1974

The Effects Of Some Salicylate Analogues On Human Blood Platelet Function

Donald Grant Mills

THE EFFECTS OF SOME SALICYLATE ANALOGUES ON HUMAN BLOOD PLATELET FUNCTION

by

Donald Grant Mills, B.Sc.Phm.

Department of Pharmacology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Canada

August 1973

This investigation was supported by a grant to Dr. R. B. Philp, Department of Pharmacology, The University of Western Ontario, from the Ontario Heart Foundation. Personal support in the form of a Graduate Student Teaching Assistantship from the Faculty of Graduate Studies, The University of Western Ontario is gratefully acknowledged.

ABSTRACT

Chemicals related to the salicylates were investigated to determine structure-activity relationships as they pertain to the inhibition of human platelet aggregation initiated by ADP and collagen, to study the effects of incubation of these compounds on such aggregation, and to define the role of platelet acetylation in this inhibition.

Utilizing a turbidometric technique and human platelet-rich plasma (PRP) at 37°C, acetylsalicylic acid (ASA), 2-propoxybenzoic acid, 2,3-diacetoxybenzoic acid, sodium salicylate and 4-aminosalicylic acid (PAS), at suitable final concentrations and without prior incubation in PRP, prevented ADP-induced second phase aggregation and inhibited collagen-induced aggregation. The minimal concentrations of the latter four compounds inhibiting second-phase ADP aggregation were 15, 43, 69 and 100 times that of ASA, respectively. Without prior incubation, 2,6-diacetoxybenzoic acid and 3-propoxybenzoic acid potentiated the second phase response of ADP-induced aggregation, while 3-acetoxybenzoic acid, 4-acetoxybenzoic acid and 2,4-diacetoxybenzoic acid had no effects on either collagen- or ADP-induced aggregation.

ASA, 2,3-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid and 2-propoxybenzoic acid were incubated in human PRP at 37°C for 5 and 10 min., and the effects on collagen aggregation and the uptake by platelets of radioactive acetate or propionate groups from ^{14}C-labelled

analogues of these compounds were studied to determine if a correlation existed between acylation and inhibition of aggregation. There was a direct relationship between the extent of inhibition and the final drug concentration. ASA was most potent, followed by 2-propoxybenzoic, 2,3-diacetoxybenzoic and 2,6-diacetoxybenzoic acid. Radioactive uptake of added activity also was dependent on the concentration of the labelled compound and the incubation time, and was highest with ASA, intermediate with 2,3-diacetoxybenzoic and 2,6-diacetoxybenzoic acid, and lowest with 2-propoxybenzoic acid. Oligomycin, a metabolic inhibitor, incubated in PRP for 15 min. at 10^{-5}M, reduced the uptake of acetate and propionate from the radioactive analogues. The amount of uptake under the experimental conditions employed did not correlate with the degree of inhibitory activity on collagen aggregation. Chloroform:methanol extraction removed about 50% of incorporated radioactivity from isolated platelet buttons. Platelet fragments produced by sonification did not incorporate radioactive label. The results suggest that structural specificity is essential to the aggregation inhibitory activity of ASA and do not support the hypothesis that acetylation of platelets by ASA is solely responsible for its inhibitory effects. They do not conflict with the suggestion that acetylation is responsible for the persistent *in vivo* effects of ASA.

To examine the effects of *in vitro* ASA on the platelets of persons suffering from gastrointestinal hemorrhage, collagen-and ADP-induced platelet aggregation were studied in 11 subjects (Group 1) having gastrointestinal hemorrhage within 96 hrs. of taking ASA, 11 (Group 2) with similar hemorrhage not associated with recent ASA ingestion, and

11 hospitalized control subjects (Group 3). ASA caused significantly greater inhibition of collagen aggregation in Group 1 than in either Group 2 ($p<0.005$) or Group 3 ($p<0.02$). A follow-up study, approximately one year later, yielded only a few patients and there was insufficient data to determine if the increased sensitivity was still present, but the results implied that this might be so. These data suggest that some individuals may have platelets which are intrinsically more sensitive to ASA.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. R. B. Philp, Professor of Pharmacology, whose patience, encouragement, and timely criticism have guided the completion of this project. For his competence as a supervisor and his provision of excellent laboratory facilities and personnel, I am grateful.

I also wish to express my appreciation to Dr. M. Hirst for the synthesis of the salicylate analogues employed in this study and for his timely guidance.

To Mr. B. Bishop for technical assistance, Mr. V. Lemieux for preparation of Figures, To Dr. I. T. Borda and to the Hematology Laboratory staff at St. Joseph's Hospital for their part in the gastrointestinal bleeding study, I extend my thanks.

Finally, I wish to thank Mrs. I. Francey whose many hours of phoning, organizing and friendly persuasion provided the blood donors who made the work possible.

TABLE OF CONTENTS

		Page
CERTIFICATE OF EXAMINATION		ii
ABSTRACT		iv
ACKNOWLEDGEMENTS		vii
TABLE OF CONTENTS		viii
LIST OF TABLES		xi
LIST OF FIGURES		xiii
I	INTRODUCTION	1
II	GENERAL REVIEW	4
	A. Early History	4
	B Blood Platelet Ultrastructure and Metabolism	8
	C. Blood Platelet Aggregation	16
	D. The Release Reaction	26
	E. Inhibitors of Platelet Aggregation	35
	F. Salicylates and the Hemostatic Mechanism	60
III	METHODS	70
	A. Selection of Blood Donors	70
	i. Normal subjects	70
	ii. Hospital patients	70
	B. Collection of Whole Blood	71
	C. Preparation of Platelet-Rich Plasma	72
	D. Platelet Counting	72
	E. Platelet Aggregation Studies	73
	F. Determination of Platelet Adhesiveness	75

		Page
	G. Source of Supply of Salicylate Analogues	75
	H. Preparation of Drugs and Reagents	78
	I. Measurement of Aggregation Curves	79
	J. Statistical Analysis	79
	K. Radioactive Label Study	79
	L. Gastric Bleeding Study - Laboratory Tests	87
IV	RESULTS	88
	A. Normal Aggregation Responses	88
	B. Effects of Salicylate Analogues on Platelet Aggregation	93
	C. Uptake by Platelets and Plasma Components of Radioactive Label From ^{14}C-ASA and its Analogues	114
	D. Salicylates and Gastrointestinal Hemorrhage	123
V	DISCUSSION	130
	A. Aggregation Responses	130
	B. Effects of Salicylate Analogues on Platelet Aggregation	131
	C. Radioactive Label Uptake	138
	D. Salicylates and Gastrointestinal Hemorrhage	140
	E. Concluding Remarks	142
VI	SUMMARY and CONCLUSIONS	145
APPENDIX I		149
APPENDIX II		151
APPENDIX III		165

Page

APPENDIX IV 157

APPENDIX V 160

APPENDIX VI 169

APPENDIX VII 178

BIBLIOGRAPHY 182

VITA 249

LIST OF TABLES

Table	Description	Page
1	Inhibitors of Platelet Aggregation	36
2	Percent Change of Adenosine Diphosphate-induced Aggregation Curve by 2-Propoxybenzoic Acid	102
3	Percent Change of Collagen-induced Aggregation Curve by Acetylsalicylic Acid	103
4	Percent Change of Collagen-induced Aggregation Curve by 2,3-Diacetoxybenzoic Acid	105
5	Percent Change of Collagen-induced Aggregation Curve by Sodium Salicylate	106
6	Percent Change of Collagen-induced Aggregation Curve by 4-Aminosalicylic Acid (PAS)	107
7	Minimum Final Concentration of the Salicylate Analogues Completely Inhibiting Adenosine Diphosphate Second Phase Aggregation	115
8	Final Concentration of Salicylate Analogues Producing Approximately 50 Percent Reduction of Collagen Curve Slope and Height	116
9	Percent of Radioactive Label Remaining in Platelet-poor Plasma Supernatant Following Exposure to the Labelled Salicylates	117

		Page
10	Total Radioactive Label Uptake by Platelets Exposed to the Labelled Salicylate Compounds	118
11	Percent of Radioactive Label in Platelet Buttons Following Exposure of Platelet-rich Plasma to Labelled Salicylate Analogues	119
12	Hemoglobin and Hematocrit Values	124
13	*In Vitro* Acetylsalicylic Acid Inhibition of Adenosine Diphosphate-induced Aggregation	126
14	Platelet Aggregation in Response to Collagen	127
15	Percent Inhibition of Collagen Aggregation by *In Vitro* Acetylsalicylic Acid	128

LIST OF FIGURES

Figure	Description	Page
1	The Chemical Structures of the Salicylate Analogues Employed in the Platelet Aggregation Study	77
2	Method of Measurement of Adenosine Diphosphate-induced Platelet Aggregation Curves	81
3	Method of Measurement of Collagen-induced Platelet Aggregation Curves	83
4	Effect of Increasing Concentrations of Adenosine Diphosphate on Human Platelet Aggregation	90
5	A Collagen-induced Human Platelet Aggregation Response	92
6	The Increasing Sensitivity to Adenosine Diphosphate of Human Platelet-rich Plasma During its Stabilization	95
7	Distribution of Final Concentrations of Adenosine Diphosphate Just Sufficient to Induce a Biphasic Aggregation Response	97
8	Effect of Increasing Concentrations of Acetylsalicylic Acid on Adenosine Diphosphate-induced Human Platelet Aggregation	100

		Page
9	Percent Inhibition of Collagen-induced Aggregation by Acetylsalicylic Acid, 2-Propoxybenzoic Acid, 2,3-Diacetoxybenzoic Acid, and 2,6-Diacetoxybenzoic Acid Not Incubated With Platelet-rich Plasma	109
10	Percent Inhibition of Collagen-induced Aggregation by Acetylsalicylic Acid, 2-Propoxybenzoic Acid, 2,3-Diacetoxybenzoic Acid and 2,6-Diacetoxybenzoic Acid Incubated For Five Minutes With Platelet-rich Plasma	111
11	Percent Inhibition of Collagen-induced Aggregation by Acetylsalicylic Acid, 2-Propoxybenzoic Acid, 2,3-Diacetoxybenzoic Acid and 2,6-Diacetoxybenzoic Acid Incubated For Ten Minutes With Platelet-rich Plasma	113
12	Effects of Incubation Time on Inhibition of Aggregation and Uptake of Radioactive Label From Acetylsalicylic Acid, 2,3-Diacetoxybenzoic Acid, 2,6-Diacetoxybenzoic Acid and 2-Propoxybenzoic Acid and Their Radioactive Analogues	122

I INTRODUCTION

"Coronary Heart Disease has reached enormous proportions, striking more at younger subjects. It will result in coming years in the greatest epidemic mankind has faced unless we are able to reverse the trend by concentrated research into its cause and prevention." This statement was made by the Executive Board of the World Health Organization in 1969 (Stamler, 1973) and reflects the concern of North Americans in general toward this rampant killer of young and old alike.

The fact that thromboembolism plays a significant role in this phenomenon is well recognized and numerous attempts have been made to reduce its morbidity and mortality with the use of anticoagulant drugs. Recognition of the differences between the formation of venous and arterial thrombi, and the vital role of blood platelets in the latter process, haveled to the study of compounds which might prevent platelets from sticking to damaged endothelial surfaces, from sticking to one another, and from forming platelet thrombi and emboli. Although a multitude of substances have been shown to inhibit platelet function *in vitro* through a variety of hypothesized mechanisms, a limited number of these have demonstrated significant actions *in vivo* and have shown promise in the prophylaxis or treatment of platelet-related thromboembolic disorders in man. Considerable research has focused on the inhibitory effects of anti-inflammatory analgesic compounds, particularly acetylsalicylic acid (ASA) which has *in vitro* inhibitory activity on human platelets and has shown some potential as an *in vivo* antiplatelet agent.

In response to suitable concentrations of aggregation stimuli, such as adenosine 5'-diphosphate (ADP) or collagen suspension *in vitro*, human platelets have been shown to release the components of a variety of intracellular granules. Some of the released substances, eg., ADP and 5-hydroxytryptamine (5-HT), are probably involved in further aggregation and the formation of a relatively stable platelet mass. Evidence has accumulated to suggest that it is this release reaction on which ASA exerts its inhibitory activity with resultant formation of an unstable, reversibly developed platelet aggregation. If corresponding phenomena take place in the human body, a better understanding of the *in vivo* ASA effects and its mechanism might lead to a better understanding of platelet function and the development of more efficient antiplatelet drugs. ASA is known to affect many systems in the human body but none of these actions can explain its inhibitory activity on platelets. Some studies have suggested that ASA acetylates platelets although the mechanism through which this reaction inhibits aggregation has not been defined. While other anti-inflammatory analgesics also inhibit platelet aggregation, both *in vitro* and *in vivo*, ASA is unique in that its effects persist in humans for up to seven days. It may be that the acetoxy group and the acetylation reaction may, in some way, be involved in the persistence of these effects *in vivo*.

Based on this background, a study was initiated to investigate the inhibitory effects on human platelet aggregation of a series of salicylate compounds, and to study the platelet uptake of a radioactive acetate or propionate group from ^{14}C-labelled salicylate derivatives in order to determine if the amount of acetate uptake was related to the degree of inhibition of *in vitro* platelet aggregation.

There is now general agreement that the administration of salicylates, particularly ASA, may in some individuals precipitate gastrointestinal hemorrhage. Many investigators have examined the effects of salicylate compounds on the gastric mucosa and have attempted to define the incidence and mechanism of ASA-induced gastric bleeding. ASA-related defects of hemostasis, including defects of blood platelet function, would be expected to intensify the bleeding tendency of susceptible individuals. In view of the importance of platelets in the arrest of bleeding, a study was undertaken in an effort to determine whether or not the platelets of individuals exhibiting gastrointestinal hemorrhage after ASA ingestion might be abnormally sensitive to ASA inhibition of platelet function, hence rendering the individual more susceptible to a bleeding episode.

II GENERAL REVIEW

A. Early History

At a session of the Académie des Sciences of Paris, on March 7, 1842, Alfred Donné stated that there existed in the blood, red and white globules and little globules (Tocantins, 1948). While Donné, because of this observation, has frequently been given credit for being the first to report the existence of blood platelets, Robb-Smith (1967) has noted that two English practitioners, George Gulliver and William Addison, may have been the first to observe these formed elements of the blood. Gulliver wrote notes and an appendix to an English translation of *Gerber's General and Minute Anatomy* in which he described minute particles which probably were platelets (Robb-Smith, 1967).

On April 15, 1842 Addison wrote that he had

> "...found the colourless corpuscles somewhat larger than the red corpuscles with which they compared, measuring 1/2800 to 1/3200 of an inch in diameter. I observed, at the same time, that the fluid, i.e. the liquor sanguinis, contained a great number of extremely minute molecules or granules, varying in size, the largest being at least eight or ten times less than the colourless corpuscles and they were in much greater abundance. Whilst examining these minute bodies, I observed the coagulation of the fibrin commence. Exceedingly delicate and perfectly cylindrical filaments or fibres crossed the field of the microscope; they gradually increased in number, intersecting one another in various ways, and at length formed a complete network, in the meshes and angles of which the molecules and colourless corpuscles were collected and drawn together. Many molecules were situate at distant intervals, along the course of the filaments and the character of the filament being less than that of the molecules, they formed nodules upon it." (Robb-Smith, 1967)

In 1873, Vulpian described the presence of colourless corpuscles in the blood, noting that these elements had the ability to stick to a cover-

glass and to accumulate in clumps (Tocantins, 1948).

Many investigators were familiar with the appearance of experimentally-induced thrombosis and noted some involvement of platelets in this occurrence. Wharton Jones, in 1851, studied the stages of experimentally-induced thrombosis in the web of a frog's foot and described the clumping of colourless corpuscles in blood vessels as he pressed them with a blunt point. Bizzozero in 1882, reported the changes observed after a crush injury to small arteries or veins in guinea-pig mesentery (French, 1965). He noted the accumulation of platelets and leucocytes at the injury site, observing that if the blood flow was strong enough, part or all of the accumulated mass was washed away and a new one developed at the injury site. In the same year, Georges Hayem concluded that platelet thrombi were intimately involved in the arrest of bleeding and that a decrease in number or absence of platelets would result in abnormal hemostasis (Tocantins, 1948). Earlier findings were expanded by Welch in 1887 (French, 1965) when he emphasized the fact that platelets accumulated at a site of thrombus formation earlier in the process than did leucocytes. Platelet aggregation was initially described by Eberth and Schimmelbusch (1886) who used the term "viscous metamorphosis" to describe this phenomenon. Since these early observations, the importance of the platelet plug in hemostasis has been confirmed by many investigators (Apitz, 1942; Zucker, 1947; Chen and Tsai, 1948; Hugues, 1953).

A number of interesting hypotheses have been developed to define the site of platelet production in the body. Tocantins (1938) has reviewed many of these, pointing out that at one time in its history,

the platelet was thought not to exist as such in the circulation but merely to precipitate from plasma when blood was shed. Other theories suggested that platelets originated from the endothelial lining of blood vessels, from erythrocytes, leucocytes or from blood platelets by direct multiplication, from the lymph and hemolymph glands, and from the spleen and bone marrow. Several of these concepts persisted until 1906, when Wright (1906) demonstrated that megakaryocytes of the bone marrow gave rise to platelets by fragmentation of their cytoplasm.

Following the turn of the century, investigators focused their efforts on the hemostatic process and few references to platelets appeared in the literature during this period. The various theories of clotting and the sequence of events leading to our present understanding have been documented by Howell (1935), Brinkhous (1959) and Poller (1969). In 1912, however, Duke proposed the bleeding time of the skin as a test for bleeding tendency, showing a close correlation between blood platelet count and the duration of the bleeding time (Tocantins, 1948). Further important contributions were made by Wright and Minot in 1917, who described irreversible aggregation in great detail, and Payling Wright (1941) when she devised a rotating glass bulb technique for the measurement of platelet adhesiveness.

The term salicylate describes a family of drugs which have in common the 2-hydroxybenzoate radical. Ancient civilizations were aware of the antipyretic properties of willow bark from which Leroux isolated the bitter glycoside salicin in 1827. It was from this glycoside that Piria prepared salicylic acid in 1838. Cahours isolated salicylic acid from oil of wintergreen in 1844 and Kolbe and Lautemann prepared it from

phenol in 1860 (Soine and Willette, 1962). Sodium salicylate was introduced as an antipyretic in 1875 by Buss (Woodbury, 1971). Acetylsalicylic acid was first prepared in 1853 by von Gerhardt, but its pharmacological properties were not discovered until 1899 by Hoffman (Soine and Willette, 1962). It was Dresser who named it "aspirin" and introduced it to medicine in 1899 (Woodbury, 1971).

It has been known for some time that salicylate administration in experimental animals and man induces a temporary hypoprothrombinemia and may be a factor in several types of bleeding episodes, including those of the gastrointestinal tract (Smith and Smith, 1966). Meyer and Howard (1943) were the first to show in man that the prothrombin content of the blood was decreased by the administration of salicylate. This finding was later confirmed both in man (Rapoport *et al.*, 1943; Shapiro, 1944) and in experimental animals (Link *et al.*, 1943; Rapoport *et al.*, 1943). In 1916, Gregersen published data which showed that positive chemical tests for blood were obtained in the feces of patients receiving salicylates. Bleeding has frequently been associated with fatal salicylate poisoning, petechial hemorrhages having been reported in the heart and lungs (Polson, 1959), skin and brain (Troll and Menton, 1945) and the thymus and adrenal glands (Stevens and Kaplan, 1945). Considerable evidence has accumulated to suggest that in certain individuals salicylate administration may precipitate marked bleeding episodes. Much of this work has been reviewed by Smith and Smith (1966). Frick (1956) has reported that salicylates increase capillary fragility with resultant bleeding.

From this historical base, the literature on platelet function and

its inhibition has proliferated since the middle of the last decade. Since it is beyond the scope of this dissertation to review in its entirety, all facets of platelet morphology and function, more emphasis has been placed on those areas directly related to the effect of salicylate compounds on platelets.

B. Blood Platelet Ultrastructure and Metabolism

Blood platelets have been shown to be complex, well-organized, anucleate, disc-shaped cells, approximately three microns in diameter and one micron in thickness (Hirsh and Doery, 1971), containing mitochondria, granules of varying electron density, vacuoles, vesicles, golgi apparatus, microtubules, microfilaments, glycogen granules (Hovig, 1968; Zucker-Franklin, 1970b), fibrinogen (Gokcen and Yunis, 1963; Morse *et al.*, 1965), and ribosomes (Booyse and Rafelson, 1968; Booyse, Hoveke and Rafelson, 1968; Ts'ao, 1971). They have been found capable of synthesis of amino acids (Puszkin *et al.*, 1970; Steiner and Baldini, 1969), fatty acids (Majerus *et al.*, 1969; Zieve and Schmukler, 1970a) and proteins (Warshaw *et al.*, 1967).

The platelet surface membrane has been reported to be similar to that of other cells in ultrastructural appearance and lipid composition (Zucker-Franklin, 1970a). It has been shown to be about 78 angstroms (A) thick and to consist of three layers, two of which are electron dense approximately 20 Å thick, and separated by a less dense layer (Born, 1968). On the outside of the membrane, an uneven layer of amorphous substance about 150 - 200 Å thick (Behnke, 1968), and found to consist of mucopolysaccharide (Behnke, 1968; Nakao and Angrist, 1968), and glycoprotein (Nachman and Ferris, 1972; Phillips, 1972) has been

observed. Barber and Jamieson (1970) have isolated the plasma membrane of human platelets and characterized its composition in some detail.

The platelet membrane can undergo changes which enable it to develop adhesiveness, aggregate, and make available membrane phospholipids for acceleration of blood coagulation (Marcus and Zucker, 1965; Horowitz and Papayoanou, 1968; Barber *et al.*, 1972). The coagulation factors V, VIII, XII, and XIII (Bounameaux, 1957; Horowitz and Fujimoto, 1965; Nachman, 1968; Karpatkin and Karpatkin, 1969; Ganguly, 1971; Berman *et al.*, 1973), some components of the fibrinolytic system (Holemans and Gross, 1961), receptors or binding sites for adenosine diphosphate (ADP) (Born, 1965), 5-hydroxytryptamine (5-HT) (Born and Gilson, 1959) and adrenaline (Mills and Roberts, 1967a; Ahtee and Michal, 1972), fibrinogen (Davey and Luscher, 1965; Nachman, Marcusand Zucker-Franklin, 1967) which is distinct from plasma fibrinogen (Ganguly, 1972; James and Ganguly, 1973), antigens (Nachman, Marcus and Zucker-Franklin, 1967), immunoglobulins (McMillan *et al.*, 1971), a thrombin-sensitive protein of molecular weight 190,000 (Baenziger *et al.*, 1971) and various reactive chemical groups (Mehrishi, 1970; 1971; Ando and Steiner, 1972; 1973) all have been found on the platelet membrane. Reddick and Mason (1973) have recently reported the presence of surface-associated particles which appeared to be protein and were embedded in the plasma membrane of human platelets. Their results suggested that these particles might, in some way, be associated with the aggregation of platelets.

Platelets have been observed to contribute to the maintenance of vascular integrity in the body by making available membrane materials (Cronkite *et al.*, 1961; Wojcik *et al.*, 1969) and platelet fragments

(Lough and Moore, 1972).

Some platelet granules, probably α-granules have been shown to contain lysosomal enzymes (Marcus *et al.*, 1966) (acid phosphatase, β-glucuronidase, β-xylosidase, β-fucosidase, α-glucosidase, cathepsin, aryl sulphatase, α- and β-galactosidase, β-N-acetyl glucosaminidase, β-N-acetyl galactosaminidase and α-β-mannosidase) (Day *et al.*, 1969; Bosmann, 1972), while in others were found ADP, adenosine triphosphate (ATP) and 5-HT (Born *et al.*, 1958; Baker *et al.*, 1959; Tranzer *et al.*, 1966; Minter and Crawford, 1967; Da Prada *et al.*, 1967; Da Prada and Pletscher, 1968; Davey and Lüscher, 1968; Holmsen, Day and Stormorken, 1969; Da Prada *et al.*, 1971). Holmsen, Day and Storm, (1969) have reported that ADP and ATP were contained in at least two and possibly three pools in the platelet. They found about 60% of total ADP and ATP in platelet granules and the remainder in the platelet cytoplasm, mitochondria and membranes. The former of these pools, they designated as the "non-metabolic pool", while the latter which turned over continuously, they called the "metabolic nucleotide pool".

Microtubules have been found in platelets in bundles of five to twenty (Behnke, 1965; Sandborn *et al.*, 1966; White *et al.*, 1966). They have been reported to measure 200 - 250 Å in diameter (Behnke, 1965; Silver, 1966; White *et al.*, 1966), to be 100 Å apart and to exist approximately 500 Å inside the platelet membrane (Silver, 1966). Platelets in which marginal bundles of microtubules have been destroyed, or are absent, have been found to be lacking in normal discoid shape, and are either irregular with numerous pseudopods or spherical (Behnke, 1967). It has been suggested that they may have a role in the

maintenance of platelet shape (Behnke, 1965; Sandborn *et al.*, 1966; White *et al.*, 1966). Neither oxidative phosphorylation nor glycolysis has been found to be necessary for maintenance of the microtubules, although glycolysis has been shown to be essential for normal platelet shape (Behnke, 1970). Zucker-Franklin (1969) has reported that the disappearance of microtubules tended to coincide with the appearance of actomyosin in the platelet.

A canalicular system has been observed to be associated with the marginal bundle of microtubules. White (1972a; 1972b) has reported the presence of two canalicular systems; an open canalicular system, which is continuous with the cell surface, and a dense tubular system, which is associated with the marginal band of microtubules. Mason and Reddick (1972) have reported that the plasma membrane appeared to be continuous with the membrane of the surface connecting system of canaliculi. They noted that these structures were closely associated with storage granules and appeared to connect to small vacuoles. Another investigator (White 1968b) also has described vesicles and vacuoles as being part of the open canalicular system. White (1972a) has found portions of the dense tubular system and channels of the open canalicular system in close proximity to each other, especially in areas where the open canaliculi were gathered in clusters. He reported that even though the distance separating the membranes of the two systems was slight, they did not fuse or communicate with each other. White (1972a) also suggested that the platelet dense tubular system was residual, smooth, endoplasmic reticulum derived from rough endoplasmic reticulum after detachment of ribosomes.

A system of microfibrils has been seen near the platelet surface (Zucker-Franklin, 1970a). Zucker-Franklin (1970b) has suggested that if these submembranous fibrils had a contractile function, they could influence movement through the canalicular system. Some studies have strongly suggested that these microfibrils were contractile (Zucker-Franklin *et al.*, 1967; Zucker-Franklin, 1970a) and were thrombosthenin A (Bettex-Galland *et al.*, 1969).

The presence of a contractile protein in normal human platelets was initially reported by Bettex-Galland and Luscher in 1959. They isolated a protein which showed many of the characteristics of muscle actomyosin and which they named thrombosthenin (Bettex-Galland and Luscher, 1961). Further studies demonstrated that as in the case of muscle actomyosin, this protein acted as an ATPase and was dependent on the presence of calcium and magnesium ions (Bettex-Galland and Luscher, 1961; Nachman, Marcus and Safier, 1967). Booyse, Zschocke and Rafelson (1972) have estimated that thrombosthenin constitutes approximately 15% of total solubilized human platelet protein. It has been found distributed throughout platelet cytoplasm (Bettex-Galland *et al.*, 1969) and may also be present on the outer surface of the platelet (Booyse and Rafelson, 1971). Thrombosthenin M, the myosin-like component of human platelets, has been studied in detail (Booyse, Hoveke, Zschocke and Rafelson, 1971) and found to be indistinguishable from muscle myosin (Adelstein *et al.*, 1971). The actin component of platelets, thrombosthenin A, has been reported to have a molecular weight of 44,000 (Luscher *et al.*, 1972) and ressemble the F-actin from striated muscle (Luscher and Bettex-Galland, 1972). Some evidence has been presented, suggesting that it consists of

two forms (Probst and Luscher, 1972). This contractile system does not appear to be preformed in intact circulating platelets, but becomes discernible only following platelet injury such as that induced by aggregating agents (Zucker-Franklin, 1969). Although the mechanisms are not completely understood, thrombosthenin probably plays a role in many aspects of normal platelet function, including clot retraction, irreversible platelet aggregation, the platelet release reaction and alterations in platelet morphology (Luscher and Bettex-Galland, 1972). An excellent review on this subject has recently been prepared by Luscher and Bettex-Galland (1972).

The formation of pseudopods by the platelet has been studied by Warren and his colleagues (Warren, 1970; 1971; Warren and Vales 1972a; 1972b), but because of its involvement in the platelet release reaction will be discussed in another section.

Normal platelet function depends on the production of energy by glycolytic and oxidative pathways (Campbell *et al.*, 1956; Detwiler and Zivkovic, 1970; Doery, Hirsh and Cooper, 1970). Either pathway may offset a decreased production by the other and glucose appears to be utilized in preference to fatty acids when the platelet is under metabolic stress (Doery, Hirsh and Cooper, 1970). An energy source has been found essential for the uptake of substrates (Zieve and Solomon, 1968), synthesis of fatty acids (Hennes *et al.*, 1964; Deykin and Dresser, 1968; Majerus *et al.*, 1969), phospholipids (Lewis and Majerus, 1969), proteins (Booyse and Rafelson, 1967a; 1967b; 1968), and glycogen (Scott, 1967; Vainer and Wattiaux, 1968; Karpatkin *et al.*, 1970), uptake and storage of 5-HT (Weissbach and Redfield, 1961; Pletscher, 1968), maintenance of the platelet intracellular ionic balance (Gorstein *et al.*, 1967),

maintenance of the platelet discoid shape (Bull and Zucker, 1965), platelet aggregation (Mürer *et al.* 1967; Kinlough-Rathbone *et al.*, 1970a), the platelet release reaction (Mürer, 1968), phagocytosis (Movat, Weiser *et al.*, 1965; Mustard and Packham, 1968; Kuramoto *et al.*, 1970), and clot retraction (Bettex-Galland and Luscher, 1960; Mürer *et al.*, 1967; Mürer, 1969a). A number of factors known to have stimulatory effects on platelet function have been shown to produce an increase in one or more of glycolysis, glycogenolysis or tricarboxylic acid activity (Hirsh and Doery, 1971). These include the aggregating agents ADP, adrenaline, thrombin and collagen (Karpatkin and Langer, 1967; Loder *et al.*, 1968; Puszkin and Jerushalmy, 1968; Kuramoto *et al.*, 1969; Steiner *et al.*, 1969; McElroy *et al.*, 1971). Karpatkin and co-workers (Karpatkin, 1969a; 1969b; Karpatkin and Strick, 1972) and Mannucci and Sharp (1967) have demonstrated that young platelets are larger and metabolically more active than older, smaller ones.

Considerable evidence has accumulated to suggest an important role for cyclic 3',5'-adenosine monophosphate (cyclic AMP) in the metabolism and normal function of blood platelets. Cyclic AMP levels are partially controlled by adenyl cyclase, an enzyme first reported to be present in platelets by Wolfe and Shulman (1969) and later found to be bound to platelet membrane (Song and Cheung, 1971). The breakdown of cyclic AMP to 5'-adenosine monophosphate is regulated by the enzyme phosphodiesterase (Song and Cheung, 1971). Amer and Mayol (1973) have recently reported the presence of two forms of phosphodiesterase in human platelets. The low Km form of the enzyme (phosphodiesterase-II) was associated with platelet membranes while the other form was soluble. They suggested

that phosphodiesterase-II might play a significant role in the platelet release reaction. Many of the platelet aggregating agents, eg. ADP, adrenaline, noradrenaline, 5-HT, thrombin and collagen have been reported by some investigators to lower platelet cyclic AMP concentrations (Salzman and Neri, 1969; Zieve and Greenough, 1969; Harwood *et al.*, 1972; Salzman *et al.*, 1972; Salzman and Weisenberger, 1972), while others (Ball *et al.*, 1970; Moskowitz *et al.*, 1971; McDonald and Stuart, 1973) have not been able to demonstrate this effect. Droller and Wolfe (1972) have described an increase in intracellular cyclic AMP in human platelets incubated with human thrombin. More frequently, however, compounds which increase intracellular levels of cyclic AMP have been shown to be inhibitors of platelet aggregation. Such agents may increase cyclic AMP levels through a stimulation of adenyl cyclase, eg. prostaglandin E_1, adenosine, 2-chloroadenosine (Wolfe and Shulman, 1969; Zieve and Greenough, 1969; Scott, 1970; Mills and Smith, 1971; Harwood *et al.*, 1972) or by an inhibition of phosphodiesterase, eg. methylxanthines papaverine, dipyridamole and its analogues RA 233, RA 433 (Cole *et al.*, 1970; Horlington and Watson, 1970; Smith and Mills, 1970; Mills and Smith, 1971; Pichard *et al.*, 1972). Haslam and Lynham (1972) have reported that adenosine and 2-chloroadenosine at low concentrations increased adenyl cyclase activity, and at high concentrations had inhibitory effects on this enzyme. They suggested that this activation and inhibition were independent processes mediated by different components of a receptor-adenylate cyclase complex. Zieve and Schmukler (1970b; 1971) have noted that incubation of washed human platelets and platelet sonicates with cyclic AMP resulted in an increase in glycogenolysis and

inhibition of glycolysis. They suggested that this inhibition may be related to the inhibition of platelet aggregation by this substance and by agents which stimulate its production. It has been suggested that a phosphokinase might be responsible for the expression of cyclic AMP effects on human platelets (Kaulen and Gross, 1972).

C. Blood Platelet Aggregation

It has been known for many years that blood platelets aggregate during the clotting process (Osler, 1874). Hellem, in 1960, found that an almost linear relationship existed between the number of red blood cells in plasma and platelet adhesiveness to glass. He described the responsible red cell factor as being acidic, dialyzable, heat stable and a relatively small molecule and gave it the designation "R factor". In 1961 Gaarder *et al.* demonstrated that this substance was adenosine diphosphate (ADP) and that it stimulated platelets to aggregate and adhere to foreign surfaces. A year later, Born (1962) suggested that the increased platelet adhesiveness observed in the presence of ADP might be related to the *in vivo* formation of hemostatic platelet plugs. Since that time, a vast number of studies have been performed in an attempt to gain a full appreciation of normal and pathological platelet function. As well as ADP, a great number of other substances have been found to aggregate blood platelets, including calcium ions (Born and Cross, 1963), 2-chloroadenosine diphosphate (Michal, 1967; Maguire and Michal, 1968), 2-methoxyadenosine-5'-diphosphate, 2-methylthioadenosine-5'-diphosphate, 2-ethylaminoadenosine-5'-diphosphate, 2-chloro-N^6-methyladenosine-5'-diphosphate (Gough *et al.*, 1972), connective tissue (Spaet *et al.*, 1962; Zucker and Borrelli, 1962), collagen (Hovig, 1963a; Wilner *et al.*, 1968),

basement membrane (Hughes and Mahieu, 1970; Ts'ao and Glagov, 1970), thrombin (Shermer *et al.*, 1961; Grette, 1962; Schmid *et al.*, 1962), trypsin, papain and pronase (Davey and Luscher, 1967), fibrinogen (Solum, 1968), adrenaline and noradrenaline (Clayton and Cross, 1963; O'Brien, 1963a; Mitchell and Sharp, 1964; Mills and Roberts, 1967a; Ahtee and Michal, 1972), 5-HT (Baumgartner and Born, 1968; White, 1970; Born *et al.*, 1972), fatty acids (Haslam, 1964; Kerr *et al.*, 1965), endotoxins (McKay *et al.*, 1958; DesPres *et al.*, 1961; Grottum *et al.*, 1969; Nagayama *et al.*, 1971), endotoxin-cupric ion complexes (Lewis and Dickson, 1971), exotoxins (Siegel and Cohen, 1962), bacterial organisms (Clawson and White, 1971), antigen-antibody complexes (Movat, Mustard *et al.*, 1965), viruses (Turpie *et al.*, 1972), triethyl tin (O'Brien, 1963b; 1964), latex particles (Glynn *et al.*, 1965; Movat, Weiser *et al.*, 1965), nicotine (Werle and Schievelbain, 1965), bile salts (Silver, 1971), heparin and protamine (Eika, 1972), polylysine (Packham *et al.*, 1970; Eika, 1972); activated factor X (Jevons and Barton, 1971), ristocetin (Howard and Firkin, 1971), arginine and lysine vasopressins (Haslam and Rosson, 1971), sodium polyanethol sulfonate (Liquoid) (Grottum *et al.*, 1969), dextrans (Dhall *et al.*, 1966; Dhall and Matheson, 1968), methionine (Buehler and Rennert, 1970), hydrogen peroxide (Higashi *et al.*, 1972) and arachidonic acid (Ingerman *et al.*, 1973).

Platelets in platelet-rich plasma (PRP), or in washed suspension, exposed to ADP, have been found to undergo a change from their normal discoid form to a spherical configuration with multiple pseudopods, and to aggregate (Zucker and Zaccardi, 1964; O'Brien, 1965; Bull and Zucker, 1965; White, 1968a). The aggregation pattern observed in PRP in response to added ADP has been shown to depend on the final concentration

of the aggregating agent employed; low concentrations (10^{-7}M) producing an aggregation which is reversible within a few minutes from its initiation (Born and Cross, 1963); higher concentrations (approximately 2×10^{-6}M) yielding a non-reversible biphasic type of aggregation response. With even higher concentrations, the two phases merge into one, the aggregation also being irreversible (Constantine, 1966; MacMillan, 1966). MacMillan (1966) and Zucker and Peterson (1968) have reported that when critical concentrations of ADP were used, the PRP of 70 - 80% of persons studied showed the biphasic response. The second phase of aggregation induced by ADP in normal human platelets has been shown to be associated with the release of a number of substances from the platelet. These include ADP (MacMillan, 1966; Mills *et al.*, 1968), ATP (Mills *et al.*, 1968), 5-HT (Zucker and Peterson, 1967; Mills *et al.*, 1968), and platelet factor 4 (Youssef and Barkhan, 1968; Thomas *et al.*, 1970). During the release reaction, platelet factor 3 availability has been found to be increased (Horowitz and Papayoanou, 1968; Zucker and Peterson, 1967).

Although the mechanism of ADP-induced aggregation is not completely understood, several theories have been proposed. Investigators have demonstrated a number of essential factors including calcium (Hovig, 1964; Skoza *et al.*, 1967; Ardlie and Mustard, 1969; Ardlie *et al.*, 1970; Cronberg and Caen, 1970; Herrmann *et al.*, 1970), magnesium (Ardlie and Mustard, 1969; Ardlie *et al.*, 1970; Cronberg and Caen, 1970), metabolic energy (Murer *et al.*, 1967; Kinlough-Rathbone *et al.*, 1970a; Mills and Lipson, 1973), fibrinogen (Cross, 1964; Solum and Stormorken, 1965), and possibly other plasma co-factors (Cronberg *et al.*, 1970; Mustard and Perry, 1972). Murer *et al.* (1967) have observed that blockade of the

energy systems of glycolysis and oxidative phosphorylation together, but not singly, inhibited ADP-induced aggregation. Platelets, incubated in a glucose free medium, have been found to lose their ability to respond to ADP, a responsiveness which could be restored by the addition of glucose to the medium (Kinlough-Rathbone *et al.*, 1970a). Spaet and Lejnicks (1966) suggested that the breakdown of ADP to adenosine monophosphate (AMP) released energy which was utilized for platelet binding reactions. Steiner and his co-workers (1969) concluded that aggregation was associated with, or possibly mediated by, a burst of activity of the citric acid cycle. Chaudhry *et al.* (1973) have reported that aggregation of human platelets was associated with stimultion of the hexose monophosphate shunt and that the increase in Kreb's cycle activity observed, occurred later and was not essential for platelet aggregation. ADP has also been found, as have other aggregating agents, to lower platelet cyclic AMP levels (Salzman and Neri, 1969; Zieve and Greenough, 1969; Hirsh and Doery, 1971; Harwood *et al.*, 1972; Salzman *et al.*, 1972), and this effect may play a role in its mechanism of action.

Several investigators have studied the essential nature of some plasma proteins for ADP-induced platelet aggregation. Born and Cross, in 1964, suggested that the substance present in plasma and required as well as calcium for the aggregation of pig platelets by ADP, was probably a protein. It was heat-labile and present in the euglobulin fraction of plasma protein and in the preparation of factor VIII. A year later, Deykin *et al.* (1965) demonstrated that at least two plasma factors, fibrinogen and a heat-stable plasma protein distinct from factor VIII, participated in ADP-induced aggregation of human platelets. Salzman *et*

al. (1966) suggested that ADP acted through inhibition of a platelet ATPase which exposed adhesive sites and permitted aggregation through intermediate secondary bridges containing calcium and fibrinogen or other proteins. More recent studies also have suggested a role for fibrinogen in ADP aggregation. Smink *et al.* (1968) proposed that during aggregation, acidic calcium-adenosine phosphate complexes were adsorbed to the platelet surface, lowering the pH and giving rise to precipitation of proteins, chiefly fibrinogen, which resulted in bridge formation between platelets. Other investigators (Shirasawa and Chandler, 1969) have suggested that the bridging substance in ADP aggregation was derived from the surface layer of the platelet, and that it contained fibrinogen preadsorbed from the plasma and released from within the platelet. Three proteins, fibrinogen, Hageman factor, and gamma globulin which supported platelet aggregation have been identified by Bang *et al.* (1970). These proteins, with a relatively high positive charged density, were reported to support aggregation by lowering of the negative platelet membrane charge, reducing electrostatic repulsion between individual platelets. Niewiarowski, Farbiszewski *et al.* (1968) proposed that ADP aggregation could be mediated through platelet factor 4, which reacted with soluble fibrin monomer complexes and fibrinogen near the platelet surface with resultant formation of platelet-platelet bridges. Hovig (1965) concluded that besides protein, neuraminic acid linked to protein might play an important role in aggregation.

A number of workers have noted that added ADP need not enter the platelet to cause aggregation (Abdulla, 1968; Boullin *et al.*, 1972) and have proposed that it was bound to ADP receptors on the platelet surface

(Born, 1965; Gaarder and Laland, 1964; Boullin *et al.*, 1972). Mustard and Perry (1973) have suggested that nucleoside diphosphokinase, an enzyme on the surface of platelets with which ADP interacts, could be the ADP receptor for platelet aggregation. Skalhegg *et al.* (1964) reported that ADP attached to the surface of platelets by amino groups and the ribose portion of the molecule, probably through hydrogen bonding. Calcium ions could then form bridges between two ADP molecules on two platelets. Both calcium and thiol groups have also been reported to be necessary for the adsorption of ADP to the platelet surface (Hampton and Mitchell, 1966). Lycette *et al.* (1970) have suggested that the availability of platelet surface phospholipids may also play a role in aggregation by ADP, exerting aggregation forces through their hydrophobic bonding groups. ADP-induced changes in platelet membrane phospholipids may play an important role in its aggregating activity (Lloyd *et al.*, 1972).

Thrombosthenin, the contractile element of blood platelets, may be involved in ADP-induced platelet aggregation. White (1967) proposed that ADP acted on an ATP- dependent calcium pump which resulted in intracellular release of calcium ions with resultant contraction of thrombosthenin and aggregation. Booyse and Rafelson (1972) have suggested that aggregation was caused by a direct action of aggregating agents on a surface- or membrane-localized thrombosthenin which existed in a contracted state. Stimulation of platelet aggregation relaxed this membrane thrombosthenin and its ATPase activity resulting in a loss of membrane permeability and and extrusion of platelet cytoplasm, which they termed cytogel and which contained thrombosthenin in a relaxed state. These investigators suggested that the extruded cytogels interacted; contraction and retraction

taking place to draw the platelets together.

Blood platelets exposed to collagen have been shown to adhere to it (Hughes, 1962), probably instantaneously (Thompson and MacKenzie, 1973), to release non-metabolic ATP and ADP (Holmsen, 1965), 5-HT (Spaet and Zucker, 1964), enzymes such as β-glucuronidase, acid phosphatase and adenylate kinase (Mills *et al.*, 1968), fibrinogen (Solum and Stormorken, 1965), platelet factor 4 (Niewiarowski, Farbiszewski *et al.* 1968), platelet factor 3 activity (Spaet and Cintron, 1965) and to aggregate (Hovig, 1963a; Wilner *et al.*, 1968). Hovig in 1963 (1963b) emphasized the importance of the release reaction in collagen-induced platelet aggregation when he suggested that released ADP was responsible for such aggregation. Hampton and Mitchell (1966) later speculated that collagen acted by releasing ADP which was adsorbed onto the platelet surface. Holmsen *et al.* (1970) have reported that protein (thrombosthenin) - bound ADP may have a function in the release reaction. More recently, Nunn (1972) and Rodman and Penick (1973) have questioned the necessity of released ADP for collagen aggregation.

