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THE AGGREGATION OF BLOOD PLATELETS

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In the circulating blood the platelets are normally carried along separately from each other and there is no evidence that they adhere to normal vascular endothelium. When the wall of a blood vessel is injured platelets adhere to it immediately (Hugues, 1953) and to each other to form aggregates on the damaged intima (Bizzozero, 1882). When the vessel wall is broken aggregates of platelets tend to seal the opening and so help to arrest bleeding (Fulton, Akers & Lutz, 1953); such aggregates have been called 'haemostatic plugs' (Roskam, 1954). When diseases, for example, atherosclerosis, damage the intima platelets again adhere to it and to each other. The aggregates so formed are called 'platelet or white thrombi', and they may increase in size until they block the flow of blood. The aggregation of platelets is, therefore, one of the immediate causes of thrombosis. Why damage to the intima should cause platelets to adhere and to aggregate is still unknown. This paper describes a method by which the aggregation of platelets may be followed quantitatively, and results obtained with the method are described; some of these have been briefly reported earlier (Born, 1962*a*, *b*; Born & Cross 1963).

METHODS

The platelets were human or porcine.

Human blood was obtained from apparently healthy volunteers, 1–2 hr after breakfast. A stainless-steel needle of 1 mm diameter, attached to 10 cm of polythene tubing, was inserted into the antecubital vein. About 45 ml. blood was allowed to flow into each of two centrifuge tubes made of cellulose acetate and each containing 0·8 ml. 19 % sodium citrate with which the blood was mixed immediately.

Pig blood was obtained from a slaughter-house. Each pig was stunned electrically and hung with its head down. The throat was cut and the freely falling blood was collected in a plastic bucket containing enough 19 % sodium citrate to give a concentration, when mixed with the blood, of 0·3 g/100 ml. The blood was taken to the laboratory within 1 hr and poured into centrifuge tubes.

The tubes were centrifuged at 500 *g* for 20 min at room temperature of about 20° C. The upper layer, which consisted of about 17 ml. per tube of plasma rich in platelets, was transferred with a siliconed Pasteur pipette into a measuring cylinder made of Perspex. The concentration of platelets in the plasma was determined by counting them in duplicate (Born & Gillson, 1959); each millilitre usually contained 2–8 $\times 10^8$ platelets. Since cooling

increases the tendency of platelets to aggregate (Zucker, 1961), the blood and the platelet-rich plasma were not cooled below room temperature.

Optical density. Changes in the optical density of platelet-rich plasma were determined, again at room temperature, as follows: A sample of 3 ml. was pipetted into a transparent tube made of cellulose acetate as supplied for use in head SW 39 of the Spinco model L ultracentrifuge. The tube was inserted into a Unicam SP 400 absorptiometer. Light at a wave-length of 600 mμ was passed through the tube. The dark current was set at infinity and the optical density of distilled water at zero. In the bottom of the tube was a small iron rod, covered in polythene, which was rotated magnetically and so stirred the plasma. When readings were taken, stirring was stopped and a keeper was applied to the magnet to prevent its field from affecting the galvanometer. All additions were made by pipetting into the plasma while it was being stirred.

The reproducibility of the method was tested by bringing about aggregation of the platelets in several samples of the same plasma. When this was done soon after obtaining the plasma, corresponding optical density readings differed by less than 5%.

Drugs. The following substances were used: cytidine, guanosine, adenosine, adenine and D(−)ribose, supplied by British Drug Houses Ltd.; adenosine monophosphate, adenosine diphosphate, adenosine triphosphate and inosine monophosphate, by Sigma Chemical Co.; purine riboside and desoxyadenosine by the California Corporation for Biochemical Research; and inosine by L. Light and Co. Ltd.

RESULTS

Plasma freshly obtained in the way we have described was turbid, and the turbidity was mostly due to the platelets. Occasionally part of the turbidity was due to fat. When platelet-rich plasma was centrifuged at 10,000 *g* for 10–15 min the platelets sedimented, whereas fat remained suspended. In the course of a day the optical density of such platelet-free plasma sometimes increased slowly; it was, therefore, determined as soon as possible. In the blood of different individuals the optical density of platelet-rich plasma was two to seven times greater than that of platelet-free plasma.

Relation between platelet concentration and optical density

The optical density of a sample of platelet-rich human plasma was determined; another sample of the same plasma was centrifuged at 10,000 *g* for 20 min and the optical density of the platelet-free plasma was also determined. The remainder of the platelet-rich plasma was then centrifuged at 500 *g* for four successive periods of 5 min. After each period the optical density was measured and the concentration of platelets was determined by direct counting. Figure 1 shows that the optical density of the platelet-rich plasma, less that of the platelet-free plasma, was proportional to the concentration of platelets. Figure 2 shows that in different individuals the optical density of platelet-rich plasma exceeded that of platelet-free plasma by an amount proportional to the concentration of platelets.

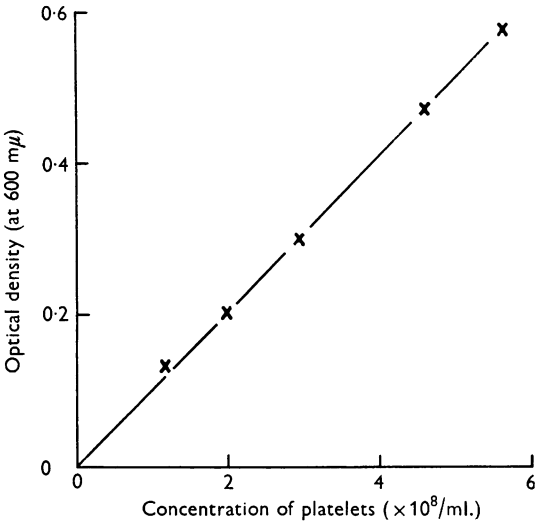

Fig. 1. Proportionality between concentration of platelets in fresh human plasma and its optical density, from which the optical density (0·115) of the platelet-free plasma has been subtracted.

