The Adrenal Cortex and Hemolysis
III. Peripheral Blood Cell Reactions

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In the course of studying the effect of the adrenal cortex and its hormones on hemolysis, a uniform pattern of leukocyte response was noted.12, 13 The reaction consisted of marked leukocytosis, neutrophilia and eosinophilia throughout the period of red cell destruction. A search of the medical literature on this aspect of hemolysis revealed a few isolated and brief remarks that various types of hemolytic anemia were accompanied by leukocytosis.2, 21, 28, 35

The present investigation was initiated to study more thoroughly the pattern of leukocyte response during hemolysis. To elucidate the mechanism of this response the peripheral blood reactions were also observed in animals with hemorrhagic anemia, in adrenalectomized and ACTH-treated rats, and in animals subjected to "stressing" procedures.

Materials and Methods

184 male rats of an inbred strain, ranging in weight from 140-240 Gm., were used. Total white cells and eosinophils in tail vein blood were counted with certified white cell pipets and certified counting chambers. Both sides of the chamber were counted and results agreed to within 10 per cent; if not, counts were repeated. A baseline level was determined just prior to the beginning of the experiment. Counts were made 4 to 6 hours after each experimental manipulation and every day for 5 days at the same time of the day. In groups receiving ACTH a baseline level was determined just before administration of the hormone and 24 hours later, prior to the injection of the hemolytic agents. Smears for differential counting were taken in almost all instances at the time the white cell and eosinophil determinations were made. Red cell and reticulocyte levels were recorded throughout the experiments.

The animals were grouped as described below:

1. Hemolytic Anemia

A) Phenylhydrazine, prepared as a 1 per cent solution in saline, was injected once into the tail vein in a dosage of 4 mg. per 100 Gm. of body weight. This quantity of phenylhydrazine caused a drop in red cells of approximately 30 per cent and was not lethal for adrenalectomized rats.

B) Immune Serum (anti-rat-red-cell rabbit serum) was administered once intravenously in a dosage of 0.3 ml. This quantity of immune serum caused an average decrease of 55 per cent in the red cell level. It had a hemolytic titre of 500 and an agglutinating titre of 5000.

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C) Tween 80 was injected into the tail vein in a dosage of 0.6 ml. per 100 Gm. of body weight. The Tween was prepared as a 20 per cent solution in saline. In this dosage the red cell level fell approximately 30 per cent.

2. Hemorrhagic Anemia

Hemorrhagic anemia was produced by cardiac puncture under light ether anesthesia. A quantity of blood was removed equivalent to 2 per cent of the animal's body weight and resulted in a red cell fall of 28 per cent.

3. Endocrine Manipulations

A) Adrenalectomized rats, 2 to 4 weeks after operation, were given the three hemolytic agents in the same manner as described above. The immune serum employed was almost uniformly lethal. Consequently an immune serum was used with a hemolytic titre of 128 and an agglutinating titre of 1280 or approximately one-fourth the strength of the immune serum given to intact rats. With this serum, of a group of 20 adrenalectomized animals only 5 survived 3 days after intravenous injection, 4 survived four days and 2, five days. These surviving animals were all quite ill during the period of observation. The Tween preparation in the dosages employed for intact animals was uniformly fatal for adrenalectomized animals and consequently the dose was halved. With the latter dose, 8 of 14 rats survived for the 5 day period of the experiment. Seven of these showed marked edema of the face and extremities which developed within a few hours after administration of the Tween and persisted for two to three days.

B) ACTH was given to intact rats receiving the three hemolytic agents; 0.4 mg. of ACTH, prepared as a 0.1 per cent solution in saline, was injected intraperitoneally four times daily for a total daily dose of 1.6 mg. It was administered for one day prior to the injection of the hemolytic agents and for 5 days thereafter.

4. "Stressing" and Control Procedures

Several groups of animals were subjected to "stressing" procedures or were studied as controls for the above animals.

A) Formaldehyde. 0.25 ml. of 4 per cent formaldehyde was injected into the tail vein.

B) Cardiac Puncture without Bleeding. Rats were lightly etherized and their hearts punctured but blood was not removed.

C) Hemoglobin. Rat blood, removed by cardiac puncture, was hemolyzed in water, filtered and injected into the tail vein. The quantity of hemoglobin was estimated at approximately 50 mg. per rat.

