Studies on Platelets

VI. Demonstration and Characterization of a Heterologous (Forssman) Platelet Agglutinin

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Recent studies have suggested that some cases of idiopathic thrombocytopenic purpura (I.T.P.) are due to destruction of platelets in the circulation, possibly by hyperimmune antibodies. We have been engaged for some time in the search for antiplatelet substances in the serum of patients with various types of thrombocytopenia as evidence for such an etiologic concept. In the course of this study, the observation was made that the serum in a case of thrombotic thrombocytopenic purpura, although devoid of activity against human platelets, would constantly and grossly agglutinate suspensions of platelets from dog, rabbit and rat. Since search of the available literature failed to disclose reports of a similar finding, the results of our studies in this case and of the nature of the heteroplatelet agglutinin were considered worthy of report.

Case History

Bernard La F., M., 44, N.E.C.H. 61-808. Admitted August 12, 1951. This patient first noticed pallor and fatigue 3 months prior to admission. Ten weeks later the pallor became extreme, jaundice was noted, and the family observed that the patient was confused and disoriented. Six to eight blood transfusions administered during the 2 weeks prior to admission failed to improve the patient’s condition.

When first seen, the patient appeared extremely ill, very pale and slightly icteric. Although conscious, he appeared confused and disoriented. Temperature was 99.2, pulse 95, respirations 22 and blood pressure 115/50. Significant findings were the presence of a few petechiae over the lower extremities, and hemorrhages and exudates in both eye grounds. Laboratory studies revealed a severe anemia (RBC 1.55 M./cu. mm.; hemoglobin 4.9 Gm. per cent; hematocrit 18 per cent) with moderate leukocytosis and neutrophilia (WBC 11,700/cu. mm. with 75 per cent polynuclears), marked thrombocytopenia (23,250 platelets/cu. mm.) and reticulocytosis (26 per cent). The peripheral blood smear showed macrocytosis and spherocytosis (approximately 12 per cent), with target cells, “helmet” cells and many nucleated red cells. The bone marrow was hypercellular with a marked increase in the number of erythroid elements (62 per cent) and megakaryocytes. Of the latter, only 6 per cent appeared to be producing platelets. Ferrocyanide hydrochloride stain demonstrated increased hemosiderin. Although efforts to demonstrate auto- and iso-, complete and incomplete red cell agglutinins and hemolysins failed, and although the Coombs test found to be negative, evidence of active hemolysis was afforded by the reticulocytosis, an elevated serum bilirubin (2.8 mg./100 ml.) and plasma hemoglobin (90 mg./100 ml.) levels, increased osmotic and mechanical fragility of the red cells, and failure of the anemia to respond to multiple transfusions. Results of various tests of the hemostatic function were compatible with the extreme thrombocytopenia: positive tourniquet test, very prolonged...
bleeding time (>20'), and high serum prothrombin/plasma prothrombin activity ratio (50 per cent). A transfusion of platelet-rich polycythemic blood, according to the technic previously described, failed to elevate the platelet count of the patient, suggesting the existence of a very active platelet destructive mechanism (fig. 1). Renal involvement was indicated by a blood urea nitrogen determination of 39 mg per cent and by the presence of albumin and granular casts in the urine; liver damage by a bromsulfalein retention of 17 per cent after 45 minutes, and by strongly positive cephalin-cholesterol flocculation and thymol turbidity tests. The result of a muscle biopsy was essentially non-contributory and no intravascular platelet thrombi were seen.

The combination of the three features: mental changes, hemolytic anemia and thrombocytopenic purpura, suggested the diagnosis of thrombotic thrombocytopenic purpura. Heparin sodium (75 mg. every 4 hours intravenously for 2 days) and ACTH (100 mg.—ap-

![Graph](https://example.com/graph.png)

**Fig. 1.—**The rate of disappearance from the circulation of injected platelets in our patient of thrombotic thrombocytopenic purpura as compared to that observed in one case of "secondary" thrombocytopenic purpura.

proximately 200 I.U.—of the Wilson preparation daily for 8 days) were tried without success. Four blood transfusions for a total of 2,000 ml. of whole blood failed to raise the red blood cell count. The patient's confusion became progressively worse; he developed diffuse neurologic signs and expired on the eleventh day of hospitalization. Autopsy findings confirmed the clinical diagnosis.

