THROMBOTIC THROMBOCYTOPENIC PURPURA

II. STUDIES ON THE HEMOLYTIC SYNDROME IN THIS DISEASE

By KARL SINGER, M.D., ARNO G. MOTULSKY, M.D., AND JACOB N. SHANBERGE, M.D.

THROMBOTIC thrombocytopenic purpura was the name suggested in 1947 for a definite disease entity which is characterized histologically by the pattern of arterial and capillary platelet thromboses, and hematologically by the simultaneous manifestations of a severe thrombocytopenic purpura as well as a hemolytic anemia. Transient leukemoid reactions are frequently seen. Furthermore, the observer may be impressed by bizarre and transitory focal neurologic signs and symptoms. Our first report summarizing the clinical features of this disease was based on 12 cases, including one of our own. Since then, several more observations have been published, bringing the number of recorded cases to 20. The correct diagnosis was considered only once ante mortem in all these instances.

This paper deals with another case of thrombotic thrombocytopenic purpura. Since the diagnosis was made while the patient was alive, some of the studies proposed in our previous communication could be carried out. The results of these investigations may be of some significance for the pathophysiology of this disorder and if corroborated in other cases, may be of help in arriving at an in vivo diagnosis. The incidence of thrombotic purpura is probably greater than the literature seems to indicate. Only by more in vivo studies will eventual clarification of the pathogenetic mechanisms be achieved.

REPORT OF CASE

S. P., a 25 year old white housewife entered Michael Reese Hospital on February 25, 1949, from another hospital where she had stayed for about two weeks. Six weeks prior to entry she started to complain of weakness and mild frontal headache. Two weeks later purple spots were noticed over her abdomen and legs and she developed hematuria and pain in the right lumbar region. Shortly thereafter, she felt severely nauseated and vomited frequently. The vomitus was bile colored and free from blood. At this time, slight jaundice was noticed. The patient was then admitted to another hospital where her red count on admission was 1.9 M. Treatment consisted of 3000 cc. of blood, iron, liver, and penicillin for a period of two weeks; little improvement was noted.

There was no history of food allergy; drugs taken before hospitalization consisted of "Dr. Hill's Cold Tablets," "Inner Aid Laxative," and an unknown amount of sulfadiazine. At the age of 2 years she had had severe bouts of epistaxis which were never explained satisfactorily and which lasted for about one year. The patient's mother and two maternal aunts had also experienced unexplained attacks of epistaxis. The patient's only sister was living and well.

Physical examination revealed a fairly well nourished, pale woman who was oriented but unable to concentrate on a given subject for any length of time. She could not follow simple commands and was very...
hazy about the onset of her illness. The sclerae were definitely icteric and numerous petechiae were present on the legs and on the roof of the mouth. Ecchymoses were noted at the site of previous needle punctures. Funduscopic examination showed slightly blurred discs. There was no lymphadenopathy except for a few small inguinal nodes. A soft systolic murmur could be heard at the apex and at the pulmonic area. The liver edge was palpated 4 cm. below the costal margin in the midclavicular line. The tip of the spleen was barely palpable. Neurologic examination was entirely negative at this time.

Laboratory examination on admission gave the following data.

Hematology: Hg 10.5 Gm. (68 per cent), RBC 3,3 mill., hematocrit (Wintrobe) 28, C.I. 1,01, MCV 86 cu. micra, reticulocites 9.6 per cent, WBC 3,600, platelets (Dameshek’s method) 33,000. The differential count showed neutrophilic segmented cells 56 per cent, nonsegmented 5 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 9 per cent. There was one nucleated red cell per 100 white cells. No spherocytosis was demonstrable on the film on this particular day. The Duke bleeding time was more than 15 minutes, the Lee-White clotting time 12 minutes, and the one-stage prothrombin time showed a normal value of 12.5 seconds. A narrow puncture revealed a marked erythroid hyperplasia of the normoblastic variety. The megakaryocytes were plentiful. The white cell series was essentially normal.

Chemistry: Blood urea nitrogen 38 mg. per cent, glucose 85 mg. per cent. Liver function tests: bilirubin 3.6 mg. per cent, cephalin and thymol flocculation negative in forty-eight hours, thymol turbidity 2.8 minutes, hematocrit (Wintrobe) 33,000.

Laboratory examination on admission gave the following data. 

