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A clinical and experimental study of platelet function in chronic renal failure

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SYNOPSIS Coagulation and platelet function studies were performed on 24 normal subjects and 29 patients with chronic renal failure due to various causes. Thrombocytopenia was uncommon in the uraemic patients but there was reduced platelet retention in glass bead columns and platelet aggregation with adenosine diphosphate (ADP) and thrombin was slower and less complete than normal. The rate of platelet disaggregation in uraemic patients was significantly reduced. The abnormalities tended to be more severe in more uraemic subjects. In normal subjects no interrelationships were observed between the various measurements of platelet activity. In patients there were significant interrelationships between the measurements of platelet aggregation with ADP and thrombin and between the measurements of aggregation and retention in glass bead columns. It is suggested that if a common pathway is involved in these reactions it is adversely affected in uraemia.

Plasma coagulation defects were uncommon and present in only five of the uraemic subjects. Impaired prothrombin consumption apparently due to defective platelet function was present in half the patients but was not detected by a kaolin activation method. Although platelet coagulation function was activated during ADP aggregation and disaggregation in normal and uraemic subjects, it did not correlate in the latter with impairment of aggregation. It is suggested that aggregation and activation of platelet coagulant activity are not necessarily related aspects of platelet function. An effect of uraemic plasma on normal platelets was demonstrated by mixing experiments consistent with a humoral cause for the uraemic platelet defects.

An abnormal bleeding tendency in patients with renal failure has been recognized for many years and has been attributed to several causes. Increased capillary fragility and thrombocytopenia have been described but recent work suggests that in uraemia the platelets do not function properly. In this condition they have been found to adhere abnormally to glass (Salzman and Neri, 1966) and to aggregate poorly with adenosine diphosphate (ADP) (Castaldi, Rozenberg, and Stewart, 1966) and thrombin (Salzman and Neri, 1966) but there is little information about the interrelationship of these abnormalities or their relationship to the biochemical changes which occur. It is also uncertain if the abnormalities are due to the production of abnormal platelets or to the direct effect of humoral or other factors.

In this paper we describe the results of a study of platelet function in chronic renal failure. The platelets of these patients were retained abnormally in glass bead columns and they aggregated poorly with ADP, and especially with thrombin. Incubation of normal platelets with uraemic plasma resulted in an altered pattern of aggregation with ADP. The interrelationship of these abnormalities and their correlation with blood urea levels are described.

Patients

Twenty-four normal subjects and 29 patients with chronic renal failure were studied, but in some instances the amount of blood obtained was insufficient to perform all the tests. The ages of the patients

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ranged from 15 to 60 years and 15 were females. Twenty suffered from chronic glomerulonephritis, five from chronic pyelonephritis, and the others from polycystic kidneys, malignant hypertension, or diabetic nephropathy. The serum urea levels ranged from 117 to 635 mg/100 ml (19·5 to 105·8 m mol/l) and serum creatinine from 3·6 to 20·5 mg/100 ml (318 to 1814 μ mol/l). Only four of the patients (Table I, nos. 5, 8, 12, 25) had signs of recent bleeding tendency. None was on regular haemodialysis. Nine had been previously treated by peritoneal dialysis but in only one patient (no. 23) was this within one week of the haematological tests.

Methods

Blood was collected from normal subjects and patients between 9.30 am and 10 am using 50 ml plastic syringes. Samples for platelet function tests and coagulation studies were taken into 0·13M trisodium citrate in plastic containers in the proportion of 1 part of citrate to 9 parts of blood and mixed by rotation on a Matburn mixer for five minutes before testing.

Platelet retention in glass bead columns and platelet aggregation were measured as previously described (Bloom and Evans, 1969) except that for the measurement of aggregation platelet-rich plasma (PRP) was adjusted to a platelet count of 250 × 10^9/l, instead of 340 × 10^9/l, with platelet-poor plasma (PPP) from the same donor. The aggregating agents used were ADP (Sigma) 0·05 ml to give a final concentration of 750 μg per 1 ($1·6 \times 10^{-6}$M) and human thrombin (Fibrindex, Ortho), 0·05 ml, to give a final concentration of 0·25 IU per ml. Changes in light transmission due to platelet aggregation and disaggregation were measured at 37°C in an EEL titrator and recorded on a Servoscribe recording potentiometer. The rate of aggregation was assessed by determining the time taken to reach 50% of the maximum fall of optical density (t 0·5OD max.). The degree of aggregation was assessed by relating the optical density at maximum aggregation to the optical density of PPP from the same blood sample. The potentiometer recorder was adjusted to a standard setting with each of the donor PPP samples, the optical density of which was taken to represent '100% aggregation' on the assumption that if all the platelets aggregated they would form one small clump which would not significantly affect light transmission. The rate of disaggregation was assessed by determining the time taken from the point of onset of disaggregation to reach the point of 50% change of optical density (t 0·5OD max disaggregation).

