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M. D. Klein
Harold M. Frost
Elias Sedlin

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A PILOT STUDY OF LAMELLAR BONE PHYSIOLOGY IN DIABETES MELLITUS

KLEIN, M.D., H. M. FROST, M.D. AND E. SEDLIN, M.D.

Medical records in the United States show that about 1 per cent of the population suffers from diabetes mellitus. Possibly another 0.7 per cent of the population with diabetes is undetected.

Drugs such as insulin or Orinase control hyperglycemia but they apparently fail to control arteriosclerosis. Lowrie, in his article about the diabetic foot affirmed: "... in spite of improved management of diabetes, foot complications are seen with greater frequency". The origin of these, and perhaps other, aspects of diabetes mellitus are still incompletely understood. This suggests that there are still unrecognized basic abnormalities in this disease, and that a search for evidence of them with a new approach might be profitable.

No studies of lamellar bone dynamics are known to us in diabetes. The closest approaches seem to be those of Bailey and Root in 1942 and in 1947 when they described the "neurotrophic joint."

In 1960, Degenhardt et al reported the results of bone biopsies done in neuropathic joints in diabetics, where they found: "... irregular absorption and formation of bone and disorganization of articular cartilage". These findings provide no information as to the behavior of lamellar bone in diabetes mellitus, but refer instead to the production of fibrous bone and cartilage in repair and reactive processes.

Lamellar bone can serve as a model biological system and biological record, in which are often imprinted clues to past and present cell regulation, dynamics and disease. We undertook a pilot investigation of lamellar bone in patients with diabetes mellitus, using recently developed quantitative methods, to see if we might find any unusual features which would justify a larger, longer-range study. We summarize here the results of this study.

The amount of bone resorbed or formed in a given amount of bone is a function of three geometrical, histologically observable properties: 1) the number of foci

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From the Orthopaedic Research Laboratory.
where these activities occur; 2) the mean size of the foci, and 3) the mean rate at which the average focus evolves. If a consistent system of units is adopted, the bone formation rate may be calculated directly from measurements of these properties.\textsuperscript{13}

It may also be measured directly with the aid of tetracycline labelled human bone,\textsuperscript{12} as a check on the above. The symbols adopted elsewhere\textsuperscript{32} for the number of places where remodelling activity occurs are $A_r$ and $A_f$ for resorption and formation respectively (Figures 1 and 2) and $S_r$ and $S_f$ for the mean sizes of each, respectively.

For the most part, regulation of mesenchymal cells determines the number of remodelling foci. Regulation of osteoclasts and osteoblasts determines the size and rate of completion of individual remodelling foci.

Thus, by means of appropriate measurements, one may study and evaluate separately the regulation of mesenchymal cell\textsuperscript{*} activation and the regulation of target cell metabolic activity. This is an important feature since target cell metabolic activity conducts the actual metabolic work. The mesenchymal cells are responsible for the size of the specialized cell populations but probably cannot themselves perform useful metabolic work.\textsuperscript{13,19,25}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A resorption space involved in internal remodelling of a human cortex. This space enlarges centrifugally. It is produced by osteoclasts which are generated by mesenchymal cell precursors. This represents a focus of resorption ($A_r$).}
\end{figure}

\textsuperscript{*}Mesenchymal cells are also variously known, depending on one's specialty, as progenitor cells, proliferating cells, stem cells and reticulum cells.
An osteoid seam in human cortex. The central canal decreases in size as new matrix is added to the inner wall of the seam. This, therefore, is a centripetal process. The new matrix is formed by osteoblasts. The osteoblasts are generated by mesenchymal cells. This represents a focus of formation (A_t). The area of the inner wall of this osteoid seam is (S_t). The lines represent the reticule of a Zeiss II integrating eyepiece.

**Materials**

1) We studied the 5th, 6th or 7th ribs from 17 patients with diabetes mellitus, known and treated for this disease here.

The cases had longstanding diabetes mellitus. All were under adequate treatment. This group, nearly evenly divided between sexes, ranged from 29 to 75 years old, the mean age being 57.7 years. Thirteen of these ribs were obtained at autopsy, the rest at surgery.

Rib was studied because of its availability, and because previous studies of this bone have provided a statistically significant norm for comparison. In all, 45 sections were made of these 17 ribs. They had a total cortical surface area of 921.1 mm^2 Data on these cases is listed in Table 1.

