EMATOLOGIC CHANGES following the subcutaneous injection of epinephrin were first described by Loeppe and Crouzon in 1903. Leukocytosis of varying degrees after parenteral administration of epinephrin has since been regularly observed by all investigators. Epinephrin-induced leukocytosis appears to occur in two distinct phases: (1) an early phase, due mainly to an increase of neutrophils and lymphocytes and only partly to an increase of monocytes and eosinophils; and (2) a late phase when neutrophilia dominates the picture in association with lymphopenia and eosinopenia. The earlier workers concentrated their observations on the early phase only and the existence of the late phase as a part of pituitary-adrenocortical activity has been recognized only during recent years. Reticulocytosis and thrombocytosis have also been reported following epinephrin. Erythrocytosis following injection has been regularly observed in experimental animals. In normal human subjects, however, while some workers have found erythrocytosis regularly, others consider that the erythrocytic response is inconstant and insignificant. Other significant events that regularly accompany the hematologic response are certain cardiovascular effects as manifested by an immediate rise in heart rate and blood pressure and a well defined contraction of the spleen.

Various theories have been advanced to explain the mechanism of this epinephrin induced pancytosis. Thus, Frey and his co-workers suggested that the leukocytosis and especially the lymphocytosis were due to mobilization of cells from the spleen. Benhamou contended that while the erythrocytosis and thrombocytosis were due to mobilization of cells from the spleen, the leukocytosis which persisted after splenectomy was due to some other mechanism. Other possible explanations suggested include altered hemodynamics leading to redistribution of the various cells; hemocrit concentration; stimulation and/or release of cells from the blood-forming tissues like the bone marrow, lymph nodes and spleen; and, finally, a humoral mechanism mediated through the pituitary-adrenocortical axis. Occasional workers have suggested that the epinephrin response might be the result of such biochemical changes as hyperglycemia, hyperkalemia or disturbed alkali reserve of blood.
ADRENALIN TEST APPLIED TO HEMATOLOGIC DISORDERS

Based on one or another of these theories, the response to epinephrin has been used as a diagnostic test in various hematologic disorders. Thus, Benda\textsuperscript{2} used the test as a measure of bone marrow function, since in aplastic anemia no rise of reticulocytes, granulocytes and thrombocytes was found, the response being mainly lympho-monocytic. Doan and Wright\textsuperscript{26} have considered the test of value in confirming the diagnosis of various hypersplenic syndromes with peripheral cytopenia. They believe the specific cytopenia in these respective syndromes is frequently due to sequestration phagocytosis of the formed elements of the blood in a pathologically hyperactive spleen. Epinephrin is stated to mobilize these sequestered cells into the peripheral circulation or via direct “arterio-venous shunt.” A temporary correction of the cytopenia may be produced when the bone marrow has been shown to be hyperplastic for the specifically deficient element or elements. The specificity of the test in panhematopenia has, however, been questioned.\textsuperscript{27} More recently, eosinopenia and lymphopenia during the later phase of epinephrin response have been used as a test of the functional integrity of the pituitary-adrenocortical system.\textsuperscript{21-23}

It may be stated in general that the early phase of the epinephrin response (observed between 5 to 45 minutes) characterized by transient pancytosis, is presumed to reflect the functional activity of the various blood forming organs. In the normal individual, at least in the late phase of the epinephrin reaction, the neutrophilia with concomitant lymphopenia and eosinopenia is thought to be a manifestation of the functional integrity of the pituitary-adrenocortical axis.

Because of the conflicting opinions as to the value of the epinephrin response, particularly in the hypersplenic and related syndromes, the present investigation was undertaken to evaluate the role of this test in the diagnosis of various hematologic disorders. Attention was directed only to the early phase of the response.

MATERIALS AND METHODS

The subjects studied in this series included 12 normal individuals and 63 patients with various hematologic disorders. Altogether 92 tests were performed on 75 subjects, 22 of them on splenectomized patients. The cases studied and their number are shown in table 1.

The epinephrin test was performed according to the technic standardized by Benda\textsuperscript{25} and by Doan and Wright.\textsuperscript{26} Under basal metabolic conditions, with the patient reclining, pulse, blood pressure and size of spleen were measured; after a steady level of pulse and blood pressure had been obtained, two control samples of capillary blood were taken for counts at intervals of 10 to 15 minutes. Depending upon the age, body weight and cardiovascular status of the subject, 0.5 to 1.0 ml. of 1:1000 solution of epinephrin chloride was injected subcutaneously. In very small children, 0.25 to 0.4 ml. was injected. After preliminary observations with the method, it was found that the optimum times for the collection of samples were 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after injection. Pulse, blood pressure and splenic size were followed throughout, particular care being taken to collect samples at the height of the systolic blood pressure and of splenic contraction. The borders of the spleen before epinephrin injection were outlined as accurately as possible with a skin marking pencil, the observations being repeated frequently during the experiment. Maximum rise of blood pressure and of splenic contraction usually occurred simultaneously. In the few instances in which leukocytosis persisted at the end of 120 minutes, another sample was taken at 180 minutes.

Erythrocyte and leukocyte counts were done according to standard technics using National Bureau of Standards certified pipets and counting chambers. Hemoglobin was estimated as alkaline hematin using the Cenco Photoelectric colorimeter. Platelet and
reticulocyte counts were done according to the method of Dameshek.28 In many instances, platelet counts were also performed by a direct method.29 Although platelet counts by our method are about twice as high as by the direct method, comparable results were always found. The values shown in the tables and figures are those obtained with Dameshek's method. Differential counts of the white blood cells were performed by counting at least 200 cells. An Arneth count on each sample of blood was performed according to the modification of Cooke and Ponder.29 Results were analyzed with reference to the absolute increase of the different cells.

