Adenosine diphosphate and the measurement of platelet adhesiveness

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SYNOPSIS Platelet adhesiveness to glass beads was measured in citrated blood at intervals up to two hours after the addition of adenosine diphosphate (A.D.P.). Adhesiveness increased for 15 minutes, fell towards the original level, and then steadily increased. The initial changes were associated with the formation and subsequent dispersal of platelet clumps. The sequence of events resembled the reported platelet aggregation, the disaggregation and secondary aggregation effect of A.D.P. and emphasizes the importance of a standard technique in the measurement of A.D.P.-induced platelet adhesiveness.

The measurement of platelet adhesiveness has received considerable attention in recent years in attempts to relate this property of platelets to the pathogenesis of such conditions as coronary thrombosis (Owren, Hellem, and Ødegaard, 1964) and Von Willebrand's disease (Ødegaard, Skålhegg, and Hellem, 1964), sometimes with conflicting or inconsistent results (Owren *et al.*, 1965). The technique usually used entails counting platelets before and after controlled exposure of blood to glass. Hellem (1960) found that platelet adhesiveness to glass was increased by a constituent of red cells. Later Gaarder, Jonsen, Laland, Hellem, and Owren (1961) identified A.D.P. as a substance in red cells which causes platelet aggregation, an observation which led to studies of platelet aggregation by A.D.P. (Born, 1962; O'Brien, 1962) and of A.D.P.-dependent platelet adhesiveness (Hellem, Ødegaard, and Skålhegg, 1963; Eastham, 1964), but little attempt has been made to determine if these are related or independent phenomena. During the course of such an investigation we noted that the proportion of apparently adhesive platelets is dependent upon the time to which blood is exposed to A.D.P. and may also be influenced by the presence of platelet aggregates.

METHODS

Platelet adhesiveness was measured by a modification of the method of Hellem *et al.* (1963) using whole blood instead of platelet-rich plasma. Blood was taken with a

plastic syringe into 3·8% w/v trisodium citrate in a plastic bottle in the proportion of 9 parts of blood to 1 of citrate. A sample was removed for an immediate platelet count (count A). In order to determine the proportion of adhesive platelets 2 ml. of blood was taken into a plastic syringe and passed through a column of glass beads by means of a Sage constant speed syringe pump. The columns, which were disposable, were made from 6 cm. lengths of polyvinyl tubing, internal diameter 0·5 cm. They were filled with 1·23 g. ($\pm$ 0·01 g.) of glass beads, 0·452–0·520 mm. diameter (Jencons, Grade 8), and sealed at each end with nylon mesh. The time taken for 2 ml. of blood to traverse the column was 47 seconds. Using this technique the following experiment was performed.

As soon as possible after venepuncture, A.D.P. (lithium salt, B.D.H.) was added to the blood in the proportion of 1 part of A.D.P. solution, 1·0 μ g./ml., to 9 parts of blood to give a final concentration of 0·1 μ g./ml. The blood was mixed by inversion and incubated at room temperature. Samples of 2 ml. were passed through a column of glass beads at intervals for up to two hours after the addition of adenosine diphosphate. A fresh column of beads was used for each 2 ml. sample. The percentage of adhesive platelets in each sample was calculated by referring the platelet count of the effluent blood to the platelet count of the original sample (count A above) after adjustment for volume of A.D.P. added.

Simultaneously with the above experiment platelet adhesiveness was estimated on the same citrated blood processed identically but without the addition of adenosine diphosphate. Platelet counts were also performed at intervals on samples containing A.D.P. but before passage through a glass bead column. Platelet counts were performed by the method of Baar (1948) but using phase contrast microscopy. When clumping was present counts of free platelets were recorded and the presence of clumps was noted.

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FIG. 1. Platelet adhesiveness, free (unclumped) platelet counts, and degree of platelet clumping at intervals after the addition of A.D.P. to citrated blood. A Before passage over beads. B After passage over beads.

RESULTS

The platelet adhesiveness of 13 samples of normal blood was investigated. The results are shown in the figure. Adjustments for haematocrit values were not made as these were all within the normal range. Platelet adhesiveness increased for 15 minutes after the addition of A.D.P. and then fell towards the original level. Subsequently there was a steady increase in adhesiveness until the experiments were terminated after two hours. Counts of free (non-clumped) platelets in citrated blood at intervals after the addition of A.D.P., but before passage through the column, are also shown in the figure. The counts fell during the first 15 minutes and considerable clumping was present but this gradually disappeared, and after 30 minutes the free platelet counts had returned to the original level. When citrated blood without A.D.P. was passed through the columns the mean platelet adhesiveness was between 10% and 13% at each time interval and no significant change occurred over a period of two hours after venepuncture.

DISCUSSION

These results show that platelet A.D.P.-dependent adhesiveness varies with the time after the addition of A.D.P. to the blood. The increase occurring during the first 15 minutes apparently represents to some extent merely filtration from the blood of platelet aggregates. The results of this part of the experiment closely resemble the findings of Eastham (1964) who rotated heparinized blood with A.D.P. and noted a fall in the platelet count during the first 30 minutes and the presence of platelet clumps on the vessel

wall. The subsequent reduction of adhesiveness was also noted by Eastham and may be due, as he implied, to the inactivation of adenosine diphosphate. It may thus parallel the disaggregation phenomenon described by O'Brien (1962) and Born (1962) after the addition of A.D.P. to stirred platelet-rich plasma. The slower evolution of the changes in our experiment was perhaps due to the fact that the blood was not stirred. The steady rise of platelet adhesiveness, which we observed after 30 minutes' incubation of blood with A.D.P., was not due to the presence of clumps and must represent a true change of adhesiveness. It may have been due to the gradual liberation of A.D.P. from platelets or red cells and in this respect resemble the secondary clumping effect of A.D.P. described by MacMillan (1966). Whatever the cause of the changes in platelet adhesiveness which we observed after the addition of A.D.P. to blood they serve to emphasize that this technique must be rigidly standardized. If grossly erroneous results are to be avoided, adhesiveness must be measured at standard times after the addition of adenosine diphosphate.

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