The mechanism through which platelets adhere to collagen and aggregate has been the subject of considerable investigation. Wilner *et al.* in 1968, using acid-soluble human collagen, found that the native structure (triple helical) of collagen was necessary for aggregation. They also suggested that free amino groups, specifically epsilon amino groups of lysine, were critical for aggregation while carboxyl groups were of relatively minor importance. Later, they (Wilner *et al.*, 1971) reported that the reactive sites of dispersed, insoluble, human, skin collagen for platelets involved about 40% of the total available

epsilon amino groups of lysine, and noted that these sites were found in the central portion of the collagen structure, probably within polar segments of the collagen chains. Other investigators (Caen *et al.*, 1970) also have recognized the importance of positive charges located on the basic groups of the collagen molecule.

Considerable evidence has accumulated to suggest that platelet glycosyl transferases may be involved in the interaction between platelets and collagen. In 1971 Barber and Jamieson (1971a) reported that the enzyme, glucosyl transferase, which was capable of transferring glucose to a collagen receptor, had been found in platelet plasma membrane. They later (1971b) isolated the enzyme and observed that it transferred glucose specifically from uridine diphosphate glucose of platelet plasma membranes to incomplete heterosaccharide chains of collagen. Inhibition of the enzyme by some known platelet aggregation inhibitors was found to parallel inhibition of platelet collagen adhesion. Results reported by Bosmann, also in 1971, suggested that two secreted glycoprotein-glycosyl transferases and two membrane glycoprotein-glycosyl transferases may function in the adhesion of collagen to platelets. Chesney and his co-workers, in 1972, noted that collagen contained glucosyl-galactose and galactose side chains linked through the galactose to hydroxylysine. They found that oxidation of the galactose residue completely abolished platelet aggregation by the collagen molecule. They suggested that integrity and accessibility of this galactose site could be essential for the formation of a collagen-enzyme-platelet membrane complex and resultant platelet aggregation. While these investigators have stressed the critical role of the carbohydrate side chain of collagen in platelet aggregation, Puett and

Cunningham (1973) have suggested that the platelet-collagen interaction which mediates the release reaction is mediated primarily by the protein moiety.

An involvement of Vitamin A in platelet to collagen adhesion has also been reported (De Luca *et al.*, 1972).

As in the case of ADP, collagen has been found to lower cyclic AMP levels in platelets and some investigators (Salzman and Neri, 1969; Zieve and Greenough, 1969; Hirsh and Doery, 1971; Harwood *et al.*, 1972; Salzman *et al.*, 1972) have suggested that this effect may be associated with its mechanism of action.

The most frequently utilized *in vitro* technique, employed to measure and quantitate platelet aggregation and its inhibition, was devised by Born in 1962 and involves a measurement of changes of optical density as aggregation takes place in a platelet-rich plasma suspension (Born, 1962; O'Brien, 1962; Born and Cross, 1963). Extensive study by Mustard and his co-workers (Packham *et al.*, 1972) has resulted in the development of a washed platelet system which has also been utilized for study by this method. Platelet suspensions must be stirred and the platelets contact one another before the aggregation phenomenon will proceed (Born, 1962; O'Brien, 1962; Zucker and Peterson, 1968). By tracing changes in optical density on a chart recorder as platelets aggregated, investigators have been able to determine the total degree and rate of the aggregation response. The maximum rate of change of optical density or the aggregation curve slope has frequently been used as a measure of the rate of aggregation (Skoza *et al.*, 1967; Mills *et al.*, 1968; Philp, 1970; Stuart, 1970).

Platelets aggregating *in vitro* also have been observed by a light scattering technique (Michal and Born, 1971), visually (Hovig, 1963a; Jerushalmy and Zucker, 1966; Niewiarowski and Thomas, 1966; Aledort and Niemetz, 1968; Peterson and Zucker, 1970) and microscopically (Brinkhous *et al.*, 1958; Mitchell and Sharp, 1964; Aledort and Niemetz, 1968; Gross *et al.*, 1968; Davies *et al.*, 1969; Sano *et al.*, 1971), and studied on isolated de-endothelialized rabbit carotid arterial ring segments (Blaisdell *et al.*, 1973), in rotating plastic loops (Chandrasekhar, 1967; Davis and Holtz, 1970; Silver, 1970; Constantine *et al.*, 1971; Michal and Silver, 1971), and in glass capillaries (Benner *et al.*, 1970). Attempts have been made to quantitate aggregation by counting the number of platelets in the supernatant of aggregated, centrifuged blood (Holdrinet *et al.*, 1969; Gordon and Gresham, 1972) and by measuring the filtration pressure exerted by aggregates in whole blood or PRP forced through a filter (Swank *et al.*, 1964; Stoltz *et al.*, 1972).

In vivo, mechanical stimulus injury (Born *et al.*, 1964; Emmons *et al.*, 1965b; Elkeles *et al.*, 1968; Baumgartner *et al.*, 1973), electrical stimulus injury (Kloeze, 1970b), biolaser units (Fleming *et al.*, 1970; Arfors *et al.*, 1972) and iontophoretic application of aggregating agents (Begent and Born, 1970; 1971) have been utilized to induce platelet aggregation which could be observed visually or with a microscope. Some investigators have injected aggregating agents into experimental animals and observed aggregation microscopically (Silver and Stehbens, 1965; Jorgensen *et al.*, 1970; Benner and Frede, 1972; Haft, Kranz *et al.*, 1972) or studied falls in *in vivo* platelet counts (Iatridis *et al.*, 1970; Simone and Choi, 1970; MacKenzie, Henderson and

Steinbach, 1971; Radegran and McAslan ,1972). Hornstra (1970) has developed a filter loop technique in which a microfilter was placed in the arterial circulation of a rat. Following injection of ADP, and measurement of changes of blood pressure before and behind the filter, he was able to calculate the degree of platelet aggregation. Broersma *et al.* (1973) have used a similar technique to study platelet aggregation in response to ADP injection in dogs.

D. The Release Reaction

In 1962 Grette demonstrated that thrombin induced a release from pig platelets of 5-HT, adenine nucleotides, amino acids and protein into the external medium, and applied the term "release-reaction" to this phenomenon (Grette, 1962). Numerous substances, many of which have been noted as aggregating agents in the preceding section, have since been found to induce, to varying degrees, the release of platelet contents in human, as well as some animal species. A partial list includes thrombin (Grette, 1962; Holmsen and Day, 1968) collagen (Hovig, 1963a; Holmsen, 1965; Mills *et al.*, 1968), adrenaline and ADP (MacMillan, 1966; Mills *et al.*, 1968), 5-HT (White, 1970), fibrinogen (Solum, 1968), proteolytic enzymes (Davey and Luscher, 1965; 1967), antigen-antibody complexes (Humphrey and Jaques, 1955; Movat, Mustard *et al.*, 1965), viruses (Jerushalmy *et al.*, 1962; Turpie *et al.*, 1972), endotoxins (DesPrez *et al.*, 1961), and latex particles (Glynn *et al.*, 1965). Because of the extensive investigation of thrombin and the relevance to this thesis of the effects of collagen and ADP on platelet function, the following discussion will concentrate on release induced by these three agents.

The platelet release reaction is now understood to involve the

extrusion of substances from platelet granules to the external medium (Holmsen, Day and Stormonken, 1969). Stormorken (1969) has suggested that blood platelets, having developed storage and releasing properties, are very similar to other secretory cells in the body. Stored substances are found to be located in α-granules, dense α-granules and very dense bodies within the platelet cytoplasm. Dense α-granules contain adenine nucleotides (Holmsen, Day and Storm, 1969) and 5-HT (Day *et al.*, 1969), while in α-granules are stored lysosomal enzymes (Day *et al.*, 1969), fibrinogen (Nachman, Marcus and Zucker-Franklin, 1967) and platelet factor 4 (Holmsen, Day and Stormorken, 1969). DaPrada *et al.* (1967) have also located 5-HT in very dense bodies in rabbit platelets. All release inducers do not liberate the contents of both granular types, for example, thrombin and collagen induce release from α-granules and dense α-granules, whereas ADP discharges only substances from the high density particles and dense α-granules (Holmsen, Day and Stormorken, 1969). Specifically, collagen induces extrusion of adenine nucleotides (Hovig, 1963b; Spaet and Zucker, 1964; Holmsen, Day and Stormorken, 1969; Thomas *et al.*, 1970), 5-HT (Spaet and Zucker, 1964; Holmsen, Day and Stormorken, 1969), fibrinogen (Solum and Stormorken, 1965; Holmsen, Day and Stormorken, 1969), platelet factor 4 (Holmsen, Day and Stormorken, 1969; Thomas *et al.*, 1970), lysosomal enzymes (Mills *et al.*, 1968; Holmsen, Day and Stormorken, 1969), permeability factors (Mustard *et al.*, 1965), antiplasmin activity (Joist *et al.*, 1971), prostaglandins (Smith *et al.*, 1973), and makes available platelet factor 3 (Spaet and Cintron, 1965). ADP releases adenine nucleotides (MacMillan, 1966; Mills *et al.*, 1968; Thomas *et al.*, 1970), 5-HT (Zucker and Peterson, 1967; Mills *et al.*, 1968; Holmsen, Day and Stormorken, 1969; Massini, 1970), platelet

factor 4 (O'Brien *et al.*, 1970b; Thomas *et al.*, 1970), prostaglandins (Smith *et al.*, 1973), and activates platelet factor 3 (Zucker and Peterson, 1967; Sixma and Nijessen, 1970). Aledort *et al.* (1971), whose results differed from those of investigators employing exogenous radioactive 5-HT, utilized a spectrophotometric assay to measure platelet 5-HT, and found very little of it released with ADP. Substances located in cytoplasm, mitochondria or membranes are retained by the platelet during the release reaction (Castaldi *et al.*, 1962; Hovig, 1962; Holmsen, Day and Stormorken, 1969). The release process is energy dependent (Murer, 1968; Kinlough-Rathbone *et al.*, 1972), obtaining ATP from either glycolytic or oxidative pathways. Simultaneous blockade of both energy pathways has been found to completely inhibit release (Murer, 1968).

Some investigators have questioned the importance of extracellular calcium ions (Ca^{++}) in the release phenomenon. Grette (1962) concluded from studies utilizing ethylenediamine tetraacetic acid (EDTA) that Ca^{++} was essential, while later investigations (Holmsen and Day, 1968; Murer, 1968) have suggested that it is not required for release. Extracellular Ca^{++} appears to catalyze the extrusion process (Murer, 1972), especially as it relates to release of lysosomal enzymes (Holmsen and Day, 1968; Holmsen, Day and Stormorken, 1969). Murer (1969b) has suggested that mobilization of intracellular Ca^{++} may play a role in the platelet release reaction.

ATP, ADP and 5-HT have been reported to be released from platelets in the same relative proportions as they are present in subcellular granules (Holmsen, Day and Storm, 1969). Platelets labelled *in vitro* with ^{14}C-adenosine or adenine and exposed to collagen or ADP released

non-radioactive ADP, while radioactive nucleotide remained within the cell (Holmsen, 1965; Ireland, 1967; Holmsen *et al.*, 1972). Intracellular ADP concentrations decreased to approximately the same degree as the extracellular levels of the nucleotide increased. The poorly-labelled pool of adenine nucleotides, designated as the "non-metabolic" pool by Holmsen, Day and Storm (1969), was located along with 5-HT within the osmiophilic α-granules containing very dense bodies. Almost no lysosomal enzymes appeared in these granules (Day *et al.*, 1969). Holmsen, Day and Storm (1969) have noted that the ATP:ADP ratio of the granular nucleotides suggested that they were released directly from the granules. The pool of ATP and ADP, which incorporated the ^{14}C-label and which was not extruded from the platelet during the release reaction, has been designated as the "metabolically active" pool (Holmsen, Day and Storm, 1969). This pool appeared to be made up of two functionally different pools; the "release energy pool" reported to consist exclusively of ATP, and the "basic metabolic pool" which took part in glycolysis and phosphorylation and supplied ATP to the "release energy pool". It was the ATP from the "release energy pool" which disappeared intracellularly during release (Holmsen, Day and Stormorken, 1969). "Release energy" ATP was completely degraded to inosine monophosphate and to inosine and hypoxanthine, and none of these breakdown products could be used by the platelet for the synthesis of ATP (Holmsen and Rozenberg, 1968). Investigations have demonstrated that this conversion of ATP to IMP was initiated immediately following the addition of a release inducer to platelets (Ireland, 1967; Holmsen, Day and Storm, 1969). Following termination of the release reaction, this conversion stopped, after which 5'-AMP deaminase in the platelet was completely inhibited (Holmsen, Day

and Stormorken, 1969). Holmsen, Day and Stormorken (1969) have reported that these results indicate that the ATP to IMP reaction may be closely associated with platelet release and suggest that the complete reaction may be mediated by 5'-AMP deaminase activity. The "release energy" ATP pool has been found to demonstrate some of the characteristics of muscle actomyosin-bound ATP, and Holmsen and his co-workers have suggested that it may be bound to platelet thrombosthenin (Holmsen, Day and Storm, 1969; Holmsen *et al.*, 1970).

Morphological changes associated with release have been shown to depend upon the aggregating or inducing agent employed. Alterations following exposure of platelets to collagen (Hovig, 1962; Rodman and Mason, 1967) were found to be more marked than those with ADP (Rodman *et al.*, 1963; Mills *et al.*, 1968; Sixma and Geuze, 1968). Addition of collagen to PRP (Hovig, 1962; 1963a) resulted in centralization of organelles, pseudopod formation and aggregation, most of these changes occurring within one minute. Disintegration of granules and disruption of plasma membranes have been reported after four minutes (Rodman and Mason, 1967). ADP caused pseudopod formation and centralization of granules, but with minimal degranulation (Mills *et al.*, 1968; Sixma and Geuze, 1968; Hardisty *et al.*, 1970); the amount of ADP, to some degree, determining the final response. Rodman and Mason (1967), employing a final concentration of two millimolar (mM) ADP, demonstrated a centralization of granules, pseudopod formation and aggregation, but no degranulation within one minute. They noted that the induced changes appeared to be transitory, in that one hour after exposure to ADP most of the platelets had returned to normal. White (1968a) used 0.25-25 mM

ADP which resulted in a change from discoid to spherical configuration, and centralization of organelles. He reported that this centralization was caused by a "contractile wave" of platelet microtubules and the canalicular sytem; the granular membranes contacting each other without fusion. Holmsen, Day and Stormorken (1969) have noted that pseudopod formation, platelet swelling and granular centralization are the initial components of ADP-induced release.

It appears unlikely that granules are discharged intact during the release reaction (Holmsen, Day and Stormorken, 1969) although conflicting results have been reported. Hovig (1968) has suggested that intact granules were extruded only very rarely and noted that this probably was not a part of the release reaction. White (1972c) has observed granules and dense bodies in the open canalicular system of platelets, utilizing polylysine and polybrene to make the platelet granules more electron opaque. Platelet stimulation by cationic polypeptides subsequently resulted in release of these granules in a relatively intact state. Libanska (1967) has suggested that the discharge of α-granule contents took place, following centralization, into a collecting system without lysis within the platelet. It must be noted that following the loss of their contents, the granules might have altered staining properties and may not be recognized as granules (Holmsen, Day and Stormorken, 1969).

Holmsen, Day and Stormorken (1969) have divided the release reaction into three phases: induction, intracellular transmission and extrusion. Although the mechanisms involved in the induction phase of the process are not well-understood, it is known that many agents, the ultimate effect of which is to induce platelet release, cause changes in

the platelet surface which result in increased platelet stickiness and some aggregation. In some way this may trigger the release response (Holmsen, Day and Stormorken, 1969). Lüscher and Bettex-Galland (1972) have suggested that the first step in the reaction possibly consists of changes in the platelet membrane which allow granular contents to be emptied into the surface-connected canalicular system. Both cyclic AMP and activation of 5'-AMP deaminase have been suggested as mediators in platelet intracellular transmission, possibly acting as an intermediate between impulses generated at the platelet membrane and the α-granules, or a system containing them (Holmsen, Day and Stormorken, 1969). A number of recent investigations have attempted to answer the question as to how granular contents are released into intracellular media. White (Lüscher and Bettex-Galland, 1972), using cytochalasin B, an inhibitor of cellular contractile mechanisms, has shown that inhibition of the platelet contractile system inhibited the release reaction; a result which implies that granular contents are extruded by contraction of the canalicular system. Weiss and Rogers (1973) have described a series of patients whose platelets were deficient in storage pools of adenine nucleotides and in electron dense granules. They found normal platelet factor 4 levels in these platelets but impaired release by collagen, and have suggested that the storage pool of ADP may also play some role in the release mechanism. During a study of platelet pseudopodia, Warren (1971) has noted multivesicular membranous sacs at or near the terminal end of the pseudopod, at the point at which this structure was in contact with basement membrane, fibrin or pseudopodia from other platelets. In some of his preparations, he observed rupture of the primary sac and release of the secondary vesicles within it (Warren, 1971). This sac

was at first closely packed with secondary vesicles, but later became swollen and finally ruptured, releasing the secondary vesicles at or near the terminal bulb of the pseudopod (Warren and Vales, 1972a) into the surrounding medium (Warren and Vales, 1972b). During this process, granules were not extruded from the platelet and still could be seen in the platelet cytoplasm (Warren and Vales, 1972a). Warren and Vales (1972a; 1972b) have postulated that the release of vesicles from mutli-vesicular membranous sacs is the morphological basis of the platelet release reaction. Smith and co-workers (1973) have recently reported the formation and release of prostaglandins E_2 and $F_{2\alpha}$ in association with the single wave of collagen aggregation and the second phase of aggregation by adrenaline and ADP. They suggested that this phenomenon might be related to the secretion of ADP and 5-HT by platelets.

In the previous section, it was noted that the aggregation, caused by critical concentrations of ADP, resulted in a biphasic aggregation response; the second phase associated with platelet release. Collagen aggregation was also reported to be related to the release reaction. Niewiarowski and Thomas (1969) have reported that both platelet factor 4 and ADP release occurred in an explosive manner at the beginning of the second phase of ADP-induced aggregation. Holmsen *et al.* (1972), utilizing platelets containing adenine nucleotides labelled with ^3H and ^{14}C, *in vitro*, found ATP and ADP with low specific radioactivity compared with that retained by the cell, to be released during the second phase of ADP aggregation. They also noted that the ATP-hypoxanthine conversion was associated with the second, not the first, phase of ADP-induced aggregation. Murakami, Yoshino *et al.* (1972), studying collagen-induced

aggregation and release, observed that the release of ADP occurred at the stage of rapid change of light transmission or a maximal aggregation.

Because of the essential nature of the platelet release reaction to the production of an irreversibly aggregated platelet plug, and due to the association of abnormal release patterns with some mild bleeding tendencies, considerable investigation has been conducted to study, usually by inhibition of platelet release, and to understand the source of malfunction in the extrusion process. Aggregation inhibitors can prevent platelet release *in vitro* either in a direct or indirect manner; that is; by inhibiting the secretion of granular material or by interfering with some stage of the induction process. Inhibitors studied most frequently have included non-steroidal, anti-inflammatory drugs such as acetylsalicylic acid (Evans *et al.*, 1967; Weiss and Aledort, 1967; Evans *et al.*, 1968; O'Brien, 1968b; Youssef and Barkhan, 1969; Doery *et al.*, 1969; Zucker *et al.*, 1969), pyrazole compounds (Packham *et al.*, 1967), tricyclic antidepressants (Mills and Roberts, 1967b; Mills *et al.*, 1968) and the pyrimido-pyrimidines (Cucuianu *et al.* 1971).

Two types of release abnormalities have been reported and their relation to *in vivo* bleeding disorders discussed. Some persons with mild bleeding tendencies and a defective release reaction have been described (Weiss *et al.*, 1969; Logan *et al.*, 1971; Papayannis *et al.*, 1971; Murakami, Odake, Matsuda *et al.*, 1972; Weiss and Rogers, 1972), and others have been found to have diminished storage pools and abnormal aggregation patterns (Weiss *et al.*, 1969; Holmsen and Weiss, 1970).

Although most of the investigation on platelet aggregation and the release reaction has been conducted *in vitro*, recently, Kobayashi and

Didisheim (1973) reported collagen-induced release of adenine nucleotides *in vivo*, following rapid injection of bovine tendon collagen into the femoral vein of rats.

E. Inhibitors of Platelet Aggregation

Many compounds have been shown to exhibit inhibitory effects on platelet aggregation and the release reaction. Some of these inhibitors, the majority of which have been studied *in vitro*, are shown in Table 1. These substances are of varied chemical nature and configuration and no common mechanism can explain all of their activities. There are, however, several aspects of platelet function on which a platelet inhibitor could possibly exert its activity. The platelet is a complex cell containing the energy systems of glycolysis and oxidative phosphorylation. It has been shown that inhibition or blockade of these systems together, but not singly, results in inhibition of ADP-induced aggregation (Murer *et al.*, 1967). Contractile proteins have been found to have a role in a number of platelet properties, including the release reaction (Luscher and Bettex-Galland, 1972). White (Luscher and Bettex-Galland, 1972), utilizing cytochalasin B, an inhibitor of cellular contractile mechanisms, has shown that inhibition of the platelet contractile system prevented the release reaction and the irreversible aggregation associated with it. Inhibitory compounds may also exert their effects through inhibition of platelet enzymes. ASA has been found to block platelet glycosyl transferase, the enzyme possibly responsible for the adhesion of platelets to collagen (Barber and Jamieson, 1971b). Alteration of platelet components through donation of a chemical moiety may be involved in the activity of some aggregation inhibitors. Platelet acetylation by ASA, for example,

Table 1. Inhibitors of Platelet Aggregation

A. Compounds administered *in vivo*.

Inhibitory Compound	Experimental Platelet System	Aggregating Agent					References
		ADP	COLL	THR	ADR	INJ	
Adenosine	rabbit-cerebral cortex surface vessels	x				x	Born *et al.* (1964)
	rat-cerebral cortex surface vessels					x	Born & Philp (1965)
	rabbit-cerebral cortex surface vessels					x	Philp & Lemieux (1968)
	hamster cheek pouch-iontophoresis	x					Begent & Born (1971)
2-Chloro-adenosine	rabbit-cerebral cortex surface vessels	x				x	Born *et al.* (1964)
N^6-substituted adenosines	rabbit PRP	x					Kikugawa *et al.* (1973)

ADP = adenosine 5' - diphosphate
COLL = collagen
THR = thrombin
ADP = adrenaline
INJ = mechanical injury

Table 1. (A) continued (Compounds administered *in vivo*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	INJ	References
Acetylsalicylic acid (ASA)	human PRP	x					Weiss & Aledort (1967)
	human PRP	x			x		MacMillan (1968)
	human PRP		x		x		O'Brien (1968b)
	human PRP	x					Zucker & Peterson (1968)
	rabbit-biolaser tech.					x	Fleming *et al.* (1970)
	human PRP				x		O'Brien *et al.* (1970a)
	rat PRP	x	x	x			Renaud & Godu (1970)
	human PRP		x				Stuart (1970)
	rabbit PRP		x				Ts'ao (1970)
	rabbit-partial ligation of abdominal aorta and vena cava					x	Ts'ao (1970)
	dog-peripheral arteries					x	Weiss *et al.* (1970)
	human dialyser membrane						Lindsay *et al.* (1972)
Benorylate	human PRP				x		O'Brien *et al.* (1970a)
Indomethacin	human PRP		x		x		O'Brien (1968b)
	human PRP				x		O'Brien *et al.* (1970a)
Phenylbutazone	human PRP		x		x		O'Brien (1968b)
	rabbit-biolaser tech.					x	Fleming *et al.* (1970)
	hyperlipaemic rat PRP	x		x			Renaud & Lecompte (1970)
Oxyphenbutazone	hyperlipaemic rat PRP	x		x			Renaud & Lecompte (1970)
Sulfinpyrazone	hyperlipaemic rat PRP	x		x			Renaud & Lecompte (1970)
	rat-filter loop tech.	x					Muirhead (1972)

Table 1. (A) continued (Compounds administered *in vivo*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	INJ	References
Dipyridamole	rabbit-cerebral cortex surface vessels					x	Emmons *et al.* (1965b)
	rabbit-cerebral cortex surface vessels					x	Elkeles *et al.* (1968)
	rabbit PRP	x					Philp & Lemieux (1969)
	rat-filter loop tech.	x					Muirhead (1972)
RA 233	human-dialyser membrane						Lindsay *et al.* (1972)
VK 744	human PRP	x					Sixma *et al.* (1972)
VK 774	human PRP	x			x		TenCate *et al.* (1972)
Fenoprofen Sodium	rabbit, guinea pig PRP	x	x				Herrmann *et al.* (1972)
	rabbit-extracorporeal shunt						Herrmann *et al.* (1972)
Reserpine	rabbit PRP	x					Zweifler (1967)
Metergoline	rabbit PRP	x	x	x			Fregnan (1972)
Heparin	human PRP		x		x		Besterman & Gillett (1972a)
Prostaglindin E_1	rabbit-cerebral cortical arteries					x	Emmons *et al.* (1967)
	rabbit PRP	x					Kinlough-Rathbone (1970b)
	rat-electrical stimulation of cortex surface vessels						Kloeze (1970b)

Table 1. (A) continued (Compounds administered *in vivo*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	INJ	References
Prostaglandin E_1 cont'd	rat-filter loop tech.	x					Hornstra (1971)
	guinea pig-platelet count fall	x					MacKenzie, Henderson & Steinbach (1971)
W-Homo-prostaglandin E_1	rat-platelet count fall	x					Kloeze (1970a)
Pyridinol-carbamate	human PRP	x					Yamazaki *et al.* (1970)
	rat-platelet count fall	x					Kobayashi & Didisheim (1972)
Brinolase	dog PRP	x	x				Roschlau & Gage (1972)
AN 162	guinea pig PRP	x					MacKenzie & Blohm (1971)
AY 16,804	rat-filter loop tech.	x					Muirhead (1972)
RMI 10,393	guinea pig PRP	x					MacKenzie *et al.* (1972)
1-(4-Chlorobenzoyl)-3-(5-tetrazolylmethyl) indole	rabbit-biolaser tech.					x	Fleming *et al.* (1970)

Table 1. (A) continued (Compounds administered *in vivo*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	INJ	References
9-[(Dialkyl-amino)alkyl]-thio-3-(di-methylamino) acridines and derivatives	rabbit PRP	x					Elslager, Haley *et al.* (1971)
Substituted 3,4-penta-dienyldi-amines	guinea pig PRP	x					Tilford *et al.* (1973)
{[(Dialkyla-mino)alkyl] thio} heter-ocyclic compounds	rabbit PRP	x					Elslager *et al.* (1972)

Table 1. Inhibitors of Platelet Aggregation

B. Compounds studied *in vitro*.

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Adenosine	human PRP	x						Born & Gross (1962)
	human PRP	x						Clayton *et al.* (1963)
	human PRP				x			O'Brien (1963a)
	rabbit PRP	x						Regoli & Clark (1963)
	human PRP	x						Born *et al.* (1965)
	washed pig & rabbit	x						Packham & Mustard (1968)
	human PRP	x						Rozenberg & Holmsen (1968)
	rabbit PRP	x						Philp & Lemieux (1969)
	human PRP	x						Salzman *et al.* (1969)
	human & rabbit PRP	x						Philp (1970)
	cat PRP	x					x	Tschopp (1970)
	human & rabbit PRP	x		x				Kien *et al.* (1971)
	human PRP	x						Mills & Smith (1971)
	human PRP	x						Murakami, Odake, Takase Yoshino (1972)
	washed human & rabbit	x						Packham *et al.* (1972)
	human PRP	x						Weisenberger *et al.* (1972)

ADP = adenosine -5'- diphosphate
COLL = collagen
THR = thrombin
ADR = adrenaline
NA = noradrenaline
5-HT = 5 - hydroxytryptamine

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
2-Chloroadenosine	human & rabbit PRP	x						Born (1964)
	human PRP	x						Born *et al.* (1965)
	human PRP	x						Mills & Smith (1971)
2-Br-adenosine 2-Fl-adenosine 2-Aza-adenosine Adenosine-1-N-oxide	human PRP	x						Born *et al.* (1965)
6-Hydroxyamino purine 2-Amino-6-hydroxyaminopurine riboside 2-Aminoadenosine	rabbit PRP	x	x	x				Kikugawa *et al.* (1972)
N6-substituted adenosines	rabbit PRP	x	x					Kikugawa *et al.* (1973)
Adenosine-monophosphate (AMP)	human PRP	x						Born & Cross (1962)
	human PRP				x			O'Brien (1963a)
	washed pig & rabbit	x						Packham & Mustard (1968)
	human PRP	x						Rozenberg & Holmsen (1968)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
AMP cont'd	human PRP	x						Salzman *et al.* (1969)
	human PRP		x	x				Bounameaux (1971)
	washed human & rabbit	x						Packham *et al.* (1972)
Cyclic AMP	human PRP	x						Marcus & Zucker (1965)
	human PRP	x						Marquis *et al.* (1969)
	human PRP	x		x	x			Salzman (1970)
	washed human			x				Mason & Read (1971)
	human PRP	x	x	x	x			Salzman & Levine (1971)
Dibutyryl-cyclic AMP	human PRP	x						Marquis *et al.* (1969)
	human PRP	x		x	x			Salzman (1970)
	washed human			x				Mason & Read (1971)
	human PRP	x	x	x	x			Salzman & Levine (1971)
Adenosine-triphos-phate	washed pig & rabbit	x						Packham & Mustard (1968)
2-Methylthio-adenosine-monophos-phate	washed human & rabbit	x						Packham *et al.* (1972)
Inosine	human PRP	x						Caen *et al.* (1972)
Hypoxanthine	human PRP	x						Caen *et al.* (1972)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Acetylsalicylic acid (ASA)	washed pig		x					Evans *et al.* (1967)
	human & rabbit PRP		x	x				Evans *et al.* (1968)
	washed human & rabbit		x	x				Evans *et al.* (1968)
	human PRP	x	x		x			O'Brien (1968a)
	human PRP		x		x			O'Brien (1968b)
	human PRP	x	x		x			Weiss *et al.* (1968)
	not given	x			x			Horowitz *et al.* (1969)
	human PRP	x	x		x			Zucker *et al.* (1969)
	human PRP	x	x		x			Al-Mondhiry *et al.* (1970)
	human PRP		x					Ball *et al.* (1970)
	guinea pig PRP	x	x					Constantine (1970)
	rabbit PRP	x	x					Peterson & Zucker (1970)
	human PRP	x	x		x			Zucker & Peterson (1970)
	rabbit PRP		x					Rosenberg *et al.* (1971)
	washed human	x	x		x			Mason *et al.* (1972)
Sodium salicylate	human & rabbit PRP		x	x				Evans *et al.* (1968)
	washed human & rabbit		x	x				Evans *et al.* (1968)
	human PRP	x	x		x			O'Brien (1968a)
	human whole blood	x						Davies *et al.* (1969)
	human PRP		x					Zucker & Peterson (1970)
Salicylic acid	washed human & human PRP		x					vonKaulla (1971)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	NA	5-HT	References
3-(2-Chloro-benzyl) salicylic acid	washed human & human PRP		x					vonKaulla (1971)
Acetic anhydride	human PRP	x	x		x			Al-Mondhiry *et al.* (1970)
Benzyl alcohol	human PRP	x						Zweifler & Sanbar (1969)
Phenol	human PRP	x						Zweifler & Sanbar (1969)
	human PRP	x						Rabiner & Molinas (1970)
Lead acetate	human & rat PRP	x	x	x				Davis & Holtz (1971)
Phenylbutazone	human, rabbit & pig PRP		x					Packham *et al.* (1967)
	human PRP		x		x			O'Brien (1968b)
	human PRP	x	x					Jobin & Gagnon (1971)
	human, rabbit & guinea pig PRP	x	x					Herrmann *et al.* (1972)
	washed human	x	x		x			Mason *et al.* (1972)
Sulfinpyrazone	human, rabbit & pig PRP		x					Packham *et al.* (1967)
	human PRP		x					Zucker & Peterson (1970)
	human PRP	x	x					Jobin & Gagnon (1971)
	washed human	x	x		x			Mason *et al.* (1972)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Indomethacin	human PRP		x		x			O'Brien (1968b)
	human PRP		x					Zucker & Peterson (1970)
Mefenamic acid	human PRP		x		x			O'Brien (1968b)
	human PRP		x					Zucker & Peterson (1970)
Flufenamic acid	human PRP		x					Zucker & Peterson (1970)
Meclofenamic acid	human PRP		x		x			O'Brien (1968b)
Fenoprefen	human, rabbit & guinea pig PRP	x	x					Herrmann *et al.* (1972)
Ibuprofen	human PRP		x		x			O'Brien (1968b)
Dipyridamole	human PRP	x						Emmons *et al.* (1965a)
	human PRP	x						Elkeles *et al.* (1968)
	human PRP	x						Born & Mills (1969)
	rabbit PRP	x						Philp & Lemieux (1969)
	human, rabbit & pig PRP	x	x	x				Cucuianu *et al.* (1971)
	washed human, rabbit & pig	x	x	x				Cucuianu *et al.* (1971)
	washed human			x				Mason & Read (1971)
	human PRP	x						Mills & Smith (1971)
RA 233	human PRP	x						Hassanein *et al.* (1970)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	NA	5-HT	References
RA 233 cont'd	human, rabbit & pig PRP	x	x	x				Cucuianu *et al.* (1971)
	washed human, rabbit & pig	x	x	x				Cucuianu *et al.* (1971)
	human PRP	x						Mills & Smith (1971)
	human PRP	x						Rozenberg & Walker (1973)
RA 433	human PRP	x				x		Elkeles *et al.* (1968)
	human PRP	x						Hassanein *et al.* (1970)
	human, rabbit & pig PRP	x	x	x				Cucuianu *et al.* (1971)
	washed human, rabbit & pig	x	x	x				Cucuianu *et al.* (1971)
	human PRP	x						Mills & Smith (1971)
	human PRP	x				x		Hampton *et al.* (1972)
VK 744	human PRP	x	x		x			Sixma & Trieschnigg (1971)
	human PRP	x				x		Hampton *et al.* (1972)
	human PRP	x						Slater *et al.* (1972)
	human PRP	x						Rozenberg & Walker (1973)
VK 774	human PRP	x				x		Hampton *et al.* (1972)
	human PRP	x						Slater *et al.* (1972)
	human PRP	x	x	x	x		x	TenCate *et al.* (1972)
Colchicine	human PRP	x	x		x			Soppitt & Mitchell (1969)
	washed human			x				Mason & Read (1971)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	NA	5-HT	References
Imipramine	human PRP				x			Rysanek *et al.* (1966)
	human PRP	x	x		x		x	Mills & Roberts (1967c)
	human PRP		x	x				Bounameaux (1971)
	human PRP	x		x				Brinson (1973)
Norimipramine	human PRP				x			Rysanek *et al.* (1966)
	human PRP	x	x		x		x	Mills & Roberts (1967c)
	guinea pig PRP	x	x					Constantine (1970)
Propazepin	human PRP				x			Rysanek *et al.* (1966)
Amitriptyline	human PRP				x			Rysanek *et al.* (1966)
	human PRP	x	x		x		x	Mills & Roberts (1967c)
	human PRP		x	x				Bounameaux (1971)
	washed human	x	x	x	x			Mason *et al.* (1972)
Nortriptyline	human PRP				x			Rysanek *et al.* (1966)
	human PRP	x	x		x		x	Mills & Roberts (1967c)
	human PRP	x		x				Brinson (1973)
Amphetamine	washed human	x	x	x	x			Mason *et al.* (1972)
Chlorpromazine	human PRP	x	x		x		x	Mills & Roberts (1967c)
	human PRP		x	x				Bounameaux (1971)
	washed human	x	x	x	x			Mason *et al.* (1972)
	human PRP	x		x				Brinson (1973)
Promethazine	human & rabbit PRP	x					x	Mitchell & Sharp (1964)
	rabbit PRP	x	x					Herrmann & Frank (1966)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Promethazine cont'd	human PRP	x	x		x		x	Mills & Roberts (1967c)
	washed human	x	x	x	x			Mason *et al.* (1972)
	human PRP	x		x				Brinson (1973)
Diphenhydramine	rabbit PRP	x	x					Herrmann & Frank (1966)
	human PRP	x	x		x		x	Mills & Roberts (1967c)
	washed human	x	x	x	x			Mason *et al.* (1972)
Methapyrilene	rabbit PRP	x	x					Herrmann & Frank (1966)
	washed human	x	x	x	x			Mason *et al.* (1972)
Trimeprazine tartrate	rabbit PRP	x	x					Herrmann & Frank (1966)
Chlorpheniramine maleate	rabbit PRP	x	x					Herrmann & Frank (1966)
Local anaesthetics	human PRP	x		x				Aledort & Niemetz (1968)
	human PRP	x	x		x			Deutsch *et al.* (1971)
	washed human	x	x	x	x			Mason *et al.* (1972)
General anaesthetics	dog PRP	x						Ueda (1971)
Glyceryl trinitrate	human PRP	x						Hampton *et al.* (1967)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	NA	5-HT	References
Propranolol	human PRP	x				x		Hampton *et al.* (1967)
	human PRP	x			x	x		Bygdeman (1968)
	human PRP	x						Bucher & Stucki (1969)
	washed human			x				Mason & Read (1971)
	human PRP	x						Gibelli *et al.* (1973)
β-Adrenergic blockers LB 46 & Ba 39089	human PRP	x						Bucher & Stucki (1969)
Phentolamine	human PRP				x			O'Brien (1963a)
	human PRP	x				x		Hampton *et al.* (1967)
	human PRP	x			x	x		Bygdeman (1968)
	washed human	x	x	x	x			Mason *et al.* (1972)
	human PRP					x		Sacchetti *et al.* (1973)
Phenoxybenz-amine	human PRP					x		Hampton *et al.* (1967)
	human PRP	x			x	x		Bygdeman (1968)
	washed human	x	x	x	x			Mason *et al.* (1972)
Tolazoline	human PRP	x				x		Hampton *et al.* (1967)
Intersain	human PRP	x				x		Hampton *et al.* (1967)
Isoxuprine	human PRP	x				x		Hampton *et al.* (1967)
Papaverine	human PRP	x				x		Hampton *et al.* (1967)
	human PRP	x						Born & Mills (1969)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Papaverine cont'd	human PRP		x	x				Bounameaux (1971)
	human PRP	x						Mills & Smith (1971)
	human PRP	x				x		Sacchetti *et al.* (1973)
Reserpine	human & rabbit PRP	x						Mitchell & Sharp (1964)
	washed human	x			x			Mason *et al.* (1972)
Compound 48/80	rat PRP	x	x	x				Ts'ao & Glagov (1972)
Metergoline	rabbit PRP	x	x	x				Fregnan (1972)
Quinidine sulphate	washed human	x	x	x	x			Mason *et al.* (1972)
Arcaine	washed human	x		x	x			Mason *et al.* (1972)
Quinacrine	washed human	x	x	x	x			Mason *et al.* (1972)
Atropine	human PRP	x						White (1969)
	washed human			x				Mason & Read (1971)
p-Chloromercuribenzoate	washed dog	x		x				Robinson *et al.* (1963)
	washed human	x	x	x	x			Mason *et al.* (1972)
p-Hydroxymercuri benzoate	human PRP	x	x	x		x	x	Harrison *et al.* (1966)
	human PRP	x	x					Al-Mondhiry & Spaet (1970)
	washed human	x	x					Al-Mondhiry & Spaet (1970)
N-ethylmaleimide	washed dog	x		x				Robinson *et al.* (1963)
	human PRP	x	x	x		x	x	Harrison *et al.* (1966)

Table 1. (B) continued (Compounds studied *in vitro*).

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
N-ethylmaleimide cont'd	human PRP	x	x					Al-Mondhiry & Spaet (1970)
	washed human PRP	x	x					Al-Mondhiry & Spaet (1970)
		x		x				Brinson (1973)
Methyl mercuric nitrate	washed dog	x		x				Robinson *et al.* (1963)
Meralluride	guinea pig & rat PRP	x						Gadd *et al.* (1971a)
Mersalyl	guinea pig & rat PRP	x						Gadd *et al.* (1971a)
Ethacrynic acid	washed human	x	x	x	x			Mason *et al.* (1972)
Prostaglandin A	human, pig & rat PRP	x						Kloeze (1967)
	human PRP	x						Marquis *et al.* (1969)
Prostaglandin E_1	human & rat PRP	x	x	x				Chandrasekhar (1967)
	human PRP	x	x	x	x		x	Emmons *et al.* (1967)
	human, pig & rat PRP	x						Kloeze (1967)
	human PRP	x						Marquis *et al.* (1969)
	human PRP		x					Ball *et al.* (1970)
	human PRP				x			Cole *et al.* (1970)
	rabbit & pig PRP	x	x	x				Kinlough-Rathbone *et al.* (1970b)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Prostaglandin E_1 cont'd	washed rabbit & pig	x	x	x				Kinlough-Rathbone *et al.* (1970b)
	human, rabbit & rat PRP	x						Seklar (1970)
	human PRP	x						Mills & Smith (1971)
	rat PRP	x						Shio *et al.* (1972)
Iso-prostaglandin E_1	human, pig & rat PRP	x						Kloeze (1967)
Prostaglandin E_2	human, rabbit & rat PRP	x						Seklar (1970)
Prostaglandin $F_{1\alpha}$	human PRP	x						Marquis *et al.* (1969)
Aminophylline	human PRP	x		x				Brinson (1972)
Theophylline	human PRP		x					Ball *et al.* (1970)
	human PRP				x			Cole *et al.* (1970)
	human PRP		x	x				Bounameaux (1971)
	rat PRP	x						Shio *et al.* (1972)
Caffeine	human PRP		x	x				Bounameaux (1971)
	washed human	x	x	x	x			Mason *et al.* (1972)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Amino acid methyl esters	human PRP	x						Salzman & Chambers (1964)
	human PRP	x						Jerushalmy *et al.* (1965)
Imidazole	human PRP	x	x			x		Davis & Phillips (1971)
Sodium cyanide	washed human	x	x	x	x			Mason *et al.* (1972)
Sodium fluoride	washed human	x	x	x	x			Mason *et al.* (1972)
EDTA	not given	x	x					Zucker & Jerushalmy (1965)
Fibrinogen degradation products	human PRP	x	x	x				Kowalski *et al.* (1963)
	washed human	x	x	x				Kowalski *et al.* (1963)
	human PRP	x	x					Jerushalmy & Zucker (1966)
	human PRP	x						Niewiarowski *et al.* (1970)
	human PRP	x		x	x	x		Stachurska *et al.* (1970)
RMI 10,393	human PRP	x	x	x	x		x	MacKenzie, Blohm, *et al.* (1971)
	human PRP	x	x	x	x			Rodman *et al.* (1971)
	washed human	x	x	x	x			Rodman *et al.* (1971)
	human PRP	x	x	x				Claxton *et al.* (1972)
	human PRP	x	x	x				MacKenzie *et al.* (1972)
Toluidine blue	human PRP	x						Mitchell & Sharp (1964)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	NA	5-HT	References
Cyproheptadine	human PRP	x	x	x	x			Goldman *et al.* (1971)
	human PRP						x	Minsker (1972)
	guinea pig PRP	x	x	x				Minsker (1972)
Chloroquin	washed human			x				Mason & Read (1971)
Guanidinosuccinic acid	not given	x			x			Horowitz *et al.* (1969)
Pyridinolcarbamate	human PRP	x						Kobayashi & Didisheim (1972)
Brinolase	human PRP	x	x					Roschlau & Gage (1972)
Lanthanum ions	human PRP	x						Holmsen *et al.* (1971)
Lobeline	guinea pig PRP	x	x	x				Gadd *et al.* (1971b)
Heparin	washed human			x				Eika (1971)
	human PRP		x					Besterman & Gillett (1972a)
Protamine	washed human			x				Eika (1971)
Polybrene	washed human			x				Eika (1971)
Histamine	rabbit PRP	x						Constantine (1965)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent ADP	COLL	THR	ADR	NA	5-HT	References
Cytochalasins	pig PRP	x						Haslam (1972)
Lysolecethin	human PRP	x	x		x			Besterman & Gillett (1971)
	human PRP	x	x		x			Besterman & Gillett (1972b)
	human PRP						x	Besterman & Gillett (1973)
Vipera aspis venom	human PRP	x	x		x			Boffa *et al.* (1972)
Penicillin G	human PRP	x	x	x				Cazenave *et al.* (1973)
	washed pig & rabbit	x	x	x				Cazenave *et al.* (1973)
Dimethylsulfoxide	human PRP	x		x	x			Holtz & Davis (1972)
Dimethyl acetamide	human PRP	x		x	x			Holtz & Davis (1972)
Sodium glycerophosphate	human PRP	x		x	x			Holtz & Davis (1972)
Lidoflazine	human PRP	x	x					deClerck (1972)
Plasmin	washed human			x				Miller & Feinstein (1973)
Proteolytic enzymes	human PRP	x						Sano & Yokoyama (1971)
	washed human			x				Sano & Yokoyama (1971)
Methysergide	human PRP						x	Cumings & Hilton (1971)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR	ADR	NA	5-HT	
Ethanol	human PRP		x			x		Davis & Phillips (1970)
Sodium chromate	human PRP	x	x		x			Kattlove & Spaet (1970)
Antithrombin III	washed human			x				Eika & Abildgaard (1970)
Rabbit liver homogenate	rabbit PRP	x	x	x				Rogowski *et al.* (1971)
Naphyl alkyl lactamimides	human PRP	x	x					Roberts *et al.* (1972)
5,10-Dihydro-3-(phenyl, thienyl, and furyl) thiazo (3,2-b) (2,4) benzodiazepines	rabbit PRP	x						Elslager, McLean *et al.* (1971)
9-[(Dialkylamino) alkyl] thio-3-(dimethylamino) acridines	rabbit PRP	x						Elslager, Haley *et al.* (1971)

Table 1. (B) continued (Compounds studied *in vitro*)

Inhibitory Compound	Experimental Platelet System	Aggregating Agent						References
		ADP	COLL	THR.	ADR	NA	5-HT	
Biphenylyloxy-alkyl amines	rabbit PRP	x	x	x	x			Lacefield *et al.*(1971)
{[(Dialkyl-amino) alkyl] thio} heterocyclic compounds	rabbit PRP	x						Elslager *et al.*(1972)

may play some role in its inhibitory effects. Drugs which block α- and β-adrenergic receptors or sulfhydryl groups (Bucher and Stucki, 1969; Mason *et al.*, 1972) have been found to be aggregation inhibitors, probably functioning through interference at specific platelet sites. Compounds which have a stabilizing action on biological membranes (eg. tricyclic antidepressants) have been found to be inhibitors of aggregation (Mills and Roberts, 1967c). Some inhibitors may also function by increasing the platelet levels of cyclic AMP, either through stimulation of adenyl cyclase, eg. prostaglandin E_1 and adenosine (Wolfe and Shulman, 1969; Zieve and Greenough, 1969; Scott, 1970; Mills and Smith, 1971; Harwood *et al.*, 1972), or by an inhibition of phosphodiesterase, eg. methylxanthines, papaverine and dipyridamole (Cole *et al.* 1970; Horlington and Watson, 1970; Smith and Mills, 1970; Mills and Smith, 1971; Pichard *et al.*, 1972). Recent investigations by Amer and his co-workers (Amer and Marquis, 1972; Amer and Mayol, 1973) have provided evidence that two forms of phosphodiesterase exist in human blood platelets. The relative proportions of the two enzymes were shifted by aggregation stimulants and inhibitors (Amer and Mayol, 1972), suggesting a possible site at which inhibitors of aggregation might function. Calcium chelation may also be involved in the inhibitory activity of some substances on platelet aggregation. A number of investigators (Hovig, 1964; Skoza *et al.*, 1967; Ardlie and Mustard, 1969; Ardlie *et al.*, 1970; Cronberg and Caen, 1970; Herrmann *et al.*, 1970) have described this ion as being an essential factor in ADP-induced aggregation.

