The Development of Platelet Factor 3 Activity by Latex Particles—An Interaction with Plasma

By Herbert I. Horowitz and Aaron J. Marcus

Boyles1 reported in 1959 that latex particles in a sharply defined range of size and concentration could substitute for platelets and brain cephalin in the generation of blood thromboplastin. These particles are polymers of phenylethyene, a relatively inert compound containing no phosphorus, and are free of lipid and protein. The report implied that platelet factor 3 activity was primarily dependent on particle size and independent of specific reacting groups. The present investigation was undertaken to gain some understanding of this phenomenon. It became apparent that platelet factor 3 activity would develop in association with latex particles only after a period of incubation with plasma constituents.

Materials and Methods

Polystyrene latex particles of the following diameters were evaluated: 0.088 μ, 0.188 μ, 0.264 μ, 0.365 μ, 0.557 μ, 1.171 μ, and 2.64 μ. The particles were suspended in a detergent, the composition of which could not be ascertained, at a concentration range of 3–10 per cent solids. Prior to use, the particles were either washed once in imidazole-buffered saline (IBS), centrifuged at 10,000 rpm and resuspended in fresh IBS by vigorous shaking, or serially diluted directly in IBS. Imidazole buffer, pH 7.3, was mixed with nine parts saline to prepare buffered saline. The suspending detergent could be obtained free of particles by centrifugation at 25,000 rpm in a Spinco model L Preparative Ultracentrifuge.

Human brain cephalin was prepared by the method of Bell and Alton.2 Stable product I was prepared from rabbit serum and plasma by the method of Spaet.3 Russell’s viper venom (Stypven) was obtained from Burroughs Welcome Company, Tuckahoe, N. Y.; the venom was diluted 1:10,000 either in the manufacturer’s diluent or normal saline, further diluted to 1:50,000 in isotonic saline and finally mixed with an equal volume of 0.025M CaCl2. “Exhausted plasma” was prepared by contact activation of intact plasma with 15 mg/ml of celite (Filter-Cel, Johns-Manville Corporation, Lampoc, Calif.) followed by incubation for 5 hours at 37 C. after adjustment of the pH to 7.0.4

The platelet factor 3 activity of latex particles was compared with appropriate controls in four clotting systems:

1. Recalcified clotting time5—particles were added to platelet-poor plasma (PPP) in glass or siliconized tubes, and the mixture clotted with 0.025M CaCl2 immediately or following incubation.

2. Thromboplastin generation test (TGT)6—particles were suspended in buffer and were used as the platelet reagent in this test, with or without prior incubation.
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3. Product I substrate time—a modification of the thromboplastin generation test, in which a stable protein intermediate, product I, is prepared from the serum and adsorbed plasma reagents of the thromboplastin generation test, and used to accelerate the recalcified clotting time of normal platelet-poor substrate plasma to which latex particles have been added as a platelet substitute.

4. Stypven time—Russell’s viper venom in a dilution of 1:100,000 was used like product I to accelerate the recalcified clotting time of plasma containing added latex particles. Stypven is an incomplete thromboplastin requiring platelet factor 3 for optimal coagulation acceleration; the Stypven time has been found to vary inversely with the concentration of available phospholipid.

The ability of latex particles to induce surface activation was evaluated by comparing the ability of PPP incubated with latex particles in siliconized glass tubes to shorten the partial thromboplastin time of exhausted plasma. Control values were obtained by incubating PPP with saline. The tests were carried out in siliconized glass tubes. This type of procedure has been found to measure activated factor XI in a specific fashion.

RESULTS

Findings with the four clotting systems are summarized in table 1. Particles of 0.188 μ were used in these tests at a concentration of 2.6 per cent. When the particles were added to plasma and the mixture tested without incubation, no platelet factor 3 activity could be detected in any test system, as compared to the activity of plasma with saline added as control. On the other hand, plasma-latex mixtures incubated for 1 hour at 37 C. showed considerable activity compared to plasma-saline mixtures incubated for a similar period. Acceleration of the recalcified clotting time was as great as that produced by a mixture of plasma and cephalin in optimal concentration, but in the other tests, platelet-like activity of the incubated plasma-latex mixtures was definitely less than that of cephalin.

