Platelets and Platelet Phosphatides in Uremia

By Gerald Altschuler, Aaron J. Marcus and Harris L. Ullman

The hemorrhagic tendency which develops in uremia is a well recognized clinical entity but the pathogenesis of this complication is not understood. In the past defective blood coagulation as well as increased vascular fragility have been suggested. The possibility of a functional blood platelet abnormality was first noted by Lewis, Ferguson and Zucker and recently Cahalane, Johnson, Monto and Caldwell reported defective blood thromboplastin formation, attributing this to a decrease in platelet factor 3 activity. In addition they found that plasma from uremic subjects reduced the platelet factor 3 activity of normal platelets. Prompted by these reports, a study was undertaken to investigate the possibility of a qualitative defect in the platelets or platelet phosphatides of uremic patients.

Materials and Methods

Twenty patients in the uremic state were selected from the medical wards of the New York Veterans Administration Hospital. No attempt was made specifically to study patients with a hemorrhagic diathesis although seven exhibited a bleeding tendency. The average blood urea nitrogen in the series was 100 mg. per 100 ml. Care was taken to exclude subjects with prerenal azotemia.

Blood for all determinations was collected by gravity flow through plastic tubing with attached siliconized needles, thereby avoiding the use of syringes.

Prothrombin consumption was measured by the method of Ware and Stragnell. After incubation for one hour and twenty minutes at 37 C., serum prothrombin times were determined in duplicate and two separate determinations were done on each subject. The normal prothrombin consumption time in this laboratory is a value over 45 seconds.

Platelet counts were done by the method of Brecher, Schneiderman and Cronkite. The normal range in this laboratory is 250,000-500,000 per cu. mm.

The thromboplastin generation test (TGT) was performed by the method of Biggs and Douglas, as modified by Spae but except that imidazole buffer (pH 7.35, 270 millimols per liter) was used as diluent. For platelet studies in the test, blood was collected in two 40 ml. siliconized tubes using 4 ml. 3.8 per cent citrate as anticoagulant in each tube. Platelets were washed four times in saline and used in serial dilution. Each time a dilution was made an aliquot was removed and the platelets enumerated. The count reported was that of the reagent tube, and of course was further diluted by the other reagents in the test. The serum used in the TGT control was from the same donor at all times and the values were consistently 9-11 seconds, 7-9 seconds, and 7-9 seconds respectively for 2, 4 and 6 minutes of incubation. Thromboplastic activity was considered poor if the 6 minute time was more than 10 seconds over the control value. The serum and plasma of each patient was tested to rule out any defect in these reagents.

For the study of the platelet phosphatides, 500 ml. of blood were collected into plastic...
bags and the packed cells later returned to the patient. The platelets were isolated and the lipids extracted as previously described except that the total phospholipid from each patient was stored in 5 ml. petroleum ether at -20 C. One milliliter aliquots were removed and taken to dryness under prepurified nitrogen and suspended in 0.3 ml. imidazole buffer. Dispersion was accomplished by means of rapid agitation with a glass rod, and the contents were arbitrarily designated as the "undiluted" specimen.

Lipid phosphorus was determined on the original 5 ml. sample according to the method of Dryer, Tammes and Routh, and was calculated for each serially diluted tube. Results were expressed as the amount of platelet lipid phosphorus in the generating tube of the TGT.

The phosphatides were examined by paper and column chromatography, utilizing methods previously reported. The residual lipid from nine patients was pooled and subjected to silicic acid column chromatography with examination of the eluates by means of phosphorus determinations and paper chromatography.

To evaluate the effect of increasing platelet concentration on prothrombin consumption, a patient with thrombocytopenia and poor prothrombin utilization was studied in the following manner: 0.9 ml. platelet-free native plasma was added to 0.1 ml. freshly prepared platelets which were serially diluted and then counted. Serum prothrombin times were measured in duplicate after one hour and twenty minutes of incubation at 37 C. Four combinations, including the patient’s plasma, patient’s platelets, plus normal plasma and normal platelets were tested. The dilutions of platelets rendering comparable prothrombin consumption were compared.

All studies were done with freshly prepared platelets.

**RESULTS**

Prothrombin consumption was compared to platelet count in 19 patients (fig. 1). Of 10 subjects with thrombocytopenia, 6 had abnormal and 4 had normal consumption. In 9 patients with normal platelet counts 5 had abnormal and 4 had normal consumption. As will be shown the platelets of the 5 patients who had abnormal prothrombin utilization and normal counts behaved normally in the thromboplastin generation test and presented chromatographic abnormalities.