We compared the above cases to another series of 139 ribs, 75 per cent obtained at thoracotomy and 25 per cent at autopsy. Sampling was limited to the 5th, 6th or 7th ribs. Indications for thoracotomy were congenital heart disease, hiatus hernia, cardiopasm, biopsies of undiagnosed solid lesions in the lung parenchyma, repair of acute thoracic trauma and
Table I

SPECIFIC SURFACE VARIATIONS IN OSTEOID SEAMS IN DIABETES MELLITUS RIBS

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Additional diagnosis</th>
<th>Cortical Area</th>
<th>No. of Sections</th>
<th>No. of Seams</th>
<th>Seam specific Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>301869</td>
<td>29</td>
<td>M</td>
<td>Uremia, Neuropathy, CHF</td>
<td>61.6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>2.</td>
<td>929523</td>
<td>32</td>
<td>F</td>
<td>Myocardial infarction</td>
<td>72.3</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>3.</td>
<td>988441</td>
<td>49</td>
<td>F</td>
<td>RHD with M.S.</td>
<td>54.5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>970954</td>
<td>52</td>
<td>F</td>
<td>RHD with M.S.</td>
<td>88.9</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>5.</td>
<td>958882</td>
<td>52</td>
<td>M</td>
<td>AS, Atelactasis, Pyothorax</td>
<td>32.0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>989221</td>
<td>55</td>
<td>M</td>
<td>AS, Ca of lung, CVA</td>
<td>71.9</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>7.</td>
<td>1002499</td>
<td>58</td>
<td>F</td>
<td>Ca. of lung, Pneumonec tomy</td>
<td>58.1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>8.</td>
<td>948209</td>
<td>58</td>
<td>F</td>
<td>CVA, Generalised AS, Oophorectomy, Lues.</td>
<td>95.6</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>9.</td>
<td>752457</td>
<td>60</td>
<td>F</td>
<td>Ca. of breast, metastases, treated with testosterone last 5 y. of life.</td>
<td>9.1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10.</td>
<td>949935</td>
<td>60</td>
<td>M</td>
<td>Uremia, AS, Renal artery thrombosis.</td>
<td>15.0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>11.</td>
<td>955962</td>
<td>60</td>
<td>M</td>
<td>Generalised AS, Ca. of lung</td>
<td>26.8</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>12.</td>
<td>270758</td>
<td>65</td>
<td>F</td>
<td>Histoplasmosis, Pneumonec tomy.</td>
<td>37.4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>13.</td>
<td>267845</td>
<td>65</td>
<td>M</td>
<td>Ca. of lung, Neuropathy, Duodenal ulcer.</td>
<td>99.5</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>14.</td>
<td>343203</td>
<td>70</td>
<td>M</td>
<td>Myocardial infarction, Gen, AS</td>
<td>61.4</td>
<td>2.8</td>
<td>27</td>
</tr>
<tr>
<td>15.</td>
<td>1018369</td>
<td>70</td>
<td>F</td>
<td>Ca. of pancreas, Icterus, Pulmonary embolism.</td>
<td>85.0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>16.</td>
<td>915340</td>
<td>71</td>
<td>M</td>
<td>Generalized AS, Neuropathy.</td>
<td>41.7</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>17.</td>
<td>385444</td>
<td>75</td>
<td>M</td>
<td>Metastatic Ca. pancreas, Cirrhosis.</td>
<td>13.8</td>
<td>5/6</td>
<td>4</td>
</tr>
</tbody>
</table>

The above table presents the 17 patients suffering from diabetes mellitus, and followed at Henry Ford Hospital. All the measurements were done by the same examiner. The additional diagnoses are listed with the idea that they might have influenced the bone remodelling rate.
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repairs of patent ductus and of aortic coarctation. Cause of death in the autopsy cases varied from acute myocardial infarct, acute CVA and cardiac arrest to accidental death and homicide. No case in this group had osteoporosis, metabolic bone disease, primary or metastatic malignancy treated with x-ray, or chemotherapeutic agents. No case had a chronic, wasting or febrile illness, clinically significant congestive heart failure, hepatic cirrhosis, ascites or edema. No case was treated with any hormonal agent and none were diabetics. We feel the ribs of this group represent normal bone. Because of the number of cases in this group, no tabular information is given concerning individual clinical features.

METHODS

Frost,¹³ and Harris, Jackson and Jowsey¹⁵ have noted that many features of lamellar bone vary from one bone to another in the same skeleton, from one quadrant to another of the same cross-section and,¹³ from one time to another in the same bone (revealed by serial tetracycline labelling). Therefore we made complete cross-sections of the ribs, cut from a standard site 12 cm. from the sternal junction. The sections were made and stained by special methods.⁷,⁸

We measured the cortical area to an accuracy of 0.4 mm² by an adaption of Chalkley's method.³⁹,²⁰ This is the total area enveloped by the periosteum, minus the area of the marrow space.²⁰

The total number of active seams in the sections was counted, reproducibility being 10 per cent.¹⁰,¹¹ Seams/mm² was found by dividing total seams by cortical area and is given the symbol (At).

