Platelet Adsorptive Properties and Platelet Extracts in Thromboplastin Generation

By Seymour Perry and Charles G. Craddock, Jr.

The role of platelets in clotting has been a subject for study for about seventy-five years, but it is only in the last decade that definite progress has been made. In 1948, Ware and his colleagues were able to demonstrate factors in platelet extracts which accelerated both prothrombin and fibrinogen conversion. With this “breakthrough,” a whole host of activities has subsequently been attributed to the platelet. To the present, at least eleven substances or properties associated with hemostasis and sixteen others have been isolated in platelet extracts. It is apparent that the platelet has not lagged behind in the ever-increasing complexity of the coagulation problem.

However, this growing list of platelet activities has not gone unchallenged. There is good evidence that the platelet is a highly adsorptive structure and that many of these factors are merely carried or are adherent to the platelet.

It is the purpose of this paper to report observations indicating the importance of platelet adsorption of coagulation factors in blood clotting. It is evident from these data that the thromboplastin generation test is greatly affected by this phenomenon.

METHOD

Blood was collected using only siliconized needles and syringes and with a minimum of stasis. Venipunctures were initiated with one syringe, which was then discarded, and only the blood obtained with a second syringe was used for the clotting studies. Bubbling in the syringe was avoided.

Platelet counts, whole-blood clotting times, prothrombin times, determination of accelerator globulin (proaccelerin) and SPCA (proconvertin) activities, prothrombin consumption, clot retraction, and the thromboplastin generation test (TGT) modified from Biggs and Douglas were performed as previously described. Fibrinogen was estimated qualitatively by two methods. Recalcification times were done according to a method recently suggested.

The original platelet suspensions of known concentration, as prepared for use in the thromboplastin generation test—to be referred to as “normal platelet suspensions”—were washed twice with saline and then resuspended in a volume of saline equal to 1/3 of the original plasma sample. It should be emphasized that this is the method generally accepted by which platelets are ordinarily prepared for use in the TGT.

“Attenuated platelets” refer to platelets collected as above, but further handled as follows: Normal platelet suspensions were incubated for 10 minutes at 37°C. or were

From the Department of Medicine, School of Medicine, University of California Medical Center and Wadsworth Veterans Administration Center, Los Angeles, California.

With technical assistance by Mary Baker, B.A., and Gloria Paul, B.A.

We are indebted to Dr. Samuel L. Rapaport for helpful suggestions and criticisms during the course of this study.

Supported in part by the U.S. Public Health Service, Parke Davis & Co., and the Gladys F. Bowyer Fund.

Submitted June 25, 1957; accepted for publication Sept. 15, 1957.

177
TABLE 1.—The Effect of Adding “Attenuated" Platelets and Platelet Extracts to Thrombopenic Blood

<table>
<thead>
<tr>
<th>Material added to 1 cc. thrombopenic whole blood</th>
<th>Clotting time</th>
<th>Prothrombin consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nothing added</td>
<td>9'30&quot;</td>
<td>10.5&quot;</td>
</tr>
<tr>
<td>0.1 cc. Normal platelet suspension</td>
<td>3'40&quot;</td>
<td>31&quot;</td>
</tr>
<tr>
<td>0.1 cc. “Attenuated” platelets</td>
<td>4'45&quot;</td>
<td>24&quot;</td>
</tr>
<tr>
<td>0.3 cc. “Attenuated” platelets</td>
<td>3'</td>
<td>26&quot;</td>
</tr>
<tr>
<td>0.1 cc. Saline platelet extract</td>
<td>3'</td>
<td>26&quot;</td>
</tr>
<tr>
<td>0.3 cc. Saline platelet extract</td>
<td>3'</td>
<td>26&quot;</td>
</tr>
</tbody>
</table>

kept at room temperature for 1½ to 4 hours. These suspensions were then centrifuged at 3500 r.p.m. for 15 minutes. The sedimented platelets were washed once with saline and then resuspended in a volume of saline equal to 3 of the original platelet-rich plasma. This suspension contains the “attenuated” platelets. The supernatant saline in which the platelets had been incubated is referred to as the “saline platelet extract.”

In those instances where qualitatively defective platelets were used, the procedure was identical.

