The Mechanism of Quinidine Purpura

By ROGER K. LARSON, M.D.

SEVERAL INSTANCES of thrombocytopenic purpura due to quinidine have been reported. Since this is a rare complication it has been considered to be a manifestation of specific sensitivity or allergy rather than drug toxicity.

In 1949 Ackroyd performed extensive studies on patients with thrombocytopenic purpura due to Sedormid sensitivity and demonstrated its vitro lysis of these patients' platelets in the presence of the drug. In 1951 Harrington et al. demonstrated a circulating antiplatelet factor in cases of primary and secondary thrombocytopenic purpuras.

Thus, recent attention has been directed to a peripheral mechanism in the production of various types of thrombocytopenias rather than to a primary disturbance of the hemopoietic tissue.

Study of a patient who developed thrombocytopenic purpura following quinidine administration afford an opportunity to study the pathogenesis of this abnormality in the light of these newer observations. These experiments have confirmed, and to some extent extended, the importance of Ackroyd's observations of a peripheral mechanism in the destruction of platelets, although in this instance quinidine rather than Sedormid was the antigenic agent.

CASE REPORT

P. G., a 61 year old Mexican female was admitted to the hospital for the first time on March 20, 1950 for the complaints of palpitation and dyspnea. Physical examination revealed a cardiac rate of 140 with a perfectly regular rhythm. A diagnosis of supraventricular paroxysmal tachycardia was made on the basis of the electrocardiogram. The tachycardia subsided spontaneously but recurred the following day. Because of a history of very frequent attacks of palpitation the patient was placed on quinidine sulfate, 0.2 Gm. four times a day. Twelve days after admission the patient was discharged free of her attacks of palpitation to continue her medication at home.

Approximately two weeks later she was re-admitted to the hospital with the complaints of bleeding from the gums and black and blue marks in the skin. This had started rather abruptly without trauma the night before entry. Physical examination revealed ecchymotic, purpuric and petechial hemorrhages over the face, trunk and extremities (fig. 1). The blood pressure was 235/100, the pulse 72, respirations 20 and temperature 37.6 C. There were petechiae in the palate and buccal mucosa and there was bleeding from the gingival margins. The liver and spleen were not palpable and there was no lymphadenopathy.

A platelet count done on admission was 8000 per cu. mm. On the second day it was 74,000 per cu. mm. and by the seventh day was 164,000 per cu. mm. (fig. 3). The rest of the laboratory data is presented in table 1.

Quinidine was suspected as the etiologic factor on admission and was therefore discontinued. The clinical improvement of the patient was as rapid as the rise in platelets (fig. 2). A sternal marrow preparation done on the seventh hospital day revealed normal erythrocytic, thrombocytic and myelocytic series. A platelet count done two months after discharge was 250,000 per cu. mm. and the patient had had no recurrence of purpura.

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The following studies were performed to elucidate the nature of this reaction:

1. **Platelet levels following administration of quinidine.** In order to establish definitely the etiology as an hypersensitivity to quinidine, 0.2 Gm. of this drug was given to the patient orally on a fasting stomach. Platelet counts were performed on capillary blood before and at one half hour intervals after the administration of quinidine. The results are shown in figure 4. A prompt fall in circulating platelets from 325,000 per cu. mm. to 25,000 per cu. mm. at the end of 2 hours was experienced. At this point the patient complained of headache and began bleeding from the gums. A tourniquet test was then markedly positive; 167 petechiae were counted per 5 cm. square. Before the administration of quinidine a tourniquet test had produced only 5 petechiae per 5 cm. square.

Shortly thereafter the platelet count began to rise and was 126,000 per cu. mm. at the end of 4 hours. Coincidental with the rise in platelet count, the bleeding from the gums ceased and the patient’s symptoms subsided.

2. **Skin sensitivity to quinidine.** Following the rather frightening results of oral administration of quinidine an intradermal test was considered too hazardous to try. A patch test was considered as a safe procedure however. Accordingly a 2 x 2 piece of gauze was thoroughly soaked in a solution of quinidine (80 mg. per 100 cc. 0.86 per cent saline) and applied to the volar surface of the forearm with a broad strip of adhesive. A similar patch soaked only in 0.85 per cent saline was applied to the other forearm as a control. The test was read at the end of 48 hours. No significant reaction was observed on either arm. Tourniquet tests of capillary fragility were performed before the application of the patches and afterwards. There was no significant increase in the number
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of petechiae following removal of the patches. The patch test was considered negative.

