Relationships between platelet function tests in normal and uraemic subjects

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SYNOPSIS In tests of platelet function in normal subjects, the following relationships were found: the greater the platelet adhesiveness the less the ability to disaggregate after challenge with adenosine diphosphate (ADP), and the greater the disaggregation after ADP, the longer the clotting time in the test for platelet factor 3 availability. Such correlations were disturbed in uraemic patients.

In recent years many platelet function tests have been devised to study platelet behaviour *in vitro*. The ability of platelets to adhere to foreign surfaces, their subsequent aggregation, and their contribution of phospholipid to the intrinsic coagulation mechanism are thought to be important factors in the formation of haemostatic plugs and of thrombi. The identification of adenosine diphosphate (ADP) as a key substance in causing aggregation and adhesiveness of platelets and release of platelet factor 3 has contributed much to the understanding of platelet function.

The present investigation was carried out to determine whether there was a relationship between platelet adhesiveness, ADP-induced platelet aggregation, and platelet factor 3 availability, in normal subjects and in uraemic patients, a group in whom a qualitative platelet defect has recently been described (Lewis, Zucker, and Ferguson, 1956; Rath, Maillard, and Schreiner, 1957; Stewart and Castaldi, 1967; Hassanein, McNicol, Kennedy, and Douglas, 1969).

Materials and Methods

Blood was collected from both normal healthy subjects and uraemic patients by clean vene-

puncture, using $1\frac{1}{2}$ in. disposable needles (21 gauge) and plastic disposable syringes. The blood was immediately mixed with 3.8% sodium citrate in 10 ml siliconized centrifuge tubes (1 part citrate to 9 parts blood). Platelet-rich plasma was prepared by centrifugation of citrated blood at 600g for five minutes at room temperature. Adenosine-5-diphosphate (ADP) was used in the form of the sodium salt as supplied by Sigma Chemical Company, St. Louis. The compound was dissolved in barbitone buffered saline, pH 7.2, and used in a final concentration of 0.25 μ g/ml.

Platelet adhesiveness was determined in whole blood by the glass bead column method of Hellem (1960) as modified by Hirsh, McBride, and Dacie (1966).

The packed cell volume (PCV) was measured from citrated blood and appropriate corrections were made in the measurement of adhesiveness, for variations in PCV using an appropriate standard curve.

Adenosine diphosphate-induced platelet aggregation was assessed by the method of Born and Cross (1963). Platelet-rich plasma (1.9 ml) was placed in a Perspex cuvette with a magnetic stirring rod on an EEL (Evans Electroselenium) titrator; 0.1 ml ADP solution was added and a stop watch was started. Changes in light transmission using a green filter (no. 604, peak length 5,200 Angstrom units) were recorded by taking optical density readings every 30 seconds for 10 minutes. All experiments were carried out at room temperature.

Assessment of Platelet Aggregation and Disaggregation

The assessments of platelet aggregation and disaggregation were expressed as follows:

30-60 PLATELET AGGREGATION

This is the fall in optical density between 30 and 60 seconds after the addition of ADP, this being the steepest part of the curve.

30-90 PLATELET AGGREGATION

The fall in optical density between 30 and 90 seconds after the addition of ADP, to include the main part of the optical density fall due to aggregation.

MAXIMUM AGGREGATION

This is measured by subtracting the lowest optical density reading from the optical density before the addition of the aggregating agent (0-600).

PERCENTAGE DISAGGREGATION

This was calculated as (increase in optical density in the five minutes from the point of maximum aggregation $\times$ 100) $\div$ (fall in optical density from before addition of the aggregating agent to the point of maximum aggregation).

The method used to measure platelet factor 3 availability was similar to that described by Spaet and Cintron (1965). To 0.9 ml of platelet-rich plasma was added 0.1 ml 5% kaolin in a siliconized graduated centrifuge tube. The two reagents were mixed by gentle shaking 10 times and then

left undisturbed in a water bath at 37°C for 30 minutes. After incubation, the platelet-rich plasma and kaolin were mixed by resuspending the kaolin using a siliconized pipette; 0.1 ml of the mixture was added to 0.1 ml 0.025 M calcium chloride and 0.1 ml Russell viper venom (10 μ g/ml) and the clotting time determined in duplicate. The kaolin cephalin clotting time (Biggs and Macfarlane, 1966) was used as a simple screening test to detect plasma coagulation defects in uraemic patients. Platelet counts were performed according to the method of Dacie (1956).