Not all aggregation inhibitors produce identical inhibitory patterns on platelet aggregation. Some compounds, such as ASA, have been found to inhibit only the release portion of the response, having no effects on the initial phase (O'Brien, 1968a; 1968b; Zucker *et al.*, 1969; Al-Mondhiry, Marcus and Spaet, 1970; Constantine, 1970; Zucker and Peterson, 1970). Other substances such as dipyridamole and its analogues and prostaglandin E_1, appear to function through an inhibition of the initial phase of aggregation (Emmons *et al.*, 1967; Elkeles *et al.*, 1968; Cucuianu *et al.*, 1971; Mills and Smith, 1971; Shio *et al.*, 1972).

Screening studies such as those of Elslager *et al.* (Elslager, McLean *et al.*, 1971; Elslager, Haley *et al.*, 1971; Elslager *et al.*, 1972), Lacefield *et al.* (1971), and Roberts *et al.* (1972), where a large number of compounds have been examined, attempt to define more clearly inhibitory mechanisms and to discover substances with potential as antiplatelet drugs.

F. Salicylates and the Hemostatic Mechanism

Considerable investigation has concentrated on the effects of salicylate compounds on the hemostatic mechanism, and specifically on platelet function and its involvement in thrombosis and bleeding disorders. As early as 1916, Gregerson reported the presence of blood in the feces of persons with rheumatic fever who were receiving salicylate therapy (Smith and Smith, 1966). In 1943 it was demonstrated that the prothrombin level in man was decreased by salicylate administration; ASA being more potent in this respect than sodium salicylate (Meyer and Howard, 1943; Shapiro *et al.*, 1943). A few years later, Bounameaux and

Cauwenberge (1954), reported that the administration of sodium salicylate to rats decreased the adherence of platelets to glass. Since that time, a great number of studies have been reported on the effects of salicylate compounds, and in particular, acetylsalicylic acid on platelet function (O'Brien, 1961; Bloom and Beamish, 1966; Weiss and Aledort, 1967; Davies *et al.*, 1968; MacMillan, 1968; O'Brien, 1968a; Weiss *et al.*, 1968; Leonards, 1969; Mielke *et al.*, 1969; Sahud and Aggeler, 1969; Samter, 1969; Zucker *et al.*, 1969; Al-Mondhiry *et al.*, 1970; Stuart, 1970). ASA *in vivo* or *in vitro* has been found to decrease aggregation with collagen (see section E.) and inhibit the second phase of ADP- or adrenaline-induced platelet aggregation (Evans *et al.*, 1968; O'Brien, 1968a; Zucker *et al.*, 1969; Mustard and Packham, 1970). This inhibition is due to an inhibitory effect on the platelet release reaction, especially the release of ADP (Weiss and Aledort, 1967; Evans *et al.*, 1968; O'Brien, 1968a; Weiss *et al.*, 1968; Berger, 1970; Mustard and Packham, 1970), and is enhanced by pre-incubation with the drug at 37°C (O'Brien, 1968a; Zucker *et al.*, 1969; Zucker, 1971). Ingestion of ASA in man has been found to inhibit platelet aggregation for up to seven days (O'Brien, 1968a; Weiss *et al.*, 1968; Stuart, 1970). Inhibition of the platelet release reaction by ASA has been measured by several means, including the determination of the release of ^{14}C-5-HT by adrenaline, connective tissue and ADP (Zucker and Peterson, 1968; Atac *et al.*, 1970; Zucker and Peterson 1970), or by measuring platelet factor 4 release induced by ADP or collagen (Youssef and Barkhan, 1969; O'Brien *et al.*, 1970b).

Although the mechanism of action of ASA on platelet function is not completely understood, a number of studies have suggested an important

role for the acetoxy group of the molecule. The drug has been shown to acetylate plasma proteins (Hawkins *et al.*, 1968; Pinckard *et al.*, 1968a; 1968b; Hawkins *et al.*, 1969; Pinckard *et al.*, 1970) and to donate an acetate group for incorporation into platelet components (Deykin and Dresser, 1968; Al-Mondhiry *et al.*, 1969; 1970; Deykin, 1970; Puszkin *et al.*, 1970; Rosenberg *et al.*, 1970; 1971; Okuma *et al.*, 1971). Using ASA labelled in the acetoxy group, Hawkins *et al.* (1968) found that the compound acetylated human serum albumin, while drug labelled in the carboxyl group resulted in very little incorporation. They later found that most of the radioactivity was taken up by albumin in a peptide designated as "peptide A", probably in a lysine residue. Pinckard *et al.* (1970) have reported the presence of at least two qualitatively different sites of acetylation on the human serum albumin molecule. Yousset and Barkhan (1969) have suggested that when ASA became attached to the platelet, it appeared to do so by the acetoxy group. When the acetoxy group of ASA waslabelled radioactively and incubated with platelets, this label was found to be taken up by the platelet into membrane, granule and soluble fractions (Al-Mondhiry *et al.*, 1969; Al-Mondhiry *et al.*, 1970). Such was not the case when the carboxyl group was labelled. Al-Mondhiry and his colleagues (Al-Mondhiry *et al.*, 1969; Al-Mondhiry *et al.*, 1970) found that both labels from ^{14}C-acetic anhydride and sodium ^{14}C-acetate, compounds with inhibitory activity and no activity on platelet aggregation respectively, were also incorporated into platelets. Based on these observations, they suggested that the ability of ASA to acetylate platelets might account for its sustained effects *in vivo* (Al-Mondhiry *et al.*, 1969), although metabolic incorporation of acetate could not be excluded (Al-Mondhiry *et al.*, 1970).

Rosenberg *et al.* (1971) utilizing acetyl-^{3}H-ASA and carboxyl-^{14}C-ASA, found that the carboxyl-^{14}C was not incorporated *in vitro* into rabbit platelets and that the degree of uptake of acetyl-^{3}H depended on the concentration of ASA and the incubation time. They reported that the degree of inhibition of aggregation, in general, paralleled the uptake of acetyl-^{3}H by the rabbit platelets. They also noted that acetylation (protein) and acetate uptake were separate processes which could be distinguished by lipid-protein labelling distribution. An *in vivo* study has also added support to the hypothesis that the acetoxy group of ASA may be involved in the sustained *in vivo* inhibitory activity on platelet aggregation. O'Brien *et al.* (1970a) examined the effects of some anti-inflammatory drugs on human platelets, noting that indomethacin, the most potent inhibitory compound examined, had no acetoxy group and its effects were short-lived. In contrast, ASA and benorylate, both with acetoxy groups, had persistent inhibitory activity; ASA with a more labile acetate radical being the more potent in this respect (O'Brien *et al.*, 1970a). Pinckard *et al.* (1970) have suggested that a single surface receptor or enzyme was permanently influenced by the acetylation by ASA. The fact that the *in vivo* effects of ASA persist for up to seven days (O'Brien, 1968a; Weiss *et al.*, 1968; Stuart, 1970) may add support to this hypothesis.

Labelled acetate has been shown to be readily incorporated into blood platelets. Deykin (1970) reported that of incorporated radioactivity, 35% appeared in granules, 8% in dense bodies, 4% in large membranes and 53% in the soluble supernatant. Of acetate taken up by rat platelet lipids, Okuma *et al.* (1971) noted that incorporation took

place primarily into phosphatidyl cholines, triglycerides, ceramides, free fatty acids and phosphatidyl ethanolamines. Puszkin *et al.* (1970) have demonstrated the presence of labelled acetate in newly synthesized proteins in human platelets.

Some of the inhibitory effects of ASA on platelet function may be related to the ability of the drug to alter platelet metabolism (O'Brien, 1968c; Ball *et al.*, 1969; Doery *et al.*, 1969; Murer and Holme, 1970). ASA has been shown to inhibit glucose uptake, increase the utilization of glycogen and oxygen uptake, and to reduce total ATP levels in a washed platelet suspension at high concentrations (50mM) (Doery *et al.*, 1969). Lower concentrations inhibited glucose uptake (Doery *et al.*, 1969), increased the level of platelet metabolic ATP (Ball *et al.*, 1969), and inhibited the collagen-induced breakdown of radioactive ATP to IMP, to an extent proportional to the degree of inhibition of release (Ball *et al.*, 1969). Some investigators (Holmsen, Ball and Storm, 1969), as noted in section D (The Release Reaction), have suggested that this conversion of ATP to IMP may provide energy for the release reaction. Muenzer *et al.* (1972) have reported that the thrombin-induced increase in oxygen consumption in human platelets was inhibited by ASA. ASA has not been reported to alter platelet cyclic AMP levels (Ball *et al.*, 1970; Amer and Marquis, 1972; Salzman and Weisenberger, 1972). Amer and Mayol (1973) described the presence of two forms of the enzyme phosphodiesterase (I and II), from human platelets existing in equilibrium. One of these forms, phosphodiesterase II, was found to be exposed during collagen aggregation, and the relative proportion of the two enzymes was shifted by aggregation stimulants and inhibitors (Amer and Marquis, 1972).

Aspirin has been found to shift this equilibrium toward phosphodiesterase I, an effect which may be related to its inhibitory activity on platelet aggregation.

A large number of enzymes including some found in platelets have been found to be inactivated by ASA. Gorog and Kovacs (1970) have reported that the drug inhibited the Ca^{++}- and Mg^{++}- activated ATPase activity of actomyosin in smooth muscle. It is possible that this compound may have similar inhibitory activity in platelets. ASA has been found to inhibit glucosyl transferase, the enzyme possibly responsible for the adhesion of platelets to collagen, in the same relative proportion that it inhibited platelet to collagen adhesion (Barber and Jamieson, 1971b). Other investigators (Ts'ao, 1970; Jenkins *et al.*, 1973) have also suggested that ASA may exert some of its inhibitory activity on the platelet release reaction through interference with the adhesion of platelets to collagen.

O'Brien (1969) has reported that ASA stabilized platelet membranes and suggested that this membrane protective effect or its activity on membrane-bound enzymes may be involved in its action on platelets.

Evidence has accumulated to demonstrate that ASA inhibits prostaglandin synthesis in platelets, an inhibition which may be associated with its activity on platelets (Smith and Willis, 1971; Kocsis *et al.*, 1973). Prostaglandin production by platelets has been found to be abolished, along with the second phase of adrenaline-induced aggregation, in human PRP, for as long as three days, following the ingestion of ASA (Kocsis *et al.*, 1973). Sodium salicylate did not produce these effects (Kocsis *et al.*, 1973). Leonardi *et al.*, (1972) have prevented the effect

of ASA on thrombin-induced platelet aggregation by preincubating citrated PRP with arachidonic acid, the precursor of prostaglandin E_2 synthesis. The inhibitory effect of ASA on the synthesis of prostaglandins by platelets would appear to represent a paradox as the prostaglandins themselves are potent aggregation inhibitors.

Sodium salicylate is not as effective an inhibitor of platelet aggregation as ASA (Evans *et al.*, 1968; O'Brien, 1968a). In some studies it was found to have no effect on collagen-induced release (O'Brien, 1968b; Weiss *et al.*, 1968), while in others it inhibited aggregation by collagen, antigen-antibody complexes, gamma globulin coated particles, and thrombin (Evans *et al.*, 1968). Davies *et al.* (1969) have reported that sodium salicylate was effective in inhibiting platelet clumping in native PRP or fresh human blood.

ASA, in relatively small doses, has been shown to result in a prolongation of the bleeding time (Beaumont *et al.*, 1955; Blatrix, 1963; Gast, 1964; Quick, 1966a; 1966b; Weiss and Aledort, 1967; Mielke *et al.*, 1969; 1973), and has been observed to worsen bleeding tendencies in persons who have a hemostatic defect (Kaneshiro *et al.*, 1969; Mielke *et al.*, 1969; Quick, 1970). Quick (1966b) also found that, unlike ASA, sodium salicylate had no effect on the bleeding time. Weiss (1968) has suggested that the increased bleeding time following the ingestion of ASA may be a result of impaired platelet aggregation due to inhibition of ADP release.

It has been known for many years that ASA may, in some individuals, produce gastrointestinal bleeding. The subject has been extensively reviewed by Smith and Smith (1966). The role of platelets, or more

specifically, ASA-inhibited platelets, is not well understood. Morris (1967) has suggested that part of the reason for gastrointestinal bleeding associated with the ingestion of ASA is related to its interference with platelet function or with an aggregating factor.

Data from a number of *in vivo* studies on the effects of salicylate compounds on platelet function and thrombus formation have been published. In some of these, ASA has been found to reduce the incidence of experimentally-induced thrombosis in dogs (Weiss *et al.*, 1970); reduce the incidence of formation of occlusive thrombi in rabbits, comparable doses of sodium salicylate being without effect (Peterson and Zucker, 1970); decrease the amount of deposit (Evans *et al.*, 1968), and inhibit thrombus formation (Herrmann *et al.*, 1972) in extracorporeal shunts in rabbits; reduce the adhesiveness of platelets to (Lindsay *et al.*, 1973), and the incidence of thrombus formation on (Lindsay *et al.*, 1972; Zucker *et al.*, 1973) dialysis membranes; markedly inhibit the production of thrombosis induced by intravenous endotoxin in hyperlipemic rats (Renaud and Godu, 1970); and reduce the incidence of adrenaline-induced myocardial necrosis in dogs (Haft, Gershengorn *et al.*, 1972). Utilizing iontophoresis to induce *in vivo* platelet aggregation in the hamster cheek pouch, Begent and Born (1971) have found that ASA had inhibitory activity. In this system, sodium salicylate was demonstrated to be several times less potent than ASA *in vivo*.

A number of thrombotic disorders have also been reported to benefit from therapy with ASA. Perhaps the largest study was completed before the present interest in ASA and platelet function developed. In 1956, Dr. L. L. Craven, a general practitioner in Glendale, California,

reported his experience with ASA in the prophylaxis of coronary and cerebral thrombosis. In a study, probably lacking stringent controls, he prescribed two ASA tablets per day and obtained results over a period of seven to ten years on 8,000 men. Dr. Craven, reporting his results in a relatively obscure journal, the Mississippi Valley Medical Journal, in which they remained buried for a number of years, stated that "Not a single case of detectable coronary or cerebral thrombosis occurred among patients who had faithfully adhered to this regimen" (Weiss, 1971). Vreeken and van Aken (1971) have noted that spontaneous aggregation of platelets probably is a cause of idiopathic thrombosis and recurrent painful toes and fingers. They have described three such cases which were aided by the use of ASA. Multiple episodes of transient monocular blindness, occurring secondary to retinal emboli, have been reported to be abolished by the administration of the compound (Mundall *et al.*, 1972). Hughes and Tonks (1968) have observed that certain types of human pulmonary infarction have responded to long-term maintenance treatment with ASA.

Based on the above evidence, one might be forced to agree with investigators who have suggested that ASA should be administered in conditions which predispose to thromboembolism (Bowie and Owen, 1969; Wood, 1972). However, all of the experimental data reported has not been so encouraging. Arfors *et al.* (1972) found that ASA had no effect on platelet reactivity at sites of biolaser-induced endothelial injury in rabbit ear chamber preparations. Moschos *et al.* (1972) gave ASA orally or intravenously to dogs, utilizing a catheter electrode to induce coronary or femoral artery thrombosis, and found that thrombus formation

in all animals receiving the drug was similar to that of the control group, although in several animals the thrombi appeared smaller. Patients with rheumatoid arthritis, who were receiving ASA, have been followed by Isomaki (1972), and found to have as many myocardial infarcts as persons taking ASA only occasionally or not at all. In a double-blind trial to study postoperative deep vein thrombosis, ASA was found to have no effect (Butterfield, 1973). It has also been noted that the drug does not effect the loss of platelets on prosthetic heart valves (Harker and Slichter, 1970).

III METHODS

A. Selection of Blood Donors

i. Normal Subjects

Male and female volunteers, who were either laboratory personnel or students in health sciences at The University of Western Ontario, donated blood for the aggregation and radioactive isotope labelling studies. These subjects were between 20 and 40 years of age, with no history of a bleeding disorder, and they had not taken any medication for at least seven days (see Appendix I). The volunteers were questioned as to drug intake, emphasis being placed on those compounds known to alter normal platelet function. Because of the essential nature of ADP-induced biphasic aggregation responses to the study, those volunteers whose PRP did not demonstrate this pattern, were excluded.

ii. Hospital Patients

To study the possible relationship between gastrointestinal (G.I.) hemorrhage and *in vitro* sensitivity of platelets to ASA inhibition, three groups of patients, most of whom were male (age 29-80, mean 50.6 yr.), admitted to St. Joseph's Hospital, London, Ontario, were selected. One group, containing 11 patients with a diagnosis of upper G.I. hemorrhage, had taken ASA within 96 hours of the onset of their bleeding episode. A second group, consisting of 11 patients with upper G.I. hemorrhage, had not taken ASA for several weeks, insofar as could be determined by care-

ful questioning. A third group comprised 11 patients who had no clinical signs of G.I. bleeding and who were admitted for a variety of reasons, none of which involved blood loss or any other condition which might affect platelet function.

Patients with upper G.I. hemorrhage were defined as those on whom the admitting diagnosis was made clinically and on the basis of melena and/or hematemesis. ASA administration was defined as the ingestion of any drug containing any quantity of the compound. Patient selections for the study were made by a gastroenterologist (Dr. I. I. Borda, Assistant Professor, Department of Medicine, The University of Western Ontario), and patient interviews were conducted by a registered nurse (Miss Cheryl Eldridge, R.N.) specially trained in interviewing procedures for a drug adverse-reaction surveillance programme. Patients taking any medication known to affect platelet function (see Appendix I) were excluded from the investigation. The blood samples were all coded and laboratory personnel conducting the various tests were unaware of the case histories of the donors.

B. Collection of Whole Blood

Whole blood was collected from a vein in the antecubital fossa of the human subjects, using a 30 ml. disposable syringe (Plastipak with Luer Lok Tip-Becton, Dickinson and Co., Clarkson, Ontario) and a 19 ga. one in. thin-walled, short bevel, or 20 ga. one in. needle (Becton, Dickinson and Co.). Nine ml. of whole blood were anticoagulated by mixing with 0.9 ml. of 3.8% trisodium citrate (McArthur Chemical Co. Ltd., Montreal, Quebec) in 13 x 100 mm. Pyrex biological test tubes (Fisher Scientific

Co. Ltd., Toronto, Ontario). All glassware contacted by whole blood or platelet-rich plasma was coated with silicone (Siliclad-Clay Adams-Canlab Laboratory Supplies Ltd., Toronto, Ontario) to retard platelet adhesion.

C. Preparation of Platelet-Rich Plasma

For the preparation and collection of platelet-rich plasma (PRP), the tubes containing the citrated whole blood were centrifuged at 750 x g for 4 min. (International Clinical Centrifuge-Model CL, International Equipment Co., Needham Heights, Massachusetts, U.S.A.). By means of a 5 ml. glass syringe, (Becton, Dickinson and Co.) to which a short section of PE 160 tubing (Clay Adams Intramedic Polyethylene Tubing-Fisher Scientific Co.) was attached, the PRP was removed and placed in a 15 ml. Pyrex conical, graduated centrifuge tube (Canlab Laboratory Supplies Ltd.). All PRP was adjusted to a final count of approximately 300,000 platelets per mm^3. If the PRP platelet count was higher than this, the PRP was diluted with the same donor's platelet-poor plasma (PPP), which was prepared by centrifugation of the remaining citrated blood at 750 x g for 15 min. When the platelet count in the PRP was less than 300,000 per mm^3, the PRP was concentrated by centrifugation and removal of an appropriate amount of PPP at the top of the centrifuge tube.

D. Platelet Counting

Platelets were counted visually by a modified method of Wright (1941), using Spencer Bright-Line hemocytometers (American Optical Instrument Co., Buffalo, New York, U.S.A.) and Dade Tri-Lyne blood

diluting pipettes (Canlab Laboratory Supplies Ltd.). The brilliant cresyl blue-EDTA platelet counting fluid had the following formula:

Brilliant Cresyl Blue	0.25 gm.
NaCl	3.38 gm.
EDTA Disodium Dihydrate	4.25 gm.
Formalin	10.00 ml.
Distilled Water q.s. ad	500 ml.

Brilliant cresyl blue dye powder was obtained from British Drug Houses (Canada) Ltd., Toronto, Ontario, NaCl from McArthur Chemical Co., Ltd., EDTA from Fisher Scientific Co., and formalin from Mallinckrodt Chemical Works, Toronto, Ontario.

E. Platelet Aggregation Studies

The platelet aggregometer is an instrument which measures changes in light transmission occasioned by the conversion of platelets from the dispersed, individual form to large clumps (aggregates) in response to an aggregation stimulus, and changes in both rate and extent of aggregation can be recorded. In the present investigation, platelet aggregation was studied by means of a Born Aggregometer (Royal College of Physicians and Surgeons, London, England), the sample container of which was modified to accept 8 x 75 mm. cuvettes (Bryston Manufacturing Ltd., Toronto, Ontario), and thus permit the use of small (0.35 ml.) PRP samples. Temperature in the cuvette holder of the aggregometer was maintained at 37 degrees centigrade (°C) by means of an attached water bath and a Grant pump and thermostatic control unit (Grant Instruments

Ltd., Cambridge, England). To follow and quantitate aggregation patterns, a Rikadenki Model B-141 recorder (Rikadenki Kogyo Co. Ltd., Tokyo, Japan) was attached to the aggregometer. Standardization of the recorder was carried out as outlined in the "Detailed Working Instructions" accompanying the Born Aggregometer with low (O.D. at 550 mμ,1cm. light path= 0.57± 0.002) and high (O.D. at 550 mμ,1cm. light path= 1.55± 0.025) density latex particle suspensions used as reference standards. PRP was pipetted in 0.35 ml. aliquots into cuvettes and allowed to stand at room temperature for approximately 30 min. before beginning the experiment. Short, magnetized sections (0.140 - 0.145 in.) of 18 ga. needle stylet wire (Becton, Dickinson and Co.) were used to stir the PRP in the aggregometer, the magnetic stirring motor of which operated at approximately 1250 r.p.m. For the ADP study, a minimum concentration of the aggregating agent, which would yield a biphasic aggregation response, was selected. PRP which did not show this biphasic aggregation pattern was discarded. To study collagen aggregation, a standard volume (0.1 ml.) of collagen suspension was added. Addition of the aggregating agents and salicylate analogues was made with micro-syringes (Agla Micrometer Syringe Outfit, Burroughs Wellcome and Co., London, England; Gilmont Instruments Inc., Great Neck, New York, U.S.A.). Prior to aggregation, each sample was incubated in the waterbath at 37°C for four minutes. Following this period, PRP samples were incubated in the aggregometer and stirred for one minute before addition of the inhibitor drugs and/or aggregating agent. To study the effects of incubation time on the inhibitory activity of the salicylate analogues, these compounds were added to the cuvette prior to incubation in the

waterbath for four or nine minutes. The samples were then incubated in the cuvette holder of the aggregometer and stirred for one minute before addition of the aggregating agent. Aggregation by ADP was allowed to continue for at least five minutes from its initiation, while that by collagen was followed for at least five minutes after the delay phase of the aggregation response. The inhibitory effects on ADP- and collagen- induced aggregation of the salicylate analogues shown in Figure 1 were studied. Each analogue was tested at various final concentrations with the PRP of 10 different donors.

F. Determination of Platelet Adhesiveness

For the determination of platelet adhesiveness, 2.5 ml. of citrated whole blood were forced vertically (by a portable infusion-withdrawal pump - Harvard Apparatus Co. Inc., Millis, Massachusetts, U.S.A.) through a 5.0 gm. column of glass beads (0.45 - 0.50 mm. diameter - Canlab Laboratory Supplies Ltd.), contained in a 2.5 ml. disposable syringe (Becton, Dickinson and Co.), at the rate of 1.0 ml. per min. The percent adhesiveness was an expression of the percentage of platelets retained on the bead column, i.e:

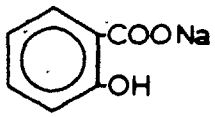
$$\% \text{ adhesive platelets} = \frac{\left(\begin{array}{c}\text{initial platelet} \\ \text{count}\end{array}\right) - \left(\begin{array}{c}\text{platelet count} \\ \text{following passage} \\ \text{through column}\end{array}\right)}{\text{initial platelet count}} \times 100$$

G. Source of Supply of Salicylate Analogues

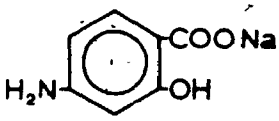
Acetylsalicylic acid (ASA) and sodium salicylate were obtained from Merck and Co., Montreal, Quebec; 4-amino salicylic acid (sodium salt)

Figure 1

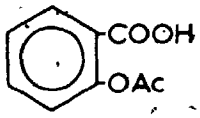
The chemical structures and molecular weights of the salicylate analogues examined in the platelet aggregation study



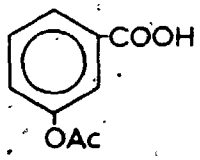
sodium salicylate
M.W. 160.11



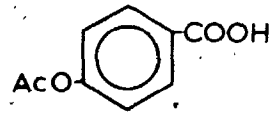
sodium PAS
M.W. 211.16



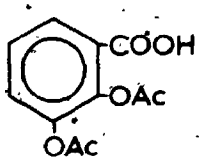
A S A
M.W. 180.15



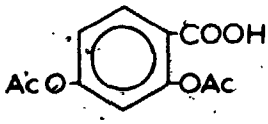
3-acetoxybenzoic acid
M.W. 180.15



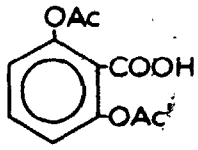
4-acetoxybenzoic acid
M.W. 180.15



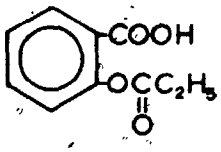
2,3-diacetoxybenzoic acid
M.W. 238.18



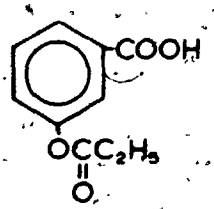
2,4-diacetoxybenzoic acid
M.W. 238.18



2,6-diacetoxybenzoic acid
M.W. 238.18



2-propoxybenzoic acid
M.W. 196.18



3-propoxybenzoic acid
M.W. 196.18

(PAS) was acquired from British Drug Houses. The remaining salicylate analogues were synthesized by Dr. M. Hirst, Department of Pharmacology, The University of Western Ontario, by the referenced methods: 3-acetoxybenzoic acid (Marshall *et al.*, 1942), 4-acetoxybenzoic acid (Chattaway, 1931), 2-propoxybenzoic acid (Hoftsee, 1952), 3-propoxybenzoic acid (Hofstee, 1952), 2,3-diacetoxybenzoic acid (Simokoriyama, 1941), 2,4-diacetoxybenzoic acid (Simokoriyama, 1941), and 2,6-diacetoxybenzoic acid (Hunt *et al.*, 1956). The radioactive analogues 1-^{14}C-acetyl salicylic acid, 2,3-di(1-^{14}C) acetoxybenzoic acid, 2,6-di(1-^{14}C) acetoxybenzoic acid and 2-(1-^{14}C) propoxybenzoic acid were also synthesized by Dr. M. Hirst (see Appendix II). The term propoxy used in this thesis denotes the moiety CH_3CH_2CO- and is an abbreviated form of propionyloxy-.

H. Preparation of Drugs and Reagents

All drugs and compounds utilized in the aggregation study were dissolved in 0.9% saline. The concentration of the adenosine 5'-diphosphate (disodium salt from equine muscle- Sigma Chemical Co., St. Louis, Missouri, U.S.A.) (ADP) stock solution was 2.5 x 10^{-4}M. It was kept frozen until used. The stock solutions of the salicylate analogues were 5.5 x 10^{-3}M, with sodium salicylate and PAS also prepared at 10^{-1}M. All of these solutions were made daily, immediately prior to their use. A fine particle suspension of collagen was prepared by a highly standardized technique, which consisted essentially of grinding minced, lyophilized and shredded bovine achilles tendon (General Biochemicals, Chagrin Falls, Ohio, U.S.A.), in a Sorval Omni Mixer (Allied Scientific Co., Ltd., Scarborough, Ontario) and removal of coarse material by centrifugation (see Appendix III).

I. Measurement of Aggregation Curves

In the present study, the slopes of the first and second phases of ADP-induced aggregation curves were measured along with the curve height at the end of the first phase and the total curve height after five minutes aggregation (Fig. 2). The slope of the aggregation curve, the total curve height after five minutes and the duration of the delay phase before aggregation were all measured in studying collagen-induced aggregation (Fig. 3).

J. Statistical Analysis

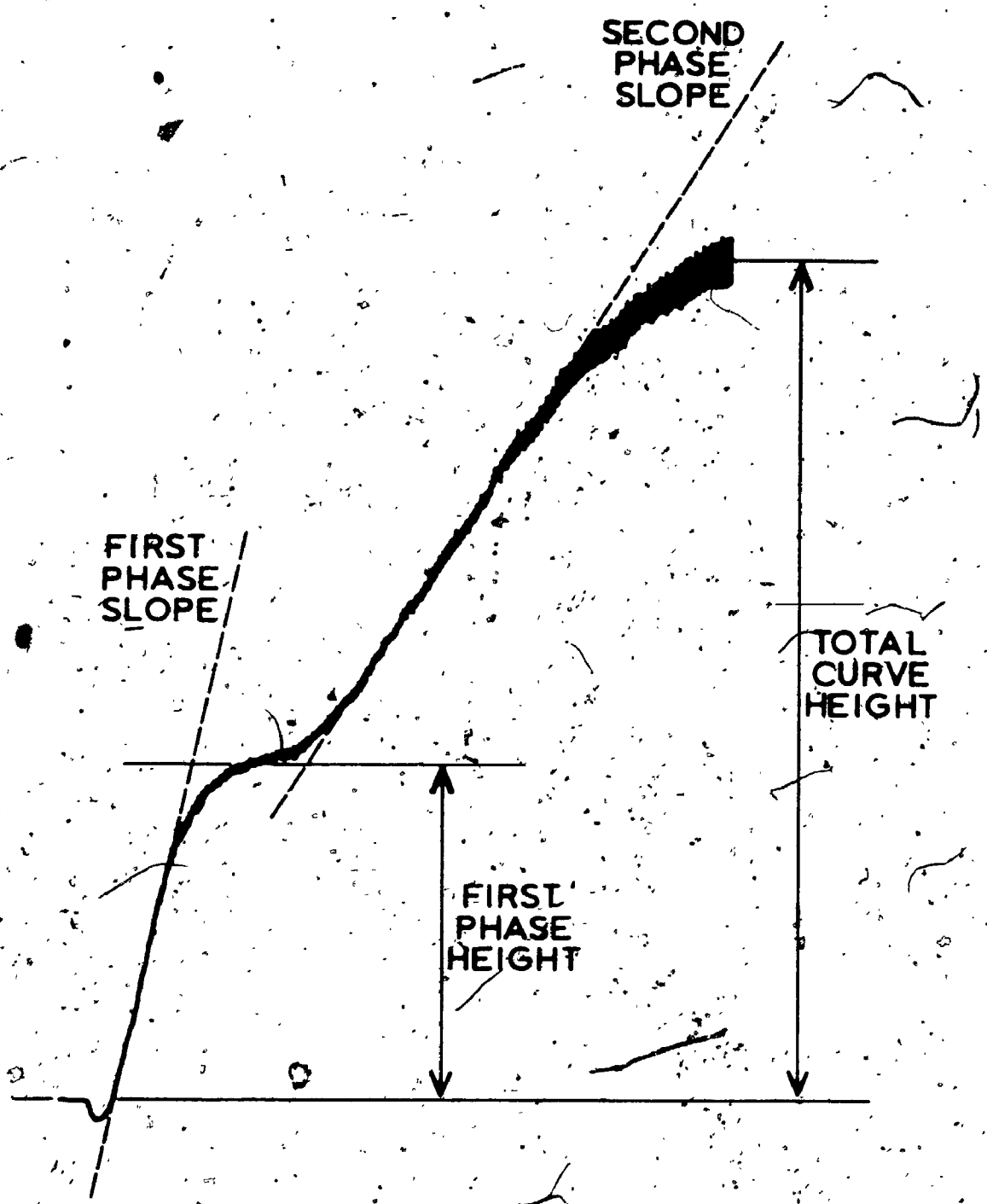
Paired t-tests (two-tail test) were utilized to determine the statistical significance of observations. The aggregation parameters of a treatment curve were compared to those of a neighbouring control sample, aggregated within 10 min. During the experimental procedure, control PRP samples were tested at the beginning of the experiment, at the end of the experiment, and after every second trial sample. Saline controls were performed periodically.

K. Radioactive Label Study

The possible relationship between the degree of inhibition of platelet aggregation and the extent of acylation of components of the platelet was investigated as follows: PRP (1.5 ml.), from five donors (whose PRP demonstrated the normal biphasic aggregation response with ADP) was pipetted into Eppendorf 1.5 ml. micro test tubes (Brinkmann Instruments, Rexdale, Ontario), and maintained at 37° by a Thermolyne Dri-Bath Heater (Fisher Scientific Co.). In cases where the analogues were incubated in the PRP, this was done at 37°.

Figure 2

Method of measurement of ADP-induced platelet aggregation curves


SECOND
PHASE
SLOPE
FIRST
PHASE
SLOPE
TOTAL
CURVE
HEIGHT
FIRST
PHASE
HEIGHT

2 3

OF/DE

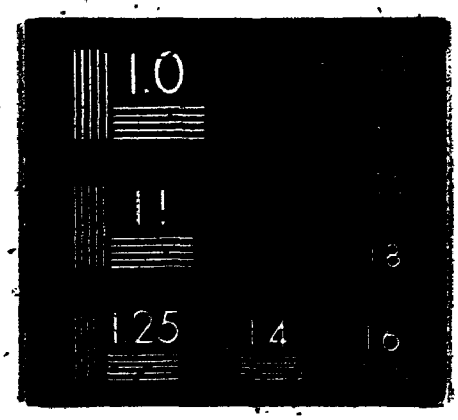
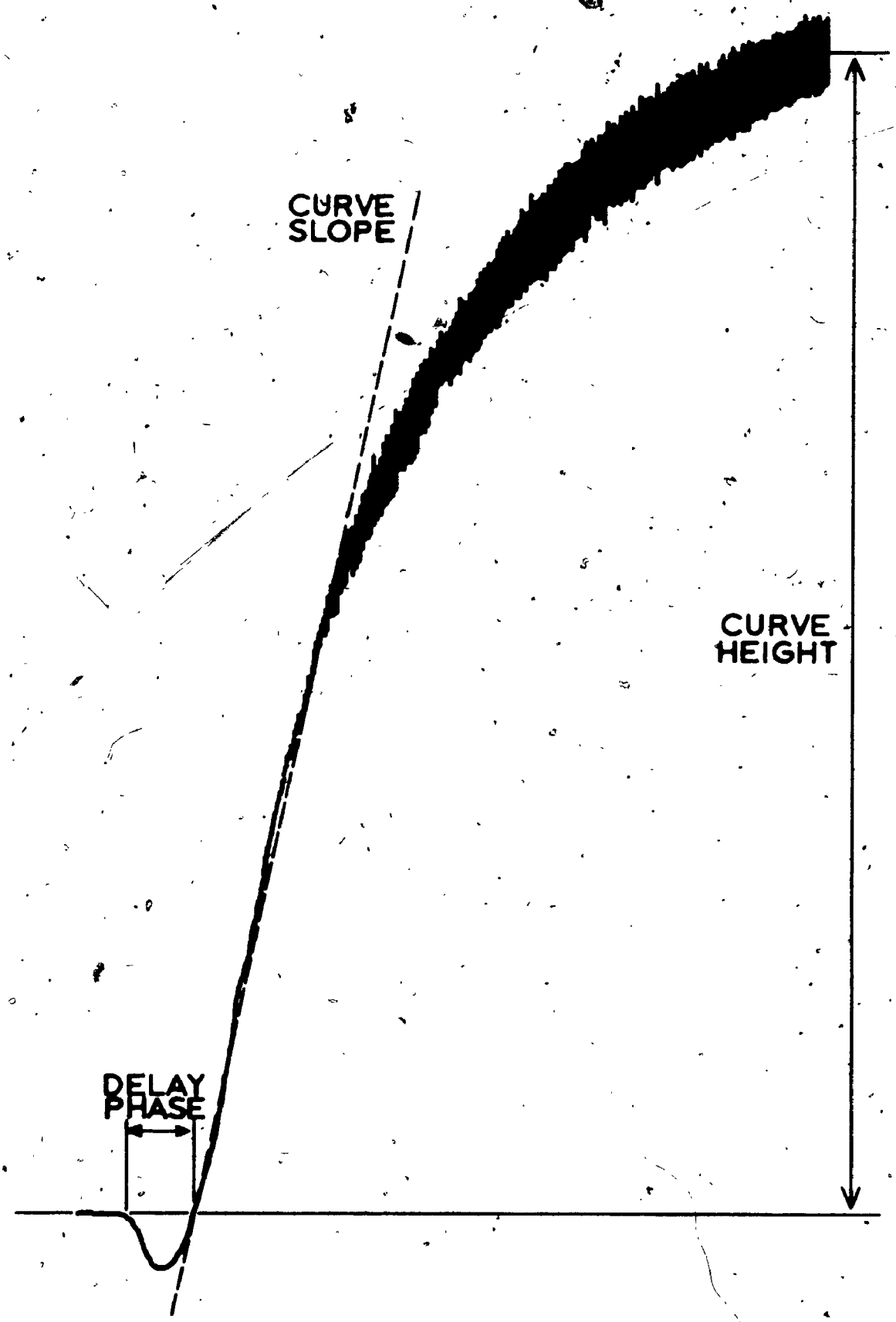


Figure 3

Method of measurement of collagen-induced platelet aggregation curves


CURVE
SLOPE
CURVE
HEIGHT
DELAY
PHASE

^{14}C-labelled acyl salicylate compounds were added to a final concentration of 12 x 10^{-5}M or 24 x 10^{-5}M. These samples were reacted for either minimal time (zero minutes incubation), 5 min. (5 min. incubation) or 10 min. (10 min. incubation). The platelets were isolated prior to determining the level of incorporation of radioactivity, by centrifugation at 15,000 r.p.m. (12,000 x g) for two minutes in an Eppendorf Microcentrifuge (Brinkmann Instruments). The PPP was removed and a 100µl. sample withdrawn with a Pedersen constriction micropipette (H. E. Pedersen, Copenhagen, Denmark) for liquid scintillation counting. The platelets were washed with a modified Tyrode's solution (in which the divalent ions were exchanged for Na^+ and K^+)(see Appendix IV) by addition of 1.5 ml. of the solution to the tube. They were re-suspended with the aid of a stirring rod (Plumper - Calbiochem, Los Angeles, California, U.S.A.), re-centrifuged as above, and the wash phase removed. This process was then repeated. The platelet button was then washed into a glass counting vial (O. H. Johns, Toronto, Ontario) with 5 ml. of XDE scintillation solution. A further 5 ml. of the scintillation fluid was then added to the vial. XDE scintillation solution was prepared according to the following formula:

1,4-dioxane	350 ml.
xylenes	350 ml.
absolute alcohol	210 ml.
naphthalene	80 gm.
PPO	5 gm.
POPOP	0.5 gm.

The 1,4-dioxane (scintanalyzed) and xylenes were obtained from Fisher Scientific Co., the absolute alcohol from Commercial Alcohol Ltd., Scarborough, Ontario, the naphthalene from British Drug Houses, and PPO and POPOP from Amersham/Searle, Oakville, Ontario.

All radioactive samples were assayed by liquid scintillation counting using a Unilux II (Nuclear Chicago Corporation, Des Plaines, Illinois). Counting was continued until a minimum of 10,000 counts above background were obtained. The raw counts were converted to disintegrations per minute (d.p.m.) after estimation of the counting efficiencies of the samples by inclusion of a calculated quantity of a standardized n-hexadecane reference sample (Amersham/Searle). The efficiencies of the PRP system and the PPP system were 86.0% and 88.5% respectively. Although the PPP yielded a slightly cloudy counting sample and the platelet button produced a sample containing solid particulate matter, there was no significant loss of counting efficiency. This was demonstrated by prior experiments in which protein-dissolving NCS scintillation fluid (Nuclear Chicago Corporation) was employed.

To study the effects of inhibition of metabolic processes on the uptake of radioactive label by human platelets, oligomycin (Sigma Chemical Co.) was prepared in 95% alcohol, in a concentration of 2.5×10^{-2}M. It was stored in a desiccator in a freezer. Oligomycin, in a final concentration of 10^{-5}M was incubated at 37° for 15 min. in PRP which had been kept at room temperature for approximately two hours following recovery from whole blood. After this incubation, labelled compounds were added to a final concentration of 24×10^{-5}M for 10 min., following which the platelet button and PPP sample were isolated for

counting as described previously.

A chloroform:methanol (2:1) extraction was performed at 37° for five minutes on labelled platelet buttons to remove that portion of the label associated with platelet lipids. Following the extraction, the button and a portion of the extract were removed for counting of the radioactive label.

To study the effects of cell disruption on the uptake of ^{14}C-acetate by human platelets, 3 ml. of PRP were subjected to sonification (90 watts) for 15 sec. with a Sonifier Cell Disruptor (Model 185 D - Heat Systems - Ultrasonics, Inc., Plainview, New York, U.S.A.). Following this procedure, any cellular material present was isolated by centrifugation in an Eppendorf microcentrifuge at 12,000 x g. for 2 min. This material, as well as a 1.0µl. sample of the supernatant, was collected for scintillation counting.