Table 1—A Summary of the Patient's Laboratory Data on the Second Hospital Admission

<table>
<thead>
<tr>
<th></th>
<th>1st Hosp. day</th>
<th>2nd Hosp. day</th>
<th>7th Hosp. day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>9.9 Gm.</td>
<td>8.7 Gm.</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>3.35 M</td>
<td>3.8 M</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>32</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>E.S.R.</td>
<td>13</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>11,000</td>
<td>11,600</td>
<td></td>
</tr>
<tr>
<td>Lymphs</td>
<td>13</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>Segmentated Cells</td>
<td>61%</td>
<td>62%</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>5%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>8,000</td>
<td>74,000</td>
<td>164,000</td>
</tr>
<tr>
<td>Clotting time capillary tube</td>
<td>5' 30&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding time</td>
<td>2'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Icterus index</td>
<td>8.8</td>
<td></td>
<td>13.8</td>
</tr>
<tr>
<td>Albumen</td>
<td>3.0 Gm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Globulin</td>
<td>3.4 Gm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceph. floe.</td>
<td>2+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thym. turb.</td>
<td>2.9 u</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPN</td>
<td></td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>16 sec.</td>
<td></td>
<td>16.6 sec.</td>
</tr>
<tr>
<td>Control</td>
<td>13.6 sec.</td>
<td></td>
<td>13.8 sec.</td>
</tr>
<tr>
<td>Urine—routine</td>
<td>Neg.</td>
<td></td>
<td>Neg.</td>
</tr>
</tbody>
</table>

Fig. 2.—The same patient approximately one month later after the purpura had subsided.
3. The *in vitro* inhibition of clot retraction by quinidine. Following a method used by Ackroyd⁹ in Sedormid purpura we studied the effect of quinidine on clot retraction. For this study all glassware was coated with silicone.⁸ The clot retraction tubes were of the type used by Ackroyd⁹ which readily permit a
quantitative measurement of the volume of clot and serum (fig. 5). The percentage of retraction was determined by dividing the volume of serum by the total volume of clot and serum. Two tubes were used for each phase of the experiment and the average obtained. The agreement between the two tubes was always close, attesting to the accuracy of the technic. The quinidine solution was prepared by dissolving 8.0 mg. of pure quinidine sulfate crystals* in 100 cc. of 0.85 per cent saline. This produced a final concentration (when mixed with the blood in the tubes) of 16 µg. per cc. which is compatible with the concentration of a clinical dose of 0.2 Gm. quinidine diluted in the total volume of extracellular fluid.

The technic was as follows. Venous blood was drawn from an antecubital vein with a silicone coated syringe and needle. The needle was then removed from the syringe and 2 cc. blood was placed in each of four clot retraction tubes. Two tubes contained 0.5 cc. of 0.85 per cent saline solution each and the remaining two tubes each contained 0.5 cc. of the quinidine solution. The concentration of the quinidine solution was 80 µg. per cc. When mixed with 2 cc. of blood the final concentration in the two tubes was then 16 µg. per cc. Paraffin coated stoppers were placed in all four tubes and the tubes mixed by inversion four times. As soon as the blood had clotted the tubes were placed in an incubator at 37 C. for 4 hours. At the end of this time the tubes were removed and allowed to remain at room temperature for another twenty hours. At the end of the 24 hour period the results were read by measuring the total height of the column in the tube, then inverting the tube and removing the clot adherent to the loop of umbilical tape attached to the stopper. After the clot had been thoroughly washed off the umbilical tape the stopper was replaced, the tube inverted again and the height of the column of serum remaining was measured. Since the diameter of the tube remained the same, the percentage difference in volume could be determined simply from the relationship between the heights of

* Supplied through the courtesy of Parke, Davis Co. Detroit, Mich.
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the two columns. The amount of retraction was then expressed as the height of the column of serum \((S)\) divided by the height of the total column \((T)\) multiplied by 100 or \(S/T \times 100 = \text{clot retraction in per cent}\).