Twenty uraemic patients were studied; of these two were in acute renal failure and 18 had chronic uraemia (Table).

Results

RELATIONSHIP BETWEEN PLATELET ADHESIVENESS AND ADP-INDUCED PLATELET AGGREGATION AND ITS REVERSAL

Normal controls

Platelet adhesiveness to glass was assessed in 17 normal controls (mean platelet-rich plasma platelet count $366,000 \pm 63,000/\text{cmm}$) together with ADP-induced platelet aggregation from the same blood sample. No correlation existed between platelet adhesiveness and platelet aggregation as measured by 30-60, 30-90, or maximum aggregation ($r = 0.0317$, $P > 0.1$; $r = 0.014$, $P > 0.1$; $r = 0.056$, $P > 0.1$ respectively). However, there was a significant negative correlation between percentage disaggregation and percentage adhesiveness ($r = -0.549$, $P < 0.05$) as shown in Figure 1. The negative correlation between percentage platelet adhesiveness and percentage disaggregation became more significant after correcting the results of platelet adhesiveness for PCV ($r = -0.611$, $P < 0.01$) shown in Figure 2.

Uraemic patients

As platelet aggregation and disaggregation were found to be dependent on the platelet-rich plasma count (Hassanein *et al*, 1969), the results from 18 uraemic patients having a platelet count within the normal range ± 2 SD were chosen. No correlation was found between platelet aggregation and disaggregation and platelet adhesiveness.

Fourteen of 18 patients showed an abnormal platelet adhesiveness-disaggregation relationship, i.e. an undue decrease in either adhesiveness or disaggregation or both. The relationship between percentage platelet adhesiveness and percentage platelet disaggregation was still insignificant when the results of platelet adhesiveness were corrected for packed cell volume. However, only eight patients, compared with 14 before correction, showed an abnormal relationship.

Subject No.	Sex	Age	Diagnosis	Blood Urea (mg per 100 ml)
1	M	20	Chronic renal failure, congenital hypoplasia of kidneys	153
2	M	31	Chronic renal failure, chronic glomerulonephritis	168
3	F	24	Chronic renal failure, congenital hypoplasia of the kidneys	155
4	M	59	Acute renal failure, bronchopneumonia	290
5	F	53	Acute renal failure, cholangitis	310
6	M	13	Chronic renal failure, chronic pyelonephritis	131
7	F	25	Spina bifida, chronic renal failure, chronic pyelonephritis	120
8	F	57	Chronic renal failure, chronic pyelonephritis myxoedema	87
9	M	58	Chronic renal failure, hydronephrosis	203
10	F	42	Chronic renal failure, chronic pyelonephritis	169
11	F	37	Chronic renal failure, renal tuberculosis	106
12	M	32	Chronic renal failure, chronic pyelonephritis	
13	F	53	Chronic renal failure, acute postrenal obstruction	300
14	M	63	Chronic renal failure, acute postrenal obstruction	650
15	M	86	Chronic renal failure, acute postrenal obstruction	920
16	M	49	Chronic renal failure, polycystic kidney	360
17	M	65	Chronic renal failure, bronchopneumonia, bronchial carcinoma	270
18	M	31	Chronic renal failure, unknown aetiology	280
19	M	55	Chronic renal failure, epiderolysis bullosa	190
20	M	23	Chronic renal failure, chronic glomerulonephritis	154

Table Clinical data of uraemic patients

Fig. 1 The relationship between platelet adhesiveness and platelet disaggregation in normal controls and in uraemic patients. The regression line and 95% confidence limits shown are derived from the control subjects.

Fig. 2 The relationship between platelet disaggregation and platelet adhesiveness after correcting for PCV in normal controls and uraemic patients. The regression line and 95% confidence limits shown are derived from the control subjects.

Fig. 3 The relationship between the Stypven time and platelet disaggregation in normal controls and uraemic patients. The regression line and 95% confidence limits shown are derived from the control subjects.

RELATIONSHIP BETWEEN FACTOR 3 AVAILABILITY ('STYPVEN' TIME) AND ADP-INDUCED PLATELET AGGREGATION AND ITS REVERSAL

Normal controls

Ten experiments were carried out in which platelet factor 3 availability was assessed together with ADP-induced platelet aggregation from the same blood sample. No correlation was found between platelet factor 3 availability (Stypven time) and platelet aggregation as measured by 30-60 sec, 30-90 sec, or maximum aggregation. However, a significant correlation was obtained between platelet factor 3 availability (Stypven time) and percentage disaggregation ($r = +0.645$, $P < 0.05$) as shown in Figure 3.

