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FACTORS INFLUENCING PLATELET FUNCTION: ADHESION, RELEASE, AND AGGREGATION

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I. INTRODUCTION

Multicellular organisms with vascular systems show similar patterns of response when the blood vessels are injured. This response consists of the accumulation of formed elements at the point of injury and stabilization of the mass of material by the formation of a coagulum of protein (51, 310, 330, 368, 543, 667, 697). In mammals, the formed element that is involved in this basic biological reaction is the blood platelet (310, 330, 697). A fundamental problem as yet unsolved is the mechanism by which the platelets, which are normally separate from each other in the circulation, are transformed in such a way that they become adherent to each other.

The mammalian platelets are non-nucleated formed elements derived from

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the megakaryocytes in the bone marrow (390). In circulating blood platelets are disc shaped (34, 159, 307). They have an outer membrane, which encloses cytoplasm and constituents such as storage granules, lysosomes, mitochondria, glycogen, a vesicular component which may be the remnants of the Golgi apparatus or endoplasmic reticulum, microfilaments, and microtubules. Platelet metabolism seems to be similar to that of nucleated cells and includes a capability for protein synthesis (263, 390, 655) and synthesis of fatty acids (384). Platelets have a mean life in the circulation of about 10 days and a half-life of about 3 days in man (1, 390, 464).

Although the platelet was originally thought to be primarily concerned with the processes of hemostasis and thrombosis, there is increasing evidence that it plays a critical part in almost all responses of the blood to injury (518). Recently the role of platelets in immunological and inflammatory reactions has been examined (272, 447). Platelets are involved in the response of the organism to injury of the endothelial lining of blood vessels, in its response to the introduction of foreign materials and chemicals into the blood stream, and in its reaction to many metabolic products that enter the blood, particularly under abnormal conditions.

The aggregation of platelets may be beneficial to the organism (hemostasis, phagocytosis of foreign material, or interaction with viruses, bacteria, or antigen-antibody complexes), but on the other hand, platelet aggregation may be harmful [the formation of platelet aggregates that obstruct flow in blood vessels (thrombosis), leading to infarction of tissues supplied by the obstructed vessel]. Although the mechanism by which platelets respond to injury to the blood or vessel walls is not clear, certain points have been established. Several compounds have been shown to promote platelet aggregation both *in vivo* and *in vitro*, and various inhibitors of platelet aggregation have also been recognized. Adenosine diphosphate (ADP) (63, 216, 390) has been established as a key compound causing the platelet changes that are essential for the platelets to be able to adhere to each other. The role of platelet aggregates in initiating or accelerating blood coagulation has been recognized (110, 250, 390, 449), and several platelet abnormalities affecting hemostasis have been described.

II. PLATELET AGGREGATION

A. Introduction

Eberth and Schimmelbusch (186) originally used the term viscous metamorphosis to describe the morphogical changes that occur when individual platelets stick to foreign surfaces and to one another. Later, Wright and Minot (689) used this term to describe the agglutination and fusion of platelets into glassy masses and strands, which they distinguished from simple agglutination of platelets without fusion. This process appeared to be associated with an increased adhesion of the platelets to each other and to foreign surfaces, the release of platelet material into the ambient fluid, and the formation of filamentous processes. Since thrombin induced these changes in platelets, Apitz

(11) believed that the platelets adhered to each other because of fibrin formation. The role of fibrinogen and fibrin was questioned when it was found that thrombin caused platelet aggregation in the blood or platelet-rich plasma of patients with afibrinogenemia (523). It was recognized in the early studies (390) that citrate, oxalate, or ethylene diamine tetraacetic acid (EDTA) prevented the platelet changes and this led to the conclusion that ionized calcium was involved in the adherence of platelets to each other.

Øllgaard (510) and Hellem (270) described a factor from red cells that caused platelet aggregation. Subsequently, Gaarder *et al.* (216) demonstrated that this factor was adenosine diphosphate (ADP). Since these observations were reported, extensive studies have confirmed the prime importance of ADP in causing platelet aggregation (63, 69, 261).

Thrombin causes the release of ADP from platelets, and Haslam (261) has suggested that this ADP is responsible for thrombin-induced platelet aggregation, because the addition of an enzyme system that converts the ADP to adenosine triphosphate (ATP) as it is released prevents thrombin-induced platelet aggregation. However, there is evidence that thrombin-induced aggregation of nonmammalian thrombocytes may not be mediated by ADP (38).

Several workers have emphasized the importance of some vessel wall constituents in initiating platelet aggregation resulting from vessel injury (80, 314, 553). Hugues (314) and Bounameaux (80) demonstrated that platelets adhere to fibers in the mesentery about injured vessels and to fragments of aorta. Kjaerheim and Hovig (349) observed that the mesenteric fibers to which the platelets adhered were collagen. Collagen causes platelet aggregation *in vitro*, and this is associated with the release of ADP from the platelets (304, 623, 704). However, it has been reported that collagen does not cause aggregation of nonmammalian thrombocytes (38).

Several other materials (table 1) have been shown to cause platelet aggregation and many of these also cause the release of platelet constituents, including adenine nucleotides. It is not known whether all these agents cause platelet aggregation through the common pathway of release of ADP followed by the effect of the released ADP on the platelets. Some of the agents may act through other mechanisms.

B. Adenosine diphosphate

After it was recognized that ADP caused platelet aggregation, other nucleotides were studied and the majority were found to be inactive. Nucleotides that have been shown to have activity are: 3-deoxy adenosine diphosphate, the 1-N-oxide of ADP, and adenosine tetraphosphate (69, 121, 216, 217, 390) (table 1). The only nucleotide more potent than ADP in causing platelet aggregation in plasma is 2-chloroadenosine diphosphate (382). Chambers *et al.* (114) have reported that uridine diphosphate (UDP), guanosine diphosphate (GDP), cytidine diphosphate, and inosine diphosphate (IDP) cause platelet aggregation. They have suggested that the reason that other investigators have found some of these compounds to be inactive is that they did not use high enough concentrations. Although it has been reported that adenosine triphosphate (ATP)

TABLE 1

Platelet-aggregating agents

Agent	Reference
Adenosine diphosphate	216, 217, 270
Adenosine tetraphosphate*	121, 610
3-Deoxyadenosine-5′-diphosphate	216, 217
1-N-Oxide of adenosine diphosphate	217
2-Chloroadenosine-5′-diphosphate	382
Adenosine triphosphate*	216, 418, 449
Cytidine-5′-diphosphate	114, 216
Uridine-5′-diphosphate	114
Guanosine-5′-diphosphate	114, 216
Inosine-5′-diphosphate	114
Epinephrine	418, 494
Norepinephrine	418, 494
Serotonin	418
Long-chain saturated fatty acids	127–129, 261, 596, 654, 694
Collagen	303, 315, 704
Thrombin	11, 237, 523
Trypsin	237, 261
Papain	155
Pronase	155
Bovine pancreatic elastase	357
Some snake venoms	153, 155, 157
Antigen-antibody complexes	3, 427, 432
Gamma-globulin-coated surfaces	199, 433, 448, 516, 518
Heat-aggregated *gamma*-globulin	47, 323, 432
Antiplatelet antibodies	432
Fibrinogen antibody	469
Antithrombosthenin antibody	469
Myxoviruses	93, 327, 375, 426, 633
Bacteria	184, 518
Bacterial endotoxin	124, 167, 170, 277, 299, 409
Staphylococcal *alpha*-toxin	43, 388, 600
Particulate material	632
Polystyrene particles	229, 429, 433, 448, 518
Quartz	52
Titanium dioxide	52
Cobalt	52
Carbon	52, 124, 184, 646
Kaolin	250, 620
Glycogen	651
Uric acid crystals	448
Thorotrast	406
Zymosan	178
Fibrinogen degradation products	28, 353, 678
Fibrin monomer	616
Enzyme from *Aspergillus oryzae*	630
Dextran	175, 666
Oxime esters	132a
Liquoid (heparinoid)	49
Triethyl tin	495
Methyl mercuric nitrate (low concentration)	545

TABLE 1—*Continued*

Agent	Reference
Thimersol	605
Fluoride	610
Nicotine	665
Digitonin	157
Dimethyl sulfoxide	158
Neuraminidase	306
Polylysine	580a

* Probably through conversion to ADP.

causes platelet aggregation in plasma (418, 449), it has been suggested that these results were due to contamination of the ATP with ADP or the conversion of the ATP to ADP by enzymes in plasma (262). On the basis of the available evidence, ADP appears to be the principal nucleotide causing platelet aggregation in physiological situations.

Several methods have been used to examine the role of ADP in platelet aggregation *in vivo*. Honour and Mitchell (295) observed that if the cortical arteries of the rabbit brain were gently pinched with a pair of fine forceps, a single white body formed and subsequently embolized with no further formation of platelet aggregates. However, if a substance that causes platelet aggregation, such as ADP, were then applied to the injured site, platelet aggregates formed and embolized. At more seriously injured sites platelet aggregates developed without local application of aggregating agents. These workers concluded that this was caused by the release of ADP from cells in the injured vessel wall. The formation of platelet aggregates was abolished by the local application of azide, fluoride, or fluoroacetate, but was not prevented by cyanide or 2,4-dinitrophenol. Since these compounds did not modify the production of platelet aggregates and emboli caused by the local application of ADP at sites of minor injury, it was suggested that they inhibited the formation or release of ADP from the injured vessel wall. These agents could also inhibit the release of ADP from platelets.

The intravenous infusion of ADP into man and experimental animals causes a rapid fall in the platelet count (70, 152, 332, 463). If the organs and small vessels receiving the direct effects of the infusion are examined immediately after its cessation, many platelet aggregates are found in arterioles, venules, and capillaries. However, shortly after cessation of the infusion, the platelet count returns to the pre-infusion level and histological examination of the tissues shows few platelet aggregates. It appears that ADP-induced platelet aggregates are unstable *in vivo*.

Several methods for studying platelet aggregation *in vitro* have been developed. Some investigators have measured the time required for the formation of macroscopic platelet aggregates in agitated systems (90, 418). The Chandler loop has also been used (116, 213); this device consists of a circular piece of plastic tubing mounted at an angle on a turn-table. The lumen is half filled with blood or

platelet-rich plasma, the loop is rotated, and the time for the formation of macroscopically visible platelet aggregates at the leading edge of the blood or plasma-air interface is measured.

At present, the most commonly used method is to measure the changes in light transmission through platelet-rich plasma or suspensions of washed platelets (63, 449, 493). When an ADP solution of low concentration is added to stirred platelet-rich plasma or a platelet suspension at 37°C, there is an abrupt increase in light transmission because of dilution. Then, as the platelets change from discs to spheres with pseudopods, light transmission decreases. This is followed by a rapid increase in light transmission which reaches a peak after 1 to 2 min. Then, with the platelets from most species, a decrease in light transmission occurs. The increase in light transmission is associated with the formation of platelet aggregates while the return to the base line represents deaggregation of the platelets.

In the study of platelet aggregation by this optical density method, the rate of stirring is important since the rate and extent of aggregation increases with the speed of stirring (63, 493). It has been found that for ADP-induced aggregation, if other conditions are kept constant, the rate of fall of optical density (*i.e.*, increase in light transmission) during the first half minute is proportional to the log of the ADP concentration (69). The minimal concentration of ADP that causes aggregation in human citrated platelet-rich plasma is about 10^{-7} M (390). Temperature influences platelet aggregation and deaggregation; the rate of aggregation is less at 20°C than at 37°C; ADP does not cause aggregation at 0°C, except when high concentrations are used (492). The pH of the suspending fluid influences the extent of aggregation. At pH 8.0, maximal aggregation occurs, but below pH 6.4 (207, 411) and above pH 10, aggregation does not take place (612). McLean and Veloso (411) showed that below pH 6.5, ADP caused the initial decrease in light transmission (shape change), but aggregation was inhibited.

ADP causes platelet aggregation in platelet-rich plasma prepared from blood taken into either heparin or citrate (63, 216, 493). EDTA or excess citrate inhibits ADP-induced platelet aggregation but the addition of calcium overcomes this inhibition (69, 612). The presence of EDTA at concentrations that inhibit aggregation does not prevent the initial ADP-induced decrease in light transmission, which is believed to be due to an initial alteration in platelet shape caused by ADP (612, 705) (see section, VI B).

Calcium is necessary for platelet aggregation. With increasing calcium concentration the initial rate of aggregation increases and the minimal concentration of ADP required to produce aggregation in citrated platelet-rich plasma is diminished (612). However, excess calcium inhibits ADP-induced platelet aggregation (71). Although there have been some experiments that suggest that magnesium can replace calcium (169, 220, 261), most of the evidence indicates that calcium is the necessary divalent metal for ADP-induced platelet aggregation (71, 305). In discussing the effects of ethylene glycol diaminoethyl tetraacetic acid (EGTA) and EDTA, Hovig (305) pointed out that magnesium can

liberate considerable amounts of calcium from calcium EDTA. This may account for some of the discrepancies in the interpretation of the effects of divalent cations on ADP-induced platelet aggregation.

In studies of suspensions of washed rabbit platelets it has been found that calcium is essential for platelet aggregation, and magnesium is involved in platelet deaggregation (12). Apparently neither divalent cation in the ionized form is required for the initial decrease in light transmission which occurs when ADP is added (612, 705).

Although in all mammalian species studied, ADP induces platelet aggregation in citrated or heparinized platelet-rich plasma (390, 398, 605), Belamarich *et al.* (37) have reported that ADP does not aggregate nonmammalian nucleated thrombocytes (turtle, chicken, alligator, smooth dogfish, and spiny dogfish). These investigators have presented evidence that ADP is not involved in thrombin-induced aggregation of the platelets of these species (38). These experiments were carried out *in vitro* and only 30 to 66 % of the cells in the thrombocyte-rich plasma used were identified as thrombocytes. The presence of other blood cells may have influenced the results. Rowsell (559), however, has reported that when chicken thrombocytes were separated from other cells by the use of phytohemagglutinin, the thrombocytes were aggregated by ADP.

C. Plasma proteins and ADP-induced aggregation

Several studies have indicated that fibrinogen may be involved in ADP-induced platelet aggregation (91, 143, 617). Born and Cross (71) observed that pig platelets that had been separated from plasma and resuspended in buffered saline were aggregated by ADP providing calcium and a heat-labile component of plasma were present. Serum did not contain this component. A similar cofactor has been found necessary for ADP-induced aggregation of washed platelets prepared from a number of mammalian species (398, 410). The plasma factor is present in highly purified fibrinogen; a concentration of about 20 mg/100 ml is required to produce the maximal effect (262, 269). Deykin *et al.* (174) have suggested that in addition to fibrinogen, a heat-stable nondialysable plasma factor acts with ADP in inducing platelet aggregation.

It is difficult to reconcile the conclusion that fibrinogen is a necessary cofactor for ADP-induced platelet aggregation with the observation that platelets in platelet-rich plasma from patients with congenital afibrinogenemia will aggregate upon the addition of ADP (243, 320, 549, 550). However, it has been pointed out that larger amounts of ADP are required than with platelets from normal subjects (320, 616). When purified fibrinogen or normal plasma is added to the afibrinogenemic plasma, normal platelet aggregation occurs upon the addition of low concentrations of ADP that usually induce aggregation in normal platelet-rich plasma (320).

The fibrinogen involved in ADP-induced platelet aggregation could be fibrinogen in the suspending fluid or fibrinogen adsorbed to the platelet surface. Plasma fibrinogen labeled with ^{131}I or ^{125}I is absorbed by washed platelets and can be removed by further washing (109, 115, 352). In addition to this exchange-

able surface fibrinogen, platelets contain intracellular fibrinogen associated with granules or mitochondria, or both (109, 471), but the plasma fibrinogen does not exchange with this inner pool. It does not seem likely that this intraplatelet fibrinogen is involved in aggregation induced by low concentrations of ADP, but under conditions such as high ADP concentrations, in which intraplatelet constituents may be released, it may take part in platelet aggregation. Platelets from patients with afibrinogenemia contain fibrinogen in the granule fraction (469). Thus the mechanism by which high concentrations of ADP cause these platelets to aggregate may be by inducing the release of this intraplatelet fibrinogen.

In many studies of platelet aggregation in suspensions of washed platelets, preparations were obtained which either would not aggregate upon the addition of ADP alone, or would aggregate only upon the addition of high concentrations of ADP (71, 261). It is possible that the procedures used removed the fibrinogen adsorbed to the platelet membrane and that the high ADP concentrations caused the release of intraplatelet fibrinogen, which then acted as a cofactor with ADP. Recently, Ardlie (12) has developed a technique for the preparation of suspensions of washed platelets from rabbits. These platelets will aggregate without the addition of a plasma cofactor upon the addition of a concentration of ADP as low as that which is effective in plasma. This method of washing does not remove much of the adsorbed fibrinogen from the platelet surface. Some of the reports (91, 352) that fibrinogen alone can produce aggregation in suspensions of washed platelets may be explained by contamination of the fibrinogen solutions with traces of prothrombin which is converted to thrombin in the suspending fluid. In our experience, dialysed fibrinogen that has been absorbed with aluminum hydroxide to remove components of the prothrombin complex does not cause aggregation of washed pig, human, or rabbit platelets when added to platelet suspensions that do not contain ADP in the suspending fluid (16).

It has also been suggested that *gamma*-M-globulin may be involved in ADP-induced aggregation of normal human platelets (632a).

D. ADP-induced secondary aggregation

Constantine (131) observed that upon the addition of ADP at a final concentration of 3×10^{-7} M to stirred citrated guinea pig platelet-rich plasma, platelet aggregation occurred in two phases: the increase in light transmission caused by ADP was followed by the usual decrease toward the initial level, but this was interrupted by a period of unchanging transmission and, after that, by a second phase of increasing light transmission. Constantine (131) pointed out that Born and Cross (70) had observed the same phenomenon in citrated cat platelet-rich plasma in 1963. MacMillan (380) made similar observations with human citrated platelet-rich plasma and reported that the critical concentration of ADP was between 10^{-6} and 2×10^{-6} M. This biphasic response has been found in only 70 to 80% of the people studied (380, 709) and has not been reported in other species except the guinea pig and cat. When secondary aggre-

gation occurs it is associated with the appearance of platelet factor 3 activity and the release of constituents such as serotonin and ADP from the platelets (380, 414, 708, 709). If high concentrations of ADP are used, it is believed that the primary and secondary phases are fused so that they cannot be distinguished from each other (380). When the secondary phase of ADP-induced platelet aggregation occurs, the platelet aggregates do not break up during the subsequent 5 min or so during which most investigators have continued their observations. However, this apparent ability of ADP to induce irreversible platelet aggregation *in vitro* may be an artifact of the experiments because none of the studies *in vivo* has shown that ADP can induce irreversible platelet aggregates in cats, people, and pigs (70, 152, 332, 463).

E. Platelet deaggregation

It was originally thought that platelet deaggregation occurred when the added ADP had been converted to other compounds (69, 262, 573). Recently, it has been recognized that platelet deaggregation is not correlated with the loss of ADP (515, 563). Deaggregation has been shown to occur in the presence of concentrations of ADP more than adequate to induce platelet aggregation (515, 563).

Washed rabbit and pig platelets suspended in a modified Tyrode's solution containing calcium, but no magnesium, aggregate upon the addition of ADP. However, little deaggregation occurs, in comparison to the amount of deaggregation that takes place when both calcium and magnesium are present (16). It appears that for washed rabbit and pig platelets, magnesium is important for deaggregation. Excess magnesium inhibits aggregation of both pig and rabbit platelets (16, 71).

Deaggregation can also be accelerated by the addition of chelating agents at the peak of aggregation (418, 515).

Platelets that have aggregated after the addition of ADP and have subsequently deaggregated, are temporarily refractory to further additions of ADP (69, 498, 515, 563). Also, if platelets are incubated with ADP without stirring for 5 to 10 min, they will not aggregate upon stirring nor upon the addition of more ADP with stirring (498, 515). This effect can be partially overcome by the addition of calcium to the suspending fiuid before incubation with the ADP (515). The mechanism by which ADP produces this refractory state is not known but it may be related to the phenomenon of platelet deaggregation and to the binding of divalent cations. Since the platelet contractile protein is thought to be involved in platelet aggregation and deaggregation, these processes may be analogous to those occurring during the contraction of muscle.

F. Mechanisms of ADP-induced aggregation

Several hypotheses have been proposed to explain ADP-induced platelet aggregation. Gaarder and Laland (217) noted that only the adenine nucleotides with an unequal number of charges (ADP, adenosine tetraphosphate) caused platelet aggregation, and they suggested that when these nucleotides became

attached to platelets they would provide more opportunity for the formation of calcium bridges between platelets than would nucleotides with an even number of charges. If this hypothesis were valid, ADP and calcium should become bound to the platelets during aggregation but it has not been possible to demonstrate this. Although Born (65) has estimated that there may be 2×10^5 receptor sites for ADP per platelet, Haslam (264) has recently found that the number must be less than 1000 per platelet.

Although it is not possible to demonstrate firm binding of ADP to platelets, there must be transient binding because ADP is converted by platelets to ATP and, under some circumstances, to adenosine monophosphate (AMP) (461, 515, 622). Born (67) has suggested that ADP forms very short-lived complexes with a component of the platelet membrane and that arguments that platelets do not "bind" ADP are invalid because they depend on the meaning of binding in terms of time.

Spaet and Lejnieks (622) found that washed human platelets suspended in heated platelet-poor plasma converted ^{14}C-ADP to ^{14}C-AMP. However, this was observed over a period of time much greater than that required for platelet aggregation and deaggregation. They proposed that the energy from the high-energy phosphate bond that was broken upon the formation of AMP from ADP was used in the process of platelet aggregation. The mechanism by which this occurred was not described. The reaction would be unique as an energy-yielding biological process. Cyanide inhibits the conversion of ADP to AMP in plasma but does not inhibit platelet aggregation (573). Although Salzman *et al.* (573, 574) have used this as evidence against the ADP-AMP hypothesis, Spaet *et al.* (621) have presented evidence that cyanide does not inhibit the ADP-to-AMP conversion occurring at the platelet membrane. However, in suspensions of washed rabbit, pig, or human platelets that are sensitive to low concentrations of ADP, little or no conversion of ^{14}C-ADP to ^{14}C-AMP occurs during the period of ADP-induced platelet aggregation (515). The predominant conversion is ^{14}C-ADP to ^{14}C-ATP. This evidence indicates that the conversion of ADP to AMP is probably unrelated to ADP-induced platelet aggregation.

Salzman and his associates (113, 573) suggested that ADP induced platelet aggregation by product inhibition of an ecto-ATPase at the platelet surface. They proposed that the continuous activity of this enzyme was necessary to maintain the platelet membrane in a nonadhesive state, and presented evidence that the ecto-ATPase of the platelet membrane is similar if not identical to the ATPase associated with the platelet contractile protein, thrombosthenin. Their hypothesis can be questioned on several points: 1) the amount of inhibition produced by ADP in the experiments reported by these authors was very slight at the concentrations of ADP required to cause platelet aggregation; 2) these investigators themselves have pointed out that the souce of the ATP for an ecto-ATPase is difficult to visualize; 3) the hypothesis does not account for deaggregation because deaggregation occurs while the concentration of ADP is more than adequate to induce aggregation (515, 563); 4) there is very little conversion of ^{14}C-ATP by washed rabbit platelets (515).

Chambers *et al.* (114) have also proposed that guanosine diphosphate (GDP), inosine diphosphate (IDP), cytidine diphosphate, and uridine diphosphate (UDP) can cause platelet aggregation through the action of a platelet nucleoside diphosphokinase which, in the presence of ATP, is able to convert the diphosphate compounds to their triphosphate forms; that, in the exchange of phosphate, ADP is produced; and that this ADP then causes platelet aggregation in the usual manner. This type of reaction might divert substrate ATP away from the platelet ecto-ATPase. It is difficult, however, to postulate a source of either external ATP or the other nucleotide diphosphates involved in this type of reaction. Although Chambers *et al.* (114) did not point it out, ADP itself would be subject to the action of the platelet nucleoside diphosphokinase. The activity of this enzyme probably accounts for the conversion of ^{14}C-ADP to ^{14}C-ATP which we have observed (515). This exchange reaction complicates the interpretation of observations concerning platelet ecto-ATPase activity and other studies of conversions of labeled nucleotides by platelets.

ADP-induced platelet aggregation is associated with alterations in platelet shape (96, 302, 307, 497, 503, 668) (section VI). Upon the addition of ADP, the platelets change from their disc form to a swollen more spherical shape and develop pseudopods. This initial change is not dependent upon the presence of calcium nor on stirring but the subsequent aggregation reaction is (96, 503, 612, 705). It has been suggested that the formation of the platelet pseudopods may involve alterations of the contractile protein (thrombosthenin), which comprises 15% of the platelet proteins and is visible by electronmicroscopy as filamentous structures in platelets (668, 710). Thrombosthenin has many similarities to actomyosin, such as an ATPase activity which is calcium- and magnesium-dependent (46, 237, 470).

On the basis of ultrastructural studies, White (668) has proposed that ADP induces a change of platelet shape by causing contraction of the contractile protein. He suggested that ADP initiates the reaction by interfering with a calcium extrusion pump in the platelet membrane or lining of the canalicular system. He stated "Inhibition of the surface membrane pump by ADP may increase platelet permeability, thereby permitting calcium ions to enter the hyaloplasm and activate the ATPase of thrombosthenin." Davey and Lüscher (156) have recently proposed the opposite of this hypothesis to account for platelet aggregation. The platelets are presumed to circulate in a contracted form maintained by a contractile protein with ATPase activity (thrombosthenin) and dependent on metabolic energy. Upon the addition of ADP, contractile activity is inhibited, but this relaxation "decays," and when the platelets contract, deaggregation occurs. They suggested that aggregation requires the binding of external calcium ions by platelet membrane phospholipid and a modification of associated proteins.

Booyse and Rafelson (57–59) suggested that platelet aggregation resulted from the combination of the actin and myosin of thrombosthenin on adjacent platelets. Some of the electron micrographs of ADP-induced platelet aggregates have shown filamentous structures bridging adherent platelets (307). Chambers

et al. (113) have reported that an antithrombosthenin preparation inhibits the ATPase activity of the surface of the platelets, and Nachman and Marcus (469) recently found that an antibody to thrombosthenin will cause platelets prepared from normal or thrombasthenic subjects (section XII B 3 a) to aggregate without a lag phase. These observations may indicate that thrombosthenin is closely associated with the platelet membrane.

Further evidence compatible with the hypothesis that the contractile protein is involved in platelet aggregation comes from the observations that lack of glucose in the suspending fluid leads to a failure of the platelets to respond to ADP, and that this response can be restored by the addition of glucose (345) (see section VII). Although the contractile protein may play a central part in platelet aggregation, its role and that of the microtubules are not clear.

In order for platelets to adhere to each other there may have to be a change in the distribution of the charge on the platelets. In the resting state platelets have a net negative charge (2), but upon exposure to ADP this is reduced (48a, 242, 246, 564a, 590). Hampton and Mitchell (246) also found that low concentrations of ADP increased the net negative charge of platelets; this has been confirmed by other investigators (48a, 564a), but Gröttum (242) has reported contradictory results. Unfortunately, the technique used for this type of study does not allow measurement of the changes of platelet charge until 5 min or more after the platelets have been exposed to ADP. At this time the platelets are likely to be deaggregating or in the refractory state, and thus this technique does not give information about charge changes immediately after the addition of ADP when shape changes, pseudopod formation, and aggregation are occurring.

Membrane phospholipid may be involved in some of the changes associated with platelet aggregation (486). Silver (603) suggested that ADP causes the "uncovering" of phospholipid micelles in platelets, that the newly available negative charges on the polar ends of the phospholipids on adjacent platelets would then be bridged by divalent cations present in the surrounding plasma, and that this bridging produces platelet aggregates.

III. INHIBITORS OF ADP-INDUCED AGGREGATION

Inhibitors of ADP-induced platelet aggregation can be grouped according to their structural similarities to ADP, their ability to combine with divalent cations, their effects on membrane structures believed to be involved in platelet aggregation, their inhibition of platelet metabolism, or their influence on contractile proteins. In addition there are several other compounds that inhibit ADP-induced platelet aggregation by mechanisms that have not yet been defined.

A. Inhibitors with structural similarities to ADP

Born *et al.* (74) found that adenosine was a strong inhibitor of ADP-induced platelet aggregation both *in vitro* and *in vivo*, and Born and Cross (69) suggested that this was because it is structurally similar to ADP and may compete with it for receptor sites on the platelet membrane. They examined a number of compounds with structural similarities to adenosine and found that 2-chloroadenosine

is the only one that is more potent as an inhibitor than adenosine (64). Compounds with other substitutions at position 2 were less inhibitory (table 2). Alterations of the adenosine molecule at other sites greatly diminished the inhibitory potency.

Species differences in the action of adenosine have been observed. Sinakos and Caen (605) have reported that adenosine does not inhibit ADP-induced platelet aggregation in rat, guinea pig, or horse plasma. In these studies, however, the investigators did not determine whether the adenosine had been degraded in the plasma or taken up by the platelets before the addition of ADP. Constantine (132) observed that aggregation of rat platelets is inhibited by adenosine in citrated plasma but not in heparinized plasma.

Skoza *et al.* (612) have supported Born's proposal that adenosine is a competitive inhibitor of ADP-induced platelet aggregation. Salzman *et al.* (573) proposed that adenosine uptake by the platelets leads to the formation of ATP and thus provides more substrate for an ATPase, which keeps the platelets in an "unsticky" state. Rozenberg and Holmsen (562) have suggested that adenosine is transported across the membrane and perhaps phosphorylated before inducing inhibition; thus inhibition could be caused by diversion of the energy required for platelet aggregation to the adenosine transport and phosphorylation processes.

A satisfactory hypothesis to explain adenosine inhibition of platelet aggregation has to take a number of observations into account. In plasma, the inhibitory effect of adenosine increases for 5 to 10 min, and then decreases (64, 69, 130, 515, 612). The decrease of the inhibitory effect of adenosine when it is incubated with plasma before ADP addition appears to be related to its loss from plasma, which occurs because of uptake by the platelets and conversion in the plasma to inosine and hypoxanthine (515, 562). In suspensions of washed rabbit platelets, the inhibitory effect of adenosine is almost immediate (515). In contrast to adenosine, incubation of 2-chloroadenosine with plasma for a period of up to 40 min does not lead to loss of its inhibitory activity (64), and Born has proposed that this is due to the stability of 2-chloroadenosine since it is not deaminated in

TABLE 2

Inhibitors of ADP-induced platelet aggregation with structural similarities to ADP

Inhibitor	Reference
Adenosine	69, 74
2-Chloroadenosine	64
Adenosine monophosphate	69, 492, 515, 573, 605, 705
Adenosine triphosphate	69, 262, 497, 515, 705
2-Oxy-6-amino purine riboside	121
Diphosphopyridine nucleotide (nicotinamide-adenine dinucleotide)	121
6-Methylaminopurine riboside	121
Flavin adenine dinucleotide	121
5′-Adamantoyl adenosine	275
2-Methylthioadenosine-5′-phosphate	411c

plasma. The inhibitory effect of adenosine given intravenously is quickly lost while that of 2-chloroadenosine is maintained (74). Although 2-chloroadenosine would appear to be an attractive compound to inhibit ADP-induced platelet aggregation *in vivo*, its toxic effects have precluded its use as a therapeutic agent (74). The importance of adenosine deaminase in limiting the inhibiting effect of adenosine in plasma is emphasized by the experiments of Michal and Thorp (411b). They found that cardiac glycosides had no effect on ADP- or serotonin-induced platelet aggregation, but the presence of cardiac glycosides enhanced the inhibiting effect of adenosine when the adenosine was incubated with the plasma. They concluded that this enhancement was caused by inhibition of the adenosine deaminase activity in plasma.

Adenosine inhibits ADP-induced platelet aggregation, but it is not as effective as other compounds in inhibiting ADP-induced changes in platelet shape and volume (461, 705). Because the effect of adenosine is almost immediate when it is added to suspensions of washed rabbit platelets, uptake of adenosine by the platelets may not be necessary for inhibition (515). Born and Mills (75) have recently found that adenosine can exert its inhibitory effect without being taken up by the platelets. Papaverine and dipyridamole (Persantine) decrease the uptake of adenosine and potentiate its inhibitory effect (75, 394). This observation has been interpreted as indicating that uptake of adenosine is not necessary for inhibition, and that the site of action of adenosine is at the membrane (75).

Adenosine monophosphate (AMP) inhibits ADP-induced platelet aggregation in human, pig, and rabbit platelet-rich plasma (69, 492, 515, 573, 705). Sinakos and Caen (605) observed that AMP in high concentrations inhibited ADP-induced platelet aggregation in rat plasma, whereas adenosine was ineffective. Furthermore, they found that cyanide, which blocks the conversion of AMP to adenosine, did not block the inhibitory effect of AMP in rat plasma, in contrast to the effect of cyanide observed by Salzman *et al.* (573) in human platelet-rich plasma. There has been confusion about the concentrations of AMP required to produce maximal inhibition. Some investigators have observed that AMP is a more potent inhibitor than adenosine (705), while others find that AMP is much less potent (69, 515, 573). Since AMP is rapidly converted to adenosine in plasma (265, 293, 322, 334), it appears that part of the reason for this variation in observed inhibitory effect is related to the length of time the AMP has been incubated with the plasma before the addition of ADP. The species studied may also introduce some variability (605). It appears that low concentrations of AMP produce an inhibitory effect in plasma through conversion of the AMP to adenosine (515, 562, 573). However, in suspensions of washed rabbit platelets in which AMP is not converted to adenosine, AMP in high concentrations is inhibitory (515). Thus, AMP produces its inhibitory effect in two ways: the action of AMP itself, and (in plasma) the conversion of AMP to adenosine. An interesting difference between AMP and adenosine is that AMP is a much better inhibitor of the ADP-induced platelet shape change than adenosine (705).

There has been conflicting evidence about the effect of ATP on platelets. Some investigators observed that ATP itself causes platelet aggregation (418,

449) whereas others found that ATP inhibits ADP-induced platelet aggregation (69, 262, 493, 515, 705). Haslam (262) has reported that ATP, in the presence of enzymes that convert ADP to ATP, inhibits ADP-induced platelet aggregation. This may mean that in experiments in which ATP has been found to cause platelet aggregation, the ATP either has been contaminated with ADP or has been rapidly converted to ADP by enzymes in the plasma. ATP causes the initial change in platelet shape although it does not cause the subsequent extensive platelet aggregation (515). In suspensions of washed rabbit platelets the inhibitory effect of ATP does not appear to be related to its conversion to ADP, AMP, or adenosine (515). The ability of ATP to bind divalent cations may account for some of its inhibitory effect.

Several other nucleotides and nucleosides have been studied in respect to inhibition of platelet aggregation (73, 275). These are listed in table 2. None of the compounds tested has been found to be as potent as adenosine, except 2-chloroadenosine.

B. Inhibition by chelating agents

Since calcium is known to be essential for ADP-induced platelet aggregation it is hardly surprising that compounds that bind calcium inhibit the reaction. Ethylene diamine tetraacetic acid (EDTA), ethylene glycol diaminoethyl tetraacetic acid (EGTA), citrate, and oxalate will inhibit ADP-induced platelet aggregation (69, 169, 305, 418, 515, 612). A number of substituted amino acids, such a tosyl arginine methyl ester (TAMe), inhibit platelet aggregation in citrated plasma (572). In studies of 80 related compounds, some of them were found to be more inhibitory than TAMe, particularly arcaine (1,4-guanidino butane) and 1,4-diguanidino diphenyl sulfone (DGPS) (328, 705). These guanidino compounds are noncompetitive inhibitors of ADP and their activity is counteracted by calcium (705). The inhibitory action appears to be specifically directed towards the reaction involving ADP and calcium, rather than towards other reactions involving calcium; for example these compounds do not inhibit clot retraction, which requires calcium. However, Skoza *et al.* (612) pointed out that "because the apparent association constant for the DGPS-ADP complex is quite low, a combination of this diguanidino compound with ADP cannot be the sole mechanism of its inhibitory action on ADP-induced platelet aggregation." Salzman and Chambers (572) suggested that these compounds might inhibit ADP-induced platelet aggregation by blocking an active enzyme site.

C. Inhibitors that affect the platelet membrane

1. Sulfhydryl group inhibitors. Thiol groups have been shown to be essential for the maintenance of platelet integrity and for certain enzymatic and biological functions of the platelets (7, 418, 492, 510). High concentrations of monoiodoacetic acid (MIA) inhibit ADP-induced platelet aggregation (610). Robinson *et al.* (544), found that the sulfhydryl inhibitors parachloromercuribenzoic acid (PCMB), N-ethyl maleimide (NEM), and methyl mercuric nitrate (MMN) inhibited the ability of washed dog platelets to agglutinate with ADP in the

presence of calcium. However, they found that MMN in low concentrations caused platelet aggregation and that this was associated with enhanced platelet ATPase activity. Concentrations of MMN that inhibited ADP-induced platelet aggregation either failed to enhance or were inhibitory to ATPase activity (544). The inhibitory effect of NEM is counteracted by L-cysteine and it has been suggested that the sulfhydryl groups on the platelet surface are included in the platelet aggregation reaction (256).

Further evidence that membrane sulfhydryl groups are probably involved in ADP-induced platelet aggregation comes from the finding that parachloromercuribenzene sulfonate (PCMBS) also inhibits ADP-induced platelet aggregation (7, 545). Since this compound is very hydrophilic and therefore penetrates cell membranes slowly, its action is primarily at the platelet surface. Both parachloromercuribenzoic acid and parachloromercuribenzene sulfonate inhibit ADP-induced platelet aggregation at equimolar concentrations and this inhibitory effect can be reversed by cysteine (7). The failure of parachloromercuribenzene sulfonate in contrast to parachloromercuribenzoic acid, to inhibit clot retraction at the concentration that inhibited ADP-induced platelet aggregation, indicates that the clot retraction activity lies beneath an outer permeability barrier, presumably the plasma membrane of the platelet, whereas the site where ADP exerts its effect is on the outer portion of the platelet membrane. Furthermore, the fact that parachloromercuribenzoic acid irreversibly inhibits clot retraction at a time when ADP aggregation may still be restored by cysteine, indicates that ADP-induced platelet aggregation and clot retraction can exist as independent processes. Both parachloromercuribenzoic acid and parachloromeribenzene sulfonate inhibited all platelet ATPase activities in osmotically lysed platelets. (7).

2. Antihistamines. Antihistamines have also been found to inhibit ADP-induced platelet aggregation (275, 418, 491, 621). Diphenhydramine, pyrilamine maleate, promethazine hydrochloride, chlorpheniramine maleate, and a number of others have been described as inhibitory. Although the mechanism of action of these compounds has not been defined, it has been suggested that they are incorporated into the membranes of cells and thus affect structure and function (621). Antihistamines, in concentrations that are effective in blocking ADP-induced platelet aggregation, cause hemolysis of red blood cells (347). At these concentrations, antihistamines also cause the release of serotonin (5-hydroxytryptamine) and adenine nucleotides from platelets (347).

3. Local anesthetics. Local anesthetics such as cocaine, lidocaine (Xylocaine), dibucaine hydrochloride (Nupercaine hydrochloride), and a number of others have been reported to inhibit ADP-induced platelet aggregation (6, 491, 492). In higher concentrations, dibucaine hydrochloride appeared to induce platelet aggregation (6). The fact that these local anesthetics cause the platelets to swell and lose potassium indicates that they change the permeability of the platelet membrane. Although their mechanism of action is not known, these compounds inhibit ATPase activity and it may be this action which leads to the swelling and alteration of platelet shape produced by these compounds. (6).

4. Antidepressants and tranquilizers. Antidepressants and tranquilizers have also been found to influence ADP-induced platelet aggregation. Ryšánek *et al.* (565) found that imipramine, desipramine (norimipramine), amitryptyline, and nortriptyline (desmethylamitriptyline) inhibited ADP-induced platelet aggregation in plasma at concentrations of 5×10^{-4} M and 5×10^{-5} M. Mills and Roberts (415) found that these 4 compounds, as well as chlorpromazine, promethazine, and diphenhydramine inhibit ADP-induced platelet aggregation, but that they inhibit only the second phase of this aggregation, not the first phase. The investigators concluded that these drugs probably inhibit the release of ADP from platelets, which is reported to occur during the second phase. These compounds have a "stabilizing action on biological membranes" (209), inhibit swelling and contraction of isolated mitochondria, and inhibit ion movement in liver slices (335, 415).