The 'release' of platelet procoagulant activity during ADP-induced aggregation was measured using Russell's viper venom (Stypven, Wellcome laboratories). Volumes of platelet-rich plasma, each of 0·05 ml, were removed from the cuvette at intervals during aggregation and added at 37°C to tubes containing 0·05 ml of pooled normal platelet-poor plasma. Simultaneously 0·2 ml of Stypven-calcium reagent was added and the clotting time determined. The Stypven-Ca reagent was prepared by mixing equal volumes of 1:50,000 Stypven and 0·025M calcium chloride.

EFFECT OF URAEMIC PLASMA ON NORMAL PLATELETS

Samples of blood from normal individuals and patients with chronic renal failure were collected within 15 minutes of each other. Platelet-poor plasma was prepared by centrifuging at 3000 rpm for 20 minutes and stored at −30°C.

For the experiments fresh platelet-rich normal plasma was adjusted to counts of 500 × 10^9/l and 330 × 10^9/l with autologous platelet-poor plasma. To 1 ml and 1·5 ml volumes of these samples respectively was added 1 ml and 0·5 ml of the stored normal or uraemic plasma. These dilutions resulted in 2 ml samples with platelet counts of 250 × 10^9/l and containing either 1 in 2 or 1 in 4 parts of stored uraemic or normal plasma. In one series of experiments aggregation with ADP, $1·6 \times 10^{-6}$M, was determined immediately. In another series the platelet-plasma mixtures were preincubated for 30 minutes at 37°C in a metabolic water bath oscillating at 80 strokes per minute.

OTHER STUDIES

Bleeding time (Ivy), clot retraction, one-stage prothrombin times, and prothrombin consumption indices were determined as described by Biggs and Macfarlane (1962). The kaolin-cephalin clotting times were determined by the method of Proctor and Rapaport (1961). Platelet factor 3 availability was measured by the method of Hardisty and Hutton (1965) and plasma fibrinogen by a modification of the method of Ratnoff and Menzie (1951) described by Varley (1967). Platelet counts were determined with a Coulter electronic counter (model F) and haemoglobin and packed cell volume by standard techniques.

Serum calcium and magnesium were determined by atomic spectrophotemetry and inorganic phosphate was measured by a Technicon AutoAnalyzer modification of the method of Fiske and Subbarow (1925). Urea and creatinine were estimated by Technicon AutoAnalyzer modifications of the methods of Marsh, Fingerhut, and Miller (1965) and Folin and Wu (1949) respectively.

Results

PLATELETS RETENTION IN GLASS BEAD COLUMNS

The whole blood platelet counts of the uraemic subjects ranged from 116 × 10^9/l to 402 × 10^9/l and only three were below 150 × 10^9/l (Table I). Retention in glass bead columns ranged from 31% to 90% (mean 70%, SD 17·3) in normal subjects (Fig. 1). Significantly lower values ($P = < 0{\cdot}001$) were obtained in patients (mean 37%, SD 18·2; range 1% to 71%).

PLATELET AGGREGATION WITH ADP (FIG. 2)

The rate of aggregation with ADP (t 0·5OD max) ranged from 20·5 sec to 34·5 sec (mean 25·8 sec, SD 3·4) in normal subjects. In patients the rate was significantly slower (mean 33·3 sec, SD 8·5; range 20 to 52·5 sec; $P = < 0{\cdot}001$).

In normal subjects the percentage aggregation ranged from 23% to 57% (mean 37%, SD 7·6). This was significantly higher ($P = < 0{\cdot}025$) than that found in patients (mean 28·3%, SD 15·7; range 0 to 65%).

The rate of platelet disaggregation (t 0·5OD max disaggregation) in normal subjects ranged from 30·5 seconds to 184 seconds (mean 69·4 sec SD 40·3) and was significantly faster ($P = < 0{\cdot}01$) than the rate observed in the patients (mean 138·3 sec, SD 105·8; range 36 to 393 sec).

Thus in uraemic patients the platelets aggregated and disaggregated more slowly with ADP and aggregation was less complete.

PLATELET AGGREGATION WITH THROMBIN (FIG. 3)

In normal subjects the rate of aggregation with thrombin (t 0·5 OD max) varied from 19 seconds to 29·5 seconds (mean 24·6 sec, SD 2·7). In patients the rates were slower varying from 17 seconds to 43·5 seconds (mean 28·6 sec, SD 8·1) but the differences from normal were not significant ($P = > 0{\cdot}05$). The percentage aggregation of platelets with thrombin in normal subjects ranged from 14 to 49% (mean 21%, SD 8·7). In uraemic subjects aggregation with thrombin was much less complete, ranging from 0% to 31% (mean 7·0%, SD 7·2). This was significantly lower than normal ($P = < 0{\cdot}001$).

The rate of disaggregation with thrombin in normal subjects ranged from 13·5 seconds to 25 seconds (mean 19 sec, SD 33·2) and was significantly faster ($P = < 0{\cdot}025$) than the rate observed in the patients (mean 27·2 sec, SD 10·9, range 13 to 47·5 sec). The pattern of results with thrombin was thus very similar to that observed with ADP.