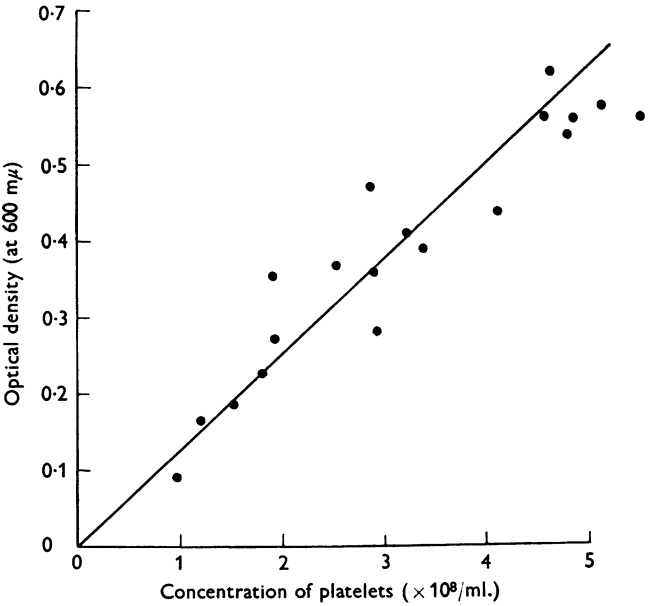

Fig. 2. Proportionality between concentrations of platelets in different people's plasmas and their optical densities; the optical densities of the platelet-free plasmas have been subtracted.

Effect of the rate of stirring on the optical density and on the concentration of platelets

When platelet-rich human plasma was stirred at up to 1000 rev/min, there were sometimes small decreases in the optical density and in the concentration of platelets; these decreases usually occurred during the first half to one hour (Born, 1962*b*). The results suggested, first, that a small proportion of the platelets was less resistant to the effects of vigorous stirring than the rest; and secondly, that at most only a very small proportion of the platelets stuck to the wall of the tube or to the stirrer.

Aggregation of platelets by added calcium

Some of the early experiments were done with pig plasma; in these, platelets were induced to aggregate by the addition of calcium chloride. Figure 3 shows such an experiment. When calcium was added to citrated

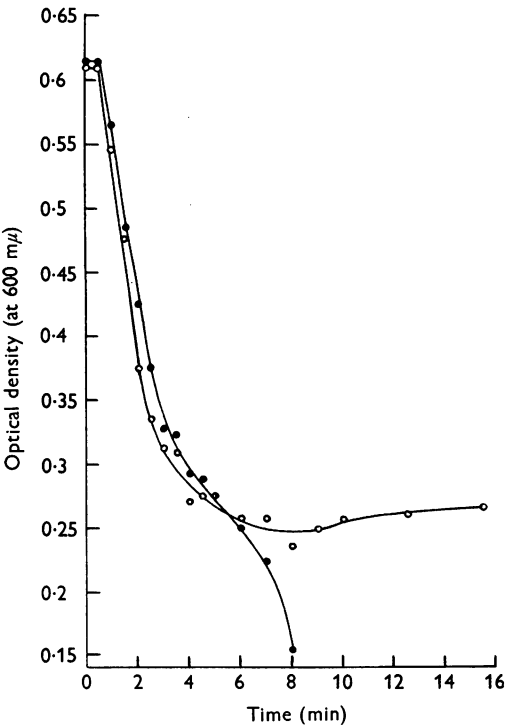

Fig. 3. Effect of adding calcium chloride ($1{\cdot}3 \times 10^{-2}$ M) on the optical density of pig's plasma containing $3{\cdot}6 \times 10^{8}$ platelets/ml. The plasma had been obtained from a pig killed the day before and had been kept overnight at room temperature. Both samples of plasma contained sodium citrate (1×10^{-2} M); one sample contained heparin at 1 u./ml. (●—●) and the other at 67 u./ml. (○—○).

plasma containing added heparin at a low concentration (1 u./ml.), within 0·5 min the optical density began to decrease and continued to fall sharply for about 5 min. Then the rate of decrease slowed; after a further 2 min the optical density fell again as the plasma clotted (curve *A*). With plasma containing added heparin at a higher concentration (67 u./ml.) when calcium was added the optical density fell just as rapidly and as far in the first 5 min as before; thereafter, however, it remained constant for 10 min and the plasma did not clot. The optical density began to decrease at least 1 min before clumping of the platelets became visible to the naked eye.

It must be emphasized that adding calcium to fresh citrated plasma, whether human or porcine, by no means always caused the platelets to aggregate before clotting occurred. When calcium did not induce aggregation in fresh plasma it sometimes did so after the plasma had been kept for 24–48 hr at room temperature; but not always even then.

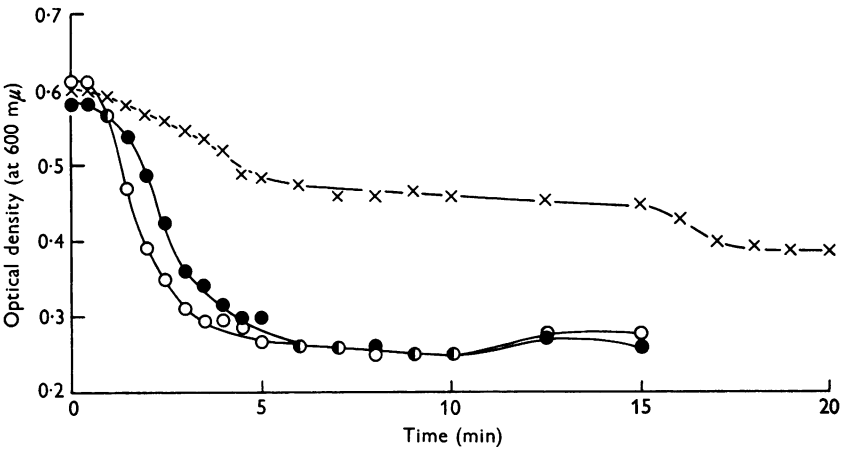

Fig. 4. Effect of stirring rate on optical density of pig's plasma containing heparin (67 u./ml.), after adding calcium chloride ($1{\cdot}3 \times 10^{-2}$ M); the plasma was the same as in Fig. 3. One sample was not stirred for 15 min, and then stirred at 1000 rev/min. (×—×). Two samples were stirred all the time, one at 500 rev/min (●—●) and the other at 1000 rev/min (○—○).

