BONE FORMATION IN PAGET'S DISEASE
A Quantitative Microscopic Study Using Tetracycline Markers

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Paget's disease of bone is a disease of unknown etiology which causes solitary or widespread lesions and pain in the human skeleton. The clinical and metabolic aspects of the disorder have recently been extensively reviewed by Nagant de Deuxchaisnes and Krane (1964). Study of the diseased tissue by histological methods (Schmorl 1932, Collins 1956) and more recently by microradiography (Engfeldt, Engström, Helander, Wilton and Zetterström 1952; Kelly, Peterson, Dahlin and Plum 1961) has shown that the bone is initially porotic and that the underlying cause of this is excessive osteoclastic resorption. Proliferative and disorganised osteoblastic activity is a characteristic feature of the subsequent repair phase, so that in the final stage the affected bone is sclerotic and structurally abnormal.

It was suggested by Henneman, Bartter, Dempsey, Carroll and Albright in 1954 that in the diseased tissue the calcium turnover was greatly in excess of the normal, and studies using the affinity of certain radio-isotopes for newly formed bone have supported this hypothesis. For example, after the injection of Ca47 and Sr85 external counting has shown that radioactivity is up to ten times greater in an affected limb than in its normal opposite (Bauer and Wendeberg 1959, Macdonald 1960), and kinetic studies based on the rate of disappearance of Ca45 from the blood stream have revealed that the calcium accretion rate and the exchangeable calcium pool are increased in the affected skeleton (Krane, Brownell, Stanbury and Corrigan 1956; Heaney and Whedon 1958; Nagant de Deuxchaisnes and Krane 1964).

Since there is no quantitative information regarding the mass of bone which is removed and replaced in this disorderly process, it was hoped that useful knowledge might be gained by the application of the recently developed microscopic techniques for the measurement of bone formation by means of tetracycline markers. Single doses of tetracycline were given at known time intervals to five individuals suffering from Paget's disease, before operative removal of samples of normal and diseased bone. The material thus obtained was used for quantitative fluorescence microscopy to determine the linear rate of bone deposition on individual surfaces, the appositional growth rate (Lee 1964), and the percentage mass of new bone formed in unit time, the bone formation rate (Lee, Marshall and Sissons 1965).

MATERIALS AND METHODS

Clinical details of the five patients are given in Table 1. Each patient received either two or three "marker" doses of tetracycline (2 grammes in forty-eight hours) at intervals of about thirty days, and a few days were allowed to elapse between the final dose and the biopsy to eliminate tetracycline staining of non-growing surfaces. Three patients (Cases 2, 4 and 5) were suffering from osteoarthritis of the hip and were treated by intertrochanteric osteotomy; at operation, bone biopsies were taken from the cortex of the femoral shafts of all three and from the iliac crests of two (Cases 2 and 4). Elective biopsies of the greater trochanter of the femur were taken from the other two patients (Cases 1 and 3).

In each instance the bone biopsies were fixed in neutral formal saline and the major part was embedded in methyl methacrylate before its preparation for microradiography and fluorescence microscopy (Jowsey 1955, Lee et al. 1965). Histological studies were carried out on undecalcified...
sections stained by the periodic acid-Schiff technique and on paraffin sections of the remaining portion of the bone which was decalcified in ethylenediamine tetra-acetic acid.

The apparatus used for quantitative fluorescence microscopy has already been described in detail (Lee 1964, Lee et al. 1965). Appositional growth rate was determined by measuring the linear separation between the markers with a calibrated ×6 eyepiece micrometer and a 4-millimetre objective; this value is expressed in μ/day. The bone formation rate is an expression of the percentage of the bone volume which is added per day to the total volume of the biopsy. This was determined by measuring, for the whole section, the area of bone enclosed by the markers and comparing it with the total area of bone; in a thin section the area components can be assumed to be proportional to the respective volume components. Two methods were used for the measurement of area components in the tissue—initially a photographic technique, and later a Leitz Integrating Eyepiece Micrometer, each method being found to give a coefficient of variation of less than 10 per cent when the repeatability was tested, and comparable results were obtained when the same tissues were measured by each technique (Lee 1963). In two patients (Cases 2 and 5) photomicrographs were taken of sufficient randomly distributed fields to amount to approximately 50 per cent of the area of the section. Prints were prepared at a magnification of ten times and the areas of bone and new bone in each print were measured by tracing on to square millimetre tracing paper. The Leitz Integrating Eyepiece Micrometer was used on the sections from three patients (Cases 1, 3 and 4) to determine the areas of bone and new bone. This instrument is based on the Rosiwal method of linear integration for area (Schuchardt 1957) and can be used to measure the areas of six components in a tissue. To achieve satisfactory repeatability it was necessary to sample not less than 50 per cent of the total area of each section.