Attenuated platelets were further incubated in a variety of media for 10 minutes at 37 C. These included normal fresh and old plasma, BaSO4-absorbed plasma, serum, etc., as will be described later. In all cases (unless otherwise noted) in which attenuated platelets were further treated in a particular plasma or serum, a volume equal to 3 of the original plasma from which they were obtained was used. After incubation, they were washed once in saline and resuspended in the same volume of saline as for use in the TGT. The number of platelets in the final suspension was estimated. The determination of the precise number of platelets in this suspension is difficult because of clumping and fragmentation. It is known, however, from carrying out this procedure in many normal individuals that these phenomena do not affect the results.

The term “qualitative platelet defect” as used in this paper refers to abnormal platelets from patients with various diseases. These platelets in normal or elevated numbers gave an abnormal TGT when used in the system, with all other clotting components being normal. The patients with a qualitative platelet defect included three with polycythemia vera, one with myeloid metaplasia and leukemia reaction, and one each with portal cirrhosis, chronic myelocytic leukemia, and thrombasthenia. Thrombopenic plasma and blood were obtained from two patients with chronic lymphocytic leukemia (platelet counts of 2,500 and 62,000 per cu. mm. respectively) and from one with acute lymphocytic leukemia (platelet count 48,000 per cu. mm.).

RESULTS

When normal platelets were incubated under the conditions described, they retained their ability to improve the clotting time and prothrombin consumption of thrombopenic blood. Similarly, the saline platelet extracts showed the same properties. These results are summarized in table 1.

The recalcification time of normal plasma was also shortened by both attenuated platelets and the platelet extracts (see table 2). It is to be noted that the property of promoting clot retraction was lost in the attenuated platelets alone, as well as those incubated in normal plasma. Saline extracts also failed to cause retraction.

The following experiments were designed to show the absorption by platelets of a factor(s) from normal plasma important in thromboplastin generation. They also demonstrate the ease with which this factor(s) can be removed from platelets by saline incubation.
Fig. 1.—Substitution of "attenuated" platelets and saline platelet extracts for platelets in the TGT. Platelets were attenuated by incubation for 10 minutes at 37°C, washing in saline, and finally resuspending in saline.

- - - - . Normal TGT, using normal platelets and all other normal reagents.
- - - - . Saline platelet extract substituted for normal platelets.
x-------x "Attenuated" platelets substituted for normal platelets.

### Table 2—Recalcification Times

<table>
<thead>
<tr>
<th>Normal plasma (platelet poor)</th>
<th>Saline</th>
<th>CaCl₂ -0.05 M</th>
<th>Preparation added</th>
<th>Amount</th>
<th>Clotting time</th>
<th>Retraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>4'45&quot;</td>
<td>in 24 hrs.</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>5'17&quot;</td>
<td>in 24 hrs.</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>Normal platelets</td>
<td>0.1</td>
<td>2'51&quot;</td>
<td>+ in 1 hr.</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>Normal &quot;attenuated&quot; platelets</td>
<td>0.1</td>
<td>1'20&quot;</td>
<td>in 24 hrs.</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>Qualitatively defective platelets</td>
<td>0.1</td>
<td>3'</td>
<td>+ in 1 hr.</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>Normal fresh platelet extract</td>
<td>0.1</td>
<td>2'</td>
<td>— in 24 hrs.</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>Normal 1-mo.-old platelet extract</td>
<td>0.1</td>
<td>3'</td>
<td>— in 24 hrs.</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>Extract from qualitatively defective platelets</td>
<td>0.1</td>
<td>2'18&quot;</td>
<td>— in 24 hrs.</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>Normal &quot;attenuated&quot; platelets incubated in normal plasma—washed once</td>
<td>0.1</td>
<td>1'31&quot;</td>
<td>— in 24 hrs.</td>
</tr>
</tbody>
</table>
The use of attenuated platelets from normal subjects in an otherwise normal system resulted in poor thromboplastin generation (fig. 1). However, saline extracts used instead of platelets in this system gave a more normal curve (fig. 1). When these same attenuated platelets were incubated in their own extract, washed once, and resuspended, they again gave good thromboplastin generation (fig. 2). However, results were poor when these platelets were treated with three-day refrigerated, or one-day frozen, normal extracts. Nevertheless, the extracts alone were able to give fairly good thromboplastin generation.