5. Heparin. It has been generally assumed that anticoagulants such as heparin do not inhibit ADP-induced platelet aggregation. Studies of platelet adhesiveness with glass bead columns and glass tubes have been inconclusive (86, 405, 422 455, 490, 524, 571, 686) but the general impression appears to be that high concentrations of heparin do diminish platelet adhesiveness (269, 455, 631). We have found that heparin at a final concentration of about 20 units/ml will inhibit ADP-induced platelet aggregation in human and rabbit citrated plasma (201). The mechanism by which heparin produces this effect has not been elucidated but it is known that heparin binds to cell membranes (365). In addition, heparin can interact with ionized calcium, and it has been shown that 80 units of heparin can bind 2 μmoles of calcium (635).

6. Fibrinogen degradation products. As previously discussed, fibrinogen has been considered by a number of investigators to be a necessary cofactor for ADP-induced platelet aggregation. Studies have been carried out on the effect of fibrinogen split products (formed by fibrinogenolysis) on platelet aggregation. It has been reported that the early products of fibrinogen digestion, which contain large fragments of fibrinogen, inhibit ADP-induced platelet aggregation (329, 355, 356, 364). The late products, which consist mostly of the small fragments D and E, are not active (282, 364). The concentration of the early products required to inhibit ADP-induced platelet aggregation is fairly high (329, 364). It is possible that fibrinogen degradation products are absorbed by the platelet surface and act as antagonists to absorbed fibrinogen (352), which is believed to be necessary for ADP-induced platelet aggregation. Some of the fibrinogen degradation products have been reported to cause platelet aggregation (28), and it appears that this is due to the formation of complexes of fibrinogen split products and fibrin monomers (353). Wilson *et al.* (678) found that two of the products from trypsin-digested fibrinogen inhibited ADP-induced aggregation, whereas one of the products accelerated it.

Although Sharp *et al.* (593a) and Bell *et al.* (38a) reported that Arvin (a purified coagulant fraction of Malayan pit-viper venom) had no effect on platelets, Prentice *et al.* (535a) found that it inhibited ADP-induced aggregation.

They attributed this inhibition to the production of fibrinogen degradation products in the circulation of patients receiving this therapy.

D. Factors influencing platelet metabolism or contractile protein

In view of the report that cyclic AMP inhibits platelet aggregation (264), the effects of compounds that cause the accumulation of cyclic AMP in cells are of some interest. Methylxanthines [caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), and aminophylline (theophylline-ethylenediamine)] inhibit and reverse platelet aggregation induced by ADP (13, 347). These compounds are believed to promote the accumulation of cyclic AMP in cells by inhibiting an enzyme (phosphodiesterase) that catalyses the hydrolysis of cyclic AMP to AMP (99).

The prostaglandin E_1 (PGE_1) is the most potent inhibitor of ADP-induced platelet aggregation *in vitro* that has yet been described (117, 190, 350, 647, 657). PGE_1 increases cyclic AMP in isolated lung, diaphragm, spleen (42), platelets (41), and other tissues, and causes maximal stimulation of adenyl cyclase of human platelets (680). Whether the increase in cyclic AMP is the mechanism by which PGE_1 inhibits ADP-induced platelet aggregation is not known. In his initial studies, Kloeze (350) reported that PGE_2 stimulated platelet aggregation and van Crevald and Pascha (647) confirmed this. However, Weeks and associates (117, 657) found that PGE_2 inhibited platelet aggregation, but that it was less potent than PGE_1.

In vitro, PGE_1 in low concentrations does not inhibit the initial decrease in light transmission, (*i.e.*, the change of platelet shape that occurs when ADP is added) but at higher concentrations, PGE_1 does inhibit this initial decrease (346). The inhibitory effect of PGE_1 on platelets appears to be dependent on its presence in the fluid surrounding the platelets because its effect is readily removed by resuspending the platelets in fresh medium (346). PGE_1 does not seem to have much effect on the conversions of ^{14}C-labeled adenine nucleotides (ATP, ADP) added to the fluid in which platelets are suspended (346). The action of PGE_1 in inhibiting ADP-induced platelet aggregation does not appear to be related to a reaction with calcium (347). In addition to an effect on cyclic AMP, prostaglandins also cause contraction or relaxation of muscle, depending upon the site of action, the concentration, and the particular prostaglandin used (41, 42). PGE_1 has a powerful vasodilator activity and it has been speculated that the action of PGE_1 on platelet aggregation may be similar to the effect of other vasodilator drugs. However, Weeks *et al.* (657) could not find any correlation between the vasodilator activity of prostaglandins and inhibition of platelet aggregation.

Emmons *et al.* (190) found that the intravenous infusion of PGE_1 into rabbits inhibited platelet aggregation and diminished the formation of thromboemboli at sites of injury. PGE_1 is rapidly inactivated when given intravenously, and, to produce an effect on platelet aggregation in rabbits *in vivo* (demonstrated as an effect on hemostatic plug formation), PGE_1 had to be given as a continuous intravenous infusion (346). In rats, a massive dose of PGE_1 (2 mg/kg intra-

venously) prevented platelet aggregation (tested *in vitro* with ADP) for as long as 30 min (117). However, in human subjects, intravenous infusion of 0.05 to 0.1 μg/kg/min for 30 min had no detectable effect on the ability of platelets to aggregate upon the addition of ADP (106). Since higher concentrations of PGE_1 produce undesirable side effects in man (106), doses which might have affected platelet aggregation were not tested.

Born *et al.*, (73) in their study of a series of analogues of adenosine, found that their effectiveness as inhibitors of platelet aggregation was correlated with their vasodilator activity in terms of the increase of forearm blood flow in man. In studies with human platelet-rich plasma, Hampton *et al.* (245) found that chromonar (intensain; [3-[2-(diethylaminoethyl)-4-methyl-2-oxo-2H-1-benzopyran-7-yl] oxy] acetic acid ethyl ester), papaverine, phentolamine, propranolol, and glyceryl trinitrate inhibited ADP-induced platelet aggregation. They suggested that these compounds may alter platelet behaviour by affecting the ion flux across the cell membrane, leading to alterations of the contractile components of the platelets.

The dependence of ADP-induced platelet aggregation on metabolic energy will be discussed in section VII.

E. Miscellaneous factors influencing ADP-induced aggregation

Several other substances have been observed to influence ADP-induced platelet aggregation and platelet adhesiveness. The intravenous infusion of polyvinylpyrrolidone and dextran in man has been reported to reduce platelet adhesiveness (575) as measured by the glass bead method of Hellem. In some experiments dextrans decreased ADP-induced platelet adhesiveness (100, 141), but dextrans have also been found to cause platelet aggregation (175). Haematoporphyrin inhibits ADP-induced platelet aggregation (163) and it has been suggested that this compound may interfere with the ADP binding site, or complex with proteins involved in the adherence of platelets to each other. Clofibrate [(*p*-chlorophenoxy)-2-methylpropionic acid ethyl ester] inhibits ADP-induced platelet aggregation (546), and it also decreases platelet adhesiveness (107, 224) as determined with glass surfaces. This compound also prolongs platelet survival in man (224). Newland (478) observed that uric acid increased the incidence of ADP-induced platelet thrombi in rats and that this was probably due to retardation of the rate of deaggregation. The administration of warfarin sodium to man was reported to cause an increase in the duration of ADP-induced platelet aggregation (351, 530). In contrast, Newland and Nordöy (480) have reported that larger doses of warfarin sodium significantly reduced ADP aggregation of platelets *in vivo* in the rat.

Dipyridamole causes a reduction in ADP-induced platelet clumping (191). Recently another pyrimido-pyrimidine has been observed to inhibit ADP-induced platelet aggregation (188). Dipyridamole may exert its effect by potentiating the action of adenosine (626). Since these compounds also inhibit the platelet release reaction, a more detailed discussion is presented in section VIII.

Although the studies are not conclusive, some of the oral contraceptives

appear to enhance platelet sensitivity to ADP-induced aggregation (189, 206, 276, 531).

White blood cells inhibit ADP-induced aggregation, apparently by inactivating ADP (510, 255a).

Hawkey (266) has described a substance found in the saliva of the vampire bat, *Desmodus rotundus*, capable of inhibiting ADP-induced platelet aggregation. The nature of this factor has not been determined but it is of some interest because its effect is long-lasting. Wounds made by these bats often continue to bleed for several hours after the bat has ceased to feed.

Zweifler and Sanbar (713) found that ethanol and isopropanol were ineffective, but the amphipathic alcohols (benzyl alcohol and phenol) diminished the adhesion of platelets to glass beads and inhibited ADP-induced platelet aggregation at a concentration of about 0.1%. The authors postulated that the inhibition produced by these alcohols might be related to the properties that give them a local anesthetic effect.

IV. EPINEPHRINE AND PLATELET AGGREGATION

Mitchell and Sharp (418) discovered that epinephrine (adrenaline) and norepinephrine (noradrenaline) caused platelets to aggregate in citrated human platelet-rich plasma. These observations were confirmed by O'Brien (494), who found that low concentrations of epinephrine appeared to give a two-phased response. Haslam (262), by using enzymes that convert ADP to AMP and adenosine, examined the possible role of ADP in epinephrine-induced platelet aggregation. A venom ADPase prepared from Russell's viper venom completely abolished the second phase of aggregation produced by 5×10^{-6} M epinephrine and greatly reduced the extent of the first phase. The addition of a high concentration of the venom ADPase 5 min after the addition of 5×10^{-6} M epinephrine caused an incomplete reversal of the second phase of epinephrine-induced platelet aggregation. Haslam (262) interpreted this to mean that ADP is involved in both phases of the aggregation of platelets by epinephrine. If epinephrine releases platelet ADP, one would expect changes in platelet shape to occur, but it has been reported that epinephrine does not increase the volume of the platelets or change their shape (96, 504, 591). However, in our opinion, the evidence is inadequate. The epinephrine in Bull and Zucker's experiment (96) was added to a very dilute suspension of platelets in which an ADP release effect may have been missed. Furthermore, it is not apparent from this paper that the platelets used actually aggregated upon the addition of the concentrations of epinephrine used, and there is considerable variation in the response of human platelets to epinephrine. O'Brien and Woodhouse (504) also have reported that epinephrine does not produce a change of shape in platelets, but their published observations indicate that epinephrine, at concentrations that cause platelet aggregation, produces a change of shape. In their electron micrograph of epinephrine-treated platelets, over two-thirds of the platelets shown have formed pseudopods (504), but in the control preparation, very few platelets have pseudopods. Mills, Robb, and Roberts (414) reported that during the initial phase of epi-

nephrine-induced platelet aggregation most of the platelets appear to have retained their disc shape, but their electron micrographs show that most of the platelets had an irregular shape with pseudopods. Recordings published by MacMillan (380) show that epinephrine does cause a change in the oscillations of the intensity of the light transmitted through platelet-rich plasma from the oscillations that are characteristic of disc-shape platelets to a pattern characteristic of swollen platelets with pseudopods. It would appear therefore, that the conclusion that epinephrine does not cause platelets to change in shape needs to be re-examined critically. Although epinephrine itself may not do this, it seems likely that the ADP that is released would produce these changes.

Ardlie *et al.* (14) found that epinephrine potentiates the action of ADP. Mills and Roberts (416) showed that epinephrine at a concentration of 5×10^{-7} M potentiated the aggregating effect of ADP and that concentrations of ADP that had virtually no effect in causing platelet aggregation did so when epinephrine was also present. They found that norepinephrine had the same effect but was less potent than epinephrine. Ardlie *et al.* (14) observed that isoproterenol also potentiates ADP-induced platelet aggregation, although this compound does not cause platelet aggregation. Mills and Roberts (416) could not confirm this potentiation.

In the studies that have been carried out, few attempts have been made to measure the ADP concentration in the suspending medium. It has generally been assumed that the amount of ADP released from platelets or damaged red cells during the preparation procedure will be insignificant because of the enzymes that convert ADP to AMP. However, in studies in which low temperatures are used in the preparation of platelet-rich plasma and the plasma samples are kept at low temperatures until tested, it is possible that the enzymatic conversion of ADP might be so slow that significant quantities might be present in the suspending fluid to affect the action of epinephrine. This could be responsible for the species differences observed in epinephrine-induced platelet aggregation; epinephrine has not been found to cause platelet aggregation in rat, rabbit, guinea pig, horse, dog, or pig platelet-rich plasma (131, 418, 446, 605). In most of these animals, ADP is converted to AMP much more rapidly than it is in human plasma (446). However, in suspensions of washed rabbit platelets from which plasma has been removed (and thereby enzymes that might rapidly convert ADP) epinephrine does induce platelet aggregation (347).

Mills and Roberts (416) found that the three effects of epinephrine on human blood platelets, namely, the initial aggregation, the second phase of aggregation, and the potentiation of ADP-induced aggregation, can all be prevented by phentolamine and dihydroergotamine. However, phentolamine did not reverse aggregation produced by epinephrine or epinephrine together with ADP, once the second phase had begun. They suggested that epinephrine acts on an *alpha*-receptor, presumed to be either in or on the platelet membrane. Since they could not find any evidence for a *beta*-receptor action (see below), they concluded that the hypothesis of Ardlie *et al.* (14) that epinephrine potentiates aggregation through an effect on adenyl cyclase was not tenable. However, they made one

observation that did not support the involvement of an *alpha*-receptor: N-(2-chloroethyl) dibenzylamine hydrochloride (Dibenamine hydrochloride) and phenoxybenzamine (Dibenzyline; dibenyline; N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl) benzylamine) did not inhibit epinephrine-induced aggregation or the potentiation by epinephrine of the ADP effect (but see below). Because of this they suggested that the epinephrine receptor on platelets may differ in some respects from the *alpha*-receptor in smooth muscle.

Bygdeman and Johnsen (101a) reported that epinephrine was 8 times more potent than norepinephrine in inducing platelet aggregation in citrated human platelet-rich plasma. They found that the aggregating effect of the catecholamines was stereospecific and that the L- form of norepinephrine was 30 times more potent than the D- form. Platelet aggregation induced by epinephrine and norepinephrine could be blocked by phentolamine, phenoxybenzamine, and Dibenamine in the concentrations of 10^{-6} M, 10^{-4} M, and 10^{-4} M respectively These observations apparently contradict those of Mills and Roberts (416). who found that phenoxybenzamine and Dibenamine were not inhibitory, but the maximum concentration tested by Mills and Roberts was only one-half of that used by Bydgeman and Johnsen (101a). Bydgeman and Johnsen reported that the *beta*-receptor blocking agents, pronethalol and propranolol were effective only at 10^{-3} M. Mills and Roberts (416) had reported similar findings for propranolol and had concluded that in high concentrations it inhibits not as a *beta*-blocker, but in the same way as compounds of the imipramine type. The uptake of norepinephrine and serotonin by the platelets was inhibited by both *alpha*- and *beta*-receptor blocking agents in concentrations of about 10^{-5} M (101a). Bydgeman and Johnsen concluded that because there was no correlation between inhibition of uptake by *alpha*-receptor blocking agents and their effect on catecholamine-induced platelet aggregation, uptake of epinephrine or norepinephrine was not a necessary step in catecholamine-mediated platelet aggregation.

Thomas (634) observed that aggregation by collagen or thrombin of platelets in human citrated platelet-rich plasma was potentiated by epinephrine in concentrations as low as 10^{-8} M. He found that aggregation induced by these substances could be reduced by the use of *alpha*-receptor blocking agents such as phentolamine and dihydroergotamine. He concluded that during the aggregation by collagen or thrombin, but not by ADP, sufficient platelet catecholamines are released to potentiate aggregation.

Recently Mills *et al.* (414) have observed that epinephrine causes the release of up to 30% of the platelet ATP and 55% of the platelet ADP. This was associated with the release of serotonin but not with the release of acid-phosphatase, β-glucuronidase, or adenylate kinase. Niewiarowski *et al.* (482) concluded that platelet aggregation induced by low concentrations of epinephrine does not cause any significant release of platelet antiheparin activity (platelet factor 4). However, higher concentrations of epinephrine do cause release of platelet factor 4 (482, 693). In addition to these effects, epinephrine diminishes the inhibiting effect of adenosine on ADP-induced platelet aggregation (14).

As discussed in the previous section, when platelets are exposed to ADP they become refractory to further additions of ADP. However, if these platelets are now exposed to epinephrine they will aggregate (498). Conversely, platelets incubated without stirring with epinephrine for 90 min will not aggregate upon the addition of further epinephrine with stirring, but the addition of ADP will cause aggregation.

V. SEROTONIN (5-HYDROXYTRYPTAMINE) AND PLATELET AGGREGATION

Mitchell and Sharp (418) found that the addition of serotonin (5-hydroxytryptamine creatinine sulphate) to human citrated platelet-rich plasma caused platelet aggregation, which was followed by deaggregation. They observed a more variable effect in rabbit citrated platelet-rich plasma; some samples were unresponsive. The addition of calcium to rabbit citrated platelet-rich plasma gave a dramatic change in the response to serotonin. Increasing the calcium concentration by as little as 2 mmoles/litre decreased the minimal amount of serotonin required to induce aggregation from 10 μg/ml to 0.1 μg/ml. This observation was confirmed by O'Brien (496). Subsequent studies have shown that serotonin causes aggregation of human and rabbit platelets but that the extent of aggregation is small, rapidly reverses, and never enters the second phase that can be demonstrated with ADP *in vitro* (31, 67). Furthermore, serotonin potentiates the aggregation brought about by ADP or epinephrine. Serotonin induces an immediate change of shape and swelling similar to that produced by ADP, (31, 503). Baumgartner and Born (30a, 31a) concluded that the aggregation brought about by serotonin is connected with its active uptake into the platelets and is caused by the release of ADP formed from ATP during the active uptake of the amine.

Promethazine hydrochloride, compound Ro-3-0837 (Roche), methysergide, and iodoacetic acid all inhibit serotonin-induced platelet aggregation (418). Diphenhydramine (496) inhibits serotonin-induced platelet aggregation at concentrations less than that required to inhibit other clumping agents. Low doses of reserpine (0.2 to 0.5 mg/kg) inhibit platelet aggregation and accelerate deaggregation (712). Low concentrations of lysergide (D-lysergic acid diethylamide; LSD-25) inhibit serotonin-induced platelet aggregation in sheep citrated platelet-rich plasma (411a). This compound does not inhibit ADP-induced platelet aggregation. Michal (411a) suggested that the platelet serotonin receptors are of the classical D type, not unlike those involved in the response of the smooth muscle of the guinea pig intestine to serotonin.

Platelets exposed to serotonin for 1 minute or more become unresponsive to ADP, epinephrine, or serotonin (31, 496, 498). The inhibitory effect of serotonin on platelet aggregation has also been demonstrated *in vivo* (294). Baumgartner and Born (31) have suggested that this could provide a new approach to the prophylaxis of thrombotic disease because exposure of platelets to high levels of serotonin would make them unresponsive to the usual aggregating agents, particularly epinephrine. They have proposed that the inhibitory effect of serotonin on platelet aggregation occurs when serotonin combines with specific

receptors on the platelet membrane, which are identical to the receptors concerned with ADP-induced platelet aggregation. Aggregation and potentiation occur as long as a sufficient portion of the receptors are free to react with the serotonin molecules. The receptors transport the serotonin through the membrane and the amine-receptor complex then dissociates. The amine diffuses through the cytoplasm and is taken up into storage organelles, while the receptor returns empty and free to react once more at the platelet surface. With increasing exposure to serotonin there is still net movement of serotonin into the platelets and the serotonin in the storage system exchanges with serotonin in the medium *via* the active transport receptors in the membrane. Thus, a diminishing proportion of the membrane receptors return free to react. This hypothesis accounts for several observations: 1) that the concentration of serotonin (3 to 3.4 $\times$ 10^{-7} M) that causes active uptake to proceed at half the maximal velocity is very similar to that (about 5 $\times$ 10^{-7} M) which causes half maximal velocity of aggregation; and 2) that platelet aggregation by serotonin is inhibited by imipramine at concentrations that also inhibit the active uptake of serotonin. They concluded that analysis of the inhibitory effect of serotonin showed that it has something in common with imipramine and similar substances that inhibit the second phase of aggregation caused by epinephrine or ADP.

Baumgartner and Born (30a, 31a) have recently found that epinephrine, in concentrations which alone did not cause platelet aggregation, enhanced the aggregation induced by serotonin. When platelets were saturated with serotonin *in vivo* aggregation *in vitro* by serotonin or by serotonin plus epinephrine was inhibited but the aggregation to ADP was unchanged. Depletion of platelet serotonin by reserpine treatment of rabbits did not change the response of the platelets to serotonin or epinephrine. However, when serotonin was injected into the reserpinized rabbits, the platelets were aggregated neither by serotonin nor by serotonin plus epinephrine.

Several studies have been carried out on the metabolism, transfer, and storage of serotonin by blood platelets (525). Platelets suspended in plasma can take up serotonin *in vitro* against concentration gradients of up to 1000:1 (72). Uptake is influenced by the type of anticoagulant; it is greater in the presence of sodium citrate than in the presence of EDTA. Serotonin present in platelets can exchange completely with serotonin in the plasma in which the platelets are suspended (72). The transfer of serotonin into platelets is believed to involve both a passive and an energy-requiring process (525). Evidence for the active process is based upon the following observations: 1) It occurs against a considerable concentration gradient and reaches a saturation level. 2) There is structural specificity in that tryptamine (serotonin is 5-hydroxytryptamine) and bufotenine (N,N-dimethylserotonin) uptake by platelets is much less than that of serotonin and does not reach a saturation level. 3) The uptake of serotonin is decreased by metabolic inhibitors and lack of glucose in the suspending medium. It has been proposed that there is a membrane carrier which may bind about 10^4 molecules of serotonin per human platelet (68).

The evidence for the passive transfer of serotonin into platelets is largely based

upon the fact that if platelets are incubated with serotonin at a low temperature, small amounts of the amine enter the platelets but the uptake is proportional to the serotonin concentration in the medium (68). The outflow of serotonin from platelets is also influenced by temperature, being greatly diminshed at low temperatures, but is not energy-dependent. The release of serotonin during the "release reaction" is energy-dependent (393).

Pletscher and others (22, 513, 525) have described in some detail the metabolism of serotonin by platelets and the sites of storage. From a number of experiments involving drugs and electron microscopy, it has been postulated that there are two subcellular transfer sites for serotonin—the platelet membrane and the intraplatelet storage granules. Pletscher (525) concluded that the serotonin transfer at the cell membrane involves active processes because it is inhibited by potassium cyanide and ouabain.

There is good evidence that within the platelets, serotonin is localized within granules that show a dense osmophilia when the platelets have been fixed with glutaraldehyde and osmium tetroxide (22, 641). The dense osmophilic serotonin organelles disappear selectively after treatment with reserpine or amphetamine-like drugs but not after imipramine treatment (149). The uptake by the granules is probably dependent on the ATP concentration and may involve the binding of serotonin to ATP. It has been found that calcium will cause release of serotonin from isolated granules, but in this experiment the role of ATP was not assessed (393). It has been shown with isolated granules from the adrenal medulla that ATP is necessary for the release of catecholamines (182).

The platelets of various species metabolize serotonin mainly to 5-hydroxytryptophol and 5-hydroxyindole acetic acid (30, 526). The enzyme responsible is monoamine oxidase, but the serotonin stored in the granules is probably unavailable to this enzyme. The endogenous platelet serotonin liberated by drugs such as reserpine, or by thrombin, is only partially converted into 5-hydroxytryptophol and 5-hydroxyindole acetic acid (525). Phenylalkylamines such as amphetamine liberate serotonin from platelets without the formation of metabolites. This is probably due to the fact that these compounds inhibit monoamine oxidase.

Reserpine, imipramine, cocaine, and chlorpromazine are among the compounds that inhibit serotonin uptake by platelets (513, 525, 625). Imipramine considerably diminishes the uptake of serotonin by platelets, and it has been proposed that since this drug has no effect on the intracellular storage of serotonin or the metabolism of serotonin its probable site of action is on the platelet membrane (31, 148, 525). Reserpine-like drugs appear to exert their effect by inhibiting uptake by the storage granules (68).

Serotonin is known to affect the movement of potassium across cell membranes. Born (66) has found that when plasma contains serotonin at a concentration of about 5 μg/ ml, the influx of potassium into platelets is increased as long as the platelets take up serotonin. This effect does not occur when the concentration of serotonin is lower (2 μg/ml) or much higher (50 μg/ml). He found that the concentration of serotonin in platelets did not affect the potassium influx. Since

increased concentrations of potassium in plasma can accelerate the accumulation of serotonin by platelets, these observations, together with those from earlier studies (577, 664), indicate that the two transport mechanisms can influence each other.

As well as being able to take up serotonin against a concentration gradient, platelets can accumulate a variety of other substances such as reserpine (615b), quinidine (615b), amino acids (696b), sugars (400a) and the diuretic drug ethacrynic acid (696c). Guanethidine is taken up by active transport, but the binding sites for it seem to be different from those for serotonin because guanethidine accumulation is independent of serotonin levels and neither the uptake nor the release of guanethidine is affected by reserpine (77a). In contrast, debrisoquine (debrisoquin; 3,4-dihydro-2(1H)-isoquinolinecarboxamidine) inhibits the uptake of serotonin by competing for a common transport process and interferes in the storage of serotonin within the platelets; it also inhibits platelet monamine oxidase activity (615a)

VI. PLATELET MORPHOLOGY

A. Normal platelet morphology

Platelets are poor subjects for light microscopy because of their size; the diameter of a cross section is of the order of 2 to 3 μ. With the development of the electron microscope and preparation methods suitable for platelets, more detailed information about platelet morphology has become available. Since platelets form pseudopods, spread, and release constituents upon contact with surfaces, the technique of handling blood samples greatly influences the structure and appearance of the platelets after fixation. Furthermore, if platelets have been exposed to anticoagulants, their morphology may be altered. In ultrathin sections, platelets that have been rapidly fixed in glutaraldehyde or osmic acid are usually disc-shaped (33, 307, 673). The longitudinal dimension of the disc form is 1.5 to 5 μ and the transverse dimension is 0.5 to 2 μ. Platelets that have not been exposed to stimuli rarely have cytoplasmic protrusions or pseudopods. The cytoplasmic matrix is surrounded by a membrane and contains a number of organelles: mitochondria, electron-dense granules (probably lysosomes), very electron-dense bodies (probably storage granules for compounds such as serotonin), vacuoles and vesicles, microtubules, filaments, glycogen, lipid inclusions, remnants of the Golgi complex, and possibly ribosomes (307).

The outer membrane of platelets has a thickness of 70 to 90 Å and has an ultrastructural appearance similar to that of other cells. Since platelets are derived from megakaryocytes, it has been suggested that the surface membrane is formed from the plasma membrane of the megakaryocyte (36). On the outer surface of the membrane there is a fluffy coat 150 to 250 Å in thickness. This coat is considered to be an integral part of the cell membrane and is believed to consist in part of protein (307) and a sulphated acid mucopolysaccharide (673). The rapid changes associated with the adhesion of platelets to each other and to surfaces undoubtedly involve this external coat.

The platelet surface contains proteins identical to some of those in plasma, including coagulation factors V, XI, and I (fibrinogen) (300, 468). Other protein clotting factors such as factor II, VII, VIII, IX, X, and XII have been identified in the peri-platelet atmosphere but are washed off with varying degrees of ease (79, 300, 337, 468). In addition, platelet lipoprotein or phospholipid (platelet factor 3) on the surface becomes available to accelerate the clotting reaction when the platelets are aggregated by ADP or other stimuli (110, 250, 389, 449).

The intracellular location of platelet fibrinogen is not established. Nachman *et al.* (471) concluded that intraplatelet fibrinogen is associated with the granule fraction, whereas Davey and Lüscher (154) presented evidence that fibrinogen was associated with vesicular elements and mitochondria. Morphological studies with ferritin-labeled antifibrinogen showed that the antibody was distributed throughout the cytoplasm (607, 674). Although the membrane fibrinogen appears to exchange with the plasma fibrinogen there is no evidence that the intraplatelet fibrinogen does this (468).

The number of mitochrondria reported in platelets has ranged between 1 and 6 per platelet section seen in the electron microscope. The mitochondria have a double surrounding membrane as in other cells but contain relatively few cristae (307). Although present in small numbers, and morphologically poorly developed, the mitochondria of the platelets seem to be important in the generation of metabolic energy. Most platelet functions, such as clot retraction, adhesiveness, aggregation, and phagocytosis of particulate matter, are completely inhibited only when both the glyoclytic and the oxidative phosphorylation pathways are blocked (see section VII).

There appear to be at least two types of granule—those involved in the storage of amines such as serotonin (641) and others that appear to be lysosome-like (307, 391). Each of these granules is surrounded by a single membrane with a thickness of 70 to 80 Å. Serotonin has been located in granules characterized by homogenous distribution of very electron-dense material (22). The ATP and ADP that are released when platelets are stimulated is believed to come from these granules. The other granules are less electron-dense and have often been referred to as the *alpha*-granules. These granules differ considerably in size and are heterogenous in appearance. Several materials are reported to be associated with these granules, including platelet factor 3 and lysosomal enzymes (391, 672). The bulk of the evidence seems to indicate that the membrane lipoprotein is the source of platelet lipid (platelet factor 3) for the clotting reaction (389).

Although most of the granules recognized as lysosomes in cells are electron-dense and surrounded by a single membrane, the types that have been shown to contain hydrolytic activity are so heterogeneous in their ultrastructural appearance that no granule seen in the electron microscope can be identified with certainty as a lysosome on the basis of morphological criteria alone. Marcus *et al.* (391) have presented considerable evidence that at least a portion of the platelet granule fraction does fulfill the biochemical criteria for lysosomes. Giudici and Turazza (226) demonstrated the presence of cathepsins and β-glucuronidase in platelets. Although Siegal and Lüscher (599) reported that the fraction

from density gradient centrifugation containing microvesicles had large amounts of cathepsins and β-glucuronidase, and suggested that the microvesicle fraction may better fulfill the requirement for lysosomes than the granule fraction, the work of Day *et al.* (164a) indicates that the *alpha*-granules are the platelet lysosomes.

Platelets fixed in glutaraldehyde and then in osmium tetroxide have vacuoles, and the membranes surrounding these vacuoles are similar to the outer platelet membrane (34, 307). In some cases the membranes are continuous so that there appear to be cavities in the platelet surface. In some platelets this is so extensive that the platelets have a spongy appearance. These channels and vacuoles have been referred to as the surface connecting system or canalicular system (34). In addition, a system of small vesicles or tubules is seen in platelets fixed with osmium tetroxide or glutaraldehyde (307). These vesicles may appear empty in the former fixative, but with the latter they appear to contain a substance of low electron-density. Various hypotheses have been proposed concerning the origin and functions of the system of vacuoles and vesicles in platelets. Some authors have suggested that these elements originate from the endoplasmic reticulum and the Golgi complex of the parent megakaryocytes (326, 585). A Golgi complex can readily be demonstrated in the perinuclear zone of the megakaryocytes and has an appearance similar to that of other cells (307). David-Ferreira (159) suggested that some of the vacuoles and vesicles result from the active incorporation of the external medium by the process of pinocytosis.

Two structures within platelets are thought to be important in maintaining platelet shape and in platelet contractility; these are the microtubules and the filaments. In glutaraldehyde-fixed human, rabbit, hamster, dog, and rat platelets a marginal bundle of fine tubules can be seen lying along the membrane in the equatorial plane of the platelet (33, 44, 267, 576, 601, 609). The microtubules are believed to be formed from filamentous parallel subunits in a longitudinal arrangement. The total diameter of a tubule, consisting of about 12 subunits, is 200 to 300 Å (33, 307). This structure is thought to be rigid and to break rather than bend. The microtubules are labile structures similar to those which have been described in other cells (307, 710). They are probably involved in maintaining the disc shape of platelets becaues they disappear when the platelets become spherical in response to low temperature, and reform when the platelets are warmed to 37°C and regain their disc shape (307, 673, 710). Incubation of platelets with colchicine, vincristine, or vinblastine sulfate causes loss of platelet microtubules and this is associated with a change in platelet shape to spheres with many pseudopods. These compounds inhibit ADP-induced secondary platelet aggregation (670, 671). The change in platelet shape produced by agents such as thrombin, ADP, or collagen has been attributed to a decrease in the circumference of the marginal band of microtubules as they move into the interior of the platelet (549, 668). It has been suggested that pseudopod formation may involve the appearance of new microtubules parallel to the axis of the pseudopod (602).

As with microtubules, filaments are rarely seen in intact platelets fixed in

osmium tetroxide, but they are frequently observed in morphologically altered platelets in hemostatic plugs or thrombi fixed in the same manner (307, 710). Filaments are seldom found in glutaraldehyde-fixed platelets, but are visible in negatively stained platelets. There is no conclusive evidence that filaments are present in intact, unstimulated platelets (710). From a morphological point of view, it is possible that these filaments are composed of contractile protein. Zucker-Franklin *et al.* have presented evidence that the filaments are thrombosthenin (710, 711). The relationship among thrombosthenin, the filaments, and the microtubules is not clearly defined. It has been suggested that the actin moiety of filaments, in its F form, is similar to the filamentous elements that form the microtubules (61). If this is so, then the actin moiety of thrombosthenin could be common to both the filaments and the microtubules. Thrombosthenin is composed of proteins with the characteristics of actin and myosin (48, 710, 711). In situations favouring the dissociation of thrombosthenin into these components (*e.g.*, high ATP concentration) the actin-like moieties may recombine in the form of microtubules. In circumstances favouring association of actin and myosin (*e.g.*, low ATP concentration) thrombosthenin would reform and contraction can occur. In other cells, the fibrils appear to be present during the formation of cytoplasmic protrusions, whereas the tubules appear to act as cytoskeletal structures associated with the maintenance of highly asymmetrical shapes (636, 637).

Platelets contain electron-dense particles that are irregular in shape and have a diameter of 150 to 300 Å. These appear as single elements interspersed between the organelles or as large aggregates in the cytoplasmic matrix. They are morphologically similar to structures identified as glycogen in other cells (307). Since these particles lose their staining properties after incubation with diastase, it probably can be concluded that they do represent glycogen (326).

The question of whether platelets contain ribosomes has not been resolved. Ribosomes are found in megakaryocytes (165, 692) and platelets are capable of synthesizing protein (655). They have been reported in platelets obtained from the rat spleen (248), but there has been no clear-cut evidence of their presence in circulating platelets (307). Since they are similar in size to glycogen granules, it is difficult to differentiate them morphologically.

B. Changes in platelet morphology induced by agents that cause platelet aggregation

Hovig (302, 307) found that rabbit platelets in citrated plasma exposed to ADP formed closely packed aggregates of platelets with pseudopods, the distance between adjacent platelets being 200 to 600 Å. At ADP concentrations less than 1 μg/ml, the structural alterations of the platelets were slight and inconsistent and there was no clear evidence of degranulation. When platelets are exposed to ADP the organelles tend to remain in the centre of the platelets and pseudopods form (549, 668). Skjørten (611) found that the addition of ADP to platelet-rich plasma *in vitro* without stirring led to platelet pseudopod formation with no evidence of platelet degranulation. However, Nolte and Breddin (488) claimed that ADP caused platelet degranulation. They used suspensions of washed

platelets to which normal plasma and ADP were added. It is possible that the washing procedure influenced the platelet structure and in addition, that the plasma added to the suspending fluid may have formed thrombin, which causes platelet degranulation. However, other authors claim that the addition of high concentrations of ADP to platelet-rich plasma causes platelet degranulation (414, 608).

Study of the sequence of changes in platelet morphology during the aggregation and deaggregation of pig and rabbit platelets *in vitro* (461) has shown changes essentially in agreement with those reported by Hovig and others (307, 549, 668). The initial increase in light transmission before platelet aggregation is associated with an alteration from a disc-shape to a form with small blebs or pseudopods. At this stage the platelets are not adherent to each other. During the next few seconds the platelets become more spherical, longer pseudopods form, and the platelets adhere to each other. During the deaggregation phase, the platelets separate from each other and the pseudopods become smaller. Eventually the platelets tend to return to their disc form. During this reaction there is little evidence of platelet degranulation, although there is some vacuole formation (461).

Hovig (307) has reinvestigated the effects of ADP on platelets in native human blood (no anticoagulant) with glutaraldehyde as a fixative. Blood samples were drawn directly into siliconized tubes containing ADP and were continuously stirred. Fixation was initiated after 30 sec. Under these conditions, platelet pseudopods could be observed but there was no clear evidence of degranulation. In zones of contact between the platelets, the fluffy coat appeared to form bridges between the platelets. The nature of these structures is not clear; they may represent protein (possibly fibrinogen or thrombosthenin) or mucopolysaccharide.

Platelet aggregates formed *in vivo* by the injection of ADP (17, 18, 332, 475) show characteristics similar to those described by Hovig (307) *in vitro*. When the platelets in these aggregates appeared degranulated, fibrin strands were present; this indicates that these platelets had been exposed to the action of thrombin. These observations indicate that ADP in concentrations sufficient to induce platelet aggregation does not cause significant morphological alteration except for pseudopod formation and some swelling of the platelets. These changes appear to be reversible.

VII. PLATELET METABOLISM

Platelets require energy to carry out their functions of aggregation (345, 440), release of constituents (345, 438), phagocytosis (459), and clot retraction (45, 137, 238, 439, 440). A major problem in studying platelet metabolism is the necessity of isolating the platelets from blood. All of the isolation procedures involve the use of anticoagulants, some of which bind divalent cations, and in many techniques the platelets have been washed to remove plasma proteins. The time required to isolate the platelets from the blood, the washing procedures, and the suspending media all influence platelet structure and function and undoubtedly affect platelet metabolism (556). This has made it virtually impossible to study platelet metabolism in what could be described as "the resting

state" since the isolation procedures themselves probably involve some platelet stimulation. This may account for the considerable variation in the results of the studies of different investigators. In addition to attempts to study platelets in the "resting state," the effects on platelet metabolism of stimuli that cause release of platelet constituents or platelet aggregation have also been examined. In these experiments the same difficulty is present, in that the base line values determined before stimulation probably do not represent the resting state of metabolism in the platelets. The isolation procedures may also influence the sensitivity of the platelets to ADP-induced platelet aggregation and to the release of platelet constituents, and thus affect the changes in metabolic rates associated with these events.

Platelet metabolism has been studied directly by measuring lactate production (45, 135, 172, 338, 370, 655), adenine nucleotide levels (45, 62, 262, 338, 340, 558, 703), oxygen consumption (171, 318, 348, 438), metabolism of substrates such as ^{14}C-glucose (655), and changes in the amount of platelet glycogen (238, 340, 589, 644, 656, 690). Platelet metabolism has been studied indirectly by observing the effects of inhibition or stimulation of metabolism on the uptake of amines such as serotonin (67, 72, 313, 527, 577, 664), potassium transport (134, 235), clot retraction (6, 45, 78, 88, 135, 238, 439, 440, 509, 702), the release of platelet constituents (345, 395, 438), ADP-induced platelet aggregation (345, 440), and the effects of epinephrine (340, 341).

Although platelets contain glycolytic (238, 390, 652, 655), Kreb's cycle (652, 655), and hexose-monophosphate-shunt enzymes (24, 655, 691), several investigators (45, 120, 338, 376) have concluded that aerobic glycolysis is the main metabolic pathway whereby platelets obtain energy. However, Haslam (263) has pointed out that platelets show a significant uptake of oxygen at 37°C (10 to 20 μl/hr/10^9 platelets) and that, while glycolysis can yield only 2 moles of ATP per mole of glucose, complete oxidation of a mole of glucose can yield 38 moles of ATP. Thus the relative contributions of aerobic glycolysis and the Kreb's cycle to energy production will depend on the efficiency of oxidative phosphorylation in platelet mitochondria. Since platelet glycolysis is more rapid under anaerobic than aerobic conditions (the Pasteur effect) (120, 690), platelet mitochondria must normally generate ATP.

Studies of the metabolism of glucose by platelets indicate that about one half of the metabolized glucose can be recovered as pyruvic or lactic acid, and about one-fifth as CO_2 and water (652). The remaining glucose is believed to be converted to glycogen, amino acids, and lipids.

Platelets form lactate from both glycogen and glucose (120, 135, 172, 338, 340, 370, 536, 690) and can metabolize other hexoses such as mannose, fructose, and galactose (655). Glucose and mannose but not fructose can support clot retraction (137).

