THE MEGAKARYOCYTES IN IDIOPATHIC THROMBOCYTOPENIC PURPURA, A FORM OF HYPERSPLENISM

By William Dameshek, M.D., and Captain Edward B. Miller, A.U.S.

In his book *Opera omnia*, published in 1775, Paul Gottlieb Werlhof devoted a chapter to "Morbus maculosus haemorrhagicus," which he had first described forty years previously. He wrote:

An adult girl, robust, without manifest cause, was attacked recently, towards the period of her menses, with a sudden severe hemorrhage from the nose, with bright but foul blood escaping together with a bloody vomiting of a very thick extremely black blood. Immediately there appeared about the neck and on the arms, spots partly black, partly violaceous or purple, such as are often seen in malignant smallpox...; moreover the number of the spots increasing and surrounding completely both of the eyes, the back of the nose and the skin around the mouth and chin, with a livid black color, like marked from bruises.

Since the bleeding began simultaneously with the menses and since there was spontaneous recovery, it is indeed probable, as most authorities have agreed since, that this was an example of idiopathic thrombocytopenic purpura. The reasons for the development of sudden, generalized bleeding from all the mucous membranes and into the skin are almost as obscure today as they were in Werlhof’s time. In the present paper, an attempt is made to develop a concept of pathogenesis centering about the failure of platelet growth from the megakaryocytes in the bone marrow, and dependent upon an abnormal inhibitory factor in a distant organ, namely, the spleen.

The great diminution in platelets in Werlhof’s disease was first recognized by Krauss in 1883 and by Denys in 1887. Hayem later confirmed and amplified these isolated observations. The relationship of the platelets to the giant cells of the bone marrow—the megakaryocytes—became known with the work of J. H. Wright in 1906 and 1910. In 1915, Frank made accurate studies of "essential thrombopenia" and postulated a marked diminution in platelet production by the megakaryocytes.* In the following year, Kaznelson suggested splenectomy as a therapeutic maneuver in a chronic relapsing case of the disease. He assumed, by analogy with hemolytic anemia, that the spleen might have an unusual thrombolytic function. The results of the first operation were brilliant, but in the next two cases only temporary increases in platelets occurred. Since that time the favorable effect of splenectomy in idiopathic thrombocytopenic purpura has been amply confirmed. The quick recovery following splenectomy of many desperately ill patients bleeding spontaneously from all the orifices is one of the most dramatic events in medicine, and must immediately implicate the spleen as of prime pathogenetic importance in the disease. In confirmation of this, the injection of splenic

* Frank is incorrectly quoted by most observers as having suggested a splenic effect on megakaryocyte platelet growth.
extracts from patients with the disease has, in the hands of several investigators, resulted in the development of thrombocytopenia in the experimental animal. Whether the diminution in platelets is due to increased thrombolysis by an abnormally active spleen or to diminished platelet production by the megakaryocytes has remained a question to the present time. Although Frank's studies of the bone marrow demonstrated a definite disorder of platelet production by the megakaryocytes, the significance of his observations has not been fully appreciated and most observers simply conclude that the spleen destroys platelets excessively. The present paper deals with a study of the megakaryocytes in bone marrow biopsies from typical cases of the disease, and from symptomatic cases in association with splenomegaly, together with their comparison with normal controls. Serial studies of the bone marrow before and after splenectomy have demonstrated the remarkable effects on platelet production which occur shortly after operation. Our findings are in accord with Frank's concepts that the fundamental defect leading to thrombocytopenia is a dysfunction of the megakaryocytes of the marrow. In turn, this appears to be due to a form of abnormal splenic activity, i.e., one of the types of "hypersplenism," and the disease may thus be considered fundamentally as a disorder of the spleen, with the bone marrow and the blood being secondarily involved.

MATERIAL

The material studied included the following:
1. Ten normal cases, all with hemoglobin values above 12.4 Gm. per 100 cc. and red cell counts above 4.0 M. per cu. mm. The platelet levels (Dameshek method) were within normal range of 400,000 to 900,000 per cu. mm.
2. Eleven cases of idiopathic thrombocytopenic purpura. Of these, 5 were acute cases in which splenectomy was performed. The usual criteria for the diagnosis of this condition were present: namely, spontaneous bleeding into the skin and from mucous membranes, low platelet count, prolonged bleeding time, normal coagulation time, poor retraction of clot, positive tourniquet test, absence of anemia other than that explainable on the degree of blood loss, and no evidence at bone marrow biopsy of leukemia or other fundamental hematopoietic disease. Bone marrow punctures were performed prior to splenectomy, and at varying intervals after splenectomy. In 6 chronic cases, single bone marrow punctures were performed.
3. Five cases of thrombocytopenia associated with well defined splenomegaly of nonleukemic and non-neoplastic origin ("symptomatic hypersplenic thrombocytopenia"), as follows: Gaucher's disease; unknown origin, probably infectious; juvenile hepatic cirrhosis; probable splenic vein thrombosis; and Felty's syndrome.
4. A miscellaneous group of hematologic conditions, in which systematic counts of the megakaryocytes were not made but in which a general impression regarding the number and condition of the megakaryocytes in the bone marrow was noted. This group included about 50 cases of idiopathic thrombocytopenic purpura, a similar number with thrombocytopenia which was "symptomatic" of an underlying splenic condition, about 300 cases of leukemia of various types, and...
several hundred cases of various types of anemia, polycythemia, and other blood dyscrasias.

METHODS

Peripheral Platelet Counts—The indirect (wet smear) technic of Dameshek, using an isotonic aqueous solution of sodium citrate containing brilliant cresyl blue, was used. The normal platelet count by this method ranges from 400,000 to 900,000 per cu. mm., with an average normal count of about 600,000 per cu. mm.

Sternal Puncture—This was performed by the introduction of a simple sternal puncture needle through the anterior lamella and into the marrow space, at a point on the sternum between the third and fourth intercostal spaces. After withdrawal of the stilet, a very small amount of marrow fluid, usually 0.3 cc. or less, was withdrawn by aspiration with a dry 5 or 10 cc. syringe. Drops of aspirated material were immediately placed, without the further use of anticoagulant or other material, on carefully cleaned new glass slides and gently spread with minimum pressure by means of another slide. The preparations were allowed to dry in air and stained first with Wright’s stain, then with Giemsa stain, following which coverslips mounted in balsam were affixed.

Megakaryocyte Counts—A rectangle 2.0 x 15 mm. was cut out of paper and placed over the slide to be studied. The megakaryocytes in this area, containing approximately 20,000 oil immersion fields, were counted and expressed in terms of a million nucleated cells. At least one-half million nucleated cells were counted, the count being facilitated by first accurately enumerating the nucleated cells in 20 oil immersion fields.

Differential Counts of the Megakaryocytes.—The identification of megakaryocytes can be either very easy or quite difficult. The typical huge adult forms are readily defined, but recognition of their precursors requires much patience and study. In studying the megakaryocytes the work and nomenclature of various Italian investigators, notably Di Guglielmo, Morone, and Torrioli and Scalfi, have been followed with slight modifications.