Latex particles were completely inert as platelet substitutes in the thromboplastin generation test at all sizes and over a wide range of concentrations when tested without prior incubation with plasma components. It proved difficult to devise a TGT test system to evaluate the platelet-like activity of latex particles following incubation with whole plasma, since the presence of even small amounts of whole plasma in the TGT test system non-specifically shortened the substrate clotting time owing to the elaboration of thrombin. Al(OH)₃-adsorbed plasma on the other hand was found to be able to interact with latex particles and develop platelet-like activity (see below) yet did not itself greatly accelerate the TGT when used as platelet reagent. Therefore the particles were incubated in Al(OH)₃-adsorbed plasma for 1 hour and the mixture used as a platelet reagent in an otherwise normal TGT. The results, shown in table 1 and figure 1, indicate that following incubation with a plasma derivative, suboptimal platelet-like activity was found in particle mixtures.

The influence of time of incubation and concentration of the particles was most readily studied in the product I time and Stypven tests. In figure 2 the Stypven time as an index of platelet factor 3 activity was followed in mixtures of 0.088 μ latex and plasma. Maximal activity was found to develop progressively during an hour’s incubation at 37 C. at a concentration of 2.6 per cent.
Table 1.—Effect of Incubation with Plasma on Platelet-like Activity of Latex Particles

<table>
<thead>
<tr>
<th>Test System</th>
<th>Unincubated*</th>
<th>Incubated†</th>
<th>Cephalin Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recalcified clotting time—glass</td>
<td>227</td>
<td>70</td>
<td>72</td>
</tr>
<tr>
<td>Recalcified clotting time—silicone</td>
<td>313</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>T.C.T.—latex in buffer</td>
<td>90</td>
<td>90</td>
<td>7</td>
</tr>
<tr>
<td>T.G.T.—latex in Al(OH)₃ plasma</td>
<td>90</td>
<td>31</td>
<td>9</td>
</tr>
<tr>
<td>Product I substrate time</td>
<td>47</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Stypven time</td>
<td>29</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

*Tested immediately after addition of 0.088 μ diameter latex.
†Incubated with plasma for 1 hour at 37°C prior to testing.
‡Substrate time after 6 minutes of incubation.

solids, with lesser activity at lower or higher latex concentration. Maximal thromboplastin-promoting properties were noted at the following concentrations: 2.6 per cent solids for 0.088 μ; 3.2 per cent for 0.188 μ; 3.0 per cent for 0.264 μ; 9.7 per cent for 3.65 μ; and 9.7 per cent for 0.557 μ particles. Larger particles were inert at all concentrations tested. When viewed by phase microscopy, both the active and inactive particle suspensions seemed to coalesce and form masses which superficially resembled clumps of platelets undergoing viscous metamorphosis, similar to human blood platelets.

Fig. 1.—Platelet factor 3 activity of 0.188 μ latex particles in the thromboplastin generation test.
Fig. 2.—Change in Stypven time on incubation of 0.088 μ latex particles with plasma.

In order to determine whether the suspending medium (presumably a detergent of unknown identity) or the particles contributed to the presence or absence of platelet factor 3 activity, studies were carried out on particles which had been washed free of this material, or diluent was used alone. Washed particles retained the ability to generate platelet factor 3 activity, whereas the diluent alone was inactive.