Patient Number	*Prothrombin Time (sec)*		*Kaolin-cephalin Time (sec)*		*Bleeding Time (min) Normal <5*	*Platelet Count per cmm × 10^3 (× 10^9/l)*
	Patient	*Control*	*Patient*	*Control*		
1	14·5	14·0	38·0	38·5	4	253
2	14·5	12·5	30·0	35·0	11·5	116
3	13·0	12·5	37·0	34·5	5	209
4	17·5	14·0	31·0	34·5	4·5	125
5	20·0	13·5	34·0	35·5	15+	175
6	13·0	12·5	34·0	37·5	7	370
7	14·0	14·0	38·0	38·5	3·5	217
8	14·5	13·0	31·0	34·0	5·5	220
9	14·5	13·0	34·5	34·0	3·5	278
10	12·5	12·0	40·0	36·5	4	265
11	13·5	13·0	35·0	33·5	—	315
12	12·0	13·0	31·5	31·0	7	253
13	12·5	13·0	31·0	32·5	6	181
14	12·5	12·5	40·0	38·0	5	227
15	13·5	13·5	34·5	36·5	3·5	402
16	14·0	13·0	38·0	38·0	2·5	300
17	12·0	13·0	36·0	38·5	2	238
18	13·0	12·5	33·5	33·0	10·5	169
19	13·0	12·5	38·0	40·0	—	164
20	16·0	13·5	32·0	35·0	—	153
21	14·0	14·0	31·5	32·0	—	214
22	12·5	12·5	37·0	37·5	—	156
23	13·0	11·5	39·5	38·5	11·0	161
24	12·5	13·5	36·5	48·0	—	162
25	12·5	13·0	31·0	33·5	15+	160
26	13·0	12·0	36·5	36·5	4·5	353
27	13·5	12·5	30·0	34·5	2	243
28	21·0	14·5	38·0	36·0	3	130
29	18·5	13·0	45·0	38·5	15−	187

Table I *Basic tests of haemostasis in patients with chronic renal failure*

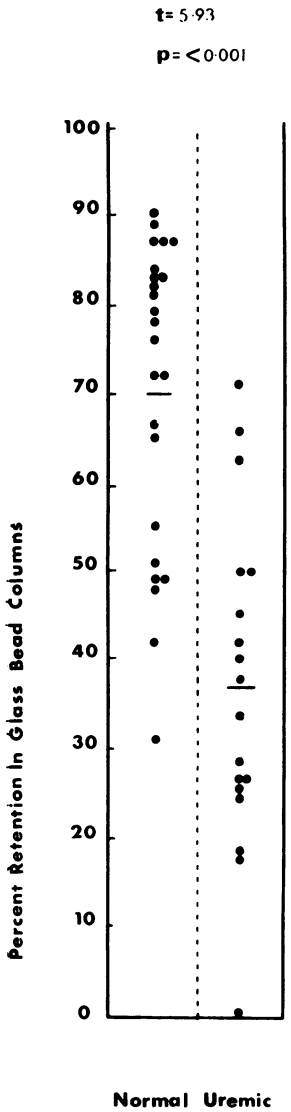

Fig. 1 *Platelet retention in glass bead columns in normal and uraemic subjects.*

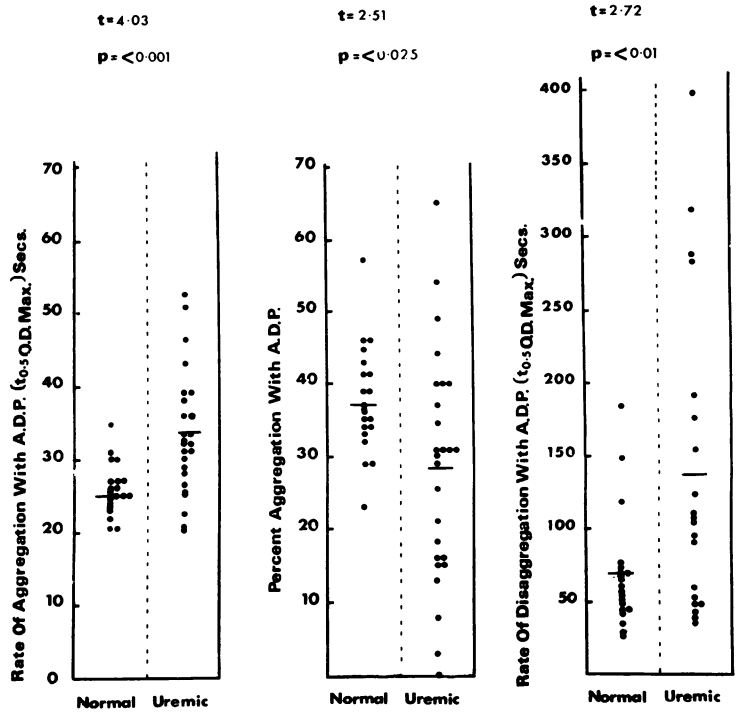

Fig. 2 *Rate and percentage of platelet aggregation and rate of disaggregation with ADP in normal and uraemic subjects.*