The predominant mode of origin of the megakaryocyte is probably from a stem cell or megakaryoblast, which in turn probably originates from the pluripotential histiocyte or hemohistioblast last (table 1). A subsidiary method of origin, about which much controversy has taken place, is that from the polykaryocyte or osteoclast. Although this cell was for many years sharply differentiated from the megakaryocyte, its close relationship to the latter was demonstrated by several Italian investigators. Di Guglielmo concluded that the polykaryocyte was derived from the fusion of primitive mononuclear histioid cells, with the resultant development of large multinucleated giant cells, which in turn became megakaryocytes. Di Guglielmo’s observations were confirmed by Bianchini, Cesabianchi, Morone, Fontana, and Gandolfo, and in this country by Rosenthal. Morone sharply differentiated the polykaryocytes from osteoclasts, but in this he was disputed by Lambin and Lamers. On the other hand, the origin of megakaryocytes from prepolykaryocytes was rejected by Lapidari and by Wujts.

Other more uncommon methods of megakaryocytic development have been cited.
Megakaryocytes in Thrombocytopenic Purpura

by various investigators. Thus Bloom\textsuperscript{29} described them as originating from nonphagocytic fixed tissue cells lining the sinusoids of the liver. Downey, Palmer, and Powell,\textsuperscript{30} by studying a case of atypical myelosis, traced the origin of megakaryocytes from reticulum and myeloblasts. Later, Downey and Nordland,\textsuperscript{31} in studying a similar case, found transitions from myeloblasts to platelet-producing megakaryoblasts in the peripheral blood, and transitions from hemocytoblasts to megakaryocytes in the spleen. No formation of megakaryoblasts from the splenic reticulum was apparent.

<table>
<thead>
<tr>
<th>A. The Usual Type</th>
<th>B. Relatively Uncommon Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megakaryoblast</td>
<td>Prepolykaryocyte</td>
</tr>
<tr>
<td>Promegakaryocyte</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>Mature Megakaryocyte</td>
<td></td>
</tr>
<tr>
<td>Mature Megakaryocyte</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>Polykaryocyte</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
</tr>
</tbody>
</table>

Megakaryocyte formation has at least two methods of derivation: (A) the usual, by way of a megakaryoblast, and (B) the relatively uncommon, from a prepolykaryocyte. Both of these stem cells appear to be derived from the pluripotential histiocyte or hemohistioblast of Ferrata. Platelet production occurs from the relatively mature types of megakaryocytes and to some extent from the promegakaryocytes.

The following megakaryocytes have been distinguished (see colored plate and photomicrographs):

**Megakaryoblast:** These are cells about twice the size of myeloblasts with a blue nongranular cytoplasm and a large somewhat irregular single nucleus, which at times is kidney shaped, with numerous nucleoli. These cells do not produce granular or nongranular platelets either normally or in cases of idiopathic thrombocytopenic purpura. In normal preparations they comprise less than 1 per cent of all the megakaryocytes.

**Promegakaryocyte:** Usually about the same size as the megakaryoblast, although occasionally much larger, with scant dark blue cytoplasm and a dense nonlobu-
lymphoid megakaryocyte: A large cell with basophilic cytoplasm, usually without

absent or rare in normal cases but were frequent in the cases of purpura. Normally, promegakaryocytes make up about one third of the total number of megakaryocytes.

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granules, and a nucleus which is relatively small in comparison with the entire cell and usually distinctly lobulated.

**Intermediate forms:** These cells are intermediate in size or intermediate in maturation between the relatively small promegakaryocyte and the huge adult forms. The cytoplasm is very heavily granulated. Platelet formation may or may not be present.

**Adult megakaryocyte:** A cell of variable size, but usually very large, containing a single large multilobed nucleus with increasing density as it matures. The cytoplasm varies in color from blue to pink and contains a variable number of characteristic azurophilic granules grouped at first in the perinuclear zone. Typical platelets which are almost always granular are frequently found in pseudopod-like structures grouped in masses about the periphery of the cell.

**Prepolykaryocyte:** A mononuclear cell which tends to occur in clusters. There is an abundant vacuolated blue cytoplasm without granules and a clear reticulated eccentric round nucleus with one or more nucleoli. The cells are seen normally and do not appear to be increased in purpura.

**Polykaryocytes:** These are probably fused syncytia of the above—that is, a large number of individual nuclei lie within one cytoplasmic body. These cells are probably identical with the osteoclasts.

**Adult megakaryocyte:** This cell is probably formed by nuclear fusion from the polykaryocyte.

**Degenerated forms:** In these cells the cytoplasm is either homogeneous and hyaline in appearance, or there is marked vacuolization of the cytoplasm, or the nucleus is hyperlobulated and its cytoplasm nongranular.

It must be recognized that the relationships of the various types of cells to each other may be more artificial than real, since they are based on a study of what appear to be transition forms. Because of this uncertainty, the megakaryoblasts and promegakaryocytes may be designated as "young forms," and the lymphoid megakaryocytes, intermediate forms, and adult types as "adult forms," with a separate designation for the degenerated cells.

**OBSERVATIONS**

A. **Normal Cases** (table 2).—In 10 normal patients with normal hematologic findings, study of the megakaryocytes revealed the following features:

1. Not more than 300 megakaryocytes per million nucleated cells were present.
2. About two thirds of the megakaryocytes contained platelets or platelet-like bodies at the peripheries of their cytoplasms.
3. Megakaryoblasts were rare.
4. Promegakaryocytes, usually producing granular platelets, were plentiful. Nongranular platelet production was rare. Approximately one half of the platelet-producing megakaryocytes were young forms.
5. Degenerated forms varied from 8 to 22 per cent of all cells.

B. **Acute Idiopathic Thrombocytopenic Purpura** (table 3).—Differential counts of the megakaryocytes were made in 5 cases of acute idiopathic thrombocytopenic purpura before splenectomy. In 4 of these, one or more marrow studies were made after splenectomy. The findings before splenectomy were as follows:
### Table 2.—Normal Controls—Differential Megakaryocyte Counts

<table>
<thead>
<tr>
<th>Name</th>
<th>Range (X10) R. B. C.</th>
<th>Platelets (per cu. mm.)</th>
<th>Promegakaryocytes</th>
<th>Lymphoid megakaryocytes</th>
<th>Intermediate megakaryocytes</th>
<th>Adult megakaryocytes</th>
<th>Degenerated forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. P.</td>
<td>4.97</td>
<td>480</td>
<td>0</td>
<td>6</td>
<td>13</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>E. DeM.</td>
<td>4.97</td>
<td>744</td>
<td>0</td>
<td>3</td>
<td>35</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>A. F.</td>
<td>4.96</td>
<td>420</td>
<td>0</td>
<td>2</td>
<td>42</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>A. E.</td>
<td>4.97</td>
<td>548</td>
<td>0</td>
<td>3</td>
<td>35</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>A. McL.</td>
<td>5.17</td>
<td>537</td>
<td>1</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>J. B.</td>
<td>3.94</td>
<td>484</td>
<td>0</td>
<td>2</td>
<td>32</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>C. C.</td>
<td>4.85</td>
<td>632</td>
<td>1</td>
<td>2</td>
<td>42</td>
<td>0</td>
<td>12</td>
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<tr>
<td>J. W.</td>
<td>4.70</td>
<td>410</td>
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<td>2</td>
<td>38</td>
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<td>6</td>
</tr>
<tr>
<td>A. C.</td>
<td>4.43</td>
<td>574</td>
<td>0</td>
<td>28</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>E. D.</td>
<td>4.46</td>
<td>597</td>
<td>0</td>
<td>26</td>
<td>0</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Average</td>
<td>6.08</td>
<td>547</td>
<td>0.3</td>
<td>2.5</td>
<td>13.8</td>
<td>0.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Range</td>
<td>3.94 - 51.7</td>
<td>420 - 744</td>
<td>0.2</td>
<td>0.6 - 13.42</td>
<td>0.2 - 2</td>
<td>0.7</td>
<td>0.6 - 16.7</td>
</tr>
</tbody>
</table>

