Ultraviolet Light: A New Stimulus for the Induction of Platelet Aggregation

Abstract. *Exposure to ultraviolet light (253.7 nanometers) causes mammalian blood platelets to aggregate. Aggregation is markedly enhanced in the presence of extracellular fibrinogen and is followed by the gradual release of relatively small amounts of nucleotide and serotonin. Aggregation is inhibited by ethylenediamine tetraacetic acid or a combination of 2-deoxy-D-glucose and antimycin A. Adenosine, apyrase, and prostaglandin E_1 produced slight inhibition. The effect of exposure to ultraviolet light is cumulative and lasting. This agent may be used to study the process of platelet aggregation after the removal of the stimulus, by delaying the addition of fibrinogen until after cessation of irradiation. Thus ultraviolet light is the first agent known which may be used to study platelet aggregation in a period following its removal.*

Blood platelets are believed to be of fundamental importance in both hemostasis and thrombosis. When a blood vessel is damaged, platelets adhere to exposed subendothelial collagen, release adenosine diphosphate (ADP) from intracellular storage granules, and then aggregate under the influence of the released ADP. A number of other agents are known to initiate the aggregation of blood platelets. Most of these agents appear to act via a mechanism involving the release of ADP (*1*). The aggregation of platelets under the influence of ADP requires the presence of ionized calcium (Ca^{++}) (*2*), fibrinogen (*3*), and metabolic energy (*4*). However, there remains the fundamental problem of how these factors interact with platelets during the process of aggregation.

Suspensions of washed pig platelets (0.5×10^6 to 1.0×10^6 platelets per cubic millimeter) in a Tyrode's solution containing albumin, calcium, and magnesium were prepared from blood taken into ACD anticoagulant (*5*). The suspensions were always shown to aggregate on the addition of ADP ($10^{-5}M$ final) before the experiments were commenced. One-milliliter samples of suspension were exposed to ultraviolet light or collagen while being stirred in quartz cuvettes at 37°C. The rate and extent of subsequent aggregation of the platelets was followed at 37°C by means of a Payton aggregometer attached to a Bausch and Lomb VO-5 recorder. The central block was adapted such that the ultraviolet light source could be used to irradiate the platelet sample at an angle of 90° to the aggregometer light source. The source of ultraviolet light was a low-pressure mercury discharge lamp (*6*) filtered to remove wavelengths other than 253.7 nm.

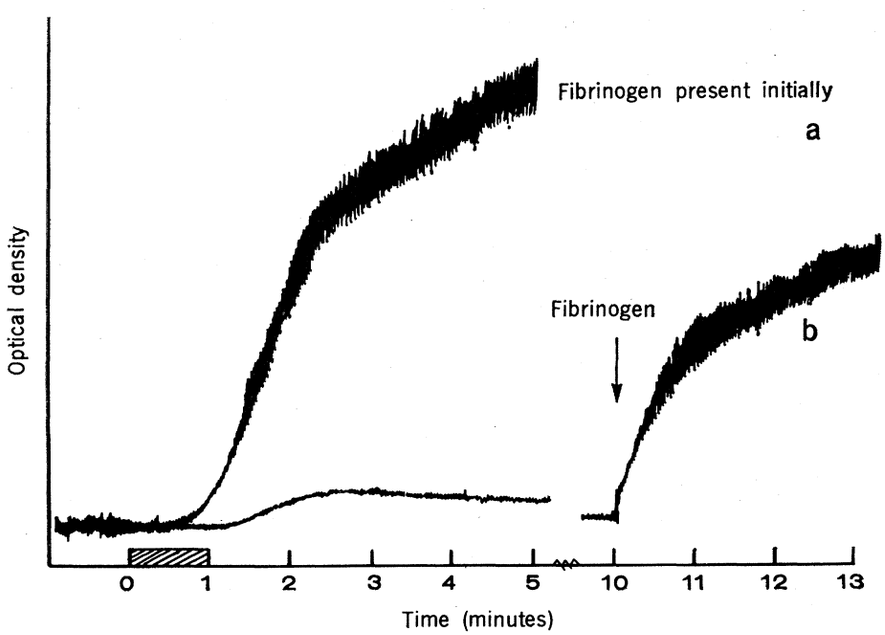

Fig. 1. Aggregation of washed platelets following 1-minute exposure to ultraviolet light. Platelets irradiated in the presence (*a*), and in the absence (*b*), of added fibrinogen. In the latter case fibrinogen was added 9 minutes after cessation of irradiation. Addition of fibrinogen to nonirradiated platelets did not cause platelet aggregation. Period of exposure to ultraviolet light is indicated by the hatched block.