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*Materials and Basic Technics*

All the procedures described were carried out with silicone coated glassware and needles, and when not otherwise indicated, in the cold room at a temperature of 4 C.

(a) **Preparation of platelet suspensions:** Blood was collected in chilled, Silicone coated syringes containing 1/10 volume of chilled 0.2 M sodium citrate solution by the two-syringe technic previously described. Blood was collected from the artery of the ear in the rabbit, the heart in the rat, the jugular or femoral vein in the dog and one of the antecubital veins in man. Blood was centrifuged at
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500 r.p.m. for 30 minutes in the cold room and the supernatant plasma, containing most of the platelets, separated by aspiration with silicone coated pipets and transferred to new silicone coated test tubes. The process was repeated until no more red cells could be separated. Platelets were then obtained by one of the two following methods: (1) centrifugation; (2) spontaneous sedimentation. In (1), the platelet-rich plasma was centrifuged at 2,500 r.p.m. for 30 minutes in the cold room, the clear supernatant plasma discarded and the button of platelets resuspended in sodium citrate solution 0.2 M. The platelets were again separated by centrifugation and resuspended in new solution. The washing was repeated 3 times. The final product was resuspended in 0.2 M solution of sodium citrate and kept in the cold room in silicone coated tubes. In (2), platelet-rich plasma, separated from the red cells by centrifugation as described, was stored at 4 C. Platelets sedimented out in approximately 2 to 3 days in the case of dog, rabbit and rat, and in approximately 5 days in the case of human thrombocytes. The supernatant plasma was then discarded, platelets resuspended in 0.2 M solution of sodium citrate and again allowed to sediment in the cold room. The procedure this time took approximately 2 days only and was repeated twice. Platelets were finally resuspended in 0.2 M solution of sodium citrate. The platelet suspensions used in this study were free of clumps by macroscopic and microscopic examination and appeared to be composed of discrete, individual thrombocytes plus occasional (1 to 2 per low power microscopic field) red cells. The platelets were shiny and able to take the supravital stain easily; they were also able to restore to normal the deficient clot retraction and utilization of prothrombin during coagulation of platelet-poor plasma. Preparations not exhibiting such properties were discarded. Suspensions used had a platelet count of approximately 500,000/ml.

(b) Preparation of serum: In order to prevent the nonspecific agglutination which occurs when thrombin evolves during the incubation of platelets in serum containing prothrombin, sera under investigation were inactivated at 56 C. for 30 minutes, decalcified by addition of 1/10 volume of 0.1 M sodium oxalate or by passage through cation-exchange resin IRC-50 and deprothrombinized by treatment with Ca$_3$(PO$_4$)$_2$ gel as described. Alternately, blood was collected in test tubes containing 1/10 volume of full strength Quick’s thromboplastin obtained from human brain. This latter procedure also yielded prothrombin-free serum. Sera were kept in deep freeze at temperatures of $-20$ C. when not immediately used.

(c) Agglutination test: 0.1 ml. of the inactivated (at 56 C. for 30 minutes), decalcified and deprothrombinized serum was added to 0.1 ml. of platelet suspension in Silicone coated test tubes 7.5 x 50 mm. and agglutination was sought after 2 and 18 hours, both macroscopically (with the help of a magnifying glass) and microscopically. The test tubes were ordinarily left standing at room temperature during the experiment. Simultaneously, normal sera similarly treated were incubated with platelet suspensions to provide negative controls.

Results

As mentioned in the introduction, the serum of the patient studied was found to contain an agglutinin against platelets of heterologous species. Studies were conducted to characterize the nature and properties of the agglutinin.
(a) Spectrum of activity of the heterologous platelet agglutinin: As indicated in table 1, the patient’s serum agglutinated suspensions of platelets from dog, rabbit and rat blood. In order to establish the specificity of such an observation, sera were collected from 30 subjects, including healthy individuals and patients with various diseases selected at random from the hospital wards. Among these were 4 cases of idiopathic thrombocytopenic purpura and 2 cases of acquired hemolytic anemia. One of the patients with I.T.P. had an associated acquired hemolytic anemia and his serum was found to contain an agglutinin against heterologous platelets active only after prolonged incubation at 4°C. This case will be discussed further. All other sera failed to show any heterologous platelet agglutinin.