RESULTS

Histological findings—Biopsies were taken from two parts of the skeleton, the proximal femoral shaft and the cortex of the iliac crest. In all five patients the tissue from those sites which were known to be in the sclerotic phase of Paget’s disease gave the familiar histological and microradiographic picture of Paget’s disease with many surfaces showing evidence of bone formation or bone resorption. The microradiographs emphasised the scalloped resorption surfaces, the mosaic cement lines, and the considerable proportion of low density bone which the tissues contained (Fig. 1). The bone of the left femur of the man of forty (Case 1), which on radiological grounds was thought to be in the porotic phase of Paget’s disease, contained many resorption spaces and less osteoblastic activity than was seen in the opposite femur which was in the sclerotic phase. In the other patients the biopsies of bone from sites which were radiologically normal were difficult to assess as some degree of porosis was present in each case. Using the descriptions given by Amprino (1937) of the human femoral neck and
Fig. 1
A microradiograph of compact bone in the sclerotic phase of Paget's disease. (×40.) (Case 5.)

Fig. 2
Figure 2—Ultraviolet photomicrograph showing three markers in forming osteones in normal femoral cortex. (×60.) (Case 2.)

Fig. 3
Figure 3—Ultraviolet photomicrograph showing three markers in compact bone in the sclerotic phase of Paget's disease. (×60.) (Case 5.)
those of Jowsey (1960, 1963) and Jowsey and Gershon-Cohen (1964) of the age changes of the human femoral diaphysis as a frame of reference, the bone from the woman of fifty (Case 2) and the man of fifty-eight (Case 4) was tentatively classified as being within normal limits, while the cortex of the woman of sixty-eight (Case 5) was undoubtedly porotic. The left iliac crest in Case 4, a man of fifty-eight who was suffering from monostotic Paget’s disease of the right innominate bone, was extremely porotic and was possibly in the early phase of the disease. **Quantitative fluorescence microscopy findings**—When undecalcified sections of cortices were viewed in ultraviolet light, the tetracycline markers were seen as yellow lines circumscribing the bone which had formed in the marker interval. In normal bone (Fig. 2) the markers were in the form of concentric rings and quantitation presented few problems, although in one patient (Case 4) who, owing to a misunderstanding, took the marker doses at irregular intervals, it was necessary to identify the individual markers for the measurement of appositional growth rate. The bone involved in the disease process showed extensive tetracycline labelling (Fig. 3) and the majority of surfaces were either undergoing bone resorption or were the site of bone formation. In some surfaces the bone had been laid down at an angle oblique to the plane of the section, so that between 30 and 50 per cent of the markers were flared; this was a serious drawback in the accurate measurement of bone formation rate since every surface must be measured in this analysis. To achieve an approximation, the midpoint of the flare was assumed to correspond to the dividing line between old bone and the bone formed in the marker period; it was considered that there were sufficient numbers of clear markers to prevent any gross errors in the final value for the ratio of new bone area to the total area of bone in the complete section. Another feature of appreciable but
indeterminate importance was the resorption of labelled and unlabelled bone within the marker interval: sharp breaks in marker continuity were seen occasionally in the tissues and comparison with microradiographs proved convincingly that some of the new bone must have been resorbed almost as soon as it was formed. In this diseased tissue two markers are clearly to be preferred to three: for example, in Case 1 the porotic phase (Fig. 4) and the sclerotic phase (Fig. 5) presented a clearer picture than Case 5 (Fig. 3).