Effect of the rate of stirring on the rate of aggregation

The influence of the rate at which platelet-rich pigs' plasma was stirred on changes in optical density as a measure of the rate of aggregation was determined by adding calcium chloride to make the platelets clump; to prevent clotting heparin was also added (67 u./ml.). Figure 4 shows that whether the plasma was stirred or not the optical density decreased during the *same* period of time. When the plasma was stirred at 500 rev/min the

decrease was only a little slower than when it was stirred at 1000 rev/min, but the magnitude of the decrease was finally the same. However, when the plasma was not stirred, the decrease was much slower; when such a sample was stirred later (after 15 min) at 1000 rev/min there was a further slow decrease which was, however, much less than in the samples which had been stirred from the beginning.

Effects of calcium on the optical density of plasma other than that due to aggregation of platelets

An experiment already published (Born, 1962*a*) showed that there was no significant change in the optical density of citrated pigs' plasma when 0·01 vol. of physiological saline or of heparin solution was added; whereas when 0·01 vol. of M-calcium chloride solution was added the optical density *increased* within the first 15 sec before decreasing as the platelets aggregated. This *immediate* increase in optical density was small but was always seen.

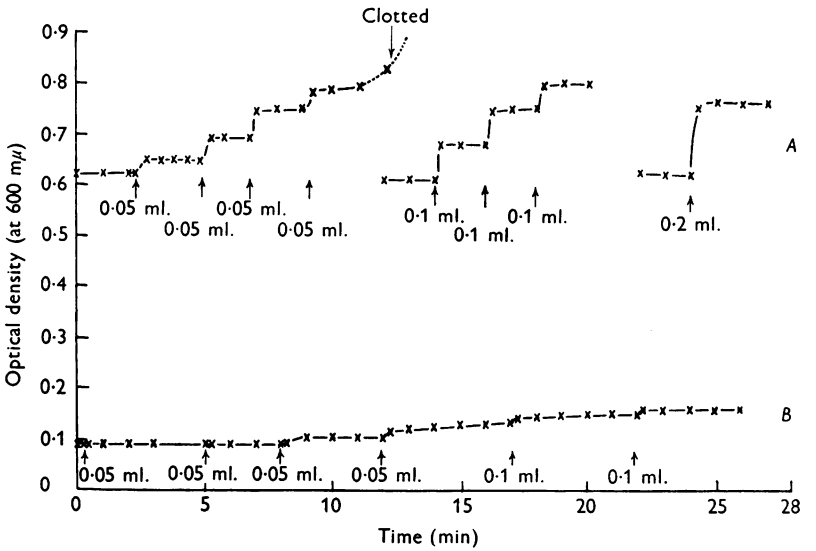

Fig. 5. Effect of adding calcium chloride in increasing the optical density of human plasma containing sodium citrate (10^{-2} M). Curves *A* represent plasma containing $4{\cdot}8 \times 10^8$ platelets/ml.; curve *B* represents plasma free of platelets. At the arrows the indicated volumes of M-$CaCl_2$ were added to plasma samples of 4 ml., which were stirred continuously at 1000 rev/min.

To investigate this effect use was made of a sample of fresh human plasma in which the platelets did not aggregate when calcium chloride was added. Figure 5 shows that the increase in optical density was proportional to the amount of calcium chloride added. When calcium chloride

was added to the same plasma after it had been centrifuged free of platelets, the optical density increased only very slightly and much less than when platelets were present.

Effects of adding adenosine diphosphate

Recently it has been found that adenosine diphosphate (ADP) causes human platelets to aggregate (Gaarder, Jonsen, Laland, Hellem & Owren, 1961); this was shown by methods which are only roughly quantitative. Therefore, the turbidimetric method here described was used to investigate this effect. When ADP was added to citrated plasma the platelets aggregated just as when calcium was added. This is shown in Fig. 6, which also shows that, as with calcium, the rate and extent of aggregation depended upon the rate of stirring.

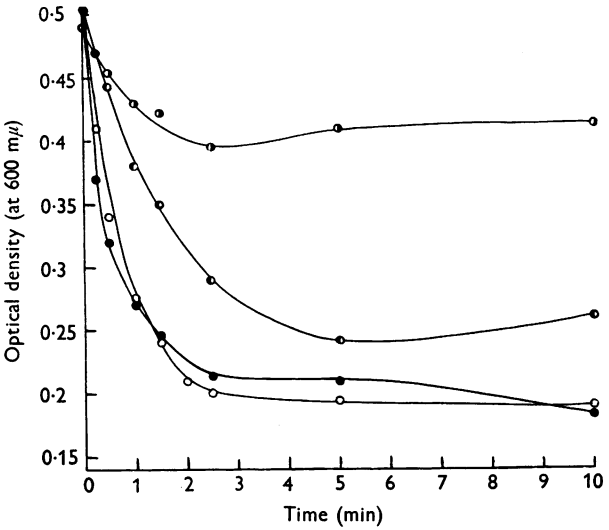

Fig. 6. Effect of the stirring rate on the optical density of human plasma, containing $4{\cdot}7 \times 10^8$ platelets/ml., after adding ADP (2×10^{-6} M). The four samples were stirred at 100 (◑—◑), 200 (◐—◐), 600 (○—○) and 1000 (●—●) rev/min.