Release of platelet constituents was assessed in platelet suspensions containing added fibrinogen (*7*) and labeled with tritiated serotonin (*8*). After equilibration to 37°C, aliquots of suspension were exposed to the stimuli for periods of 0 to 10 minutes. Release was then arrested by the addition of a one-tenth volume of isotonic 2 percent ethylenediamine tetraacetic acid (EDTA) in saline, and a platelet-free supernatant was prepared by 2 minutes' sedimentation in an Eppendorf centrifuge. Nucleotide release was assessed by measurement of the absorbance at 260 nm of an aliquot of the supernatant fraction after addition of an equal volume of 7 percent perchloric acid and further centrifugation. Serotonin release was assessed by counting the radioactivity of 0.1 ml of the platelet-free supernatant fraction in a liquid scintillation spectrometer. The supernatant fraction was also assayed for lactate dehydrogenase (LDH) activity as an index of the extent of cell lysis or release of cytoplasmic material (*9*).

After periods of irradiation of 1 to 4 minutes in the presence of added fibrinogen, aggregation of platelets was initiated and progressed even after irradiation with ultraviolet light was discontinued (Fig. 1, *a*). Aggregation was generally maximal within 4 minutes. When platelets were exposed to ultraviolet light for periods of 2 to 10 minutes there was a small and gradual release of both tritiated serotonin and nucleotide (Fig. 2). Assay of LDH activity indicated that negligible lysis occurred as a result of ultraviolet-induced aggregation, although up to 5 percent of LDH was liberated after aggregation for 10 minutes by collagen (Fig. 2).

Aggregation by ultraviolet light was completely inhibited by the addition of 5 m*M* EDTA. Addition of Ca^{++} was required for aggregation of platelets isolated in a calcium-free medium. Aggregation did not occur in the ab-

sence of extracellular fibrinogen unless prolonged periods of irradiation were used. Addition of the same concentration of albumin in place of fibrinogen did not facilitate aggregation. Calcium and fibrinogen could be added up to at least 10 minutes after platelet irradiation with aggregation not occurring until both these agents were present (Fig. 1, *b*). This result suggests that a change in the platelet may be induced by ultraviolet light in the absence of these two factors and that it endures for a considerable period of time.

The role of energy production in ultraviolet-induced aggregation was examined by incubation of platelet suspensions with metabolic inhibitors for a period of 10 minutes before irradiation. A combination of both the glycolytic inhibitor, 2-deoxy-D-glucose ($7 \times 10^{-3}M$), and the inhibitor of oxidative metabolism, antimycin A ($2.1 \times 10^{-5}M$), produced marked inhibition of aggregation induced by ultraviolet light, ADP, or collagen. The addition of either inhibitor alone produced only slight inhibition of aggregation by all three agents.

The release of platelet nucleotide and the requirement for ionized calcium, fibrinogen, and metabolic energy indicated that ultraviolet-induced aggregation might be acting by an ADP-mediated mechanism. This possibility was explored in two ways: by observing the effect of known inhibitors of ADP-induced aggregation and the effect of adding apyrase (Sigma Chemical Co.), an enzyme capable of rapidly degrading any ADP released into the suspending medium.

The effect of adding inhibitors either before or after irradiation was examined in order to ensure that they were not destroyed during irradiation. Prostaglandin E_1 (PGE_1; $4.4 \times 10^{-5}M$), one of the most potent inhibitors of ADP- and collagen-induced aggregation known, slightly inhibited ultraviolet light–induced aggregation (Fig. 3). Collagen-induced aggregation was completely inhibited by PGE_1. Adenosine at a final concentration of 10^{-4} or $10^{-5}M$ was found to inhibit aggregation, but to a significant extent only when added prior to irradiation. This may be explained by the fact that adenosine strongly absorbs ultraviolet light of the wavelength used in these experiments. If adenosine was added after irradiation of the platelet suspension, only a slight inhibition of aggregation was observed.

