Evidence Of Periodic Changes In The Rate Of Formation Of Individual Osteons In Human Bone

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EVIDENCE OF PERIODIC CHANGES IN THE RATE OF FORMATION OF INDIVIDUAL OSTEONS IN HUMAN BONE

M. KELIN, M.D. AND HAROLD M. FROST, M.D.

INTRODUCTION

This article reports an entirely unexpected phenomenon which appeared during the performance of an extensive series of measurements of the sizes of osteoid seams and of actively forming new osteons in human rib. To our knowledge no comparable observation has been reported previously within the field of bone physiology. The phenomenon provides evidence that at the local, cellular level, osteonal osteoblastic activity is not continuous but occurs as series of successive, separate bursts.

MATERIALS

The middle third of the 5th, 6th or 7th rib was obtained from 139 metabolically normal patients. Over two-thirds of the ribs were obtained at thoracotomy for indications such as patent ductus, cardiospasm, hiatus hernia, biopsy of lung parenchymal lesions and trauma. The rest were obtained at autopsy, causes of death being trauma, acute poisoning, fatal vascular incidents, air embolism, suicide and homicide.

None of these patients had a known chronic illness, endocrine disturbance, chronic fever or metastatic malignancy. None were receiving hormone therapy. Adequate records or autopsies were available in all cases. It is believed that this series of ribs represents metabolically normal bone. The case material is summarized in Table I.

We wish to express our appreciation to E. S. Zawadski, M.D., and R. H. Horn, M.D., for generously supplying the material and for giving us access to the relevant autopsy records.

Table I

<table>
<thead>
<tr>
<th>Mean Age Group</th>
<th>No. of Cases</th>
<th>Male</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.81</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>15.8</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>24.9</td>
<td>14</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>34.7</td>
<td>20</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>44.2</td>
<td>20</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>55.1</td>
<td>29</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>64.4</td>
<td>16</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>75.6</td>
<td>13</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>78</td>
<td>61</td>
</tr>
</tbody>
</table>

Age and sex distribution on the 139 cases, on which the measurements were done.
Figure 1

An actively forming osteon seen in a mineralized cross section of human bone at 450X. The central, black, circular density is the shrunken soft tissue content of the Haversian canal. The two double sets of India Ink marks bracket the osteoid seam, which is a doughnut of unmineralized, newly formed osteoid lining the wall of the Haversian canal. The quadruple set of marks identifies the inner wall of the seam; this is the circumference that was measured in the study. The two arrows show the direction in which the canal narrows as new osteoid matrix is added to the inner wall of the seam. The large, parallel lines are the grid lines of the Zeiss integrating eyepiece II, filled in with India ink to make them clearer.
PERIODIC CHANGES

METHODS

Four things will be considered under Methods: preparation of the sections, measurements of cross sectional circumferences of the osteoid seams, construction of a size-frequency histogram with this data, and determination of the average number of lamellae per osteon in these ribs.

I: Sections

Fresh, mineralized, cross sections, oriented within five degrees of perpendicularity to the long axis of the ribs, were made and stained with basic fuchsin by special methods. The sections averaged 50 microns in thickness. There were 468 of them, averaging more than three per case.

II: Circumferences of Osteoid Seams

New osteon formation occurs in a cylindrical space which has been previously prepared for it by resorptive activity. The long axis of this space lies parallel to the “grain” of the bone. The bone matrix which is synthesized in making a new osteon is then deposited on the walls of this space. Thus, the direction of osteon formation is centripetal, as is shown in Figure 1. An osteoid seam is a constant feature of new osteon formation in normal human bone, and in cross section it resembles a doughnut consisting of hyaline material lining the wall of the central canal. This is also shown in Figure 1. The canal in the center of the osteoid seam is the Haversian canal, and as the osteon is made the canal becomes progressively smaller, reaching its minimum diameter when the osteon has been completely formed. The Haversian canal also is cylindrical, so that its inner circumference also decreases with time from its maximum value at the moment when new bone formation begins, to a minimum value when the osteon is completely made. It is the circumference of this wall that was measured in this study, and which is marked on Figure 1.

