The Intercellular Substances of Bone Marrow. II. Variations in Granulocytic and in Erythrocytic Hyperplasia

By ROBERT E. CARTER AND WILLIAM L. JACKSON

In a previous report, preliminary studies of the viscosity and the mucopolysaccharide content of the intercellular substances of normal rabbit bone marrow were presented. Separation of cellular and noncellular components of femoral marrow was accomplished by centrifugation after mechanical disruption of the marrow stroma. The viscosity of the noncellular portion of the marrow was found to decrease with increasing animal age, and chemical analysis confirmed the presence of mucopolysaccharides in the intercellular substances.

This report presents observations made on the intercellular substances in granulocytic and in erythrocytic marrow hyperplasia in rabbits. Previous studies of whole bone marrow in anemic rabbits have been made by Jastro-witz, by Huggins, McFayden and Wiege, by Krause, and by Dietz and Steinberg. Where comparable analytic procedures were used, these studies demonstrated a consistent decrease in marrow lipid content. The demonstration of an inverse relationship between the lipid and the water content of the marrow was consistent with increased cellularity. In one group of animals reported by Dietz and Steinberg, granulocytic marrow hyperplasia was noted during the initial period of acute anemia following acetylphenylhydrazine hemolysis. In animals receiving acetylphenylhydrazine for three weeks or longer and where a chronic state of anemia was maintained, erythrocytic hyperplasia as well as immaturity of the granulocytic cell series was demonstrated. Considerable difference in marrow lipid content was noted between these two groups of animals, but may have been related to differences in total cellularity of the marrow rather than to an inherent difference in marrow composition in granulocytic opposed to erythrocytic hyperplasia. A decrease in lipid content was noted in each instance but was more marked in the case of animals showing erythrocytic hyperplasia.

To our knowledge, granulocytic marrow hyperplasia has not been studied to the same extent as has the marrow from anemic animals. Marrow from human cases of granulocytic leukemia was analyzed by Dietz and Steinberg and showed decreased lipid content and increased water content, as did the marrow from anemic rabbits in earlier experiments.

Methods

Female New Zealand rabbits were used. All details of animal maintenance and sacrifice and all methods of marrow analysis were identical with the previous study carried out in...
our laboratory.1 Mucopolysaccharide extraction was done by the method of Drybye and
Kirk7 with modifications previously noted,1 and estimation of total mucopolysaccharide-
content was made by the turbidimetric method of Diferranti,8 using chondroitin sulfate
standards. In addition, hexosamine, uronic acid and neutral sugar measurements were
made on the purified mucopolysaccharide material, using the method of Boas9 for hexos-
amine determination, the method of Dische10,11 for uronic acid determination and the
method of Dubois12 for neutral sugar determinations. The results obtained appeared to
confirm the validity of the turbidimetric method in the present experiments. Analysis of
the exact composition of the polysaccharide material extracted is continuing. Improved
handling of samples for turbidimetric analysis, and the use of a Coleman spectrophotometer
instead of the previously employed Beckman instrument, resulted in a higher apparent
mucopolysaccharide content in the intercellular substances in control animals in the
present experiments compared with values previously reported.1 These higher values re-
sulted from decreasing the time between addition of the cetyltrimethyl ammonium bromide
and the measurement of optical density in the spectrophotometer as well as from more
precise temperature control throughout the entire reaction. The values for the total muco-
polysaccharide content of the noncellular marrow component given in this report represent
the improved methodology throughout and include re-analysis of previous samples where
appropriate. Total mucopolysaccharide values, estimated by the turbidimetric method,
and the uronic acid values are reported in this paper.

Granulocytic marrow hyperplasia was produced in two groups of animals, one group
whose ages ranged from 2 to 6 months, the second group whose ages ranged from 12 to 18
months. Five cc. of a mixture of equal parts of turpentine and oil was injected subcutaneous-
ly over the back. After a week, a second injection was made at the same site. An abscess
developed in the area after the first injection, and the animals were sacrificed an average
of 10 days later when the overlying skin began to break down.