This same procedure was followed exactly with a control using blood from a young healthy adult male who had never received quinidine.

The results are shown in Table 2. Clot retraction proceeded normally in the patient's blood incubated with saline but was completely inhibited by the presence of quinidine. No such difference was observed in the control.

4. The \textit{in vitro effect of quinine on clot retraction}. The same experiment as outlined in the preceding study was performed using quinine sulfate instead of quinidine. The same final concentration of 16 \(\mu\)g. per cc. was used. The results are presented in Table 3. Quinine, an optical isomer of quinidine, produced no inhibition of clot retraction. The remarkable specificity of the reaction was thus demonstrated.

5. The \textit{demonstration of a factor in the patient's serum in addition to the quinidine necessary for inhibition of clot retraction}. If there were some practical method of completely separating the patient’s plasma from the cellular elements, resuspending the latter in saline and adding all the elements necessary for clotting in pure form, it could readily be determined if quinidine alone was effective in producing the inhibition of clot retraction, or if it required the presence of some other factor present in the patient’s plasma. Since this would be technically difficult the much simpler procedure of attempting to reproduce the phenomenon in another person's blood was decided upon. To do this four sets of paired tubes were used. The first two tubes each contained 0.5 cc. of 0.85 per cent saline solution. The second pair of tubes contained 0.5 cc. of a quinidine-in-saline solution of the same concentration as used in the previous experiments. The third set of tubes each contained 0.5 cc. of platelet-poor serum removed from the

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|l|l|l|}
\hline
 & \textbf{Patient} & & \textbf{Control} & & \\
\hline
 & \textbf{Saline} & \textbf{Quinidine} & \textbf{Saline} & \textbf{Quinidine} & \\
\hline
\textbf{Total height in mm.} & 30 & 26 & 28 & 29 & 27 & \\
\textbf{Serum height in mm.} & 19 & 0 & 0 & 17 & 17 & \\
\textbf{% of retraction} & 63% & 0% & 0% & 61% & 63% & \\
\hline
\textbf{Average} & 63.5% & 0% & 61.5% & 61% & \\
\hline
\end{tabular}
\caption{Effect of Quinidine in Vitro on Clot Retraction in the Patient and a Control}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|}
\hline
 & \textbf{Saline} & \textbf{Quinine} & \\
\hline
\textbf{Total height in mm.} & 29 & 27.5 & 30 & \\
\textbf{Serum height in mm.} & 16 & 16 & 19 & \\
\textbf{% of retraction} & 55% & 58% & 63% & \\
\hline
\textbf{Average} & 57% & 61% & \\
\hline
\end{tabular}
\caption{Effect of Quinine on Clot Retraction in the Patient in Vitro}
\end{table}
purpura patient's blood and the fourth two tubes each contained 0.5 cc. of the
purpura patient's serum with quinidine in the concentration of 80 μg. per cc.
The serum was prepared in the following manner. About 15 cc. of blood was
drawn from the patient with the quinidine sensitivity and allowed to clot. As
soon as the clot had retracted sufficiently to express some serum (about 2 hours),
the latter was pipetted off and placed in a centrifuge tube. This was then centri-
fuged at 2500 r.p.m. for 15 minutes and the supernatant serum again pipetted
off. One-half cc. of this serum was placed in each of the clot retraction tubes in
the third group and the remainder was placed in a test tube to which quinidine
was added to make the concentration 80 μg. per cc. One-half cc. of this mixture
was then added to each of the fourth set of two tubes.

After the tubes had been set up in this manner, blood was drawn from a con-
trol, a young adult who had never had quinidine, and 2 cc. was carefully added to
each tube. The tubes were then handled in the same manner as in the previous
experiments.

The results are presented in table 4. It is readily apparent that neither quini-
dine when added alone nor the patient's serum when added alone produced any
significant difference in clot retraction when compared to the tubes with saline.