However, the effect of ADP differed from that of calcium in two respects: (1) aggregation could be brought about by ADP in *all* samples of citrated (and, for that matter, heparinized) human and porcine plasma; and (2) adding ADP did not lead to clotting.

Relation between aggregation and concentration of ADP

Figure 7 shows that as the concentration of added ADP in the plasma was increased from 5×10^{-7} to $1{\cdot}25 \times 10^{-5}$ M the rate at and extent to which the platelets aggregated also increased. The initial rates of aggregation, as

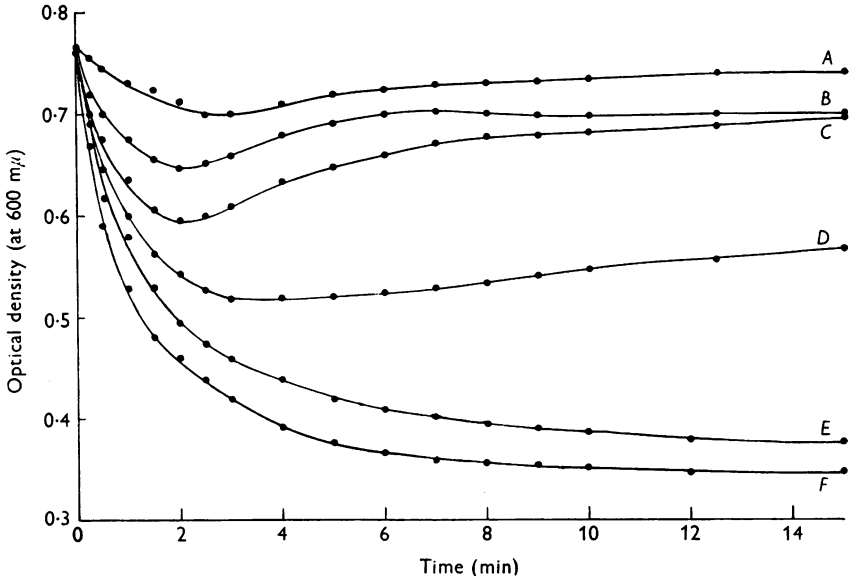

Fig. 7. Effect of adding ADP at increasing concentrations on the optical density of human plasma containing $5{\cdot}4 \times 10^8$ platelets/ml. The plasma was stirred at 1000 rev/min. The final concentrations of ADP were as follows (M): *A*, 5×10^{-7}; *B*, $1{\cdot}25 \times 10^{-6}$; *C*, $1{\cdot}9 \times 10^{-6}$; *D*, $2{\cdot}5 \times 10^{-6}$; *E*, 5×10^{-6}; and *F*, $1{\cdot}25 \times 10^{-5}$.

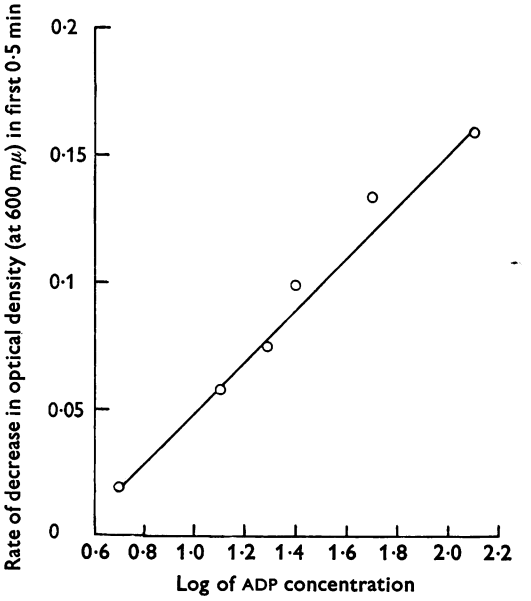

Fig. 8. Relation between the logarithm of the concentration of added ADP and the initial rate of decrease in optical density, plotted from results shown in Fig. 7. Ordinate: initial velocity as decrease in optical density in the first 0·5 min. Abscissa: logarithm of ADP concentration.

indicated by the decreases in optical density in the first $\frac{1}{2}$ min, were directly proportional to the logarithm of the concentration of ADP (Fig. 8).

Relation between aggregation and concentration of platelets

Human plasma was centrifuged at 500 *g* for successive periods of 5 min in order to diminish progressively the concentration of suspended platelets. After each period a sample of the plasma was mixed with the same concentration of ADP (1×10^{-6} M). The resulting changes in optical density were calculated as percentages of the optical densities of the samples before ADP was added; from each optical density reading the optical density of platelet-free plasma had been subtracted. In Fig. 9 the percentage changes are plotted against time: with the highest concentration