D) ACTH. ACTH was administered to intact rats in the same manner as described above.

E) Saline. Physiological saline was injected intravenously in doses of 0.5 to 1.0 ml.

F) Untreated Controls. A group of 23 rats without any treatment were used for the determination of variations in white blood cell and eosinophil levels from day to day. Counts were made at the same intervals as in the above groups of rats.

RESULTS

1. Total Leukocytes

The intravenous injection of the three hemolytic agents into intact rats caused an immediate (4–6 hours) and prolonged (3–4 days) significant leukocytosis. The absolute figures are presented in table 1. In figure 1 the percentage change is recorded. A decrease in red cells due to all hemolytic agents was noted within 4–6 hours and continued gradually to the lowest level at 3–4 days.

Hemorrhagic anemia caused an immediate slight and non-significant leukocytosis 4–6 hours after bleeding. The white cell level was not significantly different from the baseline level for the succeeding five days (table 1 and fig. 1).
TABLE 1.—Absolute Leukocyte Counts and Standard Error

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Baseline 10⁶</th>
<th>6 hours 10⁶</th>
<th>1 day 10⁶</th>
<th>2 days 10⁶</th>
<th>3 days 10⁶</th>
<th>4 days 10⁶</th>
<th>5 days 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylhydrazine</td>
<td>20</td>
<td>15.3 ± 4.83</td>
<td>5.85 ± 6.6</td>
<td>6.83 ± 8.7</td>
<td>4.85 ± 6.8</td>
<td>5.26 ± 6.0</td>
<td>20.0 ± 2.7</td>
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<tr>
<td>Immune serum</td>
<td>6</td>
<td>11.6 ± 1.1</td>
<td>21.9 ± 17.0</td>
<td>21.0 ± 1.8</td>
<td>2.0 ± 1.6</td>
<td>6.9 ± 9.0</td>
<td>16.3 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>18</td>
<td>10.1 ± 4.2</td>
<td>11.1 ± 17.6</td>
<td>21.0 ± 1.8</td>
<td>2.4 ± 1.4</td>
<td>16.4 ± 1.0</td>
<td>10.6 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Cardiac hemorrhage</td>
<td>10</td>
<td>10.2 ± 0.2</td>
<td>20.7 ± 8.6</td>
<td>11.7 ± 8.6</td>
<td>9.9 ± 6.8</td>
<td>9.1 ± 1.6</td>
<td>1.5 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Adrenalin-hydrazine</td>
<td>14</td>
<td>18.3 ± 2.2</td>
<td>10.6 ± 4.5</td>
<td>4.3 ± 11.7</td>
<td>4.8 ± 0.2</td>
<td>6.0 ± 9.4</td>
<td>4.0 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>Adrenalin-immune serum</td>
<td>5</td>
<td>19.6 ± 2.3</td>
<td>22.9 ± 23.2</td>
<td>2.5 ± 21.7</td>
<td>2.0 ± 26.4</td>
<td>5.9 ± 29.5</td>
<td>0.3 ± 1.3</td>
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</tr>
<tr>
<td>Adrenalin-Tween</td>
<td>7</td>
<td>16.0 ± 0.7</td>
<td>14.5 ± 2.4</td>
<td>2.4 ± 14.8</td>
<td>2.5 ± 14.7</td>
<td>2.1 ± 11.4</td>
<td>2.4 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>ACTH-hydrazine</td>
<td>14</td>
<td>11.3 ± 0.3</td>
<td>14.4 ± 4.5</td>
<td>3.8 ± 13.3</td>
<td>2.5 ± 11.4</td>
<td>3.4 ± 3.4</td>
<td>3.6 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>ACTH-immune serum</td>
<td>6</td>
<td>11.9 ± 0.9</td>
<td>15.1 ± 3.3</td>
<td>3.3 ± 14.0</td>
<td>4.3 ± 17.6</td>
<td>2.2 ± 3.2</td>
<td>2.4 ± 3.2</td>
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<tr>
<td>ACTH-Tween</td>
<td>10</td>
<td>11.8 ± 0.9</td>
<td>17.9 ± 4.0</td>
<td>4.0 ± 14.7</td>
<td>1.0 ± 13.0</td>
<td>1.4 ± 10.5</td>
<td>1.4 ± 10.5</td>
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</tr>
<tr>
<td>Cardiac puncture</td>
<td>10</td>
<td>8.7 ± 1.4</td>
<td>11.4 ± 1.5</td>
<td>8.5 ± 0.9</td>
<td>9.3 ± 0.9</td>
<td>8.9 ± 0.7</td>
<td>9.0 ± 0.3</td>
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<tr>
<td>Formaldehyde</td>
<td>14</td>
<td>8.4 ± 0.7</td>
<td>11.0 ± 2.0</td>
<td>7.1 ± 2.8</td>
<td>7.8 ± 1.4</td>
<td>1.0 ± 1.4</td>
<td>1.4 ± 1.4</td>
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</tr>
<tr>
<td>Hemoglobin</td>
<td>7</td>
<td>9.7 ± 0.7</td>
<td>10.9 ± 1.0</td>
<td>9.4 ± 0.9</td>
<td>9.5 ± 0.9</td>
<td>8.3 ± 1.0</td>
<td>1.0 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>4</td>
<td>12.8 ± 2.1</td>
<td>13.7 ± 2.1</td>
<td>9.5 ± 1.4</td>
<td>9.2 ± 0.7</td>
<td>9.7 ± 1.1</td>
<td>9.3 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>12</td>
<td>11.4 ± 0.5</td>
<td>14.2 ± 2.4</td>
<td>11.9 ± 1.6</td>
<td>1.2 ± 10.7</td>
<td>1.11 ± 13.6</td>
<td>1.2 ± 12.6</td>
<td>0.2 ± 1.4</td>
</tr>
<tr>
<td>Untreated</td>
<td>23</td>
<td>10.6 ± 0.2</td>
<td>11.11 ± 0.99</td>
<td>9.9 ± 0.9</td>
<td>9.1 ± 10.4</td>
<td>0.6 ± 10.4</td>
<td>1.11 ± 10.9</td>
<td></td>
</tr>
</tbody>
</table>

