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Topical Review

Activation Time of Blood Platelets*

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Summary. Blood platelets react rapidly in their hemostatic function. Determination of the reaction or activation time of individual platelets is difficult because it requires that physical and/or chemical effectors of activation are nonlimiting. Analysis of experimental conditions shows that the best estimate of mean activation time comes from *in vivo* measurements. Thus, the constancy of height-to-length ratio of a growing thrombus, and its change with flow rate, provide additional evidence for the activation time hypothesis.

The hemostatic effectiveness of platelets depends on their ability to react rapidly. This has been demonstrated experimentally in many different ways. Thus, when a small blood vessel is cut, platelets can be seen microscopically to adhere and aggregate on the margins within a few seconds (Roskam et al., 1959). In an intact blood vessel, ADP applied iontophoretically induces the formation of platelet thrombi or "white bodies" (Begent & Born, 1970). The rate at which platelets aggregate *in vivo* can be estimated by measuring on cine-film the growth rate of hemostatic plugs in cut vessels or of white bodies in normal vessels (Begent & Born, 1970). Both estimates have given us similar values for an accession rate of about 10^4 platelets/sec; and when the rate of growth diminishes after about 1 min, the hemostatic plug or thrombus is made up of the order of 10^6 platelets. These observations and the calculations provide no information about the time taken by individual platelets to react. In this paper we consider ways in which the reaction time of individual platelets may be estimated from various experimental approaches.

As circulating platelets normally show no tendency to adhere to intact vessel walls a change must have come over those that adhere and aggregate where a vessel is injured. This change from a nonadhesive to an adhesive condition may usefully be referred to as platelet *activation.* Presumably, activation consists of a sequence of physical and biochemical events analogous to the activation of muscle (Pringle, 1978). The sequence is still being worked out, but several similarities with muscle are already established. Thus, it is known that in platelets an early event is an increased influx of sodium unaccompanied by an equivalent amount of chloride (Feinberg et al., 1977) and that activation is associated with an increase of free calcium in platelets (Käser-Glanzmann et al., 1977; Le Breton, Sandler & Feinberg, 1976).

As activation is indicated by adhesiveness, the change must involve one or more constituents of the platelets' surface membrane. There is evidence that the essence is the exposure of surface receptors for fibrinogen, which has long been known to be an essential and specific plasma co-factor for platelet aggregation (Born & Cross, 1964; Cross, 1964; Born, 1968). The activation time of platelets may then be defined as the interval between the encounter of platelets with an activating agent, such as ADP, and their ability to react with plasma fibrinogen.

The rapidity with which circulating platelets become activated under conditions requiring their hemostatic function poses the problem of how such a short activation time can be determined. Although fibrinogen binding by platelets may well increase when they change shape, no method has yet been devised for measuring directly the time course for an increase in specific fibrinogen binding sites as it occurs. Therefore, present approaches depend on timing effects observed when platelets interact with each other or with another surface under conditions in which external physical or chemical constraints are made as nearly nonlimiting as possible.

* *Dedicated with admiration and affection to the memory of Walther Wilbrandt, a very dear friend.*

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Until recently, it seemed reasonable to suppose that the activation of platelets was accompanied by the gross morphological changes (MacMillan & Oliver, 1965) which are quantifiable morphometrically (Born, 1970; Morgenstern & Kho, 1977; Born et al., 1978) or photometrically (Michal & Born, 1971; Latimer, Born & Michal, 1977). A prominent component of this change is the extrusion of long thin spikes, and it seemed reasonable to assume that the ability of platelets, like that of other cells with fixed surface charges, to approach closely enough for adhesion is facilitated by the extrusion of pseudopodia (Bangham & Pethica, 1960). However, recent electron-microscopical observations indicate that platelets can adhere to vessel walls and to each other without any obvious deviations from their normal morphology (Born, 1977). Therefore, activation as defined above is likely to precede the gross morphological changes which at 37° have a time constant of the order of 1 sec (Born, 1970).

The gross morphological changes of platelets associated with light-scattering effects can be timed and quantified in an aggregometer (Michal & Born, 1971; Latimer et al., 1977). In this apparatus it is possible to inject an activating agent into platelet-rich plasma very rapidly from spring-loaded syringes. We have tried to establish the time required for such an agent to be uniformly distributed in the plasma by injecting instead the dye Evans Blue (T 1824) and following the effect on light transmission. The time was also about 1 sec. This is short enough to be no handicap to the determination of the time constant of aggregation, but limits the resolution of effects occurring in shorter times.

The experimental determination of platelet activation time presents problems which have not yet been solved. Open questions are, how rapidly platelets are individually activated by agents such as ADP; and whether the activation time is constant under varying conditions or may be altered by them. It might have been expected that these questions would be easier to answer *in vitro* than *in vivo*, but that has not yet been so. *In vitro*, one possibility is to observe how rapidly single platelets change in a property that can be quantified microscopically when they abruptly encounter an activating agent in a flow system. We are not aware that such an approach has yielded a value for the activation time. Another approach is the photometric determination of an overall time constant for the shape changes which precede aggregation. However, in view of what has already been said, this is likely to indicate no more than an upper limit. This applies even more to the timing of aggregation because that depends on successful, i.e., adhesive collisions in shear flow which itself gives rise to additional time constants: one for the dispersion of the activating agent and others associated with the collision process.