Incubation of attenuated platelets in normal fresh human plasma resulted in restoration of good thromboplastin generation (fig. 3). Attenuated platelets after incubation in normal plasma were washed once and again “attenuated” by saline incubation for 10 minutes at 37 C. This saline extract in turn demonstrated excellent activity in the TGT (fig. 4).

In an attempt to further clarify the nature of the factor(s) adsorbed by...
platelets, attenuated platelets were tested by TGT after incubation in aged human plasma (deficient in accelerator globulin) and in plasma from a dicumarolized patient. Both of these plasmas restored the activity of the attenuated platelets to normal in the TGT. Normal fresh serum, on the other hand, was ineffective (fig. 5), as was BaSO₄-adsorbed normal human plasma. Curiously, incubation in prothrombin-free beef plasma normalized the curve.

In view of the above, the following studies were made to determine whether the deficiency in patients with a qualitative platelet defect resides in the platelet or in the plasma. Not only do these platelets give poor thromboplastin generation, but also the saline extracts of these platelets are ineffective (fig. 6). Normal attenuated platelets provide a normal TGT curve following incubation in plasmas from patients with qualitative platelet defects. However, incubation of normal attenuated platelets in extracts of these defective platelets fails to restore activity (fig. 7).

Incubation of qualitatively defective platelets in normal plasma resulted in good thromboplastin generation in six of seven patients studied. These included
Fig. 4.—The effect on the TGT of an extract obtained from platelets after these were first "attenuated" and then incubated in normal plasma. The saline extract obtained from this second incubation was used.

- - - - - Normal curve.

- - - - Saline extract obtained after "attenuated" platelets are incubated in normal plasma at 37 C.

x--x--x Normal "attenuated" platelets.

one patient each with thrombasthenia, portal cirrhosis, and chronic myelocytic leukemia, and three with polycythemia vera. The one exception was a patient with myeloid metaplasia with a platelet count of 294,000 per cu. mm. He was remarkable, also, in demonstrating normal thromboplastin generation when his platelets were incubated in his own plasma. Treatment of qualitatively defective platelets with extracts from normal platelets did not give a good TGT curve in any case. An example is shown in figure 8. Qualitatively defective platelets from two patients with polycythemia vera and one with myeloid metaplasia—all with elevated platelet counts—upon incubation in either their own or normal plasmas, resulted in good thromboplastin generation when substituted for normal platelets in the TGT.

The activity of the saline platelet extract in the TGT was lost upon heating
PLATELET PROPERTIES AND EXTRACTS IN THROMBOPLASTIN GENERATION

Fig. 5.—Effect of incubating normal "attenuated" platelets in fresh serum. Platelet activity appears greatly reduced, at least in the initial 3 or 4 minutes.

--- Normal platelets.

- "Attenuated" platelets in normal fresh serum.

X Normal "attenuated" platelets.

at 56 °C. for 10 minutes (fig. 9a) and after adsorption with BaSO₄ (fig. 9b). Prothrombin times of the saline platelet extracts were invariably over 5 minutes. Accelerator globulin activity was less than 10 per cent. SPCA determinations gave an average of 35 seconds as against 21 seconds as the average control. Tests for fibrinogen were negative.
Fig. 6.—Comparison of normal saline platelet extract with one obtained from qualitatively defective platelets. Patient had chronic myelocytic leukemia and a platelet count of 219,450 per cu. mm.

- --- - Normal platelets.
- - - Extract from normal platelets.
x---x Extract from qualitatively defective platelets.

**Discussion**

Although most investigators recognize the importance of the platelet in the hemostatic mechanism, they do not agree on its exact role. For example, it is generally accepted that, in the presence of normal platelets, a clot will retract. Hemorrhagic disorders associated with poor clot retraction have been described. However, the significance of this particular platelet property has been challenged. The importance of serotonin in clotting is also controversial. At present, it is thought to be merely carried by the platelet and of questionable value in hemostasis.
PLATELET PROPERTIES AND EXTRACTS IN THROMBOPLASTIN GENERATION

Fig. 7.-Effect of incubating "attenuated" platelets in saline extracts of qualitatively defective platelets. Thromboplastin generation was poor and, in fact, appears to be reduced.