The circumferences were measured with a Zeiss integrating eyepiece II, using the method of Chalkely, Cornfield and Park as applied to the use of this eyepiece by Hennig in 1956. This eyepiece has a series of five parallel, straight lines in its reticule which are optically superimposed on the image formed by the microscope objective, as shown in Figure 1. When a circle (which the inner wall of an osteoid seam appears to be) lies in the field, some of the reticule lines may cut across its circumference. The number of such cuts (four in the figure) is proportional to the absolute value of the circumference, to the total length of the reticule lines in the object space, and to the perpendicular distance between adjacent reticule lines as measured in the object space.

The measurements required that each osteoid seam in a section be identified under a low power objective. It was then measured under the high dry objective at 360X. With a single superimposition of the eyepiece reticule on a seam there is some uncertainty in the absolute value of the circumference that is obtained. This uncertainty may be reduced by increasing the number of times (i.e., “throws”) each osteoid seam circumference is measured and averaging the result. A new measurement is made by rotating the eyepiece while vision is asserted (to make the procedure random), or by moving the section a fixed amount with the aid of the vernier scale on the mechanical stage of the microscope. Each seam in this study was measured with five throws, providing an average number of 14 cuts (i.e., “hits”) per seam. A total of 5,170 seams in the 468 sections were measured in this way.

The circumference of each seam in millimeters was calculated with this formula, adapted from Hennig:

\[
S = \frac{II \cdot a \cdot H}{2 \cdot T}
\]

where \(S\) is the seam circumference, \(a\) the perpendicular spacing in millimeters in object space between the lines in the reticule of the eyepiece, \(H\) the total number of hits and \(T\) the total number of throws. The value of “\(a\)” was measured with a Zeiss stage micrometer at the working magnification.

III: The Histogram

The data obtained in this way were arrayed vertically on ruled paper in order of increasing circumference. On the horizontal rows a check mark was made each time a circumference of corresponding size was found. The resolution of the array was 28, meaning that
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Table II

NUMERICAL VALUES OF FREQUENCIES AND SIZES BY AGE DECADES

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>0-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>70-80</th>
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<tr>
<td>35</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>12</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>16</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td>23</td>
<td>15</td>
<td>18</td>
<td>175</td>
<td></td>
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<tr>
<td>153</td>
<td>22</td>
<td>15</td>
<td>10</td>
<td>12</td>
<td>46</td>
<td>35</td>
<td>30</td>
<td>323</td>
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</tr>
<tr>
<td>0.093</td>
<td>300</td>
<td>68</td>
<td>25</td>
<td>18</td>
<td>30</td>
<td>100</td>
<td>61</td>
<td>13</td>
<td>615</td>
</tr>
<tr>
<td>0.187</td>
<td>315</td>
<td>79</td>
<td>53</td>
<td>21</td>
<td>38</td>
<td>117</td>
<td>68</td>
<td>9</td>
<td>700</td>
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<tr>
<td>0.280</td>
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<td>43</td>
<td>18</td>
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<td>78</td>
<td>33</td>
<td>6</td>
<td>362</td>
</tr>
<tr>
<td>0.373</td>
<td>54</td>
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<td>13</td>
<td>36</td>
<td>15</td>
<td></td>
<td>176</td>
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<tr>
<td>0.467</td>
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<td>79</td>
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<td>0.560</td>
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<td>6</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>0.654</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Total o.s. per decade</td>
<td>2138</td>
<td>605</td>
<td>372</td>
<td>181</td>
<td>270</td>
<td>875</td>
<td>517</td>
<td>212</td>
<td>5170</td>
</tr>
</tbody>
</table>

568
there were 28 rows spanning the sizes of the largest and the smallest osteoid seam. A histogram was then constructed from this data, and is shown in Figure 2. Going away from the origin, the X axis plots increasing sizes of circumference while the Y axis represents increasing frequencies with which various sizes of circumference were found.