The platelet factor 3 activity developed by the plasma-latex mixtures was labile, slowly disappearing over 2 hours of incubation at 37 C. In the data presented in table 2, the plasma-latex mixture was diluted 1:10 and added to normal platelet-poor substrate immediately before testing, so that the presence of other coagulation factors in the latex plasma would not influence the final Stypven time. Under these circumstances a mixture of particles and plasma shortened the Stypven time from 39.4 to 22.6 seconds during a period of 1 hour incubation. Following high speed centrifugation, activity could not be found in the latex precipitate or in the supernatant plasma. The activity was still absent on resuspension and did not return after further incubation. In addition, particles which had already developed platelet factor 3 activity would not do so again in fresh plasma, and plasma which had supported such change would not induce this activity with fresh particles. These findings indicate that the development of platelet factor 3 activity by latex particles involves an interaction with some plasma constituent which is apparently consumed during the process. It is possible that the latex particles are coated and cannot further interact with plasma.

In an effort to define further the nature of the plasma component required,
Table 2.—Loss of PF-3 Activity by Plasma-Latex Suspension after Centrifugation and Resuspension

<table>
<thead>
<tr>
<th>Incubation Mixture</th>
<th>Unincubated* (sec.)</th>
<th>Incubated† (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Plasma—saline</td>
<td>39.0</td>
<td>35.5</td>
</tr>
<tr>
<td>2. Plasma—latex†</td>
<td>39.4</td>
<td>22.6</td>
</tr>
<tr>
<td>3. Plasma—latex from 2 centrifuged, resuspended</td>
<td>44.3</td>
<td>45.0</td>
</tr>
<tr>
<td>4. Fresh plasma, latex from 2</td>
<td>39.0</td>
<td>47.6</td>
</tr>
<tr>
<td>5. Plasma from 2, fresh latex</td>
<td>42.5</td>
<td>52.0</td>
</tr>
<tr>
<td>6. Fresh plasma, fresh latex</td>
<td>40.7</td>
<td>21.3</td>
</tr>
</tbody>
</table>

*Tested immediately after addition.
†Incubated for 1 hour at 37 C. prior to testing.
‡Two and six-tenths per cent suspension of 0.088 μ latex particles.

the modified Stypven test was used to evaluate the development of platelet factor 3 activity in various plasma derivatives. Particles were incubated at 37 C. with PPP, PPP adsorbed with Al(OH)₃, serum, and native serum obtained by allowing native PPP to clot in silicone-coated tubes. The incubation mixtures were then diluted 1:10 with buffered saline, 0.1 ml. of the dilutions were added to 0.1 ml. of normal PPP and finally 0.2 ml. of Stypven-CaCl₂ were added. As seen in table 3, platelet factor 3 activity developed in plasma but was not found in serum. This difference could not be attributed to the prothrombin content of the plasma since the same activity could develop in prothrombin-poor Al(OH)₃-adsorbed plasma but not in prothrombin-rich native serum. Latex platelet factor 3 activity developed readily in heparinized plasma, but not in EDTA plasma or plasma heated at 60 C. for 5 minutes.

The latex particles did not induce surface activation of intact PPP nor did they increase the degree of contact activation of PPP which could be achieved in untreated glass tubes. The inability of the particles to induce contact activation is shown in table 4.

**Discussion**

In the early stages of blood coagulation, phospholipids derived from platelets interact with plasma coagulation factors to form a prothrombin activator. This contribution of the platelets may be defined as platelet factor 3 activity. The ethanolamine and serine phosphoglycerides isolated from human platelets are capable of replacing the platelet factor 3 activity of whole platelets in certain in vitro clotting systems. Phospholipids from erythrocytes, brain or other sources of phosphatides can also be shown to possess platelet factor 3 activity. However, the platelet lipid seems to be more readily available for interaction with the plasma clotting factors, though the precise mechanism for this availability phenomenon is unknown. Platelet factor 3 activity in vivo may be a collective function of all the platelet lipids or it may occur in the form of lipoprotein. Recent studies have indicated that the platelet factor 3 activity can be a property of any phospholipid provided it has a certain micellar configuration, particle size and surface charge.
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Table 3.—Effect of Incubation with Plasma or Serum on Platelet-like Activity of Latex Particles

