A Plasma Factor for Platelet Adhesiveness and Clot Retraction Acceleration

By J. Philip Savitsky, M.D.

Changes in platelet adhesiveness have been noted during the course of many different clinical conditions. Increased platelet adhesiveness has been correlated by some investigators with a predisposition to thromboembolic phenomena and vascular disease, but other work has not confirmed this suggestion. It has been shown that there is a very close relation between the level of the platelet adhesiveness and the rate of initiation of clot retraction as measured by the clot retraction time. The number and morphology of the platelets and the extent of clot retraction were found to be unrelated to the platelet adhesiveness and the clot retraction time.

These observations suggested that there might be a substance outside the platelets which makes them adhesive and accelerates the initiation of clot retraction. Wright has suggested that fibrin, which is constantly being formed intravascularly, coats the platelets, making them more adhesive. No evidence, however, for this hypothesis has been offered and it has been observed that the fibrinogen level has no relation to platelet adhesiveness or clot retraction time.

This present investigation attempts to demonstrate the existence of a factor circulating in the blood outside the platelets which acts to increase platelet adhesiveness and accelerate clot retraction. Some of the properties of this factor are studied and their relationship to the "viscous metamorphosis" of the platelets, described by Wright and Minot in 1917, is clarified.

Methods and Materials

Platelet Adhesiveness

The platelet adhesive index was determined by the glass wool method of Moolten and Vroman. Various substances were added to the blood to be tested during the period of 10 minutes between the time of drawing the blood and the start of the procedure. The determination was then done exactly as originally described. The normal index varies from 0.90 to 1.25 and is reproducible with less than 10 per cent error.

Clot Retraction Time

The clot retraction time was measured by the citrated venous blood method previously described. In order to investigate the effect on the clot retraction time of substances added to the blood, sufficient blood was drawn into 3.8 per cent sodium citrate (in the ratio of 9:1) for the experiments and controls. The citrated blood was placed in a flask and 2 ml. of the blood was immediately placed in the tubes containing the test substance and in control tubes. After 5 to 10 minutes, 0.1 ml. of 5 per cent CaCl2H2O was added to each tube. It is advisable to prepare the calcium solution on the day of use. Results became unreliable if more than 20 minutes passed between the drawing of the blood and the addition of the calcium. The blood clotted in 3 to 5 minutes. When fresh serum was the substance added, the clotting took place more rapidly and the succeeding step was done more quickly.

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PLASMA FACTOR FOR PLATELET ADHESIVENESS

Before gross clotting occurred, about 2 minutes after the calcium was added, blood was drawn up in a hemoglobin pipet and deposited as a single drop in a tube containing castor oil. Siliconized capillary pipets which delivered 60 to 80 drops of water per ml. were also used. It is very important that the proper pipet be utilized in transferring the blood from the tube to the castor oil since drops that are too large or too small will give unreliable results. The tubes of castor oil were kept at 21 C. and the test was done in quadruplicate. The tubes were observed and the clot retraction time taken as the time from the clotting of the recalcified blood in the tube to the exudation of serum from the drop in the castor oil. If three of the four determinations were within 3 minutes of each other, the mean value of these three was used. If the blood in the tube is already in the accelerated phase of the clotting process before the drops are transferred to the castor oil, there may be an extremely slow retraction in the drops in castor oil.

RESULTS

Serum from a patient with a high platelet adhesiveness was added to normal blood in vitro and the platelet adhesiveness determined. One-half cc. of serum added to 2 cc. of normal citrated blood was capable of raising the adhesive index of the normal blood almost to the level of the platelet adhesive index of the patient from whom the serum was taken. This property of increasing the platelet adhesiveness when transferred in vitro was also found to be present in the plasma, cerebrospinal, ascitic, and pleural fluids of individuals having an elevated platelet adhesiveness. The ability to raise the platelet adhesiveness disappeared if the serum was heated to 56 C. for 20 minutes or remained at 4 C. for several days. It was preserved, however, when stored at -60 C. in carbon dioxide. Table 1 shows some typical observations in which 0.5 cc. of serum or spinal fluid was added to 1.8 cc. blood and 0.2 cc. 3.8 per cent sodium citrate.

Sera from individuals with a normal platelet adhesiveness when added to normal blood had no effect on the adhesive index. Figure 1 illustrates the results of adding varying amounts of high adhesiveness serum to 2 cc. of normal blood. At a 1/10 dilution the serum loses its property of raising the adhesiveness.

