ANTITHROMBOTIC AGENTS

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June 2, 1977

"The little that we have done seems as
naught when we look forward and behold
how much remains for us to do"

Goethe

CONTENTS

I. Introduction

Hemostasis involves a complicated and incompletely understood sequence of events leading to the formation of a thrombus. A greatly simplified scheme is presented in Figure 1. Vessel wall injury evokes the formation of a platelet plug (primary hemostasis) and also activates the coagulation system to form thrombin which cleaves fibrinogen to form a fibrin clot. The fibrinolytic system, activated by the conversion of plasminogen to plasmin, degrades the fibrin in time to allow the inflammatory process to heal the wound (1, 2).

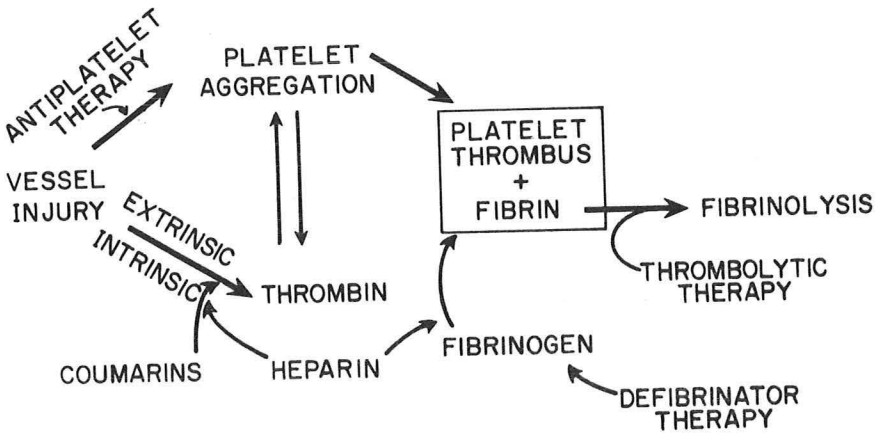

Figure 1. Simplified Scheme of Hemostasis and Modes of Antithrombotic Therapy

As shown in Figure 1, several modalities may be employed to impede a thrombotic event. Thus, the traditional anticoagulants impede the evolution of thrombin and, in the case of heparin, retard thrombin's ability to cleave fibrinogen. Platelet-inhibitory drugs are of potential value in retarding platelet thrombus production. Defibrinators, such as ancrod and reptilase, degrade fibrinogen to such a state of hypofibrinagenemia that the ability to form a stable fibrin clot is impaired. Finally, thrombolytic therapy, by enhancing fibrinolysis would be of theoretical value in hastening removal of the fibrin clot.

Although both platelets and coagulation contribute to thrombus formation, the relative contribution of each depends upon the location of the thrombus in various thrombotic disease states of man and other animals. Thus, as shown in Figure 2, the red, or venous stasis thrombus

is composed primarily of fibrin and red cells, although platelets are also present and may play a role as will be discussed later. One would expect that the major therapeutic thrust would be the dissolution and prevention of fibrin. On the other hand, the white, or arterial thrombus, has as its origin a platelet nidus at sites of atherosclerosis and arterial narrowing with progressive accretion of a platelet-fibrin mass. With increasing stasis, a red thrombus attaches secondarily. One would expect antiplatelet therapy to be of major value, and anticoagulants to be of value only in preventing extension of fibrin from the platelet thrombus (2).

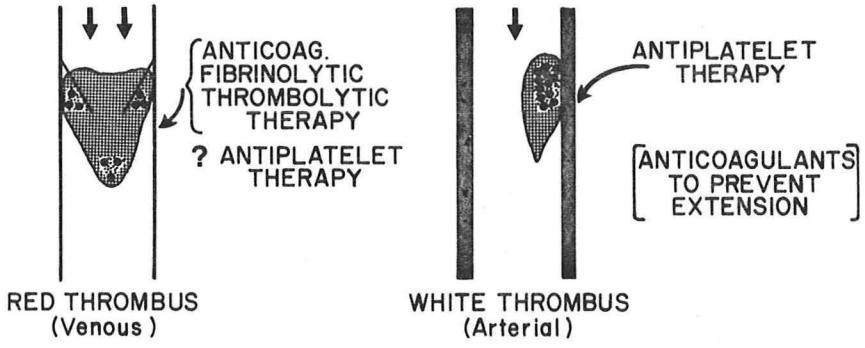

Figure 2. Contribution of Platelets and Coagulation to the Venous and Arterial Thrombus

Thus, Harker and Slichter (3) found that the increased consumption of fibrinogen and platelets in venous thrombosis was correctable by heparin. In arterial thrombosis platelets were primarily consumed and certain anti-platelet drugs increased platelet survival.

The following is a discussion of the present status of antithrombotic therapy.

II. Heparin

A. Structure

Heparin is a naturally occurring mucopolysaccharide found in several body tissues, chiefly liver, lung, and intestine. The precise

chemical and physical structure of heparin is being increasingly clarified; one can state that it is a linear heterogenous molecule with a molecular weight of 10-12,000, and it is the most highly sulfated and anionic substance known in the animal kingdom (4-8).

B. Mode of action

1. Historical Background

In order to understand the action of heparin it is best to review the process which heparin retards, i.e., coagulation, and to then historically document the discoveries which led to our present concepts of how heparin impedes coagulation.

Coagulation is a process whereby protein clotting factors become activated in succession to convert fibrinogen to fibrin. The most potent of these activated factors is the proteolytic enzyme thrombin, which specifically cleaves arginine-glycine amino acid bonds on the fibrinogen molecule, splitting off fibrinopeptides A and B. The resultant "fibrin monomer" rapidly polymerizes with itself to form a fibrin clot which is covalently bonded by factor XIII, or fibrin stabilizing factor (9-11) (Figure 3).

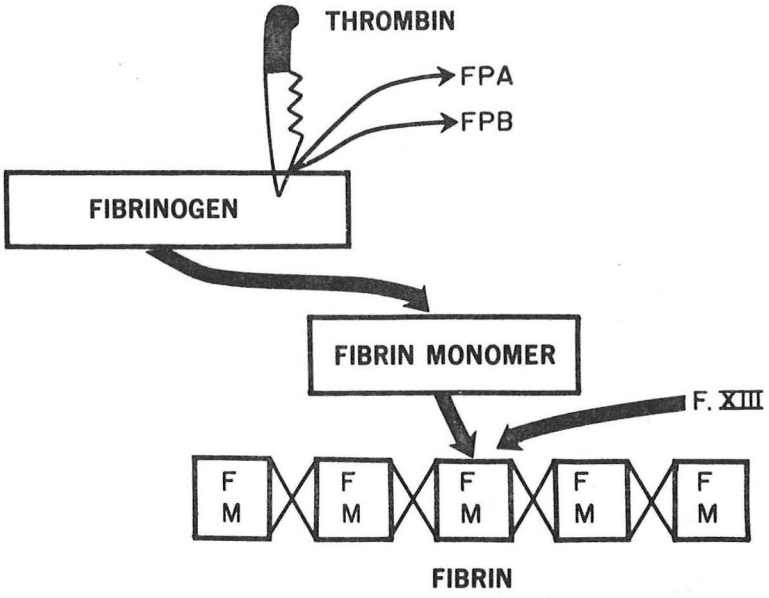

Figure 3. Action of Thrombin on Fibrinogen

The clotting of fibrinogen by thrombin was fundamentally known in the 1870's (12). At the turn of the century it was discovered that a

naturally occurring plasma protein caused thrombin to gradually lose its ability to clot fibrinogen, and this inhibitor was named "antithrombin". In 1916 McClean isolated heparin from the liver and demonstrated its potent anticoagulant properties (15). In 1939 Brinkhous, et al., showed that heparin was effective only in the presence of a plasma component which they termed heparin cofactor (16). The culmination of work by several investigators beginning in the 1950's has ultimately led to the conclusion that antithrombin and heparin cofactor are properties of a single molecular species, and that heparin accelerates 50 to 100 fold the rate at which this inhibitor neutralizes thrombin (17-23) (Figure 4).

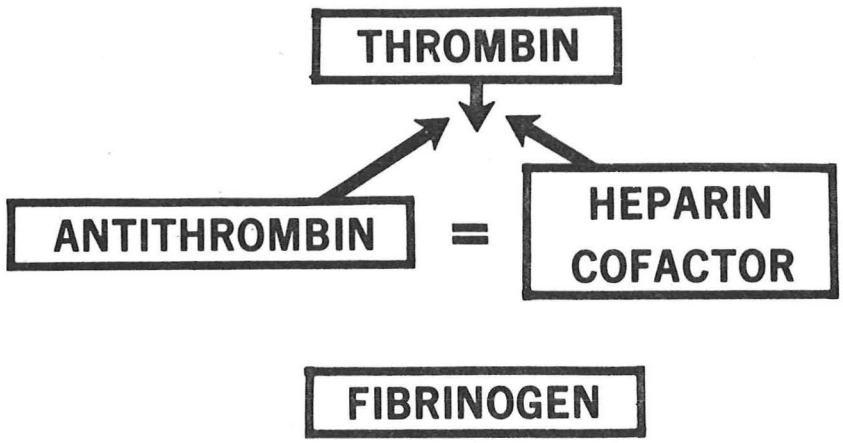

Figure 4. Impedance of Thrombin by Antithrombin (Heparin Cofactor)

2. Biochemical Model

The biochemical interaction of thrombin and antithrombin and the enhancement by heparin have been elucidated by the elegant work of Rosenberg (20-23). Thrombin's proteolytic capacity is chiefly derived from a highly reactive serine amino acid residue. Hence, thrombin may be classified as a serine protease. Since thrombin is known to have a narrow specificity for cleaving specific arginine bonds, it was subsequently proven that antithrombin contains a reactive arginine site. This reactive arginine site interacts with thrombin's active serine center, and through this complex formation antithrombin may be viewed as a "sacrificial lamb," offering to thrombin a substitute for fibrinogen's arginine

sites and preventing clot formation (Figure 5). Heparin, being highly acidic, binds to antithrombin's lysyl residues and greatly accelerates the rate of complex formation, probably by inducing a conformational alteration of antithrombin which renders its arginine site more accessible to the serine site on thrombin (Figure 6).