Italized figures are significantly different (P less than 0.05) from the baseline value of the same row.

![Graph](image)

**FIG. 1.**—Percentage change of leukocytes following the intravenous administration of phenylhydrazine, immune serum and Tween 80 and after cardiac hemorrhage.

In adrenalectomized rats, the leukocytosis was prolonged only when phenylhydrazine was used. The leukocyte level was significantly higher than the level observed in intact rats receiving phenylhydrazine with or without ACTH (table 1 and fig. 2). The red cell decrease in the adrenalectomized animals receiving phenylhydrazine was significantly greater than the red cell fall in the other two groups (49 per cent compared with 30 per cent and 10 per cent). When immune serum and Tween were administered to adrenalectomized rats, the leukocyte levels were not significantly different from the baseline levels after 6 hours. The absence of persistent leukocytosis in these animals may have been due to the
reduced doses of the hemolytic agents, to the lesser degree of anemia, or to their moribund state. In both series of animals, the maximum red cell fall was 20 per cent.

Significant leukocytosis persisted for one to two days in intact animals treated with ACTH and the hemolytic agents. Examination of table 1 reveals that the elevated white cell levels continued for three to four days, but apparently the administration of ACTH prevented significant increases.

In animals subjected to “stressing” or control procedures, there was observed an immediate slight leukocytosis, in most instances significantly different from the baseline levels. Thereafter white cell counts returned to baseline values for the duration of the experiment (table 1 and fig. 2).

2. Eosinophils

Eosinophilia was pronounced following the injection of the three hemolytic agents into intact rats and lasted three to five days (table 2). Cardiac hemorrhage also caused eosinophilia persisting for 2 days. The injection of phenylhydrazine or Tween, and cardiac bleeding caused a 4–6 hour eosinopenia; the administration of immune serum was followed immediately by eosinophilia (fig. 3).

A perusal of table 2 shows that eosinopenia was the immediate (4–6 hour) response to all injections or manipulations except in three instances. Immune serum caused an initial and continuing eosinophilia in rats with or without adrenals. In adrenalectomized rats, the immediate eosinopenia induced by phenylhydrazine and Tween was replaced by an eosinophilia. Thirdly, the intravenous injection of saline caused a transient slight eosinophilia six hours after its administration. All other “stressing” or control procedures resulted in immediate eosinopenia followed by a return to baseline levels within one day (table 2 and fig. 4).