Reports concerning the metabolism of citrate by platelets are contradictory. Rossi (555) did not find that ^{14}C-citrate was converted to $^{14}CO_2$ by rat platelets, whereas Rock and Nemerson (547) observed this conversion in washed human platelets. Possibly the different methods of platelet preparation or the species

difference may be responsible for this discrepancy. However, Rock and Nemerson (547) could not show that metabolism of citrate prevented the decline in platelet ATP levels, although Karpatkin (338) has reported that citrate increases lactate production and maintains platelet ATP. The concentration of citrate used in both these studies was very high (40 to 50 mM) but the system used by Karpatkin (338) contained EDTA as well as citrate; it is likely that the ionized calcium concentration was much lower than in Rock and Nemerson's platelet suspensions. Lack of calcium affects platelet glycolysis [as reported by Rossi (555)] and may also inhibit the release of platelet constituents, including ATP. (See section VIII.)

Platelets oxidize ^{14}C-palmitate to $^{14}CO_2$ (181, 547, 551), but Rock and Nemerson (547) did not find that this was associated with maintenance of the total ATP level in the platelets. Measurement of total ATP, however, does not give any information concerning changes in the metabolic ATP pool. A large proportion of platelet ATP is in the storage granules and does not appear to take part in metabolism (289). Both insulin (338) and epinephrine (338, 340) stimulate lactate formation by washed platelets, but the insulin effect requires glucose whereas the epinephrine effect does not.

Platelets incubated in plasma have been reported to maintain their ATP level (558). However, when platelets are incubated in an artificial medium, the ATP level usually falls (340, 556, 703).

Karpatkin and Langer (340) have proposed that if the adenylate control hypothesis (21a) is applicable to platelets, reasonable correlations exist between the observed and predicted glycogenolytic and glycolytic rates. They suggested that the three regulator enzymes known to be extremely sensitive to changes in adenine nucleotides may be important in platelets: (a) phosphorylase b and a; (b) hexokinase; and (c) phosphofructokinase. They speculated that a decrease in platelet intracellular ATP and ADP associated with an increase in intracellular P_i, would lead to glycogenolysis and increased phosphorylase activity. Scott (589) observed increased phosphorylase activity when platelets were transferred to a relatively unphysiologic environment (*i.e.*, buffered salt solutions) and suggested that even slight manipulation could cause this increase.

Oxygen consumption by platelets has been estimated by using both oxygraphic and gasometric techniques. The observations of a number of investigators have been summarized recently by Kitchens and Newcomb (348). The reported values of the oxygen consumption of "unstimulated" platelets range between 4 and 30 mμmoles of oxygen per minute per 10^9 platelets. Oxygen consumption is suppressed by the presence of glucose in the suspending medium (348). Anticoagulants, type of buffer, pH, and ionic strength affect platelet respiration (348).

The aggregation of platelets by ADP has been reported by some investigators to stimulate lactate formation (136, 172, 338). Karpatkin (338) originally reported that ADP did not increase lactate formation in the presence of glucose, but Karpatkin and Langer (340) recently reported that when washed human platelets are exposed to ADP there is increased glycogen depletion and lactate production and net decline in the adenine nucleotides both in the presence and

absence of glucose. Warshaw *et al.* (655) could not find any evidence that ADP affected the oxidation of glucose. Holmsen (289) reported that during ADP-induced platelet aggregation, the nucleoside triphosphates decreased.

Incubation of citrated platelet-rich plasma with 2-deoxy-D-glucose together with either antimycin or oligomycin for 60 min results in almost complete inhibition of ADP-induced platelet aggregation (440). 2-Deoxy-D-glucose, antimycin, or oligomycin incubated alone in platelet-rich plasma does not inhibit ADP-induced platelet aggregation. We have found that when washed rabbit platelets are suspended in Tyrode's solution without added glucose the ability of platelets to respond to ADP gradually diminishes (345). The sensitivity of the platelets to aggregation by ADP can be restored by incubating the platelets with glucose for 30 to 60 sec (345). Since this effect of added glucose can be blocked by the presence of antimycin, 2,4-dinitrophenol, or oligomycin in the suspending fluid, oxidative phosphorylation is probably involved and a source of metabolic energy is necessary for ADP-induced platelet aggregation (345).

Thrombin appears to enhance the rate of lactate formation by platelets (45, 135, 172, 338, 655). Thrombin increases the rate of oxidation of glucose to CO_2, and this effect lasts longer than the stimulation of lactate formation (655). When platelets are exposed to thrombin there is also increased glycogen consumption (340), loss of high-energy phosphate compounds (45, 262, 338, 703), and an increased uptake of extracellular orthophosphate (340). These changes in metabolism are associated with the release into the ambient fluid of adenine compounds. Mürer (438) observed that when platelets are incubated with 2-deoxy-D-glucose and antimycin in combination, thrombin does not cause the release of platelet constituents, but when these inhibitors are used separately they produce little inhibition of thrombin-induced release. We have found that washed rabbit platelets suspended in a medium containing no glucose rapidly lose their ability to aggregate upon the addition of thrombin (345). This ability can be restored by adding glucose to the suspending medium. Lack of glucose also inhibits thrombin-induced release of platelet adenine nucleotides (345). Thus the evidence from several studies indicates that the release of platelet constituents requires energy, which can be supplied by metabolism of glucose through the glycolytic pathway and the Kreb's cycle. It should be pointed out that this is analogous to the release of material from other cell systems in which a dependence on metabolic energy has been demonstrated (182).

Hussain and Newcomb (318) originally reported a burst of oxygen consumption by thrombin-stimulated platelets in their experiments with an oxygen electrode. In a subsequent study from the same laboratory, it was explained that this was an artifact which occurred because thrombin caused the formation of fibrin, which affected the electrode and gave the appearance of oxygen consumption (348). Detwiler (171) also found no evidence that thrombin caused a quick burst of oxygen consumption. He (171) concluded that the apparent effect of thrombin in causing increased oxygen uptake was due to the formation of platelet aggregates, which interfered with the function of the electrode. However, Mürer (438) has reported that thrombin does cause an initial burst of oxygen uptake. It ap-

pears possible that the increased uptake reported by Mürer (438) is related to one or other of these effects producing an artifact. The observation of Warshaw *et al.* (655) that thrombin stimulates oxidation of glucose by platelets is difficult to interpret in the light of this evidence, unless the increase in oxygen utilization is less than the electrode can detect. As Detwiler (171) has pointed out, this effect could have been due to thrombin causing an alteration in the rate of entry of substrate into the platelets or the rate at which it was diluted by endogenous substrates. Both of these factors would influence the formation of labeled CO_2 from ^{14}C-glucose. Thus, there is no satisfactory evidence that thrombin stimulates increased oxygen consumption by platelets.

Corn (136) could not demonstrate any effect of connective tissue on platelet glycolysis, but other investigators (180, 370, 536) have found that collagen increases platelet lactate formation. Puszkin and Jerushalmy (536) observed that both collagen and connective tissue caused platelet aggregation and increased lactate formation. However, the increased lactate production caused by connective tissue was arrested after 15 min. Their studies showed that this arrest was probably due to the binding of divalent cations by mucopolysaccharides in the connective tissue. Chelation of divalent cations inhibits glycolysis both in resting platelets and in platelets stimulated with collagen (370).

Holmsen (289) found an increase in fructose-1,6-diphosphate radioactivity when ^{32}P-labeled platelets were stimulated with collagen. He concluded that fructose-6-phosphokinase is the main rate-limiting glycolytic enzyme, and that the accumulation of its product stimulates glycolysis through lowering of ATP or increase of AMP (291). Doery *et al.* (180) found a 30% increase in soluble hexokinase activity in platelets stimulated with collagen and concluded that this redistribution of enzyme activity contributed to the increased lactate production they observed.

Karpatkin and Langer (340) have reported that epinephrine stimulates platelet glycolysis, but not as extensively as thrombin. An interesting observation in these studies was that the amount of AMP formed in the epinephrine-stimulated platelets was much greater than in platelets stimulated with either thrombin or ADP.

Several studies have demonstrated that the minimal requirements for clot retraction are the presence of fibrinogen, thrombin, cations, glucose or mannose, and metabolically active platelets (45, 78, 137, 260, 377, 378, 509, 702). Bettex-Galland and Lüscher (45) observed that inhibition of hexokinase suppressed clot retraction but that inhibition of oxidative phosphorylation was without effect. They concluded that the energy for clot retraction is largely derived from glycolysis. Gross (238), however, found that inhibitors of respiration such as malonate and cyanide inhibited clot retraction, but the effect was not as great as when the glycolytic inhibitors fluoride and iodoacetate were used. Recently Mürer *et al.* (440) and Mürer (439), reported that 2-deoxy-D-glucose, antimycin, or oligomycin did not prevent clot retraction when used alone, but that the combination of 2-deoxy-D-glucose with either antimycin or oligomycin was effective. Mürer (439) concluded that the effect of the inhibitors of glycolysis and respiration on

clot retraction was due to a reduction in ATP concentration. These studies indicate that metabolic energy is involved in clot retraction and that both the glycolytic and oxidative phosphorylation pathways may contribute. Aledort and Niemetz (6) found that dibucaine inhibited clot retraction but not platelet aggregation. Dibucaine caused platelet potassium loss and inhibited ATPase activity. They concluded that platelet aggregation can occur independently of clot retraction, platelet potassium loss, or ATPase activity. Aledort *et al.* (7) have shown that the sulfhydryl group inhibitor, parachloromercuribenzoic acid, inhibits clot retraction, whereas parachloromercuribenzene sulfonate, which does not readily penetrate the platelet membrane, is ineffective.

Hellem (268), studying platelet retention in glass bead columns (a process that probably involves surface-induced release of platelet constituents as well as ADP-induced aggregation), found that it was necessary to block both the glycolytic and the oxidative phosphorylation pathways to inhibit platelet retention.

Platelets, like most cells, maintain large transmembrane gradients of potassium. Gorstein *et al.* (235) suggested that this requires the participation of an active transport mechanism for potassium influx. Cooley and Cohen (134) concluded that glycolysis was crucial for maintaining high platelet potassium levels. However, Gorstein *et al.* (235) obtained evidence that either glycolysis or oxidative phosphorylation could provide the energy for maintaining platelet potassium levels.

Platelets concentrate serotonin by an active transport mechanism (67, 72, 313, 527, 577, 664). Inhibition of serotonin uptake can be achieved with fluoride, 2,4-dinitrophenol, or cardiac glycosides (72, 577, 664). It has been suggested that the energy required for serotonin uptake is probably provided by both glycolysis and oxidative phosphorylation (525). Since platelets suspended in a medium containing no glucose lose serotonin, maintenance of platelet serotonin must be dependent on metabolic energy (528).

VIII. RELEASE OF PLATELET CONSTITUENTS

A. Introduction

Thrombin, trypsin, collagen, polystyrene particles, emulsions of long-chain saturated fatty acids, antigen-antibody complexes, *gamma*-globulin-coated surfaces, snake venoms, serotonin, and epinephrine are believed to induce platelet aggregation by causing the release of platelet constituents, particularly ADP. With the aid of an enzyme system in which the released ADP was converted to ATP, Haslam (261, 262) concluded that this is the case for thrombin, fatty acids, collagen, serotonin, and epinephrine. In addition to adenine nucleotides, the constituents that may be released include potassium, serotonin, histamine, epinephrine, free amino acids, small amounts of lipoprotein and protein, mucopolysaccharides, enzymes such as acid phosphatase and β-glucuronidase, and platelet factors 3 and 4 (95, 157, 237, 291, 297, 414, 482, 541, 696). The platelet release reaction bears a resemblance to the release or secretion mechanism that has been described for the adrenal medulla (182), the acinar cells of the pancreas (358),

leukocytes (683), mast cells (419), and the posterior hypophysis of the pituitary gland (412).

Examination of the release reaction is complicated by the fact that particulate stimuli such as antigen-antibody complexes, viruses, and polystyrene particles can also be phagocytosed by the platelets.

B. Thrombin-induced release

The early studies of the effect of thrombin on platelets were primarily concerned with evaluating the reaction which has been described as "viscous metamorphosis" (11, 523). Bigelow (50) demonstrated in 1954 that thrombin released serotonin from platelets. This was confirmed by a number of other investigators (218, 237, 700, 701) and it soon became apparent that the platelet changes associated with viscous metamorphosis were also associated with a release of platelet constituents. Because only some of the contents of the platelets were released, Grette (237) concluded that this process differed from lysis and was in effect a selective "release reaction."

Thrombin also causes the release of prealbumin, albumin, platelet specific *alpha-* and *beta*-globulins, fibrinogen, *gamma*-globulin, two lipoprotein factors (one of which appears to be a *beta*-lipoprotein), acid phosphatase, and β-glucuronidase (157, 291). Holmsen and Day (290) found that washed human platelets suspended in a medium containing calcium released little acid phosphatase, but large amounts of β-glucuronidase. It appears that not all lysosomal enzymes participate in the release reaction. Of the enzymes not associated with the granules, lactic dehydrogenase was not released and only very small amounts of cytochrome *c* oxidase were released. According to these investigators (290), the release reaction does not lead to irreversible changes in the platelets, and the thrombin-stimulated release of specific contents from the platelets occurs by extrusion of material located in special granules, just as for catecholamines in the adrenal medulla. They suggested that the difference between the release of acid phosphatase and β-glucuronidase might be explained by the observation of Siegal and Lüscher (599) that acid phosphatase and β-glucuronidase are located in different platelet granules. Mills *et al.* (414) found that thrombin caused the release of about 10% of the platelet β-glucuronidase but only 2 to 4% of the platelet acid phosphatase and adenylate kinase. They suggested that thrombin might have at least two effects: initiation of the release reaction and activation of lysosomal enzymes. In addition to the substances listed above, thrombin also stimulated the release of mucopolysaccharides from the platelets (541) and a factor other than histamine and serotonin that causes contraction of smooth muscle and increases vessel permeability (452, 518).

There has been considerable investigation of the substrate on the platelet surface with which thrombin interacts, but Grette (237) and others (153, 466, 567) concluded that it was not fibrinogen. Jackson and associates (425, 580) came to the opposite conclusion based on their finding that thrombin caused the release of platelet constituents in the presence of chelating agents such as EDTA, but that platelets treated with trypsin in the presence of EDTA remained morpho-

logically intact and would not aggregate when subsequently incubated with thrombin and calcium. The addition of fibrinogen to the trypsin-treated platelets, together with thrombin and calcium, led to the release of serotonin and platelet aggregation. Davey and Lüscher (153, 154) found that some snake venoms that caused release of platelet constituents and platelet aggregation, did not convert fibrinogen to fibrin and conversely, some venoms that clotted fibrinogen did not cause platelet aggregation. The venom of *Trimeresurus purpureomaculata* caused extensive release of platelet constituents but very little clotting of fibrinogen, but the venom of *Ankistirodon rhodostoma* caused rapid clotting of fibrinogen but no release of platelet constituents or platelet aggregation. They suggested that fibrinogen was not the substrate for thrombin, but the evidence is not conclusive because some venoms (196) cause clotting by a different alteration of the fibrinogen molecule than that produced by thrombin. Acetylated thrombin, which has esterolytic but not coagulant activity, is inactive toward platelets (390). Coagulase thrombin, an enzyme formed from prothrombin by the action of staphylocoagulase, which clots fibrinogen, does not cause the release of platelet constituents or platelet aggregation (618). Also, platelets from subjects with afibrinogenemia (section XII B 3 d) are sensitive to the action of thrombin, (8, 104, 243, 550, 568, 614) but this evidence may be questioned because this condition probably involves an alteration in the fibrinogen molecule, rather than a complete lack of this protein. Thrombin can hydrolyze peptide bonds in other proteins as well as fibrinogen (193), and alters the activity of other clotting factors, including factor XIII (373). Thrombin activates the factor XIII associated with platelets, and it has been suggested that this may be the substrate for thrombin on the platelets (344).

Metabolic energy is involved in some of the platelet functions that are stimulated by thrombin, such as clot retraction and the release reaction. If inhibitors of oxidative phosphorylation and glycolysis are added in combination, thrombin-induced release of platelet constituents is decreased by as much as 80% (438).

The role of divalent cations in the release of platelet constituents by thrombin has not been clearly defined. Grette (237) found that at 15°C, calcium was necessary for thrombin-induced release of platelet constituents, but at 37°C, calcium was not necessary. Jackson *et al.* (325) concluded that divalent cations were not necessary. Zucker and Borrelli (700) originally found that thrombin caused release of serotonin from platelets in EDTA plasma but, more recently, Zucker and Jerushalmy (705) reported that EDTA partially inhibited thrombin-induced release. Since the release of constituents from other cells is dependent upon divalent cations, particularly calcium (182), it would be unusual if the platelet reaction did not require divalent cations. We have recently examined the divalent cation requirement in suspensions of washed rabbit and pig platelets and found that the release induced by low concentrations of thrombin is blocked by EDTA (347). If platelets treated with thrombin in the presence of EDTA are washed and resuspended, release occurs upon the addition of calcium. Markwardt (393) has recently reported similar observations. This is analogous to the release reac-

tion of leukocytes, which can be primed by exposure of the leukocytes to leucocidin and triggered by the addition of calcium to the medium (683).

The reasons for the failure of a number of investigators to demonstrate a role for divalent cations in thrombin-induced release of platelet constituents are not clear. It is possible that in some experiments insufficient EDTA was used to chelate all the free and bound divalent cations. In addition, most thrombin preparations contain calcium. Another variable among the different investigations has been the concentration of thrombin used. Chelation of divalent cations does not completely inhibit the release of platelet constituents by high concentrations of thrombin (347). In addition, the crude thrombin preparations used in most studies may have more than one effect on platelets.

Hovig (302, 307) found that in the platelet aggregates induced by thrombin there was no evidence of polymerized fibrin between the platelets; they had pseudopods and the surface membranes of most of them appeared to be intact. Some platelets were disrupted, had lost most of their constituents, and had breaks in their outer membranes. Rodman *et al.* (548, 550) suggested that there were 4 stages in the morphological changes induced by thrombin: 1) an initial lag period in which no morphological changes occurred, 2) pseudopod formation, centralization of granules, and platelet aggregation, 3) disintegration of platelet surface membranes with disappearance of granules and swelling of the pseudopods, and 4) lysis of the platelets. It is apparent from all the studies that have been carried out that the morphological changes are dependent on the thrombin concentration.

It has generally been assumed that thrombin causes irreversible platelet aggregation. However, aggregation induced by low concentrations of thrombin (0.25 unit/ml) in human platelet-rich plasma is reversible if fibrin formation does not occur (262). The intravenous or intra-arterial infusion of thrombin into animals causes platelet aggregation before fibrin formation occurs. In these experiments, occasional fusion of the platelets into a single mass was reported (587, 595, 648). Intravenous thrombin administration to pigs caused platelet aggregation, and a fall in the platelet count, but after 1 to 2 hr the platelets returned to the circulation (464), and the labeled platelets that reappeared were those which had disappeared from the circulation in response to thrombin. These observations were interpreted as indicating that the thrombin-induced aggregates were unstable after the fibrin component had been lysed by the action of the fibrinolytic mechanism. Thus thrombin-induced platelet aggregates may be reversible both *in vivo* and *in vitro* under appropriate circumstances.

C. Effect of other enzymes

Trypsin, papain, and pronase release adenine nucleotides (155, 237, 261) and potassium (95, 696) but not free amino acids from platelets (95). Trypsin releases serotonin (580), but chymotrypsin is inactive (155, 357). Bovine pancreatic elastase releases nucleotides (357). Subtilisin releases serotonin but not nucleotides (29). Snake venoms have a variable effect: the venoms from *Trimeresurus okinavensis*, *Trimeresurus purpureomaculata*, and *Crotalus terrificus* release

adenine nucleotides and serotonin. In some cases, this effect seems to be indirect through the formation of thrombin, while in others it is caused by a noncoagulant venom component, apparently a protein-polysaccharide complex (155, 157).

D. Collagen-induced release

After the observations of Hugues (314) and Bounameaux (80) that platelets adhere to connective tissue, it was discovered that connective tissue suspensions cause platelets to aggregate when they are shaken with citrated or heparinized platelet-rich plasma (303). Subsequently it was demonstrated that suspensions of purified collagen caused platelet aggregation (315, 704), and that the factor released from platelets that induced aggregation was ADP (304, 704).

Hovig (305) concluded that this release reaction depended on calcium. However, Zucker and Jerushalmy (705) reported that EDTA only partially inhibits connective tissue-induced release of nucleotides from platelets. Our own experiments indicate that if adequate concentrations of EDTA are used, or washed platelets are suspended in a medium free of calcium and magnesium, collagen will not induce the release of platelet constituents (347). Metabolic energy appears to be involved because washed rabbit platelets do not release nucleotides upon stimulation with collagen unless glucose is present in their suspending medium (347, 461). Thus in many respects the collagen-induced release of platelet constituents appears to be similar to that produced by thrombin.

Davey and Lüscher (157) have reported that whereas connective tissue causes the release of amino acids, nucleotides, and serotonin from platelets, it does not liberate acid phosphatase or β-glucuronidase. In contradiction of these findings, Mills *et al.* (414) reported that a large amount of β-glucuronidase, and small amounts of acid phosphatase and adenylate kinase were released. These differences may be attributable to differences in the concentration of the collagen used. We have found that collagen causes the release from the platelets of factors (in addition to serotonin and histamine) that increase vessel permeability (447, 518). Collagen makes platelet factor 3 available for the clotting reaction and induces the release of a large portion of the platelet factor 4 (482).

Morphological studies of platelets adherent to collagen fibers have shown that the distance between the platelet surface membrane and the surface of the collagen is less than that between the surface membranes of aggregated platelets (307, 309). Electron micrographs of platelets in contact with collagen fibers *in vivo* have shown a pattern of breaks in the platelet membrane matching the cross striations of the collagen fibers (308, 309, 333). Platelets adhering to collagen become swollen and lose some or all of their organelles. In some experiments it has been found that platelets in contact with small fibers of collagen completely enclose the fibers (448). This may represent phagocytosis. The fact that platelets adhere to collagen fibers in the presence of EDTA (305, 623) indicates that divalent cations are not involved in the adherence of platelets to the fibers.

Some studies of the properties of collagen bear on its reaction with platelets.

Collagen, connective tissue, and hyaluronidase-treated connective tissue all cause platelet aggregation, but their effects on lactate production differ (see section VII and 536). Both the basement membrane and collagen have similar effects on platelets (699). Removal of telopeptides by pepsin does not affect adhesion or the platelet-aggregating effect of collagen, but it does lengthen the time before the onset of aggregation (679). Treatment of collagen with collagenase destroys its ability to aggregate platelets (676, 704). Heated collagen is also ineffective, possibly because the helical structure is lost (676, 704). If D-glucosamine is present with tropocollagen (collagen subunits) during fibril formation, the platelet-aggregating activity of the repolymerized collagen fibers is impaired. This observation has led to the suggestion that carbohydrate impurities in collagen may be involved in the platelet aggregation reaction (367). Blocking the carboxyl groups of collagen (*e.g.*, by acetylation) has little effect, but the *epsilon*-amino groups of lysine on collagen have to be free if platelets are to be aggregated by it (676). Collagen taken from older persons is less potent as an aggregating agent than collagen from young persons (25). It has been postulated that the masking of free amino groups in aged collagen as a result of cross linkage could be responsible, at least in part, for the loss of the platelet-aggregating activity of this protein.

Hirsh *et al.* (283) have found that young platelets adhere to collagen more readily than do older platelets. This is in accord with the increased platelet adhesiveness and turnover found in persons with a history of thromboembolic disease (see section XII C 2).

Collagen may have an indirect effect on platelets through its ability to activate Hageman factor (factor XII of the blood coagulation sequence) (483, 675). This property of collagen appears to be dependent on the free carboxyl groups of the glutamic and aspartic acids. Activation of factor XII leads to thrombin generation, and this thrombin can cause release of platelet constituents and platelet aggregation.

E. Gamma-globulin-induced release

Immunized animals may develop thrombocytopenia after the intravenous injection of the antigen. The same signs can be produced by the intravenous injection of preformed antigen-antibody complexes. Achard and Aynaud (3) reported in 1909 that platelets in the blood of a horse immunized with dog serum were agglutinated by addition to the blood of the corresponding antigen (*i.e.*, the dog serum). Dragstedt *et al.* (183) demonstrated that species-unrelated antigen-antibody complexes caused the release of histamine from rabbit platelets. McIntire *et al.* (407) found that calcium was required for this reaction, but concluded that the clotting mechanism was not involved. Platelets were shown to be the major source of the histamine released from rabbit blood cells during immunological reactions (317).

Platelets will interact with *gamma*-globulin in the presence of surfaces or particles that adsorb *gamma*-globulin, or in the presence of antigens with which *gamma*-globulin can form immune complexes. Polystyrene particles (229, 429,

433, 448, 518) or glass surfaces (199, 516, 518) coated with plasma or *gamma*-globulin cause the release of constituents from washed pig or human platelets. With polystyrene or glass surfaces this activity could be attributed to immunoglobulin G (461). Although albumin did not affect this reaction, the presence of fibrinogen reduced the effect of the *gamma*-globulin-coated surfaces (461, 516). However, Mueller-Eckhardt and Lüscher (433), with lower concentrations of fibrinogen, did not find that it inhibited the reaction, although serum was inhibitory. We have shown that *gamma*-globulin-coated surfaces release platelet serotonin and nucleotides including ADP (199, 229, 429, 448, 516, 518), and nucleotide release has been confirmed by Mueller-Eckhardt and Lüscher (433).

Gamma-globulin-coated surfaces do not cause release of constituents from washed platelets from rabbits unless serum is added (446). Henson and Cochrane (273) found that zymosan particles coated with plasma did not cause release of histamine from washed rabbit platelets unless components of complement were present. The complement components up to C3 or perhaps C5 were necessary for this reaction. On the basis of the experiments with rabbit platelets, one could conclude that complement is involved in this reaction, but it has not been possible to demonstrate a role for complement in similar experiments with suspensions of washed platelets from pigs or man (432, 433, 518). However, polystyrene particles coated with *gamma*-globulin that had been treated so that it would not react with complement did not react with platelets (433). (This observation may be open to question because it was not established that this altered *gamma*-globulin would coat polystyrene particles.)

Heat-aggregated *gamma*-globulin also causes platelet aggregation and the release of platelet constituents (47, 323, 432).

Most studies of the effects of complexes of antigens and antibodies on platelets have centered around the release of histamine from rabbit platelets and the role of complement in this reaction (26, 168, 232, 233, 323, 606). Although washed platelets from rabbits will not release constituents or aggregate upon exposure to antigen-antibody complexes without the addition of complement (273, 446), washed platelets from pigs (427, 518) or people (427, 432) do not require added complement for this reaction. Movat *et al.* (427) found that the addition of antigen-antibody complexes to washed pig or human platelets caused the release of serotonin, histamine, and ADP. Since aggregation could be inhibited by AMP, they concluded that platelet aggregation was caused by the released ADP. Mueller-Eckhardt and Lüscher (432) have confirmed that antigen-antibody complexes cause the release of ADP from human platelets and have reported that complement is not invloved. Although they could not find complement activity in washed human platelets, two other groups have reported complement-like activity in human platelets (472, 518).

Both Henson and Cochrane (273) and Des Prez and Bryant (168) have distinguished the reaction involving rabbit platelets and preformed antigen-antibody complexes or particles coated with *gamma*-globulin, from the reaction involving rabbit platelets, soluble antigens, and soluble antibodies. It should be pointed out that in the latter type of experiment, antigen-antibody complexes

are being formed in the presence of platelets, rather than being added to them. Des Prez and Bryant (168) concluded that the release of rabbit platelet serotonin by this reaction was dependent on complement. Henson and Cochrane (273) reported that the antigen-antibody complex or particulate reaction required only the components of complement up to C3 or possibly C5 for the release of histamine from rabbit platelets and platelet clumping. In contrast, the reaction with soluble antigen and antibody required all the components of complement, up to at least C6, for histamine release. They concluded that in this system platelet clumping could occur without histamine release (the relationship between the release of nucleotides and histamine has not been established). An important difference between these studies is that Des Prez and Bryant (168) were working with platelet-rich plasma containing various anticoagulants whereas Henson and Cochrane (273) used suspensions of washed rabbit platelets to which they added plasma that had been treated in different ways. The conditions under which the complexes were formed in these experiments may have affected their characteristics. Henson and Cochrane (273) have suggested that both these reactions may be manifestations of what is referred to by immunologists as immune adherence. However, these studies do not rule out the possibility that platelet aggregation is due to the release of platelet nucleotides, as appears to be the case with washed human and pig platelets.

With washed pig platelets, the release reaction induced by *gamma*-globulin-coated surfaces or antigen-antibody complexes is dependent upon metabolic energy (347). Henson and Cochrane (273) found that histamine release from washed rabbit platelets by *gamma*-globulin-coated particles was also dependent upon metabolic energy.

Mueller-Eckhardt and Lüscher (432) observed that antigen-antibody complexes caused the release of platelet constituents in the presence of excess EDTA and they concluded that calcium ions were not necessary for this type of release reaction. Henson and Cochrane (273) found that calcium was required for antigen-antibody-complex-induced release of rabbit platelet histamine and it has also been suggested that magnesium is required for this reaction (233). Our studies (347) indicate that antigen-antibody complexes or *gamma*-globulin-coated surfaces do not induce release of constituents from washed pig or human platelets if EDTA is added to the test system in excess of the concentration required to chelate divalent cations.

Antigen-antibody complexes and *gamma*-globulin-coated surfaces also cause the release from washed human or pig platelets of a factor or factors (in addition to serotonin and histamine) that increases vessel permeability and causes contraction of smooth muscle (447, 452, 518).

There have been no reports that these reactions cause the release of other platelet constituents such as lysosomal enzymes, amino acids, or lipoproteins. However, the ultrastructural changes that occur when platelets are exposed to these stimuli indicate that these constituents are also released. The addition of antigen-antibody complexes to suspensions of washed pig or human platelets produces morphological changes similar to those caused by thrombin and collagen

(427, 459, 518). The platelets become swollen, develop extensive pseudopods, become less electron dense and have fewer organelles. Some platelets have breaks in their membranes. As well as causing release of platelet constituents, antigen-antibody complexes can be phagocytosed by platelets (427, 459) (see section XI).

Antiplatelet antibodies also induce the release of platelet constituents such as adenine nucleotides, and produce platelet aggregation (432). Antisera that have a high complement fixing activity have the greatest effect (432).

Schoenbechler and Sadun (582) and Schoenbechler and Barbaro (581) found that leukocytes from the blood of rabbits infected with *Schistosoma mansoni* will, in the presence of the antigen, release histamine from washed rabbit platelets. Henson (271) has also found that leukocytes can augment the release of histamine from washed rabbit platelets. He concluded that the interaction of leukocytes and lymphocytes from sensitized animals with the antigen causes the release or formation of a factor that induces the release of platelet histamine.

F. Plasma proteins and the release reaction

As discussed in the preceding section, if glass surfaces or polystyrene particles are coated with *gamma*-globulin, both release of platelet constituents and platelet aggregation occur (199, 429, 432, 448, 516, 518) with washed pig or human platelets. When the surfaces are coated with fibrinogen or albumin there is little release of platelet constituents. Whereas fibrinogen in the suspending medium inhibits the effect of *gamma*-globulin-coated surfaces on the platelets, albumin has no effect (516, 518). Fibrinogen-coated surfaces enhance the adhesion of platelets to the surfaces (199, 518, 709a). Zucker and Vroman (709a) reported that only fibrinogen promoted the adherence of platelets in plasma to glass surfaces. They concluded that *gamma*-globulin in plasma did not cause the interaction of platelets with glass surfaces, but these studies were carried out with citrated platelet-rich plasma and it has been found that citrate inhibits the reaction of platelets with *gamma*-globulin (in the form of antigen-antibody complexes) (446). Also, in Zucker and Vroman's investigation, the presence of albumin in the serum and plasma undoubtedly influenced the results.

Coating the surfaces of extracorporeal shunts with *gamma*-globulin or fibrinogen enhances thrombus formation from flowing blood in rabbits (199). The greatest effect was found when the surfaces of the shunt were coated with *gamma*-globulin. Albumin-coated surfaces, on the other hand, had no enhancing effect. There is some evidence that if the materials used for prosthetic devices in vascular surgery preferentially bind albumin, they are much less thrombogenic (see section XII C 3).

G. Endotoxin and platelet aggregation

Bacterial endotoxin has been reported to have an effect on platelets similar to that of antigen-antibody complexes (167). Endotoxin clumps rabbit, and dog, but not human platelets; causes the release of histamine from rabbit platelets, and serotonin from rabbit or dog platelets; and makes platelet factor 3 available

(170, 277, 299, 409). When endotoxin is injected intravenously, it rapidly and selectively accumulates with the platelets rather than with the other formed elements (83, 274). It has been demonstrated that a heat-labile plasma factor is necessary for endotoxin-induced injury of washed rabbit platelets, and Des Prez (166) concluded that the heat-labile plasma factor involved in this reaction was not complement but was similar to properdin. Spielvogel (624) has presented evidence that endotoxin may interact with rabbit platelets through the mechanism of immune adherence, but Mueller-Eckhardt and Lüscher (435) concluded from their studies with washed human platelets that endotoxin does not cause an immunological platelet injury.

Endotoxin can be phagocytosed by platelets and its interaction with platelets can cause degranulation (162, 624).

H. Other particulate material, bacteria, and viruses

Platelets will adhere to quartz, titanium dioxide, cobalt and carbon particles (52). Kaolin causes aggregation in human citrated platelet-rich plasma (250), makes platelet factor 3 available (250, 620), and induces release of serotonin (620). Glycogen liberates histamine and serotonin from rabbit platelets (651). Uric acid crystals release serotonin and nucleotides (448). In a number of animal experiments it was observed that the platelet count fell after intravenous injection of micro-organisms or particles (178, 184, 569, 632). Cohen *et al.* (124) showed that the thrombocytopenia produced in animals by the injection of inert particles such as India ink was due to destruction of the platelets. Recently Van Aken *et al.* (646) reported that carbon particles were cleared at a slower rate when transient thrombocytopenia had been induced by ADP infusion. They concluded that the aggregation of blood platelets is probably essential in the transport of carbon to the reticuloendothelial system. It should be pointed out that the inhibiting effect of Thorotrast (thorium dioxide) and endotoxin (234) on the clearance of carbon (39) could be mediated through the effect of these agents in reducing the number of circulating platelets, rather than through a direct action on the reticuloendothelial system.

Several investigators have demonstrated that platelets adhere to bacteria (301, 552) but there is no evidence that platelets can phagocytose bacteria (459). When bacteria are added to suspensions of washed platelets in the presence of *gamma*-globulin, platelet constituents are released and platelet aggregation occurs (518). It is likely that the interaction of bacteria with platelets may involve some aspects of the antigen-antibody reaction, although the bacteria themselves may have some specific effects on platelets. Staphylococcal toxins damage human platelets and can cause platelet aggregation (43, 388, 600). Staphylococcal *alpha*-toxin is believed to be responsible for these effects.

Terada *et al.* (633) found that both live and dead influenza viruses were rapidly absorbed by human platelets. The infusion of a virus suspension produced a rapid and sustained fall of the platelet count. There seems little doubt that myxoviruses also can cause platelet aggregation (93, 327, 375, 426). We have found that the addition of viruses to a suspension of washed platelets in the

presence of *gamma*-globulin releases platelet constituents (518). Schulz and Landgräber (586) demonstrated that human platelets absorbed influenza virus onto their surface and that the platelets phagocytosed this virus. The interaction between this virus and platelets led to platelet degranulation and aggregation. The Chitungunya virus (which can cause a viral hemorrhagic fever in man) can interact with human platelets. Platelets stabilize this virus against thermal inactivation at 37°C (361). There is some evidence that viruses may interact with the glycoprotein on the platelet surface (520).

I. Fatty-acid-induced release

Intravenous infusion of some fatty acids causes a fall in the platelet count which is associated with the formation of platelet aggregates (127, 128, 129, 596). Haslam (261) found that addition of the sodium salts of palmitate, stearate, arachidate, behenate, or lignocerate to a suspension of washed human platelets induced platelet aggregation. Since the aggregating effect of the fatty acids could be abolished by the addition of pyruvate kinase and phosphoenolpyruvate to the suspending medium, he concluded that the mechansim by which fatty acids cause platelet aggregation is through the release of platelet ADP. Stearate and longer-chain saturated fatty acids release serotonin and histamine from rabbit platelets in heparinized plasma (596). The importance of proteins in this mechanism was observed by Haslam (261); he found that the presence of 4% human albumin in the suspending fluid of the human platelets prevented platelet aggregation by 0.1 mM behenate. Haslam pointed out that the effects of fatty acids are demonstrable only if a portion of them is not bound to albumin. There have been contradictory reports concerning the effects of polyunsaturated fatty acids. Two groups of investigators observed that polyunsaturated fatty acids did not cause platelet aggregation (342, 383), whereas Warner *et al.* (654) found that linolenate caused platelet aggregation. Variations in concentration of the fatty acids used and the influence of albumin on the binding of fatty acids may have affected the results. Low concentrations of stearic acid that do not cause aggregation of human platelets enhance aggregation in the presence of epinephrine (15).

Upon examination by electronmicroscopy, platelets in aggregates induced by fatty acids frequently possess intact external membranes, many pseudopods, and some alteration in electron density; the granules and mitochondria maintain their structural appearance (654). However, some of the platelets appear to have lost their granules and it has been suggested that the fatty acids may cause some platelet degranulation. According to Shore and Alpers (596), calcium ions are necessary for fatty-acid-induced release of platelet constituents and platelet aggregation.

The relevance of these studies *in vitro* to the effects of fatty acids *in vivo* is not clear. The intravenous infusion of fatty acids into rabbits (694), dogs (164, 287) and bats (615) causes thrombocytopenia and the formation of intravascular thrombi. Zbinden (694) found that lauric-acid-induced platelet aggregates were retained in the lungs, and with small doses of lauric acid, platelet aggregation

was reversible. The evidence from a number of studies indicates that fatty acids bound to albumin have little activity, but it has been postulated that when all the albumin-binding sites become saturated, unbound fatty acids would be present in plasma. Hoak *et al.* (285) concluded that fatty acids bound to albumin were thrombogenic, but less so than unbound fatty acids. Further support for the concept that free fatty acids may be involved in the formation of thromboemboli has been obtained in animal experiments. The subcutaneous injection of ACTH (adrenocorticotropin; corticotropin) into rabbits caused thrombosis which was associated with high plasma free fatty acid levels (286).

Free fatty acids activate factor XII (77, 127, 392) and therefore their effects on platelets might be due to the generation of thrombin, rather than to a direct effect of the fatty acids on the platelets. However, free fatty acids can aggregate platelets in the presence of heparin concentrations that inhibit blood coagulation (596). Also, fatty acids aggregate washed platelets (261, 287), including platelets from persons with factor XII deficiency (287). This evidence indicates that fatty acids affect platelets directly, and possibly affecting them through activation of the coagulation mechanism.

J. ADP-induced release

Born and Cross (70), Constantine (131), and MacMillan (380) observed that with certain concentrations of ADP, the reversal phase of platelet aggregation was interrupted by a second phase of platelet aggregation, which continued until the increase of light transmission equaled the change produced by larger concentrations of ADP (see section II D). In studies in which potato apyrase was used to convert ADP to AMP, Haslam (262) found two cycles of aggregation and concluded that the secondary aggregation could be attributed to ADP-induced release of platelet nucleotides. Mills *et al.* (414) also observed two phases of ADP-induced platelet aggregation, and determined that the second phase was associated with the release into the plasma of up to 30 % of the platelet ATP and 55 % of the ADP. Labeled serotonin (414, 645, 708) and very small amounts of acid phosphatase and adenylate kinase are also released during the second phase but this is probably not part of the specific release reaction (414). Mills *et al.* concluded from their morphological evidence that during the second phase of ADP-induced platelet aggregation, degranulation occurred.

K. Epinephrine-induced release

Haslam (262) found that enzymes that dephosphorylated ADP inhibited both phases of epinephrine-induced aggregation; he agreed with O'Brien's conclusion (496) that epinephrine releases ADP from platelets. Epinephrine also releases serotonin, ATP, and β-glucuronidase from platelets (414).

L. Serotonin-induced release

Haslam (262) observed that venom ADPase caused extensive inhibition of serotonin-induced platelet aggregation and concluded that the aggregation induced by serotonin was mediated by ADP. However, he suggested that the

lack of a lag phase before aggregation began indicated that ADP was released by a mechanism different from that involved in the release induced by collagen or thrombin. Born (67) has pointed out that aggregation induced by serotonin is "small and rapidly reversed and never enters the second phase." This could be interpreted as evidence that serotonin does not induce release.