In marked contrast with its potent agglutinating activity against heterologous platelets, the serum of our patient failed to agglutinate suspensions of human platelets. Attempts were made to demonstrate a human platelet agglutinin by varying the conditions of the experiment. The serum was incubated with human platelet suspensions at various dilutions (1:2 to 1:128) to discover possible prozone phenomena; and at various pH levels (pH 5 to 9). In no instance could agglutination be demonstrated. The addition of varying amounts of guinea pig complement to the mixture of serum and suspension of human platelets in saline solution failed to produce platelet lysis, since the drop of approximately 50 per cent observed in the platelet count of the mixture after 2 hours of incubation was no higher than that observed in a mixture of human platelets and complement alone. Attempts to demonstrate the presence of incomplete antibodies against human platelets in the serum failed in all cases since the addition of bovine and human albumin to the serum-platelet mixture was followed by prompt agglutination in the control mixture containing normal serum. Since no platelets of the patient could be obtained, a direct test (Coombs) for the detection of platelet-coating antibodies could not be performed. Normal human platelets, therefore, were incubated with the patient’s serum in an attempt to detect coating of the normal platelets by antibodies, not otherwise detectable, present in the patient’s serum (indirect Coombs test). The experiment failed when the incubated normal platelets agglutinated heavily during the washings necessary to remove all traces of serum and could not be resuspended uniformly in the antihuman rabbit serum. Evidence was obtained, however, that the function of normal human platelets was unaffected by incubation with the patient’s serum. After

<table>
<thead>
<tr>
<th>Dilution of serum</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human platelets</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dog platelets</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rabbit platelets</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rat platelets</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
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</table>
such incubation for one hour, platelets were still able to restore the clot retraction and prothrombin consumption of platelet-poor citrated normal human plasma following recalcification.

(b) Properties of the heterologous platelet agglutinin: The ability of the serum to agglutinate heterologous platelets was not greatly affected by inactivation of the serum at 56 C. for 30 minutes, by passing through Seitz filter or cation-exchange resin IRC-50, or by absorption with Ca3(PO4)2 gel. Changes in the temperature of incubation of the serum-platelet mixture failed to influence the activity of the agglutinin in the wide range of 5 to 45 C. When the serum-platelet mixture was incubated at 56 C., flocculation of the platelets occurred.

(c) Attempts to elute the agglutinin from the platelet agglutinates: Clumps obtained by incubating serum of the patient with dog platelets for 18 hours at room temperature were separated by centrifugation at 2,000 r.p.m. for 5 minutes, washed 3 times with cold saline solution, and finally resuspended in a volume of saline one-half that of the original serum-platelet mixture. The washings failed to show any agglutinating property against dog platelets. Attempts were then made to elute the agglutinin from the resuspended clumps by various technics: (1) incubation at 56 C. for one hour; (2) acidification to pH 3; and (3) alkalinization to pH 9. At the end of each treatment, the platelets were again separated by centrifugation and the supernatant saline solution again incubated at room temperature with suspension of platelets in 0.2 M solution of sodium citrate. No agglutination was found in any case after 18 hours of incubation.

(d) Presence of a heterophil (Forssman) red cell agglutinin in the patient's serum: As mentioned in the Case History, the serum of the patient did not exhibit any agglutinating activity against his own or human compatible red cells. It did, however, agglutinate sheep red cells at a dilution of 1:112 with Davidsohn's technique. This sheep red cell agglutinin could be absorbed completely by guinea pig kidney antigen, but not by beef erythrocytes antigen, the finding indicating the Forssman-like nature of this antibody. The agglutinin could be eluted from the clumped red cells by washing these three times with cold saline solution and finally resuspending them in a volume of warm (56 C.) saline solution one-half the volume of the original red-cell-serum mixture. These resuspended red cells were incubated at 56 C. for 2 hours, the cells separated by centrifugation, and the supernatant saline separated. This eluate agglutinated sheep red cells at a dilution of 1:14 (a considerable loss in titer when compared to the original dilution of 1:112), but failed to agglutinate suspensions of dog platelets.