The initial base-line values for the different cells varied widely from case to case and it became obvious that results expressed in terms of percentage would be fallacious. For example, although a rise of platelets from 10,000 to 20,000 represents a 100 per cent increase, this actually is not particularly significant, if the errors of the platelet counting methods are kept in mind. On the contrary, a rise in platelets from 600,000 to 900,000, although representing only a 50 per cent increase, has a far greater significance since there is an absolute increment of 300,000 per cu. mm.

In each instance, extensive clinical and laboratory studies including bone marrow examination were performed before the epinephrin test.

Splenic puncture was performed in 6 cases of splenic pancytopenia according to the technic recently described,30 with a view to study the possibility of splenic sequestration of a particular cellular element.

In 5 cases, epinephrin was injected directly into the splenic artery during operation for splenectomy and serial studies were made on splenic venous blood, before and after epinephrin. Details of the technic employed to obtained blood samples from the splenic artery and vein have been described recently.31

### Table 1

<table>
<thead>
<tr>
<th>Type of case</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (8 males and 4 females, including 2 children)</td>
<td>12</td>
</tr>
<tr>
<td>Hereditary spherocytosis (before splenectomy)</td>
<td>6</td>
</tr>
<tr>
<td>Hereditary spherocytosis (after splenectomy)</td>
<td>7</td>
</tr>
<tr>
<td>Acquired hemolytic anemia (before splenectomy)</td>
<td>2</td>
</tr>
<tr>
<td>(after splenectomy).</td>
<td>1</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura (before splenectomy)</td>
<td>10</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura (after splenectomy)</td>
<td>8</td>
</tr>
<tr>
<td>Splenic pancytopenia (before splenectomy)</td>
<td>2</td>
</tr>
<tr>
<td>(after splenectomy)</td>
<td></td>
</tr>
<tr>
<td>Aplastic anemia (before splenectomy)</td>
<td>9</td>
</tr>
<tr>
<td>(after splenectomy)</td>
<td>2</td>
</tr>
<tr>
<td>Leukosarcoma and leukemia</td>
<td>9</td>
</tr>
<tr>
<td>Leukemia (splenectomized)</td>
<td>1</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>2</td>
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<tr>
<td>Myelofibrosis with myeloid metaplasia</td>
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</tr>
<tr>
<td>Hodgkin's disease</td>
<td>3</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Gaucher's disease</td>
<td>1</td>
</tr>
<tr>
<td>Mediterranean anemia (after splenectomy)</td>
<td>1</td>
</tr>
<tr>
<td>Refractory &quot;adynamic&quot; anemia with normoblastic marrow</td>
<td>3</td>
</tr>
<tr>
<td>Splenic cyst (epidermal)</td>
<td>2</td>
</tr>
</tbody>
</table>

### Results

#### A. Normal Response

The age of the normal subjects varied from 4 to 60 years. All subjects complained of varying degrees of palpitation and sweating with a feeling of ap-
prehension and nervousness, the symptoms appearing usually within 2 to 5 minutes and lasting from 30 to 40 minutes. A rise in systolic blood pressure was noticed in each case, the rise varying from 10 to 60 mm. of mercury with a mean average rise of 26 mm. of mercury. The rise in diastolic blood pressure varied from 0 to 20 mm. with a mean average rise of 8 mm. The time of maximum rise of blood pressure varied from 5 to 40 minutes, with a mean average time of 17.6 minutes. The pulse rate rose from 10 to 40 beats per minute with a mean average rise of 15, the time of maximum rise of pulse rate varying from 3 to 35 minutes with a mean average of 19 minutes.

Red Cells

A variable degree of erythrocytosis was observed in 10 cases; in 2 cases no rise in red cell count was seen. The rise in red cell level varied from 100,000 to 830,000, representing a mean average rise of 6.6 per cent above the base-line level. The time of maximum rise varied from 4 to 75 minutes after injection with a mean average of 30 minutes. Fluctuation in hemoglobin levels ran parallel to the red cell count in all the cases.

Reticulocytes

A slight increase in reticulocytes was noted in all except 2 cases. This rise varied from 0.2 to 0.6 per cent (with a mean average of 0.25 per cent). The time of maximum rise varied from 20 to 40 minutes, with a mean average rise of 35 minutes.

Platelets

All cases showed a varying degree of rise in platelet level. Net increase varied from 130,820 to 417,040 with a mean average increase of 287,720 (a mean rise of 56 per cent above base line value). Highest values were obtained between 20 and 75 minutes after epinephrin injection, the mean average peak period being 38 minutes.

Total Leukocytes

A rise in the total leukocyte count was observed in each case. The net rise varied from 3,900 to 14,250 with a mean average rise of 6677 (a mean rise of 90 per cent above base-line level). The time of maximum rise varied widely from as short a period as 9 minutes to as long a period as 120 minutes. The mean average time of leukocytic peak was, however, 37 minutes.

Neutrophils

Varying degrees of neutrophilia were seen in all cases. The total net rise varied from 687 to 5790 with a mean average rise of 3641, representing a mean rise of 82.5 per cent above base-line counts. The time of maximum rise was extremely variable; in 2 cases it was within 10 minutes, in 6 cases it was between 20 to 32 minutes and in 4 cases it was between 69 to 120 minutes. The mean average time of neutrophilic peak was 46 minutes.
Lymphocytes

A rise in lymphocyte count was also a constant feature of the epinephrin response. The net rise in lymphocyte count varied from 719 to 7676, with a mean average increase of 3044 cells, representing a mean rise of 170 per cent above base-line level. A definite lymphocytopenia was seen in 2 cases after 90 minutes, the lymphocyte counts dropping 56 per cent and 59 per cent below base-line level.

Monocytes

Varying degrees of monocytosis were seen in all the cases. The net rise of monocytes varied from 74 to 947, with a mean average rise of 518, representing a mean rise of 130 per cent. The maximum rise of monocytes was seen during a period varying from 10 to 180 minutes, with a mean average peak period of 68 minutes.