M. Miscellaneous agents that induce release

Some dextrans release platelet serotonin (666) and both dextran and dextran sulfate release histamine (244). Liquoid (heparinoid, polyethanolsulfonate) (49), triethyl tin (495), methyl mercuric nitrate (at low concentrations) (545), thimerosal (605), fluoride (610) and an enzyme from *Aspergillus oryzae* (630) cause platelet aggregation, probably by causing the release of ADP. Several agents cause a nonspecific release of platelet constituents that may reflect lysis of the platelets. Among these agents are digitonin (digitin), triton X-100, desoxycholate, and lysolecithin (157). Dimethyl sulfoxide causes release of protein, β-glucuronidase, acid phosphatase, and nucleotides (158). Bee venom, which contains both surface-active basic polypeptides and phospholipase A, has a lytic effect on platelets (157).

Tidball (635) has reported that washed rabbit platelets suspended in a balanced salt buffer spontaneously release all their histamine, but that this does not occur when the platelets are suspended in sodium chloride solution. He showed that ionized calcium was necessary for the reaction. Since washed rabbit platelets suspended in Tyrode's solution release serotonin unless a protein is present in their suspending fluid (516), Tidball's observation may be explicable on this basis.

Asymmetric acidic anions (derivatives of salicylic, resorcylic, anthranilic, and thiophencarbonic acid) induce fibrinolytic activity in human and animal plasma at low concentrations (418a). Most of these compounds induce platelet aggregation *in vitro*. Some of them, when used in high concentrations, lyse the platelets. Modifications of the molecules that reduced their activation of fibrinolysis also diminished their effect on platelets. Although it has not been established that these compounds cause platelet aggregation by the release of platelet ADP, this may be the case because their aggregating effect was inhibited by adenosine and adenosine monophosphate.

Neuraminidase has been reported to cause platelet aggregation, not by ADP release, but by the selective removal of sialic acid from the platelet membrane (306).

Polylysine causes aggregation (580a) but the reaction is instantaneous and does not appear to involve appreciable release of platelet constituents (461).

IX. INHIBITORS OF THE PLATELET RELEASE REACTION

Many compounds and conditions have been shown to inhibit the platelet release reaction. It is not possible at this stage to classify all these inhibitors in respect to the mechanism by which they inhibit the release reaction but they can be grouped to some extent.

A. Chelation of divalent cations

As discussed earlier (section VIII B), there has been some controversy about the role of divalent cations in the platelet release reaction. It would appear, however, that when high concentrations of EDTA are used, the release reaction induced by low concentrations of thrombin, antigen-antibody complexes, collagen, or *gamma*-globulin-coated surfaces, is blocked (347). Calcium is necessary for the fatty acid-induced release of platelet constituents (596).

High concentrations of aggregating stimuli appear to be able to induce some release of platelet constituents in the absence of ionized calcium (347). Many investigators seem to have been unaware that the concentration of aggregating agents affects the apparent dependence of the release reaction on ionized calcium.

B. Metabolic inhibitors

As described earlier (sections VIII B, D, and E), energy is required for the release reaction induced by thrombin, collagen, or antigen-antibody complexes (345, 438). The combination of 2-deoxy-D-glucose and antimycin in the suspending medium inhibits the release of platelet constituents induced by thrombin or polystyrene (438). There have been several reports that sulfhydryl blocking agents inhibit platelet aggregation induced by thrombin (545, 705), connective tissue (705), serotonin (418), or ADP (418). N-Ethyl maleimide and monoiodoacetate inhibit the release of serotonin induced by thrombin, *gamma*-globulin-coated polystyrene, or antigen-antibody complexes (434). The effect of N-ethyl maleimide can be overcome with L-cysteine (256).

C. Adenine compounds

In section III A it was pointed out that AMP and adenosine inhibit ADP-induced platelet aggregation. There has been much less study of the effect of these compounds on the release reaction. However, relatively high concentrations of adenosine (10^{-4} M) inhibit thrombin-induced release of platelet constituents (347, 705). Adenosine monophosphate also inhibits the release of platelet serotonin induced by thrombin or collagen (705). ATP at a concentration of 5×10^{-4} M inhibits thrombin-induced release of platelet constituents (347).

D. Prostaglandin (PGE_1)

PGE_1 inhibits platelet aggregation induced by thrombin, collagen, antigen-antibody complexes, or *gamma*-globulin-coated polystyrene particles as well as that induced by ADP (190, 346). Its effects have been demonstrated both *in vitro* and *in vivo* (190, 346). PGE_1 in concentrations ranging between 10^{-4} and 10^{-5} M, inhibits the release of platelet serotonin and nucleotides by thrombin, collagen, antigen-antibody complexes, or *gamma*-globulin-coated polystyrene particles, but the concentration of PGE_1 required to inhibit the release reaction is greater than the concentration that inhibits ADP-induced aggregation (10^{-8} M) (346). It appears that PGE_1 has two effects on platelets: the inhibition of ADP-induced platelet aggregation, and inhibition of the platelet release reaction. In this respect it is similar to adenosine.

E. Colchicine

Colchicine binds to the protein component of microtubules (61), prevents cell division (385), and inhibits the release of constituents from stimulated mast cells (225) and leukocytes (386). When leukocytes (386), other cells (98), or platelets (670) are exposed to colchicine, the microtubule system is disrupted. It has been proposed that this effect on microtubules is responsible for colchicine's inhibition of the release reaction (671).

Colchicine, at a final concentration of 10^{-3} to 10^{-4} M, inhibits the release of platelet nucleotides and serotonin induced by thrombin or collagen (347).

Although some investigators were not able to show that colchicine had an effect on clot retraction (670, 710a), Shepro *et al.* (594a), with higher concentrations and longer incubation times, observed that colchicine was inhibitory with bovine platelets.

F. Methylxanthines

As well as inhibiting ADP-induced platelet aggregation, the methylxanthines inhibit the aggregation induced by thrombin or collagen. The methylxanthines at final concentrations of 10^{-3} to 10^{-4} M inhibit the release of platelet nucleotides and serotonin from pig and rabbit platelets induced by thrombin, collagen, or antigen-antibody complex (347). Methylxanthines also inhibit the antigen-induced release of histamine from human leukocytes (369).

G. Imipramine and amitriptyline

Mills *et al.* (414, 415) have shown that imipramine and amitriptyline inhibit the second phase of ADP-induced aggregation at concentrations less than those which interfere with ADP-induced platelet aggregation and also inhibit the release of platelet serotonin and nucleotides induced by thrombin, epinephrine, or collagen.

H. Di-isopropyl fluorophosphonate (DFP) and other orthophosphonates

Di-isopropyl fluorophosphonate (DFP) inhibits the release of platelet serotonin and nucleotides induced by thrombin, collagen, antigen-antibody complex or *gamma*-globulin-coated surface (155, 462). The fact that the administration of high doses of DFP to rabbits prolongs the survival of the platelets in the circulation (118) indicates that there may be some relation between inhibition of the release reaction of platelets and platelet survival. This is of some relevance in studies that involve labeled DFP for estimation of platelet survival because the amounts of DFP used must be kept below levels that influence the interaction of platelets with stimuli.

Ward and Becker (653) have examined the effect of a series of orthophosphonate compounds on the leukocyte response to chemotactic factors. These compounds are relatively specific inhibitors of esterase activity in cell membranes. We found that these compounds inhibit the release of platelet serotonin and nucleotides induced by thrombin, collagen, or antigen-antibody complex (347).

This evidence, and that from the studies with DFP, may indicate that esterase activity is involved in the release reaction.

I. Salicylaldoxime

Gocke (232) showed that salicylaldoxime ($HOC_6H_4CH{=}NOH$) inhibited antigen-antibody-complex-induced release of histamine from rabbit platelets, and Mills and Levine (417) reported that this compound interfered with the action of the third component of hemolytic complement. In suspensions of washed human or pig platelets we found that not only does salicylaldoxime inhibit antigen-antibody-complex-induced release of platelet constituents, but also it blocks the action of thrombin or collagen on platelets (461). Although salicylaldoxime inhibits thrombin-induced release, it does not block the action of thrombin on fibrinogen (461).

J. Adrenergic alpha-receptor antagonists

The *alpha*-adrenergic blocking agents, phentolamine and dihydroergotamine, when added to citrated platelet-rich human plasma, inhibit the aggregating effects of collagen, thrombin, or epinephrine (416, 494, 634). The *beta*-adrenergic blocker, propranolol, is much less potent than *alpha*-adrenergic antagonists in inhibiting the second phase of aggregation induced by thrombin (634). Although the release of platelet constituents was not examined in all these experiments, it seems likely that these compounds inhibit the release reaction.

K. Nonsteroidal anti-inflammatory drugs and related compounds

In early studies it was found that sulfinpyrazone (1,2-diphenyl-4-[2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione) prolonged platelet survival in man and caused a significant decrease in platelet turnover (457, 464, 613). The adhesiveness of platelets from patients treated with sulfinpyrazone was less than that observed when no therapy was being given (613). In subsequent studies in rabbits, it was demonstrated that sulfinpyrazone and phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidinedione) inhibited platelet aggregation induced by collagen, antigen-antibody complexes, or *gamma*-globulin-coated surfaces (519). This inhibition of surface-induced platelet aggregation appeared to be the result of a diminished release of platelet constituents such as adenine nucleotides and serotonin (519).

The administration of either sulfinpyrazone or phenylbutazone to rabbits, in doses sufficient to cause marked suppression of the platelet-collagen reaction, impaired hemostatic plug formation at the ends of transected mesenteric vessels (519). Phenylbutazone and sulfinpyrazone prolonged platelet survival in rabbits to more than twice the normal time and reduced platelet turnover by nearly 50% (519).

Acetylsalicylic acid (aspirin) has been recognized to be associated with hemorrhagic episodes in man (32). Aspirin decreases platelet stickiness (221), and the administration of acetylsalicylic acid to persons with hemorrhagic disorders prolonged the bleeding time (537). Sodium salicylate inhibits ADP-induced platelet aggregation and decreases platelet stickiness (423). We subsequently

demonstrated that acetylsalicylic acid had an effect similar to that of the pyrazole compounds on platelet function, platelet survival, hemostasis, and thrombus formation in extracorporeal shunts (199, 202, 203). That is, acetylsalicylic acid inhibits platelet aggregation induced by collagen, antigen-antibody-complex, or *gamma*-globulin-coated-polystyrene and it appears to do this by inhibiting the release of platelet constituents. Zucker and Peterson's (709) demonstration that acetylsalicylic acid also inhibits the secondary aggregation induced by ADP in human citrated platelet-rich plasma, indicates that acetylsalicylic acid probably inhibits ADP-induced release of platelet constituents.

In the original studies with the pyrazole compounds it was reported that they did not inhibit thrombin-induced release of platelet constituents or platelet aggregation (519). However, it was found that acetylsalicylic acid inhibited thrombin-induced platelet aggregation and release of platelet constituents providing low concentrations of thrombin were used (203). Zucker and Peterson (709) could not show any effect of acetylsalicylic acid on thrombin-induced release of labeled serotonin from platelets, but the concentration of thrombin used was higher than in our studies. When we re-examined the effect of the pyrazole compounds on thrombin-induced release of platelet constituents, we found that the pyrazole compounds did inhibit the effects of low concentrations of thrombin (517).

Although these compounds inhibit the release of platelet constituents induced by collagen, antigen-antibody complexes, *gamma*-globulin-coated surfaces, or ADP, they do not inhibit the primary platelet aggregation induced by ADP (203, 519, 659, 660, 709). In this way they differ from inhibitors such as PGE_1 and adenosine.

The action of the nonsteroidal anti-inflammatory compounds on the release of platelet constituents and platelet aggregation has been confirmed by a number of investigators and it has been shown that they also inhibit epinephrine-induced platelet aggregation (381, 500–502, 660). O'Brien (502) observed that meclofenamate [N-(2,6-dichloro-3-trifluoromethyl phenyl) anthranilate], acetylsalicylic acid, and indomethacin were the most potent inhibitors among the compounds he studied.

There has been controversy as to whether sodium salicylate is as potent as acetylsalicylic acid in inhibiting the release of platelet constituents. In our studies with suspensions of washed rabbit and pig platelets we found that sodium salicylate was almost as potent as acetylsalicylic acid in inhibiting the release reaction (203). However, Weiss *et al.* (660) and O'Brien (501) have reported that sodium salicylate is a much less potent inhibitor of human platelet aggregation induced by collagen or epinephrine. They also found that platelets remain abnormal in their response to collagen or epinephrine for several days after a single oral dose of acetylsalicylic acid (501, 660). Thus the effect of acetylsalicylic acid is not correlated with its concentration in the plasma, in contrast to the pyrazole compounds. The difference between the effects of acetylsalicylic acid and sodium salicylate and the long lasting effect of acetylsalicylic acid may be related to the acetylation of proteins by acetylsalicylic acid (10, 522).

Weiss *et al.* (660) could not demonstrate any effect of acetylsalicylic acid on the platelet nucleotide levels, platelet surface charge, or rate of ADP conversion in plasma. Recently it has been observed that very high concentrations of acetylsalicylic acid inhibit glucose uptake and lactate production by human platelets (179a).

These anti-inflammatory compounds have an effect on the action of endotoxin *in vivo* (200). Indomethacin, phenylbutazone, and acetylsalicylic acid prevent the acute shock caused by intravenous endotoxin infusions in dogs (194, 195, 278). Sodium salicylate, acetylsalicylic acid, phenylbutazone, and sulfinpyrazone block the thrombocytopenia that is associated with the rise in venous pressure and fall in arterial pressure when rabbits are given endotoxin (200). There is no satisfactory explanation for the effects of these drugs on endotoxin-induced platelet aggregation. If endotoxin acts through an antigen-antibody reaction, the effects of these drugs could be mediated by their inhibition of antigen-antibody reactions (94).

It has been shown that fatty-acid-induced thrombocytopenia in rabbits can be inhibited by treating the animals with phenylbutazone, but acetylsalicylic acid was without effect (695). In addition, there is evidence from studies of renal transplants that these nonsteroidal anti-inflammatory compounds inhibit the formation of platelet aggregates in the microcirculation during treatment of rejection episodes (see section XII C 4).

L. Phosphatidyl serine

In earlier studies (451) it was demonstrated that the intravenous infusion of phosphatidyl serine into experimental animals caused a hemostatic defect. It has subsequently been shown that this material inhibits platelet aggregation induced by collagen, thrombin, antigen-antibody complexes, or *gamma*-globulin-coated particles. This inhibition of aggregation is associated with inhibition of the release of platelet constituents (487).

M. Heparin and sulfated polysaccharides

Heparin inhibits thrombin-induced platelet aggregation (122) and the release of platelet constituents (201). When heparin is used in concentrations adequate to inhibit the action of thrombin, it does not affect platelet aggregation induced by collagen, antigen-antibody complexes, or *gamma*-globulin-coated surfaces (201). However, if the concentration of heparin is increased, collagen-induced platelet aggregation is inhibited (201, 275, 503a, 560). Hovig (303) had earlier found that high concentrations of heparin decrease the response of platelets in rabbit citrated platelet-rich plasma to aggregation by tendon extract. High concentrations of heparin (10 units/ml) inhibit the release of platelet constituents induced by collagen, antigen-antibody complexes, or *gamma*-globulin-coated surfaces (201). At this concentration heparin does not inhibit ADP-induced platelet aggregation in rabbit citrated platelet-rich plasma (201). Heparin in high concentrations inhibits epinephrine-induced aggregation (503a). It has

been shown that a sulphonic polysaccharide similar to heparin inhibits platelet aggregation in recalcified citrated bovine plasma (21).

Heparin prolongs platelet survival and decreases platelet turnover in dogs (560). This effect is dependent on the dose of heparin, and when very high doses of heparin are given, platelet survival is shortened, hematomas form, and platelets appear in the lymph (560). In rabbits, very high doses of heparin inhibit not only collagen and thrombin-induced platelet aggregation, but also ADP-induced platelet aggregation (201). Rabbits given high doses of heparin do not form hemostatic plugs at the ends of transected mesenteric vessels during the 30 min after transection (204). These observations indicate that heparin, as well as being an anticoagulant inhibiting the effect of thrombin on platelets, also affects the platelet aggregation induced by either collagen or ADP. In addition, it has effects on platelet survival and hemostasis in experimental animals. Heparin also prolongs platelet survival in man (455).

It has been shown that an aortic mucopolysaccharide will inhibit thrombin-induced platelet aggregation (436). In contrast, a chondroitin-4-sulphate-peptide had no effect on thrombin-induced platelet aggregation but potentiated collagen-induced platelet aggregation (436).

N. Glucosamine

LeGrand *et al.* (367) have shown that platelet aggregation induced by purified repolymerized collagen fibers is inhibited if D-glucosamine has been incubated with the tropocollagen during the polymerization of the collagen fibers. They showed that collagen produced in this fashion did not cause the usual release of platelet constituents.

O. Dipyridamole and related compounds

The effect of dipyridamole on thrombus formation and platelet function has been studied (191, 192). This compound was used because it has been reported to inhibit the breakdown of adenosine in plasma (97). The administration of dipyridamole to rabbits decreased platelet adhesiveness and thus inhibited thrombus formation in injured cerebral cortical vessels (191). In subsequent studies, Philp and Lemieux (521) could not demonstrate a significant effect of dipyridamole on thrombus formation in injured cortical vessels of rabbits and rats. Didisheim (176), however, found that dipyridamole prevents thrombus formation at sites of vessel injury in the rat and in arteriovenous shunts made of Teflon. He and his co-workers also showed that the antithrombotic effect of this compound was not due to its hypotensive effect (177). Recently it has been reported that the administration of dipyridamole to man reduces the incidence of thromboembolism in subjects with prosthetic devices (629). Emmons *et al.* (192) found that this compound inhibits the platelet aggregation in human plasma induced by ADP or epinephrine. We have found that it inhibits collagen-induced platelet aggregation in human and rabbit plasma and that it also inhibits thrombin- and collagen-induced release of serotonin and nucleotides from rabbit platelets (144).

P. Fibrinogen degradation products

Degradation products of fibrinogen can inhibit platelet aggregation. The early products of fibrinogen degradation inhibit thrombin-induced release of platelet serotonin (329). These products also inhibit platelet aggregation induced by ADP or connective tissue particles (356) and the release of adenine nucleotides from platelets induced by thrombin, kaolin, or connective tissue (352). Purified degradation products of fibrinogen (D, E, or the high-molecular-weight component) in final concentrations of 0.1 to 2.0 mg/ml do not aggregate platelets or influence aggregation by ADP or connective tissue suspensions (139); they cause a slight decrease in the adhesion of platelets to glass. Evidently the effects are dependent on the concentration of degradation products, and in the studies in which inhibition has been demonstrated relatively high concentrations have been used. In experiments with rabbits, low concentrations of fibrinogen breakdown products did not interfere with platelet aggregation induced by ADP or collagen but did inhibit that induced by thrombin (280).

One conflicting piece of evidence in respect to the effects of the breakdown products of fibrinogen has been the observation by Barnhart *et al.* (28) that these products cause platelet aggregation. Niewiarowski (481) and also Kopeć *et al.* (353), as a result of the studies of Shainoff and Page (592), examined the complexes that can form between fibrinogen and its degradation products. These complexes can be precipitated by materials which are often referred to as "paracoagulating" agents, such as protamine sulphate. There is evidence (481) that some of these complexes can cause platelet aggregation and this may be the explanation for the observations of Barnhart *et al.* (28).

X. MECHANISM OF RELEASE REACTION

The selective release of constituents from formed elements appears to be a fairly general biological phenomenon. This reaction has been studied in exocrine, endocrine, and neuroendocrine glands, mast cells, polymorphonuclear leukocytes, and platelets (157, 182). With all of these it appears that the selective release of cell constituents is related to the discharge of compounds directly from membrane-limited granules within the interior, without discharge of the granules themselves. The secretory release reaction is dependent upon divalent cations, particularly calcium. It has been suggested that calcium can facilitate the approximation of the granule membrane with the plasma membrane by causing a change in the physical-chemical state of the cytoplasm in such a way as to enhance granule movement (182). Woodin *et al.* (682) have shown that the addition of calcium to leukocytes stimulated with leucocidin causes the enzyme-containing granules to become attached to the plasma membrane. Against this simple explanation is the fact that most of the secretory or release reactions have been shown to be energy-dependent (182, 438). The membrane changes associated with the cellular release reactions appear to involve membrane phospholipids, particularly phosphatidyl inositols (182, 486, 684).

If the granules are isolated from the cells, the release of their constituents, such as catecholamines, can be stimulated by ATP (507, 529, 642). This has

led to speculation that there may be some relation between ATP and an ATPase which has been found in association with some of the granule membranes. Douglas (182) has proposed that the secretory release mechanism may be analogous in many ways to the events that are involved in muscle contraction. For example, Davies (161) has suggested that calcium ions within the stimulated muscle neutralize mutually repulsive charges, thereby allowing approximation of the ATPase to substrate ATP. In respect to the platelet, this is of some interest since Grette (237) originally suggested that the platelet release reaction involved a process analogous to contraction.

The release of constituents from the platelet granules is a rapid, energy-dependent process. It has been proposed that the release reaction may involve the contraction of an internal canalicular membrane system (291). The diverse compounds that inhibit the release reaction may operate through a common mechanism such as inhibition of the contractile response of the release mechanism. Markwardt *et al.* (394) have provided evidence that this may be the case for papaverine. Prostaglandin (PGE_1), which inhibits the release reaction (346), also affects contractile protein (42), possibly through its effect on adenyl cyclase and cyclic AMP (680, 696a). The methylxanthines, which inhibit the platelet release reaction, also influence the discharge of leukocyte granule contents, and their mode of action may be through their effects on phosphodiesterase and cyclic AMP, which can influence contractile mechanisms (369). Although the effect of the sulfhydryl inhibitors could be related to a variety of mechanisms, they do inhibit contractile protein (343), and it has been shown that N-ethylmaleimide binds to platelet thrombosthenin (264). The critical role of calcium in the release reaction is also compatible with the suggestion that the contractile system is involved. All this evidence indicates that the release reaction involves a primary alteration of the membrane, the active participation of divalent cations (particularly calcium), metabolic energy, and possibly the contractile mechanism. This would appear to hold true for the release of nucleotides and substances such as vasoactive amines (serotonin and histamine). However, the release of other cellular constituents such as lysosomal enzymes may involve other mechanisms (157).

The initial nucleotide release from platelets appears to be from a pool that is not metabolically active (288, 292, 321). In contrast to other cell systems, in which the principal nucleotide released is ATP, platelets appear to release both ATP and ADP. However, it is possible that some of the ADP arises from the breakdown of ATP after its release from the platelets.

Holmsen and Day (291) have concluded that the stimuli that release serotonin, nucleotides, and some lysosomal enzymes do not lyse the platelets. Disruption of the platelet membrane by compounds that lyse cells leads to the release of most of the platelet contents. The question as to whether the stimuli that cause selective release also can cause lysis when used in higher concentrations has not been resolved. Electronmicroscopy of platelet aggregates formed both *in vitro* and *in vivo* indicates that thrombin, antigen-antibody complexes, collagen, and *gamma*-globulin-coated surfaces can cause complete lysis of the platelets with

loss of almost all the cytoplasmic constituents. Actual breaks in the membrane are evident (307, 309, 459). It therefore seems likely that there are at least two components to the release reaction from platelets: 1) the selective release of platelet constituents, which may or may not involve a uniform pattern of response for all stimuli and in which the release from amine storage granules may be different from that for lysosomal enzymes; and 2) lysis of platelets with a more generalized release of platelet constituents. Although the platelets may be able to recover from a stimulus in which there is a selective release, it seems unlikely that the platelets can recover from lysis.

XI. PLATELET PHAGOCYTOSIS

When platelets are exposed to polystyrene particles or antigen-antibody complexes, not only does platelet aggregation occur, but phagocytosis of the particles and degranulation of the platelets also takes place (427, 429). This subject is discussed in more detail in another review (459). There are similarities between platelets and leukocytes in their phagocytic reactions. Leukocytes also aggregate when they phagocytose particulate matter such as antigen-antibody complexes, and cellular constituents are released during this process (9, 428).

In vivo, when platelets are stimulated by intravenous injection of antigen-antibody complexes, endotoxin, carbon particles, thorium dioxide, polystyrene particles, *etc.*, the platelet count falls, platelet aggregates form, and examination of the platelet aggregates by electron microscopy often shows particulate matter contained in what appears to be vacuoles within the platelets (162, 427, 429, 459, 632, 646). It has been observed that platelets that have phagocytosed particulate matter are themselves phagocytosed by macrophages and polymorphonuclear leukocytes (429, 459). Leukocytes and macrophages tend to adhere to aggregated platelets (418). It may be that during the process of phagocytosis or the release reaction the platelets become chemotactic to the white blood cells.

Recently Behnke (34) and White (669) have suggested that some of the uptake of particles by platelets is due to a process other than phagocytosis. Behnke (34) has shown that small proteins and particles added to platelet-rich plasma *in vitro* or infused into animals appear in the canalicular system of platelets without apparent alteration in platelet morphology. White (669) has extended these studies to thorium dioxide and concluded that it is taken up through the canalicular system rather than by the process of phagocytosis. However, the evidence published in his paper is contradictory in that it shows that platelets incubated with thorium dioxide for a period of 20 to 30 min actually do undergo degranulation. It may well be that the uptake observed in these investigations is really not different from phagocytosis.

XII. PLATELET AGGREGATION IN TRANSFUSION, HEMOSTASIS, AND THROMBOSIS

A. Transfusion

Platelet transfusions are of therapeutic benefit to patients with thrombocytopenia or platelet defects. Among the problems in the development of useful

techniques have been the short life span of platelets *in vivo*, the limited period of viability during storage, the failure of many transfused platelets to survive in the recipient, and the development of platelet antibodies in patients given multiple transfusions (126, 142, 219, 638). The development by Aster and Jandl (20) of an acid-citrate-dextrose anticoagulant solution of low pH made it possible to prepare platelets for transfusion which were largely viable in the recipient's circulation. In contrast, platelets prepared from blood taken into EDTA rapidly disappear from the recipient's circulation. During storage, changes occur in platelet characteristics such as oxygen consumption (197, 241), glucose utilization (197), lactate production (197), and ATP levels (241). Unfortunately it has not been possible to correlate these changes with the viability of the transfused platelets. Freireich and associates (208) have used ADP to isolate platelets from fresh platelet-rich plasma; the aggregates that form are readily sedimented, but deaggregation occurs and the individual platelets are viable in the recipient's circulation. All of the evidence indicates that to correct the defect in thrombocytopenia effectively, transfusions of viable platelets are necessary (324).

One of the problems with repeated platelet transfusions has been the development of platelet antibodies as the result of isoimmunization (125, 142, 254). In idiopathic thrombocytopenic purpura, in which there is an increased rate of platelet destruction, presumably by an autoantibody, transfused platelets have little beneficial effect (255). The platelet antigen system (413, 597, 598) may be involved in the development of these isoantibodies. Some infants have been born with thrombocytopenia due to isoantibodies produced by the mother (4). Treatment with transfusion of incompatible platelets or by exchange transfusion containing incompatible platelets did not lead to any improvement in the thrombocytopenic purpura. Transfusion of compatible maternal platelets led to sustained improvement in the platelet count and cessation of hemorrhage.

It appears that in the transfusion of platelets, consideration has to be given to the anticoagulant and method of preparation of the platelet concentrate. Nonviable platelets are sequestered and removed in the spleen and liver (19, 23, 185). In the situation in which platelet antibodies are present, it is likely that platelet aggregates are formed and sequestered in these organs (23, 185). The platelets are probably phagocytosed by the cells of the reticuloendothelial system.

B. Hemostasis

1. Introduction. The role of platelets and platelet aggregation in hemostasis has been extensively reviewed (308, 310, 445, 460, 554, 557). When there is a defect in the coagulation mechanism leading to impaired thrombin generation, the platelet mass that forms at a site of injury is unstable. Since the effect of ADP in causing the platelets to adhere to each other appears to be transient *in vivo*, permanent platelet aggregates probably cannot be formed unless fibrin is produced to stabilize them. In normal animals, the initial platelet aggregate is transformed to a mass of fibrin as the platelets separate from each other and undergo dissolution (308). When the interaction of platelets with collagen is impaired, or the platelets do not react normally to ADP, the platelet mass which

forms is defective. It thus appears that the formation of an effective hemostatic plug involves the interaction of platelets with connective tissue, the aggregating action of ADP on platelets, and a normal coagulation mechanism.

The response of platelets *in vivo* to the stimuli that cause platelet aggregation is influenced by congenital or acquired platelet defects. There are two broad classifications which can be considered: 1) a lack of circulating platelets (thrombocytopenia); and 2) abnormal circulating platelets. Among the functions that may be abnormal are platelet aggregation induced by ADP, thrombin, collagen, or epinephrine, the availability of the platelet phospholipid (platelet factor 3) for coagulation, the adherence of platelets to collagen, clot retraction, platelet adhesiveness, platelet survival, platelet phagocytosis, possibly the platelet release reaction, and the activity of a number of enzymes present in platelets.

2. Thrombocytopenia. A reduction in the number of circulating platelets can be caused by a reduced rate of production or by an increased rate of destruction. A number of factors regulate thrombopoiesis and the circulating platelet count. In animals that have been thrombocytopenic for a period of time, there is increased incorporation of precursor material into platelets, whereas in animals in which thrombopoiesis has been inhibited (for example, by hypertransfusion with platelets), there is a decreased incorporation of precursor material (185, 253). Since young platelets adhere more readily to collagen (283) and are metabolically more active than older platelets (339), it is possible that in situations in which platelet production is increased, the reactions of the circulating platelets may be slightly different from those of platelets in a normal state. This may explain some of the reported abnormalities in platelet function that have been found in individuals with low platelet counts.

The mechanisms that cause thrombocytopenia can be divided into two categories—congenital and acquired. Some persons are born with a congenital defect responsible for their low platelet count. In some cases this defect can be corrected temporarily by the transfusion of fresh normal plasma (583, 649).

In a variety of clinical disorders acquired thrombocytopenia occurs: for example, aplastic anemia, in which there is clearly defective platelet production (588). In other circumstances, in which the thrombocytopenia is associated with a decreased number or defective production of megakaryocytes in the bone marrow, the low platelet count probably results from the megakaryocyte defect. In some forms of thrombocytopenia, immune mechanisms may be involved in producing the low platelet count (19, 147, 255). In the syndrome of autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura) the number of megakaryocytes in the bone marrow is not abnormal, but the number of platelets in the blood is reduced. Most investigators believe that in this disorder there is accelerated destruction of the platelets (19, 150, 185, 474).

Many of the stimuli that have been described in the previous section as being capable of causing platelet aggregation can induce thrombocytopenia in experimental animals and in man. These stimuli include thrombin, viruses, bacteria, endotoxin, antigen-antibody complexes, and particulate materials such as thorium dioxide (124, 146, 178, 184, 406, 408, 464, 511, 569, 632). These stimuli

may produce a situation in which the low platelet count is due to an increased rate of platelet destruction. Thus, some forms of thrombocytopenia may be the result of factors that can cause platelet aggregation. The thrombocytopenic effect of some of these agents can be inhibited by compounds that block platelet aggregation (200, 447).

A consequence of thrombocytopenia is an increased susceptibility to bleeding, but there has been some controversy about the level of circulating platelets required to prevent bleeding (379, 619). If animals are rendered thrombocytopenic to the point at which there is increased bleeding from injured vessels, an arrest of bleeding can be produced only by the transfusion of fresh viable platelets (84, 324, 685). Gaydos *et al.* (222) found that hemorrhage rarely occurred when the platelet count exceeded 20,000/mm^3 of blood. This indicates that a relatively small number of platelets is required to maintain hemostasis. It was observed that when the platelet turnover was less than about 10,000/mm^3/day, spontaneous bleeding was likely to occur (457). It should be pointed out, however, that in studies such as this there is no assessment of qualitative platelet changes or the importance of vessel wall factors.

3. Congenital and acquired platelet defects. Among the congenital disorders that are presumed to have a platelet abnormality are thrombasthenia, von Willebrand's disease, thrombocytopathia (thrombopathy), afibrinogenemia, and an abnormal platelet response to collagen. Possibly there are other conditions, not yet clearly defined, in which platelet abnormalities are involved. There are also cases in which thrombocytopathia, abnormal platelet response to collagen, or increased fibrinolysis, occur in conjunction with disease or the administration of certain drugs.

A. THROMBASTHENIA: A severe hemorrhagic disorder associated with defective clot retraction was described in 1918 by Glanzmann (227). Since that time more than 100 cases have been reported in the literature (102, 140). This is an inherited disorder which usually manifests itself early in childhood. The platelets are unable to aggregate when exposed to ADP, thrombin, collagen, norepinephrine, or serotonin (102, 249, 319, 698). Despite the abnormal response to aggregating agents, platelets from subjects with thrombasthenia have been found to release serotonin and ADP (in association with loss of granules) after exposure of the platelets to thrombin or collagen (103, 249, 446, 698, 707). This evidence may mean that their lack of response to ADP is responsible for their failure to aggregate upon the addition of stimuli that cause the release of ADP. Platelet factor 3 (platelet membrane phospholipid) does not become available when these platelets are exposed to ADP (105, 249). These platelets show a defective adherence and spreading on glass surfaces (103, 105, 270) and have a low fibrinogen level (105, 467, 698). Nachman and Marcus (469) found that an antithrombosthenin antibody caused thrombasthenic platelets to aggregate. Normal platelets in platelet rich plasma would aggregate when exposed to a fibrinogen antibody, but the platelets from subjects with thrombasthenia appeared to be less responsive (469). Taylor and Zucker (632a) found that thrombasthenic platelets were not aggregated by antifibrinogen. Many platelet func-

tions and constituents have been found to be normal. These include change of shape upon the addition of ADP (707), serotonin content and uptake (362), platelet survival *in vivo* (473) and electrophoretic mobility (706). However, the electrophoretic mobility of thrombasthenic platelets does not change upon the addition of ADP (244a). Although some investigators have observed that the morphology of platelets appears to be normal in this condition, several authors have reported that the morphology is abnormal and is characterized by fewer mitochondria, mitochondrial swelling, a reduced number of *alpha*-granules, hypervacuolization, and platelet steatosis (102, 105, 307, 397). This condition has also been described in dogs (179) and abnormal or swollen platelets were a predominant feature. Gross and his colleagues (239, 240, 371, 372) have described a number of platelet enzyme defects in association with thombasthenia. They found persons with subnormal levels of glyceraldehyde phosphate dehydrogenase and pyruvate kinase, but because these enzyme defects have not been found in all patients with thrombasthenia, it has been postulated that there is more than one type of condition. The defect in clot retraction indicates that the platelets may have a defective contractile mechanism. Platelets from patients with thrombasthenia appear to be unable to adsorb normal amounts of plasma proteins of high molecular weight (632a). These studies indicate that platelet bound *gamma*-M-globulin and fibrinogen are important in the retraction and lysis of clots formed from diluted whole blood.

B. VON WILLEBRAND'S DISEASE: von Willebrand (650) described an autosomal bleeding disorder in a family living in the Aland Islands which was associated with a prolonged bleeding time. Subsequent studies demonstrated that many of these patients have a low level of factor VIII activity (485), and that platelet function may also be abnormal (363, 658). However, there has been no clear evidence of abnormal platelet aggregation induced by ADP, thrombin, collagen, epinephrine, or other aggregating stimuli (140, 658). In studies with glass bead columns, the degree of platelet retention has been found to be reduced, although a number of investigators have not been able to demonstrate this (259, 570). A significant observation is that the transfusion of plasma from either normal persons or patients with hemophilia A corrects the bleeding defect (484, 584). This has led to the conclusion that the abnormality in von Willebrand's disease is probably related to a missing plasma factor (140, 363, 658). Studies of hemostatic plugs in subjects with von Willebrand's disease reveal that the platelets fail to form a mass adequate to bridge the gap across the transected vessel (331). Jørgensen and Borchgrevink (331) suggested that the platelets did not adhere normally to the injured endothelium.

C. THROMBOCYTOPATHIA (THROMBOPATHY): Thrombocytopathia is a term used to describe a bleeding syndrome which is either inherited or acquired and is manifested by an abnormality in platelet factor 3 activity (81, 390, 643). It may or may not be associated with a prolonged bleeding time. There has been some scepticism about the nature of this disorder—probably generated by the inadequacies of the assay procedures used. Detailed studies by a number of workers have shown that persons with this condition have abnormal prothrombin con-

sumption and a defect in platelet factor 3 (81, 140, 390, 643). In most cases of congenital thrombocytopathia (relatively rare) the platelets are not deficient in platelet factor 3 but are merely unable to make it available when they are stimulated by substances such as ADP (251). In some circumstances the factor-3 content of platelets may also be abnormal (514, 661). Abnormal platelet factor-3 activity is found in some conditions in which giant platelets are present (336) and also in association with some forms of thrombocytopenia (56, 650a). Several acquired conditions with this type of disorder have also been described: these include macroglobulinemia (514), hepatic cirrhosis, (85, 87, 92, 387), leukemia (214), congenital heart disease (5), disseminated lupus erythematosis (662), myeloproliferative disorders (123, 252, 400), uremia (119, 173, 298, 538), and multiple transfusions (60, 512). It has also been reported that the administration of compounds such as dextran interferes with the making available of platelet factor 3 from platelets (205, 663). It is possible that other drugs also may have this effect.

D. AFIBRINOGENEMIA: Patients who lack normal fibrinogen (afibrinogenemia) usually have a prolonged bleeding time (243, 320). Platelet retention in glass bead columns is low (243, 320). The platelets do not aggregate upon the addition of low concentrations of ADP, but high concentrations of ADP have been found to aggregate the platelets from some subjects with this disorder (140, 243, 320, 550). The addition of small amounts of fibrinogen corrects the abnormality. This evidence is in keeping with the fact that fibrinogen appears to be necessary for ADP-induced platelet aggregation. In addition to a congenital defect in fibrinogen, the appearance of fibrinogen breakdown products in plasma also influences platelet function (139, 354, 481) (see sections III C6 and IX P). As was pointed out in the previous discussion, some of these products can actually cause platelet aggregation, although others are inhibitory.

E. ABNORMAL RESPONSE TO COLLAGEN: Hirsh *et al.* (281) described a hemorrhagic disorder in a 12-year-old girl in which there was a long bleeding time and no abnormality in blood coagulation. The principal defect in this subject was an abnormal response to collagen-induced platelet aggregation when platelet-rich plasma was tested *in vitro*. O'Brien (499) also described a group of subjects with a hemorrhagic disorder in which there appeared to be abnormal collagen-induced platelet aggregation. The majority of these patients also showed an abnormal aggregation response to epinephrine. Recently Sahud and Aggeler (566) have reported a hemorrhagic disorder in a 45-year-old woman in which there was abnormal platelet aggregation to collagen and epinephrine and an abnormal adhesion of platelets to collagen fibrils. The defective collagen-induced platelet aggregation was associated with impaired release of ADP when the platelets were exposed to collagen. In all of these studies the authors have reported that the patients were not taking drugs that interfere with the platelet-collagen reaction. However, in view of the long-lasting effect of acetylsalicylic acid on platelet function (section IX K) the data should be interpreted with caution (500, 501, 660).

It is apparent from the studies that have been carried out concerning the effect

of drugs on platelet function that some bleeding disorders may be induced by medications (140). Among the compounds that have to be considered are clofibrate (112, 224, 230), acetylsalicylic acid (203, 660), dipyridamole (192), guaiacol glyceryl ether (604), pyrazole compounds (519), nialamide (isonicotinic acid 2-[2-(benzylcarbamoyl)-ethyl] hydrazide) (223), reserpine (712), polyvinylpyrrolidone (575), and dextran (205, 663). If these compounds are administered to animals or subjects with hemorrhagic disorders they may cause a severe aggravation of the bleeding tendency (460).

F. SCURVY: Wilson *et al.* (677) and Cetingil *et al.* (111) have reported impaired platelet aggregation associated with human scurvy. Born and Wright (76) found that platelets taken from scorbutic guinea pigs showed diminished platelet retention to glass surfaces. Although diminished platelet adhesiveness has been shown in scurvy, Harrison and Honour (257) found that the hemostatic plugs that formed in scorbutic guinea pigs had a normal integrity. Both a vessel wall defect and an abnormal platelet function may be involved in this condition.

C. Thrombosis

1. Introduction. The role of platelets in thromboembolic disease has been discussed in a number of articles (211, 212, 445, 447, 450). Platelet thromboemboli can arise from either intravascular stimuli or alterations in the vessel wall. In earlier sections (VIII E, G, and H) of this review the mechanisms by which endotoxin, bacteria, viruses, and antigen-antibody complexes can cause platelet aggregation were discussed. Experimentally it has been shown that intravascular platelet aggregates can cause organ dysfunction and permanent tissue injury (332, 447). There is some evidence that these aggregates can cause vessel injury (228, 312, 332, 539) and thus set up foci for further platelet aggregation.

