Thrombocytopenic Purpura Due to Quinidine

II. Serologic Mechanisms

By Frederick G. Bolton

Our knowledge of the probable mechanisms of thrombocytopenia in cases of thrombocytopenic purpura due to acquired sensitivity to drugs derives largely from the work of Ackroyd on patients who developed purpura after taking sedormid. He showed that agglutination and lysis of platelets in the blood of these patients was caused by adding sedormid to their blood in vitro, that this was due to a lytic factor present in serum or plasma from these patients and that platelets from normal individuals could replace the patients’ platelets in the reaction. Complement was fixed during this lysis of platelets, but in the absence of platelets, complement could not be fixed. The lytic factor was stable to heating for 20 minutes at 56 C. and in the absence of complement still caused platelet agglutination in the presence of sedormid. Addition of complement restored the property of causing platelet lysis.

More recently Ackroyd has shown that platelets from normal individuals or from these patients failed to form a stable compound with sedormid, nor was the lytic factor—sedormid complex present in a stable state. The anti-platelet lytic factor existed in the globulin fraction of serum. In the presence of sedormid, this factor formed a complex with platelets; this could then be separated from serum, washed with sedormid solution and could fix complement. If this complex was washed with saline, dissociation apparently took place, as the platelets could then no longer fix complement. Dialysis of the complex against saline also resulted in dissociation, thereby providing a means of isolating the lytic factor.

Despite the impetus given to the investigation of purpura by Ackroyd’s work, there have been few demonstrations of similar mechanisms with other drugs. In 1948, Grandjean had described a 40–44 per cent fall in the platelet count of platelet-rich plasma obtained from a woman who had recovered from thrombocytopenic purpura due to quinine when quinine was added to the plasma in vitro. Quinine caused no change in the platelet count in platelet-rich plasma from a normal individual.

Bigelow and Desforges described investigations in two cases of thrombocytopenic purpura due to quinidine. In the first case, quinidine was able to cause agglutination of platelets in platelet-rich plasma from the patient but not from normal individuals. A similar observation was made with blood from their second patient. They were, furthermore, able to demonstrate that platelet-poor plasma from this patient was able to cause agglutination of normal platelet-rich plasma in the presence of quinidine. Bolton and Young described cases of
thrombocytopenic purpura due to quinidine, sulphamezathine and quinidine and showed that the addition of the appropriate chemical to platelet-rich plasma from their patients when in remission was able to cause agglutination and lysis of platelets in the first two cases, and only agglutination in the third case. Platelet-poor plasma from this patient was able to cause agglutination of platelets in normal platelet-rich plasma in the presence of quinidine. Quinidine could not be replaced by its stereoisomer, quinine, in these experiments.

Subsequently, Larson showed that the addition of quinidine to whole blood taken from a woman with quinidine purpura, presumably during remission, prevented clot retraction, whereas normal blood retracted well with added quinidine as did the patient's blood in the absence of quinidine. Serum from the patient could inhibit clot retraction of normal blood if quinidine was added. He also found that quinidine was able to cause platelet agglutination in platelet-rich plasma from the patient and that quinine was not able to replace quinidine in these investigations.

Barkham and Tocantins described their investigations on the blood of a patient with quinidine purpura. Addition of quinidine to platelet-rich plasma from the patient caused a rapid decrease in the number of platelets, apparently without agglutination of platelets. Quinidine, but not quinine, inhibited clot retraction of the patient's blood; they were not able to show that serum from their patient inhibited clot retraction of normal blood in the presence of quinidine. They described the morphologic changes of platelet lysis but were unable to demonstrate such changes if washed normal platelets replaced platelets from the patient.

A further case of quinidine purpura with experimental studies was reported by Weisfuse, Spear and Sass. Once again, platelet-poor plasma from their patient was able to cause platelet agglutination in normal platelet-rich plasma in the presence of quinidine, but not in the presence of quinine. Washing of platelets previously treated with quinidine apparently removed the quinidine, for these platelets were not then agglutinable by the patient's plasma. They stated, without giving experimental details, that the agglutinin was completely absorbed from the plasma onto the platelets by incubation of the plasma with platelets. Presumably quinidine was necessary for this reaction. The agglutinin was heat labile at 65 C. and was equally potent at 4 C., 25 C. and 37 C. The addition of an unstated amount of complement failed to induce platelet lysis with agglutinating plasma. The agglutinin was precipitated by half saturation with ammonium sulphate.

More difficult to interpret is the account given by Lopez Garcia and Sainz de la Maza of a precipitin reaction between the serum of a patient with quinidine thrombocytopenic purpura and diluted serum from a normal individual who had recently been given quinidine.

The experiments to be described in this paper concern the mechanisms involved in the serologic reactions of the blood of a patient with thrombocytopenic purpura due to quinidine. The effect of quinidine on clot retraction was not studied as it seemed likely that any effect obtained would be mediated through the platelets.
Experiments and Results

All glassware used in agglutination and lysis tests was silicone coated. That for complement-fixation work was not coated. Blood was always taken in silicone-coated syringes.

