Total Cell Counts of the Bone Marrow of Normal Albino Rats from 1 to 50 Weeks of Age

By William T. Burke and Charles Harris

HAVING DEMONSTRATED that profound changes occur in the cellular distribution of the bone marrow of the albino rat at different ages,1 it was decided to supplement these findings by the enumeration of the total counts of bone marrow cells per mg. of bone marrow in parallel groups of rats. After reviewing some of the literature in this field,27 a technic was devised which has proved to be reproducible.

TECHNIC

In each group, 10 albino rats of the Wistar strain bred at the Fels Research Institute were sacrificed with ether vapor. The left and right femurs of each animal were exposed as described in a previous study7 and a polyethylene tube of appropriate diameter (O. D. 1. 70 mm. or 1.57 mm.) was inserted as far as possible into the marrow cavity. The tube was clamped with a hemostat and withdrawn. The marrow sample was ejected into a previously weighed, 10 x 75 mm. test tube and the tube reweighed. As rapidly as possible, 2 cc. of 3.5% Vinisil (polyvinylpyrrolidone-Abbott), treated with an antifoam silicone preparation, was pipetted into the test tube. Having experimented with the more common bone marrow diluents, i.e., serum, normal saline, 0.1 N HCl and 0.88 M Sucrose, we decided to use 3.5% Vinisil solution primarily because with this diluent, clumped cells were better separated from each other and from the stroma. In order to maintain a suitable cell count when less than 5 mg. of marrow were removed, the Vinisil dilution was lowered to 1 cc. and in some instances, in rat pups, 0.5 cc. was used. The test tube was placed in the shaking apparatus for 2 minutes after which a sample of marrow-cell suspension was diluted 1:20 with 0.1 N HCl in a white cell pipette. After shaking in a pipette shaker for 2 minutes, a regular white cell count was performed in an Ao Bright-line counting chamber. Duplicate samples were taken from each test tube representing left and right femurs, respectively.

The shaking apparatus in which the first dilution was agitated was constructed using a Champion motor, Ohmite rheostat and stand (fig. 1). A cork sleeve was made to hold the test tube. The motor shaft was fitted with a rubber strap to which was attached a soft plastic tip which tapped the bottom of the test tube with each revolution of the motor shaft. The rheostat was wired in series and the resistance regulated to produce rapid but not too violent agitation. Table 1 illustrates the effect of shaking time on the total cell count. The exact agitation rate is unknown, but it is the same for each specimen. The 2-minute interval was chosen as the maximum interval that would produce minimal cell destruction and maximum dispersion of marrow cells. Smears of the cell suspension revealed that cell fragmentation was negligible in samples agitated for the one and two-minute intervals but was increased progressively after longer periods.

Each cell count is treated with the following formula:

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When the initial dilution with Vinisil is 2 cc., the multiplication factor is 2000. The counting chamber factors equal the correction for the number of squares counted at the pipette dilution. If 4 squares are counted and 1:20 dilution used, the counting chamber factor equals 50.
equivalent to about 5000 cells per mg. of bone marrow. In rat pups, however, when only 1 to 2 mg. are recovered and diluent is reduced to 1 cc. or occasionally 0.5 cc., the multiplication factor jumps to 25,000 or 50,000, reducing the precision of the technic accordingly. Another contribution to error might be small differences in homogeneity of the cell suspension.

Table 2 demonstrates that the standard deviations increase considerably at the 2-week and 6 to 8-week periods. This change may be accounted for by interanimal variations, since the rate of increasing marrow population between 1 and 4 weeks of age is 30,000 cells per mg. each day, whereas the rate of decreasing population between 6 and 10 weeks of age is about 20,000 cells per mg. a day. Significantly, the lowest standard deviation is achieved in animals 50 weeks of age when the cell population of the bone marrow is relatively constant.

Total marrow counts and differential marrow counts for the same age groups that we have previously described can now be related. Figure 2 shows that the marked increase in lymphocyte-like cells at 2 weeks of age more than compensates for the drop in nucleated red cells and, in fact, accounts for the increase in total marrow count.