The effects of *in vivo* ASA administration on the *in vitro* uptake of ^{14}C-acetate from ^{14}C-ASA was determined in healthy human volunteers. Before administration of ASA to these individuals, their platelet function was studied with ADP to assure that their platelets were reacting in the normal biphasic pattern. Radioactive uptake by these platelets was examined with a final concentration of 24 x 10^{-5}M ^{14}C-ASA incubated for 10 min. The subjects were then given 2 ASA tablets (600 mg.) (Life Brand, Carnegie Laboratories, Mississauga, Ontario), and 2 hrs. later, another blood sample was collected. PRP was prepared to determine the effects of the ingested ASA on ADP-induced platelet aggregation, and on the uptake of ^{14}C-acetate from labelled ASA at a final concentration of 24 x 10^{-5}M and incubated for 10 min.

L. Gastric Bleeding Study - Laboratory Tests

In the gastrointestinal bleeding study, a number of laboratory tests were performed to determine if significant abnormalities were present in the hemostatic mechanism. Bleeding time was determined by the Ivy technique (Ivy *et al.*, 1935). The hemoglobin concentration was measured by the cyanmethemoglobin method (Dacie and Lewis, 1963), in a Coulter Hemoglobinometer (Coulter Electronics Co., Mississauga, Ontario). A microhematocrit technique (McGovern *et al.*, 1955) was utilized to determine the hematocrit value. Prothrombin time was measured using a commercial thromboplastin - calcium chloride reagent (Simplastin, Warner - Chilcott Laboratories, Scarborough, Ontario) (Lenahan and Phillips, 1969). Activated Thrombofax (Ortho Diagnostics Corp., Toronto, Ontario) was used in the determination of partial thromboplastin time (Lenahan and Phillips, 1969). The thrombin clotting time was evaluated utilizing topical bovine thrombin (Parke-Davis Laboratories, Detroit, Michigan, U.S.A.) (Owen *et al.*, 1969). The fibrinogen titre was measured, the fibrinogen standard being supplied by Dade (Dade Laboratories Inc., Miami, Florida, U.S.A.) (Clauss, 1957). Euglobulin lysis time was determined using the method described by Buckell (1958). Fibrinogen degradation products were estimated by the method of Pitcher (1971). Platelet factor 3 assays were performed by the technique of Hardisty and Hutton (1965). Platelet aggregation was studied as described previously.

IV RESULTS

A. Normal Responses

The mean, platelet count, before adjustment, of the 160 plasma samples used in the aggregation studies, was found to be 410,000 per $mm^3 \pm 102{,}000$ SEM.

Platelet aggregation curves obtained with ADP and collagen were similar to those described by many other investigators (Constantine, 1966; MacMillan, 1966; Zucker and Peterson, 1968). When a suitable concentration of ADP was used to induce aggregation, the response took place in a biphasic manner (Fig. 4-B). Concentrations of ADP below this level produced a relatively small, reversible aggregation response (Fig. 4-A) which was lacking the second aggregation phase, while addition of concentrations, greater than that producing the biphasic pattern, resulted in a merging of the two phases and a monophasic, irreversible aggregation (Fig. 4-C). A typical platelet reponse to the collagen suspension, shown in Figure 5, consisted of an irreversible, monophasic aggregation following a delay in onset.

One of the peculiar features of the platelet-rich plasma system is that the sensitivity to aggregating agents of PRP appears to be reduced when it is first collected. One must allow PRP to stand for approximately 30 min. to avoid changes in sensitivity during experimental procedures.

Figure 4

Effect of increasing concentrations of ADP on human platelet aggregation to produce

A. monophasic reversible aggregation

B. biphasic irreversible aggregation

C. monophasic irreversible aggregation

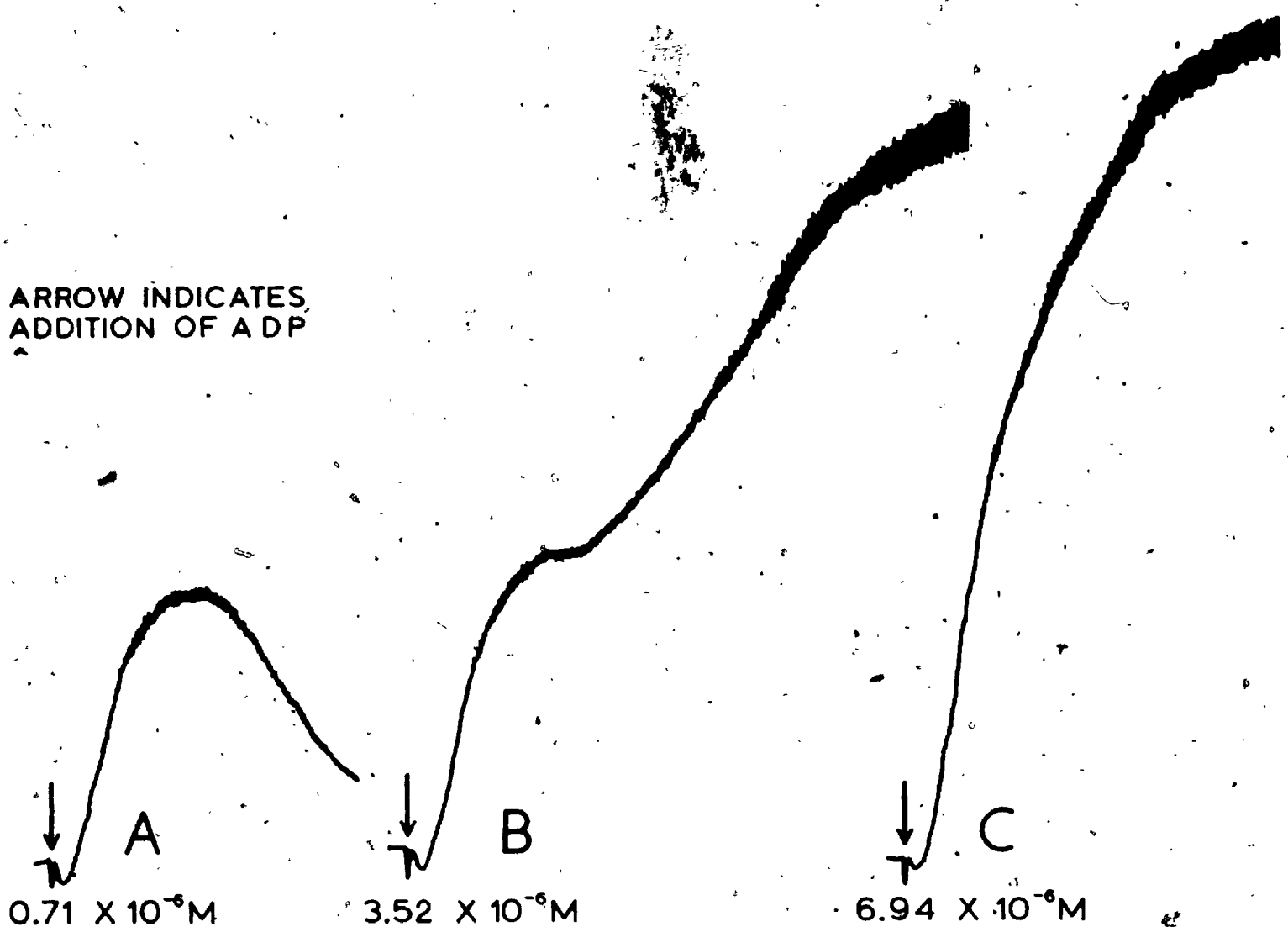

ARROW INDICATES ADDITION OF ADP
A
B
C
0.71 X 10⁻⁶M
3.52 X 10⁻⁶M
6.94 X 10⁻⁶M
FINAL ADP CONCENTRATION

Figure 5

Collagen-induced human platelet aggregation response showing a delay phase followed by a monophasic irreversible aggregation

ARROW INDICATES
0.10 ml. COLLAGEN SUSPENSION

Figure 6 illustrates these changes as they occur in response to ADP. Curve A was produced by PRP which was aggregated immediately after collecting it from whole blood. Ten minutes after withdrawal of the PRP, the aggregating activity had increased (Fig. 6-B), and by 20 min., when the plasma activity had stabilized, the sensitivity had increased to the point at which the two aggregation phases had merged (Fig. 6-C). It can be seen from Figure 6 that commencement of an experiment to study aggregating agent sensitivity or inhibitory action, prior to the time when the PRP has stabilized, could lead to misinterpretation of results.

The critical concentration of ADP necessary to induce a biphasic aggregation response was found to vary from one individual and from one PRP sample to another; the mean value being 4.4×10^{-6}M (±2.15 SEM) for 108 PRP samples from 64 different individuals. Figure 7 shows the distribution of these values.

B. Effects of Salicylate Analogues on Platelet Aggregation

To study the inhibitory effects of ASA on ADP-induced aggregation, a final concentration of ASA which would just inhibit the second-phase response was employed. Because of the high degree of activity shown by ASA, an activity which was nearly all-or-none in nature, it was not possible to establish dose-response relationships with ASA on ADP aggregation. For the ASA-collagen aggregation study, and to demonstrate the effects of the remaining analogues on both ADP- and collagen-induced aggregation, fixed final concentrations of the salicylate compound were utilized. These were 55×10^{-5}M, 35×10^{-5}M, 24×10^{-5}M, 12×10^{-5}M, and 6×10^{-5}M in the collagen study and 69×10^{-5}M, 43×10^{-5}M, 29×10^{-5}M, 15×10^{-5}M and 7.5×10^{-5}M in the ADP study. The

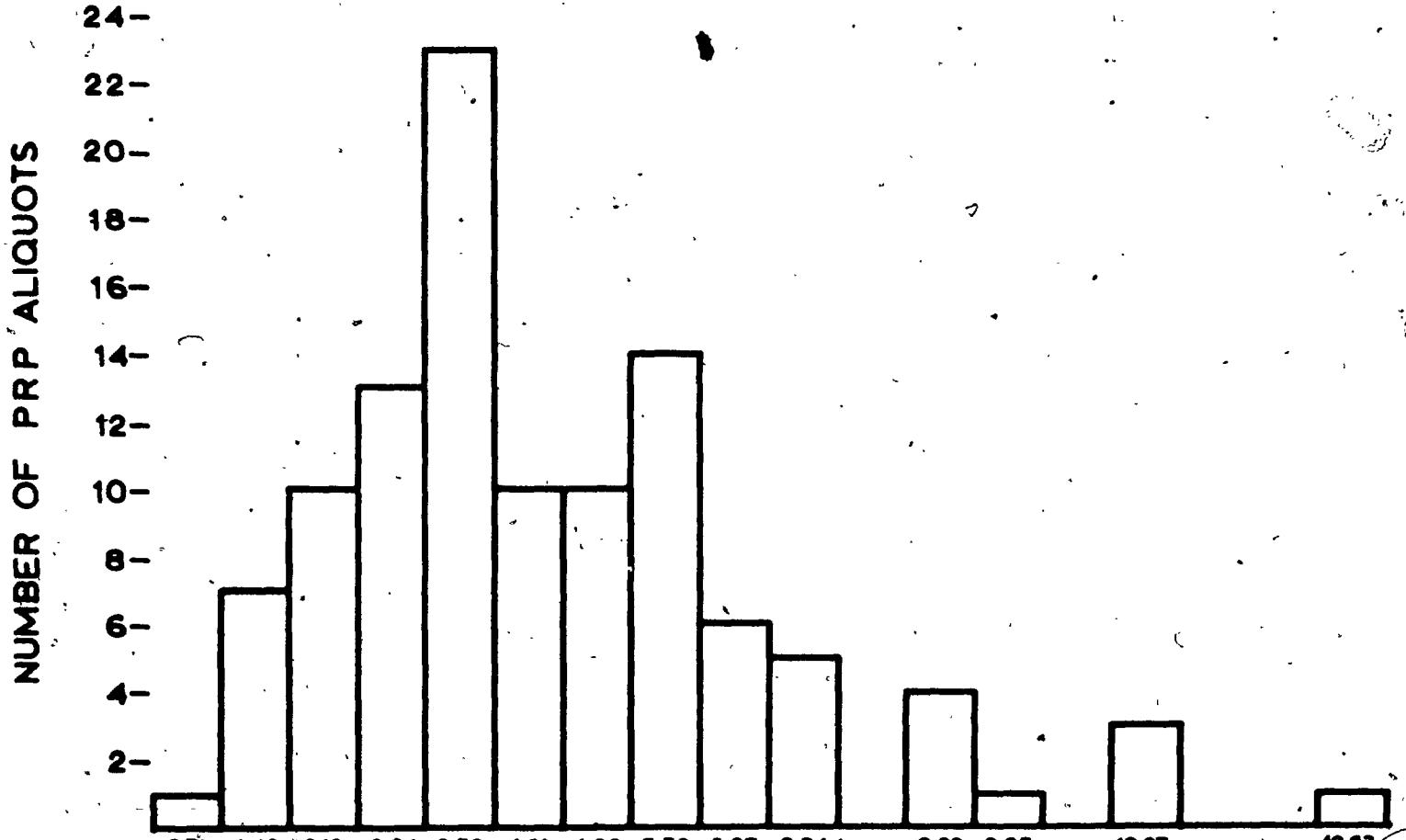
Figure 6

The increasing sensitivity to ADP of human PRP during its stabilization, showing reaction of PRP aggregated immediately after recovery from whole blood (Curve A), 10 min. after recovery (Curve B) and 20 min. after recovery (Curve C)

C
B
A

Figure 7

Distribution of final ADP concentrations just sufficient to induce biphasic aggregation


NUMBER OF PRP ALIQUOTS
24
22
20
18
16
14
12
10
8
6
4
2
0.71
1.42
2.12
2.84
3.52
4.21
4.90
5.59
6.27
6.94
8.29
8.95
10.27
12.23
FINAL ADP CONCENTRATION X 10⁻⁶ M

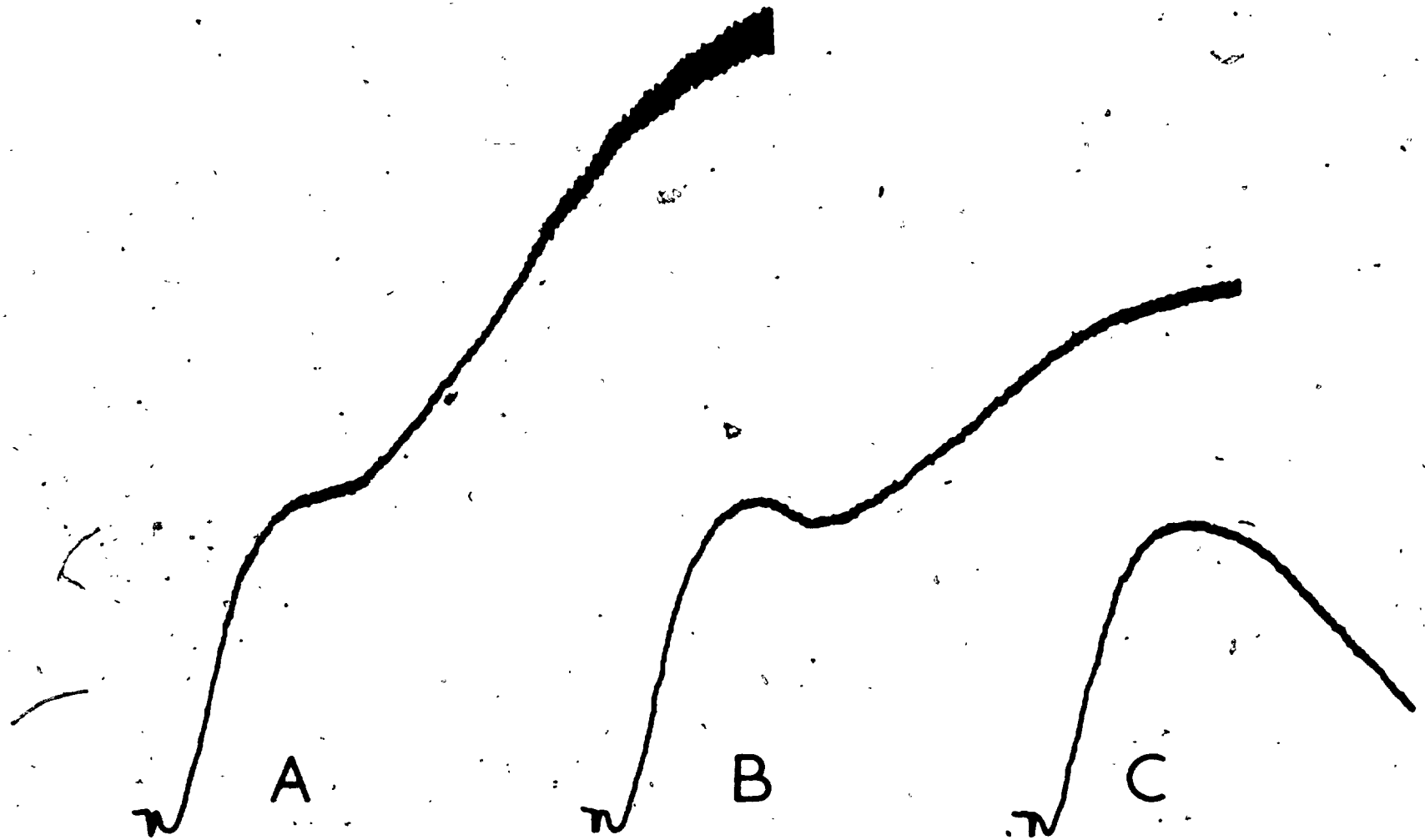
inhibitory effects of higher final concentrations (10^{-2}M, 6.3 x 10^{-3}M, 4.3 x 10^{-3}M, 2.2 x 10^{-3}M, 1.1 x 10^{-3}M) of sodium salicylate and PAS on collagen aggregation were also determined.

When the salicylate analogues were added to PRP, immediately prior to the addition of ADP, only ASA exhibited considerable inhibitory activity. The mean, threshold, concentration, i.e., the lowest concentration which completely inhibited the second phase of ADP-induced aggregation, was found to be 5.2 x 10^{-5}M (± 1.07 SEM). Although there was some individual variation among PRP samples, in general, ASA concentrations above 10^{-4}M tended to totally inhibit the second wave of ADP aggregation. Figure 8 shows the effects of increasing concentrations of ASA over a narrow range of final concentrations on ADP-induced aggregation; the lower concentration reducing the second phase and higher ones inhibiting it completely. ASA had only minimal effects on the first phase of ADP aggregation. It produced a slight inhibition of first phase slope, but had no effects on the first phase height. At concentrations up to 69 x 10^{-5}M, neither sodium salicylate nor PAS demonstrated any statistically significant inhibitory activity on ADP aggregation. This was also true of the monoacetate analogues, 3-acetoxybenzoic acid and 4-acetoxybenzoic acid (see Appendix V).

Of the diacetoxy compounds, only 2,3-diacetoxybenzoic acid demonstrated much inhibitory activity. At a final concentration of 69 x 10^{-5}M, it reduced the second phase slope and total curve height of ADP-induced aggregation responses by 71.8% ± 20.62 SEM ($P<0.025$) and 31.6% ± 10.09 SEM ($P<0.02$) respectively. In this aggregation study, in which the salicylate analogue was not incubated in the PRP, the 2,6-diacetoxy

Figure 8

Effect of increasing concentrations of ASA on ADP-induced human platelet aggregation, showing a control curve (A), a decrease in second phase aggregation at a final ASA concentration of 3.10×10^{-5}M (B) and complete inhibition of second phase aggregation at 6.10×10^{-5}M (C)


A
B
C
CONTROL CURVE
3.10 X 10⁻⁵M
6.10 X 10⁻⁵M
FINAL ASA CONCENTRATION

derivative had just the opposite effect. At a concentration of 69 x 10^{-5}M, it increased, or potentiated significantly, the second phase slope (63.1% ± 22.66 SEM, P<0.01) and the total curve height (29.0% ± 9.86 SEM, P<0.02) of the aggregation response. The 2,4-diacetoxy analogue also had slight potentiating activity (see Appendix V).

The two propoxy analogues, 2-propoxybenzoic acid (PSA) and 3-propoxybenzoic acid were both found to have significant effects on *in vitro* ADP-induced platelet aggregation. The 2-propoxy compound displayed inhibitory activity and was second in potency only to ASA. At a final concentration of 69 x 10^{-5}M, the second phase was completely abolished in all cases, and the total curve height was reduced by 35.2% ± 9.54 SEM (P<0.01) (Table 2). In contrast, the 3-propoxy derivative potentiated the second phase of the aggregation response, increasing the slope by 86.6% ± 22.05 SEM (P<0.001), and the total curve height by 33.7% ± 4.08 SEM (P<0.001), at the same concentration (see Appendix V). Added to PRP in a final concentration of 69 x 10^{-5}M, neither the 3-propoxy analogue nor the 2,6-diacetoxy derivative alone, induced platelet aggregation.

In the study of the inhibitory effects of salicylate analogues on collagen aggregation, only ASA demonstrated much activity without incubation of the drug prior to addition of the aggregating agent. The effects of increasing ASA concentrations are shown in Table 3. At these concentrations, and without incubation, ASA did not prolong significantly the collagen curve delay phase. The 2,3-diacetoxybenzoic acid derivation at 55 x 10^{-5}M reduced the collagen curve slope by 34.4% ± 8.79 SEM (P<0.01) and decreased the curve height by 25.1% ± 9.04 SEM (P<0.025)

Table 2. Percent Change of ADP induced Aggregation Curve by 2-Propoxybenzoic Acid (% ± SEM)

Final 2-Propoxybenzoic Acid Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5 x 10^{-5}M	↑1.8 ± 4.65 (NS)	↑8.2 ± 3.12 ($p<0.01$)	↑13.1 ± 14.96 (NS)	↑4.1 ± 4.01 (NS)
15 x 10^{-5}M	↑1.3 ± 5.92 (NS)	↑3.1 ± 5.79 (NS)	↑7.8 ± 18.49 (NS)	↓4.0 ± 7.80 (NS)
29 x 10^{-5}M	↑10.7 ± 7.29 (NS)	↑7.5 ± 7.61 (NS)	↑30.2 ± 18.00 (NS)	↑11.3 ± 9.31 (NS)
43 x 10^{-5}M	↑11.7 ± 6.51 (NS)	↑16.6 ± 7.29 (NS)	↑74.4 ± 16.98 ($p<0.05$)	↑16.3 ± 8.38 (NS)
69 x 10^{-5}M	↑ 2.1 ± 9.47 (NS)	↑5.0 ± 11.72 (NS)	↑100.0 ± 0.00 ($p<0.001$)	↑35.2 ± 9.54 ($p<0.01$)

Table 3. Percent Change of Collagen-induced Aggregation Curve by ASA (% ± SEM)

Final ASA Concentration	Curve Slope	Curve Height	Delay Phase
6 x 10^{-5}M	+8.7 ± 6.37 (NS)	+9.9 ± 5.26 (NS)	+0.6 ± 0.23 (NS)
12 x 10^{-5}M	+29.6 ± 5.99 ($p<0.001$)	+15.8 ± 6.18 ($p<0.05$)	+5.6 ± 7.77 (NS)
24 x 10^{-5}M	+44.9 ± 9.37 ($p<0.001$)	+35.0 ± 9.72 ($p<0.001$)	+0.0 ± 8.77 (NS)
35 x 10^{-5}M	+56.7 ± 7.28 ($p<0.001$)	+43.2 ± 8.12 ($p<0.001$)	+12.1 ± 9.44 (NS)
55 x 10^{-5}M	+66.2 ± 5.10 ($p<0.001$)	+49.1 ± 7.32 ($p<0.001$)	+12.3 ± 13.18 (NS)

(Table 4). At high final concentrations (10^{-2}M), both sodium salicylate and PAS, were found to inhibit collagen-induced aggregation. Sodium salicylate reduced the collagen curve slope by 82.2% ± 3.88 SEM ($P<0.001$), decreased the curve height by 53.8% ± 8.14 SEM ($P<0.001$), and prolonged the curve delay phase by 127.8% ± 17.75 SEM ($P<0.001$) (Table 5). PAS reduced the collagen curve slope by 48.5% ± 6.26 SEM ($P<0.001$), decreased the curve height by 24.9% ± 5.07 SEM ($P<0.001$), and produced a slight, but non-significant prolongation of the delay phase (Table 6). At concentrations similar to those utilized with ASA, with no incubation, sodium salicylate, PAS, 3-acetoxybenzoic acid, 4-acetoxybenzoic acid, 2,4-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid, 2-propoxybenzoic acid and 3-propoxybenzoic acid demonstrated no significant activity on collagen-induced platelet aggregation (see Appendix VI).

Figures 9, 10 and 11 show the effects of no incubation, 5 min. incubation, and 10 min. incubation, on the inhibitory activity of ASA, 2,3-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid and 2-propoxybenzoic acid (PSA), at various concentrations, on collagen-induced aggregation. Both the 2,6-diacetoxy analogue and the 2-propoxy compound required at least 5 min. incubation time before they exhibited any significant inhibitory activity (Fig. 10). ASA and the 2,3-diacetoxy compound were found to have significant activity with no incubation. After 10 min. incubation, ASA was still the most potent inhibitory analogue although at a concentration of 55 x 10^{-5}M, the 2-propoxy compound demonstrated similar activity (Fig. 11). At the highest concentration studied, the 2,6-diacetoxy analogue was about one-half as potent, after 10 min., as ASA, and the 2,3-diacetoxy derivative, which showed significant

Table 4. Percent Change of Collagen-induced Aggregation Curve by 2,3-Diacetoxybenzoic Acid (% ± SEM)

Final 2,3-Diacetoxybenzoic Acid Concentration	Curve Slope	Curve Height	Delay Phase
6×10^{-5}M	+4.3 ± 7.26 (NS)	+6.1 ± 3.45 (NS)	+3.6 ± 6.89 (NS)
12×10^{-5}M	+11.4 ± 7.73 (NS)	+5.2 ± 5.98 (NS)	+0.6 ± 9.23 (NS)
24×10^{-5}M	+14.7 ± 10.64 (NS)	+10.2 ± 6.78 (NS)	+1.6 ± 7.66 (NS)
35×10^{-5}M	+26.4 ± 9.07 ($p<0.01$)	+14.6 ± 6.73 (NS)	+5.7 ± 11.61 (NS)
55×10^{-5}M	+34.4 ± 8.79 ($p<0.01$)	+25.1 ± 9.04 ($p<0.025$)	+19.8 ± 16.15 (NS)

Table 5. Percent Change of Collagen-induced Aggregation Curve by Sodium Salicylate (% ± SEM)

Final Sodium Salicylate Concentration	Curve Slope	Curve Height	Delay Phase
1.1×10^{-3}M	+7.1 ± 4.99 (NS)	+3.4 ± 2.84 (NS)	+8.7 ± 5.72 (NS)
2.2×10^{-3}M	+18.8 ± 4.79 ($p<0.005$)	+7.4 ± 3.14 ($p<0.05$)	+18.6 ± 11.21 (NS)
4.3×10^{-3}M	+47.5 ± 7.66 ($p<0.001$)	+23.8 ± 6.82 ($p<0.01$)	+33.8 ± 13.54 ($p<0.05$)
6.3×10^{-3}M	+65.6 ± 5.82 ($p<0.001$)	+32.3 ± 7.46 ($p<0.005$)	+69.4 ± 23.84 ($p<0.02$)
10.0×10^{-3}M	+82.2 ± 3.88 ($p<0.001$)	+53.8 ± 8.14 ($p<0.001$)	+127.8 ± 17.75 ($p<0.001$)

Table 6. Percent Change of Collagen-induced Aggregation Curve by PAS (% ± SEM)

Final PAS Concentration	Curve Slope	Curve Height	Delay Phase
1.1×10^{-3}M	+6.3 ± 5.55 (NS)	+1.4 ± 1.91 (NS)	+3.1 ± 8.25 (NS)
2.2×10^{-3}M	+5.3 ± 6.76 (NS)	+1.6 ± 2.22 (NS)	+2.5 ± 8.34 (NS)
4.3×10^{-3}M	+16.9 ± 5.21 ($p<0.01$)	+4.0 ± 2.56 (NS)	+4.8 ± 9.89 (NS)
6.3×10^{-3}M	+27.6 ± 5.97 ($p<0.005$)	+10.5 ± 3.18 ($p<0.02$)	+10.7 ± 14.95 (NS)
10.0×10^{-3}M	+48.5 ± 6.26 ($p<0.001$)	+24.9 ± 5.07 ($p<0.001$)	+56.9 ± 26.18 (NS)

Figure 9

Percent inhibition of collagen-induced aggregation by ASA, 2,3-diacetoxybenzoic acid (2,3 DA), 2,6-diacetoxybenzoic acid (2,6 DA) and 2-propoxybenzoic acid (PSA) not incubated with PRP

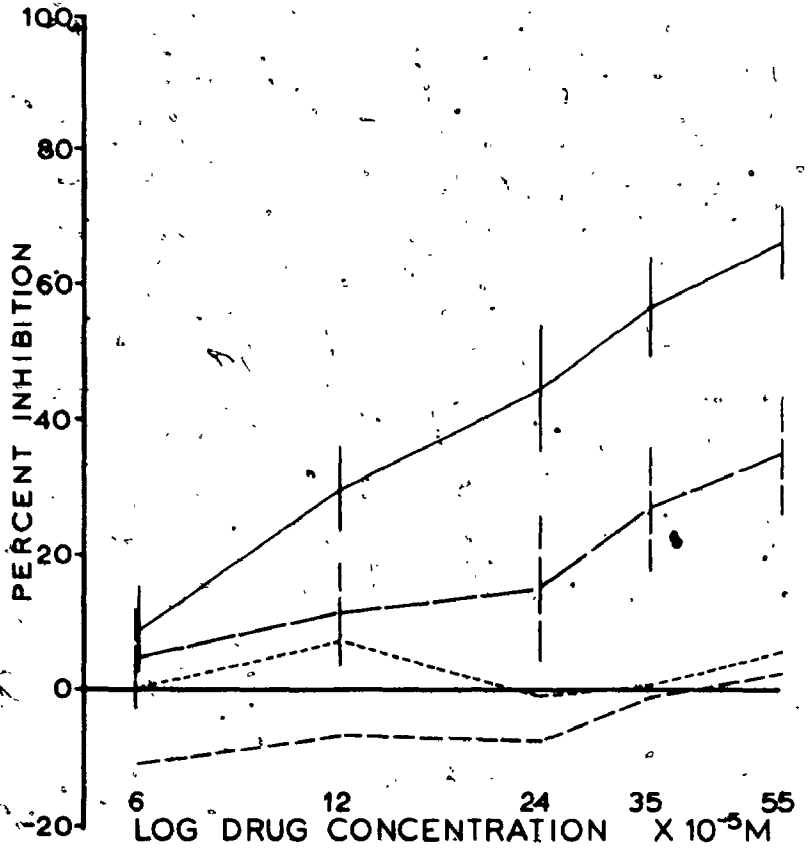
p values for curve slopes (A) and heights (B)*

Final Analogue Concentration ($x10^{-5}M$)

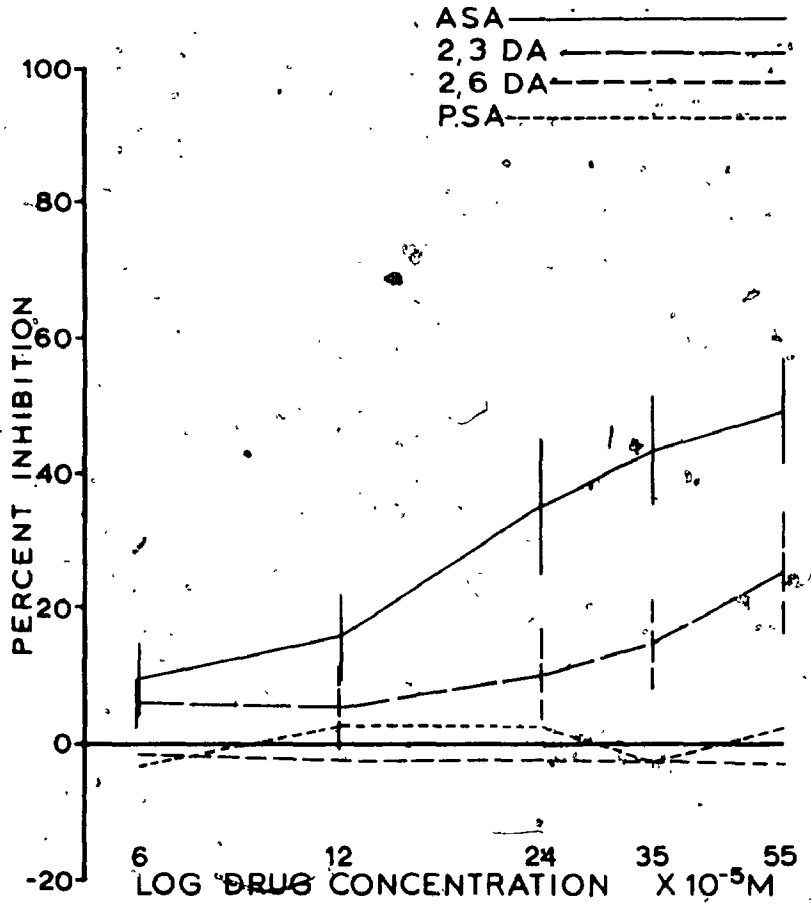
	6		12		24		35		55	
Drug	A	B	A	B	A	B	A	B	A	B
ASA	NS	NS	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2,3 DA	NS	NS	NS	NS	NS	NS	<0.01	NS	<0.01	<0.025
2,6 DA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PSA	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS

* analogue treated response vs controls

NS not significant



CURVE SLOPE



CURVE HEIGHT

Figure 10

Percent inhibition of collagen-induced aggregation by ASA, 2,3-diacetoxybenzoic acid (2,3 DA), 2,6-diacetoxybenzoic acid (2,6 DA), and 2-propoxybenzoic acid (PSA) incubated five minutes with PRP

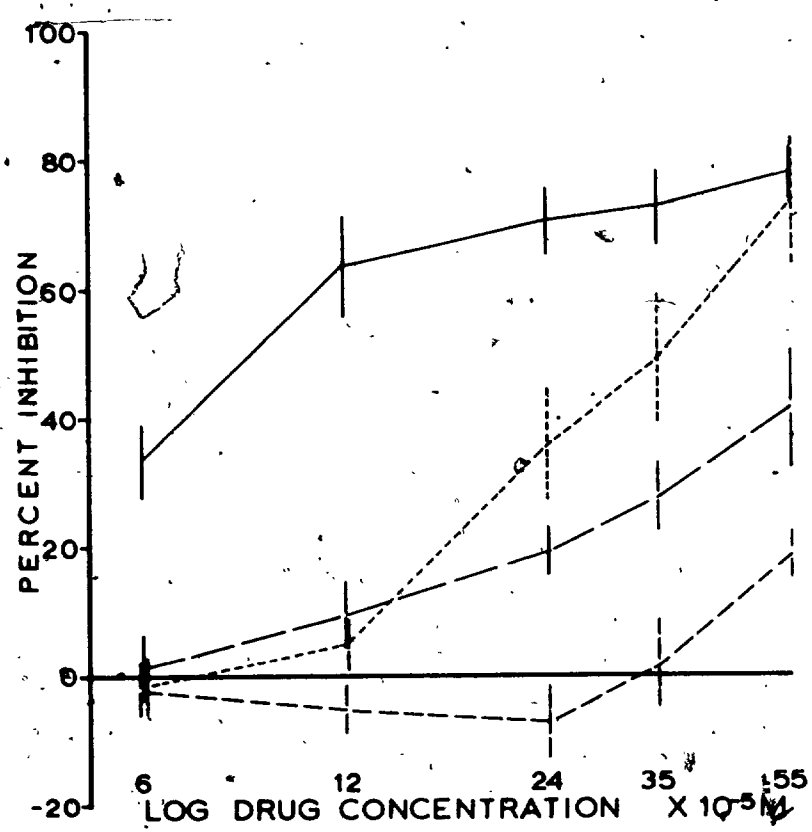
p values for curve slopes (A) and heights (B)*

Final Analogue Concentration ($x10^{-5}M$)

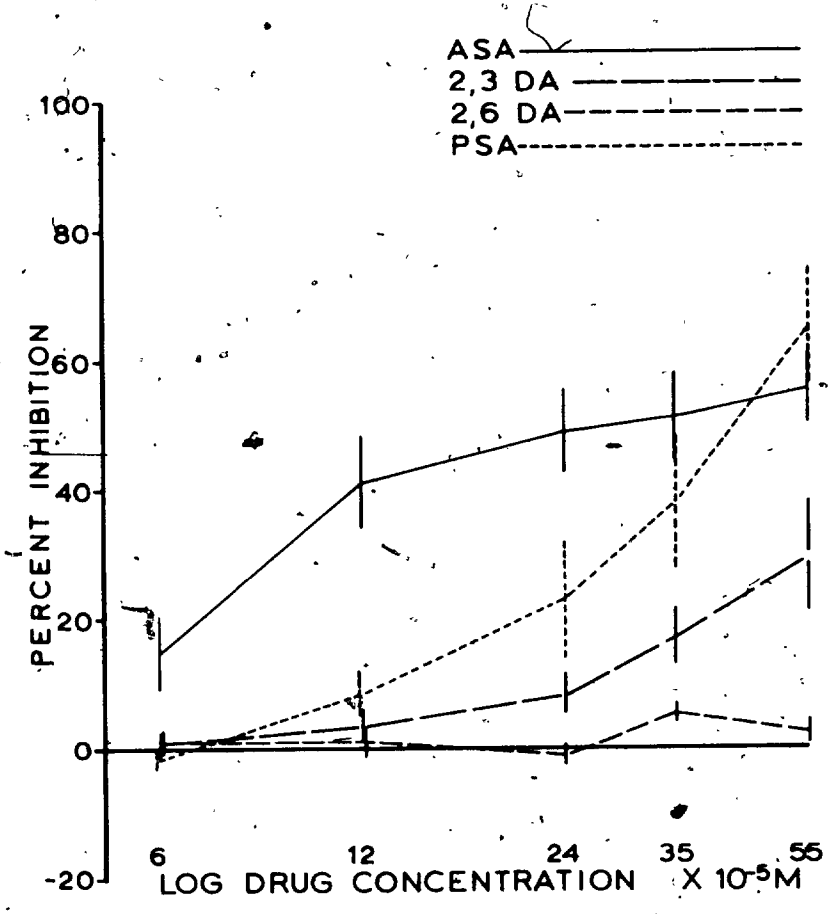
	6		12		24		35		55	
Drug	A	B	A	B	A	B	A	B	A	B
ASA	<0.001	<0.025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2,3 DA	NS	NS	NS	NS	<0.001	<0.02	<0.005	<0.001	<0.005	<0.005
2,6 DA	NS	NS	NS	NS	NS	NS	NS	<0.001	<0.005	NS
PSA	NS	NS	NS	<0.05	<0.005	<0.05	<0.001	<0.005	<0.001	<0.001

* analogue treated response vs control

NS not significant



CURVE SLOPE



CURVE HEIGHT

Figure 11

Percent inhibition of collagen-induced aggregation by ASA, 2,3-diacetoxybenzoic acid (2,3 DA), 2,6-diacetoxybenzoic acid (2,6 DA), and 2-propoxybenzoic acid (PSA) incubated ten minutes with PRP

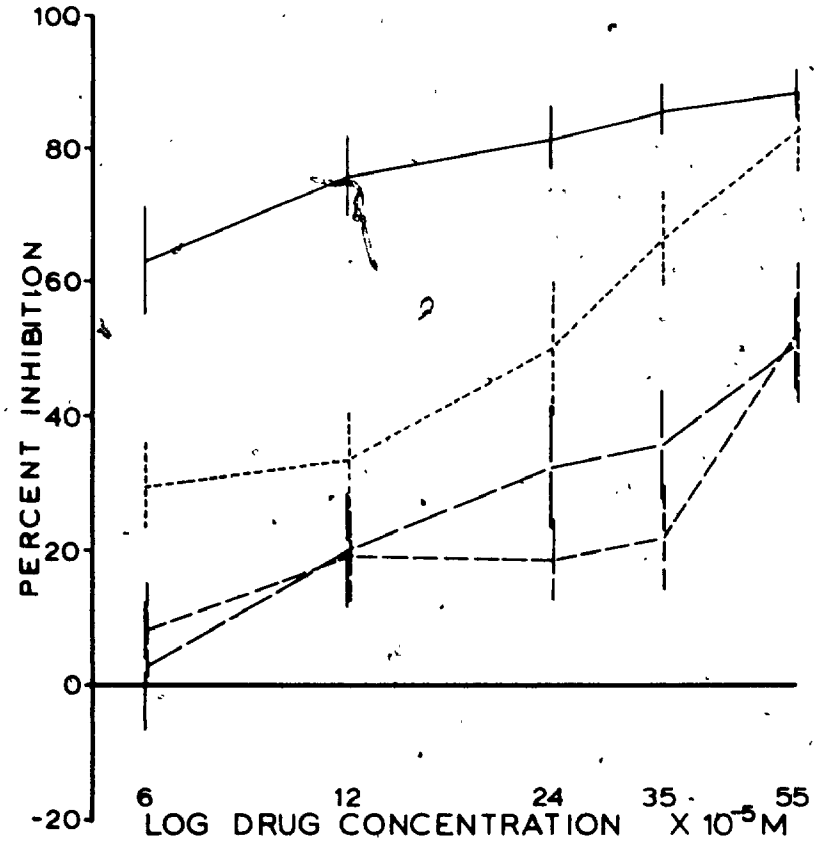
p values for curve slopes (A) and heights (B)*

Final Analogue Concentration ($\times 10^{-5}$M)

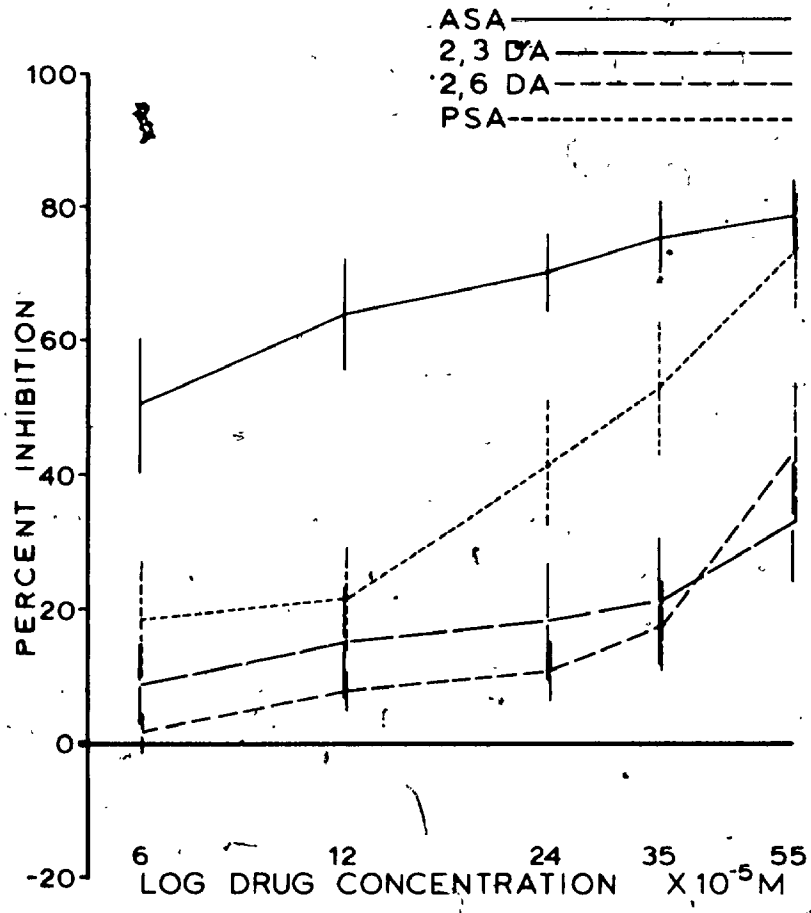
	6		12		24		35		55	
Drug	A	B	A	B	A	B	A	B	A	B
ASA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2,3 DA	NS	NS	<0.05	NS	<0.01	NS	<0.001	<0.05	<0.001	<0.005
2,6 DA	NS	NS	<0.01	<0.02	<0.02	<0.02	<0.02	<0.02	<0.005	<0.001
PSA	<0.001	<0.05	<0.001	<0.01	<0.005	<0.005	<0.001	<0.001	<0.001	<0.001

* analogue treated response vs control

NS not significant



CURVE SLOPE



CURVE HEIGHT

inhibitory effects without incubation, was the least potent of the four compounds, after 10 min.

The relative potencies of the inhibitory compounds are presented in Table 7 which lists the analogues according to the minimum concentration completely inhibiting the second phase of ADP-induced aggregation. Table 8 describes the relative potencies of the most potent inhibitory compounds incubated for 10 min., on collagen aggregation curve slope and height.