Considerable attention has been given to the role of the vessel wall in the initiation of thrombi. Friedman and Van den Bovenkamp (215) and Constantinides (133) have reported that occlusive arterial thrombi in man are almost always associated with breaks in the lining of atherosclerotic plaques. The exposed connective tissue and other plaque contents would serve as stimuli for platelet aggregation. There is little doubt that loss of endothelial cells, or separation of endothelial cells, with exposure of basement membrane or collagen, will lead to the adherence of platelets at the injured site (82, 210, 639).

There is some controversy as to whether platelet masses can form at sites of vessel injury where the subendothelial tissues are not exposed. Sawyer *et al.* (578) have claimed that reversal of the vessel wall potential difference between the outer and inner layers will lead to thrombus formation. However, Martin (396), with a similar technique, has shown that the electric current used in such studies causes vessel wall injury. In his experiments, the thrombi were associated with alterations of the endothelium and exposure of subendothelial structures. Stehbens (627) and Ashford and Freiman (18) have published observations indicating that platelets may adhere to endothelium. Although this has been observed in many electronmicroscopic studies, it has been pointed out that some of the platelets in the aggregate are probably adherent to subendothelial struc-

tures at an area remote from the plane of section (210,). Ashford and Freiman (18) have proposed a mechanism to explain accumulation of platelets at points of injury where the endothelium is apparently not ruptured. They suggest that the endothelium becomes permeable to plasma proteins, with resultant activation of the procoagulant factors by the subendothelial tissues. Local evolution of thrombin would induce platelet aggregation and fibrin formation. They also suggested that in some circumstances platelet masses may adhere to the endothelium by a mechanism that does not involve thrombin. Since endothelial cells have been observed to phagocytose platelets (388a), it seems apparent that platelets can interact with endothelium.

2. Platelet function and thromboembolic disorders. Because of the experimental evidence showing a prime role for platelets in the formation of thrombi, there have been several attempts to examine a possible relationship between platelets and thromboembolic disorders. Since the adherence of platelets to surfaces and the adherence of platelets to each other is crucial in thrombus formation, most of these techniques have been centred around attempts to measure what is generally referred to as "platelet adhesiveness" (258, 269, 458). A variety of methods for measuring this have been developed: Wright in 1941 (686) described a method with a roller flask; Moolton and Vroman (421) used glass wool; Hellem (270) developed a technique with glass beads; O'Brien (491) and Salzman (570) have also developed methods involving glass bead columns.

There is ample evidence that the platelet count rises in the immediate postoperative period (444, 508, 593, 687). Wright (687) found that the rise in the platelet count was paralleled by an increase in the percentage of adhesive platelets. Similar changes in platelet count and platelet adhesiveness occur after parturition (687). The increased platelet adhesiveness found during the first and second day after an operation may be due to changes in the red cells that influence platelet adhesiveness in the test *in vitro* (40, 269). Bygdeman *et al.* (101) proposed that the increased platelet adhesiveness during the postoperative period could be used to diagnose deep-vein thrombosis before the development of physical signs. However, Negus *et al.* (476) could not show any direct relationship between the changes in platelet adhesiveness and the development of thrombosis in the postoperative period. Regardless of whether there is a clear relation between the changes in platelet adhesiveness and the clinical evidence of thrombosis, there seems little doubt that increased platelet adhesiveness is associated with postoperative thrombocytosis.

Moolton *et al.* (422), with their glass wool technique, found that subjects with venous thrombosis showed greater platelet retention than control subjects. Others have also found elevated values for platelet adhesiveness in association with acute venous thrombosis (53, 187, 316). With the Hellem technique, Hirsh and McBride (284) observed increased platelet adhesiveness in association with venous thrombosis and pulmonary embolism. In studies of platelet survival, it was found that subjects with a history of venous thromboembolic episodes had a shorter platelet survival and greater platelet turnover than control subjects (457). There appears to be a relationship among increased platelet adhesive-

ness, platelet survival, and turnover, and clinical manifestations of venous thromboembolic disease. In considering this relationship, it should be remembered that probably only a small proportion of the thromboemboli that occur give rise to clinical manifestations.

Although the results from studies of platelet adhesiveness in subjects who have had clinical evidence of complications of atherosclerosis are varied, the bulk of the evidence seems to indicate that blood from these subjects shows an increased platelet retention when it is passed through columns or tubing containing glass surfaces (296, 401, 404, 442, 477, 564). However, other investigators with the glass bead technique could not find any apparent difference between the mean platelet adhesiveness values of the control and atherosclerotic subjects (187). Rozenberg and Stormorken (564), and Rozenberg and Firkin (561) could not find any differences in ADP-induced platelet aggregation between subjects who had a previous history of myocardial infarction and control subjects. Murphy and Mustard (442) found that subjects who had a clinical history of complications of atherosclerotic vessel disease showed a shorter platelet survival and greater platelet turnover than subjects who had not yet had these manifestations. Furthermore, these groups could be subdivided on the basis of family history and there were differences between the persons with no family history of atherosclerosis and those with a positive family history. There was also a good correlation between platelet retention, assessed by the glass wool technique, and platelet survival and turnover. Since young platelets show increased adhesiveness to surfaces (199, 283) and appear to be metabolically more reactive than older platelets (339), the increased platelet turnover found in association with thromboembolic diseases may be of some significance. However, it is not known whether the increased platelet turnover is secondary to some other factors initiating the thromboembolic disease or is a primary factor leading to the increased incidence of thrombosis.

Investigations with chromium^{51}-labeling methods have not shown any differences in platelet survival between subjects who have had clinical manifestations of thrombosis and those who have not (150, 151). However, it should be pointed out that the chromium^{51}-labeling technique probably alters platelet function and that in most of these studies the number of persons investigated was small, and the estimates of platelet survival were done visually from the graphs, instead of mathematically. Recently Abrahamsen (1), with chromium^{51}, has found that some subjects with a history of thrombotic disorders show a shorter platelet survival than control subjects.

Patients with clinical manifestations of hypertensive cardiovascular disease show increased platelet adhesiveness (532). Moolten *et al.* (420) reported that subjects with diabetes had increased platelet adhesiveness when compared with control subjects. Later, Bridges *et al.* (89) made similar observations and they also found that the administration of glucose increased platelet adhesiveness. Shaw *et al.* (594) were able to confirm the results of Bridges *et al.* (89). However, Ödegaard *et al.* (505) could not find any increase in adhesiveness in 25 diabetic patients when their blood was passed through a column of glass beads. Recently

Hellem (269) has reported that whereas platelet adhesiveness in whole blood was normal, in platelet-rich plasma prepared from blood taken from diabetic subjects, it was increased if the patients were taking insulin.

Moolton *et al.* (422) reported that there was increased platelet adhesiveness in patients with clinical manifestations of carcinoma. Increased platelet adhesiveness has been found in subjects with homocystinuria (402), a disorder which is associated with repeated thromboembolic complications.

Platelets in blood taken from patients with multiple sclerosis stick more readily than normal to each other and to glass surfaces (108, 688). It was suggested that a rise in the unsaturated fatty acid concentration in the plasma could be a factor in causing the increased platelet stickiness (688). Bolton *et al.* (55) suggested lysolecithin in low-density lipoproteins in the plasma of patients with multiple sclerosis as a factor responsible for the increased platelet stickiness. Farbiszewski and Worowski (205a) reported that *beta*-lipoprotein increases the sensitivity of platelets to ADP- and thrombin-induced aggregation.

Evans and Irvine (198) found that surgical patients who had an increased platelet adhesiveness when their blood was passed through glass bead columns, had a smaller chance that their femoral-popliteal by-pass grafts would remain patent. This study appears to demonstrate in man a direct relation between platelet adhesiveness and susceptibility to thrombus formation in flowing blood. Therefore, if the platelet adhesiveness in such subjects could be reduced by compounds such as acetylsalicylic acid, the patency of grafts might be enhanced. In experimental studies in animals, Evans and Mustard (199) presented some evidence that this may be possible.

Several studies have been directed to examination of the relation between smoking and thrombosis (231, 437, 441, 443, 454, 579); the smoking of a cigarette has been reported to increase platelet retention when blood from the smoker is passed through glass bead columns. Other studies have failed to demonstrate this acute effect, but have indicated that there may be a difference in platelet adhesiveness between habitual smokers and nonsmokers. In a study of platelet survival in controlled circumstances, when a person stopped smoking, platelet survival was prolonged and platelet turnover reduced (454). Nicotine has been observed to cause platelet aggregation *in vitro* and to potentiate the activity of ADP (665).

The effect of dietary fat on blood coagulation and thrombosis has been a subject of considerable speculation and study (366, 403). McDonald and Edgill (403) found that platelets from blood taken from persons given low fat diets showed less adhesiveness to glass than platelets from blood taken from subjects given their normal diet. Horlick (296) found that feeding high fat meals appeared to increase platelet retention in glass bead columns. Subjects maintained in a metabolic unit and fed a controlled diet showed the shortest platelet survival and greatest platelet turnover when they were given diets rich in egg yolk and dairy fat (453). The longest platelet survival and the lowest platelet turnover was observed in the subjects who were given the low fat diet. A number of studies and animal experiments indicate that dietary fat influences experimental throm-

bosis (160, 311, 465). Rats given diets rich in butter showed an increased susceptibility to thrombosis and myocardial infarction (311). Mustard *et al.* (465) found that swine given high fat diets containing egg yolk formed more deposits in extracorporeal shunts than swine given low fat diets or diets rich in unsaturated fats. Rabbits fed a diet supplemented with coconut oil and cholesterol show increased platelet adhesiveness in glass bead columns (399). Furthermore, these animals formed increased deposits in extracorporeal shunts. Thus the animal experiments seem to confirm that there is a relation among dietary fat, platelet adhesiveness as measured with glass bead columns, and extent of thrombus formation.

Nordöy *et al.* (489) found a relation between the type and quantity of dietary fat given to rats and the stability of ADP-induced platelet aggregates *in vivo*. After 5 or 6 weeks feeding, the animals given a 40% saturated fat diet had a higher incidence of ADP-induced platelet aggregates in their small vessels after an infusion of ADP than animals given a 10% saturated fat diet. When the 40% fat diet included cottonseed oil, the incidence of platelet aggregates after the infusion of ADP was reduced. These observations indicate that dietary fat may influence the rate at which ADP-induced platelet aggregates break up. Recently Renaud *et al.* (540) have reported that rats fed a diet rich in butter are more susceptible to endotoxin-induced platelet aggregation than rats given a diet rich in corn oil. In these studies, the basic change in platelet function induced by the diet appeared to be an increased susceptibility of the platelets to aggregation by thrombin. There was a reduction in the susceptibility of the platelets in the butterfat-fed animals to aggregation induced by ADP or collagen.

Hampton and Mitchell (246) have introduced the measurement of changes in platelet electrophoretic mobility as a new approach to the study of platelets in persons with a history of thrombosis. They found that patients who have had ischemic heart disease or peripheral arterial disease have platelets that are abnormally sensitive to ADP as determined by the concentration of ADP required to cause the maximal increase in platelet electrophoretic mobility. These investigators (247) found that there is a factor in the plasma that can affect normal platelets so that they exhibit the high electrophoretic mobility shown by platelets from subjects with a history of ischemic heart disease. They concluded that there were two components—a low-density lipoprotein in which the active component is lecithin, and an enzyme that converts lecithin to lysolecithin (54). According to these investigators, the lysolecithin that is formed induces the abnormal platelet sensitivity to ADP.

Abnormalities associated with platelets have been reported in a number of other disorders. During attacks of migraine headaches the platelets lose serotonin but do not lose nucleotide (279). Shortened platelet survival and increase platelet turnover has been found in subjects with a history of gout (456). Platelets from patients with gout show increased susceptibility to surface effects (542) and aggregating agents such as ADP (479); in view of the susceptibility of these subjects to vascular disease complications these observations may be of some significance. Recently it has been reported that the serotonin content of platelets

from a group of rheumatoid patients was much lower than that in normal subjects (138).

3. Platelet aggregation and vascular prosthetic devices. A very clear demonstration of the role of surfaces in the initiation of thrombosis is seen with vascular prosthetic devices (198, 199, 359). In reconstructive vascular surgery, thrombosis is a major problem. Materials of which prosthetic devices are made can cause platelet aggregation (199) and if they absorb plasma proteins onto their surfaces, this effect is modified. *Gamma*-globulin is one of the proteins that can be absorbed, and this protein makes surfaces thrombogenic (199, 516). Albumin-coated surfaces are much less thrombogenic. There have been several attempts to produce nonthrombogenic surfaces which have inhibiting effects on platelet aggregation or blood coagulation (236, 359, 681). Some of these techniques have involved the binding of heparin to the surfaces (236, 359).

Prosthetic devices that cause red cell damage and hemolytic anemia may accelerate platelet destruction (360, 628). Presumably the release of red cell contents, including ADP, would lead to intravascular platelet aggregation and some of the aggregated platelets could be removed from the circulation.

4. Platelet aggregation and organ transplantation. Sensitization of dogs with a small number of allogenic platelets results in accelerated rejection of renal transplants (424). In these studies, vascular damage was the prominent histological finding in the rejected kidneys.

There is good evidence that in the rejection episodes occurring more than 4 days after transplantation of a kidney in man, the renal circulation is obstructed by platelet aggregates (535). Biopsy of kidneys during these episodes has shown the arteries and glomerular capillaries to contain aggregated platelets which, in some cases, completely obstruct the lumen of the vessels (534, 535). Platelet aggregates have also been found in some of the transplanted livers and hearts (431), but the significance of these in terms of the function of these transplanted organs has not been established. The organization of platelet-rich thrombi in arteries leads to intimal thickenings containing phagocytic cells, foam cells, smooth muscle cells, and connective tissue (333). In the vessels of transplanted organs such intimal thickening also occurs (533) and it seems likely that this is related to organization of the platelet thrombi. In the heart lesions, these areas of intimal thickening are focal and sometimes produce considerable narrowing of the lumen of major coronary arteries (431). The mechanism by which the platelet aggregates are formed in the transplanted organs has not been established, but studies of renal transplants have provided some evidence about this.

There appear to be three processes that affect the function and survival of the transplanted kidney (534). These are an initial acute rejection, which occurs in the first few hours after the transplantation, the acute unmodified rejection, which occurs during the first 2 to 3 days after the transplantation, and the rejection episodes that occur during the next 50 days. In addition, in some patients, there is a chronic deterioration in renal function, which is associated with thickening of the glomerular basement membrane.

The acute unmodified rejection of transplanted kidneys in man has not been

shown to involve the blood platelets, probably because it is difficult to obtain suitable tissue for examination. However, it has been observed in experiments with sensitized dogs that platelets are the first formed element to accumulate in the vasculature of the transplanted organ in such an episode (374). It was suggested that this was due to the formation of antigen-antibody complexes within the transplant.

During the rejection episodes that occur 4 or 5 days or later after transplantation, the platelet count falls and, by using chromium^{51}-labeled platelets, it has been possible to demonstrate accumulation of platelets in the transplanted kidney (430). Morphological examination of biopsies from these kidneys has shown the arteries, arterioles, and glomerular capillaries to contain aggregated platelets, many of which completely occlude the lumen of the smaller vessels. The stimulus or stimuli leading to the formation of platelet aggregates in these episodes could be damage to the endothelium with exposure of subendothelial tissues such as collagen and basement membrane, the interaction of antibody with the endothelial cells creating an antigen-antibody complex with which the platelets can interact, or the interaction of antibody with the basement membrane that has been exposed, leading to platelet interaction with the basement membrane and an antigen-antibody complex associated with it. There is no good evidence to establish the primary nature of the vessel wall process causing the formation of the platelet aggregates, although it seems likely that antigen-antibody complexes either with the endothelium or with the basement membrane are involved because there is increased *gamma*-globulin associated with the endothelium or basement membrane (534). Lowenhaupt and Nathan (374a) have suggested that the primary grafts release antigen and thereby initiate the production of circulating antibody. Antibody binding to antigen on the graft induces platelet aggregation. Obstruction of the blood vessels and their injury leads to ischemic death of the graft cells.

By increasing the dose of hydrocortisone and azathioprine in subjects showing this rejection reaction, one can return the platelet count to the pre-rejection level. In experiments in which chromium^{51}-labeled platelets have been used, the radioactivity leaves the kidney and reappears in the circulation. (It has been shown *in vitro* that 6-mercaptopurine and cortisone can inhibit the interaction of platelets with surface stimuli such as collagen (430).) The intravenous administration of phenylbutazone can cause release of platelets from the transplanted kidney (430). Release of platelets from the kidney is associated with some restoration of renal function.

The observation that platelet aggregates in transplanted kidneys can be reversed, indicates that the platelet mass is forming and breaking down continuously and that if one interferes with the primary stimulus, the balance can be shifted so that the platelet mass is not maintained. However, it seems likely that if the platelet aggregates are allowed to persist long enough for some degree of transformation, with fibrin formation, to occur, this process cannot be reversed by such therapy. In these circumstances fibrinolytic therapy might break

up the mass. There is no evidence available at the present moment about this possibility.

REFERENCES

1. Abrahamsen, A. F.: Survival of Cr^{51}-labelled human platelets. Universitets-forlaget, Oslo, 1969.
2. Abramson, H. A.: The electrophoresis of the blood platelets of the horse with reference to their origin and to thrombus formation. J. Exp. Med. 47: 677–681, 1928.
3. Achard, C. and Aynaud, M.: Les globulins dans l'anaphylaxie. C. R. Séances Soc. Biol. 67: 83–84, 1909.
4. Adner, M. M., Fisch, G. R., Starobin, S. G. and Aster, R. H.: Use of "compatible" platelet transfusions in treatment of congenital isoimmune thrombocytopenic purpura. N. Engl. J. Med. 280: 244–247, 1969.
5. Alagille, D., Heim de Balsac, R., Guéry, J., Passelecq, J., Blondeau, P. and Dubost, C.: Les thrombopathies associées aux cardiopathies congénitales; étude de l'hémostase dans cinquante cas. Rev. Fr. Etud. Clin. Biol. 3: 322–331, 1958.
6. Aledort, L. M. and Niemets, J.: Dissociation of platelet aggregation from clot retraction, potassium loss, and adenosine triphosphatase activity. Proc. Soc. Exp. Biol. Med. 128: 658–661, 1968.
7. Aledort, L. M., Troup, S. B. and Weed, R. I.: Inhibition of sulfhydryl-dependent platelet functions by penetrating and non-penetrating analogues of parachloromercuribenzene. Blood 31: 471–479, 1968.
8. Alexander, B., Goldstein, R., Rich, L., LeBolloc'h, A. G., Diamond, L. K. and Borges, W.: Congenital afibrinogenemia; a study of some basic aspects of coagulation. Blood 9: 843–865, 1954.
9. Allison, F. and Lancaster, M. G.: Studies on factors which influence the adhesiveness of leukocytes in vitro. Ann. N.Y. Acad. Sci. 116: 936–944, 1964.
10. Al-Mondhiry, H., Marcus, A. and Spaet, T. H.: Acetylation of human platelets by aspirin. Fed. Proc. 28: 576, 1969.
11. Apitz, K.: Ueber Profibrin. IV. Die Agglutination von Blutplättchen durch Profibrin. Z. Gesamte Exp. Med. 105: 89–94, 1939.
12. Ardlie, N. G.: ADP-induced platelet aggregation without a plasma cofactor. Fed. Proc. 27: 321, 1968.
13. Ardlie, N. G., Glew, G., Schultz, B. G. and Schwartz, C. J.: Inhibition and reversal of platelet aggregation by methylxanthines. Thromb. Diath. Haemorrh. 18: 670–673, 1967.
14. Ardlie, N. G., Glew, G. and Schwartz, C. J.: Influence of catecholamines on nucleotide-induced platelet aggregation. Nature (London) 212: 415–417, 1966.
15. Ardlie, N. G., Kinlough, R. L., Glew, G. and Schwartz, C. J.: Fatty acids and in vitro platelet aggregation Aust. J. Exp. Biol. Med. Sci. 44: 105–110, 1966.
16. Ardlie, N. G. and Mustard, J. F.: Unpublished observations.
17. Ashford, T. P. and Freiman, D. G.: The role of endothelium in the initial phases of thrombosis. Amer. J. Pathol. 50: 257–273, 1967.
18. Ashford, T. P. and Freiman, D. G.: Platelet aggregation at sites of minimal endothelial injury. Amer. J. Pathol. 53: 599–607, 1968.
19. Aster, R. H.: Observations on survival time, sites of sequestration, and production rate of platelets in idiopathic thrombocytopenic purpura. Blood 28: 1014–1015, 1966.
20. Aster, R. H. and Jandl, J. H.: Platelet sequestration in man. I. Methods. J. Clin. Invest. 43: 843–855, 1964.
21. Aznar, J., Lasso, M. and Fernández Plá, A.: Inhibition of platelet aggregation by a sulphonic polysaccharide. Nature (London) 213: 1251–1252, 1967.
21a. Atkinson, D. E.: Regulation of enzyme activity. Ann. Rev. Biochem. 35: 85–124, 1966.
22. Bak, I. J., Hassler, R., May, B. and Westermann, E.: Morphological and biochemical studies on the storage of serotonin and histamine in blood platelets of the rabbit. Life Sci. 6: 1133–1146, 1967.
23. Baldini, M.: Platelet circulation and survival. Bibl. Haematol. No. 29, part 1, pp. 107–122, 1968.
24. Balogh, K., Jr. and Cohen, R. B.: Histochemical demonstration of diaphorases and dehydrogenases in normal human leukocytes and platelets. Blood 17: 491–496, 1961.
25. Bankowski, E., Niewiarowski, S. and Galasinski, W.: Platelet aggregation by human collagen in relation to its age. Gerontologia 13: 219–226, 1967.
26. Barbaro, J. F.: The release of histamine from rabbit platelets by means of antigen-antibody precipitates. II. The role of plasma in the release of histamine. J. Immunol. 86: 377–381, 1961.
27. Barkhan, P.: Platelet survival studies in man with diisopropylphosphorofluoridate ($DF^{32}P$): some observations in patients with hypocoagulability, "hypercoagulability" and platelet disorders. Brit. J. Haematol. 12: 25–36, 1966.
28. Barnhart, M. I., Cress, D. C., Henry, R. L. and Riddle, J. M.: Influence of fibrinogen split products on platelets. Thromb. Diath. Haemorrh. 17: 78–98, 1967.
29. Barthel, W., Hoffmann, A., Markwardt, F. and Wittwer, E.: Ueber den Einfluss von Subtilisin auf die Abgabe biogener Amine aus Blutplättchen. Med. Pharmacol. Exp. 14: 120–124, 1966.
30. Bartholini, G., Pletscher, A. and Bruderer, H.: Formation of 5-hydroxytryptophol from endogenous 5-hydroxytryptamine in isolated blood platelets. Nature (London) 203: 1281–1287, 1964.
30a. Baumgartner, H. R.: 5-Hydroxytryptamine uptake and release in relation to aggregation of rabbit platelets. J. Physiol. (London) 201: 409–423, 1969.
31. Baumgartner, H. R. and Born, G. V. R.: Effects of 5-hydroxytryptamine on platelet aggregation. Nature (London) 218: 137–141, 1968.

31a. Baumgartner, H. R. and Born, G. V. R.: The relation between the 5-hydroxytryptamine content and aggregation of rabbit platelets. J. Physiol. (London) **201**: 397–408, 1969.

32. Beaumont, J. L. and Willie, A.: Influence sur l'hémostase, de l'hypertension artérielle, des antivitamines K, de l'héparine et de l'acide acétyl salicylique. Sangue **26**: 880–891, 1955.

33. Behnke, O.: Further studies on microtubules. A marginal bundle in human and rat thrombocytes. J. Ultrastruct. Res. **13**: 469–477, 1965.

34. Behnke, O.: Electron microscopic observations on the membrane systems of the rat blood platelet. Anat. Rec. **158**: 121–137, 1967.

35. Behnke, O.: Electron microscopical observations on the surface coating of human blood platelets. J. Ultrastruct. Res. **24**: 51–69, 1968.

36. Behnke, O.: An electron microscope study of the megacaryocyte of the rat bone marrow. I. The development of the demarcation membrane system and the platelet surface coat. J. Ultrastruct. Res. **24**: 412–433, 1968.

37. Belamarich, F. A., Fusari, M. H., Shepro, D. and Kien, M.: In vitro studies of aggregation of non-mammalian thrombocytes. Nature (London) **212**: 1579–1580, 1966.

38. Belamarich, F. A., Shepro, D. and Kien, M.: ADP is not involved in thrombin-induced aggregation of thrombocytes of a non-mammalian vertebrate. Nature (London) **220**: 509–510, 1968.

38a. Bell, W. R., Pitney, W. R. and Goodwin, J. F.: Therapeutic defibrination in the treatment of thrombotic disease. Lancet **1**: 490–493, 1968.

39. Benacerraf, B. and Sebestyen, M. M.: Effect of bacterial endotoxins on the reticuloendothelial system. Fed. Proc. **16**: 860–867, 1957.

40. Bennett, P. N.: Role of erythrocytes in the acute platelet response to operation. J. Clin. Pathol. (London) **21**: 695–697, 1968.

41. Bergström, S.: Prostaglandins: Members of a new hormonal system. Science **157**: 382–391, 1967.

42. Bergström, S., Carlson, L. A. and Weeks, J. R.: The prostaglandins: a family of biologically active lipids. Pharmacol. Rev. **20**: 1–48 1968.

43. Bernheimer, A. W. and Schwartz, L. L.: Effects of staphylococcal and other bacterial toxins on platelets *in vitro*. J. Pathol. Bacteriol. **89**: 209–223, 1965.

44. Bessis, M. and Breton-Gorius, J.: Les microtubules et les fibrilles dans les plaquettes étalées. Nouv. Rev. Fr. Hematol. **5**: 657–662, 1965.

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

46. Bettex-Galland, M. and Lüscher, E. F.: Thrombosthenin—a contractile protein from thrombocytes. Extraction from human blood platelets and some of its properties. Biochim. Biophys. Acta **49**: 536–547, 1961.

47. Bettex-Galland, M. and Lüscher, E. F.: Untersuchungen über die Auslösung der viskösen Metamorphose der menschlichen Blutplättchen durch Immunkomplexe. Pathol. Microbiol. **27**: 533–541, 1964.

48. Bettex-Galland, M., Portzehl, H. and Lüscher, E. F.: Dissoziation des Thrombosthenins in seine zwei Komponenten: Untersuchung ihrer Adenosintriphosphatase-aktivität. Helv. Chim. Acta **46**: 1595–1598, 1963.

48a. Betts, J. J., Betts, J. P. and Nicholson, J. T.: Significance of ADP, plasma and platelet concentration in platelet electrophoretic studies. Nature (London) **219**: 1280–1282, 1968.

49. Bettex-Galland, M., Lüscher, E. F., Simon, G. and Vassalli, P.: Induction of viscous metamorphosis in human blood platelets by means other than by thrombin. Nature (London) **200**: 1109–1110, 1963.

50. Bigelow, F. S.: Serotonin activity in blood; measurements in normal subjects and in patients with thrombocythemia hemorrhagica, and other hemorrhagic states. J. Lab. Clin. Med. **43**: 759–773, 1954.

51. Bizzozero, J.: Ueber einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. Virchows Arch. Pathol. Anat. Physiol. Klin. Med. **90**: 261–332, 1882.

52. Bloom, G., Gustavson, K. H. and Swensson, A.: On the reaction of the thrombocytes to submicroscopic particles in vitro. Acta Haematol. **13**: 57–63, 1955.

53. Bobek, K. and Čepelák, V.: Laboratory diagnosis of venous thrombosis. Acta Med. Scand. **161**: 121–133, 1958.

54. Bolton, C. H., Hampton, J. R. and Mitchell, J. R. A.: Nature of the transferable factor which causes abnormal platelet behaviour in vascular disease. Lancet **2**: 1101–1105, 1967.

55. Bolton, C. H., Hampton, J. R. and Phillipson, O. T.: Platelet behaviour and plasma phospholipids in multiple sclerosis. Lancet **1**: 99–104, 1968.

56. Bonnin, J. A.: The management of thrombocytopenic states with particular reference to platelet thromboplastic function. I. Idiopathic and secondary thrombocytopenic purpura. Brit. J. Haematol. **7**: 250–260, 1961.

57. Booyse, F. and Rafelson, M. E.: Cell-free synthesis of contractile protein of human platelets: its location and role in cellular adhesiveness. Blood **30**: 553, 1967.

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

59. Booyse, F. M. and Rafelson, M. E.: Studies on human platelets. III. A contractile protein model for platelet aggregation. Blood **33**: 100–103, 1969.

60. Borchgrevink, C. F.: Platelet adhesion in vivo in patients with bleeding disorders. Acta Med. Scand. **170**: 231–243, 1961.

61. Borisy, G. G. and Taylor, E. W.: The mechanism of action of colchicine. Binding of colchicine-^{3}H to cellular protein. J. Cell Biol. **34**: 525–533, 1967.

62. Born, G. V. R.: Changes in the distribution of phosphorus in platelet-rich plasma during clotting. Biochem. J. **68**: 695–704, 1958.

63. Born, G. V. R.: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature (London) **194**: 927–929, 1962.

64. Born, G. V. R.: Strong inhibition by 2-chloroadenosine of the aggregation of blood platelets by adenosine diphosphate. Nature (London) **202**: 95–96, 1964.
65. Born, G. V. R.: Uptake of adenosine and of adenosine diphosphate by human blood platelets. Nature (London) **206**: 1121–1122, 1965.
66. Born, G. V. R.: The effect of 5-hydroxytryptamine on the potassium exchange of human platelets. J. Physiol. (London) **190**: 273–280, 1967.
67. Born, G. V. R.: The platelet membrane and its functions. Plenary Session Paper. *In* XII Congress of International Society for Hematology, New York, 1968, pp. 95–101.
68. Born, G. V. R. and Bricknell, J.: The uptake of 5-hydroxytryptamine by blood platelets in the cold. J. Physiol. (London) **147**: 153–161, 1959.
69. Born, G. V. R. and Cross, M. J.: The aggregation of blood platelets. J. Physiol. (London) **168**: 178–195, 1963.
70. Born, G. V. R. and Cross, M. J.: Effect of adenosine diphosphate on the concentration of platelets in circulating blood. Nature (London) **197**: 974–976, 1963.
71. Born, G. V. R. and Cross, M. J.: Effects of inorganic ions and of plasma proteins on the aggregation of blood platelets by adenosine diphosphate. J. Physiol. (London) **170**: 397–414, 1964.
72. Born, G. V. R. and Gillson, R. E.: Studies on the uptake of 5-hydroxytryptamine by blood platelets. J. Physiol. (London) **146**: 472–491, 1959.
73. Born, G. V. R., Haslam, R. J., Goldman, M. and Lowe, R. D.: Comparative effectiveness of adenosine analogues as inhibitors of blood-platelet aggregation and as vasodilators in man. Nature (London) **205**: 678–680, 1965.
74. Born, G. V. R., Honour, A. J. and Mitchell, J. R. A.: Inhibition by adenosine and by 2-chloroadenosine of the formation and embolization of platelet thrombi. Nature (London) **202**: 761–765, 1964.
75. Born, G. V. R. and Mills, D. C. B.: Potentiation of the inhibitory effect of adenosine on platelet aggregation by drugs that prevent its uptake. J. Physiol. (London) **202**: 41p–42p, 1969.
76. Born, G. V. R. and Wright, H. P.: Platelet adhesiveness in experimental scurvy. Lancet **1**: 477–478, 1967.
77. Botti, R. E. and Ratanoff, O. D.: The clot-promoting effect of soaps of long chain saturated fatty acids. J. Clin. Invest. **42**: 1569–1577, 1963.
77a. Boullin, D. J. and O'Brien, R. A.: The accumulation of guanethidine by human blood platelets. Brit. J. Pharmacol. **35**: 90–102, 1969.
78. Bounameaux, Y.: Recherches sur le mécanisme de la rétraction du caillot et de la métamorphose visqueuse de plaquettes. Experientia **12**: 355–356, 1956.
79. Bounameaux, Y.: Dosage de facteurs coagulation contenus dans l'atmosphere plasmatique des plaquettes humaines. Rev. Fr. Étude Clin. Biol. **2**: 52–63, 1957.
80. Bounameaux, Y.: L'accolement des plaquettes aux fibres sous endothéliales. C. R. Séances Soc. Biol. **153**: 865–867, 1959.
81. Bowie, E. J. W. and Owen, C. A.: Thrombopathy. Seminars Hematol. **5**: 73–82, 1968.
82. Bränemark, P.-I. and Ekholm, R.: Adherence of blood cells to vascular endothelium. Blut, Z. Gesamte Blutforschung **16**: 274–288, 1968.
83. Braude, A. I., Carey, F. J. and Zalesky, M.: Studies with radioactive endotoxin. II. Correlation of physiologic effects with distribution of radioactivity in rabbits injected with lethal doses of *E. coli* endotoxin labelled with radioactive sodium chromate. J. Clin. Invest. **34**: 858–866, 1955.
84. Brecher, G. and Cronkite, E. P.: The effects of platelet transfusions in dogs made pancytopenic by x-radiation. N. Y. State J. Med. **53**: 544–547, 1953.
85. Breddin, K.: Hämorrhagische Diathesin bei Lebererkrankungen unter besonderer Berüchsichtigung der Thrombocytenfunktion. Acta Haematol. **27**: 1–16, 1962.
86. Breddin, K.: Experimental and clinical investigations on the adhesion and aggregation of human platelets. *In* Platelets in Haemostasis. Exp. Biol. Med. **3**: 14–23, 1965.
87. Breddin, K. and Bürck, K. H.: Zur Klinik der Thrombozytenfunktionsstörungen unter besonderer Berücksichtingung der Ausbreitungsfähigkeit der Thrombozyten an silikonisierten Glasflächen. Thromb. Diath Haemorrh. **9**: 525–545, 1963.
88. Breddin, K. and Langbein, H.: Über den Einfluss verschiedener Stoffwechselhemmer auf die Thrombozytenfunktion. Thromb. Diath. Haemorrh. **10**: 29–41, 1963.
89. Bridges, J. M., Dalby, A. M., Millar, H. D. and Weaver, J. A.: An effect of D-glucose on platelet stickiness. Lancet **1**: 75–77, 1965.
90. Brinkhous, K. M., LeRoy, E. C., Cornell, W. P., Brown, R. C. Haslehurst, J. L. and Vennart, G. P.: Macroscopic studies of platelet agglutination; nature of thrombocyte agglutinating activity of plasma. Proc. Soc. Exp. Biol. Med. **98**: 379–383, 1958.
91. Brinkhous, K. M., Read, M. S. and Mason, R. G.: Plasma thrombocyte agglutinating activity and fibrinogen. Synergism with adenosine diphosphate. Lab. Invest. **14**: 335–342, 1965.
92. Brøns, K. and Gormsen, J.: Studies on platelet factor 3 activity. Scand. J. Haematol. **5**: 300–312, 1968.
93. Broun, G. O., Jr. and Broun, G. O., Sr.: Reactions between platelets and viruses. *In:* Proceedings of VIII International Congress for Hematology, Tokyo 1960, vol. 3, p. 1756, Pan Pacific Press, Tokyo, 1962.
94. Brown, J. H. and MacKey, H. K.: Inhibition of antigen-antibody reactions in vitro by nonsteroidal anti-inflammatory agents. Proc. Soc. Exp. Biol. Med. **127**: 112–114, 1968.
95. Buckingham, S. and Maynert, E. W.: The release of 5-hydroxytryptamine, potassium and amino acids from platelets. J. Pharmacol. Exp. Ther. **143**: 332–339, 1964.
96. Bull, B. S. and Zucker, M. B.: Changes in platelet volume produced by temperature, metabolic inhibitors and aggregating agents. Proc. Soc. Exp. Biol. Med. **120**: 296–301, 1965.

97. Bunag, R. D., Douglas, C. R., Imai, S. and Berne, R. M.: Influence of a pyrimidopyrimidine derivative on deamination of adenosine by blood. Circ. Res. 15: 83–88, 1964.
98. Burton, P. R.: Effects of various treatments on microtubules and axial units of lung-fluke spermatozoa. Z. Zellforsch. Mikroskop. Anat. 87: 226–248, 1968.
99. Butcher, R. W. and Sutherland, E. W.: Adenosine 3′,5′-phosphate in biological materials. I. Purification and properties of cyclic 3′,5′-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3′,5′-phosphate in human urine. J. Biol. Chem. 237: 1244–1250, 1962.
100. Bygdeman, S., Eliason, B. and Gullbring, B.: Effects of dextran infusion on the adenosine diphosphate induced adhesiveness and the spreading capacity of human blood platelets. Thromb. Diath. Haemorrh. 15: 451–456, 1966.
101. Bygdeman, S., Eliason, R. and Johnson, S.-R.: Relationship between post-operative changes in adenosine diphosphate-induced platelet adhesiveness and venous thrombosis. Lancet 1: 1301-1302, 1966.
101a. Bygdeman, S. and Johnsen, Ø.: Studies on the effect of adrenergic blocking drugs on catecholamine-induced platelet aggregation and uptake of noradrenaline and 5-hydroxytryptamine. Acta Physiol. Scand. 75: 129–138, 1969.
102. Caen, J. P., Castaldi, P. A., Leclerc, J. C., Inceman, S., Larrieu, M. J., Probst, M. and Bernard, J.: Congenital bleeding disorders with long bleeding time and normal platelet count. I. Glanzmann's thrombasthenia (report of fifteen patients.) Amer. J. Med. 41: 4–26, 1966.
103. Caen, J. and Cousin, C.: Le trouble d'adhesivité "in vivo" des plaquettes dans la maladie de Willebrand et les thrombasthénies de Glanzmann. Nouv. Rev. Fr. Hemat. 2: 685–694, 1962.
104. Caen, J. and Inceman, S.: Considérations sur l'allongement du temps de saignement l'afibrinogénémie congenitale. Nouv. Rev. Fr. Hematol. 3: 614–615, 1963.
105. Caen, J. P., Vainer, H. and Gautier, A.: Thrombasthenia. Thromb. Diath. Haemorrh. Suppl. 26: 223–237, 1967.
106. Carlson, L. A., Irion, E. and Orö, L.: Effect of infusion of prostaglandin E_1 on the aggregation of blood platelets in man. Life Sci. 7: 85–90, 1968.
107. Carson, P., McDonald, L., Pickard, S., Pilkington, T., Davies, B. and Love, F.: Effect of atromid on platelet stickiness. J. Atheroscler. Res. 3: 619–622, 1963.
108. Caspary, E. A., Prineas, J., Miller, H. and Field, E. J.: Platelet stickiness in multiple sclerosis. Lancet 2: 1108–1109, 1965.
109. Castaldi, P. A. and Caen, J.: Platelet fibrinogen. J. Clin. Pathol. (London) 18: 579–585, 1965.
110. Castaldi, P. A., Larrieu, M. J. and Caen, J.: Availability of platelet factor 3 and activation of factor XII in thrombasthenia. Nature (London) 207: 422–424, 1965.
111. Cetingil, A. I., Ulutin, O. N. and Karaca, M.: A platelet defect in a case of scurvy. Brit. J. Haematol. 4: 350–354, 1958.
112. Chakrabarti, R., Fearnley, G. R. and Evans, J. F.: Effects of clofibrate on fibrinolysis, platelet stickiness, plasma-fibrinogen, and serum-cholesterol. Lancet 2: 1007–1009, 1968.
113. Chambers, D. A., Salzman, E. W. and Neri, L. L.: Characterization of "Ecto-ATPase" of human blood platelets. Arch. Biochem. Biophys. 119: 173–178, 1967.
114. Chambers, D. A., Salzman, E. W. and Neri, L. L.: Platelet nucleotide metabolism and platelet aggregation. *In* Platelets in Hemostasis. Exp. Biol. Med. 3: 62–70, 1968.
115. Chambers, D. A., Salzman, E. W., Neri, L. L. and Sivertsen, U.: Platelet incorporation of fibrinogen I^{125}. *In* XII Congress of International Society for Hematology, New York, p. 200, 1968.
116. Chandler, A. B.: In vitro thrombotic coagulation of the blood. A method for producing a thrombus. Lab. Invest. 7: 110–114, 1958.
117. Chandrasekhar, N.: Inhibition of platelet aggregation by prostaglandins. Blood 30: 554, 1967.
118. Chang, Y.: The effect of diisopropylfluorophosphonate (DFP) on platelet survival in the rabbit. Blood 29: 891–895, 1967.
119. Cheney, K. and Bonnin, J. A.: Haemorrhage, platelet dysfunction and other coagulation defects in uraemia. Brit. J. Haematol. 8: 215–222, 1962.
120. Chernyak, N. B.: Processes of formation and utilization of energy in human blood platelets. Clin. Chim. Acta 12: 244–257, 1965.
121. Clayton, S., Born, G. V. R. and Cross, M. J.: Inhibition of the aggregation of blood platelets by nucleosides. Nature (London) 200: 138–139, 1963.
122. Clayton, S. and Cross, M. J.: The aggregation of blood platelets by catecholamines and by thrombin. J. Physiol. (London) 169: 82P, 1963.
123. Cohen, I., Djaldetti, M., Karp, M. and Vries, A.: Thrombocytosis with impaired platelet adhesiveness and platelet factor 3 availability. New Istanbul Contrib. Clin. Sci. 9: 20–25, 1966.
124. Cohen, P., Braunwald, J. and Gardner, F. H.: Destruction of canine and rabbit platelets following intravenous administration of carbon particles or endotoxin. J. Lab. Clin. Med. 66: 263–271, 1965.
125. Cohen, P., Gardner, F. H. and Barnett, G. O.: Reclassification of the thrombocytopenias by the Cr^{51}-labeling method for measuring platelet life span. N. Engl. J. Med. 264: 1294–1299 and 1350–1355, 1961.
126. Conley, C. L.: Blood platelets and platelet transfusions. Arch. Intern. Med. 107: 635–638, 1961.
127. Connor, W. E.: The acceleration of thrombus formation by certain fatty acids. J. Clin. Invest. 41: 1199–1205, 1962.
128. Connor, W. E., Hoak, J. C. and Warner, E. D.: Massive thrombosis produced by fatty acid infusion. J. Clin. Invest. 42: 860–866, 1963.
129. Connor, W. E. and Poole, J. C. F.: The effect of fatty acids on the formation of thrombi. Quart. J. Exp. Physiol. 46: 1–7, 1961.