Table 1 shows the effect of increasing the platelet concentration on prothrombin consumption in a patient who originally had thrombocytopenia and poor prothrombin utilization. It is seen that improvement in prothrombin consumption appears directly proportional to the amount of platelets added to native plasma in both the uremic and normal subject in our system.

Prior to the evaluation of the platelets in the TGT the serum and plasma of each subject were studied and were normal in all patients. The thromboplastin generation of uremic platelets was compared with four normal controls (fig. 2). It was found that normal and uremic platelets behaved similarly when comparable amounts were tested. The azotemic platelets tolerated dilution as well as normals. Each lost activity at similar concentration and there was no indication that more uremic platelets were needed to give the same thromboplastic activity as the controls.

Uremic and normal platelet lipids were compared in a manner similar to that used for the whole platelet. The actual amount of platelet lipid phosphorus in the 0.1 ml. added to the generation mixture is reported. It was found that the amount of "cephalin" required for thromboplastic activity was essentially the same for the uremic patients and normal controls. In addition, an anticoagulant effect was observed in both groups when the concentration of lipid was high (fig. 3).
Paper chromatographic study of the uremic platelet phosphatides revealed the same qualitative pattern previously reported for normal platelet phospholipids. The fractions found were, in order of Rf value; phosphatidylethanolamine, phosphatidylserine, lecithin, sphingomyelin, and inositol phosphatide. The results of column chromatography of pooled uremic "cephalin" with paper identification of each fraction were quite similar to those found in earlier studies on normal platelets (fig. 4). Ninety-one per cent of the lipid applied to the column was recovered.

Table 1.—Serum Prothrombin Time of Platelet-Free Native Plasma (Poor prothrombin consumption in a patient with thrombocytopenia was corrected by increasing the platelet concentration. Normal platelets and plasma were used as controls in the combinations indicated)

<table>
<thead>
<tr>
<th>Combination</th>
<th>Seconds</th>
<th>Count (mm.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's Platelets + Normal Plasma</td>
<td>39</td>
<td>304,000</td>
</tr>
<tr>
<td>Patient's Platelets + Patient's Plasma</td>
<td>86</td>
<td>608,000</td>
</tr>
<tr>
<td>Normal Platelets + Patient's Plasma</td>
<td>40</td>
<td>397,000</td>
</tr>
<tr>
<td>Normal Platelets + Normal Plasma</td>
<td>59</td>
<td>700,000</td>
</tr>
<tr>
<td>Patient's Plasma</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Normal Plasma</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Original Platelet Count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Prothrombin Time of Patient's Whole Blood</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

*Performed after dilution.
Fig. 2.—Thromboplastin generation of platelets of uremic subjects and normal controls. Points on the solid line represent the average clotting time after 6 minutes of incubation in the TGT (thromboplastin time) at the indicated range of platelet count in 20 uremic patients. The broken line represents the same study in 4 normal controls.

DISCUSSION

The most common abnormality in our study was thrombocytopenia, which is in agreement with other workers such as Kühlback who reported a low platelet count in 23 of 30 patients with uremia. Larrain and Adelson found thrombocytopenia in three patients with acute post-traumatic renal insufficiency and Rath, Maillard and Schreiner noted thrombocytopenia in 25 of 45 subjects in renal failure.

Impaired prothrombin consumption and thrombocytopenia can be explained in some patients on the basis of a quantitative platelet deficit; but instances of poor prothrombin utilization with normal platelet counts require further clarification. This phenomenon was also reported by Rath and co-workers who observed that 15 patients with platelet counts above 150,000 per cu. mm. demonstrated poor prothrombin utilization. In evaluating these results the possibility of a qualitative platelet defect was mentioned, but the authors found the thromboplastin generation tests difficult to interpret because of variable abnormalities in the plasma and serum reagents of the patients studied. Cahalane et al. reported that at comparable dilutions the platelets from
Fig. 3.—Thromboplastic activity of phospholipid extracts from uremic and normal platelets. The points represent the average thromboplastin time for the given range of phosphorus content in 16 uremic and 4 normal patients.

Uremic patients did not support thromboplastin generation as effectively as platelets from control subjects and felt that this supported the concept that a qualitative defect existed. In our series, platelets from the group of uremic patients with normal counts and poor prothrombin utilization behaved in a manner similar to those of normal subjects: they did not show a more rapid loss of activity attributable to dilution. In fact, ability to generate blood thromboplastin at similar platelet concentration as normal was independent of prothrombin utilization. Our data indicate that a qualitative platelet defect is not present and does not explain the prothrombin consumption paradox.