It was observed, also, that the eosinophil levels in adrenalectomized rats receiv-
## Table 2.—Absolute Eosinophil Counts and Standard Error

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Baseline</th>
<th>6 hours</th>
<th>1 day</th>
<th>2 days</th>
<th>3 days</th>
<th>4 days</th>
<th>5 days</th>
</tr>
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<tr>
<td>Phenylhydrazine</td>
<td>20</td>
<td>183 ± 20</td>
<td>119 ± 38</td>
<td>494 ± 67</td>
<td>413 ± 87</td>
<td>694 ± 81</td>
<td>84 ± 64</td>
<td>17 ± 14</td>
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<tr>
<td>Immune serum</td>
<td>6</td>
<td>153 ± 45</td>
<td>305 ± 70</td>
<td>418 ± 101</td>
<td>319 ± 62</td>
<td>160 ± 32</td>
<td>32 ± 21</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Tween 80</td>
<td>18</td>
<td>120 ± 15</td>
<td>95 ± 21</td>
<td>85 ± 50</td>
<td>79 ± 45</td>
<td>84 ± 48</td>
<td>77 ± 38</td>
<td>22 ± 17</td>
</tr>
<tr>
<td>Cardiac hemorrhage</td>
<td>14</td>
<td>103 ± 17</td>
<td>54 ± 36</td>
<td>99 ± 64</td>
<td>99 ± 54</td>
<td>33 ± 13</td>
<td>30 ± 12</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>Adren-phenylhydrazine</td>
<td>14</td>
<td>402 ± 81</td>
<td>734 ± 150</td>
<td>183 ± 87</td>
<td>168 ± 108</td>
<td>820 ± 110</td>
<td>560 ± 254</td>
<td>663 ± 304</td>
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<td>Adren-immune serum</td>
<td>5</td>
<td>247 ± 44</td>
<td>379 ± 88</td>
<td>515 ± 190</td>
<td>688 ± 185</td>
<td>466 ± 202</td>
<td>499 ± 257</td>
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<tr>
<td>Adren-Tween 80</td>
<td>7</td>
<td>378 ± 68</td>
<td>544 ± 157</td>
<td>224 ± 33</td>
<td>330 ± 83</td>
<td>306 ± 103</td>
<td>420 ± 97</td>
<td>33 ± 87</td>
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<td>10</td>
<td>121 ± 18</td>
<td>48 ± 9</td>
<td>90 ± 60</td>
<td>91 ± 34</td>
<td>93 ± 165</td>
<td>49 ± 191</td>
<td>50 ± 121</td>
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<tr>
<td>ACTH-immune serum</td>
<td>6</td>
<td>125 ± 28</td>
<td>490 ± 100</td>
<td>814 ± 81</td>
<td>238 ± 56</td>
<td>56 ± 103</td>
<td>54 ± 167</td>
<td>36 ± 36</td>
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<td>ACTH-Tween 80</td>
<td>10</td>
<td>175 ± 18</td>
<td>148 ± 38</td>
<td>669 ± 111</td>
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<td>262 ± 62</td>
<td>62 ± 275</td>
<td>75 ± 173</td>
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<td>Cardiac puncture</td>
<td>10</td>
<td>165 ± 26</td>
<td>134 ± 31</td>
<td>172 ± 46</td>
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<td>174 ± 36</td>
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<td>46 ± 46</td>
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<td>Formaldehyde</td>
<td>14</td>
<td>233 ± 33</td>
<td>54 ± 48</td>
<td>178 ± 30</td>
<td>192 ± 49</td>
<td>162 ± 49</td>
<td>120 ± 56</td>
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<td>Hemoglobin</td>
<td>7</td>
<td>191 ± 32</td>
<td>109 ± 66</td>
<td>192 ± 42</td>
<td>127 ± 56</td>
<td>162 ± 41</td>
<td>86 ± 80</td>
<td>77 ± 140</td>
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<td>316 ± 31</td>
<td>129 ± 50</td>
<td>250 ± 74</td>
<td>143 ± 74</td>
<td>467 ± 148</td>
<td>341 ± 110</td>
<td>305 ± 109</td>
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<tr>
<td>Saline</td>
<td>12</td>
<td>283 ± 44</td>
<td>334 ± 55</td>
<td>218 ± 34</td>
<td>236 ± 30</td>
<td>219 ± 164</td>
<td>238 ± 21</td>
<td>21 ± 22</td>
</tr>
<tr>
<td>Untreated</td>
<td>23</td>
<td>230 ± 40</td>
<td>233 ± 45</td>
<td>243 ± 52</td>
<td>242 ± 30</td>
<td>33 ± 163</td>
<td>35 ± 136</td>
<td>64 ± 167</td>
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</table>

Italicized figures are significantly different (P less than 0.05) from the baseline value of the same row.