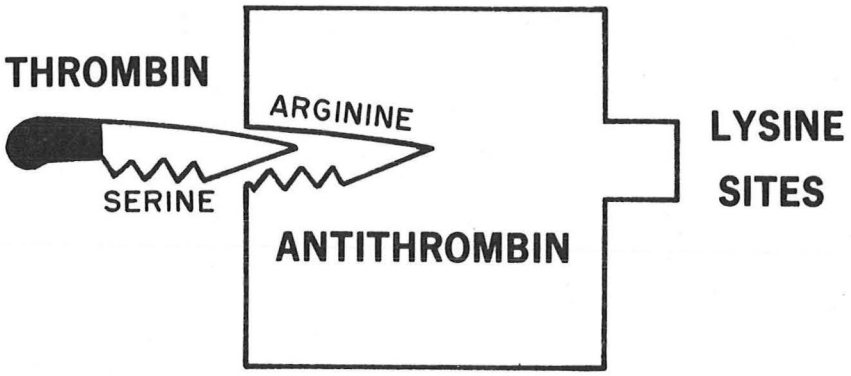

Figure 5. Interaction of Thrombin and Antithrombin
From Reference 18

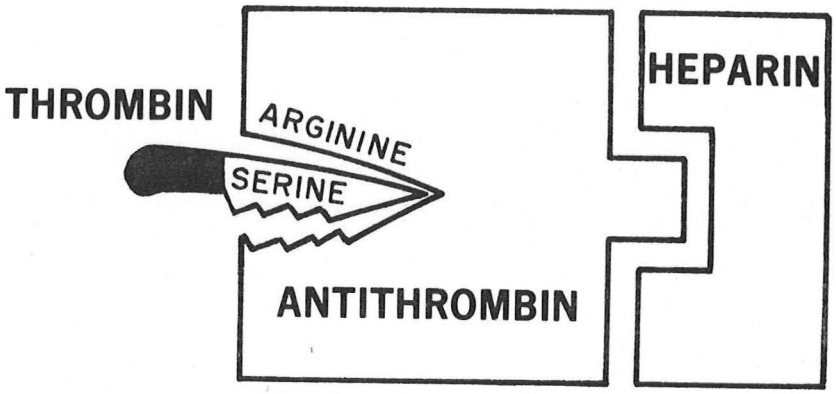

Figure 6. Binding of Heparin to Antithrombin
From Reference 18

3. The expanded role for antithrombin (heparin cofactor)

Thrombin is not the only serine protease in the classical coagulation cascade; indeed, the majority of the coagulation factors,

once activated, are serine proteases, and it has long been suspected that each had a plasma inhibitor. Rosenberg has now demonstrated that antithrombin is not specific for thrombin but is in fact the naturally occurring inhibitor for activated factors XII, XI, X, IX, and VII. Thus antithrombin's activity, and its augmentation by heparin, is operative throughout the coagulation cascade.

C. Indications

The model for heparin action is consistent with the fact that heparin therapy does not "dissolve" clots but arrests thrombin's potential for further clot formation. Indications for heparin therapy:

1. Deep vein thrombophlebitis

2. Pulmonary embolism

3. Prevention of extension of arterial thrombosis or embolus

4. Prophylaxis in those susceptible to thrombosis

5. Disseminated intravascular coagulation.

D. Contraindications:

1. Cardiovascular (endocarditis, hypertension)

2. Hematologic (hemophilia, purpura, thrombocytopenia, paroxysmal nocturnal hemoglobinuria)

3. Gastrointestinal (ulcer)

4. Surgical (especially brain, spinal cord, or eyes)

As with all therapeutic modalities, many contraindications to heparin therapy are relative, and one must weigh the potential benefits in any particular high-risk situation.

E. Management of heparin therapy - the present controversy.

Although heparin has been in use for over forty years it is still unsettled whether laboratory control or route of administration offers any advantage. In fact, it is now unclear how heparin preparations should be meaningfully assayed.

1. Problems in heparin standardization

Heparin preparations are presently standardized in units based on prolongation of the clotting time in sheep plasma. However, all

heparin made commercially must be assumed to be heterogenous within and between batches. Furthermore, it has been shown that United States and British Pharmocopoeia assays can give potency ratios which differ significantly for different batches of plasma (24, 25). This has caused difficulty in assigning proper unitage of batches for clinical use.

The most pressing problem is to answer the question "what is the relevant biologic activity of heparin?" Are we most interested in its enhancement of antithrombin effect, and if so, at what level in the coagulation cascade? How important is its interaction with platelets and the kinin system? Once these questions have been answered, it is becoming apparent that different types of heparin have different proportions of these known biologic activities. For example, the ability to augment antithrombin's inhibition of activated factor X has been shown to differ between bovine and porcine preparations and between preparations of differing molecular weight (26, 27). However, studies which have measured effects of different preparations on routine coagulation tests generally have shown no clinically significant differences (28-30). An International Task Force on Heparin Standardization has been organized and is addressing itself to these issues as well as studying different batches reported to cause thrombocytopenia.

2. Problems in laboratory control

Based on animal studies by Wessler (31) heparin therapy has been regulated to maintain the Lee White clotting time (32) at twice baseline control values. Because the Lee White method is cumbersome, imprecise, and time-consuming, there has been a consistent effort in recent years to better define and monitor a thrombotic event. Basu, et al. (33), on the basis of animal studies by Zucker, et al. (34), monitored continuous heparin therapy by maintaining the activated partial thromboplastin time (A.P.T.T.) at 1½ to 2½ times control; a shorter A.P.T.T. correlated with recurrence of thrombosis. The partial thromboplastin used in this study was not a commercially prepared reagent but was made according to the method of Bell and Alton (35). However, since that publication, commercially prepared reagents have been used in heparin management on the assumption that their sensitivity to heparin would be identical to that prepared by Basu. Recent work has shown that commercial A.P.T.T. reagents vary widely in their in vitro and in vivo sensitivity, supporting earlier work by Soloway (36-38). There is some suggestion that laboratory control boundaries may be important. Thus, in the Urokinase Pulmonary Embolism Trial (39), bleeding was more likely with a prolonged clotting time, and in Basu's study a short A.P.T.T. correlated with recurrence. However, Salzman et al. in their pulmonary embolism trial found that there was no difference in the A.P.T.T. in patients with major bleeding complications from heparin as compared with non-bleeders (40).

Other tests have been developed for both documentation and monitoring of thrombotic states. When blood clots, thrombin cleaves fibrino-

peptide A (F.P.A.) and B from fibrinogen; the resultant fibrin monomer may polymerize to a clot or form complexes with fibrinogen or plasmin-mediated split products (F.S.P.). Antithrombin is utilized, and platelets release platelet factor 4, or heparin neutralizing activity (H.N.A.). Tests measuring these different elements have been of variable usefulness. Nossel has devised a radioimmunoassay for F.P.A. and has found it to be increased in various thrombotic states; however, it is also increased in certain inflammatory disorders (41). Gurevich's group has championed the serial dilution protamine sulfate test, which measures fibrin monomer and early fibrin split products (42), and Bynum and Wilson have found that rising levels during heparin therapy correlate with recurrence of thromboembolism (43). Reduced antithrombin activity has been found in old age, with oral contraceptive use, in myocardial infarction, deep venous thrombosis, and disseminated intravascular coagulation, but it is unsettled whether its measurement is helpful in heparin management (44, 45). Thus, Sagar et al. (46) found that low preoperative antithrombin levels in patients receiving low dose heparin for hip repair correlated with postoperative thrombosis, but normal or even high levels did not protect patients against thrombosis. Several groups have found increased H.N.A. in various disorders, including occlusive peripheral vascular disease (47), coronary and cerebral thrombosis (48), and thromboembolism (49), and O'Brien et al. have shown that surgical patients who develop thrombosis have higher H.N.A. levels than those without thromboses (50).

Finally, a variety of other clotting tests - blood recalcification time, plasma recalcification time, polybrene neutralization test, thrombin clotting time, whole blood activated partial thromboplastin, and plasma heparin assay - have been advocated in heparin management (51-55). While these tests may fairly reliably measure heparin levels, their efficacy in preventing bleeding or recurrent thrombosis awaits controlled clinical trials.

3. Continuous versus intermittent administration

Whether continuous or intermittent heparin administration offers an advantage in heparin management has been the subject of much investigation. The results are conflicting and controversial. Bauer's early studies, employing intermittent therapy with avoidance of nighttime doses, achieved a low 1.5% incidence of bleeding complications, unequaled in the literature (56). Basu's studies using the A.P.T.T. were performed in patients only on continuous therapy; there was no group on intermittent therapy (33). In a study by Salzman et al. continuous infusion appeared safer than intermittent therapy but was no less effective for prevention of thromboembolism (40). A similar advantage of continuous infusion was shown by Glazier and Crowell (57). Neither study was well designed regarding objective criteria of thrombosis (58). Other studies have shown no advantage for continuous therapy, and

other factors, such as age, sex, total heparin dose, and associated hemostatic defects, may be important (58-61).