(e) Absorption of the heterologous platelet agglutinin: Commercial guinea pig kidney and beef erythrocyte antigens were diluted (as specified by Davidsohn) with saline solution and then centrifuged at 2,000 r.p.m. for 10 minutes, to separate all fluid. An equal volume of the patient's serum was added to the tightly packed antigen. The mixture, after complete mixing with a stirring rod, was incubated at 37 C. for 3 minutes, centrifuged at 2,000 r.p.m. for 5 minutes, and the supernatant tested for presence of agglutinating effect against dog platelets suspended in 0.2 M solution of sodium citrate. Table 2 shows that the absorption of the patient's serum with guinea pig kidney antigen was followed by complete loss of its ability to agglutinate heterologous platelets; this, on the
contrary, was partially preserved after absorption of the serum with beef erythrocyte antigen. These findings classify the heteroplotelet agglutinin as a Forssman-like antibody.

(f) Absorption of the heterophil (Forssman) agglutinin by heterologous red cells and platelets: Inactivated, decalcified and deprothrombinized serum of the patient was incubated with an equal volume of packed washed sheep red cells at 37 C. for 2 hours. At the end of this period, the serum was separated by centrifugation. The procedure was repeated until the supernatant serum was no longer able to agglutinate sheep red cells. It was then incubated with an equal volume of a suspension of dog platelets in 0.2 M sodium citrate solution. No agglutination of platelets was noted after 2, 18, or 24 hours of incubation. In the reverse experiment, inactivated, decalcified and deprothrombinized serum of the patient was adsorbed with packed dog platelets. After this procedure was repeated six times, the supernatant serum was found to be still able to agglutinate weakly dog platelets and sheep red cells, and the experiment was abandoned.

(g) Attempts to purify the heterophil (Forssman) platelet agglutinin: A fractionation of the serum of the patient was accomplished by the technic of Reid and Jones. This technic consists of separating the water-soluble from the salt-soluble proteins of the serum by complete adsorption of the serum salts on ion-exchange resins. After treatment of the serum with the resin, the salt soluble fraction precipitates and can be separated by centrifugation. Subsequently, the precipitate can be redissolved in saline solutions of increasing ionic strength.

One volume of serum was added to a mixture containing one volume of wet IRA-400 cation-exchange resin in the OH cycle and 1/3 volume of wet IRC-50 cation-exchange resin in the H cycle. Serum and resin were mixed carefully by means of a stirring rod for at least one hour. The pH of the mixture was maintained between 6.5 and 8 by checking at frequent intervals with nitrazine paper. The pH had a tendency to fall below 6.5 and therefore frequent additions of small amounts of IRA-400 were found necessary. At the end of one hour, the
resin was allowed to separate by spontaneous sedimentation, and the supernatant fluid was removed. To the supernatant, a new mixture of resins was added in the same volumetric proportion as previously specified and the stirring continued for another hour. At the end of this time, it was usually found that the sodium content of the serum was below 10 mEq./liter, a figure which is apparently sufficient for a complete separation of the water soluble from the salt soluble globulin fraction. When the resin was separated by sedimentation, the supernatant fluid appeared milky in color. By centrifugation at 2,000 r.p.m. for 30 minutes, the supernatant could be divided into a whitish sediment containing the salt-soluble globulins, and a clear supernatant, containing albumin and water soluble globulin. The sediment was washed repeatedly with cold distilled water. Finally, solutions of buffered NaCl of increasing ionic strength from 0.001 to

