Editorial

The Mechanism and Prevention of Platelet Adhesion and Aggregation Considered in Relation to Arterial Thrombosis

By J. R. O'Brien

There is now much evidence to suggest that the "white head" of an acute arterial thrombus consists primarily if not exclusively of a mass of platelets, while the "red tail" consists mainly of red cells trapped in fibrin. Thus it is conceivable that an inhibitor of fibrin formation in vivo could prevent the clinical effects of thrombus formation, but current anticoagulant treatment is by no means completely effective in this respect. However, if one could prevent the platelet mass from forming, it is possible that thrombosis would not occur. Accordingly, some observations and ideas relevant to the formation and prevention of the "white head" of a thrombus will be discussed.

Since platelet deposition occurs locally and not diffusely, there must be a local precipitating cause which is probably an area of damage to the vessel endothelium. Turbulence may play a role and abnormally "sticky" platelets, if such exist, could enhance the process. The initial platelet mass or white head of the thrombus is thus formed in two steps. Firstly, platelets stick to the damaged or abnormal surface, and then more platelets adhere to those already stuck, eventually forming an obstructing mass. These initial steps will be considered separately. Any subsequent fibrin formation is not discussed.

Considering first the process of platelet adhesion to the vessel wall, the nature of the damage to the endothelium is not known; but we do know that platelets stick to damaged endothelium, to other damaged cells, to collagen and to glass in one or two seconds. Only platelets are able to adhere so rapidly and are probably specifically adapted for this purpose. (Polymorphonuclear leukocytes also stick, but much more slowly.) Platelets contain almost as much ATP as a muscle cell, and it is possible that a series of enzyme systems may control the "stickiness" of their surface. If platelets are stored in plasma they gradually lose their property of stickiness, which may mean that a high energy state has become dissipated. In fact, on storage, platelets lose their ability to aggregate (a phenomenon related to adhesion) first in response to tri-alkyl tins, later to thrombin and lastly to ADP, suggesting a sequential decay of a series of enzyme systems. Even at 0°C. platelets will stick to foreign surfaces. Calcium (and possibly magnesium) is essential for adhesion; ADP encourages such adhesion, whereas AMP and ATP are inhibitory. A number of local anesthetics such as cocaine, anti-histamines, and anti-malarial agents such as quinine, are inhibitory and may be called anti-adhesive drugs. It may be relevant that...
these pharmacologically active drugs have effects on mitochondria\(^8\) and may be incorporated into biological membranes.\(^9\)

It is not known what forces bind a platelet to a foreign surface, and they may differ in detail for different surfaces: for example, adhesion to collagen probably has some special features;\(^{34}\) however, these forces are likely to be similar and are probably electrostatic. The platelets carry a negative charge as do the foreign surfaces, and these two surfaces may be held together by the two positive charges of calcium acting as a cationic ligand or binding molecule.\(^2\) However red cells also carry a negative charge and they do not stick, which emphasizes our lack of understanding of the process of adhesion. Although the proteins of the clotting mechanism almost certainly play no part in this rapid process, it is possible that other plasma proteins may. Certainly the lipophilic surfaces of yeasts have to be coated (opsinized) with serum before washed platelets will stick to them.\(^8\)

The second phase in the formation of the "white head" of the thrombus consists of the adhesion of more platelets to those already stuck. As platelets in the blood stream do not normally stick to each other, it is probable that the surface or the environment of a platelet which has stuck must change rapidly, because within a few seconds of the platelets sticking, more platelets stick to those already stuck, in preference to adhering directly to the endothelium. A few important studies\(^{10,14}\) of this situation in vivo are in broad agreement with the more extensive work on the in vitro adhesion of one platelet to another in plasma which may be called platelet aggregation. The forces holding two platelets together are unknown. They are likely to be similar to those holding platelets to a foreign surface but there are important differences; for example, aggregation does not occur at 0°C whereas platelets adhere to glass at this temperature. A number of ways of producing aggregation are now known. ADP at the high dilution of \(M \times 10^{-7}\) or 100,000 molecules per platelet causes immediate aggregation.\(^5,25\) Of the biologically active amines, adrenaline is immediately effective in high dilution;\(^21,28\) noradrenaline is equally effective if used ten times stronger; isoprenaline is inactive. 5-Hydroxytryptamine (5-HT or serotonin) produces immediate but slight aggregation only. The effect of the catecholamines is inhibited specifically and immediately by the adrenaline \(\alpha\) blocking agent phentolamine, and 5-HT is specifically inhibited by the anti-serotonin Deseryl.\(^{28}\) These observations suggest that specific receptor sites are involved in these phenomena. Thrombin (and it will be noted that this is the first time the coagulation mechanism has been implicated) causes aggregation after a delay of 5–10 seconds. If collagen is added to platelet-rich plasma some platelets stick immediately to the fibres and after a delay of about 60 seconds all the platelets aggregate.\(^{15}\) The tri-alkyl tins also cause aggregation but only after a delay of some minutes.\(^{27}\) It is evident that substances producing aggregation immediately may act through different pathways from those that are effective only after a delay.