4. Summary

It is clear that despite the accumulated effort, it is not known how heparin preparations should be assayed and compared in biologic activity, or whether laboratory control or route of administration (i.e., continuous vs. intermittent) is important in heparin management. A rigorous prospective controlled clinical trial at this institution has begun in an attempt to answer these difficult problems.

F. Low dose ("mini-dose") Heparin - Indications and Biochemical Rationale

It has been established that small subcutaneous doses of heparin offers prophylaxis against thrombophlebitis in most surgical patients and in selected medical patients (low flow states) (62-68). More importantly, low dose heparin has been shown to reduce the incidence of fatal pulmonary embolism in patients undergoing various surgical procedures (69-71). Its use is now recommended in patients over 40 undergoing general abdominothoracic or gynecologic surgery,in patients undergoing these procedures who are under 40 but are predisposed to thrombosis,and in hospitalized medical patients at risk (heart failure, immobilization) (72, 73). Its use has generally been ineffective or inconclusive in patients undergoing hip or prostatic surgery (62, 67), and cannot be recommended in cerebral surgery. The most commonly employed dose is 5000 units 2 hours pre-op, followed by the same dose q. 8 - 12 hrs until the patient is ambulatory.

The question asked is, "How can small doses of heparin, in amounts that will not even budge the whole blood clotting time, possibly provide effective protection against thromboembolism?" The answer is reflected in the manner by which thrombin is evolved. Activated X (X-a), in addition to factor V, calcium, and phospholipid, converts prothrombin to thrombin. There is a 50-fold amplification of the coagulation process, such that 1 unitof X-a leads to the evolution of 50 units of thrombin (Figure 7.)

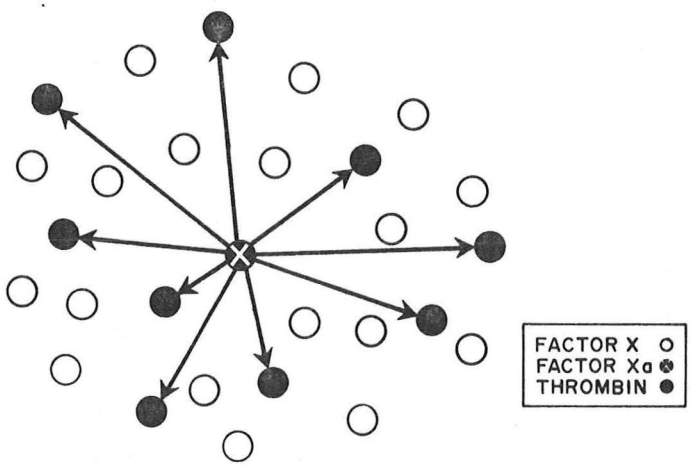

Figure 7. Conversion to Thrombin by activated Factor X

Thus, much less antithrombin (the inhibitor to X-a) and less heparin, is required to halt the clotting process by inhibition at the X-a level than if large amounts of thrombin have been evolved (Figure 8).

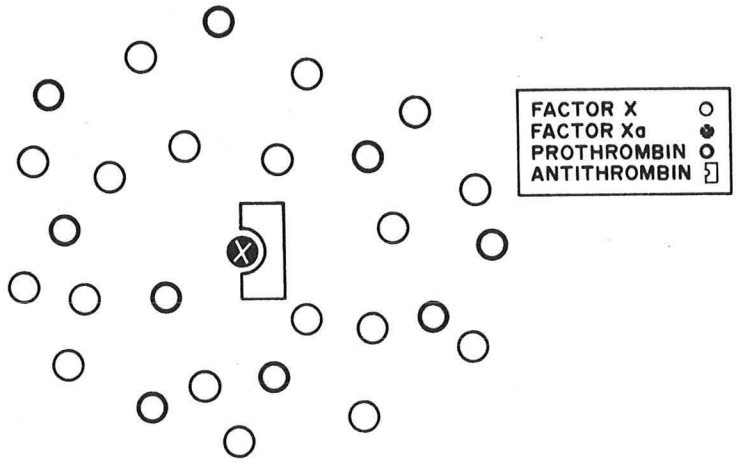

Figure 8. Inhibition of X-a by Antithrombin

Therein lies the beauty and limitations of low dose heparin. During and after surgery a state of "hypercoagulability" is initiated but remains non-thrombotic as long as the rate of X-a formation is matched by neutralization by antithrombin and low doses of heparin. Once "spillover" of X-a occurs, however, vast quantities of thrombin are formed which cannot be neutralized by these heparin doses (74). Thus, full dose heparin therapy is needed for an established thrombosis.

G. Side Effects

1. Hemorrhage. This is the most common and most serious side effect, occurring in 1.5-20% in most published series.

2. Activation of lipoprotein lipase.

3. Interference with elaboration of aldosterone.

4. Alopecia (rare).

5. Osteopenia (rare).

6. Thrombocytopenia (? incidence).

Thrombocytopenia in relation to heparin therapy deserves special mention. There has been a remarkable recent increase in reports of this phenomenon (75-78). While the mechanism is probably immunologic, hypofibrinogenemia and elevated titers of fibrinogen-fibrin degradation products have also been reported (78-79). Heparin-induced thrombocytopenia can be demonstrated by the use of the platelet aggregometer, a modified spectrophotometer. As shown in Figure 9, direct aggregation of platelets by patient's serum displays a decrease in optical density as more light can now be transmitted through the platelet-rich plasma (Curve A). After heparin is withdrawn, patient serum requires heparin to aggregate platelets (Curve B). Normal serum does not cause these changes (Curve C).

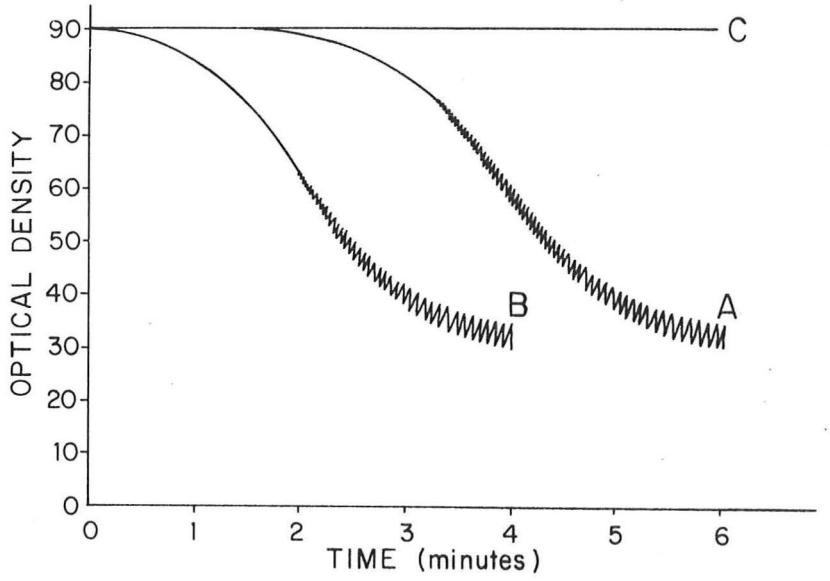

Figure 9

III. Coumadin® (sodium warfarin)

A. Structure

Sodium warfarin, or Coumadin, is one of the anticoagulants derived from 4-hydroxycoumarin. Specifically, it is the sodium salt of 3 (α-acetonyl-benzyl)-4 hydroxy-coumarin (80). The minimal structural requirements for activity are an intact 4-hydroxycoumarin residue with the three position substituted by a carbon residue or a hydrogen atom (81).

B. Mode of Action

1. Historical background

In the 1930's Karl Link was a young biochemist at the University of Wisconsin Agricultural Experiment Station. His group was pressed into

action under pressure by farmers whose bulls were bleeding to death after castration or de-horning. Shofield had described this disease in cattle in Canada in 1922 (82). This was the so-called "sweet-clover disease", a hemorrhagic, often fatal disorder caused by the ingestion of spoiled sweet clover hay. Shofield had been able to control the disease by the withdrawal of the spoiled hay from the diet and by the injection of serum freshly drawn from normal cattle. Subsequently, Quick reported that this disease was associated with a low level of prothrombin (83). But it was Link and his group who isolated 3,3'-methylene-bis (4-hydroxycoumarin) (Dicumarol) as the agent responsible for the hypoprothrombinemia (84), and soon Dicumarol was being given to patients at the Mayo Clinic for thrombophlebitis (85).

About the time that Sweet Clover Disease was being described in Canada, a Danish chemist, Henrik Dam, noted a bleeding tendency as an incidental finding in chickens fed a synthetic diet. His studies led to the conclusion that the chicks' diet lacked a substance necessary for normal coagulation. He called this substance "Koagulations-vitamin", or vitamin K (86). In 1940, Edward Doisy, a St. Louis University biochemist, isolated and characterized the vitamins K (87), and for their achievements Dam and Doisy were awarded the Nobel Prize in 1943, the only time the prize has been given for work in coagulation. Once vitamin K was found to induce hypoprothrombinemia (88), it use in obstructive jaundice and hemorrhagic disease of the newborn was established.

When Dicoumarol and Vitamin K were discovered only prothrombin could be implicated, simply because factors VII, IX and X had not yet been discovered. When they were, it became clear that these two compounds were diametrically opposed in their actions, and as the rationale for coumarins was born, so was the rationale for their antidote.