Erythrocytic hyperplasia was produced in young (age two to six months) animals
only. Blood was withdrawn by cardiac puncture on successive days until between 40 to 70
ml. had been let over a four to seven day period. Animals were sacrificed at intervals after
the cessation of bleeding, shown in table 1. The hemoglobin, total white blood cell count,
reticulocyte count and differential count of the peripherally circulating leukocytes were
determined on the animals at the time of sacrifice.

Suitable control animals were sacrificed periodically throughout these studies. The older
group of turpentine injected animals were studied at the same time as the older group of
normal animals reported previously.1 The younger animals injected with turpentine or
subjected to bleeding were studied at a time considerably later than the group of young
normals previously reported and hence a second group of young controls was included
in the present experiments.

EXPERIMENTAL RESULTS

The changes observed in the marrow of rabbits bled by cardiac puncture
are presented in tables 1 and 2 and in figure 1. Five groups of animals were
studied, and details of the intervals of bleeding, the amount of blood re-

| Table 1.—Bleeding and Sacrifice Schedule of Rabbits Showing
| Erythrocytic Marrow Hyperplasia
<table>
<thead>
<tr>
<th>Group</th>
<th>Total Blood Removed (ml.)</th>
<th>Period of Bleeding (days)</th>
<th>Period from Last Bleeding to Sacrifice (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>40</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>60</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>IV</td>
<td>65</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>V</td>
<td>70</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>
Table 2.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>2.55 ± 0.27*</td>
<td>2.69 ± 0.38</td>
<td>2.39 ± 0.50</td>
<td>2.32 ± 0.14</td>
<td>2.07 ± 0.13</td>
<td>2.15 ± 0.34</td>
</tr>
<tr>
<td>Marrow cell count (Gm. marrow x 10^9)</td>
<td>1.65 ± 0.11</td>
<td>1.73 ± 0.42</td>
<td>2.98 ± 0.50</td>
<td>3.54 ± 0.42</td>
<td>2.46 ± 0.46</td>
<td>2.18 ± 0.60</td>
</tr>
<tr>
<td>Marrow density (Gm./cm.³)</td>
<td>1.011 ± 0.007</td>
<td>1.011 ± 0.009</td>
<td>1.017 ± 0.033</td>
<td>1.044 ± 0.021</td>
<td>1.002 ± 0.017</td>
<td>1.033 ± 0.026</td>
</tr>
<tr>
<td>Relative volume of marrow components</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>noncellular</td>
<td>0.726 ± 0.025</td>
<td>0.603 ± 0.089</td>
<td>0.482 ± 0.054</td>
<td>0.326 ± 0.070</td>
<td>0.527 ± 0.054</td>
<td>0.566 ± 0.090</td>
</tr>
<tr>
<td>cellular</td>
<td>0.214 ± 0.016</td>
<td>0.397 ± 0.089</td>
<td>0.518 ± 0.054</td>
<td>0.664 ± 0.070</td>
<td>0.473 ± 0.054</td>
<td>0.434 ± 0.090</td>
</tr>
<tr>
<td>Marrow fat content (Gm./Gm. total marrow wt.)</td>
<td>0.185 ± 0.024</td>
<td>0.154 ± 0.056</td>
<td>0.123 ± 0.040</td>
<td>0.089 ± 0.030</td>
<td>0.101 ± 0.010</td>
<td>0.149 ± 0.041</td>
</tr>
<tr>
<td>Viscosity of noncellular</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>marrow component</td>
<td></td>
<td></td>
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<tr>
<td>shear (centipoises)</td>
<td>66.8 ± 2.2</td>
<td>39.5 ± 7.0</td>
<td>34.2 ± 4.0</td>
<td>17.0 ± 11.0</td>
<td>28.3 ± 10.0</td>
<td>39.7 ± 6.3</td>
</tr>
<tr>
<td>drag (centipoises)</td>
<td>44.8 ± 2.4</td>
<td>33.7 ± 15.0</td>
<td>18.3 ± 3.1</td>
<td>10.5 ± 5.4</td>
<td>25.5 ± 4.7</td>
<td>36.0 ± 18.0</td>
</tr>
<tr>
<td>Mucopolysaccharide content of noncellular marrow component</td>
<td></td>
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</tr>
<tr>
<td>turbidimetric (µg./Gm.)</td>
<td>739 ± 64</td>
<td>715 ± 185</td>
<td>1180 ± 104</td>
<td>1710 ± 255</td>
<td>950 ± 106</td>
<td>1090 ± 152</td>
</tr>
<tr>
<td>uronic acid (µg./Gm.)</td>
<td>226 ± 18</td>
<td>238 ± 29</td>
<td>242 ± 26</td>
<td>350 ± 58</td>
<td>268 ± 35</td>
<td>268 ± 35</td>
</tr>
<tr>
<td>Hemoglobin (Gm./100 ml. blood)</td>
<td>13.4</td>
<td>9.5</td>
<td>10.0</td>
<td>9.5</td>
<td>9.3</td>
<td>=</td>
</tr>
<tr>
<td>Reticulocytes (per cent)</td>
<td>3.0</td>
<td>8.7</td>
<td>21.1</td>
<td>29.8</td>
<td>10.6</td>
<td>=</td>
</tr>
<tr>
<td>Peripheral white cell count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cells/mm.³ x 10^9)</td>
<td>10.6</td>
<td>7.2</td>
<td>9.1</td>
<td>16.5</td>
<td>14.7</td>
<td>13.5</td>
</tr>
</tbody>
</table>