Hematology: Hg 10.5 Gm. (68 per cent), RBC 3,3 mill., hematocrit (Wintrobe) 28, C.I. 1,01, MCV 86 cu. micra, reticulocytes 9.6 per cent, WBC 3,600, platelets (Dameshek’s method) 33,000. The differential count showed neutrophilic segmented cells 56 per cent, nonsegmented 3 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 9 per cent. There was one nucleated red cell per 100 white cells. No spherocytosis was demonstrable on the film on this particular day. The Duke bleeding time was more than 15 minutes, the Lee-White clotting time 12 minutes, and the one-stage prothrombin time showed a normal value of 12.5 seconds. A narrow puncture revealed a marked erythroid hyperplasia of the normoblastic variety. The megakaryocytes were plentiful. The white cell series was essentially normal.

Chemistry: Blood urea nitrogen 38 mg. per cent, glucose 85 mg. per cent. Liver function tests: bilirubin 3.6 mg. per cent, cephalin and thymol flocculation negative in forty-eight hours, thymol turbidity 2.8 minutes, hematocrit (Wintrobe) 33,000.

Laboratory examination on admission gave the following data. 

Hematology: Hg 10.5 Gm. (68 per cent), RBC 3,3 mill., hematocrit (Wintrobe) 28, C.I. 1,01, MCV 86 cu. micra, reticulocytes 9.6 per cent, WBC 3,600, platelets (Dameshek’s method) 33,000. The differential count showed neutrophilic segmented cells 56 per cent, nonsegmented 3 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 9 per cent. There was one nucleated red cell per 100 white cells. No spherocytosis was demonstrable on the film on this particular day. The Duke bleeding time was more than 15 minutes, the Lee-White clotting time 12 minutes, and the one-stage prothrombin time showed a normal value of 12.5 seconds. A narrow puncture revealed a marked erythroid hyperplasia of the normoblastic variety. The megakaryocytes were plentiful. The white cell series was essentially normal.

Chemistry: Blood urea nitrogen 38 mg. per cent, glucose 85 mg. per cent. Liver function tests: bilirubin 3.6 mg. per cent, cephalin and thymol flocculation negative in forty-eight hours, thymol turbidity 2.8 minutes, hematocrit (Wintrobe) 33,000.

Laboratory examination on admission gave the following data. 

Hematology: Hg 10.5 Gm. (68 per cent), RBC 3,3 mill., hematocrit (Wintrobe) 28, C.I. 1,01, MCV 86 cu. micra, reticulocytes 9.6 per cent, WBC 3,600, platelets (Dameshek’s method) 33,000. The differential count showed neutrophilic segmented cells 56 per cent, nonsegmented 3 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 9 per cent. There was one nucleated red cell per 100 white cells. No spherocytosis was demonstrable on the film on this particular day. The Duke bleeding time was more than 15 minutes, the Lee-White clotting time 12 minutes, and the one-stage prothrombin time showed a normal value of 12.5 seconds. A narrow puncture revealed a marked erythroid hyperplasia of the normoblastic variety. The megakaryocytes were plentiful. The white cell series was essentially normal.

Chemistry: Blood urea nitrogen 38 mg. per cent, glucose 85 mg. per cent. Liver function tests: bilirubin 3.6 mg. per cent, cephalin and thymol flocculation negative in forty-eight hours, thymol turbidity 2.8 minutes, hematocrit (Wintrobe) 33,000.

Laboratory examination on admission gave the following data. 

Hematology: Hg 10.5 Gm. (68 per cent), RBC 3,3 mill., hematocrit (Wintrobe) 28, C.I. 1,01, MCV 86 cu. micra, reticulocytes 9.6 per cent, WBC 3,600, platelets (Dameshek’s method) 33,000. The differential count showed neutrophilic segmented cells 56 per cent, nonsegmented 3 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 9 per cent. There was one nucleated red cell per 100 white cells. No spherocytosis was demonstrable on the film on this particular day. The Duke bleeding time was more than 15 minutes, the Lee-White clotting time 12 minutes, and the one-stage prothrombin time showed a normal value of 12.5 seconds. A narrow puncture revealed a marked erythroid hyperplasia of the normoblastic variety. The megakaryocytes were plentiful. The white cell series was essentially normal.

Chemistry: Blood urea nitrogen 38 mg. per cent, glucose 85 mg. per cent. Liver function tests: bilirubin 3.6 mg. per cent, cephalin and thymol flocculation negative in forty-eight hours, thymol turbidity 2.8 minutes, hematocrit (Wintrobe) 33,000.