### Key to Abbreviations in Tables

- M. = Millions
- R.B.C. = Red blood cell count
- Platelets = Blood platelet count, thousands per cu. mm.
- Plts. = Platelets
- Gran. = Granular or granularity
- Nongran. = Nongranular
- Megas. = Megakaryocytes

### Table 3.—Acute Idiopathic Thrombocytopenic Purpura—Differential Megakaryocyte Counts

<table>
<thead>
<tr>
<th>Name</th>
<th>Promegakaryocytes</th>
<th>Lymphoid megakaryocytes</th>
<th>Intermediate megakaryocytes</th>
<th>Adult megakaryocytes</th>
<th>Degenerated forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. K.</td>
<td>2</td>
<td>9</td>
<td>17</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>L. Z.</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>I. G.</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>R. L.</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>M. D.</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Average</td>
<td>2.8</td>
<td>8.0</td>
<td>7.2</td>
<td>6.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Range</td>
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<td>5 - 9</td>
<td>0 - 17</td>
<td>1 - 14</td>
<td>0 - 1</td>
</tr>
<tr>
<td>Normal</td>
<td>0 - 2.0</td>
<td>0 - 6</td>
<td>0 - 6</td>
<td>0 - 6</td>
<td>0 - 2</td>
</tr>
</tbody>
</table>

### Key to Abbreviations in Tables

- Eos. = Eosinophilic
- Vacuol. = Vacuolated
- Hyal. = Hyaline
- Nuc. = Nucleated
- Blasts = Megakaryoblasts
- Promegas. = Promegakaryocytes
- Intermed. = Intermediate forms

### Megakaryocytes per million nuc. cells (%) Table

<table>
<thead>
<tr>
<th>Name</th>
<th>Promegakaryocytes</th>
<th>Lymphoid megakaryocytes</th>
<th>Intermediate megakaryocytes</th>
<th>Adult megakaryocytes</th>
<th>Degenerated forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. K.</td>
<td>546.9</td>
<td>18</td>
<td></td>
<td></td>
<td>144</td>
</tr>
<tr>
<td>L. Z.</td>
<td>501.3</td>
<td>19</td>
<td></td>
<td></td>
<td>144</td>
</tr>
<tr>
<td>I. G.</td>
<td>743.9</td>
<td>9</td>
<td></td>
<td></td>
<td>144</td>
</tr>
<tr>
<td>R. L.</td>
<td>366.4</td>
<td>18</td>
<td></td>
<td></td>
<td>144</td>
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<tr>
<td>M. D.</td>
<td>460.0</td>
<td>8</td>
<td></td>
<td></td>
<td>144</td>
</tr>
<tr>
<td>Average</td>
<td>523.7</td>
<td>14.4</td>
<td></td>
<td></td>
<td>144</td>
</tr>
<tr>
<td>Range</td>
<td>8 - 19</td>
<td>8 - 19</td>
<td></td>
<td></td>
<td>144</td>
</tr>
<tr>
<td>Normal</td>
<td>50 - 86</td>
<td>50 - 86</td>
<td></td>
<td></td>
<td>144</td>
</tr>
</tbody>
</table>
MEGAKARYOCYTES IN THROMBOCYTOPENIC PURPURA

Figs. 7-14. Idiopathic Thrombocytopenic Purpura

Fig. 7. Sternal puncture smear (X 65). Large numbers of megakaryocytes, about 25 in one low power field, and about 5 to 8 times the normal number.

8. Sternal trephine biopsy section (X 350). Increase in megakaryocytes, which show diminished granularity of cytoplasm and lack of platelet production.


10. Sternal puncture smear (X 1000). Mature megakaryocyte. Well defined granularity of cytoplasm; rare platelet bodies; no definite platelet formation.


14. Sternal puncture smear (X 1500). Adult megakaryocyte; no platelet formation; marked degeneration of cytoplasm.
1. The number of megakaryocytes per million nucleated blood cells was on the average about three times greater than in the normal.

2. Only 14.4 per cent of the megakaryocytes showed obvious platelet production, as contrasted with the normal cases in which approximately two thirds produced platelets.

3. Megakaryoblasts were definitely increased, but the proportion of promegakaryocytes was decreased.

4. Adult megakaryocytes produced fewer platelets than in normal or chronic cases. Most of the platelet production appeared to derive from promegakaryocytes.
and was often abnormal in type, with the production of large nongranulated platelets.

5. Degenerated and mitotic forms were not increased.

In the 4 cases which were studied both before and after splenectomy, the megakaryocytes per million nucleated red cells remained at about the original level or became somewhat increased. However, following operation, platelet production became sharply increased, from the average of 16 per cent before splenectomy to 73 per cent. The huge masses of platelets proceeding from previously unproductive megakaryocytes, and which often occupied large parts of the microscopic field, offered a striking contrast to the findings before splenectomy.

C. Chronic Idiopathic Thrombocytopenic Purpura.—In 6 cases of thrombocytopenic purpura of mild to moderate severity and often present for many years, the following were noted:

1. The number of megakaryocytes per million nucleated blood cells ranged from 450 to 1,565—far above the normal average of 183.
2. A third or less of the megakaryocytes showed evidence of platelet production.
3. Megakaryoblasts were increased.
4. Promegakaryocytes were much less plentiful than in the normal, but nongranular platelet production was frequently seen.
5. There was very little platelet production from adult megakaryocytes.
6. Degenerated forms were slightly increased over normal.

D. Symptomatic "Hypersplenic" Thrombocytopenic Purpura (table 5)—The megakaryocytes were studied in 5 cases in which persistent thrombocytopenia and leukopenia were associated with well defined splenomegaly. The cases were as follows: (1) Gaucher's disease, (2) unknown origin, probably infectious, (3) juvenile hepatic cirrhosis, (4) probable splenic vein thrombosis, (5) Felty's syndrome (rheumatoid arthritis with leukopenia). It was believed that these cases might be considered examples of a form of simple "hypersplenism," i.e., an increase in the normal splenic function.

The findings were as follows:

1. An increase in megakaryocytes, in about the same proportion as that seen in the acute essential cases.
2. A normal proportion of platelet-producing cells.
3. No increase in megakaryoblasts or decrease in promegakaryocytes.
5. No increase in degenerated forms.

E. Miscellaneous Cases.—A general impression of the number and type of megakaryocytes in the sternal marrow as obtained both by the puncture and trephine methods has been recorded in most of the hematologic cases studied in our laboratory since 1928. Except in poorly spread preparations, containing thick clumps of marrow tissue, and in which megakaryocytes might be numerous, the average number of megakaryocytes per low power microscopic field was just over 1, the range being from 0 to 5. Platelet formation at the edges of the megakaryocytes was visible in at least one half of the cells observed.