C. Uptake by PRP Components of Radioactive Label from ^{14}C-ASA and its Analogues

When the ^{14}C-labelled compounds, ASA, 2,3-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid and 2-propoxybenzoic acid (PSA) were added to, or incubated with PRP, and the platelets isolated by the technique described in the Methods section, radioactivity was found in both the platelet button and the PPP supernatant. The majority of the radioactive label remained in the latter (Table 9). Acetate or propionate incorporation into isolated platelet buttons was found to be dependent on the concentration of the labelled compound, and the duration of its incubation in PRP (Table 10). With all four ^{14}C-labelled compounds, the incorporation of radioactivity into platelets increased significantly over the ten minute incubation period (Table 11). The rate of acylation was highest with ASA, reaching an incorporation of 0.07% ± 0.007 SEM of added activity with no incubation and 0.23% ± 0.024 SEM following 10 min. incubation. Lower incorporations were obtained from the other compounds: Similar degrees of platelet labelling were produced by the 2,6-diacetoxy derivative (0.11% ± 0.009 SEM with 10 min. incubation), and the

Table 7. Minimum Final Concentration of Salicylate Analogues Completely Inhibiting ADP Second Phase Aggregation

Compound	Analogue Concentration
ASA	1×10^{-5}M
2-propoxybenzoic acid	15×10^{-5}M
2,3-diacetoxybenzoic acid	45×10^{-5}M
sodium salicylate	69×10^{-5}M
PAS	100×10^{-5}M

Table 8. Final Concentration of Inhibitor Incubated for 10 min., Giving Approximately 50% Reduction of Collagen Curve

Compound	Curve Slope	Curve Height
ASA	3×10^{-5}M	6×10^{-5}M
2-propoxybenzoic acid	24×10^{-5}M	35×10^{-5}M
2,6-diacetoxybenzoic acid	50×10^{-5}M	55×10^{-5}M
2,3-diacetoxybenzoic acid	55×10^{-5}M	100×10^{-5}M

Table 9. Percent of radioactive label remaining in PPP supernatant following exposure of PRP to the labelled salicylate compounds

Final Drug Conc. ($\times 10^{-5}$M)	Incubation Time (min.)	% Added label remaining in PPP			
		^{14}C-ASA	^{14}C-2,3-DA	^{14}C-2,6-DA	^{14}C-PSA
12	0	99.93 ± 0.007	99.96 ± 0.005	99.97 ± 0.003	99.97 ± 0.007
24	0	99.93 ± 0.007	99.97 ± 0.001	99.97 ± 0.004	99,95 ± 0.007
12	5	99.87 ± 0.014	99.93 ± 0.015	99.92 ± 0.009	99.96 ± 0.005
24	5	99.87 ± 0.017	99.94 ± 0.005	99.91 ± 0.010	99.95 ± 0.009
12	10	99.80 ± 0.036	99.89 ± 0.015	99.87 ± 0.013	99.93 ± 0.017
24	10	99.77 ± 0.024	99.89 ± 0.014	99.89 ± 0.009	99.92 ± 0.014

^{14}C-ASA = 1-^{14}C-acetylsalicylic acid

^{14}C-2,3-DA = 2,3-di(1-^{14}C) acetoxybenzoic acid

^{14}C-2,6-DA = 2,6-di(1-^{14}C) acetoxybenzoic acid

^{14}C-PSA = 2-(1-^{14}C) propoxybenzoic acid

Table 10. Total uptake of radioactive label by platelets following exposure of PRP to ^{14}C-ASA, ^{14}C-2,3-diacetoxybenzoic acid, ^{14}C-2,6-diacetoxybenzoic acid or ^{14}C-2-propoxybenzoic acid as determined by ^{14}C disintegrations per minute (dpm)

Final Drug Conc. (x10^{-5}M)	Incubation Time (min.)	Uptake of Radioactive label by Platelets mean dpm ± SEM			
		^{14}C-ASA	^{14}C-2,3-DA	^{14}C-2,6-DA	^{14}C-PSA
12	0	14.0 ± 1.69	10.3 ± 1.18	6.6 ± 0.71	6.6 ± 1.76
24	0	29.5 ± 2.84	16.7 ± 1.68	14.6 ± 1.80	21.7 ± 3.44
12	5	27.2 ± 2.78	18.3 ± 3.54	18.8 ± 2.33	9.3 ± 1.58
24	5	52.6 ± 7.12	31.4 ± 2.98	42.6 ± 4.75	20.3 ± 3.06
12	10	41.3 ± 7.72	29.8 ± 4.31	30.0 ± 2.96	15.6 ± 3.86
24	10	92.2 ± 9.14	57.1 ±10.45	51.2 ± 3.69	35.5 ± 3.52

N.B. dpm calculated in terms of ^{14}C-ASA basic activity

Table 11. Percent of radioactive label in platelet button following exposure of PRP to the labelled salicylate analogues

Final Drug Conc. ($\times 10^{-5}$M)	Incubation Time (min.)	% Uptake of radioactive label by platelets (mean ± SEM)			
		^{14}C-ASA	^{14}C-2,3-DA	^{14}C-2,6-DA	^{14}C-PSA
12	0	0.07 ± 0.007	0.04 ± 0.005	0.03 ± 0.003	0.03 ± 0.007
24	0	0.07 ± 0.007	0.03 ± 0.001	0.03 ± 0.004	0.05 ± 0.007
12	5	0.13 ± 0.014	0.07 ± 0.015	0.08 ± 0.009	0.04 ± 0.005
24	5	0.13 ± 0.017	0.06 ± 0.005	0.09 ± 0.010	0.05 ± 0.009
12	10	0.20 ± 0.036	0.11 ± 0.015	0.13 ± 0.013	0.07 ± 0.017
24	10	0.23 ± 0.024	0.11 ± 0.014	0.11 ± 0.009	0.08 ± 0.014

^{14}C-ASA = 1-^{14}C-acetylsalicylic acid
^{14}C-2,3-DA = 2,3-di(1-^{14}C) acetoxybenzoic acid
^{14}C-2,6-DA = 2,6-di(1-^{14}C) acetoxybenzoic acid
^{14}C-PSA = 2-(1-^{14}C) propoxybenzoic acid

2,3-diacetoxy isomer (0.11% ± 0.014 SEM with 10 min. incubation) and least incorporation occurred with the 2-propoxy compound (0.08% ± 0.014 SEM with 10 min. incubation). Figure 12 shows the effects of incubation time on the inhibition of aggregation and the uptake of radioactive label from ASA, 2,3-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid, and 2-propoxybenzoic acid and their radioactive analogues incubated with PRP at a concentration of 24 x 10^{-5}M for 10 min.

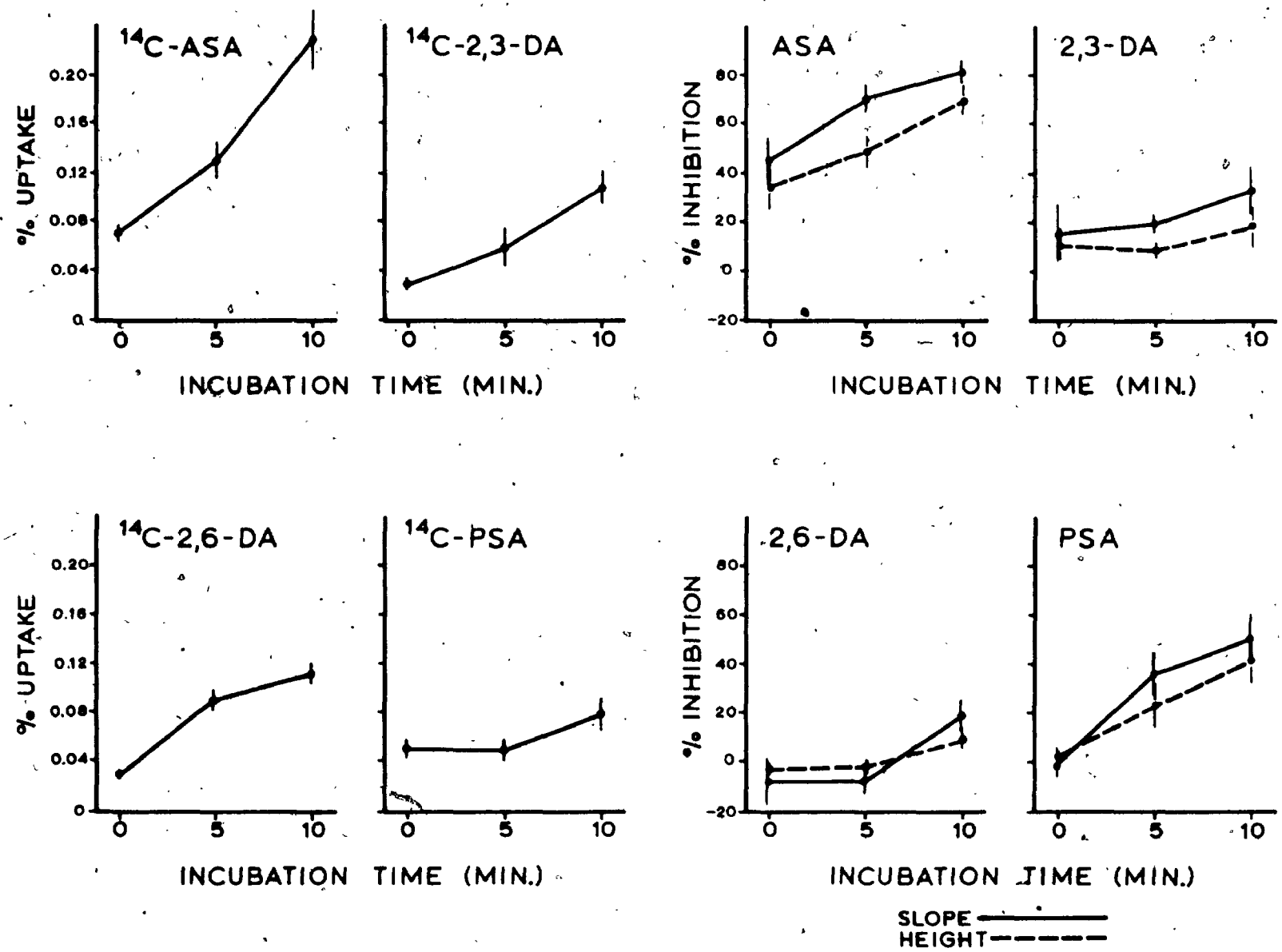
Oligomycin significantly inhibited, to varying degrees, the platelet uptake of labelled acetate from the three acetate-labelled compounds studied at a final concentration of 24 x 10^{-5}M, and incubated in PRP for 10 min. Incorporation was decreased by 50.2% ± 7.85 SEM ($P<0.05$) with ASA, 59.2% ± 3.97 SEM ($P<0.01$) with 2,3-diacetoxybenzoic acid, and 33.9% ± 10.47 SEM ($P<0.05$) with 2,6-diacetoxybenzoic acid. Uptake from 2-propoxybenzoic acid was also reduced, 29.0% ± 8.69 SEM, but not significantly ($P<0.10$), following incubation with this metabolic inhibitor.

After 10 min. incubation with the 24 x 10^{-5}M concentration of the labelled analogues, a 5 min. chloroform:methanol (2:1) extraction caused a reduction of approximately 50% of radioactivity remaining in the platelet button. Following the procedure, 52.4% ± 6.78 SEM of activity from ASA, 54.8% ± 7.91 SEM from 2,3-diacetoxybenzoic acid, 53.4% ± 5.65 SEM from 2,6-diacetoxybenzoic acid, and 51.8% ± 12.10 SEM from 2-propoxybenzoic acid, were still present in the isolated button.

Sonification resulted in the destruction of approximately 90% (as determined by counting) of platelets in the PRP sample. The procedure reduced ^{14}C-acetate uptake from ^{14}C-ASA by 84.2% ± 3.55 SEM, from

Figure 12

The effects of incubation time on the inhibition of aggregation and the uptake of radioactive label from ASA, 2,3-diacetoxybenzoic acid (2,3-DA), 2,6-diacetoxybenzoic acid (2,6-DA) and 2-propoxybenzoic acid (PSA) and their radioactive analogues incubated in PRP at concentration of 24×10^{-5}M for 10 min.


^{14}C-ASA
^{14}C-2,3-DA
ASA
2,3-DA
^{14}C-2,6-DA
^{14}C-PSA
2,6-DA
PSA
% UPTAKE
% INHIBITION
0.20
0.16
0.12
0.08
0.04
0
80
60
40
20
0
-20
0
5
10
INCUBATION TIME (MIN.)
SLOPE
HEIGHT

^{14}C-2,3-diacetoxybenzoic acid by 64.6% ± 16.11 SEM, and from ^{14}C-2,6-diacetoxybenzoic acid by 87.5% ± 1.54 SEM.

Administration of 600 mg. of ASA to 5 healthy volunteers, whose PRP showed normal biphasic aggregation patterns with ADP, resulted in the presence of characteristic, small, monophasic, reversible aggregation responses two hours later. At this time, isolated platelet buttons from the PRP of 4 of these individuals, also demonstrated a decreased (not significant) capacity (by 26.5% ± 12.84 SEM) to incorporate radioactive label from ^{14}C-ASA, incubated for 10 min. in the PRP.

D. Salicylates and Gastrointestinal Hemorrhage

In the study of gastrointestinal bleeding and the role of abnormal platelet function in this phenomenon, several interesting results were obtained. As might be anticipated, both "bleeder" groups had significantly lower hemoglobin and hematocrit values than the "non-bleeder" control group (Table 12). There were no significant differences in these values between the two "bleeder" groups. No statistically significant differences were found among the three groups, with respect to bleeding time, whole blood platelet count, prothrombin time, partial thromboplastin time, fibrinogen titer, euglobulin lysis time, thrombin time, fibrinogen degradation products, and platelet factor 3 release. There was also no significant difference among the groups with respect to the platelet adhesiveness, although this value was lower than that of the controls (45% ± 9.8 SEM) in both the "bleeders", who had not taken ASA (41% ± 6.4 SEM), and in the group who had taken ASA (36% ± 6.7 SEM).

The effects of *in vitro* ASA on ADP-induced aggregation in the

Table 12. Hemoglobin and Hematocrit Values

(Means ± SEM)

Group	N	Hemoglobin (gm)	Hematocrit (%)
Controls	11	14.9 ± 0.51	44.2 ± 1.41
G.I. Bleeders (no ASA)	11	12.7 ± 0.84 * $p < 0.05$	37.4 ± 2.3 * $p < 0.025$
G.I. Bleeders (with ASA)	11	12.5 ± 0.52 * $p < 0.005$	37.1 ± 1.20 * $p < 0.005$

* vs. controls

"bleeder" study, are shown in Table 13. In both "bleeder" groups, significantly less ADP was required to induce second phase aggregation, and more ASA was required to inhibit this second phase, than in the control group.

The results of the collagen-induced aggregation study of G.I. "bleeders" are shown in Tables 14 and 15. No significant differences were found in the responses to collagen among the three study groups, although the platelets of the patients in the two "bleeder" groups were slightly more reactive (Table 14). There were, however, significant differences with respect to percent inhibition of collagen aggregation by *in vitro* ASA (Table 15). ASA, at both 3.5 and 5.5 x 10^{-4}M, inhibited the rate and degree of collagen aggregation to a significantly greater extent in the ASA "bleeder" group than in the control group, or in the "bleeder" group not having taken ASA. At a final concentration of 5.5 x 10^{-4}M, the drug inhibited the slope of the collagen aggregation curve by 82.5% ± 4.70 SEM, in the "bleeders" who had taken ASA, and by 69.5% ± 5.08 SEM and 64.2% ± 7.26 SEM, respectively, in the control group, and in the "bleeders" who had not taken the drug. The collagen aggregation curve height was inhibited by 67.5% ± 5.91 SEM in the ASA "bleeders", and only 52.1% ± 7.44 SEM in the controls, and 45.9% ± 6.95 SEM in the "bleeders" not having taken ASA.

In the follow-up study, it was possible to examine only the platelets of two of the individuals, whose bleeding episode a year previously had been associated with ASA intake, and four of the "bleeders" who had not taken the drug. A year after the initial investigation, the increased sensitivity of the "bleeders" platelets to ADP no longer

Table 13. *In Vitro* ASA Inhibition of ADP-induced Aggregation

Group	N	ADP concentration ($X10^{-6}M$) required for release (bi-phasic aggregation) mean ± SEM	ASA concentration ($x10^{-5}M$) required to inhibit release (no second-phase aggregation)
Controls	11	9.8 ± 1.99	5.5 ± 1.29
G.I. Bleeders (no ASA)	8	2.4 ± 0.27 ($p<0.005$)*	10.0 ± 1.34 ($p<0.05$)*
G.I. Bleeders (with ASA)	11	2.7 ± 0.41 ($p<0.005$)*	12.6 ± 2.05 ($p<0.01$)*

* vs. controls

Table 14. Platelet Aggregation in Response to Collagen

Group	N	Slope (Mean ± SEM)	Height (mm) (Mean ± SEM)	Delay (sec) (Mean ± SEM)
Controls	11	4.2 ± 0.63	206.8 ± 26.32	130.2 ± 25.62
G.I. Bleeders (no ASA)	11	5.8 ± 0.37	247.2 ± 7.73	85.3 ± 6.45
G.I. Bleeders (with ASA)	11	5.8 ± 0.43	238.9 ± 9.85	81.5 ± 11.33

Table 15. Percent Inhibition of Collagen Aggregation by *In Vitro* ASA in Final Concentrations of 3.5 (A) and 5.5 (B) x 10^{-4}M

(Means ± SEM)

		Slope		Height (mm)		Delay (sec)	
Group	N	A	B	A	B	A	B
Controls	11	59.8± 9.11	69.5± 5.08	49.2± 8.02	52.1± 7.44	6.2± 15.50	9.2± 20.3
G.I. Bleeders (no ASA)	11	53.5± 7.34	64.2± 7.26	33.4± 7.53 (p<0.02)*	45.9± 6.95	24.8± 13.22	49.2± 20.24
G.I. Bleeders (with ASA)	11	77.4± 4.60 p<0.1* p<0.02†	82.5± 4.70 p<0.01* p<0.05†	59.1± 6.19 p<0.05†	67.5± 5.91 p<0.02* p<0.05†	42.8± 14.85	28.1± 18.13

* vs. controls

† vs. G.I. "bleeders" (no ASA)

existed, and the amount of ADP required to induce a biphasic aggregation response was similar to that found in the initial control group, or in normal healthy volunteers. One individual, whose platelets failed to show the normal biphasic aggregation pattern in the initial study, demonstrated the same response one year later.

Although the recalled groups were too small for statistical comparison, the platelets of the ASA "bleeders" again appeared more sensitive to *in vitro* ASA inhibition of collagen-induced aggregation than those of the other group. *In vitro* ASA (5.5×10^{-4}M) inhibited the slope of the collagen aggregation curve by 71.8% ± 3.03 SEM in the "bleeders" in whom ASA intake had been implicated as an important factor in their bleeding episode, and only 57.9% ± 21.39 SEM in the "bleeders" who did not take ASA. The collagen aggregation curve height was inhibited by 60.8% ± 0.58 SEM in the ASA "bleeders", and only 51.2% ± 18.95 SEM in the "bleeders" not having taken ASA.

V DISCUSSION

A. Aggregation Responses

The platelet aggregation responses obtained with ADP and collagen were similar to those described by many other investigators (Constantine, 1966; MacMillan, 1966; Zucker and Peterson, 1968); i.e., suitable concentrations of ADP produced an irreversible biphasic aggregation, and collagen yielded an irreversible monophasic pattern. Although it was not always possible to determine the reason why a PRP sample did not show the biphasic pattern with ADP, and was, therefore discarded from the study, there is little doubt that drug intake, especially acetylsalicylic acid, was responsible in many cases. Even though volunteers were warned, in advance, against intake of drugs which affected platelet function, especially ASA, and were questioned again at the time of blood sampling, they sometimes admitted to having taken "only one" when shown abnormal aggregation responses. It became evident during preliminary studies, that to be consistent, all PRP samples had to be tested for a normal response to ADP aggregation, even though collagen might have been employed to aggregate that particular sample. The all-or-none inhibitory effect of ingested ASA on the second phase of ADP-induced aggregation, makes this aggregation system a better indicator of ASA intake than collagen aggregation. Most people did not consider ASA as a drug and considerable effort was necessary to exclude their use of this compound.

In the assessment of ADP and collagen aggregation, and the determination of curve slope and height, only the measurement of the first phase height of the ADP-induced aggregation curve presented any difficulty. The nature of that portion of the aggregation curve, just prior to the release reaction, makes it difficult to determine the exact point at which the first phase of aggregation ends and the second phase begins. This does not represent a serious limitation in the present study as it has been shown by many investigators (O'Brien, 1968a; 1968b; Zucker *et al.*, 1969; Al-Mondhiry, *et al.*, 1970; Constantine, 1970; Zucker and Peterson, 1970) that ASA does not have inhibitory effects on this initial stage of aggregation. The decreased ability of platelets to aggregate immediately following their collection from whole blood has not been explained, although previously described (Mannucci, 1971). It seems likely that this phenomenon is in some way related to the effects of the centrifugation procedure or the effects of pipetting the plasma.

B. Effects of Salicylate Analogues on Platelet Aggregation

It has been found that blood concentrations of up to 0.5 mM of ASA could be achieved in human subjects by ingestion of 2 to 4 ASA tablets (Levy and Leonards, 1966). The highest final concentrations of salicylate analogues (excluding PAS and sodium salicylate at 10^{-2}M), utilized in the present study, closely approximate this value. The other final salicylate concentrations employed in the aggregation and radioactive label uptake investigations were lower than this value.

ASA is a relatively potent inhibitor of the second phase of

ADP-induced aggregation in human PRP. The differences between the final concentration which has no inhibitory activity, and that which completely prevents second phase aggregation, is small. This fact; combined with the normal variation found among PRP samples, exclude the possibility of presenting meaningful dose-response data of this inhibitor on ADP-induced aggregation. For this reason, the threshold inhibitory concentration of ASA, i.e., the concentration which just inhibited ADP-induced second phase aggregation was determined. The other inhibitory salicylate analogues were less potent than ASA and demonstrated increased inhibitory activity over a much wider range of increasing concentrations.

No explanation is available to elucidate the aggregation-enhancing effect observed with the 2,4-diacetoxy and 2,6-diacetoxy compounds, and the 3-propoxy derivative on ADP-induced aggregation. Following a 10 min. incubation period in PRP, the 2,6-analogue was found to cause significant inhibition of collagen-induced aggregation. Philp *et al.* (1973) have recently described a similar reaction with low concentrations of another aggregation inhibitor, dipyridamole. Greil and co-workers (1972) have reported that addition of isotonic sodium chloride solution in a final concentration of 50 mM to PRP, just prior to addition of ADP, resulted in an enhanced aggregation response. Although the salicylate analogues in the present study were dissolved in 0.9% saline, its final concentration in PRP never exceeded 20mM, and periodic controls with saline failed to show the degree of potentiation of ADP aggregation seen with the 3 salicylate compounds.

Collagen-induced aggregation was significantly inhibited by ASA and some of its analogues in a dose-dependent manner. Although incubation of ASA with PRP enhanced its inhibitory effects, the compound also was found to be significantly active when added to PRP, just prior to the aggregating agent. The other analogues which showed inhibitory activity demonstrated it in a similar manner; that is, the inhibition was dose-dependent and increased with incubation time. Inhibition by ASA of collagen aggregation differed somewhat from its effect on the ADP response. Small doses of ASA, which completely inhibited the second phase of ADP aggregation (the release reaction), had no inhibitory effects on the collagen response. While inhibition of ADP aggregation approached an all-or-none phenomenon, collagen aggregation was gradually reduced over a wide range of increasing ASA concentrations and increasing incubation times. Even with 10 min. incubation, and at a final concentration of 55×10^{-5}M, ASA did not completely inhibit the collagen-induced reaction. It is possible to explain some of the differences on the basis that the collagen stimulus was more potent in causing aggregation than was ADP. Although the second phase of ADP aggregation and collagen aggregation have been reported to be caused by the release of platelet constituents, the reactions are different, in that collagen induces the extrusion of lysosomal enzymes while ADP does not (Holmsen, Day and Stormorken, 1969). Based on the present theories of aggregation, it appears likely that the initial surface reaction between platelets and ADP or collagen are dissimilar. Several investigators (Gaarder and Laland, 1964; Born, 1965; Boullin *et al.*, 1972) have proposed the

presence of receptors on the platelet surface for the ADP molecule. Evidence is accumulating to suggest that glycosyl transferases of platelet membrane may be involved in the adhesion of platelets to collagen (Barber and Jamieson, 1971a; 1971b; Bosmann, 1971; Chesney *et al.*, 1972); a reaction which precedes the release phenomenon. Considering these basic differences in platelet aggregation and the release response to ADP and collagen, it follows that the patterns of inhibition of these reactions by ASA would not be identical.

There is, at the present time, no theory which completely explains the mechanism whereby the inhibitory effects of ASA on platelet aggregation increase with its incubation in PRP. The platelet population being aggregated and/or inhibited in the present investigation is probably heterogeneous in nature; that is, it is made up of both young and old platelets alike. Karpatkin and co-workers (Karpatkin, 1969a; 1969b; Karpatkin and Strick, 1972) and Mannucci and Sharp (1967) have demonstrated that young platelets are larger and metabolically more active than older, smaller ones. It may be that the younger platelets must be exposed to ASA for a longer period of time before they are inhibited. If ASA is acting on an energy-dependent system in the platelet, it may be that gradual depletion of energy stores is involved in the augmented inhibitory effects seen with increased incubation of the drug.

It is possible to make several conclusions with regard to the inhibitory activity of the salicylate compounds studied on the basis of structure-activity relationships. The compounds demonstrating inhibitory effects on platelet aggregation by ADP or collagen all had a hydroxyl or a substituted hydroxyl group in the ortho (2) position, relative to

the carboxyl group. When the substituted hydroxyl function was located in the three position (3-acetoxybenzoic acid) or the four position (4-acetoxybenzoic acid), the resulting compound showed no inhibitory activity. Substitutions on the benzene ring of the parent ASA compound resulted in a derivative with reduced activity. Such was the case with the diacetoxy compounds.

It appears that the acetoxy group or the propoxy group, in the case of 2-propoxybenzoic acid, is essential for significant inhibitory activity. Neither of the non-acetylated compounds, sodium salicylate, nor PAS, demonstrated inhibitory effects at concentrations similar to those used with ASA, although they inhibited collagen aggregation at much higher final concentrations. The mere presence, however, of an acetoxy group on the analogues was not sufficient to impart inhibitory activity. If the acetoxy group is vital to the inhibitory activity of ASA, these results suggest that it must be present at a specific receptor site to exert its activity. Although it is known that the acetate function is readily hydrolyzed from its parent ASA structure in the blood stream (Smith and Smith, 1966), it would appear that ASA is in an intact form when acting at a platelet receptor. While the radioactive label from sodium ^{14}C-acetate is taken up by platelets, this compound is not an aggregation inhibitor (Al-Mondhiry *et al.*, (1970). Rosenberg *et al.* (1971) also reported that acetate uptake from hydrolyzed ASA (10^{-4}M) was not accompanied by inhibition of aggregation. Al-Mondhiry *et al.* (1970) found radioactive label in platelets from ASA, tagged in the acetoxy group, but not the carboxyl function. They also reported

that "cold" ASA, incubated with human PRP, inhibited the platelet binding of acetyl-labelled ASA. Based on these observations, one would speculate that the acetate group is released once the compound reaches the platelet receptor in question, allowing the salicylic acid moiety to leave the platelet. It must be noted that the acetoxy group of ASA might be influencing the properties of the intact molecule, in such a way as, to allow it to get to a locus of activity. If this were the case, the 2,3-, 2,4- and 2,6-diacetoxy compounds might be less able to assume a suitable relationship with a platelet receptor until the acetate at position three, four or six were removed, probably by a hydrolytic process in the plasma. Even with this acetate removed, these compounds would contain a second hydroxyl group in the ring structure, and this might contribute to their reduced degree of potency.

Calcium chelation may also be involved in the inhibitory acitivity of ASA on ADP or collagen aggregation. This has not been examined in the present investigation, and it remains to be determined exactly what role chelation of calcium may play. A number of investigators (Hovig, 1964; Skoza *et al.*, 1967; Ardlie and Mustard, 1969; Ardlie *et al.*, 1970; Cronberg and Caen, 1970; Herrmann *et al.*, 1970) have described calcium ions as an essential factor in ADP-induced aggregation. The chemical configuration of ionized ASA with its two negative charges, lends itself to chelation of the divalent calcium cation.

As has been noted previously, anti-inflammatory analgesics, in general, have been found to inhibit platelet aggregation. The possibility exists that their activity on these two phenomena are related to the same mechanism. Done (1960) has compared the antirheumatic

properties of a series of salicylate analogues. He found that among the hydroxylated benzoic acid derivatives investigated, it was the compounds, with hydroxyl substitution in the ortho position, which were therapeutically active. These were also the compounds with the highest dissociation constants (lowest pKa values) and the compounds which had the greatest ability to form stable chelates. Neither the 3-hydroxybenzoic acid, nor the 4-hydroxybenzoic acid, showed any antirheumatic activity. Clarke and his co-workers (1958), studying the role of phenolic compounds in the chemotherapy of rheumatic fever, also noted that monohydroxy derivatives, not forming chelate rings, did not have antirheumatic activity. They also examined the antirheumatic activity of a series of dihydroxy compounds and observed that 2,3-dihydroxybenzoic acid and 2,6-dihydroxybenzoic acid had significant antirheumatic properties, while 2,4-dihydroxybenzoic had weak activity. The antirheumatic action of these hydroxy compounds, with respect to structure-activity relationships, correspond to the inhibitory effects of their acetylated analogues as aggregation inhibitors. Clarke *et al.*(1958) also found that increased antirheumatic potency was present in the phenolic compounds in which double chelate rings were possible. This relationship was not present in the aggregation study, as the diacetoxy compounds were less potent than ASA.

It is interesting to note that the system of platelet phosphodiesterases, enzymes involved in the regulation of platelet cyclic AMP, and altered by ASA (Amer and Marquis, 1972), are dependent on the presence of calcium and, to a lesser degree, magnesium (Amer and Mayol, 1973).

C. Radioactive Label Study

It is apparent from the results that intact platelets are required for uptake of the radioactive label from the labelled salicylate compounds. Destruction of platelets by sonification reduced the amount of incorporated radioactivity in proportion to the degree of cell disruption.

The largest proportion of added radioactivity remained in the PPP supernatant either bound to plasma components or in a free form. Of the activity present in the platelet button, a considerable degree (approximately 50%) was associated with lipids or possibly phospholipids, as determined by chloroform:methanol extraction.

Judgement based purely on the radioactive label study would suggest that acetylation of platelet components is not the only factor involved in the mechanism of aggregation inhibition by these compounds. If this were the case, considering the percent uptake of radioactive label in the platelet buttons, after 10 min. incubation, one would expect the 2,3-diacetoxy compound and the 2,6-diacetoxy derivative to be equipotent, with respect to inhibition of collagen-induced aggregation. Such was not the case; the former compound showing approximately twice the inhibitory activity of the 2,6-analogue. Since the percent uptake of radioactivity from these compounds was approximately one-half of that from ^{14}C-ASA, if acetylation were solely responsible for inhibition of aggregation, the degree of inhibition with the two diacetoxy compounds should have been approximately 50% of that with ASA. Certainly this was not the case. It should be noted that without incubation in PRP, and at a final concentration of 29×10^{-5}M, conditions, similar to those in which uptake of the radioactive label took place (see table 11),

2,6-diacetoxybenzoic acid, significantly potentiated ADP-induced aggregation. Uptake from the 2-propoxy derivative was lowest of all compounds studied. This analogue was, however, a more potent aggregation inhibitor than either of the diacetoxy compounds. Oligomycin, incubated for 15 min. in PRP which had been allowed to stand at room temperature for two hours, reduced, considerably, the uptake of ^{14}C-acetate or propionate. This reduction probably is associated with a decrease in the uptake of ^{14}C-acetate or propionate into metabolic pools of the platelet. Radioactive uptake under these conditions also did not correlate with the degree of inhibitory activity demonstrated by the four analogues on collagen-induced aggregation. In relation to the findings with ASA, the uptake of propionate from ^{14}C-propoxybenzoic acid was lower than would be expected from its effects on collagen aggregation. Correlation coefficients, relating ^{14}C-acetate or propionate uptake in PRP or in oligomycin-treated PRP to inhibition of collagen aggregation, were not significant.

In the present study, approximately 50% of radioactive label was extracted with chloroform:methanol (2:1), presumably from lipids or phospholipid material. Although this might appear to be a large proportion of label to be incorporated into platelet lipids, Rosenberg and his colleagues (1971) found greater than 50% of radioactivity from 10^{-4}M ^{3}H-ASA in this platelet component.

Although Rosenberg *et al.* (1971) suggested that in rabbit PRP, inhibition by ASA of collagen-aggregation was related to the degree of acetylation of the platelet, their results were not conclusive. Utilizing ^{3}H-ASA and sodium ^{3}H-acetate, they differentiated between

protein acetylation and acetate uptake into rabbit platelets. ^{3}H-ASA labelled protein to a significantly greater degree than did sodium ^{3}H-acetate, while the opposite held true for the labelling of platelet lipids. They stated that acetylation, not acetate uptake, was related to aggregation inhibition. However, it should be noted that, in their study, total acetate incorporation from ^{3}H-ASA both into platelet lipid and protein fractions correlated with inhibition of aggregation, not acetylation of platelet proteins. The degree of incorporation into protein fractions remained relatively constant over a period of one hour, while the degree of aggregation inhibition increased by approximately 40%.

A number of investigators (Al-Mondhiry *et al.*, 1969; O'Brien *et al.*, 1970a; Pinckard *et al.*, 1970) have suggested that platelet acetylation by ASA is responsible for the prolonged inhibitory effects observed *in vivo* with this compound. The results of the present study do not discount the possibility that this may be the case, but neither do they support the concept that platelet acetylation is totally responsible for the inhibitory effects on aggregation demonstrated *in vitro*.

D. Salicylates and Gastrointestinal Hemorrhage

In the study of *in vitro* ASA on blood platelets of gastrointestinal "bleeders", both of the "bleeder" groups had significantly decreased hemoglobin and hematocrit values. None of the other laboratory findings, excluding platelet aggregation, was significantly different in the three study groups. The greater reactivity of platelets from all patients who had recent gastrointestinal hemorrhage, as indicated by their higher sensitivity to ADP, and the fact that this sensitivity was absent after

one year, suggest that it was probably due to the presence of more new, metabolically active platelets released in response to the stimulus of hemorrhage. Such increased sensitivity of young platelets to aggregating stimuli has been reported by Karpatkin (1969a; 1969b) and by Mannucci and Sharp (1967). This higher degree of aggregating activity in both "bleeder" groups indicates that any residual effects of the ingested ASA were minimal.

Of the 33 patients studied, only three did not show a biphasic response to ADP, and these all appeared in the "bleeder" group which had not taken ASA. Since this pattern of aggregation is characteristic of that seen following recent ingestion of ASA (Evans *et al.*, 1968; O'Brien, 1968b; Mustard and Packham 1970; Zucker, 1971), the possibility that these patients took ASA prior to their bleeding episode and did not report it cannot be excluded. It is also possible, however, that these individuals may have a congenital defect of platelet function. One individual, whose platelets failed to show the normal biphasic aggregation pattern in the initial study, demonstrated the same response one year later, and would, thus, appear to be suffering from a chronic platelet defect.

A number of investigators have demonstrated a synergistic effect between alcohol consumption and intake of ASA in gastrointestinal hemorrhage (Jennings, 1965; Needham *et al.*, 1971). This effect does not appear to have been a significant factor in the present study, as only two of the eleven "bleeders" who had taken ASA consumed in excess of three bottles of beer per day, and a similar incidence of alcohol intake was observed in the other two groups.

In the initial investigation, it was found that the platelets of those patients, with bleeding associated with recent ASA intake, were more sensitive to inhibition by ASA of collagen aggregation *in vitro*, in spite of a somewhat greater aggregation response to collagen, than those of the other two groups. This raises the possibility that these people may have some intrinsic difference in their platelets or in some plasma factor(s) involved in collagen-induced aggregation. Although there was insufficient data to determine, conclusively, whether this difference was transitory or permanent, there was a suggestion from the results of the follow-up study that the latter might be the case. It may be that they are also more sensitive to ASA *in vivo*, and thus, having taken the drug, their normal platelet plug formation is defective, contributing to the blood loss from any mucosal injury.

A platelet-collagen interaction is one of the initial steps in the formation of the platelet plug (Hovig, 1963a; 1963b; Spaet and Zucker, 1964). Individuals who are unusually sensitive to the impairment of this interaction by ASA might not form a solid platelet plug at the site of gastric erosion after taking ASA, and, hence, might be more susceptible to hemorrhage. The fact that almost 30% of the non-ASA "bleeders" showed a defect of aggregation supports the contention that impaired platelet function might render an individual more susceptible to hemorrhage, following gastric mucosal erosion from any cause.

E. Concluding Remarks

The clinical problems of thrombosis and hemorrhage represent two extremes in the spectrum of activity of the hemostatic process. The epidemic proportions of thromboembolic disease in man has led to the

investigation of agents which inhibit normal hemostasis and have potential as antithrombotic drugs. No investigation of such compounds is complete without a consideration of their propensity to induce bleeding. Experience with ASA illustrates this tendency, in that while it has shown some promise as an antithrombotic agent, the substance also appears to be a causative factor in many cases of gastrointestinal hemorrhage. Should it be possible to identify a population of individuals more sensitive to the hemorrhagic properties of such drugs, as was suggested by the results of the clinical studies, this would enable clinicians to screen out susceptible persons before implementing therapy which might expose them to undue risk. Long experience with the oral anticoagulants has demonstrated the dangers of manipulating the protective hemostatic mechanism.

Of the salicylate analogues, excluding ASA, only 2-propoxybenzoic acid demonstrated sufficient inhibitory activity on platelet aggregation to justify further investigation. Extracorporeal shunts or injured cerebral cortex vessels could be utilized to determine its *in vivo* effects on platelet aggregation and thrombus formation. Experiments to determine the ulcerogenic effects of this substance on the gastric mucosa of experimental animals would also be of interest.

Based on the report of Clarke *et al.* (1958), in which they noted increased antirheumatic potency in phenolic compounds capable of forming double chelate rings, one might also examine the *in vivo* antiplatelet effects of 2,3-diacetoxy- and 2,6-diacetoxybenzoic acid. Clarke and his colleagues described some dosage and toxicity data from humans for these compounds. Further investigation to define the involvement

of calcium chelation in the mechanism of action of ASA on platelet function should also be undertaken. It would be interesting to determine if ASA could remove a radioactive calcium label from platelets and from what site the label came.

The finding that ingestion of ASA resulted in a reduced, although not statistically significant, uptake of the radioactive label from ^{14}C-ASA *in vitro*, suggests that platelet sites are partially filled during the *in vivo* exposure. A study including more subjects would determine if there is, in fact, significant uptake of acetate into human platelets *in vivo*. It is noteworthy that Al-Mondhiry *et al.* in 1970 observed that "cold" ASA or sodium acetate, incubated with human PRP, inhibited platelet binding of acetyl-labelled ASA or labelled acetate *in vitro*. Administration of labelled ASA to experimental animals would also be useful in determining the extent of *in vivo* acetate uptake.

It has been suggested that some patients suffering from gastrointestinal hemorrhage might have an intrinsic platelet defect. A study which included a large number of patients would define the incidence of platelet abnormalities in this condition.

SUMMARY and CONCLUSIONS VI

1. The aggregation responses obtained in the present study were similar to those described by other investigators. Platelet aggregation in PRP at 37°C took place in a biphasic, irreversible manner when suitable concentrations of ADP were used as the aggregating stimulus. A typical response to collagen suspension consisted of an irreversible, monophasic aggregation following a delay in onset.

2. When ASA was added immediately prior to the addition of ADP, the threshold, inhibitory concentration, i.e., the lowest concentration which completely inhibited the second phase of ADP-induced aggregation, was found to be 5.2×10^{-5}M ($\pm$ 1.07 SEM).

3. ASA did not have inhibitory effects on the first phase of the ADP-induced aggregation response.

4. At concentrations similar to ASA, and without prior incubation in PRP, 2-propoxybenzoic acid and 2,3-diacetoxybenzoic acid also had inhibitory effects on ADP aggregation; the former compound being the more potent in this respect. Under similar experimental conditions, sodium salicylate, PAS, 3-acetoxybenzoic acid and 4-acetoxybenzoic acid were without statistically significant inhibitory activity, while 2,6-diacetoxybenzoic acid and

3-propoxybenzoic acid significantly potentiated the second phase response.

5. In a study of the inhibitory effects of salicylate analogues on collagen aggregation, only ASA demonstrated much activity without incubation of the drug, prior to addition of the aggregating agent.

6. At high final concentrations (40-60 times that of ASA), both sodium salicylate and PAS were found to inhibit collagen-induced aggregation.

7. The inhibitory activity of ASA, 2,3-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid and 2-propoxybenzoic acid was found to increase with incubation in PRP, prior to addition of collagen suspension. After 10 min. incubation, ASA was still the most potent inhibitory analogue, although at a concentration of 55 x 10^{-5}M, the 2-propoxy compound demonstrated similar activity. At the highest concentration studied, the 2,6-diacetoxy analogue was about one-half as potent, after 10 min., as ASA, and the 2,3-diacetoxy derivative, which showed significant inhibitory effects without incubation, was the least potent of the four compounds.

8. Structure-activity studies suggested that an ortho relationship between a carboxyl group and an acetoxy function was essential for the inhibitory activity of the analogues examined. Introduction of a second acetoxy group to the ASA molecule resulted in compounds with decreased potency as inhibitors of platelet aggregation. Acetylated derivatives were more potent inhibitors

of aggregation than non-acetylated ones.

9. When the ^{14}C-labelled compounds, ASA, 2,3-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid and 2-propoxybenzoic acid were added to, or incubated with, PRP and the platelets isolated, acetate or propionate incorporation was found to be dependent on the concentration of the labelled compound and the duration of its incubation in PRP.

10. The degree of acylation was highest with ASA, intermediate with 2,3-diacetoxybenzoic acid and 2,6-diacetoxybenzoic acid and lowest with 2-propoxybenzoic acid. Platelet incorporation of the ^{14}C-label from these four compounds did not correlate with their activity as inhibitors of collagen aggregation.

11. Oligomycin significantly inhibited the platelet uptake of labelled acetate and reduced the propionate incorporation from 2-propoxybenzoic acid. Label uptake from the radioactive analogues by oligomycin-treated platelets did not correlate with the activity of these compounds as aggregation inhibitors.

12. A five minute chloroform:methanol extraction reduced, by approximately 50%, the amount of radioactivity remaining in the platelet button, and suggested that a large percentage of incorporated radioactivity was associated with platelet lipids.

13. Destruction of platelets by sonification reduced the amount of incorporated radioactivity in proportion to the degree of cell disruption.

14. From these results, one would conclude that the mere presence of an acetoxy group on a salicylate analogue does not confer, to

that molecule, significant inhibitory activity against platelet aggregation. It does appear, however, that the acetoxy group provides the ASA molecule with properties which make this compound a more potent inhibitor than the non-acetylated analogues.

15. In a study to examine the effects of *in vitro* ASA on the platelets of persons suffering from gastrointestinal hemorrhage, *in vitro* ASA caused significantly greater inhibition of collagen aggregation in the PRP of "bleeders" in whom *in vivo* ASA was considered to be a causative factor in their bleeding episode. Data from this investigation and a follow-up study suggested that some individuals may have platelets which are intrinsically more sensitive to ASA.

APPENDIX I

Drugs contraindicated in the aggregation and gastrointestinal bleeding studies

Drugs Contraindicated in Aggregation and Bleeding Studies

-anti-inflammatory analgesics

-mefenamic acid

-phenothiazines

-antihistamines

-local anesthetics

-atropine

-α- and β- blockers

-dipyridamole

-oral contraceptives

APPENDIX II

Methods of preparation of salicylate analogues

Method of preparation of ^{14}C-labelled salicylate analogues

1-^{14}C-acetylsalicylic acid

-salicylic acid (13.16 mg.), 1-^{14}C-acetic anhydride in benzene (0.1 ml.) (ICN Research Products, Montreal, Quebec, cat. no. 12022), pyridine (1 drop), and benzene washings (0.1 ml.) were mixed together

-mixture stirred for 1/2 hr.

-inactive acetic anhydride (0.25 ml.) added

-mixture stirred for 1/2 hr.

-ASA (99 mg.) added

-mixture dissolved in benzene (0.3 ml.) and crystallized by addition of benzene-petroleum ether (30-60)

-crystals filtered and recrystallized from benzene-petroleum either (30-60)

-recovery-68.6 mg.