Collisions in shear flows have two time constants. The first is the interval between collisions for each suspended particle. In the aggregometer cuvette, the magnetically stirred bar maintains a shear flow in the plasma which increases the frequency of platelet-to-platelet collisions well above that due to Brownian motion. A proportion of collisions between activated platelets results in their mutual adhesion, whereby the light transmission through the plasma is increased. When the stirring rate is high enough, i.e., 1,000 rpm or more, to be nonlimiting with respect to the optical effect associated with aggregation, the average time between collisions can be estimated as follows. The total collision frequency, b, i.e., the number of collisions between all the platelets per unit time per unit volume, is given by

$$b=\tfrac{4}{3}\Gamma(2R_i)^3 c_i^2$$

where Γ is the shear rate, R_i is the platelet radius and c_i is the concentration of single platelets. If we take as typical values $\Gamma=30\ \mathrm{sec}^{-1}$ for the aggregometer cuvette, $R_i=1\ \mu\mathrm{m}$ and $c_i=200{,}000/\mathrm{mm}^3$ and then calculate the collision frequency per plasma volume occupied by each platelet, we find that the typical interval between successive collisions for individual platelets on average is about 1.5 sec. The blebs and spikes that are extruded from the surface of platelets following activation have the effect of increasing the collison frequency, other factors remaining the same, by increasing the effective size R_i of each platelet without a significant change in its volume (Born, 1970).

The second time constant is the duration of contact during collision. The shear Reynolds Number $\mathrm{Re}_s=\Gamma a^2/\nu$, where a is the particle radius and ν is the kinematic viscosity. If Re_s is small enough, a spherical particle rotates with an angular velocity of one-half of the shear rate (Poe & Acrivos, 1975). A pair of particles maintains approximately the same rate of rotation during collision. Glancing collisions are comparatively brief and the longest collision without adhesion corresponds to a rotational span close to π, i.e., one-half revolution, which occupies the time of the reciprocal of the shear rate, i.e., Γ^{-1}. If it takes a finite time for two particles to adhere on collision, then the proportion of collisions which result in mutual adhesion, i.e., the collision efficiency, decreases as the shear rate is raised such that Γ^{-1} approaches or exceeds the adhesion delay time. A decrease in collision efficiency with increasing shear rates can be caused also in another way, i.e., by the forces which tend to separate colliding particles and to disperse their aggregates. These forces increase with aggregate size, viscosity of the suspending fluid, and

shear rate. To distinguish between these mechanisms experimentally, it is necessary to make measurements of collision efficiency under conditions of constant shear rate but different viscosities. Such experiments do not seem to have been done with platelets.

The closest approach so far towards determining platelet activation time has made use of techniques for inducing the hemostatic process *in vivo*. In these experiments, adhering thrombi of platelets were produced in vessels of the microcirculation of anesthetized animals either by the microiontophoretic application of ADP or by microinjury with laser radiation (Arfors, Cockburn & Gross, 1976; Begent & Born, 1970). The common feature of these techniques was the introduction of activating agents in the immediate vicinity of platelets without affecting their distribution or flow significantly. The thrombi grew exponentially with time, the growth time constant depending *inter alia* on the velocity of blood flow in the vessels (Begent & Born, 1970; Arfors, et al., 1976).

Such a thrombus grows as a mound shaped like half a grain, with the long axis parallel to that of the vessel. While small, the thrombus lies in a region of almost constant fluid shear and has little effect on the flow of blood. Initially, too, there are spaces between the platelets so that the pressure gradient may be assumed to cause a flow of plasma through the thrombus. If these extracellular spaces made up a major proportion of the thrombus volume, its growth could be accounted for by filtration of arriving platelets and their specific mutual adhesion (Fig. 1 *A*). This is not what happens, for the streamlines can be seen microscopically to be deflected by the thrombus. Therefore, the part of the flow bringing platelets into contact with the thrombus is limited upstream to a tunnel smaller in cross-section but proportional to that of the thrombus (Fig. 1 *B*). Platelets which make contact with the thrombus have a transit time which depends on their position in the flow during approach, with those furthest from the vessel wall generally having the shortest transit time. If activation of a platelet is initiated just before it makes contact, then the surface of the thrombus is available for adhesion throughout the time of transit; and if this time is longer than the activation time, the platelet has a high probability of adhering.

There remains a question as to whether the platelet membrane sites which determine adhesion are so few or distributed so asymmetrically that adhesion has to await not only activation but also rotation of the platelet into a favorable position. Any diminution in adhesion probability due to this would apply only to very small thrombi.