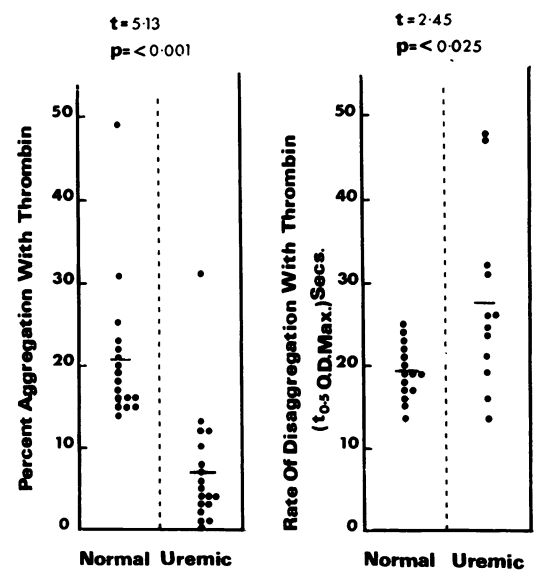

Fig. 3 *Percentage platelet aggregation and rate of disaggregation with thrombin in normal and uraemic subjects.*

INTERRELATIONSHIP OF THE MEASUREMENTS OF PLATELET AGGREGATION AND RETENTION

Bloom and Evans (1969) found that in normal subjects the rate of aggregation with both ADP and thrombin correlated with the number of platelets retained in glass bead columns. In the present study, however, there was no significant relationship in normal subjects between the percentage of the platelets retained in the columns and the rate or degree of platelet aggregation with ADP or thrombin. Nor did the rate or degree of platelet aggregation with ADP correlate with the rate or degree of aggregation with thrombin. Similarly, in uraemic subjects, there was no significant relationship between retention in the columns and the rate of aggregation with ADP and thrombin but there was a significant correlation between the retention of platelets and the percentage aggregation with ADP ($P = < 0.01$) and thrombin ($P = < 0.01$). Furthermore the rate of aggregation with ADP correlated significantly ($P = < 0.05$) with the rate of aggregation with thrombin and the percentage aggregation with ADP correlated significantly ($P = < 0.001$) with the percentage aggregation with thrombin.

THE EFFECT OF URAEMIC PLASMA ON NORMAL PLATELETS

The effect of stored normal or uraemic plasma on ADP aggregation of fresh normal platelets is shown in Tables II and III. When platelets were mixed with stored uraemic plasma the rates of aggregation and disaggregation tended to be slower but the percentage aggregation tended to be greater than when they were mixed with stored normal plasma (Table II). When the mixtures were first incubated at 37°C for 30 minutes before the addition of ADP the most consistent effects of the uraemic plasma were increased percentage aggregation and decreased rate of disaggregation (Table III). The effect on disaggregation was most marked in mixtures containing the higher proportions of uraemic plasma.

Experiment Number[1]	*Stored Plasma Added to Fresh Normal PRP*	*Rate of Aggregation (sec)*	*Rate of Disaggregation (sec)*	*Percentage Aggregation*
1	(a) Normal plasma (1ml)	20·5	61·5	42·0
	(b) Uraemic plasma (1 ml)	24·0	217·5	45·0
2	(a) Normal plasma (1 ml)	27·5	—	79·0
	(b) Uraemic plasma (1 ml)	21·5	—	87·0
3	(a) Normal plasma (1 ml)	21·0	35·5	41·0
	(b) Uraemic plasma (1 ml)	26·0	53·5	48·0
4	(a) Normal plasma (0·5 ml)	29·5	—	70·0
	(b) Uraemic plasma (0·5) ml	31·0	—	70·0
5	(a) Normal plasma (0·5 ml)	13·0	25·5	14·0
	(b) Uraemic plasma (0·5 ml)	15·0	40·0	27·5
6	(a) Normal plasma (0·5 ml)	26·0	22·0	24 0
	(b) Uraemic plasma (0·5 ml)	21·0	20·0	29·0

Table II *The ADP-induced aggregation of fresh normal platelets after the addition of stored normal or uraemic platelet-poor plasma without incubation*

[1]In experiments 1, 2, and 3 1 ml of stored normal (a) or uraemic PPP (b) was added to 1 ml of fresh normal PRP. In experiments 4, 5, and 6 0·5 ml of stored normal (a) or uraemic (b) PPP was added to 1·5 ml of fresh normal PRP. Final platelet count 250 × 10^9/1 and final volume 2 ml in each case.

