Volume and Cellular Constitution of Bone Marrow in Guinea Pigs Hypoxic from Birth

By G. HUDSON

Measurements of bone marrow volume have been carried out in normal guinea pigs, and the results have been employed in quantitative studies of hemopoiesis. Similar measurements have also been made in guinea pigs maintained at a simulated altitude of 20,000 feet for 14 days. In the latter experiments, no evidence was found to suggest that either an increase in the marrow spaces or an extension of the red marrow at the expense of the fatty (yellow) marrow had taken place in response to the erythropoietic stimulus. On the other hand, interpretation of the findings was complicated by the fact that this degree of hypoxia had led to retardation of skeletal growth. Furthermore, the fatty marrow (which had still not been replaced by hemopoietic marrow even after 4 to 5 weeks of severe hypoxia) was already established before the onset of the stimulus, and it appeared possible that the peripheral regions of the marrow might participate more readily in the response to hypoxia, if the stimulus was applied from the time of birth. In the present investigation, therefore, a quantitative study of the volume and cellular constitution of the bone marrow was carried out in guinea pigs born and reared at "14,000 ft.", a simulated altitude at which retardation of skeletal growth was not observed. In this way, it was possible to study the response of the marrow in quantitative terms and to consider the relative importance of the various possible ways in which an increase in total marrow cell population might be accommodated.

Materials and Methods

The animals studied were albino guinea pigs of the Mill Hill strain, originated by Dunklin and Hartley. Each weighed approximately 400 Gm. The experimental (hypoxic) group consisted of 10 animals which had been born and reared in the decompression chamber at a barometric pressure corresponding to an altitude of 14,000 feet. They were the offspring of healthy female guinea pigs which had been maintained at the same simulated altitude for 7 to 14 days before parturition. The exposure was continuous except for a short period each day, when the chamber was cleaned, the animals weighed and the food and drinking water renewed. The control group consisted of 10 animals which had been born and reared under conditions which, apart from the barometric pressure, appeared to be identical with those of the experimental group. In each group there were six males and four females. The mean birth weight of the hypoxic animals was 83 Gm. (range 68 to 91 Gm.), while that of the controls was 84 Gm. (55 to 110 Gm.) When the body weight of 400 Gm. had been attained, each animal was killed by exsanguination from the common carotid artery under thiopeptonate sodium anesthesia.

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The method for obtaining the absolute counts of cells was essentially that described by Yoffey. A plug of marrow removed from the medullary cavity of the right humerus was shaken with a small quantity of serum obtained from the same animal; a uniform cell suspension resulted, and the volume dilution was calculated. Normal hemocytometric methods were used to count the number of nucleated cells per unit volume of the suspension. Dry smears were also made from the suspension and were fixed and stained with MacNeal's tetrahcrone strain. Differential counts were made at right angles to the axis of the slide and 1000 cells classified. The method and morphologic criteria for classification were those at present in use in this laboratory. The results were finally expressed as absolute numbers of cells per cubic millimeter of the marrow.

The rest of the procedure for examining the marrow and for measuring its volume was essentially that used in earlier studies. In the limb bones of the right side, the distribution of red and fatty marrow was studied, the sites of junction between the two being determined as accurately as possible. Specimens of marrow were removed for histologic examination or were submitted to a confirmatory test for specific gravity. The coccygeal vertebrae were examined by transillumination. On the basis of the observations on the opposite limbs, the left limb bones were divided into red marrow and fatty marrow bones: these and the rest of the skeleton were macerated. The volume of bone substance in each division of the macerated skeleton was measured by means of a density bottle using air-free distilled water at constant temperature. The bones were then thoroughly impregnated in a saturated solution of agar kept at boiling point. The solution was allowed to set, thus filling all the spaces in the interior. The total volume of the bones was then measured, care being taken to remove agar from such spaces as the tooth sockets, tympanum, etc. The difference between the two volume measurements represented the total volume of agar taken up, and this, when corrected for the volume of agar present in bone spaces which would not normally contain marrow, was a measure of the bone marrow volume. From the results, the volumes of total, red and fatty marrow could be calculated.

