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Haemostatic mechanism in uraemia

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SYNOPSIS The haemostatic mechanism was investigated in 20 patients with renal failure, of whom nine had evidence of a bleeding tendency. A defect of platelet function was the most common finding. The effect of dialysis on the bleeding state is briefly discussed, and a scheme for the routine investigation of haemostasis in renal failure is put forward.

The occurrence of a bleeding diathesis as a complication of renal failure has frequently been described. Recent advances in the management and prognosis of acute and chronic renal disease have made it desirable to undertake both a clinical appreciation and a thorough laboratory investigation of the incidence and nature of this complication.

In patients in whom a bleeding tendency exists, or where surgery or needle biopsy is indicated, it would be a great advantage to know whether correction of the biochemical abnormalities would lessen the risk of severe haemorrhagic complications. The object of this study was to investigate the haemostatic mechanism in an unselected group of uraemic patients. An attempt was made to relate the laboratory results to the occurrence of clinical bleeding, and to the safety of biopsy techniques. In addition, some cases were re-assessed after alleviation of the uraemic state by dialysis.

A number of workers have attempted to define the nature of the bleeding tendency, and a variety of abnormalities have been reported. Commonly, either thrombocytopenia (Altschuler, Marcus, and Ullman, 1960; Willoughby and Crouch, 1961) or a qualitative platelet disorder (Willoughby and Crouch, 1961; Cahalane, Johnson, Monto, and Caldwell, 1958; Castaldi, Rozenburg, and Stewart, 1966; Salzman and Neri, 1966) have been found. Combined defects of platelet function and coagulation factors have been reported (Cheney and Bonnin, 1962; Rath, Mailliard, and Schreiner, 1957; Kendall, Lowenstein, and Morgen, 1961), and in particular Cheney and Bonnin (1962) detected a factor VII deficiency in 13 out of 33 uraemic subjects. Because of the 'vascular' nature of the bleeding tendency, a capillary abnormality has been postulated (Kendall *et al.*, 1961; Kuhlbach, 1957).

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MATERIALS AND METHODS

Studies were made on an unselected group of 20 patients suffering from renal failure due to a variety of causes, and including a wide range of degree and duration of uraemia (Table I). A careful history was taken from each patient, and an examination made for evidence of bruising or active bleeding.

Platelet factor 3 availability (PF3a) was determined by a method based on the kaolin clotting time of dilutions of platelet-rich plasma. To calculate the PF3a index, the platelet count of a mixture of test platelet-rich plasma and control platelet-poor plasma is compared with that of a dilution of normal platelet-rich plasma which gives the same kaolin clotting time (Hardisty and Hutton, 1965). Platelet aggregation was measured by Born's method (1962) and platelet adhesiveness by Salzman's technique (1962), and by a modification of Hellem's method (1960) using a 1 g bead column through which was passed 2 ml of blood. The dilute blood clot lysis (Fearnley, Balmforth, and Fearnley, 1957) was used to measure fibrinolytic activity and plasminogen was assayed according to Alkjaersig, Fletcher, and Sherry (1959). Factor VII was assayed as described by Hall, Rapaport, Ames, and Degroot (1964) and all other methods were performed as described by Hardisty and Ingram (1965).

RESULTS

HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS These were carried out on the same day as the haemostatic investigation, and the results are shown in Table II. Apart from the biochemical disturbances, the most consistent feature was the normocytic, normochromic anaemia. The platelet count was often below the normal range, but was below 100,000/cu mm in only three cases. Platelet morphology was normal in every case.