Experiment Number[1]	*Stored Plasma Added to Fresh Normal PRP*	*Rate of Aggregation (sec)*	*Rate of Disaggregation (sec)*	*Percentage Aggregation*
1	(a) Normal plasma (1 ml)	16·0	34·0	28·0
	(b) Uraemic plasma (1 ml)	18·0	116·0	35·0
2	(a) Normal plasma (1 ml)	16·0	37·5	27·0
	(b) Uraemic plasma (1 ml)	17·0	36·5	33·0
3	(a) Normal plasma (1 ml)	17·0	182·5	14·0
	(b) Uraemic plasma (1 ml)	12·0	280·0	30·0
4	(a) Normal plasma (0·5 ml)	22·0	18·0	21·0
	(b) Uraemic plasma (0·5 ml)	20·0	22·0	30·0
5	(a) Normal plasma (0·5 ml)	19·0	38·5	13·0
	(b) Uraemic plasma (0·5 ml)	11·5	43·0	21·0
6	(a) Normal plasma (0·5 ml)	12·0	28·5	16·0
	(b) Uraemic plasma (0·5 ml)	13·0	33·0	25·0

Table III *The ADP-induced aggregation of fresh normal platelets after 30 minutes' incubation with stored normal or uraemic platelet-poor plasma*

[1]Final platelet count 250 × 10^9/1 and final volume 2 ml in each case.
Basic experimental details as in Table III.

PLATELET AGGREGATION AND RETENTION AND RENAL FUNCTION

There was a significant negative correlation (Fig. 4) between the urea levels and the whole blood platelet count ($P = < 0.05$), the percentage of platelets retained in the glass bead columns ($P = < 0.05$), and the percentage aggregation with ADP ($P = < 0.05$). No significant relationship was detected between the urea levels and the rate or percentage aggregation with thrombin or the rate of aggregation with ADP.

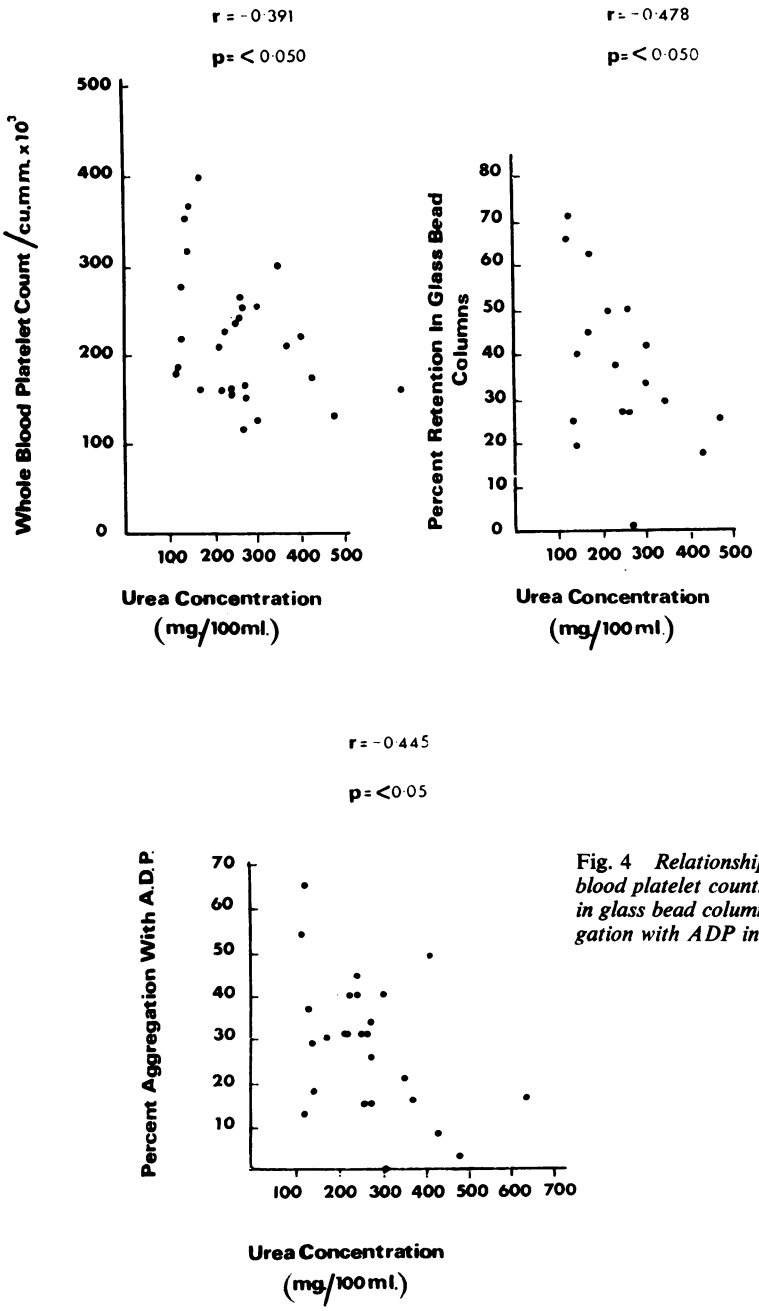

Fig. 4 *Relationships between serum urea levels and whole blood platelet counts, percentage platelet retention in glass bead columns, and percentage platelet aggregation with ADP in uraemic patients.*

There was also no significant correlation between the levels of creatinine and the rates or percentages of platelet aggregation with ADP or thrombin or the number of platelets retained in the glass bead columns.