Results

Absolute counts of marrow cells.—The results of these counts are summarized in table 1. The mean total count of nucleated cells was significantly higher in the hypoxic group than in the control and this increased cellularity was associated with a higher count of erythroid cells. In subclassifying the erythroid cells, difficulties in differentiation were encountered similar to those discussed by Yoffey, but it is noteworthy that each of these subgroups yielded a significantly higher mean figure for the hypoxic animals. The differences between the two groups of animals with respect to the other classified cells did not satisfy the requirements for significance.

The appearance of the marrow.—The general appearance of the marrow in both groups were similar to those of normal 400 Gm. guinea pigs, which have already been described and illustrated. In both groups, fatty (yellow) marrow was present in the distal part of the limbs and coccyx, whereas the rest of the marrow was actively hemopoietic (red). On histologic examination, the fatty marrow plug of the lower tibia or a metatarsal appeared to consist almost entirely of fat cells, although some evidence of residual hemopoietic activity in the fatty marrow was seen at the growing ends of diaphyses. In both groups, the hemopoietic marrow of the femur contained a few fat vacuoles but these appeared to be less numerous in the hypoxic animals.

The volume of bone marrow.—The results of these measurements are summarized in table 2. There were no significant differences between the mean figures for the bone marrow volume in the hypoxic and control groups. On
BONE MARROW IN GUINEA PIGS HYPOXIC FROM BIRTH

Table 1.—Summary of Cell Counts

<table>
<thead>
<tr>
<th>Main Classification</th>
<th>Hypoxic group</th>
<th>Control group</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nucleated</td>
<td>$2,220 \pm 190^*$</td>
<td>$1,990 \pm 190$</td>
<td>2.7</td>
</tr>
<tr>
<td>Myeloid</td>
<td>$681 \pm 96$</td>
<td>$758 \pm 126$</td>
<td>1.5</td>
</tr>
<tr>
<td>Erythroid</td>
<td>$795 \pm 161$</td>
<td>$555 \pm 99$</td>
<td>4.0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>$346 \pm 64$</td>
<td>$303 \pm 76$</td>
<td>1.4</td>
</tr>
<tr>
<td>Monocytes</td>
<td>$105 \pm 23$</td>
<td>$93 \pm 17$</td>
<td>1.3</td>
</tr>
<tr>
<td>Damaged</td>
<td>$208 \pm 50$</td>
<td>$195 \pm 30$</td>
<td>0.7</td>
</tr>
<tr>
<td>Subclassification:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythroblasts</td>
<td>$27.7 \pm 14.7$</td>
<td>$15.3 \pm 9.2$</td>
<td>2.3</td>
</tr>
<tr>
<td>Proerythroblasts</td>
<td>$694 \pm 136$</td>
<td>$502 \pm 93$</td>
<td>3.7</td>
</tr>
<tr>
<td>Basophilic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polychromatic</td>
<td>$73.4 \pm 31.9$</td>
<td>$37.7 \pm 24.0$</td>
<td>2.8</td>
</tr>
</tbody>
</table>

$^*$± standard deviations.

The absolute counts of megakaryocytes, macrophages, plasma cells and unidentified cells have not been included. The t-values are those of the standard error test for small samples; they were calculated following the procedure given by Herdan. Here, a t-value exceeding 2.1 indicates significance at the 0.05 level of probability.