-basic activity-396,343 c.p.m. per mg. (38.1 μc/mM)

2-(1-^{14}C)-propoxybenzoic acid

-1-^{14}C-sodium propionate (250μC) and propionyl chloride (1.4 mg.) in benzene (1 ml.) mixed together

-mixture was warmed and pyridine (1 drop) and salicylic acid (13 mg.) added

-mixture heated at 60° for 1 hr.

-mixture left at room temperature and propionic anhydride (15 mg.) added

-inactive 2-propionyl ester (140 mg.) added

-5.38 mg. of labelled ester isolated after recrystallization from benzene-petroleum ether

-basic activity-857,630 c.p.m. per mg. (88.9 μc/mM)

2,3-di(1-^{14}C)acetoxybenzoic acid

-2,3-dihydroxybenzoic acid (10.67 mg.), 1-^{14}C-acetic anhydride in benzene (0.1 ml.), pyridine (1 drop) and benzene (0.1 ml.) mixed together

-mixture stirred for 1 hr.

-acetic anhydride (0.1 ml.) added and mixture for additional 1/2 hr.

-inactive 2,3-diacetoxybenzoic acid (17 mg.) added and mixture taken to dryness

-crystals recrystallized twice from benzene-petroleum ether (30-60)

-recovery-27 mg.

-basic activity-3,011,125 c.p.m. per mg. (388 μc/mM)

2,6-di(1-^{14}C)acetoxybenzoic acid

-2,6-dihydroxybenzoic acid (8.96 mg.), 1-^{14}C-acetic anhydride in benzene (0.1 ml.), pyridine (1 drop), and benzene (0.1 ml.) mixed together

-mixture stirred for 1 hr.

-inactive 2,6-diacetoxybenzoic acid (23.90 mg.) added

-mixture taken to dryness

-compound recrystallized from benzene-petroleum ether (30-60) over a period of several days

-recovery-32.8 mg., basic activity-641,381 c.p.m. per mg. (81.6 μc/mM)

Synthesis of salicylate analogues

The salicylate analogues were synthesized by the referenced methods.

Analogue	Reference	Melting Point (°C)	
		found*	cited literature
3-acetoxy-benzoic acid	Marshall *et al.*, 1942	118-131	129-131
4-acetoxy-benzoic acid	Chattaway, 1931	190-191	189-190
2,3-diacetoxy-benzoic acid	Simokoriyama, 1941	136-138	146-170
2,4-diacetoxy-benzoic acid	Simokoriyama, 1941	136-139	136-137
2,6-diacetoxy-benzoic acid	Hunt *et al.*, 1956	112-113	113-114
2-propoxy-benzoic acid	Hofstee, 1952	94-95	95
3-propoxy-benzoic acid	Hofstee, 1952	85-86	86

*All melting points were performed on a Kofler Thermopan Melting Point Apparatus by Dr. M. Hirst.

APPENDIX III

Method of preparation of collagen suspension

Method of preparation of collagen suspension

-minced, lyophilized and shredded bovine achilles tendon (0.25 gm.) (General Biochemicals) was added along with 25 ml. of 0.9% saline to the cup (submerged in ice) of a Sorval Omni Mixer (Allied Scientific Co. Ltd.)

-mixer turned to speed 6 for 2 min., to speed 8 for 2 min. and finally to speed 9 for 3 min.

-a second aliquot (0.25 gm.) of collagen and saline (25 ml.) was added to the cup and the grinding process repeated

-at frequent intervals during the cutting process, the mixer was stopped and any attached collagen strands were removed from the cutting blades and returned to the ground mixture

-the coarse collagen mixture was centrifuged at 750 x g. for 4 min.

-supernatant was taken off and again centrifuged at 750 x g. for 4 min.

-supernatant was checked for activity and refrigerated for use

APPENDIX IV

Calculations for the preparation of modified Tyrode's solution

Calculations for modified Tyrode's solution

original formula (gm./L.)		molecular and atomic weights	
NaCl	8.000	NaCl=58.45	Na=22.99
KCl	0.200	KCl=74.55	Cl=35.46
$NaHCO_3$	1.000	$NaHCO_3$=84.00	K=39.10
$NaH_2PO_4.H_2O$	0.050	$NaH_2PO_4.H_2O$=137.99	
$CaCl_2.6H_2O$	0.438	$CaCl_2.6H_2O$=219.09	
$MgCl_2.6H_2O$	0.203	$MgCl_2.6H_2O$=203.33	
		Ca=40.08	Mg=24.32

ions present in original formula

Na^+ from NaCl = $\frac{8 \times 22.99 \times 1000}{58.45 \times 22.99}$ = 136.87 mM

Na^+ from $NaHCO_3$ = $\frac{1.0 \times 22.99 \times 1000}{84.00 \times 22.99}$ = 11.90 mM

Na^+ from $NaH_2PO_4.H_2O$ = $\frac{0.05 \times 22.99 \times 1000}{137.99 \times 22.99}$ = 0.36 mM

K^+ from KCl = $\frac{0.2 \times 39.10 \times 1000}{74.55 \times 39.10}$ = 2.68 mM

Ca^{++} from $CaCl_2.6H_2O$ = $\frac{0.438 \times 40.08 \times 1000}{219.09 \times 40.08}$ = 2.00 mM

Cl^- from $CaCl_2.6H_2O$ = $\frac{2 \times 35.46 \times 0.438 \times 1000}{219.09 \times 35.46}$ = 4.00 mM

Mg^{++} from $MgCl_2.6H_2O$ = $\frac{0.203 \times 24.32 \times 1000}{203.33 \times 24.32}$ = 1.00 mM

Cl^- from $MgCl_2.6H_2O$ = $\frac{2 \times 0.203 \times 35.46 \times 1000}{203.33 \times 35.46}$ = 2.00 mM

total ion replacement required for $CaCl_2.6H_2O$ and $MgCl_2.6H_2O$

= 2.00 mM + 4.00 mM + 1.00 mM = 2.00 mM = 9.00 mM

ratio of K^+ / Na^+ in original formula

$$= \frac{2.68 \text{ mM}}{(136.87 + 11.90 + 0.36) \text{ mM}} = \frac{2.68}{149.13} = 0.0179$$

NaCl needed

$$= \frac{9 \times 1000}{101.79} = 8.84 \text{ mM} = 0.517 \text{ gm.}$$

KCl needed

$$= \frac{9 \times 1.79}{101.79} = 0.158 \text{ mM} = 0.012 \text{ gm.}$$

modified formula (gm./L.)

NaCl	8.517
KCl	0.212
$NaHCO_3$	1.000
$NaH_2PO_4.H_2O$	0.050

APPENDIX V

Percent change of ADP-induced aggregation curve by sodium salicylate, PAS, 3-acetoxybenzoic acid, 4-acetoxybenzoic acid, 2,3-diacetoxybenzoic acid, 2,4-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid and 3-propoxybenzoic acid

Percent Change of ADP-induced Aggregation Curve by Sodium Salicylate (% ± SEM)

Final Sodium Salicylate Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5×10^{-5}M	+9.1 ± 6.74 (NS)	+7.9 ± 6.66 (NS)	+17.4 ± 15.36 (NS)	+4.1 ± 6.10 (NS)
15 $\times 10^{-5}$M	+9.7 ± 7.70 (NS)	+11.6 ± 5.39 (NS)	+ 5.8 ± 13.20 (NS)	+5.2 ± 6.30 (NS)
29 $\times 10^{-5}$M	+1.7 ± 6.87 (NS)	+1.8 ± 5.37 (NS)	+14.4 ± 17.32 (NS)	+3.1 ± 7.77 (NS)
43 $\times 10^{-5}$M	+4.2 ± 3.79 (NS)	+7.0 ± 2.31 ($p<0.02$)	+12.3 ± 9.15 (NS)	+3.1 ± 2.82 (NS)
69 $\times 10^{-5}$M	+3.4 ± 3.24 (NS)	+5.2 ± 3.46 (NS)	+11.3 ± 12.71 (NS)	+0.4 ± 2.75 (NS)

Percent Change of ADP-induced Aggregation Curve by PAS (% ± SEM)

Final PAS Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5 x 10^{-5}M	↑8.3 ± 5.70 (NS)	↑7.1 ± 7.69 (NS)	↑18.3 ± 20.29 (NS)	↑2.0 ± 6.78 (NS)
15 x 10^{-5}M	↑2.4 ± 5.81 (NS)	↑3.9 ± 5.76 (NS)	↑20.1 ± 10.85 (NS)	↑4.2 ± 6.34 (NS)
29 x 10^{-5}M	↑19.9 ± 9.27 (NS)	↑22.4 ± 9.05 (NS)	↑31.9 ± 16.45 ($p<0.05$)	↑17.9 ± 5.70 ($p<0.005$)
43 x 10^{-5}M	↑12.9 ± 12.03 (NS)	↑13.2 ± 10.20 (NS)	↑4.4 ± 13.41 (NS)	↑1.4 ± 8.60 (NS)
69 x 10^{-5}M	↑20.6 ± 7.17 ($p<0.02$)	↑15.9 ± 7.91 (NS)	↑8.4 ± 17.55 (NS)	↑6.2 ± 8.78 (NS)

Percent Change of ADP-induced Aggregation Curve

by 3-Acetoxybenzoic Acid (% ± SEM)

Final 3-Acetoxybenzoic Acid Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5 x 10^{-5}M	+9.4 ± 6.79 (NS)	+17.1 ± 7.03 (NS)	+34.9 ± 8.18 ($p < 0.005$)	+14.5 ± 2.29 ($p < 0.001$)
15 x 10^{-5}M	+10.5 ± 8.89 (NS)	+6.3 ± 8.36 (NS)	+44.2 ± 12.85 ($p<0.005$)	+18.9 ± 7.39 ($p<0.025$)
29 x 10^{-5}M	+7.0 ± 6.32 (NS)	+12.9 ± 6.83 (NS)	+40.1 ± 16.82 ($p<0.05$)	+21.5 ± 8.72 ($p<0.025$)
43 x 10^{-5}M	+7.4 ± 4.83 (NS)	+19.8 ± 6.96 ($p<0.025$)	+51.1 ± 12.73 ($p<0.005$)	+24.6 ± 4.55 ($p<0.001$)
69 x 10^{-5}M	+9.5 ± 9.34 (NS)	+8.5 ± 6.33 (NS)	+12.7 ± 17.45 (NS)	+9.5 ± 9.21 (NS)

Percent Change of ADP-induced Aggregation Curve by

4-Acetoxybenzoic Acid (% ± SEM)

Final 4-Acetoxybenzoic Acid Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5×10^{-5}M	↑4.0 ± 4.69 (NS)	↑3.3 ± 5.49 (NS)	↑22.9 ± 10.19 (NS)	↑6.2 ± 5.92 (NS)
15 $\times 10^{-5}$M	↑9.8 ± 4.43 (NS)	↑10.2 ± 5.28 (NS)	↑27.4 ± 11.65 (NS)	↑14.5 ± 5.12 ($p<0.02$)
29 $\times 10^{-5}$M	↑0.3 ± 5.60 (NS)	↑3.4 ± 7.05 (NS)	↑88.2 ± 46.66 (NS)	↑21.3 ± 7.80 ($p<0.02$)
43 $\times 10^{-5}$M	↑0.6 ± 5.59 (NS)	↑13.4 ± 7.53 (NS)	↑77.7 ± 23.91 ($p<0.01$)	↑28.6 ± 7.33 ($p<0.001$)
69 $\times 10^{-5}$M	↑5.7 ± 7.24 (NS)	↑3.3 ± 10.75 (NS)	↑6.8 ± 21.65 (NS)	↑14.4 ± 7.08 (NS)

Percent Change of ADP-induced Aggregation Curve by

2,3-Diacetoxybenzoic Acid (% ± SEM)

Final 2,3-Diacetoxybenzoic Acid Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5 x 10^{-5}M	↓5.8 ± 8.13 (NS)	↑8.2 ± 8.92 (NS)	↑25.4 ± 11.87 (NS)	↑1.7 ± 2.27 (NS)
15 x 10^{-5}M	↓2.1 ± 7.44 (NS)	↑6.0 ± 9.75 (NS)	↑31.7 ± 9.70 ($p<0.02$)	↑9.1 ± 5.31 (NS)
29 x 10^{-5}M	↑0.6 ± 10.39 (NS)	↑14.2 ± 11.69 (NS)	↑1.0 ± 9.55 (NS)	↑3.7 ± 6.55 (NS)
43 x 10^{-5}M	↓3.4 ± 10.31 (NS)	↓3.1 ± 11.53 (NS)	↑8.3 ± 34.83 (NS)	↓3.3 ± 12.63 (NS)
69 x 10^{-5}M	↓4.3 ± 9.96 (NS)	↓8.7 ± 9.93 (NS)	↓71.8 ± 20.62 ($p<0.025$)	↓31.6 ± 10.09 ($p<0.02$)

Percent Change of ADP-induced Aggregation Curve

by 2,4-Diacetoxybenzoic Acid (% ± SEM)

Final 2,4-Diacetoxybenzoic Acid Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5×10^{-5}M	↑4.1 ± 6.40 (NS)	↑0.8 ± 5.19 (NS)	↑13.0 ± 17.39 (NS)	↑8.3 ± 7.35 (NS)
15×10^{-5}M	↑17.3 ± 6.99 ($p<0.02$)	↑15.5 ± 6.45 ($p<0.05$)	↑59.9 ± 15.01 ($p<0.02$)	↑27.0 ± 5.40 ($p<0.001$)
29×10^{-5}M	↑3.9 ± 7.98 (NS)	↑6.3 ± 5.30 (NS)	↑42.6 ± 24.11 (NS)	↑12.6 ± 8.58 (NS)
43×10^{-5}M	↑3.7 ± 5.80 (NS)	↑16.3 ± 8.28 (NS)	↑61.2 ± 39.05 (NS)	↑21.9 ± 11.92 (NS)
69×10^{-5}M	↑18.1 ± 9.89 (NS)	↑23.2 ± 8.29 ($p<0.02$)	↑46.7 ± 37.00 (NS)	↑28.0 ± 9.15 ($p<0.01$)

Percent Change of ADP-induced Aggregation Curve by

2,6-Diacetoxybenzoic Acid (% ± SEM)

Final 2,6-Diacetoxybenzoic Acid Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5×10^{-5}M	↑5.0 ± 4.46 (NS)	↑7.3 ± 5.98 (NS)	↑36.3 ± 4.57 ($p<0.001$)	↑14.6 ± 3.89 ($p<0.005$)
15×10^{-5}M	↑0.9 ± 4.61 (NS)	↑8.0 ± 4.92 (NS)	↑38.1 ± 10.44 ($p<0.025$)	↑16.5 ± 3.86 ($p<0.005$)
29×10^{-5}M	↑15.2 ± 5.93 ($p<0.05$)	↑23.3 ± 7.50 ($p<0.02$)	↑37.5 ± 8.47 ($p<0.01$)	↑29.4 ± 8.24 ($p<0.001$)
43×10^{-5}M	↑0.2 ± 7.63 (NS)	↑11.4 ± 10.03 (NS)	↑56.9 ± 17.58 ($p<0.01$)	↑24.4 ± 9.18 ($p<0.005$)
69×10^{-5}M	↑12.1 ± 8.80 (NS)	↑33.2 ± 11.37 ($p<0.02$)	↑63.1 ± 22.66 ($p<0.01$)	↑28.0 ± 9.86 ($p<0.02$)

Percent Change of ADP-induced Aggregation Curve by

3-Propoxybenzoic Acid (% ± SEM)

Final 3-Propoxybenzoic Acid Concentration	First Phase Slope	First Phase Height	Second Phase Slope	Total Curve Height
7.5×10^{-5}M	↑5.2 ± 4.40 (NS)	↑3.2 ± 3.33 (NS)	↑32.2 ± 8.27 ($p<0.02$)	↑7.1 ± 2.58 ($p<0.02$)
15×10^{-5}M	↑13.9 ± 3.80 ($p<0.01$)	↑15.9 ± 4.08 ($p<0.05$)	↑54.4 ± 7.55 ($p<0.005$)	↑22.9 ± 4.18 ($p<0.001$)
29×10^{-5}M	↑12.0 ± 4.44 ($p<0.05$)	↑15.1 ± 5.40 ($p<0.05$)	↑37.6 ± 9.31 ($p<0.05$)	↑19.6 ± 4.69 ($p<0.005$)
43×10^{-5}M	↓0.1 ± 5.90 (NS)	↑11.5 ± 7.27 (NS)	↑87.4 ± 32.90 ($p<0.01$)	↑26.1 ± 5.31 ($p<0.005$)
69×10^{-5}M	↑11.5 ± 4.58 (NS)	↑25.5 ± 6.52 ($p<0.005$)	↑86.6 ± 22.05 ($p<0.001$)	↑33.7 ± 4.08 ($p<0.001$)

APPENDIX VI

Percent change of collagen-induced aggregation curve by sodium salicylate, PAS, 3-acetoxybenzoic acid, 4-acetoxybenzoic acid, 2,4-diacetoxybenzoic acid, 2,6-diacetoxybenzoic acid, 2-propoxybenzoic acid and 3-propoxybenzoic acid

Percent Change of Collagen-induced Aggregation Curve by Sodium Salicylate (% ± SEM)

Final Sodium Salicylate Concentration	Curve Slope	Curve Height	Delay Phase
6 x 10^{-5}M	+0.9 ± 9.09 (NS)	+0.0 ± 2.26 (NS)	+6.5 ± 12.28 (NS)
12 x 10^{-5}M	+7.6 ± 7.70 (NS)	+1.6 ± 2.61 (NS)	+2.0 ± 9.62 (NS)
24 x 10^{-5}M	+0.2 ± 5.31 (NS)	+2.7 ± 2.06 (NS)	+12.4 ± 11.49 (NS)
35 x 10^{-5}M	+2.5 ± 5.06 (NS)	+6.8 ± 3.75 (NS)	+22.6 ± 12.62 (NS)
55 x 10^{-5}M	+4.7 ± 6.85 (NS)	+4.2 ± 2.85 (NS)	+10.5 ± 7.99 (NS)

Percent Change of Collagen-induced Aggregation Curve by PAS (% ± SEM)

Final PAS Concentration	Curve Slope	Curve Height	Delay Phase
6 x 10^{-5}M	↓5.4 ± 6.96 (NS)	↓1.4 ± 1.96 (NS)	↓4.1 ± 8.31 (NS)
12 x 10^{-5}M	↓10.9 ± 5.89 (NS)	↓1.9 ± 2.10 (NS)	↓2.0 ± 6.69 (NS)
24 x 10^{-5}M	↓0.9 ± 4.92 (NS)	↓0.6 ± 3.73 (NS)	↓1.3 ± 5.18 (NS)
35 x 10^{-5}M	↓4.4 ± 7.17 (NS)	↓4.4 ± 2.84 (NS)	↓1.5 ± 8.88 (NS)
55 x 10^{-5}M	↓6.2 ± 3.62 (NS)	↓1.1 ± 3.03 (NS)	↓8.4 ± 12.35 (NS)

Percent Change of Collagen-induced Aggregation Curve by 3-Acetoxybenzoic Acid (% ± SEM)

Final 3-Acetoxybenzoic Acid Concentration	Curve Slope	Curve Height	Delay Phase
6×10^{-5}M	↑1.6 ± 4.55 (NS)	↑0.8 ± 1.71 (NS)	↓2.5 ± 7.41 (NS)
12×10^{-5}M	↑2.4 ± 4.58 (NS)	↑3.0 ± 2.92 (NS)	↓6.4 ± 2.48 (NS)
24×10^{-5}M	↑13.9 ± 5.11 ($p<0.05$)	↑1.0 ± 2.78 (NS)	↓19.7 ± 5.77 ($p<0.02$)
35×10^{-5}M	↑8.4 ± 8.70 (NS)	↓0.8 ± 3.06 (NS)	↓5.9 ± 8.29 (NS)
55×10^{-5}M	↓4.9 ± 6.12 (NS)	↓4.2 ± 2.85 (NS)	↓9.2 ± 7.16 (NS)

Percent Change of Collagen-induced Aggregation

Curve by 4-Acetoxybenzoic Acid (% ± SEM)

Final 4-Acetoxybenzoic Acid Concentration	Curve Slope	Curve Height	Delay Phase
6 x 10^{-5}M	↑2.1 ± 4.71 (NS)	↓2.9 ± 2.26 (NS)	↓6.1 ± 3.02 (NS)
12 x 10^{-5}M	↑7.5 ± 5.52 (NS)	↓0.5 ± 3.40 (NS)	↓11.0 ± 4.29 (NS)
24 x 10^{-5}M	↓1.9 ± 4.64 (NS)	↓2.7 ± 4.00 (NS)	↓9.7 ± 6.41 (NS)
35 x 10^{-5}M	↑3.6 ± 5.66 (NS)	↓1.2 ± 2.55 (NS)	↓6.2 ± 7.39 (NS)
55 x 10^{-5}M	↑0.3 ± 6.74 (NS)	↑0.0 ± 1.61 (NS)	↑0.9 ± 5.69 (NS)

Percent Change of Collagen-induced Aggregation Curve by 2,4-Diacetoxybenzoic Acid (% ± SEM)

Final 2,4-Diacetoxybenzoic Acid Concentration	Curve Slope	Curve Height	Delay Phase
6×10^{-5}M	+1.7 ± 4.07 (NS)	+1.4 ± 2.44 (NS)	+2.3 ± 6.13 (NS)
12×10^{-5}M	+5.3 ± 6.39 (NS)	+0.5 ± 3.18 (NS)	+5.2 ± 6.80 (NS)
24×10^{-5}M	+0.6 ± 9.32 (NS)	+2.7 ± 2.39 (NS)	+9.3 ± 9.88 (NS)
35×10^{-5}M	+4.5 ± 5.67 (NS)	+4.0 ± 2.41 (NS)	+15.0 ± 8.11 (NS)
55×10^{-5}M	+5.4 ± 6.34 (NS)	+1.7 ± 3.03 (NS)	+11.4 ± 9.61 (NS)

Percent Change of Collagen-induced Aggregation Curve

by 2,6-Diacetoxybenzoic Acid (% ± SEM)

Final 2,6-Diacetoxybenzoic Acid Concentration	Curve Slope	Curve Height	Delay Phase
6×10^{-5}M	+10.9 ± 4.93 ($p<0.05$)	+1.4 ± 1.68 (NS)	+8.5 ± 5.93 (NS)
12×10^{-5}M	+6.3 ± 7.27 (NS)	+2.5 ± 1.69 (NS)	+11.5 ± 8.20 (NS)
24×10^{-5}M	+7.5 ± 9.22 (NS)	+2.2 ± 1.86 (NS)	+19.4 ± 5.14 ($p<0.01$)
35×10^{-5}M	+1.2 ± 7.50 (NS)	+2.5 ± 4.03 (NS)	+18.6 ± 7.80 ($p<0.05$)
55×10^{-5}M	+2.5 ± 7.72 (NS)	+2.8 ± 4.03 (NS)	+24.3 ± 12.93 (NS)

Percent Change of Collagen-induced Aggregation Curve by 2-Propoxybenzoic Acid (% ± SEM)

Final 2-Propoxybenzoic Acid Concentration	Curve Slope	Curve Height	Delay Phase
6×10^{-5}M	↓0.1 ± 4.64 (NS)	↑3.1 ± 2.08 (NS)	↑0.7 ± 6.80 (NS)
12×10^{-5}M	↓7.1 ± 2.49 ($p<0.05$)	↓2.9 ± 1.88 (NS)	↑6.8 ± 4.67 (NS)
24×10^{-5}M	↑0.8 ± 5.34 (NS)	↓2.5 ± 1.65 (NS)	↓1.4 ± 6.31 (NS)
35×10^{-5}M	↓0.7 ± 8.48 (NS)	↓2.9 ± 1.70 (NS)	↑0.2 ± 8.61 (NS)
55×10^{-5}M	↓5.8 ± 7.87 (NS)	↓2.2 ± 4.13 (NS)	↑4.9 ± 7.59 (NS)

Percent Change of Collagen-induced Aggregation Curve by

3-Propoxybenzoic Acid (% ± SEM)

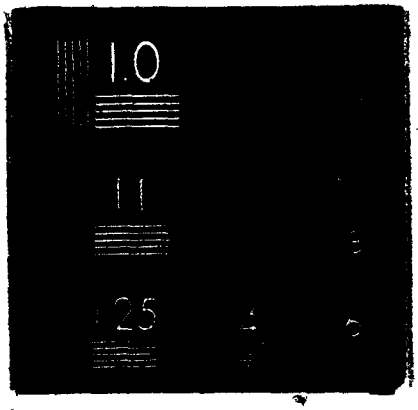
Final 3-Propoxybenzoic Acid Concentration	Curve Slope	Curve Height	Delay Phase
6×10^{-5}M	+8.0 ± 3.29 ($p<0.05$)	+4.0 ± 2.86 (NS)	+0.2 ± 7.08 (NS)
12×10^{-5}M	+1.5 ± 4.80 (NS)	+1.9 ± 3.10 (NS)	+13.9 ± 5.56 (NS)
24×10^{-5}M	+5.3 ± 3.73 (NS)	+5.5 ± 2.06 (NS)	+10.0 ± 8.97 (NS)
35×10^{-5}M	+9.8 ± 3.46 (NS)	+4.2 ± 2.81 (NS)	+18.8 ± 11.25 (NS)
55×10^{-5}M	+12.7 ± 2.66 (NS)	+2.4 ± 2.39 (NS)	+22.4 ± 8.43 (NS)

APPENDIX VII

Representative case histories from patients included in the gastrointestinal hemorrhage study.

3 3

OF/DE



Bleeding associated with ASA

patient: D.M. height: 5'9"

age: 34 weight: 133 lb.

date of birth: September 24, 1938

- patient admitted March 21, 1972 complaining of melena for past 2-3 days and with feeling of dizziness and weakness
- patient admitted to taking approximately 4-6 ASA tablets through previous week
- patient took 222's (x2) the day prior to admission
- this is third episode of bleeding since 1960, at which time X-rays were negative
- X-rays also negative in 1967 during hospitalization
- gastroscopy was performed March 22, 1972 which revealed a small acute prepyloric ulcer with several erosions in the stomach
- patient received 2 units of blood March 21, 1972
- medications in hospital: Valium 5 mg. I.M. x 3, Valium 10 mg. and atropine 0.6 mg. I.M. pre-op for gastroscopy, Magnolax and cascara
- blood taken for platelet studies on March 29, 1972

Bleeding not associated with ASA

patient: J.H. height: 5'5"

age: 45 weight: 140 lb.

date of birth: August 19, 1925

- patient admitted August 8, 1971 after having massive hematemesis and collapsing
- patient complained of "acid stomach" for past 2-3 yr.
- stomach "bothering" him over past few days
- drinks occasionally
- no history of taking ASA
- no history of previous bleeding or melena
- patient found to have melena on rectal examination
- on no other medications prior to admission other than antacids
- upper G.I. series showed defect in greater curvature of stomach
- blood sample taken for platelet studies on August 16, 1971

Control subject - no bleeding

patient: W.S. height: 6'

age: 44 weight: 215 lb.

date of birth: July 6, 1927

- patient admitted August 16, 1971 with chief complaint of chest pain of 5 days duration
- pain was central retrosternal and aggravated by inspiration
- three EEG's normal
- no history of constipation, diarrhea, melena or hematemesis
- occasional drinker, smoked cigarettes for 25 yr.
- no medications prior to admission other than occasional Bromo Seltzer
- took 6 nitroglycerin before admission
- medications in hospital: Demerol, Maalox, Darvon Plain, tetracycline
- blood sample taken for platelet studies on August 25, 1971

BIBLIOGRAPHY

Abdulla, Y. H. (1968), Platelet aggregation after inhibition of ADP release. J. Atherosclerosis Res., 8, 855-858.

Adelstein, R. S., Pollard, T. D. and Kuehl, W. M. (1971), Isolation and characterization of myosin and two myosin fragments from human blood platelets. Proc. Nat. Acad. Sci. USA., 68, 2703-2707.

Ahtee, L. and Michal, F. (1972), Effects of sympathomimetic amines on rabbit platelet aggregation *in vitro*. Br. J. Pharmacol., 44, 363P-364P.

Aledort, L. M., Gilbert, H. S. and Puszkin, E. (1971), Patterns of endogenous platelet serotonin release. Transfusion, 11, 266-269.

Aledort, L. M. and Niemetz, J. (1968), Dissociation of platelet aggregation from clot retraction, potassium loss and adenosine triphosphatase activity. Proc. Soc. Exp. Biol. Med., 128, 658-661.

Al-Mondhiry, H., Marcus, A. J. and Spaet, T. H. (1969), Acetylation of human platelets by aspirin. Fed. Proc., 28, 576.

Al-Mondhiry, H., Marcus, A. J. and Spaet, T. H. (1970), On the mechanism of platelet function inhibition by acetylsalicylic acid. Proc. Soc. Exp. Biol. Med., 133, 632-636.

Al-Mondhiry, H. and Spaet, T. H. (1970), Inhibition of platelet adhesion to collagen by sulfhydryl inhibitors. Proc. Soc. Exp. Biol. Med., 135, 878-882.

Amer, M. S. and Marquis, N. R. (1972), The effect of prostaglandins, epinephrine, and aspirin on cyclic AMP phosphodiesterase activity of human blood platelets and their aggregation. In "Prostaglandins in Cellular Biology". Ramwell, P. W. and Pharriss, B. B. (eds.), Plenum Press, New York, 1972, pp. 93-109.

Amer, M. S. and Mayol, R. F. (1973), Studies with phosphodiesterase III. Two forms of the enzyme from human blood platelets. Biochim. Biophys. Acta, 309, 149-156.

Ando, Y. and Steiner, M. (1972), Quantification of sulfhydryl and disulfide groups in platelet membranes. Fed. Proc., 31, 813.

Ando, Y. and Steiner, M. (1973), Quantitative and qualitative analysis of disulfide bonds in platelet membrane proteins. Fed. Proc., 32, 288.

Apitz, K. (1942), Die Bedeutung der Gerinnung und Thrombose für die Blutstillung. Virchows Arch. (Pathol. Anat.), 308, 540-614.

Ardlie, N. G. and Mustard, J. F. (1969), Divalent cations, platelet membrane fibrinogen, and platelet aggregation. Blood, 34, 538.

Ardlie, N. G., Nishizawa, E. E. and Guccione, M. (1970), Effect of Ca^{++} and Mg^{++} on platelet function. Fed. Proc., 29, 423.

Arfors, K. E., Bergqvist, D., Bygdeman, S., McKenzie, F. N. and Svensjö, E. (1972), The effect of inhibition of the platelet release reaction on platelet behaviour *in vitro* and *in vivo*. Scand. J. Haematol., 9, 322-332.

Atac, A., Spagnuolo, M. and Zucker, M. B. (1970), Long-term inhibition of platelet functions by aspirin. Proc. Soc. Exp. Biol. Med., 133, 1331-1333.

Baenziger, N. L., Brodie, G. N. and Majerus, P. W. (1971), A thrombin-sensitive protein of human platelet membranes. Proc. Natl. Acad. Sci. USA, 68, 240-243.

Baker, R. V., Blaschko, H. and Born, G. V. R. (1959), The isolation from blood platelets of particles containing 5-hydroxytryptamine and adenosine triphosphate. J. Physiol. (Lond.), 149, 55P-56P.

Ball, G., Brereton, G. G., Fulwood, M., Ireland, D. M. and Yates, P. (1970), Effect of prostaglandin E_1 alone and in combination with theophylline or aspirin on collagen-induced platelet nucleotides including adenosine 3',5'-cyclic monophosphate. Biochem. J., 120, 709-718.

Ball, G., Fulwood, M., Ireland, D. M. and Yates, P. (1969), Effect of some inhibitors of platelet aggregation on platelet nucleotides. Biochem. J., 114, 669-671.

Bang, N. U., Heidenreich, R. O. and Matsuda, M. (1970), Plasma protein requirements for human platelet aggregation. Thromb. Diath. Haemorrh., Suppl. 42, 37-48.

Barber, A. J. and Jamieson, G. A. (1970), Isolation and characterization of plasma membranes from human blood platelets. J. Biol. Chem., 245, 6357-6365.

Barber, A. J. and Jamieson, G. A. (1971a), Collagen-glucosyl transferase of human platelet membrane. Fed. Proc., 30, 540.

Barber, A. J. and Jamieson, G. A. (1971b), Platelet collagen adhesion-characterization of collagen glucosyltransferase of plasma membranes of human blood platelets. Biochim. Biophys. Acta, 252 533-545.

Barber, A. J., Triantaphyllopoulos, D. C. and Jamieson, G. A. (1972), Differing procoagulant activities in the extracted lipids of high and low density platelet plasma membranes. Thromb. Diath. Haemorrh., 28, 206-212.

Baumgartner, H. R. and Born, G. V. R. (1968), 5-Hydroxytryptamine in rabbit platelets and their aggregation. J. Physiol. (Lond.), 194, 92P-93P.

Baumgartner, H. R., Grimm, L. and Zbinden, G. (1973), Effect of generation of mural platelet thrombi on circulating blood in rabbit aorta. Experientia, 29, 442-445.

Beaumont, J. L., Caen, J. and Bernard, J. (1955), Action hemorragipare de l'acide acetylsalicylique au cours des maladies du sang. Bull. Soc. Med. Hop. Paris, 71, 1087-1092.

Begent, N. and Born, G. V. R. (1970), Growth rate *in vivo* of platelet thrombi, produced by iontophoresis of ADP, as a function of mean blood flow velocity. Nature (Lond.), 227, 926-930.

Begent, N. A. and Born, G. V. R. (1971), Comparison of the effects of drugs on the aggregation of hamster platelets *in vivo* and *in vitro*. Br. J. Pharmacol., 43, 580-592.

Behnke, O. (1965), Further studies on microtubules. A marginal bundle in human and rat thrombocytes. J. Ultrastruct. Res., 13, 469-477.

Behnke, O. (1967), Some possible practical implications of the lability of blood platelet microtubules. Vox Sang., 13, 502-507.

Behnke, O. (1968), Electron microscopical observations on the surface coating of human blood platelets. J. Ultrastruct. Res., 24, 51-69.

Behnke, O. (1970), Effects of some chemicals on blood platelet microtubules, platelet shape and some platelet functions *in vitro*. Scand. J. Haematol., 7, 123-140.

Benner, K. U. and Frede, K. E. (1972), Platelet aggregation induced by ADP *in vivo*: continuous and quantitative measurements in different vascular beds. Microvasc. Res., 4, 264-272.

Benner, K. U., Frede, K. E. and Lauterjung, K. L. (1970), *In vitro* investigations with a new method for continuous and quantitative measurements of platelet aggregates in flowing blood. Pfluegers Arch. Eur. J. Physiol., 320, 142-151.

Berger, S. (1970), Platelet function: a review Part II. Abnormal function. Can. Med. Assoc. J., 102, 1379-1383.

Berman, H. J., Tangen, O. and Lestrup, E. (1973), Characterization of clotting factors on the platelet surface by gel filtration. Fed. Proc., 32, 219.

Besterman, E. M. M. and Gillett, M. P. T. (1971), Inhibition of platelet aggregation by lysolecithin. Atherosclerosis, 14, 323-330.

Besterman, E. M. M. and Gillett, M. P. T. (1972a), Heparin effects on irreversible platelet aggregation. Lancet, ii, 282-283.

Besterman, E. M. M. and Gillett, M. P. T. (1972b), A comparison of the effects of saturated and polyunsaturated lysolecithin fractions on platelet aggregation and erythrocyte sedimentation. Atherosclerosis, 16, 89-94.

Besterman, E. M. M. and Gillett, M. P. T. (1973), Influence of lysolecithin on platelet aggregation initiated by 5-hydroxytryptamine. Nature (New Biol.), 241, 223-224.

Bettex-Galland, M. and Lüscher, E. F. (1959), Extraction of an actomyosin-like protein from human throbocytes. Nature (Lond.), 184, 276-277.

Bettex-Galland, M. and Lüscher, E. F. (1960), Studies on the metabolism of human blood platelets in relation to clot retraction. Thromb. Diath. Haemorrh., 4, 178-195.

Bettex-Galland, M. and Lüscher, E. F. (1961), Thrombosthenin- a contractile protein from thrombocytes. Its extraction from human blood platelets and some of its properties. Biochim. Biophys. Acta, 49, 536-547.

Bettex-Galland, M., Lüscher, E. F. and Weibel, E. R. (1969), Thrombosthenin- electron microscopical studies on its localization in human blood platelets and some properties of its subunits. Thromb. Diath. Haemorrh., 22, 431-449.

Blaisdell, R. K., Stemerman, M. B., Cintron, J. R. and Spaet, T. H. (1973), An *in vitro* method for evaluating platelet-subendothelial interaction. Fed. Proc., 32, 836.

Blatrix, C. (1963), Allongement du temps de saignement sous l'influence de certain medicaments. Nouv. Rev. Fr. Hematol., 3, 346-350.

Bloom, A. L. and Beamish, M. R. (1966), Acetylsalicylic acid and bleeding-time. Lancet, ii, 1319-1320.

Boffa, M. C., Josso, F. and Boffa, G. A. (1972), The action of Vipera aspis venom on blood clotting factors and platelets. Thromb. Diath. Haemorrh., 27, 8-18.

Booyse, F. M., Hoveke, T. P. and Rafelson, M. E. Jr. (1968), Studies on human platelets II. Protein synthetic activity of various platelet populations. Biochim. Biophys. Acta, 157, 660-663.

Booyse, F. M., Hoveke, T. P., Zschocke, D. and Rafelson, M. E. Jr. (1971), Human platelet myosin. Isolation and properties. J. Biol. Chem., 246, 4291-4297.

Booyse, F. M. and Rafelson, M. E. Jr. (1967a), Stable messenger RNA in the synthesis of contractile protein in human platelets. Biochim. Biophys. Acta, 145, 188-191.

Booyse, F. M. and Rafelson, M. E. Jr. (1967b), *In vitro* incorporation of amino-acids into the contractile protein of human blood platelets. Nature (Lond.), 215, 283-284.

Booyse, F. M. and Rafelson, M. E. Jr. (1968), Studies on human platelets. I. Synthesis of platelet protein in a cell-free system. Biochim. Biophys. Acta, 166, 689-697.

Booyse, F. M. and Rafelson, M. E. Jr. (1971), Human platelet contractile proteins: localization, properties and function. Ser. Haematol., 4, 152-174.

Booyse, F. M. and Rafelson, M. E. Jr. (1972), Mechanism and control of platelet-platelet interactions III. A relaxation-contractile model for platelet aggregation. Microvasc. Res., 4, 207-213.

Booyse, F. M., Zschocke, D. and Rafelson, M. E. Jr. (1972), Microquantitation of thrombosthenin in human platelets by single radical immunodiffusion. J. Lab. Clin. Med., 79, 344-352.

Born, G. V. R. (1962), Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature (Lond.), 194, 927-929.

Born, G. V. R. (1964), Strong inhibition by 2-chloroadenosine of the aggregation of blood platelets by adenosine diphosphate. Nature (Lond.), 202, 95-96.

Born, G. V. R. (1965), Uptake of adenosine and of adenosine diphosphate by human blood platelets. Nature (Lond.), 206, 1121-1122.

Born, G. V. R. (1968), The platelet membrane and its function. In "Metabolism and Membrane Permeability of Erythrocytes and Thrombocytes". Deutsch, E., Gerlach, E. and Moser, K. (eds.), Georg Thieme Verlag, Stuttgart; 1968, pp. 294-302.

Born, G. V. R. and Cross, M. J. (1962), Inhibition of the aggregation of blood platelets by substances related to adenosine diphosphate. J. Physiol. (Lond.), 166, 29P-30P.

Born, G. V. R. and Cross, M. J. (1963), The aggregation of blood platelets. J. Physiol. (Lond.), 168, 178-195.

Born, G. V. R. and Cross, M. J. (1964), Effects of inorganic ions and of plasma proteins on the aggregation of blood platelets by adenosine diphosphate. J. Physiol. (Lond.), 170, 397-414.

Born, G. V. R. and Gillson, R. E. (1959), Studies on the uptake of 5-hydroxytryptamine by blood platelets. J. Physiol. (Lond.), 146, 472-491.

Born, G. V. R., Haslam, R. J., Goldman, M. and Lowe, R. D. (1965), Comparative effectiveness of adenosine analogues as inhibitors of blood-platelet aggregation and as vasodilators in man. Nature (Lond.), 205, 678-680.

Born, G. V. R., Honour, A. J. and Mitchell, J. R. A. (1964), Inhibition by adenosine and by 2-chloroadenosine of the formation and embolization of platelet thrombi. Nature (Lond.), 202, 761-765.

Born, G. V. R., Ingram, G. I. C. and Stacey, R. S. (1958), The relationship between 5-hydroxytryptamine and adenosine triphosphate in blood platelets. Br. J. Pharmacol., 13, 62-64.

Born, G. V. R., Juengjaroen, K. and Michal, F. (1972), Relative activities on and uptake by human blood platelets of 5-hydroxytryptamine and several analogues. Br. J. Pharmacol., 44, 117-139.

Born, G. V. R. and Mills, D. C. B. (1969), Potentiation of the inhibitory effect of adenosine on platelet aggregation by drugs that prevent its uptake. J. Physiol. (Lond.), 202, 41P-42P.

Born, G. V. R. and Philp, R. B. (1965), Effects of adenosine analogues and of heparin on platelet thrombi in non-lipaemic and lipaemic rats. Br. J. Exp. Pathol., 46, 569-576.

Bosmann, H. B. (1971), Platelet adhesiveness and aggregation; the collagen:glycosyl, polypeptide:N-acetylgalactosaminyl and glycoprotein: galactosyl transferases of human platelets. Biochem. Biophys. Res. Commun., 43, 1118-1124.

Bosmann, H. B. (1972), Identification, purification and characteristics of glycosidases, of human blood platelets. Biochim. Biophys. Acta, 258, 265-273.

Boullin, D. J., Green, A. R. and Price, K. S. (1972), The mechanism of adenosine diphosphate induced platelet aggregation: binding to platelet receptors and inhibition of binding and aggregation by prostaglandin E_1. J. Physiol. (Lond.), 221, 415-426.

Bounameaux, Y. (1957), Dosage de facteurs coagulation continus dans l'atmosphere plasmatique des plaquettes humaine. Rev. Fr. Etude Clin. Biol., 2, 52-63.

Bounameaux, Y. (1971), A multitest study of anti-aggregating agents. Thromb. Diath. Haemorrh., Suppl. 45, 89-94.

Bounameaux, Y. and van Cauwenberge, H. (1954), Action de la cortisone, de l'ACTH et du salicylate de soude sur les thrombocytes et la coagulation sanguine. Sang, 25, 889.

Bowie, E. J. W. and Owen, C. A. (1969), Aspirin, platelets and bleeding Circulation, 40, 757-760.

Brinkhous, K. M. (1959), Blood clotting: the plasma procoagulants. Annu. Rev. Physiol., 21, 271-298.

Brinkhous, K. M., LeRoy, E. C., Cornell, W. P., Brown, R. C., Hazlehurs J. L. and Vennart, G. P. (1958), Macroscopic studies of platelet agglutination; nature of thrombocyte agglutinating activity of plasma. Proc. Soc. Exp. Biol. Med., 98, 379-383.

Brinson, K. (1972), Effect of aminophylline on blood platelet reactions Atherosclerosis, 16, 233-239.

Brinson, K. (1973), Effect of sulfhydryl inhibitors and membrane-active drugs on platelet reactions. Atherosclerosis, 17, 139-145.

Broersma, R. J., Dickerson, G. D. and Sullivan, M. S. (1973), The deter mination of platelet aggregation by filtration pressure in circula ting dog blood. Thromb. Diath. Haemorrh., 29, 201-210.

Bucher, H. W. and Stucki, P. (1969), The effect of various beta-recepto blocking agents on platelet aggregation. Experientia, 25, 280-282

Buckell, M. (1958), The effect of citrate on euglobulin methods of estimating fibrinolytic activity. J. Clin. Pathol., 11, 403-405.

Buehler, B. and Rennert, O. (1970), A new approach to the study of platelet aggregation. Fed. Proc., 29, 623.

Bull, B. S. and Zucker, M. B. (1965), Changes in platelet volume produc by temperature, metabolic inhibitors, and aggregating agents. Pr Soc. Exp. Biol. Med., 120, 296-301.

Butterfield, W. J. H. (1973), Aspirin and postoperative venous thrombosis. Lancet, i, 48.

Bygdeman, S. (1968), Effect of α- and β- receptor blocking agents on platelet aggregation and uptake of noradrenaline. Acta Physiol. Scand., 73, 28A-29A.

Caen, J. P., Jenkins, C. S. P., Michel, H. and Bellanger, R. (1972), Adenosine inhibition of human platelet aggregation by ADP. Nature (New Biol.), 239, 211-213.

Caen, J. P., Legrand, Y. and Sultan, Y. (1970), Platelet-collagen interactions. Thromb. Diath. Haemorrh., Suppl. 40, 181-197.