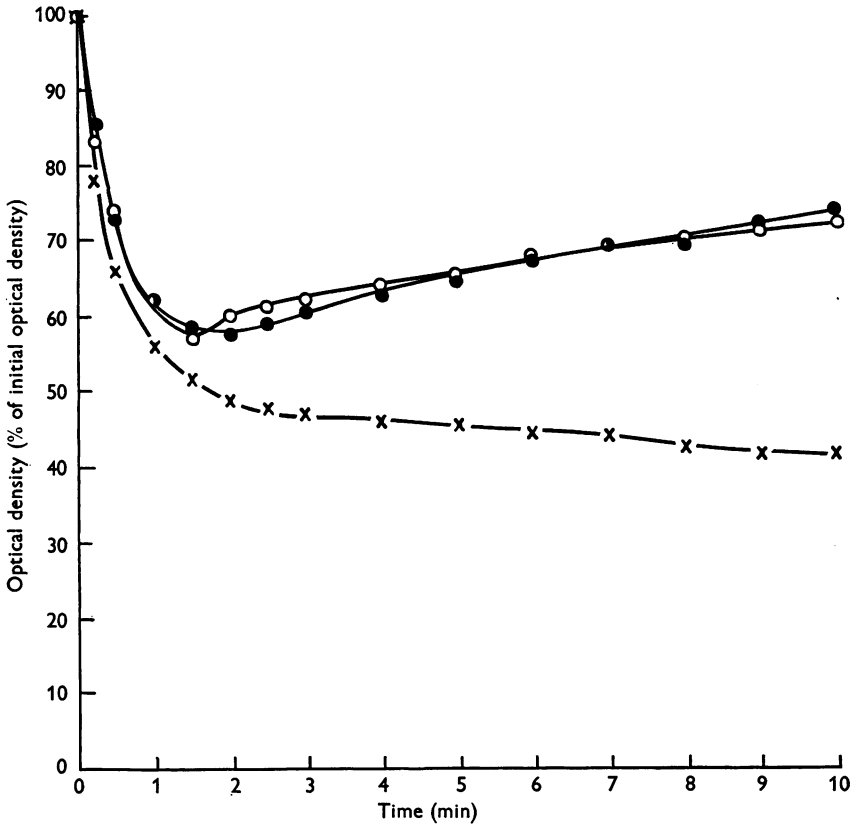

Fig. 9. Relation between concentration of platelets and optical density after adding ADP (1×10^{-6} M) to human plasma. The optical density is shown as percentage of the difference between the optical densities of the platelet-rich plasmas and the platelet-free plasma. The concentrations of platelets were as follows ($\times 10^8$/ml.): 1·4 (○—○); 2·1 (●—●); and 2·9 (×—×).

of platelets aggregation was fastest and did not reverse within 10 min; with lower concentrations aggregation was slower and began to reverse within 3 min.

Effects of ethylenediaminetetraacetate on aggregation by ADP

When human blood was mixed with 0·03 vol. 5% disodium ethylenediaminetetraacetate (EDTA) as anticoagulant, instead of with citrate, adding ADP to the plasma did not make the platelets aggregate. However, when either calcium chloride or magnesium chloride was added subsequently, at a concentration of $3{\cdot}3 \times 10^{-3}$ M, the platelets aggregated; in this plasma adding calcium chloride alone had only a slight effect (Fig. 10).

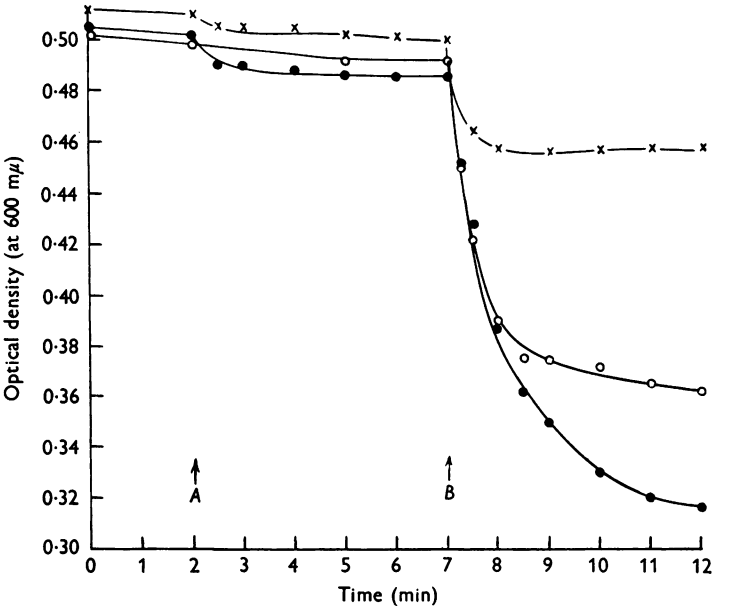

Fig. 10. Effects of calcium and magnesium on the optical density of human plasma containing ethylenediaminetetraacetate (4×10^{-3} M); the plasma contained $4{\cdot}2 \times 10^{8}$ platelets/ml. At *A*, ADP was added to two samples (○—○ and ●—●) but not to the third (×—×). At *B*, $CaCl_2$ was added to two samples (×—× and ○—○) and $MgCl_2$ to the third (●—●), both at a final concentration of $3{\cdot}3 \times 10^{-3}$ M.

The aggregation of platelets in plasma containing EDTA therefore required the addition of both ADP and of either calcium or magnesium. The EDTA added to the plasma bound its calcium; when magnesium was added later some of the calcium was presumably released. This experiment, therefore, did not allow us to decide which of these cations was required for aggregation.

Reversal of aggregation

Figures 7 and 9 both show that when ADP was added the optical density sometimes increased again after its initial fall, suggesting that under some conditions aggregated platelets were able to disperse; this was confirmed microscopically. The dispersion was always seen when aggregation had been brought about by low concentrations of ADP (Born, 1962*b*). This observation suggested that the dispersion of the aggregates might be due to the break-down of ADP to AMP and other substances.