A. Demonstration of platelet agglutination and lysis of normal platelets by quinidine and platelet-poor plasma from the patient (Table 1)

Venous blood was drawn from the patient, while still thrombocytopenic, in silicone-coated syringes and was mixed with one-ninth of its volume of 1 per cent disodium ethylenediamine tetra-acetate (sequestrene) in 0.7 per cent sodium chloride. Platelet-poor plasma was prepared by centrifuging at 3,000 r.p.m. for 30 minutes. Normal platelet-rich plasma was prepared by adding to 10 ml of blood from a normal individual 0.1 ml of heparin (10 mg per ml.) and then centrifuging at 1,000 r.p.m. for 15 minutes. The supernatant, which contained approximately 500,000 platelets per cu.mm., was used.

An experiment (Table 1) was set up with dilutions of quinidine and quinine sulphate in mixtures of normal platelet-rich plasma, patient’s platelet-poor plasma, and saline. The tests were left two hours at room temperature and then examined microscopically, drops of the incubates being placed on glass slides and viewed under the 4 mm. dry objective. Platelet agglutination was recorded as follows in this and subsequent experiments:

++ half to all platelets agglutinated per field.
+ one third to one half platelets agglutinated per field.
± small numbers of 3-4 platelets in clusters per field.
– none to one cluster of 2-3 platelets per field.

It was shown separately that saturation of normal platelet-poor plasma with quinidine sulphate did not cause platelet agglutination when mixed with an equal volume of normal platelet-rich plasma.

From this experiment it may be concluded that the plasma of the patient contained a factor which would agglutinate normal platelets only in the presence of quinidine. No agglutination resulted if quinidine was replaced by quinine.

It was noticed that loss of characteristic platelet morphology took place particularly in those tubes showing marked platelet agglutination. Although this was considered to represent platelet lysis, a further experiment was designed to elaborate this point.

B. The effect of anticoagulants on complement and on platelet agglutination and lysis by quinidine and patient’s plasma (Table 2)

Using sheep red cells, amboceptor and guinea-pig complement, concentrations of heparin, sequestrene and sodium citrate were found which were just able to inhibit lysis of the standardized hemolytic system. It was therefore possible to calculate the relative anticomplementary activities of the anticoagulant used in this experiment. Thus, blood con-

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Table 1.—Agglutination of Normal Platelets by Patient’s Plasma and Quinidine

<table>
<thead>
<tr>
<th>Dilutions of quinidine sulphate (20 mg.%) in saline 0.02 ml.</th>
<th>Dilutions of quinine sulphate (20 mg.%) in saline 0.02 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>next 1/2 1/4 1/8 1/16 1/32 1/64 1/128</td>
<td>next 1/2 1/4 1/8 1/16 1/32 1/64 1/128</td>
</tr>
<tr>
<td>Normal platelet-rich plasma ml.</td>
<td>0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1</td>
</tr>
<tr>
<td>Patient’s platelet-poor plasma ml.</td>
<td>0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1</td>
</tr>
<tr>
<td>Saline (ml. of 0.9%)</td>
<td>0.02 0.12</td>
</tr>
<tr>
<td>Agglutination</td>
<td>++ ++ ++ ++ ++ + + ±</td>
</tr>
</tbody>
</table>

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Table 2.—Effect of Anticoagulants on Agglutination and Lysis of Patient’s Platelets by Quinidine in the Presence of Patient’s Plasma

<table>
<thead>
<tr>
<th>Elapsed time after addition of quinidine or saline (minutes)</th>
<th>Platelet-rich plasma</th>
<th>Platelet-rich plasma</th>
<th>Platelet-rich plasma</th>
<th>Platelet-rich plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citrate</td>
<td>Heparin</td>
<td>Sequestrene</td>
<td>Citrate</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>Quinidine</td>
<td>Saline</td>
<td>Quinidine</td>
</tr>
<tr>
<td>5</td>
<td>All platelets separate and normal</td>
<td>All platelets separate and normal</td>
<td>All platelets separate and normal</td>
<td>All platelets separate and normal</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>separate normal</td>
<td>separate normal</td>
<td>separate normal</td>
<td>separate normal</td>
</tr>
<tr>
<td>30</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
</tr>
<tr>
<td>40</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
</tr>
<tr>
<td>60</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
</tr>
<tr>
<td>210</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
<td>Agglutinates+</td>
</tr>
</tbody>
</table>

Table 2 shows that agglutination and lysis of platelets occurred, demonstrable both in wet preparations and subsequently by examination of stained films. It can be seen that if heparinized blood was used there was slight platelet agglutination but that this was superseded by rapid lysis of platelets, leading to virtual disappearance of the cells. In citrate, there was first agglutination and later a certain amount of lysis, whereas in sequestrene, there was no apparent lysis, but intense agglutination of platelets. No changes occurred in the control tubes. These changes ran parallel with the anticomplementary nature of the anticoagulants used. Heparin was the least anticomplementary and in the heparinized sample lysis was marked. Sequestrene was very anticomplementary and in blood taken in sequestrene, no lysis occurred.

C. Demonstration of complement fixation by interaction of patient’s serum, quinidine and normal platelets (Table 3)

Platelets: Platelet-rich plasma from a normal individual with sequestrene as anticoagulant was centrifuged at 3,000 r.p.m. for 30 minutes. The platelets were washed once in...
normal saline and were resuspended in normal saline to give a concentration of approximately 32 million per ml.