130. CONSTANTINE, J. W.: Temperature and the aggregation of rabbit platelets by adenosine diphosphate. Nature (London) 205: 1075–1077, 1965.
131. CONSTANTINE, J. W.: Aggregation of guinea-pig platelets by adenosine diphosphate. Nature (London) 210: 162–164, 1966.
132. CONSTANTINE, J. W.: Aggregation and adhesion of rat platelets. Nature (London) 214: 1084–1086, 1967.
132a. CONSTANTINE, J. W. AND HOCHSTEIN, F. A.: Aggregation of rabbit and guinea-pig platelets by oxime esters. Nature (London) 210: 164–167, 1966.
133. CONSTANTINIDES, P.: Plaque fissures in human coronary thrombosis. J. Atheroscler. Res. 6: 1–17, 1966.
134. COOLEY, M. H. AND COHEN, P.: Potassium transport in human blood platelets. J. Lab. Clin. Med. 70: 69–79, 1967.
135. CORN, M.: Effect of thrombin on glycolysis of fresh and stored platelets. J. Appl. Physiol. 21: 62–66, 1966.
136. CORN, M.: Effect of thrombin, adenosine diphosphate, connective tissue, and endotoxin on platelet glycolysis. Nature (London) 212: 508, 1966.
137. CORN, M., JACKSON, D. P. AND CONLEY, C. L.: Components of blood necessary for clot retraction. Bull. Johns Hopkins Hosp. 107: 90–104, 1960.
138. CRAWFORD, N.: Some observations on the blood serotonin levels in rheumatoid arthritis with a study of platelet serotonin absorption. Clin. Chim. Acta 23: 139–146, 1969.
139. CRONBERG, S.: Effect of fibrinolysis on adhesion and aggregation of human platelets. Thromb. Diath. Haemorrh. 19: 474–482, 1968.
140. CRONBERG, S.: Investigations in haemorrhagic disorders with prolonged bleeding time but normal number of platelets. With special reference to platelet adhesiveness. Acta Med. Scand. Suppl. 486: 1–54, 1968.
141. CRONBERG, S., ROBERTSON, B., NILSSON, I. M. AND NILÉHN, J.-E.: Suppressive effect of dextran on platelet adhesiveness. Thromb. Diath. Haemorrh. 16: 384–394, 1966.
142. CRONKITE, E. P. AND JACKSON, D. P.: Use of platelet transfusions in hemorrhagic disease. *In* Progress in Hematology, vol. II, ed. by L. M. Tocantins, p. 239–257, Grune & Stratton, New York, 1961.
143. CROSS, M. J.: Effect of fibrinogen on the aggregation of platelets by adenosine diphosphate. Thromb. Diath. Haemorrh. 12: 524–527, 1964.
144. CUCUIANU, M. AND MUSTARD, J. F.: Unpublished observations.
145. CUTHBERTSON, W. F. J. AND MILLS, D. C. B.: Some factors influencing platelet clumping. J. Physiol. (London) 169: 9P–10P, 1963.
146. DALLDORF, F. G., PATE, D. H. AND LANGDELL, R. D.: Pulmonary capillary thrombosis in experimental pneumococcal septicemia. Arch. Pathol. 85: 149–161, 1968.
147. DAMESHEK, W. AND MILLER, E. B.: The megakaryocytes in idiopathic thrombocytopenic purpura, a form of hypersplenism. Blood 1: 27–51, 1946.
148. DAPRADA, M. AND PLETSCHER, A.: Isolated 5-hydroxytryptamine organelles of rabbit blood platelets: physiological properties and drug-induced changes. Brit. J. Pharmacol. 34: 591–597, 1968.
149. DAPRADA, M., TRANZER, J. P. AND PLETSCHER, A.: Effect of drugs and cations on ultrastructure and 5-hydroxytryptamine content of blood platelets. J. Pharmacol. Exp. Ther. 158: 394–398, 1967.
150. DAVEY, M. G.: The Survival and Destruction of Human Platelets. S. Karger, Basel, New York, 1966.
151. DAVEY, M. G. AND LANDER, H.: The behaviour of infused human platelets during the first twenty-four hours after infusion. Brit. J. Haematol. 10: 94–109, 1964.
152. DAVEY, M. G. AND LANDER, H.: Effect of adenosine diphosphate on circulating platelets in man. Nature (London) 201: 1037–1039, 1964.
153. DAVEY, M. G. AND LÜSCHER, E. F.: Action of some coagulant snake venoms upon blood platelets. Nature (London) 207: 730–732, 1965.
154. DAVEY, M. G. AND LÜSCHER, E. F.: Platelets proteins. *In* Biochemistry of Blood Platelets, ed. by E. Kowalski and S. Niewiarowski. pp. 9–20, Academic Press, London, New York, 1967.
155. DAVEY, M. G. AND LÜSCHER, E. F.: Actions of thrombin and other coagulant and proteolytic enzymes on blood platelets. Nature (London) 216: 857–858, 1967.
156. DAVEY, M. G. AND LÜSCHER, E. F.: Biochemical aspects of platelet function and hemostasis. Seminars Hematol. 5: 5–17, 1968.
157. DAVEY, M. G. AND LÜSCHER, E. F.: Release reactions of human platelets induced by thrombin and other agents. Biochim. Biophys. Acta 165: 490–506, 1968.
158. DAVEY, M. G. AND LÜSCHER, E. F.: Effects of dimethyl sulphoxide and of vitamin A on human platelets. Thromb. Diath. Haemorrh. 19: 12–17, 1968.
159. DAVID-FERREIRA, J. F.: The blood platelet: electron microscopic studies. Int. Rev. Cytol. 17: 99–148, 1964.
160. DAVIDSON, E., HOWARD, A. N. AND GRESHAM, G. A.: The nature of the coagulation defect in rats fed diets which produce thrombosis or experimental atherosclerosis. Brit. J. Exp. Pathol. 43: 166–171, 1962.
161. DAVIES, R. E.: A molecular theory of muscle contraction: calcium-dependent contractions with hydrogen bond formation plus ATP-dependent extensions of part of the myosin-actin cross-bridges. Nature (London) 199: 1068–1074, 1963.
162. DAVIS, R. B.: Electron microscopic changes in blood platelets induced by bacterial lipopolysaccharide. Exp. Mol. Pathol. 5: 559–574, 1966.
163. DAVIS, R. B. AND SCHWARTZ, S.: Effects of "haematoporphyrin" on adhesiveness and aggregation of rabbit platelets. Nature (London) 214: 186–187, 1967.
164. DAY, H. J., FEWELL, W. AND SOLOFF, L. A.: Thrombosis in the dog produced by single rapid infusions of long chain saturated fatty acids. Amer. J. Med. Sci. 253: 83–93, 1967.
164a. DAY, H. J., HOLMSEN, H. AND HOVIG, T.: Subcellular particles of human platelets. Scand. J. Haematol. Suppl. 7: 1–35, 1969.

165. DeBruyn, P. P.: The fine structure of the megakaryocyte of the bone marrow of the guinea pig. Z. Zellforsch. Mikroskop. Anat. 64: 111-118, 1964.
166. Des Prez, R. M.: The effects of bacterial endotoxin on rabbit platelets. V. Heat labile plasma factor requirements of endotoxin-induced platelet injury. J. Immunol. 99: 966-973, 1967.
167. Des Prez, R. M. and Bryant, R. E.: Effects of bacterial endotoxin on rabbit platelets. IV. The divalent ion requirements of endotoxin-induced and immunologically induced platelet injury. J. Exp. Med. 124: 971-982, 1966.
168. Des Prez, R. M. and Bryant, R. E.: Two mechanisms of immunologically-induced injury to rabbit platelets. J. Immunol. 102: 241-252, 1969.
169. Des Prez, R. M., Bryant, R. E., Katz, J. A. and Brittingham, T. E.: Platelet aggregation by magnesium ion. Thromb. Diath. Haemorrh. 17: 516-531, 1967.
170. Des Prez, R. M., Horowitz, H. I. and Hook, E. W., Jr.: Effects of bacterial endotoxin on rabbit platelets. I. Platelet aggregation and release of platelet factors in vitro. J. Exp. Med. 114: 857-874, 1961.
171. Detwiler, T. C.: Failure of thrombin to affect platelet respiration. Arch. Biochem. Biophys. 120: 721-722, 1967.
172. DeVreker, G. G. and DeVreker, R. A.: Aspects of platelet metabolism during aggregation. Rev. Belge Pathol. Méd. Exp. 31: 79-85, 1965.
173. de Vries, S. I., ten Cate, J. W., den Hartog-Veerman, M. E. and van Dooren, M. M.: Quelques remarques sur la thrombocytopathie acquise chez les malades urémique. Schweiz Med. Wochenschr. 98: 1658-1660, 1968.
174. Deykin, D., Pritsker, C. R. and Scolnick, E. M.: Plasma cofactors in adenosine diphosphate-induced aggregation of human platelets. Nature (London) 208: 296-298, 1965.
175. Dhall, D. P. and Matheson, N. A.: In vitro clumping of human platelets by dextran. Thromb. Diath. Haemorrh. 19: 70-76, 1968.
176. Didisheim, P.: Inhibition by dipyridamole of arterial thrombosis in rats. Thromb. Diath. Haemorrh. 20: 257-266, 1968.
177. Didisheim, P., Owen, C. A. and Bowie, E. J. W.: Effect of induced hypotension on thrombosis in the extracorporeal microcirculation of the rat. J. Clin. Invest. 48: 21a, 1969.
178. Dineen, J. J., Perillie, P. E. and Finch, S. C.: Zymosan-induced thrombocytopenia in the rat. J. Reticuloendothelial Soc. 5: 161-174, 1968.
179. Dodds, W. J.: Familial canine thrombocytopathy. Thromb. Diath. Haemorrh. Suppl. 26: 241-248, 1967.
179a. Doery, J. C. G., Hirsh, J. and de Gruchy, G. C.: Aspirin: its effect on platelet glycolysis and release of adenosine diphosphate. Science 165: 65-67, 1969.
180. Doery, J. C. G., Hirsh, J., Loder, P. B. and de Gruchy, G. C.: Distribution of platelet hexokinase and the effect of collagen. Nature (London) 216: 1317-1318, 1967.
181. Donabedian, R. K. and Nemerson, Y.: Fatty acid oxidation by human platelets and its stimulation by thrombin. Clin. Res. 16: 302, 1968.
182. Douglas, W. W.: Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. Brit. J. Pharmacol. 34: 451-474, 1968.
183. Dragstedt, C. A., Wells, J. A. and Rocha E Silva, M.: Inhibitory effect of heparin upon histamine release by trypsin, antigen, and proteose. Proc. Soc. Exp. Biol. Med. 51: 191-192, 1942.
184. Dudgeon, L. S. and Goadby, H. K.: The examination of the tissues and some observations on the blood platelets of rabbits at intervals of five minutes, and later, after intravenous inoculations of *Staphylococcus aureus* and Indian ink. J. Hyg. 31: 247-256, 1931.
185. Ebbe, S.: Megakaryocytopoiesis and platelet turnover. Ser. Haematol. I, 2: 65-98, 1968.
186. Eberth, J. C. and Schimmelbusch, C.: Die Thrombose nach Versuchen u. Leichenbefunden. Ferdinand Enke, Stuttgart, Germany, 1888.
187. Eisen, M. E., Tyson, M. C., Michael, S. and Baumann, F.: Adhesiveness of blood platelets in arteriosclerosis obliterans, thromboangiitis obliterans, acute thrombophlebitis, chronic venous insufficiency and arteriosclerotic heart disease. Circulation 3: 271-274, 1951.
188. Elkeles, R. S., Hampton, J. R., Honour, A. J., Mitchell, J. R. A. and Prichard, J. S.: Effect of a pyrimidopyrimidine compound on platelet behaviour in vitro and in vivo. Lancet 2: 751-754, 1968.
189. Elkeles, R. S., Hampton, J. R. and Mitchell, J. R. A.: Effect of oestrogens on human platelet behaviour. Lancet 2: 315-318, 1968.
190. Emmons, P. R., Hampton, J. R., Harrison, M. J. G., Honour, A. J. and Mitchell, J. R. A.: Effect of prostaglandin E_1 on platelet behaviour in vitro and in vivo. Brit. Med. J. 2: 468-472, 1967.
191. Emmons, P. R., Harrison, M. J. G., Honour, A. J. and Mitchell, J. R. A.: Effect of a pyrimido-pyrimidine derivative on thrombus formation in the rabbit. Nature (London) 208: 255-257, 1965.
192. Emmons, P. R., Harrison, M. J. G., Honour, A. J. and Mitchell, J. R. A.: Effect of dipyridamole on human platelet behaviour. Lancet 2: 603-606, 1965.
193. Engel, A., Alexander, B. and Pechet, L.: Activation of trypsinogen and plasminogen by thrombin preparations. Biochemistry 5: 1543-1551, 1966.
194. Erdös, E. G.: Effect of nonsteroidal anti-inflammatory drugs in endotoxin shock. Biochem. Pharmacol. Suppl. pp. 283-291, Pergamon Press, 1968.
195. Erdös, E. G., Hinshaw, L. B. and Gill, C. C.: Effect of indomethacin in endotoxin shock in the dog. Proc. Soc. Exp. Biol. Med. 125: 916-919, 1967.
196. Esnouf, M. P. and Tunnah, G. W.: The isolation and properties of the thrombin-like activity from Ancistrodon rhodostoma venom. Brit. J. Haematol. 13: 581-590, 1967.
197. Estes, J. W., McGovern, J. J., Goldstein, R. and Rota, M.: Stability of certain coagulation factors and metabolic activities of platelets in stored blood. J. Lab. Clin. Med. 59: 436-444, 1962.

198. Evans, G. and Irvine, W. T.: Long-term arterial-graft patency in relation to platelet adhesiveness, biochemical factors and anticoagulant therapy. Lancet 2: 353–355, 1966.
199. Evans, G. and Mustard, J. F.: Platelet surface reaction and thromboeis. Surgery 64: 273–280, 1968.
200. Evans, G. and Mustard, J. F.: Inhibition of the platelet-surface reaction in endotoxin shock and the generalized Shwartzman reaction. J. Clin. Invest. 47: 31a, 1968.
201. Evans, G. and Mustard, J. F.: Unpublished observations.
202. Evans, G., Nishizawa, E. E., Packham, M. A. and Mustard, J. F.: The effect of acetylsalicylic acid (aspirin) on platelet function. Blood 30: 550, 1967.
203. Evans, G., Packham, M. A., Nishizawa, E. E., Mustard, J. F. and Murphy, E. A.: The effect of acetylsalicylic acid on platelet function. J. Exp. Med. 128: 877–894, 1968.
204. Evans, G., Packham, M. A., Nishizawa, E. E. and Mustard, J. F.: The effect of the platelet-collagen reaction and blood coagulation on hemostasis. J. Clin. Invest. 46: 1053, 1967.
205. Ewald, R. A., Eichelberger, J. W., Young, A. A., Weiss, H. J. and Crosby, W. H.: The effect of dextran on platelet factor 3 activity: in vitro and in vivo studies. Transfusion (Philadelphia) 5: 109–119, 1965.
205a. Farbiszewski, R. and Worowski, K.: Enhancement of platelet aggregation and adhesion by β-lipoprotein. J. Atherscler. Res. 8: 988–990, 1968.
206. Fearnley, G. R., Chakrabarti, R. and Evans, J. F.: Effect of ethyloestrenol combined with phenformin or with metformin on platelet stickiness and serum-cholesterol in patients with occlusive vascular disease. Lancet 2: 1004–1007, 1968.
207. Flatow, F. A., Jr. and Freireich, E. J.: The increased effectiveness of platelet concentrates prepared in acidified plasma. Blood 27: 449–459, 1966.
208. Flatow, F. A., Levin, R. H. and Freireich, E. J.: Preparation of platelet concentrates for transfusion employing adenosine diphosphate. Transfusion (Philadelphia) 6: 205–212, 1966.
209. Freeman, A. R. and Spirtes, M. A.: Effects of chlorpromazine on biological membranes. II. Chlorpromazine-induced changes in human erythrocytes. Biochem. Pharmacol. 12: 47–53, 1963.
210. French, J. E.: Atherosclerosis in relation to the structure and function of the arterial intima, with special reference to the endothelium. Int. Rev. Exp. Pathol. 5: 253–353, 1966.
211. French, J. E.: Thrombosis in arteries: the role of the blood platelets. Pathol. Microbiol. 30: 653–664, 1967.
212. French, J. E.: Electron microscopy of thrombus formation. *In:* Modern Trends in Pathology, ed. by T. Crawford, pp. 208–237, Butterworth, London, 1967.
213. French, J. E. and Poole, J. C. F.: Electron microscopy of the platelets in artificial thrombi. Proc. Roy. Soc. Ser. B Biol. Sci. 157: 170–176, 1963.
214. Friedman, I. A., Schwartz, S. O. and Leithold, S. L.: Platelet function defects with bleeding. Early manifestations of acute leukemia. Arch. Intern. Med. 113: 177–185, 1964.
215. Friedman, M. and Van den Bovenkamp, G. J.: The pathogenesis of a coronary thrombus. Amer. J. Pathol. 48: 19–44, 1966.
216. Gaarder, A., Jonsen, J., Laland, S., Hellem, A. J. and Owren, P. A.: Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. Nature (London) 192: 531–532, 1961.
217. Gaarder, A. and Laland, S.: Hypothesis for the aggregation of platelets by nucleotides. Nature (London) 202: 909–910, 1964.
218. Gaintner, J. R., Jackson, D. P. and Maynert, E. W.: The action of thrombin on platelet 5-hydroxytryptamine. Bull. Johns Hopkins Hosp. 111: 185–197, 1962.
219. Gardner, F. H. and Cohen, P.: The value of platelet transfusions. Med. Clin. N. Amer. 44: 1425–1439, 1960.
220. Garvin, J. E.: Factors affecting the adhesiveness of human leucocytes and platelets in vitro. J. Exp. Med. 114: 51–73, 1961.
221. Gast, L. F.: Influence of aspirin on haemostatic parameters. Ann. Rheum. Dis. 23: 500–504, 1964.
222. Gaydos, L. A., Freireich, E. J. and Mantel, N.: The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. N. Engl. J. Med. 266: 905–909, 1962.
223. Gibinski, K., Walendowski, J. and Zazgórnik, J.: Blood platelet adhesiveness and the protective action of nialamide in atheroclerotic dysbasia. J. Atheroscler. Res. 4: 461–464, 1964.
224. Gilbert, J. B. and Mustard, J. F.: Some effects of atromid on platelet economy and blood coagulation in man. J. Atheroscler. Res. 3: 623–633, 1963.
225. Gillespie, E., Malawista, S. E. and Levine, R. J.: Inhibition by colchicine of histamine release from rat peritoneal mast cells. Fed. Proc. 26: 786, 1967.
226. Giudici, G. and Turazza, G.: Activités lysosomiques des plaquettes du sang humain. Hémostase 4: 91–94, 1964.
227. Glanzmann, E.: Hereditäre hämorrhagische Thrombasthenie. Ein Beitrag zur Pathologie der Blutplättchen. Jahrb. Kinderh. 88: 113–141, 1918.
228. Glynn, M. F., Jørgensen, L. and Buchanan, M. R.: Platelet aggregation, renal function and hypertension. Fed. Proc. 25: 544, 1966.
229. Glynn, M. F., Movat, H. Z., Murphy, E. A. and Mustard, J. F.: Study of platelet adhesiveness and aggregation with latex particles. J. Lab. Clin. Med. 65: 179–201, 1965.
230. Glynn, M. F., Murphy, E. A. and Mustard, J. F.: Effect of clofibrate on platelet economy in man. Lancet 2: 447–448, 1967.
231. Glynn, M. F., Mustard, J. F., Buchanan, M. R. and Murphy, E. A.: Cigarette smoking and platelet aggregation. Can. Med. Ass. J. 95: 549–553, 1966
232. Gocke, D. J.: In vitro damage of rabbit platelets by an unrelated antigen-antibody reaction. II. Studies of the plasma requirement. J. Immunol. 94: 247–252, 1965.

233. GOCKE, D. J. AND OSLER, A. G.: In vitro damage of rabbit platelets by an unrelated antigen-antibody reaction. I. General characteristics of the reaction. J. Immunol. 94: 236–246, 1965.
234. GOOD, R. A. AND THOMAS, L.: Studies on the generalized Shwartzman reaction. II. The production of bilateral cortical necrosis of the kidneys by a single injection of bacterial toxin in rabbits previously treated with thorotrast or trypan blue. J. Exp. Med. 96: 625–641, 1952.
235. GORSTEIN, F., CARROLL, H. J. AND PUSZKIN, E.: Electrolyte concentrations, potassium flux kinetics, and the metabolic dependence of potassium transport in human platelets. J. Lab. Clin. Med. 70: 938–950, 1967.
236. GOTT, V. L., WHIFFIN, J. D. AND DUTTON, R. C.: Heparin bonding on colloidal graphite surfaces. Science 142: 1297–1298, 1963.
237. GRETTE, K.: Studies on the mechanism of thrombin-catalyzed hemostatic reactions in blood platelets. Acta Physiol. Scand. 56(suppl. 195): 5–93, 1962.
238. GROSS, R.: Metabolic aspects of normal and pathological platelets. *In* Blood Platelets, ed. by S. A. Johnson, R. W. Monto, J. W. Rebuck and R. C. Horn, pp. 407–421, Little, Brown and Co., Boston, 1961.
239. GROSS, R.: Platelet enzymes. Thromb. Diath. Haemorrh. Suppl. 26: 143–154, 1967.
240. GROSS, R., GEROK, W., LÖHR, G. W., VOGELL, W., WALLER, H. D. AND THEOPOLD, W.: Über die Natur der Thrombasthenie. Thrombopathie Glanzmann Naegeli. Klin. Wochenschr. 38: 194–206, 1960.
241. GROSS, R., LÖHR, G. W. AND WALLER, H. D.: Physiologische und klinische Probleme der Thrombozytensubstitution. *In* Proceedings of VII Congress of International Society for Blood Transfusion, Rome, 1958, pp. 917–924, S. Karger, Basel, 1959.
242. GRÖTTUM, K. A.: Influence of aggregating agents on electrophoretic mobility of blood platelets from healthy individuals and from patients with cardiovascular diseases. Lancet 1: 1406–1408, 1968.
243. GUGLER, E. AND LÜSCHER, E. F.: Platelet function in congenital afibrinogenemia. Thromb. Diath. Haemorrh. 14: 361–373, 1965.
244. HAINING, C. G.: Histamine release in rabbit blood by dextran and dextran sulfate. Brit. J. Pharmacol. 10: 87–94, 1955.
244a. HAMPTON, J. R. AND HARDISTY, R. M.: Platelet electrophoretic behaviour in thrombasthenia. Nature (London) 213: 400, 1967.
245. HAMPTON, J. R., HARRISON, M. J. G., HONOUR, A. J. AND MITCHELL, J. R. A.: Platelet behaviour and drugs used in cardiovascular disease. Cardiovasc. Res. Center Bull. (Baylor Univ. Houston) 1: 101–107, 1967.
246. HAMPTON, J. R. AND MITCHELL, J. R. A.: Effect of aggregating agents on the electrophoretic mobility of human platelets. Brit. Med. J. 1: 1074–1077, 1966.
247. HAMPTON, J. R. AND MITCHELL, J. R. A.: A transferable factor causing abnormal platelet behaviour in vascular disease. Lancet 2: 764–768, 1966.
248. HAN, S. S. AND BAKER, B. L.: The ultrastructure of megakaryocytes and blood platelets in the rat spleen. Anat. Rec. 149: 251–267, 1964.
249. HARDISTY, R. M., DORMANDY, K. M. AND HUTTON, R. A.: Thrombasthenia. Studies on three cases. Brit. J. Haematol. 10: 371–387, 1964.
250. HARDISTY, R. M. AND HUTTON, R. A.: Platelet aggregation and the availability of platelet factor 3. Brit. J. Haematol. 12: 764–776, 1966.
251. HARDISTY, R. M. AND HUTTON, R. A.: Bleeding tendency associated with a new abnormality of platelet behaviour. Lancet 1: 983–985, 1967.
252. HARDISTY, R. M. AND WOLFF, H. H.: Haemorrhagic thrombocythemia: a clinical and laboratory study. Brit. J. Haematol. 1: 390–405, 1955.
253. HARKER, L. A.: Kinetics of thrombopoiesis. J. Clin. Invest. 47: 458–465, 1968.
254. HARRINGTON, W. J., MINNICH, V. AND ARIMURA, G.: The autoimmune thrombocytopenias. *In* Progress in Hematology, vol. 1, ed. by L. M. Tocantins, pp. 166–192, Grune & Stratton, New York, 1956.
255. HARRINGTON, W. J., SPRAGUE, C. C., MINNICH, V., MOORE, C. V., AULVIN, R. C. AND DUBACH, R.: Immunologic mechanisms in idiopathic and neonatal thrombocytopenic purpura. Ann. Intern. Med. 38: 433–469, 1953.
255a. HARRISON, M. J. G., EMMONS, P. R. AND MITCHELL, J. R. A.: The effect of white cells on platelet aggregation. Thromb. Diath. Haemorrh. 16: 105–121, 1966.
256. HARRISON, M. J. G., EMMONS, P. R. AND MITCHELL, J. R. A.: The effect of sulfhydryl and enzyme inhibitors on platelet aggregation in vitro. Thromb. Diath. Haemorrh. 16: 122–133, 1966.
257. HARRISON, M. J. G. AND HONOUR, A. J.: Haemostatic plugs in experimental scurvy. Nature (London) 216: 1119–1120, 1967.
258. HARTMANN, R. C.: Platelet adhesiveness: the retention of platelets on glass surfaces. Thromb. Diath. Haemorrh. Suppl. 26: 281–302, 1967.
259. HARTMANN, R. C.: Tests of platelet adhesiveness and their clinical significance. Seminars Hematol. 5: 60–72, 1968.
260. HARTMANN, R. C. AND CONLEY, C. L.: Clot retraction as a measure of platelet function. I. Effects of certain experimental conditions on platelets in vitro. Bull. Johns Hopkins Hosp. 93: 355–369, 1953.
261. HASLAM, R. J.: Role of adenosine diphosphate in the aggregation of human blood platelets by thrombin and by fatty acids. Nature (London) 202: 765–768, 1964.
262. HASLAM, R. J.: Mechanisms of blood platelet aggregation. *In* Physiology of Hemostasis and Thrombosis, ed. by S. A. Johnson and W. H. Seegers, pp. 88–112, Charles C Thomas, Springfield, Ill., 1967.
263. HASLAM, R. J.: Biochemical aspects of platelet function. Plenary Session Paper. *In* XII Congress of International Society for Hematology, New York, pp. 198–214, 1968.
264. HASLAM, R. J.: Personal communication.
265. HASLAM, R. J. AND MILLS, D. C. B.: The adenylate kinase of human plasma, erythrocytes and platelets in relation to the degradation of adenosine diphosphate in plasma. Biochem. J. 103: 773–784, 1967.

266. HAWKEY, C.: Inhibitor of platelet aggregation present in saliva of the vampire bat *Desmodus rotundus*. Brit. J Haematol. 13: 1014–1020, 1967.
267. HAYDON, G. B. AND TAYLOR, D. A.: Microtubules in hamster platelets. J. Cell Biol. 26: 673–676, 1965.
268. HELLEM, A. J.: The role of platelet metabolism in adenosine diphosphate-induced platelet adhesiveness. Thromb. Diath. Haemorrh. Suppl. 26: 193–196, 1967.
269. HELLEM, A. J.: Platelet adhesiveness. Ser. Haematol. I, 2: 99–145, 1968.
270. HELLEM, A. J.: The adhesiveness of human blood platelets *in vitro*. Scand. J. Clin. Invest. 12(suppl 51): 1–117, 1960.
271. HENSON, P. M.: The role of complement and leukocytes in the immunological release of vasoactive amines from platelets. Fed. Proc. 28: 1721–1728, 1969.
272. HENSON, P. M. AND COCHRANE, C. G.: Immunological induction of increased vascular permeability. I. A rabbit passive cutaneous anaphylactic reaction requiring complement, platelets and neutrophils. J. Exp. Med. 129: 153–165, 1969.
273. HENSON, P. M. AND COCHRANE, C. G.: Immunological induction of increased vascular permeability. II. Two mechanisms of histamine release from rabbit platelets involving complement. J. Exp. Med. 129: 167–184, 1969.
274. HERRING, W. B., HERION, J. C., WALKER, R. I. AND PALMER, J. G.: Distribution and clearance of circulating endotoxin. J. Clin. Invest. 42: 79–87, 1963.
275. HERRMANN, R. G. AND FRANK, J. D.: Effect of adenosine derivatives and anti-histaminics on platelet aggregation. Proc. Soc. Exp. Biol. Med. 123: 654–660, 1966.
276. HILDEN, M., AMRIS, C. J. AND STARUP, J.: The haemostatic mechanism in oral contraception. Acta Obstet. Gynecol. Scand. 46: 562–571, 1967.
277. HINSHAW, L. B., JORDON, M. M. AND VICK, J. A.: Mechanism of histamine release in endotoxin shock. Amer. J. Physiol. 200: 987–989, 1961.
278. HINSHAW, L. B., SOLOMON, L. A., ERDÖS, E. G., REINS, D. A. AND GUNTER, B. J.: Effects of acetylsalicylic acid on the canine response to endotoxin. J. Pharmacol. Exp. Therap. 157: 665–671, 1967.
279. HINTERBERGER, H., ANTHONY, M. AND VAGHOLKAR, M. K.: Platelet 5-hydroxytryptamine and adenine nucleotides, serum arginylesterase and plasma 11-hydroxycorticosteroids in migraine. Clin. Sci. (London) 34: 271–276, 1968.
280. HIRSH, J., BUCHANAN, M., GLYNN, M. F. AND MUSTARD, J. F.: Effect of streptokinase on hemostasis. Blood 32: 726–737, 1968.
281. HIRSH, J., CASTELAN, D. J. AND LODER, P. B.: Spontaneous bruising associated with a defect in the interaction of platelets with connective tissue. Lancet 2: 18–21, 1967.
282. HIRSH, J., FLETCHER, A. P. AND SHERRY, S.: Effect of fibrin and fibrinogen proteolysis products on clot physical properties. Amer. J. Physiol. 209: 415–424, 1965.
283. HIRSH, J., GLYNN, M. F. AND MUSTARD, J. F.: The effect of platelet age on platelet adherence to collagen. J. Clin. Invest. 47: 466–473, 1968.
284. HIRSH, J. AND MCBRIDE, J. A.: Increased platelet adhesiveness in recurrent venous thrombosis and pulmonary embolism. Brit. Med. J. 2: 797–799, 1965.
285. HOAK, J. C., CONNOR, W. E. AND WARNER, E. D.: Thrombogenic effects of albumin-bound fatty acids. Arch. Pathol. 81: 136–139, 1966.
286. HOAK, J. C., POOLE, C. F. AND ROBINSON, D. S.: Thrombosis associated with mobilization of fatty acids. Amer. J. Pathol. 43: 987–998, 1963.
287. HOAK, J. C., WARNER, E. D. AND CONNOR, W. E.: Platelets, fatty acids and thrombosis. Circ. Res. 20: 11–17, 1967.
288. HOLMSEN, H.: Collagen-induced release of adenosine diphosphate from blood platelets incubated with radioactive phosphate in vitro. Scand. J. Clin. Lab. Invest. 17: 239–246, 1965.
289. HOLMSEN, H.: Changes in the radioactivity of P^{32}-labelled acid-soluble organophosphates in blood platelets during collagen- and adenosine diphosphate-induced platelet aggregation. Scand. J. Clin. Lab. Invest. 17: 537–548, 1965.
290. HOLMSEN, H. AND DAY, H. J.: Thrombin induced platelet release reaction and platelet lysosomes. Nature (London) 219: 760–761, 1968.
291. HOLMSEN, H., DAY, H. J. AND STORMORKEN, H.: The blood platelet release reaction. Scand. J. Haematol. Suppl. 8: 1–26, 1969.
292. HOLMSEN, H., DAY, H. J. AND STORM, E.: 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, 1969
293. HOLMSEN, H., STORMORKEN, H. AND GOOTE, T. M.: Kinetic studies on the breakdown of adenosine diphosphate in human plasma. Scand. J. Clin. Lab. Invest. 17(suppl. 84): 138–150, 1965.
294. HONOUR, A. J. AND MITCHELL, J. R. A.: Platelet clumping in vivo. Nature (London) 197: 1019–1020, 1963.
295. HONOUR, A. J. AND MITCHELL, J. R. A.: Platelet clumping in injured vessels. Brit. J. Exp. Pathol. 45: 75–87, 1964.
296. HORLICK, L.: Platelet adhesiveness in normal persons and subjects with atherosclerosis. Effect of high fat meals and anticoagulants on the adhesive index. Amer. J. Cardiol. 8: 459–470, 1961.
297. HOROWITZ, H. I.: Non-sedimentable platelet factor 3 during coagulation and after immune platelet injury. Thromb. Diath. Haemorrh. Suppl. 17: 243–246, 1965
298. HOROWITZ, H. I., COHEN, B. D., MARTINES, P. AND PAPAYOANOU, M. F.: Defective ADP-induced platelet factor 3 activation in uremia. Blood 30: 331–340, 1967.
299. HOROWITZ, H. I., DES PREZ, R. M. AND HOOK, E. W.: Effects of bacterial endotoxin on rabbit platelets. II. Enhancement of platelet factor 3 activity in vitro and in vivo. J. Exp. Med. 116: 619–633, 1962.
300. HOROWITZ, H. I. AND FUJIMOTO, M. M.: Association of factors XI and XII with blood platelets. Proc. Soc. Exp. Biol. Med. 119: 487–492, 1965.

301. HOULIHAN, R. B.: Studies on the adhesion of human blood platelets and bacteria. *In* Morphologic Hematology. Blood, special issue No. 1: 142–154, 1947.
302. HOVIG, T.: The ultrastructure of rabbit blood platelet aggregates. Thromb. Diath. Haemorrh. 8: 455–471, 1962.
303. HOVIG, T.: Aggregation of rabbit blood platelets produced in vitro by saline "extract" of tendons. Thromb. Diath. Haemorrh. 9: 248–263, 1963.
304. HOVIG, T.: Release of a platelet aggregating substance (adenosine diphosphate) from rabbit blood platelets induced by saline "extract" of tendons. Thromb. Diath. Haemorrh. 9: 264–278, 1963.
305. HOVIG, T.: The effect of calcium and magnesium on rabbit blood platelet aggregation in vitro. Thromb. Diath. Haemorrh. 12: 179–200, 1964.
306. HOVIG, T.: The effect of various enzymes on the ultrastructure, aggregation, and clot retraction ability of rabbit blood platelets. Thromb. Diath. Haemorrh. 13: 84–113, 1965.
307. HOVIG, T.: The ultrastructure of blood platelets in normal and abnormal states. Ser. Haematol. I: 2: 3–64, 1968.
308. HOVIG, T., DODDS, W. J., ROWSELL, H. C. AND MUSTARD, J. F.: The transformation of hemostatic platelet plugs in normal and factor IX deficient dogs. Amer. J. Pathol. 53: 355–374, 1968.
309. HOVIG, T., JØRGENSEN, L., PACKHAM, M. A. AND MUSTARD, J. F.: Platelet adherence to fibrin and collagen. J. Lab. Clin. Med. 71: 29–40, 1968.
310. HOVIG, T., ROWSELL, H. C., DODDS, W. J., JØRGENSEN, L. AND MUSTARD, J. F.: Experimental hemostasis in normal dogs and dogs with congenital disorders of blood coagulation. Blood 30: 636–668, 1967.
311. HOWARD, A. N. AND GRESHAM, G. A.: The dietary induction of thrombosis and myocardial infarction. J. Atheroscler. Res. 4: 40–56, 1964.
312. HUGHES, A. AND TONKS, R. S.: The role of micro-emboli in the production of carditis in hypersensitivity experiments. J. Pathol. Bacteriol. 77: 207–217, 1959.
313. HUGHES, F. B. AND BRODIE, B. B.: The mechanism of serotonin and catecholamine uptake by platelets. J. Pharmacol. Exp. Therap. 127: 96–102, 1959.
314. HUGUES, J.: Accolement des plaquettes aux structures conjonctives périvasculaires. Thromb. Diath. Haemorrh. 8: 241–255, 1962.
315. HUGUES, J. AND LAPIÈRE, C. M.: Nouvelles recherches sur l'accolement des plaquettes aux fibres de collagéne. Thromb. Diath. Haemorrh. 11: 327–354, 1964.
316. HUME, M.: Platelet adhesiveness and other coagulation factors in thrombophlebitis. Surgery 59: 110–116, 1966.
317. HUMPHREY, J. H. AND JAQUES, R.: The release of histamine and 5-hydroxytryptamine (serotonin) from platelets by antigen-antibody reactions (in vitro). J. Physiol. (London) 128: 9–27, 1955.
318. HUSSAIN, Q. Z. AND NEWCOMB, T. F.: Thrombin stimulation of platelet oxygen consumption rate. J. Appl. Physiol. 19: 297–300, 1964.
319. INCEMAN, S., CAEN, J. AND BERNARD, J.: La "métamorphose visqueuse" des plaquettes de thrombasthenie de Glanzmann. (à propos de huit cas). Nouv. Rev. Fr. Hématol. 3: 575–586, 1963.
320. INCEMAN, S., CAEN, J. AND BERNARD, J.: Aggregation, adhesion and viscous metamorphosis of platelets in congenital fibrinogen deficiencies. J. Lab. Clin. Med. 68: 21–32, 1966.
321. IRELAND, D. M.: Effect of thrombin on the radioactive nucleotides of human washed platelets. Biochem. J. 105: 857–867, 1967.
322. IRELAND, D. M. AND MILLS, D. C. B.: Detection and determination of adenosine diphosphate and related substances in plasma. Biochem. J. 99: 283–296, 1966.
323. ISHIZAKA, T. AND ISHIZAKA, K.: Biological activities of aggregated γ-globulin. V. Agglutination of erythrocytes and platelets. J. Immunol. 89: 709–716, 1962.
324. JACKSON, D. P., SORENSEN, D. K., CRONKITE, E. P., BOND, V. P. AND FLIEDNER, T. M.: Effectiveness of transfusions of fresh and lyophilized platelets in controlling bleeding due to thrombocytopenia. J. Clin. Invest. 38: 1689–1697, 1959.
325. JACKSON, D. P., ZIEVE, P. D. AND MORSE, E. E.: Studies on the reactions of human platelets to thrombin. *In* Physiology of Hemostasis and Thrombosis, ed. by S. A. Johnson and W. H. Seegers, pp. 22–43, Charles C Thomas, Springfield, Ill., 1967.
326. JEAN, G. AND RACINE, L.: Ultrastructure of normal human thrombocytes. *In* Proceedings of 5th International Congress for Electron Microscopy, Philadelphia, 1961, pp. 2–8. Academic Press, New York, 1962.
327. JERUSHALMY, Z., KOHN, A. AND DE VRIES, A.: Interaction of myxoviruses with human blood platelets in vitro. Proc. Soc. Exp. Biol. Med. 106: 462–466, 1961.
328. JERUSHALMY, Z., SKOZA, L. ZUCKER, M. B. AND GRANT, R.: Inhibition by guanidino compounds of platelet aggregation induced by adenosine diphosphate. Biochem. Pharmacol. 15: 1791–1803, 1966.
329. JERUSHALMY, Z. AND ZUCKER, M. B.: Some effects of fibrinogen degradation products (FDP) on blood platelets. Thromb. Diath. Haemorrh. 15: 413–419, 1966.
330. JØRGENSEN, L. AND BORCHGREVINK, C. F.: The platelet plug in normal persons. I. The histological appearance of the plug 15 to 20 minutes and 24 hours after the bleeding and its role in the capillary haemostasis. Acta Path. Microbiol. Scand. 57: 40–56, 1963.
331. JØRGENSEN, L. AND BORCHGREVINK, C. F.: The haemostatic mechanism in patients with haemorrhagic diseases. A histological study of wounds made for primary and secondary bleeding time tests. Acta Pathol. Microbiol. Scand. 60: 55–82, 1964.
332. JØRGENSEN, L., ROWSELL, H. C., HOVIG, T., GLYNN, M. F. AND MUSTARD, J. F.: Adenosine diphosphate-induced platelet aggregation and myocardial infarction in swine. Lab. Invest. 17: 616–644, 1967.
333. JØRGENSEN, L., ROWSELL, H. C., HOVIG, T. AND MUSTARD, J. F.: Resolution and organization of platelet-rich mural thrombi in carotid arteries of swine. Amer. J. Pathol. 51: 681–719, 1967.