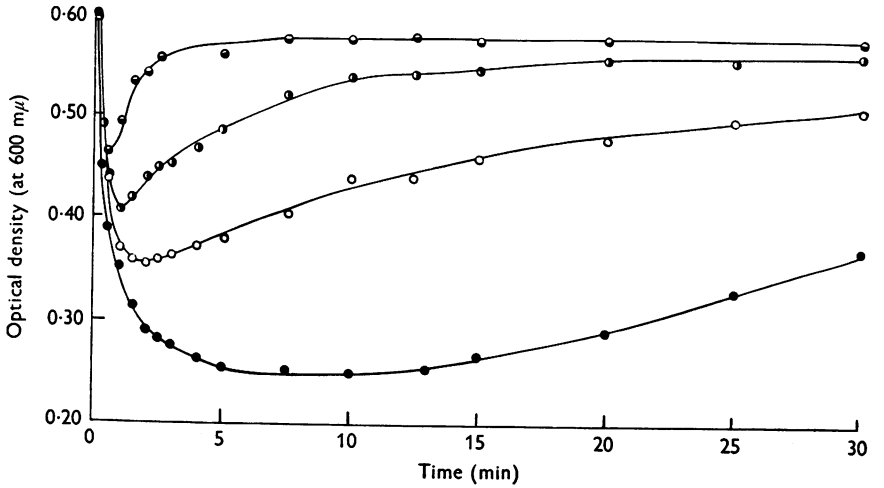

Fig. 11. Effect on the optical density of human plasma of adding AMP (2×10^{-6} M) at increasing intervals of time before adding ADP (2×10^{-6} M); the plasma contained $4{\cdot}1 \times 10^{8}$ platelets/ml. Control, i.e. no AMP added (●—●); AMP added: 2·5 min (○—○); 5 min (◑—◑); and 40 min (◒—◒), before the addition of ADP. The ADP was added at zero time.

Effects of adenosine monophosphate and adenosine

Neither adenosine monophosphate (AMP) nor adenosine caused platelets to aggregate. However, AMP and adenosine diminished and reversed the aggregation brought about by ADP. Their inhibitory effect increased the longer the intervals of time between their addition and the subsequent addition of ADP. This is shown for AMP in Fig. 11; similar results with adenosine have been published (Born & Cross, 1963). The inhibitory effect of AMP was small when it was added *after* ADP.

When AMP was added 5 min before ADP, in increasing concentrations, the aggregation of platelets was decreased more and more (Fig. 12). In inhibiting the aggregation brought about by ADP, the effectiveness of adenosine was ten times greater than that of AMP (Fig. 13). The concentra-

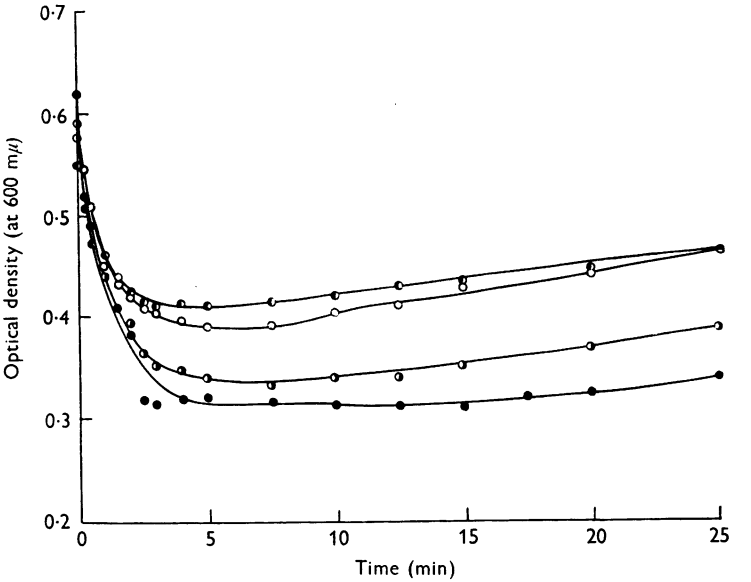

Fig. 12. Effect of adding AMP at increasing concentration 5 min before adding ADP (1×10^{-5} M) on the optical density of human platelet-rich plasma. The concentrations of AMP were as follows (M): nil (●—●); 1×10^{-5} (◑—◑); 1×10^{-4} (○—○); and 5×10^{-4} (◐—◐).

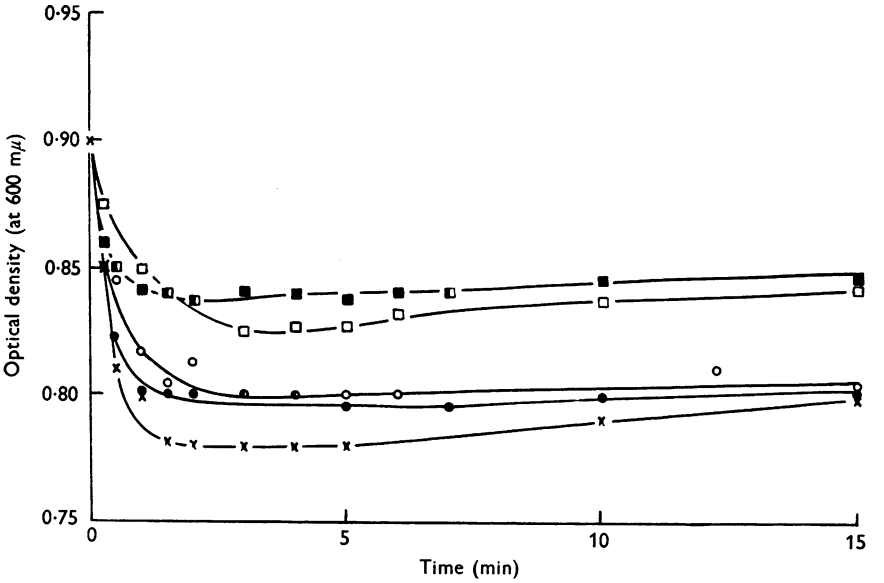

Fig. 13. Comparison between AMP and adenosine added 5 min before ADP in inhibiting its effect on the optical density of human plasma, containing $7{\cdot}2 \times 10^8$ platelets/ml. ADP only at 2×10^{-6} M (×—×); AMP as well at 5×10^{-6} M (○—○) and at 2×10^{-5} M (□—□); adenosine as well (instead of AMP as well) at 5×10^{-7} M (●—●), and at 2×10^{-6} M (■—■).

tions of AMP and adenosine required to diminish aggregation to a similar extent varied from plasma to plasma; in general, aggregation was inhibited significantly only when the concentration of AMP was at least equal to that of the ADP added afterwards.