Considering the close relation of clot retraction time and platelet adhesiveness, studies were done on the effect of the addition of high adhesiveness serum on the clot retraction time of normal blood. Table 2 demonstrates sample observations during a period of an hour following the addition of the serum to normal blood. It is evident that the high adhesiveness serum acts as a clot retraction accelerator and does not seem to act enzymatically. Further studies showed that the plasma, cerebrospinal, ascitic, and pleural fluids all contained a clot retraction

<table>
<thead>
<tr>
<th>Characteristic of Platelet Adhesiveness Factor</th>
<th>Adhesive index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Patient with active multiple sclerosis</td>
<td>1.87</td>
</tr>
<tr>
<td>2. Normal individual</td>
<td>1.05</td>
</tr>
<tr>
<td>3. Serum from 1 plus blood from 2</td>
<td>1.80</td>
</tr>
<tr>
<td>4. Serum from 1 at 56 C. for 20 min. plus blood from 2</td>
<td>0.99</td>
</tr>
<tr>
<td>5. Serum from 1 at 4 C. for 5 days plus blood from 2</td>
<td>1.00</td>
</tr>
<tr>
<td>6. Cerebrospinal fluid from 1 plus blood from 2</td>
<td>1.78</td>
</tr>
<tr>
<td>7. Cerebrospinal fluid from 1 at 56 C. plus blood from 2</td>
<td>0.96</td>
</tr>
</tbody>
</table>
accelerator in patients with a high platelet adhesiveness and a rapid clot retraction time. The ability to speed up the initiation of clot retraction was destroyed by heating to 56 C. for 20 minutes or standing at 4 C. for 5 days. These properties are the same as those for the platelet adhesiveness factor.

![Graph showing initial adhesive index of serum](image.png)

**Fig. 1.**—The results of adding varying quantities of high adhesiveness serum to normal blood.

**Table 2.**—The Effect of High Adhesiveness Serum on Clot Retraction Time of Normal Blood

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>Control</th>
<th>0.5 cc. serum added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive index</td>
<td>Clot retraction time</td>
<td>Adhesive index</td>
</tr>
<tr>
<td>0</td>
<td>1.20</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>1.81</td>
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<td>15</td>
<td>20</td>
<td>1.80</td>
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<td>30</td>
<td>22</td>
<td>1.86</td>
</tr>
<tr>
<td>45</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.38</td>
<td>20</td>
</tr>
</tbody>
</table>

The heat lability of this factor, the disappearance of activity over several days, and the preservation at -60 C. suggested the hypothesis that the factor for platelet adhesiveness and clot retraction acceleration may be related to complement. To test this hypothesis, an unrelated antigen-antibody system, devised to remove complement or any substance bound in an antigen-antibody reaction from serum, was added to the high adhesiveness serum, and 0.1 cc. of a 1. mg./cc. egg albumen solution and 0.1 cc. of a 0.8 mg. antibody X/cc. rabbit
anti egg albumen serum were added to 1 cc. of high adhesiveness serum and incubated at 37 C. for 1 hour. The precipitate was removed and the supernatant fluid was studied for the presence of the platelet adhesiveness factor. Table 3 shows the results in a typical experiment which was repeated many times with similar findings. The antigen-antibody reaction removed the factor for platelet adhesiveness.

The removal of the clot retraction accelerator by an antigen-antibody reaction was demonstrated in the guinea pig using the method described by Seltzer, Baron, and Fusco for the removal of complement in vivo. In this method, a

| Table 3.—The Removal of Platelet Adhesiveness Factor by an Antigen-Antibody Reaction |
|---------------------------------|---------------------------------|
| Adhesive index                  |                                 |
| 1. Patient with pemphigus       | 2.05                            |
| 2. Normal individual            | 1.04                            |
| 3. Serum 1 at 37 C. 1 hour plus blood 2 | 1.90                            |
| 4. Serum 1 plus egg albumen plus rabbit anti egg albumen at 37 C. 1 hour; then supernate plus blood 2 | 1.07                            |
| Controls                        |                                 |
| 5. 0.1 cc. egg albumen plus blood 2 | 1.11                            |
| 6. 0.1 cc. rabbit antiserm plus blood 2 | 0.98                            |
| 7. 0.1 cc. rabbit antiserm plus 1 cc. serum 1 at 37 C. 1 hour; 1/2 cc. of mixture added to blood 2 | 1.80                            |
| 8. 0.1 cc. egg albumen plus 1 cc. serum 1 at 37 C. 1 hour; 1/2 cc. of mixture added to blood 2 | 1.87                            |

| Table 4.—Changes in Clot Retraction Time Following in Vivo Antigen-Antibody Reaction |
|---------------------------------|---------------------------------|
| Complement removed             | Clot retraction time |
| G.P. #1                        | %                       | min. |
| 0                              | 25                      |
| 90                             | 70                      |
| G.P. #2                        | %                       | min. |
| 0                              | 23                      |
| 85                             | 65                      |
| G.P. #3                        | %                       | min. |
| 0                              | 30                      |
| 95                             | 80                      |

