OBSERVATIONS ON THE BLOOD CYTOLOGY IN EXPERIMENTAL SYPHILIS

I. THE PERIOD OF DISEASE ACTIVITY

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Among the outstanding features of the blood picture in experimental syphilis of the rabbit, Pearce (1) has reported the following: A slight increase in the total white count; unchanged or lowered lymphocyte values during the earlier phase of the disease, with increased values during the period of regression and healing of lesions; and a marked increase in the number of monocytes during the period of disease activity. These findings were presented in terms of percentage deviation from preinoculation mean values, and the results, which were considered from the standpoint of the clinical course of the disease, were charted with particular reference to the time in weeks after inoculation.

With these observations as a background, and in connection with our investigations of various aspects of host reactions to environmental influences, including disease conditions, the study of the blood of syphilitic rabbits was continued. Special attention was again paid to the cytological levels during the active phase of the disease as compared with preinoculation values, while additional comparisons were made with the findings in normal rabbits during the same period. Furthermore, observations were also made in the period of latency when all clinical manifestations of the disease have healed and the animal appears to be "normal." The results of this study are reported in this and the subsequent paper (2). In the latter paper (2), a comparison is made between the cytological findings in experimental and human syphilis (3).
Material and Methods

The rabbits employed were all purchased from dealers and were of the types commonly utilized in laboratories, that is, grays, browns, and Flemish crosses. At the beginning of the experiment, they averaged 4 to 6 months of age. Each animal was housed in an individual cage in a well ventilated room receiving sunlight. The diet consisted of hay, oats, commercial food pellets, and a free supply of water.

Weekly blood examinations of 20 normal rabbits were begun on October 2 and continued to November 13, 1930; on this date the 20 animals were compared as to weight, color, physical condition, and blood formula, and a balanced division was made into an experimental and a control group of 10 animals each. On November 14, 1930, the animals comprising the experimental group were inoculated in the right testicle with 0.3 cc. of a saline emulsion of an actively growing syphilitic lesion from an animal infected with the Nichols strain of Treponema pallidum. Weekly blood examinations of the two groups were continued to February 26, 1931, when the main experiment was terminated. Selected animals were, however, retained for other studies. Before the termination of the experiment, three of the inoculated animals were utilized for tissue studies (4) and one of the control group died of an intercurrent infection. These four animals were withdrawn from present consideration and the results, therefore, comprise observations on the blood cytology of an experimental group of seven and a control group of nine animals.

Each blood examination consisted of a total red and white cell count made with standardized pipettes and a differential count of 100 cells by the neutral red supravital technique. The Ringer-heparin method of Casey and Helmer (5) was employed for such platelet counts as were made. At the start of the experiment, hemoglobin estimations were made by the Newcomer method, but these are not reported here because a routine check of our hemoglobinometer revealed slight inaccuracies. All animals of the two groups were examined on the same day and the examinations were conducted on the same day of consecutive weeks.

For the purpose of analysis, two periods were considered: The preinoculation period from October 2 to November 13, 1930, during which there were seven counts on each animal, and the postinoculation period from November 14, 1930, to February 26, 1931, with 15 counts on each animal (Table I). The results are analyzed on the basis of all counts made on the experimental and control groups during these two periods. The mean values for all the blood elements of each group were determined for each period from all the counts on the particular group during the particular time interval. The following comparisons were made: (1) The mean values for 49 counts on the experimental group during the preinoculation period were compared with the mean values for 63 counts on the control group for the same period. (2) The mean values for the 49 counts on the experimental group during the preinoculation period were compared with the mean values for the 105 counts on the same group during the postinoculation period. (3) The mean
values for the 63 counts on the control group during the preinoculation period were compared with the means for the 135 counts on the same group during the postinoculation period. (4) A last comparison was made between the mean values in the postinoculation period for the 105 counts on the experimental group and the 135 counts on the control group. At the time these experiments were begun, platelet counting was not a routine part of our hematological procedure and platelet determinations were not made every week. We have, however, 19 platelet counts on the experimental group and 27 counts on the normal control group made on the same days of 3 consecutive weeks in the preinoculation period. These are compared in the same manner as the other blood elements with 42 determinations on the experimental group and 54 on the control group made on the same days and during the same 6 weeks of the postinoculation period.