Laboratory examination on admission gave the following data. 

Hematology: Hg 10.5 Gm. (68 per cent), RBC 3,3 mill., hematocrit (Wintrobe) 28, C.I. 1,01, MCV 86 cu. micra, reticulocytes 9.6 per cent, WBC 3,600, platelets (Dameshek’s method) 33,000. The differential count showed neutrophilic segmented cells 56 per cent, nonsegmented 3 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 9 per cent. There was one nucleated red cell per 100 white cells. No spherocytosis was demonstrable on the film on this particular day. The Duke bleeding time was more than 15 minutes, the Lee-White clotting time 12 minutes, and the one-stage prothrombin time showed a normal value of 12.5 seconds. A narrow puncture revealed a marked erythroid hyperplasia of the normoblastic variety. The megakaryocytes were plentiful. The white cell series was essentially normal.

Chemistry: Blood urea nitrogen 38 mg. per cent, glucose 85 mg. per cent. Liver function tests: bilirubin 3.6 mg. per cent, cephalin and thymol flocculation negative in forty-eight hours, thymol turbidity 2.8 minutes, hematocrit (Wintrobe) 33,000.

Laboratory examination on admission gave the following data. 

Hematology: Hg 10.5 Gm. (68 per cent), RBC 3,3 mill., hematocrit (Wintrobe) 28, C.I. 1,01, MCV 86 cu. micra, reticulocytes 9.6 per cent, WBC 3,600, platelets (Dameshek’s method) 33,000. The differential count showed neutrophilic segmented cells 56 per cent, nonsegmented 3 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 9 per cent. There was one nucleated red cell per 100 white cells. No spherocytosis was demonstrable on the film on this particular day. The Duke bleeding time was more than 15 minutes, the Lee-White clotting time 12 minutes, and the one-stage prothrombin time showed a normal value of 12.5 seconds. A narrow puncture revealed a marked erythroid hyperplasia of the normoblastic variety. The megakaryocytes were plentiful. The white cell series was essentially normal.
especially in the myocardium. Here and there a subintimal accumulation of an amorphous eosinophilic material was encountered, accompanied by an endothelial proliferation with or without a superimposed thrombus. The thrombi consisted apparently of platelets. It could not be determined from serial sections whether or not the thrombi were attached to the wall of the vessels at the site of intimal damage. Associated with these thrombotic lesions were perivascular hemorrhages and in the myocardium small focal infarcts. No increase of megakaryocytes was found in the lungs.

**Hematologic Investigations**

From the first day of observation the patient presented the conspicuous syndrome of a severe thrombocytopenic purpura coexisting with a rather marked hemolytic anemia. This peculiar combination together with the transitory focal neurolologic signs led us to the diagnosis of thrombotic thrombocytopenic purpura.

**Hemoglobin, Erythrocytes, Reticulocytes, and Platelets.** Frequent observations of the various hematologic factors were performed. The results of these studies are charted in figure 1. The patient had a severe normochromic anemia. The MCV was 86-98 cu. micra, the MCHg was 33-36.7 per cent. The reticulocyte count, which was always high, increased gradually to more than 25 per cent. The platelet count never exceeded 33,000 and was almost zero shortly before death. The patient’s red cell count could not be raised by transfusions. Since 5 liters of blood were given within five weeks (3 during her stay in another hospital and 2 while in our care) the conclusion seemed justified that the hemolytic agent operating in this disease destroyed rapidly and indiscriminately not only the patient’s own cells but the transfused normal cells as well.

**White Cell and Differential Counts. Spherocytosis and Nucleated Red Cells.** The white cell count varied between 5,600 and 12,000, corrections being made for the nucleated red cells present. The distribution of the various types of white cells was essentially normal except for a shift to the left, the nonsegmented neutrophils fluctuating between 6 and 30 per cent. Furthermore, some immature white cells appeared intermittently in the peripheral blood (table 1). This “leukemoid reaction” increased in severity during the last days of the illness. The number of nucleated red cells also became more conspicuous during the course of the disease.

At the beginning of our investigations spherocytosis was not present on the smear but later on became apparent, although changing in degree from day to day. Spherocytes have been observed previously only once in this disease.