**IV: Number of Lamellae Per Osteon**

In this part of the study, 350 osteons were selected at random from 48 sections of 24 of the cases. They were examined between the crossed polars of a Zeiss GFL polarizing microscope. The number of lamellae were counted in each osteon by counting the number of anisotropic rings. The anisotropic part of a lamella is that which appears bright, as shown in Figure 3. The data of this part of the study are summarized in Table III.

**SIZE AND FREQUENCY DISTRIBUTION OF OSTEOID SEAMS IN HUMAN RIBS**

5207 values

![Histogram](image)

Figure 2

This is the histogram constructed from the raw data. The Y axis is the real number of times a seam with a given circumference was found. The X axis is the circumferences of the seam in millimeters. Each bar is 0.0232 mm larger than the one to its left. The frequency peaks occur every 0.093 mm.
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RESULTS

The histogram in Figure 2 reveals seven definite maxima and minima in the frequencies with which various sizes of circumferences were found. The frequency maxima are distributed every .093 mm. from the origin of the graph, and seem to occur as a linear function of the circumference of the seam.

The average number of anisotropic lamellae in finished osteons was 7.9.

DISCUSSION

The circumferences of the osteoid seams in this study normally change in a unidirectional manner with time by decreasing towards zero as a limit. Therefore the frequency maxima in the histogram mean that the circumferences were changing in size more slowly at these points than they were in the frequency minima. In effect, if the rate of decrease in the circumference of a seam slows at some stage of its existence, there is a greater probability of observing this seam than there is during a fast stage. Therefore, more seams will be found with sizes that correspond to slow stages than to fast ones. In fact, the frequencies shown in Figure 2 are inversely proportional to the rate of decrease in seam circumference at the corresponding stages of osteon formation.

For this reason, the frequency peaks in the histogram mean that there are corresponding, cyclic and inversely proportional changes in the rate of decrease in seam circumference during the production of the single, average osteon. Simple geometric considerations mean there must be corresponding changes in the rate of production of new bone matrix, speaking in terms of the individual osteon. These periodic changes in rate appear to be a linear function of the size of the seam circumference. This raises the previously unexplored possibility that the changing size of the osteoid seam somehow affects the rate of osteon formation, possibly by crowding the layer of osteoblasts. Or the osteoblasts which make a single osteon might be synchronized and "pulsed" by some mechanism.

There is reasonably good but not perfect agreement between the number of frequency maxima in the histogram, and the number of anisotropic lamellae in the average completed osteon in the same bones. This raises the possibility that the physical differences in lamellar bone which are responsible for the alternating isotropic and anisotropic pattern are causally related to the periodic changes in the rate of production of the bone matrix of the osteon. Whether this hypothetical relationship is direct or indirect must remain an unanswered question for the moment.

Table III

<table>
<thead>
<tr>
<th>No. of lamellae/HS</th>
<th>Standard deviation</th>
<th>Standard error</th>
<th>No. of cases</th>
<th>No. of H.S. examined</th>
<th>No. of lamellae examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.9</td>
<td>0.51</td>
<td>0.01</td>
<td>24</td>
<td>360</td>
<td>2878</td>
</tr>
</tbody>
</table>

A page from the book is shown in the adjacent image.
Figure 3

A mineralized cross section of human bone seen between crossed polars of a polarizing microscope. There are alternating bright and dark, parallel bands. These are the anisotropic and isotropic lamellae respectively. One lamella is composed of one anisotropic and one isotropic part, so that the thickness of the average lamella can be obtained by measuring the average separation of adjacent bright bands.
This study suggests the possibility that the periodicity found in osteon production might also exist in other forms of lamellar bone production, for example in circumferential and endosteal lamellar bone formation.

SUMMARY

The circumferences were measured of 5,170 osteoid seams involved in new osteon formation in 139 normal human ribs. The measurements were made on mineralized cross sections with a Zeiss integrating eyepiece II, using a minor modification of Chalkley, Cornfield and Park's method. A size-frequency histogram was constructed from this data which revealed seven regularly spaced frequency maxima and minima. This is interpreted to mean that the average osteon in ribs is formed in about seven "bursts" of osteoblastic activity.

REFERENCES