Thrombin has been shown to convert ATP in platelets into ADP which is then released, and further degradation may subsequently occur.\(^{11,19}\)
Adrenaline and tri-ethyl tin added to platelet-rich plasma also cause an aggregating activity to appear in the plasma and this activity is probably due to ADP released from the platelets.\textsuperscript{27,28} (Tri-ethyl tin is known to stimulate ATP-ase in liver mitochondria.\textsuperscript{1}) Aggregation induced by thrombin, adrenaline and tri-ethyl tin is inhibited by adenosine and AMP and enhanced by ATP. Thus it might be argued that aggregation, however produced, is due to the liberation or the presence of ADP. However, under special conditions platelets will aggregate on the addition of adrenaline but will not aggregate on the addition of ADP.\textsuperscript{29} This observation suggests that the release of ADP from the platelets by adrenaline may not be the cause of adrenaline-induced aggregation. Of the three compounds causing immediate aggregation, namely ADP, adrenaline and 5-HT, the latter two are known to be actively transported into the platelet against a concentration gradient,\textsuperscript{7,12} which presumably involves the expenditure of energy. If adrenaline or 5-HT comes into contact with platelets it is likely that uptake involving a transport system will start immediately, and the energy is probably derived from the conversion of ATP to ADP. Platelets are known to inactivate added ADP, and this is likely to involve an immediate transfer of high energy adenosine phosphate in the cell membrane. It is possible that collagen, thrombin and tri-ethyl tin after a delay also stimulate ATP-ase and the breakdown of ATP, since ADP is liberated. Aggregation, induced by all the six compounds mentioned, is inhibited by the addition of AMP or adenosine\textsuperscript{6} and it is remarkable that ATP inhibits ADP-induced aggregation but enhances aggregation induced by the other five.\textsuperscript{28e} It has been suggested that Benadryl and similar anti-adhesive drugs interfere with a phospho-protein kinase in mitochondria that couples one phosphate group from ATP on to protein to produce ADP and phosphoprotein.\textsuperscript{17} A similar process might occur in platelets. Many details are still obscure but it seems probable that a surface change permitting aggregation comes about when ADP is in an appropriate compartment, or when ATP is degraded to ADP. These suggested mechanisms lack detailed biochemical proof, and indeed it is not known whether all the aggregating compounds result in the same kind of stickiness. Viscous metamorphosis and the tight packing of aggregated platelets that occurs in the presence of thrombin, and the release of granules and substances other than ADP\textsuperscript{11} will not be discussed, although clearly these processes may be of considerable physiological importance.

How far these observations in vitro are related to events in vivo is not clear. Serious damage to a vessel wall is likely to expose collagen to which platelets have been shown to stick in vivo and presumably ADP is subsequently released thus favoring platelet aggregation and the formation of the platelet plug. Damaged endothelial cells may attract platelets and they may also initiate the clotting process. Although fibrin formation does not usually occur in the “white head,” thrombin might be formed locally in sufficient concentration to alter the platelet surface. Hemostasis in cut small blood vessels is achieved by platelet adhesion and aggregation. Adrenaline shortens the bleeding time\textsuperscript{16} and the anti-adrenaline drug phentolamine prolongs it:\textsuperscript{28} thus,
adrenaline released locally or centrally might play a part in thrombus formation. Damaged cells may liberate ADP. If liberated ADP is proved to play a part in these events in vivo, there are a number of systems for inactivating it. In vitro plasma, platelets and white cells can all inactivate ADP, and they may also inactivate it in vivo, thus preventing excessive thrombus formation or generalized platelet aggregation.

It is going to be difficult to determine whether one or several of these possible mechanisms are involved in thrombus formation. Nevertheless, if the observations and processes discussed above are related to the physiological events, it is likely that treatment which prevents platelet adhesion and aggregation could prevent the formation of a thrombus. Such treatment might cause bleeding, but could possibly be adjusted to avoid it. How could this be achieved? Possibly the damaged endothelial surface could be rendered benign and non-attractive to platelets by the use of cocaine or the other anti-adhesive drugs. There are claims that nialamide in some special circumstances is also effective, but these have not been confirmed. Platelets can be rendered non-adhesive to surfaces and to each other by adding so high a concentration of cocaine or the other anti-adhesive drugs that the drug would be lethal to the intact animal. Adenosine or AMP injected into the bloodstream would be rapidly removed. Therapeutically tolerable doses of phentolamine had no demonstrable effect on platelets or on the bleeding time. Macromolecules such as dextran and low molecular weight polybrene also interfere with platelet adhesion and aggregation. Monoiodo-acetate, after half an hour, also renders platelets non-adhesive. Clearly, none of these methods is clinically practicable. Any attempt to devise an effective blocking agent is profoundly handicapped by the fact that it is not known what unique features of the platelet surface are responsible for its "stickiness" to surfaces or in what way ADP or the other possible aggregating compounds alter the platelet surface to permit platelet-to-platelet adhesion. Furthermore, the mechanisms thought to be involved in platelet aggregation are concerned in many other vital processes. Evidently it will be difficult to find a therapeutically acceptable inhibitor but it might be very worthwhile.

REFERENCES


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