At 2 to 4 weeks of age, the increase of normoblasts by 800,000 cells per
### Table 2.—Absolute Counts of Femoral Bone Marrow of Normal Rats from 1 to 50 Weeks of Age (cells/mg. x 1000)

<table>
<thead>
<tr>
<th>Age Weeks</th>
<th>cells/mg. Bone Marrow</th>
<th>Blasts</th>
<th>Promyelocytes</th>
<th>Myelocytes</th>
<th>Metamyelocytes</th>
<th>Mature Granulocytes</th>
<th>Total Granulocytes</th>
<th>Nucleated Red Blood Cells</th>
<th>Lymphocyte-Like Cells</th>
<th>Plasma Cells</th>
<th>Reticulum Cells</th>
<th>M:E Ratio</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>2060 ± 227</td>
<td>27 ± 13</td>
<td>132 ± 42</td>
<td>268 ± 61</td>
<td>402 ± 53</td>
<td>1,359 ± 116</td>
<td>222 ± 74</td>
<td>0</td>
<td>8 ± 6</td>
<td>0.29</td>
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<tr>
<td>2</td>
<td>2644 ± 244</td>
<td>55 ± 23</td>
<td>108 ± 31</td>
<td>222 ± 37</td>
<td>373 ± 65</td>
<td>1,007 ± 83</td>
<td>1,192 ± 117</td>
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<tr>
<td>4</td>
<td>2939 ± 205</td>
<td>29 ± 17</td>
<td>229 ± 73</td>
<td>400 ± 107</td>
<td>641 ± 131</td>
<td>1,822 ± 157</td>
<td>385 ± 93</td>
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<tr>
<td>6</td>
<td>3062 ± 368</td>
<td>25 ± 16</td>
<td>254 ± 68</td>
<td>478 ± 102</td>
<td>717 ± 124</td>
<td>1,761 ± 81</td>
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<td>8</td>
<td>2791 ± 338</td>
<td>19 ± 11</td>
<td>266 ± 17</td>
<td>628 ± 157</td>
<td>896 ± 154</td>
<td>1,398 ± 22</td>
<td>413 ± 140</td>
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<td>10</td>
<td>2463 ± 194</td>
<td>15 ± 0</td>
<td>249 ± 53</td>
<td>764 ± 190</td>
<td>1,010 ± 198</td>
<td>1,167 ± 163</td>
<td>207 ± 65</td>
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<td>50</td>
<td>2310 ± 158</td>
<td>18 ± 0</td>
<td>371 ± 74</td>
<td>673 ± 60</td>
<td>1,042 ± 72</td>
<td>1,039 ± 93</td>
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<td>30 ± 16</td>
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Each age group represents averages of 10 animals.
Differential values extrapolated from previous study.¹
mg. of marrow is exactly counterbalanced by a loss of 800,000 lymphocyte-like cells, the increase in total count caused mainly by increasing numbers of granulocytes. This supports our original supposition that the lymphocyte-like cells may turn into normoblasts.¹

The next significant change occurs between 6 and 10 weeks of age when the number of granulocytes double and a simultaneous but greater drop in the number of nucleated red cells occurs, forcing the total count down.

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**Bone Marrow Total Nucleated Cell Counts (50-week-old group)**

<table>
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<tr>
<th>No.</th>
<th>mg. of Marrow Recovered</th>
<th>Femur</th>
<th>Duplicate Counts per Femur (cells/mg. x 1000)</th>
<th>Average Counts for Left and Right Femurs (cells/mg. x 1000)</th>
<th>Average of Combined Femoral Marrow Counts (cells/mg. x 1000)</th>
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*Group Average: 2,310,000 ± 158,000 cells/mg. marrow*
Morphologic studies indicate that many of the new granulocytes are derived from lymphocyte-like cells, and this concept is supported by the decrease that occurs in the number of lymphocyte-like cells while the granulocyte count is rising.¹

In the method described above, the error, which may be as high as 20 per cent of the total count, can be traced mainly to the precision of the technic. Despite this, definite trends may be established when groups of 10 or more animals are studied. Although absolute values may not be achieved by this technic, we believe that the order of magnitude of the changes, as well as the direction they take, are properly indicated.

**SUMMARY**

A method is described by which the total nucleated cell count of femoral bone marrow of the rat can be estimated and cell population expressed in terms of differential counts. Normal values of total nucleated cell counts and the cellular distributions are given for seven age groups. These data indicate considerable change in bone marrow total cell population in rats one to 10 weeks of age.

**SUMMARIO IN INTERLINGUA**

Es describite un methodo que permitte le estimation del total numeration de cellulas nucleate in le medulla femoral de rattos e le expression del stato del population de cellulas in le forma de numerationes differential. Es citate le valores normal del total numeration de cellulas nucleate e del distribution cellular pro septe gruppus de etate. Le datos indica qu el occurre considerabile alteraciones in le total population cellular del medulla in rattos inter le prime e le septime septimana de lor vita.

**REFERENCES**

Total Cell Counts of the Bone Marrow of Normal Albino Rats from 1 to 50 Weeks of Age

WILLIAM T. BURKE and CHARLES HARRIS