Anemias: In the deficiency syndromes, notably pernicious anemia, megakaryo-
cytes were usually diminished, at times markedly so. In anemia due to actual
disease or involvement of the bone marrow, including aplasia, leukemia, lympho-
sarcoma, etc., the megakaryocytes were conspicuously reduced. This was particu-
larly true in acute leukemia, in which the almost total lack of megakaryocytes
contrasted sharply with the presence of extreme cellular hyperplasia. At times

Table 4.—Chronic Idiopathic Thrombocytopenic Purpura—Differential Megakaryocyte Counts

<table>
<thead>
<tr>
<th>Name</th>
<th>R.B. C.</th>
<th>Platelets</th>
<th>Promegakaryocytes</th>
<th>Lymphoid megakaryocytes</th>
<th>Intermediate</th>
<th>Adult</th>
<th>Degenerated forms</th>
<th>Eosin.</th>
<th>Vascular</th>
<th>Hyal.</th>
<th>Mitotic figures</th>
<th>Megakaryocytes per million nucleated cells</th>
<th>Platelets per c.c.m.</th>
</tr>
</thead>
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<tr>
<td>H. H.</td>
<td>4.23</td>
<td>143,800</td>
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<td>9</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>41</td>
<td>1</td>
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<tr>
<td>R. J.</td>
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<td>150,000</td>
<td>0</td>
<td>3</td>
<td>15</td>
<td>3</td>
<td>2</td>
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<td>1</td>
<td>5</td>
<td>6</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>S. R.</td>
<td>4.40</td>
<td>184,400</td>
<td>2</td>
<td>4</td>
<td>13</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>43</td>
<td>1</td>
</tr>
<tr>
<td>A. D.</td>
<td>3.73</td>
<td>172,900</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<td>3</td>
<td>9</td>
<td>56</td>
<td>0</td>
<td>1</td>
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<td>A. M.</td>
<td>4.54</td>
<td>90,800</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>16</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>R. P.</td>
<td>4.74</td>
<td>204,000</td>
<td>3</td>
<td>9</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>15</td>
<td>3</td>
<td>10</td>
<td>14</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>156,000</td>
<td>1.4</td>
<td>4.2</td>
<td>8.2</td>
<td>2.4</td>
<td>3.4</td>
<td>4.8</td>
<td>2.2</td>
<td>3.4</td>
<td>8.2</td>
<td>45.6</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td>90-184</td>
<td>0-3</td>
<td>1-9</td>
<td>1-15</td>
<td>0-8</td>
<td>0-6-15</td>
<td>0-5-1-10</td>
<td>3-16</td>
<td>26-50-0.2</td>
<td>0-3-5</td>
<td>0-12</td>
</tr>
<tr>
<td>Normal range</td>
<td></td>
<td>410-744</td>
<td>0-2</td>
<td>0-6-13-42-0-2</td>
<td>0-7-6</td>
<td>0-7-0-9</td>
<td>0-12-45</td>
<td>0-2-14</td>
<td>0-4</td>
<td>0-4-16</td>
<td>0-2</td>
<td>99-270</td>
<td>50-86</td>
</tr>
</tbody>
</table>

Table 5.—Symptomatic Hyperplastic Thrombocytopenic Purpura—Differential Megakaryocyte Counts

<table>
<thead>
<tr>
<th>Name</th>
<th>Bone</th>
<th>Promegakaryocytes</th>
<th>Lymphoid megakaryocytes</th>
<th>Intermediate</th>
<th>Adult</th>
<th>Degenerated forms</th>
<th>Eosin.</th>
<th>Vascular</th>
<th>Hyal.</th>
<th>Mitotic figures</th>
<th>Megakaryocytes per million nucleated cells</th>
<th>Platelets per c.c.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. S.</td>
<td>1</td>
<td>24</td>
<td>16</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>V. G.</td>
<td>1</td>
<td>3</td>
<td>24</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>E. P.</td>
<td>0</td>
<td>2</td>
<td>13</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. W.</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>31</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>U. S.</td>
<td>2</td>
<td>6</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>2</td>
<td>15</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Average</td>
<td>1.0</td>
<td>4.4</td>
<td>23.4</td>
<td>1.6</td>
<td>9.2</td>
<td>6.6</td>
<td>4.4</td>
<td>0.4</td>
<td>24.0</td>
<td>18.4</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Range</td>
<td>0-2</td>
<td>2-6-9-41</td>
<td>0-3</td>
<td>4-16</td>
<td>4-11</td>
<td>10-0</td>
<td>0-2-15</td>
<td>31-37</td>
<td>0-1</td>
<td>0-5-9</td>
<td>0-3</td>
<td>258-885</td>
</tr>
</tbody>
</table>

this finding was of considerable diagnostic value, particularly in the differential
diagnosis of thrombocytopenic purpura. In anemia due to increased blood loss,
whether by hemorrhage or increased hemolysis, the megakaryocytes were usually
conspicuously increased in number, with abundant platelet formation being
visible from their cytoplasm.

Polycythemia: In polycythemia vera, one of the most outstanding features of the

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bone marrow was the great megakaryocytic hyperplasia, which in certain cases seemed to dominate the entire marrow picture. In secondary polycythemia, the megakaryocytes appeared to be normal in number.

Leukemias: The striking diminution and even complete lack of the megakaryocyte in acute leukemia has already been mentioned. In chronic myelogenous leukemia, these giant cells were often greatly increased, particularly in the earlier stages of the disease. In chronic lymphatic leukemia, there was a gradual reduction in the number of these cells as the marrow became progressively infiltrated with lymphocytes.

Hemorrhagic diseases: No particular changes, either quantitative or qualitative in type, were observed in hemophilia, vascular types of purpura, or in other less well defined hemorrhagic conditions.

DISCUSSION

A. Origin of the Blood Platelets.—Idiopathic or essential thrombocytopenic purpura is primarily a disorder in which the blood platelets are conspicuously reduced, as a result of which blood escapes from capillaries with ensuing purpura and other hemorrhagic manifestations. Knowledge regarding the origin of the blood platelets is comparatively recent, dating from the writings of James Homer Wright in 1906 and 1910. Howell had previously (in 1890) named the giant cells of the bone marrow "megakaryocytes" to distinguish them from the multinucleated giant cells apparently related more closely to the bone and which were called polykaryocytes or osteoclasts. In 1906, Wright for the first time described the origin of the blood platelets from the megakaryocytes of the bone marrow. By the use of his special eosin-methylene blue stain (now called Wright's stain) he could clearly recognize the platelets in stained fixed tissue and differentiate them from other histologic elements. In 1910, described his studies of the bone marrow of the cat, mouse, rabbit, guinea pig, white mouse, opossum, and man with particular reference to the megakaryocytes and the platelets. He found that the giant cells of the marrow often contained granules which were most numerous in the pseudopodial processes of cytoplasm projecting into the sinusoids of the marrow. In some megakaryocytes or their pseudopods, one or more small groups of granules were separated by a zone of hyaline cytoplasm from the rest of the cell. These masses of granules with their intervening cytoplasm had the identical staining reactions of platelets. He furthermore found that bodies identical in appearance with blood platelets were often found near pseudopods and that detached pseudopods were at times seen in blood channels. He therefore concluded that megakaryocytes produced platelets by direct budding from pseudopods which had entered the circulation of the marrow. Bunting in 1909 confirmed Wright's findings in the rabbit and furthermore showed that an increase in megakaryocytes induced by bleeding and the use of turpentine and saponin was associated with an increase in platelets. He also found that both the granules of the megakaryocytes and the platelets give identical staining reactions with the supravital dyes brilliant cresyl blue and neutral red. Smith, Robinson, and Tyson showed that the oxidase reactions of the granules of both the megakaryocytes and platelets were negative.
FURTHER EVIDENCE SUBSTANTIATING THE ORIGIN OF PLATELETS FROM MEGAKARYOCYTES