Eosinophils

Varying degrees of eosinophilia were seen in all except 2 cases. The net rise in eosinophils varied from 45 to 382, with a mean average of 142, representing an average rise of 150 per cent above base-line counts. The maximum rise of eosinophils was observed between 9 to 120 minutes, the mean average time of maximum response being 32 minutes. In 3 cases distinct eosinopenia was seen after 90 minutes.

Arneth Count (Cooke and Ponder’s modification)

In only 1 case, occurring 148 minutes after epinephrin injection, did cells of group 1 and 2 show increases significant enough to cause a levodeviation of the weighted mean from 2.56 to 1.27. In all the cases, early neutrophilia and that occurring within 2 hours after epinephrin injection was due to an increase of mature cells similar to those already present in circulation during the base-line studies, and the pattern of the Arneth count did not show any significant change. We do not have sufficient data to consider the cytologic pattern in the late phase of epinephrin response.

The leukocytosis in the early phase was always due to increase of mature forms of neutrophils, lymphocytes, monocytes and eosinophils. Younger or immature forms did not appear in peripheral circulation after epinephrin.

Relation between Hemopoietic Response and Rise of Blood Pressure

In different cases there was no correlation between the degree of hemopoietic response and the degree of rise of blood pressure. In each case, however, the time of maximum cellular response usually ran parallel to the time of maximum rise of blood pressure.

There was no significant variation in the response with reference to sex and age. The response in females and children was similar to that obtained in adult males. Essential features of normal response are shown in tables 2, 3 and 4; a typical normal response is shown in figure 1.
TABLE 2.—Pattern of Erythrocytic Response to Epinephrin*

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Number of cases</th>
<th>Maximum rise observed</th>
<th>Time of maximum rise in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range of variation</td>
<td>Mean</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>0–830,000</td>
<td>315,000</td>
</tr>
<tr>
<td>Hereditary spherocytosis (before splenectomy)</td>
<td>6</td>
<td>0–500,000</td>
<td>328,000</td>
</tr>
<tr>
<td>Hereditary spherocytosis (after splenectomy)</td>
<td>7</td>
<td>0–1,000,000</td>
<td>327,000</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura (before splenectomy)</td>
<td>10</td>
<td>60,000–560,000</td>
<td>288,000</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura (after splenectomy)</td>
<td>8</td>
<td>0–850,000</td>
<td>323,000</td>
</tr>
<tr>
<td>Hypersplenic pancytopenia (before splenectomy)</td>
<td>8</td>
<td>300,000–790,000</td>
<td>467,000</td>
</tr>
<tr>
<td>Hypersplenic pancytopenia (after splenectomy)</td>
<td>2</td>
<td>390,000–550,000</td>
<td>470,000</td>
</tr>
<tr>
<td>Aplastic anemia (before splenectomy)</td>
<td>9</td>
<td>0–680,000</td>
<td>325,000</td>
</tr>
<tr>
<td>Aplastic anemia (after splenectomy)</td>
<td>2</td>
<td>140,000–500,000</td>
<td>320,000</td>
</tr>
</tbody>
</table>

* Hemoglobin values in all cases ran parallel to the rise in red cell level.

TABLE 3.—Pattern of Platelet Response to Epinephrin

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Number of cases</th>
<th>Maximum rise observed</th>
<th>Time of maximum rise in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range of variation</td>
<td>Mean</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>130,800–417,000</td>
<td>287,720</td>
</tr>
<tr>
<td>Hereditary spherocytosis (before splenectomy)</td>
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<td>176,300–563,800</td>
<td>318,458</td>
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<tr>
<td>Hereditary spherocytosis (after splenectomy)</td>
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<td>119,800–720,500</td>
<td>408,253</td>
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<tr>
<td>Idiopathic thrombocytopenic purpura (before splenectomy)</td>
<td>10</td>
<td>0–33,800</td>
<td>4,728</td>
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<tr>
<td>Idiopathic thrombocytopenic purpura (after splenectomy)</td>
<td>8</td>
<td>3,800–1,779,500</td>
<td>596,742</td>
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<tr>
<td>Hypersplenic pancytopenia (before splenectomy)</td>
<td>8</td>
<td>16,600–591,000</td>
<td>250,945</td>
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<tr>
<td>Hypersplenic pancytopenia (after splenectomy)</td>
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<td>251,300–285,000</td>
<td>268,150</td>
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<tr>
<td>Aplastic anemia</td>
<td>9</td>
<td>1,100–8,300</td>
<td>3,124</td>
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<tr>
<td>Aplastic anemia (after splenectomy)</td>
<td>2</td>
<td>4,960–9,900</td>
<td>7,430</td>
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### Table 4.—Pattern of Leukocytic Response to Epinephrin