Effects of other substances

Adenosine triphosphate (ATP) inhibited aggregation by ADP; this has already been described (Born, 1962*b*).

Other substances related to ADP were also tested for possible effects on platelet aggregation; they were adenine, desoxyadenosine, inosine monophosphate, inosine, guanosine, purine riboside, cytidine, and ribose. None of them, in concentrations of up to 5×10^{-4} M, caused aggregation. Their effectiveness in *inhibiting* aggregation brought about by ADP was compared in each experiment with that of adenosine, because (1) the effectiveness of adenosine itself varied from one plasma to another, and (2) because adenosine was the most potent inhibitor we found.

None of these substances, except purine riboside and desoxyadenosine, inhibited in molar concentrations up to 100 times greater than a concentration of adenosine which reduced aggregation by about 30%. Purine riboside had about 0·5% and desoxyadenosine about 0·25% of the activity of adenosine.

Effect of adding ADP repeatedly

When ADP was added repeatedly to the same sample of platelet-rich plasma its effectiveness in causing aggregation decreased; an example of this is shown in Fig. 14 in which the decrease was emphasized by adding twice the concentration of ADP the second time.

DISCUSSION

The method here described for observing quantitatively the aggregation of platelets is simple, but even in this simple form it has allowed us to make several new observations. There is no doubt that the method can be modified and improved, as O'Brien (1962) has already shown.

In the circulating blood platelets presumably collide frequently with each other and with the vessel wall, particularly where the flow of blood is turbulent. In our experiments this condition was imitated by stirring platelet-rich plasma vigorously. In some samples of plasma vigorous stirring alone brought about a small decrease in optical density during the first $\frac{1}{2}$ hr; this suggested that in citrated plasma a small proportion of the platelets either aggregate or break up under the influence of stirring alone. That the platelets in any one plasma may *differ* in their tendency to

clump is also suggested by our finding that platelets which remained suspended in plasma after brief centrifugation aggregated less under the influence of ADP than the platelet population as a whole.

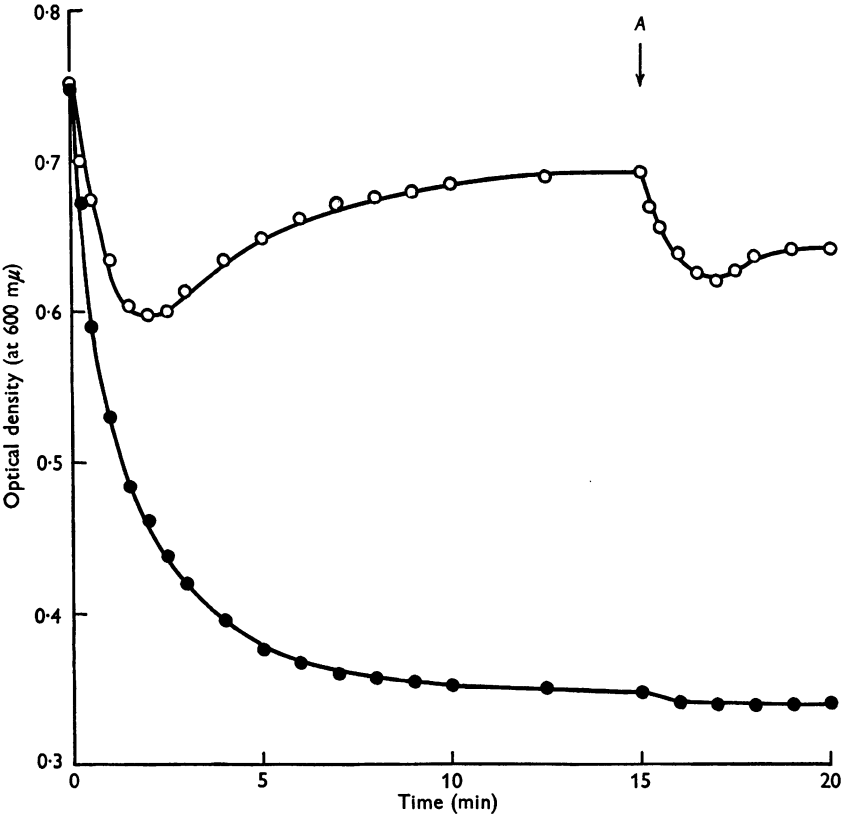

Fig. 14. Effect on the optical density of human plasma containing $5{\cdot}4 \times 10^8$ platelets/ml. of adding ADP repeatedly. At zero time, ADP was added at 2×10^{-6} M (○—○) and at $1{\cdot}25 \times 10^{-5}$ M (●—●). At *A*, ADP was added at 4×10^{-6} M (○—○) and at $2{\cdot}5 \times 10^{-6}$ M (●—●).

Rapid aggregation of a large proportion of the platelets in citrated or heparinized plasma can be brought about sometimes by adding calcium and always by adding ADP. *Aggregation by calcium* proved difficult to study because it varied from plasma to plasma, a variability which itself needs investigating. The turbidimetric method has, however, revealed another effect of calcium which is of interest. This is the small increase in the optical density of platelet-rich plasma which was observed when calcium chloride was added. The increase depended on the presence of platelets; whether it was due to a change in the platelets or whether the platelets

acted as nuclei for the precipitation or adsorption of calcium compounds we cannot yet say.