Campbell, E. W., Small, W. J. and Dameshek, W. (1956), Metabolic activity of human blood platelets. J. Lab. Clin. Med., 47, 835-843.

Castaldi, P. A., Firkin, B. G., Blackwell, P. M. and Clifford, K. I. (1962), An electron microscopic study of the changes in platelets during viscous metamorphosis. Blood, 20, 566-580.

Cazenave, J.-P., Packham, M. A., Guccione, M. A. and Mustard, J. F. (1973), Effects of penicillin G on platelet aggregation, release, and adherence to collagen. Proc. Soc. Exp. Biol. Med., 142, 159-166.

Chandrasekhar, N. (1967), Inhibition of platelet aggregation by prostaglandins. Blood, 30, 554.

Chattaway, F. D. (1931), Acetylation in aqueous alkaline solutions. J. Chem. Soc., 2495-2496.

Chaudhry, A. A., Sagone, A. L. Jr., Metz, E. N. and Balcerzak, S. P. (1973), Relationship of glucose oxidation to aggregation of human platelets. Blood, 41, 249-258.

Chen, T. I. and Tsai, C. (1948), The mechanism of haemostasis in peripheral vessels. J. Physiol. (Lond.), 107, 280-288.

Chesney, C., Harper, E. and Colman, R. W. (1972), Critical role of the carbohydrate side chain of collagen in platelet aggregation. Fed. Proc., 31, 242.

Clarke, N. E., Clarke, C. N. and Mosher, R. E. (1958), Phenolic compounds in chemotherapy of rheumatic fever. Am. J. Med. Sci., 235, 7-22.

Clauss, A. (1957), Gerinnungsphysiologische Schnelemethode zür Bestimmung des Fibrinogens. Acta Haematol., 17, 237-247.

Clawson, C. C. and White, J. G. (1971), Platelet interaction with bacteria. I. Reaction phases and effects of inhibitors. Am. J. Pathol., 65, 367-380.

Claxton, G. P., Grisar, J. M., Roberts, E. M. and Fleming, R. W. (1972), Synthesis of α-[p-(fluoren-9-ylidene-methyl)phenyl]-2-piperidine-ethanol, an inhibitor of platelet aggregation. J. Med. Chem., 15, 500-504.

Clayton, S., Born, G. V. R. and Cross, M. J. (1963), Inhibition of the aggregation of blood platelets by nucleosides. Nature (Lond.), 200, 138-139.

Clayton, S. and Cross, M. J. (1963), The aggregation of blood platelets by catecholamines and by thrombin. J. Physiol. (Lond.), 169, 82P-83P.

de Clerck, F. (1972), Effect of lidoflazine (R7904) on human platelet function *in vitro*. Thromb. Diath. Haemorrh., 28, 228-236.

Cole, B., Robison, G. A. and Hartmann, R. C. (1970), Effects of prostaglandin E_1 and theophylline on aggregation and cyclic AMP levels of blood platelets. Fed. Proc., 29, 316.

Constantine, J. W. (1965), Inhibition of adenosine diphosphate-induced platelet aggregation by histamine. Nature (Lond.), 207, 91.

Constantine, J. W. (1966), Aggregation of guinea pig platelets by adenosine diphosphate. Nature (Lond.), 210, 162-164.

Constantine, J. W. (1970), Aggregation patterns and release of adenosine diphosphate from guinea pig platelets. Atherosclerosis, 12, 25-30.

Constantine, J. W., Purcell, I. M. and Gotthelf, M. (1971), Platelets, coagulation and *in vitro* thrombus formation. J. Pharmacol. Exp. Ther., 176, 76-82.

Cronberg, S. and Caen, J. P. (1970), Mg^{++}- and Ca^{++}- induced platelet aggregation and ADP. Thromb. Diath. Haemorrh., 24; 432-437.

Cronberg, S., Kubisz, P. and Caen, J. P. (1970), Demonstration of a plasmatic cofactor different from fibrinogen necessary for platelet release by ADP and adrenaline. Thromb. Diath. Haemorrh., 24, 409-418.

Cronkite, E. P., Bond, V. P., Fliedner, T. M., Paglia, D. A. and Adamik, E. R. (1961), Studies on the origin, production and destruction of platelets. In "Blood Platelets", Johnson, S. A., Monto, R. W., Rebuck, J. W. and Horn, R. C. (eds.), Little, Brown and Company, Boston, 1961, pp. 595-609.

Cross, M. J. (1964), Effect of fibrinogen on the aggregation of platelets by adenosine diphosphate. Thromb. Diath. Haemorrh., 12, 524-527.

Cucuianu, M. P., Nishizawa, E. E. and Mustard, J. P. (1971), Effect of pyrimido-pyrimidine compounds on platelet function. J. Lab. Clin. Med., 77, 958-974.

Cumings, J. N. and Hilton, B. P. (1971), Effects of methysergide on platelets incubated with reserpine. Br. J. Pharmacol., 42, 611-619.

Dacie, J. V. and Lewis, S. M. (1963), "Practical Haematology", J. A. Churchill Ltd., London, 1963, pp. 32-56.

DaPrada, M. and Pletscher, A. (1968), Isolated 5-hydroxytryptamine organelles of rabbit blood platelets: physiological properties and drug-induced changes. Br. J. Pharmacol., 34, 591-597.

DaPrada, M., Pletscher, A. and Tranzer, J. P. (1971), Storage of ATP and 5-hydroxytryptamine in blood platelets of guinea-pigs. J. Physiol. (Lond.), 217, 679-688.

DaPrada, M., Pletscher, A., Tranzer, J. P. and Knuchel, H. (1967), Subcellular localization of 5-hydroxytryptamine and histamine in blood platelets. Nature (Lond.), 216, 1315-1317.

Davey, M. G. and Luscher, E. F. (1965), Actions of some coagulant snake venoms on blood platelets. Nature (Lond.), 207, 730-732.

Davey, M. G. and Luscher, E. F. (1967), Actions of thrombin and other coagulant and proteolytic enzymes on blood platelets. Nature (Lond.), 216, 857-858.

Davey, M. G. and Luscher, E. F. (1968), Release reactions of human platelets induced by thrombin and other agents. Biochim/ Biophys. Acta, 165, 490-506.

Davies, D. T. P., Hughes, A. and Tonks, R. S. (1968), Salicylates, platelets and adenine nucleotides. Arch. Pharmacol. Exp. Pathol., 259, 163.

Davies, D. T. P., Hughes, A. and Tonks, R. S. (1969), The influence of salicylate on platelets and whole blood adenine nucleotides. Br. J. Pharmacol., 36, 437-447.

Davis, J. W. and Phillips, P. E. (1970), The effect of ethanol on human platelet aggregation *in vitro*. Atherosclerosis, 11, 473-477.

Davis, J. W. and Phillips, R. E. (1971), The effect of imidazole on human platelet aggregation. Blood, 38, 417-421.

Davis, R. B. and Holtz, G. C. (1970), Alterations of platelet aggregation in the Chandler Tube: II. Electron microscopic observations. Am. J. Clin. Pathol., 54, 683-691

Davis, R. B. and Holtz, G. C. (1971), Effects of lead acetate on platelet aggregation, ultrastructure and serotonin release. Thromb. Diath. Haemorrh., 26, 455-466.

Day, H. J., Holmsen, H. and Hovig, T. (1969), Subcellular particles of human platelets. Scand. J. Haematol., Suppl. 7, 1-35.

De Luca, L., Barber, A. J. and Jamieson, G. A. (1972), Possible role of vitamin A in platelet collagen adhesion. Fed. Proc., 31, 242.

Des Prez, R. M., Horowitz, H. I. and Hook, E. W. (1961), Effects of bacterial endotoxin on rabbit platelets. I. Platelet aggregation and release of platelet factors *in vitro*. J. Exp. Med., 114, 857-874.

Detwiler, T. C. and Zivkovic, R. V. (1970), Control of energy metabolism in platelets. A Comparison of aerobic and anaerobic metabolism in washed rat platelets. Biochim. Biophys. Acta, 197, 117-126.

Deutsch, E., Lechner, K., Moser, K. and Stockinger, L. (1971), The action of an aniline derivative (AN 162) on blood coagulation and on platelets. Thromb. Diath. Haemorrh., 26, 145-166.

Deykin, D. (1970), Subcellular distribution of platelet lipids labelled by acetate-1-^{14}C. Fed. Proc., 29, 442.

Deykin, D. and Desser, R. K. (1968), The incorporation of acetate and palmitate into lipids by human platelets. J. Clin. Invest., 47, 1590-1602.

Deykin, D., Pritzker, C. R. and Scolnick, E. M. (1965), Plasma co-factors in adenosine diphosphate-induced aggregation of human platelets. Nature (Lond.), 208, 296-298.

Dhall, D. P., Harper, D. R., McKenzie, F. N. and Matheson, N. A. (1966), Aggregation of human platelets by dextrans. Nature (Lond.), 210, 745-746.

Dhall, D. P. and Matheson, N. A. (1968), *In vitro* clumping of human platelets by dextran. Thromb. Diath. Haemorrh., 19, 70-76.

Doery, J. C. G., Hirsh, J. and Cooper, I. (1970), Energy metabolism in human platelets: interrelationships between glycolysis and oxidative metabolism. Blood, 36, 159-168.

Doery, J. C. G., Hirsh, J. and De Gruchy, G. C. (1969), Aspirin, its effect on platelet glycolysis and release of adenosine diphosphate. Science, 165, 65-67.

Done, A. K. (1960), The nature of the antirheumatic action of salicylates. Clin. Pharmacol. Ther., 1, 141-148.

Dröller, M. J. and Wolfe, S. M. (1972), Thrombin-induced increase in intracellular cyclic 3' ,5'-adenosine monophosphate in human platelets. J. Clin. Invest., 51, 3094-3103.

Eberth, J. C. and Schimmelbusch, C. (1886), Experimentelle Untersuchungen über Thrombose. Virchows Arch. (Pathol. Anat.), 103, 39-87.

Eika, C. (1971), Inhibition of thrombin induced aggregation of human platelets by heparin. Scand. J. Haematol., 8, 216-222.

Eika, C. (1972), On the mechanism of platelet aggregation induced by heparin, protamine and polybrene. Scand. J. Haematol., 9, 248-257.

Eika, C. and Abildgaard, U. (1970), Inhibition of thrombin induced aggregation of human platelets by antithrombin III. Scand. J. Haematol., 7, 460-464.

Elkeles, R. S., Hampton, J. R., Honour, A. J., Mitchell, J. R. A. and Prichard, J. S. (1968), Effect of a pyrimido-pyrimidine compound on platelet behavior *in vitro* and *in vivo*. Lancet, ii, 751-754.

Elslager, E. F., Haley, N. F., McLean, J. R., Perricone, S. C., Potoczak, D., Veloso, H., Worth, D. F. and Wheelock, R. H. (1971), Inhibitors of platelet aggregation.2.9-{[(Dialkylamino)alkyl]thio} -3-(dimethylamino) acridines and related acridine derivatives. J. Med. Chem., 14, 782-789.

Elslager, E. F., Haley, N. F., McLean, J. R., Potoczak, D., Veloso, H. and Wheelock, R. H. (1972), Inhibitors of platelet aggregation 3. {[(djalkylamino)alkyl]thio} heterocyclic compounds. J. Med. Chem., 15, 61-65.

Elslager, E. F., McLean, J. R., Perricone, S. C., Potoczak, D., Veloso, H., Worth, D. F. and Wheelock, R. H. (1971), Inhibitors of platelet aggregation J. 5,10-dihydro-3-(phenyl, thienyl, and furyl)thiazolo[3,2-b] [2,4] benzodiazepines and related compounds. J. Med. Chem., 14, 397-401.

Emmons, P. R., Hampton, J. R., Harrison, M. J. G., Honour, A. J. and Mitchell, J. R. A. (1967), Effect of prostaglandin E_1 on platelet behaviour *in vitro* and *in vivo*. Br. Med. J., 2, 468-472.

Emmons, P. R., Harrison, M. J. G., Honour, A. J. and Mitchell, J. R. A. (1965a), Effect of dipyridamole on human platelet behaviour. Lancet, ii, 603-606.

Emmons, P. R., Harrison, M. J. G., Honour, A. J. and Mitchell, A. J. (1965b), Effect of a pyrimidopyrimidine derivative on thrombus formation in the rabbit. Nature (Lond.), 208, 255-257.

Evans, G., Nishizawa, E. E., Packham, M. A. and Mustard, J. F. (1967), The effect of acetylsalicylic acid (aspirin) on platelet function. Blood, 30, 550.

Evans, G., Packham, M. A., Nishizawa, E. E., Mustard, J. F. and Murphy, E. A. (1968), The effect of acetylsalicylic acid on platelet function. J. Exp. Med., 128, 877-894.

Fleming, J. S. Bierwagen, M. E., Losada, M., Campbell, J. A. L., King, S. P. and Pindell, M. H. (1970), The effects of three anti-inflammatory agents on platelet aggregation, *in vitro* and *in vivo*. Arch. Int. Pharmacodyn. Ther., 186, 120-128.

Fregnan, G. B. (1972), The inhibitory effect of metergoline (an anti-5-hydroxytryptamine agent) on rabbit platelet aggregation. Pharmacology, 7, 115-125.

French, J. E. (1965), The structure of natural and experimental thrombi. Ann. R. Coll. Surg. Engl., 36, 191-200.

Frick, P. G. (1956), Hemorrhagic diathesis with increased capillary fragility caused by salicylate therapy. Am. J. Med. Sci., 231, 402-406.

Gaarder, A., Jonsen, J., Laland, S., Hellem, A. and Owren, P. A. (1961), Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. Nature (Lond.), 192, 531-532.

Gaarder, A. and Laland, S. (1964), Hypothesis for the aggregation of platelets by nucleotides. Nature (Lond.), 202, 909-910.

Gadd, R. E. A., Clayman, S. and Hebert, D. (1971a), The effect of several diuretics on adenosine-diphosphate induced platelet aggregation *in vitro*. Experientia, 27, 1339-1340.

Gadd, R. E. A., Clayman, S. and Hebert, D. (1971b), Effect of lobeline on guinea pig platelet aggregation induced by adenosine diphosphate, collagen and thrombin. Pharmacol. Res. Commun., 3, 279-286.

Ganguly, P. (1971), Isolation and properties of a thrombin-sensitive protein from human blood platelets. J. Biol. Chem., 246, 4286-4290.

Ganguly, P. (1972), Isolation and some properties of fibrinogen from human blood platelets. J. Biol. Chem., 247, 1809-1816.

Gast, L. F. (1964), Influence of aspirin on haemostatic parameters. Ann. Rheum. Dis., 23, 500-504.

Gibelli, A., Montanari, C., Bellani, D., Mandelli, V. and Sacchetti, G. (1973), Beta-blocking drugs and human platelet aggregation *in vitro*. Experientia, 29, 186-187.

Glynn, M. F., Movat, H. Z., Murphy, E. A. and Mustard, J. F. (1965), Study of platelet adhesiveness and aggregation, with latex particles. J. Lab. Clin. Med., 65, 179-201.

Gokçen, M. and Yunis, E. (1963), Fibrinogen as a part of platelet structure. Nature (Lond.), 200, 590-591.

Goldman, B., Aledort, L. M., Puszkin, E. and Burrows, L. (1971), Cyproheptadine, a new platelet deaggregating agent. Circulation, 44, Suppl. II-68.

Gordon, J. L. and Gresham, G. A. (1972), The measurement of platelet aggregation in small blood samples. Atherosclerosis, 15, 383-386.

Gorog, P. and Kovacs, I. B. (1970), Effects of anti-inflammatory compounds on actomyosin-adenosinetriphosphate interaction. Biochem. Pharmacol., 19, 2289-2294.

Gorstein, F., Carroll, H. J. and Puszkin, E. (1967), Electrolyte concentrations, potassium flux-kinetics and the metabolic dependence of potassium transport in human platelets. J. Lab. Clin. Med., 70, 938-950.

Gough, G., Maguire, M. H. and Penglis, F. (1972), Analogues of adenosine 5'-diphosphate - new platelet aggregators. Influence of purine ring and phosphate chain substitutions on the platelet-aggregating potency of adenosine 5'-diphosphate. Molec. Pharmacol., 8, 170-177.

Gregersen, J. P. (1916), Occult blood in the stools. J. A. M. A., 67, 84.

Greil, W., Patscheke, H. and Brossmer, R. (1972), Effect of lithium and other monovalent cations on the ADP-induced platelet aggregation in human platelet rich plasma. FEBS Lett., 26, 271-273.

Grette, K. (1962), Studies on the mechanism of thrombin-catalyzed hemostatic reactions in blood platelets. Acta Physiol. Scand., Suppl. 195, 5-93.

Gross, R., Niemeyer, G. and Reuter, H. (1968), Localization of inhibition of platelet aggregation by incubation of platelet rich EDTA-plasma at 37°C. Thromb. Diath. Haemorrh., 19, 1-11.

Grottum, K. A., Hjort, P. F. and Jeremic, M. (1969), Interaction of bacterial endotoxin and liquoid with blood platelets: aggregation and decrease in the electrokinetic charge. Thromb. Diath. Haemorrh., 22, 192-202.

Haft, J. I., Gershengorn, K., Kranz, P. D. and Oestreicher, R. (1972), Protection against epinephrine-induced myocardial necrosis by drugs that inhibit platelet aggregation. Am. J. Cardiol., 30, 838-843.

Haft, J. I., Kranz, P. D., Albert, F. J. and Fani, K. (1972), Intravascular platelet aggregation in the heart induced by norepinephrine: microscopic studies. Circulation, 46, 698-708.

Hampton, J. R., Harrison, M. J. G., Honour, A. J. and Mitchell, J. R. A. (1967), Platelet behaviour and drugs used in cardiovascular disease. Cardiovasc. Res., 1, 101-107.

Hampton, J. R., Harrison, M. J. G., Honour, A. J., Mitchell, J. R. A. and Prichard, J. S. (1972), Assessment of anti-thrombotic agents: effects of dipyridamole analogues on platelet behaviour. Cardiovasc. Res., 6, 696-701.

Hampton, J. R. and Mitchell, J. R. A. (1966), Modification of the electrokinetic response of blood platelets to aggregating agents. Nature (Lond.), 210, 1000-1002.

Hardisty, R. M. and Hutton, R. A. (1965), The kaolin clotting time of platelet-rich plasma: a test of platelet factor 3 availability. Br. J. Haematol., 11, 258-268.

Hardisty, R. M., Hutton, R. A., Montgomery, D., Rickard, S. and Trebilcock, H. (1970), Secondary platelet aggregation: a quantitative study. Br. J. Haematol., 19, 307-319.

Harwood, J. P., Moskowitz, J. and Krishna, G. (1972), Dynamic interaction of prostaglandin and norepinephrine in the formation of adenosine 3',5'-monophosphate in human and rabbit platelets. Biochim. Biophys. Acta, 261, 444-456.

Harker, L. A. and Slichter, S. J. (1970), Studies of platelet and fibrinogen kinetics in patients with prosthetic heart valves. N. Engl. J. Med., 283, 1302-1305.

Harrison, M. J. G., Emmons, P. R. and Mitchell, J. R. A. (1966), The effect of sulfhydryl and enzyme inhibitors on platelet aggregation *in vitro*. Thromb. Diath. Haemorrh., 16, 122-133.

Haslam, R. J. (1964), Role of adenosine diphosphate in the aggregation of human blood-platelets by thrombin and by fatty acids. Nature (Lond.), 202, 765-768.

Haslam, R. J. (1972), Inhibition of blood platelet function by cytochalasins: effects on thrombosthenin and on glucose metabolism. Biochem. J., 127, 34P.

Haslam, R. J. and Lynham, J. A. (1972), Activation and inhibition of blood platelet adenyl cyclase by adenosine and by 2-chloroadenosine. Life Sci., 11, 1143-1154.

Haslam, R. J. and Rosson, G. M. (1971), Effect of vasopressin on human blood platelets. J. Physiol. (Lond.), 219, 36P-38P.

Hassanein, A.A., Turpie, A. G. G., McNicol, G. P. and Douglas, A. S. (1970), Effect of RA 233 on platelet function *in vitro*. Br. Med. J., 2, 83-86.

Hawkins, D., Pinckard, R. N., Crawford, I. P. and Farr, R. S. (1969), Structural changes in human serum albumin induced by ingestion of acetylsalicylic acid. J. Clin. Invest., 48, 536-542.

Hawkins, D., Pinckard, R. N. and Farr, R. S. (1968), Acetylation of human serum albumin by acetylsalicylic acid. Science, 160, 780-781.

Hellem, A. J. (1960), The adhesiveness of human blood platelets *in vitro*. Scand. J. Clin. Lab. Invest., Suppl. 51, 1-117.

Hennes, A. R., Awai, K. and Hammarstrand, K. (1964), Studies of incorporation of radioactivity into lipids of human blood. V. Pattern of fatty acid radioactivity in lipid fractions of platelets. Biochim. Biophys. Acta, 84, 610-612.

Herrmann, R. G. and Frank, J. D. (1966), Effect of adenosine derivatives and antihistaminics on platelet aggregation. Proc. Soc. Exp. Biol. Med., 123, 654-660.

Herrmann, R. G., Lacefield, W. B. and Crowe, V. G. (1970), Effect of ionic calcium and magnesium on human platelet aggregation. Proc. Soc. Exp. Biol. Med., 135, 100-103.

Herrmann, R. G., Marshall, W. S., Crowe, V. G., Frank, J. D., Marlett, D. L. and Lacefield, W. B. (1972), Effect of a new anti-inflammatory drug, fenoprofen, on platelet aggregation and thrombus formation. Proc. Soc. Exp. Biol. Med., 139, 548-552.

Higashi, O., Kikuchi, Y. and Konno, K. (1972), A case of thrombasthenia with a study of platelet aggregation by hydrogen peroxide (H_2O_2). Tohoku J. Exp. Med., 106, 399-409.

Hirsh, J. and Doery, J. C. G. (1971), Platelet function in health and disease. Prog. Hematol., 7, 185-234.

Hofstee, B. H. J. (1952), Specificity of esterases. II. Behavior of pancreatic esterases I and II towards a homologous series of n-fatty acid esters. J. Biol. Chem., 199, 365-371.

Holdrinet, A., Ewals, M. and Haanen, C. (1969), A simple test for measuring platelet aggregation. Thromb. Diath. Haemorrh., 22, 174-191.

Holemans, R. and Gross, R. (1961), Fibrinolytic activities present in human blood platelets. Nature (Lond.), 189, 238-239.

Holmsen, H. (1965), Collagen-induced release of adenosine diphosphate from blood platelets incubated with radioactive phosphate *in vitro*. Scand. J. Clin. Lab. Invest., 17, 239-246.

Holmsen, H. and Day, H. J. (1968), Thrombin-induced platelet release reaction and platelet lysosomes. Nature (Lond.), 219, 760-761.

Holmsen, H., Day, H. J. and Setkowsky, C. A. (1972), Behaviour of adenine nucleotides during the platelet release reaction induced by adenosine diphosphate and adrenaline. Biochem. J., 129, 67-82.

Holmsen, H., Day, H. J. and Storm, E. (1969), Adenine nucleotide metabolism of blood platelets. VI. Subcellular localization of nucleotide pools with different functions in the platelet release reaction. Biochim. Biophys. Acta, 186, 254-266.

Holmsen, H., Day, H. J. and Stormorken, H. (1969), The blood platelet release reaction. Scand. J. Haematol., Suppl. 8, 1-26.

Holmsen, H. and Rozenberg, M. C. (1968), Adenine nucleotide metabolism of blood platelets. I. Adenosine kinase and nucleotide formation from exogenous adenosine and AMP. Biochim. Biophys. Acta, 155, 326-341.

Holmsen, H., Storm, E. and Day, H. J. (1970), Protein bound ADP in platelets: a possible function in the release reaction. Fed. Proc., 29, 316.

Holmsen, H. and Weiss, H. J. (1970), Hereditary defect in the platelet release reaction caused by a deficiency in the storage pool of platelet adenine nucleotides. Br. J. Haematol., 19, 643-649.

Holmsen, H., Whaun, J. and Day, H. J. (1971), Inhibition by lanthanum ions of ADP-induced platelet aggregation. Experientia, 27, 451-453.

Holtz, G. C. and Davis, R. B. (1972), Inhibition of human platelet aggregation by dimethylsulfoxide, dimethylacetamide, and sodium glycerophosphate. Proc. Soc. Exp. Biol. Med., 141, 244-248.

Horlington, M. and Watson, P. A. (1970), Inhibition of 3',5'-cyclic-AMP phosphodiesterase by some platelet aggregation inhibitors. Biochem. Pharmacol., 19, 955-956.

Hornstra, G. (1970), Method to determine the degree of ADP-induced platelet aggregation in circulating rat blood (Filter-loop technique). Br. J. Haematol., 19, 321-329.

Hornstra, G. (1971), Degree and duration of prostaglandin E_1-induced inhibition of platelet aggregation in the rat. Eur. J. Pharmacol., 15, 343-349.

Horowitz, H. I. and Fujimoto, M. M. (1965), Association of factors XI and XII with blood platelets. Proc. Soc. Exp. Biol. Med., 119, 487-492.

Horowitz, H. I. and Papayoanou, M. F. (1968), Activation of platelet factor 3 by adenosine 5' diphosphate. Thromb. Diath. Haemorrh., 19, 18-28.

Horowitz, H. I., Stein, I. M., Cohen, B. D. and White, J. G. (1969), A comparison of the effects of guanidinosuccinic acid and aspirin on platelet factor-3 activation and platelet aggregation. Blood, 34, 537.

Hovig, T. (1962), The ultrastructure of rabbit blood platelet aggregates. Thromb. Diath. Haemorrh., 8, 455-471.

Hovig, T. (1963a), Aggregation of rabbit blood platelets produced *in vitro* by saline 'extract' of tendons. Thromb. Diath. Haemorrh., 9, 248-263.

Hovig, T. (1963b), Release of platelet-aggregating substance (adenosine diphosphate) from rabbit blood platelets induced by saline "extract" of tendons. Thromb. Diath. Haemorrh., 9, 264-278.

Hovig, T. (1964), The effect of calcium and magnesium on rabbit blood platelet aggregation *in vitro*. Thromb. Diath. Haemorrh., 12, 179-200.

Hovig, T. (1965), The effect of various enzymes on the ultrastructure, aggregation, and clot retraction ability of rabbit blood platelets. Thromb. Diath.Haemorrh., 13, 84-113.

Hovig, T. (1968), The ultrastructure of blood platelets in normal and abnormal states. Ser. Haematol., 1, (No.2), 3-64.

Howard, M. A. and Firkin, B. G. (1971), Ristocetin- a new tool in the investigation of platelet aggregation. Thromb. Diath. Haemorrh., 26, 362-369.

Howell, W. H. (1935), Theories of blood coagulation. Physiol. Rev., 15, 435-470.

Hughes, J. (1962), Accolement des plaquettes aux structures conjunctives perivasculaires. Thromb. Diath. Haemorrh., 8, 241-255.

Hughes, A. and Tonks, R. S. (1968), Some observations on the use of aspirin in certain thrombotic diseases. Br. J. Pharmacol., 33, 219P-220P.

Hugues, J. (1953), Contribution à l'etude des facteurs vasculaires et sanguins dans l'hemostase spontanee. Arch. Int. Physiol., 61, 565-711.

Hugues, J. and Mahieu, P. (1970), Platelet aggregation induced by basement membranes. Thromb. Diath. Haemorrh., 24, 395-408.

Humphrey, J. H. and Jaques, R. (1955), Release of histamine and 5-hydroxytryptamine (serotonin) from platelets by antigen-antibody reactions (*in vitro*). J. Physiol. (Lond.), 128, 9-27.

Hunt, S. E., Jones, J. I. and Lindsey, A. S. (1956), 4-Hydroxy-isophthalic acid. J. Chem. Soc., 3099-3117.

Iatridis, S. G., Ferguson, J. H. and Iatridis, P. G. (1970), Intestinal alkaline phosphatase as a thrombogenic and platelet-altering factor in rabbits. Thromb. Diath. Haemorrh., 24, 191-202.

Ingerman, C., Smith, J. B., Kocsis, J. J. and Silver, M. J. (1973), Arachidonic acid induces platelet aggregation and platelet prostaglandin formation. Fed. Proc., 32, 219.

Ireland, D. M. (1967), Effect of thrombin on the radioactive nucleotides of human washed platelets. Biochem. J., 105, 857-867.

Isomaki, H. A. (1972), Aspirin and myocardial infarction in patients with rheumatoid arthritis. Lancet, ii, 831.

Ivy, A. C., Shapiro, P. R. and Melnick, P. (1935), The bleeding tendency. Surg. Gynecol. Obstet., 60, 781-784.

James, H. L. and Ganguly, P. (1973), Studies on the identity of human platelet fibrinogen using ^{125}I-labeled plasma fibrinogen. Fed. Proc., 32, 313.

Jenkins, C. S. P., Packham, M. A., Guccione, M. A. and Mustard, J. F. (1973), Modification of platelet adherence to protein-coated surfaces. J. Lab. Clin. Med., 81, 280-290.

Jennings, G. H. (1965), Causal influences in haematemesis and melaena. Gut, 6, 1-13.

Jerushalmy, Z., Adler, A., Rechmic, J., Kohn, A. and De Vries, A. (1962), Effect of myxoviruses on the clotting and clotretracting activities of human blood platelets '*in vitro*'. Pathol. Biol., 10, 41-48.

Jerushalmy, Z., Skoza, L. and Zucker, M. B. (1965), Inhibition of ADP-induced platelet clumping. Fed. Proc., 24, 402.

Jerushalmy, Z. and Zucker, M. B. (1966), Some effects of fibrinogen degradation products (FDP) on blood platelets. Thromb. Diath. Haemorrh., 15, 413-419.

Jevons, S. and Barton, P. G. (1971), Biochemistry of blood platelets. Interaction of activated factor X with platelets. Biochemistry, 10, 428-434.

Jobin, F. and Gagnon, F. T. (1971), Inhibition of human platelet aggregation by a dibenzazepine compound (GP 44296) and by N-(2,6-dichlorophenyl)-o-aminophenylacetic acid (GP 45840). Can. J. Physiol. Pharmacol., 49, 479-481.

Joist, J. H., Niewiarowski, S. and Mustard, J. F. (1971), Release of antiplasmin during thrombin or collagen induced platelet aggregation. Thromb. Diath. Haemorrh., Suppl. 47, 113-119.

Jorgensen, L., Hovig, T., Rowsell, H. C. and Mustard, J. F. (1970), Adenosine diphosphate-induced platelet aggregation and vascular injury in swine and rabbits. Am. J. Pathol., 61, 161-176.

Kaneshiro, M. M., Mielke, C. H. Jr., Kasper, C. K. and Rapaport, S. I. (1969), Bleeding time after aspirin in disorders of intrinsic clotting. N. Engl. J. Med., 281, 1039-1042.

Karpatkin, M. H. and Karpatkin, S. (1969), *In vivo* and *in vitro* binding of factor VIII to human platelets. Thromb. Diath. Haemorrh., 21, 129-133.

Karpatkin, S. (1969a), Heterogeneity of human platelets I. Metabolic and kinetic evidence suggestive of young and old platelets. J. Clin. Invest., 48, 1073-1082.

Karpatkin, S. (1969b), Heterogeneity of human platelets II. Functional evidence suggestive of young and old platelets. J. Clin. Invest., 48, 1083-1087.

Karpatkin, S., Charmatz, A. and Langer, R. M. (1970), Glycogenesis and glyconeogenesis in human platelets. Incorporation of glucose, pyruvate, and citrate into platelet glycogen: glycogen synthetase and fructose-1,6-diphosphate activity. J. Clin. Invest., 49, 140-149.

Karpatkin, S. and Langer, R. (1967), Human platelet glycogenolysis and glycolysis during simulated thrombus initiation. Blood, 30, 552-553.

Karpatkin, S. and Strick, N. (1972), Heterogeneity of human platelets. V. Differences in glycolytic and related enzymes with possible relation to platelet age. J. Clin. Invest., 51, 1235-1243.

Kattlove, H. E. and Spaet, T. H. (1970), The effect of chromium on platelet function *in vitro*. Blood, 35, 659-668.

von Kaulla, K. N. (1971), Inhibitors of platelet aggregation with dual action. The example of fibrinolytic organic anions. Thromb. Diath. Haemorrh., Suppl. 45, 83-87.

Kaullen, H. D. and Gross, R. (1972), Demonstration of adenosine 3',5'-monophosphate dependent protein-phosphokinase in human platelets. Klin. Wochenschr., 50, 1114-1115.

Kerr, J. W., Pirrie, R., MacAulay, I. and Bronte-Stewart, B. (1965), Platelet-aggregation by phospholipids and free fatty acids. Lancet, i, 1296-1299.

Kien, M., Belamarich, F. A. and Shepro, D. (1971), Effect of adenosine and related compounds on thrombocyte and platelet aggregation. Am. J. Physiol., 220, 604-608.

Kikugawa, K., Iizuka, K., Higuchi, Y., Hirayama, H. and Ichino, M. (1972), Platelet aggregation inhibitors.2. Inhibition of platelet aggregation by 5'-,2-,6-, and 8-substituted adenosines. J. Med. Chem., 15, 387-390.

Kikugawa, K., Iizuka, K. and Ichino, M. (1973), Platelet aggregation inhibitors. 4. N^6-substituted adenosines. J. Med. Chem., 16, 358-364.

Kinlough-Rathbone, R. L., Packham, M. A. and Mustard, J. F. (1970a), The effect of glucose on adenosine diphosphate-induced platelet aggregation. J. Lab. Clin. Med., 75, 780-787.

Kinlough-Rathbone, R. L., Packham, M. A. and Mustard, J. F. (1970b), The effect of prostaglandin E_1 on platelet function *in vitro* and *in vivo*. Br. J. Haematol., 19, 559-571.

Kinlough-Rathbone, R. L., Packham, M. A. and Mustard, J. F. (1972), The effect of glucose on the platelet response to release-inducing stimili. J. Lab. Clin. Med., 80, 247-255.

Kloeze, J. (1967), Influence of prostaglandins on platelet adhesiveness and platelet aggregation. In "Prostaglandins" (Nobel Symp. 2.) Bergstrom, S. and Samuelsson, B. (eds.). Almqvist and Wiksell, Stockholm, 1967, pp. 241-252.

Kloeze, J. (1970a), Prostaglandins and platelet aggregation *in vivo*. I. Influence of PGE_1 and w-homo-PGE_1 on transient thrombocytopenia and of PGE_1 on the LD_{50} of ADP. Thromb. Diath. Haemorrh., 23, 286-292.

Kloeze, J. (1970b), Prostaglandins and platelet aggregation *in vivo* II. Influence of PGE_1 and PGE_{1_α} on platelet thrombus formation induced by an electric stimulus in veins on the rat brain surface. Thromb. Diath. Haemorrh., 23, 293-298.

Kobayashi, I. and Didisheim, P. (1972), Effect of pyridinolcarbamate (PDC) on platelet aggregation *in vitro* and *in vivo*. Fed. Proc., 31, 818.

Kobayashi, I. and Didisheim, P. (1973), Collagen-induced release of adenine nucleotides from platelets *in vivo*. Fed. Proc., 32, 220.

Kocsis, J. J., Hernandovich, J., Silver, M. J., Smith, J. B. and Ingerman, C. (1973), Duration of inhibition of platelet prostaglandin formation and aggregation by ingested aspirin or indomethacin. Prostaglandins, 3, 141-144.

Kowalski, E., Kopec, M. and Wegrzynowicz, Z. (1963), Influence of fibrinogen degradation products (FDP) on platelet aggregation, adhesiveness and viscous metamorphosis. Thromb. Diath. Haemorrh., 10, 406-423.

Kuramoto, A., Steiner, M. and Baldini, M. (1969), Stimulation of platelet glycolysis. Blood, 34, 522.

Kuramoto, A., Steiner, M. and Baldini, M. G. (1970), Metabolic basis of platelet phagocytosis. Biochim. Biophys. Acta, 201, 471-478.

Lacefield, W. B., Herrmann, R. G., Mills, J., Mills, W. M. and Frank, J. D. (1971), Inhibitors of blood platelet aggregation. I. Biphenylyloxyalkylamines. J. Med. Chem., 14, 133-135.

Lenahan, J. G. and Phillips, G. E. (1969), Seminar on the coagulation of blood. Warner-Lambert Research Institute, Morris Plains, N. J., 1969, pp. 10-24.

Leonardi, R. G., Alexander, B. and White, F. (1972), Prevention of the inhibitory effect of aspirin on platelet aggregation. Fed. Proc., 31, 248.

Leonards, J. R. (1969), Platelets and aspirin. Br. Med. J., 4, 301.

Levy, G. and Leonards, J. R. (1966), Absorption, metabolism and excretion of salicylates. In "The Salicylates, A Critical Bibliographic Review". Smith, M. J. H. and Smith, P. K. (eds.), Interscience Publishers Inc., New York, 1966, pp. 5-48.

Lewis, A. F. and Dickson, R. C. (1971), Platelet aggregation: induction by means of a complex between endotoxin and copper (Cu^{2+}). Can. J. Biochem., 49, 1236-1244.

Lewis, N. and Majerus, P. W. (1969), Lipid metabolism in human platelets. II. *De Novo* phospholipid synthesis and the effect of thrombin on the pattern of synthesis. J. Clin. Invest., 48, 2114-2123.

Libanska, J. (1967), Ultrastructure of human blood platelets during the clotting process *in vivo*. In "Biochemistry of Blood Platelets". Kowalski, E. and Niewiarowski, S. (eds.), Academic Press, New York, 1967, pp.61-79.

Lindsay, R. M., Prentice, C. R. M., Ferguson, D., Burton, J. A. and McNicol, G. P. (1972), Reduction of thrombus formation on dialyser membranes by aspirin and RA 233. Lancet, ii, 1287-1290.

Lindsay, R. M., Prentice, C. R. M., Ferguson, D., Muir, W. M. and McNicol, G. P. (1973), A method for the measurement of platelet adhesiveness by use of dialysis membranes in a test-tube. Br. J. Haematol., 24, 377-389.

Link, K. P., Overman, R. S., Sullivan, W. R., Huebner, C. F. and Scheel, L. D. (1943), Studies on the hemorrhagic sweet clover disease. XI. Hypoprothrombinemia in the rat induced by salicylic acid. J. Biol. Chem., 147, 463-474.

Lloyd, J. V., Nishizawa, E. E., Haldar, J. and Mustard, J. F. (1972), Changes in ^{32}P-labelling of platelet phospholipids in response to ADP. Br. J. Haematol., 23, 571-585.

Loder, P. B., Hirsh, J. and DeGruchy, G. C. (1968), The effect of collagen on platelet glycolysis and nucleotide metabolism. Br. J. Haematol., 14, 563-573.

Logan, L. J., Rapaport, S. I. and Maher, I. (1971), Albinism and abnormal platelet function. N. Engl. J. Med., 284, 1340-1345.

Lough, J. and Moore, S. (1972), Platelet embolic injury; the healing process. Exp. Mol. Pathol., 17, 132-144.

Lüscher, E. F. and Bettex-Galland, M. (1972), Thrombosthenin, the contractile protein of blood platelets. New facts and problems. Pathol. Biol., 20, (Suppl.), 89-101.

Lüscher, E. F., Probst, E. and Bettex-Galland, M. (1972), Thrombosthenin: structure and function. Ann. N. Y. Acad. Sci., 201, 122-130.

Lycette, R. M., Danforth, W. R., Koppel, J. L. and Olwin, J. H. (1970), Aggregation of human blood platelets and its correlation with their uptake of phospholipid-specific dyes. Am. J. Clin. Pathol., 54, 692-698.

MacKenzie, R. D. and Blohm, T. R. (1971), Effects of N-(2-diethylaminoethyl)-N-(2-hydroxy-2-phenylethyl)-3,5-dichloroaniline (AN162) on platelet function and blood coagulation. Thromb. Diath. Haemorrh., 26, 577-587.

MacKenzie, R. D., Blohm, T. R., Auxier, E. M., Henderson, J. G. and Steinbach, J. M. (1971), Effects of α(p-(fluoren-9-ylidene-methyl)phenyl)-2-piperidineethanol (RMI 10,393) on platelet function. Proc. Soc. Exp. Biol. Med., 137, 662-667.

MacKenzie, R. D., Blohm, T. R. and Steinbach, J. M. (1972), Effects *in vivo* of α[p(fluoren-9-ylidenemethyl) phenyl]-2-piperidineethanol (RMI 10,393) on platelet aggregation and blood coagulation. Biochem. Pharmacol., 21, 707-717.

MacKenzie, R. D., Henderson, J. G. and Steinbach, J. M. (1971), The evaluation of a method for adenosine diphosphate induced platelet aggregation in the guinea pig. Thromb. Diath. Haemorrh., 25, 30-40.

MacMillan, D. C. (1966), Secondary clumping effect in human citrated platelet-rich plasma produced by adenosine diphosphate and adrenaline. Nature (Lond.), 211, 140-144.

MacMillan, D. C. (1968), Effect of salicylates on human platelets. Lancet, i, 1151.

Maguire, M. H. and Michal, F. (1968), Powerful new aggregator of blood platelets- 2-chloroadenosine-5'-diphosphate. Nature (Lond.), 217, 571-573.

Majerus, P. W., Smith, M. B. and Clamon, G. H. (1969), Lipid metabolism in human platelets. Evidence for a complete fatty acid synthesizing system. J. Clin. Invest., 48, 156-164.

Mannucci, P. M. (1971), In "Round-the-Table Conference on Normal and Modified Platelet Aggregation". Vermylen, J., de Gaetano, G. and Verstraete, M. (eds.), Almqvist and Wiksell, Stockholm, 1971, p.55.

Mannucci, P. M. and Sharp, A. A. (1967), Platelet volume and shape in relation to aggregation and adhesion. Br. J. Haematol., 13, 604-617.

Marcus, A. J. and Zucker, M. B. (1965), "The Physiology of Blood Platelets". Grune and Stratton, Inc., New York, 1965.

Marcus, A. J., Zucker-Franklin, D., Safier, L. B. and Ullman; H. L. (1966), Studies on human platelet granules and membranes. J. Clin. Invest., 45, 14-28.

Marquis, N. R., Vigdahl, R. L. and Tavormina, P. A. (1969), Platelet aggregation I. Regulation by cyclic AMP and prostaglandin E_1. Biochem. Biophys. Res. Commun., 36, 965-972.

Marshall, E. R., Kuck, J. A. and Elderfield, R. C. (1942), Lactones related to the cardiac aglycones. X. Synthesis of simple hydroxylated β-substituted $\Delta^{\alpha,\beta}$-butenolides. J. Org. Chem., 7, 444-456.

Mason, R. G. and Read, M. S. (1971), Effects of some membrane-active and other compounds on thrombin-induced platelet aggregation. Experientia, 27, 1218-1220.

Mason, R. G., Read, M. S., Saba, S. R. and Shermer, R. W. (1972), Apparent similarity of mechanisms of platelet adhesion and aggregation. Differentiation of these functions from clot retraction. Thromb. Diath. Haemorrh., 27, 134-140.

Mason, R. G. and Reddick, R. L. (1972), Morphologic study of the plasma membrane and internal structure of platelets by freeze-etch techniques. Fed. Proc., 31, 242.

Massini, P. (1970), Specific activity of ^{14}C-labelled serotonin released from human blood platelets. Experientia, 26, 87-88.

McDonald, J. W. D. and Stuart, R. K. (1973), Regulation of cyclic AMP levels by prostaglandin E_1, J. Lab. Clin. Med., 81, 838-849.

McElroy, F. A., Kinlough-Rathbone, R. L., Ardlie, N. G., Packham, M. A. and Mustard, J. F. (1971), The effect of aggregating agents on oxidative metabolism of rabbit platelets. Biochim. Biophys. Acta, 253, 64-77.

McGovern, J. J., Jones, A. R. and Steinberg, A. G. (1955), The hematocrit of capillary blood. N. Engl. J. Med., 253, 308-312.

McKay, D. G., Shapiro, S. S. and Shanberge, J. N. (1958), Alterations in the blood coagulation system induced by bacterial endotoxins. II. *In vitro*. J. Exp. Med., 107, 369-376.

McMillan, R., Smith, R. S., Longmire, R. L., Yelenosky, R., Ried, R. T. and Craddock, C. G. (1971), Immunoglobulins associated with human platelets. Blood, 37, 316-322.

Mehrishi, J. N. (1970), Phosphate groups (receptors?) on the surface of human blood platelets. Nature (Lond.), 226, 452-453.

Mehrishi, J. N. (1971), The human blood platelet as a molecular mosaic- its role in aggregation and thrombus formation. Thromb. Diath. Haemorrh., 26, 370-377.

Meyer, O. O. and Howard, B. (1943), Production of hypoprothrombinemia and hypocoagulability of the blood with salicylates. Proc. Soc. Exp. Biol. Med., 53, 234-237.