Additional evidence for a qualitative platelet defect in uremia was suggested recently by Geiger, Rath and Chung, who reported on chromatographic analysis of platelet phospholipids in one patient. A variation from the normal distribution of the phosphatides, particularly in the lysolecithin and sphingomyelin fractions was found. Our column chromatographic studies and paper chromatographic identification shows an essentially normal distribution of the platelet phosphatides. In addition, lipid from uremic patients' platelets showed activity similar to normals when used in the TGT.

To account for some of the differences in results, the following possibilities
Fig. 4.—Column chromatography of uremic platelet phosphatides. A total of 65 mg. phospholipid was placed on a column containing 10 Gm. siliceic acid. Superimposed on the phosphorus analyses are the results of paper chromatographic identification of the individual fractions in order of their respective Rf values. PE, phosphatidylethanolamine; PS, phosphatidylserine; LEC, lecithin; SPH, sphingomyelin; INOS, inositol phosphatide.

are mentioned: the technics used in measuring prothrombin utilization vary in different laboratories. Secondly, we have noted that serial dilution of platelets without enumeration of each specimen is not reliable. Moreover, the numerical definition of thrombocytopenia is not uniform, and techniques of blood collection differ. In the studies of phospholipids, technical variations in extraction and separation might influence the results. Finally, there is the possibility that different types of uremic patients are being evaluated in the various laboratories.

In conclusion it is our feeling that the contribution of platelets to the hemorrhagic disorder in the uremic state is a quantitative phenomenon, and we could find no evidence of a qualitative disturbance in the blood platelets of uremic patients.

SUMMARY

1. The platelets of 20 uremic subjects were studied, utilizing the following procedures; phase platelet count, serum prothrombin time, thromboplastin
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generation test with evaluation of plasma, serum, and platelet reagents.
2. Platelet phospholipids from these subjects were used as reagents in the
thromboplastin generation test and examined by means of silicic acid paper
and column chromatography.
3. Thrombocytopenia was the most common abnormality encountered and
was associated with either normal or abnormal prothrombin consumption. Some
patients demonstrated defective prothrombin utilization despite normal plate-
let counts, but their platelets had normal thromboplastic activity, as did those
of all patients studied.
4. The paper chromatographic pattern of the phosphatides in all subjects was
the same as that reported for normal platelets. Similar results were obtained
on column chromatographic analysis of a pooled extract from nine of the
uremic patients.
5. On the basis of these studies it was not possible to demonstrate a qualita-
tive platelet defect in uremia.

SOMMARIO IN INTERLINGUA

1. Esseva studiate le plachettas de 20 subjectos con uremia. Le technicas
usate esseva numeration del plachettas phasic, tempore de prothrombina
del sero, e generation de thromboplastina con evalutation del reagentes
plasmatic, soral, e plachettal.
2. Phospholipidos plachettal ab iste subjectos esseva usate como reagentes
in le test del generation de thromboplastina e examinate per medio de
chromatographia a papiro a acido silicic e per medio de chromatographia
columnal.
3. Thrombocytopenia esseva le anormalitate incontrate le plus frequentemence, e illo esseva associate con normal e etiam con anormal consumption
de prothrombina. Plure patientes monstrava defectos del utilisation de
prothrombina in despecto de normal numerationes plachettal, sed lor plachettas
habeva un normal activitate thromboplastic. precisemente como omne le
patientes studiate.
4. Le configuration del chromatographia a papiro pro le phosphatidas in
omne le subjectos del presente serie esseva identic con illo reportate pro
plachettas normal. Simile resultatos esseva obtenite per le analyse a
chromatographia columnal in un pool de extracto ab novem del patientes con
uremia.
5. Super le base de iste studios il non esseva possibile demonstrar un defecto
qualitative del plachettas in patientes uremic.

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The incorporation P³² into human platelets has been studied by using as suspending fluid A.C.D. solution, sodium citrate, saline solution and homologous plasma. The radioactive phosphorus was bound to the platelets with a greater stability and at a higher concentration when the platelets were suspended in A.C.D. than in other fluids.—P. d. N.


Significant finding in the cytochemical make-up of normal megakaryocytes was the presence in the cytoplasm of a large amount of chondroitin sulphuric acid type of acidic mucopolysaccharide.—J. B. C.
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GERALD ALTSCHULER, AARON J. MARCUS and HARRIS L. ULLMAN