**TABLE II**

**RESULTS OBTAINED IN FIVE PATIENTS SUFFERING FROM PAGET’S DISEASE**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Skeletal site</th>
<th>Structural change</th>
<th>Mean appositional growth rate* and standard deviation (microns per day)</th>
<th>Mean bone formation rate† and standard deviation of samples (per cent per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal bone</td>
<td>Paget’s disease</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>M</td>
<td>Left femur</td>
<td>Paget’s disease: porotic</td>
<td>0.9 : 0.4</td>
<td>0.14 : 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right femur</td>
<td>Paget’s disease: sclerotic</td>
<td>1.4 : 0.4</td>
<td>0.63 : 0.05</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>F</td>
<td>Left femur</td>
<td>Normal</td>
<td>0.8 : 0.5</td>
<td>0.04 : 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right iliac crest</td>
<td>Paget’s disease: sclerotic</td>
<td>1.0 : 0.4</td>
<td>0.44 : 0.06</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>F</td>
<td>Left femur</td>
<td>Paget’s disease: sclerotic</td>
<td>1.0 : 0.5</td>
<td>0.40 : 0.10</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>M</td>
<td>Left femur</td>
<td>Normal</td>
<td>1.0 : 0.4</td>
<td>0.04 : 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right femur</td>
<td>Normal</td>
<td>1.0 : 0.4</td>
<td>0.03 : 0.02</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Left iliac crest</td>
<td>Paget’s disease: sclerotic</td>
<td>1.0 : 0.5</td>
<td>0.34 : 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right iliac crest</td>
<td>Paget’s disease: sclerotic</td>
<td>0.8 : 0.3</td>
<td>0.13 : 0.07</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>F</td>
<td>Left femur</td>
<td>Osteoporosis</td>
<td>0.7 : 0.3</td>
<td>0.04 : 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right femur</td>
<td>Paget’s disease: sclerotic</td>
<td>0.9 : 0.5</td>
<td>0.31 : 0.08</td>
</tr>
</tbody>
</table>

* Mean value obtained from not less than sixty observations.
† Mean value obtained from samples of 50 per cent of the total area of the biopsy.

**Appositional growth rate**—There were adequate numbers of clear markers in both the normal and the diseased tissue to allow accurate microscopic measurement of appositional growth rate. The essential problem in this estimation is the expression of the final value obtained from the measurement since the rate of deposition declines as the osteone is forming. In absolute terms, therefore, a mean value obtained from a sample of growing surfaces is of doubtful value. To avoid this problem some authors have calculated a mean osteone completion time (Vanderhoeft, Kelly and Peterson 1962; Frost 1964) or used a mathematical analysis of the marker diameters to determine a coefficient for growth regression (Lee 1964, Manson and Waters 1965). Obviously neither of these sophisticated techniques could be applied to disorganised diseased tissue and the best alternative seemed to be to measure the widest separation between markers in individual surfaces as they appeared during transverse scans of the section; it must be assumed that the final figure would be an expression of maximal osteoblastic function in the normal and the diseased tissues.

The values obtained from not less than sixty observations on each biopsy are given as a mean and a standard deviation in Table II. In normal bone the values ranged from 0.8 to 1 μ per day; in Paget’s disease the values in each patient were the same or slightly higher. In two cases (2 and 5) the increase of about 20 per cent was statistically significant (P>0.001).
Bone formation rate—The values obtained in this estimation in each biopsy are given in Table II. In the "normal" or non-Pagetic bone the values were about 0.04 per cent per day with a standard deviation of about \( \pm 0.02 \). This wide standard deviation is probably the result of inhomogeneity of remodelling within the tissue, since the quantitative techniques gave good repeatability for the individual samples (Lee 1963). The results obtained in the right iliac crest of the man of fifty-eight (Case 4) who had Paget's disease of the left innominate bone were about three times the normal femoral values, as was the bone formation rate in the porotic phase of the left femur of the man of forty (Case 1). The values in the sclerotic phase of the disease varied between 0.3 and 0.6 per cent per day and the standard deviations of the samples were comparatively lower than those in the normal tissues; this is probably an expression of homogeneity in the remodelling process.

DISCUSSION

An interesting feature of the material used in this investigation was the variation in the rate of remodelling in bone biopsies taken from normal and diseased bone in the same skeleton. The timing of administration of tetracycline markers was therefore of importance because it was necessary to label enough new bone in the normal tissue for accurate measurement while keeping the confusion of the picture seen in the diseased tissue to a minimum. In retrospect it seems that two marker doses given with a thirty-day interval provide the most satisfactory compromise.