2. Biochemical model

As with the heparin model, it is best to describe Coumadin's biochemical activity in terms of the process it is antagonizing, namely, the promotion of vitamin-K-dependent activity of factors II, VII, IX, and X, otherwise grouped as the "prothrombin complex".

It had long been supposed that vitamin K was responsible for the synthesis of the prothrombin complex. This concept was shaken when it was discovered that proteins immunologically similar to these clotting factors but lacking procoagulant activity could be found in vitamin-K-deprived animals (89-92). Vitamin K does not induce the synthesis of these factors, but a vitamin-K-dependent post ribosomal modification is essential for their calcium-binding properties, their specific adsorption onto barium salts, and for their physiologic activation (90-93). Vitamin K apparently is required for incorporation of a second carboxyl group onto the glutamic acid side chain of prothrombin (89, 94). The coumarins apparently antagonize the ability of vitamin K to effect these biochemical modifications.

C. Indications

Coumadin has no effect on the evolution of the arterial thrombus arising through the interaction of platelets with an abnormal vessel wall. Its use is confined to prevention of the formation and extension of the "red" or stasis thrombus. Coumadin does not have an immediate antithrombotic effect, and if such therapy is required heparin must be used. Coumadin is therefore used to continue treatment initiated by heparin or to prevent future thrombosis, as follows:

1. Deep vein thrombosis and pulmonary embolism.

2. Prevention of systemic embolism in patients with mitral stenosis and atrial fibrillation or artificial heart valves.

3. Prophylaxis against thromboembolism in patients undergoing hip fracture repair.

4. Prophylaxis against thrombosis in high risk patients.

5. Of questionable benefit in patients with cerebrovascular disease (96).

D. Contraindications

1. Unreliable patient

2. Bleeding

3. Certain operations (lung, CNS, eye)

4. Pregnancy

5. Miscellaneous (hypertension, liver disease, S.B.E., peptic ulcer disease).

As with heparin, one must weigh the risk of Coumadin therapy against the need for therapy, and if the need is urgent enough certain contraindications e.g., non-bleeding peptic ulcer disease, may be waived and the patient treated with caution.

E. Therapy

1. Initiation of therapy

Coumadin affects only the vitamin-K-dependent levels of prothrombin complex factors; it does not alter clotting factor catabolism. Therefore, the effect of Coumadin is dependent only on the rate of decline of activity of these factors. Due to different half-lives, factor VII

will decay most rapidly, followed by factors IX, X, and II in that order. It has been shown however, that the antithrombotic effect of Coumadin is related to the depression of factor IX and X activity, not factor VII, and an antithrombotic effect is not reached until the fourth or fifth day. Thus, the traditionally employed large loading dose may quickly cause an early prolongation of the prothrombin time to a "therapeutic range," but this only reflects acutely depressed factor VII levels, occasionally to the point of bleeding, and such doses do not hasten the onset of a true antithrombotic effect (97-99). The safest dose appears to be 15 mgm p.o. daily until a therapeutic range (1½ - 2½ times control) is approached, then reducing the dose to a maintenance level. If heparin has been initiated, it should not be withdrawn until after five days of Coumadin therapy.

2. Variables complicating Coumadin therapy

The variables complicating Coumadin administration can virtually be predicted, knowing the basic pharmacology of Coumadin and vitamin K as diagrammed in Figure 10.

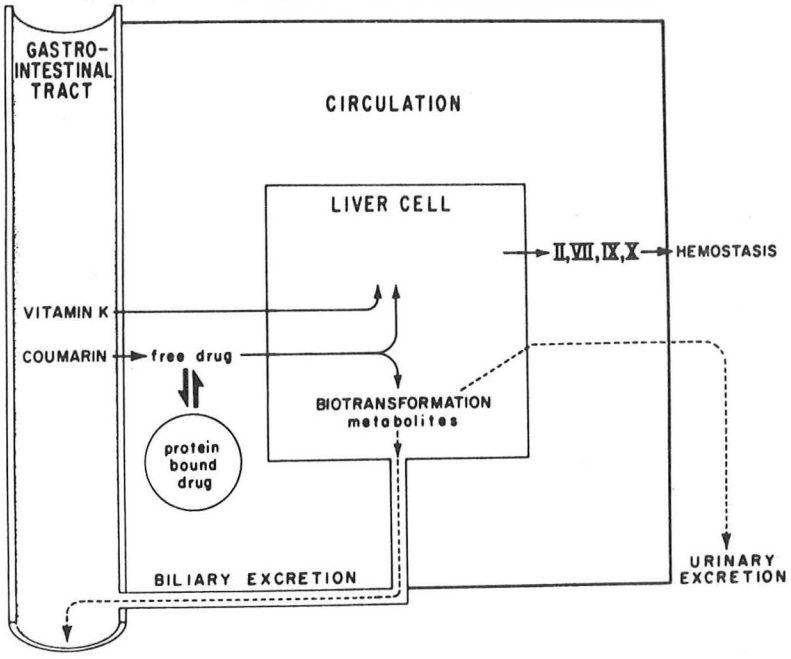

Figure 10. Pharmacology of Coumadin and Vitamin K
From Reference 97

Vitamin K is absorbed from the intestine into the blood stream, and subsequently enters the hepatocyte to modify precursor proteins into clotting factors II, VII, IX and X. Man obtains his vitamin K from two principal sources; probably dietary intake of leafy vegetables is more important than intestinal bacterial synthesis. Naturally-occurring vitamin K is fat-soluble, requiring bile for its absorption, but there are synthetic

water-soluble analogues available for therapy.

The ability of Coumadin to antagonize the action of vitamin K is dependent on the concentration of free, unbound Coumadin in the hepatocyte. The concentration of free Coumadin is dependent on the completeness of absorption, the degree of binding to plasma proteins, and the rate of biodegradation. Coumadin is rapidly and completely absorbed by passive diffusion from the upper gastrointestinal tract, and its peak plasma concentration is reached in three to nine hours. Once absorbed, Coumadin is highly bound to albumin (in the range of 97%). The bound portion of the drug is pharmacologically inactive and is protected from being metabolized in the liver. Only the unbound, or free drug is active in the hepatocyte and susceptible to biodegradation. The binding of Coumadin is readily reversible and therefore the percentage of bound to free drug is constant over a wide range of concentrations. The bound drug serves as a reservoir and is gradually released as biodegradation lowers the concentration of the free drug. Biodegradation of Coumadin consists largely of extensive hydroxylation in the hepatic microsomes. The rate of biodegradation appears to be genetically determined and varies greatly, accounting for the wide range of individual maintenance doses (97, 100, 101).

Based on the previous discussion, one can easily foresee the following variables complicating Coumadin therapy:

a. Those affecting vitamin K concentration at the hepatocyte

   1) Nutritional deficiency states (prolonged)

   2) Biliary obstruction, malabsorption

   3) Broad spectrum antibiotics (with debilitated patients and/or parenteral alimentation)

b. Those affecting action of free Coumadin at the hepatocyte

   1) Drugs displacing Coumadin from albumin binding site - e.g., phenylbutazone, clofibrate

   2) Drugs enhancing biodegradation of Coumadin; e.g., phenobarbital, glutethimide

   3) Drugs competing for similar biodegradation; e.g., Dilantin, tolbutamide

   4) Drugs inhibiting biodegrading enzymes - chloramphenicol

   5) Drugs interacting at site of coumarin action; e.g., vitamin K, salicylates

c. Those affecting the ability of the liver to synthesize clotting factors

1) Liver disease

2) Hepatotoxic drugs

3) Estrogens - increase activity

4) Anabolic steroids - decrease activity

d. Those drugs increasing clotting factor catabolism d-thyroxine, anabolic steroids

e. Those imposing additional hemostatic defects

1) Underlying bleeding disorders

2) Drugs - aspirin, phenylbutazone

Since literally scores of drugs interact with Coumadin, it is best to refer to excellent review articles (97, 100, 102) before prescribing the drug for a patient on Coumadin.

F. Side effects

1. Hemorrhage

2. Miscellaneous (rash, alopecia, leukopenia, leukemoid reaction, thrombocytopenia, jaundice, gastrointestinal distress)

3. "Purple toe" syndrome (103)

4. Coumadin necrosis.

Management of toxicity must be individualized. When the prothrombin time is outside the therapeutic range, with little or no bleeding, simply discontinuing the drug for a brief period of days will often suffice. With more bleeding symptoms Vitamin K_1 given orally or parenterally will correct the prothrombin time, and will also render the patient refractory to Coumadin for the next several days. When severe hemorrhage occurs plasma must be immediately administered, since it will take several hours for the vitamin K to begin to exert its effect. The use of prothrombin complex concentrates is associated with a high risk of hepatitis and disseminated intravascular coagulation can occur, especially in the setting of liver disease (104). Finally, it should be remembered that bleeding in the face of a therapeutic prothrombin time often indicates a structural lesion, such as ulcer or tumor, and such bleeding warrants investigation.

IV. Defibrinators (Ancrod and Reptilase)

A. Background and mode of action

Ancrod (Arvin) and reptilase are the purified coagulant enzymes which can be isolated from the venoms of the Malayan (Agkistrodan rhodostoma) and South American (Bothrops atrox) pit vipers respectively (105, 106). Their evaluation as antithrombotic agent was based on observations that the bites of these vipers caused severe hypofibrinogenemia, but relatively little bleeding (107, 108).