<table>
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<tr>
<th>Subjects</th>
<th>Number of cases</th>
<th>Total WBC</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range of variation in maximum rise</td>
<td>Mean maximum rise</td>
<td>Time of maximum rise in minutes</td>
<td>Range of variation in maximum rise</td>
<td>Mean maximum rise</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>3,000-14,250</td>
<td>6,677</td>
<td>Mean maximum rise</td>
<td>37.1</td>
<td>687-5,790</td>
</tr>
<tr>
<td>Hereditary spherocytosis (before splenectomy)</td>
<td>6</td>
<td>2,100-9,950</td>
<td>845-4,443</td>
<td>10-90</td>
<td>262-6,272</td>
<td>10-90</td>
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<tr>
<td>Hereditary spherocytosis (after splenectomy)</td>
<td>7</td>
<td>3,400-9,900</td>
<td>154-5,367</td>
<td>10-60</td>
<td>1,105-11,13</td>
<td>30-82</td>
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<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>10</td>
<td>1,800-13,100</td>
<td>3,035</td>
<td>17.3</td>
<td>539-9,986</td>
<td>10-60</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenia (after splenectomy)</td>
<td>8</td>
<td>2,200-21,000</td>
<td>1,44-7,094</td>
<td>28-60</td>
<td>9,178</td>
<td>54</td>
</tr>
<tr>
<td>Hypersplenic pancytopenia (after splenectomy)</td>
<td>2</td>
<td>2,450-5,600</td>
<td>2,013-2,469</td>
<td>25-30</td>
<td>4,025</td>
<td>20</td>
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<tr>
<td>Aplastic anemia</td>
<td>9</td>
<td>1,550-5,650</td>
<td>3,610</td>
<td>5-60</td>
<td>44-1,442</td>
<td>5-60</td>
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<tr>
<td>Aplastic anemia (after splenectomy)</td>
<td>2</td>
<td>6,200-6,220</td>
<td>6,210</td>
<td>10-50</td>
<td>1,077-1,674</td>
<td>20-40</td>
</tr>
</tbody>
</table>

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B. Response in Various Hematologic Disorders

Hereditary Spherocytosis (Congenital Hemolytic Anemia)

Thirteen epinephrin tests were performed on 7 patients. In 6 cases the test was done before and after successful splenectomy. In the seventh case the test was done one year after splenectomy. Essential features of response in the 2 groups, before and after splenectomy, are given in tables 2 to 4. Responses in a case of hereditary spherocytosis before and after splenectomy are shown in figures 2a and b. The only significant difference between the two groups was a slightly higher peak of platelet level in the splenectomized group. The net rise of red cells, reticulocytes, total white cells, neutrophils, lymphocytes, monocytes and eosinophils was of similar magnitude in both groups. The response in the pre-splenectomy group was almost identical with that obtained in the normal group. After splenectomy the response was also essentially normal except for a slightly higher rise of thrombocyte level. All the patients had varying degrees of splenomegaly (3 to 7 cm. below the costal margin). Following epinephrin injection, a marked splenic contraction was observed in each case, the tip of the spleen becoming just barely palpable at the height of splenic contraction. The time of maximum contraction of spleen varied from 10 to 30 minutes, the mean average time being 20 minutes. The maximum rise of systolic blood pressure usually coincided with the time of maximum splenic contraction. At this time, too, the peak lymphocyte response usually occurred. Lymphocytopenia following epinephrin injection was observed between 90 to 120 minutes in 4 cases prior to splenectomy and in 1 case after splenectomy. Eosinopenia was seen in only 1 case before splenectomy in the 120 minute period.

Fig. 1.—Patient A. H. C., 22 years old, male. Normal epinephrin response.
Fig. 2a.—Patient S. S., 8 years old, female. Congenital hemolytic anemia, before splenectomy.

Fig. 2b.—Patient S. S., 8 years old, female. Congenital hemolytic anemia, after splenectomy.
Arnet count: no significant increase in the cells of group 1 and 2 was seen in any of the cases either before or after splenectomy. The weighted mean also showed no deviation from the base-line counts at any stage after epinephrin injection.

Neutrophilia and lymphocytosis, as also monocytosis and eosinophilia, were
always due to an increase of mature cells and leukocytosis was never characterized by the presence of younger forms.

**Acquired Hemolytic Anemia**

Epinephrin tests were done in 3 cases of acquired hemolytic anemia, 1 of which was splenectomized one and a half years previously. The response in the first case was essentially normal. In the other 2 cases, 1 of which was splenectomized, the response was also normal except for greater rise of total white cells and neutrophils. Marked splenic contraction was noted in both the cases.

**Idiopathic Thrombocytopenic Purpura**

Epinephrin tests were performed in 10 patients before splenectomy and in 8 patients after splenectomy. The range of variation and the time of maximum cellular responses in the two groups are shown in tables 2 to 4. Response in a case of idiopathic thrombocytopenic purpura, before and after splenectomy, is shown in figures 3a and b. Comparison of the data shows that the net rise of red cells, reticulocytes, lymphocytes, monocytes and eosinophils was similar in magnitude in both groups, conforming closely to the response seen in normal subjects. Total leukocytic and neutrophilic responses were, however, higher after splenectomy. The remarkable feature of the response in the nonsplenectomized group was the almost complete lack of rise in the platelet level; the mean maximum rise of thrombocytes in this group was only 4728, in sharp contrast to the net rise of 596,742 in the splenectomized group and 287,720 in the normal group. No significant change in Arneth count was seen in any of the cases and leukocytosis was always due to an increase of mature cells.

**Hypersplenic Pancytopenia**

Eight patients with either “primary” or “secondary” hypersplenism with splenomegaly were studied in this group. In 2 cases the test was repeated after successful splenectomy. The range of variation and the time of maximum cellular responses are shown in tables 2 to 4. Compared to the normal, the response in both groups, before and after splenectomy, was of lower magnitude with reference to reticulocytes and the different leukocytes. The response of the platelets was also lower than normal except 1 case (fig. 4) in which the maximum rise of thrombocytes was greater than normal. Erythrocytosis in this series was, however, slightly greater than normal but, contrary to expectation, there was no significant difference in the degree of erythrocytosis in the two groups before and after splenectomy. The only significant difference between the two groups was the greater rise of total leukocytes and neutrophils after splenectomy. All cases showed a moderate to marked splenic contraction. The time of maximum splenic contraction varied from 14 to 30 minutes, the mean time of maximum splenic contraction being 21.6 minutes. The mean time of lymphocytic peak corresponded with the time of maximum splenic contraction. Splenic puncture was performed in 5 cases and in no case was there any evidence of abnormal sequestration of neutrophils and thrombocytes. Bone marrow examination in all the cases showed hypercellularity. In some cases, “maturation arrest” was evidently present as judged by the large number of relatively early cells without
any apparent progression to mature forms. In other cases, the morphology of the marrow was entirely normal, except for the hyperplasia, suggesting that the blood cytopenia might be on the basis of “blocked delivery” of cells from the marrow to the blood. The response to epinephrin in the two groups was different.