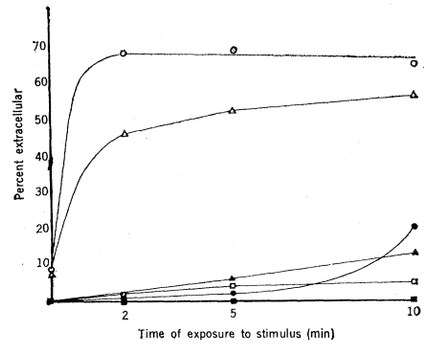

Fig. 2. Release of [H^3]serotonin (open and solid circles), adenine nucleotides (open and solid triangles), and LDH (open and solid squares) during exposure to collagen (open symbols) or ultraviolet light (solid symbols). Aggregation was initiated by both agents within 1 to 2 minutes (see Fig. 1, *a*, and Fig. 3A). The results represent mean values from three experiments, each assay being performed in duplicate.

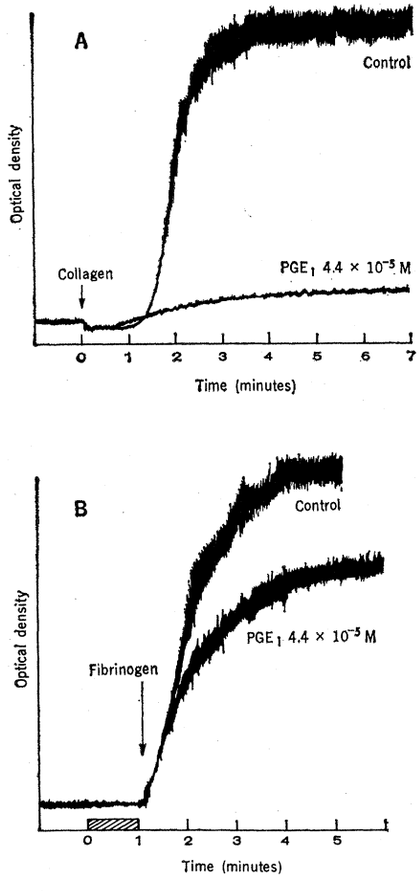

Fig. 3. The effect of PGE_1 on collagen-induced (A) and ultraviolet light–induced (B) platelet aggregation. The PGE_1 was incubated with the platelet suspension for 3 minutes prior to the addition of the stimulus. Final concentration of PGE_1 was $4.4 \times 10^{-5}M$. The period of exposure to ultraviolet light is indicated by the hatched block.

The addition of apyrase at a final concentration of 2 mg/ml also exerted a slight inhibitory effect of ultraviolet-induced aggregation, although this concentration was adequate to markedly inhibit aggregation induced by collagen or ADP ($10^{-6}M$).

The absence of appreciable inhibition of ultraviolet-induced aggregation by PGE_1, adenosine, or apyrase suggests that ultraviolet light–induced aggregation occurs by a mechanism in which released ADP plays either a minor or negligible role. Indeed, the release of nucleotide appeared to be confined to the period following maximal aggregation (Fig. 1, *a*, and Fig. 2), suggesting that this release may be secondary to the ultraviolet light–induced aggregation. The requirement for ionized calcium, fibrinogen, and metabolic energy indicates that the mechanism may involve processes in common with the mechanism of ADP-induced aggregation.

One of the primary effects of ultraviolet light on proteins is the cleavage of disulfide bonds (*10*) and the initiation of sulfhydryl-disulfide exchange (*11*). There is also evidence that membrane sulfhydryl groups are involved in ADP-induced platelet aggregation (*12*), and it may be that ultraviolet light is initiating platelet aggregation by a direct effect at these sites. Alternatively, peroxidation of platelet lipid may be involved, since ultraviolet light is also known to induce such a reaction in other tissues (*13*).

In conclusion, it would appear that ultraviolet irradiation may provide a useful technique for the further study of the mechanism of platelet aggregation. Of particular interest is the observation that ultraviolet light may be used to study platelet aggregation after removal of the stimulus by delaying the addition of fibrinogen until after cessation of irradiation. The platelet system may also provide a useful model to study the effect of ultraviolet light on other cells such as mast cells in which it is thought to induce the release of catecholamines from intracellular granules (*14*).