The modes of action of these coagulants have been well described by Pizzo et al. (109). Both cause a lowering of plasma fibrinogen by converting it to fibrin which, in turn, is assumed to be rapidly digested by plasmin before significant vascular occlusion and organ dysfunction occur (110-116). Both have thrombin-like actions but with important differences. As described earlier, thrombin cleaves fibrinopeptide A and fibrinopeptide B from fibrinogen's α and β chain respectively (the γ chain is unaffected). Ancrod cleaves the entire α chain, including fibrinopeptide A, but does not cleave fibrinopeptide B from the β chain (Figure 11). Also, Ancrod, unlike thrombin, does not activate factor XIII (fibrin-stabilizing factor). Thus ancrod promotes the formation of a non-crosslinked fibrin which is very vulnerable to plasmin digestion. Reptilase cleaves only fibrinopeptide A from fibrinogen, but, like thrombin, also activates factor XIII, causing fibrin crosslinking. This promotes a fibrin clot which has a greater susceptibility to lysis

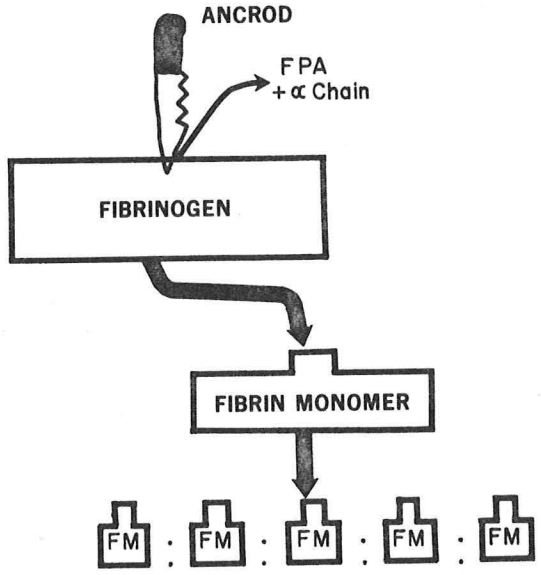

Figure 11. Action of Ancrod on Fibrinogen

than a thrombin-induced clot, but a lesser susceptibility to lysis than an ancrod-induced clot (Figure 12).

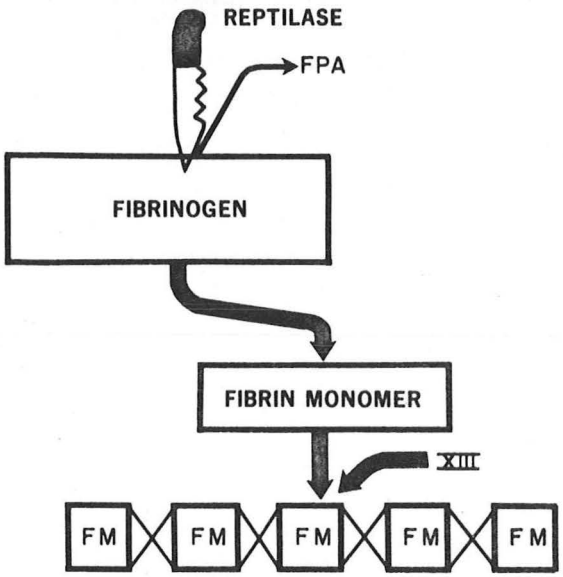

Figure 12. Action of Reptilase on Fibrinogen

B. Clinical effectiveness

Clinical studies have concentrated mainly on venous thrombosis. Three controlled studies have evaluated ancrod as a therapeutic agent in established venous thrombosis (117-119). Ancrod was shown to be as effective as heparin in preventing further thrombus extension, but neither produced increased thrombolysis assessed by repeat venography. No benefit was shown in two double blind studies of ancrod treatment for sickle cell crisis (120, 121). Reptilase has, in one small uncontrolled study, shown to be effective in deep vein thrombosis (122). Ancrod and reptilase have been reported, in small, uncontrolled studies, to be efficacious in central retinal vein occlusion (122, 123).

C. Side Effects

Spontaneous bleeding has been rare during ancrod and reptilase therapy, but bleeding is a problem in surgical patients. Specific antivenom is available for antidotal therapy.

D. Conclusions

Defibrinators, such as ancrod and reptilase, are effective antithrombotic agents by causing controlled hypofibrinagenemia. However, they are still investigational in this country, and their use seems to offer no clinical advantage over heparin.

V. Thrombolytic Drugs

The goal of thrombolytic therapy is to lyse thrombi by digesting their fibrin framework. The most important thrombolytic drugs are the plasminogen activators, streptokinase and urokinase. These agents cause clot lysis by converting plasminogen to plasmin, which degrades fibrinogen and fibrin. Because streptokinase is soon to be released commercially (and urokinase soon thereafter), these drugs merit discussion.

A. Chemistry

Streptokinase is a product of B-hemolytic streptocci, and is thus antigenic to man. Dosage must be large enough to exceed neutralization of antibodies found in response to previous infection. Urokinase can be isolated from human urine or tissue cultures of human embryonic kidney cells. It is not antigenic to man, thus circulating antibodies are not a problem (1).

B. Mode of Action (Figure 13)

Endogenous plasminogen activator is found mainly in vascular endothelium; urine contains trace amounts, termed urokinase (124-126). Once released, it activates plasminogen to plasmin which in turn digests fibrin, releasing degradation products. Plasminogen exists in a two-phase system: as a soluble phase form in plasma and other body fluids, and as a gel phase form in thrombi (plasminogen is bound to the fibrin interstices during clot formation) (127). When activator is present in the circulation the resultant plasmin is inhibited by antiplasmins, the most important of which is antithrombin (20). Any excess plasmin results in lysis of fibrinogen as well as proteolysis of factors V and VIII. On the other hand, activator also diffuses into thrombi; uninhibited plasmin released within the thrombus directly lyses fibrin (128). Thus, controlled infusions of streptokinase and urokinase result in transiently reduced levels of fibrinogen and factors V and VIII while thrombi are being digested.

-Dissimilar consequences of plasminogen activation in plasma and in a thrombus.

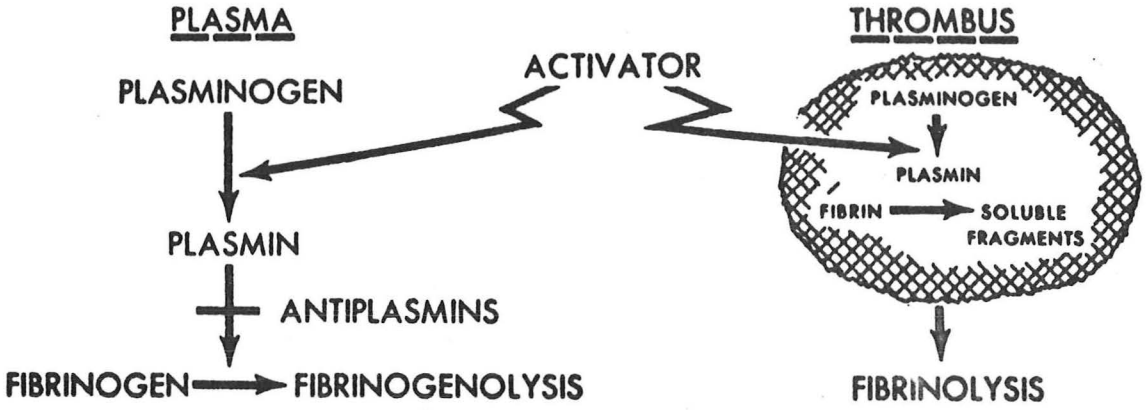

Figure 13. Fibrinolysis. From Reference 128.

C. Clinical Effectiveness

Acute major pulmonary embolism. Two trials organised by the National Heart and Lung Institute have compared the effects of urokinase (infused for 12 or 24 hours), streptokinase (infused for 24 hours) and heparin on the rate of resolution of massive or submassive pulmonary emboli (39, 129). All three regimens using thrombolytic drugs produced greater resolution at 24 hours than did heparin, and 24 hours of infusion of either agent gave no significant advantage over 12 hours of urokinase (Figure 14) (39, 129, 130). However, at the end of one week the lung scan improvement was no greater than with heparin alone (130). There was no difference in mortality between the heparin group and any of the plasminogen-activator groups. However, the patients had to survive long enough to have angiography performed, giving them a relatively better prognosis for survival. Thus the design of the studies made it unlikely that a reduction of mortality could have been shown. Gallus and Hirsh recommend thrombolytic therapy for 1) those patients with severe pulmonary artery obstruction at diagnosis, 2) those patients with poor recovery during heparin therapy and, 3) those patients with underlying cardiac or respiratory disease who have reduced cardiopulmonary reserve (1).

Results of Thrombolytic Therapy in Pulmonary Embolism 12 to 24 Hours after Therapy Begun (NHLI Studies).[11,12]

	PHASE I (UPET)*		PHASE II (USPET)†		
	HEPARIN	12-HR UROKINASE	12-HR UROKINASE	24-HR UROKINASE	24-HR STREPTOKINASE
Change in angiographic severity score based on 4-point system	0.54	1.78	1.66	1.76	1.70
Decrease in relative percentage perfusion defect on lung scans	8.3	24.1	20	29.2	18.5
Change in pulmonary artery pressure (mm Hg)	−1.1	−6.2	−7.28	−7.53	−5.34
Change in cardiac index (l/min/m²)	−0.05	+0.02	+0.06	+0.30	+0.60

*Urokinase pulmonary-embolism trial.

†Urokinase-streptokinase pulmonary-embolism trial.