The usual statistical procedures were employed for calculating the standard error of the mean and the standard error of the difference of the mean. For the purposes of this paper, a difference is considered significant when the probability of its occurrence by chance is less than 1 in 100 \( t = 2.5, \ P = 0.01 \).

**RESULTS**

The results are indicated by the data given in Tables II and III in which are presented the mean blood cell values obtained from all counts on the experimental and control animals during the preinoculation and postinoculation periods. Text-fig. 1 represents these values graphically.

A comparison of the mean values for the two groups in the preinoculation period revealed no significant differences. (Red blood cells: Difference = 180,000 ± 132,000; \( t = 1.4 \). Platelets: Difference = 0 ± 42,000. White blood cells: Difference = 307 ± 366; \( t = 0.8 \). Neutrophils: Difference = 9 ± 235; \( t = 0.04 \). Basophils: Difference = 66 ± 101; \( t = 0.7 \). Eosinophils: Difference = 28 ± 31;
### TABLE II

**Mean Blood Cell Values for All Counts on Experimental and Control Groups in the Preinoculation Period**

<table>
<thead>
<tr>
<th>Group</th>
<th>Red blood cells</th>
<th>Platelets</th>
<th>White blood cells</th>
<th>Neutrophils</th>
<th>Basophils</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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<tr>
<td></td>
<td>thousands</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>5,554 ±88</td>
<td>475 ±35</td>
<td>8,097 ±261</td>
<td>4,165 ±177</td>
<td>754 ±78</td>
<td>164 ±26</td>
<td>2,392 ±160</td>
<td>623 ±48</td>
</tr>
<tr>
<td>Control</td>
<td>5,374 ±99</td>
<td>475 ±24</td>
<td>8,404 ±257</td>
<td>4,156 ±152</td>
<td>820 ±64</td>
<td>136 ±17</td>
<td>2,643 ±137</td>
<td>649 ±40</td>
</tr>
</tbody>
</table>

### TABLE III

**Mean Blood Cell Values for All Counts on Experimental and Control Groups in the Postinoculation Period**

<table>
<thead>
<tr>
<th>Group</th>
<th>Red blood cells</th>
<th>Platelets</th>
<th>White blood cells</th>
<th>Neutrophils</th>
<th>Basophils</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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<tbody>
<tr>
<td></td>
<td>thousands</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>5,245 ±48</td>
<td>720 ±39</td>
<td>8,978 ±236</td>
<td>5,073 ±173</td>
<td>821 ±47</td>
<td>154 ±15</td>
<td>1,937 ±83</td>
<td>996 ±47</td>
</tr>
<tr>
<td>Control</td>
<td>5,085 ±43</td>
<td>612 ±18</td>
<td>7,483 ±148</td>
<td>3,447 ±126</td>
<td>601 ±28</td>
<td>107 ±9</td>
<td>2,854 ±81</td>
<td>465 ±25</td>
</tr>
</tbody>
</table>
The changes in the blood cytology after inoculation are evident by comparing the mean values for the experimental group in the postinoculation period with the mean findings for the same group in the preinoculation period. After inoculation, there was a significant increase in the total white cell count, the platelet count, and the absolute numbers of neutrophils and monocytes, but the total red cell count and the

**Text-Fig. 1.** The blood cytology in the active phase of experimental syphilis. Mean blood cell values for all counts on the experimental and control groups in the preinoculation and postinoculation periods.
absolute number of lymphocytes were significantly decreased. No significant changes in the absolute numbers of basophils or eosinophils were noted after inoculation. (Red cell count: Difference = 297,000 ± 102,000; t = 2.9, P = 0.01-. Platelets: Difference = 245,000 ± 52,000; t = 4.7, P = 0.01-. White cell count: Difference = 881 ± 351; t = 3.3, P = 0.01-. Neutrophils: Difference = 806 ± 247; t = 3.3, P = 0.01-. Basophils: Difference = 67 ± 92; t = 0.7. Eosinophils: Difference = 10 ± 30; t = 0.3. Lymphocytes: Difference = 456 ± 180; t = 2.5, P = 0.01-. Monocytes: Difference = 373 ± 68; t = 5.5, P = 0.01-.)