Table 2.—Summary of Marrow Volume Measurements and Other Data

<table>
<thead>
<tr>
<th>Mean values</th>
<th>Hypoxic group</th>
<th>Control group</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Total marrow vol. (ml.)</td>
<td>$6.70 \pm 0.32$</td>
<td>$6.59 \pm 0.38$</td>
<td>0.7</td>
</tr>
<tr>
<td>B. Red marrow vol. (ml.)</td>
<td>$6.00 \pm 0.29$</td>
<td>$5.83 \pm 0.29$</td>
<td>1.3</td>
</tr>
<tr>
<td>C. Total skeleton vol. (ml.)</td>
<td>$13.84 \pm 0.66$</td>
<td>$13.87 \pm 0.75$</td>
<td>0.1</td>
</tr>
<tr>
<td>D. Red marrow skeleton vol. (ml.)</td>
<td>$12.32 \pm 0.60$</td>
<td>$12.29 \pm 0.59$</td>
<td>0.1</td>
</tr>
<tr>
<td>A C × 100 (%)</td>
<td>$48.4 \pm 1.2$</td>
<td>$47.5 \pm 1.3$</td>
<td>1.6</td>
</tr>
<tr>
<td>B D × 100 (%)</td>
<td>$48.7 \pm 1.3$</td>
<td>$47.4 \pm 1.3$</td>
<td>2.2</td>
</tr>
<tr>
<td>B A × 100 (%)</td>
<td>$89.6 \pm 1.2$</td>
<td>$88.3 \pm 1.2$</td>
<td>2.4</td>
</tr>
<tr>
<td>Age (days)</td>
<td>$50 \pm 6.2$</td>
<td>$48 \pm 6.6$</td>
<td>0.7</td>
</tr>
<tr>
<td>Spleen weight (Gm.)</td>
<td>$0.68 \pm 0.29$</td>
<td>$0.49 \pm 0.06$</td>
<td>1.9</td>
</tr>
</tbody>
</table>

$^*$± standard deviations.

The figures in rows C and D relate to the volume of the macerated skeleton after agar impregnation. For the significance of the t-values see footnote to table 1.

the other hand, the red marrow volume of the hypoxic animals formed a significantly greater percentage both of the total marrow volume and of the total volume of the red-marrow skeleton (i.e., that part of the skeleton which had contained red marrow).

**Total cell population of the marrow.**—If the figures for the absolute counts (per cubic millimeter) are multiplied by the corresponding values for red marrow. In this way, the mean total nucleated cell population of the hypoxic group was computed as approximately $13.3 \times 10^6$ and that of the controls as marrow volume, a figure is obtained for the total number of cells in the whole $11.6 \times 10^6$. Similarly, the mean erythroid cell population of the marrow was estimated as approximately $4.77 \times 10^6$ in the hypoxic animals and $3.24 \times 10^6$ in the controls. These estimates must only be regarded as approximate, in view of the nature of the underlying assumption, viz. that humeral marrow was
typical of the hemopoietic marrow as a whole. The presence of damaged cells must also be considered. It should however, be noted that, relative to the body weight, the above figures are of the same order as those obtained by other methods, both in normal animals of other species and in normal man.

Other findings.—The two groups appeared comparable with regard to age and total skeleton volume (table 2). The liver weights were also very similar, the mean values being 17.4 Gm. (14.7 to 21.7) and 17.5 (15.6 to 20.9) for the hypoxic and control groups, respectively. In the hypoxic group, the weight of the spleen showed a striking range of variation (0.44 to 1.46 Gm.), as compared with that of the controls (0.44 to 0.58 Gm.), but the t-value for the difference between the means was only 1.9 (table 2). There were no apparent differences between the results obtained from male and those obtained from female guinea pigs.

DISCUSSION

Although the values obtained for the total cell populations of the marrow can only be regarded as very approximate, they appear to indicate that the hypoxic animals had 45 to 50 per cent more nucleated red cells in their bone marrow than had the normal control guinea pigs. There would seem to have been an increase in cell population at each stage of development studied (from proerythroblast to orthochromatic erythroblast).

This increase in erythroid cell population was largely accommodated by increasing the number of cells in each unit volume of red marrow; there were about 43 per cent more erythroid cells per cubic millimeter in the hypoxic animals. This closer packing of cells was accompanied by a diminution in the number of fat vacuoles in the red marrow, but there was no significant reduction in the absolute numbers of other marrow cell groups. It would seem that the total populations of myeloid cells, lymphocytes and monocytes were very similar for the two groups of animals. These findings do not preclude the possibility that changes in the absolute counts, e.g., of the marrow lymphocytes, may be present during the early stages of adaptation to lowered barometric pressure.