Figure 14. Results of Pulmonary Embolism Trials
From Reference 130

Venous thrombosis. Streptokinase has been shown to accelerate lysis of recent venous thrombi, preserving valve function and thus minimizing chances of postphlebitic disability (117, 131, 132). Such valve preservation is not possible with conventional anticoagulants. However, the chance of successful lysis is reduced if the thrombus is over 4 days old (132). Therapy is limited since many thrombi have been present for a long time, or have occurred within 10 days of surgery (when thrombolytic agents are contraindicated).

Arterial occlusion. Uncontrolled studies have demonstrated that over 50% of limb artery occlusions less than three days old can be partially or completely lysed by streptokinase (133-135). Thrombolysis by streptokinase can occur with chronic occlusions but is probably beneficial only in those with exacerbations of symptoms within four months of treatment (136-137). Since streptokinase infusions act slowly over three to four days, and since limb viability is often a concern, thrombolytic therapy for arterial occlusions appears reserved for patients in whom surgery is contraindicated.

Myocardial Infarction. Results of trials of thrombolytic therapy in myocardial infarction have neither totally proved nor disproved an advantage. In general, studies conducted on general medical wards have shown a significant reduction in mortality in the treated group; studies performed in coronary care units have shown no significant benefit, but the mortality in the control groups was low (1, 138, 139).

D. Dosage, Side-effects, Contraindications and Antidotes

For streptokinase infusion, a large loading dose (generally 250,000 units) is used, followed by a maintenance dose. Urokinase is generally given as a loading dose (2000 units/lb) followed by a maintenance dose. Heparin is administered after therapy to prevent rethrombosis.

The major side effect is bleeding, and treatment is contraindicated within 10 days of surgery and within 24 hours of arterial puncture. Mild local bleeding will respond to pressure. More serious bleeding requires stopping therapy and possibly reversal with epsilon aminocaproic acid along with fibrinogen infusions. Mild temperature elevations have occurred in 24% of patients given streptokinase and 16% of patients given urokinase.

E. Conclusions

Plasminogen activators will accelerate lysis of major pulmonary emboli and recent venous thrombosis, yet risk of bleeding is greater than with conventional anticoagulants. Thrombolytic therapy of recent arterial occlusion can be considered if surgery is not possible. Their usefulness in myocardial infarction is not settled.

VI. Antiplatelet Drugs

A. Summary of platelet plug formation.

A detailed review is beyond the scope of this text, and several excellent articles are available (140-143). Briefly, endothelial disruption leads to platelet adhesion to subendothelial structures. This initiates the release reaction, resulting in the release of platelet adenosine diphosphate (ADP) from storage granules. This causes further ADP release and platelet aggregation (Figure 15).

Prostaglandins also contribute in a major way to the release reaction. When platelets are stimulated by a variety of release inducers, arachidonic acid is converted by a cyclooxygenase to a labile cyclic endoperoxide which induces platelet release and aggregation and is a precursor of prostaglandins E_2 and $F_{2\alpha}$ (Figure 16). However, cyclic endoperoxide also is transformed to prostacyclin, which (strangely) inhibits platelet aggregation. Thus, aspirin, which inhibits arachidonic acid, may produce effects which counteract each other, perhaps explaining aspirin's weak clinical performance (189).

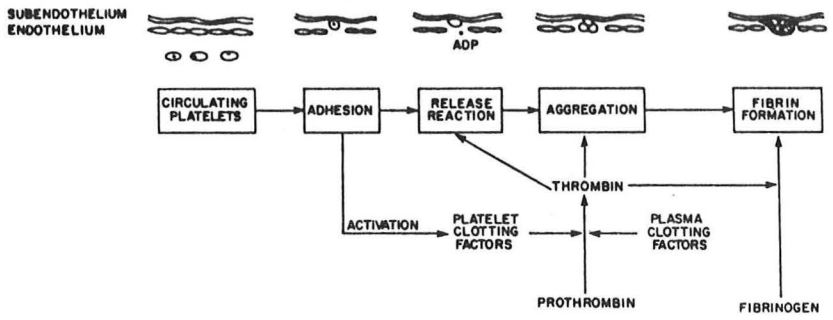

Figure 15. Scheme of platelet plug formation. From Reference 140.

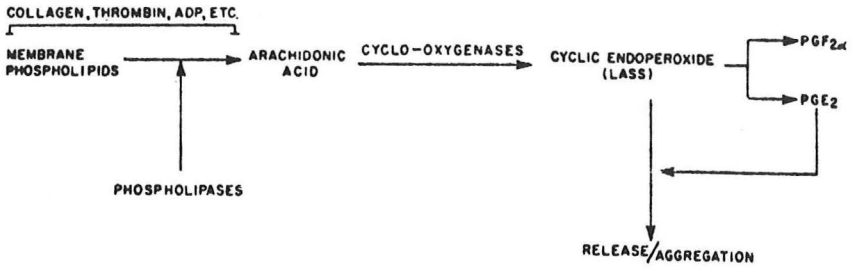

Figure 16. Role of prostaglandins in platelet plug formation. From Reference 141

B. Rationale for use of antiplatelet drugs

1. Arterial thrombi are chiefly composed of platelets.

2. Platelets may play a role in initiation of venous thrombi (144, 145).

3. Certain thrombotic states are associated with a reduced platelet survival.

4. Certain agents can correct reduced platelet survival in thrombotic states (146).

C. Specific agents

1. Aspirin

a. Mode of action. Aspirin inhibits the platelet release reaction induced by adrenaline, ADP, and other substances, probably by inhibiting arachidonic acid (147). Aspirin prolongs the bleeding time in normal volunteers (148), and its effect lasts five to seven days.

b. Clinical effects.

Cerebrovascular Disease. Although case reports have suggested a beneficial effect of aspirin in amaurosis fugax (149, 150), any endorsement of this drug in transient ischemic attacks must await results of multicenter trials in progress (151).

Myocardial Infarction. Although retrospective studies have suggested that myocardial infarction occurs less frequently in aspirin users (152), a prospective randomized study did not demonstrate a significant effect on survival post-infarction by aspirin (153). In that study, however, those admitted to the study within 6 weeks of infarction did show an effect on survival. Until large prospective trials in England and America are completed, the use of aspirin in myocardial infarction cannot be recommended.

Venous Thrombosis. Although Harker and Slichter found reduced platelet survival in venous thrombosis (3), the use of aspirin in preventing venous thrombosis has been disappointing in clinical trials. Although positive results have been found when venous thrombosis was diagnosed clinically (154, 155), results have been negative (156, 157), or inconclusive (158, 159) when the diagnosis was made with ^{125}I-fibrinogen leg scanning or venography. Studies with aspirin combined with dipyridamole have been conflicting (160, 161). In summary, aspirin cannot be recommended as an effective prophylactic agent for venous thrombosis.

Prosthetic Heart Valves. In patients with substitute heart valves taking warfarin, the addition of aspirin alone (162) or aspirin plus dipyridamole in two patients (163) has been shown to reduce the incidence of thromboembolism. More studies need to be done before aspirin can be recommended in this situation.

Thrombocytosis and Spontaneous Platelet Aggregation. Three case report series have demonstrated that aspirin can abolish peripheral ischemia in patients with thrombocytosis and spontaneous platelet aggregation, usually in the setting of a myeloproliferative disorder (164-166).

c. Conclusions

Aside from the rare disorder of thrombocytosis, spontaneous platelet aggregation and ischemia, the use of aspirin in most thrombotic states cannot yet be recommended.

2. Sulfinpyrazone

a. Mode of action. Sulfinpyrazone is a uricosuric agent, and was the first drug shown to inhibit platelet function (167). Although sulfinpyrazone inhibits the release reaction, it does not prolong the bleeding time in normal volunteers (168). The mechanism of the inhibitory effect is not known. Unlike aspirin, the effect of sulfinpyrazone on platelet function is reversible and only present for as long as the drug is present in the circulation.

b. Clinical effects

Cerebrovascular Disease. In a small controlled study by Evans and Gent (169), sulfinpyrazone was shown to reduce the frequency of amaurosis fugax; however the important parameters of stroke and death were not examined. Blakely and Gent (170) showed that the drug reduced mortality from vascular causes in patients with a past history of stroke, but autopsies were not done. Thus sulfinpyrazone, while showing promise cannot yet be recommended in cerebrovascular disorders.

Venous Thrombosis. Steele et al. (171) found that, in patients with reduced platelet survival and recurrent venous thrombosis resistant to anticoagulants, sulfinpyrazone could normalize platelet survival and decrease the number of recurrences. However, the diagnosis of recurrence in some patients may have been based on clinical grounds.

Prosthetic Heart Valves. Steele et al. (163) showed that the addition of sulfinpyrazone to a warfarin regimen improved

platelet survival and diminished thromboembolic recurrence in six patients. Once again, more studies and patient numbers are needed.

Arterio-venous Shunts. Kaegi et al., in two prospective, controlled studies (172, 173) showed that sulfinpyrazone reduces the frequency of shunt thrombosis in patients with arterio-venous shunts used for chronic haemodialysis.

c. Conclusions

Sulfinpyrazone is of value in preventing shunt thrombosis in patients undergoing chronic hemodialysis, and it is of possible value with oral anticoagulants in patients with recurrent venous thrombosis refractory to anticoagulants alone.

3. Dipyridamole

a. Mode of action. Initially used as a vasodilator, dipyridamole inhibits the platelet release reaction only at concentrations above the usual pharmacologic doses. Dipyridamole does not prolong the bleeding time.

b. Clinical effects

Cerebrovascular Disease. Acheson et al., in evaluation of patients with transient ischemic attacks or stroke, found that dipyridamole did not reduce the frequency of further transient ischemic attacks on strokes and did not affect mortality (174).