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Nitrogen(mgs/100 ml)</th>
<th>Anti sheep red cell agglutinin titer</th>
<th>Anti dog platelet agglutinin titer</th>
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</thead>
<tbody>
<tr>
<td>Water soluble</td>
<td></td>
<td>660</td>
<td>0</td>
</tr>
<tr>
<td>Soluble in NaCl at ionic strength</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001 M</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.01 M</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.025 M</td>
<td>10</td>
<td>1:28</td>
<td>0</td>
</tr>
<tr>
<td>0.05 M</td>
<td>340</td>
<td>1:56</td>
<td>1:8</td>
</tr>
<tr>
<td>0.075 M</td>
<td>280</td>
<td>1:56</td>
<td>1:16</td>
</tr>
<tr>
<td>0.1 M</td>
<td>80</td>
<td>1:7(?)</td>
<td>1:2(?)</td>
</tr>
<tr>
<td>0.115 M</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.15 M</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2 M</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Agglutination read after 18 hours of incubation at 25 C. Due to the insufficient nitrogen content no satisfactory electrophoretic patterns of the various fractions could be obtained.

0.2 M through 0.01, 0.025, 0.05, 0.075, 0.10 and 0.15 M were added to the precipitate in a volume approximately 1/50 that of the serum originally employed. Each solute was again centrifuged at 2,500 r.p.m. until clear. All fractions were then incubated against suspensions of dog platelets in 0.2 M sodium citrate and sheep red cells, with the results indicated in table 3. It is evident that most of the agglutinating activity was concentrated in the salt soluble globulin fraction, and particularly in those fractions soluble in buffered saline of ionic strength equal to 0.025 M to 0.075 M. According to Haberman and Hill,11 these fractions, as demonstrated by parallel electrophoretic studies, contain mainly the β- and part of the γ-globulin fraction. Notwithstanding these results, it still remains doubtful whether the agglutinating activity of the patient's serum was in fact limited to those fractions characterized by high protein content. It is evident, however, that no agglutinating activity was present in the water soluble fraction which contains the albumin and euglobulins. In no fraction was a higher titer observed than in the original serum.
Diagnosis
1 Infectious mononucleosis
2 Infectious mononucleosis
3 Infectious mononucleosis
4 Infectious mononucleosis
5 Cirrhosis of liver
6 Idiopathic thrombocytopenic purpura
7 Idiopathic thrombocytopenic purpura
8 Aplastic anemia

(h) Agglutination of dog platelets by sera with demonstrable infectious mononucleosis and Forssman-type of red cell antibodies: Since the patient's serum not only agglutinated heterologous platelets but also sheep red cells, the heterologous platelet agglutinating activity was studied in five sera containing a Forssman antibody against sheep red cells and four sera containing heterophil antibody of the infectious mononucleosis type. As shown in table 4, six of these sera agglutinated dog platelets, among them all those exhibiting the highest heterophil titer.

Table 4.—Ability of Human Sera Containing Forssman and Infectious Mononucleosis Type Heterophil Antibodies to Agglutinate Heterologous Platelets (Dog)

<table>
<thead>
<tr>
<th>s</th>
<th>Diagnosis</th>
<th>Titer against sheep red cells</th>
<th>Titer against dog platelets</th>
<th>Activity after absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infectious mononucleosis</td>
<td>1:2048</td>
<td>1:64</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Infectious mononucleosis</td>
<td>1:1024</td>
<td>1:16</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Infectious mononucleosis</td>
<td>1:2048</td>
<td>1:32</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Infectious mononucleosis</td>
<td>1:512</td>
<td>1:8</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Cirrhosis of liver</td>
<td>1:1024</td>
<td>1:32</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>1:112</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>1:448</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>1:224</td>
<td>1:4</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Aplastic anemia</td>
<td>1:112</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

*Guinea pig kidney antigen.
†Beef erythrocytes antigen.
‡Positive 1:4 after 18 hours of incubation of the test tubes at 4 C.

DISCUSSION

The ability of human sera to agglutinate heterologous platelets does not seem to have been reported in the literature available to us. Its occurrence must be rather rare, since of the 30 other cases studied by us, only one, a patient with I.T.P. and acquired hemolytic anemia, exhibited agglutinin ability against dog platelets, detectable only after incubation at 4 C. for 12 hours. On the contrary, ability to agglutinate heterologous platelets seems to be a common property of sera exhibiting a significantly high titer of heterophil antibody of the infectious mononucleosis or Forssman type.