*Denotes standard error of estimate of the mean.
Fig. 1.—Changes in the viscosity of the noncellular marrow components in erythrocytic marrow hyperplasia. Each point after the beginning of bleeding represents a specific group of animals (groups I through V). Details of the total volume of blood removed and the time from cessation of bleeding to sacrifice for each group are given in table 1.

moved, and the time between cessation of bleeding and sacrifice are summarized in table 1. Table 2 gives data on marrow changes seen in these groups. Certain data from table 2 are graphically presented in figure 1. Erythrocytic hyperplasia of the marrow was confirmed by examination of microscopic sections and by the appearance of a moderate peripheral normoblastosis in those groups sacrificed shortly after the completion of bleeding. Reticulocytosis in the peripheral blood reached 29.8 per cent in group III, the group in which a total of 60 ml. of blood was removed over a five day period and which was sacrificed five days after completion of bleeding. As shown in table 2, all bled groups showed increased marrow cell counts, cell volumes and decreased marrow lipid content. The viscosity of the noncellular marrow component decreased sharply as marrow hyperplasia increased and recovered slowly as marrow hyperplasia decreased during the recovery phase from the acute anemia. At the time of decreased viscosity of the noncellular component, there was a moderate increase in its mucopolysaccharide content. While the significance of this difference between the bled and control animals cannot be assessed by conventional statistical means due to the small sample size in the bled groups, no overlapping between the range of experimental and control values was seen in the case of groups II and III, suggesting that this consistent elevation of noncellular mucopolysaccharide material was in fact a significant finding at the time of peak erythrocytic hyperplasia.
Unfortunately, hemoglobin and reticulocyte counts were not performed on the three animals in group V, the group sacrificed 15 days after bleeding, and it cannot be stated with certainty that their hemoglobin levels were returning to normal. However, the decreasing reticulocyte count seen in the immediately preceding group IV, which was sacrificed nine days after bleeding, together with the decreasing marrow cell count and cellular component volume seen in group V, testify to the continuing recovery of the animals from the period of acute anemia.