Sera: Serum from the patient and from a patient with idiopathic thrombocytopenic purpura (I.T.P.) were inactivated at 56 C. for 30 minutes.

Quinidine: 0.25 per cent quinidine sulphate in 0.9 per cent saline.

Complement: Lyophilized guinea-pig complement titrated against 1 per cent sensitized sheep cells in 0.85 per cent saline, the cells having previously been sensitized with an equal volume of amboceptor containing two minimum hemolytic doses (M.H.D.); 2 M.H.D. of complement were used in each test in 0.5 ml.

Sera, platelets, quinidine, saline and complement were mixed in the proportions given in table 3 and were incubated for 1 hour at 37 C. with occasional shaking. Sensitized sheep cells were then added and mixed; after further incubation for 30 minutes at 37 C. the tubes were examined for hemolysis. The following notation was used for this experiment and subsequently:

NH—No hemolysis
PH—Partial hemolysis
CH—Complete hemolysis

Complement fixation occurred when normal platelets, patient's serum and quinidine were allowed to interact. In the absence of quinidine, serum and platelets together did not fix complement; in the presence of quinidine, a certain number of platelets were needed to fix complement with the patient's serum. With 0.1 ml. of serum, more than 300,000 platelets were required.

In an identical experiment, serum from a patient with idiopathic thrombocytopenic purpura (I.T.P.) failed to fix complement in the presence of platelets and quinidine. Subsequent experiments with normal sera always failed to demonstrate fixation of complement in the presence of quinidine and normal platelets.

Table 3.—Complement Fixation by Normal Platelets, Quinidine and Serum from the Patient

<table>
<thead>
<tr>
<th>Patient's serum</th>
<th>Platelet suspension</th>
<th>Platelet suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>neat</td>
<td>1/2</td>
</tr>
<tr>
<td>neat</td>
<td>NH</td>
<td>NH</td>
</tr>
<tr>
<td>1/2</td>
<td>NH</td>
<td>NH</td>
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<tr>
<td>1/4</td>
<td>NH</td>
<td>NH</td>
</tr>
<tr>
<td>1/8</td>
<td>CH</td>
<td>PH</td>
</tr>
<tr>
<td>1/16</td>
<td>CH</td>
<td>CH</td>
</tr>
</tbody>
</table>

NH—No hemolysis; PH—Partial hemolysis; CH—Complete hemolysis.

Control tests set up at same time. Measurements in ml.

<table>
<thead>
<tr>
<th>Platelets</th>
<th>0.15</th>
<th>0.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinidine</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Patient's serum</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>ITP serum</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Saline</td>
<td>0.35</td>
<td>0.25</td>
</tr>
<tr>
<td>Complement</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sensitized cells</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Result</td>
<td>NH</td>
<td>CH</td>
</tr>
</tbody>
</table>
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Table 4.—Absorption of Plasma Agglutinating Factor by Platelets in the Presence of Quinidine

<table>
<thead>
<tr>
<th>Supernatant dilution</th>
<th>neat</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>A + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A + Quinidine</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B + Quinidine</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C + Quinidine</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A' + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A' + Quinidine</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B' + Saline</td>
<td>±</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B' + Quinidine</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C' + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C' + Quinidine</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1. The role of quinidine in the union of the plasma factor with normal platelets (Table 4)

In this experiment, efforts were made to absorb the abnormal plasma factor by normal platelets both in the presence and absence of quinidine.

Platelets were prepared from normal blood using sequestrene as an anticoagulant, washed once with a large volume of saline and then resuspended in as small an amount of saline as possible.

To 1 ml. of platelet-poor plasma from the patient were added 0.1 ml. of platelet suspension and 0.1 ml. of saline; to a second 1 ml. were added 0.1 ml. of platelet suspension and 0.1 ml. of 0.01 per cent quinidine sulphate solution. The two samples were each mixed and allowed to stand for 1 hour at room temperature; they were then centrifuged at 3,000 r.p.m. for 15 minutes. 0.3 ml. of each supernatant was set aside and to the remainder, platelets and saline or quinidine were again added. In this way absorption was carried out three times.

```
Patient's Plasma

1 ml. + 1 ml. 0.1 ml. saline + 0.1 ml. quinidine
0.1 ml. platelets + 0.1 ml. platelets

A 0.3 ml. Supernatant
0.1 ml. saline + 0.1 ml. quinidine
0.1 ml. platelets + 0.1 ml. platelets

B 0.3 ml. Supernatant
0.1 ml. saline + 0.1 ml. quinidine
0.1 ml. platelets + 0.1 ml. platelets

Supernatant C
```

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Each supernatant was titrated in doubling dilutions in saline, to which were added equal volumes of saline or 0.01 per cent quinidine sulphate and normal platelet-rich plasma. After mixing they were allowed to stand at room temperature and were then read microscopically, results being given in table 4.

The platelet agglutinating factor in plasma could be absorbed out of the patient's plasma by platelets only if quinidine was present in the system. In the absence of quinidine, it was apparent that no union of plasma factor with normal platelets took place.