In the cases with normal maturation an essentially normal response was obtained (fig. 4), whereas in the case with maturation arrest the response was remarkably poor (fig. 5a). However, in this case the response became normal after splenectomy, when the bone marrow presented a normal appearance (fig. 5b). The response in these different cases with pancytopenia could thus be correlated with the qualitative changes in the bone marrow. The granulocytic response in the cases with essentially normal marrow (blocked delivery?) was essentially normal, whereas in the cases with maturation arrest, the response was poor, comparable to that seen in cases with granulocytic hypoplasia (figs. 4, 5a, 6).

Hypoplastic and Aplastic Anemia

Nine patients were studied in this group. In 2 cases the test was repeated after splenectomy. Details of the response are shown in tables 2 to 4. In contrast to the normal response, the net rise of reticulocytes, thrombocytes, total leukocytes and neutrophils was remarkably poor, although a slight response in lymphocytes and red cells took place. The net rise of lymphocytes was somewhat less than normal, the mean average maximum rise being 2454, per cu. mm. in contrast to 3044 per cu. mm. in the normal group. The net rise of erythrocytes
**Fig. 5a.**—Patient S. W., 41 years old, male. Epinephrine response in splenic pancytopenia before splenectomy. Bone marrow was hypercellular with maturation arrest of granulocytes. Megakaryocytes were of diminished activity.

**Fig. 5b.**—Patient S. W., 41 years old, male. Splenic pancytopenia, after splenectomy. Bone marrow was hypercellular with normal granulopoiesis. Megakaryocytes were active.

before and after splenectomy was essentially of normal pattern. After splenectomy the response improved with reference to total white cells, lymphocytes and monocytes, but there was no change in the levels of reticulocytes, neutro-
phils and thrombocytes. The response in a typical case of aplastic anemia is shown in figure 6. The bone marrow in these cases always showed marked hypoplasia with acute paucity of cells not responding to epinephrin injection. The net rise in neutrophils and thrombocytes in this series was 741 and 3124 respectively, in contrast to 3641 and 287,720 in the normal series. Poor response of reticulocytes, neutrophils and thrombocytes after splenectomy could possibly be correlated with the inactivity of the marrow persisting after splenectomy.

**Leukemia and Leukosarcoma**

Nine patients with different types of leukocytic proliferative disorders were studied in this series. In 2 cases of chronic granulocytic leukemia with massive splenomegaly, very slight contraction of the spleen was seen. In both these cases there was a great increase of total white cells following epinephrin injection, the increase involving both immature and mature granulocytes. In one case the net rise of total granulocytes was 65,000 and in the other 29,450. The net rise of red cells was 160,000 and 450,000 and the net rise of thrombocytes was 104,000 and 35,000, respectively. There was no elevation of reticulocyte, lymphocyte, monocyte or eosinophil counts over the base-line levels.

In one case of lymphocytic leukemia the net rise of lymphocytes was 12,500, the variation in other cellular elements being essentially normal.

Three cases of lymphosarcoma with massive splenomegaly were studied. Two cases had peripheral leukopenia, with the marrow almost completely replaced by lymphocytic cells. The third case, with only partial infiltration of the marrow, had a normal total white cell count with lymphocytosis. The response in the first 2 cases was identical, being characterized by erythrocytosis, lymphocytosis (involving both mature and immature forms) and monocytosis, with
only a slight increase of neutrophils and thrombocytes. The third case, with partial infiltration of the marrow, on the other hand, had an essentially normal response except for the appearance of a few atypical lymphocytes. All the cases showed only slight splenic contraction, yet the erythrocytic response was greater than normal.

In a case of follicular lymphoblastoma of the spleen with normal blood and bone marrow picture the response was essentially normal. The splenic puncture in this case showed many immature cells with fine nuclear chromatin and nucleoli, which failed, however, to appear in the peripheral blood after epinephrin. The spleen in this case showed no significant contraction, although the normal response of pancytosis was seen.

In 3 cases of aleukemic (leukopenic) leukemia, 2 of which were granulocytic and 1 plasmocytic, epinephrin injection failed to mobilize any immature cells in the peripheral circulation. The response of the different cellular elements reflected closely the bone marrow changes. Thus in cases with extreme preponderance of myeloblasts, promyelocytes and myelocytes with diminished thrombopoiesis in the bone marrow, the neutrophilic response after epinephrin was poor. Mature neutrophils and thrombocytes were obviously not available in the system for mobilization in the peripheral circulation due to their greatly diminished production.

In one of the cases of aleukemic granulocytic leukemia where the blood and bone marrow picture were unaffected by splenectomy, the epinephrin test showed similar response before and after operation.

Polycythemia

In 2 cases of polycythemia, the net rise of all the cellular elements was greater than normal. There was marked splenic contraction in both cases. The response in one case is shown in figure 7.

Myelofibrosis with Myeloid Metaplasia

In 1 case with marked splenomegaly the response was essentially normal with a moderate degree of splenic contraction. The absolute numbers of neutrophilic myelocytes and erythroblasts, which were already present in the peripheral circulation during base-line studies, also increased. In another case with slight splenomegaly the general cellular response was poor, although the absolute numbers of erythroblasts increased after epinephrin.

The different responses in the 2 cases could probably be explained by the different degrees of extramedullary hematopoiesis present. In the first case, which showed splenomegaly, there was probably a larger amount of hemopoietic tissue present than in the second case which exhibited only slight enlargement of the spleen.