Table 4—The Effect of Quinidine Alone, Patient's Serum Alone and Quinidine with Patient's
Serum in Vitro on Clot Retraction in Control Blood

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Saline</th>
<th>Quinidine</th>
<th>Serum</th>
<th>Serum</th>
<th>Quinidine + Serum</th>
<th>Quinidine + Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total height in mm.</td>
<td>28</td>
<td>29</td>
<td>28</td>
<td>28</td>
<td>31</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>Serum height in mm.</td>
<td>17</td>
<td>18</td>
<td>17</td>
<td>14</td>
<td>18.5</td>
<td>17.5</td>
<td>0</td>
</tr>
<tr>
<td>% of retraction</td>
<td>61%</td>
<td>62%</td>
<td>61%</td>
<td>61%</td>
<td>60%</td>
<td>63%</td>
<td>0%</td>
</tr>
<tr>
<td>Average</td>
<td>62%</td>
<td>61%</td>
<td>62%</td>
<td>0%</td>
<td>63%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

When the patient's serum and quinidine were added to the control blood together,
however, complete inhibition of clot retraction resulted.

6. The in vitro effect of quinidine on the enumeration of platelets. For this study
the following technic was used. Two silicone coated graduated test tubes were
each placed in an ice bath. One cc. of citrate solution was added to the first tube
and 1 cc. of citrate solution containing 80 μg. of quinidine was added to the
second tube. Blood was then drawn from an antecubital vein of the patient with
a silicone coated syringe and needle. The needle was removed from the syringe
and 2 cc. of blood was allowed to run down the side of each tube and mix with
the solution. The tubes were then replaced in the ice bath. Two hours later
counts were performed on each tube after thorough mixing. Red cell pipets and
counting chambers were used for the counts and Rees-Ecker solution was used
for the diluting fluid. The counts after correcting for the dilution with citrate
solution were as follows: 214,000 per cu. mm. in the control tube and 243,000
per cu. mm. in the tube containing quinidine. Some clumping of platelets was
observed in the counting chamber in the case of the latter but not the former.
DISCUSSION

The interpretation of the data presented rests largely on the significance of clot retraction as a measure of platelet activity. Clot retraction is known to vary directly with the concentration of platelets\textsuperscript{10,11} and has been a time honored test in the study of patients with thrombocytopenia. On the other hand clot retraction is known to be influenced by at least three other factors, namely, packed cell volume, fibrinogen concentration and thrombin concentration.\textsuperscript{8} In regard to the experiments performed in this study, the first factor, packed cell volume, can immediately be eliminated as a source of error since the significant differences were always contrasted to a control done on the same blood with presumably the same hematocrit. The dilution factor was always the same, i.e., 2 cc. of blood to 0.5 cc. of solution. The second factor, fibrinogen concentration, is also easily eliminated as a source of error since clot retraction bears an inverse relationship to fibrinogen concentration. In other words, to produce the results indicated in the data the addition of quinidine to blood in vitro would have to increase the concentration of fibrinogen. Since fibrinogen is formed in the liver this would be impossible in an in vitro preparation and can be dismissed from further consideration.

The third factor, thrombin concentration, is not so easily dismissed. Although it may be assumed that the prothrombin concentration was constant in all the samples of blood from the same patient at the start of the experiment it cannot be proven that the quinidine did not interfere with the conversion of prothrombin to thrombin or did not destroy or inactivate the thrombin after it was formed. That it did not inactivate, destroy or prevent the formation of all the thrombin is evidenced by the fact that the blood clotted normally. This, however, is not significant in view of the fact that only minute amounts of thrombin are necessary for blood to clot. The strongest argument against a primary action on thrombin concentration is the fact that no effect of quinidine on clot retraction was observed in the control. If a pure chemical reaction were involved it would be logical to expect the same action in the control. Since this was not the case it is highly improbable that changes in thrombin concentration were responsible for the effects observed in this experiment.

Assuming that the differences in percentage of clot retraction roughly paralleled the level of platelet activity, the profound effect of quinidine on this patient's platelets in the peripheral blood was demonstrated. No effect of quinidine on the platelets of the control was observed. This suggests a mechanism of specific sensitivity. The specificity of this sensitivity was even more strikingly demonstrated by the complete ineffectiveness of quinine, the levoratory isomer of quinidine, in producing the same reaction.