E. Nonabsorption of plasma factor by leukocytes or red cells in the presence of quinidine (Table 5)

Since platelet preparations usually contain small numbers of red cells and leukocytes, it was necessary to know that results of experiment 1) were not attributable to absorption by red cells or leukocytes. Packed 5-times washed group O red cells and packed twice washed leukocytes from defibrinated group O blood (the red cells having been sedimented by added dextran) were used. The red cell preparation was uncontaminated by significant numbers of leukocytes or platelets; the leukocyte preparation contained approximately one leukocyte to four red cells.

The procedures of the previous experiments were carried out using red cells and leukocytes instead of platelets as absorbing agents.

Results are given in table 5, i and ii; they indicated that there was no agglutinin absorption by red cells or leukocytes in the presence of quinidine.

**Table 5.—Nonabsorption of Plasma Agglutinating Factor by Red Cells or Leukocytes in the Presence of Quinidine**

<table>
<thead>
<tr>
<th>Supernatant dilution</th>
<th>next</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Red cell absorption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A + Quinidine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>B + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B + Quinidine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>C + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C + Quinidine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>A' + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A' + Quinidine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>B' + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B' + Quinidine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C' + Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C' + Quinidine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

| **II. Leukocyte absorption** |
| A + Saline           | -    | -   | -   | -   | -    |
| A + Quinidine        | ++   | ++  | ++  | +   | -    |
| B + Saline           | -    | -   | -   | ±   | -    |
| B + Quinidine        | ++   | ++  | ++  | +   | -    |
| C + Saline           | -    | -   | -   | -   | -    |
| C + Quinidine        | ++   | ++  | ++  | +   | -    |
| A' + Saline          | -    | -   | -   | -   | -    |
| A' + Quinidine       | ++   | ++  | +   | ±   | -    |
| B' + Saline          | -    | -   | -   | -   | -    |
| B' + Quinidine       | ++   | ++  | +   | ±   | -    |
| C' + Saline          | -    | -   | -   | -   | -    |
| C' + Quinidine       | ++   | ++  | ++  | +   | -    |
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F. Fixation of complement by platelets which had absorbed serum agglutinating factor in the presence of quinidine (Table 6)

Ten ml. of inactivated serum from the patient were saturated with quinidine sulphate powder and were mixed with approximately 7,500 million normal platelets which had been prepared from normal sequestrene blood, washed in saline and resuspended in a minimum of saline. The mixture was allowed to stand at room temperature for 2½ hours; the platelets were then washed twice with saline saturated with quinidine sulphate. They were then tested for complement fixing ability by incubation for 1 hour at 37 C, with complement alone, with complement and quinidine and with complement, quinidine and patient’s serum together. The hemolytic system described earlier was used. Suitable controls were also set up.

Table 6 shows that complement was fixed by the platelets which from experiment D are known to have absorbed agglutinin.

From these investigations it was clear that platelets, quinidine and a plasma or serum factor from the patient united to form a complex which could be separated by centrifuging from the reaction mixture. It seemed likely from the result of the absorption experiment D, that quinidine provided a link between the humoral factor and the platelets. Future experiments were designed to investigate the nature of this linkage.

G. The effect of quinidine on normal platelets (Table 7)

Approximately 10 million platelets from normal sequestrene blood were divided into two aliquots, and washed with saline. One aliquot was suspended in 10 ml. of 0.25 per cent

| Table 6.—Complement Fixation by Platelets Which Have Absorbed Serum Factor in the Presence of Quinidine |
| Measurements in ml. |
| Experimental platelets | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Normal platelets (7 million per ml.) | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Patient’s serum | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Quinidine sulphate 0.1% | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Saline | 0.2 | 0.1 | 0.1 | 0.25 | 0.35 | 0.1 |
| Complement | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Sensitized cells | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Result | NH | NH | PH | PH | NH | CH CH | CH |

| Table 7.—The Effect of “Quinidinization” and Subsequent Washing with Saline of Normal Platelets on Their Ability to Fix Complement |
| Saline treated platelets ml. | neat | 0.15 | 0.15 |
| Diluted in saline | 1.10 | 0.15 | 0.15 |
| “Quinidinized” platelets ml. | neat | 0.15 | 0.15 |
| Diluted in saline | 1.10 | 0.15 | 0.15 |
| Inactivated patient’s serum ml. | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Quinidine sulphate (0.25%) ml. | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Saline ml. | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Complement ml. | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Sensitized cells ml. | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Results | CH | CH | CH | NH | NH | CH | CH | CH | CH |
quinidine sulphate in saline; the other was suspended in 10 ml. of 0.9 per cent saline. After one hour at room temperature, the platelets were deposited by centrifuging and were each washed three times with normal saline, being suspended between each washing. Finally each batch of platelets was suspended in 1.0 ml. of saline and tested for complement fixing ability by the method previously described. The results are shown in table 7.

Evidently no significant amounts of quinidine were bound by platelets treated in such a way, nor were the platelets affected in their power to act as antigen in the presence of quinidine in the test.

H. The effect of dialysis on a mixture of quinidine and the patient’s plasma (Table 8)

Six ml. of platelet-poor plasma from the patient were mixed with 0.6 ml. of 0.01 per cent quinidine sulphate in saline. Two ml. aliquots were dialyzed in cellophane bags against a large volume of 0.9 per cent saline, which was changed several times during dialysis, at 4 C. Dialysis of the first aliquot was stopped after 24 hours, of the second after 48 hours and of the third after 66 hours. The contents of the dialysis bags were frozen until tested.