Myocardial Infarction. Gent et al.(175), in a small study, found no effect of dipyridamole on mortality after myocardial infarction.

Venous Thrombosis. There is little data. Two studies have shown no effect on the incidence of postoperative venous thrombosis (176, 154). However, the diagnosis of recurrence was based on clinical grounds, and no study has been done using objective diagnostic tests such as ^{125}I fibrinogen leg scanning or venography.

Prosthetic Heart Valves. Harker and Slichter (146) found that dipyridamole prevented valve-related platelet consumption. Sullivan et al. showed that treatment with dipyridamole and oral anticoagulants reduced the incidence of embolism from aortic or mitral prosthetic valves of the older type (177). Other studies have had similar findings (178, 179). A study by Taguchi et al. suggests that large doses of dipyridamole together with aspirin may also reduce the incidence of systemic embolism in this situation (180).

c. Conclusions

Combined with oral anticoagulants, dipyridamole prevents systemic embolism in patients with prosthetic heart valves.

4. Hydroxychloroquine

a. Mode of action. Hydroxychloroquine is an antimalarial drug which is also of value in rheumatoid arthritis and systemic lupus erythematosis. Although it weakly inhibits the platelet release reaction, it does not prolong the bleeding time (181).

b. Clinical effects. Although results of two studies demonstrated reduction in venous thrombosis using hydroxychloroquine, the designs of the studies have been criticized (182, 183, 151). Thus, although these results are promising they need confirmation before hydroxychloroquine can be recommended in this disorder.

5. Clofibrate

a. Mode of action. Clofibrate is a hypolipidemic drug which also inhibits platelet function (181, 184).

b. Clinical effects

Cerebrovascular disease. In one study by Acheson and Hutchinson, clofibrate did not prevent further transient ischemic episodes and stroke or death (185).

Myocardial Infarction. Two studies have shown reduced mortality in patients with angina pectoris given clofibrate, but both have been attacked on methodologic grounds (186, 187, 151), and a third study by the Coronary Drug Project Research Group failed to show any benefit in patients with proven myocardial infarction (188).

PRESENT VALUE OF ANTIPLATELET DRUGS

	Cerebro-vascular Disease	Coronary Artery Disease	Venous Thrombosis	Prosthetic Valves	A-V Shunts
Aspirin	?	?	?	?	-
Sulfinpyrazone	?	?	?	?	Yes
Dipyridamole	No	-	-	Yes*	-
Hydroxychloroquine	-	-	?	-	-
Clofibrate	-	?	-	-	-

Yes - Firm data supports use of drug

No - Firm data does not support use of drug

? - Data inconclusive or more studies needed

\- - Little or no data

* - In combination with oral anticoagulants

BIBLIOGRAPHY

1. Gallus AS, Hirsh J: Drugs 12:41, 1976.

2. Deykin D: N Eng J Med 276:622, 1967.

3. Harker LA, Slichter SJ: N Eng J Med 287:999, 1972.

4. Genton, E: Ann Int Med 80:77, 1974.

5. Freeman, L: Am J Card 14:3, 1964.

6. Goodman L, Gilman A: The Pharmacologic Basis of Therapeutics, 4th ed. New York, NY, Macmillan Co, 1970.

7. Bradshaw RA, Wessler S (editors): Heparin: Structure, Function and Clinical Implications. New York, NY, Plenum Press, 1975. page 3.

8. Dietrick CP, Silva ME: J Biol Chem 250:6841, 1975.

9. McKee PA, et al.: Proc Nat Acad Sci 66:738, 1970.

10. Bailey K, et al: Nature (London) 167:233, 1951.

11. Laki K, Gladner JA: Physiol Review 44:127, 1964.

12. Hammersten, O: Pflueger Arch Ges Physiol 19:563, 1879.

13. Contejean C: Arch Physiol Norm Pathol 7:45, 1895.

14. Morowitz P: The Chemistry of Blood Coagulation. Springfield, Massachusetts., Charles C. Thomas, 1968.

15. McClean, J: Am J Physiol 41:25, 1916.

16. Brinkhous KM et al: Am J Physiol 125:683, 1939.

17. Waugh DF, Fitzgerald MA: Am J Physiol 184:627, 1956.

18. Monkhouse FC et al: Circ Res 3:397, 1955.

19. Abildgaard U: Scand J Clin Lab Invest 21:89, 1968.

20. Rosenberg RD, Damus PS: J Biol Chem 248:6490, 1973.

21. Rosenberg RD: New Eng J Med 292:146, 1975.

22. Rosenberg RD: Circulation 49:603, 1973.

23. Rosenberg RD: Annual Symposium, American Association of Bood Banks, page 25, 1975.

24. Borzvic M, Bangham DR: Heparin: Structure, Function and Clinical Implications (Ed by RA Bradshaw and S Wessler), New York NY, Plenum Press, 1975. Page 163.

25. Bangham DR: Thrombos Haemostas 35:472, 1975.

26. Yin ET et al: J Lab Clin Med 81:298, 1973.

27. Johnson EA et al: Thrombos Haemostas 35:586, 1976.

28. Gomez-Perez F: J Clin Pharm 12:413, 1972.

29. McMahan FG, Jain AK, Ryan JR, Lefton TE: Clin Pharm Therap 17:80, 1975.

30. Baltes BJ, Diamond S, D'Agostino RJ: Clin Pharm Therap 14:287, 1973.

31. Wessler S, Morris LE: Circulation 12:563, 1956.

32. Lee RI, White PD: Am J Med Sci 146:495, 1913.

33. Basu et al: New Eng J Med 287:324, 1972.

34. Zucker S et al: J Lab Clin Med 73:320, 1969.

35. Bell WM, Alton HG: Nature (London) 17:880, 1954.

36. Shapiro GA, Huntzinger S, Wilson JE: Am J Clin Path (In Press 1977).

37. Teien AN, Abildgaard U: Thrombo Haemostas 35:592, 1976.

38. Soloway HB et al: Am J Clin Path 58:405, 1972.

39. Urokinase Pulmonary Embolism Trial. Circulation (Suppl. II) 1-108, 1973.

40. Salzman EW, Deykin D, Shapiro RM, Rosenberg R: New Eng J Med 292:1046, 1975

41. Nossel HL et al: J Clin Invest 54:43, 1974.

42. Niewiarowski S, Gurewich V: J Lab Clin Med 77:665, 1971.

43. Bynum LJ, Wilson JE: Clin Res 24 (1):53A, 1976.

44. Fagerhol MK, Abildgaard U: Scand J Haem 7:10, 1970.

45. Von Kaulla, von Kaulla KN: Am J Clin Path 48:69, 1967.

46. Sagar S, Stamatakis JD, Higgins AF: Lancet 1:1151, 1976.

47. Cotton RC et al: Atherosclerosis 16:337, 1972.

48. Cotton RC et al: Atherosclerosis 8:959, 1968.

49. Farbiszewski R et al: Thromb et Diath Haemorrh 19:578, 1968.

50. O'Brien JR et al: J Lab Clin Med 83:342, 1974.

51. Gullian GS et al: Am J Clin Path 65:390, 1975.

52. Blakely JA: Canad Med Asso J 99:1072, 1968.

53. Grann VR et al: Am J Clin Path 58:26, 1972.

54. Penner JA: Am J Clin Path 61:645, 1974.

55. Baden et al: Am J Surg 124:797, 1972.

56. Bauer G: Am J Card 14:29, 1964.

57. Glazier RL, Browell EB: JAMA 236:1365, 1976.

58. Wilson JE: Medical Grand Rounds, Parkland Memorial Hospital, 31 March 1977.

59. Bynum LJ, Wilson JE: Clin Res 24(1):32A, 1976.

60. Mant et al: National Meeting, Am Soc Hem Abst. page 94, 1975.

61. Jick H et al: New Eng J Med 279:284, 1968.

62. Gallus AS et al: New Eng J Med 288:545, 1973.

63. Sharnoff JG: Surg Gyn Obst 123:303, 1966.

64. Kakkar VV et al: Lancet 2:669, 1971.

65. Williams HT: Lancet 2:950, 1971.

66. Gordon-Smith IC et al: Lancet 1:1133, 1972.

67. Kakkar VV: Lancet 2:101, 1972.

68. Nicolaides AN et al: Lancet 2:890, 1972.

69. Kakkar VV et al: Lancet 2:45, 1975

70. Sherry S: New Eng J Med 293:300, 1975.

71. Sagar S et al: Brit Med J 4:257, November 1, 1975.

72. A.H.A. Recommendations. Circulation 55:423a, 1977.

73. Sherry S: Am Rev Resp Dis 114:661, 1976.

74. Wessler S, Yin ET: Circulation 47:671, 1973.

75. Rhodes GR et al: Surg Gyn Obst 136:409, 1973.

76. Fratantoni JC et al: Blood 45:395, 1975.

77. Babcock RB et al: New Eng J Med 295:237, 1976.

78. Bell WR et al: Ann Int Med 85:155, 1976.

79. Klein HG, Bell WR: Ann Int Med 80:477, 1974.

80. Deckert FW: South Med J 67:1191, 1974.

81. Goodman L, Gilman A: The Pharmacologic Basis of Therapeutics, 5th edition. New York NY, Macmillan Co., 1970. page 1355.