Further evidence substantiating the origin of platelets from megakaryocytes has been gained from experimental studies in which the number of megakaryocytes and the fragments issuing from their pseudopods was correlated with the number of blood platelets as modified by repeated bleeding (Bunting\textsuperscript{35}), the action of benzol (Weiskotten, Wyatt, and Gibbs\textsuperscript{38}), the use of antiplatelet serum (Bedson and Johnston\textsuperscript{37}), the use of saponin (Firket and Campos\textsuperscript{38}), and by "blocking" of the reticulo-endothelial system (Volterra\textsuperscript{39}). Injection of colloidal materials, notably india ink, in mice was followed by thrombocytosis, the appearance of megakaryocytes in the peripheral blood, and a simultaneous increase in their number in the spleen and bone marrow. This so-called "blocking" of the reticulo-endothelial system in inducing thrombocytosis may be unimportant as compared with the actual effects of the colloidal particles on the platelets themselves. One of us\textsuperscript{4} showed in unpublished experiments (1933-35) that the injection of various types of colloidal materials was followed by an extreme thrombocytopenia due to a "sweeping up" of the colloidal particles by all the platelets in the circulation, and the resultant massing of agglutinated platelets as thrombi. This was followed by active regeneration of platelets from megakaryocytes, as described by Volterra.\textsuperscript{39}

Other origins of the platelets have been described. Brown\textsuperscript{41} in 1913 found that under conditions of excessive demand, platelet production from monocytes could occur. Bunting\textsuperscript{35} in 1920 claimed that in epidemic influenza in which thrombocytopenia was present, platelets or platelet-like bodies were formed from lymphocytes in the blood stream. Howell and Donahue\textsuperscript{42} (1937), on the basis of arterial and

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
Disease & Promegaka-
\begin{tabular}{c}
ryocytes
\end{tabular} & Lympho-
\begin{tabular}{c}
nid megak-
\end{tabular} & Intermediate & Adult & Degenerated forms & Mitotic figures & Megakaryocytes per m. nuc. cells & Percentage with plts. \\
\hline
\textbf{Normals}} & & & & & & & & & \\
Average & 0.5 & 1.5 & 31.8 & 0.3 & 2.2 & 2.2 & 9.3 & 2.1 & 25.1 & 11.0 & 2.4 & 2.1 & 0.4 & 8.4 & 0.3 & 182.5 & 99-270 & 68.6 \\
Range & 0-2 & 0-6 & 13-42 & 0-2 & 0-7 & 0-6 & 7-18 & 0-9 & 12-45 & 2-32 & 0-14 & 0-4 & 0-16 & 0-2 & 50-86 \\
\hline
\textbf{Acute idiopathic} & & & & & & & & & \\
\textbf{thrombocytopenic purpura}} & & & & & & & & & \\
Average & 2.8 & 8.0 & 7.2 & 6.2 & 2.0 & 8.6 & 1.0 & 5.0 & 4.2 & 40.6 & 1.2 & 1.8 & 0.8 & 11.4 & 0.8 & 523.7 & 366-743 & 14.4 \\
Range & 5-9 & 0-17 & 2-13 & 1-3 & -14 & 0-5 & 0-9 & 0-9 & 14-50 & 0-3 & 0-3 & 0-8 & 0-17 & 0-2 & 8-19 & 8-19 \\
\hline
\textbf{Chronic idiopathic} & & & & & & & & & \\
\textbf{thrombocytopenic purpura}} & & & & & & & & & \\
Average & 1.4 & 4.2 & 8.2 & 2.4 & 8.4 & 2.2 & 2.2 & 3.4 & 8.2 & 45.6 & 0.8 & 1.2 & 0.8 & 14.2 & 0 & 836.5 & 450-1565 & 22 \\
Range & 0-3 & 1-9 & 1-15 & 0-8 & 0-6 & 1-2 & 1-2 & 1-2 & 1-10 & 3-16 & 26-50 & 0-2 & 0-3 & 0-1-2 & 0 & 10-34 \\
\hline
\textbf{Symptomatic hyper-} & & & & & & & & & \\
\textbf{splenic purpura}} & & & & & & & & & \\
Average & 1.0 & 4.4 & 23.4 & 1.6 & 9.2 & 6.6 & 4.4 & 0.4 & 24.0 & 18.4 & 0.2 & 0.4 & 0.0 & 6.0 & 1.0 & 493.9 & 258-885 & 61.8 \\
Range & 0-2 & 2-6 & 9-41 & 0-3 & 4-16 & 4-11 & 0-10 & 0-2 & 13-31 & 8-37 & 0-1 & 0-1 & 0 & 5-9 & 0-3 & 51-72 \\
\hline
\end{tabular}
\caption{Summary of Differential Megakaryocyte Counts}
\end{table}
venous platelet counts, histologic studies, and perfusion experiments, concluded that new platelets were added to the blood in capillary areas of the lungs and were destroyed in the systemic capillaries.

Originally based on purely morphologic grounds, the evidence from both histologic and experimental studies that platelets are formed from the megakaryocytes of the bone marrow can hardly be more complete. Under abnormal conditions, as in myeloid metaplasia, these giant cells may also develop in the spleen, the lungs, and in other sites. "Vicarious" platelet production from lymphocytes, monocytes, etc., is a possibility under conditions of unusual demand. The literature on this subject is carefully reviewed by Tocantins.43

B. Variations in the Blood Platelet Count; Certain Regulatory Mechanisms.—The number of platelets in the circulating blood probably depends upon several factors, none of which has been adequately studied. Probably of greatest importance is the total number of megakaryocytes and their degree of activity. In conditions with greatly increased megakaryocytes, as in polycythemia vera and certain cases of chronic myelogenous leukemia, the platelet count becomes considerably increased. Megakaryocytic activity is also stimulated by excessive blood loss, whether due to hemorrhage or to increased hemolysis. The operative removal of various organs, such as the uterus, the stomach, etc., is followed by a transient thrombocytosis,44-46 which may, however, be a function of bleeding rather than dependent upon actual removal of the organ. When megakaryocytes are conspicuously reduced, as in acute leukemia, aplastic anemia, destructive lesions of the bone marrow, and certain deficiency disorders such as pernicious anemia, the blood platelets are also greatly diminished. In these abnormal situations, the relationship of megakaryocytes to platelets seems to be clear and well defined. However, no such clarity exists when one considers the normal blood platelet count. The mechanisms dealing with the stimulation of platelet growth and budding, the delivery of platelets from the bone marrow to the blood, the life span of the platelet, and its mode of destruction and disintegration remain quite obscure.