The mean specific surface of the osteoid seam is the surface area of the Haversian canal wall of the average osteoid seam in forming osteons (Figure 2) in cross-sections 1 mm. thick. Geometrically it is the area of the inner wall of a cylinder. This was measured by another adaption of Chalkley's method to an accuracy of 0.02 mm².⁷,²¹

We calculated the arithmetic means, standard deviations and mean standard errors in the usual way. The dimensional basis for these calculations assigns the values to cross-sections exactly 1 mm. thick.

The diabetic measurements are listed individually in Table I. In Table II, a comparison of normal and diabetic measurements is provided with their respective standard deviations and mean standard errors.

RESULTS AND DISCUSSION

1) Haversian systems are formed centripetally, after creation of a resorption space by osteoclasts.¹⁴ In a forming osteon, the osteoid seam begins with a large central canal and surface area, and forms until the minimum diameter of the Haversian canal and surface area of the seam is reached as the osteon is completed. Thus the specific surface of osteoid seam is an approximate function of the degree of completion of the osteon at the moment of observation.

Table II

<table>
<thead>
<tr>
<th>Index</th>
<th>NORMAL</th>
<th>DIABETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Mean area per cross-section in mm²</td>
<td>19.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Seams/mm²</td>
<td>0.36</td>
<td>0.264</td>
</tr>
<tr>
<td>Seam specific surface mm²/mm²</td>
<td>0.32</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*The P test of the null hypothesis: P<.05
**The P test of the null hypothesis: P<.05
In the 17 diabetics with a mean age of 57.7 years the mean seam specific surface is 0.41 mm.$^2$ Normal seam specific surface at this age is 0.32 mm.$^2$ This difference is just statistically significant at the 5 per cent level.

This increased specific surface in diabetics could mean that the kinetics of protein formation in the bone of diabetics differs from normal. More cases would be needed to be sure that this difference is not spurious, and a different approach to the kinetic problem might be valuable.

While the terminal illnesses of these patients could have superimposed unknown changes, note that the mean value of cases$^{2,3,4,7,13,14}$ is 0.48 mm$^2$, higher than the mean of the entire group. Four of these six cases had no chronic illness, and their ribs were obtained at elective thoracotomy. In cases 2 and 14, sudden death occurred due to fatal infarctions in previously healthy diabetics (who were however being managed with insulin or tolbutamide).

The abnormal specific surface should be viewed as an index of the metabolism of osteoblasts, and not as an index of mesenchymal cell activation.

2) The 198 seams counted in the 45 rib sections of diabetics are 43 per cent less than normal for this amount of bone (925.1 mm$^2$) at mean age of 57.7 years; the values being 0.36/mm$^2$ for normal ribs and 0.21 mm$^2$ in the diabetics.

The low number of osteoid seams indicates that less new bone is formed in internal remodelling in adult diabetics than in normals.

From this, we infer that a low bone remodelling rate exists in diabetes mellitus. This could account for the consistently noted increase in brittleness of these bones during the grinding of their sections. It is speculatively possible that there is an analogous derangement in other tissues in this disease.

The decrease in seams also indicates that there is less mesenchymal cell activation in diabetics than in normals. The decrease is probably not an index of the metabolism of osteoblasts.

3) The cortical cross section areas of the ribs of diabetic patients did not differ significantly from normal (see Table II). The meaning of this measurement is that the average diabetic rib cross-section in this study has (for 1 mm. thick sections) 19.5 mm$^2$ of bone left after the medullary space is subtracted from the whole.

One may deduce that this group of diabetics was neither unusually resistant nor susceptible to the osteoporosis of ageing which is found in normal persons.

SUMMARY

It has been shown that the average size of osteoid seams in ribs of 17 diabetic subjects is increased, compared to normals of the same age.
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The numbers of osteoid seams in the ribs of diabetic subjects is decreased compared to normals of the same age.

No osteoporosis was found in the ribs of diabetic patients. Our data suggest a fault in the bone of diabetic subjects in both mesenchymal cell physiology and in the activity of the protein forming bone cells.

The model system we have used seems promising and appropriate for further investigation of diabetics. It could be called to the attention of interested investigators with profit to our understanding of this disease.

ACKNOWLEDGMENTS

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REFERENCES