The chemical condition for platelet activation is the concentration of activating agent(s) within and immediately around a growing thrombus. Thrombi

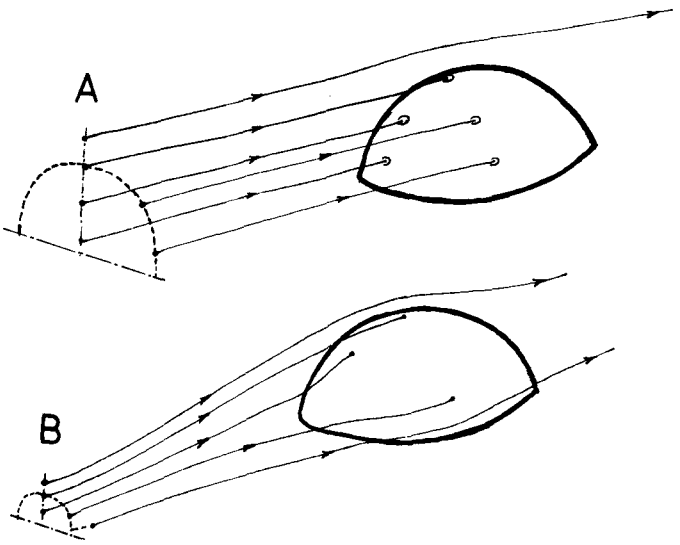

Fig. 1. Streamlines of platelets approaching a mural platelet thrombus under two different assumed conditions. (*A*): The thrombus is sufficiently permeable to plasma for the streamlines to be straight, so that platelets adhere to the thrombus through filtration. (*B*): The thrombus is sufficiently impermeable to deflect the streamlines, so that platelets brought into contact with the thrombus arise from a smaller cross-section upstream than in *A* (dashed lines)

can grow around a highly localized source of activating agent in the vessel wall (Begent & Born, 1970; Richardson, 1973). Immediately around such a source the plasma contains the agent at a concentration which is effective for activation. A growing thrombus may continue to be surrounded by a thin layer of plasma in which the concentration remains effective. On the other hand, activation may be initiated through actual contact of arriving platelets with the thrombus. Both of these possibilities are taken into account in a theory proposed for platelet thrombogenesis (Richardson, 1973) which yielded an activation time of 0.1–0.2 sec. The theory depends on two assumptions: first, that the ratio of height to length of a growing thrombus is constant; and second, that platelets have a characteristic activation time. Evidence for the first assumption is shown in Fig. 2, based on results obtained with the iontophoretic technique (Begent, 1971). For two different blood flow velocities, the height of thrombi increased in proportion to their length. For a given length, however, the height was less at the greater blood flow velocity. This supports the second assumption of the theory. The transit time is not uniform for all platelets passing a thrombus, being shortest for those furthest from the vessel wall. As the shear rate increases with increasing blood flow velocity, the platelets more able to escape capture are those passing over the top of the thrombus rather than those passing its sides. Where the transit time is less than the activation time,

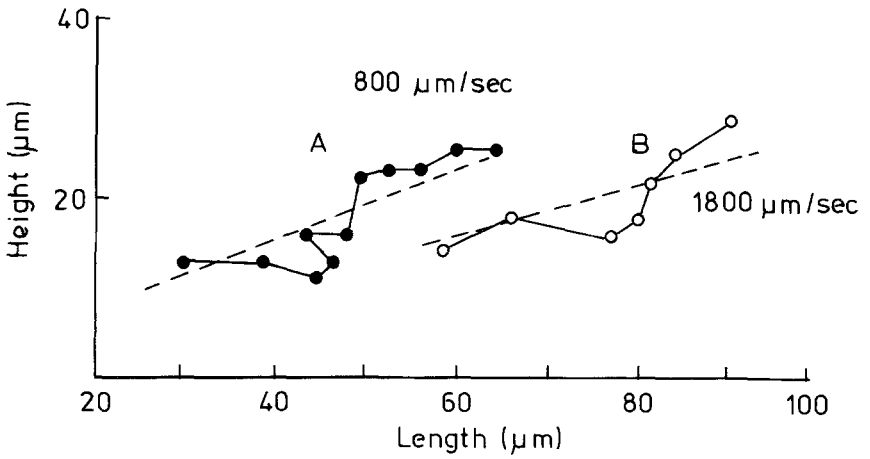

Fig. 2. Relation between height and length of platelet thrombi growing in normal venules as a result of the iontophoretic application of ADP (Begent, 1971) for two different mean blood flow velocities (data taken from notes of Dr. Begent for experiments and recorded in thesis)

platelets are unable to adhere at all. An expected consequence is that the higher the blood flow velocity the smaller the height of a thrombus compared with its length, in accordance with experimental results (Fig. 2). It might be countered that platelets passing over the top of a growing thrombus would be prevented from adhering by the higher shear stresses there. However, the shear stress at the top of a small thrombus is not much greater than at its base on the vessel wall. Furthermore, platelet thrombi can form at even higher flow rates (Begent & Born, 1970).

Our explanation is also consistent with the fact (Poole, Sanders & Florey, 1958) that in a large vessel such as the aorta, in which the blood flow velocity is very high, platelets tend to form monolayers on damaged areas of wall without aggregating into mural thrombi.

These considerations on platelet interaction times, with the additional information on height-to-length ratios of thrombi growing under different blood flow velocities, support the conclusion that there is a platelet activation time of the order of 0.1 sec.

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