A similar comparison was made between the mean values for the control group in the preinoculation and postinoculation periods. In the postinoculation period as compared with the preinoculation period, there was a slight statistically insignificant rise in the absolute number of lymphocytes and a significant increase in the platelet value. All the other blood cell elements described a change to lower levels. The mean values for the red cell count, total white cell count, and absolute numbers of neutrophils, basophils, and monocytes were significantly lower than the preinoculation mean findings. The change in the number of eosinophils was not significant. (Red blood cells: Difference = 290,000 ± 108,000; t = 2.7, P = 0.01-. Platelets: Difference = 137,000 ± 30,000; t = 4.6, P = 0.01-. White blood cells: Difference = 922 ± 297; t = 3.1, P = 0.01-. Neutrophils: Difference = 709 ± 200; t = 3.5, P = 0.01-. Basophils: Difference = 219 ± 69; t = 3.2, P = 0.01-. Eosinophils: Difference = 29 ± 19; t = 1.5. Lymphocytes: Difference = 210 ± 160; t = 1.3. Monocytes: Difference = 184 ± 47; t = 3.9, P = 0.01-.)

A final comparison between the mean values for the various blood cells of the two groups in the postinoculation period revealed striking differences. The red cell count, total white cell count, platelet count, and the numbers of neutrophils, basophils, eosinophils, and monocytes of the experimental group were all significantly higher than the corresponding values for the control group, while the absolute number of lymphocytes were significantly lower. (Red blood cells: Difference = 160,000 ± 64,000; t = 2.5, P = 0.01-. Platelets: Difference = 108,000 ± 43,000; t = 2.5, P = 0.01. White blood cells: Difference = 1,495 ± 278; t = 5.4, P = 0.01-. Neutrophils: Difference
= 1,627 ± 214; t = 7.6, P = 0.01-. Basophils: Difference = 219 ± 55; t = 4.0, P = 0.01-. Eosinophils: Difference = 48 ± 17; t = 2.7, P = 0.01-. Lymphocytes: Difference = 917 ± 116; t = 7.9, P = 0.01-. Monocytes: Difference = 531 ± 53; t = 9.9, P = 0.01-.)

**DISCUSSION**

For the purpose of this analysis, the mean of all counts on the experimental group during the 3½ month period after inoculation was taken to represent the findings during the active phase of the disease. In the great majority of rabbits inoculated intratesticularly with such a strain of *Tr. pallidum* as the Nichols' strain, this period covers the time of active clinical manifestations of the infection; that is, the primary orchitis, critical edema, metastatic orchitis, and generalized lesions. No attempt was made to correlate the various clinical stages of active syphilitic infection and the blood cell values during these stages. This was done in the preliminary report of Pearce previously referred to, in which the findings were charted with particular reference to the time in weeks after inoculation and to the clinical course of the disease. A future publication will discuss this matter in greater detail. The trends of the various blood cells during the 3½ month period after inoculation may have described up and down swings, but the mean represents in general the several blood cell levels during the clinically active course of the disease since all the animals of the group presented some clinical evidence of infection at the end of the observation period.

The use of control animals counted at the same time as the experimental group served the purpose of indicating that the changes observed after inoculation may be ascribed to the disease rather than to spontaneous variations with time. Thus a change in the mean cell level of the experimental group after inoculation was considered to be significant when the following conditions were fulfilled: First, the mean value in the postinoculation period must be significantly different from the mean value in the preinoculation period, and second, the mean value in the postinoculation period must be significantly different from the mean value obtained in the control group in the postinoculation period, and furthermore, this difference must be in the same direction as observed in the first condition. These requirements were
fulfilled by the following blood components: Total white blood cells, platelets, neutrophils, lymphocytes, and monocytes. The fact that no significant differences were observed between the mean values for the experimental group and the control group in the preinoculation period indicates that the two groups were satisfactorily balanced with respect to their blood cytology before inoculation.