Miscellaneous Conditions

In a case with a large epidermal splenic cyst there was no visible or measurable contraction of the spleen following epinephrin. The cytologic response, was nevertheless, essentially normal. Blood and bone marrow pictures in this case were also normal.
In 3 cases of Hodgkin's disease and in 1 case of reticulum-cell sarcoma with splenomegaly and essentially normal bone marrow, the epinephrin response was also normal.

In 3 cases of refractory "adynamic" anemia with cellular normoblastic marrow the variation of cytologic response was within normal limits.

In a case of Mediterranean anemia, splenectomized two years previously, there was no rise in red cell count following epinephrin. Reticulocytes, thrombo-

Fig. 7.—Patient N. W., 45 years old, male. Epinephrin response in polycythemia vera
<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Splenic artery</th>
<th>Splenic vein before epinephrin</th>
<th>Splenic vein showing the maximum values after epinephrin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RBC in millions</td>
<td>Hematocrit %</td>
<td>Platelets X 10,000</td>
</tr>
<tr>
<td>1</td>
<td>S. S.</td>
<td>8 yrs.</td>
<td>F</td>
<td>Hereditary spherocytosis</td>
<td>12.2</td>
<td>3.8</td>
<td>39.5</td>
</tr>
<tr>
<td>2</td>
<td>S. E.</td>
<td>8 yrs.</td>
<td>M</td>
<td>Hereditary spherocytosis</td>
<td>11.3</td>
<td>3.5</td>
<td>32.0</td>
</tr>
<tr>
<td>3</td>
<td>M. D.</td>
<td>10 mos.</td>
<td>F</td>
<td>Hereditary spherocytosis</td>
<td>14.8</td>
<td>4.6</td>
<td>44.0</td>
</tr>
<tr>
<td>4</td>
<td>T. M.</td>
<td>2⅓ yrs.</td>
<td>M</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>13.3</td>
<td>4.91</td>
<td>46.8</td>
</tr>
<tr>
<td>5</td>
<td>S. R.</td>
<td>8 yrs.</td>
<td>M</td>
<td>Hypersplenic cytopenia (secondary to congenital stenosis of portal vein)</td>
<td>12.9</td>
<td>4.66</td>
<td>39.0</td>
</tr>
</tbody>
</table>
Epinephrin Response in Splenectomized Subjects

Response in 22 patients splenectomized for various disorders was essentially similar to or slightly greater than that shown by normal subjects. Details of response have already been described under different disorders and are also shown in tables 2 and 4. Comparison of the responses in 3 patients before and after splenectomy are shown in figures 2a and b, 3a and b, 5a and b.

C. Results of Injection of Epinephrin into Splenic Artery

Cytologic studies of splenic arterial and venous blood and the results of the injection of epinephrin into the splenic artery are shown in table 5. The differences in the values of splenic arterial and venous blood before epinephrin were hardly significant. Five to 10 minutes following injection of 0.2 to 0.7 ml. of epinephrin the splenic venous blood counts showed an increase but this was pan-cellular in type and not confined to the particular cell type deficient in the peripheral blood. For example, in cases of hereditary spherocytosis there was an increase, not only of the red cells, hemoglobin and hematocrit values but also of the platelets and leukocytes which showed normal values in the peripheral blood and were, therefore, hardly likely to be sequestered in the spleen. Similarly, in a case of idiopathic thrombocytopenic purpura, the post-epinephrin venous blood showed an increase not only of thrombocytes but also of red cells and white cells. Thus any possible splenic contribution to the epinephrin response was not selectively confined to any particular cell. The hematocrit value and all the various cellular counts in the splenic parenchymal blood were usually higher than splenic arterial and venous blood, irrespective of the nature and degree of peripheral cytopenia. This particular finding might indicate that the spleen is a reservoir of concentrated cellular elements, but not necessarily a “sequestrator” of any specific type. Disproportionately higher lymphocyte counts in the splenic parenchymal blood probably point to the splenic origin of these cells. Thus the higher counts in the splenic venous blood after epinephrin were obviously due to mobilization of these cells from the spleen.

D. Reactions to Epinephrin

Reactions were similar to those seen in normals. Patients with enlarged spleen sometimes complained of slight pain over the spleen and a few patients also complained of an uncomfortable feeling over the legs. Only one patient with chronic lymphocytic leukemia developed a few extrasystoles which disappeared within an hour.

Injection of epinephrin into the splenic artery was followed by immediate rise of systolic blood pressure, synchronous with sharp contraction of spleen and splenic artery.

Discussion

A. Analysis of Epinephrin Response

Normal Epinephrin Response

Leukocytosis and thrombocytosis were constant features of the normal epinephrin test. Erythrocytosis, though less constant, was also observed in most of
the cases. Regarding the sequence of cellular responses, the only significant finding was that the lymphocytic peak was usually the first to appear. Leukocytosis was due to an increase of the same type of mature cells already present in the circulation during base-line studies and immature forms of leukocytes were never observed after epinephrin.

**Positive Response**

The epinephrin test, in order to be diagnostic of abnormal "splenic sequestration" should elicit a response which must be greater than the expected normal response or which should, at least, restore to a normal level the existing peripheral pancytopenia. Our results in the various hematologic disorders have been interpreted with these criteria of a positive response in mind.