Michal, F. (1967), The aggregation of blood platelets by 2-chloroadenosine diphosphate. Aust. J. Exp. Biol. Med. Sci., 45, 31P.

Michal, F. and Born, G. V. R. (1971), Effect of the rapid shape change of platelets on the transmission and scattering of light through plasma. Nature (New Biol.), 231, 220-222.

Michal, F. and Silver, M. J. (1971), Inhibition of aggregation and plug formation of platelets subjected to ultrasound and tested in the revolving plastic loop. Br. J. Pharmacol., 42, 635P-636P.

Mielke, C. H. Jr., Kaneshiro, M. M., Maher, I. A., Weiner, J. M. and Rapaport, S. I. (1969), The standardized normal Ivy bleeding time and its prolongation by aspirin. Blood, 34, 204-215.

Mielke, C. H. J. Jr., Ramos, J. C. and Britten, A. F. H. (1973), Aspirin as an antiplatelet agent: template bleeding time as a monitor of therapy. Am. J. Clin. Pathol., 59, 236-242.

Miller, J. L. and Feinstein, M. B. (1973), Inhibition by plasmin of thrombin-induced aggregation of washed human platelets. Fed. Proc., 32, 289.

Mills, D. C. B. and Lipson, C. (1973), Platelet energy charge: effects of aggregating agents and of metabolic poisons. Fed. Proc., 32, 732.

Mills, D. C. B., Robb, I. A. and Roberts, G. C. K. (1968), The release of nucleotides, 5-hydroxytryptamine and enzymes from human blood platelets during aggregation. J. Physiol. (Lond.), 195, 715-729.

Mills, D. C. B. and Roberts, G. C. K. (1967a), Effects of adrenaline on human blood platelets. J. Physiol. (Lond.), 193, 443-453.

Mills, D. C. B. and Roberts, G. C. K. (1967b), Inhibition of the platelet release reaction by amitriptyline. Biochem. J., 105, 30P.

Mills, D. C. B. and Roberts, G. C. K. (1967c), Membrane active drugs and the aggregation of human blood platelets. Nature (Lond.), 213, 35-38.

Mills, D. C. B. and Smith, J. B. (1971), The influence on platelet aggregation of drugs that affect the accumulation of adenosine 3',5'-cyclic monophosphate in platelets. Biochem. J., 121, 185-196.

Minsker, D. H. (1972), Inhibition of platelet aggregation by cyproheptadine (C); dependency upon incubation time. Fed. Proc., 31, 599.

Minter, B. and Crawford, N. (1967), The subcellular distribution of serotonin in blood platelets before and after incubation with serotonin. Biochem. J., 105, 22P-23P.

Mitchell, J. R. A. and Sharp, A. A. (1964), Platelet clumping *in vitro*. Br. J. Haematol., 10, 78-93.

Morris, C. D. W. (1967), Acetylsalicylic acid and platelet stickiness. Lancet, i, 279-280.

Morse, E. F., Jackson, D. P. and Conley, C. L. (1965), Role of platelet fibrinogen in the reactions of platelets to thrombin. J. Clin. Invest., 44, 809-816.

Moschos, C. B., Lahiri, K., Peter, A., Jesrani, M. U. and Regan, T. J. (1972), Effect of aspirin upon experimental coronary and non-coronary thrombosis and arrhythmia. Am. Heart J., 84, 525-530.

Moskowitz, J., Harwood, J. P., Ried, W. D. and Krishna, G. (1971), The interaction of norepinephrine and prostaglandin E_1 on the adenyl cyclase system of human and rabbit blood platelets. Biochim. Biophys. Acta, 230, 279-285.

Movat, H. Z., Mustard, J. F., Taichman, N. S. and Uriuhara, T. (1965), Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytosis of antigen-antibody complexes. Proc. Soc. Exp. Biol. Med., 120, 232-237.

Movat, H. Z., Weisel, W. J., Glynn, M. F. and Mustard, J. F. (1965), Platelet phagocytosis and aggregation. J. Cell. Biol., 27, 531-543.

Muenzer, J., Weinbach, E. C. and Wolfe, S. M. (1972), Thrombin-stimulation of oxygen consumption in human platelets. Fed. Proc., 31, 241.

Muirhead, C. R. (1972), The effect of inhibitors of ADP induced platelet aggregation in circulating blood of the rat. Fed. Proc., 31, 654.

Mundall, J., Quintero, P., von Kaulla, K. N., Harmon, R. and Austin, J. (1972), Transient monocular blindness and increased platelet aggregability treated with aspirin. Neurology, 22, 280-285.

Murakami, M., Odake, K., Matsuda, T., Manmi, S., Takase, M., Imura, M. and Toyama, T. (1972), Bleeding tendency with platelet dysfunction and albinism- a case report. Thromb. Diath. Haemorrh., 27, 461-471.

Murakami, M., Odake, K., Takase, M. and Yoshino, K. (1972), Potentiating effect of adenosine on other inhibitors of platelet aggregation. Thromb. Diath. Haemorrh., 27, 252-262.

Murakami, M., Yoshino, K., Takase, M. and Odake, K. (1972), Adenine nucleotide metabolism of human platelets during collagen-induced aggregation. Thromb. Diath. Haemorrh., 27, 416-424.

Murer, E. H. (1968), Release reaction and energy metabolism in blood platelets with special reference to the burst in oxygen uptake. Biochim. Biophys. Acta, 162, 320-326.

Mürer, E. H. (1969a), Clot retraction and energy metabolism of platelets. Effect and mechanism of inhibitors. Biochim. Biophys. Acta, 172, 266-276.

Mürer, E. H. (1969b), Thrombin-induced release of calcium from blood platelets. Science, 166, 623.

Mürer, E. H. (1972), Factors influencing the initiation and the extrusion phase of the platelet release reaction. Biochim. Biophys. Acta, 261, 435-443.

Mürer, E. H., Hellem, A. J. and Rozenberg, M. C. (1967), Energy metabolism and platelet function. Scand. J. Clin. Lab. Invest., 19, 280-282.

Mürer, E. H. and Holme, R. (1970), A study of the release of calcium from human blood platelets and its inhibition by metabolic inhibitors, N-ethylmaleimide and aspirin. Biochim. Biophys. Acta, 222, 197-205.

Mustard, J. F., Movat, H. Z., Macmorine, D. R. L. and Sényi, A. (1965), Release of permeability factors from the blood platelet. Proc. Soc. Exp. Biol. Med., 119, 988-991.

Mustard, J. F. and Packham, M. A. (1968), Platelet phagocytosis. Ser. Haematol., 1, 168-184.

Mustard, J. F. and Packham, M. A. (1970), Factors influencing platelet function: adhesion, release and aggregation. Pharmacol. Rev., 22, 97-187.

Mustard, J. F. and Perry, D. W. (1972), ADP-induced secondary platelet aggregation. Fed. Proc., 31, 268.

Mustard, J. F. and Perry, D. W. (1973), Interaction of platelets with nucleoside diphosphates. Fed. Proc., 32, 844.

Nachman, R. L. (1968), Platelet proteins. Semin. Hematol., 5, 18-31.

Nachman, R. L. and Ferris, B. (1972), Studies on the proteins of human platelet membranes. J. Biol. Chem., 247, 4468-4475.

Nachman, R. L., Marcus, A. J. and Safier, L. L. (1967), Platelet thrombosthenin: subcellular localization and function. J. Clin. Invest., 46, 1380-1389.

Nachman, R. L., Marcus, A. J. and Zucker-Franklin, D. (1967), Immunologic studies of proteins associated with subcellular fractions of normal human platelets. J. Lab. Clin. Med., 69, 651-658.

Nagayama, M., Zucker, M. B. and Beller, F. K. (1971), Effects of a variety of endotoxins on human and rabbit platelet function. Thromb. Diath. Haemorrh., 26, 467-473.

Nakao, K. and Angrist, A. A. (1968), Membrane surface specialization of blood platelet and megakaryocyte. Nature (Lond.), 217, 960-961.

Needham, C. D., Kyle, J., Jones, P. F., Johnston, S. J. and Kerridge, D. F. (1971), Aspirin and alcohol in gastrointestinal hemorrhage. Gut, 12, 819-821.

Niewiarowski, S., Farbiszewski, R., Poplawski, A. and Lipinski, B. (1968), Further investigations on the role of platelet factor 4 (PF-4) in the mechanism of platelet aggregation. In "Metabolism and Membrane Permeability of Erythrocytes and Thrombocytes". Deutsch, E., Gerlach, E. and Moser, K. (eds.), Georg Thieme Verlag, Stuttgart, 1968, pp.314-317.

Niewiarowski, S., Ream, V. J. and Thomas, D. P. (1970), Effect of fibrinogen derivatives on platelet aggregation. Thromb. Diath. Haemorrh., Suppl. 42, 49-59.

Niewiarowski, S. and Thomas, D. P. (1966), Platelet aggregation by ADP and thrombin. Nature (Lond.), 212, 1544-1547.

Niewiarowski, S. and Thomas, D. P. (1969), Platelet factor 4 and adenosine diphosphate released during human platelet aggregation. Nature (Lond.), 222, 1269-1270.

Nunn, B. (1972), The role of adenosine diphosphate (ADP) in collagen-induced platelet aggregation. Br. J. Pharmacol., 46, 579P-580P.

O'Brien, J. R. (1961), The adhesiveness of native platelets and its prevention. J. Clin. Pathol., 14, 140-149.

O'Brien, J. R. (1962), Platelet aggregation. Part II. Some results from a new method of study. J. Clin. Pathol., 15, 452-455.

O'Brien, J. R. (1963a), Some effects of adrenaline and anti-adrenaline compounds on platelets *in vitro* and *in vivo*. Nature (Lond.)., 200, 763-764.

O'Brien, J. R. (1963b), The effect of the alkyl tins on platelet aggregation. Thromb. Diath. Haemorrh., 9, 330-334.

O'Brien, J. R. (1964), A comparison of platelet aggregation produced by seven compounds and a comparison of their inhibitors. J. Clin. Pathol., 17, 275-281.

O'Brien, J. R. (1965), Effects of adenosine diphosphate and adrenalin on mean platelet shape. Nature (Lond.), 207, 306-307.

O'Brien, J. R. (1968a), Effects of salicylates on human platelets. Lancet, i, 779-783.

O'Brien, J. R. (1968b), Effect of anti-inflammatory agents on platelets. Lancet, i, 894-895.

O'Brien, J. R. (1968c), Aspirin and platelet aggregation. Lancet, i, 204-205.

O'Brien, J. R. (1969), Cell membrane damage, platelet stickiness and some effects of aspirin. Br. J. Haematol., 17, 610-611.

O'Brien, J. R., Finch, W. and Clark, E. (1970a), A comparison of an effect of different anti-inflammatory drugs on human platelets. J. Clin.Pathol., 23, 522-525.

O'Brien, J. R., Finch, W. and Clark, E. (1970b), Platelet-bound and soluble platelet factor 4: effects of aggregating agents, of aggregation, and of aspirin. Proc. Soc. Exp. Biol. Med., 134, 1128-1131.

Okuma, M., Steiner, M. and Baldini, M. G. (1971), Incorporation of acetate and fatty acids into lipids of rat platelets. Proc. Soc. Exp. Biol. Med., 136, 842-847.

Osler, W. (1874), An account of certain organisms occurring in the liquor sanguinis. Proc. R. Soc. Lond. (Biol.), 22, 391-398.

Owen, C. A., Bowie, E. J. W., Didisheim, P. and Thompson, J. H. (1969), "The Diagnosis of Bleeding Disorders"., Little and Brown Co., Boston, 1969, pp.89-90.

Packham, M. A., Guccione, M. A., Perry, D. W., Kinlough-Rathbone, R. L. and Mustard, J. F. (1972), AMP inhibition of reactions of ADP with washed platelets from humans and rabbits. Am. J. Physiol. (Lond.), 223, 419-424.

Packham, M. A., Jenkins, C. S. P., Lloyd, J. V., Kinlough-Rathbone, R. L. and Mustard, J. F. (1970), Comparison of polylysine and ADP-induced platelet aggregation. Fed. Proc., 29, 423.

Packham, M. A. and Mustard, J. F. (1968), Inhibition of platelet aggregation by adenine compounds. Fed. Proc., 27, 321.

Packham, M. A., Warrior, E. S., Glynn, M. F., Senyi, A. S. and Mustard, J. F. (1967), Alteration of the response of platelets to surface stimuli by pyrazole compounds. J. Exp. Med., 126, 171-188.

Papayannis, A. G., Watson-Williams, E. J. and Israels, M. C. G. (1971), Platelet functional abnormalities: a report of two familial defects in interactions between collagen and platelets and ADP release. Blood, 38, 745-758.

Payling Wright, H. (1941), The adhesiveness of blood platelets in normal subjects with varying concentrations of anticoagulants. J. Pathol. Bact., 53, 255-262.

Peterson, J. and Zucker, M. B. (1970), The effect of adenosine monophosphate, arcaine and anti-inflammatory agents on thrombosis and platelet function in rabbits. Thromb. Diath. Haemorrh., 23, 148-158.

Phillips, D. R. (1972), Surface structure of the human platelet membrane. Fed. Proc., 31, 412.

Philp, R. B. (1970), Species-dependent inhibition of platelet aggregation by adenosine and dipyridamole: paradoxical potentiation in the rat. Thromb. Diath. Haemorrh., 23, 129-139.

Philp, R. B., Francey, I. and McElroy, F. (1973), Effects of dipyridamole and five related agents on human platelet aggregation and adenosine uptake. Thromb. Res., 3, 35-50.

Philp, R. B. and Lemieux, V. (1968), Comparison of some effects of dipyridamole and adenosine on thrombus formation, platelet adhesiveness and blood pressure in rabbits and rats. Nature (Lond.), 218, 1072-1074.

Philp, R. B. and Lemieux, V. (1969), Interactions of dipyridamole and adenosine on platelet aggregation. Nature (Lond.), 221, 1162-1164.

Pichard, A. L., Hanoune, J. and Kaplan, J. C. (1972), Human brain and platelet cyclic adenosine 3',5'-monophosphate phosphodiesterase: different response to drugs. Biochim. Biophys. Acta, 279, 217-220.

Pinckard, R. N., Hawkins, D. and Farr, R. S. (1968a), Permanent alteration of human serum albumin (HSA) by acetylsalicylic acid (ASA). Fed. Proc., 27, 543.

Pinckard, R. N., Hawkins, D. and Farr, R. S. (1968b), *In vitro* acetylation of plasma proteins, enzymes and DNA by aspirin. Nature (Lond.), 219, 68-69.

Pinckard, R. N., Hawkins, D. and Farr, R. S. (1970), The inhibitory effect of salicylate on the acetylation of human albumin by acetylsalicylic acid. Arthritis Rheum., 13, 361-368.

Pitcher, P. M. (1971), Preparation of a rapid slide test for the detection of fibrinogen degradation products (FDP) in serum and a primary report on its use as a clinical screening test. Oslo Meeting Int. Soc. Thromb. Haemostasis, 1971.

Pletscher, A. (1968), Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. Br. J. Pharmacol. Chemother., 32, 1-16.

Poller, L. (ed.) (1969), "Recent Advances in Blood Coagulation". J. and A. Churchill Ltd., London, 1969.

Polson, C. J. (1959), Poisoning by salicylates. In "Clinical Toxicology". Polson, C. J. and Tattersall, K. N. (eds.), English Universities Press Ltd., London, 1959, pp. 173-180.

Probst, E. and Luscher, E. F. (1972), Studies on thrombosthenin A, the actin-like moiety of the contractile protein from blood platelets. I. Isolation, characterization and evidence for two forms of thrombosthenin A. Biochim. Biophys. Acta, 278, 577-584.

Puett, D. and Cunningham, L. W. (1973), Effect of collagen modification on platelet aggregation. Fed. Proc., 32, 614.

Puszkin, E., Aledort, L. and Puszkin, S. (1970), The labeling of dicarboxylic amino acids and their amides by glucose and acetate in human platelets. J. Lab. Clin. Med., 75, 234-243.

Puszkin, E. and Jerushalmy, Z. (1968), Effect of connective tissue and collagen on platelet lactate production: role of acid mucopolysaccharides. Proc. Soc. Exp. Biol. Med., 129, 346-350.

Quick, A. J. (1966a), Effect of aspirin on the bleeding time. Fed. Proc., 25, 498.

Quick, A. J. (1966b), Salicylates and bleeding: the aspirin tolerance test. Am. J. Med. Sci., 252, 265-269.

Quick, A. J. (1970), Hemophilia and aspirin. J. A. M. A., 213, 1689.

Rabiner, S. F. and Molinas, F. (1970), The role of phenol and phenolic acids on the thrombocytopathy and defective platelet aggregation of patients with renal failure. Am. J. Med., 49, 346-351

Radegran, K. and McAslan, C. (1972), Circulatory and ventilatory effects of induced platelet aggregation and their inhibition by acetylsalicylic acid. Acta Anaesthesiol. Scand., 16, (Suppl.), 76-84.

Rapoport, S., Wing, M. and Guest, G. M. (1943), Hypoprothrombinemia after salicylate administration in man and rabbits. Proc. Soc. Exp. Biol. Med., 53, 40-41.

Reddick, R. L. and Mason, R. G. (1973), Freeze-etch observations on the plasma membrane and other structures of normal and abnormal platelets. Am. J. Pathol., 70, 473-488.

Regoli, D. and Clark, V. (1963), Prevention by adenosine of the effect of adenosine diphosphate on the concentration of circulating platelets. Nature (Lond.), 200, 546-548.

Renaud, S. and Godu, J. (1970), Thrombosis prevention by acetylsalicylic acid in hyperlipemic rats. Can. Med. Assoc. J., 103, 1037-1040.

Renaud, S. and Lecompte, F. (1970), Thrombosis prevention by coagulation and platelet aggregation inhibitors, in hyperlipemic rats. Thromb. Diath. Haemorrh., 24, 577-586.

Robb-Smith, A. H. T. (1967), Why the platelets were discovered. Br. J. Haematol., 13, 618-637.

Roberts, E. M., Grisar, J. M., MacKenzie, R. D., Claxton, G. P. and Blohm, T. R. (1972), Naphthylalkyl lactamimides as inhibitors of blood platelet aggregation. J. Med. Chem., 15, 1270-1273.

Robinson, C. W. Jr., Mason, R. G. and Wagner, R. H. (1963), Effect of sulfhydryl inhibitors on platelet agglutinability. Proc. Soc. Exp. Biol. Med., 113, 857-861.

Rodman, N. F. and Mason, R. G. (1967), Platelet-platelet interaction. Relationship to hemostasis and thrombosis. Fed. Proc., 26, 95-105.

Rodman, N. F., Mason, R. G. and Brinkhous, K. M. (1963), Some pathogenetic mechanisms of white thrombus formation: agglutination and self-destruction of the platelet. Fed. Proc., 22, 1356-1362.

Rodman, N. F., Mason, R. G. and Brinkhous, K. M. (1971), Effect of a new antiaggregating chemical on the structure and function of the human platelet. Am. J. Pathol., 65, 103-114.

Rodman, N. F. and Penick, G. D. (1973), The role of ADP in platelet aggregation. Fed. Proc., 32, 843.

Rogowski, W., Galasinski, W., Bankowski, E. and Rzeczycki, W. (1971), Studies on liver factors which exert an inhibitory effect on platelet aggregation. The identification and characterization of platelet aggregation inhibitors from rabbit liver. Thromb. Diath. Haemorrh., 26, 135-144.

Roschlau, W. H. E. and Gage, R. (1972), The effects of brinolase (fibrinolytic enzyme from aspergillus oryzae) on platelet aggregation of dog and man. Thromb. Diath. Haemorrh., 28, 31-48.

Rosenberg, F. J., Gimber-Phillips, P. E., Groblewski, G. E., Davison, C., Phillips, D. K., Goralnick, S. J. and Cahill, E. D. (1971), Acetylsalicylic acid: inhibition of platelet aggregation in the rabbit. J. Pharmacol. Exp. Ther., 179, 410-418.

Rosenberg, F. J., Groblewski, G., Davison, C., Phillips, D. and Gimber-Phillips, P. (1970), Acetylsalicylic acid: inhibition of platelet aggregation in the rabbit. Fed. Proc., 29, 382.

Rozenberg, M. C. and Holmsen, H. (1968), Adenine nucleotide metabolism of blood platelets. II. Uptake of adenosine and inhibition of ADP induced platelet aggregation. Biochim. Biophys. Acta, 155, 342-352.

Rozenberg, M. C. and Walker, C. M. (1973), The effect of pyrimidine compounds on the potentiation of adenosine inhibition of aggregation, on adenosine phosphorylation and phosphodiesterase activity of blood platelets. Br. J. Haematol, 24, 409-418.

Rysanek, K., Svehla, C., Spankova, H. and Mlejnkova, M. (1966), The effect of drugs inhibiting the uptake of biogenic amines on adrenaline induced platelet aggregation. Experientia, 22, 320-321.

Sacchetti, G., Bellani, D., Montanari, C. and Gibelli, A. (1973), Effects "*in vitro*" of some cardiovascular drugs and other agents on human platelet aggregation. Thromb. Diath. Haemorrh., 29, 190-195.

Sahud, M. A. and Aggeler, P. M. (1969), Platelet dysfunction-differentiation of a newly recognized primary type from that produced by aspirin. N. Engl. J. Med., 280, 453-459.

Salzman, E. W. (1970), Inhibition of platelet aggregation by cyclic AMP and dibutyryl cyclic AMP. Fed. Proc., 29, 316.

Salzman, E. W., Ashford, T. P., Chambers, D. A. and Neri, L. L.(1969), Platelet incorporation of labelled adenosine and adenosine diphosphate. Thromb. Diath. Haemorrh., 22,304-315.

Salzman, E. W. and Chambers, D. A. (1964), Inhibition of ADP-induced platelet aggregation by substituted amino-acids. Nature (Lond.), 204, 698-700.

Salzman, E. W., Chambers, D. A. and Neri, L. L. (1966), Possible mechanism of aggregation of blood platelets by adenosine diphosphate. Nature (Lond.), 210, 167-169.

Salzman, E. W., Kensler, P. C. and Levine, L. (1972), Cyclic 3',5'-adenosine monophosphate in human blood platelets. IV. Regulatory role of cyclic AMP in platelet function. Ann. N. Y. Acad. Sci., 201, 61-71.

Salzman, E. W. and Levine, L. (1971), Cyclic 3',5'-adenosine monophosphate in human blood platelets II. Effect of N^6-2'-O-dibutyryl cyclic 3',5'-adenosine monophosphate on platelet function. J. Clin. Invest., 50, 131-141.

Salzman, E. W. and Neri, L. L. (1969), Cyclic 3',5'-adenosine monophosphate in human blood platelets. Nature (Lond.), 224, 609-610.

Salzman, E. W. and Weisenberger, H. (1972), Role of cyclic AMP in platelet function. Adv. Cyclic Nucl. Res., 1, 231-247.

Samter, M. (1969), The acetyl- in aspirin. Ann. Intern. Med., 71, 208-209.

Sandborn, E. B., Le Buis, J. J. and Bois, P. (1966), Cyctoplasmic mictrotubules in blood platelets. Blood, 27, 247-252.

Sano, T., Boxer, M. G. J., Boxer, L. A. and Yokoyama, M. (1971), Platelet sensitivity to aggregation in normal and diseased groups- A method for assessment of platelet aggregability. Thromb. Diath. Haemorrh., 25, 524-531.

Sano, T. and Yokoyama, M. (1971), Inhibitory effect of proteolytic enzymes on platelet aggregation induced by ADP or thrombin. Experientia, 27, 1179-1181.

Schmid, H. J., Jackson, D. P. and Conley, C. L. (1962), Mechanism of action of thrombin on platelets. J. Clin. Invest., 41, 543-553.

Scott, R. B. (1967), Activation of glycogen phosphorylase in blood platelets. Blood, 30, 321-330.

Scott, R. E. (1970), Effects of prostaglandins, epinephrine and NaF on human leukocyte, platelet and liver adenyl cyclase. Blood, 35, 514-516.

Sekhar, N. C. (1970), Prostaglandins and platelet function. Thromb. Diath. Haemorrh., Suppl. 42, 305-314.

Shapiro, S. (1944), Studies on prothrombin VI. The effect of synthetic vitamin K on the prothrombinopenia induced by salicylate in man. J. A. M. A., 125, 546-548.

Shapiro, S., Redish, M. H. and Campbell, H. A. (1943), Studies on prothrombin: IV. The prothrombinopenic effect of salicylate in man. Proc. Soc. Exp. Biol. Med., 53, 251-254.

Shermer, R. A., Mason, R. G., Wagner, R. H. and Brinkhous, K. M. (1961), Studies on thrombin-induced platelet agglutination. J. Exp. Med., 114, 905-920.

Shio, H., Ramwell, P. W. and Jessup, S. J. (1972), Prostaglandin E_2: effects on aggregation, shape change and cyclic AMP of rat platelets. Prostaglandins, 1, 29-36.

Shirasawa, K. and Chandler, A. B. (1969), Fine structure of the bond between platelets in artificial thrombi and in platelet aggregates induced by adenosine diphosphate. Am. J. Pathol., 57, 127-152.

Siegel, I. and Cohen, S. (1962), Action of staphylococcal toxin on human platelets. J. Infect. Dis., 114, 488-502.

Silver, M. D. (1966), Microtubules in the cytoplasm of mammalian platelets. Nature (Lond.), 209, 1048-1050.

Silver, M. D. and Stehbens, W. E. (1965), Platelet reaction to adenosine diphosphate *in vivo*. Nature (Lond.), 205, 91-92.

Silver, M. J. (1970), Platelet aggregation and plug formation: a model test system. Am. J. Physiol., 218, 384-388.

Silver, M. J. (1971), Bile salts and platelet aggregation. Experientia, 27, 566-567.

Simokoriyama, V. M. (1941), Über die Bildung der partiellen Acetate von Flavonen, Flavanonen, Anthrachinonen und dergleichen Verbindungen. Bull. Chem. Soc. Japan, 16, 284-291.

Simone, J. V. and Choi, S. I. (1970), Neuraminidase induced thrombocytopenia. Fed. Proc., 29, 623.

Sixma, J. J. and Geuze, J. J. (1968), Degranulation of human platelets during ADP-induced aggregation. Vox Sang., 15, 309-314.

Sixma, J. J., Annemieke, M. C., Trieschnigg, S., De Graff, S. and Bouma, B. N. (1972), *In vivo* inhibition of human platelet function by VK 744. Scand. J. Haematol., 9, 226-230.

Sixma, J. J. and Nijessen, J. G. (1970), Characteristics of platelet factor 3 release during ADP-induced aggregation. Comparison with 5-hydroxytryptamine release. Thromb. Diath. Haemorrh., 24, 206-213.

Sixma, J. J. and Trieschnigg, A. M. C. (1971), Inhibition of the function of human blood platelets *in vivo* by VK 744. Scand. J. Haematol., 8, 417-424.

Skalhegg, B. A., Hellem, A. J. and Ödegaard, A. E. (1964), Investigations on adenosine diphosphate (ADP) induced platelet adhesiveness *in vitro*. Part II. Studies on the mechanism. Thromb. Diath. Haemorrh., 11, 305-316.

Skoza, L., Zucker, M. B., Jerushalmy, Z. and Grant, R. (1967), Kinetic studies of platelet aggregation induced by adenosine diphosphate and its inhibition by chelating agents, guanidino compounds and adenosine. Thromb. Diath. Haemorrh., 18, 713-775.

Slater, S. D., Turpie, A. G. G., Douglas, A. S. and McNicol, G. P. (1972), Effect *in vitro* on platelet function of two compounds developed from the pyrimido-pyrimidines. J. Clin. Pathol., 25, 427-432.

Smink, D. A., Kruisheer, H. E. J. and Böttcher, C. J. F. (1968), Physicochemical interaction between adenosine phosphates and fibrinogen as a cause of platelet aggregation. Nature (Lond.), 217, 374-375.

Smith, J. B., Ingerman, C., Kocsis, J. J. and Silver, M. J. (1973), Formation of prostaglandins during the aggregation of human blood platelets. J. Clin. Invest., 52, 965-969.

Smith, J. B. and Mills, D. C. B. (1970), Inhibition of adenosine 3':5' cyclic monophosphate phosphodiesterase. Biochem. J., 120, 20P.

Smith, M. J. H. and Smith, P. K., eds. (1966), "The Salicylates. A Critical Bibliographic Review". John Wiley and Sons, New York, 1966.

Smith, J. B. and Willis, A. L. (1971), Aspirin selectively inhibits prostaglandin production in human platelets. Nature (New Biol.), 231, 235-237.

Soine, T. O. and Willette, R. E. (1962), Analgesic agents. In "Textbook of Organic Medicinal and Pharmaceutical Chemistry". Wilson, C. O. and Gisvold, O. (eds.), J. B. Lippincott Company, Philadelphia, 1962, P.586.

Solum, N. O. (1968), Aggregation of human platelets by bovine platelet fibrinogen. Scand. J. Haematol., 5, 474-485.

Solum, N. O. and Stormorken, H. (1965), Influence of fibrinogen on the aggregation of washed human blood platelets induced by adenosine diphosphate, thrombin, collagen and adrenaline. Scand. J. Clin. Lab. Invest., Suppl. 84, 170-182.

Song, S. Y. and Cheung, W. Y. (1971), Cyclic 3',5'-nucleotide phosphodiesterase. Properties of the enzyme of human blood platelets. Biochim. Biophys. Acta, 242, 593-605.

Soppitt, G. D. and Mitchell, J. R. A. (1969), The effect of colchicine on human platelet behavior. J. Atherosclerosis Res., 10, 247-252.

Spaet, T. H. and Cintron, J. (1965), Studies on platelet factor-3 availability. Br. J. Haematol., 11, 269-275.

Spaet, T. H., Cintron, J. and Spivack, M. (1962), Some properties of the platelet-connective tissue mixed agglutination reaction. Proc. Soc. Exp. Biol. Med., 111, 292-295.

Spaet, T. H. and Lejnieks, I. (1966), Studies on the mechanism whereby platelets are clumped by adenosine diphosphate. Thromb. Diath. Haemorrh., 15, 36-51.

Spaet, T. H. and Zucker, M. B. (1964), Mechanism of platelet plug formation and role of adenosine diphosphate. Am. J. Physiol., 206, 1267-1274.

Stachurska, J., Latallo, Z. and Kopec, M. (1970), Inhibition of platelet aggregation by dialysable fibrinogen degradation products (FDP). Thromb. Diath. Haemorrh., 23, 91-98.

Stamler, J. (1973), Epidemiology of coronary heart disease. Med. Clin. North Am., 57, 5-46.

Steiner, M. and Baldini, M. (1969), Protein synthesis in aging blood platelets. Blood, 33, 628-633.

Steiner, M., Kuramoto, A. and Baldini, M. (1969), Effect of aggregating agents on platelet energetics. Blood, 34, 852.

Stevens, D. L. and Kaplan, D. B. (1954), Salicylate intoxication in children. Am. J. Dis. Child., 70, 331-335.

Stevenson, M. M. and Yoder, I.I. (1970), Studies of platelet aggregation, plasma adenosine diphosphate breakdown and blood coagulation in magnesium deficient calves and rats. Thromb. Diath. Haemorrh., 23, 299-305.

Stoltz, J. F., Larcan, A., Streiff, F. and Nicolas, A. (1972), Standardization of a new technique for studying platelet aggregation, based on the filtration pressure exerted by aggregates on a given filter. Acta Haematol. (Basel), 48, 292-299.

Stormorken, H. (1969), The release reaction of secretion. Scand J. Haematol., Suppl. 9, 1-24.

Stuart, R. K. (1970), Platelet function studies in human beings receiving 300 mg. of aspirin per day. J. Lab. Clin. Med., 75, 463-471.

Swank, R. L., Roth, J. G. and Jansen, J. (1964), Screen filtration pressure method and adhesiveness and aggregation of blood cells. J. Appl. Physiol., 19, 340-346.

Ten Cate, J. W., Gerritsen, J. and van Geet-Weijers, J. (1972), "*In vitro*" and "*in vivo*" experiences with VK 774. A new platelet function inhibitor. Pathol. Biol. 20, (Suppl.), 76-81.

Thomas, D. P., Niewiarowski, S. and Ream, V. J. (1970), Release of adenine nucleotides and platelet factor 4 from platelets of man and four other species. J. Lab. Clin. Med., 75, 607-618.

Thompson, R. J. and MacKenzie, R. D. (1973), A method for preparing and staining the platelet-collagen interaction for microscopic observation. J. Clin. Pathol., 26, 78-80.

Tilford, C. H., MacKenzie, R. D., Blohm, T. R. and Grisar, J. M. (1973), Substituted 3,4-pentadienyldiamines as inhibitors of platelet aggregation. J. Med. Chem., 16, 688-693.

Tocantins, L. M. (1938), The mammalian blood platelet in health and disease. Medicine, 17, 155-260.

Tocantins, L. M. (1948), Historical notes on blood platelets. Blood, 3, 1073-1082.

Tranzer, J. P., Da Prada, M. and Pletscher, A. (1966), Ultrastructural localization of 5-hydroxytryptamine in blood platelets. Nature (Lond.), 212, 1574-1575.

Troll, M. M. and Menton, M. L. (1945), Salicylate poisoning. Am. J. Dis. Child., 69, 37-43.

Ts'ao, C. (1970), Ultrastructural study of the effect of aspirin on *in vitro* platelet-collagen interaction and platelet adhesion to injured intima in the rabbit. Am. J. Pathol., 59, 327-345.

Ts'ao, C. (1971), Rough endoplasmic reticulum and ribosomes in blood platelets. Scand. J. Haematol., 8, 134-140.

Ts'ao, C. H. and Glagov, S. (1970), Platelet adhesion to subendothelial components in experimental aortic injury. Role of fine fibrils and basement membrane. Br. J. Exp. Pathol., 51, 423-427.

Ts'ao, C. and Glagov, S. (1972), Effects of compound 48/80 on platelets: structural alterations and inhibition of aggregation. Scand. J. Haematol., 9, 73-82.

Tschopp, T. B. (1970), Aggregation of cat platelets *in vitro*. Thromb. Diath. Haemorrh., 23, 601-620.

Turpie, A. G. G., Chernesky, M. A. and Larke, R. P. B. (1972), Interaction of Newcastle Disease virus (NDV) with platelets. Fed. Proc., 31, 248.

Ueda, I. (1971), The effects of volatile general anesthetics on adenosine diphosphate-induced platelet aggregation. Anesthesiology, 34, 405-408.

Vainer, H. and Wattiaux, R. (1968), Glycogen synthetase activity in blood platelets. Nature (Lond.), 217, 951-952.

Vreeken, J. and van Aken, W. G. (1971), Spontaneous aggregation of blood platelets as a cause of idiopathic thrombosis and recurrent painful toes and fingers. Lancet, ii, 1394-1397.

Warren, B. A. (1970), The ultrastructure of platelet pseudopodia and the adhesion of homologous platelets to tumor cells. Br. J. Exp. Pathol., 51, 570-580.

Warren, B. A. (1971), The platelet pseudopodium and its involvement in aggregation and adhesion to vessel walls. Br. J. Exp. Pathol., 52, 378-387.

Warren, B. A. and Vales, O. (1972a), The release of vesicles from platelets following adhesion to vessel-walls *in vitro*. Br. J. Exp. Pathol., 53, 206-215.

Warren, B. A. and Vales, O. (1972b), The adhesive dendritic pseudopodium of the platelet and the release reaction. Microvasc. Res., 4, 159-178.

Warshaw, A. L., Laster, L. and Schulman, N. R. (1967), Protein synthesis by human platelets. J. Biol. Chem., 242, 2094-2097.

Weisenberger, H., Grube, H., Koenig, E. and Pelzer, H. (1972), Isolation and identification of the platelet aggregation inhibitor present in the onion, Allium cepa. FEBS Lett., 26, 105-108.

Weiss, H. J. (1968), Bleeding-time after aspirin ingestion. Lancet, i, 527-528.

Weiss, H. J. (1971), In "Aspirin, Platelets and Stroke. Background For a Clinical Trial". Fields, W. S. and Hass, W. K. (eds.). Warren H. Green Inc., St. Louis, 1971, pp. 96-97.

Weiss, H. J. and Aledort, L. M. (1967), Impaired platelet/connective-tissue reaction in man after aspirin ingestion. Lancet, ii, 495-497.

Weiss, H. J., Aledort, L. M. and Kochwa, S. (1968), The effect of salicylates on the hemostatic properties of platelets in man. J. Clin. Invest., 47, 2169-2180.

Weiss, H. J., Chervenick, P. A., Zalusky, R. and Factor, A. (1969), A familial defect in platelet function associated with impaired release of adenosine diphosphate. N. Engl. J. Med., 281, 1264-1270.

Weiss, H. J., Danese, C. A. and Voleti, C. D. (1970), Prevention of experimentally induced arterial thrombosis by aspirin. Fed. Proc., 29, 381.

Weiss, H. J. and Rogers, J. (1972), Thrombocytopathia due to abnormalities in platelet release reaction-studies on six unrelated patients. Blood, 39, 187-196.

Weiss, H. J. and Rogers, J. (1973), Platelet factor 4 in platelet disorders- storage location and the requirement of endogenous ADP for its release. Proc. Soc. Exp. Biol. Med., 142, 30-35.

Weissbach, H. and Redfield, B. G. (1961), Studies on the uptake of serotonin by platelets. In "Blood Platelets". Johnson, S. A., Monto, R. W., Rebuck, J. W. and Horn, R. C. (eds.), Little, Brown and Company, Boston, 1961, pp 393-405.

Werle, E. and Schievelbein, H. (1965), Activity of nicotine and inactivity of kallikrein and kallidin in aggregation of blood platelets. Nature (Lond.), 207, 871-872.

Wharton Jones, T. (1851), On the state of the blood and blood-vessels in inflammation. Guy's Hosp. Rep. (Second Series), 7, 1-100.

White, J. G. (1967), The muscular system of platelets. Blood, 30, 539-540.

White, J. G. (1968a), Fine structural alterations induced in platelets by adenosine diphosphate. Blood, 31, 604-622.

White, J. G. (1968b), The transfer of thorium particles from plasma to platelets and platelet granules. Am. J. Pathol., 53, 567-575.

White, J. G. (1969), Effects of atropine on platelet structure and function. Scand. J. Haematol, 6, 236-245.

White, J. G. (1970), A biphasic response of platelets to serotonin. Scand. J. Haematol., 7, 145-151.

White, J. G. (1972a), Interaction of membrane systems in blood platelets. Am. J. Pathol., 66, 295-312.

White, J. G. (1972b), The sarcoplasmic reticulum of platelets. Fed. Proc., 31, 654.

White, J. G. (1972c), Exocytosis of secretory organelles from blood platelets incubated with cationic polypeptides. Am. J. Pathol., 69, 41-55.

White, J. G., Silver, M. J. and Krivit, W. (1966), Microtubules in human platelets and the platelet-fibrin meshwork: a possible mechanism for clot retraction. Blood, 28, 984.

Wilner, G. D., Nossel, H. L. and Le Roy, E. C. (1968), Aggregation of platelets by collagen. J. Clin. Invest., 47, 2616-2621.

Wilner, G. D., Nossel, H. L. and Procupez, T. L. (1971), Aggregation of platelets by collagen: polar active sites of insoluble human collagen. Am. J. Physiol., 220, 1074-1079.

Wojcik, J. D., Van Horn, D. L., Webber, A. J. and Johnson, S. A. (1969), Mechanism whereby platelets support the endothelium. Transfusion, 9, 324-335.

Wolfe, S. M. and Shulman, N. R. (1969), Adenyl cyclase activity in human platelets. Biochem. Biophys. Res. Commun., 35, 265-272.

Wood, L. (1972), Treatment of atherosclerosis and thrombosis with aspirin. Lancet, ii, 532-533.

Woodbury, D. M. (1971), Analgesic-antipyretics, anti-inflammatory agents, inhibitors of uric acid synthesis. In "Pharmacological Basis of Therapeutics". 4th edition. Goodman, L. S. and Gilman, A. (eds.), The MacMillan Company, New York, 1971, pp. 314-347.

Wright, H. P. (1941), The adhesiveness of blood platelets in normal subjects with varying concentrations of anti-coagulants. J. Pathol. Bacteriol., 53, 255-262.

Wright, J. H. (1906), The origin and nature of the blood platelets. N. Engl. J. Med., 154, 643.

Wright, J. H. and Minot, G. R. (1917), The viscous metamorphosis of blood platelets. J. Exp. Med., 26, 395-409.

Yamazaki, H., Kobayashi, I. and Shimamoto, T. (1970), Enhancement of ADP-induced platelet aggregation by exercise test in coronary patients and its prevention by pyridinolcarbamate. Thromb. Diath. Haemorrh., 24, 438-449.

Youssef, A. and Barkhan, P. (1968), Release of platelet factor 4 by adenosine diphosphate and other platelet-aggregating agents. Br. Med. J., 1, 746-747.

Youssef, A. H. and Barkhan, P. (1969), Inhibition by aspirin of release of antiheparin activity of human platelets. Br. Med. J., 3, 394-396.

Zieve, P. D. and Greenough, W. B. (1969), Adenyl cyclase in human platelets: activity and responsiveness. Biochem. Biophys. Res. Commun., 35, 462-466.

Zieve, P. D. and Schmukler, M. (1970a), Stimulation by glucose of fatty acid synthesis in human platelets. Am. J. Physiol., 219, 1009-1013.

Zieve, P. D. and Schmukler, M. (1970b), Metabolic effects of cyclic AMP on human platelets. Blood, 36, 853.

Zieve, P. D. and Schmukler, M. (1971), The effect of cyclic AMP on glycogenolysis and glycolysis in human platelets. Biochim. Biophys. Acta, 252, 280-284.

Zieve, P. D. and Solomon, H. M. (1968), Uptake of amino acids by the human platelet. Am. J. Physiol., 214, 58-61.

Zucker, M. B. (1947), Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypoprothrombinemic rats, Am. J. Physiol., 148, 275-288.

Zucker, M. B. (1971), Effects of aspirin and other anti-inflammatory agents on platelets. In "Aspirin, Platelets and Stroke. Background For a Clinical Trial". Fields, W. S. and Hass, W. K. (eds.), Warren H. Green, Inc., St. Louis, 1971, pp. 74-83.

Zucker, M. B. and Borrelli, J. (1962), Platelet clumping produced by connective tissue suspensions and by collagen. Proc. Soc. Exp. Biol. Med., 109, 779-787.

Zucker, M. B. and Jerushalmy, Z. (1965), Effects of inhibitors of platelet aggregation on platelet degranulation and release. Blood, 25, 603.

Zucker, M. B. and Peterson, J. (1967), Serotonin, platelet factor 3 activity, and platelet-aggregating agent released by adenosine diphosphate. Blood, 30, 556.

Zucker, M. B. and Peterson, J. (1968), Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicylic acid ingestion. Proc. Soc. Exp. Biol. Med., 127, 547-551.

Zucker, M. B. and Peterson, J. (1970), Effect of acetylsalicylic acid, other nonsteroidal anti-inflammatory agents, and dipyridamole on human blood platelets. J. Lab. Clin. Med., 76, 66-75.

Zucker, M. B., Peterson, J. and Grant, R. (1969), Inhibition of some functions of platelets in human citrated platelet-rich plasma by acetylsalicylic acid. Fed. Proc., 28, 576.

Zucker, M. B. and Zaccardi, J. B. (1964), Platelet shape change induced by adenosine diphosphate and prevented by adenosine monophosphate. Fed. Proc., 23, 299.

Zucker, W. H., Morton, B. D., and Erlandson, S. E. (1973), Ultrastructural aspects of blood-artificial surface reactions in experimental and clinical hemodialysis. Fed. Proc., 32, 836.

Zucker-Franklin, D. (1969), Microfibrils of blood platelets: their relationship to microtubules and the contractile protein. J. Clin. Invest., 48, 165-175.

Zucker-Franklin, D. (1970a), The ultrastructure of megakaryocytes and platelets. In "Regulation of Hematopoiesis". Vol. 2. Gordon, A. S. (ed.), Appleton-Century - Crofts, 1970, pp. 1553-1586.

Zucker-Franklin, D. (1970b), The submembranous fibrils of human blood platelets. J. Cell Biol., 47, 293-299.

Zucker-Franklin, D., Nachman, R. L. and Marcus, A. J. (1967), Ultrastructure of thrombosthenin, the contractile protein of human blood platelets. Science, 157, 945-946.

Zweifler, A. J. (1967), Impairment of platelet function and thrombus growth in reserpine-treated rabbits. J. Lab. Clin. Med., 70, 16-22.

Zweifler, A. J. and Sanbar, S. S. (1969), Inhibition of platelet adhesiveness and aggregation by benzyl alcohol and phenol. Thromb. Diath. Haemorrh., 21, 362-366.