BLOOD COAGULATION AND FIBRINOLYSIS STUDIES The results of various tests of the coagulation and

TABLE I

CLINICAL MATERIAL

Case Number	*Age*	*Sex*	*Diagnosis*	*Duration of Uraemia*	*Bleeding Symptoms*	*Dialysis*	*Surgery without Haemorrhage*
1	65	F	Chronic calculi, pyelonephritis	3 mth	None	P	Abdominal
2	65	M	Chronic nephritis	1 yr	None	—	Bone marrow
3	50	M	Calculi, nephrectomy	3½ yr	None	—	Abdominal
4	70	M	Diabetic nephropathy	4 yr	None	—	
5	39	M	Nephrocalcinosis	6 mth	None	—	Liver biopsy
6	41	M	Nephrosis	3 mth	None	—	Renal biopsy
7	44	M	Gold toxicity	1 mth	None	—	Bone marrow
8	28	F	Malignant hypertension	1 mth	None	—	Renal biopsy
9	31	M	Obstructive uropathy	2 yr	None	—	
10	22	F	Acute renal failure, septicaemia	2½ wk	None	H-P	Dental extractions
11	49	M	Acute renal failure	10 days	None	—	
12	55	M	Acute renal failure, septicaemia	1 wk	Melaena	H	
13	15	M	Congenital hydronephrosis	15 yr	Bruising	—	Abdominal
14	48	F	Acute renal failure, P.N.H.	1 wk	Bleeding PV Haematuria	P	
15	34	F	Chronic glomerulonephritis	1 yr	Epistaxis	—	Renal biopsy
16	41	M	Hydronephrosis	10 yr	Epistaxis Melaena	H	Abdominal
17	33	M	Malignant hypertension	3 wk	Bruising	P	Renal biopsy
18	26	M	Malignant hypertension	1 wk	Purpura Epistaxis	P	Renal biopsy
19	31	F	Nephrosis	9 mth	Multiple haematoma	P	Renal biopsy
20	51	F	Primary amyloidosis	6 yr	Bruising	—	Liver biopsy

TABLE II

HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS

Case No.	*Hb (g/%)*	*PCV (%)*	*MCHC (%)*	*Retics (%)*	*WBC per (cu mm)*	*Platelet Count × 1,000 (cu mm)*	*E.S.R. (West.) (mm/1 hr)*	*Plasma Na (mEq/l)*	*Plasma YCo_3 (mEq/l)*	*Plasma K (mEq/l)*	*Blood Urea (mg/%)*	*Serum Proteins (g/%)*	*Serum Ca (mg/%)*
1	10·8	31	35	—	6,300	304	80	141	24	4·3	74	8·0	—
2	9·0	28	32	—	6,600	234	40	136	15	5·4	224	6·5	8·2
3	13·3	40	33	—	11,000	152	—	128	16	2·7	66	6·9	9·6
4	9·8	30	33	—	7,500	239	110	132	21	5·0	230	7·5	7·2
5	9·8	36	27	—	7,000	291	—	137	26	3·2	124	6·7	9·5
6	11·8	36	33	—	7,900	130	60	135	24	3·7	234	6·4	—
7	9·0	30	30	—	1,300	205	41	141	23	3·3	195	5·8	—
8	8·7	27	32	16·5	5,900	159	79	138	26	4·1	80	5·2	—
9	9·0	29	31	—	6,500	171	122	128	28	3·5	475	7·1	—
10	10·5	34	31	—	31,000	326	85	138	29	4·9	84	6·0	7·2
11	9·5	29	33	2·0	15,000	630	—	142	22	4·5	330	5·9	—
12	12·7	39	33	0·6	13,500	78	60	136	22	5·5	250	5·6	8·6
13	9·2	28	33	—	6,700	205	81	127	12	4·4	390	6·8	6·2
14	9·5	31	31	2·0	2,800	73	—	136	28	3·5	135	6·5	—
15	8·7	30	29	—	10,100	181	122	127	19	4·5	212	7·1	—
16	8·2	24	34	1·4	5,400	112	—	143	14	5·1	380	6·1	8·2
17	9·8	29	34	3·0	8,400	130	—	136	28	6·6	180	6·9	—
18	7·0	20	35	4·4	8,000	42	35	132	20	3·5	530	4·2	—
19	5·2	15	35	1·0	11,400	102	—	135	21	5·3	232	7·0	—
20	7·2	22	33	—	7,900	133	105	142	14	4·8	320	7·3	—

fibrinolytic mechanisms are shown in Table III. Case 15 had an isolated moderate deficiency of factor VII (17%) and case 19, who had been receiving heparin therapy until 24 hours before testing, had a slight prolongation of the prothrombin and partial thromboplastin times. The stypven time was normal in all of 17 cases tested, indicating that factor X levels were not reduced. The factor VIII and fibrinogen levels were often above the normal range, while the dilute clot lysis times were longer than normal in all but four cases. There was no obvious correlation between these findings, or with the plasma plasminogen levels, which were usually within normal limits and significantly below them in only two cases.