The data from the turpentine injected animals are presented in table 3, together with the values from appropriate control groups. In the older animals, creation of a turpentine abscess was followed by increased marrow cellularity, a decreased lipid content and a significant increase in the viscosity of the noncellular component of the marrow. The increase in the peripheral total white blood cell count was moderate although not striking. A marked increase in the mucopolysaccharide content of the noncellular marrow component was seen in the turpentine-injected animals compared with the control group. Granulocytic hyperplasia of the marrow, indicated by the increased marrow cell counts and the increased volume of the cellular component, was confirmed by examination of sections of the marrow where between 80 and 90 per cent of the cells were developing granulocytes.

It was more difficult to produce turpentine abscesses in the younger animals studied. Attempts to improve abscess formation by increasing the turpentine dose resulted in animal death from pneumonia. With small turpentine doses, abscess could be produced as in the older animals, but they were small and tended to heal rapidly. Where abscesses could be produced, the younger
animals showed a marrow pattern similar to that seen in the older rabbits but the changes were less striking. The increase in the cellular component and the decrease in the marrow lipid content were greater on the average than in the older animals, but the increase in viscosity was smaller and not statistically significant with the sample sizes employed. The increase in the mucopolysaccharide content of the noncellular component was less pronounced and amounted to only a threefold increase compared with the control animals.

**DISCUSSION**

The finding of a decreased marrow lipid content with increased cellularity, both in granulocytic and in erythrocytic hyperplasia, agrees with previous studies. The decreased lipid content paralleled the decrease in the volume of the noncellular marrow component. A slight increase in the ratio of lipid per gram of marrow to the volume of the noncellular component was seen in the animals showing granulocytic marrow hyperplasia, and at the peak of the erythrocytic response in the bled animals, but was not significant statistically.

The demonstration of a decreased viscosity but an increased mucopolysaccharide content in the intercellular materials in erythrocytic hyperplasia is in accord with our previous impression that the mucopolysaccharide substances make no significant contribution to the viscosity as measured in these experiments. Similarly, the decreased fat content but increased viscosity in the intercellular substances in granulocytic marrow hyperplasia indicates that ether-extractable fat is not the significant determinant of viscosity in these experiments.

It is interesting to speculate that the increased viscosity of the intercellular substances seen in granulocytic marrow hyperplasia reflects a basic change necessary to support active granulocyte production. The migratory ability of these cells would still permit their entry into marrow sinusoids. Such a concept might be consistent with the finding of decreased viscosity in intercellular substances in erythroid marrow hyperplasia, in view of the lack of motility of the erythrocytic elements and previous evidence suggesting liquefaction of intercellular substances around mature cells and their extrusion into sinusoids. However, measurements as conducted in the present experiments are too crude to support such previous hypotheses of access of marrow cells to the peripheral circulation. It appears established that differences in the composition of marrow intercellular substances exist in various hyperplastic states, but the significance of these differences, if any, must await further study.

**SUMMARY**

Rabbits were injected with turpentine to produce granulocytic marrow hyperplasia, or were bled to produce erythrocytic marrow hyperplasia. Both granulocytic and erythrocytic marrow hyperplasia were associated with decreased marrow lipid content. The viscosity of the intercellular substances
and the mucopolysaccharide content of this material were significantly increased in granulocytic marrow hyperplasia. In erythrocytic marrow hyperplasia, the viscosity of the intercellular substances was markedly reduced, while the mucopolysaccharide content was slightly elevated compared with control animals.

**SUMMARIO IN INTERLINGUA**

Conilios recipeva injectiones de terebinthina pro producer hyperplasia granulocytic del medulla, o ilos esseva sanguinate pro produced hyperplasia erythrocytic del medulla. Ambe iste hyperplasias esseva associate con un reducere contento de lipido in le medulla. In hyperplasia granulocytic del medulla le viscositate del substantias intercellular e le conteto de mucopolysaccharido in iste material esseva augmentate significativemente. In hyperplasia erythrocytic del medulla, le viscositate del substantias intercellular esseva marcatemente reducite, durante que le conteto del mucopolysaccharido esseva levemente elevate in comparation con illo observate in le animales de controlo.

**REFERENCES**

INTERCELLULAR SUBSTANCES OF BONE MARROW II

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