334. JØRGENSEN, S.: Breakdown of adenine and hypoxanthine nucleotides and nucleosides in human plasma. Acta Pharmacol. Toxicoli 12: 294–302, 1956.
335. JUDAH, J. D., AHMED, K. AND MCLEAN, A. E. M.: Possible role of ion shifts in liver injury. *In* Ciba Foundation Symposium on Cellular Injury, ed. by A. V. S. de Reuck and J. Knight, pp. 187–208, Churchill, London, 1964.
336. KÁNSKA, B., NIEWIAROWSKI, S., OSTROWSKI, L., POPLAWSKI, A. AND PROKOPOWICZ, J.: Macrothrombocytic thrombopathia. Clinical, coagulation and hereditary aspects. Thromb. Diath. Haemorrh. 10: 88–100, 1963.
337. KARPATKIN, M. H. AND KARPATKIN, S.: In vivo and in vitro binding of factor VIII to human platelets. Thromb. Diath. Haemorrh. 21: 129–133, 1969.
338. KARPATKIN, S.: Studies on human platelet glycolysis. Effect of glucose, cyanide, insulin, citrate, and agglutination and contraction on platelet glycolysis. J. Clin. Invest. 46: 409–417, 1967.
339. KARPATKIN, S.: Heterogeneity of human platelets. 1. Metabolic and kinetic evidence suggestive of young and old platelets. J. Clin. Invest. 48: 1073–1082, 1969.
340. KARPATKIN, S. AND LANGER, R. M.: Biochemical energetics of simulated platelet plug formation. Effect of thrombin, adenosine diphosphate, and epinephrine on intra- and extracellular adenine nucleotide kinetics. J. Clin. Invest. 47: 2158–2168, 1968.
341. KARPATKIN, S. AND SISKIND, G. W.: Effect of antibody binding and agglutination on human platelet glycolysis: comparison with thrombin and epinephrine. Blood 30: 617–624, 1967.
342. KERR, J. W., PIRRIE, R., MACAULEY, I. AND BRONTE-STEWART, B.: Platelet aggregation by phospholipids and free fatty acids. Lancet 1: 1296–1299, 1965.
343. KIELLEY, W. W.: The biochemistry of muscle. Ann. Rev. Biochem. 33: 403–430, 1964.
344. KIESSELBACH, T. H. AND WAGNER, R. H.: Fibrin-stabilising factor: a thrombin-labile platelet protein. Amer. J. Physiol. 211: 1472–1476, 1966.
345. KINLOUGH, R. L., PACKHAM, M. A. AND MUSTARD, J. F.: Glucose and platelet aggregation. Fed. Proc. 28: 509, 1969.
346. KINLOUGH, R. L., PACKHAM, M. A. AND MUSTARD, J. F.: The effect of prostaglandin E_1 on platelet function in vitro and in vivo. In preparation.
347. KINLOUGH, R. L., PACKHAM, M. A. AND MUSTARD, J. F.: Unpublished observations.
348. KITCHENS, C. S. AND NEWCOMB, T. F.: Human platelet respiration. J. Appl. Physiol. 25: 581–585, 1968.
349. KJAERHEIM, A. AND HOVIG, T.: The ultrastructure of haemostatic blood platelet plugs in rabbit mesenterium. Thromb. Diath. Haemorrh. 7: 1–15, 1962.
350. KLOESE, J.: Influence of prostaglandins on platelet adhesiveness and platelet aggregation. *In* Proceedings of 2nd Nobel Symposium, Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 241–252, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
351. KNIERIEM, H. J. AND CHANDLER, A. B.: The effect of warfarin sodium on the duration of platelet aggregation. Thromb. Diath. Haemorrh. 18: 766–778, 1967.
352. KOPEĆ, M., BUDZYŃSKI, A., STACHURSKA, J., WEGRZYNOWICZ, Z. AND KOWALSKI, E.: Studies on the mechanism of interference by fibrinogen degradation products (FDP) with the platelet function. Role of fibrinogen in the platelet atmosphere. Thromb. Diath. Haemorrh. 15: 476–490, 1966.
353. KOPEĆ, M., WEGRZYNOWICZY, Z., BUDZYNSKI, A. Z., LATALLO, Z. S., LIPINSKI, B. AND KOWALSKI, E.: Interaction of fibrinogen degradation products (FDP) with platelets. *In* Platelets in Hemostasis. Exp. Biol. Med. 3: 73–79, 1968.
354. KOWALSKI, E.: Fibrinogen derivatives and their biologic activities. Seminars Hematol. 5: 45–59, 1968.
355. KOWALSKI, E., BUDZYNSKI, A. Z., KOPEĆ, M., LATALLO, Z. S., LIPINSKI, B. AND WEGRZYNOWICZ, Z.: Studies on the molecular pathology and pathogenesis of bleeding in severe fibrinolytic states in dogs. Thromb. Diath. Haemorrh. 12: 69–86, 1964.
356. KOWALSKI, E., KOPEĆ, M. AND WEGRZYNOWICZ, Z.: Influence of fibrinogen degradation products (FDP) on platelet aggregation, adhesiveness and viscous metamorphosis. Thromb. Diath. Haemorrh. 10: 406–423, 1964.
357. KOWALSKI, E., KOPEĆ, M., WEGRZYNOWICZ, A., HURWIC, M. AND BUDZYNSKI, A. Z.: A lysosomal concept of the platelet release reaction and viscous metamorphosis. Thromb. Diath. Haemorrh. 16: 134–152, 1966.
358. LACY, P. E., HOWELL, S. L., YOUNG, D. A. AND FINK, C. J.: New hypothesis of insulin secretion. Nature (London) 219: 1177–1179, 1968.
359. LAGERGREN, H., EGBERG, N., ERIKSSON, J. C. AND GILLBERG, G.: Preparation and evaluation of "nonthrombogenic" surfaces. J. Thorac. Cardiovasc. Surg. 56: 381–387, 1968.
360. LANDER, H., KINLOUGH, R. L. AND ROBSON, H. N.: Reduced platelet survival in patients with Starr-Edwards prostheses. Brit. Med. J. 1: 688–689, 1965.
361. LARKE, R. P. B. AND WHEELOCK, E. F.: Stabilization of Chikungunya virus infectivity by human blood platelets. Bacteriol. Proc. Abstract No. V 229, p. 184, 1969.
362. LARRIEU, M. J., CAEN, J., LELONG, J. C. AND BERNARD, J.: Maladie de Glanzmann. Etude clinique, biologique et pathogénique. A propos de cinq observations. Nouv. Rev. Fr. Hematol. 1: 662–693, 1961.
363. LARRIEU, M. J., CAEN, J. P., MEYER, D. O., VAINER, H., SULTAN, Y. AND BERNARD, J.: Congenital bleeding disorders with long bleeding time and normal platelet count. II. Von Willebrand's disease (report of thirty-seven patients). Amer. J. Med. 45: 354–372, 1968.
364. Larrieu, M. J., Inceman, S. and Marder, V.: Action des produits de dégradation du fibrinogène sur les fonctions plaquettaires. Nouv. Rev. Fr. Hematol. 7: 691–704, 1967.
365. LAZZARINI-ROBERTSON, A., Jr.: Effects of heparin on the uptake of lipids by isolated human and animal arterial endothelial type cells. Angiology 12: 525–534, 1961.

366. LEE, K. T., KIM, D. N., SHAPER, A. G. AND THOMAS, W. A.: Coagulation and clot-lysis studies in groups eating high and low fat diets. Exp. Mol. Pathol. 3: 500–521, 1964.
367. LEGRAND, Y., CAEN, J. P. AND ROBERT, L.: Effect of glucosamine on platelet-collagen reaction. Proc. Soc. Exp. Biol. Med. 127: 941–943, 1968.
368. LEVIN, J. AND BANG, F. B.: A comparison between human blood platelets and Limulus amebocytes. Blood 28: 984–985, 1966.
369. LICHTENSTEIN, L. M. AND MARGOLIS, S.: Histamine release in vitro: inhibition by catecholamines and methylxanthines. Science 161: 902–903, 1968.
370. LODER, P. B., HIRSH, J. AND DE GRUCHY, G. C.: The effect of collagen on platelet glycolysis and nucleotide metabolism. Brit. J. Haematol. 14: 563–573, 1968.
371. LÖHR, G. W., WALLER, H. D. AND GROSS, R.: Beziehungen zwischen Plättchenstöffwechsel und Retraktion des Blutgerinnsels unter besonderer Berücksichtigung der Thrombopathie Glanzmann-Naegeli. Deut. Med. Wochenschr. 86: 897–904, 1961.
372. LÖHR, G. W., WALLER, H. D. AND GROSS, R.: Beziehungen zwischen Plättchenstöffwechsel und Retraktion des Blutgerinnsels unter besonderer Berücksichtigung der Thrombopathie Glanzmann-Naegeli. Deut. Med. Wochenschr. 86: 946–950, 1961.
373. LORAND, L. AND KONISHI, K.: Activation of fibrin-stabilizing factor of plasma by thrombin. Arch. Biochem. 105: 58–67, 1964.
374. LOWENHAUPT, R. AND NATHAN, P.: Platelet accumulation observed by electron microscopy in the early phase of renal allotransplant rejection. Nature (London) 220: 822–825, 1968.
374a. LOWENHAUPT, R. AND NATHAN, P.: The participation of platelets in the rejection of dog kidney allotransplants: hematologic and electron microscopic studies. Transplantation Proc. 1: 305–310, 1969.
375. LU, W. C.: Agglutination of human platelets by influenza (PR8 strain) virus and mumps virus. Fed. Proc. 17: 446, 1958.
376. LUGANOVA, I. S., SEITS, I. F. AND TEODOROVICH, V. I.: Metabolism in human thrombocytes. Biokhimiya 23: 405–411, 1958.
377. LÜSCHER, E. F.: Glukose als Cofactor bei der Retraktion des Blutgerinnsels. Experientia 12: 294, 1956.
378. LÜSCHER, E. F.: Retraction activity of the platelets: biochemical background and physiological significance. *In* Blood Platelets, ed. by S. A. Johnson, R. W. Monto, J. W. Rebuck and R. C. Horn, Jr., pp. 445–453, Little, Brown and Co., Boston, 1961.
379. MACFARLANE, R. G.: A critical review: the mechanism of haemostasis. Quart. J. Med. 10: 1–29, 1941.
380. MACMILLAN, D. C.: Secondary clumping effect in human citrated platelet-rich plasma produced by adenosine diphosphate and adrenaline. Nature (London) 211: 140–144, 1966.
381. MACMILLAN, D. C.: Effect of salicylates on human platelets. Lancet 1: 1151, 1968.
382. MAGUIRE, M. H. AND MICHAL, F.: Powerful new aggregator of blood platelets—2-chloroadenosine-5′-diphosphate. Nature (London) 217: 571–573, 1968.
383. MAHADEVAN, V., SINGH, H. AND LUNDBERG, W. O.: Effects of saturated and unsaturated fatty acids on blood platelet aggregates in vitro. Proc. Soc. Exp. Biol. Med. 121: 82–85, 1966.
384. MAJERUS, P. W., SMITH, M. B. AND CLAMON, G. H.: Lipid metabolism in human platelets. I. Evidence for a complete fatty acid synthesizing system. J. Clin. Invest. 48: 156–164, 1969.
385. MALAWISTA, S. E.: Colchicine: A common mechanism for its anti-inflammatory and anti-mitotic effects. Arthritis Rheum. 11: 191–197, 1968.
386. MALAWISTA, S. E. AND BENSCH, K. G.: Human polymorphonuclear leukocytes: demonstration of microtubules and effect of colchicine. Science 156: 521–522, 1967.
387. MANDEL, E. E. AND LAZERSON, J.: Thrombasthenia in liver disease. N. Engl. J. Med. 265: 56–61, 1961.
388. MANOHAR, M., MAHESWARAN, S. K., FROMMES, S. P. AND LINDORFER, R. L.: Platelet damaging factor, a fifth activity of staphylococcal α-toxin. J. Bacteriol. 94: 224–231, 1967.
388a. MARCHESI, V. T.: Some electron microscopic observations on interactions between leukocytes, platelets and endothelial cells in acute inflammation. Ann. N.Y. Acad. Sci. 116: 774–788, 1964.
389. MARCUS, A. J.: The role of lipids in blood coagulation. *In* Advances in Lipid Research, ed. by R. Paoletti, and D. Kritchevsky, pp. 1–37, Academic Press, New York, 1966.
390. MARCUS, A. J. AND ZUCKER, M. B.: The Physiology of Blood Platelets. Grune & Stratton, Inc., New York, 1965.
391. MARCUS, A. J., ZUCKER-FRANKLIN, D., SAFIER, L. B. AND ULLMAN, H. L.: Studies on human platelet granules and membranes. J. Clin. Invest. 45: 14–28, 1966.
392. MARGOLIS, J.: Activation of Hageman factor by saturated fatty acids. Aust. J. Exp. Biol. Med. Sci. 40: 505–513, 1962.
393. MARKWARDT, F.: Influence of drugs and enzymes on platelet 5-hydroxytryptamine. Ann. Med. Exp. Biol. Fenn. 46: 407–415, 1968.
394. MARKWARDT, F., BARTHEL, W., GLUSA, E. AND HOFFMANN, A.: Untersuchungen über den Einfluss von Papaverin auf Reaktionen der Blutplättchen. Arch. Pharmacol. Exp. Pathol. (Naunyn-Schmiedebergs) 257: 420–431, 1967.
395. MARKWARDT, F., BARTHEL, W. AND HOFFMANN, A.: Über die Beziehungen zwischen Stoffwechsel und Serotoninabgabe der Blutplättchen bei Einwirkung von Thrombin. Naturwissenschaften 51: 639, 1964.
396. MARTIN, M.: Formation of thrombus and thrombolysis in animal experiments. Angiologica 5: 80–94, 1968.
397. MARX, R. AND JEAN, G.: Studien zur Pathogenese der Thrombasthenie Glanzmann-Naegeli. Klin. Wochenschr. 40: 942–953, 1962.

398. Mason, R. G. and Read, M. S.: Platelet response to six agglutinating agents: species similarities and differences. Exp. Mol. Pathol. 6: 370–381, 1967.
399. Mathues, J. K., Wolff, C. E., Cevallos, W. H. and Holmes, W. L.: Platelet adhesiveness and thrombosis in rabbits on an atherogenic diet. Med. Exp. 18: 121–128, 1968.
400. McClure, P. D., Ingram, G. I. C., Stacey, R. S., Glass, V. H. and Matchett, M. O.: Platelet function tests in thrombocythaemia and thrombocytosis. Brit. J. Haematol. 12: 478–498, 1966.
400a. McDonagh, R. P., Jr., Burns, M. J., Delaimi, K. E. and Faust, R. G.: Sugar penetration into human blood platelets. J. Cell. Physiol. 72: 77–79, 1968.
401. McDonald, L.: Studies on blood coagulation and thrombosis and on the action of heparin in ischemic heart disease. Amer. J. Cardiol. 9: 365–371, 1962.
402. McDonald, L., Bray, C., Field, C., Love, F. and Davies, B.: Homocystinuria, thrombosis, and the blood-platelets. Lancet 1: 745–746, 1964.
403. McDonald, L. and Edgill, M.: Dietary restriction and coagulability of the blood in ischaemic heart-disease. Lancet 1: 996–998, 1958.
404. McDonald, L. and Edgill, M.: Changes in coagulability of the blood during venous phases of ischaemic heart-disease. Lancet 1: 1115–1118, 1959.
405. McDonald, L. and Edgill, M.: Action of heparin in ischaemic heart disease. Lancet 1: 844–847, 1961.
406. McGehee, W. G., Hjort, P. F. and Rapaport, S. I.: Effects of thorium dioxide upon blood clotting and platelets. II. In vivo studies in rabbits. Thromb. Diath. Haemorrh. 16: 354–369, 1966.
407. McIntire, F. C., Roth, L. W. and Richards, R. K.: The in vitro release of histamine from the blood cells of sensitized rabbits: relationship to blood coagulation mechanisms. Amer. J. Physiol. 159: 332–336, 1949.
408. McKay, D. G. and Margaretten, W.: Disseminated intravascular coagulation in virus diseases. Arch. Intern. Med. 120: 129–152, 1967.
409. McKay, D. G., Shapiro, S. S. and Shanberge, J. N.: Alterations in the blood coagulation system induced by bacterial endotoxins II. In vitro. J. Exp. Med. 107: 369–376, 1958.
410. McLean, J. R., Maxwell, R. E. and Hertler, D.: Fibrinogen and adenosine diphosphate-induced aggregation of platelets. Nature (London) 202: 605–606, 1964.
411. McLean, J. R. and Veloso, H.: Change of shape without aggregation caused by ADP in rabbit platelets at low pH. Life Sci. 6: 1983–1986, 1967.
411a. Michal, F.: D-Receptor for serotonin on blood platelets. Nature (London) 221: 1253–1254, 1969.
411b. Michal, F. and Thorp, R. H.: Enhanced adenosine inhibition of platelet aggregation in the presence of cardiac glycosides. Nature (London) 209: 208–209, 1966.
411c. Michal, F., Maguire, M. H. and Gough, G.: Nature (London) 222:1073–1074, 1969.
412. Mikiten, T. M. and Douglas, W. W.: Effect of calcium and other ions on vasopressin release from rat neurohypophyses stimulated electrically in vitro. Nature (London) 207: 302, 1965.
413. Milgrom, F., Campbell, W. A. and Witebsky, E.: Serological specificity of platelets. Vox Sang. 15: 418–426, 1968.
414. Mills, D. C. B., Robb, I. A. and Roberts, G. C. K.: The release of nucleotides, 5-hydroxytryptamine and enzymes from human blood platelets during aggregation. J. Physiol. (London) 195: 715–729, 1968.
415. Mills, D. C. B. and Roberts, G. C. K.: Membrane active drugs and the aggregation of human blood platelets. Nature (London) 213: 35–38, 1967.
416. Mills, D. C. B. and Roberts, G. C. K.: Effects of adrenaline on human blood platelets. J. Physiol. (London) 193: 443–453, 1967.
417. Mills, S. E. and Levine, L.: The inhibition of immune haemolysis by salicylaldoxime. Immunology 2: 368–383, 1959.
418. Mitchell, J. R. A. and Sharp, A. A.: Platelet clumping in vitro. Brit. J. Haematol. 10: 78–93, 1964.
418a. Molinas, F. and von Kaulla, K. N.: On the effect on human thrombocytes of synthetic organic fibrinolytic compounds. Thromb. Diath. Haemorrh. 21: 46–64, 1969.
419. Mongar, J. L. and Schild, H. O.: Cellular mechanisms in anaphylaxis. Physiol. Rev. 41: 226–270, 1962.
420. Moolten, S. E., Jennings, P. B. and Solden, A.: Dietary fat and platelet adhesiveness in arteriosclerosis and diabetes. Amer. J. Cardiol. 11: 290–300, 1963.
421. Moolton, S. E. and Vroman, L.: The adhesiveness of blood platelets in thromboembolism and hemorrhagic disorders. I. Measurement of platelet adhesiveness by glass-wool filter. Amer. J. Clin. Pathol. 19: 701–709, 1949.
422. Moolten, S. E., Vroman, L., Vroman, G. M. S. and Goodman, B.: Role of blood platelets in thromboembolism. Arch. Intern. Med. 84: 667–710, 1949.
423. Morris, C. D. W.: Acetylsalicylic acid and platelet stickiness. Lancet 1: 279–280, 1967.
424. Morrison, F. S. and Baldini, M. G.: Antigenic relationship between blood platelets and vascular endothelium. Blood 33: 46–56, 1969.
425. Morse, E. E., Jackson, D. P. and Conley, C. L.: Role of platelet fibrinogen in the reactions of platelets to thrombin. J. Clin. Invest. 44: 809–816, 1965.
426. Motulsky, A. G.: Platelet agglutination by influenza virus. Clin. Res. Proc. 1: 100, 1953.
427. Movat, H. Z., Mustard, J. F., Taichman, N. S. and Uriuhara, T.: Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytosis of antigen-antibody complexes. Proc. Soc. Exp. Biol. Med. 120: 232–237, 1965.
428. Movat, H. Z., Uriuhara, T., Macmorine, D. R. L. and Burke, J. S.: A permeability factor released from leukocytes after phagocytosis of immune complexes and its possible role in the Arthus reaction. Life Sci. 3: 1025–1032, 1964.

429. MOVAT, H. Z., WEIBER, W. J., GLYNN, M. F. AND MUSTARD, J. F.: Platelet phagocytosis and aggregation. J. Cell Biol. 27: 531–543, 1965.
430. MOWBRAY, J. F.: Methods of suppression of immune responses. *In* Proceedings of IX International Congress for Internal Medicine. Excerpta Medica Int. Congr. Ser. No. 137: 106–110, 1966.
431. MOWBRAY, J. F.: Personal communication.
432. MUELLER-ECKHARDT, C. AND LÜSCHER, E. F.: Immune reactions of human blood platelets. I. A comparative study on the effects on platelets of heterologous antiplatelet antiserum, antigen-antibody complexes, aggregated gammaglobulin and thrombin. Thromb. Diath. Haemorrh. 20: 155–167, 1968.
433. MUELLER-ECKHARDT, C. AND LÜSCHER, E. F.: Immune reactions of human blood platelets. II. The effect of latex particles coated with gammaglobulin in relation to complement activation. Thromb. Diath. Haemorrh. 20: 168–179, 1968.
434. MUELLER-ECKHARDT, C. AND LÜSCHER, E. F.: Immune reactions of human blood platelets. III. Inhibition of immune reactions by sulfhydryl reagents. Thromb. Diath. Haemorrh. 20: 327–335, 1968.
435. MUELLER-ECKHARDT, C. AND LÜSCHER, E. F.: Immune reactions of human blood platelets. IV. Investigations on the problem of an immunologically induced effect of endotoxin on human platelets. Thromb. Diath. Haemorrh. 20: 336–344, 1968.
436. MUIR, H. M. AND MUSTARD, J. F.: Enhancement of platelet aggregation by glycosaminglycans (mucopolysaccharides). *In* Le rôle de la paroi artérielle dans l'athérogénèse. Colloq. Int. Centre Nat. Rech. Sci., No. 169, pp. 589–596, 1968.
437. MURCHISON, L. E. AND FYFE, T.: Effects of ciagrette smoking on serum-lipids, blood-glucose, and platelet adhesiveness. Lancet 2: 182–184, 1966.
438. MÜRER, E. H.: Release reaction and energy metabolism in blood platelets with special reference to the burst in oxygen uptake. Biochim. Biophys. Acta 162: 320–326, 1968.
439. MÜRER, E. H.: Clot retraction and energy metabolism of platelets. Effect and mechanism of inhibitors. Biochim Biophys. Acta 172: 266–276, 1969.
440. MÜRER, E. H., HELLEM, A. J. AND ROZENBERG, M. C.: Energy metabolism and platelet function. Scand. J. Clin. Lab. Invest. 19:280–282, 1967.
441. MURPHY, E. A.: Thrombozyten, Thrombose und Gerinnung. *In* Nikotin Pharmakologie und Toxikologie des Tabakrauches, ed. by H. Schievelbein, pp. 178–192, Georg Thieme, Stuttgart, 1968.
442. MURPHY, E. A. AND MUSTARD, J. F.: Coagulation tests and platelet economy in atherosclerotic and control subjects. Circulation 25: 114–125, 1962.
443. MURPHY, E. A. AND MUSTARD, J. F.: Smoking and thrombosis. *In* Towards a Less Harmful Cigarette. Nat. Cancer Inst. Monogr. 28: 47–56, 1968.
444. MUSTARD, J. F.: Changes in the platelet levels of non-transfused patients following surgical operations. Acta Haematol. 17: 257–262, 1957.
445. MUSTARD, J. F.: Hemostasis and thrombosis. Seminars Hematol. 5: 91–106, 1968.
446. MUSTARD, J. F.: Unpublished observations.
447. MUSTARD, J. F., EVANS, G., PACKHAM, M. A. AND NISHIZAWA, E. E.: The platelet in intravascular immunological reactions. *In* Cellular and Humoral Mechanisms in Anaphylaxis and Allergy, ed. by H. Z. Movat, pp. 151–163, S. Karger, New York, 1969.
448. MUSTARD, J. F., GLYNN, M. F., NISHIZAWA, E. E. AND PACKHAM, M. A.: Platelet-surface interactions: relationship to thrombosis and hemostasis. Fed. Proc. 26: 106–114, 1967.
449. MUSTARD, J. F., HEGARDT, B., ROWSELL, H. C. AND MACMILLAN, R. L.: Effect of adenosine nucleotides on platelet aggregation and clotting time. J. Lab. Clin. Med. 64: 548–559, 1964.
450. MUSTARD, J. F., JØRGENSEN, L., HOVIG, T., GLYNN, M. F. AND ROWSELL, H. C.: Role of platelets in thrombosis. *In* Pathogenesis and Treatment of Thromboembolic Diseases. Thromb. Diath. Haemorrh. Suppl. 21: 131–158, 1966.
451. MUSTARD, J. F., MEDWAY, W., DOWNIE, H. G. AND ROWSELL, H. C.: Effects of intravenous phospholipid containing phosphatidyl serine on blood clotting with particular reference to the Russell's viper venom time. Nature (London) 196: 1063–1065, 1962.
452. MUSTARD, J. F., MOVAT, H. Z., MACMORINE, D. R. L. AND SENYI, A.: Release of permeability factors from the blood platelet. Proc. Soc. Exp. Biol. Med. 119: 988–991, 1965.
453. MUSTARD, J. F. AND MURPHY, E. A.: Effect of different dietary fats on blood coagulation, platelet economy, and blood lipids. Brit. Med. J. 1: 1651–1655, 1962.
454. MUSTARD, J. F. AND MURPHY, E. A.: Effect of smoking on blood coagulation and platelet survival in man. Brit. Med. J. 1: 846–849, 1963.
455. MUSTARD, J. F. AND MURPHY, E. A.: Blood platelet economy during moderate and intensive heparin therapy. Blood 22: 1–8, 1963.
456. MUSTARD, J. F., MURPHY, E. A., OGRYZLO, M. A. AND SMYTHE, H. A.: Blood coagulation and platelet economy in subjects with primary gout. Can. Med. Ass. J. 89: 1207–1211, 1963.
457. MUSTARD, J. F., MURPHY, E. A., ROBINSON, G. A., ROWSELL, H. C., OZGE, A. AND CROOKSTON, J. H.: Blood platelet survival. Thromb. Diath. Haemorrh. Suppl. 13: 245–275, 1964.
458. MUSTARD, J. F. AND PACKHAM, M. A.: The assessment of platelet adherence. J. Atheroscler. Res. 7: 405–407, 1967.
459. MUSTARD, J. F. AND PACKHAM, M. A.: Platelet phagocytosis. Ser. Haematol. I, 2: 168–184, 1968.
460. MUSTARD, J. F. AND PACKHAM, M. A.: The biochemistry of primary hemostasis. Plenary Session Paper. XII Congress of International Society for Hematology, New York, pp. 306–314, 1968

461. MUSTARD, J. F. AND PACKHAM, M. A.: Unpublished observations.
462. MUSTARD, J. F., PACKHAM, M. A. AND SENYI, A.: The effect of DFP on platelet function in vitro and in vivo. J. Lab. Clin. Med. 70: 1003–1004, 1967.
463. MUSTARD, J. F., ROWSELL, H. C., LOTZ, F., HEGARDT, B. AND MURPHY, E. A.: The effect of adenine nucleotides on thrombus formation, platelet count and blood coagulation. Exp. Mol. Pathol. 5: 43–60, 1966.
464. MUSTARD, J. F., ROWSELL, H. C. AND MURPHY, E. A.: Platelet economy (platelet survival and turnover). Brit. J. Hematol. 12: 1–24, 1966.
465. MUSTARD, J. F., ROWSELL, H. C., MURPHY, E. A. AND DOWNIE, H. G.: Diet and thrombus formation: quantitative studies using an extracorporeal circulation in pigs. J. Clin. Invest. 42: 1783–1789, 1963.
466. NACHMAN, R. L.: Immunologic studies of platelet protein. Blood 25: 703–711, 1965.
467. NACHMAN, R. L.: Thrombasthenia: immunologic evidence of a platelet protein abnormality. J. Lab. Clin. Med. 67: 411–419, 1966.
468. NACHMAN, R. L.: Platelet proteins. Seminars Hematol. 5: 18–31, 1968.
469. NACHMAN, R. L. AND MARCUS, A. J.: Immunological studies of protein associated with the subcellular fractions of thrombasthenic and afibrinogenaemic platelets. Brit. J. Haemat. 15: 181–189, 1968.
470. NACHMAN, R. L., MARCUS, A. J. AND SAFIER, L. B.: Platelet thrombosthenin: subcellular localization and function. J. Clin. Invest. 46: 1380–1389, 1967.
471. NACHMAN, R. L., MARCUS, A. J. AND ZUCKER-FRANKLIN, D.: Immunologic studies of proteins associated with subcellular fractions of normal human platelets. J. Lab. Clin. Med. 69: 651–658, 1967.
472. NAGAKI, K., FUJIKAWA, K. AND INAI, S.: Studies on the fourth component of complement. II. The fourth component of complement in guinea pig and human platelets. Biken J. 8: 129–141, 1965.
473. NAJEAN, Y., ARDAILLOU, N., CAEN, J., LARRIEU, M. J. AND BERNARD, J.: Survival of radiochromium-labeled platelets in thrombocytopenias. Blood 22: 718–732, 1963.
474. NAJEAN, Y., ARDAILLOU, N., DRESCH, C. AND BERNARD, J.: The platelet destruction site in thrombocytopenic purpuras. Brit. J. Haematol. 13: 409–426, 1967.
475. NATHANIEL, E. J. H. AND CHANDLER, A. B.: Electron microscopic study of adenosine diphosphate-induced platelet thrombi in the rat. J. Ultrastruct. Res. 22: 348–359, 1968.
476. NEGUS, D., PINTO, D. J. AND BROWN, N.: Platelet adhesiveness in postoperative deep-vein thrombosis. Lancet 1: 220–224, 1969.
477. NESTEL, P. J.: A note on platelet adhesiveness in ischemic heart diseases. J. Clin. Pathol. (London) 14: 150–151, 1961.
478. NEWLAND, H.: Antagonism of the anti-thrombotic effect of warfarin by uric acid. Amer. J. Med. Sci. 256: 44–52, 1968.
479. NEWLAND, H. R. AND CHANDLER, A. B.: Blood uric acid levels and ADP-induced platelet aggregation. Fed. Proc. 27: 321, 1968.
480. NEWLAND, H. AND NORDÖY, A.: Effect of large doses of warfarin sodium on haemostasis and on ADP-induced platelet aggregation in vivo in the rat. Cardiovasc. Res. 1: 363–370, 1967.
481. NIEWIAROWSKI, S.: Physiologic implications of fibrinolysis. Plenary Session Paper, XII Congress of International Society for Hematology, New York, pp. 330–338, 1968.
482. NIEWIAROWSKI, S., POPLAWSKI, A., LIPINSKI, B. AND FARBISZEWSKI, R.: The release of platelet clotting factors during aggregation and viscous metamorphosis. *In* Platelets in Hemostasis. Exp. Biol. Med. 3: 121–128, 1968.
483. NIEWIAROWSKI, S., STUART, R. K. AND THOMAS, D. P.: Activation of intravascular coagulation by collagen. Proc. Soc. Exp. Biol. Med. 123: 196–200, 1966.
484. NILSSON, I. M., BLOMBÄCK, M. AND BLOMBÄCK, B.: von Willebrand's disease in Sweden; its pathogenesis and treatment. Acta Med. Scand. 164: 263–278, 1959.
485. NILSSON, I. M., BLOMBÄCK, M. AND VON FRANCKEN, I.: On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time. Acta Med. Scand. 159: 35–57, 1957.
486. NISHIKAWA, E. E. AND HALDAR, J.: Phospholipid synthesis during platelet aggregation. Fed. Proc. 26: 760, 1967.
487. NISHIKAWA, E. E., HOVIG, T., LOTZ, F., ROWSELL, H. C. AND MUSTARD J. F.: Effect of a natural phosphatidyl serine fraction on blood coagulation, platelet aggregation and haemostasis. Brit. J. Haematol. 16: 487–499, 1969.
488. NOLTE, A. AND BREDDIN, K.: Elektronenmikroskopische Untersuchungen an normalen und agglutinierten Thrombozyten. Thromb. Diath. Haemorrh. 15: 93–108, 1966.
489. NORDÖY, A., HAMLIN, J. T., CHANDLER, A. B. AND NEWLAND, H.: The influence of dietary fats on plasma and platelet lipids and ADP-induced platelet thrombosis in the rat. Scand. J. Haematol. 5: 458–473, 1968.
490. NORDÖY, A. AND ÖDEGAARD, A. E.: The influence of citrate and heparin on the adhesiveness of rat platelets and human platelets measured in vitro. Scand. J. Clin. Invest. 15: 399–404, 1963.
491. O'BRIEN, J. R.: The adhesiveness of native platelets and its prevention. J. Clin. Pathol. (London) 14: 140–149, 1961.
492. O'BRIEN, J. R.: Platelet aggregation. I. Some effects of the adenosine phosphates, thrombin and cocaine upon platelet adhesiveness. J. Clin. Pathol. (London) 15: 446–452, 1962.
493. O'BRIEN, J. R.: Platelet aggregation. II. Some results from a new method of study. J. Clin. Pathol. (London) 15: 452–455, 1962.
494. O'BRIEN, J. R.: Some effects of adrenaline and anti-adrenaline compounds on platelets in vitro and in vivo. Nature (London) 200: 763–764, 1963.
495. O'BRIEN, J. R.: The effect of the alkyl tins on platelet aggregation. Thromb. Diath. Haemorrh. 9: 330–334, 1963.
496. O'BRIEN, J. R.: A comparison of platelet aggregation produced by seven compounds and a comparison of their inhibitors. J. Clin. Pathol. (London) 17: 275–281, 1964.

497. O'Brien, J. R.: Effects of adenosine diphosphate and adrenaline on mean platelet shape. Nature (London) 207: 306–307, 1965.
498. O'Brien, J. R.: Changes in platelet membranes possibly associated with platelet stickiness. Nature (London) 212: 1057–1058, 1966.
499. O'Brien, J. R.: Platelets: a Portsmouth syndrome? Lancet 2: 258, 1967.
500. O'Brien, J. R.: Aspirin and platelet aggregation. Lancet 1: 204–205, 1968.
501. O'Brien, J. R.: Effects of salicylates on human platelets. Lancet 1: 779–783, 1968.
502. O'Brien, J. R.: Effect of anti-inflammatory agents on platelets. Lancet 1: 894–895, 1968.
503. O'Brien, J. R. and Heywood, J. B.: Effect of aggregating agents and their inhibitors on the mean platelet shape. J. Clin. Pathol. (London) 19: 148–153, 1966.
503a. O'Brien, J. R., Shoobridge, S. M. and Finch, W. J.: Comparison of the effect of heparin and citrate on platelet aggregation. J. Clin. Pathol. (London) 22: 28–31, 1969.
504. O'Brien, J. R. and Woodhouse, M. A.: Platelets: their size, shape and stickiness in vitro: degranulation and propinquity. *In* Platelets in Hemostasis. Exp. Biol. Med. 3: 90–102, 1968.
505. Ödegaard, A. E., Skalhegg, B. A. and Hellem, A. J.: Increased activity of "anti-Willebrand factor" in diabetic plasma. Thromb. Diath. Haemorrh. 11: 27–37, 1964.
506. Ödegaard, A. E., Skalhegg, B. A. and Hellem, A. J.: Investigation on adenosine diphosphate (ADP) induced platelet adhesiveness in vitro. Part III. The inactivation of ADP in plasma. Thromb. Diath. Haemorrh. 11: 317–326, 1964.
507. Oka, M., Ohuchi, T., Yoshida, H. and Imaizumi, R.: Stimulatory effect of adenosine triphosphate and magnesium on the release of catecholamines from adrenal medullary granules. Jap. J. Pharmacol. 17: 199–207, 1967.
508. Olef, I.: The differential platelet count: its clinical significance. Arch. Intern. Med. 57: 1163–1185, 1936.
509. Øllgaard, E.: Clot retraction as a quantitative test of the function and agglutinability of the blood platelets. Acta Haematol. 6: 220–230, 1951.
510. Øllgaard, E.: Macroscopic studies of platelet aggregation. Nature of an aggregating factor in red blood cells and platelets. Thromb. Diath. Haemorrh. 6: 86–97, 1961.
511. Oski, F. A. and Naiman, J. L.: Effect of live measles vaccine on the platelet count. N. Engl. J. Med. 275: 352–356, 1966.
512. Ottolander, G. J. H. den and Bleijenberg, A.: Factors which influence the bleeding time. Thromb. Diath. Haemorrh. 8: 511–523, 1962.
513. Paasonen, M. K.: Platelet 5-hydroxytryptamine as a model in pharmacology. Ann. Med. Exp. Biol. Fenn. 46: 416–422, 1968.
514. Pachter, M. R., Johnson, S. A., Neblett, T. R. and Truant, J. P.: Bleeding, platelets, and macroglobulinemia. Amer. J. Clin. Pathol. 31: 467–482, 1959.
515. Packham, M. A., Ardlie, N. G. and Mustard, J. F.: The effect of adenine compounds on platelet aggregation. Amer. J. Physiol. 217: 1009–1017, 1969.
516. Packham, M. A., Evans, G., Glynn, M. F. and Mustard, J. F.: The effect of plasma proteins on the interaction of platelets with glass surfaces. J. Lab. Clin. Med. 73: 686–697, 1969.
517. Packham, M. A. and Mustard, J. F.: The effect of pyrazole compounds on thrombin-induced platelet aggregation. Proc. Soc. Exp. Biol. Med. 130: 72–75, 1969.
518. Packham, M. A., Nishizawa, E. E. and Mustard, J. F.: Response of platelets to tissue injury. *In* Biochem. Pharmacol. Suppl. pp. 171–184, Pergamon Press, Oxford, London, 1968.
519. Packham, M. A., Warrior, E. S., Glynn, M. F., Senyi, A. S. and Mustard, J. F.: Alteration of the response of platelets to surface stimuli by pyrazole compounds. J. Exp. Med. 126: 171–188, 1967.
520. Pepper, D. S. and Jamieson, G. A.: Isolation of a glycoprotein fraction from human platelet membranes which inhibits viral haemagglutination. Nature (London) 219: 1252–1253, 1968.
521. Philip, R. B. and Lemieux, V.: Comparison of some effects of dipyridamole and adenosine on thrombus formation, platelet adhesiveness and blood pressure in rabbits and rats. Nature (London) 218: 1072–1074, 1968.
522. Pinckard, R. N., Hawkins, D. and Farr, R. S.: In vitro acetylation of plasma proteins, enzymes and DNA by aspirin. Nature (London) 219: 68–69, 1968.
523. Pinniger, J. L. and Prunty, F. T. G.: Some observations on the blood-clotting mechanism; role of fibrinogen and platelets with reference to a case of congenital afibrinogenemia. Brit. J. Exp. Pathol. 27: 200–210, 1946.
524. Pitney, W. R. and Potter, M.: Retention of platelets by glass bead filters. J. Clin. Pathol. (London) 20: 710–716, 1967.
525. Pletscher, A.: Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. Brit. J. Pharmacol. Chemother. 32: 1–16, 1968.
526. Pletscher, A., Bartholini, G. and DaPrada, M.: Metabolism of monoamines by blood platelets and relation to 5-hydroxytryptamine liberation. *In* Mechanisms of Release of Biogenic Amines. ed. by U. S. von Euler, S. Rosell and B. Uvnäs, pp. 165–175, Pergamon Press, Oxford, London, 1966.
527. Pletscher, A., Burkard, W. P., Tranzer, J. P. and Gey, K. F.: Two sites of 5-hydroxytryptamine uptake in blood platelets. Life Sci. 6: 273–280, 1967.
528. Pletscher, A., DaPrada, M. and Bartholini, G.: Temperature dependence of the passive in and outflow of 5-hydroxytryptamine in blood platelets. Biochem. Pharmacol. 15: 419–424, 1966.
529. Poisner, A. M. and Trifaró, J. M.: The role of ATP and ATPase in the release of catecholamines from the adrenal medulla. 1. ATP-evoked release of catecholamines, ATP and protein from isolated chromaffin granules. Mol. Pharmacol. 3: 561–571, 1967.