**Differential Fragility and Mechanical Fragility Tests.** Anomalies of red cells often manifest themselves in a pathologic resistance to various hemolytic agents. Hypotonic saline solutions, lysolecithin, saponin, and acids have been used in such studies. By means of these “differential fragility tests” characteristic reaction patterns have been obtained in some of the hemolytic syndromes. The differential fragility tests are based on the concept that pathologic erythrocytes, when injured by different procedures, react differently. It seemed of interest to determine whether a characteristic fragility pattern could be established for the hemolytic syndrome occurring in thrombotic purpura, and the following investigations were undertaken:

(a) Hypotonic saline fragility (method of Suess, Limentani and Dameshek): As can be seen from figure 2, the osmotic fragility, although sometimes definitely in-
creased, showed great variations on different days. Since the hypotonic fragility depends to a certain extent on the shape of the red cells, these results reflect the irregular appearance of spherocytosis previously mentioned. It is, therefore, obvious that single determinations of this test may lead to unwarranted conclusions. Most observers report a normal osmotic resistance in the cases of thrombotic purpura.

The findings recorded by Muirhead et al., however, also reveal the fluctuations from normal to pathologic values.

(b) Lysolecithin fragility (method of Singer): Lysolecithin is a hemolytic agent which may be extracted from normal human or animal serum. It originates there through the action of an enzyme which splits off one fatty acid chain from the lipids lecithin and cephalin.

It has been demonstrated repeatedly that the spherocytes of familial hemolytic
THROMBOTIC THROMBOCYTOPENIC PURPURA

Table 1.—Appearance of Spherocytosis, Nucleated Red Cells, and Leukemoid Reactions in Peripheral Blood

<table>
<thead>
<tr>
<th>Date</th>
<th>Spherocytosis*</th>
<th>Nucleated Red Cells</th>
<th>Blasts</th>
<th>Promyelocytes</th>
<th>Myelocytes</th>
<th>Metamyelocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>per 100 White Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February 26</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 2</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 3</td>
<td>++</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>March 5</td>
<td>++</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 7</td>
<td>++</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>March 8</td>
<td>++</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>March 9</td>
<td>++</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>March 10</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 12</td>
<td>+++</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March 14</td>
<td>+++++</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>March 15</td>
<td>++</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>March 16</td>
<td>+++</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>March 17</td>
<td>++</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>March 18</td>
<td>++</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

* Grading of Spherocytosis: + one occasional spherocyte seen; ++ one to two spherocytes per oil immersion field; +++ several spherocytes per oil immersion field; ++++ numerous spherocytes.

Fig. 1.—Osmotic Fragility
jaundice often show an increased saline and lysolecithin fragility, whereas the
spherocytes in acquired hemolytic anemia have a normal resistance to lysolecithin,
although the saline fragility may be increased. As can be seen from figure 3, the
lysolecithin fragility in our patient remained normal although the saline fragility
was definitely increased on the same day. A determination with the cells of a case
of hereditary spherocytosis, showing increased lysolecithin fragility, was per-
determined simultaneously.

(c) Heat fragility. Hegglin and Maier demonstrated diminished "heat resist-
ance" of the erythrocytes in paroxysmal nocturnal hemoglobinuria. When blood
is placed in an incubator at 37 C for six hours, gross hemolysis of the clotted blood

is considered specific evidence for the presence of this disorder. According to Minot
and Castle, however, this test does not depend on the heat resistance of the eryth-
rocytes but is caused by the accelerated acid production at the increased temperature
in the incubator. Addition of alkali to the blood inhibits the effect of incubation. Thus, the "heat resistance test" is really a modification of Ham's "acid fragility
test."

Since in the Marchiafava-Micheli syndrome, hemoglobinemia may not always be
demonstrable although a hemolytic anemia exists, and since in this disease throm-
boctopenia may be observed, the patient's erythrocytes were tested for acid he-
molysis. The result was negative.

(d) Mechanical fragility (method of Shen, Castle and Fleming). It has been
shown that the erythrocytes of various types of hemolytic anemias, regardless of
their etiology, reveal an increased destructibility when rotated with glass beads un-
der standard conditions. The mechanical fragility of the red cells of our patient was
definitely increased to about twice the normal value (table 2).
An attempt was then made to demonstrate the assumed hemolytic agent in the serum of the patient by means of the mechanical fragility test. Normal cells (after three washings with isotonic saline) were incubated with the patient's serum, and equally treated erythrocytes of this patient were incubated with normal serum for two hours. As can be seen from table 2, it was not possible to increase the mechanical fragility of the normal red cells by this procedure. This negative finding, however, does not rule out the existence of such a hemolytic agent in the plasma. This interpretation is supported by the following experiments. It is often assumed that "coating" or "coating" of the red cells with globulin immune bodies leads to injury of the corpuscles. When normal Rh positive erythrocytes were incubated for two to six hours with a serum containing potent second degree (albumin) antibodies, the heavy coating of the cells was readily demonstrable by means of the Coombs developing test. However, the adsorption of the globulin to the red cells did not enhance their mechanical destructibility in vitro (table 2). Thus, these findings seem to indicate that under the conditions of our experiments the mechanical fragility test may not be suitable for revealing an existing hemolytic activity in the plasma.