A control of the patient’s plasma with a corresponding amount of saline instead of quinidine was dialyzed in two portions, one for 24 hours and the other for 66 hours.

The volumes of fluid in the dialysis bags were measured.

Quinidine-plasma mixtures (original volumes 2.0 ml.)

<table>
<thead>
<tr>
<th>Time of Dialysis</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours dialysis</td>
<td>3.1</td>
</tr>
<tr>
<td>48 hours dialysis</td>
<td>3.1</td>
</tr>
<tr>
<td>66 hours dialysis</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Plasma-saline mixtures (original volumes 2.0 ml.)

<table>
<thead>
<tr>
<th>Time of Dialysis</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours dialysis</td>
<td>2.0</td>
</tr>
<tr>
<td>66 hours dialysis</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Doubling dilutions of the dialyzed mixtures were made and tested for platelet agglutinating power by addition of an equal volume of saline or quinidine and of normal sequestrene platelet-rich plasma. Microscopic examination was made after one hour at room temperature. An undialyzed mixture of patient’s plasma with one tenth of a volume of 0.01 per cent quinidine sulphate was similarly titrated, saline being added instead of more quinidine.

It is evident from table 8 that dialysis removed the platelet agglutinating activity of the quinidine plasma mixture, after 24 hours dialysis. This was due to removal of quinidine, for addition of quinidine restored the titer to as high as, or curiously, perhaps even a little higher than that of an undialyzed plasma-quinidine mixture.

**Table 8.**—The Effect on Platelet-Agglutination of Dialysis of a Patient’s Plasma-Quinidine Mixture

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Neat</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 24-hour dialysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 48-hour dialysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3 66-hour dialysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 Plasma-saline mixture + saline 24-hour dialysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 66-hour dialysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 No. 3 with 1 volume of 0.01% quinidine sulphate added to each tube</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7 No. 5 with 1 volume of 0.01% quinidine sulphate added to each tube</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>8 Undialyzed plasma quinidine mixture + saline</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
The plasma factor was not removed by dialysis; if there was an association between quinidine and plasma factor, it did not withstand the forces of such dialysis.

I. Dissociation of platelet-quinidine-serum factor complex by dialysis (Table 9)

Approximately 5,000 million platelets from normal sequestre blood were mixed with 5 ml. of serum from the patient to which had been added 0.5 ml. of sequestre solution, and 1 ml. of 0.25 per cent quinidine sulphate solution in saline. The mixture was kept at room temperature for five hours with frequent gentle shaking. The platelets were then deposited by centrifuging and were washed twice with saline saturated with quinidine sulphate. They were suspended in 1 ml. of quinidine-saline and were dialyzed in a cellophane bag against a large volume of saline at 4C. for 24 hours. At the end of this time the contents of the bag were centrifuged to separate the platelets from the supernatant. Complement fixation tests were set up as indicated in Table 9, the platelets being suspended in 1 ml. of saline after draining them free of supernatant fluid.

This experiment demonstrated that dialysis had split the platelet-quinidine-serum factor complex into its component parts, quinidine passing through the dialyzing bag and leaving behind serum factor separated from platelets. Complement was not fixed by these platelets until both patient’s serum and quinidine were added. Similarly the supernatant fluid was able to fix complement only when both platelets and quinidine were included in the test.

J. Nature of the serum or plasma factor (Table 10)

The previous experiment showed a way of isolating the serum factor in a reasonably pure state. The supernatant fluid from the dialyzing bag was subjected to filter-paper

<table>
<thead>
<tr>
<th>Dialyzed platelets</th>
<th>Normal platelets (7,000 million per ml.)</th>
<th>0.075 0.075 0.075</th>
<th>0.075 0.075</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialyzed supernatant</td>
<td>Inactivated patient’s serum</td>
<td>0.05 0.05</td>
<td>0.05 0.05</td>
</tr>
<tr>
<td>Quinidine sulphate 0.25%</td>
<td>Saline</td>
<td>0.1 0.05</td>
<td>0.05 0.05</td>
</tr>
<tr>
<td>Complement</td>
<td>Sensitized cells</td>
<td>0.25 0.25</td>
<td>0.25 0.25</td>
</tr>
<tr>
<td>Result</td>
<td>CH CH CH CH CH CH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 10.**—Results on Platelet Agglutinin Titer of Fractionating Patient’s Plasma with Ammonium Sulphate

<table>
<thead>
<tr>
<th>Volume after dialysis</th>
<th>With quinidine</th>
<th>With saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated patient’s plasma</td>
<td>—</td>
<td>++ ++ ++ ++ ± − −</td>
</tr>
<tr>
<td>25% saturation fraction</td>
<td>lost in error</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>33% saturation fraction</td>
<td>1.7 ml.</td>
<td>++ ++ + ± − −</td>
</tr>
<tr>
<td>40% saturation fraction</td>
<td>0.4 ml.</td>
<td>++ ++ + ± − −</td>
</tr>
<tr>
<td>50% saturation fraction</td>
<td>1 ml.</td>
<td>++ + — — − −</td>
</tr>
<tr>
<td>Remaining supernatant</td>
<td>Concentrated from 8 to 6 ml.</td>
<td>— — — — — —</td>
</tr>
</tbody>
</table>
TABLE 11.—The Heat-Destruction of Complement-Fixing Ability of Patient’s Serum

Measurements in ml.