As noted above, a potent stimulator of megakaryocytic and platelet production is blood loss, whether by hemorrhage or by hemolysis. Since blood destruction by hemolysis is constantly taking place, it is possible that this process results in a constant stimulation of megakaryocytic platelet growth. In women, the menstrual cycle appears to be closely related to variations in the count. With onset of the catamenia, as one of us17 showed a number of years ago, there is an immediate and striking rise in platelets to about twice their original level. This may be interpreted as indicative of an endocrinial relationship, but it may simply be due to the stimulating effect of hemorrhage into the endometrial mucosa and its sudden separation from the uterus. In any event, the platelet count of menstruating women is subject to regular peaks and valleys, determined largely by the menstrual cycle, and is quite in contrast with that of men and nonmenstruating women. The low megakaryocyte and platelet counts in pernicious anemia, and the prompt response in platelet count following treatment with liver extract—often in advance of the reticulocyte increase—suggest that "liver extract substance" is required for megakaryocyte platelet growth.
The mechanisms which have to do with the delivery of platelets from the marrow to the blood are also obscure. One fact is, however, certain: the operative removal of the normal spleen is followed, not only by a marked temporary increase in platelet count, but by a sustained high platelet level which may be present as long as twenty years after splenectomy. This is quite in contrast with the transient platelet increase which occurs following the removal of an organ such as the uterus, and suggests that the normal spleen exerts a regulatory (inhibitory?) effect on either the growth of the platelets or their delivery from the marrow to the blood. Support for this hypothesis is given by the findings in many cases of splenomegaly, whether due to cirrhosis of the liver, splenic vein thrombosis, chronic infection, Gaucher’s disease, Boeck’s sarcoid, Felty’s syndrome, or whatever. In these conditions, leukopenia, granulocytopenia, and thrombocytopenia are usually present, suggesting that the large spleen exerts an unusually marked inhibitory effect on both leukocytic and platelet formation and/or delivery. Evidence indicating that the thrombocytopenia is due to an inhibition of delivery rather than to disturbed formation is brought out by the findings in the bone marrow which show an increase in megakaryocytes with a normal degree of platelet production (table 5). Following splenectomy in these cases, the platelets rise quickly to high levels, where they remain indefinitely.

C. The Marrow Findings in Idiopathic Thrombocytopenic Purpura.—The above observations lead us to interpretations regarding the marrow findings in essential or idiopathic thrombocytopenic purpura and their relationship to the spleen and splenectomy. Frank in 1915 was the first to hypothesize that the low platelet counts might be due to a dysfunction of the megakaryocytes. His actual studies of the bone marrow were reported fully in 1915 in his authoritative review of the hemorrhagic diseases in Schittenhelm’s Handbuch der Krankheiten des Blutes und der Blutbildenden Organe. In this article, he noted diminished granularity and greatly diminished platelet production from megakaryocytes, together with the presence of degenerative changes. The article is illustrated with several excellent drawings in color. Frank’s very complete studies have unfortunately received little attention, particularly in this country, where the concept of an undue thrombocytolysis by the spleen seems to have gained wide credence. This concept, which was originated by Kaznelson of Prague in 1916, was based on the finding of an enlarged spleen in a case of chronic thrombocytopenic purpura; studies of the bone marrow were not made. In 1917, Minot, with the help of J. H. Wright, studied the bone marrow of a fatal case of the disease. The megakaryocytes were plentiful and perhaps even slightly increased above normal. From the available preparations, we could not tell if there was any definitely altered histologic appearance of the cells. These important statements were made: ‘We can suppose that, though these giant cells are plentiful in numbers, they became affected so that they are unable to allow platelets to be cut off from them in normal fashion. . . . Is it not possible that at times, with or without hypertrophy of the spleen, its physiologic activity is altered so as to cause bone marrow depression?’ Seeliger (1924) in 2 cases and Gasper (1926) in 1 case found increased megakaryocytes. Weiner and Kaznelson in 1926 stated that megakaryocytes were abundant in all
their cases. Since they found the structure of the megakaryocytes normal, they postulated an unusual thrombolytic function of the spleen. They found more platelets in smears made directly from the extirpated spleen than in the splenic blood. No actual evidence of thrombocytolysis was, however, brought forth.

Jedlička and Altschuler in 1925 studied the marrow from 2 cases of chronic essential purpura. The megakaryocytes were numerous and showed increased vacuolization and deficiency in granularity. In an autopsied case, Schmincke found increased megakaryocytes; their cytoplasms were free of granules and many contained leukocytes and lymphocytes. The latter finding was interpreted as evidence of phagocytosis. Gerlach reported an autopsied case with numerous megakaryocytes in the marrow. Large pyknotic nuclei, with little cytoplasm, containing few or no granules were present; pseudopods were rare. Nickerson and Sunderland, studying autopsy material, found that the megakaryocytes were either increased or decreased in number. The predominance of young forms suggested a functional hyperplasia. These observers found that the platelets and the megakaryocytic granules were very fragile and unusually susceptible to destruction both by postmortem change and the various processes involved in fixing the hydrating tissues prior to staining.

Studying the sternal biopsies of 4 cases of idiopathic thrombocytopenic purpura, Willi believed that the megakaryocytes were not pathologic; however, no

<table>
<thead>
<tr>
<th>Case</th>
<th>Dates</th>
<th>Blasts</th>
<th>Promegakaryocytes</th>
<th>Lymphoid</th>
<th>Intermediate</th>
<th>Adult</th>
<th>Degenerated forms</th>
<th>Megakaryocytes per million nor.</th>
<th>Total</th>
<th>Percentage with plts.</th>
<th>Blood-plts. per cu. mm.</th>
<th>State</th>
</tr>
</thead>
</table>
| A. K. | 9-14-40 | 0 | 9 | 17 | 7 | 1 | 7 | 0 | 5 | 0 | 35 | 2 | 1 | 0 | 14 | 1 | 434.9 | 18 | 45,000 | Before Xylo.
| 9-23-40 | 0 | 6 | 7 | 17 | 6 | 0 | 3 | 5 | 21 | 0 | 12 | 0 | 0 | 0 | 13 | 0 | 449.4 | 22 | 29,000 | 42,300
| 10-2-40 | 0 | 0 | 6 | 2 | 6 | 10 | 0 | 0 | 30 | 34 | 0 | 0 | 0 | 12 | 0 | 327.6 | 42 | 29,000 | 42,300
| 10-13-40 | 0 | 4 | 6 | 0 | 6 | 8 | 10 | 0 | 46 | 12 | 0 | 4 | 0 | 4 | 0 | 739.8 | 68 | 459,000 | 459,000
| L. Z. | 5-10-40 | 1 | 5 | 9 | 6 | 1 | 9 | 0 | 0 | 9 | 45 | 3 | 3 | 0 | 9 | 0 | 301.3 | 19 | 25,000 | Before splenec.
| 5-16-40 | 0 | 2 | 15 | 2 | 7 | 3 | 0 | 0 | 50 | 13 | 0 | 0 | 0 | 8 | 2 | 324.3 | 72 | 111,000 | 242,000
| 5-17-40 | 0 | 6 | 12 | 0 | 0 | 0 | 0 | 0 | 58 | 12 | 0 | 4 | 0 | 10 | 0 | 60 | 60 | 48 hrs. post |
| I. G. | 8-3-38 | 2 | 9 | 1 | 3 | 3 | 7 | 0 | 9 | 5 | 30 | 0 | 2 | 0 | 9 | 2 | 743.9 | 9 | 20,000 | Before splenec. 5 days post
| 8-9-38 | 0 | 0 | 41 | 0 | 3 | 0 | 9 | 0 | 32 | 6 | 0 | 0 | 0 | 9 | 0 | 895.6 | 85 | 440,000 | 440,000
| R. L. | 8-8-39 | 8 | 8 | 9 | 13 | 3 | 4 | 5 | 4 | 1 | 14 | 1 | 3 | 0 | 17 | 1 | 366.4 | 18 | 15,000 | Before splenec.
| 8-24-39 | 6 | 4 | 30 | 0 | 6 | 2 | 3 | 0 | 30 | 8 | 0 | 1 | 10 | 0 | 408.3 | 69 | 240,000 | 24 hrs. post
| 10-3-39 | 0 | 11 | 2 | 1 | 3 | 9 | 4 | 3 | 13 | 41 | 0 | 2 | 0 | 11 | 0 | 444.9 | 22 | 154,000 | Mild relapse
platelet formation was seen. In a case undergoing spontaneous recovery, the peripheral platelet count having reached 207,000 per cu. mm., Krujko56 found at marrow biopsy numerous normal appearing megakaryocytes surrounded by large numbers of platelets.