Summarizing the results of these investigations, the erythrocytes of our patient showed an increased osmotic fragility of varying degree, a normal lyssolecithin and a normal acid (heat) fragility, but a definitely increased mechanical fragility. This pattern is often seen in the acquired type of hemolytic anemia (idiopathic and symptomatic). Since in these latter disorders the Coombs antiglobulin test is usually positive, an examination of the red cells of the patient with Coombs antiglobulin serum was performed.

Results of the Coombs Antiglobulin Test. This test demonstrates the presence of immune bodies which form a globulin "coating" on the surface of the red cells. The autoimmune bodies present in the spherocytic hemolytic anemias differ from the antibodies directed against the Rh-Hr systems. Although it has been shown that a positive Coombs test may occur in hereditary spherocytosis, such a finding is
exceptional. In acquired hemolytic anemia of the idiopathic or the symptomatic type, a positive Coombs test is observed usually. In our case the test was performed on three different days and was always completely negative. The sera used gave a positive result with known "coated" cells. Therefore, the result of a negative test in our case of thrombotic thrombocytopenic purpura may indicate that the hemolytic mechanism was neither mediated nor accompanied by globulin antibodies.

**Pigment Studies.** The patient's serum bilirubin fluctuated between 2.4 and 3.8 mg. per cent. Bilirubin was never found in the urine. Moreover, several tests for urine urobilinogen revealed no marked increase, an unexpected negative finding in the presence of a severe hemolytic anemia. A quantitative determination of the fecal urobilinogen (method of Watson\(^4\)) was performed on a stool specimen obtained following a five-day period of constipation. The total amount of urobilinogen found was 590 mg., or 118 mg. per day. From this figure and from the total circulating hemoglobin of the patient, the hemolytic index: 

\[
\text{hemolytic index} = \frac{\text{average pigment output/day}}{\text{total hemoglobin in grams}} \times 100
\]

was estimated to be 43 mg. in contrast to a normal value of 10–20 mg. Since the patient showed definite retention jaundice and had a rather high reticulocyte count, this relatively slight increase of the hemolytic index was difficult to evaluate. This phenomenon may partly be due to the effect of constipation with increased resorption of fecal pigments. However, recently Sborow, Jay and Watson\(^7\) have shown that aureomycin interferes with the formation of urobilinogen in the feces by suppressing the intestinal coliform bacteria which are responsible for the transformation of bilirubin into urobilinogen. Thus, the fecal urobilinogen values of normal individuals during aureomycin medication may fall into the range of levels seen only in complete biliary obstruction. Since the stool specimen was obtained while the patient was treated with aureomycin, it is more likely that the relatively low output of urobilinogen may be explained by the administration of the antibiotic. Unfortunately, no determination of bilirubin in the feces was made.

**Bone Marrow Studies.** Two sternal punctures and one biopsy (by means of the Tuerkel needle) were done. The marrow showed a marked erythroid hyperplasia, the erythroid-myeloid ratio being 2:1. The following special investigations were undertaken.

(a) Formation of platelets by the megakaryocytes: Since the syndrome under discussion is characterized by severe thrombocytopenia, the production of platelets by the megakaryocytes was studied in detail. Dameshek and Miller\(^8\) have demonstrated that in idiopathic thrombocytopenic purpura only 8–19 per cent of all giant cells show evidence of platelet formation, whereas in normal marrows such an activity was present in 50–86 per cent. We examined 150 megakaryocytes and found production of platelets in 85 (57 per cent). Thus, in thrombotic thrombocytopenic purpura the manufacture of thrombocytes is apparently not inhibited.

(b) Hemosiderin content of the marrow (method of Rath and Finch\(^9\)): Large amounts of hemosiderin were visualized, a finding consistent with the presence of a hemolytic anemia as well as of a rapid destruction of the great numbers of transfused cells.