Of the two parameters used to assess osteoblastic function, the appositional growth rate appeared to be more reliable in both normal and diseased tissue because the measurements were made on well-defined markers. In addition it was valid to compare results obtained in normal and diseased bone in different parts of the skeleton, as the range of appositional growth has been shown to be constant throughout the normal animal skeleton (Marotti 1963, Lee 1964, Manson and Waters 1965). The measurement of the maximum distance between the markers meant that the results obtained were biased towards the upper limits of osteoblastic function, although the value of 1 \( \mu \) per day for appositional growth in normal bone was similar to that obtained by Frost (1963) and Epker, Hattner and Frost (1964) in their comprehensive study of human bone formation. When the figures obtained in normal and diseased bone were compared, the difference was found to be slight; this suggests that there might be an upper limit to the rate at which bone matrix can be deposited on the individual surface.

Many factors lead to uncertainty in the interpretation of the values obtained for bone formation rate in both the normal and diseased tissue. In the normal tissue the clarity and definition of the markers gave reliability to the quantitative technique, but there was wide variation between individual samples taken from a single section. The variation was a reflection of the anatomical inhomogeneity of internal remodelling which is a prominent feature of the animal skeleton (Vanderhoef et al. 1962, Marotti 1963, Lee et al. 1965) and the human skeleton (Urist, Zaccalini, Macdonald and Skoog 1962; Frost 1963, 1964). The figures obtained in a small biopsy represent neither the whole bone nor the skeleton, and it would be incorrect to compare values for normal and diseased tissue if they were obtained from different parts of the skeleton. An additional factor to consider in the interpretation of the values obtained in the normal bones was the proximity of the biopsy site to an osteoarthritic hip (Cases 2, 4 and 5) around which there is alteration of mechanical stress and almost certainly an intensification of internal remodelling. Finally the normal tissue might be in an early stage of Paget's disease, histologically unrecognisable as yet, but manifest as an increase in internal remodelling. The values of about 0.04 per cent per day obtained for normal bone in the present investigation are therefore put forward with reservation, but it is of interest that they are of the same order as the figure of 0.02 per cent per day that Frost (1963) obtained in his study of the bone formation rate in the femoral diaphysis of a man of fifty-seven.
In the tissues affected by Paget's disease the first problem which arose in the interpretation of the figures for bone formation rate was the degree of inaccuracy in the quantitative technique. The use of an approximation in those surfaces which contained flared markers and the resorption of labelled and unlabelled bone in the marker period inevitably introduced an error which could not be assessed. It seems more likely that the small standard deviation of the sample values reflected homogeneity of the remodelling process within the biopsy sample rather than accurate quantitation. The divergent results obtained in the sclerotic and porotic phases of the disease suggest that the homogeneity of the remodelling rate is focal and that values would vary with the biopsy site as the disease progresses along a bone. The figures obtained in the sclerotic phase were of the order of 0.4–0.6 per cent per day or about ten times that found in unaffected bone. This comparative figure is of the same order as the ratio determined by the radioisotope studies of Bauer and Wendeberg (1959) and Macdonald (1960), although the value obtained by external counting reflects exchange processes within previously formed bone in addition to uptake by new bone.

The application of the tetracycline marker technique to the study of Paget's disease allows one broad generalisation to be made with confidence. While about ten times more bone is added to the diseased tissue than to the normal in unit time, the rate of deposition on the individual surface is increased only slightly. This suggests that the osteoblast in Paget's disease is behaving as a normal cell and is not the primary factor in the abnormal process.

SUMMARY

1. Normal and diseased bone was obtained by biopsy from five patients suffering from Paget's disease. The tissue was studied by histology, microradiography and quantitative fluorescence microscopy using tetracycline markers. Study of the morphological changes showed that two of the biopsies could be regarded as normal, while one was osteoporotic; two biopsy specimens were in the porotic phase of Paget's disease and the remaining five were in the sclerotic phase.

2. The tetracycline markers were used to measure the linear rate at which bone was deposited on individual surfaces (appositional growth rate) in \( \mu \) per day and the percentage volume of new bone added to the total volume of bone per day (bone formation rate). The values obtained for appositional growth rate in all the biopsies were of the order of 1 \( \mu \) per day, but slightly higher values were obtained in the diseased tissue of each individual. The bone formation rate in normal bone from the proximal femur was about 0.04 per cent per day, about 0.13 per cent per day in the porotic phase, and about 0.4 per cent per day in the sclerotic phase of Paget's disease.

3. Although these values must be accepted with some reservation, there seems to be no doubt that there is an upper limit of about 1 \( \mu \) per day to the rate of deposition of bone on an individual bone surface; this suggests that in Paget's disease the osteoblast behaves as a normal cell.

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REFERENCES


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