. . . . . Normal platelets.
. - - - "Attenuated" platelets incubated in extracts of qualitatively defective platelets.
. x-x-x Normal "attenuated" platelets.

With the description of accelerator activity in platelets, called Platelet Factor 1, it was felt that this was different from plasma accelerator globulin. Recently, Hjort, Rapaport, and Owren have shown that this is merely adsorbed plasma proaccelerin.

Under the conditions of this study, it has been demonstrated that whatever the role of platelets in hemostasis may be they adsorb or combine with a factor or factors in plasma, and this phenomenon is necessary for optimal thromboplastin generation as measured by the TGT.

In the light of these observations, descriptions of various factors or properties attributed to platelets must be viewed with caution. The statement recently made, that "many of the platelet and plasma factors, although reputedly independent, may later prove to be identical," seems quite appropriate.

After "attenuation" by saline incubation for 10 minutes, platelets can be restored to normal function in the TGT by incubation in normal fresh or old plasma, in saline extracts from normal platelets, in dicumarolized plasma, and in plasmas from patients with thrombocythemia, thrombocytopenia, and qual-
SEYMOUR PERRY AND CHARLES G. CRADDOCK, JR.

Fig. 8.—Qualitatively defective platelets incubated in extract from normal platelets resulted in poor thromboplastin generation.

- - - - - Normal platelets.

. . . . Qualitatively defective platelets incubated in normal platelet extract.

x-----------x Qualitatively defective platelets, all other constituents being normal.

These results seem to be at variance with the studies reported by Bonnin, who showed inhibition of thromboplastic activity after platelets were incubated in plasma or sera from patients with thrombocytopenia. Only normal platelets were used for incubation, however, in the various plasmas and sera, and the conditions of the experiments were not comparable to those in the present study.

On the other hand, the general effect of preincubation of platelets on the recalcification and clotting time of plasma, found in the present investigation, is in agreement with the findings of Hougie. Again, the platelet suspensions were not comparable to those used here.

The data pertaining to the nature of the plasma factor(s) adsorbed by platelets does not permit a conclusive statement. Accelerator globulin activity appears unlikely since attenuated platelets incubated in aged human plasma result in good thromboplastin generation. Also, saline platelet extracts showed little accelerator globulin activity, although they were very effective in the TGT. No
Fig. 9a.—Effect on TGT of heating saline platelet extract to 56 C. for 10 minutes. The extracts were substituted for platelets, all other constituents being normal.

- - - - - Normal platelets.
- - - - Heated platelet extract.
×— ×— × Unheated platelet extract.

explanation is apparent for the effect of prothrombin-free beef plasma in improving the activity of attenuated platelets in the TGT.

Prothrombin and SPCA, as determined by usual technics based on their influence on prothrombin conversion, could not be detected in saline platelet
extracts which were active in the TGT. As previously mentioned, plasma from a dicumarolized patient restored normal platelet activity to attenuated platelets. These observations are evidence against these factors being the active plasma substances adsorbed by platelets. This is in agreement with the findings of others showing no significant prothrombin or SPCA activity in platelet extracts. However, the differences between the test systems employed to study thromboplastin generation and those measuring effect on prothrombin conversion do not permit dogmatic statements in this regard.

The thromboplastin component of platelets has been shown to remain with platelet fragments after damage to the platelet. This would indicate that the change in platelet activity in the TGT, brought about by saline incubation, does not involve loss of platelet thromboplastic substances. Furthermore, the results obtained after incubation of attenuated platelets in aged plasma (AHG deficient) and fresh serum (containing PTC and PTA) would not suggest that these platelet co-factors are important in this effect.

The recently described Product I is not a consideration in these results since it is thought to be present in serum; it is adsorbed by prothrombin adsorbents and is inactivated by decalcifying agents.

Platelet Factor 2 may be eliminated since it is heat-stable and the platelet extracts were readily inactivated by heating at 56 C. for 10 minutes.

FIG. 9b.—Thromboplastin generation of BaSO$_4$-adsorbed saline platelet extract.