(c) Demonstration of platelet thromboses: An attempt was made to substantiate
### Table 3 — Differential Diagnosis of the Diseases Showing Thrombocytopenic Purpura together with Hemolytic Anemia

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Osmotic Fragility</th>
<th>Lysolecithin Fragility</th>
<th>Acid (Heat) Fragility</th>
<th>Coombs Developing Test</th>
<th>Severity of Thrombocytopenia</th>
<th>Focal Neurologic Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombotic Thrombocytopenic Purpura</td>
<td>normal or increased</td>
<td>normal*</td>
<td>negative*</td>
<td>negative*</td>
<td>usually severe</td>
<td>transitory, frequent</td>
</tr>
<tr>
<td>Idiopathic Acquired Hemolytic Anemia</td>
<td>increased or normal</td>
<td>usually normal</td>
<td>negative</td>
<td>usually positive</td>
<td>usually slight but</td>
<td>absent</td>
</tr>
<tr>
<td>Symptomatic Hemolytic Anemia as seen in Hodgkin's disease, leukemia, teratoma, etc.</td>
<td>increased or normal</td>
<td>normal</td>
<td>negative</td>
<td>usually positive</td>
<td>may be severe</td>
<td>may be present due to underlying disorder. Not transitory</td>
</tr>
<tr>
<td>Drugs, e.g. sulfanilamide</td>
<td>often increased</td>
<td>unknown</td>
<td>negative</td>
<td>negative</td>
<td>may be severe in crisis only</td>
<td>absent</td>
</tr>
<tr>
<td>Familial Hemolytic Jaundice (crisis)</td>
<td>increased</td>
<td>increased</td>
<td>negative</td>
<td>usually negative, occasionally positive</td>
<td>usually not severe</td>
<td>absent</td>
</tr>
<tr>
<td>Paroxysmal Nocturnal Hemoglobinuria (Marchiafava Micheli Syndrome)</td>
<td>normal</td>
<td>normal</td>
<td>positive</td>
<td>negative</td>
<td>may be severe</td>
<td>may be present due to thrombosis. Not transitory</td>
</tr>
<tr>
<td>Liver Cirrhosis</td>
<td>often decreased</td>
<td>often decreased</td>
<td>negative</td>
<td>negative</td>
<td>may be severe only in untreated cases. Slight to severe</td>
<td>present (subacute combined disease). Not transitory</td>
</tr>
<tr>
<td>Unreated Pernicious Anemia</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* It is realized that definite statements cannot be made from a single observation.
the diagnosis by demonstration of platelet thromboses in a marrow biopsy preparation. Neither endothelial proliferation in the blood vessels nor platelet thrombi were seen. Although our attempt was unsuccessful, we believe that this promising approach should be tried again in other cases.

Discussion

Differential Diagnosis. Since the recognition of cases with thrombotic purpura in vivo is a prerequisite for elucidation of the pathogenesis of this disorder, a discussion of the differential diagnosis in the light of our results seems appropriate. In our first communication the clinical picture was described in detail. It was emphasized that the syndrome of thrombocytopenic purpura (petechiae and ecchymoses associated with reduced platelet count, increased bleeding time and poor clot retraction) is usually the first one to be considered. Therefore, the differential diagnosis was outlined in relation to the great variety of disorders which may show this symptom complex.

In this paper we wish to stress the fact that the purpuric manifestations are almost always accompanied by a hemolytic anemia. Provided that this combination is ascertained properly, it is possible to narrow the differential diagnosis to a relatively small number of diseases (table 3).

The hemolytic syndrome in thrombotic purpura is often not very conspicuous. However, retention jaundice (hyperbilirubinemia without bilirubinuria) is a constant finding. Erythroid hyperplasia of the marrow and a rather high reticulocyte level, indicating increased production and release of erythrocytes, are also readily demonstrable. Although this latter reaction pattern is encountered following severe hemorrhage, in the presence of retention jaundice it should create sufficient suspicion to consider an existing hemolytic syndrome. An increased excretion of fecal urobilinogen as well as the normal liver function tests will lead then to the correct evaluation. Spherocytosis may be seen only intermittently and must be searched for with care. Since spherocytes can be interpreted as representing the prehemolytic state of the red cells, their inconstant appearance points to a variable intensity of the operating hemolytic mechanism during the course of the illness. As can be inferred from the effect of the transfusions, this hemolytic mechanism is of the "extracorpuscular" variety, destroying indiscriminately the patient's own as well as the foreign normal erythrocytes. Survival time studies of transfused red cells were not performed, because the patient had received 3000 cc. of blood shortly before coming into our observation.