<table>
<thead>
<tr>
<th>Incubation Mixture</th>
<th>Unincubated*</th>
<th>Incubated†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latex (sec.)</td>
<td>Saline (sec.)</td>
</tr>
<tr>
<td>Plasma</td>
<td>52</td>
<td>57</td>
</tr>
<tr>
<td>Serum</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>Al(OH)₃ plasma</td>
<td>61</td>
<td>65</td>
</tr>
<tr>
<td>Native serum</td>
<td>52</td>
<td>51</td>
</tr>
</tbody>
</table>

*Tested immediately after addition.
†Incubated 2 hours at 37 C. prior to testing.

Table 4.—Lack of Contact Activation by Latex Particles Using “Exhausted Plasma” to Test for Activation Product

<table>
<thead>
<tr>
<th>Recalcified Clotting Time (sec.)</th>
<th>PPP-Saline</th>
<th>PPP-Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unincubated</td>
<td>91.0</td>
<td>93.2</td>
</tr>
<tr>
<td>60' Incubated</td>
<td>95.2</td>
<td>96.8</td>
</tr>
</tbody>
</table>

Biochemical studies of platelet phosphatides with clotting activity have shown the presence of one saturated and one unsaturated fatty acid in addition to the ethanolamine or serine component in the molecule. Only 34 per cent of the ethanolamine phosphoglycerides of human platelets are in the diester form, the remaining 66 per cent being present as plasmalogen, a class of phospholipids of uncertain function. The ethanolamine phosphoglycerides obtained synthetically or from animal sources may not have these structural properties. Conclusions as to the clotting activity of phosphatides from various sources other than platelets should take these biochemical findings into consideration.

The development of platelet factor 3 activity by latex particles has been shown to be dependent upon some type of interaction (probably an adsorption phenomenon) between the particles and a component in the plasma. It requires an incubation period at 37 C. and is labile, disappearing on further incubation or after centrifugation. It appears to be dependent on the size of the particles and their concentration. When compared to activity obtainable with platelets or phospholipid it is somewhat incomplete. Boyles did not report the need for pre-incubation of these particles with plasma in order for the clot-promoting properties to develop. On the basis of our observations it appears that his methods probably involved some type of pre-incubation phenomenon of the particles and plasma before they could be shown to replace platelets in the TCT. Admittedly our studies are not strictly comparable since the precise diameter particles (0.144 µ) with which he found maximal activity could not be obtained and tested by us. However he reported some activity with particles of 0.09 µ diameter, a size which we found to be inert in the absence of incubation with plasma but capable of developing considerable activity with pre-incubation.

The precise nature of the plasma constituent required for this interaction
with polystyrene particles will require further elucidation. It is present in plasma, heat-labile, not completely adsorbed by Al(OH)₃ and inhibited by EDTA. Plasmas from four patients with congenital acanthocytosis contained significantly less of the plasma constituent than did normals, suggesting perhaps that the factor is associated with plasma lipids or β-lipoproteins. It is clear, however, that untreated latex particles cannot substitute for platelets in coagulation tests.

**SUMMARY**

Platelet factor 3 activity of polystyrene latex particles was found to be absent when the particles were tested without prior incubation with plasma. Following such incubation, the particles developed considerable but sub-optimal activity. An interaction between the particles and some plasma component(s) during the incubation period which gives rise to a labile type of activity has been postulated.

**SUMMARIO IN INTERLINGUA**

Esseva trovate que le activitate factor-plachettal-3 de partículas de latex polystyrenic es absente quando le partículas es testate sin incubation anterior con plasma. Post tal incubation, le partículas disveloppa un considerabile sed non un optimal grado de activitate. Un interaction inter le partículas e certe componentes de plasma durante le periodo de incubation con le resultato de un typo labil de activitate ha essite postulate.

**REFERENCES**

12. Quick, A. J., Georgatsos, J. G., and
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