**Fig. 3.** Percentage change of eosinophils following the intravenous administration of phenylhydrazine, immune serum and after cardiac hemorrhage.

ing phenylhydrazine or immune serum were significantly higher than the levels in the corresponding groups of intact rats. Tween 80 in adrenalectomized animals caused a transient rise of eosinophils which was not significantly different from the baseline.
The eosinophil response of intact rats treated with ACTH and the three hemolytic agents was similar to the response observed in the corresponding groups which did not receive ACTH. The levels, however, tended to be lower and returned to baseline values sooner than in rats without ACTH (fig. 4).

3. Neutrophils and Lymphocytes

In general, the neutrophil levels in all groups of rats paralleled the total white cell changes (table 3). Neutrophilia was immediate in all the series. The absence of the adrenals and the use of ACTH did not influence this initial increase of neutrophils. In most instances of hemolysis, the neutrophilia tended to persist and often was significantly elevated.

The lymphocytic responses were variable (table 4). Phenylhydrazine and immune serum elicited a lymphocytosis in intact rats which lasted for four days. Tween and cardiac hemorrhage caused a slight lymphopenia which lasted for the period of observation. In adrenalectomized rats phenylhydrazine induced a lymphocytosis on top of the increased lymphocyte levels which appeared following adrenalectomy. Tween, on the other hand, caused a lymphopenia in rats without adrenals.

![Graph showing percentage change of eosinophils in adrenalectomized and ACTH-treated rats following phenylhydrazine hemolysis and after various "stressing" and control procedures.](image-url)
In animals receiving ACTH, phenylhydrazine and immune serum elicited an immediate significant lymphocytosis and Tween a significant lymphopenia. The continued administration of ACTH seemed to depress the lymphocyte levels (table 4).

Animals subjected to “stress” and control procedures showed no clear pattern of lymphocyte response. Only the administration of ACTH produced a significant lymphocytosis from the first to the fourth day.

4. Reticulocytes

Reticulocytosis was observed in all animals subjected to the hemolytic agents and cardiac hemorrhage. Elevation of peripheral reticulocytes began after two to three days and gradually increased up to the fifth day of observation, reaching a level between 15 per cent and 30 per cent. In general, the more severe the anemia, the higher the reticulocytosis. Extirpation of the adrenals and the adminis-
tration of ACTH did not affect the extent of reticulocyte response. In some rats, three to four days after the injection of saline, formaldehyde and hemoglobin a slight reticulocytosis up to 4 or 5 per cent was noted.

**DISCUSSION**

The peripheral white blood cell changes during hemolysis formed a fairly uniform pattern of response. Leukocytosis and neutrophilia were immediate and lasted up to four days; eosinophilia was marked and persistent after the first six hours; reticulocytosis appeared two to three days after the inception of hemolysis and increased rapidly for the remaining days of the experiment.

The observations suggested that the peripheral blood cell reactions were associated with the continuing destruction of red cells and that the products of cellular disintegration stimulated the bone marrow to provide myeloid and erythroid elements. This was concluded from several data. The rapid rise in leukocytes coincided with the period of rapid red cell destruction, and the elevated white cell levels gradually subsided as the erythrocyte fall slowly reached its zenith. The one factor common to the three dissimilar and unrelated hemolytic agents was hemolysis. Lastly, when anemia was produced by cardiac bleeding without red cell destruction or when non-hemolytic substances were administered, such as formaldehyde, saline, or hemoglobin, the peripheral white cell changes were transient and characteristic of "stress" reactions. Steinberg and Martin have suggested that the white blood cell alterations following treatment with various materials may be due to destruction of leukocytes, the products of which are the hemopoietic stimulant. It was possible that the hemolytic agents employed in the present experiments injured white cells as well as erythrocytes.