In the fifth experiment the necessity of a factor or factors, present in the patient's platelet-free serum in addition to quinidine in order to affect the platelets was demonstrated. This could be schematized as follows:

\[
\text{quinidine + serum factor—antiplatelet factor}
\]

This again is compatible with the most commonly accepted theory of the mechanism of specific sensitivity, i.e., an antigen-antibody type of reaction. It is obvious
MECHANISM OF QUINIDINE PURPURA

that sensitization of the platelets per se is not involved in this reaction since it could be produced in blood with no previous contact with quinidine.

It seems justifiable to speculate that quinidine acting as a haptene induced a state of sensitivity in this patient with the production of antibodies. The shock tissue for the antigen-antibody combination was apparently the blood platelets. This induced a state of thrombocytopenia with its attendant clinical picture of purpura.

Finally, a disparity is noted between the effects of quinidine on the platelet count when administered in vivo and when incubated with the blood in vitro. Although at the end of 2 hours in vivo the platelet count had fallen from 325,000 per cu. mm. to 25,000 per cu. mm., in the test tube containing quinidine the count was still 243,000 per cu. mm. at the end of 2 hours. There was no significant difference between it and the control tube. Clumping of the platelets, however, was noted in the chamber when the platelets from the tube containing quinidine solution were counted and no clumping was observed in the control. This suggests that agglutination might have been the method of destruction rather than lysis as was the case with Sedormid.

So far no mention has been made of a possible effect of quinidine on the megakaryocytes in the bone marrow. Until recently most thrombocytopenic purpuras due to drugs were considered to be the result of a toxic action of the drug on the bone marrow with arrested production of platelets. This, of course, is still a theoretical possibility and may even play a role in the case reported. Since platelets are considered to be merely fragments of cytoplasm of the megakaryocytes it is logical to assume that any circulating antiplatelet factor might also damage the cytoplasm of the megakaryocytes and thus inhibit the production of new platelets. Indeed, Hirsch and Dameshek studied the bone marrow in detail in a case of thrombocytopenic purpura due to quinidine sensitivity and reported inhibition of platelet formation by the megakaryocytes. That this does not play the major role was indicated by the rate of disappearance of the platelets from the circulation when quinidine was administered to the patient. The physiologic rate of platelet production has been variously estimated at 40,000 to 100,000 per cu. mm. per day. Taking the highest estimate this would correspond to an hourly rate of production of approximately 4000 platelets per cu. mm. per hour. If the thrombocytopenia in our case was primarily due to inhibition of platelet production the platelet count should have fallen at the end of 2 hours from a level of 325,000 per cu. mm. to only 317,000 per cu. mm. Instead it fell to 25,000 per cu. mm. Increased destruction or removal of platelets from the circulation must be invoked to explain the discrepancy.

SUMMARY AND CONCLUSIONS

A case of thrombocytopenic purpura was studied and evidence of a specific hypersensitivity mechanism was found. The following experimental observations and their conclusions were discussed.

1. A rapid fall in circulating platelets occurred in the patient after a test dose of the drug. This fall was too rapid to be accounted for by inhibition of the bone marrow megakaryocytes alone.
Inhibition of clot retraction could be produced in the patient's blood by the addition of minute quantities of quinidine in vitro. This was assumed to demonstrate a specific peripheral action of quinidine on this patient's platelets.

3. No inhibition of clot retraction could be produced by the addition of quinine (an optical isomer of quinidine) to the blood of this patient in vitro. This was further evidence of the marked specificity of the reaction.

4. Complete inhibition of clot retraction could be produced in the blood of a normal individual by the addition of serum from the patient together with quinidine. No inhibition occurred if one or the other was added alone. This was assumed to demonstrate that quinidine did not affect the platelets directly but did so through a combined action with some other element in platelet-free serum. It was felt that this was strong support for an antigen-antibody type of reaction.

5. No significant fall in platelet concentration by the addition of quinidine in vitro could be demonstrated. This was interpreted as evidence against a complete lysis of the platelets as the method of destruction. The disparity between the platelet counts at the end of 2 hours in vivo and in vitro suggested that the platelets were injured in such a manner as to effect their rate of removal by the reticulo-endothelial system and their ability to produce clot retraction.

REFERENCES

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