Transitory focal neurologic signs (facial weakness, hemiparesis, aphasia, apraxia etc.) lasting for a few days only, form the third outstanding feature of the disease. Involvement of the nervous system is encountered in other similar syndromes (table 3), but the characteristic waxing and waning of the symptomatology is found in thrombotic purpura only. Histologic examinations of the various organs including the brain reveal that the formation of platelet thrombi in the smallest vessels takes place in a succession of attacks. It is likely that by opening up of collateral vessels restoration of function occurs relatively rapidly following such paroxysms of capillary occlusions.
THROMBOTIC THROMBOCYTOPENIC PURPURA

Table 3 contains a compilation of the pertinent data concerning the diseases in which the simultaneous appearance of thrombocytic and hemolytic manifestations is observed. Some of these entities, like pernicious anemia, cirrhosis of the liver, and paroxysmal nocturnal hemoglobinuria, are excluded quite readily. Recent studies of the survival time of the megalocyte have established pernicious anemia as a hemolytic syndrome. The thrombocytopenia seen in severe untreated cases responds promptly to liver extract, the rise in platelets even precedes the elevation of the reticulocytes in the peripheral blood. In cirrhosis, hemolytic anemia and thrombotic thrombocytic purpura may sometimes dominate the picture. Even in the presence of accelerated erythrocyte disintegration, the icterus is usually also of the regurgitation type. Furthermore, pernicious anemia and cirrhosis are chronic diseases and show quite characteristic hematologic and biochemical alterations. In the Marchiafava-Micheli syndrome the hemoglobinuria and the acid fragility test are invaluable aids for differentiation. Diminution of the thrombocytes is only rarely very severe.

Interestingly, neurologic signs may be produced by thromboses of larger cerebral vessels. The mechanism responsible for these thromboses is not understood.

In hereditary spherocytosis, severe thrombocytopenia is present during the crisis, but usually the family history, the constant spherocytosis, and the typical differential fragility tests lead to the correct evaluation.

The idiopathic and particularly the symptomatic types of acquired hemolytic anemias, and sometimes the reactions to drugs (e.g., sulfanilamide) may provide really great difficulties in differential diagnosis. Thrombocytopenia is not as unusual in these disorders as was previously believed. Whether thrombotic purpura is caused by a hypersensitivity to drugs is a moot question. Symptomatic hemolytic anemia due to leukemia, Hodgkin's disease, or neoplasms resembles most closely the disease under discussion. The leukemoid reaction seen in "generalized platelet thromboses" can be misinterpreted easily. If our observation of a negative Coombs test should be corroborated in the future, this immunologic tool may become especially useful since in the idiopathic and symptomatic varieties of hemolytic anemia the erythrocytes usually show a positive antiglobulin test. Furthermore, only in thrombotic purpura are fleeting neurologic signs seen. It was on these grounds that the diagnosis of Hodgkin's disease was rejected in our case, although the patient showed roentgenologically a mediastinal mass.

Pathogenesis. The pathogenesis of the disease remains unknown. The following phenomena require an adequate explanation: (a) The mechanism causing formation of hyaline thrombi predominantly located in the capillaries and small arterioles; (b) the endothelial proliferations within the blood vessels; (c) the increased rate of destruction of the erythrocytes by an extrinsic agent; and (d) the fluctuations in the symptomatologic manifestations (leukemoid reactions, spherocytosis, neurologic signs).

The thrombi are usually considered to be composed of platelets. This opinion is not based on direct cytochemical evidence but on the observations that the staining reactions of the hyaline material differ from those obtained with erythrocytes, leukocytes, bacteria, fibrin, hemosiderin, and hemoglobin. In spite of this evi-
dence, Wiener maintains that the thrombi consist of 'partially lysed autoconglutinated red cells.' In patients who have developed autoimmune bodies against their own erythrocytes, causing an acquired hemolytic anemia, thromboses may be formed by 'intravascular conglutination' due to the disintegration and precipitation of the red corpuscles. From this point of view, the difference between the pathologic and clinical patterns of the 'immunologic' acquired hemolytic anemias and the 'generalized vascular thromboses' would be one of degree only, and thrombotic purpura would cease to be a special entity. The finding of a negative Coombs test in our patient does not seem to support Wiener's interpretation.