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7. A 6 percent solution of human fibrinogen in 0.9 percent saline was adsorbed twice for 10 minutes with a 1/10 volume of 25 percent (weight/volume) aluminum hydroxide suspension in 0.9 percent saline, with centrifugation after each adsorption. The resultant fibrinogen solution was dialyzed overnight against 0.9 percent saline and adjusted to a 5 percent solution. An 0.1-ml aliquot of this solution was added to each 0.9 ml of platelet suspension where noted.
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15. This study was supported by the Medical Research Council of Canada and the Canadian Heart Foundation. We thank Miss P. Traynor, M. Skunca, and D. W. Perry for valuable technical assistance.

1 February 1971; revised 18 March 1971 ■

Boron: Possible Role in Plant Metabolism

Abstract. *Leaves of boron-deficient oil palm showed a total absence of the leucoanthocyanins usually present, well before onset of pathological symptoms. The association of boron with flavonoid synthesis is consistent with the otherwise anomalous situation that this element is essential for higher plants but not for animals or lower plants.*

Despite considerable work carried out on boron nutrition of plants over the past 60 years, the metabolic role of this element remains obscure (*1*). Boron deficiency has been correlated with the accumulation of phenolic compounds in plants (*2*) and, since these have included caffeic and chlorogenic acid (*3*) (compounds biogenetically close to the monomer lignin), it has been suggested that boron is necessary for the lignin polymerization process. There is a recognized association between flavonoid content and lignin production. Our results show that the role of boron may be more closely associated with the biosynthesis of flavonoids.

For this study seedlings of oil palm (*Elaeis guineensis*) were grown in water culture with complete nutrient media (+B) and complete nutrient media minus boron (−B) to the seventh leaf stage when symptoms of "little leaf" appeared in the plants grown in the −B media. At this stage most plants were harvested, and leaves 1 (oldest) to 7 (youngest) were separately excised; another group of boron-deficient plants were supplied with boron for a further period of 3 weeks. Leaf samples from all groups were examined for polyphenols by the method of Bate-Smith (*4*). For each treatment there were four replications. The common flavonols and flavones were not detected, and of the common cinnamic acids only *p*-coumaric acid was identified, in traces throughout. In boron-deficient leaves, caffeic and chlorogenic acids did not accumulate, but an unidentified compound, fluorescing turquoise under ultraviolet light, accumulated. The relative amounts of leucocyanidins, as indicated by the amount of cyanidin in the acid hydrolyzates, are shown in Table 1. Particularly striking was the total absence of leucocyanidin in these leaves of boron-deficient plants where no morphological symptoms of boron deficiency were visible (and would not be expected for some months).

Table 1. Relative concentration of leucocyanidin* (detected as cyanidin) in leaves of seedlings growing in + B and − B media.

Leaf number	Concentration of leucocyanidin in leaf						
	1	2	3	4	5	6	7
+ B seedlings	3	3	3	3	3	3	3
− B seedlings	0	0	0	0	0	0	0
− B seedlings subsequently treated with B	1	1	2	2	2	2	2

* Scored visually as follows: 0, below the limit of detection; 1, trace of color; 2, distinct spot confined; and 3, spot tailing on the paper. In the first two sets of samples there was no variation between the four replicates; slight variation occurred in the last set of samples.

Additional evidence for correlation between the presence of boron and leucoanthocyanin synthesis has been obtained from field trials on the nutrition of the pine *Pinus merkusi*, grown in soil of initially low boron content. Needles from all plants contained leucodelphinidin, but the content was markedly increased in those plants grown in soil with boron added. Quercetin and myricetin were also present but there was no clear correlation between the presence of these compounds and the leucodelphinidin content.

"Bud rot" and "little leaf," reported to be two stages of the same disease of the oil palm (*5*), have been ascribed by some investigators to boron deficiency (*6*) and by others to pathogenic (*5*) or insect attack on primordia (*6, 7*). Both views may be correct, because increased susceptibility might occur as a secondary effect of the absence of leucoanthocyanins since such polyphenols are associated with the resistance to viral (*8*), fungal (*9*), and insect attack (*10*). Furthermore, this effect could occur at any stage in the life of the plant when available boron was exhausted, because this element is not translocated in the oil palm (*11*) and the deficiency would show up first in the apical region.

One of the long-standing mysteries associated with the role of boron is that very little or no boron is required by animals, microorganisms, or algae, but that boron is essential to higher plants. If boron is required only or mainly for flavonoid synthesis, the anomaly mentioned above is explained, since these compounds are found only in the higher plants.

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