- - - - - Curve with normal platelets.
- - - Saline platelet extract substituted for platelets.
- - - Saline platelet extract after absorption with BaSO$_4$. 
Thrombin does not seem to play a role since it is not adsorbed by BaSO₄. The saline platelet extracts showed no activity in the TGT after BaSO₄ adsorption. The recently described Stuart factor is thought to be readily removed from platelets by a single washing. Platelets prepared for use in the TGT were normally washed twice. Finally, the possibility exists that saline incubation interferes with an interaction between platelets and calcium. However, such a reaction should proceed normally in serum containing no calcium-binding agent. In this study, incubation of attenuated platelets in fresh serum failed to restore their activity in the TGT.

Thus, under the conditions of these studies, the results do not appear to be reconcilable with any known explanation of the initiation of the first stage of clotting. It may be that the mechanism responsible was not investigated in these experiments or, perhaps, yet another unrecognized factor is involved. The factor(s) is highly active in saline extracts of platelets, is heat labile, is adsorbed by BaSO₄, and is absent in serum and in extracts of qualitatively defective platelets. The activity of saline extracts in the TGT does not completely deteriorate with storage after freezing. However, such stored extracts fail to normalize the defective action of attenuated platelets. Platelets thought to be qualitatively defective, as measured in the TGT, show good activity in the test after incubation in both their own and in normal plasmas.

The data presented suggest that an important role of the platelet in thromboplastin generation involves the adsorption of a factor(s) from the plasma. The ease with which this factor(s) is removed from, and readsorbed by, the platelet indicates a surface phenomenon. The abnormal thromboplastin generating activity of qualitatively defective platelets from patients with bleeding disorders may be due to alteration in the adsorptive characteristics of the platelet surface.

The observation that saline platelet extracts contribute to good thromboplastin generation, and improve prothrombin consumption and the clotting time of thrombocypenic blood, suggests the possibility that such extracts may have value in the treatment of appropriate clinical conditions.

**Summary**

1. Simple incubation of normal platelets in saline for 10 minutes at 37°C markedly diminishes their activity in the thromboplastin generation test. This loss of activity is due to the removal of a factor(s) from the platelet and is present in the saline extract.
2. These “attenuated” platelets retain their effect on the recalcification time, prothrombin consumption, and clotting time of whole blood.
3. Attenuated platelets, as well as platelets thought to be qualitatively defective, regain normal activity in the TGT after incubation in normal plasma or in plasmas from patients with qualitative platelet defects.
4. Attenuated platelets, in contrast to qualitatively defective platelets, are restored to normal, as measured by the TGT, after treatment with saline extracts of normal platelets. Attenuated platelets, however, do not function normally after incubation with saline extracts of qualitatively defective platelets.
SEYMOUR PERRY AND CHARLES G. GRADDOCK, JR.

(5) The possible mechanisms involved are discussed, and it is concluded that the phenomenon involves the adsorption of plasma factor(s) by the platelet. The nature of the plasma factor(s) is not known.

SUMMARIO IN INTERLINGUA

1. Le simple incubation de plachettas normal in un solution salin durante 10 minutas a 37 C resulta in un reduction marcate de lor activitate in le test del generation de thromboplastina. Iste perdita de activitate es effectuate per le elimination de un factor (o plure factores) ab le plachettas. Le factor (o factores) se retrova in le extracto plachetatal salin.

2. Le plachettas assi “attenuate” retine lor effecto super le tempore de recalcification, super le consumption de prothrombina, e super le tempore de coagulation de sanguine integre.

3. Plachettas attenuate—e etiam plachettas considerate como qualitativemente defective—recovrar lor normal activitate in le test del generation de thromboplastina post incubation in plasma normal o in plasmas ab patientes con qualitative defectos plachettal.

4. Attenuate plachettas—per contrasto con plachettas que es qualitativemente defective—recovrar lor normal activitate in le test del generation de thromboplastina post que illos es tractate con un extracto salin de plachettas normal. Tamen, attenuate plachettas non functiona normalmente post que illos es incubate in extractos salin de plachettas que es qualitativemente defective.

5. Le mechanismos possibile de iste phenomenos es discutite. Le conclusion es presentate que il se tracta del adsorption de un factor o factores plasmatic per le plachettas. Le natura del factor (o factores) non es cognoscite.

REFERENCES


Platelet Adsorptive Properties and Platelet Extracts in Thromboplastin Generation

SEYMOUR PERRY and CHARLES G. CRADDOCK, JR.