**Response in Various Hematologic Disorders**

In hereditary spherocytosis and acquired hemolytic anemia the response both before and after splenectomy was essentially similar, conforming to the normal pattern. In idiopathic thrombocytopenic purpura the response before splenectomy was characterized by the absolute and invariable lack of thrombocytic response. This finding suggests that platelets were not available anywhere in the system for mobilization in the peripheral circulation. The megakaryocytes in the bone marrow in all these cases were presumably inactive, their appearance suggesting that few platelets were being produced. The lack of thrombocyte production could thus be correlated with the lack of rise of platelet level with epinephrin. The pattern of platelet response in our series was different from that obtained by Doan and Doan and Wright who found an increase in platelet level after epinephrin and interpreted the response as evidence of mobilization of thrombocytes sequestered in the spleen. Other workers, however, have failed to confirm their findings, and our own observations strongly suggest that platelets were not available anywhere in the system for mobilization in the peripheral circulation. The lack of availability might be due either to the suppression of megakaryocytic activity or to the excessive destruction of platelets in the peripheral circulation. On the contrary, in all the cases successfully splenectomized the thrombocytic response was always either similar to or greater than the normal response, reflecting clearly in each instance both the increased activity of the megakaryocytes and the presence of available platelets within the general circulation or in the bone marrow for mobilization by epinephrin. In a case of idiopathic thrombocytopenic purpura not responding to splenectomy the thrombocytic response was poor, reflecting again the inactivity of the megakaryocytes and indicating that the fundamental cause of the disease had not been removed.

In the splenic pancytopenia group, neither the epinephrin test nor the splenic puncture and splenic imprint studies showed any evidence of abnormal sequestration of cells. The response to epinephrin, on the other hand, could be clearly correlated with the activity of the bone marrow at the moment. Neutropenia with maturation arrest showed a much poorer response than a similar case with normal maturation with blockage in the release or delivery of the cells. The response in cases with maturation arrest of the granulocytic elements was
similar to that shown in cases with hypoplasia of the granulocytic elements (figs. 5, 6). The epinephrin test thus could not differentiate between the neutropenia due to maturation arrest and that due to hypoplasia of the marrow.

Correlation of the marrow picture with the epinephrin response in the group of hypoplastic and aplastic anemias was clearer. In the group of cases of leukemia and leukosarcoma, there was also a close correlation between bone marrow activity and the epinephrin test. In polycythemia vera and in myelofibrosis with extramedullary hematopoiesis there was a similar correlation between the epinephrin test and the functional activity of the formative tissue. A normal bone marrow thus appears to be an essential prerequisite for a normal epinephrin response. A hypoplastic marrow, on the other hand, produces very little response with reference to neutrophils and thrombocytes simply because these cells are not being produced in the marrow and thus are presumably depleted in the various sinusoidal areas throughout the body. Cases of splenic pancytopenia with maturation arrest react functionally like an aplastic marrow with reference to epinephrin response. Aleukemic leukemia also reacts in a similar fashion with reference to neutrophils and thrombocytes, probably because the production and perhaps delivery of these cells is always seriously interfered with by the abnormal leukemic proliferation. Polycythemia vera results in an exaggerated response reflecting in all probability not only the myeloid hyperplasia commonly present, but the unusually high blood and splenic volume. The exaggerated response of neutrophils and lymphocytes in chronic granulocytic and chronic lymphocytic leukemia respectively is similarly explained by the marrow picture which always shows an increased number of these cells.

B. Mechanism of Epinephrin Response

Various possibilities may be considered as to the mechanism of the epinephrin response. In splenectomized individuals there is always an epinephrin response which is either similar to or slightly greater than that obtained in normals. This finding, which has been reported by previous workers, definitely excludes the possibility that the response is mediated through mobilization of cells solely from the spleen. Studies on blood samples from splenic arterial and venous blood and the results of injection of epinephrin into the splenic artery show that splenic contribution to the response is, in general, pancellular in type and not confined to any particular cell, being obviously independent of the nature and degree of peripheral cytopenia. Wright et al. stated that post-epinephrin samples of splenic venous blood showed a specific rise of the particular cell deficient in the peripheral blood as a result of splenic contraction mobilizing this specifically sequestered cell. Analysis of their data, however, shows that they also obtained a general pancellular response with epinephrin. To cite one example from their series, (case number 1 in table 1), a case of essential thrombocytopenic purpura, showed not only an increase in platelets but also granulocytes and hematocrit reading. The granulocytes rose from an initial base-line value of 6156 to 59,760 and the hematocrit from 41.0 to 51.0 during the time when the platelets rose from 48,120 to 167,900. Similarly, in case 16 of the same table, with hereditary spherocytosis, there was at least a threefold increase of granulocytes and thrombocytes. These observations clearly show that in the hypersplenetic syndromes the response to
epinephrin indicates that the spleen does not specifically sequester any particular cell (providing of course that the epinephrin response indicates a splenic reaction).

Since different blood cells show an unequal rise following epinephrin, the possibility of hemoconcentration as a factor in the response appears to be excluded. No significant changes in blood volume were found in normal people and in individuals with splenomegaly following epinephrin injection. Bone marrow stimulation as a cause of the post-epinephrin pancytosis is hardly likely since immature granulocytes or nucleated red cells do not as a rule appear in the peripheral circulation.

As regards a possible hormonal mechanism mediated through the pituitary-adrenocortical system, this is probably responsible only for the late phase of the reaction. ACTH does not produce the transitory pancytosis seen in the early phase of the epinephrin test, with which we are concerned in this paper. It is possible that the neutrophilia with concomitant lymphopenia and eosinopenia seen in some of our cases at 90 to 120 minutes might have been due to an early ACTH effect.

The exclusion of these possibilities leaves for consideration the theory of redistribution of the various cells brought about by altered hemodynamics to explain the early phases of the epinephrin response. Increased cardiac and general vascular activity leading to dilatation of quiescent capillaries with liberation of trapped leukocytes, re-entry into the blood stream of cells adherent to vessel walls, vasoconstriction of the deeper vessels and sinusoidal organs like liver, kidney, lungs and spleen and lymph nodes are all possible factors in producing the cellular changes noted in the epinephrin response. Epinephrin has also been shown to contract lymph nodes, thus leading to an increase in thoracic lymph flow. It is conceivable that mature cells ready to be released from the bone marrow may likewise be mobilized into the peripheral circulation. Whatever might be the source of new cells appearing in the peripheral circulation and giving rise to the characteristic epinephrin response, the essential prerequisite for the response appears to be a properly functioning bone marrow and lymphatic tissue capable of producing the different cells in the normal fashion. Thus, the epinephrin response in the various hypersplenic syndromes is probably a reflection of the functional status of the bone marrow, the splenic contribution to this response being at most a minor one.