Uraemic patients

Twelve uraemic patients having a normal kaolin cephalin clotting time and a platelet-rich plasma platelet count within the normal range were chosen to show this relationship. The significant correlation found in normal controls between the Stypven time and percentage disaggregation was absent in uraemic patients ($r = +0.389$, $P < 0.1$). The results with eight of the 12 uraemic patients fell outside the 95% confidence limit of the normal, i.e. they showed either prolonged Stypven times or diminished disaggregation or both.

Discussion

PLATELET FUNCTION

The role of platelets in haemostasis and thrombosis has become a subject of much interest in recent years and appropriate tests have been devised to measure platelet function *in vitro*. Platelet adhesiveness is a measure of the ability of platelets to adhere to a foreign surface, while platelet aggregation is the property of platelets to adhere to each other. Platelets also contribute to the coagulation mechanism through the release of platelet factor 3 which catalyses the formation of the intrinsic prothrombin activator. Aggregation, adhesion, and release of platelet factor 3 can therefore be considered as important platelet functions which can be assessed in the laboratory. An attempt has been made to find a relationship between these three functions in healthy subjects and in uraemic patients in whom a qualitative platelet defect has been recently recognized.

Platelet adhesiveness to glass appears to be mediated through the release of ADP from red cells during their passage through the glass bead column (Hellem, 1960 and 1964; Gaarder, Jonsen, Laland, Hellem, and Owren, 1961), a step which is followed by aggregation between the platelets themselves (Wright, 1941; Salzman, 1963). A relationship might therefore be anticipated between the results obtained in tests of platelet adhesiveness and those found in tests of ADP-induced aggregation. When the two types of tests were performed from the same blood sample in normal subjects, no correlation was found between platelet adhesiveness and platelet aggregation. However, platelet adhesiveness was found to be negatively correlated with the ability of platelets to disaggregate following the addition of ADP in the turbidimetric system, ie, the more adhesiveness, the less disaggregation. In the majority of uraemic patients a negative correlation between platelet adhesiveness and disaggregation was not found.

Adenosine diphosphate has been found to activate platelet factor 3 (Hardisty and Hutton, 1966b; Horowitz and Papayouanou, 1968) and kaolin makes platelet factor 3 available by a reaction which involves platelet aggregation and which is inhibited by ADP antagonists such as adenosine and AMP (Hardisty and Hutton, 1965 and 1966a). It is possible that kaolin acts by releasing ADP from platelets, resulting in platelet aggregation and availability of platelet factor 3.

Hardisty and Hutton (1965 and 1966a), measured the changes in optical density and in the Stypven time of platelet-rich plasma aggregated by ADP; they found that aggregation is rapidly followed by a reduction in the Stypven time which reaches a maximum after 20 minutes. The effect on the Stypven time was found to depend on the rate, degree, and duration of platelet aggregation. They suggested that the membrane changes which lead to platelet aggregation also

provide a catalytic surface for the interaction of coagulation factors; inhibition of platelet aggregation by adenosine was associated with a proportionate inhibition of the fall in Stypven time, and phenothiazine derivatives, which inhibit ADP, halted the effect of ADP on the Stypven time but did not reverse it (Hardisty and Hutton, 1966b). The relationship found in the present communication between platelet disaggregation and the Stypven time in normal controls may be explained in terms of the findings cited above. Aggregated platelets disaggregate because of degradation of ADP by plasma and platelet enzymes (Hellem and Owren, 1964; Salzman, Chambers, and Neri, 1966), and hence where disaggregation is early and substantial, rapid degradation of ADP may be expected to have occurred. Substances which accelerate ADP degradation also reduce platelet factor 3 availability (Hardisty and Hutton, 1966b; Horowitz and Papayouanou, 1968) and therefore both reduced platelet factor 3 availability and accelerated disaggregation might be expected in platelet-rich plasma with a high capacity to inhibit adenosine diphosphate.

In uraemic patients no correlation between disaggregation and platelet factor 3 availability was found, and most patients showed diminished disaggregation or prolonged Stypven time or both.

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