The endothelial proliferations of the wall of the blood vessels are commonly interpreted as being stimulated by the preceding thrombotic occlusions. Usually, thromboses and endothelial reactions are seen together, but occasionally thrombosis without proliferations, and vice versa, may be encountered. In our present case a subintimal amorphous eosinophilic material was occasionally found. Very recently, Gore has described similar changes in his cases and has expressed the belief that these vascular lesions may be one of the causes of thrombus formation. In his opinion, the thrombus may 'grow' by apposition along the vessel and if then cut away from the site of origin an endothelial proliferation may not be seen. Restudy of the slides of our first case did not reveal any eosinophilic material. The evidence available as to whether the damage to the blood vessels is the primary or the secondary event still remains equivocal.

In this respect it may be of interest that the siblings of patients with thrombotic purpura show a rather remarkable incidence of the rare diffuse vascular diseases. A brother of one of the patients of Baer et al. died of periarteritis nodosa; the sister of our first patient succumbed to a typical generalized lupus erythematosus one year following the publication of our paper. Hypersensitivity to sulfanilamide was demonstrated by Engel et al., and allergic reactions are often mentioned in the records of cases with thrombotic purpura. In the light of the experiments by Rich, who produced periarteritis nodosa with sensitization to sulfonamides and iodine, the possible significance of a hypersensitivity reaction to drugs or bacterial agents must be considered. Ehrich and Seifter believe that iodine may play a role in bringing on generalized platelet thromboses. No conclusive evidence, however, is furnished to verify a causal relationship between the intake of an iodine-containing medication and the appearance of the disease in their case. Shwartzman et al. produced lesions similar to those found in 'generalized platelet thromboses' experimentally in animals by inducing the Shwartzman phenomenon. In these experiments, hemorrhage and leukocytic infiltrations of the walls of the blood vessels precede the formation of thrombi which are located primarily in the venules. In the human disease the arterioles and capillaries are predominantly affected.

One may speculate that all the pathologic changes in thrombotic purpura could be caused by one single agent which might injure all the structures involved, namely platelets, endothelial lining of capillaries, and red cells. Such an agent may be a 'toxin' or may result from a specific antigen-antibody reaction. It should be realized, however, that such an assumption is not yet based on any established facts. Bernheim found no platelet-agglutinating antibodies in cadaver blood from a case
of this disease, and the negative Coombs test in our patient also points against such a postulation. Further investigations, however, should be conducted to elucidate the role of a possible immunologic or allergic mechanism.

Treatment. Splenectomy is the most successful procedure in the treatment of thrombocytopenic purpura as well as of the spherocytic hemolytic anemias. In thrombotic purpura, death followed shortly after the performance of this operation in three instances. The negative Coombs test seems to indicate that production of globulin antibodies is absent, although this abnormality is demonstrable in the immunologic acquired hemolytic anemias. On the other hand, Muirhead et al. emphasize that splenectomy has not received a fair trial in thrombotic purpura because the operated patients were all in the moribund state. If in the future the diagnosis should be made early in the course of the disease the physician will be faced with a difficult decision.

Equally difficult is the decision whether heparin, which may prevent thrombus formation, should be administered to a patient with a severe bleeding tendency. Aureomycin was not effective in our patient in the dosage of 2 Gm. per day. No other promising treatment seems to be available at the present time.

Summary

Thrombotic thrombocytopenic purpura (generalized platelet thromboses) is an acute febrile disease characterized by a diagnostic triad: thrombocytopenic purpura, hemolytic anemia, and transitory focal neurologic signs. Twenty cases with this condition have been reported so far. Another case is described in which the diagnosis was made ante mortem on the basis of the above criteria. Detailed hematologic, immunologic, and pigmentary studies revealed a hemolytic process unaccompanied by evidence of autoimmunization. The differential diagnosis, etiologic factors, and pathologic physiology of the disease are discussed together with the possibility that a hypersensitivity reaction might be present.

ACKNOWLEDGMENT

We are very much indebted to Dr. William Brams who permitted us to study his private patient.

REFERENCES

SINGER, MOTULSKY, AND SHANBERGE

448

THROMBOTIC THROMBOCYTOPENIC PURPURA

40 Pierce, P.: Personal communication.
THROMBOTIC THROMBOCYTOPENIC PURPURA: II. STUDIES ON THE HEMOLYTIC SYNDROME IN THIS DISEASE

KARL SINGER, ARNO G. MOTULSKY and JACOB N. SHANBERGE