Experiments most closely related to the present ones were done by Young, Ervin and Yuile. They noted a leukocytosis, following a brief leukopenia, which persisted for three days following hemolysis due to incompatible whole blood transfusion in dogs. The donor red cells were destroyed within 90 minutes but destruction of the recipient's red cells continued for a number of days due to the presence of the incompatible plasma. Hueper has described marked leukocytosis in dogs following the injection of macromolecular substances. Shortly after a single intravenous injection of glycogen, there appeared a significant leukopenia followed by a marked leukocytosis which lasted up to 24 hours. A significant decrease in red cell level also occurred in the first 24 hours. Repeated injections of glycogen induced a continuous leukocytosis and also an anemia. Prior to Hueper's investigation, Elvidge, in a series of papers, described a marked and prolonged leukocytosis after a transient leukopenia following the intravenous injection of India ink, quartz particles, carmine and Trypan Blue. All of these substances caused a "blood crisis" when given once and produced anemia when injected repeatedly. Furr et al. administered to animals clostridium welchii lecithinase which produced profound hemolysis. They recorded the appearance of marked leukocytosis within 24 hours. Observations on the peripheral blood beyond this time were not reported. A few isolated clinical case reports have also been found in which rapid red cell destruction was accompanied by leukocytosis. Unfortunately, in many cases of hemolytic anemia, the data were incomplete, the time sequences were not carefully recorded, or unknown and...
variable factors have complicated the hematologic picture of the peripheral blood. In animal experiments designed to study the effect of various agents on the peripheral white cell reactions, the period of observation has often been limited to the first few hours and red cell levels have not been reported.\textsuperscript{17, 20, 21, 23, 25, 32}

Hemoglobin did not seem to be the causative agent for the hemolytic leukocytic response. The injection of hemoglobin produced a reaction similar to that observed with all other nonspecific substances. In a small series of animals injected repeatedly with hemoglobin (not recorded here), the peripheral leukocyte response was the same as that found after a single injection of hemoglobin, i.e., the second, third and fourth injections did not elicit leukocytosis. In another series of rats, also not recorded here, hemolysed blood including its laked red cells, was administered to intact rats and caused a peripheral blood cell reaction characteristic of nonspecific reagents. It was understood, of course, that the exogenous administration of hemoglobin once daily was not the same as continuous hemolysis of red cells and liberation of hemoglobin within the animal.

The white cell response to hemolysis could not be attributed to hyperfunction of the adrenal. "Stressing" and control procedures, such as the injection of formaldehyde or hemoglobin or ACTH, all elicited a standard response: immediate leukocytosis, neutrophilia, eosinopenia, and a return to baseline values within twenty-four hours. No matter what the manipulation was, this stereotyped response was transient and different from the persistent cell changes observed during hemolysis. These ephemeral peripheral white cell changes have been described many times as characteristic of adrenal cortex hyperfunction or "stress."\textsuperscript{4, 5, 6, 16, 26, 27, 29, 30, 31} It might be concluded that the red cell destruction, continuing for three to four days, produced by hemolytic agents, constituted a "stress" situation throughout this period. Consequently, the leukocytosis and neutrophilia might be regarded as a response to persistent "stress." Several observations in the present investigation militated against this concept.

First, the hemolytic leukocytic pattern observed in intact rats occurred in adrenalectomized animals when phenylhydrazine was used. Indeed, the total white cell and eosinophil levels were significantly higher than the levels in the corresponding intact groups. Such results eliminated the adrenal cortex as a factor in the production of the hemolytic peripheral blood cell pattern. Immune serum and Tween in adrenalectomized rats did not induce the leukocytic response seen in intact animals. As mentioned previously, this might have been due to the decreased dosage of the hemolytic agents, the lesser anemia or the moribund state of the animals. Second, there was no anatomical evidence that adrenal hyperfunction occurred, i.e., there was no increase in the weight of the adrenals nor significant decrease in the weight of the thymus in animals with anemia. Lastly, the repeated intraperitoneal injections of ACTH over a period of 6 days caused only an initial leukocytosis and eosinopenia with return to baseline values within 24 hours.