The differences observed in the control group of normal rabbits between the mean values for the preinoculation and postinoculation periods indicate the spontaneous variations which may occur in time. It will be noted that in the postinoculation period the values for red cells, total white cells, neutrophils, basophils, eosinophils, and monocytes were lower, and the values for lymphocytes and platelets were higher than the mean values for the preinoculation period. The preinoculation counts were made in the mid-fall of 1930 while the postinoculation period was essentially the winter of 1930-31. The changes in the cell values of the control group in the postinoculation period as compared with the preinoculation period coincide very closely with the seasonal trends of blood cells as observed in this laboratory over many years.

On the basis of the foregoing analysis, it is evident that during the 3½ month period of disease activity, the blood cytology in experimental syphilis is characterized by an increase in the total white cell count, the platelet count, the neutrophil and monocyte counts, and a decrease in the number of lymphocytes. These changes after inoculation gain in significance when compared with the findings in the controls, since with the exception of the blood platelets, the identical blood elements of control uninoculated animals were altered in the opposite direction. It will be recalled that three animals of the original experimental group were withdrawn from consideration because they were employed for tissue studies. The inclusion of these animals in the analysis would certainly have increased the significance of the changes after inoculation since they were selected for tissue studies on the basis of the marked activity of their syphilitic lesions and the striking alterations in their blood cytology.

The change in the red blood cell level after inoculation is not definite. The mean red blood cell values of the two groups in the preinoculation period showed no statistically significant difference. In the
postinoculation period the mean value for the inoculated group was significantly lower than the preinoculation mean value. In the same time interval, however, the mean value for the control group also was significantly lower than the control preinoculation level. Thus both experimental and control groups described identical changes in time, and consequently it is probable that this change may be interpreted as a result of adaptation to environmental conditions. It is quite possible, however, that the change in the red cell count after inoculation represents an actual decrease which lacks significance in the present analysis only because of the comparatively small statistical sample. On the same basis, significant changes in the eosinophils and basophils might be demonstrated in a larger sample.

With respect to the changes in the total white cells and the neutrophils, lymphocytes, and monocytes, these findings confirm the observations of Pearce. The tables published by Bessemans and Lambin (6) indicate that their syphilitic rabbits showed a higher relative number of neutrophils and monocytes and a lower relative number of lymphocytes than the values which they obtained in a study on the blood cytology of normal rabbits. The authors, however, did not draw attention to this aspect of their investigations.

Of particular interest is the marked increase in the number of circulating monocytes during the period of disease activity. Supravital studies have demonstrated the presence of large numbers of monocytes in tissue scrapings from actively developing syphilitic lesions (4). The increased numbers of monocytes in the peripheral blood and local lesions of syphilitic rabbits indicates that they are an important participant in the cellular reaction to Tr. pallidum. An occasional clasmocyte was seen in the peripheral blood, but this finding was too infrequent to be of significance in the present study.

SUMMARY

Weekly observations were made on the blood cytology of seven syphilitic and nine normal control rabbits. Each animal was examined seven times prior to and fifteen times after inoculation of the experimental group. Comparisons were made between the mean blood cell values obtained from all counts on the experimental and control groups in the preinoculation and postinoculation periods.
The mean blood cell formula of the syphilitic group for the 3½ month period after inoculation was significantly different from the preinoculation mean values observed in the same group in the following respects: higher total white cell count, platelet count, neutrophil count, and monocyte count, and lower lymphocyte count.

The mean blood cell formula of the syphilitic group for the 3½ month period after inoculation was significantly different from the mean blood cell formula of the normal control group in the same time interval in the following respects: higher total white cell count, platelet count, neutrophil and monocyte counts, and lower lymphocyte count.

From these results it was concluded that during the period of disease activity, the blood cytology of rabbits infected with *Tr. pallidum* is characterized by an increase in the total white cell count, the platelet, neutrophil, and monocyte counts, and a decrease in the lymphocyte count from normal values. These changes were statistically significant.

**BIBLIOGRAPHY**