Klima57 studied 17 cases by the method of sternal puncture. Megakaryocytes were found increased in the majority. This was particularly true of the chronic cases, in which nongranulated megakaryocytes were often found. Rosenthal58 stated that 'the megakaryocytes are present in normal numbers or increased in thrombocytopenic purpura hemorrhagica. The diminution in platelets is either due to increased destruction in the spleen, or is the result of lack of fragmentation of the megakaryocytes.' Scott59 investigated the sternal marrow in 1 case and stated that although the megakaryocytes were certainly more abundant in most films, qualitative changes in the cells were not striking.

Recently Limarzi and Schleicher,60 employing a technic whereby films are made from the nucleated cell layer from the sternal marrow at puncture, found a marked megakaryocytic hyperplasia. In the acute cases younger forms of megakaryocytes predominated, while in the less acute phases adult types appeared to be increased. Pathologic or 'toxic' forms of megakaryocytes, cells with degenerative types of nuclei and hyaline cytoplasm and an absence of azurophilic granules, were frequently observed. Splenectomy resulted in a reversion of the marrow picture to normal values. They concluded that splenectomy removed a factor inhibitory to the maturation of megakaryocytes and that the differential diagnosis of purpuric states could be satisfactorily made from bone marrow studies.

Fieschi and Villalobos,61 studying the bone marrow in 8 cases, found changes in the megakaryocytes consisting of a disturbance in maturation of the cells and of a deficiency in platelet formation and detachment. There was also a lack of platelets in the blood of the marrow. The megakaryocyte counts showed an increase in young forms and an asynchronism in the maturation of the nucleus and protoplasm. Distinct hyperlobulation was present. Degenerated forms were inconstant and never very numerous.

Lawrence and Knutti,62 in 6 cases of idiopathic thrombocytopenic purpura, found the bone marrow sections normal in number in 4 cases. In the other 2, the megakaryocytes were decreased. Morphologic variations existed in the megakaryocytes of 4 of the cases. These authors concluded that at least two different varieties of the disease were present and suggested that splenectomy was of greater value in those cases with normal bone marrows. On the other hand, Heinild63 in 4 cases was unable to demonstrate qualitative characteristic alterations of the megakaryocytes. Wiseman, Doan, and Wilson64 reported bone marrow findings before and after splenectomy and found practically no change. The numbers of megakaryocytes are given but are not compared with normals, and the time intervals after splenectomy are not stated.

The majority of the reports in the literature thus give the impression that the megakaryocytes are increased in essential thrombocytopenic purpura. The only real attempt to compare their numbers with those of normal cases is that of Limarzi and Schleicher,60 who found a definite increase. With reference to qualitative
changes, most reports deal with fixed material, usually obtained at autopsy. Here, unless special care is taken in technic, the platelets and granules are at least partially destroyed, and classification of the types of megakaryocytes becomes very difficult. For the proper study of the megakaryocyte pattern and platelet production, the use of biopsy material with the preparation of well spread, well stained smears directly from touch or aspirated material is essential.

Our own observations of the marrow in idiopathic thrombocytopenic purpura agree closely with those of Frank, who found a great diminution in megakaryocytic platelet production corresponding with the marked platelet diminution in the blood. However, since the actual number of megakaryocytes is often greatly increased, although very few of them are producing platelets, the total concentration of platelet-producing tissue may not be as severely depleted as would appear at first glance. This might indicate not only a disturbance in the megakaryocyte platelet production, but an inhibition of delivery as well.

D. The Nature of Idiopathic Thrombocytopenic Purpura.—Several basic facts are paramount in any discussion of the nature of the disease known as idiopathic or essential thrombocytopenic purpura:

1. The blood platelets are considerably reduced.
2. The bone marrow shows normal or increased numbers of megakaryocytes.
3. The megakaryocytes show a greatly diminished productivity of platelets.
4. Splenectomy leads in most cases to a great increase in platelet production by megakaryocytes, and
5. To a great increase in blood platelets.

At first glance, it might appear paradoxical that the blood is practically depleted of platelets while the bone marrow contains normal or even increased numbers of megakaryocytes. Careful inspection of the megakaryocytes reveals what appears to be an adequate explanation of this discrepancy: the megakaryocytes show very little if any platelet production, which is often abnormal in type, proceeding from immature forms. The low blood platelet count is therefore in all probability due to a diminished production of platelets from megakaryocytes, although a diminished delivery of platelets from megakaryocytes may also be a factor. The qualitative changes in platelets as seen in the blood—large forms, often bizarre in shape—may be due to their abnormal production by the earlier forms of megakaryocytes.

These observations must be aligned with the undeniably dramatic effects of splenectomy. Often within an hour, and almost always within a few hours after this operation, the platelet count begins to rise and quickly reaches abnormally high values. Simultaneously the marrow shows striking changes: within a few hours platelet budding is seen proceeding from most megakaryocytes; within two days huge masses of platelets are often seen overrunning whole microscopic fields.

If these observations are correct, it would appear that only one conclusion as to the pathogenesis of the disease is tenable: i.e., that it is due fundamentally to an abnormality of the spleen, which exerts an unusual effect upon the production of platelets from the megakaryocytes in the marrow. This postulates a hormonal relationship between the spleen and the bone marrow. Several indications of such a relationship...
are already at hand:47,48 (1) following splenectomy in the normal human and animal the white cell and platelet counts rise and Howell-Jolly bodies appear in the red cells; (2) in many conditions associated with a large spleen, the leukocyte, granulocyte, and platelet counts usually are diminished, although the bone marrow is hyperplastic; (3) following splenectomy in such conditions, the leukocyte, granulocyte, and platelet counts rise to normal or high values. These findings suggest that the spleen may secrete materials with effects upon the production of red cells, granulocytes, and platelets in the marrow and their delivery to the circulating blood. Direct proof of such a hormonal relationship has thus far not been established, although a number of observers in the past few years have demonstrated a possible platelet-inhibiting hormone in acetone extracts from the spleens of cases of idiopathic thrombocytopenic purpura.9-13 Recent experiments in our laboratory65 have demonstrated that the injection in dogs of homogenized saline extracts of fresh whole spleen from cases of idiopathic thrombocytopenic purpura is followed by an extreme reduction in the platelet count, the marrow simultaneously showing very large numbers of megakaryocytes.

The hypothesis originally advanced by Kaznelson7 that the spleen required removal because of its unusual thrombolytic effect is unconvincing, since historically the spleen shows little, if any, deviation from the normal. Frank16 attacked the thrombolytic hypothesis vigorously. How, he asked, can the sudden platelet increase take place unless a sufficient number of mother cells are already present in the marrow ready to take on platelet formation? Furthermore, Frank went on, actual thrombocytolysis in the extirpated spleen was never demonstrated, even by Kaznelson. The splenic morphology is often diverse in the presence of the thrombocytopenic condition. The more recent studies of Wiseman, Doan, and Wilson,64 utilizing the supravital technic and seeming to point to a phagocytic activity of the splenic clasmatocytes upon platelets, require confirmation.