The adrenal cortex seemed to be concerned only with the early eosinopenia. In intact rats, there was a 4–6 hour eosinopenia following almost every type of manipulation; in adrenalectomized rats there was a 4–6 hour eosinophilia. In contrast, immune serum and saline caused a 6 hour eosinophilia in intact rats. Obviously, there were factors other than the adrenal which determined the eosinophil level during the first 4–6 hours after treatment and certainly beyond this period.\textsuperscript{11, 15}
The appearance of eosinophilia in the various series of experiments remained unexplained. During hemolysis the elevated eosinophil levels tended to parallel the rise in leukocytes; they were very high and lasted for three to four days. This could not be considered as a "rebound" phenomenon. It was regarded, rather, as an effect of total bone marrow response to red cell destruction, i.e., the production and release of both myeloid and erythroid cells.

The six hour eosinophilia following the intravenous administration of saline and immune serum to intact rats was also unexplained. As little as 0.5 ml. of physiological saline intravenously induced a rise in eosinophils, a phenomenon noted by Kass, Lundgren and Finland. In addition, saline caused a marked transient leukocytosis. It appeared that physiological salt solution could not be considered a completely innocuous substance. The procedure of intravenous injection, however, was not eliminated as a possible cause of these reactions in this instance. Immune serum, in intact rats, elicited an eosinophilia in contrast to the eosinopenia caused by the other hemolytic agents. This might be explained as the result of the injection of foreign protein, as described by Biggart, or of the administration of antibody. However, in experiments not presented here, lyophilized human plasma injected intraperitoneally produced a typical 6 hour eosinopenia. It could also be assumed that the immediate intravascular destruction of red cells might have liberated sufficient histamine to negate the eosinopenic effect of the adrenal cortex. Another possibility was that the foreign protein of the immune serum blocked the reticulo-endothelial system and thus permitted a rise in eosinophils, a hypothesis recently put forward by Esselier, Jeanneret and Morandi.

Total leukocyte and neutrophil patterns were not influenced by the presence or absence of the adrenals. Others have commented upon the lack of dependence between neutrophils and adrenal function. The lymphocyte responses were too variable to recognize any specific pattern of reaction. Only with the use of ACTH was there a persistent lymphopenia from the first to the fourth days of its administration, a response noted often by other investigators.

Summary

Hemolysis was induced in rats by the intravenous administration of phenylhydrazine, immune hemolytic serum and Tween 80. During the period of red cell destruction, which lasted for three to four days, there was a marked leukocytosis, neutrophilia and eosinophilia which also persisted for three to four days. With phenylhydrazine and Tween 80 there was an initial transitory eosinopenia; with immune serum there was an immediate eosinophilia.

In contrast, anemia produced by cardiac hemorrhage was accompanied by a transient slight leukocytosis and eosinopenia. Within one day the white cell level returned to baseline levels, but eosinophilia appeared and persisted for two days. The injections of formaldehyde, ACTH, and saline, and cardiac puncture without bleeding caused a slight ephemeral leukocytosis and eosinopenia.

The peripheral blood cell response observed during hemolysis occurred in the presence or absence of the adrenals, and with or without the administration of ACTH.
It was concluded that hemolysis elicits a distinct pattern of cellular response in the peripheral blood which is not mediated through the adrenal cortex.

**SUMMARIO IN INTERLINGUA**

Hemolyse esseva inducite in rattos per le administration intravenose de phenylhydrazina, de sero hemolytic immun, e de Tween 80°. Durante le periodo de destruction del erythrocytos (que durava inter tres e quatro dies) nos observava marcate leucocytosis, neutrophilia, e eosinophilia (que etiam persisteva pro tres o quatro dies). Con phenylhydrazina e Tween 80° il habeva inicialmente un eosinopenia transiente; con le sero hemolytic immun le eosinophilia se declarava immediatamente.

Del altere latere, anemia producita per hemorrhagia cardiac esseva accompaniata per leve e transiente leucocytosis e eosinopenia. Intra un die le nivello leucocytic retornava al stato pre-experimental, sed eosinophilia se declarava e persisteva pro duo dies. Le injection de formaldehydo, ACTH, o solution salin e le execution de non-sanguinose puncturas cardiac causava leve e ephemere leucocytosis e eosinopenia.

Le responsa de peripheric celulas sanguinee, que esseva observate durante le hemolose, occurreva in le absentia como in le presentia del adrenales; illo occurreva sin como con le administration de ACTH.

Nos conclude que hemolyse evoca un distincte complexo de responsas cellular in le sanguine peripheric e que isto non es mediate per le cortice adrenal.

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