<table>
<thead>
<tr>
<th></th>
<th>0.075</th>
<th>0.075</th>
<th>0.075</th>
<th>0.075</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets 7,000 million per ml.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient’s serum (heated 56 C. 30 min.)</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient’s serum (heated 65-70 C. 30 min.)</td>
<td></td>
<td></td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Quinidine 0.25%</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>0.05</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Complement</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Sensitized cells</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Result</td>
<td>NH</td>
<td>CH</td>
<td>CH</td>
<td>NH</td>
</tr>
</tbody>
</table>

electrophoresis at pH 8.6. It contained a protein in low concentration having the same mobility as the γ-globulin of a normal serum run concurrently. No other protein was detected.

This corroborated the following results obtained by fractionation of the patient’s plasma by ammonium sulphate. Saturated ammonium sulphate solution was added to sequestrene plasma of the patient to produce 25, 33, 40 and 50 per cent saturation. After each addition the mixtures were allowed to stand at 4 C. for five minutes and then the precipitates were separated by centrifuging at 4 C. for 15 minutes at 3,000 r.p.m. The deposits so obtained were dissolved in 1 ml. of 0.9 per cent saline and dialyzed overnight against saline at 4 C. The supernatant remaining after the last precipitation was likewise dialyzed.

The fractions were then tested for platelet agglutinating power by titrating them in doubling dilutions in saline with an added equal volume of saline or 0.01 per cent quinidine sulphate solution and an equal volume of normal platelet-rich plasma. Platelet agglutination was read and recorded as before, that is, after 1 hour incubation at room temperature.

The fractions after dialysis were also subjected to filter-paper electrophoresis at pH 8.6. In the 25 per cent fraction, fibrinogen only was detected. The globulins were all present in the 33, 40 and 50 per cent fractions but the highest concentration of γ-globulin was in the 33 per cent fraction, the concentration falling in the other fractions. Albumin was also present in these fractions but was present in the highest concentration in the supernatant after 50 per cent saturation; there was very little globulin present in this fraction.

Results of fractionation (table 10) established that the agglutinating power in the plasma lay in the fractions containing globulins and of those, allowing for differing degrees of concentration of dialysates, that fraction containing the most γ-globulin was probably the most potent.

K. The effect of heating on complement-fixing power of the patient’s serum (Table 11)

It has already been seen that heat inactivation at 56 C. for 30 minutes does not inhibit the complement fixing ability of the patient’s serum in the presence of quinidine, platelets and added complement.

The serum was heated for 30 minutes at 65-70 C. and a complement fixation test was set up as shown in table 11.

It will be seen that this amount of heating inhibited the complement fixing ability of the patient’s serum.

L. Thermal range of agglutinating activity of patient’s plasma (Table 12)

Doubling dilutions of the patient’s and of normal sequestrene plasma were made in saline. Equal volumes of dilutions, of 0.01 per cent quinidine sulphate solution in 0.9 per cent saline and of normal platelet-rich plasma were mixed and allowed to stand at 37 C. for one hour and were then examined microscopically for agglutination. Similar titrations were set up at 4 C. and at 22 C.

Table 12 indicates that there was no essential difference in activity at these three temperatures.
M. Noninhibition of agglutinating power of plasma of patients by previous addition of quinidine (Table 13)

The effect of the previous addition of quinidine in high concentration to the plasma was studied on the agglutinating titer of the patient's plasma for platelets in the presence of quinidine.

Two ml. of the patient's platelet-poor plasma was shaken with 200 mg. of quinidine sulphate powder and the mixture was allowed to stand for one hour at room temperature. Undissolved quinidine was separated by centrifuging and a portion of supernatant was set aside. A further 200 mg. of quinidine was added and the process was repeated as before.

Normal platelet-poor plasma was treated in the same way. Both samples were subsequently titrated by mixing doubling dilutions in saline with equal volumes of normal platelet-rich plasma and saline or 0.01 per cent quinidine solution. After one hour at room temperature, the degree of platelet agglutination was recorded microscopically.

Table 13 demonstrates that there was no inhibition of potency of agglutinating plasma by this procedure.

**Table 12.**—Thermal Range of Agglutinating Power of Patient's Plasma

<table>
<thead>
<tr>
<th>Plasma dilution</th>
<th>neat</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>Patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 C.</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>22 C.</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 C.</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 C.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22 C.</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>37 C.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 13.**—Noninhibition of Plasma Agglutinating Power by Previous Addition of Quinidine to Plasma

<table>
<thead>
<tr>
<th>Plasma dilution</th>
<th>neat</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>Normal plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ quinidine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal plasma after 1 addition of quinidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ quinidine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal plasma after 2 additions of quinidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ quinidine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient's plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ quinidine</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient's plasma after 1 addition of quinidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ saline</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ quinidine</td>
<td>++</td>
<td>++</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient's plasma after 2 additions of quinidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ saline</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ quinidine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
TABLE 14.—Nonabsorption of Serum Factor by Blood Vessels in Presence of Quinidine, Tested by Complement Fixation

Measurements in ml.