PLATELET FUNCTION TESTS Platelet function was studied in various ways and the results are given in Table VI. The bleeding time was significantly prolonged in only one patient (case 13), and in no case did the amount of blood lost appear to be excessive, although this was not measured quantitatively. The initial rate of platelet aggregation by adenosine diphosphate (ADP) was normal in all cases tested, but

TABLE III

COAGULATION AND FIBRINOLYSIS STUDIES

Case Number	P.T. (Quick) (sec)	P.T.T. (sec)	Factor VIII (%)	Fibrinogen (mg/%)	Dilute Blood Clot Lysis (hours)	Plasma Plasminogen (units/ml)	Factor IX (%)	Factor XIII
1	12·2	28·0	140	650	>8	—	—	—
2	12·1	31·0	230	570	>8	5·5	—	—
3	13·0	34·5	190	675	3½	3·9	—	Normal
4	14·5	34·5	200	1,100	>8	4·6	150	Normal
5	14·7	36·5	175	350	4½	3·8	—	Normal
6	12·9	42·0	145	600	—	2·2	—	Normal
7	13·2	48·5	240	445	20	3	100	Normal
8	11·8	47·0	145	400	>8	3·9	150	Normal
9	12·3	46·5	130	480	>24	2·0	90	Normal
10	12·8	28·5	150	890	7	—	—	Normal
11	14·2	35·5	—	—	8	—	—	—
12	13·3	41·5	320	450	>8	3·9	140	—
13	13·3	36·5	600	740	4½	5·7	205	Normal
14	13·9	42·5	265	690	—	4·2	—	Normal
15	17·9	45·0	105	815	>8	—	—	Normal
16	12·3	34·0	540	595	>8	2·9	125	—
17	11·9	36·5	165	630	>30	4·3	—	—
18	14·3	39·0	85	600	>24	3·2	100	—
19	16·0	49·0	—	—	>8	3·2	—	—
20	14·9	38·0	210	865	>7	4·8	—	—
Normal range	11·5-15·0	30-45	50-200	200-500	<7	2-5	50-200	

TABLE IV

RESULTS OF PLATELET FUNCTION TESTS

Case No.	Bleeding Time (Ivy) (min)	PF3a Test (%)		Platelet Adhesiveness (%)		Platelet Aggregation with 10^{-5}M ADP
		$-^1FT^4$	$+^1FT^4$	Salzman	Hellem	
1	4	80		20	—	Normal
2	4	63		30	—	Normal
3	3½	200		10	—	Normal
4	8	200		0	—	Normal
5	4	125		43	41	Normal
6	6½	115		12	49	Normal
7	3½	52		10	63	Normal
8	8	40	45	1	32	Normal
9	6	40		0	28	Normal
10	3	64		27	41	Normal
11	3	90		48	—	Normal
12	3	110		0	—	Normal
13	11	23	70	0	29·5	Normal but marked disaggregation
14	—	5	40	0	—	Normal but moderate disaggregation
15	7½	93		42	49	—
16	3	28		1·5	26	Normal
17	4½	6	55	11·5	—	Normal but moderate disaggregation
18	—	100		—	—	—
19	—	—	—	—	—	—
20	6	49		0	23	Normal but moderate disaggregation
Normal range	<7	>40	>40	>20	>20	

$^1FT^4$ = freezing and thawing four times.

in four patients the normal rapid aggregation was followed by unusually rapid disaggregation. This phenomenon has been observed in a number of other patients without renal disease, and is discussed elsewhere (Hardisty and Hutton, 1967).