C. Mechanism of Splenic Contraction

Marked splenic contraction was seen in all cases of hemolytic anemia, splenic pancytopenia, polycythemia vera and myelofibrosis with myeloid metaplasia, whereas in a case of splenic cyst and one of follicular lymphoblastoma of the spleen the contraction was insignificant. In cases of leukemia the contraction was quite variable. There was no significant correlation between the degree of hemopoietic response and the degree of splenic contraction. The maximum degree of contraction, however, always synchronized with the maximum rise of blood pressure. While splenic contraction is said to be produced by the contraction of the smooth muscle fibers in the capsule and trabeculae of the spleen, it is unlikely that the sharp and vigorous contraction of the spleen following epinephrin
ADRENALIN TEST APPLIED TO HEMATOLOGIC DISORDERS

is brought about by the contraction of the very thin muscle fiber of the human spleen. Histologic examination of the enlarged spleens showing sharp splenic contraction with epinephrin also failed to show any increase of muscle fibers either in the capsule or in the trabeculae of the spleen. Following epinephrin injection into the splenic artery there was always a simultaneous contraction of the spleen and the splenic artery synchronous with the rise of blood pressure. In fact, the contraction of the splenic artery was so sharp and vigorous that it often precluded any subsequent blood samples being taken from the artery. It has been shown that the property common to all irritants capable of causing splenic contraction is their ability to provoke a rise of blood pressure. These various facts lead us to believe that the observed splenic contraction might be due to a marked constriction of the splenic artery and its intrasplenic branches, with a resultant passive deflation of the organ. The preponderance of elastic tissue in the capsule and the trabeculae of the spleen is admirably suited for this passive deflation. The fact that following epinephrin injection there is always a decreased blood volume in the essentially non-muscular organs like lungs and kidneys is in line with our idea of the mechanism of splenic contraction. Thus, epinephrin contraction following epinephrin injection probably represents only one part of altered hemodynamics. The essentially normal response in splenectomized subjects suggests that the role of the spleen, if any, must be a minor one in the elicitation of the epinephrin response.

D. Value of Epinephrin Test

In all probability a significant epinephrin response with reference to any particular cell indicates its optimum production by the formative tissue involved and its availability in the system for mobilization into the peripheral circulation. A poor epinephrin response, on the other hand, may denote a lack of availability of the cell in question either because of deficient production or of peripheral destruction. It seems quite certain that the nature of the deficient production (whether due to aplasia, leukemic infiltration or maturation arrest) cannot be determined by this test. Thus the epinephrin response reflects either the functional status of the blood forming organs or the degree of the available reserve cellular forces within the body. A positive epinephrin test in hypersplenic pancytopenia may indicate only that the various cells are being normally produced in the bone marrow and are thus available to the peripheral circulation, although the possibility cannot be excluded that splenic contraction may contribute some cells to the circulation in an occasional case. A negative epinephrin test does not, however, rule out the diagnosis of hypersplenic pancytopenia since this may be due, not to diminished delivery, but to maturation arrest of marrow elements, as a result of exaggerated splenic inhibition of the marrow. Further, the epinephrin response is not diagnostic of aplastic anemia. Thus, in our hands, the test has provided only indirect corroborative evidence regarding the functional status of the blood forming tissues. We believe, therefore, that the test per se is not diagnostic of any specific disorder and that the results of the test should always be interpreted in the light of more direct information gained by examination of the bone marrow and other studies.
SUMMARY

1. The epinephrin test was performed in 12 normal subjects and 63 patients with various hematologic disorders to evaluate its role in the diagnosis of hypersplenic syndromes and aplastic anemias. Attention was directed solely to the early phases of the reaction, that is to the pre-adrenocortical stimulatory effect, which has been claimed to reflect the functional activity of the spleen and bone marrow.

2. The epinephrin response in normal subjects was characterized by transitory pancytosis. Leukocytosis and thrombocytosis were conspicuous features whereas erythrocytosis and reticulocytosis were not regularly seen. Leukocytosis was due to an increase of mature forms of neutrophils, lymphocytes, monocytes and eosinophils. The Arment count did not show any significant variation after epinephrin. There was no correlation in different cases between the degree of hemopoietic response and the degree of rise of blood pressure, although in each case the time of maximum cellular response usually ran parallel to the time of maximum rise of blood pressure.

3. The response in splenectomized individuals was essentially similar to or slightly greater than that seen in normal subjects. In splenomegalic conditions there was no correlation between the degree of splenic contraction and the degree of hemopoietic response.

4. Results of epinephrin injection into the splenic artery showed that the increase of cellular elements was in general pancellular in type, and not confined to any particular cell deficient in the peripheral blood; it was obviously independent of the nature and degree of peripheral cytopenia.

5. The splenic contraction following epinephrin may possibly be due to a marked constriction of the splenic artery and its intrasplenic branches with the resultant passive deflation of the organ.

6. In each case there was a close correlation between the epinephrin response and the functional activity of the bone marrow.

7. The epinephrin test per se is not diagnostic of any clinical condition. In the hypersplenic syndromes interpretation of negative tests may actually be misleading. At most, the test provides indirect corroborative evidence regarding the functional status of the blood forming tissues and to some extent of the "reservoir" of blood cells present not only in the bone marrow, but in various tissues throughout the body. The exact degree of splenic participation in the epinephrin response is not known, but our studies indicate that it is minimal.

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* Only the more comprehensive and significant articles are included.
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The Adrenalin (Epinephrin) Test as Applied to Hematologic Disorders

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