Aggregation by ADP was investigated more fully because it occurred in all plasmas. We have shown that the *initial rate* of fall in optical density was proportional to the logarithm of the ADP concentration, and that high concentrations of ADP brought about a maximal decrease in the optical density which did not decrease further when yet more ADP was added. Presumably all platelets were then aggregated but, since the relation between the optical density on the one hand and number and sizes of the aggregates on the other has still to be established, we do not know whether the platelets had aggregated into many small clumps or into fewer large ones.

Whatever the exact relation between the fall in optical density and the kind of aggregates formed, it was established conclusively that the subsequent rise in optical density represents *dispersal of the aggregates*. This is most simply accounted for by the suggestion (Born, 1962*b*) that added ADP is broken down to other substances such as AMP and adenosine which not only do not cause aggregation (Gaarder *et al.* 1961) but are potent inhibitors of the aggregation brought about by ADP. Jørgensen (1956) showed that human plasma contains enzymes which catalyse the break-down of ADP to AMP. It is not possible to calculate from his results whether the rate of break-down of ADP is high enough to account for the observed rate of dispersal of the aggregates.

It may be, therefore, that the break-down of ADP that is crucial to the dispersal occurs on the surface of the platelets themselves. Other results accord with this idea. Thus, O'Brien (1962) added ADP to unstirred platelet-rich plasma; when stirring was started the rate at which the platelets then aggregated decreased the longer the interval between the addition of ADP and the beginning of the stirring. This suggests that ADP absorbed on to the platelets had broken down before they were made to collide with each other. Furthermore, we found that the effectiveness of ADP, added repeatedly in small concentrations to the same plasma, progressively diminished; this would be explained if some of the ADP added first was bound to the platelets as AMP by the time ADP was added again.

These considerations suggest the hypothesis that ADP causes aggregation by becoming associated with 'aggregating sites' on the surface of platelets. The inhibiting effects of added AMP and adenosine would then be explained by assuming that they compete with ADP for these sites. Moreover, the conversion of ADP to these substances at or near these very sites might be expected to result in dispersal of the aggregates.

Apart from AMP and adenosine, the only other substances related to

ADP which inhibited its aggregating effect were ATP, desoxyadenosine, and purine riboside, but they were much less potent. Inosine and its monophosphate had no effect, although they are also products of the break-down of ADP in human plasma (Jørgensen, 1956). The ineffectiveness of adenine and of desoxyadenosine suggested that the ribose component was essential for inhibition. The ineffectiveness of purine riboside indicated that the 6-amino group was required. The ineffectiveness of cytidine suggested that a purine base was necessary. All these findings suggest that the effect of ADP in bringing about platelet aggregation, and the effect of AMP and adenosine in inhibiting it, are highly specific. This conclusion is supported by the very low concentrations at which these substances act. Several basic drugs, including cocaine, mepyramine, and imipramine have also been shown to diminish the effect of ADP in causing aggregation, but they do so only in relatively high concentrations (O'Brien, 1961, 1962; Born, 1962*c*). Their effects are, therefore, probably non-specific.

Aggregation by ADP was inhibited by EDTA and this inhibition was reversed when calcium or magnesium was added; it seems that one of these metals is required for the aggregating effect of ADP. Moreover, calcium alone sometimes caused platelets to aggregate in citrated plasma. It may be that the basic mechanism is the same, in that calcium forms bridges between the phosphate groups of molecules of ADP bound to the platelets by their adenosine portions.

The ADP may be added, as in our experiments, or derived from ATP (Born, 1958) which is in the interior of platelets or perhaps at their surfaces. Käser-Glanzmann & Lüscher (1962) have shown that in the presence of thrombin washed platelets release enough ADP to account for their aggregation. ADP may also be released from walls of vessels when the cells are damaged and their ATP is broken down; in this way the adhesion of the first few platelets may come about. If thrombin is then formed on the surface of these platelets more ADP would be released and more platelets would adhere. This would account for the process of thrombogenesis.

The aggregation of platelets by ADP was first observed by Hellem (1960) who found that platelets were adsorbed on glass from citrated plasma only if erythrocytes were present; he isolated a substance from erythrocytes which induced this adsorption and which was later identified as ADP (Gaarder *et al.* 1961). It is difficult to think of any physiological significance of this effect of ADP in red cells, except perhaps in the spleen. 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.

SUMMARY

1. A turbidimetric method is described for following quantitatively the aggregation of blood platelets *in vitro*. The method depends on the decrease in optical density of plasma which occurs when the platelets in it aggregate.

2. When calcium chloride was added to platelet-rich plasma there was an immediate small *increase* in optical density. No such increase was observed when calcium chloride was added to platelet-free plasma.

3. In citrated human or pig plasma platelets could be made to aggregate *sometimes* by adding calcium and *always* by adding adenosine diphosphate. The plasma had to be stirred vigorously in order to achieve maximal aggregation.

4. The intial rate of aggregation of platelets by added ADP was proportional to the logarithm of its concentration.

5. Platelets which remained suspended in plasma after brief centrifugation aggregated less under the influence of ADP than did the platelet population as a whole.

6. Platelets that had aggregated in the presence of low concentrations of ADP dispersed again after a few minutes. When ADP was added repeatedly its effectiveness in causing aggregation decreased.

7. The aggregation of platelets in plasma containing sodium ethylenediaminetetraacetate required not only ADP but also calcium or magnesium ions.

8. Adenosine and its monophosphate inhibited aggregation by ADP; adenosine was about ten times more active than its monophosphate. The inhibitory effect increased the longer the time between their addition to plasma and the subsequent addition of ADP.

9. Adenosine triphosphate, desoxyadenosine, and purine riboside also inhibited this aggregation but very much less than adenosine. Adenine, inosine monophosphate, inosine, guanosine, cytidine and ribose were ineffective.

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