82. Shofield FS: Canad Vet Rec 3:74, 1922.

83. Quick AJ: Am J Physiol 118:260, 1937.

84. Link KP: Harvey Lect 39:162, 1943-44.

85. Allen EV et al: JAMA 120:1009, 1942.

86. Dam H: Biochem J 29:1273, 1935.

87. Doisy EA: Science 91:58, 1940.

88. Dam H: Biochem J 30:1075, 1936.

89. Nelsestuen GL et al: J Biol Chem 249:6347, 1974.

90. Stenflo J, Ganrot PD: J Biol Chem 247:8160, 1972.

91. Stenflo J: J Biol Chem 247:8167, 1972.

92. Brozovic M: Brit J Haematol 32:9, 1976.

93. Nelsestuen GL, Suttie JW: Biochemistry 11:4961, 1972.

94. Nelsestuen GL, Suttie JW: J Biol Chem 247:8176, 1972.

95. Nelsestuen GL et al: Mayo Clin Proc 49:941, 1974.

96. Cervantes FD, Schneiderman LJ: Arch Int Med 135:875, 1975.

97. Deykin D: New Eng J Med 283:691-694, 801-803, 1970.

98. Deykin et al: Am J Physiol 199:1161, 1960.

99. O'Reilly RA, Aggeler PM: Circulation 38:169, 1968.

100. Koch-Weser J, Sellers EM: New Eng J Med 285:487-498, 547-558, 1971.

101. Zieve PD, Solomon HM: J Lab Clin Med 73:103, 1969.

102. Macleod SM, Sellers EM: Drugs 11:461, 1976.

103. Feder W, Auerbach R: Ann Int Med 55:911, 1961.

104. Davey RJ et al: Am J Med 60:719, 1976.

105. Esnouf MP, Tunnah GW: Br J Haematol 13:581, 1967.

106. Klobusitzky D, Konig P: Arch Exp Pathol Pharmakol 182:387, 1936.

107. Reid HA, Chan KE, Thean PC: Lancet 1:621, 1963.

108. Olsson P, Blombäck M, Egberg N, et al: Thromb Diath Haemorrh Suppl 47: 389, 1971.

109. Pizzo SV, Schwartz ML, Hill RL, Mckee PA: J Clin Invest 51:2841, 1972.

110. Chan KE, Reid HA: Lancet 1:461, 1964.

111. Ashford A, Ross JW, Southgate P: Lancet 1:486, 1968.

112. Bell WR, Pitney WR, Goodwin JF: Lancet 1:490, 1968.

113. Pitney WR, Bell WR, Bolton G: Br J Haematol 16:165, 1969.

114. Pitney WR: Thromb Diath Haemorrh Suppl 45:43, 1971.

115. Sharp AA: Thromb Diath Haemorrh Suppl 45:69, 1971.

116. Blomback M, Egberg N, Gruder E, et al: Thromb Diath Haemorrh Suppl 45: 51. 1971.

117. Kakkar VV, Flanc C, Howe CT, et al: Br Med J 1:860, 1969.

118. Davies JA, Merric MV, Sharp AA, Holt JM: Lancet 1:113, 1972.

119. Tibbutt DA, Williams EW, Walker MW, et al: Br J Haematol 27:404, 1974.

120. Mann JR, Deeble TJ, Breeze GR, Stuart J: Lancet 1:934, 1972.

121. Haddock DRW, Botoney-Ahulu FID, Janosi M, et al: J Trop Med Hygiene 76: 274, 1973.

122. Egberg N, Blomback M, Johnsson H, et al: Thromb Diath Haemorrh Suppl 47: 379, 1971.

123. Bowell RE, Marmion VJ, McCarthy CF: Lancet 1:173, 1970.

124. Todd AS: J Path Bact 78:281, 1959.

125. Warren BA: Br J Exp Path 44:365, 1963.

126. Kwaan HC: Fed Proc 25:52, 1966.

127. Alkjaersig N, Fletcher AP, Sherry S: J Biol Chem 233:86, 1958.

128. Sherry S: Disease a Month May 1969.

129. Urokinase-Streptokinase Embolism Trial. JAMA 229:1606, 1974.

130. Fratantoni JC, Ness P, Simon TC: New Eng J Med 293:1073, 1975.

131. Johansson E, Ericson K, Zetterquist: Acta Med Scand 199:89, 1976.

132. Kakkar VV, Sagar S, Lewis M: Lancet 2:674, 1975.

133. Schmutzler R, Koller F: Recent Advances in Blood Coagulation, edited by Poller, London, Churchill. page 299. 1969.

134. Amery A, Deloof W, Vermylen J, Verstraete M: Brit Med J 4:639, 1970.

135. Dotter CT, Rosch J, Scaman AJ: Radiology III:31, 1974.

136. Poliwoda H, Alexander K, Buhl V, et al: New Eng J Med 280:689, 1969.

137. Verstraete M, Vermylen J, Donati M: Ann Int Med 74:377, 1971.

138. Simon TL, Ware JH, Stengle JM: Ann Int Med 79:712, 1973.

139. European Collaborative Study. Lancet 2:624, 1975.

140. Weiss HJ: Am Heart J 92:86, 1976.

141. Weiss HJ: New Eng J Med 293:531, 1975.

142. Deykin D: New Eng J Med 290:144, 1974.

143. Deykin D: National AABB Symposium, page 5, 1975.

144. Paterson JC: Thrombosis. Edited by Sherry S, Brinkhous KM, Genton E, et al. Washington, D.C., National Academy of Sciences, 1969. page 321.

145. Sevitt S: Ibid. page 29.

146. Harker LA, Slichter SJ: New Eng J Med 283:1302, 1970.

147. Roth GJ, Majerus PW: J Clin Invest 56:624, 1975.

148. Mielke CH, Kaneshiro MM, Maher IA, et al: Blood 34:204, 1969.

149. Harrison MJG, Marshall J, Meadows JC, Russell RWR: Lancet 2:743, 1971.

150. Mundall J, Quintero P, Von Kaulla KN, et al: Neurology 22:280, 1972.

151. Genton E, Gent M, Hirsh J, Harker LA: New Eng J Med 293:1174-78, 1236-1240, 1296-1300, 1975.

152. Boston Collaborative Drug Surveillance Group: Brit Med J 1:440, 1974.

153. Elwood PC, Cochrane AL, Burr ML, et al: Brit Med J 1:436, 1974.

154. Salzman EW, Harris WH, DeSanctis RW: New Eng J Med 284:1287, 1971.

155. Loew D, Wellmer B, Baer V, et al: Deutsche Med Woch 99:565, 1974.

156. Medical Research Council. Lancet 2:441, 1972.

157. Wood EH, PrenticeCRM, McGrouther DA, et al: Thromb Diath Haemorrh 30: 18, 1973.

158. Harris WH, Salzman EW, Athanasoulis C, et al: J Bone Joint Surg 56A: 1552, 1974.

159. Clagett CP, Brier DF, Rosoff CB, et al: Surg Forum 25: 473, 1974.

160. Dechavanne M, Ville D, Viala JJ, et al: Haemostasis 4:94, 1975.

161. Renney JTG, O'Sullivan EF, Burke PF: Brit Med J 1:992, 1976.

162. Altman R, Boullon F, Rouvier J, et al: J Thor Card Surg 72:127, 1976.

163. Steele P, Weilly H, Davies H, et al: Circulation 51:358, 1975.

164. Vreeken J, van Aken WG: Lancet 2:1394, 1971.

165. Bierme R, Bonen R, Buiraud B, Pris J: Lancet 1:432, 1972.

166. Preston FE, Emmanuel IG, Winfield DA, Malia RG: Br Med J 3:548, 1974.

167. Smythe HA, Ogryzlo MA, Murphy EA, Mustard JF: Canad Med Ass J 92: 818, 1965.

168. Packham MA, Mustard JF: Platelets, Drugs and Thrombosis. Editors: Hirsh, Cade Gallus, Schonbaum. Basel, Karger, 1975. page 111.

169. Evans G, Gent M: Ibid, page 258.

170. Blakely JA, Gent M, Ibid, page 284.

171. Steele PP, Weily HS, Genton E: New Eng J Med 288:1148, 1973.

172. Kaegi A, Pineo GF, Shimizu A, et al: New Eng J Med 290:304, 1974.

173. Kaegi A, Pineo GF, Shimizu Z: Circulation 52:497, 1975.

174. Acheson J, Danta G, Hutchinson EC: Br Med J 1:614, 1969.

175. Gent AE, Brook CGD, Foley TH, Miller TN: Br Med J 4:366, 1968.

176. Browse N, Hall JH: Lancet 2:718, 1969.

177. Sullivan JM, Harken DE, Gorlin R: New Eng J Med 284:1391, 1971.

178. Meyer JS, Charney JZ, Rivera VM, Mathew NJ: Stroke 2:541, 1971.

179. Arrants JE, Hairston P: Am Surg 38:432, 1972.

180. Taguchi K, Matsumura H, Washizu T, et al: J Card Surg 16:8, 1975.

181. Kinlough-Rathbone RL: op. cit., page 124.

182. Carter AE, Eban R, Perrett RD: Br Med J 1:312, 1971.

183. Carter AE, Eban R: Brit Med J 3:94, 1974.

184. Carvalho ACA, Colman RW, Lees RJ: Circulation 50:570, 1974.

185. Acheson J, Hutchinson EC: Atherosclerosis 15:177, 1972.

186. Scottish Society of Physicians. Br Med J 4:775, 1971.

187. Group of Physicians of the Newcastle-upon-Tyne Region. Br Med J 4: 767, 1975.

188. Coronary Drug Project Research Group. JAMA 231:360, 1975.

189. Moncada S, Hibbs EA, Vane JR: Lancet 1:18, 1977.