530. Poller, L., Thomson, J. M. and Priest, C. M.: Coumarin therapy and platelet aggregation. Brit. Med. J. 1: 474–476, 1969.
531. Poller, L., Thomson, J. M., Tabiowo, A. and Priest, C. M.: Progesterone, oral contraception and blood coagulation. Brit. Med. J. 1: 554–556, 1969.
532. Poplawski, A., Skorulska, M. and Niewiarowski, S.: Increased platelet adhesiveness in hypertensive cardiovascular disease. J. Atheroscler. Res. 8: 721–723, 1968.
533. Porter, K. A.: Morphological aspects of renal homograft rejection. Brit. Med. Bull. 21: 171–175, 1965.
534. Porter, K. A.: Rejection in treated renal allografts. J. Clin. Pathol. (London) Suppl. 20: 518–523, 1968.
535. Porter, K. A., Dossetor, J. B., Marchioro, T. L., Peart, W. S., Rendall, J. M., Starzl, T. E. and Terasaki, P. I.: Human renal transplants. 1. Glomerular changes. Lab. Invest. 16: 153–181, 1967.
535a. Prentice, C. R. M., Turpie, A. G. G., Hassanein, A. A., McNicol, G. P. and Douglas, A. S.: Changes in platelet behaviour during Arvin therapy. Lancet 1: 644–647, 1969.
536. Puszkin, E. and Jerushalmy, Z.: Effect of connective tissue and collagen on lactate production: possible role of acid mucopolysaccharides. Proc. Soc. Exp. Biol. Med. 129: 346–351, 1968.
537. Quick, A. J.: Salicylates and bleeding: the aspirin tolerance test. Amer. J. Med. Sci. 252: 265–269, 1966.
538. Rabiner, S. F. and Hrodek, O.: Platelet factor 3 in normal subjects and patients with renal failure. J. Clin. Invest. 47: 901–912, 1968.
539. Reeves, J. T., Jokl, P., Merida, J. and Leathers, J. E.: Pulmonary vascular obstruction following administration of high-energy nucleotides. J. Appl. Physiol. 22: 475–479, 1967.
540. Renaud, S., Kinlough, R. and Mustard, J. F.: Platelet aggregation in rats with a thrombotic tendency. Circulation 38 (suppl. 6): 19, 1968.
541. Riddell, P. E. and Bier, A. M.: Electrophoresis of S^{35}-labelled material released from clumping platelets. Nature (London) 205: 711–712, 1965.
542. Riddle, J. M., Bradshaw, D. L. and Bluhm, G. B.: Platelets in primary gout—an electron microscopic study. Fed. Proc. 28: 509, 1969.
543. Robb-Smith, A. H. T.: Why the platelets were discovered. Brit. J. Haematol. 13: 618–637, 1967.
544. Robinson, C. W., Jr., Kress, S. L., Wagner, R. H. and Brinkhous, K. M.: Platelet agglutination and deagglutination with a sulfhydryl inhibitor, methyl mercuric nitrate: relationships to platelet ATPase. Exp. Mol. Pathol. 4: 457–464, 1965.
545. Robinson, C. W., Jr., Mason, R. G. and Wagner, R. H.: Effect of sulfhydryl inhibitors on platelet agglutinability. Proc. Soc. Exp. Biol. Med. 113: 857–861, 1963.
546. Robinson, R. W. and LeBeau, R. J.: Platelet adhesiveness and aggregation with chlorophenoxyisobutyric ester. Amer. J. Med. Sci. 253: 106–112, 1967.
547. Rock, R. C. and Nemerson, Y.: Energetics of human blood platelets: uncoupling of oxidative phosphorylation in intact cells. J. Lab. Clin. Med. 73: 42–53, 1969.
548. Rodman, N. F.: Sequential changes in platelet ultrastructure in a white thrombus model. *In* Physiology of Hemostasis and Thrombosis, ed. by S. A. Johnson and W. H. Seegers. pp. 266–287, Charles C Thomas, Springfield, Ill., 1967.
549. Rodman, N. F. and Mason, R. G.: Platelet-platelet interaction: relationship to hemostasis and thrombosis. Fed. Proc. 26: 95–105, 1967.
550. Rodman, N. F., Jr., Mason, R. G., Painter, J. C. and Brinkhous, K. M.: Fibrinogen—its role in platelet agglutination and agglutinate stability. A study of congenital afibrinogenemia. Lab. Invest. 15: 641–656, 1966.
551. Rosenzweig, A. and Ways, P.: The oxidation of long-chain fatty acids by the formed elements of human blood. Blood 27: 57–64, 1966.
552. Roskam, J.: Erreurs anciennes et perspectives nouvelles dans l'étude de l'adhesion, de l'agrégation et de la métamorphose visqueuse des plaquettes, facteurs d'hémostase. Thromb. Diath. Haemorrh. 10:253–266, 1963.
553. Roskam, J.: Du rôle de la paroi vasculaire dans l'hémostase spontanée et la pathogénie des états hémorragiques. Thromb. Diath. Haemorrh. 12: 338–352, 1964.
554. Roskam, J.: Historical remarks on the relationship of platelets to hemostasis. *In* Biochemistry of Blood Platelets, ed. by E. Kowalski and S. Niewiarowski, p. 3–8, Academic Press, New York, 1967.
555. Rossi, E. C.: Effects of ethylenediaminetetraacetate (EDTA) and citrate upon platelet glycolysis. J. Lab. Clin. Med. 69: 204–216, 1967.
556. Rossi, E. C.: Some effects of artificial media upon glycolysis in rat platelets. Blood 30: 758–766, 1967.
557. Rossi, E. C.: Platelets and hemostasis. J. Chronic Dis. 21: 607–613, 1969.
558. Rossi, E. C. and Clatanoff, D. V.: Maintenance of phosphonucleotides in platelets suspended in plasma. J. Lab. Clin. Med. 64: 42–50, 1964.
559. Rowsell, H. C.: Hemostasis in the chicken and turkey. Fed. Proc. 27: 627, 1968.
560. Rowsell, H. C., Glynn, M. F., Mustard, J. F. and Murphy, E. A.: Effect of heparin on platelet economy in dogs. Amer. J. Physiol. 213: 915–922, 1967.
561. Rozenberg, M. C. and Firkin, B. G.: The rate of platelet aggregation in haemorrhagic disease and thrombosis. Scand. J. Haematol. 3: 5–18, 1966.
562. Rozenberg, M. C. and Holmsen, H.: Adenine nucleotide metabolism of blood platelets. II. Uptake of adenosine and inhibition of ADP-induced platelet aggregation. Biochim. Biophys. Acta 155: 342–352, 1968.
563. Rozenberg, M. C. and Holmsen, H.: Adenine nucleotide metabolism of blood platelets. IV. Platelet aggregation response to exogenous ATP and ADP. Biochim. Biophys. Acta 157: 280–288, 1968.
564. Rozenberg, M. C. and Stormorken, H.: Comparison of glass adhesiveness and rate of aggregation of blood platelets. Scand. J. Clin. Lab. Invest. 19: 82–85, 1967.

564a. RUTTY, D. A. AND VINE, T. L.: Electrokinetic mobility of blood-platelets. Lancet 1: 206, 1969.
565. RYŠÁNEK, R., SVEHLA, C., SPÁNKOVÁ, H. AND MLEJNKOVÁ, M.: The effect of tricyclic antidepressive drugs on adrenaline and adenosine diphosphate induced platelet aggregation. J. Pharm. Pharmacol. 18: 616–617, 1966.
566. SAHUD, M. A. AND AGGELER, P. M.: Platelet dysfunction—differentiation of a newly recognized primary type from that produced by aspirin. N. Engl. J. Med. 280: 453–454, 1969.
567. SALMON, J. AND BOUNAMEAUX, Y.: Étude des antigéns plaquettaires et, en particulier, du fibrinogéne. Thromb. Diath. Haemorrh. 2: 93–110, 1958.
568. SALMON, J., VERSTRAETE, M. AND BOUNAMEAUX, Y.: Fibrinogéne plaquettaire et afibrinogénémie. Arch. Int. Physiol. Biochem. 65: 632–634, 1957.
569. SALVIDIO, E. AND CROSBY, W. H.: Thrombocytopenia after intravenous injection of India ink. J. Lab. Clin. Med. 56: 711–716, 1960.
570. SALZMAN, E. W.: Measurement of platelet adhesiveness. A simple in vitro technique demonstrating an abnormality in von Willebrand's disease. J. Lab. Clin. Med. 62: 724–735, 1963.
571. SALZMAN, E. W.: The limitation of heparin therapy after arterial reconstruction. Surgery 57: 131–138, 1965.
572. SALZMAN, E. W. AND CHAMBERS, D. A.: Inhibition of ADP-induced platelet aggregation by substituted amino acids. Nature (London) 204: 698–700, 1964.
573. SALZMAN, E. W., CHAMBERS, D. A. AND NERI, L. L.: Possible mechanism of aggregation of blood platelets by adenosine diphosphate. Nature (London) 210: 167–169, 1966.
574. SALZMAN, E. W., CHAMBERS, D. A. AND NERI, L. L.: Incorporation of labelled nucleotides and aggregation of human blood platelets. Thromb. Diath. Haemorrh. 15: 52–68, 1966.
575. SANBAR, S. S., ZWEIFLER, A. J. AND SMET, G.: In-vivo and in-vitro effects of polyvinylpyrrolidone on platelet adhesiveness in human blood. Lancet 2: 917–919, 1967.
576. SANBORN, E. B., LEBIUS, J. AND BOIS, P.: Cytoplasmic microtubules in blood platelets. Blood 27: 247–252, 1966.
577. SANO, I., KAKIMOTO, Y. AND TANIGUCHI, K.: Binding and transport of serotonin in rabbit blood platelets and action of reserpine. Amer. J. Physiol. 195: 495–498, 1958.
578. SAWYER, P. N., REARDON, J. H. AND OGONIAK, J. C.: Irreversible electrochemical precipitation of mammalian platelets and intravascular thrombosis. Proc. Nat. Acad. Sci. U. S. A. 53: 200–207, 1965.
579. SCHIEVELBEIN, H. AND WERLE, E.: Freisetzung von 5-hydroxytryptamin durch Nikotin. Psychopharmacologia 3: 35–43, 1962.
580. SCHMID, H. J., JACKSON, D. P. AND CONLEY, C. L.: Mechanism of action of thrombin on platelets. J. Clin. Invest. 41: 543–553, 1962.
580a. SCHNEIDER, W., KÜBLER, W. AND GROSS, R.: Untersuchungen über den In-vitro-Einfluss der kationischen Polypeptide Protaminsulfat und Trasylol auf Aggregation und Retraktion menschlicher Blutplättchen. Thromb. Diath. Haemorrh. 20: 314–326, 1968.
581. SCHOENBECHLER, M. J. AND BARBARO, J. F.: The requirement for sensitized lymphocytes in one form of antigen-induced histamine release from rabbit platelets. Proc. Nat. Acad. Sci. U. S. A. 60: 1247–1251, 1968.
582. SCHOENBECHLER, M. J. AND SADUN, E. H.: In vitro histamine release from blood cellular elements of rabbits infected with *Schistosoma mansoni*. Proc. Soc. Exp. Biol. Med. 127: 601–606, 1968.
583. SCHULMAN, I., PIERCE, M., LUKENS, A. AND CURRIMBHOY, Z.: Studies on thrombopoiesis. I. A factor in normal human plasma required for platelet production; chronic thrombocytopenia due to its deficiency. Blood 16: 943–957, 1960.
584. SCHULMAN, I., SMITH, C. H., ERLANDSON, M., FORT, E. AND LEE, R. E.: Vascular hemophilia: a familial hemorrhagic disease in males and females characterized by combined antihemophilic globulin deficiency and vascular abnormality. Pediatrics 18: 347–361, 1956.
585. SCHULZ, H., JÜRGENS, R. AND HIEPLER, E.: Die Ultrastruktur der Thrombozyten bei der konstitutionellen Thrombopathie (von Willebrand-Jürgens) mit einem, Beitrag zur submikoskopischen Orthologie der Thrombozyten. Thromb. Diath. Haemorrh. 2: 300–323, 1958.
586. SCHULZ, H. AND LANDGRÄBER, E.: Elektronenmikroskopische Untersuchungen über die Adsorption und Phagocytose von Influenza-Viren durch Thrombocyten. Klin. Wochenschr. 44: 998–1006, 1966.
587. SCHULZ, H. AND RABANUS, B.: Die kapilläre Plättchenthrombose im elektronenmikroskipischen Bild. Beitr. Pathol. Anat. Allg. Pathol. 131: 290–311, 1965.
588. SCOTT, J. L., CARTWRIGHT, G. E. AND WINTROBE, M. M.: Acquired aplastic anemia: an analysis of thirty-nine cases and review of the pertinent literature. Medicine 38: 119–172, 1959.
589. SCOTT, R. B.: Activation of glycogen phosphorylase in blood platelets. Blood 30: 321–330. 1967.
590. SEAMAN, G. V. F.: Surface potential and platelet aggregation. *In* Platelets: Their Role in Hemostasis and Thrombosis. Thromb. Diath. Haemorrh. Suppl. 26: 53–68, 1967.
591. SEAMAN, G. V. F. AND BROOKS, D. E.: Effect of aggregating agents on platelet geometry and electrokinetic charge. Fed. Proc. 28: 576, 1969.
592. SHAINOFF, J. R. AND PAGE, I. H.: Significance of cryoprofibrin in fibrinogen-fibrin conversions. J. Exp. Med. 116: 687–707, 1962.
593. SHAPIRO, S., SHERWIN, B. AND GORDIMED, H.: Postoperative thromboembolization: platelet count and prothrombin time after surgical operations; simple method for detecting reductions and elevations of prothrombin concentration (or activity) of blood plasma. Ann. Surg. 116: 175–183, 1942.
593a. SHARP, A. A., WARREN, B. A., PAXTON, A. M. AND ALLINGTON, M. J.: Anticoagulant therapy with a purified fraction of Malayan pit viper venom. Lancet 1: 493–499, 1968.
594. SHAW, S., PEGRUM, G. D., WOLFF, S. AND ASHTON, W. L.: Platelet adhesiveness in diabetes mellitus. J. Clin. Pathol. (London) 20: 845–847, 1967.

594a. Shepro, D., Belamarich, F. A. and Chao, F. C.: Retardation of clot retraction after incubation of platelets with colchicine and heavy water. Nature (London) 221: 563–565, 1969.
595. Shirasawa, K.: Electron and light microscopic observations on experimental thrombosis. Acta Pathol. Jap. 16: 1–36, 1966.
596. Shore, P. A. and Alpers, H. S.: Platelet damage induced in plasma by certain fatty acids. Nature (London) 200: 1331–1332, 1963.
597. Shulman, N. R., Aster, R. H., Pearson, H. A. and Hiller, M. C.: Immunoreactions involving platelets. VI. Reactions of maternal isoantibodies responsible for neonatal purpura. Differentiation of second platelet antigen system. J. Clin. Invest. 41: 1059–1069, 1962.
598. Shulman, N. R., Marder, V. J., Hiller, M. C. and Collier, E. M.: Platelet and leukocyte isoantigens and their antibodies: serologic, physiologic and clinical studies. Progr. Hematol. 4: 222–304, 1964.
599. Siegal, A. and Lüscher, E. F.: Non-identity of the alpha-granules of human blood platelets with typical lysosomes. Nature (London) 215: 745–747, 1967.
600. Siegel, I. and Cohen, S.: Action of staphylococcal toxin on human platelets. J. Infec. Dis. 114: 488–502, 1964.
601. Silver, M. D.: Cytoplasmic microtubules in rabbit platelets. Z. Zellforsch. Mikroskop. Anat. 68: 474–480, 1965.
602. Silver, M. D.: Microtubules in the cytoplasm of mammalian platelets. Nature (London) 209: 1048–1050, 1966.
603. Silver, M. J.: Role of calcium ions and phospholipids in platelet aggregation and plug formation. Amer. J. Physiol. 209: 1128–1136, 1965.
604. Silverman, J. L. and Wurzel, H. A.: The effect of glyceryl guaiacolate on platelet function and other coagulation factors in vivo. Amer. J. Clin. Pathol. 51: 35–40, 1969.
605. Sinakos, Z. and Caen, J. P.: Platelet aggregation in mammalians (human, rat, rabbit, guinea-pig, horse, dog)—a comparative study. Thromb. Diath. Haemorrh. 17: 99–111, 1967.
606. Siqueira, M. and Nelsen, R. A., Jr.: Platelet agglutination by immune complexes and its possible role in hypersensitivity. J. Immunol. 86: 516–525, 1961.
607. Sixma, J. J.: De localisatie van fibrinogen in blood plaatjes. Thesis, Utrecht, 1966, cited in ref. (307).
608. Sixma, J. J. and Geuze, J. J.: Degranulation of human platelets during ADP-induced aggregation. Vox Sang. 15: 309–314, 1968.
609. Sixma, J. J. and Molenaar, I.: Microtubules and microfibrils in human platelets. Thromb. Diath. Haemorrh. 16: 153–162, 1966.
610. Skålhegg, B. A., Hellem, A. J. and Ödegaard, A. E.: Investigations on adenosine diphosphate (ADP) induced platelet adhesiveness in vitro. II. Studies on the mechanism. Thromb. Diath. Haemorrh. 11: 305–316, 1964.
611. Skjørten, F.: Studies on the ultrastructure of pseudopod formation in human blood platelets. I. Effect of temperature, period of incubation, anticoagulants and mechanical forces. Scand. J. Haematol. 5: 401–414, 1968.
612. Skosa, L., Zucker, M. B., Jerushalmy, Z. and Grant, R.: 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, 1967.
613. Smythe, H. A., Ogryzlo, M. A., Murphy, E. A. and Mustard, J. F.: The effect of sulfinpyrazone (anturan) on platelet economy and blood coagulation in man. Can. Med. Ass. J. 92: 818–821, 1965.
614. Sokal, G.: Etude morphologique des plaquettes sanguines et de la métamorphose visqueuse au moyen d'antisérums fluorescents antifibrinogéne et antiplaquettes. Acta Haematol. 28: 313–325, 1962.
615. Soloff, L. A. and Wiedeman, M. P.: Biomicroscopic observations of vasoconstrictive and thrombogenic effects produced by long-chain saturated fatty acids within the vasculature of the wing of an unanaesthetized bat. Nature (London) 199: 495–496, 1963.
615a. Solomon, H. M., Ashley, C., Spirt, N. and Abrams, W. B.: The influence of debrisoquin on the accumulation and metabolism of biogenic amines by the human platelet, in vivo and in vitro. Clin. Pharmacol. Ther. 10: 229–238, 1969.
615b. Solomon, H. M. and Zieve, P. D.: The accumulation of organic bases by the human platelet. J. Pharmacol. Exp. Ther. 155: 112–116, 1967.
616. Solum, N. O.: Platelet aggregation during fibrin polymerization. Scand. J. Clin. Lab. Invest. 18: 577–587, 1966.
617. Solum, N. O. and Stormorken, H.: 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) 17: 170–182, 1965.
618. Soulier, J. P., Prou-Wartelle, O. and Halle, L.: Study of thrombin-coagulase. Thromb. Diath. Haemorrh. 17: 321–334, 1967.
619. Spaet, T. H.: Vascular factors in the pathogenesis of hemorrhagic syndromes. Blood 7: 641–652, 1952.
620. Spaet, T. H. and Cinteron, J.: Studies on platelet factor-3 availability. Brit. J. Haematol. 11: 269–275, 1965.
621. Spaet, T. H., Erichson, R. B. and Spielvogel, A. R.: The hemostatic sequence. *In* Physiology of Hemostasis and Thrombosis, ed. by S. A. Johnson and W. H. Seegers, pp. 154–178. Charles C Thomas, Springfield, Ill., 1967.
622. Spaet, T. H. and Lejnieks, I.: Studies on the mechanism whereby platelets are clumped by adenosine diphosphate. Thromb. Diath. Haemorrh. 15: 36–51, 1966.
623. Spaet, T. H. and Zucker, M. B.: Mechanism of platelet plug formation and role of adenosine diphosphate. Amer. J. Physiol. 206: 1267–1274, 1964.
624. Spielvogel, A. R.: An ultrastructural study of the mechanisms of platelet-endotoxin interaction. J. Exp. Med. 126: 235–249, 1967.
625. Stacey, R. S.: Uptake of 5-hydroxytryptamine by platelets. Brit. J. Pharmacol. 16: 284–295, 1961.
626. Stafford, A.: Potentiation of adenosine and the adenine nucleotides by dipyridamole. Brit. J. Pharmacol. Chemotherap. 28: 218–227, 1966.

627. Stehbens, W. E.: Early intravascular platelet aggregation. *In* 4th European Conference on Microcirculation, Cambridge, 1966, ed. by H. Harders, pp. 80–84, S. Karger, Basel, New York, 1966.
628. Stohlman, F., Jr., Sarnoff, S. J., Case, R. B. and Ness, A. T.: Hemolytic syndrome following the insertion of a Lucite ball valve prosthesis into the cardiovascular system. Circulation 13: 586–591, 1956.
629. Sullivan, J. M., Harken, D. E. and Gorlin, R.: Pharmacologic control of thrombo-embolic complications of cardiac-valve replacement. A preliminary report. N. Engl. J. Med. 279: 576–580, 1968.
630. Svärd, P. O.: The effect of an enzyme preparation from *Aspergillus oryzae* on the aggregation of rabbit platelets in vitro. Scand. J. Haematol. 3: 277–289, 1966.
631. Swank, R. L.: The screen filtration pressure method in platelet research. Significance and interpretation. Ser. Haematol. I, 2: 146–167, 1968.
632. Tait, J. and Elvidge, A. R.: Effect upon platelets and on blood coagulation of injecting foreign particles into the blood stream. J. Physiol. (London) 62: 129–144, 1926.
632a. Taylor, F. B. and Zucker, M. B.: Prolonged clot lysis time and absence of platelet γ M-globulin in patients with thrombasthenia. Nature (London) 222: 99, 1969.
633. Terada, H., Baldini, M., Ebbe, S. and Madoff, M. A.: Interaction of influenza virus with blood platelets. Blood 28: 213–228, 1966.
634. Thomas, D. P.: The role of platelet catecholamines in the aggregation of platelets by collagen and thrombin. *In* Platelets in Haemostasis. Exp. Biol. Med. 3: 129–134, 1968.
635. Tidball, M. E.: Interrelation of calcium and heparin with respect to spontaneous release of histamine. Amer. J. Physiol. 213: 407–410, 1967.
636. Tilney, L. G. and Porter, K. R.: Studies on microtubules in Heliozoa. I. The fine structure of Actinosphaerium nucleofilum (Barrett), with particular reference to the axial rod structure. Protoplasma 60: 317–344, 1965.
637. Tilney, L. G. and Porter, K. R.: Studies on the microtubules of Heliozoa. II. The effect of low temperature in these structures in the formation and maintenance of axopodia. J. Cell Biol. 34: 327–343, 1967.
638. Tocantins, L. M., Harrington, W. J., Smith, C. H., Cronkite, E. P., Conley, C. L. and Gardner, F. H.: Discussion on transfusions of platelets and platelet substitutes. Blood 13: 1089–1096, 1958.
639. Transer, J. P. and Baumgartner, H. R.: Filling gaps in the vascular endothelium with blood platelets. Nature (London) 216: 1126–1128, 1967.
640. Transer, J. P., Da Prada, M. and Pletscher, A.: Ultrastructural localization of 5-hydroxytryptamine in blood platelets. Nature (London) 212: 1574–1575, 1966.
641. Transer, J. P., Da Prada, M. and Pletscher, A.: Electron microscopic study of the storage site of 5-hydroxytryptamine in blood platelets. Advan. Pharmacol. 6:(part A) 125–128, 1968.
642. Triparó, J. M. and Poisner, A. M.: The role of ATP and ATPase in the release of catecholamines from the adrenal medulla. II. ATP-evoked fall in optical density of isolated chromaffin granules. Mol. Pharmacol. 3: 572–580, 1967.
643. Ulutin, O. N.: Primary thrombocytopathy. Israel J. Med. Sci. 1: 857–860, 1965.
644. Vainer, H. and Wattiaux, R.: Glycogen synthetase activity in blood platelets. Nature (London) 217: 951–952, 1968.
645. Valdorf-Hansen, J. F. and Zucker, M. B.: Temperature dependence of ^{14}C-serotonin release from human platelets. In XII Congress of International Society for Hematology, New York, p. 196, 1968.
646. Van Aken, W. G., Groote, T. M. and Vreeken, J.: Platelet aggregation: an intermediary mechanism in carbon clearance. Scand. J. Haematol. 5: 333–338, 1968.
647. van Creveld, S. and Pascha, C. N.: Influence of the prostaglandins E_1 and E_2 on aggregation of blood platelets. Nature (London) 218: 361–362, 1968.
648. Vassalli, P., Simon, G. and Rouiller, C.: Ultrastructural study of platelet changes initiated in vivo by thrombin. J. Ultrastruct. Res. 11: 374–387, 1964.
649. Vildosola, J. and Emparanza, E.: Hereditary familial thrombocytopenia. *In* X International Congress for Paediatrics, Lisbon, ed. by M. Cordeiro, p. 36, 1962.
650. Von Willebrand, E. A.: Hereditäre Pseudohämophili. Finska Läkaresallak Handl. 68: 87–112, 1926.
650a. Vossen, M. E. M. H., Stadhouders, A. M., Kurstjens, R. and Haanen, C.: Observations on platelet ultrastructure in familial thrombocytopathic thrombocytopenia. Amer. J. Pathol. 53: 1021–1039, 1968.
651. Waalkes, T. P. and Coburn, H.: Comparative effects of glycogen and antigen-antibody reactions on serotonin and histamine in the rabbit. Proc. Soc. Exp. Biol. Med. 101: 122–125, 1959.
652. Waller, H. D., Löhr, G. W., Grignani, F. and Gross, R.: Über den Energiestoffwechsel normaler menschlicher Thrombozyten. Thromb. Diath. Haemorrh. 3: 520–547, 1959.
653. Ward, P. A. and Becker, E. L.: Mechanisms of the inhibition of chemotaxis by phosphonate esters. J. Exp. Med. 125: 1001–1020, 1967.
654. Warner, E. D., Hoak, J. C. and Connor, W. E.: The role of fatty acids in platelet aggregation and thrombosis. Thromb. Diath. Haemorrh. Suppl. 26: 249–259, 1967.
655. Warshaw, A. L., Laster, L. and Shulman, N. R.: The stimulation by thrombin of glucose oxidation in human platelets. J. Clin. Invest. 45: 1923–1934, 1966.
656. Weber, E. and Unger, W.: Über Veränderungen des Glykogengehaltes in Blutplättchen während der Gerinnung. Biochem. Pharmacol. 12: 23–30, 1964.
657. Weeks, J. R., Chandra Sekhar, N. and Ducharme, D. W.: Relative activity of prostaglandins E_1, A_1, E_2 and A_2 on lipolysis, platelet aggregation, smooth muscle and the cardiovascular system. J. Pharm. Pharmacol. 21: 103–108, 1969.
658. Weiss, H. J.: Von Willebrand's disease—diagnostic criteria. Blood 32: 668–679, 1968.

659. Weiss, H. J. and Aledort, L. M.: Impaired platelet connective-tissue reaction in man after aspirin ingestion. Lancet 2: 495–497, 1967.
660. Weiss, H. J., Aledort, L. M. and Kochwa, S.: The effect of salicylates on the hemostatic properties of platelets in man. J. Clin. Invest. 47: 2169–2180, 1968.
661. Weiss, H. J. and Eichelberger, J. W., Jr.: The detection of platelet defects in patients with mild bleeding disorders. Use of a quantitative assay for platelet factor 3. Amer. J. Med. 32: 872–883, 1962.
662. Weiss, H. J. and Eichelberger, J. W., Jr.: Secondary thrombocytopathia: platelet factor 3 in various disease states. Arch. Intern. Med. 112: 827–834, 1963.
663. Weiss, J. A.: The effect of clinical dextran on platelet aggregation, adhesion, and ADP release in man: in vivo and in vitro studies. J. Lab. Clin. Med. 69: 37–46, 1967.
664. Weissbach, H. and Redfield, B. G.: Studies on the uptake of serotonin by platelets. *In* Blood Platelets, ed. by S. A. Johnson, R. W. Monto, J. W. Rebuck and R. C. Horn, Jr., pp. 393–405. Little, Brown and Co., Boston, 1961.
665. Werle, E. and Schievelbein, H.: Activity of nicotine and inactivity of kallikrein and kallidin in aggregation of blood platelets. Nature (London) 207: 871–872, 1965.
666. Westerholm, B.: Dextran-induced release of 5-hydroxytryptamine from rabbit platelets. Acta Physiol. Scand. 67: 236–242, 1966.
667. Wharton-Jones, W. T.: On the state of the blood and blood vessels in inflammation. Guys Hosp. Rep. Ser. 2: 7, 1, 1851.
668. White, J. G.: Fine structural alterations induced in platelets by adenosine diphosphate. Blood 31: 604–621, 1968.
669. White, J. G.: The transfer of thorium particles from plasma to platelets and platelet granules. Amer. J. Pathol. 53: 567–575, 1968.
670. White, J. G.: Effects of colchicine and vinca alkaloids on human platelets. I. Influence on platelet microtubules and contractile function. Amer. J. Pathol. 53: 281–291, 1968.
671. White, J. G.: Effects of colchicine and vinca alkaloids on human platelets. III. Influence on primary internal contraction and secondary aggregation. Amer. J. Pathol. 54: 467–478, 1969.
672. White, J. G. and Krivit, W.: The ultrastructural localization and release of platelet lipids. Blood 27: 167–186, 1966.
673. White, J. G. and Krivit, W.: An ultrastructural basis for the shape changes induced in platelets by chilling. Blood 30: 625–635, 1967.
674. White, J. G., Krivit, W. and Vernier, R. L.: The platelet-fibrin relationship in human blood clots: an ultrastructural study utilizing ferritin-conjugated anti-human fibrinogen antibody. Blood 25: 241–257, 1965.
675. Wilner, G. D., Nossel, H. L. and LeRoy, E. C.: Activation of Hageman factor by collagen. J. Clin. Invest. 47: 2608–2615, 1968.
676. Wilner, G. D., Nossel, H. L. and LeRoy, E. C.: Aggregation of platelets by collagen. J. Clin. Invest. 47: 2616–2621, 1968.
677. Wilson, P. A., McNicol, G. P. and Douglas, A. S.: Platelet abnormality in human scurvy. Lancet 1: 975–978, 1967.
678. Wilson, P. A., McNicol, G. P. and Douglas, A. S.: Effect of fibrinogen degradation products on platelet aggregation. J. Clin. Pathol. (London) 21: 147–153, 1968.
679. Wojteka-Lukasik, E., Sopata, I., Wise, J., Skonieczna, M., Neidzwiecka-Namyslowska, I. and Kowalski, E.: Adhesion of platelets to collagen devoid of telopeptides. Thromb. Diath. Haemorrh. 18: 76–79, 1967.
680. Wolfe, S. M. and Shulman, N. R.: Adenyl cyclase activity in human platelets. Biochem. Biophys. Res. Comm. 35: 265–272, 1969.
681. Wong, P. S. L., Merrill, E. W. and Salzman, E. W.: A non-thrombogenic hydrogel cast from solution. Fed. Proc. 28: 441, 1969.
682. Woodin, A. M., French, J. E. and Marchesi, V. T.: Morphological changes associated with the extrusion of protein induced in the polymorphonuclear leucocyte by staphylococcal leucocidin. Biochem. J. 87: 567–571, 1963.
683. Woodin, A. M. and Wieneke, A. A.: The participation of calcium, adenosine triphosphate and adenosine triphosphatase in the extrusion of granule proteins from the polymorphonuclear leucocyte. Biochem. J. 90: 498–509, 1964.
684. Woodin, A. M. and Wieneke, A. A.: Role of leucocidin and triphosphoinositide in the control of potassium permeability. Nature (London) 220: 283–286, 1968.
685. Woods, M. C., Gamble, F. N., Furth, J. and Bigelow, R. R.: Control of the postirradiation hemorrhagic state by platelet transfusions. Blood 8: 545–553, 1953.
686. Wright, H. P.: The adhesiveness of blood platelets in normal subjects with varying concentrations of anti-coagulants. J. Pathol. Bacteriol. 53: 255–262, 1941.
687. Wright, H. P.: Changes in the adhesiveness of blood platelets following parturition and surgical operations. J. Pathol. Bacteriol. 54: 461–468, 1942.
688. Wright, H. P., Thompson, R. H. S. and Zilkha, K. J.: Platelet adhesiveness in multiple sclerosis. Lancet 2: 1109–1110, 1965.
689. Wright, J. H. and Minot, G. R.: The viscous metamorphosis of the blood platelets. J. Exp. Med. 26: 395–409, 1917.
690. Wu, R., Sessa, G. and Hamerman, D.: P_i transport and glycolysis in leucocytes and platelets. Biochim. Biophys. Acta 93: 614–624, 1964.

691. WURZEL, H., MCCREARY, T., BAKER, L. AND GUMERMAN, L.: Glucose-6-phosphate dehydrogenase activity in platelets. Blood 17: 314–318, 1961.
692. YAMADA, E.: The fine structure of the megakaryocyte in the mouse spleen. Acta Anat. 29: 267–290, 1957.
693. YOUSSEF, A. AND BARKHAN, P.: Release of platelet factor 4 by adenosine diphosphate and other platelet-aggregating agents. Brit. Med. J. 1: 746–747, 1968.
694. ZBINDEN, G.: Lauric acid-induced thrombocytopenia and thrombosis in rabbits. Thromb. Diath. Haemorrh. 18: 57–65, 1967.
695. ZBINDEN, G.: Modification of the fatty acid-induced thrombocytopenia by anticoagulants and compounds which inhibit in vitro platelet aggregation. J. Pharmacol. Exp. Ther. 159: 163–171, 1968.
696. ZIEVE, P. D., GAMBLE, J. L. AND JACKSON, D. P.: Effects of thrombin on the potassium and ATP content of platelets. J. Clin. Invest. 43: 2063–2069, 1964.
696a. ZIEVE, P. D. AND GREENOUGH, W. B., III: Adenyl cyclase in human platelets: activity and responsiveness. Biochem. Biophys. Res. Comm. 35: 462–466, 1969.
696b. ZIEVE, P. D. AND SOLOMON, H. M.: Uptake of amino acids by the human platelet. Amer. J. Physiol. 214: 58–61, 1968.
696c. ZIEVE, P. D. AND SOLOMON, H. M.: Effects of diuretics on the human platelet. Amer. J. Physiol. 215: 650–654, 1968.
697. ZUCKER, M. B.: Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypothrombinemic rats. Amer. J. Physiol. 148: 275–288, 1947.
698. ZUCKER, M. B.: Platelet adhesion, release and aggregation. Thromb. Diath. Haemorrh. Suppl. 13: 301–303, 1964.
699. ZUCKER, M. B.: ADP- and collagen-induced platelet aggregation in vivo and in vitro. Thromb. Diath. Haemorrh. Suppl. 26: 175–184, 1967.
700. ZUCKER, M. B. AND BORRELLI, J.: Quantity, assay and release of serotonin in human platelets. J. Appl. Physiol. 7: 425–431, 1955.
701. ZUCKER, M. B. AND BORRELLI, J.: Concentration of serotonin in serum in thrombocytopathia, pseudohemophilia and thrombocytosis. Amer. J. Clin. Pathol. 26: 13–20, 1956.
702. ZUCKER, M. B. AND BORRELLI, J.: Viscous metamorphosis, clot retraction and other morphologic alterations of blood platelets. J. Appl. Physiol. 14: 575–580, 1959.
703. ZUCKER, M. B. AND BORRELLI, J.: Changes in platelet adenosine triphosphate concentration and phosphate distribution during viscous metamorphosis and clot retraction. *In* Blood Platelets, ed. by S. A. Johnson, R. W. Monto, J. W. Rebuck and R. C. Horn, Jr., pp. 383–391, Little, Brown and Co., Boston, 1961.
704. ZUCKER, M. B. AND BORRELLI, J.: Platelet clumping produced by connective tissue suspensions and by collagen. Proc. Soc. Exp. Biol. Med. 109: 779–787, 1962.
705. ZUCKER, M. B. AND JERUSHALMY, Z.: Studies on platelet shape and aggregation; effect of inhibitors on these and other platelet characteristics. *In* Physiology of Haemostasis and Thrombosis, ed. by S. A. Johnson and W. H. Seegers, pp. 249–265, Charles C Thomas, Springfield, Ill., 1967.
706. ZUCKER, M. B. AND LEVINE, R. U.: Microelectrophoresis of washed and unwashed human blood platelets, with additional studies of platelets from patients with von Willebrand's disease and thrombasthenia. Thromb. Diath. Haemorrh. 10: 1–8, 1963.
707. ZUCKER, M. B., PERT, J. H. AND HILGARTNER, M. W.: Platelet function in a patient with thrombasthenia. Blood 28: 524–534, 1966.
708. ZUCKER, M. B. AND PETERSON, J.: Serotonin, platelet factor 3 activity, and platelet-aggregating agent released by adenosine diphosphate. Blood 30: 556, 1967.
709. ZUCKER, M. B. AND PETERSON, J.: Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicyclic acid ingestion. Proc. Soc. Exp. Biol. Med. 127: 547–551, 1968.
709a. ZUCKER, M. B. AND VROMAN, L.: Platelet adhesion induced by fibrinogen adsorbed onto glass. Proc. Soc. Exp. Biol. Med. 131: 318–320, 1969.
710. ZUCKER-FRANKLIN, D. AND BLOOMBERG, N.: Microfibrils of blood platelets: their relationship to microtubules and the contractile protein. J. Clin. Invest. 48: 167–175, 1969.
710a. ZUCKER-FRANKLIN, D. AND NACHMAN, R. L.: Microtubules and microfilaments of blood platelets. J. Cell Biol. 35: 149a, 1967.
711. ZUCKER-FRANKLIN, D., NACHMAN, R. L. AND MARCUS, A. J.: Ultrastructure of thrombosthenin, the contractile protein of human blood platelets. Science 157: 945–946, 1967.
712. ZWEIFLER, A. J.: Impairment of platelet function and thrombus growth in reserpine-treated rabbits. J. Lab. Clin. Med. 70: 16–22, 1967.
713. ZWEIFLER, A. J. AND SANBAR, S. S.: Inhibition of platelet adhesiveness and aggregation by benzyl alcohol and phenol. Thromb. Diath. Haemorrh 21: 362–366, 1969.