CLASSICAL PERSPECTIVES

Light on platelets

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It is gratifying that the editors of the *Journal of Physiology* wish to make available on-line a paper published by the late Michael Cross and myself more than 40 years ago (Born & Cross, 1963). The paper is based on the first method – optical aggregometry – permitting reproducible and rapid measurements of platelet aggregation *in vitro*, and reports some basic results. The method had been presented to the Physiological Society and published in *Nature* the year before (Born, 1962). The introduction of the simple, rather obvious method presumably accounts for the popularity of these papers, as with other papers introducing novel techniques (e.g. Lowry's for protein determination). The publications initiated a long series of papers reporting extensive new information about platelets and their doings.

At the time the *Journal of Physiol*ogy paper appeared the only known physiological function of platelets was haemostasis, in which individually circulating platelets rapidly adhere and aggregate in injured blood vessels, the resulting plug slowing and arresting the bleeding. The aggregation process is induced by changes in the normal haemodynamic and/or biochemical environment of circulating platelets. Although intravascular aggregation of platelets was recognized when they were discovered by Bizzozero in 1882, elucidation of the process made little progress until it could be investigated *ex vivo* by optical aggregometry. The urgency increased when it became clear that platelet aggregation in arterial, e.g. coronary, thrombosis is induced in essentially the same way as a consequence of haemorrhage through fissures in atheromatous plaques (Davies & Thomas, 1983; Richardson *et al.* 1989).

My excellent coworker and friend Michael Cross died in an aeroplane crash in the Great Smoky Mountains in Tennessee on 9th July 1964.

I first became properly aware of platelets as an Army Medical Officer posted close to atom-bombed Hiroshima in defeated Japan in 1946, where many people were still dying from thrombocytopenic haemhorrages induced by radiation. My interest in the mechanism of platelet aggregation was awakened through the coming together of two observations. One was the exceptionally large concentrations of ATP in platelets, discovered while providing evidence that 5-hydroxytryptamine or serotonin, the vasoconstrictor factor in platelets, is bound ionically to ATP (Born *et al.* 1956). The other was the platelet-aggregating effect of ADP (Gaarder *et al.* 1961). I suggested that these findings are connected through the formation of ADP from ATP released from platelets and from other cells involved in vascular injury (Born, 1962). This turned out to be an oversimplification as far as the platelets themselves are concerned, which release ADP from both the same and a different pool from that containing most of the ATP. Nevertheless, the hypothesis that ADP contributes significantly to haemostatic and thrombotic platelet aggregation has been supported experimentally (Begent *et al.* 1982; Begent & Born, 1983; McClure *et al.* 1988) and by clinical trials (CAPRIE Steering Committee, 1996).

The idea of optical aggregometry occurred to me from making so-called turbidimetric measurements of ribonuclease activity in *Streptomyces* culture filtrates for the Oxford D.Phil. In this method, a light beam is passed through platelet-rich, anticoagulated plasma, the optical density of which decreases, that is, light transmission increases, when the platelets are induced to aggregate. The only interpretable parameter is the initial rate of aggregation, which is proportional to the logarithm of the added aggregating agent, in that paper ADP. This method permitted *ex vivo/in vitro* quantification and analysis of determinants and inhibitors of platelet aggregation. This work has been extensively reviewed (see Born, 2002; Born & Patrono, 2005; Reynolds & Tansey (eds) 2005 for references). Aggregate formation was related to the optical changes and accounted for by classical light-scattering theory. Essential cofactors, viz. calcium and fibrinogen, were discovered; and the suggestion that fibrinogen forms 'bridges' linking aggregating platelets was later confirmed by electronmicroscopy and by the characterization of fibrinogen receptors. The first aggregation inhibitors were discovered, viz. ATP and adenosine, looked at initially because of their chemical relationship to pro-aggregatory ADP. A second phase of aggregation was discovered (by Macmillan & Oliver, 1965) and shown to be caused by the release of aggregating agents, viz. ADP and thromboxane A2, from the platelets themselves. The discovery (by Weiss & Aledort, 1967; Evans *et al.* 1968) that this release reaction is inhibited by Aspirin initiated its highly successful 'second life' for the prevention of arterial thromboses responsible for myocardial infarction and stroke.

The main avenue of platelet research was followed with more than 40 co-workers for over 20 years. During that time several intriguing side roads presented themselves for investigation. Here are two of them. First, the non-adherence of platelets in normal vessels was shown to be due to electrostatic repulsion (rather than to prostacyclin). This led to the finding that the surface of vascular endothelium has a much greater density of free negative charges (from sialoproteins and proteoglycans) than the surfaces of other cells including erythrocytes; and that electrostatic repulsion contributes significantly to the ability of red cells to flow through capillaries (Born & Palinksi, 1989). Secondly, the inhibitory action of adenosine on platelet aggregation is potentiated by dipyridamole, which inhibits adenosine uptake by platelets. The trypanosome parasite responsible for African sleeping sickness is incapable of synthesizing nucleotides *de novo* but only from nucleosides like adenosine taken up from surrounding tissue fluids. This brought up the idea of developing trypanocidal drugs to act as inhibitors of adenosine uptake by these organisms. The demonstration that dipyridamole has a weak effect of this kind on *Trypanosoma brucei* (James & Born, 1980) initiated extensive research on purine acquisition by these organisms, with the discovery of many transporters with different substrate specificities. Differences between mammalian and trypanosome transporters are being exploited to find agents blocking

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the latter but not the former (for review see el Kouni, 2003).

The 1963 *Journal of Physiol*ogy paper ended as follows: 'There are reasons for believing that red cells and platelets end their existence in the spleen. It is therefore possible that ADP released from red cells disintegrating in the spleen causes the circulating platelets to aggregate there and so to become trapped and to disintegrate in their turn.' This suggestion for a physiological function of aggregation remains possible. As it has turned out, a much more important suggestion was made in the 1962 *Nature* paper: 'If it can be shown that ADP takes part in the aggregation of platelets in blood vessels it is conceivable that AMP or some other substance could be used to inhibit or reverse platelet aggregation in thrombosis.' Indeed, inhibition of aggregation was the most important discovery made with optical aggregometry because it established the therapeutic possibility of preventing arterial thrombosis by antiplatelet drugs and initiated the era of their beneficial use against myocardial infarction and stroke.

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Original classic paper

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