Three of these four patients had decreased PF3a and the fourth was close to the lower limit of normal. In each case the PF3a index was partially corrected by rapidly freezing and thawing the platelets four times. One patient (case 16) had a reduced PF3a index, but the platelets were not retested after disruption.

Platelet adhesiveness to glass measured by Salzman's method was below normal limits in 12 of 18 cases tested. Using a modification of Hellem's method, however, platelet adhesiveness was normal in all of ten cases tested, including eight patients with reduced adhesiveness measured by Salzman's method.

Praga and Cortellaro (1967), using a rotating bulb

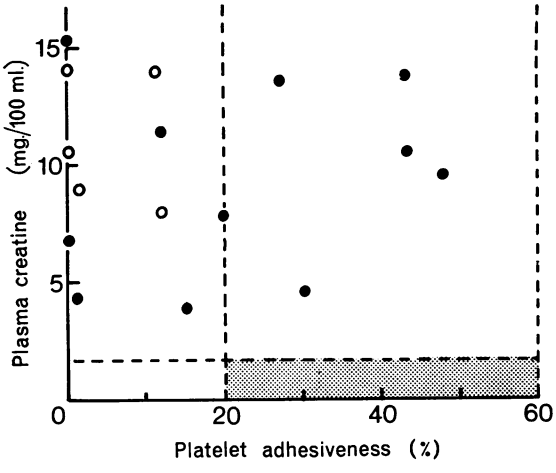

FIG. 1. *Platelet adhesiveness (Salzman) and creatinine levels.*
● *non-bleeders*
○ *bleeders*
Shaded area represents normal range.

method, have reported a significant inverse correlation between platelet adhesiveness and serum creatinine level in 17 patients with chronic renal failure. As can be seen from Fig. 1 we have found no such correlation in the present group using Salzman's technique.

The effect of uraemic plasma on platelet function was studied in two ways: first, by using fresh citrated platelet-poor plasma in which crystalline urea had been dissolved, and secondly by using citrated platelet-poor plasma from a uraemic patient (case 20) with bleeding symptoms and a defect in several of the platelet function tests. In each case, 5 ml of uraemic plasma, or as control 5 ml of normal plasma, was added to 10 ml of fresh normal citrated blood. The blood samples were left at room temperature for one hour before centrifuging, and so tests were begun one and a half to two hours after adding the test plasma. Similar results were obtained in three such experiments, one of which is reported in Table V, and these show that neither urea nor uraemic plasma were able to confer an abnormality on normal platelets in the system used.

Laboratory tests were performed before and after dialysis in two patients (cases 12 and 16). The low PF3a index in case 16 was completely corrected and clinical improvement was marked, his epistaxis and melaena ceasing abruptly. This patient was given haemodialysis as a preparatory measure for a renal plastic operation. The biochemical improvement was good, and blood loss at operation next day was normal. Dialysis was without effect on the abnormal Salzman test in both cases. No significant effect was observed in other tests of the haemostatic mechanism, all of which were normal.

DISCUSSION

One of the most difficult problems which arose during this study was to decide what constituted abnormal bleeding. Many patients with renal disease have hypertension, which itself is associated with an increased incidence of epistaxis. This bleeding symptom was seen in three patients, all of whom were hypertensive (cases 15, 16, 18). Two of these had other clinical signs of bleeding and all three had one or more abnormalities of laboratory tests.

Six other patients had some clinical evidence of bleeding, and in five of these an abnormality of platelet function was found.

Of the remaining 11 patients without evidence of abnormal bleeding, six had reduced platelet adhesiveness measured by Salzman's method in the absence of any other abnormality. Of these six cases, four had normal adhesiveness measured by Hellem's method. The complete lack of correlation between the Salzman and Hellem tests in uraemia is paralleled in von Willebrand's disease and there is no satisfactory explanation at present for either situation. The Salzman test is clearly an extremely sensitive method for measuring platelet adhesiveness, and it is possible that platelets can exhibit reduced adhesiveness in this system without clinical bleeding manifestations becoming apparent. Because it is non-specific, and fails to correlate well with clinical bleeding, the Salzman test is of limited value in the diagnosis of haemorrhagic disorders.