OTHER PLATELET FUNCTION TESTS IN RENAL FAILURE

Measurements of the availability of platelet factor 3 after contact with kaolin were made in only seven patients and the value did not differ significantly from normal (mean 110%, SD 21·4). Platelet function in blood coagulation was also assessed by determining the prothrombin consumption index (PCI). The kaolin-cephalin clotting times and prothrombin times were normal in all patients except five (nos. 4, 5, 20, 28, 29) in whom the prothrombin times were slightly prolonged (Table I). The PCI determinations from these patients were excluded from the analyses in order to avoid as far as possible the effect of plasma coagulation defects on prothrombin consumption. The PCI was determined in 21 uraemic patients with normal coagulation tests. The values ranged from 7% to 120% and in 11 patients was over 20% (the upper limit of normal in this laboratory). No significant correlation was detected between the PCI and the rates or percentages of platelet aggregation with ADP or thrombin or with the number of platelets retained in the glass bead columns. Nor did the PCI correlate significantly with the blood urea or creatinine levels. There was a tendency for higher platelet counts to be associated with lower values for the PCI but the correlation was not statistically significant ($P = > 0{\cdot}05$).

Clot retraction was measured in 12 patients but was not abnormal, ranging from 48% to 68% (mean 56%, SD 5·5). In normal subjects the range for the method used was 44-64%. The bleeding time was determined in 22 patients and was prolonged (> 5 minutes) in nine (Table I). There was no correlation between the bleeding times and any of the other measurements of platelet function, but it is noteworthy that three of the four patients with a bleeding tendency had prolonged bleeding times.

THE 'RELEASE' OF PLATELET PROCOAGULANT ACTIVITY DURING ADP-INDUCED AGGREGATION

This series of experiments was carried out in five pairs of normal subjects and uraemic patients. There was a reduction in the Stypven clotting time during aggregation which continued during disaggregation. The overall reduction of the Stypven time during aggregation and disaggregation, however, was not significantly different from normal in the uraemic patients and there was no significant differences between the final (minimum) clotting times (Table IV).

Subject[1]	*Stypven Clotting Times (sec)*		
	At Time 0	*At Maximum Aggregation*	*Final (minimum)*
N 1	36·5	30	25
N 2	40	31	25
N 3	38	23	21·5
N 4	47	37	28
N 5	42	29·5	23
N mean	40·7	30·1	24·5
U 1	48·5	33	25
U 2	43	26	17·5
U 3	35	24	18·5
U 4	42	37·5	30
U 5	44	33	23
U mean	42·5	30·7	22·8

[1]U = patients with chronic renal failure, N = paired normal control

Table IV *The Stypven times of platelet-rich plasma during ADP-induced platelet aggregation in health and chronic renal failure*

When the platelet-rich plasma was stirred without the addition of ADP only small and inconsistent shortening of the Stypven times occurred in healthy subjects and patients. Similar results were observed when ADP was added to platelet-rich plasma without stirring.

RELATIONSHIP BETWEEN BIOCHEMICAL MEASUREMENTS AND PLATELET ACTIVITY

The plasma fibrinogen concentration in the patients (range 3·62 g/l to 8·99 g/l; mean 6·23, SD 1·81) was significantly higher ($P = < 0{\cdot}001$) than the levels found in the normal subjects (range 2·0 g/l to 5·47 g/l, mean 3·57 g/l, SD 0·96). In the patients the mean serum calcium level was 1·99 m mol/l, SD 0·40 (7·9 mg/100 ml, SD 1·57), the inorganic phosphate concentration was 2·6 m mol/l, SD 0·7 (8·12 mg/100 ml, SD 2·2), and the magnesium concentration was 1·14 m mol/l, SD 0·17 (2·77 mg/100 ml, SD 0·41). There was no significant correlation between these values and any of the measurements of platelet activity.

Discussion

The results of the present study confirm the presence of defective platelets in chronic renal failure. Thrombocytopenia was not a prominent feature. The main abnormalities observed were reduced retention in glass bead columns, reduced rate of aggregation and disaggregation with ADP and thrombin and reduced degree of aggregation. Impaired prothrombin consumption apparently due to defective platelet function was present in about 50% of patients. In general the abnormalities were more severe in the more uraemic subjects.

The determination of platelet retention in glass bead columns was introduced by Hellem (1960) as an index of platelet adhesiveness and was subsequently found to be abnormal in renal failure (Hellem, Odegaard, and Skalhegg, 1964). It is possible that this technique measures the filtration of platelet aggregates rather than adhesion to glass. In a previous study (Bloom and Evans, 1969), a relationship was detected in normal individuals between the initial rates of platelet aggregation with ADP and thrombin and the percentage of platelets retained in glass bead columns. This was not observed in the present study. The reason for the discrepancy is not certain but a considerably lower concentration of ADP and relatively higher concentration of thrombin, compared to platelet number, was used in the present study. In uraemic subjects the percentage aggregation with ADP and thrombin did correlate with the percentage of platelets retained in the columns. It seems likely that platelet aggregation plays a part in the filtration of platelets in glass bead columns but the relationship is difficult to demonstrate in normal individuals and is apparently not a close one.