guinea pig received an injection of egg albumen and rabbit antiserum to egg albumen followed an hour later by a similar injection. The antigen-antibody reaction removes the complement almost entirely and it does not return for about eight hours. Thirty minutes after the second injection, a blood sample was drawn and the clot retraction time determined. In conjunction with the originators of this method, the following observations were made as shown in table 4. The details of the method are described elsewhere. The amount of complement removed indicates the fact that the antigen-antibody reaction actually took place in vivo.
The total serum complement was then measured in twenty-five patients with concomitant determinations of the platelet adhesiveness and the clot retraction time. The complement was determined by the method of Mayer, et al.\textsuperscript{10, 11} and summarized by Fischel, Pauley, and Lesh,\textsuperscript{12} using the spectrophotometric determination of the 50 per cent hemolytic end point. No correlation whatsoever was found between the platelet adhesiveness, clot retraction time, and total serum complement level.

The properties of the clot retraction accelerator were further investigated. In addition to its heat lability, it was found to be nondialyzable and insoluble in water; it precipitated with the globulins between 40 and 50 per cent saturation of ammonium sulfate and was adsorbed on barium sulfate (50 mg./cc.) and calcium phosphate gel. Whereas the ability to elevate the platelet adhesiveness was lost after 1/10 dilution, the clot retraction accelerating effect was present up to 1/1000 dilution. This suggested that the calcium ion increased the effect of this factor as the platelet adhesiveness is determined in a citrated system whereas clot retraction takes place in the presence of calcium.

Although the total complement level had no relation to platelet adhesiveness or clot retraction time, the possibility remained that one of the components of complement was the active substance. Of the four components, only component 1, or midpiece, is both heat labile and totally insoluble in water. The methods described by Pillemer, et al.\textsuperscript{13} for the separation of midpiece were applied to the clot retraction accelerator. Precipitation by 1.4 M (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} and precipitation by dialysis against buffer of pH 5.4 and ionic strength 0.02 failed to separate the clot retraction accelerator from the other serum proteins. This suggested that the factor was not complement or one of its components although the factor was removed in an antigen-antibody reaction.

Wright and Minot in 1917 described a phenomenon which they termed the “viscous metamorphosis” of the platelets\textsuperscript{7} and they suggested the presence of a serum factor responsible for the changes. To study this, platelets were separated on a cation exchange resin,\textsuperscript{14} eluted with isotonic saline, centrifuged in siliconized tubes at 5000 rpm for 30 minutes, and washed with saline and centrifuged again. The platelet masses in the tube were resuspended in a few drops of saline. Following the technic of Wright and Minot, the platelets were observed under the microscope in the presence of various substances plus calcium. The platelets collected in this manner showed a small amount of “viscous metamorphosis” without the addition of the serum factor. It was observed that the factor for viscous metamorphosis of the platelets had the same properties as the clot retraction accelerator. A partial separation of the clot retraction accelerator was done as follows: The fraction of serum precipitating between 40 and 50 per cent (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} was dialyzed overnight against distilled water. The insoluble material was resuspended in the same volume of saline and adsorbed with 50 mg./cc. of BaSO\textsubscript{4}. Following elution from the BaSO\textsubscript{4} with citrate in saline, it was redialyzed against distilled water and the precipitate redissolved in saline. This extract contained nearly all the ability to cause the viscous metamorphosis of the platelets.

\footnote{With the aid of Dr. John Gruhn and the Montefiore Hospital Blood Bank using the Fenwall Blood Pack, Fenwal Laboratories, Inc., Ashland, Mass.}
No systematic investigation was pursued into the relation of platelet adhesiveness, clot retraction time, and viscous metamorphosis of the platelets to the clotting mechanism. It was observed, however, that the measurement of the clot retraction time became unreliable and prolonged if the drop of blood was removed from the tube late in the clotting process during the accelerated phase. No clot accelerating activity was found in the separated clot retraction accelerator.

**DISCUSSION**

The experimental data indicate that there exists a substance in the blood, external to the platelets, which acts on them to increase their adhesiveness. This factor also causes the viscous metamorphosis of the platelets and an acceleration of the clot retraction time. It seems to have no effect on the extent of the clot retraction after it has fully retracted, nor does it appear to have any directly accelerating effect on the coagulation mechanism.

The viscous metamorphosis of the platelets was described in 1917 by Wright and Minot. They demonstrated that the addition of serum in the presence of calcium to a concentrated saline solution of platelets on a slide resulted in pseudopodia formation, some ameboid movement, and a rapid fusion of masses of platelets. In these masses the platelets lost their individual identities and merged to form large amorphous, hyalin-like clumps. This process differed from simple agglutination, in that in agglutination such large masses are not formed and the platelets retain much of their individual identities. The serum factor was heat labile, a globulin, and adsorbed on BaSO₄. Platelet adhesiveness appears to be the same basic phenomenon as viscous metamorphosis except that the adhesiveness is determined in a citrated system so that the fusion into masses does not occur. Budtz-Olsen in his monograph on clot retraction has suggested that the mechanism of retraction, as observed microscopically, consists of this fusion of the platelets into large masses which pull the fibrin clot together. The nature of this clot retraction accelerator and its mechanism of action are not known but it probably functions as an activator of the clot retraction mechanism.