<table>
<thead>
<tr>
<th>Normal platelets (3,000 million per ml.)</th>
<th>0.15</th>
<th>0.15</th>
<th>0.15</th>
<th>0.15</th>
<th>0.15</th>
<th>0.15</th>
<th>0.15</th>
<th>0.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's serum (0.5 ml. + 0.7 ml. saline)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1/2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1/4</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Patient's serum absorbed with blood vessel and saline</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1/2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1/4</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Patients' serum absorbed with blood vessel and quinidine</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1/2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1/4</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Quinidine 0.25%</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Saline</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Complement</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sensitized cells</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Result</td>
<td>NH</td>
<td>PH</td>
<td>CH</td>
<td>NH</td>
<td>PH</td>
<td>CH</td>
<td>NH</td>
<td>PH</td>
</tr>
</tbody>
</table>

TABLE 15.—Inability of Blood Vessels to Replace Platelets in Complement Fixation

Measurements in ml.

<table>
<thead>
<tr>
<th>Normal platelets 7,000 million per ml.</th>
<th>0.15</th>
<th>0.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel homogenate</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Patient's serum</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Quinidine 0.25%</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Saline</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Complement</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sensitized cells</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Result</td>
<td>NH</td>
<td>PH</td>
</tr>
</tbody>
</table>

N. Failure to demonstrate absorption of serum factor by a blood vessel preparation
(Tables 14 and 15)

There being the possibility that platelets and blood vessels are related antigenically, an attempt was made to replace platelets by a blood vessel preparation in the complement fixation reaction and further, to attempt to discover whether serum factor from the patient could be absorbed by this preparation in the presence of quinidine.

Whole choroid plexus removed at autopsy was used as a source of blood vessels. It was believed that this source would be relatively free of other tissues. Then 1.66 Gm. wet weight of choroid plexus, which had previously been perfused with saline to remove blood cells, was ground with sand and 3 ml. of saline until reasonable homogeneity had been attained. Absorption of patient's inactivated serum was attempted as follows, by adding successive amounts of vessel homogenate in the presence of saline or quinidine.
THROMBOCYTOPENIC PURPURA DUE TO QUINIDINE

Complement fixation tests were made to compare the potency of these sera with untreated patient’s serum against normal platelets and also tests were made to see if the blood vessel homogenate could fix complement with patient’s serum and quinidine.

Table 14 indicates that the blood vessel preparation could not absorb serum factors in the presence of quinidine and table 15 that it could not fix complement with patient’s serum and quinidine in the proportions used.

O. Prausnitz-Küstner reaction.²¹

A further attempt was made to demonstrate a relationship between blood vessels and platelets in the serologic reaction described. If blood vessel endothelium and platelets are closely related antigenically, then intradermal injection of patient’s serum in the presence of quinidine in a normal person might be expected to affect the adjacent blood vessels in some detectable way.

When 0.1 ml. amounts of Seitz-filtered patient’s serum were injected intradermally in 3 sites in the leg of a person whose platelets were known to fix complement in the presence of patient’s serum and quinidine, no reaction occurred in the skin in the next 24 hours. Thereupon, 0.1 ml. of 0.1 per cent quinidine solution was injected intradermally into one of these sites and also in a fresh site. Quinine sulphate in similar amounts was injected with another of the serum sites and also in a fresh site. No reaction of any kind was noticed during the next 72 hours in any of these tests. Capillary fragility tests (by subjecting the limb to pressure by sphygmomanometer of between systolic and diastolic pressure for 5 minutes) were negative at 6 and 24 hours after the last injection.

Discussion

From the experiments detailed above, it has been demonstrated that in a case of quinidine thrombocytopenic purpura an abnormal factor was present in the
plasma (or serum) which caused agglutination and lysis of platelets from normal individuals or from the patient in the presence of quinidine. This reaction did not occur if quinidine was exchanged for quinine.

The nature of the anticoagulant used in these experiments affected the ability of the plasma to cause platelet agglutination and particularly platelet lysis. Different degrees of agglutination or lysis were shown to be related to the anti-complementary nature of the anticoagulant. The varied use of anticoagulants together with the possibility of variation in human complement in the blood used may help to explain discrepancies in the literature where only agglutination or lysis were demonstrable.9, 10

Complement fixation tests revealed that complement was fixed when normal platelets, quinidine and the patient’s serum were incubated with complement. In the absence of any one of the three factors, complement was not fixed. The factor present in serum or plasma could be absorbed by normal platelets only in the presence of quinidine. Platelets having this absorbed factor were able to fix complement.

Normal platelets treated with quinidine and washed with saline were not able to fix complement in the presence of patient’s serum until more quinidine was added; quinidine could be dialyzed out readily from a patient’s plasma-quinidine mixture. No firm union was demonstrated between these three factors. When platelets having the serum factor and quinidine absorbed on to them were dialyzed against saline, the complex of platelet-quinidine-serum dissociated into its three constituents, again showing the lability of union between the components. The serum factor liberated by this procedure was shown by electrophoresis to be present in the γ-globulin fraction. To some extent this finding was confirmed separately by fractionation of the patient’s plasma by ammonium sulphate. The complement fixing ability of serum factor was destroyed by heating to 65–70 C. for 30 minutes.