Platelet aggregation in uraemia has been the subject of several recent studies. Defective aggregation with ADP was found by Castaldi *et al* (1966), Holdrinet, Ewals, and Haanen (1968), and De Vries, ten Cate, den Hartog-Veerman, and van Dooren (1968). Salzman and Neri (1966) failed to demonstrate abnormal aggregation with ADP but found defective aggregation with thrombin. Three of the uraemic subjects studied by Rozenberg and Firkin (1966) showed defective platelet aggregation but it is not clear if this was with ADP or thrombin or with both. In the present study the effect of both ADP and thrombin was impaired in uraemic patients. The thrombin aggregation curves closely resembled those of Salzman and Neri (1966), who used similar concentrations of thrombin, and it is probable that the different results with ADP represent differences in experimental technique.

Thrombin not only causes platelet aggregation it also releases certain platelet contents such as adenine nucleotides. It has been suggested that thrombin-induced aggregation is mediated through the release of platelet ADP (Haslam, 1964). In the present study no correlation was detected between aggregation with ADP and thrombin in normal individuals but in uraemic subjects there was a positive correlation between the rate, and especially the extent, of platelet aggregation with ADP and thrombin. It seems possible therefore that if a common pathway is involved in both ADP and thrombin-induced aggregation, it is adversely affected in uraemia.

The role of disaggregation in haemostasis is not clear but it seems likely that haemostasis would be favoured if disaggregation were slow. Studies of disaggregation in renal failure are few. Rapid disaggregation after ADP was reported by De Vries *et al* (1968) and occasionally by Hutton and O'Shea (1968) but diminished disaggregation after ADP was noted by Hassanein, McNicol, and Douglas (1970). The disaggregation curves after the addition of thrombin illustrated by Salzman and Neri (1966) suggest slow disaggregation but the authors did not comment on this. Our findings support those of Hassanein *et al* (1970) and Salzman and Neri (1966). Disaggregation may occur because of degradation of ADP by plasma and platelet enzymes (Hellem and Owren, 1964; Salzman, Chambers, and Neri, 1966). There is evidence that ADP breakdown is impaired in renal failure (Gan and Firkin, 1968) and this could account for our present findings. If, however, the disaggregation phase of reversible aggregation is an important factor in uraemic bleeding, a faster than normal disaggregation rate would be expected. Our results therefore throw some doubt on the significance of disaggregation in haemostasis in renal failure.

Plasma coagulation defects are relatively uncommon in chronic renal failure and were present in only five of our patients. On the other hand, even when coagulation tests were normal, prothrombin consumption was impaired in half the patients. Under these circumstances abnormal prothrombin consumption was probably due to defective platelet procoagulant function. Although this was not detected by the kaolin-activation method it could have been due to the defective reaction of platelets to thrombin.

The results of the present study confirm the observation of Hardisty and Hutton (1966) that platelet coagulant activity is made available during aggregation. No consistent differences were observed, however, between the results with Stypven in normal subjects and patients, although in the latter platelet aggregation was impaired. Our findings suggest that although procoagulant activity is made available during platelet aggregation with ADP these are not necessarily parallel indices of platelet function.

In acute renal failure thrombocytopenia is relatively common and circulating fibrin degradation products (FDP) may suggest the possibility of intravascular coagulation. Although FDP may affect platelets it is uncertain if they play an important part in the pathogenesis of the platelet defects in chronic renal failure. It is possible that in some patients the most reactive platelets are at times deposited in the renal vasculature leaving a less

reactive peripheral population. Thrombocytopenia, however, was uncommon in the present study and mixing experiments suggested an effect of uraemic plasma on normal platelets. Urea has been reported to inhibit platelet aggregation (Hellem *et al*, 1964) and when administered orally to normal subjects it caused impaired platelet adhesiveness (Eknoyan, Wacksman, Glueck, and Will, 1969). In one study, however, infusion of urea into normal subjects did not induce platelet defects (Castaldi *et al*, 1966), and it has even been reported to enhance platelet aggregation (Salzman and Neri, 1966). It seems possible, therefore, that the platelet defects in uraemia are due to accumulation of some substances other than urea. The fact that the platelet abnormalities may be corrected by haemodialysis (Castaldi *et al*, 1966) also supports a humoral hypothesis but whether the substances concerned act upon the bone marrow or peripheral platelets is uncertain. Horowitz, Cohen, Martinez, and Papayoanou (1967) described an inhibitory effect of uraemic plasma on factor 3 activity of normal platelets and suggested that this may be due to guanidino-succinic acid, but De Vries *et al* (1968) were unable to confirm this. An inhibitory effect of phenolic compounds has also been suggested (Rabiner and Molinas, 1970). On the other hand, Lewis, Zucker, and Ferguson (1956) failed to detect any effect of uraemic plasma on normal platelets. Similarly Salzman and Neri (1966) were unable to detect an inhibitory effect of uraemic plasma on thrombin aggregation. Salzman and Neri concluded that the defect resides in the platelets rather than in the plasma. While thrombopoiesis may be impaired, most of the evidence seems to indicate that humoral factors are largely responsible for the platelet defects found in renal failure. Identification of these factors could well be of value both in the study of normal haemostasis and in the prevention of thrombosis.