The marrow volume measurements indicated either that no increase had occurred in the volume of bone marrow in response to the stimulus of hypoxia, or that any such absolute increase was too small to be detected by the method employed. There was in fact evidence to suggest that the latter was the case; when considered in relation to either the volume of the skeleton containing it or to the total marrow volume, the red marrow volume of the hypoxic animal formed a significantly higher percentage (table 2). These findings may be compared with those of other observers which indicate that the marrow cavities may increase in size in association with increased activity in the marrow cells, e.g., following bleeding in the dog; during seasonal variations in hemopoiesis in the dermal bones of the nine-banded armadillo; and in certain human anemias in which the bony changes may sometimes be demonstrated by means of roentgenograms. In the conditions of the present experiments at least, such changes would appear to be of little importance in accommodating the increase in cell population.
A notable finding was that fatty marrow occupied its normal centrifugal position in the skeletons of the hypoxic animals. Assuming that all the marrow of the newborn mammal is hemopoietic, it may be concluded that the original red marrow of the distal limbs and coccyx had actually been converted into inactive, fatty marrow even in the presence of a potent erythropoietic stimulus. It may be calculated that a further increase of about 40 per cent in the total erythroid cell population would have resulted if the marrow in these areas had remained hemopoietic (and the total populations of the other cell types had remained constant). It would appear from these and previous observations that, after birth, conditions in the centrifugal parts of the skeleton are unfavorable to the processes of hemopoiesis. The circumstances and conditions under which the appearance of fatty marrow in these regions of the skeleton may be prevented or under which the fatty marrow may be completely reconverted into hemopoietic tissue would seem to merit further study.

While a study of extramedullary hemopoiesis did not come within the scope of the present investigation, it has to be borne in mind as a further mechanism for increasing the total numbers of red cell precursors in the whole organism. In view of the fact that extramedullary hemopoiesis has been observed in the spleens of hypoxic animals, it is noteworthy that the spleens of the experimental group showed a striking range of variation in weight, but the mean increase was only of doubtful significance.

**SUMMARY**

A quantitative study of the volume and cellular constitution of the bone marrow was carried out in 10 guinea pigs of approximately 400 Gm. body weight, which had been born and reared at a simulated altitude of 14,000 feet, and in 10 normal controls.

The hemopoietic marrow of the experimental group was more cellular and showed a marked increase in the number of erythroid cells per unit volume. No significant changes were demonstrated in the absolute counts of other cells, but fat vacuoles appeared less numerous. The fatty (yellow) marrow occupied its normal centrifugal position in the skeletons of both groups.

No absolute increase in the volume of bone marrow was detected in the experimental group, but the red marrow volume formed a significantly greater proportion both of the volume of the skeleton containing it and of the total marrow volume.

It was concluded that the marrow of the hypoxic animals had a total population of erythroid cells about 40 to 50 per cent greater than normal; this increase was largely accommodated by closer packing of cells in the red marrow.
revelava un marcate augmento del numero de cellulas erythroide. Nulle altera-
tiones significative esseva demonstrate in le numeration absolute de altere
cellulas, sed le numero del vacuolos grasse esseva inferior in le gruppo ex-
perimental. Le medulla grasse (jalne) occupava su normal position centrifugal 
in le skeletos de ambe gruppos.
Nulle augmento absolute in le volume de medulla ossee esseva detegite 
in le gruppo experimental, sed le volume del medulla rubie formava un 
significativamente plus grande proportion tanto del volume que contineva 
lo como etiam del total volume de medulla.
Le conclusion esseva que le medulla del animales vivente sub conditiones 
de hypoxia habeva un population total de cellulas erythroide excedente le 
norma per circa 40 a 50 pro cento. Iste augmento esseva absorbite in grande 
mesura per un plus forte compaction de cellulas in le medulla rubie.

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