Houlihan in 1947 described an adhesive factor found in both plasma and serum of dogs which modified the adhesion of dog platelets to bacteria. The method used was the extent of clumping of platelet-bacteria mixtures in vitro. This adhesion factor was found to be heat labile, adsorbed nonspecifically, and present in high and fairly constant titer. This adhesion factor in dogs, although determined by a different method, is similar to the factor described for human platelet adhesiveness.

The observations that the clot retraction accelerator is heat labile and removed by an antigen-antibody reaction in vitro and in vivo suggested some relationship to the complement system. No correlation was found between the total serum complement level and the level of platelet adhesiveness and clot retraction time. The heat lability, insolubility in water, and precipitation between 40 and 50 per cent saturation of (NH₄)₂SO₄ all speak against any individual component of the complement system being involved. There is no other known substance removed from serum in an antigen-antibody reaction. Whether the clot retraction accelerator is heat labile and removed by an antigen-antibody reaction in vitro and in vivo suggested some relationship to the complement system. No correlation was found between the total serum complement level and the level of platelet adhesiveness and clot retraction time. The heat lability, insolubility in water, and precipitation between 40 and 50 per cent saturation of (NH₄)₂SO₄ all speak against any individual component of the complement system being involved. There is no other known substance removed from serum in an antigen-antibody reaction.
tion accelerator actually participates in any immunologic phenomena or is only removed nonspecifically by the antigen-antibody reaction has not been determined.

It has been observed for many years that foreign substances, particularly bacteria, adhere to platelets. Antibodies have been implicated in this phenomenon although Houlihan's observations question this relation. Anaphylactic shock results in thrombocytopenia with agglutinated platelets found in large quantities in the viscera. Stetson\textsuperscript{17} has pointed out that the primary lesion in both the Arthus and Schwartzman phenomena is the formation of platelet-leukocyte thrombi. In vivo observations using the rabbit ear chamber by Ebert and coworkers\textsuperscript{18} demonstrated that, in the Arthus phenomenon, platelet thrombi formed and the platelets began to stick to the endothelium immediately following the antigen injection. Following this, a mixture with leukocytes occurred with formation of white thrombi which occluded the vessels and formed the basis for the gross lesion. Similarly, Ebert and Wissler\textsuperscript{19} using the same technic showed that during experimental serum sickness large platelet clumps are formed.

Numerous other observations have been made of the relation of white thrombi and platelet thrombi to antigen-antibody reactions. No cogent explanation or satisfactory mechanism has been proposed up to the present to account for the changes in the platelets in the immunologic response of the organism. The observations recorded here may help to explain these phenomena. A possible explanation is that the removal of the adhesiveness factor in the antigen-antibody reaction lowers the platelet adhesiveness and changes the surface properties of the platelets so that they tend to agglutinate in the blood vessels. Some indirect evidence in support of this hypothesis is the fact that heparin is known to induce platelet clumps and emboli in vivo while concomitantly lowering the platelet adhesiveness.\textsuperscript{2, 20, 21} Similarly, the addition of heparin in vitro markedly lowers the platelet adhesiveness, at the same time producing clumps of agglutinated platelets in citrated blood.\textsuperscript{22} In idiopathic thrombocytopenic purpura, there is an extremely low platelet adhesiveness in addition to the thrombocytopenia\textsuperscript{2} and there seems to be good evidence that much platelet agglutination is taking place.\textsuperscript{23-25} In studying platelet adhesiveness to bacteria, Copley and Houlihan\textsuperscript{26} have emphasized that platelet agglutination or the sticking of platelet to platelet differs entirely from platelet adhesiveness or the sticking of platelets to other substances such as glass, bacteria, or endothelium.

**CONCLUSIONS**

There is a substance in the blood, external to the platelets, which acts on the platelets to increase the platelet adhesiveness, accelerate clot retraction, and cause visous metamorphosis of the platelets. The clot retraction accelerator is heat labile, precipitates with the euglobulins, and is adsorbed on BaSO\textsubscript{4} and Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2}. It is removed from the blood by antigen-antibody reactions although it does not appear to be related to complement nor to any of the components of complement.

**REFERENCES**

PLASMA FACTOR FOR PLATELET ADHESIVENESS


A Plasma Factor for Platelet Adhesiveness and Clot Retraction Acceleration

J. PHILIP SAVITSKY