Allergic states, in which there is a direct effect upon the megakaryocytes without splenic mediation, may be at the basis of some cases of thrombocytopenic purpura.66,67 By and large, however, idiopathic thrombocytopenic purpura must be considered as primarily a disease of the spleen with secondary effects upon the bone marrow megakaryocytes, thus resulting in a disorder of the blood. The disease may thus be considered a form of “hypersplenism” or “hypersplenemic thrombocytopenia,” and as such may be compared with the hypersplenism of “spleenic neutropenia.”68-70 The cause of the abnormal splenic activity and what initiates it still remain obscure. Were this obscurity to be lifted, it might be possible to modify the action of the spleen without actually having recourse to its removal.

E. The Diagnostic Value of the Bone Marrow Biopsy in Thrombocytopenic Purpura.— A reduction in platelets—thrombocytopenia—may be due to various mechanisms which may be classified as follows: (1) Actual disease of the entire bone marrow—aplasia, hypoplasia, infiltration by abnormal cells or tissues, fibrosis, liver extract deficiency, etc.; here the platelets are reduced, together with the granulocytes and the red cells, and pancytopenia is usually present. (2) Selective involvement of the megakaryocyte platelet mechanism by the action of chemicals, infections, and allergens. (3) The “hypersplenism” of many cases of splenomegaly: Gaucher’s
disease, Felty's syndrome, Boeck's sarcoid, cirrhosis of the liver, etc.; in these cases leukopenia and granulocytopenia are also common. (4) The abnormal 'hyper-splenism' of idiopathic thrombocytopenic purpura. The differential diagnosis between these conditions usually offers little difficulty, but occasionally one is hard put to it to discriminate definitely between an idiopathic case and one which is secondary or symptomatic of a more fundamental disorder. Since this may be important from the standpoint of splenectomy, the diagnostic value of the bone marrow biopsy must be considered.

In the first group, which may be called 'secondary thrombocytopenia,' the bone marrow shows either aplasia, hypoplasia, leukemic infiltration, sarcomatous invasion, or fibrotic replacement. The megakaryocytes are conspicuously diminished; in extreme cases, none of these cells is seen. The great reduction in these cells is the outstanding feature which differentiates the condition from the 'idiopathic' disease; the few remaining megakaryocytes are essentially normal in size, shape, appearance, and platelet production.

In the allergic type, and in infectious thrombocytopenias, the megakaryocytes are selectively involved, while the rest of the marrow is essentially normal. Schwartz has shown that bone marrow eosinophilia is a common finding in the allergic group, even in the absence of blood eosinophilia. In the toxic and infectious types, there may be a definite reduction in the megakaryocytes, with the granulocytes often showing marked toxic changes.

In the symptomatic thrombocytopenia of 'hypersplenism' associated with a large spleen, the megakaryocytes are numerous and platelet production appears to be normal. The low platelet count and the response in platelets following splenectomy must therefore be due either to an inhibition of delivery of platelets from the marrow to the blood by the unusually active enlarged spleen, or to unusual phagocytosis of platelets by that organ. Our histologic studies of the spleen indicate that the first of these explanations is probably the correct one.

In the idiopathic group there is usually an increase, often great, in the megakaryocytes, which, however, show greatly diminished platelet production. The ability to recognize this abnormality may be of prime importance in deciding whether splenectomy will be of value in a given case.

SUMMARY AND CONCLUSIONS

1. The megakaryocytes of the sternal bone marrow at biopsy were studied in 11 cases of idiopathic thrombocytopenic purpura and compared with those of 10 normal cases, 5 of thrombocytopenic purpura associated with various types of splenomegaly, and of a large group of miscellaneous hematologic conditions, including leukemia, associated with a reduction in platelets.

2. Megakaryocyte counts expressed in terms of a million nucleated red cells and differential counts of megakaryocytes were performed. The megakaryocytes were classified as megakaryoblasts, promegakaryocytes, and mature forms, and were further subdivided into those showing granularity, platelet production, degenerated forms, and mitoses.

3. In the normal cases, not more than 300 megakaryocytes per million nucleated red cells were present, and an average of 68.6 per cent showed platelet production.
4. In acute idiopathic thrombocytopenic purpura, although the platelets in the circulating blood were rare, megakaryocytes were increased, being present in a proportion of 366 to 743 per million nucleated red cells. Platelet production was, however, greatly diminished and found in only 8 to 19 per cent of all megakaryocytes. Following splenectomy, there was a striking increase in platelet production, which was now present in 69 to 85 per cent of all cells; the large masses of new platelets in the marrow were often very striking.

5. In chronic idiopathic thrombocytopenic purpura, the megakaryocytes were considerably increased over normal values, but showed great diminution in platelet production; following splenectomy, extreme degrees of platelet production from megakaryocytes took place.

6. In splenomegaly of nonleukemic origin (cirrhosis, splenic vein thrombosis, Gaucher’s disease, Felty’s syndrome), the megakaryocytes were somewhat increased, but platelet production was normal.

7. In aplastic anemia, lymphosarcoma, acute leukemia, and other diseases invading or destroying the bone marrow, the megakaryocytes were conspicuously reduced, the few remaining cells present being of normal morphology.

8. The origin of the blood platelets from megakaryocytes, certain regulatory mechanisms for platelet production and delivery, and the possible relationship of the spleen to these mechanisms are discussed.

9. The findings of increased megakaryocytes and greatly diminished platelet production in the marrow before splenectomy and the striking increase in platelet production after splenectomy indicate a definite pathogenetic relationship of the spleen to the disease. Idiopathic thrombocytopenic purpura is probably a form of hypersplenism (splenic thrombopenia) in which, through a possible hormonal mechanism, the megakaryocytes of the bone marrow are inhibited from normal platelet production and delivery.

10. The marrow findings in idiopathic thrombocytopenic purpura are sufficiently characteristic to be of diagnostic value in differentiating the disease from leukemia and other conditions associated with a low blood platelet count.

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MEGAKARYOCYTES IN THROMBOCYTOPENIC PURPURA


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Megakaryocytes in thrombocytopenic purpura

1. Mature megakaryocyte. Note granularity of cytoplasm, pseudopods, and the presence of platelets, which are situated chiefly at the periphery of the cell and in the pseudopods.

2. Megakaryoblast.

3. Mature megakaryocyte or intermediate form, from a case of chronic idiopathic thrombocytopenic purpura. There is well defined granularity of the cytoplasm, but no platelet formation.

4. Mature megakaryocyte from a case of chronic idiopathic thrombocytopenic purpura. There is almost complete lack of granularity and marked vacuolization and degeneration of the cytoplasm.

5. Intermediate form of megakaryocyte from an acute case of idiopathic thrombocytopenic purpura. Granule formation, without platelet development, is evident and there are some questionable nuclear bodies (asynchronism of development) in the cytoplasm.

6, 7. Lymphoid megakaryocytes from an acute case of idiopathic thrombocytopenic purpura. These are characterized by blue cytoplasmic, lack of granularity, and lack of platelet formation.

8. Promegakaryocyte from an acute case of idiopathic thrombocytopenic purpura; 24 hours after splenectomy. Nongranular platelet formation is present around the periphery of the cell, with a streamer containing a group of newly formed platelets.

9, 10. Intermediate form and mature megakaryocyte from an acute case of idiopathic thrombocytopenic purpura; 48 hours after splenectomy. There is a striking productivity of granular (functioning) platelets, seemingly with the entire cytoplasm almost ready to break up into platelets.

All illustrations drawn with the aid of a camera lucida at a magnification of 1200 times.
THE MEGAKARYOCYTES IN IDIOPATHIC THROMBOCYTOPENIC PURPURA, A FORM OF HYPERSPLENISM

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