TABLE V

EFFECT OF UREA AND URAEMIC PLASMA ON NORMAL PLATELET FUNCTION

Addition Made to Normal Blood	*Blood Urea (mg %) Final Concentration*	*Platelet Count (10^3 cu mm)*	*PF3a Index (%)*	*ADP Aggregction*	*Platelet Adhesiveness (Hellem) (%)*
Nil	30	175	100	Normal	28
Normal platelet-poor plasma	34	135	93	Normal	31
Normal platelet-poor plasma and urea	649	131	68	Normal	29
Uraemic platelet-poor plasma (case 20)	220	128	80	Normal	27·5

Using qualitative methods, pletelet aggregation by ADP has been reported as being normal in uraemic subjects (Hardisty and Hutton, 1965). The use of a more sensitive technique, as here, has revealed some abnormalities of platelet behaviour in response to ADP, which in some patients seem to be related to the bleeding tendency.

No abnormality of platelet function developed in blood incubated for two hours with urea or uraemic plasma. These results indicate that neither urea alone, nor any other toxic substance in uraemic plasma, can rapidly induce a functional defect by direct action on the platelet, and they are in agreement with the findings of Castaldi *et al.* (1966), who noted that platelet function was not impaired by infusing urea into a normal person to a level of 200 mg per 100 ml blood. None of these observations exclude the possibilities that abnormal platelet function only develops after prolonged exposure to a biochemically abnormal atmosphere, as would occur during the course of renal disease, or that functionally abnormal platelets are formed in the bone marrow. If the former explanation is correct, the abnormality would have to develop during the life span of the platelets, reported as being normal in renal disease (Stewart, in press) and would therefore not be expected to increase with the duration of the uraemia beyond about 10 days.

Thrombocytopenia is a common finding in uraemia and it may develop as part of a general marrow hypoplasia such as is often found in renal disease. In this series the platelet count was below the normal range in eight cases, but was less than 50,000 per cu mm in only one patient. Regardless of the degree of thrombocytopenia, the bleeding tendency was more severe when platelet function was also impaired (cases 14, 16, 17, 20) than when it was normal (cases 12, 6, 18). The severe bleeding state of case 19 was probably due to a combination of mild thrombocytopenia and the effect of the heparin therapy she was receiving.

High levels of fibrinogen and factor VIII have often been reported in renal disease, but the mechanism by which this occurs is still in dispute. Chronic or acute infections, often present in renal disease, will stimulate factor VIII and fibrinogen production, and the prolonged clot lysis times reported here may indicate an excess of substrate fibrinogen rather than decreased fibrinolytic activity. The usually normal plasminogen levels lend support to this concept.

Eight patients were dialysed, including six with clinical evidence of bleeding. Only one case showed any clear-cut improvement, with sudden cessation of the bleeding signs and correction of a previously low PF3a index.

CONCLUSION

Although the laboratory tests were not of much value in predicting the haemostatic response to surgery in the cases reported here, it is nevertheless advisable to investigate haemostasis before undertaking surgical procedures in patients suffering from renal diseases. The coagulation mechanism was normal in all but two of our patients, but reports of isolated or combined deficiencies of clotting factors have been reported, and so prothrombin time and partial thromboplastin time tests should be performed to check the adequacy of the coagulation mechanism.

The poor correlation between platelet function tests and surgical bleeding may be partly explained by the fact that patients were generally dialysed and sometimes transfused before surgery but after laboratory tests had been performed. Although with one exception these procedures had no immediate effect on the bleeding symptoms where present, the possibility that some toxic substance responsible for the bleeding was thereby removed or diluted cannot be excluded. In addition it would be an advantage to the surgeon to know whether or not a platelet abnormality was present before surgery, so that suitable measures could be taken should surgical bleeding occur. To this end a bleeding time and a test of the availability of platelet factor 3 seem to provide the most adequate information.

The observation here that Salzman's platelet adhesiveness test was abnormal in patients with or without bleeding symptoms, while Hellem's test was always normal, seems to detract from the value of these methods as routine screening tests of haemostatic function.

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