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References

Biggs, R., and MacFarlane, R. G. (1962). *Human Blood Coagulation and Its Disorders*, 3rd ed. Blackwell, Oxford.

Bloom, A. L., and Evans, E. P. (1969). Relationship of platelet retention in glass bead columns to the rate of aggregation with adenosine diphosphate and thrombin. *J. clin. Path.*, **22**, 560-562.

Castaldi, P. A., Rozenberg, M. C., and Stewart, J. H. (1966). The bleeding disorder of uraemia. *Lancet*, **2**, 66-69.

De Vries, S. I., ten Cate, J. W., den Hartog-Veerman, M. E., and van Dooren, M. M. (1968). Quelques remarques sur la thrombocytopathie acquise chez les malades urémiques. *Schweiz. med. Wschr.*, **98**, 1658-1660.

Eknoyan, G., Wacksman, S. J., Glueck, H. I., and Will, J. J. (1969). Platelet function in renal failure. *New Engl. J. Med.*, **280**, 677-681.

Fiske, C. H., and Subbarow, Y. (1925). The colorimetric determination of phosphorous. *J. biol. Chem.*, **66**, 375-400.

Folin, O., and Wu, H. (1947). In *Practical Physiological Chemistry*, edited by P. B. Hawk, B. L. Oser, and W. H. Sammerson, 12th ed. Churchill, London.

Gan, I. E. T., and Firkin, B. G. (1968). Adenosine diphosphate (ADP) breakdown in human plasma with reference to platelet aggregation in uraemia. *Thrombos. Diathes. haemorrh.* (*Stuttg.*), **19**, 438-450.

Hardisty, R. M., and Hutton, R. A. (1965). The kaolin clotting time of platelet-rich plasma. A test of platelet factor-3 availability. *Brit. J. Haemat.*, **11**, 258-268.

Hardisty, R. M., and Hutton, R. A. (1966). Platelet aggregation and the availability of platelet factor 3. *Brit. J. Haemat.*, **12**, 764-776.

Haslam, R. J. (1964). Role of adenosine diphosphate in the aggregation of human blood platelets by thombin and fatty acids. *Nature* (*Lond.*), **202**, 765-768.

Hassanein, A. A., McNicol, G. P., and Douglas, A. S. (1970). Relationships between platelet function tests in normal and uraemic subjects. *J. clin. Path.*, **23**, 402-406.

Hellem, A. J. (1960). Adhesiveness of human blood platelets in vitro. *Scand. J. clin. Lab. Invest.*, **12**, Suppl. 51.

Hellem, A. J., Odegaard, A. E., and Skalhegg, B. A. (1964). Platelet adhesiveness in chronic renal failure. In *Proceedings of the Xth Congress of the International Society of Haematology, Stockholm.*

Hellem, A., and Owren, R. A. (1964). The mechanism of the hemostatic function of blood platelets. *Acta Haemat.* (*Basel*), **31**, 230-238.

Holdrinet, A., Ewals, M., and Haanen, C. (1968). 'Platelet function and von Willebrands' disease. (Letter). *Lancet*, **1**, 1150.

Horowitz, H. I., Cohen, B. D., Martinez, P., and Papayoanou, M. F. (1967). Defective ADP-induced platelet factor-3 activation in uremia. *Blood*, **30**, 331-340.

Hutton, R. A., and O'Shea, M. J. (1968). Haemostatic mechanism in uraemia. *J. clin. Path.*, **21**, 406-411.

Lewis, J. H., Zucker, M. B., and Ferguson, J. H. (1956). Bleeding tendency in uremia. *Blood*, **11**, 1073-1076.

Marsh, W. H., Fingerhut, B., and Miller, H. (1965). Automated and manual direct methods for the determination of blood urea. *Clin. Chem.*, **11**, 624-627.

Proctor, R. R., and Rapaport, S. I. (1961). The partial thromboplastin time with kaolin. *Amer. J. clin. Path.*, **36**, 212-219.

Rabiner, S. F., and Molinas, F. (1970). The role of phenol and phenolic acids on the thombocytopathy and defective platelet aggregation of patients with renal failure. *Amer. J. Med.*, **49**, 346-351.

Ratnoff, O. D., and Menzie, C. (1951). A new method for the determination of fibrinogen in small samples of plasma. *J. Lab. clin. Med.*, **37**, 316-320.

Rozenberg, M. C., and Firkin, B. G. (1966). The rate of platelet aggregation in haemorrhagic disease and thrombosis. *Scand. J. Haemat.*, **3**, 5-18.

Salzman, E. W., Chambers, D. A., and Neri, L. L. (1966). Possible mechanism of aggregation of blood platelets by adenosine diphosphate. *Nature* (*Lond.*), **210**, 167-169.

Salzman, E. W., and Neri, L. L. (1966). Adhesiveness of blood platelets in uremia. *Thrombos. Diathes. haemorrh.* (*Stuttg.*), **15**, 84-92.

Varley, H. (1967). *Practical Clinical Biochemistry*, 4th ed. Heinemann, London.