Mustard, J.F., H.C. Rowsell, H.A. Smythe, A. Senyi and E.A. Murphy 1967. The Effect of Sulfinpyrazone on Platelet Economy and Thrombus Formation in Rabbits. Blood, 29: 859.

62. O'Brien, J.R. 1964. A Comparison of Platelet Aggregation Produced by Seven Compounds and a Comparison of their Inhibitors. Jour. Clin. Path., 17: 275.

63. O'Brien, J.R. 1963. Some Effects of Adrenaline and Antiadrenaline Compounds on Platelets in Vitro and in Vivo. Nature, 200: 763.

64. O'Brien, J.R. 1961. The Adhesiveness of Native Platelets and its Prevention. J. Clin. Path., 14: 140.

65. Packham, M.A., E.S. Warrior, M.F. Glynn, S. Senyi and J.F. Mustard 1967. Alteration of the Response of Platelets to Surface Stimuli by Pyrazole Compounds. J. Exp. Med., 126: 171.

66. Rittenhouse, E.K., E.A. Hessel, C.S. Ito and K.A. Merendino 1972. Effect of Dipyridamole on Microaggregate Formation in the Pump Oxygenator. Ann. Surg., 175: 1.

67. Robertson, A.L. and W. Insul 1967. Dissection of Normal and Atherosclerotic Human Artery with Proteolytic Enzymes in Vitro. Nature, 214: 5090.

68. Rowsell, H.C., B. Hegardt, J. Dodds and H.G. Downie 1965. Thrombus Formation in Thrombocytopenic Swine. Fed. Proc., 24: 154.

69. Rozenberg, M.C., C.M. Ledwidge, D. Wilcken and M. McKeon 1971. The Inhibition of Adenosine Phosphorylation in Platelets by Dipyridamole. J. Lab. Clin. Med., 77: 88.

70. Salzman, E.W. 1972. Cyclic AMP and Platelet Function. New Eng. J. Med., 286: 358.

71. Salzman, E.W. 1965. Limitations of Heparin Therapy after Arterial Reconstruction. Surgery, 57: 131.

72. Spaet, T.H. and R.B. Erichson 1966. The Vascular Wall in the Pathogenesis of Thrombosis. Thromb. Diath. Haemorrh. Suppl., 21: 67.

73. Spaet, T.H. and M.B. Zucker 1964. Mechanism of Platelet Plug Formation and Role of Adenosine Diphosphate. Amer. J. Physiol., 206: 1267.

74. Sullivan, J.M., D.E. Harken and R. Gorlin 1971. Pharmacologic Control of Thromboembolic Complications of Cardiac Valve Replacement. New Eng. J. Med., 284: 1391.

75. Thomas, D.P. 1968. The Role of Platelet Catecholamines in the Aggregation of Platelets by Collagen and Thrombin. Exp. Biol. Med., 3: 129.

76. Warren, B.A. 1971. The Platelet Pseudopodium and its Involvement in Aggregation and Adhesion to Vessel Walls. Br. J. Exp. Path., 52: 378.

77. Weiss, H.J. and L.M. Aledort 1967. Impaired Platelet-Connective Tissue Reaction in Man after Aspirin Ingestion. Lancet, 2: 495.

78. 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.

79. Weiss, H.J. and J. Rogers 1971. Fibrinogen and Platelets in the Primary Arrest of Bleeding. New Eng. J. Med., 285: 369.

80. Wilner, G.D., H.L. Nossel and E.C. LeRoy 1968. Aggregation of Platelets by Collagen. J. Clin. Invest., 47: 2616.

81. Winfrey, E.W. and J.H. Foster 1964. Low Molecular Weight Dextran in Small Artery Surgery. Arch. Surg., 83: 78.

82. Zucker, M.B. 1947. Platelet Agglutination and Vasoconstriction as Factors in Spontaneous Hemostasis in Normal, Thrombocytopenic, Heparinized, and Hypoprothrombinemic Rats. Amer. J. Physiol., 148: 275.

83. Zucker, M.B. and J. Borrelli 1962. Platelet Clumping Produced by Connective Tissue Suspensions and by Collagen. Proc. Soc. Exp. Biol. Med., 109: 779.

84. Zucker, M.B. and J. Peterson 1970. Effect of Acetylsalicylic Acid, Other Non-Steroidal Anti-Inflammatory Agents, and Dipyridamole on Human Blood Platelets. J. Lab. Clin. Med., 76: 66.

85. Zucker, M.B. and J. Peterson 1968. Inhibition of Adenosine Diphosphate Induced Secondary Aggregation and Other Platelet Functions by Acetylsalicylic Acid Ingestion. Proc. Soc. Exp. Biol. Med., 127: 547.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS | DATE

Laurie Martin, 3345 Yale Station — 3-11-76

[illegible] 100 York St New Haven — 2/19/81

" — 2/25/81