Concentrations of quinidine of the order of 0.3 mg. per liter were able to cause platelet agglutination in vitro in these experiments. This level is surpassed in normal persons receiving doses of 0.6 g. of quinidine by mouth, in whom blood levels of 2–4 mg. per liter of plasma have been recorded.18-20

The mechanism of this serologic reaction in vitro in quinidine purpura is similar to that demonstrated so clearly by Ackroyd in sedormid purpura. No significant differences have been detected between the two systems.

Hemorrhages into the skin were produced by Ackroyd in two of his patients by the application of sedormid to the skin. At the time of this test, the patients were not thrombocytopenic and it must be concluded that this result was due either to the direct action of sedormid on the skin vessels or to a local thrombocytopenic effect. Passive transfer of sensitivity by means of a Prausnitz-Küstner reaction was not achieved by Ackroyd or in the single experiment reported here. Breu and Zollner22 described a case of fatal anaphylaxis to quinidine with associated thrombocytopenia in which passive transfer of serum from the patient together with the incriminated quinidine-containing drug caused marked swelling and reddening at the site of injection in the donor. Local purpura evidently was not produced by these maneuvers and unfortunately insufficient controls are described fully to interpret their results. It was not demonstrated that there was
an antigenic relationship between platelets and a blood vessel preparation, but in the absence of suitable positive controls for these preparations, it is impossible to conclude that this did not exist.

The experiments described above demonstrated well-defined evidence of an antigen-antibody reaction, as in Ackroyd's work. Although the antibody seems to be more or less well-defined as a γ-globulin, the nature of the antigen is by no means as clear. The antibody does not attach itself to platelets unless quinidine is present; quinidine does not attach itself firmly to either platelet or antibody. Specific inhibition by a high concentration of quinidine does not occur, but otherwise an analogy may be said to exist with the artificially conjugated antigens of which Landsteiner's azodyes were the prototype. In these azoprotein-antibody reactions, the protein moiety conferred antigenicity on the otherwise nonantigenic azodye, which Landsteiner termed "hapten." However, he found that specific inhibition was produced when the azodye was added to the antibody before carrying out precipitin tests. The platelet in quinidine purpura may enter into loose combination with quinidine and confer antigenicity on it with ensuing antibody formation. In vitro this may lead to platelet destruction and the same mechanism may well apply in vivo. Here the platelet-quinidine-antibody complex must be stable enough for the reaction to proceed to platelet destruction.

It is of interest that in the experiments described, quinidine could not be replaced by a stereo-isomer, quinine. This is in agreement with previous reports. In other types of sensitivity to quinine, quinidine and other related dextrorotatory compounds could not induce skin reactions, whereas quinine and some of its related levorotatory compounds were able to cause skin reactions. There was considerable difference in cross-reactions among the various patients described.

Conclusions

1. Investigations of serologic reactions in a case of quinidine purpura showing a strong concentration of platelet antibody are described.
2. In the presence of quinidine, but not of its isomer quinine, the antibody in the patient's blood was able to cause platelet agglutination and, in the presence of complement, lysis of both normal platelets and platelets from the patient.
3. The platelet-quinidine-antibody complex fixed complement.
4. Platelets in the presence of quinidine were able to absorb antibody; this complex could be separated by centrifugation and it was then capable of fixing complement.
5. By serologic testing, no union was demonstrable between normal platelets and quinidine and between plasma from the patient and quinidine.
6. Dialysis of the platelet-quinidine-antibody complex against saline readily split the complex into its three constituents.
7. The antibody lay in the γ-globulin fraction; it was destroyed by heating at 65–70 C. for 30 minutes.
8. The antibody was found to be inactive against blood vessels.
9. The mechanism by which quinidine confers antigenicity, presumably upon platelets, was not elucidated in these experiments.
SUMMARIO IN INTERLINGUA

1. Investigationes del reactiones serologic in un caso de purpura debite a quinidina es describite. Le caso eseva characterisate per un alte concentration de anticorpore plachettal.

2. In le presentia de quinidina—sed non de su isomero, quinina—le anticorpore in le sanguine del patiente eseva capace a causar agglutination plachettal e, in le presentia de complemento, lyse de plachettas normal e de plachettas ab le patiente.

3. Le complexo de plachettas-quinidina-anticorpore se monstrava capace a fixar complemento.

4. Plachettas in le presentia de quinidina eseva capace a absorber anticorpore. Le complexo eseva separabile per centrifugation e postea se monstrava capace a fixar complemento.

5. Per medio de tests serologic il non eseva possibile demonstrar un union de plachettas normal con quinidina e de plasma ab le patiente con quinidina.

6. Dialyse del complexo de plachettas-quinidina-anticorpore contra solution salin resultava promptemente in le discomposition del complexo in su tres partes constituite.

7. Le anticorpore eseva in le fraction de globulina gamma. Illo eseva destruite per calefaction a 65–70 C durante 30 minutas.

8. Esseva constatate que le anticorpore eseva inactive contra vasos sanguitsee.

9. Le experimentos hic reportate non pote servir e elucidar le mechanisnto per le qual quinidina imparti antigenicitate a (probabilemente) le plachettas.

REFERENCES


THROMBOCYTOPENIC PURPURA DUE TO QUINIDINE

Thrombocytopenic Purpura Due to Quinidine: II. Serologic Mechanisms

FREDERICK G. BOLTON