Absorption studies with guinea pig kidney and beef erythrocyte antigen demonstrated that the heterophil anti-platelet antibody detected in the serum of our patient was of the Forssman variety. The relation of this agglutinin to the Forssman antibody against sheep red cells also found in the serum of the patient is very interesting. The two could probably be considered similar, since (1) absorption studies showed that both agglutinins could be absorbed out with guinea pig kidney but not with beef erythrocyte antigen; (2) sera absorbed with sheep red cells no longer agglutinated dog platelets; and (3) fractionation studies showed recovery of the two agglutinins in identical serum fractions. Further support of this concept comes from the observation that sera with heterophil antibodies of the Forssman and non-Forssman variety tend to agglutinate...
heterologous platelets. This is not an absolute statement since, as shown in table 4, low heterophil titer against sheep red cells may be accompanied by inability to agglutinate heterologous platelets. This may be due to the level of the titer, since it has been our impression in the course of this investigation that much higher heterophil titers are required to agglutinate platelets than red cells, an observation probably worthy of further study.

We do not believe that the presence of the Forssman red cell and platelet agglutinin in the serum of our patient explains in any way the hemolytic anemia and thrombocytopenia. Whether, on the other hand, the existence of a destructive process against red cells (hemolytic anemia) and platelets (thrombocytopenia with short survival of the injected platelets) could bring about the presence of the Forssman type agglutinin is worth discussing. Heterophil antigens are widely distributed in animal tissues. In 1934, Bernstein described the presence of an elevated heterophil titer, of unspecified variety, in one case of thrombocytopenic purpura. Commenting on this finding, Tocantins mentioned that acute destruction of platelets “in vivo,” such as determined by the injection of antiplatelet serum in animals, may result in an increase of the nonspecific antibody content of the serum. Tocantins’ concept could well be applied to our patient where evidence of rapid destruction of platelets was available. We searched, however, for the occurrence of heterophil anti-platelet antibody in other patients with I.T.P., where the rapid destruction of platelets is demonstrated by the extremely rapid disappearance of injected platelets from the circulation. None of these cases presented a platelet heterophil antibody, although the majority of them had a significant anti-sheep red cell Forssman antibody. The possibility exists that the titer of the antiplatelet antibody was too low to permit its detection by our methods. It must be stated that the case under discussion here was an example of the rather ill-defined condition known as thrombotic thrombocytopenic purpura, and the findings in such a case might vary considerably in their immunologic context from those in typical cases of idiopathic thrombocytopenic purpura. Whether the occurrence of a heterophil platelet antibody is a characteristic feature of thrombotic thrombocytopenic purpura can only be decided by the study of future cases.

A last possibility should be given consideration. Virus infections are known to be responsible for the development in patients of iso- and hetero-red cell agglutinins. This is true, as an example, in diseases strongly suspected or known to be caused by a virus, for example infectious mononucleosis (hetero-red cell agglutinin) and atypical pneumonia (cold iso-red cell agglutinin). The possibility exists that in our patient also the development of the hetero-platelet agglutinin was initiated by a virus infection. It must be said, however, that no immunologic nor pathologic evidence of such possibility could be found in the course of our studies in this case.

**Summary**

1. The serum of a patient with thrombotic thrombocytopenic purpura was found to contain an agglutinin active against sheep red cells and heterologous platelets (dog, rabbit and rat), but not against human platelets. The activity of the agglutinin was not affected by absorption with gels, treatment with ion-
exchange resins, filtration through Seitz filters or heating of the serum at 56 C. The antibody was found to be present in the salt soluble fraction of the serum proteins. The agglutinin could not be eluted from the agglutinated platelets, was absorbed completely by guinea pig kidney and incompletely by beef erythrocyte antigen (Forssman's variety).

2. Ability to agglutinate heterologous platelets was found to be a property of sera exhibiting a high titer of heterophil antibodies of the Forssman or non-Forssman variety.

3. It is not likely that the presence of such heterophil agglutinin was of importance in the pathogenesis of the hemolytic anemia and thrombocytopenia of the patient; rather, it could be an effect of the active lytic process of red cells and platelets. Whether the finding had diagnostic significance in the ill-defined syndrome of thrombotic thrombocytopenic purpura remains to be established with the observation of further cases.

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EDWARD ADELSON and MARIO STEFANINI